Methods and Protocols for Drug Stability Studies

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Abstract

Stability is a significant quality attribute for drug molecules and pharmaceutical preparations. Investigation of drug stability is essential to improve quality, safety and efficacy. The drug toxicity and adverse effects prevented by proper evaluation of parameters are related to stability. The toxic effects could be due to degraded impurities, drug metabolites or functional groups of drug molecules. Therefore, stability studies are planned to identify and maintain the product quality, throughout the shelf life. The major role of such studies is to predict shelf life, determine the suitable storage condition and suggest the label instructions. Stability studies are deemed as prerequisite for the recognition and endorsement of pharmaceuticals. Stability studies should comply the guidelines of the ICH, the WHO or other agencies deemed fit. These guidelines postulate the outline for the execution of stability studies on both drug and dosage form. The aim of these guidelines is not to constrain the experimentation but to execute the proper and meaningful experiments. The scope of these guidelines is limited to pharmaceutical dosage forms and any feed impregnated with medicinal product. Stability studies are necessary for the development and registration of newer drug.

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4.1 Introduction

Stability studies of drugs are carried out to identify the time in which the pharmaceuticals maintain their physical, chemical, microbiological and pharmacokinetic properties and characteristics. Bright light, sunlight, radiations, temperature, humidity and certain other environmental factors cause the drug to degrade. Despite the product is not expired. While developing pharmaceuticals, principal stages include analysis and stability studies. These studies are mandatory for the identification and maintenance of transparency and potency until its expiry date. To maintain the product integrity and suitability, it should be prevented from the exposure of any environmental factor up to a maximum extent. Drug stability studies are also carried out for the newly developed drugs and dosage forms to identify the expiry of the certain dosage forms. Similarly, physically and chemically active substances in the formulation and the nature of container-closures used and the storage conditions [1].

The decomposition procedure and methods of degradability of active substances are usually accessible in the literature along with adequate analytical methods. Therefore, stability studies are generally limited to the dosage form. Shelf life of the product means that any substance reduces to 90% of its original concentration. In pharmaceutical terms, shelf life is used to indicate the stability of the product, and it is denoted as expiry date. Expiration differs for each pharmaceutical preparation.

Besides that, many factors affect the stability of a pharmaceutical product like microbiological changes including the growth of microorganisms and variations in preservative efficacy. Likewise, the statistics obtained while testing is an imperative prerequisite for regulatory approval of any drug formulation or dosage form [2]. The testing of pharmaceutical products is a multifaceted task, and it requires substantial funds, ample time and appropriate scientific proficiency and capability to obtain apposite excellence, effectiveness and harmlessness pharmaceuticals. Technical and viable triumph of any pharmaceutical merchandise lies in the successful product development alongside appropriate stability of the product [3].

There are certain factors that influence the stability of the drug including stability of the active ingredient, physical and chemical interaction among the active ingredients and excipients, particular manufacturing steps, “dosage form, container/closure” used for packaging and “environmental (light, heat and moisture) circumstances” that come across during shipment, post marketing storage and handling. Moreover, oxidization, hydrolysis and reduction are the most likely degradation reactions that occur in the pharmaceutical products. These physical changes are observed when there is fluctuation in the erstwhile explained factors that influence the stability [4].

4.2 Importance of Conducting Drug Stability Studies

These types of studies are patient centred, and patients are undergoing from the particular ailment for which the drug is developed. The degradation of the drug product is converted into harmful chemical, loss of claimed therapeutic effect that might result into catastrophic results including death. For instance, glyceryl trinitrate tablets indicated for angina and cardiac arrest or certain antibiotics can lead to dose dumping. Due to these reasons, firstly, it has been a legal obligation to carry out these stability studies and present the data to the regulatory agencies or supervisory watchdog prior to approval of a new drug. Secondly, the stability studies provide confidence to the manufacturer that the product will retain all its claimed attributes including efficacy and effectivity for as long as it is in the market [5].

4.3 Types of Drug Stabilities

Particular pharmacopoeia "US Pharmacopeia and British Pharmacopoeia" explains the following standards [6, 7].

4.3.1 Physical Stability

This type of stability covers the physical properties like appearance, colour, dissolution, palatability and suspendability. The physical stability is conducted to check uniformity and release rate; henceforth adequate physical stability is essential for the optimal efficacy and safety of the product.

4.3.2 Chemical Stability

In fact, this type of stability is conducted to determine the potential of a drug to resist its degradation due to the chemical reactions triggered by air, atmosphere, temperature, etc.

4.3.3 Microbiological Stability

To identify the presence of potential harmful microbial growth and to attain the desired sterility, such type of studies is conducted.

4.3.4 Therapeutic Stability

This type of stability is conducted to ensure that therapeutic effect remains unchanged.

4.3.5 Toxicological Stability

This type of stability is conducted to check the formation of certain toxic compounds, due to the degradation of the product. This includes photostability testing, forced degradation studies and degradation product studies (flowchart shown in Fig. 4.1). According to the time frame, stability studies [8] are classified in Table 4.1.

4.4 Methods for the Stability Studies of Drugs

Stability testing is a procedure performed for all the pharmaceutical products at various stages of the product development. It is suggested to follow the test protocols stated in the official compendia, because these are officially accepted and extensively

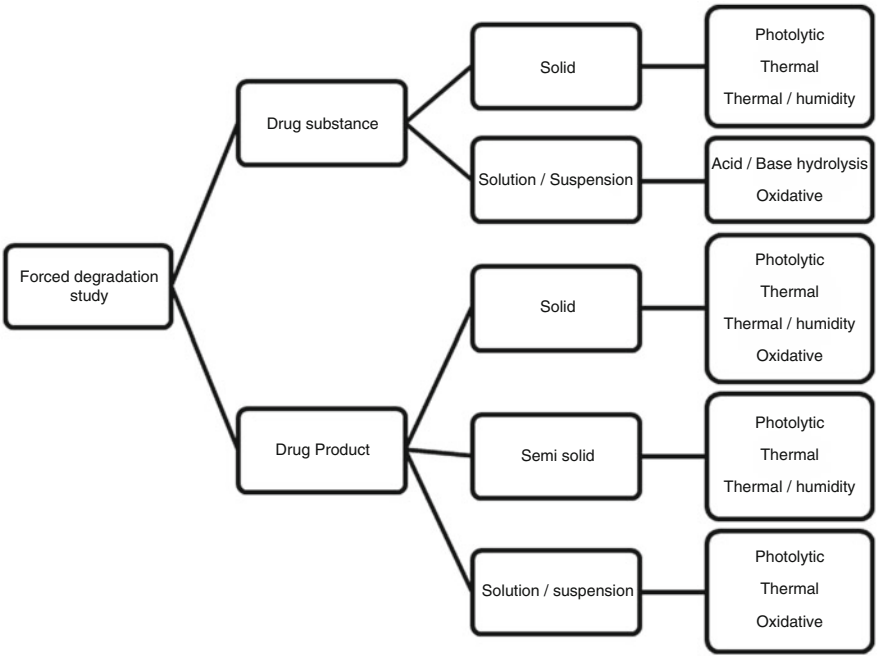

Fig. 4.1 Schematic representation of forced degradation study of both the drug product and drug substance

Table 4.1 Showing the type storage and duration of study

Type of study	Storage conditions	Study period (months)
Long term	25 ± 2 °C and 60 ± 5% RH or 30 ± 2 °C and 65 ± 5% RH	12
Intermediate	30 ± 2 °C and 65 ± 5% RH	06
Accelerated	40 ± 2 °C and 75 ± 5% RH	06

researched and practised, and this is the reason these are ultimately better accepted [9]. However, if any alternate protocol is followed, it must be validated twice. The following methods and procedure are employed for the stability studies.

4.4.1 Real-Time Stability Testing

Real-time stability testing is usually implemented for extended period of time to permit and help to analyse substantial degradation of the drug under the recommended storage conditions. The time period for undergoing test product solely is contingent to the stability of the product. This clearly states that the drug is not decomposed for this time period from inter-assay variation. The testing is usually performed in such a way that the analyst can perform the testing at regular interval and appropriate frequency so that the analyst can distinguish the daily degradation of the drug. The extent of degradation is compared by standard single batch (reference batch), of which stability studies are carried out before or stability attributes have been well known. In this regard, the component materials and the instruments operated should be in the consistency throughout the stability testing. The studies shall not be reproducible if there is a change in both reagents and instruments. In order to change either reagents or instruments, it should be again standardized and optimized [10].

4.4.2 Accelerated Stability Testing

Such type of testing is executed at relatively higher temperatures and that decomposes the product. The data originated from such study is used to assess the shelf life and help to compare the relative stability of alternative formulations. Such type of studies are helpful to predict the shelf life; hence it cuts the duration to identify the stability of the drug. Moreover, along with the temperature, further stress conditions are also applied such as strenuous moisture, light, pH and gravity. As the name suggested, such instability studies are taken place in a very reduced time as compared to real-time testing. The stability projections are carried out at four distinct stress temperatures [11].

The accelerated stability studies are easily calculated by the Arrhenius equation:

$$K = Ae\frac{-Ea}{RT}$$

where:

K = specific rate constant
A = frequency factor or Arrhenius factor
Ea = energy of activation
R = real gas constant 4.184 J/mol.k
T = absolute temperature

In this method, the drugs are placed at different temperatures such as 40 °C, 60 °C, 70 °C, 80 °C, 100 °C, etc. These studies are also carried out at room temperature (25 °C) and at fridge (0–4 °C) temperatures [12]. The specimen is sampled and examined and has undergone stability testing at different time intervals. At first year, the sampling is performed at 3 months' and 6 months' interval the following year and yearly afterward. Frequent sampling and testing is required for the rapidly degraded products. The purpose of increasing the temperature and providing stress to the products is to ascertain the decomposition potential of the substances.

Conferring to the existing International Committee of Harmonization (ICH) guidelines, 40% products must be kept at controlled room temperature. As per ICH and World Health Organization (WHO) guidelines, the storage condition for accelerated stability studies is 40 °C ± 2 ° C; 75% RH ± 5% RH. If the product is unstable on the recommended temperature and humidity, intermediate conditions are used, i.e. 30 °C ± 2 °C; 65% RH ± 5% RH. The Food and Drug Administration (FDA) suggested the sampling testing for 0, 2, 4 and 6 months, respectively. The WHO prescribes for 0, 1, 2, 3, 4 and 6 months. The ICH prescribes the test to be performed for every 3 months in a year, 6 months in 2 years and yearly thereafter. Moreover, these accelerated tests are mainly executed for stability and moisture absorption testing. This test is implemented for the entire pharmaceutical preparations, but primarily this is a test utilized for dispersed systems like pharmaceutical emulsion and suspension testing [13].

4.4.3 Retained Sample Stability Testing

It is a conventional type of testing for each marketed finished product. In this category of testing, the stability is done by randomly selecting one batch for a year. If the number of samples surpasses 50, then sampled from two batches. For the newly marketed batches, every batch should be sampled later on that may reduce from 2% to 5%. Such stability studies are conducted to forecast the shelf life. The probable extreme shelf life of every product is to be 5 years which is predictable to the test samples at 3, 6. 9, 12, 18, 24, 36, 48 and 60 months. This testing method is also known as constant interval method. In fact this type of testing is fundamentally more accurate because it trials the product not only in the idealized retained sample storage conditions but also in the actual marketplace [14].

4.4.4 Cyclic Temperature Stress Testing

This method of testing is not frequently exercised. In this technique, cyclic temperature stress tests are planned, and attempts are made to provide similar type of conditions that the product may face in the market. The sampling and testing are

deliberately carried out by a 24-hour cycle. Based on temperature and storage conditions and predicted by physicochemical degeneration of the drug product, the lowest and extreme temperatures are noted for product. In order to forecast the shelf life, the 20-cycle testing is recommended [15].

4.5 Protocol of Drug Stability Testing

The protocols and the procedures for stability studies are prerequisite intended for initiating and conducting testing. The protocol should be in written documented form that must address mechanisms and key steps for conducting the appropriate stability studies. Furthermore, the protocol is different for novel drug and previously marketed drug [16]. In order to attain accuracy and precision, the analytical procedures and steps should essentially be validated and verified. Specified assay nominated for stability studies should be performed. The recommended tests for related drug products should be validated prior the testing is performed. Likewise, the approval and rejection standards for each analytical result in addition to the presence of degradation products should also be predetermined. Preferably it should be fixed in numerical limits [17]. Optimum stability protocol should address the following parameters.

4.5.1 Number of Batches

The protocol for selecting the number of batches to perform stability studies is the following:

1. Stability testing is carried out in batches as performing the stability studies in a single step is difficult; hence, they are divided into batches.
2. For a stable product, the stability studies are executed on a single batch.
3. For the unstable and newly registered products, studies are carried out on three consecutive batches. If any one of the batch displays instability, then the studies shall be performed for six consecutive batches, and if the instability persists, then all the batches have to be rejected as they cannot pass the stability testing [18].

The preliminary batches are not full-scale production batches. For the first three batches with subsequent approval from the regulatory bodies, long-term studies following the same protocol as in approved drug applications should be performed. The laboratory data are not accepted for the primary stability data. Random sampling technique should be used for the selection of samples from the batches [19].

4.5.2 Containers and Closures

The choice of containers and closures is extremely crucial for the stability studies. The product should be packed in such a container that it not only protects the product from the harmful environment but also made up of a suitable material that could not harm the product itself. Usually the packaging of the drugs is borne in materials like aluminium strip packs, blister packs, Alu-Alu packs, HDFE bottles, etc. Possibly the secondary packaging is present. Testing should be performed on each of the container and closure of the product since the inappropriate shippers and packing materials can degrade the drug. Prototype containers can be used for the bulk packing. The finished goods products must be placed in tested suitable containers because inappropriate containers can contaminate the product; hence shelf life of the product can be affected [20].

4.5.3 Orientation of Storage of Containers

Drug products like solutions and semi-solid should essentially be placed upright, so that the drug comes in contact with the containers. This aids to determine any chemical change occurring in the drug that can lead to the degradation of the drug. Such type of degradation might be due to either absorption or loss of water.

4.5.4 Sampling Time Points

The testing is vital at specific time intervals to ascertain and authenticate the stability of the new drug substance. The testing should be scheduled monthly in the first year, then after 6 months for the second year and then yearly thereafter throughout the predicted shelf life. However, in the case of accelerated stability studies the testing should be conducted in a minimum of three time points, for instance, 0, 3 and 6 months. If the testing should be done for the different batch size and strength of the same product, then retained stability testing method should be followed, i.e. a smaller number of time points. This testing plan is merely depending on the bracketing and matrixing statistical designs. Bracketing is the design only when the samples on the certain design factors such as strength and package size are tested at all the three time points as in full design. The factors that can be matrixed can include the strength, batches, container sizes and intermediate time points [21]. Sampling plan covers the number of samples to be placed in stability chambers. This plan merely depends on the number of sampling points and the product to be required for each test to be performed as depicted in Table 4.2.

Table 4.2 Plan for stability testing of new products

Temperature and relative humidity	Sampling time point (months)	Method
25 °C/60%	3, 6, 9, 12, 18, 24, 36	Long term
30 °C/35%	3, 6, 9, 12, 18, 24, 36	Long term
30 °C/65%	3, 6, 9, 12, 18, 24, 36	Long term
30 °C/75%	3, 6, 9, 12, 18, 24, 36	Long term
40 °C/75%	3, 6, 9, 12, 18, 24, 36	Accelerated

Table 4.3 Stability test storage conditions for drug products

Storage	Stability study	Storage conditions for			
		ICH		WHO	
		Temperature and relative humidity	Time (months)	Temperature and relative humidity	Time (months)
Room temperature	Long term	25 ± 2 °C and 60 ± 5%	12	25 ± 2 °C and 60 ± 5%	12
		or			
		30 ± 2 °C and 65 ± 5%			
	Intermediate	30 ± 2 °C and 65 ± 5%	6	–	–
	Accelerated	40 ± 2 °C and 75 ± 5%	6	–	–
Refrigerator	Long term	5 ± 3 °C and –	16	5 ± 3 °C	–
	Accelerated	25 ± 2 °C and 60 ± 5%	6	–	–
Freezer	Long term	−20 ± 5 °C and –	12	−20 ± 5 °C	–

4.5.5 Test Storage Conditions

The storage conditions are selected solely on the basis of the climatic zones in which the finished product has to be marketed. The WHO and the ICH have designed general recommendation on the storage conditions [21]. The ICH and the WHO explain the storage conditions for drug products, as shown in Table 4.3.

4.5.6 Mean Kinetic Temperature

In accordance to ICH guidelines, "*Mean Kinetic Temperature* is a single calculated temperature, which degrades the same amount of the drug as degraded, by the different temperatures during the particular time period." It is a valuable tool for the stability studies. It aids to analyse the degradation of stability sample. It is predictable from the temperatures of stability chambers [22].

4.5.7 Test Parameters

The test parameters used in the stability studies must be designed to estimate the stability of samples. The test of sample is generally carried out to determine the quality, purity, efficacy and identity. Therefore, appearance, quantitative assay, degradation potential, dissolution and moisture content are benchmark tests for the stability studies. Microbiological tests comprise of sterility, microbial count and preservative measures whenever applicable. Besides that, stability testing parameters also includes the determination of heavy metals, residue of ignition, residual solvents, etc. Depending upon the nature of the product, several other tests are also performed that are discussed in ICH guidance Q6A [23].

4.6 Stability Test Equipment

Stability chamber is used for the stability testing. Stability chamber is the particular environmental compartment intended to maintain the optimal storing environment and approximate the product stability based on real-time, accelerated and long-term protocols. Various sizes of the stability chambers are available. Usually smaller chambers are selected for accelerated testing; however, for long-term testing, the walk-in chambers are ideal. Walk-in chambers are up to the size of rooms. Rooms are designed to ensure the optimum storage condition, uninterrupted for years. They are equipped with appropriate recording, safety and alarm devices. Besides that, photostability chambers are also designed having two types of light sources (near-UV fluorescent and artificial daylight lamps) and can be operated with both modes with and without temperature and humidity control. It is essential to achieve entire exposure of 1.2 million lux hours [5].

4.7 Climate Zones

The stability studies are implemented and followed globally (Table 4.4). Due to the environmental differences, one standard cannot be practicable to conduct the stability studies. For this reason, the ICH has divided the world into five climatic zones depending on their climatic conditions, temperature and humidity levels of the stability chambers, which are adjusted to carry out stability studies [24].

Table 4.4 Classification of climatic zones

Zone	Type of climate	Temperature	Relative humidity
I	Temperate zone	21 °C ± 2 °C	45% ± 5%
II	Mediterranean/subtropical zone	25 °C ± 2 °C	60% ± 5%
III	Hot-dry zone	30 °C ± 2 °C	35% ± 5%
IVa	Hot humid/tropical zone	30 °C ± 2 °C	65% ± 5%
IVb	Hot/higher humidity	30 °C ± 2 °C	75% ± 5%

4.8 Applications of Stability Studies

The main objectives and application of stability testing are explained below.

4.8.1 For Drug Development

Once the formulation and manufacturing process of product have been established, the manufacturer conducted frequent accelerated stability tests that help to predict the product stability. Similarly simultaneous real-time studies essentially be commenced; the aim of such study is to confirm the product stability [25].

4.8.2 For Approval from Regulatory Bodies

In order to register drug in the respective drug regulatory authority, it is essential to support your application with stability studies. Usually the results of both accelerated and real-time studies are submitted in the dossier. When the product needs to be diluted or reconstituted prior to use (e.g. a powder for injection or an oral suspension), stability data essentially should explain optimal storage period and surrounding environment for such dosage forms [6].

4.8.3 Post-Registration Period

In order to validate the tentative expiry date and the storage conditions, the manufacturer should continue on-going real-time stability studies. Other results of on-going stability studies are verified in the course of Good Manufacturing Practices inspections. The government regulatory bodies and watchdog agencies to conform the quality and safety of products carry out follow-up inspection and testing programmes [26].

4.9 Conclusion

The physical, chemical and microbial drug stability is essential for ensuring the quality of pharmaceutical formulations. The objective of stability evaluation is for providing evidence about the integrity of drug product affected under various environmental factors, like temperature, pH, radiations, light and humidity. Due to integrated research and with escalating knowledge and awareness, the regulatory requirements for the pharmaceutical products are increasingly stringent to attain the required objectives. Therefore, the stability tests should confirm the appropriate scientific parameters. It would require understanding and application of up-to-date regulatory prerequisites in accordance with climatic zones.

Conflict of Interest Authors declare no conflict of interest.

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