WWW.IJRPR.COM

**International Journal of Research Publication and Reviews**

Journal homepage: www.ijrpr.com ISSN 2582-7421

# Stability Indicating Method Development and Force Degradation Study of Drug: A Review

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**ABSTRACT**

The FDA and ICH recommendations suggest that stability testing data is required. Forced degradation experiments are useful in figuring out the process through which different pharmacological compounds degrade. These experiments can be used to identify the degradant that are created, and provide information on potential degradation pathways and by-products of the active components. The information in this review has been condensed to the greatest extent possible in order to provide knowledge about the various regulatory guidelines available for studies of forced degradation, techniques for carrying out stress studies under various accelerated conditions, stability and degraded products produced under various stress conditions, and the significance of stability studies.

**Key words- Stability testing, Forced degradation, Regulatory guidelines.**

## 1. INTRODUCTION

Chemical stability of drugs is a major concern as it affects drug safety and efficacy. FDA and ICH recommendations state that stability data are needed to understand how the quality of active ingredients and drug products changes over time under the influence of different environmental conditions. [1] Forced degradation, including degradation of drugs and drugs under conditions other than accelerated conditions, assesses the stability of molecules and results in degradation products. [2] Forced degradation or stress testing is used to demonstrate specificity when establishing methods to demonstrate stability, especially when knowledge of possible degradation products is minimal. These investigations also include degradation pathways and potential degradation products that may occur during storage. In areas where understanding of chemical behaviour can be used to improve therapeutic products. Studies on formulation development, manufacturing, packaging, and forced degradation are also useful in drug development. [3] Samples generated by forced decomposition can be used to create stability indication techniques. This technique is later used to analyse samples generated by accelerated and long-term stability studies. [4] Stability assays are techniques used in the pharmaceutical industry to analyse stability samples. The International Conference on Harmonization (ICH) recommendations have made it clearer that the development of stability-indicating testing techniques (SIAM) is a prerequisite. According to the guidelines, forced degradation tests should be performed under various conditions such as pH, light, oxidation and dry heat to separate the drug from the degradation products. This method should enable the analysis of various degradation products. [5] Stability indicator assays are used in the forced degradation analysis of pharmaceuticals and drugs. This is a method that can detect changes in the concentration of active pharmaceutical ingredients (APIs) in pharmaceuticals. [6] Stability studies to clarify the intrinsic stability of drug substances and drug products are called stress studies. [7] According to the 1987 guidelines, stability determination methods are "based on the characteristic structural, chemical or biological properties of each active ingredient in the drug, distinguishing each active ingredient from its degradation products, Active ingredients can be accurately measured for content, and in the 1998 Directive Draft, this definition reads: in that it can detect changes in the chemical, physical, or microbiological properties of drug substances and drug products over time and accurately measure levels of active substances, degradation products, and other constituents of interest without interference. Specific validated quantitative analytical methods. Previous stability labelling techniques have focused on estimating the amount of drug substance present in commercial formulations. Therefore, this category of test methods is called special stability test methods. These techniques estimate the amount of active ingredient in the presence of excipients, degradation products, and other impurities without separating them. [8] Long-term (12 months) and accelerated stability studies are also included in the stability study (6 months). However, mid-term exams (6 months) can be conducted in a friendlier environment than expedited exams. This evaluation provides an indication of the practical capabilities of forced degradation and its use in developing stability indication methods.[4]

## 2. REGULATORY STATUS OF STABILITY-INDICATING ASSAYS

International stability guidelines

1.1. ICH Guidelines

1.2. FDA ( Food and Drug Administration )

1.3. ANVISA (National Health Surveillance Agency)

1.4. EMA Guidelines (European Medicines Agency)

1.5. USP ( US Pharmacopoeia )

1.6. Japanese Pharmacopoeia

### *2.1 CH Guidelines*

The EU, Japan, and the US have incorporated the ICH principles into their legal systems, but other countries also use them. These rules have legal force because they reflect general inspection trends.

Quality, Safety, Efficacy, and Interdisciplinary Guidelines are the titles of these regulations (also known as QSEM). WHO revised these guidelines in 1996 . This is because the ICH guidelines do not take into account the extreme climatic conditions of many countries and in all countries he has not yet used the products distributed by WER, so only new active substances and products have been recorded. Guidance documents develop various test conditions and specifications for active pharmaceutical ingredients (APIs), drug substance, drug products, and excipients. The codes and titles of the stability studies covered by ICH guidelines are listed below.

#### *2.1.1. ICH Q1A (R2): Stability testing of new drug substances and products*

These regulations state that information about new molecular entities and related medicinal products must be included in the registration application. Section 2.1.2 of the Q1A guidelines. Testing of stability for novel medicinal compounds and their products is covered in section ICH Q1A. These recommendations are useful when developing techniques to assess the stability of pharmaceuticals. Degradation is dependent on the individual drug molecules and the makeup of the drug products, claims Q1A. Several accelerated conditions were proposed in order to carry out these forced decomposition analyses on pharmacological compounds and their derivatives. The impacts of oxidation, photolysis, a wide range of pH (solution/suspension), humidity (75% relative humidity), and temperature (>50°C) were those circumstances.

#### *2.1.2. Q1B: Photo stability testing of new drug substances and products*

These techniques are typically employed in the drug development stage to estimate the photo stability of drug molecules. These recommendations offer information on how to evaluate the photo stability of compounds being researched for stability studies. Need for forced degradation of pharmaceuticals and regulatory rules, respectively, both specified forced degradation of drug molecules and their products. In confirmatory research, forced degradation studies are useful for detecting photolytic degradants.

#### *2.1.3. Q1C: Stability testing for new dosage forms*

Following the initial application for novel drug substances and products, the original applicant made a suggestion about the stability of new dosage forms, which is addressed by these guidelines. The idea of stability for a new dosage should be based on the parent stability guideline.

#### *2.1.4. Q1D: Bracketing and matrixing design*

The usage of matrixing instability research and bracketing is suggested in this recommendation. The usage of matrixing instability research and bracketing is suggested in this recommendation. Bracketing is defined as the process of creating a schedule for stability in which only samples are consistently evaluated to the limits of layout variables like strength, container size, or full design filling.

The process of matrixing is the creation of a stability schedule that specifies which subsets of the total number of samples should be examined for all possible factor combinations at a given time point and which subsets should be verified later.

#### *2.1.5. Q1E: Evaluation of stability data*

This guideline for a re-assessment length for the drug substance or drug product shelf life that extends beyond the time period covered by "long-term storage conditions information accessible from the stability research" explains when and how to take extrapolation into consideration.

#### *2.1.6. Q1F: Stability data package for registration applications in climatic Zone III and IV:*

These documents were approved by the ICH Steering Committee in February 2003, and the ICH regions then had to abide by them. The storage specifications for stability testing in climatic zones III (hot and dry) and IV are established by this guideline (hot and humid). It outlines standardised international stability testing procedures in order to increase access to medications by minimising the number of storage situations.

#### *2.1.7. ICH Q2B: validation of analytical procedures:*

The protocols to be followed for the validation of various analytical protocols are detailed in the ICH Q2B guidelines. For further information on using samples for forced deterioration investigations, see ICH Q2B, Part II, section 1.2.2. It highlights that samples should be stressed under various accelerated conditions, such heat and humidity, and then utilised to determine specificity. Additionally, these recommendations are helpful for quantifying the degradants created.

#### *2.1.8. ICH Q3A: impurities in new drug substances:*

The identification of contaminants found in novel pharmacological compounds is described in ICH Q3A guidelines. This section offers information on a variety of topics, including impurity identification, kinds, and specifications, analytical procedures, and report creation. More crucially, it is thought helpful to ensure safety for clinical investigations if contaminants are either completely missing or present in tiny amounts in a batch of a new therapeutic molecule.

#### *2.1.9. ICH Q3B: impurities in new products:*

Information about analytical processes is available in ICH Q3B. Validating particular or non-specific degradation products under diverse stress settings is crucial for analytical procedures.

#### *2.1.10. Q5C: Stability testing of biotechnological/biological products:*

This advice only applies to clearly defined polypeptides and proteins, as well as the products and derivatives that are created using r-DNA technology or that have been separated from bodily fluids, tissues, cell cultures, or other sources.

### *2.2. FDA (Food and Drug Administration)*

In order to analyse the photo stability of newer medication compounds and their products, the FDA is providing recommendations (Q1B). The FDA states that degradation studies should be carried out under typical development circumstances. It protects samples against degradation when samples are exposed to light. These guidelines aid in the development of SIM and also serve to condense validation data, which is useful for confirmatory investigations. According to these guidelines, it is not necessary to conduct confirmatory research on degradation products. A SIM must be extremely specific and be able to measure the amount of active ingredient present, the type of degradation products resulting from this process, and other components present in dosage form without any interference, as per Section 211.166(a)(3). For investigations on forced degradation, stress conditions such as pH, temperature, and oxygen are employed.

### *2.3. ANVISA (National Health Surveillance Agency)*

It discusses about the prerequisites for forced degradation and stability. ANVISA was established to defend against hazards brought on by the manufacture and use of diverse drug products while promoting public health. In order to improve the quality of life for the populace, ANVISA coordinates between states, districts, and municipalities using the guiding principles of the Brazilian Unified Health System.

### *2.4. EMA Guidelines (European Medicines Agency)*

It serves as a general guideline in the chemistry of active compounds. It includes information on the many types of research carried out, the methods employed, and the conclusions drawn from the investigation. Testing for API and dosage forms' stability is discussed in section 2.1.2. It includes

information on the retest and expiration dates for drugs. Degradation pathways, method validation, intrinsic stability, and method development are also determined. Additionally, it mandates the execution of stability studies for delicate substances like photosensitive and hygroscopic drugs.

### *2.5. USP (US Pharmacopoeia)*

These guidelines state that, in the absence of degradation standards or contaminants, the specificity can be calculated by comparing the data to the outcomes of the analytes, which contain the contaminants or degradative products, when using a different procedure under the same accelerated conditions.

### *2.6. Japanese Pharmacopoeia*

It specifies that the suggested method must be precise, able to recognise the analyte in the sample, and able to calculate its concentration. If reference standard impurities are not accessible for comparative investigations, samples will be subjected to stress conditions, and degradation products may be used for further research.

## 3. OBJECTIVES OF FORCED DEGRADATION STUDIES

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

- To assess the intrinsic stability of a drug substance in formulation.
- To identify degradation pathways of drug substances and drug products.
- Understanding the degradation mechanism and how each of these components might catalyse, accelerate, or mediate one or more of the many degradation reactions, such as hydrolysis, oxidation, photolysis (photolysis), or some other undesired conversion of the therapeutic substance or drug product.
- To distinguish degradation products produced by non-drug items in a formulation from those related to drug products.
- Data from stability studies is used to assist commercialization, registration applications, and clinical trials.
- To generate a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions
- To clarify the structure of degradation products
- To generate more stable formulations.
- Process development, design and optimization of manufacturing process.
- Understand the chemical properties of drug molecules
- Define the drug substance's retest period or the drug product's shelf life, together with the proper storage conditions.

## 4. FORCED DEGRADATION STUDY

### *4.1. Time to perform force degradation study*

For new medicinal compounds, forced degradation is essential since it affects the stability and shelf life of the pharmaceuticals [9]. Stress tests should be carried out in Phase III of the regulatory submission procedure, according to FDA guidance. Forced degradation experiments should be performed in various pH solutions, with oxygen and light present, as well as at high temperatures and humidity levels, in order to determine the stability of the pharmacological ingredient. Studies on forced degradation are performed on a single batch. An annual report should be submitted with the summary of the results [4] . These studies are most helpful if carried out initially in early stages of development or phase I clinical trials as this offers timely suggestions for improvements in the production process, ensures there is adequate time for degradation product identification, proper selection of stability indicating method, degradation product identification, and optimization of stress conditions which will help later in the production process.[10]

### *4.2. Requirements*

#### *4.2.1. IND Stage*

In Phase 1 or Phase 2 INDs, reporting of forced degradation research circumstances or outcomes is not necessary. However, to aid in the development of stability indicating methods, early research are advised. To assess the possible chemical behaviour of the active, studies can be done on the API and experimental formulations to look for degradation by thermolysis, hydrolysis, oxidation, and photolysis. The findings of one-time forced degradation experiments should, according to a draught guidance paper, be included in Phase 3 INDs.

#### *4.2.2. NDA Stage*

At the NDA stage, complete studies of the API and DP degradation are required, including the isolation and/or characterisation of important degradation products and a complete written report of the studies' findings.[7]

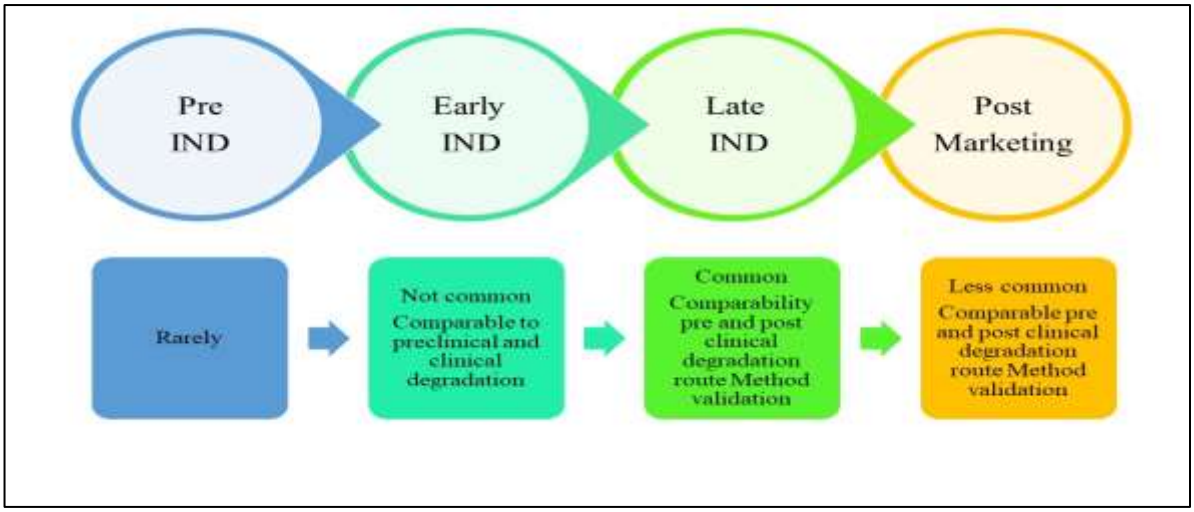


Figure no.1: Forced Degradation Submitted Data

### *4.3. Limit for degradation*

The regulatory authorities' recommendations specify the upper and lower limits for degradation products. For the purpose of validating chromatographic tests, degradation of pharmacological compounds between 5% and 20% has been approved as appropriate. For use in analytical validation for small pharmaceutical compounds, when acceptable stability limitations of 90% of label claim are common, some pharmaceutical professionals believe 10% degradation is ideal.[9,4] In order to make it easier to identify the compounds that are seen during the degradation, spiked samples of a mixture of drug ingredients and known degradation products are typically used to monitor drug product stability. The drug molecule is thought to have degraded if the drug sample exhibits any changes in its physical or chemical makeup, or changes in its activity during the shelf life.

Forced degradation does not necessarily produce a degradation product. If no degradation is observed following exposure to stress conditions other than those listed in the protocol for accelerated stability, the study may be discontinued. This demonstrates the stability of the chemical that is being tested. Under-stressing a sample could result in insufficient degradation products, while over-stressing could cause the development of secondary degradation products that wouldn't be detected in conventional shelf-life stability investigations. Due to variations in materials and concentrations, the protocols for producing product-related degradation for drug substance and drug product may be different. It is advised that stressed samples be provided for method development for stress testing in solution for a maximum of 14 days (or for oxidative tests, for a maximum of 24 hours).

### *4.4. Strategy for selection of degradation conditions*

The complexity of drug molecules' structural makeup makes it challenging to provide a standard set of circumstances for a forced degradation study. The specified stress conditions ought to be consistent with the product's breakdown. The specified condition should take into account the

product's characteristics as well as how they change during typical production, storage, and use processes. Acid and base hydrolysis, heat degradation, photolysis, oxidation, and possibly freeze-thaw cycles and shear are forced degradation variables that are required.[11]

How much do you want to deteriorate a material, is the initial query. In reality, aim for an acceptable sample with a broad deterioration range of 2 to 30%. For a stability indicating assay, use the sample for method development or method evaluation. To prevent secondary degradation, the high end is the intended aim. Can go further if the degradation pattern is straightforward—that is, if only one or two peaks occur. If significant non-specific degradation is seen with several peaks, many must descend further or may draw the conclusion that the material is extremely stable under the specified stress setting.[10]

The pH, temperature, and particular oxidising agents to be utilised are not specified in the regulatory standards. Although Q1B states that the light source should generate both visible and ultraviolet (UV, 320-400 nm) wavelengths and that exposure levels should be justified, the design of photolysis experiments is left to the applicant's decision. The initial experiment should seek to identify the circumstances that cause the medication to degrade by about 10%. Table 2 lists a few situations that are frequently utilised for forced degradation research.[4] But other researcher have discovered that it is practicable to start under harsh conditions (80°C or even higher, 0.5N NaOH, 0.5N HCl, 3% H2O2) and test at shorter (2, 5, 8, and 24 hrs, etc.) numerous time points, allowing for a rough assessment of degradation rates. Early testing may allow for the separation of primary degradants from their secondary degradation products. Better identification of the degradation route is made possible by this method. When formulations or procedures are altered, studies should be conducted again because this could result in the creation of novel degradation products.

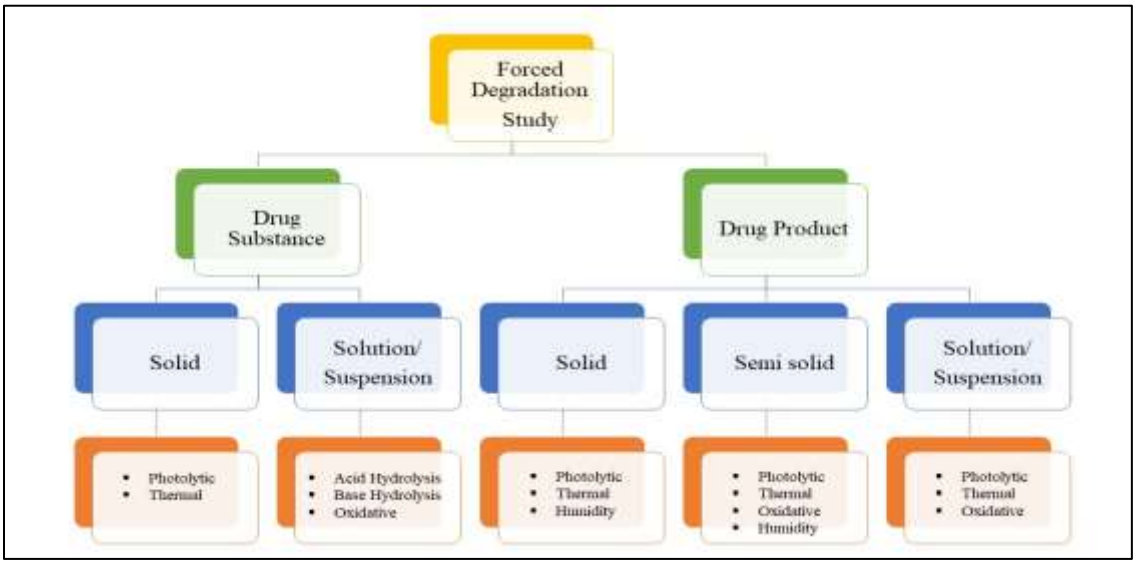


Figure no. 2 : Schematic representation of degradation studies of drug substance and drug products under different stress conditions.

### *4.5. Selection of drug concentration*

The regulatory guideline is silent on the drug concentration that should be employed for the degradation research. The trials should begin with a concentration of 1 mg/mL, as advised. It is typically possible to identify even small amounts of breakdown products when employing a drug concentration of 1 mg/mL. It is advised that some investigations on drug degradation be conducted at a concentration where the drug is anticipated to be present in finished formulations.

### *4.6. Degradation conditions*

#### *4.6.1. Hydrolytic Conditions*

One of the most frequent chemical processes for deterioration across a broad pH range is hydrolysis. In the chemical process of hydrolysis, a chemical substance is broken down by interaction with water. Ionisable functional groups within the molecule are catalysed during a hydrolytic research in acidic and basic conditions. [4] The type and quantity of acid or base to be taken should be chosen based on how stable the drug component is. For base hydrolysis, sodium hydroxide or potassium hydroxides (0.1-1 M) are recommended, but hydrochloric acid or sulphuric acid (0.1-1 M) is assumed to be the most suited. [11]

By refluxing the drug in 0.1 N HCl/0.1 N NaOH, the hydrolytic breakdown of a new drug in acidic and alkaline conditions can be investigated. If reasonable deterioration is observed, testing can be stopped at this point. However, if under these circumstances no degradation is observed, the medicine should be refluxed in a stronger acid or alkali for a longer period of time. Alternately, acid/alkali strength can be decreased with a decrease in reaction temperature if 100% degradation is observed after exposing the medications to the first environment. [12]

Co-solvents can be used to dissolve compounds for stress testing in HCl or NaOH if they are not easily dissolved in water. The structure of the drug substance is used to guide the choice of co-solvent. Normal stress testing trials begin at room temperature, and if no degradation occurs, increased temperature (50–70°C) is next applied. A maximum of seven days should be allowed for stress testing. To stop further degradation, the degraded sample is subsequently neutralised with the appropriate acid, base, or buffer. [4]

**Table 1. Conditions used for stability studies**

| Degradation type | Experimental conditions | Storage Conditions | Sampling time (days) |
|---|---|---|---|
| **Hydrolysis** | Control API ( No acid or base) | 40°C, 60°C | 1,3,5 |
| | 0.1 M 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 |

### *4.6.2. Oxidative conditions*

Studies of forced degradation used to assess a compound's oxidative degradation resistance. Autoxidation is a free radical reaction that requires free radical initiator to begin the chain reaction. The majority of medication ingredients are discovered to be auto oxidizers. Initiators of free radicals are needed for the oxidation process. In stress studies, hydrogen peroxide is frequently used to oxidise drug substances, but other oxidising agents can also be used, including metal ions, oxygen, and radical initiators like 2,2-Azobisisobutyronitrile, ACVA (Azobiscyan-valeric acid), and AMPD (Azobis methyl propionamide hydrochloride). According to reports, exposing the solutions to 0.1%–3% hydrogen peroxide for seven days at neutral pH and room temperature, or up to a maximum 20% degradation, may produce the correct degradation products. [4] An electron transfer process is used in the oxidative breakdown of medicinal substances to create reactive anions and cations. An electron transfer process is used in the oxidative breakdown of medicinal substances to create reactive anions and cations. [13]

#### *4.6.3. Photolytic conditions*

Drug molecules may undergo photolytic breakdown when exposed to light. The amount of light absorbed by the drug molecule and the intensity of incident light both affect how quickly photo degradation occurs. By exposing the drug material or drug product to both visible and UV light, photolytic degradation is carried out. Both oxidative and non-oxidative photolytic reactions can lead in photolytic breakdown. [13] Photo stability tests are carried out to produce the drug substance's core degradants by exposing it to ultraviolet or fluorescent light. According to the ICH Q1B protocols, the photolytic tests should be conducted by exposure to light using either a mix of cool white and ultraviolet fluorescent lamps or one of the xenon and metal halide lamps. Exposure energy needs to be at least 200W/m2 of UV and 1.2 million lux/h of fluorescent light, and if breakdown isn't evident, the intensity needs to be increased five times. The medication can be classified as photostable if there is still no sign of degradation. [7] The most generally accepted wavelength of light to produce photolytic deterioration is between 300 and 800 nm. The suggested maximum illumination is 6 million lux h. By using a free radical pathway, light stress conditions can cause photo oxidation. [4]

#### *4.6.4. Neutral condition*

Neutral hydrolysis is carried out by refluxing the drug in water for 12 hrs. If no degradation is found then reflux time should be increases and if total degradation is seen then time and temperature of the study should decreases.

#### *4.6.5. Thermal conditions*

Thermal deterioration should be tested under more demanding circumstances than those suggested by ICH Q1A accelerated testing (for example, dry heat and wet heat). Different processes, including pyrolysis, hydrolysis, decarboxylation, isomerization, rearrangement, and polymerization, are involved in thermal degradation. [14] Samples of dry and wet heat should be applied to solid-state drug ingredients and drug products. Drug products in liquid form should be exposed to dry heat. Studies may be carried out at higher temperatures for a shorter time. At temperatures between 40 and 80 degrees Celsius, these studies should be carried out. Thermal stress tests are often carried out at 70 °C and high humidity over a period of 1-2 months. [9]

Effect of temperature on thermal degradation of a substance is studied through Arrhenius equation:

K= Ae-Ea/RT

Where k is specific reaction rate, A is frequency factor, Ea is energy of activation, R is gas constant (1.987 cal/ deg mole) and T is absolute temperature. [12]

The drug powder should be heated in an oven to a higher temperature for the dry heat degradation, and the drug solution should be refluxed for many hours for the wet heat degradation.

### *4.7. Factors affecting degradation*

The various factors that lead to drug substance degradation are listed below.

As follows: [12]

#### *4.7.1. Temperature-*

Temperature variations occasionally have a negative impact on the drug's stability. The rate at which drugs hydrolyse typically increases as the temperature rises.

#### *4.7.2. Moisture-*

Water-soluble compounds may dissolve in the presence of moisture. This causes modifications to the molecule's physical and chemical properties.

#### *4.7.3. pH-*

The pace at which medicines are hydrolysed has a substantial impact on pH. Utilizing buffer solutions with the highest degree of pH stability during drug formulation assists in reducing this effect.

#### *4.7.4. Excipients-*

It was noted that some excipients might have a high water content. This moisture could result in a higher water content in the formulation, which would then impact the drug's stability. In some circumstances, poor stability is a result of chemical interactions between the excipients and the drug itself.

#### *4.7.5. Light-*

Some drugs are photo labile, meaning they break down when exposed to light. By contrasting a substance's stability in the presence of light and stability when stored in the dark, the sensitivity to photolytic degradation can be evaluated. It is important to keep in mind that the photolabile compounds should be kept in dark, amber glass containers.

#### *4.7.6. Oxygen-*

Some medications' oxidation is accelerated by the presence of oxygen. Purging nitrogen or carbon dioxide from the storage container stabilises medications whose rate of breakdown is accelerated in the presence of oxygen.

## 5. STABILITY INDICATING ASSAY METHOD

An analytical technique called a stability indicating method (SIM) is used to quantify the reduction of active pharmaceutical ingredient (API) in a drug product as a result of deterioration. A stability-indicating method is "a validated quantitative analytical approach that can detect the changes with time in the pertinent properties of the drug substance and drug product," according to an FDA guidance paper.[15] Without the influence of additional degradation products, contaminants, or excipients, a stability-indicating approach accurately assesses changes in the concentration of the active components. Stress tests are conducted to show the developed method's specificity in measuring changes in drug substance concentration when knowledge of possible degradation products is limited. The pre-formulation studies, stability studies, and creation of appropriate storage requirements are all supported by the development of an appropriate stability indicating method. [4]

### *5.1. Preparation of sample*

A crucial step in the stability indicating assay procedure is the appropriate sample preparation. The choice of the approach depends heavily on understanding the drug's structure and the process by which it degrades. Stress testing is performed on the prepared sample, thus knowledge of its based products, degradation profile, and associated contaminants from earlier samples will aid in the development of an effective stability indicating assay method. [8] The API is forcedly degraded at conditions that are more severe than accelerated degradation settings in order to provide samples for the stability indicating assay procedure. It involves drug degradation under the previously mentioned hydrolytic, oxidative, photolytic, and thermal conditions. In order to produce degradation products that are expected to occur under actual storage conditions, forced degradation of API in solid state and solution form is conducted. Then, a SIM is developed using this sample. [4]

### *5.2. Method selection*

The choice of method is based on the method's selectivity and specificity, or how sensitively it can evaluate the supplied material. The choice of technique is based on a thorough review of the literature for a sample that is likely to be used, on which methods have already been created. It depends on the technique's capacity to distinguish between the drug's active pharmaceutical ingredients (API) and its degraded result, as well as any related contaminants. [8]

### *5.3. Method development and optimization*

The initial stage in creating a technique is to ascertain the pKa value, log P, solubility, and max of the relevant medication. It is usual practise for the separation of pharmaceuticals to develop a reverse phase approach employing HPLC.[9] For the first stages of separation, mobile phase made up of acetonitrile, water, and methanol can be utilised in a variety of ratios. Based on the analyte's solubility, methanol or acetonitrile should be chosen for the organic phase. In order to achieve a satisfactory separation of peaks, the water: organic phase ratio can initially be set at 50:50 and relevant adjustments can be made as trials advance.[4] The process of developing a method find a certain column and a mobile phase that can effectively separate the active component and its related product. Developing a method may involve a trial-and-error process to improve the parameters and produce successful separation.[8] If the degrades peak is seen while the drug peak's area under the curve and its percentage are unaffected, the method established is regarded as homogenous. It can be determined that a drug peak is homogenous if the area percentage of the drug peak and

degradant peaks stay constant. If it is discovered that the co-eluting degradant was not created under accelerated and long-term storage circumstances, it would be acceptable. Then, by adjusting the flow rate, injection volume, column type, and mobile phase ratio, the procedure is made to work best for isolating closely eluting peaks. The method created for the study will be put through validation in accordance with ICH criteria when these parameters have been optimised.

### *5.4. Method validation*

The ICH principles are followed when validating a method. Each newly developed unique approach has its accuracy, precision, linearity, LOQ, LOD, robustness, and ruggedness evaluated. RSD value should be under 2% in accordance with ICH guidelines. [8] The degradants that are discovered to be above the identification threshold (about 0.1%) must be isolated, identified, and quantitated. The method is updated and revalidated if it does not meet the validation's acceptance requirements.

### *5.5. Analytical Methods used for developing SIM*

Potency, purity, and biological activity will be the defining characteristics of stability-indicating procedures. The development of the SIM is made simpler by improvements in analytical instrument methods.[8] Previous research have demonstrated that even at very low concentrations, a variety of analytical techniques are available to isolate, recognise, and characterise the impurities created in the degradation experiments.[9] Reverse-phase high-performance liquid chromatography is the most popular method of analysis for a stability indicator assay (HPLC). Due to its compatibility with both aqueous and organic solutions, high precision, sensitivity, and capacity to identify polar molecules, RP-HPLC was chosen. Additionally, a well-known approach is developed by choosing the proper column type, column temperature, and modifying the pH of the mobile phase.[11] Stability indicating techniques can include a wide range of techniques, such as electrophoresis (SDS-PAGE, immunoelectrophoretic, Western blot, isoelectrofoccusing), high-resolution chromatography (e.g., reversed phase chromatography, SEC, gel filtration, ion exchange, and affinity chromatography), peptide mapping, TLC, HPLC-DAD, HPLC-UV, HPTLC, HPLC-MS, LC-MS/MS, LC-NMR.[4] According to ICH criteria, degradation product identification and characterisation must be done using formal stability results. The identification and characterisation of the degradation products can be done using conventional techniques (like column chromatography) or hyphenated ones (like LC-MS, LC-NMR). These methods can help shed more light on the impurity's structure, expanding our understanding of potential structural alerts for genotoxicity and enabling stricter controls on such impurities. [1]

In cases where drug related impurities cannot be isolated in its purest form, a great variety of hyphenated chromatographic and spectroscopic techniques, including HPLC-DAD (High Performance Liquid Chromatography- Photodiode Array ultraviolet Detector), LC-MS (Liquid Chromatography-Mass Spectrometry), LC-NMR (Liquid Chromatography-Nuclear Magnetic Resonance), and GCMS (Gas Chromatography. The RRT (relative retention time), UV spectra, and mass spectra (MS/MS or MSN) are compared using HPLC-DAD and LC-MS. [14]

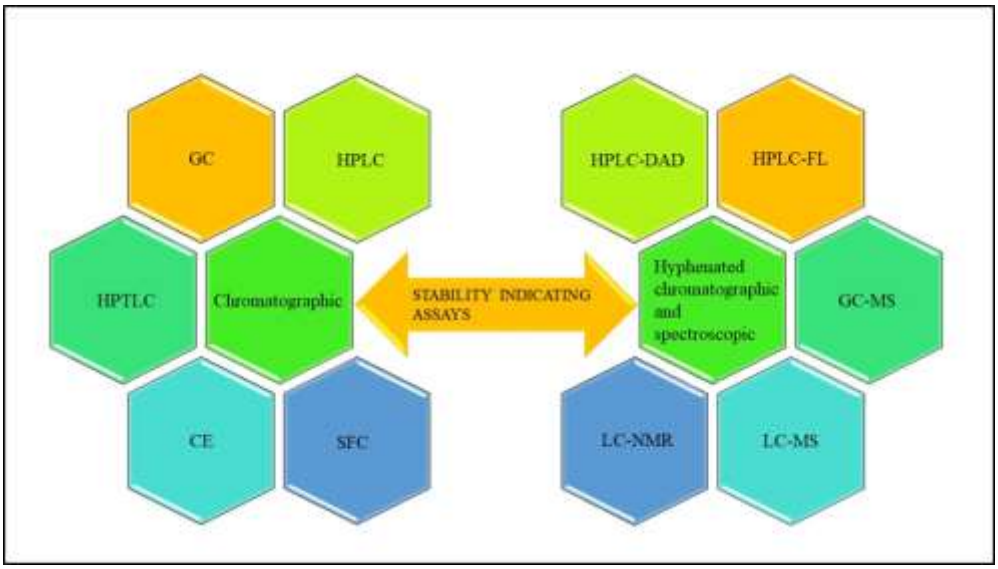


**Figure no. 1 Stability indicating assays for pharmaceuticals**

## 6. RELATION BETWEEN FORCED DEGRADATION AND STABILITY

In comparison to standard stability testing, more items are created in forced deterioration investigations. Due to their limited potential, actual degradation products can be challenging to find during stability testing. Studies on forced deterioration reduce this issue from this viewpoint. If no degradation products were created, the medicinal ingredient is thought to be stable under the specified stress conditions, thus the protocol can be seen as a stability signalling method. The correct storage conditions for certain medications can also be studied with the aid of forced degradation analysis. More importantly, forced degradation experiments are helpful in figuring out the process through which different pharmacological compounds degrade. [9]

## 7. CONCLUSION

The forced decomposition experiments offer a tool to assess a drug's stability, help identify the degradants that are created, and provide information on potential degradation pathways and by-products of the active components. They also help explain the structure of the degradants. In order to create stability-indicating and degradant-monitoring methodologies as part of a validation programme, forced degradation experiments are essential. This information is generally utilised to create stability-indicating analytical techniques, but it can also be used to create formulations, packaging, and formal stability studies. Any approach used to promote degradation has the goal of causing the desired quantity, between 5% and 20%. An appropriate sample for the creation of a stability indicating method would be produced by a forced degradation study that was adequately planned and carried out.

The information in this review has been condensed to the greatest extent possible in order to provide knowledge about the various regulatory guidelines available for studies of forced degradation, techniques for carrying out stress studies under various accelerated conditions, stability and degradants produced under various stress conditions, and the significance of stability studies.

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