ISSN-0975-2366
Research Article https://doi.org/10.31838/ijpr/2020.12.01.059

Spectrophotometric Determination of Candesartan Cilexetil and Atenolol In Pure and Pharmaceutical Forms

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Received: 19.09.19, Revised: 19.10.19, Accepted: 19.11.19

ABSTRACT
The present work aimed to estimate two antihypertensive drugscontent using the proposed analytical UV-Spectrophotometric methods. The two antihypertensive drugs; candesartan cilexetil(CAN) and Atenolol (ATE) were estimated in pure form and pharmaceutical tablets. Firstly, using different blanks for each drug composed of methanol: distilled water (8:2) and 0.1M hydrochloric acid HCl were applied and scanned at specific wavelengths (λ max) of 254 nm and of 224 nm for Candesartan cilexetil and Atenolol, respectively. The methods were confirmed for linearity through preparation a series of certain concentration and measure their absorptions and correlation coefficient, and then these methods were validated by studying the accuracy and precision. The linearity and relative standard deviation RSD% for candesartan cilexetil and atenolol were (5-60)µg/ml, (3-40) µg/ml, (0.4-2.46) and (0.48-0.74), respectively. The relative error RE% for candesartan cilexetil and atenolol were (0.26-1.07) and (0.33-1.6).The percentage of recoveries were found to be in the range of 98.9%-101% and 98.4-99.6% for candesartan cilexetil and atenolol, respectively. Validation experiments were also accomplished to reveal the low limit of detection LOD, and limit of quantification LOQ for candesartan cilexetil and atenolol and were found to be (0.14) (0.09) and LOQ (0.48) (0.3), respectively. Hence, the simple, accurate methods can be used for routine analysis of candesartan cilexetil and atenolol in bulk, and their pharmaceutical tablets form.

Keywords: Candesartan Cilexetil**,** Atenolol**,** Antihypertensive drugs, UV- Spectroscopy**.**

INTRODUCTION

Candesartancelixetil(CAN) is (±)-1-Hydroxyethyl 2-ethoxy-1- [p-(o-1H-tetrazole-5-ylphenyl) benzyl]-7-benzimidazolecarboxylate,cyclohexyl carbonate ($C_{33}H_{34}N_6O_6$) as shown **in Fig.1A**. It is a potent and highly selective angiotensin II receptor blocker for AT1 subtype angiotensin II receptor antagonist [1]. It is presented as an ester prodrug form -Candesartan cilexetil. After absorption from the gastrointestinal tract, it hydrolysed to give its actively therapeutic metabolite; Candesartan. CAN is insoluble in water and moderately soluble in methanol [2]. It has an evaluated bioavailability of 14% [3] Its plasma protein binding is up to 99%, and it is excreted mainly in urine and bile in unchanged form with slight amount of inactive metabolites. The half-life of CAN is around nine hours. CAN is indicated as a hypotensive drug in case of hypertension, congestive heart failure, diabetic neuropathy [4,5,6,7].It may be given alone or in combination with another antihypertensive medication such as with hydrochlorothiazide to get an additive hypotensive effect. These formulations are commercially marketed as Atacand HCT®, Hytacand® and Biopress Plus ®.In the literature survey, several methods have been reported to determine CAN in bulk and in formulation, High - performance liquid chromatography (HPLC) [8], voltammetry [9], high performance thin layer[10], NMR[11], spectrofolumetry [12] , conductometry[13], UV spectrophotometry [14,15,16,17], spectrophotometric method [18, 19].Atenolol (ATE) is (2-[4-[(2RS)-2-hydroxy-3-[(1-methylethyl) amino] propoxy] phenyl] acetamide, as shown in Fig 1 B., ATE is α1- selective α- adrenergic receptor blocker .it acts as antihypertensive agent by lowering the systolic and diastolic pressure by 15-20% and decrease the rate of cardiovascular death. It can be used alone or in combination with another agent for management of heart failures such as cardiac arrhythmias, myocardial infarction and angina. ATE is used in management of alcohol withdrawal, in migraine, in anxiety states and tremors [20].ATE has a rapid but incomplete absorption after oral administration. About 35-50% of an oral dose being eliminated unchanged in urine. It is hepatically metabolised, and approximately 6–16% of it is bounded to plasma protein [21]. Different analytical methods have been

reported for estimation of ATE in urine, plasma or pharmaceutical formulations, such as gas chromatography [22]. HPLC [23,24,25], liquid chromatography (LC)[26], voltammetric[27,28], electrophoresis [29], spectrofluorometric[30], ultraviolet and visible spectrophotometric methods [31, 32, 33]. The goal of this study is to estimate Candesartan cilexetil and Atenolol in pure form and different pharmaceutical preparations marketed in Iraqi pharmacy shop using suggested analytical spectrophotometric methods.

MATERIALS AND METHODS

Chemicals and Instruments

Methanol and Hydrochloric acid (HCl) were obtained from the local market, manufactured by media pharmaceuticals, India. Distilled water was obtained from the laboratories of the college of pharmacy, university of Kerbala, Iraq. Standard samples of Candesartan celextile and Atenolol were obtained from Sammara Drug industry. The tablet dosage form of both CAN and ATE that were purchased from the Iraqi market, including Aderan®-16 mg, Novatan®-50mg and 100mg(Ajanta Pharma Limited, India), Atenolol Actavis®-50 mg and 100 mg, ActavisPharma), and Candelog®8 mg (Micro Labs Ltd., India).

Instruments

A double beam UV-visible spectrophotometer (model 1800, Shimadzu, Japan), with a pair of 1.0 cm quartz cell and with a scan range of 200–400 nm was used. The spectrophotometer connected to a computer loaded with UV Probe 2.32 software hasbeen used. All weights were taken using electronic balance, Denver, Germany.

General procedure for Candesartan cilexetil and Atenelol Estimation

perpetration of solvent (8:2 methanol- distilled water) and 0.1 M HCl diluted acid

For assay of CAN, the solvent used was prepared by diluting 80 ml of methanol with 20 ml of distilled water to get the solvent ratio of (8: 2). While, for assay of ATE, 2.1 ml of 11.9 M concentrated were pipetted to a 250 ml volumetric flask, and then the volume was completed up to mark by D.W.

Preparation of standard stock solution of CAN

CAN stock solution (100 µg /ml) was prepared by taking 2500µg of pure CAN and dissolved in methanol, then, transferring to 25 ml volumetric flask and making the volume to mark using a solvent of (8:2 methanol-DW).the final solution was used for more dilution

Procedure for Pharmaceutical Preparation

Preparation of (Candelog)® 80 µg /ml, (Aderan)® 160 µg /ml solutions

Twenty tablets of each tablet dosage form of CAN;Aderan-16 mg and Candelog- 8 mg, were separately weighted and then grinded using glass mortar. A quantity of tablet powder equivalent to 0.1347 g and 0.1297g for each cited drug was transferred to 100 ml volumetric flask, and the solvent was added to the mark. The solution was stirred for 15 minutes until the drug was dissolved then filtered using Whatman filter paper no. 42 to remove any insoluble substances. After filtration, the solution was diluted with diluent to get a final concentration of 80 µg /ml and 160 µg /ml for Candelog and Aderan, respectively.

Assay Procedure for Commercial CAN Formulations

Assay of (CANDELOG)®-8mg tablets

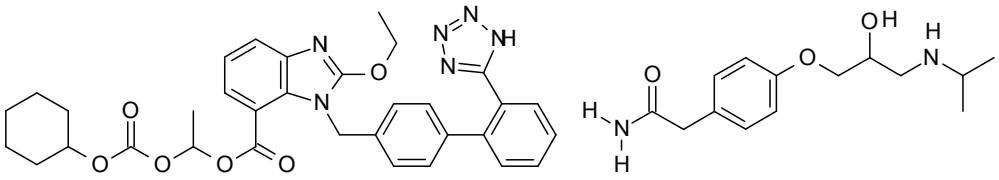
0.5ml, 1ml, 2ml were withdrawn from CANDELOG (80 µg/ml) stock solution then they were transferred to three (10 ml) volumetric flasks to prepare the concentrations of (4, 8, 16) µg/ml respectively. The volume was made up to the mark with solvent **(**8:2 methanol-DW).

Assay of (ADERAN)®-16mg tablets

0.25ml, 0.5ml, 1ml were withdrawn from ADERAN (160 µg/ml) stock solution, and they were transferred to three (10ml) volumetric flasks to prepare the concentrations of (4, 8, 16) µg/ml respectively. The volume was made up to the mark with solvent **(**8:2 methanol-DW).

Preparation of standard stock solution of ATE

10 mg of pure ATE was weighed, then it was dissolved in 0.1 M HCl. The solution was transferred to 100 ml volumetric flask, and the volume was made up to mark with solvent to get ATN stock solution of 100µg/mlthat was used for more dilution**.**



A **B**

Fig 1: Chemical structure of A- Candesartan Cilexetil B- Atenelol

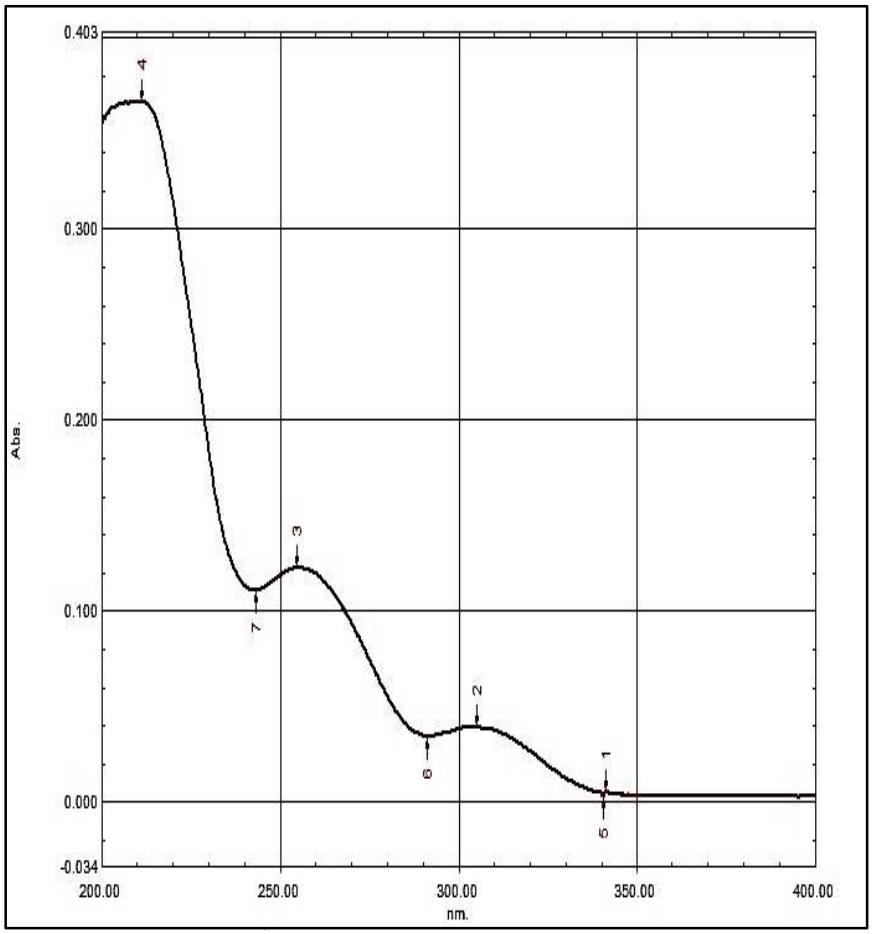


Fig 2: UV spectrum of CAN (5 µg/ml) in pure form

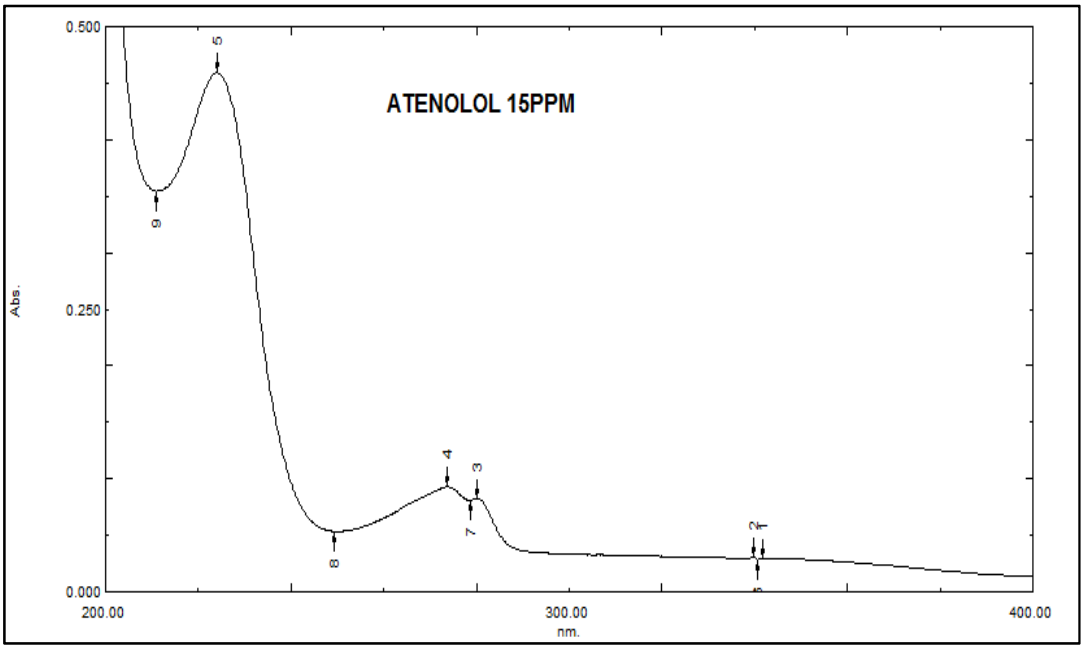


Fig 3: UV spectrum of ATN (15 µg/ml) in pure form

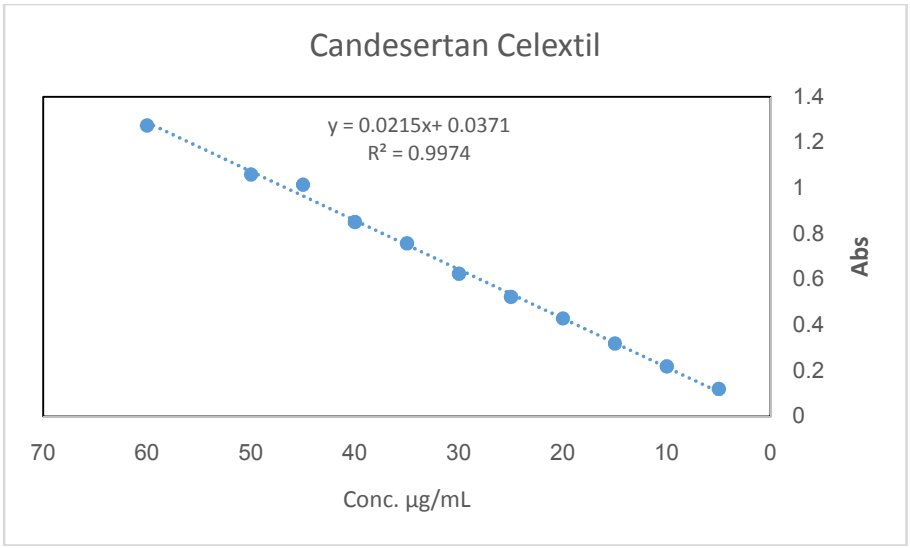


Fig 4. Linearity curve for the determination of CAN in pure form

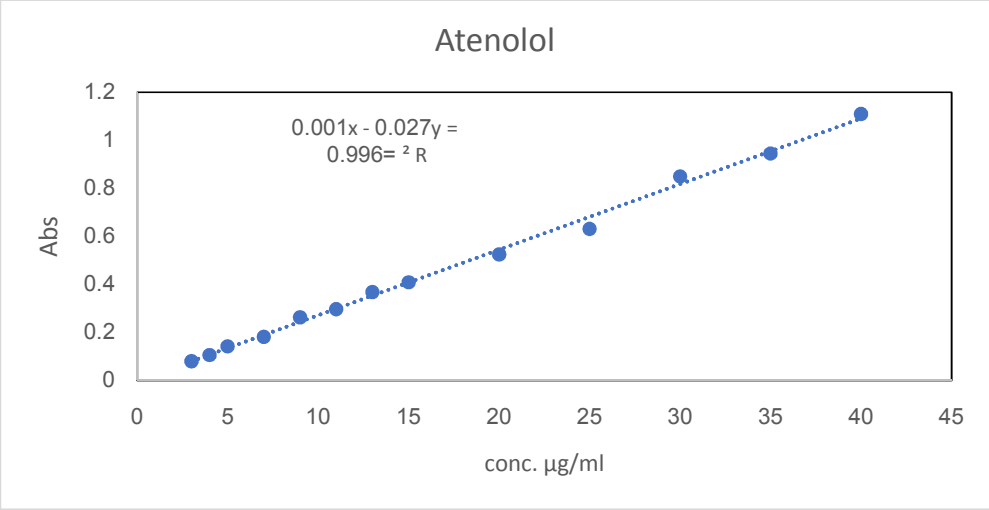


Fig. 5 Linearity curve for the determination of ATE in pure form

Procedure for Pharmaceutical Preparation

Sample Preparation of Atenolol (500 µg/ml and 1000 µg/ml) Solutions

Ten tablets of each marketed drug Atenolol Actavis ®50mg and Atenolol Actavis ®100 mg, (Novaten) ® 50 mg and (Novaten)® 100 mg were separately weighed and powdered using a glass mortar. A quantity of tablet powder equivalent to 0.2254 g, 0.4512g, 0.231g and 0.4053 g for each cited dosage form was transferred to 100 ml volumetric flask then the solvent was made to the mark. The solution was stirred using Ultrasonic device for 3 min, andthen it was filtered using Whatman filter paper no. 42 to remove insoluble substances. After that, the solution was diluted to attain the final concentration of 500 µg/ml and 1000 µg/ml for each dosage form.

Assay Procedure for Commercial ATE Formulations

Atenolol Actavis® -50 mg and Novaten® -50 mg

1ml, 1.25 ml were separately withdrawn from atenolol (Actavis) ® and Novaten (Ajanta) ® stock solution (500 µg/ml) and then were transferred to two volumetric flasks of 25ml in size to prepare the concentrations of 20 µg/ml 25 µg/ml, respectively. The prepared solutions were made up to mark with 0.1 M HCl.

Atenolol (Actavis)® -100mg and Novaten (Ajanta)-100mg :-

0.5 ml, 0.625ml were separately withdrawn from atenolol (Actavis) ® and Novaten (Ajanta) ® (1000 µg/ml) stock solution and then were transferred to two volumetric flasks of 25 ml in size to prepare the concentrations of 20 µg/ml25 µg/ml, respectively. The prepared solutions were made up to the mark.

Table 1: Optical Characteristic And Validation for determination of CAN and ATE in pure form

No	Parameters	CAN	ATN
1	ƛmax (nm)	254	224
2	LOQ (µg/ml)	0.48	0.3
3	LOD (µg/ml)	0.14	0.09
4	Regression Equation (y= a x+ b)	y=0.0215x+0.0371	y=0.0273x-0.0019
5	Correlation coefficient (R^2)	0.9974	0.9963
6	Percentage linearity (R^2%)	99.22	99.63
7	Slope	0.0215	0.0271
8	Intercept	0.0377	0.0019
9	Linearity range (µg/ml)	5-60	3-40

Table 2: Accuracy and precision results for CAN and ATE standard solutions

RE: relative error. RSD: relative standard deviation

Drug	Conc. taken µg/ml	Conc. founded (X) µg/ml	SD	%RSD	Recovery%	RE%
CAN	4	3.957	0.003	2.46%	98.9%	1.07%
	8	7.948	0.0036	1.74%	99.3%	0.65%
	16	15.929	0.0046	1.216%	99.5%	0.44%
	30	29.92	0.0036	0.5%	99.7%	0.26%
	50	50.53	0.0045	0.4%	101%	1.06%
ATE	10	9.84	0.002	0.74%	98.4%	1.6%
	20	19.8	0.0026	0.48%	99%	1%
	30	29.9	0.0045	0.55%	99.6%	0.33%

RESULTS AND DISCUSSION

Determination of λ max for Candesartan cilexetiland Atenolol in pure form

Separately, By a suitable dilution of stock solution for both CAN and ATE. (100µg/ml), stock solutions of 5µg/ml and 15 µg/ml of CAN and ATE, respectively were prepared and scanned in the wavelength range (200-400nm) against blank. It was found that the λ max that was selected as working wavelength was 254nm and 224 nm for CAN and ATE, respectively, as shown in **Fig 2 and 3.**

Method Validation

Linearity Studies For CAN and ATE Analytical Method

Linearity study for CAN in pure form

Fordeterminingthe linearity, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, and 6 ml were separately transferred from 100 µg/ml of CAN stock solution to 10 ml volumetric flasks, then made up to the mark with solvent (8:2 methanol: DW) to prepare the concentration range of (5- 60 µg/ml). These solutions were scanned against blank reagent (8:2 methanol:DW) at λ max 254 nm. The calibration curve was obtained by plotting concentration of CAN on X-axis, and their respective absorbance is on Y-axis. As shown in Fig 4, The calibration curve was found to be linear in concentration range of 5- 60 µg/ml with a high correlation coefficient (R^2) equal to 0.9974. The correlation coefficient, intercept, and slope for calibration data of CAN are tabulated in table 1.

Linearity studyfor ATE in pure form

For determination of linearity, 0.75 , 1, 1.25 , 1.75 , 2.25 , 2.75 , 3.25 , 3.75 , 5 , 6.25 , 7.5 , 8.75 and 10 mlwere separately transferred from 100 µg/ml of ATE stock solution to 10 ml volumetric flasks, then made up to the mark with 0.1M HCL to prepare the concentration range of (3-40 µg/ml). These solutions were scanned against a blank reagent blank (0.1M HCl) at λ max 224 nm. The calibration curve was obtained by plotting concentration of ATE on X-axis, and their respective absorbance is on Y-axis. As shown in **Fig 5**, The calibration curve was found to be linear in concentration range of 3-40 µg/ml with high correlation coefficient (R^2) equal to 0.9963. The correlation coefficient, intercept, and slope for calibration data of ATE are tabulated in table 1.

Table 3: Accuracy and Precision For Determination of CAN and ATE in Pharmaceutical Tablet Dosage Forms

RE: relative error. RSD: relative standard deviation

Companies	Drug	Conc. Taken µg/ml	Conc. founded (X) µg/ml	RSD%	Recovery%	RE%	Recovery average
Micro Labs Ltd.,	Candelog-8mg	4	3.95	4.5%	98.75%	1.25%	99.68
		8	7.948	1.73%	99.3%	0.65%	
		16	16.164	0.94%	101%	1%	
Ajanta pharma Ltd .	Aderan-16mg	4	3.95	1,41%	98.75%	1.25%	100.48
		8	8.089	1.42%	101.1%	1.11%	
		16	16.258	1.22%	101.6%	1.6%	
Actavis Pharma	Atenolol Actavis-50mg	20	19.7	0.48%	98.5%	1.5%	98.85
		25	24.8	0.443%	99.2%	0.8%	
	Atenolol Actavis-100mg	20	19.63	0.37%	98.1%	1.85%	98.75
		25	24.86	0.31%	99.4%	0.56%	
Ajanta Pharma Ltd.	Novaten-50mg	20	19.66	0.97%	98.3%	1.7%	98.35
		25	24.6	0.29%	98.4%	1.6%	
	Novaten-100mg	20	19.739	0.48%	98.69%	1.3%	98.74
		25	24.7	0.44%	98.8%	1.2%	

Table 4: Determination of CAN and ATE by suggested and standard method

Drug	Suggested methods		Standard method	
	Recovery%(Xi)1	$(Xi1-X1)^2$	Recovery % (Xi)2	$(Xi2-X2)^2$
Candelog-8mg	99.68	0.2916	98.35	0.1156
Aderan -16mg	100.48	1.795	99.26	0.36
Atenolol Actavis-50mg	98.85	0.0841	98.185	0.2601
Atenolol Actavis-100mg	98.75	0.1521	98.24	0.202
Novaten-50mg	98.35	0.6241	98.82	0.0169
Novaten-100mg	98.74	0.16		
	X1= 99.14	∑= 3.107	X2= 98.69	∑= 0.9546
T value (exp.)= 1.179		F value (exp.)= 2.437		

Accuracy and precision

The precision and accuracy were studied. Accuracy represented by per cent relative error (RE%) and precision, as relative standard deviation (RSD%). These two parameters were estimated for series of three determination at five levels of pure CAN (4,8,16, 30 and 50) µg/ml and three levels of pure ATE (10,20,30) µg/ml.As shown in Table2, the recovery percent values for each concentration used for both CAN and ATE were very close to 100% and with low RE%. Also, standard deviation values were minimal. Therefore, the results indicate the accuracy of the suggested methods. The results showed that relative standard deviations values were low and revealed the repeatability of suggested analytical methods.

Limit of detection and limit of quantification (LOD and LOQ)

LOD and LOQ were determined and calculated using the following equations:

LOD = 3 S/kWhere S is the standard deviation and k is the slope in the calibration curve.

LOQ =10 S/K

As shown in table1, the LOD and LOQ were found to be (0.14 µg/ml, and 0.48 µg/ml), respectively for

CAN and (0.09 µg/ml, and 0.3 µg/ml), for ATE. The values revealed that the methods used were sensitive as they were within the permitted levels.

Assay of Commercial Tablet Dosage Form

The selected commercial formulations with different brand names of CAN and ATE were assayed. For CAN formulations, the series of three determinations at three concentration levels of (4,8 and 16 µg/ml) were estimated for which the % recovery was found to be in range of 98.75 to 101.6. While for ATE tablet formulations, the series of three determinations at two concentration levels of (20 and 25 µg/ml) were estimated in which the % recovery was found to be 98.85- 98.75- 98.35 and 98.74. The results were listed in Table 3demonstrated the high percentage recoveries and low RSD%. The values for tablet formulations were very close as citedin label claim, signifying that no additives and excipients effect used in tablet preparation for each determined drug. Besides, the recovery results of suggested methods were compared with those obtained by UV spectrophotometric British Pharmacopeia (2012) through the application of t- test and F-test at 95% confidence level.The results of the two tests (as in table 4)indicated that there is no significant difference between the results of present methods and the conventional methods. Therefore, the methods used were suitable for routine analysis of these constituents in their tablet pharmaceutical preparation.

CONCLUSION

In conclusion, the suggested methods represent secure, opportuneness, accurate and linear data for determination of candesartan cilexetil and atenolol in pure form and tablet formulation. Also, according to statistical tests were used to evaluate the proposed methods showed that the methods are efficient for analysis of samples and to detect the quality control of therapycontaining either candesartan cilexetil or atenolol.

ACKNOWLEDGEMENTS

The authors thanks Sammara drug laboratories for providing gift samples of candesartan cilexetil and atenolol. We are thankful the staff of pharmaceutical chemistry department for their support and advice to complete this work.

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