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Stability Toolkit for the Appraisal of Bio/Pharmaceuticals' Level of Endurance (STABLE) as a Framework and Software to Evaluate the Stability of Pharmaceuticals

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Abstract

The Stability Toolkit for the Appraisal of Bio/Pharmaceuticals' Level of Endurance (STABLE) is introduced and proposed as a comprehensive tool and software to evaluate the stability of active pharmaceutical ingredients (APIs) under various stress conditions. In the pharmaceutical industry, stability testing is a critical step in the drug development process, ensuring the quality, safety, and efficacy of APIs. Traditional stability tests—such as real-time, accelerated, and forced degradation testing—often face challenges, including inconsistent interpretation and implementation across different regions and organizations. STABLE addresses these challenges by providing a standardized and holistic approach to assessing drug stability across five key stress conditions: oxidative, thermal, acid-catalyzed hydrolysis, base-catalyzed hydrolysis, and photostability. Beyond its role as an evaluation tool, STABLE also serves as a practical guide for chemists, encouraging a more complete and thoughtful approach to stability studies. While many investigations focus solely on acid- and base-catalyzed hydrolysis, other critical conditions—such as photostability—are often underexplored or entirely omitted. By highlighting the importance of evaluating all relevant degradation pathways, STABLE promotes more robust and informed stability testing protocols. The index utilizes a color-coded scoring system to quantify and compare stability, facilitating consistent assessments across different APIs. This paper discusses the methodology of STABLE, including the scoring system and specific criteria applied under each condition. This tool is introduced to reflect intrinsic degradation susceptibility under forced conditions. The software is freely available as an open-source tool at bit.ly/STABLE2025, enabling broader accessibility and implementation across the pharmaceutical research community.

Keywords: stability toolkit for the assessment of bio/pharmaceuticals' level of endurnce; STABLE; stress testing; forced degradation; metrics

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1. Introduction

Stability studies help in selecting proper formulations, determining storage conditions, and establishing shelf life. Many drugs are susceptible to hydrolytic degradation, especially in the presence of water or under acidic or basic conditions [1]. Drugs containing ester,

amide, or lactam functional groups are particularly prone to hydrolysis. Oxidation is another common degradation pathway for drugs, particularly those containing oxidation-sensitive functional groups like alcohols, aldehydes, or thiol [2]. Exposure to oxygen, peroxides, or metal ions can accelerate oxidative degradation. Light exposure can cause photochemical degradation of drugs, leading to the formation of reactive species and subsequent degradation products [3]. Drugs with chromophores or unsaturated bonds are more susceptible to photolytic degradation [4]. Elevated temperatures can accelerate chemical degradation reactions, such as hydrolysis, oxidation, and decarboxylation. Drugs with low thermal stability are more prone to thermal degradation during storage [5].

Stability testing is a critical key point during drug development that helps ensure the quality, safety, and efficacy of APIs [6,7]. Stability testing is required by regulatory agencies like the FDA and ICH to understand how the quality of an active pharmaceutical ingredient (API) changes over time under the influence of environmental factors such as heat, light, and humidity [8]. The different types of drug stability testing include real-time stability testing, accelerated stability testing, forced degradation testing, and comparative stability testing. Real-time stability testing involves storing drug products under recommended conditions and testing them periodically. Accelerated stability testing exposes products to harsh conditions to predict stability over time more quickly [9]. Forced degradation testing intentionally exposes products to extreme conditions to assess their stability under stress. Comparative stability testing compares the stability of different batches of the products.

Forced degradation (stress) testing is essential for understanding degradation pathways, intrinsic stability, and generating stability-indicating assay methods (SIAMs) [10]. Stress testing involves degradation of drug products and substances under more severe conditions than accelerated conditions to generate degradation products that can be studied. Common stress factors include acid-/base-catalyzed hydrolysis, thermal degradation, photolysis, and oxidation [5]. Stress testing should be performed early in drug development to optimize conditions, select suitable methods, and improve manufacturing processes [8].

In the last decade, special attention has been paid to the introduction of evaluation tools in order to standardize the process/method of evaluation. Recent assessment metrics and software, such as Analytical Green Star Area (AGSA) [11], Modified GAPI (MoGAPI) [12], ComplexMoGAPI [13], Click Analytical Chemistry Index (CACI) [14], and Carbon Footprint Reduction Index (CaFRI) [15], play a significant role in evaluating and improving the greenness, sustainability, applicability, and practicality of analytical methods. While regulatory guidelines provide frameworks for stability testing, variations in interpretation and implementation across different regions or organizations can introduce inconsistencies in predicting drug stability [9]. Addressing these limitations requires a comprehensive approach to testing the stability of active pharmaceutical ingredients (APIs). In this regard, developing a new tool, the Stability Toolkit for the Appraisal of Bio/Pharmaceuticals' Level of Endurance (STABLE), to help assess the stability of APIs is an urgent need. To address this need, we here present and propose an innovative tool that enables the evaluation of drugs and chemicals based on experimental data. The presented tool, available as a free source at bit.ly/STABLE2025, assess five different aspects of the drug stability including oxidative, thermal, acid-catalyzed hydrolysis, base-catalyzed hydrolysis, and photostability. STABLE is built on established forced degradation protocols [16], and serves as a complementary tool, rather than an alternative to existing guidelines. Incorporating such a tool into stability testing protocols could significantly enhance the consistency and reliability of drug stability studies, while also highlighting areas for potential improvements in susceptible drug development.

2. Stress Conditions and STABLE

Choosing appropriate stress conditions that mimic real-world scenarios can be challenging, as the conditions need to be consistent with the product's decomposition under normal manufacturing, storage, and usage conditions. It is not necessary that forced degradation would result in a degradation product. The study can be terminated if no degradation is seen after exposure to stress conditions. On the other hand, over-stressing a sample may lead to the formation of a secondary degradation product that would not be seen in long-term stability studies. Degradation of drug substances between 5% and 20% has been accepted as reasonable for stability studies and validation of SIAMs.

STABLE provides a facility to evaluate the stability of APIs under different stress conditions. The index assesses stability under five conditions, including hydrolytic conditions (including acidic and base-catalyzed hydrolysis), oxidative, photolytic, and thermal degradation. Each condition is represented in STABLE with a color and score. If the tested compound has sufficient stability, the section will be colorful. If the drug has moderate stability, the section will be gray, and if the drug is unstable, the color will be black. The following figure shows the STABLE scores of four cases of drugs with high stability, low stability, moderate stability, and mixed stability under the five tested conditions (Figure 1). It is worth mentioning that mixed stability is the most expected result of commercially available APIs. The point system employed in the STABLE metric is empirical and assumes linear degradation kinetics of APIs. While this assumption does not reflect the often complex, non-linear kinetics observed in real-world degradation processes, it provides a practical and standardized framework for comparative assessment. This simplification is considered acceptable within the context of STABLE's design goals, which prioritize ease of use, generalizability across a broad range of compounds, and the ability to generate consistent, interpretable stability scores across multiple stress conditions. In the following sections, we will discuss how these colors and the scores were assigned to each case.

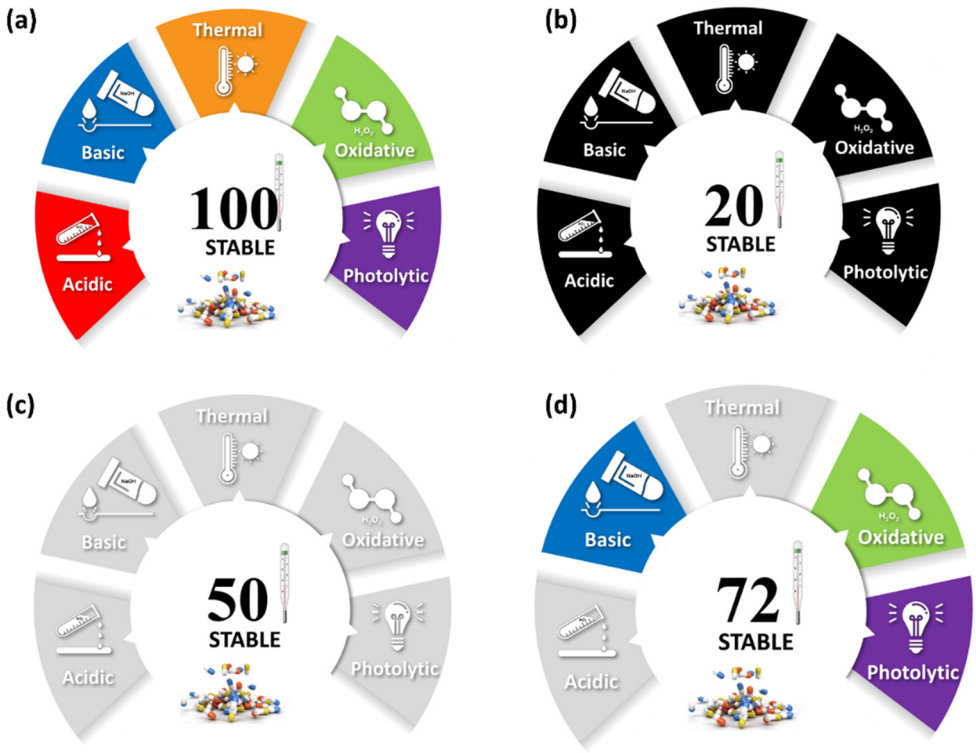


Figure 1. Four proposed cases of APIs with maximum (**a**), minimum (**b**), average (**c**), and mixed stability (**d**).

2.1. Acid-Catalyzed Hydrolysis

Acid-catalyzed hydrolysis is a major degradation pathway for many APIs, especially those containing ester, amide, lactone, or other acid-labile functional groups. Exposure to acidic environments during storage or processing can significantly compromise the stability of such compounds. Acid stress testing is commonly employed to evaluate the chemical stability of drug substances under forced degradation conditions. Typically, hydrochloric acid (HCl) solutions in the range of 0.1–1 mol/L are used to simulate acid-induced stress and generate primary degradation products within a suitable window for analytical characterization.

This testing helps uncover degradation mechanisms and pathways, providing insights into the chemical liabilities of a molecule and supporting the development of stability-indicating methods. To avoid further degradation post-treatment, the stressed samples are neutralized using an appropriate base or buffer prior to analysis.

Drugs can be categorized based on their susceptibility to acid-catalyzed hydrolysis, ranging from highly labile to exceptionally stable. Such classification aids in defining appropriate stress conditions and informs formulation design and storage recommendations.

To standardize the evaluation of acid-induced degradation, we present a scoring system that quantifies drug stability under acidic conditions. The system considers four parameters: HCl concentration, reaction time, temperature, and observed percentage of degradation. Higher points indicate greater stability under acidic stress (Table 1). Notably, drugs demonstrating $\leq$10% degradation under harsh conditions (e.g., >5 mol/L HCl for 24 h under reflux) are assigned the maximum score, reflecting exceptional acid resistance.

Table 1. Different conditions of acid-catalyzed hydrolysis and the points gained in STABLE.

Parameter	Points
HCl Conc (in mol/L)	
0.0001–0.005	1
>0.005–0.05	2
>0.05–0.1	3
>0.1–0.5	4
>0.5–1.5	5
>1.5–2.5	6
>2.5–4.99	7
$\geq$5.0	8
Time (in hours)	
0.1–1	1
>1–5	2
>5–12	3
>12–24	4
Temperature (in °C)	
20–35	1
>35–65	2
>65–95	3
>95	4
Degradation (%)	
>90	1
>80–90	2
>70–80	3
>60–70	4
>50–60	5
>40–50	6

Table 1. *Cont.*

Parameter	Points
>30–40	7
>20–30	8
>10–20	9
≤10	10

2.2. Base-Catalyzed Hydrolysis

Base-catalyzed hydrolysis represents a key degradation pathway for many APIs, particularly those containing ester, amide, lactam, or other base-labile functional groups. Exposure to alkaline conditions during manufacturing, formulation, or storage can significantly compromise drug stability. Stress testing under basic conditions, commonly using sodium hydroxide (NaOH) or potassium hydroxide (KOH) at concentrations ranging from 0.1 to 1 mol/L, is a standard approach to force degradation and uncover potential degradation products.

These studies are instrumental in understanding hydrolytic mechanisms and in identifying vulnerable molecular regions. The results support the development of stability-indicating analytical methods and guide formulation strategies. Following stress testing, the sample is typically neutralized with an appropriate acid or buffer to halt further degradation prior to analytical evaluation.

A drug's susceptibility to base-catalyzed hydrolysis can vary widely. Therefore, classifying compounds based on their performance under alkaline conditions provides essential information for risk assessment and stability profiling. Drugs that remain stable even under severe basic conditions, such as 5 mol/L NaOH for 24 h under reflux, can be considered highly resistant to base-induced degradation.

To facilitate standardized evaluation, a scoring system is provided in Table 2. This system quantifies a drug's stability by assigning points across four parameters: NaOH concentration, exposure time, temperature, and the percentage of degradation observed. Higher points indicate greater chemical stability under base-catalyzed hydrolysis stress. This framework supports the systematic comparison of compounds and can be used to estimate relative hydrolytic susceptibility.

Table 2. Different conditions of base-catalyzed hydrolysis and the points gained in the STABLE.

Parameter	Points
NaOH Conc (in mol/L)	
0.0001–0.005	1
>0.005–0.05	2
>0.05–0.1	3
>0.1–0.5	4
>0.5–1.5	5
>1.5–2.5	6
>2.5–4.99	7
≥5.0	8
Time (in hours)	
0.1–1	1
>1–5	2
>5–12	3
>12–24	4

Table 2. *Cont.*

Parameter	Points
Temperature (in °C)	
20–35	1
>35–65	2
>65–95	3
>95	4
Degradation (%)	
>90	1
>80–90	2
>70–80	3
>60–70	4
>50–60	5
>40–50	6
>30–40	7
>20–30	8
>10–20	9
≤10	10

2.3. Thermal Hydrolysis

Temperature and humidity are critical factors affecting the stability of APIs. Elevated temperatures can accelerate hydrolytic degradation, particularly in compounds containing hydrolysis-prone functional groups such as esters, amides, and lactones. The presence of moisture under thermal conditions further exacerbates degradation, contributing to both chemical and physical instabilities. These include reduced drug potency, altered dissolution properties, and compromised dosage accuracy.

Thermal stress can also induce physical changes such as clumping, discoloration, or caking, which may affect drug performance or patient compliance. Additionally, high temperature and humidity may facilitate microbial growth in susceptible formulations, posing risks to product safety. Moreover, excessive heat and moisture can deteriorate packaging materials, leading to compromised barrier properties and moisture ingress, which in turn accelerate the degradation of the pharmaceutical product.

To mitigate these risks, understanding the thermal and moisture sensitivity of APIs and formulations is essential. Stress testing under thermal hydrolysis conditions, typically involving prolonged exposure to water at elevated temperatures, helps to evaluate the compound's resilience and supports the design of suitable storage and packaging strategies.

Table 3 introduces a scoring system to quantify drug stability under thermal hydrolysis stress. The scoring criteria include three parameters: duration of exposure, temperature, and the percentage of degradation observed. Higher scores indicate greater resistance to degradation. Drugs that demonstrate minimal degradation (≤10%) even after extended exposure (e.g., water reflux for over five days) receive the maximum score, reflecting excellent thermal stability.

Table 3. Different conditions of thermal hydrolysis * and the points gained in the STABLE.

Parameter	Points
Time (in hours)	
0.1–1	1
>1–5	2
>5–12	3
>12–24	4
>24–48	5

Table 3. *Cont.*

Parameter	Points
>48–120	6
>120	7
Temperature (in °C)	
20–35	1
>35–65	2
>65–95	3
>95	4
Degradation (%)	
>90	1
>80–90	2
>70–80	3
>60–70	4
>50–60	5
>40–50	6
>30–40	7
>20–30	8
>10–20	9
≤10	10

* Neutral pH was assumed in thermal hydrolysis unless otherwise indicated.

2.4. Oxidative Degradation

Oxidation is a significant and often unavoidable degradation pathway for many APIs, especially those containing oxidation-prone functional groups such as alcohols, aldehydes, phenols, thiols, or unsaturated bonds. During storage and manufacturing, exposure to oxidizing agents, including molecular oxygen, hydrogen peroxide, peroxides, or trace metal ions, can initiate or accelerate oxidative degradation, leading to loss of potency or formation of toxic impurities.

Oxidative stress testing is commonly conducted using hydrogen peroxide (H_2O_2), typically at neutral pH and elevated temperatures, to simulate long-term oxidative conditions and generate degradants for characterization. Such forced degradation studies help identify sensitive structural motifs and support the development of stability-indicating analytical methods. These insights are essential for selecting appropriate antioxidants, excipients, packaging materials, and storage conditions during drug development.

To standardize the evaluation of oxidative stability, a quantitative scoring system is presented in Table 4. This framework assesses a compound's resistance to oxidative degradation based on three experimental parameters: hydrogen peroxide concentration, exposure time, and temperature. Percentage degradation is an additional fourth-parameter, included to directly measure oxidative susceptibility. Higher scores represent greater oxidative stability. Compounds that show minimal degradation (≤10%) even under severe oxidative stress (e.g., 30% H_2O_2 for 24 h at 95 °C under reflux) are awarded the highest score, reflecting strong resistance to oxidative breakdown.

Table 4. Different conditions of oxidative degradation and the points gained in the STABLE.

Parameter	Points
H_2O_2 Conc (in %)	
0.005–0.05	1
>0.05–0.1	2
>0.1–0.5	3

Table 4. *Cont.*

Parameter	Points
>0.5–1.5	4
>1.5–2.5	5
>2.5–5	6
>5–30	7
>30	8
Time (in hours)	
0.1–1	1
>1–5	2
>5–12	3
>12–24	4
Temperature (in °C)	
20–35	1
>35–65	2
>65–95	3
>95	4
Degradation (%)	
>90	1
>80–90	2
>70–80	3
>60–70	4
>50–60	5
>40–50	6
>30–40	7
>20–30	8
>10–20	9
≤10	10

2.5. Photodegradation

Photodegradation is a critical degradation pathway for many APIs, particularly those containing photo-labile functional groups such as aromatic rings, conjugated systems, or chromophores. Light exposure, especially within the ultraviolet (UV) and visible spectrum (3800–800 nm), can trigger photochemical reactions leading to drug degradation, formation of reactive intermediates, and loss of potency or safety.

Photostability testing, as outlined in ICH Q1B guidelines, is essential to ensure that light exposure does not cause unacceptable changes in the quality of drug substances or products. Standard testing involves subjecting samples to a minimum of 1.2 million Lux hours of light and 200 watt hours/m^2 of UV energy, with extended exposure up to 6 million Lux hours used to stress-test light stability, as per the ICH Q1B guidelines [17]. Photolysis stress testing commonly employs fluorescent or UV lamps to mimic daylight or sunlight exposure and induce degradant formation for structural elucidation and risk assessment.

Molecules bearing chromophores or extended π-systems are particularly vulnerable to photolytic degradation, as these moieties absorb light energy and initiate radical or ionic degradation pathways. Understanding a compound's photostability helps define suitable formulation strategies and drives the selection of protective packaging (e.g., amber vials, light-resistant blisters) to preserve product integrity throughout shelf life.

To enable a structured assessment of photostability, Table 5 presents a scoring system that evaluates drug resistance to photodegradation. The scoring incorporates four parameters: light source type, light exposure intensity (in million Lux hours), duration of exposure (in hours) andpercentage degradation which captures the actual extent of photolysis. Higher scores reflect greater photostability. An additional point is awarded if the compound exhibits ≤ 10% degradation under the maximum standard

exposure of 6×10^6 Lux hours. This multidimensional scoring model offers a consistent and quantitative tool for evaluating photostability, supporting the development of light-protected formulations and packaging solutions, and ensuring compliance with regulatory expectations.

Table 5. Different conditions of photodegradation and the points gained in the STABLE.

Parameter	Points
Type	
Sunlight	1
UV	4
Lux (in million Lux hours)	
0.1–1.2	1
>1.2–5.99	2
≥6.00	3
Time (in hours)	
0.1–1	1
>1–5	2
>5–12	3
>12–24	4
Degradation (%)	
>90	1
>80–90	2
>70–80	3
>60–70	4
>50–60	5
>40–50	6
>30–40	7
>20–30	8
>10–20	9
≤10	10

3. Case Studies

Panobinostat is an FDA-approved drug under the trade name Farydak® for the management of multiple myeloma. The drug has also been investigated in the therapeutic regimens of human immunodeficiency virus (HIV). The stability of Panobinostat has been studied by Bhukya and Beda using LC-MS/MS. Various degradation studies were performed to evaluate its stability under different stress conditions. Acidic degradation was conducted by adding 0.18 mol/L HCl and heating the mixture for 72 h at 80 °C. Basic degradation involved treating the drug with 0.27 mol/L NaOH for 6 h at 80 °C. For oxidative degradation, 3% hydrogen peroxide was used at room temperature for 1 h. Photolytic degradation was tested by exposing the drug stock solution to UV (1.2×10^6 Lux hours) for 7 days. Thermal stability was assessed by refluxing the drug at 80 °C for 48 h. The results indicated that 2.3% degradation was observed under acidic conditions, while 3.11% degradation occurred under basic conditions. Thermal hydrolysis caused 0.38% degradation, and oxidative degradation resulted in 3.18%. Finally, photolytic degradation led to 0.3% degradation. The STABLE score of Panobinostat was determined to be 80 (Figure 2), indicating significantly high stability under acidic, basic, thermal, oxidative and photolytic conditions [18].

Daclatasvir is an antiviral medication used primarily to treat hepatitis C. It works by inhibiting the NS5A protein in the hepatitis C virus (HCV), which is crucial for the virus's replication and assembly. By blocking this protein, daclatasvir helps to reduce the amount of virus in the body, aiding in the clearance of the infection [19]. Zaman et al. [19]. developed a stability-indicating assay method for the determination of daclatasvir stability

under different stability conditions. Acidic degradation was conducted by adding 5.0 mol/L HCl and heating the mixture for 168 h at room temperature. Basic degradation involved treating the drug with 1.0 mol/L NaOH for 168 h at room temperature. For oxidative degradation, 30% hydrogen peroxide was used at room temperature for 168 h. Photolytic degradation was tested by exposing the drug stock solution to UV (1.2×10^6 Lux hours) for 168 h. Thermal stability was assessed by exposing the drug at 80 °C for 168 h. The results indicated that 1.91% degradation was observed under acidic conditions, while 22.64% degradation occurred under basic conditions. Thermal hydrolysis caused 2.07% degradation, and oxidative degradation resulted in 0.59%. Finally, photolytic degradation led to 1.42% degradation. The STABLE score of daclatasvir was determined to be 84 (Figure 3).

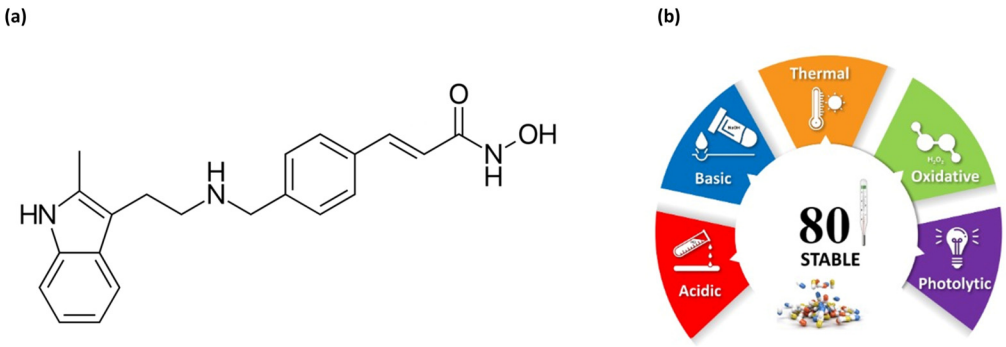


Figure 2. Chemical structure (**a**) and STABLE score (**b**) of Panobinostat.

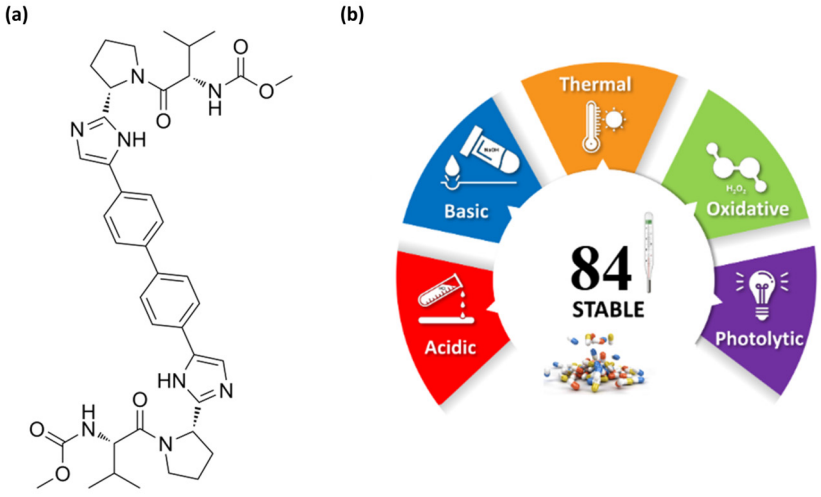


Figure 3. Chemical structure (**a**) and STABLE score (**b**) of Daclatasvir.

Molnupiravir is an antiviral medication developed to treat COVID-19. It was originally developed by Merck & Co. (Rahway, NJ, USA) in collaboration with Ridgeback Biotherapeutics. Molnupiravir works by inhibiting the replication of the virus, specifically targeting the RNA-dependent RNA polymerase enzyme that the virus uses to replicate its genetic material [20]. Faruk et al. [21] developed RP-UHPLC to study the stress degradation of molnupiravir. Acidic degradation was conducted by adding 0.1 mol/L HCl and heating the mixture for 2 h at 100 °C. Basic degradation involved treating the drug with 0.1 mol/L NaOH for 1 h at 60 °C. For oxidative degradation, less than 5% hydrogen peroxide was used at 80 °C for 2 h. Photolytic degradation was tested by exposing the drug stock solution to UV (1.2×10^6 Lux hours) for 72 h. Thermal stability was assessed by exposing the drug at 100 °C for 2 h. The STABLE score of molnupiravir was determined to be 64.

As indicated in the pictogram in Figure 4, molnupiravir is susceptible to base-catalyzed hydrolysis and exhibits intermediate stability toward thermal hydrolysis. The results indicated that 33% degradation was observed under acidic conditions, while 100% degradation occurred under basic conditions. Thermal hydrolysis caused 21% degradation, oxidative degradation resulted in 6%, while photolytic degradation led to 2.5% degradation. These findings suggest that careful control of pH and temperature conditions is essential during formulation, storage, and handling to maintain the drug's stability and efficacy.

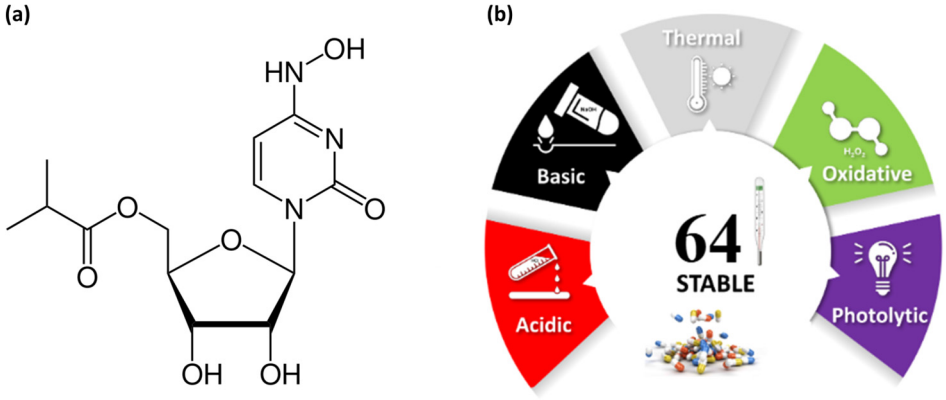


Figure 4. Chemical structure (**a**) and STABLE score (**b**) of Molnupiravir.

Tedizolid is designed to overcome resistance seen with other antibiotics, such as linezolid. It is particularly useful for treating infections caused by resistant strains of bacteria, like MRSA, making it an important option in the fight against antibiotic-resistant infections. Michalska et al. [22] developed LC with DAD and tandem MS detection for the study stability of tedizolid. Acidic degradation was conducted by adding 0.1 mol/L HCl and heating the mixture for 72 h at 70 °C. Basic degradation involved treating the drug with 0.1 mol/L NaOH for 3 h at 25 °C. For oxidative degradation, less than 5% hydrogen peroxide was used at 30 °C for 144 h. Photolytic degradation was tested by exposing the drug stock solution to UV (1.2×10^6 Lux hours) for 48 h. Thermal stability was assessed by exposing the drug at 90 °C for 24 h. The results indicated that 12.38% degradation was observed under acidic conditions, while 58.78% degradation occurred under basic conditions. Thermal hydrolysis caused 9.43% degradation, and oxidative degradation resulted in 6.88%. Photolytic degradation led to 60% degradation. The STABLE score of tedizolid was determined to be 68. As shown in the pictogram in Figure 5, tedizolid demonstrates moderate stability toward base-catalyzed hydrolysis and photolytic degradation. This indicates the need for protective measures against pH increase and light exposure during formulation and storage.

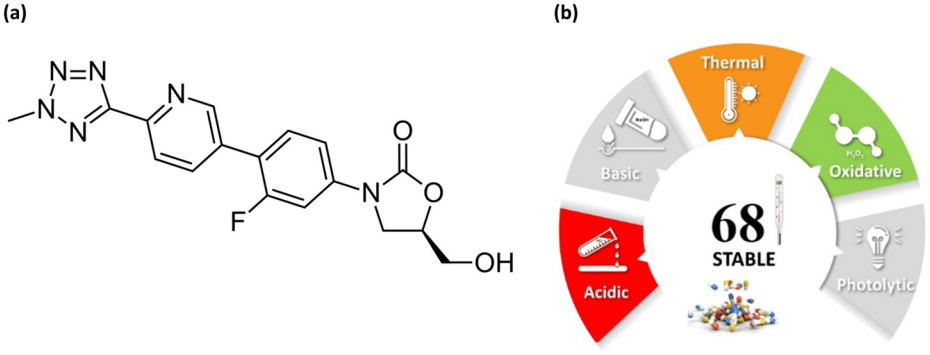


Figure 5. Chemical structure (**a**) and STABLE score (**b**) of Tedizolid.

Apixaban is an oral anticoagulant, often referred to as a "blood thinner." It works by inhibiting a protein called Factor Xa, which plays a key role in the blood clotting process. Salakolusu et al. [23] studied forced degradation of apixaban. Acidic degradation was conducted by adding 1 mol/L HCl and heating the mixture for 48 h at 25 °C. Basic degradation involved treating the drug with 1 mol/L NaOH for 48 h at 25 °C. For oxidative degradation, 30% hydrogen peroxide was used at 25 °C for 48 h. Photolytic degradation was tested by exposing the drug stock solution to UV (1.2×10^6 Lux hours) for 48 h. Thermal stability was assessed by exposing the drug at 100 °C for 48 h. The results showed that 17% degradation occurred under acidic conditions and 16% under basic conditions. Thermal hydrolysis led to 1% degradation of the compound, while oxidative stress caused a similar 1% degradation. Lastly, exposure to light (photolytic conditions) resulted in 1% decomposition. The STABLE score of apixaban was determined to be 82 (Figure 6).

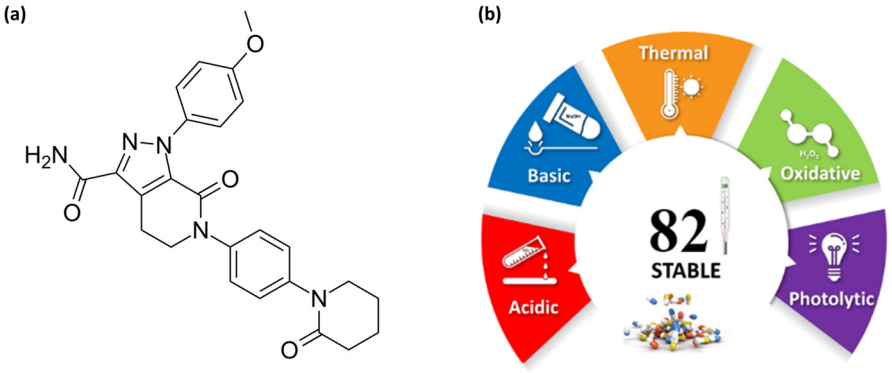


Figure 6. Chemical structure (**a**) and STABLE score (**b**) of Apixaban.

4. Comparison with Established Regulatory Guidelines in Forced Degradation

Upon comparison with established forced degradation benchmarks outlined in major regulatory documents—such as ICH Q1A(R2) [24], ICH Q1B [17], FDA guidance [25], EMA [26], and WHO guidelines [27]—the STABLE metric introduces a standardized, quantitative framework specifically developed to evaluate pharmaceutical stability under five distinct stress conditions. In contrast, regulatory guidelines predominantly provide qualitative procedural frameworks aimed at guiding forced degradation studies within the broader context of stability assessment.

The STABLE approach represents a holistic methodology that encompasses five key degradation pathways: acid-catalyzed hydrolysis, base-catalyzed hydrolysis, thermal degradation, oxidative degradation, and photodegradation. Each pathway is addressed with equal emphasis and evaluated using a quantitative scoring system. Conversely, existing guidelines often address these degradation routes with varying degrees of focus. For example, ICH Q1B concentrates exclusively on photostability, while ICH Q1A(R2) recommends testing susceptibility to hydrolysis over a broad pH range without specifying precise concentrations. Similarly, ICH Q1A(R2) advises thermal stress testing in 10 °C increments above the accelerated testing temperature (e.g., 50 °C, 60 °C), yet it lacks detailed guidance on exposure durations or scoring criteria.

While regulatory guidelines are indispensable for ensuring compliance with registration requirements and for establishing minimum expectations in stability testing, the STABLE metric should be viewed as a complementary tool. Rather than replacing existing approaches, STABLE enhances the rigor and reproducibility of forced degradation studies by introducing a quantitative dimension that is currently absent from most regulatory frameworks.

The STABLE methodology offers several practical advantages for comprehensive stability profiling. Quantifying the intrinsic susceptibility of APIs to degradation under forced conditions enables a more systematic and comparative evaluation. This is particularly important given that certain degradation pathways—most notably oxidative degradation and photodegradation—are frequently under-investigated or entirely overlooked in conventional practice.

Moreover, the inclusion of a STABLE pictogram provides an immediate visual summary of an API's degradation profile, highlighting specific vulnerabilities across the five stress conditions. This facilitates rapid interpretation and supports evidence-based decisions regarding formulation strategies and packaging requirements. For instance, APIs identified as photolabile may be prioritized for protection using amber glass containers, while thermally labile compounds may warrant controlled storage conditions. While the calculated scoring in this work was retrospective to demonstrate tool feasibility, STABLE serves as a roadmap for prospective forced degradation studies to ensure a comprehensive assessment of different aspects of the stability of APIs under different stress conditions.

Accordingly, the STABLE metric represents a significant methodological advancement in the field of pharmaceutical stability testing. By offering a standardized, quantitative, and visual assessment across multiple degradation pathways, it fills critical gaps in current regulatory approaches. When used in conjunction with existing guidelines, STABLE contributes to more comprehensive, reproducible, and informative forced degradation studies, thereby supporting enhanced pharmaceutical development, risk assessment, and quality assurance processes.

5. Conclusions

The Stability Toolkit for the Appraisal of Bio/Pharmaceuticals' Level of Endurance (STABLE) represents a significant advancement in the evaluation of pharmaceutical compound stability. By incorporating a multifaceted approach that considers oxidative, thermal, acid-catalyzed hydrolysis, base-catalyzed hydrolysis, and photostability, the STABLE provides a comprehensive analysis of potential degradation pathways. This tool addresses the limitations of traditional stability testing methods by offering a standardized and quantifiable metric for assessing drug stability, thereby reducing inconsistencies across different testing protocols. The color-coded scoring system not only simplifies the interpretation of stability data but also highlights areas where formulation improvements can be made. Establishing a correlation between forced degradation scores and long-term stability would require extensive longitudinal studies across multiple drug classes, which is beyond the scope of the current work but represents an important direction for future research. Moreover, stress testing is mainly conducted in the solution state, which does not necessarily correlate with solid-state stability. However, STABLE can facilitate better decision-making in drug manufacturing and storage by providing critical insights into the stability of APIs, ultimately enhancing the quality, safety, and efficacy of products reaching the market. Future work will focus on refining the STABLE methodology and exploring its application in various drug formulations to further validate its utility in pharmaceutical development.

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References

1. Zelesky, T.; Baertschi, S.W.; Foti, C.; Allain, L.R.; Hostyn, S.; Franca, J.R.; Li, Y.; Marden, S.; Mohan, S.; Ultramari, M.; et al. Pharmaceutical Forced Degradation (Stress Testing) Endpoints: A Scientific Rationale and Industry Perspective. *J. Pharm. Sci.* **2023**, *112*, 2948–2964. [CrossRef] [PubMed]
2. Singh, S.; Junwal, M.; Modhe, G.; Tiwari, H.; Kurmi, M.; Parashar, N.; Sidduri, P. Forced Degradation Studies to Assess the Stability of Drugs and Products. *TrAC Trends Anal. Chem.* **2013**, *49*, 71–88. [CrossRef]
3. Tamizi, E.; Jouyban, A. Forced Degradation Studies of Biopharmaceuticals: Selection of Stress Conditions. *Eur. J. Pharm. Biopharm.* **2016**, *98*, 26–46. [CrossRef] [PubMed]
4. Bhaskar, R.; Ola, M.; Agnihotri, V.; Chavan, A.; Girase, H. Current Trend in Performance of Forced Degradation Studies for Drug Substance and Drug Product's. *J. Drug Deliv. Ther.* **2020**, *10*, 149–155. [CrossRef]
5. Jain, D.; Basniwal, P.K. Forced Degradation and Impurity Profiling: Recent Trends in Analytical Perspectives. *J. Pharm. Biomed. Anal.* **2013**, *86*, 11–35. [CrossRef] [PubMed]
6. Roberto de Alvarenga Junior, B.; Lajarim Carneiro, R. Chemometrics Approaches in Forced Degradation Studies of Pharmaceutical Drugs. *Molecules* **2019**, *24*, 3804. [CrossRef] [PubMed]
7. EL-Malla, S.F.; Mansour, F.R.; Elbastawissy, A.B.B.; Elagamy, S.H. Development of a Stability Indicating High-Performance Liquid Chromatography Method for Determination of Cenobamate: Study of Basic Degradation Kinetics. *BMC Chem.* **2024**, *18*, 74. [CrossRef] [PubMed]
8. Blessy, M.; Patel, R.D.; Prajapati, P.N.; Agrawal, Y.K. Development of Forced Degradation and Stability Indicating Studies of Drugs—A Review. *J. Pharm. Anal.* **2014**, *4*, 159–165. [CrossRef] [PubMed]
9. González-González, O.; Ramirez, I.O.; Ramirez, B.I.; O'Connell, P.; Ballesteros, M.P.; Torrado, J.J.; Serrano, D.R. Drug Stability: ICH versus Accelerated Predictive Stability Studies. *Pharmaceutics* **2022**, *14*, 2324. [CrossRef] [PubMed]
10. Bakshi, M.; Singh, S. Development of Validated Stability-Indicating Assay Methods—Critical Review. *J. Pharm. Biomed. Anal.* **2002**, *28*, 1011–1040. [CrossRef] [PubMed]
11. Mansour, F.R.; Bedair, A.; Belal, F.; Magdy, G.; Locatelli, M. Analytical Green Star Area (AGSA) as a New Tool to Assess Greenness of Analytical Methods. *Sustain. Chem. Pharm.* **2025**, *46*, 102051. [CrossRef]
12. Mansour, F.R.; Płotka-Wasylka, J.; Locatelli, M. Modified GAPI (MoGAPI) Tool and Software for the Assessment of Method Greenness: Case Studies and Applications. *Analytica* **2024**, *5*, 451–457. [CrossRef]
13. Mansour, F.R.; Omer, K.M.; Płotka-Wasylka, J. A Total Scoring System and Software for Complex Modified GAPI (ComplexMoGAPI) Application in the Assessment of Method Greenness. *Green Anal. Chem.* **2024**, *10*, 100126. [CrossRef]
14. Mansour, F.R.; Bedair, A.; Locatelli, M. Click Analytical Chemistry Index as a Novel Concept and Framework, Supported with Open Source Software to Assess Analytical Methods. *Adv. Sample Prep.* **2025**, *14*, 100164. [CrossRef]
15. Mansour, F.R.; Nowak, P.M. Introducing the Carbon Footprint Reduction Index (CaFRI) as a Software-Supported Tool for Greener Laboratories in Chemical Analysis. *BMC Chem.* **2025**, *19*, 121. [CrossRef] [PubMed]
16. Baertschi, S.W.; Alsante, K.M.; Reed, R.A. *Pharmaceutical Stress Testing*; Baertschi, S.W., Alsante, K.M., Reed, R.A., Eds.; CRC Press: Boca Raton, FL, USA, 2016; ISBN 9780429136085.
17. International Council for Harmonisation Photostability Testing of New Drug Substances and Products Q1B. ICH Harmon. *Tripart. Guidel.* 1996. Available online: https://database.ich.org/sites/default/files/Q1B%20Guideline.pdf (accessed on 17 June 2025).
18. Bhukya, V.N.; Beda, D.P. Implementation of Green Analytical Principles to Develop and Validate the HPLC Method for the Separation and Identification of Degradation Products of Panobinostat, and Its Characterization by Using LC-QTOF-MS/MS and Its in-Silico Toxicity Prediction Usin. *Green Anal. Chem.* **2024**, *8*, 100090. [CrossRef]
19. Zaman, B.; Hassan, W. Development of Stability Indicating HPLC–UV Method for Determination of Daclatasvir and Characterization of Forced Degradation Products. *Chromatographia* **2018**, *81*, 785–797. [CrossRef]
20. Kannouma, R.E.; Kamal, A.H.; Hammad, M.A.; Mansour, F.R. Incorporation of Eggshell Waste in the Preparation of Carbon Quantum Dot Nanoprobes for the Determination of COVID-19 Antiviral Drug; Molnupiravir. *Microchem. J.* **2024**, *200*, 110397. [CrossRef]
21. Faruk, O.; Kumar, D.; Noor, D.; Shara, A.; Rouf, S.; Kumar, U. Full Factorial Design Assisted RP-UHPLC Method Development and Study of Stressed Degradation of Molnupiravir. *Drug Anal. Res.* **2023**, *7*, 9–22. [CrossRef]
22. Michalska, K.; Widyńska, W.; Bus, K.; Bocian, W.; Tyski, S. The Solution and Solid-State Degradation Study Followed by Identification of Tedizolid Related Compounds in Medicinal Product by High Performance Liquid Chromatography with Diode Array and Tandem Mass Spectrometry Detection. *J. Pharm. Biomed. Anal.* **2020**, *194*, 113783. [CrossRef] [PubMed]
23. Salakolusu, S.; Sharma, G.V.R.; Katari, N.K.; Puppala, U.; Kaliyapermal, M.; Vijay, R.; Doddipalla1, R.; Geereddi, M.K.R. Identification, Isolation, and Structural Characterization of Novel Forced Degradation Products of Apixaban Using Advanced Analytical Techniques. *J. Sep. Sci.* **2022**, *45*, 3942–3954. [CrossRef] [PubMed]

24. International Council for Harmonisation International Conference on Harmonisation (ICH). *Guidance for Industry: Q1A(R2) Stability Testing of New Drug Substances and Products*; ICH: Geneva, Switzerland, 2003; Volume 4, p. 24.
25. *Food and Drug Administration Guidance for Industry ANDAs: Stability Testing of Drug Substances and Products*; Food and Drug Administration: Silver Spring, MD, USA, 2012.
26. EMA. *Guideline on Stability Testing: Stability Testing of Existing Active Substances and Related Finished Products*; EMA: Amsterdam, The Netherlands, 2023; Volume 31, pp. 1–18.
27. Information, W.D. Stability Testing of Drug Substances and Drug Products. *WHO Drug Inf.* **1999**, *13*, 180. [CrossRef]

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