



Potential Application of Ai-Assisted HPLC Method Development for Emerging Pharmaceuticals: Finerenone as a Case Study

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Abstract: Traditional HPLC method development relies on trial-and-error, one-factor-at-a-time approaches that struggle to capture complex, multivariate interactions among method parameters, often resulting in suboptimal separations and transfer failures. While Design of Experiments and Analytical Quality by Design frameworks address some of these shortcomings under ICH Q2(R2) and Q14 guidelines, they remain vulnerable to noise and nonlinear retention behavior. This review examines how artificial intelligence and machine learning tools—including artificial neural networks, support vector regression, and quantitative structure-retention relationship modeling—are reshaping chromatographic method development, using finerenone, a novel non-steroidal mineralocorticoid receptor antagonist for cardiorenal protection, as a representative case study. It surveys finerenone's physicochemical and pharmacokinetic profile, forced-degradation behavior, and existing bioanalytical and stability-indicating RP-HPLC methods, and evaluates how AI-assisted approaches can accelerate method optimization in line with Green and White Analytical Chemistry principles. The review further discusses regulatory considerations for validating machine learning models in GxP environments, including the OECD (Q)SAR framework, FDA and EMA guidance, and Predetermined Change Control Plans, alongside persistent challenges such as data scarcity, model interpretability, and chiral separation limitations. Finally, it outlines emerging directions—transfer learning, hybrid mechanistic-ML models, explainable AI, and autonomous closed-loop chromatography—as pathways toward more sustainable, intelligent analytical workflows for emerging pharmaceuticals.

Keywords: HPLC method development , Artificial intelligence, Finerenone, Quantitative structure-retention relationship (QSRR) , Analytical Quality by Design (AQbD)

1. Introduction

High-performance liquid chromatography (HPLC) is the primary analytical tool across the pharmaceutical lifecycle^[1]. However, traditional method development has historically relied on empirical, trial-and-error, one-factor-at-a-time (OFAT) approaches.^[14] While intuitive, OFAT is inherently inefficient and cannot capture complex, multivariate interactions between critical method parameters such as mobile phase pH, organic modifier ratio, flow rate, and temperature which often leads to suboptimal separations, poor reproducibility, and method failures during transfer^[13]. To resolve these limitations, statistical Design of Experiments (DoE) and systematic Analytical Quality by Design (AQbD) frameworks have been established under international regulatory guidelines (including ICH Q2(R2) and Q14). By defining a prospective Analytical Target Profile (ATP) and mapping critical parameter interactions, AQbD identifies a robust Method Operable Design Region (MODR) that ensures long-term method reliability and regulatory flexibility.^[1] Classical statistical designs are susceptible to statistical fragility under high noise conditions and frequently fail to model highly non-linear or atypical retention behaviours. Therefore, Artificial Intelligence (AI) and Machine Learning (ML) methods, such as artificial neural networks (ANNs), support vector regression (SVR), and quantitative structure-retention relationship (QSRR) calculations, have emerged as effective tools for modeling

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complex chromatographic systems and in silico prediction of retention behaviors.^[1] Finerenone (BAY 94-8862) provides a compelling case for AI-assisted chromatographic method development, as a clinically important model for emerging pharmaceuticals.^[6] Finerenone is a novel, first-in-class, highly selective non-steroidal mineralocorticoid receptor antagonist (MRA) approved for cardiorenal protection in patients with chronic kidney disease associated with type 2 diabetes.^[5] Unlike older steroidal MRAs such as spironolactone and eplerenone, finerenone features a non-steroidal dihydronaphthyridine core that acts as a bulky physical antagonist to prevent the transcription of proinflammatory and profibrotic genes, achieving over 500-fold selectivity for the mineralocorticoid receptor and avoiding androgenic adverse effects.^[7] Pharmacokinetically, it undergoes rapid absorption and extensive CYP3A4 (90%) and CYP2C8 (10%) mediated first-pass biotransformation to inactive metabolites. Despite its short plasma half-life of 2 to 3 hours, finerenone demonstrates persistent 24-hour therapeutic and hemodynamic efficacy with once-daily morning dosing.^[7] The need for sensitive, selective and robust stability-indicating HPLC separations arises from the requirement to control its tablet formulations and trace-level unspecified impurities in biological matrices.^[8] To conform these separation objectives with Green and White Analytical Chemistry (GAC and WAC) principles, solvent consumption, waste generation and run times are minimized, which are quantitatively evaluated using metrics like AGREE, MoGAPI and BAGI.^[1] The rapid predictive power of AI with human experimental validation creates a sustainable, high-performance and eco-friendly analytical workflow that avoids resource-intensive trial-and-error campaigns.^[14]

2. Finerenone: Chemical And Analytical Profile

- **Chemical Nomenclature and Structural Details:** As a pure S-enantiomer dihydronaphthyridine derivative (namely(4S)-4-(4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyridine-3-carboxamide).^[7,11] It explains its unique “bulky-passive” binding mode that elongates the helix 12 of mineralocorticoid receptor to impede coactivator recruitment,^[10] giving it selectivity over 500-fold compared to older steroidal alternatives.^[5,7]
- **Physicochemical Properties:** Discussing its polarity (log D = 2.40), base-dependent solubility (pKa of 6.06 with an equilibrium solubility of 0.024 mg/mL at pH 6.8),^[7,8] BCS Class II, and its 91.7% plasma protein binding capacity.^[7]
- **Pharmacokinetic properties:** In brief, it has 43.5% oral bioavailability (due to the strong first-pass metabolism in the gut wall), CYP3A4-predominant biotransformation and short elimination half-life of 2–3 hours.^[7]
- **Analytical techniques:** Comparison between the green fluorimetric nano-sensing technique using the Inner Filter Effect (IFE) quenching of nitrogen and sulfur co-doped carbon quantum dots (N,S-CQDs)^[11] and the stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) validated method on C18 column at pH 7.0.^[9]
- **Forced Degradation and Chemical Stability Matrix:** Documentation and evaluation of the high susceptibility of finerenone to alkaline hydrolysis (~49% degradation), moderate susceptibility to acidic hydrolysis and oxidation, and excellent stability under photolytic and dry heat thermal conditions.^[8]

3. Existing Hplc Methods For Finerenone

The literature depicts an increasing trend in the development of liquid chromatographic methods for finerenone, ranging from high-sensitivity bioanalytical characterizations to systematic, stability-indicating assays for pharmaceutical formulations.^[11,8] These methods can be broadly categorized as bioanalytical assays for clinical pharmacokinetics and reverse-phase high-performance liquid chromatography (RP-HPLC) methods optimized for routine quality control, stability testing, and impurity profiling.^[8]

A. Bioanalytical HPLC-MS/MS methods

For the quantification of finerenone in complex biological matrices for clinical and pharmacokinetic evaluations ultra-trace sensitivity and high specificity are required.^[8]

- Rohde et al.^[8](2021) developed and validated a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) methods for quantifications of finerenone in human venous and capillary plasma. The bioanalytical assay used an autosampler temperature of 10 °C and a mobile phase of acetonitrile and ammonium acetate (pH 3.0 adjusted with formic acid).^[11] This approach has been successfully employed to map the capillary-venous drug concentration equilibration during the absorption phase of early clinical trials.^[8,7]
- Bioanalytical methods such as mass spectrometry are characterized by nanomolar sensitivity but are associated with costly instrumentation, complex sample preparation (including protein precipitation with organic solvents) and specialised labour, making them less accessible for standard pharmaceutical quality control laboratories.^[8,11]

B. Stability-Indicating RP-HPLC Methods

Several stability-indicating RP-HPLC-UV methods have been developed to support the manufacturing lifecycle and shelf-life assessment of commercial tablet formulations (e.g., Kerendia or Nexifinerenone).^[8,11] These methods are developed to resolve the active pharmaceutical ingredient (API) from its degradation products under various forced stress conditions.^[34,8]

- An AQbD framework for the development, validation, and stability assay of finerenone in bulk drug substances using the RP-HPLC method was proposed by **Reddy et al (2024)**. The robustness of method performance and regulatory compliance with AQbD requires the development of ATP and mapping of the interactions of critical parameters.^[8,14]
- **Rishipathak, D. (2025)** Coupled RP-HPLC assay with LC-MS characterization identified and profiled the chemical structures of the degradation products of finerenone.^[8]

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- **Mukthinuthalapati et al.** have successfully validated the forced degradation studies of finerenone in pharmaceutical tablet dosage forms with the isocratic RP-HPLC-UV methods developed by them.
- **Marie, Yassin and Elshenawy (2025)** Development and optimization of a highly selective stability indicating RP-HPLC method for analysis of a new tablet dosage form for unspecified impurities (related substances) (2021) & **Imran et al. (2023)** The method employs a Phenomenex C18 column (250 × 4.6 mm, 5 µm) and is performed isocratically at 40° C with a mobile phase of water:acetonitrile:triethylamine (45:55:1 v/v/v) adjusted to pH 7.0 at a flow rate of 0.8 mL/min with UV detection at 252 nm to elute finerenone at 4.437 ± 0.05 min. The linear range of the assay is 8–30 µg/mL and as sensitive as 0.2–1.4 µg/mL for unspecified impurities, consistent with the stringent identification criteria of the ICH Q3(B) guidelines.^[8]

C. Routine Assay RP-HPLC Methods

For routine, high-throughput assays where profiled degradation is not the main objective, simpler isocratic RP-HPLC methods are developed: Varade et al. (2024), Naazneen (2024) and Metkari (2024) described rapid isocratic RP-HPLC methods for routine quantitative determination of finerenone in bulk drugs and commercial tablet formulations. Commonly, C18 stationary phases and aqueous organic solvent mixtures are used, which verify label claims without any need for complicated gradients or mass spectrometry coupling.^[8]

4. Traditional Hplc Method Development Challenges

A. Empirical and One-Factor-at-a-Time (OFAT) Optimization Inefficiency

Traditional HPLC method development has been based on empirical, trial-and-error screening. The most common approach is a One-Factor-at-a-Time (OFAT) strategy, in which one experimental parameter (e.g., mobile phase pH, organic modifier ratio, or temperature) is varied while all others are held constant.^[1] Although intuitive, this approach suffers from several major disadvantages:

- **Labor- and time-consuming:** It involves a large number of laboratory experiments, leading to long method development cycles and to a heavy dependence on the subjective experience of the analyst.^[16]
- **Inability to discover interactions:** The parameters of HPLC are highly interdependent. The linearity of the OFAT approach does not allow the investigation or the quantification of the synergistic multivariate interactions between the key factors (e.g., the combined effect of pH and organic phase ratio on analyte ionization and retention).^[1]
- **Suboptimal solutions:** Due to the highly nonlinear nature of the chromatographic responses, the linear single-variable adjustments used in the optimization often cause the analyst to miss the true global optimum and to obtain suboptimal separations.^[1,20]

B. Narrow Robustness Windows and Method Fragility

The focus of conventional HPLC method development is usually limited to achieving an acceptable separation under a single fixed set of parameters, rather than mapping out a robust operating space.^[31] This leads to a number of operational risks:

- **Narrow Robustness Profile:** Methods developed with empirical trial and error often display narrow robust behaviour toward small, unavoidable instrumental variations.^[13]
- **High Failure Rates during Method Transfer:** Small differences in dwell volumes, gradient profiles or mixer configurations between instrument brands often cause chromatographic deviations, resulting in out-of-specification (OOS) or out-of-trend (OOT) results.^[31,22]
- **Frequent Revalidation Costs:** Conventional validation guidelines (e.g. ICH Q2(R1)) treat validation as a static, one-time event, with little focus on lifecycle management or continuous improvement.^[31,33] Therefore, a transferring or changing a parameter of a traditional method typically triggers a burdensome and costly revalidation protocol.^[13,15]

C. Separation and Selectivity Challenges of Complex Matrices

Modern pharmaceutical and biological analysis often encounters severe separation challenges that are not systematically addressed by conventional method development:

- **Co-elution and Spectral Overlap:** Complex matrices such as biological fluids or tablet formulations often present high-noise chemical interferences, co-eluting impurities, and overlapping peaks which are difficult to separate without extensive manual trial-and-error optimization.^[1]
- **Analyte Stability:** Labile compounds can undergo chemical, photolytic, or oxidative degradation during preparation or analysis leading to baseline noise, peak tailing or appearance of unexpected degradant peaks.^[1]
- **Subtle Energetic Differences in Isomeric Separations:** For chiral pharmaceuticals, enantiomeric method development is highly complex because resolving enantiomers depends on very minor free energy differences in the formation of transient diastereomeric complexes with a chiral selector, which requires tedious stationary and mobile phase screening.^[12]

D. Environmental and Economic Unsustainable

The high experimental burden of the traditional trial-and-error sweeps directly violates the principles of Green and White Analytical Chemistry (GAC and WAC):^[12]

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- **Excessive Solvent Usage:** Iterative scouting runs consume huge volumes of hazardous and expensive organic modifiers, such as acetonitrile.^[14] One conventional HPLC instrument can produce 1.0 to 1.5 litres of chemical waste on a daily basis, representing serious disposal and environmental safety hazards.^[29]
- **Low Operational Throughput:** Long analytical run-times and long column equilibration periods during the trial-and-error optimization severely limit sample throughput, while consuming large electrical energy and instrument resources.^[14,29]

E. Technical Bottlenecks in Data Extraction and Processing

- **Multi-Dimensional Data Discarding:** State-of-the-art Photodiode Array (PDA) detectors fitted to modern HPLC systems record full three-dimensional (time-wavelength-absorbance) spectra, but conventional vendor software routinely reduces this data to a single wavelength, discarding up to 90% of recorded information.^[21]
- **Proprietary Data Silos:** Chromatographic raw data often resides in vendor-specific proprietary software formats, which limits interoperability with automated data processing, machine learning algorithms, or open-source chemometric toolkits.^[21]
- **Manual Peak Tracking:** Manually tracking and labelling overlapping peaks across a large series of development runs is tedious and error-prone, especially when the elution order is changed by varying the compositions of the mobile phases.^[23]

5. Ai/ML Approaches In Hplc Method Development

A. Paradigm Shift from Empirical to In-Silico Modeling

Traditional HPLC method development has been empirical, trial-and-error, one-factor-at-a-time (OFAT) screening, which is highly inefficient and does not capture multivariate interactions.^[1] Although statistical Design of Experiments (DoE) and systematic Analytical Quality by Design (AQbD) improve screening efficiency, they are statistically fragile in high-noise environments and cannot extrapolate outside of predefined experimental boundaries. In contrast, Artificial Intelligence (AI) and Machine Learning (ML) act as powerful data-driven tools to model highly complex, non-linear, and multidimensional chromatographic spaces.^[1] These applications operate at two resolutions: signal-level modeling (processing entire raw detector arrays for denoising, baseline correction, and peak deconvolution) and feature-level modeling (utilizing extracted analytical outputs like retention times (Rt), resolution (Rs), and retention factors (k) for optimization).^[21,2]

B. Signal Preprocessing and Peak Deconvolution

Raw chromatographic data require mathematical cleaning to remove instrumental anomalies prior to modeling:

- **Baseline Correction:** Gradient-induced baseline drift is effectively corrected by the asymmetrically reweighted penalized least squares (arpls) algorithm or sparsity-assisted BEADS (baseline estimation and denoising using sparsity).^[19]
- **Retention Time Alignment:** Correction of instrument and column aging shifts, where pixel-level alignment techniques such as Correlation-Optimized Warping (COW)^[23] or Dynamic Time Warping (DTW)^[23] are employed.
- **Peak Deconvolution:** Resolution of overlapping analyte and impurity peaks is achieved by Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS).^[23] Open-source Python libraries like a MOCCA (Multivariate Online Contextual Chromatographic Analysis)^[24] automate this deconvolution to provide “actionable analytics” for closed-loop autonomous optimization in the absence of human intervention.^[21]

C. Molecular Representations and QSRR modeling

Quantitative Structure-Retention Relationship (QSRR) modeling is the core of in silico retention prediction, where the chemical structure of analytes is correlated with their retention behavior:

- **Featurization:** Structures of molecules are converted into machine-readable forms, including molecular descriptors (1D constitutional, 2D topological, 3D steric/shape parameters, and quantum mechanical [QM] descriptors), molecular graphs, or binary molecular fingerprints (e.g., ECFPs or MACCS keys).^[3]
- **Feature Selection:** Redundant features are eliminated by Genetic Algorithms (GA), Random Forest Gini importance, or stepwise linear regression to address the high collinearity.^[3]

D. Machine Learning Algorithms and Deep Architectures

A wide range of supervised learning algorithms are used, depending on the size of datasets and the degree of separation:

- **Support Vector Regression (SVR):** Particularly effective with radial basis function (RBF) kernels for predicting retention indices in small-to-medium localized datasets.^[1]
- **Random Forests (RF) & Gradient Boosting (GB):** Great ensemble techniques for the modelling of complex, non-linear and highly diverse analyte classes with robust generalization and built-in feature importance ranking.^[3]
- **Artificial Neural Networks (ANNs):** Process highly non-linear parameter interactions (e.g. analyte ionization states across pH, temperature and solvent gradients). [1,18] ANN-consensus models are used to predict full Efficient Enantioseparation-Mobile Phase (EES-MP) profiles in sustainable chiral separations.^[12]
- **Graph Neural Networks (GNNs):** Deep architectures (e.g., Graph Isomorphism Networks [GIN]) learn representations directly from molecular graphs to predict retention times without manual descriptor engineering.^[3]

E. Data Scarcity and Transfer Learning

While large databases such as METLIN-SMRT (80,019 molecules) and CMRT (chiral molecules) provide extensive training data,^[3] project-specific datasets in pharmaceutical quality control are often small and scarce.^[1] To address this

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bottleneck, analytical scientists are applying transfer learning:

1. A deep learning architecture (e.g., GNN) is pre-trained on a large, publicly available dataset (such as METLIN-SMRT) to learn general structural and chemical features.^[3,30]
2. The model's feature-extraction layers are frozen and the output layers fine-tuned (retrained) with a very small, project-specific in-house dataset. This results in drastic improvement in prediction accuracy while decreasing lab workload and chemical waste.^[3,30]

F. Explainable AI (XAI) and Regulatory Compliance

In an effort to be accepted by regulators in compliant clinical and GxP pharmaceutical settings, AI-assisted methods must progress from opaque "black boxes" to auditable workflows.^[1] Post hoc Explainable AI (XAI) frameworks, such as SHAP (Shapley Additive Explanations) and LIME (Local Interpretable Model-agnostic Explanations), are incorporated to calculate and rank the quantitative contributions of individual molecular descriptors. Such predictions can thus be computationally transparent, chemically meaningful, and aligned with international OECD (Q)SAR assessment frameworks.^[30]

6. AI-Assisted Predictive Optimization

The optimization of chromatography has evolved from empirical one-factor-at-a-time (OFAT) screening and statistical Design of Experiments (DoE) to data-driven, AI-assisted prediction modeling.^[1] The core of predictive optimization is Bayesian Optimization (BO), a sequential, model-based global algorithm that combines machine learning with adaptive sampling.^[25] BO relies on three key components: a probabilistic surrogate model (typically a nonparametric Gaussian Process [GP]), an acquisition function (e.g., Expected Improvement), and an optimization algorithm. GP regression represents complex, non-linear relationships, quantifies localized standard deviations, and allows the acquisition function to dynamically balance the exploration of uncertain regions with the exploitation of known high-value zones to locate global optima with minimal experimental iterations.^[25] For example, replacing a complex mechanistic model with a Gaussian Process Regression (GPR) model for a ternary protein separation reduced run time by 70.8% and increased productivity by 50.1%.^[1]

When optimizing conflicting objectives—such as maximizing chromatographic separation resolution and sample throughput while minimizing solvent waste and analysis cost—multi-objective BO algorithms map out a non-dominated Pareto front of optimal trade-offs.^[17] In data-scarce scenarios, Multi-Task Bayesian Optimization (MTBO) trains a shared multi-task GP simultaneously on auxiliary data from related reactions, which reduces predictive uncertainty and dramatically decreases the number of iterations required to locate global optima. To handle categorical variables alongside continuous parameters, algorithms like Gryffin utilize Bayesian neural networks as surrogates.^[17] These algorithms are increasingly embedded within closed-loop autonomous platforms that couple automated flow or batch reactors with robotics and real-time Process Analytical Technology (PAT) tools. Real-time PAT signals are processed through automated deconvolution software to calculate performance metrics, which are immediately fed back to update active learning surrogates without human intervention.^[17,18]

Predictive tools such as the YesChat HPLC Method Developer are used by analytical laboratories to provide first method design baselines, but human-expert laboratory validation is used to resolve elution order and peak tailing discrepancies.^[29] To tackle extreme non-linearity in chiral HPLC, researchers deploy an ANN-consensus model optimized via the chaotic competitive-learning neural network algorithm (CCLNNA) to accurately simulate full Efficient Enantioseparation-Mobile Phase (EES-MP) profiles across diverse solvent ratios, avoiding extensive empirical column and mobile phase screening.^[12] Finally, to ensure GxP compliance and model generalizability, development is transitioning toward Physics-Informed Bayesian Optimization (PIBO). PIBO integrates data-driven models with first-principles physical knowledge—such as mechanistic thermodynamic laws, physics-infused kernels, or structured probabilistic priors—to constrain search spaces within physical reality, enhance predictive accuracy, and accelerate convergence toward the true global optimum.^[25]

7. Integration With Aqbd

The Paradigm Shift and Regulatory Alignment Implementing Analytical Quality by Design (AQbD) represents a proactive, science- and risk-based lifecycle methodology that replaces traditional, empirical "Quality-by-Testing" (QbT) and reactive one-factor-at-a-time (OFAT) trial-and-error schemes in liquid chromatography.^[1] Based on international regulatory guidelines such as ICH Q14 (which suggests an "enhanced approach" for method development), ICH Q2(R2) (which updates validation parameters to include lifecycle and risk management), and ICH Q10 (for pharmaceutical quality systems), AQbD creates a systematic workflow in which robustness is inherently built into the analytical method.^[31] This methodology helps to minimize out-of-specification (OOS) and out-of-trend (OOT) errors by scientifically correlating variables and thereby ensuring consistent analytical quality and performance.^[13]

A. Step-by-Stage Implementation Workflow

The AQbD process shares many similarities with product-level QbD, where quality target product profiles (QTPP) are translated into analytical objectives through a systematic series of steps:^[13]

- **Analytical Target Profile (ATP):** The fundamental component that defines the intended, target requirements (e.g. analytical purpose, sensitivity, selectivity and precision) that the method has to meet to provide quality reportable values.^[1]
- **Critical Quality/Analytical Attributes (CQAs/CAAs):** Quantifiable chromatographic responses (e.g. peak resolution, symmetry, tailing factor and retention times) that are directly indicative of method performance.^[1]
- **Critical Method Parameters (CMPs):** Operating input variables (e.g. mobile phase pH, organic modifier ratio, column chemistry, temperature and flow rate) that have a significant impact on the CQAs.^[1]

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- **Risk Assessment:** Systematic tools such as Ishikawa (fishbone) diagrams, Failure Mode and Effects Analysis (FMEA) and 5x5 risk estimation matrices for systematic evaluation, prioritization and isolation of the most critical parameters (CMPs) affecting chromatographic performance prior to screening runs.^[1]

B. Multivariate DoE and the Method Operable Design Region (MODR)

Rather than evaluating variables in isolation, AQbD employs statistical Design of Experiments (DoE) and Response Surface Methodology (RSM)—typically employing Central Composite Designs (CCD) or Box-Behnken Designs (BBD)—to quantitatively map complex, non-linear interactions among multiple CMPs.^[1] This multi-parameter investigation defines the Method Operable Design Region (MODR), a validated, multidimensional operating space. The MODR mathematically demonstrates that minor changes to input parameters meet ATP criteria providing regulatory flexibility that allows analysts to adjust method parameters without formal regulatory resubmissions or expensive revalidation protocols.^[13]

C. Control Strategy and Lifecycle Management

One of the primary outputs of the AQbD framework is the establishment of a sound analytical control strategy to monitor performance during the entire method lifecycle.^[1] This is accomplished through routine System Suitability Testing (SST) and continuous performance verification. In line with USP General Chapter <1220> (“Analytical Procedure Lifecycle”) and post-approval change management guidelines (ICH Q12), AQbD considers the analytical method to be a dynamic, continuously monitored process rather than a static validated event to ensure long-term method transferability and reliability across laboratories.^[31]

D. Computational and AI-Driven AQbD Integration

A significant recent advance in pharmaceutical development is the integration of Artificial Intelligence (AI), Machine Learning (ML) and QSRR modelling into the AQbD loop.^[14,30] Rather than extensive in-lab DoE sweeps, GNNs, Support Vector Regression (SVR) and neural networks can be trained on high-throughput data to simulate and map virtual MODRs in silico. This hybrid modeling minimizes empirical laboratory runs by mathematically predicting retention behaviours, separations and peak purities. This predictive synergy greatly reduces solvent usage, analysis run times and waste generation, connecting AQbD directly to the ecological and functional goals of Green and White Analytical Chemistry (GAC-WAC).^[14]

8. Ai-Based Robustness Assessment

A. Robustness in Analytical Lifecycle and Regulatory Standards

Analytical robustness is no longer viewed as a post-optimization static check, but as a dynamic parameter throughout the method lifecycle, in line with current regulatory standards set by ICH Q2(R2) and ICH Q14.^[14] In conventional liquid chromatography, robustness is evaluated by measuring the extent to which a method can tolerate small deliberate changes in operating parameters, such as mobile phase pH, flow rate, column temperature, organic solvent ratio, and detection wavelength.^[32] These small changes are typically tested in the lab and critical attributes such as retention times, peak symmetry, resolution, and tailing factors are monitored to ensure that their relative standard deviations (%RSD) are strictly below 2%.^[32] These variables are systematically mapped into a multidimensional Method Operable Design Region (MODR) under the Analytical Quality by Design (AQbD) framework, ensuring method ruggedness across different analysts, instruments, and laboratories. However, classical response surface designs and polynomial regression models are statistically fragile in high-noise environments and are limited to their extrapolation boundaries, which does not allow the prediction of robustness outside the predefined experimental space.^[1]

B. Technical vulnerabilities of algorithmic chromatographic models

Artificial intelligence (AI) and machine learning (ML) algorithms (e.g., ANNs, SVR, and GNNs) provide powerful data-driven capabilities for modeling highly non-linear chromatographic systems. However, their implementation in GxP-regulated laboratories is often hampered by their inherent weaknesses:^[1,2]

- **Domain shift and system transferability:** Predictive models are highly sensitive to slight changes in column chemistry, instrument setup (e.g., dwell volumes or mixer profiles), and environmental factors. A model trained on one particular HPLC system typically suffers a substantial loss in prediction accuracy when applied to a different instrument or a different batch of column.^[2]
- **Data scarcity and overfitting:** Data sets in pharmaceutical quality control are typically localized and project-specific, containing only a few dozen analytes or degradation products.^[2,3] These small data sets make it difficult to train complex high dimensional models that are prone to over-fitting, i.e. the learning algorithm learns the process noise and cannot generalize to new chemical structures.^[3,18]
- **Extrapolation failures:** Purely empirical machine learning architectures lack intrinsic chemical or thermodynamic reasoning, compared to physics-based mechanistic models.^[20,25] Consequently, predictions of chromatographic retention or separation behaviour outside the experimental limits of the training data set are highly inaccurate and physically impossible predictions.^[1]

C. Algorithmic Strategies for Developing and Validating Robustness of Models

To overcome the limitations of the model fragility and domain shift, modern chromatographic studies exploit some advanced computational strategies:

- **Consensus and Ensemble Architectures:** Instead of a single optimized neural network, which may be sensitive to small splits of the data, researchers exploit ANN-consensus models. For example, in chiral mobile phase selection, multiple neural

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networks are optimized by custom metaheuristics such as the chaotic competitive-learning neural network algorithm (CCLNNA). The best performing networks are combined and their outputs aggregated using median statistics, where the median absolute deviation (MADe) is used as a robust estimator of uncertainty, to stabilize the model, limit overfitting and provide reliable confidence intervals for the predicted separations.^[12]

- **Multi-System and Cross-Column Descriptors:** To avoid domain shift between different stationary phases, machine learning based Quantitative Structure-Retention Relationship (QSRR) models incorporate stationary phase selectivity descriptors directly into their input matrices.^[2,30] Combining analyte structural features with the Hydrophobic Subtraction Model (HSM) parameters of the column—hydrophobicity (H), steric hindrance (S), hydrogen-bond acidity (A), hydrogen-bond basicity (B), and ionic interaction (C)—the model achieves robust predictive accuracy across a range of commercial C18 columns from different suppliers without requiring prior retention data on the target stationary phase.^[2]
- **Transfer Learning and Meta-Learning:** Analytical laboratories tackle the issue of limited local data by pre-training deep learning models on large, public databases like METLIN-SMRT (80,019 small molecules) or CMRT (chiral retention data).^[30] In this transfer learning approach, the model learns generalized structural and chemical representations from the big data, followed by freezing the feature-extraction layers.^[3,30] The output layers are then fine-tuned on a very small target dataset of in-house compounds, greatly improving predictive stability and reducing overfitting in data-poor settings.^[3]

D. Probabilistic Uncertainty Quantification and Virtual MODR Verification

A key milestone in AI-assisted robustness assessment is the use of probabilistic surrogate models to map out and validate virtual design spaces.^[30,24] During autonomous optimization loops, Gaussian Process (GP) regression is widely deployed because it naturally quantifies prediction uncertainty.^[25] GP models estimate a localized standard deviation ($\sigma(x)$) alongside the predicted mean value ($\mu(x)$) for every point in the multi-dimensional parameter space.^[24]

This allows active learning algorithms (such as Bayesian Optimization) to guide experiments using acquisition functions (e.g., Expected Improvement [EI] or q-Noisy Expected Improvement [qNEI]) that mathematically balance exploration of highly uncertain regions with the exploitation of known optimal zones.^[25] After optimization, the GP's localized uncertainty can be plotted across the design space, allowing analysts to visually identify "risk zones" where model confidence is lowest. This enables targeted, calibration-free verification experiments to confirm the physical boundaries of the virtual MODR.^[24] Additionally, the coupling of such data-driven surrogates with physics-informed hybrid architectures (e.g., the General Rate Model) guarantees that the predicted design space boundaries are consistent with fluid dynamics conservation laws and thermodynamic mass balances, thereby ruling out non-viable proposals for operation.^[1,25]

E. Explainable AI (XAI) as a Quality Safeguard

To be GxP compliant and thus acceptably regulatory, AI-assisted HPLC methods must be auditable and scientifically transparent, as per OECD (QSAR assessment frameworks).^[30] Opaque "black-box" systems erode user trust and complicate root-cause investigations of out-of-specification (OOS) results.^[28,1] To bridge statistical predictions and chromatographic theory, laboratories deploy Explainable AI (XAI) frameworks such as SHAP (Shapley Additive Explanations) and LIME (Local Interpretable Model-agnostic Explanations).^[2]

Based on a QSRR retention time prediction, SHAP provides a precise, mathematically well-founded quantification of the contribution of any molecular and quantum mechanical descriptor (e.g., hydrogen-bond acidity, polarity, solvent-accessible surface area) to the predicted elution behavior. XAI allows analytical chemists to verify that the underlying logic of the model aligns with known principles of chromatographic separation mechanisms, by sorting the descriptor importance and providing a mapping of whether a structural feature has a positive or negative impact on retention. Ensuring that algorithmic outputs are chemically plausible and grounded in physical chemistry is one of the crucial safeguards against statistical artifacts, enhancing method reliability throughout its commercial lifecycle.^[30]

9. Validation And Regulatory Considerations

A. Evolving Regulatory Frameworks: The Transition to Lifecycle Validation

The validation of pharmaceutical liquid chromatography has transitioned from a static, checkbox-style exercise under the historical ICH Q2(R1) guideline to a science- and risk-based lifecycle framework defined by the revised ICH Q2(R2) and ICH Q14 guidelines.^[33] Under this modernised paradigm, validation spans a continuous three-stage lifecycle: Stage 1 (Method Design and Development), Stage 2 (Method Performance Qualification), and Stage 3 (Continued Method Performance Verification).^[33] The process begins with the development of an Analytical Target Profile (ATP) which describes the purpose of the method, the sensitivity, selectivity and precision requirements prospectively. Potential variables and analytical risks are systematically prioritized using tools such as Ishikawa diagrams and Failure Mode and Effects Analysis (FMEA).^[14] Additionally, System Suitability Testing (SST) has been transitioned from an implicit parameter to an explicitly recommended routine component of validation to monitor ongoing performance.^[33]

B. Analytical Quality by Design (AQbD) and Post-approval flexibility

The incorporation of AQbD principles in the ICH Q8 to Q12 series revolutionizes HPLC method development by adopting a proactive strategy for ensuring robustness in the separation.^[1,33] Using statistical Design of Experiments (DoE), analysts investigate multivariate interactions between critical method parameters (e.g. pH, mobile phase ratio, temperature) to determine a Method Operable Design Region (MODR).^[1] The boundaries of this MODR are formalized in ICH Q12 as Established Conditions (ECs).^[31,35] The establishment of an approved MODR is important as it grants regulatory flexibility to the

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laboratories to implement routine chromatographic changes in the validated space to adapt to column aging or instrument drift without the need for formal regulatory re-submissions or costly revalidation campaigns.^[13] This lifecycle-oriented approach is in line with the USP General Chapter <1220> (“Analytical Procedure Lifecycle”) to ensure a consistent and reliable performance between different analysts and equipment.^[1]

C. Case Study: Validation of Finerenone Assays and Impurity Profiling

The application of modern validation principles is demonstrated in stability-indicating assays for the cardiorenal pharmaceutical finerenone (BAY 94-8862). A validated, reversed-phase isocratic HPLC method uses a Phenomenex C18 (250 × 4.6 mm, 5 µm) column maintained at 40°C, with a mobile phase of water:acetonitrile:triethylamine (450:550:10 v/v/v) adjusted to pH 7.0. The method is run at a flow rate of 0.8 mL/min with UV detection at 252 nm and finerenone elutes at 4.437 ± 0.05 min. The unspecified tablet impurities are validated as per ICH Q3(B) guidelines against stringent limits derived from the maximum daily therapeutic dose of 20 mg, which leads to a reporting threshold of 0.1% (0.0005 mg/mL) and an identification threshold of 0.2% (0.001 mg/mL). The method is highly linear over the ranges of 8–30 µg/mL for the API assay and 0.2–1.4 µg/mL for related substances, with excellent recoveries (99.74% and 99.11%, respectively) and precision (%RSD < 2%). Specificity is confirmed via forced degradation: finerenone is highly susceptible to alkaline hydrolysis (~49% degradation in 0.1 N NaOH at 80°C for 2 h), moderately labile under acidic hydrolysis (~24% degradation in 0.1 N HCl at 80°C for 2 h) and oxidative stress (~6% in 30% H₂O₂ for 30 min), and highly stable under photolytic and dry heat conditions (~0.8% degradation).^[8]

Alternatively, an eco-friendly spectrofluorometric nanosensing platform has been validated for biological and tablet matrices. This system utilizes nitrogen and sulfur co-doped carbon quantum dots (N,S-CQDs) and measures quenching via the inner filter effect (IFE) at an emission of 419 nm (excitation at 344 nm). It has a linear range of 0.30–9.00 µg/mL, limit of detection (LOD) of 0.098 µg/mL, intermediate precision RSD of 1.15% and high selectivity in spiked human plasma with recoveries of 99.07%–100.67%.^[11]

D. Validation and Regulatory Credibility of AI/ML Chromatographic Models

The implementation of predictive machine learning and Quantitative Structure-Retention Relationship (QSRR) models in GxP pharmaceutical laboratories will necessitate new validation and data governance strategies.^[1,30] For computational models to withstand regulatory scrutiny, their development must be in accordance with the OECD (Q)SAR Assessment Framework, which requires a well-defined analytical endpoint, an unambiguous algorithm, a defined chemical applicability domain, transparent performance indicators (R², RMSE) and mechanistic chemical interpretability.^[30] Regulatory agencies have developed risk-based credibility policies to govern these digital technologies: the U.S. FDA’s January 2025 Draft Guidance on AI establishes a rigorous framework based on the model’s Context-of-Use (COU), while the EMA’s September 2024 Reflection Paper mandates strict data governance, human-in-the-loop oversight and bias mitigation.^[27]

Because conventional validation assumes static, deterministic software, adaptive machine learning models that evolve post-deployment must be validated under GAMP 5 (Second Edition) guidelines. To enable compliant, real-time computational updates, laboratories implement Predetermined Change Control Plans (PCCPs).^[35] A PCCP establishes predefined, pre-approved boundaries within which an active learning model is permitted to optimize its parameters (such as peak deconvolution or retention time prediction), allowing continuous performance updates without requiring a full, resource-intensive revalidation cycle for every minor update.^[36]

10. Limitations and Challenges Of Ai-Driven Hplc Method Development

- **Lack of Data, Quality and Standardization Issues:** The development of robust AI models for chromatography is severely limited by the lack of large, high-quality and standardized databases.^[1,2] Most available datasets are small, in-house collections compiled for localized project needs, increasing the risk of overfitting and random correlations.^[3] Additionally, public literature databases are biased toward positive results and negative data points are seldom reported.^[20,4] Fragmented data architectures, variations in reporting experimental parameters (e.g., precursor concentrations or exact column configurations), and non-standard raw data formats across labs limit reproducibility and stymie training of large-scale models.^[1]
- **The “Black Box” Barrier and Interpretability Limits:** The inner mathematical decision layers of state-of-the-art deep learning architectures like deep neural networks (DNNs), high-dimensional artificial neural networks (ANNs), and graph neural networks (GNNs) are notoriously opaque. This “black box” nature complicates GxP software validation and compromises user trust. Regulations require analytical scientists to provide a clear, chemically plausible, and mechanistic rationale for why separations occur, meaning purely data-driven predictions are often insufficient for formal submissions.^[1]
- **Domain Shift, Extrapolation Failures, and Chiral Hurdles:** Empirical machine learning models are structurally limited to interpolating within the boundaries of their training design space, often leading to physically impossible predictions when extrapolated.^[18] Consequently, models are not robust to domain shift, where small, unavoidable changes regarding the age of the equipment, ambient temperature, dwell volumes of the instrument, or the column batches lead to a degradation of the predictive accuracy.^[1] Also, separation of chiral enantiomers remains a significant bottleneck; common 0D, 1D and 2D molecular descriptors are not enough to model the 3D stereo-electronic, spatial, and conformational flexibilities necessary to model the small free-energy differences that drive enantioselective stationary phase interactions.^[3]
- **Resource and Expertise Barriers:** Designing, validating, and maintaining adaptive machine learning algorithms requires specialized expertise bridging data science and analytical chemistry. The computational resources necessary to run deep

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learning and reinforcement learning models are often beyond the budgets and technical infrastructure of smaller, routine quality control or academic laboratories. ^[34,1]

11. Future Perspectives: Toward Intelligent, Autonomous and Sustainable Chromatography

- **Transfer Learning, Meta-Learning and Hybrid Architectures:** To avoid localized data scarcity, future research is actively moving toward transfer learning and meta-learning. Under these frameworks, deep learning models are first pre-trained on massive public datasets (such as METLIN-SMRT or CMRT) to extract generalised structural and chemical features, and are subsequently fine-tuned using small, in-house target datasets. Concurrently, there is a prominent shift toward physics-informed and hybrid modeling.^[3] Hybrid models embed mechanistic first-principles equations (such as the General Rate Model or Lumped Kinetic Model) within machine learning layers to ensure that predicted design spaces remain thermodynamically plausible and compliant with Quality by Design (QbD) principles.^[30]
- **Adaptive Validation and Explainable AI (XAI) Integration:** To meet stringent GxP and OECD (Q)SAR regulatory standards, future AI-assisted workflows will routinely embed post hoc Explainable AI (XAI) interpretability layers—such as SHAP and LIME—to calculate and rank the exact contributions of individual molecular descriptors.^[1] To accommodate models that continuously learn and adapt post-deployment, analytical development is transitioning toward continuous, lifecycle-based verification under GAMP 5 (Second Edition) and the FDA's Predetermined Change Control Plans (PCCPs), which establish predefined change boundaries to prevent model drift without requiring a complete revalidation cycle for minor updates.^[35]
- **Autonomous Closed Loop Systems and Green Chemistry (GAC-WAC) Convergence:** The ultimate aim of chromatographic development is the creation of fully autonomous, closed-loop systems.^[1] These systems will feature state-of-the-art robotics, microfluidics, online Process Analytical Technologies (PAT), instant peak deconvolution libraries (e.g., MOCCA or HappyTools), and active optimization algorithms (e.g., Bayesian Optimization or Reinforcement Learning) to autonomously design, execute, and optimize separation methods in real time.^[1] Crucially, future algorithm designs will mathematically prioritize environmental and economic sustainability by directly embedding Green and White Analytical Chemistry (GAC and WAC) metrics (e.g., MoGAPI, AGREE, and BAGI scores) into active learning loops. This will reconcile computational efficiency with waste minimization, hazardous solvent reduction, and energy-conserving laboratory practices.^[30]

12. Conclusion

AI-assisted method development represents a meaningful evolution beyond traditional OFAT and even classical DoE/AQbD approaches, offering the capacity to model complex, nonlinear chromatographic behavior that conventional statistical designs often fail to capture. Using finerenone as a case study illustrates both the promise and the practical hurdles of this shift: existing bioanalytical and stability-indicating RP-HPLC methods provide a strong foundation, but fully realizing AI's potential will require overcoming data scarcity, black-box interpretability concerns, and domain-shift limitations, particularly for challenging separations like chiral resolution. Regulatory frameworks such as the OECD (Q)SAR Assessment Framework, FDA and EMA guidance, and Predetermined Change Control Plans are beginning to establish pathways for validating adaptive models within GxP environments, but broader adoption will depend on closing the gap between computational prediction and mechanistic, regulator-acceptable justification. Looking forward, hybrid mechanistic-ML architectures, explainable AI, and autonomous closed-loop systems—aligned with Green and White Analytical Chemistry principles—are poised to drive HPLC method development toward faster, more sustainable, and more robust workflows for emerging pharmaceuticals like finerenone.

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