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Studies on Liquid-Liquid Extraction of Lanthanum from Sulfuric Acid Solutions Using PRIMENE-JMT

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Summary: Rare earth elements have been increasingly used in the field of chemical engineering, metallurgy, nuclear energy, optical, magnetic and laser materials, high temperature superconductors and secondary batteries, red phosphorous. Lanthanum, one of the most abundant of the lanthanides, is an important element of mischmetal and hydrogen-absorbing alloy. The RE elements occur together in nature in some minerals like monazite, bastaensite, xenotime and others, the high value of these elements depends on their effective separation into high purity compounds. This paper is concerned firstly with the achieved optimum conditions for the extraction of lanthanum from sulfuric acid solution using primene-JMT/kerosene. The extractant concentration 0.1M primene-JMT/kerosene at an O/A phase ratio of 1:1 and a contact time of 5 minutes gives extraction efficiency 94.6 % at pH 1.1. The best diluent to use is kerosene at room temperature. In the stripping experiments, 6M nitric acid was found to be the best stripping reagent. Finally, an application of the proposed method La_2O_3 92.23% is obtained from a rare earth concentrate of 12.42 % La_2O_3 prepared industrially from Egyptian chemical processing plant of monazite sand of the Nuclear Materials Authority

Introduction

There has been a significant increase in the use and demand for various rare earth element compounds, particularly for high purity rare earth elements. REE's have been used in the field of chemical engineering, metallurgy, nuclear energy, optical, magnetic, luminescence and laser materials, high-temperature superconductors and secondary batteries, catalysis, red phosphors, among others [(1)]. Lanthanum, one of the most abundant elements of the lanthanides, is an important element of mischmetal and hydrogen-absorbing alloy [(1)]. The REE's occur together in nature in some minerals like bastaenasite, monazite, xenotime and others [(2)]. The high value of these elements depends on their effective separation into high purity compounds. The separation of the natural RE mixtures into the individual elements is very difficult to achieve, due to very low separation factors involving the adjacent RE elements. Among the trivalent lanthanides, Ce, and Eu can be separated through changes in their oxidations state. The Ce(III) is oxidized to Ce(IV) and the Eu(III) is reduced to Eu(II) [(3,4)]. The separation of the other RE elements, usually carried out by solvent extraction or ion

exchange, is based on systematic differences in their basicity, which decreases from La to Lu [2]. Generally, a mixture of rare earths is first separated into groups of light rare earths (La, Ce, Pr, Nd), middle rare earths (Sm, Eu, Gd) and heavy rare earths (Tb, Dy, Ho, Er, Tm, Yb, Lu, Sc, Y). This separation is favoured by the relatively higher separation factor of the adjacent elements within a lanthanides group. The separation into these three groups is usually accomplished by solvent extraction, using di-2-ethylhexylphosphoric acid (DEHPA) as the extractant [2,5]. The extraction behaviour of rare earths has been studied since 1950s. There are several reports on the separation of rare earth elements in different media and extractants, such as phosphonic and phosphinic acids with 2-ethylhexylphosphonic acid mono-2-ethylhexyl ester(HEHEHP) and DEHPA being the most used [6,7], neutral phosphate, such as tri-n-butylphosphate (TBP) and tri-n-octylphosphine oxide (TOPO) [8,9], carboxylic acid derivative [10], amines [11], and others. The majority of these studies focus on the fundamentals, which include the determination of separation and extraction parameters, evaluation of kinetics and reaction mechanism, among others. There are no reports about using primene-JMT as extractant for individual rare earth elements except for yttrium [12]. In this work the use of primene-JMT for the separation of lanthanum from light rare earth sulfate was investigated.

Experimental

Reagents and analysis

Primene-JMT (trialkyl methylamine) was obtained from Rohm& Hass Company, England. Odorless kerosene (non-aromatic) was obtained from Misr Petroleum Company, Egypt. Stock solutions of lanthanum were prepared from its oxide (La_2O_3) Fluka in concentrated sulfuric acid and diluted with distilled water. Arsenazo III is analytical grade purity from Fluka. All other chemicals were Prolabo products and were used as received.

Lanthanum was determined spectrophotometrically using the colorimetric determination "Metertech Inc" model SP-8001, UV-Visible spectrophotometer. The other lanthanides concentration was determined by Atomic Absorption Spectroscopy (AAS) PERKIN-ELMER AAnalyst100. All measurements were carried out at laboratory temperature.

.Batch experiments

The effect of the main parameters such as aqueous phase composition, lanthanum concentration, equilibration time, diluents, and nature and concentration of primene-JMT on the lanthanum extraction. The experiments were carried out in mechanically agitated beakers containing equal volumes (10 ml) of the aqueous and organic phases containing the desired concentrations of lanthanum (5.22×10^{-3} mol.L) and extractant, at (25 ± 1) ºC. Following contact; the phases were separated by means of a separation funnel. The metal concentration in the aqueous phase was determined spectrophotometrically using arsenazo III [(13)].

Results and discussion

Effect of different concentrations of primene-JMT

Firstly the solvent was equilibrated by contacting it with sulfuric acid solution for 10 minutes. To 10 ml of the sulfate liquor of lanthanum, 10 ml of a solution of primene-JMT in kerosene was added with concentrations ranging from 0.02 to 0.15M. The solution was then shaken for 10 minutes at room temperature to attain equilibrium state. It is clear from Fig.1 that the distribution coefficient increases from 7.5 to 12.7 by increasing the primene-JMT from 0.025M to 0.1M in kerosene, after it remains constant. Accordingly 0.1M solution of primene-JMT in kerosene is considered as an optimum value

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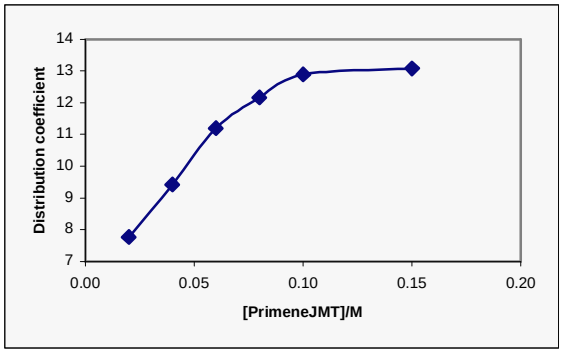

Fig.1 Effect of different concentrations of primene-JMT on the distribution coefficient of lanthanum

Effect of equilibration time on the distribution coefficient of lanthanum

The effect of contact time on the attainment of an equilibrium state was studied at intervals between 1–30 minutes, while the other factors were kept as previously mentioned. The results obtained are shown in Fig.2. It is obvious that contact time of 5 minutes is quite adequate for efficient lanthanum distribution coefficient and by

increasing the shaking time the distribution coefficient is decreased, and this behaviour may be due to the entrainment of some organic phase droplets into aqueous phase, which causes decrease in the extractive power of extractant.

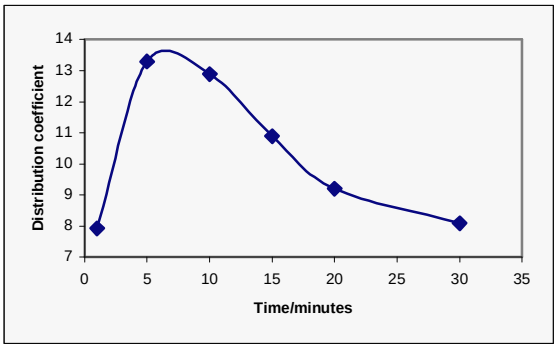

Fig.2 Effect of contact time on the distribution coefficient of lanthanum

Effect of pH on the distribution coefficient of lanthanum

The distribution coefficient has been studied by varying the pH values of the sulfate liquor from 0.7 to 5.9, using either sulfuric acid or sodium hydroxide. Other factors were fixed at 1:1 (v/v) organic to aqueous phase ratio, 0.1M primene-JMT in kerosene, contact time 5 minutes and the experiments were carried out at room temperature. The results obtained are shown in Fig. 3. It is clear that the pH 1.1 of the sulfate liquor can be taken as an optimum pH value. Fortunately, that is the same pH of the parent sulfate liquor under examination. The reason for the decrease in lanthanum distribution coefficient may be result from the formation of non-extractable lanthanum species as a result of complication with components of aqueous phase. This occurs, in sulfate media due to equilibria between SO_4^{2-}, HSO_4^-, and H_2SO_4 resulting in the formation of lanthanum complexes with these anions.

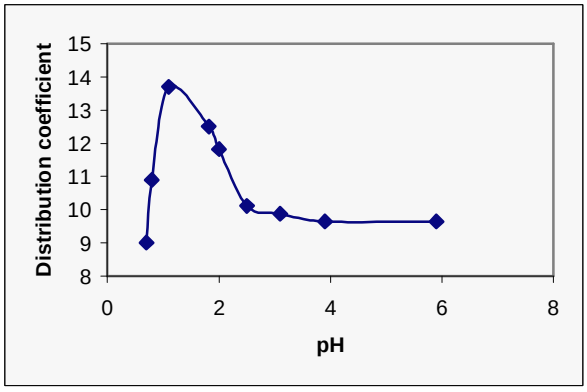

Fig.3 Effect of pH on the distribution coefficient of lanthanum.

Effect of diluent on the distribution coefficient of lanthanum

The distribution coefficient of lanthanum has been studied using different organic diluents, namely chloroform, carbon tetrachloride, toluene, benzene, *o*-xylene, and kerosene. The other factors were fixed at the values at which maximum distribution coefficient occurred i.e. 1:1 (v/v) organic to aqueous phase ratio, room temperature, 0.1M primene-JMT in all diluents, and a contact time of 5 minutes. Results are presented in Table 1. Kerosene was preferred as the diluent for further lanthanum(III) extraction studies.

Table (1): Extraction of lanthanum(III) by primene-JMT as a function of diluents

Diluents	Dielectric constant	Distribution coefficient	Extraction percent, [%]
Benzene	2.3	2.93	75.3
Toluene	2.23	2.62	72.36
O-xylene	2.28	2.03	66.93
Chloroform	5.1	5.35	84.24
Carbon tetrachloride	2.23	3.29	76.69
Kerosene	2	13.89	94.52

0.1M Primene-JMT; Phase ratio (Org:Aq) 1:1; time 5 min; room temperature.

Effect of stripping agents

Stripping is the reverse of the extraction, so it should be promoted by these factors that affect extraction negatively. Lanthanum(III) stripping from loaded organic solvent, were investigated using various stripping agents, such as HCl, H_2SO_4 and HNO_3, in the range 0.5–9M and O:A ratio of 1:1. The results are presented in Fig.4. From the results, it is clear that 6M HNO_3 is the most effective acid for the quantitative stripping of lanthanum(III). Variation of the stripping period showed that 5 minutes equilibration time were sufficient for quantitative recovery of lanthanum(III) from organic phase. Lanthanum ions can be stripped from lanthanum-loaded primene-JMT solution into 6M HNO_3, where 10 % oxalic acid was added gradually to the obtained strip liquor rich in lanthanum(III), and was left for 24 hours where lanthanum oxalates were obtained.

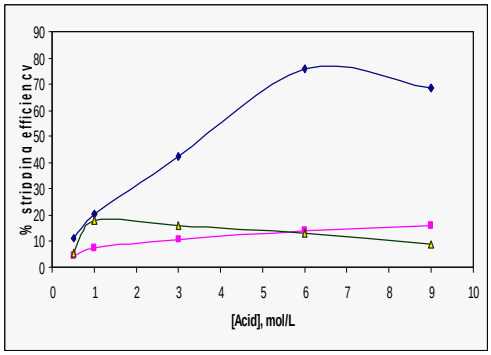

Fig. 4 Effect of different acidic stripping agents on stripping process

Natural ore application

In the application studies, the extraction of lanthanum from rare earth hydrous oxide concentrate, which reacted with sulfuric acid according to the following overall reactions:

$$RE_2O_3 + 3H_2SO_4 \rightarrow RE_2(SO_4)_3 + 3H_2O \quad (1)$$

$$2RE(OH)_3 + 3H_2SO_4 \rightarrow RE_2(SO_4)_3 + 6H_2O \quad (2)$$

5 g rare earth concentrate was heated with 1L 0.2M sulfuric acid at ≈ 80^{o}C for two hour to allow complete dissolution of all rare earth elements, and then filtrated. The filtrate contained most rare earth elements. Chemical analysis of rare earth concentrate was estimated by energy dispersive analysis with X-rays (EDAX).

For this work a REE concentrate containing (%) La_2O_3 12.42, Y_2O_3 2.16, Gd_2O_3 1.89, Eu_2O_3 1.71, Sm_2O_3 2.81, Nd_2O_3 16.91, Pr_2O_3 5.38, Ce_2O_3 31.29, and Na_2O 24.81 is used. The mentioned concentrate was produced industrially from the chemical treatment of monazite sand by Nuclear Materials Authority in Egypt. According to the high percent of Ce_2O_3 31.29 % which affect the liquid-liquid extraction of lanthanum, cerium should be first removed following the procedure given by Morais et al [(14)].

Liquid-liquid extraction of lanthanum rich fraction.

The obtained mother liquid solution is evaporated to such a consistency that it would solidify upon cooling. The almost quantity of lanthanum separates in the first fraction. After removal of cerium the mother liquid solution is evaporated and cooled many times to obtain fraction (I) which is rich in lanthanum starting from 12.42 % Ln_2O_3 and up to 33.55 % Ln_2O_3. The above fraction rich in lanthanum is dissolved in 0.2M sulfuric acid. Solvent extraction of lanthanum from sulfate liquor was carried out

in three stages with a 0.1M primene-JMT in kerosene at the obtained bench scale results, followed by stripping in two stages with 6M nitric acid at an organic aqueous phase ratio of 1:1. Recovery of the strip liquors was 92–93% for lanthanum. Lanthanum was precipitated as lanthanum oxalate and ignited to lanthanum oxide. The final product is a 92.23% Ln_2O_3 together with smaller amounts of 1.5% Pr_2O_3, 0.95% Gd_2O_3, 3.42 % Nd_2O_3 and 0.76% Sm_2O_3.

Conclusion

An application of the proposed method, a final lanthanum oxide of ca. 92.23% is obtained from a lanthanum rich rare earth concentrate of 12.42% La_2O_3 produced industrially from the chemical processing plant of monazite at the Nuclear Materials Authority, Egypt.

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Kietic Spectrophotometric Determination of some Angiotensin Converting Enzyme Inhibitors using Potassium Permanganate.

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Summary: A kinetic spectrophotometeric method has been developed for the determination of some angiotensin converting enzyme inhibitors (ACEI) namely, enalapril maleate (EN), ramipril (RM), lisinopri (LS) and fosinopril sodium (FOS) in the bulk powder and in pharmaceutical formulations. The procedure is based on kinetic investigation of the oxidation reaction of those drugs with alkaline potassium permanganate at room temperature at 610 nm. The fixed time method was utilized for constructing the calibration graph to determine the concentration of the drugs. The absorbance – concentration plot is rectilinear over the range of 5 - 40 µg ml^{-1} for EN, , 5-35 µg ml^{-1} for both RM and LS and 15-60 µg ml^{-1} for FOS with percentage mean recovery (99.81 ± 0.30, 99.95 ± 0.57, 99.60 ± 0.56 and 99.75 ± 0.24) respectively. Different experimental parameters affecting the development and stability of the colour were carefully studied and optimized. The results obtained were in good agreement with those obtained by potentiometric method for EN, RM and LS and HPLC method for FOS.

Key words: enalapri maleate, ramipril, lisinopril, fosinopril sodium, Potassium permanganate, spectrophotometric method, kinetic.

Introduction

Enalapril maleate1-[N-[(S)-1-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline 1-ethylester, ramipril; (2S,3aS,6aS)-1-[(S)-N-[(S)-1-carboxy-3-phenylpropyl]-alanyl]-octahydrocylopenta [b]pyrole-2-carboxylic acid 1-ethyl ester, lisinopril; (S)-1-[N^2-(1-Carboxy-3-phenylpropyl]-L-lysyl]-L-proline and fosinopril sodium (4S)-4-Cyclohexyl-1-[[[(RS)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)-phos-phinyl]acetyl]-L-Proline propionate, are an active inhibitors of angiotensin converting enzyme (ACE) with antihypertensive activity[1-2]. Those drugs used in the treatment of all forms of hypertension and heart failure [3].

Different methods published for their determination such as spectrophotometric methods [4-15], atomic absorption spectroscopy [16, 17], capillary electrophoresis [18 - 21], HPLC [22 - 30], GC [31 - 33], radioimmunoassay [34]

Kinetic- based methods of pharmaceutical analysis are not widely applied, although they offer the advantage of simplicity, low costs, and wide availability in quality control laboratories. Furthermore eliminating additives interferences, which probably affects other methods such as titrimetry and direct spectrophotometric methods [35]

In the present work, simple, valided kinetically based method is proposed for the determination of some ACEI namely EN, RM, LS and FOS by measuring the absorbance at 610 nm after their oxidation with alkaline $KMnO_4$ at ambient temperature 25 ± 5 C^0

Experimental

Apparatus: Shimadzu 1601 U V Spectrophotometer, Japan.

Materials and reagents*:*

All chemicals and reagents were of analytical grade

Enalapril maleate was assayed for purity according to the official method[1] to contain 99.84 ± 0.43 and Renetic tablets B.No (0311057) nominally containing enalapril maleate (5 mg) were manufactured and supplied by MSD, Co, Egypt, ramipril was assayed for purity according to the official method [1] to contain 99.91 ± 0.61 and tritace tablets batch number (14E03) nominally containing ramipril (5 mg) ,were manufactured and supplied by Hochest, Co, Egypt, lisinopril was assayed for purity according to the official method[1] to contain 99.17 ± 0.36and zystril tablets batch number (1204148) nominally containing lisinopril (10 mg), were manufactured and supplied by Sedico,Co, Egypt and Fosinopril sodium its purity was found to be 99.96 ± 0.39 according to the HPLC manufactural method[37] and Monopril tablets nominally containing fosinopril sodium (10 mg), were manufactured and supplied by Bristol-Mayers squib- Co, Egypt. Potassium permanganate (Merck, Germany), 8 mM (0.1264% w/ v) in water. Sodium hydroxide used as 0.5 M (EL-Nasr Chemical Company, Egypt)

Stock solutions:

Stock solutions of EN, RM, LS (0.01 %w/ v) and FOS solution (0.02 %w/v) were prepared by dissolving the specified amount of each drug in water. The solutions were stable for one week if kept in a refrigerator.

Procedures

For calibration graph

Aliquots of the stock solutions equivalent to (0.1 - 0.3 mg) of EN, (0.1 - 0.25 mg) of RM, (0.1 - 0.25 mg) of LS and (0.2 - 0.5 mg) of FOS, were transferred into 10-ml volumetric flasks, 1-ml of 0.5 M NaOH for EN and 1.5-ml for RM, while 2-ml for both LS and FOS were added to each flask, followed by the addition of 3-ml of 8 mM of potassium permanganate solution. The content of each flask were mixed and the volumes were completed to the mark with water and allowed to stand at ambient temperature for 30 min. for all drugs, except for FOS 25 min. were found to be adequate. For calibration curve the values of absorbance were plotted against the drug concentrations, alternatively, the corresponding regression equations were driven.

Procedure for tablets

For EN in Ezapril tablets and RM in Tritace tablets. Ten tablets were weighed and pulverized. A quantity of each powdered tablets equivalent to 10 mg of EN or RM were accurately weighed and transferred into 50 ml conical flask. The powdered tablets were dissolved in 30 ml of methanol using magnetic stirrer for 30 min., then the extracts were filtered and the residue washed with methanol (5 ml x 4), the combined filtrate and washing was evaporated and the residues were dissolved in water and transferred quantitatively into 50 ml volumetric flasks. The volumes were completed to the mark with water and the method proceeded as mentioned under 2.2.1

For LS in Zystril tablets. A quantity of powdered tablets equivalent to 10 mg was accurately weighed and transferred into 250 ml conical flask, then extracted with 200 ml of chloroform using magnetic stirrer for 30 min. The solution was filtered and the residue washed with chloroform (10 ml x 5). The filtrate was evaporated; the residue was dissolved in water and transferred quantitavely into 50-ml volumetric

flask. The volume was completed to the mark with water and proceeded as directed under 2.2.1

For FOS in Monopril tablets. A quantity of powdered tablets equivalent to10 mg FOS was accurately weighed, transferred into 50-ml conical flask, then extracted with 30 ml chloroform using magnetic stirrer for 10 min. The solution was filtered and washed with chloroform (5 ml x 4). The combined chloroformic extracts containing interfering povidone were rejected. The residue left on the filter paper was dried and extracted with 50 ml methanol for 30 min. using magnetic stirrer, the solution was filtered and washed with methanol (5 ml x 4). The extract was evaporated and the residue was dissolved in water into 50 ml volumetric flask. The volume was completed to the mark with water and proceeded as under 2.2.1

The procedures were repeated applying the standard addition technique. The recovered concentration of labeled and added EN, RM, LS and FOS were calculated

Results and Discussion

The UV absorption spectra of EN, RM, LS and FOS shown in [Figure-1] suffer from low intensity and lack of well-defined maxima, typical of unconjugated phenyl moiety; make it not useful for characterization or quantitative analysis of these compounds. This led to study the reaction of EN, RM, LS and FOS with alkaline $KMnO_4$ in an attempt to develop a simple and reliable method for their determination in bulk and in pharmaceutical formulations.

Kinetic and optimization of the reaction condition:

The reaction between EN, RM, LS, FOS and $KMnO_4$ in alkaline medium yields a colour due to the production of manganate ion, which absorbs at 610 nm [Figure-2]. As the intensity of the colour increases by time, it was deemed useful to elaborate kinetically determination of these drugs in bulk and in pharmaceutical preparations.

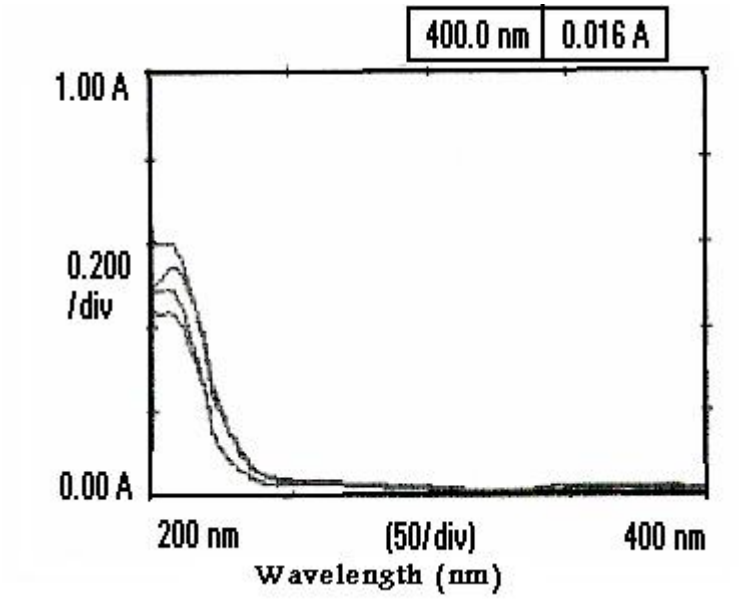

Figure 1: Absorbance spectra of EN(25 µg ml^{-1}), RM(25 µg ml^{-1}), LS(25 µg ml^{-1}) and FOS(25 µg ml^{-1}) in water

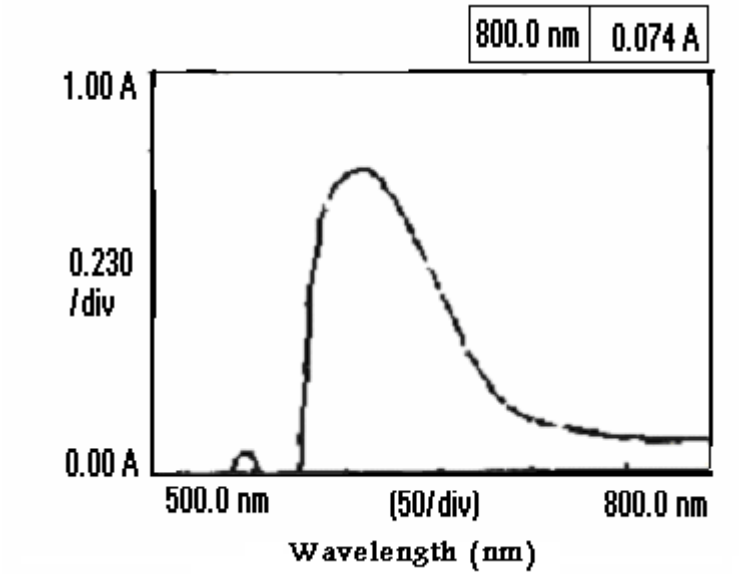

Figure 2: absorption spectrum of manganate ion obtained after the reaction of $KMnO_4$ with FOS (50 µg ml^{-1}) .

The reaction was studied under various conditions of reagent concentration, alkalinity and temperature; the effect of different solvents was also studied. Water was used to dissolve the drug since $KMnO_4$ oxidizes other solvents with the production of green manganate ions.

At room temperature the reaction rate increased substantially by time [Figure- 3], as revealed by the increase in the intensification of the developed colour and subsequent increase in the slopes of the calibration graphs [Table -1] indicating high analytical sensitivity. Heating the solution was found to increase the rate of the reaction but MnO_2 was precipitated. Therefore, room temperature was selected as the optimum temperature.

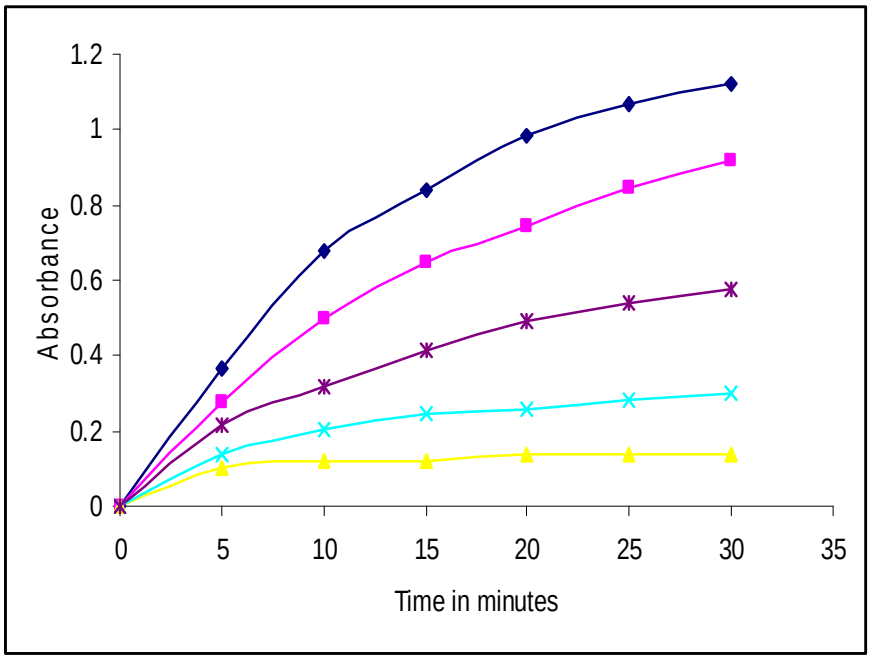

Figure 3: Absorbance versus time graph for the reaction between EN (5 – 40 µg ml^{-1}) and potassium permanganate

The reaction rate and maximum absorbance increased by time, and with increasing $KMnO_4$ concentration. It was found that 3 ml of 8 mM of $KMnO_4$ solution was adequate for the maximum absorbance.

The influence of NaOH concentration on the reaction rate was also studied. It was found that increasing the amount of 0.5 M NaOH would increase the absorbance of the reaction product up to 1 ml, 1.5 ml for EN and RM respectively and 2 ml for both LS and FOS, after which further increase does not affect the absorbance of the reaction product. Trials were made through oxidation with $KMnO_4$ in neutral and acidic medium, but no oxidation was observed.

The rate of the reaction was also found to be dependent on the drug concentration.

The rates were followed at room temperature with various concentrations of the cited drugs in the range of 5 – 40 µg ml^{-1} for EN, 5- 35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} of FOS keeping $KMnO_4$ and NaOH concentrations constant. The results in [Table- 1] clearly indicate that the reaction obeys the following equation:-

$$\text{Rate} = K' [\text{drug}]^{n} \quad (1)$$

Where K′ is the pseudo- order rate constant and n is the order of the reaction.

The rate of the reaction ($\Delta A/\Delta t$) may be estimated by the variable time method measurement [36], where A is the absorbance and t is the time in seconds. Taking logarithms of rates and concentrations [Table-2] and equation (1) is transformed into:

$$\log (\text{rate}) = \log \Delta A/\Delta t = \log K' + n \log [\text{drug}]. \quad (2)$$

For EN

Regression of log (rate) versus log [EN] gave the regression equation:

$\log \text{rate} = 0.817 \log C - 0.2056$ ($r = 0.9909$)

Hence $K' = 1.605$ Sec^{-1} and the reaction is first order $n = 0.817$ with respect to EN concentration.

For RM

Regression of log (rate) versus log [RM] gave the regression equation:

$\log \text{rate} = 1.3805 \log C + 2.2147$ ($r = 0.9955$).

Hence $K' = 163.95$ Sec^{-1} and the reaction is first order $n = 1.3805$ with respect to RM concentration.

For LS

Regression of log (rate) versus log [LS] gave the regression equation:

$\log \text{rate} = 0.8001 \log C - 0.3293$ ($r = 0.9948$).

Hence $K' = 2.135$ Sec^{-1} and the reaction is first order $n = 0.8001$ with respect to LS concentration.

Table 1: Calibration equations at different fixed time for EN, RM, LS and fos over the concentration ranges 5-40 µg ml^{-1}, 5-35 µg ml^{-1}, 5-35 µg ml^{-1} A and 15 – 60 µg ml^{-1} respectively

Time (min.)	Regression equations			
	EN	RM	LS	FOS
5	A = 0.0125 C + 0.0141 r = 0.9919	A = 0.00139 C + 0.041 r = 0.9932	A = 0.0173 C + 0.0393 r = 0.9968	A = 0.009C +0.0381 r = 0.9987
10	A = 0.0155C + 0.0321 r = 0.9970	A = 0.0179C + 0.0230 r = 0.9954	A = 0.0201C + 0.0356 r = 0.9984	A = 0.0114C -0.0258 r = 0.9987
15	A = 0.0181C + 0.0351 r = 0.9985	A = 0.0219C + 0.0093 r = 0.9975	A = 0.0222C + 0.0224 r = 0.9988	A = 0.0128 C -0.0330 r = 0.991
20	A = 0.0201C + 0.0396 r = 0.9991	A = 0.0245C + 0.0031 r = 0.9988	A = 0.0235C + 0.004 r = 0.9991	A = 0.0142C-0.0290 r = 0.9992
25	A = 0.0219C + 0.0381 r = 0.9991	A = 0.0265C + 0.0169 r = 0.9990	A = 0.0249C + 0.0109 r = 0.9995	A = 0.0143C+0.0198 r = 0.9995
30	A = 0.0240C + 0.0312 r = 0.9998	A = 0.0289C + 0.0083 r = 0.9996	A = 0.0263C + 0.0097 r = 0.9996	A = 0.0146 C +0.0189 r = 0.9993
35	A = 0.0261C + 0.0291 r = 0.9992	A = 0.0289 C + 0.0079 r = 0.9990	A = 0.0269 C + 0.0078 r = 0.9992	

For FOS

Regression of log (rate) versus log [FOS] gave the regression equation:

Log rate =1.2173 log C +1.2549 (r = 0.9571)

Hence K′ = 17.98 Sec^{-1} and the reaction is first order n =1.2173 with respect to FOS concentration.

Table 2: logarithms of rates for different concentrations of drugs (mol l^{-1}) applying the suggested method

log ΔA/Δt	.log [EN]
-4.313	-4.994
-3.986	-4.693
-3.820	-4.391
-3.650	-4.215
log ΔA/Δtl	log [RM]
-4.585	-4.921
-4.194	-4.620
-3.852	-4.444
-3.737	-4.319
-3.641	-4.215
-3.516	-4.148
log ΔA/Δt	log [LS]
-4.313	-4.946
-4.027	-4.645
-3.891	-4.469
-3.783	-4.344
-3.706	-4.247
-3.695	-4.168
-3.631	-4.101
Log ΔA/Δt	log [FOS]
-4.310	-4.592
-4.170	-4.466
-3.960	-4.291
-3.914	-4.166
-3.787	-4.069
-3.464	-3.989

3.2. Evaluation of the kinetic methods

The quantitation of the cited drugs under the optimized experimental conditions outlined above would result in a pseudo-first order with respect to their concentrations. Where, $KMnO_4$ concentration was at least 75 times the concentration of EN, RM and LS drugs, and 25 times for FOS. While NaOH

concentration was at least 400 times the initial concentration of EN, 600 times of RM, 800 times of LS and 266.7 times for FOS.

However, the rate will be directly proportional to cited drugs in a pseudo-first order rate equation as follows:

Rate = K′ [drug] ……..Eq. (3)

Where, K′ is the pseudo-first order rate constant. Several experiments were then carried out to obtain drug concentration from the rate data according to equation (3). Fixed time method, rate constant method and fixed absorbance method were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the intercept and the correlation coefficient (r).

Fixed-Time method:

Reaction rates were determined for different concentrations of drugs at a preselected fixed- time, which was accurately determined, the absorbance versus initial concentration of drugs were established at fixed times of 5, 10, 15, 20, 25 and 30 min. for EN, RM and LS and 5, 10, 15, 20, and 25 min. for FOS. With the regression equations assembled in [Table- 1].

It is clear that the slope increases by time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time of 30 min. for EN, RM and LS and 25 min. for FOS which was therefore chosen as the most suitable time interval for measurement.

After optimizing the reaction conditions, the fixed time method was applied to the determination of the drugs concentrations in bulk powder over the concentration range 5– 40 µg ml^{-1} for EN, 5-35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} for FOS. Analysis of the data gave the following regression equations:

A = 0.0246 C – 0.0017	r = 0.9995	EN
A = 0.029 C+ 0.0053	r= 0.9997	RM
A = 0.027 C – 0.0019	r = 0.9999	LS
A = 0.0156 C – 0.0007	r = 0.9996	FOS

Where A is the absorbance at 610 nm and C is the concentration in µg ml^{-1}.

Table 3: Values of k′calculated from slopes of log a versus t graph multiplied by -2.303 for different concentration of drugs.

$K'sec^{-1}$	[EN] mol L^{-1}
$-0.344x10^{-4}$	$4.061x10^{-5}$
$-0.346x10^{-4}$	$6.092x10^{-5}$
$-0.390x10^{-4}$	$8.122x10^{-5}$
$K'sec^{-1}$	[RM] mol L^{-1}
-0.167×10^{-4}	1.200×10^{-5}
-0.223×10^{-4}	$2.401x\ 10^{-5}$
-0.360×10^{-4}	3.601×10^{-5}
-0.365×10^{-4}	$4.802x\ 10^{-5}$
$K'sec^{-1}$	[LS] mol L^{-1}
$-0.375x10^{-4}$	$3.397x10^{-5}$
$-0.342x10^{-4}$	$4.530x10^{-5}$
$-0.329x10^{-4}$	$5.662x10^{-5}$
$-0.275x10^{-4}$	$6.795x10^{-5}$
$-0.267x10^{-4}$	$7.927x10^{-5}$
$K'sec^{-1}$	[FOS] mol L^{-1}
$-0.128\ x10^{-4}$	$3.415\ x10^{-5}$
$-0.133x10^{-4}$	5.123×10^{-5}
$-0.175\ x10^{-4}$	$6.830\ x10^{-5}$

Rate-Constant method*:*

Graphs of log absorbance versus time for EN concentration in the range of $4.061x10^{-5}$ to $8.122x10^{-5}$ M, $1.200x10^{-5}$ – $4.802\ x10^{-5}$ M of RM, $3.397x10^{-5}$ – $7.927\ x10^{-5}$ M of LS and $3.415\ x10^{-5}$ – $6.830\ x10^{-5}$ M of FOS were plotted and all appear to be rectilinear. The pseudo-first order rate constants (K′) corresponding to different drug concentrations (C) were calculated from the slopes multiplied by -2.303 and are presented in [Table- 3]

For EN

Regression of (C) versus K′ gave the equation:

$K' = -0.133C - 3x10^{-5}$ (r = 0.8847)

For RM

Regression of (C) versus K′ gave the equation:

$K' = -0.6088\ C + 1\ x10^{-5}$ (r =0.9494)

For LS

Regression of (C) versus K′ gave the equation:

$K' = 0.2499C - 5x10^{-5}$ (r =0.9767)

For FOS

Regression of (C) versus K′ gave the equation:

$K' = -1.376\ C - 7x10^{-5}$ (r = 0.9103)

The values of correlation coefficient (r) are indicative of poor linearity, probably because of inconsistency of K'

Fixed-Absorbance method

Reaction rates were recorded for different drugs concentrations in the range of $4.061x10^{-5}$-$8.122x10^{-5}$ M of EN, $6.002x10^{-5}$-$8.403x10^{-5}$ M of RM, $5.661x10^{-5}$-$7.927x10^{-5}$ M LS and 6.830 $x10^{-5}$-10.245 $x10^{-5}$ M of FOS. A preselected value of the absorbance 0.5 for EN and 0.6 for RM, LS and FOS was fixed and the time was measured in seconds. The reciprocal of time (1/t) versus the initial concentration of drugs was plotted as shown [Table- 4]

Table 4: Values of reciprocal of time taken at fixed absorbance (0.5) for en and (0.6) for rm, ls and fos for different rates of variableconcentrations of drugs.

1/t sec^{-1}	[EN] mol L^{-1}
$6.29x10^{-4}$	$4.061x10^{-5}$
$1.85x10^{-3}$	$6.091x10^{-5}$
$3.03x10^{-3}$	$8.122x10^{-5}$
1/t sec^{-1}	[RM] mol L^{-1}
$9.26x10^{-4}$	$6.002x10^{-5}$
$1.52x10^{-3}$	$7.203x10^{-5}$
$2.38x10^{-3}$	$8.403x10^{-5}$
1/t sec^{-1}	[LS] mol L^{-1}
$6.67x10^{-4}$	$5.662x10^{-5}$
$1.51x10^{-3}$	$6.795x10^{-5}$
$2.38x10^{-3}$	$7.927x10^{-5}$
1/t sec^{-1}	[FOS] mol L^{-1}
7.092 $x10^{-4}$	6.830 $x10^{-5}$
1.587 $x10^{-3}$	$8.538x10^{-5}$
3.03 $x10^{-3}$	10.245 $x10^{-5}$

The following equations for calibration graphs were obtained by linear regression

1/t= 59.124 C - 0.0018 r = 0.9997 for EN

1/t= 60.557 C - 0.0028 r = 0.9944 for RM

1/t= 75.629 C - 0.0036 r = 0.9999 for LS

1/t= 67.957 C - 0.004 r = 0.9903 For FOS

The ranges of EN, RM, LS and FOS concentration giving the most acceptable calibration graphs with the above equations were (20-40 µg ml^{-1}), (25-35 µg ml^{-1}), (25-35 µg ml^{-1}) and (40-60 µg ml^{-1}) respectively. These narrow ranges could be considered a disadvantage for fixed absorbance method.

Application

In the present work different kinetic methods were studied to determine the EN, RM, LS and FOS concentration by fixed time method, rate constant method and fixed absorbance method. The fixed time method was chosen to be applied for the determination of EN, RM, LS and FOS in the bulk powder and in dosage form. The concentrations of the cited drugs were calculated using the corresponding regression equations at fixed times. The most acceptable values of correlation coefficient and intercept were obtained for a fixed time of 30 min. except FOS 25 min.. The method could be applied over the concentration range 5 – 40 µg ml^{-1} for EN, 5 – 35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} for FOS. The results obtained for the analysis of the cited drugs in bulk powder were compared with BP [1] method for EN, RM, and LS and the reported HPLC method [37] for FOS (Table- 5). The student t-test and F-test values at 95% confidence level did not exceed the theoretical values indicating no significant difference between the performance of the two methods regarding accuracy and precision. More over the method was successfully applied for the analysis of the studied drugs in pharmaceutical formulations and standard addition technique was also applied. The validity of the method was accessed by statistical analysis of the regression data [Table- 6].

Table 5: t-testes of significane of the proposed $KMnO_4$ method for the determination of en, rm, ls and fos.

Statistical terms	EN		RM		LS		FOS	
	official method[1]	$KMnO_4$ method	Official method[1]	$KMnO_4$ method	official method[1]	$KMnO_4$ method	Reported method [37]	$KMnO_4$ method
Mean*	99.84	99.95	99.91	99.81	99.17	99.60	99.96	99.75
SD	0.43	0.57	0.61	0.30	0.36	0.56	0.39	0.24
SE	0.18	0.25	0.25	0.13	0.15	0.25	0.16	0.11
N	6	5	6	5	6	5	6	5
V*	0.18	0.32	0.37	0.09	0.13	0.31	0.15	0.06
t-test	(2.262*)	0.357		0.355		1.475		1.082
F-ratio	(5.19*)	1.78		4.11		2.38		2.5

*The values between parentheses are the theoretical value of t and F test.
*mean : mean recovery percent
* v : variance

Table 6: Results obtained by the proposed method for the determination of en, rm, ls and fos in bulk powder and dosage forms.

Items	EN	RM	LS	FOS
Linearity range (µg ml^{-1})	5-40 µg ml^{-1}	5-35 µg ml^{-1}	5-35 µg ml^{-1}	15-60 µg ml^{-1}
Regression equation	A= 0.0246C-0.0017	A= 0.029C+0.0053	A= 0.027C-0.0019	A= 0.0156-0.0007
correlation coefficient (r)	0.9995	0.9997	0.9999	0.9996
$Sb^{(1)}$	0.0142	0.016	0.014	$7.72X10^{-3}$
$Sa^{(2)}$	0.667	0.740	0.646	0.693
SD of the estimation	0.407	0.403	0.352	0.300
Accuracy (mean ± S.D)				
1-Drug in bulk	99.95 ± 0.57	99.81± 0.30	99.60± 0.56	99.75 ± 0.24
2-Drug in dosage forms	99.97±0.10	99.33± 0.28	99.81± 0.20	99.48 ± 0.27
3-Drug added	99.44±0.36	99.46± 0.58	99.73 ± 0.91	99.67 ± 0.36

(1) = Standard deviation of intercept
(2) = Standard deviation of slope

Conclusion

The kinetically – based method in this work for the quantitative determination of some ACEI EN, RM, LS, and FOS is accurate, simple, non expensive and offers a contribution for routine analysis of the cited drugs in the bulk powder and in pharmaceutical formulations.

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Kietic Spectrophotometric Determination of some Angiotensin Converting Enzyme Inhibitors using Potassium Permanganate.

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Summary: A kinetic spectrophotometeric method has been developed for the determination of some angiotensin converting enzyme inhibitors (ACEI) namely, enalapril maleate (EN), ramipril (RM), lisinopri (LS) and fosinopril sodium (FOS) in the bulk powder and in pharmaceutical formulations. The procedure is based on kinetic investigation of the oxidation reaction of those drugs with alkaline potassium permanganate at room temperature at 610 nm. The fixed time method was utilized for constructing the calibration graph to determine the concentration of the drugs. The absorbance – concentration plot is rectilinear over the range of 5 - 40 µg ml^{-1} for EN, , 5-35 µg ml^{-1} for both RM and LS and 15-60 µg ml^{-1} for FOS with percentage mean recovery (99.81 ± 0.30, 99.95 ± 0.57, 99.60 ± 0.56 and 99.75 ± 0.24) respectively. Different experimental parameters affecting the development and stability of the colour were carefully studied and optimized. The results obtained were in good agreement with those obtained by potentiometric method for EN, RM and LS and HPLC method for FOS.

Key words: enalapri maleate, ramipril, lisinopril, fosinopril sodium, Potassium permanganate, spectrophotometric method, kinetic.

Introduction

Enalapril maleate1-[N-[(S)-1-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline 1-ethylester, ramipril; (2S,3aS,6aS)-1-[(S)-N-[(S)-1-carboxy-3-phenylpropyl]-alanyl]-octahydrocylopenta [b]pyrole-2-carboxylic acid 1-ethyl ester, lisinopril; (S)-1-[N^2-(1-Carboxy-3-phenylpropyl]-L-lysyl]-L-proline and fosinopril sodium (4S)-4-Cyclohexyl-1-[[[(RS)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)-phos-phinyl]acetyl]-L-Proline propionate, are an active inhibitors of angiotensin converting enzyme (ACE) with antihypertensive activity[1-2]. Those drugs used in the treatment of all forms of hypertension and heart failure [3].

Different methods published for their determination such as spectrophotometric methods [4-15], atomic absorption spectroscopy [16, 17], capillary electrophoresis [18 - 21], HPLC [22 - 30], GC [31 - 33], radioimmunoassay [34]

Kinetic- based methods of pharmaceutical analysis are not widely applied, although they offer the advantage of simplicity, low costs, and wide availability in quality control laboratories. Furthermore eliminating additives interferences, which probably affects other methods such as titrimetry and direct spectrophotometric methods [35]

In the present work, simple, valided kinetically based method is proposed for the determination of some ACEI namely EN, RM, LS and FOS by measuring the absorbance at 610 nm after their oxidation with alkaline $KMnO_4$ at ambient temperature 25 ± 5 C^0

Experimental

Apparatus: Shimadzu 1601 U V Spectrophotometer, Japan.

Materials and reagents*:*

All chemicals and reagents were of analytical grade

Enalapril maleate was assayed for purity according to the official method[1] to contain 99.84 ± 0.43 and Renetic tablets B.No (0311057) nominally containing enalapril maleate (5 mg) were manufactured and supplied by MSD, Co, Egypt, ramipril was assayed for purity according to the official method [1] to contain 99.91 ± 0.61 and tritace tablets batch number (14E03) nominally containing ramipril (5 mg) ,were manufactured and supplied by Hochest, Co, Egypt, lisinopril was assayed for purity according to the official method[1] to contain 99.17 ± 0.36and zystril tablets batch number (1204148) nominally containing lisinopril (10 mg), were manufactured and supplied by Sedico,Co, Egypt and Fosinopril sodium its purity was found to be 99.96 ± 0.39 according to the HPLC manufactural method[37] and Monopril tablets nominally containing fosinopril sodium (10 mg), were manufactured and supplied by Bristol-Mayers squib- Co, Egypt. Potassium permanganate (Merck, Germany), 8 mM (0.1264% w/ v) in water. Sodium hydroxide used as 0.5 M (EL-Nasr Chemical Company, Egypt)

Stock solutions:

Stock solutions of EN, RM, LS (0.01 %w/ v) and FOS solution (0.02 %w/v) were prepared by dissolving the specified amount of each drug in water. The solutions were stable for one week if kept in a refrigerator.

Procedures

For calibration graph

Aliquots of the stock solutions equivalent to (0.1 - 0.3 mg) of EN, (0.1 - 0.25 mg) of RM, (0.1 - 0.25 mg) of LS and (0.2 - 0.5 mg) of FOS, were transferred into 10-ml volumetric flasks, 1-ml of 0.5 M NaOH for EN and 1.5-ml for RM, while 2-ml for both LS and FOS were added to each flask, followed by the addition of 3-ml of 8 mM of potassium permanganate solution. The content of each flask were mixed and the volumes were completed to the mark with water and allowed to stand at ambient temperature for 30 min. for all drugs, except for FOS 25 min. were found to be adequate. For calibration curve the values of absorbance were plotted against the drug concentrations, alternatively, the corresponding regression equations were driven.

Procedure for tablets

For EN in Ezapril tablets and RM in Tritace tablets. Ten tablets were weighed and pulverized. A quantity of each powdered tablets equivalent to 10 mg of EN or RM were accurately weighed and transferred into 50 ml conical flask. The powdered tablets were dissolved in 30 ml of methanol using magnetic stirrer for 30 min., then the extracts were filtered and the residue washed with methanol (5 ml x 4), the combined filtrate and washing was evaporated and the residues were dissolved in water and transferred quantitatively into 50 ml volumetric flasks. The volumes were completed to the mark with water and the method proceeded as mentioned under 2.2.1

For LS in Zystril tablets. A quantity of powdered tablets equivalent to 10 mg was accurately weighed and transferred into 250 ml conical flask, then extracted with 200 ml of chloroform using magnetic stirrer for 30 min. The solution was filtered and the residue washed with chloroform (10 ml x 5). The filtrate was evaporated; the residue was dissolved in water and transferred quantitavely into 50-ml volumetric

flask. The volume was completed to the mark with water and proceeded as directed under 2.2.1

For FOS in Monopril tablets. A quantity of powdered tablets equivalent to10 mg FOS was accurately weighed, transferred into 50-ml conical flask, then extracted with 30 ml chloroform using magnetic stirrer for 10 min. The solution was filtered and washed with chloroform (5 ml x 4). The combined chloroformic extracts containing interfering povidone were rejected. The residue left on the filter paper was dried and extracted with 50 ml methanol for 30 min. using magnetic stirrer, the solution was filtered and washed with methanol (5 ml x 4). The extract was evaporated and the residue was dissolved in water into 50 ml volumetric flask. The volume was completed to the mark with water and proceeded as under 2.2.1

The procedures were repeated applying the standard addition technique. The recovered concentration of labeled and added EN, RM, LS and FOS were calculated

Results and Discussion

The UV absorption spectra of EN, RM, LS and FOS shown in [Figure-1] suffer from low intensity and lack of well-defined maxima, typical of unconjugated phenyl moiety; make it not useful for characterization or quantitative analysis of these compounds. This led to study the reaction of EN, RM, LS and FOS with alkaline $KMnO_4$ in an attempt to develop a simple and reliable method for their determination in bulk and in pharmaceutical formulations.

Kinetic and optimization of the reaction condition:

The reaction between EN, RM, LS, FOS and $KMnO_4$ in alkaline medium yields a colour due to the production of manganate ion, which absorbs at 610 nm [Figure-2]. As the intensity of the colour increases by time, it was deemed useful to elaborate kinetically determination of these drugs in bulk and in pharmaceutical preparations.

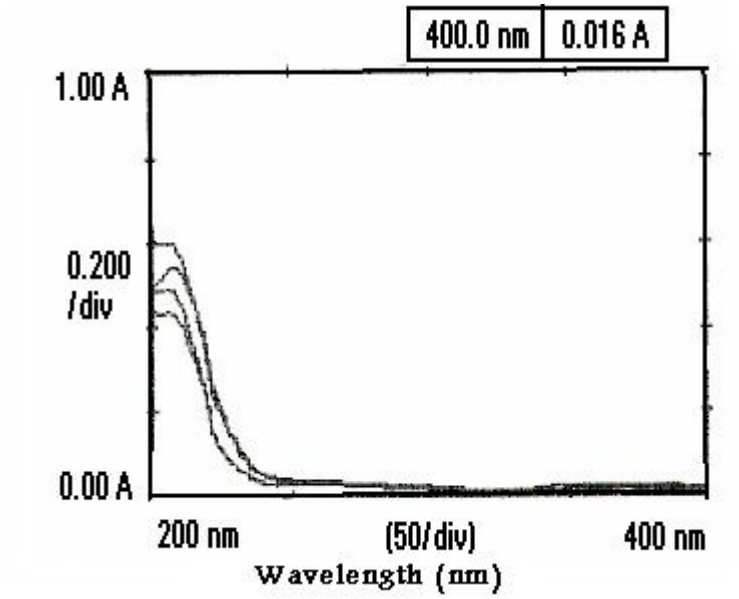

Figure 1: Absorbance spectra of EN(25 µg ml^{-1}), RM(25 µg ml^{-1}), LS(25 µg ml^{-1}) and FOS(25 µg ml^{-1}) in water

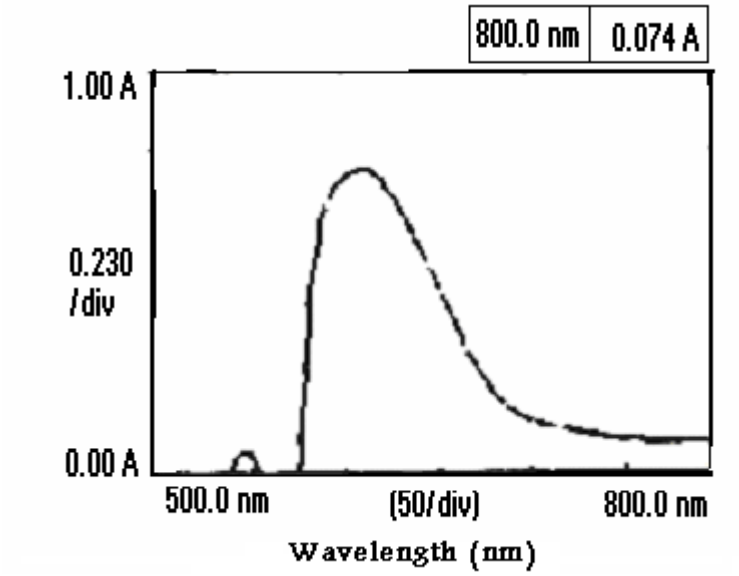

Figure 2: absorption spectrum of manganate ion obtained after the reaction of $KMnO_4$ with FOS (50 µg ml^{-1}) .

The reaction was studied under various conditions of reagent concentration, alkalinity and temperature; the effect of different solvents was also studied. Water was used to dissolve the drug since $KMnO_4$ oxidizes other solvents with the production of green manganate ions.

At room temperature the reaction rate increased substantially by time [Figure- 3], as revealed by the increase in the intensification of the developed colour and subsequent increase in the slopes of the calibration graphs [Table -1] indicating high analytical sensitivity. Heating the solution was found to increase the rate of the reaction but MnO_2 was precipitated. Therefore, room temperature was selected as the optimum temperature.

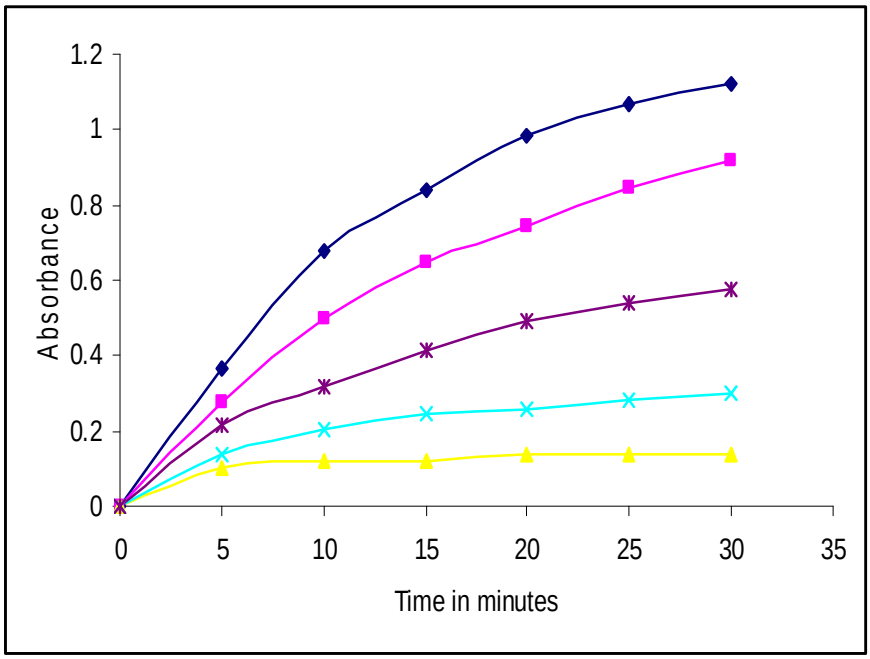

Figure 3: Absorbance versus time graph for the reaction between EN (5 – 40 µg ml^{-1}) and potassium permanganate

The reaction rate and maximum absorbance increased by time, and with increasing $KMnO_4$ concentration. It was found that 3 ml of 8 mM of $KMnO_4$ solution was adequate for the maximum absorbance.

The influence of NaOH concentration on the reaction rate was also studied. It was found that increasing the amount of 0.5 M NaOH would increase the absorbance of the reaction product up to 1 ml, 1.5 ml for EN and RM respectively and 2 ml for both LS and FOS, after which further increase does not affect the absorbance of the reaction product. Trials were made through oxidation with $KMnO_4$ in neutral and acidic medium, but no oxidation was observed.

The rate of the reaction was also found to be dependent on the drug concentration.

The rates were followed at room temperature with various concentrations of the cited drugs in the range of 5 – 40 µg ml^{-1} for EN, 5- 35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} of FOS keeping $KMnO_4$ and NaOH concentrations constant. The results in [Table- 1] clearly indicate that the reaction obeys the following equation:-

$$\text{Rate} = K' [\text{drug}]^{n} \quad (1)$$

Where K′ is the pseudo- order rate constant and n is the order of the reaction.

The rate of the reaction ($\Delta A/\Delta t$) may be estimated by the variable time method measurement [36], where A is the absorbance and t is the time in seconds. Taking logarithms of rates and concentrations [Table-2] and equation (1) is transformed into:

$$\log (\text{rate}) = \log \Delta A/\Delta t = \log K' + n \log [\text{drug}]. \quad (2)$$

For EN

Regression of log (rate) versus log [EN] gave the regression equation:

$\log \text{rate} = 0.817 \log C - 0.2056$ ($r = 0.9909$)

Hence $K' = 1.605$ Sec^{-1} and the reaction is first order $n = 0.817$ with respect to EN concentration.

For RM

Regression of log (rate) versus log [RM] gave the regression equation:

$\log \text{rate} = 1.3805 \log C + 2.2147$ ($r = 0.9955$).

Hence $K' = 163.95$ Sec^{-1} and the reaction is first order $n = 1.3805$ with respect to RM concentration.

For LS

Regression of log (rate) versus log [LS] gave the regression equation:

$\log \text{rate} = 0.8001 \log C - 0.3293$ ($r = 0.9948$).

Hence $K' = 2.135$ Sec^{-1} and the reaction is first order $n = 0.8001$ with respect to LS concentration.

Table 1: Calibration equations at different fixed time for EN, RM, LS and fos over the concentration ranges 5-40 µg ml^{-1}, 5-35 µg ml^{-1}, 5-35 µg ml^{-1} A and 15 – 60 µg ml^{-1} respectively

Time (min.)	Regression equations			
	EN	RM	LS	FOS
5	A = 0.0125 C + 0.0141 r = 0.9919	A = 0.00139 C + 0.041 r = 0.9932	A = 0.0173 C + 0.0393 r = 0.9968	A = 0.009C +0.0381 r = 0.9987
10	A = 0.0155C + 0.0321 r = 0.9970	A = 0.0179C + 0.0230 r = 0.9954	A = 0.0201C + 0.0356 r = 0.9984	A = 0.0114C -0.0258 r = 0.9987
15	A = 0.0181C + 0.0351 r = 0.9985	A = 0.0219C + 0.0093 r = 0.9975	A = 0.0222C + 0.0224 r = 0.9988	A = 0.0128 C -0.0330 r = 0.991
20	A = 0.0201C + 0.0396 r = 0.9991	A = 0.0245C + 0.0031 r = 0.9988	A = 0.0235C + 0.004 r = 0.9991	A = 0.0142C-0.0290 r = 0.9992
25	A = 0.0219C + 0.0381 r = 0.9991	A = 0.0265C + 0.0169 r = 0.9990	A = 0.0249C + 0.0109 r = 0.9995	A = 0.0143C+0.0198 r = 0.9995
30	A = 0.0240C + 0.0312 r = 0.9998	A = 0.0289C + 0.0083 r = 0.9996	A = 0.0263C + 0.0097 r = 0.9996	A = 0.0146 C +0.0189 r = 0.9993
35	A = 0.0261C + 0.0291 r = 0.9992	A = 0.0289 C + 0.0079 r = 0.9990	A = 0.0269 C + 0.0078 r = 0.9992	

For FOS

Regression of log (rate) versus log [FOS] gave the regression equation:

Log rate =1.2173 log C +1.2549 (r = 0.9571)

Hence K′ = 17.98 Sec^{-1} and the reaction is first order n =1.2173 with respect to FOS concentration.

Table 2: logarithms of rates for different concentrations of drugs (mol l^{-1}) applying the suggested method

log ΔA/Δt	.log [EN]
-4.313	-4.994
-3.986	-4.693
-3.820	-4.391
-3.650	-4.215
log ΔA/Δtl	log [RM]
-4.585	-4.921
-4.194	-4.620
-3.852	-4.444
-3.737	-4.319
-3.641	-4.215
-3.516	-4.148
log ΔA/Δt	log [LS]
-4.313	-4.946
-4.027	-4.645
-3.891	-4.469
-3.783	-4.344
-3.706	-4.247
-3.695	-4.168
-3.631	-4.101
Log ΔA/Δt	log [FOS]
-4.310	-4.592
-4.170	-4.466
-3.960	-4.291
-3.914	-4.166
-3.787	-4.069
-3.464	-3.989

3.2. Evaluation of the kinetic methods

The quantitation of the cited drugs under the optimized experimental conditions outlined above would result in a pseudo-first order with respect to their concentrations. Where, $KMnO_4$ concentration was at least 75 times the concentration of EN, RM and LS drugs, and 25 times for FOS. While NaOH

concentration was at least 400 times the initial concentration of EN, 600 times of RM, 800 times of LS and 266.7 times for FOS.

However, the rate will be directly proportional to cited drugs in a pseudo-first order rate equation as follows:

Rate = K′ [drug] ……..Eq. (3)

Where, K′ is the pseudo-first order rate constant. Several experiments were then carried out to obtain drug concentration from the rate data according to equation (3). Fixed time method, rate constant method and fixed absorbance method were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the intercept and the correlation coefficient (r).

Fixed-Time method:

Reaction rates were determined for different concentrations of drugs at a preselected fixed- time, which was accurately determined, the absorbance versus initial concentration of drugs were established at fixed times of 5, 10, 15, 20, 25 and 30 min. for EN, RM and LS and 5, 10, 15, 20, and 25 min. for FOS. With the regression equations assembled in [Table- 1].

It is clear that the slope increases by time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time of 30 min. for EN, RM and LS and 25 min. for FOS which was therefore chosen as the most suitable time interval for measurement.

After optimizing the reaction conditions, the fixed time method was applied to the determination of the drugs concentrations in bulk powder over the concentration range 5– 40 µg ml^{-1} for EN, 5-35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} for FOS. Analysis of the data gave the following regression equations:

A = 0.0246 C – 0.0017	r = 0.9995	EN
A = 0.029 C+ 0.0053	r= 0.9997	RM
A = 0.027 C – 0.0019	r = 0.9999	LS
A = 0.0156 C – 0.0007	r = 0.9996	FOS

Where A is the absorbance at 610 nm and C is the concentration in µg ml^{-1}.

Table 3: Values of k′calculated from slopes of log a versus t graph multiplied by -2.303 for different concentration of drugs.

$K'sec^{-1}$	[EN] mol L^{-1}
$-0.344x10^{-4}$	$4.061x10^{-5}$
$-0.346x10^{-4}$	$6.092x10^{-5}$
$-0.390x10^{-4}$	$8.122x10^{-5}$
$K'sec^{-1}$	[RM] mol L^{-1}
-0.167×10^{-4}	1.200×10^{-5}
-0.223×10^{-4}	$2.401x\ 10^{-5}$
-0.360×10^{-4}	3.601×10^{-5}
-0.365×10^{-4}	$4.802x\ 10^{-5}$
$K'sec^{-1}$	[LS] mol L^{-1}
$-0.375x10^{-4}$	$3.397x10^{-5}$
$-0.342x10^{-4}$	$4.530x10^{-5}$
$-0.329x10^{-4}$	$5.662x10^{-5}$
$-0.275x10^{-4}$	$6.795x10^{-5}$
$-0.267x10^{-4}$	$7.927x10^{-5}$
$K'sec^{-1}$	[FOS] mol L^{-1}
$-0.128\ x10^{-4}$	$3.415\ x10^{-5}$
$-0.133x10^{-4}$	5.123×10^{-5}
$-0.175\ x10^{-4}$	$6.830\ x10^{-5}$

Rate-Constant method*:*

Graphs of log absorbance versus time for EN concentration in the range of $4.061x10^{-5}$ to $8.122x10^{-5}$ M, $1.200x10^{-5}$ – $4.802\ x10^{-5}$ M of RM, $3.397x10^{-5}$ – $7.927\ x10^{-5}$ M of LS and $3.415\ x10^{-5}$ – $6.830\ x10^{-5}$ M of FOS were plotted and all appear to be rectilinear. The pseudo-first order rate constants (K′) corresponding to different drug concentrations (C) were calculated from the slopes multiplied by -2.303 and are presented in [Table- 3]

For EN

Regression of (C) versus K′ gave the equation:

$K' = -0.133C - 3x10^{-5}$ (r = 0.8847)

For RM

Regression of (C) versus K′ gave the equation:

$K' = -0.6088\ C + 1\ x10^{-5}$ (r =0.9494)

For LS

Regression of (C) versus K′ gave the equation:

$K' = 0.2499C - 5x10^{-5}$ (r =0.9767)

For FOS

Regression of (C) versus K′ gave the equation:

$K' = -1.376\ C - 7x10^{-5}$ (r = 0.9103)

The values of correlation coefficient (r) are indicative of poor linearity, probably because of inconsistency of K'

Fixed-Absorbance method

Reaction rates were recorded for different drugs concentrations in the range of $4.061x10^{-5}$-$8.122x10^{-5}$ M of EN, $6.002x10^{-5}$-$8.403x10^{-5}$ M of RM, $5.661x10^{-5}$-$7.927x10^{-5}$ M LS and 6.830 $x10^{-5}$-10.245 $x10^{-5}$ M of FOS. A preselected value of the absorbance 0.5 for EN and 0.6 for RM, LS and FOS was fixed and the time was measured in seconds. The reciprocal of time (1/t) versus the initial concentration of drugs was plotted as shown [Table- 4]

Table 4: Values of reciprocal of time taken at fixed absorbance (0.5) for en and (0.6) for rm, ls and fos for different rates of variableconcentrations of drugs.

1/t sec^{-1}	[EN] mol L^{-1}
$6.29x10^{-4}$	$4.061x10^{-5}$
$1.85x10^{-3}$	$6.091x10^{-5}$
$3.03x10^{-3}$	$8.122x10^{-5}$
1/t sec^{-1}	[RM] mol L^{-1}
$9.26x10^{-4}$	$6.002x10^{-5}$
$1.52x10^{-3}$	$7.203x10^{-5}$
$2.38x10^{-3}$	$8.403x10^{-5}$
1/t sec^{-1}	[LS] mol L^{-1}
$6.67x10^{-4}$	$5.662x10^{-5}$
$1.51x10^{-3}$	$6.795x10^{-5}$
$2.38x10^{-3}$	$7.927x10^{-5}$
1/t sec^{-1}	[FOS] mol L^{-1}
7.092 $x10^{-4}$	6.830 $x10^{-5}$
1.587 $x10^{-3}$	$8.538x10^{-5}$
3.03 $x10^{-3}$	10.245 $x10^{-5}$

The following equations for calibration graphs were obtained by linear regression

1/t= 59.124 C - 0.0018 r = 0.9997 for EN

1/t= 60.557 C - 0.0028 r = 0.9944 for RM

1/t= 75.629 C - 0.0036 r = 0.9999 for LS

1/t= 67.957 C - 0.004 r = 0.9903 For FOS

The ranges of EN, RM, LS and FOS concentration giving the most acceptable calibration graphs with the above equations were (20-40 µg ml^{-1}), (25-35 µg ml^{-1}), (25-35 µg ml^{-1}) and (40-60 µg ml^{-1}) respectively. These narrow ranges could be considered a disadvantage for fixed absorbance method.

Application

In the present work different kinetic methods were studied to determine the EN, RM, LS and FOS concentration by fixed time method, rate constant method and fixed absorbance method. The fixed time method was chosen to be applied for the determination of EN, RM, LS and FOS in the bulk powder and in dosage form. The concentrations of the cited drugs were calculated using the corresponding regression equations at fixed times. The most acceptable values of correlation coefficient and intercept were obtained for a fixed time of 30 min. except FOS 25 min.. The method could be applied over the concentration range 5 – 40 µg ml^{-1} for EN, 5 – 35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} for FOS. The results obtained for the analysis of the cited drugs in bulk powder were compared with BP [1] method for EN, RM, and LS and the reported HPLC method [37] for FOS (Table- 5). The student t-test and F-test values at 95% confidence level did not exceed the theoretical values indicating no significant difference between the performance of the two methods regarding accuracy and precision. More over the method was successfully applied for the analysis of the studied drugs in pharmaceutical formulations and standard addition technique was also applied. The validity of the method was accessed by statistical analysis of the regression data [Table- 6].

Table 5: t-testes of significane of the proposed $KMnO_4$ method for the determination of en, rm, ls and fos.

Statistical terms	EN		RM		LS		FOS	
	official method[1]	$KMnO_4$ method	Official method[1]	$KMnO_4$ method	official method[1]	$KMnO_4$ method	Reported method [37]	$KMnO_4$ method
Mean*	99.84	99.95	99.91	99.81	99.17	99.60	99.96	99.75
SD	0.43	0.57	0.61	0.30	0.36	0.56	0.39	0.24
SE	0.18	0.25	0.25	0.13	0.15	0.25	0.16	0.11
N	6	5	6	5	6	5	6	5
V*	0.18	0.32	0.37	0.09	0.13	0.31	0.15	0.06
t-test	(2.262*)	0.357		0.355		1.475		1.082
F-ratio	(5.19*)	1.78		4.11		2.38		2.5

*The values between parentheses are the theoretical value of t and F test.
*mean : mean recovery percent
* v : variance

Table 6: Results obtained by the proposed method for the determination of en, rm, ls and fos in bulk powder and dosage forms.

Items	EN	RM	LS	FOS
Linearity range (µg ml^{-1})	5-40 µg ml^{-1}	5-35 µg ml^{-1}	5-35 µg ml^{-1}	15-60 µg ml^{-1}
Regression equation	A= 0.0246C-0.0017	A= 0.029C+0.0053	A= 0.027C-0.0019	A= 0.0156-0.0007
correlation coefficient (r)	0.9995	0.9997	0.9999	0.9996
$Sb^{(1)}$	0.0142	0.016	0.014	$7.72X10^{-3}$
$Sa^{(2)}$	0.667	0.740	0.646	0.693
SD of the estimation	0.407	0.403	0.352	0.300
Accuracy (mean ± S.D)				
1-Drug in bulk	99.95 ± 0.57	99.81± 0.30	99.60± 0.56	99.75 ± 0.24
2-Drug in dosage forms	99.97±0.10	99.33± 0.28	99.81± 0.20	99.48 ± 0.27
3-Drug added	99.44±0.36	99.46± 0.58	99.73 ± 0.91	99.67 ± 0.36

(1) = Standard deviation of intercept
(2) = Standard deviation of slope

Conclusion

The kinetically – based method in this work for the quantitative determination of some ACEI EN, RM, LS, and FOS is accurate, simple, non expensive and offers a contribution for routine analysis of the cited drugs in the bulk powder and in pharmaceutical formulations.

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Kietic Spectrophotometric Determination of some Angiotensin Converting Enzyme Inhibitors using Potassium Permanganate.

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Summary: A kinetic spectrophotometeric method has been developed for the determination of some angiotensin converting enzyme inhibitors (ACEI) namely, enalapril maleate (EN), ramipril (RM), lisinopri (LS) and fosinopril sodium (FOS) in the bulk powder and in pharmaceutical formulations. The procedure is based on kinetic investigation of the oxidation reaction of those drugs with alkaline potassium permanganate at room temperature at 610 nm. The fixed time method was utilized for constructing the calibration graph to determine the concentration of the drugs. The absorbance – concentration plot is rectilinear over the range of 5 - 40 µg ml^{-1} for EN, , 5-35 µg ml^{-1} for both RM and LS and 15-60 µg ml^{-1} for FOS with percentage mean recovery (99.81 ± 0.30, 99.95 ± 0.57, 99.60 ± 0.56 and 99.75 ± 0.24) respectively. Different experimental parameters affecting the development and stability of the colour were carefully studied and optimized. The results obtained were in good agreement with those obtained by potentiometric method for EN, RM and LS and HPLC method for FOS.

Key words: enalapri maleate, ramipril, lisinopril, fosinopril sodium, Potassium permanganate, spectrophotometric method, kinetic.

Introduction

Enalapril maleate1-[N-[(S)-1-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline 1-ethylester, ramipril; (2S,3aS,6aS)-1-[(S)-N-[(S)-1-carboxy-3-phenylpropyl]-alanyl]-octahydrocylopenta [b]pyrole-2-carboxylic acid 1-ethyl ester, lisinopril; (S)-1-[N^2-(1-Carboxy-3-phenylpropyl]-L-lysyl]-L-proline and fosinopril sodium (4S)-4-Cyclohexyl-1-[[[(RS)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)-phos-phinyl]acetyl]-L-Proline propionate, are an active inhibitors of angiotensin converting enzyme (ACE) with antihypertensive activity[1-2]. Those drugs used in the treatment of all forms of hypertension and heart failure [3].

Different methods published for their determination such as spectrophotometric methods [4-15], atomic absorption spectroscopy [16, 17], capillary electrophoresis [18 - 21], HPLC [22 - 30], GC [31 - 33], radioimmunoassay [34]

Kinetic- based methods of pharmaceutical analysis are not widely applied, although they offer the advantage of simplicity, low costs, and wide availability in quality control laboratories. Furthermore eliminating additives interferences, which probably affects other methods such as titrimetry and direct spectrophotometric methods [35]

In the present work, simple, valided kinetically based method is proposed for the determination of some ACEI namely EN, RM, LS and FOS by measuring the absorbance at 610 nm after their oxidation with alkaline $KMnO_4$ at ambient temperature 25 ± 5 C^0

Experimental

Apparatus: Shimadzu 1601 U V Spectrophotometer, Japan.

Materials and reagents*:*

All chemicals and reagents were of analytical grade

Enalapril maleate was assayed for purity according to the official method[1] to contain 99.84 ± 0.43 and Renetic tablets B.No (0311057) nominally containing enalapril maleate (5 mg) were manufactured and supplied by MSD, Co, Egypt, ramipril was assayed for purity according to the official method [1] to contain 99.91 ± 0.61 and tritace tablets batch number (14E03) nominally containing ramipril (5 mg) ,were manufactured and supplied by Hochest, Co, Egypt, lisinopril was assayed for purity according to the official method[1] to contain 99.17 ± 0.36and zystril tablets batch number (1204148) nominally containing lisinopril (10 mg), were manufactured and supplied by Sedico,Co, Egypt and Fosinopril sodium its purity was found to be 99.96 ± 0.39 according to the HPLC manufactural method[37] and Monopril tablets nominally containing fosinopril sodium (10 mg), were manufactured and supplied by Bristol-Mayers squib- Co, Egypt. Potassium permanganate (Merck, Germany), 8 mM (0.1264% w/ v) in water. Sodium hydroxide used as 0.5 M (EL-Nasr Chemical Company, Egypt)

Stock solutions:

Stock solutions of EN, RM, LS (0.01 %w/ v) and FOS solution (0.02 %w/v) were prepared by dissolving the specified amount of each drug in water. The solutions were stable for one week if kept in a refrigerator.

Procedures

For calibration graph

Aliquots of the stock solutions equivalent to (0.1 - 0.3 mg) of EN, (0.1 - 0.25 mg) of RM, (0.1 - 0.25 mg) of LS and (0.2 - 0.5 mg) of FOS, were transferred into 10-ml volumetric flasks, 1-ml of 0.5 M NaOH for EN and 1.5-ml for RM, while 2-ml for both LS and FOS were added to each flask, followed by the addition of 3-ml of 8 mM of potassium permanganate solution. The content of each flask were mixed and the volumes were completed to the mark with water and allowed to stand at ambient temperature for 30 min. for all drugs, except for FOS 25 min. were found to be adequate. For calibration curve the values of absorbance were plotted against the drug concentrations, alternatively, the corresponding regression equations were driven.

Procedure for tablets

For EN in Ezapril tablets and RM in Tritace tablets. Ten tablets were weighed and pulverized. A quantity of each powdered tablets equivalent to 10 mg of EN or RM were accurately weighed and transferred into 50 ml conical flask. The powdered tablets were dissolved in 30 ml of methanol using magnetic stirrer for 30 min., then the extracts were filtered and the residue washed with methanol (5 ml x 4), the combined filtrate and washing was evaporated and the residues were dissolved in water and transferred quantitatively into 50 ml volumetric flasks. The volumes were completed to the mark with water and the method proceeded as mentioned under 2.2.1

For LS in Zystril tablets. A quantity of powdered tablets equivalent to 10 mg was accurately weighed and transferred into 250 ml conical flask, then extracted with 200 ml of chloroform using magnetic stirrer for 30 min. The solution was filtered and the residue washed with chloroform (10 ml x 5). The filtrate was evaporated; the residue was dissolved in water and transferred quantitavely into 50-ml volumetric

flask. The volume was completed to the mark with water and proceeded as directed under 2.2.1

For FOS in Monopril tablets. A quantity of powdered tablets equivalent to10 mg FOS was accurately weighed, transferred into 50-ml conical flask, then extracted with 30 ml chloroform using magnetic stirrer for 10 min. The solution was filtered and washed with chloroform (5 ml x 4). The combined chloroformic extracts containing interfering povidone were rejected. The residue left on the filter paper was dried and extracted with 50 ml methanol for 30 min. using magnetic stirrer, the solution was filtered and washed with methanol (5 ml x 4). The extract was evaporated and the residue was dissolved in water into 50 ml volumetric flask. The volume was completed to the mark with water and proceeded as under 2.2.1

The procedures were repeated applying the standard addition technique. The recovered concentration of labeled and added EN, RM, LS and FOS were calculated

Results and Discussion

The UV absorption spectra of EN, RM, LS and FOS shown in [Figure-1] suffer from low intensity and lack of well-defined maxima, typical of unconjugated phenyl moiety; make it not useful for characterization or quantitative analysis of these compounds. This led to study the reaction of EN, RM, LS and FOS with alkaline $KMnO_4$ in an attempt to develop a simple and reliable method for their determination in bulk and in pharmaceutical formulations.

Kinetic and optimization of the reaction condition:

The reaction between EN, RM, LS, FOS and $KMnO_4$ in alkaline medium yields a colour due to the production of manganate ion, which absorbs at 610 nm [Figure-2]. As the intensity of the colour increases by time, it was deemed useful to elaborate kinetically determination of these drugs in bulk and in pharmaceutical preparations.

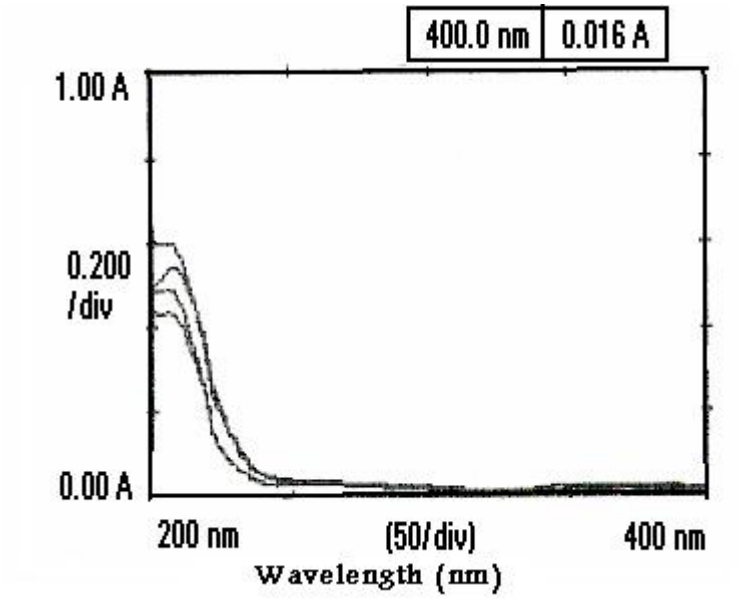

Figure 1: Absorbance spectra of EN(25 µg ml^{-1}), RM(25 µg ml^{-1}), LS(25 µg ml^{-1}) and FOS(25 µg ml^{-1}) in water

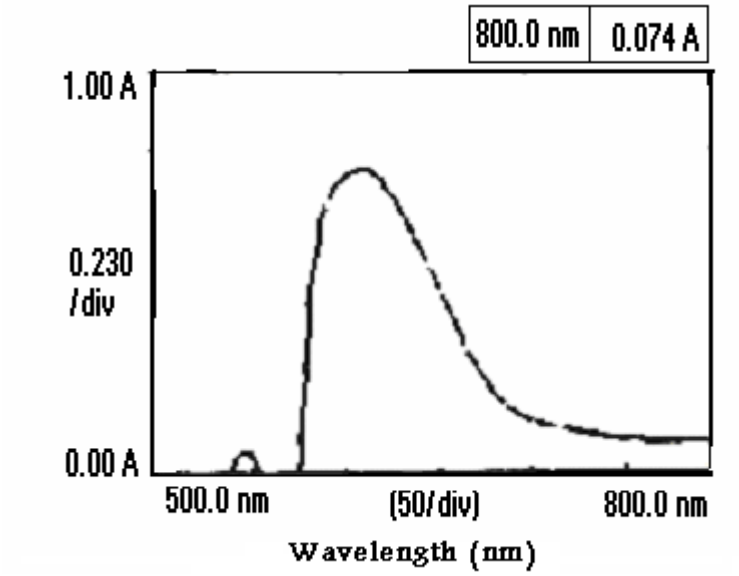

Figure 2: absorption spectrum of manganate ion obtained after the reaction of $KMnO_4$ with FOS (50 µg ml^{-1}) .

The reaction was studied under various conditions of reagent concentration, alkalinity and temperature; the effect of different solvents was also studied. Water was used to dissolve the drug since $KMnO_4$ oxidizes other solvents with the production of green manganate ions.

At room temperature the reaction rate increased substantially by time [Figure- 3], as revealed by the increase in the intensification of the developed colour and subsequent increase in the slopes of the calibration graphs [Table -1] indicating high analytical sensitivity. Heating the solution was found to increase the rate of the reaction but MnO_2 was precipitated. Therefore, room temperature was selected as the optimum temperature.

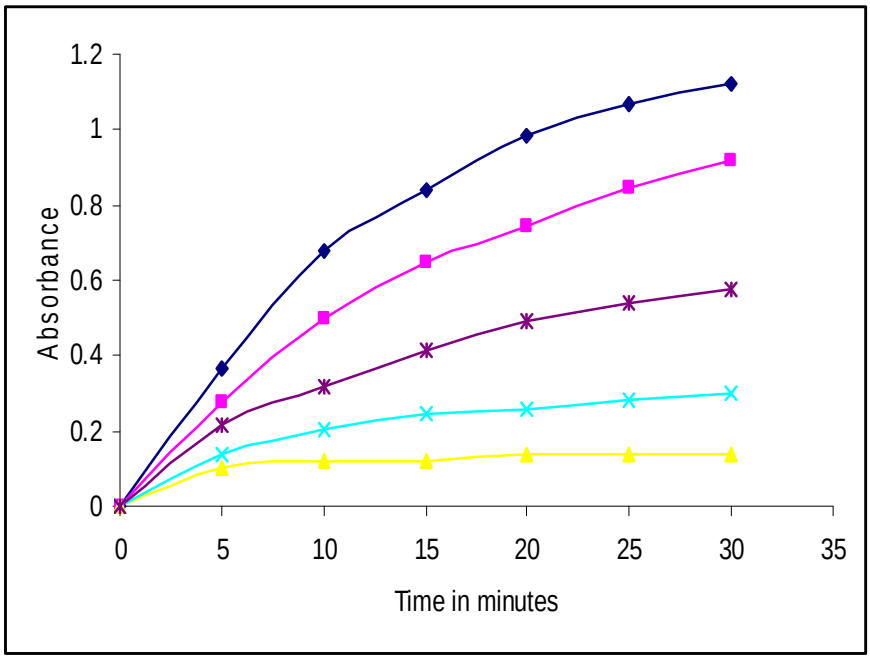

Figure 3: Absorbance versus time graph for the reaction between EN (5 – 40 µg ml^{-1}) and potassium permanganate

The reaction rate and maximum absorbance increased by time, and with increasing $KMnO_4$ concentration. It was found that 3 ml of 8 mM of $KMnO_4$ solution was adequate for the maximum absorbance.

The influence of NaOH concentration on the reaction rate was also studied. It was found that increasing the amount of 0.5 M NaOH would increase the absorbance of the reaction product up to 1 ml, 1.5 ml for EN and RM respectively and 2 ml for both LS and FOS, after which further increase does not affect the absorbance of the reaction product. Trials were made through oxidation with $KMnO_4$ in neutral and acidic medium, but no oxidation was observed.

The rate of the reaction was also found to be dependent on the drug concentration.

The rates were followed at room temperature with various concentrations of the cited drugs in the range of 5 – 40 µg ml^{-1} for EN, 5- 35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} of FOS keeping $KMnO_4$ and NaOH concentrations constant. The results in [Table- 1] clearly indicate that the reaction obeys the following equation:-

$$\text{Rate} = K' [\text{drug}]^{n} \quad (1)$$

Where K′ is the pseudo- order rate constant and n is the order of the reaction.

The rate of the reaction ($\Delta A/\Delta t$) may be estimated by the variable time method measurement [36], where A is the absorbance and t is the time in seconds. Taking logarithms of rates and concentrations [Table-2] and equation (1) is transformed into:

$$\log (\text{rate}) = \log \Delta A/\Delta t = \log K' + n \log [\text{drug}]. \quad (2)$$

For EN

Regression of log (rate) versus log [EN] gave the regression equation:

$\log \text{rate} = 0.817 \log C - 0.2056$ ($r = 0.9909$)

Hence $K' = 1.605$ Sec^{-1} and the reaction is first order $n = 0.817$ with respect to EN concentration.

For RM

Regression of log (rate) versus log [RM] gave the regression equation:

$\log \text{rate} = 1.3805 \log C + 2.2147$ ($r = 0.9955$).

Hence $K' = 163.95$ Sec^{-1} and the reaction is first order $n = 1.3805$ with respect to RM concentration.

For LS

Regression of log (rate) versus log [LS] gave the regression equation:

$\log \text{rate} = 0.8001 \log C - 0.3293$ ($r = 0.9948$).

Hence $K' = 2.135$ Sec^{-1} and the reaction is first order $n = 0.8001$ with respect to LS concentration.

Table 1: Calibration equations at different fixed time for EN, RM, LS and fos over the concentration ranges 5-40 µg ml^{-1}, 5-35 µg ml^{-1}, 5-35 µg ml^{-1} A and 15 – 60 µg ml^{-1} respectively

Time (min.)	Regression equations			
	EN	RM	LS	FOS
5	A = 0.0125 C + 0.0141 r = 0.9919	A = 0.00139 C + 0.041 r = 0.9932	A = 0.0173 C + 0.0393 r = 0.9968	A = 0.009C +0.0381 r = 0.9987
10	A = 0.0155C + 0.0321 r = 0.9970	A = 0.0179C + 0.0230 r = 0.9954	A = 0.0201C + 0.0356 r = 0.9984	A = 0.0114C -0.0258 r = 0.9987
15	A = 0.0181C + 0.0351 r = 0.9985	A = 0.0219C + 0.0093 r = 0.9975	A = 0.0222C + 0.0224 r = 0.9988	A = 0.0128 C -0.0330 r = 0.991
20	A = 0.0201C + 0.0396 r = 0.9991	A = 0.0245C + 0.0031 r = 0.9988	A = 0.0235C + 0.004 r = 0.9991	A = 0.0142C-0.0290 r = 0.9992
25	A = 0.0219C + 0.0381 r = 0.9991	A = 0.0265C + 0.0169 r = 0.9990	A = 0.0249C + 0.0109 r = 0.9995	A = 0.0143C+0.0198 r = 0.9995
30	A = 0.0240C + 0.0312 r = 0.9998	A = 0.0289C + 0.0083 r = 0.9996	A = 0.0263C + 0.0097 r = 0.9996	A = 0.0146 C +0.0189 r = 0.9993
35	A = 0.0261C + 0.0291 r = 0.9992	A = 0.0289 C + 0.0079 r = 0.9990	A = 0.0269 C + 0.0078 r = 0.9992	

For FOS

Regression of log (rate) versus log [FOS] gave the regression equation:

Log rate =1.2173 log C +1.2549 (r = 0.9571)

Hence K′ = 17.98 Sec^{-1} and the reaction is first order n =1.2173 with respect to FOS concentration.

Table 2: logarithms of rates for different concentrations of drugs (mol l^{-1}) applying the suggested method

log ΔA/Δt	.log [EN]
-4.313	-4.994
-3.986	-4.693
-3.820	-4.391
-3.650	-4.215
log ΔA/Δtl	log [RM]
-4.585	-4.921
-4.194	-4.620
-3.852	-4.444
-3.737	-4.319
-3.641	-4.215
-3.516	-4.148
log ΔA/Δt	log [LS]
-4.313	-4.946
-4.027	-4.645
-3.891	-4.469
-3.783	-4.344
-3.706	-4.247
-3.695	-4.168
-3.631	-4.101
Log ΔA/Δt	log [FOS]
-4.310	-4.592
-4.170	-4.466
-3.960	-4.291
-3.914	-4.166
-3.787	-4.069
-3.464	-3.989

3.2. Evaluation of the kinetic methods

The quantitation of the cited drugs under the optimized experimental conditions outlined above would result in a pseudo-first order with respect to their concentrations. Where, $KMnO_4$ concentration was at least 75 times the concentration of EN, RM and LS drugs, and 25 times for FOS. While NaOH

concentration was at least 400 times the initial concentration of EN, 600 times of RM, 800 times of LS and 266.7 times for FOS.

However, the rate will be directly proportional to cited drugs in a pseudo-first order rate equation as follows:

Rate = K′ [drug] ……..Eq. (3)

Where, K′ is the pseudo-first order rate constant. Several experiments were then carried out to obtain drug concentration from the rate data according to equation (3). Fixed time method, rate constant method and fixed absorbance method were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the intercept and the correlation coefficient (r).

Fixed-Time method:

Reaction rates were determined for different concentrations of drugs at a preselected fixed- time, which was accurately determined, the absorbance versus initial concentration of drugs were established at fixed times of 5, 10, 15, 20, 25 and 30 min. for EN, RM and LS and 5, 10, 15, 20, and 25 min. for FOS. With the regression equations assembled in [Table- 1].

It is clear that the slope increases by time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time of 30 min. for EN, RM and LS and 25 min. for FOS which was therefore chosen as the most suitable time interval for measurement.

After optimizing the reaction conditions, the fixed time method was applied to the determination of the drugs concentrations in bulk powder over the concentration range 5– 40 µg ml^{-1} for EN, 5-35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} for FOS. Analysis of the data gave the following regression equations:

A = 0.0246 C – 0.0017	r = 0.9995	EN
A = 0.029 C+ 0.0053	r= 0.9997	RM
A = 0.027 C – 0.0019	r = 0.9999	LS
A = 0.0156 C – 0.0007	r = 0.9996	FOS

Where A is the absorbance at 610 nm and C is the concentration in µg ml^{-1}.

Table 3: Values of k′calculated from slopes of log a versus t graph multiplied by -2.303 for different concentration of drugs.

$K'sec^{-1}$	[EN] mol L^{-1}
$-0.344x10^{-4}$	$4.061x10^{-5}$
$-0.346x10^{-4}$	$6.092x10^{-5}$
$-0.390x10^{-4}$	$8.122x10^{-5}$
$K'sec^{-1}$	[RM] mol L^{-1}
-0.167×10^{-4}	1.200×10^{-5}
-0.223×10^{-4}	$2.401x\ 10^{-5}$
-0.360×10^{-4}	3.601×10^{-5}
-0.365×10^{-4}	$4.802x\ 10^{-5}$
$K'sec^{-1}$	[LS] mol L^{-1}
$-0.375x10^{-4}$	$3.397x10^{-5}$
$-0.342x10^{-4}$	$4.530x10^{-5}$
$-0.329x10^{-4}$	$5.662x10^{-5}$
$-0.275x10^{-4}$	$6.795x10^{-5}$
$-0.267x10^{-4}$	$7.927x10^{-5}$
$K'sec^{-1}$	[FOS] mol L^{-1}
$-0.128\ x10^{-4}$	$3.415\ x10^{-5}$
$-0.133x10^{-4}$	5.123×10^{-5}
$-0.175\ x10^{-4}$	$6.830\ x10^{-5}$

Rate-Constant method*:*

Graphs of log absorbance versus time for EN concentration in the range of $4.061x10^{-5}$ to $8.122x10^{-5}$ M, $1.200x10^{-5}$ – $4.802\ x10^{-5}$ M of RM, $3.397x10^{-5}$ – $7.927\ x10^{-5}$ M of LS and $3.415\ x10^{-5}$ – $6.830\ x10^{-5}$ M of FOS were plotted and all appear to be rectilinear. The pseudo-first order rate constants (K′) corresponding to different drug concentrations (C) were calculated from the slopes multiplied by -2.303 and are presented in [Table- 3]

For EN

Regression of (C) versus K′ gave the equation:

$K' = -0.133C - 3x10^{-5}$ (r = 0.8847)

For RM

Regression of (C) versus K′ gave the equation:

$K' = -0.6088\ C + 1\ x10^{-5}$ (r =0.9494)

For LS

Regression of (C) versus K′ gave the equation:

$K' = 0.2499C - 5x10^{-5}$ (r =0.9767)

For FOS

Regression of (C) versus K′ gave the equation:

$K' = -1.376\ C - 7x10^{-5}$ (r = 0.9103)

The values of correlation coefficient (r) are indicative of poor linearity, probably because of inconsistency of K'

Fixed-Absorbance method

Reaction rates were recorded for different drugs concentrations in the range of $4.061x10^{-5}$-$8.122x10^{-5}$ M of EN, $6.002x10^{-5}$-$8.403x10^{-5}$ M of RM, $5.661x10^{-5}$-$7.927x10^{-5}$ M LS and 6.830 $x10^{-5}$-10.245 $x10^{-5}$ M of FOS. A preselected value of the absorbance 0.5 for EN and 0.6 for RM, LS and FOS was fixed and the time was measured in seconds. The reciprocal of time (1/t) versus the initial concentration of drugs was plotted as shown [Table- 4]

Table 4: Values of reciprocal of time taken at fixed absorbance (0.5) for en and (0.6) for rm, ls and fos for different rates of variableconcentrations of drugs.

1/t sec^{-1}	[EN] mol L^{-1}
$6.29x10^{-4}$	$4.061x10^{-5}$
$1.85x10^{-3}$	$6.091x10^{-5}$
$3.03x10^{-3}$	$8.122x10^{-5}$
1/t sec^{-1}	[RM] mol L^{-1}
$9.26x10^{-4}$	$6.002x10^{-5}$
$1.52x10^{-3}$	$7.203x10^{-5}$
$2.38x10^{-3}$	$8.403x10^{-5}$
1/t sec^{-1}	[LS] mol L^{-1}
$6.67x10^{-4}$	$5.662x10^{-5}$
$1.51x10^{-3}$	$6.795x10^{-5}$
$2.38x10^{-3}$	$7.927x10^{-5}$
1/t sec^{-1}	[FOS] mol L^{-1}
7.092 $x10^{-4}$	6.830 $x10^{-5}$
1.587 $x10^{-3}$	$8.538x10^{-5}$
3.03 $x10^{-3}$	10.245 $x10^{-5}$

The following equations for calibration graphs were obtained by linear regression

1/t= 59.124 C - 0.0018 r = 0.9997 for EN

1/t= 60.557 C - 0.0028 r = 0.9944 for RM

1/t= 75.629 C - 0.0036 r = 0.9999 for LS

1/t= 67.957 C - 0.004 r = 0.9903 For FOS

The ranges of EN, RM, LS and FOS concentration giving the most acceptable calibration graphs with the above equations were (20-40 µg ml^{-1}), (25-35 µg ml^{-1}), (25-35 µg ml^{-1}) and (40-60 µg ml^{-1}) respectively. These narrow ranges could be considered a disadvantage for fixed absorbance method.

Application

In the present work different kinetic methods were studied to determine the EN, RM, LS and FOS concentration by fixed time method, rate constant method and fixed absorbance method. The fixed time method was chosen to be applied for the determination of EN, RM, LS and FOS in the bulk powder and in dosage form. The concentrations of the cited drugs were calculated using the corresponding regression equations at fixed times. The most acceptable values of correlation coefficient and intercept were obtained for a fixed time of 30 min. except FOS 25 min.. The method could be applied over the concentration range 5 – 40 µg ml^{-1} for EN, 5 – 35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} for FOS. The results obtained for the analysis of the cited drugs in bulk powder were compared with BP [1] method for EN, RM, and LS and the reported HPLC method [37] for FOS (Table- 5). The student t-test and F-test values at 95% confidence level did not exceed the theoretical values indicating no significant difference between the performance of the two methods regarding accuracy and precision. More over the method was successfully applied for the analysis of the studied drugs in pharmaceutical formulations and standard addition technique was also applied. The validity of the method was accessed by statistical analysis of the regression data [Table- 6].

Table 5: t-testes of significane of the proposed $KMnO_4$ method for the determination of en, rm, ls and fos.

Statistical terms	EN		RM		LS		FOS	
	official method[1]	$KMnO_4$ method	Official method[1]	$KMnO_4$ method	official method[1]	$KMnO_4$ method	Reported method [37]	$KMnO_4$ method
Mean*	99.84	99.95	99.91	99.81	99.17	99.60	99.96	99.75
SD	0.43	0.57	0.61	0.30	0.36	0.56	0.39	0.24
SE	0.18	0.25	0.25	0.13	0.15	0.25	0.16	0.11
N	6	5	6	5	6	5	6	5
V*	0.18	0.32	0.37	0.09	0.13	0.31	0.15	0.06
t-test	(2.262*)	0.357		0.355		1.475		1.082
F-ratio	(5.19*)	1.78		4.11		2.38		2.5

*The values between parentheses are the theoretical value of t and F test.
*mean : mean recovery percent
* v : variance

Table 6: Results obtained by the proposed method for the determination of en, rm, ls and fos in bulk powder and dosage forms.

Items	EN	RM	LS	FOS
Linearity range (µg ml^{-1})	5-40 µg ml^{-1}	5-35 µg ml^{-1}	5-35 µg ml^{-1}	15-60 µg ml^{-1}
Regression equation	A= 0.0246C-0.0017	A= 0.029C+0.0053	A= 0.027C-0.0019	A= 0.0156-0.0007
correlation coefficient (r)	0.9995	0.9997	0.9999	0.9996
$Sb^{(1)}$	0.0142	0.016	0.014	$7.72X10^{-3}$
$Sa^{(2)}$	0.667	0.740	0.646	0.693
SD of the estimation	0.407	0.403	0.352	0.300
Accuracy (mean ± S.D)				
1-Drug in bulk	99.95 ± 0.57	99.81± 0.30	99.60± 0.56	99.75 ± 0.24
2-Drug in dosage forms	99.97±0.10	99.33± 0.28	99.81± 0.20	99.48 ± 0.27
3-Drug added	99.44±0.36	99.46± 0.58	99.73 ± 0.91	99.67 ± 0.36

(1) = Standard deviation of intercept
(2) = Standard deviation of slope

Conclusion

The kinetically – based method in this work for the quantitative determination of some ACEI EN, RM, LS, and FOS is accurate, simple, non expensive and offers a contribution for routine analysis of the cited drugs in the bulk powder and in pharmaceutical formulations.

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Kietic Spectrophotometric Determination of some Angiotensin Converting Enzyme Inhibitors using Potassium Permanganate.

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Summary: A kinetic spectrophotometeric method has been developed for the determination of some angiotensin converting enzyme inhibitors (ACEI) namely, enalapril maleate (EN), ramipril (RM), lisinopri (LS) and fosinopril sodium (FOS) in the bulk powder and in pharmaceutical formulations. The procedure is based on kinetic investigation of the oxidation reaction of those drugs with alkaline potassium permanganate at room temperature at 610 nm. The fixed time method was utilized for constructing the calibration graph to determine the concentration of the drugs. The absorbance – concentration plot is rectilinear over the range of 5 - 40 µg ml^{-1} for EN, , 5-35 µg ml^{-1} for both RM and LS and 15-60 µg ml^{-1} for FOS with percentage mean recovery (99.81 ± 0.30, 99.95 ± 0.57, 99.60 ± 0.56 and 99.75 ± 0.24) respectively. Different experimental parameters affecting the development and stability of the colour were carefully studied and optimized. The results obtained were in good agreement with those obtained by potentiometric method for EN, RM and LS and HPLC method for FOS.

Key words: enalapri maleate, ramipril, lisinopril, fosinopril sodium, Potassium permanganate, spectrophotometric method, kinetic.

Introduction

Enalapril maleate1-[N-[(S)-1-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline 1-ethylester, ramipril; (2S,3aS,6aS)-1-[(S)-N-[(S)-1-carboxy-3-phenylpropyl]-alanyl]-octahydrocylopenta [b]pyrole-2-carboxylic acid 1-ethyl ester, lisinopril; (S)-1-[N^2-(1-Carboxy-3-phenylpropyl]-L-lysyl]-L-proline and fosinopril sodium (4S)-4-Cyclohexyl-1-[[[(RS)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)-phos-phinyl]acetyl]-L-Proline propionate, are an active inhibitors of angiotensin converting enzyme (ACE) with antihypertensive activity[1-2]. Those drugs used in the treatment of all forms of hypertension and heart failure [3].

Different methods published for their determination such as spectrophotometric methods [4-15], atomic absorption spectroscopy [16, 17], capillary electrophoresis [18 - 21], HPLC [22 - 30], GC [31 - 33], radioimmunoassay [34]

Kinetic- based methods of pharmaceutical analysis are not widely applied, although they offer the advantage of simplicity, low costs, and wide availability in quality control laboratories. Furthermore eliminating additives interferences, which probably affects other methods such as titrimetry and direct spectrophotometric methods [35]

In the present work, simple, valided kinetically based method is proposed for the determination of some ACEI namely EN, RM, LS and FOS by measuring the absorbance at 610 nm after their oxidation with alkaline $KMnO_4$ at ambient temperature 25 ± 5 C^0

Experimental

Apparatus: Shimadzu 1601 U V Spectrophotometer, Japan.

Materials and reagents*:*

All chemicals and reagents were of analytical grade

Enalapril maleate was assayed for purity according to the official method[1] to contain 99.84 ± 0.43 and Renetic tablets B.No (0311057) nominally containing enalapril maleate (5 mg) were manufactured and supplied by MSD, Co, Egypt, ramipril was assayed for purity according to the official method [1] to contain 99.91 ± 0.61 and tritace tablets batch number (14E03) nominally containing ramipril (5 mg) ,were manufactured and supplied by Hochest, Co, Egypt, lisinopril was assayed for purity according to the official method[1] to contain 99.17 ± 0.36and zystril tablets batch number (1204148) nominally containing lisinopril (10 mg), were manufactured and supplied by Sedico,Co, Egypt and Fosinopril sodium its purity was found to be 99.96 ± 0.39 according to the HPLC manufactural method[37] and Monopril tablets nominally containing fosinopril sodium (10 mg), were manufactured and supplied by Bristol-Mayers squib- Co, Egypt. Potassium permanganate (Merck, Germany), 8 mM (0.1264% w/ v) in water. Sodium hydroxide used as 0.5 M (EL-Nasr Chemical Company, Egypt)

Stock solutions:

Stock solutions of EN, RM, LS (0.01 %w/ v) and FOS solution (0.02 %w/v) were prepared by dissolving the specified amount of each drug in water. The solutions were stable for one week if kept in a refrigerator.

Procedures

For calibration graph

Aliquots of the stock solutions equivalent to (0.1 - 0.3 mg) of EN, (0.1 - 0.25 mg) of RM, (0.1 - 0.25 mg) of LS and (0.2 - 0.5 mg) of FOS, were transferred into 10-ml volumetric flasks, 1-ml of 0.5 M NaOH for EN and 1.5-ml for RM, while 2-ml for both LS and FOS were added to each flask, followed by the addition of 3-ml of 8 mM of potassium permanganate solution. The content of each flask were mixed and the volumes were completed to the mark with water and allowed to stand at ambient temperature for 30 min. for all drugs, except for FOS 25 min. were found to be adequate. For calibration curve the values of absorbance were plotted against the drug concentrations, alternatively, the corresponding regression equations were driven.

Procedure for tablets

For EN in Ezapril tablets and RM in Tritace tablets. Ten tablets were weighed and pulverized. A quantity of each powdered tablets equivalent to 10 mg of EN or RM were accurately weighed and transferred into 50 ml conical flask. The powdered tablets were dissolved in 30 ml of methanol using magnetic stirrer for 30 min., then the extracts were filtered and the residue washed with methanol (5 ml x 4), the combined filtrate and washing was evaporated and the residues were dissolved in water and transferred quantitatively into 50 ml volumetric flasks. The volumes were completed to the mark with water and the method proceeded as mentioned under 2.2.1

For LS in Zystril tablets. A quantity of powdered tablets equivalent to 10 mg was accurately weighed and transferred into 250 ml conical flask, then extracted with 200 ml of chloroform using magnetic stirrer for 30 min. The solution was filtered and the residue washed with chloroform (10 ml x 5). The filtrate was evaporated; the residue was dissolved in water and transferred quantitavely into 50-ml volumetric

flask. The volume was completed to the mark with water and proceeded as directed under 2.2.1

For FOS in Monopril tablets. A quantity of powdered tablets equivalent to10 mg FOS was accurately weighed, transferred into 50-ml conical flask, then extracted with 30 ml chloroform using magnetic stirrer for 10 min. The solution was filtered and washed with chloroform (5 ml x 4). The combined chloroformic extracts containing interfering povidone were rejected. The residue left on the filter paper was dried and extracted with 50 ml methanol for 30 min. using magnetic stirrer, the solution was filtered and washed with methanol (5 ml x 4). The extract was evaporated and the residue was dissolved in water into 50 ml volumetric flask. The volume was completed to the mark with water and proceeded as under 2.2.1

The procedures were repeated applying the standard addition technique. The recovered concentration of labeled and added EN, RM, LS and FOS were calculated

Results and Discussion

The UV absorption spectra of EN, RM, LS and FOS shown in [Figure-1] suffer from low intensity and lack of well-defined maxima, typical of unconjugated phenyl moiety; make it not useful for characterization or quantitative analysis of these compounds. This led to study the reaction of EN, RM, LS and FOS with alkaline $KMnO_4$ in an attempt to develop a simple and reliable method for their determination in bulk and in pharmaceutical formulations.

Kinetic and optimization of the reaction condition:

The reaction between EN, RM, LS, FOS and $KMnO_4$ in alkaline medium yields a colour due to the production of manganate ion, which absorbs at 610 nm [Figure-2]. As the intensity of the colour increases by time, it was deemed useful to elaborate kinetically determination of these drugs in bulk and in pharmaceutical preparations.

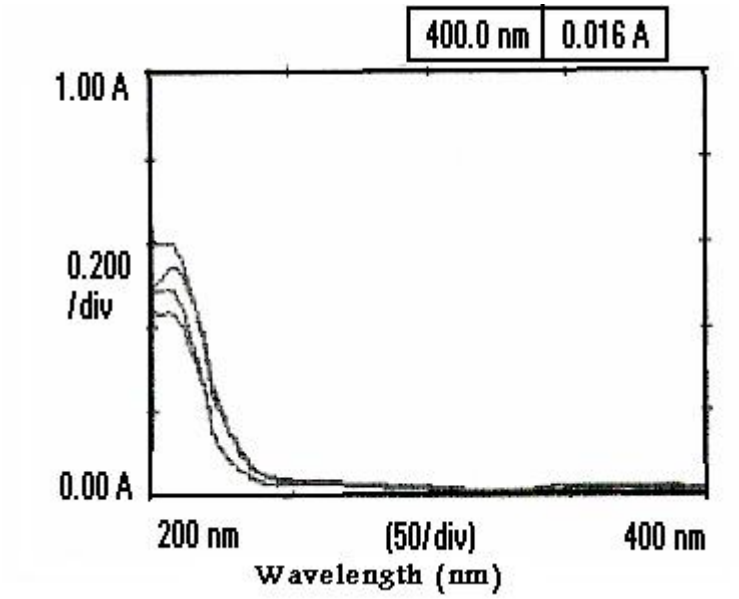

Figure 1: Absorbance spectra of EN(25 µg ml^{-1}), RM(25 µg ml^{-1}), LS(25 µg ml^{-1}) and FOS(25 µg ml^{-1}) in water

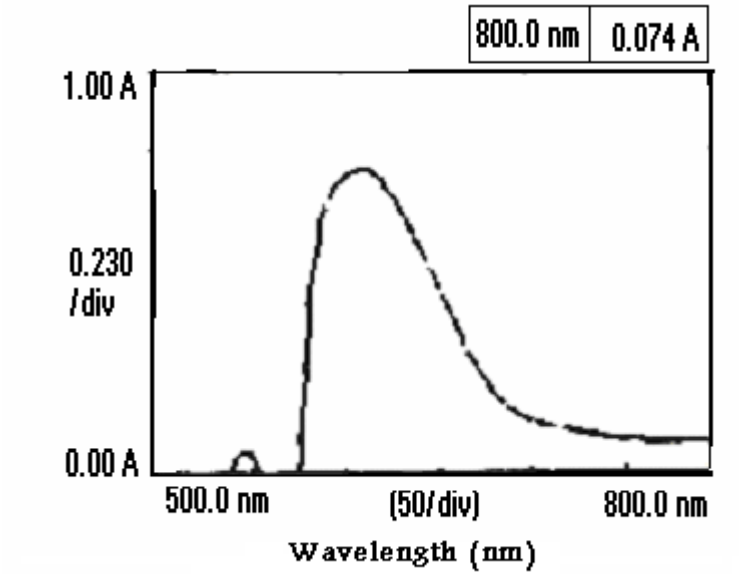

Figure 2: absorption spectrum of manganate ion obtained after the reaction of $KMnO_4$ with FOS (50 µg ml^{-1}) .

The reaction was studied under various conditions of reagent concentration, alkalinity and temperature; the effect of different solvents was also studied. Water was used to dissolve the drug since $KMnO_4$ oxidizes other solvents with the production of green manganate ions.

At room temperature the reaction rate increased substantially by time [Figure- 3], as revealed by the increase in the intensification of the developed colour and subsequent increase in the slopes of the calibration graphs [Table -1] indicating high analytical sensitivity. Heating the solution was found to increase the rate of the reaction but MnO_2 was precipitated. Therefore, room temperature was selected as the optimum temperature.

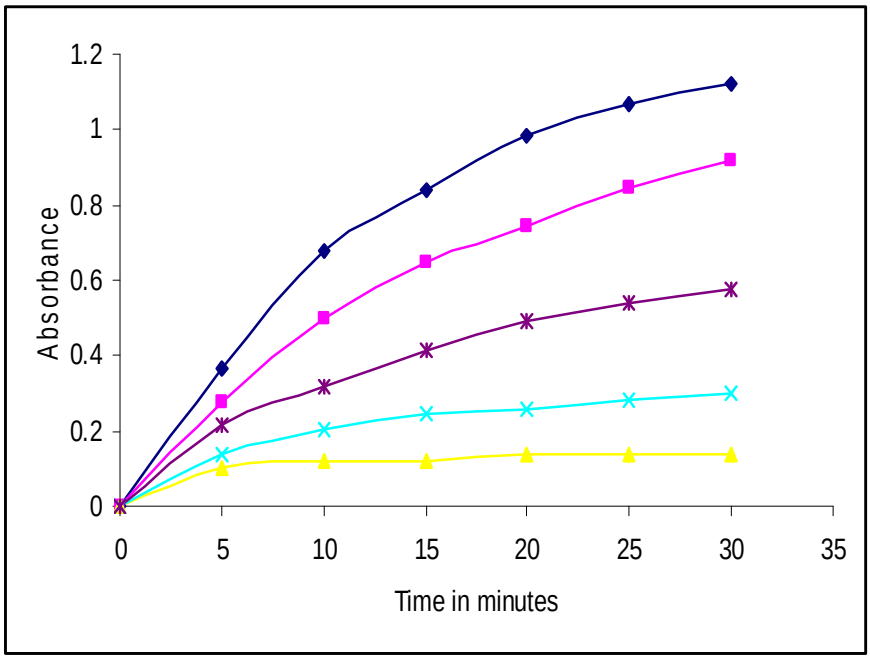

Figure 3: Absorbance versus time graph for the reaction between EN (5 – 40 µg ml^{-1}) and potassium permanganate

The reaction rate and maximum absorbance increased by time, and with increasing $KMnO_4$ concentration. It was found that 3 ml of 8 mM of $KMnO_4$ solution was adequate for the maximum absorbance.

The influence of NaOH concentration on the reaction rate was also studied. It was found that increasing the amount of 0.5 M NaOH would increase the absorbance of the reaction product up to 1 ml, 1.5 ml for EN and RM respectively and 2 ml for both LS and FOS, after which further increase does not affect the absorbance of the reaction product. Trials were made through oxidation with $KMnO_4$ in neutral and acidic medium, but no oxidation was observed.

The rate of the reaction was also found to be dependent on the drug concentration.

The rates were followed at room temperature with various concentrations of the cited drugs in the range of 5 – 40 µg ml^{-1} for EN, 5- 35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} of FOS keeping $KMnO_4$ and NaOH concentrations constant. The results in [Table- 1] clearly indicate that the reaction obeys the following equation:-

$$\text{Rate} = K' [\text{drug}]^{n} \quad (1)$$

Where K′ is the pseudo- order rate constant and n is the order of the reaction.

The rate of the reaction ($\Delta A/\Delta t$) may be estimated by the variable time method measurement [36], where A is the absorbance and t is the time in seconds. Taking logarithms of rates and concentrations [Table-2] and equation (1) is transformed into:

$$\log (\text{rate}) = \log \Delta A/\Delta t = \log K' + n \log [\text{drug}]. \quad (2)$$

For EN

Regression of log (rate) versus log [EN] gave the regression equation:

$\log \text{rate} = 0.817 \log C - 0.2056$ ($r = 0.9909$)

Hence $K' = 1.605$ Sec^{-1} and the reaction is first order $n = 0.817$ with respect to EN concentration.

For RM

Regression of log (rate) versus log [RM] gave the regression equation:

$\log \text{rate} = 1.3805 \log C + 2.2147$ ($r = 0.9955$).

Hence $K' = 163.95$ Sec^{-1} and the reaction is first order $n = 1.3805$ with respect to RM concentration.

For LS

Regression of log (rate) versus log [LS] gave the regression equation:

$\log \text{rate} = 0.8001 \log C - 0.3293$ ($r = 0.9948$).

Hence $K' = 2.135$ Sec^{-1} and the reaction is first order $n = 0.8001$ with respect to LS concentration.

Table 1: Calibration equations at different fixed time for EN, RM, LS and fos over the concentration ranges 5-40 µg ml^{-1}, 5-35 µg ml^{-1}, 5-35 µg ml^{-1} A and 15 – 60 µg ml^{-1} respectively

Time (min.)	Regression equations			
	EN	RM	LS	FOS
5	A = 0.0125 C + 0.0141 r = 0.9919	A = 0.00139 C + 0.041 r = 0.9932	A = 0.0173 C + 0.0393 r = 0.9968	A = 0.009C +0.0381 r = 0.9987
10	A = 0.0155C + 0.0321 r = 0.9970	A = 0.0179C + 0.0230 r = 0.9954	A = 0.0201C + 0.0356 r = 0.9984	A = 0.0114C -0.0258 r = 0.9987
15	A = 0.0181C + 0.0351 r = 0.9985	A = 0.0219C + 0.0093 r = 0.9975	A = 0.0222C + 0.0224 r = 0.9988	A = 0.0128 C -0.0330 r = 0.991
20	A = 0.0201C + 0.0396 r = 0.9991	A = 0.0245C + 0.0031 r = 0.9988	A = 0.0235C + 0.004 r = 0.9991	A = 0.0142C-0.0290 r = 0.9992
25	A = 0.0219C + 0.0381 r = 0.9991	A = 0.0265C + 0.0169 r = 0.9990	A = 0.0249C + 0.0109 r = 0.9995	A = 0.0143C+0.0198 r = 0.9995
30	A = 0.0240C + 0.0312 r = 0.9998	A = 0.0289C + 0.0083 r = 0.9996	A = 0.0263C + 0.0097 r = 0.9996	A = 0.0146 C +0.0189 r = 0.9993
35	A = 0.0261C + 0.0291 r = 0.9992	A = 0.0289 C + 0.0079 r = 0.9990	A = 0.0269 C + 0.0078 r = 0.9992	

For FOS

Regression of log (rate) versus log [FOS] gave the regression equation:

Log rate =1.2173 log C +1.2549 (r = 0.9571)

Hence K′ = 17.98 Sec^{-1} and the reaction is first order n =1.2173 with respect to FOS concentration.

Table 2: logarithms of rates for different concentrations of drugs (mol l^{-1}) applying the suggested method

log ΔA/Δt	.log [EN]
-4.313	-4.994
-3.986	-4.693
-3.820	-4.391
-3.650	-4.215
log ΔA/Δtl	log [RM]
-4.585	-4.921
-4.194	-4.620
-3.852	-4.444
-3.737	-4.319
-3.641	-4.215
-3.516	-4.148
log ΔA/Δt	log [LS]
-4.313	-4.946
-4.027	-4.645
-3.891	-4.469
-3.783	-4.344
-3.706	-4.247
-3.695	-4.168
-3.631	-4.101
Log ΔA/Δt	log [FOS]
-4.310	-4.592
-4.170	-4.466
-3.960	-4.291
-3.914	-4.166
-3.787	-4.069
-3.464	-3.989

3.2. Evaluation of the kinetic methods

The quantitation of the cited drugs under the optimized experimental conditions outlined above would result in a pseudo-first order with respect to their concentrations. Where, $KMnO_4$ concentration was at least 75 times the concentration of EN, RM and LS drugs, and 25 times for FOS. While NaOH

concentration was at least 400 times the initial concentration of EN, 600 times of RM, 800 times of LS and 266.7 times for FOS.

However, the rate will be directly proportional to cited drugs in a pseudo-first order rate equation as follows:

Rate = K′ [drug] ……..Eq. (3)

Where, K′ is the pseudo-first order rate constant. Several experiments were then carried out to obtain drug concentration from the rate data according to equation (3). Fixed time method, rate constant method and fixed absorbance method were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the intercept and the correlation coefficient (r).

Fixed-Time method:

Reaction rates were determined for different concentrations of drugs at a preselected fixed- time, which was accurately determined, the absorbance versus initial concentration of drugs were established at fixed times of 5, 10, 15, 20, 25 and 30 min. for EN, RM and LS and 5, 10, 15, 20, and 25 min. for FOS. With the regression equations assembled in [Table- 1].

It is clear that the slope increases by time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time of 30 min. for EN, RM and LS and 25 min. for FOS which was therefore chosen as the most suitable time interval for measurement.

After optimizing the reaction conditions, the fixed time method was applied to the determination of the drugs concentrations in bulk powder over the concentration range 5– 40 µg ml^{-1} for EN, 5-35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} for FOS. Analysis of the data gave the following regression equations:

A = 0.0246 C – 0.0017	r = 0.9995	EN
A = 0.029 C+ 0.0053	r= 0.9997	RM
A = 0.027 C – 0.0019	r = 0.9999	LS
A = 0.0156 C – 0.0007	r = 0.9996	FOS

Where A is the absorbance at 610 nm and C is the concentration in µg ml^{-1}.

Table 3: Values of k′calculated from slopes of log a versus t graph multiplied by -2.303 for different concentration of drugs.

$K'sec^{-1}$	[EN] mol L^{-1}
$-0.344x10^{-4}$	$4.061x10^{-5}$
$-0.346x10^{-4}$	$6.092x10^{-5}$
$-0.390x10^{-4}$	$8.122x10^{-5}$
$K'sec^{-1}$	[RM] mol L^{-1}
-0.167×10^{-4}	1.200×10^{-5}
-0.223×10^{-4}	$2.401x\ 10^{-5}$
-0.360×10^{-4}	3.601×10^{-5}
-0.365×10^{-4}	$4.802x\ 10^{-5}$
$K'sec^{-1}$	[LS] mol L^{-1}
$-0.375x10^{-4}$	$3.397x10^{-5}$
$-0.342x10^{-4}$	$4.530x10^{-5}$
$-0.329x10^{-4}$	$5.662x10^{-5}$
$-0.275x10^{-4}$	$6.795x10^{-5}$
$-0.267x10^{-4}$	$7.927x10^{-5}$
$K'sec^{-1}$	[FOS] mol L^{-1}
$-0.128\ x10^{-4}$	$3.415\ x10^{-5}$
$-0.133x10^{-4}$	5.123×10^{-5}
$-0.175\ x10^{-4}$	$6.830\ x10^{-5}$

Rate-Constant method*:*

Graphs of log absorbance versus time for EN concentration in the range of $4.061x10^{-5}$ to $8.122x10^{-5}$ M, $1.200x10^{-5}$ – $4.802\ x10^{-5}$ M of RM, $3.397x10^{-5}$ – $7.927\ x10^{-5}$ M of LS and $3.415\ x10^{-5}$ – $6.830\ x10^{-5}$ M of FOS were plotted and all appear to be rectilinear. The pseudo-first order rate constants (K′) corresponding to different drug concentrations (C) were calculated from the slopes multiplied by -2.303 and are presented in [Table- 3]

For EN

Regression of (C) versus K′ gave the equation:

$K' = -0.133C - 3x10^{-5}$ (r = 0.8847)

For RM

Regression of (C) versus K′ gave the equation:

$K' = -0.6088\ C + 1\ x10^{-5}$ (r =0.9494)

For LS

Regression of (C) versus K′ gave the equation:

$K' = 0.2499C - 5x10^{-5}$ (r =0.9767)

For FOS

Regression of (C) versus K′ gave the equation:

$K' = -1.376\ C - 7x10^{-5}$ (r = 0.9103)

The values of correlation coefficient (r) are indicative of poor linearity, probably because of inconsistency of K'

Fixed-Absorbance method

Reaction rates were recorded for different drugs concentrations in the range of $4.061x10^{-5}$-$8.122x10^{-5}$ M of EN, $6.002x10^{-5}$-$8.403x10^{-5}$ M of RM, $5.661x10^{-5}$-$7.927x10^{-5}$ M LS and 6.830 $x10^{-5}$-10.245 $x10^{-5}$ M of FOS. A preselected value of the absorbance 0.5 for EN and 0.6 for RM, LS and FOS was fixed and the time was measured in seconds. The reciprocal of time (1/t) versus the initial concentration of drugs was plotted as shown [Table- 4]

Table 4: Values of reciprocal of time taken at fixed absorbance (0.5) for en and (0.6) for rm, ls and fos for different rates of variableconcentrations of drugs.

1/t sec^{-1}	[EN] mol L^{-1}
$6.29x10^{-4}$	$4.061x10^{-5}$
$1.85x10^{-3}$	$6.091x10^{-5}$
$3.03x10^{-3}$	$8.122x10^{-5}$
1/t sec^{-1}	[RM] mol L^{-1}
$9.26x10^{-4}$	$6.002x10^{-5}$
$1.52x10^{-3}$	$7.203x10^{-5}$
$2.38x10^{-3}$	$8.403x10^{-5}$
1/t sec^{-1}	[LS] mol L^{-1}
$6.67x10^{-4}$	$5.662x10^{-5}$
$1.51x10^{-3}$	$6.795x10^{-5}$
$2.38x10^{-3}$	$7.927x10^{-5}$
1/t sec^{-1}	[FOS] mol L^{-1}
7.092 $x10^{-4}$	6.830 $x10^{-5}$
1.587 $x10^{-3}$	$8.538x10^{-5}$
3.03 $x10^{-3}$	10.245 $x10^{-5}$

The following equations for calibration graphs were obtained by linear regression

1/t= 59.124 C - 0.0018 r = 0.9997 for EN

1/t= 60.557 C - 0.0028 r = 0.9944 for RM

1/t= 75.629 C - 0.0036 r = 0.9999 for LS

1/t= 67.957 C - 0.004 r = 0.9903 For FOS

The ranges of EN, RM, LS and FOS concentration giving the most acceptable calibration graphs with the above equations were (20-40 µg ml^{-1}), (25-35 µg ml^{-1}), (25-35 µg ml^{-1}) and (40-60 µg ml^{-1}) respectively. These narrow ranges could be considered a disadvantage for fixed absorbance method.

Application

In the present work different kinetic methods were studied to determine the EN, RM, LS and FOS concentration by fixed time method, rate constant method and fixed absorbance method. The fixed time method was chosen to be applied for the determination of EN, RM, LS and FOS in the bulk powder and in dosage form. The concentrations of the cited drugs were calculated using the corresponding regression equations at fixed times. The most acceptable values of correlation coefficient and intercept were obtained for a fixed time of 30 min. except FOS 25 min.. The method could be applied over the concentration range 5 – 40 µg ml^{-1} for EN, 5 – 35 µg ml^{-1} for RM and LS and 15-60 µg ml^{-1} for FOS. The results obtained for the analysis of the cited drugs in bulk powder were compared with BP [1] method for EN, RM, and LS and the reported HPLC method [37] for FOS (Table- 5). The student t-test and F-test values at 95% confidence level did not exceed the theoretical values indicating no significant difference between the performance of the two methods regarding accuracy and precision. More over the method was successfully applied for the analysis of the studied drugs in pharmaceutical formulations and standard addition technique was also applied. The validity of the method was accessed by statistical analysis of the regression data [Table- 6].

Table 5: t-testes of significane of the proposed $KMnO_4$ method for the determination of en, rm, ls and fos.

Statistical terms	EN		RM		LS		FOS	
	official method[1]	$KMnO_4$ method	Official method[1]	$KMnO_4$ method	official method[1]	$KMnO_4$ method	Reported method [37]	$KMnO_4$ method
Mean*	99.84	99.95	99.91	99.81	99.17	99.60	99.96	99.75
SD	0.43	0.57	0.61	0.30	0.36	0.56	0.39	0.24
SE	0.18	0.25	0.25	0.13	0.15	0.25	0.16	0.11
N	6	5	6	5	6	5	6	5
V*	0.18	0.32	0.37	0.09	0.13	0.31	0.15	0.06
t-test	(2.262*)	0.357		0.355		1.475		1.082
F-ratio	(5.19*)	1.78		4.11		2.38		2.5

*The values between parentheses are the theoretical value of t and F test.
*mean : mean recovery percent
* v : variance

Table 6: Results obtained by the proposed method for the determination of en, rm, ls and fos in bulk powder and dosage forms.

Items	EN	RM	LS	FOS
Linearity range (µg ml^{-1})	5-40 µg ml^{-1}	5-35 µg ml^{-1}	5-35 µg ml^{-1}	15-60 µg ml^{-1}
Regression equation	A= 0.0246C-0.0017	A= 0.029C+0.0053	A= 0.027C-0.0019	A= 0.0156-0.0007
correlation coefficient (r)	0.9995	0.9997	0.9999	0.9996
$Sb^{(1)}$	0.0142	0.016	0.014	$7.72X10^{-3}$
$Sa^{(2)}$	0.667	0.740	0.646	0.693
SD of the estimation	0.407	0.403	0.352	0.300
Accuracy (mean ± S.D)				
1-Drug in bulk	99.95 ± 0.57	99.81± 0.30	99.60± 0.56	99.75 ± 0.24
2-Drug in dosage forms	99.97±0.10	99.33± 0.28	99.81± 0.20	99.48 ± 0.27
3-Drug added	99.44±0.36	99.46± 0.58	99.73 ± 0.91	99.67 ± 0.36

(1) = Standard deviation of intercept
(2) = Standard deviation of slope

Conclusion

The kinetically – based method in this work for the quantitative determination of some ACEI EN, RM, LS, and FOS is accurate, simple, non expensive and offers a contribution for routine analysis of the cited drugs in the bulk powder and in pharmaceutical formulations.

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Thermal Analysis of Some Anti-Diabetic Pharmaceutical Compounds

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Summary: Thermal analysis of some interesting antidiabetic compounds was achieved. Thermogravimetry, derivative thermogravimetry (TG, DTG) and differential thermal analysis (DTA) were used to study the thermal behavior of pioglitazone hydrochloride, rosiglitazone maleate, glibenclamide and glimepiride. The results obtained are useful for the identification of these compounds, with special reference to their stability and also permitted interpretations concerning their thermal decomposition**.**

Introduction

The most widely used techniques are differential thermal analysis (DTA), and thermogravimetry/derivative thermogravi-metry (TG/DTG). Several methods have been reported for the determination of the studied drugs including chromatographic (1-4), electrochemical (5-7) and titrimetric (8-9) methods

The main objective of this study is to investigate the thermal behavior of pioglitazone hydrochloride (PTZ**)**, rosiglitazone maleate (RGZ)**,** glibenclamide (GBD) and glimepiride (GMP) raw materials using the TG , DTG and DTA techniques.

Experimental

Instrumentation

The measurements were made with simultaneous DTA-TG apparatus thermal analyzer (Shimadzu DTG -60). The weight of samples is ranging from 2.2 to about 6.6 mg, using a platinum pan. Measurement were carried out from ambient to 900 ºC in dynamic nitrogen atmosphere with the flow rate of 30 mL min^{-1} and heating rate of 10 ºC min^{-1}. The flow rate was measured using an electron flow meter, (Jack - Scientific, model # ADM1000).

Results and discussion

The TG-DTG and DTA curves of pioglitazone hydrochloride, rosiglitazone maleate, glibenclamide and glimepiride are shown in Figs. (1-5). The TG curves of pioglitazone and rosiglitazone show approximate stability with decomposition in four thermal decomposition stages while glibenclamide and glimepiride show decomposition in three steps. The results obtained from Fig. (5) suggested the following sequence of thermal stability: pioglitazone hydrochloride < rosiglitazone maleate <glibenclamide < glimepiride.

The DTA curve of pioglitazone (Fig. 1) shows a small endothermic peak at 187.13 ^{o}C due to fusion of the drug followed by another one at 252.05 ^{o}C and abroad one at 370.05 ^{o}C. Four exothermic peaks were observed: two small at 220.13 and 270.75 ^{o}C and two sharp peaks at 444.47 and 498.20 ^{o}C corresponding to the third and fourth steps in the DTG curve.

The DTA curve of rosiglitazone (Fig.2) shows an endothermic peak at 124.42 ^{o}C attributed to the melting of the compound followed by another endothermic one at 192.75 ^{o}C and a broad flattened one between 317.05 and 418.08 ^{o}C. Two small exothermic peaks are present at 144.26 and 233.49 ^{o}C and a big one at 556.49 ^{o}C which corresponds to the last step in the TG-DTG curves.

DTA curve of glibenclamide (Fig. 3) shows a sharp endothermic peak at 174.71 ^{o}C due to the melting of the drug which is in agreement with the value obtained from literature (172-174 ^{o}C), this peak is followed by a small and broad endothermic one (196.00-286.60 ^{o}C) which corresponds to the first step in the TG-DTG curves. A small endothermic peak at 350.81 ^{o}C which corresponds to the second step in the TG-DTG curve, is observed. The DTA curve shows a broad exothermic peak at 578.38 ^{o}C which is due the final pyrolysis of the drug and corresponds to the third step in the TG-DTG curve.

The DTA curve of glimepiride (Fig. 4) shows a sharp endothermic peak at 211.68 ^{o}C that corresponds to melting followed by a broad and flat exothermic peak between 220. 25 and 515.78 ^{o}C which is followed by a huge

and broad exothermic peak (515.78- 681.88 ^{o}C) corresponding to the third step in the TG-DTG curve.

By comparing the melting temperatures of the examined compounds obtained by using DTA and those stated in the literature(10), it is clear that the results of the DTA method are comparable with the literature figures and can be used for melting point determination for pioglitazone, rosiglitazone, glibenclamide and glimepiride.

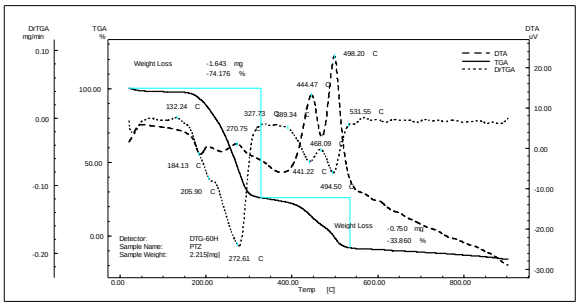

Fig.(1) TG-DTG & DTA for pioglitazone

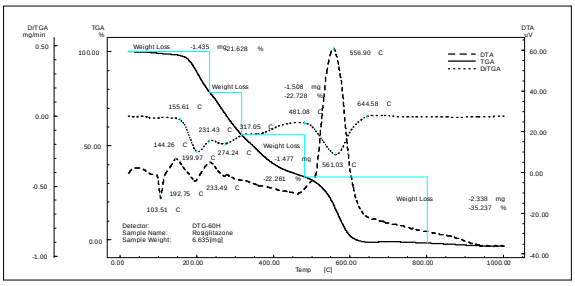

Fig.(2) TG-DTG & DTA for rosiglitazone

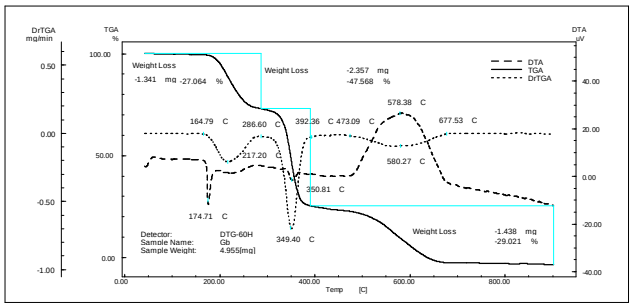

Fig.(3) TG-DTG & DTA for glibenclamide

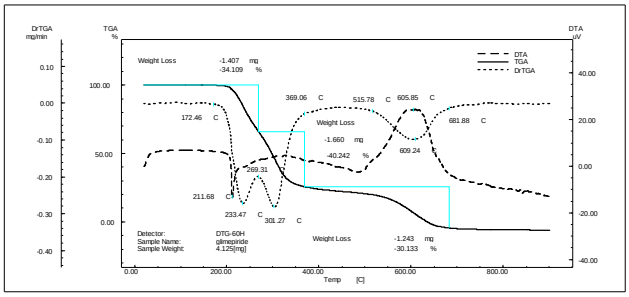

Fig.(4) TG-DTG & DTA for glimepiride

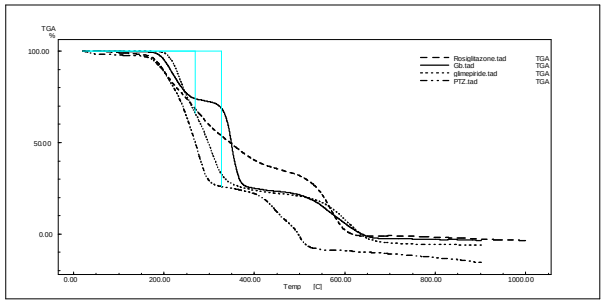

Fig. (5) TG curves for PTZ, RGZ, GBD and GMP

Conclusion

The TG-DTG and DTA curves permitted studies on the thermal stability and the thermal decomposition of some antidiabetic agents. The results demonstrated differences in thermal stability between the four drugs and suggested the following sequence of stability pioglitazone hydrochloride< rosiglitazone maleate <glibenclamide < glimepiride. The results justify the use of DTA as a routine technique for the identification of pioglitazone hydrochloride, glibenclamide and glimepiride, through the melting point.

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