Available online on 15.04.2020 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Force Degradation Study of Tenofovir Disoproxil Fumarate by UV-Spectrophotometric Method

Debaje Priyanka D*, Chavan Harishchandra H.

Department of Pharmaceutics, Sandip Institute of Pharmaceutical Sciences, Nashik

ABSTRACT

Tenofovir disoproxil Fumarate (TDF), acyclic phosphonate nucleotide analogue, used as antiretroviral agents in the treatment of HIV-1 infection. A stability indicating UV -spectrophotometric method is simple, an accurate and economic, precise and reproducible method has been used for the estimation of Tenofovir disoproxil Fumarate in bulk and tablets dosage form in present work. The wavelength selected for the absorption correction method was 260 nm. The linearity range of 2-10µg/ml proved that it obeyed Beer's Law and the correlation coefficient (r2) was found to be 0.999 at 260 nm. The drug was subjected to acid, alkali, peroxide, UV and Heat degradation. The force degradation studies of Tenofovir disoproxil Fumarate was done on Stress degradation by hydrolysis under alkaline condition by using 0.1N NAOH was found to be 10.6%. Stress degradation by hydrolysis under acidic condition by using 0.1N HCl and product degradation was found to be 10.95%. Oxidative degradation was done by using hydrogen peroxide and product degradation was found to be 12.22%. Neutral hydrolytic degradation was found to be 12.26%. Forced degradation studies of drug reveal good stability under the chosen experimental conditions.

Keywords: Tenofovir disoproxil Fumarate, UV Spectroscopy, Forced Degradation study

ARTICLE INFO: Received 16 Jan 2020; Review Completed 10 March 2020; Accepted 17 march 2020; Available online 15 April. 2020

Cite this article as:

Debaje P D, Harishchandra H. C, Force Degradation Study of Tenofovir Disoproxil Fumarate by UV-Spectrophotometric Method, Asian Journal of Pharmaceutical Research and Development. 2020; 8(2):21-25.
DOI: http://dx.doi.org/10.22270/ajprd.v8i2.679

***Address for Correspondence:**

Debaje Priyanka Datta, Department of Pharmaceutics, Sandip Institute of Pharmaceutical Sciences, Nashik

INTRODUCTION

Tenofovir Disoproxil Fumarate belongs to the class of antiretroviral drugs as nucleotide reverse transcriptase inhibitors which block reverse transcriptase an enzyme crucial to viral production in HIV infected people. After oral absorption, Tenofovir Disoproxil Fumarate is rapidly converted to Tenofovir and then undergoes subsequent phosphorylation by cellular enzymes to the active Tenofovir Diphosphate, which inhibits the activity of HIV-1 reverse transcriptase.

Tenofovir DF belongs to a class (group) of HIV drugs called nucleoside reverse transcriptase inhibitors (NRTIs). NRTIs block an HIV enzyme called reverse transcriptase. (An enzyme is a protein that starts or increases the speed of a chemical reaction.) By blocking reverse transcriptase, NRTIs prevent HIV from multiplying and can reduce the amount of HIV in the body [1].

Tenofovir DF is also effective against HBV and approved by FDA for the treatment of chronic HBV infection in adults and children 12 years of age and older. For information on the HBV-related use of Tenofovir DF in people with HIV, please refer to the HBV section of the Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents.

Chemically, Tenofovir disoproxil Fumarate (TDF) is Fumaric acid salt of the bisisopropoxycarbonyloxymethyl ester derivative of Tenofovir. Chemically it is 9-[(R)-2-[[bis[[(isopropoxycarbonyl) oxy] methoxy] phosphinyl] methoxy] propyl] adenine Fumarate (1:1).It is white to light-yellow crystalline powder and it is soluble in water and in Dimethyl Sulfooxide (DMSO). The chemical structure for Tenofovir disoproxil Fumarate was show in Fig.1.[2]

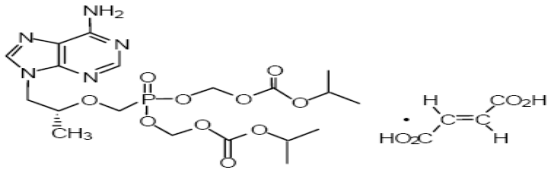

Figure 1: Structure of Tenofovir disoproxil Fumarate

It is not official in any of the pharmacopoeias. This is listed in the Merck Index and Martindale: The complete drug reference. Literature review reveals that several methods have been reported for the estimation of TDF in tablets, high-performance liquid chromatographic methods, liquid chromatography-mass spectrometry, and high-performance thin-layer liquid chromatographic methods (HPTLCs). Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development, manufacturing, and packaging, in which knowledge of chemical behavior can be used to improve a drug product. So the present work is to carry out the force degradation studies along with its pH degradation studies. The method was validated according to the ICH (Q2A1995) guidelines (8) Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development, manufacturing, and packaging, in which knowledge of chemical behavior can be used to improve a drug product. The available regulatory guidance provides useful definitions and general comments about degradation studies. The International Conference on Harmonization (ICH) guidelines indicates that stress testing is designed to determine the intrinsic stability of the molecule by establishing degradation pathway in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedure used. ICH guidelines stability testing of new drug substances and products' Q1A (R2) and (Q1B) requires that stress testing should be carried out to elucidate the substance. It suggests that the degradation products that are formed under the variety of condition should include the effect of temperature, appropriate oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH value. [3-4-5]

Force Degradation Study [6-7-8]

Forced degradation is the process of subjecting drug compounds to extreme chemical and environmental conditions to determine product breakdown levels and preliminary degradation kinetics, and to identify potential degradation products. They are used to facilitate the development of analytical methodology, to gain a better understanding of active pharmaceutical ingredient (API) and drug product (DP) stability, and to provide information about degradation pathways and degradation products. It is particularly useful when little information is available about potential degradation products Forced degradation studies are also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc.

Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used. But these guidelines are very general in conduct of forced degradation and do not provide details about the practical approach towards stress testing.

Objective of forced degradation studies

Forced degradation studies are carried out to achieve the following purposes:

- To establish degradation pathways of drug substances and drug products.
- To differentiate degradation products that is related to drug products from those that are generated from non-drug product in a formulation.
- To elucidate the structure of degradation products.
- To determine the intrinsic stability of a drug substance in formulation.
- To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and drug product.

Overview of Regulatory guidance

According to the available guidance, forced degradation studies are carried out for the following reasons:

- Development and validation of stability-indicating methodology.
- Determination of degradation pathways of drug substances and drug products.
- Structure elucidation of degradation products.
- Determination of the intrinsic stability of a drug substance molecule. Degradation studies have several defining characteristics.
- They are carried out in solution and/or the solid state.
- Involve conditions more severe than accelerated testing.
- Are typically carried out on one batch of material.
- Include conditions that analyze thermolytic, hydrolytic, oxidative, and photolytic degradation mechanisms in the drug substance and drug product (as appropriate).
- It is not part of the formal stability program.

Factors affecting forced degradation

The characteristic factors which affect API for forced degradation are Time, Temperature and/ or with humidity, Acid/base Stress testing, photo degradation and pH variation (high and low).

Thermal and/or humidity stress testing:

This test is initiated as the drug substance is place in thermal/humidity conditions for extended period of time which consequently causes the active substance to forcefully degrade to its primary constituents.

Acid/base stress testing:

This test is used to evaluate the forced degradation of an API which involves exposure of a drug substance in basic or acidic environment for longer period of time causes degradation to its primary constituents. Acid/base hydrolysis take place in labile carbonyl functional groups for e.g. esters (lactones), amides (lactams), alcohols, carbamates, imides, imines and aryl amines.

Degradation by UV light:

Several UV-unstable products have a major problem of Ultraviolet-visible degradation. UV-unstable products consist of synthetic and natural polymers which crack or disintegrate when exposed to constant sunlight. Continuous exposure is a more severe problem than discontinuous exposure because the attack is dependent on the extent and degree of exposure.

Degradation conditions

Typical stress tests include four main degradation mechanisms: heat, hydrolytic, oxidative, and photolytic degradation. Selecting suitable reagents such as the concentration of acid, base, or oxidizing agent and varying the conditions (e.g., temperature) and length of exposure can achieve the preferred level of degradation. Overstressing a sample may lead to the formation of secondary degradants that would not be seen in formal shelf-life stability studies and under- stressing may not serve the purpose of stress testing. Therefore, it is necessary to control the degradation to a desired level.

Hydrolytic condition

Hydrolysis is one of the most common degradation chemical reactions over wide range of pH. Hydrolysis is a solvolytic process in which drug reacts with water to yield breakdown products of different chemical compositions. Water either as a solvent or as moisture in the air comes in contact with pharmaceutical dosage forms is responsible for degradation most of the drugs.

Oxidation conditions

Hydrogen peroxide is widely used for oxidation of drug substances in forced degradation studies but other oxidizing agents such as metal ions, oxygen, and radical initiators (e.g.,azobisisobutyronitrile, AIBN) can also be used. Selection of an oxidizing agent, its concentration, and conditions depends on the drug substance. The mechanism of oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations. Amines, sulphides and phenols are susceptible to electron transfer oxidation to give N-oxides, hydroxylamine, sulphones and sulphoxide .

Photo degradation

According to ICH Q1B guideline for photo degradation, samples should be exposed to light providing an overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours/square meter with spectral distribution of 320-400nm to allow direct comparisons to be made between the drug substance and drug product.

The Photolytic Degradation can occur through Nonoxidative or Oxidative Photolytic Reaction. The Nonoxidative Photolytic Reaction Include Isomerization, Dimerization, Cyclization, Rearrangements, Decarboxylation and Hemolytic Cleavage of X-C Hetero Bonds, N-Alkyl Bond (Dealkylation and Deamination), So2- C Bonds Etc and While Oxidative Photolytic Reaction occur through either Singlet Oxygen (1o2) Or Triplet Oxygen (3o2) Mechanism.

Thermal Condition [9]

In general, rate of a reaction increase with increase in temperature. hence, the drugs are susceptible to degradation at higher temperature. Many apis are sensitive to heat or tropical temperatures. for example, vitamins, peptides, Etc.

Table: 1 Thermal conditions

Degradation Type	Experimental Condition	Storage Conditions	Sampling Time(Days)
Hydrolysis	Control API (No Acid Or Base) 0.1n Hcl 0.1n Naoh Acid Control (No Api) Base Control (No Api) Ph: 2,4,6,8 3% H2o2	 40°C, 60°C 40°C, 60°C 40°C, 60°C 40°C, 60°C 40°C, 60°C 25°C, 60°C	 1, 3, 5 Days 1, 3, 5 Days 1, 3, 5 Days 1, 3, 5 Days 1, 3, 5 Days 1, 3, 5 Days
Oxidation	Peroxide Control Azobisisobutyronitrile Aibn Control	25°C, 60°C 40°C, 60°C 40°C, 60°C	1, 3, 5 Days 1, 3, 5 Days 1, 3, 5 Days
Photolytic	Light, 1xich Light, 3xich Light Control	Na Na Na	1, 3, 5 Days 1, 3, 5 Days 1, 3, 5 Days
Thermal	Heat Chamber Heat Chamber Heat Chamber Heat Chamber Heat Control	60°C 60°C/ 75% Rh 80°C 80°C/ 75% Rh Room Temp.	1, 3, 5 Days 1, 3, 5 Days 1, 3, 5 Days 1, 3, 5 Days

MATERIAL AND METHOD [10-11]

Instruments

Absorption spectral measurements were carried out with a UV-Visible spectrophotometric method, Agilent model and software is Cary 60 win, with 1 cm matched quartz cells, Digital weighing balance, sonicator was utilized for present work.

Chemicals

Reagents were used 0.1 N NaOH (sodium hydroxide), 0.1 N HCl (hydrochloric acid) and DI (de-ionized) water), hydrogen peroxide. All of the reagents used were of analytical grade.

Preparation of solution [10-12]

Preparation of stock solution of drug

Stock solution of Tenofovir disoproxil Fumarate was prepared by weighing 10mg of Tenofovir disoproxil Fumarate and dissolved it in water to made up to the volume 100ml. The concentration of the prepared stock solution was 100µg/ml.

Preparation of standard solution

Different aliquots were taken from stock solution and diluted with methanol to prepare a series of concentrations from 2-10µg/ml.

Calibration curve for the Tenofovir disoproxil Fumarate

Calibration standard were prepared by dissolving working standard into the solvent to yield the concentrations of 2, 4, 6, 8, 10µg/ml and was determined for absorbance. Aliquots (0.2, 0.4, 0.6, 0.8, 1.0 ml) from standard solution of Tenofovir disoproxil Fumarate were pipetted out into the 10 ml volumetric flask and the volume was made upto10 ml with water. The absorbances were measured for five times each at 260 nm against reagent blank. The calibration curve was constructed by plotting absorbance v/s concentration (µg/ml). Regression coefficient was also measured.

Table: 2 Calibration curve for the Tenofovir disoproxil Fumarate

Sr. no.	Concentration µg/ml	Absorbance
1	2	0.331
2	4	0.512
3	6	0.676
4	8	0.850
5	10	1.032

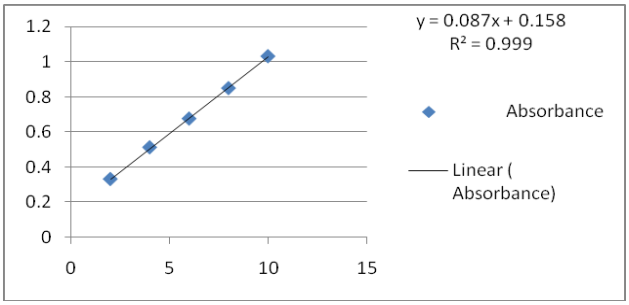

Figure 1: Calibration curve of Tenofovir disoproxil Fumarate

Method of Degradation Studies [13-14-15]

Tenofovir disoproxil Fumarate was subjected to various stress conditions to affect their degradation. Degradation method such as acid, alkali, oxidative and neutral hydrolyte was attempted.

Degradation under alkali catalyzed hydrolytic condition:

To 20mg of Tenofovir disoproxil Fumarate in 100ml volumetric flask, 10 ml water was added. To this solution, 10ml 0.1N NaOH was added. The above solution was kept for 4 hours at 40°C.volume was made up to the mark. Afterward the absorbance of the solution were analyze separately at wavelength maximum at 260nm.

Degradation under acid catalyzed hydrolytic condition:

To 20 mg of Tenofovir disoproxil Fumarate in 100ml volumetric flask, 10 ml water was added .To this solution, 10 ml 0.1N HCL was added. The above solution was kept for 4 hours at 40°C volume was made up to the mark. Afterward the absorbance of the solution were analyze separately at wavelength maximum at 260nm.

Degradation under oxidative condition:

To 20 mg Tenofovir disoproxil Fumarate, 10 ml of water was added. To this solution, 10 ml of 3% hydrogen peroxide was added. The above solution was kept at room temperature for 1hours. Volume was made up to 100ml with water. Afterward the absorbance of the solution were analyze separately at wavelength maximum at 260nm.

Degradation under neutral hydrolytic condition:

To 20 mg of Tenofovir disoproxil Fumarate in 100ml volumetric flask, 10 ml water was added. The above solution was kept 4 hours at 40°C volume was made up to the mark. Afterward the absorbance of the solution were analyze separately at wavelength maximum at 260nm.

Table: 3 Results of Stress Degradation Studies

Sr. no.	Degradation condition	Chemical Used	Temp (^{0}c)	%Degradation
1	Alkali	0.1N NaOH	40^{0}c	10.6%
2	Acid	0.1N HCL	40^{0}c	10.95%
3	Neutral hydrolyte	water	40^{0}c	12.26%
4	oxidative	3% Hydrogen peroxide	at room temperature	12.22%

RESULT AND DISCUSSION

Tenofovir disoproxil Fumarate was freely soluble in water and Dichloromethane. Water was choose as a solvent. The drug has maximum absorbance at 260nm.The optical characteristic of drug was found to be Beer's law limits 2-10µg/ml, Correlation coefficient is 0.999.The drug sample was analyzed by UV spectroscopy using water as solvent. The force degradation studies of Tenofovir disoproxil

Fumarate was done on Stress degradation by hydrolysis under alkaline condition by using 0.1N NaOH was found to be 10.6%. Stress degradation by hydrolysis under acidic condition by using 0.1N HCl and product degradation was found to be 10.95%. Oxidative degradation was done by using hydrogen peroxide and product degradation was found to be 12.22%. Neutral hydrolytic degradation was found to be 12.26%. Forced degradation studies of drug reveal good stability under the chosen experimental conditions.

CONCLUSION

The proposed method is found to be simple, sensitive and reproducible and hence it can be used in appreciable determination of Tenofovir disoproxil Fumarate in bulk. Linearity was observed in the concentration range of 2 - 10ug/ml. The regression obtained for Tenofovir disoproxil Fumarate was stability indicating UV spectrophotometric method has been developed for quantitative 0.999. The method is accurate, precise, reproducible & economical and it was also selective for the desirable range. The result was found to be within the specified range as per the ICH guidelines. Stability testing study includes oxidation, alkylation, hydrolysis and acidic conditions.

REFERENCE

1. ICH, Stability Testing of New Drug Substances and Products. International Conference on Harmonization, IFPMA, Geneva, 1993.
2. ICH, Impurities in New Drug Products. International Conference on Harmonization, IFPMA, Geneva, 1996.
3. Budavari S, editor. The Merck Index. 13th ed. White House Station, NJ: Merck and Co. Inc; 200; 1628.
4. ICH, Q2A, Text on Validation of Analylical procedures, International Conference on Harmonization, Geneva,octomber 1994:1-5.
5. Christen, G.D.Analytical Chemistry 6th edition, John Wiley and Sons. 2003; 6:13l-l32
6. Kasture, A.V.; Mahadik, K.R.; Wadodkar, S.G. pharmaceutical Analysis Nirali Prakashan 1:5-7, 28-30.
7. Sharma B. Instrumental Method of analysis. 21st edition, Goel Publishing House. 2006: 6
8. Havele S., Dhaneshwar S. Stress Studies of Tenofovir Disoproxil Fumarate by HPTLC in Bulk Drug and Pharmaceutical Formulation. 2011: 6
9. Bhattacharyya S., Choudhury P. etb al. Estimation and Validation of Stability Indicating UV Spectrophotometric Method For The Determination Of Guaifenesin In Presence Of Its Degradant Products. 2013; 5:262-268.
10. Bojjagani R. Madasu S. Method Development and Validation of Forced Degradation Studies of Pioglitazone Hydrochloride by Using UV Spectroscopy.2012; 4:1750-1757.
11. Hossain F., Bhadra S. et al. The ICH guidance in practice: Stress degradation studies on aceclofenac and development of a validated stability-indicating reversed-phase HPLC assay in tablet dosage form.2013; 5:131-146.
12. Blessy M., PatelR.et al. Development of forced degradation and stability indicating studies of drugs. A review.2013; 5: 159–165.
13. Khagga B., Balaram V. et al. Development and Validation of Forced Degradation Studies of Atazanavir Using RP-HPLC and Characterization of Degradants by LC-MS/MS. 2015; 33(03):11-15.
14. S. Singh and M. Bakshi, "Guidance on conduct of stress tests to determine inherent stability of drugs," Pharmaceutical Technology Online 2000; 24:1–143
15. S. Singh and M. Bakshi, "Development of validated stability indicating assay methods critical review," Journal of Pharmaceutical and Biomedical Analysis, 2002; 28(6):1011–1040
16. Reynolds D., Facchine K. et al. Available guidance and best practices for conducting forced degradation studies, Pharm. Technol. 2002;26(3):48–56.
17. El-Saharty Y., Refaat M., et al. Stability- Indicating spectrophotometric and densitometric methods for determination of aceclofenac. Drug Dev. Ind. Pharm. vol. 28 No. 5, 2000; 28(5):571–582.
18. Grosa G, Grooso ED, Russo R, Allergrone G. Simultaneous, stability-indicating, HPLC-DAD determination of guaifenesin and methyl and propyl-parabens in cough syrup .J Pharm Biomed Anal.2006; 41:298-803.