

# REVIEW ON FORCED DEGRADATION STUDIES

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## **Abstract**

Forced degradation studies play a critical role in modern pharmaceutical development by providing comprehensive insights into the intrinsic stability of drug substances and drug products. These studies involve the intentional exposure of active pharmaceutical ingredients (APIs) and finished dosage forms to extreme stress conditions such as acidic and alkaline hydrolysis, oxidation, thermal stress, photolysis, and humidity in order to induce controlled degradation. Unlike conventional long-term or accelerated stability studies, forced degradation is designed to rapidly elucidate degradation pathways, mechanisms, and potential degradation products that may arise during manufacturing, storage, or distribution. The information obtained from stress testing is essential for impurity profiling, formulation development, excipient compatibility assessment, packaging selection, and the establishment of appropriate control strategies. Furthermore, forced degradation studies are fundamental to the development and validation of stability-indicating analytical methods, particularly chromatographic techniques such as HPLC and UPLC, ensuring accurate quantification of APIs in the presence of degradation products. Regulatory agencies, guided by International Council for Harmonisation (ICH) recommendations, recognize forced degradation as an integral component of pharmaceutical development and regulatory submissions, although specific experimental conditions remain flexible and science-driven. This review provides a comprehensive overview of the principles governing forced degradation studies, outlines methodological considerations for designing effective stress testing protocols, and discusses analytical perspectives for the detection, separation, and characterization of degradation products using advanced techniques such as LC–MS and NMR. Emphasis is placed on regulatory expectations, analytical challenges, and ensuring drug quality, safety and regulatory compliance.

## **Keywords**

**Degradation conditions, Degradation product, Forced degradation, Stability indicating method, Stress testing.**

## INTRODUCTION

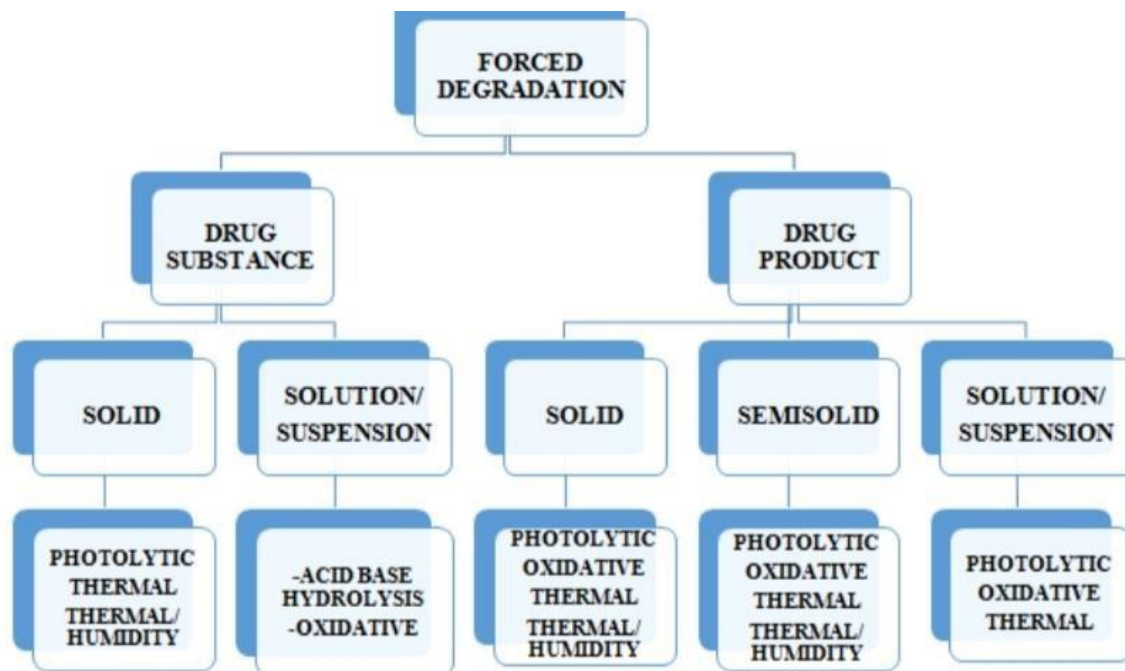


**Figure [1]** An illustrative flow diagram different forced degradation conditions to be used for drug substance drug product.

In order to improve the quality, safety and efficacy of medication formulation stability testing of pharmaceutical goods is a complex collection of process. Requiring significant expense, time consumption, and scientific skill. The pharmaceutical analysis and tests that are necessary to ascertain and ensure the identity, potency, and purity of ingredients as well as those of the formed products are among the most crucial tasks during the developmental stages. The capacity of a specific formulation in a particular container/closure system to maintain its physical, chemical, microbiological, toxicological, protective, and information characteristics is referred to as a pharmaceutical product's stability. Thus, stability testing assesses how the environment affects a drug substance's or a manufactured product's quality in order to anticipate how long it will last, identify the best storage options, and recommend labeling guidelines include the active ingredient(s)' stability, interactions between the active ingredient(s) and excipients, manufacturing procedures used, dosage form type, packaging container/closure system, and environmental conditions for light, heat, and moisture encountered during [35] transportation, storage, and handling. Additionally, factors like reactant concentration, pH, radiation, catalysts, raw material usage, and the interval between production and use of the product all affect the stability of degradation reactions like oxidation, reduction, hydrolysis, or racemization, which can be extremely important for pharmaceutical products. A pharmaceutical product's stability may be impacted by changes to its appearance, consistency, content uniformity, clarity (solution), moisture content, particle size, shape, and members of the European Union, while WHO guidelines are intended for global product development and are widely used in international health setting. 34

### Objective of forced degradation studies

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

1. To establish degradation pathways of drug substances and drug products.

2. To differentiate degradation products that are related to drug products from those that are generated from non-drug product in a formulation.
3. To elucidate the structure of degradation products.
4. To determine the intrinsic stability of a drug substance in formulation.
5. To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and drug product.
6. To establish stability indicating nature of a developed method.
7. To understand the chemical properties of drug molecules.
8. To generate more stable formulations.
9. To produce a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.
10. To solve stability-related problems.

## Drug Concentration for Degradation Process

The regulatory guidelines do not specify the exact drug concentration required for degradation studies. However, it is commonly advised to start forced degradation studies using a concentration of 1 mg/mL, as this level is usually adequate to identify even low levels of degradation products. Addition advisable conduct degradation studies at concentrations that align with the drugs expected levels in the final formulations.

## Types of Degradation Hydrolytic conditions

Hydrolysis is one of the most common degradation chemical reactions over wide range of pH. Hydrolysis is a solvolytic process in which drug reacts with water to yield breakdown. Products of different chemical compositions. Water either as a solvent or as moisture in the air comes in contact with pharmaceutical dosage forms is responsible for degradation .most of the drugs. For example, aspirin combines with water and hydrolyzed to salicylic acid and acetic acid .Hydrolytic study under acidic and basic condition involves catalysis of ionizable functional groups present in the molecule. Acid or base stress testing involve which generates primary degradants in desirable range. The selection of the type and Concentrations of acid or base depends on the stability of the drug substance. Hydrochloric acid or sulfuric acids (0.1–1 M) for acid hydrolysis and sodium hydroxide or potassium Hydroxide (0.1–1M) for base hydrolysis are suggested as suitable reagents for hydrolysis[18].The hydrolytic stress testing normally is conducted at room temperature with or without co-solvent and if no degradation appears, continues under higher temperature of 50°C to 70°C. Stress testing normally should not exceed more than 7 days. The degraded sample is then neutralized before injection using suitable acid, base or buffer, to avoid further decompositions forced degradation of a drug substance by exposure to acidic or basic conditions. 23, 27

## Oxidation conditions.

Degradation, hydrogen peroxide is broadly used. Apart from this as metal ions, oxygen, and radical initiators: azobisisobutyronitrile, AIBN can also be used. Drug structure will allow selecting concentration and condition of oxidizing agents. An electron transfer mechanism occurs in the oxidative degradation of drug substance[8].Many drug substances undergo auto-oxidation i.e. oxidation under normal storage

condition and involving ground state elemental oxygen. Therefore it is an important degradation pathway of many drugs. Auto-oxidation is a free radical reaction that requires free radical initiator to begin the chain reaction. Hydrogen peroxide, metal ions and trace level of impurities in a drug substance act as initiators for drug substance. Selection of an oxidizing agent, its concentration and condition depends on the drug substance. It is mentioned that Drug solutions are subjected to 0.1%-3% hydrogen peroxide at neutral pH and room temperature for seven days or up to a maximum 20% degradation could potentially generate relevant degradation products. 26, 22

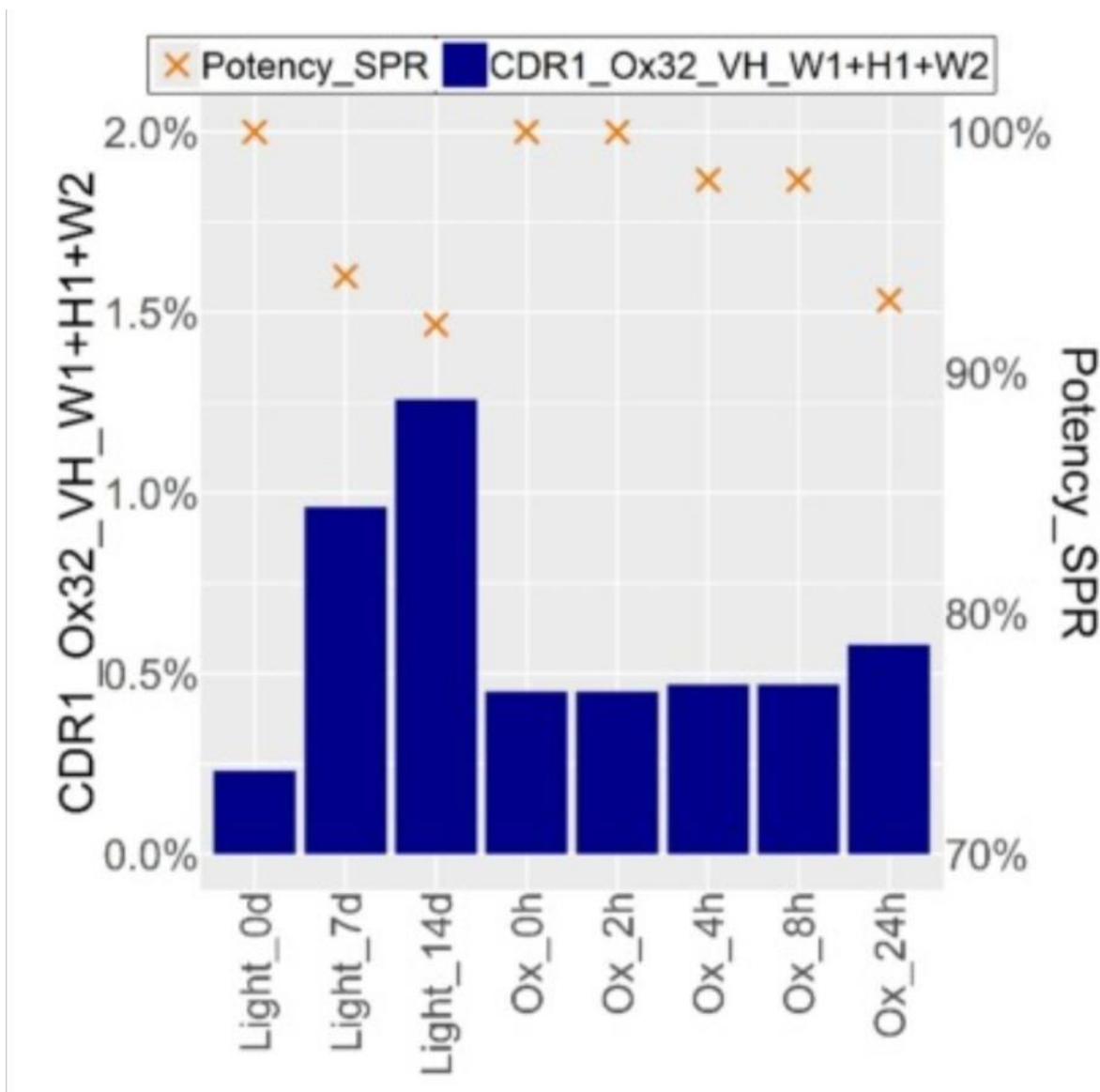


**Figure [2]** Relative Quantitative di oxidation levels in CRDS

### Photolytic conditions.

The photo Stability testing of drug substances must be evaluated to demonstrate that light exposure does not result in unacceptable change. Photo stability studies are performed to generate primary

degradants of drug substance by exposure to UV or fluorescent conditions. Some recommended conditions for photostability testing are described in ICH guidelines. Samples of drug substance and solid/liquid drug product should be exposed to a minimum of 1.2million lx h and 200 W h/m<sup>2</sup> light. The most commonly accepted wavelength of light is in the range of 300– 800 nm to cause the photolytic degradation. The maximum illumination recommended is 6 million lx h. Light stress conditions can induce photo oxidation by free radical mechanism. Functional groups like carbonyls, nitro aromatic, Oxide, alkenes, aryl chlorides, weak C–H and O–H bonds sulphide sand polyenes are likely to introduce Thermal degradation (e.g., dry heat and wet heat) should be carried out at more strenuous conditions than the recommended ICH, Q1A accelerated testing conditions.<sup>6</sup>

## Thermal conditions.

Samples of solid state drug substances and drug products should be exposed to dry or wet heat, while liquid drug products should be exposed to dry heat. Studies may be conducted at higher temperatures for a shorter period. Effect of temperature on thermal degradation of a substance is studied through the Arrhenius equation:  $k=Ae^{-E_a/RT}$  Where k is specific reaction rate, A is frequency factor,  $E_a$  is energy of activation, R is gas constant (1.987 Cal/deg mole) and T is absolute temperature. Thermal degradation study is carried out at 40–80°C <sup>21, 24</sup>

Table[1] stress conditions

| Typesof conditions | Experimental conditions         | Storage conditions |
|--------------------|---------------------------------|--------------------|
| Hydrolytic         | Control no acid or base         | 40°C,60°C          |
|                    | 0.1Msodium hydroxide            | 40°C,60°C          |
|                    | Acid control (no API)           | 40°C,60°C          |
|                    | Base control (no API)           | 40°C,60°C          |
| Oxidation          | PH:2,4,6,8                      | 40°C,60°C          |
|                    | 3%H <sub>2</sub> O <sub>2</sub> | 25°C,60°C          |
|                    | Peroxide control                | 25°C,60°C          |
|                    | AIBN control                    | 40°C,60°C          |
| Photolytic         | Light1×ICH                      | Not applicable     |
|                    | Light 2×ICH                     | Not applicable     |
|                    | Light control                   | Not applicable     |
| Thermal            | Heat chamber                    | 60°C               |
|                    | Heat chamber                    | 60°C/75RH          |
|                    | Heat chamber                    | 80°C               |
|                    | Heat chamber                    | 80°C/75RH          |
|                    | Heat control                    | Room temperature   |

## Selection of experimental conditions

There are many examples in the literature of experimental conditions for conducting forced degradation studies and the structural multiplicity of drug molecules that makes it not possible to identify a generic set of conditions for a forced degradation study.In designing forced degradation studies, it must be

remembered that more strenuous conditions than those used for accelerated studies (25°C/60% RH or 40°C/75% RH) should be used. At a minimum, the following conditions should be investigated: [1] acid and base hydrolysis, hydrolysis at various pH, thermal degradation, photolysis, and oxidation. For the drug substance and drug product, the scheme could be used as a guide. Excess degradation of a sample may lead to further degrade impurities and form secondary degradants that would not be seen in real-time stability studies. Excess degradation may mislead mass balance results also because of differences in response factors of unknown impurities'. The initial experiments should be focused on determining the conditions that degrade the drug by approximately 10%. The conditions generally employed for forced degradation are summarized in Table [1]. However, some scientists have found it practical to begin at extreme conditions (80°C or even higher, 0.5N NaOH, 0.5N HCl, 3% H<sub>2</sub>O<sub>2</sub>) and testing at shorter [2, 5, 8, and 24 hrs, etc.] multiple time points, thus allowing for a rough evaluation of rates of degradation. Forced degradation studies used for degradation of drug product or drug substance are mentioned below.

### Time to perform forced degradation studies

Knowing when to conduct forced degradation experiments is crucial for the creation of novel medicinal substances and products. According to FDA guidelines, phase III of the regulatory submission procedure is when stress testing should be carried out. To ascertain the stability of the drug material, stress tests should be conducted in various pH solutions, with oxygen and light present, and at high temperatures and humidity levels. A single batch is used for these stress tests. A yearly report summarizing the findings should be submitted.<sup>8</sup> However, it is strongly advised to begin stress testing on medicinal substances early in the preclinical phase or phase I of clinical trials. This will allow enough time to identify degradation products, clarify structure, and optimize stress settings. Additionally, an early stress study provides prompt suggestions for manufacturing process enhancements and appropriate stability indicating analytical procedure selection.

### Methods

Seven different plant extracts were quantified and the stability of the constituents was estimated by forced degradation studies. The separation of the phytoconstituents was performed on a Phenomenex C18 column with a mobile phase of 80% acetonitrile and 20% (25 mM) ammonium acetate (pH 3) at a flow rate of 1 mL min<sup>-1</sup> detected at 380 nm.

### Stability indicating method

The active pharmaceutical ingredient (API) in drug products degrades over time, and the s. Stability indicating method (SIM) is an analytical technique used to quantify this degradation. A stability-indicating method is a validated quantitative analytical procedure that can be used to determine how the stability of drug substances and drug products changes over time, according to an FDA guideline document. Without interference from other degradation products, contaminants, or excipients, a stability-indicating method correctly gauges variations in active component concentration. Stress tests are conducted to show the developed method's specificity in measuring changes in drug substance concentration when knowledge of probable 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. The most popular combination of the RP-HPLC with a UV detector allows for the separation and quantification of contaminant. The following are the steps involved in the development of SIM on HPLC which meets the regulatory requirements.

### Sample generation:

The API is forced to deteriorate under conditions that are more severe than accelerated degradation settings in order to generate samples for SIM. It involves drug breakdown under the previously stated hydrolytic, oxidative, photolytic, and thermal conditions. In order to produce degradation products that are likely to develop under actual storage conditions, forced degradation of API in solid state and solution form is conducted. The development of a SIM then uses this sample.

### **Method development and optimization:**

The foundation for developing an HPLC method is laid by numerous physiochemical parameters of the drug, such as its pKa value, log P, solubility, and maximal rate of absorption. The selection of the mobile phase and sample solvent is aided by log P and solubility, while the mobile phase's pH is aided by the pKa as the degradation occurs in an aqueous solution, the reverse phase column is the ideal choice to begin the separation of sample components. For the first stages of separation, the mobile phase made up of acetonitrile, water, and methanol can be utilized 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 decent separation of peaks, the water: organic phase ratio can initially be set at 50:50 and relevant adjustments can be made as trials advance. If the additional buffer is needed to improve peak symmetry and separation, it can be added. Mobile phase buffer should be compatible with MS if the approach is to be extended to liquid chromatography-mass spectrometry (LC-MS), such as trifluoroacetic acid and ammonium formate. The selectivity of the approach is impacted by column temperature variation since various analytes react differently to temperature fluctuations. To achieve good consistency, a temperature in the 30–40°C range is ideal. The drug peak should be pushed further up the chromatogram since this separates all degradation products. In order to acquire the degradant peak eluting after the drug peak, a sufficient run time after the drug peak must be permitted. When developing a technique, it is possible that the drug peak conceals an impurity or degradation peak that co-elutes with the drug. Peak purity analysis, which establishes the method's specificity, is necessary for this. Online direct analysis employing photodiode array (PDA) detection is possible. PDA offers data on the homogeneity of the spectral peak, but it does not apply to degradants whose UV spectra are comparable to those of drugs. The indirect technique entails altering the chromatographic parameters, such as the mobile phase ratio and column, which will impact peak separation. Then, the original spectra are contrasted with the spectrum of the altered chromatographic condition. It can be determined that a drug peak is homogenous if both the degradant peaks and the drug peak's area % remain constant. If it is discovered that the co-eluting degradant was not created under rapid and long term storage circumstances, it would be acceptable. Then, by altering the flow rate, injection volume, column type, and mobile phase ratio the technique is made to work best for separating closely eluting peaks.

### **Method validation:**

The created SIM is then validated in accordance with USP/ICH guidelines for method linearity, accuracy, precision, specificity, quantitation limit, detection limit, ruggedness, and robustness. The degradants discovered to be above the identification threshold (about 0.1%) must be isolated, identified, and quantitated. The technique is updated and revalidated if it does not meet the acceptance requirements for validation.<sup>23</sup>

## Factors effecting degradation

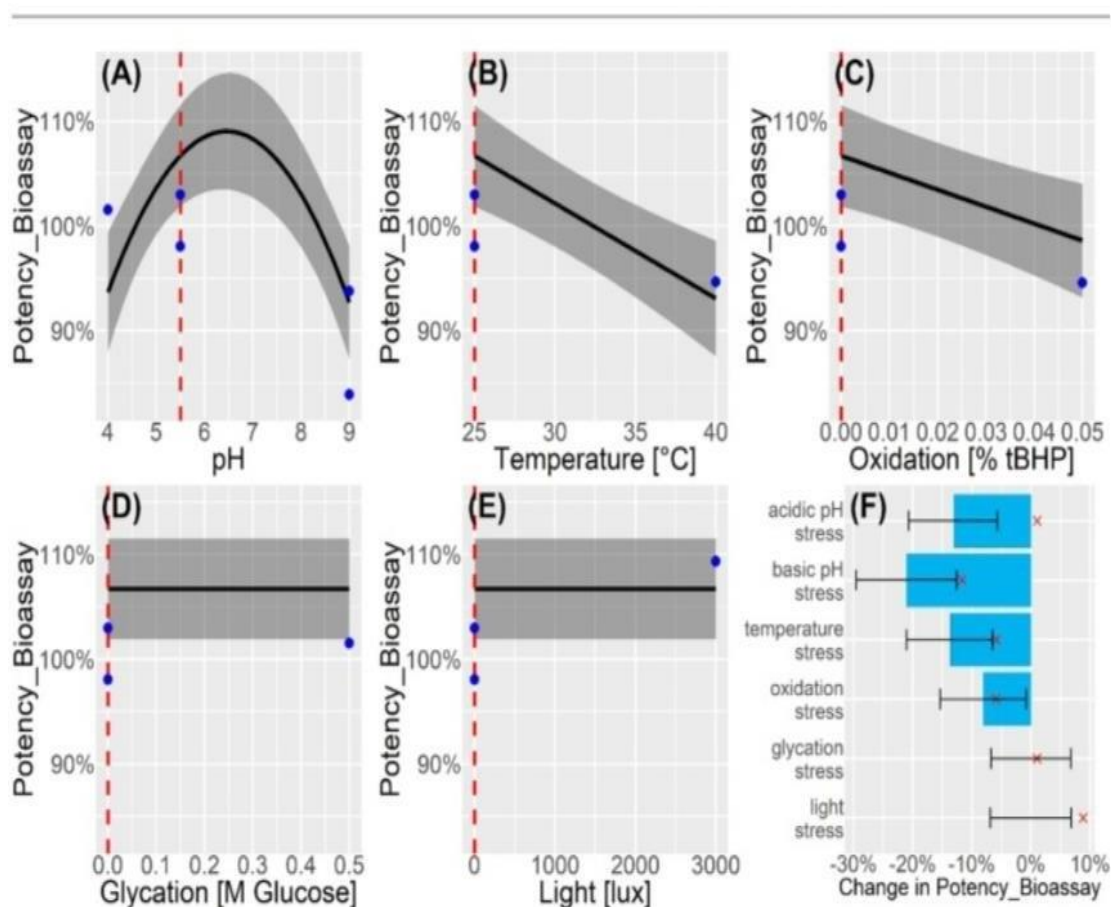


**Figure [3]** Bioassay potency in relation to stress conditions

## Moisture

Water-soluble compounds may dissolve when moisture is present. The molecule undergoes chemical and physical changes as a result.

## Excipients

It was noted that certain excipients can have high water content. This moisture could result in higher water content in the formulation, which would subsequently impact the drug's stability. Reduced stability is sometimes the result of chemical reactions between the medication substance and the excipients.

## Temperature

Temperature variations can occasionally have a negative impact on the drug's stability. Generally speaking, higher temperatures accelerate the rate of medication breakdown

## Light

Certain medications have a tendency to break down when exposed to light because they are photolabile. Its stability in the presence of light and stability while stored in the dark can be used to test for photolytic decomposition susceptibility. It is important to keep in mind that the photolabile compounds ought to be kept in dark, amber glass container.

## Conclusion

Forced degradation studies play a fundamental role in pharmaceutical drug substance development, converging analytical chemistry, organic reaction mechanistic chemistry, regulatory compliance and patient safety science. This experimental and regulatory knowledge illustrates that a scientifically sound stress-testing program, which will systematically test hydrolytic, oxidative, photolytic, thermal, and humidity-induced degradation, will lead to a degradation target of 5 to 20% (industry accepted), and will lead to structurally elucidated impurity profiles that will be confirmed by LC-MS/MS analysis and supported by kinetic modelling, will provide information that cannot be replaced by any other part of the pharmaceutical development program. The imminent change to the consolidated ICH Q1 framework requires that stability scientists shift from regulatory compliance scenario as the goal to mechanistic understanding as the scientific benchmark and include predictive in silicone tools, advanced hyphenated analytical platforms, and impurity assessment based on risk in programs that can serve medicines throughout their commercial life cycle.

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