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STABILITY TESTING OF ACTIVE PHARMACEUTICAL INGREDIENT [API]

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ABSTRACT
Stability plays an important role in drug development process. It explains several factors that affect the quality of a drug substance or drug products varies with time under the influence of environmental factors such as temperature, humidity and light. Stability studies play a vital role to decide the re-test period and shelf life for the drug substance and recommended storage conditions of API. ICH and EMEA guidelines define stability data package for new drug substance or drug product that is sufficient for a registration application within the three regions of the EC, Japan and United States.
KEY WORDS: Stability, ICH, API, Labelling, Retest period

INTRODUCTION

It is considered good practice to test the stability of drug substances according to the International Conference on Harmonization (ICH) and Committee for Proprietary Medicinal Products [CPMP] guidelines, The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions.

In a standard stability program, a stress study is first carried out to determine the drug substance's degradation path and to establish suitable analytical methods. Drug substance stability studies are then conducted to define stability under long-term and accelerated storage conditions.

In the next phase of the development plan, the drug substance is formulated into a drug product and compatibility of the drug substance with excipients and container parts is then tested. When suitable conditions are determined, long-term and accelerated studies commence with the drug product. The data obtained from these studies are used to define the optimal storage conditions and corresponding retest period or shelf life for the drug substance.

Objective of stability studies:

The purpose of stability testing is to provide evidence on how the quality of a drug substance [API]

- under the influence of a variety of environmental factors such as temperature, humidity, and light, and varies with time
- to establish a re-test period for the API (drug substance)
- to develop the understanding of the degradation pathway of the API which may influence the quality of drug product.

COMMON TERMINOLOGY USED DURING THE STABILITY STUDIES

What is a Commitment Batch?

Production batches of a drug substance for which the stability studies are initiated or completed post approval through a commitment made in the registration application.

What is Drug Substance?

The unformulated drug substance that may subsequently be formulated with excipients to produce the dosage form.

What is the meaning of Pilot scale batch?

A batch of a drug substance manufactured by a procedure fully representative of and simulating that to be applied to a full production scale batch.

What is the meaning of Primary Batch?

A batch of a drug substance used in a formal stability study, from which stability data are submitted in a registration application for the purpose of establishing a re-test period or shelf life, respectively. However, a primary batch may be a production batch.

What is the Production Batch?

A batch of a drug substance manufactured at production scale by using production equipment in a production facility as specified in the application.

What is significant change?

Failure to meets its specification or 5% assay variation from its initial value.

What is Re-test Date?

The date after which samples of the drug substance should be examined to ensure that the material is still in compliance with the specification and thus suitable for use in the manufacture of a given drug product.

What is Re-test period

The period of time during which the drug substance is expected to remain within its specification and, therefore, can be used in the manufacture of a given drug product, provided that the drug substance has been stored under the defined conditions. After this period, a batch of drug substance destined for use in the manufacture of a drug product should be re-tested for compliance with the specification and then used immediately.

A batch of drug substance can be re-tested multiple times and a different portion of the batch used after each re-test, as long as it continues to comply with the specification.

WHAT IS CLIMATIC ZONES AND HOW THE STABILITY CONDITIONS [TEMPERATURE AND RELATIVE HUMIDITY] ARE ESTABLISHED BASED ON THE CLIMATIC ZONES

The four zones in the world that are distinguished by their characteristic prevalent annual climatic conditions. This is based on the concept described by W. Grimm (*Drugs Made in Germany*, 28:196-202, 1985 and 29:39-47, 1986).

Climatic zones

– Partition of the world into three temperature classes based on kinetic averaging of monthly temperatures, &

subdivision of the hottest class into predominantly wet or predominantly dry
– Zones (Futscher & Schumacher 1972):
• I Temperate (21ºC/45%RH)
• II Subtropical (25ºC/60%RH with possibly high RH)
• III Hot & dry (30ºC/35%RH)
• IV Hot & wet (30ºC/70%RH)
– The temperatures above are *kinetic* averages

International Climatic Zones & Conditions results are tabulated below:

Climatic Condition	Zone-I Temperature	Zone-II Mediterranean (sub-tropical)	Zone-III Hot / dry or Hot/moderate RH	Zone-IV Very hot / humidity
Mean Annual Temp	< 20°C	20.5-24°C	>24°C	>24°C
Kinetic Mean Temp	21°C	26°C	31°C	31°C
Mean Annual RH	45%	60%	40%	70%

STABILITY TESTING CONDITIONS (Zone I and II):

Long-term conditions : 25°C ± 2°C/60% RH ± 5%
Accelerated conditions : 40°C ± 2°C/75% RH ± 5%
Intermediate conditions : 30°C ± 2°C/65% RH ± 5%

STABILITY TESTING CONDITIONS (Zone III and IV):

Long-term conditions : 30°C ± 2°C/65% RH ± 5% RH
Accelerated conditions : 40°C ± 2°C/75% RH ± 5%

No intermediate storage conditions for stability studies are recommended for climatic Zone III and IV. Therefore, the intermediate storage conditions are not relevant.[1,2]

STRESS TESTING

Stress testing can help identity the likely degradation products which can help to establish.

- The degradation pathways.[i.e degradation impurities and based on these studies the Pharma professional can understand how the Drug substance will behave during stability studies and how the product is stored in different environmental conditions].
- The intrinsic stability of the molecule.
- Validate the stability indicating power of the analytical procedures used.

Stress testing is to be carried out on a single batch. It should include the effect of temperature i.e. Testing at 50/60°C (i.e 10°C increment) above that accelerated testing, Humidity (75% or greater) where appropriate oxidation & photolysis on the drug substance has to be performed.

To achieve the objective of stress study, Stress study of the API is to be performed in several conditions

a. Stress study in oxidation condition by using Hydrogen peroxide
b. Stress study in acidic condition by using Hydrochloric acid.
c. Stress study in alkali condition by using Sodium Hydroxide.
d. Stress study by heat treatment by using the 80°C temperature for 48 hours.
e. Stress study in Sunlight by taking in account the ICH Q1B guideline

If the significant levels of impurities are not present in the sample of API then a particular level of impurities are to spike in the stample and stress study is to performed for the aforementioned conditions to understand the degradation pathways for the API.

Photo stability testing should be an integral part of stress testing.[1, 2]

Examining degradation products under stress conditions is useful in establishing degradation pathways and developing and validating suitable analytical procedures. However, it is not necessary to identify all the impurities in stress studies for all but it is necessary to demonstrate that they are not formed under accelerated or long term storage conditions.

The peak purity is one of the important criteria which is to be considered during stress study to ensure that there is no interference of the other impurities peak with the main peak of API.

Moreover during stress study the time period for each test should not be short because no large degradation will be observed in the short period. In addition to this strengthening of the test conditions to make sure the height or peak area of principal peak is decreased up to 20% during stress study.

SELECTION OF BATCHES

Data from formal stability studies should be provided on at least three primary batches of the drug substance. The batches should be manufactured to a minimum of pilot scale by the same synthetic route as, and using a method of manufacture and procedure that simulates the final process to be used for, production batches. The overall quality of the batches of drug substance placed on formal stability studies should be representative of the quality of the material to be made on a production scale.

Following points are to considered during submission of the information in the registration dossier to the International Regulatory Agencies

a. Data from 3 primary batches required (Batch number, date of manufacturing and size of each batch should be stated).
b. Primary batches could be from pilot / plant scale.
c. Plant / Pilot batches should be similar (process, equipment, route should be similar).

CONTAINER CLOSURE SYSTEM

Container closure is the sum of packaging components that together contain and protect the drug substance. This includes primary packaging components and secondary packaging components.

The stability studies should be conducted on the drug substance packaged in a container closure system that is the same as or simulates the packaging proposed for storage and distribution.

Packaging for API

Primary packaging material

Generally Low density polyethylene bag EU (LDPE bag) with twist tied with a plastic fastener is used as a primary packaging material. The grade of the primary packaging material should complies with EU Directive No. 2002/72/EEC and clause # 3.2.2 and clause # 3.1.3 of European Pharmacopoeia level of plastic additives phenolic antioxidants, non phenolic antioxidants and amides and sterates should be well below the prescribed level in Pharmacopoeias. Primary packaging material should also

comply with FDA regulations like CFR title 21.177.1520, olefin polymer.

Secondary Packaging material

Generally Triple laminated aluminum bag or LDPE black bag with heat sealed or twist tied are used as a second packaging material.

Tertiary packaging material:

HDPE Drum is used as a tertiary packaging material.[3,4]

STABILITY SPECIFICATION

Specification – Release specification

The combination of physical, chemical, biological, and microbiological tests and acceptance criteria that determine the suitability of a drug product at the time of its release.

Specification – stability specification

The combination of physical, chemical, biological, and microbiological tests and acceptance criteria that determine the suitability of a drug substance throughout its re-test period, or that a drug product should meet throughout its shelf life.

Note : Generally release and stability specification are different, in these case API manufacturer should confirm that analytical method are same and stability results obtained during the stability studies are in compliance with the release specification and there is no out of specification is observed.

Stability specification should have the following information's

- List of Tests.
- Validated testing procedure.
- Acceptance criteria (at the time of release/shelf life)
- Impurities.
- Micro limits [If API is sterile in nature].
- Stability studies should include testing of those attributes of the drug substance that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy.
- The testing should cover: chemical, physical, biological & microbiological parameters.
- Validated stability-indicating analytical procedures should be applied.
- The appropriate physical, chemical and microbial properties of the product susceptible to change during storage should be determined over the period of the proposed in-use shelf life.
- If possible, testing should be performed at intermediate time points and at the end of the proposed in-use shelf life on the final remaining amount of the product in the container.

Physical test: Description, colour, clarity, particle size etc.

Chemical test: Residue on ignition, Heavy metal, Loss on drying/Water, pH, Specific optical rotation, related substances, Assay and Residual solvents.

Microbial test: Total viable count, sterility, BET.

TESTING FREQUNCY

For long term stability studies, stability samples are to be analyzed every three months for the first year, every six month for second year. Annually thereafter through the proposed re-test period.

For accelerated storage condition, stability samples are to be analyzed a miminum of three time points [eg. 0, 3 and 6 months]. A six months study is acceptable for accelerated storage conditions by various international authorities for the registration dossier.

For Intermediate stability studies, stability samples are to be analyzed a minimum of four time points [eg.: 0, 3, 6,9,12 months] if significant changes are observed at accelerated storage condition.

STORAGE CONDITIONS

General case

Study	Storage Condition	Minimum time period covered by data at submission
Long Term*	25±2°C/60 ±5%RH	12 months
Intermediate**	30±2°C/65 ±5%RH	6 months
Accelerated	40±2°C/75 ±5%RH	6 months

*It is up to the applicant to decide whether long term stability studies are performed at 25 ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH.

**If 30°C ± 2°C/65% RH ± 5% RH is the long-term condition, there is no intermediate condition.

Any "significant change" occurs at any time during 6 months at accelerated storage conditions. Additional testing at intermediate storage condition should be conducted and evaluated against significant change criteria. Testing at the intermediate storage condition should include all tests, unless otherwise justified.

"Significant change" for a drug substance is defined as failure to meet its specification.

Drug substances intended for storage in a refrigerator

Study	Storage Condition	Minimum time period covered by data at submission
Long Term	5°C ±3°C	12 months
Accelerated	25°C±2°C/60% RH ±5%RH	6 months

If significant change occurs between 3 and 6 months' testing at the accelerated storage condition, the proposed re-test period should be based on the real time data available at the long term storage condition.

If significant change occurs within the first 3 months' testing at the accelerated storage condition, a discussion should be provided to address the effect of short term excursions outside the label storage condition, e.g., during shipping or handling.

Drug substances intended for storage in a freezer

Study	**Storage Condition**	**Minimum time period covered by data at submission**
Long Term	**-20°C ±5°C**	**12 months**

There is no accelerated study for above case.
Drug substances intended for storage below -20°C
Drug substances intended for storage below -20°C should be treated on a case-by-case basis.
Any "significant change" occurs during 6 months accelerated study. Additional testing at intermediate storage should be conducted.
"Significant change" for a drug substance is defined as failure to meet its specification.[3,4,5]

STABILITY COMMITMENT

- When Initial long term data on primary batches may not cover the proposed re-test period granted at the time of approval, a commitment should be made to continue the stability studies, post approval studies in order to firmly establish the re-test period. If long-term batches cover the proposed retest period then commitment is considered unnecessary.
- If the submission includes data from stability studies on at least three production batches, a commitment should be made to continue these studies through the proposed re-test period.
- If submission of the less than 3 production batches, a commitment is made to continue the long term studies during the proposed shelf life and place additional production batches on long term studies through the proposed shelf life.
- If submission does not include stability data on production batches, a commitment should be made to place first 3 production batches on long term studies through the proposed re-test period**.**

EVALUATION OF STABILITY DATA TO ESTABLISH RETEST PERIOD/SHELF LIFE

The main purpose for evaluation to establish the re-test period, applicable to all future batches of the drug substance manufactured under similar circumstances. The degree of variability of individual batches affects the confidence that a future production batch will remain within specification throughout the assigned re-test period.

Extrapolation of data

If real time data are supported by results from studies conducted under accelerated or intermediate storage conditions, the re-test period may be extended beyond the end of real time studies.
The extrapolated retest period may be up to twice, but should not be more than 12 months beyond the period covered by real time data, depending on the change over time, variability of data observed, proposed storage conditions and extent of statistical analyses performed.

Example for assigning the re-test period or shelf life is given below:

Sr. No.	Stability condition	X = Period covered by long-term data	Y= Proposed retest period or shelf life
01.	Accelerated (6 months OK)	Long Term (9 months)	Y=2x Shelf life/re-test date is 18 months.
	Accelerated (6 months OK)	Long Term (12 months OK)	Y=2x Shelf life/re-test date is 24 months.
	Accelerated (6 months OK)	Long Term (18 months OK)	Y=x+12 Shelf life/re-test date is 30 months.
	Accelerated (6 months OK)	Long Term (24 months OK)	Y=x+12 Shelf life/re-test date is 36 months.
	Accelerated (6 months OK)	Long Term (36 months OK)	Y=x No extrapolation beyond 36 months.
02.	Accelerated (6 months not OK)	Intermediate (12 months OK)	Y=1.5x Shelf life/re-test date is18 months.
	Accelerated (6 months not OK)	Intermediate (9 months OK)	Y=1.5x Shelf life/re-test date is 13.5 months.
	Accelerated (6 months not OK)	Intermediate (9 months not OK) & if long term (9 months OK)	Y=x+3 Shelf life/re-test date is12 months.
	Accelerated (3 months not OK)	Intermediate (12 months OK) & Long Term (12 months OK)	Y=1.5x (not exceeding x+6 months) Shelf life/retest date: 18months
	Accelerated (6 months not OK)	Long Term (6 months OK)	Y=2x (not exceeding x+12 months) Shelf life/retest date: 12 months

LABELING CONSIDERATION FOR DRUG SUBSTANCE:

Following point's are to be considered for the storage statement on labels:

- A storage statement should be based on the stability evaluation.
- Avoid use of "ambient condition" or "Room temperature"
- Need direct link between the label storage statement & the demonstrated stability.
- A retest period for drug substance should be derived from stability information and displayed on the container label.

Testing condition	Required labeling statement	Additional labeling statement, where relevant.
25±2°C/60 ±5%RH 40±2°C/75 ±5%RH [If stability results are within the specified limits]	Does not require any special storage conditions.	Do not refrigerate or freeze.
25±2°C/60 ±5%RH 30±2°C/65 ±5%RH [If stability results are within the specified limits]	Do not store above 30°C Or Store below 30°C	Do not refrigerate or freeze.
25±2°C/60 ±5%RH (long term) [If stability results are within the specified limits]	Do not store above 25°C Or Store below 25°C	Do not refrigerate or freeze.
5±3°C (long term) [If stability results are within the specified limits]	Store in refrigerator	Do not freeze
Below zero [If stability results are within the specified limits]	Store in a freezer	---

Other specific storage conditions

Sr. No.	Storage Problem	Additional labeling statements* depending on the packaging
1	Sensitivity to moisture.	Keep the container*** tightly closed.
2	Sensitivity to moisture.	Store in the original package.
3	Sensitivity to light.**	Store in the original package.
4	Sensitivity to light.**	Keep the container*** in the outer carton.

An explanation for the labeling statement should be given in the package leaflet and on the outer packaging, where space permits.

**Details of evaluation and included in the committee for proprietary medicinal products (CPMP) / ICH guideline on photo stability testing.

*** The actual name of the container should be used, eg. Bottle, blister.[5,6]

PHOTOSTABILITY

The intrinsic photostability characteristics of new drug substances and products should be evaluated to demonstrate that, as appropriate, light exposure does not result in unacceptable change. Normally, photo stability testing is carried out on a single batch of material. A systematic approach to photostability testing is recommended covering, as appropriate, studies such as:

i) Tests on the drug substance;

ii) Tests on the exposed drug product outside of the immediate pack; and if necessary ;

iii) Tests on the drug product in the immediate pack; and if necessary;

iv) Tests on the drug product in the marketing pack.

Light Source:

The light sources described below may be used for photostability testing. The applicant should either maintain an appropriate control of temperature to inimize the effect of localized temperature changes or include a dark control in the same environment unless otherwise justified.

Option 1

Any light source that is designed to produce an output similar to the D65/ID65 emission standard such as an artificial daylight fluorescent lamp combining visible and ultraviolet (UV) outputs, xenon, or metal halide lamp.

D 65 = Out door; ID 65 = Indoor

For a light source emitting significant radiation below 320 nm, an appropriate filter(s) may be fitted to eliminate such radiation.

Option 2

Sample should be exposed to both the cool white fluorescent and near ultraviolet lamp.

01. A cool white fluorescent lamp designed to produce an output similar to that specified in ISO 10977(1993) ;
02. A near UV fluorescent lamp having a spectral distribution from 320 nm to 400 nm with a maximum energy emission between 350 nm and 370 nm; a significant proportion of UV should be in both bands of 320 to 360 nm and 360 to 400 nm.

Procedure

For confirmatory studies, samples should be exposed to light providing an overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours/square meter to allow direct comparisons to be made between the drug substance and drug product.

Samples may be exposed side-by-side with a validated chemical actinometric system to ensure the specified light exposure is obtained, or for the appropriate duration of time when conditions have been monitored using calibrated radiometers/lux meters. If protected samples (e.g., wrapped in aluminum foil) are used as dark controls to evaluate the contribution of thermally induced change to the total observed change, these should be placed alongside the authentic sample.[6,7,8]

Presentation of samples:

Care should be taken to ensure that the physical characteristics of the samples under test are taken into account and efforts should be made, such as cooling and/or placing the samples in sealed containers, to ensure that the effects of the changes in physical states such as sublimation, evaporation or melting are minimized. All such precautions should be chosen to provide minimal interference with the exposure of samples under test. Possible interactions

between the samples and any material used for containers or for general protection of the sample, should also be considered and eliminated wherever not relevant to the test being carried out. As a direct challenge for samples of solid drug substances, an appropriate amount of sample should be taken and placed in a suitable glass or plastic dish and protected with a suitable transparent cover if considered necessary. Solid drug substances should be spread across the container to give a thickness of typically not more than 3 millimeters. Drug substances that are liquids should be exposed in chemically inert and transparent containers.

Analysis of samples:

At the end of the exposure period, the samples should be examined for any changes in physical properties (e.g., appearance, clarity, or color of solution) and for assay and degradants by a method suitably validated for products likely to arise from photochemical degradation processes. Where solid drug substance samples are involved, sampling should ensure that a representative portion is used in individual tests. Similar sampling considerations, such as homogenization of the entire sample, apply to other materials that may not be homogeneous after exposure. The analysis of the exposed sample should be performed concomitantly with that of any protected samples used as dark controls if these are used in the test. [9,10]

CONCLUSION

From the above work it can be concluded a successful stability study will establish the retest period and shelf life for drug substance and appropriate storage conditions. Every manufacturer of the API should perform the stability studies as per the ICH/EMEA guidelines for USA, Europe and Japan region and understand the regulatory requirements versus scientific requirements. It is the responsibility of the API manufacturer to choose the correct storage condition and re-test date so that impact on drug product quality can be minimized wrt degradation impurities.

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