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Review Article

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Comparison of Analytical Parameters Required for Validation Forced Degradation Studies

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Abstract Force degradation studies or stress testing are commonly employed in pharmaceutical industries in order to develop a stability indicating method (SIM). In forced degradation studies, drug substance (API) or a drug product is subjected to various extremities of pH, humidity, hydrolysis and oxidation, photolytic and thermal conditions which in turn produce various degrading products known as degradants. This gives an understanding about the degradation pathways of drug substance and drug products. International Conference on Harmonization (ICH) guidelines Q1A, Q1B, Q2B, Q3A and Q3B regulate the certain conditions of pH, light, oxidation, acid-base hydrolysis, dry heat etc. for carrying out the stress testing. Forced degradation studies exploit the chemical structure of a molecule thereby helping in development of the product formulation, manufacturing and packaging. The studies ensure the appropriate stability of final pharmaceutical products in initial stages of pharmaceutical development. The present review article discusses about various regulatory aspects, methodology for forced degradation studies, various factors affecting the stress decomposition and also about the analytical methods helpful for development of stability indicating method.

Keywords Forced Degradation Studies, Stability Indicating Method, ICH Guidelines, Stress Testing

Introduction

Forced degradation studies are synonymous to forced decomposition studies, stress testing, stress studies and stress decomposition studies [1] which is performed when a few data is available about potential degradation products, in order to demonstrate specificity when developing stability-indicating methods. Force degradation studies also throws light on the degradation pathways as well as degradation products that are likely to be formed during storage. These studies are of much use in pharmaceutical development including formulation development, manufacturing, and packaging, where knowledge of chemical behavior can be used to improve a drug product [2]. It was after the introduction of International Conference on Harmonization (ICH) guideline entitled 'Stability Testing of New Drug Substances and Products' (Q1A) in 1993, whereby forced degradation/ stress studies became a formal regulatory requirement. As per the guideline, stress testing for drug substances was defined as follows- "The studies are performed to elucidate intrinsic stability characteristics. Such testing is part of the development strategy and is normally carried out under more severe conditions than those used for accelerated tests. Stress testing is conducted to provide data on forced decomposition products and decomposition mechanisms for the drug substance. The severe conditions that may be encountered during distribution can be covered by stress testing of definitive batches of drug substance" [3, 4].

To predict the shelf life of the drug substance or product, mainly two types of stability studies are performed. Long term stability studies which are done for duration of 12 months and accelerated stability studies performed for a

period of 6 months. Intermediate stability and controlled room temperature stability studies are also conducted for 6 months at conditions milder than accelerated studies. In force degradation studies, the drug is subjected to various extreme stress conditions of pH, light, oxidation, dry heat, acid-base hydrolysis etc. and therefore, the degradants are formed more quickly as compared to stability studies [5, 6].

Objectives of Forced Degradation Studies

Forced degradation studies are performed for-

- Developing degradation pathways for drug substances and drug products.
- Differentiating products that are generated from non-drug product in a formulation from the degradation products that are related to drug products.
- Elucidating the structure of degradants.
- Identifying the intrinsic stability of a drug substance in formulation.
- Exploiting the degradation mechanisms of the drug substance and drug product (hydrolysis, oxidation, thermolysis or photolysis).
- Indicating the steadiness of a developed methodology.
- Understanding the chemical properties of drug molecules.
- Formulating comparatively stable formulations.
- Developing a degradation profile identical to that mentioned in stability study under ICH conditions.
- Fixing stability-related difficulties [2, 7-9].

Regulatory Guidelines

Various issues related to stress testing are addressed in numerous regulatory guidance documents which provide useful definitions and insights about degradation studies [2].

1. **ICH Guidelines-**

   ICH guidelines that give details about forced degradation studies are ICH Q1A, Q1B, and Q2B, Q3A, Q3B, M4Q (R1).

   a. **ICH Q1A** – testing of stability for new drug molecules and their products.

   This guidelines help in determining the intrinsic stability of drug as well as in designing methods for determining the stability of drugs. ICH Q1A states that the drug degradation is dependent on respective drug molecules and the nature of drug products. The conditions for carrying out the forced decomposition analyses on drug substances and their product are effects of temperature (>50°C), humidity (≥75% relative humidity), oxidation, photolysis, and wide pH range (in case of solution/suspension) [6, 10-13].

   b. **ICH Q1B** - Photostability testing of new drug substances and drug products.

   These guidelines recommend methods to assess the photo stability of drug substances and drug products. Section II and Section III of the guidelines give information about the forced degradation conditions for drug substance and drug product respectively. Forced degradation studies exposure levels are not defined. Photo stability testing is utilized for solid or in solution/suspension. These samples are further helpful to develop a stability indicating method [1, 14, 15].

   c. **ICH Q2B** – validation of analytical procedures: Methodology

   The ICH Q2B guidelines highlight the protocols to be followed for the validation of different analytical protocols. Part II, Section 1.2.2 of ICH Q2B gives explanation on usage of samples for forced degradation studies. As per the guideline, the samples should be subjected to stress under different accelerating circumstances of humidity and heat and further used for the determination of specificity. Specificity is the foundation for determining whether the analytical method is stability indicating or not [16]. Additionally, these guidelines helps in quantitative determination of the degradants produced [6, 14, 17].

d. **ICH Q3A** - impurities in new drug substances
This guideline give details about the determination of impurities and contaminants present in new drug molecules. It highlights different chemical aspects like the identification of impurities and its types, specification of impurities, analytical protocols, and documentation in form of reports. If in a batch of new drug molecule, no impurity is found or if impurity is present in very minute trace amounts then it is considered helpful to ensure safety prospects toward clinical studies [1, 6, 18, 19].

e. **ICH Q3B**- impurities in new products
As per ICH Q3B (R2)2 guideline impurities in new drug products are defined as "degradation products of the drug substance or reaction products of the drug substance with an excipient and/or the container-closure system". Such impurities related to drug substances are generally regarded to be less toxic [20]. Impurities classified under this guideline include impurities from degradation products of the drug substance, from interactions between drug substance and excipients or components of primary packaging materials [21]. ICH Q3B guideline is not applicable for new drug products under the clinical research stages of development [22].

f. **ICH M4Q(R1)** – the common technical document for the registration of pharmaceuticals for human use: Module 3: Quality
Types of studies performed, procedures used, and outcomes of the studies are included in ICH M4Q (R1). Storage conditions, storage life, and the probable date for reassessment are mentioned under this guideline. Results of stability analysis (including analytical procedures along with the validation data) are marked under section 3.2.S.7.3 [6, 10].

Table 1: ICH Guidelines for Stability Study [16]

Q1A(R2)	Stability Testing of New Drug Substances and Products
Q1B	Stability Testing: Photo stability Testing of New Drug Substances and Products.
Q1C	Stability Testing for New Dosage Forms.
Q1D	Bracketing and Matrixing Designs for Stability Testing of Drug Substances and Drug Products.
Q1E	Evaluation of Stability Data.
Q1F	Stability Data Package for Registration in Climatic Zones III and IV
Q3A(R2)	Impurities in New Drug Substances
Q3B(R2)	Impurities in New Drug Products
Q3C(R4)	Impurities: Guideline for Residual Solvents

2. EMA Guidelines

It is a regulation employed in chemistry of active substances. It comprises of type of studies performed, methods used, and the analysis results. The stability testing for API and dosage forms is delineated under the Section 2.1.2. It includes the information about date of re-performing the test and expiry date of substances. Analytical method development, validation of method, degradation pathways, and intrinsic stability are also determined. Performing stability studies for sensitive compounds like hygroscopic and photosensitive drugs is additionally necessary under EMA guidelines [6, 10].

3. FDA Guidelines

Food and Drug Administration provides regulation for photostability analysis of newer drug molecules and their products (Q1B). As per the rules, degradation studies should be performed under normal development conditions. It involves the degradation pathway of samples upon light exposure. Stability Indicating Method

(SIM) is developed on the basis of FDA guidelines. No confirmatory studies are required for degradation products. Section 211.166(a)(3) of regulation focuses on the specificity of a SIM capable of quantifying presence of amount of active ingredient, the kind of degradation products thus obtained with and other components present in dosage form without any interference under stress conditions of pH, temperature, and oxygen [6, 10].

4. **National Health Surveillance Agency (ANVISA)**

Agência Nacional de Vigilância Sanitária (ANVISA) is the National Health Surveillance Agency in Brazil. It highlights the stability requirements and conditions for forced degradation. For protection from risks due to the production and use of various drug products and for promotion of public health, ANVISA was formulated. It includes a variety of detailed documents describing about Forced Degradation Studies and the way they should be designed and managed, and which relevant data should be evaluated [6, 23-25].

Aspects of Forced Degradation Studies

Time to Perform Forced Degradation Studies

According to the FDA guidelines, stress testing should be performed during the phase III of regulatory submission process of new drug substance and new drug product. To work out the stability of the drug substance, stress studies should be carried out in different pH solutions, within the presence of oxygen and light, and at elevated temperatures and humidity levels. These stress studies should be done on a single batch and the results should be summarized and submitted in an annual report. However, it is highly recommended to perform stress testing on a drug substance during pre clinical or phase I clinical trial so that there is sufficient time for identifying degradation products and elucidating the structure as well as optimizing the stress conditions, also there is enough time to improvise manufacturing process and to select analytical procedures for stability testing [7, 17, 19, 26, 27].

FDA encourages forced degradation studies to be performed [2, 17, 19]

A. During pre-IND:

- During formulation Studies: stability indicating quality attributes, degradation routes.
- For pre-clinical studies: degradants, identification of toxic components.

B. During clinical development:

- Comparing pre-clinical to clinical quality
- Comparing pre- to post- manufacturing changes
- In-use stability

C. Post-marketing:

- Identifying new stresses
- Manufacturing changes
- Additional indications

Limits for degradation

Forced degradation are often considered as a key source in drug development process as it helps in understanding the degradation chemistry of drug substances and drug products. This data about the degradation chemistry can be further used to develop stability- indicating analytical methods as well as for formulation and packaging development and the design of the official stability studies. It is generally prescribed to use appropriate conditions to attain 5-20% degradation. However, if degradation is higher than 20%, than it is labeled as abnormal and should be further investigated [1].

Degradation studies of a drug substance

Following should be presented to FDA at the time of registration-

- Stressing the drug substance in solution or suspension at alkaline and acidic pH and under oxidation conditions.
- Subjecting the drug substance to stressing at alkaline and acidic pH and under oxidation conditions.

- Stressing the solid bulk drug substance at temperature and temperature + humidity conditions in excess of accelerated conditions.
- Subjecting the solid bulk drug substance to stressing at temperature and also temperature along with humidity conditions in excess of accelerated conditions.
- Stressing the drug substance photolytically in the solid state or in solution excess.
- Photolytic stressing of the drug substance in the solid state or in excess solution.
- Demonstration of the specificity of stability indicating methods with forced degraded samples. Full characterization of the degraded products by means of NMR, mass spectrometry (MS), UV analysis
- If available, Chemical and physical properties of the degradation products.
- The mechanism and kinetics [1, 28, 29].

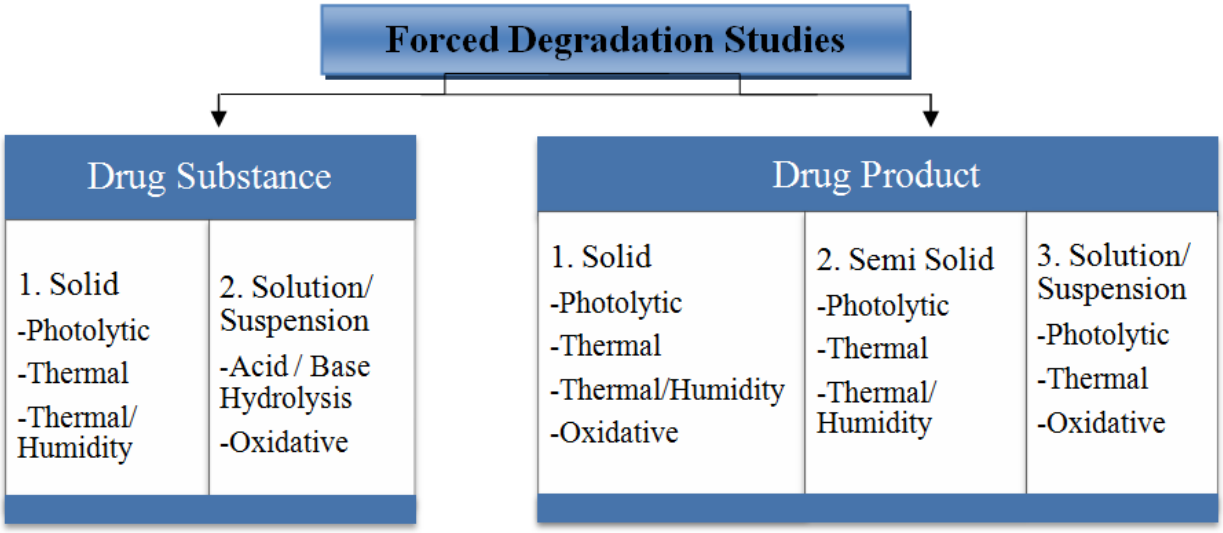
Selection of conditions for Forced Degradation Studies

Due to the complexity of the structure of drug molecules it is time consuming to build the generic set of conditions for a forced degradation study. The chosen conditions for stress should be in compliance with the product's decomposition. The condition selected must include products property and its degradation under normal manufacturing, storage and use conditions [17, 30].

Table 2: Conditions for Forced Degradation Studies [7]

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		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)	25˚C, 60˚C	1,3,5
	AIBN control	25˚C, 60˚C	1,3,5
		25˚C, 60˚C	1,3,5
Photolytic	Light 1 × ICH	NA	1,3,5
	Light 3 × ICH	NA	1,3,5
	Light 3 × ICH	NA	1,3,5
Thermal	Heat chamber	60˚C	1,3,5
	Heat chamber	60˚C /75% RH	1,3,5
	Heat chamber	60˚C	1,3,5
	Heat chamber	60˚C /75% RH	1,3,5

Table 3: Various Stress Conditions for Degradation studies of Drug (substance and product)



Forced Degradation Studies				
Drug Substance		**Drug Product**		
1. Solid	2. Solution/ Suspension	**1.** Solid	**2.** Semi Solid	**3.** Solution/ Suspension
• Photolytic • Thermal • Thermal/ Humidity	• Acid / Base Hydrolysis • Oxidative	• Photolytic • Thermal • Thermal/humidity • Oxidative	• Photolytic • Thermal • Thermal/humidity	• Photolytic • Thermal • Oxidative

Degradation Studies

Force degradation study is usually accomplished by four major degradation mechanisms viz heat, hydrolytic, oxidative, and photolytic degradation. In order to obtain the projected level of degradation it is necessary to settle on appropriate concentration of acid, base, or oxidizing agent and vary the conditions (like temperature) and length of exposure. Over stressing and under stressing of the sample must be avoided so as to control the degradation to a desired level. Over stressing of sample produces secondary degradants which are not visible in shelf life stability studies. Therefore, recommended degradation limit is between 5-20% degradation [31].

1. Hydrolysis:

The most common reaction occurring at the wide pH range (acidic as well as alkaline) is hydrolysis. Hydrolysis is a solvolytic process whereby a drug reacts with water to yield breakdown products with varying chemical compositions. Water in the form of moisture or as solvent is the major contributing factor for degradation of most of the drugs. [30] The catalysis of ionizable functional groups in the molecule forms the core of acidic and basic hydrolysis. Forced degradation of a drug substance occurs upon the interaction of the drug with acid and base to generate primary degradants in the desirable range. Hydrochloric acid or sulphuric acids (0.1-1 M) are considered to be suitable for acid hydrolysis whereas for basic hydrolysis sodium hydroxide or potassium hydroxides (0.1-1M) are suggested [17, 32]. Phenomenon of co-solvency can be used for poorly soluble drugs. However, if the drug does not undergo degradation in any of the conditions, then the drug is subjected to stressing for longer duration of time under stronger concentration of acid/alkali. Whereas in case of complete degradation of drug in initial condition, the

strength of acid/alkali can be reduced with a reduction in reaction temperature. Hydrolysis of most of the drugs relies upon the relative concentration of hydronium and hydroxyl ions, for example- Anatrazole is degraded in basic pH and Doxophylline shows degradation in acidic pH. Therefore, pH at which each drug is optimally stable can be determined [31, 33]. Time period of study should not exceed 7 days. After completion of the study the samples should be neutralized with buffer or acid/base to avoid further decomposition [6]. The analysis should be done as soon as possible after the termination of the test [34].

2. Oxidation

Most of the drugs are auto oxidizers i.e. undergo self oxidation upon normal storage and involving ground state elemental oxidation. Such drugs require free radical initiator to commence the chain reaction [6]. Hydrogen peroxide, trace level of impurities, metal ions and radical initiators like azobisisobutyronitrile (AIBN) are commonly employed for oxidation [1]. Hydrogen peroxide is widely used as an oxidation initiator in the concentration of 3-30% at a temperature not more than 40 ˚C for 2-8 days. The oxidative forced degradation testing is generally carried out in 3% H_2O_2 for 6 hours at room temperature which can be further increased/ decreased accordingly [30, 35]. Still if the drug does not undergo oxidation reaction should be conducted in 10% H_2O_2 for 24 hours. For a drug which not oxidizing in 10% H_2O_2 concentration, more extreme conditions of 30% H_2O_2 for 24 hours may be tried. The drug is said to be "practically stable'' if no degradation is observed even after subjecting the drug to the extreme condition [16]. Oxidative degradation occurs due to transfer of electron to form reactive anions and cations. Amines, sulphides and phenols undergo electron transfer oxidation to give N-oxides, hydroxylamine, sulphones and sulphoxide [36]. The functional group with labile hydrogen like benzylic carbon, allylic carbon, and tertiary carbon or α – positions with respect to hetro atom is susceptible to oxidation to form hydro-peroxides, hydroxide or ketone [37, 38].

3. Photo degradation

To confirm that the light exposure does not affect the drug substance, photo stability is conducted. In photo stability studies, primary degradants of drug substance are produced upon exposure to UV or fluorescent conditions. **[27, 17]** This study becomes an integral part of degradation testing particularly for photo-labile compounds. **[32]** ICHQ1B guidelines lay down the conditions for carrying out photo stability testing of samples (drug substances and products). The degradation is achieved by exposing the samples of drug substance, and solid/liquid drug product to a minimum of 1.2 million lux hours and 200-watt hours per square meter light to a combination of both white and UV light in the wavelength range of 300-800 nm. **[30, 32]** 6 million lux hours is the highest illumination recommended [7]. Photolytic degradation can occur by either of the two mechanisms; non-oxidative or oxidative photolytic reaction [31].

i. Non-oxidative photolytic reaction includes reactions such as dimerization, rearrangements, cyclization, isomerization, decarboxylation and hemolytic cleavage of X-C hetero bonds, N-alkyl bond (dealkylation and deamination), SO_2- C bonds etc.
ii. Oxidative photolytic reaction takes place either through singlet oxygen [$^1O_2$] (reacts with the unsaturated compounds like dienes, alkenes, polynuclear aromatic hydrocarbon to form photo-oxidative degradants) or triplet oxygen [$^3O_2$] (reacts with the free radical present in the drug molecule to form peroxide) mechanisms. **[31]** For example photodegradtion of Barnidipin by formation of singlet oxygen [30].

The samples exposed to photolytic degradation should be analyzed for any changes in appearance, clarity, color of solution, and for assay and degradants [33].

4. Thermal conditions

Several drugs, vitamins, peptides etc are susceptible to degradation at higher temperature i.e. they are thermolabile in nature [6]. The rate of reaction increases with an increase in the temperature which further results in the formation of the degradation products. Thermal stress studies are carried at temperature between 40–80°C. Temperature of 70°C is widely used to carry out thermal stress studies for 1–2 months at low and high humidity. A temperature

above 80°C is not suitable as it may not produce predictive degradation pathway. Solid drugs are exposed to both dry and wet heat conditions, while liquids are subjected only to dry heat for the shorter time periods. Arrhenius equation explains the effect of temperature on thermal degradation of a substance-

$$k = Ae^{-Ea/RT}$$

Where, k is the specific reaction rate, A is the frequency factor, e is the base of natural logarithms, Ea is the energy of activation, R in the universal gas constant (1.987 cal/ deg/mole), and T is the absolute temperature in Kelvin [12, 31, 39, 40].

5. Humidity Conditions

Humidity conditions play an important role in determining the potential degradants in the finished product and active pharmaceutical ingredient. Ideally, 90% humidity for the time span of one week is suggested for the establishment of forced degradation samples [17, 41].

Termination of Study

Stress decomposition studies are discontinued after adequate exposure of drug substance and product to various stress conditions. Activation energy of drug molecules ranges between 12–24 kcal/mol. It is not mandatory that a drug molecule undergoes degradation under every single stress condition. In case if a drug is stable and dose not degrade under any of the above mentioned stress conditions, specificity of an analytical method can be established by spiking the drug substance or placebo with known impurities and establishing adequate separation [33].

Factors Affecting Degradation

i. Moisture - water-soluble substances easily dissolves in presence of moisture causing physic-chemical changes within the molecule.
ii. Excipients – Some excipients contain large amount of water which can further affect the drug stability by increasing water level in formulation. Also drug-excipients chemical interaction can sometimes lead to reduced stability.
iii. Temperature – An increase in temperature is directly proportional to rate of hydrolysis of the drug. Changes in temperature are usually associated with damaging effect on the stability of the drug.
iv. pH - To minimize the effects of pH, the formulations of drugs are prepared in buffer solution at pH showing maximum stability.
v. Oxygen – Availability of oxygen triggers the process of oxidation in some drugs. This can be overcome by purging nitrogen or carbon dioxide in the storage container.
vi. Light – photolabile drugs have the property to decompose when they are exposed to light. Therefore, photolabile compounds should be stored in amber glass containers and should be stored in the dark [6, 42].

Stages of Stability Studies

It is very crucial to perform stability studies at every stage of the drug life cycle beginning from the early stage of product development to late stage follow up studies. There are 6 different stages [42]:

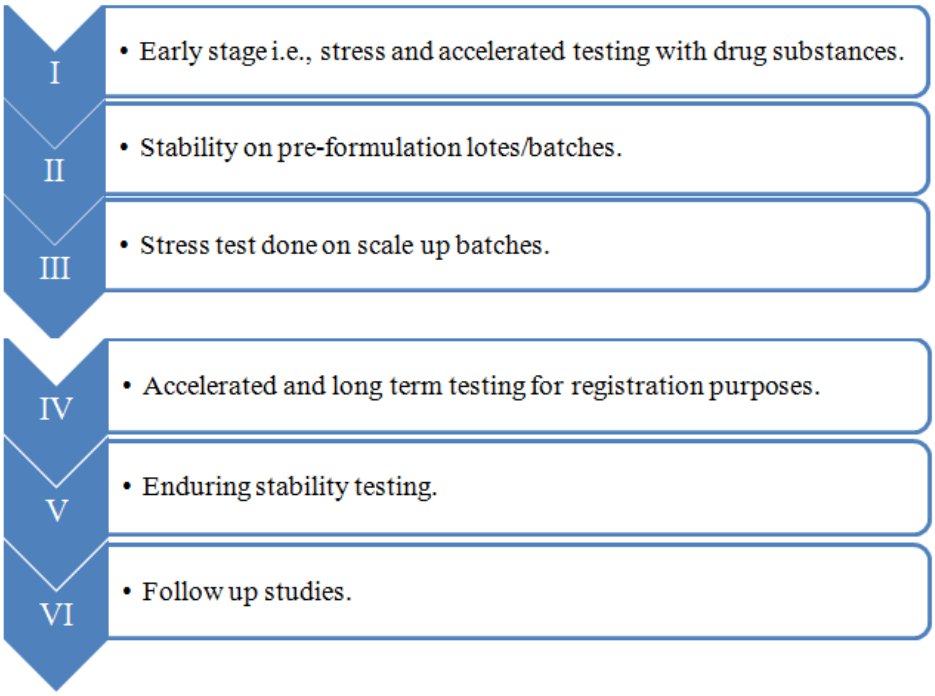


Figure 1: Stages of Stability Studies

Stability Indicating Method (SIM)

Stability indicating method (SIM) is a quantitative method that monitors the relationship between the concentrations of the drug with respect to change in time. It determines the decrease in concentration of drug present and drug product. As the concentration of the drug substance changes during the degradation studies; notably, no interference is observed from the excipients or other degradation products. Force degradation is performed to identify specificity of the developed method to measure the changes in concentration of drug substance when little information is available about potential degradation product [7]. Hence, the SIM helps in preformulation studies and also to predict the storage conditions of the drug [6]. A stability-indicating method is defined as an analytical method that accurately quantitates the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities [43].

The Reverse Phase High Performance Liquid Chromatography (RP-HPLC) coupled with a UV detector is extensively used analytical tool for separation and quantifying the impurities [39]. Sample generation, method development and optimization and method validation are three important steps for development of SIM on HPLC.

Sample Generation

The API which is force degraded at extreme conditions of oxidation, hydrolysis, photolytic and thermal degradation is used as sample for developing stability indicating method. The purpose of carrying out forced degradation of API (in solid state and solution form) is to generate degradation products which are thought to be formed in realistic storage conditions. This sample is further used to develop a SIM [44].

Method Development and Optimization

Prior knowledge about various physiochemical properties of the drug like pKa value, log-P, solubility and absorption maximum is essential, as it forms the basis for developing HPLC method. Selection of mobile phase and solvent depends upon the Log P and solubility whereas pH is dependent on the pKa value of drug [45]. As the separation takes place in aqueous solution, reverse phase column is preferred. Different ratios of methanol, water and acetonitrile are used for mobile phase during initial stages of separation. Based upon the solubility of analyte,

either methanol or acetonitrile is chosen for organic phase. In the beginning, the ratio of water: organic phase is kept 50:50 which can be later modified to obtain good separation. Further, peak symmetry and peak separation can be enhanced by adding a buffer.

In case of selecting Liquid Chromatography–Mass Spectrometry (LC–MS) as a SIM, the selected mobile phase and buffer should be compatible with the Mass Spectrometry, for example triflouro acetic acid and ammonium formate. Separation should be carried out at an optimum temperature of 30^0 C-40^0 C in order to obtain good reproducibility. **[46]** Sometimes, a drug peak hides the impurity or the degradant peak that co-elutes with the drug. In such a case, a peak purity analysis is carried out either by direct analysis using photodiode array (PDA) detection or indirectly by altering chromatographic conditions like mobile phase ratio, column, etc. which affects the peak separation. The method is then optimized for separating closely eluting peaks by changing flow rate, injection volume, column type and mobile phase ratio [7].

Method Validation

USP/ICH guidelines are referred to validate the developed stability indicating method (SIM). Validation is performed for linearity, accuracy, precision, specificity, limit of quantification, limit of detection, ruggedness and robustness of the method. It is required to isolate, identify and quantitate the degradants found to be above identification threshold (usually 0.1%). If the method does not fall within the acceptance criteria for validation, the method is modified and revalidated [32, 47, 48].

Table 4: Comparison of Analytical Parameters Required for Validation [49]

USP General Chapter (1225)	ICH Q2A Guidelines	FDA Reviewer Guidance
Accuracy	Accuracy	Accuracy
Precision	Precision	Precision
No	Repeatability	Repeatability
		Injection Analysis
No	Intermediate Precision	Intermediate Precision
No	No	Reproducibility
Specificity	Specificity	Specificity/ Selectivity
Detection Limit	Detection Limit	Detection Limit
Quantitation Limit	Quantitation Limit	Quantitation Limit
Linearity	Linearity	Linearity
Ruggedness	No	No
Robustness	Robustness	Robustness
System Suitability	System Suitability	System Suitability
		Sample Solution Stability

Analytical Tools for Degradant Separation and Identification

Various methods that can be used for the identification and characterization of forced degradation products.

Table 5: Methods for Identification and Separation of Force Degradation Products [43]

Conventional Techniques	Hyphenated Techniques
Thin Layer Chromatography (TLC)	Gas chromatography-liquid chromatography (GC-MS)
Solid Phase Extraction(SPE)	Liquid Chromatography–Mass Spectrometry (LC–MS)
Accelerated Solvent Extraction (ASE)	Capillary Electrophoresis- Mass Spectrometry (CE-MS)
Flash Chromatography (Low Pressure Liquid Chromatography)	Liquid Chromatography-Nuclear Magnetic Resonance (LC-NMR)
Supercritical Liquid Extraction (SLE)	Liquid chromatography-Fourier Transfer Infrared (LC-FTIR)
Mass Spectrometry (MS)	
Nuclear Magnetic Resonance (NMR)	
High Performance Liquid Chromatography (HPLC)	

Conclusion

Forced degradation studies play a vital role in drug formulation development process. A well designed degradation study is helpful in developing the drug degradation pathway, elucidating the chemical structure of drug molecule as well as in identifying the degradants. Analysis of chemical and physical stability of drug substance and drug product is an important feature of forced degradation studies. This data further facilitates to develop formulation manufacturing conditions, storage conditions and determine the expiry date of a drug formulation. Various ICH guidelines provide deep regulatory insights about the degradation studies. Appropriately designed and well executed forced degradation study would generate a suitable sample for development of stability indicating method.

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