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Stability Indicating HPLC Method Development: A Review

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ABSTRACT

High performance liquid chromatography (HPLC) is an important analytical tool for the stability analysis of pharmaceutical products. HPLC methods must be able to isolate, detect, and quantify various drug-related compounds that may arise during storage or manufacturing, as well as detect and identify any drug-related impurities that may be introduced during synthesis. This article discusses features and issues related to the design of HPLC methods that demonstrate the stability of pharmaceuticals. It promotes the understanding of pharmaceutical and pharmaceutical product chemistry and supports the development of analytical methods that demonstrate stability. Several chromatographic parameters were evaluated to optimize the detection of all potential contaminants. Appropriate solvents and mobile systems must be found that provide appropriate stability and compatibility with the components of interest and potential impurities and contaminants. The system should be carefully evaluated for its ability to distinguish between primary and secondary degradants. Forced degradation studies of the new chemical industry and pharmaceutical products are important to help, develop and demonstrate the specificity of these methods of demonstrating stability. Practical advice is given for creating a mandatory violation protocol at any stage of drug development and avoiding common pitfalls that can complicate data interpretation.

INTRODUCTION

Forced degradation exhibit's specificity when developing stability-indicating methods, particularly when the little information is available about potential degradation products. These studies also provide information about the degradation pathways and degradation products that could form during storage. Drug Stability testing requires precision of an analytical method that quantifies the active pharmaceutical ingredients (API) without interfering from degradation products, process impurities and other potential impurities. International Conference on Harmonization (ICH) guideline, what is required to establish stability-indicating assay method (SIAM) has become clear and mandatory. These guidelines explain forced degradation study below different conditions, like pH, light, oxidation, dry heat, etc. and the separation of drug from degradation products. High performance liquid chromatography (HPLC) is the most common, a precision analysis method that is often used for quantification and qualitative analysis of drug products and their use determine the stability of the drug product. [1,2]

IMPORTANCE OF STABILITY INDICATING HPLC METHOD

Quality control has become a difficult part of pharmaceutical production to reduce Batch-to-batch change and ensures quality. Today, stability is the key and the most important thing quality requirements for pharmaceutical products. Stable preparations have a direct emphasis on the quality of the product, assuring its precise delivery. In addition, the shelf life of drug formulation is based on research studies under normal and stress conditions. The ICH Q1A(R2) Drug Stability Testing Guidelines emphasize the analysis of samples of Active pharmaceutical products, which are under pressure, should be carried out, to establish their inherent stability characteristics, thus leading to identification of the degradation products through the use of validated stability-indicating analytical methods. Stability Indicating Assay Methods (SIAMs) is a specific method that analyses drugs in the presence of its degradation products, excipients and additives. [3,4,5]. The purpose of stability testing is to provide evidence on how a drug is stable or drugs that change over time under the influence of various environmental factors, such as temperature, humidity and light, and to establish a test time for the drug Shelf life of the substance or medicinal product and recommended storage conditions.

The FDA and ICH guidelines state the requirements for stability testing data to understand how to do so drug quality and drug product degradation studies requirements and scientific

needs during the development of medicine, it is not considered a requirement for formal stability program. It has become mandatory to perform stability study of a new drug before applying for registration. Stability study included long term studies (12 months) and accelerated (6 months) studies. But (6 months) can be done and milder conditions than those used in rapid studies. So, the study of degradation products as separation, identification and quantification will take even longer. Compared to stability studies, forced degradation studies help to create the most degradants things in a short time period, which is more than a few weeks. [6,7]

STABILITY INDICATING METHOD DEVELOPMENT STRATEGIES

Provide methods to produce the specific hydrolysis products required to support those methods. There is no sustainable development process "if not everything" that involves the analysis of growth plans for sustainability indicators. It is wise to consider the development of this system in a broad way before starting the actual research. Bakshi and Singh discussed several important issues related to the development of the system. Dolan [8] provided feedback on the stability of the measurements. Discussing Smella [9] from an organizational point of view.

Step I – Physicochemical properties of the drug

Knowledge of the physical and chemical properties of the API is essential to support process development. Information on various resources has been collected to extract relevant information from the planning program or literature review to support drug discovery, industrial drug specifications, spectral libraries and drug discovery reports. Information on the selection of materials for the study of dissociation constants, partition coefficients, fluorescence properties (if applicable), chromatographic behaviour, spectral properties, redox potential to prepare the initial test conditions and resistance, or information suggesting dissociation methods useful. [10,11]. Dissociation constants can be used to develop an effective test method to determine the optimal mobile pH for good separation. Fluorescence properties, spectroscopy, chromatography, and redox data can be used to determine the best way to measure the assay of interest. The structure of the analyte, particularly functional groups, reflects the potential active degradation sites and the drug's susceptibility to hydrolysis, oxidation, and pyrolysis. A compatibility test is done to determine the stability of the state when mixed with additives and lubricants and to determine the interaction of the

drug with the active ingredient (inactive). A first-level test should be conducted to determine the pattern for subsequent tests based on previous experience.

Step II – Setting up the initial HPLC conditions

The initial experimental conditions can be adapted from official or unofficial methods and documents as a starting point. Official methods published in the United States Pharmacopeia (USP) are considered appropriate and may be used for stability testing if they are shown to be stable and suitable for the intended purpose. New methods should be developed if there is no suitable method. The setting of the test mode should be based on the characteristics of the API and the impurity if known. Proper column and mobile system options are critical. So much information about different HPLC columns is available nowadays that it is possible to choose the right column for each API type. [21] One of the most useful sources of information about columns is the supplier's catalogue. Get appropriate separation conditions by selecting columns and mobile phase combinations. The development of computer-aided procedures can be of great help in rapidly improving the initial HPLC conditions. Since the goal at this time is to quickly develop HPLC conditions for such a test development process, scientists should focus on separating the important factors related substances instead of trying to achieve good resolution for all related substances. A proper experimental condition at the beginning will save a lot of time in subsequent development stage. [12]

Step III – Preparation of the samples required for method development

The SIMs development process to be developed by placing the API in a state that exceeds the standard used for stability testing. In addition to showing the specifications of the SIMs, resistance testing, also known as forced degradation, can also be used to provide information about degradation pathways in products that may develop during storage and help support the development of design, manufacturing and packaging. It's hard to get a realistic model during development. The concentration of API produces samples that contain products that can be produced under optimal safety conditions, which are used to produce SIMs. [13] In general, the goal of these studies is to reduce API by 5-10%. Perform forced degradation studies by thermolysis, hydrolysis, oxidation, photolysis and/or combined conditions. Each sample must be analysed using standard HPLC conditions with an appropriate detector, preferably a PDA detector. Although the most common dosage form – solid (tablet / capsule), semi-solid (cream / cream) or solution (cough syrup / ophthalmic solution) – is used solid phase extraction (SPE) for sample preparation, and especially for lead samples and as an alternative

to liquid-liquid extraction in many US Environmental Protection Agency (EPA) methods. [14]

Step IV – Developing Separation – Stability Indicating Chromatography

Prerequisites the most important factor in choosing a SIM-based chromatography base for a new device is to ensure the separation and identification of substances that have been degraded by solution. For this reason, a 1: 1 dilution of water: organic solvent is a good starting point as it increases the solubility of many of those related substances and ensures good dissolution of solid formulations. The second step is to obtain separation conditions so that we can identify as many different peaks as possible in the experimental sample set. The most common dissociation variables are solvent type, pH of the mobile phase, column type, and temperature. [15]

Isocratic or Gradient mode:- The choice between phase mode or phase mode depends on the number of active ingredients to be dissolved or separated. To determine whether calibration is required or whether a stoichiometric condition is appropriate, an initial slope is generated which is the ratio of the total calibration time to the difference in calibration time between the first and last component is calculated. Usually, the confirmation mode is used to produce the product and the gradient mode is used to check the stability. This is because isometric methods often involve passwords. No wear is checked unless the product initially molds within 15 minutes. Over time, a product is created that must be controlled. This requires a gradient method to properly dissolve the mixture. Therefore, the gradient method is a stable or structured method.

Solvent type:- The type of solvent (methanol, acetonitrile, tetrahydroflurane) affects the selectivity. The choice between methanol and acetonitrile depends on the solubility of the analyte and the buffer used. Tetrahydrofuran is the least polar of the three solvents and often leads to large selectivity changes and is incompatible with the short-wavelength detection required by most active materials. [10,16].

Mobile phase pH: -When filtering a sample in 100 mobile phase (organic), if the sample is placed in a vacuum volume, separation does not occur due to insufficient sample retention, but retention occurs because the solvent strength in the mobile phase decreases. Collisions between dissolved molecules between the conjugate and the mobile phase preparation: d. If Harry was confused. Separation should be attempted if there is another organic solvent of

different polarity or a mixture of the two. The target bandgap (K') for the melt should be 4 to 9 and the run time is about 15 minutes or up to 20 minutes for most conventional or static products. [10]

Role of the column and column temperature:- The heart of the HPLC system is the column. Column changes have the greatest impact on analytical design during method development. The three main components of an HPLC column are the material (plant), matrix, and stationary phase. Modern reverse-phase HPLC columns are designed using a column jacket coated with a spherical silica gel coated with a hydrophobic solid surface. The reaction of chlorosilanes with hydroxyl groups on the surface of silica gel introduces stationary phase into the matrix. In general, the characteristics of the stationary phase have the greatest influence on capacity, selectivity, efficiency and leaching factors. There are many types of solid matrices, including silica, polymer, alumina, and zirconium. Silica is the most commonly used matrix in HPLC columns. The silica matrix is hard, easily flexible, forms a solid bead, and does not bond under pressure. Silica is stable in most organic solvents and low pH systems. A short-term solution for solid silica support is to dissolve at a pH of 7 or higher. Recently, silica-based columns have been developed for use at high pH values. The shape, size and shape of the particles support the separation effect of silica. The smaller the particles, the more theoretical pages and the higher the separation efficiency. However, using smaller particles increases the reflux pressure during chromatography and increases the permeability of the column. For this reason, development work uses more than three or five pillars. The narrower particle distribution of the silica particles results in greater accuracy. Therefore, different combinations of columns of similar phase from different manufacturers or columns from the same company may have different separation properties due to different matrix manufacturing processes. The character of the place has been proven. [17]

Column temperature:- Column temperature control is important for long-term reproducibility of methods because temperature can affect selectivity. Expected temperatures in the range of 30-40°C are usually sufficient for good measurements. Using high temperatures can be beneficial for many reasons. First, working at high temperatures reduces the viscosity of the mobile system and reduces the pressure across the shaft. Low system pressure results in faster flow rates and faster scanning. Temperature can also affect the selection process because researchers react differently to different temperatures. Finally, using a horizontal beam eliminates the differences due to normal temperature changes around the axis. Temperature is a variable that can affect selection, but the effect is small. Also, k

generally decreases with increasing temperature of the neutral compound, but it is often referred to in the analysis of partial ionization. If there is a big difference in size or shape, it will be damaged. In general, it is better to use melting point than temperature to control selectivity. The effect is more dramatic. An increase of 1°C decreases k by 1% to 2%, but both the ionic and neutral samples show large temperature changes. The temperature may change during the process.

Peak purity:- Analysis of the maximum purity (or maximum asymmetry) of the central peak is an important part of SIM's validation to determine the presence of contamination under the central peak. Linear analysis can be performed using a PDA16, LC-MS17 or LC-NMR detector. However, PDA works best with hydrophobic materials with a different UV spectrum than the drug itself. If the molecular weight is similar to Detromere, or if the existing API prevents ionization digester, the digester will not work. Indirect estimation of peak purity can be obtained by changing one or more chromatographic parameters (column, mobile phase, gradient method, etc.) that affect the separation selectivity. The resulting impurity profile is compared to the original method. If the two classes have the highest rate of decomposition and the percentage of the main elements is the same in both classes, then one can logically conclude that all the main elements are fixed. This type of automated method has been successfully used in sieves with different dimensions with different columns and devices to analyse the parameters for impurity analysis [22,23,24]. Other methods for further separation methods have similar objectives as the chapter on LiLi Chromatography, Thin Layer Chromatography (TLC), Natural Phase HPLC, Capillary Electrophoresis (CE) and Supercritical Liquid Chromatography (SFC). [18]

Step V - Method optimization

After obtaining suitable separation, experimental conditions should be optimized to obtain the desired separation and sensitivity. The experimental conditions for the stability indicating assay are achieved through a deliberate/systematic examination of parameters such as pH (if ionic), components and proportions of the mobile phase, gradient, flow rate, temperature, sample volume, injection volume and diluent solvent type. [12]

Step VI – Validation of Analytical Methods

Methods should be validated in accordance with USP/ICH guidelines to demonstrate accuracy, precision, specificity, linearity, range, limit of detection, limit of quantification,

robustness and robustness of the method. A verification plan should be developed and acceptance criteria defined. If a degradation product exceeds the identification threshold (usually 0.1%), it must be isolated, identified, characterized and identified. [19,20]. There are several techniques that can be used to identify and characterize contaminants and degradation products such as HPLC with photodiode array detector (PDA), infrared spectroscopy (IR), elemental analysis, mass spectrometry (MS), nuclear magnetic resonance (NMR), GC/MS, LC/MS, LC/MS/MS Similar to LC/NMR method development and validation, it is a cyclical activity. If new problems with the method are discovered during the validation process or the results do not meet the acceptance criteria, the method must be modified and revalidated before the method can be used.

FORCED DEGRADATION STUDIES IN STABILITY-INDICATING METHOD DEVELOPMENT: -

Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus results degradation products that can be studied to determine drug stability. The ICH guidelines state that stress testing aims to identify the likely degradation products which helps in determining the internal stability of drug molecules and establishing degradation pathways, and to validate the stability indicating procedures used. The chemical stability of pharmaceutical molecules is a critical topic because it affects people safety and efficacy of drugs. The study of degradation products like separation, identification and quantitation would take even more time. Compared to stability studies, forced degradation studies help in generating degradants in a shorter period of time, mainly a few weeks. Samples taken from forced degradation can be used to develop the stability indicating method which can be applied later for the analysis of samples generated from accelerated and long term stability studies. [25]

OBJECTIVES OF FORCED DEGRADATION STUDY: -

a) To establish methods of degradation drug substances and drug product.

b) To distinguish degradation products related to drug products and existing ones produced from non-drug products and processing.

c) To elucidate the structure of degradation products.

d) To determine the internal stability of the drug in the formulation.

e) Exposure to degradation processes such as hydrolysis, oxidation, thermolysis or photolysis of drug substance and drug product.

f) To establish stability indicating nature of the developed method.

g) To understand the chemical properties of drug molecules.

h) To create a stable system.

i) To produce degradation profile as shown in a stability study under ICH conditions.

j) To solve problems related to stability. [26,27,28]

DEGRADATION CONDITIONS: -

A typical study involves four main degradation processes: heat, hydrolysis, oxidative and photolytic damage. The choice of reagents is appropriate according to the dosage acid, base or oxidizing agent that changes in conditions (such as temperature) and length of exposure the preferred level of degradation can be obtained. [29,30]

Table No. 1: Conditions generally employed for forced degradation

Stress Type	Condition	Time
Acid hydrolysis	0.1N HCL (upto 5.0 N)	1-7 Days
Base hydrolysis	0.1N NaOH/KOH (upto 5.0 N)	1-7 Days
Thermal hydrolysis	70°C	1-7 Days
Oxidative solution	O2+ Initiator (AIBN) in ACN/water, 80/20, 40°C, RT, protected from light	Few hrs to 7 days
Thermal	70°C	Upto 2 weeks
Thermal/Humidity	70°C/75%RH	Upto 2 weeks

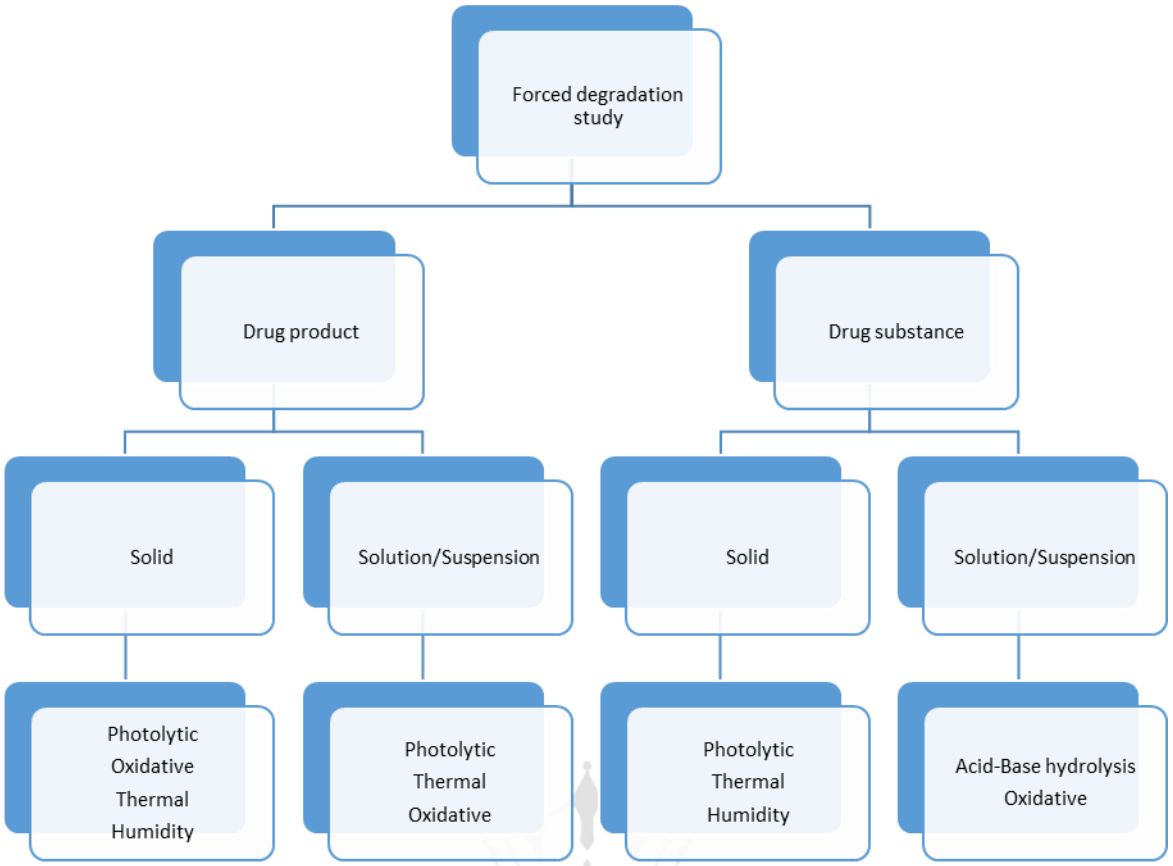

Figure No. 1: An Illuminative Diagram Showing the Distinct Forced Degradation to be used for Drug Substance and Drug Product [7]

CONCLUSION:-

A stability indicating method (SIMs Method) is an analytical procedure capable of distinguishing the main (intact) active pharmaceutical ingredient (API) from all degradation (decomposition) products formed under defined storage conditions during the stability assessment period.

Forced degradation studies are essential in the development of stability-indicating and degradant-monitoring methods as part of a validation protocol. It also gives an overview of the study of degradation products. The use of well-designed and executed forced degradation study will promote a representative sample that will in turn help to establish stability-indicating HPLC method.

Chromatographic parameters should be evaluated to improve the SIM-HPLC method to detect all relevant potential degradation products. A suitable sample solvent and mobile phase should be found that will ensure sufficient stability and compatibility. It includes not only the desired component, but also impurities and degradants. The resulting SIM-HPLC is therefore very suitable for finding degradants and impurities in pharmaceuticals.

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