GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(REVIEW ARTICLE)

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Futuristic review on progress in force degradation studies and stability indicating assay method for some antiviral drugs

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GSC Biological and Pharmaceutical Sciences, 2021, 16(01), 133–149

Publication history: Received on 15 May 2021; revised on 13 July 2021; accepted on 15 July 2021

Article DOI: https://doi.org/10.30574/gscbps.2021.16.1.0172

Abstract

Force degradation studies of drug substance give perceptive knowledge about the intrinsic stability of the molecule as well as possible degradants which formed during the shelf life of drug and thus, aid within the successive development of its stable formulation. A number of analytical methods with hyphenated techniques are required for the identification, determination and characterization of degraded product and impurities produce during different conditions of stress studies; Chromatographic methodology play a vital role in the field of impurity and degradation profiling .This review summarizes the current regulatory requirements guidelines for the laboratory performance of forced degradation and its application for the development of stability indicating method. There are number of strategies have been implemented for the quantitative assessment of antiviral drugs. This study will provide detailed literature on stability-indicating HPLC/ RP-HPLC approaches for the development and validation of various antiviral drugs.

Keywords: Intrinsic stability; Degradants; Forced degradation; Stability indicating methods

1. Introduction

The stability of pharmaceutical product requires more attention because the stability get directly affects the safety, purity and efficacy of drug products. Stability parameter of active pharmaceutical drugs and their formulations are determined during the early stage of drug development process [1]. The International Council for Harmonization (ICH) and Food and Drug Administration (FDA) provided the different guidelines and requirements for stability testing data [2]. According to different guidelines generally two types of stability testing studies, like, long-term stability studies and accelerated stability studies. In case of long-term studies, the require time for completion of study is about 12 months. Generally long-term stability studies useful for identification and separation of degraded products. In case of accelerated stability testing required around 6 months, Intermediate stability testing is also proceeding for 6 months at conditions milder than accelerated studies [3].

2. Search area

This review summarizes the current regulatory requirements for the practical performance of forced degradation and its application for the development of stability indicating method. There are numerous strategies have been implemented for the quantitative assessment of antiviral drugs.This study will provide detailed literature on stability-indicating HPLC/ RP-HPLC approaches for the development and validation of various antiviral drugs as well as gives a basic idea to the researchers who are working in the area of product development and finish product testing.

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3. Discussion

Authors selected many references published in the reputed journals on degradation studies, impurity profiling, and different stability indicating assay methods and took the important information from that is published in 1998-2021. The article are divided into various section such as regulatory requirements for the practical performance of forced degradation and development of stability indicating method detailed literature on stability- indicating HPLC/ RP-HPLC approaches for the development and validation of various antiviral drugs.

4. Force degradation studies

Force degradation studies are very useful to determine the reason of degradation routes and stability of pharmaceutical product under various stress producing conditions. In the force degradation studies the characterization of the degradants is usually performed with help of ICH guidelines. To performed stability studies and development of stability indicating method (SIM) various analytical equipment are used such as HPLC-UV and HPLC- (PDA) are two common equipment's, while characterization and structural information of degradant products LC-MS is most authentic method [4] .

5. Objective of forced degradation studies

Forced degradation studies establish degradation pathways of drug molecule and their pharmaceutical products. For the structural elucidation of degradant force degradation studies play major role and also applicable for determination of intrinsic stability of a drug substance in pharmaceutical formulation. Forced degradation reveal the degradation mechanisms of the drug product and drug substance.These studies provided information about stability indicating nature of a developed method [5-7].

6. Worldwide status of regulatory requirements on degradation studies

For providing information about forced degradation many guidelines are mentioned like International Committee for harmonization (ICH), Food and drug administration (FDA), European Medicines Agency (EMA), United States Pharmacopeia (USP), Japanese Pharmacopoeia (JP), and Agencia National de Vigilancia Sanitaria (ANVISA). [8-9]

6.1. ICH Guidelines

In ICH guidelines ICH Q1A, Q1B, & Q2B, Q3A, Q3B, M4Q (R1) discusses about Forced degradation studies.

6.2. ICH Q1A – Testing of Stability for New drug molecules & Products.

ICH Q1A guideline gives information about stress testing of a drug product as the studies base on drug component and drug products. To carry out forced degradation evaluation on drug substances & Products at several accelerated conditions were mentioned. Those conditions were effect of humidity (% Relative humidity), oxidation, temperature, and photolysis & range of pH solution / suspension) [10-11].

6.3. ICH Q1B – Photostability Testing of New drug substances and drug products

These guidelines use for check out the photostability behavior of drug substance in the development stage. The guideline provides information about the approaches to evaluate photostability of the drug and its formulation to be used in development of stability indicating methods [11, 12].

6.4. ICH Q2B – Validation of analytical procedures: Methodology

The ICH Q2 guideline gives the information about the protocols to be followed by validation of different analytical protocols. Sample usages for forced degradation studies mentioned in ICH Q2B, Part II, Section 1.2.2. Samples should be to stress under different accelerating condition like heat & humidity used for determination of specificity. In additional, essential for the quantitative determination of the degradation produced [11, 13-15].

6.5. ICH Q3 -Impurities in New drug substances

These guidelines give the knowledge about the determination of impurity present in new drug molecules. In these Section gives guidelines about different aspect like identification of impurities, types of impurities & specification impurities analytical protocols of impurities. Mostly it provided important about, validation of chromatographic method

of new batch of drug molecule. According to guidelines in case of trace molecules, stability limit should be more than 90% and hence about 10% degradation is sufficient [12, 16].

6.6. ICH Q3B – Impurities in New Products

This guideline gives knowledge about analytical methods. It is important for an analytical method to validate the specific or non - specific degradation products under various stress conditions [17]

6.7. ICH M4Q (R1) – The common technical document for the registration of pharmaceuticals for human use: Module 3: Quality

This guideline gives knowledge about various types of studies performed, used procedures and various outcomes of the studies. In outcomes of stability checking covers in Section 3.2.S.7.3. In these guidelines results gives in form of graphic, tabular, narrative format with the validation data [18].

7. FDA Guidelines (Food and drug administration)

This guideline explains how to analyze the photo stability of newer drug molecules and products (Q1B). Forced degradation studies, according to the FDA, should be carried out in normal development conditions. It talks about how samples degrade when they're exposed to light. These guidelines also aid in the development of Stability indicating method (SIM) and the summarization of data from validation studies, which are confirmatory studies. According to section 211.166(a) (3) of these guidelines, a SIM should be highly precise and capable of quantifying the amount of active ingredient present, whether these types of degradation products occur, as well as other components present in dosage form, without interference under stress. pH, temperature, and oxygen are three stress conditions that are needed for forced degradation studies [13,19].

8. EMA Guidelines (European Medicines Agency)

These rules are essential for the chemistry of active substances. It includes information on the types of experiments that were conducted, the protocols that were used, and the results that were obtained as a result of the study. In section 2.1.2 of this guideline, stability checking for API and dosage type is discussed. It also contains information on retest dates and substance expiration dates. It also determined the analytical methods for implementation and evaluation, as well as intrinsic stability and degradation pathways. It also conducts stability tests on sensitive compounds such as photosensitive and hygroscopic drugs [8,20].

9. USP Pharmacopoeia: validation of compendia procedures

This guideline gives the knowledge about the If no degradation criterion or contaminants are available, the specificity should be calculated by comparing the data to the results obtained degradation product or contaminants using a different method under the same accelerated conditions [3,19].

10. Japanese Pharmacopoeia

This guideline notes that the proposed method must be precise in order to classify and detect the amount of analyte present in the sample. Samples may be subjected to stress conditions for comparative studies, and degradation products can be used for further studies if reference standards are not available [19].

11. ANVISA (National Health Surveillance agency)

This guideline explains how to meet the criteria for stability and forced degradation. These guidelines protect manufacturers from liability for risks posed by and uses of different drug products. It was created with the aim of promoting public health. It also encourages states, districts, and municipalities to promote the Brazilian unified health system's principles in order to enhance overall quality of life [19].

12. Factors Affecting Degradation

The following are the various factors that cause drug product degradation. They are as follows [20-23]:

12.1. Moisture

Water soluble substances can dissolve if there is moisture present or when comes in contact with any moisture; the molecules undergo physical and chemical changes as a result of formulation prone to degradation and lose its properties.

12.2. Temperature

Temperature fluctuations may have a negative impact on the drug's stability. The rate of drug hydrolysis improves as the temperature rises.

12.3. Excipients

Some excipients have been found to have high water content. This moisture can cause an increase in the amount of water in the formulation, affecting the drug's stability. Chemical interactions between the excipients and the drug material can lead to decreased stability in some cases.

12.4. pH

The rate of drug degradation by hydrolysis is significantly affected by pH. To minimize this effect, drug formulations are carried out with buffer solutions of the highest pH stability.

12.5. Oxygen

Oxidation of certain drugs is increased when oxygen is present. Purging nitrogen or carbon dioxide from the storage container stabilizes drugs that decompose more quickly in the presence of oxygen.

12.6. Light

Some drugs are photolabile, meaning they degrade when exposed to light. The susceptibility to photolytic decomposition can be determined by comparing the stability of the substance in the presence of light to the stability of the substance when stored in the dark. It is also important to note that photolabile compounds should be kept in amber glass containers and kept in the dark.

13. Time to perform forced degradation

When it comes to the production of new drug compounds and new drug products, it's vital to know when to conduct forced degradation studies. Stress testing should be done during the phase III of the regulatory submission period, according to FDA guidelines. To assess stress experiments should be conducted in various pH solutions, with oxygen and light present, and at elevated temperatures and humidity levels [6]. The stress tests are carried out in a single batch. The findings should be summarized and reported on annually. Stress research on drug substance should be started early in the preclinical process or phase I of clinical trials to provide enough time for identifying degradation products and structure elucidation, as well as optimizing the stress response. Early stress analysis also provides timely guidelines for making improvements in the manufacturing process and the correct range of stability-indicating analytical procedures [19, 24].

14. Limits for Forced degradation studies

The majority of regulatory guidelines listed degradation limits, such as 5% -20% agreed requirements for chromatographic assay validation. Stability should be greater than 90%, with 10% being sufficient for deterioration. In general, spiked sample mixtures of known degradation drug content & drug products are used to observe drug product stability, deciding the product that is controlled during the degradation process [25].

15. Sources of degradation products

Impurities may come from a variety of sources, including degradation. Drug molecules degrade due to chemical instability under various stress conditions such as heat, humidity, isolation, pH, storage, and transportation processes. Forced degradation should be accomplished through a variety of methods, including oxidation, heat, photolysis, and hydrolysis [6, 10].

16. Plan for selection of forced degradation condition

The intrinsic stability of a drug substance and its product should be calculated under normal conditions such as pH and high temperatures. Along with this, the drug molecules were subjected to additional stress in order to investigate its stability. The solution containing the sample was refluxed for a specific amount of time to investigate the forced degradation. If the degradation substance and product were to be monitored at that period, the process would be interrupted, and further detection, isolation, and monitoring of the degradation product would be carried out. If no signs of degradation are monitored, the reaction time to monitor any signs of degradation will increase as time passes. [3, 6, 19, 26-28] The following conditions were used in the forced degradation studies presented in Table 1 and Figure 1**.**

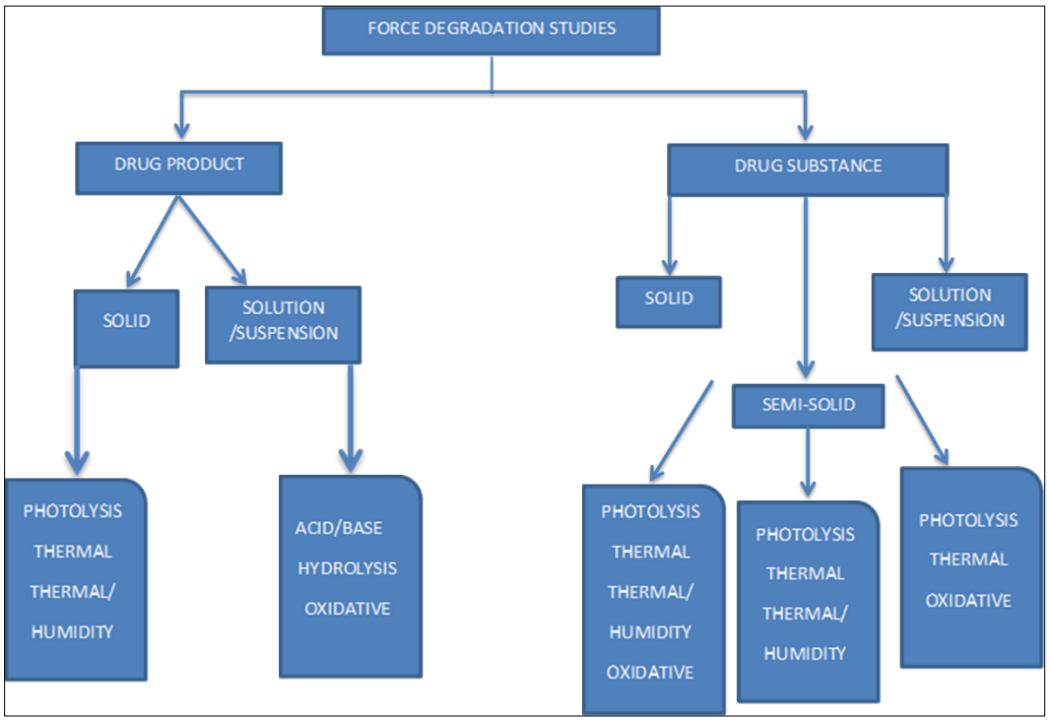

Figure 1 Representation of force degradation studies for drug product and drug substance

Table 1 Condition used for forced degradation

Degradation type	Experimental condition	Storage condition	Sampling time	References
Hydrolysis	Control API (no acid or base)	40 °C, 60 °C	1,3,5 Days	[3, 6, 19, 26-28]
	0.1N HCl	40 °C, 60 °C	1,3,5 Days	
	0.1N NaOH	40 °C, 60 °C	1,3,5 Days	
	Acid Control (no API)	40 °C, 60 °C	1,3,5 Days	
	Base Control (no API)	40 °C, 60 °C	1,3,5 Days	
	pH: 2,4,6,8	40 °C, 60 °C	1,3,5 Days	
Oxidative	3% H_2O_2	25 °C, 40 °C	1,3,5 Days	
	Peroxide Control	25 °C, 40 °C	1,3,5 Days	
	Azobisisobutyronitrile	40 °C, 60 °C	1,3,5 Days	
	AIBN Control	40 °C, 60 °C	1,3,5 Days	
Photolytic	Light, 1 × ICH	NA	1,3,5 Days	
	Light, 3 × ICH	NA	1,3,5 Days	
	Light Control	NA	1,3,5 Days	
Thermal	Heat Chamber	60 ^{0}C	1,3,5 Days	
	Heat Chamber	60 °C/ 75% RH	1,3,5 Days	
	Heat Chamber	80 °C	1,3,5 Days	
	Heat Chamber	80 °C / 75% RH	1,3,5 Days	
	Heat Control	Room temp	1,3,5 Days	

Table 2 Summary of stability-indicating RP-HPLC methods of different antiviral drugs

Sr.no	Name of Drug	Sample	Column	Mobile phase	Flow rate	Detection	Linearity	References
1	Atazanavir sulphate	API	C18Phenomenex (250 mm×4.6mm, 5 µm)	Methanol: water (900:100)	0.5 ml/min	249 nm	10–90 µg/ml	[35]
	Atazanavir sulphate	Capsule	Agilent TC C18 (2) 250 × 4.6 mm, 5 µ	0.02 M ammonium dihydrogen phosphate buffer: acetonitrile: methanol (30:25:45 v/v)	1 ml/min	288nm	5-50 µg/ml.	[36]
2	Abacavir	API+ Tablet	C18- column, Phenomenex Luna, (250×4.6 mm, 5 µm)	Water: acetonitrile (80:20)	1 ml/min	285 nm	600–1600 ng/ml	[37]
	Abacavir sulfate	Tablet	Grace C18 column	Methanol and 10ml of potassium dihydrogen orthophosphate (38:62 v/v).	0.9 ml/min	255nm	10-50 mg/mL	[38]
3	Acyclovir	API	Nova Pack C18 (250 x 4.6) mm, 5µ	Buffer, methanol and acetonitrile (50:20:30, v/v/v)	1.0mL/min	264nm	80-120 µg/ml	[39]
	Acyclovir	API+ Ointment	Agilent column (150mmx 4.6mm, 5µm)	Acetonitrile, Methanol and phosphate buffer (16:20:64 v/v)	1.0 mL/min	290nm	20-100 µg/ml.	[40]
4	Adefovir Dipivoxil	API+Tablet	Nucleodur C18 column (15 cm × 4.6 mm i.d.)	Acetonitrile-citrate buffer (10 mM at pH 5.2) 36:64 (%v/v)	1.5mL/min	260nm	0.5–16 µg/mL	[41]
5	Boceprevir	API	HiQsil C-18 Column (150mm x 4.6 mm i.d., particle size 5 µm)	Methanol :10mM dihydrogen phosphate: triethaylamine (90:10:0.5) (%v/v/v)	1.0mL/min	215nm	50-250 µg/mL	[42]
6	Baloxavir marboxil	Tablet	HSS C18 (100 x 2.1 mm, 1.8 mm)	Buffer 0.1% orthophosphoric acid and acetonitrile (50:50)	0.3ml/min.	240nm	0.69 -2.10 mg/ml	[43]

7	Cobicistat	API+ Tablet	Hypersil BDS C-18 (150mm x 4.6mm 5µ)	Water: Acetonitrile (90:10)	1.0 ml/min	240nm	7.5 - 45µg/mL	[44]
8	Darunavir ethanolate	API+ Tablet	X-Bridge C18 (150 × 4.6 mm ×3.5 µm)	0.01M ammonium formate (pH.3.0) buffer + acetonitrile in the ratio of 55:45 (v/v)	1.0 ml/min	265nm	0.16 to 0.24 mg/mL	[45]
	Darunavir ethanolate	Raw material + Tablet	RP C18 column (Symmetry, 5 μm, mm, Waters)	acetonitrile water (50:50, v/v)	1.0 ml/min	267nm	6.0 to 21.0 μg/mL	[46]
9	Dolutegravir sodium	API+ Tablet	ODS C_{18} column (150 × 4.6 mm, 5 µm particle size)	acetonitrile: water (pH 7.5) in the ratio of 80:20 v/v	1 ml/min	260nm	5–35 µg/mL.	[47]
	Dolutegravir sodium	API+ Tablet	C8 column (150 × 4.6 mm), 5µm	0.1% trifluoroacetic acid in water as MP: A, methanol as MP: B.	0.9 ml/min	240nm	0.1-0.5/mL	[48]
10	Didanosin	Tablet	Zebra Eclipse XDB-C-18 (4.6×250×5µm)	methanol: water (30:70)	1.0 ml/min	246nm	2-12µg/ml w	[49]
11	Efavirenz	Tablet	Agilent eclipse XDB C18 column	Acetonitrile: Water (60:40 v/v)	1.2 ml/min	240nm	2.5-200.0µg/mL	[50]
	Efavirenz	API	RP Spherisorb C-8 (Waters) column (250x4.6 mm, 10 µm),	Acetonitrile: potassium dihydrogen phosphate (pH 2.9, 25mM) - (60:40% v/v)	1.0 ml/min	247nm	0.02 - 20.00 µg mL^{-1}	[51]
	Efavirenz	API +Tablet	Novapak phenyl column, 150 x 3.9 mm dia with 4 µm particle Size	Buffer solution pH 6.0 and acetonitrile in proportion (55:45 v/v)	1.0 ml/min	247nm	0.05 - 0.15 mg/mL	[52]
12	Emtricitabine	API	Intersil ODS-3V column	10 mM sodium phosphate buffer and methanol (85:15)	1.0 ml/min	280nm	0.002 to 0.5 mg/mL.	[53]
13	Etravirine	API	Zorbax Eclipse Plus C18 (250 x 4.6 mm; 5 µm particle size)	acetonitrile and 10 mM ammonium acetate buffer (pH 4.5) in the ratio of 90:10 v/v.	1.0 mL/min	271nm	15-45 µg/ml.	[54]

	Etravirine	API	hypersil ods C18, (150*4.6 mm, 5 µ)	Acetonitrile	1.0 ml/min	271nm	10-60 µg/ml	[55]
14	Famciclovir	API	RP-C18 column 250 mm × 4.6 mm i. d., 5-µm	Methanol: KH2PO4 buffer (pH 3.0, with ortho phosphoric acid) (35:65, v/v)	1.0 ml/min	242nm	10 - 50.00 µg mL^{-1}	[56]
	Famciclovir	API + Tablet	C18- column Hypersil BDS (250×4.6 mm, 5 µ)	0.05M potassium dihydrogen orthophosphate dihydrogen orthophosphate buffer and acetonitrile (80:20)	1.0 ml/min	220nm	0.025-0.150mg/ml	[57]
	Famciclovir	API	C18- column, Symmetry c18(150×4.6 mm, 5 µ)	Mixture of phosphate buffer (0.02M pH: 5.0±0.050 with dilute orthophosphoric acid acetonitrile (80:20)	1.0 ml/min	220nm	20-30 µg/ml	[58]
15	Foscarnet	API+ Injection	C18- column, ODS (150×4.6 mm, 5 µ)	Buffer and acetonitrile (40:60)	1.1 ml/min	225nm	0.25–1.5 µg/ml	[59]
16	Ganciclovir	API	BDS C18, 150mm X four.6mm, 5µm	Phosphate buffer and acetonitrile 70:30 (v/v);	1.0 ml/min	245nm	20 - 30 µg mL	[60]
17	Imiquimod	API	C18 column (Inertsil-ODS3 4.6 × 250 mm, 5 µm)	Phase-A disodium hydrogen phosphate buffer with ortho-phosphoric acid. mobile phase-B methanol and acetonitrile	1.2 ml/min	226nm	0% to 150%	[61]
18	Lamivudine	API + Tablet	IntersilC-18 (250 X 4.6mm, 5µm) column	Acetonitrile, water (50:50% v/v)	1.0 ml/min	270nm	40-120 µg/ml	[62]
19	Oseltamivir	API	Kromasil C (18), 5 microm, 250 mm x 4.6 mm	Acetonitrile and triethylamine)	1.0 ml/min	215nm	70-130 microg/ml	[63]
20	Ribavirin	Capsule+ Plasma	CPS Hypersil cyano column (4.6 × 250 mm × 5 µm).	50 mM phosphate buffer of pH 4 (adjusted by using phosphoric acid).	0.8 ml/min	240 nm	5–200 µg/mL	[64]

	Ribavirin	API	Promosil C18; stainless steel column (250 × 4.6 mm, 5 µm particle size)	0.02 M potassium dihydrogen phosphate of pH 4.7	1.1 ml/min	207nm	2.0-40 µg/ml	[65]
21	Simeprevir	API	Discovery® HS C18 column	Acetonitrile.	1.0ml/min	288nm	1.5 – 45 µg/mL.	[66]
	Simeprevir	API	C18 column (250×4.6, 5 mm	orthophosphoric acid and acetonitrile (55:45 % v/v)	1.0 ml/min	300nm	25–150 µg/ml	[67]
22	Tenofovir	Solid lipid nanoparticles	C18- column Agilent (250× 4.6 mm×5 µm)	20 mM potassium dihydrogen orthophosphate and acetonitrile, (50:50)	1.0 ml/min	260nm	10–60 µg/ml	[68]
23	Valganciclovir	API and Tablet	C18- column, HSS (100×2.1 mm, 1.8 m)	0.01N potassium dihydrogen orthophosphate and acetonitrile (55:45)	0.3 ml/min	254 nm	25–150 µg/m	[69]
	Valganciclovir	API	C18- column, Hypersil gold (250×4.6 mm, 5 µm	Acetonitrile and potassium dihydrogen orthophosphate buffer (pH 5.0) in the ratio of 5:95	0.6 ml/min	252 nm	2–500 µg/ml	[70]
	Valganciclovir	API	C18- column, Hibar (250×4.6 mm, 5 µ)	10 volumes of methanol and 90 volumes of ammonium acetate	1 ml/min	254 nm	18–72 µg/ml	[71]
	Valganciclovir	API	C18- column, µ-Bondapack (250×4.6 mm, 5 µ)	0.01N sodium dihydrogen phosphate buffer (pH 5.0) and acetonitrile in the ratio of 600:400	1.2 ml/min	254 nm	5–30 µg/ml	[72]
24	Zanamivir	API	C18- column, Agilent TC 250×4.6 mm, 5 µ)	Methanol:0.02M phosphate buffer, pH -3.5 (50:50)	1.0 ml/min	230nm	2–12 µg/ml	[73]
25	Zidovudine	API+ Tablet	Phenomenex, (250 × 4.6 mm) Luna 5m C18 Column.	Methanol +Water (89:11v/v)	1.0 ml/min	266nm	25-350 µg/ml	[74]

17. Forced degradation condition

17.1. Hydrolysis

The drug reacts with water under various pH conditions during hydrolysis (both acidic and alkaline). The drug compounds are generally prepared at 50–60°C with 0.1N hydrochloric acid, sulphuric acid, or 0.1N sodium hydroxide. The strength of the acid or alkali used in the experiment determines the molecule's stability. The intensity of acid or alkali solutions should be kept between 0.1 and 1 N. Notably, the analysis does not last longer than 7 days. To prevent decomposition, samples should be neutralized with buffer or acid/base after being exposed to stress conditions.

17.2. Oxidation

Auto-oxidizers are existing in the majority of drug substances. For the oxidation phase, they need free radical initiators. Free radical initiators consist of hydrogen peroxide, trace impurities, and metal ions. The transfer of electrons is involved in this form of degradation .0.1–3% of hydrogen peroxide is a frequent initiator for oxidation forced degradation studies. These research need to be carried out at 40°C for 1–7 days. If greater than 20% degradants are produced, then it needs to be considered as abnormal.

17.3. Thermal condition

Some drugs are viewed to be thermolabile in nature. By increasing the temperature, the rate of response additionally tends to increase which in turn leads to the production of degradation products. These researches have to be performed at 40–80°C. The periods of thermal stress studies generally lasts for 1–2 months and are carried out at 70°C and at high humidity. The drug molecules which are solid in nature are subjected to each dry and wet heat conditions, whilst liquids are exposed to dry heat for the shorter duration of time. Due to the elevated temperature, the drug molecule undergoes degradation and given by Arrhenius equation:

$$k = \frac{Ae - Ea}{RT}$$

Where,

K: Specific reaction rate, A: Frequency factor, Ea: Energy of activation, R: Gas constant (1.987 cal/ deg/mole), and T: Absolute temperature in Kelvin.

17.4. Photolytic conditions

In photolytic degradation studies, the drug substances are exposed to UV or fluorescent conditions. In this study, the drug components or drug products (solid/ liquid) are exposed to the light supply in accordance to the ICH Q1B protocols. The generally used radiation vary for degradation research is about 300–800 nm. In photolytic condition, the degradation happens due to oxidation via free radical mechanism or non-oxidation process. Non-oxidative degradation process involves with isomerization, dimerization, etc., amongst others. On the other hand, oxidative photolytic response includes mechanism involving singlet/triplet oxygen states. Singlet oxygen reacts with unsaturated compounds to produce photooxidative decomposition products, while triplet oxygen follows free radical mechanism, to produce peroxide. Notably, it is proven that light also catalyzes oxidation reactions. In non-oxidative process, numerous types of reactions are discovered such as the hemolytic breakage of C-X hetero bonds, deamination, and cleavage of C-S bonds are observed.

17.5. Stability Indicating Method

A stability indicating method (SIM) is an analytical procedure used to quantitate the decrease in the amount of the active pharmaceutical ingredient (API) in drug product due to degradation. According to an FDA guidance document, a stability-indicating method is a validated quantitative analytical procedure that can be used to detect how the stability of the drug substances and drug products changes with time. A stability-indicating method accurately measures the changes inactive ingredients concentration without interference from other degradation products, impurities and excipients [29]. Stress testing is carried out to demonstrate specificity of the developed method to measure the changes in concentration of drug substance when little information is available about potential degradation product. The development of a suitable stability indicating method provides a background for the pre-formulation studies, stability studies and the development of proper storage requirements. The RP-HPLC is a most widely used analytical tool for separation and quantifying the impurities and it is most frequently coupled with a UV detector [30-31].

18. Development of stability indicating method

Though the necessities with respect to stability indicating technique have been spelt out in regulatory documents, records on the fundamental steps to be observed for the improvement and validation of stability-indicating methods is neither provided in the regulatory guidelines nor in the pharmacopoeias [30, 32-33].

Step 1: Critical study of the drug structure to assess the likely decomposition route

These have to be the first element every time one takes up the project on establishment of a SIM. Much information can clearly be gained from the structure, with the aid of study of the functional groups and other key components.

Step 2: Collection of information on physicochemical properties

Before method development is taken up, it is commonly essential to recognize more than a few physicochemical parameters like pKa, log P, solubility, absorptivity and wavelength most of the drug .

Step 3: Stress (forced decomposition) studies

As described above in forced degradation section, these researches have to be carried out in accordance with ICH Q1A guideline. Stress conditions are (i) 10°C increments above the accelerated temperatures (e.g. 50°C, 60°C, etc.), (ii) humidity where appropriate (e.g. 75% or greater), (iii) hydrolysis throughout a wide range of pH values, (iv) oxidation and (v) photolysis.

Step 4: Preliminary separation studies on stressed samples

The simplest of separation way is to begin with a reversed-phase octadecyl column and perform HPLC separation using UV/PDA detector system. Another way is to go for LC-MS separation. Using these chromatographic techniques, one need to comply with the changes in all the stress samples at a variety of time periods. The effects need to be critically compared with the blank solutions injected in a comparable manner. It has to be found whether or not the fall in drug peak is quantitatively followed through a corresponding rise in the degradation product peaks.

Step 5: Final method development and optimization

Ensuing to fundamental chromatographic examinations, the RT and relative retention times (RRT) of substance shaped should be arranged for each response condition. Exceptional consideration is then paid to these viewpoints whose RT or RRT is close. PDA spectra or LC-MS profile of such viewpoints are gotten and altogether assessed to check whether the product is same or different. To isolate or separate close or co-eluting pecks, the technique is streamlined, by changing the pH, ratio of solvent system, flow rate, mode, temperature, and column.

Step 6: Identification and characterization of degradation products, and preparation of standards

To perceive the resolved products, a conventional way is to isolate them and determine the structure via spectral (MS, NMR, IR, etc.) and elemental analysis. However, this strategy is tedious and time consuming when more than one degradation products are formed. Against it, the modern method is to use hyphenated LC methods coupled with mass spectrometry. This approach integrates in a single instrument approach; this method integrates in a single instrument approach, analytical HPLC, UV detection, full scan mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS-MS) and presents a fair concept on identification of resolving components. These days an in addition integrated method is becoming popular whereby LC-MS or LC-MS-MS is employed to obtain molecular weight and fragmentation information, and in addition detailed structural data is got via LC-NMR analysis.

Step 7: Validation

Validation of analytical methods, in general, has been extensively included in the ICH guidelines Q2A and Q2B, in the FDA guidance and through USP [10-13]. The main focus of validation at this stage is on institution of specificity/selectivity, followed through different parameters like accuracy, precision, linearity, range, robustness, etc. The limits of detection and quantitation are additionally determined for degradation products to assist in establishment of the mass balance.

19. Stability indicating RP-HPLC/ HPLC approaches for different antiviral drugs

In the present year, due to the appearing of new viruses, Hence of development and treatment of antiviral drugs also gain equal importance. Before the use of antiviral drugs should undergoes validation process to produce safe and efficient formulations. The various methods have been used for the quantitative determination of antiviral drugs such as UV, capillary electrophoresis, and Different chromatographic methods likes GC and HPLC, LC-MS, GC-MS. In this review, authors concentrate on stability, suggesting HPLC /RP-HPLC methods for the accurately and effective development and validation of selected antiviral drugs such as, Atazanavir sulfate[35-36], Abacavir[37-38], Acyclovir[39], Adefovir Dipivoxil[41], Boceprevir[42], Baloxavir marboxil[43],Cobicistat[44], Darunavir ethanolate[45-46], Dolutegravir sodium[47-48], Didanosin[49], Efavirenz[50-52], Emtricitabine[53], Etravirine[54-55], Famciclovir[56-58], Foscarnet[59], Ganciclovir[60], Imiquimod[61], Lamivudine[62], Oseltamivir[63], Ribavirin[64-65], Simeprevir[66-67], Tenofovir[68], Valganciclovir[69-72], Zanamivir[73] and Zidovudine[74].The selective stability indicating RP-HPLC/ HPLC approaches for different antiviral drugs is summaries in Table.2

20. Conclusion

Force degradation studies of drug substance give perceptive knowledge about the intrinsic stability of the molecule as well as possible degradants which formed during shelf life of drug and thus, aid within the successive development of its stable formulation. This review summarizes the current regulatory requirements for the practical performance of forced degradation and its application for the development of stability indicating method. There are numerous strategies have been implemented for the quantitative assessment of antiviral drugs.This study will provide detailed literature on stability- indicating HPLC/ RP-HPLC approaches for the development and validation of various antiviral drugs as well as gives a basic skill to the researchers who are working in the area of newer product development and quality control testing.

Compliance with ethical standards

Acknowledgments

Authors are thankful to Dr. Rajendra Gode College Pharmacy, Malkapur, for providing adequate facilities and unqualified support to carry out this review.

Disclosure of conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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