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Stability-indicating bivariate spectrophotometric method for determination of candesartan cilexetil in presence of its alkaline induced degradation product application to tablets and content uniformity testing

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ABSTRACT

A simple, sensitive, selective and precise stability-indicating method for the determination of candesartan cilexetil (CC) in presence of its alkaline degradate and in tablets was developed and validated. The method is based on determination of CC by the bivariate calibration depending on simple mathematic algorithm which provide simplicity and rapidity. The method showed good linearity in the range of 1-12 μg mL^{-1} at 225 and 2-12μgmL^{-1} at 250 nm with mean percentage recovery 100.29±0.64.CC can be determined in the presence of up to 80 % of its alkaline degradate, the selectivity of the method was checked using laboratory prepared mixtures. The proposed method has been successfully applied to the analysis of CC in bulk and in commercial tablets without interference from additives or excipients and the results were satisfactory compared with a reference method. Also, the suggested method was successfully applied to the content uniformity testing.

KEYWORDS

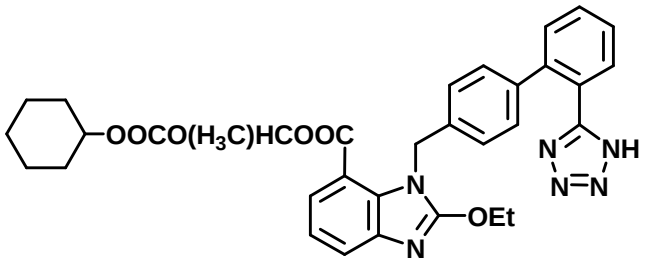
Candesartan cilexetil;
Candesartan;
Stability-indicating;
Bivariate method.

INTRODUCTION

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

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 sus-



Candesartan cilexetil (CC)

Molecular formula; $C_{33}H_{34}N_6O_6$

Molecular weight: 610.67

ceptible 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 bivariate method is simple, rapid, selective, less expensive and less time consuming compared with other published chromatographic methods. The focus of the present work study was 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 dosage forms.

EXPERIMENTALE

Instruments

The spectrophotometric measurements were made with Ultrospec 2000, UV/VIS Spectrophotometer, Amersham Pharmacia Biotech with Swift II Application, Biochrom Ltd, Cambridge U.K. The solutions were recorded in 1-Cm matched quartz cells against methanol as a solvent blank over the range 200-400 nm.

Materials

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

- Atacand 16 tablets, manufactured by AstraZeneca – Egypt under license of AstraZeneca, Sweden. The Batch No. was 90123.
- 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 2N NaOH and refluxed with stirring at 80°C for 3hrs. Complete hydrolysis was followed via TLC using chloroform/ methanol (80/20 v/v) as a developing system. The solution was neutralized with 4N HCl solution till pH 3, then the degradate was extracted with chloroform (6X 20 mL). The extract was evaporated at room temperature and the degradate powder was collected and elucidated by IR spectroscopy.

Chemicals and reagents

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

- Stock standard solutions of candesartan cilexetil and its alkaline degradate containing 1mg mL^{-1} were prepared separately in methanol.
- Working solutions were prepared (100 $\mu g\ mL^{-1}$) 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.

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PROCEDURE

Construction of calibration graphs for the bivariate spectrophotometric method

Into two separate sets of 10-mL volumetric flasks, aliquots equivalent to 10-120 μg mL^{-1} of CC and its alkaline degradate were transferred from their working solutions (100 μg mL^{-1}) in methanol. The volume was completed with methanol. The regression equations, at 225nm and 250nm, for CC and its alkaline degradate were computed.

Analysis of bulk substance

The method mentioned above was applied to the determination of the purity of CC raw material, and the percent recoveries were calculated by application in the bivariate equations.

Analysis of pharmaceutical dosage forms

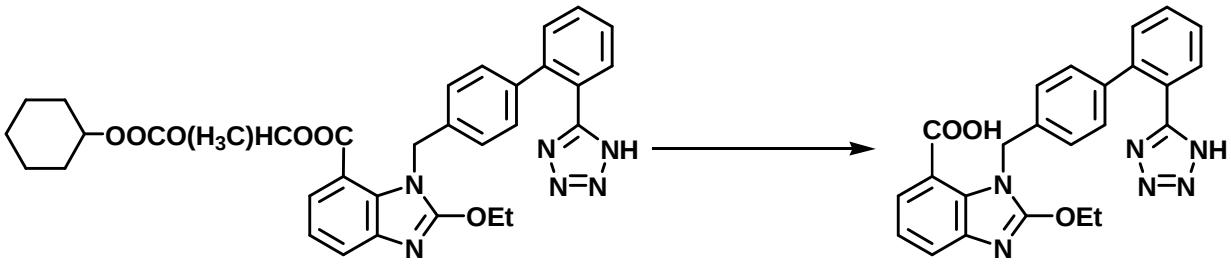
Fourteen tablets of each Candesar 8 tablets and Atacand 16 tablets were powdere d and mixed well; an accurately weighed amount of the powder equivalent to 50 mg of candesartan cilexetil of each was transferred into two separate 100 mL volumetric flasks. 75 mL of methanol were added, sonicated for 30 min., completed to volume with methanol, to obtain 0.5 mg / mL 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 application in the bivariate equations.

Content uniformity testing

The same procedure applied for the analysis of CC in tablets was followed using one tablet as a sample. Ten tablets were analyzed and the uniformity of their contents was tested by applying the official of USP guidelines.

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 structure of the alkaline degradate was elucidated by IR, where 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 3388Cm^{-1} indicating the presence of a hydroxyl group of the carboxylic acid (due to hydrolysis, Figure 1). Only one spot of the degraded product seen on TLC under UV lamp (254 nm) with R_f =0.24 (R_f for candesartan cilexetil was 0.78)

The focus of the present work was 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 sever overlapping (Figure 2) which interfere with the direct determination of candesartan cilexetil.

In the present work, CC was determined and resolved from its alkaline degradate by using the bivariate calibration spectrophotometric method[26,27]. The method is based on a simple mathematic algorithm, in which the data used derives from four linear regression calibration equations : Two calibrations for each component at two wavelengths selected using the method of Kaiser[28]. The method has been successfully applied to resolve different binary mixtures, such as Nifuroxazide and Drotaverine HCl[29]. The advantages of bivariate calibration method is its simplicity and the fact that derivatization procedures are not necessary; unlike other chemometric techniques, there is no need for full spectrum information and no data processing is required.

The linear calibration regression function for the spectrophotometric determination of an analyte A, at a selected wavelength (i) is given by:

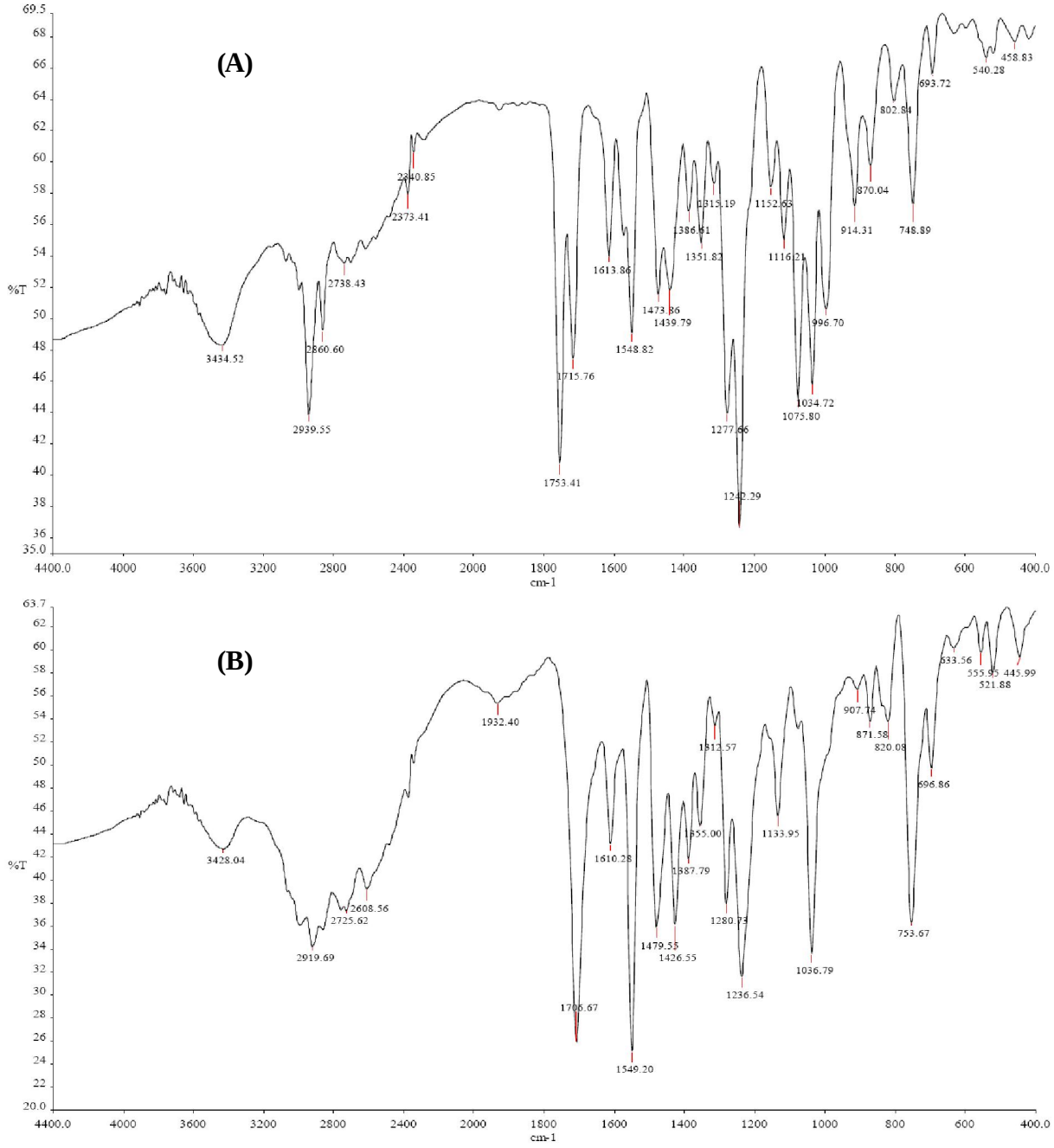


Figure 1 : IR spectra of candesartan cilexetil (A) and its degradation product candesartan (B) in methanol.

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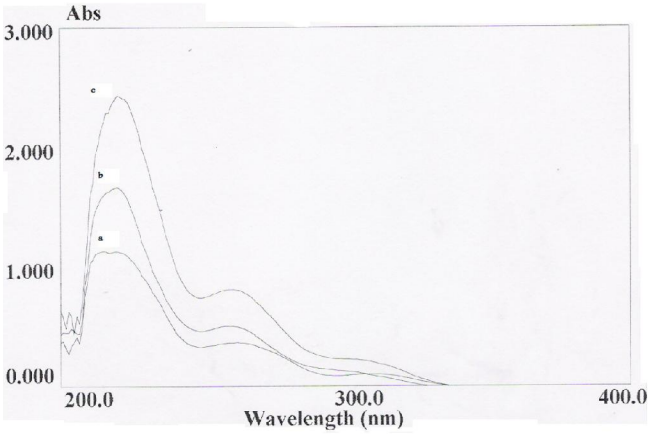

Figure 2 : Absorption spectra of candesartan cilexetil (a), Candesartan (b) 10 µgml^{-1} each in methanol and their mixture (c).

$$A_{Ai} = m_{Ai}.C_A + e_{Ai}$$

where m_{Ai} is the slope of linear regression, C_A is the concentration of analyte A and e_{Ai} is the intercept value. If the measurements for the binary mixture (A, B) are performed at two selected wavelengths (λ_1, λ_2) we have a two equations set:

$$A_{AB1} = m_{A1} C_A + m_{B1} C_B + e_{AB1}$$

$$A_{AB2} = m_{A2} C_A + m_{B2} C_B + e_{AB2}$$

The resolution of such equations set allows the evaluation of C_A and C_B values:-

$$C_A = \frac{m_{B2}(A_{AB1} - e_{AB1}) + m_{B1}(e_{AB1} - A_{AB2})}{m_{B2} m_{A1} - m_{B1} m_{A2}}$$

$$C_B = \frac{A_{AB1} - e_{AB1} - m_{A1} C_A}{m_{A1}}$$

where e_{AB1} and e_{AB2} are the sum of the intercepts of the linear calibration regression equations at the selected two wavelengths ($e_{AB1} = e_{A1} + e_{B1}$), m_A and m_B are the slopes of the linear regression equations at the two selected wavelengths and C is the concentration of CC and its alkaline degradate.

These simple mathematic algorithms allow the resolution of the two compounds by measuring the absorbance of CC and its degradate at the two wavelengths and using the parameters of the linear regression functions evaluated individually for each component at the same wavelengths. The method of Kaiser[28] was used for the selection of optimum wavelength set which assured the best sensitivity for the quantitative determination of the cited drug. In order to apply this method, select the signals of the two components locate (225, 230, 235, 240, 245, 250, 255, 260, 265nm) wavelengths. The calibration curve equations and their respective linear regression coefficients are obtained directly with the aim of ensuring the linearity between the signal and the concentrations. The slope values of the linear regression were estimated for the drug and its alkaline degradate at the selected wavelengths and used for the determination of the sensitivity matrices K, proposed by Kaiser's method[28]. A series of sensitivity matrices K, were calculated for each binary mixture and for every pair of the pre- selected wavelengths:

$$K = \begin{bmatrix} m_{B1} & m_{A1} \\ m_{B2} & m_{A2} \end{bmatrix} \quad \textbf{(A is CC, B is the degradate)}$$

where : $m_{A1,2}$ and $m_{B1,2}$ are the sensitivity parameters (slope) of the regression equations of A and B at the two selected wavelengths (225, 250 nm). The determinants of these matrices were calculated and shown in TABLE 1. The wavelength set was selected for which the highest matrix determinant value was obtained.

TABLE 1 : Application of the method of kaiser for the selection of the wavelength set for the determination of candesartan cilexetil.

λ1/λ2	225	230	235	240	245	250	255	260	265
225	0	13.24	33.19	38.19	37.96	40.60	38.18	30.30	14.0
230		0	18.58	23.80	23.70	25.37	23.30	17.50	5.5
235			0	6.08	6.10	6.62	4.90	1.33	5.8
240				0	0.13	1.77	1.32	4.03	9.28
245					0	0.43	1.44	4.1	9.27
250						0	1.56	4.42	9.9
255							0	2.97	8.79
260								0	5.89
265									0

The absolute values of determinations of sensitivity (K x10-5) , The bold value represent the highest matrix determinant value obtained at the wavelength set 225and 250

For bivariate determination of CC in presence of its degradate, 225nm and 250nm were used; at these selected wavelengths the one- component calibration curves were obtained in the range of 2- 12 µgmL^{-1} for CC and its alkaline degradate, using the following linear regression calibration formula:

For CC A = 0.0037 + 0.0623 C (r = 0.9997) at 225 nm

A = 0.0005 + 0.030 C (r = 0.9992) at 250 nm

For Deg A = 0.0043 + 0.0763 C (r = 0.9999) at 225 nm

A = 0.0067 + 0.0414 C (r = 0.9996) at 250 nm

where : A is the absorbance at the selected wavelength, C is the concentration in μg mL^{-1} and (r) is the regression coefficient.

Different solvents were tried to resolve their overlapping as methanol, ethanol, butanol, acetonitrile, 0.05N NaOH and 0.05NHCl, the best regression calibration lines were obtained in methanol solvent.

Validation of the method

Concentration ranges and calibration graphs

Under the above described experimental conditions, linear relationship were established by plotting the concentration of CC and the alkaline degradate against absorbance at 225 nm and 250 nm in the range of 1-12 μg mL^{-1} for 225nm and 2-12 μg mL^{-1} at 250nm. The high values of correlation coefficient (r) and small intercepts indicate good linearity of the calibration graphs. Statistical analysis of the CC data gave small values of the standard deviation of the residuals ($S_{Y/X}$), of slop (S_b), of intercept (S_a) and the% RSD and % relative error (% Er), as shown in TABLE 2.

TABLE 2 : Performance data of the proposed bivariate method for the determination of candesartan cilexetil.

Parameter	Values	
	At 225nm	At250nm
Range	1-12μgL^{-1}	2-12μgmL^{-1}
Slope	0.0623	0.03
Intercept	0.0037	0.0005
Correlation Coefficient	0.9997	0.9992
LOD	0.28	0.42
LOQ	0.86	1.27
$S_{y/x}$ (Standard deviation of residuals)	$2.764x10^{-4}$	$1.397x10^{-4}$
S_a (Standard deviation of intercept)	0.0038	0.00535
S_b (Standard deviation of slope)	$7.4039x10^{-4}$	$5.26356x10^{-4}$

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 Q 2 B recommendation[30] below which the calibration graph is none linear and the limit of detection was determined by establishing the minimum level at which the analyte can be reliably detected (S/N =3). The values are demonstrated in TABLE 2.

Accuracy and precision

The proposed method was evaluated by studying the accuracy as percent relative error (% Er) and precision as percent relative standard deviation (% RSD) using three preparations with suitable concentration, as shown in TABLE 3, the intraday (n=3) and interday (n=3) accuracy calculated as % Error was found to be 0.36-0.47 % and 0.22-0.33 % for CC respectively. The repeatability of the assay was found to be within 0.63-0.82 % (n=3) at 4, 8, 12 μg mL^{-1} The reproducibility of the assay at the same concentration levels was found to be 0.38-0.65 % (n=3).

TABLE 3 : Accuracy and precision data for candesartan cilexetil using the proposed bivariate method.

Parameter	CC concentration (μgmL^{-1})		
	4	8	12
Intraday			
%Recovery	99.52	100.28	101.08
	100.60	98.98	99.55
	99.49	99.76	100.80
Mean±S.D	99.87±0.63	99.67±0.65	100.48±0.82
%RSD	0.63	0.65	0.81
%Er	0.36	0.38	0.47
Interday			
% Recovery	101.14	98.74	99.61
	100.06	99.77	100.10
	100.28	99.93	100.35
Mean±S.D	100.49±0.57	99.48±0.65	100.02±0.38
%RSD	0.57	0.65	0.38
%Er	0.33	0.38	0.22

N.B. Each result is the average of three separate determinations
Intraday: within the day
Interday: consecutive days

Applications

Determination of CC bulk material

The results of the proposed method for determination of the purity of CC were favorably compared with those obtained using the reference method[22]. The latter method depends on measuring the first derivative (D^1) of CC, 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 2 methods using the Student's t- test and Variance ratio, F-test (TABLE 4)[31]. The proposed procedure offers additional advantages over the reference procedure in that the proposed is more sensitive with good accuracy and precision and con-

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sidered as a stability – indicating method for determination of candesartan cilexetil in presence of its alkaline degradation product.

TABLE 4 : Statistical analysis of the results obtained by the proposed and reference methods.

Parameter	Proposed method			Reference method[22]	
	Taken μgmL^{-1}	Found μgmL^{-1}	Found %	Taken μgmL^{-1}	Found %
	2	2.017	100.85	8	100.38
	4	4.050	101.25	16	99.17
	6	5.982	99.70	24	100.01
	8	7.983	99.79	28	101.70
	10	9.983	99.83	32	101.34
	12	12.038	100.32	38	101.32
n		6			6
Mean±S.D		100.29±0.64			100.65±0.97
Variance		0.41			0.94
Student's-t-value		0.75(2.228)			
Variance ratio F- value		2.29(5.05)*			

Tabulated values at P = 0.05[31]*

Analysis of laboratory prepared mixtures

The absorption spectra of different prepared mixtures were measured at 225nm and 250 nm, the concentration of CC was calculated using the parameters of the linear regressions function evaluated for CC and its degradate at the same wavelengths and substituting in the previous equations for C_A and C_B. The results obtained in TABLE 5 showed that the method is valid for the determination of CC in presence of up to 80 % of its alkaline degradate.

TABLE 5 : Determination of candesartan cilexetil in laporatory prepared mixtures by the proposed bivariate spectrophotometric method.

Alkaline product		Candesartan Cilexeti		
Added (μgml^{-1})	%	Taken (μgml^{-1})	Found (μgml^{-1})	Found %
2	20	8	7.994	99.93
4	40	6	5.938	98.97
6	50	6	6.050	100.83
6	60	4	3.986	99.65
8	80	2	2.016	100.80
Mean±S.D				100.04±0.79

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

Tablet analysis

The proposed bivariate method was applied to the determination of CC in its commercial tablets, the results were shown in TABLE 6. The validity of the method was assessed by applying the standard addition technique (TABLE 7), 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 tab-

TABLE 6 : Assay of candesartan cilexetil in formulation using the proposed and reference methods.

Parameters	Proposed method			Reference method[22]	
	Taken μgmL^{-1}	Found μgmL^{-1}	Found %	Taken μgmL^{-1}	Found %
Atacand 16	6	5.991	99.85	10	99.35
Tablets.					
	8	8.034	100.43	16	99.47
	10	9.967	99.67	24	100.43
	30	100.70	12	12.032	100.27
Mean± S.D		100.06±0.35			99.99±0.68
Student's-t- value		0.13			
Variance ratio F- value		3.77			
Candesar 8					
Tablets.	6	6.046	100.77	10	99.88
	8	7.938	99.23	16	100.70
	10	10.033	100.33	20	100.35
	12	11.926	99.38	30	100.57
Mean±S.D		99.93±0.74			100.13±0.63
Student's-t- value		0.41			
Variance ratio F- value		1.38			

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[31].

TABLE 7 : Assay of candesartan cilexetil in formulation by application of standard addition method using the proposed bivariate method.

Preparation	Amt.taken μgmL^{-1}	CC. added μgmL^{-1}	Amt. found μgmL^{-1}	Found %
Atacand 16	2	4	5.968	99.47
Tablets.	4	6	9.971	99.71
	6	2	7.905	98.81
	6	6	12.016	100.13
Mean±S.D				99.53±0.55
Candesar 8	2	4	5.953	99.22
Tablets.	4	6	9.843	98.43
	6	2	7.936	99.20
	6	6	12.018	100.15
Mean±S.D				99.25±0.70

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

let formulations. The results for the determination of CC in tablets obtained by the proposed method were compared with the D^1 method[22]. Statistical analysis of the results was performed with regard to accuracy and precision using Student 't-test and F- ratio; as presented in TABLE 6, there is no significant difference between the proposed and the reference methods with regard to accuracy and precision[31].

Content uniformity testing

Due to the high precision of the proposed method and its ability to rapidly estimate the concentration of CC in a single tablet extract with sufficient accuracy, the method is ideally suited for content uniformity testing which is a time consuming process when using conventional assay techniques. The steps of the test were adopted according to the USP[32] procedure. The acceptance value (AV) was calculated for each of the commercially available tablets and it was found to be smaller than the maximum allowed acceptance value (LI). The results demonstrated excellent drug uniformity, as shown in TABLE 8.

TABLE 8 : Results of content uniformity testing of CC tablets using the proposed bivariate method.

Parameter	Percentage of the label claim	
	Atacand 16	Candesar 8
Data	99.51	100.55
	101.14	99.49
	100.22	98.98
	99.55	100.60
	100.94	100.54
	99.52	100.35
	98.74	99.76
	99.77	100.12
	99.93	101.08
	100.54	99.61
Mean±S.D	99.99±0.73	100.11±0.64
%RSD	0.73	0.64
%Error	0.23	0.20
Acceptance value (AV)[32]	1.75	1.54
Max.allowed Av(LI)[32]	15	

CONCLUSION

The proposed bivariate method provides simple, accurate and reproducible quantitative analysis for the determination of candesartan cilexetil in pharmaceutical tablets and in presence of its alkaline- induced degradation product, it is considered as a stability – indicating one. Thus, it can be used for the quality control of CC in the commercial tablets with excellent application of content uniformity test. Moreover, the method is fast and feasible and has the advantages of being lower costing.

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