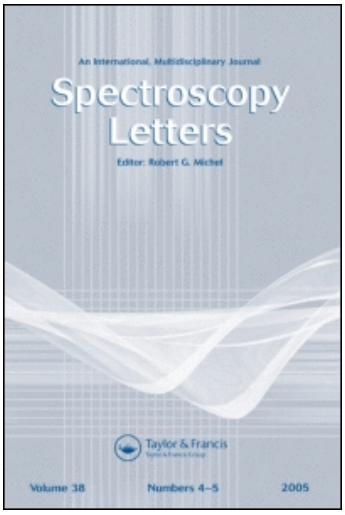

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SPECTROPHOTOMETRIC DETERMINATION OF ENALAPRIL MALEATE AND RAMIPRIL IN DOSAGE FORMS

Key Words: Enalapril maleate; Ramipril; *p*-Chloranilic acid; Picric acid; Bromocresol green; Dosage forms.

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ABSTRACT

Three sensitive and accurate spectrophotometric methods have been developed for the assay of enalapril maleate and ramipril, each in its dosage forms. These methods depend on the reaction of the drugs with *p*-chloranilic acid, the reaction with picric acid, and the ion-pair salt formation with bromocresol green. The proposed methods have been applied to the analysis of these drugs in their commercial tablets. The results obtained were precise and accurate.

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INTRODUCTION

Enalapril maleate (EN): (S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, (Z)-2-butenedioate, and **ramipril (RM)**: (2S, 3aS, 6aS)-1-[(S)-N-[(S)-1-carboxy-3-phenlypropyl] alanyl] octahydrocyclopenta[b]pyrrole-2-carboxylic acid, 1-ethyl ester, are angiotensin converting enzyme (ACE) inhibitors which constitute an important group of compounds that are used in the treatment of hypertension[1]. The literature presents some methods for EN determination in pharmaceutical preparations. These include colorimetric reaction with bromothymol blue[2], fourth derivative spectrophotometric technique[3], infra-red method[4], GC[5], and HPLC[6, 7]. On the other hand, only a few methods have been reported for RM determination. These include HPLC[8,9], radioimmunoassay[10], and a GC method[5] for its detection. No spectrophotometric method has been reported for RM assay in dosage forms.

The reported methods require multi-step extraction procedures and selective detectors. The problem in the assay of EN and RM is that there is no current precise, specific, and simple measurement of these potent drugs when they are formulated at a low dosage level (5 mg/tablet). Both drugs exhibit a very low UV absorption with $E_{1\%}$ (257 nm)= 15 and $E_{1\%}$ (254 nm) = 4.5 for EN and RM, respectively. This weak absorption means that a conventional UV spectrophotometric assay is susceptible to interference from excipients due to the high excipient to drug ratio. Therefore, the aim of this work was to develop simple and accurate spectrophotometric methods for the analysis of EN and RM in dosage forms.

This paper presents three sensitive, rapid, and accurate methods for the determination of EN and RM either in bulk powder or in tablets. These methods depend

on the measurement of color intensity produced by the reaction of either drug with *p*-chloranilic acid (*p*CA), picric acid, and bromocresol green (BCG). The methods are very adaptable to use in quality control and content uniformity assay of these drugs.

EXPERIMENTAL

Instruments

A Perkin-Elmer Model 550 UV-VIS spectrophotometer and a Hitachi Model 561 recorder were used. The spectra of the sample and reference solutions were recorded in 1-cm cells. A Schött-Gerate pH meter Model CG 710 was used for pH measurements.

Reagents and Chemicals

All chemicals and solvents used were of analytical grade. EN and RM were of pharmaceutical grade. Renitec® tablets (Swiss Pharma, Egypt) containing 5 mg enalapril maleate/ tablet and Tritace® tablets (Hoechst, Egypt) containing 5 mg ramipril /tablet were obtained from the local market. *p*-Chloranilic acid (E. Merck, Germany; 3 mg/ml in acetone), picric acid (Aldrich Chem.Co. USA; 0.8 mg/ml in chloroform), and bromocresol green (Adrich Chem. Co. USA; 1 mg/ml in distilled water) were used. McIlvaine buffer solutions[11] of pH 2.0 and 3.2 were used.

Standard Solutions and Calibration Graphs

Method A: Reaction with *p*CA

About 50 mg of each drug were accurately weighed, transferred into two separate 50-ml volumetric flasks, then dissolved in 10 ml either of DMSO for EN or DMF for RM. Each solution was neutralized to phenolphthalein indicator using 0.1N NaOH solution; then the solutions were completed to volume with the corresponding solvent.

Different aliquots of standard solutions, with the range stated in Table 1, were transferred into two sets of 10-ml volumetric flasks. The solutions were adjusted to constant volume with the corresponding solvent. To each flask, 2 ml of *p*CA solution were added and the mixtures were left to stand at room temperature for the specified time (Table 1). The flasks were completed to volume with acetone and the absorbance of the resulting color was measured at 534 and 524 nm for EN and RM, respectively, against a reagent blank.

Method B: Reaction with Picric Acid

Standard solutions containing 0.4 mg/ml in chloroform were used. Various portions of the standard solutions, within the specified range (Table 1), were transferred into two sets of 10-ml volumetric flasks. The solutions were adjusted to a constant volume with chloroform. Then 5 ml of picric acid solution were added to each flask, and the mixtures were left to stand at room temperature for the specified time (Table 1). The flasks were completed to volume with chloroform and the absorbance was measured at 370 nm against a reagent blank.

Method C: Reaction with BCG

Standard solutions of 0.2 mg/ml of EN in distilled water and 0.1 mg/ml of RM in ethanol were prepared. Accurate volumes from standard solutions, within the range shown in Table 1, were transferred into two sets of 60-ml separating funnels containing 5 ml of McIlvaine buffer solutions of pH 3.2 for EN and pH 2.0 for RM. Then 5 ml or 2 ml of BCG solution were added for EN and RM, respectively. The contents of each separator were mixed and then extracted with chloroform (3 x 3 ml). The chloroformic extracts were passed over anhydrous sodium sulfate, collected in 10-ml volumetric flasks,

TABLE 1

Assay Parameters for the Determination of EN and RM by the Proposed Methods

Parameter	Method A		Method B		Method C	
	EN	RM	EN	RM	EN	RM
Conc. range (mg%)	8 - 22	4 - 12.8	1.6 - 3.6	0.4 - 3.2	0.4 - 2.4	0.1 - 0.5
λ_{max} (nm)	534	524	370	370	412	412
Solvent	DMSO	DMF	Chloroform	Chloroform	Dist. Water	Ethanol
Time	10	30	40	0	--	--
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	$1.39x10^3$	$2.19x10^3$	$6.45x10^3$	$1.04x10^4$	$1.94x10^4$	$7.45x10^4$
Intercept (a)	-0.057	0.045	$-2.3x10^{-3}$	0.02	$5.6x10^{-3}$	$-5.0x10^{-4}$
Slope (b)	0.028	0.053	0.131	0.251	0.395	1.789
Correlation coefficient (r)	0.9999	0.9997	0.9996	0.9999	0.9995	0.9997
RSD*	0.24	1.03	0.70	0.22	1.20	1.01

*Mean of five separate determinations.

and completed to volume with chloroform. The absorbance of each solution was measured at 412 nm against a reagent blank.

Analysis of Dosage Forms

Twenty tablets from each drug preparation were weighed and finely powdered. A weight of the powder, equivalent to 25 mg of each drug, was extracted with the appropriate solvent for each method (Table 1), filtered into a 25-ml volumetric flask, and completed to volume using the same solvent. Different aliquots from the filtrate, within the range of each method, were treated according to the corresponding procedure. In the case of Method A, EN was extracted first with about 15 ml of methanol. Then the extract was evaporated, the residue was dissolved in DMSO, and the volume was completed to 25 ml with DMSO.

RESULTS AND DISCUSSION

Method A

*p*CA, a π-acceptor, is known to yield radical ions with various donors.[12] EN or RM, being an amine that has a pair of unshared electrons, acts as electron donors to the acceptor *p*CA. Such interaction in polar solvents as DMSO or DMF gives a highly colored radical anion.

$$\underset{\text{n-donor}}{D} + \underset{\pi\text{-acceptor}}{A} \rightleftharpoons \underset{\text{complex}}{(D \rightarrow A)} \rightleftharpoons \underset{\text{radical anions}}{D^{+} + A^{-}}$$

Moreover, EN and RM molecules contain carboxylic groups which can also act as electron donors, but these groups must be in the ionized form to interact with *p*CA. Figure 1 shows the absorption curves of the color products of the reaction of RM with

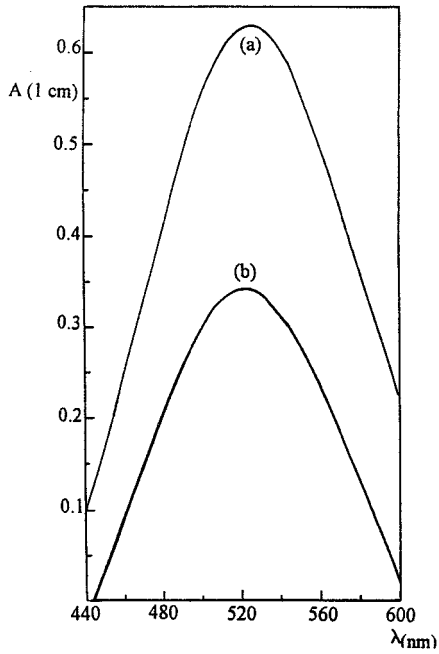

FIG. 1. Absorption spectra of the colored chromogen formed by the reaction of *p*CA with (a) 11.2 mg% neutralized RM and (b) 11.2 mg% non-neutralized RM.

*p*CA. It is obvious that the color intensity obtained from the reaction of the neutralized drug is nearly double the color intensity obtained from the non-neutralized drug. Therefore, the availability of the electrons on the carboxylate anion and the nitrogen of the secondary amino group of EN or RM provides an electron-rich moiety. Indeed, the reaction also can be explained as a proton transfer from *p*CA to the drug molecules with the formation of a purple colored anion of *p*CA. The reaction time, the volume of *p*CA solution, and the type of solvent were selected as a compromise between optimum sensitivity, stability, and obedience to Beer's law. In order to ascertain the stoichiometry

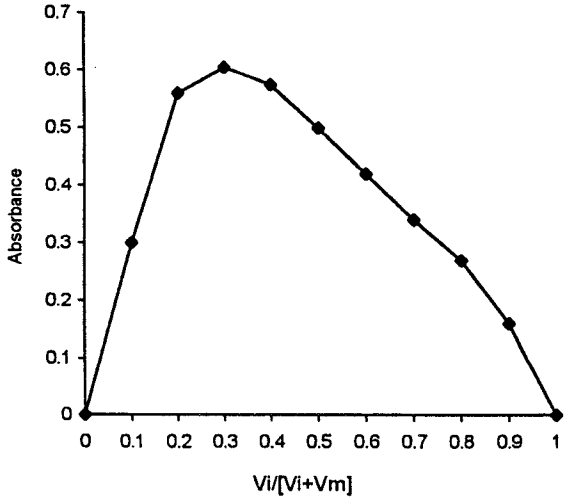

FIG. 2. Continuous variation plot for neutralized EN with *p*CA (4 x 10^{-3} M).

of the reaction between EN and *p*CA, Job's method[13] has been adopted. The formation of a 2:1 ratio of EN:*p*CA (Fig. 2) supported the previous explanation of the mechanism of the reaction.

Method B

Picric acid has been used to determine some basic drugs.[14] EN and RM, being amine derivatives, form salts with picric acid in organic solvents (chloroform). These salts were found to have an intense color due to the formation of a negatively charged picrate ion with a maximum absorbance at 370 nm (Fig. 3). The reaction conditions were studied in order to achieve high sensitivity and stability of the produced color. The addition of picric acid solution results in the instant formation of a yellow-colored chromogen at room temperature in the case of RM, while the maximum color in the case of EN was attained after 40 min.

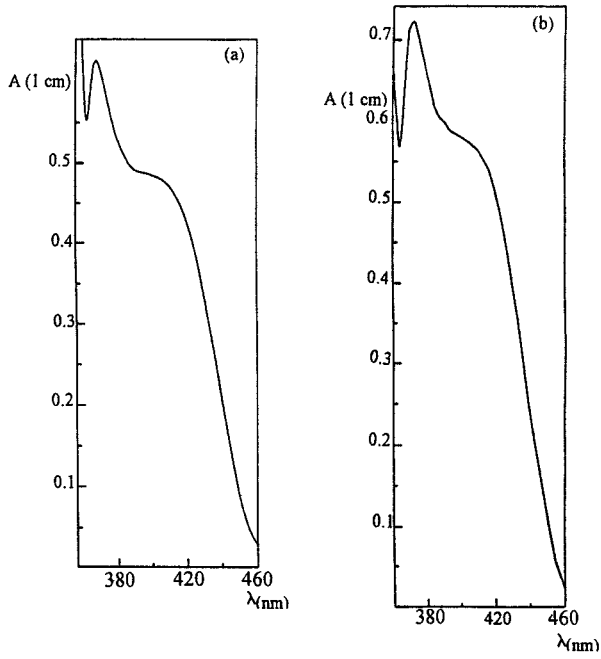

FIG. 3. Absorption spectra of (a) EN picrate salt (3.0 mg%) and (b) RM picrate salt (2.8 mg%).

Method C

EN and RM, containing basic nitrogen, were determined by the formation of highly colored ion pair complexes with BCG (an acid dye).[15] The formed complex was easily extracted with chloroform and exhibited maximum absorption at 412 nm (Fig. 4). Maintaining the pH of the solution at 3.2 and 2.0 was found to provide the optimum condition for sensitivity and stability of the color formation for EN and RM, respectively. The formed chromogen in each case was stable for at least 30 minutes. Job's method[13] was applied to study the reaction stoichiometry between EN or RM and BCG. The method showed a ratio of 1:1 under the described procedures.

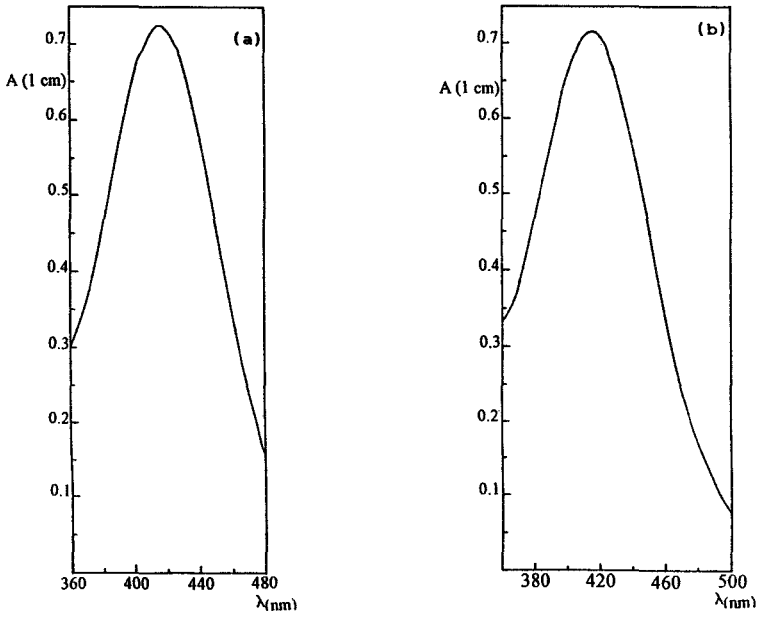

FIG. 4. Absorption spectra of the colored product formed by the reaction of BCG with (a) 1.8 mg% EN and (b) 0.4 mg% RM.

For all three methods, the graphs obtained by plotting the absorbances of the developed colors versus concentration were found to be linear using the experimental conditions described in Table 1. Molar absorptivities, slopes, intercepts, and correlation coefficients obtained by the linear least squares treatment of the results were shown in Table 1. The values of correlation coefficients and the negligible intercepts indicated the good linearity of the calibration graphs.

In order to study the precision of the proposed methods, five replicate determinations at different concentration levels were carried out. The relative standard deviations (RSD) were less than 2%, indicating good reproducibility of the methods (Table 1).

TABLE 2

Assay of EN and RM in Dosage Form Using the Proposed Methods

Sample	Method, Mean* ± S.D.		
	*p*CA	Picric Acid	BCG
Powder EN	99.9 ± 0.24	100.0 ± 0.70	99.9 ± 1.20
Renitec® tablets	101.8 ± 0.53	101.2 ± 0.85	101.6 ± 0.56
t**	0.609	0.966	--
F	1.116	2.304	--
Powder RM	100.0 ± 1.03	99.9 ± 0.22	100.0 ± 1.01
Tritace® tablets	101.4 ± 1.07	101.1 ± 0.82	100.3 ± 0.79
t**	1.731	1.571	--
F	1.834	1.077	--

* Mean of five separate determinations.

** Theoretical values for t- and F tests at p = 0.05 are 2.31 and 6.39, respectively.

Dosage Form Analysis

The proposed methods have been applied to the analysis of EN and RM in commercial tablets. The results (Table 2) obtained by the developed methods are of comparable accuracy and reproducibility (t- and F-tests). Experimental data show that tablet excipients exerted no interferences in any of the mentioned procedures (Table 2). The conventional UV method cannot be applied due to the lack of the characteristic features of absorption spectra combined with very weak UV absorption. In addition,

both drugs are present at low concentrations in tablets. On the other hand, the proposed methods are sensitive, simple, and accurate. They can be applied for either content uniformity or routine quality control.

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