CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3627600

Available online at: http://www.iajps.com Review Article

A REVIEW ON FORCED DEGRADATION AND STABILITY INDICATING STUDIES

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Article Received: November 2019 **Accepted:** December 2019 **Published:** January 2020

Abstract:

Forced degradation is a process in which different stress conditions are applied over drug substances and which turn different degradation products are produced Forced degradation studies show the chemical behavior of the molecule which in turn helps in the development of formulation and package. Forced degradation is a powerful tool used routinely in pharmaceutical development in order to develop stability indicating methods that lead to quality stability data and to understand the degradation pathways of the drug substances and drug products. ICH guidelines demonstrate certain degradation conditions like light, oxidation, dry heat, acidic, basic, hydrolysis etc. ICH Q1A, QIB and Q2B exemplify the forced degradation studies.

Key words: *Forced degradation, stress conditions, drug substances, drug product and ICH guidelines.*

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Please cite this article in press G. Indira Priyadarshini et al., ***A Review On Forced Degradation And Stability Indicating Studies..,*** *Indo Am. J. P. Sci, 2020; 07(01).*

1.INTRODUCTION:

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[1].

The FDA and ICH guidance's state the requirement of stability testing data to understand how the quality of a drug substance changes with time under the influence of various environmental factors. Knowledge of the stability of molecule helps in selecting proper formulation and package as well as providing proper storage conditions and shelf life, which is essential for regulatory documentation. Forced degradation or stress testing studies are part of the development strategy and are an integral component of validating analytical methods that indicate stability and detect impurities. This relates to the specificity section of the validation studies. It is important to recognize that forced degradation studies are not designed to establish qualitative or quantitative limits for change in drug substance or drug product[2].

The main purpose of forced degradation testing studies is to evaluate the overall photosensitivity of the material for method development purposes and/or degradation pathway elucidation. This testing may involve the drug substance alone and/or in simple solutions/suspensions to validate the analytical procedures. For development and validation purposes, it is appropriate to limit exposure and end the studies if extensive decomposition occurs. For photo stable materials, studies may be terminated after an appropriate exposure level has been used. The stability studies include long term studies (12months) and accelerated stability studies (6months).But intermediate studies (6months) can be performed at conditions milder than that used in accelerated studies. So the study of degradation products like separation, identification and quantitation would take even more time. As compared to stability studies, forced degradation studies help in generating degradants in much shorter span of time, mostly a few weeks[3].

Outcomes of forced degradation studies[4]

Forced degradation studies offer the following information

a. Determination of likely degradants,
b. Determination of degradation pathways,
c. Determination of intrinsic stability of the drug molecule,
d. Determination of validated stability indicating analytical methods.

Objective of forced degradation studies[5]:

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 are 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
- To establish stability indicating nature of a developed method.
- To understand the chemical properties of drug molecules.
- To generate more stable formulations.
- To produce a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.
- To solve stability-related problems.

2. REGULATORY GUIDELINES

Various International guidelines recommended forced degradation studies ICH guidelines sometimes apply only to the marketing applications for new products and do not cover the part during clinical development. The ICH guidelines that are applicable to forced degradation studies are[6,7]

a. ICH Q1A: Stability Testing of New Drug Substances and Products,

b. ICH Q1B: Photo stability Testing of New Drug Substances and Products,

c. ICH Q2B: Validation of Analytical Procedures: Methodology.

Origin of Degradation Products

Degradation is considered as one of the chief sources for impurities. Under different stress conditions, namely humidity, heat, pH, isolation, storage, and transportation processes, drug molecules may undergo degradation because of chemical instability. Forced degradation can be carried out through various pathways including hydrolysis, oxidation, heat, and photolysis. It is also observed from different studies that under different stress conditions it is possible to produce all possible types of degradants.

Selection of experimental conditions: There are many examples in the literature of experimental conditions for conducting forced degradation studies and the structural multiplicity of drug molecules makes it not possible to identify a generic set of conditions for a forced degradation

study. For an early phase molecule, using a set of normal conditions by first intention makes sense since very little may be known about the intrinsic stability. If early stability data are available which suggest the molecule is labile at a particular condition (e.g., high pH), the conditions can be modified to take into account the instability (e.g., reduced temperature or time of study). Once a set of conditions have been found, they may be repeated whenever a new stability-indicating method is required during development. Therefore, for later-phase molecules, the forced degradation conditions are defined by the earlier work. By reprocess the same forced degradation conditions throughout development a consistent approach is maintained. Some conditions mostly used for forced degradation studies are presented in Table

Table-1-Conditions mostly used for forced degradation studies

Degradation type	Experimental conditions	Storage conditions	Sampling time (days)
Hydrolysis	Control API (no acid or base)	40°C, 60°C	1,3,5
	0.1M HCl	40°C, 60°C	1,3,5
	0.1 M NaOH	40°C, 60°C	1,3,5
	Acid control (no API)	40°C, 60°C	1,3,5
	Base control (no API)	40°C, 60°C	1,3,5
	pH: 2,4,6,8	40°C, 60°C	1,3,5
Oxidation	3%H_2O_2	25°C, 60°C	1,3,5
	Peroxide control	25°C, 60°C	1,3,5
	Azobisisobutyronitrile (AIBN)	40°C, 60°C	1,3,5
	AIBN control	40°C, 60°C	1,3,5
Photolytic	Light 1 × ICH	NA	1,3,5
	Light 3 × ICH	NA	1,3,5
	Light control	NA	1,3,5
Thermal	Heat chamber	60°C	1,3,5
	Heat chamber	60°C/75% RH	1,3,5
	Heat chamber	80°C	1,3,5
	Heat chamber	80°C/75% RH	1,3,5
	Heat control	Room temp.	1,3,5

3.DEGRADATION CONDITIONS

Hydrolytic conditions[8]:

Hydrolysis is one of the most common degradation chemical reactions over a wide range of pH. Hydrolysis is a chemical process that includes decomposition of a chemical compound by reaction with water. Hydrolytic study under acidic and basic condition involves catalysis of ionizable functional groups present in the molecule. Acid or base stress testing involves forced degradation of a drug substance by exposure to acidic or basic conditions which generates primary degradants in desirable range. The selection of the type and concentrations of acid or base depends on the stability of the drug substance. Hydro chloric acid or sulfuric acids (0.1–1M) for acid hydrolysis and sodium hydroxide or potassium hydroxide (0.1–1M) for base hydrolysis are suggested as suitable reagents for hydrolysis. If the compounds for stress testing are poorly soluble in water, then co-solvents can be used to dissolve the min HCl or NaOH. The selection of co-solvent is based on the drug substance structure. Stress testing trial is normally started at room temperature and if there is no degradation, elevated temperature (50–70ºC) is applied. Stress testing should not exceed more than 7days. The degraded sample is then neutralized using suitable acid, base or buffer, to avoid further decomposition.

Oxidation conditions:

Many drug substances undergo autoxidation i.e. oxidation under normal conditions and involving ground state elemental oxidation. Autoxidation is a free radical reaction that requires free radical initiator to begin the chain reaction. Hydrogen peroxide, metal ions, traces level of impurities in a drug substance act as initiator for oxidation.

The oxidative stress testing is initially carried out in 3% H_2O_2 at room temperature for 6 h and it can be increased or decreased to achieve sufficient degradation. The time can also be increased up to 24 hr 3% or decreased up to 30 min with 1% of H_2O_2 The mechanism of oxidative degradation of drug substance involves an electron transfer

mechanism to form reactive anions and cations. The mechanism of oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations. Drugs In pharmaceuticals most common form of oxidative decomposition is oxidation through a free radical chain process.

Example- Amines, sulphide, and phenols are susceptible to electron transfer oxidation. Drugs which prone to oxidation are hydrocortisone, methotrexate, adinazolam, catecholamine conjugated dienes, heterocyclic aromatic rings, nitraso and nitrate derivatives.

a) Rapamycin, an immunosuppressant drug reacts with oxygen to form a complex mixture of monomeric and oligomeric products. Formation of the majority of the rapamycin degradation products could be rationalized with free radical-mediated auto oxidation reactions involving alkenes and alcohol sites.

b) Auto-oxidation of ascorbic acid studies reveals that cupric ion known to oxidize ascorbic acid rapidly in to dehydro ascorbic acid and potassium cyanide.

Photolytic conditions: The photo stability testing of drug substances must be evaluated to demonstrate that a light exposure does not result in unacceptable change. Photo stability studies are performed to generate primary degradants of drug substance by exposure to UV or fluorescent conditions. Some recommended conditions for photostability testing are described in ICH guidelines. Samples of drug substance and solid/liquid drug product should be exposed to a minimum of 1.2million l×h and 200W h/m^2 light. The most commonly accepted wavelength of light is in the range of 300– 800 nm to cause the photolytic degradation. The maximum illumination recommended is 6 million l×h. Light stress conditions can induce photo oxidation by free radical mechanism. Functional groups like carbonyls; nitro aromatic, N-oxide, alkenes, aryl chlorides, weak C–H and O–H bonds, sulfides and polyenes are likely to introduce drug photosensitivity.

Thermal conditions: Thermal degradation (e.g., dry heat and wet heat) should be carried out at more strenuous conditions than recommended ICH Q1A accelerated testing conditions. Samples of solid-state drug substances and drug products should be exposed to dry and wet heat. Liquid drug products should be exposed to dry heat. For a shorter period studies may be conducted at higher temperatures. Through the Arrhenius equation the effect of temperature on thermal degradation of a substance can be studied. Where k is specific reaction rate, A is frequency factor, Ea is energy of activation, R is gas constant (1.987cal/deg mol) and T is absolute temperature. Thermal degradation study is carried out at 40-80℃.

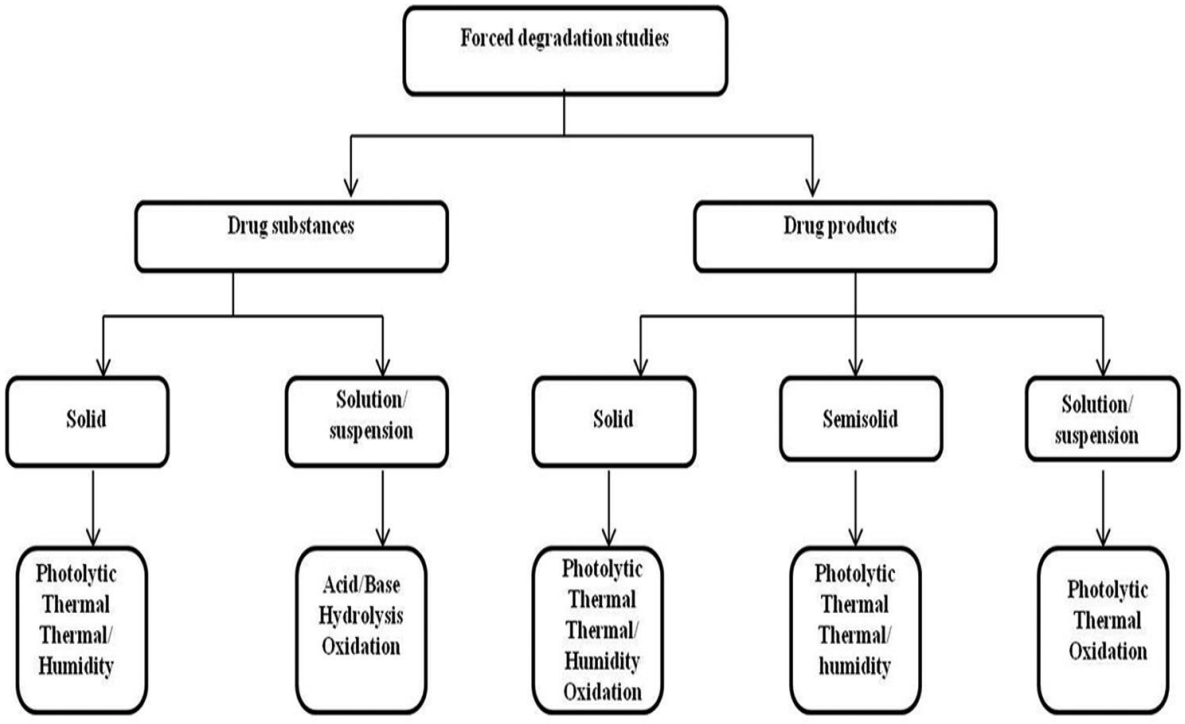

Fig-1-Schematic representation of degradation studies of drugs and drug products under different stress conditions

4.STRESS CONDITIONS[9-13]

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. A generic approach for stress testing has been proposed to achieve purposeful degradation that is predictive of long-term and accelerated storage conditions. The generally recommended degradation varies between 5-20% degradation. This range covers the generally permissible 10% degradation for small molecule pharmaceutical drug products, for which the stability limit is 90%-110% of the label claim. Although there are references in the literature that mention a wider recommended range (e.g., 10-30%), the more extreme stress conditions often provide data that are confounded with secondary degradation products.

Table-2-The four climate zones (ICH Stability guidelines)

Zone I	Temperate	21°C ± 2°C/ 45% RH ± 5% RH
Zone II	Subtropical and Mediterranean	25°C ± 2°C/ 60% RH ± 5% RH
Zone III	Hot and Dry	30°C ± 2°C/ 35% RH ± 5% RH
Zone IV	Hot and Humid	30°C ± 2°C/ 65% RH ± 5% RH

Table-3-Type of study with Storage conditions (ICH Stability guidelines)

Study	Storage condition	Min. time period
Long term	25°C ± 2°C/ 60% RH ± 5% RH or 30°C ± 2°C/ 65% RH ± 5% RH	12 months
Intermediate	30°C ± 2°C/ 65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/ 75% RH ± 5% RH	6 months

Table-4-Drug products intended for storage in refrigerator (ICH Stability guidelines)

Study	Storage condition	Min. time period
Long term	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/ 60% RH ± 5% RH	6 months

Table-5-Drug products intended for storage in freezer

Study	Storage condition	Min. time period
Long term	-20°C ± 5°C	12 months

5.STABILITY INDICATING METHOD[14-18]

Characterization by HPLC

Various methods can be used for the identification and characterization of forced degradation products. Most chosen method of analysis for a stability indicating assay is reverse-phase high-performance liquid chromatography (HPLC). RP-HPLC is chosen because its compatibility with aqueous and organic solutions, high precision, sensitivity and ability to detect polar compounds. Further a prominent method development is done by selecting appropriate column type, column temperature, and making an

adjustment to mobile phase pH. Early elution of highly polar compounds and retention of non polar compounds achieved through gradient elution. Various environmental condition stressed samples can also elute by gradient elution. Solvents and mobile phase should be compatible with the drug substances. Acidic and basic stressed samples should be neutralised before analysis Dilution of samples should be carried out depending on the analytical technique used. The calculation should not include blank. Based on stability results degradation product identification and characterization shall be performed with ICH requirements.

Other analytical methods for developing SIM

Stability-indicating methods will be characterized by potency, purity and biological activity. The selection of tests is product specific. Stability indicating methods may include various methods like electrophoresis (SDS-PAGE, immunoelectrophoresis, Western blot, isoelectro focusing), high-resolution chromatography (e.g., reversed phase chromatography, SEC, gel filtration, ion exchange, and affinity chromatography) and peptide mapping. The analytical method of choice should be sensitive enough to detect impurities at low levels (i.e., 0.05% of the analyte of interest or lower) and the peak responses should fall within the range of detector's linearity. The analytical method should be capable of capturing all the impurities formed during a formal stability study at or below ICH threshold limits. Degradation product identification and characterization are to be performed based on formal stability results in accordance with ICH requirements. Conventional methods (e.g., column chromatography) or hyphenated techniques (e.g., LC–MS, LC–nuclear magnetic resonance (NMR)) can be used in the identification and characterization of the degradation products. Use of these techniques can provide a better insight in to the structure of the impurities that could add to the knowledge space of potential structural alerts for genotoxicity and the control of such impurities with tighter limits. It should be noted that structural characterization of degradation products is necessary for those impurities formed during formal shelf-life stability studies and above the qualification threshold limit. New analytical technologies that are continuously being developed can also be used when it is appropriate to develop stability indicating method. The unknown impurity, which is observed during the analysis, pharmaceutical development, stress studies and formal stability studies of the drug substances and drug product, can be separated and analyzed by using various chromatographic techniques like reversed phase high performance liquid chromatography (RP-HPLC), thin layer chromatography (TLC), gas chromatography (GC), capillary electrophoresis (CE), capillary electrophoresis chromatography (CEC) and supercritical fluid chromatography (SFC). An excellent combination of hyphenated chromatographic and spectroscopic technique such as HPLC- photodiode array ultra violet detector (DAD), LC–MS, LC–NMR and GC–MS are used when degradants cannot be isolated in pure form. HPLC-DAD and LC–MS are used to compare the relative retention time (RRT), UV spectra, mass spectra (MS/MS or MSN).

6.CONCLUSION:

Forced degradation studies provide knowledge about possible degradation pathways and degradation products of the active ingredients and help elucidate the structure of the degradants. A well-designed stress study can provide insight in choosing the appropriate formulation for a proposed product prior to intensive formulation development studies. Forced degradation studies also facilitate the chemical and physical stability analysis of drug substances and drug products. This assists to develop formulation manufacturing conditions, storage conditions and determine the expiry date of a drug formulation. The forced decomposition studies provide a means to estimate the stability of drug stability and aids in the identification of the degradants produced. Degradants produced in forced degradation studies may or may not produce in storage conditions, but they are used to select appropriate storage conditions. It will help to develop SIM.

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