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ANALYTICAL SPECTROPHOTOMETRIC STUDY OF CANDESARTAN CILEXETIL IN TABLETS FORMULATIONS

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***ABSTRACT*:** *Spectrophotometric method was developed and applied for determination of Candesartan Cilexetil (CDC) in tablets formulations. This new method was applied for determination (CDC) in several Syrian trademark drugs. The proposed method is based on the formation of colored complex between 2, 6-Dichloroquinone chlorimide (DCQC) with CDC. Under optimized conditions, it shows a maximum absorption at 467nm. The analytical parameters and theirs effects are investigated. Beer's law was obeyed in the range of 20 – 100 µg/mL, with correlation coefficient R^2 = 0.9988. The average recovery of CDC was between 98.09 and 102.86%. The limit of detection (LOD) and limit of qualification (LOQ) were 2.49 and 7.55 µg/mL, respectively. The proposed method has been successfully applied to the analysis of the studied drug in pure form and pharmaceutical formulations.*

Keywords: *Candesartan Cilexetil (CDC), 2, 6-Dichloroquinone chlorimide (DCQC), Spectrophotometry, pharmaceutical formulations.*

1. INTRODUCTION

Candesartan cilexetil (CDC) is Angiotensin II receptor Antagonist. Chemically it is 2-Ethoxy-3-[21-(1H-tetrazol-5-yl) biphenyl-4-ylmethyl]- 3Hbenzoimidazole-4-carboxylic acid 1- cyclohexyloxycarbonyloxy ethyl ester, Figure 1 [1].

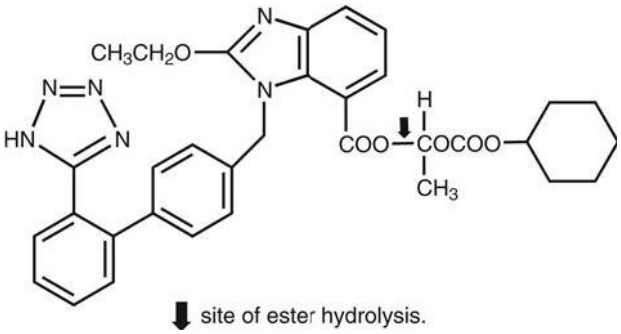

Figure 1: Structural formula of candesartan cilexetil

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 [2]. The typical dose of Candesartan cilexetil is 16 mg per day in patients who are not volume depleted. It may be given once or twice daily with total daily doses ranging from 8 mg to 32 mg [3]. Candesartan cilexetil is hydrolyzed to Candesartan during absorption from the gastrointestinal tract [4].

Angiotensin II receptor antagonists are a group of drugs also known as angiotensin receptor blockers (ARBs), AT1 receptor antagonists or sartans. ARBs modulate the reninangiotensin- aldosterone system; their main use is in hypertension (high blood pressure), diabetic nephropathy (kidney damage due to diabetes) and congestive heart failure [5]. 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 [6].

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 [7, 8] and Capillary electrophoresis [9]. There seems to be very few spectrophotometric methods have been reported for estimation of Candesartan cilexetil in tablet dosage form. A spectroscopic method depends upon the reaction of bromocresol green (BCG) or bromocresol purple (BCP) with candesartan in phosphate buffered solution to form stable colored ion-pair complex, which was extracted in chloroform, the yellow colored complexes were determined at λ_{max} 415, 405 nm with BCG, BCP, respectively [10].

2. MATERIALS AND METHODS

2.1 Apparatus

UV/VIS double beam spectrophotometer V-630 (Jasco, Japan) equipped with 1 cm quartz cells; ultrasonic bath (Daihan, Korea); analytical balance TE64 (Sartorius, Germany) with accuracy ±0.1 mg; digital pipettes (Accumax); pH-meter 744 (Metrohm, Swiss).

2.2 Reagents and Materials

All reagents and chemicals used were of analytical reagent or pharmaceutical grade.
Ethanol (≥99.0%, Sigma-Aldrich), 2,6-Dichloroquinone chlorimide (DCQC) (≥95.0%, Mw=210.45 $g.mol^{-1}$, Sigma-Aldrich, USA), Candesartan cilexetil (CDC) was obtained from (Oubari Pharmaceutical, Al-Mansourah, Aleppo, Syria) with 99.5% purity.

Syrian commercial dosage forms of Candesartan cilexetil:

- Desartan tablet (Unipharma) 16 and 8 mg of Candesartan cilexetil.
- Cansartan tablet (Oubari) 16 and 8 mg of Candesartan cilexetil.

2.3 Preparation of solutions

2.3.1 Standard Stock Solution of CDC

Standard Stock solution of Candesartan cilexetil (1mg/mL) was prepared by dissolving 100 mg of CDC in 100mL of Ethanol.

2.3.2 Standard Stock Solution of DCQC

Standard Stock solution of 2,6-Dichloroquinone chlorimide (0.4mg/mL) was prepared by dissolving 40 mg of DCQC in 100mL of Ethanol.

2.3.3 Preparation of calibration curve

To prepare calibration standards of CDC, compatible volumes of stock standard solutions were diluted with distilled water into volumetric flasks (25 mL) to obtain final drug concentration of (20, 40, 60, 80, 100) µg/mL.

2.3.4 Pharmaceutical Samples

Two Syrian trademark products with two different doses for each were studied:
- 10 tablets of Desartan and Cansartan tablet**s (8mg)** were powdered, an accurate weight equivalent to one tablet

was transferred into 25ml volumetric flask and dissolved in ethanol. Then the solution was swirled, sonicated for 20 minutes and diluted to volume with ethanol. 5mL of the solutions were transferred into 25ml volumetric flask, 2mL of DCQC and 5mL of phosphate buffered solution (pH=10.0) were added, then diluted to volume with distilled water.

- 10 tablets of Desartan and Cansartan tablet**s (16mg)** were powdered, an accurate weight equivalent to one tablet was transferred into 25ml volumetric flask and prepared as described above. 2mL of the solutions were transferred into 25ml volumetric flask, 2mL of DCQC and 5mL of phosphate buffered solution (pH=10.0) were added, then diluted to volume with ethanol.

3. RESULTS AND DISCUSSIONS

3.1. Spectrophotometric analysis of CDC with DCQC

This method is based on the reaction of Candesartan cilexetil (CDC) with the reagent DCQC in phosphate buffered solution (pH = 10.0) to form a stable red complex. The absorption spectrum of the complex was measured in the range of 350– 600 nm against the blank solution. The complex has a maximum absorbance at **467 nm** as shown in Figure 2.

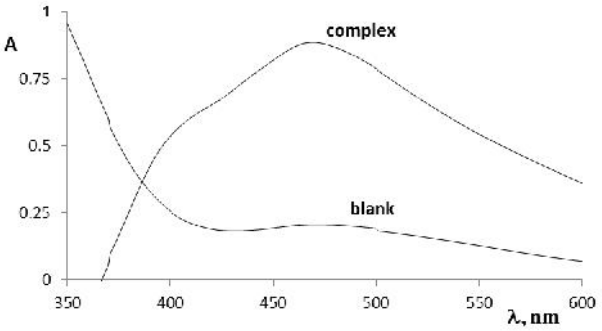

Figure 2: Spectrum of CDC-DCQC complex at the optimum conditions

3.2 Optimization of the reaction conditions

The optimum conditions of the method were carefully studied to achieve complete reaction formation, highest sensitivity, and maximum absorbance.

3.2.1 Effect of Reagent Concentration

The effect of reagent concentration was studied by measuring the absorbance of solutions containing a fixed concentration of CDC (2mL, 80µg/mL), varied amounts of DCQC reagent. Maximum color intensity of the complex was achieved with (2 mL, 32 µg/mL) of DCQC solution, Figure 3.

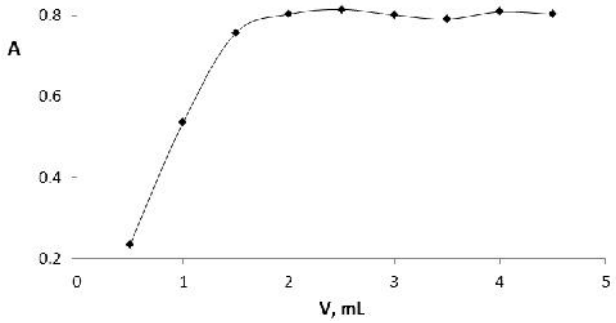

Figure 3: Effect of reagent volume on the CDC-DCQC complex

3.2.2 Effect of Time and Temperature

The optimum reaction time was investigated from 5 to 35 min by following the colored development at ambient temperature (25 ± 2◦C). Complete color intensity was attained after 20 min of mixing the complex as shown in Figure 4. The effect of temperature on colored complex was investigated by measuring the absorbance values at different temperatures. We have found that temperature does not effect on the complex formation speed and color intensity. So the best temperature for studied complex was found at room temperature (25 ± 2◦C) and the absorbance remains stable for at least 7 days.

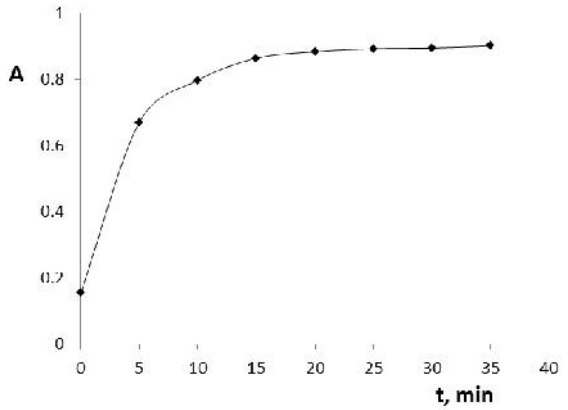

Figure 4: Effect of time on the CDC-DCQC complex

3.2.3 Effect of pH

The optimum pH value of CDC-DCQC complex was studied in aqueous phosphate buffered solutions of pH range (8.0 -11.0). The absorbance intensity was measured at λ_{max} = 467nm, Figure 5. The highest absorbance for studied complex was found between (9 – 11) of pH value, so we have chosen pH=10.0 as an optimum value.

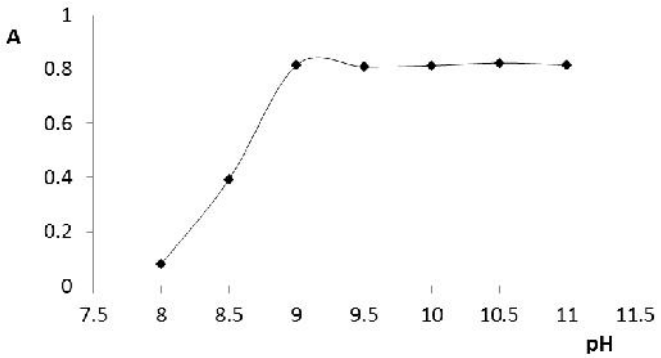

Figure 5: Effect of pH value on the CDC-DCQC complex

3.3 Stoichiometric Relationship

The stoichiometric ratio between drug and reagent in the complex CDC-DCQC was determined by Job's method of the continuous variation, and Molar ratio method as following:

3.3.1 Job's method of continuous variation

Job's method of continuous variation was employed: 1.6×10^{-3} M standard solution of CDC and 1.9×10^{-3} M solution of DCQC, were used. A series of solutions were prepared in which the total volume of CDC and DCQC was kept at 4.0 mL and diluted with distilled water to 25 ml.

The absorbance was measured at λ_{max}, the result indicates the existence of 1:1 molar ratio (CDC: DCQC), (Figure 6).

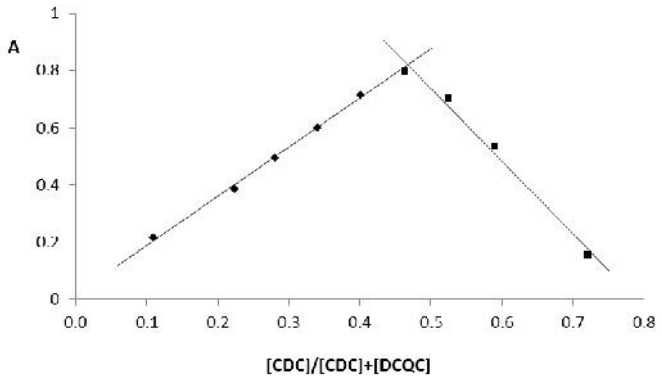

Figure 6: Job's method of continuous variation of CDC-DCQC complex

3.3.2 Molar ratio method

The stoichiometry of CDC-DCQC complex by molar ratio method was studied according to the following equation: A_{max} = f([DCQC]/[CDC]). The result confirms the stoichiometry of complex CDC-DCQC that is 1:1; Where the concentration of CDC is constant (1.3×10^{-4} M), and the concentrations of DCQC are changed from (0.38 to 3.4) $\times10^{-4}$ M (Figure 7).

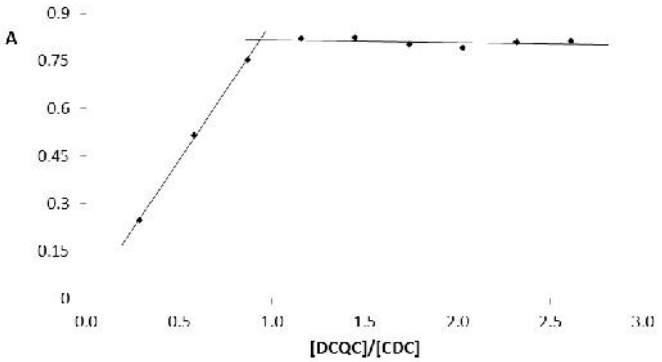

Figure 7: Molar ratio method of CDC-DCQC complex

4. METHOD VALIDATION

4.1 The linearity

Under the experimental conditions described, standard calibration curve was constructed by measuring a series of different concentrations of standard solutions of candesartan cilexetil and plotting absorbance versus concentration, Figure 8. The correlation coefficient was 0.9988 indicating good linearity, in the concentration range of 20 – 100 µg/ml.

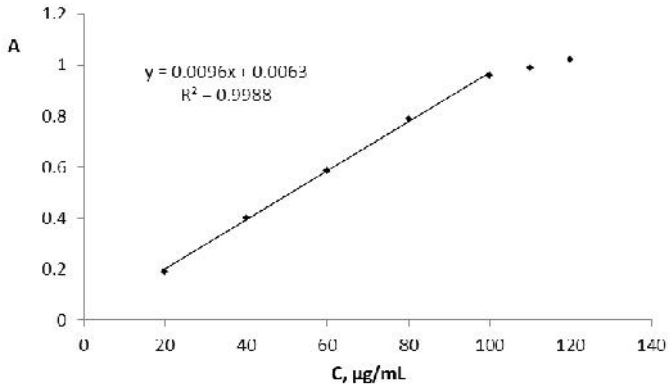

Figure 8: Calibration curve for determination of CDC-DCQC complex

The analytical results for accuracy and precision are listed in Table 1. All measurements were carried out using five replicate measurements (n= 5).

Table 1: Evaluation of the accuracy and precision of candesartan cilexetil determination

(µg/mL)		SD (µg/mL)	RSD%	Confidence Limits* $\bar{x} \pm \frac{t \mid SD}{\sqrt{n}}$ (µg/mL)	Recovery %
X Taken	X Found				
20	19.62	0.531	2.71	0.66 ± 19.62	98.09
40	41.14	0.600	1.46	0.75 ± 41.14	102.86
60	60.12	0.555	0.92	0.69 ± 60.12	100.19
80	81.07	0.473	0.58	0.59 ± 81.07	101.34
100	99.04	0.374	0.38	0.47 ± 99.04	99.04

* the tabulated *t* value at 95% confidence limit for 4 degrees of freedom (n =5) is 2.78.

The analytical results for accuracy and precision show that the proposed method has good repeatability and reproducibility.

4.2 Sensitivity

The mean molar absorptivity, Sandell sensitivity, detection and quantification limits are calculated from the standard deviation of the absorbance measurements obtain from Beer's law and recorded in Table 2.

Table 2: analytical parameters of candesartan cilexetil determination

Parameter	Value
λ_{max} (nm)	467
Beer's law limits (µg/mL)	20 – 100
Molar absorptivity, ε (L/mol.cm)	5952.01
Regression equation	Y = 0.0096X + 0.0063
Slope (b)	0.0096

Intercept (C)	0.0063
R^2	0.9988
LOD (µg/mL)	2.49
LOQ (µg/mL)	7.55
Sandell sensitivity SS (µg/cm^2)	0.205

The detection and quantification limits for the proposed method were calculated using the following equations:

$$\text{LOD} \quad \text{X } 3.3 \quad \text{SD} \ / \text{m}$$

$$\text{LOQ} \quad \text{X } 10 \quad \text{SD} \ / \text{m}$$

Where SD = the standard deviation, and m = the slope of the calibration curve.

4.3 Analysis of Pharmaceutical Formulations

The proposed method has been successfully applied to the determination of CDC in pharmaceutical dosage forms in two trade mark Syrian products: Desartan (Unipharma), Cansartan (Oubari), Table 3.

Table 3: The obtained results for pharmaceuticals samples

Trade mark	Dose	$\bar{x}^*$ (mg/dose)	RSD%	Recovery %
Desartan	16 mg/Tab	15.91	1.20	99.45
Desartan	8 mg/Tab	8.05	1.38	100.65
Cansartan	16 mg/Tab	16.12	1.35	100.74
Cansartan	8 mg/ml	8.09	1.42	101.13

To check the validity of the proposed method, pharmaceutical dosage forms were tested for possible interference with standard addition method (Tables 4 and 5). There was no significant difference between slopes of calibration curves and standard addition methods. Therefore, it is concluded that the excipients in pharmaceutical dosage forms of CDC did not cause any interference in the analysis of CDC.

Table 4: The recovery of candesartan cilexetil in Desartan

	*Sample µg/mL	*Added µg/mL	*Found µg/mL	RSD%	Recovery %
Desartan 8 mg	32	20	52.58	0.85	101.12
	32	40	72.46	0.77	100.64
	32	60	92.49	0.73	100.53
Desartan 16 mg	25.6	20	45.44	0.68	99.64
	25.6	40	65.49	0.60	99.83
	25.6	60	85.73	0.49	100.16

* n = 5

Table 5: The recovery of candesartan cilexetil in Cansartan

	*Sample µg/mL	*Added µg/mL	*Found µg/mL	RSD%	Recovery %
Cansartan 8 mg	32	20	51.75	0.79	99.53
	32	40	72.35	0.72	100.49
	32	60	92.55	0.68	100.60
Cansartan 16 mg	25.6	20	45.67	0.64	101.16
	25.6	40	65.34	0.60	99.61
	25.6	60	85.53	0.41	99.92

* n = 5

5. CONCLUSION

This paper describes the application to form a stable colored complex for determination of candesartan cilexcetil (CDC) in pure forms and pharmaceutical formulations. Tests of repeatability and accuracy were successfully done in order to validate the method. The proposed method is found to be simple, sensitive selective, accurate and economical when compared to quantitative methods by HPLC and LC-MS. Moreover, procedure is free from tedious experimental steps such as heating. The most attractive feature of this method is their relative freedom from interference by the usual diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical formulations. Therefore, the validated method could be useful for routine quality control assay of the studied drug in pure forms and pharmaceutical formulations.

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