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A Stability Indicating Assay Method For Candesertan Tablets By High Performance Liquid Chromatography For Stability Studies

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ABSTRACT

A simple and stability indicating HPLC assay procedure had been developed and validated for candesertan tablets. The mobile phase consisted of buffer(6.8g KH_2PO_4 /1000ml water): methanol : in the ratio of (40:60) isocratic elution is carried out under ambient condition at flow rate of 1.0ml min^{-1} and detector was set at 220nm. The column selected was thermohypersil, C18, 5µm packing, 4.6mm×250mm and injection volume was 20µl. The procedure separated candesertan and potential degradation product. The retention time of candesertan is 11.3 min and asymmetry was 1.17. The instrument precision obtained was 0.18 %.The procedure provided a linear response in the range of 50-150% of target concentration(r=1.000). Forced degradation study shows, response of main drug is reduced in acid, alkali, peroxide thermal and sunlight degradation. The method was validated for accuracy, robustness and solution stability was obtained up to 14 hrs.

KEYWORDS

Candesertan;
Cardiovascular drug;
Force degradation;
Validation;
Stability studies.

INTRODUCTION

Candesertan is antihypertensive agent and angiotensin II receptor antagonist. Candesartan competes with angiotensin II for binding at the AT1 receptor subtype. As angiotensin II is a vasoconstrictor which also stimulates the synthesis and release of aldosterone, which results in a decreases in systemic vascular resistance[1]. Candesertan is chemically 2-ethoxy-3-[[4-[2-(2H-tetrazol-5-yl)phenyl] phenyl]methyl]-3H-benzoimidazole-4-carboxylic acid(Figure 1). Literature survey reveals that it is unofficial in U.S.P[2] and B.P[3]. Several techniques such as using fluorescence detector[4-5], spectrophotometry[6], voltametry[7] and HPLC[8]] have been reported for estimation of candesertan in pharmaceutical formulation and in biological samples.

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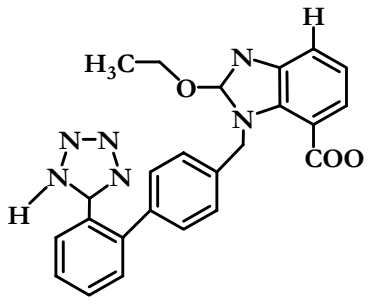

Figure 1: Chemical structure of candesertan

EXPERIMENTAL

Chromatographic conditions

The HPLC system used for this study consist of solvent delivery pump with autoinjector, and photodiode array detector and pentium 4 computer with data integrating software. The wavelength of the detector was set at 220 nm. Separation was carried out on a hypersil C-18, 250×4.6mm i.d, 5µm(Thermohypersil, U.K) using buffer(6.8 g KH_2PO_4 /1000ml water): methanol in the proportion of 40:60, v/v respectively as a mobile phase, at a flow rate of 1ml/min. The mobile phase was filtered through nylon membrane filter(0.45mm) and ultrasonically degassed prior to use. Chromatography was carried out at room temperature maintained at 20-24^{0}C.

Preparation of solutions

A working standard solution containing 500µg ml^{-1}. Candesertan was prepared by dissolving candesertan reference standard in mobile phase. A blend of candesertan tablets equivalent to 50mg of candesertan is transferred to 100ml volumetric flask. 20ml of mobile phase was added and sonicated for 5 minutes with immediate shaking and diluted with mobile phase to volume and mix. This solution was centrifuged at about 2000 RPM for 10 minute, and upper clear solution was used for injection.

Validation parameters

Method validation was performed as per USP 27-NF22[9]. The following validation parameters were addressed: specificity, precision, linearity, accuracy and solution stability of candesertan in mobile phase.

Specificity

Stress testing of the drug substance can help in identifying the likely degradation products, which in turn help's to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedure used[10] stress testing is done by exposing the candesertan to following conditions(TABLE 1).

TABLE 1: Summary of stress testing conditions for candesertan

S.No.	Degradation	Conditions	Figure No.
1	Acid	2ml, 2 M HCl and heated at 70^{0}C for 1 hr	2A
2	Alkali	2ml, 2 M NaOH and heated at 70^{0}C for 1 hr.	2B
3	Peroxide	1ml, 30% $H_2O_2$and heated at 70^{0}C for 1hr.	2C
4	Thermal	Heated at 70^{0}C for 1hr.	2D
5	Sunlight	Exposed for 2 hrs.	2E

Precision

The system precision was determined by performing six replicate injections of standard solutions. The method precision was determined by performing six consecutive assays of candesertan by preparing six independent samples.

Accuracy

The accuracy was evaluated by the recovery of candesertan at three different levels(80,100 and 120%) using three preparations for each level tested three times.

Linearity

The linearity of detector response for candesertan standard was determined by preparing and injecting solutions in the concentration range of 250-750µg/ml(50-150% of assay conc.) of candesertan standard.

Robustness

The robustness study helps us in demonstrating that transferring the methodology can be done successfully or not. In this study we had compared the results between normal operating conditions and by deliberately changing certain parameters like changing analyst, instrument, column(Inertsil, C18, 250 ×4.6mm, 5m).

Solution stability

The solution stability study was performed by

injecting a standard solution in duplicate at different time intervals, the peak areas were compared with the initial areas.

RESULT AND DISCUSSION

Under the chromatographic conditions employed, the sample showed sharp peaks of drug and good resolution with degradant peaks. The retention time of the drug was found to be 11.3±0.1min. The method developed was validated for specificity, precision, accuracy linearity, robustness and stability as per USFDA guidelines. The results of validation parameters are given in TABLE 2.

TABLE 2: Summary of the performance parameters of the HPLC procedure for candesertan

S.No.	Parameters	Observed value
1	System suitability	
	a. Theoretical plates	8155
	b. Tailing Factor	1.17
2	Instrument Precision	RSD 0.18%
3	Method Precision	Label Claim 93.64%
4	Linearity and range	Correlation coefficient(r)=1.0000
5	Accuracy	Mean recovery 93.15%
6	Specificity	Peak Purity of candesertan peak after degradation was 100%.
7	Robustness	Difference from original condition 0.44%
8	Solution stability	14 hrs

The result obtained from degradation study shows peak purity of candesertan was 100% as calculated by PDA detector, proving that no degradation product is interfering with the main peak. (Figure 2A-E). The % residual drug was calculated in comparison with the standard, which is 55.71, 62.16, 79.89, 91.15 and 86.87% for acid, alkali, peroxide, thermal and sunlight degradation respectively. System precison of 0.18% RSD and method precision shows a mean of 93.64% label claim with a RSD of 0.59% was obtained. The mean recovery data for each level is within accepted values(92.90, 93.10 and 93.38% label claim for 80, 100 and 120% level respectively). Therefore, these results indicated a good

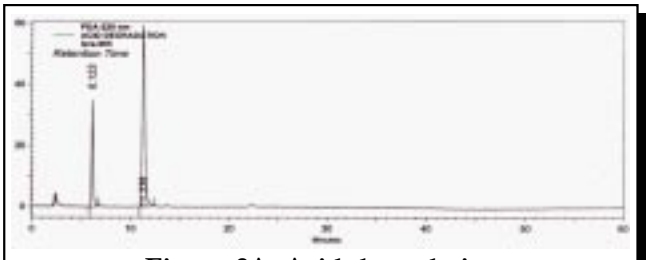

Figure 2A: Acid degradation

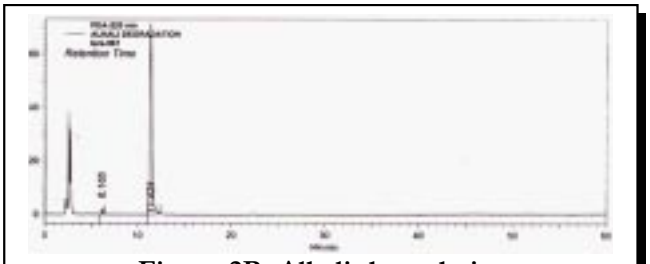

Figure 2B: Alkali degradation

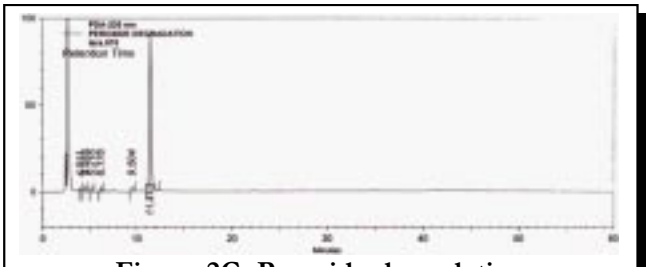

Figure 2C: Peroxide degradation

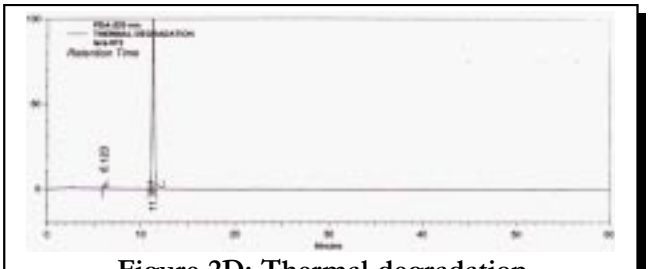

Figure 2D: Thermal degradation

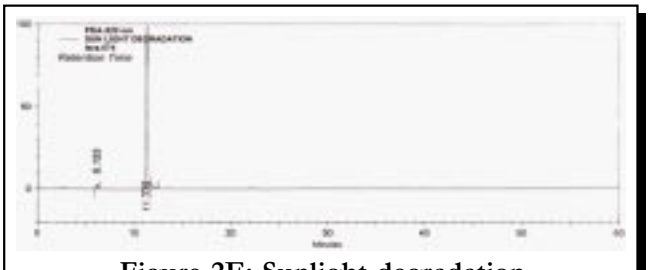

Figure 2E: Sunlight degradation

accuracy of the method for candesertan. The mean recovery was 93.15% label claim and % RSD was 0.26. A calibration curve was constructed using characteristic parameters for regression equation(y=a+bx) of the HPLC method obtained by least squares

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treatment of the results confirmed the good linearity of the method developed(r=1.000). The result obtained during robustness shows that by changing deliberately, some internal and external parameters of the method does not influence the results. Solution stability of 14 hrs was observed.

There was no cost effective method reported as per our knowledge which can be used on regular basis for quantification of candesertan, most of the reported method involves drugs in combinations or plasma matrix. we used previously reported data such as pKa value of candesertan which is 5.3[11], for which buffer choice of KH_2PO_4 is taken as it gives basic pH, helping ionization of the drug and various trials of organic phase methanol was tried to get desired retention time, so that all degradation products are well separated and peak shape of candesertan has good asymmetry. The previous procedures mention uses of short C18 columns as 4.6mm×12.5-15cm, where it was observed that it creates back pressure and resolution problem as in stability samples lot of impurities are generated and large number of samples are to be analyzed, A thermohypersil C18 column, 4.6mm×25.0cm, 5µ is selected as it resolved degradation peaks generated by stability samples. Though candesertan has lambda max at 238nm but the degradation peaks shows high response at 220nm so this wavelength proved to be ideal for stability indicating method.

CONCLUSION

A stability-indicating, rapid, cost effective and reliable HPLC assay method was developed for the assay of candesertan tablets useful for long and short term stability samples. This chromatographic assay fulfilled all the requirements such as specificity, precision, accuracy, linearity, robustness and solution stability up to 14 hrs. The peak shape obtained though out the study shows asymmetry of 1.17 as well as the retention time of the candesertan is 11.3(±0.1) min, showing a good column life and fast analysis of large numbers of samples in short time period, making method suitable for routine sample analysis and preferably for samples with short term stability and long term stability studies.

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