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Spectrophotometric and spectrofluorimetric methods for the determination of ramipril in its pure and dosage form

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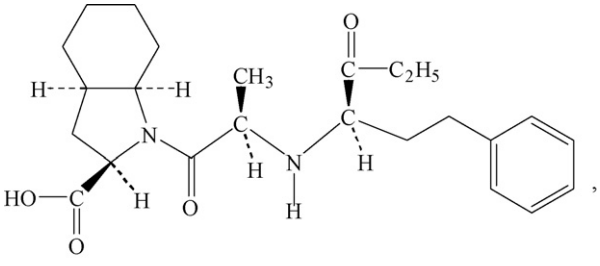
Abstract

This paper describes three sensitive spectrophotometric and spectrofluorimetric methods for determination of ramipril in its pure form and pharmaceutical tablets. The first method is based on the oxidation of the drug with 1-chlorobenzotriazole reagent (CBT) in strong alkaline medium followed by measuring the absorbance at 350 nm. The method obeys Beer's law over concentration range 15–50 μg ml^{-1}. For the second and third, both are non-extractive methods based on the formation of ternary complex between copper (II), eosin and ramipril in the presence of methylcellulose as surfactant. Spectrophotometrically, under the optimum condition, the ternary complex showed an absorption maximum at 543 nm. The method obeys Beer's law over concentration range of 20–80 μg ml^{-1}. A fluorescence quenching method for the determination of ramipril by forming this ternary complex was also investigated for the propose of enhance the sensitivity of the determination. The methods are simple, sensitive, and accurate. The results obtained are reproducible with a coefficient of variation less than 2%. The proposed have been successfully applied to the assay of ramipril in tablets. The results compare favorably with official method.

Keywords: Ramipril; Spectrophotometry; 1-CBT; Ternary complex formation

1. Introduction

Ramipril, 2-[*N*-[(*S*)-1-ethoxy carbonyl-3-phenyl propyl]-L-alanyl]-(1*S*, 3*S*, 5*S*)-2-azabicyclo [3,3,0]-octane-3-carboxylic acid [CAS:87333-19-5] is a prodrug [1]



Ramipril

which is rapidly hydrolyzed with the cleavage of an ester group through hepatic metabolism forming an active metabolite i.e., ramiprilat. This prodrug itself is a poor inhibitor of angiotensin converting enzyme (ACE) but its active metabolite has a higher affinity for ACE, thus blocking the conversion of the angiotensin I to the angiotensin II, a highly potent vasoconstrictor and there by leading to a reduction in vasopressor activity and a decrease in peripheral vascular resistance [2,3]. The drug is officially listed in British Pharmacopoeia [4], which describes a potentiometric titration procedure for its assay in bulk and dosage form. The estimation of ramipril along with hydrochlorothiazide in binary mixture was performed by derivative compensation technique [5] as well as zero crossing derivative technique [6,7] Two extractive spectrophotometric methods have been reported for the assay of drug in commercial dosage forms, which are based on the formation of ternary complex of the drug with Cu (II) and eosin [8] and Fe (III) and ammonium thiocyanate [9]. The drug content in pharmaceutical formulations has been determined spectrophotometrically in visible region based on the charge transfer reaction of ramipril with π-acceptors such as 7,7,8,8-tetracyanoquinodimethane and *p*-chloranilic acid and subsequently measuring the absorbance at 840 and 520 nm, respectively [10]. Two extractive spectrophotometric methods [11] have been recommended based on extractive ion-pair complex of the drug with picric acid and bromocresol green. Potassium permanganate oxidizes ramipril in alkaline medium resulting in the formation of bluish green coloured complex peaking at 610 nm [12]. The quantitation of ramipril [13] has been done

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by spectrophotometry and fluorimetry utilizing the reaction of the drug with 7-fluoro-4-nitrobenzo-2-oxo-1,3-diazole which exhibits maximum absorbance at 460 nm, and maximum fluorescence intensity at 530 nm after excitation at 465 nm. More recently, a kinetic sopectrophotometric method based on the reaction of the carboxylic acid group of the drug with a mixture of potassium iodate (KIO_3) and potassium iodide (KI) in aqueous medium has been reported [14].

Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost. This study presents new spectrophotometric methods for the determination of ramipril in tablets. The first method is based on the reaction of ramipril with 1-chlorobenzotriazol (CBT) which is an *N*-halogen compound that contain cyclic three nitrogen chain, has the capability to undergo certain chemical reactions which prove its usefulness in organic synthesis, thus, 1-CBT oxidizes alcohols to aldehydes and ketones, hydrazo to azo compounds and 1-amino-4,5-diphenyl triazole to diphenylacetylene, and 1-CBT is converted to benzotriazole hydrochloride [15].

1-CBT reagent was used for assay of some sulphur compounds as thiourea, allythiourea, phenylthiourea, tolythiourea, thioacetamide, thiobezamide, diethylthiocarbamate, ethylphenyldithiocarbamate, diisopropyldithiocarbamate, and methionine [16], some of phenothiazine derivative [17] and certain sulphur containing drugs, cefaclor, cefadroxil [18] cefotaxime and cefuroxime [19]. Recently, 1-CBT was used for determination of lisinopril in tablet [20]. The second and third methods are based on the formation of ternary complex between ramipril, Cu (II) and eosin. The complex was measured either photometrically or fluorimetrically. The advantages of these methods over the extractive spectrophotometric and AAS method [8] – the author previous work with others – is in the use of methylcellulose as surfactant at pH 5 to increase the solubility and intensity of the formed complex and to avoid the separation process which is a time consuming process and need more precautions especially the AAS method which required evaporation the solvent to dryness in a boiling water bath before aspirating the solution in the atomic absorption spectrometer. Moreover, the quenching effect on the fluorescence intensity of eosin due to the formation of this ternary complex was also used for the determination of ramipril. The applicability of the developed methods was evaluated through the determination of ramipril in the bulk form or tablets.

2. Experimental

2.1. Apparatus

Shimadzu 260 UV recording spectrophotometer. Shimadzu RF-1501 spectrofluorophotometer.

2.2. Materials and reagents

Chemical were of the highest purity available from their sources.

(1) 1-Chlorobenzotriazole was prepared by method of Johnson et al. [21] and recrystallized from dichloromethane, m.p. (105–106 °C). 0.1% (w/v) prepared by dissolving 0.1 g CBT in 10 ml DMF and diluting to 100 ml with distilled water.
(2) Eosin (PS PARIC Scientific limited), 0.1% solution in distilled water.
(3) Copper (II) chloride, 0.2% solution in distilled water.
(4) Methylcellulose, 0.3% (w/v) in distilled water, 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.
(5) Acetate buffer pH 5 (dissolve 13.6 g of sodium acetate and 6 ml of glacial acetic acid in sufficient water to produce 1000 ml).
(6) Ramipril and Tritace tablets containing 2.5 mg per tablet from Hoechst orient Egypt, Cairo.

2.3. Standard solutions

Solution of 0.5 mg ml^{-1} was prepared by dissolving 25-mg ramipril in 5 ml methanol and the volume to 50 ml was completed with distilled water. The solution was used to prepare calibration curves and quality control samples. Quality control samples prepared at three concentration levels of 0.2, 0.3 and 0.4 mg ml^{-1} applying method 1 (using 1-CBT as reagent) and 0.25, 0.50 and 0.75 mg ml^{-1} applying method 2 (formation of ternary complex) the solution is stable for a least 1 week if kept stored in a cool and dark place [22].

2.4. General procedures and calibration graphs

2.4.1. Method 1 (photometric method using 1-CBT)

Appropriate volumes of the standard solution in the concentration range stated in Table 1 were placed in a series of 10 ml volumetric flasks, treated with 3 ml 0.1% CBT and 2 ml 0.1 N NaOH. The solution allowed to stand for 30 min at room temperature then completed to volume with distilled water. The absorbance was measured at 350 nm against a reagent blank.

2.4.2. Method 2 (photometric method based on ternary complex formation)

Appropriate volumes of the standard solution in the concentration range stated in Table 1 were placed in a series of 25 ml volumetric flasks. A 2 ml of methyl cellulose, 1.5 ml of the buffer solution pH 5, 3 ml of eosin solution and 3 ml of 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 a warm water-bath (60 ± 5 °C) for 5 min. The solution was then cooled to room temperature. The absorbance of the solution (solution A) was measured at 543 nm against a similarly prepared eosin copper (II) chloride and buffer solution (solution B).

2.4.3. Method 3 (fluorimetric method based on ternary complex formation)

Volume of 0.1 ml of the above solution (solution A) in method 2 was pipetted into 10 ml volumetric flask and diluted to volume with water. The difference in the relative fluorescence intensities

Table 1
Optical characteristics and statistical data of the regression equations for determination of ramipril using the proposed methods

Parameters	Photometric methods		Fluorimetric method
	Method 1 (using CBT)	Method 2 (ternary complex formation)	Method 3 (ternary complex formation)
Linearity range ($\mu g\,ml^{-1}$)	15–50	20–80	2–8
Molar absorptivity ($mol^{-1}\,l\,cm^{-1}$)	3.1×10^3	4.6×10^3	–
Sandell's sensitivity ($\mu g\,cm^{-1}$)	7.8×10^{-2}	9.5×10^{-2}	–
Regression equation			
Intercept (a)	0.0102	0.0291	41.281
Slope (b)	0.0130	0.0162	4.771
Correlation coefficient (r)	0.9989	0.9999	0.9991

between solutions A and B at a 543 nm emission wavelength with excitation at 300 nm was measured.

The concentration of ramipril in the analyte solution can be determined by reference to corresponding calibration graphs, which had been constructed previously according to the regression equations (Table 1).

2.5. Procedure for assay of tablets

Twenty tablets of Tritace tablets were powdered and quantity of the powder equivalent to 25 mg of ramipril was dissolved by shaking with 5-ml methanol followed by 30 ml of water. The solution was filtered through filter paper into a 50 ml volumetric flask and then diluted to volume with water. The assay of ramipril content was completed as described in Section 2.4.

3. Result and discussion

3.1. Method 1 (photometric method using 1-CBT)

The organic positive halogen compounds have been used as oxidizing agent for the oxidation of a variety of organic compounds. The oxidation reactions generally involve the loss of the hydrogen from –C–H, O–H, –N–H, or –S–H bonds. Though the reactions involving addition of oxygen have also been reported. These reactions have found extensive application in the determination of organic compounds [23].

1-CBT was used to oxidise ramipril with the production of yellow colour reaction product with λ_{max} at 350 nm Fig. 1.

For optimization the reaction condition of 1-CBT with the studied drug, several factors have been carefully studied. Concerning the effect of pH, it was found that the reaction proceeds only in alkaline pH and maximum colour intensity produced using 2 ml of 0.1 N NaOH. The amount of the reagent necessary to obtain a linear graph for a drug concentration was studied. Maximum and reproducible colour intensity was produced when the amount of the reagents mentioned in the construction of the calibration graphs have been used. Higher concentration of reagents did not affect the colour intensity.

3.2. Method 2 (non-extractive procedure based on ternary complex formation in presence of surfactant)

Ternary complexes of general formula ($L_NM_XS_Y$) have been widely used in spectrophotometric analysis [8,9,24–27]. For the ternary complexes dealt with in this paper is that the main ligand L is the cited drug ramipril, the second ligand S is eosin and M is copper (II) metal, respectively. This triple complexes is extractable with chloroform, whereas, the binary systems (metal–drug and metal–eosin) cannot be extracted in that way.

The author previous manuscript (with others) describe an extractive spectrophotometric and AAS methods for determination of ramipril through ternary complex formation with Cu (II) and eosin [8]. The method was based on the extraction of the ternary complex with chloroform and measuring the absorbance either spectrophotometrically at 535 nm or atomic absorption spectrometrically after evaporation of chloroform to dryness. The aim of the proposed method is to avoid the extraction process and increasing the sensitivity of the method (the spectrophotometric one) by using methycellose as surfactant and measuring the absorption directly without extraction at 543 nm spectrophotometrically and at 543 nm wavelength with excitation at 300 nm fluorimetrically.

The absorption spectra of the ternary complex (drug–Cu–eosin) (solution A) and the blank solution (Cu–eosin) (solution B) were scanned in the range 400–600 nm. It was found that,

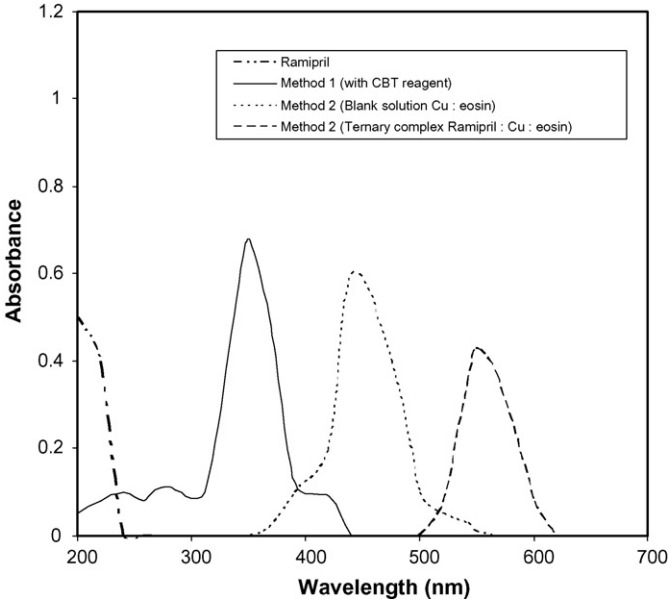

Fig. 1. Absorption spectra of coloured reaction products of: 50 $\mu g\,ml^{-1}$ ramipril with 1-CBT, in alkaline medium applying method 1 and of 0.25 $\mu g\,ml^{-1}$ with eosin and in the presence of methyl cellulose applying method 2

Table 2
Intra-day assay: test of precision of the proposed methods for the determination of ramipril

Proposed methods	Concentration ($\mu g\ ml^{-1}$)		Recovery ± R.S.D.[a] (%)	SAE[b]	CL[c]
	Theoretical	Nominal ± S.D.[a]			
Method 1 (photometric method using CBT)	20	19.8 ± 0.113	99.00 ± 0.57	0.051	0.141
	30	30.2 ± 0.321	100.67 ± 1.06	0.144	0.399
	40	39.5 ± 0.364	98.75 ± 0.92	0.163	0.451
Method 2 (photometric method using Cu:eosin)	25	24.3 ± 0.321	97.2 ± 1.321	0.144	0.398
	50	49.0 ± 1.012	98.02 ± 2.065	0.453	1.254
	75	74.0 ± 1.066	98.66 ± 1.441	0.477	1.321
Method 3 (fluorimetric method using Cu:eosin)	3	2.96 ± 0.054	98.67 ± 1.824	0.024	0.067
	5	4.86 ± 0.037	97.20 ± 0.761	0.017	0.046
	7	6.89 ± 0.066	97.80 ± 0.958	0.029	0.082

[a] Mean for five determinations.
[b] SAE, standard analytical error.
[c] CL, confidence limit at 95% confidence level and four degrees of freedom ($t = 2.776$).

on addition of the cited drugs to eosin–Cu (II) solution, a difference in absorbance was observed around 543 nm (Fig. 1). The absorption difference was proportional to the concentration of ramipril.

To optimize the assay parameters, the effects of pH, reaction time, effect of temperature, concentration of surfactant, eosin and copper (II) chloride on the absorbance of the ternary complex formed were studied. The effect of pH on the absorbance of the ternary complex studied at 543 nm. The absorbance of the drug–Cu (II)–eosin complex solution was investigated over the pH ranges 6–8. 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 ± 5 °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, methyl cellulose, benzalkonium chloride, Tween 20 and Tween 80. Among the surfactants studied, best results were obtained in the presence of methyl cellulose. The effect of concentration of the reagents, eosin and copper (II) chloride, on the absorbance of the ternary complex was studied. Maximum and reproducible colour intensity was produced when the amount of the reagents mentioned in the construction of the calibration graphs have been used. Higher concentration of reagents did not affect the colour intensity. The colour formed under the above-mentioned optimum conditions was stable for at least 1 h.

3.3. Method 3 (study of the fluorimetric method)

It was found that the formation of the ternary complex reduced the fluorescence of solution B, so, a fluorescence quenching method for the determination of ramipril was developed. On addition of studied drug to solution B, the relative fluorescence intensity of the solution B decreases significantly and the magnitude of the decrease was proportional to the concentration of the drug. In the development of the procedure for fluorimetric measurements, the same conditions as for the spectrophotometric method were adopted. The spectrophotometric and fluorimetric characteristics are summarized in Table 1.

Table 3
Inter-day assay: test of precision of the proposed methods for the determination of ramipril

Proposed methods	Concentration ($\mu g\ ml^{-1}$)		Recovery ± R.S.D.[a] (%)	SAE[b]	CL[c]
	Theoretical	Nominal ± S.D.[a]			
Method 1 (photometric method using CBT)	20	19.8 ± 0.101	99.0 ± 0.510	0.045	0.125
	30	29.7 ± 0.431	99.0 ± 1.451	0.193	0.534
	40	39.3 ± 0.252	98.3 ± 0.641	0.113	0.312
Method 2 (photometric method using Cu:eosin)	25	24.4 ± 0.292	97.6 ± 1.197	0.131	0.362
	50	49.2 ± 0.982	98.4 ± 1.996	0.439	1.217
	75	73.8 ± 0.875	98.4 ± 1.183	0.390	1.081
Method 3 (fluorimetric method using Cu:eosin)	3	2.95 ± 0.061	98.3 ± 1.729	0.027	0.076
	5	4.86 ± 0.055	97.20 ± 1.132	0.025	0.068
	7	6.91 ± 0.070	98.7 ± 1.013	0.031	0.087

[a] Mean for five determinations.
[b] SAE, standard analytical error.
[c] CL, confidence limit at 95% confidence level and four degrees of freedom ($t = 2.776$).

Table 4
Determination of ramipril by standard addition method

Proposed methods	Concentration ($\mu g\,ml^{-1}$)			Recovery ± R.S.D.[a] (%)	SAE[†]	CL[‡]
	Theoretical	Spiked	Nominal ± S.D.[a]			
Tritace tablets						
Method 1	15	15	29.83 ± 0.311	99.43 ± 1.043	0.139	0.385
Method 2	25	25	49.01 ± 0.442	98.02 ± 0.902	0.198	0.548
Method 3	3	3	5.91 ± 0.063	98.85 ± 1.066	0.028	0.078

[a] Mean for five determinations.
[†] SAE, standard analytical error.
[‡] CL, confidence limit at 95% confidence level and four degrees of freedom ($t = 2.776$).

Table 5
Determination of ramipril using the proposed methods compared with BP method [4]

	Proposed methods			Official method [4][a]
	Method 1	Method 2	Method 3	
Mean ± S.D.	99.81 ± 1.154	100.38 ± 1.028	100.52 ± 1.010	99.83 ± 0.921
N	9	9	9	5
t	0.04 (2.179)	0.98 (2.228)	1.24 (2.228)	
F	1.57 (3.84)	1.25 (4.53)	1.20 (4.53)	

Values in parentheses are the tabulated values of t and F at $p = 0.05$.
[a] Potentiometric titration method.

3.4. Quantification, accuracy and precision of the proposed methods

A linear correlation was found between absorbance and concentration in the ranges given in Table 1. The correlation coefficients, intercepts and slopes for the calibration data for the cited drug are calculated using the least-squares method.

The accuracy and precision of the proposed methods was established by measuring the content of ramipril in pure form at three different concentration levels. The intra-day precision of the proposed methods was performed by carrying out five independent analyses at each concentration level within 1 day (Table 2). In the same manner, the inter-day precision was also evaluated by measuring the cited drugs content at each concentration level on five consecutive days by the proposed methods (Table 3). The results of standard deviation (S.D.), relative standard deviation (R.S.D.) and recoveries by the proposed methods in Tables 2 and 3 can be considered to be very satisfactory. Thus the proposed methods are very effective for the assay of ramipril in tablets. The validity of the proposed methods was presented by recovery studies using the standard addition method. For this purpose, a known amount of reference drug was spiked to formulated tablets and the nominal value of drug was estimated by the proposed methods. Each level was repeated five times. The results (Table 4) were reproducible with low S.D. and R.S.D. No interference from the common excipients was observed. The proposed methods were compared with the official, potentiometric titration method [4]. The results obtained showed that the calculated t- and F-values did not exceed the theoretical values (95% confidence limits for five degree of freedom) (Table 5) from which we can conclude that the proposed methods do not differ significantly from reference method. The proposed methods were also, applied to commercial tablets contain ramipril with mean recoveries 99.81 ± 1.154, 100.38 ± 1.028 and 100.52 ± 1.010, applying methods 1, 2 and 3, respectively. The results show that there is no interference from any excipients.

4. Conclusion

The proposed methods are quite simple and do not require any pretreatment of the drug and tedious extraction procedure. The methods have wider linear range with good accuracy and precision. Hence the data presented in the manuscript by spectrophotometric and spectrofluorimetric methods for the determination of ramipril in its pure and dosage form demonstrate that the proposed method is accurate, precise and linear and thus can be extended for routine analysis of ramipril in pharmaceutical industries, hospitals and research laboratories.

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