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KINETIC SPECTROPHOTOMETRIC DETERMINATION OF DRUGS BASED ON OXIDATION BY ALKALINE $KMnO_4$

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ABSTRACT

Simple, accurate and precise spectrophotometic methods for quantitative determination of four drugs viz., Ambroxol hydrochloride (AMB), Deferiprone (DEF), Guaifensin (GUA), Rivaroxaban (RIV) 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 5-30 µg ml^{-1} for AMB; 4-24 µg ml^{-1} for DEF ;12.0-72 µg ml^{-1} for GUA; and 5-30 µg ml^{-1} for RIV. Recovery studies using pure samples and formulations in the Beer's Law limits have been carried out. Excellent recoveries indicate the methods are accurate and precise. The methods have been validated in terms of ICH guidelines. Statistical analysis in terms of student's t- test and variance F- tests demonstrate high accuracy and precision and suggest the methods can be applied in bulk drug and pharmaceutical industries.

KEYWORDS: Spectrophotometry, Quantitative determination, Alkaline $KMnO_4$, Ambroxol hydrochloride, Deferiprone, Guaifensin, Rivaroxaban.

I. INTRODUCTION

1. Ambroxol hydrochloride

Ambroxol hydrochloride (AMB) [trans-4-[(2-amino-3,5- dibromobenzyl)amino]cyclohexanol hydrochloride] (Fig.1a) is a semi-synthetic derivative of vasicine obtained from Indian shrub Adhatodavasica. It is a metabolic product of bromhexine and possesses mucokinetic (improvement in mucus transport) and secretolytic (liquifies secretions) properties. It promotes the removal of tenacious secretions in the respiratory tract and reduces mucus stasis

(arresting the secretion of mucus).[1] Different methods have been reported for the analysis of AMB including HPLC[2], GC[3], HPTLC[4], FT Raman spectroscopy.[5]

2. DEFERIPRONE

Deferiprone or 1,2-dimethyl-3-hydroxypyrid-4-one (Fig.1b) is an active iron chelator and superoxide radical scavenger which belongs to the new class of chelating agents, *i.e.* alpha-ketohydroxypyridines. Iron overload which can occur as a consequence of chronic transfusion therapy in patients with *β*-thalassemia and sickle cell diseases or due to excessive dietary iron uptake in patients with chronic anemia and hereditary hemochromatosis, causes organ damages and its chelators such as deferiprone (DFP) should be used to remove excess iron from various parts of body.[6-10] Intestinal absorption of DFP and one of its analogs was investigated by Taher *et al.*[11] DFP and other chelators could be used to complex cations other than iron which are used for different purposes in biomedical sciences. Zinc complexes of DFP and some related compounds were synthesized and further investigated to improve the bioavailability of zinc after oral administration.[12]

3. GUAIFENSIN

Guaifenesin (GUA), 3-(2-Methoxyphenoxy)-1,2-propanediol;(Fig1.c) is reported to increase the volume and reduce the viscosity of tenacious sputum and is used as expectorant for productive cough.[13] Different methods have been reported for the analysis of GUA including HPLC[14–24], GC[25, 27], capillary electrophoresis, mass spectrometry[27], X-ray diffraction[28], voltammetry.[25]

4. RIVAROXABAN

Rivaroxaban (RIV) (Fig.1d) is the first orally active direct factor Xa inhibitor; a coagulation factor in the blood coagulation pathway leading to thrombin generation and clot formation[26] and.[27] RIV is well absorbed from the gut and exerts maximum inhibition of factor Xa within four hours after administration. Its effect lasts 8–12 h, however factor Xa activity does not return to normal until 24 h. Currently, there is no specific way to reverse the anticoagulant effect of RIVA in the case of major bleeding[28] and.[29] Chemically, RIV is (S)-5-chloro-*N*-thiophene-2-carboxamide (Fig 1d). It is a white, crystalline powder that is freely to very soluble in DMF, acetonitrile, chloroform and practically insoluble in water. Only few methods of analysis have been reported for RIV in its dosage forms using the following techniques: spectrophotometry,[30, 31] and[32] TLC-densitometry[30] and[33] and HPLC.[30, 34, 35,36, 37,38] and [339] However, proper method validation to pharmaceutical industry standards has

not been reported. A sensitive and specific bioanalytical assay has also been reported for assessment of the pharmacokinetics of RIV using LC/MS/MS in human.[40]

A comparision of various techniques used for estimation of above drugs in terms of sensitivity and reproducibility are presented in Table-4.

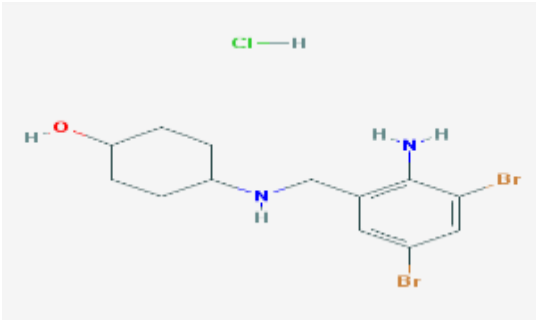

Fig1. a: Ambroxol hydrochloride

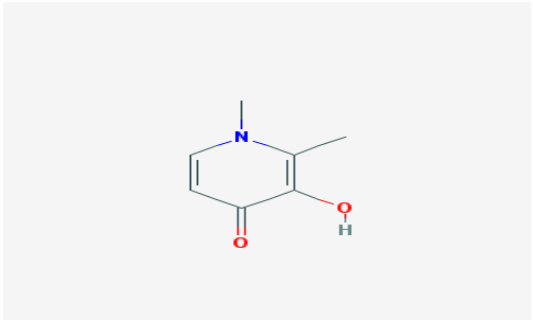

Fig1. b: Deferiprone

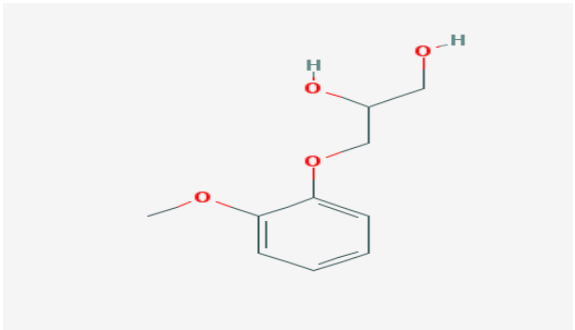

Fig1.c: Guaifensin

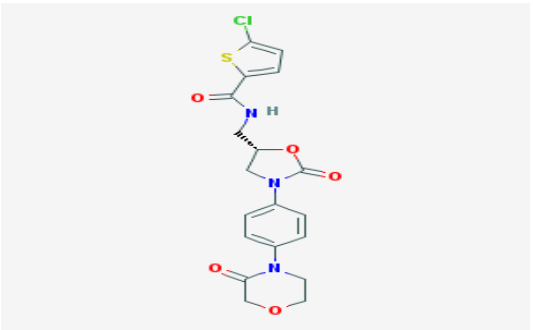

Fig1.d: Rivaroxaban

Figure 1: Structures of the Drugs

II. EXPERIMENTAL

2.1. Instrumentation: The UV-VIS spectra of the study have been recorded on ELICO SL 210 double beam Spectrphotometer using quartz cells of 10 mm path length.

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

2.2. Materials: Analytical grade $KMnO_4$, NaOH and triple distilled water was used for preparing solutions for the study.

2.2.1. Preparation of Drug Solution: A stock solution of each drug containing 2000 µg ml^{-1} was initially prepared and further diluted to get working concentrations.

2.2.2. $KMnO_4$ 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.

2.2.3. Standardization of $KMnO_4$: The standardization of $KMnO_4$ solution is carried out by titration against a standard solution of Sodium oxalate. The reaction is

$2MnO_4^-(aq) + 8H^+(aq) + 5e^- \rightarrow 2Mn^{2+} + 4H_2O(l)$

$2KMnO_4 + 5Na_2C_2O_4 + 8H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 5Na_2SO_4 + 10CO_2 + 8H_2O$

2.2.3. NaOH Solution: 0.5*M* 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.

III. 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.

IV. PROCEDURE FOR KINETIC STUDY

A stock solution of each drug containing 1000 µg ml^{-1} was prepared as mentioned earlier. The drug solutions were further diluted to get required concentrations for the kinetic study.

8 ml of this 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 7 ml, 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.2were constructed.

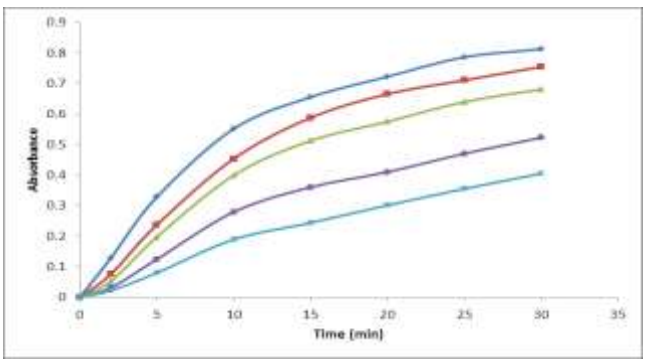

Figure 2: Absorbance –Time curves for the reaction of Drugs with Alkaline KMnO4

V. PROCEDURE FOR CALIBRATION

5.1. 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 5-30 μg ml^{-1} of AMB; 4-24 μg ml^{-1} of DEF ;12.0-72 μg ml^{-1} of GUA; and 5-30 μg ml^{-1} of RIV test solutions were pipetted into a series of 10ml standard flasks. 1 ml of 0.5*M* NaOH followed by 1.0 ml of 0.01*M* 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.

5.2. 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.

VI. 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 7.5, 12.5 ,17.5and 22.5μg ml^{-1} of AMB; 6,10 ,15 and 18 μg ml^{-1} of DEF; 6.18,30 and 42 μg ml^{-1} of GUA; 8, 12,16 and 24μg ml^{-1} of RIV 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.

VII. PROCEDURE FOR ANALYSIS OF PHARMACEUTICALS

7.1. Ambroxol Hydrochloride: Each tablet of Mucolite contains 30 mg Ambroxol as active substance. The total content of ten tablets was weighed and grounded to a fine powder using a pestle and a mortar. Powder was dissolved in NaOH -water filtered through a filter paper and diluted to the mark in a 100 ml calibrated flask. Convenient aliquots from this solution were taken for the determination of Ambroxol (5.0– 30.0 μg ml^{-1}). Kinetics runs were performed using 7, 12, 17 and 20 μg ml^{-1} of Ambroxol other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

7. 2. Defriprone: An accurately weighed four capsules (Kelfer) were taken and the granules in the capsules were finely powdered, weighed, a portion equivalent to 250 mg was transferred quantitatively into 100 ml volumetric flask, sonicated for 15 min, completed to

volume with distilled water. A stock solution containing 2.5 mg ml^{-1} was further diluted to get required concentration(4µg ml^{-1}) for pharmaceutical analysis. Kinetics runs were performed using 4,6, 14 and 22 µg ml^{-1} of pseudoephedrine other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

7. 3. Guaifensin: For analysis of commercial formulations, two tablets (Barkeit) were taken and powdered. Tablet powder equivalent to 400 mg of Formulation were transferred into 100 ml volumetric flask and dissolved in 0.5 *M* NaOH. Then the solution was sonicated for 30 minutes and filtered. 1.25ml from the filtrate were taken and further diluted with 0.5 *M* NaOH to form 12 µgml^{-1}. Kinetics runs were performed using 13, 18, 21 and 25 µgml^{-1} of Guaifensin other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

7.4. Rivaroxaban: In order to see the feasibility of proposed method for estimation of Rivaroxaban in marketed pharmaceutical formulations, twenty tablets (Xarelto-10mg) were weighed, average weight determined and crushed into fine powder. A quantity of tablet powder equivalent to 10 mg of Rivaroxaban was transferred into 100 ml volumetric flask containing 50 ml water, shaken manually for 10 min, volume was adjusted to mark with same solvent and filtered through Whatmann filter paper No.1. The appropriate aliquots were transferred to 10 ml volumetric flask, volume was adjusted to the mark with same solvent to obtain concentration of 2.5 µg ml^{-1}. Kinetics runs were performed using 7, 11,17 and 19 µg ml^{-1} of Rivaroxaban, other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

VIII. RESULT AND DISCUSSION

8.1. Construction of calibration

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

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

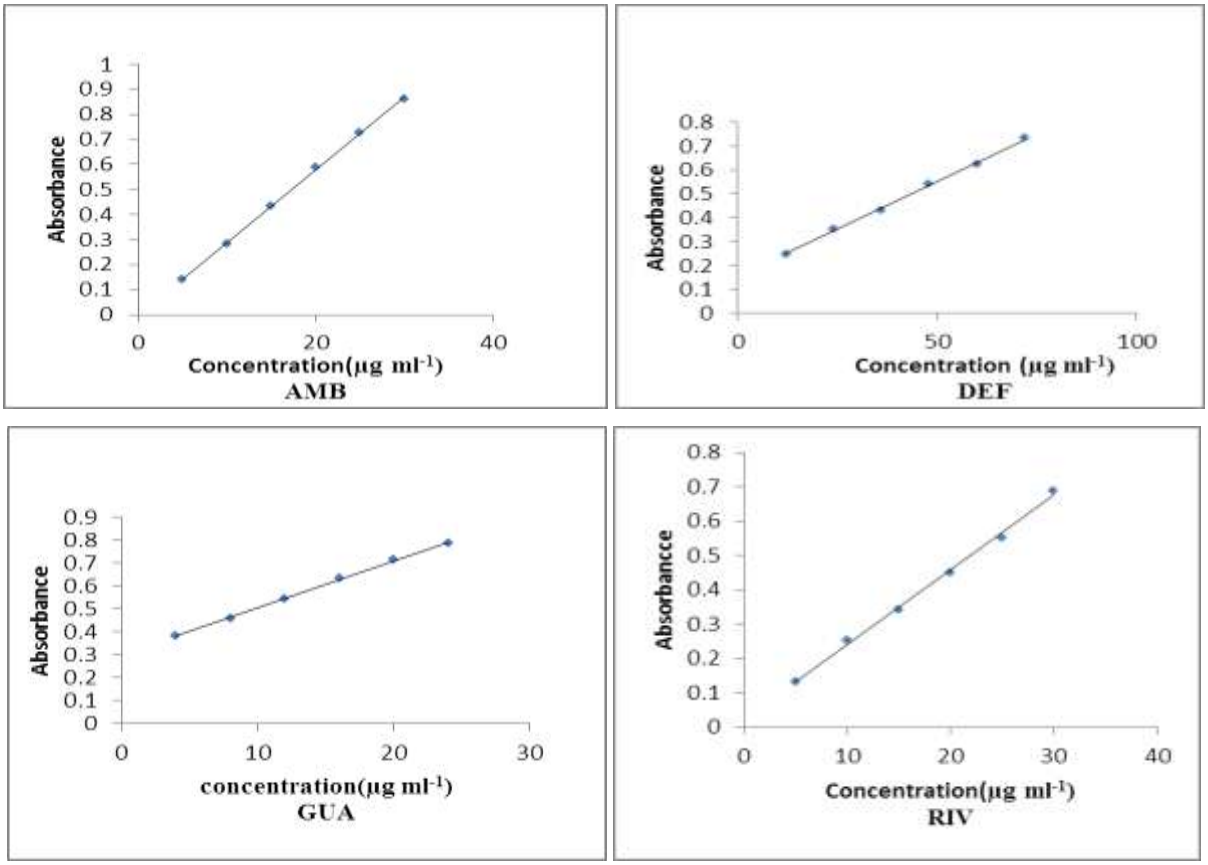

Figure 3: Calibration curves of the drugs

Table 1: Analytical parameters for determination of drugs by oxidation with alkaline $KMnO_4$

Name of the drug property	AMB	DEF	GUA	RIV
λ_{max}	610	610	610	610
Beer's law limits(µg ml^{-1})	5-30	4-24	12-72	5-30
Sandell's sensivity (µg cm^{-2})	0.0344	0.125	0.5	0.476
Std.dev. of intercepts	0.00115	0.00321	0.00104	0.00098
LOD (µg ml^{-1})	0.130	1.324	1.716	1.54
LOQ (µg ml^{-1})	0.396	4.01	5.2	4.66
Slope,b	0.029	0.008	0.002	0.0021
Intercept,a	-0.004	0.154	0.297	0.024
Correlation co-efficient	0.999	0.997	0.998	0.996
Regression equation $Y=c+bx^*$				

X= Concentration of the Drug, (µg ml^{-1})

8.2. 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]. As mentioned earlier, limit of detection is the minimum limit that can be detected but not necessarily quantified, is determined for each drug.

LOD is determined from the standard deviation of y-intercepts of regression lines of replicate determinations.

LOD = 3.3 s/S

Where s = standard deviation of intercept (n=5)

S = slope of linearity plot

LOQ the minimum concentration of analyte using calibration curve is also determined.

LOQ = 10s/S

Limits of Linearity of calibration curves are mentioned in the Table 1 under the title Beer's law limit. To test the selectivity, known excipients of each drug are added to the pure drug sample and recovery experiments were performed. Ruggedness is resistance of method for a small change in variables like instrument and analyst or both. To test the ruggedness of the method absorbance data was collected using 3 different instruments and 2 analysts. No significant changes were observed either by change of instrument or analyst, hence the method may be treated as rugged.

Table 2: Recovery Studies to evaluate Accuracy and Precision for the Determination of Drugs by Redox Reaction with Alkaline $KMnO_4$

Name of the drug	Amount taken (µg ml-1)	Amount found (µg ml-1)	% recovery	RSD%	Proposed method Mean ± SD	Ref method Mean ± SD	t-test	F-test
AMB	7.5 12.5 17.5 22.5	7.51 12.49 17.48 22.51	100.1% 99.92% 99.88% 100.04%	0.07503	99.96±0.075	98.63±0.0435	2.99	2.77
DEF	6 10 15 18	5.99 10.01 15.01 17.98	99.83% 100.1% 100.06% 99.88%	0.10706	99.94±0.107	98.7±0.63	2.36	0.057
GUA	6	6.01	100.16%					

	18 30 42	17.98 30.01 41.99	99.88% 100.1% 99.97%	0.1259	100.02±0.126	99.61±1.48	0.674	0.014
RIV	8 12 16 24	7.99 12.03 16.02 23.97	99.87% 100.25% 100.12% 99.87%	0.1889	100.02±0.189	99.6±0.745	0.434	0.128

IX. FACTORS EFFECTING ABSORBANCE

9.1. 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.

9.2. 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 0.5 *M* 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.

9.3. 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.

9.4. 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.

X. ANALYSIS OF PHARMACEUTICALS

To test the applicability of the method developed, solution of pharmaceutical tablets containing drug in the Beer's Law limit were chosen and kinetics of the reaction were studied. For this study 7.5, 12.5, 17.5 and 20 µg ml^{-1} of AMB; 6. 10, 14 and 22 µg ml^{-1} of DEF; 13,18, 21 and 25 µg ml^{-1} of GUA; 7, 11,16 and 19 µg ml^{-1} of RIV 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. Absorbance-time curves were drawn, initial rate and fixed time methods were used to assess the recovery of the drug in pharmaceuticals. To assess the precision each tablet analysis 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 applicability of the methods for pharmaceutical analysis. Further t-test and F-test values have also been calculated using a standard reference method. The closeness of t-test and F-test values are less than that they permissible range indicating excellent applicability of the methods for pharmaceutical analysis[Table 3].The excellent recovery studies indicate that methods developed can be applied to pharmaceutical analysis without hesitation.

As defined earlier Sandell's sensitivity of the analyte capable of producing a change 0.001 absorbance units is a measure of sensitivity of the method. Lower the Sandell's sensitivity higher is the sensitivity of the method developed. The Sandell's sensitivity values of drugs presented in [Table 1] indicate that AMB has lowset Sandell's snstivity and hence is highest sensitivity of the method, they are in the order AMB<DEF<RIV < GUA.

Table 3: Application of proposed method for the analysis of studied drugs in pharmaceutical formulations by redox reaction with alkaline $KMnO_4$

Name of the drug	Amount taken (µg ml-1)	Amount found (µg ml-1)	% recovery	RSD%	Proposed method Mean ± SD	Ref method Mean ± SD	t-test	F-test
AMB (Mucolite-30mg)	7 12 17 20	7.01 12.02 16.99 19.99	100.14% 100.16% 99.94% 99.95	0.1019	100.02±0.102	98.7±0.68	1.86	0.022
DEF (Kelfer-250mg)	4 6 14 22	3.98 5.97 14.02 22.01	99.5% 99.5% 100.14% 100.04%	0.3437	99.79±0.343	98.7±0.72	3.07	0.453
GUA (Barkeit-200mg)	13 18 21 25	13.01 17.98 21.01 24.99	100.07% 99.88% 100.04% 99.96%	0.0850	99.98±0.085	98.8±0.88	3.25	0.322
RIV (Xarelto-10mg)	7 11 17 19	6.98 11.01 17.02 18.99	99.71% 100.09% 100.11% 99.94%	0.3028	99.85±0.3024	99.01±0.54	2.99	1.42

Table 4: Comparision of various techniques

Name of the drug	Method	Sensitivity	Recovery
AMB	1)Spectrophotometry (CT complexes) 2)HPLC 3)RP-HPLC 4)Micellar liquid chromatography	5-40 µg ml^{-1} 2-12 ng ml^{-1} 50-150 µg ml^{-1} 1-20 µg ml^{-1}	98.63% 98.3% 95.6% 98.33%
DEF	1)HPLC 2)RP-HPLC	1-120 µg ml^{-1} 0.25-10 µg ml^{-1}	98.7% 97.52%
GUA	1)UV Spectrophotometry 2)RP-HPLC	5-40 µg ml^{-1} 50-90 µg ml^{-1}	99.61% 99.96%
RIV	1)uv spectrophotometry 2)UPLC 3)Area under curve method	0.5-50 µg ml^{-1} 100-500 µg ml^{-1} 2-12 µg ml^{-1}	98.6% 99.6% 99.7%

XI. CONCLUSION

$KmnO_4$, an oxidizing agent in alkaline medium is found to oxidise drugs like AMB, DEF, GUA and RIV which are soluble in basic medium. One of the oxidizing products namely manganate ion absorbs maximally at 610 nm, whose absorbance is the function of concentration of the drug. Kinetics of the reaction is followed for quantification, construction of calibration, validation and optimization of the method.

XII.AKNOWLEDGEMENT

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