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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF EMTRICITABINE IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Emtricitabine in tablet dosage form. **Methods:** The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Emtricitabine in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in analytical grade methanol, soluble in water and very slightly soluble in acetonitrile. So, the analytical grade methanol is used as a diluent in method. The melting point of Emtricitabine was found to be 137-138˚C (uncorrected). It showed absorption maxima 225 nm in analytical grade methanol. On the basis of absorption spectrum the working concentration was set on 6 µg/ml (PPM). The linearity was observed between 2-10 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 98.00 and 98.33% for three levels respectively. The % RSD for precision was found to be 1.38% and for Ruggedness is 0.95%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Emtricitabine in tablet dosage form. The method could be considered for the determination of Emtricitabine in quality control laboratories.

KEYWORDS: Emtricitabine, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Emtricitabine was granted FDA approval on 2 July 2003. Emtricitabine is an analogue of cytidine. The chemical name of emtricitabine is 4-amino-5-fluoro-1[(2R, 5S)-2 (hydroxymethyl) -1,3oxathialon-5yl]ne.[1] Emtricitabine is indicated in combination with other medications for the treatment of HIV-1 infections. It is administered once daily so it has a long duration of action Emtricitabine is a nucleoside reverse transcriptase inhibitor for the prevention and treatment of HIV infection in adults and children.[2] The drug works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. By interfering with this process, which is central to the replication of HIV, emtricitabine can help to lower the amount of HIV, or "viral load", in a patient's body and can indirectly increase the number of immune system cells (called T cells or CD4+ T-cells). Both of these changes are associated with healthier immune systems and decreased likelihood of serious illness.[3]

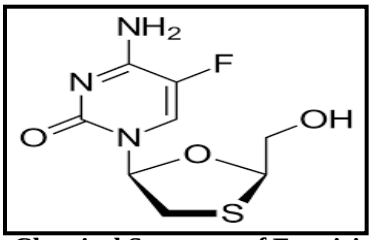

Fig. 1: Chemical Structure of Emtricitabine.

The available literature studies describe utilization of few analytical methods for the evaluation of Emtricitabine in

tablet dosage form. These methods are based on various techniques such as HPLC[4] and LC[5] methods as well as HPLC in plasma[6-9], HPTLC[10] and AUC methods for simultaneous estimation[11] were existed for estimation of Emtricitabine. But, some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Emtricitabine in tablet dosage form. So, the aim of work was to develop and validate an analytical method by UV-Visible Spectrophotometer for the estimation of Emtricitabine.

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.
Melting point apparatus of Equiptronics were used.

- **Materials**

Emtricitabine was obtained as a gift sample. Emtricitabine tablets were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

Method Development

A. Determination of λ max (10 PPM)[13, 14, 15]

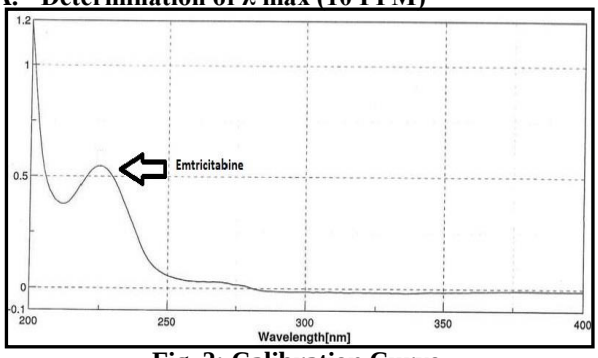

Fig. 2: Calibration Curve.

50 mg weighed amount of Emtricitabine was dissolved into 100 ml of volumetric flask with analytical grade methanol. Pipette out 0.6 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

B. Preparation of Working concentration

Preparation of Standard stock solution

Standard stock was prepared by dissolving 50 mg of Emtricitabine in 100 ml of analytical grade methanol to get concentration of 500 µg/ml (PPM).

Preparation of Standard solution

Pipette out 0.6 ml from standard stock solution and diluted up to 50 ml with analytical grade methanol to get concentration of 6 µg/ml (PPM).

C. Procedure for UV reading.

Blank Solution: (For Auto zero)

Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[13]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Emtricitabine was accurately weighed and transferred into the 100 ml of volumetric flask, added 70 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade methanol. Filtered the solution through nylon syringe filter 0.45µ. Pipette out 0.6 ml of the filtered solution and diluted up to 50 ml with analytical grade methanol. The absorbance was measured at 225 nm. The absorbance was recorded.

Table 1: Absorbance of Dosage Form.

Vinmec Healthcare Pvt. Ltd. (200 mg)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0000
2	Standard	0.6524
3	Sample	0.6479

Table 2: Dosage Form Specifications.

No.	Brand / Company	M.D.	E.D.	Batch No.	Avg wt (g)	Assay (%)
1	Emtricitabine Stada ® Vinmec Heathcare LTD (200 mg)	08/2023	07/2026	D01227	0.260	99.31

E. Method of validation[12,14,15]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 2 - 10 PPM at 225 nm. The

calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample Concentration	Absorbance
1	2 PPM	0.2158
2	4 PPM	0.4161
3	6 PPM	0.6417
4	8 PPM	0.8547
5	10 PPM	1.1054
Correlation coefficient		0.9986 ~ 0.999

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100 ml of volumetric flask with analytical grade methanol and measuring the absorbance at 225nm.

Table 4: Accuracy Studies.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.79	98.75
100	1	0.98	98.00
120	1.2	1.18	98.33

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.6325
2	Sample Solution 2	0.6454
3	Sample Solution 3	0.6527
4	Sample Solution 4	0.6445
5	Sample Solution 5	0.6554
MEAN		0.6461
SD		0.0089
% RSD		1.3801 ~ 1.38

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.6432	0.6427	98.51	0.9515 ~ 0.95
		0.6421			
2	Analyst 2	0.6435	0.6424	99.84	
		0.6412			

RESULTS

1. Solubility of Emtricitabine

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	Methanol	Freely Soluble
2	Water	Soluble
3	Acetonitrile	Very Slightly Soluble

2. Melting point of Emtricitabine

The melting point of Emtricitabine was found to be 137 - 138˚C (uncorrected).

3. Results for linearity for assay method of Emtricitabine

The linearity of method was determined at concentration level ranging from 2 to 10µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.9986 ~ 0.999.**

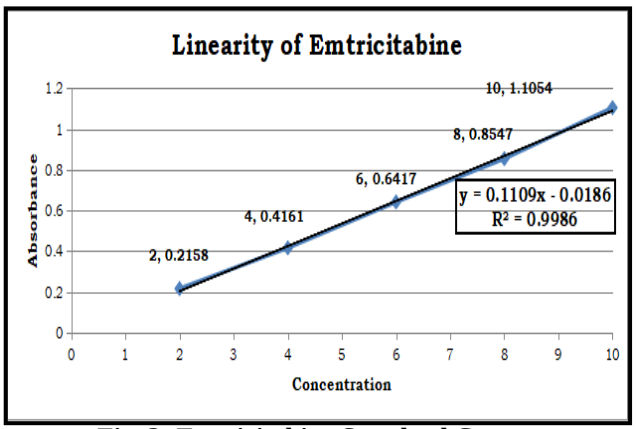

Fig. 3: Emtricitabine Standard Curve.

4. Results for accuracy for assay method of Emtricitabine

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy

results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Emtricitabine

The % RSD for different sample of precision was found to be 1.3801 ~ 1.38 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Emtricitabine

The % RSD for different sample of ruggedness was found to be 0.9515 ~ 0.95 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Emtricitabine in tablet form has been developed. From the spectrum of Emtricitabine, it was found that the maximum absorbance was 225 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 2 - 10 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Emtricitabine in solid dosage forms. Hence, the method could be considered for the determination of Emtricitabine in quality control laboratories.

ABBREVARTIONS

1. FDA - Food and Drug Administration.
2. DNA - Deoxyribonucleic Acid.
3. HIV - Human Immunodeficiency Virus.
4. RNA - Ribonucleic Acid.
5. PPM - Parts per Million.
6. nm – Nanometer.
7. HPLC - High Performance Liquid Chromatography.
8. UV - Ultra violet.
9. MS - Mass Spectroscopy.
10. LC - Liquid Chromatography.
11. ICH - International Council for Harmonization.
12. RSD - Relative Standard Deviation.
13. SD - Standard Deviation.
14. Qty – Quantity.
15. °C - Degree Celsius.
16. M.D. - Manufacturing Date.
17. E.D. - Expiry Date.
18. µg/ml - Microgram per milliliter.
19. Avg – Average.
20. Wt – Weight.
21. g – gm.

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