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REVIEW ARTICLE

FORCED DEGRADATION: REVIEW ON ANALYTICAL PERSPECTIVESAND STRATEGIES

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ABSTRACT:

Forced degradation (or stress testing) is a degradation of new drug substance and drug product at conditions more severe than accelerated conditions. It typically involves the exposure of drug substances to heat, humidity and light for solid-state studies. For the solution-state studies the drug substance is exposed to a range of pH values i.e. acid and base degradation study. The experimental samples produced are then used to demonstrate that a proposed analytical method is "Stability Indicating," i.e., the method is capable of detecting the loss in content of the active component and subsequent increase in degradation products. Ideally, loss in content of the active component and increase in degradation products should be monitored by a single analytical method. However, in some cases, this is not possible and separate assay and impurity methods have to be developed. It is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation pathways and degradation products of drug substance and helps in elucidation of the structure of the degradation products. In addition, the regulatory guidance is very general and does not explain about the performance of forced degradation studies.Forced degradation plays an important role in the development of analytical methods, setting specifications, and design of formulations under the quality-by-design (QbD) paradigm. Thus, this review article discusses the current trends related to performance of forced degradation studies by providing a strategy for conducting studies on degradation mechanisms which is helpful for development of stability indicating method (i.e. SIAM).

KEYWORDS: Degradation conditions; Degradation product; Forced degradation; Stability indicating method; Stress testing

INTRODUCTION

The work on stability was initiated by the World Health Organization (WHO) in 1988, Following the WHO process for consultation a general text on stability and the WHO Guidelines on stability testing for well-established drug substances in conventional dosage forms were adopted in 1994 and 1996 respectively. In 2000, discussions were initiated between the International Conference on Harmonization (ICH) Expert Working Group Q1 (stability) and WHO in order to harmonize the number of stability tests and conditions undertaken worldwide.

Forced degradation studies are generally used to identify reactions which may occur to degrade drug substance or drug product. Usually conducted before final formulation, forced degradation uses external stresses to rapidly screen material stabilities.Longer term storage tests are usually used to measure similar properties when final formulations are involved because of the stringent FDA regulations. These tests are generally more expensive (because of the time involved) than forced degradation which is therefore used for rapid selection and elimination tests.[1]

Most common stressconditions which are used in forced degradation are as follows[1]

a) Acid/Base: Chemical processes are often catalysed by the presence of acids and bases.

b) Temperature: In accordance to Arrhenius kinetics, increasing temperature increases the rate of any degradation process. Temperature is often used in conjunction with other stresses to increase reaction rates.
c) Oxidation (generally 0.1% to 3% hydrogen peroxide solution used)
d) UV-Visible treatment (The exposure time should not be less than 1.2 million lux hours and the light intensity should not be less than 200 watt hours per square meter.)
e) Humidity treatment (at 90% Relative humidity using saturated solution of potassium nitrate)

The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used [2]

Degradation factor	Conditions
Thermal	≥ 60 °C
Humidity	≥ 75% RH
Acid	0.1N HCl
Base	0.1N NaOH
Oxidative	Oxygen gas, or 3% H_2O_2
Photolytic	Metal halide, Hg, Xe lamp, or UV-B fluorescent
Metal ions (optional)	0.05M Fe^{2+} or Cu^{2+}

FIGURE 1: STRESS CONDITIONS (COMMONLY USED IN STUDY)

OBJECTIVES:

a) To establish degradation pathways of drug substances and drug products.
b) To differentiate degradation products that are related to drug products from those that are generated from non-drug product in a formulation.
c) To elucidate the structure of degradation products.
d) To determine the intrinsic stability of a drug substance in formulation.
e) To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and drug product.
f) To establish stability indicating nature of a developed method.
g) To understand the chemical properties of drug molecules.
h) To generate more stable formulations.
i) To produce a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.
j) To solve stability-related problems.[3]

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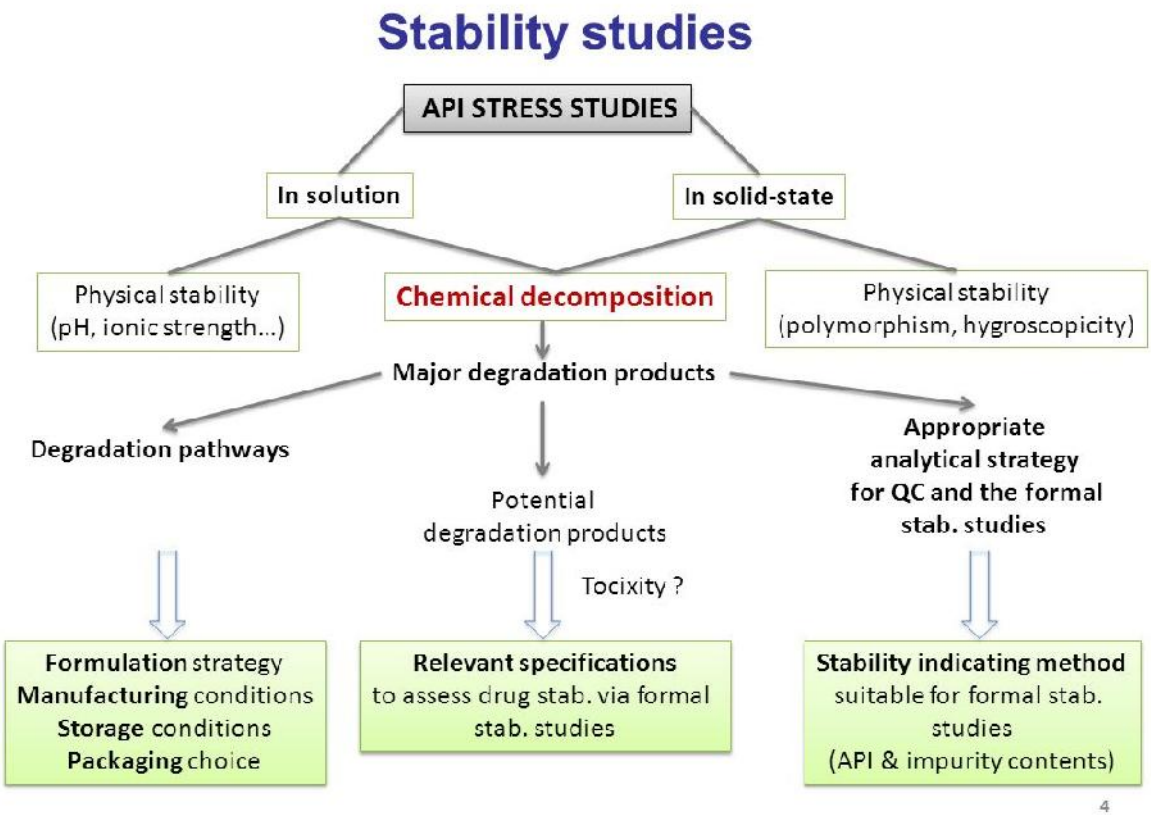

FIGURE 2: STRESS STUDY

FDA PERSPECTIVES:

The nature of the stress testing depends on the individual drug substance and the type of drug product (e.g., solid oral dosage, lyophilized powders, and liquid formulations) involved.[4]TheInternational Conference on Harmonization (ICH) Q1B guideline provides guidance for performing photo stability stress testing; however, there are no additional stress study recommendations in the ICH stability or validation guidelines.[5]

There is also limited information on the details about the study of oxidation and hydrolysis. The drug substance monographs of Analytical Profiles of Drug Substances and Excipients provide some information with respect to different stress conditions of various drug substances.[6]

Photo stability testing should be an integral part of stress testing, especially for photo-labile compounds. Some recommended conditions for photo stability testing are described in ICH Q1B Photo stability Testing of New Drug Substances and Products [5]. Samples of drug substance, and solid/liquid drug product, should be exposed to a minimum of 1.2 million lux hours and 200 watt hours per square meter light. The same samples should be exposed to both white and UV light. To minimize the effect of temperature changes during exposure, temperature control may be necessary. The light-exposed samples should be analysed for any changes in physical properties such as appearance, clarity, color of solution, and for assay and degradants. The decision tree outlined in the ICH Q1B can be used to determine the photo stability testing conditions for drug products. The product labelling should reflect the appropriate storage conditions. It is also important to note that the labeling for generic drug products should be concordant with that of the reference listed drug (RLD) and with United States Pharmacopeia (USP) monograph recommendations, as applicable.

Recently, ANVISA (the Brazilian National Health Surveillance Agency) published the RDC53/2015 regulation outlining specific requirements for product registration and post-approval change submissions with regard to reporting, identification and qualification of degradation products. These are generally aligned with industry practices, however, the scope and depth of such requirements are expanded beyond the ICH guidelines detailing specific requirements and recommendations for Forced Degradation Studies. The extension of Forced Degradation Studies

requirements necessitated a review and update of current accepted practices, and assessment of its impact to previously filed and registered products to ensure compliance.

ANVISA primarily focused on registrational filing requirements for Forced Degradation Studies, specifically to obtain:

1) Identification of functional groups susceptible to major degradation pathways and subsequent mechanistic understanding,
2) Assessment of specific Forced Degradation experimental conditions and extent of degradation (Articles 4, 5 and 6 of the RDC53/2015),
3) The requirement for specific information to be captured within a formal report,
4) Consideration of degradants resulting from drug product manufacture and storage,
5) Safety qualification of degradation products,
6) Compliance with the new regulation for approved products and post-approval change submissions. [12, 13]

Various detection types can be used to analyze stressed samples such as UV and mass spectroscopy. The detector should contain 3D data capabilities such as diode array detectors or mass spectrometers to be able to detect spectral non-homogeneity. Diode array detection also offers the possibility of checking peak profile for multiple wavelengths. The limitation of diode array arises when the UV profiles are similar for analyte peak and impurity or degradant peak and the noise level of the system is high to mask the co-eluting impurities or degradants. Compounds of similar molecular weights and functional groups such as diastereoisomers may exhibit similar UV profiles. In such cases, attempts must be made to modify the chromatographic parameters to achieve necessary separation. An optimal wavelength should be selected to detect and quantitate all the potential impurities and degradants. Use of more than one wavelength may be necessary, if there is no overlap in the UV profile of an analyte and impurity or degradant peaks. A valuable tool in method development is the overlay of separation signals at different wavelengths to discover dissimilarities in peak profiles.[7]

Issues not specifically addressed in regulatory guidance:

• Exact experimental conditions for forced degradation studies (temperatures, duration, and extent of degradation, etc.) are not specified.
• Experimental design is left to the applicant's discretion. **[8, 18]**

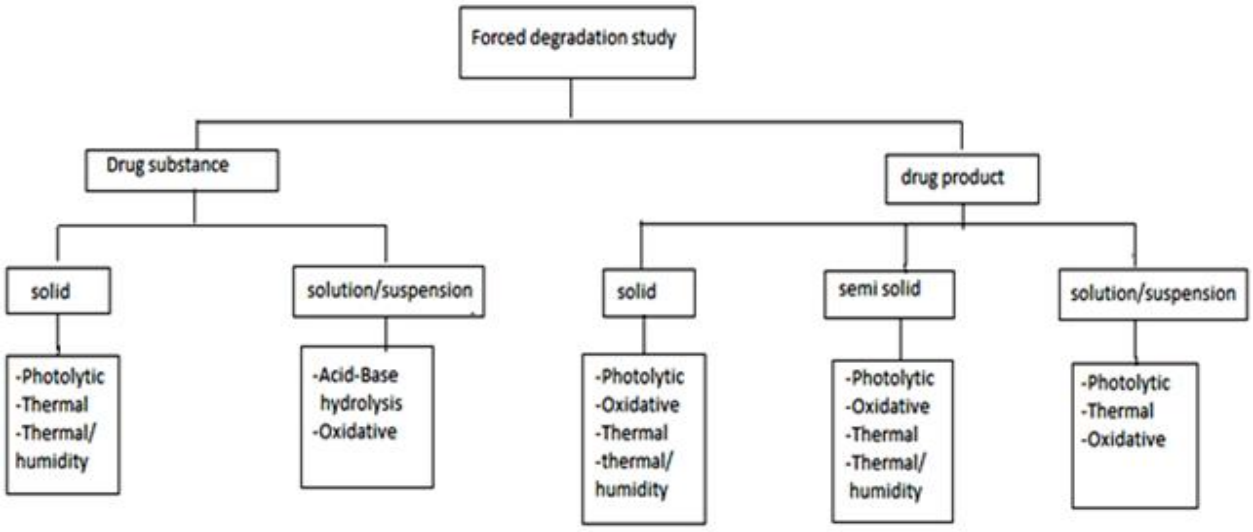

Figure 3: List of some common conditions used in conducting forced degradation studies for drug substances

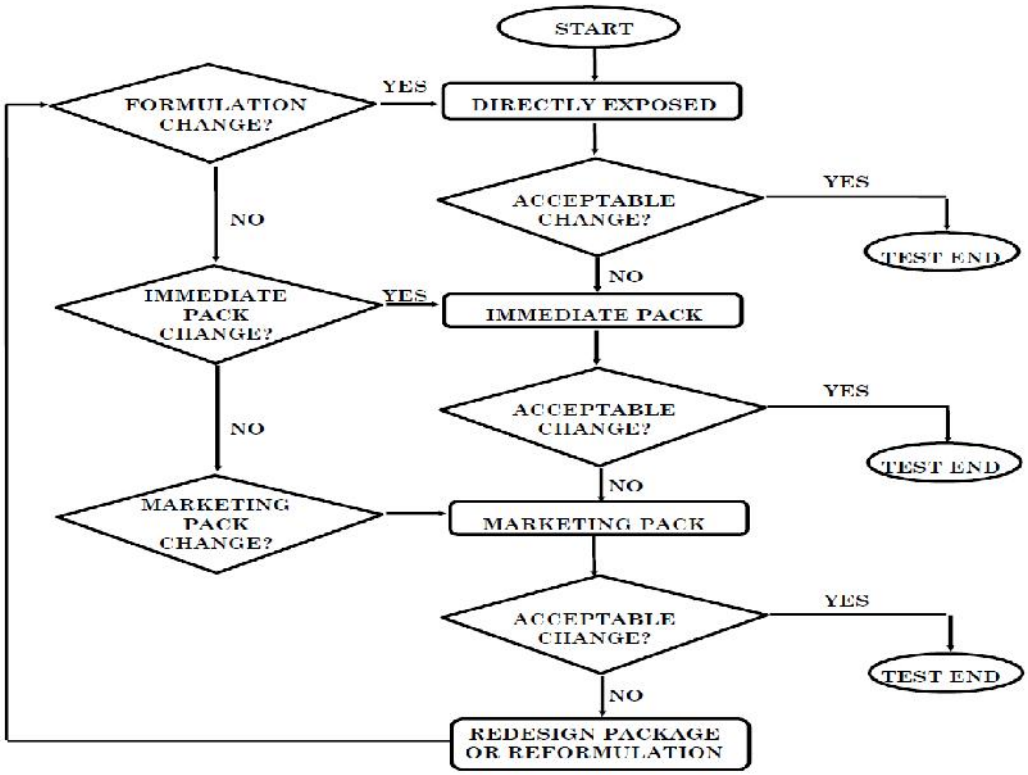

FIGURE4: DECISION FLOW CHART FOR PHOTOSTABILITY TESTING OF DRUG PRODUCTS

A systematic approach to photo stability testing is recommended covering, as appropriate, studies such as:

i) Tests on the drug substance;

ii) Tests on the exposed drug product outside of the immediate pack; and if necessary;

iii) Tests on the drug product in the immediate pack; and if necessary;

iv) Tests on the drug product in the marketing pack.

The extent of drug product testing should be established by assessing whether or not acceptable change has occurred at the end of the light exposure testing as described in the Decision Flow Chart for Photostability Testing of Drug Products.[5]

STABILITY INDICATING ANALYTICAL METHOD (SIAM):

Force degradation is required to demonstrate the specificity when developing SIMs and for this reason, it should be perform prior to implementing the stability studies. Force degradation of drug standard and excipients is carried out under different conditions to determine whether the analytical method is stability indicating. The main contemporary goal of stability indicating methods is to provide information about condition for stress testing so as to establish the stability of drug substances and product. This paper reviews the regulatory aspects for development of stability indicating methods. SIMs are used to differentiate the API from its potential decomposition product. Regulatory guidance in ICH Q1A (R2) ICH Q3B (R2) Q6A and FDA 21 CFR section 211 requires validated stability indicating methods.

A proposed analytical method is “Stability Indicating,” i.e., the method is capable of detecting the loss in content of the active component and subsequent increase in degradation products. Ideally, loss in content of the active component and increase in degradation products should be monitored by a single analytical method. However, in some cases, this is not possible and separate assay and impurity methods have to be developed. A SIAMs is an estimative analytical method used to detect a trace level amount or residual levels of the API present due to degradation or designing of its synthesis route. As per the FDA regulations, a SIAMs is defined as a completely

validated method that accurately and precisely measures API free from potential interferences like degradants, biproducts, intermediates, and exicipients and the FDA recommend that all assay content methodologies for stability studies be stability indicating. [8]

The ICH guidelines that are applicable to forced degradation studies are:

ICH Q1A – Stability Testing of New Drug Substances and Products [4]

ICH Q1B – Photostability Testing of New Drug Substances and Products [5]

ICH Q2B – Validation of Analytical Procedures: Methodology [9]

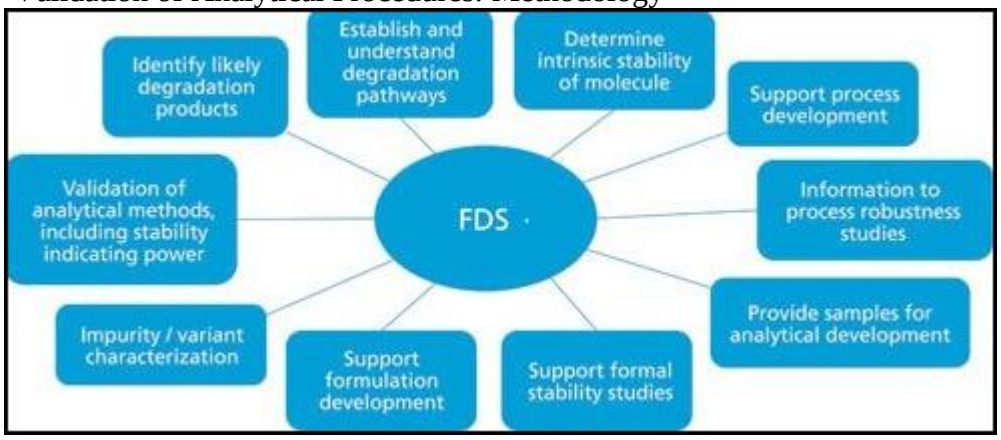

FIGURE 5: PURPOSE OF FORCED DEGRADATION STUDIES

Guideline	Title	Publication date
Q1A(R2)	Stability Testing of New Drug Substances and Products	February 2003
Q1B	Stability Testing: Photostability Testing of New Drug Substances and Products	November 1996
Q1C	Stability Testing for New Dosage Forms	November 1996
Q1D	Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products	February 2002
Q1E	Evaluation for Stability Data	February 2003

FIGURE6: ICH GUIDELINES FOR STABILITY TESTING

HOW MUCH FORCED DEGRADATION IS ENOUGH?

It is the subject of much discussion amongst pharmaceutical scientists. In general, values anywhere in 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. Unduly overstressing the drug substance may produce aberrant results. [10, 11]

PHOTOSTABILITY TESTING REQUIREMENT:

1) **LUX METER:**
   The lux (symbol: lx) is the SI unit of illuminance and luminous emittance, In photometry, this is used as a measure of the intensity.
2) **UV METER:**
   UV light radiometers for measurement of UV light irradiance or UV light exposure within specific wavelength regions.
3) **PHOTOSTABILITY CHAMBER:**
   Photostability chambers are specifically designed to perform near UV and visual light testing as per the requirement. For confirmatory studies, samples should be exposed to light providing an overall illumination of not less than 1.2 million lux hours and an integrated near ultravioletenergy of not less than 200 watt hours/square meter to allow direct

comparisons to bemade between the drug substance and drug product. Samples may be exposed side-by-side with a validated chemical actinometric systemto ensure the specified light exposure is obtained, or for the appropriate duration oftime when conditions have been monitored using calibrated radiometers/lux meters.If protected samples (e.g., wrapped in aluminium foil) are used as dark controls toevaluate the contribution of thermally induced change to the total observed change,these should be placed alongside the authentic sample.[5]

FIGURE 7: LUX METERFIGURE 8: UV METER

CHARACTERISATION OF DEGRADANTS:

Most of the degradants can be separated by preparative chromatography and further subjected to structural elucidation by mass spectrometry using electron spray ionization technique. Typically, complete structural elucidation is only performed for significant degradants which are obtained during forced degradation study. This will help to confirm the degradation mechanism and understand the key degradation mechanism. Such identified degradants may require initial In-Silico assessment for toxicity and subsequent qualification, if observed at high levels in accordance with ANVISA RDC-53/2015 and ICH Q3B (R2) guidelines.

Additionally, these are the most likely degradants to be formed (if at all) in the long term stability study, and are, therefore, necessary for the selectivity validation element of the stability indicating analytical HPLC method. Small amounts of the material will need to be synthesized or isolated, with their purity obtained for RF determination and accuracy studies.

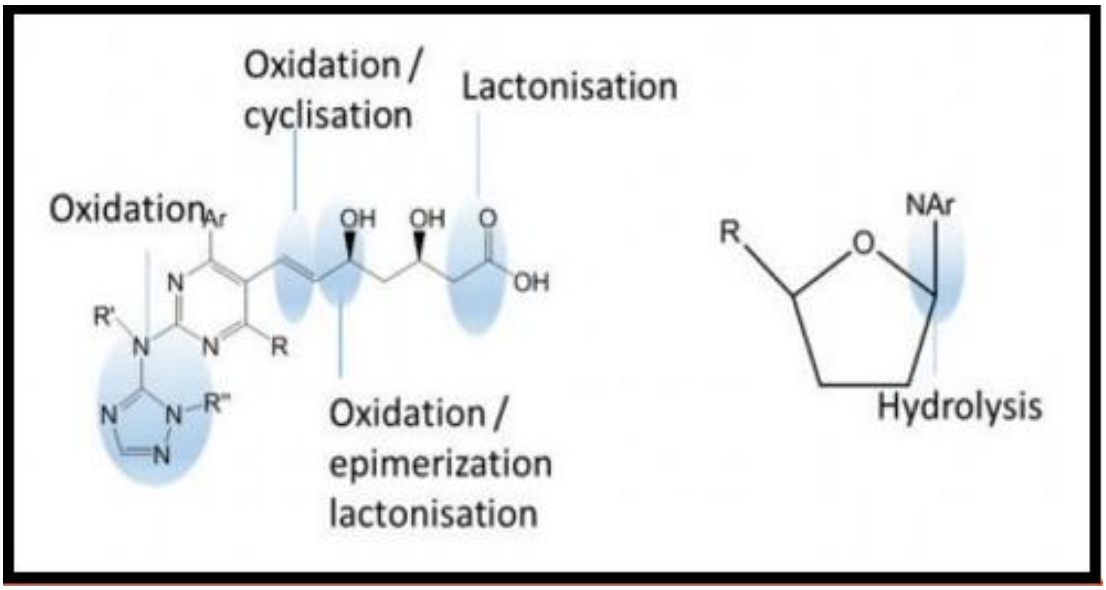

FIGURE 9: MOLECULAR 'SOFT SPOTS' BASED ON FUNCTIONAL GROUP

THERMAL DEGRADATION:

High temperature (>80˚C) may not produce predictive degradation pathway [14]

Effect of temperature on thermal degradation of a drug 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. [15, 16]

Nowadays some researchers uses microwave as new tool for thermal degradation study. During the past ten years microwave-assisted chemistry has emerged as a very efficient and powerful technology to heat reaction mixtures in dedicated sealed reaction vessels/reactors. [17]

PREDICTION OF DEGRADATION PATHWAYS AND PRODUCT[26]

A valuable first step of the process is an in-silico (computer software) and in-cerebro (chemistry knowledge) prediction of the potential reactive functional groups and degradation pathways of the drug molecule.

A. Predictive softwares (in-silico prediction)
   1. CAMEO (Computer-Assisted Mechanistic Evaluation of Organic reactions):
      Historical degradation predictions involved the use of for modelling and predicting organic chemical reactivities, software developed by William L. Jorgensen. [19, 20]
   2. DELPHI (Degradation Expert Leading to Pharmaceutical Insight):
      It was another historical expert system, capable of predicting reaction products under given conditions. In contrast to CAMEO, DELPHI was specifically designed to predict reactivity and degradation of molecules [21] and proceeded beyond a primary reactive degradant to subsequent degradants of degradants.
   3. ZENETH (In-silico software):
      This In-silico software released in 2010 is the only commercially available program designed to predict degradation pathways of pharmaceutical compounds. It was developed by Lhasa Ltd. in consortium with a group of pharmaceutical companies and based on the framework of Meteor, a metabolite-prediction software program by Lhasa.[21] Zeneth predicts degradation under the influence of reaction conditions and optionally in the presence of other compounds such as excipients.

B. In-Cerebro prediction
   In-Cerebro tools of great utility have been published in reviews and books in the primary literature. [22-25]in these references, the major mechanisms of chemical decomposition of pharmaceuticals have been examined in the context of common functional groups. The major mechanisms of chemical decomposition of pharmaceuticals include hydrolysis, dehydration, oxidation, isomerization/epimerization, decarboxylation, dimerization, polymerization, and photolysis and transformation products involving reaction with excipients/salt forms.

C. Drug degradation database
   The concept of a drug degradation database was developed at Pfizer and was initially designed to contain structure-elucidation data to allow scientists to retrieve data readily based on change of structure and degradation-chemistry conditions.

ANALYTICAL TOOLS: DEGRADANT SEPARATION&IDENTIFICATION

1) Thin layer chromatography (TLC): TLC is fast, easy and inexpensive tool.
2) Solid phase extraction (SPE): fast way to enrich and to simplify a sample matrix.
3) Accelerated solvent extraction (ASE)
4) Low-pressure LC (LPLC): Flash chromatography (FC) is the example.
5) Supercritical fluid extraction:Using carbon CO_2&counter current chromatography
6) Mass Spectrometry (MS): Essential tool in all structure elucidation workflows.
7) Nuclear Magnetic Resonance (NMR): Extremely powerful tool.
8) High Performance Liquid Chromatography (HPLC): Routine technique.
9) GC-MS: First Hyphenated Technique.

10) LC-MS: LC-MS and its variants are most popular.
11) Capillary Electrophoresis- Mass Spectrometry (CE-MS)

SUMMARY:

Forced degradation studies provide invaluable insight in investigating degradation products and pathways of drug substances and products. Even though the ICH and FDA guidance documents only call for the inclusion of these studies in Phase III of the regulatory submission process, it is strongly recommended these studies be started as early as possible to be able to provide valuable information that can be used to assess the inherent stability of a drug, and to improve formulations and the manufacturing process. Given that no specific set of conditions will be applicable to all drug substances and products, the pharmaceutical scientist should ensure the stress conditions are consistent with product decomposition under normal manufacturing, storage, and intended use conditions. Recommended stress factors include high and low pH, elevated temperature, photolysis, and oxidation. Care should be taken to avoid understressing or unduly over-stressing the drug substance or product, for this may lead to aberrant and non-representative results. A degradation level of approximately 10% of the drug substance should be optimal for method optimization.

CONCLUSION:

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. The review described above discusses the importance of forced degradation in drug development stage. A properly designed and executed forced degradation study would generate an appropriate sample for development of stability indicating method.

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