IJPSR (2012), Vol. 3, Issue 09 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 May, 2012; received in revised form 07 June, 2012; accepted 17 August, 2012

STABILITY INDICATING HPLC METHOD DEVELOPMENT: A REVIEW

Bhoomi P. Shah*, Suresh Jain, Krishna K. Prajapati and Nasimabanu Y. Mansuri

Department of Quality Assurance, Baroda College of Pharmacy, Vadodara, Gujarat, India

Keywords:
High performance liquid chromatography,
Stability indicating method,
Drug substance,
Forced degradation

Correspondence to Author:

Bhoomi P. Shah

Department of Quality Assurance, Baroda College of Pharmacy, Vadodara, Gujarat, India

E-mail: bhumi0610@gmail.com

ABSTRACT

High performance liquid chromatography is one of the most accurate methods widely used for the quantitative as well as qualitative analysis of drug product 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 product. This article discusses the strategies and issues regarding the development of stability indicating HPLC system for drug substance**.** A number of key chromatographic factors were evaluated in order to optimize the detection of all potentially relevant degradants. The method should be carefully examined for its ability to distinguish the primary drug components from the impurities. New chemical entities and drug products must undergo forced degradation studies which would be helpful in developing and demonstrating the specificity of such stability indicating methods. At every stage of drug development practical recommendations are provided which will help to avoid failures.

INTRODUCTION: Most of the optimization of HPLC method development have been focused on the optimization of HPLC conditions. This article will look at this topic from other perspectives. Forced degradation or stress testing is undertaken to demonstrate specificity when developing stability-indicating methods, particularly when 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.

Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development, manufacturing and packaging, in which knowledge of chemical behavior can be used to improve a drug product. Stability testing of drug substance requires an accurate analytical method that quantitates active pharmaceutical ingredients (API) without interference from degradation products, process impurities and other potential impurities [1]. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability-indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry heat, etc. and separation of drug from degradation products.

QUICK RESPONSE CODE

IJPSR:
ICV- 4.57

Website:
www.ijpsr.com

Stability Indicating Method Development Statergies: There is no "one set fits all" formula for developing stability indicating analytical method. Before beginning with actual experimentation it would be advantageous to view method development from a broader perspective. Bakshi and Singh [2] reviewed and discussed some critical issues about developing stability indicating methods. Dolan [3] made comments and suggestions on stability indicating assays. Smela [4] discussed from regulatory point of view about stability indicating analytical methods. The method development process can be visualized from a high-level process map perspective better to define the general steps encountered to achieving the end product, stability-indicating method. The following is a discussion of a general idea for designing stability indicating analytical method.

There are 3 critical components for a HPLC method are: sample preparation, HPLC analysis and standardization (calculations). During the preliminary method development stage, all individual components should be investigated before the final method optimization. This gives the scientist a chance to critically evaluate the method performance in each component and streamline the final method optimization.

Define Method Objectives: There is no absolute end to the method development process. The question is what is the "acceptable method performance"? The acceptable method performance is determined by the objectives set in this step. This is one of the most important considerations often overlooked by

scientists. In this section, the different end points (i.e., expectations) will be discussed.

A. **Analytes**: For a related substance method, determining the "significant and relevant" related substances is very critical. With limited experience with the drug product, a good way to determine the significant related substances is to look at the degradation products observed during stress testing. Significant degradation products observed during stress testing should be investigated in the method development. Based on the current ICH guidelines on specifications, the related substances method for active pharmaceutical ingredients (API) should focus on both the API degradation products and synthetic impurities, while the same method for drug products should focus only on the degradation products. In general practice, unless there are any special toxicology concerns, related substances below the limit of quantitation (LOQ) should not be reported and therefore should not be investigated. In this stage, relevant related substances should be separated into 2 groups:

   - Significant related substances: Linearity, accuracy and response factors should be established for the significant related substances during the method validation. To limit the workload during method development, usually 3 or less significant related substances should be selected in a method.
   - Other related substances: These are potential degradation products that are not significant in amount. The developed HPLC conditions only need to provide good resolution for these related substances to show that they do not exist in significant levels.

B. **Resolution (Rs):** A stability indicating method must resolve all significant degradation products from each other. Typically the minimum requirement for baseline resolution is 1.5. This limit is valid only for 2 Gaussian-shape peaks of equal size. In actual method development, Rs = 2.0 should be used as a minimum to account for day to day variability, non-ideal peak shapes and differences in peak sizes.

C. **Limit of Quantitation (LOQ):** The desired method LOQ is related to the ICH reporting limits. If the corresponding ICH reporting limit is 0.1%, the method LOQ should be 0.05% or less to ensure the results are accurate up to one decimal place. However, it is of little value to develop a method with an LOQ much below this level in standard practice because when the method is too sensitive, method precision and accuracy are compromised.

D. **Precision, Accuracy**: Expectations for precision and accuracy should be determined on a case by case basis. For a typical related substance method, the RSD of 6 replicates should be less than 10%. Accuracy should be within 70 % to 130% of theory at the LOQ level.

E. **Analysis time:** A run time of about 5-10 minutes per injection is sufficient in most routine related substance analyses. Unless the method is intended to support a high-volume assay, shortening the run time further is not recommended as it may compromise the method performance in other aspects (e.g., specificity, precision and accuracy).

F. **Adaptability for Automation:** For methods that are likely to be used in a high sample volume application, it is very important for the method to be "automatable". The manual sample preparation procedure should be easy to perform. This will ensure the sample preparation can be automated in common sample preparation workstations.

Understand the Chemistry/Physicochemical Properties of Drug: Knowledge of the physicochemical properties of API is very useful tool. Information about dissociation constants and partition coefficients which are used to develop an efficient sample extraction scheme and determine the optimum PH in mobile phase to achieve good separation. Information about fluorescent properties (if any), chromatographic behavior, Spectro-photometric properties, oxidation-reduction potentials are used to determine the best means of measuring and quantifying the analyte. These all properties useful in setting up preliminary experimental condition and also helpful in selecting the condition of stress studies or possibly in proposing degradation mechanism [5].

Compatibility studies are performed to assess the stability of the when mixed with common excipients and lubricants as well as to determine any interaction between the drug and the (inactive) raw materials [6].

Initial Hplc Conditions: Official methods published in the United States Pharmacopeia (USP) are considered validated and can be used for stability testing if it is proved stability indicating and suitable for intended purposes. Establishing experimental conditions should be based on the properties of API and impurities if known. Proper column and mobile phase selection is very critical.

Copious information about various HPLC columns is from catalogs of vendors and and it is possible to select a right column for any kind of API [7]. Select the appropriate mobile phase combination and column for the separation. Computer assisted method development can be very helpful in developing the preliminary HPLC conditions quickly. A proper experimental condition at the beginning will save a lot of time in subsequent development stage [8].

Sample Preparation For Method Development: Forced degradation also referred as SIMS, also can be used to provide information about degradation pathways and products that could form during storage and help facilitate formulation development, manufacturing, and packaging. It is hard to get actual representative samples in the early stage of development. Stressing the API generates the sample that contains the products most likely to form under most realistic storage conditions, which is in turn used to develop the SIM [9].

For most samples, storage for as long as two weeks at 80 °C, or six weeks at 60 °C, is used. Samples should be stored in appropriate vessels that allow sampling at timed intervals and that protect and preserve the integrity of the sample. Thermostated and humidity-controlled ovens also should be employed. Generally, the goal of these studies is to degrade the API 5–10 %. Any more than this and relevant compounds can be destroyed, or irrelevant degradation products produced (for example, degradation products of the degradation products). Any less, and important products might be missed.

Experience and data obtained from studies performed previously on related compounds also should be used when developing new protocols. The goal of these studies is to degrade the API 5-10 %.. Each forced degradation sample should be analyzed by using the preliminary HPLC conditions with suitable detector, most preferably PDA detector. While the typical dosage form-solid (tablet/capsule), semisolid (ointment/cream), or solution (cough syrup/ ophthalmic solution)- utilizes a solid-phase extraction (SPE) for sample preparation, especially for biosamples and as an alternative to liquid-liquid extractions in many U.S. Environmental Protection Agency (EPA) methods [10].

Developing Separation- Stability-indicating Chromatography Conditions: In selecting initial chromatographic conditions for a SIM of a new entity, most important is to make sure that degradants are in solution, separated, and detected. To this effect, a diluents of 1:1 water: organic solvent is a good starting point as it will increase the likelihood of solubility of most related materials and ensure proper disintegration of solid dosage forms [11]. The second step is to obtain separation conditions that allow the determination of as many distinct peaks as possible from the set of test samples. The most common separation variables include solvent type, mobile phase PH, column type and temperature [12].

Isocratic or Gradient Mode: It is usually preferred to work in isocratic conditions, whereby the mobile phase composition remains constant. The system and column are equilibrated all the time and does not suffer from fast chemical changes. However, the demands from HPLC analysis has increased and the samples are usually complex in nature, the HPLC systems has evolved into very robust reliable machines, and the columns are manufactured to provide thousands of injections, therefore, in recent years the majority of the chromatographic runs has been based on a composition gradient in the mobile phase.

In a gradient work the solvent strength is increased with time during the chromatographic run.Selection of isocratic or gradient mode depends on the number of active components to be resolved or separated. In deciding whether a gradient would be required or whether isocratic mode would be adequate, an initial gradient run is performed, and the ratio between the total gradient time and the difference in gradient time between the first and last components are calculate.

The calculated ratio is <0.25, isocratic is adequate; when the ratio is >0.25, gradient would be beneficial [13]. Generally, Isocratic mode is used for product release and gradient mode for stability assessment because the isocratic method has generally a say less than 15 minutes, and no degradation product would be monitored, assuming that none are formed initially. With time the degradation products are formed and must be monitored, which requires a gradient method to resolve completely the mixture.

The gradient method, then, would be the stability or regulatory method.

Solvent type: The starting solvent selected for a given separation can be chosen by matching the relative polarity of the solvent to that of the sample. This is done as a first approximation by selecting the solvent to match the most polar functional group on the sample molecule (e.g. alcohols for OH, amines for NH_2, etc.). From this attempt, the separation can be refined by the following procedure:

1. If the sample appears at the solvent front then the solvent is too polar to allow the adsorbent to retard the sample. Go to a solvent higher up (lower polarity) on the scale.

2. Conversely if the sample does not appear in a reasonable time go to a solvent or solvent blend lower down (higher polarity) on the scale.

Solvent type (methanol, acetonitrile and tetrahydro-furan) will affect selectivity. The choice between methanol and acetonitrile may be dependent on the solubility of the analyte as well as the buffer used. Tetrahydrofuran is least polar among these three solvent, often responsible for large changes in selectivity and is also incompatible with the low wavelength detection required for most pharmaceutical compounds [7, 12].

Mobile phase pH: Most pharmaceutical compounds contains ionisable functionalities such as amino,pyridine and carboxylic acid.Introduction of new packing material that are stable over a wide range of pH up to pH 12 allow for a broader applicability of a mobile phase pH as a retention/selectivity adjustment parameter [14].

When the sample is eluted with a mobile phase of 100% (organic), there is no separation, as the sample is eluted in the void volume. This is because the sample is not retained; but retention is observed when the mobile phase solvent strength is decreased to allow equilibrium competition of the solute molecules between the bonded phase and the mobile phase. When the separation is complex, that is, many components are to be separated, and when the

solvent strength is decreased and there is still no resolution between two close peaks, another organic solvent of a different polarity or even a mixture of two organics may need to be tried to effect separation. Additionally, mobile phase optimization can be enhanced in combination with bonded phase optimization (i.e., substituting C18/C8 with cyano or phenyl). A goal for the band spacing of a solute (K') should be in the range of 4 to 9 and a run time of about 15 minutes or 20 minutes at most for most routine product release or stability runs [7].

Role of the Column and Column Temperature:

Selecting an HPLC Column: The heart of a HPLC system is the column. Changing a column will have the greatest effect on the resolution of analytes during method development. Choosing the best column for application requires consideration of stationary phase chemistry, retention capacity, particle size, and column dimensions. The three main components of an HPLC column are the hardware (column housing), the matrix, and the stationary phase. Generally, modern reverse phase HPLC columns are made by packing the column housing with spherical silica gel beads which are coated with the hydrophobic stationary phase.

The stationary phase is introduced to the matrix by reacting a chlorosilane with the hydroxyl groups present on the silica gel surface. In general, the nature of stationary phase has the greatest effect on capacity factor, selectivity, efficiency and elution. Identifying the best stationary phase for separation is the most critical step of column selection, and decision should be based on sample solubility and the chemical differences among the compounds of interest .

There are several types of matrices for support of the stationary phase, including silica, polymers, alumina, and zirconium. Silica is the most common matrix for HPLC columns. Silica matrices are robust, easily derivatized, manufactured to consistent sphere size, and does not tend to compress under pressure. Silica is chemically stable to most organic solvents and to low pH systems. One short coming of a silica solid support is that it will dissolve above pH 7. In recent years, silica supported columns have been developed for use at high pH.

The nature, shape and particle size of the silica support effects separation. Smaller particle results in a greater number of theoretical plates, or increased separation efficiency. However, the use of smaller particles also results in increased backpressure during chromatography and the column more easily becomes plugged. For this reason 5Å columns are more frequently used than 3Å columns in development work. Narrower particle size distribution of the silica particles also results in better resolution. Hence, similar phase columns from different manufacturers or different lots of columns from the same manufacture may have very different separation properties due to differing methods of matrix preparation.

The nature of the stationary phase will determine whether a column can be used for normal phase or reverse phase chromatography. Normal phase chromatography utilizes a polar stationary phase and a non-polar mobile phase (e.g. Ultra IBD, Allure® Basix, and Allure® PFP Propyl). Generally, more polar compounds elute later than non-polar compounds. Types of columns suitable for normal phase chromatography include underivatized silica, nitrile, amino (or amino propyl), glycerol and nitro columns. Chiral separation is usually performed under normal phase conditions.

Since highly polar and ionic compounds are retained on normal phase columns, a guard column or silica gel sample purification should be used to extend the column life. In reverse phase chromatography the stationary phase is non-polar and the mobile phase is polar, causing polar peaks to generally elute earlier than non-polar peaks. To create a stationary phase for reverse phase chromatography on silica support, the free silanols are reacted with a chlorosilane with hydrophobic functionality to introduce the non-polar surface.

Due to steric constraints, only about 1/3 of the surface silanols are derivatized. The remaining free silanols can interact with analytes, causing peak tailing. Typically, after the derivitization of a column with the desired stationary phase, the column is further reacted with chlorotrimethylsilane to end cap the remaining free silanols and improve the column efficiency. Common stationary phases are C4 (butyl), C8 (MOS), C18 (ODS), nitrile (cyanopropyl), and phenyl (phenylpropyl)

columns. In general, longer alkyl chains, higher phase loading, and higher carbon loads provide greater retention of non-polar analytes. Selectivity is most influenced by the amount of accessible surface area of the derivatized silica gel particles and the carbon load. Thus it is often a benefit to not only have columns with different stationary phases, but columns with the same phase from different manufacturers.

Commonly used reverse phase columns and their uses are listed below.Propyl (C3), Butyl (C4), and Pentyl (C5) phases are useful for ion-pairing chromatography (C4) (vide infra)and peptides with hydrophobic residues, and other large molecules. C3–C5 columns generally retain non-polar solutes more poorly when compared to C8 or C18 phases. Examples include Zorbax SB-C3, YMC-Pack C4, and Luna C5. These columns are generally less stable to hydrolysis than columns with longer alkyl chains. Octyl (C8, MOS) phases have wide applicability. This phase is less retentive than the C18phases, but is still quite useful for pharmaceuticals, nucleosides, and steroids.

Octyl columns are also useful for peptides, peptide mapping and small hydrophilic proteins when bonded to 300 Å silica particles. Examples include Zorbax SB-C8, Luna C8, and YMC-Pack-MOS. Octadecyl (C18, ODS) columns are the most widely used and tend to be the most retentive for non-polar analytes. This phase is useful in ion-pairing chromatography and has wide applicability (same as C8 in addition to vitamins, fatty acids, environmental compounds).Examples include Zorbax SB-C18, YMC- Pack ODS and Luna C18.Xterra RP-C18 and Zorbax Extend-C18 columns have been formulated to tolerate high pH systems (pH>7, normally up to pH 11).

Varying the pH can dramatically affect selectivity and resolution of polar analytes, especially for ionizable compounds. Phenyl (Ph) columns offer unique selectivity from the alkyl phases and are generally less retentive than C8 or C18 phases. Phenyl columns are commonly used to resolve aromatic compounds. Examples include Zorbax SB-Phenyl, YMC-Pack Phenyl and Luna Phenyl- Hexyl. Nitrile (CN or cyano) columns are polar and can be used for both reverse and normal phase applications. This phase is often used to increase retention of polar analytes.

The nitrile derivatization allows for rapid column equilibration. Examples include Zorbax SB- CN, Luna-CN, and YMC-Pack CN. Standard C18 Columns and similar stationary phases will undergo phase collapse at highly aqueous mobile phases, typically at less than 5-10% organic composition; this will decrease analyte-stationary phase interaction. Collapsed phases are also difficult to re-equilibrate. To prevent phase collapse, C18 columns with a polar group embedded in the alkyl chain have been developed to help solvate the hydrophobic chain in >90% aqueous mobile phases. Examples include Zorbax SB-Aq, Synergi Hydro-RP andYMC-Pack ODS-Aq [15].

Column Temperature: Column temperature control is important for long-term method reproducibility as temperature can affect selectivity. A target temperature in the range of 30-40°C is normally sufficient for good reproducibility.

Temperature has been an overlooked operational parameter in HPLC, and the potential advantages of elevated column temperatures, particularly enhanced kinetic and transport properties, which are based on the decrease of the viscosity of mobile phase and increase of the analyte diffusivity at higher temperature [16]. In most instances, the objective of using elevated or high temperature is to increase the speed of separation to obtain higher efficiencies and faster results, though there are some situations where selectivity can be manipulated through change of temperature [17].

Temperature-programmed HPLC can be used as an alternative to using solvent gradient elution for variation of solvent strength during the run, and this is expected to be of particular utility with small-bore columns which have low thermal mass [18]. A number of papers have considered the effect of change of temperature on retention [16, 19-22]. The effect of temperature on retention factor *k* can be described by the van't Hoff equation, the retention factor decreases with increase of temperature.

In one review paper, Dolan [23] pointed out that temperature can be programmed quite simply in HPLC operating systems, and that during method development changes in temperature can be more convenient than solvent composition or pH changes.

Temperature programming for HPLC is now being promoted commercially and comparisons are available on the effects of temperature gradients and solvent gradients using a range of columns and a test set of analytes spanning neutral, acidic and basic molecules [24]. Use of elevated temperatures can bring benefits to HPLC, particularly in instances where columns are stable over an extended temperature range. When working with mixtures of analytes in different compound classes, selectivity can be dependent on temperature.

Peak Purity: An essential requisite of a separation analysis is the ability to verify the purity of the separated species, that is, to ensure that no coeluting or comigrating impurity contributes to the peak response. The confirmation of peak purity should be performed before quantitative information from a chromatographic or electrophoretic peak is used for further calculations.

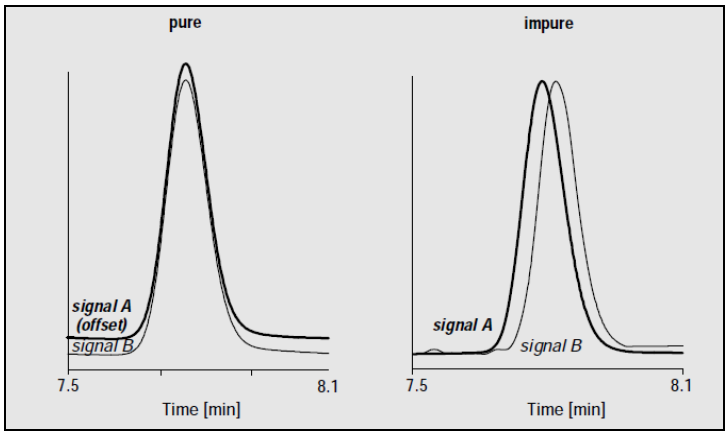

FIGURE 1: NORMALISED SIGNALS FOR PURE AND IMPURE PEAK

Peak purity (or peak homogeneity) analysis of the main peak, to assess for the presence of impurities under the main peak, is an essential part of the validation of a SIM. Direct evaluation can be performed in-line by employing PDA detection [25], LC-MS [26], or LC-NMR. However, PDA only works well for degradants that have a different UV spectrum from that of the drug LC-MS evaluation will not work if the degradant has the same molecular weight, as is the case for diastereomers, or if the ionization of the degradant is suppressed by the co-eluting API. Indirect evaluation of peak purity can be accomplished by changing one or more chromatographic parameters (column, mobile phase, gradient composition, etc.) that will significantly impact the separation selectivity.

The resulting impurity profile is then compared against that of the original method. If the number of degradant peaks is the same in both separations, and if the are percent of the main component is the same in both separations, then there can be reasonable confidenc that all the degradants have been resolved from the main component. Automated versions of this approach have been successfully utilized in a multi-dimensional screening with instrumentation capable of systematically evaluating several different columns and eluents for impurity analysis [27-29].

Other approaches use alternate separation techniques such as thin-layer chromatography (TLC), normal-phase-HPLC, capillary electrophoresis (CE), or supercritical fluid chromatography (SFC), with similar goals as explained in general terms by Lee Polite in a chapter on liquid chromatography [30].

Method Optimization: Method is optimized to get better sensitivity after separation. The mobile phase and stationary phase compositions need to be taken into account. Note that the optimization of mobile phase parameters is always considered first as this is much easier and convenient than stationary phase optimization.To minimize the number of trial chromatograms involved, only the parameters that are likely to have a significant effect on selectivity in the optimization must be examined.

Primary control variables (factors) in the optimization of liquid chromatography (LC) methods are the different components of the mobile phase determining acidity, solvent strength, gradient, flow rate, temperature, sample amounts, injection volume, and diluents solvent type [8]. This is used to find the desired balance between resolution and analysis time after satisfactory selectivity has been achieved. The parameters involved include column dimensions, column-packing particle size and flow rate. These parameters may be changed without affecting capacity factors or selectivity.

Validation of Analytical Methods: Validation is followed according to ICH guidelines. In validation, accuracy, precision, specificity, linearity, range, detection limit, quantitation limit, ruggedness, and robustness of the method are done. It is necessary to isolate, identify, characterize, and qualify the

degradation products if they are above the identification threshold (usually 0.1%) [31, 32]. Analytical method validation is now required by regulatory authorities for marketing authorizations and guidelines have been published. It is important to isolate analytical method validation from the selection and development of the method.

Method selection is the first step in establishing an analytical method and consideration must be given to what is to be measured, and with what accuracy and precision. Method validation must have a written and approved protocol prior to use [33].

Method development and validation can be simultaneous, but they are two different processes, both downstream of method selection. Analytical methods used in quality control should ensure an acceptable degree of confidence that results of the analyses of raw materials, excipients, intermediates, bulk products or finished products are viable. Before a test procedure is validated, the criteria to be used must be determined.

Analytical methods should be used within good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols set out in the International Conference on Harmonization (ICH) guidelines (Q2A and Q2B) [1, 2]. The US Food and Drug Administration (FDA) [3, 4] and US Pharmacopoeia (USP) [5] both refer to ICH guidelines.

Forced Degradation Studies in Stability-Indicating Method Development: Stability indicating method must be able to monitor a change in the chemical, physical, and microbiological properties of drug product over time.

The ability of the method to monitor a change in the chemical properties of the drug over time, invariably calls for a forced degradation (stress testing) study to be done on the drug substance and drug product.

Forced degradation on the drug substance and product will (in addition to establishing specificity) 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 (eg, excipients);

(3) Structure elucidation of degradation products;

(4) Determination of the intrinsic stability of a drug substance molecule in solution and solid state; and

(5) Reveal the thermolytic, hydrolytic, oxidative, and photolytic degradation mechanism of the drug substance and drug product [34, 35].

From the foregoing, it is obvious that forced degradation plays a key role not just in the development of stability-indicating methods, but also in providing useful information about the degradation pathways and degradation products that could form during storage [34]. One of the guidance documents, *Q1A (R2) – Stability Testing of New Drug Substances and Products*, states: "Stress testing is likely to be carried out on a single batch of the drug substance. The testing should include the effect of temperatures (in 10°C increments (i.e., 50°C, 60°C) above that for accelerated testing), humidity (i.e., 75% relative humidity or greater) where appropriate, oxidation, and photolysis on the drug substance.

The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension." the guidance does not specify pH, temperature ranges, specific oxidizing agents, or conditions to use, the number of freeze-thaw cycles, the following will provide some suggestions for performing forced degradation studies based upon available guidance from the ICH and FDA, thus narrowing these guidance generalities to practicalities.

Appropriate Timing: The stress studies should assess the stability of the drug substance in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels.

These studies are most beneficial if done initially in early development, ie, during the preclinical development or Phase I clinical trials. A forced degradation study on the drug substance at this stage will provide timely recommendations for improvements in the manufacturing process, ensure proper selection of stability-indicating analytical techniques, and ensure there is sufficient time for degradation product identification, degradation pathways elucidation, and optimization of stress conditions [36]. Such a proactive approach will help avert any surprises later in the development process.

How Much Is Enough: The question of how much stressing is enough has been the subject of much discussion amongst pharmaceutical scientists. In general, values anywhere between 5% to 20% degradation of the drug substance have been considered as reasonable and acceptable for validation of chromatographic assays [37, 38].

However, for small pharmaceutical molecules for which acceptable stability limits of 90% of label claim is common, pharmaceutical scientists have agreed that approximately 10% degradation is optimal for use in analytical validation [8].

In the event that the experimental conditions generate little or no degradants due to the exceptional stability of the molecule, an evaluation should be made to verify if the drug substance has been exposed to energy in excess of the energy provided by accelerated storage (i.e., 40°C for 6 months).

Experimental Design: In designing forced degradation studies, it must be remembered that more strenuous conditions than those used for accelerated studies (25°C/60% RH or 40°C/75% RH) should be used. At a minimum, the following conditions should be investigated:

(1) Acid and base hydrolysis,

(2) Hydrolysis at various ph,

(3) Thermal degradation,

(4) Photolysis, and

(5) Oxidation. For the drug substance and drug product, the scheme shown in **Figure 2** could be used as a guide [35].

The initial experiments should be focused on determining the conditions that degrade the drug by approximately 10%. The conditions generally employed for forced degradation are summarized in **Table 1**.

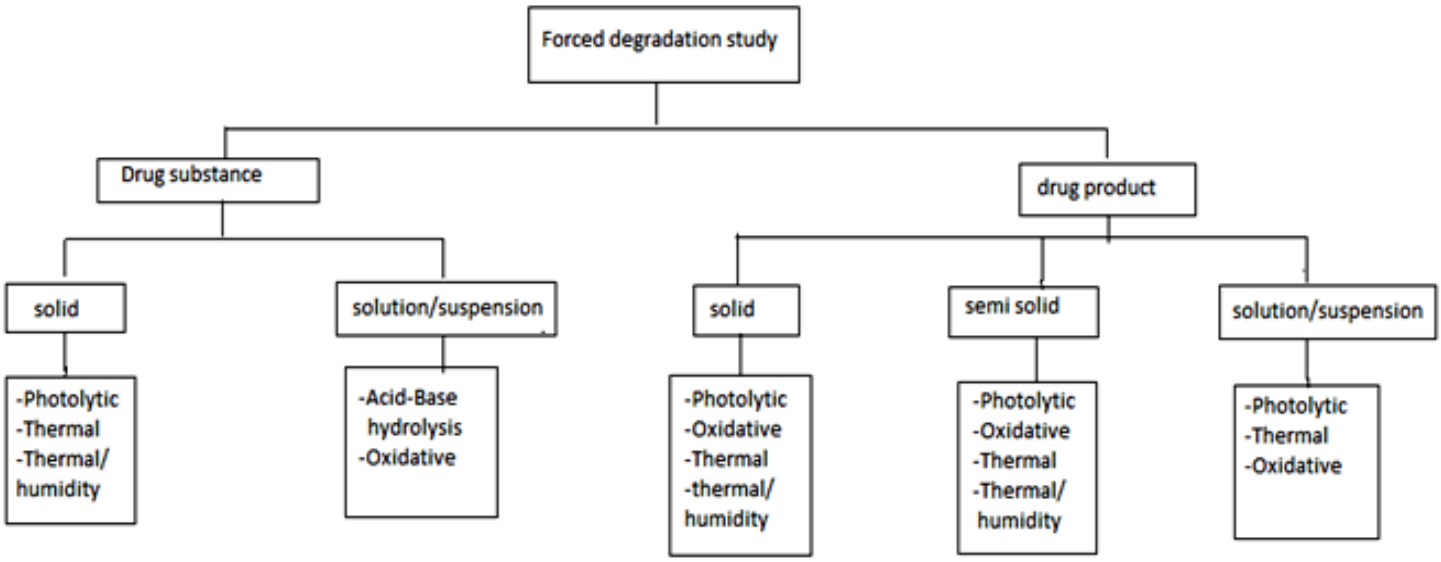

FIGURE 2: AN ILLUSTRATIVE DIAGRAM SHOWING THE DIFFERENT FORCED DEGRADATION CONDITION TO BE USED FOR DRUG SUBSTANCE AND DRUG PRODUCT

TABLE 1: CONDITIOS GENERALLY EMPLOYED FOR FORCED DEGRADATION [39]

Degradation Type	Experimental Condition	Storage Condition	Sampling Time
	Control API (no acid or base)	40 ^{0}C, 60^{0}C	1,3,5 days
Hydrolysis	0.1N HCL	40 ^{0}C, 60^{0}C	1,3,5 days
	0.1N NAOH	40 ^{0}C, 60^{0}C	1,3,5 days
	Acid Control(no API)	40 ^{0}C, 60^{0}C	1,3,5 days
	Base Control(no API)	40 ^{0}C, 60^{0}C	1,3,5 days
	pH: 2,4,6,8	40 ^{0}C, 60^{0}C	1,3,5 days
	3% H_2O_2	25 ^{0}C, 60^{0}C	1,3,5 days
Oxidation	Peroxide Control	25 ^{0}C, 60^{0}C	1,3,5 days
	Azobisisobutyronitrile (AIBN)	40 ^{0}C, 60^{0}C	1,3,5 days
	AIBN Control	40 ^{0}C, 60^{0}C	1,3,5 days
Photolytic	Light, 1X ICH	NA	1,3,5 days
	Light, 3X ICH	NA	1,3,5 days
	Light Control	NA	1,3,5 days
Thermal	Heat chamber	60 ^{0}C	1,3,5 days
	Heat chamber	60 ^{0}C /75% RH	1,3,5 days
	Heat chamber	80 ^{0}C	1,3,5 days
	Heat chamber	80 ^{0}C /75% RH	1,3,5 days
	Heat control	Room Temp.	1,3,5 days

However, some scientists have found it practical to begin at extreme conditions (80°C or even higher, 0.5N NaOH, 0.5N HCl, 3% H2O2) and testing at shorter (2, 5, 8, and 24 hrs, etc) multiple time points, thus allowing for a rough evaluation of rates of degradation. Testing at early time points may permit distinction between primary degradants and their secondary degradation products. This strategy allows for better degradation pathway determination. It must be noted that a forced degradation study is a "living process" and should be done along the developmental time line as long as changes in the stability-indicating methods, manufacturing processes, or formulation changes are ongoing.

Forced degradation is only considered complete after the manufacturing process is finalized, formulations established, and test procedures developed and qualified. The conditions listed in Table 1 are by no means exhaustive and should be adjusted by the researcher as needed to generate ~10% degradation of the API.

The nature (inherent stability/instability) of the particular drug substance will determine in which direction to adjust the stress conditions. Also, the aforementioned conditions could be used to stress the drug substance or drug product either in the solid or liquid/suspension form as applicable.

For oxidative degradation with H_2O_2, at least one of the storage conditions should be at room temperature. Heating H_2O_2 solution increases the homolytic cleavage of the HO-OH bond to form the alkoxy radical. The alkoxy radical is very reactive and may come to dominate the observed degradation pathway. Adding a small quantity of methanol in a confirmatory stress experiment quenches the alkoxy radical and rules out species produced by this more aggressive oxidizing agent.

Also, the formation of peroxycarboxymidic acid has been observed when acetonitrile is used as a cosolvent in H_2O_2 stress studies (in basic conditions). The peroxycarboximidic acid has activated hydroxylation reactivity, which is not representative of H_2O_2. To circumvent these problems, some research scientists always perform a parallel or alternative oxidative study using azobisisobutyronitrile (AIBN), which is a less reactive oxidant and has been shown to produce more representative degradants.

CONCLUSION: Stability-indicating method is an analytical procedure that is capable of discriminating between the major active (intact) pharmaceutical ingredients (API) from any degradation (decomposition) product(s) formed under defined storage conditions during the stability evaluation period.

Forced degradation studies are indispensable in the development of stability-indicating and degradant-monitoring methods as part of a validation protocol. Forced degradation studies also provide invaluable insight in investigating degradation products The use of properly designed and executed forced degradation study will generate a representative sample that will in turn help to develop stability-indicating HPLC method.

Chromatographic factors should be evaluated to optimize the stability indicating HPLC method for detection of all potentially relevant degradants. An appropriate sample solvent and mobile phase must be found that afford suitable stability and compatibility with the component of interest, as well as the impurities and degradants. Therefore, resulting stability indicating HPLC is truly fit for finding the degradants and impurities in pharmaceutical products.

REFERENCES:

1. FDA Guidance for Industry: Analytical Procedures and Methods Validation (draft guidance), August 2000.
2. Monika Bakshi and Saranjit Singh: Development of validated stability-indicating assay methods--critical review. J. Pharm. Biomed. Anal. 2002; 28(6):1011-1040
3. JohnW.Dolan:Stability-IndicatingAssays.LC Troubleshooting. LCGC North America, 2002; 20(4):346-349.
4. Michael J. Smela: Regulatory Considerations for Stability Indicating Analytical Methods in Drug Substance and Drug Product Testing. American Pharmaceutical Review. 2005; 8(3): 51-54.
5. K. Huynh-Ba: Development of Stability indicating methods; In:Handbook of Stability Testing in Pharmaceutical Development, Springer 2009, 153.
6. http://www.cvg.ca/images/HPLC_Method_Development.pdf- Effective HPLC method development.
7. Donald D. Hong and Mumtaz Shah: Development and validation of HPLC Stability-indicating Assays, In: Sens T. Carstensen, C.T.Rhodes, editors Drug Stability-Principle & Practice. 3rd Edition. New York: Marcel Dekker Inc. 2008; p. 332.
8. Changhe Wen: Designing HPLC Methods for Stability Indication and Forced Degradation Samples For API, Collected from American Pharmaceutical Reviewat.http://www.americanpharmaceuticalreview.com
9. Swartz M. and Krull I.: "Developing and Validating Stability- Indicating Methods". LCGC North America, 2005; 23(6):586- 593.
10. Supplement to LC/GC. Current trends and developments in sample preparation, May1998.
11. K. Huynh-Ba (ed.): Development of Stability indicating methods.Handbook of Stability Testing in Pharmaceutical Development; Springer 2009: 154.
12. John W. Dolan: "Stability-Indicating Assays". LC Troubleshooting 2005: 275.
13. LR Snyder, JL Glajch, JJ Kirkland: Practical HPLC method Development. New York: John Wiley; 1988; 227-251
14. Yuri V. Kazakevich, Rosario LoBrutto: HPLC for Pharmaceutical Scientists; page no 158
15. Seble Wagaw, Jason Tedrow, Tim Grieme, Lalit Bavda, Weifeng Wang,ShekharViswanath et al:HPLC Guide.http://www.chemgroups.northwestern.edu/scheidt/PDFsHPLC_guide.pdf
16. Chen and Cs. Horváth: Anal. Methods Instrum*,* 1;1993: 213–222 .
17. J. Li and P.W. Carr: *Anal. Chem.* 69;1997:837–847
18. T. Greibrokk and T. Andersen: Journal of Chromatography*., A* 1000;2003: 743–755.
19. Chen and Cs. Horváth: Journal of Chromatography*, A*705;1995: 3–20 .
20. M.H. Chen and C. Horváth: Journal of Chromatography*, A* 788;1997: 51–61.
21. Li, Y. Hu, and P.W. Carr: *Anal. Chem.* 69; 1997:3884–3888.
22. T. Kondo and Y. Yang: Anal. Chim. Acta. 494; 2003: 157–166 .
23. J.W. Dolan: Journal of chromatography*, A* 965; 2002: 195–205.
24. S.J. Marin et al.: journal of. Chromatography*., A* **1030**; 2004:255–262.
25. Bryant DK, Kingwood MD, Belenguer: A Determination of liquid chromatographic peak purity by electro spray ionization mass spectrometry. J Chromatogr A 1996; 721:41–51.
26. Cameron G, Jackson PE, Gorenstein MV: A new approach to peak purity assessment using photodiode array detection. ChemAus; 1993: 288–289.
27. Ruan J, Tattersall P, Lozano R, Shah P, The role of forced degradation studies in stability indicating HPLC method development. Am Pharm Rev 2006; 9:46–53.
28. Stepensky D, Chorny M, Dabour Z, Schumacher I ,Long-term stability study of Ladrenaline injections: kinetics of sulfonation and racemization pathways of drug degradation. J Pharm Sci, 2004; 93:969–980.
29. Xiao KP, Xiong Y, Liu FZ, Rustum AM: Efficient method development strategy for challenging separations of pharmaceutical molecules using advanced chromatographic technologies. J Chromatogr A, 2007; 1163:145–156.
30. Polite L, Liquid chromatography: basic overview. In: Miller J, Crowther JB (eds) Analytical chemistry in a GMP environment: a practical guide. John Wiley & Sons, New York, 2000.
31. The United States Pharmacopoeia, USP 28-NF 23, <1225>, 2005.
32. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Quality Guidelines, http://www.ich.org/products/guidelines/quality/ article/ quality- guidelines.html
33. G.A. Shabir: Validation of HPLC Chromatography Methods for Pharmaceutical Analysis. Understanding the Differences and Similarities Between Validation Requirements of FDA, the US Pharmacopeia and the ICH. *J. Chromatogr. A.* 987(1-2);2003: 57-66.
34. ICH guidelines Q1A (R2). Stability Testing of New Drug Substances and Products (revision 2), November 2003.
35. Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Motto MG: Available guidance and best practices for conducting forced degradation studies. Pharm Tech; 2002:48-56.
36. Kats M.:Forced degradation studies: regulatory considerations and implementation. BioPharm Int; July 2005.
37. Szepesi G: Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. Journal of Chromatography. 1989;464:265-278.
38. Carr GP, Wahlich JC: A practical approach to method validation in pharmaceutical analysis. J Pharm Biomed Anal.1990;86:613-618.
39. Georg nagva:Forced degradation studies as a integral part of HPLC stability indicating method development,drug delivery technology.june 2010.

How to cite this article:
Shah BP, Jain S, Prajapati KK and Mansuri NY: Stability Indicating HPLC Method Development: A Review. *Int J Pharm Res Sci*. 3(9); 2978-2988.