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A REVIEW ON THE SCIENTIFIC AND REGULATORY CONSIDERATION IN SIGNIFICANCE OF THE DEVELOPMENT AND VALIDATION OF DRUG SUBSTANCES AND DRUG PRODUCTS: FORCED DEGRADATION AND STABILITY STUDIES

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ABSTRACT

The intricate scientific and regulatory considerations involved in the development and validation of drug substances and products include forced degradation and stability studies. These studies evaluate the deterioration of drugs under different stressful conditions to ensure their safety, effectiveness, and quality. It is important that the selection of stress conditions, analytical methods, and statistical analysis are reflective of real-world exposure and validated for accuracy. Regulatory bodies like the US FDA and European Medicines Agency require stability testing for approval. Forced degradation and stability studies are crucial in ensuring the safety, effectiveness, and quality of Drugs.

KEYWORDS: Oxidation, photolytic degradation, thermal, degradation, Potency, Degradants, accelerated conditions, Stress testing, purity.

• INTRODUCTION

The safety and effectiveness of a drug product is closely tied to the chemical stability of its pharmaceutical molecules. This is a major concern, as evidenced by FDA and ICH guidelines that stipulate the need for stability testing data to assess how drug substances and products change over time in response to various environmental factors. Understanding the stability of a molecule is important for selecting the right formulation and packaging, as well as providing proper storage conditions and shelf life. Such information is also essential for regulatory purposes. Forced degradation is a process that involves subjecting drug substances

and products to conditions more severe than those used in accelerated conditions. This generates degradation products that can be studied to determine the molecule's stability.[36] The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used.[2-4] However, these guidelines are very general in conduct of forced degradation and do not provide details about the practical approach towards stress testing. Although forced degradation studies are a regulatory requirement and sciatic necessity during drug development, it is not considered as a requirement for formal stability program.[5]

It has become mandatory to perform stability studies of new drug moiety before filing in registration dossier. The stability studies include long-term studies (12 months) and accelerated stability studies (6 months). But intermediate studies (6 months) can be performed at conditions milder than that used in accelerated studies. So the study of degradation products like separation, identification and quantitation would take even more time.[6] As compared to stability studies, forced degradation studies help in generating degradants in much shorter span of time, mostly a few weeks. The samples generated from forced degradation can be used to develop the stability indicating method, which can be applied latter for the analysis of samples generated from accelerated and long-term stability studies. This review provides a proposal on the practical performance of forced degradation and its application for the development of stability indicating method. The quality of a drug substance and a drug product may change over time due to a range of environmental factors, according to FDA and ICH standards.[7]

Forced deterioration in solid-state research comprises exposing the medicinal substance to heat, heat and humidity, and light. For solution state research, a variety of pH values is applied to the medicinal substance.[8]

By exposing the molecules, stability tests can be performed, which help in determining the ideal formulation (such as a solid, liquid, or semisolid), as well as the rules for packing, storage, and shelf life required for the regulatory document.[9-10]

ICM Standards The statement claims that the goal of stress testing is to identify the degradation product that assists in figuring out how to degrade a molecule, creating degradation routes, and validating stability indicating methods. Prior to submitting

registration dossiers, stability studies of novel drug moieties and compounds must now be finished.[11]

Using forced decomposition experiments, the stability of the molecule, different degradative pathways, and validation of the stated stability processes are assessed in accordance with the International Committee for Harmonization (ICH) criteria. The Food and Drug Administration (FDA) and International Conference on Harmonization (ICH) guidelines give a clear explanation of the complexities of drug molecules that degrade, the numerous products that are created with respect to time changes under the influence of various environmental elements, and a comprehension of stability data.[12,13]

There have been reports of both accelerated and long-term stability examinations. Expedited stability tests typically around 6 months, whereas long-term trials last about 12 months. Furthermore, under more loose settings, six-month trials on intermediate stability are conducted.[14]

- **Objective of forced degradation studies**

Following are some of the reasons to carry out the forced degradation studies:[15]

Stability related problems are solved by these studies.

- These studies produce formulations that are more stable.
- These studies elucidate the structures of degradation products.
- These studies establish the degradation pathways of drug substances and drug products.
- These studies establish the characteristics of stability that indicate the nature of a developed method.
- determination of the intrinsic stability of the drug substances in the formulation
- These studies aid in understanding the chemical properties of drug molecules.
- Degradation mechanisms such as hydrolysis, oxidation, photolysis,or thermolysis of drug substances and drug products are understood by these studies.

Overview of regulatory authorities[16]

- **ICH QIB-Photo stability testing of new drug substances and drug product**

One of the most common ways of determining whether a substance can withstand the effects of light is to determine how recently it was formed. In the sections on the necessity of such degradation as well as the regulatory requirements, the subject of the forced degradation of

drug molecules and their compounds was discussed. Researchers have the opportunity to obtain vital information regarding the security and consistency of a treatment. Forced degradation studies are used as part of confirmatory research to find the products of photolytic breakdown.

- **ICH Q1A-Testing of stability for new drug molecules and their product**

The ICH Q1A standard addresses the assessment of the stability of innovative pharmaceutical compounds and the derivatives of those compounds. The photo stability of molecules must be evaluated using assays that involve forced decomposition in order to properly identify any photolytic breakdown products that may be produced when the molecules are subjected to light. These parameters can be used to determine how safe a medicine is over the long term. Testing of Stability for New Drug Molecules and Their Products. The formulation of methodologies to evaluate drug stability could benefit from these suggestions. According to the information provided in Q1A, the rate of degradation is contingent not only on the individual drug molecules but also on the composition of the drug products.[1] These pharmacological compounds and their derivatives were subjected to extensive forced decomposition studies, which also made use of many different settings that accelerated the process. These parameters included high temperatures (over 50°C), high humidity (above 75% relative humidity), oxidation, photolysis, and various pH levels; however, this list is not exhaustive (solution or suspension). These accelerated conditions should be taken into account as recommendations for product development when designing appropriate stability testing techniques to assure stability in the development and design of the product. This will ensure that the product will remain stable over time.[19-21]

- **ICH Q2B: Analytical Procedure Validation: Methodology**

The ICH Q2B guidelines outline the procedures that must be followed to validate the various analytical protocols. It emphasizes the importance of stressing samples under various accelerating conditions such as humidity and heat before using them to determine sample specificity. Furthermore, following these guidelines can greatly assist in quantifying the degradants produced**.**

- **ICH, Q3A, Impurities in new drug substances**

The ICH Q3A guidelines help identify contaminants in newly developed drug molecules. This section covers a wide range of topics, such as impurity identification, impurity types,

analytical protocols, and report generation. More importantly, it is thought that if impurities are either completely absent or present in trace amounts in a batch of a new drug molecule, it will aid in ensuring safety prior to clinical trials. This is because either of these conditions indicates that the impurities are not hazardous.[17,18]

- **ICH Q3B Impurities in new products**

ICH Q3B provides information about analytical procedures. It is important for an analytical procedure to validate the specific or non-specific degradation products under various stress conditions.[17,18]

- **EMA Guidelines**

The chemistry of active substances may be understood on a more fundamental level thanks to this overarching idea. It provides details on the many different kinds of study that were conducted, as well as the methodologies that were used and the conclusions that were made from the examination. We address the testing of the API and dosage forms to ensure their stability. It provides details on the testing dates as well as the expiration dates for the medications. The paths of degradation, the validity of the approach, the inherent stability, and the development of the method are also determined. In addition to this, it necessitates the performance of stability tests on sensitive chemicals, such as hygroscopic and photosensitive pharmaceuticals.[17]

- **FDA Guidelines**

FDA is providing guidelines for photo stability analysis of newer drug molecules and their products (Q1B). According to the FDA, degradation studies should be conducted using normal development conditions. It covers the degradation pathway of samples when they exposed are to light. These guidelines help to develop SIM and summarize the data of validation, which are in turn helpful for confirmatory studies. These guidelines insist on the fact that there is no necessity to carry out the confirmatory studies for degradation products. According to the Section 211.166(a) (3), a SIM should be highly specific and must be able to quantify the amount of active ingredient present, the type of degradation products thus obtained with and other components present in dosage form without any interference under stress conditions. Stress conditions used for forced degradation studies are pH, temperature, and oxygen.[17]

- **USP Pharmacopoeia: Validation of Compendia Procedures**

If degradation standards or contaminants are not available, the specificity can be assessed by comparing the data with the results obtained from the analytes (which contain the contaminants or degradation products) using a different process under the same accelerated conditions. This comparison will show the difference between the data and the analyte results.[17]

- **Japanese Pharmacopoeia**

It states that, in order to satisfy these standards, the suggested technique must be precise, capable of identifying and measuring the amount of analyte present in the sample, and should be repeatable. The samples will be subjected to a number of demanding conditions, and the degradation products may be used in further investigations if reference standard impurities are not easily accessible for use in comparative studies.[17]

- **National Health Surveillance Agency (ANVISA)**

It discusses the requirements for stability and forced degradation. ANVISA was created to promote public health and protect against risks associated with the manufacture and use of various pharmaceutical products. ANVISA coordinates states, districts, and municipalities in accordance with the principles of the Brazilian Unified Health System in order to improve people's quality of life.[17]

- **Inception of degradation products**

The main cause of development of impurities in drug substance or product is due to its degradation. The chemical instability of the drug substance under the conditions of heat, solvent, humidity, pH, and light encountered during manufacture, isolation, drying, purification, storage, transportation is the main cause of its degradation. The chemical reactions like oxidation, hydrolysis, heat and photolysis occurred in the drug substance and main route of degradation.[22,23]

- **Limits of degradation**

Degradation of pharmacological compounds between 5% and 20% has been approved for the validation of chromatographic tests, according the ICH Guidelines. A degradation product need not always be produced when forced degradation occurs. Stress studies should be stopped if no degradation is observed following exposure to stressful conditions for drug

substances or drug products. Maximum 14 days are advised for stress testing in order to produce stressed samples for method development.

- **Strategy for selection of degradation condition**

For the development of stability-indicating processes for therapeutic components and medicinal products, forced degradation is employed to generate representative samples. The stress condition should be chosen based on how the product decomposes under typical manufacturing, use, and storage settings, which vary for each drug substance and drug product. Stress variables that have been advocated for forced deterioration study include thermo-, photo-, acid-, and alkali-hydrolysis, as well as oxidation. Fig.1 contains all of the conditions for force decline.

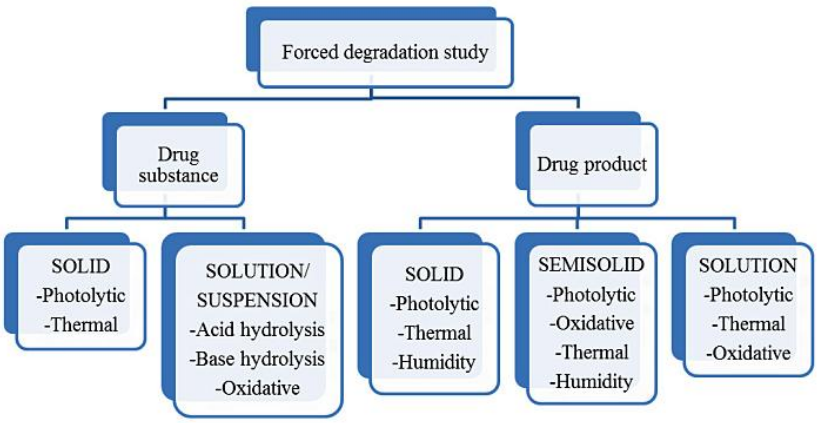

Fig. 1: A protocol of degradation conditions used for drug substance and drug product.

The regulatory standards include no mention of the pH, temperature, thermal condition, or oxidizing agent used.

Conditions of degradation chosen.[7],[8] Normal conditions such as high temperature and pH may previously be used to determine a drug's intrinsic stability. The medicinal compounds' stability was later studied by applying additional stress. In order to analyse the degradation, the drug sample-containing solution was refluxed for a predefined period. If any degradation products were discovered at this time, the procedure would be halted and additional work on isolating, identifying, and describing the discovered degradation products would be performed. If no signs of degradation were found, the reaction time would be increased to look for any signs of degradation because of the increased duration.[12],[19],[21],[26-30]

- **Hydrolysis**

The hydrolysis of a medication alters the pH of the reaction and the water content of the mixture (both acidic and alkaline). 0.1 N sodium hydroxide, 0.1 N hydrochloric acid, or 0.1 N sulfuric acid are commonly used to treat pharmaceutical compounds at 40-60 °C. The strength of the acid or alkali employed in the experiment determines how stable the molecules are. The optimum acid or alkali strength range should be between 0.1 N and one N solution. It should be noted that the trial can last no more than seven days. To prevent decomposition, materials must be neutralized with a buffer or acid/base. Look at Table 1.

Table 1: Conditions For Forced Degradation Studies.

Degradation type	**Experimental Condition Control API (No acid or base)**	**Storage condition 40 °C, 60 °C**	**Sampling time 1, 3, 5 days**
Hydrolysis	0.1N NaOH Acid Control (no API) Base Control (no API) pH: 2,4,6,8	40 °C, 60 °C 40 °C, 60 °C 40 °C, 60 °C 40 °C, 60 °C 25 °C, 60 °C	1,3,5 days 1,3,5 days 1,3,5 days 1,3,5 days 1,3,5 days
Oxidation	3% H2O2 Peroxide control	25 °C, 60 °C	1,3,5 days
	Azobisisobutyronitrile (AIBN)	40 °C, 60 °C	1,3,5 days
photolytic	Light, 1X ICH Light, 3X ICH Light Control	NA NA NA	1,3,5 days 1,3,5 days 1,3,5 days
Thermal	Heat chamber Heat chamber Heat chamber Heat chamber Heat control	60°C 60 °C /75% RH 80 °C 80 °C /75% RH Room Temp.	1,3,5 days 1,3,5 days 1,3,5 days 1,3,5 days 1,3,5 days

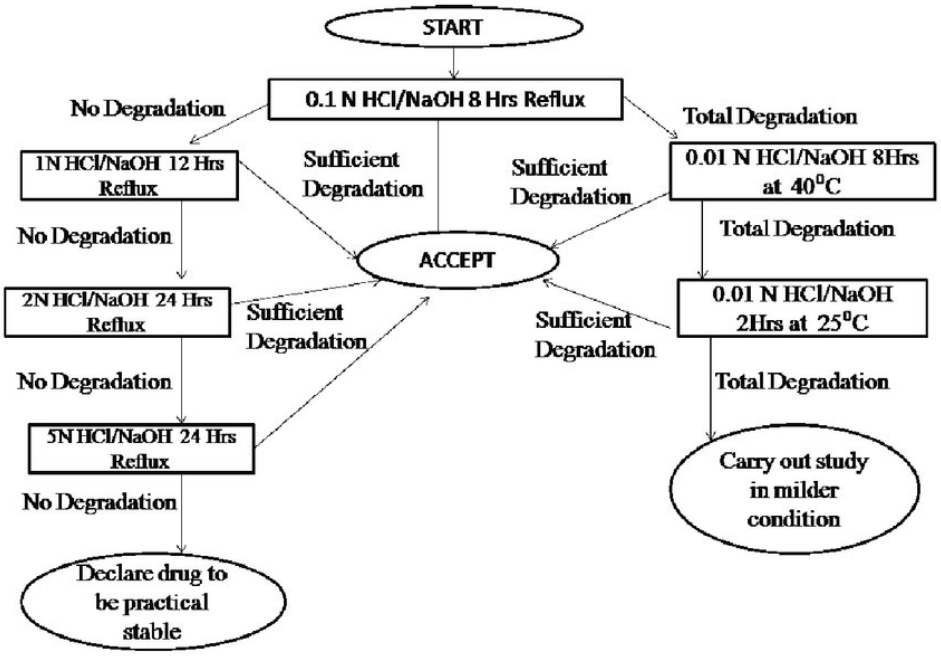

Fig. 2: Flow Chart of Hydrolytic Degradation.

- **Oxidation**

The vast majority of pharmaceutical substances have been demonstrated to be auto-oxidizers. The oxidation process requires free radical initiators. Hydrogen peroxide, trace quantities of pollutants, and metal ions are present as free radical initiators. The transport of electrons is involved in this form of degradation. A common initiator for oxidation induced degradation study is 0.1-3% hydrogen peroxide. These studies should be carried out for 1-7 days at 4.0°C. If more than 20% of the degradants are produced, this should be considered abnormal. Refer to Table-1.

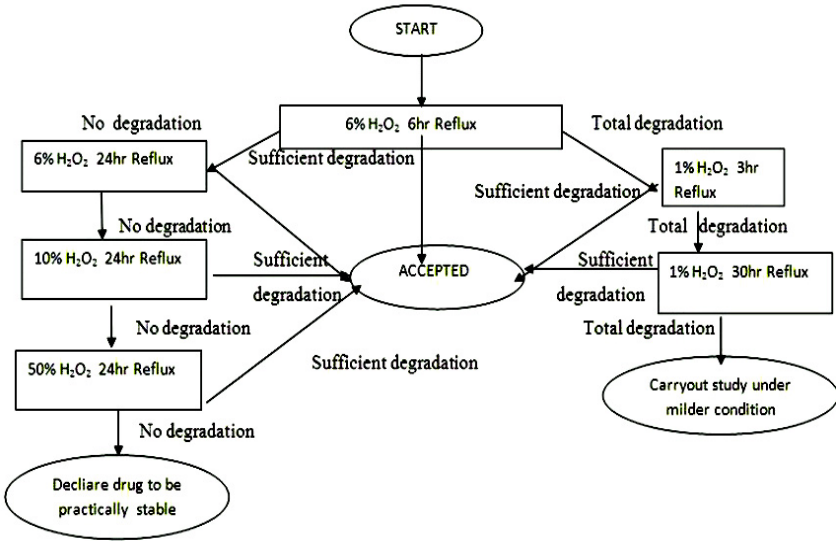

Fig. 3: Flow chart of oxidative degradation.

- **Thermal condition**

It has been found that a number of drugs have a heat labile tendency. Degradation products are created as a result of the reaction's pace accelerating as temperature rises. The temperature range for these studies should be between 40 and 80 degrees Celsius. Thermal stress studies are normally carried out at 70°C and high humidity over a period of one to two months. Drug molecules in solid form are subjected to both dry and wet heat conditions, whereas liquids are exposed to dry heat for a shorter amount of time. The Arrhenius equation predicts that the drug's molecule will deteriorate as a result of the high temperature.

$$Ae\text{-}Ea/RT = k.$$

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

- **Stress Conditions**

Overstressing a sample may lead to the formation of secondary degradants that would not be seen in formal shelf-life stability studies and under-stressing may not serve the purpose of stress testing. Therefore, it is necessary to control the degradation to a desired level. A generic approach for stress testing has been proposed to achieve purposeful degradation that is predictive of long-term and accelerated storage conditions. The generally recommended degradation varies between 5-20% degradation. This range covers the generally permissible 10% degradation for small molecule pharmaceutical drug products, for which the stability limit is 90%-110% of the label claim. Although there are references in the literature that mention a wider recommended range (e.g., 10-30%), the more extreme stress conditions often provide data that are confounded with secondary degradation products. For more information see. Table-2

Table 2: The four climate zones (ICH Stability guidelines).

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

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

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

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

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

Table 5: Drug products intended for storage in freezer.

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

- **Photolytic conditions**

In studies on photolytic degradation, fluorescent or UV light is employed to break down the therapeutic chemicals. The solid or liquid drug compounds or drug products in this study are exposed to a light source in accordance with the ICH Q1B requirements. For degradation investigations, a common radiation range is between 300 and 800 nm. In a photolytic condition, oxidising fluorescent or UV light is employed in photolytic degradation studies to breakdown pharmaceutical substances. In this study, the drug compounds or drug products (solid or liquid) are exposed to a light source in accordance with ICH Q1B requirements.

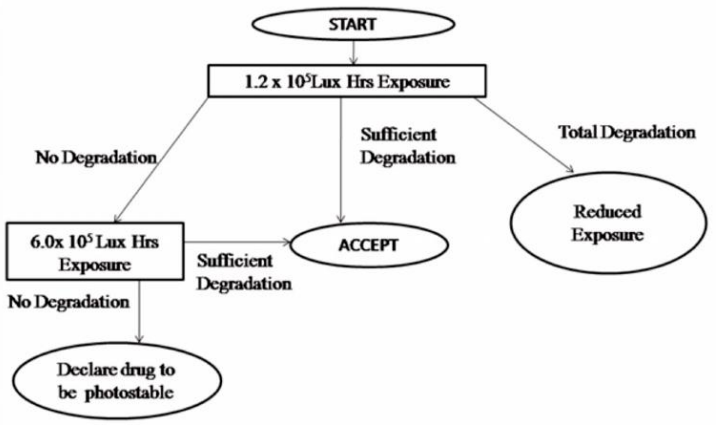

Fig. 4: Flow Chart of Photolytic Degradation.

- A typical radiation range for degradation investigations is 300 to 800 nm. Degradation in a photolytic scenario is generated by oxidation via a free radical mechanism or by a non-oxidation process. Non-oxidative destruction includes methods such as isomerization, dimerization, and others. The oxidative photolytic reaction, on the other hand, is founded on the singlet/triplet oxygen state mechanism. Singlet oxygen reacts with unsaturated molecules to generate photo-oxidative breakdown products, whereas triplet oxygen reacts with free radicals to make peroxide. It's also vital to understand that light promotes oxidation. Non-oxidative processes exhibit a wide range of reactions, including the homolytic breakage of C-S bonds, deamination, and the rupturing of C-X heterobonds. Examine Table 1.

- **Humidity**

The process of degradation is significantly influenced by humidity. High humidity can increase the rate of degradation, while low humidity levels can slow down the process In experiments involving forced degradation, the pharmacological substance is left in a 90% humidity environment for a week, which usually leads to degradation. One of the crucial factors to consider when identifying potential degradants in finished goods and API is humidity. As it is known, humidity can accelerate the process of degradation in drugs and other substances, as was seen in experiments.

- **Aspect of degradation**

Following are the different factor which causes degradation of drug substances, they are:

- **Moisture**

In the presence of moisture, water-soluble substances may get dissolved. This leads to physical and chemical changes within the molecule.[28]

- **Excipients**

It was observed that some excipients may contain high amounts of water. This posed a major challenge for scientists to develop novel strategies to minimize the water activity of these excipients. This moisture may lead to an increased water level in the formulation, which later affects the stability of the drug. In some cases, chemical interactions that occur between the excipients and the drug material often result in decreased stability. To counteract these issues, formulation scientists must be aware of the properties of the excipients they are using and adjust their processing parameters accordingly.[28]

- **Temperature**

The stability of the medication can occasionally be negatively impacted by temperature fluctuations. This can have a significant impact on the quality of the medication, reducing its effectiveness or causing it to spoil. Temperatures should be raised and maintained within a safe range to keep drugs stable and safe for use. Temperature control is thus critical to ensuring the quality and safety of medicines. Usually, a rise in temperature speeds up the hydrolysis of drugs.[28,29]

- **pH**

The rate of medication degradation via hydrolysis is affected significantly by pH. According to research, an increase in pH results in a decrease in medication stability due to hygroscopicity. To minimize this effect, medication formulations are conducted out utilizing buffer solutions with the highest stability. These buffer solutions assist keep the medications stable at a pH that reduces hydrolysis, extending their shelf life and effectiveness.[28,29]

- **Oxygen**

The presence of oxygen increases the oxidation of some drugs. The increased oxidation leads to a reduction for drug active in the body and can reduce the effectiveness of the treatment Drugs with an increased rate of decomposition in the presence of oxygen are stabilized by purging nitrogen or carbon dioxide from the storage container. This approach of purging the storage container with inert gases helps prevent oxidation and further degradation of the drug.[28,29]

- **Light**

Some drugs are photo labile and tend to decompose when they are exposed to light. The susceptibility to photolytic decomposition can be tested by comparing its stability in the presence of light with its stability when stored in the dark. It is to be remembered that the photo labile compounds should be stored in amber glass containers and should be stored in the dark. To extend their shelf life, photo labile compounds should be handled carefully and in accordance with standard safety protocols.[28,29]

Sample preparation in degradation study

During forced decomposition and stability studies, active pharmaceutical ingredients are subjected to various stresses under accelerated conditions, such as photolytic, thermal, oxidative, and hydrolytic conditions. The effects of these stresses are then studied over a

predetermined period of time in order to determine their effect on the pharmaceutical product's physical and chemical properties Due to stress conditions, several degradation products are expected to be produced, which can be compared to the degradation products (if any) that are obtained from regular storage conditions.[7]

- **Hydrolytic Conditions**

In acid hydrolysis, drug molecules are dissolved in sulfuric acid or hydrochloric acid (0.1–1 M). Drug molecules are dissolved in 0.1–1 M potassium hydroxide or sodium hydroxide during base hydrolysis. At room temperature, samples undergo stress for 2 to 7 days. To stop further degradation, stressed samples were neutralized using the appropriate acids or bases.

- **Oxidation Conditions**

Drug molecules are stressed with 0.1–3% hydrogen peroxide. Samples are stressed for not more than 7 days at room temperature and samples are neutralized with suitable agents.

- **Photolytic Conditions**

Photolytically stressed sample solutions are exposed to as little as 1.2 million l h and 200 W h/m2 of light at 300-800 nm.

- **Thermal Conditions**

Solids are exposed to wet heat and liquids are exposed to dry heat. Thermal stress conditions are applied for shorter period.[31]

- **Stability indicating method**

The FDA describes the stability indicated method as a quantitative one and keeps track of how changes in time will affect the drug's concentration. The reduction in drug and drug product concentrations will be assessed. Notably, the degradation studies reveal that the drug molecules' concentration can change. This information is useful in helping manufacturers predict the shelf life of their drugs, allowing them to estimate the time frame in which their drug will remain safe and effective for consumers During the degradation studies, it is noted that the concentration of the drug substance changes; notably, no interference from the excipients or other degradation products is noted. The SIM is therefore useful for preformulation studies and for forecasting the storage conditions for drugs.

- **Development and optimization of stability indication techniques**

The initial stage in creating a method is to ascertain the pKa value, log P, solubility, and composition of the relevant medication. For the separation of pharmaceuticals, it is usual practice to develop a reverse phase HPLC method. Methanol, acetonitrile, and water, among other frequently used solvents, are utilized as mobile phases in various ratios and combinations. The organic phase, such as acetonitrile or methanol, is chosen based on the drug's solubility profile. Earlier reports or procedures based on trial and error are typically used to choose the mobile phase and its proportion. The organic and aqueous phases are kept at a 50:50 ratio at the start of the experiment, and the solvent ratios for the mobile phase can be further optimised to provide the best peak resolution. Buffers can occasionally be used to achieve adequate baseline separation and peak symmetry. To achieve good reproducibility of the results, the column temperature is occasionally increased to 30–40 °C. To get good resolution, degradant peaks are pushed in the chromatogram. Peak purity analysis is triggered when degradant peaks elute concurrently with or are obscured by drug peaks. With the use of PDA detector-equipped HPLC, direct analysis can be carried out. The mobile phase can be changed in proportion, making it simpler to resolve and analyze the degradant peaks. If the degradation product peak is seen while the drug peak's area under the curve and its percentage are unaffected, the method established is regarded as homogenous. To a certain extent, these co-eluting degradants with the medicine are acceptable as long as they weren't noticed during rapid and long-term storage trials. Additionally, the process can be improved by adjusting the parameters, including the volume of sample injected, the kind of column employed, and the velocity at which the mobile phase is flowing. The mobile phase can be adjusted to accommodate the presence of these co-eluting degradants by making sure that any changes have no effect on the peak area and percentage of the drug under study The method created for the study will be put through ICH-recommended validation after these parameters have been optimised.[7]

- **Characterization of degradants**

Previous investigations using LC-MS, LC-UV, and LC-Previous have shown that a number of analytical techniques can be used to separate, categorise, and identify the impurities produced by degradation studies even at very low concentrations. The study's degradants were found and described using hyphenated techniques like LC-MS and LC-nuclear magnetic resonance spectroscopy (LC-NMR).[7] The structural characterization of the degradants and impurities is essential since it helps predict their stability over time, which is more critical.

While reversed-phase HPLC, TLC, gas chromatography, and supercritical fluid chromatography can be used to separate and extract degradants in their pure form, thin-layer chromatography (TLC), electrophoresis, colorimetry, and gel filtering procedures can be utilised to identify contaminants. Notably, the pathways travelled by degradants are determined using LC-MS/MS technology. The degradative pathways can be located using the observed fragmentation patterns. Degradant structures are clarified by synthesising or isolating procedures after the discovery of degradant pathways, and they are then further characterised by NMR techniques.[17],[7],[13],[26],[32],[33]

- **Method validation**

Following development, the stability indicating technique is validated in accordance with ICH guidelines for specificity, accuracy, precision, detection limit, quantitation limit, linearity, range, and robustness of the method. This process ensures that the technique is suitable for its intended purpose and that results are reproducible under normal operating conditions Once validated, this method is used for routine analyses of drug substances and related products, as well as in studies that evaluate the effect of various parameters on drug stability The degradation products that are discovered to be above the identification threshold (about 0.1%) must be isolated, identified, and quantitated. The main objective of these analyses is to provide information about the drug stability profile, which allows the necessary adjustments to be made in order to obtain a stable and reliable drug product The method is updated and revalidated if it does not meet the validation's acceptance requirements.[34],[35]

- **Preliminary Separation Studies on Stressed Samples**

A preliminary analysis of the several stress samples is performed to determine how much and what kind of degradation products were produced under various situations. Because the mobile introduces the beginning staves, either water-methanol or water-acetonitrile must be preferred. Using selection chromatographic conditions (such as selection of wavelength and selection of mobility and flow in HPLC analysis), OUSI would track variations in the signal (e.g., selection time). To ensure the accuracy of the results, it is important to select the correct mobile phase in order to capture the widest range of stress degradation products The resulting results should be carefully compared to those of the similarly injected blank solutions. A preliminary analysis of the several stress samples is performed to determine how much and what kind of degradation products were produced under various situations. These results can be used to guide the selection of mobile phases and column chemistries to ensure that the

most appropriate conditions are being used for analysis Because the mobile introduces the beginning stages, either water-methanol or water-acetonitrile must be preferred. One should track the variations in the strain samples over time by using various chromatographic conditions (such as selection of wavelength, selection of mobile phase, and flow in HPLC analysis). The resulting results should be carefully compared to those of the similarly injected blank solutions. By doing this, one can identify and quantify the degree of degradation products in the strain samples.

- **Final Method Development and Optimization**

Subsequent to preliminary chromatography studies, the RT and relative retention times (RRT) of all products formed should be tabulated for every reaction condition. A PDA The RT and relative retention times (RRT) of all generated products should be tabulated for each reaction condition after preliminary chromatographic experiments. To ascertain whether the products are same or different, PDA spectra or LC-MS profiles of such components are obtained and critically calculated. The strategy is improved by changing the mobile phase proportion, gradient, pH, flow rate, solvent type, temperature, and ultimately the column and its type in order to distinguish adjacent or co-eluting peaks. spectra or LC-MS profile of such components is obtained and critically calculated to determine whether the products are similar or different. To separate close or co-eluting peaks, the tactic gets improved; by make change the mobile phase proportion, gradient, pH, flow rate, solvent type, temperature, and therefore the column and its type.

- **Methods for Isolation and Identification of Degradation**

Several of the methods typically succeed in keeping impurities under control. Preparative high-performance liquid chromatography, thin-layer chromatography (TLC), and flash chromatography (column chromategraphy) are three of the most popular techniques (HPLC). The precise method to be employed will depend on the kind of impurity or degradant. The extent is contained in the original component, which needs to be isolated. Extraction techniques are occasionally used for the isolation of contaminants on the basis that impurities and pharmaceutical substances have varying solubilities in different solvents. These techniques are beneficial in their own right, each providing a unique set of capabilities that can be used to isolate and identify the contaminant or impurity Contaminants that have been separated depending on their acidity, basicity, or neutrality may be removed. The extraction procedure will typically have an impact on liquid-liquid extraction when one form is aqueous

and the other is a non-polar organic substance. Contaminants that are acidic, basic, or neutral can be eliminated by carefully regulating the pH of the aqueous phase. This method has the advantage of allowing for the fast extraction of impurities depending on their distinctive properties, allowing for the purification of a particular chemical.[36-40]

- **Separation Methods:** The following separation methods are often used.

❖ Thin-layer chromatography (TLC)
❖ Gas chromatography (GC)
❖ High-pressure liquid chromatography (HPLC)
❖ Capillary electrophoresis (CE)
❖ Supercritical fluid chromatography (SFC)

- **TLC**: by using a variety of different plates and mobile phases. The main problems with this method are defined resolution, ease of detection, and ease of quantification. The biggest advantages are that it is easy to use and cheap.
- **Gas Chromatography:** It was an excellent method for determining the cost of something. It can give the resolution, selectivity, and ease of measurement that are needed. The main problem was that the sample had to be volatile or be made volatile through a process called derivatization. This method worked very well for organic impurities that could be easily moved around.
- **High-Pressure Liquid Chromatography:** High-performance liquid chromatography is what most people call it now. Chromatographers use both of these words, which can be written as HPLC. Because of the need for different detectors like fluorescence, electrometry, mass spectrometry, etc., this was a useful method that has been used more and more in pharmaceutical chemistry.
- **Capillary Electrophoresis:** It was a helpful method when a very low volume of samples wasIt was useful when only a small number of samples were available but a high resolution was needed. The biggest risk was making sure that the samples that were injected could be used again. available and high resolution was required. The primary hazard was assuring reproducibility of the injected samples.
- **Supercritical Fluid Chromatography:** It can find and separate some of the same things that GC and HPLC can, but it doesn't matter as much how volatile the sample is. The ratio of a molecule's mass to its charge can be used in mass spectrometry (MS) to find and identify it. Gas chromatography-mass spectrometry is the name for this method (GC-MS). It is used to look at things that are still being worked on, like proteins, peptides, and small

molecules. So far, the best way to use it is to try it out. GC-MS has been shown to be a good way to find and separate small molecules, proteins, and peptides. This is because it can look at volatile samples.[41]

- **Hyphenated Methods:** The following hyphenated methods are often used effectively to watch impurities

❖ GC–MS
❖ LC-MS
❖ LC–DAD–MS
❖ LC–NMR
❖ LC–DAD–NMR–MS
❖ LC–MS–MS[46]

- **Outcomes of Forced Degradation Studies** : Forced degradation studies provide the following information.[42]

❖ Resolution of acceptable degradants
❖ Resolution of degradation pathways
❖ Resolution of intrinsic stability of the drug molecule
❖ Resolution of validated stability-indicating method.

CONCLUSION

In conclusion, forced degradation studies are an essential aspect of the drug development process, as they provide vital information on the stability and quality of drug substances and products. These studies help researchers identify active ingredient degradation pathways, develop more specific and reliable stability-indicating methods, and determine formulation storage conditions and manufacturing processes. Moreover, forced degradation studies provide insights into the stereochemical stability of drug substances, the chemical and physical stability of crystal forms, and mass balance issues in formulations. By conducting these studies earlier in the development process, researchers can obtain sufficient time to learn more about the molecule's stability and ensure that their drug products are safe and efficacious for patients. While there is no specific regulatory guidance for forced degradation studies, it is recommended to use suitable conditions to achieve 5-20% degradation to generate enough data to be useful, while still preserving enough of the sample for further analysis and experimentation. Overall, incorporating forced degradation studies into the

development process is critical for developing effective and reliable drug products that meet regulatory standards and are safe and effective for patients.

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