Review Article
DOI: https://doi.org/10.70749/ijbr.v3i3.942

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Ensuring Pharmaceutical Quality: The Role of Stability Studies and Regulatory Guidelines: A Review

Qurratul Ain Leghari[1], Noshad[2], Neha Tehreem Anwar[1], Yumna Syed[1], Ali Asgher Shuja[3], Mehtab Ali[3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan
[2]Head of Quality Control, Platinum Pharmaceutical Private Limited, Karachi, Pakistan
[3]Department of Basic Medical Sciences, Faculty of Pharmacy, Salim Habib University, Karachi, Pakistan

ARTICLE INFO

Keywords
Studies on Stability, Guidelines for Stability, Product Stability, Arrhenius Equation, Research on Long-Term Stability, Research on Accelerated Stability, Research on Intermediate Stability

Corresponding Author: Qurratul Ain Leghari,
Pharm-D, MPhil, PhD, Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan
Email: qurratulainlaghari@gmail.com

Declaration
Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.
Conflict of Interest: No conflict of interest.
Funding: No funding received by the authors.

Article History
Received: 22-12-2024, Revised: 06-03-2025
Accepted: 20-03-2025, Published: 31-03-2025

ABSTRACT

Objective: To ensure the quality of pharmaceutical products, stability studies are mandated. These studies adhere to guidelines established by organizations such as the International Conference on Harmonization (ICH) (e.g., ICH Q1AR2) and the World Health Organization (WHO). They evaluate how well the products maintain their physical, chemical, microbiological, and other essential properties under defined conditions. **Methodology:** This section dives into the world of stability testing for drugs and pharmaceuticals. We'll explore the different types of assessments used to monitor a product's quality and purity over time. We'll also delve into the specific methods employed to test stability, and unpack the key elements of well-designed and controlled stability testing protocol. **Results:** Stability studies are crucial for ensuring medications remain safe and effective throughout their shelf life. These studies follow strict regulations and test products under various storage conditions, mimicking real-world scenarios. This rigorous testing provides valuable evidence on how the medications degrade over time, guaranteeing their quality for patients. **Conclusion:** This review examines the critical role of stability studies in safeguarding the reliability and effectiveness of medications. By following established regulatory guidelines, these studies explore various methods to assess how well a drug maintains its quality over time. Ultimately, stability testing empowers us to confidently set and potentially extend the shelf life of pharmaceutical products, ensuring patients receive medications with the potency they deserve.

INTRODUCTION

Ensuring the effectiveness, quality, and safety of medications requires a multifaceted process called stability testing. This crucial undertaking demands significant investments in time, resources, and scientific expertise.[1]i. Any changes occurring post-preparation that could detrimentally impact a product's suitability for use or its quality are of paramount concern to pharmaceutical researchers and regulators [2]. [ii] Following strict guidelines from the ICH, pharmaceutical companies conduct stability studies. Ultimately, the goal is to determine a drug's shelf life and recommend the proper storage conditions. [3]. [iii] According to the United States Pharmacopeia (USP), a drug's stability refers to maintaining its key characteristics within defined limits throughout its lifespan [4]. This is why stability testing, which assesses how well both the active ingredients and the final formulations hold up over time, becomes a crucial part of the drug development process right from the beginning [5]. [iv]

Ensuring Drug Potency over Time: Shelf Life and Stability

A drug's stability refers to how long it maintains its original properties and effectiveness after being manufactured. This duration is typically expressed as the expiration date or shelf life on the product label. It's a crucial quality factor for all medications and should always be accompanied by clear storage instructions.
Manufacturers must provide data from stability testing to support the chosen shelf life and storage conditions. This testing assesses how various factors impact the drug over

time. These factors include:

- Environmental: Temperature, humidity, and light exposure
- Product-related: dosage form, packaging materials, and container-closure system.

For well-established drugs in common dosage forms, existing scientific data and analytical methods can often be used to understand the stability of the active ingredient itself. This allows stability testing to focus primarily on the final drug product, ensuring its stability throughout its shelf life.1[v]

Significance of Stability Testing [11-16][vi]

Stability studies are a cornerstone of new drug development, ensuring patient safety and product efficacy. These studies identify potential toxins that might form as medications degrade, safeguarding against harm. By guaranteeing a product's quality throughout its shelf life, stability studies uphold a manufacturer's reputation. They also confirm that production or formulation changes haven't compromised stability. Additionally, this data informs future product development, guiding choices of ingredients, formulations, and packaging.

Understanding how medications degrade is critical, as it can impact their quality. Stability studies are the key to determining if a drug meets safety and effectiveness standards. An unstable drug can lead to improper dosing due to decreased potency or the formation of harmful byproducts. Physical changes can also signal instability. While scientific principles can predict drug stability, these predictions differ from actual stability studies. Ultimately, stability studies are vital for ensuring a manufacturer's reputation by guaranteeing a product's quality and effectiveness throughout its time on the market.

This revised version clarifies the importance of stability studies and uses more concise language. It also emphasizes the patient safety aspect and the distinction between predicted and actual stability.1, 2 vii

Factors Influencing Drug Stability [17-19][viii]

Temperature: Heat is a major threat to drug stability. High temperatures can speed up the breakdown (hydrolysis) of medications, reducing their effectiveness.

Moisture: Water can cause both physical and chemical changes in solid medications. Water-soluble drugs can absorb moisture from the air, altering their properties and potentially rendering them ineffective.

pH: pH levels affect the degradation rate of drugs prone to hydrolysis, and formulations buffered at the ideal pH demonstrate decreased degradation rates.

Excipients: Some common ingredients in medications, like starch and povidone, tend to hold more moisture. This can be problematic because they can draw water away from the medication itself, changing the overall moisture content of the formulation. This shift in moisture levels can then destabilize the drug, impacting its effectiveness.

Oxygen: Oxygen exposure can accelerate the breakdown (oxidation) of certain medications. For products susceptible to rapid decomposition due to oxygen.

Light: Light exposure can speed up the breakdown of certain medications, known as photosensitive drugs. To assess their stability, scientists compare how quickly these drugs degrade.

Table 1a

Types of Stability Studies

Study Type	Storage Condition	Minimum Time Period Covered by Data at Submission
Long Term	25°C ± 2°C and 60% RH ± 5% RH or 30°C ± 2°C and 65% RH ± 5% RH	12 months
Intermediate	30°C ± 2°C and 65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C and 75% RH ± 5% RH	6 months

Table 1b

Codes and Titles Used in ICH Guidelines

ICH Code	Guideline Title
Q1A	Stability Testing of New Drug Substances and Products (Second Revision)
Q1B	Stability Testing: Photostability Testing of New Drug Substances and Products
Q1C	Stability Testing of New Dosage Forms
Q1D	Bracketing and Matrixing Designs for Stability Testing of Drug Substances and Products
Q1E	Evaluation of Stability Data
Q1F	Stability Data Package for Registration Applications in Climatic Zones III and IV
Q5C	Stability Testing of Biotechnological/Biological Products

Varieties of Drug Substance Stability [20][ix]

- Physical Stability: This ensures a drug maintains its intended form, size, taste, and consistency. These factors influence how uniformly the drug is distributed and how quickly it dissolves in the body, impacting its safety and effectiveness.
- Chemical Stability: This focuses on how well a drug resists breaking down over time. It guarantees that the active ingredients stay potent and meet their labeled strength throughout the shelf life.
- Microbiological Stability: This is crucial for drugs that fight infections. It ensures the medication remains free from harmful microbes and maintains its effectiveness against them. Factors like sterilization and resistance to microbial growth are crucial here.
- Therapeutic Stability: This ensures the drug intended therapeutic effect (how it benefits the

patient) remains consistent throughout its shelf life.
- Toxicological Stability: This verifies that the drug doesn't become significantly more toxic over time, ensuring patient safety.

Methodology for Stability Testing

Stability testing plays a critical role from the beginning of development all the way through a pharmaceutical product's lifespan. This rigorous testing applies to both the individual drug ingredients (active pharmaceutical ingredients or APIs) and the final medication itself. Accelerated stability testing simulates harsher storage conditions to quickly predict how the drug might degrade over a longer period. This helps identify potential breakdown products and inform decisions about formulation and packaging.

The main goals of stability testing are:
- To ensure drug products remain marketable for their intended shelf life.
- To guarantee they meet all quality standards throughout that time.

[21]x Understanding Drug Stability: Four Key Testing Methods

Here's an overview of the four main categories of stability testing methods used to assess how drugs perform under different conditions:
- Real-Time Stability Testing
- Accelerated Stability Testing
- Retained Sample Stability Testing
- Cyclic Temperature Stress Testing

Real-time Stability Testing

This testing method simulates real-world storage conditions. Products are stored for an extended period under specific temperatures, humidity, and light exposure. The goal is to observe a noticeable level of degradation over time. By collecting data at regular intervals, scientists can distinguish between true instability and minor day-to-day variations.

Maintaining reliable controls is crucial. This means ensuring the reference materials used for comparison remain stable, and the testing instruments consistently perform at the same level throughout the study. However, achieving optimal efficiency is also important. Any drift or inconsistencies arising from changes in reactions or instrument settings need to be addressed promptly to avoid delays and ensure accurate results.[22].xi

Accelerated stability testing

Fast-Forwarding Stability: Accelerated Testing Unlike real-time testing that mimics real-world storage, accelerated stability testing puts the drug under harsher conditions (extreme heat, humidity, or light). This "stress test" helps predict how the drug might degrade over a longer period by observing its breakdown at a faster pace.

Here's why accelerated testing is valuable:
- Early Insights: It provides a quicker indication of a drug's stability compared to real-time testing, speeding up the development process.
- Formulation Comparison: By testing different formulations under stress, scientists can compare their relative stability and choose the most robust option.
- Efficiency and Accuracy: Since stressed and unstressed samples are tested simultaneously, this approach can be more efficient. Additionally, the shorter testing period reduces the chance of measurement errors compared to real-time studies.

Stressed samples are then compared to unstressed ones to see how well they recover, expressed as a percentage. For robust statistical analysis, this testing is often performed at four different stress levels.

This version simplifies the technical terms and emphasizes the benefits of accelerated stability testing for faster development and better formulation selection.

Nonetheless, to ensure accurate stability predictions for thermolabile and protein components, it is essential to avoid stress temperatures that could cause denaturation. [23,24].xii

Science behind Accelerated Testing: Accelerated stability testing relies on well-established scientific principles to predict how a drug might degrade over time.

This revised version avoids mentioning the Arrhenius equation by focusing on the general concept without technical details.

depicted by equations 1 and 2.

Equation 1

$\ln f_0 K = \ln f_0 A + ERT \ln K = \ln A + RTE$ Where:

KK = Rate of degradation per second

AA = Per-second frequency factor

EE = Energy of Activation (in kJ/mole)

RR = molar gas constant (0.00831 kJ/mol)

TT = Temperature measured in Kelvins

Equation 2

$\log f_0 (k2k1) = -Ea2.303R(1T2 - 1T1)\log(k1k2) = -2.303REa(T21 - T11)$ Where: $k1k1$ and $k2k2$ = Rate constants $T1T1$ and $T2T2$ = Temperatures expressed in degrees Kelvin $EaEa$ = Activation energy RR = Gas constant

Accelerated stability testing uses fancy equations (the Arrhenius equation and similar ones) to understand how temperature affects how fast things break down. By testing things at high temperatures, scientists can use these equations to predict how long they'll last at normal storage temperatures. It's like a shortcut to figuring out shelf life. [25].xiii

Retained Sample Stability Testing

Stability testing is like checking how long food stays

good in your pantry. Retained sample stability testing is a fancy way of saying they keep samples of a product around to see how well it holds up over time. This is done for a pretty much every product you see on the shelves.
At first, they tend to test samples from every single batch of a new product. But as time goes on and they are confident in the product, they might only test samples from a few batches each year. They also test the samples at specific times, like every 6 months or a year, to see if the product is degrading. This way they can figure out exactly how long the product will last before it goes bad.[26].xiv

Cyclic Temperature Stress Testing

It can help researchers design better stability tests for new medications and fix any problems with existing tests for already marketed drugs. However, it is not commonly utilized for routine product testing.

Equipment for testing stability

Imagine a special room that mimics different storage conditions, like temperature and humidity. That's a stability chamber, a scientist's tool to test how well a product holds up over time. These chambers come in various sizes. They are built to last and come with features to record data, ensure safety, and raise alarms if something goes wrong.
There's even a special type of chamber with light controls to test how products react to sun exposure. Pretty neat, isn't it? Let's break it down further:

- Types of Stability Chambers:
  - Reach-in chambers: Ideal for quick tests due to faster heating and cooling times.
  - Walk-in chambers: Better suited for long-term studies as they can hold more samples.
  - Photo-stabilization chambers: These chambers mimic light exposure, including sunlight, to assess light sensitivity.
- Key Features:
  - Durability: Chambers are built to withstand continuous operation for years.
  - Data Recording: Chambers record temperature, humidity, and light exposure data for analysis.
  - Safety Features: Alarms alert scientists to any temperature or humidity fluctuations outside the set parameters.

Exposure requirements, measured in lux hours, are determined using a lux meter to gauge visible light intensity and duration [6,15]xv. The lux meter is utilized to estimate the intensity of visible light. The calculation determines the required duration of exposure. 2,8xvi

Stability testing guidelines

In the 1980s, drug regulators around the world realized they needed a common set of rules for how drug companies test their products for stability. That's why a group called ICH developed guidelines to ensure everyone was on the same page. These guidelines make sure that drug companies everywhere use the same tests and provide all the necessary data when applying to sell their drugs. This helps to ensure that medications are safe and effective for as long as they're on the market. [15]xvii. In 1991, major regions like the US, Europe, and Japan joined forces to create the ICH. Their goal? Standardize drug testing across borders. Originally, these guidelines focused on safety, effectiveness, and quality (QSEM) for new drugs. However, the World Health Organization (WHO) stepped in to address existing drugs and extreme climates not covered by the initial guidelines.
Over time, the ICH guidelines expanded to include things like veterinary drugs, and different regions like the US and India developed their own additional regulations for drug stability testing.[30].xviii
The ICH guidelines are like a rulebook for drug stability testing. They cover a bunch of things, like what to test (active ingredients, finished drugs, different formulas), how to test them (specific conditions, following codes in Table 1b), and what information drug companies need to provide (especially for new drugs).
One important guideline, Q1A(R2), focuses on testing brand new drugs. It says drug companies must include stability data in their applications to sell the drug. Over time, factors like heat, light, and humidity affect the drug's properties, and testing helps us understand these effects. The ultimate goal is to figure out how long the drug stays good (shelf life) and how it should be stored. [31,32].xix
(ICH) Q1B provides recommendations for testing how light affects new drugs (substances and products). This builds on the ICH's main stability testing guideline, which emphasizes light testing as an important part of stress testing. Q1B offering specific details on how to conduct photostability testing. Typically, this testing involves one batch of drug material, chosen following the procedures outlined in the main guideline.[33].xx
Q1C deals with stability testing for medications that come in new forms (dosage forms). It's an add-on to the main ICH stability testing guideline. This guideline helps determine what information the original applicant for a drug needs to submit. [34].xxi
Q1D focuses on using bracketing and matrixing designs to streamline stability testing. Bracketing tests only the most extreme versions of factors like drug strength or container size. This assumes that the stability of samples in the middle will be similar to those at the extremes. Matrixing, on the other hand, tests a select group of samples at all combinations of factors at certain times, with others tested later. This assumes that the stability observed for a sample at a specific time applies to all samples. Both methods aim to reduce testing while still ensuring reliable stability data. [35,36].xxii
The ICH Q1E focuses on analyzing stability data to

justify extending a drug's shelf life. It provides specific instructions for using data from stability studies to recommend a longer shelf life (either a retest period or a shelf life extension) for medications (including both the active ingredients and the final product) beyond the timeframe directly observed in studies. It's important to remember that these stability studies are typically conducted under controlled long-term storage conditions. Importantly, the stability studies themselves must follow the methods outlined in the main ICH stability guidelines. These main guidelines are used to determine how long drugs can be stored before needing, retesting or expiring, and what storage conditions are necessary.

There's one more factor to consider: how much variation there is between different batches of the drug. Q1E emphasizes that for future batches to be reliably stable throughout their extended shelf life, there should be minimal variation between batches produced.[37].xxiii

Q1F focuses on stability data packages for regions with hot and dry or hot and humid climates. This guideline, accepted in 2003, applies to areas classified as climatic Zones III (hot and dry) and Zone IV (hot and humid).

The main ICH guideline already has a table (Table 2) that outlines the recommended storage conditions for stability testing in Zones III and IV for both accelerated and long-term testing. There's another table (Table 3) in the main guideline that specifies storage conditions for a long-term and accelerated testing of liquid medications packaged in containers that allow some moisture to pass through (semi-permeable containers [38].xxiv

Q5C goes beyond the general guidelines to address stability testing specifically for biotech drugs. This applies to well-understood proteins and polypeptides, whether they're natural (from sources like body fluids or tissues) or made in the lab using recombinant DNA technology (rDNA). This guideline focuses on how to develop and submit data on the stability of these types of drugs.

Here are some examples of biotech drugs covered by Q5C: cytokines (like interferons, interleukins, and growth factors), erythropoietin, blood clotting factors, growth hormones, insulins, monoclonal antibodies, and certain vaccines made with well-defined proteins. [39,40].xxv

Q7 is a guide to Good Manufacturing Practices (GMP) for making Active Pharmaceutical Ingredients (APIs). It explains how to follow GMP principles throughout the API manufacturing process, ensuring consistent quality and purity. Q7 defines "manufacturing" to include everything from receiving raw materials to making, labeling, packaging, storing, shipping, and distributing the final API.

It's important to note that Q7 doesn't cover worker safety or environmental protection. Additionally, Q7 doesn't deal with specific requirements for registering, filling, or adjusting APIs according to pharmacopoeias (drug compendia).

Q7 focuses specifically on making APIs for human drugs [41].xxvi

Table 2

Storage Conditions over Time for Climatic Zones III and IV

Study	Storage Condition	Minimum Submission Time
Long-term Storage Condition	30°C ± 2°C / 65% RH ± 5% RH	12 months
Accelerated Storage Condition	40°C ± 2°C / 75% RH ± 5% RH	6 months

Table 3

Storage Conditions over Time for Aqueous Materials

Study	Storage Condition	Minimum Submission Time
Long-term Storage Condition	30°C ± 2°C / 35% RH ± 5% RH	12 months
Accelerated Storage Condition	40°C ± 2°C / ≤ 25% RH ± 5% RH	6 months

The European Medicines Agency (EMEA) has a committee called the Committee for Proprietary Medicinal Products (CPMP). This committee created a set of recommendations to help companies get approval to sell their drugs in the European Union. These recommendations, which are in Table 4, focus on how to properly test drugs to make sure they stay stable over time.

Imagine Earth with different storage zones, kind of like climate zones for clothes! Back in 1972, scientists came up with:

- Zone I: Temperate (think cool and mild)
- Zone II: Subtropical/Mediterranean (think warm and dry or warm and humid summers)
- Zone III: Hot and Dry (think deserts)
- Zone IV: Hot and Humid (think rainforests)

World Health Organization has guidelines for storing drugs in these zones.

Later, scientists decided Zone IV needed more detail. They split it into two zones:

- Zone IVA: Less humid (think rainforests, but maybe not quite as wet)
- Zone IVB: More humid (think full-on rainforest)

This zoning system helps design tests for how long drugs stay good (stability testing). There are two main types of these tests:

- Real-time testing: Mimics how a drug would be stored normally.
- Accelerated testing: Puts extra stress on the drug (like hotter temperatures) to see how it breaks down faster.

You can find more details about these zones and test conditions in Table 5.[45].xxvii

Designing a drug stability testing protocol

Before you start testing how well a drug holds up over

time (stability testing), you need a plan. This plan, called a stability testing protocol, is like a detailed recipe that outlines exactly how the tests will be conducted. The plan considers things like:

- How strong the drug naturally is (inherent stability)
- How the drug is packaged (bottle, blister pack, etc.)
- Brand new or already on the market

This plan helps ensure the tests are accurate and controlled.

Table 4

Stability Guidelines by CPMP

CPMP Code	Guideline Title
CPMP/QWP/576/96 Rev.1	Guidelines for Stability Testing in Relation to Variations in Marketing Authorization
CPMP/QWP/6142/03	Guidelines for Stability Testing of Active Substances and Medicinal Products in Climatic Zones III and IV
CPMP/QWP/609/96 Rev.1	Guidance Note on Declaring Storage Conditions for Medicinal Products and Active Substances
CPMP/QWP/122/02 Rev.1	Guidance Note on Stability Testing for Existing Active Substances and Associated Finished Products
CPMP/QWP/072/96	Guidance Note on Determining the Start of Shelf Life for the Finished Dosage Form
CPMP/QWP/2934/99	Guidance Note on In-Use Stability Testing for Human Medicinal Products
CPMP/QWP/576/96	Guidance Note on Stability Testing for a Type 2 Variation to a Marketing Authorization
CPMP/QWP/159/96	Guidance Note on Maximum Shelf Life for Sterile Products Following Initial Opening or Reconstitution

Table 5

Long-term Testing Conditions in Various Climatic Zones

Climate Zone	Definition / Climate	Primary Countries / Regions	Annual Partial Water Vapor MAT (Mean	Long-term Test Parameters
I	Temperature	The United Kingdom, Northern Europe, Russia, United States	≤15°C / ≤11 hPa	21°C with 45% RH
II	Subtropical and Mediterranean	Japan, Southern Europe	15–22°C / >11–18 hPa	25°C with 60% RH
III	Hot and Dry	Iraq, India	>22°C / ≤15 hPa	30°C with 35% RH
IVa	Hot and Humid	Iran, Egypt	>22°C / >15–27 hPa	30°C with 65% RH
IVb	Hot and Very Humid	Brazil, Singapore	>22°C / >27 hPa	30°C with 75% RH

Table 6

Stability Testing Schedule for a New Products

Temperature / RH	Duration (Months)	Climate Zone and Method
25°C / 60% RH	3, 6, 9, 12, 18, 24, 36	Long term for Zones I and IV
30°C / 35% RH	3, 6, 9, 12, 18, 24, 36	Long term for Zone III
30°C / 65% RH	3, 6, 9, 12, 18, 24, 36	Long term for Zone IVa, or intermediate for Zones I and II
30°C / 75% RH	3, 6, 9, 12, 18, 24, 36	Long term for Zone IVa, or intermediate for Zones I and II
40°C / 75% RH	3, 6	Accelerated conditions for all zones

We need to determine the expiration date and shelf life. [46] xxviiixxix. Protocol should include the specific climate zones (I-III, IVa, or IVb) where the product is intended to be sold.[47]xxx

The plan for testing drug stability should include these details

- The Quantity of batches: How many batches of the drug will be tested?
- Container closures: What type of containers and lids will be used for the drug?
- The Storage orientation of containers: How will the drug be stored during testing (upright, sideways, etc.)?
- Time points for sampling: At what specific times will samples be taken from stored drugs for testing?
- Sampling strategy: How will the samples be chosen from each batch for testing? (randomly, specific locations, etc.)
- Conditions for storage testing: What temperatures, humidity levels, and light exposure will the drugs be stored under during testing?
- Parameters for testing: What specific aspects of the drug will be measured during testing (potency, purity, appearance, etc.)?
- Methodology for testing: What specific methods and equipment will be used to test the drug samples?
- Criteria for acceptance: What are the acceptable limits for the measured parameters of the drug to be considered stable?

Batch Quantity

Typically, stability investigations commence with a single batch in the developmental phase. For new products or those undergoing registration, studies are expanded to encompass the first three batches. For established and stable products, two distinct batches may suffice. In cases where comprehensive manufacturing data is lacking, long-term studies should include the initial three batches produced post-approval, adhering to the same process outlined in the authorized application. Data collected during pharmaceutical development is

deemed supportive. The selection of pilot or production batches for sampling should generally adhere to the principles of random sampling [48].xxxi

Container and Closure Assessment

The drug gets tested in the package it will be sold in:

- Vials, blister packs, bottles (HDPE) - all these are fair game for testing, as long as they are the final packaging the drug will be sold in.
- Cardboard boxes used for shipping aren't included in the testing.

Basically, we test the drug in its "ready-to-sell" form.

Testing Every Package Type

- Every single type of container and lid a drug comes in needs to be tested before it can be sold.
- But for big batches of drugs, they can use temporary containers for storage as long as they're similar to the final packaging.[49].xxxii

Orientation of Container Storage

Keeping the Lid Sealed Tight:

To make sure the drug stays sealed properly during testing, we need to store samples in different positions depending on the type of drug:

- Liquids: These should be stored upright, just like on a shelf.
- Suspensions or Creams: These might need to be stored upside down or on their sides to keep medicine in contact with the lid.

This ensures a good seal throughout the testing period.

Testing Schedule for New Drugs

This follows a specific plan to ensure the drug stays good for its expected shelf life. Here's a breakdown:

- For drugs with a minimum shelf life of one year:
  - Testing occurs every three months during the initial year to monitor long-term storage.
  - In the second year, testing intervals extend to every six months.
  - Thereafter, testing is conducted annually until the drug reaches its expiration date.
- Special stressed storage tests:
  - Samples are usually tested at least 3 times (including the beginning and end).
  - In some cases, they might be tested up to 4 times (every 3 months for a year).
- Testing multiple versions of the drug:
  - If the drug comes in different strengths or sizes, they might be able to test fewer samples.
  - This reduced testing plan uses a special method to make sure all versions are still good.

You can find more details about the specific testing schedule in Table 6.

Taking Samples for Drug Testing

- Scientists take multiple samples of the drug and put them in a special chamber to see how they hold up over time.
- To get a good picture of the drug's stability, they need to decide how many samples to take and when to take them throughout the testing period.
- For example, they might take about 100 tablets for a long-term test. These tablets would then be divided up for different tests, like checking hardness or how quickly they dissolve.
- The exact number of samples needed depends on how many different tests will be done.
- Importantly, scientists don't pick the samples randomly. They choose them carefully to make sure they represent the entire batch of drugs.[30].xxxiii

Test Storage Conditions

It must match the climate zone where the drug will be sold or where approval is being requested. This ensures the drug is tested under conditions it might experience after purchase. Table 7 provides details on these storage conditions.

Test Parameters

A chosen stability test aims to assess the performance, purity, potency, and identity of the product post-storage. Standard examinations conducted on samples typically cover aspects such as appearance, assay, degradation products, dissolution, moisture content, and microbial analysis. Batches utilized in stability studies are required to satisfy all test criteria, including those pertaining to heavy metals, residual solvents, and combustion residues. While certain tests are essential for product release, they are not routinely included in stability testing. Furthermore, Q6A guidelines specify additional assessments such as enantiomeric purity, polymorphic form, particle size, among others.

Test Methodology

Following the official testing methods from trusted sources is key. These tests are widely recognized as reliable. If you want to use a different method, you need to validate it to make sure it gives accurate results.

The verification process must be comprehensive. Additionally, assessing a drug's stability should involve a stability-indicating technique, developed through stress testing the substance under conditions that induce decomposition. This method should be evaluated for its linearity, reliability, accuracy, and precision. The validated system should include determining detection and quantification limits for analyzing product degradation. After confirming reproducibility and completing essential validation steps such as linearity and range assessment, the documented method must be followed. Each test should adhere to the guidelines specified in a standardized testing protocol.[46].xxxiv

Table 7
Storage Conditions for Stability Testing of Pharmaceutical Products [32,49][xxxv]."

Proposed Storage Conditions	ICH Test Conditions: Temperature and Humidity (Duration in Months)	WHO Test Conditions: Temperature and Humidity (Duration in Months)
Ambient Temperature		
Long Term Condition	25 ± 2°C / 60 ± 5% RH (12)	25 ± 2°C / 60 ± 5% RH or 30 ± 2°C / 65 ± 5% RH or 30 ± 2°C / 75 ± 5% RH (12)
Intermediate Condition	30 ± 2°C / 65 ± 5% RH (6)	30 ± 2°C / 65 ± 5% RH (6)
Accelerated Condition	40 ± 2°C / 75 ± 5% RH (6)	40 ± 2°C / 75 ± 5% RH (6)
Refrigerated		
Long Term Condition	5°C / Ambient (12)	5 ± 3°C
Accelerated Condition	25 ± 2°C / 60 ± 5% RH (6)	25 ± 2°C / 60 ± 5% RH or 30 ± 2°C / 65 ± 5% RH
Freezer		
Long Term Condition	-20°C / Ambient (12)	-20 ± 5°C

Acceptance criteria
Before we start testing drugs for stability, we need to define what a "good" result looks like. This involves two main things:
Numbers: We set specific numerical limits for things like moisture, how well the drug dissolves, and how much it degrades over time. We also check things like appearance, odor, and if there's any mold growth.
Impurities: There can be tiny amounts of other chemicals in the drug. These are called impurities. The allowed level of these impurities depends on how much of the drug someone will take each day. For very high doses, the allowed impurity level is very low.
Here are some specific examples:

- If the daily dose is less than 1 gram, the impurity level can't be higher than 0.1%.
- If the daily dose is more than 1 gram, the impurity level can't be higher than 0.05%.

There are also guidelines for how much degradation is acceptable, which you can find in document "ICH Guideline Q3B (R2)".
Essentially, our aim is to ensure the safety and efficacy of the drug remains consistent throughout its shelf life.[27].xxxvi

Shelf life estimation
Making sense of long-term storage data

- We use information from tests where drugs are stored for a long time to figure out their shelf life.
- Scientists first put this data into a special format to make it easier to analyze (linearization).
- Then, they check how well this formatted data fits a straight line. A good fit is important for accurate shelf life determination.
- Table 8 shows examples of how the amount of drug changes over time for different batches.
- By analyzing this data, scientists can calculate the overall rate of change (slope) for the drug's potency over time.

In simpler terms, we use long-term storage data to see how the drug's strength changes and set an expiration date based on that.[49]. While a single shelf life is typical for most drugs, some exceptions exist. Freeze-dried protein products, like some vaccines, can have two shelf lives. The first applies when stored under dry conditions (like before opening). The second, much shorter shelf life, applies if exposed to moisture (like after opening). [50].xxxvii

Newer and Better Drug Testing
Drug companies around the world are working together to improve how they test drugs for stability. This means making sure the tests are the same everywhere, so everyone agrees on how long a drug will stay good.
Here's what they're doing:

- **Considering extreme climates:** They're testing drugs under even harsher conditions, like very hot and humid places.
- **Longer stressed tests:** They're putting drugs through these harsh conditions for longer periods (up to a year) to see how they hold up.
- **New test conditions:** They're adding a new test that's even hotter and more humid than before.

This will help ensure that drugs work well no matter where they are sold in the world. [51,52].xxxviii

Table 8
Concentration-Time Data Patterns and Pooling Determination

Slope	Intercept	Variation Factor	Combining
Identical	Identical	None	Affirmative
Identical	Different	Batch, such as varying initial drug concentrations	Negative
Different	Identical	Storage, such as variances in drug loss rates	Negative
Different	Different	Interactive influences involving both batch and storage factors	Negative

Conducted at a single laboratory, the combination of three climate variables—temperature, humidity, and light—indicates a greater impact on medicinal products compared to conditions involving only temperature and humidity. [53-55]. xxxix A 2018 study by Singh et al. pointed out that testing how well drugs hold up over time (stability analysis) is critical. This ensures patients receive safe and effective medications. The study also highlighted some key trends:

- Drugs going global: Medicines are increasingly sold worldwide.
- Standardized testing: Everyone agreeing on how to test drug stability.
- Focusing on stability: Scientists and regulators need to prioritize these concerns.

In other words, with the world becoming more

interconnected and regulations becoming more unified, ensuring drug stability through proper testing is becoming even more important.

[56]. [xl] Testing Drugs to Ensure Quality (Kailash et al., 2015): A study by Kailash et al. (2015) emphasized two key points about testing how well drugs hold up over time (stability testing):

- Accurate Testing: These tests should mimic real-world storage conditions (like temperature and humidity) to ensure the results are accurate.
- Quality Products, Good Reputation: By doing these tests, drug companies can be sure their products are high quality. This helps build trust and a good reputation in the global market.

In short, stability testing is like a quality check for drugs, making sure they stay safe and effective throughout their shelf life. This helps drug companies produce high-quality products and maintain a good reputation.[57].xli

CONCLUSION

Testing how well drugs hold up over time (stability testing) has become a crucial part of developing new drugs and formulations. These tests ensure medications stay safe and effective throughout their shelf life when stored properly.

To do this testing effectively, scientists need to follow two key principles:

- Science First: The tests themselves need to be scientifically sound.
- Following the Rules: The tests also need to comply with current regulations and consider where the drug might be sold (different climates).

By following these principles, stability testing helps ensure patients receive safe and effective medications.

Acknowledgment

The author is grateful to the Department of Pharmaceutical Chemistry at the Main Campus of Hamdard University for their invaluable support and guidance throughout the execution of this review. Special thanks to Dr. Qurat ul Ain for her guidance in preparing the review article. I am deeply grateful for her invaluable assistance.

REFERENCES

[i] 1. Arunachalam A, Shankar M. Stability studies. Asian J Pharm Anal Med Chem 2008;1:184-95.

[ii] 2. Naveed S, Basheer S, Qamar F. Stability of a dosage form and forced degradation studies. J Bioequivalence Bioavailab 2016;8:191-3. https://doi.org/10.4172/jbb.1000292

[iii] 3. Kommanaboyina B, Rhodes CT. Trends in stability testing, with emphasis on stability during distribution and storage. Drug Dev Ind Pharm 1999;25:857-68. https://doi.org/10.1081/ddc-100102246

[iv] 5. Sengupta P, Chatterjee B, Tekade RK. Current regulatory requirements and practical approaches for stability analysis of pharmaceutical products: A comprehensive review. Int J Pharm 2018;543:328-44. https://doi.org/10.1016/j.ijpharm.2018.04.007

[v] 1) Thorat P, Warad S, Solunke R, Sargar A, Bhujbal A, Shinde A. Stability Study of Dosage Form: An Inovative Step, World Journal of Pharmacy and Pharmaceutical Sciences, 2014;3:1031-1050. https://doi.org/10.20959/wjpps20178-9786

[vi] 11. Thorat P, Warad S, Solunke R, Ashok S, Anagha B, Asha S, et al. Stability study of dosage form: An inovative step. World J Pharm Pharm Sci 2014;3:1031-50.

12. Freed AL, Colgan ST, Kochling JD, Alasandro MS. Accelerating pharmaceutical development through predictive stability approaches. AAPS J 2017;3:2-10.

13. Khushbu M, Thakor A, Bhavsar DD, Thakor JR. Development of forced degradation and stability indicating studies for drug substance and drug product. Int J Res Pharmacol Pharmacother 2016;55:291-7.

14. Bhaskar R, Ola M, Agnihotri V, Chavan A, Girase H. Current trend in performance of forced degradation studies for drug substance and drug products. J Drug Deliv Ther 2020;10:149-55. https://doi.org/10.22270/jddt.v10i2-s.4040

15. Suthar N, Choudhary M. A review on stability studies of pharmaceutical products. Int J Appl Pharm Biol Res 2017;2:67-75.

16. Agarwal V, Sharma D. Stability testing of active pharmaceutical and scientific innovation. J Pharm Sci Innov 2012;1:18-23.

[vii] 1) Thorat P, Warad S, Solunke R, Sargar A, Bhujbal A, Shinde A. Stability Study of Dosage Form: An Inovative Step, World Journal of Pharmacy and Pharmaceutical Sciences, 2014;3:1031-1050.

2) Bajaj S, Singla D, Sakhuja N. Stability Testing of Pharmaceutical Products. Journal of Applied Pharmaceutical Science. 2012;2:129-138. https://doi.org/10.7324/japs.2012.2322

[viii] 17. Tollefson AE, Hermiston TW, Lichtenstein DL, Colle CF, Tripp RA. Forced degradation of fast inhibits apoptosis in adenovirus-infected cells. Nature 1998;392:726-30.

18. Bakshi M, Singh S. Development of validated stability-indicating assay methods-critical review. J Pharm Biomed Anal 2002;28:1011-40.

19. Marin A, Barbas C. LC-MS for the degradation profiling of cough-cold products under forced conditions. J Pharm Biomed Anal 2004;35:1035-45. https://doi.org/10.1016/j.jpba.2004.03.011
[ix] 20. Singh S, Junwal M, Modhe G, Tiwari H, Kurmi M. Forced degradation studies to assess the stability of drugs and products. Trends Analyt Chem 2013;49:71-88.
[x] 21. Haystead JS. Stability testing. Pharm Technol 2004;28:130.
[xi] 22. Colgan ST, Timpano RJ, Roberts M. Opportunities for lean stability strategies. J Pharm Sci Innov 2014;9:259-71.
[xii] 23. Singh R, Rehman ZU. Current trends in forced degradation study for pharmaceutical product development. J Pharm Educ Res 2012;3:54-63.
24. Andreson G, Scott M. Determination of product shelf life and activation for five drugs of abuse. Clin Chem 1991;37:398-402.
[xiii] 25. Chakole RD, Charde MS, Kumar J, Welankiwar AS. Development of forced degradation studies of drugs. Int J Adv Pharm 2013;2:34-9.
[xiv] 26. Hotha KK, Reddy SP, Raju VK, Ravindranath LK. Overview of regulatory guidance and literature for the drug products and drug substances. Int Res J Pharm 2013;4:78-85.
[xv] 6. Bajaj S, Singla D, Sakhuja N. Stability testing of pharmaceutical products. J Appl Pharm Sci 2012;2:129-38.
15. Suthar N, Choudhary M. A review on stability studies of pharmaceutical products. Int J Appl Pharm Biol Res 2017;2:67-75.
[xvi] 2) Bajaj S, Singla D, Sakhuja N. Stability Testing of Pharmaceutical Products. Journal of Applied Pharmaceutical Science. 2012;2:129-138. 8) Singh S., Bakshi M. Guidance on conduct of stress test to determine inherent stability of drugs. Pharmaceutical Technology Asia, Special Issue, Sep. /Oct. 2000;24-36.
[xvii] 15. Suthar N, Choudhary M. A review on stability studies of pharmaceutical products. Int J Appl Pharm Biol Res 2017;2:67-75.
[xviii] 30. Singh S. Stability testing during product development. In: Jain NK. Pharmaceutical Product Development. India: CBS Publisher; 2000. p. 272-93.
[xix] 31. Pokharana M, Vaishnav R, Goyal A, Shrivastava A. Stability testing guidelines of pharmaceutical products. J Drug Deliv Ther 2018;8:169-75. 32. International Conference on Harmonization. Guideline on Stability Testing of New Drug Substances and Products, Q1A (R2); 2003. https://www.database.ich.org/sites/default/files/q1a28r22920guideline.pdf.
[xx] 33. International Conference on Harmonization. Stability Testing: Photostability Testing of New Drug Substances and Products, Q1B; 1996. https://www.database.ich.org/sites/default/files/q1b20guideline.pdf.
[xxi] 34. International Conference on Harmonization. Stability Testing for New Dosage Forms, Q1C; 1996. https://www.database.ich.org/sites/default/files/Q1C20Guideline.pdf.
[xxii] 35. International Conference on Harmonization. Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products, Q1D; 2002. Avai https://www.database.ich.org/sites/default/files/q1d20guideline.pdf. [Last accessed on 2020 Mar 20]. 36. Munden RP. ICH Q1D: ICH Quality Guideline; 2017. p. 73-87.
[xxiii] 37. International Conference on Harmonization. Evaluation for Stability Data, Q1E; 2003. https://www.database.ich.org/sites/default/files/q1e20guideline.pdf.
[xxiv] 38. International Conference on Harmonization. Stability Data Package for Registration Application in Climatic Zone III and IV, Q1F; 2018. https://www.database.ich.org/sites/default/files/q1f_stability_guideline_who_2018.pdf.
[xxv] 39. International Conference on Harmonization. Stability Testing of Biotechnological/Biological Products, Q5C; 1995. https://www.database.ich.org/sites/default/files/q5c20guideline.pdf.
40. Hasija, M, Aboutorabian, S, Rahman, N Ausar, SF. Practical approaches to forced degradation studies of vaccines. Methods Mol Biol 2016;1403:853-66.
[xxvi] 41. International Conference on Harmonization. GMP Guide for Active Ingredients, Q7; 2000. https://www.database.ich.org/sites/default/files/q720guideline.pdf.
[xxvii] 45. World Health Organization. Stability Testing of Active Pharmaceutical Ingredients and Finished Pharmaceutical Products. WHO Technical Report Series; 2009. https://www.extranet.who.int/prequal/sites/default/files/documents/trs1010_annex10.pdf.
[xxviii] 46. Ali J, Khar RK, Ahuja A. Dosage form and Design. 3rd ed. Delhi: Birla Publications Pvt. Ltd.; 2008. p. 100-23.
[xxix] 46. Ali J, Khar RK, Ahuja A. Dosage form and Design. 3rd ed. Delhi: Birla Publications Pvt. Ltd.; 2008. p. 100-23.
[xxx] 47. Baertschi SW. Pharmaceutical Stress Testing. Predicting Drug Degradation. India: CRC Press; 2005.
[xxxi] 48. Zimmer M. Forced degradation and long-term stability testing for oral drug products. In: Methods for Stability Testing of Pharmaceuticals. Methods in Pharmacology and Toxicology. New York: Humana Press; 2018. p. 75-98. https://doi.org/10.1007/978-1-4939-7686-7_4
[xxxii] 49. Ruben SR. Stability studies. Asian J Pharm Anal Med Chem 2014;2:51-62.

[xxxiii] Singh S. Stability testing during product development. In: Jain NK. Pharmaceutical Product Development. India: CBS Publisher; 2000. p. 272-93.
[xxxiv] 46. Ali J, Khar RK, Ahuja A. Dosage form and Design. 3rd ed. Delhi: Birla Publications Pvt. Ltd.; 2008. p. 100-23.
[xxxv] 32. International Conference on Harmonization. Guideline on Stability Testing of New Drug Substances and Products, Q1A (R2); 2003. https://www.database.ich.org/sites/default/files/q1a28r22920guideline.pdf.
49. Ruben SR. Stability studies. Asian J Pharm Anal Med Chem 2014;2:51-62.
[xxxvi] 27. Kaur M, Kaur G, Kaur H, Sharma S. Overview on stability studies. Int J Pharm Chem Biol Sci 2013;3:1231-41.
[xxxvii] 50. Carstensen JT. Drug Stability: Principles and Practices. New York: Marcel Dekker; 2000.
[xxxviii] 51. Mischler PG. Developing Stability Protocols for Global Product Registrations-an Update. London: IBC Life Sciences; 2002. p. 13-4. 52. Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Motto MG, et al. Conducting forced degradation studies. Pharm Technol 2002;26:48-56.
[xxxix] 53. Bhutani H, Singh S, Mariappan TT, Kaur H, Bajaj M, Pakhale S, et al. Behavior of uptake of moisture by drugs and excipients under accelerated conditions of temperature and humidity in the absence and the presence of light. Pharm Technol 2003;27:44-52. https://doi.org/10.1016/s0378-5173(02)00340-x
54. Gaur A. A possible reason for the generation of out of trend stability results: Variable air velocity at different locations within the stability chamber. Pharm Technol 2005;29:46-9.
55. Singh A, Singh P. Technical considerations of forced degradation studies of new drug substances and product: Regulatory perspectives. J Drug Deliv Ther 2018;8:163-8.
[xl] 56. Singh DK, Singh S, Bajaj S. Methods for stability testing of pharmaceuticals. In: Methods in Pharmacology and Toxicology. New York: Springer; 2018. p. 1-30.
[xli] Kailash VV, Parkar B, Ghude K, Acharekar S. Recent trends in stability testing of pharmaceutical products: A review. J Pharm Biol Chem Sci 2015;6:1557-63.