

Analytical Approaches to Mass Balance Determination During Forced Degradation Testing



Maja Hadzieva Gigovska*

Research & Development, ALKALOID AD, Blvd. Aleksandar Makedonski, North Macedonia

Submission: September 17, 2026; **Published:** September 29, 2026

***Corresponding author:** Maja Hadzieva Gigovska, Research & Development, ALKALOID AD, Blvd. Aleksandar Makedonski, North Macedonia

Abstract

Forced degradation studies constitute an essential component of pharmaceutical development, facilitating the understanding of degradation pathways, intrinsic stability characteristics, and the development of stability-indicating analytical methods. A critical aspect of these studies is the establishment of mass balance, which ensures that the amount of drug substance lost during degradation is quantitatively accounted for through the identification and measurement of degradation products. Accurate mass balance assessment enhances confidence in analytical methods and supports regulatory submissions. However, incomplete degradation product characterization, analytical limitations, and complex degradation pathways often hinder successful mass balance achievement. This review examines the principles of mass balance determination during forced degradation testing, the analytical approaches used to quantify the active pharmaceutical ingredient and its degradation products, and the practical considerations that influence the interpretation of mass balance results.

Keywords: Mass balance, Forced degradation, Stability-indicating methods, Degradation products, Pharmaceutical analysis, ICH guidelines

Abbreviations: ICH – International Council for Harmonisation, API – Active Pharmaceutical Ingredient, AQBd – Analytical Quality by Design, GMP – Good Manufacturing Practice, TLC – Thin-Layer Chromatography, GC – Gas Chromatography, HPLC – High-Performance Liquid Chromatography, UHPLC – Ultra-High-Performance Liquid Chromatography, LC-MS – Liquid Chromatography-Mass Spectrometry, HRMS – High-Resolution Mass Spectrometry, CE – Capillary Electrophoresis, SFC – Supercritical Fluid Chromatography, NMR – Nuclear Magnetic Resonance, UV – Ultraviolet, DAD – Diode-Array Detector, MS – Mass Spectrometry.

Introduction

Mass balance is a fundamental principle extensively applied in pharmaceutical development; particularly for assessing drug substance and drug product stability; as well as for evaluating degradation pathways and degradation products during stability studies.

According to the International Council for Harmonization (ICH); mass balance is defined as the process of adding the assay value and the levels of degradation products to determine whether the total approaches 100% of the initial amount of the drug substance; taking into account analytical variability and experimental error [1]. In practical terms mass balance reflects the quantitative relationship between the loss of the Active Pharmaceutical Ingredient (API) and the formation of degradation products during stability or forced degradation studies.

Mass balance serves as an important indicator of the suitability of stability-indicating analytical methods. The achievement of acceptable mass balance provides evidence that the analytical procedure is sufficiently specific and capable of detecting; separating; and quantifying both the drug substance and its degradation products. Consequently, it contributes to a better understanding of degradation pathways; facilitates the identification of potential drug-excipient interactions; and supports the overall assessment of pharmaceutical product stability. Furthermore, the concept has applications beyond stability testing; including potency assignment of reference standards; assessment of synthetic reaction efficiency; and evaluation of material losses occurring during manufacturing processes [2,3].

The conventional approach for mass balance determination is based on the simultaneous quantification of the drug substance and all detectable impurities using selective and stability-indicating analytical methods. High-Performance Liquid Chromatography (HPLC) coupled with ultraviolet detection remains the most commonly employed technique; however, advanced techniques such as Ultra-High-Performance Liquid Chromatography (UHPLC); Liquid Chromatography-Mass Spectrometry (LC-MS); High-Resolution Mass Spectrometry (HRMS); and Nuclear Magnetic Resonance (NMR) spectroscopy are increasingly utilized to improve the identification and characterization of degradation products. These techniques enhance the ability to account for API loss through comprehensive impurity profiling and degradation product analysis [4,5].

Despite its apparent simplicity, establishing satisfactory mass balance is often challenging. Numerous factors may contribute to discrepancies between the loss of the parent compound and the measured levels of degradation products. These include the formation of multiple degradation products through complex reaction pathways; incomplete detection due to loss of chromophore groups; evaporation of volatile degradation products; adsorption losses during sample preparation; insufficient chromatographic resolution; co-elution with the parent compound; unknown or inappropriate response factors; and the absence of reference standards for degradation products. In addition, analytical variability in assay determination and impurity quantification may significantly influence mass balance calculations [2,3,5].

Forced degradation studies represent one of the most important applications of mass balance assessment. These studies expose drug substances and drug products to various stress conditions; including acidic; alkaline; oxidative; thermal; and photolytic environments; to accelerate degradation processes and reveal intrinsic stability characteristics. In this context, mass balance provides valuable information regarding the completeness of degradation product characterization and the adequacy of analytical methodologies employed during pharmaceutical development. A satisfactory mass balance generally indicates that the majority of degradation pathways have been adequately elucidated and that significant degradation products have been identified and quantified.

Although the importance of mass balance has been recognized for decades; relatively few studies have systematically investigated the critical factors influencing its determination. Most published reports discuss mass balance as part of stability-indicating method development rather than as an independent analytical challenge. Consequently, there remains a need for a more comprehensive understanding of the sources of variability affecting mass balance calculations and for the development of scientifically sound approaches that improve the reliability of the obtained results.

In recent years; growing attention has been directed toward risk-based methodologies and the principles of Analytical Quality by Design (AQbD) for the evaluation of mass balance. Such approaches enable the systematic identification; assessment; and control of factors that may affect analytical performance and result reliability. Therefore, the determination of mass balance should not be regarded merely as a mathematical calculation; but rather as a multidisciplinary process requiring a thorough understanding of degradation chemistry; analytical science; statistics; and risk management principles. Systematic; science-based; and holistic approach is essential for achieving reliable mass balance data and ensuring the successful development; regulatory approval and lifecycle management of pharmaceutical products.

Concept of Mass Balance in Forced Degradation Studies

Mass balance can be calculated and expressed in different ways. The loss of the Active Pharmaceutical Ingredient (API) and the formation of degradation products may be reported either on a mass basis or in terms of the amount of substance (moles). The term mass balance implies a direct correlation between the quantity of material lost and the quantity of degradation products formed. When all starting materials and degradation products are quantitatively determined; mass balance can be assessed based on a gravimetric approach. However, when degradation results in products with significantly different molecular weights; or when certain degradation products cannot be quantified accurately; expressing mass balance in terms of the amount of substance (molar balance) may provide a more scientifically meaningful assessment.

The concept of mass balance is derived from the law of conservation of mass; one of the fundamental principles of physics and chemistry. According to this law, the total mass within a closed system remains constant over time; irrespective of the physical or chemical processes occurring within the system [6]. Consequently, in any chemical reaction occurring in a closed system; the total mass of the reactants must be equal to the total mass of the products. In contrast; apparent deviations from mass conservation may be observed in open systems; where matter or energy can enter or leave the system during the course of the reaction.

In the context of forced degradation studies, this principle forms the basis for evaluating mass balance. Ideally, the reduction in the amount of the parent drug substance should correspond to the cumulative amount of degradation products generated under the applied stress conditions. Therefore, mass balance serves as an important indicator of the completeness of degradation product detection; identification; and quantification.

For the purpose of illustrating the concept, a simplified degradation process is presented in (Figure 1) Schematic illustration of the chemical degradation reaction of the active substance

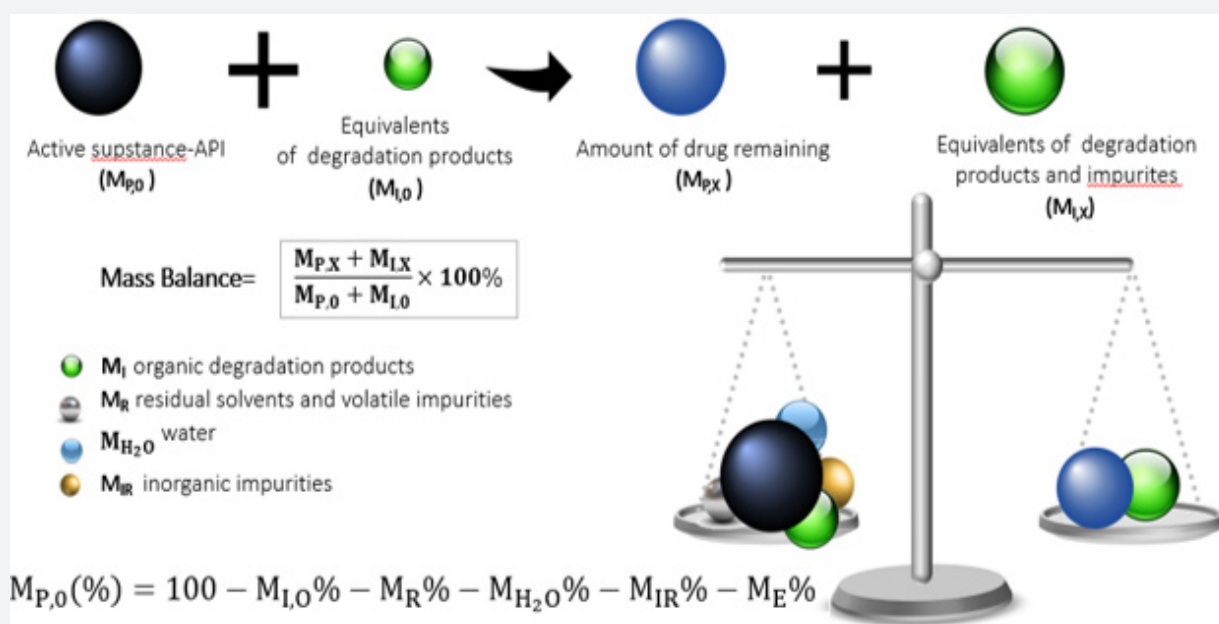


Figure 1:
where P represents the parent drug substance and I represent impurities or degradation products.

Mass balance may be presented as an absolute deficit expressed in terms of the amount of substance (mole); according to the following equation:

$$\frac{N_{P,0} - N_{P,x}}{a} - \frac{N_{I,x} - N_{I,0}}{b} \quad (1)$$

Where:

$N_{P,0}$ denotes the amount of active pharmaceutical ingredient (API) at the initial time point (time zero);

$N_{P,x}$ denotes the amount of active pharmaceutical ingredient (API) at a given time point x;

$N_{I,0}$ denotes the total amount of impurities at the initial time point (time zero);

$N_{I,x}$ denotes the total amount of impurities at a given time point x;

a and b are stoichiometric coefficients.

The mass balance; expressed as the absolute mass deficit; can be calculated using the following equation:

$$\frac{M_{P,x} + M_{I,x}}{M_{P,0} + M_{I,0}} \times 100\% \quad (2)$$

Where:

$M_{P,0}$ is the assay value (content) of the active pharmaceutical ingredient (API) at the initial time point (time zero);

$M_{P,x}$ is the assay value (content) of the active pharmaceutical ingredient (API) at time point x;

$M_{I,0}$ is the total impurity content at the initial time point (time zero);

$M_{I,x}$ is the total impurity content at time point x.

When expressed as a relative deficit; mass balance can be calculated using the following equation:

$$\frac{(M_{P,0} - M_{P,x}) - (M_{I,x} - M_{I,0})}{M_{P,0} + M_{P,x}} \times 100\% \quad (3)$$

Where:

$M_{P,0}$ is the content of the parent drug substance (API) at the initial time point (time zero);

$M_{P,x}$ is the content of the parent drug substance (API) at time point x;

$M_{I,0}$ is the total impurity content at the initial time point (time zero);

$M_{I,x}$ is the total impurity content at time point x.

Following the determination of the Active Pharmaceutical Ingredient (API) content; the next critical parameter in mass balance evaluation; as evident from Equations 2 and 3; is the determination of impurities.

According to the International Council for Harmonisation (ICH); an impurity is defined as any component present in a drug substance that is not the chemical entity designated as the active pharmaceutical ingredient and that consequently affects the purity of the drug substance or drug product. According to ICH Q3A; impurities in drug substances are classified into three

principal categories: organic impurities; inorganic impurities; and residual solvents [7].

The ICH Q3A guideline does not address extraneous contaminants; which should be controlled through Good Manufacturing Practice (GMP); nor does it specifically cover polymorphic forms and enantiomeric (chiral) impurities. Nevertheless, these components may significantly influence the overall purity profile and should be considered whenever relevant.

The primary criterion in selecting an appropriate analytical method for impurity determination is the ability to adequately distinguish and quantify the components of interest. This requirement generally limits the selection to spectroscopic techniques; separation methods; or combinations of both. To ensure effective impurity control; analytical methodologies with high sensitivity; selectivity; and specificity are employed for impurity detection; identification; quantification; and characterization.

In contemporary pharmaceutical analysis, hyphenated techniques combining separation and detection capabilities are

routinely utilized. Commonly employed separation techniques include thin-layer chromatography (TLC); gas chromatography (GC); high-performance liquid chromatography (HPLC); capillary electrophoresis (CE); and supercritical fluid chromatography (SFC). These techniques are frequently coupled with spectroscopic detectors such as Ultraviolet (UV); Diode-Array (DAD); Mass Spectrometric (MS); or Nuclear Magnetic Resonance (NMR) systems to facilitate comprehensive impurity profiling.

An overview of the most frequently applied analytical techniques and the corresponding regulatory acceptance criteria for different classes of impurities is provided in (Table 1).

Analysis of the variables included in Equations 2 and 3 demonstrates that accurate determination of the initial API content requires not only a validated; precise; accurate; and reproducible assay method; but also, comprehensive characterization of all impurity classes; including organic impurities; inorganic impurities; chiral impurities; residual solvents; and water content. Failure to account for any of these components may compromise the accuracy of the mass balance calculation.

Table 1: Overview of Analytical Methods and the Permitted Limits for Each Group of Impurities.

Organic Impurities					Inorganic Impurities	Residual Solvents		
Subtype	Process-related impurities	Degradation products	Enantiomeric impurities	Genotoxic Impurities	Heavy Metals, Ligands, Residues, Catalysts	Class		
						I	II	III
Most Commonly Used Analytical Techniques Acceptance Limit / Guideline	HPLC, GC, LC/MS, GC/MS, TLC	HPLC, GC, LC/MS, GC/MS, TLC	HPLC, GC, CE	HPLC, GC, LC/MS, GC/MS, NMR, IC	Pharmacopoeial limit tests (semi-quantitative determination), ICP-OES, ICP-MS	GC; loss on drying and thermogravimetric analysis as non-specific methods		
	ICH Q3A (0.05-0.15%)	ICH Q3A (0.05-0.15%)	ICH Q3A, ICH Q6 (0.05-0.15%)	ICH M7, S9, FDA guidelines	ICH Q3D	ICH Q3C		

TLC: Thin-Layer Chromatography

GC: Gas Chromatography

HPLC: High-Performance Liquid Chromatography

LC-MS: Liquid Chromatography-Mass Spectrometry

GC-MS: Gas Chromatography-Mass Spectrometry

NMR: Nuclear Magnetic Resonance Spectroscopy

IC: Ion Chromatography

ICP-OES: Inductively Coupled Plasma Optical Emission Spectroscopy

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

CE: Capillary Electrophoresis

The determination of water content is particularly important when the drug substance exhibits hygroscopic properties; undergoes moisture-induced degradation; or exists as a stoichiometric hydrate. Under such circumstances, the accurate quantification of water becomes a critical factor affecting the accuracy of assay determination and; consequently; the reliability of mass balance calculations [8].

Water content may be determined using several analytical approaches; including Karl Fischer titration; gravimetric methods

based on loss on drying; and distillation techniques. Each approach possesses specific advantages and limitations with respect to sensitivity; selectivity; precision; and applicability to different sample types. The fundamental characteristics; advantages; and disadvantages of these methods are summarized in (Table 2).

Given the complexity of impurity profiling and the necessity to accurately quantify all components contributing to the overall material balance; impurity determination remains one of the most challenging aspects of mass balance assessment. Comprehensive impurity characterization is therefore essential for obtaining

meaningful mass balance data; supporting degradation pathway elucidation; and demonstrating the suitability of stability-indicating analytical methods used during pharmaceutical development.

Conversely, poor mass balance may indicate the presence of undetected degradation products; volatile degradation components; extraction losses; or analytical limitations.

Forced Degradation Studies

Forced degradation studies are conducted to establish time [11].

Table 2: Basic Characteristics of Different Types of Methods for Water Determination.

Characteristics	Titrimetric Method				Distillation	Gravimetry
	Volumetric titration		Coulometric Titration		Method II	Method II
	Method Ia	Method Ib	Method Ic	Loss on drying		
	2.5.12	Ph. Eur.	2.5.32 Ph. Eur. <921> USP		2.2.13 Ph. Eur. <921>USP	2.2.32 Ph. Eur. <921> USP or <731>
Principle of analysis	Quantitative analysis based on a stoichiometric reaction between one mole of iodine (I ₂) and one mole of water.		Iodine is generated electrolytically at the anode and reacts with water; for 1 mole of I, $2 \times 96,485 \text{ C mol}^{-1}$ are required.	The measured sample is evaporated, and the evaporated water is titrated.	Moisture is removed from the sample by distillation as an azeotropic mixture with toluene and is then measured at a known temperature	The total amount of volatile compounds is determined, including water and residual solvents that evaporate under the experimental conditions (most commonly at 105 °C).
Calibration and Standardization	Required. The titer must be determined immediately before use.		Not required. The determination is based on Faraday's law of electrolysis.		Not required	Calibrated balance and oven.
Instrument maintenance	Reagents must be replaced frequently and stored protected from air and light.		Reagents must be replaced frequently and stored protected from air and light.			/
Reagents	Short shelf life.		Long shelf life.	Longest shelf life.	/	/
Solubility	The sample must be dissolved.		The sample must be dissolved.	Suitable for substances that are insoluble in the titration solvent but thermally stable.		
Working range	200 µg-50 mg		10 µg-5 mg			A larger sample quantity is required (>100 mg).
Accuracy	mH ₂ O>5mg±0,5%		>400 µg ±0,5%		/	/
Precision	mH ₂ O>5mg±0,5%		>400 µg ~1%			

During forced degradation experiments, samples are intentionally subjected to a range of stress conditions designed to accelerate chemical degradation. These typically include exposure to acidic and alkaline environments to induce hydrolytic

the intrinsic stability profile of a drug substance; thereby facilitating formulation development; manufacturing process design; and packaging selection. In addition, these studies enable the identification of potential degradation products and degradation pathways; while providing essential information for the development and validation of stability-indicating analytical methods for drug substances and pharmaceutical dosage forms [9-12]. Compared with conventional stability studies; which are often time-consuming and costly; forced degradation studies generate relevant stability data within a relatively short period of

degradation; oxidizing agents to promote oxidative degradation; elevated temperature and humidity; and Ultraviolet (UV) or visible light irradiation to assess photostability. Prior to the experimental design, an evaluation of the chemical structure of the drug substance and its functional groups is recommended as this information can provide valuable insight into potential

degradation liabilities. For example, esters; amides; and lactones are generally susceptible to hydrolysis; whereas compounds containing heteroatoms such as nitrogen and sulfur; as well as

aldehydes and ketones; are more prone to oxidative degradation. Similarly, alkenes; aromatic compounds; and heterocyclic derivatives frequently exhibit photosensitivity [13]. Typical stress

conditions applied in forced degradation studies are summarized in (Table 3).

Table 3: Overview of Literature Data on Strategies for Selecting Conditions for Conducting a Forced Degradation Study.

Type of Studies	Acid Degradation	Base	Oxidative Degradation	Thermal Degradation	Photodegradation	Literature Reference(s)
Experimental Conditions	0.1 M HCl	0.1 M NaOH	3% H ₂ O ₂	60 °C	Metal halide, mercury vapor, xenon, or UV-B fluorescent lamp(s)	[22]
Exposure Time	1-10 days	1-10 days	1-3 days	1-10 days	1-10 days	
Experimental Conditions	0.1 M HCl 40 or 60 °C	0.1 M NaOH 40 or 60 °C	3% H ₂ O ₂ 25 or 60 °C	60 or 80 °C	> 1.2 million lux hours; UV exposure. 200 Wh/m ² > 2x	[3] [19]
Exposure Time	1, 3, 5 days	1, 3, 5 days	1, 3, 5 days	1, 3, 5 days	1, 3, 5 days	
Experimental Conditions	0.1 M-1M HCl or H ₂ SO ₄	0.1 M-1M NaOH, LiOH, KOH	3% H ₂ O ₂	> 50 °C	> 1.2 million lux hours; UV exposure. 200 Wh/m ² > 2x	[9]
Exposure Time	To 14 days	To 14 days	To 14 days	2-3 weeks	/	
Experimental Conditions	0.01-5 M HCl	0.01-5 M NaOH	1-3% H ₂ O ₂	40-60 °C	6-1.2 million lux hours; UV exposure. UV > 200 Wh/m ² > 2x	[23]
Exposure Time	24 h	24 h	30 min-24 h	Up to 1 month	/	
Experimental Conditions	0.1 M HCl 40 or 60 °C	0.1 M NaOH 40 or 60 °C	3% H ₂ O ₂ 25 or 40 °C AIBN	60 °C	> 1.2 million lux hours; UV exposure. UV > 200 Wh/m ² > 2x	[10,11,14]
Exposure Time	1, 3, 5 days	1, 3, 5 days	1, 3, 5 days	1, 3, 5 days	1, 3, 5 days	

A degradation extent of approximately 5-20% is generally considered acceptable for forced degradation studies [3,14]. However, for small-molecule drug substances; a broader degradation range of 10-30% is often recommended; with approximately 10% degradation frequently regarded as optimal. Nevertheless; no universally accepted criterion exists; and the target degradation level should be evaluated on a case-by-case basis. Failure to achieve the desired level of degradation does not necessarily indicate an unsuccessful study; rather; it may demonstrate the inherent stability of the molecule under the applied stress conditions. Conversely, excessive exposure to stress conditions may result in the formation of secondary degradation products that are unlikely to occur under normal storage conditions; potentially leading to misleading conclusions. On the other hand, insufficient stress exposure may fail to generate detectable degradation products. Consequently, forced degradation protocols should be tailored to the specific physicochemical characteristics of the molecule under

investigation; and all experimental conditions should be reported in detail as the conclusions drawn are directly dependent on the applied stress conditions.

Mass balance determination plays a crucial role in forced degradation studies as it provides evidence of the completeness of degradation product characterization and contributes to a comprehensive understanding of degradation pathways. Achievement of satisfactory mass balance indicates that the loss of the parent drug substance has been adequately accounted for by the detected degradation products; thereby supporting the reliability and scientific validity of the study.

Current International Council for Harmonisation (ICH) guidelines [1,15] provide general recommendations regarding the conduct of forced degradation studies; however, the specific stress conditions should be selected based on the physicochemical and structural characteristics of the drug substance. In practice, it is generally recommended to begin stress testing under relatively

mild conditions and gradually increase the severity of the applied stress. This approach minimizes the risk of altering the degradation mechanism and avoids practical complications associated with sample neutralization; handling; or solubilization when highly concentrated acidic or alkaline reagents are employed [11].

Notably, current regulatory guidelines do not specify a recommended working concentration for treated samples during forced degradation studies. Consequently, the selection of an appropriate sample concentration remains the responsibility of the analyst and should be scientifically justified based on the objectives of the study; the sensitivity of the analytical method; and the physicochemical properties of the analyte.

Recommended Conditions for Forced Degradation Studies

A sample concentration of approximately 1 mg/mL is generally recommended to ensure adequate detection and characterization of low-level degradation products formed during stress testing. Such concentrations typically provide sufficient analytical sensitivity while maintaining chromatographic performance and facilitating the quantification of degradation impurities [3,14]. When the objective is to evaluate a pharmaceutical dosage form; certain degradation studies; particularly binary-mixture studies involving drug substance and excipients; should be performed using concentrations representative of the final formulation composition in order to better simulate product-specific degradation pathways.

Experimental conditions should be designed to achieve approximately 5-20% degradation of the Active Pharmaceutical Ingredient (API); which is generally considered sufficient to demonstrate meaningful degradation without producing excessive secondary degradation products. Most forced degradation experiments are therefore conducted at API concentrations close to 1 mg/mL and under stress conditions carefully optimized to achieve this target degradation range [16].

To facilitate correct interpretation of degradation profiles; several control samples should be analyzed during forced degradation studies. These include untreated reference standards; untreated test samples; stress reagents; and solvent blanks. Subsequently, stressed solvent blanks and stressed reagents should also be examined. This approach enables differentiation between degradation products originating from the API and artifacts generated from the solvent system or stress reagent itself. For pharmaceutical dosage forms; placebo samples should be subjected to identical stress conditions. Such studies facilitate the identification of degradation products derived from excipients and provide valuable information regarding drug-excipient compatibility [10].

Experimental Conditions for Forced Degradation Studies

Hydrolytic Degradation

Hydrolysis is among the most common degradation pathways encountered in pharmaceutical products and may occur over a wide pH range. Acidic and alkaline hydrolysis studies provide important information regarding the susceptibility of ionizable functional groups and the formation of primary degradation products.

The selection of hydrolytic stress conditions should be based on the intrinsic stability characteristics of the molecule under investigation. Hydrochloric acid (HCl) and Sulfuric Acid (H₂SO₄) are commonly employed for acidic hydrolysis, whereas Sodium Hydroxide (NaOH) and Potassium Hydroxide (KOH) are typically used for alkaline hydrolysis. Concentrations generally range from 0.1 M to 1.0 M.

Hydrolytic studies are usually performed in solution. For partially soluble or insoluble compounds; suitable co-solvents may be employed to facilitate dissolution or suspension in the acidic or alkaline medium. Acetonitrile is frequently reported as the preferred co-solvent because of its chemical inertness; whereas methanol should be used cautiously owing to its potential reactivity with electrophilic functional groups or reactive degradation intermediates; particularly under acidic conditions.

Stress studies generally begin at room temperature. If the desired extent of degradation is not achieved through prolonged exposure or increased reagent concentration; temperature may be introduced as an additional stress factor. Temperatures up to approximately 70 °C are commonly employed; however, elevated temperatures may promote water evaporation and cause deviations from Arrhenius behavior; potentially complicating data interpretation. Hydrolysis studies are generally not recommended to exceed seven days; and stressed samples should be appropriately neutralized before analysis [11,17,14,18].

Oxidative Degradation

Oxidative degradation is based on electron-transfer mechanisms leading to the formation of reactive intermediates; including radical; anionic; and cationic species. The most widely used oxidizing agent in forced degradation testing is hydrogen peroxide (H₂O₂) [3,19].

Alternative oxidizing systems involving metal ions; molecular oxygen; or radical initiators such as azobisisobutyronitrile (AIBN) may also be utilized when appropriate. The choice of oxidizing reagent and concentration depends on the chemical structure and oxidation susceptibility of the molecule [16].

Typically, oxidative degradation studies employ 0.1-3% hydrogen peroxide under near-neutral pH conditions for periods of up to seven days; aiming to achieve not more than 20% degradation. Particular caution should be exercised when conducting oxidative studies at temperatures above 30 °C; as elevated temperatures may promote cleavage of the peroxide O-O

bond and subsequent formation of hydroxyl radicals; which are considerably stronger oxidizing agents than hydrogen peroxide itself.

Photolytic Degradation

Photolytic degradation studies are performed to assess the susceptibility of drug substances and drug products to light-induced degradation and to identify the primary photodegradation products formed following exposure to ultraviolet or visible light.

Current recommendations are described in ICH Q1B; which specifies that samples should be exposed to a minimum overall illumination of 1.2 million lux hours and an integrated near-ultraviolet energy of 200 Wh/m² [15]. The wavelength range most commonly employed for photodegradation studies is 300-800 nm [19].

Photodegradation may proceed via direct photochemical reactions or indirectly through photooxidative mechanisms. Additionally, light can act as a catalyst; accelerating oxidative degradation processes and altering degradation pathways [20,11,18].

Thermal Degradation

Thermal degradation studies evaluate the impact of elevated temperature on the stability of pharmaceutical materials. These studies may involve degradation mechanisms such as hydrolysis; dehydration; isomerization; epimerization; decarboxylation; molecular rearrangement; and polymerization reactions.

Thermal stress studies may be conducted under either dry or humid conditions; typically, within a temperature range of 40-80 °C and relative humidity levels between 60% and 75% RH. Most commonly, samples are exposed to approximately 70 °C under controlled humidity conditions for periods ranging from one to two months.

Exposure to temperatures exceeding 80 °C is generally discouraged because such conditions may induce unrealistic degradation pathways; generate misleading degradation profiles; and promote the formation of secondary degradation products that are unlikely to occur during normal storage conditions [3,21].

Relevance to Mass Balance Assessment

Forced degradation studies represent a critical component of mass balance evaluation because they generate the degradation products required to establish a quantitative relationship between API loss and impurity formation [22-23]. Properly designed stress conditions enable the identification; characterization; and quantification of degradation products while minimizing the formation of irrelevant secondary degradants. Therefore, careful selection of stress factors; reagent concentrations; temperature; duration of exposure; and appropriate controls is essential for obtaining reliable mass balance data and demonstrating the

suitability of stability-indicating analytical methods.

Conclusion

Mass balance assessment is a central element of forced degradation testing because it links the measured loss of the active pharmaceutical ingredient with the formation and quantification of degradation products. A meaningful mass balance therefore depends not only on an accurate assay result; but also, on comprehensive impurity profiling and appropriate consideration of water; residual solvents; inorganic components; volatile products; and sample-preparation losses. The reliability of the final result is strongly influenced by the selectivity; sensitivity; and quantitative capability of the analytical procedures used.

No single analytical technique can address every degradation pathway or all potential sources of mass imbalance. HPLC or UHPLC with UV or diode-array detection remains suitable for routine quantitative analysis; whereas complementary techniques such as LC-MS; HRMS; GC; NMR; Karl Fischer titration; and gravimetric methods can provide essential information when degradation products have weak chromophores; are volatile; co-elute with other components; or differ substantially from the parent compound in their detector response. The use of orthogonal analytical techniques and experimentally justified response factors can substantially improve the completeness and scientific credibility of mass balance evaluation.

Forced degradation conditions should be selected on a case-by-case basis according to the physicochemical properties and degradation liabilities of the molecule. Stress exposure should be sufficient to generate relevant degradation products without causing extensive secondary degradation that is unlikely to occur during manufacture or storage. Appropriate controls; including unstressed samples; reagent blanks; solvent blanks; stressed blanks; and placebo samples where applicable; are necessary to distinguish true drug-related degradation products from analytical or matrix-related artifacts.

Overall, mass balance should be interpreted as an integrated scientific assessment rather than as a single numerical acceptance criterion. A result approaching 100% can support the adequacy of a stability-indicating method; but it should always be evaluated together with analytical variability; recovery; detector response; degradation chemistry; and the possibility of undetected or volatile products. Future improvements in high-resolution mass spectrometry; multidetector platforms; automated impurity tracking; and risk-based analytical development are expected to strengthen degradation-product characterization and make mass balance investigations more robust. A systematic strategy combining sound stress-study design; validated quantitative methods; orthogonal detection; and transparent interpretation provides the most reliable basis for understanding degradation pathways and supporting pharmaceutical development.

References

1. International Council for Harmonisation (2003) ICH Q1A(R2): Stability Testing of New Drug Substances and Products. International Council for Harmonisation, Geneva Switzerland.
2. Baertschi SW, Reed RA, Alsante KM (2011) Role of mass balance in pharmaceutical stress testing. In: Baertschi SW Alsante KM Reed RA (Eds) Pharmaceutical Stress Testing: Predicting Drug Degradation. 2nd Edition Informa Healthcare London United Kingdom pp. 245-265.
3. Blessy M, Patel RD, Prajapati PN, Agrawal YK (2014) Development of forced degradation and stability indicating studies of drugs: A review. J Pharm Anal 4(3): 159-165.
4. Hokanson GC (1994) A life cycle approach to the validation of analytical methods during pharmaceutical product development. Part II: Changes and the need for additional validation. Pharm Technol 18(10): 92-100.
5. Alsante KM, Huynh BK, Baertschi SW, Reed RA, Landis MS, et al. (2014) Recent trends in product development and regulatory issues on impurities in active pharmaceutical ingredient and drug products. Part 1: Predicting degradation-related impurities and impurity considerations for pharmaceutical dosage forms. AAPS PharmSciTech 15(1): 237-251.
6. Baertschi SW, Pack BW, Hoaglund HCS, Nussbaum MA (2013) Assessing mass balance in pharmaceutical drug products: New insights into an old topic. Trends Anal Chem 49: 126-136.
7. International Council for Harmonisation (2006) ICH Q3A(R2): Impurities in New Drug Substances. International Council for Harmonisation, Geneva, Switzerland.
8. Zhou L, Socha JM, Vogt FG, Chen S, Kord AS (2010) A systematic method development strategy for water determinations in drug substances using Karl Fischer titration. Am Pharm Rev.
9. Alsante KM, Ando A, Brown R, Ensing J, Hatajik TD, Kong W, et al. (2007) The role of degradant profiling in active pharmaceutical ingredients and drug products. Adv Drug Deliv Rev 59(1): 29-37.
10. Chakole RD, Charde MS, Kumar J, Welankiwar AS (2013) Development of forced degradation studies of drugs. Int J Adv Pharm 2(3): 34-39.
11. Patel R, Patel M, Dubey N, Patel B (2016) Forced degradation studies: An essential tool in pharmaceutical development. Int J Pharm Sci Rev Res pp. 2683-2691.
12. Singh S, Junwal M, Modhe G, Tiwari H, Kurmi M, et al. (2013) Forced degradation studies to assess the stability of drugs and products. Trends Anal Chem 49: 71-88.
13. Swarbrick J (Ed.) (2005) Encyclopedia of Pharmaceutical Technology. 3rd Edition, Informa Healthcare, New York, USA.
14. Sharma MK, Murugesan M (2017) Forced degradation study: An essential approach to develop stability-indicating methods. J Chromatogr Sep Tech 8(1): 349.
15. International Council for Harmonisation (1996) ICH Q1B: Photostability Testing of New Drug Substances and Products. International Council for Harmonisation, Geneva, Switzerland.
16. Shete S, Dhaneshwar S, Bhatt L (2014) Forced degradation studies: Regulatory guidance, characterization of drugs, and their degradation products. Int J Pharm Sci Rev Res.
17. Ngwa G (2010) Forced degradation as an integral part of HPLC stability-indicating method development. Drug Deliv Technol 10(5): 56-59.
18. Klick S, Muijselaar PGHM, Waterval J, Eichinger T, Korn C, et al. (2005) Toward a generic approach for stress testing of drug substances and drug products. Pharm Technol 29(2): 48-66.
19. Baertschi SW (2006) Pharmaceutical Stress Testing: Predicting Drug Degradation. CRC Press, Boca Raton, Florida, USA.
20. Deokate UA, Gorde AM (2014) Forced degradation and stability testing: Strategies and analytical perspectives. Int J Pharm Sci Rev Res.
21. World Health Organization (2009) Stability testing of active pharmaceutical ingredients and finished pharmaceutical products. WHO Technical Report Series No 953 Annex 2 World Health Organization Geneva Switzerland.
22. Singh R, Rehman Z (2012) Current trends in forced degradation study



This work is licensed under Creative Commons Attribution 4.0 License
DOI: 10.19080/GJPPS.2026.12.5558478

Your next submission with Juniper Publishers will reach you the below assets

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats (Pdf, E-pub, Full Text, Audio)
- Unceasing customer service

Track the below URL for one-step submission

<https://juniperpublishers.com/online-submission.php>

for pharmaceutical product development. J Pharm Educ Res 3: 54-63.

23. Alsante KM, Martin L, Baertschi SW (2003) A stress-testing benchmarking study. Pharm Technol 27(2): 60-72.

