IJAPB International Journal of Advances in Pharmacy and Biotechnology

Journal homepage: http://ijapbjournal.com/

Review Article

Guidelines on Stability Studies of Pharmaceutical Products and Shelf Life Estimation

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ARTICLE INFO

Article history:
Received 11 March 2020
Received in revised form 18 March 2020
Accepted 25 March 2020
doi.org/ 10.38111/ijapb.20200601004

Keywords:
Stability Studies, Stability Testing, Stability Guidelines, Shelf Life, Pharmaceutical Products.

ABSTRACT

The primary aim of carrying out stability studies of drug products is to determine the expiration date and to promise the product standardized for efficacy, safety and elegance throughout its shelf-life. ICH, WHO, ASEAN and separate agencies issued the guidelines for stability studies, which are requisite to be demeanour in a deliberate way and are wise as pre-requisite for regulatory fill and approval of any medicinal product. Stability investigating provides the collection on stability strikingness of drug product including the belief of various environmental factors, packaging method etc. ensuring that it remain within planted specification to hold its degree and present the desirable performance consistently and in a way comforting for the usefulness of its intentional use all finished the retest or expiry date. This canvas types of stability studies, guidelines issue for stability investigation and forecasting of shelf life of medicine products along with flowing trends in stability studies.

1. Introduction

Stability of products is a sign of its significant characteristic, ensuring the identity, strength, quality and purity of drug substances enduring within the predefined limit as specified in the pertinent specification.[1] The degradation in the form of physical, chemical, or biological changes occur is determined by pharmaceutical analysis and stability studies that are required to pledge standard for efficacy, safety and elegance which pledge patients that the pharmaceutical formulated dosage form constantly executes and in a manner acceptable for the purpose for which it is recommended.[2]

Pharmaceuticals product stability may be referred to the capability of a dosage form in a specific packaging system without losing its potency and other characteristics as specified in specification throughout its shelf life. Suggesting proper storage condition on the instruction of label arise from stability testing after evaluation on deterioration rate on quality of active ingredients in a pharmaceutical product when they subjected to stressful environmental condition. Moreover, for regulatory acceptance of drug products, the stability studies data is a compulsory requirement.[3]

Stability testing is an intricate process to assign the expiration date to marketable medicine which is affected by a variety of factors counting by all physical, chemical and biological like the interaction between ingredients used in a formulated product, dosage form types, packaging system and light, temperature and humidity in environments encountered during storage and shipment. In addition to, solvolysis, oxidation, reduction, hydrolysis, racemization and other chemical mechanism are associated to the drug deterioration which is accelerated by a subsequent condition like a concentration of reactants, pH, radiation etc., additionally the starting materials used and time period from the time of manufacturing to the consumption of the product. Such degradation is measure by physical, chemical and biological stability assessment such as changes or non-compliance in appearance, content uniformity, Colour, organoleptic characteristics, hardness, friability, disintegration, dissolution,

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sedimentation and resuspendability, weight, moisture content, particle shape and size, pH, package integrity, loss of potency (active ingredient), loss of excipients (antimicrobial preservatives, antioxidants), microbial growth in non-sterile products, preservation of sterility, preservative efficacy changes etc.[4, 5]

2. Reason for Implication of Stability Testing

The principal rationale on stability testing subsists the focus on the wellbeing of the consumer's health and to sustain the concern medication of superior efficacy, safety and elegance which assure the consumer that the products perform consistently and in a manner satisfactory for the reason of its intended use throughout its shelf life, stability testing arises. Even though slight losing the potency of active substances up to the level of eighty-five percent of that label claimed might be fatal to patients after following therapeutic failure apart from considering the degradation of unstable products into toxic decomposition. E.g. Cardiac arrest by unstable Nitroglycerine tablets an anti-angina agent. Since of this concern, the regulatory body divulges a legal requirement to submit stability testing data before approval of a new product.[6, 7]

Almost all pharmaceutical products are not shipped directly to the pharmacy from the manufacturer therefore the robustness of product must be assessed on stability studies. Thus, the second important concern of stability testing is to maintain robustness for use with admiration to all functionally pertinent characteristics for as lengthy as the drug products are on the market.[5] Stability testing at the developmental phase provides the full confirmation that how physical, chemical and biological factor like compatibility between ingredients used in a formulated product, dosage form types, packaging system and light, temperature and humidity in environments influences on the quality of drug products and moreover data generated from stability study is subjected to determine the expiry date and storage condition of products before they are released into the market. As well as benefits of stability studies are to validate the claimed shelf life through the principle of kinetic and predictive studies, and to confirm that no alterations have been imparted in the formulation or manufacturing method which may affect the stability of the drug.[5-7]

Furthermore, stability data on the formulated product; containing new active substances, known active substances (including reformulation), different strength or dosage form, changes in packaging system etc. is an imperative pre-requisite for regulatory acceptance or amendment in manufacturing & marketing authorizations certificate of any formulation.[4]

3. Stability Testing Methods

Stability testing is usual grooming executed on drug products and is conducted at various phases of the product development. In embryonic stages, for an assessment of "worst-case", in an organization to pioneer the operation of degradation products that might exist after long-term store, the drug product is subjected to accelerated stability studies (at somewhat moderate temperatures and/or humidity). Those suggested for long-term storage, investigating under the conditions at slightly lofty temperature is used to establish a products shelf life and expiration dates.

The generalization essay of medicine stability testing is to wage medication of fine property which free supporter to the patients that the products usable in marketplace performs over and over again in copasetic kind for the design of its witting use throughout the phase of their expiration dates.[2]

On the fundament of aim and steps pursued, stability testing measures know been classified into the succeeding cardinal types: Real Time stability testing, Accelerated stability testing, Retained sample stability testing and Cyclic temperature stress testing.

3.1 Real-time stability testing

In order to create set degradation salience under storage assumption advisable by ICH/WHO/FDA, etc., the real-time stability studies are performed ordinarily for extended duration of the test period. Since the stability of products is to decide the periods of a test, thus testing periods must be long enough to specify precisely that no quantifiable degradation takes place and should allow one to characterize degradation from inter-assay deviation. During the testing, the collection is composed of an intelligent oftenness in such a way that trend analysis is competent to tell disequilibrium from day-to-day vagueness. To increase the consistency of data interpretation, the lone batch of reference substances of established stability characteristic should be included in every assay. Throughout stability testing period, the stability of the reference material also embraces the stability of reagents as well as uniformity of the performance of the instruments that are used for testing. Nevertheless, system performance and control for drift and discontinuity ensuing from alters in both reagents and instrumentation ought to be scrutinized.[8, 9]

3.2 Accelerated stability testing

This nervy testing stability method is utilized for the reasoning of product stability through the storage of product at various inebriated (warmer than ambient) temperatures and to determine the quantity of heat required to cause accelerated degradation of the product. This message enables to foretell stability of medicine product, its shelf life and to see storage condition by providing a substance of examination deciding formulations and thus diminution the development schedule. In gain to elevated temperature, hassles conditions are subjected during accelerated stability testing are humidity, light, pH, package and manufacturing process.[2]

In this method, the samples are assayed after subjecting to stress, refrigerated after stressing concurrently. Additional, a comparison is made between stressed product and unstressed material within the same assay in accelerated stability study and the recovery of stressed sample is articulated as an unstressed sample recovery percent. Relatively stability projection of thermolabile and proteinaceous components by denaturing stress temperatures is evaded. A commonsensible statistical discourse in accelerated stability projections based on the Arrhenius equation unremarkably requires that at least four stress temperatures be used. Many accelerated stability testing models are based on the Arrhenius equation (1) and modified Arrhenius equation (2)[8-10]

$$lnk = lnA + \frac{\Delta E}{RT} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where k= degradation rate/s, A = frequency factor/s, ΔE = activation energy (kJ/mol), R = universal gas constant (0.00831 kJ/mol), T=absolute temperature (Kelvin).

$$\log\left(\frac{k2}{k1}\right) = \frac{-Ea}{2.303R}\left(\begin{matrix}1 & 1\\ T2 & T1\end{matrix}\right) \quad \ldots\ldots\ldots\ldots\ldots (2)$$

Where *k1* and *k2* are rate constants at temperatures *T1* and *T2* expressed in

degree Kelvin; *Ea* is the activation energy; *R* is the gas constant.

Both equations explained the effect of storage temperatures on the degradation rate. Using the Arrhenius equation, protrusion of stability from the degradation rates observed at high temperatures for some degradation processes can be determined. Activation energy an independent variable, in the equation is equal to energy barricade that has to be surpassed for degradation reaction to ensue. Thus, when activation energy is known, then the degradation rate at low temperature can be projected from those at "stress" temperature.[10]

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 having some activation energy.[8] Although the Q rule[10] and bracket tables[8] methods for estimation of shelf life are not accepted by ICH or FDA, though some pharmaceutical companies are utilizing these methods.[2]

Q Rule: The Q rule affirms that product degradation rates increase by a constant factor Q_{10} when the storage temperature is increased by 10°C. The value of Q_{10} is frequently set at 2, 3, or 4 (to represent low, average and high estimates respectively) because these correspond to sensible activation energies. Q_{10} factor can be calculated from the following equation:

$$Q_{10} = \frac{k_{(T+\Delta T)}}{k_T} \ldots\ldots\ldots\ldots\ldots\ldots \text{(i)}$$

For an arbitrary change in temperature, ΔT=T2-T1, $Q_{\Delta T}$ is given by:

$$Q_{\Delta T} = \frac{k_{(T+\Delta T)}}{k_T} \ldots\ldots\ldots\ldots\ldots\ldots \text{(ii)}$$

The rate constant alters exponentially with temperature and is proportional to $(Q_{10})^n$, for larger shifts in temperature where n equals the temperature change (ΔT) divided by 10, which is obtained from equation (i) and (ii) as below:

$$Q_{\Delta T} = Q_{10}{}^{(\frac{\Delta T}{10})} \ldots\ldots\ldots\ldots\ldots\ldots \text{(iii)}$$

The time required to attain 90% of the original drug concentration after the storage at a higher or lower temperature is given by:

$$t_{90}(T_2) = \frac{t_{90}(T_1)}{Q_{10}{}^{(\Delta T/10)}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(iv)}$$

This model fallaciously presumes that Q value does not diverge with temperature.

An estimate of t_{90} (T_2) is independent of the order of a reaction. A Q_{10} value of 2 provides the most traditional estimate of the increase in shelf life with decreasing temperature, and Q_{10} value of 4 will estimate and result calculated with this value are considered to be possible diminish in shelf life with rising temperature.[10]

For e.g. If 90% of Phencyclidine (PCP) is recovered after 26 days at 55°C then the stability of PCP at 5°C under refrigerated condition may be estimated as PCP is probably stable for 832 days, maybe stable for 6318 days and possibly stable for 26624 days when Q_{10} is 2, 3 and 4 respectively.[8]

Bracket Tables: The bracket table system supposes that the activation energy is between two limits (e.g., between 10 and 20 kcal), for a given analyte. As a result, a table (eg.**Table-1:** Bracket table for Phencyclidine) may be drawn illustrating days of stress at different stress temperatures. The use of a 10 to 20 kcal bracket table is logical because broad experience indicates that, activation energies for analytes and reagents of interest in pharmaceutical and clinical laboratories lies in this range.[2, 8, 11]

Since the bracket table does not specify the stability requirement at stress temperature of 55°C, so data can be taken from 47.5°C. While PCP stability assumed to be 26 days which exceeds 6 months stability requirement (16.6 days) for 10 kcal model as well as 3 years (9 days) for 20 kcal model as per bracket table, thus it can be interpreted that PCP is probably stable for at least six months and possibly for 3 years respectively.[8]

Table-1: If 90% of Phencyclidine (PCP) is recovered after 26 days at 55°C then the stability of PCP at 5°C under refrigerated condition may be estimated as per following bracket table[8]

Stress Temp. (°C)	Days of stress to predict stability at 5°C for							
	6 Months		1 Year		2 Year		3 Year	
	20 kcal	**10 kcal**	**20 kcal**	**10 kcal**	**20 kcal**	**10 kcal**	**20 kcal**	**10 kcal**
14.5	55.3	100	111	201	221	402	332	603
25	16.1	54	32	108	64	217	97	326
35.5	5.1	30.6	10	61	20	122	31	183
47.5	1.5	16.6	3	32	6	66	9	100
60	0.5	9.2	0.9	18	1.9	37	2.8	55

3.3 Retained sample stability testing

For all marketed medicine whose stability data is essential, the manufacturer commonly practices of selecting a minimum one batch per year as stability sample for retained storage and those for new product, stability sample of every batch is taken which on later stage might be reduced to only 5% to 2% of marketed batches. When more than 50 batches are marketed, then stability sample from 2 batches are suggested to be taken. In this study, frequency of testing ought to be enough to set up the stability profile of the retained product and testing interval should be every three months over 1st year, every six months over 2nd year and annually thereafter throughout the estimated expiry date. i.e. sample will be tested at 3, 6, 12, 18, 24, 36, 48 and 60 months for a product having a shelf life of 5 years.

This typical method for determining stability profile on retained samples is termed as constant interval method. Since in this study, samples are subjected to storage under ideal condition because of which they never experience the stressful situation in the market during shipping and storage. Thus to make the quality of the product modified method known as stability testing by evaluation of market sample was used, which involve assessing quality attributes of that product available in the market place.[2, 12]

3.4 Cyclic Temperature Stress Testing

This method is not a conventional pharmaceutical stability testing technique for marketed products but is an awfully functional component in the gamut of tests obtainable for stability testing used in development or troubleshooting. This try is premeditated in such a way that examines mimics the same marketplace healthiness where the product is subjected during transport and storage. The period of a cycle is standardized to 24 hours since the diurnal rhythm on earth is 24 hours, which the marketed medicines are most belike to have much stipulation during storage. The

option of peak and extreme temperatures for the cyclic stress testing are definite on a product by product assumption and considering the grievous features same advisable storage temperatures and distinct physiochemical degradation properties of the products. Twenty cycles are also most advisable in this experimentation, but in more cases, ten cycles are enough. Cyclic temperature stress testing is useful, especially for liquid formulations like- emulsion, suspension etc. However, problems in tablets packed in bottles such as weakening of colouring or ink from the label, loosening of cap etc. are clearly plain from this test.[2, 13]

4. Guidelines for Stability Testing

The various guiding principle for stability study has been established (by WHO, ICH, FDA, EMA etc.) and the regulatory bodies in numerous countries have made requirements in the drug regulation for the manufacturer to assure that the optimally stable products are manufactured and marketed so that patients receive medication of quality, efficacy and safety throughout the products shelf life. Its principle rationale was to bring uniformity in testing from manufacturer to manufacturer.[14]

The assessed on the stability of medicinal products was started for the first time in 1988 by WHO. Later on, in 2000, a discussion between WHO and ICH results in harmonization in a number of stability test and condition to register the products all around the globe.[15] The ICH was established in 1991 and was an association formed with inputs from both regulatory and industry from the European Commission, Japan and the United States of America. Later in the year 1996, WHO has modified the version of ICH Q1A guideline.[16] In 2004, the World Health Organization released the guidelines for stability studies in a global environment.[17]

The United States Food and Drug Administration (USFDA) espoused all ICH guidelines (Human product), except Q1F. As well as the European Medicines Agency (EMA), also has included all ICH stability guidelines, except Q5C. Japan has adopted all of them in its regulations.[14] The codes and titles covered under ICH guidance have been outlined in the **Table-2**.[14, 18, 19] ICH guidelines were also extensive later on for veterinary products where codes and title are presented in **Table-3**.[20]

Table-2: Codes and titles used in ICH Guidelines for human products

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
Q1F	Stability data package for Registration Applications in Climatic Zones III and IV
Q5C	Stability testing of Biotechnological/Biological Products

Series of stability testing guidelines also have been issued by the Committee for Proprietary Medicinal Products (CPMP), Committee for Veterinary Medicinal Products (CVMP) under the European Agency for the Evaluation of Medicinal Products (EMEA) have also been issued to support those looking for marketing authorization for medicinal products in European Union. These are listed in **Table-4** and stability guidelines listed by WHO are shown in **Table-5.**[14, 21] The DDA (Department of Drug Administration) which is the drug regulating authority for Nepal situated at Bijulibazar, Kathmandu also has been implementing the WHO guidelines on stability for applying marketing authorization of the product.[22]

Table-3: Codes and titles used in ICH Guidelines for veterinary products

ICH Code	Guideline title
VICH GL3 (R)	Stability testing of new veterinary drug substances and medicinal products.
VICH GL4	Stability testing of new veterinary dosage form
VICH GL5	Photostability testing of new veterinary drug substances and medicinal products.
VICH GL8	Stability testing for medicated premixes
VICH GL17	Stability testing of biotechnological/ biological veterinary medicinal products.
VICH GL45	Bracketing and Matrixing Designs for Stability Testing of New Veterinary Drug Substances and Medicinal Products
VICH GL51	Statistical Evaluation of Stability Data

Table-4: CPMP Guidelines for stability studies

CPMP Codes	Guideline Titles
CPMP/QWP/576/96 Rev. 1	Guideline on Stability Testing for Applications for Variations to a Marketing Authorization
CPMP/QWP/6142/03	Guideline on Stability Testing for Active Substances and Medicinal Products Manufactured in Climatic Zone III and IV to be marketed in the EU
CPMP/QWP/609/96 Rev. 1	Note for Guidance on Declaration of Storage Conditions for Medicinal Products particulars and Active Substances
CPMP/QWP/122/02 Rev. 1	Note for Guidance on Stability Testing of Existing Active Substances and Related Finished Products
CPMP/QWP/072/96	Note for Guidance on Start Shelf Life of the Finished Dosage Form
CPMP/QWP/2934/99	Note for the Guidance for In-Use Stability Testing of Human Medicinal Products
CPMP/QWP/576/96	Note for Guidance on Stability Testing for a Type 2 Variation to a Marketing Authorization
CPMP/QWP/ 159/96	Note for Guidance on Maximum Shelf-Life for Sterile Products after First Opening or Following Reconstitution
EMA/CHMP/CVMP/QWP/441071/2011-Rev.2	Stability Testing for Applications for Variations to Marketing Authorization
EMEA/CVMP/424/01	In-Use Stability Testing of Veterinary Medicinal Products (Excluding Immunological Veterinary Medicinal Products)
EMEA/CVMP/846/99-Rev.1	Stability Testing of Existing Active Substances and Related Finished Products

Source: https://www.ich.org/page/quality-guidelines

5. Climatic Zones for Stability Studies

Since the different country has different climatic condition and temperature are different in different places. Thus stability testing program should be designed considering the intended market and the climatic condition in the region in which drug product will be used. For this purpose, the world has been alienated into four zones (Zone I to IV)

depending on their climatic conditions in order to estimate the deterioration rate of product and the expiration date precisely. Based on average yearly temperature and relative humidity data, the long term or real-time stability testing conditions, intermediate and accelerated stability testing conditions have been derived.[16, 23]

Table-5: WHO Guidelines for stability studies

WHO Codes	Guideline Titles
WHO Technical Report Series, No. 863, 1996, Annex 5	Guidelines for Stability Testing of Pharmaceutical Products Containing Well Established Drug Substances in Conventional Dosage Forms
WHO Technical Report Series, No. 953, 2009, Annex 2	Stability Testing of Active Pharmaceutical Ingredients and Finished Pharmaceutical Products
WHO Technical Report Series, No. 962, 2006, Annex 3	Guidelines on Stability Evaluation of Vaccines
Technical Guidance Series (TGS)-2	Establishing component stability for in vitro diagnostic medical devices. This document provides recommendations for establishing the stability of components for IVDs.
WHO Technical Report Series No. 999, 2016, Annex 5	Guidelines on the Stability Evaluation of Vaccines for Use Under Extended Controlled Temperature Conditions
Working document QAS/16.694/Rev. 1	Stability Testing of Active Pharmaceutical Ingredients and Finished Pharmaceutical Products

According to ASEAN guidance, climatic zone IV was subdivided into zones IVa and IVb to suit conditions in a nation where there was extreme humidity condition of 75% RH throughout the year like ASEAN countries and some parts of South America.[14, 15]

The standard climatic zones for stability studies have been presented in the **Table-6.**[14-16, 23]

Table-6: ICH Climatic zones and long-term stability conditions

Climatic Zone	Climate/ Definition	Major Countries/ Region	MAT*/Mean annual partial water vapour pressure	Long-term testing conditions
I	Temperate	UK, Russia, Northern Europe, USA	<15°C/ <11hPa	21°C/ 45%RH
II	Subtropical &Medi-terranean	Japan, Southern Europe	>15-22°C/ >11-18 hPa	25°C/ 60%RH
III	Hot & Dry	Iraq, India	>22°C/ <15 hPa	30°C/ 35%RH
IVa	Hot & humid	Iran, Egypt	>22°C/ >15-27 hPa	30°C/ 65%RH
IVb	Hot & very Humid	Brazil, Singapore	>22°C/ >27 hPa	30°C/ 75%RH

*MAT - Mean annual temperature measured in open air.

6. Protocol for Stability Testing

The stability testing protocol is a requirement for preliminary stability testing and is a written document which portrays the necessary part of stability study like-tests to be performed and planned schedule of testing. The protocol is required for batches of clinical, formulation development, registration and marketed product to develop a stability profile of the product. The protocol depends on types of dosage form and proposed container closure system. As well as protocol depends on the drugs formulated newly or is already is in the market. The protocol should also include the regions where the medicine is planned to be marketed that are proposed by ICH, namely climatic zones I-IV and extreme tropical zones, IVb by ANSEAN.[1] A well-designed stability study protocol should include the following information:

6.1 Number of batches

For batches at developmental stages, for registration of novel product or unstable established product, for stable and well-established batches only single batch, 1st three production batches and even two batches are subjected to stability studies respectively. The first three batches of drug product manufactured post-approval, if not submitted in original drug application, should be set on long-term studies using the identical protocol as in approved drug application.

Those data on laboratory scale batches generated at the developmental stage are not considered as primary stability data; rather, it can be used as supporting information. Generally, the selection of batches must compose a random sample from the population of pilot or production batches. [24]

6.2 Containers and Closures

The selection of containers and closure is crucial, and stability studies are done in an immediate containers-closures system intended for marketing. The packaging materials include aluminum strip packs, blister packs, HDPE bottles etc. which may also include secondary packs except for shippers. Products in all different types of containers/closures, whether meant for distribution or for physician and promotional samples, are to be tested separately. If bulk containers stimulate the actual packaging, then testing in prototype containers is acceptable.[24]

6.3 The Orientation of Storage of Containers

To permit for the full interaction of the product with the container-closure, samples of liquid or semisolid form like a solution, suspension, emulsion etc. are kept upright and placed either inverted or tilted. This orientation helps to know when the drug comes in contact with the containers and the closure consequences in the leaching of chemical substances from the closure components or adsorption of product components into the container-closure system.[24]

6.4 Testing time points

Testing frequency should be enough to establish the stability profile of drug product where the testing point interval at the long-term storage condition should be each three months over the 1st year, every six months over the 2nd year and yearly thereafter throughout the estimated expiration date. But for the accelerated storage conditions, at least 3 testing points, including the initial and ending points, for e.g. initial, three, and six months are suggested. If the accelerated stability data show a trend toward a significant failure, then the testing frequency should be amended to include more frequent testing either by adding samples at the last time point or by counting a 4th time point in order to determine the actual failure occurring time period, in the stability study design. If significant changes occur at the accelerated storage condition, then testing at the intermediate storage condition is necessary where a minimum of 4 test

points, including the initial and end time points, is suggested, for e.g. 0, 6, 9 and 12 months. For stability studies of new products, the test program has been presented in **Table-7.** [1, 16]

The testing frequency can be reduced, or testing of certain factor combinations can be excluded on the basis of reduced design, i.e. matrixing or bracketing if can be justified.[16] Bracketing is the design of a stability schedule such that only samples on the extreme of certain design factors, e.g., strength, package size, are tested at all time points as in a full design. On another aggregation, matrixing involves testing of a subset of the complete size of possible samples for all combinations at a specific time point. Subsequently, another subset of samples for every factor combination is studied. The factors that can be matrixes include batches, strengths with identical formulation, container sizes, fill sizes and intermediate time points.[26]

6.5 Test parameters

The stability testing protocol is a subset of product specification which consists of the list of tests similar to product specification. The stability study should include those tests that scrutinize the quality, purity, potency and identity, which might be distorted during storage. Therefore, appearance, assay, dissolution, degradation products, moisture content for the moisture-sensitive product, pH and microbiological test are standard test executed on stability test samples. Some tests are not requisite to be repetitive during stability testing as those tests that are requisite at the period of product release.[1] In addition to, ICH guidance Q6A also discussed other various tests like the purity of enantiomers, particle size and polymorphic form etc.

6.6 Test storage conditions

Stability studies on the finished product should provide adequate information on storage, subsequent use, as well as reconstitution or dilution of the product, if appropriate. The expiration date on labelling is based on storage condition at which long term testing is conducted. The storage condition is selected on the basis of the climatic zones where the product is anticipated to be marketed or proposed to be filed for regulatory acceptance[1]. The storage condition recommended by ICH and WHO are given in **Table-8**.[16, 27]

7. Impurities in New Drug Products

As per ICH Q3B guidelines, the impurities presents in new drug products also referred as degradation products of the drugs substances or reaction products of the drug substance with an excipient and/or immediate container closure system. It is not necessary to monitored and specified in new drug products of those impurities present in the new drug substance unless they too are degradation product. Degradation might occur during manufacturing and/or stability studies of the drug product. The specification for a drug product should include a list of degradation products expected to occur during manufacture of the commercial product and under recommended storage conditions.

Table-7: Test Schedule for stability testing of new products[1, 25]

Environment	Sampling Time Points (Months)[26]	Method & Climatic zone
25°C/ 60% RH	3, 6, 9, 12, 18, 24 and annually through the proposed shelf-life	Long term for zones I & II
30°C/ 35% RH	3, 6, 9, 12, 18, 24 and annually through the proposed shelf-life	Long term for zones III
30°C/ 65% RH	3, 6, 9, 12, 18, 24 and annually through the proposed shelf-life	Long term for zone III & IVa, or intermediate condition for zones I & II
30°C/ 75% RH	3, 6, 9, 12, 18, 24 and annually through the proposed shelf-life	Long term for zone IVb
40°C/ 75% RH	3, 6	Accelerated condition for all zones

"Specified degradation products" defines to those degradation products included in the specification of product with specific acceptance criteria where it can be identified or unidentified. Specified identified degradation products should be included along with specified unidentified degradation products estimated to be present at a level greater than (>) the identification threshold given in **Table-9**. Those for potent or toxic or having unexpected pharmacological effects at levels not more than (≤) the identification threshold, the quantitation/ detection limit of the analytical measures should be commensurate in a way to control the degradation products. The procedures utilize or assumption made to established the level of the unidentified degradation product should be clearly defined.[29]

Table-8: Stability studies storage conditions for drug products.

Intended Storage condition	Stability Test Method	Storage Condition			
		WHO		ICH	
		Temperature/humidity	Period in months	Temperature/ humidity	Period in months
Room Temperature	Long term[a]	25±2°C/60±5%RH or 30±2°C/65±5%RH or 30±2°C/75±5%RH	12	25±2°C/60±5% RH	12
	Intermediate[b]	30±2°C/65±5%RH	6	30±2°C/65±5% RH	6
	Accelerated	40±2°C/75±5%RH	6	40±2°C/75±5% RH	6
Refrigerated	Long term	5±3°C	12 or 6	5±3°C	12
	Accelerated[a]	25±2°C/60±5%RH or 30 ±2°C/65±5%RH or 30 ±2°C/75±5%RH	6	25±2°C/60±5% RH	6
Freezer	Long term	–20 °C ± 5 °C	12 or 6	–20 °C ± 5 °C	12

[a]It will be applicant decision to performed long term stability and as well accelerated stability studies at either 25±2°C/60% RH ±5% RH or 30°C ±2°C/65% RH ±5% RH as given in room temperature and refrigerated storage condition respectively. [b]If 30 °C ± 2 °C/65% RH ± 5% RH or 30 °C ± 2 °C/75% RH ± 5% RH is the long-term condition, there is no intermediate condition.

8. Expiration Date or Shelf Life

Every drug product bears expiration date on their label, which clearly states the time where the product remains stable and can be dispensed when stored under the manufacturer's recommended storage condition. The expiration date established the limit beyond which it is expected that the product might not be fit for use. Where an expiration date is stated only in terms of the month and the year, it is a depiction that the proposed expiration date is the end day of the month. If the product is not stored as per storage condition instructed by the manufacturer, then the product may degrade more hastily. To ensure the product stability to the labelled expiration date, the strict adherence to the instructed storage requirement is necessary. Thus the expiration date mentioned in the label applies when these products are store only as per requirement set by the manufacturer until it is dispensed to consumer.[2,30]

Table-9: Reporting, Identification, and Qualification Thresholds for Impurities in New Drug Products[29]

Maximum Daily Dose	Reporting Threshold	Identification Threshold	Qualification Threshold
>2g/day	0.10%	0.10%	0.15%
>100mg-2g	0.1/0.05%	0.2% or 2mg Total Daily Intake (TDI), whichever is lower	0.2% or 3mg TDI, whichever is lower
10mg-100mg	0.05%	0.2% or 2mg TDI, whichever is lower	0.5% or 200µg TDI, whichever is lower
1mg-10mg	0.05%	0.5% or 20µg TDI, whichever is lower	1.0% or 50µg TDI, whichever is lower
<1mg	0.05%	1.0% or 5µg TDI, whichever is lower	1.0% or 50µg TDI, whichever is lower

9. ESTIMATION OF SHELF LIFE

9.1 Based on Real-Time Testing

The data obtained from the studies of long term storage condition is used for the determination of shelf life. For this, the data is linearized at first and test for goodness of fit is applied from where the linearized data is analyzed to see the matching of slope and intercepts. The different potential in the pattern of the concentration-time data of the 3 batches is given in **Table-10.** The data is pooled accordingly and used for estimation of the common slope.[6]

Table -10: Pattern of concentration-time data and pooling decision

Slope	Intercept	Variation Factor	Pooling
Identical	Identical	Nil	Yes
Identical	Different	Batch, e.g. unequal initial drug concentrations	No
Different	Identical	Storage, e.g. difference in the rate of drug loss	No
Different	Different	Interactive Forces-Both batch and storage factor	No

For the dedication of the importance of distinction in case of slope or intercept, statistical tests like t-test should be applied. The statistics are to be had within the form of best 5 data points, i.e. zero, 3, 6, 9 and twelve months, either pooled from the 3 batches or from the 3 individual batches if they may be no longer fit for pooling. In case data isn't in shape for pooling, stability approximations are to be made on the worst batch. The shelf life/expiry date is decided from the regression line of this 5 point records-based totally on the calculation of 95% one-sided confidence limit. For interpretation, the shelf life, 90% drug concentration is taken into consideration as the lowest specification criteria and the point where the extension line cuts the 95% confidence limit line is taken as an expiry date.

Due to the fact shelf life derived from the intersection of the lower 90% confidence bound and 90% potency value has a 95% confidence level; therefore there is simplest a 5% hazard that our estimate of the shelf life will be too excessive.[24, 29]

Maximum pharmaceutical merchandise is characterized via only 1 shelf life. However, in a few cases, a product may additionally have two, e.g. a freeze-dried (lyophilized) protein product can also have handiest one shelf life, say 2 years, for the product stored in the dry circumstance and a 2nd shelf life, say 2 days, for the product whilst it has been reconstituted with the perfect vehicle and is prepared for injection.[7]

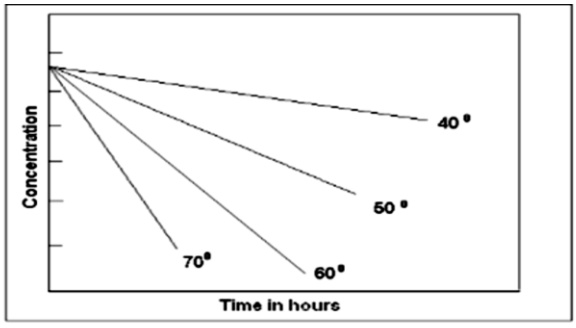

Fig. 1: Arrhenius plot for predicting drug stability at room temperature

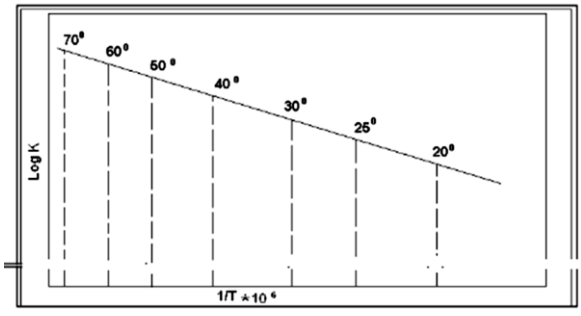

Fig. 2: Arrhenius plot for predicting drug stability at roomtemperature

9.2 Based on accelerated testing

Shelf life can be predicted based on the principle of chemical kinetics demonstrated by Garret and Carper method as follow:

In this method, the determination of shelf-life is carried out by the Arrhenius plot. The Arrhenius equation is applied for the mathematical estimation of shelf life, which embrace the effect of temperature of chemical reaction on rate constant k, at thermodynamic temperature 1/T

as observed in a straight line. The value of k is obtained by extrapolation of the slope of this line which is determined from the results of temperature. This k value is substituted extraneous. After a given time, the amount of decomposition is shown by order of a reaction. Preliminary tests are there for indispensable to determine this order.

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

Log K=logA-Ea/2.303*RT

Where, k = rate constant; R= gas constant=1.987cal/mole; T= absolute temperature; A= frequency factor and Ea= energy of activation

For zero order reaction, expiration date observed at 25°C. C=Initial potency–minimum potency/reaction rate at 25°C.

$T_x= Y_0-Y_x/K_0$

For first order reaction, expiration date at 25°C. C (tx) = log initial potency – log minimum potency/reaction rate at 25°C

$T_x= log0-logY_x/K_1$

Where, Y_0= initial potency; Y_x= final potency; K_0= zero order reaction and K_1= first order reaction

As per Garret and Carper method

1. At least three temperatures, such as **40ºC, 50 ºC and 60ºC** are used to store several samples of the drug product**.**

2. For a few weeks, the **drug content at all three storage points are determined, and the average is calculated.**

3. A graph is plotted between time and **log percent of drug remaining, a**t each temperature If the decomposition is **first order** this gives a linear line. If it is **zero order, percent drug remaining versus time** will give a straight line.

4. Next, the **log K or log of reaction constant on Y axis and 1/T x 10-3 on X axis is taken** and best fit line is drawn. E**xtrapolate** this line, the Arrhenius plot to get **k at 25 ºC** and from this we can calculate the shelf-life.[24, 32]

10. Current Trends in Stability Testing

The Current trends in stability studies are to characterize the environment for stability testing for worldwide marketing, especially amongst the multinational pharmaceutical industries. To be in compliance with this and to establish a single set of condition covering extreme environmental conditions, the pharmaceutical industries are orienting their protocols. Extending in the duration of the accelerated testing period from six to twelve months and conducting further tests at 50°C/75% RH for three months are some examples of specific changes for global testing. As all tests are done in one laboratory, to avoid reiteration of stability testing for other areas as well as for efficient and optimum utilization of resources, the concept behind these changes has grown up. In addition, drug product degradation is mainly caused by an amalgamation of 3 environmental factors, viz., temperature, humidity and light rather than by temperature and humidity conditions only.[2, 31-33]

11. Conclusion

The stability testing is a crucial aspect for new drug product and formulation during the pharmaceutical development phase, which is an important component of it. The stability studies are the key practical technique that is capable of distinguishing active drug substance from any degraded product as well as estimate the shelf life under defined storage condition. The degradation studies should be conducted during the developmental phase of the drug product to gain enough information about the stability profile of the molecule. Over with time and with escalating knowledge and awareness, the regulatory necessities have been made ever strict about attaining the above goal in all probable situation to which the product might be subjected during its shelf life. To include the recommended storage condition and expiration date on the label and moreover to ensure the safety and efficacy of medicine throughout its shelf life, stability tests are carried out. Therefore, the stability tests should be carried out following proper scientific principles and after understanding of the current regulatory provisions and as per the climatic zones.

Acknowledgements

Authors are thankful to the Biogain Remedies Pvt. Ltd., especially Abhishek Ghimire, Factory Operations Director for providing space, encouragement and needed things to carry out this work.

Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

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