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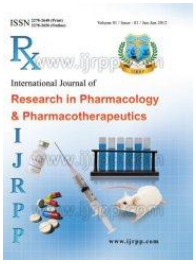

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Review article ***Open Access***

A review article – development of forced degradation and stability indicating studies for drug substance and drug product

Mrs. Khushbu A. Thakor, Dhara D. Bhavsar, Jagruti R. Thakor

[1]*Quality Assurance department, Smt. Bnb. Swaminarayan Pharmacy college,Salvav.*
[2]*Pharmacology Department, Smt. Bnb. Swaminarayan Pharmacy college,Salvav.*
[3]*Pharmaceutical chemistry department, Smt. Bnb. Swaminarayan Pharmacy college,Salvav.*
***Corresponding author: Mrs. Khushbu A. Thakor**

ABSTRACT

The objective of the review article is to give detailed description and guidance of the forced degradation studies as per regulatory guidelines. Forced degradation study provide information about the degradation pathways and degradation products of the drug substance and helps in the elucidation of the structure of the degradation products. forced degradation study provide the chemical behaviour 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.

Keywords: Degradation, Purity, Potency

INTRODUCTION

Forced degradation studies are also named as forced decomposition studies, stress decomposition studies, stress testing, stress studies [1].

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 [2].

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 [3].

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 [4].

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 [5].

The ICH guideline states that the 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 to validate the stability indicating procedure [6].

Before filling in registration dossier, it has become mandatory to perform stability studies of new drug moiety or molecule [7].

The stability studies include long term studies (12 months) and accelerated stability studies (6 months) but intermediate studies (6 months) can be performed at conditions milder than that conditions used in accelerated studies [8].

Forced degradation studies help in generating degradant products in much shorter span of time, (few weeks) as compared to degradation study [9].

OBJECTIVE OF FORCED DEGRADATION STUDIES [10]

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

- Stability related problems are solved by this studies.
- More stable formulations are generated by this studies.
- Structure of degradation products are elucidated by this studies.
- Degradation pathways of drug substances and drug products are established by this studies.
- Stability indicating nature of a developed method are established by this studies.
- Determination of the intrinsic stability of the drug substances in the formulation.
- Chemical characteristics of drug molecules are understood by this studies.
- Degradation mechanisms such as hydrolysis, oxidation, photolysis or thermolysis of drug substance and drug product are understood by this studies.

OVERVIEW OF REGULATORY GUIDANCE [11]

Various international guidelines have described the forced degradation studies. The international council for harmonization of technical requirements for registration of the pharmaceutical drugs for human use (ICH) has published a set of guidelines, this guidelines have been discussed, studied and adopted by the American, European and Japanese regulatory authorities. ICH guidelines those are applied to forced degradation studies are:

- ICH Q1A- Stability Testing of New Drug Substances and Products
- ICH Q1B- Photo stability Testing of New Drug Substances and Products
- ICH Q1C- Stability Testing of New Dosage Forms
- ICH Q2B- Validation of Analytical Procedures: Methodology

When to perform forced degradation

Its very important to know when to perform forced degradation studies for the development of new drug substance and new drug product [12]. FDA guideline says that stress testing should be performed in phase III of regulatory submission process. Stress testing should be carried out in different pH solutions like acid and alkali hydrolysis, in presence of oxygen and light [13], and at different temperature and humidity levels to determine the stability of drug substance and drug product. These studies should be carried out on a single batch. The results should be summarized and submitted in an annual report [14]. For obtaining the sufficient time and for the identification of the degradation products and structure elucidation as well as optimization of stress conditions, the starting stress testing of drug substance for preclinical phase or phase I of clinical trials is conducted [15]. An early or starting study also gives important guidance for improvements in manufacturing of the drug substance and process [16].

Origin 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 [17-18].

Limits of degradation

Degradation of drug substances between 5% to 20% 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. A general protocol of degradation conditions used for drug substance and drug product is shown in below

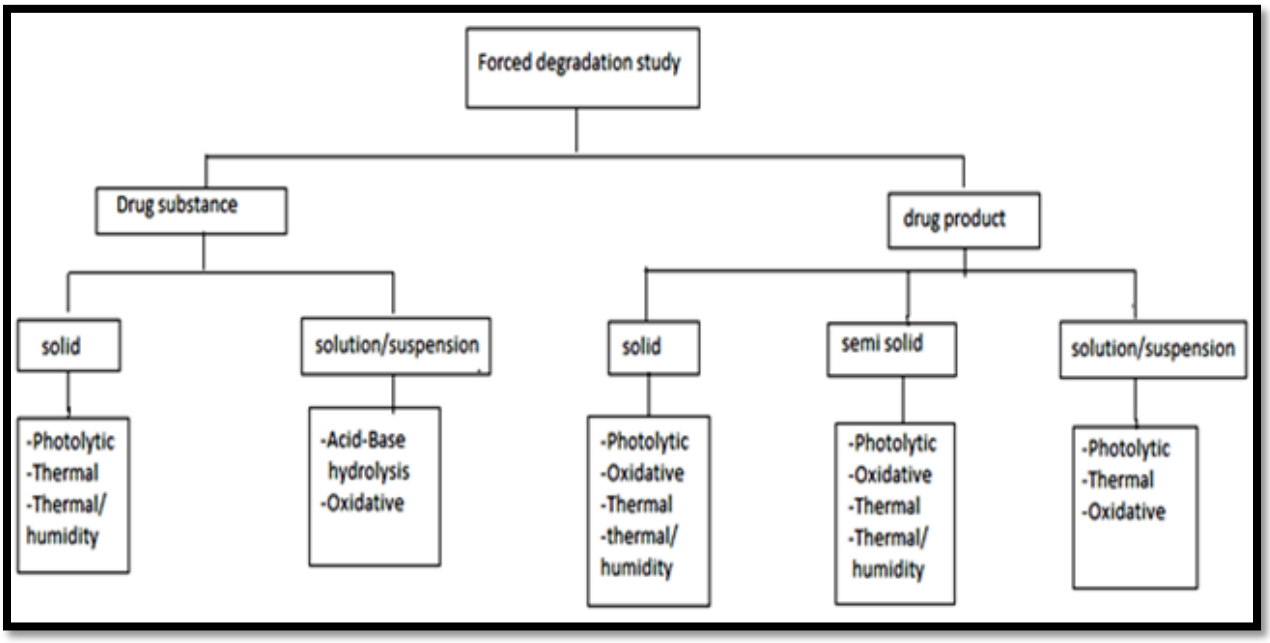

Fig: Various stress conditions used for degradation of drug substance and drug product

Stress factors suggested for forced degradation studies include acid and alkali hydrolysis, thermal degradation, photolysis, oxidation. There is no specification in regulatory guidelines about the conditions of pH, temperature and thermal condition and oxidizing agent used.

Some conditions mostly used for forced degradation studies are presented in below table

Degradation type	Experimental conditions	Storage conditions	Sampling time (days)
	Control API (no acid or base)	40°C, 60°C	1,3,5
	0.1 M HCl	40°C, 60°C	1,3,5
hydrolysis	0.1 M 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
	3% H_2O_2	25°C, 60°C	1,3,5
Oxidation	Peroxide control	25°C, 60°C	1,3,5
	Azobisisobutyronitrile(AIBN)	40°C, 60°C	1,3,5
	AIBN control	40°C, 60°C	1,3,5

	Light 1 × ICH	NA	1,3,5
Photolytic	Light 3 × ICH	NA	1,3,5
	Light control	NA	1,3,5
	Heat chamber	60°C	1,3,5
	Heat chamber	60°C/75% RH	1,3,5
Thermal	Heat chamber	80°C	1,3,5
	Heat chamber	80°C/75% RH	1,3,5
	Heat control	Room temp.	1,3,5

Selection of drug concentration

How much concentration of drug should be used for degradation study has not been specified in regulatory guidance. The stress condition should be carried out in the concentration of 1 mg/ml. even in minor concentration drug should be degraded. It is suggested that degradation study should be carried out at a concentration which the drug is expected to be present in the final formulations.

Conditions for degradation

Hydrolytic conditions

Hydrolysis is one of the most common degradation chemical reactions carried out over a wide range of pH. In hydrolysis process drugs are come in contact with water and reactions occurs and gives the different products with different chemical composition. Water reacts with the pharmaceutical dosage form either in the form of solvent or as a atmospheric moisture and that is responsible for the degradation of the hydrolysis products. For examples Aspirin reacts with water and hydrolysed to salicylic acid and acetic acid. HCl and NaOH are employed for generating acidic and alkaline stress samples, respectively. The hydrolytic degradation of a new drug in acidic and alkaline condition can be studied by refluxing the drug in 0.1 N HCl/NaOH. If reasonable degradation is seen, testing can be stopped at this point. If degradation is not seen under these condition, the drug should be refluxed in condition higher concentration of 0.1N NaOH/0.1N HCl.

Procedure for conducting hydrolytic degradation

Conduct the following forced degradation studies to obtain degraded samples wherever degradation possible from about 1% to 30%. For acid stress reflux with 0.1N HCl at 60°C for 30 minutes. For alkaline stress reflux with 0.1N NaOH at 60°C for 30 minutes.

Oxidative condition

Hydrogen peroxide is widely used for oxidation of drug substances in forced degradation studies but some other oxidizing agents such as metal ions, oxygen and radical initiators (azobisisobutyronitrile, AIBN) can also be used. Many drug substances undergo auto-oxidation i.e. oxidation under normal storage condition and involving ground state elemental oxygen. Therefor it is an important degradation pathway of many drugs. Auto-oxidation is a free radical reaction that requires free radical initiator to begin the chain reaction. Hydrogen peroxide, metal ions and trace level of impurities in a drug substance act as initiators for drug substance. Selection of an oxidizing agent, its concentration and condition depends on the drug substance. It is mentioned that the drug solutions are subjecting to 0.1%-3% hydrogen peroxide at neutral pH and room temperature for seven days or upto a maximum 20% degradation could potentially generate relevant degradation products. The mechanism of oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations. Amines, sulphides and phenols are susceptible to electron transfer oxidation to give N-oxides, hydroxylamine, sulphones and sulphoxide.

Procedure for conducting oxidative degradation

Conduct the following forced degradation studies to obtain degraded samples wherever degradation possible from about 1% to 30%. For oxidation stress: treat with 1% H_2O_2at less than 30°C for 30 minutes. The oxidative stress testing is initially carried out in 3% H_2O_2atroom temperature for 6 hour and it can be increased/decreased to achieve sufficient degradation. Co-solvent can be used to dissolve and extract the drug, where necessary.

Thermal condition

Rate of chemical reaction increases with increase in temperature. Hence, the drugs are susceptible to degradation at higher temperature. Many active pharmaceutical ingredients are sensitive to heat or tropical temperature. For examples, vitamins, peptides, etc. thermal degradation involves different reactions like hydrolysis, polymerization, decarboxylation. Effect of temperature on thermal degradation of a substance is studied through Arrhenius equation:

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

Where K is specific rate reaction, A is frequency factor, Ea is energy of activation, R is gas constant (1.987 cal/deg mole) and T is absolute temperature. Thermal degradation is carried out at 40°C to 80°C. The most widely accepted temperature at 70°C at low and high humidity for 1-2 months. High temperature (>80°C) may not produce predictive degradation pathway.

Procedure for conducting thermal degradation

Conduct the following forced degradation studies to obtain degraded samples wherever degradation possible from about 1% to 30%. 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.

Photolytic condition

Exposure of drug molecules may produce photolytic degraded products. The rate of photo degradation depends upon the intensity of incident light and quantity of light absorbed by the drug molecule. Photolytic degradation is carried out by exposing the drug substance to a UV light and Visible light. The most commonly accepted wavelength of light is in the range of 300-800 nm to cause the photolytic degradation. Samples of drug substance and drug product should be exposed to a minimum of 1.2 million lx h and 200 W h/m^2light. The maximum illumination recommended is 6 million lx h.

Procedure for conducting photolytic degradation

Conduct the following forced degradation studies to obtain degraded samples wherever degradation possible from about 1% to 30%. Expose the drug substance or drug productto ultraviolet radiation up to minimum of 200 watts hour/$m^2$and minimum of 1.2 million lx hour for visible light and photo stability chamber. If photo stability chamber is not available, expose the drug substance and drug product to intense the ultraviolet radiation (both at longer and shorter wavelengths) up to minimum of 7 days in UV cabinet.

Humidity

Humidity the key factor for establishing the potential degradants in the finished product and active pharmaceutical ingredients. Normally 90% humidity for duration of one week shall be recommended for the establishment of forced degradation samples.

Stability indicating method

According to the FDA guidance document, a stability indicating method is a validated quantitative analytical procedure that can be used to detect how the stability of the drug substances and drug products changes with time. A stability indicating method accurately measures the changes in active ingredients concentration without interference from other degradation products, impurities and excipients. The development of a suitable stability indicating method provides background information of active ingredients about the solubility study, pre-formulation study, concentration changes with time, development of suitable storage conditions. The RP-HPLC is most widely used analytical tool for separation and quantifying the impurities and most important coupled with UV detector.

Sample generation

For generating samples for SIM the API is forced at conditions more severe than accelerated degradation conditions. It involves degradation of drug at hydrolytic, oxidative, photolytic and thermal conditions as discussed earlier. The forced degradation of API in solid state and solution form is carried out with an aim to generate degradation products which are likely to be formed in realistic storage conditions. This sample is then used to develop an SIM.

Method development and optimization

Before starting the method development, various physiochemical properties like pKa value, log P,

solubility and absorption maximum of drug must be known. Log P and solubility helps for selecting the mobile phase and solvent system while pKa value helps for determination of pH of the mobile phase.

Reverse phase column is a preferred choice to start the separation of sample components as the degradation is carried out in aqueous solution. Water, Methanol, acetonitrile can be used as mobile phase in various ratios for the initial stages of separation. Selection between methanol and acetonitrile for organic phase is based on the solubility of the analyte. Initially the ratios should be kept at 50:50 and suitable and different trials were taken to obtain good separation of peaks. Latter buffer can be added if it is required to obtain better peak separation and symmetry. Variation in column temperature affects the selectivity of the method as analytes respond differently to temperature changes. A temperature in the range of 30-40°C is suitable for the good reproducibility. The method is then optimized for separating closely eluting peaks by changing flow rate, injection volume, column type and mobile phase ratio.

Method validation

The developed SIM 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.

Other analytical methods for developing sim

Stability indicating methods will be characterized by the safety, purity, potency and biological activity. Stability indicating methods may include various methods like electrophoresis, high resolution chromatography (i.e. gel filtration, ion exchange, affinity chromatography, size exclusion chromatography) and peptide mapping. Whatever analytical methods selected for stability purpose, it should sensitive enough to detect the impurities at low level and peak responses should fall within the range of detector's linearity. The analytical method should be capable of detecting all the impurities formed during a formal stability study or below ICH threshold limit. IR (infra-red spectroscopy), MS (Mass spectroscopy), NMR (Nuclear magnetic resonance) helps for structure identification, characterization and interpretation of impurities. The unknown impurities, which is observed during the analysis, pharmaceutical development of drug substance and drug product, can be separated and analysed by using various chromatographic techniques like reverse phase high performance liquid chromatography (RP-HPLC), thin layer chromatography (TLC), super critical fluid chromatography (SFC), gas chromatography (GC). An excellent combination of hyphenated chromatographic and spectroscopic technique such as LC-NMR, LC-MS, GC-MS, HPLC-photodiode array ultraviolet detector (DAD) are used when degradants cannot be isolated in pure form.

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.

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