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Spectrophotometric Determination of Candesartan Cilexetil in Presence of Its Alkaline Induced Degradation Product

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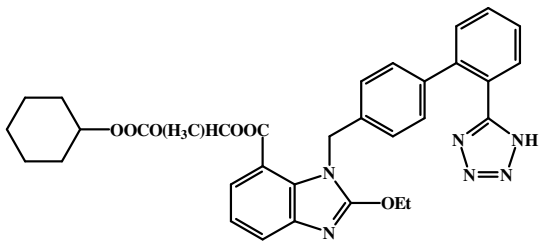
The present work describes a simple stability-indicating second derivative spectrophotometric assay method for determination of an antihypertensive drug, candesartan cilexetil in presence of its alkaline degradation product, candesartan. The method was satisfactory validated with respect to linearity, precision, accuracy, selectivity and sensitivity. The response was linear in the concentration range of 4-32 μg mL^{-1} (r = 0.99995) at wavelength 291.2 nm, which was the zero crossing point of candesartan in methanol. The detection and quantitation limits were 0.33 and 1.00 μg mL^{-1}, respectively. The suggested method was successfully applied for the analysis of candesartan cilexetil in bulk and in commercial tablets. The results were favourably compared statistically to that obtained by a reference method.

Key Words: Candesartan cilexetil, Candesartan, Second derivative spectrophotometry, Stability-indicating method.

INTRODUCTION

Candesartan cilexetil belongs to the class of angiotensin receptor antagonists and acts by competitively to angiotensin II receptor type I, thus preventing actions of angiotensin II.



Structure of candesartan cilexetil, m.f. $C_{33}H_{34}N_6O_6$; m.w. 610.67

The drug finds most significant clinical use in the treatment of hypertension of all grades[1]. Chemically, candesartan cilexetil is an ester prodrug of its active metabolite candesartan (C.V. 11974), to which it owes its therapeutic effect, by the action of some endogenous esterases[2].

The chemical stability of candesartan cilexetil has been studied in plasma and bioanalytical samples[3]. Under these conditions the drug was found to be susceptible to hydrolysis resulting the removal of cilexetil moiety. Few methods for the determination of candesartan cilexetil have been reported in literature. HPLC methods were reported for determination of candesartan cilexetil or candesartan with some angiotensin II receptor antagonists with or without hydrochlorothiazide as a diuretic drug[4-6]. Also, HPLC methods were reported for determination of candesartan cilexetil in tablets, as a single component[3,7-9], in combination with candesartan and a metabolite (M II)[10] in human plasma and urine and with hydrochlorothiazide simultaneously in pharmaceutical formulations[11-13]. Capillary electrophoresis methods were reported for simultaneous analysis of several angiotensin II receptor antagonists including candesartan cilexetil[14-17]. Other methods such as voltametry[18-20] and HPTLC-densitometry[21] were reported for determination of candesartan cilexetil.

The only spectrophotometric methods reported for determination of candesartan cilexetil were the first order derivative for it in tablets[22] as a single component or simultaneously with hydrochlorothiazide[23] or for simultaneous determination of candesartan and hydrochlorothiazide in tablets[24].

Till present, no stability-indicating methods were reported for determination of candesartan cilexetil in presence of its alkaline degraded product, candesartan. The scientific novelty of the present work is that the suggested spectrophotometric second derivative method is simple, rapid, selective, less expensive and less time consuming compared with other published chromatographic methods. The focus of the present work study is to develop and validate a simple stability-indicating method for determination of candesartan cilexetil in presence of its alkaline degradate (candesartan) for the quality control of candesartan cilexetil in its pharmaceutical preparations.

EXPERIMENTAL

The spectrophotometric measurements were made with Ultrospec 2000,UV/Vis spectrophotometer, Amersham Pharmacia Biotech with Swift II Application, Biochrom Ltd., Cambridge U.K. The second derivative spectra of the solutions were recorded in 1 cm matched quartz cells against solvent blank over the range 200-400 nm. The optimal condition for recording the spectra to achieve good reproducibility included scan speed at 6000 nm/min, slit width at 2 nm. The ordinate maximum and minimum settings were + 0.40 and -0.20 for the proposed method and + 0.40 and -0.40 for the reference first derivative method, respectively.

Pure standard: Candesartan cilexetil was kindly supplied from Jazeera Pharmaceutical Industries(JPI), Riyadh, Saudi Arabia. It was used as received without purification (its purity was 99.98 %).

Pharmaceutical dosage forms: (1) Atacand 16 tablets, manufactured by AstraZeneca-Egypt under license of Astra Zeneca, Sweden. The Batch No. was 90123. (2) Candesar 8 tablets, produced by PHARAONIA Pharmaceuticals, Pharo Pharma (Egypt), under license of Takeda Pharmaceutical Company Ltd. The Batch No. was 1409002.

Degraded product: 0.4 g of candesartan cilexetil powder was transferred into 250 mL stoppered flask, dissolved in 25 mL methanol, completed to 100 mL with 2 N NaOH and refluxed with magnetic stirring at 80 ºC for 3 h. Complete hydrolysis was followed *via* TLC using chloroform:methanol (80/20 v/v) as a developing system. The solution was neutralized with 4 N HCl solution till pH 3, then the degraded product was extracted with chloroform (6 × 20 mL). The extract was evaporated at room temperature and the degradate powder was collected and elucidated by IR spectrometry.

All chemicals used throughout this work (methanol, chloroform, HCl and NaOH) were of BDH, Poole, UK and the solvents were of spectroscopic grade.

Standard solutions: (a) Stock standard solutions of candesartan cilexetil and its alkaline degradate containing 1 mg mL^{-1} were prepared separately in methanol. (b) Working solutions were prepared (100 µg mL^{-1}) by suitably diluting the stock standard solutions.

Laboratory prepared mixtures: Solutions containing different ratios of candesartan cilexetil and its alkaline product were prepared to contain 20-80 % of alkaline degradate.

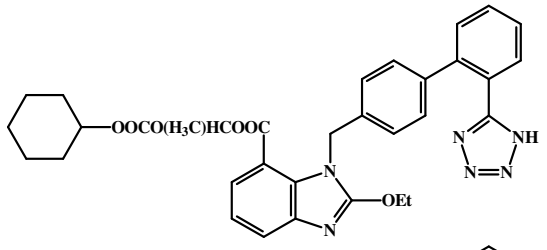
Construction of calibration graph for D^2 spectrophotometric method: Aliquots of candesartan cilexetil working solution (100 µg mL^{-1}) equivalent to 40-320 µg were accurately transferred into a series of 10 mL volumetric flasks. The volume was completed to the mark with methanol. The second derivative absorption spectra of the UV-spectrum of each solution were recorded against methanol, as a blank, according the conditions described before. Calibration graph was obtained by plotting the peak amplitude at 291.2 nm (corresponding to zero-crossing of the degraded product) of D^2 spectra *versus* the corresponding concentration of candesartan cilexetil and the regression equations were computed.

Application to pharmaceutical dosage forms: Fourteen tablets of each Candesar 8 tablets and Atacand 16 tablets were powdered and mixed well. An accurately weighed amount of the powder equivalent to 50 mg of candesartan cilexetil of each was transferred into two separate 100 mL volumetric flasks. 75 mL of methanol were added, sonicated for 0.5 h, completed to volume with methanol, to obtain 0.5 mg mL^{-1} stock solution, and filtered. The solution was diluted to the same concentrations of the appropriate working solutions and proceeded according to the procedure mentioned above. The nominal content of candesartan cilexetil in each tablets was calculated from the calibration graph prepared simultaneously or applying the regression equation.

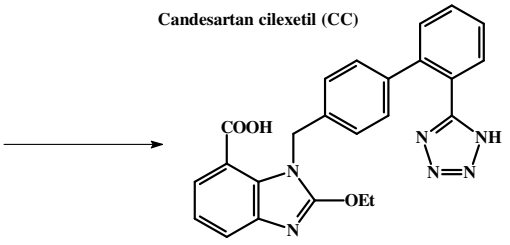
Analysis of the laboratory prepared mixtures: The second derivative absorption spectra of the UV-spectrum of each mixture of candesartan cilexetil and its alkaline product (20-80 % of the latter) were recorded as mentioned before. The concentration of candesartan cilexetil was calculated by referring to the calibration graph or the regression equation prepared early.

RESULTS AND DISCUSSION

Candesartan is marketed as the cyclohexyl 1- hydroxyethyl carbonate (cilexetil) ester, known as candesartan cilexetil. Candesartan cilexetil is metabolized completely by esterases in the intestinal wall during absorption to the active candesartan moiety. The use of a prodrug form increases the bioavailability of candesartan. Upon refluxing candesartan cilexetil with alkali, the carboxylic acid (candesartan) was obtained.



Candesartan cilexetil (CC)



Candesartan (CV-11974)

So the determination of candesartan cilexetil in presence of its alkaline degradation was essential. The International Conference on Harmonization (ICH) guideline entitled "stability testing of new drugs substances and products" requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substances[25]. An ideal stability-indicating method is one that quantifies the standard drug alone and also resolves its degradation products.

The complete degradation was produced which confirmed by using the above mentioned TLC system. The R_f values of the compounds were found to be 0.78 and 0.24 for the candesartan cilexetil and Candesartan, respectively. The structure of the degradate was elucidated by IR spectrometry. The IR spectrum of candesartan cilexetil showed a characteristic band at 1750 cm^{-1}, indicating the presence of carbonyl group while the IR spectrum of the degradate showed the same band but

shifted to 1705 cm^{-1} and a new broad band at 3200 cm^{-1} indicating the presence of a hydroxyl group of carboxylic acid (due to hydrolysis).

The focus of the present work is to develop an accurate, specific, reproducible and sensitive stability-indicating method for the determination of candesartan cilexetil in presence of degradation product. The zero order absorption spectra of candesartan cilexetil and its alkaline degradate showed similarity and several overlapping (Fig. 1) which interfere with the direct determination of candesartan cilexetil.

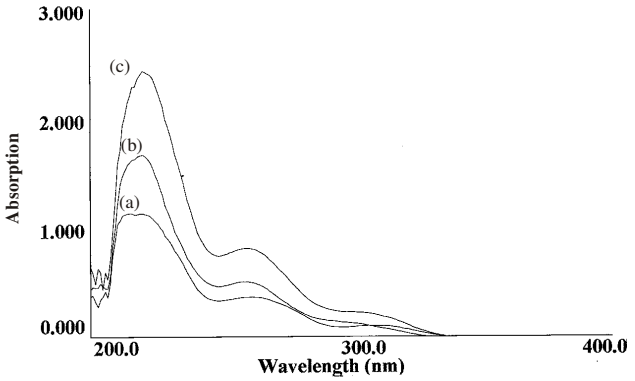


Fig. 1. Absorption spectra of candesartan cilexetil (a), Candesartan (b) 10 μg mL^{-1} each in methanol and their mixture (c)

Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands and for eliminating the effect of baseline shifts and baseline tilts by using the first or higher derivatives of absorbance with respect to wavelength[26]. A rapid simple and low cost spectrophotometric method based on measuring the peak amplitude of D^2 spectrum of candesartan cilexetil at 291.2 nm (corresponding to zero crossing of the degradate) was developed with good selectivity without interference of alkaline degradate, as shown in Fig. 2.

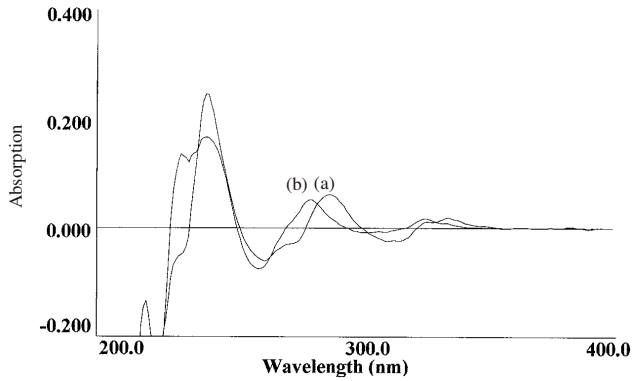


Fig. 2. Second deritvative spectra of (a) 5 μg mL^{-1} candesartan cilexetil and (b) 20 μg mL^{-1} candesartan in methanol

Different solvents were tried to resolve their overlapping as methanol, ethanol, butanol, acetonitrile, 0.05 N NaOH and 0.05 N HCl, in each of these solvents derivatization was done; zero-crossing of degradate corresponding to peak of candesartan cilexetil was obtained by applying D^2 technique using methanol as a solvent. The spectra showed good resolution and a linear correlation was obtained at 291.2 nm between the peak amplitude and the corresponding concentration in the range of 4-32 μg mL^{-1}; from which the linear regression equation was computed and found to be:

$$D^2 = - 0.0008 + 0.0047C \quad (r = 0.99995)$$

where, D^2 is the peak amplitude at 291.2 nm, C is the concentration of candesartan cilexetil in μg mL^{-1} and (r) is the correlation coefficient. The high value of correlation coefficient and small intercept indicate good linearity of the calibration graph. Statistical analysis of the data gave small values of standard deviation of the residuals (S y/x), of slope (Sb) and of intercept (Sa), % Er and % RSD as indicated in Table-1.

TABLE-1
PERFORMANCE DATA OF THE PROPOSED D^2 METHOD FOR DETERMINATION OF CANDESARTAN CILEXETIL

Parameter	Value
Linearity (μg mL^{-1})	4-32
LOD (μg mL^{-1})	0.33
LOQ (μg mL^{-1})	1.00
Correlation coefficient (r)	0.9995
Slope	0.0047
Intercept	-0.0008
$S_{y/z}$ (Standard deviation of residuals)	2.2×10^{-6}
S_a (Standard deviation of intercept)	4.70×10^{-6}
Sb (Standard deviation of slope)	2.32×10^{-5}
% RSD	0.71
% Er (%RSD /√n)	0.25

The proposed method is valid for determination of candesartan cilexetil in up to 80 % of its alkaline degradation in different laboratory prepared mixtures, as presented in Table-2.

TABLE-2
DETERMINATION OF CANDESARTAN CILEXETIL IN LAPORATORY PREPARED MIXTURES BY THE PROPOSED SECOND DERIVATIVE SPECTROPHOTOMETRIC METHOD

Alkaline product		Candesartan cilexet		
Added (μg mL^{-1})	%	Taken (μg mL^{-1})	Found (μg mL^{-1})	Found (%)
5.0	20	20.0	19.832	99.16
10.0	40	15.0	15.132	100.88
12.5	50	12.5	12.451	99.61
15.0	60	10.0	10.078	100.78
20.0	80	5.0	5.062	101.24
Mean ± SD			100.33 ± 0.90	

N.B. Each result is the average of three separate determinations.

Accuracy and precision: The proposed method was evaluated by studying the accuracy as per cent relative error (% Er) and precision as per cent relative standard deviation (% RSD) using three preparations with suitable concentration, in presence of a fixed concentration of alkaline degradate, as shown in Table-3. The intraday (n = 3) and interday (n = 3) accuracy calculated as % relative error was found to be 0.19-0.58 % and 0.34-0.47 % for candesartan cilexetil, respectively. The repeatability of the assay was found to be within 0.33-1.00 % (n = 3) at 10, 20, 30 μg mL^{-1}, the reproducibility of the assay at the same concentration levels was found to be within 0.59- 0.81 % (n = 3).

TABLE-3
ACCURACY AND PRECISION DATA FOR CANDESARTAN CILEXETIL USING D^2 METHOD

Alkaline product	Candesartan cilexetil		
Added (μg mL^{-1})	Taken (μg mL^{-1})	Found (μg mL^{-1})	Found (%)
Intraday[a]			
10	10	9.868	98.68
	10	9.887	98.87
	10	9.933	99.33
Mean ± SD			98.96±0.33
% RSD			0.33
% Er			0.19
10	20	19.924	99.62
	20	20.254	101.27
	20	20.188	100.94
Mean ± SD			100.61±0.87
% RSD			0.87
% Er			0.50
10	30	30.306	101.02
	30	30.612	102.04
	30	30.006	100.02
Mean ± SD			101.03±1.01
% RSD			1.00
% Er			0.58
Interday[b]			
16	10	10.189	101.89
	10	10.075	100.75
	10	10.038	100.38
Mean ± SD			101.00±0.79
% RSD			0.78
% Er			0.45
16	20	19.614	98.07
	20	19.902	99.51
	20	19.880	99.40
Mean ± SD			98.99±0.80
% RSD			0.81
% Er			0.47
16	30	30.612	102.04
	30	30.303	101.01
	30	30.303	101.01
Mean ± SD			101.35±0.60
% RSD			0.59
% Er			0.34

N.B. Each result is the average of three separate determinations.
[a]The intraday (n=3)Average of three different concentrations repeated three times within day. [b]The interday (n=3),Average of three different concentrations repeated three times in three successive day

The results of the proposed method for determining the purity of candesartan cilexetil were favourably compared with those obtained using the reference method[22]. The latter method depends on measuring the first derivative (D^1) of candesartan cilexetil, as a single component, at 270.1 nm. Statistical analysis of the results obtained by the proposed and reference methods showed no significant differences in the performance of the two methods using the Student's t- test and Variance ratio, F-test (Table-4)[27]. The proposed procedure offers additional advantages over the reference procedure in that the proposed is more sensitive with good accuracy and precision and considered as a stability-indicating method for determination of candesartan cilexetil in presence of its alkaline degradation product.

Limit of quantitation (LOQ) and limit of detection (LOD): The limit of quantitation was determined by establishing the lowest concentration that can be measured according to ICH Q2B recommendation[28] below which the calibration graph is none linear and was found to be 1.00 μg mL^{-1}. The limit of detection was determined by establishing the minimum level at which the analyte can be reliably detected (S/N = 3). It was found to be 0.33 μg mL^{-1}.

TABLE-4
STATISTICAL ANALYSIS OF THE RESULTS OBTAINED BY THE PROPOSED AND REFERENCE METHODS

Proposed method			Reference method [22]	
Taken (μg mL^{-1})	Found (μg mL^{-1})	Found (%)	Taken (μg mL^{-1})	Found (%)
4	4.021	100.53	8	100.38
8	8.128	101.60	16	99.17
12	11.958	99.65	24	100.01
16	16.234	101.46	28	101.70
20	20.276	101.38	32	101.34
24	24.425	101.77	38	101.32
28	28.129	100.46	–	–
32	32.300	100.94	–	–
n		8		6
Mean ± SD		100.97±0.72		100.65±0.97
Varince		0.52		0.94
Student's t-value		0.71 (2.18)*		
Variance ratio F-value		1.81 (3.97)*		

*Tabulated values at P = 0.05 [27].

Tablet analysis: The proposed D^2 method was applied to the determination of candesartan cilexetil in its commercial tablets, the results were shown in Table-5. The validity of the method was assessed by applying the standard addition technique (Table-6), the results of analysis of the commercial tablets and the recovery study (standard addition method) suggested that there are no interference from any excipients which are normally present in tablet formulations.

TABLE-5
ASSAY OF CANDESARTAN CILEXETIL IN FORMULATION USING THE PROPOSED AND REFERENCE METHODS

Parameter	Proposed method			Reference method [22]	
	Taken (μg/mL)	Found (μg/mL)	Found (%)	Taken (μg/mL)	Found (%)
Atacand 16 tablets	10	10.015	100.15	10	99.35
	16	16.094	100.59	16	99.47
	20	20.008	100.04	24	100.43
	24	23.959	99.83	30	100.70
Mean ± SD		100.15±0.32		99.99±0.68	
Student's t-test		0.43			
Variance ratio F-value		4.47			
Candesar 8 tablets	10	10.024	100.24	10	99.88
	16	16.024	100.15	16	100.70
	20	19.898	99.49	24	100.35
	24	23.971	99.88	30	100.57
Mean ± SD		99.94±0.34		100.13±0.63	
Student's t-test		0.53			
Variance ratio F-value		3.43			

N.B. Each result is the average of three separate determinations.
Tabulated t-test and F test are 2.45 and 9.28 at P = 0.05 respectively.

The results for the determination of candesartan cilexetil in tablets obtained by the proposed method were compared with the D^1 reference method[22]. Statistical analysis of the results was performed with regard to accuracy and precision

TABLE-6
ASSAY OF CANDESARTAN CILEXETIL IN FORMULATION BY APPLICATION OF STANDARD ADDITION METHOD USING THE PROPOSED D^2 METHOD

Preparation	Taken (µg/mL)	Added (µg/mL)	Found (µg/mL)	Found (%)
Atacand 16 tablets	10	10	20.026	100.13
	12	16	28.028	100.10
	20	10	29.973	99.91
	24	8	31.955	99.86
Mean ± SD				100.00±0.13
Candesar 8 tablets	10	10	19.994	99.97
	12	16	27.994	99.98
	20	10	30.009	100.03
	24	8	32.058	100.18
Mean ± SD				100.04±0.10

N.B. Each result is the average of three separate determinations.

using Student 't- test and F- ratio, as presented in Table-5, there is no significant difference between the proposed and the reference methods with regard to accuracy and precision.

Conclusion

The present work is concerned with the determination of candesartan cilexetil in presence of its alkaline -induced degradation product. In this work a simple, sensitive and rapid method is described for determination of candesartan cilexetil in pure form or in pharmaceutical formulations.

Reviewing literature in hand no other spectrophotometric methods concerned with the determination of candesartan cilexetil in presence its alkaline degradation and no synthetic mixture were prepared to check the specificity of the method.

D^2 spectrophotometric methods are well established teqnique that are able to enhance the resolution of overlapping bands.This method has the advantages of being lower costing, rapid and is suitable for quality control laboratories, where economy and time are essential.

From the results obtained, it is concluded that the suggested method showed high sensitivity, accuracy, specicifity and reproducibility and can be used as stability indicating method.

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