ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Studies on forced degradation and solid state stability of tenofovir disoproxil orotate

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Article History:

Received on: 14 May 2021
Revised on: 10 Jun 2021
Accepted on: 14 Jun 2021

Keywords:

Tenofovir disoproxil orotate,
HPLC method,
Forced degradation

ABSTRACT

In order to develop a stability regulatory system for drug substances and degraded products, a forced degradation study is an essential part in the design of the method. As per ICH Guidelines Q1A in 1993, it was established as an essential requirement for the regulatory system to assess the stability of drugs and their degradation products under the degradation studies by force. These analytical methods are helpful in the development of stability, indicating the method by conducting the studies on forced degradation with their mechanism of degradation. Drug products by degradation and new drug substance by forced degradation conditions are more severe than a demonstration of specificity of stability indicating methods. The analytical method development is facilitated by those techniques for better understanding of (API) active pharmaceutical ingredients and (DP) drug products stability.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v12i4.4891

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INTRODUCTION

According to the ICH recommendations, stress testing is designed to discover potential degradation products, which assists in estimating the inherent stability of the molecule and developing degradation routes, as well as confirming the stability indicating methodologies utilised. However, in the case of forced degradation, these guidelines are very general. The chemical stability of the molecule influences drug product safety and efficacy. The information on molecule stability is used to select the best formulation, package, storage conditions, and shelf life (Alsante *et al.*, 2003; Harmon and Boccardi, 2005). Many pharmaceutical drug substances and products are photosensitive and undergo photodegradation upon exposure to outdoor or indoor photon sources (Baertschi *et al.*, 2006; Brummer, 2011), they have been estimated the drug molecule intrinsic stability. Forced degradation studies can be used to estimate the intrinsic stability of a drug molecule. Forced degradation studies, which create degradation products that may be analysed to identify the molecule's stability, can be used to examine potentially polymorphic or enantiomeric compounds, as well as differences in drug degradation and excipient interferences (Rockville, 2004; Dorman *et al.*, 1997) showed the ICH guidelines and degradation routes for stress testing to potential degradation products, which aids in determining the molecule's intrinsic stability. Photostability studies were carried out to generate primary degradants of drug substances through UV or fluorescent exposure. Some recommended photostability testing conditions were described in the ICH guidelines. Drug substance and solid/liquid drug product samples should be exposed to a minimum of 1.2 million lx h and 200 W h/ m2 light, with the most commonly accepted wavelength of light caus-

ing photolytic degradation being 300-800 nm (ICH Q3C (R3) Impurities, 2002; European Medicines Agency, 2003). Degradation of the drug product and new drug substance by forced degradation under conditions is more severe and accelerated. Stress testing, stress studies, stress decomposition studies, forced decomposition studies etc., are known to be the introduction of forced degradation studies. It is necessary to follow ICH recommendations while conducting forced degradation tests under proper circumstances such as light, oxidation, dry heat, acidic, basic, hydrolysis, and so on. Moreover, from the degradation products, the drug gets separated. The time and different environmental factors varies the quality of a drug substance and drug product obtained from forced degradation studies and it should be mandated by FDA and ICH guidance (Kovaříková *et al.*, 2004; Maheswaran, 2012). The HPLC method stability indicating nature has been assessed by detecting the solution state and the solid state samples were degraded and then the photo diode-array detector was analyzed (Oyler *et al.*, 2012; Prekodravac *et al.*, 2011) have been expressed that the forced degradation is promoted during phase I of a clinical trial to allow for the identification of degradation products, structural elucidation, and optimization of stress settings. The determination of an assay by HPLC method of Tenofovir disoproxil orotate has been developed and conducted the analytical method as per the guidelines of ICH and performance of the method characteristics met the requirements for the analytical application (Ranjit and Rehman, 2012; Singh and Bakshi, 2000). The identification of each contaminant is essential in terms of both chemistry and safety prospects. Chemical possibilities include impurity categorization and identification, report creation, an impurity catalogue in the specification, and a brief description of analytical methodologies. The safety prospects contain explicit instructions for qualifying those contaminants that were not present at much lower levels in a batch of a new therapeutic ingredient (Trabelsi *et al.*, 2005).

MATERIALS AND METHODS

Reagents and chemicals

Triethylamine HPLC grade, acetonitrile and Orthophosphoric acid (AR grade), Sodium hydroxide, hydrochloric acid, Hydrogen peroxide (LR grade) were obtained from Qualigen (Mumbai, India). The HDPE bottle and the polypropylene cap meet the general Pharmaceutical European requirements for plastic primary packaging material. Tenofovir disoproxil orotate standard and Tenofovir disoproxil orotate sample in house material (Baertschi and Alsante, 2005).

Instrumentation and Chromatographic Conditions

Waters Alliance HPLC system module 2695 with 2998 PDA detectors with wavelength range 190-700 and an automatic sample injector were used In this study. The data processing and management for chromatographic peak integration was obtained by using the Empower 2 software. YMC Make Column YMC Pack ODS-AQ (250mm x4.6mm x 5μm particle size) was used in this study as a stationary phase. Analytical Balance Mettler Toledo, photostability chamber newtronics, and PH meter Metrohm.

Sample standard solution

About 50 mg of Tenofovir disoproxil orotate is accurately weighed and transferred to a 50 ml volumetric flask for dilution with the diluents. Again 10.0ml of this solution is further diluted to 50 ml with diluent.

Studies on forced degradation

Studies on forced degradation are undertaken to degrade the sample consciously; these studies are helpful to evaluate analytical methods ability to appraise the functioning ingredient and its products of degradation without interference. In order to determine the HPLC stability indicating nature, the Tenofovir disoproxil orotate sample will be stressed by acid, base, hydrogen peroxide, heat, light (Fluorescent overall illumination with UV) to produce about 10% - 30% degradation of active pharmaceutical ingredient. The breakdown samples will be analyzed for determining the peak purity by using a photodiode array detector. The degradation study will be designed in the following parts.

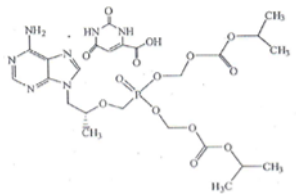

Figure 1: Structuren of Tenofovir disoproxil orotate

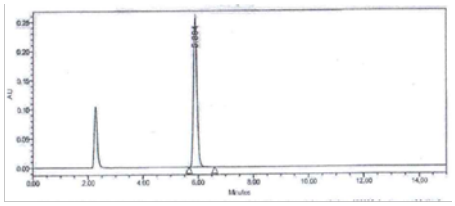

Figure 2: Humidity Exposed Tenofovir Disoproxil Orotate Control sample solution Chromatogram

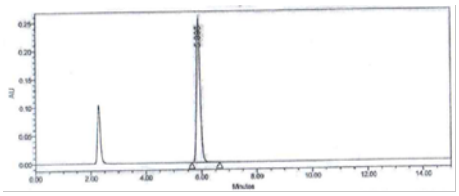

Figure 3: Humidity Exposed Tenofovir Disoproxil Orotate sample solution Chromatogram

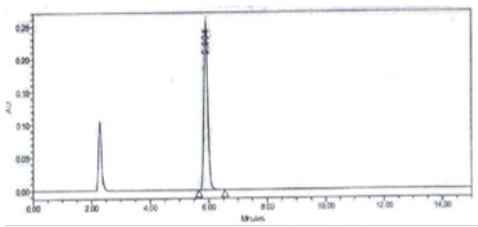

Figure 4: Heat at 105^{o}C for 24hrs Exposed Tenofovir Disoproxil Orotate sample solution Chromatogram

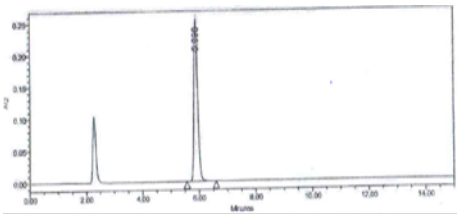

Figure 5: Photostability Tenofovir Disoproxil Orotate Control sample solution Chromatogram

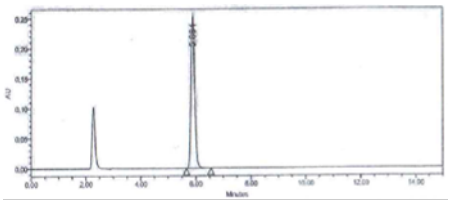

Figure 6: Photostability Tenofovir Disoproxil Orotate sample solution Chromatogram

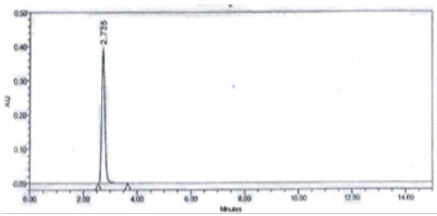

Figure 7: 6% H_2O_2 at RT-24 hours Blank solution Chromatogram

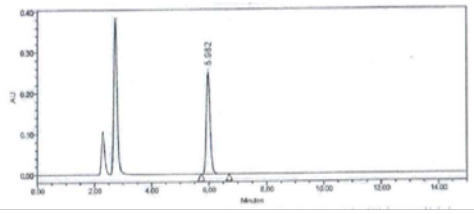

Figure 8: 6% H_2O_2 at RT-24 hours Tenofovir Disoproxil Orotate sample solution Chromatogram

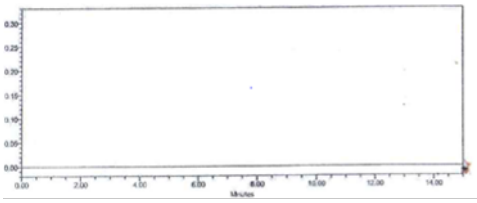

Figure 9: Blank 0.1N HCl Chromatogram

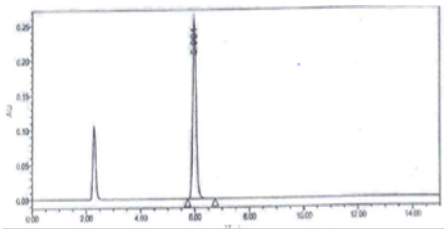

Figure 10: 0.1N HCl Tenofovir Disoproxil Orotate Control sample Chromatogram

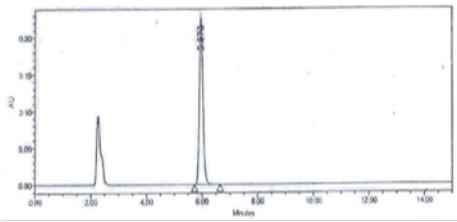

Figure 11: 0.1N HCl Tenofovir Disoproxil Orotate sample Chromatogram

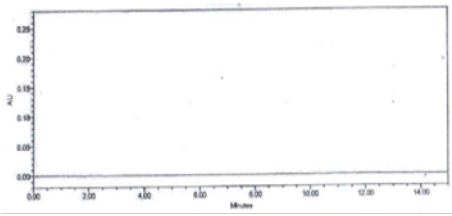

Figure 12: 0.1NaOH Blank solution Chromatogram

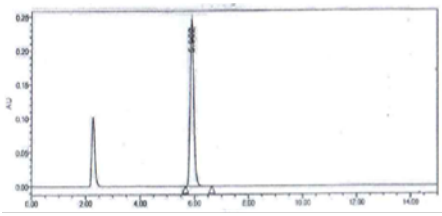

Figure 13: 0.1NaOH Tenofovir Disoproxil Orotate Control sample solution Chromatogram

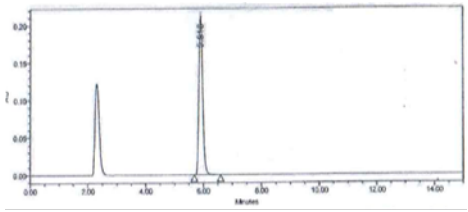

Figure 14: 0.1NaOH Tenofovir Disoproxil Orotate sample solution Chromatogram

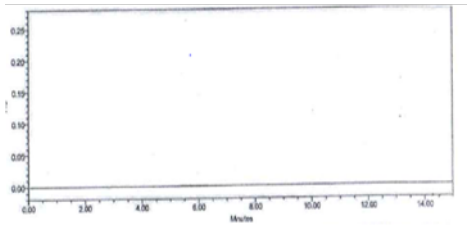

Figure 15: Heat at 60°C-24 Hrs Blank solution Chromatogram

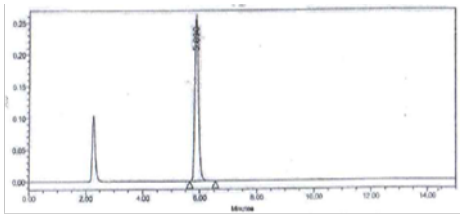

Figure 16: Heat at 60°C-24 Hrs Tenofovir Disoproxil Orotate Control Sample solution Chromatogram

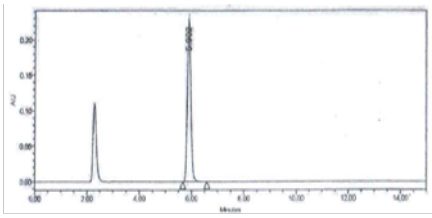

Figure 17: Heat at 60°C-24 Hrs Tenofovir Disoproxil Orotate Sample solution Chromatogram

Solution state degradation

Acid Hydrolysis

10 ml of this solution is required to transfer into a 50 ml volumetric flask, add 15 ml of diluent and 2.5 ml of 1.0 N Hydrochloric acid solution (final strength of an acid is equivalent to about 0.1N HCl). Keep the solution at room temperature for 24 hours and then neutralize with 2.5 ml of 1.0 N Sodium hydroxide solution and dilute to volume with diluents and then results are listed in Table 2 and Figures 9, 10 and 11.

Base Hydrolysis

10 ml of this solution is required to transfer into 50 ml volumetric flask, add 15.0 ml of diluent, add 2.5 ml of 1.0 N Sodium hydroxide solution (final strength of Base is equivalent to about 0.1N NaOH). Keep the solution at room temperature for 24 hours and then neutralize with 2.5 ml of 1.0 N Hydrochloric acid solution and dilute to volume with diluents and results are then listed in Table 3 and Figures 12, 13 and 14.

Oxidation

10 ml of this solution is required to transfer into a 50 ml volumetric flask, add 15 ml of diluent, add 2.5 ml of 20% Hydrogen peroxide (final strength of Hydrogen peroxide is equivalent to about 6%). Keep the solution at room temperature for 24 hours and dilute to volume with diluents and results are then listed in Table 4 and Figures 7 and 8.

Heat Degradation

10 ml of this solution is required to transfer into a 50 ml volumetric flask, add 15.0 ml of diluents. Keep the solution at 60°C temperature for 24 hours and dilute to volume with diluents and then the results are listed in Table 5 and Figures 7 and 8.

Blank Preparation

For the above degradations studies, prepared a blank solution without addition of sample, keep under the same condition and analyze as per the method.

Solid state degradation

Perform the solid state degradation study by exposing the Tenofovir disoproxil orotate sample to light (an overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy, not less than 200-watt hours/square meter fluorescent light and UV light), heat (at 105°C for 24 hours) and humidity (90% + 5), Analyze the thermal and photo exposed samples along with control sample for determining the peak purity by using a photodiode array detector.

Note: if the sample is prone to degrade in any stress condition, the degradation will be carried out between 10% - 30% degradation in that condition (European Medicines Agency, 2003).

Method development

System Suitability

System suitability is an integral part of the method of analysis. A standard solution was prepared and analysed as per the proposed method. Inject blank followed by standard solution six times into the chromatograph and record the chromatograms. The system is suitable, if and only if the number of theoretical plates for Tenofovir disoproxil peak should be not less than 5000. The tailing factor should be

Table 1: System suitability

S.No	Parameter	Tailing factor for Tenofovir disoproxil orotate peak from standard solution	%RSD for peak area of Tenofovir disoproxil orotate from standard solution	Number of theoretical plates for Tenofovir disoproxil orotate from standard solution
1	Forced Degradation	1.2	0.3	9304
2	Forced Degradation	1.2	0.2	9632
3	Forced Degradation	1.1	0.6	10408
	Minimum	1.1	0.2	9304
	Maximum	1.2	0.6	10408

Table 2: Assay values for acid hydrolysis samples

Sample	Assay(%w/w on anhydrous basis)	Purity angle	Purity Threshold
Control sample	99.3	0.28	1.11
AT RT-24 hours(0.1N HCl)	89.0	0.28	1.14

Table 3: Assay values for base hydrolysis samples

Sample	Assay(%w/w on anhydrous basis)	Purity angle	Purity Threshold
Control sample	98.9	0.27	1.02
AT RT-24 hours(0.1N NaOH)	83.0	0.22	1.01

Table 4: Assay values for oxidation samples

Sample	Assay(%w/w on anhydrous basis)	Purity angle	Purity Threshold
Control sample	99.3	0.28	1.11
AT RT-24 hours 6% H_2O_2	96.4	0.25	1.01

Table 5: Assay values for heat (60°C) degraded samples

Sample	Assay(%w/w on anhydrous basis)	Purity angle	Purity Threshold
Control sample	100.0	0.27	1.02
Heat at 60° C hours - 24	90.5	0.26	1.02

not more than 2.0. The %RSD for the Tenofovir disoproxil peak area should be not more than 0.85. System suitability results are listed in Table 1.

Photostability degradation

Transfer about 200 mg of the sample in a Petri dish, spread it uniformly and completely wrap with aluminium foil and display to the illumination of light at least 1.2 million lux hours an integrated near UV energy of at least 200-watt hours/ square meter. After exposure, about 50 mg of Tenofovir disoproxil orotate sample is accurately weighed and transferred into 50 ml volumetric flask and dilute to volume with diluent. 10 ml of this solution is required to transfer into a 50 ml volumetric flask and make up to the mark for dilution.

Heat at 105°c by exposure

Transfer the sample around 200 mg into a Petri dish, spread it uniformly, expose it to 105°c heat for one day; after this, transfer accurately weighed around 50 mg sample of Tenofovir disoproxil orotate into a 50 ml volumetric flask to dissolve and dilute with diluent to the volume. Again 10.0 ml of this solution is diluted to 50 ml with diluent.

Humidity stress study

Transfer the sample around 200 mg into a Petri dish, spread it uniformly and expose it In a desiccator

Table 6: Assay values for the solid state stability sample

Sample	Assay(%w/w on anhydrous basis)	Purity angle	Purity Threshold
Control sample	100.0	0.27	1.02
Photostability control sample-A1.2 million Lux hours exposure to white fluorescent light and 200 Wh/sq.mt exposure to UV light.	99.2	0.28	1.02
Photostability sample-A1.2 million Lux hours exposure to white fluorescent light and 200 Wh/sq.mt exposure to UV light.	99.2	0.32	1.02
Heat at 105°C 24-hours	99.4	0.26	1.01
Humidity Control sample at 25°C with 90% RH for 24 hours.	99.3	0.26	1.01
sample at 25°C with 90% RH for 24 hours.	99.7	0.23	1.01

at 25°C With 90% RH+ 5% RH for 24 hrs. After exposure, transfer accurately weighed around 50 mg sample of Tenofovir disoproxil orotate into a 50 ml volumetric flask to dissolve dilute with diluent to the volume. Again 10.0 ml of this solution is diluted to 50 ml with diluent.

Solid State stability

Significant degradation was observed when Tenofovir disoproxil orotate sample solutions were exposed to light (1.2 million Lux hours and 200 Wh/sq.mt exposure to white fluorescent light and UV light) and heat at 105°C and Humidity at 90% RH for the typically degraded sample, the API peak purity test. The Tenofovir disoproxil peak passed the peak purity testing, leading that the peak is spectrally homogeneous. In other words, none of the degradants formed during the stress study co-elute with the Tenofovir disoproxil peak. The results are listed in Table 6 and Figures 1, 2, 3, 4, 5 and 6.

RESULTS AND DISCUSSION

Method development

System Suitability

System suitability is an integral part of the method of analysis. A standard solution was prepared and analysed as per the proposed method. Inject blank followed by standard solution six times into the chromatograph and record the chromatograms.

The system is suitable, if and only if the number of theoretical plates for Tenofovir disoproxil peak should be not less than 5000. The tailing factor should be not more than 2.0. The %RSD for the Tenofovir disoproxil peak area should be not more than 0.85. System suitability results are listed in Table 1.

Forced Degradation Studies

The HPLC method stability indicating nature can be able to assess by taking the Tenofovir disoproxil orotate sample and stressed by acid, base, oxidation, heat, light, humidity, the degraded samples were analyzed using a photodiode array detector. Passed the testing of peak purity for the tenofovir disoproxil orotate peak; this understands that the peak is spectrally homogeneous. In other words, during the stress study, no measurable degradants was formed, co-elute with the Tenofovir disoproxil orotate peak.

CONCLUSIONS

Significant degradation has been observed. The peak purity of Tenofovir disoproxil orotate is passing purity angle is less than purity threshold. There is no interference of degradant peaks with the Tenofovir disoproxil peak. By compiling the results of degradation studies obtained, the prominent way to develop degradation pathways and to identify the degradation products of the active ingredients are forced degradation studies; further, it facilitates the elucidation of the structure of degradants. The chemical and physical stability analysis of drug substances and drug products can be facilitated by forced degradation studies. The forced degradation studies help during the development of formulation, manufacturing conditions, storage conditions and also determines the date of expiry of a drug formulation.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of

interest for this study.

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