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REVIEW ARTICLE: DEVELOPMENT AND VALIDATION OF UV VISIBLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF EMTRICITABINE IN BULK DOSAGE FORM

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ABSTRACT

Simple, rapid, sensitive, precise and specific UV spectrophotometric method for the determination of Emtricitabine (EMB) in bulk drug and pharmaceutical dosage form was developed and validated. A simple double beam UV spectrophotometric method has been developed and validated with different parameters such as linearity, precision, repeatability, limit of detection (LOD), Limit of Quantification (LOQ), accuracy as per ICH guidelines. UV-visible spectrophotometric method, measurement of absorption at the maximum wavelength in 10 ml methanol and volume make with water solvent system as reference EMB were found to be at 225 nm respectively. The drug obeyed the Beer's law and showed good correlation. Beer's law was obeyed in concentration range 3-21 for Emtricitabine respectively with correlation coefficient was 0.999. The LOD and LOQ of EMB were found to be 0.3900(µg/ml) and 1.2936(µg/ml) respectively. Percentage Assay of EMB in tablets. The proposed method is precise, accurate and reproducible and can be used for routine analysis of EMB in bulk and tablet dosage form.

KEYWORDS: Emtricitabine, method development, validation, UV Spectroscopy.

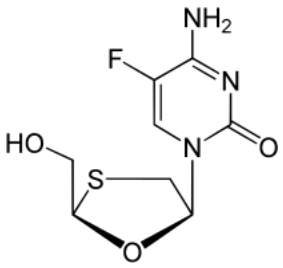
INTRODUCTION

Emtricitabine is used in combination with other medicines for the treatment of the infection caused by human immunodeficiency virus (HIV). HIV is the virus that causes acquired

immune deficiency syndrome (AIDS).

Emtricitabine will not cure or prevent HIV infection or AIDS. It helps keep HIV from reproducing and appears to slow down the destruction of the immune system. This may help delay problems that are usually related to AIDS or HIV disease from occurring. Emtricitabine will not keep you from spreading HIV to other people. This product is available in the capsule dosage form and solution form.

Structure



Iupac Name

4-amino-5-fluoro-1[(2R, 5S)-2(hydroxyl methyl)-1,3 oxathialon-5yl]ne.

Category

Antiretroviral agent.

General Properties

CAS number	143491-57-0
IUPAC Name	4-amino-5-fluoro-1[(2R,5S)-2-(hydroxymethyl)-1,3 oxathialon-5yl]ne
Molecular formula	$C_8H_{10}FN_3O_3S$
Molecular weight	247.244g/mol
Melting point	136-140
Physical state	Crystalline powder
Description	White colored
Purity	99.92%
Solubility	Freely soluble in methanol, slightly soluble in water, sparingly soluble in 0.1N NaOH, sparingly soluble in 0.1N Hcl.
Storage condition	Stored in tightly closed container. Stored at $2\text{-}8^0$ C.

Mechanism of action

Emtricitabine works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. Emtricitabine is a synthetic nucleoside analogue of Cytidine. It is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate, which is responsible for the inhibition of HIV-1 reverse transcriptase. It competes with the natural

substrate deoxycytidine 5'-triphosphate and incorporates into nascent viral DNA resulting in early chain termination. Therefore emtricitabine inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate deoxycytidine 5'-triphosphate and by its incorporation into viral DNA. By inhibiting HIV-1 reverse transcriptase, 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.

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Emtricitabine helps to block HIV reverse transcriptase, a chemical in your body (enzyme) that is needed for HIV to multiply.

Pharmacokinetics

Rapidly absorbed [(26)] (mean absolute bioavailability of 93% for capsules, and 75% for solution). Food does not affect absorption. Widely distributed throughout the body, Protein binding is Very low (less than 4%). Minimally transformed (13%), most appears unchanged in urine (86%). The biotransformation of emtricitabine includes oxidation of the thiol moiety to form the 3′-sulfoxide diastereomers (~ 9% of dose) and conjugation with glucuronic acid to form 2′-O-glucuronide (~ 4% of dose). In vitro studies indicate emtricitabine is not an inhibitor or cytochrome P450 enzymes.

Pharmacodynamics

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Emtricitabine helps to block HIV reverse transcriptase, a chemical in your body (enzyme) that is needed for HIV to multiply. Emtricitabine is always used with other anti-HIV medicines to treat people with HIV infection. Emtricitabine may also help to increase the number of T cells called CD4 cells.

Side effects

Some of its side effects include:

- Rare: Lactic acidosis.
- Liver dysfunction,
- Worsening of Hepatitis B infection.

Toxicity

- Toxicological Information
- Heptatoxicity.

Materials

Drug sample used

Emtricitabine was obtained as a gift sample from Dr.REDDY'S pharmaceuticals pvt. Ltd., Hyderabad.

Formulaton used

"EMTRIVA "tablets containing 200 mg of Emtricitabine was purchased from local pharmacy.

Chemical and solvents

S.NO	CHEMICAL	MAKE	PURITY
1.	Distilled water	milli-Q	-
2.	Methanol	Fischer-scientific	99.9%
3.	Sodium hydroxide	Fischer-scientific	97.78%
4.	Hydrochloric acid	Fischer-scientific	38-41%

Instrumentation

Instruments employed for the study were,

- **WENSAR** weighing scales limited (weighing balance).
- **ELICO- Double beam SL -210**/ UV –Visible spectrophotometer with pair of 10mm matched quartz cells.

Specifications of instrument

A) WENSAR weighing digital balance

Specifications	
Weighing capacity	500gms
Minimum display	0.1mg
Standard deviation	< 0.1mg
Operation temperature range	5 to 40^0C

B) UV –Visible Spectrophotometer

Model: ELICO – DOUBLE BEAM SL-210.

Chromatographic Conditions

Specifications	
Light source	20W halogen lamp, Deuterium lamp tungsten lamp. Light source position automatic adjustment Mechanism.
Monochromator	Concave holographic grating with 1200 lines\mm
Detector	Silicon photodiode
Stray Light	>0.05% T at(220nm: NaI 10g\1)
Wavelength range	190-1100nm
Spectral Band Width	1.8nm
Wavelength Accuracy	±0.5nm automatic wavelength calibrationmechanism
Recording range	Absorbance :-3.99~3.99Abs
	Transmittance :-3.99~399%
Photometric Accuracy	±0.005Abs(at 1.0 Abs),±0.010Abs(at 1.5 Abs)

Method development

Based on the solubility and physical parameters of the drug the standard stock solution of the drug was prepared and wavelength maxima were determined. The λ_{max} was found to be 225nm.

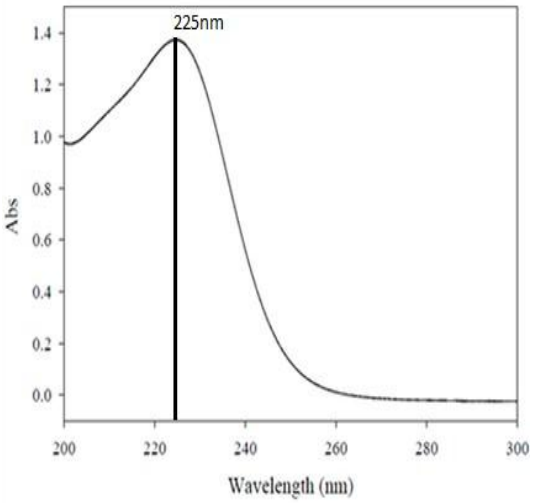
Based on the absorbance maxima of the drug, different dilutions were prepared and the formulation estimation was carried out.

Selection of Solvent

The solubility of emtricitabine was determined in a variety of solvents as per Indian pharmacopoeia standards. Solubility test for emtricitabine was carried out in different polar solvents. From the solubility studies, methanol was selected as suitable solvent for proposed method.

Selection of λ_{max}

The standard stock solution was further diluted with methanol to get 10 µg/ml concentrations. The solution was scanned between 200 and 400nm range using methanol as blank. From the UV Spectra 225 nm was selected as λmax for analysis of Emtricitabine.



Preparation of Reagents

Preparation of Stock 1 solution

Weigh accurately 100mg of emtricitabine in a 100ml volumetric flask and dilute with up to the mark to get concentration of 1000µg/ml.

Preparation of Stock 2 solutions

Take 1.0ml of above stock-1 solution and dilute with methanol in 100ml volumetric flask to get concentration of 10µg/ml.

Preparation of Stock 3 solutions

From stock-1 solution, take 1.0ml and dilute with methanol in 10ml volumetric flask to get concentration of 100µg/ml.

Preparation of NaOH solution (0.1N)

Accurately weighed 4.0gms of NaoH and dissolved in few ml of water and final volume is makeup to 1000 ml with distilled water and standardized.

Preparation of HCL solution

Accurately measured 8.0ml of HCL and diluted in few ml of water and final volume is make up to 1000ml with distilled water and standardized.

Preparation of sample solution

To determine the content of Emtricitabine in conventional tablet (label claim: 200 mg Emtricitabine per tablet), twenty tablets were weighed; their mean weight was determined and finely powdered. Tablet powder equivalent to 100 mg of Emtricitabine was weighed and

transfer into 100 ml volumetric flask then dissolved with methanol to mark. It was kept for ultra-sonication for 30 min; this was filtered through Whatman filter paper No. 41 and then the final dilution was made with methanol to get the final stock solution of 1000 µg/ml. From this stock solution, various dilutions of the sample solution were prepared and analyzed.

Method Validation Parameters

Linearity

In this methanolic stock solution of Emtricitabine (0.2-1.0ml of 10 µg /ml) were transferred in to 100 ml volumetric flask and made up to the mark with methanol. The absorbance of different concentration solutions were measured at 225nm against blank. The samples were found to be linear from 2-10 µg /ml. The calibration curve was plotted using concentration Vs absorbance. The curve obtained was linear in the concentration range of 2- 10 µg /ml.

Preparation of 2µg/ml solution

From stock-3 solution, take 1.0ml and dilute with methanol in 50 ml volumetric flask to get concentration of 2µg/ml.

Preparation of 4µg/ml solution

From stock-3 solution, take 2.0ml and dilute with methanol in 50ml volumetric flask to get concentration of 4µg/ml.

Preparation of 6µg/ml solution

From stock-3 solution, take 3.0ml and dilute with methanol in 50ml volumetric flask to get concentration of 6µg/ml.

Preparation of 8µg/ml solution

From stock-3 solution, take 4.0ml and dilute with methanol in 50ml volumetric flask to get concentration of 8µg/ml.

Preparation of 10µg/ml solution

From stock-3 solution, take 5.0ml and dilute with methanol in 50ml volumetric flask to get concentration of 10µg/ml.

Recovery Studies

To the pre-analyzed formulation, a known quantity of standard solution (2, 4 and 6 µg/ml solution) was added and the contents were mixed well, finally made up to the volume with

distilled water. Absorbance was measured at 225 nm. Amount present was calculated from slope and intercept. Then the % recovery was determined by using the following formula.

$$\% \text{ Recovery} = \frac{N \sum xy - \sum x \sum y}{N \sum x^2 - (\sum x)^2} 100$$

Where, N = Number of observations
X = Amount Added in microgram/ml
Y = Amount recovered in microgram/ml.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Preparation of calibration curve from the serial dilutions of standard was repeated for six times. The limit of detection and limit of quantification was calculated by using the average value of slope(s) and standard deviation of intercept.

$$\textbf{Limit of detection:} = \frac{3.3\,\sigma}{S}$$

Units - (mcg/ml)

Where: σ= the standard deviation of the response.

S = the slope of the calibration curve.

$$\textbf{Limit of quantification} = \frac{10\times\,\sigma}{S}$$

Unit- (mcg/ml)

Where: σ= the standard deviation of the response
S = the slope of the calibration curve.

Repeatability

Repeatability of the method was checked by repeating the measurement of formulation six times.

Precision

To evaluate the precision of the methods, pure drug solution (Within the working limits) was analyzed and being repeated six times of two different days.

Preparation of 6µg/ml solution

From stock-3 solution take 3.0ml and dilute with methanol in 50ml volumetric flask to get concentration of 6µg/ml.

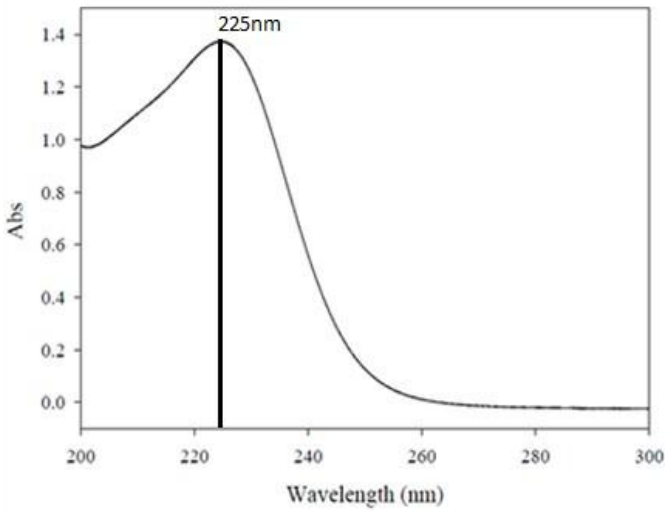
Ruggedness

Interday variation of the proposed methods was carried by change in the analyst at a concentration equal to the standard concentration and ruggedness was tested. % relative standard deviation in each method was calculated.

Preparation of 6µg/ml solution

From stock-3 solution, take 3.0ml and dilute with methanol in 50ml volumetric flask to get concentration of 6µg/ml.

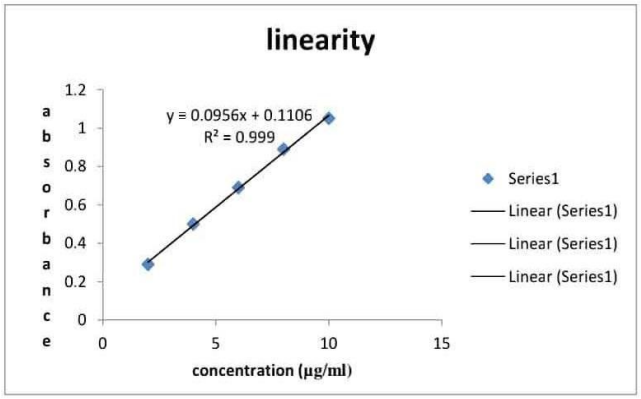
Validation Parameters



Calibraton of emitricitabine by u.v method

S.NO	CONCENTRATION (µg/ml)	ABSORBANCE
1.	2	0.230
2.	4	0.545
3.	6	0.727
4.	8	0.958
5.	10	1.051

Linearity



Solubility

S.NO	SOLVENT	20mg DISSOLVED IN µg/ml	STATUS
1	Water	20	Slightly soluble
2	methanol	20	Freely soluble
3	0.1 N NaOH	20	Sparingly soluble
4	0.1N HCL	20	Sparingly soluble

Optical characteristics

PARAMETERS	METHOD VALUES
Wavelength λ(nm)	225nm
Beer's law limit(µg/ml)	3-21
Sand ell's sensitivity (µg/cm^2/0.001 AU)	0.02296
Molar absorbtivity(L mol^{-1}cm^{-1})	1.0677×10^4
Correlation Co-efficient (r)	0.999
Regression equation (Y=mx+c)	Y=0.0956X-0.1106
Slope(m)	0.0956
Intercept(c)	0.1106
LOD(µg/ml)	0.3900
LOQ(µg/ml)	1.2936
Standard error of mean of regression line	0.0113

Precision

System Precision: Standard solution prepared of desired concentration and injected five times

	Injection	Absorbance
Concentration 6%	1	0.6783
	2	0.6810
	3	0.6809
	4	0.6776
	5	0.6810
	6	0.6769
Statistical Analysis	Mean	0.67985

	SD	0.00444
	% RSD	0.6472

Intraday precision

	Injection	Absorbance
Concentration 6%	1	0.6808
	2	0.6806
	3	0.6799
	4	0.6811
	5	0.6790
	6	0.6777
Statistical Analysis	Mean	0.67985
	SD	0.00444
	% RSD	0.6472

Interday precision

	Injection	Absorbance
Concentration 6%	1	0.6783
	2	0.6810
	3	0.6809
	4	0.6776
	5	0.6810
	6	0.6769
Statistical Analysis	Mean	0.67928
	SD	0.00554
	% RSD	0.00817

Accuracy (Recovery)

Accuracy

Accuracy expresses the closeness of agreement between the value, which is accepted either as conventional true value or and accepted reference value (International Standard e.g. pharmacopoeal standard) and the value found (mean value) by applying the test procedure a number of times.

To study reliability, suitability and accuracy of the method, recovery studies were carried out, by adding a known quantity of the standard to the pre analyzed sample and recovery study was done. The recovery was carried out at 80%, 100% and 120% level and the contents were determined from the respective chromatogram. From the results obtained we can conclude that the method was accurate.

Recovery studies

Recovery	Target in µg/ml	Spiked in µg/ml	Total in µg/ml	Amount found in µg/ml	%Recovery	Mean	%RSD
50%	20	10	30	29.818	99.39	99.78	0.43
	20	10	30	30.07	100.24		
	20	10	30	29.92	99.73		
100%	20	20	40	39.707	99.27	99.41	0.26
	20	20	40	39.707	99.257		
	20	20	40	39.890	99.72		
150%	20	30	50	49.66	99.32	99.94	0.57
	20	30	50	50.03	100.07		
	20	30	50	50.22	100.44		

Ruggedness

Defined by the USP as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst.

	Injection	Absorbance
Concentration 6%	1	0.6783
	2	0.6810
	3	0.6809
	4	0.6776
	5	0.6812
	6	0.6770
Statistical Analysis	Mean	0.67932
	SD	0.00190
	% RSD	0.28140

Limit of Detection (Lod)

Limit of detection is the lowest concentration of the analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions.

$$\text{Limit of detection} = \frac{\sigma}{S} \times 3.3$$

The minimum concentration at which the analyte can be detected is determined from the

linearity curve by applying the formula.

The lowest concentration of EMTRICITABINE that can be detected was determined from standard curve was 0.3900 µg/ml.

Limit of quantification (loq)

Limit of quantitation is the lowest concentration of the analyte in a sample that can be estimated quantitatively by injecting decreasing amount of drug, with acceptable precision and accuracy under the stated experimental conditions of the method. Limit of quantitation can be obtained from linearity curve by applying the following formula

$$\text{Limit of quantification} = \frac{\sigma}{\text{x } 10\ S}$$

The lowest concentration at which peak can be quantified is called LOQ was found to be 1.2936 µg/ml.

The LOD value is found to be 0.3900µg/ml based on the concentration of the LOD, LOQ value is calculating the fallowing formula LOQ=3.3×LOD. LOQ value is found to be 1.2936µg/ml.

Results of lod and loq data

LOD	0.3900µg/ml
LOQ	1.2936µg/ml

CONCLUSION

Emtricitabine is a drug useful for the treatment of HIV infections. It is currently marketed by Gilead.

The method adopted for our studies are

1. Simple UV –Spectroscopic method
2. The drug samples were analyzed by UV spectroscopy using methanol as solvent.

The developed UV Spectrophotometric method for the determination of Emtricitabine has the advantage of being fast, simple, inexpensive and applicable over the wide concentration range with high precision and accuracy. The method was validated as per the guidelines laid

by ICH. The result of the validation tests was found to be satisfactory and therefore this method can be applied successfully to analyzed drug formulations.

Hence it can be concluded that the proposed new method is an good approach for obtaining reliable results and which is suitable for doing analysis on Emtricitabine in Research institutions, Quality control department in industries, Approved testing laboratories, Bio-pharmaceutics and Bio-equivalence studies and in Clinical pharmacokinetic studies.

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ABBREVATIONS

EMB-Emtricitabine, LOD-Limit of detection, LOQ-Limit of quantification.

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