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Spectrophotometric and AAS determination of ramipril and enalapril through ternary complex formation

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Abstract

Two sensitive, spectrophotometric and atomic absorption spectrometric procedures are developed for the determination of two antihypertensive agents (enalapril maleate and ramipril). The spectrophotometric procedures for the two cited drugs are based on ternary complex formation. The first ternary complex (copper(II), eosin, and enalapril) was estimated by two methods; the first depends on its extraction with chloroform measuring at 533.4 nm. Beer's law was obeyed in concentration range from 56 to 112 μg ml^{-1}. The second method for the same complex depends on its direct measurement after addition of methylcellulose as surfactant at the pH value 5 at 558.8 nm. The concentration range is from 19 to 32 μg ml^{-1}. The second ternary complex (iron(III), thiocyanate, and ramipril) was extracted with methylene chloride, measuring at 436.6 nm, with a concentration range 60–132 μg ml^{-1}. The direct atomic absorption spectrometric method through the quantitative determination of copper or iron content of the complex was also investigated for the purpose of enhancing the sensitivity of the determination. The spectrophotometric and atomic absorption spectrometric procedures hold their accuracy and precision well when applied to the determination of ramipril and enalapril dosage forms.

Keywords: Enalapril maleate; Ramipril; Ternary complex; Spectrophotometry; Atomic absorption spectrometry

1. Introduction

The official drugs enalapril maleate (1-[*N*-[(*S*)-1-carboxy-3-phenylpropyl]-L-anayl]-L-proline 1′-ethyl ester, maleate(1:1)) and ramipril ([2*S*-1-(*R*,*R*)2α, 3aβ, 6aβ]-1-[[2-ethoxy carbonyl)-3-phenyl propyl]amino]-1-oxopropyl, octahydro-cyclopenta [b] pyrrole-2-carboxylic acid) are antihypertensive agents which their metabolites are an active inhibitor of angiotensin-I converting enzyme (ACE). The two cited drugs are official in USP 24 [1] and B.P. [2], respectively. The methods of analysis for the bulk drug are high-performance liquid chromatography (HPLC) for enalapril and potentiometric titration procedure for ramipril. The few reported methods in the literature for the determination of enalapril are HPLC [3–6], capillary electrophoresis [7], spec-

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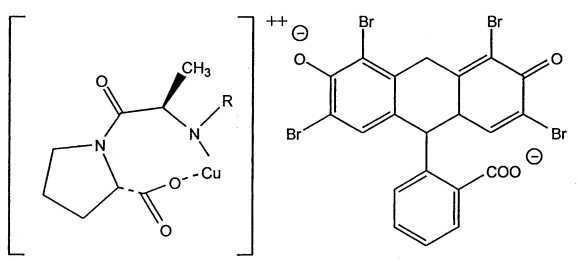
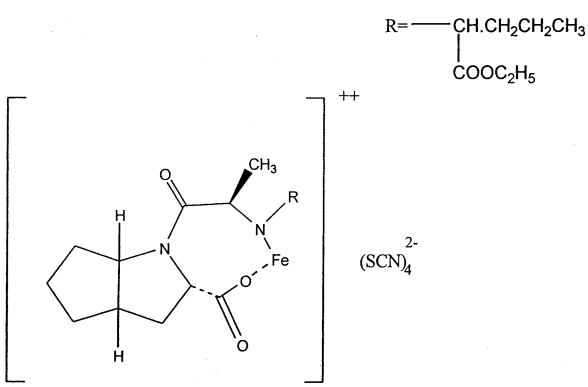
trophotometry [8,9] and selective membrane electrode [10–12]. For ramipril selective membrane electrode [11–13], HPLC [14,15], gas chromatography [16], radioimmunoassay [17], spectrophotometry [18] derivative and derivative compensation techniques [19].

An inspection of both the available methods and the official ones for the cited drugs reveals that most of them cumbersome or involve the use of expensive equipment and reagents. Although atomic absorption spectrometry AAS is a rapid method and has very low detection limits which cannot be reached by most of other methods, it has not been applied yet to the determination of enalapril and there is only one paper—two of the authors earlier work—describe atomic absorption spectrometric method for determination of ramipril [18].

This paper reports simple, sensitive and accurate spectrophotometric and atomic absorption spectrometric methods for the analysis of two antihypertensive agents, enalapril maleate and ramipril.



Enalapril - Cu - eosin complex



Ramipril - Fe - thiocyanate complex

Scheme 1.

2. Experimental

2.1. Instrumentation

Shimadzu 260 recording spectrophotometer: Shimadzu atomic absorption spectrophotometer, model AA-640-13 was used.

2.2. Materials and reagents

Chemicals used were of the highest purity available from their sources.

1. Eosin (Merck, Darmastate, Germany) was prepared as a 0.5% w/v solution of distilled water;
2. Ferric(III) chloride solution was prepared as a 6% w/v solution in distilled water;
3. Copper(II) chloride solution was prepared as 0.1 and 0.4% w/v solution in distilled water;
4. Ammonium thiocyanate solution was prepared as 6% w/v solution in distilled water;
5. Acetate buffer pH 5 (dissolve 13.6 gm of sodium acetate and 6 ml of glacial acetic acid in sufficient water to produce 1000 ml);
6. Methylcellulose, 0.3% w/v in distilled, was prepared by dissolving the appropriate amount in hot water (80 °C) with stirring for 10 min then chilling to 5 °C for 30 min.
7. Enalapril maleate pure drug (purity 99.49%) and Ezapril tablets containing 10 mg enalapril maleate per tablets from Kahira Pharm. & Chem. Ind. Co., Cairo, Egypt;
8. Ramipril pure drug (purity 99.32%) and Tritace® tablets containing 2.5 mg ramipril per tablet from Hoechst Orient, Cairo, Egypt.

2.3. Standard solutions

Solution of 0.4 mg ml^{-1} was prepared by dissolving 10 mg enalapril or ramipril in distilled water in 25 ml volumetric flask.

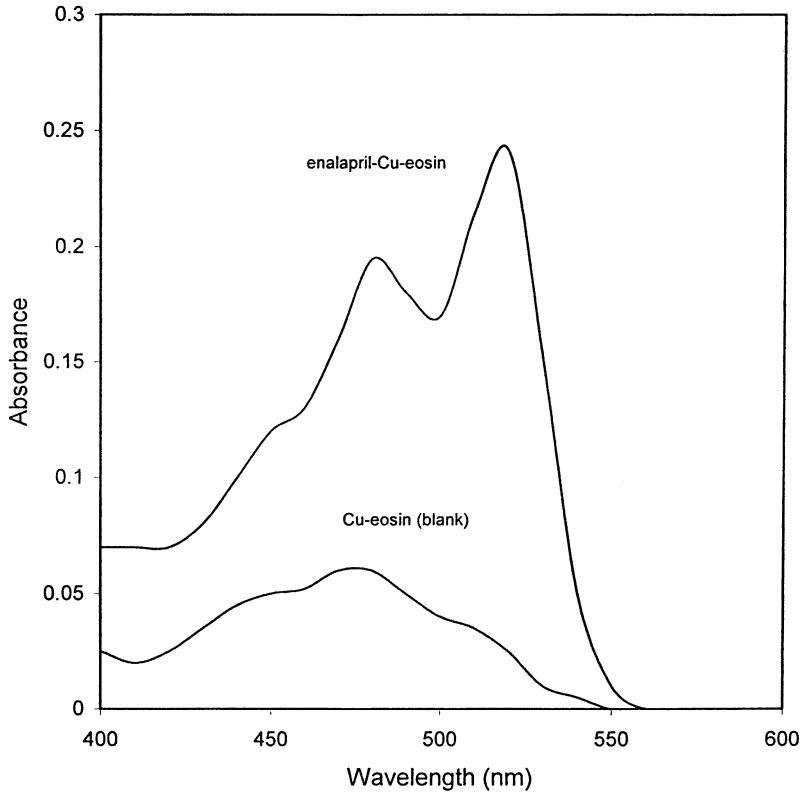


Fig. 1. Absorption spectrum of the ternary complex of 64 μg ml^{-1} enalapril with eosin and Cu(II) – extractive method.

2.4. General procedure

2.4.1. Spectrophotometric method

2.4.1.1. Extractive procedure for enalapril. Accurately measured aliquots of the standard drug solution in concentration range 0.56–1.12 mg enalapril were placed in 50 ml separating funnels. Two milliliters of 0.4% Cu(II) chloride solution was added followed by 1.5 ml of 0.5% eosin solution and 0.5 ml buffer solution of pH 5. The complex was extracted with 2×4 ml portions of chloroform. The solution was shaken for 1 min each time, and the chloroform layer was passed through a layer of anhydrous sodium sulphate into a 10 ml volumetric flask. The volume of the chloroform layers was made up to 10 ml, and the absorbance was measured at 533.4 nm against blank in which the drug is omitted.

2.4.1.2. Extractive procedure for ramipril. Accurately measured aliquots of the standard drug solution in concentration range 0.60–1.32 mg ramipril were placed in 50 ml separating funnels. Half milliliter buffer solution of pH 5 followed by a 3 ml of 6% iron(III) chloride solution and a 5 ml of 6% ammonium thiocyanate solution were added. The complex was extracted with 2×4 ml portions of methylene chloride. The solution was shaken for 1 min each time, and the methylene chloride layer was passed through a layer of anhydrous sodium sulphate into a 10 ml, and the absorbance was measured at 436.6 nm against blank in which the drug is omitted.

2.4.1.3. Spectrophotometric procedure using surfactants for enalapril. Appropriate volumes of the standard solution in the concentration range 0.5–0.8 mg of enalapril maleate were pipetted into two sets of 25 ml volumetric flasks. A 2 ml of 0.3%

methyl cellulose, 1.5 ml of the buffer solution of pH 5, 3 ml of the 0.5% eosin solution, and 3 ml of 0.1% copper(II) chloride, were added to the flasks, in this order. The mixture was diluted to volume with water, homogenized by shaking and immersed in warm water-bath (60 ± 0.5 °C) for 5 min. The solution was then cooled to room temperature. The absorbance was measured at 558.8 nm against blank in which the drug is omitted.

2.4.2. Atomic absorption spectrometric method

The procedure as under extractive spectrophotometric method above as far as 'the volume of chloroform (or methylene chloride) layers was made up to 10 ml' was applied. The organic extract was evaporated to dryness on a boiling water bath.

Then the residue was dissolved in 2 ml 0.1N HCl and 0.5 ml conc. HCl, for enalapril and ramipril, and the volume was completed to 25 and 10 ml with distilled water, respectively. A blank (omitting the addition of the drug) was prepared and the absorption was measured for both the agents at the following condition:

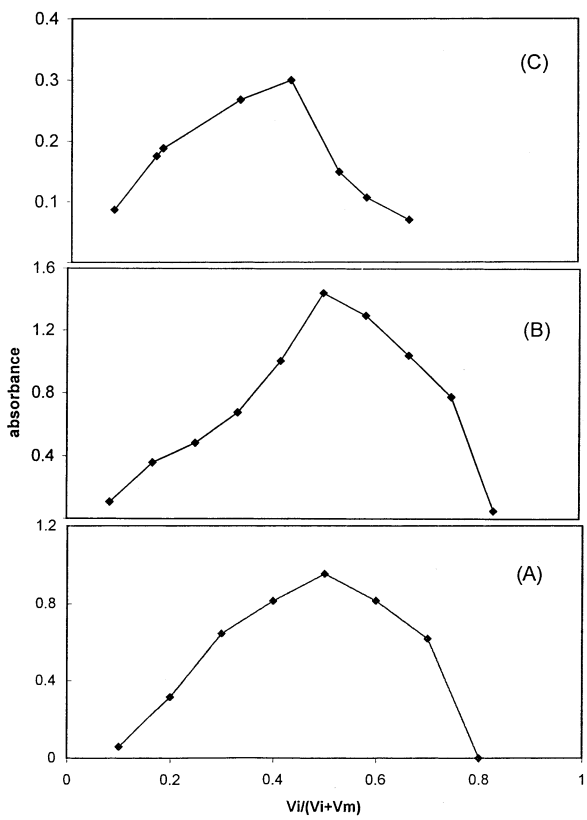


Fig. 2. (A) Continuous variation plots for enalapril:Cu(II) (1×10^{-3} M) complex ratio in the presence of excess eosin (5 ml, 0.5%), Vi = drug, and Vm = Cu(II); (B) Continuous variation plots for enalapril:eosin (1×10^{-3} M) complex ratio in the presence of excess Cu(II) (2 ml, 0.4%), Vi = drug, and Vm = eosin; and (C) Continuous variation plots of eosin:Cu(II) 1×10^{-3} M) complex ratio in the presence of excess enalapril (100 μg ml^{-1}), Vi = eosin, and Vm = Cu(II).

	Enalapril	Ramipril
Analysis wavelength	3247 Å	2483 Å
Lamp current	7 mA	9 mA
Slit width	3.8 Å	1.9 Å
Burner height	4 mm	4 mm
Burner slot, flame	10 cm, air–C_2H_2	10 cm, air–C_2H_2
Support gas flow	10 l min^{-1}	10 l min^{-1}
Fuel gas flow	2.3 l min^{-1}	2.5 l min^{-1}
Absorption sensitivity	0.13 ppm	0.15 ppm

The concentration of the consumed iron or copper was calculated from calibration graph of standard iron or copper chloride solution.

2.5. Assay of pharmaceutical tables

Twenty tablets were powdered and the quantity of the powder equivalent to 20 mg of enalapril or ramipril was extracted by shaking with 10 ml water. The extracts were filtered into a 25 ml volumetric flask and then diluted to volume. The assay for enalapril and ramipril content was completed as described in Section 2.4.

3. Results and discussion

Ternary complexes of general formula ($L_N M_X S_Y$) have been widely used in spectrophotometric analysis [20–24]. For the ternary complexes dealt with in this paper is that their main

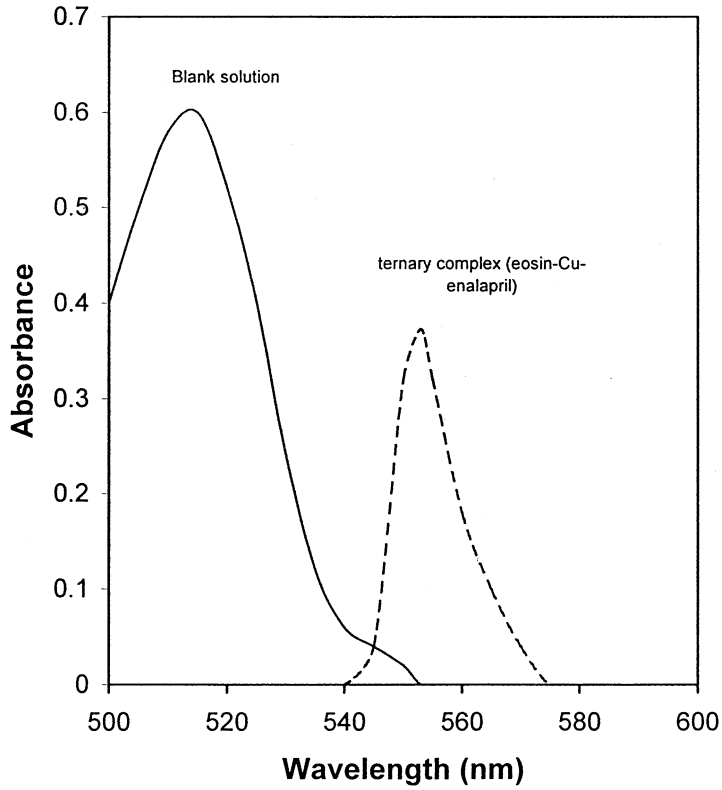


Fig. 3. Absorption spectrum of the tenary complex of 25 μg ml^{-1} enalapril with eosin and Cu(II) in the presence of methylcellulose as surfactant and at pH 5.

ligand *L* is the cited drugs enalapril or ramipril, the second ligand *S* is eosin or thiocyanate ion and *M* is copper(II) or iron(III) metal, respectively (Scheme 1). These triple complexes are extractable with organic solvents (chloroform or methylene chloride), whereas the binary systems (metal-drug and metal-second ligand eosin or thiocyanate) cannot be extracted in that way.

3.1. Spectrophotometric procedure for enalapril

3.1.1. Extractive procedure

The effects of the reagent concentrations (eosin and copper), pH, temperature, time, order of addition of reagents and solvents with respect to maximum sensitivity, minimum blank, adherence to Beer's law, and stability have been studied through control experiments. The optimum conditions were established by varying one variable and observing its effect on the absorbance of the coloured product.

1. Two milliliters of 0.4% w/v copper(II) chloride solution and 1.5 ml of 0.5% w/v eosin solution was found optimum to maximize the colour intensity.
2. It was found that two extractions each for 1 min were necessary for the quantitative estimation of the complex.
3. The colour of the ternary complex in the chloroform was quit stable for at least 24 h.

To prove the formation of a ternary complex between copper(II) (A), eosin (B) and enalapril (C), the interaction of the three components may be considered as

$$\text{either} \quad AB + C \rightleftharpoons AC + B \tag{1}$$

$$\text{or} \quad AB + C \rightleftharpoons ABC \tag{2}$$

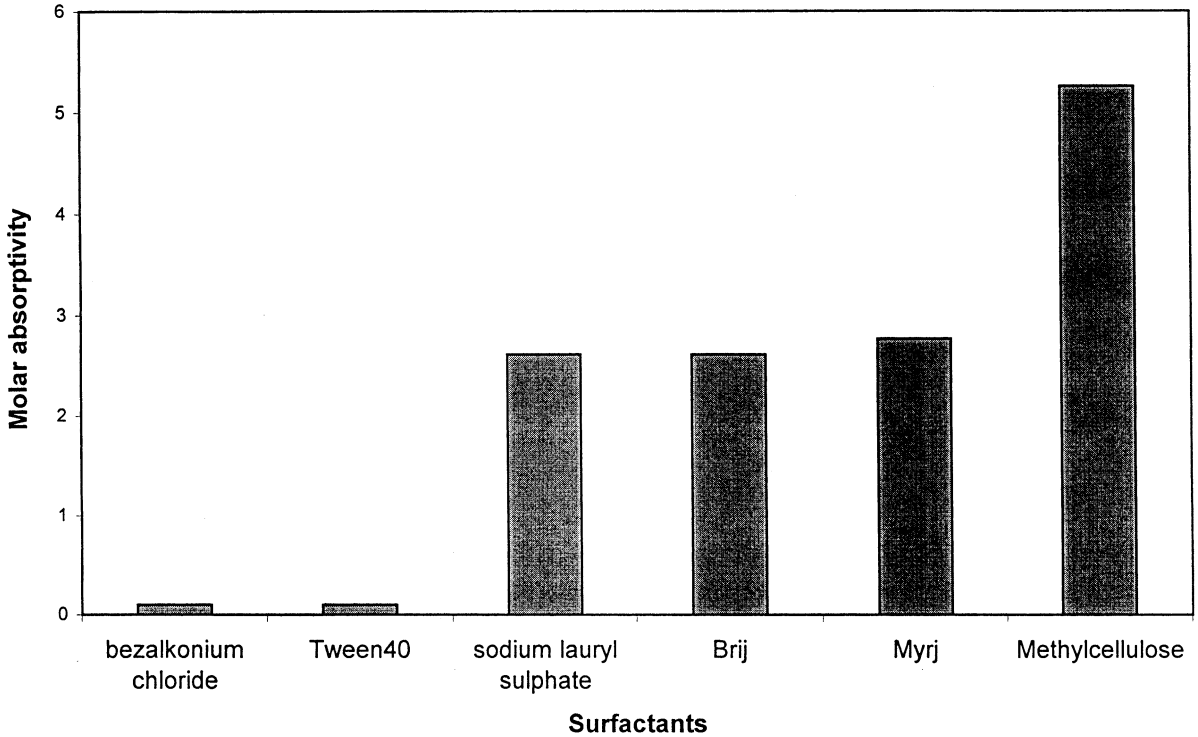


Fig. 4. Effect of different surfactants (2 ml of 0.5% w/v aqueous solution) on the apparent molar absortivity of the enalapril–Cu(II)–eosin ternary complex.

A series of absorption spectra have been done for each component, separately, and to their mixtures under the experimental conditions, discussed above, in both aqueous and organic solvent. The spectra revealed that aqueous solution of eosin (B) absorbs in the visible region at $\lambda_{max} = 499$ nm, while neither copper(II) chloride (A) nor the enalapril (C) have absorbance in the visible region. The mixture (AB) has the same maximum absorbance as that of (A) and (B) separately, also the mixture (AC) has the same maximum absorbance as that of (A) and (C), separately.

According to these considerations, and to the finding that the complexes formed have absorption maximum at 533 nm, the absorbance was not be additive, but it would be a ternary complex system, ABC, having different properties from that of AB or AC. Practically, extraction of aqueous solutions of the separate components with chloroform gave no absorption maxima in the visible region, while that of the ternary mix-

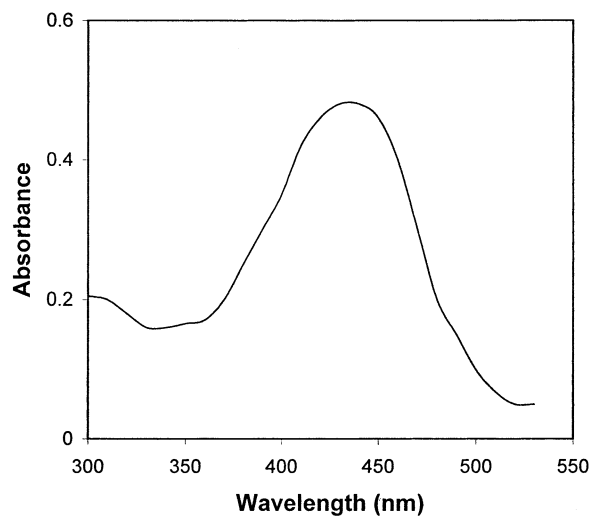


Fig. 5. Absorption spectrum of the ternary complex of 132 μg ml^{-1} ramipril with Fe(III) and thiocyanate – extractive method.

Table 1
Optical characteristics and statistical data of the regression equations for ternary complex formation with enalapril maleate and ramipril

Parameters	Enalapril maleate			Ramipril	
	Spectrophotometric methods		Atomic absorption procedure	Extractive spectrophotometric methods	Atomic absorption procedure
	Extractive procedure	Procedure using surfactant			
Beer's law ($\mu g\,ml^{-1}$)	56–112	20–32	2.4–5.6	60–132	24–80
Molar absorptivity ($mol^{-1}\,cm^{-1}$)	2.1×10^{3}	5.6×10^{3}	–	1.5×10^{3}	–
Sandell's sensitivity (μcm^{-2} per 0.001 A)	4.3×10^{-4}	1.1×10^{-3}	–	3.6×10^{-4}	–
Regression equation					
Intercept (a)	0.0331	0.4757	−1.50	3.7×10^{-3}	0.302
Slope (b)	4.9×10^{-3}	0.0321	8.75	8.7×10^{-3}	0.745
Correlation coefficient (r)	0.9949	0.9990	0.9949	0.9976	0.9967
Variance(S_{σ}^{2})	1.27×10^{-4}	3.27×10^{-4}	1.02×10^{-2}	1.33×10^{-5}	2.24×10^{-3}
Detection limit ($\mu g\,ml^{-1}$)	1.412	0.587	0.251	2.735	1.372

ture, in the same solvent, gave a predominant absorption spectrum with λ_{max} at 533 nm (Fig. 1).

3.1.2. Constitution of the ternary complex

The nature of the ternary complex (enalapril–Cu(II)–eosin) was determined using Job's method of continuous variation [25]. The results of applying this method (extractive procedure) can be summarized as follows: the [Cu(II):enalapril] ratio in the presence of excess eosin was 1:1 (Fig. 2a), while the [eosin:enalapril] ratio in the presence of excess Cu(II) chloride was 1:1 (Fig. 2b) and the [eosin:Cu(II)] ratio in the presence of excess drug was 1:1 (Fig. 2c). Hence the composition of the ternary complex formed may be expressed as drug–Cu(II)–eosin (1:1:1) (Scheme 1).

3.1.3. Spectrophotometric procedure using surfactants

The absorption spectra of the ternary complex (enalapril–Cu–eosin) (Solution A) and the blank solution (Cu–eosin) (Solution B) were scanned in the range 500–600 nm. It was found that, on addition of the enalapril to eosin–Cu(II) solution, a difference in absorbance was observed at 558 nm (Fig. 3). The absorption difference was proportional to the concentration of enalapril.

To optimize the assay parameters, the effects of pH, reaction time, effect of temperature, concentration of surfactant, and eosin and copper(II) sulphate on the absorbance of the ternary complex formed were studied. The effect of pH on the absorbance of the ternary complex studied at 558 nm. The absorbance of the drug–Cu(II)–eosin complex solution was investigated over a pH 2.2–9.7. The optimum absorbance was achieved at pH 5.

In order to examine the effect of temperature and reaction time on the absorbance of the ternary complex, the above mentioned procedure was carried out at different temperatures (room temperature, 50, 60, 70 and 80 °C) using thermostatic water bath. Maximum and constant absorbance was obtained at 60 °C after 5 min from the addition of the reaction contents, excessive heat decrease the absorbance sharply.

The effect of surfactants on the absorbance of the solution of the ternary complex was examined

using various dispersing agents, such as sodium lauryl sulphate, methylcellulose, benzalkonium chloride, Tween 40, myrj and brij. Among the surfactants studied, best results were obtained in the presence of methylcellulose. The histogram in Fig. 4 shows the effect of each dispersing agent on the apparent molar absorptivity of the ternary complex solution examined.

The effect of concentration of the reagents, eosin and copper(II) sulphate, on the absorbance of the ternary complex was studied. The optimum result was obtained using 3 ml of 0.5% eosin and 3 ml of 0.1% copper(II) chloride solutions. The colour formed under the above mentioned optimum conditions was stable for at least 1 h.

3.2. Spectrophotometric procedure for ramipril

Ramipril react with iron(III) chloride in the presence of ammonium thiocyanate in acidic medium. The reaction takes place at room temperature and pH 5, and the complex formed between ramipril and the iron(III) ion was extracted with chloroform. Absorption spectra were recorded over the range 300–600 nm. The pink complex shows one absorbance maximum at 477 nm (Fig. 5). The effects of the reagent concentrations (iron(III) and thiocyanate), pH, temperature, time, order of addition of reagents and solvents with respect to maximum sensitivity, minimum blank, adherence to Beer's law and stability, have been studied through control exper-

Table 2
Evaluation of the accuracy and precision of the proposed methods

Compared method	Added ($\mu g\,ml^{-1}$)	Found $\pm$ SD[a]	RSD(%)	SAE[b]	Confidence limits[c]
Enalapril					
Spectrophotometry					
(a) Extractive procedure	70	72.01 ± 0.53	0.736	0.238	72.01 ± 0.66
	80	79.21 ± 1.13	1.427	0.507	79.21 ± 1.41
	90	93.00 ± 1.07	1.151	0.480	93.00 ± 1.33
Mean			1.105	0.408	
(b) Procedure using surfactant	20	19.51 ± 0.36	1.845	0.161	19.51 ± 0.45
	25	25.22 ± 0.50	1.982	0.224	25.22 ± 0.63
	30	31.73 ± 0.58	1.828	0.260	31.73 ± 0.72
Mean			1.885	0.215	
Atomic absorption procedure					
	30	28.92 ± 0.45	1.556	0.202	28.92 ± 0.56
	40	40.27 ± 0.58	1.440	0.260	40.27 ± 0.72
	50	52.02 ± 1.02	1.960	0.457	52.02 ± 1.27
Mean			1.65	0.306	
Ramipril					
Extractive spectrophotometric procedure					
	70	68.90 ± 0.78	1.132	0.349	68.90 ± 0.97
	80	79.00 ± 1.02	1.291	0.457	79.00 ± 1.27
	90	89.28 ± 1.11	1.243	0.498	89.28 ± 1.38
Mean			1.222	0.435	
Atomic absorption procedure					
	30	29.20 ± 0.57	1.952	0.256	29.20 ± 0.71
	50	48.98 ± 0.89	1.817	0.399	48.98 ± 1.11
	70	69.35 ± 1.11	1.586	0.498	69.35 ± 1.38
Mean			1.785	0.384	

[a] Mean $\pm$ standard deviation for five determinations.
[b] SAE, standard analytical error.
[c] Confidence limits at $P = 0.95$ and 4 degree of freedom.

Table 3
Determination of enalapril and ramipril in commercial tablets using the proposed procedures compared statistically with an official method

	Normal amount (mg/tablet)	Found in sample[a] (mg/tablet)	Recover ± SD (%)	*t*-test[c]	*F*-test[d]
Enalapril (Ezapril tablet, Kahira Co)	10				
Spectrophotometric					
Extractive procedure		9.906	99.06 ± 0.81	0.68	1.48
Procedure using surfactant		9.852	98.52 ± 0.52	0.17	3.63
Atomic absorption procedure		9.893	98.93 ± 0.77	0.47	1.66
Official method[b]		9.863	98.63 ± 0.99		
Ramipril (Tritace tablet, Hochest Orient Egypt)	2.5				
Extractive spectrophotometric procedure		2.434	97.37 ± 0.59	0.7	1.80
Atomic absorption procedure		2.438	97.55 ± 0.46	0.17	1.09
Official method[b]		2.440	97.60 ± 0.44		

[a] Average of five determination.
[b] USP 24 [1] and BP [2], for enalapril and ramipril, respectively.
[c] Tabulated *t*-value for $P = 0.05$ and 8 degree of freedom is 2.306.
[d] Tabulated *F*-value for $P = 0.05$ and $f_1 = f_2 = 4$ is 6.39.

iments. The optimum conditions were established by varying one variable and observing its effect on the absorbance of the coloured product

1. Half milliliter buffer solution of pH 5, 3 ml of 6% w/v iron(III) chloride solution and 5 ml of 6% w/v thiocyanate solution was found optimum to maximize the colour intensity with the same order of addition;
2. It was found that two extractions each for 1 min were necessary for the quantitative estimation of the complex;
3. The colour of the ternary complex in the methylene chloride was quit stable for at least 24 h.

3.3. Study of the atomic absorption spectrometric method

It was not practical to aspirate the organic solvent of the ternary complex in the atomic absorption spectrometer, the high chlorine/carbon ratio would lead to the formation of a large quantity of HCl in the flame, which would damage the instrument [26,27]. It was better to extract the ternary complex with organic solvent (chloroform or methylene chloride), evaporate, and then dissolve the ternary complex residue with HCl, which could be aspirated directly in the atomic absorption spectrometer.

The effects of the reagent concentrations (eosin and copper for enalapril determination, iron(III) and thiocyanate ions for ramipril determination), pH, temperature, time, order of addition of reagents and solvents with respect to maximum sensitivity, minimum blank, adherence to Beer's law and stability, have been studied through control experiments. The optimum conditions were established by varying one variable and observing its effect on the absorbance of metal ion. It was found that the optimum experimental conditions are the same as in the extractive spectrophotometric procedures and incorporated into the general procedures.

Concerning the stoichiometric relationships, the molar ratio method indicated a molar ratio 1:1 cited drugs to Cu(II) or Fe(III). According to this ratio it was founds that

$3.07\ \mu g\ ml^{-1}\ Cu(II) = 24\ \mu g\ ml^{-1}$ enalapril
$8.58\ \mu g\ ml^{-1}\ Fe(III) = 32\ \mu g\ ml^{-1}$ ramipril

3.4. Calibration graph and statistical analysis

By using the above spectrophotometric and AAS procedures a linear regression equation was obtained. The regression plot showed a linear dependence of the absorbance over the Beer's law range given in Table 1. The mean molar absorptivity (ε), Sandell sensitivity (S) correlation coefficients, intercepts, slopes, variance and detection limit obtained by the linear square treatment of the results were listed in Table 1. The good linearity of the calibration graph and the negligible scatter of the experimental points were clearly evident from the values of the correlation coefficient and variance.

3.5. Accuracy and precision of the methods

To test the accuracy and precision of the methods, five successive measurements on the sample solution were carried out on three different drug concentrations. The small RSD% and SAE indicate high precision and good accuracy (Table 2).

3.6. Application of the analysis of commercial tablets

The proposed methods have been applied for the analysis of enalapril and ramipril in their commercial tablets together with the official USP 24 [1] and BP [2], respectively. The recovery of the drugs was tested by the standard addition method to the solution of the extracted tablets. These determinations were carried out on the same batch of samples. The results obtained were compared statistically by Student's t-test and variance ratio F-test (Table 3). The experimental values did not excess the theoretical values in either test, which indicates that there was no significant difference between the methods compared.

3.7. Interference

Applying the two procedures either using iron–thiocyanate or copper–eosin to form ternary complexes, interferents are compounds that have the ability to form extractable metal complexes or ion-association complexes with eosin as acidic dye. Thus as far as drugs are concerned, other ACE inhibitors as perindopril, captopril, benazapril and fosinopril give positive reaction due to the formation of such ternary complexes. Moreover, many β-blockers as propranol, atenolol and pindolol are able to form extractable ion-association complexes with eosin. However, such compounds are not usually present with examined drugs, and hence are not likely to cause analytical problems.

On the other hand, tablet fillers such as lactose, starch and stearic acid which represent a potential source of interference, do not interfere in the proposed methods.

4. Conclusion

The ternary complex formed under the abovementioned conditions and measured either spectrophotometrically (the extractive method and the method using surfactant at pH 5) or atomic absorption spectrometry can be regarded as an ion-association complex between the metal-drug cation and the eosin or thiocyante anion.

Although the present method is more time consuming (AAS method more than spectrophotometric methods) in comparison to other methods it have the advantages of high sensitivity, accuracy, precision and convenience. Moreover, the reproducibility of the results is superior to those obtained from other methods as spectrophotometry [8,9] and gas chromatography [16], where enalapril and ramipril can be determined in 0.02–0.13 mg. Therefore, the methods should be useful for routine analytical and quality control assay of the investigated drugs in dosage forms with low cost and depending upon the availability of chemicals and the equipment.

References

[1] The United States Pharmacopoeia, 24 Revision, The National Formulary, United States Pharamacopeial Convention, Inc, 2000, p. 638.
[2] The British Pharmacopoeia, vol. 2, HM Stationery Office, London, 2000, pp. 1331–1333.

[3] U.P. Halkar, N.P. Bhandari, S.H. Rane, Indian Drugs 35 (3) (1998) 168–169.
[4] G.Z. Yin, S.Y. Gao, Yaowa Fenxi Zazhi 16 (4) (1996) 227–229 in Chinese.
[5] C. Yu, H. Zhang, Y.C. Hong, G.L. Chen, S.M. Zhang, Yaowu Fenxi Zazhi 16 (6) (1996) 389–391 in Chinese.
[6] H.H. Trabelsi, S. Bouabdallah, S. Sabbah, F. Raouafi, K. Bouzouita, J. Chromatogr. A 871 (1-2) (2000) 189–199.
[7] H.F. Chen, J. Wang, Yaowu Fenxi Zazhi 18 (4) (1998) 245–248 in Chinese.
[8] C.V.N. Prasad, R.N. Saha, P. Parimoo, Pharm. Pharmacol. Commun. 5 (6) (1999) 383–388.
[9] S.M. Blaih, H.H. Abdin, F.A. El-Yazbi, R.A. Shaalan, Spectrosc. Lett. 33 (1) (2000) 91–102.
[10] H.Y. Aboul-Enein, R.I. Stefan, J.F. van Staden, Analysis 27 (1) (1999) 53–56.
[11] R.I. Stefan, J.F. van Staden, H.Y. Aboul-Enein, Electroanalysis (NY) 11 (16) (1999) 1233–1235.
[12] H.Y. Aboul-Enein, A.A. Bunaciu, C. Bala, S. Fleschin, Anal. Lett. 30 (11) (1997) 1999–2008.
[13] R.I. Stefan, J.F. van Staden, G.E. Baiulescu, H.Y. Aboul-Enein, Chem. Anal. (Warsaw) 44 (3A) (1999) 417–422.
[14] H.Y. Aboul-Enein, S.A. Baker, Drug Dev. Ind. Pharm. 18 (9) (1992) 1022–1022.
[15] H.Y. Aboul-Enein, C. Thiffaut, Anal. Lett. 24 (12) (1991) 2217–2224.
[16] D. Chmidt, A. Keller, Z. Fresenius, Anal.Chem. (Ger.) 320 (7) (1985) 731.
[17] H.G. Eckert, G. Munescher, R. Oekonomopuls, H. Strecker, H. Urbach, H. Wissmann, Arzneim-Forsch 35 (II(8) (1985) 1251–1256.
[18] H.E. Abdellatef, M.M. Ayad, E.A. Taha, J. Pharm. Biomed. Anal. 18 (1999) 1021–1027.
[19] H.H. Abdin, F.A. El-Yazbi, R.A. Shaalan, S.M. Blaih, STP Pharm. Sci. 9 (6) 587–591.
[20] M.M. Ayad, H.E. Abdellatef, E.A. Taha, S.M. Soliman, Zagazig, J. Pharm. Sci. (Egypt) 8 (2) (1999) 7–12.
[21] A.M. El-Walily, S.F. Belal, R.S. Bakry, J. Pharm. Biomed. Anal. 14 (1996) 561–569.
[22] P.B. Issopouls, P.T. Economou, Fresenius J. Anal. Chem. 343 (1992) 518–522.
[23] P.B. Issopouls, P.T. Economou, Fresenius J. Anal. Chem. 345 (1993) 595–599.
[24] Y. Fujita, I. Mori, K. Fujita, T. Tanaka, Y. Koshiyama, H. Kawabe, Chem. Pharm. Bull. 34 (1986) 2236–2238.
[25] J. Rose, Advanced Physico-Chemial Experiments, Pitman, London, 1964, p. 54.
[26] N. Cristina, G. Agustin, Anal. Chem. 57 (1985) 34–38.
[27] C. Nerin, J. Cacho, A. Garania, Anal. Lett. 18 (B15) (1985) 1887–1891.