ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
18 (1999) 1021–1027

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Spectrophotometric and atomic absorption spectrometric determination of ramipril and perindopril through ternary complex formation with eosin and Cu(II)

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Received 3 February 1998; received in revised form 4 April 1998; accepted 5 April 1998

Abstract

Two sensitive, spectrophotometric and atomic absorption spectrometric procedures are developed for the determination of ramipril and perindopril. Both methods are based on the formation of a ternary complex, extractable with chloroform, between copper(II), eosin and the two cited drugs. Spectrophotometrically under the optimum condition, the ternary complexes showed an absorption maximum at 535 nm, with apparent molar absorptivities of 6.55 and 4.00×10^3 $mol^{-1}cm^{-1}$ and Sandell's sensitivities of 5.80×10^{-2} and 1.04×10^{-1}μg cm^{-2} for perindopril and ramipril, respectively. The solution of ternary complex obeyed Beer's law in concentration ranges 10–60 and 20–100 μg ml^{-1} for perindopril and ramipril, respectively. The proposed method was applied to the determination of the two cited drugs in pharmaceutical tablets. The atomic absorption spectrometric method, directly through the quantitative determination of copper content of the organic extract 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 perindopril dosage forms.

Keywords: Perindopril; Ramipril; Ternary complex; Eosin; Copper sulphate; Spectrophotometry; Atomic absorption spectrometry

1. Introduction

The non official drugs perindopril (*tert*-butylamine salt of [[2*S*-1-(*R*,*R*)2α,3aβ,7aβ]-1-[2-(1-ethoxycarbonyl) butyl] amino]-oxopropyl, octahydo-1H indole-2-carboxylic acid and ramipril ([2*S*-1-(*R*,*R*)2α, 3aβ,6aβ]-1-[[2-(ethoxy carbonyl)-3-phenylpropyl]amino]-1-oxopropyl, octahydrocyclo-penta [b] pyrrole-2-carboxylic acid) are antihypertensive agents which their metabolites are an active inhibitor of angiotensin I-converting enzyme (ACE) [1]. The few reported methods in the literature for the determination of perindopril are gas chromatography [2], gas chromatography mass spectrometry (GC-MS) [3], radioimmunoassay [4] and derivatization-gas chromato-

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0731-7085/99/$ - see front matter
PII S0731-7085(98)00113-7

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

Parameters	Spectrophotometric		Atomic absorption spectrometric	
	Perindopril	Ramipril	Perindopril	Ramipril
Beer's law limits (μg ml^{-1})	10–60	20–100	1–6	2–10
Molar absorptivity (mol^{-1} cm^{-1})	6.55×10^{3}	4.00×10^{3}	—	—
Sandell's sensitivity[a]	5.8×10^{-2}	1.04×10^{-1}	—	—
Regression equation:				
Slope (*b*)	0.0162	0.0095	12.536	7.987
Intercept (*a*)	0.018	0.001	0.9281	0.7452
Correlation coefficient (*r*)	0.9998	0.9998	0.9997	0.9997

[a] μg cm^{-1} per 0.001 A.

graphy [5] and for ramipril high performance liquid chromatography [6,7] gas chromatography [8] and radioimmunoassay [9].

This paper reports simple, sensitive and accurate spectrophotometric and atomic absorption spectrometric methods for the determination of the two cited drugs. The two methods are based on the chelate-forming ability of the salt of the carboxylic group and the nearest nitrogen atom in these drugs with copper with the subsequent formation of a ternary complex with eosin (sodium 2,4,5,7-tetrabromofluorescein).

2. Experimental

2.1. Instrumentation

Shimadzu 260 UV recording spectrophotometer.

Shimadzu atomic absorption spectrophotometer, model AA-460-13.

2.2. Materials and reagents

Chemicals used were of the highest purity available from their sources. Eosin (Merck, Darmstadt, Germany) was prepared as a 0.1% w/v solution in distilled water. Copper(II) sulphate solution was prepared as a 0.2% w/v solution in distilled water. Perindopril and Coversyl® tablets containing 4 mg perindopril per tablet from Servier Egypt Industries, Cairo. Ramipril and Tritace® tablets containing 2.5 mg ramipril per tablet from Hoechst orient Egypt, Cairo.

2.3. Standard solutions:

Solutions of 1 mg ml^{-1} were prepared by dissolving 25 mg of perindopril or ramipril in distilled water in a 25-ml volumetric flask and diluting to volume. The solutions were stable for at least 1 week if they had been stored in a cool (≤ 25°C) and dark place.

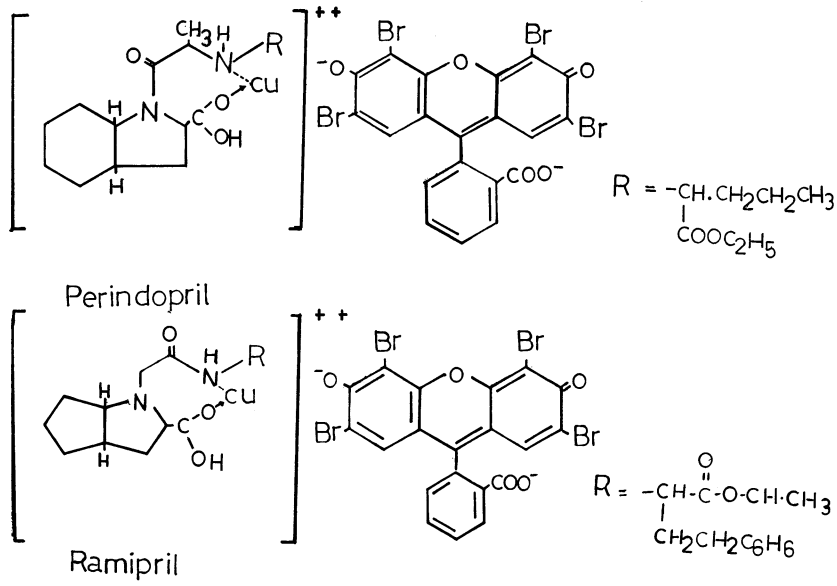
2.4. General procedure

2.4.1. Spectrophotometric method

Appropriate volumes of the standard solution in the concentration range stated in Table 1 were placed in 50 ml separating funnels. The volume of each solution was adjusted to 10 ml with distilled water. Three milliliters of copper(II) sulphate solution was added followed by 1 ml of eosin solution. The complex was extracted with 3×3 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 535 nm against blank in which the drug is omitted.

2.4.2. Atomic absorption spectrometric method

The procedure as under spectrophotometric method above as far as ' The volume of the



Scheme 1.

chloroform layers was made up to 10 ml' was applied. The chloroformic extract was evaporated to dryness on a boiling water bath. The residue was dissolved in 100 ml 0.1 N HCl. A blank (omitting the addition of the drug) was performed and the absorption at the following conditions:

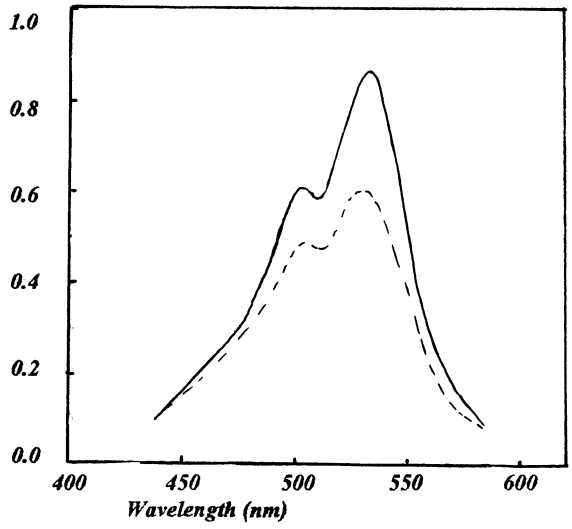


Fig. 1. Absorption spectra of the ternary complex of 50 μg ml^{-1} perindopril (—) and 70 μg ml^{-1} rampipril (----) with eosin and Cu(II).

wavelength 327.7 nm, lamp current 7 mA, slit width 3.8 A, air pressure 10 l min^{-1} and acetylene pressure 2.3 l min^{-1}. The concentration of the consumed copper was calculated from calibration graph of standard copper sulphate solution.

2.5. Assay of pharmaceutical tablets

Twenty tablets were powdered and a quantity of the powder equivalent to 25 mg of perindopril and ramipril was extracted by shaking with 10 ml of water, followed by another two extractions, each with 5 ml of water. The extracts were filtered through Whatman No 44 filter paper into a 25-ml volumetric flask and then diluted to volume. The assay for perindopril 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 [10–16]. The particularity of the ternary complexes dealt with in this paper is that their main ligand L is the non-official drug perindopril or ramipril, the second ligand S is eosin and M is

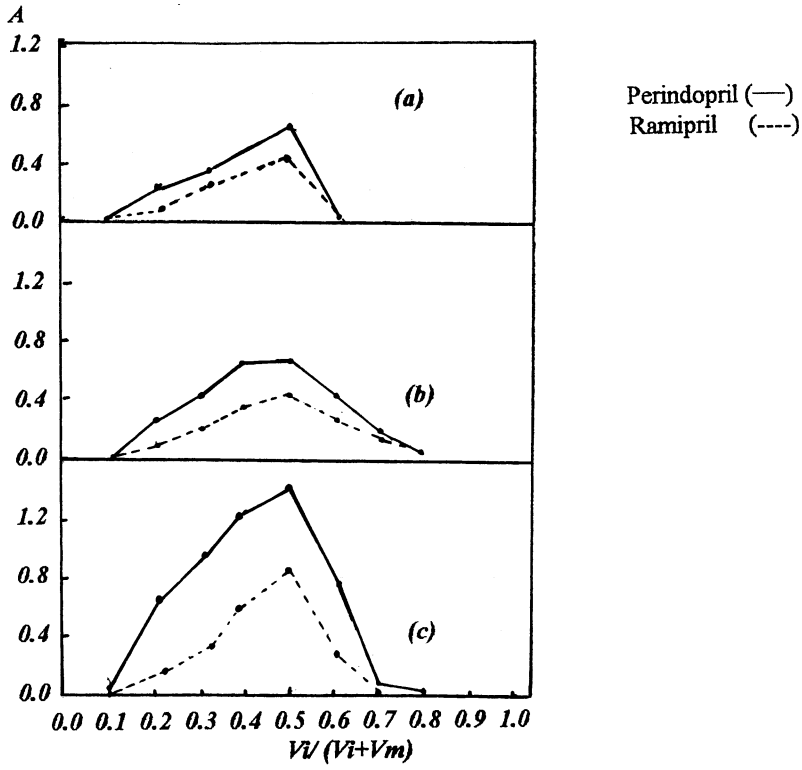


Fig. 2. (a) Continuous variation plots for drug: Cu(II) (1×10^{-3} M) complex ratio in the presence of excess eosin (2×10^{-2} M), Vi = drug; Vm = Cu(II). (b) Continuous variation plots for drug: eosin (1×10^{-3} M) complex ratio in the presence of excess Cu(II) (2×10^{-2}), Vi = drug; Vm = eosin. (c) Continuous variation plots for Cu(II): eosin (2×10^{-3} M) complex ratio in the presence of excess drug (2×10^{-3} M), Vi = eosin; Vm = Cu(II).

copper(II) metal (Scheme 1). These triple complexes are extractable with chloroform, whereas the binary systems (copper–drug and copper–eosin) cannot be extracted in that way.

3.1. Optimum experimental condition

The experimental conditions were established by varying one variable and observing its effect on the absorbance of the coloured product:

1. Three milliliters of 0.3% w/v copper(II) sulphate solution and 1 ml of 0.1% w/v eosin solution were found optimum to maximize the colour intensity.
2. Variation of the pH of the aqueous phase in the range from 6 to 8 has no effect on the intensity of the absorbance of the complex. At pH 9, however, the eosin-copper reagent start to precipitate and at pH bellow 6 the red colour of the ternary complex disappeared.
3. It was found that three extractions each for 1 min were necessary for the quantitative estimation of the complex.
4. The colour of the ternary complex in the chloroform was quit stable for at least 24 h.
5. It was not practical to aspirate the chloroformic solution 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 [17,18]. It was thus better to extract the ternary complex with chloroform, evaporate, and then dissolve the ternary complex residue with dilute HCl which could be aspirated directly in the atomic absorption spectrometer.

Table 2
Evaluation of the accuracy and precision of the two proposed procedures

Compared method	Added[a] μg ml^{-1}	Found ± SD[b]	RSD (%)	Range of error (%)	Confidence limits[c]
Perindopril					
Spectrophotometric	10	11.01 ± 0.121	1.099	0.089–0.054	11.01 ± 0.1500
	20	20.05 ± 0.025	0.125	0.005–0.011	20.05 ± 0.0305
	30	30.33 ± 0.081	0.267	0.061–0.036	30.33 ± 0.1000
	Mean		0.497	0.052–0.034	
Atomic absorption spectrometric	1	1.01 ± 0.013	1.288	0.099–0.006	1.01 ± 0.0167
	2	1.98 ± 0.029	1.465	0.045–0.013	1.98 ± 0.0361
	3	2.87 ± 0.036	1.254	0.089–0.016	2.87 ± 0.0444
	Mean		1.322	0.78–0.012	
Ramipril					
Spectrophotometric	20	18.9 ± 0.200	1.058	0.098–0.090	18.9 ± 0.2500
	40	38.8 ± 0.158	0.407	0.095–0.071	38.8 ± 0.1970
	60	57.9 ± 0.200	0.345	0.098–0.090	57.9 ± 0.2500
	Mean		0.603	0.097–0.084	
Atomic absorption spectrometric	2	1.97 ± 0.028	1.421	0.088–0.013	1.97 ± 0.3610
	4	3.88 ± 0.040	1.031	0.057–0.018	3.88 ± 0.0500
	6	5.87 ± 0.063	1.073	0.062–0.028	5.87 ± 0.0777
	Mean		1.175	0.069–0.020	

[a] Concentration in the final measured solution.
[b] Mean ± standard deviation for five determination.
[c] Confidence limits at $p = 0.95$ and four degrees of freedom.

3.2. Ternary complex formation

To prove the formation of a ternary complex between copper(II) (A), eosin (B) and the drugs (ramipril or perindopril) (C), the interaction of the three component may be considered as

$$\text{either } AB + C \leftrightarrow AC + B \quad (1)$$

$$\text{or } AB + C \leftrightarrow ABC \quad (2)$$

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 λ_{max} 507 nm, while neither copper(II) sulphate (A) nor the drugs (C) have absorbance maximum 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 535 nm, the reaction could 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 mixture, in the same solvent, gave a predominant absorption spectrum with λ_{max} at 535 nm (Fig. 1).

Furthermore, the reaction of copper with ramipril or perindopril, was studied in the absence of eosin at different pH values and no visible reaction was observed, in the presence of eosin, however, an instantaneous reaction occurs, which indicate the ionic character of the reaction.

3.3. Constitution of the ternary complex

The nature of the ternary complex (drug–Cu(II)–eosin) was determined using Job's method of continuous variation [19]. The results of apply-

Table 3
Determination of perindopril and ramipril in commercial tablets using the proposed methods

	Normal amount (mg)	Found (%)	Recovery (%)	RSD (%) ($n = 5$)
Spectrophotometric				
Coversyl tablets	4	4.047	101.2	1.657
Tritace tablets	2.5	2.446	97.84	1.992
Atomic absorption spectrometric				
Coversyl tablets	4	4.033	100.8	1.423
Tritace tablets	2.5	2.468	98.7	1.025

ing this method can be summarized as follows: the [Cu(II): drug] ratio in the presence of excess eosin was 1:1 (Fig. 2a), while the [eosin: drug] ratio in presence of excess Cu(II) sulphate was 1:1 (Fig. 2b) and the [Cu(II): eosin] 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).

3.4. Quantification, accuracy and precision

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 two cited drugs are calculated using the least-squares method.

The precision and accuracy of the two methods were tested by estimating five replicates of the two cited drugs within the Beer's law limits. The percentage standard deviation and the percentage range of error at 95% confidence level are given in Table 2.

The utility of each method was verified by means of replicate measurements of pharmaceutical formulations and recovery experiments. Recoveries were determined by adding standard drug to the pre-analyzed mixture of pharmaceutical preparations. The results of recovery experiments by the proposed methods are listed in Table 3. The commonly used additive and excipients in the preparation of tablets such as starch, lactose, talc, stearic acid and magnesium carbonate were found not to interfere in the analysis.

4. Conclusion

The data given above reveal that the proposed methods are simple, accurate and sensitive (atomic absorption spectrometric method > spectrophotometric method) with good precision and accuracy. With these methods, one can do the analysis at low cost without losing accuracy. The proposed methods can be used as alternative methods to reported ones for the routine determination of perindopril and ramipril in the pure form and in pharmaceutical formulations depending upon the availability of chemicals and the equipment.

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