REVIEW ARTICLE

Digital Transformation of Antiviral Drug Stability Studies: Integration of Quality by Design, Liquid Chromatography Coupled with Tandem Mass Spectrometry, and Artificial Intelligence-Based Predictive Modelling

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Abstract

Antiviral drugs remain essential in combating emerging and re-emerging viral infections; however, many possess chemically labile functional groups that predispose them to hydrolytic, oxidative, thermal, and photolytic degradation. Conventional stability programs, guided by International council for harmonisation of technical requirements for pharmaceuticals for human use (ICH) frameworks, rely on empirical forced degradation studies and long-term storage testing supported by stability-indicating analytical techniques, such as liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). While LC–MS/MS provides superior sensitivity, structural elucidation capability, and impurity profiling performance, traditional approaches are time-intensive, experimentally demanding, and largely retrospective. This review highlights the integration of artificial intelligence (AI) and machine learning (ML) with LC–MS/MS-based degradation profiling to enable predictive and digitally transformed stability programs in antiviral drug development. Classical ML algorithms, including Random Forest, Support Vector Machines, Gradient Boosting, and k-Nearest Neighbors, are discussed for degradation risk classification and kinetic modeling. Advanced deep learning approaches, such as artificial neural networks for degradation rate prediction, convolutional neural networks for MS/MS spectral interpretation, and graph neural networks for molecular instability mapping are presented as transformative tools for identifying degradation hotspots and simulating impurity formation pathways. A closed-loop AI–LC–MS/MS framework is proposed in which molecular structure input drives predictive degradation modeling, simulated impurity structures are generated, LC–MS/MS provides experimental confirmation, and iterative model retraining enhances predictive accuracy. Integration of Quality by Design strategies with LC–MS/MS platforms enables optimized method performance, improved impurity detection, and enhanced understanding of degradation mechanisms. Incorporation with predictive shelf-life modeling, real-time stability monitoring, and risk-based testing aligns this approach with ICH Q1A, Q2, Q12, and Q14 principles. Collectively, AI-enabled digital stability systems represent a paradigm shift toward proactive, lifecycle-based, and data-driven antiviral drug stability assessment.

Key words: Antiviral drugs, artificial intelligence, digital stability programs, ICH guidelines, liquid chromatography coupled with tandem mass spectrometry, predictive degradation modeling

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INTRODUCTION

Viral infections continue to represent one of the most significant global public health challenges of the 21st century. The recurrent emergence of novel viral pathogens, the re-emergence of previously controlled infections, and the increasing viral resistance to available therapies have intensified the demand for effective antiviral pharmacotherapy.[1] The global impact of outbreaks, such as those caused by severe acute respiratory syndrome coronavirus 2, Human immunodeficiency virus, Hepatitis B virus, and Influenza A virus underscores the critical importance of robust antiviral drug development and lifecycle management strategies.[2] These pathogens are associated with substantial morbidity, mortality, and socioeconomic disruption, particularly in low- and middle-income countries.[3] The therapeutic management of viral infections relies heavily on small-molecule antiviral agents, many of which contain chemically labile functional groups designed to interact selectively with viral enzymes or replication machinery.[4] While these structural features enhance pharmacological specificity, they often increase susceptibility to hydrolytic, oxidative, thermal, or photolytic degradation.[5] For instance, nucleotide analogues, such as Remdesivir and protease inhibitors, such as Ritonavir contain ester, amide, or heterocyclic moieties that may undergo degradation under stress conditions.[6,7] Degradation not only reduces therapeutic potency but may also generate potentially toxic or pharmacologically inactive impurities. Consequently, ensuring chemical stability throughout manufacturing, storage, and distribution is essential to guarantee drug safety, efficacy, and regulatory compliance.[8]

Stability testing programs, guided by international regulatory frameworks, such as ICH Q1A and Q2 guidelines, are therefore integral to pharmaceutical development. These programs typically involve forced degradation studies, accelerated stability testing, long-term storage evaluation, and analytical method validation.[9] Stability-indicating analytical techniques, most notably liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS), are employed to monitor active pharmaceutical ingredient (API) integrity and detect degradation products at trace levels.[10] While these traditional stability programs have proven effective, they are inherently experimental, time-consuming, and resource-intensive. They rely heavily on empirical stress testing and retrospective data interpretation, often identifying degradation pathways only after significant laboratory investigation.[11]

Moreover, the increasing structural complexity of modern antiviral agents and the growing volume of analytical data generated by high-resolution LC–MS/MS systems present new challenges.[12] Data interpretation, impurity identification, and kinetic modeling demand sophisticated analytical expertise and computational resources. Conventional approaches may struggle to fully exploit the multidimensional datasets produced during stability studies, limiting predictive insight and proactive risk assessment.[13]

In this context, there is a compelling need for predictive and digitally enabled stability frameworks that can complement experimental methodologies. Artificial intelligence (AI) and machine learning (ML) technologies offer transformative potential in this.[14] By leveraging molecular descriptors, historical stability datasets, and mass spectral information, AI models can predict degradation hotspots, simulate impurity formation pathways, and estimate shelf-life parameters before extensive laboratory testing.[15] Such predictive systems can reduce experimental burden, accelerate development timelines, and enhance regulatory decision-making.

The integration of AI-driven predictive modeling with LC–MS/MS-based experimental validation represents a paradigm shift from reactive stability assessment toward proactive, data-informed pharmaceutical lifecycle management.[16] Quality by design (QbD) is a systematic, science-based framework for pharmaceutical development that focuses on understanding and controlling variability through pre-defined objectives, risk assessment, and structured experimentation. In analytical and stability studies, the integration of QbD with advanced techniques, such as LC–MS/MS and AI enables robust stability-indicating methods, improved degradation profiling, and more predictive, data-driven stability assessment.[11]

This review explores the emerging convergence of advanced analytical techniques, QbD, and AI in the context of antiviral drug stability, highlighting opportunities, challenges, and future directions for digital transformation in pharmaceutical analysis may be limited.

CHEMICAL INSTABILITY CHALLENGES IN ANTIVIRAL DRUGS

The chemical stability of antiviral drugs is strongly influenced by their structural complexity and the presence of reactive functional groups essential for antiviral activity. Many antiviral agents are nucleoside or nucleotide analogues, prodrugs, or enzyme inhibitors designed to undergo intracellular activation.[17] While these structural features enhance therapeutic efficacy, they frequently introduce chemical liabilities that predispose the molecules to degradation under environmental or physiological stress conditions. Understanding the intrinsic instability mechanisms of representative antiviral drugs, such as Remdesivir, Famciclovir, and Oseltamivir [Figure 1] is critical for designing robust stability-indicating analytical methods and predictive degradation models.[18]

Ester hydrolysis

Ester functional groups are commonly incorporated into antiviral agents either as part of prodrug strategies or to enhance

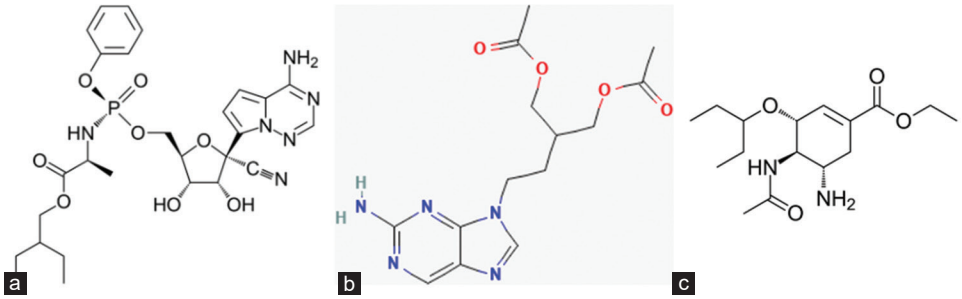

Figure 1: Molecular structures of (a) Remdesivir, (b) Famciclovir, and (c) Oseltamivir

membrane permeability.[19] However, esters are inherently susceptible to hydrolytic cleavage under acidic, basic, or even neutral aqueous conditions. For example, Remdesivir contains ester moieties within its phosphoramidate prodrug structure. These groups are designed to facilitate intracellular activation but are chemically labile during formulation and storage.[20] Hydrolytic degradation may occur through nucleophilic attack by water molecules, leading to cleavage of the ester bond and formation of inactive or partially active metabolites. The rate of hydrolysis is influenced by pH, temperature, ionic strength, and solvent composition, making ester-containing antivirals particularly sensitive during accelerated stability studies.[21]

Similarly, Oseltamivir possesses an ethyl ester functionality that can undergo hydrolysis to yield the corresponding carboxylic acid derivative. While this transformation is pharmacologically relevant *in vivo* (as part of metabolic activation), pre-mature hydrolysis during storage can compromise dosage accuracy and product performance.[22] From a mechanistic perspective, ester hydrolysis proceeds through acid- or base-catalyzed pathways involving tetrahedral intermediates. Predictive modeling of hydrolytic susceptibility requires consideration of steric hindrance, electronic effects, and local molecular environment parameters that can be quantitatively described using molecular descriptors in AI-based degradation prediction systems.[23]

Amide cleavage

Amide bonds are generally more stable than esters; however, they may undergo cleavage under extreme pH conditions or prolonged exposure to elevated temperatures. Amide hydrolysis involves nucleophilic attack at the carbonyl carbon, resulting in the formation of carboxylic acids and amines.[24]

In nucleotide analogues, such as Remdesivir, amide linkages contribute to structural rigidity and target specificity. Nevertheless, forced degradation studies have demonstrated that under strong acidic or basic stress, amide cleavage may occur, generating structurally distinct degradation products detectable by LC–MS/MS.[25]

Famciclovir, a prodrug of penciclovir, contains amide and heterocyclic functionalities that may undergo degradation under hydrolytic or thermal stress. Although relatively stable under recommended storage conditions, extreme environments can promote bond cleavage or rearrangement reactions.[24]

From an analytical perspective, amide cleavage products often exhibit distinct mass-to-charge (m/z) ratios and fragmentation patterns, facilitating identification through MS/MS. Incorporating AI-assisted fragmentation prediction tools can further enhance structural elucidation and impurity profiling in such cases.[26]

Oxidative vulnerability

Oxidative degradation represents a major stability concern for antiviral drugs containing electron-rich heterocycles, secondary amines, or unsaturated systems. Oxidative stress may arise from exposure to oxygen, peroxides in excipients, trace metal contaminants, or light-induced radical formation.[27]

Famciclovir contains purine-based structures that are potentially susceptible to oxidative transformation. Oxidation may lead to hydroxylated derivatives or ring-opened products, altering pharmacological activity and potentially introducing toxicological risks.[28]

In Oseltamivir, the presence of amine and unsaturated moieties can facilitate oxidation under peroxide stress. Oxidative degradation mechanisms often involve radical intermediates, electron transfer reactions, or peroxide-mediated transformations.[29]

Predictive assessment of oxidative vulnerability can be supported by computational evaluation of electron density distribution, highest occupied molecular orbital (HOMO) energy levels, and electrophilicity indices. ML models trained on historical oxidative degradation datasets can classify molecules according to oxidative risk and predict probable degradation sites before experimental validation.[30]

Photolabile groups

Exposure to ultraviolet (UV) or visible light can initiate photochemical reactions in antiviral drugs containing

conjugated systems or chromophoric functional groups. Photodegradation may involve bond cleavage, isomerization, or rearrangement reactions.

For instance, heterocyclic rings present in Remdesivir and Famciclovir can absorb UV radiation, triggering photochemical instability under uncontrolled light exposure. Photodegradation products often exhibit altered chromatographic retention behavior and distinct MS/MS fragmentation profiles, necessitating comprehensive spectral analysis.[31]

Photostability testing under ICH Q1B guidelines is therefore essential for antiviral drug products, particularly those formulated in transparent packaging or exposed to light during manufacturing. AI-based spectral pattern recognition systems can assist in rapidly identifying photodegradation products by comparing experimental mass spectra with predicted fragmentation libraries.[15]

ROLE OF LC–MS/MS IN DEGRADATION PROFILING

LC–MS/MS represents the most advanced and widely adopted analytical platform for stability-indicating evaluation of antiviral drugs. Its integration of chromatographic separation with mass-selective detection enables comprehensive characterization of degradation products formed under various stress conditions. In modern pharmaceutical stability programs, LC–MS/MS serves not only as a confirmatory analytical tool but also as a critical data source for emerging AI-driven predictive models.[12]

Structural elucidation

One of the principal roles of LC–MS/MS in degradation profiling is the structural elucidation of unknown impurities. Following chromatographic separation, the mass spectrometer provides molecular ion information that allows accurate determination of the molecular weight of degradation products.[32] High-sensitivity detection enables identification of even trace-level impurities that may form during hydrolytic, oxidative, or photolytic stress testing. For antiviral drugs, such as Remdesivir, Famciclovir, and Oseltamivir, even minor structural modifications can significantly influence therapeutic performance and safety.[33] LC–MS/MS facilitates differentiation between parent drug and transformation products by identifying characteristic mass shifts, isotopic distributions, and molecular ion patterns.[12] This capability is essential for meeting regulatory requirements that mandate identification and qualification of impurities above specified thresholds.

Fragmentation analysis

MS/MS further enhances structural characterization through fragmentation analysis. In MS/MS experiments [Figure 2], selected pre-cursor ions undergo collision-induced dissociation, generating product ions that reveal substructural information. The fragmentation pattern provides insight into bond cleavage pathways and helps localize degradation sites within the molecule. Ester hydrolysis products typically exhibit loss of alkoxy fragments, while amide cleavage results in distinct carboxylic or amine-derived fragment ions.[34] Oxidative transformations frequently produce characteristic mass increments corresponding to oxygen addition. The interpretative value of these fragmentation pathways makes LC–MS/MS indispensable for confirming degradation mechanisms proposed during forced degradation studies.[33]

The increasing adoption of computational tools has further strengthened fragmentation analysis. Deep learning-based spectral prediction algorithms can now assist in annotating complex MS/MS spectra, reducing interpretation time and supporting automated impurity identification workflows.

High-resolution mass spectrometry (HRMS)

HRMS, typically implemented using Orbitrap or Q-TOF systems, significantly enhances confidence in structural assignment. Accurate mass measurements with parts-per-million precision allow determination of the exact elemental composition of degradation products.[35] This is particularly important for structurally complex antiviral drugs, where small mass differences may correspond to distinct chemical modifications. HRMS enables differentiation between closely related impurity species, confirmation of oxidative additions, and detection of minor degradation products that may otherwise remain unresolved. The precision of HRMS datasets also makes them highly valuable for training ML models aimed at predicting degradation patterns.[36]

Impurity tracking and profiling

Beyond structural identification, LC–MS/MS plays a critical role in monitoring impurity formation during stability studies.[33] By analyzing samples collected at multiple time points under defined stress conditions, it becomes possible to track the decline of the parent compound and the corresponding growth of degradation products. Such impurity profiling supports establishment of mass balance and elucidation of dominant degradation pathways. Chromatographic overlays often reveal emerging peaks corresponding to stress-specific degradation products, enabling differentiation between hydrolytic, oxidative, thermal, and photolytic degradation routes.[37]

When integrated with chemometric tools, LC–MS/MS datasets can be subjected to multivariate analysis to detect subtle impurity trends. This data-driven approach enhances early identification of stability risks and supports more informed formulation optimization.

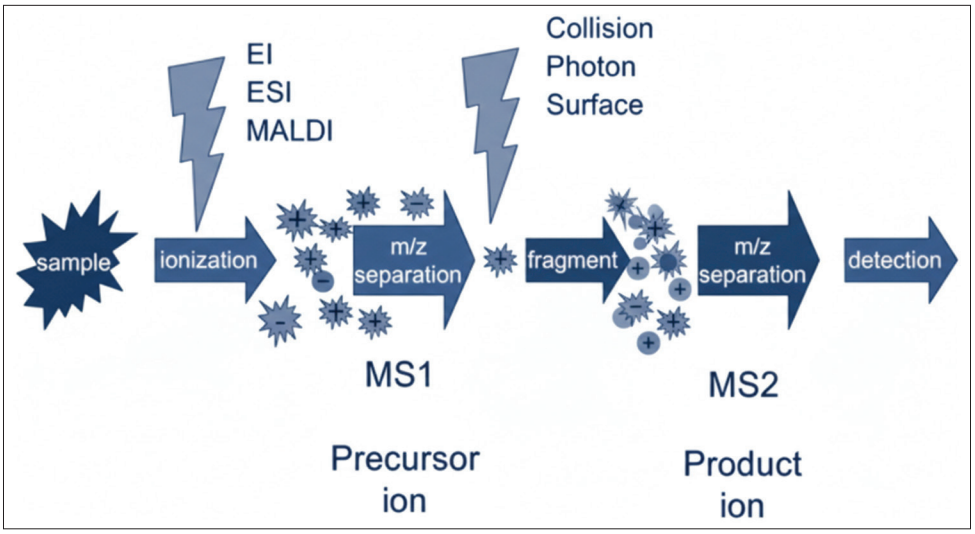

Figure 2: Schematic representation of tandem mass spectrometry workflow

Degradation kinetic studies

LC–MS/MS also facilitates degradation kinetic evaluation through accurate quantification of both the API and its degradation products over time.[12] Time-resolved data allow calculation of degradation rate constants, determination of reaction order, and estimation of activation energy using Arrhenius modeling. Such kinetic studies are fundamental for shelf-life assignment and prediction of long-term stability based on accelerated testing conditions. In digitally enabled stability programs, these kinetic datasets can be incorporated into ML regression models to predict impurity growth trajectories and remaining shelf life with improved efficiency.[38]

Limitations of LC–MS/MS in stability programs

Despite its analytical sophistication, LC–MS/MS-based degradation profiling is associated with several limitations. Method development can be time-intensive, requiring extensive optimization of chromatographic gradients, mobile phase composition, column selection, and ionization parameters.[36] Comprehensive forced degradation studies necessitate multiple stress experiments and replicate analyses, increasing laboratory workload and instrument utilization. Furthermore, modern high-resolution systems generate large and complex datasets, including detailed fragmentation trees and chromatographic profiles that demand expert interpretation. Manual analysis of such multidimensional data can be laborious and may introduce variability in interpretation.[39]

These limitations highlight the need for integration of AI and ML strategies into stability workflows. By leveraging the rich datasets generated through LC–MS/MS, predictive algorithms can assist in degradation pathway forecasting, automated spectral annotation, and kinetic modeling. Consequently, LC–MS/MS evolves from a predominantly reactive analytical platform into a central component of a predictive, digitally integrated stability assessment framework for antiviral drug development.

QbD IN STABILITY-INDICATING ANALYTICAL METHOD DEVELOPMENT

Analytical target profile (ATP)

The ATP represents the foundational element of the analytical QbD (AQbD) framework and defines the intended purpose and performance requirements of an analytical method. Similar to the quality target product profile used in formulation development, the ATP establishes pre-defined objectives that the analytical procedure must consistently achieve throughout its lifecycle. In the context of stability-indicating methods, the ATP specifies the ability of the analytical method to accurately detect, separate, and quantify the API and its potential degradation products under various stress conditions.[40]

For stability studies of antiviral drugs, the ATP typically includes criteria, such as adequate chromatographic resolution between the API and degradation products, sufficient sensitivity to detect impurities at regulatory threshold levels, and reliable quantification of degradation products during forced degradation and long-term stability testing.[41] In LC–MS/MS-based stability assessment, the ATP may further incorporate requirements for mass accuracy, fragmentation consistency, and detection limits necessary for confident structural elucidation of degradation products.[38] Establishing a well-defined ATP ensures that analytical development is guided by clearly defined scientific objectives and regulatory expectations from the earliest stages of method development.

Critical method attributes (CMAs)

CMAs are measurable analytical characteristics that directly influence the ability of the analytical method to meet the objectives defined in the ATP. Identification and control of CMAs are essential for ensuring that the analytical method consistently delivers reliable and accurate results throughout its lifecycle.[42]

In LC–MS/MS-based degradation profiling, several CMAs play a crucial role. Chromatographic resolution is one of the most important attributes, as it ensures effective separation between the parent drug and its degradation products. Insufficient resolution can lead to peak overlap and inaccurate impurity quantification. Sensitivity is another key attribute, particularly for detecting trace-level degradation products that may arise during stability studies. Analytical methods must be capable of detecting impurities at concentrations consistent with regulatory impurity thresholds.

Peak purity assessment is also critical for confirming that chromatographic peaks represent single chemical entities rather than co-eluting impurities.[43] In mass spectrometry-based methods, mass accuracy becomes a vital CMA because precise mass measurements facilitate confident identification of degradation products and confirmation of elemental composition. Maintaining control over these attributes ensures that the analytical method provides reliable degradation profiling throughout stability studies.[35]

Critical method parameters (CMPs)

CMPs refer to experimental variables that influence the performance of the analytical method and, consequently, affect the CMAs. Identification and optimization of CMPs are central to the AQbD approach, as variations in these parameters may significantly impact chromatographic separation, sensitivity, and reproducibility.[44]

In LC–MS/MS methods used for stability studies, several CMPs must be carefully evaluated. Mobile phase composition strongly affects chromatographic retention, peak shape, and ionization efficiency in mass spectrometry. Selection of appropriate solvents, buffer systems, and pH conditions is therefore essential for achieving optimal separation of the API and degradation products. Column chemistry and stationary phase characteristics also play an important role in determining selectivity and retention behaviour.[34]

Gradient profile and flow rate are additional parameters that influence chromatographic resolution and analysis time. In the mass spectrometer, ionization conditions, such as electrospray voltage, nebulizer gas flow, and source temperature significantly affect signal intensity and sensitivity. Other MS parameters, including collision energy and scan settings, influence fragmentation patterns used for structural elucidation of degradation products.[32] Systematic evaluation of these parameters enables the identification of optimal analytical conditions that ensure robust and reliable method performance.

Risk assessment in AQbD

Risk assessment is an integral component of the QbD framework and serves to identify factors that may potentially affect analytical method performance. By systematically evaluating sources of variability, risk assessment allows researchers to prioritize critical variables and focus experimental efforts on parameters that have the greatest impact on method reliability.[41]

Several tools are commonly used in analytical risk assessment. The Ishikawa diagram, also known as a fishbone diagram, provides a visual representation of potential factors influencing analytical performance.[45] These factors may include instrument settings, mobile phase composition, column characteristics, and sample preparation procedures. Another widely used method is failure mode and effects analysis, which quantitatively evaluates potential failure points by assigning scores based on severity, probability of occurrence, and detectability.[46] This approach generates a risk priority number that helps identify parameters requiring further investigation.

Risk prioritization enables efficient allocation of experimental resources and supports systematic method optimization. In stability-indicating methods, risk assessment is particularly important because analytical variability may obscure detection of degradation products or lead to inaccurate impurity quantification.

Design of experiments (DoE)

DoE is a statistical methodology that allows simultaneous evaluation of multiple analytical parameters and their interactions. Unlike traditional one-factor-at-a-time approaches, DoE enables a more comprehensive understanding of how various experimental variables influence analytical performance.[47]

In AQbD, factorial designs are commonly employed during early-stage screening experiments to identify significant factors affecting method performance. These designs allow researchers to evaluate the influence of multiple variables simultaneously while minimizing the number of experimental runs required. Response surface methodology is often used in subsequent optimization studies to determine the optimal operating conditions for analytical parameters. This approach generates mathematical models that describe relationships between experimental factors and analytical responses, such as resolution, retention time, or signal intensity.[44]

When applied to LC–MS/MS-based degradation profiling, DoE facilitates systematic optimization of chromatographic and mass spectrometric parameters. By evaluating interactions between mobile phase composition, column temperature, gradient conditions, and ionization settings, DoE enables development of highly robust stability-indicating methods with improved analytical reliability.

Method operable design region (MODR)

The MODR, also referred to as the analytical design space, represents the multidimensional combination of method parameters that consistently produce acceptable analytical performance. Within this region, variations in analytical parameters do not significantly affect the method's ability to meet ATP requirements.[48]

Establishing the MODR provides several advantages for analytical method development. First, it ensures robustness by identifying parameter ranges within which the method remains stable and reliable. Second, it provides regulatory flexibility, as adjustments made within the design space are generally not considered regulatory changes. This aligns with regulatory expectations for lifecycle management of analytical methods.[42]

In LC–MS/MS stability studies, defining the MODR ensures that minor variations in chromatographic conditions, ionization parameters, or instrument settings do not compromise detection of degradation products. Consequently, MODR-based analytical methods demonstrate improved reliability during long-term stability testing and routine quality control.[33]

Integration of QbD with AI-based stability modeling

The integration of QbD principles with AI-based predictive modeling represents an emerging paradigm in pharmaceutical stability assessment. While QbD provides a structured framework for systematic analytical method development, AI technologies enable predictive interpretation of complex analytical datasets.

ML algorithms can analyze large LC–MS/MS datasets generated during stability studies to identify degradation trends, predict impurity formation pathways, and estimate degradation kinetics. When combined with QbD-based experimental design strategies, these predictive models can guide targeted method optimization and reduce experimental burden.

Furthermore, AI-driven predictive models can utilize datasets generated through DoE to identify optimal analytical conditions and detect hidden relationships between analytical parameters and degradation outcomes. This synergy between QbD and AI enables a data-driven approach to analytical development, where experimental design, predictive modeling, and stability assessment operate within an integrated framework.

Ultimately, the combination of QbD methodology with AI-assisted analytical systems enhances analytical robustness, improves degradation pathway understanding, and supports the development of proactive and digitally enabled pharmaceutical stability programs.

AI IN PREDICTIVE DEGRADATION MODELING

The integration of AI into pharmaceutical stability assessment represents a transformative shift from reactive degradation analysis to predictive, data-driven modelling.[14] Traditionally, degradation pathways of antiviral drugs were elucidated only after extensive forced degradation studies followed by LC–MS/MS characterization.[33] In contrast, AI-based predictive degradation modeling enables the identification of instability hotspots, simulation of stress conditions, and estimation of impurity formation risk before comprehensive experimental testing.[40] When combined with high-quality LC–MS/MS datasets, AI systems create a closed-loop framework in which predictions are continuously refined through experimental validation.

ML models

Conventional ML algorithms have demonstrated strong potential in modeling degradation susceptibility and predicting impurity formation patterns. These models typically operate on structured datasets containing molecular descriptors, physicochemical parameters, and experimentally derived stability data.[49]

Random forest models are particularly useful for degradation prediction because they handle non-linear relationships and complex variable interactions efficiently. By constructing multiple decision trees and aggregating their outputs, random forest algorithms can classify antiviral molecules according to hydrolytic or oxidative risk and rank structural features based on importance. This interpretability is valuable for identifying functional groups contributing most significantly to instability.[50]

Support vector machines (SVM) are effective in binary and multiclass classification tasks, such as distinguishing stable from unstable compounds under specific stress conditions.[51] SVM models perform well with high-dimensional descriptor data and can identify optimal decision boundaries even when the dataset is limited. In antiviral drug stability assessment, SVM models can predict degradation probability under acidic, basic, or oxidative stress conditions.

Gradient boosting algorithms, including XGBoost and LightGBM frameworks, enhance predictive performance by sequentially correcting errors made by prior models.[52] These models are particularly advantageous in regression tasks, such as predicting degradation rate constants or impurity growth percentages. Their ability to capture subtle non-linear patterns makes them suitable for stability kinetics modeling.

k-nearest neighbors (kNN) operates by classifying a compound based on similarity to structurally related molecules in the dataset. Although comparatively simple, kNN can be effective in early-stage risk assessment when structurally analogous antiviral compounds with known stability profiles are available.[53]

Collectively, these ML approaches provide robust tools for classification, regression, and risk prediction in degradation modeling workflows.

Deep learning approaches

Deep learning architectures extend beyond traditional ML by enabling automated feature extraction and handling of complex, high-dimensional data, such as mass spectra and molecular graphs.[54]

Artificial neural networks (ANNs) are widely applied in predicting degradation kinetics. By learning non-linear relationships between molecular descriptors and experimentally observed degradation rates, ANNs can estimate rate constants under varied stress conditions.[55] These models are particularly useful for shelf-life prediction and accelerated stability modeling.

Convolutional neural networks (CNNs), originally developed for image processing, are increasingly applied to mass spectrometry data interpretation. When MS/MS spectra are treated as intensity-distribution patterns, CNNs can automatically detect fragmentation signatures associated with specific degradation pathways.[56] This significantly accelerates impurity identification and reduces manual spectral interpretation burden.

Graph neural networks (GNNs) represent one of the most advanced approaches for molecular instability mapping. In GNN models, molecules are treated as graphs where atoms represent nodes and chemical bonds represent edges.[57] This architecture enables the model to learn directly from molecular structure without relying solely on pre-defined descriptors. GNNs can predict reactive sites, identify likely bond cleavage locations, and estimate degradation propensity under oxidative or hydrolytic stress. For structurally complex antivirals, such as Remdesivir and Famciclovir, GNN-based mapping can reveal instability hotspots that align with experimentally observed LC–MS/MS degradation data.[58]

Molecular descriptor-based prediction

Molecular descriptor-based modeling forms the quantitative backbone of AI-driven degradation prediction. These descriptors numerically represent chemical properties influencing stability.

The pKa of ionizable groups strongly affects hydrolytic susceptibility, as protonation state influences electrophilicity and nucleophilic attack probability. Antiviral compounds with ester functionalities may exhibit accelerated hydrolysis under pH conditions favoring protonated intermediates.[51]

The HOMO–LUMO energy gap provides insight into molecular reactivity. A smaller gap generally indicates higher chemical reactivity and potential susceptibility to oxidative or photochemical degradation.[59] Computational chemistry tools can calculate these energy levels, which are then incorporated into ML models.

The electrophilicity index quantifies a molecule's ability to accept electrons, providing predictive insight into oxidation-prone sites. Molecules containing electron-rich heterocycles may demonstrate elevated oxidative risk when electrophilicity parameters indicate vulnerability.

Steric hindrance influences accessibility of reactive functional groups. Bulky substituents surrounding ester or amide bonds may reduce hydrolysis rates by limiting solvent accessibility. Descriptor-based models integrate steric parameters, such as solvent-accessible surface area to refine predictive accuracy.[34]

By combining these descriptors with supervised learning algorithms, predictive degradation modeling can move from qualitative risk assessment to quantitative probability estimation.

AI-based forced degradation simulation

Beyond risk prediction, AI systems can simulate forced degradation scenarios computationally. This represents a major innovation in digital stability programs.

Predictive models can estimate oxidative stress susceptibility by identifying electron-dense regions likely to undergo radical attack. Simulation outputs may suggest probable oxidation products, enabling targeted LC–MS/MS validation rather than broad empirical screening.[60]

Hydrolytic breakdown simulation can identify ester or amide bonds most vulnerable under acidic or basic conditions. Such predictions assist in prioritizing stress experiments and optimizing pH-dependent stability studies.[61]

Impurity formation risk can be modeled through generative algorithms that propose likely degradation structures

based on reaction rules and structural similarity to known degradation products. These predictions can then be cross-validated experimentally.[62]

The integration of AI-based simulation with LC–MS/MS validation creates a predictive–experimental feedback loop.[14] Molecular input data are processed through AI models to generate degradation hypotheses, which are then experimentally verified. The resulting data refine and retrain the models, progressively enhancing predictive accuracy.

DIGITAL STABILITY PROGRAMS IN ANTIVIRAL DRUG DEVELOPMENT

The evolution of pharmaceutical stability assessment from conventional, time-bound studies toward digitally enabled, predictive systems represents a major paradigm shift in antiviral drug development.[63] Traditional stability programs are largely empirical, relying on pre-defined storage conditions and periodic testing schedules. While scientifically robust, these approaches can be resource-intensive and slow to adapt to emerging risks. Digital stability programs integrate AI, advanced analytics, and real-time monitoring technologies to create a proactive, lifecycle-oriented stability framework that aligns with modern regulatory expectations.[64]

Predictive shelf-life modeling

Predictive shelf-life modeling uses statistical, kinetic, and ML approaches to estimate product stability beyond experimentally observed time points.[65] In conventional stability studies, degradation data collected under accelerated conditions are extrapolated using Arrhenius-based kinetic models to estimate long-term shelf life. Digital stability programs extend this concept by incorporating multivariate datasets, including formulation variables, environmental parameters, packaging effects, and historical degradation trends.[66]

ML regression algorithms can be trained on LC–MS/MS-generated impurity profiles to predict degradation rate constants and future impurity levels with improved precision. These models can integrate stress-specific degradation pathways, such as hydrolysis or oxidation and generate probabilistic shelf-life estimates. This approach reduces dependency on prolonged real-time studies while maintaining scientific rigor and regulatory defensibility.

Big data analytics in stability studies

Modern LC–MS/MS systems generate extensive multidimensional datasets, including high-resolution mass spectra, fragmentation trees, chromatographic peak distributions, and time-resolved impurity trends. Digital stability platforms leverage big data analytics to process and interpret these complex datasets efficiently.[67]

Advanced chemometric techniques and ML classification models can identify hidden patterns in impurity growth, detect early deviation from expected stability profiles, and cluster degradation pathways based on structural similarity. Integration of historical stability datasets across multiple antiviral products enhances predictive capability and supports cross-product risk assessment. The transition from isolated batch-wise analysis to aggregated data intelligence enables continuous improvement of predictive degradation models.[68]

Real-time stability monitoring

Real-time stability monitoring represents a significant advancement over traditional periodic sampling approaches.[69] Through integration of process analytical technology, inline sensors, and automated LC–MS systems, it becomes possible to monitor degradation behavior dynamically during manufacturing and storage.

Digital systems can continuously analyze environmental parameters, such as temperature and humidity and correlate them with degradation kinetics. AI-driven anomaly detection algorithms can identify unexpected impurity formation in real time, enabling early corrective action. Such proactive monitoring enhances product quality assurance and reduces the likelihood of out-of-specification results during routine stability testing.[70]

Continuous manufacturing integration

Continuous manufacturing platforms generate a steady stream of production and analytical data. When integrated with digital stability programs, this data flow can support predictive degradation modeling at scale.[71] Process parameters, such as temperature profiles, solvent exposure times, and drying conditions can be linked to impurity formation trends observed in LC–MS/MS analysis.

AI algorithms can model relationships between manufacturing variables and chemical instability, allowing predictive adjustment of process parameters to minimize degradation. This integration enhances robustness, supports real-time release testing, and aligns with the pharmaceutical industry's transition toward continuous processing technologies.[72]

AI-enabled risk-based stability testing

Risk-based stability testing moves beyond fixed testing intervals and stress conditions toward adaptive, data-driven study design. AI models can evaluate molecular structure, formulation composition, packaging characteristics, and

historical degradation data to predict likely instability pathways. Based on this risk assessment, stress testing conditions can be prioritized, and unnecessary experiments minimized.[73]

For antiviral drugs with known susceptibility to hydrolytic or oxidative degradation, predictive models can guide selection of specific stress conditions rather than applying uniform forced degradation protocols. This targeted approach reduces experimental burden while maintaining comprehensive stability coverage.[74]

Regulatory alignment and ICH framework

Digital stability programs must operate within established international regulatory frameworks to ensure acceptability and compliance.

The guideline ICH Q1A provides foundational principles for stability testing of new drug substances and products. While traditionally focused on defined storage conditions and time points, its framework allows incorporation of scientifically justified predictive modeling approaches when supported by robust data.[9]

ICH Q2 outlines validation requirements for analytical procedures. AI-assisted LC–MS/MS methods must demonstrate accuracy, precision, specificity, robustness, and reproducibility consistent with Q2 principles. Model validation and performance verification become essential components of digital stability systems.

ICH Q14 emphasizes science- and risk-based analytical development.[75] The integration of ML for method optimization and degradation prediction aligns strongly with Q14's emphasis on knowledge management and method lifecycle control.

ICH Q12 supports a structured approach to pharmaceutical product lifecycle management. Digital stability platforms, with continuous data integration and predictive analytics, are particularly compatible with Q12 principles by enabling post-approval change management and ongoing stability assurance.[76]

INTEGRATION FRAMEWORK: CLOSED-LOOP AI–LC–MS/MS SYSTEM

The true novelty of AI-driven degradation modeling lies not merely in prediction but in the establishment of a closed-loop predictive experimental framework. In this system, AI and LC–MS/MS operate synergistically, continuously refining degradation understanding through iterative feedback. Rather than functioning as isolated tools, computational models and analytical platforms become components of an integrated digital stability ecosystem.[77]

The workflow begins with molecular structure input. The chemical structure of the antiviral drug is encoded in machine-readable formats, such as Simplified Molecular Input Line Entry System or molecular graphs. Structural descriptors, quantum chemical parameters, and physicochemical properties are computed automatically. These include ionization constants, electron density distribution, steric accessibility, and frontier molecular orbital energies.[78]

Based on this input, AI algorithms predict degradation hotspots. ML and GNN models analyze bond environments to identify regions most susceptible to hydrolysis, oxidation, photolysis, or thermal stress.[79] Reactive functional groups, such as esters, amides, and electron-rich heterocycles are ranked according to predicted vulnerability. The model may output probability scores for specific degradation pathways under defined stress conditions.[16]

Subsequently, simulated impurity structures are generated. Reaction-rule engines and generative AI models propose likely degradation products derived from predicted bond cleavage or oxidative transformation events. These computationally generated impurity structures are assigned theoretical molecular weights and predicted MS/MS fragmentation patterns.[80]

The next stage involves LC–MS/MS confirmation. Experimental forced degradation studies are designed in a targeted manner based on predicted vulnerabilities. HRMS validates the presence or absence of predicted impurities and refines structural assignments through fragmentation analysis. This targeted approach reduces experimental redundancy and enhances analytical efficiency.[81]

Model retraining follows experimental validation. Newly generated LC–MS/MS datasets, including confirmed impurity structures and kinetic data, are fed back into the AI model. Continuous retraining improves predictive accuracy, reduces bias, and adapts the model to molecule-specific behaviors. Over time, the system evolves into a highly specialized predictive engine for antiviral drug stability.[82]

Using the refined dataset, shelf-life prediction is performed. ML regression models incorporate kinetic parameters, environmental factors, and impurity growth trends to estimate long-term stability. Predictive modeling may reduce reliance on extended real-time studies while maintaining scientific robustness.[83]

Finally, regulatory documentation is generated within a structured digital framework. Data traceability, model validation metrics, and experimental confirmations are compiled to support regulatory submissions. This integrated system aligns predictive modeling outputs with established ICH stability requirements, enhancing transparency and defensibility. This closed-loop architecture transforms stability assessment from a reactive analytical exercise into a continuously learning, data-driven system.

QbD principles further strengthen the proposed closed-loop AI–LC–MS/MS framework by ensuring systematic experimental design and analytical robustness. Within this framework, risk assessment tools can identify critical degradation pathways, while design-of-experiments strategies optimize analytical parameters for impurity detection. The resulting design space supports consistent analytical performance and provides structured datasets that improve the training and validation of ML models.

CHALLENGES AND REGULATORY CONSIDERATIONS

Despite its transformative potential, implementation of AI-integrated stability systems presents several scientific and regulatory challenges. Data bias in AI models represents a primary concern. ML algorithms are highly dependent on training datasets. If degradation datasets are limited in chemical diversity or stress conditions, predictive accuracy may decline for novel antiviral structures. Bias toward historically studied molecules may limit generalizability.[84]

Validation of AI predictions is another critical requirement. Regulatory acceptance demands demonstration of model reliability, reproducibility, and statistical robustness. Performance metrics, such as accuracy, sensitivity, specificity, and external validation against independent datasets must be clearly documented.

Regulatory acceptability remains an evolving area. While international guidelines encourage science- and risk-based approaches, explicit frameworks for AI model qualification are still under development. Clear documentation of algorithm architecture, training data sources, and validation procedures will be essential for regulatory review.[85]

Data integrity is central to digital stability programs. Compliance with data governance principles, audit trails, cybersecurity measures, and traceability requirements must be ensured. Integration with validated laboratory information management systems and secure data repositories becomes essential.[45] Model explainability is particularly important in pharmaceutical contexts. Black-box algorithms may face resistance if they cannot provide mechanistic justification for predictions. Techniques, such as feature importance analysis, SHAP values, and interpretable modeling frameworks should be incorporated to provide transparency regarding predictive drivers.[86-88] Addressing these challenges is essential for successful implementation of AI–LC–MS/MS systems within regulatory-compliant pharmaceutical environments. Regulatory frameworks increasingly encourage the adoption of QbD principles in analytical method development and stability assessment.

FUTURE PERSPECTIVES

The future of digital stability assessment in antiviral drug development is closely linked to advances in generative AI and integrated analytical automation. Generative AI for impurity prediction represents a major emerging opportunity. Large language models and reaction-based generative networks can propose plausible degradation products by learning chemical transformation rules from extensive reaction databases. Such systems may eventually predict entire degradation pathways without pre-defined reaction templates.

Digital twins for stability modeling constitute another forward-looking concept. A digital twin is a virtual replica of a physical product that dynamically simulates degradation behavior under varying environmental conditions. By integrating kinetic models, molecular descriptors, and real-time sensor data, digital twins could continuously predict stability outcomes throughout the product lifecycle.

Integration with automated LC–MS platforms will further enhance efficiency. Robotic sample preparation, automated injection sequences, and AI-assisted spectral interpretation can create fully autonomous degradation profiling systems. Such platforms would accelerate data acquisition while minimizing manual intervention.

AI-driven real-time release testing represents the ultimate goal of digital transformation. By combining predictive degradation modeling, process analytics, and real-time impurity monitoring, it may become possible to assure product stability and quality without relying exclusively on traditional long-term stability studies.

Collectively, these advancements indicate that the future of antiviral drug stability assessment lies in the convergence of predictive modeling, automated analytical technologies, and regulatory-aligned digital infrastructure. The closed-loop AI–LC–MS/MS system thus forms the conceptual foundation for next-generation stability science.

CONCLUSION

The chemical instability of antiviral drugs presents significant challenges for ensuring product safety, efficacy, and regulatory compliance. Although LC–MS/MS remains the gold standard for degradation profiling and impurity characterization, traditional stability programs are predominantly empirical and resource-intensive. The integration of AI into stability assessment introduces a transformative, predictive dimension to pharmaceutical analysis. ML and deep learning models enable identification of degradation-prone sites, simulation of stress-induced transformations, and quantitative prediction of impurity growth and shelf life. The closed-loop AI–LC–MS/MS framework establishes a continuously learning system in which computational predictions and experimental validation

operate synergistically. The incorporation of QbD principles into AI-enabled stability workflows provides a structured, risk-based framework that enhances analytical robustness, regulatory compliance, and predictive accuracy in modern pharmaceutical stability programs. When embedded within digitally enabled, ICH-aligned stability programs, this approach supports risk-based testing, lifecycle management, and real-time decision-making. Despite regulatory and validation challenges, AI-driven predictive degradation modeling represents a forward-looking strategy that can enhance efficiency, scientific robustness, and innovation in antiviral drug development.

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