

Indirect spectrophotometric determination of lamivudine

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Abstract: Indirect Spectrophotometric method for trace determination of Lamivudine has been developed. The method is Base on decrease in absorbance of alkaline potassium permanganate in presence of Barium ion. The calibration Curve, Which is a plot of decreasing absorbance value of potassium permanganate solution at 540 nm against increasing concentration of Lamivudine is linear up to 2.5 ppm.

(Keywords : Absorbance / trace determination / color reduction / spectrophotometric method.)

Introduction

Antiretroviral medications prevent HIV from multiplying, thus protecting the cells that the virus would otherwise target.

Types of antiretroviral drugs

1. Nucleoside reverse transcriptase inhibitors (NRTIs)
 2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
 3. Protease inhibitors (PIs)
 4. Entry inhibitors
 5. Integrase inhibitors
- NRTIs block the action of an enzyme called viral reverse transcriptase, which is necessary for HIV to replicate.

Examples of NRTIs include:

- * Abacavir
- * Lamivudine
- * Emtricitabine
- * Stavudine
- * Tenofovir
- * Zidovudine

Lamivudine, commonly called 3TC, used to prevent and treat HIV/AIDS. It is typically used in combination with other antiretroviral such as zidovudine and abacavir. Lamivudine is a nucleoside reverse transcriptase inhibitor and works by blocking the HIV reverse transcriptase and hepatitis B virus polymerase. Lamivudine has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV/AIDS.

Some of the analytical methods available for determination of Lamivudine are as follows :

1. UV Spectrophotometric Method for Assay of the Anti-Retroviral Agent Lamivudine in Active Pharmaceutical Ingredient and in its Tablet Formulation.¹
2. Two visible spectrophotometric methods have been developed for the determination of Lamivudine (LMV) in pure and tablet forms. Method-A is based on oxidation of 3-methyl-2-benzathiazolinone hydrazone (MBTH) in the presence of iodoso benzene diacetate (IBDA). Method-B depends on diazonium salt formation and consequent reaction with resorcinol producing colored product.²
3. Spectrophotometric Method for Determination of Lamivudine in Pure and Tablet Dosage Form by Formation of a Colored Hydrazone Using 2,4 dinitrophenyl Hydrazine³
4. Development and validation of analytical method for the estimation of lamivudine in rabbit plasma.⁴
5. A Validated RP - HPLC Method for Simultaneous Estimation of Lamivudine and Tenofovir Disoproxil Fumarate in Pure and in Tablet Dosage Form⁵

Different methods for determination of Lamivudine mentioned above are easy to carry out but they have some limitations. For instance some methods are

- 1) Time consuming
- 2) Require organic reagents and solvents
- 3) Some methods require expensive apparatus.

Therefore it is a matter of practical interest to develop relatively simple, rapid, economic, ecofriendly and sensitive method for determination of Lamivudine.

In the present paper a simple, precise and low cost indirect Spectrophotometric method for trace determination of Lamivudine developed. Trace level determination is one the most important factor in analytical chemistry. Trace level determination methods can be used for cleaning process.

Regulatory Agency / FDA have specific guideline for validation of cleaning processes. Cleaning method validation is the methodology used to assure that the cleaning procedure removes residue of APIs, excipient and detergents used for cleaning process. Cleaning process ensure the quality of next product.

Principle:

Lamivudine is Oxidation sensitive and Potassium permanganate if strong oxidizing agent hence addition of Lamivudine in Alkaline permanganate solution tends to reduce permanganate which results in decrease in absorbance of alkaline permanganate.⁶

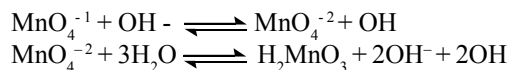
Alkaline permanganate Mn (VII) is reduced quantitatively to manganese (VI) after addition of Lamivudine in the presence of Ba (II) ions. Thus addition of Lamivudine decreases color intensity of alkaline potassium permanganate solution.

In the present work a simple and rapid

spectrophotometric method for trace level determination of Lamivudine based on reduction of color intensity of alkaline permanganate solution is developed. Reduction of permanganate (Mn VII) to manganate (Mn VI) and subsequently to manganese dioxide (Mn IV) are the stages through which reaction proceeds.

The reduction of permanganate solution in alkaline solution highly depends on the condition of reaction like concentration of hydroxyl ions, temperature, etc.

Mechanism^{7,8} of the permanganate oxidation in alkaline solution has been thoroughly investigated and it was assumed that hydroxide radical which are real oxidising agent are intermediately formed.



The effect of hydroxide ion concentration on the velocity of two reduction steps in alkaline solutions is well recognizable from the two chemical equations.

As temperature increases rate of the above reaction is greatly enhanced. Hence hydroxide ion concentration and temperature are two important parameters in the reduction of alkaline potassium permanganate solution.

The correct choice of the alkalinity of the solution is not sufficient to ensure that only one of the two reduction steps is reached. A definite course of reaction, however, is ensured under suitable conditions when barium (II) salts are added.

Role of Ba (II) ions:

Barium permanganate [$\text{Ba}(\text{MnO}_4)_2$] is readily water soluble. Solubility of barium manganate (BaMnO_4) is $2.46 \times 10^{-10} \text{ gm/dm}^3$ at 25°C . If barium nitrate or barium chloride is added to alkaline

permanganate solution, the barium manganate formed during the reaction precipitates so rapidly that under optimum conditions it cannot be further reduced¹⁰. The barium manganate forms deposits very well and settles down.

Experimental, Material and Method:

Absorbance measurements were made on a SPECTROCHEM spectrophotometer using optical glass cell with 10 nm path length. AR grade chemicals and deionized water after distillation were employed throughout the work. Potassium permanganate solution was standardized by volumetric method. A working solution of 2×10^{-3} M KMnO_4 was prepared by dilution. Lamivudine API received from DIVINE HEALTH CARE.

Procedure for the determination of Lamivudine:

$5 \times 10^{-3} \text{ dm}^3$ of 2×10^{-3} M KMnO_4 solution were placed in $50 \times 10^{-3} \text{ dm}^3$ volumetric flask, $5 \times 10^{-3} \text{ dm}^3$ of 1×10^{-3} M $\text{Ba}(\text{NO}_3)_2$, $2.5 \times 10^{-3} \text{ dm}^3$ of 1×10^{-2} M NaOH were added to it, followed by a suitable aliquot of Lamivudine Solution. The volume was made up with deionized distilled water. The flask was kept in boiling water bath for 20 minutes. After cooling solution was centrifuged and absorbance was measured at 540 nm against distilled water as blank.

Results and Discussion:

The present method is based on the decrease in absorbance at 540 nm (λ_{max}) of permanganate solution on addition of Lamivudine. Reduction of color intensity of permanganate solution by Lamivudine was studied in presence of different concentrations of sodium hydroxide in the range of 0.0001M to 0.01M and optimum base concentration range for reproducible decrease in absorbance of permanganate solution was found to be 0.0003M to 0.0009M NaOH. All analytical

work was carried out using 0.0005 M. NaOH. [Table 1, Fig.1A & B]

The effect of the barium nitrate concentration on the decrease in the color intensity of permanganate solution due to Lamivudine was studied. The optimum barium nitrate concentration range for reproducible decrease in the absorbance was found to be 0.00004 M to 0.00018 M. All analytical work was carried out using 0.0001M $\text{Ba}(\text{NO}_3)_2$. [Table 2, Fig.No.2]

It was found that reduction of permanganate was complete after placing the volumetric flask in boiling water bath for 20 minutes. After cooling the solution, reduced color intensity retains its absorbance at the same value for at least 3 hours. All the analytical work was carried out by placing volumetric flask in boiling water bath for 20 minutes. (Table 3 A & B)

Optimum Range and Accuracy:-

It has been found that the calibration curve obtained by plotting the decreasing absorbance values of 2×10^{-4} M KMnO_4 solution against Lamivudine concentration is linear up to $2.5 \mu\text{g}$ of Lamivudine per 10^{-3} dm^3 . (Table 4, Fig.3). Slope of Calibration curve is 0.0339, Hence calibration sensitivity is 0.0339.

Precision and accuracy of the method were tested by analyzing eleven samples each containing $1.5 \mu\text{g}$ per 10^{-3} dm^3 of Lamivudine. The amount of Lamivudine for a decrease in the absorbance by 0.001 was found to be $0.034 \mu\text{g}$ per 10^{-3} dm^3 . Hence Sandell's sensitivity is 0.034. Mean absorbance at 540 nm was 0.244 with a standard deviation of 0.0018 and % relative standard deviation of 0.743. The average of eleven determinations carried out using 1.5 ppm of Lamivudine is 1.503 ppm with % relative standard deviation is 0.743%

Table-1 Effect of NaOH concentration.Concentration of KMnO_4 2×10^{-4} MConcentration of $\text{Ba}(\text{NO}_3)_2$ 1×10^{-4} M

Concentration of Lamivudine – 1.5 ppm

Sr. No	Conc. Of NaOH (in Molar)	Optical Density
1	0.0001	0.260
2	0.0002	0.251
3	0.0003	0.244
4	0.0004	0.243
5	0.0005	0.243
6	0.0006	0.243
7	0.0007	0.242
8	0.0008	0.240
9	0.0009	0.238
10	0.001	0.235
11	0.005	0.214
12	0.01	0.186

Figure 1A & B Effect of NaOH concentration.Concentration of KMnO_4 2×10^{-4} MConcentration of $\text{Ba}(\text{NO}_3)_2$ 1×10^{-4} M

Concentration of Lamivudine – 1.5 ppm

Fig. 1 A

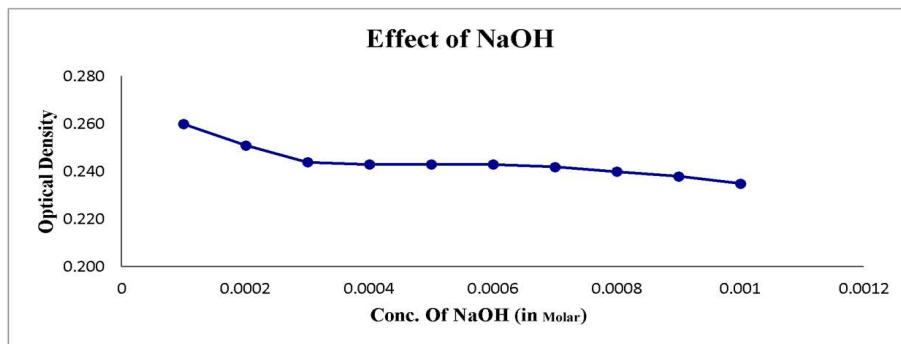


Fig. 1 B

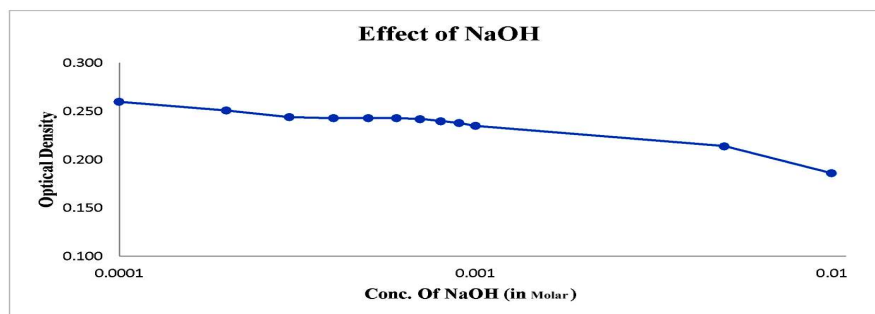


Table-2 Effect of $\text{Ba}(\text{NO}_3)_2$ concentration.Concentration of KMnO_4 2×10^{-4} MConcentration of NaOH 5×10^{-4} M

Concentration of Lamivudine – 1.5 ppm

Sr. No.	Conc. Of $\text{Ba}(\text{NO}_3)_2$ (in Molar)	Optical Density
1	0.00002	0.250
2	0.00004	0.244
3	0.00006	0.244
4	0.00008	0.243
5	0.0001	0.243
6	0.00012	0.243
7	0.00014	0.243
8	0.00016	0.241
9	0.00018	0.240
10	0.0002	0.238

Figure 2 Effect of $\text{Ba}(\text{NO}_3)_2$ concentration.Concentration of KMnO_4 2×10^{-4} MConcentration of NaOH 5×10^{-4} M

Concentration of Lamivudine – 1.5 ppm

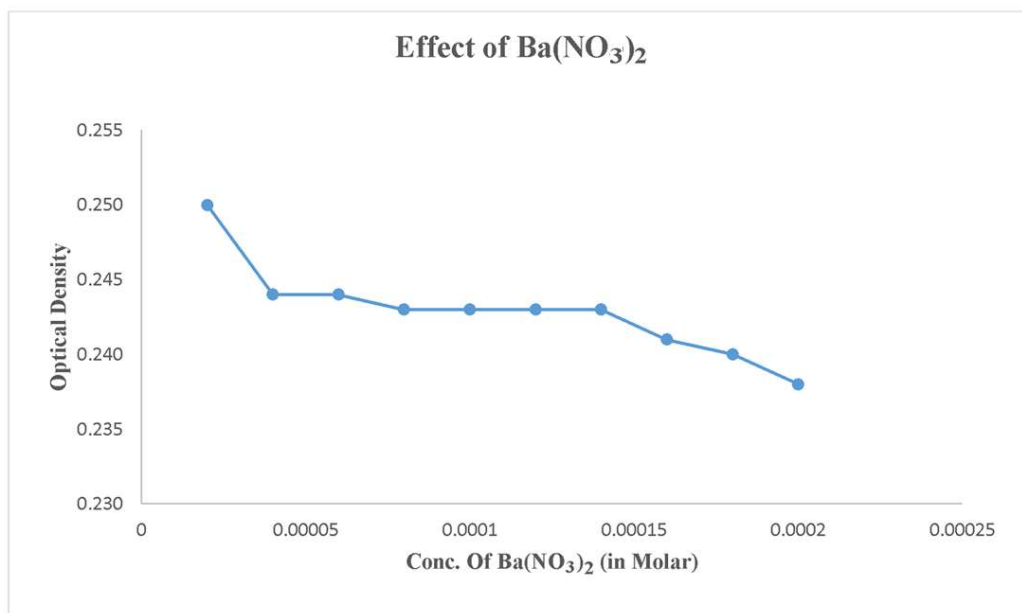


Table 3 (A) Effect of Temperature

Concentration of KMnO_4 2×10^{-4} M
 Concentration of $\text{Ba}(\text{NO}_3)_2$ 1×10^{-4} M
 Concentration of NaOH 5×10^{-4} M
 Concentration of Lamivudine – 1.5 ppm Effect of Temperature

Sr. No.	Temperature in Degree	Optical Density
1	30	0.290
2	40	0.278
3	50	0.259
4	60	0.250
5	70	0.246
6	80	0.244
7	90	0.244
8	98	0.241

Table 3 (B) Effect of Temperature

Concentration of KMnO_4 2×10^{-4} M
 Concentration of $\text{Ba}(\text{NO}_3)_2$ 1×10^{-4} M
 Concentration of NaOH 5×10^{-4} M
 Concentration of Lamivudine – 1.5 ppm Effect of Time in boiling water bath

Sr. No	Effect of Time in Boiling Water bath in Min	Optical Density
1	5	0.258
2	10	0.248
3	15	0.244
4	20	0.243
5	25	0.243
6	30	0.241

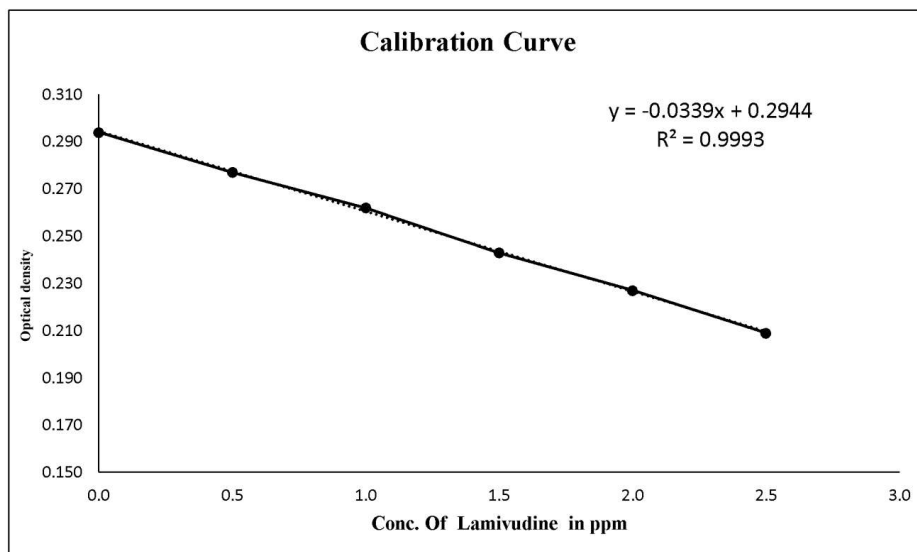
Table 4 - Calibration Curve

Concentration of KMnO_4 2×10^{-4} M Concentration of $\text{Ba}(\text{NO}_3)_2$ 1×10^{-4} M
 Concentration of NaOH 5×10^{-4} M Time in Water Boiling Water Bath – 20 min

Sr. No	Conc. Of Lamivudine in ppm	Optical Density
1	0.0	0.294
2	0.5	0.277
3	1.0	0.262
4	1.5	0.243
5	2.0	0.227
6	2.5	0.209

Figure -3 Calibration CurveConcentration of KMnO_4 2×10^{-4} MConcentration of $\text{Ba}(\text{NO}_3)_2$ 1×10^{-4} MConcentration of NaOH 5×10^{-4} M

Time in Water Boiling Water Bath – 20 min

**Table 5****Reproducibility Of method**Concentration of KMnO_4 2×10^{-4} MConcentration of $\text{Ba}(\text{NO}_3)_2$ 1×10^{-4} MConcentration of NaOH 5×10^{-4} M

Concentration of Lamivudine – 1.5 ppm

Flask No	Absorbance	Lamivudine found In ppm	%Error
1	0.243	1.500	0.000
2	0.246	1.519	-1.235
3	0.241	1.488	0.823
4	0.243	1.500	0.000
5	0.244	1.506	-0.412
6	0.245	1.512	-0.823
7	0.245	1.512	-0.823
8	0.246	1.519	-1.235
9	0.243	1.500	0.000
10	0.242	1.494	0.412
11	0.241	1.488	0.823
Mean	0.244	1.503	-0.224
Standard Deviation	0.0018	0.0112	NA
Relative Standard Deviation (%)	0.743	0.743	NA

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