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STABILITY INDICATING CHROMATOGRAPHIC METHODS FOR ACTIVE PHARMACEUTICAL INGREDIENTS AND FORMULATIONS – A REVIEW

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ABSTRACT

The article highlights on the development of validated stability indicating methods for active pharmaceutical ingredients and formulations. International Council on Harmonization (ICH) prescribed different stress test conditions and degradation studies for testing the stability of active pharmaceutical ingredients and formulations is emphasized. A systematic approach for the development of stability indicating chromatographic methods is also discussed.

Key words: Stability indicating methods, Active pharmaceutical Ingredients, Formulations.

INTRODUCTION

A Stability Indicating Method (SIM) is a quantitative analytical procedure used to detect a decrease in the amount of the active pharmaceutical ingredient (API) present due to degradation. According to FDA guidelines, a SIM is defined as a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences like degradation products, process impurities, excipients, or other potential impurities, and the FDA recommends that all assay procedures for stability studies be stability indicating. During stability studies, liquid chromatography (LC) is used routinely to separate and quantitate the analytes of interest. There are three components necessary for implementing a SIM: sample generation, method development, and method validation. Stability testing is a routine procedure performed on drug substances and products. It is involved at various stages of product development. In early stages, accelerated stability testing (at relatively high temperatures and/or humidities) can be used as a "worst case" evaluation to determine what types of degradation products may be found after long-term storage. Testing under more gentle conditions (those recommended for long-term shelf storage), and slightly elevated temperatures, can be used to determine a product's shelf life and expiration dates.

In these types of studies, the product is analyzed at intervals for various parameters, which may include assay of the active ingredient, measurement of known degradation products, dissolution time, appearance, etc. Additionally, samples from production lots of approved products are retained for stability testing in case of product failure in the field. Retained samples can be tested alongside returned samples to ascertain if the problem was manufacturing or storage related In recent times, there is an increased tendency towards the development of stability indicating methods [1–3], using the approach of stress testing as enshrine in the International Conference on Harmonization (ICH) guideline Q1AR(2) [4]. Even this approach is being extended to drug combinations [5,6] to allow accurate and precise quantitation of multiple drugs, their degradation products, and interaction products, if any.

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Chromatographic method

Because of requirement of separation of multiple components during analysis of stability samples, chromatographic methods have taken precedence over the conventional methods of analysis. Other than separation of multiple components, the advantage of chromatographic methods is that these possess greater accuracy and sensitivity for even small quantities of degradation products produced. Various chromatographic methods that have been used are thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), gas chromatography (GC), HPLC and newer technique like capillary electrophoresis (CE). TLC is a simple technique that has been used in the past for developing Stability Indicating Analytical Methods (SIAMs) [4-7]. Its disadvantages, such as variability and non-quantitative nature, limit its use as a basic technique for SIAM development. However, it is very much used, especially during initial degradation [8] and stress studies to study the number of degradation products formed, to identify the products formed through matching studies using standards and even for isolation where preparative TLC is employed. A large number of publications have appeared in the last decade on the use of HPTLC for stability-indicating method development.

This technique overcomes the shortcomings of TLC, and is reliable, fast and accurate for quantitative drug analysis. Moreover, many samples can be run simultaneously using a small quantity of mobile phase, thus minimizing analysis time and cost per analysis. Unfortunately, its limitation is that the equipment is not routinely available in every laboratory. GC is stability-indicating but it is not very versatile, as the drug substance may be nonvolatile or thermally unstable. Further any attempt to increase the volatility of the drug and components by increasing the temperature may lead to degradation or racemization. Therefore, there are very few reports on the use of GC for the purpose of establishment of SIAMs. In comparison, HPLC has been very widely employed. It has gained popularity in stability studies due to its high-resolution capacity, sensitivity and specificity. Non-volatile, thermally unstable or polar/ionic compounds can also be analyzed by this technique. Therefore, most of the SIAMs have been established using HPLC.

***Stress testing of Active pharmaceutical ingredient* (API)**

Stress testing of the API can help identify the likely degradation products, which, in turn, can help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual API and the type of FPP involved. For an API the following approaches may be used: when available, it is acceptable to provide the relevant data published in the scientific literature to support the identified degradation products and pathways; when no data are available, stress testing should be performed. Stress testing may be carried out on a single batch of the API. It should include the effect of temperature (in 10 °C increments (e.g. 50 °C, 60 °C,etc.) above the temperature used for accelerated testing), humidity (e.g. 75% relative humidity (RH) or greater) and, where appropriate, oxidation and photolysis on the API. The testing should also evaluate the susceptibility of the API to hydrolysis across a justified range of pH values when in solution or suspension. Assessing the necessity for photostability testing should be an integral part of a stress testing strategy. More details can be found in other guidelines. Results from these studies will form an integral part of the information provided to regulatory authorities.

Selection of batches

Data from stability studies on at least three primary batches of the API should normally be provided. The batches should be manufactured to a minimum of pilot scale by the same synthesis route as production batches, and using a method of manufacture and procedure that simulates the final process to be used for production batches. The overall quality of the batches of API placed on stability studies should be representative of the quality of the material to be made on a production scale. For existing active substances that are known to be stable, data from at least two primary batches should be provided.

Storage conditions

In general an API should be evaluated under storage conditions (with appropriate tolerances) that test its thermal stability and, if applicable, its sensitivity to moisture. The storage conditions and the lengths of studies chosen should be sufficient to cover storage and shipment.

Active pharmaceutical ingredients intended for storage below -20°C

APIs intended for storage below -20 °C should be treated on a case-by-case basis.

Stability commitment

When the available long-term stability data on primary batches do not cover the proposed re-test period granted at the time of approval, a commitment should be made to continue the stability studies post-approval in order to firmly establish the re-test period or shelf-life. Where the submission includes long-term stability data on the number of production batches covering the proposed re-test period, a post-approval commitment is considered unnecessary. Otherwise one of the following commitments should be made:

• If the submission includes data from stability studies on the number of production batches a commitment should be made to continue these studies through the proposed re-test period.
• If the submission includes data from stability studies on fewer than the number of production batches ,a commitment should be made to continue these studies through the proposed re-test period and to place additional production batches, to a total of at least three, in long-term stability studies through the proposed re-test period.
• If the submission does not include stability data on production batches, a commitment should be made to place the first two or three production batches on long-term stability studies through the proposed re-test period. The stability protocol used for long-term studies for the stability commitment should be the same as that for the primary batches, unless otherwise scientifically justified.

Stability study of Finished Pharmaceutical Product (FPP)

General

The design of the stability studies for the FPP should be based on knowledge of the behavior and properties of the API, information from stability studies on the API and on experience gained from preformulation studies and investigational FPPs.

Selection of batches

Data from stability studies should be provided on at least three primary batches of the FPP. The primary batches should be of the same formulation and packaged in the same container closure system as proposed for marketing. The manufacturing process used for primary batches should simulate that to be applied to production batches and should provide product of the same quality and meeting the same specification as that intended for marketing. In the case of conventional dosage forms with APIs that are known to be stable, data from at least two primary batches should be provided. Two of the three batches should be at least pilot-scale batches and the third one can be smaller, if justified. Where possible, batches of the FPP should be manufactured using different batches of the API(s). Stability studies should be performed on each individual strength, dosage form and container type and size of the FPP unless bracketing or matrixing is applied.

Container closure system

Stability testing should be conducted on the dosage form packaged in the container closure system proposed for marketing. Any available studies carried out on the FPP outside its immediate container or in other packaging materials can form a useful part of the stress testing of the dosage form or can be considered as supporting information, respectively.

Storage conditions

Specification

Stability studies should include testing of those attributes of the FPP that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The testing should cover, as appropriate, the physical, chemical, biological and microbiological attributes, preservative content (e.g. antioxidant or antimicrobial preservative) and functionality tests (e.g. for a dose delivery system). Analytical procedures should be fully validated and stability-indicating. Whether and to what extent replication should be performed will depend on the results of validation studies. Shelf-life acceptance criteria should be derived from consideration of all available stability information. It may be appropriate to have justifiable differences between the shelf-life and release acceptance criteria based on the stability evaluation and the changes observed on storage. Any differences between the release and shelf-life acceptance criteria for antimicrobial preservative content should be supported by a validated correlation of chemical content and preservative effectiveness demonstrated during development of the pharmaceutical product with the product in its final formulation (except for preservative concentration) intended for marketing. A single primary stability batch of the FPP should be tested for effectiveness of the antimicrobial preservative (in addition to preservative content) at the proposed shelf-life for verification purposes, regardless of whether there is a difference between the release and shelf-life acceptance criteria for preservative content.

Testing frequency

For long-term studies, frequency of testing should be sufficient to establish the stability profile of the FPP. For products with a proposed shelf-life of at least 12 months, the frequency of testing at the long-term storage condition should normally be every three months over the first year, every six months over the second year and annually thereafter throughout the proposed shelf-life. At the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g. 0, 3 and 6 months), from a six-month study is recommended. Where an expectation (based on development experience) exists that results from accelerated testing are likely to approach significant change criteria, testing should be increased either by adding samples at the final time point or by including a fourth time point in the study design. When testing at the intermediate storage condition is called for as a result of significant change at the accelerated storage condition, a minimum of four time points, including the initial and final time points (e.g. 0, 6, 9 and 12 months), from a 12-month study is recommended. Reduced designs, i.e. matrixing or bracketing, where the testing frequency is reduced or certain factor combinations are not tested at all, can be applied if justified.

In general an FPP should be evaluated under storage conditions with specified tolerances that test its thermal stability and, if applicable, its sensitivity to moisture or potential for solvent loss. The storage conditions and the lengths of studies chosen should be sufficient to cover storage,

Shipment and subsequent use with due regard to the climatic conditions in which the product is intended to be marketed. Photostability testing, which is an integral part of stress testing, should be conducted on at least one primary batch of the FPP if appropriate. More details can be found in other guidelines. The orientation of the product during storage, i.e. upright versus inverted, may need to be included in a protocol where contact of the product with the closure system may be expected to affect the stability of the products contained, or where there has been a change in the container closure system.

Storage condition tolerances are usually defined as the acceptable variations in temperature and relative humidity of storage facilities for stability studies. The equipment used should be capable of controlling the storage conditions within the ranges defined in these guidelines. The storage conditions should be monitored and recorded. Short-term environmental changes due to opening of the doors of the storage facility are accepted as unavoidable. The effect of excursions due to equipment failure should be assessed, addressed and reported if judged to affect stability results. Excursions that exceed the defined tolerances for more than 24 hours should be described in the study report and their effects assessed. The long-term testing should cover a minimum of six or 12 months at the time of submission and should be continued for a period of time sufficient to cover the proposed shelf-life. For an FPP containing an API that is known to be stable and where no significant change is observed in the FPP stability studies at accelerated and long-term conditions for at least 6 months data covering a minimum of six months should be submitted. Additional data accumulated during the assessment period of the registration application should be submitted to the authorities if requested. Data from the accelerated storage condition and from the intermediate conditions, where appropriate, can be used to evaluate the effect of short-term excursions outside the label storage conditions (such as might occur during shipping).

General case

In this case the initial application should include a minimum of six months' data from a 12-month study at the intermediate storage condition. In general "significant change" for an FPP is defined as:

• A change from the initial content of API(s) of 5% or more detected by assay, or failure to meet the acceptance criteria for potency when using biological or immunological procedures.

• Any degradation product exceeding its acceptance criterion.

• Failure to meet the acceptance criteria for appearance, physical attribute and functionality test (e.g. color, phase separation, resuspendability 100% caking, hardness, dose delivery per actuation). However, some changes in physical attributes (e.g. softening of suppositories, melting of creams, and partial loss of adhesion for transdermal products) may be expected under accelerated conditions. [7,8]

Forced degradation studies

The next step in the development of stability studies is the conduct of forced degradation studies to generate degradation products of the drug. The ICH guideline Q1A suggests the following conditions to be employed: (i) 10 °C increments above the accelerated temperatures (e.g. 50 °C, 60 °C, etc.), (ii) humidity where appropriate (e.g. 75% or greater), (iii) hydrolysis across a wide range of pH values, (iv) oxidation and (v) photolysis. However, the guideline provides no details on how hydrolytic, photolytic and oxidative studies have to be actually performed. On the other hand, the information is available in literature but in a staggered way, with suggested approaches differing a lot from one another [9-11].

A comprehensive document providing guidance on the practical conduct and issues related to stress testing under variety of ICH prescribed conditions has been published lately. This report from the authors proposes a classification scheme and offers decision trees to help in the selection of the right type of stress condition in a minimum number of attempts. The hydrolytic degradation of a new drug in acidic and alkaline conditions can be studied by refluxing the drug in 0.1 N HCl/NaOH for 8 h. If reasonable degradation is seen, testing can be stopped at this point. However, in case no degradation is seen under these conditions, the drug should be refluxed in acid/alkali of higher strengths and to initial conditions, acid/alkali strength can be decreased along with decrease in the reaction temperature. In a similar manner, degradation under neutral conditions can be started by refluxing the drug in water for 12 h. Reflux time should be increased if no degradation is seen. If the drug is found to degrade completely, both time and temperature of study can be decreased. To test for oxidation, it is suggested to use hydrogen peroxide in the concentration range of 3–30%. The photolytic studies should be carried out by exposure to light, using either a combination of cool white and ultraviolet fluorescent lamps, or one among the xenon and metal halide lamps. Exposure energy should be minimum of 1.2 million lux h fluorescent light and 200W h/m2 UV and if decomposition is not seen, the intensity

should be increased by five times. In case still no decomposition takes place, the drug can be declared photostable. A minimum of four samples should be generated for every stress condition, viz. the blank solution stored under normal conditions, the blank subjected to stress in the same manner as the drug solution, zero time sample containing the drug which is stored under normal conditions and the drug solution subjected to stress treatment.

The comparison of the results of these provides real assessment of the changes. Furthermore, it is advised to withdraw samples at different time periods for each reaction condition. By doing so, one can get a clear idea on the number of products formed, their relative strengths and whether they are stable or unstable, resulting further in newer products. This information is essential in establishment of Stability indicating method. The studies should be initiated at a concentration of 1 mg/ml. If solubility is a limitation, varying amounts of methanol may be used to get a clear solution or even the testing can be done on a suspension. By using drug concentration of 1 mg/ml, it is usually possible to get even minor decomposition products in the range of detection [12]. If several degradation products are formed in different conditions, the establishment of stability indicating method may involve a lot of development work. For this, repeat injections of reaction solutions might be required. Therefore, the volume of samples subjected to stress studies should be in sufficient quantity and also enough sample volume should be drawn at each period. The withdrawn samples can be stored in cold cabinets to stop further reaction. The aliquots might be diluted or neutralized before injecting into HPLC.

Table 1. General case

Study	Storage condition	Minimum time period covered by data at submission
Long-term [a]	25 °C ± 2 °C/60% RH ± 5% RH or 30 °C ± 2 °C/65% RH ± 5% RH or 30 °C ± 2 °C/75% RH ± 5% RH	12 months or 6 months
Intermediate [b]	30 °C ± 2 °C/65% RH ± 5% RH	6 months
Accelerated	40 °C ± 2 °C/75% RH ± 5% RH	6 months

[a] Whether long-term stability studies are performed at 25 °C ± 2 °C/60% RH ± 5% RH or 30 °C ± 2 °C/65% RH± 5% RH or 30 °C ± 2 °C/75% RH ± 5% RH is determined by the climatic condition under which the API is intended to be stored. Testing at a more severe long-term condition can be an alternative to testing condition, i.e. 25 °C/60% RH or 30 °C/65% RH.

[b] If 30 °C ± 2 °C/65% RH ± 5% RH or 30 °C ± 2 °C/75% RH ± 5% RH is the long-term condition there is no intermediate condition.

Table 2. Active pharmaceutical ingredients intended for storage in a refrigerator

Study	Storage condition	Minimum time period covered by data at submission
Long-term	5 °C ± 3 °C	12 months
Accelerated [a]	25 °C ± 2 °C/60% RH ± 5% RH or 30 °C ± 2 °C/65% RH ± 5% RH or 30 °C ± 2 °C/75% RH ± 5% RH	6 months

[a] Whether accelerated stability studies are performed at 25 ± 2 °C/60% RH ± 5% RH or 30 °C ± 2 °C/65% RH ±5% RH or 30 °C ± 2 °C/75% RH ± 5% RH is based on a risk-based evaluation. Testing at a more severe long term condition can be an alternative to storage testing at 25 °C/60%RH or 30 °C/65%RH.

Table 3. Active pharmaceutical ingredients intended for storage in a freezer

Study	Storage condition	Minimum time period covered by data at submission
Long-term	-20 °C ± 5 °C	12 months

Table 4. General case

Study	Storage condition	Minimum time period covered by data at submission
Long-term [a]	25 °C ± 2 °C/60% RH ± 5% RH or 30 °C ± 2 °C/65% RH ± 5% RH or 30 °C ± 2 °C/75% RH ± 5% RH	12 months or 6 months
Intermediate [b]	30 °C ± 2 °C/65% RH ± 5% RH	6 months
Accelerated	40 °C ± 2 °C/75% RH ± 5% RH	6 months

[a] Whether long-term stability studies are performed at 25 °C ± 2 °C/60% RH ± 5% RH or 30 °C ± 2 °C/65% RH ± 5% RH or 30 °C ± 2 °C/75% RH ± 5% RH is determined by the climatic zone in which the FPP is intended to be marketed . Testing at a more severe long-term condition can be an alternative to storage at 25 °C/60% RH or 30 °C/65% RH.

[b] If 30 °C ± 2 °C/65% RH ± 5% RH or 30 °C ± 2 °C/75% RH ± 5% RH is the long-term condition, there is no intermediate condition.

CONCLUSION

Proper implementation of a SIM relies on three critical aspects; generation of the sample, development and validation of the method. The use of a properly designed and executed forced degradation study will generate a representative sample that will in turn help to ensure that the resulting method adequately reflects long-term stability. HPLC is a widely used analytical technique in SIAM. New technology and hyphenated advanced detector techniques are extremely valuable in providing increased levels of resolution and specificity and faster analysis times resulting in higher sample throughput. Although specificity does play a central role, all of the remaining pertinent validation parameters also must be evaluated in order to properly validate a SIM in a regulated environment and ultimately ensure the method accomplishes its intended purpose.

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