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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 five drugs viz., piperacillin (PIP), Argatroban (ARG), Levocetrizine (LCTZ), Methocarbamol (MET), Diclofenac Sodium(DCS), 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 20-120 µg ml^{-1} for PIP; 5-30 µg ml^{-1} for ARG ;10-60 µg ml^{-1} for LCTZ; and 10-60 µg ml^{-1} for MET; 7-42 µg ml^{-1} for DCS. 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 $KMnO_4$, Drugs.

I. INTRODUCTION

1. Piperacillin

Piperacillin (PIP) is a derivative of α-acylureido-substituted penicillin, (2S, 5R, 6R)-6-[[(2R)-2-[[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl] amino]-2-phenyl-acetyl]amino]- 3,3-dimethyl-7-oxo-4-thia-1- azabicyclo [3.2.0.] heptane-2-carboxylic acid (Fig.1a). That has been shown effective in the treatment of many serious infections associated with Gram-positive and Gram-negative organisms, including Pseudomonas aeruginosa, Proteus, Klebsiella pneumoniae and Serratia marcescens. It is a β-lactam antibiotic, susceptible to hydrolysis by

a range of β-lactamases, including the plasmid-mediated enzymes. These enzymes inactivate β-lactam antibiotics by opening the β-lactam ring. Piperacillin has been determined by many analytical methods, such as capillary zone electrophoresis[1], cyclic voltammetry[2], spectrophotometry[3], potentiometric titration[4] and especially by high performance liquid chromatography (HPLC)[5-12].

2. Argatroban

Argatroban [((2R,4R)-4-methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl)-L-arginyl-2-piperidine-carboxylic acid mono hydrate] (Fig.1c) is a direct thrombin inhibitor under clinical development as adjunctive therapy to thrombolytic agents in acute myocardial infarction (AMI). Recent clinical trials have shown argatroban to be especially effective when administered in conjunction with a thrombolytic agent within 6 hours of the onset of AMI symptoms. Biochemical studies have shown that argatroban is a potent and selective thrombin inhibitor, Argatroban is a direct thrombin inhibitor that reversibly binds to the thrombin active site. Argatroban does not require the co-factor antithrombin III for antithrombotic activity. Argatroban exerts its anticoagulant effects by inhibiting thrombin-catalyzed or -induced reactions. Argatroban is capable of inhibiting the action of both free and clot-associated thrombin.[13-22]

3. Levocetrizine

Levocetirizine (LCTZ), as the active enantiomer of cetirizine dihydrochloride, (2-[4-[(R)-(4-Chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]-acetic acid dihydrochloride,)(Fig.1b) is a third generation non-sedative antihistamine . It works by blocking H_1 histamine receptors It is used in the treatment of the allergic rhinitis and conjunctivitis, hay fever, pollinosis—control sneezing, runny but not blocked nose, and red, watering, and itchy eye. It has the advantages of higher efficacy, less side effects, and longer duration over other antihistamines, and has begun to replace cetirizine in clinical therapy stepwise. It has been chemically proved that the half dosage form of LCTZ (2.5 mg) has comparable antihistaminic activity to normal amount (5.0 mg) of cetirizine in the treatment of allergic rhinitis and chronic idiopathic urticaria. Different methods have been reported for the analysis of LCTZ incuding liquid chromatography [26] HPLC [23] RP-HPLC[28].

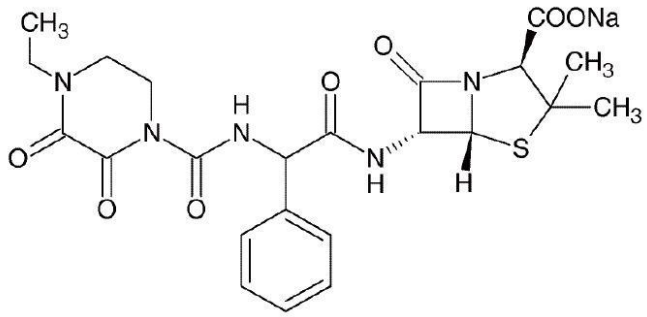
4. Methocarbamol

MET is chemically, (RS)-2-hydroxy-3- (2-methoxyphenoxy) propyl carbamate. MET is used as a centrally acting skeletal muscle relaxant The mechanism of action of methocarbamol is currently unknown, but may involve the inhibition of carbonic anhydrase. The muscle relaxant effects of methocarbamol are largely attributed to central depressant effects; however, peripheral effects of methocarbamol to prolong muscle refractory period have also been reported.The review of literature revealed that various analytical methods involving UV Spectrophotometry, RP-HPLC, Stability indicating HPLC, HPTLC has been reported for MET in single form and in combination with other drugs.[29-37]

5. Diclofenac Sodium

Diclofenac sodium 2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetate(Fig.1e) is a Non-Steroidal AntiInflammatory Drug (NSAID) used to reduce inflammation, and as an analgesic reducing pain, in medical conditions such as osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. The mechanism of action is related to the inhibition of the arachidonate metabolites synthesis through cyclooxygenase inhibition. The pharmacological effects of this drug are thought to be related to the inhibition of the conversion of arachidonic acid to prostaglandins, which are the mediators of the inflammatory process. Several types of analytical procedures have been proposed for the analysis of diclofenac in pharmaceutical formulation. These procedures include potentiometry [38-39], fluorimetry.[40-41] HPLC, GC [42-43], gravimetry [44], UV -spectrophotometry [45-49] and spectrofluorometric methods .[50]

Fig.1a: PIPERACILLIN



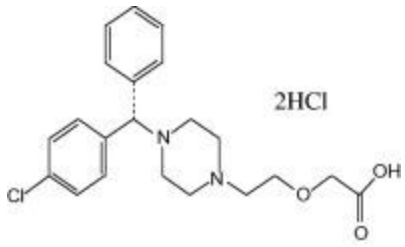


Fig.1b: LEVOCETRIZINE

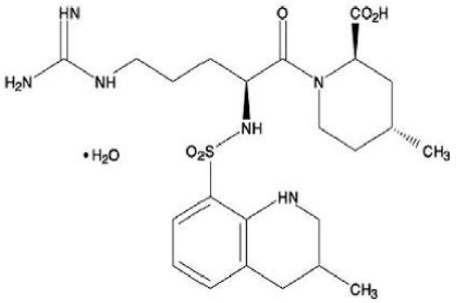


Fig.1c: ARGATROBAN

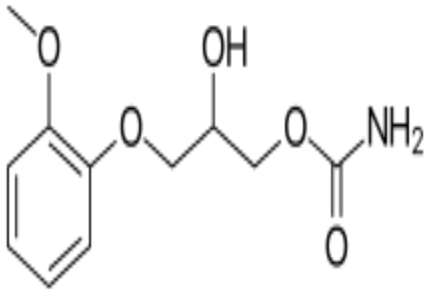


Fig.1d: METHOCARBAMOL

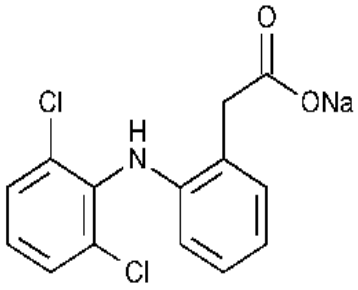


Fig.1e: DICLOFENAC SODIUM

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. 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

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

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

2.2.3. NaOH Solution: 0.5*M* NaOH solution is prepared by dissolving 20 gms of NaOH in 1000ml 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 into 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 the remaining volume with distilled water. Absorbance-time curves Fig.2 were constructed.

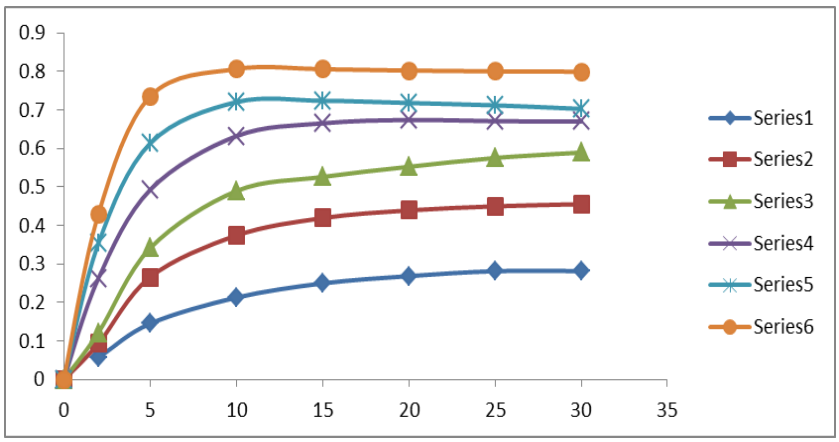


Figure 2: Absorbance –Time curves for the reaction of Drugs with Alkaline $KMnO_4$

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 20-120 µg ml^{-1} of PIP; 10-60 µg ml^{-1} of ARG ;5-30 µg ml^{-1} of LCTZ; 10-60 µg ml^{-1} of MET and 7-42 µg ml^{-1} DCS 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 coloured 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 30,50,70 and 90 µg ml^{-1} of PIP; 7.5,12.5,17.5 and 22.5 µg ml^{-1} of ARG; 15,25,35 and 45 µg ml^{-1} of LCTZ; 12.5,24.5,36.5 and 48.5 µg ml^{-1} of MET; 9.5,14.5,19.5 and 24.5 µg ml^{-1} DCS 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. Piperacillin: Mix about 8 vials of Pipralin injections each containing 4gm/vial of piperacillin were combined and diluted with distilled water to the required concentrations of working range. Kinetics runs were performed using 22, 32, 42 and 52 µg ml^{-1} of piperacillin, other experimental details being common. .Initial rate and fixed time of 15 min were chosen to estimate the amount found.

7.2. Argatroban: Argatroban injection is a sterile non-pyrogenic, clear, colourless, to pale yellow isotonic solution. It is available as a single use polyolefin bag containing 250 mg of argatroban in 250 ml sodium chloride solution (1mg/ml).convenient aliquots were taken from this for the determination of Argatroban. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

7.3. Levocetrizine: In order to see the feasibility of proposed method for estimation of Levocetrizine in marketed pharmaceutical formulations, twenty tablets (CetUp-10mg) were weighed, average weight determined and crushed into fine powder. A quantity of tablet powder equivalent to 10 mg of Levocetrizine 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 12,24,36 and 48 µg ml^{-1} of Levocetrizine, other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

7.4. Methocarbamol: For analysis of commercial formulations, two tablets (Robaxin) were taken and powdered. Tablet powder equivalent to 750 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. 2.5ml from the filtrate were taken and further diluted with 0.5 *M* NaOH to form 10 µgml^{-1}. Kinetics runs were performed using 12, 27, 42 and 57 µgml^{-1} of methocarbamol, other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

7.5. Diclofenac sodium: An accurately weighed four tablets (Diclonac) were taken and finely powdered, weighed, a portion equivalent to 200 mg was transferred quantitatively into 100 ml volume with distilled water. A stock solution containing 2.5 mg ml^{-1} was further diluted to get required concentration (7µg ml^{-1}) for pharmaceutical analysis. Kinetics runs were performed using 9,14, 19 and 24 µg ml^{-1} of Diclofenac sodium, 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].

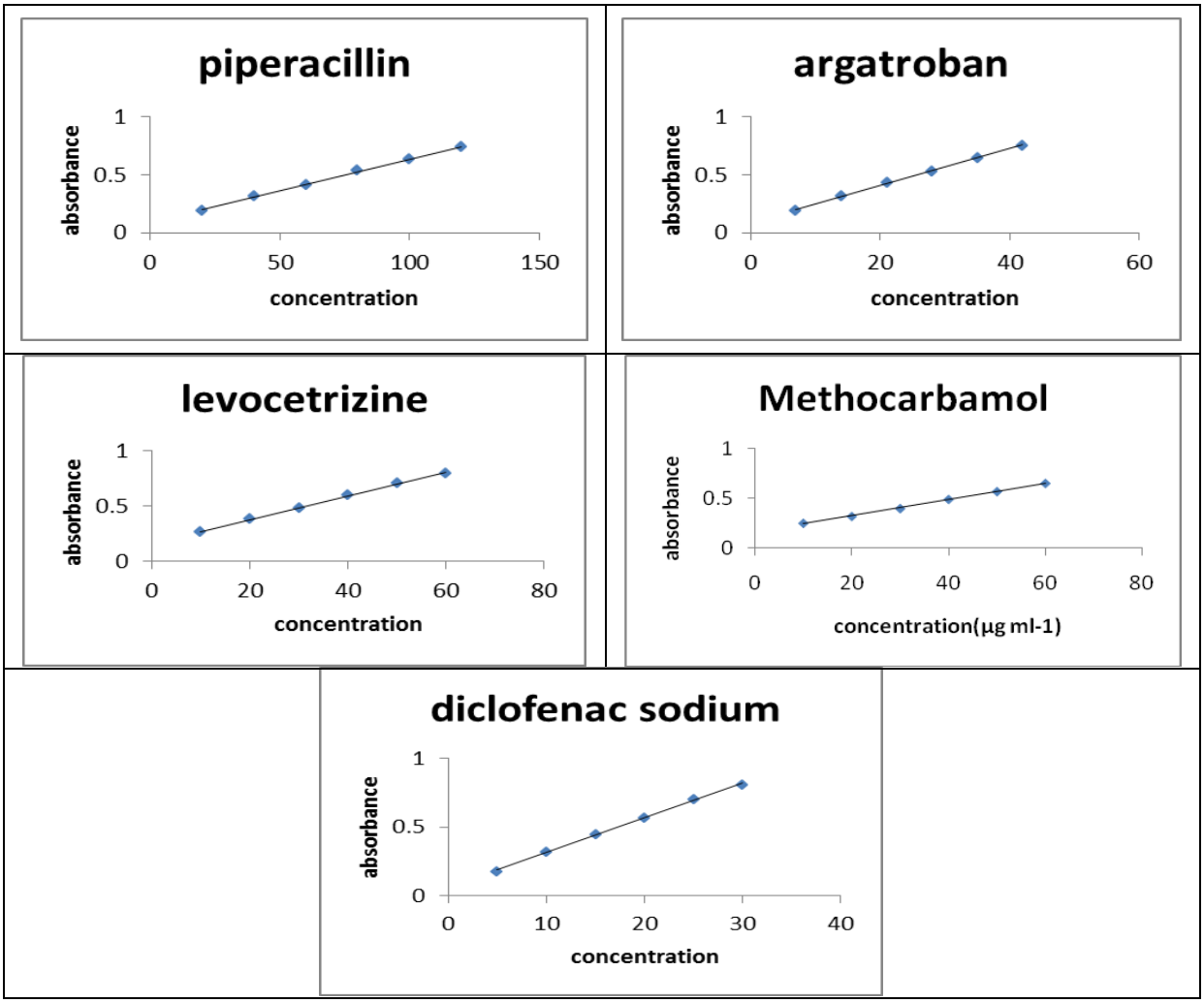


Figure 3: Calibration curves of the drugs

Table 1: Analytical Parameters for Determination of Drugs by Oxidation with Alkaline $KMnO_4$

Name of the drug property	PIP	ARG	LCTZ	MET	DCS
λ_{max}	610	610	610	610	610
Beer's law limits (µg ml^{-1})	20 - 120	5 - 30	10 - 60	10 - 60	7 - 42
Sandell's sensivity (µg cm^{-2})	0.2	0.0666	0.1	0.125	0.03703
Std. dev. of intercepts	0.021213	0.006723	0.00403	0.00336	0.002828
LOD (µg ml^{-1})	13.992	0.72806	1.3322	1.38682	0.37224
LOQ (µg ml^{-1})	42.4	2.20625	4.037	4.2025	1.128
Slope, b	0.005	0.016	0.01	0.008	0.025
Intercept, a	0.091	0.084	0.162	0.165	0.059
Correlation co-efficient	0.998	0.998	0.999	0.998	0.998

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.

$LOD = 3.3\ \sigma/S$

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

S = slope of linearity plot

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

$LOQ = 10\ \sigma/S$

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 ($\mu g\ ml^{-1}$)	Amount found ($\mu g\ ml^{-1}$)	% Recovery	RSD%	Proposed method Mean ± SD	Ref method Mean ± SD	t-test	F-test
PIP	30	29.99	99.96%	0.0294	99.99 ± 0.0294	99.84 ± 0.055	4.61	0.286
	50	50.01	100.02%					
	70	70.01	100.01%					
	90	89.98	99.97%					
ARG	7.5	7.51	100.01%	0.114	99.98 ± 0.114	99.8 ± 0.05	3.045	5.16
	12.5	12.48	99.84%					
	17.5	17.49	99.94%					
	22.5	22.51	100.04%					
LCTZ	15	15.01	100.06%	0.0607	99.99 ±	99.61 ±	1.866	0.0022

	25	24.98	99.92%		0.0607	1.28		
	35	35.01	100.02%					
	45	44.99	99.97%					
MET	12.5	12.52	100.16%	0.0943	100.025 ± 0.09434	99.37 ± 0.166	5.942	0.3229
	24.5	24.49	99.96%					
	36.5	36.51	100.02%					
	48.5	48.48	99.96%					
DCS	9.5	9.49	99.89%	0.0899	99.97 ± 0.0899	100.78 ± 0.507	2.74	0.030
	14.5	14.51	100.06%					
	19.5	19.51	100.05%					
	24.5	24.48	99.91%					

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 $0.2x10^{-2}M$ 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 22, 32, 42 and 52 µg ml^{-1} of PIP; 7, 12, 17 and 20 µg ml^{-1} of ARG; 12,24, 36 and 48 µg ml^{-1} of LCTZ; 12, 27,42 and 57 µg ml^{-1} of MET; 9,14,19 and 24 µg ml^{-1} DCS 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 the 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 DCS has lowset Sandell's snstivity and hence is highest sensitivity of the method, they are in the order DCS<ARG<LCTZ < MET<PIP.

Table 3: 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
PIP	22	22.01	100.04%	0.05619	99.99 ± 0.0561	99.01 ± 0.029	3.904	3.69
	32	31.98	99.93%					
	42	42.02	100.04%					
	52	51.98	99.96%					
ARG	7	7.01	100.14%	0.1186	100.04 ± 0.118	99.7 ± 0.08	4.91	1.89
	12	12.02	100.16%					
	17	16.99	99.94%					
	20	19.99	99.95%					
LCTZ	12	12.02	100.16%	0.0988	100.02 ± 0.0988	98.96 ± 0.8	2.94	0.666
	24	23.99	99.95%					
	36	36.01	100.02%					

	48	47.98	99.95%					
MET	12	12.01	100.08%	0.0763	100.012 ± 0.0763	98.7 ± 0.172	2.87	0.197
	27	27.02	100.07%					
	42	41.97	99.92%					
	57	56.99	99.98%					
DCS	9	8.98	99.77%	0.0788	100.007 ± 0.0788	99.8 ± 0.36	0.952	0.046
	14	13.99	99.92%					
	19	19.02	100.10%					
	24	24.01	100.04%					

XI. CONCLUSION

$KMnO_4$, an oxidizing agent in alkaline medium is found to oxidise drugs like PIP, ARG, LCTZ, MET and DCS 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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