Stability indicating study by using different analytical techniques

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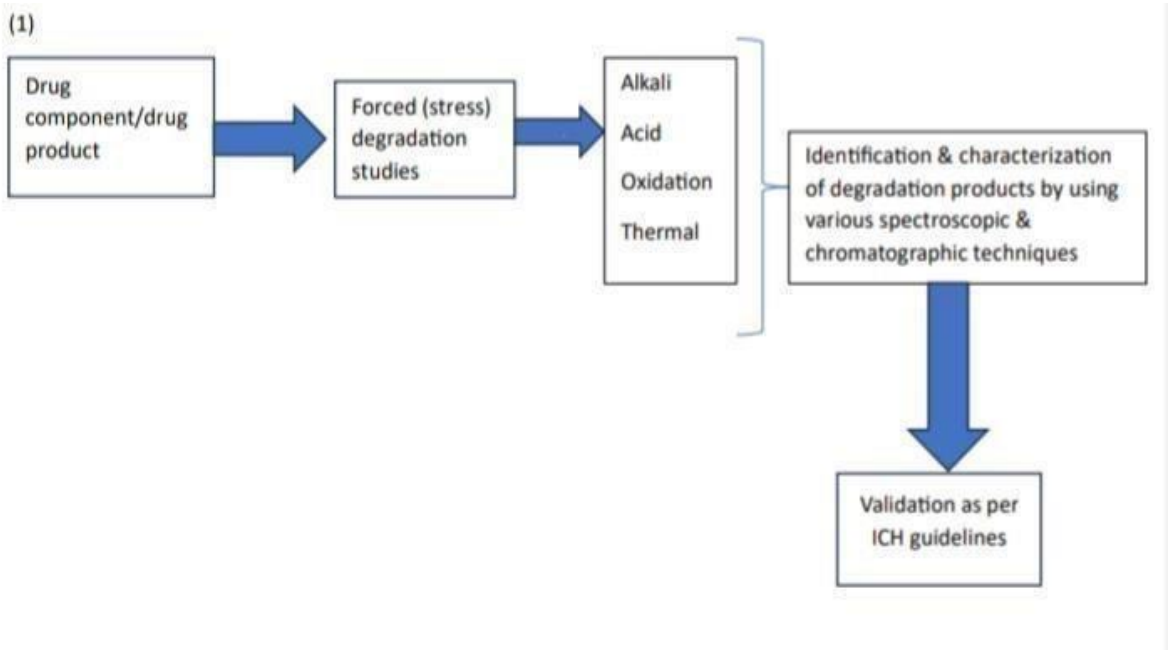
Abstract- **This write up provides a review on the development of validated stability-indicating assay methods (SIAMs) for drug products. The imperfections of described methods concerning to regulatory requirements are highlighted. A systematic approach for the development of stability-indicating methods is demonstrated in this review article. The main current goal of stability-indicating assay methods (SIAMs) is to gives information about the conditions for stress testing so as to demonstrate the stability of drug substances and drug products. This paper reviews the regulatory aspects for development of stability-indicating methods. SIAMs are used to discriminate the active pharmaceutical ingredient (API) from its potential decomposition products and substances. Regulatory guidelines in ICH Q1AR2, Q3BR2, Q6A and FDA 21 CFR Section 211 requires validated stability-indicating methods. Forced degradation is required to establish specificity when developing SIAMs and for this reason, it should be performed prior to accomplishment of stability studies. Forced degradation of the drug standard and excipients is carried out under various conditions to determine whether the analytical method is stability-indicating. The approaches for the development of stability-indicating methods are discussed.**

Keywords: **Stability Indicating assay method, Regulatory guidelines, stress testing.**

Introduction:

Since stability is a crucial component of pharmaceutical products' quality, testing for stability is essential to the creation of new medications. By showing how the quality of a drug substance or drug product changes over time under the influence of various environmental factors like temperature, humidity, and light, stability testing is used to establish a retest period for a drug substance or a shelf life for a drug product, as well as recommended storage conditions [3].The stability of the drug component or drug product appears to be crucial as it impacts purity, potency, and safety. Any alteration in the stability of a medicine has the potential to produce hazardous substances or lessen its efficacy. Therefore, it is essential to understand how different environmental factors affect a drug's component or product, since this will enable us to determine its purity profile (3) "It is a validated quantitative analytical procedure which is used to detect the changes in the properties of the drug products and the drug substances," is how the FDA defines a stability indicating method.[4] Drug ingredients and drug products can undergo changes in their chemical, microbiological, and physical properties, which can be detected using stability indicating methods. A stability indication method needs to be specific, repeatable, and validated for the use for which it is intended. It also needs to be able to track changes in the attributes of the therapeutic substance and drug product. (4)Stability testing of pharmaceutical items is a complex collection of procedures that need a large amount of money, time, and scientific expertise in order to improve the quality, efficacy, and safety of a prescription formulation. Among the most important jobs during the development stages are the pharmaceutical analysis and stability tests that are required to determine and assure the identity, potency, and purity of ingredients as well as those of the created products. The stability of a pharmaceutical product is its ability to retain its physical, chemical, microbiological, toxicological, protective, and informational properties in a given container or closure system .(5) Stability testing, then, evaluates the impact of the environment on the quality of a manufactured product or therapeutic ingredient in order to determine optimal storage conditions, forecast shelf life,and suggest labelling guidelines. These components include dosage form type, manufacturing processes, interactions between the active ingredient(s) and excipients, stability of the active ingredient(s), packaging container/closure system, and light, heat and moisture conditions encountered during handling, storage, and transportation. Furthermore, the stability of degradation reactions such as oxidation, reduction, hydrolysis, or racemization can be greatly impacted by variables such as reactant concentration, pH, radiation, catalysts, raw material usage, and the time elapsed between product production and use.(5)



Regulatory Status of Stability-Indicating Assays:

The ICH guidelines have been incorporated as law in the EUROPEAN UNION (EU), Japan and in the US, but in actuality, apart from these other countries are also using them. As these guidelines consider the current inspectional tendencies, they carry the de facto force of regulation. ICH defines SIAM Stability Indicating assay method) as quantitative analytical techniques that are establish on the characteristic structural, chemical or biological properties of each component of a drug product and that will differentiate each active constitute from its degradation products so that the active constitute content can be accurately measured. USFDA defines SIAM as Validated quantitative analytical methods that can analyze the changes with time in the physical, chemical or microbiological properties of the drug substance and drug product and that are particular so that the contents active constitute degradation products, and other components of interest can be accurately measured without interference or difficulties. Regulatory guidelines for stability testing is shown in Table[1].

Regulatory guidelines several International guidelines suggested forced degradation studies ICH guidelines sometimes execute only to the marketing applications for new drug products and do not include the part during clinical development. The ICH guidelines that are relevant to forced degradation studies are [1] .

ICH Q1A: Stability testing of new drug substances and products.

ICH Q1B: Photo stability testing of new drug substances and products. ICH Q2B: Validation of analytical procedures: Methodology.

1.ICH Q1A (Stress testing): Recommended conditions for representation forced degradation studies on drug substances and drug products. The recommendations are to inspect the results of temperature (above that for accelerated testing, i.e., >50 C), humidity (75% relative humidity), oxidation and photolysis. Wide pH range should be considered in the testing of solution or suspension. Eventually the stability-indicating method developed from these products samples.[1]

2.ICH Q1B: Recommended approaches to assessing the photo stability of drug substances and drug products. For drug substance and drug product forced degradation conditions are specified in photo stability testing can be accelerated. These samples are then utilized to develop a stability indicating method. Some of the degradation products formed during forced degradation studies may not really be experiential to form during stability studies in which case they need not be examined further.[1]

3.ICH Q2B: Gives guidance to validate the analytical methodology. To demonstrate specificity, in section B 1.2.2 (impurities not available) there is a suggestion to utilize samples from forced degradation studies. Whether the analytical method is stability indicating or not specificity‘ is a key factor.[1] l

4.ICH Q3A (R2): [16] With respect to both chemistry and safety prospects the identification of each impurity is required. The chemical anticipation include classification and identification of impurities, report creation, catalogue of impurities in the specification and a concise discussion of analytical methods. The safety prospects include particular guidance for qualifying those impurities that were not present at significantly lower levels in batch of a new drug substance and drug products.[1]

Table: Regulatory Guidelines for stability testing

Guidelines	Title

Q1A(R2)	Stability testing of new drug substances and products.
Q1B	Stability testing: photo stability testing of new drug substances and products.
Q1C	Stability testing for new dosage forms.
Q1D	Bracketing and matrixing designs for stability testing of new drug substances and products.
Q1E	Evaluation of stability data.
Q1F	Stability data package for registration applications in climatic zones III and IV
Q5C	Quality of Biotechnological products: stability testing of Biotechnological/biological products.

Regulatory aspects of stability study:
Guidelines about the requirements of GMP'S(good manufacturing practices)for the industry .all some organizations like international conference on harmonization(ICH),Food and drug administration (FDA) and world health organization (WHO) have issues of Guidelines for stability testing API and drug products . Stability testing in United States containing 21CFR, part 21,section166 and the Guidelines of ICH put in the United States , Japan and Europe. The WHO guidelines are related to the development of product (7).

Objective of stability study:
- Stability studies are performing the or established the storage conditions and shelf life of API and product .
- In recently adopted the stability Guidelines ,the committee for proprietary medicinal products (CPMP)indicate that the to provide stability testing of evidence that the how the quality of product varies with time under the influence of the variety environmental factors such as temperature, humidity and light. The stability of API is not fixed or not likely change but it means control.(6)
- To identify the processes by which drug compounds and drug products degrade.
- To distinguish degradation products that are produced from the non-drug product in a formulation from those that are related to drug products.
- To ascertain a pharmacological substance's intrinsic stability inside the formulation.
- To identify the drug substance's and drug product's degradation processes, such as hydrolysis, oxidation, thermolysis, or photolysis
- To produce a deterioration profile resembling what would be seen in an official stability study carried out under ICH guidelines.
- To create formulas that are more stable. It also aids in figuring out when a specific formulation will expire.

UV spectrometer Parameter for stability indicating study-
A Shimadzu UV-visible spectrophotometer (UV1800, Shimadzu Corporation, Kyoto, Japan) was used for all maximum absorbance measurements with matched quartz or sample cells.[21]
Stress Degradation studies include following parameters:
1. Thermal Degradation
2. Photolytic Degradation
3. Acid degradation
4. Base degradation
5. Oxidative degradation

- **Thermal Degradation**: It is recommended to conduct thermal deterioration (such as dry heat and wet heat) under more demanding conditions than those suggested by ICH Q1A accelerated testing settings. Dry and wet heat should be applied to samples of drug compounds and drug products in a solid form. Dry heat should be applied to liquid medication products. Studies at higher temperatures may be carried out for a shorter amount of time. The Arrhenius equation can be used to study how temperature affects a substance's thermal deterioration.[22]

- **Degradation by UV light:** As natural and manufactured polymers break or dissolve when exposed to prolonged sunlight, UV degradation is a major issue for many UV unstable items. Intermittent exposure is not as dangerous as continuous exposure because the attack depends on the level and duration of exposure. Degradation brought on by UV or visible light exposure. Normal Exposure circumstances: Samples should be exposed three times to 200 W-hr./m2 UV and 1.2 million lux-hr. visible light.[23]
- **Acid Degradation:** Acid degradation were carried out by weighing the sample to be determined at different volumetric flask. If the compound for stress degradation study are poorly soluble in water the cosolvents are used to dissolved the sample in solvents like methanol to get the standard concentration. Then the sample solution were stressed by using 0.1N HCl on water bath at 80°C for for 2 hr. Samples were scanned in UV range at 200-400nm and spectra were noticed.[24]
- **Base Degradation:** For Base degradation study the sample solution diluted by using 0.1NaOH. Then the solutions were stressed at 80°C for 2 hr and spectra were scanned. [24]
- **Oxidative Degradation:** One of the most prevalent ways that drugs deteriorate. Oxidative degradations should normally be carried out in the dark and at room temperature, or between 25 and 30°C. It is not advised to utilize temperatures higher than 30°C because the solvent's decreased oxygen concentration could actually lower the reaction rate in solution at higher temperatures. However, some compounds may degrade At higher temperatures as a result of the start of free radical reactions. Normal stress condition: Apply 3% hydrogen peroxide to the area while it's dark, at room

Temperature, and stirring continuously. Stress for a whole day, or until a 5–20% deterioration is Attained.[23]

Table 1. Conditions mostly used for forced degradation studies

Degradation Type	Experimental conditions	Storage conditions	Sampling Time (days)
Hydrolysis	Control API(no acid or base) 0.1M HCl 0.1M NaOH Acid control (no API) Base control (no API) pH 2,4,6,8	 40ºc,60ºc 40ºc,60ºc 40ºc,60ºc 40ºc,60ºc 40ºc,60ºc	1,3,5 1,3,5 1,3,5 1,3,5 1,3,5 1,3,5
Oxidation	3%H_2O_2 Peroxide control azobisisobutyronitrile AIBN AIBN control	25ºc,60ºc 25ºc,60ºc 25ºc,60ºc 25ºc,60ºc 25ºc,60ºc	1,3,5 1,3,5 1,3,5 1,3,5 1,3,5
Photolytic	Light 1+ICH Light 3+ICH Light 3+ICH	NA NA NA	1,3,5 1,3,5 1,3,5
Thermal	Heat chamber Heat chamber Heat chamber Heat chamber	60ºc 60ºc/75%RH 60ºc 60ºc/75ºc	1,3,5 1,3,5 1,3,5 1,3,5

STABILITY INDICATING HPLC METHOD DEVELOPMENT:

High-performance liquid chromatography is one of the most accurate methods widely used for the quantitative as well as qualitative analysis of drug products and is used for determining drug product stability. Stability-indicating HPLC methods are used to separate various drug-related impurities that are formed during the synthesis or manufacture of drug products. This article discusses the strategies and issues regarding the development of a stability indicating HPLC system for drug substances. A number of key chromatographic factors were evaluated. High-performance liquid chromatography (HPLC) is an integral analytical tool for assessing drug product stability. HPLC methods should be able to separate, detect, and quantify the various drug-related degradants that can form during storage or manufacturing, as well as any drug-related impurities that may be introduced during synthesis. Forced degradation studies (chemical and physical stress testing) of new chemical entities and drug products are essential to help develop and demonstrate the specificity of such stability-indicating methods. In addition to demonstrating specificity, forced degradation studies can be used to determine the degradation pathways and degradation products of the APIs that could form during storage

and facilitate formulation development, manufacturing, and packaging. Procedures for the preparation of specific degradation products needed for method validation often emerge from these studies. For marketing applications, current FDA and ICH guidance recommends inclusion of the results, including chromatograms of stressed samples, demonstration of the stability-indicating nature of the analytical procedures, and the degradation pathways of the API in solid state, solution, and drug product. The chemical structures of significant degradation products and the associated procedures for their isolation and/or characterization are also expected to be included in the filing. The experimental protocol for performing forced degradation studies will depend on the active ingredients and formulation involved because the chemistry of each compound is different. In general, a target of approximately 10% degradation of the API during forced degradation, or exposure to energy in slight excess of what is typically used in accelerated storage, is recommended. In this way, the "worst-case" degradation products can be studied. The following will provide some suggestions for performing forced degradation studies based upon available guidance from the ICH and FDA.(18) Forced degradation is the degradation of a new drug substance or drug product under conditions more severe than accelerated conditions. It is required to demonstrate the specificity of stability-indicating methods and also provide insight into the degradation pathways and degradation products of the drug substance and help in the elucidation of the structure of the degradation products. Forced degradation studies show the chemical of the molecule, which in turn helps in the development of formulations and packages. In addition, the regulatory guidance is very general and does not explain the performance of forced degradation studies. Thus, this review discusses the current trends in the performance of forced degradation studies by providing a strategy for conducting studies on degradation mechanisms and also describes the analytical methods helpful for the development of stability-indicating methods.(19)

Type of solvent: The relative polarity of the solvent and the sample can be compared to determine whic h starting solvent is best for a particular separation. As it chosen as a preliminary approximation by using the solvent in order to align with the polar functional group on the sample molecule (amines for NH2, alcohols for OH, etc. such). This attempt allows for improved separation. through the subsequent process:

1.When the sample is visible at the solvent front, Because the solvent is too polar, the adsorbent cannot to impede the specimen. Visit a solvent who is higher up. Positioned lower on the scale.(9)

2.In contrast, switch to a solvent or solvent blend with a lower polarity on the solvent if the sample doe s not appear in a reasonable amount of time. size.

Type of solvent (tetrahydrofuran, acetonitrile, and methanol) furan) is going to impact selectivity. Selecting between Acetonitrile and methanol could rely on the solubility of both the employed buffer and the analyte.Of these three, tetrahydrofuran is the least polar. solvent, frequently in charge of significant changes in additionally lacks selectivity and is incompatible significantly wavelength detection needed for the majority of pharmaceutical ingredients.(10)

Mobile phase pH: Ionisable functionalities like amino, pyridine, and carboxylic acid are present in the majority of pharmaceutical compounds. The debut of new packaging materials that hold up well under a variety of pH values up to pH 12 enable a greater range of applications for pH of the mobile phase as a retention/selectivity modelement 14Upon eluting the sample using a mobile phase There is no separation because the sample is 100% (organic). formed in the empty space. This is because retention is seen when the mobile phase solvent strength is lowered to allow for sample retention rather than sample retention occurring. Equilibrium rivalry between the molecules of the solute amid the mobile phase and the bonded phase.(11)

Column Temperature: Since temperature has an impact on selectivity, controlling column temperature is crucial for long term method reproducibility. A goal Normal operating temperature is between 30 and 40 °C. adequate to ensure high reproducibility.(12) Temperature has been disregarded in operations parameter in HPLC, as well as the possible benefits of high column temperatures, especially improved properties of kinetics and transport, which are founded on the reduction of the mobile phase's viscosity and the analytes diffusivity rising at higher16 degrees Celsius in temperature Generally speaking, the goal of utilizing a high or elevated temperature is meant to boost the separation speed to achieve greater efficiencies and quicker outcomes, although there are some circumstances where Selectivity can be adjusted by altering the warmth.(13) Commercial temperature programming for HPLC is currently being promoted, and comparisons between the effects of solvent and temperature gradients are available.(14)gradients with a test set of and a variety of columns analytes including basic, acidic, and neutral molecule s 24. Utilizing higher temperatures has advantages for HPLC, especially when there are columns involved stable across a wide range of temperatures. Once working with analyte mixtures in various Compound classes may influence selectivity. the temperature.(15)

Peak Purity: The capacity to confirm the separated species' purity, or to make sure that no coeluting or comigrating impurity plays a role in the maximum answer. Verification of peak purity ought to be carried out prior to quantitative data from a Electrophoretic or chromatographic peaks are utilized in additional computations.(16)

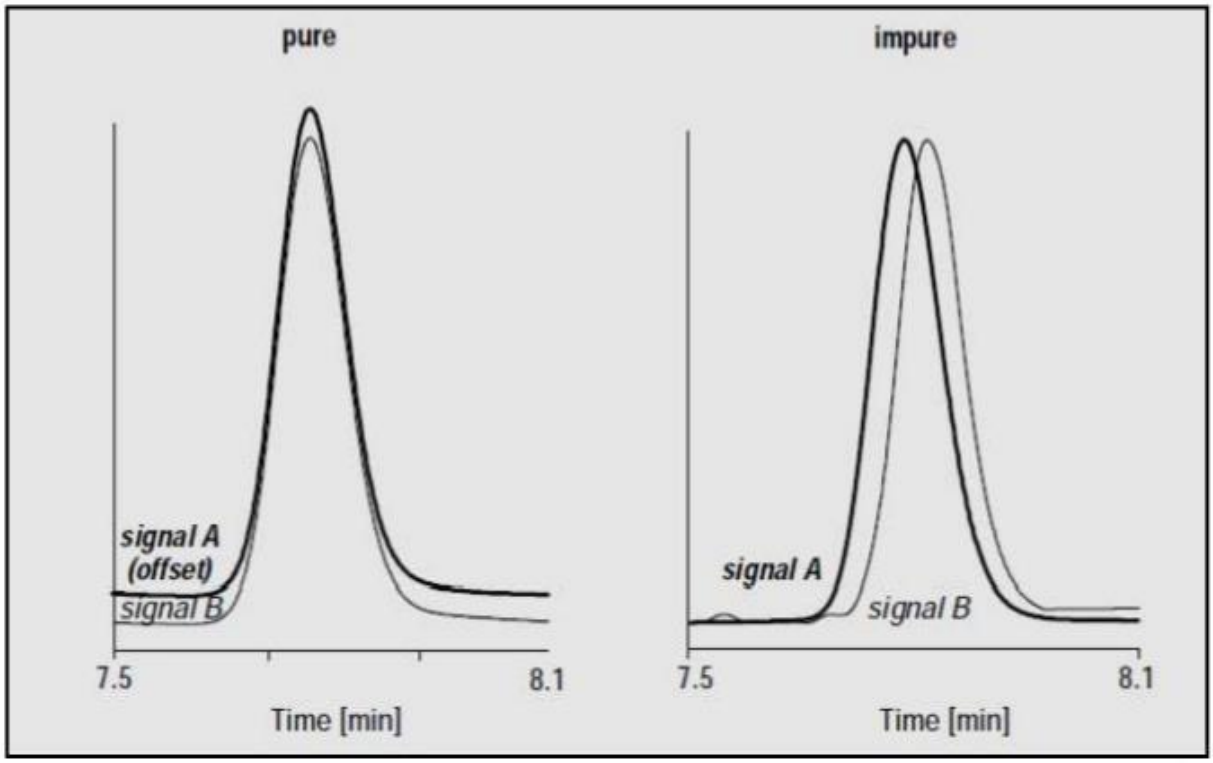


FIGURE 1: NORMALISED SIGNALS FOR PURE AND IMPURE PEAK

An integral component of the validation of peak purity (also known as peak homogeneity) analysis is determining whether impurities are present beneath the main peak. an SMS. Direct assessment can be carried out in accordance with using LC-NMR, LC-MS 26, or PDA detection 25. PDA, however, is only effective with degradants that possess a UV spectrum distinct from the medication 's The evaluation of LC-MS won't function if the degradants has the same molecular weight, similar to what diastereomers or if the degradant's ionization is suppressed by the API that co-eluents.(17)

Stability indicating assay method (SIAM)-

The stability indicating assay method is used for the analysis of stability samples in pharmaceuticals industry. With the emergence of ICH guidelines, the requirement of setting up of stability indicating assay method (SIAM) has become more clearly requested. The guidelines explicitly need to conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry heat, etc. and separation and quantification of drug from degradation products.

Stability of a pharmaceutical ingredient depends on many factors which majorly include the following factors:

Temperature: High temperature expedite oxidation, reduction and hydrolysis reaction which lead to drug degradation

pH: Acidic and alkaline pH impact the rate of decomposition of most drug products. Depending on the amount of ionization drug degradation enlarges.

Moisture: Water help in catalysis of the degradation process.

Light: Influence drug stability through energy or thermal heat which lead to oxidation of components.

Drug Incompatibility: Some other ingredients which are existing in finished pharmaceutical drug product can manifest reactions between ingredients itself or between these components and cover of the container.

Stability protocol: The stability protocol contain for testing sample store one or more storage conditions .The protocol specifications can differentiate significantly from one product to another.(8)

Stability indicating assay method (SIAM)

The stability indicating method (SIM) is an analytical technique used to evaluate the degradation of the active pharmaceutical ingredient (API) in medicinal products over time. As per an FDA guideline document, a stability Indicating method is a quantitative analytical procedure that has been validated and can be utilized to ascertain the temporal variations in the stability of drug substances and drug products. A stability indicating approach accurately measures fluctuations in the concentration of the active component without influence from other degradation products, impurities, or excipients. Stress tests are carried out to demonstrate the specificity of the devised method in assessing changes in drug ingredient concentration when there is little knowledge about likely degradation products. The development of a suitable stability indicating approach underpins the preformulation studies, stability studies, and establishment of suitable storage requirements. Contaminant separation and quantification are possible with the most

widely used RPHPLC and UV detector combo. The procedures for developing SIM on HPLC that complies with legal requirements are as follows.

1) sample generation -

In order to produce samples for SIM, the API is compelled to degrade under circumstances more harsh than accelerated degradation settings. It involves drug degradation in the hydrolytic, oxidative, photolytic, and thermal conditions mentioned earlier. Forced degradation of API in solid state and solution form is carried out to generate degradation products that are anticipated to emerge under real storage conditions. This sample is then used in the construction of a SIM.

2)Method development and optimization-

Many of the drug's physiochemical characteristics, including its pKa value, log P, solubility, and maximal rate of absorption, set the groundwork for the development of an HPLC approach. Log P and solubility help in the selection of the mobile phase and sample solvent, whereas pKa value helps in determining the pH of the mobile phase. The reverse phase column is the best option to start the sample component separation process because the degradation happens in an aqueous solution. The mobile phase, which consists of acetonitrile, water, and methanol in different ratios, can be used during the initial phases of separation. Methanol or acetonitrile should be selected for the organic phase based on the solubility of the analyte. The water: organic phase ratio can be first set at 50:50 to obtain a respectable separation of peaks, and as trials progress, pertinent modifications can be made. If the procedure is to be extended to liquid chromatography mass spectrometry (LC-MS), mobile phase buffer compatible with MS (trifluoroacetic acid and ammonium formate) can be added if additional buffer is required to improve peak symmetry and separation. Because different analytes respond differently to temperature changes, column temperature variation affects the approach's selectivity. It is best to have the temperature between 30 and 40°C to obtain optimum consistency. Depending on the analyst's solubility, either acetonitrile or methanol should be used for the organic phase. To achieve a reasonable peak separation, the water: organic phase ratio can be initially adjusted to 50:50. As the trials continue, relevant adjustments can be made. The mobile phase buffer compatible with MS (trifluoroacetic acid and ammonium for mate) can be added if more buffer is needed to improve peak symmetry and separation if the process is to be extended to liquid chromatography mass spectrometry (LC-MS). The selectivity of the technique is impacted by column temperature variation since various analytes react to temperature variations in different ways. For the optimal consistency, the temperature should be between 30 and 40°C.PDA provides information on the spectral peak's homogeneity, however it is in applicable to degradants with UV spectra similar to pharmaceuticals. Peak separation will be impacted by the indirect procedure, which involves changing the chromatographic parameters such the mobile phase ratio and column. Subsequently, the original and modified chromatographic condition spectra are compared. If the area percentage of the drug peak and the degradant peaks both stay constant, then the drug peak is homogeneous. It would be acceptable if it turned out that the coeluting degradant was not produced in conditions of rapid and extended storage. The process is then optimized t separate closely eluting peaks by adjusting the injection volume, column type, .mobile phase ratio, and flow rate.

3)Method validation:

Following creation, the SIM is verified for linearity, accuracy, precision, specificity, quantitation limit, . detection limit, robustness, and ruggedness in compliance with USP/ICH requirements. It is necessary to isolate, identify, and quantify the degradants that are found to be higher than the identification threshold (about 0.1%).If the technique fails to meet the validation acceptance conditions, it is changed and revalidated.

Advantages of HPLC over other methods used for SIAM development

- HPLC is the most widely used technique for the separation of analytes, impurities, and degradation of drug products.
- Limit of detection of analyte for HPLC is low as correlated to other techniques, i.e., it possesses greater sensitivity when juxtapose with other techniques used for development of SIAM.
- HPLC needed fewer time for separation and quantification of analytes and degradation Products.
- It gives upgrade resolution between the analytes and impurities with extensive variety of stationary phases.
- It is having more sensitivity for the detection of significant amounts of analytes and degradants as various detectors can be used for the determination of response.
- Another main advantage of HPLC is its reusable columns which can be utilized several times for the determination which makes it cost effective technique.
- The method is completely automated and quantification can also be performed using this technique.
- HPLC has been very widely immersed. It has gained popularity in stability studies due to its high-resolution capacity, sensitivity and specificity. Non-volatile, thermally unstable components can also be analyzed by using this technique. Thus, most of the SIAM have been established well using HPLC.

Conclusion:

Stability indicating method is an analytical technique that can distinguish between the primary active (unaltered) pharmaceutical components (API) against any deterioration product(s) of (decomposition) generated under specified storage circumstances when assessing stability epoch. In order to develop stability-indicating and degradant monitoring methods as part of a validation protocol, forced degradation studies are essential. Studies on forced degradation also offer priceless discernment in examining degradation products .The application of carefully planned and carried out forced degradation research will produce a sample is representative of the turn assist in creating the HPLC method that indicates stability. To maximize the stability indicating HPLC method for the detection of all potentially relevant degradants, chromatographic factors should be assessed. The right mobile phase and sample solvent must be discovered that provide appropriate stability and compatibility along with the interesting component and the contaminants and deteriorations. Thus, as a result steadiness signifying HPLC is a great tool for determining the contaminants and degradants in pharmaceutical goods.

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