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A REVIEW ON STABILITY INDICATING ASSAY METHOD

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ABSTRACT

Stability-indicating assay method (SIAM) is an analytical procedure that is capable of distinguishing between the active pharmaceutical ingredient (API) from any degradation products formed under defined storage/ stressed conditions during the stability evaluation period. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions like pH, light, oxidation, dry heat, etc. and separation of drug from degradation products. The method is expected to allow analysis of individual degradation products. International Conference on Harmonization (ICH) guidelines are required for establishment of SIAM. Stress testing is the main tool that is used to predict stability problems, develop analytical methods, and identify degradation products. The products generated from stress testing may be useful in developing and validating a suitable stability-indicating analytical method for the analysis of the drug substance and the drug product, expediting the availability of the completed analytical method.

Keywords:

Stability indicating assay method, ICH guidelines, forced degradation, stress conditions, active pharmaceutical ingredients etc

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INTRODUCTION

According to the regulatory definition, a stability-indicating method is one of a number of quantitative analytical methods that are based on the characteristic structural, chemical, or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured [1]. Stress testing of the drug substance can help identify the likely degradation products, which can in turn 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 drug substance and the type of drug product involved [2]. Stress testing is the main tool that is used to predict stability problems, develop analytical methods, and identify degradation products/pathways. Since there are no detailed regulatory guidelines that direct how stress testing is to be done, nor has there ever been a text/reference book on the subject, stress testing has evolved into an artful science that is highly dependent on the experience of the company or the individuals directing the studies. Stress testing is conducted to provide data on forced decomposition products and decomposition mechanisms for the drug substance. The severe conditions that may be encountered during distribution can be covered by stress testing of definitive batches of drug substance. From the ICH definition, it is clear that there is now a [regulatory] differentiation between “accelerated testing” and “stress testing.” Stress testing is distinguished by both the severity of the conditions and the focus or intent of the results. Stress testing, which is also often referred to as “forced degradation,” is an investigation of the “intrinsic stability” characteristics of the molecule, providing the foundation for developing and validating analytical methods and for developing stable formulations. [3]. The purpose of stability studies is to monitor possible changes to a product or material over time and at different storage conditions, it is expected that all analytical methods applied in the study should be stability indicating and that only those methods that are truly stability-indicating should be used [4]. Chemical stability of the molecules affects the quality of the drug substances or products. The quality of the substances may be changes by some environmental or storage conditions like light, heat, oxidation and hydrolysis. These environmental conditions produce the various types of the degradation products from pharmaceutical substances which shows different properties than original one. Therefore, the SIAM is used to determine these degradation products occurring from the various

pharmaceutical products by using various analytical instruments. The analytical instruments are help to isolate, identify and quantify the degradation products and showing the stability indicating power of the analytical methods. Forced degradation is the process of degradation where stress conditions are applied to obtain the degradation products more severe than long term and accelerated stability testing. Within few weeks the stability of the drug products are identified in specific time interval [5]. The SIAM is an analytical method which used to resolve the drug substances from its degradation products. For this analysis the high performance liquid chromatography (HPLC) method mostly used. The various hyphenated techniques are used to identify and quantify the degradation products from pharmaceutical drug substances or products. The main purpose of this analytical method is to separate or resolve, identify and quantify the major API from its degradation products. Forced degradation studies typically involve the exposure of representative samples of drug substance or drug product to the relevant stress conditions of light, heat, humidity, acid/base hydrolysis, and oxidation. These experiments play an important role in the drug development process. The results of forced degradation studies can facilitate SIM development, drug formulation design, selection of storage conditions and packaging, better understanding of potential liabilities of drug molecule chemistry, and solving of stability-related problems [6].

OBJECTIVES OF STRESS STUDIES

1. To establish degradation pathways of drug substances and drug products.
2. To differentiate degradation products occurred from drug substances or products.
3. Structural elucidation of degradation products
4. To determine the quantity of degradation of produced
5. To develop and validate method according to ICH guidelines.
6. To study the various degradation mechanisms like hydrolysis, oxidation, thermal and photo degradation.
7. To determine intrinsic stability of the molecules [5].

FORCED DEGRADATION STUDIES

- **Experimental Approach To Forced Degradation Studies**

1. Timeline for Conducting Studies

For SIAM no any regulatory guidelines are available in ICH and USP. Starting from forced degradation experiments at early stage is highly encouraged. There are good reasons for initiating forced degradation studies on drug substances at Phase I. There are good reasons

for initiating forced degradation studies early, it requires material and time that may not be available in early development, and it is perfectly acceptable from a regulatory point of view to delay these experiments until after the initial clinical assessment. Forced degradation studies on drug substance and drug product should be completed prior to registrational stability studies and it would be useful to have identified major degradants by that time. Finally, when the synthetic process and formulation are locked, just prior to the start of registrational studies of drug substance and drug product, the forced degradation work is repeated as part of registrational analytical method validation. In summary, the decision to start forced degradation early or late in development is one that should be driven by quality risk assessment and depends, among other factors, on the chemistry of the molecule, the formulation approach, material availability and portfolio prioritization[6].

2. Study Protocol

A general protocol for conducting forced degradation studies according to the type of test material (drug substance, solid or liquid drug product) and type of degradation (hydrolysis, oxidation, etc.). It is essentially based upon the protocol described in available guidance and best practices for conducting forced degradation studies [6].

3. Specific Limits for Degradants

The desired target extent of degradation is approximately 5–20%. This is achieved by varying the stress conditions, for example exposure time, temperature, or concentration of stressing agents like acid, base, oxidizer etc. Generally two conditions may happen first is overstressing and second is under stressing. Overstressing may destroy the compound or may lead to further degradation of the relevant primary degradants. Under stressing may fail to generate important degradation products. The degradation studies should be terminated after the maximum recommended time/stress conditions, if sufficient degradation has not been achieved. It is unnecessary and even unwise to try to degrade the drug at all cost as it would only increase the complexity of the method development with little or no benefit in the quality of the data generated by the method. The concentration of drug in the stressed sample solution may affect the target level of degradation. A more dilute sample concentration of drug may not produce more extensive degradation products than more concentrated solution. Therefore it shows that lowering the drug concentration may help to increase degradation [6].

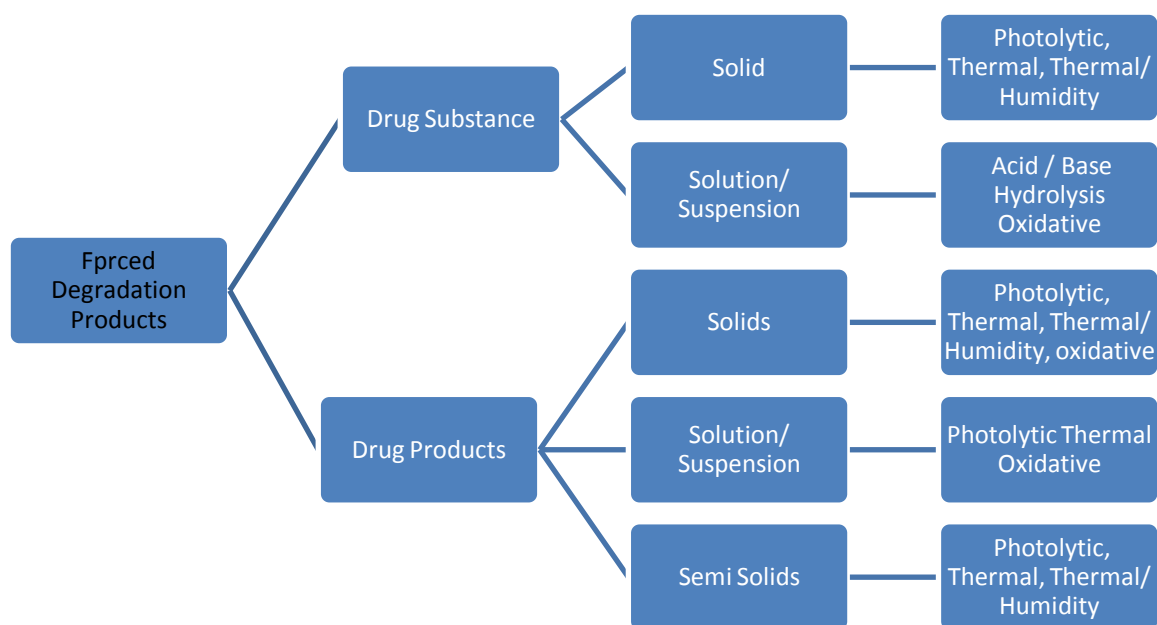
4. Conditions for Degradation [6]

Table 1: Stress conditions for drug substance

Type of stressing	Conditions	Time
Acid hydrolysis	1 mg/ml in 0.1 N (up to 1 N) HCl; RT or higher	1–7 days
Base hydrolysis	1 mg/ml in 0.1 N (up to 1 N) NaOH; RT or higher	1–7 days
Oxidation	0.3% (up to 3%) H ₂ O ₂ ; RT; protected from light	Few hours to 7 days
Thermal hydrolysis (control)	Aqueous Solution; 70°C	1–7 days
Thermal	70°C	Up to 2 weeks
Thermal/ Humidity	70°C/75% RH	Up to 2 weeks

Table 2: Stress conditions for drug substance

Type of stressing	Conditions	Time
Thermal	70°C	Up to 3 weeks
Thermal/humidity	70°C/75%RH	Up to 3 weeks
Photo-degradation	Fluorescent and UV light (Option 1 or Option 2)	>2× ICH

**Fig. 1:- flow chart describing various stress conditions for degradation of drug substances and drug products [5].**

- **Special Considerations in Conducting Stress Testing [6]**

- a. Polymorphism**

The physical form of the API can affect both its physical and chemical stability. The potential for chemical stability shows difference in forced degradation studies when a new polymorphic form is occurs during development. Surprisingly, this is not directly

addressed in regulations but only touched upon in the FDA guidance for industry on ANDA. On a practical point of view, solvates and hydrates present a particular challenge in terms of conducting forced degradation and should be stressed in closed and open containers as different rates of hydrolysis may be observed

b. Low Solubility Drugs

Usually, organic co- solvents are used to enhance the solubility of poorly water soluble drugs. In stress testing in force degradation studies following organic co-solvents are used depending on their stress types:

Acid hydrolysis- DMSO, acetic acid, and propionic acid are useful for acidic conditions

Alkaline hydrolysis- Glyme and 1,4-dioxane facilitate reactions in base

Neutral condition- DMSO, N-methylpyrrolidone (NMP), and acetonitrile (ACN) work under neutral conditions Photolysis- ACN is the co-solvent of choice for photochemical reaction Avoid methanol for $-\text{CO}_2\text{H}$, amide, $-\text{OH}$, ArNH_2

c. Combination Drugs

Drug products that contain more than one active ingredient should be submitted to stress testing and formation of degradation products produced by drug–drug and drug–excipient interactions. Degradants of each of the active ingredients are typically well characterized by the time. The development of a combination product starts and forced degradation of each API may not need to be repeated. In one example (atorvastatin and amlodipine combination tablet), the tablet itself, in addition to each drug separately, was submitted to forced degradation, hence evaluating additional degradation that may be caused by reactions between the two actives and/or their synthetic impurities. In combination drugs stress study; they can be not only resolve from each other but also their degradation products. Each degradation product must be identify and characterizes from the API.

d. Characterization of Degradants

Primary degradation pathways need to be established for the full characterization of new drug substances. In practice, primary degradants obtained in stress conditions are often identified. A decision to isolate and characterize a degradation product based on results obtained from formal stability studies of the drug substance and drug product. Only peaks that occur at or above the ICH identification thresholds from formal stability studies of the drug substance and drug product need to be identified.

STABILITY INDICATING HPLC METHOD DEVELOPMENT

As discussed in the introduction, the accepted definition of a SIM for a traditional pharmaceutical is a chromatographic method which is able to separate the reportable degradants generated upon long term storage of the product. Traditionally, the stability-indicating quality of the method is demonstrated by using stressed samples or long-term stability samples. If a single method is to be used for quality control and stability of an API, the method should also be able to separate process-related impurities. Stress testing is not the only avenue available for evaluating the validity of the method for stability determination. When available, naturally aged samples or other degraded samples may be more representative of the product's degradation. In their analysis, many of the published methods that claimed to be stability-indicating fell short of meeting the current regulations by conducting no stress testing or stress testing at only a few of the recommended conditions. Methods published in recent years however, seem to mostly follow the ICH guidelines with a general protocol of stress testing for acid and base hydrolysis, oxidation and light and heat stress.

1) Method Scope

The developments of SIMs are building on knowledge throughout drug development. SIMs may be required at different phases of development and the purpose of the method at these different stages is an important consideration. It is not only difficult but also beneficial to conduct an extensive SIM development in the early phase of drug development. As the project develops, the synthesis and degradation pathways become better understood, further method development should be performed. Finally, for formal stability studies a more rugged development should be embarked upon for filing purposes. At early stages, methods need to have a broad gradient because impurities/degradants of the compound may not be known and may alter with changes in synthetic route form or dosage formulation. IND stability studies play an important role in development not only data gathering but also supporting clinical evaluation. Method improvement is expected as the chemistry evolves and formulation is initiated. At later stages in development the formal long-term stability studies are designed to be confirmatory in nature with the researcher already having a good understanding of the impurities/degradants expected to form at significant amounts. Also, practical experience with the method, along with further method development enables a more subjective insight into the known impurity/degradant profile.

2) Preliminary Requirements

a. Samples Required for Method Development

Representative samples of the synthetic process with enriched impurities (e.g., mother liquors or reaction mixtures) and individual intermediates, if available, are required to start the development of a selective method. If these impurities can be obtained individually through isolation or suitably characterized from the solution mixture they can serve as markers for positive identification. Other samples from crude batches that have not yet undergone final crystallization, or any other batch containing a large number of process-related impurities, are also useful in testing out the method.

b. Physico-chemical Properties of the Drugs

Information about the compound and the formulations is essential in helping to frame the development of the method, primarily to determine whether HPLC/UV is appropriate to select the diluents and the chromatographic mode. There may be significant background information already available from previous discovery/development scientist reports or experiences. In some cases degradation studies have been already undertaken, albeit with different objectives (such as pre-nomination compound screenings or exploratory development work) but they may be useful in selecting the conditions of the stress studies or possibly in proposing degradation mechanisms.

c. Functional Group Effects

Some level of structural understanding of the compound, especially functional groups present that may undergo chemical transformation. Other valuable information includes the pKa, pH solubility curve, solubility in common solvents, and log P. They give a valuable insight in solubility and likely structural arrangements in solution. They also guide the selection of chromatographic conditions, including pH of the mobile phase and choice of organic modifiers. Having the pH of a buffered mobile phase >1.5 units away from its pKa (to avoid mixed ionization state) is generally accepted, even though the better column performance/selectivity may be at a pH closer to the pKa. Also due consideration of the pKa of likely

d. Related Structures

Even for new drug entities, a lot of understanding in this area can be gained from browsing the literature looking at similar compounds which may or may not have been used in drug development. Small changes in chemical structures, whether backbones or functional groups,

can have a profound effect on the reactivity so any information on related compounds should always be used as a guide only and not as extrapolation.

3) Method Development Approach

a. Stability-Indicating Chromatography Conditions

Principles of chromatography method development, including wavelength, diluent, column and mobile phase selection, have been discussed in a number of chromatography books. An effective solvent and column screen, with relevant samples, cannot be overstated as a valuable foundation for method development. In selecting initial chromatographic conditions for a SIM of a new entity, most important is to make sure that degradants are in solution, separated, and detected. To this effect, a diluent of 1:1 water:organic solvent is a good starting point as it will increase the likelihood of solubility of most related materials and ensure proper disintegration of solid dosage forms. When choosing conditions for method evaluation, broad gradients are appropriate as they maximize the separation of early eluting peaks and increase the opportunity of detecting late eluting peaks. Mass Spectrometry/Evaporative Light Scattering Detector/Charged Aerosol Detector (MS/ELSD/CAD) compatible conditions are beneficial, as they assist in developmental understanding especially in early development. Most pharmaceuticals have a usable chromophore, allowing for UV detection. UV spectra may be different between the API and the impurities/degradants. Consideration of likely impurities and degradants as to whether they have a chromophore is important for both mass balance reasons as well as experimental setup (choice of detector(s)). At the column scouting phase, the use of a photo-diode array (PDA) detector will increase the likelihood of detecting degradants with different UV spectrum to that of the API. Alternatively, a wavelength in the lower UV range: 210–254 nm may be appropriate. The method sensitivity to impurities compared to the main peak is important to understand when choosing the wavelength. A signal to noise of 10–1 for limit of quantitation (LOQ) and 3–1 for limit of detection (LOD) are expected with a typical LOQ being 0.05%, although this may vary depending on the known relative response factor (RRF) of the impurities. This can usually be achieved by appropriate adjustment of wavelength, detector settings, sample concentration, and injection volume. Final selection of a specific UV wavelength is crucial for detection of all relevant degradants. If the λ_{\max} of the parent compound is relatively high (e.g., above 280 nm), it should not automatically be selected as the UV detection wavelength, since impurities/degradants may have a significantly different λ_{\max} . Alternatively, a dual

wavelength detector can be used at both a high λ_{max} and a lower wavelength. At a later stage in development when most or all of the degradants' and impurities' UV spectra are known, any specific wavelength may be justified.

b. Peak Purity

Peak purity analysis of the main peak is used to assess for the presence of impurities under the main peak which is an essential part of the validation of a SIM. Determination of peak purity is more difficult than it seems as one can never be certain that a peak is truly pure. Determination of peak purity by the two evaluation techniques such as direct and indirect evaluation.

1. **Direct Evaluation:** it can be performed in-line by employing PDA detection LC-MS or LC-NMR. However, PDA only works well for degradants that have a different UV spectrum from that of the drug. LC-MS evaluation will not work if the degradant has the same molecular weight, as is the case for diastereomers or if the ionization of the degradant is suppressed by the co-eluting API.
2. **Indirect Evaluation:** It can be performed by changing one or more chromatographic parameters (column, mobile phase, gradient composition, etc.) that will significantly impact the separation selectivity. The resulting impurity profile is then compared against that of the original method. If the number of degradant peaks is the same in both separations and if the percent area of the main component is the same in both separations then there can be reasonable confidence that all the degradants have been resolved from the main component. Automated versions of this approach have been successfully utilized in a multi-dimensional screening with instrumentation capable of systematically evaluating several different columns and eluents for impurity analysis.

Other approaches use alternate separation techniques such as thin-layer chromatography (TLC), normal-phase-HPLC, capillary electrophoresis (CE), or supercritical fluid chromatography (SFC).

4) Method Optimization

Once a method is considered appropriate, the chromatographic conditions and runtime efficiency may be further improved upon by using predictive software. The resolution is affected by temperature and gradient composition but unaffected by small changes in chromatographic conditions. This approach has the advantage of predicting the robustness zone for the chromatographic parameters and is consistent with the Quality by Design (QbD)

approach to pharmaceutical development. At that stage, if all degradants that need to be monitored have similar polarities, it may be advantageous to evaluate whether an isocratic method would be suitable, instead of a longer gradient method [6].

SELECTION OF CHROMATOGRAPHIC CONDITION

There are a number of HPLC methods available to the development chemist, perhaps the most commonly applied method is reversed-phase. Reversed-phase and reversed-phase coupled with ion-pairing probably account for more than 85% of the applications for a typical pharmaceutical compound. The typical pharmaceutical compound is considered to be an API of less than 1,000 daltons, either soluble in water or in an organic solvent. The water-soluble API is further differentiated as ionic or nonionic which can be separated by reversed-phase. Similarly, the organic soluble API can be classed as polar and non polar and equally separated by reversed-phase. In some cases, the non-polar API may have to be separated using adsorption or normal phase HPLC, in which case the mobile phase would be a non polar organic solvent. For those “special” compounds that do not fall into this category API>1000 daltons, isomers or enantiomers, other chromatographic modes may be necessary for separation. include ion-exchange and chiral chromatography. In this discussion of developing a stability-indicating HPLC method, only reversed-phase will be discussed [1].

CONDITIONS FOR DEGRADATION

Oxidation

Oxidation may be performed using several conditions. Hydrogen peroxide is the most commonly used oxidant. The peroxide concentration may be adjusted as necessary to obtain 5–20% degradation. One disadvantage of using H₂O₂ is that it is non-selective and relatively unpredictable in its results. Stress with hydrogen peroxide often leads to secondary degradation of the primary degradants making results interpretation more difficult. Radical initiators such as AIBN (2, 2-azobis isobutyronitrile), ACVA (azobis-cyan valeric acid), and AMPD (azobis methyl propionamidinedihydrochloride) are alternatives for oxidation studies but are less commonly used. They are generally more selective than peroxides and can be used to confirm or invalidate the peroxide results. An appropriate temperature for the reaction is 40⁰C. The test may be stopped after 5–20% degradation or after 7 days if no degradation is observed [6].

Hydrolysis

Generally hydrolysis degradation is performed using acid(HCl) and alkali (NaOH) solution. If the compound is poorly water-soluble then organic co-solvents may be used in combination with acid or base. Organic solvents that have been commonly used for stress-testing studies are discussed previously. Stress is typically first initiated at room temperature; if no degradation occurs, an elevated temperature is applied above the accelerated condition. A thermal control (i.e., drug in neutral solution at the same stress temperature) should also be run to identify any degradation due to temperature alone. Maximum stress time should not exceed 7 days. The degraded test samples are often neutralized using acid/base/buffer to avoid further decomposition. However, if the degradation is a pH-based equilibrium, this may remove the desired degradants. As always, when conducting stress testing, the analyst should be wary of possible side reactions that may affect the drug, for example, methanol should be avoided for compounds containing $-\text{COOH}$, $-\text{COOR}$, amide groups [6].

Thermal

The goal of thermal studies is to force the degradation of the drug substances to determine the primary degradation products. Based on the Arrhenius equation a 10°C increase in temperature result in a doubling in a reaction rate and decrease in reaction time by a factor of 2. Using this rule of thumb, 1 year at 30°C is equivalent to 3 weeks at 70°C . Increasing the energy of system may produce the products not seen under ICH stability guidelines because of there is more energy of activation barrier to produce that cannot be formed under common ICH stability. Thermolytic degradation is usually degradation caused by exposure to temperatures high enough to induce bond breakage that is pyrolysis. The storage condition of the stress condition is more severe than accelerated stability condition [8].

Table.Relationship between temperature and storage period in forced degradation studies

Temperature	Period of storage
30°C	1 year
40°C	6 months
50°C	12 weeks
60°C	6 weeks
70°C	3 weeks
80°C	11 days

Photolytic degradation

The purpose of forced degradation testing studies is to evaluate the overall photosensitivity of the material for method development purposes and/or degradation pathway elucidation. This

testing may involve the drug substance alone and/or in simple solutions/suspensions to validate the analytical procedures. In these studies, the samples should be in chemically inert and transparent containers. In these forced degradation studies, a variety of exposure conditions may be used, depending on the photosensitivity of the drug substance involved and the intensity of the light sources used. For development and validation purposes it is appropriate to limit exposure and end the studies if extensive decomposition occurs. For photo stable materials, studies may be terminated after an appropriate exposure level has been used. The design of these experiments is left to the applicant's discretion although the exposure levels used should be justified [7]. Photolytic degradation is the degradation that results from exposure to ultraviolet or visible light in the wavelength range of approximately 300–800 nm. Exposure to radiation at wavelengths <300nm is not needed because a pharmaceutical compound would not experience such exposure during its life cycle [8].

CONCLUSION

Forced degradation studies provide the techniques about how the stress testing can be applied to the pharmaceuticals. Stress testing provides the information about the stress mechanisms such as hydrolysis (acid/ base), oxidation, thermal degradation, photo degradation. According to ICH guidelines the stability can be started from long term and accelerated stability testing but for stability indicating method there is no special guidelines provided by the ICH. The forced degradation provides the potential degradation pathway for determining the degradants. With the help of this method we can isolate the impurities as well as degradants, elucidation of the possible structures of the degradants and detection of the quantity of it. This information is help to improve the stability related problems. Due to this method the stability can be determined within the week.

REFERENCES

1. Carstensen J. T., Rhodes C. T., DRUG STABILITY, Principles and practices in pharmaceuticals, third edition, vol. 107, 2000, p. 329.
2. ICH Harmonized Tripartite Guideline (2003) Stability Testing of New Drug Substances, Q1A (R2).
3. ICH Harmonized Tripartite Guidelines, Validation of Analytical Procedures: Methodology (Q2B)
4. ICH Harmonized Tripartite Guidelines., Text on validation of analytical procedures. Q2A.
5. Blessy M., Ruchi D. Patel, Prajesh N. Prajapati, Agrawal Y. K., Development of forced degradation and stability indicating studies of drugs—A review, Journal of Pharmaceutical Analysis, 2014, 4(3):159–165.
6. Kim Huynh-Ba, Handbook of Stability Testing in Pharmaceutical Development, U.S.A., published by Springer, July 2008, 139-62.
7. ICH Guidance for Industry, Q1B: Photo stability Testing of New Drug Substances and Product, International Conference on Harmonization.
8. Baertschi S. W., Reynolds D. W., Pharmaceutical Stress Testing, U.S.A., Taylor & Francis Group, 2005.