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Current Insights and Analytical Advances in Forced Degradation and Impurity Profiling: A Comprehensive Review

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Abstract:

Impurities, particularly those linked to the active pharmaceutical ingredient (API), can compromise a drug's quality, safety, and efficacy by inducing deterioration or interactions. The effective mitigation and control of impurities in pharmaceuticals hinge on establishing safety-based limits for these impurities. Conducting a forced degradation study is a crucial component in the evaluation of degradation impurities that complies with regulatory standards for both drug substances and products. This process is formalized as a regulatory requirement in the ICH Guideline. It is very significant to characterize the sources of impurities prior to the delving into subsequent processes like analytical techniques and acceptance criteria, This characterization ensures the formulation of regulatory requirements and management plans that can be defined and adhered to. Additionally, it scrutinizes strategies for the identification, control, and determination of impurities in drug substances. Analytical techniques such as HPLC and LC-MS/MS enable the quantification of impurities at trace levels. Ultimately, the study underscores the significance of impurity analysis using HPLC along with LCMS application for elucidating the structure of unknown stress degradation compounds.

Key words: Impurity profiling, HPLC, LCMS, degradation impurities, Characterization, Analytical method development

1. Introduction to Pharmaceutical impurities:

The primary objective of the pharmaceutical industry is to safeguard public health by ensuring patients have access to appropriate medicines [1] with the correct dosage and efficacy at an affordable cost. Consequently, the safety and efficacy of pharmaceuticals are pivotal considerations in drug therapy [2]. The safety of a drug product relies on the toxicological properties not only of the active drug substance but also on the impurities it contains [3, 4].

Pharmaceutical impurities are undesired substances that can be present in drug formulations and can originate from various sources during the drug development and manufacturing process [5]. Effective control and monitoring of impurities are essential to ensure the safety, efficacy, and quality of pharmaceutical products [6].

1.1 Classification of Pharmaceutical Impurities:

1.1.1. Organic Impurities:

Organic impurities represent a class of unintended substances that can be present in pharmaceuticals, arising from various stages of drug development and manufacturing processes. Understanding and controlling these impurities are crucial for ensuring the safety, efficacy, and overall quality of pharmaceutical products.

Types of Organic Impurities:

Organic impurities encompass diverse categories, including starting materials, intermediates, degradation products, and residual reagents or catalysts. Starting materials and intermediates can persist through synthesis, while degradation products may form during manufacturing or storage, potentially compromising the stability of the active pharmaceutical ingredient (API) [7]. Residual reagents and catalysts from the synthesis process pose additional challenges, as they may have adverse toxicological effects.

1.1.2. Inorganic Impurities:

Inorganic impurities constitute a category of unintentional substances that may be present in pharmaceutical products [8]. These impurities can originate from various sources during the drug development and manufacturing processes, and their control is vital to ensuring the safety, efficacy, and quality of pharmaceuticals [9].

***Types of Inorganic Impurities*:**

Inorganic impurities encompass a range of substances, with notable examples including heavy metals such as lead, mercury, arsenic, and cadmium. These impurities can find their way into pharmaceuticals through raw materials, catalysts, or other processes during manufacturing [10].

1.1.3. Residual Solvents:

Residual solvents refer to unintended substances that may persist in pharmaceutical products as remnants from the manufacturing process [11]. Managing and controlling residual solvents are critical aspects of ensuring the safety, efficacy, and overall quality of pharmaceuticals.

***Classes of Residual Solvents*:**

Residual solvents are typically categorized into three classes based on potential health risks. They are

Class 1: Includes solvents with known carcinogenic or toxic properties.
Class 2: Consists of solvents with low to moderate toxicity levels.
Class 3: Encompasses solvents with low toxic potential.

***1.1.4. Isomeric and Chiral Impurities*:**

Isomeric and chiral impurities represent specialized categories of unintended substances in pharmaceuticals, often requiring precise control due to their potential impact on drug efficacy and safety. Understanding and managing these impurities are critical for ensuring the quality of pharmaceutical products [12].

1.2 Degradation impurities:

Degradation impurities, also known as degradation products, are substances that are formed during the degradation of a drug substance or drug product. These impurities can arise due to various factors, including exposure to environmental conditions such as light, heat, humidity, or chemical reactions over time [13]. The presence of degradation impurities in pharmaceuticals can impact the safety, efficacy, and overall quality of the product. The common types of degradation impurities include

Oxidation Products:

Oxidation products are a class of degradation impurities that can emerge during the exposure of pharmaceuticals to oxygen. This chemical reaction, known as oxidation, can significantly impact the safety, efficacy, and stability of drug substances and products. Understanding the formation, impact, and control of oxidation products is crucial in pharmaceutical development [14].

***Hydrolysis Products*:**

These are formed through the reaction of the drug with water, resulting in the cleavage of chemical bonds. Hydrolysis products can include acids or bases depending on the reaction conditions [15].

***Photo-degradation Products*:**

These are arising from the exposure of the drug substance to light. Light-induced reactions can lead to the formation of new compounds with altered chemical structures [16].

***Thermal Degradation Products*:**

These are occurring due to the exposure of the drug substance to elevated temperatures. High temperatures can induce chemical reactions that result in the formation of new compounds [17].

The representative image visualizing the formation of degradation impurities in any pharmaceutical product was presented in figure 1.

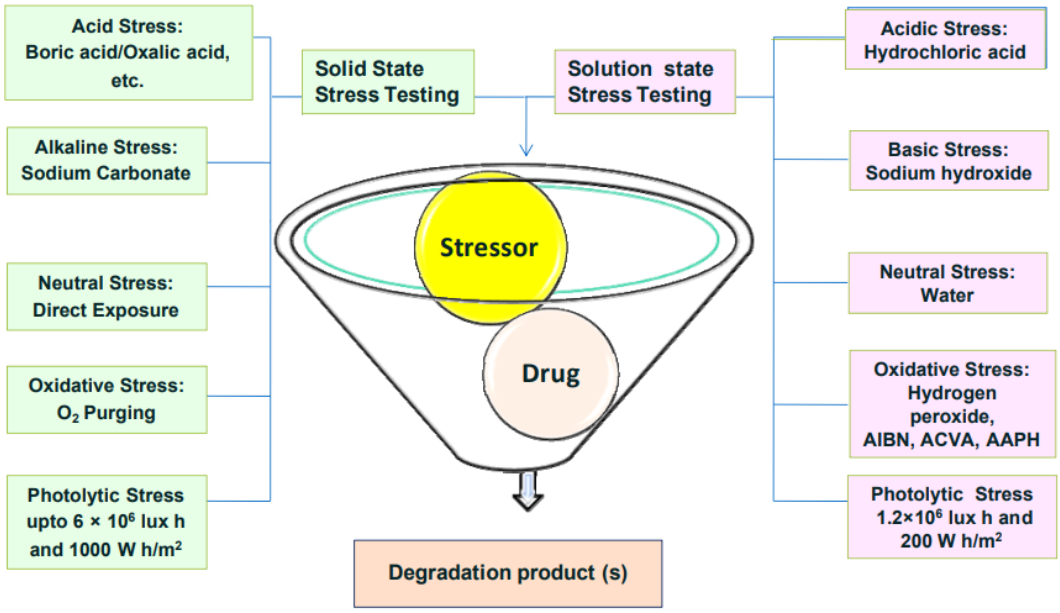


Figure 1: Representative image showing the formation of degradation impurities in a pharmaceutical product

2. Qualification of impurities:

Qualification involves obtaining and assessing data to establish the biological safety of a specific impurity or a designated impurity profile at the relevant level(s). It is advisable for applicants to offer a justification for setting impurity acceptance criteria, encompassing safety considerations when applicable [18].

An impurity is deemed qualified under the following circumstances:

- If the observed level and proposed acceptance criterion for the impurity are within the range observed in an FDA-approved human drug product.
- When the impurity functions as a significant metabolite of the drug substance.
- If the observed level and the proposed acceptance criterion for the impurity are scientifically justified by existing literature.
- When the observed level and proposed acceptance criterion for the impurity are below the level adequately assessed in comparative in vitro genotoxicity studies.

Recommended qualification thresholds, based on the maximum daily dose (outlined in Table No. 1 for drug substance and Table No. 2 for drug product), can be found in ICH Q3A [19] and ICH Q3B [20]. Table 1: Drug substance impurities thresholds

Maximum daily dose	Threshold Reported[a,b]	Threshold Identified[a,b]	Qualification threshold[a,b]
≤ 2g/day	0.05%	0.10% or 1.0 mg/day intake (whichever is less)	0.15% or 1.0 mg/day intake (whichever is less)
≥ 2g/day	0.03%	0.05%	0.05%

a Higher reporting threshold should be scientifically justified.

b Lower threshold can be appropriate if the impurities are unusually toxic.

Table 2: Thresholds for degradation products in drug products

Daily dose[a]	**Threshold Reported[a,b]**
≤1 g >1 g	0.1% 0.05%
Daily dose[a]	**Threshold Identified[a,b]**
<1 mg 1 mg–10 mg >10 mg–2 g >2 g	1.0% or 5 µg TDI, whichever is lower 0.5% or 20 µg TDI, whichever is lower 0.2% or 2 mg TDI, whichever is lower 0.10%
Daily dose[a]	**Qualification threshold[a,b]**
<10 mg 10 mg–100 mg >100 mg–2 g >2 g	1.0% or 50 µg TDI, whichever is lower 0.5% or 200 µg TDI, whichever is lower 0. 2% or 3 mg TDI, whichever is lower 0.15%

a Thresholds for degradation products are expressed either as a percentage of the drug substance or as total daily intake (TDI) of the degradation product. Lower thresholds can be appropriate if the degradation product is unusually toxic.

b Higher thresholds should be scientifically justified

Table 2: Thresholds for degradation products in drug products

3. Significance of Impurity Analysis in Pharmaceuticals:

The pharmaceutical industry is undergoing rapid expansion in the quest to discover and develop new drugs, whether derived from natural sources or synthesized through chemical processes. Amidst this evolution, a consistent principle endures: the imperative for pharmaceutical products to attain the highest possible purity. Purity has maintained its paramount status as a critical criterion ensuring the quality of medications. It is essential to acknowledge that no medication can be deemed entirely risk-free, and even a minute dosage may yield fatal consequences. The historical trajectory, from the era of Paracelsus in the first half of the 16th century to the groundbreaking work of Ehrlich, reflects a shift from the use of natural substances in their entirety to the utilization of pure extracts or synthetic compounds. Ehrlich, notably awarded the Nobel Prize in 1909, pioneered significant advancements in pharmaceutical research during the early 20th century. In the preceding century, heightened attention has been directed towards scrutinizing and evaluating the purity of natural substances. Researchers have challenged the notion that apparent quantities, measured by weight, truly represent virtual quantities once impurity alloys are considered. This heightened scrutiny underscores the evident progress in pharmaceutical research over the past century, contributing significantly to the enhancement of human health and overall quality of life [21].

Drug regulatory bodies are increasingly focusing on pharmaceutical contaminants falling within the 0.01% to 0.1% range. The term "impurities" refers to undesired substances that can be found in active pharmaceutical ingredients (APIs), emerge during the formulation process, or result as byproducts of API degradation or the API's use in drug manufacturing. The presence of these unwanted substances, even in trace amounts, poses a potential risk to the effectiveness and safety of pharmaceutical products [22].

The impurity profile of a drug substance is influenced by a multitude of variables, encompassing the quality of raw materials, reagents, and solvents utilized in synthesis, the conditions of the reactions, the purification steps employed, and the duration of storage for the drug substance. Even minor alterations in these factors can exert a significant impact on the

distribution of impurities. Detection of impurities is imperative in drug material produced in developmental and commercial batches, as well as under stress conditions. When contaminants are identified at concentrations of 0.1% or higher, or in specific instances 0.2% or higher relative to the prescribed daily dose, their structures need to be elucidated. Once identified, these impurity structures are synthesized. To facilitate selective analytical quantification in drug material and/or products, a reference impurity standard is established, enabling the development of a precise analytical procedure [23].

For regulatory purposes, API impurities can be categorized into three groups: "organic impurities, inorganic impurities, and residual solvents." Organic impurities may originate from various sources, including raw materials (often isomeric impurities), synthetic intermediates (resulting from incomplete reactions or the use of excess reagents), byproducts, degradation products, reagents, ligands, and catalysts. While these compounds, ligands, and catalysts are typically not present, their occasional occurrence can pose challenges as contaminants in APIs. Pharmaceutical products may contain inorganic impurities such as "equipment residues, reagents, catalysts, heavy metals, drying agents, and filter aids." Despite the infrequency of impurities from reagents, ligands, and catalysts, their presence can still present challenges if manufacturers are not vigilant. The primary sources of heavy metal contamination are the water used in operations and the reactors, especially when stainless steel reactors are employed for processes involving acidification or acid hydrolysis [24].

The advent of recommendations from ICH, US-FDA, WHO, and the European Committee for Directorates has markedly heightened the necessity for the establishment of a stability-indicating assay. These recommendations explicitly mandate the separation of the drug ingredient from degradation products. The guidelines prescribe the execution of forced decomposition experiments encompassing various conditions such as pH variations, exposure to light, oxidation, dry heat, among others. A stability-indicating test is precisely defined as "a validated quantitative analytical procedure capable of discerning changes over time in the pertinent properties of the drug substance and drug product." Typically, the validation process for a stability-indicating assay comprises two distinct phases [25].

The examination of drug material involves a thorough investigation of forced breakdown, and a stability-indicating test is crafted based on insights into the drug's degradation behavior. During this phase, the primary objective of validation is to establish specificity and selectivity, followed by the incorporation of additional criteria such as accuracy, precision, linearity, range, and robustness. This established technique is then employed to analyze stability samples, determining the retest or expiration date of bulk medicine. When the stability-indicating test is applied to diverse matrices, such as formulations, the focus shifts to demonstrating the assay's applicability in the presence of excipients or other components. In this scenario, key metrics including specificity, selectivity, accuracy, and precision undergo revalidation. ICH has standardized necessary conditions through two recommendations. The first offers a concise overview and definition of validation criteria for various testing methodologies, while the second delves deeper, incorporating essential experimental data and statistical interpretation. These recommendations serve as a cornerstone for regulatory agencies and industries globally, emphasizing the imperative of accurate validation for all stakeholders involved in the submission process [26].

4. Significance of Impurity Profiling through Contemporary Analytical Methods:

In contemporary times, the pharmaceutical industry stands as a vital component of the global economy, wielding a profound impact on the daily lives of people worldwide. A substantial portion of the industry's investments is directed towards Research and Development (R&D) for innovative medication compounds, a process that demands over $1 billion and 10-15 years for the creation of a new medicinal compound. The past decade has witnessed the authorization of approximately 280 therapeutic novel molecular entities by the FDA (2010-2020), underscoring the paramount importance of regulators in overseeing the quality, safety, and efficacy of these medications. To address the evolving challenges, there has been a surge in the utilization of analytics in the drug development process. This has prompted continuous technological advancements to meet the demands of ensuring medication quality. Presently, pharmaceutical analysis plays a pivotal role in providing dependable data for diverse purposes, including research on medication safety, facilitating development and monitoring processes, and ensuring post-market regulatory compliance [27]. Figure 2 illustrates the framework for estimating impurities in APIs.

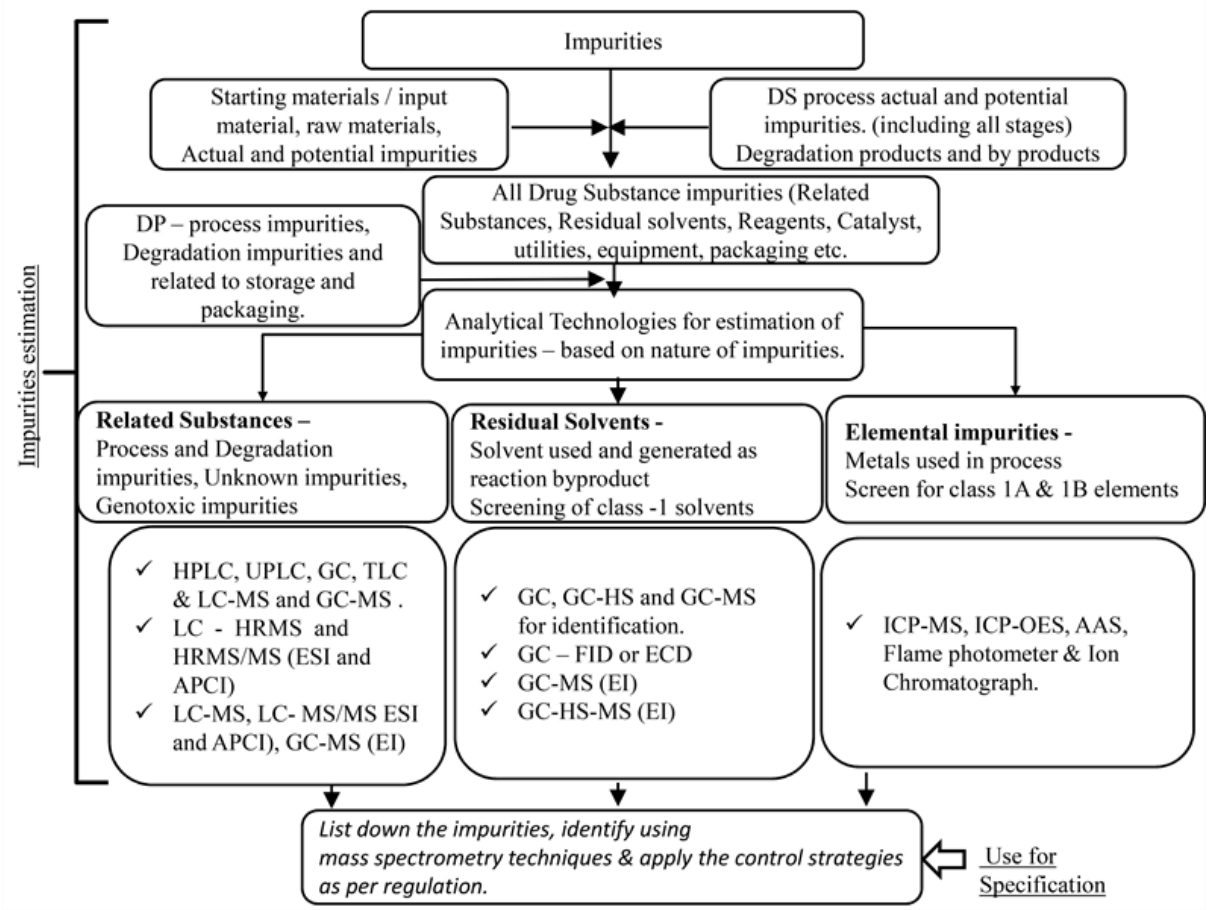


Figure 2: Framework for estimating impurities in APIs

The pressing need to establish an analytical technique for a newly introduced medicinal ingredient or formulation stands out as a critical concern. Various analytical methods, including titrimetric, spectrophotometric, qualitative, and activity procedures, are employed to ensure the adherence to quality standards in drugs. The realms of artificial, medical specialty, pharmaceutical, and clinical analysis heavily rely on the analytical insights derived from pharmaceutical research. This reliance fosters the development of highly cost-effective medication treatments. The absence of suitable analytical methods would render the measurement of crucial elements such as impurity levels, degradation products, or medication dosages impossible. These contaminants significantly contribute to the subpar quality of drugs in the current market. Therefore, in the creation of new medicines, the identification and characterization of potential contaminants become imperative. Spectroscopic tests, including NMR, IR, and MS, play a crucial role in characterizing the structure of contaminants or degradation products present within the medication constituents [28].

Impurities should be rigorously assessed, even when present at levels of 0.1% or higher. Recognizing the pharmaceutical industry's growing demand for advanced analytical techniques, the focus of this review was selected to address these requirements. Numerous contemporary analytical methods, including UPLC, LC-MS, LC-Q-TOF, GCMS, HPTLC, and LC-NMR, were cited among others. The discussion encompassed a range of topics, including impurity sources, types, management strategies, identification, regulatory considerations, degradation products, and stability-indicating assay methods [29].

4.1 Significance of HPLC in Pharmaceutical Impurity Analysis:

HPLC is a powerful analytical technique widely employed in the pharmaceutical industry for the analysis of impurities in drug substances and formulations. Its significance lies in its ability to separate, identify, and quantify various components in complex mixtures with high precision and sensitivity. In the context of pharmaceutical impurity analysis, HPLC plays a pivotal role in ensuring the quality, safety, and efficacy of drugs [30, 31]. The key futures of HPLC includes

***High Resolution and Sensitivity*:**

HPLC offers exceptional resolution, enabling the separation of closely related impurities. This high resolution is crucial for identifying and quantifying impurities, even at low concentrations. The sensitivity of HPLC allows for the detection of impurities in trace amounts, ensuring a comprehensive analysis.

***Versatility*:**

HPLC is versatile and can be applied to a diverse range of compounds, including polar and non-polar substances. This versatility is essential in pharmaceutical impurity analysis, where impurities can vary widely in their chemical nature.

***Quantitative Analysis*:**

HPLC facilitates accurate and reproducible quantification of impurities. Precise measurement of impurity levels is crucial for assessing the quality of drug substances and formulations, aligning with regulatory requirements.

***Speed and Efficiency*:**

HPLC provides rapid analysis, allowing for the quick assessment of impurity profiles. This efficiency is vital for pharmaceutical companies to streamline quality control processes and expedite decision-making in drug development.

***Stability-Indicating Method*:**

HPLC methods can be designed to be stability-indicating, meaning they can effectively separate and quantify impurities generated during the drug's shelf life. This is essential for ensuring the stability and potency of pharmaceutical products over time.

***Regulatory Compliance*:**

Regulatory authorities, such as the FDA, recommend HPLC as a standard method for impurity analysis in pharmaceuticals. Adherence to these standards ensures regulatory compliance and facilitates the approval process for new drug applications.

Keep this in consideration; various authors utilized HPLC for developing stability indicating method for quantification of impurities in formulations. The summary of the methods reported during 2019-2023 were tabulated in table 3.

S No	Drug	No. of impurities studied	Stress conditions studied	No. of DPs reported	Reference
1	Cilnidipine	impurities A, and B	Acid, base, Oxidative, photolytic and thermal	6	32
2	Leflunomide	impurities A, and B	Acid, base, Oxidative, photolytic and thermal	7	33
3	Tolfenamic	impurities A, B, and C	Alkaline, Neutral, Oxidative, Thermal, Photolytic	1	34
4	Cardiazol	impurities A, and B	Acid, base, peroxide and temparature	6	35
5	pantoprazole	pantoprazole N-oxide, N-oxide of sulfone,	Oxidative, Photolytic	18	36
6	Tirofiban hydrochloride	impurities A, and C	Acid, base, hydrolysis, UV, Thermal, Oxidative	No DPs reported	37
7	darolutamide	impurities 1, 2, and 3	Acid, base, thermal, peroxide and UV	5	38

8	Eptifibatide	impurities 1, and 2	Acid, base, Oxidative, photolytic and thermal	8	39
9	Agomelatine	Agomelatine AGL-5D, Desmethyl impurity, 5th Isomer, 4D and hydroxy impurity	Acid, base, aqueous, peroxide, dry heat, humidity, UV	5	40
10	Dacomitinib	impurities 1, and 2	Acid, base, thermal, peroxide and photolytic	4	41
11	Neostigmine Methylsulfate	impurities A, B, and C	Acid, Alkali and Oxidative	5	42
12	diltiazem	EP impurity-A, B, C and E	Acid, base, thermal, peroxide, photolytic and humidity	9	43
13	Niraparib	impurity 1, Impurity 1 Acyl Glucuronide, and impurity 2	Acid, base, thermal, peroxide and UV	8	44
14	Tigecycline	impurity 1, Impurity 2	Acid, base, Oxidative, photolytic and thermal	5	45
15	Velpatasvir Copovidone	impurity 1, Impurity 2	Acid, base, Oxidative, photolytic and thermal	8	46
16	Acalabrutinib	impurities 1, 2, and 3	Acid, base, Oxidative, photolytic and thermal	7	47
17	Lamotrigine	impurities B, C, and D	Acid, base, thermal, peroxide, photolytic and humidity	6	48
18	Alpelisib	Impurity 1, 2, 3 and 4	Acid, base, peroxide, thermal, UV light	4	49
19	Brigatinib	Impurity A, B, C and D	Acid, base, peroxide, thermal, UV light	7	50
20	Gliclazide	Impurity A, C and F	Acid, base and peroxide	15	51

Table 3: Analytical HPLC stability indicating methods reported for quantification of impurities along with APIs.

5. Application of LC-MS for Characterization of Stress Degradation Products:

LCMS is a powerful analytical technique widely employed in the pharmaceutical industry for the characterization of stress degradation products. Stress testing, involving the intentional exposure of a drug substance or product to various stress conditions, is a critical step in the drug development process. LC-MS, with its ability to separate, identify, and quantify compounds, plays a crucial role in elucidating the degradation pathways and characterizing the resulting degradation products [51-54]. The key applications of LCMS include

***Identification of Degradation Products*:**

LC-MS allows for the accurate identification of degradation products formed under stress conditions. The technique provides high-resolution chromatographic separation coupled with mass spectrometric detection, enabling precise determination of the molecular weight and structure of degradation products.

***Structural Elucidation*:**

LC-MS facilitates the structural elucidation of degradation products by providing information about their elemental composition, fragmentation pattern, and possible chemical structures. This is essential for understanding the chemical transformations undergone by the drug substance under stress.

***Selective and Sensitive Detection*:**

LC-MS offers selective and sensitive detection of degradation products even at low concentrations. This is crucial for detecting trace levels of impurities, ensuring a comprehensive assessment of the drug's stability.

***Quantification of Degradation Products*:**

The quantitative capabilities of LC-MS enable the determination of the levels of degradation products. This quantitative information is vital for assessing the extent of degradation and establishing degradation kinetics.

***Comparative Analysis*:**

LC-MS allows for the comparison of stressed and unstressed samples, facilitating a comprehensive understanding of changes induced by stress conditions. Comparative analysis aids in differentiating degradation products from inherent impurities.

***Regulatory Compliance*:**

Regulatory authorities often require a thorough characterization of stress degradation products as part of the drug approval process. LC-MS, as a well-established and widely accepted technique, helps meet regulatory requirements [55].

The summary of stress degradation studies along with characterization of stress degradation products reported during 2019-2023 were tabulated in table 4.

S No	Drug	Stress conditions studied	No. of DPs characterized	Reference
1	Diclofenac sodium	Alkaline hydrolysis, Acid, Neutral, Oxidation, Photolysis	7	57
2	Fosamprenavir	Acid, base, thermal, peroxide and UV	6	58
3	Venetoclax	Acid, base, Oxidative, photolytic and thermal	3	59
4	Alectinib Hydrochloride	Acid, base, thermal, peroxide and UV	4	60
5	Bilastine	Acid, base, thermal, peroxide and UV	2	61
6	Ibrutinib	Acid and alkaline	10	62
7	Imidazole and Triazole	pH, light, oxidation, dry heat, acidic, basic, hydrolysis	4	63
8	Bedaquiline	Acid, base, peroxide, water	8	64
9	Vandetanib	acid, alkali and oxidation	2	65
10	Evodiamine	Acidic hydrolytic, basic hydrolytic, oxidative, solid photolytic, and thermal stress	1	66
11	Netarsudil	Acid, base, thermal, peroxide and UV	6	67
12	Felodipine	Acid, base, thermal, peroxide and UV, Humidity	2	68
13	Ertugliflozin	Acid, oxidative	5	69
14	Bedaquiline	Acid, base, thermal, peroxide and UV	6	70

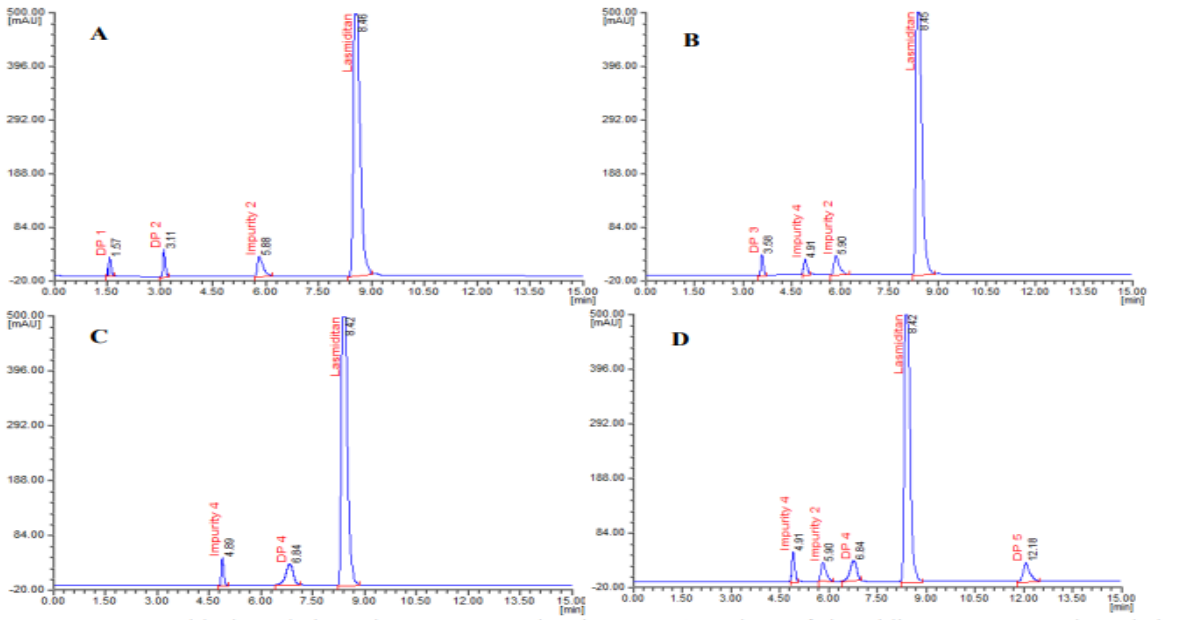
15	Dordaviprone	photolytic	4	71
16	Lasmiditan	Acid, base, thermal, peroxide and UV	5	72
17	Letermovir	Acid, base, thermal, peroxide and UV	5	73

Table 4: Studies reported during 2019-2023 for LCMS/MS characterization of stress degradation compounds

The characterization of compounds using mass fragmentation patterns is a fundamental aspect of mass spectrometry. Mass spectrometry provides information about the molecular weight and structural composition of compounds. When a sample is ionized and introduced into a mass spectrometer, the resulting mass spectra can reveal valuable insights into the chemical nature of the molecules present.

Mass fragmentation patterns occur when ions generated from the sample undergo fragmentation in the mass spectrometer. This process involves the breaking of chemical bonds within the ions, resulting in the formation of smaller fragments. The mass-to-charge ratios (m/z) of these fragments are then measured, and the pattern of fragment peaks in the mass spectrum is indicative of the compound's structure. By analyzing the mass fragmentation pattern, researchers can deduce the possible structural elements of a compound. This aids in elucidating the connectivity of atoms and the arrangement of functional groups.

In the process of identification of stress degradation compounds, researcher initially optimizes a method for resolution of degradation products along with analyte in the study. Further, the LCMS analysis of the degradation products was evaluated. The mass fragmentation spectra of the peaks representing degradation products were simulated and the interpretation of the mass fragmentation spectrum confirms the structure of that particular degradation product. The representative LCMS chromatograms of lasmiditan reported in literature [72] that shows the formation of five degradation products were presented in figure 3. Figure 4 shows the mass fragmentation spectra of five DPs reported in literature [72]. The authors [72] finalized the structure of DPs based on the mass fragmentation spectrum of DP and the reported structure of DPs was shown in figure 5.



A) Acid degradation chromatogram showing DP 1 and 2 of lasmiditan; **B)** Base degradation chromatogram showing DP 3 of lasmiditan; **C)** Peroxide degradation chromatogram showing DP 1 of lasmiditan; **D)** UV light degradation chromatogram showing DP 4 and 5 of lasmiditan

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Figure 3: Stress degradation chromatograms of lasmiditan reported in literature [72]

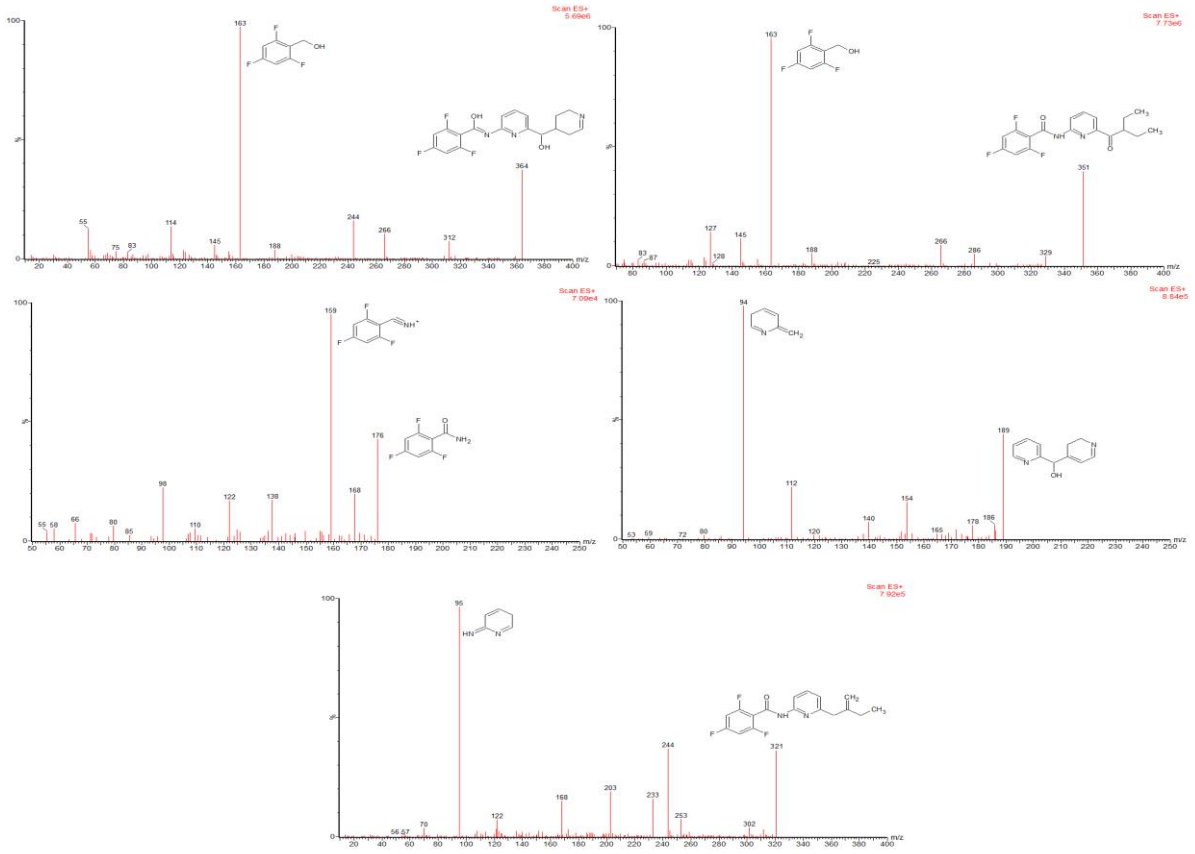

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Figure 4: Mass fragmentation spectra of degradation product 1 to 5 of lasmiditan reported in literature [72]

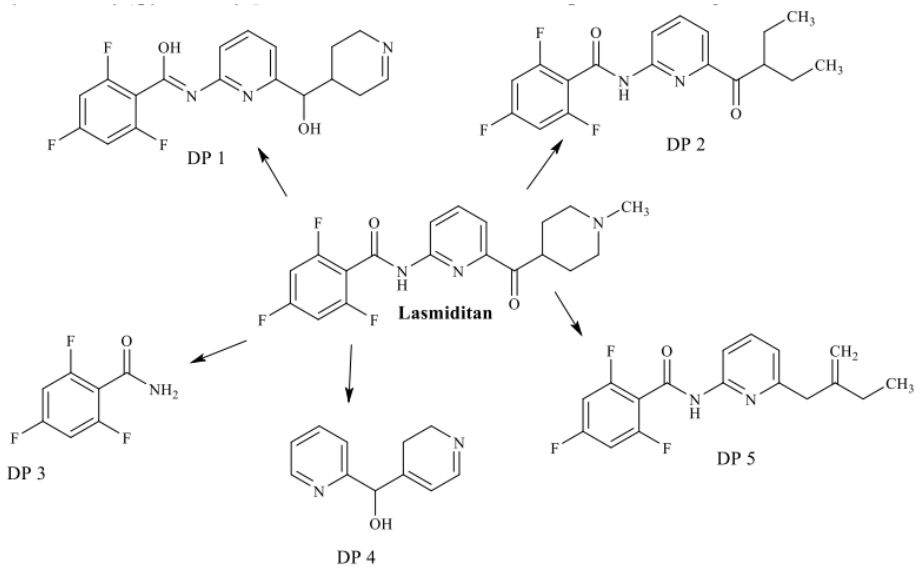

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Figure 5: Structures elucidated for degradation product 1 to 5 of lasmiditan reported in literature [72]

The structural characterization of DPs was reported based on individual fragments identified in mass spectra. The probable molecular mass and accurate mass measurements were correlated with structure of main compound and the

possible structure of each fragment along with structure of DP was proposed. Figure 5 presents the possible mechanism of formation of each mass fragment and on correlation with all these fragments, structure of DP proposed by reported by Rajesh *et al*., 2023 [73] for letermovir pure drug.

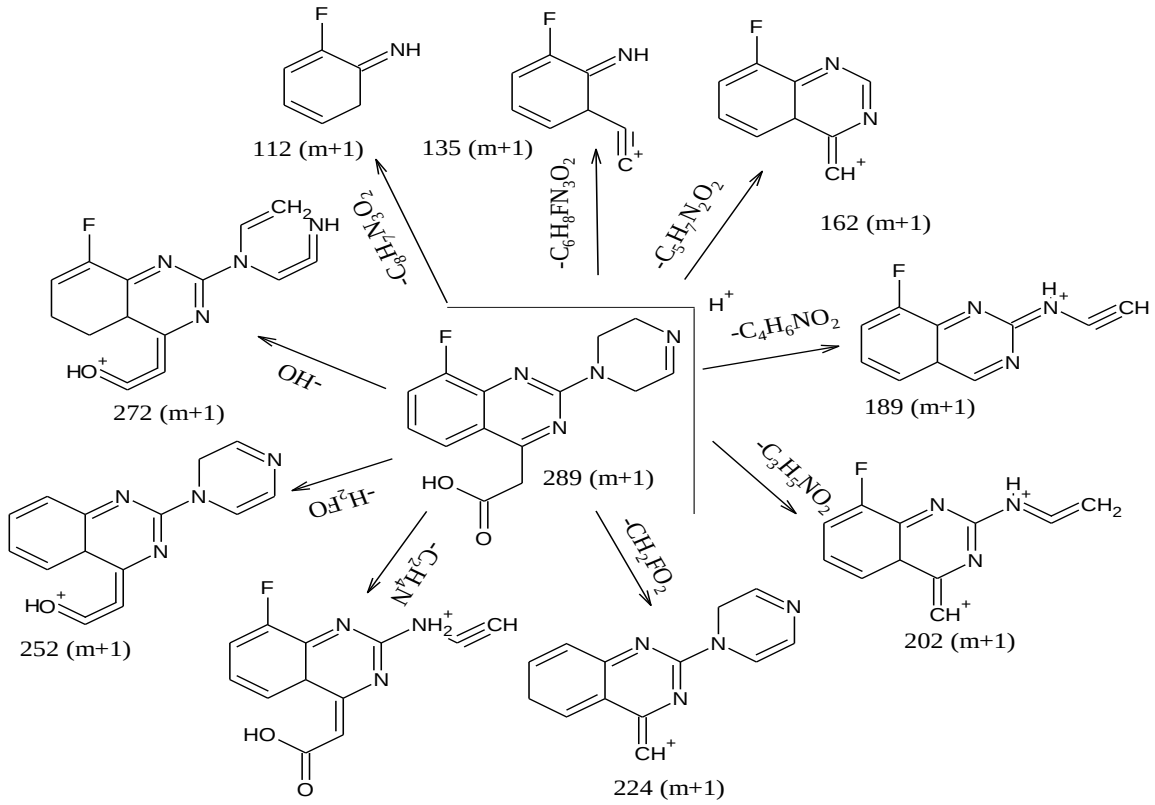

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Figure 6: Schematic representation of structural characterization of degradation products of letermovir reported by Rajesh *et al*., 2023 [73]

Limitations of the study:

LC-MS is a powerful analytical technique widely used for the characterization of degradation products in pharmaceuticals, environmental samples, and other complex mixtures. However, like any analytical technique, LC-MS has its limitations. The limitations specific to the characterization of degradation products includes

Sensitivity Issues:

Detection of low-abundance degradation products can be challenging, especially in the presence of a complex matrix. Some degradation products may be present at very low concentrations, requiring highly sensitive instrumentation.

Matrix Effects:

Complex sample matrices, such as biological fluids or environmental samples, can affect the ionization and detection of degradation products. Co-eluting matrix components may interfere with the accurate identification and quantification of degradation products.

Ionization Variability:

The efficiency of ionization can vary among different compounds. Some degradation products may ionize less efficiently than others, leading to underrepresentation or complete absence in the mass spectrum.

Chemical Diversity:

LC-MS may not be equally effective for all types of degradation products. Compounds with diverse chemical properties (polarity, volatility, etc.) may exhibit different behaviors during chromatographic separation and mass spectrometric analysis.

Resolution Limitations:

The chromatographic resolution of closely related degradation products can be challenging. Co-elution of peaks may occur, making it difficult to distinguish between structurally similar compounds.

Structural Elucidation:

While mass spectrometry provides valuable information about the mass and fragmentation patterns of compounds, it may not always provide sufficient structural information for complex degradation products. Additional techniques such as NMR spectroscopy may be required for comprehensive structural elucidation.

Stability of Degradation Products:

Some degradation products may be unstable and prone to further degradation during the analysis process, leading to inaccurate characterization or underestimation of their presence.

Sample Preparation Challenges:

The complexity of sample preparation, especially for complex matrices, can impact the efficiency and reproducibility of the analysis. Proper sample preparation is crucial for the accurate detection and characterization of degradation products.

Quantification Challenges:

Quantifying degradation products accurately can be challenging, especially without suitable reference standards. Calibration curves may not be linear across a wide concentration range, and matrix effects can impact quantification accuracy.

Economic aspects:

The economic aspects of Liquid Chromatography-Mass Spectrometry (LC-MS) characterization of degradation products are essential considerations, especially in pharmaceutical research and development. The economic aspects to be mindful of includes

Instrumentation Costs:

Acquiring and maintaining LC-MS instrumentation involves significant upfront and ongoing costs. High-quality instruments with advanced capabilities for accurate degradation product characterization may require substantial financial investment.

Consumables and Reagents:

The cost of consumables, such as columns, solvents, and reagents, can contribute significantly to the overall expense. Proper handling and disposal of these consumables also incur additional costs.

Training and Expertise:

Personnel's training is crucial for the effective use of LC-MS equipment and data interpretation. Investing in training programs for analysts to ensure proper handling of the instrumentation can impact the economic efficiency of the characterization process.

Sample Preparation Costs:

Sample preparation is a critical step in LC-MS analysis. The cost of sample preparation methods, including labor, materials, and equipment, should be considered. Efficient and cost-effective sample preparation methods can contribute to overall economic efficiency.

Analysis Time and Throughput:

The time required for LC-MS analysis influences the overall efficiency and cost-effectiveness. Improving throughput, either through the use of faster analysis methods or automation, can have economic implications, especially in high-throughput environments.

Data Analysis Software:

The use of specialized software for data analysis and interpretation may incur additional costs. Proper software licenses and updates are essential for accurate data processing and reporting.

Reference Standards and Quality Control:

The availability and cost of reference standards for degradation products are important economic considerations. Ensuring the quality control of standards and establishing validation protocols contribute to the reliability of the results.

Regulatory Compliance:

Adhering to regulatory requirements may involve additional costs for documentation, validation studies, and compliance with Good Laboratory Practices (GLP) or Good Manufacturing Practices (GMP) standards. Meeting regulatory standards is crucial, especially in the pharmaceutical industry.

Sample Storage and Stability:

Proper storage conditions for samples to maintain stability can impact costs. Some samples may require specific storage conditions, and ensuring their stability over time may involve additional resources.

Outsourcing vs. In-House Analysis:

Organizations may choose to outsource LC-MS analysis to external service providers rather than investing in in-house capabilities. The economic feasibility of outsourcing depends on factors such as sample volume, frequency of analysis, and the availability of suitable service providers.

Return on Investment (ROI):

Assessing the overall economic impact of LC-MS characterization involves evaluating the return on investment. This includes considering the value of the information obtained, the impact on decision-making in research and development, and potential cost savings in downstream processes.

Balancing the economic aspects of LC-MS characterization involves optimizing the workflow, utilizing resources efficiently, and making informed decisions based on the specific needs and goals of the analytical process. It is crucial for organizations to carefully evaluate the cost-effectiveness of their analytical strategies in the context of their overall research and development objectives

Conclusion and Recommendations:

The rapid expansion of the pharmaceutical industry is fuelled by the quest for new therapeutic agents, whether derived from natural origins or chemically synthesized pharmacological compounds. As a result, ensuring the purity and absence of impurities has been a longstanding fundamental criterion for guaranteeing the quality of medications. It is crucial to recognize that no medication can be deemed entirely safe, and even a minimal dose may lead to fatal effects. The assertion by researchers that apparent amounts, as measured by weight, do not represent virtual quantities when factoring in impurities has catalyzed a heightened interest in studying and evaluating the purity of natural substances over the past century. Undoubtedly, the remarkable progress in medical science during the last century, driven by pharmaceutical research, has significantly elevated public health and overall well-being.

Drug regulatory agencies are increasingly focusing on the detection of trace contaminants in pharmaceuticals, particularly those present in the range of 0.01% to 0.1%. Impurities in pharmaceuticals refer to unintended compounds that may originate from active APIs, emerge as by-products of the formulation process, or result from the degradation of APIs or their precursors used in drug manufacturing. The presence of even minute quantities of these undesired substances could compromise the effectiveness and safety of pharmaceutical products. To address this concern, drug

registration authorities have proposed specific methods for the detection and quantification of impurities. The impurity profile of a drug substance is influenced by various factors, including the purity of starting materials, reaction conditions, purification methods, and the duration of storage for the drug substance.

Developing an analytical method for a novel pharmaceutical ingredient or formulation is a formidable task. Various analytical techniques, such as titration, spectroscopy, qualitative testing, and activity assays, are employed to ensure that the drug adheres to the necessary quality standards. The analytical insights derived from pharmaceutical research find extensive applications in artificial, medical specialty, pharmaceutical, and clinical analyses, fostering the creation of highly cost-effective pharmaceutical solutions. Importantly, without these established analytical methods, there would be no official means of quantifying impurity levels, degradation products, or medication dosages in the event of their accumulation. The subpar quality of drugs in the current market is largely attributed to the presence of contaminants. Identifying and characterizing potential contaminants during drug development is a critical step. The structure of the actual contaminant or degradation product within the medication constituent should be elucidated through spectroscopic tests, including NMR, IR, and MS.

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