Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(2):122-133
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Validated kinetic spectrophotometric determination of candesartan cilexetil and olmesartan medoxomil using alkaline permanganate oxidation

Ekram Mahmoud Hassan*, Marwa Khaled El Jamal* and Mahmoud Abdelhady El Sayed

Department of Pharmaceutical Analysis, Faculty of Pharmacy, Beirut Arab University, Lebanon

---

ABSTRACT

A simple and sensitive kinetic method is described for the determination of two hypotensive drugs belonging to the angiotensin II antagonist Candesartan Cilexetil (CAN) and Olmesartan Medoxomil(OLM). This method is based upon a kinetic investigation of drug oxidation with alkaline potassium permanganate. All variables affecting color development have been investigated and the conditions optimized. The kinetic rate was obeying pseudo-first order reaction. Among the methods applied were the Initial rate, Rate constant, Fixed-concentration and Fixed-time methods. Accounting for the applicability, the sensitivity, values of correlation coefficient (r) and intercept (a), the Fixed-time method is selected for these two drugs assay. The absorbance-concentration plots were rectilinear within the range of 5-30 ug.mL^{-1}for CAN and20-60 ug.mL^{-1}for OLM. The statistical data for the results challenged for the robustness of the fixed-time method.

Keywords: Candesartan Cilexetil; Olmesartan Medoxomil; Kinetic; Spectrophotometry; Determination.

INTRODUCTION

The hypotensive drugs Candesartan Cilexetiland Olmesartan Medoxomilare belonging to the angiotensin II antagonists. These are prodrugs that are rapidly and completely de-esterified to the active metabolites candesartan and olmesartan by both arylesterase and albumin during gastrointestinal absorption.

Candesartan Cilexetil, [1-[[(cyclohexyloxy)carbonyl]oxy]ethyl 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1H-benzimidazole-7-carboxylate],was determined in dosage formulations and biological fluids by different methods. These included UV and ratio derivativespectrophotometry[1] ,HPTLC and difference spectrophotometry [2],solid phase extraction coupled to electrospray tandem mass spectrometry[3],HPLC [4-5],HPLC tandem mass spectrometry [6-7],densitometry[8].

On the other hand several methods have been reported for Olmesartan Medoxomil, [(5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazole-5-carboxylate],determination. These were direct spectrophotometry[19-11],difference and derivative spectrophotometry [12,13], fluorimetry[14],capillary zone electrophoresis[15].

Recently kinetic methods have been reported for the assay of many pharmaceutical compounds like ampicillin [16], tobramycin [17,18], cisapride [19], ipratropium bromide [20], atenolol [21], norfloxacin [22].

In the present work, kinetically based method is proposed for the determination of CAN and OLM through absorbance measurement at 609 nm after oxidation reaction with alkaline permanganate. Although the poor selectivity of the proposed methods, yet it is more simple, time saving and more economic compared with HPLC and other sophisticated chemometric methods. These facts encourage to apply such methods in drug quality control laboratories.

MATERIALS AND METHODS

Apparatus

Spectrophotometer: The spectroph-otometric measurements were carried out on a Jasco V-530 double beam UV-Vis Spectrophotometer connected to a computer loaded with Jasco UVPC software and an HP Deskjet 5652 printer. The absorption spectra were measured using 1 cm quartz cells. For the derivative, the absorption spectra were recorded on the same spectrophotometer, with 1 cm quartz cells and supported with Jasco Spectra Manager software for GULLIVER Ver. 1.53,and the same printer.

Balance: Adventurer TM, Ohaus Corporation Pine Brook, NJ USA, sensitivity = 0.1 mg.

Orbital Shaker: Dissolution was done using Wiggen Hauser Shaker OS-150.

Water Bath:A thermostatically controlled water bath (Gemmy Industrial Corp. Instruments,Spain) was used to control the temperature of the reaction mixture.

MATERIALS AND REAGENTS

Authentic samples: Candesartan Cilexetil & Olmesartan Medoxomil from PHARO Pharma, Alexandria, Egypt were used as working standards.

Standard solutions: In two separate 100-mL flasks, accurate weights of 20 mg of CAN or OLM were transferred and dissolved in 0.01 M NaOH (with little warming for CAN dissolution.) and diluted to volume with the same solvent (20 mg% w/v).For working standarddilution was made 10 times (20 $mg.mL^{-1}$).

Calibration graphs: Into separate series of 10-ml volumetric flasks, aliquots of 5-20 $\mu g.mL^{-1}$ for CAN or 20-60 $\mu g.mL^{-1}$for OLM were transferred. To CAN flasks, 3 ml 0.5 M NaOH and 4 ml 0.01 M $KMnO_4$ and to OLM flasks, 4 ml 0.5 M NaOH and 3 ml 0.01 $KMnO_4$were added. The flasks were immersed in a thermostatic water bath at 70ºCfor 30 min (CAN) or at 30ºCfor 30 min (OLM).Both series were cooled to room temperature and volumes completed with water. The absorbances were measured at 609 nm using the corresponding blank simultaneously prepared.The corresponding regression equation, relating final concentration versus corresponding absorbance were derived.

Tablet Assay: Ten tablets of each drug were separately weighed, powdered and mixed. A weight equivalent to 20 mg of CAN or OLM was transferred into separate small flasks (50-mL capacity).The Tablet base was extracted with water by decantation through filter paper till negative test with alkaline permanganate. The drug residue was quantitatively collected onto filter paper, then dissolved by 0.01 M sodium hydroxide into separate 50-mL volumetric flask and completed to volume with the same solvent. Oneml aliquot of each drug was transferred to 10-ml volumetric flask and procedure completed as under calibration graph starting from "aliquots of 5-20 $ug.mL^{-1}$for CAN or 20-60 $ug.mL^{-1}$for OLM….."

RESULTS AND DISCUSSION

Optimization of reaction conditions:

The reaction of CAN and OLM with potassium permanganate in alkaline medium yielded a green color with manganate ion formation, exhibiting λ_{max} at 609 nm (Fig. 1).

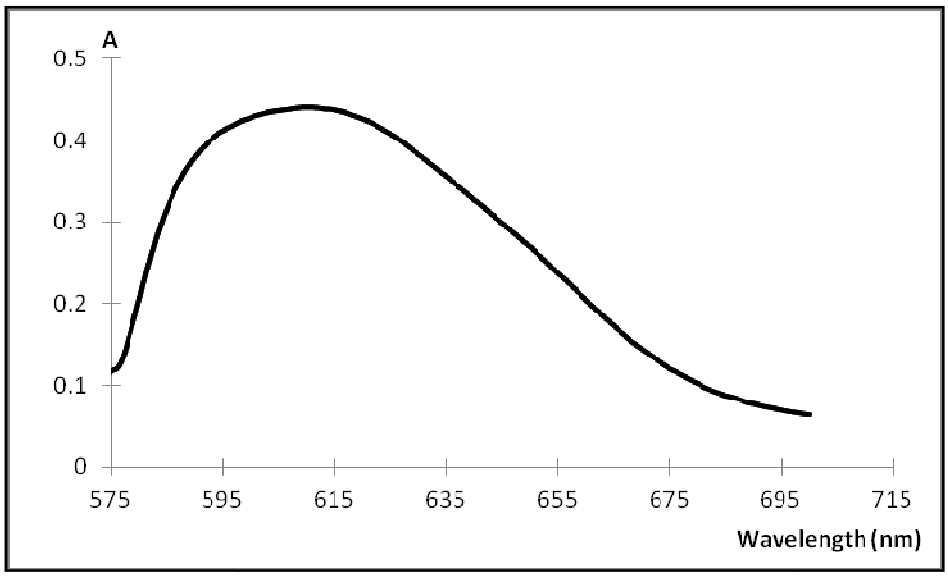


Fig. 1: Absorption spectrum of 20 µg.mL^{-1} of OLM with 3 mL 0.01 mol.L^{-1} $KMnO_4$,4 mL 0.5 mol.L^{-1} NaOH at 30°C for 30 min

As the intensity of color increases with time, it was deemed useful to elaborate a kinetically based method for the determination of CAN or OLM in tablets.In order to come to this conclusion, the reaction was investigated under various conditions of reagent concentration and alkalinity. It was found that the use of 4.0x10^{-3} M and 3.0x10^{-3} M of $KMnO_4$ were adequate for reaction with CAN and OLM, respectively (Fig 2).

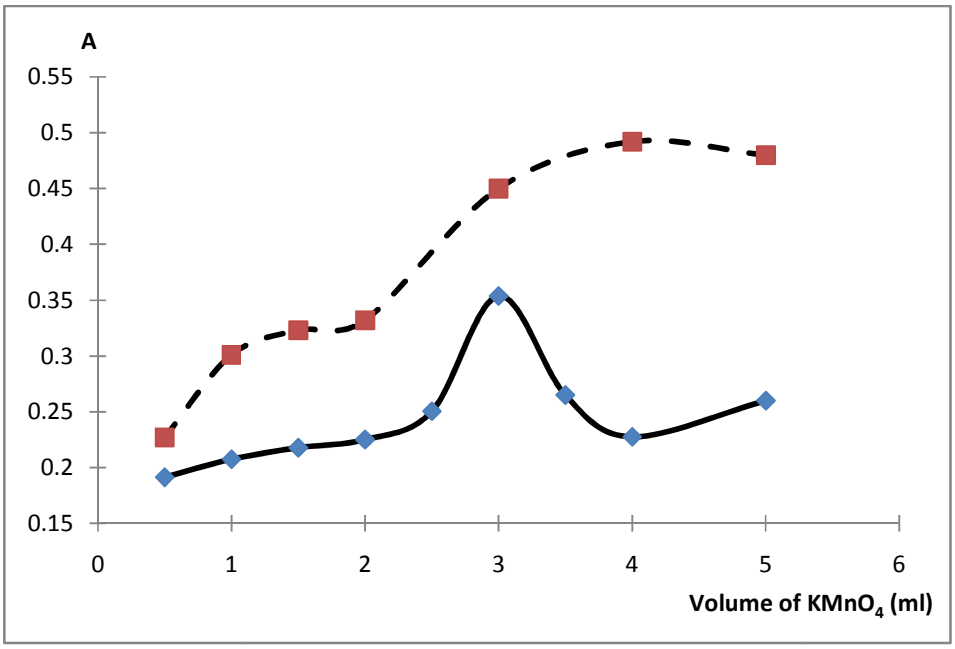


Fig. 2: Effect of volume of $KMnO_4$ (4x10^{-3} M for CAN,3x10^{-3} M for OLM) on the absorbance of the reaction product of 20 µg.mL^{-1} of either CAN (- - -) or OLM (—) at 609 nm

The influence of NaOH concentration on the reaction rate was investigated. The reaction rate increased using 0.15 M NaOH or 0.2 M of NaOH for CAN and OLM, respectively (Fig 3).

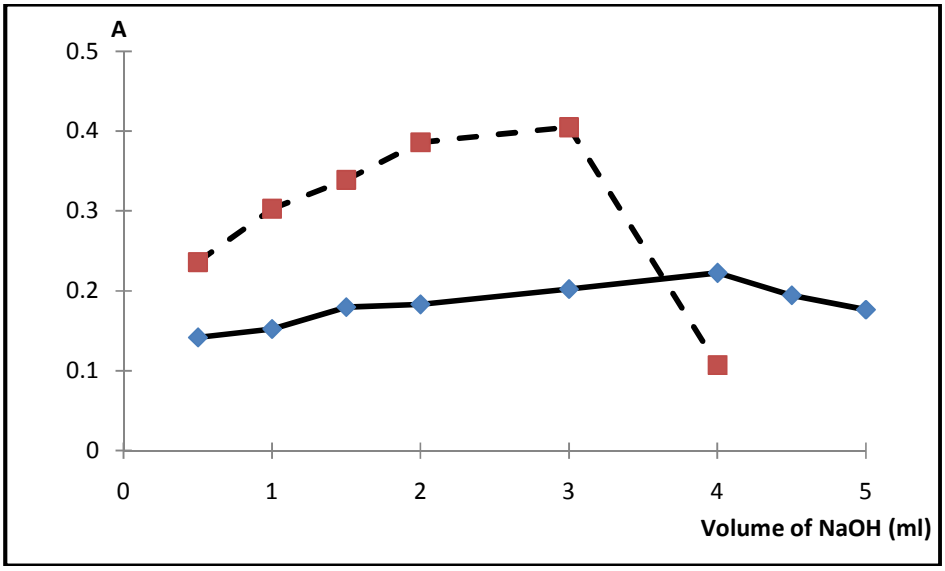


Fig. 3: Effect of volume of NaOH(0.15 M for CAN and 0.2 M for OLM) on the absorbance of the reaction product of 20 µg.mL^{-1} of either CAN (- - -) or OLM (—) at 609 nm

Sodium hydroxide concentrations higher than 3 mL for CAN or 4 mL for OLM resulted in lower absorbancevalues.Optimum temperature for the reaction with alkaline $KMnO_4$ was found to be 70 ºC for CAN and 30 ºC for OLM (Fig4).

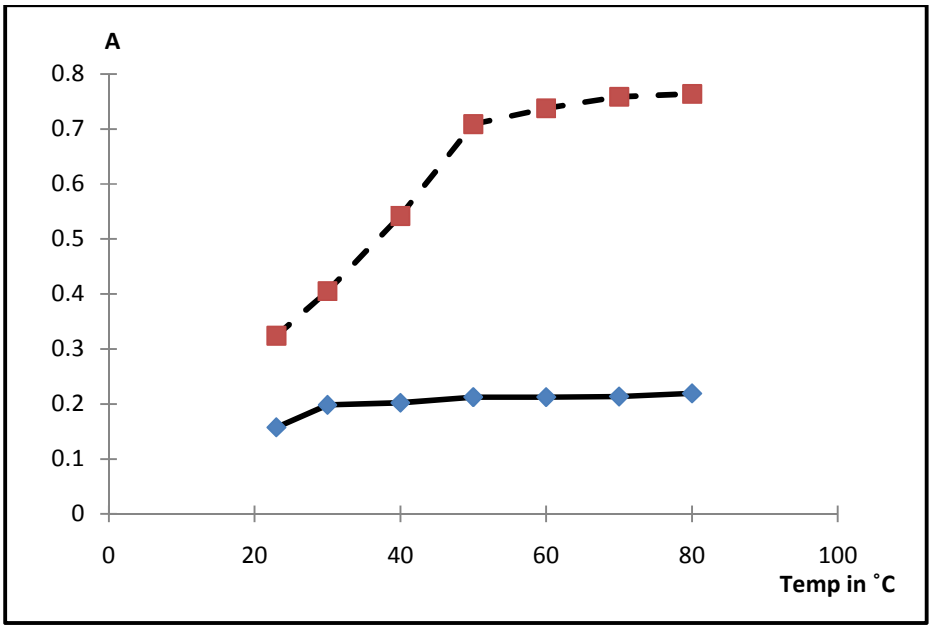


Fig. 4: Effect of temperature (70°C for CAN and 30°C for OLMon the absorbance of the reaction product of 20 µg.mL^{-1} either CAN (- - -) or OLM (—) at 609 nm

Above these temperatures, no significant change in absorbance was observed.The stoichiometry of the reaction was investigated using Job's method under the specified conditions mentioned above. The molar ratio was found to be 1:2 drug to $KMnO_4$ for both investigated compounds (Fig 5).

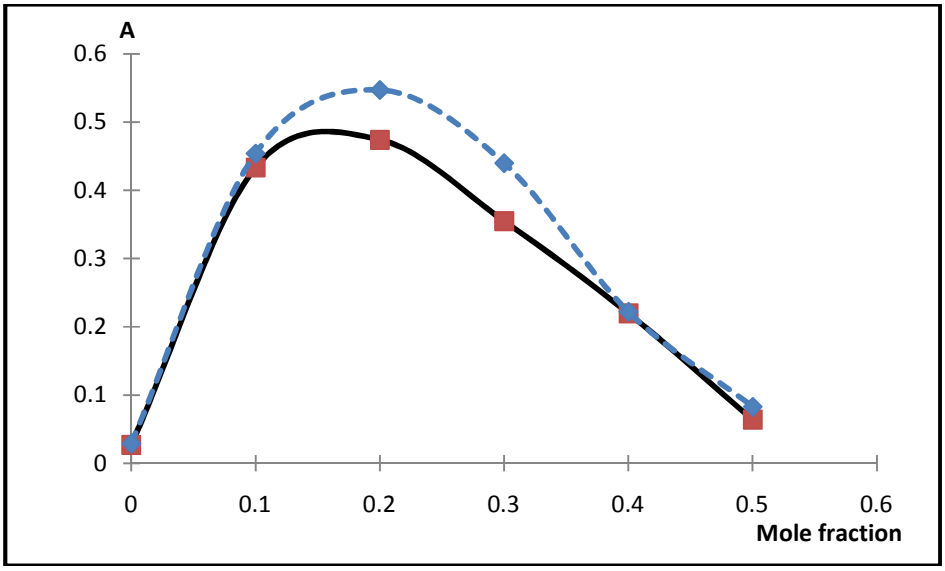
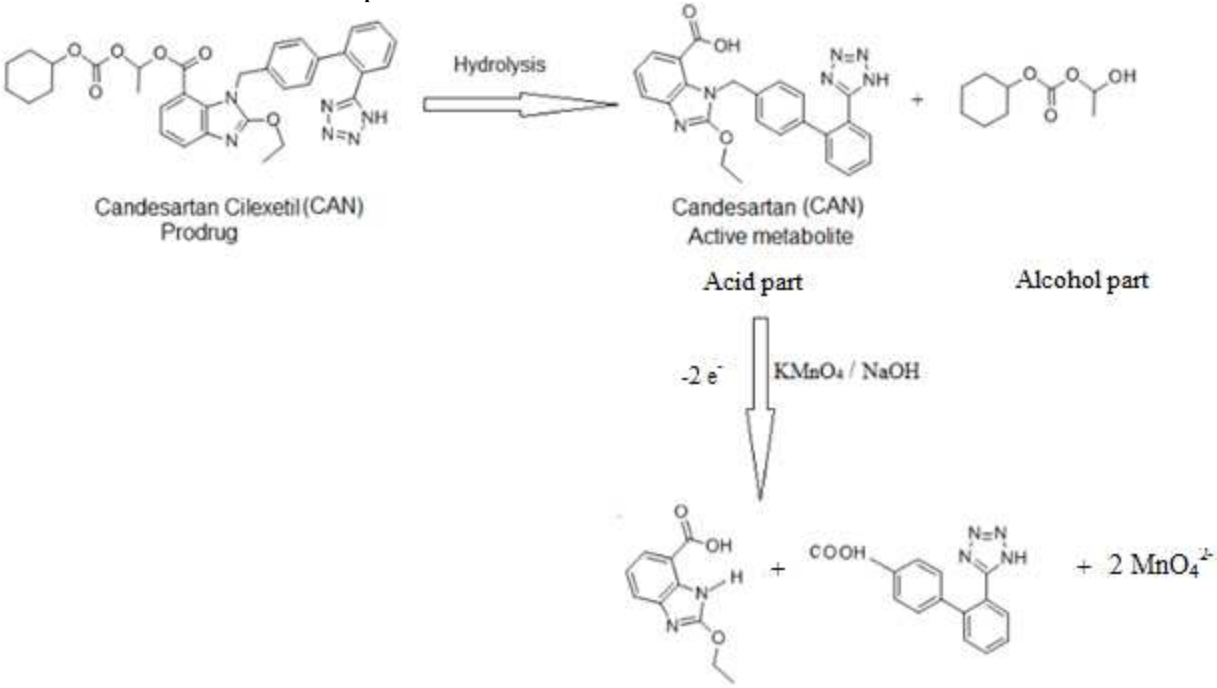


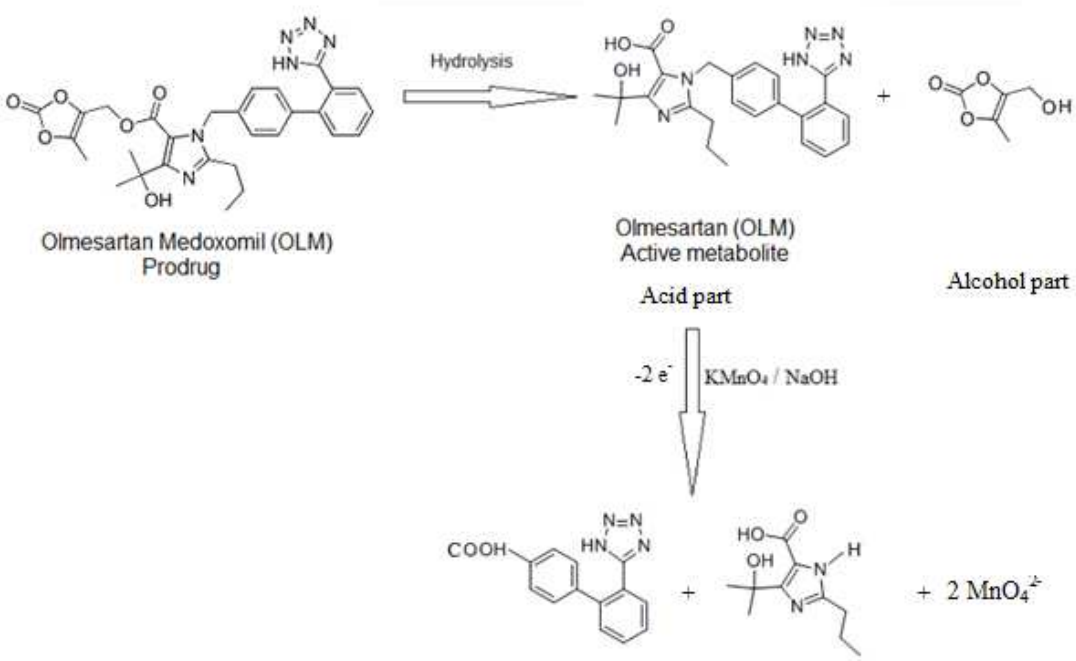
Fig. 5: Continous variation plot for the determination of the molar ratio of the reaction of CAN (—) and OLM (- - -) with $KMnO_4$

Therefore each drug molecule donates 2 electrons to two permanganate ions in alkaline medium giving manganate ions (scheme 1&2).

Scheme 1: Proposed reaction between CAN and $KMnO_4$ in alkaline medium



Scheme 2: Proposed reaction between OLM and $KMnO_4$ in alkaline medium



Kinetic study of the reaction:

The kinetic reaction was found to be drug concentration dependent. The initial rates of the reactions were determined from absorbance-time plot for different CAN or OLM concentrations (Fig 6&7).

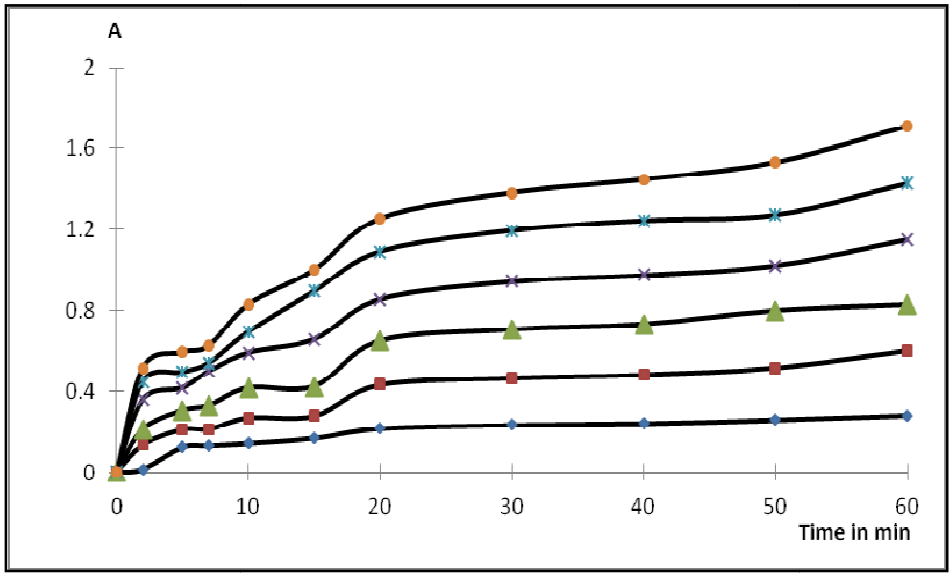


Fig. 6: Absorbance-time curve for the reaction of CAN (5-30 µg.mL^{-1}) with $KMnO_4$ and NaOH

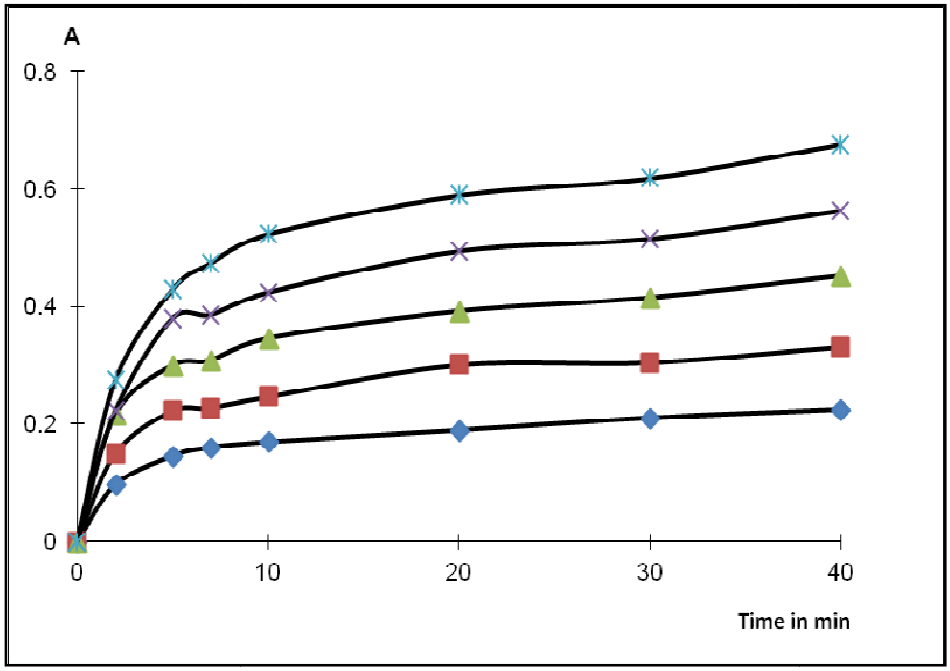


Fig. 7: Absorbance-time curve for the reaction of OLM (20-60 µg.mL^{-1}) with $KMnO_4$ and NaOH

Keeping the concentration of $KMnO_4$ and NaOH at constant concentration, the reaction rate was found to obey the following equation:

Rate = K´ $[C]^n$ …… (1)

Where k´ is the pseudo-order rate constant and n is the order of reaction. From (Fig. 6&7) thereaction rate may be estimated by the variable-time method [23],measured as $\frac{\Delta A}{\Delta T}$, where A is the absorbance and t is the time in seconds.Taking logarithms of rates and concentrations of the above equation (1):

Log (rate) = log $\frac{\Delta A}{\Delta T}$ = log k´+ n log [C] …….. (2)

The corresponding regression equations using least squares method gave the following equations:

For CAN: Log (rate) = 2.4 + 1.2 log C (Fig 8)

With correlation coefficient (r) = 0.994 and with, k´ = 251 S $^{-1}$ and the order of reaction is first order (n ~1).

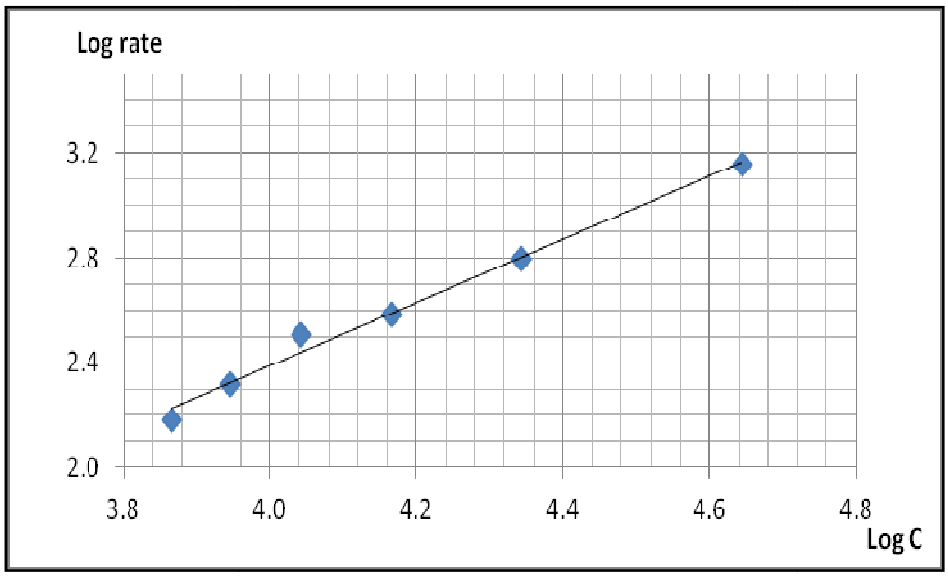


Fig. 8: Log rate versus log C of CAN

For OLM: Log (rate) = 2.87 + 1.28 log C (Fig 9)

With correlation coefficient (r) = 0.995 and with, k´= 741.31 S^{-1} and the reaction is first order (n~1).

Therefore, the oxidation of the investigated drugs was obeying pseudo-first order reaction.

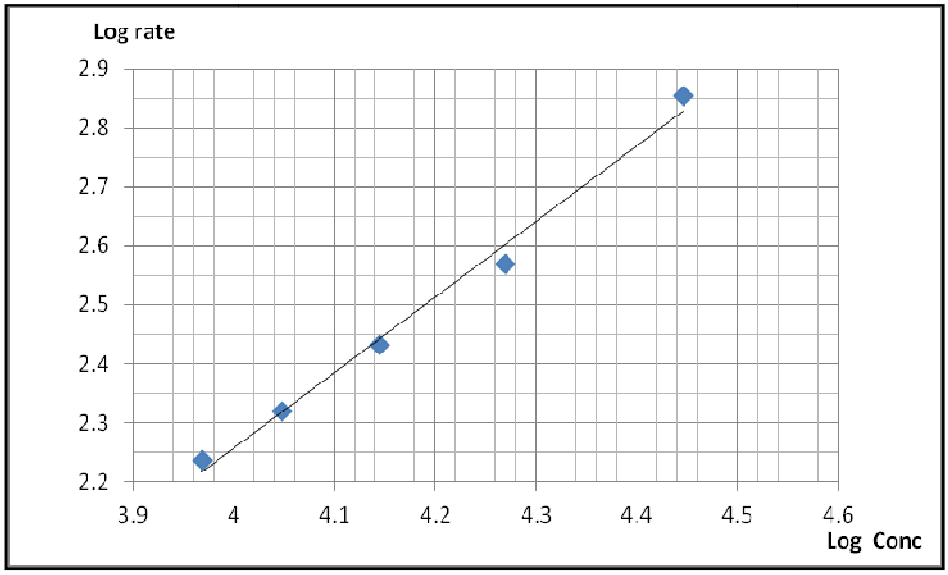


Fig. 9: Log rate versus log C of OLM

Methods validation:

Rate-constant, Initial rate, Fixed-concentration and Fixed-time methods were investigated. Taking into account the applicability, the sensitivity, the correlation coefficient (r) and the intercept, the most suitable method was selected for the precise determination of the drugs CAN and OLM.

Rate-constant method:
Graphs of Log (Absorbance) versus time, over the concentration ranges of $2.27x10^{-5}$-$1.362\ x10^{-4}$ mole.$L^{-1}$for CAN (Fig. 8) and $3.58x10^{-5}$-$1.074\ x10^{-4}$ mole.$L^{-1}$for OLM (Fig. 9) , were plotted and all appeared to be rectilinear. Pseudo- first order rate constants corresponding to different concentrations of CAN and OLM, were calculated from the slopes multiplied by (-2.303) and presented in table 1.

Regression of [C] versus k ´ gave the equations:

k´= -0.026+61.1 C r = 0.802, for CAN

k´= -0.01525-6.3 C r = 0.376, for OLM

The value of the correlation coefficients (r) indicate poor linearity, which is probably due to changes in the rate constant (k´), with the slight changes in the temperature of the reactions.

Table 1: Values of k´, calculated from slopes of log A versus time graphs multiplied by (- 2.303), for different concentrations of CAN and OLM

Drug	K´(s^{-1})	C, [mole.L^{-1}]
CAN	-0.00272	$2.27x10^{-5}$
	-0.02181	$4.54\ x10^{-5}$
	-0.02080	$6.81x10^{-5}$
	-0.01819	$9.08x10^{-5}$
	-0.01914	$1.135x10^{-4}$
	-0.01964	$1.362x10^{-4}$
OLM	-0.01573	$3.58x10^{-5}$
	-0.01564	$5.37x10^{-5}$
	-0.01495	$7.16x10^{-5}$
	-0.01617	$8.95x10^{-5}$
	-0.01603	$1.07x10^{-4}$

Initial rate method: The initial rates of the reactions were determined from the absorbance-time plots (Fig. 6&7), by measuring the slopes of the initial tangents to the absorbance-time curves at different concentrations of the investigated drugs, and are summarized in table 2.

Regression of the initial rates versus [C] gave the equations:

$\upsilon = \Delta A/\Delta t = - 0.0209 + 2091.8$ C, r = 0.993 for CAN, k´= 0.953 S^{-1}

$\upsilon = \Delta A/\Delta t = 0.0107 + 1189.9$ C, r = 0.978 for OLM, k´=1.025 S^{-1}

The values of the correlation coefficients (r) indicate poor linearity, indicating that the first step is too fast and not rate determining.

Table 2: Values of slopes calculated for different concentrations of CAN at 70°C with constant concentration of sodium hydroxide (0.15 mole.L^{-1}) and potassium permanganate ($4x10^{-3}$ mole.L^{-1}) and OLMat 30°C with constant concentration of sodium hydroxide (0.2 mole.L^{1}) and potassium permanganate ($3x10^{-3}$ mole.L^{-1})

Drug	C,mol.L^{-1}	Slope,S^{-1}
CAN	$2.27x10^{-5}$	0.0080
	$4.54\ x10^{-5}$	0.0675
	$6.81x10^{-5}$	0.1065
	$9.08x10^{-5}$	0.1800
	$1.135x10^{-4}$	0.2245
	$1.362x10^{-4}$	0.2585
OLM	$3.58x10^{-5}$	0.0485
	$5.37x10^{-5}$	0.0750
	$7.16x10^{-5}$	0.1080
	$8.95x10^{-5}$	0.1110
	$1.07x10^{-4}$	0.1370

Fixed concentration method: The procedure for each of CAN and OLM was followed up at different concentration levels by recording the time in seconds required for the absorbance to reach a preselected value. This preselected value was chosen as it gave the widest calibration range. The reciprocals of time (1/Δt) were plotted versus the initial concentration of CAN and OLM and the equations of calibration graphs are given in table 3.The values of the correlation coefficients indicate poor linearity, which is considered a disadvantage.

Table 3: Value of (1/Δt) taken at fixed absorbance* for different concentrations of CAN at 70°C with constant concentration of sodium hydroxide (0.15 mol.L^{-1}) and potassium permanganate (4x10^{-3} mol.L^{-1}) and OLMat 30°C with constant concentration of sodium hydroxide (0.2 mol.L^{-1}) and potassium permanganate (3x10^{-3} mol.L^{-1})

Drug	Δt (min)	1/Δt(S^{-1})	C,mol.L^{-1}	Regression equation	Regression coefficient (r)
CAN	16.00	0.00104	4.54 x10^{-5}	1/Δt = -0.0101 + 220.264 C	r = 0.989
	4.80	0.00347	6.81x10^{-5}		
	1.60	0.01042	9.08x10^{-5}		
	1.20	0.01389	1.135x10^{-4}		
	0.80	0.02083	1.362x10^{-4}		
OLM	24.32	0.0123	3.58x10^{-5}	1/Δt = -0.00442 + 155.06 C	r = 0.964
	3.63	0.00921	5.37x10^{-5}		
	1.81	0.00459	7.16x10^{-5}		
	1.36	0.000685	1.07x10^{-5}		

**The preselected absorbance values for CAN is 0.3 and for OLM is 0.2.*

Fixed time method: Reaction rates were determined for different concentrations of CAN and OLM. At a preselected fixed-time, which was accurately determined, the absorbance was measured. Calibration graphs of absorbance versus concentration of CAN and OLM were established at fixed times of 2, 5, 7, 10, 20, 30, 40, 50 and 60 min for CAN in the concentration ranges of 2.27x10^{-5}-1.362x10^{-4} mol.L^{-1} (5-30 µg.mL^{-1}) and at fixed times of 2, 5, 7, 10, 20, 30 and 40 min for OLM in the concentration range of 3.58x10^{-5}-1.074x10^{-4} mol.L^{-1} (20-60 µg.mL^{-1}) with the regression equations assembled in table 4.It is clear that the slopes increase with time and the most suitable values for the correlation coefficient (r) and the intercept (a) were obtained for a fixed-times of 30 min for CAN and OLM (table 4).This was therefore chosen as the most suitable time interval for measurement.

Table 4: Regression equations at different fixed times for CAN at 20,30,40,50 and 60 min in the concentration ranges of 2.27x10^{-5}-1.362x10^{-5} mol.L^{-1} (5-30 µg.mL^{-1}) and for OLM at 20,30 and 40 min in the concentration ranges of 3.58x10^{-5}-1.074x10^{-4} mol.L^{-1} (20-60 µg.mL^{-1})

Drug	Time (min)	Regression equation*	Correlation coeffecient (r)
CAN	20	A=0.0126+0.042C	0.9993
	30	A=0.00487+0.0466C	0.9997
	40	A=-0.024+0.0486C	0.9996
	50	A=0.0153+0.0505C	0.9997
	60	A=0.0056+0.0568C	0.9993
OLM	20	A=-0.0032+0.00991 C	0.9996
	30	A=0.00325+0.01024C	0.9998
	40	A=-0.0036+0.0113C	0.9999

**Regression equation calculated using concentrations in µg.mL^{-1}.*

Statistical evaluation of the regression line (table 5) gave small values for the standard deviation of residuals ($S_{y/x}$), standard deviation of the slope S_b. These small values reflect the high reproducibility of the proposed method.The limit of detection LOD and quantitation LOQ were calculated using statistical treatment of calibration data. These statistical data challenged for the robustness of the fixed-time method under the optimum reaction condition for carrying it in the assay of CAN and OLM.

Pharmaceutical applications: Direct application of the proposed method to the determination of CAN and OLM in pharmaceutical preparations resulted in high % recoveries. This might be due to the interaction of excipients in the formulations (especially lactose, hydroxymethyl and hydroxypropyl cellulose which contain hydroxyl groups) with alkaline permanganate. Other solvents as, acetone and isopropanol,were tried. Such solvents failed to correct for the interference due to their ability to dissolve excipients together with the drug. Best results were obtained by successive extraction of the tablets with water and subsequent rejection of the water extract. The determined drugs,

CAN and OLM, are insoluble in water but soluble in alkaline NaOH. The results obtained (table 6) were compared with the first-derivative spectrophotometric method (D_1-method) [228-246 nm] for CAN or [246-272 nm] for OLM. The student t- test and variance ratio-F-test values at 95% confidence level did not exceed the theoretical values [24], indicating no significant difference in accuracy and precision of the proposed kinetic spectrophotometric method and the D_1- method.

Table 5: Analytical parameters for the determination of CAN and OLM using the fixed-time method

Parameters	CAN	OLM
λ_{nm}	609	609
Linearity range (µg.mL^{-1})	5-30[a]	20-60[b]
a	4.87 x 10^{-3}	3.25 x 10^{-3}
b	4.66 x 10^{-2}	1.02 x 10^{-2}
r	0.9997	0.9997
S_a	1.19x10^{-2}	5.09 x10^{-3}
S_b	6.10x10^{-3}	1.2 x10^{-4}
$S_{y/x}$	1.18 x10^{-3}	4.32x10^{-5}
% Error	1.72	0.379
LOD (µg.mL^{-1})	1.030	1.492
LOQ (µg.mL^{-1})	3.34	4.97

[a] 6 points, at 5-µg.mL^{-1} intervals
[b] 5 points, at 10-µg.mL^{-1} intervals

s

Table 6: Determination of CAN and OLM in pharmaceutical preparations using the fixed-time method and D_1-method

Drug	Pharmaceutical preparation	Mean Recovery ± SD[a] RSD %[b] Er %[c]	
		Fixed-time method	**D_1-method**
CAN	Atacand® 16 mg	99.47 ± 1.18 1.18 -0.53	100.64 ± 1.45 1.44 0.64
		t** = 0.95 F** = 1.53	
OLM	Olmetec® 20 mg	101.10 ± 0.68 0.67 1.10	99.70 ± 0.70 0.70 -0.30
		t** = 2.11 F** = 1.06	

[a] Mean ± SD for the five determinations; [b] % Relative standard deviation; [c] % Relative error
***Theoretical values of t- and F- at P = 0.05 are 2.13 and 6.93,respectively.*

CONCLUSION

In conclusion the developed kinetic method is simple for the quantitation of CAN and OLMin their tablet formulations. The method has the advantage of using inexpensive instrument and easily available reagents. Therefore the proposed method can be frequently used in the quality control for the investigated drugs in the research laboratory belonging to pharmaceutical insudtries.

REFERENCES

[1] N Erk,*Pharmazie*,**2003**, 58(11),796-800.
[2] RM Youssef, HM Maher, EM Hassan, EI EL- Kimary, MA Barary,*Int. J. Appl. Chem.*, **2010**, 6(2), 233-246.
[3] M Levi, G Wuerzner, EEzan, APruvost,*J. Chromatogr., B: Anal. Technol. Biomed. Life Sci*.,**2009**, 877(10), 919-926.
[4] SS Qutab; SN Razzaq, M Ashfaq, ZA Shuja, IU Khan, *Acta Chromatogr*.,**2007**, (19), 119-129.
[5] DVS Rao, P Radhakrishnanand, MV Suryanarayan, V Himabindu, *Chromatographia*,**2007**, 66(7-8), 499-507.
[6] HG Lou, Z.R.Ruan, BJiang, *J. Liq. Chromatogr. Relat. Technol*.,**2012**, 35(8), 1027-1037.
[7] B Dogan, B Ulsu, SA Ozkan, *Pharmazie*,**2004**, 59(11), 840-844.
[8] M Stolarczyk, A Maslanka, JKrzek,*J. liq. Chromatogr. Relat.Technol*.,**2008**, 31(13-16), 1982-1902.
[9] M Celebrier, S Altinoz, *J. Fac. Pharm. Hacettepe Univ*.,**2007**, 27(2), 119-130.
[10] NJ Shah, Shah, BN Shah, RR Shah, NM Patel, *Ind. J. Pharm. Sci*.,**2007**, 69 (6), 834-836.

[11] S Caglar, A Onal, *J. Anal. Chem.*,**2010**, 65 (3), 239-243.
[12] PMehulkumar, VRamesh, V Vinay Kumar, R Srinivas, VPrakash Diwan 1, *Asian J, Research Chem.***2009**, 2(2): April.- July,.
[13] M Celebier, S Altinoz, *Pharmazie*,**2007**, 62 (6), 419-422.
[14] BA Moussa, MF Mohamed, NF Youssef,*Journal of applied Spectroscopy*, 2011, 77(6), 869-877.
[15] PD Bari, AR Rote, *Chromatographia*,**2009**, 69 (11-12), 1469-1472.
[16] JQ Yuan,SM Li; HWang, JYu, WTJing, *Yaowu fenxi zazhi***2006,** 26, 761-763.
[17] HGLou, ZRRuan, B Jiang,*J. Liq. Chromatogr. Relat. Technol*. **2012,** 35, 1027-1037.
[18] BDogan, B Ulsu, SAOzkan, *Pharmazie* **2004,** 59, 840-844.
[19] MStolarczyk, AMaslanka, JKrzek, *J. liq. Chromatogr. Relat.Technol*. **2008,** 31, 1982-1902.
[20] RM Youssef, HMMaher, EM Hassan, EIEL- Kimary, MA Barary,; *Int. J. Appl. Chem*. **2010,** 6, 233-246.
[21] SCSiTripti Sharmal, D Gowrishankar, Tripti Sharma et al., *J. of Pharm. Res.***2010,** 3, 1553-1555.
[22] M Celebier, S Altinoz,*Pharmazie***2007,** 62, 419-422.
[23] D Pérez-BenditO, M Silva,"*Kinetic Methods in Analytical Chemistry''* J. Wiley and Sons, New York (**1988**), chapter II, p. 44-45.
[24] JN Miller, JC Miller,, *Statistics and Chemometrics for Analytical Chemistry*, 5th Edition,Pearson Prentice Hall, London (**2005**).