CHAPTER - 1
GENERAL INTRODUCTION
General Introduction

1.1 Introduction and importance of stability-indicating analytical methods in pharmaceutical research and development:

The efficacy and safety of pharmaceuticals can not be ensured unless the quality of the pharmaceuticals is maintained during their specified shelf lives. New drug applications need to submit scientific data that guarantee the stability of the product over a specified time period when maintained under specific storage conditions. If you look at the label on your favorite prescription or over-the-counter (OTC) pharmaceutical product, you will see a use before or expiry date [1]. Before this date, the product should remain fully effective under normal storage conditions. The product’s shelf life is determined using standardized storage conditions of controlled temperature and humidity, which can be translated into accepted product lifetimes. All of these aspects are therefore the part of stability programs [2, 3].

The International Conference on Harmonisation of the Technical requirements for the Registration of Pharmaceuticals for Human Use (ICH) was organized in order to harmonize stability-testing requirements for new drug applications within the European Union (EU), the United States, and Japan. ICH guidelines for stability testing of New Drug Substances and Products and for photo stability testing of New Drug Substances and Products were officially adopted in October 1993 and November 1996, respectively [4, 5].
1.2 Requirements of stability testing and development of stability-indicating analytical methods (SIAM’S):

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enables recommended storage conditions, re-test periods and shelf lives to be established.

The ICH guideline primarily addresses the information required in Registration Applications for new molecular entities and associated drug products. This guideline does not currently seek to cover the information required for abbreviated or abridged applications, variations, clinical trial applications, etc. The choice of test conditions defined in the guideline is based on the analysis of the effects of climatic conditions in the three areas of the EC, Japan and USA. The mean kinetic temperature in any region of the world can be derived from climatic data.

Information on the stability of the drug substance is an integral part of the systematic approach to stability evaluation. The goal of stability program depends on the stage of development of the drug product. At very beginning of product development, it is necessary to understand the inherent stability of the drug substance and its interaction with the proposed excipients. At this stage the effect of pH, moisture, air (oxygen), and light on the stability of the drug substance also studied. The accelerated testing on drug substance and drug product provides the information to the intrinsic stability of the
molecule/formulation and may establish the likely degradation pathways. The formulation group also has the responsibility for recommending to the toxicology group about the stability of drug substance in the vehicle used in the animal trails. On the analytical side, the analytical research group supports the pre-formulation stability program, which ultimately responsible for developing and validating the stability-indicating assays that, will be included in New Drug Application [NDA].

In the preclinical formulation stage, the selection of a stable drug product formula is the primary goal. The temporary preclinical formula is included in the Investigational New Drug application [IND]. The goal of the stability program in the clinical trail stage is to ascertain that the drug product batches tested in the clinical trails are stable, and these data will be subsequently included in the NDA. At the NDA approval stage, the validated stability-indicating analytical method will be transferred to the quality control group, to ascertain that it works well in the hands of those who have to monitor the stability of the marketed product.

The marketed product stability program fulfills the commitment of part of the NDA and also ensures that the marketed drug products are stable (potent) until the expiry date stamped on the product label. Usually, the first three marketed batches and at least one batch per year are subjected to stability program.
1.2.1 ICH activity and quality guidelines:

The ICH was established in 1990 as a joint regulatory/industry project to improve, through harmonisation, the efficiency of the process for developing and registering new medicinal products in Europe, Japan and the United States of America, to make these products available to patients with a minimum of delay. The six parties involved in ICH process represent the regulatory bodies and pharmaceutical industry in the three regions, Europe, Japan and the USA. The parties involved from these three regions are – European Commission (EC), European Federation of Pharmaceutical Industries and Associations (EFPIA); Ministry of Health, Labor and Welfare (MHLW), Japan Pharmaceutical Manufacturers Association (JPMA); and US Food and Drug Administration (FDA), and the Pharmaceutical Research and Manufacturers of America (PhRMA). Each of the six co-sponsors has two seats on the ICH Steering Committee (SC) which oversees the harmonization activities. There are three observers: World Health Organisation (WHO), the European Free Trade Area (EFTA) and Drugs Directorate Health, Canada. The International Federation of Pharmaceutical Manufacturers Association (IFPMA) also has two seats on the ICH SC as non-voting members. ICH meetings have been held every alternate year starting from 1991 to 1997. The last ICH was held at Tokyo, Japan in December 2005.

The fourth International Conference on Harmonisation, Brussels, 16-18 July 1997, marked the completion of the first phase of activities. In the first phase the exercise was directed towards
elimination of redundant and duplicates technical requirements for registration in individual countries, and laying down of minimum standards applicable uniformly irrespective of where the product is manufactured and/or marketed in the three regions. This was done with development of over 40 guidelines covering Efficacy, Quality and Safety aspects of drug development. The list of guidelines under the head ‘Quality’ is given in Table: 1.1 [6].
### Table: 1.1 List of topics, codes and corresponding quality guidelines developed by ICH

<table>
<thead>
<tr>
<th>Topics / Code</th>
<th>Quality guidelines</th>
</tr>
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<tbody>
<tr>
<td>Q1A</td>
<td>Stability Testing of New Drug Substances and Products</td>
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<tr>
<td>Q1A(R2)</td>
<td>Stability Testing of New Drug Substances and Products</td>
</tr>
<tr>
<td>Q1B</td>
<td>Stability Testing: Photo stability Testing of New Drug Substances and Products</td>
</tr>
<tr>
<td>Q1C</td>
<td>Stability Testing for New Dosage Forms Annex to the ICH Harmonised Tripartite Guideline on Stability Testing for New Drugs and Products</td>
</tr>
<tr>
<td>Q1D</td>
<td>Bracketing and Matrixing Designs for Stability Testing of Drug Substances and Drug Products</td>
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<td>Q1E</td>
<td>Evaluation of Stability Data</td>
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<tr>
<td>Q1F</td>
<td>Stability Data Package for Registration in Climatic Zones III and IV</td>
</tr>
<tr>
<td>Q2A</td>
<td>Text on Validation of Analytical Procedures</td>
</tr>
<tr>
<td>Q2B</td>
<td>Validation of Analytical Procedures: Methodology</td>
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<tr>
<td>Q3A</td>
<td>Impurities in New Drug Substances</td>
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<tr>
<td>Q3A(R)</td>
<td>Impurities in New Drug Substances</td>
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<tr>
<td>Q3B</td>
<td>Impurities in New Drug Products</td>
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<tr>
<td>Q3B(R)</td>
<td>Impurities in New Drug Products Questions and Answers on Attachment 2 of Q3B(R): Illustration of Reporting Degradation Product Results for Identification and Qualification in an Application (Draft)</td>
</tr>
<tr>
<td>Q3C</td>
<td>Impurities: Guideline for Residual Solvents</td>
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<tr>
<td>Q3C(M)</td>
<td>Impurities: Residual Solvents (Maintenance) PDE for N-Methylpyrrollidone (NMP)</td>
</tr>
<tr>
<td>Q3C(M)</td>
<td>Impurities: Residual Solvents (Maintenance) PDE for Tetrahydrofuran</td>
</tr>
<tr>
<td>Q5A</td>
<td>Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin</td>
</tr>
<tr>
<td>Q5B</td>
<td>Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of R-DNA Derived Protein Products</td>
</tr>
<tr>
<td>Q5C</td>
<td>Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products</td>
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<tr>
<td>Q5D</td>
<td>Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products</td>
</tr>
<tr>
<td>Q5E</td>
<td>Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process</td>
</tr>
<tr>
<td>Q6A</td>
<td>Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances</td>
</tr>
<tr>
<td>Q6B</td>
<td>Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products</td>
</tr>
<tr>
<td>Q7</td>
<td>Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients</td>
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<tr>
<td>Q7A</td>
<td>Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients</td>
</tr>
<tr>
<td>Q8</td>
<td>Pharmaceutical Development</td>
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<td>Q9</td>
<td>Quality Risk Management</td>
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The pharmacopoeial authorities have been closely involved with the work of ICH since the outset and harmonization between the major Pharmacopoeias, which started before ICH, has proceeded in parallel. The ICH Steering Committee receives regular reports on pharmacopoeial harmonization at its meetings.

In the second phase of activities, a process has been drawn up for updating and supplementing the current guidelines, when necessary, and monitoring their use, so that benefits of harmonisation achieved so far will not be lost in the future. Another important objective of this phase was to harmonize the format and content of the application documents for new product applications.

In a view expressed by a representative of US FDA - ‘the success of the first phase of ICH has changed FDA from what was a largely a self-reliant domestic arm of the federal government to an internationally-minded organization that actively seeks out and promotes world-wide work sharing and co-operation in protecting the public health’. Evidently, the individual regulatory authorities are also opening up subsequent to their active participation in the ICH process. The excellent activities done by ICH in the areas of common medical terminology, electronic adverse drug reporting, electronic pharmaco vigilance and electronic transmission of regulatory information is certainly a leap towards use of information and communication technologies in the transfer of regulatory information. Finally, the ICH has proved that industry and regulators can work constructively together in the interest of public interest when science
is regarded as very important.

The ICH has implemented a forum for exchange of information with the countries of Central and Eastern Europe and hence they will automatically benefit from the harmonization effort. The EFTA countries, which include Iceland, Norway, Liechtenstein and Switzerland, on the other hand have the honor of being observer to the ICH SC. Among these, Switzerland, which is the base of pharmaceutical majors, namely Novartis and Hoffman La Roche, has the open policy of accepting data generated by following the ICH guidelines. The same is also true of another observer country, i.e., Canada.

Feeling that it is increasingly important to ensure that the objectives and outcome of ICH are well understood and widely disseminated, an ICH Global Cooperation Group has been formed, which held its first meeting on 5 October 1999. It developed an action plan to facilitate the purpose of more actively disseminating information outside the EU, Japan and the US, and identifying needs for ICH products, in close co-operation with WHO.

WHO has already been involved in disseminating the guidelines and encouraging their wide-spread adoption and use. It has adopted on itself the role to build a communication bridge between ICH and the 170 non-participating member states. WHO has been an observer member of ICH steering committee since the inception of the ICH. Taking on it the global responsibilities, it circulates the ICH documents to its member countries through the network of national
information officers. This is done under the belief that wide dissemination and easy access to ICH documents stands to improve the professional knowledge of all regulators and has an important educational role. WHO has strongly advocated that ICH should be extended beyond its current 17 members to include all 191 member states. It believes that involving all countries will serve the purpose, both of harmonization and of increasing access to pharmaceuticals worldwide. WHO is also conducting an exercise to evaluate the global applicability of the ICH guidelines. The plan is to suggest adoption of those ICH guidelines, which are found to be globally applicable and for those which are not, draft a normative global guideline, using the ICH guideline as a base. In 1996, WHO published ‘Guideline on stability testing of pharmaceutical products containing well-established drug substances in conventional dosage forms’, as it found that ICH guideline ‘Stability Testing of New Drug Substances and Products’ (Q1AR2) did not address all climatic regions around the globe. WHO is of the considered view that less developed countries will need to evaluate the advantages and disadvantages of implementation of ICH guidelines, considering the costs and resource needs of regulatory agencies and drug-development in local circumstances.

1.2.2 Stability testing of New Drug substances:

The expert Working Group of the International Conference on Harmonisation of the Technical Requirements for Registration of Pharmaceuticals for Human Use developed a guideline on stability
testing for a registration application within the European Union, Japan and the United States. The goal of the ICH stability guideline was to exemplify the core stability data package required for new drug substances and products in the European Union, Japan and the United States such that the data generated in any of the regions is mutually acceptable in the other two. The guideline applies to the information required for the registration applications of new molecular entities and drug products, but not to abbreviated or abridged applications, clinical trial applications, and so on. The test conditions were selected based on the climatic conditions in three areas so that test data provides evidence on the variation in quality with time under the influence of a variety of representative environmental factors. These data in turn allow recommended storage conditions and shelf lives to be established.

**Drug substance:** The primary stability studies for the drug substance show that it will remain within specification during the retest period. Long-term (12-month) and accelerated testing are performed on at least three batches. Batches can be manufactured at a minimum of pilot scale, but should use the same synthetic route and a method of manufacture that simulates the final process to be used at manufacturing scale. In addition, supporting stability on laboratory-scale batches may be submitted. The quality of the batches placed on stability should be representative of the quality of –

(a) material used in preclinical and clinical studies
(b) material to be made at a manufacturing scale.
The first three batches made post approval should also be placed on long-term stability using the product registration protocol. Testing should cover physical, chemical, and microbiological properties susceptible to change during storage and likely to affect product quality, safety, and/or efficacy. Validated stability-indicating methods should be used. The number of replicates to be run depends on the results of validation studies, and limits should be derived from material used in preclinical and clinical studies, including both individual and total upper limits for impurities and degradation products. The length of the studies and the storage conditions should cover storage, shipment, and subsequent use, although use of the same conditions as for the drug product will facilitate comparative review and assessment. Other conditions should be included as scientifically justified. Temperature-sensitive drugs should be stored at the labeled long-term storage temperature, and accelerated testing should be conducted at 15°C above the designated long-term storage temperature with appropriate relative humidity conditions.

At the time of regulatory submission, a minimum of 12 months at 25°C ± 2°C/60% RH ± 5 and 6 months at 40°C ± 2°C and /75% RH ± 5% is required. If significant changes are noted at the elevated temperature, additional testing at an intermediate condition, such as 30°C ± 2°C/60% RH ± 5%, should be conducted. The registration application should include a minimum of 6 months of data from a 12-month study at the intermediate condition. Significant change at 40°C and 75% RH is defined as failure to meet specification. Long-term-
testing should be continued to cover all retest periods. Normally, testing under long-term conditions is performed every 3 months for the first year, every 6 months for the second year, and then annually. Containers employed in the long-term stability study should be the same or simulate actual packaging used for storage and distribution. As the application is pending review, accumulated stability data should be submitted. Accelerated or intermediate temperature data may be used to support shipping conditions and evaluate the effect of short-term excursions outside the label storage conditions.

Because long-term stability is used to establish appropriate retest periods, it should be noted that the degree of inter batch variability affects the confidence that a future batch will remain within specifications for the entire retest period. As a rule, determination of the time at which the 95% one-sided confidence limit for the mean degradation curve intersects the acceptable lower specification limit is acceptable, combining data into one overall estimate to account for variability. Before combining the data, apply appropriate statistical tests (e.g., $p$ test) to be sure it is allowable. If inappropriate to combine data, the retest period may depend on the minimum time a batch is actually measured to remain in specification. The nature of the degradation relationship determines the need for transformation of the data for linear regression analysis. This relationship can generally be fitted to a linear, quadratic, or cubic function on an arithmetic or logarithmic scale. Use statistical methods to test the goodness of fit of the data on all batches and combined batches, where appropriate, to
the assumed degradation curve. If the data show little degradation or variability, a retest period can be justified without statistical analysis and a limited extrapolation of real-time data may be undertaken when supported by the accelerated data. Any extrapolation must be justified, because it assumes that the same mechanism of degradation will continue beyond the observed data; this evaluation should include assay, degradation products, and any other appropriate attributes.

The storage temperature range should be based on the stability data and used in accordance with the national or regional requirements. Specific labeling requirements should be stated, particularly for drugs that cannot freeze; terms such as ambient and room temperature are to be avoided.

1.2.3 Stress testing route to the development of stability-indicating analytical methods (SIAMs):

Studies under taken to elucidate the intrinsic stability of the drug substance. Such testing is part of the development strategy and is normally carried out under more severe conditions than those used for accelerated testing. A more detailed description of stress testing is provided near the beginning of the ICH stability guideline, under the “Drug substance” heading:

“Stress testing of the drug substance can help identify the likely degradation products, which can turn help, establish the degradation pathways and the intrinsic stability of the molecules and validate the stability indicating power of the analytical procedures used”. The
nature of the stress testing will depend on the individual drug substance involved.

Stress testing is likely to be carried out on single batch of drug substance. It should include the effect of temperatures (in 10°C increments (e.g., 50°C, 60°C etc.) above that for accelerated testing), humidity (e.g., 75% RH 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 wide range of pH values when in solution or suspension. Photo stability testing should be an integral part of stress testing. The standard conditions for photo stability testing are described in ICH Q1B.

Examining the degradation products under stress conditions is useful in establishing the degradation pathways, developing, and validating suitable analytical procedures. However, it may not be necessary to examine specifically for certain degradation products if it has been demonstrated that they are not formed under accelerated or long term storage conditions.

Results from these studies will form an integral part of the information provided by the regulatory authorities. The description of stress testing was slightly modified in the revised stability guideline from the original description in ICH Q1A. The original Q1A description contains this additional paragraph:

Stress testing is conducted to provide data on forced decomposition products and decomposition mechanisms for the drug substance. The severe conditions that may be encountered during
distribution can be covered by stress testing of definitive batches of drug substance.

From the ICH definition, it is now clear that there is now a (regulatory) differentiation between “accelerated testing” and “stress testing”. Stress testing is distinguished by both severity of the conditions and the focus or intent of the results. Stress testing, which is also often referred as “forced degradation,” is an investigation of the “intrinsic stability” characteristics of the molecule, providing the foundation for developing and validating analytical methods. Stress testing studies are intended to discover stability issues, and are therefore predictive in nature. Stress testing studies are not part of the “validated” formal stability program. Rather, pharmaceutical stress testing is a research investigation requiring a scientific expertise and judgment.

It is interesting to consider some of the conditions that have historically been employed to consider in the stress testing of pharmaceuticals, documented both in the “Analytical Profiles of Drug Substances” [7] and by Singh and Bakshi [8]. Acidic stress conditions can be found to vary from 0.1 N HCl at 40°C for 1 week (with “negligible degradation”) [9], to 0.1 N HCl at 65°C for 21 days (71.6% degradation) [10], to 0.1 N HCl at 105°C for 2 months (with “considerable degradation”), to 4 N HCl under refluxing conditions for 2 days (66% degradation) [11], to 6.5 N HCl at 108°C for 24 hr (50% degradation), to concentrated HCl at room temperature (56.5% degradation) [12]. Similar elevated temperatures, times, and base
strength have been employed for basic stress conditions. For example, conditions can be found to vary from 0.1 N NaOH at 40°C for 1 week (with negligible degradation) [13], to 0.1 N NaOH at 65°C for 21 days (68% degradation) [14], to 0.1 N NaOH under refluxing conditions for 2 days (68% degradation) [15], to 1 N NaOH under boiling conditions for 3 days (7.2% degradation) [16], to 5 N NaOH under refluxing conditions for 4 hr (100% degradation) [17]. In terms of oxidative degradation studies, hydrogen peroxide has been employed at strengths from 0.3% to 30% [18]. Studies were often conducted at elevated temperatures, e.g., 37°C for 6 hr [3% hydrogen peroxide, 60% degradation [19]], 50°C for 72 hr (3% hydrogen peroxide, 6.6% degradation), and even refluxing conditions for 30 min (3% hydrogen peroxide, extensive degradation) [20] or 6 hr (10% hydrogen peroxide, no significant degradation) [21].

As these examples illustrate that historically there has been tremendous variation in the conditions employed in acid/base and oxidative stress testing studies. There has also been tremendous variation in defining the appropriate “endpoint” of the stress testing studies, i.e., length of time (and temperature) or amount of degradation that is sufficient to end the stress exposure. Perhaps the most dramatic variability in stress testing conditions is observed in the photo- stressing of drugs [22], where the lamps and exposures range from short wavelength Hg arc lamps (254 nm, UVC range), fluorescent light, artificial light, halogen lamps to xenon lamps. The variability of exposure to type of light during pharmaceutical photo-
stability studies has also been documented by surveys of practices in the pharmaceutical industry [23-26].

From the information provided above, it is apparent that stress-testing conditions have varied greatly from compound to compound and from investigator to investigator. Extremely harsh conditions have been commonly used in the past to ensure degradation, even if the conditions far exceeded plausible exposures.

More recently, several articles relevant to stress testing have appeared in the pharmaceutical literature. A paper by Singh and Bakshi [8] in 2000 provides the most thorough collection of references to various degradation studies of drug products, documenting the diversity of conditions and approaches to stress testing. This paper attempts to provide a classification system (Extremely labile, Very labile, Labile, Stable) based on a defined systematic approach. It is not clear from the article on what basis (scientific or otherwise) the classification system was devised; however, the paper does define “endpoints” to stressing (albeit, fairly harsh endpoints), allowing for the conclusion that a particular compound may be regarded as “stable” under a certain set of conditions.

In 1992 (and again in 1994), Boccardi provided some needed guidance on oxidative stress testing by asserting that most pharmaceutical oxidative degradation was the result of autoxidation and that hydrogen peroxide was not a very good reagent to mimic autoxidation processes [27, 28]. Boccardi was the first to describe the use of radical initiators such as azobisisobutyronitrile (AIBN) for
oxidative pharmaceutical stress testing, and he provided a simple procedure with mild conditions, which he termed as “The AIBN Test.” In 1996, Baertschi [29] presented and discussed an approach to stress testing that had defined limits of harshness and exposure time. In 1998, Weiser [30], while discussing the role of stress testing in analytical method development, suggested a set of conditions for performing stress testing that was arguably milder than many of the historical studies cited above. In 2001, Alsante et al. [31] provided a guide to stress testing studies that suggested defined limits to the stress conditions of 1 N HCl and 1 N NaOH for a maximum of 1 week at room temperature. In 2002, the views of the Pharmaceutical Research and Manufacturer’s Association (PhRMA) were summarized in an article on forced degradation studies published in Pharmaceutical Technology [32]. The PhRMA article did not discuss specifics of conditions of stress, but rather focused more on what kinds of stress testing should be performed for drug substances and products and on the regulatory requirements.

Recent publications on the topic of stress testing/forced degradation studies reveal that there is still a tremendous variability in the conditions employed. A few examples will be discussed here, although this discussion is not intended to be an exhaustive review of the literature.

A degradation study of haloperidol utilized 1 M HCl and 1 M NaOH (refluxed for 5 hr), and 30% hydrogen peroxide (70°C for 5 hr) for the most stressful conditions of the study [33]. These conditions
appear to have been chosen to enable production of known degradation products (six degradation products shown) to facilitate HPLC method validation efforts. A degradation study of ibuprofen produced 13 degradation products, several of which had never been detected before [34]. In this study, oxidative studies were carried out utilizing potassium permanganate (0.05 M) at room temperature up to 16 hr in 0.5 M NaOH; up to 33% hydrogen peroxide at room temperature for 22 hr; and potassium dichromate (0.1 N) at room temperature up to 14 days in 0.5 M HCl. Solid-state studies utilized 50ºC up to 8 months and 100ºC up to 16 hr to detect volatile degradation products. An NMR study of the aqueous degradation of isophosphoramid mustard was conducted in buffered aqueous solutions in the pH range of 1-13 [35]. The degradation of sumatriptan in 0.1 N HCl, 0.1 N NaOH, and in 3% hydrogen peroxide was studied using LC/MS and LC/MS/MS [36]. The solutions were heated at 90ºC for 30 min to 9 hr. Photostability was assessed by exposure to UV irradiation at 254 min for 24 hr (no indication of irradiation intensity). A study of the major oxidative degradation products of SCH 56592 was conducted by exposure of the drug substance in the solid state to 150ºC for 12 days with identification of the major products using LC-MS and LC-NMR [37]. Singh et al. describe stress degradation studies of ornidazole [38] and prazosin, terazosin, and doxazosin [39] under conditions designed to be in “alignment” with the ICH Stability guideline (Q1AR). In the case of ornidazole, significant degradation was seen under acidic conditions of
0.1 M HCl to 5 M HCl at 80°C for 12-72 hr, although no degradation products were detected (presumably because of degradation to non-chromophoric products). Studies under basic conditions of 0.1 M NaOH at both 80°C and 40°C revealed complete degradation at time zero. Milder studies were then conducted at pH 8 and 40°C. Oxidative studies involved 3% and 30% hydrogen peroxide at room temperature for 24 and 48 hr, with losses of 8% and 53% of the parent, respectively. Photo-degradation studies utilized Option 2 of the ICH photostability guideline with exposures up to 30 days at 7000 lux (over 5 million lux-hr exposure). Similar conditions were employed for prazosin, terazosin, and doxazosin.

In these recent examples of stress testing studies, it is apparent that there is still a great diversity of conditions employed to induce degradation, although the diversity is arguably less than was observed prior to publication of the ICH guidances. This continued diversity of approach could be interpreted in a couple of ways. One interpretation is that stress-testing studies are inherently a research undertaking, and, therefore, flexibility and scientific judgment are required, leading to diverse conditions and approaches. Another interpretation is that there is (appropriately or inappropriately) very little guidance (either regulatory or in the scientific literature) on the specifics of the conditions or appropriate endpoints of pharmaceutical stress testing.
1.3 Techniques employed in literature reports for the development of SIAMs:

If one critically evaluates the literature reports, titrimetric, spectrophotometric and chromatographic techniques have been commonly employed in the analysis of stability samples.

a) Titrimetric and spectrophotometric:

In these methods, usually the objective is the analysis of drug alone in the matrix of impurities, degradation products, impurities, etc., and also other drugs in case of the combination products. Their advantage is low cost and simplicity, though some times they are not sensitive. Due to limitation of specificity there are hardly any reports these days on the use for the assay of stability samples. However, a few reports involving derivative spectroscopy have been published lately.

b) Chromatographic:

Because of very nature of requirement of separation of multiple components during analysis, chromatographic methods have taken precedence over conventional methods of analysis. Other than separation of multiple components, the advantage of chromatographic methods is that these possess greater accuracy and sensitivity for even small quantities of degradation products performed. Various chromatographic methods that have been used are thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), gas chromatography (GC), HPLC (High Performance Liquid
Chromatography) and newer technique like capillary electrophoresis (CE).

In comparison, HPLC has been very widely employed. It has gained popularity in stability studies due to its high-resolution capacity, sensitivity and specificity. Non-volatile, thermally unstable or polar/ionic compounds can also be analyzed by this technique. Therefore, most of the SIAMs have been established using HPLC.

1.3.1 Steps involved during the development of stability-indicating analytical methods (SIAMs):

A SIAM is a quantitative analytical procedure used to detect a decrease in the amount of the active pharmaceutical ingredient (API) present due to degradation. According to FDA guidelines, a SIAM is defined as a validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences like degradation products, process impurities, excipients, or other potential impurities, and the FDA recommends that all assay procedures for stability studies be stability-indicating [40]. During stability studies, liquid chromatography (LC) is used routinely to separate and quantitate the analytes of interest. There are three components necessary for implementing a SIAM: sample generation, method development, and method validation.
**Step 1: Generation of the Sample:**

Stressing the API in both solutions and in solid-state form generate the sample that contains the products most likely to form under most realistic storage conditions, which is in turn used to develop the SIAM. In simplest terms, the goal of the SIAM is to obtain baseline resolution of all the resulting products (the API and all the degradation products) with no coelutions.

Table: 1.2 lists some common conditions used in conducting forced degradation studies for drug substances [41].

**Table: 1.2**

<table>
<thead>
<tr>
<th>Sample condition</th>
<th>Time/Exposure</th>
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<tbody>
<tr>
<td>Solid / 60 - 70ºC</td>
<td>7 – 10 days</td>
</tr>
<tr>
<td>Solid / 60 - 70ºC / 75% RH</td>
<td>7 days</td>
</tr>
<tr>
<td>Solid / simulated sunlight exposure</td>
<td>2 – 3 weeks x ICH confirmatory</td>
</tr>
<tr>
<td>0.1 to 2 N HCl solutions either at RT or at 60 - 70ºC</td>
<td>1 – 3 days</td>
</tr>
<tr>
<td>0.1 to 1 N NaOH solutions either at RT or at 60 - 70ºC</td>
<td>1 – 3 days</td>
</tr>
<tr>
<td>Dilute hydrogen peroxide (0.3 to 3%) at RT or at 60 - 70ºC</td>
<td>1 – 3 days</td>
</tr>
<tr>
<td>Solution in Water</td>
<td>1 – 3 days</td>
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</tbody>
</table>

Samples should be stored in appropriate vessels that allow sampling at timed intervals and that protect and preserve the integrity of the sample. Thermo stated and humidity-controlled ovens should
also be employed. Generally, the goal of these studies is to degrade the API by 5-20%. 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.

**Step 2: Developing the LC Method:**

After the sample is generated through the use of a properly designed and executed forced degradation, it can be used to develop the LC method. These days, LC method development often is performed on gradient systems capable of automated column and solvent switching, and temperature control. Systems and software that automate the process, some with decision making built-in, also have been reported [42]. Scouting experiments often are run, and then conditions are chosen for further optimization. Resolving power, specificity, and speed are key chromatographic method attributes to keep in mind during method development. However, excellent resources are available to anyone not already schooled in the art [43].
1.3.2 Selectivity Manipulated during Method Development:

The selectivity can be manipulated by a combination of different factors that including the solvent composition, type of column stationary phase (C8, C18, phenyl and cyano and mobile phase buffers and pH (acidic/basic). Chromatographic methods are most part are comfortable changing solvents and column stationary phases to generate a separation. However, advances in LC column technology recently have made possible the use of pH as a true selectivity tool for the separation of ionizable compounds [44, 45]. These hybrid chemistry columns take advantage of the best of both the silica and polymeric column worlds. They are manufactured using a classical sol-gel synthesis that incorporates carbon in the form of methyl groups, resulting in columns that are mechanically strong, with high efficiency, and operate over an extended pH range.

The graphics in Fig: 1.1 illustrate why pH can be such a useful tool.
Acidic pH containing compounds are more retained at low pH; while basic compounds are more retained at higher pH (neutral compounds are not effected). At pH values are used normally (pH 4-8); a slight change in pH would result in a dramatic shift in retention (up-slope or down-slope of curve). However, by operating at pH extremes, not only is there a 10-30-fold difference in retention that can be exploited in method development, the method can be made more robust as well, a desirable outcome with validation in mind. Indeed, the selectivity differences afforded by a change in pH are the equivalent to a 20% change in the organic solvent composition, and often are underutilized.
1.3.3 Evaluating Specificity by during method development:

Another key parameter to evaluate during method development is specificity. The United States Pharmacopoeia (USP) and various International Conference on Harmonisation (ICH) guidelines define specificity as the ability of a method to assess unequivocally the analyte of interest in the presence of potential interferences [46, 47]. In the past, it has been acceptable to evaluate resolution, peak shape, and tailing factors to measure and document specificity. However, starting with USP 24, and as a direct result of the ICH process, it was recommended that a peak purity test based upon photodiode-array (PDA) detection or mass spectrometry (MS) be used to demonstrate that a given peak was pure.

Modern PDA technology is a powerful tool for evaluating specificity. PDA detectors can collect spectra across a range of wavelengths at each data point collected across a peak, and through software manipulations involving multidimensional vector algebra, they compare each of the spectra to determine peak purity. In this manner, PDA detectors today can distinguish minute spectral and chromatographic differences not readily observed by simple overlay comparisons [48-50]. To be successful, three components are required:

- A UV chromophore, or some absorbance in the wavelength range selected
- Some degree of chromatographic resolution.
- Some degree of spectral difference.
Fig: 1.2 shows an example of a partial reversed-phase LC separation, where, by the appearance, the peaks certainly are well-resolved, sharp, and symmetrical.

An examination of peak 2 indicated the peak was pure. However, a close examination of the spectral information related to peak one reveals a different situation.

In Fig: 1.3, the calculated peak purity (in green) is plotted against the noise threshold (in blue), both superimposed on the red chromatographic trace. The purity plot clearly indicates a co-elution in the front of the peak as the purity plot exceeds the threshold, requiring more method development.

PDA detectors can be limited on occasion in evaluating peak purity, governed by the three required components mentioned previously, as well as the noise of the system and disparate levels of absorbance responses. That is, the more similar the spectra, and the lower the
relative absorbances, the more difficult it can be to distinguish co-eluted compounds. MS detection overcomes many of these limitations and in most laboratories; it has become the detection method of choice for even routine method development.

**Fig: 1.3 The calculated peak purity (in green) is plotted against the noise threshold (in blue), both super imposed on the red chromatographic trace.**

MS has come a long way from the days in which many companies had a dedicated central MS lab and staff. Modern mass spectrometers are smaller, simpler, and operate from the same software used to operate the chromatographic system or other detectors commonly utilized, decreasing the learning curve. MS can provide unequivocal peak purity information, exact mass, and structural and quantitative information depending upon the type of instrument used. MS is also a very useful tool to track peaks as they
move around in response to selectivity manipulations in method development. However, only the combination of both PDA and MS on a single instrument and software platform provides the type of valuable orthogonal information required when evaluating specificity and developing SIAMs.

**Step 3: Validation of SIAM’s**

Different methods have different requirements when it comes to validation. The USP recognizes four method categories and defines the analytical performance characteristics that must be measured to validate each method type [51]:

- Category 1: Analytical methods for the quantification of major components of bulk drug substances or active ingredients.
- Category 2: Analytical methods for the determination of impurities in bulk drug substances or degradation compounds.
- Category 3: Analytical methods for the determination of performance characteristics.
- Category 4: Identification tests

Table: 1.3 summarizes each category and the analytical performance characteristics that must be investigated.
Table: 1.3 Data elements required for assay validation (as per USP)

<table>
<thead>
<tr>
<th>Analytical performance characteristics</th>
<th>Assay Category I</th>
<th>Assay Category II Limit tests</th>
<th>Assay Category III</th>
<th>Assay Category IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quantitative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td>Yes</td>
<td>Yes</td>
<td>*</td>
<td>No</td>
</tr>
<tr>
<td>Precision</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Specificity</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>*</td>
</tr>
<tr>
<td>Detection limit</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>*</td>
</tr>
<tr>
<td>Quantitation</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>*</td>
</tr>
<tr>
<td>Linearity</td>
<td>Yes</td>
<td>No</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Range</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*May be required, depending on the nature of the specific test

SIAM falls into the quantitative division of Category 2, and as such, all analytical performance parameters must be determined, except for the limit of detection, limit of quantification would apply instead, because SIAMs need to be quantitative.

According to ICH guideline on validation of analytical methods [52], the objective of an analytical procedure is to demonstrate that it is suitable for its intended purpose. One should keep in mind that stress testing methods are screening methods used to help in understanding the degradation chemistry of the drug and, therefore, do not need to (nor, in general, can they) be validated to the extent of final control methods. The concepts in the ICH guideline on validation of analytical methods are a good starting point for validation of stress testing methods, however, the overall validation should be significantly abbreviated when compared to the validation of final control methods, as stress testing methods are investigational.
methods. Accuracy normally should not be a problem with stress testing methods as long as the response of the detector is linear and samples are completely dissolved prior to analysis. The specificity of the methods can not be fully validated because one does not know all of the possible degradation products during initial stress testing. Specificity can be addressed by using any known impurities and degradation products produced in the method development samples. Precision (repeatability) of the assay of the main component can be evaluated by preparing a limited number of assay samples and using simple statistics to estimate the standard deviation. Estimation of intermediate precision and reproducibility should normally not be necessary for stress testing methods. Detection and quantitation limits for degradation products can be determined by using parent compound and assuming that the response of all degradation products will be similar. Although there is no requirement to reach any specific detection limit, a reasonable is 0.1% since the aim of stress testing is to detect the major degradation products in samples which is approximately 10 – 20% degraded. The linearity of the method should be validated over ranges for both assay and impurity determination. A typical assay range might be from 50% to 150% of nominal sample concentration, while a typical range for impurity determination might cover a range from the quantitation limit to a few percent. If one wishes to quantitate impurities vs. the parent peak, then linearity (range) should be demonstrated from the quantitation limit to at least 120% of nominal sample concentration.
One of the most important aspects of stress testing is the analysis of samples using a suitable analytical method, which, in many cases, is reverse-phase HPLC. This necessitates the development of an HPLC method capable of measuring both the loss of parent compound as well the levels of degradation products or impurities formed in stress conditions.

1.3.4 Role of Mass Balance during SIAM development

Mass balance correlates the measured loss of a parent drug to the measured increase in the amount of degradation products. It is a good quality control check on analytical methods to show that all degradation products are adequately detected and do not interfere with quantitation of the parent drug (i.e., stability-indicating methods). Regulatory agencies use mass balance to assess the appropriateness of the analytical method as a stability-indicating method and determine whether all degradants have been accounted for [53].

In mass balance calculations, the loss of parent drug or the amount of drug remaining is determined from a sample assay, and the measured increase in degradation products is determined by a related substances method. The fundamental approach for determining mass balance is to quantitate the decomposition peaks using degradation methods and then reconcile the measured loss in the parent drug with the amount of degradation products. If the loss in potency can be
reasonably accounted for by the amount of degradants measured, then mass balance is achieved.

The assessment of degradation in pharmaceutical products involves two aspects of analytical measurement. Firstly, a specific or selective analytical method must be available for accurate assay of parent drug compound, in order to measure any loss. Second, methodology should be in place for quantification of the degradation products formed. Ideally, when degradation occurs, the measured amount of parent drug lost should correlate well with the measured increase in degradation products. This correlation is referred to as “mass balance” [54]. More recently, the International Conference on Harmonization (ICH) has provided definition of “mass balance; material balance” as follows:

The process of adding together the assay value and levels of degradation products to see how closely these add up to 100% of initial value, with due consideration of the margin of analytical precision. The concept is useful scientific guide for evaluating data, but it is not achievable in all circumstances. The focus may instead be on assuring the specificity of the assay, the completeness of the investigation of route of degradation, and the use, if necessary, of identified degradants as indicators of the extent of degradation via particular mechanism [55]. The analyst must balance time and resource demands to provide the information necessary to understand degradation without going to extreme measures of quantify components of little interest.
Mass balance in pharmaceutical analysis is very important for several reasons. By demonstrating the degradative losses of parent drug correlate well with the measured increase in degradation products unaccounted for. Conversely, if one observes, for example, a 20% loss of parent drug but only measures a 5% increase in degradation products, it is likely that additional degradation products formed are not accurately determined by the given method(s). Because unknown degradation products could potentially be toxic or otherwise compromise the safety of drug, it is important to have methods that detect all major degradation products. Thus, safety is the major reason for the study of mass balance.

Mass balance is also useful in method validation. In order to demonstrate that analytical methods are stability-indicating, unstressed and stressed materials are often compared. Any increase in degradation a product that correlates well with loss of parent drug, aids in demonstrating that the methods can accurately assess degradation.

Mass balance is also important in understanding alternative degradation pathways. For example, consider a situation where both acid-catalyzed and oxidative degradation produces a substantial loss of parent compound in stress-testing studies. If good mass balance is achieved for the acid-catalyzed degradation, but not for the oxidative degradation, further work to better understand the oxidative degradation pathway(s) is warranted. It may be that the poor mass balance in the latter case results from important oxidative products
that are unaccounted for or from structures, which need to be more fully elucidated to understand response factor differences. Mass balance is an important consideration in assessing degradation pathways of pharmaceutical products. Often, response factor differences between degradation products and the parent compound are responsible for mass balance problems. RRFs should, therefore, be incorporated, when possible, in the quantification of degraded samples.

1.3.5 Application of SIAMs

Stability studies are used to establish the re-test period for the active ingredient – that is the length of time it can be stored and used without analyzing immediately before use – and the shelf life of the finished product. The release and shelf life specifications for the product may differ to accommodate degradation of active ingredient or other acceptable changes, which may occur on storage. The International Conference on Harmonization (ICH) drug stability test guideline Q1A (R2) requires that analysis of stability samples should be done through the use of validated stability-indicating analytical methods (SIAMs). Additional guidance is given only for photo stability testing. It also recommends carrying out the stress testing on drug substance to establish its inherent stability characteristics and to support the suitability of proposed analytical procedure. The validated SIAMs will be used extensively for testing the stability samples of drug substances.
1.4 Scope and Objectives of research work

The present research work focuses on the development of novel stability-indicating analytical methods for some active pharmaceutical ingredients. The work also includes the validation of the developed methods as per ICH requirements and demonstrates the suitability of developed methods to assess the stability of active pharmaceutical ingredient (API). The list of active pharmaceutical compounds taken for research study was listed in the below Table: 1.4.
**Table: 1.4 list of active pharmaceutical substances**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Chemical name</th>
<th>Structure</th>
<th>Therapeutic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\left[\left[(1R)-1\right]-3\right]-\left[(1E)-2\right]-\left(7\text{-Chloro}2\text{-Quinolyl}\right)\text{ethenyl}\left[\text{phenyl}\right]-3-\left[2\text{-1-hydroxy-1-methyethyl}\right]\text{phenyl}\left[\text{propyl}\right]-\text{sulfanyl}\left[\text{methyl}\right]\text{cyclopropane acetic acid, mono-sodium salt.}$</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>anti asthametic drug</td>
</tr>
<tr>
<td>2</td>
<td>$(\pm)-5-\left[4\text{-}\left(2\text{-Ethyl-2-pyridinyl}\right)\text{ethoxy}\right]\text{benzyl}-2,4\text{-thiazolidinedione hydrochloride}$</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>antidiabetic drug</td>
</tr>
<tr>
<td>3</td>
<td>$2,2\text{-dichloro-N}\left[\left(1S,2R\right)-1\right]-\left(\text{fluoromethyl}\right)-2\text{-hydroxy-2-}\left[4\text{-methylsulfonyl}\right]\text{phenyl} \text{ethyl} \text{acetamide}$</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>antibacterial drug</td>
</tr>
<tr>
<td>4</td>
<td>$(6R,7R)-7-\left[\left(Z\right)-2\text{-}\left(2\text{-Aminothiazol-4-yl}\right)\text{carboxymethylethoxy}\right]\text{imino} \text{acetyl} \text{amino}-8\text{-Oxo-3-}\left[1\text{-pyridinio}\right]\text{methyl}5\text{-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylatepentahydrate}$</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>antibacterial drug</td>
</tr>
</tbody>
</table>

Novel stability-indicating analytical methods were developed for above active pharmaceutical ingredients. The developed methods can be successfully implemented during the quality monitoring and also well employed for the assessment of quality during its storage and stability.
1.5 References:


2. Guideline for the Submitting Documentation for the Stability of Human Drugs and Biologics, Center for Drugs and Biologics, Food and Drug Administration, February **1987**.


6. ICH Website: [www.ich.org](http://www.ich.org)


22. See Table V in Singh, S.; Bakshi, Guidance on conduct of stress tests to determine inherent stability of drugs, M.; Pharma Technol Online April, 2000, 1.


49. Young, P.M.; Gorenstein, M.V.; LCGC 1994, 12, 832.


