STABILITY INDICATING METHOD DEVELOPMENT FOR DRUG STABILIZATION: A REVIEW

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ABSTRACT
The regulatory issues of stability indicating method development are reviewed in this work. The API is distinguished from any potential breakdown products using SIMs. Validated stability indicating methods are required by regulatory guidance in ICH Q1A (R2), ICH Q3B (R2), Q6A, and FDA 21 CFR section 211. To establish whether the analytical approach indicates stability, force degradation of drug standard and excipients is conducted under various situations by the help of HPTLC, HPLC with UV, and MS. Forced degradation studies give information regarding crucial degradation pathways and active ingredient degradation products, as well as aid in elucidating the structure of the degradants. There is discussion of the methods for developing a stability indicating method by various instrumentation techniques.

KEYWORDS: Stability Indicating Method, UV Spectroscopy, forced Degradation Studies, Method Validation.

INTRODUCTION
A Stability-indicating assay method can be defined as “Validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and drug products are specific so that the content of active ingredients and degradation products can be accurately measured without interference”.[15]

Stability studies are performed to determine shelf life and storage conditions for APIs and products. In recently adopted stability guidelines, the committee for a proprietary medicinal
product (CPMP) suggests the objective of stability testing is to provide evidence on how much quality of an API varies with time under influence of the variety of environmental factor such as temperature, humidity and light. The stability of API refers to "managed and allowed modification," not "fix" or "not likely change." Bringing up the conditions for force degradation, stress agent concentration, and stress time must result in degradation of at least 10-20% of the parent constituent. As a requirement of regulatory authorities to give insights that may be used in the formulation of other products, stability testing is carried out for the welfare of the patient and to protect the producer's reputation.\textsuperscript{[15]}

A Stability Indicating Method (SIM) is a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from process impurities, excipients, and degradation products, according to FDA guidance (Guidance for Industry, Analytical Procedures and Methods Validation, FDA, 2000). All assay techniques for stability should, according to the FDA, be stability indicating. A stability indicating method's major goal is to keep track on the outcomes of stability investigations in order to ensure their safety, effectiveness, and quality. It also serves as a potent investigative tool for examining outcomes that are out-of-trend (OOT) (Swartz et al., 2004) or out-of-spec (OOS) (CDER, 2006) in quality control processes.\textsuperscript{[4]}

Generally forced degradation/stress testing is used to generate the samples for stability-indicating assay methods. Forced degradation/stress testing is defined as “the stability testing of drug substance and drug product under conditions exceeding those used for accelerated stability testing”.\textsuperscript{[2]} Degradation can be achieved by exposing the drug, for extended period of time, to extremes of pH (HCl or NaOH solutions of different strengths), at elevated temperature, to hydrogen peroxide at room temperature, to UV light, and to dry heat (in an oven) to achieve degradation to an extent of 5–20%. Generally, trial and error experimentation are used during these experiments. A systematic technique should be used instead of the trial-and-error method because it is typically more expensive, labour- and time-intensive. A thorough review of the literature revealed that using experimental design in forced deterioration trials can result in cost and labour savings by minimising trial and error experimentation.\textsuperscript{[3,4]}

The general steps in the experimental design strategy are the choice of variables, choice of response, preference of design, preparation of experimental domain, preparation of
experimental matrix, generation of polynomial equation, choice of essential/most affecting variables using one-way ANOVA, Pareto chart, or normal/half normal plots, and finally choice of optimal region by surface response.

**Why are stability indicating method necessary?**

- The GMP requires a formal written stability testing program whose results are used to establish storage conditions, expiration dates of Drug Products/Substances and further mandates the use of reliable, meaningful and specific test methods.
- When there is a desire to document drug product stability, such procedures are applicable. This information is utilized to determine, adhere to, or expand retest intervals or expiration dates for drug substance or drug product.\(^4\)

1. **Sample generation**

For generating samples for SIM, the API is force degraded at conditions more severe than accelerated degradation conditions. It involves drug degradation under the previously mentioned hydrolytic, oxidative, photolytic, and thermal conditions. The purpose of forcing API degradation in solid state and solution form is to produce degradation products that are likely to occur under realistic storage conditions.\(^{21}\) This sample is then used to develop an SIM.

2. **Method Development and Optimization**

Before starting the method development, various physiochemical properties like pKa value, log P, solubility and absorption maximum of the drug must be known, for it lays a foundation for HPLC method development. Log P and solubility helps select mobile phase and sample solvent while pKa value helps determine the pH of the mobile phase.\(^6\)

As deterioration occurs in aqueous solution, reverse phase columns are a popular alternative to begin the separation of sample components. For the first stages of separation, mobile phase made up of acetonitrile, water, and methanol can be utilised in a variety of ratios. Based on the analyte's solubility, methanol or acetonitrile is chosen for the organic phase. Initially the water: organic phase ratio can be kept at 50:50 and suitable modifications can be made as trials proceed to obtain a good separation of peaks. Latter buffer can be added if it is required to obtain better peak separation and peak symmetry. If the method is to be extended to liquid chromatography–mass spectrometry (LC–MS), then mobile phase buffer should be MS compatible like triflouroacetic acid and ammonium formate. Variation in column temperature
affects the selectivity of the method as analytes respond differently to temperature changes. A
temperature in the range of 30–40°C is suitable to obtain good reproducibility.[31] The drug
peak should be pushed further up the chromatogram since this separates all degradation
products. Additionally, a long enough run time must be allowed after the drug peak to acquire
the peak elution of the degradants.[6]

When developing a technique, it is possible that the drug peak masks an impurity or
degradation peak that co-elutes with the drug. Peak purity analysis, which establishes the
method's specificity, is necessary for this. Online direct analysis employing photo diode array
(PDA) detection is possible. PDA offers data on the homogeneity of the spectral peak, but it
does not apply to degradants whose UV spectra are comparable to those of drugs. The
indirect technique entails altering the chromatographic variables, such as the mobile phase
ratio and column, which will impact peak separation. The spectrum of altered
chromatographic condition is then compared with the original spectra. If the degradant peaks
and area percentage of the drug peak remain same, then it can be confirmed that the drug
peak is homogeneous.[32] The degradant that co-elutes with the drug would be acceptable if it
is not found to be formed in accelerated and long-term storage conditions.[1] Then, by
adjusting the flow rate, injection volume, column type, and mobile phase ratio, the procedure
is made to work best for separating closely eluting peaks.

3. Method validation
The developed SIM is then validated according to USP/ICH guideline for linearity, accuracy,
precision, specificity, quantitation limit, detection limit, ruggedness and robustness of the
method. It is necessary to isolate, identify, and quantify the degradants found to be above the
detection threshold (about 0.1%). If the method does not meet the validation acceptance
requirements, it is updated and revalidated.[32]

4. Other analytical methods for developing SIM
Methods that indicate stability will have high biological activity, high potency, and high
purity. The tests that are chosen are product-specific. Numerous techniques, such as
electrophoresis (SDS-PAGE, immune-electrophoresis, Western blot, isoelectrofocusing),
high-resolution chromatography (such as reversed phase chromatography, SEC, gel filtration,
ion exchange, and affinity chromatography), and peptide mapping, can be used to determine
stability.[33] The chosen analytical technique must be sensitive enough to pick up
contaminants at low concentrations (i.e., 0.05% of the target analyte or less), and the peak
responses must fall within the linearity range of the detector. The analytical approach must be able to detect every impurity created during a formal stability study at or below ICH threshold levels. Degradation product identification and characterization are to be performed based on formal stability results in accordance with ICH requirements. Conventional methods (e.g., column chromatography) or hyphenated techniques (e.g., LC–MS, LC–nuclear magnetic resonance (NMR)) can be used in the identification and characterization of the degradation products. Use of these techniques can provide a better insight into the structure of the impurities that could add to the knowledge space of potential structural alerts for genotoxicity and the control of such impurities with tighter limits.\[6,33\] It should be noted that structural characterization of degradation products is necessary for those impurities formed during formal shelf-life stability studies and above the qualification threshold limit.\[34\]

When it is acceptable to build a stability signalling approach, new analytical technologies that are always being developed can also be utilised. The unknown impurity can be separated and analysed using a variety of chromatographic techniques, including 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 analysis (SCFA). When degradants cannot be extracted in pure form, a great hyphenated chromatographic and spectroscopic approach is utilised, such as HPLC photodiode array ultraviolet detector (DAD), LC-MS, LC-NMR, and GC-MS. HPLC-DAD and LC–MS are used to compare the relative retention time (RRT), UV spectra, mass spectra (MS/MS or MSN).\[35\] Singh and Rehman.\[23\] discussed the role of hyphenated systems for the isolation of degradants and impurities.

**Steps involved in stability indicating UV spectroscopy method development**

1. Preliminary solubility studies of drug
2. Preparation of standard stock solution
3. Preparation of calibration curve
4. Sample preparation
5. Method validation
6. Linearity
7. Precision
8. Accuracy
9. LOD and LOQ
10. Forced degradation studies

![Diagram](Figure: The Various Parameters of ICH Q2 R1 Guidelines.)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>r2 ≥0.99, similar response ratios</td>
</tr>
<tr>
<td>Precision-System</td>
<td>RSD&lt;2%</td>
</tr>
<tr>
<td>Precision-Method</td>
<td>RSD&lt;2%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>FDA 98-102%, EPA 50-150%</td>
</tr>
<tr>
<td>Specificity</td>
<td>No interference</td>
</tr>
<tr>
<td>Detection Limit</td>
<td>&gt;2 times base line</td>
</tr>
<tr>
<td>Quantitative Limit</td>
<td>Signal-to-Noise=10:1</td>
</tr>
<tr>
<td>Range</td>
<td>Concentration where data can be reliably detected</td>
</tr>
<tr>
<td>Linearity</td>
<td>r2 ≥0.99, similar response ratios</td>
</tr>
<tr>
<td>Precision-System</td>
<td>RSD&lt;2%</td>
</tr>
<tr>
<td>Precision-Method</td>
<td>RSD&lt;2%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>FDA 98-102%, EPA 50-150%</td>
</tr>
</tbody>
</table>

**Method validation**

Method validation is completed to ensure that an analytical methodology is accurate, specific, reproducible and rugged over the specified range that an analyte will be analysed. Acceptance criteria for the different characteristics of validation by ICH are shown in table 1.

Method validation provides an assurance of reliability during normal use, and is sometime referred to as "the process of providing documented evidence that the method does what it is intended to do” and parameters are shown in table 2.\[18,19\]

**Precision**
Precision is a measure of how repeatable an analytical method is when used routinely. It is typically stated as the percent relative standard deviation for a large enough sample size to be statistically significant. The ICH states that three different levels of accuracy should be used: repeatability, intermediate precision, and reproducibility. Repeatability is the outcome of the approach being used repeatedly under the same circumstances over a short period of time (inter-assay precision).

It should be determined from a minimum of six determinations at 100% of the test or target concentration, or from a minimum of nine determinations covering the procedure's stated range (for instance, three levels, three repetitions each). The results of internal lab differences brought on by chance occurrences such various days, analysts, equipment, etc. are referred to as intermediate precision. Experimental design should be used to determine intermediate precision so that the impacts (if any) of the different variables can be tracked. Results of cross-laboratory research are said to as reproducible. The standard deviation, relative standard deviation, coefficient of variation, and the confidence interval should all be included in the documentation for precision research’s.\(^{[1],[2]}\)

**Accuracy**

Accuracy is the measure of exactness of an analytical method, or the closeness of agreement between the value which is accepted either as a conventional, true value or an accepted reference value and the value found. It is determined by spiking samples in a blind trial and measuring the percentage of analyte recovered by assay. In order to determine the accuracy of the drug substance test, results are compared to an analysis of a standard reference material or to a different, well-established method. The accuracy of the drug product test is assessed by analysing synthetic mixes that have been laced with known amounts of component parts. Accuracy for impurity quantification is assessed by analysing samples (drug substance or drug product) spiked with known impurity concentrations.\(^{[15]}\) (If there are no impurities, refer to specificity.)

**Specificity**

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. It determines the level of interference from things like other active ingredients, excipients, contaminants, and degradation products to make sure that a peak response is only caused by one active ingredient a lack of co-elution. Resolution, plate count (efficiency), and tailing
factor are used to quantify and record specificity in a separation. Modern photodiode array detectors can also assess specificity by statistically comparing the spectra gathered across a peak to determine peak homogeneity. The ICH uses the term "specificity" as well and categorises it into two groups: identification and assay/impurity tests.

**Limit of detection**

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantitated. The limit test determines if an analyte is above or below a given value. It is expressed as a concentration at a particular signal-to-noise ratio, typically a two- or three-to-one ratio. The signal-to-noise ratio convention has been acknowledged by the ICH, but it also specifies two other ways to establish LOD: non-instrumental visual approaches and a method for computing the LOD. Visual non-instrumental methods may include LOD’s determined by techniques such as thin layer chromatography (TLC) or titrations. LOD’s may also be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula:

\[
\text{LOD} = 3.3(\text{SD}/S)
\]

Based on the standard deviation of the blank, the residual standard deviation of the regression line, or the standard deviation of the y-intercepts of regression lines, the standard deviation of the answer can be calculated. A sufficient quantity of samples should be assessed at the limit to validate the level, and the method used to calculate LOD should be validated and documented. LOD values are always individual to a certain set of experimental variables. The detection limits of a method will change if something alters the method's sensitivity, including the instrument, sample preparation, etc.

**Limit of quantification**

The Limit of Quantification (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Like LOD, LOQ is expressed as a concentration, with the precision and accuracy of the measurement also reported. Sometimes a signal-to-noise ratio of ten-to-one is used to determine LOQ. This signal-to-noise ratio is a good rule of thumb, but it should be remembered that the determination of LOQ is a compromise between the concentration and the required precision and accuracy. In other words, precision improves as LOQ concentration level falls. A larger concentration must be recorded for LOQ if greater
precision is needed. The analytical technique and its intended application require this trade-off. The ICH has acknowledged a signal-to-noise ratio of ten to one as common and, like LOD, specifies two more methods that can be used to calculate LOQ: visual non-instrumental methods and a method for computing the LOQ. The calculation method is again based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula:

$$\text{LOQ} = 10(\text{SD}/S)$$

Again, the standard deviation of the response can be determined based on the standard deviation of the blank, on the residual standard deviation of the regression line, or the standard deviation of y-intercepts of regression lines. The value of LOQ is almost 10 times higher than that of the blank. The LOQ was found with in limit concentrations that 0.211μg. The maximum limit of LOD value must be not more than 2 μg, from the standard references.

**Linearity and Range**

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range reported as the variance of the slope of the regression line. Range is the interval between the upper and lower levels of analyte (inclusive) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. Typically, the range is stated in the same units as the test findings that were derived using the procedure. The ICH regulations outline a minimum of five concentration levels as well as a few minimum specified ranges. 80-120% of the target concentration is the minimum specified range for test. The minimal range for an impurity test is between each impurity's reported level and 120% of the specification. The range ought to be in line with the restricted level (for harmful or more strong contaminants).

**Ruggedness**

According to the USP, ruggedness is measured by the percentage of RSD that may be used to replicate results that were achieved under various situations. These circumstances include various labs, analysts, tools, reagents, days, etc. Ruggedness was not particularly covered by the ICH in the definitions and terminology guide. However, this seeming exclusion is actually a semantic issue because ICH instead decided to treat ruggedness as a component of precision, as previously mentioned.

**Robustness**
Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. The robustness of a method is evaluated by varying method parameters such as percent organic, pH, ionic strength, temperature, etc., and determining the effect (if any) on the results of the method. As documented in the ICH guidelines, robustness should be considered early in the development of a method. In addition, if the results of a method or other measurements are susceptible to variations in method parameters, these parameters should be adequately controlled and a precautionary statement included in the method documentation.

Table 2: Typical performance characteristics and related validation tests for measured product attributes.[1]

<table>
<thead>
<tr>
<th>Type of measured product attribute</th>
<th>Identity</th>
<th>Impurity (purity) Other quantitative measurements (1)</th>
<th>Assay Content/potency Other quantitative measurements (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical Procedure performance characteristics to be demonstrated (2)</td>
<td></td>
<td>Quantitative Limit</td>
<td></td>
</tr>
<tr>
<td>Specificity (3) Specificity test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Working range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suitability of Calibration model</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lower range limit Verification</td>
<td>-</td>
<td>Ql (dl)</td>
<td>Dl</td>
</tr>
<tr>
<td>Accuracy (4) Accuracy test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Precision (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Intermediate Precision test</td>
<td>-</td>
<td>+ (5)</td>
<td>-</td>
</tr>
</tbody>
</table>

- signifies that this test is not normally evaluated
+ signifies that this test is normally evaluated
( ) signifies that this test is normally not evaluated, but in some complex cases recommended

QL, DL: Quantitation Limit, Detection Limit

(1) Other quantitative measurements can follow the scheme of impurity testing, if the working range is close to the detection or quantitation limits of the technology, otherwise following the assay scheme is recommended.
(2) Some performance characteristics can be substituted with technology inherent justification or qualification in the case of certain analytical procedures for physicochemical properties.

(3) A combined approach can be used alternatively to evaluating accuracy and precision separately

(4) Lack of specificity of one analytical procedure could be compensated by one or more other supporting analytical procedures.

(5) Reproducibility and intermediate precision can be performed as a single set of experiments.[1]

**Forced degradation studies**

ICH guidelines entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of active substance. Stability studies of was carried out under extreme conditions acidic, alkaline, hydrolytic, oxidative, thermolytic and photolytic as per stability indicating assays.[17]

**Objective of forced degradation studies**

Forced degradation studies are carried out to achieve the following purposes:

1. To establish degradation pathways of drug substances and drug products.
2. To differentiate degradation products that are related to drug products from those that are generated from non-drug product in a formulation.
3. To elucidate the structure of degradation products.
4. To determine the intrinsic stability of a drug substance in formulation.
5. To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and drug product.[1,2]
6. To establish stability indicating nature of a developed method.
7. To understand the chemical properties of drug molecules.
8. To generate more stable formulations.
9. To produce a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.
10. To solve stability-related problems.[3]
Scheme 1: An illustrative flowchart describing various stress conditions used for degradation of drug Substance and Drug product.

Table 3: Conditions mostly used for forced degradation studies.

<table>
<thead>
<tr>
<th>Degradation type</th>
<th>Experimental conditions</th>
<th>Storage conditions</th>
<th>Sampling time(days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis</td>
<td>Control API (no acid or base)</td>
<td>40 °C, 60 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>0.1 M HCl</td>
<td>40 °C, 60 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>0.1 M NaOH</td>
<td>40 °C, 60 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>Acid control (no API)</td>
<td>40 °C, 60 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>Base control (no API)</td>
<td>40 °C, 60 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>pH: 2,4,6,8</td>
<td>40 °C, 60 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td>Oxidation 3%</td>
<td>3% H2O2</td>
<td>25 °C, 60 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>Peroxide control</td>
<td>25 °C, 60 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>Azobisisobutyronitriile (AIBN)</td>
<td>40 °C, 60 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>AIBN control</td>
<td>40 °C, 60 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td>Photolytic Light</td>
<td>Light 1* ICH</td>
<td>NA</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>Light 3* ICH</td>
<td>NA</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>Light control</td>
<td>NA</td>
<td>1,3,5</td>
</tr>
<tr>
<td>Thermal</td>
<td>Heat chamber</td>
<td>60 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>Heat chamber</td>
<td>60 °C /75%</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>Heat chamber</td>
<td>80 °C</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>Heat chamber</td>
<td>80 °C /75%</td>
<td>1,3,5</td>
</tr>
<tr>
<td></td>
<td>Heat control</td>
<td>Room temp</td>
<td>1,3,5</td>
</tr>
</tbody>
</table>

Acid/Alkali Degradation: (Hydrolytic conditions)
Over a wide pH range, hydrolysis is among the most prevalent chemical processes involved in degradation. In the chemical process of hydrolysis, a chemical substance is broken down by interaction with water. Ionizable functional groups present in the molecule are catalysed during hydrolytic research in acidic and basic conditions. Acid or base stress testing entails exposing a pharmacological material to acidic or basic conditions in order to force degradation that produces primary degradants in a desired range. The type and concentration of acid or base to use depends on how stable the drug ingredient is. As acceptable reagents
for hydrolysis, sodium hydroxide or potassium hydroxide (0.1-1 M) are proposed for base hydrolysis and hydrochloric acid or sulfuric acids (0.1-1 M) for acid hydrolysis.\textsuperscript{[6],[7]} Co-solvents can be employed to dissolve chemicals for stress testing in HCl or NaOH if they are insufficiently soluble in water. The structure of the drug substance is used to guide the choice of co-solvent. Normal stress testing trials begin at room temperature, and if no degradation occurs, higher temperature (50–70 °C) is next applied. A maximum of seven days should be allowed for stress testing. To stop further degradation, the degraded sample is subsequently neutralised with the appropriate acid, base, or buffer.

**Oxidative degradation:**

Hydrogen peroxide is widely used for oxidation of drug substances in forced degradation studies but other oxidizing agents such as metal ions, oxygen, and radical initiators (e.g., azobisisobutyronitrile, AIBN) can also be used. The drug substance determines the type of oxidising agent to use, as well as its concentration and environmental factors. According to reports, exposing the solutions to 0.1–3% hydrogen peroxide for seven days at neutral pH and room temperature, or up to a maximum 20% degradation, may produce relevant degradation products.\textsuperscript{[7]} The oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations. Amines, sulfides and phenols are susceptible to electron transfer oxidation to give N-oxides, hydroxylamine, sulfones and sulfoxide.\textsuperscript{[8]} The functional group with labile hydrogen like benzylic carbon, allylic carbon, and tertiary carbon or α-positions with respect to heteroatom is susceptible to oxidation to form hydro peroxides, hydroxide or ketone.\textsuperscript{[9],[10]}

**Dry Heat Degradation/Wet Heat Degradation:**

Thermal degradation (e.g., dry heat and wet heat) should be carried out at more strenuous conditions than recommended ICH Q1A accelerated testing conditions. In contrast to liquid drug products, samples of solid-state drug substances and drug products should be subjected to both dry and moist heat. Studies might be carried out for a shorter amount of time at a higher temperature.\textsuperscript{[7]} Effect of temperature on thermal degradation of a substance is studied through the Arrhenius equation:

$$k=Ae^{-\frac{E_a}{RT}}$$

where $k$ is specific reaction rate, $A$ is frequency factor, $E_a.$ is energy of activation, $R$ is gas constant (1.987 Cal/deg mole) and $T$ is absolute temperature.\textsuperscript{[10],[14],[15]} Thermal degradation study is carried out at 40–80 °C.
Photolytic degradation:
The photo stability testing of drug substances must be evaluated to demonstrate that a light exposure does not result in unacceptable change. The chief degradants of a pharmacological substance are produced by photo stability tests when exposed to UV or fluorescent light. The ICH guidelines outline a few suggested circumstances for photostability testing. A minimum of 1.2 million lx h and 200 W h/m² of light should be applied to samples of drug material and solid/liquid drug product. The photolytic deterioration is most frequently attributed to light with a wavelength between 300 and 800 nm.\textsuperscript{11,12} The maximum illumination recommended is 6 million lx h.\textsuperscript{10} Light stress conditions can induce photo oxidation by free radical mechanism. Functional groups like carbonyls, nitro aromatic, N-oxide, alkenes, aryl chlorides, weak C-H and O-H bonds, sulfides and polyenes are likely to introduce drug photosensitivity.\textsuperscript{13}

Chromatographic analysis of forced degraded samples
After degradation, each sample obtained under each forced degradation condition was diluted appropriately with mobile phase to get a final concentration of 10 μg/mL; the resulting solution was injected in the column under described chromatographic condition. The resulting chromatogram was examined for the area of the drug peak and the emergence of secondary peaks. The presence of secondary peaks and a decrease in the drug peak's surface area were thought to be signs of deterioration. The % degradation was calculated as:\textsuperscript{17}

\[
\text{% degradation} = \left(\frac{\text{area of unstressed} - \text{area of stressed}}{\text{area of unstressed}}\right) \times 100
\]

CONCLUSION
The stability indicating approach, which takes a lot of sample preparation and solvent consumption and is therefore time-consuming, can be employed with HPTLC, HPLC with UV, and MS. Since the UV method of detection is used in both procedures. Instead of immediately using HPLC or HPTLC methods, it is always preferable to use a quick, affordable, and efficient UV spectrophotometry method that at the very least provides an idea of degradation and is always useful for planning any stability indicating method by HPLC or HPTLC that cuts down on time. The primary objective of any forced degradation approach is to create the required level of degradation, i.e., 5-20%. A properly conceived and carried out forced degradation study would yield an adequate sample for the development of a stability signalling method. These verified techniques can be used for regular quality checks.
REFERENCE


2. ICH Q2 (R1) Validation of Analytical Procedures, International Conference on Harmonization


