Development of stability indicating studies for pharmaceutical products: an innovative step

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Abstract

A stability study is a routine procedure which ensures the maintenance of pharmaceutical product safety, quality and efficacy throughout the shelf life. These pharmaceutical products are followed by the guidelines issued by International Conference on Harmonization (ICH), World Health Organization (WHO) or other agencies. Stability testing provides evidence that the quality of a drug substance or drug product under the influence of various environmental factors changes with time. Importance of various methods followed for stability testing of pharmaceutical products, guidelines issued for stability testing and other aspects related to stability of pharmaceutical products have been presented in a concise manner in the present review. This review article includes introduction about stability studies types of stability studies and chemical reactions takes place during degradation etc. This article also includes the forced degradation studies and shelf life estimation of pharmaceutical products.

Keywords: Stability, Pharmaceutical, Environmental, Chemical reaction, Forced degradation, Shelf life.

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Introduction

Stability studies are carried out at various stages of the drug development process. At early stages of drug development, accelerated stability studies are performed to determine the rate of degradation of the product if stored for longer period under specific conditions. After that, forced degradation study is carried out to check the effect of external stressed conditions on the drug product.[1]

"The capability of a particular formulation in a specific container/closed system, to remain within its physical, chemical, microbiological, therapeutic, and toxicological specifications throughout its shelf life" defines stability. "Stability is official defined as "the time lapse during which the drug product retains the same properties & characters that is processed at the time of manufacture. This testing evaluates the effects of environmental factors on the quality of the drug substance or a formulated product which is utilized for prediction of its shelf life, determine storage and labeling instructions. In a stability study, the effects of variation in temperature, time, humidity, light intensity and partial vapor pressure over pharmaceutical product are investigated.

In current good manufacturing practices (cGMP) the word "current" refers to the present good manufacturing practices regulations, not the past or future regulations. cGMP standards refer to conditions under which the product is produced, not the condition of the final product. Products may be deemed to be adulterated if they are not produced in conformance with cGMP.[2]

Stability studies are necessary for the following reason

1. Product instability of active drug may lead to under medication due to lowering concentration of the drug in dosage form.
2. During decomposition of active drug toxic products may be formed.
3. Instability may be due to changing in physical appearance though the principles of kinetics are used in predicting the stability of drug there different between kinetics and stability study.[3]

Types of stability studies

Stability studies are mainly of four types:

- Long term stability
- Intermediate stability
- Accelerated stability
- In-use stability

Table 1: Types of Stability Studies

Study	Storage condition	Minimum time period covered by data at submission
Long term	25°C±2°C and 60% RH±5% RH or 30°C±2°C and 65% RH±5% RH	12 months
Intermediate	30°C±2°C and 65% RH±5% RH	6 months
Accelerated	40°C±2°C and 75% RH±5% RH	6 months

Stability studies are used for testing the drug product for longer periods under varying conditions of temperature and humidity. If the drug is to be distributed in different geographical regions and if shipping is required for transportation, in that case long term stability studies are of prime importance. Long term stability studies are performed by testing the sample at specific time intervals and conditions of external parameters are changed accordingly. Main objective of this study is to determine shelf-life of the drug product.

1. **Intermediate stability:** Studies conducted at 30°C and 65% RH and designed to moderately increase the rate of chemical degradation or physical changes for a drug substance or drug product intended to be stored long term at 25°C.
2. **Accelerated testing:** These studies include use of exaggerated storage conditions designed to study increased rate of physical and chemical degradation. This is part of the formal stability studies. Data from these studies is uses to carry out long term stability studies i.e. to determine shelf-life of the drug product
3. **In-use stability:** This type of stability studies is mainly for the one dose or multi-dose drugs. The chemical composition and physical stability of these drugs are such that due to repetitive opening and closing, it gets degraded due microbial contamination. The purpose of in use stability testing is to establish where applicable a period of time during which a multi-dose product can be used until retaining quality within an accepted specification once the container is opened.[1]

Guidelines for stability testing

To assure that optimally stable molecules and products are manufactured, distributed and given to the patients, the regulatory authorities in several countries have made provisions in the drug regulations for the submission of stability data by the manufacturers. Its basic purpose was to bring in uniformity in testing from manufacturer to manufacturer. These guidelines include basic issues related to stability, the stability data requirements for application dossier and the steps for their execution. Such guidelines were initially issued in 1980s. These were later harmonized (made uniform) in the International Conference on Harmonization (ICH) in order to overcome the bottle neck to market and register the products in other countries. The ICH was a consortium formed with inputs from both regulatory and industry from European commission, Japan and USA. The World Health Organization (WHO), in 1996, modified the guidelines because the ICH guidelines did not address the extreme climatic conditions found in many countries and it only covered new drug substances and products and not the already established products that were in circulation in the WHO umbrella countries. A technical monograph on stability testing of drug substances and products existing in India has also been released by India Drug Manufacturers Association. Further, different test condition and requirements have been given in the guidance documents for active pharmaceutical ingredients drug products or formulations and excipients. The codes and titles covered under ICH guidance have been outlined in the Table 2.[4]

Table 2: Code and Title used in ICH Guidelines

ICH Code and their Guideline title
Q1A Stability testing of New Drug Substances and Products (Second revision).
Q1B Stability testing Photo stability testing of New Drug Substances and products.
Q1C Stability testing of New Dosage Forms.
Q1D Bracketing and Matrixing Designs for stability testing of Drug substances.
Q1E Evaluation of stability data.
Q1F Stability data package for Registration Applications in Climatic Zones III and IV.
Q5C Stability testing of Biotechnological/Biological Products.

Table 3: ICH Q1A Summary of Stability Parameters

Study	Storage Condition	Minimum Time Period	Comments
General Case: Long-term	25 °C±2°C/60% RH±5% RH or 30°C 2°C/65% RH±5%±RH	12 months	Must cover retest or shelf life period at a minimum and includes storage, shipment and subsequent use.
General Case: Intermediate	30°C±2°C/65% RH±5% RH	6 months	Must cover retest or shelf life period at a minimum and includes storage, shipment and subsequent use.
General Case: Accelerated	40°C±2°C/75% RH±5% RH	6 months	Period at a minimum and includes storage, shipment and subsequent use.
Refrigeration: Long-term	5°C±3°C	12 months	Must cover retest or shelf life period at a minimum and includes storage, shipment and subsequent use.
Refrigeration: accelerated	25°C±2°C/60% RH±5% RH	6 months	Must cover retest or shelf life period at a minimum and includes storage, shipment and subsequent use.
Freezer: Long term	-20°C±5°C	12 months	Must cover retest or shelf life period at a minimum and includes storage, shipment and subsequent use.

Type of stability of drug substance

1. Physical stability: The original physical properties, including appearance, palatability, uniformity, dissolution and suspend ability are retained. Physical degradation reactions include:
- Precipitation
- Racemization
- Epimerization
- Sorption
- Leaching
2. Chemical stability: Each active ingredient retains its chemical integrity and labeled potency within the specified limits. Chemical degradation reaction includes:
- Hydrolysis
- Oxidation reduction
- Photolysis
3. Microbiological stability: Sterility or resistance to microbial growth is retained according to the Specified requirements. Antimicrobial agents retain effectiveness within specified limits.
4. Therapeutic stability: The therapeutic effect remains unchanged.
5. Toxicological stability: No significant increase in toxicity occurs.[3]

Photo Stability Studies: The photo stability studies are carried out to demonstrate that the appropriate light exposure dose not results into unacceptable change in dosage form. Light can influence the active principle in a drug formulation, as well as the final product or package. The other effects include cloudy appearance of the products, a loss in viscosity of formulation, precipitation of active principle, alteration in dissolution rate. The drug which undergo light induce degradation are called as photo stable drug e.g. chlorpromazine, tetracycline.[5]

Force Degradation Studies or Stress Testing: Forced degradation is carried out to produce representative samples for developing stability-indicating methods for drug substances and drug products. The choice of stress conditions should be consistent with the product's decomposition under normal manufacturing, storage, and use conditions which are specific in each case. The stress factors suggested for forced degradation studies include acid and base hydrolysis, thermal degradation, photolysis and oxidation. The initial trial should have the aim to come upon the conditions that degrade the drug by approximately 10%.[6]

Table 4: Conditions mainly used for forced degradation studies

Degradation Type	Experimental Conditions	Storage Condition	Sampling Time (days)
Hydrolysis	Control API (no acid or base)	40°C, 60°C	1,3,5
	0.1 M HCl	40°C, 60°C	1,3,5
	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
Oxidation	3% H_2O_2	25°C, 60°C	1,3,5
	Peroxide control	25°C, 60°C	1,3,5
	Azobsisobutyronitrile (AIBN)	25°C, 60°C	1,3,5
	AIBN control	25°C, 60°C	1,3,5
Photolytic	Light 1 X ICH	NA	1,3,5
	Light 3 X ICH	NA	1,3,5
	Light control	NA	1,3,5
Thermal	Heat chamber	60°C	1,3,5
	Heat chamber	60°C/75% RH	1,3,5
	Heat chamber	80°C	1,3,5
	Heat chamber	80°C/75% RH	1,3,5
	Heat chamber	Room temperature	1,3,5

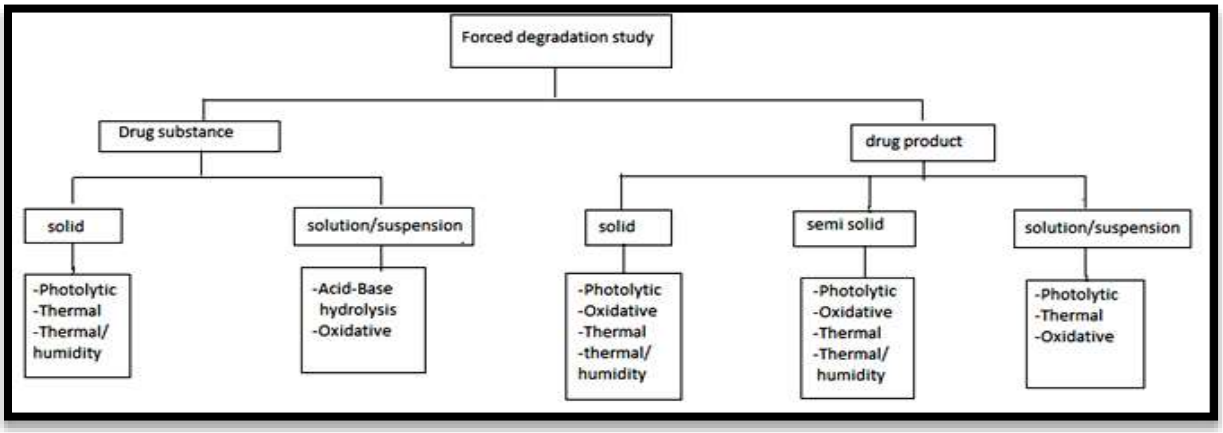

Fig. 1: Different Forced Degradation to be used for Drug Substance and Drug Product

Factors influencing stability of dosage form

1. **pH:** pH plays a significant role in the active ingredient's solubility and thus in its bioavailability. The rate of degradation is much higher at extreme values. The optimum pH is often the same as the pH at which a given molecule is most soluble. Buffers are often included in pharmaceutical product formulations, and provide very good stability. However the formulation of preparations using these pharmaceutical products may change their pH and their stability.
2. **Surfactants:** Surfactants can be used to protect the active ingredient in hydrolytic groups such as hydroxyls, and limit their degradation. The different types of surfactants (anionic, cationic or non-ionic) may however form micelles in solution, thus trapping the active ingredient molecules and changing their bioavailability in solution.
3. **Temperature:** Temperature is one of the most important factors in drug stability. An increase of 10° C in storage temperature may lead to a 2 to 5 fold increase in the speed of degradation reactions. For certain molecules, physicochemical stability is only optimal within a small temperature range, outside of which increased degradation is observed. For most active ingredients, the kinetics of degradation reactions follows the Arrhenius law. Thus, when performing stability studies at elevated temperatures (at 40° C, for example), it is possible to determine the formulation's stability at ambient temperature.
4. **Oxygen:** The presence of oxygen in a preparation may cause instability via the oxidation of one of its components. Formulation (antioxidants) and manufacturing techniques (under nitrogen) need to be determined accordingly. Selecting an appropriate container and ensuring its integrity are also important elements to consider in order preventing the infiltration of oxygen over time.

5. **Light:** Light is a parameter that may cause chemical instability in photosensitive molecules. If preventive measures are implemented during manufacturing (selection of appropriate packaging material), it is important to check that they are maintained over time.[7]

Study strategy

Stability testing includes long-term studies, where the product is stored at room temperature and humidity conditions, as well as accelerated studies where the product is stored under conditions of high heat and humidity. Proper design, implementation, monitoring and evaluation of the studies are crucial for obtaining useful and accurate stability data. Stability data for the drug substance are used to determine optimal storage and packaging conditions for bulk lots of the material. The stability studies for the drug product are designed to determine the expiry date (or shelf life).

Stress studies at elevated temperature (e.g., 50°C, 60°C and 70°C) for several weeks may be performed to assess thermal stability. The solid stability should also be performed in the presence and absence of water vapor to assess the dependence of stability on humidity. [8] Drug products will be exposed to temperature conditions both inside and outside of label storage conditions. Long-term and accelerated stability studies will be completed per ICH guidelines.[9]

Table 5: Long-term stability study per ICH Q1A

Storage condition	Testing condition
Controlled room temperature 20–25°C	25°C and 60% RH for 12 months
Refrigerated condition 2–8°C	5°C for 12 months
Freezer condition –20 to –10°C	–20°C for 12 months

Table 6: Accelerated stability study per ICH Q1A

Storage condition	Testing condition
Controlled room temperature 20–25°C	40°C and 75% RH for 6 months
Refrigerated condition 2–8°C	25 °C and 60% RH for 6 months
Freezer condition –20 to –10°C	5°C for 6 months

Various steps in stability studies

1) **Formulating the preparation:** As soon as a stability study is planned, the formulation of the preparation and the container selected, both need to be validated. When planning a stability study, it is assumed as a preliminary condition that the available literature has been consulted in order to establish that the formulation contains no known incompatibilities, and that there are no known content/container interactions involved. If no data is available, preliminary studies should be conducted.
   - Data on the active ingredient
   - All stability studies should be conducted with an active ingredient issued from the same batch
   - Packaging item.
2) **Choice of concentration tested:** The concentration of active ingredient in the preparation to be studied will always depend on the product's intended therapeutic use. When preparations are recognized as clinically effective only at a single concentration, the stability study will be conducted at that concentration. Since the preparation's stability may vary as a function of this dilution, it is necessary to study the stability of the preparation at a minimum of two concentrations: one low and one high. Pharmacists must first identify the effective therapeutic range and then select the higher and upper concentrations. If the difference between the low and high concentrations is too significant (more than a factor of 10) an intermediate concentration stability study may be considered, depending on the clinical interest.
3) **Number of Testing:** In stability studies the test batch must include at least 3 units, so as to obtain a minimum of 3 independent measurements, and it is preferable to create one preparation unit per sampling time-point. If it is not possible to use different units for each sampling time-point (for a very expensive drug, for example), one unit may be used for all sampling time. Testing should then be repeated 3 times, on preparations from different batches.
4) **Storage Conditions**
   - **Ambient Temperature:** So long as the active ingredient is not known to be heat unstable, testing is performed at temperatures near 25°C. If the pharmacist has access to an environmental chamber, the units should be kept in the temperature conditions recommended by the ICH, namely 25°C±2°C.
   - **Refrigeration:** If the data in the literature recommend refrigeration, or indicate that the principle active is known to be thermo sensitive, or if analyses performed at 25°C show a rapid degradation of the molecule, a study at 5°C will be considered.
   - **Freezing-Thawing:** If the data in the literature recommend freezing, or if the active ingredient degrades rapidly at ambient temperature and/or after refrigeration, a stability study on the frozen preparation will be programmed at approximately -20°C. Regular temperature recording must be performed in order, as far as possible, to maintain a temperature of -20°C±5°C.

- **Residual Moisture:** If the pharmacist has a climate chamber, stability testing is performed at 25°C while maintaining the residual moisture at 60% ± 5%, in order to achieve the conditions recommended by the ICH.

5) **Light:** In the absence of data from the literature regarding the possible photosensitivity of the active ingredient, the use of day/night ambient light is recommended. If the molecule is known to be photosensitive, tests will be carried while protecting the preparation with suitable packaging (amber bottle, opaque packaging, and opaque outer packaging) and appropriate storage conditions, sheltered from the light.
6) **Duration of Study:** Real-time studies are recommended, while limiting the storage period to 1 year in order to stay within acceptable limits with regard to normal hospital practices. Upstream accelerated aging studies may help to get an idea of the molecules' degradation pathways. In such case, pharmacists will work to the methodology dictated by the ICH.
7) **Sampling time point:** Subsequent to this, sampling time points are calculated with reference to the maximum planned duration. We recommend establishing a minimum of 5 sampling time points between the initial time T0 and the maximum duration. We propose sampling frequencies corresponding to about 1/24th, 1/12th, ¼, ½ and ¾ of the maximum duration. These frequencies may be adjusted slightly to fit around a reasonable working schedule.
8) **Analysis to be performed:** An assay of the active ingredient and monitoring of the appearance of degradation products are performed systematically. The other analyses to be performed are determined according to the pharmaceutical dosage-form used for the stability study. In the case of sterile preparations, the physicochemical stability study may be supplemented by a microbiological stability study.[7]

Method development and validation

Before starting the method development, various physiochemical properties like pKa value, log P, solubility and absorption maximum of the drug must be known, for it lays a foundation for HPLC method development. Log P and solubility helps select mobile phase and sample solvent while pKa value helps determine the 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. Methanol, water and 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 water and organic phase ratio can be kept at 50:50 and suitable modifications can be made as trials proceed to obtain a good separation of peaks. Latter buffer can be added if it is required to obtain better peak separation and peak symmetry. If the method is to be extended to liquid chromatography–mass spectrometry (LC–MS), then mobile phase buffer should be MS compatible like triflouroacetic acid and ammonium acetate. 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 to obtain good reproducibility. It is better to push the drug peak further in chromatogram a site results in separation of all degradation products. Also a sufficient run time after the drug peak is to be allowed to obtain the degradants peak eluting after the drug peak. During the method development it may happen that the drug peak may hide an impurity or degradant peak that co-elutes with the drug. This requires peak purity analysis which determines the specificity of the method. Direct analysis can be done online by using photodiode array (PDA) detection. PDA provides information of the homogeneity of the spectral peak but it is not applicable for the degradants that have the similar UV spectrum to the drug. Direct method involves change in the chromatographic conditions like mobile phase ratio, column, etc. which will affect the peak separation. The spectrum of altered chromatographic condition is then compared with the original spectra. If the degradant peaks and area percentage of the drug peak remain same, then it can be confirmed that the drug peak is homogeneous. The degradant that co-elutes with the drug would be acceptable if it is not found to be formed in accelerated and long term storage conditions. The method is then optimized for separating closely eluting peaks by changing flow rate, injection volume, column type and mobile phase ratio. The developed method validated according to USP/ICH guideline for linearity, accuracy, precision, specificity, quantitation limit, detection limit, ruggedness and 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.[10]

Table 7: Objective, Type and Use of stability Testing

Objective	Type of study	Use
To select adequate formulation and container closure system.	Accelerated	Development of the product
To determine shelf life and storage condition.	Accelerated real time	Development of the product and of the registration dossier
To substantiate the claimed shelf life	Real time	Registration dossier
To verify that no changes have been introduced in the formulation or manufacturing process that can adversely affect the stability of the product	Accelerated real time	Quality assurance in general, including quality control

Drug shelf life estimation

The shelf-life of a drug product is the time that the average drug characteristic (e.g., potency) remains within an approved specification after manufacture. The United States Food and Drug Administration (FDA) require indication for every drug product of a shelf-life on the immediate container label. Since the true shelf-life of a drug product is typically unknown, it has to be estimated based on assay results of the drug characteristic from a stability study usually conducted during the process of drug development. Furthermore, the FDA requires that the estimated shelf-life be so constructed that it is statistically evident that the estimated shelf-life is less than the true shelf-life, i.e., the estimated shelf-life should be a conservative (negatively biased) estimator.

Shelf life can be estimated by

- FDA method
- Direct method
- Inverse method
- Simulation results
- Shelf life estimation under batch to batch variation.[11]

Conclusion

Stability studies are capable of differentiating active drug ingredient from any degradation product formed under defined storage conditions. 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. Over a period of time and with increasing experience and attention, the regulatory requirements have been made increasingly stringent to achieve the above goal in all possible conditions to which the product might be subjected during its shelf life.

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