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A Review Article on Development of Forced Degradation and Stability Indicating Studies for Drug Substance and Drug Product

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ABSTRACT:

HPLC is an analytical technique widely used for identification, separation, detection and quantification of various drugs and its related degradants. HPLC Process development is important in case of drug discovery, drug development and in analysis of pharmaceutical products. High performance liquid chromatography is one of the most accurate methods widely used for the quantitative as well as qualitative analysis of drug product and is used for determining drug product stability. Stability indicating HPLC methods are used to separate various drug related impurities that are formed during the synthesis or manufacture of drug product. The objective of the review article is to give detailed description and guidance of the forced degradation studies as per regulatory guidelines. Forced degradation or alternatively referred as stress testing and it demonstrates specificity when developing stability indicating methods, especially when little is known about potential degradation products. Forced degradation study provides information about the degradation pathways and degradation products of the drug substance that could form during storage, transportation. Force degradation study also helps in the elucidation of the structure of the degradation products. Forced degradation study provide the chemical behavior and chemical nature of the molecule which ultimately helps in the development of formulation during manufacturing and packaging specification, thus this review article provide knowledge of the current trends in performance of forced degradation study and establishing the analytical methods that helpful for development of stability indicating method. The stability of drug product and or drug substance is a critical parameter which may affect purity, potency and safety.

KEY WORDS: Degradation, Purity, Potency, Degradants, accelerated conditions, Stress testing.

INTRODUCTION:

It may be defined that Analytical chemistry is the study of separation, quantification and chemical components identification of natural and artificial materials constituted with one or more compounds or elements. Analytical chemistry is separated into two main categories, qualitative analysis that is to say the identification with regard to the chemical components exist in the sample, whereas quantitative analysis estimates the amount of certain element or compound in the substance i.e., sample.

Pharmaceutical analysis plays a very outstanding role in the examination of pharmaceutical formulations and bulk drugs regarding the quality control and assurance. Rapid increase in pharmaceutical industries and production of drug in and around the world bring forward a rise in inevitable demand to seek novel and systematic analytical techniques in the pharmaceutical industries.

As a consequence, analytical method development has become the basic activity of analysis. [1-4]

Forced degradation studies are also named as forced decomposition studies, stress decomposition studies, stress testing, stress studies.[5] According to FDA guidance document, stability indicating method is defined as a validated quantitative analytical procedure that accurately and precisely measure active ingredients (drug substance or drug product) that free from excipients, process impurities and degradation products or other potential impurities.[6]

The FDA and ICH guidance state that the under the influence of various environmental factors the how the quality of a drug substance and drug product changes with time. [7]

Forced degradation involves the exposure of drug substance to heat, heat and humidity and light for solid state studies. For solution state studies the drug substance is exposed to range of pH values. [8]

Exposing the molecules for stability study that help in the selecting the proper formulation (i.e. solid, liquid, and semisolid) and packaging directions, storage conditions and shelf life that is requirement for the regulatory document. [9]

The ICH Guideline States that stress testing is intended to identify the degradation product which helps in determination of the intrinsic stability of the molecule and establishing degradation pathways and validate the stability indicating procedure.[10] before filling in registration dossier, it has become mandatory to perform stability studies of new drug moiety and molecules.[11]

As per the International Committee for Harmonization (ICH) guidelines, the stability of the molecule, different degradative pathways, and validation of the developed stability procedures are studied using forced decomposition studies. The details of drug molecules that undergo degradation and the different products that are formed with respect to time changes under the impact of different environmental parameters and understanding of stability data are well explained using the Food and Drug Administration (FDA) and ICH guidelines. [12, 13]

Two kinds of studies, namely, long-term and accelerated stability studies have been reported. In case of long-term studies, the duration of study is about 12 months while accelerated stability studies take around 6 months. Intermediate stability studies are also conducted for 6 months at conditions milder than accelerated studies. [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.
- More stable formulations are generated by these studies.
- Structure of degradation products are elucidated by these studies.
- Degradation pathways of drug substances and drug products are established by these studies.
- Stability indicating natures of a developed method are established by these studies.
- Determination of the intrinsic stability of the drug substances in the formulation.
- Chemical characteristics of drug molecules are understood by these studies.

Degradation mechanisms such as hydrolysis, oxidation, photolysis or thermolysis of drug substance and drug product are understood by this studies.

Overview of regulatory authorities [16]

ICH Q1B – Photo stability testing of new drug substances and drug products

These methods are used to estimate the photo stability nature of drug molecules normally in the development stage. These guidelines provide knowledge about how to assess the photo stability of molecules that are under study for stability studies. Forced decomposition of drug molecules and their products were described in sections need of forced degradation of drugs and regulatory guidelines, respectively. Forced degradation studies find application for the detection of photolytic degradants in confirmatory studies.[17, 18]

ICH Q1A – Testing of stability for new drug molecules and their products

Intrinsic stability of drug is determined using these guidelines. Q1A Guidelines of Section 2.1.2 of Q1A guidelines. (Under section ICH Q1A-testing of stability for new drug molecules and their products). These guidelines are helpful in designing methods for determining the stability of drugs. According to Q1A, degradation depends on respective drug molecules and the nature of drug products.[1] To conduct these forced decomposition analyses on drug substances and their products several accelerated conditions were mentioned. Those conditions were effects of temperature (>50°C), humidity (≥75% relative humidity), oxidation, photolysis, and diverse range of pH (solution/suspension). [19-21]

ICH Q2B – Validation of analytical procedures: Methodology

The ICH Q2B guidelines provide information about the protocols to be followed for the validation of different analytical protocols. ICH Q2B, Part II, Section 1.2.2 explains about usage of samples for forced degradation studies. It emphasizes that the samples should be subjected to stress under different accelerating conditions such as humidity and heat and further used for the determination of specificity. In addition, these guidelines are useful for the quantitative determination of the degradants produced. [17, 18]

ICH Q3A Impurities in new drug substances

ICH Q3A guidelines provide information about the determination of contaminants present in new drug molecules. This section provides insights about different aspects such as the identification, types and specification of impurities, analytical protocols, and generation of reports. More importantly, if the impurities are either completely absent or present in trace amounts in batch of a new drug molecule is considered helpful to ensure safety toward clinical studies. [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

It is a guideline used in chemistry of active substances. It covers the data for type of studies performed, procedures used, and outcomes thus obtained from the analysis. The Section 2.1.2 explains about the stability testing for API and dosage forms. It contains the data of retest date and expiry date of substances. Development of analytical method, validation of method, degradation pathways, and intrinsic stability are also determined. It also mandates on conducting stability studies for sensitive compounds such as photosensitive and hygroscopic drug. [17]

FDA Guidelines

FDA is providing guidelines for photostability 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 also 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

According to these guidelines, if degradation standards or contaminants are not available, the specificity can be estimated in comparison of the data with the results obtained from the analytes (containing the contaminants or degradative products) using an alternative procedure under the same accelerated conditions. [17]

Japanese Pharmacopoeia

It states that the proposed method should be specific, be able to identify and estimate the amount of analyte present in the sample. For comparative studies, if reference standard impurities are not available, samples will be exposed to stress conditions and degradation products may be used for further studies. [17]

National Health Surveillance Agency (ANVISA)

It mentions about the requirements regarding stability and forced degradation. ANVISA was developed to promote public health and protect from risks caused by the production and use of various drug products. ANVISA coordinates states, districts, and municipalities, according to the Brazilian Unified Health System principles, so as to enhance the quality of life of the people. [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

According to ICH Guideline degradation of drug substances between 5% to20% has been accepted for the validation of chromatographic assays. It is not necessary that forced degradation would result in a degradation products. If no degradation is seen after drug substance or drug product has been exposed to stress condition than stress study should be terminated. It is recommended that maximum of 14 days for stress testing in solution to provide stressed samples for method development.

Strategy for selection of degradation condition

Forced degradation is carried out to produce representative samples for developing stability indicating methods for drug substances and drug products. The criteria of selecting stress condition should be depend upon the products decomposition under normal manufacturing, uses condition and storage specifications which are specific and different for each drug substance and drug product. Stress factors suggested for forced degradation studies include acid and alkali hydrolysis, thermaldegradation, photolysis, oxidation. All force degradation condition mention Table-1. There is no of specification in regulatory guidelines about the conditions of pH, temperature and thermal condition and oxidizing agent used.

Table-1: A general protocol of degradation conditions used for drug substance and drug product shown in below [24]

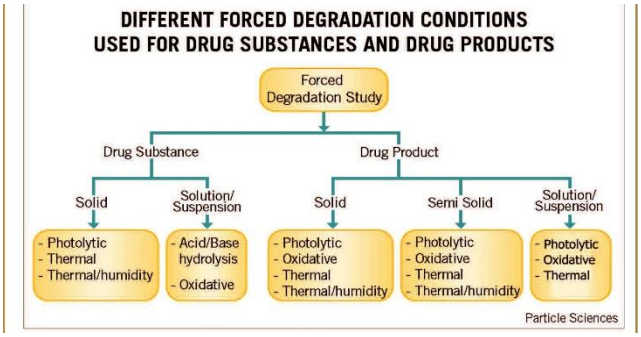

Table-2: Frequently used conditions for forced degradation studies [25]

Type of degradation	Experimental conditions	Storage condition	Sampling time (days)
Hydrolysis	Control API (no acid/base)	40°C, 60°C	1,3,5
	0.1N HCl	40°C, 60°C	1,3,5
	0.1N 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% H2 O2	25°C, 60°C	1,3,5
	Peroxide control	25°C, 60°C	1,3,5
	Azobisisobutyronitrile (ABIN)	25°C, 60°C	1,3,5
	ABIN control	25°C, 60°C	1,3,5
Photolysis	Light 1 X ICH	NA	1,3,5
	Light 3 X ICH	NA	1,3,5
	Light 3 X ICH	NA	1,3,5
Thermolysis	Heat chamber	60°C	1,3,5
	Heat chamber	60°C/75°C % RH	1,3,5
	Heat chamber	60°C	1,3,5

Selection of degradation conditions 7, 8, 12, 19, 21, 26-30

Earlier, intrinsic stability of drugs can be determined using normal conditions such as high temperature and pH. Later, the drug molecules were subjected to additional stress to study the stability. To study the degradation, the solution containing the drug sample was refluxed for a definite time. During this time, if any degradation products were observed, the process would be stopped; further isolation, identification, and characterization of the observed degradation products will be carried out. If no degradation was observed, the reaction time would be increased to observe any signs of degradation due to the extension of time.

Hydrolysis

In hydrolysis, the drug reacts with water under different pH conditions (both acidic and alkaline). In general, the drug substances are treated with 0.1N hydrochloric acid or sulphuric acid or 0.1N sodium hydroxide at 40–60°C. The stability of molecule depends on the strength of acid or alkali used in the study. The strength of acid or alkali should be maintained between 0.1 N and 1 N solution. Notably, the duration of the study should not exceed 7 days. After subjected to stress conditions, the samples should be neutralized with buffer or acid/base to avoid decomposition See Table-2.

Oxidation

Most of the drug substances are found to be auto-oxidizers. They require free radical initiators for oxidation process. Hydrogen peroxide, trace level of impurities, and metal ions act as free radical initiators. This type of degradation involves the transfer of electrons. 0.1–3% of hydrogen peroxide is a common initiator for oxidation forced degradation studies. These studies should be conducted at 4.0°C for 1–7 days. If more than 20% degradants are produced, then it should be considered as abnormal See Table-2.

Thermal condition

Several drugs are seen to be thermo labile in nature. By increasing the temperature, the rate of reaction also tends to increase which in turn leads to the production of degradation products. These studies should be conducted at 40–80°C. The duration of thermal stress studies usually lasts for 1–2 months and are conducted at 70°C and at high humidity. The drug molecules which are solid in nature are subjected to both dry and wet heat conditions, while liquids are exposed to dry heat for the shorter duration of time. Due to the elevated temperature, the drug molecule undergoes degradation and given by Arrhenius equation

See Table-2:

k = Ae-Ea/RT

Where k: Specific reaction rate, A: Frequency factor, Ea: Energy of activation, R: Gas constant (1.987 cal/ deg/mole), and T: Absolute temperature in Kelvin.

Photolytic conditions

In photolytic degradation studies, the drug substances are exposed to UV or fluorescent conditions. In this study, the drug substances or drug products (solid/ liquid) are exposed to the light source according to the ICH Q1B protocols. The commonly used radiation range for degradation studies is about 300–800 nm. In photolytic condition, the degradation occurs due to oxidation through free radical mechanism or non- oxidation process. Non-oxidative degradation process involves with isomerization, dimerization, etc., among others. On the other hand, oxidative photolytic reaction involves mechanism involving singlet/triplet oxygen states. Singlet oxygen reacts with unsaturated compounds to produce photooxidative decomposition products, while triplet oxygen follows free radical mechanism, to produce a peroxide. Notably, it is shown that light also catalyzes oxidation reactions. In non-oxidative process, several types of reactions are observed such as the homolytic breakage of C-X hetero bonds, deamination, and cleavage of C-S bonds are observed See Table-2.

Humidity

Humidity plays an important role in degradation process. In forced degradation studies, the drug substance is exposed to 90% humidity for 1 week which tends to cause degradation. Humidity is one of the important parameters to establish the possible degradants in finished products and API.

Aspect of degradation

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

1. Moisture

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

2. Excipients

It was observed that some excipients may contain high content of water. This moisture may lead to increased water level in formulation which later affects the stability of the drug. In some cases, Chemical interactions that occur between the excipients and the drug material often results in decreased Stability. 28

3. Temperature

Changes in temperature at times show deleterious effect on the stability of the drug. Increase in temperature usually causes increases the rate of drug hydrolysis. 28, 29

4. pH

pH shows a significant effect on the degradation rate of drugs by hydrolysis. To reduce this effect, formulations of the drugs are carried out using buffer solutions of pH with maximum stability. 28, 29

5. Oxygen

Presence of oxygen increases the oxidation in some drugs. Drugs with increased rate of decomposition in the presence of oxygen are stabilized by purging nitrogen or carbon dioxide in the storage container. 28, 29

6. Light

Some drugs are photolabile 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 and stability when stored under dark. It is to be remembered that the photolabile compounds should be stored in amber glass containers and should be stored in the dark. 28, 29

Sample preparation in degradation study

During forced decomposition and stability studies, active pharmaceutical ingredient is subjected to various stresses under accelerated conditions such as photolytic, thermal, oxidative, and hydrolytic conditions. Due to stress conditions, several degradation products are expected to be produced, which can be compared to the degradative products (if any) that are obtained from regular storage conditions.7

Hydrolytic Conditions

Drugs molecules are dissolved in hydrochloric acid or sulfuric acid (0.1–1 M) in acid hydrolysis. In base hydrolysis drug molecules are dissolved in 0.1–1 M of potassium hydroxide or sodium hydroxide. Samples are subjected to stress for 2–7 days at room temperature. Stressed samples were neutralized with relevant acids or bases to prevent additional degradation.

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

Sample solutions that are subjected to photolytic stress by exposing them to as minimal as of 1.2 million × l h and 200 W h/m2 light of 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

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

Type of study with Storage conditions (ICH Stability guidelines)

Long term 25°C ± 2°C/ 60% RH ± 5% RH or 30°C ± 2°C/ 65% RH ± 5% RH12 months

Intermediate 30°C ± 2°C/ 65% RH ± 5% RH 6 months

Accelerated 40°C ± 2°C/ 75% RH ± 5% RH 6 months

Drug products intended for storage in refrigerator (ICH Stability guidelines)

Long term 5°C ± 3°C 12 months

Accelerated 25°C ± 2°C/ 60% RH ± 5% RH 6 months

Drug products intended for storage in freezer

Long term -20°C ± 5°C 12 month

Stability indicating method

FDA defines the stability indicated method as a quantitative method and monitors how the concentration of the drug will get affected with respect to change in time. The decrease in concentration of drug present and drug product will be determined. Notably, the concentration of the drug molecules is shown to vary during the degradation studies. It is observed that the concentration of the drug substance changes during the degradation studies; notably, no interference is observed from the excipients or other degradation products. Hence, the SIM helps in preformulation studies and also to predict the storage conditions of the drug.

Stability indicating method development and optimization

Before developing a method, the first step is to determine the pKa value, log P, solubility, and λ of the respective drug. Development of a reverse phase method using HPLC is a common practice for the separation drugs. The commonly used solvents such as methanol, acetonitrile, and water are used as mobile phases in different combinations and proportions. With respect to the solubility profile of the drug, the organic phase such as methanol or acetonitrile is chosen. The choice of the mobile phase and its proportion is usually determined from earlier reports or by trial and error methods. At the onset of the experiment, the organic and aqueous phases are maintained at 50:50, and further optimization can be done on the proportions of the solvents for the mobile phase such that an ideal resolution of the peaks are obtained. In certain cases, buffers can be used for good baseline separation and peak symmetry. At times, the column temperature is adjusted to 30–40°C to get good reproducibility of the results. Degradant peaks are pushed in the chromatogram to get good resolution. Sometimes degradants peaks elute along with the drug peak or hidden by drug peaks, which in turn leads to peak purity analysis. Direct analysis can be done using HPLC that are equipped with PDA detectors. By changing the proportion of the mobile phase, it becomes easier to resolve and analyze the degradants peaks. The method developed is considered as homogeneous if the degradants peak is observed where the area under the curve of drug peak and its percentage are not affected. These degradants, which coelute with drug, are acceptable to some extent provided; they were not observed in accelerated and long-term storage studies. Further, the method can be optimized by modifying the parameters such as the rate of flow of mobile phase, volume of sample injected, type of column used, and by changing the proportion of the mobile phase used in the analysis. After optimization of these parameters, the method developed for the study will be subjected to validation as per the ICH guidelines. 7

Characterization of degradants

Earlier reports have shown that multiple analytical techniques are available to isolate, identify, and characterize the impurities that are produced in the degradation studies even at a very low concentration. The degradants isolated in the study were identified and characterized by hyphenated methods such as LC–MS and LC–nuclear magnetic resonance spectroscopy (LC– NMR).7 More importantly, the structural characterization of the degradants/impurities become necessary as they play a vital role in the determination of shelf-life stability. Detection of impurities can be done by thin layer chromatography (TLC), electrophoresis, colorimetric, and gel filtration techniques, while separation and isolation of degradants in pure form can be done using reversed-phase HPLC, TLC, gas chromatography, and supercritical fluid chromatography. Notably, the determination of degradants pathways is carried out using LC-MS/MS technique. The degradative pathways can be determined on the basis of fragmentation patterns that are observed. After determination of degradant pathways, structure elucidations of degradants are done by synthesizing or isolating methods and further characterized by employing LC-MS, LC-UV, and LC- NMR techniques. 17,7,13,26,32,33

Method validation

The developed stability indicating method is then validated

according to ICH guideline for specificity, accuracy, precision, detection limit, quantitation limit, linearity, range, robustness of the method. It is required to isolate, identify and quantitate the degradants found to be above identification threshold (usually 0.1%). If the method does not fall within the acceptance criteria for validation, the method is modified and revalidated. 34, 35

CONCLUSION

Forced degradation studies of the new drug substances and drug products are important to help for developing and for determining specificity of stability indicating methods and also helps to determine the degradation pathways and degradation products of active ingredients and structure elucidation of the degradants. They were also useful in the investigation of the chemical and physical stability of crystal forms, the stereochemical stability of the drug substance alone, mass balance issues in formulations. It is better to start degradation studies earlier in the drug development process to have sufficient time to gain more information about the stability of the molecule. This information will further helpful in the formulation manufacturing process and determine the storage condition. As there is no specific regulatory guidance for forced degradation, it is recommended to use appropriate conditions to achieve 5-20% degradation.

Authors Contribution statement

Ms. Dharti Patel and Ms. Miral Patel conceptualized and gathered the data with regard to this topic. Dr. Keyur Ahir and Dr. Sumer Singh reviewed these data and provided necessary inputs towards the designing of the manuscript. All authors were actively contributed towards the final manuscript.

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