A REVIEW ON FORCED DEGRADATION STUDIES AND ITS IMPORTANCE IN ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

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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. It is required to demonstrate the 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 degraded products. Forced degradation studies show the chemical behavior of the molecule which in turn helps in elucidation of the structure of the degraded products. Forced degradation studies show the chemical behavior of the molecule which in turn helps in development of formulation and package. In addition, the regulatory guidance is very general and does not explain about the performance of forced degradation studies. Thus, the present review discusses the current trends in performance of forced degradation studies by providing a strategy for conducting studies on degradation mechanisms and also describes the analytical methods helpful for development of stability indicating method.

Keywords: Forced Degradation, Accelerated, Stability Indications, Regulatory

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INTRODUCTION

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. Forced degradation studies provide data to support identification of possible degradants; degradation pathways and intrinsic stability of the drug molecule and validation of stability indicating analytical procedures. A draft guidance document suggests that results of one-time forced degradation studies should be included in Phase 3 INDs (Investigational New Drugs). NDA (New Drug Application) registration requires data of forced degradation studies as forced degradation products, degradation reaction kinetics, structure, mass balance, drug peak purity, etc. This forced degradation study provides information about degradation pathways of API alone and in drug product, any possible polymorphic or enantiomeric substances and difference between drug related degradation and excipient interferences [1,2].

The Regulatory perspectives of forced degradation

1. From a regulatory perspective, forced degradation studies provide data to support the following:
   - Identification of possible degradants.
   - Degradation pathways and intrinsic stability of the drug molecule.
   - Validation of stability indicating analytical procedures.

2. Issues addressed in regulatory guidance’s include:
   - Forced degradation studies are typically carried out using one batch of material. Forced degradation conditions are more severe than accelerated stability testing such as >50°C; ≥75% relative humidity; in excess of ICH light conditions; high and low pH, oxidation, etc.
   - Photo stability should be an integral part of forced degradation study design. Degradation products that do not form in accelerated or long term stability may not have to be isolated or have their structure determined.
   - Mass balance should be considered.

3. Issues not specifically addressed in regulatory guidance:
   - Exact experimental conditions for forced degradation studies (temperatures, duration, and extent of degradation, etc.) are not specified.
   - Experimental design is left to the applicant’s discretion. [2, 3]
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 those 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 [4,5].
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 [6].

EXPERIMENTAL DESIGN

In designing forced degradation studies, it must be remembered that more strenuous conditions than those used for accelerated studies (25°C/60% RH or 40°C/75% RH) should be used. At a minimum, the following conditions should be investigated:

1. Acid and base hydrolysis,
2. Hydrolysis at various pH,
3. Thermal degradation,
4. Photolysis, and
5. Oxidation.

For the drug substance and drug product, the scheme shown in Figure 1 could be used as a guide. The initial experiments should be focused on determining the conditions that degrade the drug by approximately 10%.

However, some scientists have found it practical to begin at extreme conditions (80°C or even higher, 0.5N NaOH, 0.5N HCl, 3% H₂O₂) and testing at shorter (2, 5, 8, and 24 hrs, etc) multiple time points, thus allowing for a rough evaluation of rates of degradation. Testing at early time
points may permit distinction between primary degradants and their secondary degradation products. This strategy allows the better degradation pathway determination.

It must be noted that a forced degradation study is a “living process” and should be done along the developmental time line as long as changes in the stability-indicating methods, manufacturing processes, or formulation changes are ongoing.

Forced degradation is only considered complete after the manufacturing process is finalized, formulations established, and test procedures developed and qualified.

The conditions listed in Table 1 are by no means exhaustive and should be adjusted by the researcher as needed to generate ~10% degradation of the API.

The nature (inherent stability/instability) of the particular drug substance will determine in which direction to adjust the stress conditions.

Also, the aforementioned conditions could be used to stress the drug substance or drug product either in the solid or liquid/suspension form as applicable.

For oxidative degradation with H₂O₂, at least one of the storage conditions should be at room temperature. Heating H₂O₂ solution increases the homolytic cleavage of the HO-OH bond to form the alkoxy radical. The alkoxy radical is very reactive and may come to dominate the observed degradation pathway. Adding a small quantity of methanol in a confirmatory stress experiment quenches the alkoxy radical and rules out species produced by this more aggressive oxidizing agent.

Also, the formation of peroxyacarboxymidic acid has been observed when acetonitrile is used as a co solvent in H₂O₂ stress studies (in basic conditions). The peroxyacarboxymidic acid has activated hydroxylation reactivity, which is not representative of H₂O₂.

To circumvent these problems, some research scientists always perform a parallel or alternative oxidative study using azobisisobutyronitrile (AIBN), which is a less reactive oxidant and has been shown to produce more representative degradants.

List of some common conditions used in conducting forced degradation studies for drug substances as shown in Table 1
Table No: 1 Conditions Generally Employed For Forced Degradation [7]

<table>
<thead>
<tr>
<th>Degradation Type</th>
<th>Experimental Condition</th>
<th>Storage Condition</th>
<th>Sampling Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control API (no acid or base)</td>
<td>40°C, 60°C</td>
<td>1,3,5 days</td>
<td></td>
</tr>
<tr>
<td>0.1N NaOH</td>
<td>40°C, 60°C</td>
<td>1,3,5 days</td>
<td></td>
</tr>
<tr>
<td>Acid Control (no API)</td>
<td>40°C, 60°C</td>
<td>1,3,5 days</td>
<td></td>
</tr>
<tr>
<td>Base Control (no API)</td>
<td>40°C, 60°C</td>
<td>1,3,5 days</td>
<td></td>
</tr>
<tr>
<td>pH: 2,4,6,8</td>
<td>25°C, 60°C</td>
<td>1,3,5 days</td>
<td></td>
</tr>
<tr>
<td>3% H₂O₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>Peroxide control</td>
<td>25°C, 60°C</td>
<td>1,3,5 days</td>
</tr>
<tr>
<td></td>
<td>Azobisisobutyronitrile (AIBN)</td>
<td>40°C, 60°C</td>
<td>1,3,5 days</td>
</tr>
<tr>
<td></td>
<td>AIBN Control</td>
<td>40°C, 60°C</td>
<td>1,3,5 days</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Light, 1X ICH</td>
<td>NA</td>
<td>1,3,5 days</td>
</tr>
<tr>
<td></td>
<td>Light, 3X ICH</td>
<td>NA</td>
<td>1,3,5 days</td>
</tr>
<tr>
<td></td>
<td>Light Control</td>
<td>NA</td>
<td>1,3,5 days</td>
</tr>
<tr>
<td>Photolytic</td>
<td>Heat chamber</td>
<td>60°C</td>
<td>1,3,5 days</td>
</tr>
<tr>
<td></td>
<td>Heat chamber</td>
<td>60°C /75% RH</td>
<td>1,3,5 days</td>
</tr>
<tr>
<td></td>
<td>Heat chamber</td>
<td>80°C</td>
<td>1,3,5 days</td>
</tr>
<tr>
<td></td>
<td>Heat chamber</td>
<td>80°C /75% RH</td>
<td>1,3,5 days</td>
</tr>
<tr>
<td></td>
<td>Heat control</td>
<td>Room Temp.</td>
<td>1,3,5 days</td>
</tr>
<tr>
<td>Thermal</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 (0.1–1 M) for base hydrolysis are suggested as suitable reagents for hydrolysis [9, 10]. If the compounds for stress testing are poorly soluble in water, then co-solvents can be used to dissolve them in 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°C) is applied. Stress testing should not exceed more than 7 days. The degraded sample is then neutralized using suitable acid, base or buffer, to avoid further decomposition.

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 up to a maximum 20% degradation could potentially generate relevant degradation products [11]. 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 [12]. The functional group with labile hydrogen like benzylic carbon, allylic carbon, and tertiary carbon or α-positions with respect to hetero atom is susceptible to oxidation to form hydro peroxides, hydroxide or ketone [13, 14].

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 photo stability testing are described in ICH guidelines. Samples of drug substance and solid/liquid drug product should be exposed to a minimum of 1.2 million lux hours and 200 Watt hours/m² light. The most commonly accepted wavelength of light is in the range of 300–800 nm to cause the photolytic degradation [15, 16]. The maximum illumination
recommended is 6 million lux hours. Light stress conditions can induce photo oxidation by free radical mechanism. 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 [17].

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 [11]. Effect of temperature on thermal degradation of a substance is studied through the Arrhenius equation:

\[
k = \frac{\text{A} e^{-\frac{E_a}{RT}}}{k}
\]

where \( k \) is specific reaction rate, \( A \) is frequency factor, \( E_a \) is energy of activation, \( R \) is gas constant (1.987 cal/deg mole) and \( T \) is absolute temperature [18,19]. Thermal degradation study is carried out at 40–80°C.

**STABILITY INDICATING METHOD**
The stability-indicating assay is a method that is employed for the analysis of stability samples in pharmaceutical industry. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability-indicating assay method (SIAM) has become more clearly mandated [20].

A stability-indicating method is defined as an analytical method that accurately quantities the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities. A method that accurately quantifies significant degradants may also be considered as stability-indicating. A proactive approach to developing a stability indicating HPLC method should involve forced degradation at the early stages of development with the key degradation samples used in the method development process.

Forced degradation should be the first step in method development. If forced degradation studies are performed early, method development and identification of primary degradation products and unknown impurities can be run in parallel. Using this process, a validated HPLC analytical assay, mechanisms of degradation, and the impurity/degradant information for filing can all be generated.
Development of stability indicating method

Though the requirements with respect to stability indicating method have been spelt out in regulatory documents, information on the basic steps to be followed for the development and validation of stability-indicating methods is neither provided in the regulatory guidelines nor in the pharmacopoeias. General steps in stability indicating method are

**Step1: Critical study of the drug structure to assess the likely decomposition route:**

This should be the first element whenever one takes up the project on establishment of a SIM. Much information can simply be gained from the structure, by study of the functional groups and other key components. There are definite functional group categories, like amides, esters, lactams, lactones, etc. that undergo hydrolysis [21], others like thiols, thioethers, etc. undergo oxidation, and compounds like olefins, aryl halo derivatives, aryl acetic acids, and those with aromatic nitro groups, N-oxides undergo photodecomposition [22].

**Step 2: Collection of information on physicochemical properties:**

Before method development is taken up, it is generally important to know various physicochemical parameters like pKa, log \( P \), solubility, absorptivity and wavelength maximum of the drug in question.

**Step 3: Stress (forced decomposition) studies:**

As described above in forced degradation section, these studies should be carried out in accordance with ICH Q1A guideline. Stress conditions are (i) 10°C increments above the accelerated temperatures (e.g. 50°C, 60°C, etc.), (ii) humidity where appropriate (e.g. 75% or greater), (iii) hydrolysis across a wide range of pH values, (iv) oxidation and (v) photolysis.

**Step 4: Preliminary separation studies on stressed samples:**

The simplest of separation way is to start with a reversed-phase octadecyl column and perform HPLC separation using UV/PDA detector system. Another way is to go for LC-MS separation. Using these chromatographic techniques, one should follow the changes in all the stress samples at various time periods. The results should be critically compared with the blank solutions injected in a similar manner. It should be observed whether the fall in drug peak is quantitatively followed by a corresponding rise in the degradation product peaks.
Step 5: Final method development and optimization:

Subsequent to preliminary chromatographic studies, the RT and relative retention times (RRT) of all products formed should be tabulated for each reaction condition. Special attention is then paid to those components whose RT or RRT is very close. PDA spectra or LC-MS profile of such components are obtained and critically evaluated to ascertain whether the products are same or different.

To separate close or co-eluting peaks, the method is optimized, by changing the mobile phase ratio, pH, gradient, flow rate, temperature, solvent type, and the column and its type [20].

Step 6: Identification and characterization of degradation products, and preparation of standards

To identify the resolved products, a conventional way is to isolate them and determine the structure through spectral (MS, NMR, IR, etc.) and elemental analysis. However, this approach is tedious and time consuming when multiple degradation products are formed. Against it, the modern approach is to use hyphenated LC techniques coupled with mass spectrometry. This strategy integrates in a single instrument approach, analytical HPLC, UV detection, full scan mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS-MS) and provides a fair idea on identity of resolving components. These days a further integrated approach is becoming popular wherein LC-MS or LC-MS-MS is employed to obtain molecular weight and fragmentation information, and further detailed structural information is obtained through LC-NMR analysis.

Step 7: Validation

Validation of analytical methods, in general, has been extensively covered in the ICH guidelines Q2A and Q2B [22, 23], in the FDA guidance [24] and by USP [25].

The main focus of validation at this stage is on establishment of specificity/selectivity, followed by other parameters like accuracy, precision, linearity, range, robustness, etc. The limits of detection and quantitation are also determined for degradation products to help in establishment of the mass balance.

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. Degradation products generated from forced degradation studies are potential degradation
products that may or may not be formed under relevant storage conditions but they assist in the developing stability indicating method. 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 in turn help improve the formulation manufacturing process and determine the storage conditions. As no specific set of conditions is applicable to all drug products and drug substances and the regulatory guidance does not specify about the conditions to be used, this study requires the experimenter to use common sense. The aim of any strategy used for forced degradation is to produce the desired amount of degradation i.e., 5–20%.

REFERENCES


3. ICH harmonised tripartite guideline stability testing of new drug substances and products Q1A (R2).


