



APPROACH ABOUT CURRENT TRENDS ON FORCED DEGRADATION STUDIES FOR STABILITY INDICATING METHODS.

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ABSTRACT:

Stability of drug substance or drug product is an essential parameter which can affect purity, potency and safety of the drug. Poor stability, impurities and toxic degradants is associated with poor efficacy, low potency and toxic effects of the drug product during treatment. So a need to establish shelf life, purity profile and chemical behavior of the drug substance under various different conditions (adjusted pH, temperature, humidity, light etc.) to determine better modifications during development and formulation of the drug. Forced degradation study involves exposure of drug substance to exaggerated conditions to establish chemical behavior of the drug substance or drug products; it is required to perform the study according to regulatory guidelines, at almost every stage of drug development. Forced degradation studies are useful to mimic decomposition of drug substance or drug product in real time stability studies, determine specificity of stability indicating method, the degradation pathways and degradation products likely to form during drug management (handling, storage and use) i.e drug stability profile. Stability indicating analytical procedures of different methods (such as: RP-HPLC, column chromatography and other spectroscopic techniques) are applied to analyze the outcomes of forced degradation conditions on drug substance which gives the qualitative and quantitative data analysis, which can be a combination of conventional and spectroscopic techniques. In this review we provide an insight about current trends on conducting forced degradation studies for stability indicating method.

KEY WORDS: FORCED DEGRADATION STUDIES, STABILITY INDICATING MEYHODS, DEGRADANTS, ICH

INTRODUCTION:^[1,2,9,19,20,23]

Stability of drug substances or drug product is a critical and essential parameter because it can directly affect purity, safety and efficacy of drug product. The FDA and ICH as regulatory bodies have established requirements of stability testing data to understand quality profile of drug product, drug product changes with time, degradation pathway or degradation kinetics under influence of various different environment conditions i.e (exposure to heat, humidity and light for solid state studies, pH range for solution state studies). The required stability testing data serves as a regulatory requirement in drug development. The Forced degradation study provides outlook of degradation pathway of drug molecules and its excipient interference, it influence the development of analytical method by setting specification and design of formulation. Forced degradation study involves stress testing measures that duplicate conditions to demonstrate specificity for development of stability indicating methods according to degradation pathway and products i.e (impurities, drug metabolites, non degradants e.t.c), so analytical indicating methods ; (RP-HPLC, Spectroscopy, Mass Spectroscopy, e.t.c) are used for qualitative and quantitative analysis of the process of forced degradation conditions. The stress testing creates a small scale time frame/exposure of drug substance to adjusted conditions to reflect drug life span under environmental conditions during drug management and use.

FORCED DEGRADATION STUDIES,^[1,10,15,19,20,23]

Also called stress testing studies; they are undertaken specifically to degrade the sample into potential products. It is done in presence of API with and without excipients, In presence of different drug substances or mixture of formulations. The studies are applied to evaluate ability of an analytical method to measure the outcome (i.e active ingredient and other degradation products with or without excipient interference) by generating potential degradation products. It includes exposure of the drug substance to adjusted or elevated conditions to observe the extent of degradation, degradation pathway and rate of process to anticipate on course of storage and use.

Purpose of degradation studies;

Forced degradation studies are mainly applied to help identify potential degradation products, degradation pathway and so the intrinsic property of drug and therefore an important role to validate stability indicating analytical procedure. **The stress studies are important and purposely applied;**

- To develop and validate stability indicating methods.
- To determine the structure of degradation products.
- To determine the degradation pathway of drug substance and drug products.
- To identify the potential metabolites, impurities from drug formulation and drug products.
- To establish drug degradation drug profile.

- To understand drug molecule chemistry.
- To solve stability related drug problem (e.g mass balance)
- To determine proper storage condition, packaging, administration and general drug management.
- To generate more stable and effective drug formulation.
- To determine intrinsic stability of the active pharmaceutical ingredient in solid state and in solution state.

FORCED DEGRADATION STUDIES:^[19,20]

Development of forced degradation studies; Stress testing is aimed to give representative samples for developing stability indicating methods for drug substance and drug products. Selection criteria for stress condition depend upon products decomposition under manufacturing process, storage and use conditions through drug life cycle which are distinct from drug to drug. Stress factors include; alkali and acid hydrolysis, thermal, photolysis and oxidation. Types of forced degradation studies are:

- **Real time stress studies:** take about 12 months and above
- **Intermediate stress studies:** take about 6 months
- **Accelerated degradation studies:** exaggerated conditions are used to study degradation within 7days.

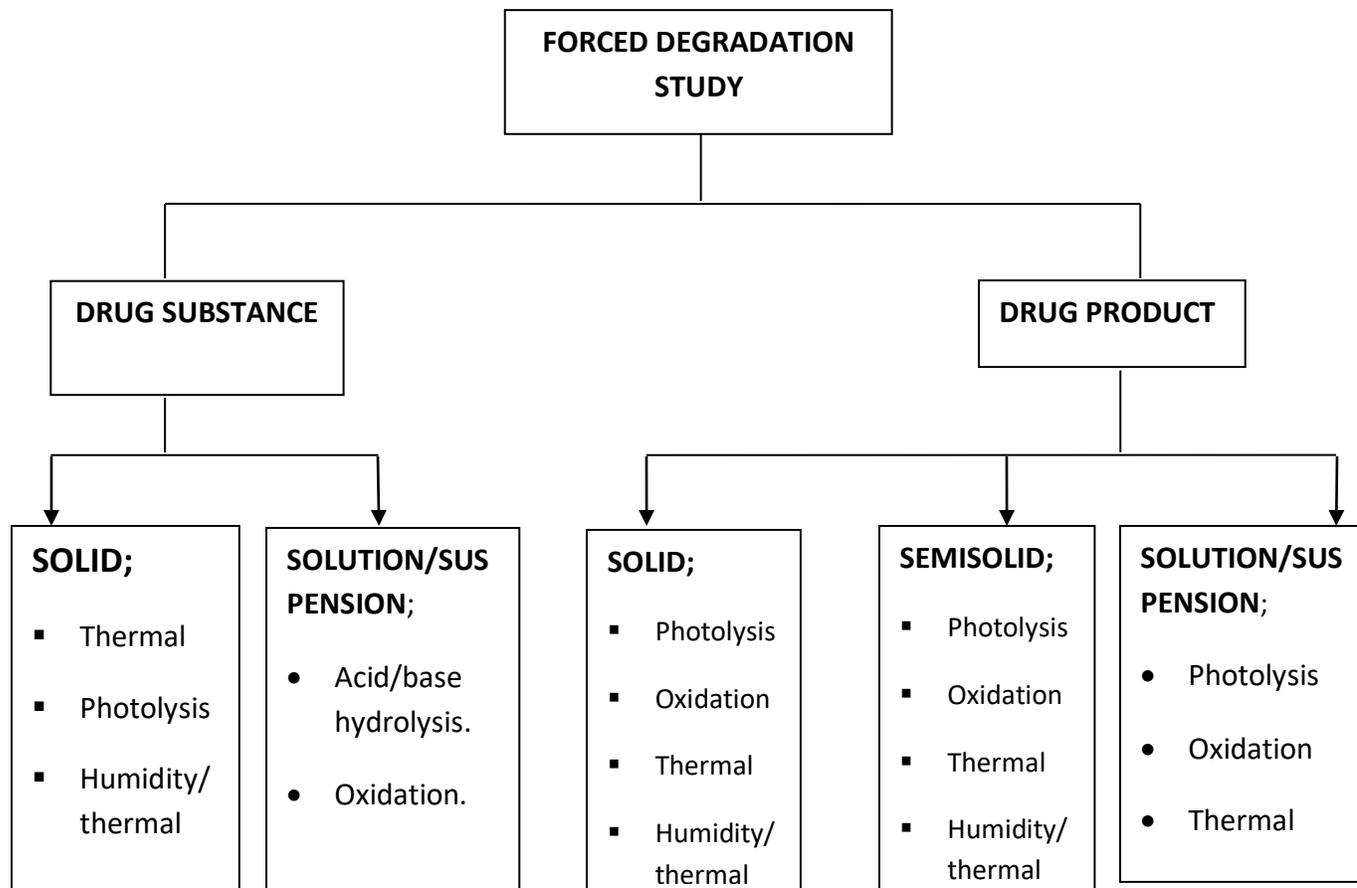


Fig 1. The illustration of flow diagram showing conditions used for forced degradation study on drug substance and drug product.

Table 1: conditions generally applied for force degradation

Sr. No	Degradation factor;	Experimental condition;	Storage conditions	Sampling time in days
1	Hydrolysis;	Control API (no acid or base)	40 ⁰ C, 60 ⁰ C	1, 3, 5
		0.1M HCL	40 ⁰ C, 60 ⁰ C	1, 3, 5
		0.1M NaOH	40 ⁰ C, 60 ⁰ C	1, 3, 5
		Acid control (no API)	40 ⁰ C, 60 ⁰ C	1, 3, 5
		Base control(no API)	40 ⁰ C, 60 ⁰ C	1, 3, 5
		pH; 2, 4, 6, 8	40 ⁰ C, 60 ⁰ C	1, 3, 5
2	Oxidation;	3% H ₂ O ₂	25 ⁰ C, 60 ⁰ C	1, 3, 5
		Peroxide control	25 ⁰ C, 60 ⁰ C	1, 3, 5
		Azobisisobutyronitrile (AIBN)	40 ⁰ C, 60 ⁰ C	1, 3, 5
		AIBN Control.	40 ⁰ C, 60 ⁰ C	1, 3, 5
3	Photolysis;	Light 1 x ICH	NA	1, 3, 5
		Light 3 x ICH	NA	1, 3, 5
		Light control	NA	1, 3, 5
4	Thermal ;	Heat chamber	60 ⁰ C	1, 3, 5
		Heat chamber	60 ⁰ C/75%RH	1, 3, 5
		Heat chamber	80 ⁰ C	1, 3, 5
		Heat chamber	80 ⁰ C/75%RH	1, 3, 5
		Heat control	Room temperature.	1, 3, 5

Experimental conditions.[2,7,10,12,14,22]

- There is no generic set of conditions established for forced degradation study, due to multiplicity in the kinds and nature of drug substance. The main objective is to subject drug molecules to adjust more accelerated or strenuous conditions.
- The following are conditions at a minimum are measured and undertaken into consideration:
 - **Acid and base hydrolysis.**
 - **Hydrolysis at different pH levels.**
 - **Thermal degradation.**
 - **Photolysis.**
 - **Oxidation.**

During stress testing it is more critical to monitor the degradation process to prevent excess or misleading results which are unrealistic in real stability studies. It is possible to start with conditions that degrade the drug to less

percentage, concentration 1mg/ml of drug can be initially used to detect minor decomposition products in range of detection, 10% degradation of the drug substance and drug product has been considered optimal during various studies from the range of 2%-30%, however some studies have applied extreme condition at the first step which extensively lead to decomposition of drug to high percentage (i.e extreme temperature or more acidic or more basic). Below are the conditions applied:

i. Hydrolytic condition:

Hydrolysis is a solvolytic process where the drug reacts with water to yield degradation products of different chemical composition; example aspirin is hydrolyzed into salicylic acid and acetic acid. Hydrolytic process under acidic or basic condition may require a catalyst for ionization and selection of the concentration of acid/base depends on stability of drug substance. Hydrochloric acid or sulphuric acid and Sodium hydroxide or potassium hydroxide are common choices for acid and base hydrolysis respectively (0.1-1M), stress testing can be done with or without a co-solvent, varying temperature for desired products within a required period.(about 7 days).

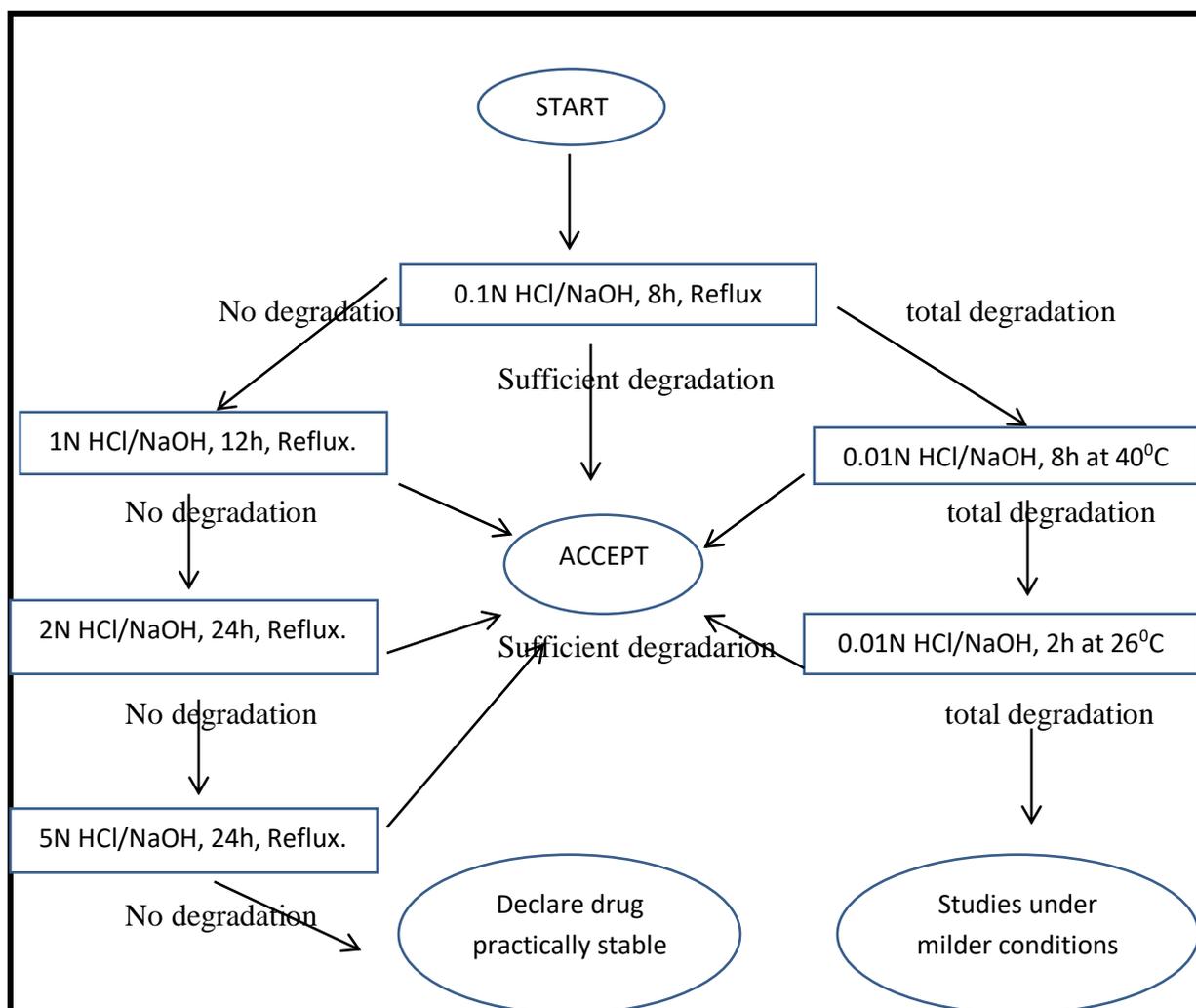


Figure 2; flow chart of hydrolytic degradation

ii. Oxidation condition;

Here, the drug substance is exposed to oxygen, hydrogen peroxide is the widely used reagent besides oxygen and other radical initiators. The drug structure influences the selection of required oxidizing agent, the oxidation process involves transfer of electrons during degradation. Many drugs undergo auto oxidation at normal storage conditions in the presence of elemental oxygen. For drug solutions, hydrogen peroxide at 0.1%-3% is used at neutral pH and room temperature for about 7 days or up to 20% degradation. Light stress has shown to induce photo oxidation by free radical mechanism.

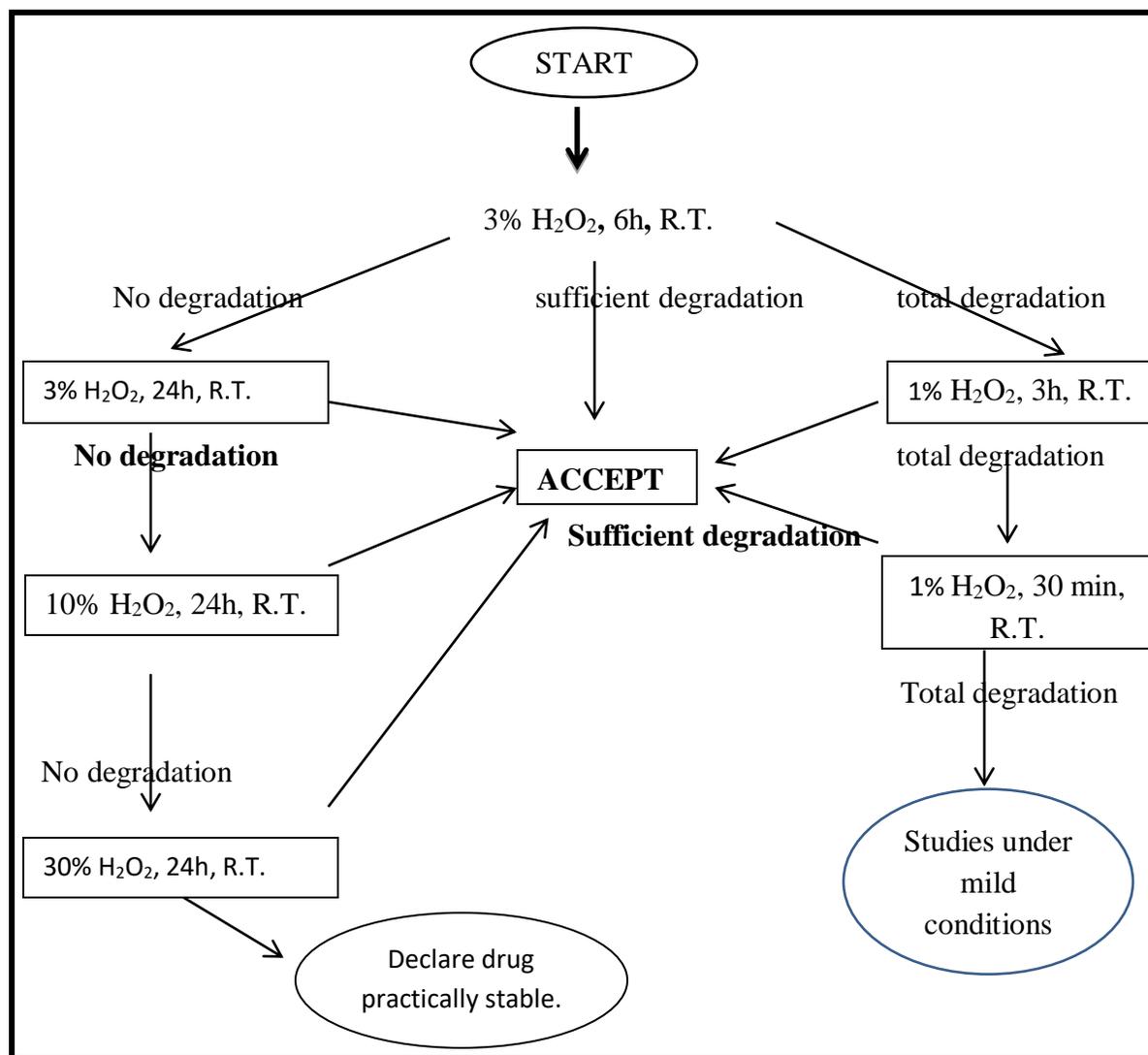


Figure 3; flow chart of oxidative degradation

iii. Photolytic condition;

The drug substance or drug product is subjected to photolytic conditions to determine the effect on drug stability, so photo stability testing is also important. Ultra Violet or fluorescent conditions are applied to generate primary degradants. 300-800nm wavelength of light is the most accepted range according to regulatory guidelines for photolytic degradation. The minimum of 1.2million lux hrs and 200 Wh/m² light and maximum of 6million lux hrs

are recommended conditions. The photolytic degradation can happen through oxidative or non-oxidative photolytic reactions, some functional groups like carbonyls, alkenes, aryl chloride among others have showed to induce drug photosensitivity.

iv. Thermal condition;

For this step the drug is exposed to different levels of heat, the heat can be wet or dry heat, solid state drug form is exposed to either dry heat or wet heat but the liquid drug products are only exposed to dry heat. Arrhenius equation is used to study the thermal degradation of the drug substance. ($K=Ae^{-E_a/RT}$), where K-restricted reaction time, A-frequency factor, E_a -activation energy, R- universal gas constant(1.987Cal/deg mole), and T –temperature. Thermal degradation study is administered at 40⁰-80⁰C, 70⁰C has showed successful degradation output for 1-2 months at low and high humidity.

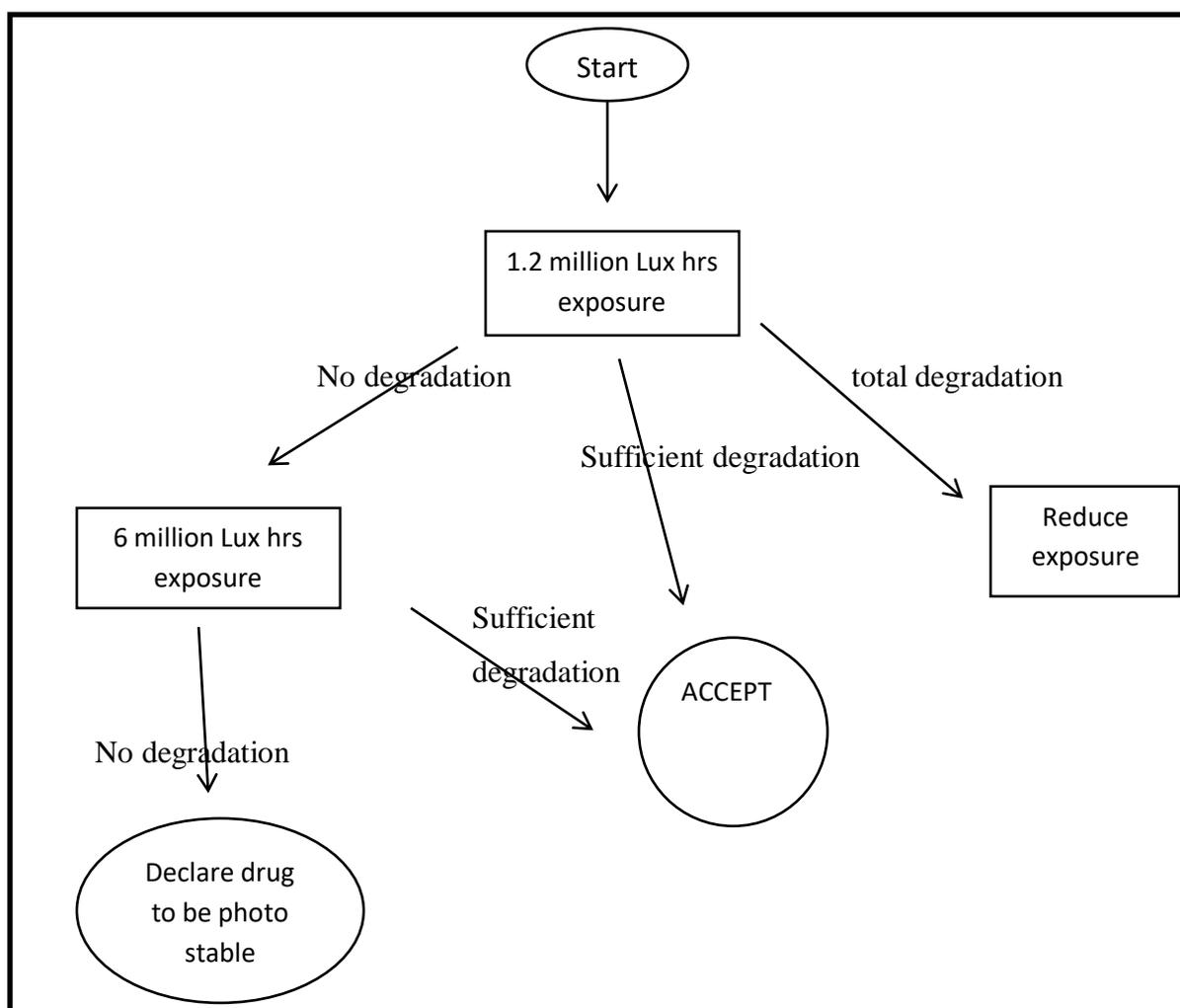


Figure 4; flow chart of thermal degradation

Factors affecting degradation of drug substance; ^[20]

Moisture; water soluble substances can dissolve leading to change in physical and chemical properties of the drug molecules.

Excipients; these additives can produce unanticipated degradants during or after degradation due to interaction with co-solvents or other additives hence impurities.

Temperature; increase in temperature generally cause increase in the rate of drug hydrolysis however extreme temperatures may cause misleading results.

pH; this may affect the drug degradation by hydrolysis and so buffer system are required to contain the effect.

Air; especially oxygen directly leads to oxidation of drug molecules hence increase the rate of decomposition of drug, nitrogen and carbon dioxide can be used to prevent the effect.

Light; this can induce photo oxidation by free radical mechanism of drug molecule example is the photo degradation of barnidipin includes the plausible formation of singlet oxygen, so photo labile drug substance are more vulnerable.

Impurities, source of impurities and types of impurities; the substance with no value in the drug substance or product are considered as impurities, they can arise from manufacturing process, packaging container or decomposition during storage. ICH terminology classifies the impurities into: **Organic, Inorganic** and **Residual solvents** (eg raw material by products, degradation products, reagents, catalysts, heavy metals, inorganic salts e.t.). Impurities can lead to change in physical and chemical stability. Filter aid, charcoal, functional groups related impurities among other kinds of impurities.

Regulatory guidelines on degradation studies and stability testing:[2,19,20,22,24]

The regulatory bodies of different regions have a set of requirements, guidelines, procedures and information about stress testing, protocols to be followed, and importance of degradation studies. For a New Drug Application (NDA), the registration needs data of forced degradation studies as forced degradation products, degradation kinetics, structure, drug peak purity, and mass balance.

Examples; FDA(Food and Drug administration) Guidelines, EMA (European Medicine Agency) Guidelines, USP(United States) Pharmacopoeia-Validation of compendia procedures, Japanese Pharmacopoeia, ANVISA (Agencia Nacional de Vigilancia Sanitaria)-National Health Surveillance Agency and ICH (International Committee for Harmonization) Guidelines.

Table 2: ICH Regulatory guideline for degradation studies and stability testing.

Guideline	Title
Q1A(R2)	Stability testing of new drug substances and products.
Q1B	Stability testing photo stability of new drug substances and products.
Q1C	Stability testing for new dosage forms.
Q1D	Bracketing and matrix designs for stability testing of new drug substances and products.
Q1E	Evaluation for stability data
Q1F	Stability data package for registration application in climatic zones iii and iv.
Q2B	Validation of analytical procedures: Methodology.
Q3	Impurities in new drug substance.
Q3B	Impurities in new products.
Q5C	Quality of biotechnological products: stability testing of biotechnological biological products.
Q6A	Specifications: test procedures and acceptance criteria for biotechnological/biological products.

STABILITY INDICATING METHODS; [2-4,5,7,11,12,14,17-19,22,23]

Various international regulatory guidelines such as ICH defines Stability Indicating Methods (SIM), that is ICH defines SIM as quantitative analytical method that is based on the characteristic structural, chemical or biological properties of each ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured. These are analytical procedures or measures, required to monitor changes in the properties (physical or chemical) of the drug substance and drug product over time, determines need to perform a forced degradation studies on drug substance and drug products. The analytical studies are conducted on drug substance to identify degradation byproducts likely to appear during long term storage (i.e potential products) and ensure the method detects and quantifies these products. They are characterized by potency, purity and biological activity, product specific and should be highly sensitive to detect impurities at low level (at 0.05% of analyte) below percentage of analyte under which ICH guidelines.

Selection of technique to use depends upon character of impurities or degradants, the idea of acidity, basicity, or neutrality of impurities and the variations in solubility of impurities and drug substances give a baseline for separation technique to separate and isolate main component from impurities.

- ❖ **The general idea for designing stability indicating method:** [3,6,8,14,16,19]
- ✓ **Understand the chemistry and physicochemical properties of drug.** It is necessary and required to have detailed knowledge of chemistry of drug such structural features and physicochemical properties such as pKa, log P, solubility, absorptivity and wavelength of maximum of the drug are under study to assess the likely decomposition route/pathway.
- ✓ **Set up preliminary chromatographic conditions.** The stress samples obtained are preliminary analyzed to review the amount and kinds of degradation products formed under different conditions. Analysis is done using different chromatographic conditions: methanol- water mobile phase system can be applied or other system, selection of wavelength, flow rate and pH. The obtained results from analyzing different strain samples is then critically compared with that of blank solutions injected in a similar manner.
- ✓ **Prepare samples required for method development.** To generate samples, severe forced degradation conditions are applied to enable drug undergo degradation into potential products likely to be formed in realistic lifecycle of drug substance or drug product under oxidative, photolytic, hydrolytic and thermal conditions.
- ✓ **Establish separation stability indicating chromatographic condition.** to separate close peaks; mobile phase, gradient, pH, flow rate, solvent type, temperature, column(type) can be changed. (Acetonitrile, methanol and water in different proportions are widely used as mobile phase, ODS column, phosphate buffer, room temperature, pH of 3, isocratic elution mode at flow rate of 1.0micronmeters/min are used commonly in RP-HPLC application).
- ✓ **Method development and optimization.** Physiochemical properties like pKa value, log P, solubility and absorption maximum of the drug should be known to determine the method development, Log P and solubility helps to determine mobile phase and solvent of the sample and pKa value determines pH condition for use of mobile phase. (to separate close peaks; mobile phase, gradient, pH, flow rate, solvent type, temperature, column(type) can be changed.)
- ✓ **Validation of analytical method.** It is done as per ICH guidelines, accuracy, precision, linearity, limit of quantification (LOQ), limit of detection (LOD), robustness and ruggedness are parameters tested. The main focus is to establish specificity/selectivity followed by other parameters. Mass balance is determined with respect to LOQ and LOD parameters, for quantitative measurement of analyte all typical validation parameters should be considered. If the analytical method does not fall in the acceptance criteria, the method is modified and revalidated.

SEPARATION TECHNIQUES USED;^[8,11]

After generation of forced degradation samples, the next step is to evaluate the degradants of changes in the drug substance and drug product formed. Separation and identification of a particular degradant from other mixed degradation products is so complex and critical for successful study, therefore the preference should be given to a highly selective method with emphasis on peak purity. The highly selective analytical method is well enough to detect impurities at low levels and able to separate main component from impurities when drug peak co-elutes and interrupted with degradation impurity peak and also separate known from unknown impurities. Separation techniques include: liquid-liquid extraction, solid phase extraction, supercritical fluid extraction, flash (column) chromatography, TLC, GC, SFC, CE, HPLC. Description:

TLC: Thin Layer Chromatography; this economical, variety of plates and mobile phases can be used but there difficulties in defined resolution, detection and ease of quantification.

Gas Chromatography (GC); this is limited to volatile substances or volatile derivate however it provides desired resolution, quantification and selectivity.

Capillary Electrophoresis (CE): this is advantageous that less volume os samples are used and high resolution is observed but reproducibility is unreliable.

Super fluid chromatography (SFC): it is advantageous and has greater application for extraction of sample and has good detection.

HPLC: High Performance Liquid Chromatography (high pressure liquid chromatography): This is the widely used conventional chromatographic technique and has shown importance with extended application of detectors such as Photodiode Array Detector etc. it is categorized into;

Normal phase-HPLC: polar material is used as stationary phase and mobile phase is relatively non polar and,

Reversed phase HPLC: It is the most applied method on industrial scale, Stationary phase used is less polar than mobile phase, ODS Column as stationary phase and mobile phase relatively non polar; non polar drugs eluted later than polar drugs. HPLC use either gradient or isocratic elution mode, special chromatographic column is applied such C18 or C8 or phenyl columns, stationary and mobile phase with a buffer system, optimum flow rate and powerful UV detectors are used. The method is considered and desired for its compatibility with organic and inorganic solution, high resolution, high selectivity/sensitivity, ability to detect polar compounds and characterization of products.

HYPHENATED METHODS: ^[2-5,7,8,11,19,23,25] These are analytical techniques that give in fast description of impurities and other degradation products. An excellent combination of conventional techniques (such as column chromatography, gas chromatography, capillary electrophoresis, supercritical fluid chromatography, liquid-liquid

extraction, solid phase extraction etc) and spectroscopic techniques (such as ultra violet(UV), infrared(IR), nuclear magnetic resonance(NMR), mass spectrometry(MS)) is used when the degradants or drug related impurities cannot be isolated in pure form. They are advanced with use of detectors such as Photodiode Array Detector so that are able to detect, identify, and characterize degradation products and impurities (known and unknown). The combination technique is essential to watch degradants and impurities, however there are limitations to peak purity i.e (1) Non-linear UV detection at higher absorbance, (2) The co-eluting peak has no chromophoric functional group distinctive for easy identification, (3) The co-eluting product peak having same UV spectra^[25]

The highly selective analytical method should be well enough to detect impurities at low levels and able to separate main component from impurities when drug peak co-elutes/interrupted with degradation impurity peak and also separate known from unknown impurities.

Examples of such combination are:

- GC-MS; Gas Chromatography – Mass Spectroscopy.
- LC-MS; Liquid Chromatography – Mass Spectroscopy
- LC-DAD-MS: Liquid Chromatography – Photodiode Array Detector – Mass Spectroscopy.
- LC-NMR; Liquid Chromatography – Nuclear Magnetic Spectroscopy
- SFC-MS; Supercritical Fluid Chromatography – mass spectroscopy
- CE-MS; Capillary Electrophoresis – Mass Spectroscopy, e.t.c.

Automation of data analysis: ^[11]

Currently there is advanced spectroscopy instrumentation that complement on analysis of samples with high precision and sensitivity/selectivity. MassChemSite and WebChem-base software has been described and proven as a tool to analyze forced degradation products. In drug discovery and new plant elucidation, the software has been applied for last-stage functionalization through C-H functionalization of a drug. MassChemSite is a desktop application that involves data input (LC-MS/MS raw data), peak detection and structure assignment, it is used purposely for automatic structural elucidation of organic compounds. The software use LC-M/MS raw data provided from different mass spectrometry vendors, structural information of products/compounds to identify, and anticipated reactions specifically given by the analyst to elucidate the structure of reaction products finally.

Advantages of HPLC over other analytical methods.

- Most specific technique for separation of analyte, impurities and degradation product.
- Greater sensitivity due to low limit of detection of analyte.
- Less time required for separation and quantification of analyte and degradation product.
- Improved resolution between the analyte and impurities.
- Provision of reusable column for several time therefore become cost effective.

- Completely automated and human errors are reduced or prevented.
- Thermally unstable compounds can be analyzed by this method.

CONCLUSION;

Stress testing is an essential tool in drug development process in order to determine potential degradation products under exaggerated conditions, the products are analyzed to characterize products and identify the degradation pathway. The stress test is much helpful to perform at every critical stage i.e from early stages of drug development to provide important information which creates room for better modifications of drug formulation and product and in turn better drug profile with good purity, safety, efficacy and effectiveness. Forced degradation study is aimed at analyzing the stability and excipient compatibility of the drug substance or drug product to develop and validate stability indicating method by determining the specifications of the method. Stability indicating methods are assay procedures or techniques applied with instrumentation and varying conditions that identify and quantify degradation products as well as presence of impurities. A combination of chromatographic techniques and spectroscopic techniques provide inclusive and in-depth analysis of products and able to identify and separate known and unknown impurities effectively as recommended by different regulatory guidelines on pharmaceutical/biological products. The products and impurities can be identified and characterized according to structural differences, functional groups, chemical behavior, degradation pathway, bonding with respect to absorbance of wavelength obtained after stress testing period, thus better accuracy, precision, robustness, ruggedness, limit of detection and limit of quantification of the method after analysis determine its development and validation.

REFERENCES:

1. Blessy M, Ruchi D, et al, "A review; Development of forced degradation studies of drugs" *Journal of Pharmaceutical Analysis*, 4(3), June **2014**, 159-165. <https://doi.org/10.1016/j.jpba.2013.09.003>
2. Mullani A.K. and Nargatti P.I: "Forced degradation study - a new approach for stress testing of drug substances and drug products", *Int J Pharm Sci & Res*, **2021**; 12(5): 2683-91. DOI link: [http://dx.doi.org/10.13040/IJPSR.0975-8232.12\(5\).2683-91](http://dx.doi.org/10.13040/IJPSR.0975-8232.12(5).2683-91)
3. Giorgio G, Erika G, et al. "Simultaneous, stability indicating, HPLC-DAD determination of guaifenesin and methyl and propyl-parabens in cough syrup", *J Pharm Biomed Anal*, **2006** Jun 7; 41(3):798-803. DOI: [10.1016/j.jpba.2006.01.026](https://doi.org/10.1016/j.jpba.2006.01.026)

4. El-Saharty Y. S et al, "Stability-Indicating Spectrophotometric and Densitometric Methods for Determination of Aceclofenac". *Drug Development and Industrial Pharmacy*, **2002**. 28(5), 571–582. DOI: [10.1081/ddc-120003453](https://doi.org/10.1081/ddc-120003453)
5. Babita Agarwal, Santosh Gandhi, "Development of Validated Stability-indicating RP-HPLC Method for Determination of Novel Directly Acting Antiviral agent and Characterization of its Degradants by LC–ESI–MS". *Indian Journal of Pharmaceutical Education and Research*. **2020**; 54(4):1159-68. doi:10.5530/ijper.54.4.212
6. Angirekula N, Mathrusri M. A, "Study of Forced Degradation behavior of Atazanavir". *Research J. Pharm. and Tech*, **2018**; vol. 11(8): 3693-3697. DOI: [10.5958/0974-360X.2018.00678.9](https://doi.org/10.5958/0974-360X.2018.00678.9).
7. Abu S. S. Rouf, Md. F. Hossain et al. "Stress degradation studies on aceclofenac and development of a validated stability-indicating reversed-phase HPLC assay in tablet dosage form", *Der Pharma Chemica*, **2013**, 5 (4):131-146. www.derpharmachemica.com
8. Saha Chandni, N. Vishal G, R. S. Chandan, "Development and validation of a UPLC-MS method for determination of atazanavir sulfate by the "analytical quality by design" approach", *Acta Pharm*, **2020** Mar 1;70(1):17-33, PMID: 31677371. DOI: [10.2478/acph-2020-0008](https://doi.org/10.2478/acph-2020-0008)
9. Jenny Desantis, Elisabeth OC, et al. "Automatic Identification of Lansoprazole Degradants under Stress Conditions by LC-HRMS with MassChemSite and WebChembase". *J. Chem. Inf. Model*. **2021**, 61(6), 2706–2719. <https://doi.org/10.1021/acs.jcim.1c00226>
10. Narsimha rao Doredla, et al. "Method Development And Validation Of Forced Degradation Studies Of Pioglitazone Hydrochloride By Using UV Spectroscopy". *Int.J.PharmTech Res.*2012,4(4), 4(4), **2019**, 01-06. [https://sphinxesai.com/2012/oct-dec/Pharmpdf/PT=54\(1750-1757\)OD12.pdf](https://sphinxesai.com/2012/oct-dec/Pharmpdf/PT=54(1750-1757)OD12.pdf)
11. Lalitha M Devi, K B Chandrasekhar, "A validated stability-indicating RP-HPLC method for levofloxacin in the presence of degradation products, its process related impurities and identification of oxidative degradant", *J Pharm Biomed Anal* , **2009** Dec 5;50(5):710-7, DOI: [10.1016/j.jpba.2009.05.038](https://doi.org/10.1016/j.jpba.2009.05.038)
12. G. Andermann,A. Richard, "Stability-indicating determination of tetrahydrozoline hydrochloride in ophthalmic solutions by high-performance liquid chromatography", *J Chromatogr*, **1984** Aug 17;298(1):189-92. DOI: [10.1016/s0021-9673\(01\)92710-4](https://doi.org/10.1016/s0021-9673(01)92710-4)
13. Anna Gumieniczek et al, "Comparative Study of Chemical Stability of Two H₁ Antihistaminic Drugs, Terfenadine and Its *In Vivo* Metabolite Fexofenadine, Using LC-UV Methods", *J Anal Methods Chem*. Article ID 5790404, 10, **2019**. <https://doi.org/10.1155/2019/5790404>

14. Khagga bhavyasri et al. “DEVELOPMENT AND VALIDATION OF FORCED DEGRADATION STUDIES OF LOPINAVIR USING RP-HPLC AND CHARACTERIZATION OF DEGRADANTS BY LC-MS/MS”, *Int. J. Chem. Sci.*: 13(1), **2015**, 551-562. www.sadgurupublications.com
15. AL-Ghobashy et al. “Evaluation of the Pattern and Kinetics of Degradation of Adalimumab using a Stability Indicating Orthogonal Testing Protocol”. *Biomedical Chromatography*, (**2019**). DOI: [10.1002/bmc.4676](https://doi.org/10.1002/bmc.4676)
16. Al-Ghobashy et al, “Analytical comparability study of anti-CD20 monoclonal antibodies rituximab and obinutuzumab using a stability-indicating orthogonal testing protocol: Effect of structural optimization and glycoengineering”. *Journal of Chromatography B*, **2020**, 1159, 122359. <https://doi.org/10.1016/j.jchromb.2020.122359>
17. Marghala Dasaratha Dhanaraju et al, “Stability indicating HPLC method for the simultaneous determination of dapagliflozin and saxagliptin in bulk and tablet dosage form”, 39 *Curr. Issues Pharm. Med. Sci.*, 31(1), 39-43. DOI: <https://doi.org/10.1515/cipms-2018-0009>.
18. Mohinish sahai et al, “Validated stability indicating HPLC approach for quantifying tricholine citrate and cyproheptadine simultaneously in syrup forms”, *Int J App Pharm*, 13(3), **2021**, 207-213. DOI: [10.22159/ijap.2021v13i3.40871](https://doi.org/10.22159/ijap.2021v13i3.40871)
19. Panchumarthy R., Swathi V, et al, “Current Trends in Performance of Forced Degradation Studies and Stability Indicating Studies of Drugs”, *IOSR Journal of Pharmacy and Biological Sciences*, 12(6), **2017**, 17-36. www.iosrjournals.org , DOI: 10.9790/3008-1206021736.
20. Chavan Arjun et al, “Current Trend in Performance of Forced Degradation Studies for Drug Substance and Drug Product’s”, *Journal of Drug Delivery & Therapeutics*. 10(2), **2020**, March-April 2020 (Supplement Issue), DOI <https://doi.org/10.22270/jddt.v10i2-s.4040>.
21. INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE, “Stability testing of new drug substances and drug products Q1A(R2)”, *ICH Harmonised Tripartite Guideline*, current step 4 version, 06/02/2003, <https://database.ich.org/sites/default/files/Q1A%28R2%29%20Guideline.pdf>
22. Saranjit Singh et al, “Forced degradation studies to assess the stability of drugs and products”, *TrAC Trends in Analytical Chemistry*, 49, **2013**, 71-88, ISSN 0165-9936, <https://doi.org/10.1016/j.trac.2013.05.006>.
23. Verma A, Singla S, Palia P, “Development of Forced Degradation and Stability Indicating Studies of Drugs- A Review”, *Asian Journal of Pharmaceutical Research and Development*. **2022**; 10(2): 83-89, DOI: <http://dx.doi.org/10.22270/ajprd.v10i211034>.

24. Ranjit Singh, Zia ur Rehman, “Current trends in forced degradation study for pharmaceutical product development”, *Ranjit Singh J Pharm Educ Res*, 3(1), **2012**, <file:///E:/article%20review/Currenttrendsinforceddegradationstudyforpharmaceuticalproductdevelopment.pdf>
25. Manish Kumar Sharma, Manoharam, “Forced Degradation Study an Essential Approach to Develop Stability Indicating Method”, *J Chromatogr Sep Tech*, **2017**, 8:1, DOI: 10.4172/2157-7064.1000349