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RP-HPLC HYDROLYTIC STABILITY STUDY OF CANDESARTAN CILEXETIL

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ABSTRACT

In this study the rate and order of hydrolytic degradation of candesartan cilexetil drug standard under both basic and acidic conditions at room temperature were investigated. The obtained samples were then analyzed by using RP-HPLC. The column used was Shim-pack 15x4.6mm i.d, particle size 4.6 µm. The mobile phase was acetonitrile: formic acid (80:20 v/v) in isocratic elution mode. UV detection was performed at 254 nm. Base degradation experiments indicated a prominent degradation peak. Moreover, the drug was found to follow first-order degradation kinetics with a rate constant k_1 0.111 min^{-1} and a half-life ($t_{1/2}$) and shelf-life (t_{90}) 62.4 minutes and 9.45 minutes, respectively. Acid treatment of the drug showed that degradation took place that follows zero-order kinetics with rate constant k_0 value of 0.0229µg/ml. min, $t_{1/2}$ and t_{90} of 21 hours and 4 hours 16 minutes, respectively. The results of hydrolytic degradation showed that the prodrug is more sensitive to basic than acidic conditions.

KEYWORDS: Candesartan, stability, degradation, RP_HPLC.

INTRODUCTION

Drug stability is a vital element in the accurate and appropriate delivery of drug therapy to patients. Both the therapeutic efficacy of the treatment and safety of the therapy can be negatively affected by drug instabilities.[1-2] The purpose of stability studies is to establish a retest period or shelf life and label storage instructions applicable to all future batches manufactured and packaged under similar circumstances based on testing a minimum of three batches of the drug substance or product. The degree of variability of individual batches affects the confidence that a future production batch will remain within acceptance criteria

throughout its retest period or shelf life. Guidance documents for conducting stability study had been introduced by many international organizations such as ICH and WHO. These guidelines were directed towards dealing with drug substances as well as drug formulations.[3-10]

Candesartan cilexetil's chemical name is (1 RS)-1-[[(cyclohexyloxy) carbonyl] oxy] ethyl-2-ethoxy-1-[[2′-(1H-tetrazol-5-yl) biphenyl-4-yl] methyl]-1H-benzimidazole-7-carboxylate. Molecular formula: $C_{33}H_{34}N_6O_6$. It is a white powder, practically insoluble in water, freely soluble in methylene chloride and slightly soluble in anhydrous ethanol.[11] The chemical structure is shown below:

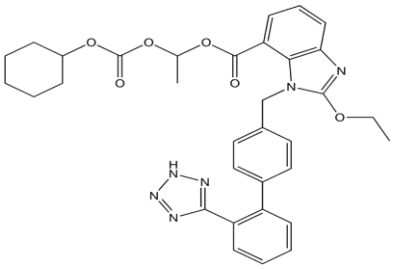

Figure 1 Candesartan cilexetil chemical structure.

Candesartan cilexetil is an angiotensin II receptor antagonist that is mainly indicated for the treatment of hypertension. It is an ester prodrug that is hydrolyzed during the absorption process from the gastrointestinal tract to the active candesartan as shown in Figure 2. The absolute bioavailability of Candesartan cilexetil following oral administration as tablets is 14%. Peak plasma concentrations of Candesartan occur about 3-4 hours after oral administration. More than 99% bound to plasma proteins. It is excreted in bile and urine mainly unchanged and a small amount as inactive metabolite. Terminal elimination half life is approximately 9 hours.[12]

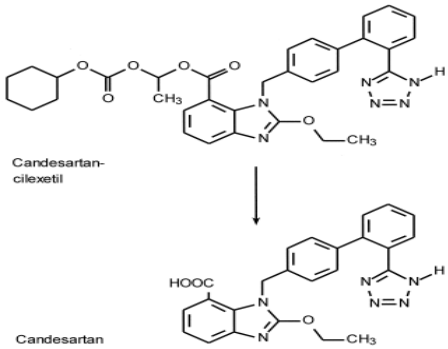

Figure 2 hydrolysis of candesartan cilexetil to the active Candesartan.

Drug stability is a major concern in the process of drug discovery, development and manufacturing. Several stability studies have been performed on Candesartan cilexetil exposing the drug to different stress conditions.[13-15] The purpose of this study is to investigate the stability of candesaratan under acid/ base treatment.

MATERIALS AND METHODS

- Hydrochloric acid 12.076 M AR (SHAMLAB Laboratory Chemicals, Syria)
- Sodium hydroxide AR (SUPERFIT LABWARE, India)
- Acetonitrile HPLC Grade (SHAMLAB Laboratory Chemicals, Syria)
- Formic acid 88% AR (Sigma-Aldrich Company, U.S.A)
- Candesartan cilexetil reference standard 99.75% purity (was kindly donated by AMIPHARMA Laboratories, Sudan).

Instrumentation

HPLC (Shimadzu Japan LCMS-2020, APCI-2020, DUIS-2020 and Shimadzu Japan LC-20AB, SIL-20A, CTO-20A, SPD-20AV), column C18 Shim-pack 150x4.6mm i.d., particle size 4.6 µm.

- Sonicator (Bandelin Sonorex, Germany)
- Sensitive balance (Voyager Ohaus, U.S.A)
- Hot air oven (Hirayama Manufacturing Corporation, Japan)
- Filter paper 45µm.
- Mobile phase flow rate1ml/min Wavelength, 254 nm, column oven temperature was 40°C and the injection volume was10µl. Microsoft office Excel 2007 was used in data.

METHODS

Preparation of Mobile Phase

A volume of 0.34ml of 88% formic acid was added to 100ml distilled water, the mixture was transferred to a 500ml volumetric flask and the volume was completed with acetonitrile, to obtain a solution of acetonitrile: formic acid 0.3% (80: 20 v/v). The mobile phase was sonicated, filtered through a 45µm membrane and degassed before use.

Preparation of Standard

An accurate weight of 50mg candesartan cilexetil was weighed and dissolved in 20ml acetonitrile. It was then sonicated. The solution was poured into a 50ml volumetric flask and

completed up to the mark; to obtain a solution of 1000µg/ml. 1ml of this solution was transferred to a 10ml volumetric flask and completed with acetonitrile to obtain a concentration of 100µg/ml.

Preparation of Samples for base induced degradation

An accurate weight of 50mg Candesartan cilexetil was weighed and dissolved in 20ml acetonitrile. The solution was sonicated and then transferred into a 50ml volumetric flask. It was then completed up to the mark with acetonitrile. 1ml of this solution was pipetted into a 10ml volumetric flask; 2ml of 0.1M sodium hydroxide was added and the mixture was completed to the mark with acetonitrile. The mixture was left to stand at room temperature and samples were collected after 15, 30, 45 and 60 minutes.

Preparation of Samples for acid induced degradation

An accurate weight of 50mg candesartan cilexetil was weighed and dissolved in 20ml acetonitrile. The solution was sonicated until complete dissolving, and transferred into a 50ml volumetric flask. It was then completed up to the mark with acetonitrile. 1ml of this solution was pipetted into a 10ml volumetric flask; 2ml of 0.1M hydrochloric acid was added and the mixture was completed up to the mark with acetonitrile. The mixture was left to stand at room temperature and samples were collected after 15, 30, 45, and 60 minutes and were then analyzed by RP_HPLC.

RESULTS AND DISCUSSION

In all the following chromatograms (a) stands for the chromatogram of the standard drug i.e. not being subjected to acid or base treatment and b-e refer to the chromatograms of the drug after, 15, 30,45 and 60 minutes after applying the specific stress factor, respectively.

Base Hydrolysis

drug samples were collected obtained by analyzing the samples collected at 15, 30, 45 and 60 minutes after base treatment and are shown in figures **3a-e)**.

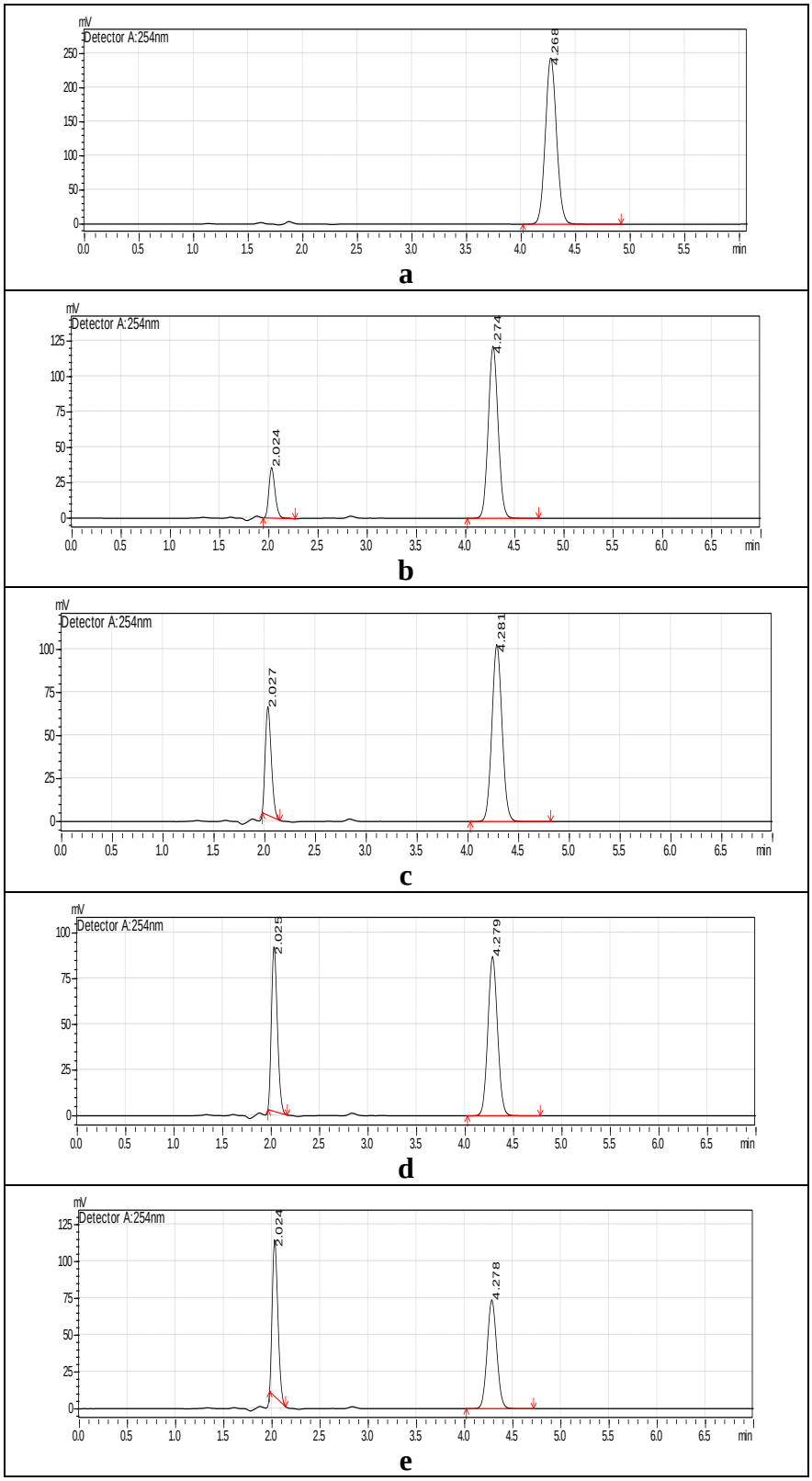

Figure 3a-e: chromatograms of candesartan before and after base treatment.

Candesartan cilexetil was eluted at t_R 4.268 minutes forming a well separated fine looking symmetrical peak with a steady baseline. It was observed during waiting for sample collection that there is production of fine bubbles in the solution of (Candesartan cilexetil

plus base) which was left to stand. The bubbles were not vigorous enough to say it was effervescence and they were reproducible i.e. they were produced every time Candesartan cilexetil was subjected to base.

From the chromatograms in **Figure 3b-e,** it is observed that an additional degradant peak was eluted at 2.0 minutes $t_{R.}$ The peak increases gradually with time.i.e. from 15 to 60 minutes, with a subsequent decrease in the candesartan cilexetil peak at 4.2 minutes $t_{R.}$. At time 45 minutes both the degradant's peak and the candesartan cilexetil peak have approximately equal peak heights, going further to time 60 minutes t_R the peak height of the degradant peak exceeded that of candesartan cilexetil. A summary of the assay content after each time interval is given in Table 1.

Table 1 Base degradation Candesartan cilexetil peaks' retention time (t_R), area and concentration.

Sample name	Retention time (minutes)	Area	Concentration(µg/ml)
Base degradation time 15	4.274	846,903	48.668
Base degradation time 30	4.281	716,118	41.153
Base degradation time 45	4.279	605,361	34.788
Base degradation time 60	4.278	513,518	29.510

Kinetic study

Candesartan cilxetil degradation was found to follow first-order degradation kinetics under basic conditions with a rate constant k_1 (slope) = -0.0111 min^{-1} as the equation of the line is:

y=-0.0111x+4.0513.

The half-life and shelf-life (t_{90}) for first order processes were calculated using the following equations:

$$t1/2 = \frac{0.693}{k1}$$

$t_{1/2}$=0.693/0.0111=62.4 min.

$$t90 = \frac{0.105}{k1}$$

t90=0.105/0.0111=9.45 min.

Therefore after approximately 62 minutes of exposure to sodium hydroxide 0.1M at room temperature the concentration of Candesartan cilexetil will drop to half its initial concentration. On calculation of the shelf life (t_{90}), it was found to be approximately.

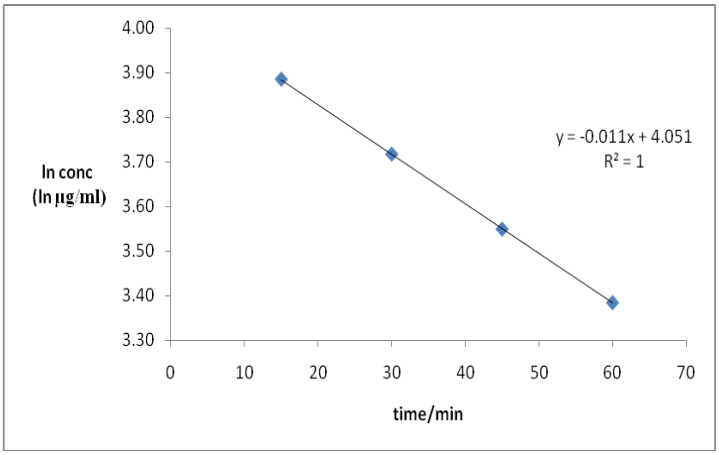

Figure 4 Plot of ln Conc. vs. time (first-order) for base degradation.

The relationship between the increasing degradant peak area and the decreasing Candesartan peak area can also be displayed graphical as shown in **Figure 5**. Since in chromatographic analysis the area under the curve is directly proportional to the concentration, we can plot the area under the curve as a representative for concentration.

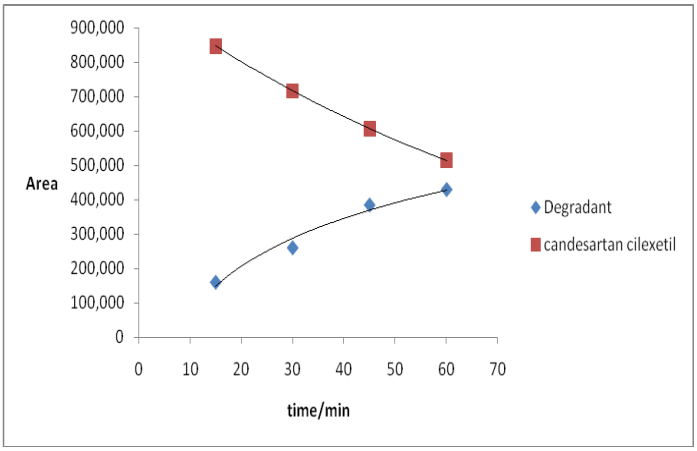

Figure 5 Plot of Candesartan cilexetil and degradant peak area with time.

The this symmetrical pattern shown in figure 5 indicates that the prodrug degrades to only one product that is to say it follows one degradation pathway under basic condition in room temperature. This is also supported by the literature which shows one degradation product in their base degradation experiments.[16]

Acid Hydolysis

Candesartan cilexetil drug solution was subjected to exactly 2ml of 0.1M hydrochloric acid, then samples were withdrawn from the solution ever 15 minute interval , collecting a sample at 15, 30, 45, 60 minutes from the point of adding the base to the solution. In acid degradation this timing interval was chosen to apply consistence and a fair comparison to the degradation pattern under basic conditions.

Figure 6a is for the drug standard before addition of the acid and the following chromatograms were obtained by analyzing the samples collected at 15, 30, 45 and 60 minutes by HPLC are shown in **Figures 6b-e**.

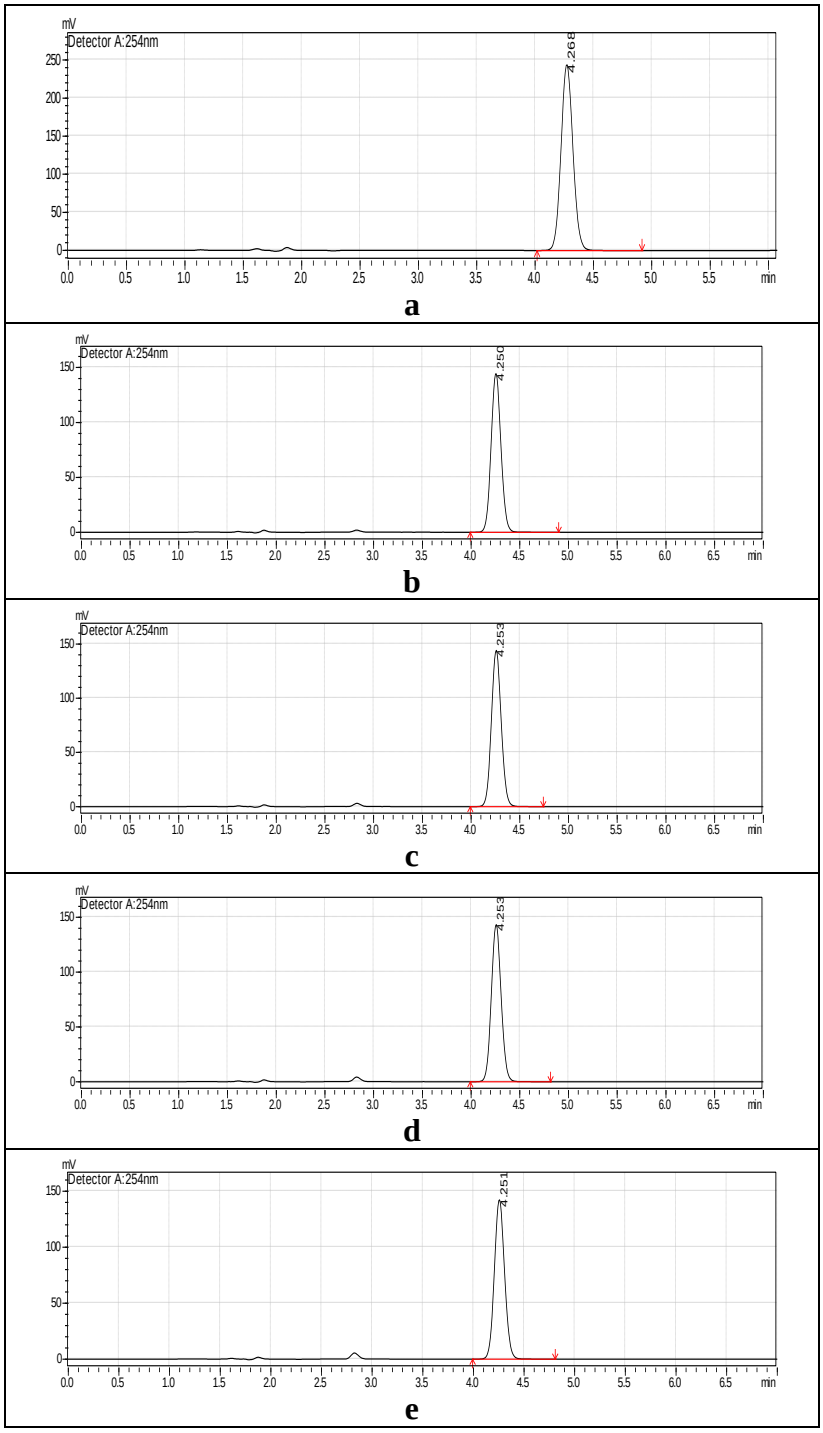

Figure 6a-e: chromatograms of candesartan before and after acid treatment.

In acid degradation experiments there was no production of bubbles in contrast to when Candesartan cilexetil solution was exposed to base.

From the chromatograms shown in **Figures 6b-e**. there was no significant degradant peak apparent in the chromatograms, in comparison to the very obvious degradant peak shown in the chromatograms obtained from base forced degradation experiments. There might be a degradant product produced but its concentration is below the detection limit of the instrument. Another explanation for lack of degradant peak is that all our readings are examined at a fixed detection wavelength of 254 nm and the degradant peak might be better quantified at another wavelength. The use of a photo-diode array detector might solve the latter suggestion for lack of degradant peak. A summary of the assay content after each time interval is given in Table 2.

Table 2 Acid degradation Candesartan cilexetil peaks' retention time (t_R), area and concentration.

Sample name	Retention time (minutes)	Area	Concentration(µg/ml)
Acid degradation time 15	4.250	1,017,458	58.469
Acid degradation time 30	4.253	1,012,005	58.156
Acid degradation time 45	4.253	1,005,896	57.805
Acid degradation time 60	4.251	999,606	57.444

Candesartan cilexetil in acidic media proceed with a zero-order pattern of degradation with a zero order rate constant k (slope)= 0.0229µg/ml min as the equation of the line is y=-0.0229x+58.826.

The half-life and shelf-life (t_{90}) for zero order processes were calculated using the following equations:

$$t1/2 = \frac{0.5[Co]}{k}$$

$t_{1/2}$ = (0.5x 58.826)/0.0229=1284.4 min.

$$t90 = \frac{0.1[Co]}{k}$$

t90= (0.1x58.826)/0.0229=256.9 min.

Therefore after approximately after 1284 minutes (i.e. 21 hours) the concentration of candesartan cilexetil under acidic stress at room temperature will fall to half its initial concentration. The concentration of the drug will drop by 10% after 257 minutes (i.e. approximately 4 hours and 16 minutes) of exposure to the acid treatment, according to the t_{90} calculation.

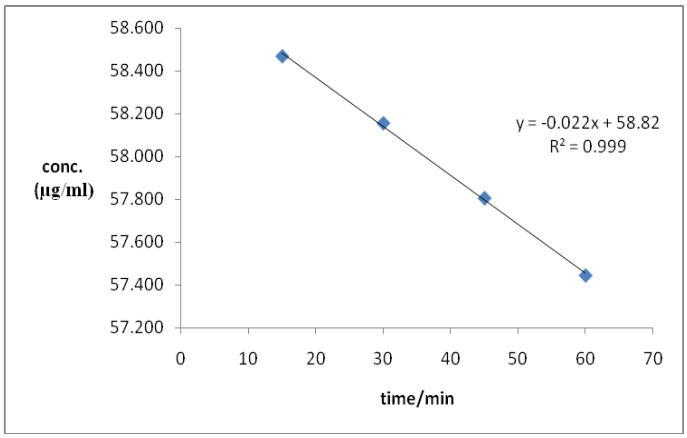

Figure 7 Plot of Conc. vs. time (zero-order) for acid degradation.

Comparison of hydrolytic degradation patterns

A comparison of the drug content as a function of time is depicted in Table 3.

Table 3: Comparison between Candesartan cilexetil in acidic and basic media.

time/min	Conc. in acidic media (µg/ml)	conc. in basic media (µg/ml)
15	58.469	48.668
30	58.156	41.153
45	57.805	34.788
60	57.444	29.510

Subjecting Candesartan cilexetil to equimolar concentrations of acid (0.1M hydrochloric acid) and base (0.1 M sodium hydroxide) allows a fair comparison of rates and order of degradation in acidic and basic medium. On analyzing our results it was obvious that Candesartan Cilexetil degrades at a faster rate under basic conditions than in acidic conditions regardless the order of reaction.

On comparison of their $t_{1/2}$, Candesartan cilexetil's $t_{1/2}$ under basic condition is 62 minutes while under acidic condition 1284 minutes (21 hours) showing a very slow degradation rate under acidic than basic stress. The shelf-life under basic and acidic condition was found to be 10 minute and 4 hours 16 minutes respectively, indicating a faster drop to 90% of drug concentration in basic than acidic stress.

CONCLUSION

Candesartan cilexetil follows first-order degradation kinetics under basic conditions at room temperature, with a first-order rate constant k_1 0.111 min^{-1} and a half-life ($t_{1/2}$) and shelf-life (t_{90}) 62.4 minutes and 9.45 minutes respectively.

Under acidic conditions also carried out at room temperature, Candesartan cilexetil follows zero-order degradation kinetics with a zero-order rate constant k_0 0.0229µg/ml. min. and a $t_{1/2}$ and t_{90} of 21 hours (1284 minutes) and 4 hours 16 minutes (257 minutes) respectively.

On comparison of both hydrolytic rates (acid and base) showed that Candesartan cilexetil is more sensitive to basic than acidic degradation.

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