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# A Brief Study on Forced Degradation Studies with Regulatory Guidance

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**Abstract:- Forced degradation studies is a stability indicating analytical method to identify the pathway of degradation of both drug molecule and product by applying various stress testing conditions than accelerated conditions. As stress degradation study is made compulsory by various countries food and drug agencies for regulatory and market approval of both "Branded drugs and Generic drugs" and products respectively. After introduction of ICH guidelines in 1993, worldwide attention gained by stress degradation evaluation report. Hence we provide a brief overview on the force degradation procedure, method development for characterization and isolation of degraded products and validation of development method.**

***Keywords:-*** *Regulatory Guidelines, Forced Degradation, Method Development, Method Validation, Limits of Impurities.*

## I. INTRODUCTION

Studies on forced degradation are sometimes referred to as forced decomposition studies, stress studies, stress decomposition studies, etc. A Physico-chemical stability property of the drug molecule plays a major role in the safety and efficacy of the product shelf life and it proportional to patient care. The evaluation of Physico-chemical stability property of the drug molecule is needful for the approval of the ICH and FDA types regulatory agencies for development and marketing of the drug product in various countries. In regulatory approval process includes selection of proper storage and package condition and selection of suitable formulation type based on the stability analysis report of the molecules. In stress degradation studies the conditions includes light, heat, humidity and oxidation etc. By studying various degradation pathway and conditions of drug molecules helps to improve the stability of a compound and product. And also it reports the various influencing environmental factors of themolecule.

## II. SOME REGULATORY GUIDELINES

### *A. International Conference on Harmonization*

- Forced degradation studies have long been employed by the industry, but in 1993 the International Conference on Harmonization (ICH) was published (Q1A)guidelines for "Stability Testing of New Drug Molecule and New Drug Products", making them a formal regulatory obligation.
- *The word "Stress Testing," which was used in the Guideline, a Definition of Drug Compounds as Follows:*

*"The stress testing studies are conducting on drug molecule help to discover potential degraded products, which may then helps to report the molecule's intrinsic stability and the pathways by which it degrades, as well as the accuracy with which the analytical techniques employed to stability measurement. The particular drug molecule and the type ofdrug dosage under test will determine the nature of the forced degradation studies."*

- *For Drug Product Concentration Testing, there is no Precise Definition:*

*"For drug product stability testing are no clearly guidelines except for photo-stability testing. The design of formal stability studies for a drug product should take into consideration for the behavior and qualities of the drug molecule, stability studies that have been done on the drug substance, as well as knowledge gained through clinical formulation studies. The choice of characteristics to be assessed in official stability studies and probablechanges in storage should both be taken into consideration."*

- As the stability stress testing gaining global attention. Hence, most of the regulatory agencies made compulsory to submit reports of forced degradation studies even for the generic drug approval. Stress testing is now included in regulatory guidelines issued by regions and countries around the world, and it also occupies a prominent place in pharmacopeia in some countries.

### *B. International Conference on Harmonization*

- *The ICH Q1A(R2) Guidance on Standard Stability Testing Describes the Following Objectives of Resistance Testing for "New Drug Substances" in Section 2.1.2*

- "Drug concentration testing can help identify potential degradation products, which in turn can help establish degradation pathways and the **intrinsic stability** of the molecule, and confirm its ability to determine the stability of the analytical procedures used.
- " The nature of resistance testing will depend on the individual drug substance and type ofmedicinal product involved.

- It goes on to make the following recommendations:

✓ "Concentration testing will likely be performed on a single batch of drug substance.
✓ It shall include the effect of temperature (in 10°C increments (e.g.50°C, 60°C, etc.) above the temperature of accelerated tests), humidity (e.g.75% RH or higher), if any, from oxidation.- Photolysis and photolysis of pharmaceutical substances.
✓ Tests should also assess the sensitivity of the drug substance to hydrolysis over a wide range of pH values when in solution or suspension.
✓ Photochemical testing should be an integral part of strength testing.
✓ However, it may not be necessary to specifically test for some degradation products if they have been shown to not form under rapid or long-term storage conditions. The results of these studies will be an integral part of the information provided to regulatory agencies."

➢ *The Relevant ICH Q1B Guideline on "Photo-Stability Testing" Defines the RequirementsAs Follows:*

- "Forced degradation experimental studies are studies performed to intentionally degrade the sample.
- These studies, which may be performed during the normal development phase on drug substances, are used to evaluate the overall photosensitivity of a material for the purposes of method development and/or brightening show decomposition pathways.

➢ *The ICH Q2(R1) Guideline is titled "Analytical Procedure Validation:*

- *Content and Methods" Section 1.2.2.Specifically Mentioned the Following:*
- "If there is no standard for impurities or degradation products, specificity can be demonstrated by comparing test results of samples containing impurities or degradation products with a second, well-characterized procedure. For example: pharmacopoeial method or other validated analytical procedure (independent procedure).Where possible, this should include samples stored under relevant pressure conditions: light, temperature, humidity, hydrolysis and acid/base oxidation.

➢ *The ICH Q3A(R2) Guidelines Described "Impurities in New Drug Substances", in Section 3.Rationale for Reporting and Controlling Impurities, Subsection 3.1 Organicimpurities, where is Described as Follows:*

- "In addition, the applicant must summarize the laboratory studies performed to detect impurities in the new drug substance.
- This summary should include test results from batches produced during development and batches from the proposed commercial process, as well as results of stress tests (see Guidance on stability of ICH Q1A) is used to determine the possibility of impurities occurring during storage.

➢ *The ICH Q3B(R2) Guideline "Impurities in New drug Products" in section 3, Analytical Procedures, also addresses stress conditions, stating:"In particular, analytical procedures need to be validated to demonstrate the specificity of identified and undetermined degradation products.*

- Where applicable, this validation should include samples stored under relevant pressure conditions: light, temperature, humidity, hydrolysis and acid/base oxidation
.

➢ *Additionally, the ICH Q5C Guidance on "Stability Testing of Biotechnology/BiologicalProducts" States:*

- "Studies under stressful conditions may be useful in determining whether inadvertent exposure to conditions other than those recommended (e.g., during transportation) is harmful to product as well as in evaluating which specific test parameters may be the best indicators of product stability.
- Drug substance or drug product under harsh conditions can help detect degradation patterns; If so, these changes should be monitored under storage conditions.
- Although the Tripartite Stability Guidelines describe conditions for accelerated and stressed research, applicants should note that these conditions may not be appropriate for biotechnology/biological products. The study, conditions need to be carefully chosen accordingly the product.

➢ *The ICH Q5E guidance titled "Comparability of biotechnology/biological products subject to variations in their manufacturing processes", where the requirement is when Considering the comparability of products, the manufacturer must evaluate, e.g. "the need for stability data, including data generated under accelerated or stressed conditions, to provide insight into potential differences in product degradation and resulting That is, potential differences in product-related substances and impurities in the product".*

➢ *Elsewhere, the Instructions State:*

- "Accelerated and stressed stability studies are often useful tools for establishing degradation profiles and providing more direct comparisons of products before and after changes.
- The results obtained can therefore reveal differences between products that require additional evaluation and also identify conditions that indicate that additional control measures need to be applied during production and during storage to eliminate these unwanted differences.

*C. ICH Common Technical Document (CTD):*

- ICH Quality ($M_4Q(R1)$) guideline in section 3.2.S.7, subsection 3.2.S.7.1 Stability Summary and Conclusion refers to:"The types of studies performed, the procedures

used, and the results ofthe studies should be summarized. The summary should include the results of, for example, studies on forced decomposition and stress conditions, as well as conclusions on storage conditions and retest dates or shelf life, if applicable.

- In the same section, subsection 3.2.S.7.3 highlights stability data: "The results of stability studies (e.g., forced degradation studies and stress conditions) should be presented in an appropriate format such as tables, graphs or notes. Information on the analytical procedures used to generate the data and the validation of these procedures should be included."

*D. FDA Guidelines*

The Food and Drug Administration (FDA) is issuing guidelines for the condition of Photolytic stress stability Analysis of Newer Drug Molecules and their Drug Product(s) like as ICH Q1B. The FDA suggests that stress testing studies be conducted by using standard development procedure and then conditions are optimized. This guidance covers the pathway for degradation ofsamples when light is exposed. This guidance assists in the development of SIMs and also summarizes validation data, which are useful for confirmatory assays. These guidelines emphasize the absence of the need to perform confirmatory studies on degradation products. Article 211.166.166a(3) requires that a SIM must be highly precise and quantify the quantity of active substance, the types of products obtained with the active substance, and an extra constituents available in the formulation without intercession in stress testing conditions. Condition of pH, Condition of temperature and Condition of oxygen are stress conditions used in forced degradation studies.

*E. European Medicines Agency*

The European Medicines Agency (EMA) has established a set of guidelines for active ingredient chemistry. These guidelines provide information on the type of research conducted, the procedures employed, and the outcomes of the analysis. Particular the Section 2.1.2 is paid attention to the analysis of API and dosage forms for stability testing, as well as information on retest and expiration dates for substances. Additionally, analytical methods have been developed, method validation has been conducted, pathways of stress testing products have been identified, and inherent stability has been determined. It is also necessary to conduct stability testing studieson delicate compounds, such as sensitive to photolytic reaction and hygrogenic drugs.

*F. National Health Surveillance Agency (ANVISA)*

ANVISA was created to safeguard public health and reduce the potential risks associated with theproduction and utilization of a variety of pharmaceutical products. It is designed to coordinate theactivities of the state, district, and municipality, in accordance with the principles of Brazil's Unified Health System, to monitor living standard of the population and it also suggest the improvement in standard of living.

➢ *USP Pharmacopoeia:*

In accordance with USP Pharmacopoeia guidelines, where degradation standards are not availableor adulterants or impurities are present, the degree of specificity parameter can be determined by the data comparison with results obtained from analytes (which contain adulterants or products of stress testing) using a different approach under the similar accelerated circumstances.

➢ *Japanese Pharmacopoeia*

The proposed procedure should be specified; enable the quantification and estimation of the analyte content of the sample, and be suitable for comparative studies. In the absence of reference standard impurities, samples shall be subjected to stress conditions and further studies may be conducted using degradation products.

➢ *Indian Pharmacopoeia (IP)*

In accordance with IP Pharmacopoeia guidelines Impurities in Drug Products has the following requirement under section of General Test 5.5 'Impurities':

Generally, dosage forms are subject to an impurity test that is tailored to the active ingredient of the product, with the necessary modifications to include stress testing products. These stress products include the active component's degraded products, formed degraded products from the reaction between the active substance and the excipient or ingrident , stress testing products from the reaction between the active compound to the direct container or closure system, and Dosage form or products of drug-drug interactions. Acceptance criteria for degradation products include both identically known and unknown stress testing products. These impurities can be identified through stability testing studies and stress testing studies, as well as examinations of regular manufacturing batch analysis. For impurities that arise during manufacture or while storing the dosage form, wider limits may be necessary, and further controls may be necessary.

## III. BENEFITS OF FORCED DEGRADATION STUDIES

- Evaluate a inherent sensitivity of molecule and product's to various variables and different combinations of variables, including temperature, humidity, pH level, oxygen concentration, light,and the presence of catalysts.
- It further evaluates the intrinsic stability properties of the active molecule, and also in addition generation of samples to identify products formed during various stress testing conditions and the delineation of pathways and mechanisms for degradation.
- It is essential in developing and validating methods to demonstrate stability of the drug substance. Applied to produce breakdown results that, in the worst-case scenario, can be used to gauge how well an analytical method development can approach is working.
- It Include Research on drugs and drug interactions in pharmaceuticals.

- Include any important circumstances that could arise during distribution and storage.
- To support exclusionary labeling, including shelf life extensions.
- Describing the degradation products produced in forced degradation samples is important tothe QbD knowledge space as it helps to understand the chemical reactivity of drugs.
- Establishing mass balance early will avoid surprises later in formal stability studies and even in forced decomposition studies.
- Forced degradation studies act as a filter to quickly select and eliminate drugs and/or their products due to their shorter duration, in contrast to formal long-term stability studies which are very costly.
- Forced degradation studies are often repeated as stability indicator methods develop.
- The same applies every time this method is repeated, regardless of technology, human consciousness or even recent dosage and form.

➢ *When should stress testing studies to be performed?*

- To carry out the required stress testing studies to develop a new molecule of drug and a newproduct of drug, the pattern required to conduct them is essential.
- As per FDA guidance, Clinical trials 3rd Phase of the submission process of regulatory are the priority phase for stress testing studies.
- To demonstrate the stability of a drug molecule, stress testing studies are required to be performed in solutions of varying level of pH, in the presence of parameters like oxygen and light, and at hightemperature and humidity.
- The stress testing study was performed on the same batch or single batch.
- The results of the stress testing studies must be reported in an annual report of the product study.
- Stability testing studies include two types of studies: stability studies of long term (12 months) and intermediate stability studies (6 months).
- However, short term stability studies (6 months) may be performed under fewer conditions than intermediate stability studies. After degradation studies, partition of components, component identification and quantification of components are performed.
- Forced degradation in preclinical or clinical trials 1st phase is recommended, allowing sufficient time to identify degradation products, elucidate structure, and optimize stress conditions.
- Improved manufacturing processes and appropriate selection of analytical procedures demonstrate stability. This is obtained from the first stress testing studies.
- As per FDA, required reports of stress testing studies performed:

✓ *In pre-IND studies:*

- During formulation Studies: Stability, indicating quality characteristics and pathwaysof degraded products during formulation studies.
- Preclinical studies: During preclinical studies, degradation substances are identified, as well as toxic components is identified.

✓ *In Molecule clinical development phase:*

- Comparing clinical quality with preclinical quality
- Comparison of changes in both pre-manufacture and post manufacture production
- In stability testing studies

✓ *Post-marketing:*

Usually stress testing studies are not preferred after product was marketed but insome case studies considered to be perform:

- Identification of any new degraded product
- Changes in manufacturing area or conditions
- Any indications other than reported in submission.

## IV. LIMITS FOR DEGRADATION

There has been a lot of debate among pharmaceutical scientists regarding the appropriate level of degradation. The acceptance percentage of the drug degradation is between 5-20 as for validated chromatographic procedure. And some other scientists of pharmaceutical area suggests that 10% of increments are optimal for pharmaceutical small molecule for analytical confirmation, where the acceptable stability limits is 90% of that stated above. It has been proposed that drug composites could be laced with a variety of well-known degradation details in order to go around the techniques employed to cover active molecule stability. For any type or class of natural goods,no specific top limitations for physiochemical changes, performance degradation, or shelf life shortening have been established. A declination product isn't always affected by forced degradation. However, if no degeneration is observed following exposure to the drug component or product to stress conditions other than those mentioned in the accelerated stability protocol, thisis a sign of the molecular stability of the tested patch. It is recommended to run a stress test for at least 14 days for style development testing and for 24 hours for oxidative tests. It is important to note that protocols for generating product-related degradation can vary between active molecule and dosage form due to differences in matrix and concentration. Excessive stress may result in a secondary degradation product, while insufficient stress may not generate satisfactory products of stability studies.

### *A. Selecting Concentration of Drug for Preparation of Stock Solution:*

The precise concentration of drug to use for stress testing studies has not mention in any of the regulatory guidelines. However, initiate the study with 1 milligrams per milliliter (mg/mL) as an initial concentration. This stock solution is often suitable for detection of minor range of impurities also. Additionally, certain stress studies conducted at a drug concentration at which the drug is expected to be included in the nomenclature of a formulation (s).

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*B. Different Conditions of Stress Testing Studies:*

➢ *Condition of Hydrolytic studies:*

Hydrolytic condition of degradation is a common chemical reaction for the stress testing studies for studying of different pH levels. In chemical mechanism, a chemical molecule interacts with water to break down the molecule into its ionizable functional groups. During a hydrolysis research, a pharmacological substance is exposed to acidic or base conditions to induce degradation that results in primary degradation of the desired range of degradants. The drug molecule stability determines based on the acid or base test. Sodium hydroxide/Potassium hydroxide are acceptable reagents for Base hydrolysis, Hydrochloric acid/Sulfuric acid are acceptable reagents for Acid hydrolysis.0.1M to 1M concentration is acceptable for hydrolysis studies. if water is not suitable for solubility of drug molecule than Co-solvent is added, it can be dissolved in acid solution(HCl) or base solution(NaOH). Normal stress testing trials start at room temperature and increases to higher temperature (50 to 70 °C) the duration stress testing is allowed of 7 days maximum. To stop further degradation, the collected stress sample is should be neutralized with a suitable buffer or acid reagents, base reagents respectively.

➢ *Condition of Oxidation:*

In stress testing studies, for studying of oxidation conditions, hydrogen peroxide is often used, And some other agents like metal ions, oxygen and radical initiators) can also be used (e.g., azobisisole, AIBN, etc.) as oxidizing agents. The drug substance dictates the oxidizing agent to be used, as well as the concentration and environmental conditions. According to some reports, suitable degradation products can be obtained by exhibit the solution to 0.1% to 3% $H_2O_2$(at pH level is neutral and at room level temperature) for maximum of 7 days or at 40°C for 1 to 7 days (at room level temperature) up to degradation of 20% maximum. During the oxidative breakdown of the active molecule, an mechanism of electron transfer is employed to create reaction anion andcation, resulting in the formation of N-oxides (see below), hydroxylamine (see below), sulfones (see below), and sulfoxide (see below). The labile functional group hydrogen can also be oxidized to produce hydroperoxides and hydroxides, and ketones.

➢ *Photolytic Degradation Condition:*

In order to confirm that exposure to Light does not cause an excessively undesirable change. Stability test of photolytic condition of the pharmacological compound should be performed. The main degradants of the pharmacological substance are formed by photo stability testing when exposed to ultraviolet (UV) or fluorescing (fluorescing) light. Below are some of the suggested test conditions in Q1B(R2)guidelines of ICH. Sample of drug molecule and sample of solid drug products /liquid drug products must be exhibit to 200 watts per square meter (Wp/m2) and 1.2 watts per square inch (Lx/h) respectively. The most common wavelength of light used for photolytic degradation is 300 to 800 nm. Maximum 6 million lux per hour (lx/h) is suggested condition for photolytic degradation. When something is photolytic, it either gets oxidized through a free radical reaction or it goes through a non-oxidizing process. The oxidative process involves isomerization, dimerization, etc. The oxidative reaction is based on two types of oxygen: singlet and triple oxygen.

Singlet oxygen causes photo-oxidation when it reacts with unsaturated compounds and the free radical reaction to make the peroxide by using triplet oxygen. Plus, light can also cause oxidation reactions. C-X hetero bonds breaks down by Homolysis. C-S bonds breaks down by Deamination. Photo-oxidation may cause by light stress. Free radical mechanisms include carbonyls, nitro aromatic N-oxides, alkenes, aryl chlorides, weak c-H and o-H bonds, and sulfide sulfide.

➢ *Thermal Degradation Condition:*

The thermal stress condition includes both dry heat and wet heat. Thermal condition should be performed at higher temperature then the ICH Q1A guideline for test condition. Bulk drug sampleand Drug dosage form should be subjected for dry heat and the liquid form of drug (or) liquids are subjected to dry heat. Higher temperatures may be used for a shorter period of time. In thermal stress testing condition the effect of temperature is determined by the following Arrhenius equation:

$$k ¼ Ae — Ea=RT$$

Where, specific reaction rate is denoted by *k*, frequency factor is denoted by *A*,energyof activation is denoted by *Ea*, gas constant (1.987 cal/deg mole) is denoted by *R*, absolute temperature is denoted by *T*,
Thermal stress testing is conducted at 40–80 °C.

➢ *Humidity*

Humidity plays a significant role in degradation. For example, in forced degradation studies, 90% humidity is used for 1 week to degrade the drug substance. Humidity is also one of the key parameters used to determine the potential degradants in final products and API.

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Table 1 Conditions Mostly Used For Stress Testing Studies

| Degradation Types | Experimental Conditions | Storage Conditions | Sampling time |
|---|---|---|---|
| Hydrolysis | Control API(no acid or base) | 40°C,60°C | 1,3,5 |
| | 0.1N HCL | 40°C,60°C | 1,3,5 |
| | 0.1N NaOH | 40°C,60°C | 1,3,5 |
| | Acid control (no API) | 40°C,60°C | 1,3,5 |
| | Base control (no API) | 40°C,60°C | 1,3,5 |
| Oxidation | 3% $H_2O_2$ | 25°C, 60°C | 1,3,5 |
| | Peroxide control | 25°C, 60°C | 1,3,5 |
| | Azobisisobutyronitrile (ABIN) | 25°C, 60°C | 1,3,5 |
| | ABIN control | 25°C, 60°C | 1,3,5 |
| Photolytic | Light 1 X ICH | NA | 1,3,5 |
| | Light 3 X ICH | NA | 1,3,5 |
| Thermal | Heat chamber | 60°C | 1,3,5 |
| | Heat chamber | 60°C/75°C% RH | 1,3,5 |
| | Heat chamber | 60°C | 1,3,5 |
| | Heat chamber | 60°C/75°C% RH | 1,3,5 |

## V. FACTORS AFFECTINGDEGRADATION

*A. For active molecule stress testing, there are a number of variables to consider, including solvents, Moisture content, excipients properties, pH level, oxygen content, Light exposure and temperature level.*

➢ *Moisture:*

Moisture can lead to the dissolution of substances that are soluble in water, which can cause physical and chemical changes in the molecule.

➢ *Excipients:*

Excipients may also contain high water content, which can lead to an increasein the water level in the formulation; hence there is chance of rise in formulation water level. This water level may impact negatively on the stability of drugs. In such cases, chemical reaction between drug and excipients decreases the stability of the dosage form.

➢ *Condition of Temperature:*

It is also affects the drug stability, As reaction rate of hydrolytic stress study increases as temperature increases

➢ *pH:*

To reduce this, buffer solutions of pH are used to ensure the drug is formulated withmaximum stability.

➢ *Oxygen:*

Oxygen can also increase the oxidation rate of certain drugs, which can cause them to decompose more quickly. To stabilize drugs with an increased rate of oxidation, nitrogen or carbon dioxide should be purged from the storage container.

➢ *Light:*

Drugs undergo photolysis when exposed to light. This can be used to determine drug sensitivity by comparing a molecule's stability under various light conditions. Keep in mind that photolytic agents should be kept in a dark environment and in gold-brown glass bottles.

*B. Stability Indicating Method(SIM)*

An FDA guidance document defines a Stability Indicating Method (SIM) as a method of quantitative analysis used to measure the degradation of an active pharmaceutical ingredient in a drug product. This method is capable of accurately measuring the concentration of the active ingredients over time, without being affected by other degradation products or excipients. Stress testing is conducted to demonstrate the accuracy of the method used to measure the concentration of the drug substance when there is limited information on the potential degradation product available. Developing a suitable Stability Indicating Method serves as a basis for pre-formulation studies and stability studies, as well as the development of appropriate storage requirements.

*C. Relation between Stress Testing Studies and Data of Stability:*

In the case of Stress testing studies, more products are produced than in normal stability testing. Due to the low potential of stability testing, it is difficult to identify the actual degradation products. From this point of view, forced degradation studies minimize this issue. If there are no degradants produced, then the active substance is considered as stable under the applied potentialconditions. The protocol can be interpreted as a stability indication. Forced degradation analysis can also be used to study the

appropriate storage conditions of various pharmaceuticals. Furthermore, Stress degradation studies helps to estimate the pathway degradation of differentactive molecules.

*D. Development Method and Optimization of Analytical Method:*

For development of chromatography method like HPLC, It is important to be aware of the molecules properties especially physico-chemical parameters like pKa, LogP and molecule solubility, as they will provide a basis for the development of the method. The suitable mobile phase and solvent are selected by the help of LogP and solubility and pH is optimized by pKa valve. A RP-HPLC method column is a recommended method for the separation of formed products, if the procedure done in the aqueous solution. Then begin with various composition of hplc grade filtered distilled water and hplc grade filtered Acetonitrile for the initial condition. If the procedure is performed done by using organic solvents the most commonly used initial condition is 50:50 composition of Methanol and Acetonitrile. And change the composition till theobservation of suitable composition for obtaining sharp peak with good resolution and theoretical plate count. In order to achieve optimal peak separation and symmetry, a more saturated buffer can be added. For LC-MS method suitable mobile phase and the buffer chemicals should be MS compatible such as THA, Ammonium formate in order to ensure the selectivity. And also sometimes analyte reacts to different conditions of temperature; hence column temperature also affects the method selectivity. For good reproducibility, a temperature range of 30-40 0C is recommended. The parameter is recommended for further peak pushing in chromatogram to separate the formed stress products. Additionally, Increasing run time of the samples also helps to observe the elution of formed products peak after the standard ingredient peak. During the time of method development, there is chance of standard peak merging with other peaks like products peak or impurity peak, which eludes the peak of the drug. The purity of peak analysis is necessaryto report the specificity of method; Directed peak analysis method can be performed with aid of PDA detection. However, this method is not applicable to degradants with same wavelength of UV spectrum of the molecule. In indirect peak analysis method some conditions should be modify such as mobile phase compositions, the stationary phase type or any peak influence parameters.After the modification of chromatographic parameters the obtain spectrum is compared with original spectrometric conditions. If the strength and proportion of stress product in the remains unchanged in the standard peak, then the standard peak considered ad homogenous. The co-luting stress products are acceptable as long as it is not produced in the both accelerated and long term conditions like storage. The optimized method conditions for separation of jointly eluted peaks byvarying the rate of osmotic, stationary phase types and composition of mobile phase.

*E. Validation of Developed SIM Method*

The stress testing method was developed and it is validated as per guidelines of ICH/ USP for condition of linearity, condition of accuracy, condition of precision, condition of specificity, quantification limits, detection limits, condition of robustness and condition of robustness.

Method validation is necessary for identification, Isolation and also to quantify formed products whose levels exceed the threshold for identification (usually known 0.1%).If performed method may not meet the criteria of acceptance for validation, and then modify the method and revalidated revised method.

## VI. CONCLUSION

Stress Stability studies are performed to identify possible mechanism of degraded product formation or pathway of degradation of the API. The performed study becomes reference for the elucidate the structure of formed products. The expected products of stress conditions may form in the process of manufacturing, transportation and also in storage condition of the product or under any of the step in the product life. The products formed during the study by applying a very potential condition in various time lines are helps to improve various conditions in the process of producing and delivering of the product. Forced degradation studies helps to give a quality and safe product to the customer. And also it helps to modify the environmental conditions like production area, packing material selection, transportation and storage conditions. The submission of forced degradation studies reports are significant to regulatory approval to move next phase of study or to launch in different market. By following different guideline of the forced degradation studies is performed to get approval ofa specific market launch.

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