

## DETERMINATION OF CANDESARTAN CILEXETIL IN TABLETS BY SPECTROFLUORIMETRY

Amir Alhaj SAKUR\*, Hanan FAEL

Department of Analytical and Food Chemistry, Faculty of Pharmacy, Aleppo University, Syria

\*Email: [dramirarticle@fastmail.fm](mailto:dramirarticle@fastmail.fm)

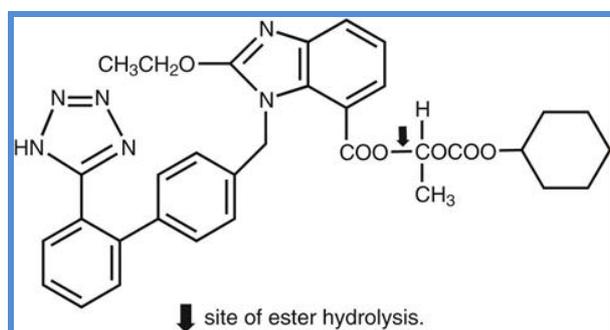
### ABSTRACT

A Simple, accurate, and highly sensitive spectrofluorimetric method is presented for the determination of candesartan cilexetil in bulk and tablets. The proposed method is based on direct measurement of candesartan cilexetil fluorescence intensity. The excitation and emission wavelengths were 260 nm and 381 nm, respectively. The method was linear over the concentration range of 3.00 – 288.60 ng/ml. The limit of detection was 0.25 ng/ml, and the limit of quantitation was 0.77 ng/ml. In order to validate the method the results were compared with those obtained by a high performance liquid chromatography method. The proposed method was successfully applied to the analysis of candesartan cilexetil in pure form and tablets with good precision and accuracy compared to the reported method as revealed by *t*- and *F*- tests. The method was shown to be highly specific in the presence of hydrochlorothiazide which is commonly combined with candesartan cilexetil in tablets.

**Keywords:** Spectrofluorimetric, candesartan cilexetil, hydrochlorothiazide, tablets.

### INTRODUCTION

Candesartan cilexetil, a nonpeptide, is chemically described as ( $\pm$ )-1-Hydroxyethyl 2-ethoxy-1-[p-(*o*1H-tetrazol-5-ylphenyl)benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (Figure 1). It is a white to off-white powder with a molecular weight of 610.67. It is practically insoluble in water and sparingly soluble in methanol.



**Figure 1:** Structural formula of candesartan cilexetil

Candesartan cilexetil, a prodrug, is hydrolyzed to candesartan during absorption from the gastrointestinal tract. Candesartan is a selective AT1 subtype angiotensin II receptor antagonist<sup>1</sup>.

Angiotensin II receptor antagonists are a group of drugs also known as angiotensin receptor blockers (ARBs), AT1 receptor antagonists or sartans. ARBs modulate the renin-angiotensin-aldosterone system; their main use is in hypertension (high blood pressure), diabetic nephropathy (kidney damage due to diabetes) and congestive heart failure.<sup>2</sup>

However, unlike angiotensin converting enzyme inhibitors, ARBs do not inhibit the breakdown of bradykinin, and thus are unlikely to cause the persistent dry cough which commonly complicates ACE inhibitor therapy<sup>3</sup>.

Scientific literature reported several analytical methods for the determination of candesartan cilexetil in human plasma, urine and dosage forms such as high performance liquid chromatography<sup>4-8</sup> and Capillary electrophoresis<sup>9</sup>. There seems to be no reports on determination of candesartan cilexetil using spectrofluorometer which shows several advantages such as high sensitivity, low detection limit, ease of use and less time consumption comparing with other analytical methods.

### MATERIALS AND METHODS

#### Instrumentation

Fluorescence signals were measured using Spectrofluorometer (SHIMAD-ZU, RF5301-PC) equipped with a xenon lamp using a standard 10mm path-length quartz cell, with 5nm slit bandwidth for the emission and excitation monochromators.

Chromatographic analysis was performed on (HITACHI – ELITE LaChrom) apparatus equipped with photodiode array detector, autosampler, and column oven. Chromatographic separation was achieved on CN column (5  $\mu$ m, 250 mm  $\times$  4.6 mm)<sup>8</sup>.

#### Chemicals and reagents

All solvents used were of analytical grade. Ethanol and acetonitrile were of HPLC grade (Merck, Germany).

Candesartan cilexetil (Dr. Reddy's Laboratories, India) stock standard solution of 2.62 mM was prepared in ethanol. The later solution was diluted with ethanol to reach a concentration of  $10.5 \times 10^{-3}$  mM. Stock standard solution was stable for several weeks at room temperature.

### Pharmaceutical formulation

The following available commercial preparations were analyzed:

- 1- Candesar® tablets (ASIA Pharmaceutical Industries) labeled to contain 4 mg, 8 mg, 16 mg and 32 mg of candesartan cilexetil per tablet.
- 2- Cansartan® tablets (Obari Pharmaceutical) labeled to contain 16 mg and 32 mg of candesartan cilexetil per tablet.
- 3- Desertic® tablets (Universal Pharmaceutical Industries) labeled to contain 16/12.5 mg and 32/12.5 mg of candesartan cilexetil and hydrochlorothiazide, respectively.

### Procedure

#### General procedure

A suitable volume of working standard solution was pipetted into a 10 ml volumetric flask, then it was completed to mark with ethanol and mixed thoroughly. The fluorescence intensity of candesartan cilexetil solutions were measured at  $\lambda_{em} = 381$  nm using an excitation wavelength of 260 nm against a blank solution of ethanol. The calibration curve was constructed by plotting fluorescence intensity against concentration. The calibration curve was then used to calculate the amount of candesartan cilexetil in tablets.

#### Procedure for pharmaceutical samples

The contents of 10 tablets were pulverized carefully. An accurately weighed amount of the powder equivalent to 4 mg of candesartan cilexetil was transferred to a 25 ml volumetric flask, dissolved in 15 ml of ethanol, swirled and sonicated for 20 minutes, then diluted to volume with ethanol. Portions of this solution were centrifuged at 4500 rpm for 10 minutes. Suitable dilution with ethanol was made to obtain the final solution.

#### Chromatographic procedure

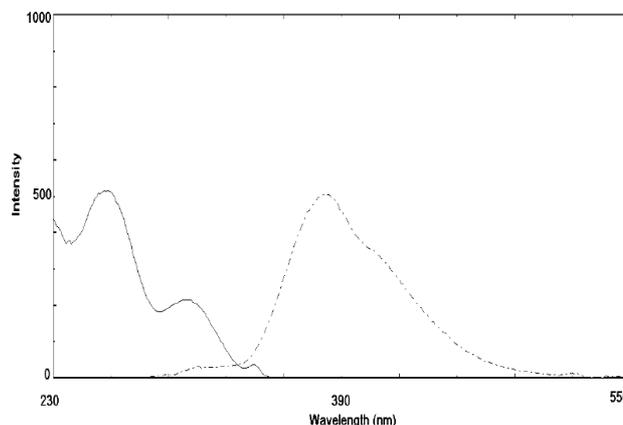
Tablets were grinded and an accurately weighed powder equivalent to 4 mg of candesartan cilexetil was transferred to a 25 ml volumetric flask, dissolved in 15 ml of mobile phase, sonicated for 20 minutes, and then diluted to volume with the mobile phase. Portions of this solution were centrifuged at 4500 rpm for 10 minutes, filtered and injected to the chromatographic system.

The mobile phase consisted of 50:50 (v/v) mixture of phosphate buffer, pH 3.0, and acetonitrile. The flow rate was 1 ml/min, the detection wavelength was 210 nm, and injection volume was 10  $\mu$ l. The Column oven temperature was kept at 25°C.<sup>8</sup>

## RESULTS AND DISCUSSION

### Excitation and emission spectra

Candesartan cilexetil solution has strong native fluorescence in ethanol, the excitation spectra was obtained showing a maximum excitation wavelength at 260 nm, then the emission spectra was scanned from 290-550 nm, showing an emission wavelength maxima at 381 nm (Figure 2).



**Figure 2:** Excitation (solid) & Emission (dotted) spectra of candesartan cilexetil in ethanol.

### Effect of organic solvents

In the aqueous medium candesartan cilexetil undergoes hydrolysis at the ester link to form candesartan. Organic solvents were used to keep candesartan cilexetil unhydrolyzed.

The effects of solvent polarity on the native fluorescence of candesartan cilexetil were investigated. Four different organic solvents with different polarities were tested (Ethanol, Methanol, Chloroform, and n-hexane). Interestingly, the fluorescence intensity was completely quenched in the nonpolar solvent n-hexane, while the fluorescence intensity was found to be unaltered when changing from the polar solvents (Ethanol and Methanol) to the less polar solvent Chloroform. Ethanol was the preferred solvent since it is non-toxic in contrast to methanol and Chloroform.

### Validation of the proposed method

#### Linearity

Under the experimental conditions described, standard calibration curve was constructed by measuring a series of twelve different concentrations of standard solutions of candesartan cilexetil and plotting fluorescence intensity versus concentration. All measurements were carried out using six replicate measurements (n= 6). The analytical parameters are listed in Table 1.

The correlation coefficient was 0.9999 indicating good linearity, in the concentration range of 3.00 – 288.60 ng/ml.

**Table 1.** Statistic and analytical parameters of candesartan cilexetil determination

Parameters	Results
$\lambda_{\text{ex}}/\lambda_{\text{em}}$ (nm)	260/381
Linear range (ng/ml)	3.00 – 288.60
Limit of detection (ng/ml)	0.25
Limit of quantitation (ng/ml)	0.77
Slope ( <i>b</i> )	3.084
Intercept on the ordinate	0.384
Correlation coefficient ( <i>r</i> )	0.9999

### Detection and quantitation limit

The limit of detection and quantitation were calculated according to the method proposed by the Analytical Methods Committee<sup>10</sup>, and were found to be 0.25 ng/ml and 0.77 ng/ml, respectively.

### Repeatability or intra-day precision

The repeatability of proposed method was estimated by measuring six replicate samples of each concentration of candesartan cilexetil prepared in one laboratory on the same day. The relative standard deviations (RSD%) were less than 4.86% indicating good precision (Table 2).

### Accuracy

Accuracy was determined in two ways: (1) analyzing the recoveries of known different amounts of candesartan cilexetil (Table 2), and (2) comparing test results from the presently proposed method with those obtained from a reference method such as HPLC<sup>8</sup>. The obtained results were statistically compared with each other (Table 3). With respect to *t*- and *F*-tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level.

**Table 2:** Results for candesartan cilexetil samples prepared in ethanol.

Taken Candesartan cilexetil (ng/ml)	Found Candesartan cilexetil (ng/ml)	Recovery % (RSD%)
3.00	2.87	95.66 (4.86)
5.00	4.93	98.60 (2.79)
10.00	9.95	99.50 (1.81)
15.00	15.09	100.60 (1.69)
20.00	19.82	99.10 (1.66)
25.00	24.71	98.84 (1.52)
50.00	49.86	99.72 (1.43)
65.00	65.37	100.57 (1.17)
130.00	130.72	100.55 (1.16)
190.00	191.18	100.62 (1.03)
260.00	260.07	100.02 (0.75)
288.60	288.71	100.04 (0.67)

\*Average of six determinations.

**Table 3.** Comparison of the determination of candesartan cilexetil in tablets by spectrofluorimetry and HPLC.

Preparation (tablet)	Labeled amount	Candesartan cilexetil found mg/tablet $\pm$ S.D <sup>a</sup> (recovery%) <sup>b</sup>		<i>t</i> and <i>F</i> test <sup>c</sup>
		Fluorescence	HPLC	
Candesar	4 mg	4.02 $\pm$ 0.05 (100.50)	4.00 $\pm$ 0.06 (100.00)	0.884, 1.440
	8 mg	8.01 $\pm$ 0.13 (100.12)	7.96 $\pm$ 0.12 (99.50)	0.750, 1.173
	16 mg	16.11 $\pm$ 0.25 (100.69)	16.30 $\pm$ 0.20 (101.87)	1.826, 1.562
	32 mg	32.32 $\pm$ 0.29 (101.01)	32.11 $\pm$ 0.25 (100.34)	1.802, 1.345
Cansartan	16 mg	16.18 $\pm$ 0.30 (101.12)	15.91 $\pm$ 0.29 (99.44)	2.204, 1.070
	32 mg	32.17 $\pm$ 0.42 (100.53)	32.50 $\pm$ 0.35 (101.56)	1.914, 1.440
Desertic <sup>d</sup>	16 mg	16.13 $\pm$ 0.32 (100.81)	16.27 $\pm$ 0.27 (101.68)	1.080, 1.404
	32 mg	32.53 $\pm$ 0.52 (101.65)	32.95 $\pm$ 0.59 (102.96)	1.970, 1.287

<sup>a</sup> Average of six determinations

<sup>b</sup> Recoveries were calculated considering that the preparations contained the amounts reported by the manufacturing laboratories.

<sup>c</sup> the tabulated *t* and *F* values at 95% confidence limit for 5 degrees of freedom (*n* = 6) are 2.571 and 5.050, respectively.

<sup>d</sup> combined with 12.5mg of hydrochlorothiazide.



Potential effects of hydrochlorothiazide, a diuretic which is commonly combined with candesartan cilexetil in tablets, were thoroughly studied with no interference shown at the selected emission and excitation wavelength for the determination of candesartan cilexetil.

### CONCLUSION

Candesartan cilexetil can be directly determined in tablets in the presence of excipients using spectrofluorimetry. Tests of repeatability and accuracy were successfully done in order to validate the method. The recovery results are statistically comparable to those obtained by reference method based on high performance liquid chromatography. It can be concluded that the proposed method is fully validated. Furthermore, the method was shown to be highly selective for Candesartan cilexetil with no interference by hydrochlorothiazide which is commonly combined with candesartan cilexetil in tablets.

### REFERENCES

1. Physician's Desk Reference (PDR) – 2009.
2. Reif M, White WB, Fagan TC, Effects of candesartan cilexetil in patients with systemic hypertension. *Am J Cardiol*, Volume 82, 1998, 961-965.
3. British National Formula (BNF), 2009.
4. Khedr A E M, Simultaneous Determination of Candesartan Cilexetil and Hydrochlorothiazide by High-Performance Liquid Chromatography, *The Journal of King Abdulaziz University - Medical Sciences*, Volume 15, No 2, 2008.
5. Stenhoff H, Lagerström P, Andersen C, Determination of candesartan cilexetil, candesartan and a metabolite in human plasma and urine by liquid chromatography and fluorometric detection, *Journal of Chromatography B: Biomedical Sciences and Applications*, Volume 731, Issue 2, 1999, 411-417
6. González L, López JA, Alonso RM, Jiménez RM, Fast screening method for the determination of angiotensin II receptor antagonists in human plasma by high-performance liquid chromatography with fluorimetric detection, *Journal of Chromatography A*, Volume 949, Issues 1-2, 2002, 49-60
7. Erk N, Simultaneous Analysis of Candesartan Cilexetil and Hydrochlorothiazide in Human Plasma and Dosage Forms Using HPLC with a Photodiode Array Detector, *Journal of Liquid Chromatography & Related Technologies*, Volume 26, Issue 15, 2003, 2581 - 2591
8. Subba Rao DV, Radhakrishnanand P, Suryanarayana MV, Himabindu V, A stability-indicating LC method for candesartan cilexetil, *Chromatographia*, ISSN 0009-5893, Volume 66, no7-8, 2007, 499-507.
9. Hillaert S, Van den Bossche W, Simultaneous determination of hydrochlorothiazide and several angiotensin-II-receptor antagonists by capillary electrophoresis, *J Pharm Biomed Anal.*, 31(2), 2003, 329-39.
10. Analytical Methods Committee, *Analyst* 112, 1987, 199

\*\*\*\*\*

