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Review on Strategies and Analytical Perspectives of Stability Indicating Assay Methods (SIAMS)



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ABSTRACT

The present review describes that strategies and analytical perspectives of stability-indicating methods. In past reported works many authors discussed stability-indicating HPLC method development but present review focused on different strategies and conditions of stability-indicating methods. Stability indicating the method is a validated quantitative analytical procedure to detect the changes in some period of the drug substance or drug product. It accurately measures the active ingredients without the degradation products, the process of impurities and excipients. Stability studies applied to new drug substances also affect the shelf life of the product. Stability indicating methods the drug sample was subjected to different stress conditions like acid, base, peroxide, thermal and photolytic conditions, the measure the amount of drug degraded in above stress conditions. The regulatory guidelines of stability indicating are contained in the ICH Q₁AR₂, Q₃BR₂, Q₆A, and FDA 21 CFR.



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INTRODUCTION

Stability indicating methods are quantitative test methods that can detect changes with time in the properties of drug substances and drug products. Information on type and amount of degradation products over time is important for the safety of drugs. Therefore US FDA (United States Food and Drug Administration) and other agencies, stressed such methods are to be well designed and validated. Stability indicating analytical method (SIAM) is 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. It is recommended that forced degradation or chemical stress studies be carried out to determine if analytical methods are stability indicating before embarking on long term stability studies¹. SIAMs are routinely developed by stressing the API (Active pharmaceutical ingredients) under conditions exceeding those normally used for accelerated stability testing. In addition to demonstrating specificity in SIAMs, chemical stress testing also referred to as forced degradation, can also be used to provide information about degradation pathways and products that could form during storage, and help facilitate formulation development, manufacturing, and packaging. In simplest terms, the goal of the SIAM is to separate all of the resulting products (the API and all the degradation products) each from the other (i.e. no co-elution's). Liquid Chromatography is a commonly used technique for SIAMs, primarily with UV detection².

In the preclinical formulation stage, the selection of a stable drug product formula is the primary goal. The temporary preclinical formula is included in the Investigational New Drug application [IND], The goal of the stability program in the clinical trial stage is to ascertain that the drug product batches tested in the clinical trials are stable, and these data will be subsequently included in the NDA. At the NDA approval stage, a validated stability-indicating analytical method is transferred to the quality control group, to ascertain that it works well in the hands of those who have to monitor the stability of the marketed product^{3,4}.

The shelf life of drugs and its importance

New drug applications need to submit scientific data that guarantee the stability of the product over a specified period when maintained under specific storage conditions. For example on the label of prescription or over-the-counter (OTC) pharmaceutical product, the

labeling text "use before or expiry date refers that before the prescribed date, the product should remain fully effective under normal storage conditions. The product's shelf life is determined using standardized storage conditions of controlled temperature and humidity, which can be translated into accepted product lifetimes^{5,6}. The performance of a drug when given as a tablet, capsule, syrup, or injection depends not only on the content of the drug substance but also on its pharmaceutical properties such as dissolution, disintegration, hardness, and so on. All of these aspects are therefore the part of the stability program.

Stability testing of new drug substances and new drug products

The expert Working Group of the International Conference on Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use developed a guideline on stability testing for a registration application within the European Union, Japan, and the United States. The test conditions were selected based on the climatic conditions in three areas so that test data provide evidence on the variation in quality with time under the influence of a variety of representative environmental factors. These data, in turn, allow recommended storage conditions and shelf lives to be established. The primary stability studies for the drug substance show that it will remain within specification during the retest period. Long-term (12-month) and accelerated testing are performed on at least three batches, these batches can be manufactured at a minimum of pilot scale, but should use the same synthetic route and a method of manufacture that simulates the final process to be used at manufacturing scale. Also, supporting stability on laboratory-scale batches may be submitted⁷.

Temperature-sensitive drugs should be stored at the labeled long-term storage temperature, and accelerated testing should be conducted at 15°C above the designated long-term storage temperature with appropriate relative humidity conditions. At the time of regulatory submission, a minimum of 12 months at 25°C ± 2°C/60% RH ± 5% and 6 months at 40°C ± 2°C and /75% RH ± 5% is required. If significant changes are noted at the elevated temperature, additional testing at an intermediate condition, such as 30°C ± 2°C/65% RH ± 5%, should be conducted. The registration application should include a minimum of 6 months of data from a 12-month study in the long term/intermediate condition. Significant change at 40°C and 75% RH is defined as the failure to meet specifications. Long-term stability testing should be continued to cover all retest periods. Normally, testing under long-term conditions is performed every 3 months for the first year, every 6 months for the second year, and then

annually. Containers employed in the long term stability study should be the same or simulate actual packaging used for storage and distribution.

Method development of stability-indicating analytical methods

Studies undertaken to elucidate the intrinsic stability of the drug substance is part of the development strategy and is normally carried out under more severe conditions than those used for accelerated testing. Stress testing is likely to be carried out on a single batch of the drug substance. It should include the effect of temperatures (in 10° C increments (e.g., 50°C, 60°C, etc.) above that for accelerated testing), humidity (e.g., 75% RH or greater) where appropriate, oxidation, and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension. Photostability testing should be an integral part of stress testing. The standard conditions for photostability testing are described in ICH QIB^{8,9}.

Techniques employed for the development of stability-indicating methods

If one critically evaluates the literature reports, titrimetric, spectrophotometric and chromatographic techniques have been commonly employed in the analysis of stability samples.

Titrimetric and spectrophotometric methods:

In these methods, usually, the objective is the analysis of drug alone in the matrix of impurities, degradation products, impurities, etc., and also other drugs in case of the combination products. Their advantage is low cost and simplicity, though sometimes they are not sensitive. Due to the limitation of specificity, there are hardly any reports these days on the use of the assay of stability samples.

Chromatographic methods:

Because of the very nature of the requirement of separation of multiple components during analysis, chromatographic methods have taken precedence over conventional methods of analysis. Other than the separation of multiple components, the advantage of chromatographic methods is that these possess greater accuracy and sensitivity for even small quantities of degradation products performed. Various chromatographic methods that have been used are thin-layer chromatography (TLC), high-performance thin-layer

chromatography (HPTLC), gas chromatography (GC), HPLC (High-Performance Liquid Chromatography) and newer technique like RRLC (Rapid Resolution liquid chromatography). 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 HPLC¹⁰.

Steps involved in stability-indicating method development

A SIAM 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 SIAM 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 interest¹¹. There are three components necessary for implementing a SIAM: sample generation, method development, and method validation.

Step 1: Generation of the Sample

Stressing the API in both solutions and solid-state form generates the sample that contains the products most likely to form under the most realistic storage conditions, which in turn is used to develop the SIAM. In simplest terms, the goal of the SIAM is to obtain baseline resolution of all the resulting products (the API and all the degradation products) with no co-elutions. Samples should be stored in appropriate vessels that allow sampling at timed intervals and that protect and preserve the integrity of the sample. Thermo stated and humidity-controlled ovens should also be employed. Generally, the goal of these studies is to degrade the API by 5-20 %. Any more than this, relevant compounds can be destroyed or irrelevant degradation products are produced (for example, degradation products of the degradation products)¹². Any less and important products might be missed. Experience and data obtained from studies performed previously on related compounds also should be used when developing new protocols.

Table No. 1: Forced degradation conditions¹⁴

Sample Condition	Time / Exposure
Solid / 60 - 100°C	24 to 240 hours
Solid / 60 - 70°C / 75% RH	24 to 240 hours
Solid/simulated sunlight	Overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200-watt hours/square meter
0.1 to 2 N HCl solutions either at RT or at 60 - 80°C	5-20% degradation
0.1 to 1 N NaOH solutions either at RT or at 60 - 80°C	5-20% degradation.
Dilute hydrogen peroxide (0.1 to 6%) at RT or at 60 - 80°C	5-20% degradation.
The solution in Water or at 60 - 80°C	5-20% degradation.

Step 2: Developing the LC Method

After the sample is generated through the use of a properly designed and executed forced degradation, it can be used to develop the LC method. These days, LC method development is often performed on gradient systems capable of the automated column and solvent switching and temperature control. Systems and software that automate the process, some with decision-making built-in, also have been reported. Scouting experiments often are run, and then conditions are chosen for further optimization. Resolving power, specificity, and speed are key chromatographic method attributes to be kept in mind during method development. However, excellent resources are available to anyone not already schooled in the art¹³.

Step 3: Evaluating Specificity during Method Development

Another key parameter to evaluate during method development is specificity. The United States Pharmacopoeia (USP) and various international conference on harmonization (ICH) guidelines define specificity as the ability of a method to assess unequivocally the analyte of interest in the presence of potential interferences. However, starting with the US and as a direct result of the ICH process, it was recommended that a peak purity test based upon photodiode-array (PDA) detection or mass spectrometry (MS) be used to demonstrate that a given peak was pure. Modern PDA technology is a powerful tool for evaluating specificity.

PDA detectors can collect spectra across a range of wavelengths at each data point collected across a peak, and through software manipulations involving multidimensional vector algebra, they compare each of the spectra to determine peak purity. In this manner, PDA detectors today can distinguish minute spectral and chromatographic differences not readily observed by simple overlay comparisons^{14,15}. To be successful, three components are required:

- A UV chromosphere or some absorbance in the wavelength range selected.
- Some degree of chromatographic resolution.
- Some degree of spectral difference.

Peak purity determination:

Comparing peak spectra is probably the most popular method to discover an impurity. If a peak is pure all UV-visible spectra acquired during the peak's elution or migration should be identical, allowing for amplitude differences due to concentration. The results obtained by comparison of these spectra against each other should be very close to a perfect 100% match. Significant deviations can be considered as an indication of impurity.

A valuable tool in peak purity analysis is the overlay of separation signals at different wavelengths to discover dissimilarities of peak profiles. The availability of spectral data in a three-dimensional matrix generated by the diode-array detector enables signals at any desired wavelength to be selected and reconstructed for peak purity determination after the analysis. A set of signals can be interpreted by the observe best when, before being displayed, it is normalized to maximum absorbance or equal areas^{16,17}.

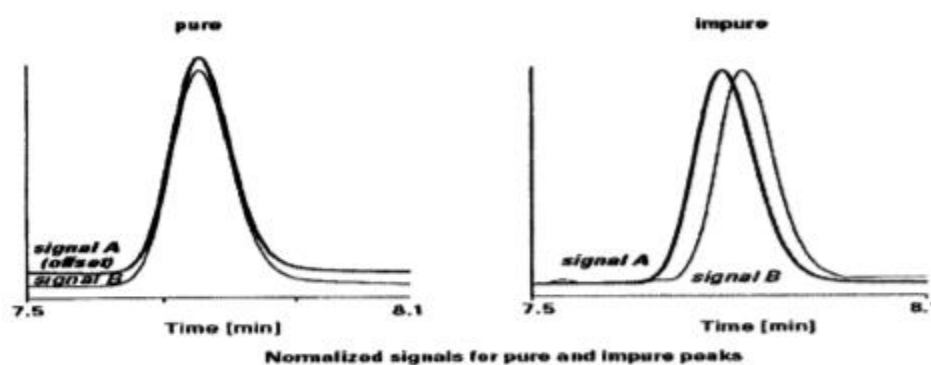


Figure No. 1: Chromatographic signals of pure and impure peak

Validation of Stability indicating assay methods

Different methods have different requirements when it comes to validation. The USP recognizes four method categories and defines the analytical performance characteristics that must be measured to validate each method type:

Category 1: Analytical methods for the quantification of major components of bulk drug substances or active ingredients.

Category 2: Analytical methods for the determination of impurities in bulk drug substances or degradation compounds.

Category 3: Analytical methods for the determination of performance characteristics.

Category 4: Identification tests.

Table No. 2: Parameters required for the validation¹⁸

Analytical performance characteristics	Category I	Category II		Category III	Category IV
		Quantitative	Limit tests		
Accuracy	Yes	Yes	*	*	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	*	Yes
Detection limit	No	No	Yes	*	No
Quantification limit	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	No	*	No
Robustness	Yes	Yes	No	Yes	No

* May be required, depending on the nature of the specific test

According to ICH guidelines on validation of analytical methods, the objective of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Stress testing methods are screening methods used to help in understanding the degradation chemistry of the drug and, therefore need not be (nor, in general, can they) validated to the extent of final control methods. The concept in the ICH guideline on validation of analytical methods is a good starting point for validation of stress testing methods, however, the overall validation should be significantly abbreviated when compared to the validation of final control methods, as stress testing methods are investigational methods¹⁸. Accuracy normally

should not be a problem with stress testing methods as long as the response of the detector is linear and samples are completely dissolved before analysis. The specificity of the methods cannot be fully validated because one does not know all of the possible degradation products during initial stress testing. Specificity can be addressed by using any known impurities and degradation products produced in the method of development samples¹⁹. Precision (repeatability) of the assay of the main component can be evaluated by preparing a limited number of assay samples and using simple statistics to estimate the standard deviation. Estimation of intermediate precision and reproducibility should normally not be necessary for stress testing methods. Detection and quantitation limits for degradation products can be determined by using parent compounds and assuming that the response of all degradation products will be similar. One of the most important aspects of stress testing is the analysis of samples using a suitable analytical method, which, in many cases, is reverse-phase LC. This necessitates the development of an LC method capable of measuring both the loss of parent compound as well as the levels of degradation products or impurities formed in stress conditions²⁰.

Mass of balance during stability-indicating method development

Mass balance correlates the measured loss of a parent drug to the measured increase in the number of degradation products. It is a good quality control check on analytical methods to show that all degradation products are adequately detected and do not interfere with the quantitation of the parent drug (i.e., stability-indicating methods). Regulatory agencies use the mass balance to assess the appropriateness of the analytical method as a stability-indicating method and determine whether all degradants have been accounted²¹.

The assessment of degradation in pharmaceutical products involves two aspects of analytical measurement. Firstly, a specific or selective analytical method must be available for accurate assay of the parent drug compound, to measure any loss. Second, the methodology should be in place for the quantification of the degradation products formed. Ideally, when degradation occurs, the measured amount of parent drug lost should correlate well with the measured increase in degradation products²². This correlation is referred to as “mass balance”. More recently, the International Conference on Harmonization (ICH) has provided definition of “mass balance; material balance” as “The process of adding together the assay value and levels of degradation products to see how closely these add up to 100% of initial value, with due consideration of the margin of analytical precision”. The concept is a useful scientific

guide for evaluating data, but it is not achievable in all circumstances. The focus may instead be on assuring the specificity of the assay, the completeness of the investigation of the route of degradation, and the use, if necessary, of identified degradants as indicators of the extent of degradation via the particular mechanism. The analyst must balance time and resource demands to provide the information necessary to understand degradation without going to extreme measures of quantifying components of little interest^{23,24}.

Mass balance is also useful in method validation. To demonstrate that analytical methods are stability-indicating, unstressed and stressed materials are often compared. Any increase in degradation of a product that correlates well with the loss of parent drug, aids in demonstrating that the methods can accurately assess degradation²⁵.

Applications of SIAMs

Stability studies are used to establish the re-test period for the active ingredient i.e., the length of time it can be stored and used without analyzing immediately before use and the shelf life of the finished product. The release and shelf-life specifications for the product may differ to accommodate the degradation of the active ingredient or other acceptable changes, which may occur in storage. The International Conference on Harmonization (ICH) drug stability test guideline Q1A (R2) requires that analysis of stability samples should be done through the use of validated stability-indicating analytical methods (SIAMs). Additional guidance is given only for photo stability testing^{26,27}. It also recommends, carrying out the stress testing on drug substances to establish its inherent stability characteristics and to support the suitability of the proposed analytical procedure. The validated SIAMs will be used extensively for testing the stability samples of both drug substances as well as drug product^{28,29,30}.

SUMMARY

The stability-indicating method is an analytical procedure that is capable of discriminating between the major active (intact) pharmaceutical ingredients (API) from any degradation (decomposition) product(s) formed under defined storage conditions during the stability evaluation period. Forced degradation studies are indispensable in the development of stability-indicating and degradant monitoring methods as part of a validation protocol. Forced degradation studies also provide invaluable insight in investigating degradation products the use of properly designed and executed forced degradation study will generate a representative

sample that will in turn help to develop a stability-indicating method. Chromatographic factors should be evaluated to optimize the stability-indicating HPLC method for the detection of all potentially relevant degradants. An appropriate sample solvent and mobile phase must be found that afford suitable stability and compatibility with the component of interest, as well as the impurities and degradants.

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