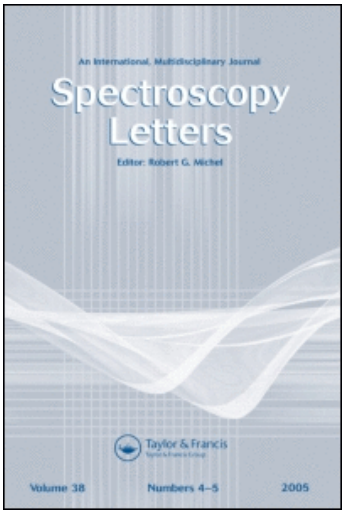

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SPECTROPHOTOMETRIC DETERMINATION OF RAMIPRIL (A NOVEL ACE INHIBITOR) IN DOSAGE FORMS

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SPECTROPHOTOMETRIC DETERMINATION OF RAMIPRIL (A NOVEL ACE INHIBITOR) IN DOSAGE FORMS

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ABSTRACT

A simple and sensitive spectrophotometric method has been developed for the determination of ramipril in its dosage forms. The method is based on reacting the drug with potassium permanganate in alkaline medium whereby a bluish green colour peaking at 610 nm is produced. The absorbance-concentration plot is rectilinear over the range $0.1-7.5\ \mu g \cdot ml^{\Sigma 1}$ ($r = 0.9992$) with minimum detectability of $0.05\ \mu g \cdot ml^{-1}$ (1.2×10^{-7} M). The molar absorptivity was $2.42 \times 10^4\ L \cdot Mol^{-1} \cdot cm^{-1}$. The different experimental parameters affecting the development and stability of the colour were carefully studied and optimized. The proposed method was further applied to the determination of ramipril in its dosage forms, whether alone or in combination with hydrochlorothiazide. The results obtained were in good agreement with those obtained by a

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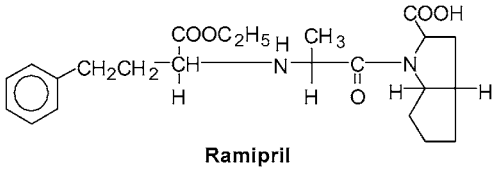
reference HPLC method. A proposal of the reaction pathway was postulated.

Key Words: Ramipril dosage forms; ACE inhibitor; Colorimetric.

INTRODUCTION

Ramipril, 2-[N-[S]-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl]-(1S,3S,5S)-2-azabicyclo[3,3,O] octane-3-carboxylic acid, is an orally active inhibitor of angiotensin converting enzyme (ACE) with antihypertensive activity [1]. It is used in the treatment of all forms of hypertension, heart failure and following myocardial infarction to improve survival in patients with clinical evidence of heart failure [2].



Ramipril

In spite of the clinical importance of ramipril, little has been published concerning its determination, viz, GC [3,4], HPLC [5–8], enzymatic assay with GC or HPLC [9], radioimmunoassay [10], derivative spectroscopy [8] ion-selective electrode potentiometry (11) and atomic absorption spectroscopy (12). All these methods are either insufficiently sensitive (8,11) or tedious and require highly sophisticated instrumentation (5–10).

The UV absorption spectrum of ramipril shows a barely discernible maxima at about 257 nm (its molar absorptivity at 257 nm is about 290 $L \cdot Mol^{-1} \cdot cm^{-1}$). This low intensity and lack of well-defined maxima in the UV region, typical of unconjugated phenyl moiety, make it not useful for quantitative analysis of the compound. This led us to study the reaction of ramipril with $KMnO_4$ in an alkaline medium in an attempt to develop a simple and reliable method for its determination in dosage forms. The results obtained were promising.

EXPERIMENTAL

Apparatus

Spectrophotometer: LKB, UV/Vis 4050.

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Materials and Reagents

Ramipril (Batch No. Z001) was kindly provided by Hoechst, AG, Frankfurt, Germany. Tritace 5 tablets (Batch No. 40 B 414), Tritace 2.5 tablets (Batch No. 204) and Tritace Comp. (Batch No. 005) were obtained from commercial sources. A stock solution was prepared by dissolving 100.0 mg of ramipril in 100.0 ml of distilled water and further diluted with the same solvent as appropriate.

Potassium Permanganate: AnalaR, BDH (England). A 0.1 M stock solution was prepared by and further diluted as appropriate.

Sodium hydroxide: Merck (Germany). 12.5 M stock solution.

Procedures

Recommended Analytical Procedure

Transfer 1.5 ml of 0.01 M $KMnO_4$ and 1.5 ml of 12.5 M . NaOH into a series of 25-ml measuring flasks. Add aliquot volumes of ramipril solution so that the final concentration is in the range of 0.1−7.5 μg/ml. Make up to volume of about 20 ml with distilled water, then heat in a boiling water bath for about 15 min. Cool then complete to the mark with distilled water. Measure the absorbance against a reagent blank at 610 nm. Plot the absorbance versus the final concentration to get the calibration graph. Alternatively, derive the regression equation.

Procedure for the Tablets

Weigh and pulverize 10 tablets. Transfer a weighed amount of the powder equivalent to 5.0 mg of ramipril into a small flask. Extract with 3 × 30 ml of water and filter into a 100 ml volumetric flask. Wash the residue and filter with few ml of water and pass the washings to the same flask. Complete to the mark with the same solvent. Transfer suitable aliquots of the solution into 25 ml volumetric flask then proceed as described previously in the Recommended Analytical Procedure section. Determine the content of the tablet either from the calibration graph or using the regression equation.

RESULTS AND DISCUSSION

Optimization of Parameters

Ramipril was found to react with $KMnO_4$ in alkaline medium producing a bluish green colour peaking at 610 nm (Fig. 1). The various experimental factors

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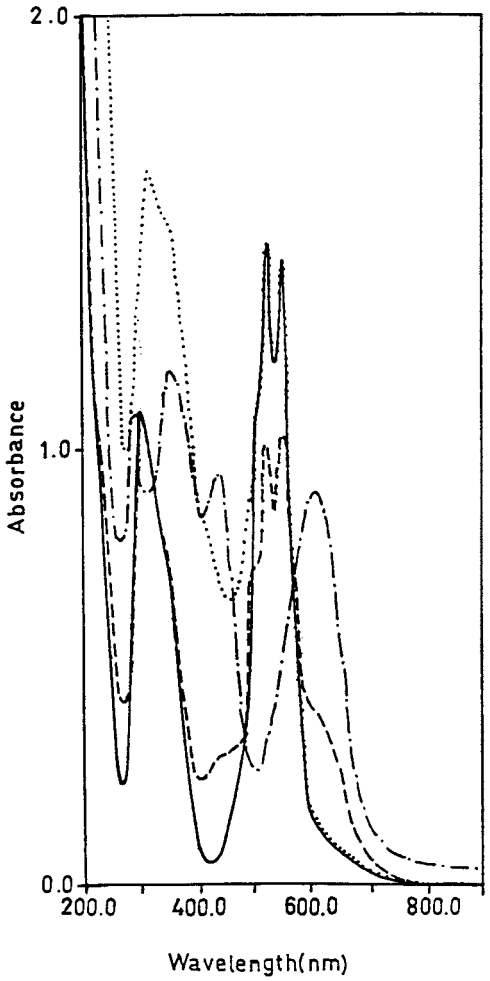

Figure 1. Absorption spectrum of: ————, $KMnO_4$ ($6 \times 10^{\Sigma 4}$ M); ----------, $KMnO_4$/NaOH; ·····, $KMnO_4$ + Ramipril (7.5 μg/ml); – • – • –•, Reaction product (7.5 μg/ml).

affecting the development and stability of the reaction product were studied and optimized. Such factors which were changed individually, include concentration of the reagents ($KMnO_4$ and NaOH), order of addition of reagents, temperature, time of heating, buffers, sensitiziers, surfactants, alkalies and foreign substances. The influence of the concentration of $KMnO_4$ was studied using different concentrations ranging from 5×10^{-4}–1×10^{-3} M final concentration. The highest result

was obtained with 6×10^{-4} M, higher concentrations of the reagent caused the colour to fade, probably the product is decomposed. Complete reaction between $KMnO_4$ and ramipril takes place only in an alkaline medium. Different concentrations of NaOH ranging from 0.05–1.5 M were tested. It was found out that, the reaction took place starting from 0.25 M upwards. However, to ensure a complete reaction, 0.75 M NaOH (final concentration) was chosen, as it gave the highest absorbance reading. Other alkalies (KOH and NH_4OH) with the same concentration were also tested. However, their effect on colour development was less than that of NaOH, therefore, the latter was used throughout the study.

The order of addition of reagents is an essential part of the experiment. Heating the $KMnO_4$/NaOH mixture before addition of ramipril produced the lowest readings. Similarly, heating ramipril with $KMnO_4$ before addition of NaOH or ramipril with NaOH before addition of $KMnO_4$ gave lower absorbance readings. The highest results were obtained upon heating the 3 ingredients altogether. Preliminary test proved that complete colour formation was achieved by heating the resulting solution. Different temperature settings were used with constant heating time. The highest absorbance readings were obtained upon heating the solution in a boiling water-bath. The time of heating is a critical part of the experiment. Heating for 15 min gave the highest absorbance readings. Excessive heating time did not produce significant increase in absorbance readings.

Different buffers at concentration of 0.01 M were tested. Addition of buffers was done before heating the resulting product. Thus acetate, borate, carbonate, oxalate and phosphate buffers were employed. In all cases, no outstanding effect was noticed.

Different sensitizers (quinine, cyclohexane-diol, fluorescein and rhodamine-B) at concentration of 5 μg/ml were tested, likewise, blank solutions for corresponding sensitizers were also simultaneously prepared. Outstanding inhibitory effects were observed as it was evident from the blank readings. It is clear that the sensitizers reacted strongly with the $KMnO_4$/NaOH system (Table 1).

In the same manner, the effect of surfactants on the colour development was studied. Three different surfactants (Brji-35; cetyltrimethylammonium bromide

Table 1. Effect of Sensitiziens on the Performance of the Proposed Method

	Absorbance		
Sensitizien	Sample	Blank	Net Result
No Sensitizen	0.594	Adjusted to 0.0	0.594
Quinine	0.525	0.398	0.127
Cyclohexanediol	0.466	0.446	0.020
Fluorescein	Turbid yellow solution for sample and blank		
Rhodamin B	0.590	0.501	0.089

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Table 2. Effect of Foreign Substances on the Performance of the Proposed Method

Foreign Substances / Concentration	% Recovery of Ramipril		
	20 $\mu g \cdot ml^{\Sigma 1}$	5 $\mu g \cdot ml^{\Sigma 1}$	1 $\mu g \cdot ml^{\Sigma 1}$
No foreign substance	100.0	100.0	100.0
Fructose	1.1	12.4	42.2
Glucose	1.3	8.3	62.1
Sucrose	0.94	9.1	62.7
Ribose	1.5	12.5	63.2
Glucosamin	15.6	20.6	64.0
Cysteine	20.4	38.6	84.9
Alanine	81.5	85.8	91.5
Methionine	1.41	17.0	71.3
Carbowax	76.9	82.4	89.8
Nicotinamide	84.4	87.6	92.6
Riboflavin	9.6	41.8	67.3
Starch	6.8	40.2	82.3

and sodium lauryl sulphate) at three different concentrations (2.5, 7.5 and 15 μg/ml) were tested by being added to the resulting product prior to heating. Corresponding blank solutions were simultaneously prepared. An inhibitory effect was evident as the blank solutions yielded high absorbance readings and consequently the net absorbance values decreased.

The effect of foreign substances was studied by adding three different concentrations (20.0, 5.0 and 1.0 μg/ml) of each of the following: fructose, glucose, sucrose, ribose, starch, glucosamine, cysteine, alanine, methionine, carbowax, nicotinamide and riboflavine. It was found out that almost all of these foreign substances greatly interfered with the product formation (Table 2). It is safe to note that these interferences could be avoided by serial dilution as evident from the 1.0 μg/ml concentration of each foreign substance tested. These substances reacted with the $KMnO_4$/NaOH system producing colored products as shown by the high readings of the blank solutions.

Analytical Performance

A series of standard solutions was prepared from the stock solution of ramipril and treated as described earlier. A typical spectrum for ramipril reaction product is shown in Figure 1 from which a calibration graph of ramipril concentration vs absorbance was extracted. A typical regression analysis of this plot resulted in the calibration equation:

$$A = 6.68 \times 10^{\Sigma 3} + 0.054\,C \qquad (R = 0.9992)$$

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where A is the absorbance, C is the concentration of the drug in $\mu g \cdot ml^{-1}$, with a correlation coefficient of 0.9992 and the percentage error of 1.374% indicating a fairly good straight line that passes through the origin with an insignificant intercept. Statistical analysis of the regression data (14) gave a standard deviation of 0.0104, 0.0114 and 2.427×10^{-3} for the residuals ($S_{x/y}$), intercept (Sa) and slope (Sb) respectively. The above equation can be used to calculate unknown ramipril concentrations over the range $0.1-7.5\ \mu g \cdot ml^{-1}$ within which the equation was found to be linear.

Application

The proposed method was applied to the determination of ramipril in its pharmaceutical formulations, Tritac 5 and Tritac 2.5 in addition to Tritac Comp. tablets. High percentage recoveries due to interference from excipients were first obtained upon direct application of the method (around 160%). To eliminate this interference, the standard addition technique was resorted to, whereby satisfactory percentage recoveries were obtained (Table 3). The results obtained were compared with those obtained from a reference method (5) adopting HPLC technique. Statistical analysis of the results using Student's t-test and Variance ratio-F-test for paired data revealed no significant difference between the two methods at the 95% confidence level for the tablets.

Interference from co-formulated drugs, such as hydrochlorothiazide was studied. Hydrochlorothiazide, being 5 times more than content of ramipril interfered seriously with the method, being oxidised by $KMnO_4/OH^-$ system. However, this interference could be almost overcome by extraction of ramipril with water, in which hydrochlorothiazide is practically insoluble. Complete overcome of the interference could be accomplished by adopting the standard addition technique. The results in Table 3 show that the proposed method is satisfactorily accurate and precise.

Table 3. Application of the Proposed and Reference Method to the Determination of Ramipril in Dosage Forms

	% Recovery	
Preparation	Proposed Method	Reference Method (5)
Tritace 5 (ramipril, 5 mg/tablet)	100.38 ±0.56	100.72 ±0.71
Tritace 2.5 (ramipril, 2.5 mg/tablet)	100.05 ±0.35	98.66 ±0.37
Tritace Comp. (ramipril, 5 mg + hydrochlorothiazide 25 mg/tablet)	99.52 ±0.47	99.31 ±0.50

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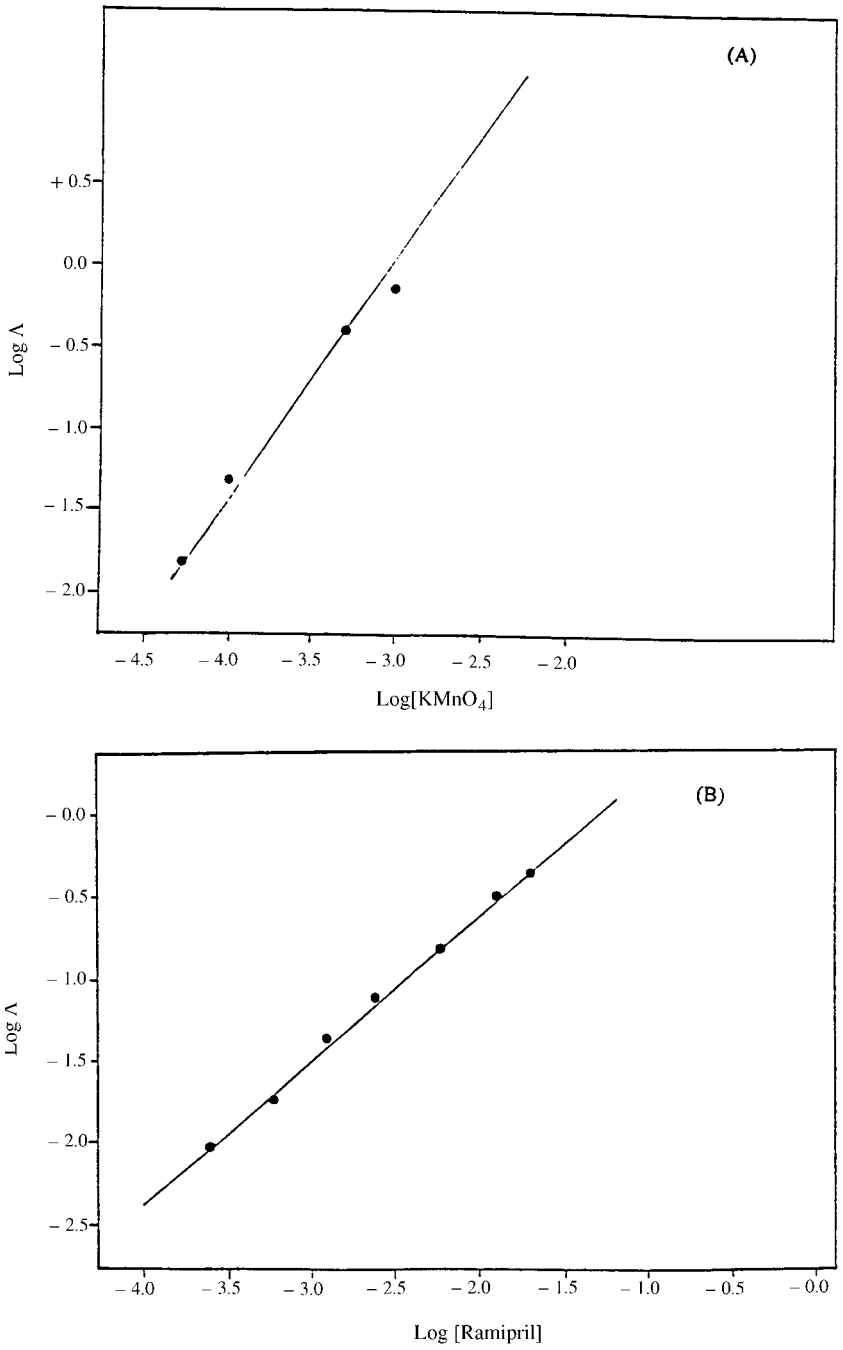

Figure 2. Bent and French Plots for the Molar Ratio: (A) log A *v*s log [$KMnO_4$] with [Ramipril] kept at $2.4 \times 105^{\Sigma 5}$ M. (B) log A *v*s log [Ramipril] with [$KMnO_4$] kept at $6 \times 10^{\Sigma 4}$ M.

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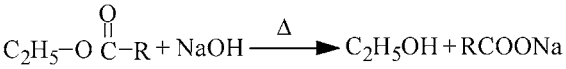
Mechanism of the Reaction

The stoichiometry of the reaction was studied adopting the Bent and French method (13). The absorbance of the reaction product was measured in presence of both excess $KMnO_4$ and in presence of excess ramipril. A plot of log absorbance versus log [$KMnO_4$] and [ramipril] gave values of the slopes of 1.85 and 0.86, respectively (Figs. 2A and 2B). Hence it is concluded that the molar reactivity of the reaction is 1.85/0.86 = 2.15, i.e., the reaction proceeds in the ratio of 2:1. Therefore, the following pathway is proposed as the reaction mechanism: Ramipril-being ethyl ester of benzatepine acetic acid derivative- is proposed to be hydrolysed upon heating in the presence of sodium hydroxide as follows:



$$C_2H_5{-}O\overset{\overset{O}{\|}}{C}{-}R + NaOH \xrightarrow{\Delta} C_2H_5OH + RCOONa$$

The resulting ethyl alcohol is oxidised by $KMnO_4/OH^-$ whereby the permanganate is reduced to the manganate ion, which is the colored species.

$$C_2H_5OH + 2MnO_4^- + 2\ OH^- \longrightarrow CH_3CHO + 2\ MnO_4^{-2} + 2\ H_2O$$

The above proposed mechanism was proved by treating ethanol with $KMnO_4$/NaOH under the same conditions whereby the bluish green-colored product with same absorption spectrum, was produced.

CONCLUSION

The present method is superior to other reported methods by showing good sensitivity, and low LOD (1.2×10^{-7} M). In addition to competitive precision and sensitivity, the new proposed procedure shows a relevant selectivity allowing analysis without separation steps, providing a suitable alternative to the many chromatographic procedures proposed.

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