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# STABILITY INDICATING METHOD USING FORCED DEGRADATION STUDY: A REVIEW

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## Abstract:

Forced degradation studies play a crucial role in assessing the chemical stability of pharmaceuticals and identifying potential degradation pathways. These studies involve subjecting drug substances or drug products to various stress conditions, including temperature, humidity, light, oxidation, and acid/base hydrolysis, to accelerate degradation and evaluate the inherent stability of the compounds. In this review, we present a comprehensive analysis of forced degradation studies conducted in the pharmaceutical industry, highlighting their importance in the development and quality control of drugs. The primary objective of forced degradation studies is to simulate and predict the degradation pathways and degradation products that may arise during the drug's shelf-life or under specific storage conditions. By subjecting the drug molecules to stress conditions, potential degradation impurities can be identified and characterized using sophisticated analytical techniques. This review covers the fundamental aspects of forced degradation studies, including the selection of appropriate stress conditions, sample preparation techniques, and analytical methodologies for impurity profiling. We discuss the factors influencing degradation, such as pH, temperature, humidity, and light exposure, and their impact on the stability of drugs. Furthermore, we delve into the regulatory requirements and guidelines governing forced degradation studies, ensuring that pharmaceutical companies comply with the established standards and produce safe and effective drugs. The significance of forced degradation studies extends beyond stability assessment, as they aid in elucidating the chemical mechanisms involved in degradation processes and provide insights into formulation development and packaging design. Additionally, the identification and quantification of degradation impurities facilitate the establishment of impurity acceptance criteria, ensuring product quality and patient safety.

**Keywords:** Forced degradation study, stability indicating method, stress conditions, impurities, stability

## Introduction to forced degradation study:

A forced degradation study is a crucial component of pharmaceutical drug development and analysis. To speed up the degrading process, it entails exposing a drug substance or drug product to a variety of stress conditions, including temperature, humidity, light, pH, oxidation, and hydrolysis. This study's objectives are to evaluate the drug's inherent stability and to pinpoint the degradation products that could arise in certain situations [1, 2].

Forced degradation/deterioration studies, sometimes called stress testing, are a crucial part of pharmaceutical development and stability testing. These studies include subjecting the active form of substance or finished goods to several stress conditions to accelerate the degradation process and evaluate the stability characteristics of the drug [3].

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The goal of investigations on forced deterioration studies is to identify and characterize the degradation pathways, degradation products, and potential impurities that may form over time in the active form of substance or finished goods. These studies help establish the stability-indicating nature of the analytical method and provide information on the drug's inherent stability and susceptibility to degradation [4].

The primary objectives of a forced degradation study are as follows: Degradation pathway determination: By subjecting the drug to different stress conditions, the study helps determine the potential degradation pathways of the drug molecule. This information is essential for understanding the drug's stability and developing appropriate storage and handling guidelines [5]. Identification of degradation products: Forced degradation exposes the drug to conditions that promote degradation, resulting in the creation of degradation products. Analytical techniques such as chromatography, spectroscopy, and mass spectrometry are used to identify and characterize these degradation products. This information aids in assessing the drug's safety and efficacy and supports the establishment of appropriate specifications for impurities [6]. Creating stability-detecting techniques: The creation of analytical techniques that indicate stability is made possible by forced degradation research. These methods should be capable of separating and quantifying the drug's degradation products and should be specific, sensitive, precise, and accurate. Stability-indicating methods are essential for monitoring the drug's stability during formulation, manufacturing, and storage [7]. Assessment of drug product shelf-life: By monitoring the degradation process under accelerated conditions, forced degradation studies help estimate the drug product's shelf-life. The creation of stability-indicating analytical techniques is made possible by forced degradation research [8]. Regulatory requirements: Regulatory authorities, such as the United States Food and Drug Administration (FDA) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), require forced degradation studies as part of the drug approval process. Critical information from these researches supports the drug's safety, effectiveness, and quality. In summary, forced degradation studies play a vital role in drug development and analysis by providing insights into a drug's stability, identifying degradation products, developing stability-indicating methods, estimating shelf-life, and meeting regulatory requirements [9].

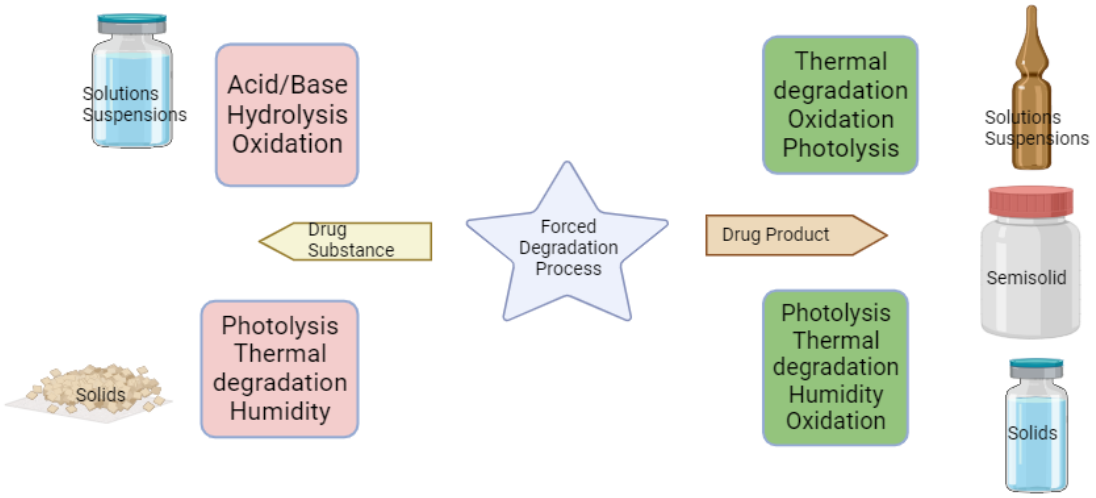


**Figure 1: Diagram showing Forced Degradation Process for Drug Substance and Drug product**

**Key steps involved in conducting forced degradation studies:**

1. Selection of Stress Conditions: Determine the stress conditions based on regulatory guidelines and the drug's known degradation mechanisms. The commonly employed stress conditions include:

ISSN 2515-8260 Volume 09 Issue 09, 2022

a. Hydrolysis: Expose the active form of substance or finished goods to acidic and alkaline ambience to simulate degradation caused by hydrolysis.
b. Oxidation: Subject the drug to oxidative stress using hydrogen peroxide, peroxides, or oxidizing agents to mimic oxidation-induced degradation.
c. Photolysis: Expose the drug to UV light or direct sunlight to evaluate its susceptibility to light-induced degradation.
d. Thermal Degradation: Heat the active pharmaceutical ingredient or finished goods to high temperatures to assess thermal stability.
2. Experimental Setup: Prepare stress samples by revealing the active form of the substance or finished goods to the selected stress conditions. Control samples (untreated) should also be prepared for comparison. Consider using different stress durations and stress levels to evaluate the degradation kinetics.
3. Analytical Method: Select a suitable analytical method (such as RP-HPLC, GC, or LC-MS) capable of separating and quantifying the drug substance, degradation products, and potential impurities. The method should be stability-indicating and be disposed to differentiate between the active form of the substance and its deterioration products.
4. Sample Analysis: Analyse the stress samples using the chosen analytical method. Monitor the changes in chromatographic peaks, retention times, and peak areas to identify degradation products and impurities. Compare the stressed samples with the control samples to assess the drug's stability.
5. Degradation Product Characterization: Isolate and identify the degradation products using complementary methodology like mass spectrometry (MS), nuclear magnetic resonance (NMR), and infrared spectroscopy (IR). Determine the structures of the deterioration products to understand the deterioration pathways and potential impurities formed during stress.
6. Reporting: Document the results of forced degradation studies, including the stress conditions employed, observed degradation products, and their structures. Summarize the degradation pathways and provide a comprehensive assessment of the drug's stability under different stress conditions.
Forced degradation studies play a critical role in determining the drug's stability profile, defining appropriate storage conditions, establishing shelf-life, and supporting regulatory submissions. These studies help ensure the safety, efficacy, also quality of pharmaceutical products throughout their shelf life [5-9].

**Guidelines for forced deteriorations study:**

Investigations into forced deteriorations are conducted to assess the stability and degradation pathways of a drug ingredient or drug item under various stress conditions. These studies help in identifying potentially harmful byproducts and determining the consistency of the molecule. Here are some general guidelines for conducting a forced degradation study: Selection of stress conditions: Choose appropriate stress conditions that are likely to cause degradation, such as temperature, humidity, light, pH, oxidation, and hydrolysis. The stress conditions must be applicable to the drug chemical or medicinal product and mimic potential real-world storage and use conditions. Sample preparation: Prepare samples of the drug ingredient or drug item at a concentration suitable for analysis. Use representative batches and ensure that the samples are homogeneous and free from impurities. Stress conditions and duration: Apply the selected stress conditions to the samples. The duration of the stress study may vary depending on the nature of the molecule and the anticipated degradation rates. Common stress conditions include exposure to elevated temperatures, humidity, light, and acidic or basic pH for specified time intervals [9, 10].
Control samples: Include control samples that are subjected to normal storage conditions and not exposed to stress conditions. These samples serve as a baseline for comparison and help in identifying degradation that occurs under accelerated stress conditions. Analysis of degradation products: Analyze the stressed samples for the presence of degradation products using appropriate

analytical methods like chromatography (HPLC, GC), spectroscopy (IR, NMR, UV-Vis), mass spectrometry, or any other suitable method. Quantify the degradation products and compare them with the control sample. Forced degradation study reporting: Document the experimental details, including stress conditions, sample preparation, analysis methods, and results obtained. Present the findings in a clear and concise manner, highlighting the identified degradation pathways and products. Include any conclusions or recommendations based on the results obtained. Impurity profiling: Perform impurity profiling of the stressed samples to identify and characterize any impurities formed during the degradation process. This aids in evaluating the drug substance's or product's quality and safety [11-13].

Stability indicating assay: Develop an assay for determining stability method that can separate the drug substance from its degradation products. This assay should be able to accurately determine the concentration of the drug ingredient in the existence of degradation products. Validation: Validate the analytical techniques used for degradation product analysis and stability-indicating assays as per relevant guidelines or regulatory requirements. Regulatory considerations: If the forced degradation study is being conducted for regulatory purposes, make sure it complies with any applicable regulatory bodies, including the United States Pharmacopoeia (USP) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). Remember, these guidelines provide a general framework, and the specific requirements for a forced degradation study may vary based on the nature of the drug substance or product and the regulatory environment [12-14].

**Stability-Indicating RP-HPLC method**:

A stability-indicating RP-HPLC (Reverse Phase High-Performance Liquid Chromatography) technique is an approach used in pharmaceutical analysis to govern the stability of a drug or pharmaceutical product over time. The method is designed to separate and quantify the essential component of a drug (API) and its associated impurities, degradation products, and potential contaminants. Additionally, it aids in assessing the stability and degradation products of a drug substance or drug product over time. It is a crucial tool in drug development and quality control processes [13-15].

The primary objective of a stability-indicating technique is to separate and quantify the active pharmaceutical ingredient (API) in the presence of its degradation products, impurities, and other potential matrix components. This method ensures that any changes in the drug's chemical structure or purity can be accurately detected and monitored during stability studies or routine quality control testing [16].

Sample Preparation: Prepare the test sample by accurately weighing the drug substance or drug product. Steps for sample extraction or dilution should be carried out, if necessary.

Mobile Phase Selection: Choose an appropriate mobile phase that provides good chromatographic separation for the drug and its impurities. The mobile phase composition typically consists of a mixture of organic solvent(s) and buffer solution. The pH of the buffer can be adjusted based on the drug's stability and solubility requirements.

Column Selection: Select a suitable RP-HPLC column based on the medication's physicochemical characteristics and the desired separation. The column should provide good resolution and peak shape for the analytes of interest.

Method Optimization: Optimize the chromatographic conditions by modifying the gradient elution programme, injection volume, column temperature, and flow rate, among other variables. The goal is to achieve a robust method that provides well-separated peaks for the analytes and meets the required system suitability criteria.

Forced Deterioration Studies: Perform forced deterioration studies on the active form of substance or finished goods to induce degradation under different stress conditions (e.g., acidic, basic,

oxidative, thermal, photolytic). This helps identify the degradation products and potential impurities that may be formed during the drug's shelf life.
Chromatographic Separation: Develop a chromatographic method that can effectively separate the active form of the substance, its adulteration, and degradation compounds. Optimize the separation by adjusting the mobile phase composition, pH, gradient program, and column temperature.
Method Validation: Validate the stability-indicating RP-HPLC technique as per relevant regulatory guidelines. This involves determining parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness.
Sample Analysis: Analyze the drug samples collected from stability studies or routine quality control tests using the validated stability-indicating method. Quantify the active form of substance, adulteration, and degradation components present in the sample.
Data Analysis: Evaluate the data obtained from sample analysis to assess the drug's stability over time. Compare the levels of impurities and degradation products at different time points and storage conditions. Calculate degradation kinetics if required.
Reporting: Prepare a comprehensive report summarizing the stability-indicating method, validation results, and stability data. The report should include information on the drug's degradation pathways, degradation products, and any observed trends in stability.
It is important to note that the construction of a stability-indicating RP-HPLC technique requires expertise in analytical chemistry, chromatography, and pharmaceutical analysis. The method should be robust, sensitive, and capable of accurately quantifying the drug and its impurities even under stressed conditions. Stability Indicating RP-HPLC methods are vital in the pharmaceutical industry for making sure that pharmaceutical medicines are safe, effective, and of high quality during their entire shelf life. These methods enable accurate determination of the drug's stability profile and the identification of potential degradation pathways, thus aiding in formulation development, manufacturing process optimization, and regulatory compliance [15-17].

**Quantification of impurities generated in forced degradation studies:**

When studying forced deterioration, the goal is to assess the durability of an active pharmaceutical ingredient or finished goods under different stress situations. Impurities can be formed during these stress conditions, and their quantification is crucial for evaluating the stability profile of the drug. The calculation of impurities obtained in a forced degradation study involves several steps:
Identification of impurities: The first step is to identify and characterize the impurities formed during the forced degradation study. Various analytical technologies such as high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) can be used to identify and characterize these impurities.
Quantification of impurities: Once the impurities are identified, the next step is to quantify them. This is typically done using a validated analytical method such as HPLC or GC. The impurities are usually compared to a suitable reference standard to determine their concentrations.
Calculation of impurity levels: The impurity levels are calculated depending on the quantification results obtained from the analytical method. The impurity levels are typically expressed as a percentage relative to the active pharmaceutical ingredient or finished goods. For example, the impurity level may be reported as a percentage of the drug substance's peak area or concentration.
Reporting and interpretation: The calculated impurity levels are then reported and interpreted based on regulatory guidelines and acceptance criteria. The significance of the impurity levels in terms of safety and efficacy of the active pharmaceutical ingredient or finished goods is evaluated. If the impurity levels exceed the acceptable limits, further investigations may be required to determine the potential impact on the drug's quality, safety, and efficacy.
It's crucial to remember that the specific calculations and reporting requirements may differ depending on the regulatory guidelines and the specific drug being studied. It is recommended to consult the appropriate regulatory guidelines and seek guidance from experts in analytical chemistry

and pharmaceutical development to ensure accurate calculations and interpretation of impurities in a forced degradation study [18-20].

**Regulatory perspectives of forced degradation:**

A. Issues addressed in regulatory guidance's include:

- One batch of material is normally used in forced deterioration studies.
- Forced deterioration circumstances are more severe than accelerated stability testing, such as temperatures above 50°C, relative humidity below 75%, light levels above ICH, high and low pH, oxidation, and so on.
- The design of a forced degradation research should include photostability.
- It may not be necessary to identify or analyse the structure of degradation products that do not occur in accelerated or long-term stability.
- It's important to think about mass balance.

B. Issues that aren't addressed in the regulatory advice:

- For forced deterioration research, exact experimental parameters (temperatures, time, and extent of degradation, for example) are not given.
- The applicant has complete control over the experimental design [21-24].

## CONCLUSION

Forced degradation experiments help determines pharmaceutical chemical stability and breakdown processes. To accelerate degradation and assess chemical stability, these experiments submit therapeutic substances or products to temperature, humidity, light, oxidation, and acid/base hydrolysis. We analyse pharmaceutical forced degradation studies in this review, emphasizing their importance in medication development and quality monitoring. Forced degradation studies aim to mimic and predict drug breakdown processes and products over shelf life or under specific storage settings. Advanced analytical methods can identify and characterize degrading contaminants in medicinal molecules under stress. This review covers forced degradation basics such stress condition selection, sample preparation, and impurity profile analysis. We explore how pH, temperature, humidity, and light exposure affect drug stability and degradation. We also examine forced degradation study regulations to ensure pharmaceutical companies manufacture safe and effective medications. Beyond stability assessment, forced degradation experiments help understand degradation chemical pathways and inform formulation and package design. Identifying and quantifying degrading impurities helps create impurity acceptability standards, assuring product quality and patient safety.

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**Conflict of Interest**

None

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