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Review Article

Review: Development of forced degradation studies of drugs

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Abstract

Forced degradation studies show the chemical behavior of the molecule which in turn helps in the development of formulation and package. A forced degradation study is an essential step in the design of a regulatory compliant stability program for both drug substances and products, and formalized as a regulatory requirement in ICH Guideline Q1A in 1993. Forced degradation is a degradation of new drug substance and drug product at conditions more severe than accelerated conditions. It is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation pathways and degradation products of the drug substance and helps in elucidation of the structure of the degradation products. Thus, this review discusses the current trends in performance of forced degradation studies by providing a strategy for conducting studies on degradation mechanisms.

1. Introduction

Forced degradation studies are also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc. Forced degradation 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 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 [1]. But 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 area regulatory requirement and scientific necessity during drug development, it is not considered as a requirement for formal stability program.

It has become mandatory to perform stability studies of new drug moiety before filing in registration dossier. The FDA and ICH guidances state the requirement of stability testing data to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors. The stability studies include long term studies (12months) and accelerated stability studies (6months).But intermediate studies (6months) 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. As compared to stability studies, forced degradation studies help in generating degradants in much shorter span of time, mostly a few weeks.

2. Objective of forced degradation studies

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

1. To establish degradation pathways of drug substances and drug products.
2. To differentiate degradation products that are related to drug products from those that are generated from non-drug product in a formulation.

3. To elucidate the structure of degradation products.

4. To determine the intrinsic stability of a drug substance in formulation.

5. To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and drug product [1,2].

6. To establish stability indicating nature of a developed method.

7. To understand the chemical properties of drug molecules.

8. To generate more stable formulations.

9. To produce a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.

10. To solve stability-related problems [3]

3. Overview of Regulatory Guidance

Force degradation studies are described various International guidelines. The International committees for Harmonization of Technical Requirements for Registration of pharmaceutical Human use (ICH) has published a set of guidelines which have been discussed, agreed upon and adopted by the American, European and Japanese regulatory authorities. In the majority of cases, the **ICH guidelines** only apply to the marketing applications for new products, i.e they do not apply during clinical development. However, since the conditions used for forced degradation are only defined in general terms, it is possible to apply them for developing stability indicating methods during clinical development. The same forced degradation conditions can then be applied to the drug substance during development and commercialization. The ICH guidelines that are applicable to forced degradation studies are [4-8]

ICH Q1A Stability Testing of New Drug Substances and Products

ICH Q1B Photo stability Testing of New Drug Substances and Products

ICH Q2B Validation of Analytical Procedures: Methodology

In ICH Q1A, section 2.1.2 (Stress Testing), there are recommended conditions for performing forced degradation studies on drug substances and drug products. The recommendations are to examine the effects of temperature (above that for accelerated testing, i.e., >50 C), humidity (75% relative humidity), 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** 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 guidances 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** gives guidance on how to validate analytical methodology and in section B 1.2.2 (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. **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 [9-15].

3.1 Forced degradation performed: It is very important to know when to perform forced degradation studies for the development of new drug substance and new drug product. FDA guidance states that stress testing should be performed in phase III of regulatory submission process. 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. These stress studies are conducted on a single batch. The results should be summarized and submitted in an annual report. However, starting stress testing early in preclinical phase or phase I of clinical trials is highly encouraged and should be

conducted on drug substance to obtain sufficient time for identifying degradation products and structure elucidation as well as optimizing the stress conditions. An early stress study also gives timely recommendations for making improvements in the manufacturing process and proper selection of stability-indicating analytical procedures [16,17].

3.2 Origin of degradation products: The main reason of appearance of impurities in drug substance or product is due to its degradation. The chemical instability of the drug substance under the conditions of heat, humidity, solvent, pH, and light encountered during manufacture, isolation, puri cation, drying, storage, transportation, and/or formulation is main cause of its degradation. It is governed by inherent chemical stability of the drug substance. The major routes of degradation of any drug substance include hydrolysis, oxidation, heat and photolysis. The stress testing helps in generation all possible degradation products that may form under different conditions [4].

3.3 Selection of experimental conditions: There are many examples in the literature of experimental conditions for conducting forced degradation studies and the structural multiplicity of drug molecules makes it not possible to identify a generic set of conditions for a forced degradation study. For an early phase molecule, using a set of normal conditions by first intention makes sense since very little may be known about the intrinsic stability. If early stability data are available which suggest the molecule is labile at a particular condition (e.g., high pH), the conditions can be modified to take into account the instability (e.g., reduced temperature or time of study). Once a set of conditions have been found, they may be repeated whenever a new stability-indicating method is required during development. Therefore, for later-phase molecules, the forced degradation conditions are defined by the earlier work. By reprocess the same forced degradation conditions throughout development a consistent approach is maintained. Some conditions mostly used for forced degradation studies are presented in Table 1.

Table 1 Conditions mostly used for forced degradation studies

Degradation type	Experimental conditions	Storage conditions	Sampling time (days)
Hydrolysis	Control API (no acid or base) 0.1M HCl 0.1 M NaOH Acid control (no API) Base control (no API) pH: 2,4,6,8	40ºC, 60º C 40ºC, 60º C 40ºC, 60º C 40ºC, 60º C 40ºC, 60º C 40ºC, 60º C	1,3,5 1,3,5 1,3,5 1,3,5 1,3,5 1,3,5
Oxidation	3%H_2O_2 Peroxide control Azobisisobutyronitrile (AIBN) AIBN control	250C, 600 C 250C, 600 C 40ºC, 60º C 40ºC, 60º C	1,3,5 1,3,5 1,3,5 1,3,5
Photolytic	Light 1 × ICH Light 3 × ICH Light control	NA NA NA	1,3,5 1,3,5 1,3,5
Thermal	Heat chamber Heat chamber Heat chamber Heat chamber Heat control	60 ^{0}C 60 ^{0}C/75% RH 80 ^{0}C 80 ^{0}C/75% RH Room temp.	1,3,5 1,3,5 1,3,5 1,3,5 1,3,5

4. Degradation conditions

4.1 Hydrolytic conditions: Hydrolysis is one of the most common degradation chemical reactions over a wide range of pH. Hydrolysis is a chemical process that includes decomposition of a chemical compound by reaction with water. Hydrolytic study under acidic and basic condition involves catalysis of ionizable functional groups present in the molecule. Acid or base stress 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. Hydrochloric acid or sulfuric acids (0.1–1 M) for acid hydrolysis and sodium hydroxide or potassium hydroxide (0.1–1M) for base hydrolysis are suggested as suitable reagents for hydrolysis [8,18]. If the compounds for stress testing are poorly soluble in water, then co-solvents can be used to dissolve the min HCl or NaOH. The selection of co-solvent is based on the drug substance structure. Stress testing trial is normally started at room temperature and if there is no degradation, elevated temperature (50–70 1C) is applied. Stress testing should not exceed more than 7days. The degraded sample is then neutralized using suitable acid, base or buffer, to avoid further decomposition.

4.2 Oxidation conditions: 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.,azobisisobutyronitrile, 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 upto a maximum 20% degradation could potentially generate relevant degradation products [18]. 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 [19]. The functional group with labile hydrogen like benzylic carbon, allylic carbon, and tertiary carbon or α-positions with respect to hetroatom is susceptible to oxidation to form hydroperoxides, hydroxide or ketone.

4.3 Photolytic conditions: The photo stability testing of drug substances must be evaluated to demonstrate that a light exposure does not result in unacceptable change. Photo stability studies are performed to generate primary degradants of drug substance by exposure to UV or fluorescent conditions. Some recommended conditions for photostability testing are described in ICH guidelines [20]. Samples of drug substance and solid/liquid drug product should be exposed to a minimum of 1.2million lx h and 200 W h/m^2 light. The most commonly accepted wavelength of light is in the range of 300– 800 nm to cause the photolytic degradation[21,22]. The maximum illumination recommended is 6 million lx h. Light stress conditions can induce photo oxidation by free radical mechanism. Functional groups like carbonyls, nitroaromatic, N-oxide, alkenes, aryl chlorides, weak C–H and O–H bonds, sulfides and polyenes are likely to introduce drug photosensitivity.

4.4 Thermal conditions: 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 [18]. Effect of temperature on thermal degradation of a substance is studied through the Arrhenius equation:

$$k = Ae^{-Ea/RT}$$

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^oC.

4.5 Humidity: Humidity is the Key factor in establishing the potential degradants in the finished product and active pharmaceutical ingredient. Normally 90% Humidity for duration of one week shall be recommended for the establishment of forced degradation samples.

6. Selection of samples

The strength and duration of the stress conditions need to be decided by experimenting to get the sample with required degradation. Simultaneously subjects the Placebo (Excipients mixture) as per the manufacturing formula to all the above stress conditions. For multi-drug product placebo formulation containing one drug substance each shall be subjected to forced degradation. Prepare test solutions using unstressed sample, placebo and the stressed samples, as per the test method and inject into the HPLC system with diode array detector. Record the chromatograms and calculate the Percentage degradation and percent net degradation as per acceptance criteria. In case of stable molecules, percent ne degradation may be difficult to achieve as per acceptance criteria. Hence based on the experiments, study can be concluded and summary of the experiments shall be documented. Demonstrate the effective separation of the analyte from the degradation product and peaks if any due to components of placebo mixture. Ensure that response of analyte peak in test solution is equal to or less than 1AU (absorbance unit). If it is more, dilute the test solution accordingly and repeat the analysis.

6.1Identification and characterization of drug products by selected analytical methods: The preferred method of analysis for a stability indicating assay is reverse-phase high-performance liquid chromatography (HPLC). RP-HPLC is preferred for several reasons, such as its compatibility with aqueous and organic solutions, high precision, sensitivity, and ability to detect polar compounds. Separation of peaks can be carried out by selecting appropriate column type, column temperature, and making adjustment to mobile phase pH Poorly-retained, highly polar impurities should be resolved from the solvent front. As part of method development, a gradient elution method with varying mobile phase composition (very low organic composition to high organic composition) may be carried out to capture early eluting highly polar compounds and highly retained non polar compounds. Stressed samples can also be screened with the gradient method to assess potential elution pattern. Sample solvent and mobile phase should be selected to afford compatibility with the drug substance, potential impurities and degradants.

Stress sample preparation should mimic the sample preparation outlined in the analytical procedure as closely as possible. Neutralization or dilution of samples may be necessary for acid and base hydrolyzed samples. Chromatographic profiles of stressed samples should be compared to those of relevant blanks (containing no active and unstressed samples to determine the origin of peaks. The blank peaks should be excluded from calculations. The amount of impurities (known and unknown) obtained under each stress condition should be provided along with the chromatograms (full scale and expanded scale showing all the peaks) of blanks, unstressed, and stressed samples Additionally, chiral drugs should be analyzed with chiral methods to establish stereo chemical purity and stability. The analytical method of choice should be sensitive enough to detect impurities at low levels (i.e., 0.05% of the analyte of interest or lower), and the peak responses should fall within the range of detector's linearity [23-27].

Degradation product identification and characterization shall be performed based on stability results in accordance with ICH requirements. Conventional methods (e.g., column chromatography) or hyphenated techniques (e.g., LC MS, LC NMR) can be used in the identification and characterization of the degradation products. It should be noted that structural characterization of degradation products is necessary for those impurities that are formed during formal shelf-life stability studies. The detector should contain 3D data capabilities such as diode array detectors or mass spectrometers to be able to detect spectral non- homogeneity. After the method finalization test method on different detectors like RI/ELSD/CE detector with the suitable method parameters and compare the data with other detectors like UV, Fluorescence etc. The UV inactive compounds can be found with this exercise. If any such type of components are there these shall be addressed based on the process and cross checking to be made by using LC-MS technique [8,9,28-30].

Use the analytical mode for major impurities /degradants and check the mass numbers or develop chromatographic conditions suitable to LC-MS and identify the mass of major degradant which are found to be forming greater than 1.0% during stress studies. Try to establish the structures of the major degradant, if possible and compare the synthetic process for justification. Diode array detection also offers the possibility of checking peak profile for multiple wavelengths. The limitation of diode array arises when the UV profiles are similar for analyte peak and impurity or degradant peak and the noise level of the system is high to mask the co-eluting impurities or degradants Compounds of similar molecular weights and functional groups such as diastereoisomers may exhibit similar UV profiles. In such cases, attempts must be made to modify the chromatographic parameters to achieve necessary separation. An optimal wavelength should be selected to detect and quantitation of all the potential impurities and degradants Use of more than one wavelength may be necessary, if there is no overlap in the UV profile of an analyte and impurity or degradant peaks. A valuable tool in method development is the overlay of separation signals at different wavelengths to discover dissimilarities in peak profiles.

7. Conclusion

Forced degradation studies of new drug substances and drug products are important to help develop and demonstrate specificity of stability-indicating methods and to determine the degradation pathways and degradation products of the active ingredients. They were also useful in the investigation of the chemical and physical stability of crystal forms, the stereochemical stability of the drug substance alone and in the drug product and mass-balance issues, and for differentiating drug substance related degradation products in formulations. The ICH not provided any formal guidance. Adequate degradation required to understand the probable degradants for the evaluation of stability indicating method.

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