

Indirect spectrophotometric determination of Emtricitabine

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Abstract: Indirect Spectrophotometric method for trace determination of Emtricitabine 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 Emtricitabine is linear up to 2.5 ppm.

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

Introduction

Emtricitabine is synthetic fluoro derivative of thiacytidine with potent antiviral activity. It is nucleoside reverse-transcriptase Inhibitor for prevention and treatment of HIV infection in Adult and children. Common name of Emtricitabine is FTC and trade name is Emtria. Emtricitabine is use with other antiretroviral agent for prevention and treatment of HIV infection. Emtricitabine is also have activity against Hepatitis B virus. The adverse events involve in treatment with Emtricitabine are diarrhea, headache, nausea and rash. Skin decolorisation is also reported as adverse event in treatment with Emtricitabine.

Emtricitabine is analogue of Cytidine. Emtricitabine inhibits enzymes that copies HIV RNA into new viral DNA, that means Emtricitabine prevent replication of HIV ultimately help to reduce amount of HIV and indirectly increase immune system.

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

1. Simple Spectrophotometric method for determination of Tenofovir fumarate and Emtricitabine in bulk powder and in Tablet.¹
2. Emtricitabine and Tenofovir can be determine with a spectrophotometric method which includes area under Curve method and Dual Wavelength method.²
3. HPLC method for determination of Emtricitabine and related degradation product.³
4. A validated Stability indicating RP-HPLC method for simultaneous determination of Tenofovir, Emtricitabine and Efavirenz and statistical approach to determine the effect of variables.⁴

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

- 1) Time consuming
- 2) Require organic reagents
- 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 Emtricitabine.

In the present paper a simple, precise and low cost indirect Spectrophotometric method for trace determination of Emtricitabine 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.

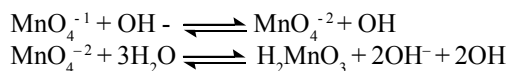
Principle:

Emtricitabine is Oxidation sensitive and Potassium permanganate if strong oxidizing agent hence addition of Emtricitabine in Alkaline permanganate solution tends to reduce permanganate which results in decrease in absorbance of alkaline permanganate.⁵

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

In the present work a simple and rapid spectrophotometric method for trace level determination of Emtricitabine 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^{6,7} 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 (8). 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 reduced⁹. 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 $2 \times 10^{-3} \text{ M KMnO}_4$ was prepared by dilution. Emtricitabine API received from DIVINE HEALTH CARE.

Procedure for the determination of Emtricitabine:

$5 \times 10^{-3} \text{ dm}^3$ of 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 $10^{-3} \text{ M Ba}(\text{NO}_3)_2$, $2.5 \times 10^{-3} \text{ dm}^3$ of 10^{-2} M NaOH were added to it, followed by a suitable aliquot of Emtricitabine 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.

Result and Discussion

The present method is based on the decrease in absorbance at 540 nm ($\lambda_{\max}$) of permanganate solution on addition of Emtricitabine.

Reduction of color intensity of permanganate solution by Emtricitabine 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.1 A & B] The effect of the barium nitrate concentration on the decrease in the color intensity of permanganate solution due to Emtricitabine 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 Ba (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 No. 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 Emtricitabine concentration is linear up to 2.5 μg of Emtricitabine per 10^{-3} dm^3 . (Table 4, Fig.3). Slope of Calibration curve is 0.0295, Hence calibration sensitivity is 0.0295. Precision and accuracy of the method were tested by analyzing eleven samples each containing 1.5 μg per 10^{-3} dm^3 of Emtricitabine. The amount of Emtricitabine for a decrease in the absorbance by 0.001 was found to be 0.030 μg per 10^{-3} dm^3 . Hence Sandell's sensitivity is 0.030. Mean absorbance at 540 nm was 0.251 with a standard deviation of 0.0012 and % relative standard deviation of 0.486. The average of eleven determinations carried out using 1.5 ppm of Emtricitabine is 1.501 ppm with % relative standard deviation is 0.486 %

Table-1 Effect of NaOH concentration.

Concentration of KMnO_4 2×10^{-4} M Concentration of Ba (NO_3)₂ 1×10^{-4} M
Concentration of Emtricitabine – 1.5 ppm

Sr. No	Conc. of NaOH (in molar)	Optical Density
1	0.0001	0.262
2	0.0002	0.256
3	0.0003	0.253
4	0.0004	0.251
5	0.0005	0.251
6	0.0006	0.251
7	0.0007	0.251
8	0.0008	0.250
9	0.0009	0.249
10	0.001	0.247
11	0.005	0.225
12	0.01	0.196

Table-1 Effect of NaOH concentration.

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

Fig. 1 A

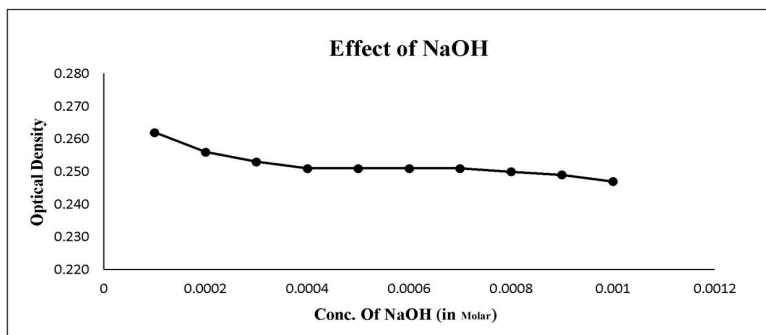
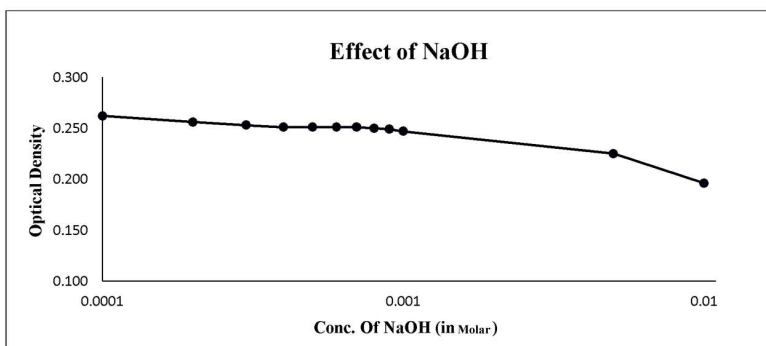


Fig. 1 B

**Table 2****Effect of $\text{Ba}(\text{NO}_3)_2$ concentration.**

Concentration of KMnO_4 2×10^{-4} M
 Concentration of NaOH 5×10^{-4} M
 Concentration of Emtricitabine – 1.5 ppm

Sr. No	Conc. Of $\text{Ba}(\text{NO}_3)_2$ (in Molar)	Optical Density
1	0.00002	0.259
2	0.00004	0.253
3	0.00006	0.251
4	0.00008	0.251
5	0.0001	0.251
6	0.00012	0.251
7	0.00014	0.251
8	0.00016	0.250
9	0.00018	0.251
10	0.0002	0.249

Table 3
Effect of $\text{Ba}(\text{NO}_3)_2$ concentration.
 Concentration of KMnO_4 2×10^{-4} M
 Concentration of NaOH 5×10^{-4} M
 Concentration of Emtricitabine – 1.5 ppm

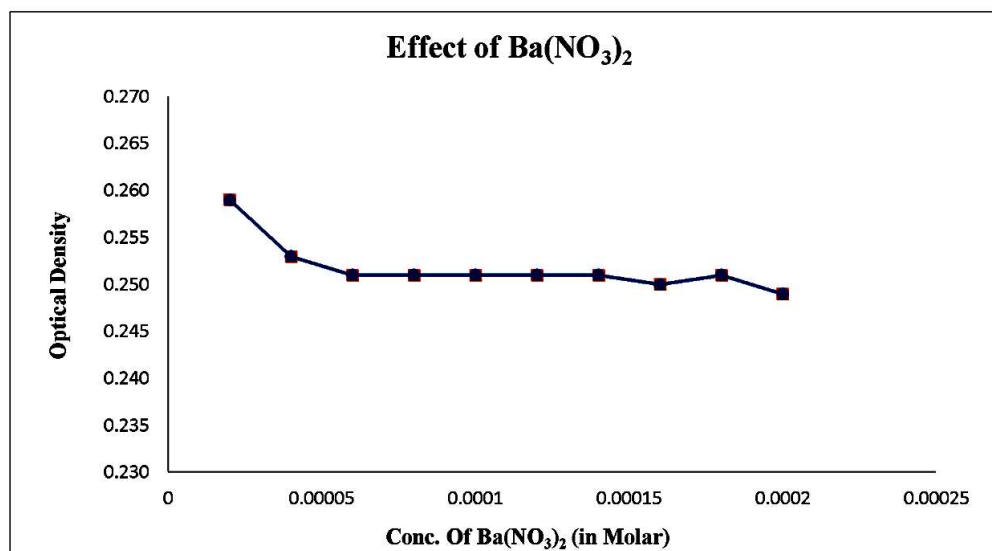


Table 3 (A)
Effect of Temperature
 Concentration of KMnO_4 2×10^{-4} M
 Concentration of $\text{Ba}(\text{NO}_3)_2$ 1×10^{-4} M
 Concentration of NaOH 5×10^{-4} M
 Concentration of Emtricitabine – 1.5 ppm Effect of Temperature

Sr. No	Temperature in Degree	Optical Density
1	30	0.290
2	40	0.280
3	50	0.263
4	60	0.254
5	70	0.252
6	80	0.252
7	90	0.251
8	98	0.251

Table 3 (B)**Effect of Temperature**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 Emtricitabine – 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	0.265
2	10	0.253
3	15	0.252
4	20	0.252
5	25	0.250
6	30	0.249

Table 4**Calibration Curve**Concentration of KMnO_4 2×10^{-4} M Concentration 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

Sr. No	Conc. of Emtricitabine in ppm	Optical Density
1	0.0	0.296
2	0.5	0.280
3	1.0	0.266
4	1.5	0.251
5	2.0	0.238
6	2.5	0.221

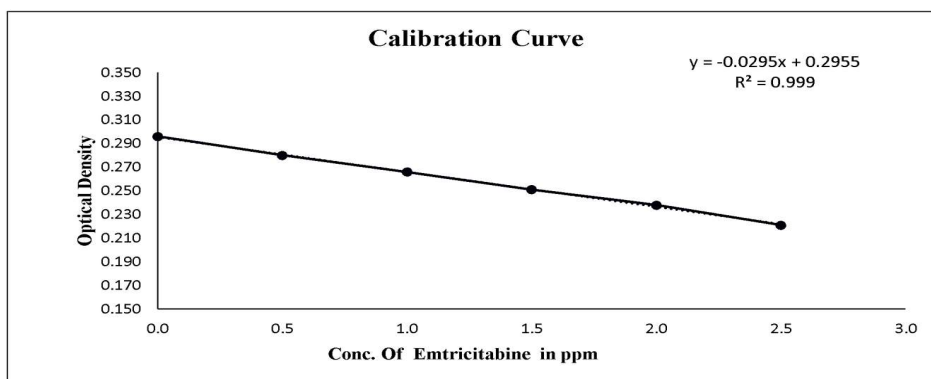
Figure - 3**Calibration Curve**Concentration of KMnO_4 2×10^{-4} M Concentration of $\text{Ba}(\text{NO}_3)_2$ 1×10^{-4} MConcentration of NaOH 5×10^{-4} M Time in Water Boiling Water Bath – 20 min

Table 5
Reproducibility Of method

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

Flask No.	Absorbance	Emtricitabine found In ppm	%Error
1	0.251	1.500	0.000
2	0.251	1.500	0.000
3	0.249	1.488	0.797
4	0.251	1.500	0.000
5	0.252	1.506	-0.398
6	0.252	1.506	-0.398
7	0.249	1.488	0.797
8	0.253	1.512	-0.797
9	0.251	1.500	0.000
10	0.252	1.506	-0.398
11	0.251	1.500	0.000
Mean	0.251	1.501	-0.398
Standard Deviation	0.0012	0.0073	NA
Relative Standard Deviation (%)	0.486	0.486	NA

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