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KINETIC SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF FOSINOPRIL SODIUM ANTIHYPERTENSIVE DRUG IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple kinetic spectrophotometric method has been described for the determination of fosinopril sodium (FOS) antihypertensive drug. The method depends on oxidation of the studied drug with alkaline potassium permanganate in presence of sodium hydroxide to produce a bluish- green colored species. The reaction is followed spectrophotometrically by measuring the rate of change of absorbance at room temperature for a fixed time of 10 min. The absorbance of the colored manganite ion is measured at 610nm. Beer's low is obeyed over the concentration range 2-30µgml^{-1}FOS using fixed time method. The relative standard deviation is 2.0 % for 5 replicates of 10µgml^{-1} of FOS. The results are validated statistically and checked through recovery studies. The method has been successfully applied for the determination of the studied drug in commercial dosage forms. Statistical comparison of the results with the reference methods show excellent agreement and indicate no significant difference in accuracy and precision.

Keywords: Fosinopril sodium- spectrophotometry- oxidation- kinetic- potassium permanganate.

1. INTRODUCTION

Fosinopril sodium(FOS) issodium (2S, 4S)-4-cyclohexyl-1-[[(R)-[(1S)-2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl)phosphoryl]acetyl]pyrrolidine-2-carboxylate (Fig 1).It is an angiotensin converting enzyme (ACE) inhibitor used for the treatment of hypertension and some types of chronic heart failure. FOS is the only phosphinate- containing ACE

inhibitor. It is marked by Bristol- Myers Squib under the trade name monopril[1]. Unlike other ACE inhibitors that are primarily excreted by kidneys, fosinopril is eliminated from the body via both renal or hepatic pathway [2] this characteristic of fosinopril makes the drug a safer choice than other ACE inhibitors for heart failure patients with impaired kidney function resulting from poor [3] perfusion as fosinopril can still be eliminated by liver preventing accumulation of the drug in the body.

Some methods have been reported for determination of fosinopril in pure form or in pharmaceutical formulations as well as in biological fluids. These methods including derivative- differential spectrophotometry comprised of measurement of the difference absorptivitiesderivatized in the first order of a tablet extract in 0.1 N NaoH relative to that of an equimolar solution in methanol [4]. Fourth derivative and multiwave UV spectrophotometry were also provided for simultaneous determination of fosinopril and hydrochlorothiazide in tablet [5,6] FOS was determined by flow injection spectrophotometry using UV- assisted digestion of the analyte using ammonium peroxodisulfate as the oxidizing agent [7]. FOS and its active metabolite fosinoprilate were determined in human plasma and serum using liquid chromatography tandem mass spectrometric method. [8,9]. A microemulsion system mixture as mobile phase has been used for the separation and analysis of fosinopril in plasma samples using liquid chromatographic method [10]. Other methods were reported such as solid state NMR, IR, High performance liquid chromatography and capillary zone electrophoresis [11-13]. The chromatographic method for the determination of FOS requires an automated system, which are not available in many research laboratories. Therefore, it was considered worthwhile to develop rapid and sensitive procedure suitable for the routine quality control analysis of the investigated drug. Spectrophotometric methods still belongs to the most frequently used analytical techniques in pharmaceutical analysis, which gives practical and significant economic advantages overother methods [14]. As a result in the present study, we provide spectrophotometric method for the determination of FOS in pure form and in pharmaceutical formulations based on oxidation of the studied drug with alkaline potassium permanganate in presence of sodium hydroxide to produce a bluish- green colored species. The reaction is followed spectrophotometrically by measuring the rate of change of absorbance at room temperature for a fixed time.

2-EXPERIMENTAL

2-1 Apparatus

A Shimadzu spectrophotometer with matched 1cm Quartz cell was used for all spectral measurements. pH metric measurements were done with Jenway 3510 pH meter. Water bath Profgerate-Werk Medingen W21 Germany was used to control the temperature.

2-2 Materials and solutions

All the materials were of analytical reagent grade. Solutions were prepared with double distilled water. Samples of fosinopril and monopril were supplied by Bristol- Myers Squib pharmaceutical Co., Cairo- Egypt, and were used without further purifications. Potasium permanganate (Merk, Germany) $6x10^{-3}$ M solution was prepared by dissolving 100mg In 100 ml double distilled water followed by boiling and filtration through sintered glass. It should be freshly prepared and molarity was checked titrimetrically. Sodium hydroxide was supplied from El Nasr chemical Co., AbuoZabbel, Egypt. Stock solution 10M was prepared by dissolving 40g of NaoH in 100 ml distilled water. Working solutions of lower concentrations were freshly prepared by appropriate dilution.

2-3 Procedures

2-3-1Recommended procedure and calibration curve

Transfer 2mL of $7.5x10^{-4}$ M $KMnO_4$ and 1 mL of 0.5M NaoH into a series of 50 mL volumetric flask add aliquot of volumes of FOS solution so that the final concentration in the range of 2-30μgml^{-1} shake and complete to the mark with distilled water. Measure the absorbance of the resulting solution at 610nm at 5 min intervals at ambient temperature (25ºC) against a reagent blank prepared simultaneously. To obtain the standard calibration curve, plot the values of absorbance against drug concentration in μgml^{-1} after 10 min.

2-3-2 Procedure for the tablets

Weight and pulverize ten tablets. Transfer a portion of the powder equivalent to 10 mg of FOS into a small beaker. Shake with 2x30 mL of acetone for 10 min. then filter into a conical flask. Wash the beaker and filter with few mL of acetone and pass the washings to the same flask. Evaporate the acetone using a rotatory evaporator at 55ºC till dryness. Dissolve the residue in 3x30 mL of water, and filter, if necessary, into 100mL volumetric flask, than complete to the mark with water. Proceed as described in the recommended procedure.

3- RESULTS AND DISCUSSION

3-1-Optimization of the reaction conditions

FOS was found to react with $KMnO_4$ in alkaline medium producing a bluish green color peaking at 610 nm (Fig 2). The spectrophotometric properties of colored product as well as the different experimental parameters affecting the color development and its stability were carefully studied and optimized. The intensity of the color produced increases gradually reaching its maximum after 10 min when it remains stable for at least 24 h. As the intensity of color increase with time it was deemed useful to elaborate a kinetically based method for the determination of FOS in bulk and in pharmaceutical forms. The reaction was investigated under various conditions of reagent concentration, alkalinity, surfactant and sensitizers. At room temperature the reaction increased substantially with time, as revealed by intensification of the developed color and subsequent increase in the slope of the calibration graph (Table1) indicating high analytical sensitivity.

3-2 The influence of $KMnO_4$

The reaction rate and absorbance increases with increasing $KMnO_4$ concentration. The absorbance was studied in the range $1x10^{-4}$ to $1x10^{-3}$ mol L^{-1} keeping all other parameter constant. It was found that $7.5x\ 10^{-4}$mol L^{-1} $KMnO_4$ is the optimum concentration for the absorbance as shown in (Fig 3)The effect of the color development was investigated by adding different volumes (0-2.6mL) of $7.5x\ 10^{-4}$ mol L^{-1} potassium permanganate to the drug under investigation. The maximum absorbance of the green color was attained with 2 mL of the reagent, and remained nearly constant even when higher volumes were added (Fig 4). There for, 2ml of the reagent was used throughout the experimental investigation.

3-3 The influence of the NaoH

Complete reaction between $KMnO_4$ and FOS takes place only in alkaline medium. The reaction rate and absorbance increase with increasing $KMnO_4$ concentration on the formation of MnO_4^{2-} was also examined at constant concentration of drug, permanganate and varying volume (0.1- 1.5mL) of 0.5 mol L^{-1}NaOH at 25ºC. The optimum absorbance was obtained with1.0 mL of 0.5 molL^{-1}NaOH, after which any increase in volume caused no significant change in absorbance. Hence 1 mL of 0.5mol L^{-1} NaOH was used throughout the recommended experiments (Fig5). Other alkalies, such as KOH and NH_4OH with the same concentration were also tested to identify the best alkaline medium. However, their effect on color development was less than that of NaOH, Therefor the latter was used during the study.

Different sensitizers (quinine, xyclohexane-diol, fluorescein and rhodamine-B), at concentration of 20 µgml^{-1} were tested by adding to the reactants mixture. Outstanding inhibitory effects were observed as these sensitizers reacted strongly with The $KMnO_4$ – NaOH system (Table2). In the same manner the effect of surfactant on the color development was studied. Different surfactant (citrimide, gelatin and sodium lauryl sulfate) at three different concentration 2.5, 7.5 and 15 µgml^{-1} were tested by adding to the reactant mixture, All tested surfactant react strongly with the $KMnO_4$ –NaOH system with inhibitory effect, as evident from the low absorbance reading (table3) Potassium permanganate is consumed by the surfactants, being reduced to reduction products other than the measured species.

3-4 Calibration graphs

After optimizing the reaction conditions, the fixed time was applied to the determination of FOS in pure form over concentration range 2-30µgml^{-1}. Liner regression analysis of the data (n=6) gave the following equation

A= 0.0797+ 0.0346C(R=0.999)

Where, A is the absorbance in 1 Cm cell. C is the concentration of the drug inµgml^{-1}. The apparent molar absorptivity was found to be 3.09x 10^4 mol^{-1} cm^{-1}. The calibration graphs were shown in Fig7.

3-5 Kinetic study of the reaction

The rate of the reactions was also found to be dependent on the concentration of FOS. The rates were followed at room temperature with various concentrations of FOS.

In the range 2-30 µg mL^{-1}, Keeping $KMnO_4$ and NaOH constant at the optimum concentration as described in the proposed procedure, from the graph shown in Fig 6.It is clear that the rate increases as the drug concentration increases, indicating that the reaction rate obeys the equation:

Rate = $K'[FOS]^n$ (1)

Where K is the pseudo first- order rate constant of the reaction and n is the order of the reaction. The rate of the reaction may be estimated by the variable- time method measured as $\Delta A/\Delta t$, where A is the absorbance and t is the measuring time in seconds[14]. Taking logarithms of the rates and concentration, as shown in table4, fig8, Eq. 1 is transformed into:

log (rate)= log$\Delta A/\Delta t$= log K' + n log [FOS] (2)

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

Log (rate) = -1.2805 + 1.0339 logC

r= 0.9977

Hence $K^{'}$ =0.0524 S^{-1}, and the reaction can be approximated to first order (n ≈ 1) with respect to drug concentration.

3-6 Evaluation of the kinetic method

The quantitation of the drug under the optimized experimental conditions outlined above would result in a pseudo- first order with respect to its concentration where $KMnO_4$ concentration was at least 50 times of the initial concentration of FOS and NaOH concentration was at least 100 times of the initial concentration of the drug. However, the rate will be directly proportional to drug concentration in a pseudo- first rate equation as follows:

Rate= $K^{'}$[Drug] (3)

Where $K^{'}$ is the pseudo-order rate constant. Several experiments were then carried out to obtain drug concentration from the rate according to Eq. (3). The rate constant, fixed concentration and fixed time methods[15] were tried and the most suitable analytical method was selected taking into account the sensitivity, applicability, correlation coefficient and the intercept.

4- Rate constant method

Graphs of log absorbance versus time for concentrations 1.4x 10^{-5} , 2.8x10^{-5} , 4.2x10^{-5} , 5.6x10^{-5},7.0x 10^{-5} and 8.4x10^{-5} mol L^{-1} FOS, were plotted applying the suggested method. The pseudo- first order rate constants corresponding to different FOS concentrations were then calculated from the slopes multiplied by – 2.303; as shown in table 5. Regression of concentration versus $K^{'}$ gave the equation:

$K^{'}$ =-3.2x10^{-3} + 66.95C r = 0.936

The value of r is indicating for poor linearity, probably because of inconstancy of $K^{'}$.

4-1 Fixed concentration method

Reaction rate was recorded for different FOS concentrations in the range of 5.6x10^{-5} to 8.4x10^{-5}mol L^{-1} applying the proposed method. A preselected value of the absorbance was fixed and the time was measured in seconds. The reciprocal of time versus the initial concentration of the cited drug was plotted (table 6). The following equation for calibration graphs were obtained by liner regression:

1/t = -4.4x10^{-3} + 76.92 C r=0.9835

4-2 Fixed time method

Reaction rate was determined for different concentration of FOS. At a preselected fixed time was accurately determined the absorbance was measured. Calibration graphs of the absorbance versus initial concentration of FOS were established at fixed time of 5,10,15,20,25 and 30 minwith the regression equation shown in Table 1. It is clear that the slope increases with time.

4-3stoichiometry of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method [16]. The absorbance of the reaction product was measured in the presence of excess of both $KMnO_4$ and FOS. A plot of log absorbance versus log [$KMnO_4$] and [FOS] gave straight lines. The ratio of the reaction was calculated by dividing the slope of $KMnO_4$ over the slope of the drug curve. It was found that the reaction proceeds in the ratioof 1:2 (FOS- $KMnO_4$).

5- Validation of the proposed method

5-1 Accuracy and precision of the proposed method

Accuracy and precision was checked according to USP validation guidelines[17] at three concentration levels within the specified range, six replicate measurements were recorded at each concentration level. The results are summarized in table 7.

5-2 Limit of detection (LOD)

LOD was calculated based on standard deviation and the slope of calibration curve. The limit of detection was expressed as:

$LOD= 3\sigma/ S$

Where σ is the standard deviation of intercept, S is the slope of calibration curve. The results were summarized in table 8 indicating good sensitivity of the proposed method. In the present work, good results were obtained where the calculated drug concentration by LOD equations were actually detected in the experiment.

5-3 Limit of quantification

LOQ was calculated based on slope and standard deviation of intercept of calibration curve. In this method, the limit of quantification is expressed as:

$LOQ = 10\sigma/S$

Results in table 8 indicate good sensitivity of the proposed method. According to USP validation guidelines[17], the calculated LOQ values should be further validated by

laboratory experiments. In our work, good results were obtained where the calculated drug concentration by LOQ equations were actually determined quantitatively in this experiment.

5-4 Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation in the experimental parameters on the analytical performance of the method[19]. In these experiments, one parameter was changed where as others were kept unchanged, and the recovery percentage was calculated each time. It was found that none of these variables significantly affected the performance of the method, the recovery values were 98.90- 100.90 ± 0.21-0.32% this provides an indication of the reliability of the proposed method during the routine application of the proposed method. Ruggedness was tested by applying the proposed method to the assay of the cited drug using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained were reproducible, as the relative standard deviations RSD did not exceed 2%.

6- Application to pharmaceutical dosage forms

The rate constant and fixed time methods of the proposed kinetic spectrophotometric method for determination of investigated FOS have been tested on commercial pharmaceutical dosage formmonopril. The concentration of the investigated drug was computed from its responding regression equation. The results of method were statistically compared with the official method [18], in respect to accuracy and precision. The obtained mean recovery values of the obtained amount ensure that there is no interference of other interactive compounds present in the tablet. The calculated and theoretical value of both the proposed and the reported methodat 95% confidence level indicates good precision and accuracy in the analysis of monopril tablet.

Table 1 Calibration equations at different fixed times for FOS in the range 2-30µg mL^{-1} applying the proposed method.

Time (min)	Regression equation	Correlation coefficient
5	A= 0.0012+0.0210C	0.992
10	A= 0.0797+ 0.0346C	0.999
15	A= 0.0212 + 0.0361C	0.994
20	A=0.0237 + 0.0375C	0.998
25	A= 0.0244 + 0.0513C	0.996
30	A= 0.0259 + 0.0586C	0.996

Table2 Effect of sensitizers on the performance of the proposed method with 20µgmL^{-1}FOS concentration.

Sensitizer	Absorbance
No sensitizer	0.712
Quinine	0.256
Cyclohexane- diol	0.129
Fluorescein	Precipitate
Rhodamine-B	0.201

Table3 Effect of surfactants on the performance of the proposed method using20µgmL^{-1}FOS concentration.

Surfactant	Concentration µgmL^{-1}	Absorbance
No surfactant		0.716
Cetrimide	2.5	0.596
Sodium lauryl sulfate	2.5	0.630
Gelatin	2.5	0.601
Cetrimide	7.5	0.211
Sodium lauryl sulfate	7.5	0.562
Gelatin	7.5	0.508
Cetrimide	15	0.117
Sodium lauryl sulfate	15	0.301
Gelatin	15	0.224

Table 4 Logarithms of the rates for different concentrations of FOS applying the proposed method.

Log ΔAlΔt	Log [drug] (mol L^{-1})
-3.442	-4.854
-3.190	-4.553
-2.991	-4.376
-2.860	-4.244
-2.781	-4.155
-2.701	-4.075

Table 5 Values of $K^{'}$ calculated from slops of log A versus t graphs for different drug concentrations.

$K^{'}$ (S^{-1})	[drug], (mol L^{-1})
$-8.4663x\ 10^{-4}$	$1.4x10^{-5}$
$-6.8323x10^{-4}$	$2.8x10^{-5}$
$-4.6241x10^{-4}$	$4.2x10^{-5}$
$-3.8372x10^{-4}$	$5.6x10^{-5}$
$-4.2558x10^{-4}$	$7.0x10^{-5}$
$-3.7788x10^{-4}$	$8.4x10^{-5}$

Table 6 Values of reciprocal of time taken at fixed absorbance for different rates of various concentrations of fosinopril applying the proposed method.

1/t (S^{-1})	[drug] (mol L^{-1})
$1.6x10^{-3}$	$5.6x10^{-5}$
$8.5x10^{-3}$	$7.0x10^{-5}$
$6.7x10^{-3}$	$8.4x10^{-5}$

Table7 Evaluation of precision of the proposed kinetic spectrophotometric method for determination of investigated FOS.

Amount taken µg mL^{-1}	Amount found µg mL^{-1}	%Recovery ±SD	± RSD[a] (%)	SAE[b]
10	9.89	98.90	0.430	0.0857
20	20.18	100.90	0.378	0.1301
30	30.13	100.43	0.269	0.1082

[a] Mean of six independent analyses.

[b] Standard analytical error.

Table 8 Analytical parameters for fixed time method of the kinetic spectrophotometric determination of FOS in pure form.

Analytical parameter	Results of fosinopril
Optical characters	
$\lambda_{max\ (nm)}$	610
Linearity range (µg mL^{-1})	2-30
Regression equation	
Intercept (a)	0.0797
Slope (b)	0.0346
Correlation Coefficient (r)	0.999

LOD(µg mL^{-1})	0.173
LOQ(µg mL^{-1})	0.519
Interday precision	99.97 ± 0.255
Intraday precision	99.92 ± 0.397

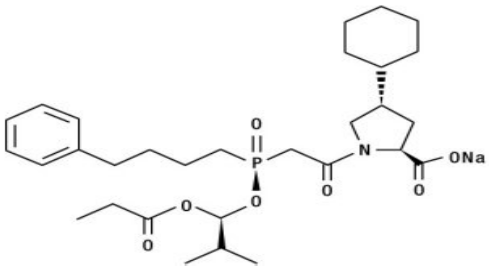

Fig 1 Chemical structure of fosinopril sodium

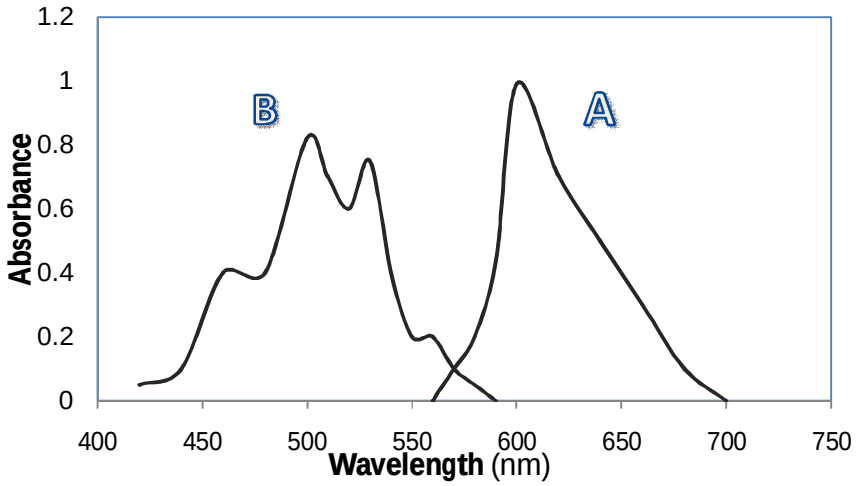

Fig 2 **Absorbance spectrum of the reaction product of FOS(** 25 **µg** mL^{-1}**) with** $KMnO_4$ **– NaOH system. (A) Reaction product. (B)** $KMnO_4$**:** $5x10^{-5}$ **M**

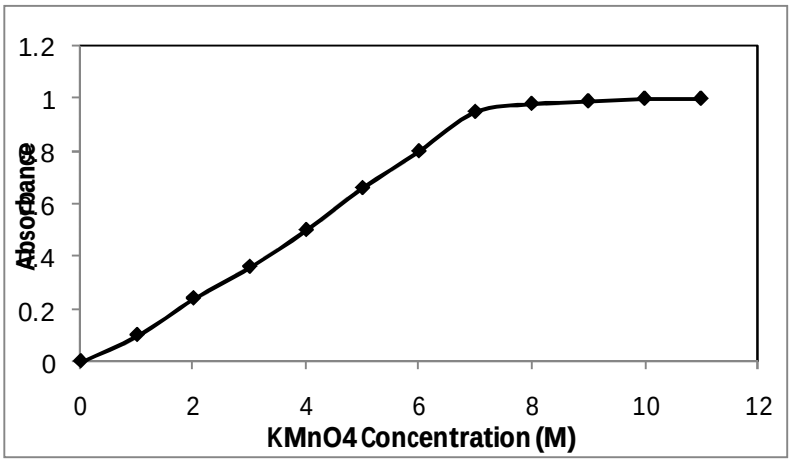

Fig 3 Effect of the concentration ranges $1x10^{-3}$ **to** $1x10^{-4}$**mol** L^{-1} **of** $KMnO_4$ **on the intensity of the color produced during the reaction (25µg** mL^{-1}**FOS)**

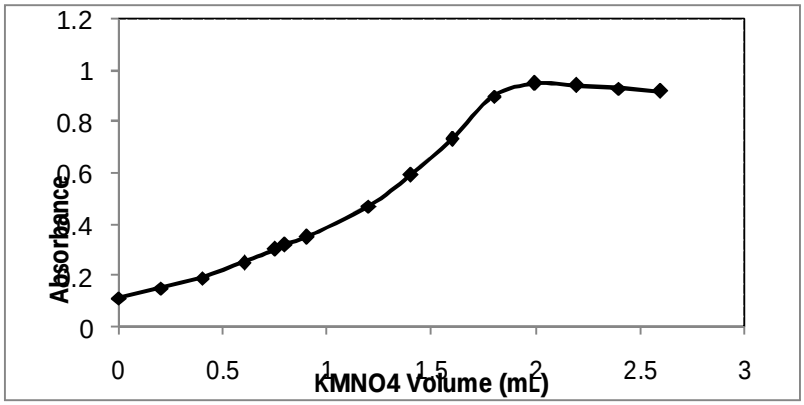

Fig 4 Effect of the volume of $7.5x10^{-4}$mol L^{-1} $KMnO_4$ on the intensity of the color product using 25µg mL^{-1}FOS.

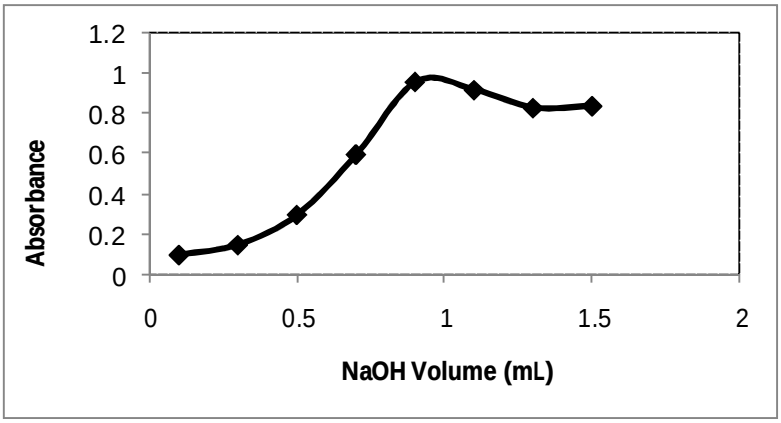

Fig 5 Effect of volume of 0.5 mol L^{-1}NaoH on the intensity of the colourproduced during the reaction of FOS 25µg mL^{-1} with 2ml of $7.5x\ 10^{-4}$ mol L^{-1} $KMnO_4$

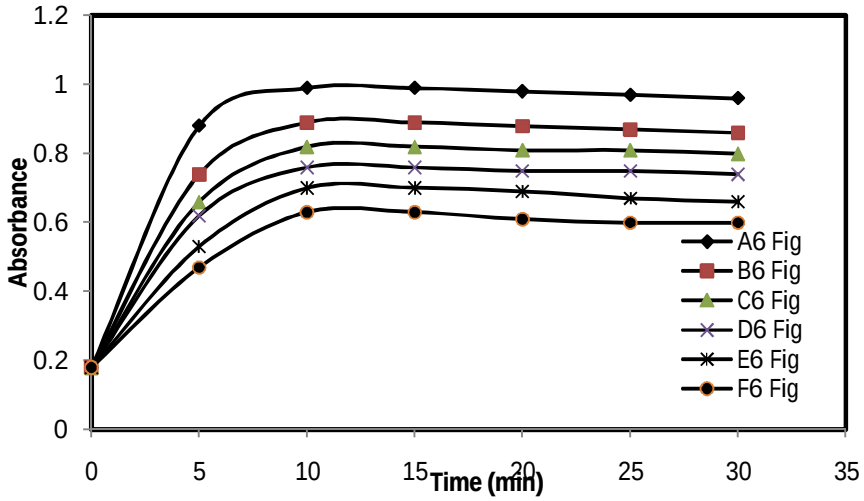

Fig 6 Absorbance versus time graphs for the reaction of FOS and alkaline $KMnO_4$ at 25°C concentration of FOS (A) $1.4x10^{-5}$, (B) $2.8x10^{-5}$, (C) $4.2x\ 10^{-5}$, (D) $5.6x10^{-5}$, (E) $7.0x10^{-5}$, (F) $8.4x10^{-5}$

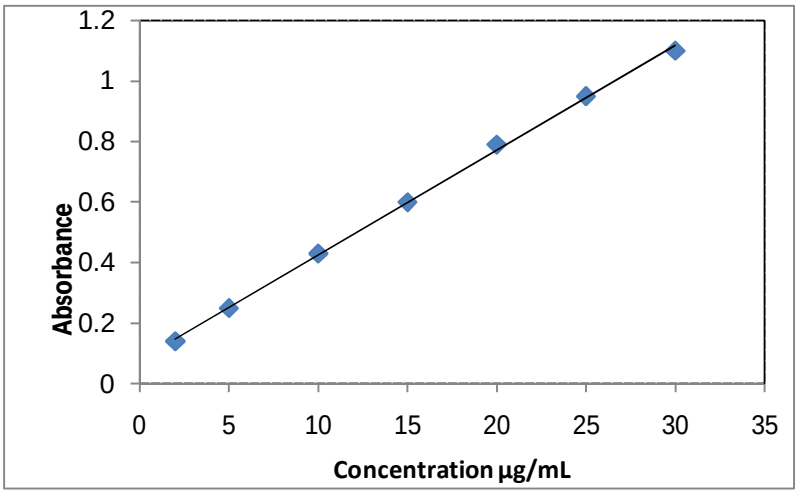

Fig 7 Spectrophotonetric calibration curve

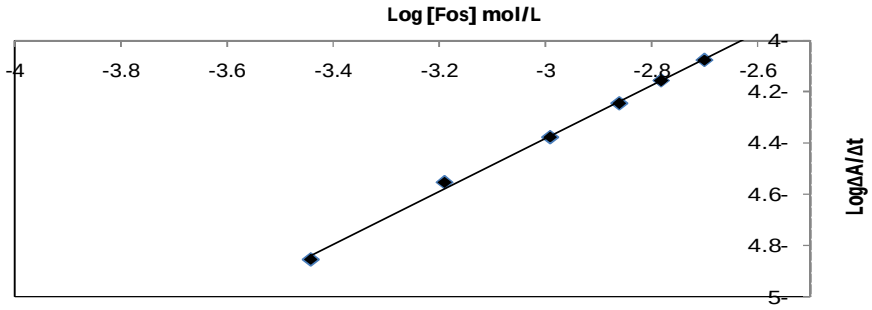

Fig 8 **Spectrophotometric plot shows logarithms of the rates for different concentrations of FOS applying the proposed method.**

7- CONCLUSION

The rate constant and fixed time methods are kinetically based spectrophotometric method can be easily applied for determination of FOS drug either in raw material or in pharmaceutical dosage forms. Applying the fixed time method, it is clear that the slope increased with time and the most acceptable values of correlation coefficient (r) and intercepts were obtained for a fixed time, which therefore chosen as the most suitable time interval for measurements. The proposed method is sensitive enough to enable determination of least amounts of drug, these advantage encourage the application of proposed method in routine quality control of investigated drug. Finally our method provide advantages of

improving selectivity, avoiding interference of colored and turbid background of samples as our method measure the increase in absorbance with timeagainst blank treated similarly, and possibility of avoiding interference of other active compound present in commercial product.

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