Kinetic Spectrophotometric Determination of Drugs Based On Oxidation by Alkaline \( \text{KMnO}_4 \)

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Abstract: Simple, accurate and precise spectrophotometric methods for quantitative determination of four drugs viz., Levofloxacin (LEV), Moxifloxacin (MOX), Pseudo Ephidrine (PSE), Torsemide (TOR) have been developed based on oxidation of the drugs by alk.\( \text{KMnO}_4 \). 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 6.25-37.5 µg ml\(^{-1}\) for LEV; 5-30 µg ml\(^{-1}\) for MOX; 6.25-37.5 µg ml\(^{-1}\) for PSE and 2.5-15 µg ml\(^{-1}\) for TOR. Recovery studies using pure samples and pharmulations 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, Determination, Drugs, \( \text{KMnO}_4 \).

I. Introduction

1.1. Levofloxacin:
Levofloxacin [Fig. 1 a], (S)-(−)-9-fluro-2,3,dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid, a third-generation fluoroquinolone, is the active levo-isomer of ofloxacin and is twice as active as the parent drug. It is active against both Gram-positive and Gram-negative bacteria. Levofloxacin is administrated to patients with urinary, respiratory or cutaneous infections, in 500 mg/day doses. Levofloxacin is mainly excreted in urine (>85%), in unaltered form [1]. Some analytical methods HPLC [2,3], spectrophotometry [4,5] and Spectrofluorimetry [6] have been described for determination of LEV.

1.2. Moxifloxacin:
Moxifloxacin (MOX) [Fig.1 b] chemically known as 1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-[(4aS, 7aS)-octahydro-6H-pyrrolo-[3, 4-b] pyridin-6-yl]-4-oxo-3-quinoilcarboxylic acid is a fourth generation fluoroquinolone broad spectrum antibiotic agent used in Conjunctivitis [7,8]. Some methods such as HPLC [9,10], Fluorimetry [11,12], spectrophotometry [13,14] and Voltammetry [15] are used for the determination of MOX.

1.3. Pseudo ephidrine:
Pseudo Ephidrine (PSE) [Fig.1 c], chemically known as (S,S)-2-methylamino-1-phenylpropan-1-ol is asympathomimetic drug that has been for the relief of nasaldecongestion [16]. Literature survey reveals that there are HPLC [17,18], Capillary Electrophoresis [19],spectrophotometry [20,21] and LC-MS [22,23] methods reported for the determination of PSE.

1.4. Torsemide:
Torsemide chemically 3-[4-[(3-methylphenyl) amino] pyridin-3-yl]sulfonyl-1-propan-2-ylurea [Fig.1 d], is a loop diuretic mainly used in the management of edema associated withcongestive heart failure. It is also used at low doses for the management of hypertension [24,25].Hydrotropy phenomenon is considered as a unique technique by which solubility for sparinglysoluble solute under normal conditions increase in the several folds[26]. Colorimetry [27], UV- Spectrophotometry [28] methods have been reported for the determination of Torsemide.
II. Experimental

2.1. Instrumentation:
The UV-VIS spectra of the study have been recorded on SHIMADZU 140 double beam Spectrophotometer, 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.

2.2. Materials:
Analytical grade KMnO₄, 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 1000 µg ml⁻¹ was initially prepared and further diluted to get working concentrations.

2.2.2. KMnO₄ Solution:
A stock solution of KMnO₄ is prepared by dissolving 0.158 gm of pure sample of KMnO₄ in 100 ml triple distilled water. The permanganate solution was standardized against oxalic acid by standard procedure.

2.2.3. Standardization of KMnO₄:
The standardization of KMnO₄ solution is carried out by titration against a standard solution of oxalic acid. Oxalic acid is a good primary standard because the compound is available in solid form, as H₂C₂O₄.H₂O, which can be prepared to a very high degree of purity and is neither hygroscopic nor efflorescent. The reaction is

\[ 2\text{MnO}_4^- (aq) + 6\text{H}^+ (aq) + 5\text{H}_2\text{C}_2\text{O}_4 (aq) \rightarrow 2\text{Mn}^{2+} + 8\text{H}_2\text{O (l)} + 10\text{CO}_2 (g) \]

2.2.3. 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.

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 KMnO₄ (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, 6 ml, 5 ml, 4 ml, 3 ml, 2 ml, 1 ml and 0.5 ml of drug solutions by making up remaining volume with distilled water. Absorbance-time curves [Fig. 2] were constructed.

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 6.25-37.5 µg ml⁻¹ of LEV, 5-30 µg ml⁻¹ of MOX, 6.25-37.5 µg ml⁻¹ of PSE, 2.5-15 µg ml⁻¹ of TOR test solutions were pipetted into a series of 10ml standard flasks. 1 ml of 0.5M NaOH 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.

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, 8, 15, 22, and 29 μg ml⁻¹ of Lev; 5, 10, 15, and 20 μg ml⁻¹ of Mox; 7, 14, 21, and 28 μg ml⁻¹ of Pse; 3, 6, 9, and 12 μg ml⁻¹ of Tor 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. Levofloxacin:
Each tablet of Levofloxacin contains 500 mg Levofloxacin 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 Levofloxacin (6.25 – 37.5 μg ml⁻¹). Kinetics runs were performed using 9, 16, 23 and 30 μg ml⁻¹ of Levofloxacin other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

7.2. Moxifloxacin:
For analysis of commercial formulations, twenty tablets 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 5μgml⁻¹. Kinetics runs were performed using 6, 11, 16 and 21 μg mL⁻¹ of Moxifloxacin other experimental details being common. Initial rate and fixed time of 15 min were chosen to estimate the amount found.

7.3. Pseudoephedrine:
An accurately weighed ten tablets were finely powdered, weighed, a portion equivalent to 120 mg was transferred quantitatively into 50ml volumetric flask, sonicated for 15 min, completed to volume with distilled water. A stock solution containing 2.4 mg ml⁻¹ was further diluted to get required concentration for pharmaceutical analysis. Kinetics runs were performed using 6, 13, 20 and 27 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.

7.4. Torsemide:
In order to see the feasibility of proposed method for estimation of Torsemide in marketed pharmaceutical formulations, twenty tablets were weighed, average weight determined and crushed into fine powder. A quantity of tablet powder equivalent to 10 mg of Torsemide 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 4, 7, 10 and 13 μg mL⁻¹ of Torsemide 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].

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.

IX. Factors Effecting Absorbance

9.1. Effect of concentration of KMnO₄:

The effect of concentration of KMnO₄ on the absorbance at preselected time, 15 min was studied in the range 0.2x 10⁻²M to 1.2x10⁻¹M by keeping the concentration of drug constant. The absorbance [Fig. 5] increased with increasing the concentration of KMnO₄ and became constant at 0.7x10⁻²M to 0.8x10⁻²M. Thus, the adoption of 1x10⁻²M KMnO₄ 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 0.01M KMnO₄ solution and varying volumes (0.2 – 1.2ml) of 0.5 M NaOH. The maximum absorbance was obtained with 0.8 ml of 0.5MNaOH, after which further increase in volume of NaOH caused no change in absorbance. Hence, 0.8 to 1.0 ml of 0.5M NaOH was used as an optimum value [Fig. 6]

9.3. Effect of prolonged time:

The effect of time on the reaction between KMnO₄ 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 MnO₂; 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 9, 16, 23 and 30 μg ml⁻¹ of Lev; 6, 11, 16 and 21 μg ml⁻¹ of Mox; 6,13, 20 and 27 μg ml⁻¹ of Pse; 4, 7, 10 and 13 μg ml⁻¹ of Tor were chosen for kinetic study other experimental details being common. Initial rate and fixed time 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 Ind has lowest Sandell’s sensitivity and hence is highest sensitivity of the method, they are in the order Tor<Lev<Mox<Pse.

XI. Conclusion

KMnO₄, an oxidizing agent in alkaline medium is found to oxidise drugs like LEV, MOX, PSE and TOR which are soluble in basic medium. One of the oxidizing products namely manganese ion absorbs maximally at

www.iosrjournals.org 11 | Page
Kinetic Spectrophotometric Determination Of Drugs Based On Oxidation By Alkaline KMnO₄

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.

Acknowledgement

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Kinetic Spectrophotometric Determination Of Drugs Based On Oxidation By Alkaline KMnO$_4$

<table>
<thead>
<tr>
<th>a. Levofloxacin</th>
<th>b. Moxifloxacin</th>
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<td>![Levofloxacin Structure]</td>
<td>![Moxifloxacin Structure]</td>
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<th>c. Pseudo Ephedrine</th>
<th>d. Torsemide</th>
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<tr>
<td>![Pseudo Ephedrine Structure]</td>
<td>![Torsemide Structure]</td>
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</table>

Figure 1: Structures of the Drugs

![Absorbance –Time curves for the reaction of Drugs with Alkaline KMnO$_4$]

Figure 2: Absorbance –Time curves for the reaction of Drugs with Alkaline KMnO$_4$

![Calibration Curves]

Figure 3: Calibration Curves

![Effect of KMnO$_4$ on Absorbance]

Figure 5: Effect of KMnO$_4$ on Absorbance
Table 1: Analytical Parameters for determination of drugs by oxidation with alkaline KMnO₄

<table>
<thead>
<tr>
<th>Name of the Drug</th>
<th>Amount Taken (µg ml⁻¹)</th>
<th>Amount Found (µg ml⁻¹)</th>
<th>% Recovery</th>
<th>RSD %</th>
<th>Proposed method Mean ± SD</th>
<th>Ref method Mean ± SD</th>
<th>t-test (*)</th>
<th>F-test (**)</th>
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<td>LEV</td>
<td>8</td>
<td>8.07</td>
<td>100.87</td>
<td>0.587</td>
<td>100.16 ± 0.588</td>
<td>[136] 99.62 ± 0.788</td>
<td>0.478</td>
<td>0.557</td>
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<td></td>
<td>15</td>
<td>14.95</td>
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<td>28.91</td>
<td>99.68</td>
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<td>MOX</td>
<td>5</td>
<td>4.98</td>
<td>99.6</td>
<td>0.978</td>
<td>99.94 ± 0.977</td>
<td>[143] 100.09 ± 0.86</td>
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<td>PSE</td>
<td>7</td>
<td>6.97</td>
<td>99.57</td>
<td>0.76</td>
<td>100.506 ± 0.765</td>
<td>[150] 102.6 ± 0.78</td>
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<td>3</td>
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* t-test and **F-test values from literature

Table 2: Recovery Studies to evaluate Accuracy and Precision for the Determination of Drugs by Redox Reaction with Alkaline KMnO₄

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<tr>
<th>Name of the Drug (Tablet)</th>
<th>Amount Taken (µg ml⁻¹)</th>
<th>Amount Found (µg ml⁻¹)</th>
<th>% Recovery</th>
<th>RSD %</th>
<th>Proposed method Mean ± SD</th>
<th>Ref method Mean ± SD</th>
<th>t-test (*)</th>
<th>F-test (**)</th>
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<td>Lev (Alevo)</td>
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<td>100.78</td>
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Table 3: Application of proposed method for the analysis of studied drugs in pharmaceutical formulations by redox reaction with alkaline KMnO₄