WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 1, 1029-1037. Research Article ISSN 2277– 7105

ESTIMATION OF BICTEGRAVIR IN BULK SAMPLES BY UV- VISIBLE SPECTROPHOTOMETRIC METHOD

Pavankumar Gangavarapu*

Sr. Director, Regulatory Affairs, Amneal Pharmaceuticals LLC, Piscataway, NJ, USA.

Article Received on
22 October 2020,

Revised on 12 Nov. 2020,
Accepted on 02 Dec. 2020

DOI: 10.20959/wjpr202101-19303

***Corresponding Author**
Dr. Pavankumar Gangavarapu
Sr. Director, Regulatory Affairs, Amneal Pharmaceuticals LLC, Piscataway, NJ, USA.

ABSTRACT

Simple, precise, economic, less time consuming UV-spectroscopic method for estimation of Bictegravir in bulk by UV spectroscopic method is developed. The absorption maximum was found to be 340 nm. The developed method was obeying Beer Lambert's law in the range of 5-25µg/ml concentration. The method has also been statistically evaluated and the results were within the regulated limits.

INTRODUCTION

Bictegravir (INN; BIC, formerly known as GS-9883)[1,2] is an antiviral drug of the integrase inhibitor class that was structurally derived from an earlier compound dolutegravir by scientists at Gilead Sciences; in vitro and clinical results were presented by Gilead in the summer of 2016[3] In 2016, bictegravir was in a Phase 3 trial as part of a single tablet regimen in combination with tenofovir alafenamide (TAF) and emtricitabine (FTC) for the treatment of HIV-1 infection[4] and the combination drug bictegravir/emtricitabine/tenofovir alafenamide (Biktarvy) was approved for use in the United States in 2018.[5]

The aim of our work was to develop a simple and efficient method for the estimation of the drug Bictegravir in bulk and to evaluate the developed method on the basis of various statistical parameters and check whether they are within the regulated limits. Currently there were no UV spectroscopic methods for the estimation of Bictegravir. Hence a simple, precise, economic and less time consuming UV-Spectrophotometric method was developed for the estimation of Bictegravir.

MATERIAL AND METHODS

In developing a quantitative method for determining an unknown concentration of a given species by Absorption Spectrophotometry, the first step is the choice of the absorption band at which absorbance measurements are made when several absorption band of suitable absorptivity are present. The band selected should favor wavelength regions that correspond to relatively high output of the light source and high spectral sensitivity of the detector (usually λ_{max}).

METHODOLOGY

Instruments

1. SHIMADZU-1700 Pharma SPEC, Ultraviolet-Visible spectrophotometer (double beam) was used for all spectral measurements.
2. Electronic balance of model Scale (High precision balance) Model SAB-203 having sensitivity of 1 mg was used for weighing
3. Chemicals and Reagents: Bictegravir, Methanol (A.R.GRADE), Veltam (Formulation).

Preparation of bictegravir stock solution

Standard bictegravir stock-I

It is prepared by dissolving 100mg drug in methanol and volume make up to 100ml with methanol to get concentration 1000µg/ml.

Standard bictegravir stock-II

It is prepared by taking 1ml of the stock-I in 10 ml volumetric flask and make up to 10ml with methanol to get concentration 100µg/ml.

Preparation of linearity curve

To construct Beer's law plot, different aliquots of Bictegravir (0.5-2.5ml) of stock-II with different concentrations (5, 10, 15, 20 and 25 µg/ml) were prepared by serial dilutions with methanol. Then absorbance of the solution was measured at 340 nm.

RESULTS AND DISCUSSION

Linearity and sensitivity of the method

Lambert's Law states "the absorbance is proportional to the thickness of the solution."

Beer's Law states "the absorbance is proportional to the concentration."

Beer-Lambert's Law can be Law can be expressed mathematically as:

$$A = \frac{\text{log intensity of the incident radiation}}{\text{Intensity of transmitted light}}$$

The absorption (A) is proportional to the concentration (C) of the absorbing species if absorptivity (a) and the thickness of the medium (b) are constant.

Validity of beer's laws

The law is not applicable for

1. Highly concentrated solutions.
2. Solutions exhibiting stray florescence or suspensions may not strictly adhere to Beer's laws.
3. If a dilute solution during measurement undergoes chemical reaction such as oxidation, reduction, hydrolysis, association, dissociation or polymerization,

Methods of least squares

Many analytical procedures involve measurements of physical parameters (e.g. absorbance of solution in Spectrophotometry), which is directly proportional to the concentration of the analyte. A series of solution of known concentration are prepared and the instrument response is measured for each standard solution. Fortunately statistics provides a mathematical relationship, which enables the chemist to calculate objectively the slope and the intercept of the "best" straight line. This process is called as regression analysis; when applied to that of a straight-line relationship, it is called the method of least squares. The numbers on the X-axis represents the concentrations of the standard solutions. The numbers on the Y-axis represents the instrument response (i.e. Absorbance).

One assumption made, is that the relationship is a linear one, and the equation for the straight line is **Y = mx + b** where 'm' is the slope of the intercept on the Y-axis.

The failure of the data points to fall exactly on the line is assumed to be cased entirely by the indeterminate errors in the instrument readings, 'y'. The sum of the squares of the deviation of actual instrument regarding from the correct values are minimized by adjusting the values of the slope, m, and the intercept, b. If a linear relationship between x and y does not exist, this puts the line through the best estimates of the true mean values.

The data for the method of least squares concerns with not only the values of x and y for the graph, but also the values of x_2, y_2, by and the sum of these terms. The quantities of C and D

are defined for convenience in presenting later formulae. The term 'n', is the number of data points (or number of determinations).

The equation given for the slope and intercept of the line is given as follows:

Slope,

$$m = \frac{\sum xy - [\sum x \cdot \sum y] / n}{C}$$

$$C = (\sum x^2 - [\sum x]^2)/ n$$

$$D = \frac{\sum y^2 - [\sum y]^2}{n}$$

Intercept

$$b = \frac{\sum y - m \sum x}{n}$$

$$\text{Slope } m = \frac{\sum xy - [\sum x \cdot \sum y]}{N/C}$$

Correlation coefficient (R^2)

It is used to measure the ability of the regression line to explain the variations in the independent variable and it is calculated from the following formula:

$$R = \frac{\sum (x - X)(y - Y)}{\sqrt{\sum (x - X)^2 \sum (y - Y)^2}}$$

where, X and Y are the means of x and y respectively.

Accuracy

The accuracy of a determination may be defined as the concordance between it and the true or the most probable value. For analytical methods there are two possible ways of determining the accuracy.

- Absolute method
- Comparative method

Absolute method

The test for accuracy of a method is carried out by taking varying amounts of the constituent and proceeding according to specified instructions. The difference between the mean of an adequate number of results and the amount of constituent actually present is usually expressed as "Parts Per Hundred".

Comparative method

In the analysis of pharmaceutical formulation or solid laboratory prepared samples of desired composition, the content of the constituent sought has been determined by two or more methods of analysis. The agreement between at least two methods of essentially different character can usually be accepted as indicating the absence of an appreciable determinate error.[6-10]

Precision

Precision may be defined as the concordance of a series of measurement of the same quality. It refers to the agreement among a group as experiment result. Precise values may be in accurate.[11-17]

The precision terms usually one encountered as

1. **Mean**
2. **Standard deviation**
3. **Average deviation**

1. MEAN

Mean of a finite number of measurements like X1, X2, X3……Xn

Is obtained designated as X.

$$\textbf{MEAN} = \frac{\textbf{X1+X2+X3+--------+Xn}}{\textbf{N}}$$

Average deviation

The average deviation of measurement of a set is the mean difference of the individual measurement.

Average deviation = **(Σ X1-MEAN)/N**

Where, Xi = individual measurements

N = Number of measurements

2. Standard deviation

Standard deviation **σ**, of an infinity set of individual data is theoretically the square root of the mean of the difference between the individual measures values xi and the mean of the infinite numbers of measurements X or μ

$$\textbf{S/}\sigma = \sqrt{(\Sigma\ (xi - \mu)^2 \setminus N)}$$

Or

$$S/\sigma = \sqrt{(\Sigma (xi - \mu)^2 \setminus N-1)}$$

The denominator is (N – 1) rather than N when the number of values is small (less than 30) the square of the standard deviation is called as the Variance (s)

A more accurate measure of the precision, known as the coefficient of variation is given by:-

$$\textbf{C.V or \% RELATIVE STANDARD DEVIATION} = \frac{S \times 100}{N}$$

Table 1: Determination of lambda max.

Wavelength (nm)	Absorbance
300	0.041
310	0.042
320	0.043
330	0.048
340	0.053
350	0.045
360	0.044
370	0.043

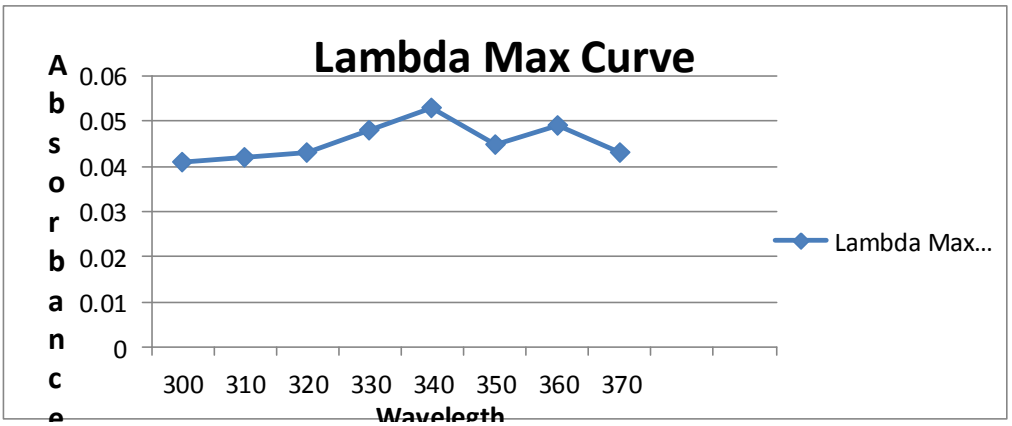

Figure 1: Absorption spectrum of bictegravir.

Table 2: Optical characteristics.

01	λ_{max} (nm)	340
02	Beer's law range (µg/ml)	5-25
03	Regression equation (y = mx + c) * Slope (m) Intercept (c)	 0.0092 0.0344
04	Correlation coefficient (r^2)	0.9981
05	Precision (%Relative Standard Deviation)	0.357

Table 3: Absorbance at optimum concentration range.

Concentration (mcg/ml)	Absorbance
5	0.044
10	0.053
15	0.061
20	0.071
25	0.081

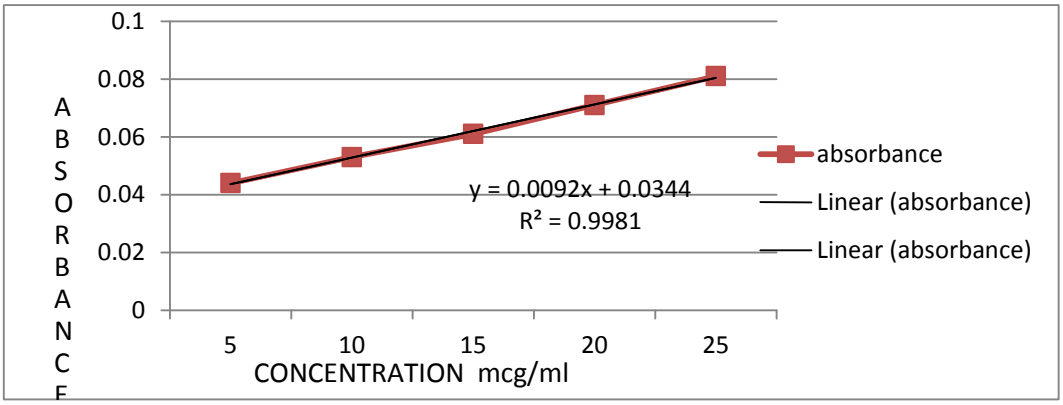

Figure 2: Linearity plot.

Method validation

Precision

The precision of the proposed method was ascertained by actual determination of nine replicates of fixed amount of the drug. Results given below in Table-4.

Table 4: Precision results.

S. No	Concentration (mcg/ml)	Absorbance	Average	SD	%RSD
1	15	0.062	0.0611	0.0011	1.91
2	15	0.061			
3	15	0.061			
4	15	0.061			
5	15	0.063			
6	15	0.061			
7	15	0.060			
8	15	0.059			
9	15	0.062			

From the optical characteristics of the proposed method it was found that the drug obeys linearity within the concentration range of 5-25 µg/ml. From the results it was found that the percent RSD is less than 2% which indicates that the method has good reproducibility. The proposed method was simple, sensitive and reliable with good precision and accuracy. Hence,

this method can be used for the routing determination of Bictegravir in bulk samples. Summary of proposed UV method for determination Bictegravir in bulk and formulations was shown in Table-7.

Table 5: Summary.

Parameter	Description/Result
Analytical method	UV- Spectrophotometric
λ_{max} (nm)	340
Beer's Law obey Limit (µg/ml)	5-25
Regression Line Equation Slope (m) Intercept (c)	 0.0092 0.0344
Correlation Coefficient (r^2)	0.9981
% RSD	1.91

CONCLUSION

The proposed UV-Spectro-photometric method enables quantitative determination of Bictegravir in bulk drug samples. Efficient UV spectrophotometric detection at the respective absorption maxima. The calibration curve was linear over the concentration range from 5-25 µg/ml for the proposed method. The relative standard deviation's (R.S.D.) was less than 10% and average recovery was above 98%. The proposed method is fast, sensitive, precise, accurate and efficient and can be used in for analysis in quality control laboratories.

ACKNOWLEDGEMENT

Author is thankful to the management Annamalai University for providing facilities to carry out this work.

CONFLICT OF INTEREST: Authors do not have any conflict of interest.

BIBLIOGRAPHY

1. "Recommended INN: List 75" (PDF). WHO Drug Information, 2016; 30(1): 102.
2. "Bictegravir - Gilead Sciences". Adis Insight. Springer Nature Switzerland AG. Retrieved, 2017; 22.
3. Highleyman L "New integrase inhibitor bictegravir looks promising in early studies". NAM aidsmap, 2016; 6.
4. "Press Release: Gilead Presents Preliminary Data on Bictegravir, an Investigational Integrase Strand Transfer Inhibitor for the Treatment of HIV | Gilead". Gilead, 2016; 20.

5. "U.S. Food and Drug Administration Approves Gilead's Biktarvy (Bictegravir, Emtricitabine, Tenofovir Alafenamide) for Treatment of HIV-1 Infection" (Press release). Gilead, 2018; 7.
6. Ohno Y; Improved photometric standards and calibration procedures at NIST; Journal of Research of the National Institute of Standards and Technology, *1997; 102: 323–331*.
7. Ninfa, Alexander J; Ballou, David P; Benore; Fundamental Laboratory Approaches for Biochemistry and Biotechnology, *2010; (2): 135*.
8. *Mavrodineanu, Radu, Schultz, J. I,Menis, Oscar, Accuracy in Spectrophotometry and Luminescence Measurements: Proceedings. Washington, D.C.: U.S. National Bureau of Standards, 1973; 5(2): 2.*
9. *Robert D, Simoni, Robert L, Hill;* A Classic Instrument: The Beckman DU Spectrophotometer and Its Inventor, Arnold O. Beckman; *The journal of biological chemistry,* 2016; *278: 49.*
10. S. Budavari, The Merck Index, 13th ed. New Jersey: Merck & Co., Inc., 2001; 612-1615.
11. A. Narayana, C. Narasimha Rao, C. Narasimha Rao, K. Sivakumar, Spectrophotometric determination of esmolol hydrochloride using analytical reagents, Indian Journal of Advances in Chemical Sciences, 2014; 2(4): 289-293.
12. Rao TSS, Tirumala R and Rao PS. Quantification of Bictegravir in human plasma using LC-MS/MS. Bioanal. Biomed, 2011; 3: 55-58.
13. Shamsa F and Amani L. Determination of sulfamethoxazole and trimethoprim in pharmaceuticals by visible and UVspectrophotometry. Iran. J. Pharm. Res, 2006; 5: 31-36.
14. Matsushima H, Takanuki KI, Kamimura H, Watanabe T and Higuchi S. Drug Metab. Dispos, 2004; 26: 240-245.
15. Nilam A.Gadhave Sanjay D. Sawant, Minal R.Ghante Atul D. Nikam IJPRD, 2011; 3(4): 87 – 92.
16. Rao N, Talluri RK, Raju MVN, Shinde A and Ramanjaneyulu DD. J Pharm Biomed Ana, 2008; 46: 94-103.
17. Rahkonen K, Parssinen P, Leppanen O, Mauriala E, Lehtonen T, Auriola M, J Pharm Biomed Ana, 2007; 43: 606-612.