Trade Science Inc.

September 2009 Volume 8 Issue 3

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 8(3) 2009 [334-341]

Spectrophotometric determination of captopril and ethamsylate in pharmaceutical preparations

N.El-Enany[1,*], F.Belal[1], M.Rizk[2]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, 35516, Mansoura, (EGYPT)

[2]Department of Analytical Chemistry, Faculty of Pharmacy, University of Helwan, Cairo, (EGYPT)

E-mail : nelenany1@yahoo.com

Received: 4th July, 2009 ; Accepted: 14th July, 2009

ABSTRACT

Two simple and sensitive methods were developed for the determination of captopril (CPL) and ethamsylate (ESL) in pharmaceutical preparations. The method is based upon investigation of the oxidation reaction of these drugs with alkaline potassium permanganate. In the first one, the absorbance of the coloured manganate radical was measured at 610 nm. Alternatively, the decrease in the absorbance of potassium permanganate after addition of the drugs was measured at 525 nm. The absorbance-concentration plots in both methods were rectilinear over the range of 4-24 μg/ mL and 2-15 μg/ mL at 610 nm and 525 nm, respectively for captopril. As for ESL the range was 1-7 μg/ mL and 0.5-4 μg/ mL at 610 nm and 525 nm respectively. The lower detection limits were 0.41 μg/ mL and 0.38 μg/ mL for captopril at 610 and 525 nm respectively. As for ESL, the lower detection limits were 0.04 μg/ mL and 0.06 μg/ mL at 610 and 525 nm respectively. The different experimental parameters affecting the development and stability of the colours were carefully studied and optimized. Both methods were further applied to the determination of CPL and ESL in formulations.

KEYWORDS

Captopril;
Ethamsylate;
Spectrophotometry;
Potassium permanganate;
Pharmaceutical analysis.

INTRODUCTION

Captopril (CPL), 1-(3-mercapto-2-methyl-1-oxoproppyl)-L-proline (S,S) Figure 1., is used therapeutically as an antihypertensive agent. It is used in the management of hypertension, in heart failure, following myocardial infraction and in diabetic nephropathy.[1] As for ethamsylate (ESL), 2,5-Dihydroxybenzenesulfonic acid (compound with N-ethylethanamine) Figure 1., it is a homeostatic agent that appears to maintain the stability of capillary walls and correct abnormal platelet adhesion. It is given for the prophylaxis and control of hemorrhages from small blood vessels.[1]

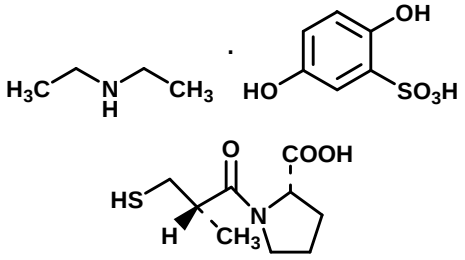


Figure 1 : Structural Formulae of captopril (CPL), and Ethamsylate (ESL)

Several methods have been reported for the quantitative determination of captopril in formulations and biological fluids, including: spectrophotometry,[2-8]

GC.MS,[9] HPLC,[10-19] electrochemistry,[20-24] chemiluminescence[25] and capillary electrophoresis.[26] The official method involves the titration of captopril with potassium iodate in acidic medium.[27]

Regarding ESL, various methods have been applied for its determination in formulations and biological fluids. These methods include spectrophotometry,[5,28,29] HPLC,[30] electrochemistry,[31-34] chemiluminescence[35] and fluorimetry.[36] A Kinetic spectrophotometric method based on oxidation of CPL and ESL with Iodine and sodium azide has been reported.[5]

The aim of the present work was to study the reaction of CPL or ESL with alkaline potassium permanganate, in an attempt to evaluate these two drugs in their different dosage forms.

EXPERIMENTAL

Apparatus

UV- VIS 1601, Shimadzu recording Spectrophotometer (P/ N 206-67001).Recording range from 0 to1.0, wavelengths 610 and 525 nm.

Reagents and Materials

All the reagents were of Analytical Reagent grade.

CPL was kindly supplied by Squibb Egypt Co. Giza, Egypt. Pharmaceutical preparations including: Capozide tablets labeled to contain 50 mg of captopril and 25 mg of hydrochlorothiazide each (Batch # E11477); and Capoten tablets labeled to contain 25 mg of CPL each (B10401204). Both are products of Squibb Egypt Co. Giza, Egypt. Ethamsylate was provided by Memphis Chemical. Co. Egypt.

Dicynone tablets each containing 250 mg of ethamsylate (Batch # 301296) and dicynone ampoules each containing 250 mg of ethamsylate/ 2 mL (Batch # 010030B); both are products of Minapharm Pharm. Co. Cairo, Egypt. All pharmaceutical preparations were obtained from commercial sources in the local market.

- Potassium permanganate (Merck, Darmstadt, Germany): 5 x 10^{-3} mol L^{-1} and 7.6 x 10^{-3} mol L^{-1}aqueous solutions were freshly prepared.
- Sodium hydroxide (BDH, UK). 0.5 mol L^{-1} aqueous solution was prepared.

Stock solution

Stock solutions were prepared by dissolving 20.0 mg of CPL or 10.0 mg of ESL in 100 mL of distilled water, and were further diluted with the same solvent as appropriate. The standard solutions were stable for one week when kept in the refrigerator.

General procedures

Construction of calibration curve

i-First Method

Transfer aliquot volumes of the standard solutions into a series of 10 mL volumetric fask. Add 1 mL of 0.5 M NaOH solution, followed by 2.6 mL of 5 x 10^{-3} M potassium permanganate solution. Shake the mixture well and complete immediately to the volume with distilled water in case of CPL while,allow the reaction mixture to stand for 15 min in case of ESL. Measure the absorbance of the resulting solutions at 610 nm against a reagent blank prepared simultaneously. Plot the values of the absorbance *vs* the final drug concentration (μg/ mL) to get the calibration graphs. Alternatively, derive the corresponding regression equations.

ii-The Second Method

Transfer aliquot volumes of the standard solutions into a series of 10 ml volumetric flasks. Add 1 mL of 0.5M NaOH solution, followed by 0.6 mL of 7.6 x 10^{-3} M KMnO4, then shake the mixture well. Complete to the volume with distilled water immediately in case of CPL and after 15 min in case of ESL. Measure the decrease in the absorbance of the resuling solutions in both cases at 525 nm. Plot the decrease in the absorbance *vs* the final content of the drugs in μg/ mL to get the calibration graphs. Alternatively, derive the corresponding regression equations.

Procedure for the Tablets

Weigh and pulverize twenty tablets. Transfer a weighed quantity of the powder equivalent to 20 mg of CPL or 10 mg of ESL into a small conical flask, extract with 3 x 30 mL of distilled water. Filter the extract into 100 mL volumetric flask. Wash the conical flask with few mLs of water. Pass the washings into the same volumetric flask and complete to the mark with the same solvent. Transfer aliquot volumes covering the working concentration range (cited in TABLE 2) into 10 mL

volumetric flasks. Proceed as described under the General procedures, adopting either methods. Determine the nominal content of the tablets either from the calibration curve or using the corresponding regression equation.

Procedure for the ampoules

Mix the contents of 5 ampoules well. Transfer aliquot volumes of the solution equivalent to 10.0 mg of ESL into 100 ml measuring flask, dilute and complete to volume with distilled water. Transfer aiquots of this solution within the concentration range cited in TABLE 2 into a series of 10 mL volumetric flasks. Proceed as described under the General procedures, adopting either methods. Determine the nominal content of the ampoules either from the calibration curve or using the corresponding regression equation.

RESULTS AND DISCUSSION

Both of CPL and ESL were found to react with KMnO4 in alkaline medium producing a bluish-green colour (manganate radical) which absorbs maximally at 610 nm (Figure 2). The absorbance of the reaction product remains stable for at least 40 min in case of CPL and 60 min in case of ESL. The spectrophotometric properties of the coloured product as well as the different experimental parameters affecting the colour development and its stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. The factors include, effect of different oxidants, effect of different solvents, concentration of the reagents (KMnO4 and NaOH), temperature, time, sensitizers and surfactants.

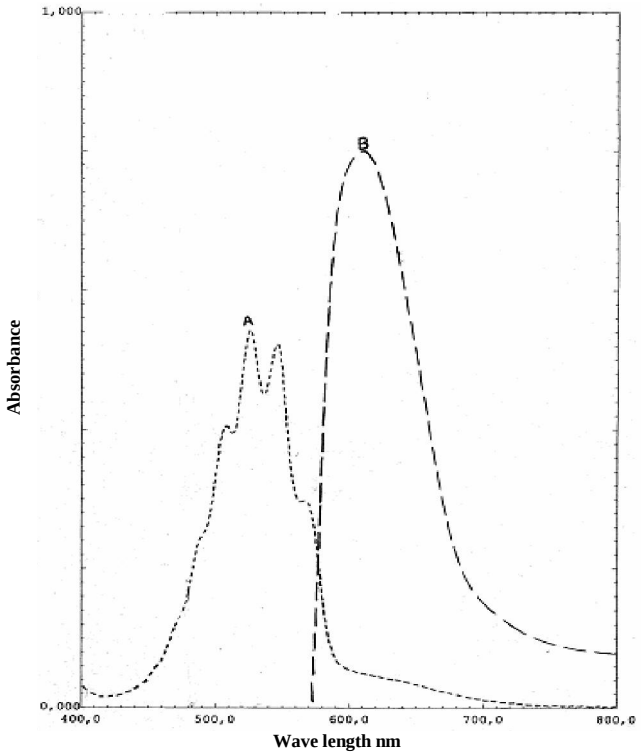


Figure 2 : Absorption spectra of :
A) KMnO4 (12.5×10^{-4} mol L^{-1}) and 0.05 mol L^{-1} NaOH,
B) Reaction product of captopril (24 µg/ mL) after reaction with KMnO4 and NaOH

Optimization of the reaction conditions

At room temperature, the green colour of the reaction product was formed immediately in case of CPL and remained stable for about 40 min. In case of ESL the colour was formed immediately and increased up to 15 min, then remained stable for one hour Heating the reaction mixture was found to increase the rate of the reaction but a brown precipitate of MnO2 was formed, therefore, room temperature (25°C) was selected as the optimum temperature.

The absorbance of the reaction products increased upon increasing KMnO4 concentration. It was found that 2.6±0.2 mL of 5 x 10^{-3} M KMnO4 was adequate for the maximum absorbance (Figure 3).

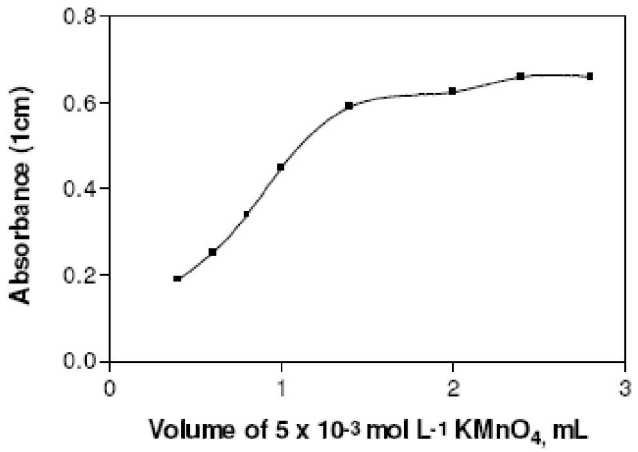


Figure 3 : Effect of volume of 5×10^{-3} mol L^{-1} KMnO4 on the absorbance of the reaction product of captopril (20 µg/ mL) at 610 nm.

Oxidation of CPL and ESL with KMnO4 was carried out in presence of NaOH. Trials were made to determine these drugs through their oxidation with KMnO4 in neutral or acidic media, but very little oxidation of the drugs was observed.

The influence of NaOH concentration on the absorbance of the reaction product was also studied using 0.1-3 mL of 0.5 M NaOH. It was found that increasing the volume of 0.5 M NaOH, would increase the absorbance of the reaction product up to 1.2 mL, further increase, resulted in very slight decrease in the absorbance of the reaction product, thus, 1 ±0.2 mL of 0.5 M NaOH was found to be the most suitable volume for maximum absorbance. (Figure 4).

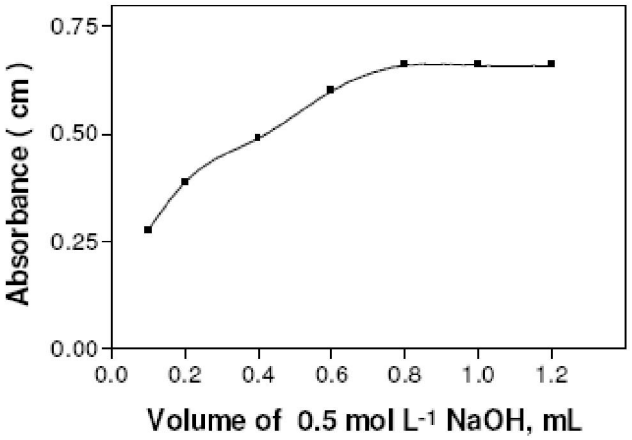


Figure 4 : Effect of volume of 0.5 mol L^{-1} NaOH on the absorbance of the reaction product of captopril (20 µg/ mL) at 610 nm.

Effect of different oxidising agents

Different oxidants have been used through out this approach such as, H2O2, potassium persulphate in alkaline medium, cerric ammonium sulphate and potassium dichromate in strong acid medium. In case of each of H2O2 and persulphate, oxidation of the drug resulted in a hypsochromic shift and hypochromic effect of the reaction product. In case of cerric ammonium sulphate and dichromate, very little oxidation was attained as revealed by the very low absorbance.

Effect of diluting solvents

The effect of diluting solvents was also studied. Using different solvents such as water, ethanol, acetonitrile, acetone, dimethyl sulphoxide and dimethyl formamide. It was found that water was the best solvent as it gave the highest absorbance readings, moreover its choice adds to the adventages of the method. However in case of dimethyl sulphoxide, dimethyl formamide and actonitrile, the absorbance of the reaction product was less than that in case of water. On the other hand, turbid green solution was obtained immediately in case of acetone and ethanol.

Effect of different sensitizers and surfactants

Different sensitizers (quinine, fluorescein and rhodamine-B), at concentration of 5 µg mL^{-1} were tested by adding to the reaction mixture before measurement of the absorbance. Outstanding inhibitory effects were observed as these sensitizers reacted strongly with KMnO4/ NaOH system. In the same manner, the effect of surfactants on the colour development was studied. Different surfactants (cetrimide, gelatine and sodium lauryl sulphate) at three concentrations, 2.5, 7.5 and 15 µg/ mL, were tested by adding to the reaction mixture prior to measurement of the absorbance. All these compounds reacted strongly with the KMnO4/ NaOH system with inhibitory effect, as evident from the low absorbance readings. Potassium permanganate is consumed by the surfactants, being reduced to reduction products other than the measured species.

An alternative spectrophotometric method for the determination of CPL and ESL based upon measuring

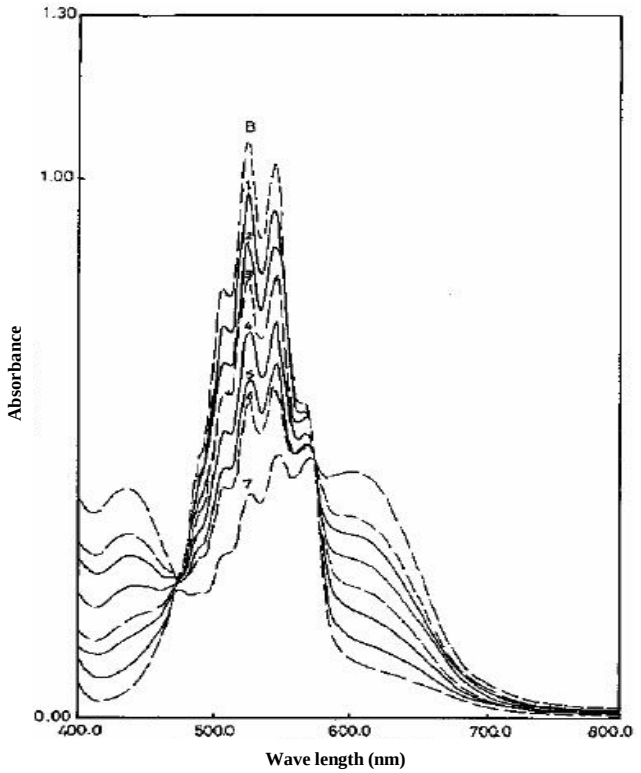


Figure 5 : Absorption spectra of captopril after reaction with KMnO4 /NaOH at 525 nm at different concentrations (µg mL^{-1}).

(B) KMnO4 (1) 2 µg/ mL (2) 4 µg/ mL
(3) 6 µg/ mL (4) 8 µg/ mL (5) 10 µg/ mL
(6) 12 µg/ mL (7) 15 µg/ mL

Full Paper

the decrease in the absorbance of KMnO4 at 525 nm (Figure 5) was also developed. The difference in the absorbance was plotted against the concentration of the drugs.

Applications

After optimizing the reaction conditions, the two proposed methods were applied to the determination of the studied compounds in pure form. The absorbance-concentration plots were rectilinear over the range 4-24 μg/ mL, 2-15 μg/ mL for CPL at 610 nm and 525 nm respectively, and over the range 1-7 μg/ mL, 0.5-4 μg/ mL for ESL at 610 nm and 525 nm respectively (TABLE 1).

Linear regression analysis of the data gave the following equations:

1- For CPL:

$A = -1.53 \times 10^{-3} \times 0.03\ C$ (r= 0.9997) at 610 nm,

$A = -1.48 \times 10^{-3} \times 0.04\ C$ (r= 0.9997) at 525 nm.

2- For ESL:

$A = -8.57 \times 10^{-4} \times 0.09\ C$ (r= 0.9999) at 610 nm,

$A = -1.12 \times 10^{-3} \times 0.14\ C$ (r= 0.9999) at 525 nm.

where A is the absorbance and C is the concentration in μg/ mL

The limits of quantification (LOQ) were determined by establishing the lowest concentrations that can be measured and were found to be 1.38 μg/ mL, 0.14 μg/ mL for CPL and ESL respectively at 610 nm and 1.27 μg/ mL,0.2 μg/ mL for CPL and ESL respectively at 525 nm. The limits of detection (LOD) were determined by establishing the minimum level at which the analytes can be reliably detected, and were found to be 0.41 μg/ mL, 0.04 μg/ mL for CPL and ESL respectively, at 610 nm and 0.38 μg/ mL, 0.06 μg/ mL for CPL and ESL respectively at 525 nm. The precision of the methods were evaluated by analyzing standard solutions of CPL and ESL. The results for pure samples were in accordance with those obtained by the reference methods [6,29] for CPL and ESL respectively.

The validity of the method was evaluated by statistical analysis of the regression data and the results are represented in TABLE 1.

Validation of the method

The method was tested for linearity, specificity, precision and reproducibility. By using the above spectrophotometric procedures, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the absorbance value on the concentration of the drugs over the range cited in TABLE 1.

The validity of the method was evaluated by statistical analysis of the regression lines regarding the standard deviation of the residuals (Sy/x), the standard deviation of the intercept (Sa) and standard deviation of the slope (Sb). The results are given in TABLE 1. The small values of the figures point out to the low scattering of the points around the calibration curve and the high precision of the proposed method.

TABLE 1 : Analytical performance data for the determination of captopril and ethamsylate in pure form.

Parameter	Proposed method at 610 nm		Proposed method at 525 nm	
	CPL	ESL	CPL	ESL
-concentration range(μg/ ml).	4-24	1-7	2-15	0.5-4
-LOQ (μg/ mL).	1.38	0.14	1.27	0.20
-LOD (μg/ mL).	0.41	0.04	0.38	0.06
- Correlation coefficient (r)	0.9997	0.9999	0.9997	0.9999
- Slope	0.03	0.09	0.04	0.14
- Intercept	-1.53×10^{-3}	-8.57×10^{-4}	-1.48×10^{-3}	-1.12×10^{-3}
$-S_{y/x}$	7.48×10^{-3}	1.19×10^{-3}	6.98×10^{-3}	2.07×10^{-3}
$-S_a$	4.56×10^{-3}	1.26×10^{-3}	5.32×10^{-3}	2.74×10^{-3}
$-S_b$	4.47×10^{-4}	2.29×10^{-4}	6.25×10^{-4}	7.22×10^{-4}
-% Error	0.41	0.14	0.59	0.28

N.B. : -Sy/x =standard deviation of the residuals.
-Sa = standard deviation of the intercept of regression line.
-Sb = standard deviation of the slope of regression line
-% Error = RSD% / √ n.

Accuracy

The accuracy of the proposed method was evaluated by analysing standard solutions of CPL and ESL. The results obtained by the proposed method were compared with those given by the reference methods[6,29] for CPL and ESL respectively.

Statistical analysis[37] of the results obtained by both methods and reference methods[6,29] using the Student t-test and Variance ratio F-test, shows no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (TABLE 2).

Precision

The within-day precision was evaluated through rep-

licate analysis of authentic samples of CPL at concentrations of 4, 8, 16, 20 µg/ mL. The percentage recoveries based on the average of four separate determinations were 99.24±0.86 thus, indicating the high precision of the method.

The inter-day precision was also evaluated through replicate analysis of authentic samples of CPL at concentration of 8 µg/ mL on four successive days. The percentage recoveries based on the average of four separate determinations were 99.74±1.03. The repeatability and reproducibility of the proposed method are fairly good as indicated by small value of standard deviation (SD).

Robustness of the method

The robustness of the method adopted is demonstrated by the constancy of the absorbance value with the deliberated minor changes in the experimental parameters such as change in the concentration of NaOH, 1 ±0.2 mL of 0.5 mol L^{-1} and change in KMnO4 concentration, 2.6±0.2 mL of 5 x 10^{-3} mol L^{-1}. These minor changes that may take place during the experimental operation didn't affect the absorbance value.

TABLE 2 : Application of the proposed method for the determination of CPL and ESL in pure form using the proposed methods.

Parameters Compound	Proposed method at		Reference Methods[6,29]
	610 nm	525nm	
1-CPL			
-Mean found (%) ±SD.	100.40 ±0.97	100.50 ±1.07	99.89±0.78
-Variance.	0.94	1.14	0.61
-Student's t-Value.	0.81 (1.48)	0.83 (1.31)	
-Variance ratio F-test.	1.55 (4.74)	1.88 (9.55)	
2-ESl			
-Mean found (%) ±SD.	100.03 ±0.03	100.24± 0.62	100.26±0.53
-Variance.	0.12	0.38	0.28
-Student's t-Value.	0.80 (2.37)	0.05 (2.37)	
-Variance ratio F-test.	2.28 (5.79)	1.37 (5.79)	

N.B. : Figures in parentheses are the tabulated values of t and F respectively (at p = 0.05)[37].

Pharmaceutical applications

The proposed methods were further applied to the determination of CPL and ESL in its tablets and ampoules. Common tablets excipients such as talc, lactose, starch, avisil, gelatine and magnesium stearate did not interfere with the assay. Hydrochlorothiazide, which is frequently co-formulated with captopril did not interfere with the proposed method. It is practically insoluble in water and is removed by filtration. The results obtained were compared with those given using reference methods.[6,29] Statistical analysis[37] of the results using Student's t-test and variance ratio F- test, revealed no significant difference between the two methods at the 95 % confidence level regarding accuracy and precision, respectively. The results obtained are abridged in TABLE 3. Moreover, the proposed methods are more

TABLE 3 : Application of the proposed method to the of CPL and ESL in dosage forms.

Preparations	% Recovery at 610 nm.	% Recovery at 525 nm.	Reference Methods[6,29]
1-Capoten tablets [a]	99.37	100.55	99.64
(CPL, 25 mg/ tablet)	98.80	99.60	100.54
	100.81	100.63	101.30
	101.08	98.48	
	99.68	101.12	
X^- ±SD.	99.95±0.96	100.0.8±1.04	100.49±0.83
t-value.	0.80 (2.45)	0.58 (2.45)	
F-value	1.34 (6.94)	1.57 (6.94)	
2-Capozide tablets[b]	100.63	99.05	99.50
(CPL, 50 mg / tablet).	99.83	100.90	101.05
	100.50	98.85	100.88
	100.44	99.41	
	101.07	100.36	
X^- ±SD.	100.49±0.45	99.71±0.88	100.48±0.85
t-value.	0.02 (2.45)	1.21 (2.45)	
F-value.	3.57 (6.94)	1.07 (6.94)	
3-Dicynone tablets[c]	98.90	99.25	99.31
(ESL, 250 mg / tablet).	99.75	99.11	100.11
	98.53	98.64	99.48
	98.45	99.06	
X^- ±SD.	98.90±0.59	99.02±0.23	99.63±0.42
t-value.	1.80 (2.57)	2.49 (2.57)	
F-value	1.99 (9.55)	3.32 (9.55)	
4-Dicynone ampoules[d]	99.20	100.30	101.44
(ESL, 250 mg / 2ml each ampoule).	101.10	99.55	101.23
	100.58	101.70	100.70
	99.38	99.93	
X^- ±SD.	100.07±0.92	100.37±0.94	101.12±0.38
t-value.	1.82 (2.57)	1.28 (2.57)	
F-value	5.90 (9.55)	6.11 (9.55)	

[a]Product of Squibb Egypt Co. Giza, Egypt (Batch # B10401204)
[b]Product of Squibb Egypt Co. Giza, Egypt (Batch # E11477)
[c]Products of Minapharm Pharm. Co. Egypt. (Batch # 301296)
[d]Products of Minapharm Pharm. Co. Egypt. (Batch # 10030B)
N.B. : Figures in parentheses are the tabulated t and F values respectively (at p = 0.05) [37].

Full Paper

simple and sensitive than the official method.[27]

Mechanism of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method [38]. The absorbance of the reaction product was alternatively measured in the presence of either excess of KMnO4 or the drug. A plot of log absorbance versus log [KMnO4] and log CPL or log ESL gave straight lines, the values of the slopes were 0.8, 1.01 for KMnO4, CPL and 1.47, 0.99 for KMnO4, ESL respectively. Hence, it is concluded that, the molar reactivity of the reaction is 0.8 / 1.01 for CPL (Figure 6) and 1.47/0.99 for ESL i.e. the reaction proceeds in the ratio of 1 : 1 in case of CPL and 1:2 in case of ESL/KMnO4.

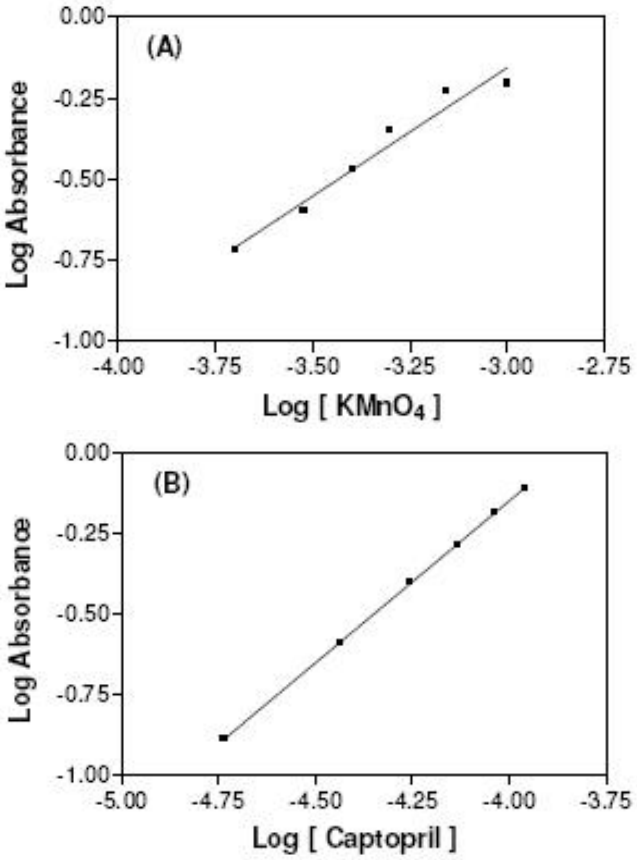
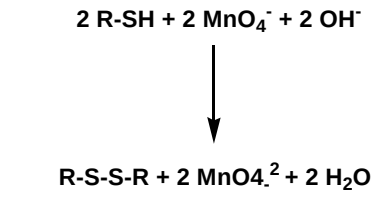
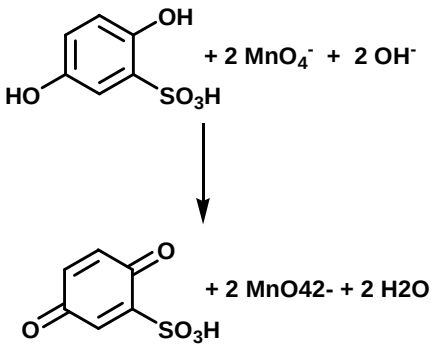


Figure 6 : Limiting logarithmic plots for the molar ratio.
(A) Log A vs. Log [KMnO4]
(B) Log A vs. Log [Captopril]

Based on the obtained molar reactivity, and depending on the presence of thiol (SH) group in CPL and hydroquinone group in ESL, the reaction pathways are proposed to proceed as shown in Scheme 1 and Scheme 2, respectively:



Scheme 1 : Proposal of the reaction pathway between CPL and KMnO4



Scheme 2 : Proposal of the reaction pathway between ESL and KMnO4

CONCLUSION

Simple and sensitive methods have been developed for the determination of captopril and ethamsylate in pharmaceutical preparations. The methods are more sensitive than other reported spectrophotometric methods. They can measure as low as 1.37 and 0.2 µg/ mL for CPL and ESL with good accuracy. The proposed method can be used for routine quality control studies. Moreover it has distinct advantages over other existing methods regarding sensitivity, time saving and minimum detection limit. Moreover, it could be applied to the determination of different pharmaceutical dosage forms.

REFERENCES

[1] K.Parfitt; Martindale, The Complete Drug Reference, 32nd Ed, The Pharmaceutical Press; Massachusts, p. 720, 836 **(1999)**.
[2] A.Sachan, D.K.Jain, P.Trived; Indian Drugs., **34**, 168 **(1997)**.
[3] N.Rahman, M.Singh, Md.NasrulHoda; Farmaco., **60**, 569 **(2005)**.
[4] A.F.M.El-Walily, O.A.Razak, S.F.Belal, R.S.Bakry; J.Pharm.Biomed.Anal., **21**, 439 **(1999)**.
[5] Y.El-Shabrawy, N.El-Enany, K.Salem; Farmaco., **59**, 803 **(2004)**.

[6] C.S.P.Sastry, K.R.Srinivas, K.M.M.K.Prasad; Anal.Lett., **29**, 1329 **(1996)**.
[7] U.Rose; J.Pharm.Biomed.Anal., **18**, 1 **(1998)**.
[8] N.El-Enany, F.Belal, M.Rizk; Int.J.Biomed.Sci., **4**, 100 **(2008)**.
[9] M.E.Franklin, R.S.Addison, P.V.Baker, W.D.J.Hooper; Chromatogr.Biomed.Appl., **705**, 47 **(1998)**.
[10] J.F.Salazar, H.Schorr, W.Herrmann, B.Herbeth, G.Siest, P.Leroy; J.Chromatogr.Sci., **37**, 469 **(1999)**.
[11] G.Battermann, K.Cabrera, S.Heizenroeder, D.Lubda; Laborpraxis., **22**, 32 **(1998)**.
[12] A.Khedr, H.El-Sherief; J.Biomed.Chromatogr., **12**, 57 **(1998)**.
[13] J.Ouyang, W.R.G.Baeyens, J.Delanghe, G.Van-der-Weken, D.De-Keukeleire, W.Van-Daele, A.M.Garcia-Campana, A.C.Calokerinos; Ibid., **12**, 160 **(1998)**.
[14] J.Russell, J.A.Mckeown, C.Hensman, W.E.Smith, J.Reglinski; J.Pharm.Biomed.Anal., **15**, 1757 **(1997)**.
[15] M.Bahmaei, A.Khosravi, C.Zamiri, A.Massoumi, M.Mahmoudian; Ibid., **15**, 1181 **(1997)**.
[16] E.Bald, S.Sypniewski; Fresenius J.Anal.Chem., **358**, 554 **(1997)**.
[17] R.J.Kok, J.Visser, F.Moolenaar, D.de-Zeeuw, D.K.-F.Meijer; J.Chromatogr.Biomed.Appl., **693**, 181 **(1997)**.
[18] G.Favaro, M.Fiorani; Anal.Chim.Acta., **332**, 249 **(1996)**.
[19] E.Bald, S.Sypniewski, J.Drzewoski, M.Stepien; J.Chromatogr.Biomed.Appl., **681**, 283 **(1996)**.
[20] R.I.Stefan, J.F.Van-Staden, H.Y.Aboul-Enein; Anal.Chim.Acta., **411**, 51 **(2000)**.
[21] R.I.Stefan, J.F.Van-Staden, H.Y.Aboul-Enein; Talanta., **51**, 969 **(2000)**.
[22] Z.Yang, S.M.Zhu; Fenxi Huaxue., **27**, 1431 **(1999)**.
[23] R.I.Stefan, J.F.Van-Staden, H.Y.Aboul-Enein; Talanta., **48**, 1139 **(1999)**.
[24] J.M.G.Fraga, A.I.J.Abizanda, F.J.Moreno, J.J.A.Leon; Ibid., **46**, 75 **(1998)**.
[25] J.Ouyang, W.R.G.Baeyens, J.Delanghe, G.Van-der-Weken, W.Van-Daele, D.De-Keukeleire, A.M.Garcia-Campana; Anal.Chim.Acta., **386**, 257 **(1999)**.
[26] S.Hillaert, W.Van-den-Bossche; J.Pharm.Biomed.Anal., **21**, 65 **(1999)**.
[27] United States Pharmacopeia 24; National Formulary 19, Rockville, USP, p. 296, 297 **(2000)**.
[28] N.El-Enany, F.Belal, M.Rizk; J.AOAC.INT., **90**, 679 **(2007)**.
[29] S.Huang, Q.Bi, N.Sun; Yaowu.Fenxi.Zazhi., **8**, 319 **(1988)**.
[30] J.Ma, Y.Liu; Yaowu.Fenxi.Zazhi., **4**, 209 **(1984)**.
[31] Z.H.Wang, D.Zhang, Y.Zhang, S.P.Zhou; Fenxi.Hauxue., **29**, 83 **(2001)**.
[32] G.J.Yang, L.T.Jin, Z.Z.Leng; Yaowu.Fenxi.Zazhi., **18**, 311 **(1998)**.
[33] Y.Wang, W.Wang, G.Qing; Ibid., **10**, 113 **(1990)**.
[34] V.Noninski, E.Sobovale, L.Dryanovska-Noninska; Farmacia., **35**, 175 **(1987)**.
[35] C.X.Zhang, J.C.Huang, M.L.Feng, Z.J.Zhang., Anal.Lett., **31**, 1917 **(1998)**.
[36] X.Wang, B.Zeng; Yaowu.Fenxi.Zazhi., **7**, 150 **(1987)**.
[37] J.C.Miller, J.N.Miller; Pearson Prentice Hall., Statistics and Chemometrics for Analytical Chemistry. 5th Ed., p.256, **(2005)**.
[38] J.Rose, Advenced Physico-Chemical Experiments, Pittman, London, p. 67, **(1964)**.