Review Article

An approach to drug stability studies and shelf life determination

ABSTRACT

The main objective of carrying out stability studies of the drug product is to determine shelf life of drug during storage. Stability of drug can be defined as “The capability of a particular formulation in a specific container/closed system, to remain within its physical, chemical, microbiological, therapeutic, and toxicological specifications throughout its shelf life”. As mentioned in the International Conference on Harmonization (ICH) guideline Q1A (R2), stability studies are commonly the activity on the critical path to regulatory filing and approval. There are different types of stability studies and different methods are applied for the determination of stability; namely real time stability testing, accelerated stability testing, retained sample stability testing and cyclic temperature stress testing. The pH and temperature are the main factors which influences the stability. The pH-rate profile *(log(k)*vs *pH)* is the pH dependence of the specific rate constant of degradation of a compounds. Forced degradation indicates specificity of stability indicating methods and it includes degradation of new drug substance and products at more severe conditions than the accelerated conditions. The different conditions used for forced degradation includes hydrolytic, oxidation, photolytic and thermal etc. The techniques utilized for evaluation of stability studies can be TLC, HPLC-DAD, HPLC-UV, HPTLC, HPLC-MS, LC-MS/MS, LC-NMR, some techniques have high sensitivity and resolution power to develop the effective stability indicating method while FDA’s method, the direct method, inverse method, simulation results and Garret and Carper method are the different methods mentioned for shelf life estimation of drugs and products. Thus stability testing of pharmaceutical products the key procedural contribution in the development program for a new drug as well as new formulation.

Keywords: Stability, Shelf life, Forced degradation, ICH guidelines, pH profile.

1. INTRODUCTION

The Shelf life of the pharmaceutical drug products is established by the stability studies. Stability testing of pharmaceuticals is known to be a complex set of procedures which involving considerable cost, time consumption and scientific expertise in order to build in quality, efficacy and safety in a drug formulation. Scientific as well as commercial success ofany 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[1]. Stability defines as “The capability of a particular formulation in a specific container/closed system, to remain within its physical, chemical, microbiological, therapeutic, and toxicological specifications throughout its shelf life”. Stability is officially defined as “the time lapse during which the drug product retains the same properties & characters that is processed at the time of manufacture[2]. There are varieties of factors influencing the stability of a pharmaceutical product; because of their involvement stability testing is termed as a complex process. These factors mainly includes stability of the active ingredient(s); interaction between active ingredients and excipients, type of dosage form and there manufacturing process followed, container/closure system used for packaging,

light, heat and moisture conditions encountered during shipment, storage and handling etc.[3].Theshelf life determination of the drug product is main objective of stability studies. The stability refers to storage time allowed before any degradation product in dosage form achieves a sufficient level to represent a risk to the patient.Based on this time, the product shelf life or expiration date is determined[4]. From a pharmaceutical development point of view, stability studies are frequently on the critical path to starting patient studies and registration stability studies, as described in the International Conference on Harmonization (ICH) guideline Q1A (R2), are commonly the activity on the critical path to regulatory filing and approval. Stability studies are also a significant resource commitment in both pre and post-approval phases[5].

1.1. Importance of Stability Studies

- Instability of active drug and products may lead to under medication of the drug due to lowering concentration in the dosage form.
- The toxic product may be formedduring decomposition of active drug.
- Changing in physical appearance through the principles of kinetics due to instability, are used in predicting the stability of the drug.
- 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.

1.2. Objectives of Stability Studies

- The purpose of stability testing is to provide evidences on how the quality of drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light.
- To select adequate (from the viewpoint of stability) formulations and container closure Systems. To determine shelf-life and storage conditions.
- To substantiate the claimed shelf-life.
- To verify that no changes have been introduced in the formulation or manufacturing process that can adversely affect the stability of the product.
- The main aim of accelerated stability study to predict the stability profile of a drug product that prediction of the shelf life of the product before launching into the market [6].

1.3. Guidelines for Stability Testing

The regulatory authorities in several countries have made provisions in the drug regulations for the submission of stability data by the manufacturers to assure that optimally stable molecules and products are manufactured, distributed and given to the patients. Its basic purpose was to bring uniformity in testing from manufacturer to manufacturer. Such guidelines were initially issued in 1980s which include basics issues related to stability, the stability data requirements for application dossier and the steps for their execution. To overcome the bottleneck to market and register the products in other countries,these were later harmonized (made uniform) in the International Council for Harmonization (ICH). The ICH was established in 1991, it was a consortium formed with inputs from both regulatory and industry from European commission, Japan and USA and various guidelines for drug substance and drug product came into existence regarding their quality, safety and efficacy. These guidelines are called as quality, safety, efficacy and multi- disciplinary (also called as Q, S, E and M) guidelines.

ICH Code	Guideline title
Q1A	Stability testing of New Drug Substances and Products (Second Revision)
Q1B	Stability testing: Photo stability testing of New Drug Substances and Products
Q1C	Stability testing of New Dosage Forms
Q1D	Bracketing and Matrixing Designs for stability testing of Drug Substances 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

Table 1: Codes and titles used in ICH Guidelines

The ICH guidelines did not address the extreme climatic conditions found in many countries, for this reasonthe World Health Organization (WHO) in 1996 modified these guidelines, also 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, United States Food and Drug Administration (USFDA) also issued a guidance document entitled 'Expiration Dating of Solid Oral Dosage Form Containing Iron'.For stability studies in global environment,in 2004 WHOalso released guidelines. ICH guidelines were also extended later for veterinary products. India Drug Manufacturers Association also a technical monograph on stability testing of drug substances and products existing in India. For active pharmaceutical ingredients, drug products or formulations and excipients different test condition and requirements have been given in the guidance documents. The codes and titles covered under ICH guidance have been outlined in the Table 1 & Table 2. Series of guidelines related to stability testing have also been issued by the Committee for Proprietary Medicinal Products (CPMP) under the European Agency for the Evaluation of Medicinal Products (EMEA) to assist those seeking marketing authorization for medicinal products in European Union. These are listed in Table 3.

Table 2: ICH Q1A Summary of Stability Parameters

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

Table 3: CPMP Guidelines for Stability

CPMP code	Guideline title
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 Zones 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 of Shelf Life of the Finished Dosage Form
CPMP/QWP/2934/99	Note for 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

Ref.: [7]

1.4. Stability Studies and their Classification

Stability studies is the essential criteria for assure the quality efficacy and integrity of the final product.

1.4.1 Physical stability studies: For intrathecal, ocular and intra-arterial routes, the physical evaluation of the solution is of particular importance. The physical changes can have deleterious effects too. A physical stability studies are also essential because tablet may become soft and ugly or it may become very hard and show very slow dissolution time as a result of which bioavailability may not be good, so. A more refined physicalevaluationis particularly important for therapeutic proteins to evaluate their kinetic profiles of aggregation by using turbidimetry, light obstruction, dynamic light scattering or microscopic analysis.

1.4.2. Chemical stability studies: Many chemical reactions involve moisture as a reactant and play the role of solvent vector in many reactions. Molecules have more kinetic energy and more decomposition is observed because moisture has better thermal conductivity than solids whichallow better heat transfer. The common cause in all these, hydrolysis or oxidation or fermentation; is moisture. The presence of moisturespeeds upall reactions. The HPLC, HPTLC or capillary electrophoresismethods are widely for evaluation of chemical instability.

1.4.3Microbiological stability studies: Microorganisms not only contaminate the formulations containing moisture but also solid dosage forms containing natural polymer because many natural polymers are source of microorganism[8].

2. STABILITY TESTING METHODS

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

2.1 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.

2.2Accelerated stability testing

In accelerated stability testing, a product is stressed at several high (warmer than ambient) temperatures to determine the amount of heat input required to cause product failure. This information is then projected to compare the relative stability of alternative formulations and to predict shelf life. In addition to temperature, the moisture, light, agitation, gravity, pH and package etc. arethe stress conditions applied during acceleratedstability testing. In thismethod the samples are subjected to stress, refrigerated after stressing, and then assayed simultaneously. Because of 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, 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. However, for thermo labile and proteinaceous components, relatively accurate stability projections are obtained when denaturing stress temperatures are avoided.For statistical reasons, the treatment in accelerated stability projections is recommended to be conducted at four different stress temperatures. The concept of accelerated stability testing is based upon the Arrhenius equation (1) and modified Arrhenius equation (2):

$$\ln K = \ln A + \frac{\Delta E}{RT} \quad (1)$$

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 (K).

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

Where *k1* and *k2* are rate constants at temperatures *T1* and *T2* 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. 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. A common practice of manufacturers in pharmaceutical industries was to utilize various shortcuts such as Q rule and bracket tables for prediction of shelf life of the products but these methods are not official either in ICH or FDA. The Q rule states that a product degradation rate decreases by a constant factor Q10 when the storage temperature is decreased by 10°C. The value of Q10 is typically set at 2, 3 or 4 because these

correspond to reasonable activation energies. This model falsely assumes that the value of Q does not vary with temperature. The bracket table technique assumes that, for a given analyte, the activation energy is between two limits (e.g., between 10 and 20 kcal). As a result, a table may be constructed showing days of stress at various stress temperatures. The use of a 10 to 20 kcal bracket table is reasonable because broad experience indicates that most analytes and reagents of interest in pharmaceutical and clinical laboratories have activation energies in this range.

2.3Retained sample stability testing

In this study, stability samples, for retained storage for at least one batch a year are selected. If the number of batches marketed exceeds 50, stability samples from two batches are recommended to be taken. At the time of first introduction of the product in the market, the stability samples of every batch may be taken, which may be decreased to only 2% to 5% of marketed batches at a later stage. In this study, the stability samples are tested at predetermined intervals i.e. if aproduct has shelf life of 5 years, it is conventional to test samples at 3, 6, 9, 12, 18, 24, 36, 48, and 60 months. This conventional method of obtaining stability data on retained storage samples is known as constant interval method. Stability testing by evaluation of market samples is a modified method which involves taking samples already in the market place and evaluating stability attributes. This type of testing is inherently more realistic since it challenges the product not just in the idealized retained sample storage conditions, but also in the actual marketplace.

2.4 Cyclic temperature stress testing

This is not a routine testing method for marketed products. In this method, cyclic temperature stress tests are designed on knowledge of the product so as to mimic likely conditions in market place storage. The period of cycle mostly considered is 24 hours since the diurnal rhythm on earth is 24 hour, which the marketed pharmaceuticals are most likely to experience during storage. The minimum and maximum temperatures for the cyclic stress testing is recommended to be selected on a product-by-product basis and considering factors like recommended storage temperatures for the product and specific chemical and physical degradation properties of the products. It is also recommended that the test should normally have 20 cycles[9].

3. FACTORS INFLUENCING STABILITY OF DOSAGE FORM

3.1 pH:pH plays a significant role in the active ingredient's solubility and thus in its bioavailability. The rate of degradation is much higher at extreme values. The optimum pH is often the same as the pH at which a given molecule is most soluble. Buffers are often included in pharmaceutical product formulations, and provide very good stability. However the formulation of preparations using these pharmaceutical products may change their pH and their stability.

3.2 Temperature: One of the most important factors in drug stabilitytemperature. An increase of 10°C in storage temperature may lead to a 2 to 5 fold increase in the speed of degradation reactions. For certain molecules, physicochemical stability is only optimal within a small temperature range, outside of which increased degradation is observed. For most active ingredients, the kinetics of degradation reactions follows the Arrhenius law. Thus, when performing stability studies at elevated temperatures (at 40° C, for example), it is possible to determine the formulation's stability at ambient temperature.

3.3 Surfactants: The active ingredient in hydrolytic groups such as hydroxyls, surfactants can be used to protect and limit their degradation. The different types of surfactants (anionic, cationic or non-ionic)

may however form micelles in solution, thus trapping the active ingredient molecules and changing their bioavailability in solution.

3.4 Oxygen: The presence of oxygen in a preparation may cause instability via the oxidation of one of its components. Formulation (antioxidants) and manufacturing techniques (under nitrogen) need to be determined accordingly. In order to preventing the infiltration of oxygen over time selecting an appropriate container and ensuring its integrity are also important elements to consider.

3.5 Light: In photosensitive molecules light is a parameter that may cause chemical instability. If preventive measures are implemented during manufacturing (selection of appropriate packaging material), it is important to check that they are maintained over time [10].

4. pH-RATE PROFILES

The pH-rate profile is the pH dependence of the specific rate constant of degradation of a compound; sometimes it called as pH-stability profile or rate-pH profile, and it is conveniently represented by a *log(k)* versus *pH* plot. The pH-rate profiles help in developing more stable solution formulations and lyophilized products alsoprovide insights into the catalytic nature of a reaction. Many drug degradation reactions follow apparent first order kinetics andusually plotted in a pH-rate profile which subject to specific and general acid-base catalysis. One should correct for general acid-base catalysis by buffer components by extrapolation to zero buffer concentration if the catalysis effect is significant. Analysis of a pH-rate profile can be started by assuming all possible pathways and writing down the corresponding rate equations (Eq. 3). The presence or absence of a certain mechanism can then be verified by analyzing the kinetic data.

$$k_{obs} = k_0 + k_H[H^+] + k_{OH}[OH^-] + k_1[\text{buffer species 1}] + k_2[\text{buffer species 2}] + \cdots$$

$$= k_0 + \sum_i k_i\, k_i \quad (3)$$

4.1 V-shaped, U-shaped, and other truncated pH-rate profiles

In the degradation of carboxylic acid derivatives, such as esters, amides, substituted ureas, etc.specific acid and base catalysis is common. In the absence of other more complicated mechanisms the pseudo-firstorder rate constant can be written as;

$$k_{obs} = k_H[H^+] + k_0 + k_{OH}[OH^-] \quad (4)$$

Here, $k_0$is the intrinsic apparent first-order rate constant, and k_H and k_{OH} are the catalytic coefficients for the hydrogen and hydroxyl ions, respectively. The resulting rate-pH profile plot consists of a straight line with slope of -1 in the acidic region, and another straight line with slope of 1 in the basic region (Fig. 1).

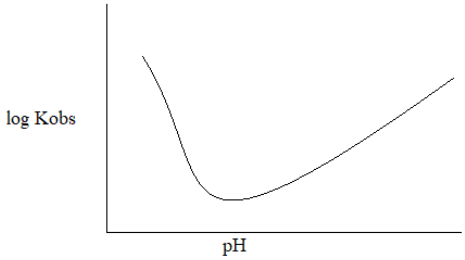

Fig.1: Schematic rate-pH profiles for reactions involving only a single reactive species with specific acid- base-catalysis.

4.2 Sigmoidal pH-rate profiles

Sigmoidal rate-pH profiles usually arise from the dissociation of the drug molecules. In the vicinity of *pH=pKa*, when plotted as a function of pHthe species distributions of a weak base or weak acid are sigmoidal. Therefore, if both the acidic and basic species of the compound can undergo degradation with differing rate constants, the rate-pH profile is expected to be sigmoidal. For example, for the decomposition of weak acid HA:

$$HA \xrightarrow{k_{HA}} product \quad (5)$$

$$A^- \xrightarrow{k_{A^-}} product \quad (6)$$

When the drug concentration is measured, a distinction between the ionized and unionized species is usually not made. The apparent rate of the reaction is

$$rate = k_{HA}[HA] + k_{A^-}[A^-]$$

$$= \frac{k_{HA}[H^+] + k_{A^-}K_a}{K_a + [H^+]}\{HA\} \quad (7)$$

Here, *K*ais the dissociation constant of HA, while *{HA}* is the total concentration of HA. Therefore, a plot of the apparent rate constant is sigmoidal with respect to pH, so long as the rate constants are not identical. The rate constant of each species can be estimated from the limits of the apparent rate constant at low and high pH, and that *pKa=pH* at the inflection point of the sigmoidal pH-rate profile plot. If the change in rate is due to ionization at a single site, the sigmoidal curve will encompass slightly more than ± 1 pH units of the expected pKa.

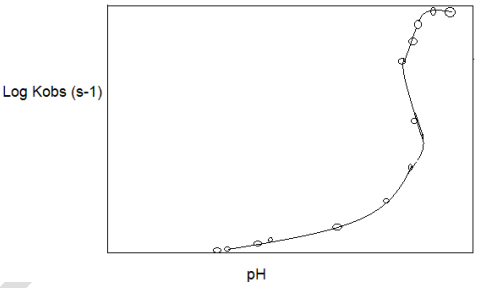

Fig. 2: Sigmoidal pH-rate profile

4.3 Bell-shaped pH-rate profiles

Bell-shaped pH-rate profiles shows a minima or maxima. Different scenario can lead to this kind of pH-rate profile. The most inherent framework arises from the presence of two ionizable functional groups in the molecule. For example, for a diprotic acid, H_2A, three species are in solution: H_2A, HA^-, and A^{2-}, where the concentration-pH profile of species HA^- is bellshaped. Based on reactivityof monoprotic species, HA, the corresponding pH-rate profile could show either maxima or a minima. In case ofacid and a base, the two ionizations are on different reactant molecules. Another one occurs when ionization is combined with a change in the rate-determining step. For example, consider a reaction: A→B→C, where A is a monoprotic acid/base. The two species of reactant A may have very different reactivity's with the rate constant of step B→C falling somewhere in between. Therefore, in one pH region (below or above its

pKa), the step A→B is the slowest, whereas B→C becomes the rate-determining step over another pH range. A bell-shaped pH-rate profile then results, with one side of the bell corresponding to the ionization while the other corresponds to the switch of the ratelimiting step.

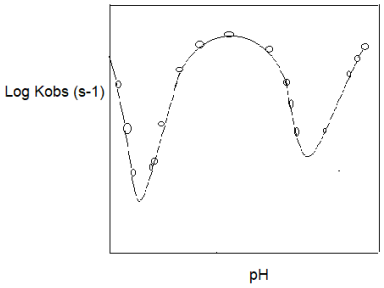

Fig. 3: Bell-shaped pH-rate profile

4.4 More complicated pH-rate profiles

The analysis of a pH-rate profile can complicatedwith the presence of multiple ionization centers, either their construction is based on same principles. Some of the features may not be fully developed in a particular pH-rate profile depending on how far their *pKa* values are isolated. For example, the pH-rate profile of aspirin shows conformation for specific acid-catalysis at pH<2 and specific base-catalysis at pH>10. The sigmoidal portion is due to the different reactivity of the neutral and ionized aspirin species and broad shoulder within some pHdue to intramolecular catalysis [11].

4.5 Influence of Temperature

Linearplotsof$Ink = f(1/T)$ were used to calculate the energy of activation (Ea), the entropy ($\Delta S^{\neq}$) and enthalpy ($\Delta H^{\neq}$) and the preexponential coefficient (A) for the partial reactions which based on the Arrhenius equation $Ink = InA - Ea/RT$. The entropy of all reactions under the influence of water (spontaneous hydrolysis) was negative, which suggest the bimolecular character of these reactions. The positive values of entropy forthereactions catalyzed by hydrogenionsindicatedapositive participation ofentropyof protonationreaction. The linear relationships of $\Delta H^{\neq} = f(\Delta H^{\neq})$ and $Ea = f(InA)$ were obtained for the degradation of protonated molecules of compounds catalyzed by hydrogen ions and spontaneous hydrolysis of molecules under the influence of water, which suggested that all reactions occurred according to the same mechanism of a bimolecular reaction[12].

5. FORCED DEGRADATION

Forced degradation is the degradation of new drug substance and products at more severe conditions than the accelerated conditions. It is essential to indicate specificity of stability indicating methods and also provides an insight into degradation pathways and degradation products of the drug substance and helps in elucidation of the structure of the degradation products[13].

Forced degradation studies are performed for following reasons:

1. To establish degradation pathways of drug substances and drug products.

2. To differentiate degradation products those are related to drug products from those that are generated from non-drug product in a formulation.
3. To elucidate the structure of degradation products.
4. To determine the intrinsic stability.
5. To reveal the degradation mechanism such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and drug product[14, 15].
6. To establish stability indicating nature of drug molecules.
7. To understand the chemical properties of drug molecules.
8. To generate more stable formulations.
9. To produce a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.
10. To solve stability-related problems[16].

5.1 Time to perform forced degradation

It is very necessary to know when to perform forced degradation studies for the development of new drug substance and new dug product. As per FDA guidelines, stress testing should be performed in phase III of regulatory submission process at different pH solutions, in the presence of oxygen and light, and at elevated temperature and humidity levels to determine the stability of the drug substance. These stress studies are conducted on a single batch. The results should be summarized and submitted in an annual report[17].

5.2 Limits for degradation

How much degradation is sufficient is the question which always has been the topic of many discussions amongst pharmaceutical scientists. Degradation of drug substances between 5% and 20% has been accepted as reasonable for validation of chromatographic assays [18, 19]. Some pharmaceutical scientists think 10% degradation is optimal for use in analytical validation for small pharmaceutical molecules for which acceptable stability limits of 90% of label claim is common [20]. Over-stressing a sample may lead to the formation of a secondary degradation product that would not be seen in formal shelf-life stability studies and under-stressing may not generate sufficient degradation products[21].

Table4: Conditions mostly used for forced degradation studies.

Degradation type	Experimental conditions	Storage conditions	Sampling time (days)
Hydrolysis	Control API (no acid or base)	40 ^{0} C, 60 ^{0} C	1,3,5
	0.1 M HCl	40 ^{0} C, 60 ^{0} C	1,3,5
	0.1 M NaOH	40 ^{0} C, 60 ^{0} C	1,3,5
	Acid control (no API)	40 ^{0} C, 60 ^{0} C	1,3,5
	Base control (no API)	40 ^{0} C, 60 ^{0} C	1,3,5
	pH: 2,4,6,8	40 ^{0} C, 60 ^{0} C	1,3,5
Oxidation	3% H_2O_2	25 ^{0} C, 60 ^{0} C	1,3,5
	Peroxide control	25 ^{0} C, 60 ^{0} C	1,3,5
	Azobisisobutyronitrile (AIBN)	40 ^{0} C, 60 ^{0} C	1,3,5
	AIBN control	40 ^{0} C, 60 ^{0} C	1,3,5
Photolytic	Light 1× ICH	NA	1,3,5
	Light 3× ICH	NA	1,3,5
	Light	NA	1,3,5
Thermal	Heat chamber	60 ^{0} C	1,3,5
	Heat chamber	60 ^{0} C/75% RH	1,3,5
	Heat chamber	80 ^{0} C	1,3,5

	Heat chamber Heat control	80 ^{0} C/75% RH Room temp.	1,3,5 1,3,5

NA: Not Applicable, Ref. [22]

5.3 Degradation prediction tools

CAMEO

CAMEO is a computer program that predicts the products of organic reactions given starting materials, reagents and conditions. The analyses cover the following key degradation conditions: basic/nucleophilic, acidic/electrophilic, radical, oxidative/reductive and photochemical as well as mechanistic interpretations of these reactions. In general, the CAMEO algorithms have been designed to give product mixtures that err on predicting more degradation products than actually observed[23].

5.4 Mechanism of Degradation

5.4.1Hydrolytic conditions

Hydrolysis is a chemical process that includes decomposition of a chemical compound by reaction with waterand it is most common degradation chemical reactions over a wide range of pH. It involves catalysis of ionizable functional groups present in the molecule under acidic and basic condition.Acid or base stress testing involves forced degradation of a drug substance by exposure to acidic or basic conditions which generates primary degradants in desirable range. Hydrochloric acid or sulfuric acids (0.1–1M) for acid hydrolysis and sodium hydroxide or potassium hydroxide (0.1–1M) for base hydrolysis are suggested as suitable reagents for hydrolysis and it depends on the stability of the drug substance. [22, 24]. If case of poorlywater soluble compounds, co-solvents can be used to dissolve them in HCl or NaOH. The selection of co-solvent is based on the drug substance structure. Stress testing trial is normally started at room temperature and if there is no degradation, elevated temperature (50–70^{0} C) is applied. Stress testing should not exceed more than 7 days. The degraded sample is then neutralized using suitable acid, base or buffer, to avoid further decomposition.

5.4.2Oxidation conditions

In forced degradation studies Hydrogen peroxide is widely used for oxidation of drug substances but other oxidizing agents such as metal ions, oxygen, and radical initiators (e.g., azobisisobutyronitrile, AIBN) can also be used.Depending on the drug substance selection of an oxidizing agent, its concentration, and conditions is proceeds. It is found that that maximum 20% degradation could potentially generate relevant degradation productswhen subjecting the solutions to 0.1–3% hydrogen peroxide at neutral pH and room temperature for seven days[22]. In oxidative degradation of drug substance an electron transfer mechanism isinvolve to give reactive anions and cations. Amines, sulfides and phenols are susceptible to electron transfer oxidation to give N-oxides, hydroxylamine, sulfones and sulfoxide[25]. The functional group with labile hydrogen like benzylic carbon, allylic carbon, and tertiary carbon or α-positions with respect to hetero atom is susceptible to oxidation to form hydro peroxides, hydroxide or ketone [26,27].

5.4.3Photolytic conditions

Photo stability studies are performed to generate primary degradants of drug substance by exposure to UV or fluorescent conditions. Some recommended conditions for photo stability testing are described in ICH guidelines [28]. Samples of drug substance and solid/liquid drug product should be exposed to a minimum of 1.2 million lx h and 200W h/m^{2} light. The most commonly accepted wavelength of light is in

the range of 300–800 nm to cause the photolytic degradation [29,30]. The maximum illumination recommended is 6 million lx h [27]. Light stress conditions can induce photo oxidation by free radical mechanism. Functional groups like carbonyls, nitro aromatic, N-oxide, alkenes, aryl chlorides, weak C–H and O–H bonds, sulfides and polyenes are likely to introduce drug photosensitivity [31].

5.4.4Thermal conditions

Thermal degradation (e.g., dry heat and wet heat) should be carried out at more strenuous conditions than recommended ICH Q1A accelerated testing conditions. Samples of solid-state drug substances and drug products should be exposed to dry and wet heat, while liquid drug products should be exposed to dry heat. Studies may be conducted at higher temperatures for a shorter period [22]. Effect of temperature on thermal degradation of a substance is studied through the Arrhenius equation:

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

Where *k* is specific reaction rate, *A* is frequency factor, *Ea* is energy of activation, *R* is gas constant (1.987 cal/deg mole) and *T* is absolute temperature (27, 32 and 33). Thermal degradation study is carried out at 40–80^0 C.

6. SOLUTION KINETICS

Chemical degradation reactions of pharmaceuticals follow the well-established treatments of chemical kinetics.

6.1 Rate equations

When a chemical reaction starts, the concentrations of reactants and products change with time until the reaction reaches completion or equilibrium. The concentrations of the reactants decrease, while those of the products increase over time. Therefore, the rate of a reaction can be represented either by the decreasing change in the concentration of a reactant or the increasing change in the concentration of a product with respect to time.

An arbitrary chemical reaction can be represented as,

$$aA + bB \rightarrow cC + dD \qquad (8)$$

Here, a, b, c and d are the stoichiometric coefficients indicating the molar ratio of the reactants and products of the reaction. The rate of change of concentration of each species can differ, depending on the stoichiometric coefficients. Hence, a unified expression of the rate is preferred, which can be obtained via normalization:

$$rate = -\frac{1}{a}\frac{d[A]}{dt} = -\frac{1}{b}\frac{d[B]}{dt} = \frac{1}{c}\frac{d[C]}{dt} = \frac{1}{d}\frac{d[D]}{dt} \qquad (9)$$

A negative sign is used for reactants so that the rate of a reaction is positive if it moves toward equilibrium or completion. The rate of a reaction often depends on the concentrations of the reactants/products when other conditions are kept identical. Consider the hydrolytic reaction of ethyl acetate under alkaline conditions:

$$CH_3COOC_2H_5 + OH \rightarrow CH_3COO^- + C_2H_5OH \qquad (10)$$

The rate of this reaction is proportional to the concentrations of each reactant species:

$$rate = -\frac{d[CH_3COOC_2H_5]}{dt} = \frac{d[C_2H_5OH]}{dt} \quad (11)$$

Here, *k*, the proportional constant, is called the specific rate constant, or just the rate constant. This hydrolytic reaction is first order with respect to either ethyl acetate or hydroxide, and is an overall second order reaction. In general, the rate of the arbitrary reaction, may be written as

$$rate = k[A]^{\alpha}[B]^{\beta} \quad (12)$$

Here, α and β are the reaction order with respect to A and B, respectively. The order of the overall reaction is n=α+β. This rate equation can be expanded to include more reactant/product species.

6.1.1 Zero-order reactions

In zero-order reactions, the rate of the reaction does not depend on the concentration of the reactant; thus, the rate is a constant:

$$rate = -\frac{d[A]}{dt} = k[A]^0 = k \quad (13)$$

Here, *A* is the reactant and k is the zero-order rate constant. In this case, the decrease in concentration of A is linear with time;

$$[A]_t = [A]_0 - kt \quad (14)$$

Here, $[A]_t$ is the concentration of *A* at time *t*, while $[A]_0$ is that at time zero, or the initial concentration.

6.1.2First-order reactions

First-order reactions appear to be the most commonly encountered in pharmaceutical stability studies. The rate of a first-order reaction is proportional to the concentration of the reactant:

$$rate = -\frac{d[A]}{dt} = k[A] \quad (15)$$

The concentration-time profile of the reactant for a first-order reaction follows an exponential decay to a limiting value, while that of the product follows an exponential increase to a different limiting value:

$$A \rightarrow C \quad (16)$$

$$[A]_t = [A]_0 \exp(-kt) \quad (17)$$

$$[C]_t = [A]_0[1 - \exp(-kt)] \quad (18)$$

The half-life, $t_{1/2}$, of the reaction is the time required for the reactant concentration to decrease to 50% of itsoriginal value; similarly, the times for the reactant concentration to decrease to 95% and 90% of its original values are designated as t_{95}, and t_{90}, respectively. These quantities can be obtained readily for a firstorder reaction if the rate constant is known:

$$t_{1/2} = \frac{In\ 2}{k}; \qquad t_{95} = \frac{In\ 0.95}{k}; \qquad t_{90} = \frac{In\ 0.9}{k} \quad (19)$$

A characteristic feature of first-order reactions is that the time required to lose the first 50% of the material ($t_{1/2}$) is the same as the time required to drop from 50% remaining to 25% remaining, from 25% remaining to 12.5% remaining, and so on.

6.1.3 Second-order reactions

Many apparently first-order reactions observed for pharmaceuticals are actually second order. Usually, two reactant molecules must collide in order to react. However, in practice, one reactant (e.g., water, hydrogen ion, hydroxyl ion, buffer species, etc.) may be in great excess so that its change in concentration is negligible, and an apparent first-order reaction is therefore observed.For a second-order reaction where two reactants are involved,

$$A + B \rightarrow C \quad (20)$$

The rate equation can be written as;

$$rate = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = k[A][B] \quad (21)$$

The rate is first-order with respect to each reactant, but the overall reaction is second order. The concentration-time profile of a second-order reaction can be represented as

$$\frac{1}{[A]_0 - [B]_0}\left(In\frac{[A]_t}{[B]_t} - In\frac{[A]_0}{[B]_0}\right) = kt \quad (22)$$

When the initial concentrations of A and B are identical, the concentration-time profile can be simplified as

$$\frac{1}{[A]_t} - \frac{1}{[A]_0} = kt \quad (23)$$

The $t_{1/2}$, t_{95}, and t_{90} values for a second-order reaction all depend upon the initial concentration of each species.

Fig. 4 plots the reactant concentration-time profiles for theoretical zero-, first-, and second-order kinetics. Table 5 summarizes the rate equations, the formula for calculating reactant concentration-time profiles, and half-lives for this simple order kinetics. The rate constants used to generate Fig. 4 were assumed to be numerically identical in all cases. Identical initial reactant concentrations were assumed for the second-order reaction in both Fig. 4 and Table 5.

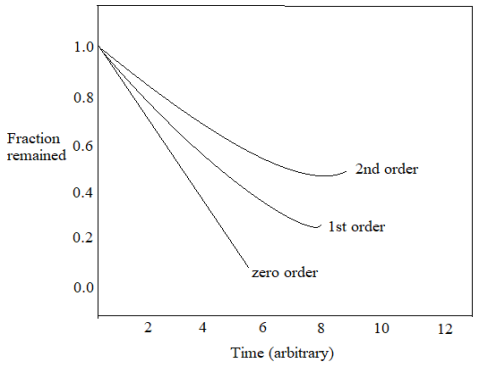

Fig 4: Reactant concentration-time profiles for theoretical zero-, first-, and second-order reactions.

Table 5: Rate Equations, Reactant Concentration-Time Profiles, and Half-Lives for Zero-, First-, and Second-Order Reactions

Reaction order	Rate equation	Concentration-time profile	Half-life

Zero	$-\frac{d[A]}{dt} = k$	$[A]_t = [A]_0 - kt$	$t_{1/2} = \frac{[A]_0}{2k}$
First	$-\frac{d[A]}{dt} = k[A]$	$[A]_t = [A]_0 \exp(-kt)$	$t_{1/2} = \frac{In\ 2}{k}$
Second	$-\frac{d[A]}{dt} = k[A]^2$	$\frac{1}{[A]_t} - \frac{1}{[A]_0} = kt$	$t_{1/2} = \frac{1}{k[A]_0}$

Ref:[11, 34]

7. ANALYTICAL TOOLS USED IN STABILITY INDICATING METHOD DEVELOPMENT:

The advancement in analytical instrument techniques makes it easier to develop the SIM. It should have good separation between the drug substance, its impurities, and degradant products and should possess high sensitivity towards analyzing of minimum concentration of drug substance. The TLC, HPLC-DAD, HPLC-UV, HPTLC, HPLC-MS, LC-MS/MS, LC-NMR, these are some techniques that have high sensitivity and resolution power to develop the effective stability indicating method. TLC method has advantages over HPLC is the volume of mobile phase required is small, large no. of the sample can be analyzed in one single plate by densitometry method. HPTLC has higher sensitivity than TLC but less sensitive than HPLC.

Table 6: Drug Examples with Analytical Instrument Used For Stability Studies

Drug examples	Analytical instrument used
Albendazole, Atazanavir Sulfate, Desloratadine, Cefexime&dicloxacilline, Temozolamide, Letrozol, Praziquantel, Prulifloxacin, BuprinorphineHCl and NalaxoneHCl, Guaifenesin& pseudoephedrine, Rizatriptan Benzoate, Doxorubicin,	HPLC-UV SIM
Rufinamide, Roflimilast, Pragabalin, Nizatidine, Naftopidil, Dexamethasone and Moxifloxacin, Levocabastine, AMLO-VAL-HCTZ, Eremantholide C, Silymerin and curcumin, Sofosbuvir and Ledipasvir, n-acetyl cysteine,	HPLC –DAD SIM
Diclofenac, Piracetam, Rivaroxaban,	UPLC SIM
Ofloxacine&ornidazoleIsoflavoneaglycone in soybean	UFLC SIM
Desonide	HPTLC SIM
Loratadine, Clobetasol,	TLC SIM
Nicardipine, AzilsartanmedoxomilPottasium,	HPLC-MS SIM
Ezetimibe, Simavastatin, Zidovudine	HPLC-MS/MS SIM

HPTLC has advantages over large no. of the sample can apply on a single plate, and the amount of mobile phase required is small, so it has costeffective analysis. HPLC -UV method is the most commonly used method for development of stability indicating method, but it has a limit of its detection ability; however it is more sensitive than TLC and HPTLC method. HPLC-PDA or DAD has a wide range of detection. One can determine the wavelength where all impurities, degradant products, and drug substance show absorbance so, it makes easy detection, separation, and quantification of all contaminants, related substances to give exact drug concentration at any time point during its storage. HPLC-MS has higher sensitivity to analyze the small quantity of analyte also. In such a way the HPLC-MS/MS shows to study the fate of a drug in human biological fluids, i.e. drug plasma concentration level. LC-NMR is also a highly sensitive technique to have the ability of separation of enantiomers in which one of them considered as an impurity of drug substance[35].

7.1Mean Kinetic Temperature(MKT)

The Mean kinetic temperature is the single calculated temperature at which the total amount of degradation over a particular period is equal to the sum of the individual degradations that would occur at various cycles of higher and lower temperature. It is an isothermal storage temperature that simulates the non-isothermal effects of storage temperature variation. The MKT takes into account seasonal and daily temperature variations during a year. It expresses the cumulative thermal stress undergone by a product at varying temperatures during storage and distribution. The concept of MKT is applied in order to provide assurance that the actual storage conditions will not affect the stability and shelf life of the product adversely. This is based upon the fact that the degradation rate constants are temperature dependent. A controlled room temperature maintained thermostatically to the usual working environment of 20°C to 25°C results in a mean kinetic temperature calculated to be not more than 25°C. This concept is applied to pharmacies, hospitals, distribution and storage areas and vehicles and warehouses. Articles may be labeled for storage at “controlled room temperature” or at “up to 25°C”, or any other relevant word/phrase based on the same mean kinetic temperature. The distribution of the countries and regions of the world into four different climatic zones has been on the basis of the mean kinetic temperature. Mean kinetic temperature is calculated by two methods, i.e. USP method and FDA method. In the USP method, MKT is derived from the average storage temperatures recorded over a 1-year period and the running average derived from the average of weekly high and low temperatures recorded over the preceding 52 weeks. This result in entering 52 data points and calculation is done by Hayne’s equation, which is derived from Arrhenius equation and relates degradation rate constants at different temperatures to the activation energy.

$$T_{MKT} = \frac{\Delta H/R}{-ln\frac{e^{-\Delta H/RT_1}+e^{-\Delta H/RT_2}+\cdots+e^{-\Delta H/RT_n}}{n}} \qquad (24)$$

where *MKT* is the mean kinetic temperature; ΔH is the energy of activation, in kJ/mole; *R* is the universal gas constant, 83.144kJ/mole (5240 kJ/mole); *T1* is the arithmetic mean of the highest and lowest temperatures recorded during the first time period (e.g., the first week); *T2* is the arithmetic mean of the highest and lowest temperatures recorded during the second time period (e.g., the second week); *Tn* is the arithmetic mean of the highest and lowest temperatures recorded during the n^{th} time period (e.g., n^{th} week), *n* being the total number of average storage temperatures recorded during the annual observation period; and alltemperatures *T* being absolute temperatures in degrees Kelvin (K).

The relative humidity (RH) is the ratio of the water vapor pressure of the environment to the saturation water vapor pressure at fixed temperature. The relative humidity can be calculated from the partial and saturation pressures of the water vapor, according to Eq. (25):

$$UR = \frac{P_D}{P_S} \times 100 \qquad (25)$$

The partial and saturation pressures of the water vapor could be estimated through Eqs. (26 & 27)

$$P_S = 0.61078 \times exp\left(\frac{17.269 \times T}{T+237.3}\right) \qquad (26)$$

$$P_D = 0.61078 \times exp\left(\frac{17.269 \times T_D}{T_D+237.3}\right) \qquad (27)$$

Where, PS=saturation pressure of the water vapor, (kPa);

PD=partial pressure of the water vapor, (kPa);

T=measured environment temperature, (°C);

TD=dew point temperature, (°C).

The storage conditions could be derived from Eq.(24&25). The storage conditions used generally should include a safety margin for temperature and RH [9,36].

7.2 HPLC

After the sample is generated through the use of a properly designed and executed forced degradation, it can be used to develop the LC method. The goal of development of method is separation of API peak from degradable product and detection of same. The separation of peaks are depends on solvent type, mode of chromatograph, mobile phase pH, column type and temperature. The choice of solvent is depend on the solubility of analyte, buffer used and UV value of solvent and safety of solvent. The experimental conditions regarding the stability indicating assay can be achieved through planned and systemic examination including pH, mode of chromatogram, flow rate of mobile phase, column type and column temperature, sample concentration and amount of sample injected, solvent used, detection wavelength, etc.[4, 37].

7.3 Calorimetry

Methodology for accelerated stability testing normally involves chemical assay of samples stored at high temperatures for appropriate time periods. Motivated largely by the desire to increase sample throughput, thermal analysis methods, particularly differential scanning calorimetry (DSC), have been applied in studies of the decomposition kinetics of explosives and in stability studies of pharmaceutical solids. However, sensitivity limitations demand high temperatures in both scanning mode and isothermal mode. In principle, the isothermal mode has the potential to provide data at more realistic temperatures. In isothermal operation, deviation of the sample signal (W) from baseline is the rate of heat production by the sample (dQ/dt) and is proportional to the reaction rate at that temperature (dn/df), where n is number of moles of parent compound, with the constant of proportionality being the heat of reaction (ΔH),

$$dQ/dt = \Delta H_r\, dn/dt \qquad (28)$$

The heat of reaction is not normally known and may be evaluated by integration of dQ/dtoverthe course of the experiment, provided the sample decomposes completely during the experiment. Thus, extremely high temperatures are required. In principle, one could run at a more moderate temperature, without decomposing the sample greatly, and be content to compare the thermal activities (dQ/dt) for a series of samples. Assuming that the heats of reaction do not vary greatly among the samples studied, this procedure would yield a comparison of reaction rates (Eq. 28). However, reproducibility of the baseline limits the sensitivity of the measurement to roughly ±50 μW for a common DSC unit^{-1}. With this sensitivity and the small sample size (≤30 mg), high temperatures are required to generate reproducible data. The recent availability of commercial high sensitivity isothermal calorimeters has dramatically increased the potential of calorimetric stability studies. With a sensitivity of ≈0.1 μW and a sample capacity of several grams, such units have more than 4 orders of magnitude greater effective sensitivity than a conventional DSC. Thus, assuming a heat of reaction in the tens of kJ/mol, such instrumentation is capable, in principle, of comparative stability studies on relatively stable materials at room temperature. High sensitivity isothermal calorimetry has found application in shelf-life stability estimation for explosives, and a brief report from this laboratory suggests that this calorimetric approach would be useful in pharmaceutical stability studies [38].

Recently Willson has described a general procedure for the determination of both thermodynamic and kinetic parameters from microcalorimetric output data [39, 40]. The procedure takes a kinetic equation for

a particular reaction, and modifies it such that it applies directly to microcalorimetric data. This is achieved by recognition of the fact that the total heat evolved during the course of a reaction (Q) is equal to the total number of moles of material reacted (A_0) multiplied by the change in molar enthalpy for that reaction (DH) (Eq. 29).

$$Q = A_0 \Delta H \qquad (29)$$

Similarly, the heat evolved at time t(q) is equal to the number of moles of material reacted (x) attime t multiplied by the change in molar enthalpy for that reaction (Eq. 30).

$$q = x \Delta H \qquad (30)$$

Eq. (30) may be substituted into a general rate expression of the form dx/dt to give an expression of the form dq/dt (or power).

For example, the general rate expression for a simple, first-order, A→B process is given by Eq. (31).

$$\frac{dx}{dt} = k(A_0 - x) \qquad (31)$$

Substitution of Eq. (30) into Eq. (31) yields,

$$\frac{dx}{dt} = k\Delta H \left(A_0 - \frac{q}{\Delta H}\right) \qquad (32)$$

This modified rate expression may be used to fit power–time data recorded using the microcalorimeter by a process of iteration. Using this method, Willson showed how it is possible to write calorimetric equations that describe a range of commonly encountered mechanisms. It is also possible, if the integrated form of the transformed calorimetric equation is known, to simulate calorimetric data using a suitable mathematical worksheet. In this way, it is possible to obtain values for reaction parameter by fitting real calorimetric data and de-convolute complex data into their component parts using the worksheet[41].

7.4 First derivative of ratio spectra spectrophotometric method (DD^1)

The main advantage of the method is that the whole spectrum of interfering substance is cancelled. Accordingly, the choice of the wavelength selected for calibration is not critical. The best results in terms of signal to noise ratio, sensitivity and selectivity followed using 5 mg/mL [42].

7.5 Chemometric methods

This method is based on UV-spectrophotometry, and the resulting heavily overlapping responses are processed by chemometrics. In this method, different chemometric approaches were applied for simultaneous determination of drugs and its degradation products, including PCR and PLS methods. These multivariate calibrations were useful in spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of single wavelength greatly improved the precision and predictive ability. For evaluation of the predictive abilities of the developed models, several diagnostic tools were used: predictive versus actual concentration plot (model and sample diagnostic); concentration residuals versus actual concentration plot (model and sample diagnostic) and root mean square error of prediction (RMSEP) (model diagnostic), the predicted concentrations of the validation samples were calculated [43].

7.6 TLC-densitometric method

Chromatographic techniques overcome the problem of overlapping absorption spectra of mixture of drugs or in presence of impurities or degradation products by separation of these components on TLC plates or chromatographic columns and determining each ingredient by scanning the corresponding chromatogram [44]. It has many applications in the field of pharmaceutical studies, which include the following: stability, impurities, synthetic drugs, pharmacokinetic, enantiomeric purity and drug monitoring in biological fluids. To improve separation of bands, it was necessary to investigate the effect of different parameters [45].

7.7 LC-MS/MS

LC-MS/MS is a superior and advanced analyticaltool for the identification and characterization of the degradation products in the APIs or a drug product. A combination of these techniques is finding increased use in the analytical structural organic chemistry. The analytical applications of HPLC and MS as well established. HPLC for resolving the mixture of compounds into its individual components, while MS as an excellent for characterization of compounds. LC-MS/MS studies were carried out in +APCI ionization mode in the mass range of 50-2000 amu. High purity helium was used as carrier gas, and nitrogen was used as a nebulizer. Mass parameters were optimized to the following values: R_f loading: 80%; capillary voltage: 80 volts; syringe volume: 250µl; spray chamber temperature: 50ºC; nebulizer pressure: 35psi; drying gas temperature: 300ºC; drying gas pressure: 10psi; vaporizer gas temperature: 350ºC; vaporizer gas pressure: 20psi; spray shield voltage: ± 600.0 volts [46].

8. DRUG SHELF-LIFE ESTIMATION

The expiration dating period or shelf-life of a drug product is defined as the time at which the average drug characteristic (e.g., potency) remains within an approved specification after manufacture. The United States Food and Drug Administration (USFDA) require that a shelf-life be indicated on the immediate container label of every drug product. Since the true shelf-life of a drug product is usually unknown, it is typically estimated based on assay results of the drug characteristic from a stability study conducted during the process of drug development [47].

Lety_j be the assay result of a pharmaceutical compound at time x_j, j =1,...,n. A simple linear regression model is usually assumed:

$$y_j = \alpha + \beta x_j + e_j, \qquad j = 1, \dots, n, \tag{33}$$

where α and β are unknown parameters, x_j's are deterministic time points selected in the stability study, and e_j's are measurement errors independently and identically distributed as $N(0,\sigma^2)$.

8.1 FDA's Method

Let$(\widehat{\alpha}\ \hat{\beta})$ be the least squares estimator of (α, β) based on $(y_j x_j)$'s under (33). For any fixed time *x*, a 95% lower confidence bound for $\alpha + \beta x$ is

$$L(x) = \widehat{\alpha} + \widehat{\beta x} - \hat{\sigma} t_{n-2} \sqrt{\frac{1}{n} + \frac{(x-\bar{x})^2}{S_{xx}}} \tag{34}$$

Where t_{n-2} is the 95th percentile of the t-distribution with n− 2 degrees of freedom, $\bar{x}$ is the average of$x_j's, \hat{\sigma}^2 = (S_{yy} - S^2 xy/\ S_{xx})/(n-2), S_{yy} = \sum_{j=1}^{n}(y_j - \bar{y})^2, S_{xx} = \sum_{j=1}^{n}(x_j - \bar{x})^2, S_{xy} = \sum_{j=1}^{n}(x_j - \bar{x})(y_j -$ $\bar{y}$,and $\bar{y}$ is the average of y_j's. FDA's shelf-life estimator is $\widehat{\theta_F} = \inf\{x \geq 0 : L(x) \leq \eta\}$, the smallest x ≥0 satisfying L(x)=η. By definition, $\widehat{\theta_F} > \theta$implies L(θ) >η and $P(\widehat{\theta_F} > \theta) \leq P(L(\theta) > \eta) = 5\%$, sinceL(θ) is a

95% lower confidence bound for α + βθ = η. This means that $\hat{\theta}_F$ is a (conservative) 95% lower confidence bound for θ.

8.2 The Direct Method

From the asymptotic theory (either n →∞or σ →0),

$$\left(\frac{\eta-\hat{\alpha}}{\hat{\beta}}-\theta\right)/\frac{\hat{\sigma}}{|\hat{\beta}|}\sqrt{\frac{1}{n}+\frac{1}{S_{xx}}\left(\frac{\eta-\bar{\alpha}}{\hat{\beta}}-\bar{x}\right)^2}\rightarrow N(0,1)\text{in law.} \qquad (35)$$

Let z be the 95th percentile of the standard normal distribution. Then an approximate (large n or small σ) 95% lower confidence bound for θ is

$$\widehat{\theta_D}=\frac{\eta-\hat{\alpha}}{\hat{\beta}}-\frac{\hat{\sigma}_z}{|\hat{\beta}|}\sqrt{\frac{1}{n}+\frac{1}{S_{xx}}\left(\frac{\eta-\hat{\alpha}}{\hat{\beta}}-\bar{x}\right)^2} \qquad (36)$$

We call this the direct method (of obtaining a shelf-life estimator).

8.3 The Inverse Method

Another shelf-life estimator can be obtained using the so-called inverse regression method. Start with

$$x_j=\alpha^*+\beta^* y_j+e_j^*,\quad j=1,\ldots,n, \qquad (37)$$

which is the same as (33) except that x_j and y_j are switched. In a stability study, however, the x_j's are deterministic time points and the y_j's are assay results and, therefore, the error term e_j^* is not independent of y_i.

8.4 Simulation Results

A simulation study is conducted to examine the finite sample performance of,$\hat{\theta}_F, \hat{\theta}_D$ and $\hat{\theta}_I$.We also study whether the asymptotic bias and mean squared error formulas are close to the bias and mean squared error given by simulation. We consider a typical stability study design: x_j=0,3,6,9,12,18, and 24 months, with 3 replications at each x_j. Thusn = 21. Values of α, β and η are chosen to be105,−0.5 and 90, respectively, so that θ = 30. To see the asymptotic effect, we consider values of σ ranging from 0.1 to 2.0.

8.5 Shelf-Life Estimation under Batch-To-Batch Variation

Drug products are usually manufactured in batches. The values of α and β in (33) may be different for different batches, referred to as batch-to-batch variation. The FDA requires testing of at least three batches, preferably more, in any stability analysis to account for this variation so that a single estimated shelf-life can be used for all future drug products [48].

8.6 Garret and Carper method

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 k, of a chemical reaction of thermodynamic temperature 1/T is a straight line. If the slope of this line is determined from the results of temperature by extrapolation, the k value obtained. This k value is substituted inappropriate. Order of reaction allows the amount of decomposition after a given time. Preliminary experiments are there for necessary to determine this order.

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

$$LogK = logA - Ea/2.303 * RT \quad (38)$$

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

If the reaction is following zero order, Expiration date at 25°C C=Initial potency–minimum potency/reaction rate at 25°C. Tx= Yo-Yx/Ko If the reaction is following the first order, Expiration date at 25°C C (tx) = log initial potency – log minimum potency/reaction rate at 25

$$T_x = log0 - logY_x/K_1 \quad (39)$$

Where, Yo= initial potency, Yx= final potency, Ko= zero order reaction, K1= first order reaction[6,49].

9. HOLD TIME STABILITY STUDIES IN PHARMACEUTICAL INDUSTRY

It is a stability establishment tool for each and every stage in the drug product manufacturing. In the drug product development, hold time stability is an important tool for establishing the in-process hold time. Hold time stability is evaluating for each stage in the product manufacturing. Hold stability study can demonstrates how much time is suitable for hold the blend or bulk stage before processing to the next stage. When appropriate, time limits for the completion of each phase of production shall be established to assure the quality of the drug product.

Hold time study protocol can be prepared on the basis of product manufacturing process of the drug product. The main contents in the protocol are, hold study stages, study time points and analytical tests.

Hold Time Study Results Evaluation

Each manufacturing stage shelf life can be determined based on the hold study results. If the hold time samples are passing at 60 days' time point then the shelf life of the particular stage can be considered up to 45 days [50].

10. CONCLUSION

For new drug and new formulation stability testing is important aspect of pharmaceutical development program which is important component of it. Stability testing of pharmaceutical products the key procedural contribution in the development program for a new drug as well as new formulation. Stability studies are capable of differentiating active drug ingredient from any degradation product formed under defined storage conditions. It is better to start degradation studies earlier in the drug development process to have sufficient time to gain more information about the stability of the molecule. This information will in turn help improve the formulation manufacturing process and determine the storage conditions. Over a period of time and with increasing experience and attention, the regulatory requirements have been made increasingly stringent to achieve the above goal in all possible conditions to which the product might be subjected during its shelf life. 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.

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