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DETERMINATION OF DRUGS BASED ON OXIDATION BYALKALINE $KMNO_4$: A KINETIC SPECTROPHOTOMETRIC STUDY

K. Damodhar Reddy, K. Sayanna and G. Venkateshwarlu*

Department of Chemistry, Osmania University, Hyderabad, Andhra Pradesh, India

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Correspondence to Author:

G. Venkateshwarlu

Department of Chemistry, Osmania University, Hyderabad-500 007, Andhra Pradesh, India
Email:venkateshwarlugoud@yahoo.com

ABSTRACT: Simple, accurate and precise spectrophotometric methods for quantitative determination of four drugs viz., Alfuzosin (ALF), Esomeprazole (ESO), Indomethacin (IND), Ketorolac Tromethamine (KET) have been developed based on oxidation of the drugs by alk.$KMnO_4$. Kinetics of the oxidation reaction is followed spectrophotometrically, as one of the reaction product, Mn(VI), absorbed at 610 nm. Initial rate and fixed time method are used for the construction of calibration curves Beer's law is obeyed in the range 12.5-100µg mL^{-1} for ALF; 25-150 µg mL^{-1} for ESO; 2.5-15 µg mL^{-1} for IND and 6.25-37.5 µg mL^{-1} for KET. Recovery studies using pure samples and pharmulations in the Beer's Law limits have been carried out and the methods have been validated in terms of ICH guidelines. Statistical analysis in terms of student's t-test and variance F- test demonstrate high accuracy and precision and suggest the methods can be applied in bulk drug and pharmaceutical industries.

INTRODUCTION: Alfuzosin is chemically known as N-[3-[4amino-6, 7-dimethoxy-quinazolin-2-yl)-methyl-amino] propyl] tetrahydro furan-2 carboxamide **Fig. 1a**, is an alpha1-adrenoreceptor blocker. It is used in the symptomatic treatment of urinary obstruction caused by benign prostatic hyperplasia and has been tried in the treatment of hypertension [1]. For the determination of ALF some analytical methods namely HPLC [2, 3] voltammetry [4,] Colorimetry [5,] spectrophotometry [6, 7] and Spectro-fluorimetry [8] has been described.

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Esomeprazole: Esomeprazole (ESO) 2-{1-[(4-cholorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl}acetic acid **Fig. 1b,** is a compound that inhibits gastric acid secretion and it is cost effective in the treatment of gastricoesophageal reflux diseases. It is S-isomer of omeprazole and is the first single optical isomer proton pump inhibitor. It provides better acid control than current racemic proton pump inhibitors and has a favourable pharmacokinetic profile relative to omeprazole [9].

A few analytical methods HPLC [10, 11] LC-MS [12, 13] and UV [14, 15] have been developed for determination of ESO.

Indomethacin: Indomethacin, **Fig.1 c,** 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid, is a non-steroidal anti-inflammatory, analgesic and antipyretic drug. Despite its high toxicity, indomethacin is a primary medicine used for the treatment of rheumatoid arthritis, gout, and

collagen disease [16]. Because of its physiological significance several methods have been developed for its quantitative determination *viz.*, HPLC [17, 18] voltammetry [19] LC-MS [20] and Spectrophotometry [21].

Ketorolac tromethamine: Ketorolac tromethamine has anti- inflammatory and analgesic activity. Chemically it is 5-benzoly- 2, 3 – dihydro -1H-pyrrolizine-1-carboxylicacid,2-(hydroxymethyl)-1,3-propanediol **Fig.1 d,** and official only in USP 22 .

Literature survey reveals that HPLC [23, 24], spectrophotometry [25] and Spectrofluorimetry [26] methods have been developed for the determination of KET.

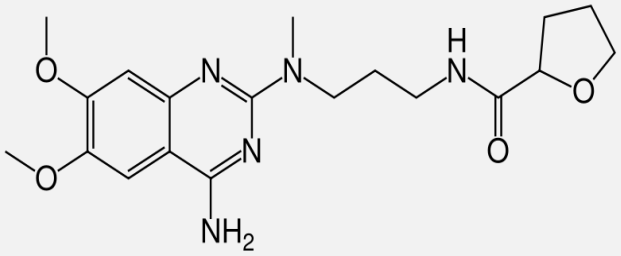
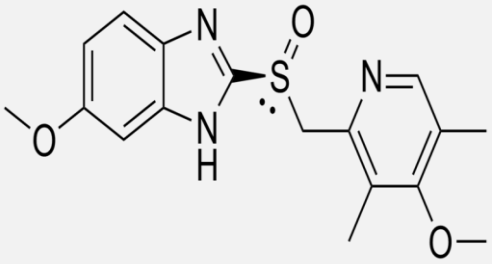
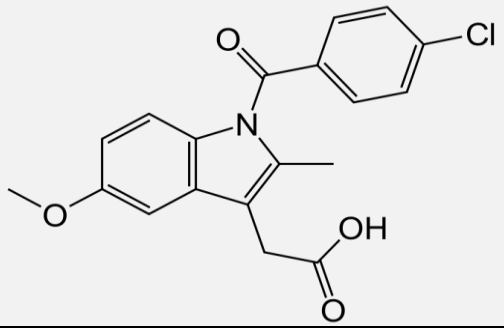
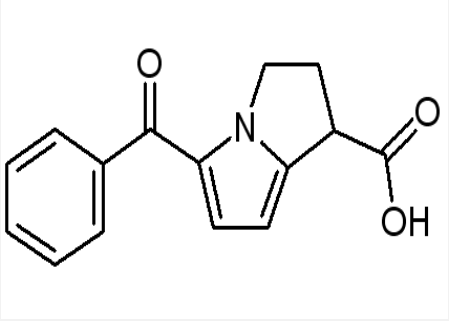
a) Alfuzosin	b) Esomeprazole
	
c) Indomethacin	**d) Ketorolac Tromethamine**
	

FIGURE 1: STRUCTURES OF THE DRUGS

EXPERMENTAL:

Instrumentation: The UV-VIS spectra of the study have been recorded on SHIMADZU 140 double beam Spectrphotometer, Thermo Nicolet 1000 and also on ELICO 159 UV-VIS single beam spectrophotometers using quartz cells of 10 mm path length.

A Dhona 200 single pan electrical balance is used for weighing the samples.

MATERIALS: Analytical grade $KMno_4$, NaOH and triple distilled water was used for preparing solutions.

Preparation of Drug Solution: A stock solution of each drug containing 1000 µg mL^{-1} was initially prepared and further diluted to get working concentrations.

The drugs analysed were produced from Dr. Reddy's laboratories Hyd, Hetero Drugs Pvt Ltd as gift samples.

$KMnO_4$Solution: A stock solution of $KMnO_4$ is prepared by dissolving 0.158 gm. of pure sample of $KMnO_4$ in 100 mL triple distilled water. The permanganate solution was standardized against oxalic acid by standard procedure.

NaOH Solution: 0.5M NaOH solution is prepared by dissolving 20 gm of NaOH in 1000 ml triple distilled water. The same is standardized by titrating against standardized HCl solution.

Method Development: The method depends on the oxidation of the drug with alkaline potassium permanganate ($1x10^{-2}$ *M)* to produce Manganate ion which absorbs at 610 nm and formed a basis for quantification of drug.

A solution of 0.45 - 0.5 *M* NaOH is used to produce required alkalinity to the solution. Linearity and calibration curves are determined from initial rate and fixed time methods.

Procedure for kinetic study: 8 ml of drug solution was transferred in to 10 ml calibrated flask, 1mL of $KMnO_4$ ($1x10^{-2}M$) and 1mL of NaOH were added. After shaking for 10 sec the solution was transferred to a cuvette and was placed in sample compartment. Similarly prepared blank solution was placed in the reference compartment. The absorbance of this sample was measured at 2, 5, 10, 15, 20, 25 and 30 min. The procedure is repeated with 6mL, 5 mL, 4 mL, 3 mL, 2 mL and 1 mL of drug solutions by making up remaining volume with distilled water. Absorbance-time curves **Fig. 2** were constructed.

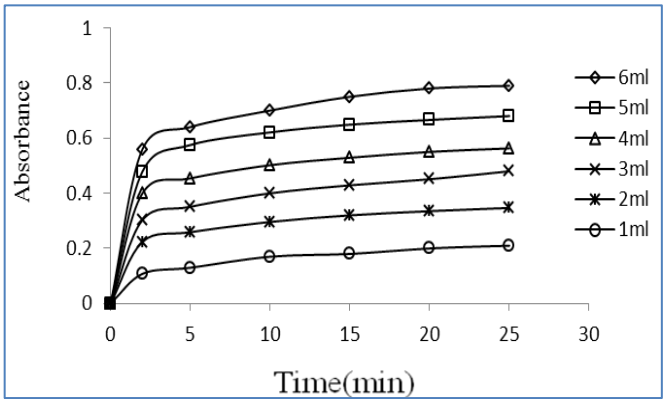


FIGURE 2: ABSORBANCE –TIME CURVES FOR THE REACTION OF DRUGS WITH ALKALINE $KMNO_4$

PROCEDURE FOR CALIBRATION

Initial rate method: The initial rates of the reaction were determined from absorbance-time curves by measuring the slopes of the initial tangent to the absorbance time curves. Aliquots of 12.5-100 µg mL^{-1} of ALF, 25-150 µg mL^{-1} of ESO, 2.5 – 15 µg mL^{-1} of IND and 6.25-37.5 µg $mL^{-1}$of KET test solutions were pipetted into a series of 10mL standard flasks. 1 mL of 0.5MNaOH followed by 1.0 mL of 0.01M potassium permanganate were added to each flask and then diluted with distilled water at room temperature. The contents of the mixture of each flask were mixed well and the increase in absorbance at 610 nm was recorded as a function of time. The initial rate of the reaction (n) at different concentrations was obtained from the slope of the tangent to the absorbance time curve.

Fixed time method: In this method, the absorbance of a green colored solution containing varying amounts of drugs as mentioned above for initial rate method were measured at a preselected fixed time, 15 min.

Procedure for assay of pure drug: To test the accuracy and precision of the methods developed, pure sample solutions containing drug in the Beer's Law limit were chosen and kinetics of the reaction were studied. For this study 15, 30, 45 and 60 µg mL^{-1} of ALF; 30, 60, 90 and 120 µg mL^{-1} of ESO; 3, 6, 9 and 12 µg mL^{-1} of IND and 7, 14, 21 and 28 µg mL^{-1} of KET were chosen for kinetic study other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

Procedure for analysis of Pharmaceuticals:

Alfuzosin: Twenty tablets were weighed accurately and crushed to fine powder. Quantity of tablet powder equivalent to 50mg of analyte was weighed and transferred to 50 mL volumetric flask and dissolved in 40 mL of distilled water by using 0.5*M*NaOH. This solution was then filtered through Whatmann filter paper No. 41. The volume was made up to 50 mL with distilled water. Kinetic runs were performed using 16, 32, 48 and 64 µg $mL^{-1}$of Alfuzosin other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

Esomeprazole: Twenty capsules of Esomeprazole magnesium were emptied and powder was weighed. An amount equivalent to 50 mg was transferred to 50 ml volumetric flask, dissolved in dilute NaOH distilled water. The absorbance of sample solution was measured as described in the calibration procedure and amount of Esomeprazole magnesium was determined by referring to the calibration curve. Kinetics runs were performed using 32, 64, 96 and 128 µg $mL^{-1}$of Esomeprazole other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

Indomethacin: For the investigation, indomethacin gastro-resistant tablets were used. Twenty tablets containing 25 mg active substance each were weighed and finely powdered.

An amount of the powder equivalent to 25.0 mg indomethacin for assay was weighed into 50.0 mL volumetric flasks and approximately 30 ml distilled water was added. The samples were sonicated for 10 min and the solutions were then diluted to volume with water, mixed well, and filtered. For assay, 5.00 ml stock solutions were diluted to give a solution containing 2.5 µg mL^{-1} of indomethacin. Kinetics runs were performed using 4, 7, 10 and 13 µg $mL^{-1}$of indomethacin other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

Ketorolac tromethamine: Twenty tablets (Ketonic–10mg) were finely grounded and mixed. An accurately weighed 50 mg of KET was taken into a 100 mL. Volumetric flask, sonicated and remaining volume is made up with distilled water. Kinetic runs were performed using 6, 13, 20 and 27 µg mL^{-1} of KET other experimental details are as mentioned earlier.

RESULT AND DISCUSSION:

Construction of calibration: The absorbances data of kinetic runs at 2 min and 15 min are used to construct calibration. The average relative response of 5 replicates was evaluated. The absorbance falling within 95% to 105% of average relative responses only are considered in construction of the calibration curve **Fig. 3.**

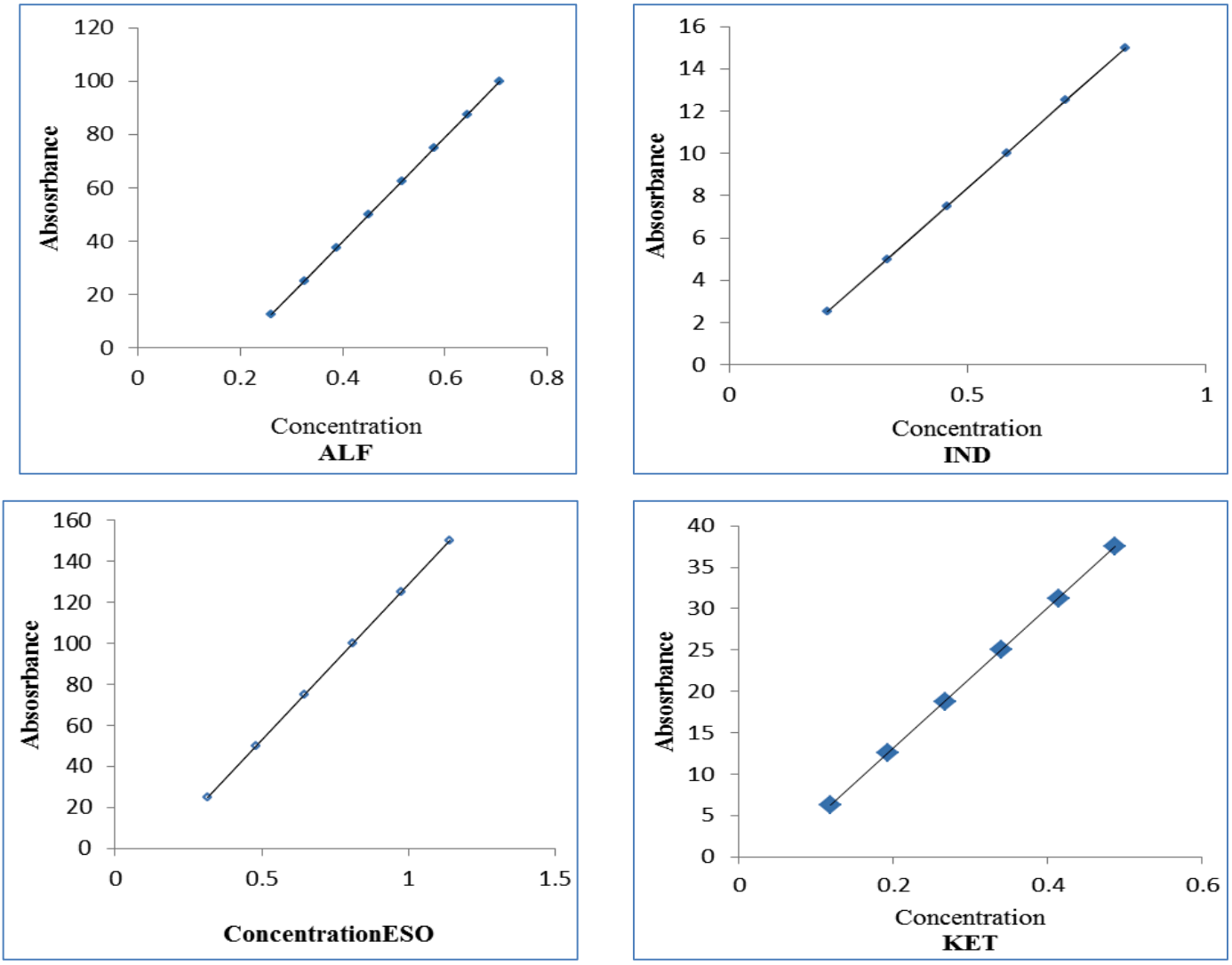


FIGURE 3: CALIBRATION CURVES OF DRUGS

The limits of Beer's law, slope, intercept, correlation coefficient, Sandell's sensitivity and regression equation for each drug are tabulated in **Table 1.**

TABLE 1: ANALYTICAL PARAMETERS FOR DETERMINATION OF DRUGS BY OXIDATION WITH ALKALINE $KMNO_4$

Name of Drug Property	Alf	Eso	Ind	Ket
λ_{max}	610	610	610	610
Beer's law limits (μgmL^{-1})	12.5-100	25-150	2.5-15	6.25-37.5
Sandell's sensitivity (μgcm^{-2})	0.196	0.151	0.022	0.085
Std. Dev. of intercepts	0.00432	0.00735	0.00296	0.0068
LOD (μgmL^{-1})	2.85	3.62	0.21	1.87
LOQ (μgmL^{-1})	8.64	10.97	0.66	5.66
Slope, b	0.0051	0.0066	0.0449	0.0118
Intercept, a	0.1973	0.1518	0.0824	0.0452
Correlation coefficient	0.9963	0.9951	0.9987	0.9982
Regression equation	0.1973+	0.1518+	0.0824+	0.0452+
$Y=a+bX^*$	0.0051X	0.0066X	0.0449X	0.0118X

X= Concentration of the Drug, ($\mu g\ mL^{-1}$)

Method validation: Each method developed for quantification of drugs has been validated in terms of precision, accuracy, limit of detection. Limit of quantification, linearity, selectivity and ruggedness. Absorbance-time curves were drawn, initial rate and fixed time methods were used to assess the recovery of the drug. To assess the precision, each experiment was repeated at least 5 times and accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2 for each drug demonstrates accuracy and precision of the methods. Further t-test and F-test values have also been calculated using a standard reference method. The t-test and F-test values are less than that their permissible range indicating high accuracy and precision of the methods **Table 2.**

TABLE 2: RECOVERY STUDIES TO EVALUATEACCURACY AND PRECISION FOR THE DETERMINATION OF DRUGS BY REDOX REACTION WITH ALKALINE $KMNO_4$

Name of the Drug	Amount taken ($\mu g\ ml^{-1}$)	Amount found ($\mu g\ ml^{-1}$)	% Recovery	RSD %	Proposed method Mean± SD	Ref method Mean± SD	t-test (*)	F-test (**)
Alf	15	15.2	101.3	0.735	100.56±0.739	[171] 100.6±0.60	0.347 (2.45)	1.519 (4.28)
	30	30.15	100.50					
	45	44.81	99.58					
	60	60.5	100.83					
Eso	30	29.98	99.93	0.139	99.98±0.139	[164] 99.80±0.08	0.834 (2.57)	3.019 (4.95)
	60	60.06	100.10					
	90	89.82	99.80					
	120	120.09	100.07					
Ind	3	3.02	100.66	0.666	99.91±0.666	[122] 99.70±0.73	1.004 (2.57)	3.237 (4.95)
	6	5.95	99.16					
	9	9.02	100.22					
	12	11.95	99.58					
Ket	7	7.06	100.86	0.894	100.68±0.900	[157] 98.28±0.76	0.291 (2.45)	1.403 (4.28)
	14	14.2	101.43					
	21	20.87	99.39					
	28	28.3	101.07					

*t- test and **F-test values from literature.

Factors affecting Absorbance:

Effect of concentration of $KMnO_4$: The effect of concentration of $KMnO_4$ on the absorbance at preselected time, 15 min was studied in the range $0.2x\ 10^{-2}M$ to $1.2x10^{-2}M$ by keeping the concentration of drug constant. The absorbance increased with increasing the concentration of $KMnO_4$ and became constant at $0.7x10^{-2}M$ to $0.8x10^{-2}M$. Thus, the adoption of $1x10^{-2}M$ $KMnO_4$ in the final solution proved to be adequate for the maximum concentration of drugs used in the determination process.

Effect of NaOH: The influence of the NaOH concentration examined by taking fixed concentration of drug, 1.0 mL of 0.01*M* $KMnO_4$ solution and varying volumes (0.2 – 1.2ml) of 0.5M NaOH. The maximum absorbance was obtained with 0.8 mL of 0.5*M*NaOH, after which further increase in volume of NaOH caused no change in absorbance. Hence, 0.8 to 1.0 mL of 0.5*M*NaOH was used as an optimum value.

Effect of prolonged time: The effect of time on the reaction between $KMnO_4$ and the drugs was studied. The absorbance of the reaction mixture was increased with time. The solutions turned turbid after 30-35 min.

Effect of temperature: At room temperature the reaction rate of four drugs increased substantially as the color development increased. Higher temperature causes precipitation of MnO_2; therefore, room temperature was selected as the optimum.

Analysis of Pharmaceuticals: To test the applicability of the method developed, solution of pharmaceutical tablets containing drug in the Beer's Law limit were prepared and kinetics of the reaction were studied.

For this study 16, 32, 48 and 64 μg mL^{-1} of ALF: 32, 64, 96 and 128 μg mL^{-1} of ESO; 4, 7, 10 and 13 μg mL^{-1} of IND; 6, 13, 20 and 27 μg mL^{-1} of KET; were chosen for kinetic study other experimental details being common **Table 3.**

Sensitivity of the methods has been determined in terms of Sandell's sensitivity. It is μg $mL^{-1}$of drug to produce a change in absorbance of 0.001 units. From the **Table 1,** it's clear that the sensitivity of the drugs for the method is in the order IND<KET<ESO<ALF.

TABLE 3: APPLICATION OF PROPOSED FOR THE ANALUSIS OF STUDIED DRUGS IN PHARMACEUTICAL FORMULATIONS BY REDOX REACTION WITH ALKALINE $KMNO_4$

Name of the Drug (Tablet)	Amount taken (μg ml^{-1})	Amount Found (μg ml^{-1})	% Recovery	RSD %	Proposed method Mean± SD	Ref method Mean± SD	t-test (*)	F-test (**)
Alf (Alfoo)	16	16.21	101.31	0.465	100.95±0.470	[171] 100.6±0.60	0.463 (2.45)	0.612 (4.28)
	32	32.21	100.65					
	48	48.22	100.44					
	64	64.88	101.37					
Eso (Nexpro)	32	32.08	100.25	0.051	100.21±0.052	[164] 99.80 ±0.08	1.047 (2.57)	0.416 (4.28)
	64	64.16	100.25					
	96	96.2	100.208					
	128	128.18	100.14					
Ind (Indocap)	4	4.02	100.5	0.550	99.90±0.550	[122] 99.70±0.73	0.573 (2.57)	0.568 (4.28)
	7	6.95	99.29					
	10	10.02	100.2					
	13	12.95	99.61					
Ket (Ketonic)	6	6.06	101	0.459	100.93±0.463	[157] 98.28±0.76	0.975 (2.57)	0.371 (4.28)
	13	13.2	101.54					
	20	20.15	100.75					
	27	27.12	100.44					

CONCLUSION: $KMnO_4$, an oxidizing agent in alkaline medium is found to oxidise drugs like ALF, ESO, IND and KET which are soluble in basic medium. One of the oxidizing products namely manganate ion absorbs maximally at 610nm, whose absorbance is a function of concentration of the drug. Kinetics of the reaction is followed for quantification, construction of calibration , validation and optimization of the method.

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