Archives of Current Research International

19(1): 1-20, 2019; Article no.ACRI.52101
ISSN: 2454-7077

An Approach to Drug Stability Studies and Shelf-life Determination

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Authors' contributions

This work was carried out in collaboration among all authors. This review work first draft of manuscript was written by author ET. Author KRG finalized the draft of the manuscript. Author MJU managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACRI/2019/v19i130147
Editor(s):
(1) Prof. Peter A. Akah, Coordinator, Natural Products Research Group, Department of Pharmacology & Toxicology, University of Nigeria, Nsukka, Nigeria.
Reviewers:
(1) Marta de Diego Glaría, Universidad de Concepción, Chile.
(2) Panchumarthy Ravi Sankar, Vignan Pharmacy College, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/52101

Review Article

Received 20 August 2019
Accepted 24 October 2019
Published 07 November 2019

ABSTRACT

The main objective of carrying out stability studies of the drug product is to establish shelf life of drug during storage. Stability of drug is 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. Stability studies are of different types and different methods are useful for the determination of stability like real-time stability testing, accelerated stability testing, retained sample stability testing and cyclic temperature stress testing. pH and temperature are the main factors influencing the stability of the drug. The pH-rate profile *(log(k)*vs *pH)* is the pH dependence of the specific rate constant of degradation of compounds. Forced degradation includes deterioration of new drug substances and products at more severe conditions than the accelerated conditions and it indicates the accuracy of stability-indicating methods. The different conditions applied during the forced degradation include hydrolytic, oxidation, photolytic and thermal stress etc. The techniques utilized for evaluation of stability studies can be LC-MS/MS, HPLC-DAD, HPLC-MS, HPLC-UV, HPTLC, TLC, LC-NMR etc.

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amongst them some techniques shows high sensitivity and resolution power to establish more effective stability-indicating method while for shelf life estimation of drugs and products the different methods mentioned are FDA's method, the direct method, inverse method, simulation results and Garret and Carper method. Thus stability testing of pharmaceutical products inputs specific scheme in the evolution of 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 involves significant cost, time and scientific proficiency to generate safety, in quality and efficacy in a drug formulation. The understanding of the drug development process and the infinite tasks and milestones that is essential to abroad development plan result in scientific as well as commercial success of any pharmaceutical product [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 are processed at the time of manufacture" [2]. The various factors affecting the stability of a pharmaceutical product; because of their involvement, stability testing is known as a complex process. These factors mostly concern the stability of the active ingredient(s); interaction of active ingredients and excipients, type of dosage form and their manufacturing process followed, container/closure system used for packaging, heat, moisture and light come across during shipment, storage and handling etc. [3]. The shelf-life determination of the drug product is the 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 formed during the decomposition of active drug.
- Changing in physical appearance through the principles of kinetics due to instability, are used to forecast the stability of the drug.
- To save the reputation of the manufacturer by confirming the product will retain strength for use concerning all functionally related aspects for as long as they are in the market.

1.2 Objectives of Stability Studies

- Stability testing aims to display clues on how the quality of drugs changes with time under the presence of numerous environmental factors including temperature, humidity and light.
- To select suitable (from the perspective of stability) formulations and container-closure systems to evaluate storage conditions and shelf-life.
- To substantiate the claimed shelf-life.
- To confirm that no modifications have been imparted in the formulation or manufacturing process that may affect the stability of the drug.
- The main purpose of stability study is to generate the stability profile of a drug product so that the prediction of the shelf life of the product can be made before launching it into the market [6].

1.3 Guidelines for Stability Testing

The availability of stability data by the manufacturers to confirm that most stable molecules and products are synthesized, distributed and provided to the patient's provisions have been made by the regulatory authorities of many countries. These guidelines were firstly issued in the 1980s which contains basic concerns relevant to stability, the stability data for application dossier and the steps for their execution. The basic purpose was to maintain uniformity in testing from manufacturer to manufacturer. These were later harmonized (made uniform) in the International Council for Harmonization (ICH) to register the products in other countries and minimize the barrier to market. The ICH was established in 1991, it was a confederacy formed with profits from both industry and regulatory from the European Commission, USA and Japan and different guidelines for drug substance and product came into the essence for their quality, safety and efficacy. These guidelines are known as a quality, safety, efficacy and multidisciplinary (also called Q, S, E and M) guidelines.

The ICH guidelines did not mention the extreme climatic conditions observed in many countries, for this, the World Health Organization (WHO) in 1996 modified these guidelines, also it only includes new drug substances and products and not the already developed products that were in dissemination in the WHO umbrella countries. In June 1997, the United States Food and Drug Administration (USFDA) also issued a navigation document entitled 'Expiration Dating of Solid Oral Dosage Form Containing Iron'. 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 present in India. Different test conditions and provisions have been given in the guidance documents for active pharmaceutical ingredients, drug products or formulations and excipients. The codes and titles covered under ICH guidelines are given in Table 1 & Table 2. Numbers of guidelines related to stability testing have also been extended by the Committee for Proprietary Medicinal Products (CPMP) under the European Agency for the Evaluation of Medicinal Products (EMEA) to support those seeking marketing authorization for drug products in European Union are listed in Table 3.

1.4 Stability Studies and their Classification

Stability studies are the essential criteria for assuring 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. 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 physical evaluation is particularly important for therapeutic proteins to evaluate their kinetic profiles of aggregation 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 the solvent vector in many reactions. Molecules have more kinetic energy and more decomposition is observed because moisture has better thermal conductivity than solids which allow better heat transfer. The common cause in all these, hydrolysis or oxidation or fermentation; is moisture.

Table 1. Codes and titles used in ICH guidelines

ICH code	Guidelines
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

Table 2. ICH Q1A summary of stability parameters

Study Type & Condition		Storage Conditions	Period (in 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]

The presence of moisture speeds up all reactions. The HPLC, HPTLC or capillary electrophoresis methods are widely for evaluation of chemical instability.

1.4.3 Microbiological stability studies

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

2. STABILITY TESTING METHOD

The stability testing is a routine process employed at different stages of drug substances product development. Accelerated stability study (at relatively high temperatures and/or humidity) is performed in initial stages, for evaluation of the nature of degradation products which may be generated after long-term storage. The long-term shelf storage testing under meticulous conditions i.e. at quite elevated temperature is recommended which determines the product's

shelf life and expiration dates. Providence of acceptable declaration that the products will remain at an acceptable level of fitness/quality throughout the time 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 is the major aim of pharmaceutical stability testing. Depends on objective and steps followed, stability testing procedures have been classified into the following types.

2.1 Real-Time Stability Testing

Longer period degradation of the test drugs to allow degradation under recommended storage conditions consists of real-time stability testing. Stability of the product decides the period of the test which should be long enough to indicate accurately that no quantitative degradation takes place and must allow one to differentiate degradation from inter-assay deviation. Data is collected during the testing at a relevant frequency so that trend analysis can differentiate instability from day-to-day uncertainty. Data interpretation accuracy can be increased by the addition of a single batch of a reference substance for which stability characteristics have been already established.

2.2 Accelerated Stability Testing

In accelerated stability testing, a subject is stressed at a distinct high (warmer than ambient) temperatures to determine the amount of heat required to cause product degradation. The comparison of the relative stability of alternative formulations and shelf life is then projected. Temperature together with the moisture, agitation, pH, light, gravity and package etc. are the stress conditions applied during accelerated stability testing. In this method, the samples are assayed simultaneously which are subjected to stress and refrigerated after stressing. The measurement system is reduced in comparison to the real-time stability testing because of the duration of the analysis is short. Further, comparison of the unstressed product with stressed material is taken within the same assay and the stressed sample recovery is expressed as a per cent of unstressed sample recovery. Relatively accurate stability of thermolabile and proteinaceous components projections are obtained by denaturing stress temperatures is avoided. For statistical reasons, the accelerated stability projections are recommended to be conducted at four different stress temperatures. The approach of accelerated stability study 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. Both equations denote the relationship between storage temperatures and degradation rate. By using the Arrhenius equation, some degradation processes can be determined by the projection of stability from the degradation rates observed at high temperatures. The degradation rate at low temperatures may be projected from those observed at "*stress*" temperatures when the activation energy is known. 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. Some methods are not official either in ICH or FDA to apply various shortcuts such as Q rule and bracket tables for prediction of shelf life of the products, this common practice used by manufacturers in pharmaceutical industries. 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 usually set at 2, 3 or 4 because these correspond to reasonable activation energies. This model maliciously considers that the value of Q does not vary with temperature. According to the bracket table technique, for a given analyte, the activation energy is between two limits. As a result, a table may be constructed showing days of stress at various stress temperatures. Broad experience shows that most analytes and reagents of interest in pharmaceutical and clinical laboratories have activation energies in the range 10 to 20 kcal hence bracket table technique uses this range.

2.3 Retained Sample Stability Testing

At least one batch a year is selected in this study, for retained storage of stability samples. Stability samples from two batches are

suggested to be taken when the number of batches marketed exceeds 50. The stability samples of each batch may be taken when they are first introduced to the market, 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 intermissions i.e. if a product has a shelf life of 5 years, it is typically tested at 3, 6, 9, 12, 18, 24, 36, 48, and 60 months. This typical method of determining stability data on retained storage samples is known as constant interval method. One modified method includes stability testing of marketed samples in which involves taking samples already in the market place and evaluating stability aspects. This method is more realistic as 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

For marketed products, this is not applied as a routine testing method. To mimic similar conditions in market place storage cyclic temperature stress tests are design to product knowledge. The diurnal rhythm on earth is 24 hours hence the period of cycle mostly design is 24 hours, which the marketed pharmaceuticals are most prone to sense during storage. Depends on the product-by-product basis the minimum and maximum temperatures for the cyclic stress testing is selected and important factors like suggested storage temperatures and specific physicochemical degradation properties of the products. Normally 20 cycles have been recommended [9].

3. FACTORS INFLUENCING STABILITY OF DOSAGE FORM

3.1 pH

In active ingredient's solubility and thus in its bioavailability pH plays an important role. At extreme conditions, the rate of degradation is much higher. The optimum pH defines the pH where a given molecule is most soluble. Buffers are also included in pharmaceutical product formulations and it provides very good stability. However, the pH and the stability of the formulation of preparations using these pharmaceutical products may changes.

3.2 Temperature

It is one of the most crucial factors in drug stability. An increase in about 10°C in storage temperature may lead to a 2 to 5 fold increase in the degradation reactions speed. For some molecules, physicochemical stability is only ideal within a narrow range of temperature, outside of this increased degradation is observed. The Arrhenius law followed for kinetics of degradation reactions for most active ingredients. 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 micelles in solution are formed by different types of surfactants (anionic, cationic or non-ionic) however; this trapping of the active ingredient molecules changes their bioavailability in solution. The surfactants can be used to protect and limit the degradation of the active ingredient in hydrolytic groups such as hydroxyls.

3.4 Oxygen

The oxidation of one of the drug components takes place by the presence of oxygen in preparation may lead to instability. Use of antioxidants and suitable manufacturing techniques e.g. under nitrogen are essential. An appropriate container with its ensured integrity is important elements to preventing the infiltration of oxygen over time.

3.5 Light

Light may cause chemical instability in photosensitive molecules is an important factor. If preventive measures are applied during manufacturing e.g. selection of appropriate packaging material, it can be prevented and 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 the 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 also provide insights into the catalytic nature of a reaction. Many drug degradation reactions follow apparent first-order kinetics and usually plotted in a pH-rate profile which subjects 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

Specific acid and base catalysis is common in case of carboxylic acid derivatives, like esters, amides, substituted ureas, etc. Rather than other more complicated mechanisms, the pseudo-first-order 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 pH-rate profile plot includes a straight line for acidic region with slope of -1 and another straight line for basic region with slope of 1. Fig. 1 shows pH-rate profiles for reactions involving only a single reactive species with specific acid- base-catalysis.

4.2 Sigmoidal pH-rate Profiles

Sigmoidal pH-rate profiles are generally the results of dissociation of the drug molecules. Species distributions of a weak base or weak acid are sigmoidal when in the vicinity of *pH=pKa*, it plotted as a function of pH. Therefore, the rate-pH profile results to be sigmoidal when both the acidic and basic species of the compound can undergo degradation at different rate constants. Consider, 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, *Ka* is the dissociation constant of HA, while *{HA}* is the total concentration of HA. The rate constants are not identical therefore, a plot of the apparent rate constant seen sigmoidal against the pH. 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. The sigmoidal curve will encircle somewhat more than ± 1 pH units of the expected pKa if the change in rate is due to ionization at a specific site. An example of sigmoidal pH-rate profile is given in Fig. 2.

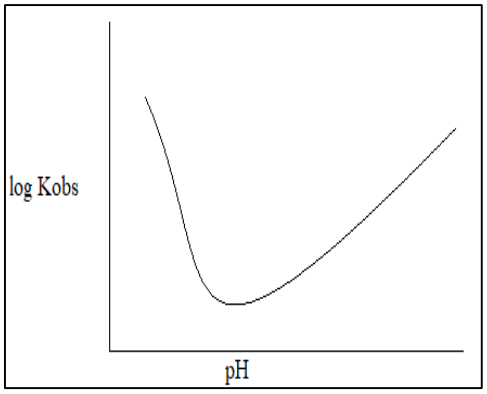

Fig. 1. pH-rate profiles for reactions consisting only a single reactive species with specific acid- base-catalysis

4.3 Bell-Shaped pH-Rate Profiles

Minima or maxima observe in Bell-shaped pH-rate profiles. The 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 bell shaped. Based on reactivity of monoprotic species, HA, the corresponding pH-rate profile could show either maxima or minima. In case of acid 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 reactivities with the rate

constant of step B→C falling somewhere in between. Therefore, in one pH region (below or above its pKa), 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 rate limiting step. An example of a sigmoidal pH-rate profile is given in Fig. 2.

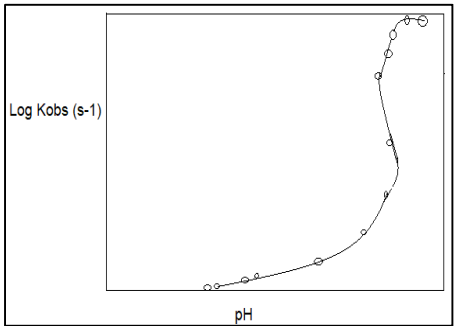

Fig. 2. Sigmoidal pH-rate profile

4.4 More Complicated pH-Rate Profiles

The analysis of a pH-rate profile can be complicated with the presence of multiple ionization centers, either their construction is based on the 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 pH due to intramolecular catalysis [11].

4.5 Influence of Temperature

Linear plots of $Ink = f\ (1/T)$ were used to calculate the energy of activation (Ea), the entropy $(\Delta S^{\neq})$ and enthalpy $(\Delta H^{\neq})$ and the pre exponential 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 for the reactions catalyzed by hydrogen ions indicated a positive participation of entropy of protonation reaction. The linear relationships of $\Delta H^{\neq}$ = $(\Delta H^{\neq})$ 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].

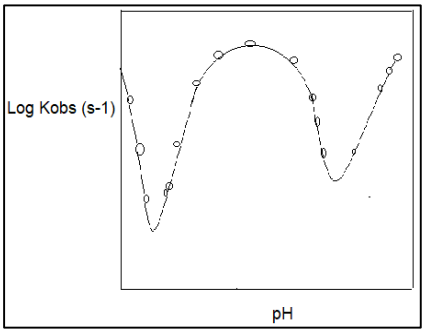

Fig. 3. Bell-shaped pH-rate profile

5. FORCED DEGRADATION

Forced degradation is the degradation of new drug compounds and related products at more severe conditions than the accelerated conditions. It is required for structure elucidation of the degradation products which indicates the specificity of stability indicating methods which is essential for understanding of degradation products of the drug substances and degradation pathways [13].

Forced degradation studies are performed for the following reasons:

1. To understand the degradation pathways of drug substances and drug products.
2. To separate degradation products in a formulation those are obtained from drug products from those that are evolved from non-drug product.
3. To explain the chemical properties of drug molecules.
4. To exemplify the structure of degradation products.
5. Intrinsic stability determination.
6. To explain the mechanism of degradation such as thermolysis or photolysis, hydrolysis, oxidation of the drug substance and product [14,15].
7. To discover more stable formulations.
8. To provide nature of methods stability indicating for drug molecules.
9. To produce a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.
10. To clarify stability-related problems [16].

5.1 Time to Perform Forced Degradation

The time to perform forced degradation studies for the development of new drug substance and new dug product is very essential. As per FDA guidelines, stress testing should be performed in phase III of regulatory submission process to determine the stability of the drug substance which carried out at elevated temperature and humidity in various pH solutions, in the presence of oxygen and light. The single batch stress studies are conducted. 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% have been accepted as reasonable for validation of chromatographic assays [18,19]. 10% degradation is sufficient for analytical validation of pharmaceutical molecules having low mol. weight as per some pharmaceutical researchers for which acceptable stability limits of 90% of label claim is common [20]. Over-stressing a sample may lead to the generation 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]. Some conditions used for forced degradation studies are given in Table 4 [22].

5.3 Degradation prediction tools

5.3.1 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 observed [23].

5.4 Mechanism of Degradation

5.4.1 Hydrolytic conditions

Hydrolysis involves the degradation of a chemical compound due to reaction with water within the chemical process and it is most common chemical reactions causes degradation over a wide range of pH. In the acidic and basic condition molecule under prone to catalysis of ionizable functional groups present within molecules. When drug substance exposes to acidic or basic conditions forced degradation

Table 4. Conditions mostly used for forced degradation studies

Degradation type	Experimental conditions	Storage conditions	Sampling time (days)
Hydrolysis	Control API (no acid or base)	40°C, 60°C	1,3,5
	0.1 M HCl	40°C, 60°C	1,3,5
	0.1 M NaOH	40°C, 60°C	1,3,5
	Acid control (no API)	40°C, 60°C	1,3,5
	Base control (no API)	40°C, 60°C	1,3,5
	pH: 2,4,6,8	40°C, 60°C	1,3,5
Oxidation	3% H_2O_2	25°C, 60°C	1,3,5
	Peroxide control	25°C, 60°C	1,3,5
	Azobisisobutyronitrile (AIBN)	40°C, 60°C	1,3,5
	AIBN control	40°C, 60°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°C	1,3,5
	Heat chamber/RH	60°C/75% RH	1,3,5
	Heat chamber	80°C	1,3,5
	Heat chamber/RH	80°C/75% RH	1,3,5
	Heat control	Room temp.	1,3,5

NA: Not applicable, RH: Relative humidity

generates primary degradants in desirable range in acid or base stress testing. For hydrolysis, Hydrochloric acid or sulfuric acids (0.1–1M) for acid hydrolysis and sodium hydroxide or potassium hydroxide (0.1–1M) for base hydrolysis are considered as convenient reagents and it mainly depends on the stability of the drug substance [22,24]. For low water-soluble compounds, co-solvents can be used to dissolve them in HCl or NaOH and selection is depends on the structure of drug substance. In stress testing, trial elevated temperature (50–70°C) is normally started when there is no degradation at room temperature. Stress testing should not exceed more than 7 days. Further degradation is avoided by neutralized the degraded sample using suitable acid, base or buffer.

5.4.2 Oxidation conditions

In forced degradation studies hydrogen peroxide is largely used for oxidation of drug substances, also other oxidizing agents like oxygen, metal ions and radical initiators (e.g., azobisisobutyronitrile, AIBN) can be used side by side. According to the drug substance, the selection of an oxidizing agent and its concentration with suitable conditions is proceeding. When the drug substances subjected to 0.1–3% hydrogen peroxide at neutral pH and room temperature results into a maximum 20% degradation potentially generate relevant to degradation products under seven days period [22]. In oxidative degradation, reactive anions and cations of drug substance are formed by an electron transfer mechanism. For example, amines, phenols and sulfides give hydroxylamine, N-oxides, sulfones and sulfoxide by electron transfer oxidation [25]. In case of the functional group-containing labile hydrogen-like benzylic carbon, allylic carbon, and tertiary carbon or α-positions concerning hetero atom is susceptible to oxidation to form hydroperoxides, hydroxide or ketone [26,27].

5.4.3 Photolytic conditions

It involves the formation of primary degradants of a drug substance by exposure to UV or fluorescent light. Some essential conditions for photostability testing are given in the ICH guidelines [28]. Minimum 1.2 million lx h and 200W h/m^2 light is applied to exposed drug substance and solid/liquid drug product. For photolytic degradation, the most commonly used wavelength of light is in the range of 300–800 nm [29,30]. The maximum illumination suggested is 6 million lx h [27]. Functional groups like carbonyls, N-oxide, alkenes, aryl chlorides, nitroaromatic, sulfides, weak C–H and O–H bonds and polyenes etc. are mostly included drug photosensitivity because free radical mechanism involves in photo-oxidation at light stress conditions [31].

5.4.4 Thermal conditions

As per recommended in ICH Q1A accelerated testing conditions the thermal degradation (e.g., dry heat and wet heat) is accomplished at quite more exhausting conditions than this recommendation. The solid-state drug substances and drug products samples should be exposed to dry and wet heat, while the liquid drug products should be exposed to dry heat. These degradations may be conducted at higher temperatures for a shorter period [22]. The Arrhenius equation is useful to study the effect of temperature on the thermal degradation of a substance.

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

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

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 reactant concentration or the increasing change in the concentration of a product concerning time.

An arbitrary chemical reaction can be represented as,

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

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:

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

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:

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

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 concerning 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 concerning 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 the 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.

6.1.2 First-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 its original 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 first-order reactions observed for pharmaceuticals are second order. Usually, two reactant molecules must collide 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 concerning 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.

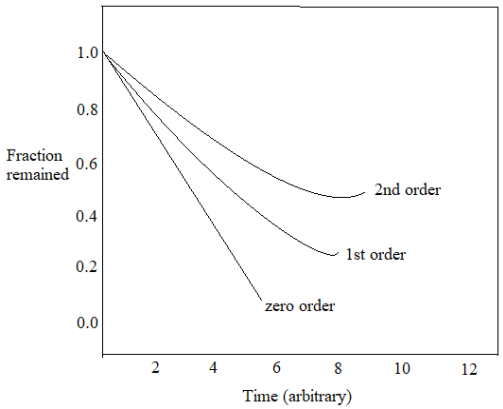

Fig. 4. Reactant concentration-time profiles for theoretical zero-, first-, and second-order reactions

7. ANALYTICAL TOOLS USED IN STABILITY INDICATING METHOD DEVELOPMENT

The stability indicating methods are easier to develop due to improvement in analytical instrument techniques. The advance methods must have well separation between the drug substance, degradant products and its impurities. It should also possess high sensitivity and specificity towards analyzing drug substance with minimum concentration. The TLC, LC-MS/MS, HPTLC, HPLC-DAD, HPLC-MS, HPLC-UV and LC-NMR, these are some effective stability-indicating the method that has high sensitivity and resolution power to develop the effective technique. HPTLC has less sensitive than HPLC but higher sensitivity than TLC. TLC method involves a small volume of mobile phase and large no. of the substances can be analyzed in one single plate by densitometry method hence it has advantages over HPLC.

In HPTLC method, several no. of the samples can apply on a single plate and the amount of mobile phase required is small, so it has cost-effective analysis hence it has advanced over other methods. Although HPLC -UV is the widely used method for the development of stability-indicating the method and is more sensitive than TLC and HPTLC method it has a limit of its detection ability. HPLC-PDA or DAD detectors can determine the wavelength over the large range where all drug substance, impurities and degradants products show absorbance hence, it causes easy detection, separation and quantification of all contaminants and related substances to give exact drug concentration at any time point during its storage. The small quantity of analyte analyzes by HPLC-MS because it has higher sensitivity. For this reason, the HPLC-MS/MS use to study the fate of a drug in human biological fluids, i.e. drug plasma concentration level and it identifies degradant products. LC-NMR is also another highly sensitive technique which can separate enantiomers in which one of them considered as an impurity of drug substance [35].

7.1 Mean 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 deals with the seasonal as well as daily temperature variations over the year. It indicates the cumulative thermal stress experience by a product at distinct temperatures during its distribution and storage. It is based upon the fact that the degradation rate constants depend on temperature. The mean kinetic temperature provides affirmation that the actual storage

conditions will not be affected by the stability and shelf life of the product negatively. Controlled room temperature at 20°C to 25°C is taken asusual working environment is maintained thermostatically so mean kinetic temperature calculated should not more than 25°C. This concept is applicable in pharmacies, hospitals, storage and distribution areas, vehicles and warehouses etc. Compounds may be labelled for storage at "controlled room temperature" or at "up to 25°C", or any other suitable word/phrase indicating same mean kinetic temperature. Two methods were used to calculated Mean kinetic temperature i.e. USP method and FDA method. USP method includes average storage temperatures recorded over 1 year and the running average derived from the average of weekly high and low temperatures recorded over the preceding 52 weeks. The calculation is done by Hayne's equation, which is derived from Arrhenius equation and this result in the introduction of 52 data points and compares the degradation rate constants at different temperatures to the activation energy.

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

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 all temperatures *T* being absolute temperatures in degrees Kelvin (K).

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

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

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]

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, Ofloxacine&ornidazole	UPLC SIM
Isoflavoneaglycone in soybean	UFLC SIM
Desonide	HPTLC SIM
Loratadine, Clobetasol,	TLC SIM
Nicardipine, Azilsartan,medoxomil, Pottasium,	HPLC-MS SIM
Ezetimibe, Simavastatin, Zidovudine	HPLC-MS/MS SIM

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

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

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

Where,

PS=saturation pressure of the water vapour, (kPa);
PD=partial pressure of the water vapour, (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

The aim of method development is the separation of active ingredient peak from degradation product peak and detection of the same. When the sample is developed by using a properly designed and accomplished forced degradation, it can be used to develop the LC method. The various factors on which separation of peaks are depends are solvent type, mobile phase pH, the form of chromatography, temperature and column type. Analyte solubility, buffer used and UV value of solvent and safety of solvent are the selection parameters of solvent. In the stability-indicating assay, planned and systemic examination of experimental conditions such as pH, the flow rate of the mobile phase, column type and column temperature, mode of the chromatogram, sample concentration and amount of sample injected, solvent used and wavelength etc. are taking place to develop method [4,37].

7.3 Calorimetry

Methodology for accelerated stability testing normally involves a chemical assay of samples stored at high temperatures for appropriate 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 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 sampled 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),

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

The heat of reaction is not normally known and may be evaluated by the integration of dQ/dt throughout 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 suggest 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).

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

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

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

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).

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

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

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

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 the reaction parameter by fitting real calorimetric data and de-convolute complex data into their 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 the interfering substance is cancelled. Accordingly, the choice of the wavelength selected for calibration is not critical. The best results are shown in terms of signal to noise ratio, sensitivity and selectivity [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 the 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 a 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 the 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 analytical tool 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 components, while MS as an excellent for characterization of compounds. For example LC-MS/MS studies of Carfilzomib which accomplished in the mass range of 50-2000amu and at +APCI ionization mode. Highly purified helium was used as carrier and nebulizer consist of nitrogen. The following optimized mass parameters are applied given values are: R_f loading: 80%; capillary voltage: 80 volts; drying gas temperature: 300°C; nebulizer pressure: 35psi; syringe volume: 250µl; spray chamber temperature: 50°C; drying gas pressure: 10psi; vaporizer gas pressure: 20psi; spray shield

voltage: ± 600.0 volts;vaporizer gas temperature: 350°C [46].

8. DRUG SHELF-LIFE ESTIMATION

The time at which the average drug characteristic (e.g., potency) of drug substance remains within an approved specification after manufacture is known as its expiration dating period or shelf-life. As per the United States, Food and Drug Administration (USFDA) a container label of each drug product must show shelf-life of that drug substance. Shelf-life usually evaluated based on assay results of the drug characteristic of a drug product as true shelf life usually unknown, it is generally from a stability study performed during the drug development process [47].

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

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

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$(\hat{\alpha}\,\hat{\beta})$ is the least squares estimator of (α, β) depends on $(y_j x_j)$'s under (33). For any fixed time *x*, a 95% lower confidence bound for *α + βx* 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 $x_j's, \hat{\sigma}^2 = (S_{yy} - S^2 xy / S_{xx})/(n-2), S_{yy} = \sum_{j=1}^{n}(y_j - \bar{y})^2, S_{xx} = \sum_{j=1}^{n}(x_j -$ *x)2,Sxy=j=1n(xj−x)(yj−y,* and 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. From definition, $\widehat{\theta_F} > \theta$ implies L(θ) >η and $P(\widehat{\theta_F} > \theta) \leq P(L(\theta) > \eta) = 5\%$, since L(θ) is a 95% lower confidence bound for α + βθ = η. It means that $\hat{\theta}_F$ is a (conservative) 95% lower confidence bound for θ.

8.2 The Direct Method

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

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

Consider 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

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

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

$$x_j = \alpha^* + \beta^* y_j + e_j^*, \quad j = 1, \dots, n, \tag{37}$$

which is the same as (33) except that x_j and y_j are converted. 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, $\hat{\theta}_F, \hat{\theta}_D \; and \; \hat{\theta}_I$. It includes whether the asymptotic bias and mean squared error formulas are close to the bias and mean squared error given by simulation. Consider a typical stability study design: x_j=0,3,6,9,12,18, and 24 months, with 3 replications at each x_j. Thus n = 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, 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 for α and β in Eq. 33 may vary for different batches, this is referred to as batch-to-batch variation. As per FDA, testing of minimum three batches are required or preferably more. Single estimated shelf-life can be applied for all future drug products in any stability testing to clarify for this variation [48].

8.6 Garret and Carper Method

In this method, shelf-life determination carried out as per Arrhenius plot. The assumption of shelf life is based on the mathematical result obtained from the application of the Arrhenius equation, which includes the effect of temperature of chemical reaction on the rate constant k, at thermodynamic temperature 1/T which observed as a straight line. The value of k obtained from the results of temperature by extrapolation from the slope of this line. This k value is substituted irrelevant. The order of reaction shows the amount of decomposition takes place in the given time. Thus the primary operations are there for essential to determine this order of the reaction.

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

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

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

If the reaction is follows zero order, expiration date observed at 25°C. C=Initial potency– minimum potency/reaction rate at 25°C.

$$T_x = Y_0 - Y_x/K_0 \quad (39)$$

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

$$T_x = log0 - logY_x/K_1 \quad (40)$$

Where, Y_o= initial potency, Y_x= final potency, K_o= zero order reaction, K_1= first order reaction [6,49].

9. HOLD TIME STABILITY STUDIES IN PHARMACEUTICAL INDUSTRY

It is a stability establishment tool for every stage in 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 is used to determine the time requirement suitable for hold the blend or bulk stage before it passes 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.

Product manufacturing process of the drug product and compounds determines the preparation of hold time study. The important criteria includes in the protocol are, study time points, hold study stages and analytical tests for drugs.

9.1 Hold Time Study Results Evaluation

Hold study results is essential at each manufacturing stage to evaluate the shelf life can of the drugs and its component. The shelf life of the specific stage is considered up to 45 days if the hold time samples are passing at 60 days' time [50].

10. CONCLUSION

Stability testing is important aspect for new drug and new formulation during 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, in turn, helps to improve the formulation manufacturing process and determine the storage conditions. Over some 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 by proper understanding of scientific principles and current regulatory requirements and as per the climatic zone.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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