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Method Development and Validation of Emtricitabine in Bulk by UV Spectroscopy

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ABSTRACT

A simple, rapid, precise and economical spectrophotometric method has been developed for quantitative analysis of emtricitabine in bulk form. The solutions of the standard were prepared in methanol. The wavelength for emtricitabine was found to be 225nm. The method can be adopted in routine analysis of emtricitabine in bulk form and it involves relatively low-cost solvents and no complex extraction techniques. The drug showed linearity in the range of 2-10µg/ml with a correlation coefficient of 0.999. The method validated for different validation parameters such as linearity, accuracy, precision, detection limit, quantitation limits, robustness, ruggedness, and the results were found to be within the acceptable limits as per the guidelines of International Conference on Harmonisation (ICH).

INTRODUCTION

Emtricitabine is a nucleoside reverse transcriptase inhibitor for the prevention and treatment of HIV infection in adults and children. The chemical name of emtricitabine is 4-amino-5-fluoro- 1[(2R, 5S)- 2(hydroxymethyl)-1,3oxathialon-5yl]ne. It has a molecular formula of $C_8H_{10}FN_3O_3S$. Emtricitabine is a white colored crystalline powder having a solubility in methanol, water, 0.1N NaOH, and 0.1N HCl. The melting point of emtricitabine is 136-140^{0}C.

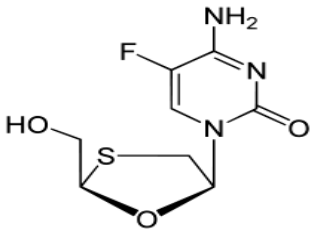

Figure 1: Structure of Emtricitabine

MATERIALS AND METHODS

Drug, solvents, chemicals:

Emtricitabine was obtained as a gift sample from Dr. REDDY'S Pharmaceutical private limited, Hyderabad. Methanol and distilled water are used as solvents for this method and were procured from the local market.

INSTRUMENTS:

Instruments employed for the study were

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

Preparation of reagents:

Preparation of stock 1 solution:

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

Preparation of stock 2 solutions:

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

Preparation of stock 3 solutions:

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

Preparation of NaOH solution (0.1N):

Accurately weighed 4 gms of NaOH, dissolved in few ml of water and final volume is makeup to 1000ml with distilled water, and standardized.

Preparation of HCl solution:

Accurately measured 8 ml of HCl and diluted in a few ml of water and final volume is made up to 1000ml with distilled water and standardized.

METHODOLOGY

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 the solvent:

The solubility of emtricitabine was determined in a variety of solvents as per Indian pharmacopeia standards. Solubility test for emtricitabine was carried out in different polar solvents. From the solubility studies, methanol was selected as a suitable solvent for the 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 200-400nm range using methanol as blank. From the UV spectra, 225nm was selected for analysis of emtricitabine.

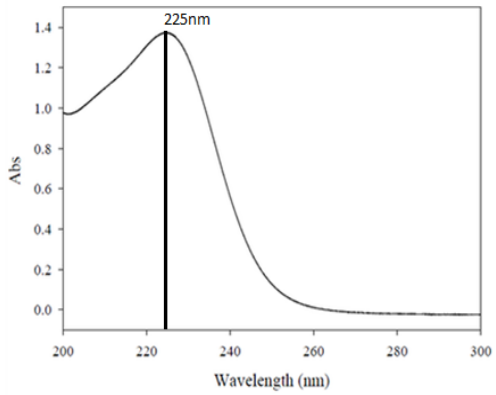

Figure 2: UV absorption spectrum of emtricitabine at 225nm

METHOD VALIDATION

Linearity:

In this methanolic stock solution of emtricitabine (0.2-1.0ml of10µg/ml) were transferred into a 100ml volumetric flask and made up to the mark with methanol. The absorbance of different concentration solutions was measured at 225nm against blank. The sample was found to be linear from 2-10µg/ml the calibration curve was plotted using concentration v_s absorbance. The curve obtained was linear in the concentration range of 2-10µg/ml.

Preparation of 2µg/ml solution:

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

Preparation of 4µg/ml solution:

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

Preparation of 6µg/ml solution:

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

Preparation of 8µg/ml solution:

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

Preparation of 10µg/ml solution:

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

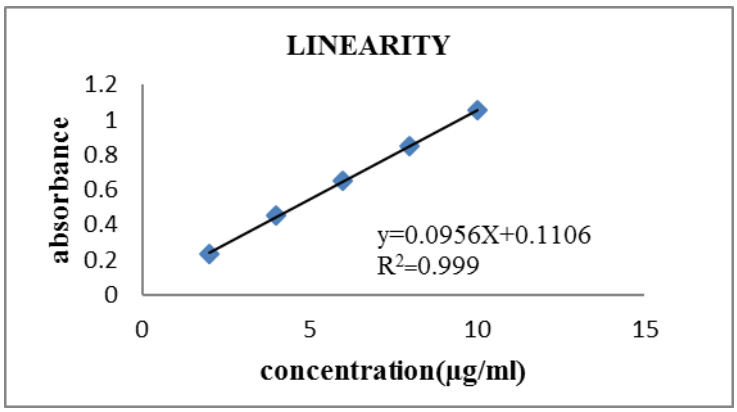

Figure 3: Calibration curve of Emtricitabine.

Precision:

To evaluate the precision of the methods, pure drug solution (within working limits) was analyzed and is repeated 6 times of two different days. The relative error (%) and relative standard deviation (%) were found to be well within the acceptance criteria of below 2, indicate the high accuracy and precision for the proposed methods.

Preparation of 6µg/ml stock solution:

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

Table 1: Precision result

Precision	% RSD
Repeatability	0.6472
Intraday precision	0.6472
Inter-day precision	0.00817

Ruggedness:

In order to determine this parameter, the analysis was performed at the same operational conditions and the same environmental conditions but using different analysis.

Table 2: Ruggedness result

Parameter	% RSD
Ruggedness	0.28140

Limit of detection (LOD) and limit of quantification (LOQ):

The detection limit and quantification limit of the method were calculated as the 0.3900µg/ml and 1.2936µg/ml respectively.

Table 3: Optical characteristics of emtricitabine by UV method

Parameters	Method values
Wavelength λ (nm)	225 nm
Beer's law limit (µg/ml)	3-21
Sandell's sensitivity (µg/cm^2/0.001AU)	0.02296
Molar absorbtivity (L mol-1 cm-1)	1.0677x10^4
Correlation co-efficient (r)	0.999
Regression equation (Y=mx+c)	Y=0.0956x0.1106
Slope (m)	0.0956
Intercept (c)	0.01106
LOD(µg/ml)	0.3900
LOQ(µg/ml)	1.2936
Standard error of mean of regression line	0.0113

RESULTS AND DISCUSSIONS

In the present work, we have developed and validated a UV spectroscopic method. The method was validated as per ICH guidelines. The linearity was found to be 2-10µg/ml for UV spectroscopic method showing the correlation coefficient of 0.999. The UV spectroscopic method was validated for linearity, precision, LOD, LOQ, and ruggedness and the results were tabulated in table 1, 2, 3. All the results were found to be within the limits as per ICH guidelines and hence the proposed method was successfully employed for the determination of Emtricitabine in its API for regular and routine analysis.

CONCLUSION

A simple, accurate, precise method was developed for the estimation of emtricitabine in bulk form. The method was validated as per ICH guidelines.

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