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GENERAL PHARMACEUTICAL CHEMISTRY

Drug stability

Lesson 14
IV term

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Stability testing of pharmaceutical products is a complex set of procedures involving considerable cost, time consumption and scientific expertise in order to build in quality, efficacy and safety in a drug formulation. The most important steps during the developmental stages include pharmaceutical analysis and stability studies that are required to determine and assure the identity, potency and purity of ingredients, as well as those of the formulated products.

Stability of a pharmaceutical product may be defined as the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, toxicological, protective and informational specifications.

In other words, it is the extent to which a product retains, within the specified limits, throughout its period of storage and use, the same properties and characteristics possessed at the time of its packaging.

Stability testing thus evaluates the effect of environmental factors on the quality of the a drug substance which is utilized for prediction of its shelf life, determine proper storage conditions and suggest labeling instructions.

Stability testing is termed as a complex process because of involvement of a variety of factors influencing the stability of a pharmaceutical product.

These factors include

- ✓ stability of the active ingredient(s);
- ✓ interaction between active ingredients and excipients,
- ✓ manufacturing process followed,
- ✓ type of dosage form,
- ✓ container/closure system used for packaging
- ✓ light, heat and moisture conditions encountered during shipment, storage and handling.

In addition, degradation reactions like oxidation, reduction, hydrolysis or racemization, which can play vital role in stability of a pharmaceutical product, also depend on such conditions like concentration of reactants, pH, radiation, catalysts etc., as well as the raw materials used and the length of time between manufacture and usage of the product.

A pharmaceutical product may undergo change in appearance, consistency, content uniformity, clarity (solution), moisture contents, particle size and shape, pH, package integrity thereby affecting its stability. Such physical changes may be because of impact, vibration, abrasion, and temperature fluctuations such as freezing, thawing or shearing etc.

The chemical reactions like solvolysis, oxidation, reduction, racemization etc. that occur in the pharmaceutical products may lead to the formation of degradation

product, loss of potency of active pharmaceutical ingredient (API), loss of excipient activity like antimicrobial preservative action and antioxidants etc.

Stability of a pharmaceutical product can also be affected because of microbiological changes like growth of microorganisms in non sterile products and changes in preservative efficacy. Potential adverse effects of instability in pharmaceutical products have been given in Table 1.

Table 1: Potential Adverse Effects of Instability in Pharmaceutical Products.

Potential Adverse Effect	**Explanation/ Reason**	**Example**	**Stability Parameter Tested**
Loss of Active Ingredient	Degradation of API in product resulting in less than 90% drug as claimed on label - unacceptable quality	Nitroglycerine tablets	Time elapsed before the drug content no longer exceeds 90%
Increase in concentration of active Ingredient	Loss of vehicle perfusion bags sometimes allow solvent to escape and evaporate so that the product within the bag shows an increase in concentration	Lidocaine gel, products in perfusion bags	Stability in final container
Alteration in bioavailability	Changes in rate and extent of absorption on storage		Dissolution/release studies
Loss of content uniformity	Loss of contents as a function of time	Suspension	Ease of re- dispersion or sedimentation volume
Decline of microbiological status	Increase in number of viable microorganisms already present in the product. Contamination because of compromised package integrity during distribution/ storage	Multiuse cream	Total bioburden after storage
Loss of pharmaceutical elegance and patient acceptability	Speckling caused by the interaction of the drug containing amine group with a minor component in the lactose resulting in the	Slight yellow or brown speckling on the surface of tablet containing spray-dried lactose	Visual Examination

	formation of a chromatophore		
Formation of toxic degradation products	Degradation of the drug component	Formation of epianhydrotetracycline from tetracycline, Protein drugs	Amount of degradation products during shelf life
Loss of package integrity	Change in package integrity during storage or distribution	Plastic screw cap losing back-off-torque	Specific package integrity tests
Reduction of label quality	Deterioration of label with time and cause the ink to run and thus adversely affect legibility	Plasticizer from plastic bottle migrates into the label	Visual examination of the label
Modification of any factor of functional relevance	Time-dependent change of any functionally relevant attribute of a drug product that adversely affects safety, efficacy, or patient acceptability or ease of use	Adhesion ageing of transdermal patches	Monitoring changes

STABILITY TESTING METHODS

Stability testing is a procedure performed on drug substances and products and is employed at various stages of the product development.

In early stages, ***accelerated stability testing*** (at relatively high temperatures and/or humidity) is used in order to determine the type of degradation products which may be found after long-term storage.

Testing under less rigorous conditions i.e. those recommended for long-term shelf storage, at slightly elevated temperatures is used to determine a product's shelf life and expiration dates.

The major aim of pharmaceutical stability testing is to provide reasonable assurance that the products will remain at an acceptable level of fitness/quality throughout the period during which they are in market place available for supply to the patients and will be fit for their consumption until the patient uses the last unit of the product.

Depending upon the aim and steps followed, stability testing procedures have been categorized into the following four types.

Real-Time stability testing

Real-time stability testing is normally performed for longer duration of the test period in order to allow significant product degradation under recommended

storage conditions. The period of the test depends upon the stability of the product which should be long enough to indicate clearly that no measurable degradation occurs and must permit one to distinguish degradation from inter-assay variation. The reliability of data interpretation can be increased by including a single batch of reference material for which stability characteristics have already been established.

Accelerated stability testing

In accelerated stability testing, a product is stressed at several high (warmer than ambient) temperatures and the amount of heat input required to cause product failure is determined. This is done to subject the product to a condition that accelerates degradation. This information is then projected to predict shelf life or used to compare the relative stability of alternative formulations.

This usually provides an early indication of the product shelf life and thus shortening the development schedule. In addition to temperature, stress conditions applied during accelerated stability testing are moisture, light, agitation, gravity, pH and package.

Retained sample stability testing

This is a usual practice for every marketed product for which stability data are required. In this study, stability samples, for retained storage for at least one batch a year are selected. In this study, the stability samples are tested at predetermined intervals i.e. if a product has shelf life of 5 years, it is conventional to test samples at 3, 6, 9, 12, 18, 24, 36, 48, and 60 months. This conventional method of obtaining stability data on retained storage samples is known as constant interval method . Stability testing by evaluation of market samples is a modified method which involves taking samples already in the market place and evaluating stability attributes. This type of testing is inherently more realistic since it challenges the product not just in the idealized retained sample storage conditions, but also in the actual marketplace.

Cyclic temperature stress testing

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

CLIMATIC ZONES FOR STABILITY TESTING

For the purpose of stability testing, the whole world has been divided into four zones (I - IV) depending upon the environmental conditions the pharmaceutical

products are likely to be subjected to during their storage. These conditions have been derived on the basis of the mean annual temperature and relative humidity data in these regions. Based upon this data, long-term or real-time stability testing conditions and accelerated stability testing conditions have been derived. The standard climatic zones for use in pharmaceutical product stability studies have been presented in the Table 2. The break-up of the environmental conditions in each zone and also the derived long-term stability test storage conditions, as given by WHO have also been presented.

Table. 2: ICH Climatic zones and long term stability conditions

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

*MAT - Mean annual temperature measured in open air.

PROTOCOL FOR STABILITY TESTING

The protocol for stability testing is a pre-requisite for starting stability testing and is necessarily a written document that describes the key components of a regulated and well-controlled stability study. Because the testing condition is based on inherent stability of the compound, the type of dosage form and the proposed container-closure system, the protocol depends on the type of drug substance or the product. In addition, the protocol can depend on whether the drug is new or is already in the market. The protocol should also reflect the regions where the product is proposed to be marketed e.g. if the product is planned to be used in climatic zones I-III, IVa and IVb, the stability program must include all these zones. A well designed stability protocol should contain the following information.

Batches

Stability studies at developmental stages are generally carried out on a single batch while studies intended for registration of new product or unstable established product are done on first three production batches, while for stable and well-

established batches, even two are allowed. In general, the selection of batches should constitute a random sample from the population of pilot or production batches.

Containers and closures

The testing is done on the product in immediate containers and closures proposed for marketing. The packaging materials include aluminium strip packs, blister packs, Alu-Alu packs, HDPE bottles etc. This may also include secondary packs, but not shippers. Products in all different types of containers/closures, whether meant for distribution or for physician and promotional samples, are to be tested separately. However, for bulk containers, testing in prototype containers is allowed, if it simulates the actual packaging.

Orientation of storage of containers

Samples of the solutions, dispersed systems and semi solid drug products for stability studies must be kept upright and positioned either inverted or on the side to allow for full interaction of the product with the container-closure. This orientation helps to determine whether the contact between the drug product or solvent and the closure results in the extraction of chemical substances from the closure components or adsorption of product components in to the container-closure.

Sampling time points

Frequency of testing should be such that it is sufficient to establish the stability profile of the new drug substance. For products with a proposed shelf life of at least 12 months, the testing frequency at the long-term storage condition should be every 3 months over the first year, every 6 months over the second year and annually thereafter throughout the proposed shelf life expiration date. In the case of accelerated storage conditions, a minimum of three time points, including the initial and end points, for example, 0, 3, and 6 months is recommended.

Sampling Plan

Sampling plan for stability testing involves, planning for the number of samples to be charged to the stability chambers and sampling out of the charged batch so as to cover the entire study.

The first step should be the development of the sampling time points followed by the number of samples needed to be drawn at each pull point for complete evaluation of all test parameters and finally adding up to get the total number of samples.

Test storage conditions

The storage conditions to be selected are based upon the climatic zone in which the product is intended to be marketed or for which the product is proposed to be filed for regulatory approval.

Test parameters

The stability test protocol should define the test parameters that would be used for evaluation of the stability samples. The tests that monitor the quality, purity, potency, and identity which could be expected to change upon storage are chosen as stability tests. Therefore appearance, assay, degradation products, microbiological testing, dissolution, and moisture are standard tests performed on stability test samples. Microbiological tests include sterility, preservative efficacy and microbial count as applicable e.g. for liquid injectable preparations. The batches used for stability study must meet all the testing requirements including heavy metals, residue on ignition, residual solvents etc.

Test methodology

It is always recommended to follow the procedures given in the official compendia, as the results obtained using the official tests, in general find better acceptance. If alternate methods are used, they are required to be duly validated.

Acceptance criteria

All analytical methods are required to be validated before initiating the stability studies. Similarly, the acceptance criteria for the analytical results as well as that for the presence of degradation products should also be fixed beforehand. The acceptance criteria for each test in the stability study is fixed in the form of numerical limits for the results expressed in quantitative terms e.g., moisture pick-up, viscosity, particle size, assay, degradation products, etc. and pass or fail for qualitative tests e.g., odour, colour, appearance, cracking, microbial growth, etc. These acceptance criteria should also include individual and total upper limits for degradation products.

CONDUCT OF STABILITY STUDIES

The stability study is conducted by keeping the drug substance or the product in their proposed final packs (e.g. Aluminium strip, blister pack, Alu-Alu pack, HDPE container etc.), or prototype containers in the case of bulk drugs, in sufficient numbers in the stability chambers set at appropriate storage conditions as per the protocol. The samples are then withdrawn, as per the stability protocol, at the prescribed sampling intervals and are then analyzed by a suitable method. In order to minimize the effect of day-to-day variability on the results, the following two approaches are followed. Samples are drawn in replicate. One of the samples is tested and others are kept at temperatures sufficiently low to prevent further drug loss and then all the samples are subjected to analysis on the same day at the end of study (i.e after withdrawl of the last sample).

Second approach is to freeze the initial samples till the expiration period and test them at appropriate times by using them as internal standards in the assays.

PRESENTATION AND RECORDING OF STABILITY DATA

Stability data is recorded in an organized, comprehensive and cumulative format. The stability data table is the means for reporting the results of the stability study in a concise format for ease of review and interpretation. The data is recorded in a proper tabular format and all-encompassing information on a batch is recorded at one place. Similar sheets are prepared for each batch.

EXPIRATION DATE/SHELF LIFE

An expiration date is defined as the time up to which the product will remain stable when stored under recommended storage conditions. Thus, an expiration date is the date beyond which it is predicted that the product may no longer retain fitness for use. If the product is not stored in accordance with the manufacturer's instructions, then the product may be expected to degrade more rapidly.

The expiration date is also defined as the date placed on the container/labels of a drug product designating the time during which a batch of the product is expected to remain within the approved shelf life specifications, if stored under defined conditions and after which it should not be used.

STABILITY TEST EQUIPMENT

The equipment used for stability testing is called stability chamber. These are specialized environmental chambers that can simulate the storage condition and enable evaluation of product stability based on real-time, accelerated and long-term protocols.

They are available in both walk-in and reach-in styles. Smaller chambers are preferred for accelerated testing, as the retention time of products is much less in these cabinets, while the walk-in chambers are preferred for long-term testing. Such chambers or rooms are engineered and qualified to ensure uniform exposure of the set conditions to all the samples in the chamber. These chambers are expected to be dependable and rugged because of the requirement of uninterrupted use for years. They are fitted with appropriate recording, safety and alarm devices. In addition, photostability chambers are also available and utilized both with and without temperature and humidity control. Two types of light sources are usually employed in photostability chambers, one is the combination of cool white and near UV fluorescent tubes, while second are the artificial daylight lamps, e.g., xenon or metal halide.

Drug Stability Testing Machine

Thermal Shock Environmental Test Chamber
Medicine Drug Stability Test Chamber

CONCLUSION

Stability testing is now the key procedural component in the pharmaceutical development program for a new drug as well as new formulation. Stability tests are carried out so that recommended storage conditions and shelf life can be included on the label to ensure that the medicine is safe and effective throughout its shelf life. Over a period of time and with increasing experience and attention, the regulatory requirements have been made increasingly stringent to achieve the above goal in all possible conditions to which the product might be subjected during its shelf life. Therefore, the stability tests should be carried out following proper scientific principles and after understanding of the current regulatory requirements and as per the climatic zone.