Stability Indicating HPLC Method Development - A Review

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ABSTRACT

High-performance liquid chromatography (HPLC) is an essential analytical tool for evaluating drug stability. HPLC methods must be able to isolate, detect, and quantify drug-related degradation products that may form during storage or production, and identify drug-related impurities that may form during synthesis. .. This article describes strategies and challenges for designing HPLC methods to demonstrate drug stability. It will deepen our understanding of drugs and medicinal chemistry and demonstrate advances in stability that reflect an analytical approach. Several important chromatographic parameters were investigated to improve the detection of potentially related degradants. It is necessary to find suitable solvent and mobile phase samples that provide sufficient stability and compatibility with each component and potential impurities and degradants. This method should be carefully considered as it has the ability to distinguish between primary and secondary decomposers. The study of forced destruction of chemicals and new drugs is essential for the development and characterization of these immobilization methods. Practical guidance is provided at each stage of drug development to develop a forced-disposal protocol and avoid common issues that might impede data interpretation.

KEYWORDS: HPLC, Forced degradation, Stability indicating method

INTRODUCTION

Drug stability testing requires an accurate analytical method to determine the amount of a pharmaceutically active substance (API) without the interference of degradants, process contaminants, and other potential contaminants. With the introduction of guidelines from the International Council for Coordination Harmonization (ICH), the need to establish a Stability Assessment Methodology (SIAM) has become increasingly clear. The guidelines specifically call for essential degradation studies to be performed under various conditions such as pH, light, oxidation and dry heat. It separates the drug from its breakdown products. Stability Demonstration Methods Stability demonstration experiments are quantitative analytical methods that can detect changes in drugs and drug properties over time. Stability tests accurately determine the active ingredient without affecting the handling of degradation products, impurities, auxiliary ingredients or other potential impurities. In addition to demonstrating specificity, forced degradation studies are used to facilitate the formulation, production and development of packaging by identifying degradation pathways and degradation products of active ingredients that may occur during storage. These studies often

STABILITY INDICATING METHOD DEVELOPMENT STRATEGIES

Provide methods to produce specific hydrolysis products that are needed to validate methods.

There is no "but if not all" sustainable development formula that relates to the analysis of growth strategies for sustainability indicators. It is advisable to look at the development of this method from a broader perspective before embarking on actual experiments. Bakshi and Singh discussed several important issues related to the development of

fixation techniques. Dolan 3 provided feedback on the stability of the measurements. Discussing Smella 4 from an organizational point of view.
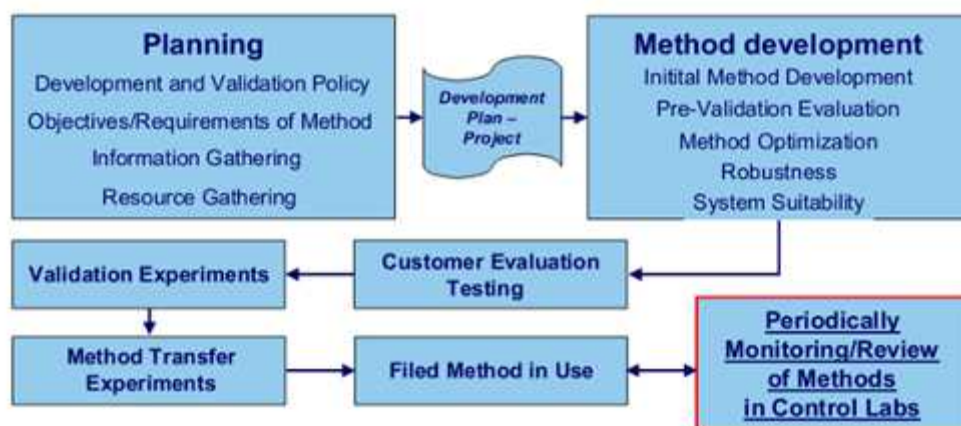

Figure 1: Overview of the Method Development Process

Step I - Understand the chemistry/ Physicochemical properties of drug

Knowledge of physical and chemical properties and API expression is essential to support method development. Information on various properties was collected to generate relevant information through systematic programs or literature searches to support drug discovery, company drug specifications, spectral libraries, and drug discovery reports. Information for element selection for study of dissociation constants, partition coefficients, fluorescence properties (if applicable), chromatographic behavior, spectral properties, redox potentials to prepare initial test conditions, and stresses, or information suggesting useful dissociation mechanisms. [5,6] Dissociation constants and dissociation constants can be used to develop efficient sampling methods to determine optimal mobile phase pH for good separation. Fluorescence properties, spectroscopy, chromatography, and redox data can be used to determine the best way to measure and 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. Compatibility testing is performed to evaluate the stability of the state when mixed with traditional adjuvants and lubricants and to determine the interaction of the drug with the raw (inactive) ingredient. First-class testing should be performed to determine procedures for subsequent experiments based on past experience.

Step II – Set up Preliminary HPLC condition

The first empirical situation can be adopted from the literature in a formal or informal manner and as a starting point. Officially published USP methods are acceptable and can be used for stability testing after stability and suitability for use have been demonstrated. If that path is not available, you must create a new path. Test conditions should be based on API properties and impurities, if known. Choosing the right tree and the right mobile phase is very important. There is a lot of information about many HPLC columns today, and you can choose the right column for each API type. Choose the column and phase group you are moving to get the correct separation position. The development of computational methods is very useful for rapidly developing initial HPLC conditions. As the goal of this phase is to rapidly develop HPLC conditions for subsequent method development experiments, scientists will focus on isolating important related substances to improve the accuracy of all related substances. A good beta level saves a lot of time in the early development phase.[8]

Step III – Preparation of samples required for method development

SIM cards are typically designed using API controls under conditions other than those used for rapid reliability testing. In addition to being displayed on the SIM card, it can generate experimental stress, also known as forced disassembly, to provide information about the product and disassembly pathway during storage to aid development. Used package. In the early stages of development, it is difficult to find real examples. Focusing on the API creates a more realistic prototype in terms of the memory used to develop SIM cards. [9] These studies typically aim to analyze 5-10% of APIs. Conduct pyrolysis, hydrolysis, oxidation, photolysis, and/or forced degradation studies under harvest conditions. Forced dissolution samples should be tested under initial HPLC conditions using appropriate reagents, preferably PDA reagents. Common formulations - solid (tablets/capsules), semi-solids (ointments/creams) or slurries (cough syrup/ophthalmic solutions) - solid phase extraction (SPE) are used for sample preparation, but it is very important that they are used specifically for the sample. One is for replacement. director. .. in many ways the US Environmental Protection Agency (EPA).[10]

Step IV – Developing Separation – Stability Indicating Chromatography
Prerequisites The most important factor in choosing a SIM card chromatography basis for a new device is to ensure the separation and identification of degraded substances in solution. To this end, a 1:1 dilution of water:organic solvent is a good starting point as it increases the solubility of most of the 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.[1]

➤ **Isocratic or Gradient Mode** The choice between step mode or step mode depends on the number of active ingredients to be dissolved or separated. To determine if calibration is required or if a stoichiometric mode is appropriate, an initial slope is generated and the ratio of the total calibration time to the difference in calibration time between the first and last components is calculated. The calculated ratio is 0.25, and a slope is useful as shown in Figure 2. Typically, conformal mode is used to launch a product, and gradient mode is used to evaluate stability. This is because isometric methods usually involve passwords. No wear is checked unless the product initially molds within 15 minutes. Over time, decomposition products are formed that must be controlled. This requires a gradient process to completely dissolve the mixture. Thus, the gradient process is a stabilization or conditioning process.
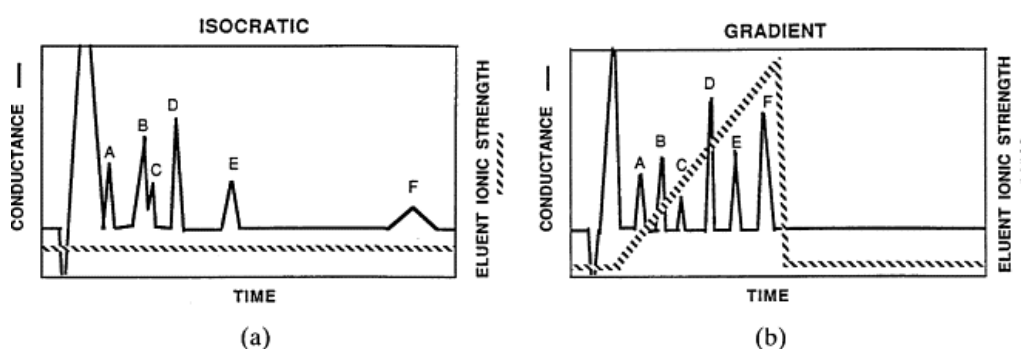

Figure 2: Isocratic or Gradient?

➤ **Solvent type** The solvent type (methanol, acetonitrile, tetrahydrofuran) affects the selectivity. The choice between methanol and acetonitrile depends on the solubility of the analysed material and the buffer used. Tetrahydrofuran is the least polar of these three solvents, often produces large changes in selectivity, and is not compatible with the short wavelength detection required for most active substances.[5, 12]

➤ **Mobile phasepH** When filtering a sample in mobile phase 100 (organic), if the sample is placed in a volume under vacuum, separation does not occur due to insufficient sample retention, but retention occurs when the solvent strength decreases in the mobile phase. Collision between dissolved molecules between the conjugate and the mobile phase preparation: d. If Harry is complicated. Separation should be attempted if there is another organic solvent of different polarity, or a mixture of the two organic substances. The target bandgap (K') for the dissolved material should be 4-9 and the run time is about 15 minutes or up to 20 minutes for most conventional or stationary products.[5]

➤ **Role of the column and column temperature** The heart of the HPLC system is the column. Column changes have the greatest impact on analytical accuracy during method development. The three main components of an HPLC column are material (shaft housing), matrix, and stationary phase. Typically, modern reverse-phase HPLC columns are produced by filling the column jacket with spherical silica gel coated with a hydrophobic solid phase. The reaction of chlorosilanes with hydroxyl groups on the silica gel surface introduces a 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 several types of solid-phase matrices, including silica, polymer, alumina, and zirconium. Silica is the most commonly used matrix in HPLC columns. The silica matrix is strong, easily deformed, formed into a rigid bead shape, and is not compressed under pressure. Silica is chemically stable in most organic solvents and low pH systems. A short-term solution for solid silica supports is to melt at pH 7 or higher. Recently, silica-based columns have been developed for use at high pH values. The type, size and shape of the particles support the silica separation effect. 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 access to the column. For this reason, development work uses more than three or five pillars. A narrower particle size distribution of the silica particles results in higher accuracy. Thus, different

combinations of similar phase columns from different manufacturers or columns from the same plant can have very different separation properties due to different matrix manufacturing processes. The character of the scene has been proven.[14]

➤ **Column temperature** Controlling column temperature is important for long-term reproducibility of methods as temperature can affect selectivity. A target temperature in the range of 30-40 °C is usually sufficient for good measurements. Using a higher temperature can be beneficial for a number of reasons. First, working at a higher ambient temperature reduces the viscosity of the mobile phase and reduces the pressure across the shaft. Low system pressure results in faster flow rates and faster analysis. Temperature can also affect selectivity models because analytes react differently at different temperatures. Finally, using a vertical oven eliminates deviations due to normal temperature fluctuations around the axis. Temperature is a variable that can affect selectivity, but the effect is relatively small. Also, k generally decreases with increasing temperature of neutral compounds, but is less pronounced in partial ionization analyses. If there is a big difference in size or shape, it will take some damage. In general, it is better to use the strength of the solvent rather than the temperature to control the selectivity. The effect is more dramatic. An increase of 1 °C decreases k from 1% to 2%, and the ionic and neutral samples show large changes in temperature. Possible temperature changes during the process.

➤ **Peak Purity** Analyzing the peak purity (or peak asymmetry) of the central peak is an important part of validating the SIM card to assess the presence of contaminants below the central peak. Direct linear evaluation can be performed using PDA16, LC-MS17 or LC-NMR detection. However, PDA only works well with hydrophobic substances that have a different UV spectrum than the drug itself. If the molecular weight is the same as that of Detromere, or if the ionization of the digester is suppressed by the existing API, the digester will not work. An indirect assessment of peak purity can be accomplished by modifying one or more chromatographic parameters (column, mobile phase, gradient structure, etc.) that significantly affect separation selectivity. The resulting impurity profile is compared to the original method. If the two classes have the same number of decomposition peaks and the percentage areas of the principal components are the same in both classes, then we can logically conclude that all decompositions are solved by the principal components. Automated versions of this approach have been successfully used on multidimensional sieves equipped with various columns and tools to systematically assess mass for the analysis of impurities 18, 19, 20. Other approaches to alternative separation techniques with similar objectives as the chapter on LiLi chromatography, thin layer chromatography (TLC), natural phase HPLC, capillary electrophoresis (CE) and supercritical liquid chromatography (SFC).[21].

Step V –Method Optimization
The experimental conditions should be optimized to get desired separations and sensitivity after getting appropriate separations. Stability-indicating assay experimental conditions will be achieved through planned/systematic examination on parameters including pH (if ionic), mobile phase components and ratio, gradient, flow rate, temperature, sample amounts, injection volume, and diluents solvent type.[8]

Step VI – Validation of analytical method
The method must be validated according to USP/ICH guidelines to show the accuracy, precision, specificity, linearity, range, detection limit, quantification limit, robustness and robustness of the method. A verification plan must be developed and acceptance criteria must be defined. If the degradation product is above the identification threshold (usually 0.1%), it is necessary to separate, identify, characterize and identify it. [22, 23] There are a variety of techniques that can be used to identify and characterize impurities and degradation products, such as HPLC with PDA (photodiode array) detector, IR (infrared) spectroscopy, elemental analysis, MS (mass spectrometry), NMR (resonance nuclear magnetic field)), GC/MS, LC/MS, LC/MS/MS, LC/NMR, etc. Method development and validation are cyclical activities. If new problems are found in the method or the results do not meet the acceptance criteria during the verification process, the method should be modified and re-verified until the method is suitable for use.

FORCED DEGRADATION STUDIES IN STABILITY-INDICATING METHOD DEVELOPMENT
Forced degradation studies usually involve exposing a drug or a representative sample of a drug to light, heat, humidity, acid/base hydrolysis, and oxidation, and other related pressure conditions. These experiments play an important role in the drug development process to promote: the development of stability indicator methods, the design of drug formulations, the selection of storage and packaging conditions, and a better understanding of the potential responsibilities of drug molecule chemistry and stability- Related issues are resolved. 9.24-26 The

mandatory degradation of APIs and products (in addition to establishing specificity) will also provide the following information:
1. Determination of degradation pathways of drug substances and drug products;
2. Discernment of degradation products in formulations that are related to drug substances versus those that are related to non-drug substances(e.g., excipients);
3. Structure elucidation of degradation products;
4. Determination of the intrinsic stability of a drug substance molecule in solution and solid state;
5. reveal the thermolytic, hydrolytic, oxidative, and photolytic degradation mechanism of the drug substance and drug product.[27]

According to the guidance documents of ICH and FDA, mandatory degradation studies are mainly used for three purposes: to provide assessment of the stability of drug substances or preparations; to clarify the possible degradation pathways of active pharmaceutical ingredients in drug substances or drug products; and to investigate applicable to drug substances and drug products. The stability index capability of the drug analysis program.

Although FDA Guide 28 and ICH Guide provide useful definitions and general comments on mandatory degradation studies, their guidelines on scope, timing, and best practices are very general and lack details. Test conditions and schedule for conducting research related to the drug development phase.

Experimental Design to Forced Degradation Studies Study protocol
A general protocol for conducting forced degradation studies, shown in figure 3 is arranged according to the type of test material (drug substance, drug product) and the type of degradation (hydrolysis, oxidation, etc.)
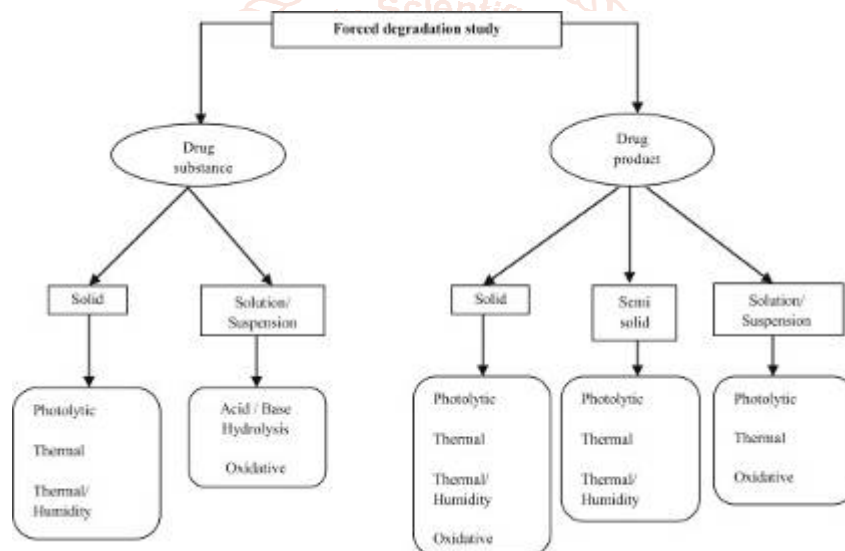

Figure 3: An illustrative diagram showing the different forced degradation conditions to be used for drug substance and drug product

Condition for stress Testing
Initial experiments should focus on determining the conditions that will degrade the drug by approximately 10%. Table 1 summarizes the different pressure conditions and exposure times commonly used for forced degradation. The concentration of the drug in the pressure sample solution will affect the final target degradation level achieved. Dilute sample concentrations generally produce more extensive degradation than more concentrated solutions, as shown in Figure 4. Therefore, reducing the drug concentration when necessary can help increase degradation.[16,17]

Table 1: Conditions generally employed for forced degradation

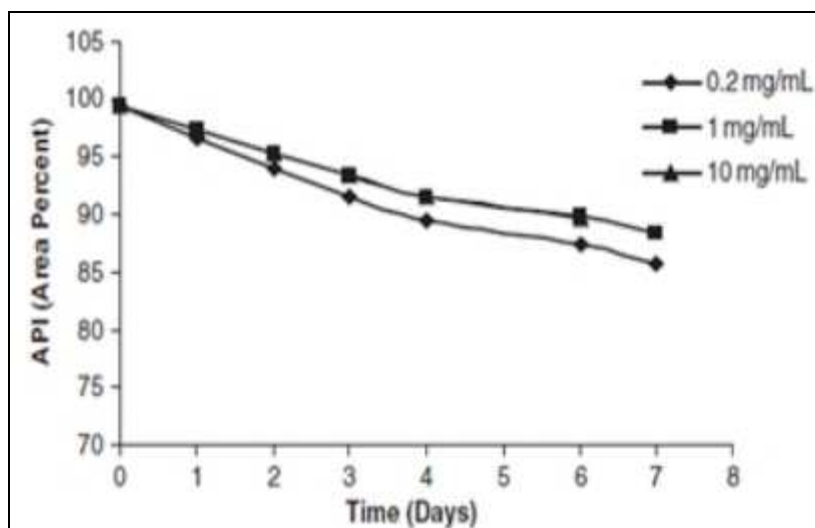| Stress Type	Condition	Time
Acid hydrolysis	0.1 N HCL (upto 5.0N)	1-7 days
Base Hydrolysis	0.1 N naOH/KOH (upto 5.0 N)	1-7 days
Thermal Hydrolysis	70 ^{0}C	1-7 days
Oxidative Solution	O_2 + Initiator (AIBN) in ACN/water, 80/20, 40^{0}C, RT, protected from light	Few hrs to 7 days
Thermal	70^{0}C	Upto 2 weeks
Thermal / Humidity	70^{0}C/75%RH	Upto 2 weeks

Figure 4: Thermal hydrolysis profile of an API (Structure not shown) at 700C: degradation vs. time at three sample concentrations

Timeline for conducting studies

The ICH guidelines do not mention any regulatory requirements for mandatory degradation studies in the first or second phase of development. There are good reasons to initiate the forced degradation study of drug substances in the first phase. The most important reason is to support the development of a highly discriminatory preliminary method because it can detect most, if not all, degradation products. . This method will have stability indicator capabilities, and at this stage only requires minimal verification. The compulsory degradation study of APIs and preparations should be completed before the registration stability study. It will be helpful to determine the main degradation products at that time.[23,24]

CONCLUSION

The ICH guidelines do not mention any regulatory requirements for mandatory degradation studies in the first or second phase of development. There are good reasons to initiate the forced degradation study of drug substances in the first phase. The most important reason is to support the development of a highly discriminatory preliminary method because it can detect most, if not all, degradation products. . This method will have stability indicator capabilities, and at this stage only requires minimal verification. The compulsory degradation study of APIs and preparations should be completed before the registration stability study. It will be helpful to determine the main degradation products at that time.

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