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ANALYTICAL HYPHENATION: A NOVEL APPROACH TO PHARMACEUTICAL STABILITY TESTING

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ABSTRACT

The pharmaceutical industry is growing quickly, especially with fixed-dose combination (FDC) formulations. This leads to a greater variety of contaminants and degradation products because of different APIs, processing techniques, and storage conditions. Chronic illnesses like diabetes and hypertension necessitate long-term medicine intake, which may result in cumulative exposure to cytotoxic or genotoxic chemicals, even if regulatory agencies enforce impurity limits (usually within 1%). Therefore, it is crucial to thoroughly identify and characterize these contaminants. For the purpose of assessing the best storage and labeling practices and guaranteeing the safety and effectiveness of drugs, stability testing. which includes forced degradation techniques including hydrolysis, oxidation, acidic, alkaline, and thermal stress is essential. Sophisticated hyphenated approaches have proven indispensable in addressing the analytical issues presented by impurity profiling. Both qualitative and quantitative impurity analysis have been transformed by the combination of spectroscopic detection and chromatographic separation. Pharmaceutical research frequently uses techniques like LC/MS, LC/NMR, LC/NMR/MS, GC/MS, and LC/MS/MS because of their sensitivity and ability to clarify structures. These techniques provide quick and precise impurity profiling, facilitating safety assessment in drug development and conforming to changing regulatory requirements. The current conventional and hyphenated analytical methods for identifying drug degradation products are described in detail in this thorough study. In order to demonstrate the advancements in impurity analysis that support pharmaceutical quality, efficacy, and safety, we look at forced-degradation approaches in addition to newly developed hyphenated procedures.

KEYWORDS: Liquid Chromatography (LC); Mass Spectroscopy (MS); Hyphenated Techniques; Force Degradation.

INTRODUCTION

Hyphenated procedures are a mixture of two methods wherein one method, generally chromatographic, is used to isolate or separate a component and the other method, such as mass, NMR, or IR spectroscopy, is used to identify the separated component. While spectroscopic procedures provide selected information for identification through comparison with standards or library spectra, chromatographic procedures are crucial for the separation of components in complex mixtures in pure chemical form. The term "hyphenation" was created by the few-decked-back scientist Hirschfield to refer to the online combination of a separation approach and one or more spectroscopic detection approaches. Pharmaceutical stability testing is a complicated set of procedures that demand a great deal of money, time, and scientific expertise in order to enhance the quality, effectiveness, and safety of a drug formulation. Among the most important responsibilities throughout the development phases are the pharmaceutical analysis and stability tests required to determine and guarantee the identity, potency, and purity of components as well as

those of the created products. A pharmaceutical product's stability is defined as its ability to retain its physical, chemical, microbiological, toxicological, protective, and informational properties in a given container or closure system.[1-7]

In order to predict how long a drug substance or manufactured product will survive, determine the optimum storage alternatives, and suggest labeling recommendations, stability testing evaluates how the environment influences the product's quality. These factors include the stability of the active ingredient or ingredients, the way the active ingredient or ingredients interact with excipients, the manufacturing processes, the type of dosage form, the packaging container or closure system, and the light, heat, and moisture conditions that are present during handling, storage, and transportation.

Furthermore, the stability of degradation reactions like oxidation, reduction, hydrolysis, or racemization—which can be crucial for pharmaceutical products—is influenced by a number of variables, including reactant concentration, pH, radiation, catalysts, raw material usage, and the time between product production and use. Changes to a pharmaceutical product's appearance, consistency, homogeneity of content, clarity (solution), moisture content, packaging integrity, pH, particle size, and shape. Among other things, impact, vibration, abrasion, and temperature fluctuations like freezing, thawing, or shearing can cause these physical changes. Chemical processes such as solvolysis, oxidation, reduction, racemization, etc., can lead to the degradation of pharmaceutical goods. Additionally, these procedures may result in the loss of part of the excipient action and efficacy of active pharmaceutical ingredients (API), including antioxidant and antibacterial preservative activities.[8-14]

Role of Hyphenated Techniques in force degradation analysis

Hyphenated approaches have drawn a lot of attention lately as they are thought to be the primary method for resolving challenging analytical issues. In addition to being used for impurity profiling and qualitative and quantitative analysis of impurity and related products, the combined power of separation technologies and spectroscopic techniques has been used primarily over the years for both quantitative and qualitative analysis of unknown compounds from complex natural product extracts or fractions.

The hyphenation need not necessarily occur between two procedures; for example, LC-PDA-MS, LC-MS-MS, LC-NMR-MS, and LCPDA-NMR-MS are examples of techniques that can be coupled with one another. The creation of many hyphenated methods has given researchers studying natural products very potent new tools that offer both online complementary spectroscopy data on an LC or GC peak of interest within a complicated combination and superb, efficient separation. The pharmaceutical industry is being overtaken by several formulations that use a variety of APIs.

Degradation of each of them can result in the creation of cytotoxic, genotoxic, and other unwanted substances. Analytical chemists now have a method to overcome the difficulty of impurity profiling and determining the safety and effectiveness of APIs thanks to the hyphenated approaches. Impurities can enter pharmaceutical products from a variety of sources, including the use of particular reactants and reagents in reactions, heavy metals, ligands, catalysts, additional materials used in the manufacturing process, such as charcoal and filter aids, and degraded end products produced during the storage of manufactured bulk drugs. When exposed to different atmospheric conditions, processes such as hydrolysis, photolytic cleavage, oxidative degradation, decarboxylation, enantiomeric impurity, and so on might result in the generation of degradants. Limits on allowable amounts of impurities found in APIs or formulations have gradually begun to be incorporated into the major pharmacopoeias, such as the British Pharmacopoeia, United States Pharmacopoeia, and Indian Pharmacopoeia. Therefore, it is now imperative to identify and quantify contaminants using a variety of hyphenated approaches. This demonstrates the necessity and extent of drug impurity profiling in pharmaceutical research.

A methodical approach to the profiling of impurities Impurity profiling is a methodical technique used to detect, clarify the structure, and determine the impurities and degradation products in pharmaceutical formulations and bulk medicinal materials both qualitatively and quantitatively. It is impossible to achieve 100% purity for any API. At first, it was sufficient to state that the substance is 99 percent pure. However, a number of regulatory bodies have recently begun to demand that the remaining 1%'s makeup be determined. This is because of a few factors, such as the fact that if a person is diagnosed with diabetes or hypertension, they must take medication for the rest of their lives.

He or she has to take the prescribed drugs or formulations for a long time. Long-term exposure to even a small number of pollutants may cause traits like cytotoxicity and genotoxicity, even if there won't be many. As a result, proper impurity profiling of all formulations is essential. The development of a number of hyphenated techniques has tremendously benefitted analytical chemists by simplifying impurity profiling and qualitative analysis in formulations of the fixed dose combination type.

REGULATORY GUIDELINES

Studying suggested recommendations has become crucial before organizing a force degradation investigation. The ICH recommendations are the most

significant. The following are the several kinds of ICH recommendations for forced deterioration studies:

- ICH Q1A: Stability testing of new drug substances and products,
- ICH Q1B: Photo stability testing of new drug substances and products,
- ICH Q2B: Validation of analytical procedures: Methodology.

ICH Q1A

Guidelines for conducting forced degradation tests on pharmaceutical ingredients and products. It is advised to examine the findings of oxidation, photolysis, humidity (75% relative humidity), and temperature (above that for accelerated testing, i.e., > 50 oC). When evaluating a solution or suspension, a wide pH range should be taken into account. These samples produced an ultimate stability-indicating approach.[15–20]

ICH Q1B

It is advised to use them to evaluate the photo stability of both drug substances and drug products. Photo stability testing can be done on solids or in solutions or suspensions. The samples are also utilized in the creation of a technique that indicates stability.

ICH Q2B

These instructions provide information on how to validate the analytical technique that has been created.

ICH Q3A (R2)

It is necessary to identify each and every contaminant for safety and chemical reasons. Chemistry-related topics covered include impurity classification and identification, how force degradation may result in their creation, and, if feasible, the development of analytical techniques. development of a report, a list of contaminants in the specification, and a succinct explanation of analytical techniques. Checking if the contaminants are under the limit is necessary for safety precautions.

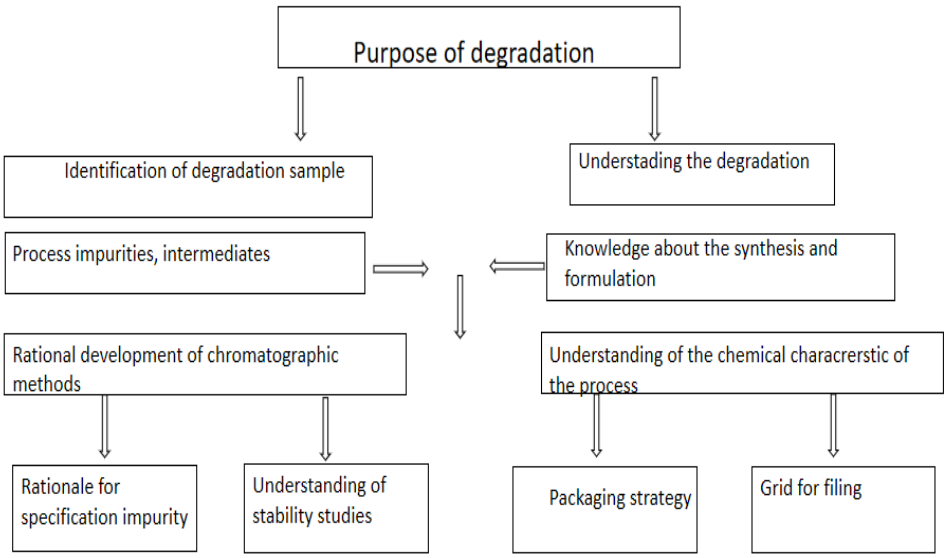

Fig. 1: Purpose of degradation study.

Types of Stability Studies

I. Long-Term Stability
II. Intermediate Stability
III. Accelerated Stability

I. Long-Term Stability

- Studies on stability during the designated shelf life or retest time under suggested storage conditions.
- In the worst-case conditions for humidity and temperature, this means storing the medication in cabinets or areas with regulated temperatures.
- Any procedure should be developed to ensure that the product maintains an acceptable level of quality for the duration of its indicated shelf life under the prescribed storage circumstances at the time it is marked. • Samples are regularly withdrawn and evaluated over a minimum of 12 months.

II. Intermediate Stability

- Studies conducted at 30°C and 65% relative humidity to somewhat accelerate the pace of physical changes or chemical degradation for a drug substance or drug product meant for long-term usage at 25°C.
- Research was done in case the expedited trials didn't work, and the temperature and humidity were between long-term and accelerated.

II. Accelerated Stability

- Research aimed at utilizing inflated storage circumstances as part of an officially established storage program to hasten the physical or chemical degradation of active pharmaceutical goods, medical equipment, or pharmaceutical products (i.e., drug products).
- A stability study may be performed to determine the shelf life of a product by accelerating the rate of breakdown, ideally by increasing the temperature of the reaction.
- These designs are experimental.
- Stability is the ability of a pharmacological product to retain its potency in a certain container closing mechanism.
- By the end of its shelf life, the drug's concentration should have decreased to 90% of its starting point.

Stability of a pharmaceutical ingredient depends on many factors which majorly include the following factors

Temperature: Drug degradation is accelerated by high temperatures that speed up oxidation, reduction, and hydrolysis reactions.

pH: The pace at which the majority of medications decompose is influenced by acidic and alkaline pH. Drug degradation rises in proportion to the degree of ionization.

Moisture: The deterioration process is catalyzed by water.

Light: Energy or thermal heat that causes substance oxidation has an impact on medicine stability.

Drug Incompatibility: Certain additional ingredients that are included in the final pharmaceutical medication product may exhibit interactions with one another or with the container's lid.

Method for choosing degradation conditions

In order to provide representative samples for the creation of stability-indicating procedures for pharmaceutical ingredients and therapeutic products, forced degradation is employed. The chosen stress parameters ought to align with the breakdown of the product under normal manufacturing, storage, and user conditions, as detailed in each scenario. Scheme 1 illustrates a standard procedure for drug substance and drug product degradation conditions. Heat degradation, photolysis, oxidation, and acid and base hydrolysis are the bare minimum of stress factors recommended for forced degradation investigations, whereas shear[21] and freeze-thaw cycles, as seen in Figure No. 2, are optional. The pH, temperature, and particular oxidizing agents that must be used are not specified in the regulatory criteria. Although Q1B stipulates that the light source must generate mixed visible and ultraviolet (UV, 320–400 nm) outputs and that exposure levels must be justified, the applicant retains discretion over the design of photolysis experiments. Finding the factors that reduce the drug's efficacy by about 10% should be the aim of the first investigation. Table 1 lists some of the scenarios that are commonly utilized for research into forced deterioration.

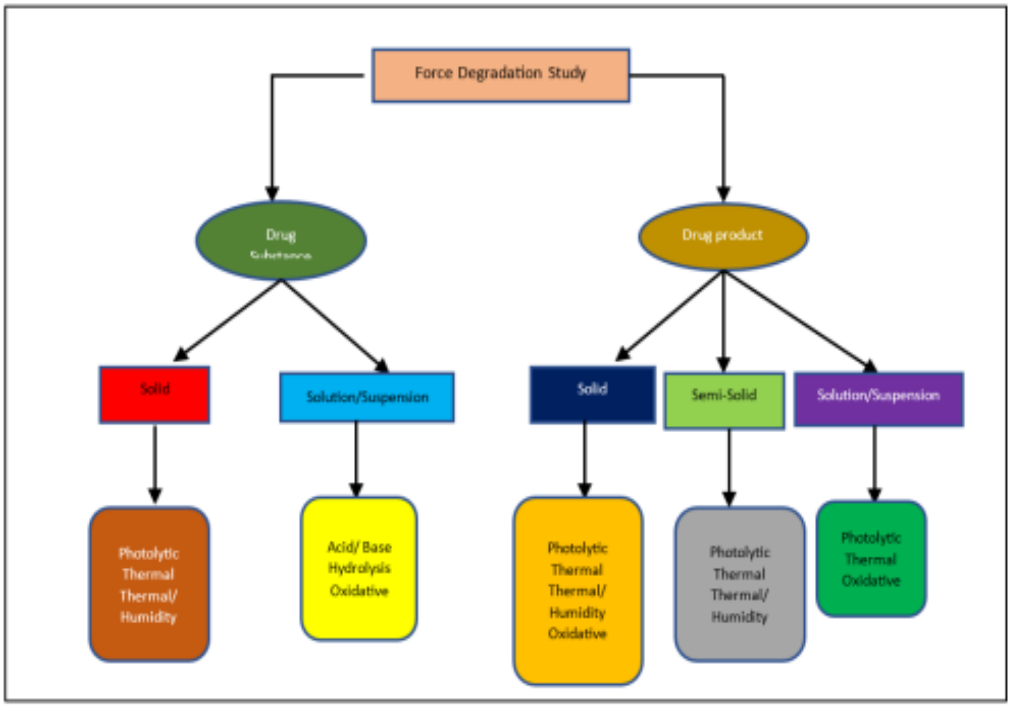

Fig 2. Force Degradation Study.

Table 1: Conditions mostly used for forced degradation studies.[22]

DEGRADATION TYPE	EXPERIMENTAL CONDITIONS	STORAGE CONDITIONS	SAMPLING TIME(DAYS)
Hydrolysis	Control (no acid or base)	40° C,60 °C	1,3,5
	0.1M sodium hydroxide	40°C,60°C	1,3,5
	0.1Mhydrochloric acid	40°C,60°C	1,3,5
	Acid controle (no API)	40°C,60°C	1,3,5
	Base controle (no API)	40°C,60°C	1,3,5
	pH-2,4,6,8	40°C,60°C	1,3,5
Oxidation	3%H2O2	25°C,60°C	1,3,5
	Peroxide control Azobisisobutyronitrile	25°C,60°C	1,3,5
	(AIBN)	40°C,60°C	1,3,5
	AIBN control	40°C,60°C	1,3,5
Photolytic	Light 1 ICH	Not applicable	1,3,5
	Light 3 ICH	Not applicable	1,3,5
	Light control	Not applicable	1,3,5
Thermal	Heat chamber	60°C	1,3,5
	Heat chamber	60°C/75%RH	1,3,5
	Heat chamber	80°C	1,3,5
	Heat chamber	80°C /75%RH	1,3,5
	Heat control	Room temperature	1,3,5 (22)

Conditions of Degradation

1. Hydrolysis: When a material interacts with water, it undergoes a chemical process called hydrolysis, often referred to as neutral deterioration. In Figure 3, the procedure is shown. Hydrolytic studies in basic and acidic environments cause the ionizable functional groups of the molecule to catalyze. Acid or base stress testing is the process of putting a pharmaceutical substance through an acidic or basic environment to force disintegration that results in the production of major degradants at an optimal level. Different kinds and amounts of bases or acids should be employed, depending on how stable the drug substance is. As seen in Fig. 4, sodium hydroxide or potassium hydroxide (0.1-1 M) are recommended for base hydrolysis, whereas sulfuric acid or hydrochloric acid (0.1-1 M) are recommended as suitable reagents for acid hydrolysis.[23]

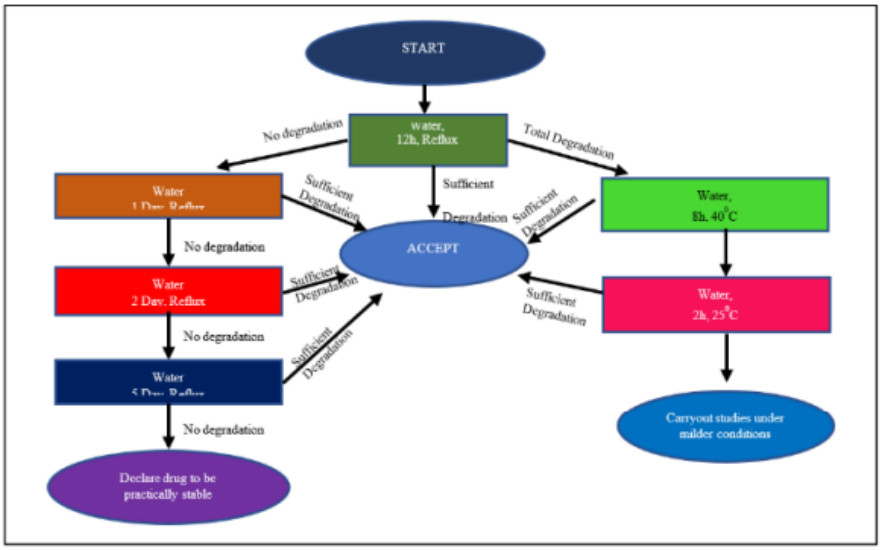

Fig 3. Hydrolytic Degradation

2. Oxidative degradation: Many chemical compounds undergo autoxidation, or oxidation under normal storage conditions, which employs ground-state elemental oxygen. It is hence a crucial route for the degradation of many medications. A free radical initiator is necessary for the free radical reaction known as autoxidation to occur. Metal ions, hydrogen peroxide, or trace contaminants in a pharmaceutical medication can all initiate autoxidation.[24] The pharmaceutical substance dictates the type of oxidizing agent to be used, as well as its concentration and exposure to the environment. Some hypotheses suggest that exposing the solutions to 0.1–3% hydrogen peroxide at neutral pH for seven days might provide beneficial degradation products, with Fig. 5 showing a maximum degradation of 20%.[25]

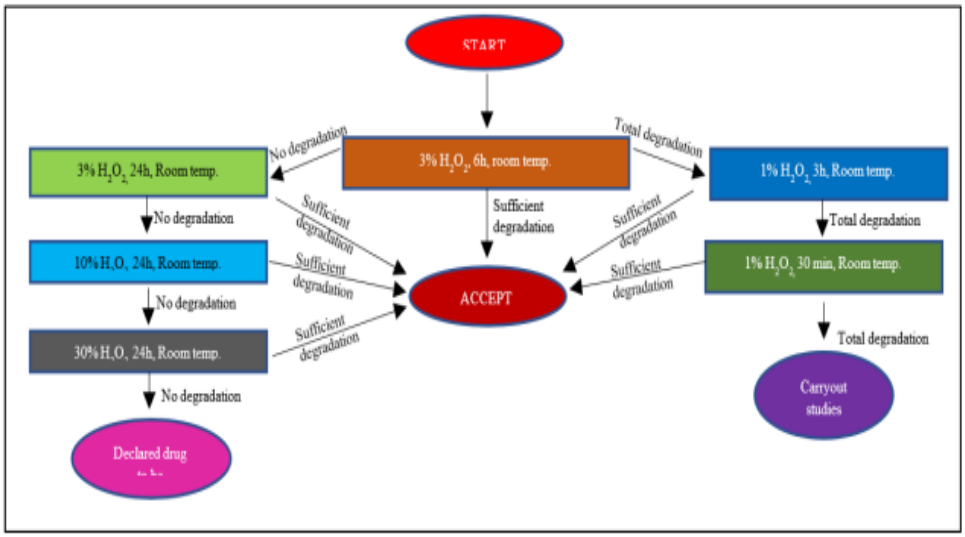

Fig 5. Oxidation Degradation.

3. Photolytic degradation: Samples must be exposed to light that has a spectral distribution of 320–400nm, an integrated near ultraviolet energy of at least 200 watt hours/square meter, and an overall illumination of at least 1.2 million lux hours in order to allow for direct comparisons between the drug substance and drug product. Samples may be exposed side by side using a validated chemical actinometric system, for the appropriate amount of time when conditions have been tracked using calibrated radiometers/lux meters, or both, to confirm that the intended light exposure is generated. Benzylic, carbon, allylic, and tertiary carbons are examples of functional groups with labile hydrogens that can be oxidized to produce hydroperoxides, hydroxides, and ketones. Both oxidative and nonoxidative photolytic mechanisms can lead to photolytic destruction, as illustrated in Fig. 6.[26]

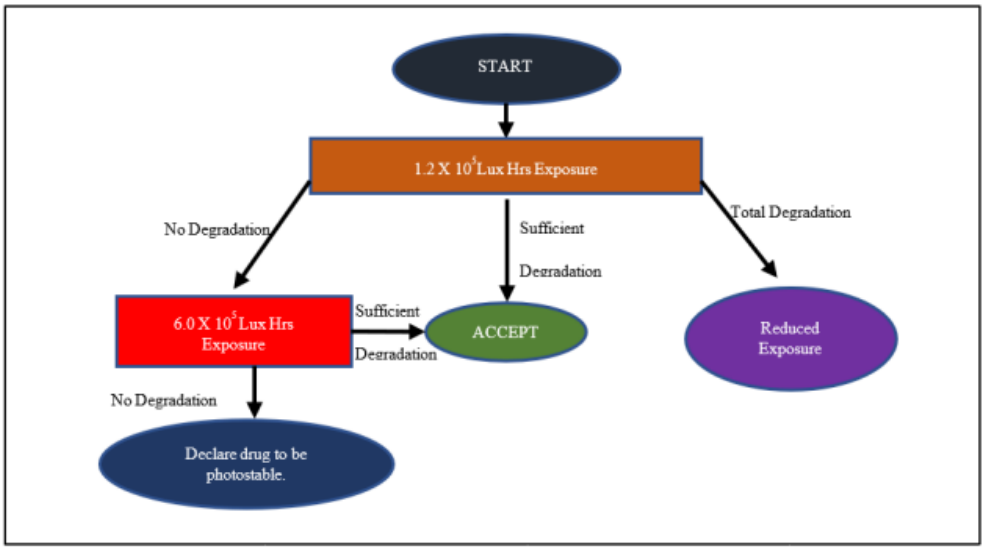

Fig 6. Photo Degradation

4. Thermal condition: The recommended accelerated ICH Q1A testing conditions should not be used for testing for thermal deterioration (such as dry heat and wet heat). Samples of both dry and wet heat should be applied to solidstate medication constituents and drug formulations. It is best to heat liquid medications until they reach a dry temperature. At greater temperatures, studies might be carried out for shorter periods of time. The Arrhenius equation can be used to ascertain if temperature affects a substance's ability to tolerate heat. The symbol k represents the rate of a certain reaction, where A is the frequency factor, Ea is the activation energy, R is the gas constant (1.987 cal/deg mol), and T is the absolute temperature. Investigations on thermal degradation are conducted between 40 and 800 C.[27]

Degradation limits

The amount of decomposition needed has been a topic of significant debate among experts in the pharmaceutical sector. It has been established that drug component degradation of 5% to 20% is appropriate for chromatographic test validation. For tiny pharmaceutical compounds, whose accepted stability limitations are usually 90% of the label claim, some pharmaceutical professionals believe that 10% degradation is ideal for use in analytical validation. Forcible deterioration may not necessarily result in a degradation product.

If no deterioration is seen following exposure to stress conditions other than those outlined in an accelerated stability protocol, the inquiry may be closed.[28] This demonstrates the stability of the chemical under investigation. While under-stressing a sample might provide inadequate degradation products, over-stressing a sample could produce a secondary degradation product that would not be detected in formal shelf-life stability testing.[29] Due to differences in matrices and concentrations, several methods may be used to create product-related degradation for drug substances and drug products. It is recommended that stressed samples be maintained in solution for a maximum of 14 days (or, for oxidative tests, for a maximum of 24 hours) in order to develop the procedure.[30]

Hyphenated analytical instruments for the detection and isolation of degradants

1.GC-MS: The first hyphenated approach, is still crucial for verifying that samples[31-34] include organic volatile IMPs and leftover solvents. However, the properties of analytes required for GC-MS, such as volatility and thermal stability, are unknown in advance for the vast majority of organic contaminants and degradants. The application of this technique in the characterisation of pollutants that are pertinent to the pharmaceutical business is therefore relatively seldom documented in the literature.

2. LC-MS: Of all the hyphenated techniques for characterizing impurities, LC-MS and its variants are the most widely utilized because, even by themselves, they may yield structural data that is almost definitive. The spectrum of sophisticated instruments with LC-MS is as follows.[35]

3. CE-MS, or capillary electrophoresis-mass spectrometry: Capillary electrophoresis (CE) and capillary electrochromatography (CEC) are important orthogonal methods for the separation of contaminants and degradation products.[36] CEC is a hybrid method that combines the high efficiency of CE with the mobile and stationary phase selectivity of LC. Systems where CE and CEC are hyphenated with MS are progressively becoming more important for the characterization of degradants, even though they are still in the exploratory stage and restricted to the development of separation techniques, the study of the utility of various CE modes, and the evaluation of the benefit of various types of mass spectrometers for the purpose.[37]

4. Liquid Chromatography-Nuclear Magnetic Resonance (LC-NMR): In 1978, the first paper was published on the relationship between LC effluent and NMR. Numerous tools have now been installed in both academic and industry laboratories. Cryoprobes, microprobes, and high-field magnets are among the technological advancements associated with modern LCNMR systems that improve instrument sensitivity and resolution. As LC attachments, magnets with field strengths of 500 MHz and greater are commonly utilized. The flow-through microprobes can be made with different inner diameters to handle different amounts of LC samples. Cryogenic chilling enables the detection of sub-microgram quantities since reducing the temperature accelerates the reaction.[38]

5. LC- FTIR: LC-FTIR, a hyphenated technique, plays a crucial role in forced degradation studies by separating drug degradation products using liquid chromatography (LC) and then analyzing their structures with Fourier-transform infrared (FTIR) spectroscopy, providing detailed chemical information that helps to identify the products and elucidate the complete degradation pathway of a drug molecule.

CONLUSION

Hyphenated approaches combine the advantages of separation and identification procedures, they have become essential tools in contemporary chemical and pharmaceutical investigation. While spectroscopic techniques like MS, NMR, and IR offer very sensitive and selective structural information, chromatographic techniques like HPLC, GC, and CE guarantee the effective separation of complicated mixtures into their pure components. Combining these techniques into a single analytical platform improves analysis speed, accuracy, and reproducibility while minimizing operator involvement and sample handling. Hirschfeld initially proposed the idea of "hyphenation," which has transformed analytical sciences by making it possible to characterize isolated analytes in real time and online.

Numerous fields, including drug development, stability research, impurity profiling, environmental monitoring, and metabolomics, can benefit from the use of these integrated systems. Crucially, they tackle the drawbacks of particular methods, such low sensitivity, insufficient selectivity, or the incapacity to completely describe intricate samples.

Hyphenated procedures are very useful in the pharmaceutical business for stability testing and forced degradation, which guarantee medication safety and regulatory compliance. Additionally, they are vital in quality control, biomarker identification, and natural product analysis, where high accuracy and dependability are critical. All things considered, hyphenated approaches are a strong and adaptable analytical method that keeps developing as instruments and data processing improve. They are essential to contemporary analytical chemistry due to their capacity to provide thorough information from a single experiment, guaranteeing their ongoing importance in the fields of research, development, and regulatory sciences.

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