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# STABILITY STUDY: REGULATORY REQUIREMENT

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**Abstract**
Stability is an essential factor of quality, safety and efficacy of a drug product. The objective of stability study is to determine the shelf life, the time period of storage at a specified condition within which the drug product still meets its established specifications. Stability study is of three types that is physical, chemical and microbial stability. Various factors like oxygen, water, temperature, pH, moisture, light and concentration affect the stability. Present work aims to represent the stability testing (ST) requirements of International Conference on Harmonization (ICH), different regulatory agencies like, World Health Organization (WHO), Association of South East Asian Nations (ASEAN) and European Agency for Evaluation of Medicinal and Health Products (EMEA) and difference of those agencies with respect to ICH guideline. Most of the stability requirements for WHO, ASEAN, and EMEA are similar to the ICH guideline, except for the parameters like selection of batches and storage conditions.
***Keywords*:** stability testing, ICH, WHO, ASEAN, EMEA

## 1. Introduction

Stability is defined as the capacity of a drug substance or drug product to remain within the established specifications which maintains its identity, strength, quality and purity throughout the retest or expiration dating period. The objective of stability study is to determine the shelf life, namely the time period of storage at a specified condition within which the drug product still meets its established specifications. Stability is an essential factor of quality, safety and efficacy of a drug product. A drug product, which is not of sufficient stability, can result in changes in physical (like hardness, dissolution rate, phase separation etc) as well as chemical characteristics (formation of high risk decomposition substances). The Chemical stability of drug is of great importance since it becomes less effective as it undergoes degradation. Also drug decomposition may yield toxic byproducts that are harmful to the patient. Microbiological instability of a sterile drug product could also be hazardous. Stability evaluation of drug substance or drug product is the key to drug quality as it determines the efficacy of any drug or dosage form. Stability assessment of drug products and drug substances are mandated by regulatory agencies across the globe. In fact, stability-testing issues are responsible for a number of audit findings by regulatory agencies. Stability testing problems are regularly cited in warning letters and sometimes results in costly product recall. Stability testing provides evidence that the quality of drug substance or drug product changes with time under the influence of various environmental conditions such as temperature, relative humidity etc. The stability study consists of a series of tests in order to obtain an assurance of stability of a drug product, namely maintenance of the drug product packed in it specified packaging material and stored in the established storage condition within the determined time period. [1,2]

## 2. Types of stability study

**2.1 Physical stability** Physical instabilities possibilities are like,

**2.1.1 Crystal formation in pharmaceutical preparations:** Causes of crystal formation in pharmaceutical formulations are like Polymorphism phenomena which are seen in Chloramphenicol, while in Saturated solution by different temperature precipitation of solute may occur and In suspension when very fine powder is used a part of suspending agent which dissolve and precipitated as crystals.

**2.1.2 Loss of volatile substances:** Loss of volatile substance from pharmaceutical dosage forms like Aromatic waters, Elixirs, Spirits and some types of tablets which contain aromatic water cases physical instabilities.

**2.1.3 Loss of water:** This can be seen in the dosage forms like, in saturated solution, by loss of water they become supersaturated and precipitate as crystals is formed. In emulsions, loss of water leads to separation of the two phases and change to other type while, in creams especially oil/water, they become dry by loss of water.

**2.1.4 Absorption of water :** In the different type of dosage form the absorption of water

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cause physical instability by different mechanism. In Powders type of dosage form Liquefaction and degradation may occur as a result of absorption of water while in case of Suppositories which base made from hydrophilic substances as Glycerin, Gelatin, poly ethylene glycol, the consistency of these forms becomes jelly-like appearance and causes physical instabilities in pharmaceutical preparations.

**2.1.5 Change in crystalline form:** Change in crystalline forms changes physical properties like solubility, melting point, bioavailability so change in crystalline form case physical instability. Example: Cocoa butter which is capable of existing in four polymorphic forms. Cocoa butter can crystallize in to six polymorphic forms designated as i-vi according to their stability and different physical characteristics. The chemical composition is identical in all forms; only the arrangement of the lipid molecules varies. The diverse polymorphs are formed under different crystallization condition.[3]

**2.2 Chemical stability:** It involves various reactions like hydrolysis, reduction or fermentation which are influenced by moisture.[3]

**2.3 Microbial stability:** Contamination from microorganisms is a big problem for all formulations containing moisture but it can be a bother in solid dosage forms also if some natural polymers are used because many natural polymers are fertile sources of microorganisms. In the type of hygienic manufacture carried out today where "Quality Assurance" is a prerequisite as per the GMP procedures, there are definite procedures to prevent microbial contamination in all formulations.[3]

## 3. Factors affecting drug stability

**3.1 Oxygen:** Oxidation is the most important part of the drug degradation. Oxygen is present everywhere in the atmosphere and exposure to oxygen will decompose the drug substance that is not in their most oxidized state through auto-oxidation. Oxygen is a diradical and most auto oxidation is a free radical reactions. Oxidation/reduction reaction involves transfer of electron, oxygen or hydrogen in substances. The process involves a radical chain reaction between molecular oxygen and the pharmaceutical drug candidate, a process known as auto-oxidation. The free radical process of auto-oxidation consists of a chain sequence involving three distinct types of reactions: initiation, propagation, and termination. The initiator produce a free radical to begin the chain initiator is used to accelerating auto-oxidation. For example, diazine. Oxidation of diazine occure by N-oxidation.[4]

**3.2 Water:** The word hydrolysis literally means "splitting by water". Drug substance having ester & amide functional groups within their structure undergoes hydrolysis reactions. For example solution of sodium acetate produces acetic acid and hydroxide ions. The reaction involving lactum group are fastest and are those followed by ester, amides and imides in that order and follows first order. These reactions are catalyzed by divalent metal ions, heat, light and high drug concentrations.[4]

**3.3 Temperature:** Temperature has a high degree of influence on all variety of chemical reactions and usually they are accelerated by raise in temperature. To evaluate stability utilizing elevated temperatures and stress conditions are selected based on the Arrhenius expression a quantitative relationship of reaction rate and temperature. Based on this estimate, a 10◦C increase in temperature results in a doubling of the reaction rate and a decrease in the reaction time by a factor of 2.[4]

**3.4 pH:** PH influences the rate of decomposition of most drugs. Most of the drugs are stable between pH 4 and 8. Weekly acidic and basic drugs show good solubility when they are ionized and they also decompose faster when they are ionized. So if the pH of a drug solution has to be adjusted to improve solubility and the resultant pH leads to instability then a way out of this tricky problem is to introduce a water miscible solvent into the product. It will increase stability by suppressing ionization, reducing the extreme pH required to achieve solubility, enhancing solubility and reducing the water activity by reducing the polarity of the solvent. For example, 20% propylene glycol is placed in chlordiazepoxide injection for this purpose.

Reactions catalyzed by pH are monitored by measuring degradation rates against pH, temperature, ionic strength and solvent concentration constant. Some buffers such as acetate, citrate, lactate, phosphate and ascorbate buffers are utilized to prevent drastic change in pH. Sometimes pH can have a very serious effect on decomposition. As little as 1 pH unit change in pH can cause a change of ten fold in rate constant. So when we are formulating a drug into a solution we should carefully prepare a pH – decomposition profile and then formulate the solution at a pH which is acceptable physiologically and stability-wise also.[4]

**3.5 Moisture:** Water catalyses chemical reactions as oxidation, hydrolysis and reduction reaction, it also promotes microbial growth.[4]

**3.6 Light:** Affects drug stability through its energy or thermal effect which lead to oxidation.[4]

**3.7 Concentration:** Rate of drug degradation is constant for the solutions of the same drug with different concentration. So, ratio of degraded part to total amount of drug in diluted solution is bigger than of concentrated solution.[4]

## 4. Parameters for stability testing

Different parameters like appearance, - assay and – degradation products should be evaluated for all dosage forms. Each dosage form is presented as a guide for the types of tests to be included in a stability study. In general, as well as preservative and antioxidant content if applicable. The microbial quality of multiple-dose sterile and non-sterile dosage forms should be controlled. Challenge tests should be carried out at least at the beginning and at the end of the shelf-life. The list of tests presented for each dosage form is not intended to be exhaustive, nor is it expected that every listed test be included in the design of a stability protocol for a particular pharmaceutical product (for example, a test for odor should be performed only when necessary and with consideration for the analyst's safety).[5]

## 5. Regulatory requirement for stability study

There are many regulatory guidelines for stability study mainly International Conference on Harmonization (ICH), World Health Organization (WHO), Association of South East Asian Nations (ASEAN) and European Agency for Evaluation of Medicinal and Health Products (EMEA).

**5.1 Objectives of the guidelines:** These guidelines seek to exemplify the core stability data package required for registration of active pharmaceutical ingredients (APIs) and finished pharmaceutical products (FPPs), replacing the previous WHO guidelines in this area. However, alternative approaches can be used when they are scientifically justified. Further guidance can be found in International Conference on Harmonization (ICH) guidelines and in the WHO guidelines on the active pharmaceutical ingredient master file Procedure. It is recommended that these guidelines should also be applied to products that are already being marketed, with allowance for an appropriate transition period, e.g. upon re-registration or upon re-evaluation. [6]

**5.2 Scope of these guidelines:** These guidelines apply to new and existing APIs and address information to be submitted in original and subsequent applications for marketing authorization of their related FPP for human use. These guidelines are not applicable to stability testing for biological. [6]

**5.3 General principles:** The purpose of stability testing is to provide evidence of how the quality of an API or FPP varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. The stability programmed also includes the study of product-related factors that influence its quality, for example, interaction of API with excipients, container closure systems and packaging materials. In fixed-dose combination FPPs (FDCs) the interaction between two or more APIs also has to be considered. As a result of stability testing a re-test period for the API (in exceptional cases, e.g. for unstable APIs, a shelf-life is given) or a shelf-life for the FPP can be established and storage conditions can be recommended. Various analyses have been done to identify suitable testing conditions for WHO Member States based on climatic data on the basis of which each Member State can make its decision on long-term (real-time) stability testing conditions.[6]

**5.4 Stability protocol/report:** Protocol include following elements

1. Information on batches tested (commercial formula)
2. Unit composition (or cross-reference)
3. Container-closure system (commercial)
4. Literature and/or supporting data
5. Stability specifications (only for Finished Pharmaceutical Products)
6. Analytical methods – stability indicating (cross-reference)
7. Stability plan (schedule)
8. Tabulated test results (including specifications)
9. Analysis/discussion of data (statistical if negative trend)
10. Re-test or shelf-life proposal (including storage condition)
11. Post approval commitments.[5]

## 6. Different regulatory guidelines for stability study

**6.1 ICH guideline:** ICH guidelines define the stability data package for a new drug substance or drug product that is sufficient for a

registration application within the three regions of the EC, Japan and the United States. ICH guideline given for drug substance and drug product.[7-10]

**6.1.1 Stability testing requirements as per ICH guidelines**

**(a) Drug substances**

***Selection of batches:*** Data should be provided on at least three primary pilot scale batches.

***Storage conditions:*** Storage condition in different cases are given in table-1

**Table: 1 storage conditions in different case of stability study**

| Different case of stability study | Storage conditions |
|---|---|
| General case | |
| Long term: | 25°C ± 2°C/60%RH ± 5% RH or 30°C±2°C/65%RH±5% RH |
| Intermediate: | 30°C ± 2°C/65% RH ±5% RH |
| Accelerated: | 40°C ± 2°C/75%RH ±5% RH |
| For drug substance intended to be stored in Refrigerator | |
| Long term | 5°C ± 3°C |
| Accelerated | 25°C ±2°C/60%RH ± 5% RH |
| For drug substance intended to be stored in freezer | |
| Long term | - 20°C ± 5°C |

*Stress testing*

Stress testing recommendations:

**Temperature:** Temperature above accelerated temperature (50˚C, 60˚C, 70˚C, etc.)

**Humidity:** 25˚C/75% RH and 25˚C/90% RH

**Oxidation:** Wide range of pH

Photo stability testing should be carried out on a single batch.

***Testing frequency***

**Long term studies: 0, 3, 6, 9, 12, 18, 24 months and annually through the proposed re-test period**.

**Accelerated:** 0, 3, 6 months

**Intermediate:** 0, 6, 9, 12 months

***Container closure system:*** Stability testing should be carried out in the same container closure system as that proposed for storage and distribution.

***Stability commitment:*** If the data does not cover the proposed retest period granted at the time of approval, a commitment should be made to continue the stability studies post approval.

***Statements/ labeling:*** A storage statement should be established based on the stability evaluation of the drug substance.

**(b) Drug products or finished pharmaceutical products:**

***Selection of batches:*** Data should be provided on at least three primary batches. Two should be at least pilot scale batches.

***Storage condition:*** Storage conditions for general case, for the product intended to be stored in refrigerator and freezer is same as that for drug substance.

Drug products packaged in semi-permeable containers:

**Accelerated:** 40°C±2°C/NMT 25%RH

*Stress testing*

**Temperature:** 50˚C or 60˚C for 1 month

**Temperature/humidity:** 40˚C/75%RH, 25˚C/80%RH

Photo stability testing should be carried out.

***Testing frequency:*** Same as that of drug substances.

***Container closure system:*** Container closure system for testing should be same as that proposed for marketing including any secondary packaging.

***Stability commitment:*** If the data does not cover the proposed shelf life granted at the time of approval, a commitment should be made to continue the long term studies through the proposed shelf life and the accelerated studies for 6 months post approval.

***Statements/ labeling:*** Same as drug substances.[7-10]

**6.2 WHO guideline:** WHO guidelines apply to new and existing API's and their related FPP's for human use. WHO guideline is derived from ICH parent guideline, they have some significant differences which define its individuality as a separate guideline. The differences were observed in the parameters like selection of batches, storage conditions and statements and labeling. [5,11]

**6.3 ASEAN guidelines:** Association of South East Asian Nations (ASEAN) guideline mainly focuses on the requirements for stability testing of drug products along with new chemical entities (NCE's). The differences were observed in stress testing, selection of batches and real time storage conditions. This guideline addresses the information to be submitted an application for marketing authorization of drug products in ASEAN countries including examples of a protocol of stability study, a report format, reduced design and extrapolation of data, and examples of types, thickness and permeability coefficient which are covered in Annexes. The drug products covered in this guideline include NCE, Generics and Variation**.** But exclude drug products containing vitamin and mineral preparations. [12]

**6.4 EMEA guidelines:** This guideline is an extension of the note for guidance on stability testing of new veterinary drug substances and medicinal products (EMEA/CVMP/VICH/899/99-Rev.1) and sets out the stability testing requirements for existing active substances and related finished products. For the purposes of this guideline, an existing active substance is one that has been authorized previously through a veterinary medicinal product within the European Community. This guideline is applicable to chemical active substances and related finished products, herbal drugs, herbal drug preparations and related herbal medicinal products and not to radiopharmaceuticals, biological and products derived by biotechnology. The guideline seeks to exemplify the core stability data package required for such active substances and finished products, but leaves sufficient flexibility to encompass the variety of different practical situations that may be encountered due to specific scientific considerations and characteristics of the materials being evaluated. Alternative approaches can be used when there are scientifically justifiable reasons. The guideline addresses the information to be submitted in registration applications for existing active substances and related finished products. Most of the parameters for drug substance and drug products are followed same as the ICH guideline like stress testing, testing frequency, stability commitment, statement/labeling. Only difference in the parameters like selection of batches and storage conditions. [13]

**6.5 Comparison of all guidelines [7-13]:** Stability testing (ST) requirements are presented by International Conference on Harmonization (ICH) and different regulatory agencies like, World Health Organization (WHO), Association of South East Asian Nations (ASEAN) and European Agency for Evaluation of Medicinal and Health Products (EMEA). Most of the stability requirements for WHO, ASEAN, and EMEA are similar to the ICH guideline, except for the parameters like selection of batches and storage conditions. These differences given in table-2

## Conclusion

From the above work it can be concluded that stability study regulatory requirements are important for defined the capacity of a drug substance or drug product to remain within the established specifications to maintain its identity, strength, quality and purity throughout the retest or expiration dating period. Various factors affect the stability of drugs are water, oxygen, temperature, pH, moisture and concentration. Review compares the stability testing (ST) requirements of International Conference on Harmonization (ICH) with other international regulatory agencies like World Health Organization (WHO), Association of South East Asian Nations (ASEAN) and European Agency for Evaluation of Medicinal and Health Products (EMEA). ICH guideline is parent guideline and from which WHO guideline is derived. ICH guideline defines stability testing requirements for new drug substances and drug products whereas WHO guideline applies to both new and existing API's and their related finished products and ASEAN guideline mainly focuses on the stability testing requirements for drug products which include generics and variations along with NCEs. The differences were observed in the following parameters: selection of batches, storage conditions and statements and labeling.

**Table:2 Comparison of stability study parameters with respect to different regulatory agencies**

| Parameters | ICH | WHO | ASEAN | EMEA |
|---|---|---|---|---|
| Selection of batches | Data from atleast three primary batches of the new Drug substance should be provided. | Data from at least two primary batches should be Provided for stability testing of existing API's. | Data on at least two pilot scale batches are acceptable for conventional dosage forms and for product Containing stable drug substances. | **Option a:** Drug substance (Official): Stability information from at least two production scale batches. **Drug product:** For conventional dosage form and when drug substances are known to be stable, stability data on at least two pilot scale batches are acceptable. |
| | | | | **Option b:** Drug substance (not official): at least three pilot scale batches use. **Drug product:** For critical dosage forms or when active substances are known to be unstable, three primary batches are taken. |

| | | | | |
|---|---|---|---|---|
| Storage conditions | Long term: 25°C± 2°C/60% RH ± 5% RH or 30°C± 2°C/65% RH ± 5% RH | Long term 25°C±2°C/60% RH ± 5% RH or 30°C± 2°C/65% RH ± 5% RH or 30°C±2°C/75% RH ± 5% RH | Product stored in permeable container: 30°C±2°C/75%RH ± 5% RH | **Drug substance:** Long term storage conditions: minimum time covered by data at the time of submission should be 6 months for both option a and b. |
| | Accelerated 25°C± 2°C/60% RH ± 5% RH | Accelerated storage: 25°C ± 2°C/60% RH ± 5% RH or 30°C±2°C/65% RH ± 5% RH or 30°C± 2°C/75% RH ± 5% RH | For NCE (real time storage), generics and variations: 30°C ± 2°C/75%RH ± 5% RH | **Drug product:** Long term storage conditions: minimum time covered by data at the time of submission should be - Option a: 6 months, Option b:12 months |
| Stress testing | Temperature: above accelerated storage conditions (50ºC, 60ºC, 70ºC) Humidity: 25˚C/75%RH and 25˚C/90%RH | Same as ICH | Stress testing conditions is 40°C± 2°C/75% RH±5%RH which is same as accelerated storage Conditions. | Same as ICH |

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