Stability testing of pharmaceutical products is a complex set of procedures involving considerable cost, time consumption and scientific expertise in order to build in quality, efficacy and safety in a drug formulation. Scientific and commercial success of a pharmaceutical product can only be ensured with the understanding of the drug development process and the myriad tasks and milestones that are vital to a comprehensive development plan. The most important steps during the developmental stages include pharmaceutical analysis and stability studies that are required to determine and assure the identity, potency and purity of ingredients, as well as those of the formulated products (Singh *et al.,* 2000).

Definition

Stability of a pharmaceutical product may be defined as the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, toxicological, protective and informational specifications (Kommanaboyina *et al.,* 1999).

In other words, it is the extent to which a product retains, within the specified limits, throughout its period of storage and use, the same properties and characteristics possessed at the time of its packaging.

Stability testing thus evaluates the effect of environmental factors on the quality of the a drug substance or a formulated product which is utilized for prediction of its shelf life, determine proper storage conditions and suggest labeling instructions. Moreover, the data generated during the stability testing is an important requirement for regulatory approval of any drug or formulation (Singh *et al.,* 2000).

Factors affecting stability

Stability testing is termed as a complex process because of the involvement of a variety of factors influencing the stability of a pharmaceutical product. These factors include;

- Stability of the active ingredient (s); interaction between active ingredients and excipients, manufacturing process followed,
- Type of dosage form,
- Container/closure system used for packaging

- Stability of the active ingredient (s); interaction between active ingredients and excipients, manufacturing process followed, Light, heat and moisture conditions encountered during shipment, storage and handling. Degradation reactions like oxidation, reduction, hydrolysis or racemization, which can play vital role in stability of a pharmaceutical product, also depend on such conditions like concentration of reactants, pH, radiation, catalysts etc., as well as the raw materials used and the length of time between manufacture and usage of the product.
- A pharmaceutical product may undergo change in appearance, consistency, content uniformity, clarity (solution), moisture contents, particle size and shape, pH, package integrity thereby affecting its stability. Such physical changes may be because of impact, vibration, abrasion, and temperature fluctuations such as freezing, thawing or shearing etc. The chemical reactions like solvolysis, oxidation, reduction, racemization etc. that occur in the pharmaceutical products may lead to the formation of degradation product, loss of potency of active pharmaceutical ingredient (API), loss of excipient activity like antimicrobial preservative action and antioxidants etc. (Carstensen *et al.,* 2000).
- Stability of a pharmaceutical product can also be affected because of microbiological changes like growth of microorganisms in non sterile products and changes in preservative efficacy (Matthews *et al.,* 1999).

Importance of stability testing

The primary reason for stability testing is the concern for the well-being of the patient suffering from the disease for which the products are designed. Apart from degradation of the unstable product into toxic decomposition products, loss of activity up to a level of 85% of that claimed on the label may lead to failure of the therapy resulting in death e.g. nitroglycerine tablets for angina and cardiac arrest. Because of this concern, it has become a legal requirement to provide data for certain types of stability tests for the regulatory agencies before approval of a new product.

The second important concern is to protect the reputation of the manufacturer by assuring that the product will retain fitness for use with respect to all functionally relevant attributes for as long as they are on the market. Other benefits of stability studies at the developmental stage

or of the marketed products are to provide a database that may be of value in selection of adequate formulations, excipients and container closure systems for development of a new product, to determine shelf life and storage conditions for development of a new product, preparation of registration dossier, to substantiate the claimed shelf life for the registration dossier and to verify that no changes have been introduced in the formulation or manufacturing process that can adversely affect the stability of the product (Singh *et al.,* 2000; Carstensen *et al.,* 2000).

Major aim of stability testing:

The major aim of pharmaceutical stability testing is to provide reasonable assurance that the products will remain at an acceptable level of fitness/quality throughout the period during which they are in market place available for supply to the patients and will be fit for their consumption until the patient uses the last unit of the product (Kommanaboyina *et al.,* 1999).

Methods of stability testing:

Stability testing is a routine procedure performed on drug substances and products and is employed at various stages of the product development. In early stages, accelerated stability testing (at relatively high temperatures and/or humidity) is used in order to determine the type of degradation products which may be found after long-term storage. Testing under less rigorous conditions i.e. those recommended for long-term shelf storage, at slightly elevated temperatures is used to determine a product's shelf life and expiration dates. Depending upon the aim and steps followed, stability testing procedures have been categorized into the following four types.

Real-Time stability testing

Real-time stability testing is normally performed for longer duration of the test period in order to allow significant product degradation under recommended storage conditions. The period of the test depends upon the stability of the product which should be long enough to indicate clearly that no measurable degradation occurs and must permit one to distinguish degradation from inter-assay variation. During the testing, data is collected at an appropriate frequency such that a trend analysis is able to distinguish instability from day-to-day ambiguity. The reliability of data interpretation can be increased by including a single batch of reference

material for which stability characteristics have already been established. Stability of the reference material also includes the stability of reagents as well as consistency of the performance of the instrument to be used throughout the period of stability testing. However, system performance and control for drift and discontinuity resulting from changes in both reagents and instrumentation must be monitored (Anderson *et al.,* 1991).

Accelerated stability testing

In accelerated stability testing, a product is stressed at several high (warmer than ambient) temperatures and the amount of heat input required to cause product failure is determined. This is done to subject the product to a condition that accelerates degradation. This information is then projected to predict shelf life or used to compare the relative stability of alternative formulations. This usually provides an early indication of the product shelf life and thus shortening the development schedule. In addition to temperature, stress conditions applied during accelerated stability testing are moisture, light, agitation, gravity, pH and package (Kommanaboyina *et al.,* 1999).

In accelerated stability testing the samples are subjected to stress, refrigerated after stressing, and then assayed simultaneously. Because the duration of the analysis is short, the likelihood of instability in the measurement system is reduced in comparison to the real-time stability testing. Further, in accelerated stability testing, comparison of the unstressed product with stressed material is made within the same assay and the stressed sample recovery is expressed as percent of unstressed sample recovery. For statistical reasons, the treatment in accelerated stability projections is recommended to be conducted at four different stress temperatures. However, for thermolabile and proteinaceous components, relatively accurate stability projections are obtained when denaturing stress temperatures are avoided (Anderson *et al.,* 1991).

The concept of accelerated stability testing is based upon the Arrhenius equation (1) and modified Arrhenius equation (Anderson *et al.,* 1991), (Connors *et al.,* 1973) (2):

$$lnK = lnA + \Delta E/RT \qquad (1)$$

Where

K = degradation rate/s;

A = frequency factor/s;

Δ*E* = activation energy (kJ/mol);

R = universal gas constant (0.00831 kJ/mol);

T=absolute temperature (K).

$$log\left(\frac{k_2}{k_1}\right) = \frac{-E_a}{2.303R}\left(\frac{1}{T_2} \quad \frac{1}{T_1}\right) \quad \textbf{(2)}$$

Where *k*1 and *k*2 are rate constants at temperatures *T*1 and *T*2 expressed in degree kelvins;

Ea is the activation energy;

R is the gas constant.

These equations describe the relationship between storage temperatures and degradation rate. Using Arrhenius equation, projection of stability from the degradation rates observed at high temperatures for some degradation processes can be determined. When the activation energy is known, the degradation rate at low temperatures may be projected from those observed at "stress" temperatures (Connors *et al.,* 1973; Lachman *et al.,* 1976; Bott *et al.,* 2007). The stress tests used in the current International Conference on Harmonization (ICH) guideline (e.g., 40% for products to be stored at controlled room temperature) were developed from a model that assumes energy of activation of about 83 kJ per mole (Anderson *et al.,* 1991).

and reagents of interest in pharmaceutical and clinical laboratories have activation energies in this range (Kommanaboyina *et al.,* 1999; Anderson *et al.,* 1991).

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 bottleneck 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. In June 1997, US FDA also issued a guidance document entitled 'Expiration dating of solid oral dosage form containing Iron. WHO, in 2004, also released guidelines for stability studies in global environment (WHO, 2004). ICH guidelines were also extended later for veterinary products.

A technical monograph on stability testing of drug substances and products existing in India has also been released by India Drug Manufacturers Association (Singh *et al.,* 2000). 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 following table.

Table 1: Codes and titles used in ICH guidelines.

ICH code	Guideline title
Q1A	Stability testing of New Drug Substances and Products (Second Revision)
Q1B	Stability testing : Photostability 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 and Products
Q1E	Evaluation of stability data
Q1E	Stability data package for Registration Applications in Climatic Zones III and IV
Q5C	Stability testing of Biotechnological/Biological Products

Protocol for stability testing

The protocol for stability testing is a pre-requisite for starting stability testing and is necessarily a written document that describes the key components of a regulated and well-controlled stability study. Because the testing condition is based on inherent stability of the compound,

the type of dosage form and the proposed container-closure system, the protocol depends on the type of drug substance or the product. In addition, the protocol can depend on whether the drug is new or is already in the market (Ali *et al.,* 2008; Cha *et al.,* 2001). The protocol should also reflect the regions where the product is proposed to be marketed e.g. if the product is planned to be used in climatic zones I-III, IVa and IVb, the stability program must include all these zones (Cha *et al.,* 2001). A well designed stability protocol should contain information about following contents;

- Batches
- Container and closures
- Orientation of storage of containers
- Sampling time points
- Sampling plan
- Test storage conditions
- Test parameters
- Methodology
- Acceptance criteria

For long-term studies, frequency of testing should be sufficient to establish the stability profile of the drug substance. For drug substances with a proposed re-test period of at least 12 months, the frequency of testing at the long term storage condition should normally be every 3 months over the first year, every 6 months over the second year, and annually thereafter through the proposed re-test period.

Storage Conditions

In general, a drug substance should be tested under those storage conditions that can be evaluated in its thermal stability and, if applicable, its sensitivity to moisture. The storage conditions chosen should be sufficient to cover storage, shipment, and subsequent use. Long term, accelerated, and, where appropriate, intermediate storage conditions for drug substances are detailed in the sections below. The general case applies if no specific information about storage of the drug substance is provided. Alternative storage conditions can be used if justified.

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

Fig 1: Description of different studies with their storage conditions

General case

If long-term studies are conducted at 25°C ± 2°C/60% RH ± 5% RH and "significant change" occurs at any time during 6 months then testing at the accelerated storage condition and additional testing at the intermediate storage condition should be conducted and evaluated according to specific 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.

Table 2: Drug substances intended for storage in a refrigerator

Study	Storage conditions	Minimum time period covered by date at submission
Long term	$5\ ^0C \pm 3\ ^0C$	12 months
Accelerated	$25^0C \pm 2^0C$ /60%RH ±5%RH	6 months

Data from refrigerated storage should be assessed according to the above section, except where explicitly noted below.

_ If a significant change occurs between 3 and 6 months during the test at the accelerated storage condition, the re-test period should be decided according to data obtained during the long term storage condition.

_ If a significant change occurs within the first 3 months during the test 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. This discussion can be supported by further testing on a single batch of the drug substance for a period shorter than 3 months but with more frequent testing than usual. It is considered unnecessary to continue to test a drug substance through 6 months when a significant change has occurred within the first 3 months.

Drug substances intended for storage in a freezer

For drug substances intended for storage in a freezer, long term study is conducted at 20 ^{o}C ±5 ^{o}C. Testing of a single batch can be conducted at an elevated temperature (e.g., 5°C ±3°C or 25°C ± 2°C) for an appropriate time period to address the effect of short term excursions outside the proposed label storage condition, e.g., during shipping or handling.

Table 3: Drug substances intended for storage in a freezer

Study	Storage conditions	Minimum time period covered by date at submission
Long term	-20 ^{0}C$\pm$5 ^{0}C	12 months

Shelf Life Determination Based on Arrhenius Plot (Garret and Carper method)

The mathematical prediction of shelf life is based on the application of the Arrhenius equation, which indicates the effect of temperature on the rate constant. The steps involve in a shelf life determination by Arrhenius plot are as follows:

1. Keep several samples of the drug product at least three temperatures, such as 40oC, 50oC and 60oC.
2. Determine the drug content at all three storage points by taking a number of samples. We do this for a few weeks.
3. At each temperature we plot a graph between time and log percent drug remaining. If the decomposition is first order this gives a straight line. If it is zero order, percent drug remaining versus time will give a straight line.
4. Next we take the log K or log of reaction constant on Y axis and 1/T x 10-3 on X axis and draw a best fit line. This line is the Arrhenius Plot, extrapolate this line to get k at 25oC and from this we calculate the shelf-life.

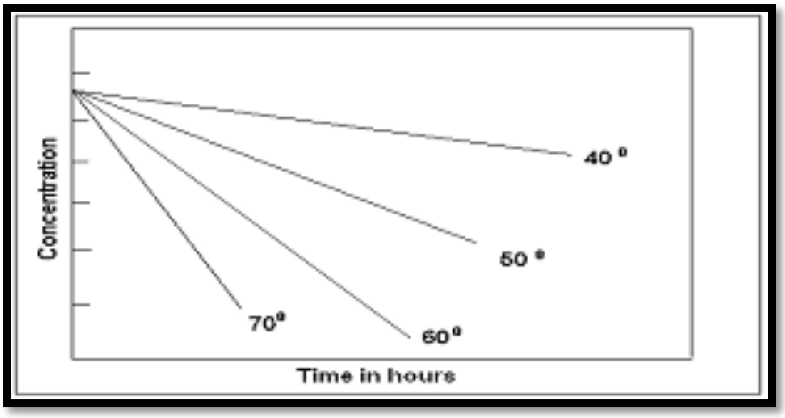

Fig 3: Graph between time and percent drug remaining

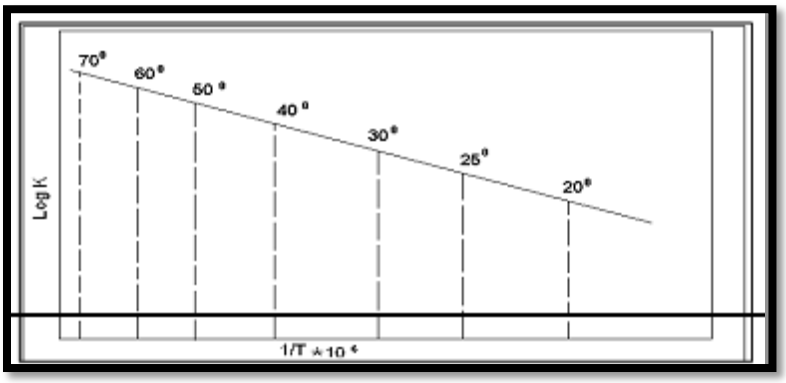

Fig 4: Arrhenius Plot

If the reaction is following zero-order Expiration date at 25oC = Initial potency – minimum potency / reaction rate at 25 °C

$$t_x = Y_o - Y_x / K_o \quad (3)$$

If the reaction is following first order

Expiration date at 25oC (tx) = Log initial potency – log minimum potency/reaction rate at 25

$$t_x = \log Y_o - \log Y_x / K_1 \quad (4)$$

Where Yo = initial potency

Yx = final potency

Ko = zero order constant

K1 = first order constant

Stability testing is now the key procedural component in the pharmaceutical development program for a new drug as well as new formulation. Stability tests are carried out so that recommended storage conditions and shelf life can be included on the label to ensure that the medicine is safe and effective throughout its shelf life. 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. Therefore, the stability tests should be carried out following proper scientific principles and after understanding of the current regulatory requirements and as per the climatic zone.