Review Article

Stability Indicating HPLC Method Development and Validation

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ABSTRACT

High performance liquid chromatography (HPLC) is an integral analytical tool in assessing drug product stability. HPLC methods should be able to separate, detect, and quantify the various drug-related degradants that can form on storage or manufacturing, plus detect and quantify any drug-related impurities that may be introduced during synthesis. Forced degradation studies 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 that could form during storage, and facilitate during formulation, development, manufacturing and packaging. 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. A review of literature reveals that a large number of methods reported over the period of last 3 – 4 decades under the nomenclature 'stability-indicating', but most of the reported methods fall short in meeting the current regulatory requirements. Hence a systematic approach for the development of validated SIAMs that should meet the current ICH and regulatory requirements. . The following will provide some suggestions for performing forced degradation studies based upon available guidance from the ICH and FDA.

Keywords: Degradation conditions, regulatory requirements, stability indicating HPLC method development, method validation

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INTRODUCTION

The stability-indicating assay is a method that is employed for the analysis of stability samples in pharmaceutical industry. With the advent of International Conference on Harmonisation (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 [1].

Stability testing of drug substance requires an accurate analytical method that quantitates active pharmaceutical ingredients (API) without interference (**Table 2**) from degradation products, process impurities and other potential impurities [1]. High performance liquid chromatography (HPLC) is an integral analytical tool in assessing drug product stability. HPLC methods would be able to separate, detect, and quantify the various drug-related degradants that can form on storage or manufacturing, plus detect and quantify any drug-related impurities that may be introduced during synthesis [2-7]. Forced degradation studies of new chemical entities and drug products are essential to develop and demonstrate the specificity of stability-indicating methods. In addition to demonstrating specificity, forced degradation studies can be used to determine the degradation pathways and degradation products that could form during storage and facilitate during formulation, development, manufacturing

and packaging [8]. A review of literature reveals that a large number of methods reported over the period of last 3 – 4 decades under the nomenclature 'stability-indicating', but most of the reported methods fall short in meeting the current regulatory requirements. Hence a systematic approach for the development of validated SIAMs that should meet the current ICH and regulatory requirements [9].

When to perform forced degradation studies?

During:

- Lead optimization.
- API method development/validation.
- IND/NDA filing [10].

Regulatory status of stability-indicating assays:

Table 1: Regulatory guidelines of ICH

Standard	Title and reference
ICHQ1A(R2)	Stability Testing of New Drug Substances and Products.
ICHQ1B	Photostability of New Drug Substances and Products.

Need For Stability Studies:

- To determine the shelf life of the drug and drug product.
- To determine the storage conditions of the drug product.
- To determine the degradants or impurities those appear as a result of aging.
- To assess the inherent stability of a drug and to improve formulations and the manufacturing process.
- To identify reactions which are involved in the degradation of a product.
- To develop and demonstrate the specificity of validated analytical methods [10].

Objectives:

1. Determination of degradation pathways of drug substance and drug products and to generate a degradation profile.
2. Discrimination of degradation products in formulations 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.
5. Reveal the thermolytic, hydrolytic, oxidative and photolytic degradation mechanism of the drug substance and drug product.
6. To develop and validate a stability indicating method.
7. To identify impurities related to drug substances or excipients.
8. To understand the drug molecule chemistry.
9. To generate more stable formulations.
10. To solve stability-related problems (e.g., mass balance) [11].

Development of validated SIAMs:

The practical steps involved in the development of SIAMs are discussed below:

Step I: critical study of the drug structure:

Much information can simply be gained from the structure, by study of the functional groups and other key components. There are definite functional group categories, like amides, esters, lactams, lactones, etc. that undergo hydrolysis, others like thiols, thioethers, etc. undergo oxidation. For information on degradation chemistry of like drugs, one can look into the treatises like Analytical Profiles of Drug Substances and the monographs provided by Connors et al., the internet search engines and the Chemweb [12].

Step II: collection of information on physicochemical properties:

Before method development is taken up, it is generally important to know various physicochemical parameters like pKa, log P, solubility, absorptivity and wavelength maximum of the drug in question. The knowledge of pKa is important as most of the pH-related changes in retention occur at pH values within 1.5 units of the pKa value. The ionization value also helps in selecting the pH of the buffer to be used in the mobile phase. The availability of the solubility data in aqueous, organic and commonly used

HPLC solvents and their combinations can thus prove to be very useful in the selection of the sample solvent and the mobile phase [12].

Step III: Stress (Forced Decomposition) Studies:

Table 2: Forced Degradation Conditions Used for Drug Substances and Drug Product

Forced Degradation Conditions				
Drug Substance		**Drug Product**		
Solid	Solution/Suspension	Solid	Semi-Solid	Solution/Suspension
Photolytic	Acid/Base Hydrolysis	Photolytic	Photolytic	Photolytic
		Thermal	Thermal	Thermal
Thermal	Oxidative	Humidity	Humidity	Oxidative
Humidity		Oxidative	Oxidative	

The ICH guideline Q1A suggests the following conditions to be employed:

Conditions for degradation:

Hydrolytic degradation:

Hydrolysis is one of the most common degradation chemical reactions over wide range of pH. Hydrolysis is a solvolytic process in which drug reacts with water to yield breakdown products of different chemical compositions. Water either as a solvent or as moisture in the air comes in contact with pharmaceutical dosage forms is responsible for degradation most of the drugs. Hydrolytic study under acidic and basic condition involves breakdown of ionisable functional groups present in the molecule [13].

Alternatively if total degradation is seen after subjecting the drugs to initial condition, acid/alkali strength can be decreased with decrease in reaction temperature. In general temperature and pH are the major determinant in stability of the drug prone to hydrolytic decomposition [13].

Procedure for conducting hydrolytic degradation:

Conduct the following forced degradation studies to obtain degraded samples as seen in flow chart-1) wherever degradation possible from about 1% to 30%. For Acid stress Reflux with 0.1N HCL at 60°C for 30 minutes. For Base stress Reflux with 0.1N NaOH at 60°C for 30 minutes. For water stress Reflux with water at 60°C for 30 minutes [13].

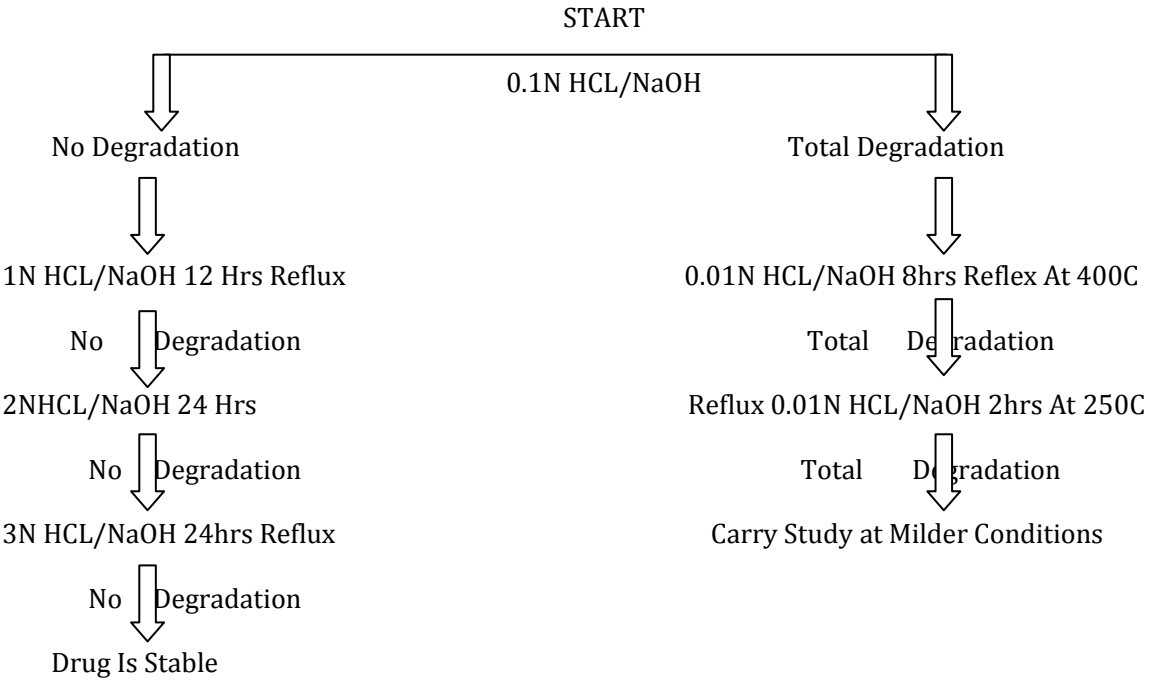

Figure 1: procedure for performing hydrolytic degradation

Oxidative degradation:

Many drug substances undergo autoxidation i.e., oxidation under normal storage condition and involving ground state elemental oxygen. Autoxidation is a free radical reaction that requires free radical initiator to begin the chain reaction. Hydrogen peroxide, metal ions, or trace level of impurities in a drug substance act as initiators for autoxidation. Hydrogen peroxide is very common oxidant to produce oxidative degradation products which may arise as minor impurities during long term stability studies. It can be used in the concentration range of 3-30% at a temperature not exceeding 40 ˚C for 2-8 days. The oxidative stress testing can be carried out initially with 3% H_2O_2 [13].

Procedure for conducting oxidative degradation:

Conduct the following forced degradation studies to obtain degraded samples wherever degradation possible from about 1% to 30%.For oxidation stress: Treat with 1% H_2O_2 at less than 30°C for 30 min. The oxidative stress testing is initially carried out in 3% H_2O_2 at room temperature for 6 hr and it can be increased/ decreased (as seen in flowchart-2) to achieve sufficient degradation[13].

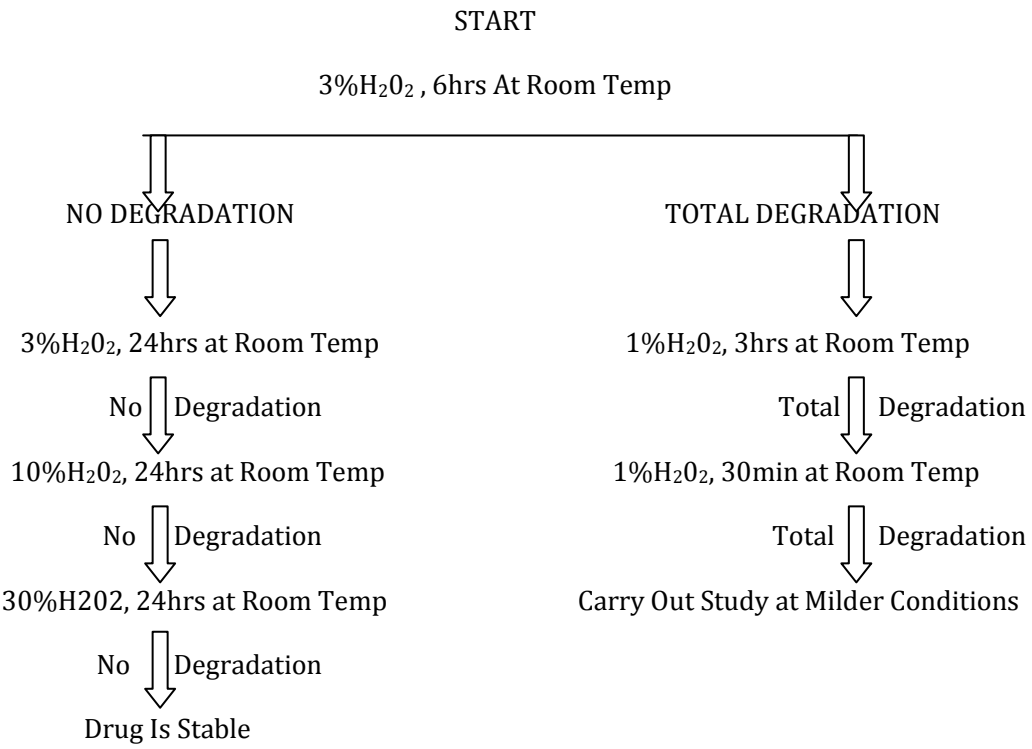

Figure 2: procedure for performing oxidative degradation

Thermal degradation:

In general, rate of a reaction increase with increase in temperature. Hence, the drugs are susceptible to degradation at higher temperature Effect of temperature on thermal degradation of a substance is studied through

Arrhenius equation:

K= Ae-Ea/RT

Where k is specific reaction rate,
A is frequency factor,
Ea is energy of activation,
R is gas constant (1.987 cal/deg mole) and T is absolute temperature [13].

Thermal degradation study is carried out at 40˚C to 80˚C (as seen in table-2). The most widely accepted temperature is 70˚C at low and high humidity for 1-2months. High temperature (>80˚C) may not produce predictive degradation pathway. The use of high-temperatures in predictive degradation studies assumes that the drug molecule will follow the same pathway of decomposition at all temperatures [13].

Procedure for conducting thermal degradation:

Conduct the following forced degradation studies to obtain degraded samples wherever degradation possible from about 1% to 30%. Preferably, the stress conditions are recommended for specificity study, however stress condition can be decided based on experimental data, or physical properties of the analyte based on literature. If melting point of API is less than 150°C, stress at 105°C or 40°C less than melting point whichever is higher. If melting point of API is more than 150°C stress at the nearest melting point and at 105°C [13].

Photolytic degradation:

Exposure of drug molecules may produce photolytic degraded products. The rate of photo degradation depends upon the intensity of incident light and quantity of light absorbed by the drug molecule. Photolytic degradation is carried out by exposing the drug substance or drug product to a combination of visible and UV light. The most commonly accepted wavelength of light is in the range of 300-800 nm to cause the photolytic degradation. The photolytic degradation can occur through non oxidative or oxidative photolytic reaction [13].

The non-oxidative photolytic reaction include isomerization, dimerization, cyclization, rearrangements, decarboxylation and hemolytic cleavage of X-C hetero bonds, N-alkyl bond(de alkylation and deamination), SO_2-C bonds etc. and while oxidative photolytic reaction occur through either singlet oxygen($1O_2$) or triplet oxygen($3O_2$) mechanism. The singlet oxygen reacts with the unsaturated bonds, such as alkenes, dienes, poly nuclear aromatic hydrocarbon to form photo oxidative degradation products whereas triplet oxygen react with free radical of the drug molecule, which than react with a triplet oxygen molecule to form peroxide. Hence, light can also act as a catalyst to oxidation reactions [13].

Procedure for conducting photolytic degradation:

Conduct the following forced degradation studies to obtain degraded samples wherever degradation possible from about 1% to 30%. Expose the tablet powder contents of capsule to ultraviolet radiation up to minimum of 200 watts hour/m^2 and minimum of 1.2 million lux hour for visible light and photo stability chamber(as seen in table-2). If photo stability chamber is not available, expose the tablet powder/content of capsule to intense ultraviolet radiation (both at longer and shorter wavelengths) up to minimum of 7 days in UV cabinet [13].

Humidity:

Humidity is the Key factor in establishing the potential degradants in the finished product and active pharmaceutical ingredient. Normally 90% Humidity for duration of one week shall be considered for the establishment of forced degradation samples [13].

Acceptance criteria:

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. 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. 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 (ie., 40°C for 6 months), then the experiment can be stopped and a note of the stability of the drug substance can be made [13].

Step IV: preliminary separation studies and identification of degradation products:

Preliminary separation:

The stress samples so obtained are subjected to preliminary analyses to study the number and types of degradation products formed, by using various chromatographic techniques like Reversed Phase High Performance Liquid Chromatography (RP-HPLC), Thin Layer

Chromatography (TLC), Gas Chromatography (GC), Capillary Electrophoresis (CE), Capillary Electrophoresis Chromatography (CEC) and Super critical Fluid Chromatography (SFC). The RP-HPLC is most widely used analytical tool for separation and quantifying the impurities and it is most frequently coupled with UV detector [14].

Table 3: Conditions generally employed for forced degradation

CONDITION EMPLOYD FOR FORCED DEGRADATION			
DEGRATION TYPE	**EXPEIMENTAL CONDITION**	**STORAGE CONDITION**	**SAMPLING TIME (Days)**
	Control API (no ACID or BASE)	4O^{0}C,6O^{0}C	1,3,5
	O.1N ACID	4O^{0}C,6O^{0}C	1,3,5
Hydrolysis	O.1N BASE	4O^{0}C,6O^{0}C	1,3,5
	Control Acid (No API)	4O^{0}C,6O^{0}C	1,3,5
	Control Base(No API)	4O^{0}C,6O^{0}C	1,3,5
	PH: 2,4,6,8	4O^{0}C,6O^{0}C	1,3,5
	3% H_2O_2	25^{0}C,4O^{0}C	1,3,5
	Peroxide Control	25^{0}C,4O^{0}C	1,3,5
	Azobisisobutyryonitrile	4O^{0}C,6O^{0}C	1,3,5
Oxidative	(AIBN)		
	AIBN Control	4O^{0}C,6O^{0}C	1,3,5
	Light 1X ICH	NA	1,3,5
Photolytic	Light 3X ICH	NA	1,3,5
	Light Control	NA	1,3,5
	Heat Chamber	6O^{0}C	1,3,5
	Heat Chamber	6O^{0}C/75%RH	1,3,5
	Heat Chamber	8O^{0}C	1,3,5
Thermal	Heat Chamber	8O^{0}C/75%RH	1,3,5
	Heat Control	Room Temp.	1,3,5

Identification:

To identify the resolved products, a conventional way is to isolate them and determine the structure through spectral (MS, NMR, IR, etc.) and elemental analysis. The modern approach is to use hyphenated LC techniques coupled with mass spectrometry. This strategy integrates in a single instrument approach, analytical HPLC, UV detection, full scan mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS-MS) and provides a fair idea on identity of resolving components. These days LC-MS or LC-MS-MS is employed to obtain molecular weight and fragmentation information, and further detailed structural information is obtained through LC-NMR analysis [14].

Step V: final method development and optimization:

Validation of analytical methods has been extensively covered in the ICH guidelines Q2A and Q2B, in the FDA guidance and by USP. Overall, there are two stages in the validation of a SIAM. First stage is early in the development cycle when drug substance is subjected to forced decomposition studies and the SIAM is established based on the knowledge of drug degradation behavior. The main focus of validation at this stage is on establishment of specificity/selectivity, followed by other parameters like accuracy, precision, linearity, etc. The limits of detection and quantification are also determined for degradation products to help in establishment of the mass balance. In the second stage, when the SIAM so developed is extended to formulations or other matrices, in the presence of excipients or formulation constituents. Here only parameters of critical importance are specificity/ selectivity, accuracy and precision are revalidated. If the SIAM is

being developed directly for a formulation, without involving the bulk drug route, then all validation parameters are necessary to be established [14].

STABILITY INDICATING HPLC METHOD:

Step1: Understanding of the physico-chemical properties of drug:

Knowledge of the physicochemical properties of the API and the formulations is essential to frame the development of the method. Information on various properties has been collected either through the appropriate information in support of drug discovery or from a search of the literature, company drug profiles, spectral libraries, and reports [14].

Structure of the analyte, especially functional group will indicate the potential active sites for degradation and the susceptibility of the drug to hydrolysis, oxidation, thermal degradation, etc. is determined. Compatibility studies are performed to assess the stability of the drug when mixed with common excipients and lubricants as well as to determine any interaction between the drug and the (inactive) raw materials [14].

Step II Set up Preliminary HPLC condition:

Preliminary experimental conditions may be adapted from official or unofficial methods and from literature as a starting point. 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 [14].

Proper column and mobile phase selection is very critical. Get the appropriate conditions by selecting columns and mobile phase combinations. Computer assisted method development can be very helpful in developing the preliminary HPLC conditions. A proper experimental condition at the beginning will save a lot of time and effort in subsequent development stage [14].

Step III Preparation of samples required for method development:

SIMs is developed routinely by stressing the API under conditions exceeding those normally used for accelerated stability testing. In addition to demonstrating specificity SIMs, stress testing, also referred to as forced degradation, 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 [14] .

It is hard to get actual representative samples in the early development. Stressing the API generates the sample that contains the products most likely to form under most realistic storage conditions. Generally, the goal of these studies is to degrade the API to 5-10%. Perform forced degradation study through thermolysis, hydrolysis, oxidation, photolysis, and or combination conditions [14].

Step IV: Developing Separate 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. 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 [15, 16].

Isocratic or Gradient Mode:

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 [15, 16].

Solvent type:

Solvent type (methanol, acetonitrile, and tetrahydrofuran) 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 [15,16].

Mobile phase pH:

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 [15, 16].

Role of the 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. In general, the nature of stationary phase has the greatest effect on capacity factor, selectivity, efficiency and elution. 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 [15, 16].

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. 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. 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 .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 [15, 16].

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 function-ality 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. Common stationary phases are C4(butyl), C8(MOS), C18(ODS), nitrile (cyanopropyl), and phenyl (phenyl propyl) columns [15, 16].

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. Use of elevated temperature can be advantageous for several reasons. First, operating at a temperature higher than ambient reduces the viscosity of the mobile phase and thus the overall backpressure on the column. Lower system pressures allow for faster flow rates and thus faster analyses. An increase of 1 C will decrease the k' by 1 to 2%, a both ionic and neutral samples are reported to show significant changes in a with temperature changes. It is recommended that the column be thermo stated to control the temperature [15, 16].

Peak Purity:

Peak purity 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 on-line by employing PDA detection, LC-MS, or LC-NMR. However,

PDA only works well for degradants that have a different UV spectrum . 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. Other approaches use alternate separation techniques such as thin- layer chromatography (TLC), normal-phase-HPLC, capillary electrophoresis (CE), or supercritical fluid chromatography (SFC) [15, 16].

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 [17].

Step VI Validation of analytical method:

The methods have to be validated according to USP/ICH guidelines to show accuracy, precision, specificity, linearity, range, detection limit, quantification limit, ruggedness, and robustness of the method. Validation protocol should be written and acceptance criteria should be defined [18].

It is necessary to isolate, identify, characterize, and qualify the degradation products if they are above the identification threshold (usually 0.1%). A variety of techniques are available to identify and characterize impurities and degradation products such as HPLC with PDA (Photodiode Array) Detector, IR(Infrared) Spectrometry, elemental analysis, MS (Mass Spectrometry) [18].

Selectivity (Specificity):

Selectivity of a method refers to the extent to which it can determine particular analyte(s) in a complex mixture without interference from other components in the mixture. The selectivity of the analytical method must be demonstrated by providing data to show the absence of interference peaks with regard to degradation products, synthetic impurities and the matrix (excipients present in the formulated product at their expected levels) [18].

The selectivity of chromatographic methods may be assessed by examination of peak homogeneity or peak purity test [18].

Linearity:

The linearity is the ability of analytical procedure to produce test results which are proportional to the concentration of analyte in samples within a given concentration range. Linearity should be determined by using a minimum of six standards whose concentration span 80 –120% of the expected concentration range [18].

Range:

The specified range is normally derived from the linearity studies. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical method has suitable levels of precision, accuracy and linearity [18].

The following minimum specified ranges should be considered:

- For the assay of the active constituent or an agricultural/veterinary chemical product: normally from 80 –120% of the test concentration/label concentration.
- For the determination of an impurity: from the specification level of the impurity to 120% of the specification [19].

Accuracy:

The accuracy of an analytical method is defined as the degree to which the determined value of analyte in a sample corresponds to the true value. Accuracy may be measured in different ways [19].

The accuracy of an analytical method may be determined by any of the following ways:

- Analyzing a sample of known concentration and comparing the measured value to the 'true 'value. However, a well characterized sample (e.g., reference standard) must be used.
- Spiked – placebo (product matrix) recovery method. In the spiked – placebo recovery method, a known amount of pure active constituent is added to formulation blank [sample that contains all other ingredients except the active(s)], the resulting mixture is assayed, and the

results obtained are compared with the expected result.

- Standard addition method. In the standard addition method, a sample is assayed, a known amount of pure active constituent is added, and the sample is again assayed. The difference between the results of the two assays is compared with the expected answer [19].

In both methods (spiked – placebo recovery and standard addition method), recovery is defined as the ratio of the observed result to the expected result expressed as a percentage. The accuracy should cover at least 3 concentrations (80, 100 and 120%) in the expected range [19].

Acceptance criteria: the expected recovery depends on the sample matrix, the sample processing procedure and on the analyte concentration [19].

The mean % recovery should be within the following ranges:

Table 4: Mean recovery Percent for Pharmaceutical Substances

% Active/impurity content	Acceptable mean recovery
≥ 10	98 –102%
≥ 1	90 –110%
0.1 – 1	80 – 120%
< 0.1	75 – 125%

Precision:

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. A minimum of 5 replicate sample determinations should be made together with a simple statistical assessment of the results, including the percent relative standard deviation [19].

The following levels of precision are recommended:

Table 5: Precision levels for different sample concentrations

Component measured in sample	Precision
> 10.0%	≤ 2%
1.0 up to 10.0%	≤ 5%
0.1 up to 1.0%	≤ 10%
< 0.1%	≤ 20%

Limit of Detection (LOD):

The detection limit of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated as an exact value.

The LOD may be determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level (lowest calibration standard) at which the analyte can be reliably detected. The average response (X) and the standard deviation (SD) calculated. The LOD is X + (3 x SD) [19].

Limit of Quantitation (LOQ):

The limit of quantitation is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions. The limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products or low levels of active constituent in a product [19].

The LOQ may be determined by preparing standard solutions at estimated LOQ concentration .The average response and the standard deviation (SD) of the n results should be calculated and the SD should be less than 20%. If the SD exceeds 20%, a new standard solution of higher concentration should be prepared. The LOQ is X + (10 x SD) [19].

TYPICAL PARAMETERS USED IN HPLC METHOD VALIDATION

System Suitability:

System suitability test involves a comparison of chromatogram with a standard trace, this allows a comparison of the peak shape, peak width, base line

resolution. Parameters to be calculated to provide a system suitability test report are number of theoretical plates (efficiency), capacity factor, separation (relative retention), resolution, tailing factor, relative standard deviation, these are measured on a peak or peaks of known retention and peak width [20].

Table 6: System Suitability Parameters of HPLC System

S.NO.	System Suitability Parameters	Acceptance Criteria
1	% RSD for five replicate injections of analyte peak in standard solution	Should be NMT 2.0%
2	USP Tailing factor	Should be NMT 2.0
3	USP Plate count	Should be NLT 2000
4	USP Resolution	Should be NL 2.0

CONCLUSION

Forced degradation studies of new drug substances and drug products are important to help develop and demonstrate specificity of stability-indicating methods and to determine the degradation pathways and degradation products of the active ingredients. They were also useful in the investigation of the chemical and physical stability of crystal forms, the stereochemical stability of the drug substance alone and in the drug product and mass-balance issues, and for differentiating drug substance-related degradation products in formulations.

Forced degradation studies are used to facilitate the development of analytical methodology, to gain a better understanding of active pharmaceutical ingredient (API) and drug product (DP) stability and to provide information about degradation pathways and degradation products. HPLC methods would be able to separate, detect, and quantify the various drug-related degradants that can form on storage or manufacturing, plus detect and quantify any drug-related impurities that may be introduced during synthesis. In addition to demonstrating specificity, forced degradation studies can be used to determine the degradation pathways and degradation products that could form during storage, and facilitate during formulation, development, manufacturing and packaging. 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. In order to harmonize the procedures of forced degradation, an automated method for forced degradation was developed, utilizing the CTC LEAP. The Automated Forced Degradation approach significantly reduces the amount of manual labor used to perform the tests and harmonizes the operational procedures of forced degradation.

REFERENCES

1. Monika Bakshi, Saranjit Singh, Development of validated stability-indicating assay methods -a critical review, Journal of Pharmaceutical and Biomedical Analysis 2002.
2. H.H. Willard, L.L.Merritt, J.A.Dean, F.A. Settle, Instrumental Methods of Analysis,7th edition, CBS Publishers and Distributers, New Delhi, Pg: 518-521, 580-610.
3. D.A. Skoog, J. Holler, T.A. Nieman, Principle of Instrumental Analysis, 5th Edition, Pg: 778-787.
4. Sharma B.K., Instrumental Methods of Chemical Analysis, GOEL Publication House, Meerut, Pg. 133-161, 68-80, 114-165, 286-320.
5. Gurdeep R.Chatwal, Instrumental Methods of Chemical Analysis, Himalaya Publication House, Pg: 1.1-1.8, 2.566-2.570.
6. Sethi P.D., HPLC-Quantitative analysis of pharmaceutical formulations; 3rd Edn, CBS publishers & distributors, 1997, Pg: 182-184.
7. Szepesi G. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. Journal of Chromatography. 1989;464:265-278.
8. Lina Liu, Timothy A Rhodes, Roy Helmy, Brian Farrer, Edwin C-Automated Kinetic Forced Degradation Development and HPLC Method Validation, Pharmaceutical Analytical Chemistry,2009.

9. ICH guidelines Q1A (R2). Stability Testing of New Drug Substances and Products (revision 2), November 2003.
10. Ranjit Singh and Zia ur Rehman Current trends in forced degradation study for pharmaceutical product development, Journal of Pharm Educ Res Vol. 3, Issue No. 1, June 2012.
11. KishoreKumar Hotha, Satti Phani Kumar Reddy, V. Kishore Raju, L.K.Ravindranath Forced degradation studies: practical approach - overview of regulatory guidance and literature for the drug products and drug substances, international research journal of pharmacy, 2013.
12. Patel Riddhiben M, Patel Piyushbhai M , Patel Natubhai M, Stability Indicating Hplc Method Development - A Review, International Research Journal Of Pharmacy2011.
13. 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.
14. Kats M. Forced degradation studies: regulatory considerations and implementation. BioPharm Int. July 2005.
15. R. Snyder, J. Kirkland, L. Glajch, Practical HPLC method development, 1997, II Ed, A Wiley International publication, Pg: 235,266-268,351-353.653-600.686-695.
16. Introduction to Method development and Validation [Online] Available at: http://www.pharmainfo.net/reviews/introduction-analytical-method-development-pharmaceutical-formulations.
17. FDA Guidance for Industry. Analytical Procedures and Methods Validation (draft guidance), August 2000.
18. Carr GP, Wahlich JC. A practical approach to method validation in pharmaceutical analysis. J Pharm Biomed Anal. 1990;86:613-618.
19. Australian Pesticides And Veterinary Medicine Authority -Guidelines for the validation of analytical methods for active constituent, agricultural and veterinary chemical products,2004.
20. Jenke DR. Chromatographic method validation: a review of common practices and procedures II. J Liq Chromat.1996;19:737-757.