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Spectrophotometric determination of benazepril in tablets

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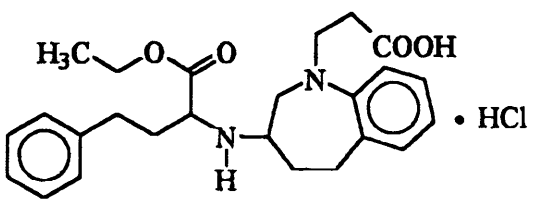
Abstract

A simple and sensitive spectrophotometric method has been developed for the determination of benazepril HCl in pharmaceutical formulations. The method is based on the reaction of the drug with potassium permanganate in the presence of sodium hydroxide to produce a bluish–green colored species measurable at 609.4 nm. The absorbance–concentration plot is linear over the range 1–8 μg ml^{-1} with minimum detectability of 0.1 μg ml^{-1} (2.17×10^{-7} M). The molar absorptivity was 4.07×10^4 l mol^{-1} cm^{-1} with correlation coefficient ($n = 6$) of 0.9991. The different experimental parameters affecting the development and stability of the color were studied carefully and optimized. The proposed method was applied successfully to the determination of benazepril in its dosage forms, the percentage recoveries $\pm$SD ($n = 9$) were 99.79 ± 1.40 and 100.50 ± 1.48 for tablets containing 10 and 20 mg, respectively. The results obtained were in good agreement with those obtained using a reference spectrophotometric method. The proposed method could be applied to the determination of benazepril in presence of the co-formulated drug, hydrochlorothiazide. A proposal of the reaction pathway was presented.

Keywords: Benazepril; Potassium permanganate; Pharmaceutical preparations; Spectroscopy; Pharmaceutical analysis

1. Introduction

Benazepril hydrochloride [(3*S*)-3-[(1*S*)-1-ethoxycarbonyl-3-phenylpropylamino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepin-1-yl] acetic acid, hydrochloride, is an angiotensin-converting enzyme (ACE) inhibitor. It acts on the renin–angiotensin aldosterone system. It inhibits the conversion of the inactive angiotensin I to the highly potent vasoconstrictor, angiotensin II. Also, it reduces the degradation of bradykinin [1]. It is used in the treatment of all forms of hypertension, congestive heart failure and postmyocardial infarction.



Benazepril Hydrochloride

Literature survey reveals few analytical methods for the determination of benazepril in pharmaceutical preparations and biological fluids, viz. spectrophotometry [2,3], HPLC [4,5], GC-MS [6,7] and enzymatic method [8]. All these methods are either insufficiently sensitive [2–5] or tedious and require highly sophisticated instrumentation and time-consuming [6–8]. Benazepril, like other ACE inhibitors, exhibits a weak benzene chromophore, so, it has low absorption in the UV region with absence of characteristic λ_{max}; as a consequence, poor sensitivity will be achieved by direct measurement of the absorbance. This has led us to develop a simple and sensitive spectrophotometric method for the determination of benazepril HCl in pharmaceutical preparations, based on the reaction of the drug with $KMnO_4$ in alkaline medium.

2. Experimental

2.1. Apparatus

1. Shimadzu UV-160 IPC, UV–Vis spectrophotometer, Japan.

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2. Rotatory evaporator: Rotavap. 110, Buchi, Brinkmann, Switzerland.

2.2. Materials and reagents

1. Benazepril HCl pure sample was kindly provided by Novartis Pharma, Basle, Switzerland (batch no. 79664). Tablets containing benazepril HCl: Cibacen 10, labeled to contain 10 mg of benazepril HCl per tablet (batch no. 010900), Cibacen 20, labeled to contain 20 mg of benazepril HCl per tablet (batch no. 002800) and Cibadrex tablets labeled to contain 10 mg of benazepril HCl and 12.5 mg of hydrochlorothiazide (batch no. 008) were obtained from commercial sources.
2. Potassium permanganate (Riedel-de Haen, Germany). A 0.05 M aqueous solution.
3. Sodium hydroxide (Winlab, UK) 5 M stock aqueous solution.
4. Standard solution of benazepril HCl was prepared by dissolving 10.0 mg in 100 ml of water and further diluting as appropriate.

2.3. Procedures

2.3.1. Recommended procedure and calibration curve

Transfer 1 ml of 0.05 M $KMnO_4$ and 7.5 ± 0.5 ml of 5 M NaOH into a series of 50-ml volumetric flasks.

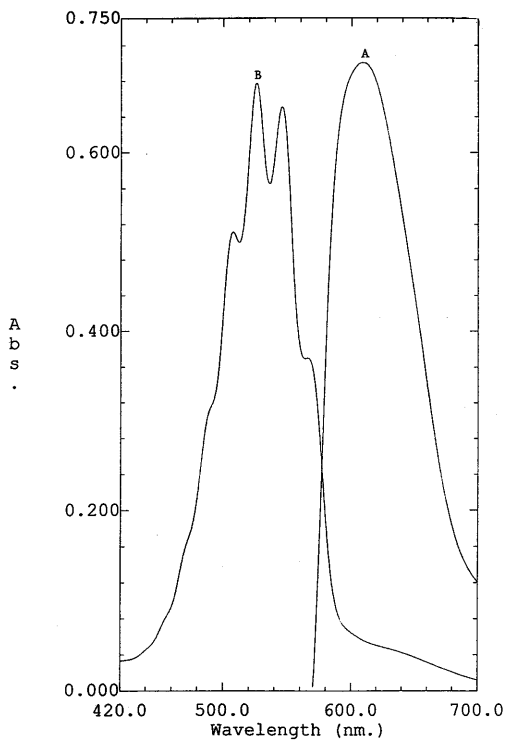


Fig. 1. Absorption spectrum of the reaction product of benazepril HCl (8.0 μg ml−1) with $KMnO_4$–NaOH system. (A) Reaction product. (B) $KMnO_4$: 5×10^{-5} M.

Add aliquot volumes of benazepril HCl solution so that the final concentration is in the range of 1–8 μg ml^{-1}, shake and complete to the mark with distilled water. Heat for 30 ± 2 min in a thermostatically-controlled water bath at 100°C, then cool. Measure the absorbance against a reagent blank at 609.4 nm. Plot the absorbance versus the final concentration to get the calibration curve. A calibration curve obtained at room temperature is simultaneously constructed. Alternatively, derive the regression equation.

2.3.2. Procedure for the tablets

Weigh and pulverize ten tablets. Transfer a portion of the powder equivalent to 10.0 mg of benazepril HCl into a small beaker. Shake with 2×30 ml of acetone for 10 min. then filter into a conical flask. Wash the beaker and filter with few ml of acetone and pass the washings to the same flask. Evaporate the acetone using a rotatory evaporator at 55°C till dryness. Dissolve the residue in 3×30 ml of water and filter, if necessary, into 100-ml volumetric flask, then complete to the mark with water. Proceed as described under Section 2.3.1. Determination of the nominal content of the tablets either form the calibration curve or using the regression equation.

3. Results and discussion

3.1. Study of the experimental conditions

Benazepril was found to react with $KMnO_4$ in alkaline medium producing a bluish–green color peaking at 609.4 nm (Fig. 1). The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development and its stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. The factors include, concentration of the reagents ($KMnO_4$ and NaOH), temperature, time of heating, sensitizers, surfactants and type of alkalies.

The influence of the concentration of $KMnO_4$ was studied using different concentrations. It was found that the reaction with the drug started at a concentration of 5×10^{-5} M in alkaline medium. Increasing the concentration of $KMnO_4$ was found to produce a proportional increase in absorbance up to 2.5×10^{-3} M. It was noticed that upon using concentrations between 1.5×10^{-3} and 2.5×10^{-3} M, the absorbance of the reaction product decreased slowly, probably due to a consecutive reaction involving the reaction product, therefore, 1×10^{-3} M was chosen as optimum concentration of potassium permanganate (Fig. 2). Complete reaction between $KMnO_4$ and benazepril takes place only in alkaline medium. Different concentrations of NaOH ranging from 0.05–1.5 M were tested. It was

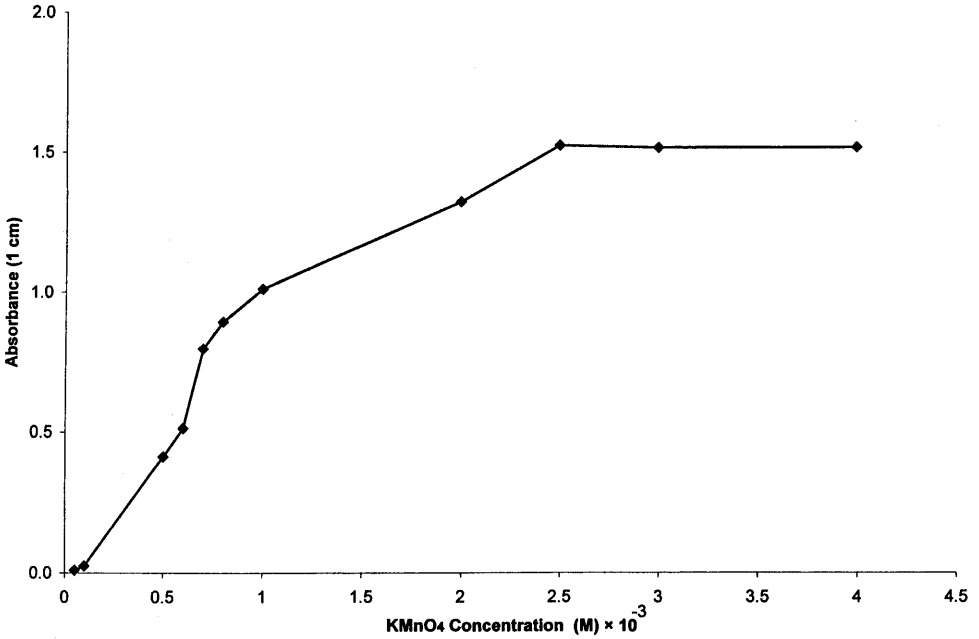


Fig. 2. Effect of the concentration of potassium permanganate on the reaction with benazepril HCl (16 μg ml^{-1}).

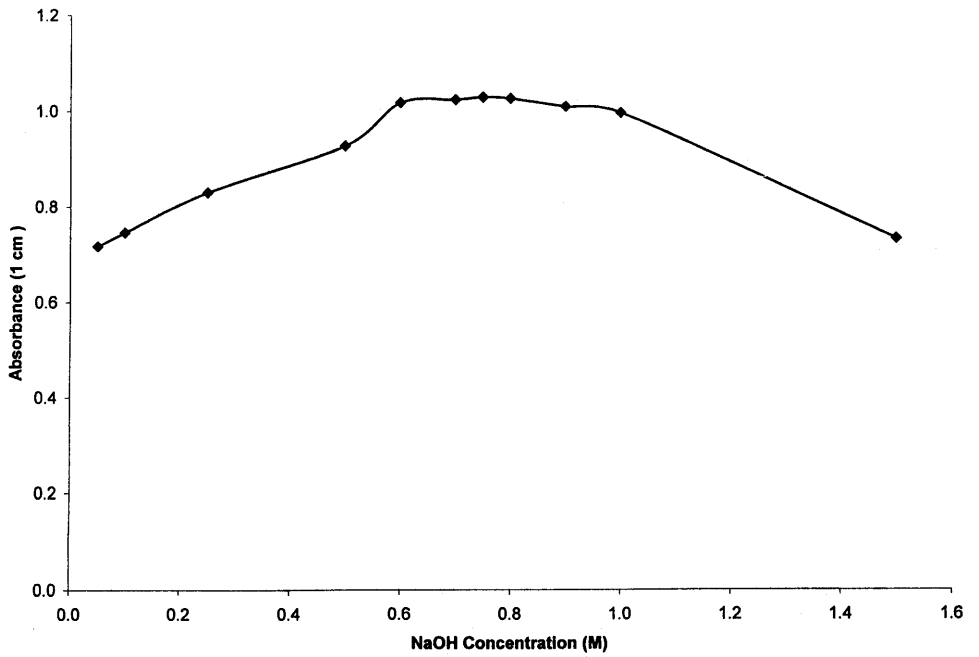


Fig. 3. Effect of concentration of sodium hydroxide on the reaction of benazepril HCl (16 μg ml^{-1}).

found that 0.75 ± 0.5 M gave the highest absorbance reading. Increasing concentration of NaOH over 0.75 ± 0.5 M would decrease the absorbance reading, so, 0.75 M was chosen as the optimum concentration of NaOH (Fig. 3). Other alkalies, such as KOH and NH_4OH with the same concentration were also tested to identify the best alkaline medium. However, their effect on color development was less than that of NaOH, therefore, the latter was used during the study.

Complete color formation was achieved by heating the resulting solution in a thermostatically-controlled water bath. Different temperature settings were used with constant heating time. Increasing temperature of the water bath was found to produce a proportional increase in absorbance up to boiling, therefore, 100°C was chosen as the optimum temperature (Fig. 4). The time of heating is an essential part of the experiment. Different time intervals were tested to as-

certain the time after which the solution attains its highest absorbance. It was found that after 30 ± 2 min, the resulting product reaches the highest absorbance (Fig. 5).

Different sensitizers (quinine, cyclohexane-diol, fluorescein and rhodamine-B), at concentrations of 5 μg ml^{-1} were tested by adding to the reactants mixture before heating. Outstanding inhibitory effects were observed as these sensitizers reacted strongly with the $KMnO_4$–NaOH system (Table 1). In the same manner, the effect of surfactants on the color development was studied. Different surfactants (cetrimide, gelatin and sodium lauryl sulfate) at three different concentrations, 2.5, 7.5 and 15 μg ml^{-1}, were tested by adding to the reactant mixture prior to heating. All tested surfactants reacted strongly with the $KMnO_4$–NaOH system with inhibitory effect, as evident from the low absorbance readings (Table 2). Potassium permanganate is con

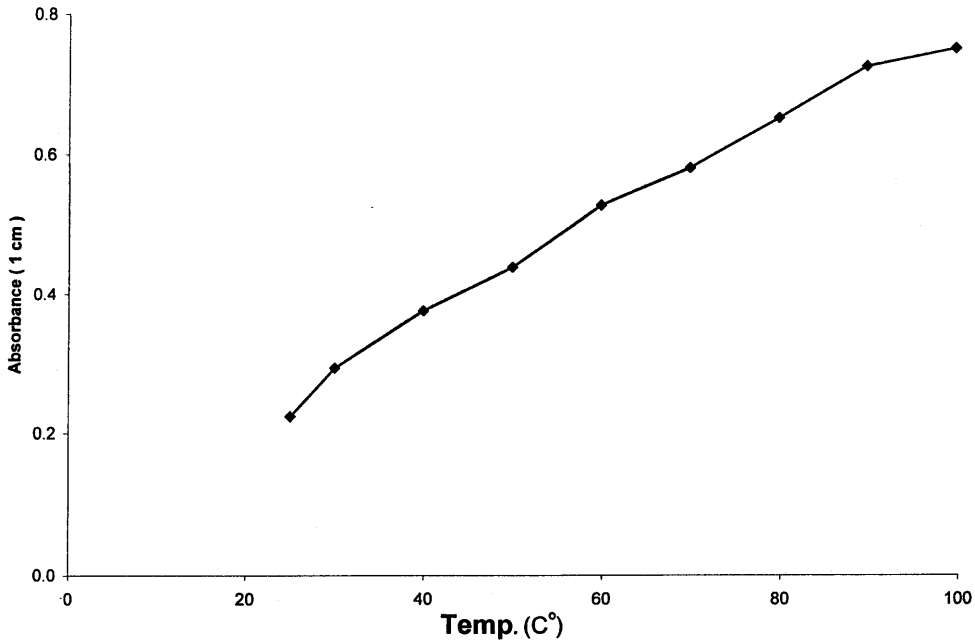


Fig. 4. Effect of temperature on the reaction of benazepril HCl (8.0 μg ml^{-1}) with $KMnO_4$–NaOH system.

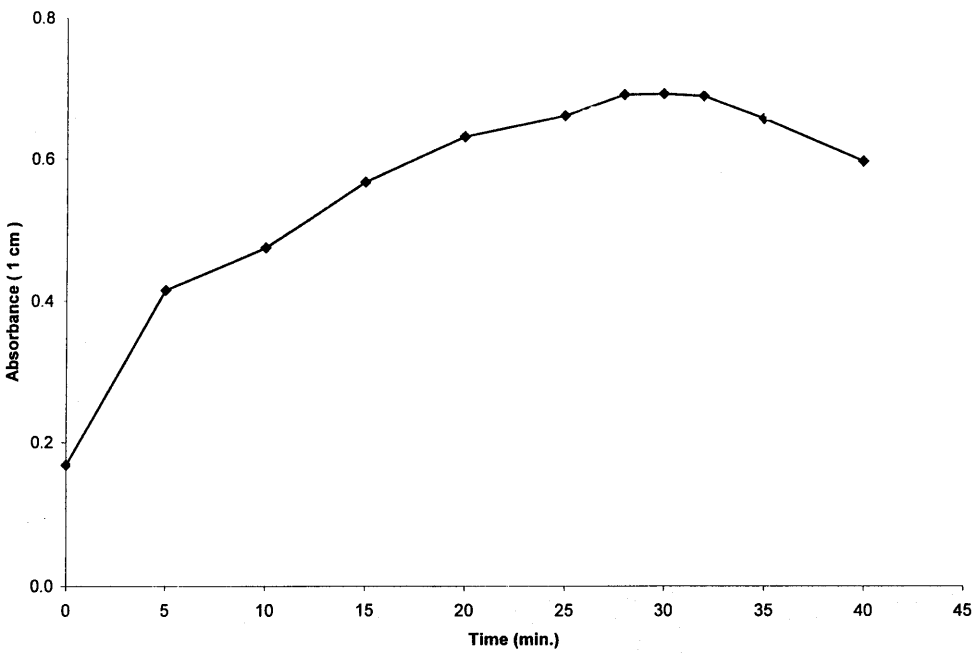


Fig. 5. Effect of time of heating on the reaction of benazepril HCl (8.0 μg ml^{-1}) with $KMnO_4$–NaOH system.

Table 1
Effect of sensitizers on the performance of the proposed method

Senzitizer	Absorbance
No sensitizer	0.632
Quinine	0.142
Cyclohexane-diol	0.071
Fluorescein	Precipitate
Rhodamine-B	0.103

Table 2
Effect of surfactants on the performance of the proposed method

Surfactant	Concentration (μg ml^{-1})	Absorbance
No surfactant	7.5	0.653
Cetrimide	2.5	0.501
Sodium lauryl sulfate	2.5	0.620
Gelatin	2.5	0.603
Cetrimide	7.5	0.252
Sodium lauryl sulfate	7.5	0.540
Gelatin	7.5	0.502
Cetrimide	15	0.112
Sodium lauryl sulfate	15	0.236
Gelatin	15	0.169

sumed by the surfactants, being reduced to reduction products other than the measured species.

3.2. Analytical performance

The absorbance–concentration plot was found to be linear over the range 1–8 μg ml^{-1}. Linear regression analysis of the data ($n = 6$) gave the following equation:

$$A = 0.015 + 11.55C \quad (R = 0.9991)$$

where A is the absorbance in 1-cm cell, C is the concentration of the drug in μg ml^{-1}.

The apparent molar absorptivity was found to be 4.07×10^4 l mol^{-1} cm^{-1} and $A^{1\%}_{1cm}$was about 885. Statistical evaluation [9] of the regression line gave the following values: standard deviation of the residuals ($S_{y/x}$) is 0.012; standard deviation of the intercept (S_a) is 9.6×10^{-4}; standard deviation of the slope is 2.1×10^{-3} while the percentage error is 1.01%. These small figures point out to the high precision of the proposed method.

To assess the concentration of free ethanol probably, to be present as a result of the degradation of benazepril, a calibration curve conducted at room temperature is constructed simultaneously with the original calibration curve as shown in Fig. 6. It is evident that, the amount of free ethanol (measure of the degradation of benazepril) is negligible.

The proposed method was applied to the determination of pure sample of benazepril HCl. The results obtained by the proposed method were compared with those given by a reference spectrophotometric method [10]. Statistical analysis [9] of the results obtained by both methods using the Student's t-test and variance ratio, F-test, reveals no significant difference in the performance of the two methods regarding accuracy and precision, respectively (Table 3).

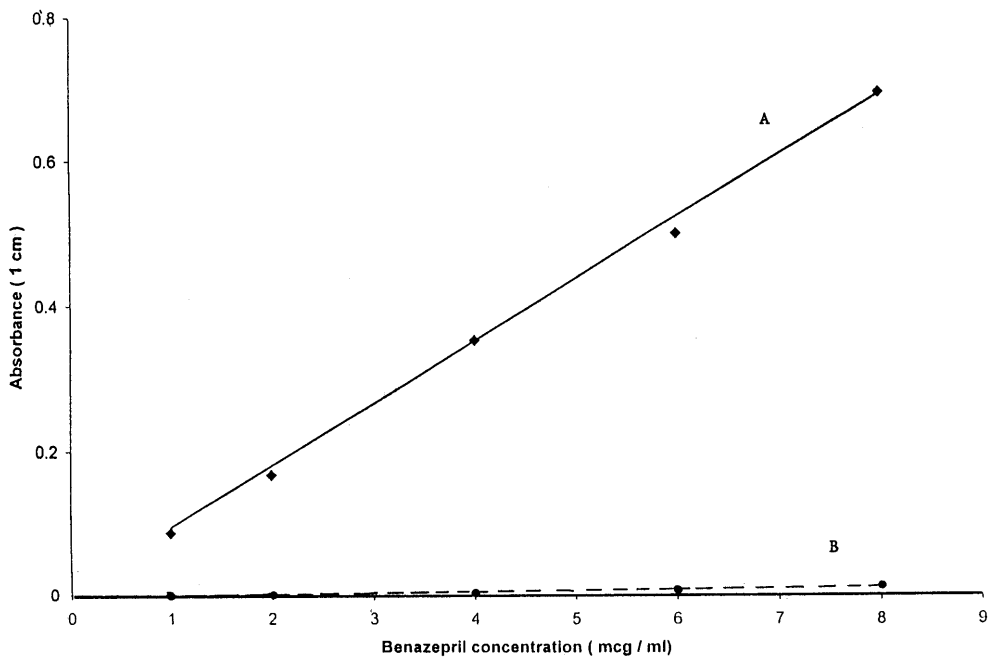


Fig. 6. Calibration curve of the reaction product of benazepril HCl and $KMnO_4$–NaOH system at 609.4 nm. (A) Obtained under the described reaction conditions. (B) Obtained at room temperature.

Table 3
Application of the proposed method and reference method to the determination of benazepril in pure sample [a]

Amount taken (μg ml^{-1})	Amount found (μg ml^{-1})	% Recovery	Reference method [10]
2.0	1.98	99.00	99.60
4.0	3.98	99.50	100.50
6.0	6.01	100.22	98.50
Mean ± SD		99.57 ± 0.61	99.87 ± 0.55
t		0.80 (2.78)	
F		7.12 (19.0)	

[a] Each result is the average of three separate determinations. The figures in parenthesis are the tabulated values of *t* and *F* at $P = 0.05$.

Table 4
Application of the proposed method and reference method to the determination of benazepril in dosage forms [a]

Preparation	Amount taken (μg ml^{-1})	Amount found (μg ml^{-1})	% Recovery	Reference method [10]
Cibacen 10® tablets (benazepril HCl-10 mg per tablet) [b]	2.0	2.04	101.9	98.94
	4.0	4.00	100.0	101.41
	6.0	5.98	99.6	99.03
Mean ± SD			100.50 ± 1.23	99.79 ± 1.40
t			0.79 (2.78)	
F			1.63 (19.0)	
Cibacen 20® tablets (benazepril HCl-20 mg per tablet) [b]	5.0	4.92	98.40	99.17
	6.0	6.06	101.0	100.23
	7.0	7.01	100.14	100.27
Mean ± SD			99.85 ± 1.32	99.89 ± 0.62
t			0.08 (2.78)	
F			4.45 (19.0)	
Cibadrex® tablets (benazepril 10 mg + hydrochlorothiazide 12.5 mg per tablet) [b]	2.0	1.98	99.0	99.31
	3.0	3.02	100.67	100.46
	4.0	3.98	99.5	98.01
Mean ± SD			99.72 ± 0.86	99.46 ± 0.93
t			0.35 (2.78)	
F			1.19 (19.0)	

[a] The figures in parenthesis are the tabulated values of *t* and *F* at $P = 0.05$. Each result is the average of three separate determinations.
[b] Product of Novartis Pharma, Basle, Switzerland.

3.3. Pharmaceutical applications

It was found that, when the proposed method was applied to the determination of benazepril in pharmaceutical preparations, a precipitate was obtained after heating the resulting solution; this might be due to the interaction of the excipients in the formulation (especially lactose and hydroxymethylcellulose which contain alcohol groups), with $KMnO_4$–^-OH system. Therefore, the tablet had to be extracted with another solvent, such as acetone. The tablet was extracted using acetone and the latter was evaporated using a rotatory evaporator till dryness. Satisfactory percentage recoveries were obtained (Table 3). The proposed method was also applied to the determination of benazepril in combination with hydrochlorothiazide, which is frequently co-formulated with benazepril in a medicinally-recommended ratio of 4:5. High percentage recoveries, around 125% due to interference from excipients, were obtained. To eliminate this interference, the standard addition technique was used. Table 4 shows the percentage recoveries obtained after applying the standard addition method. The results obtained were compared with those obtained from a reference method [10]. Statistical analysis of the results using Student's *t*-test and variance ratio *F*-test, revealed no significant difference between the two methods at the 95% confidence level regarding accuracy and precision, respectively.

3.4. Mechanism of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method [11]. The absorbance of the reaction product was measured in the presence of excess of both $KMnO_4$ and benazepril. A plot of log absorbance versus log[$KMnO_4$] and [benazepril] gave straight lines, the values of the slopes of

1.80 and 1.0, respectively (Fig. 7). Hence, it is concluded that, the molar reactivity of the reaction is 1.80/1, i.e. the reaction proceeds in the ratio of 2:1. Therefore, the following pathway is proposed as the reaction mechanism. Benazepril-being ethyl ester of benzatepine acetic acid derivative, is proposed to be hydrolyzed upon heating in the presence of sodium hydroxide as follows:

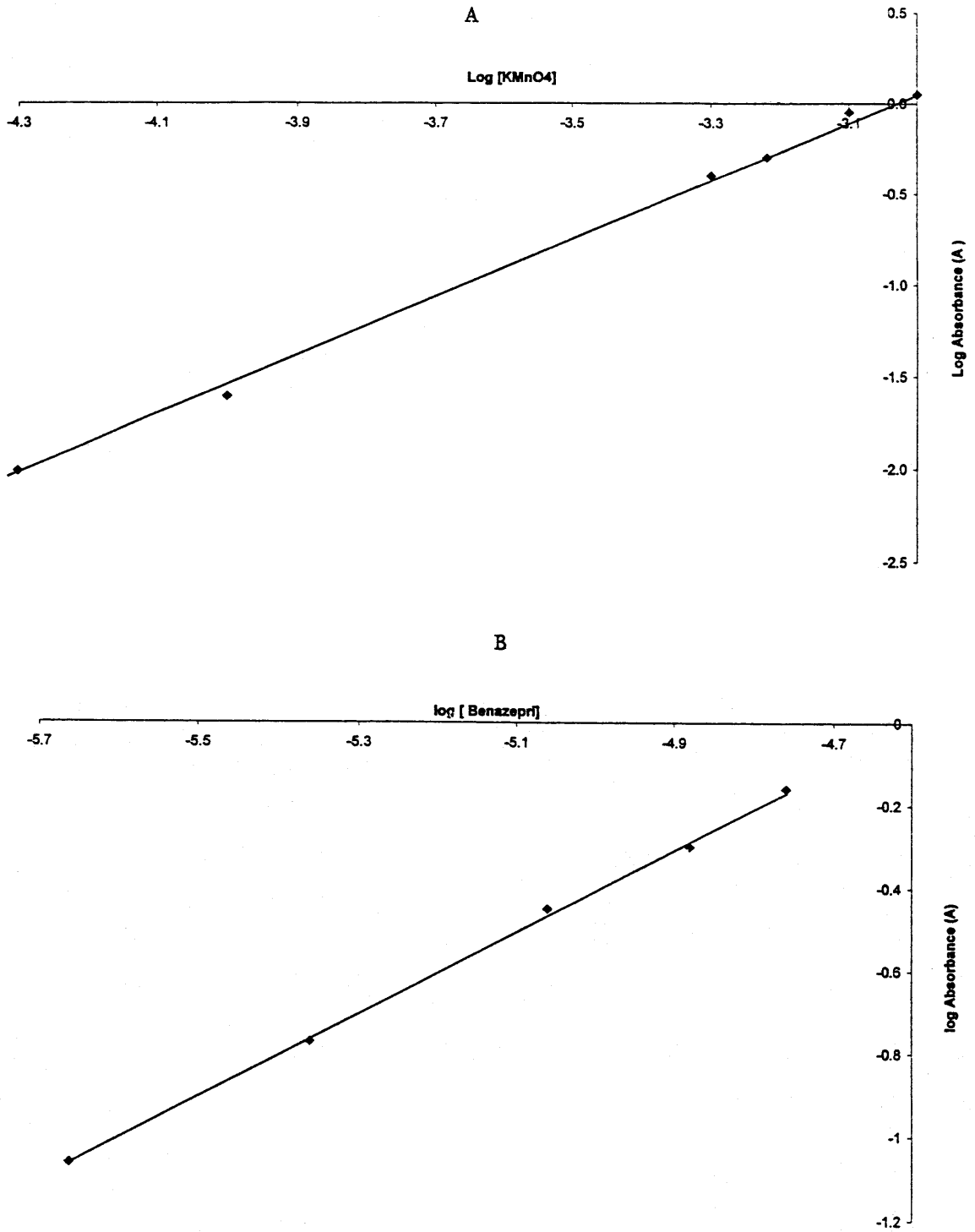
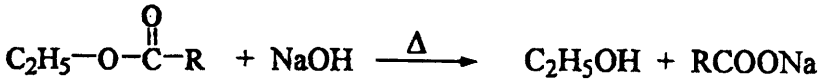


Fig. 7. Limiting logarithmic plots for the molar ratio. (A) Log A vs. log[$KMnO_4$] with [Benazepril] kept at 1.7×10^{-5} M. (B) Log A vs. log[Benazepril] with [$KMnO_4$] kept at 1×10^{-3} M.



The resulting ethyl alcohol is oxidized by ($KMnO_4$–NaOH), whereby, the permanganate is reduced to the manganate ion, which is the colored species.

$$C_2H_5OH + 2MnO_4^- + 2OH^-$$

$$\rightarrow CH_3CHO + 2MnO_4^{2-} + 2H_2O$$

The above proposed reaction pathway was confirmed by treating ethanol with $KMnO_4$–NaOH under the same reaction conditions whereby a bluish–green colored reaction product with the same absorption spectrum, was obtained.

4. Conclusions

A simple and sensitive method has been developed for the determination of benazepril HCl in pharmaceutical preparations. The method is more sensitive than other reported spectrophotometric methods. It can measure as low as 1 μg ml^{-1} with good accuracy. The minimum detectability is 0.1 μg ml^{-1} ($\sim 2.17 \times 10^{-7}$ M). The proposed method can be used for routine quality control studies.

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