International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 643-645 www.ijprajournal.com ISSN: 2456-4494

RP- HPLC Method Development and Validation For the Simultaneously Estimation of Emtricitabine and Tenofovir Alafenamide Fumarate in It's Dosage Form Using Internal Standard Method

Puja Ramesh Rathod*1, Dr.Prachi Udapurkar2, Dr. L. D. Hingane.
Aditya Pharmacy College, Nalwandi Naka, Beed, Department of Quality Assurance.

Submitted: 10-07-2022 Accepted: 21-07-2022

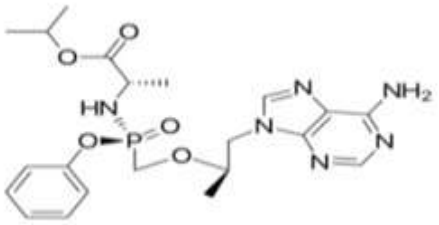
ABSTRACT
New Analytical method was developed for the estimation of Emtricitabine and Tenofovir Alafenamide Fumarate in drug product by liquid chromatography. The chromatographic separation was achieved on Cosmosil C18 column (250mm×4.6ID, 5µm) at ambient temperature. The separation achieved employing a mobile phase consists of Methanol:water(80:20v/v). The flow rate was 0.8ml/ minute and ultra violet detector at 252nm. The average retention time for Emtricitabine and Tenofovir Alafenamide Fumarate found to be 4.277min and 5.293min. The proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 10-50µg/ml for Emtricitabine and 15-75µg/ml for Tenofovir Alafenamide Fumarate.

Keywords: Emtricitabine and Tenofovir Alafenamide Fumarate, HPLC, Methanol and validation.

I. INTRODUCTION

Analytical chemistry may be defined as the science and art of determining the components of materials in the terms of the elements or compound contained. Analytical chemistry seeks ever improved means of measuring the chemical composition of natural and artificial materials. The techniques of this science are used to identify the substances which may be present in a material and to determine the exact amounts of the identified substances. Analytical chemistry is important is nearly all aspects of chemistry, for example, agricultural, clinical, environmental, forensic, manufacturing metallurgical and pharmaceutical chemistry. Analytical techniques play an important role in assuring and maintaining the quality of substance and are critical components of Q.A/Q.C. the reliability, utility, accuracy, interception and specificity of the measurements are the responsibility of an analytical chemist. In general terms pharmaceutical analysis comprises of those procedures which are necessary to determine the identity, strength, quality and purity of drugs and chemicals. There are two types of branches of analytical chemistry.

TENOFOVIR ALAFENAMIDE FUMARATE
Structure

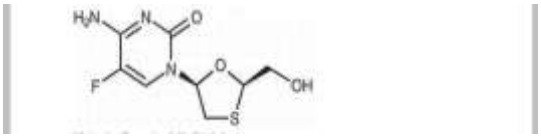


Molecular Formula: C46H62N12O14P2
Molecular Weight: - 1069 g/mol

Pharmacology –Tenofovir alafenamide (TAF) is a phosphonoamidate prodrug of the nucleotide analog tenofovir (TFV). TAF was designed to circulate systemically as the prodrug and undergo conversion to TFV intracellularly, achieving higher active metabolite concentrations in peripheral blood mononuclear cells and lower plasma TFV exposures than tenofovir disoproxil fumarate (tenofovir DF; TDF) does. LTAF has also demonstrated in vitro and in vivo activity against HBV. The TAF terminal half-life is 0.51 hours. The active metabolite, TFV-DP, has an intracellular half-life of 150 to 180 hours.

EMTRIICITABINE
Structure:-

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 643-645 www.ijprajournal.com ISSN: 2456-4494



Molecular Formula: C8H10FN3O3S
Molecular Weight: - 247.25g/mol

Pharmacology –Emtricitabine is a synthetic nucleoside analogue of cytidine with activity that is specific to human immunodeficiency virus (HIV-1 and HIV-2) and hepatitis B virus (HBV). Emtricitabine is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate, which competitively inhibits HIV-1 reverse transcriptase, resulting in DNA chain termination. Emtricitabine is a weak inhibitor of mammalian DNA polymerase α, β and ε and mitochondrial DNA polymerase γ.Emtricitabine did not exhibit cytotoxicity to peripheral blood mononuclear cells (PBMCs), established lymphocyte and monocyte-macrophage cell lines or bone marrow progenitor cells in vitro. There was no evidence of toxicity to mitochondria in vitro or in vivo.

OBJECTIVE :
• Development of RP-HPLC method
• To perform the Assay of EMT and TAF in pharmaceutical dosage form.
• To validate the RP-HPLC method as per ICH guidelines.

RP-HPLC Method
Selection of mobile phase and its composition Selection of suitable detection wavelength Selection of suitable chromatographic parameters Selection of pH Selection of Internal standard. Analysis of laboratory mixture and marketed formulation by proposed method Validation of developed analytical method as per ICH and USP guidelines. Statistical Interpretation of the data.

Determination of Wavelength:
100 mg of the standard drug Emtricitabine & Tenofovir Alafenamide was taken in a separate 100 ml volumetric flask and dissolved in 30 ml of methanol and the volume was made up to the rmark. The solutions have been further diluted to get 10 µg / ml with water. The solution was scanned within the 200-400 nm range using water as blank. For Emtricitabine & Tenofovir Alafenamide, The individual and overlain spectra for the drugs Emtricitabine & Tenofovir Alafenamide were recorded in the range of 200-800 nm and the maximum absorption was found to be, 280 nm of Emtricitabine and 261 nm of Tenofovir Alafenamide. The isoabsorbptive point was found to be 260 n.

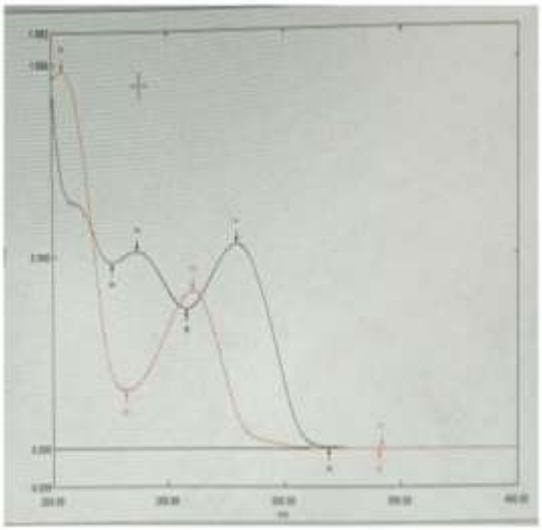


Fig.3: Overlain spectrum of Emtricitabine & Tenofovir Alafenamide

Assay of the marketed Formulation:
The sample solution was prepared as mentioned injected into six replicatesand the peak ratio was measured.

METHOD VALIDATION
Mobile phase consisting of Acetonitrile:Buffer (KH2PO4) pH 4.2 adjusted with triethylaminein the ratio 43:57. at 260 nm with a flow rate 1.0 ml/min was used for the present method.The method was validated for the parameters such as system suitability, linearity and range,precision, accuracy, robustness, ruggedness limit of quantitation (LOQ) and limit of detection(LOD) as

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 4 July-Aug 2022, pp: 643-645 www.ijprajournal.com ISSN: 2456-4494

per ICH guidelines

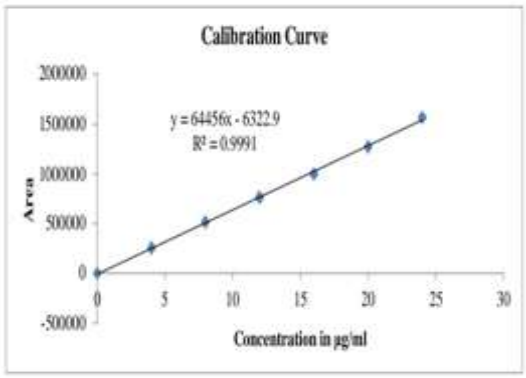


Fig. 8: Calibration Curve of Emtricitabine

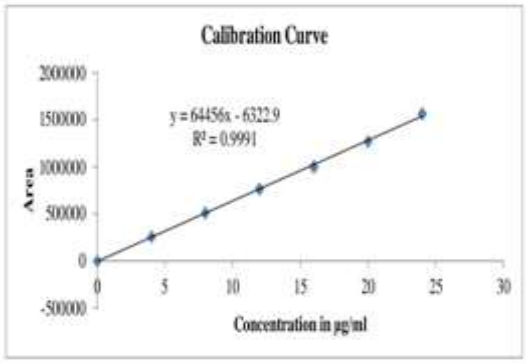


Fig 9: Calibration Curve of Tenofovir Alafenamide

Assay of the marketed Formulation:

The developed method analyzed the market formulation TAFERA –EM , and the percentageassay was found using the formula described in 6.5.2. The percentage assay of Emtricitabine& Tenofovir Alafenamide was found to be 99.87% w / w and 99.69% w / w respectively.

II. CONCLUSION

A validated simple and robust method for simultaneous estimation of EMT & TAF byRP-HPLC Method using Diclofenac Na as Internal Standard. The HPLC method was validated as per ICH guidelines for specificity, linearity,precision, accuracy, robustness, ruggedness, LOD and LOQ.All the validation parameters were found to be well within the acceptable limit. Theassessment of robustness and ruggedness of the method indicates that the methodremains unaffected by slight changes in chromatographic conditions.

REFERANCES

[1]. Sharma Bk. instrumental method of chemical analysis, 21th edition, goel publicationhouse, meerut, 2002, p- 3-5, 10.
[2]. Sethi Pd. high performance liquid chromatography: quantitative analysis ofpharmaceutical formulations, 2001; p- 3 - 5, 10.
[3]. Chatwal Gr, Anand Sk, instrumental method of chemical analysis, 5th edition,himalaya publishing house, delhi, 2004, p- 1.6 - 1.7, 2.156 - 2.158.
[4]. Skoog Da, Holler Fj, Timothy A, Nieman Nw. principle of instrumental analysis,eastern press, bangalore, , 2004, 5.p- 678 to 688, 695- 696.
[5]. Swartz Me, Krull is. analytical method development and validation, 2009, p- 26 -27.
[6]. ICH Harmonized guideline: validation of analytical procedures: methodology, Q2b(2005).
[7]. Hingson S.P.analytical chemistry; oxford university press; 1 st ed.; 2005: 3-5.
[8]. Mendham J., Denney R.C., Barnes J.D., Thomas M.J.K.K.; Vogel's textbook ofquantitative chemical analysis; published by pearson education; 6 th edition,; 2004: 1-2, 5-6.
[9]. Gray N., Calvin M., Bhatia B.K.; instrumental method of analysis; cbs publishers anddistributors; 1 st edition; 2009: 5-10.
[10]. Christian G.D.; analytical chemistry; 6 th edition; 2007, 1-5.
[11]. Day R.A., Underwood A.L.; quantitative analysis; phi learning pvt. ltd.; 6 thedition;2009: 1-2.
[12]. Willard H., Merritt L., Dean J., Settle F., 1986, Instrumental methods of analysis,CBS distributors. 7, 629-63
[13]. Hingson S.P.; analytical chemistry; oxford university press; 1 st ed.; 2005: 3-5.
[14]. Mendham J., Denney R.C., Barnes J.D., Thomas M.J.K.K.; Vogel's textbook ofquantitative chemical analysis; published by pearson education; 6 th edition,; 2004: 1-2, 5-6.
[15]. Curell G.; analytical instrumentation performance characteristics & amp; quality;wiley india pvt. ltd.; 2010: 1-publication 2 nd edition; 2004: 35-41.