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IMPORTANCE OF FORCED DEGRADATION STUDY IN PHARMACEUTICAL INDUSTRY— A REVIEW

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ABSTRACT

Forced degradation is the process or state in which new drug substance and drug products are with the conditions which are more severe than accelerated stability conditions. The stability of drug substance is a critical parameter which may affect purity, potency and safety. Changes in drug stability can risk patient safety by formation of a toxic degradation product or deliver a lower dose than expected. Therefore it is essential to know the purity profile and behavior of a drug substance under various environmental conditions. The real and accelerated stability of the dosage forms are generally studied in pharmaceutical industry but force degradation study has its importance before starting a new formulation. It is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation pathways of degradation products of the drug substance and helps in elucidation of the structure of the degradation products. Forced degradation studies show the chemical behavior of the molecule which in turn helps in the development of formulation and selection of packaging material. ICH guidelines demonstrate certain degradation conditions like light, oxidation, dry heat, acidic, basic, hydrolysis etc. ICH Q1A, QIB and Q2B exemplify the forced degradation studies. the present review discusses the current process and scenario in performance of forced degradation studies, its method and its importance in new formulation development.

KEYWORD: Degradation condition, Degradation product, Forced degradation, Stability.

INDRODUCTION

A forced degradation study also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc. is the intentional degradation of the API or Drug Product to an appropriate extent by means of various stress conditions such as pH, temperature, light, oxidizing agents, mechanical stress etc. According to FDA guideline, a Force Degradation Study is defined as a validated analytical procedure that accurate and precisely measure active ingredients (drug substance or drug product) free from process impurities, excipients and degradation products. It is a quantitative test method that can detect possible degradants and impurities of drug substance (API) and drug products, normally using HPLC.[18] The main objective of stability indicating method is to monitor results during stability studies in order to guarantee safety, efficacy and quality. Before performing studies, a stability method is necessary so that any possible degradants generated during storage conditions (such as 30°C/60% RH and 40°C/75% RH) can be separated, detected and quantified. Forced degradation studies are used to identify reactions which may occur to degrade a processed product. It is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The stability studies include long term studies (12months) and accelerated stability studies (6months). As compared to stability studies, forced degradation studies help in generating degradants in much shorter span of time, mostly a few weeks.[15] 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. As stated by United States Food and Drug Administration guidelines, a Stability-Indicating method] is defined as a validated analytical procedure that accurately and precisely measures active ingredients free from potential interferences like degradation products, process impurities, excipients, or other potential impurities, and the FDA recommends that all assay procedures for stability studies be stability indicating. The definition in the draft guideline of 1998 read as Validated Quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference. Stability-Indicating Method are validated quantitative test methods that can detect changes with time in the chemical, physical, or microbiological properties of drug substances or drug products. They are specific so that the quantity of the active ingredient,

degradation products and other components of interest may be accurately measured without interference in the material being tested. A degradation product is a molecule resulting from a change in the active ingredient brought about over time as a result of processing or storage. The regulation requires a formal written stability testing program whose results are used to establish storage conditions and expiration dates of drug products and further mandates the use of reliable, meaningful, and specific test methods. A drug application is expected to contain a full description of the drug substance or drug product including its physical and chemical characteristics and stability as well as such specifications and analytical methods as are necessary to assure the identity, strength, quality, purity and bioavailability of the drug product, and stability data with proposed expiration dating. If such documentation is generated to support a regulatory submission such as an Investigational New Drug Application (IND), Drug Master File (DMF) or generated to satisfy cGMP requirements for a non-application drug substance or drug product. These data are used to establish, confirm or extend retest intervals (usually) or expiration dating periods (if unstable) for drug substances and expiration dating periods for drug products.[10]

FORCED DEGRADATION STUDIES

The ICH guideline Q1A on Stability Testing of New Drug Substances and Products gives indications for the testing of parameters which are may be susceptible to change during long storage and are likely to affect quality, safety and efficacy must be done by validated stability indicating testing methods. It is mentioned that forced degradation studies or stress testing at temperatures in 10 °C increments above the accelerated temperatures, extremes pH and under oxidative and photolytic conditions have to be carried out on the drug substance so to set up the stability characteristics and degradation pathways to back up the suitability of the proposed analytical procedures.

Objective of forced degradation studies

Forced degradation studies are carried out to achieve the following purposes:[14, 12, and15]

1. To establish degradation pathways of drug substances and drug products.
2. To differentiate degradation products that is 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 establish degradation pathways and intrinsic stability of the drug molecule.
11. To validate stability-indicating analytical procedures.
12. To identify impurities related to drug substances or excipients.
13. To distinguish degradation products in formulations that is related to drug substances from those that are related to non-drug substances (e.g., excipients).
14. To solve stability-related problems (e.g., mass balance).
15. To generate a degradation profile that mimics what would be observed in a formal stability study under ICH conditions.
16. To facilitate improvements in the manufacturing process and formulations in parallel with accelerated pharmaceutical studies".
17. To choose the correct storage conditions, appropriate packaging and better understanding of the potential liabilities of the drug molecule chemistry".[16]
18. To facilitate improvements in the manufacturing process and formulations in parallel with accelerated pharmaceutical studies.[16]

Regulatory overview

ICH guidelines - regulatory overview

The ICH (The International Committee for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) has achieved harmonization in many areas of quality e.g. in conducting of stability studies or in providing definition of relevant thresholds for impurity testing. ICH has published several guidelines which have been discussed, agreed upon and adopted by the regulatory authorities of the ICH regions United States, Europe and Japan. When it comes to the topic "forced degradation" the most ICH guidelines emphasize the importance of conducting forced degradation studies, but provide only very general and limited information on the experimental stress conditions and only general guidance on how to conduct forced degradations studies. For example, the guidelines do not provide specific information and recommendations on the stress conditions e.g. pH,

temperature ranges, specific oxidizing agents, or conditions to use. Furthermore, the guidelines mostly refer to new drug substances and drug products and do not refer to drug substances and clinical development.

Following ICH guidelines are in place and applicable when searching for guidance with regard to conducting forced degradation studies.

ICH Q1A – Stability Testing of New Drug Substances and Products.[16, 4]

ICH Q1B – Photo stability Testing of New Drug Substances and Products.[2]

ICH Q2B – Validation of Analytical Procedures: Methodology.[3]

ICH Q3A – Impurities in New Drug Substances.[16]

ICH Q3B – Impurities in New Products.[16]

M4Q (R1) – The common Technical Document (CTD).[9]

ICH Q1A – Stability Testing of New Drug Substances and Products.[16, 4]

In ICH Q1A (Stress Testing), there are recommended conditions for performing forced degradation studies on drug substances and drug products. The condition are to examine the effects of temperature (above that for accelerated testing, i.e. (>50°C), humidity (≥75% RH), oxidation and photolysis. Testing in solution should also be performed across a wide pH range either as a solution or suspension. These samples are then used to develop a stability-indicating method.

ICH Q1B – Photo stability testing of New Drug Substances and Products.[2]

ICH Q1B gives recommended approaches to assessing the photo stability of drug substances and drug products. Forced degradation conditions are specified in Section II (drug substance) and Section III (drug product). Exposure levels for forced degradation studies are not defined, although they can be greater than that specified for confirmatory (stability) testing. The actual design of photo stability studies is left to the applicant; however, scientific justification is required where light exposure studies are terminated after a short time, e.g., where excessive degradation is observed. Photo stability testing can be performed on the solid or in solution/suspension. These samples are then used to develop a stability indicating method. Both guidance's, Q1A and Q1B, note that some of the degradation products formed during forced degradation studies may not actually be observed to form during stability studies, in which case they need not be examined further.

ICH Q2B–Validation of Analytical Procedures.[3]

ICH Q2B gives guidance on how to validate analytical methodology and in section B (impurities not available) there is a recommendation to use samples from forced degradation studies to prove specificity. Specificity is a key factor in determining whether or not the analytical method is stability indicating. Co-elution of peaks or components being retained on the column will underestimate the amount of degradation products formed and could compromise quality and increase risk to the patient.[3]

Q3A (R2) requires identification of each impurity with respect to both chemistry and safety perspectives. The chemistry perspectives include classification and identification of impurities, report generation, listing of impurities in specification and a brief discussion of analytical procedures while the safety perspectives include specific guidance for qualifying those impurities that were not present or were present at substantially lower levels in batch of a new drug substance and used in safety and clinical studies.[13]

Different forced degradation conditions utilized for drug substances and finished dosage form is shown in the "Fig. 1"[20] and Conditions commonly applied for forced degradation is tabulated in Table 1".

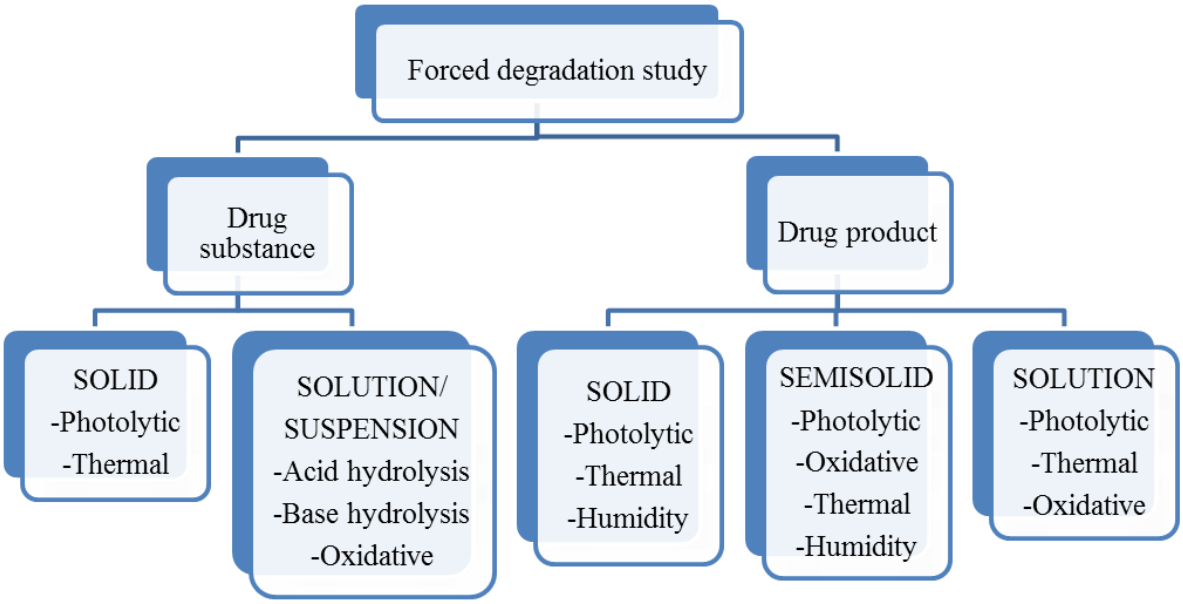

Table 1: Condition usually used for forced degradation study.[10]

Degradation Type	Experimental condition	Storage condition	Sampling time
Hydrolysis	0.1N HCL	40°C, 60°C	1,3,5 days
	0.1N NaOH	40°C, 60°C	1,3,5 days
	pH:2,4,6,8	40°C, 60°C	1,3,5 days
Oxidative	3%H_2O_2	25°C, 60°C	1,3,5 days
	Peroxide control	25°C, 60°C	1,3,5 days
	Azobisisobutyronitrile(AIBN)	40°C, 60°C	1,3,5 days
Photolytic	Light,1 X ICH	NA	1,3,5 days
	Light,3 X ICH	NA	1,3,5 days
	Light control	NA	1,3,5 days
Thermal	Heat environment	60°C	1,3,5 days
	Heat environment	60°C/75%RH	1,3,5 days
	Heat environment	80°C	1,3,5 days
	Heat environment	80°C/75% RH	1,3,5 days
	Heat control	Room temperature	1,3,5 days

Appropriate Time to perform forced degradation

The forced degradation studies are not performed earlier it is very vital to conduct them during the phase III (FDA guidance states) to demonstrate the stability of a drug substance, potential degradation pathways and capability and suitability of proposed analytical procedures. Stress studies should be done in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels to determine the stability of the drug substance. The forced degradation studies conducted in a single batch. These studies are most useful if done initially in early development or phase I clinical trials which provides timely recommendations for improvement in the manufacturing process, ensure there is sufficient time for degradation product identification, proper selection of stability indicating analytical technique, degradation product identification and optimization of stress conditions which will help later in manufacturing process.

Limit of degradation

Usually degradation of drug substance between 5-20% is considered as reasonable and acceptable for validation of chromatographic assays. Some Pharmaceutical scientists have agreed that approximately 10% degradation is optimal for use in analytical validation. For small pharmaceutical molecules for which acceptable stability limits of 90% of label claim is common. In the event that the experimental conditions generate no or little degradation due to the experimental stability of the molecule, an evaluation should be made to verify if the drug

substance has been exposed to energy in excess of the energy provided by accelerated storage (i.e., 40°C for six months).[10]

Selection of drug concentration

Which concentration of the drug should be used for the degradation study has not been specified in regulatory guidance. It is recommended that the studies should be initial concentration of 1 mg/ml. By using drug concentration of 1mg/ml it is generally possible to get even minor decomposition products in the range of detection. It is also suggested that some degradation studies should be done at a concentration which the drug is expected to be present in the final formulations.[15]

Degradation condition:

Typical stress tests include four main degradation mechanisms: heat, hydrolytic, oxidative, and photolytic degradation. Selecting suitable reagents such as the concentration of acid, base, or oxidizing agent and varying the conditions (e.g., temperature) and length of exposure can achieve the preferred level of degradation. Over stressing 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.[6]

To know how much degradation is enough in stress testing forced degradation can be Classified into following types.[10]

1. Deceptive**:** Good degradation level (<15%) without any relevant degradants.
2. Predictive: Good degradation level (<15%) with one or more relevant degradants.
3. Useless**:** Between 15 to 100% degradation without any relevant degradants

1. Hydrolytic condition

Hydrolytic degradation (Acidic and basic hydrolysis): Hydrolytic degradation is one of the most frequent degradation chemical reactions over a wide range of pH. Hydrolysis is a chemical process that includes decomposition of a chemical compound by reaction with H2O. Hydrolytic degradation under acidic and basic condition involves catalysis of ionizable

functional groups present in the molecule. Base or acid degradation testing involves forced degradation of a drug substance by exposure to acidic or basic conditions 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. HCl or H2SO4 (0.1 to 1 M) for acid hydrolysis and NaOH (or) KOH (0.1–1 M) for base hydrolysis are suggested as suitable reagents for hydrolysis. If the compounds for stress testing are poorly soluble in water, then co-solvents can be used to dissolve them in hydrochloric acid or Sodium hydroxide. Stress testing trial is normally started at room temperature and if there is no degradation, elevated temperature (50 – 70 °C) is applied. Stress testing should not exceed more than seven days. The degraded sample is then neutralized using suitable acid, base or buffer, to avoid further decomposition.[14, 8]

Procedure

For Acid hydrolytic study reflux with 0.1 N HCL at 60°C for 30 minutes. For Base stress reflux with 0.1N NaOH at 60°C for 30 min. For water stress Reflux with water at 60°C for 30 minutes.

2. Oxidative degradation

Hydrogen peroxide is widely used for oxidation of drug substances in forced degradation studies but other oxidizing agents such as metal ions, oxygen, and radical initiators (e.g., azobisisobutyroni-trile, AIBN) can also be used. Selection of an oxidizing agent, its concentration, and conditions depends on the drug substance. It is reported that subjecting the solutions to 0.1–3% hydrogen per-oxide at neutral pH and room temperature for seven days or up to a maximum 20% degradation could potentially generate relevant degradation products.[15] The oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations. Amines, sulfides and phenols are susceptible to electron transfer oxidation to give N-oxides, hydroxylamine, sulfones and sulfoxide.[7] The functional group with labile hydrogen like benzylic carbon, allelic carbon, and tertiary carbon or α-positions with respect to heteroatom is susceptible to oxidation to form hydro peroxides hydroxide or ketone.[14]

Procedure

Treat with 3% H_2O_2 at less than 30°C for 30 min. The oxidative stress testing is initially carried out in 1% H_2O_2 at room temperature for 6 hr and it can be increased/ decreased to achieve sufficient degradation. Stress agent is changed to achieve degradation if necessary.

3. Thermal degradation

Thermal degradation (e.g., dry heat and wet heat) should be carried out at more strenuous conditions than recommended ICH Q1A accelerated testing conditions. Samples of solid-state drug sub- stances and drug products should be exposed to dry and 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.[19]

$$A = ke^{-Ea/RT}$$

Where k is specific reaction rate, A is frequency factor, Ea is energy of activation, R is gas constant (1.987cal/degmole) and T is absolute temperature. Thermal degradation study is carried out at 40–80°C.[14, 15]

Procedure

Thermal degradation can be conducted based on physical properties of API i.e. Melting Point. If melting point of API is less than 150°C, stress at 105°C or 40°C less than melting point whichever is higher. If melting point of API is more than 150°C stress at the nearest melting point and at 105°C.

4. Photolytic degradation

Photo stability studies are performed to generate primary degradants of drug substance by exposure to ultra violet or fluorescent conditions. The rate of photo degradation depends upon the intensity of incident light and quantity of light absorbed by the drug molecule. Samples of drug substance and solid/liquid drug product should be exposed to a minimum of 200 W h/m2 light. The most commonly accepted wavelength of light is in the range of 300 - 800 nm to cause the photolytic degradation. Light stress conditions can induce photo oxidation by free radical mechanism. ICH guideline options requirements widely regarded. UV exposure - NLT 200 watts (sq meters). Visible exposure- NLT 1.2 Million lux-hrs. Functional groups like carbonyls, nitro aromatic, N-oxide, alkenes, aryl chlorides, weak C-H and O-H bonds, sulfides and polyenes are likely to introduce drug photosensitivity. Options per ICH Q1B: Any light source with output similar to D65/ID65 emission standard, such as (i) artificial daylight fluorescent lamp combining visible and UV outputs (ii) xenon lamps or (iii) metal-halide lamps. A cool white fluorescent lamp per ISO 10977 and a near UV fluorescent lamp

having a spectral distribution 320 - 400 nm with a maximum energy emission 350 - 370 nm. A significant portion should be in both bands 320 - 360 nm and 360 - 400 nm.[14]

Procedure

Expose the tablet powder/content of capsule to intense ultraviolet radiation (both at longer and shorter wavelengths) up to minimum of 7 days in UV cabinet.

Acceptance criteria

All requirements of the software are to be met while evaluating peak purity. The purity angle should be less than purity threshold.[5] Peak purity not less than 0.995.[21] If peak purity not observed within the limit this molecule is sensitive for specific condition.

Mass balance of all stressed samples shall be verified by calculating

Mass balance: (% assay of stressed sample +% impurities) X 100/ % assay of unstressed sample.

-mass balance is to be achieved at least up to 95% levels.[11]

-if the mass balance is less than the required criteria investigation to be done and justified.[11]

Analytical tools for separation and identification of degradant

A. Convectional technique[15]

1. Thin layer chromatography (TLC).
2. Solid phase extraction (SPE)
3. Accelerated solvent extraction (ASE)
4. Low-pressure Liquid Chromatography (Flash chromatography)
5. Supercritical fluid extraction (SFE) countercurrent chromatography (CCC)
6. Mass Spectrometry (MS).
7. NMR: NMR spectroscopy is an extremely powerful tool for the analysis of drug degradation products. In order to perform NMR-based structure elucidation of drug degradant products, it is common practice to isolate sufficient material (>1 mg) for NMR analysis.

8. HPLC

HPLC is routine technique for separation of degradants. The normal UV HPLC detectors these days allow for simultaneous measurement at multiple wavelengths, and some of them

even give output of ratio plots at two wavelengths. This technique has also been promoted for peak purity testing during development of SIMs.

B. Hyphenated technique[15]

1. GC-MS.
2. LC-MS.
3. Capillary Electrophoresis- Mass Spectrometry (CE-MS).
4. Liquid chromatography-Fourier Transfer Infrared (LC-FTIR)
5. LC-NMR: The advantages of using NMR in combination with HPLC in comparison to HPLC-MS coupling are (1) both HPLC and NMR are conducted in solution and no transfer from one phase to another, as from the liquid to vapour phase in HPLC-MS; (2) NMR measurements are not limited by vaporization and hence by molecular weight; (3) in many cases the structure information by NMR spectra is more extensive, especially when the stereochemistry of the molecule is considered.

Evaluation of forced degradation

Peak purity

Peak purity is comparison of the reference standard to the API in the sample stressed by "forced degradation". it is used as a support in stability indicating method development. The spectral uniqueness of a compound is used to establish peak purity when co eluting compounds are present. Limitations to peak purity arise when co eluting peaks are spectrally similar, or below the detection limit, or a peak has no chromophore, or when they are not resolved at all.

Mass Balance

Mass balance is calculated by adding the assay value and the amounts of impurities and degradants to evaluate the closeness to regulatory guidance for forced degradation, It is recommended to use appropriate conditions to achieve 5-20% degradation. Success to formulation depends on degradation study and it absolutely depends on skill of researcher, so force degradation study is very important tools for the new formulation development.

CONCLUSION

Forced degradation studies provide knowledge about possible degradation pathways and degradation products of the active ingredients and help elucidate the structure of the degradants. It is essential to help to develop and demonstrate specificity of stability-

indicating methods. Forced degradation is important part of the formulation development process as it provides knowledge about the degradation, chemistry of drug substances and drug products. This knowledge is used to develop analytical method, formulation development, packaging development and the design of the official stability studies. They were also useful in the investigation of the chemical and physical stability of crystal forms, the stereo chemical stability of the drug substance alone and in the drug product and mass-balance issues, and for identifying drug related degradation products in formulations. Stress testing has played a critical role in the drug development process, there is no formal regulatory guidance for forced degradation, it is recommended to use appropriate conditions to achieve 5-20% degradation. Success to formulation depends on degradation study and it absolutely depends on skill of researcher, so force degradation study is very important tools for the new formulation development.

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