RESEARCH PAPER

Chemometric-Assisted Optimization of Chromatographic Methods for Drug Analysis

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Abstract

Chromatographic techniques, especially high-performance liquid chromatography (HPLC), gas chromatography (GC), and their hyphenated forms, are the most widely used tools for the identity, purity, assay, and stability testing of pharmaceuticals. Developing a method that separates all relevant analytes with adequate resolution, sensitivity, and robustness is a multivariate problem in which mobile-phase composition, pH, temperature, gradient profile, flow rate, and stationary-phase chemistry interact in nonlinear ways. Traditional one-factor-at-a-time (OFAT) development is slow, ignores these interactions, and often yields methods that fail under small, routine variations. Chemometrics offers a structured alternative. Experimental design, response surface methodology, multi-criteria desirability functions, evolutionary algorithms, and multivariate data-analysis tools such as principal component analysis, partial least squares, and multivariate curve resolution make method development systematic, data-driven, and defensible to regulators. This paper reviews the theoretical foundations of these tools, outlines a generic chemometric workflow for chromatographic method optimization, discusses how each tool maps to a typical development problem in drug analysis, and highlights current challenges and future directions, including analytical quality by design (AQbD) and machine-learning-guided optimization.

Keywords: Chemometrics, Chromatography, HPLC, Design of Experiments, Response Surface Methodology, Analytical Quality by Design, Multivariate Curve Resolution, Pharmaceutical Analysis.

How to cite this article: Aishwarya Singh Chauhan1 S. Sakthivel2 Andreena Joseph3 Dr. Sarita Agrahari4 Yashada Chandrkant Shinde5 DUDEKULA HUSSAINAIAH6. Chemometric-Assisted Optimization of Chromatographic Methods for Drug Analysis. Int J Drug Deliv Technol. 2026;16(87s):341-347. DOI: 10.25258/ijddt.16.87s.35.

I. Introduction

Chromatography is the backbone of pharmaceutical quality control. Regulatory submissions rely on chromatographic procedures to quantify active pharmaceutical ingredients (APIs), to profile related substances and degradation products, to monitor residual solvents, and to support bioanalytical studies in pharmacokinetics [1]. Because these results directly affect patient safety and product release decisions, the analytical procedure must be demonstrably fit for its intended purpose, a principle that underpins current international guidance on analytical procedure development and validation [2], [3].

The difficulty is that a chromatographic separation is governed by many interacting variables. In reversed-phase HPLC, retention and selectivity depend on the organic modifier type and proportion, the buffer pH and ionic strength, column temperature, gradient shape, flow rate, and the column's surface chemistry [1]. For ionizable drug molecules, a small change in pH near a compound's pKa can shift retention dramatically and even reverse elution order. Such interactions mean that the optimum for one factor changes as the others change, so a sequence of single-factor adjustments may never reach the true optimum, and it provides little information about how the method behaves under small variations.

Chemometrics, defined broadly as the application of mathematical and statistical methods to chemical data, addresses this problem on two fronts [4]. First, it supplies efficient strategies for planning experiments, so that maximum information is obtained from a minimum number of runs [5], [6]. Second, it supplies tools for extracting information from the resulting, often complex, data, including overlapping peaks, drifting baselines, and multichannel detector signals [7]. Together these capabilities turn method development from a craft based mainly on analyst experience into a reproducible, documented, and statistically supported process.

The aim of this paper is informational. It surveys the principal chemometric techniques used to optimize

chromatographic methods for drug analysis, explains how they are combined in a practical workflow, and discusses their benefits and limitations. The paper is organized as follows. Section II reviews the theoretical background and literature. Section III describes a generic methodology for chemometric-assisted optimization. Section IV discusses how the tools are applied to common problems in drug analysis. Section V outlines challenges and future directions, and Section VI concludes.

II. Literature Review

2.1 Experimental Design as the Foundation

The statistical design of experiments (DoE) is the cornerstone of chemometric optimization. A design specifies in advance which combinations of factor levels will be run so that the effects of individual factors and their interactions can be estimated with known precision [6], [8]. Factorial and fractional factorial designs are typically used first, in a screening role, to identify which of many candidate factors matter. Plackett–Burman designs, introduced in the 1940s, are especially economical for this purpose because they estimate the main effects of up to N−1 factors in N runs [9]. In chromatographic development, screening might involve factors such as pH, buffer concentration, percentage of organic solvent, temperature, flow rate, and gradient time, with responses such as critical-pair resolution, retention time, tailing factor, and theoretical plates.

Once the influential factors are known, response surface methodology (RSM) is used to model the relationship between factors and responses and to locate optimum conditions. RSM was formalized by Box and Wilson as a sequential strategy of fitting low-order polynomial models and moving toward the optimum region [10]. The most common RSM designs in chromatography are central composite designs (CCD) and Box–Behnken designs (BBD). The CCD augments a two-level factorial with axial and center points to estimate quadratic curvature. The BBD uses a rotatable arrangement of midpoints of the edges of the factor space and does not include extreme corner combinations, which can be an advantage when extreme combinations are impractical or damage the column [11], [12]. Myers, Montgomery, and Anderson-Cook provide a comprehensive treatment of these designs and of the associated model-fitting and diagnostic procedures [13], and general principles of experimental design are covered in the classic text by Box, Hunter, and Hunter [14].

Reviews of experimental design in analytical chemistry emphasize that design choice should reflect the objective. Screening, optimization, and robustness testing each call for different design families [15], [16]. Hibbert specifically reviewed the use of experimental design in chromatography and noted that practical issues, such as hard-to-change factors and constraints on solvent composition, influence which design is most suitable [16].

2.2 Multi-Response Optimization

A chromatographic method rarely has a single goal. Analysts want adequate resolution of the critical pair, short run time, good peak symmetry, high sensitivity, and low solvent consumption simultaneously, and these goals conflict. Improving resolution by lengthening the gradient, for instance, lengthens analysis time.

Derringer and Suich's desirability function is the most widely used approach to this problem [17]. Each response is transformed into an individual desirability between 0 (unacceptable) and 1 (fully acceptable) according to whether it should be maximized, minimized, or kept on target. The individual values are combined through a geometric mean into a single overall desirability, which is then maximized over the factor space. This reduces a multi-objective problem to a single-objective one while allowing the analyst to weight responses by importance.

An alternative is to treat the problem as true multi-objective optimization and search for the Pareto front of non-dominated solutions. Evolutionary algorithms such as NSGA-II are well suited to this task [18]. Rather than collapsing responses into one number, they present a set of trade-off solutions from which the analyst chooses.

Historically, chromatographers also developed specialized optimization strategies. The interactive mixture-design statistical technique of Glajch and colleagues optimized both solvent strength and selectivity in reversed-phase HPLC by exploring ternary and quaternary solvent mixtures [19]. Schoenmakers' work on the optimization of chromatographic selectivity formalized criteria for evaluating separation quality across entire chromatograms [20]. The book by Berridge on automated optimization of HPLC separations anticipated many of the ideas now implemented in commercial software [21].

Sequential simplex optimization, based on the geometric simplex designs of Spendley, Hext, and Himsworth and the algorithm of Nelder and Mead, offers another model-free route [22], [23]. A simplex of experiments moves across the factor space by reflecting away from the worst response, and it requires no assumption about the functional form of the response surface. Deming and Morgan described its application to analytical chemistry, and it remains useful when the response is hard to model or when instrument automation allows sequential experiments [24]. More recently, Bayesian optimization has emerged as a sample-efficient strategy for expensive-to-evaluate functions. It builds a probabilistic surrogate model of the response and chooses each next experiment by balancing exploration and exploitation [25].

2.3 Data Preprocessing and Exploratory Analysis

Raw chromatographic data are rarely ready for modeling. Noise, baseline drift, and retention-time shifts between runs all degrade the quality of the information. The Savitzky–Golay filter, which smooths and differentiates data by local polynomial least-squares fitting, is a foundational preprocessing method for noisy signals [26]. Penalized least-squares smoothing, described by Eilers as "a perfect smoother," offers a flexible alternative that also underlies several baseline-correction approaches [27]. When multiple chromatograms must be compared, as in impurity profiling or stability studies, retention-time misalignment can be corrected with algorithms such as parametric time warping [28].

After preprocessing, principal component analysis (PCA) provides an unsupervised overview of multivariate data. It projects the data onto a small number of orthogonal components that capture the largest variance, revealing groupings, trends, and outliers [29], [30]. In method development, PCA can be applied to a set of chromatograms or to diode-array spectra to see whether samples cluster by column type, pH, or degradation condition, or to detect an anomalous run.

For quantitative modeling, partial least squares (PLS) regression relates a block of predictors (for example, spectra or full chromatograms) to one or more responses (for example, concentrations), and it handles collinear and noisy variables well [31], [32]. PLS is especially valuable for building calibration models in situations where chromatographic resolution is incomplete but a multichannel detector provides additional selectivity.

2.4 Resolving Overlapping Peaks: MCR and PARAFAC

Even a well-optimized method may leave co-eluting peaks, for example when a degradation product elutes within the main API peak. Second-order data, such as HPLC with diode-array detection (HPLC-DAD) or LC-MS, produce a matrix for each sample (retention time × wavelength or m/z), which opens the door to mathematical resolution of overlaps.

Multivariate curve resolution (MCR), particularly the alternating least squares variant (MCR-ALS), decomposes the data matrix into pure concentration profiles and pure spectra under constraints such as non-negativity and unimodality [33], [34]. Tauler showed how MCR-ALS can be extended to multiple matrices, which improves the reliability of the resolution and allows quantification even in the presence of uncalibrated interferents [33]. Parallel factor analysis (PARAFAC) decomposes three-way arrays (samples × time × wavelength) into trilinear components and, when the trilinearity assumption holds, provides unique solutions without additional constraints [35]. Automated peak detection and deconvolution programs have also been developed for multi-overlapped chromatographic signals [36].

These tools are complementary to optimization. Optimization aims to achieve physical separation, while resolution tools provide a mathematical safety net, and in some cases, they allow shorter, faster separations that would not be acceptable if baseline resolution had to be achieved physically.

2.5 Analytical Quality by Design and Robustness

The pharmaceutical industry's quality-by-design philosophy, originally articulated for drug product development in ICH Q8(R2), has been extended to analytical methods [37]. Analytical quality by design (AQbD) begins by defining an analytical target profile (ATP), which states what the method must achieve. It then identifies critical method attributes and critical method parameters through risk assessment and screening designs, builds response surface models, and defines a method operable design region (MODR), a multidimensional zone within which the method meets the ATP [38], [39]. Working inside the MODR gives regulatory flexibility, since movement within the region is not a change to the method but part of its validated operating space. Orlandini, Pinzauti, and Furlanetto reviewed how QbD concepts, with DoE at their core, have been applied to separation methods [40], and Tomé and colleagues summarized recent advances in AQbD-based LC method development [41].

Robustness evaluation is the final element of the picture. Guidance by Vander Heyden and coworkers describes how to select factors, levels, and designs for robustness and ruggedness testing, and how to interpret effects statistically, so that system suitability limits can be set on a rational basis [42]. The 2023 ICH Q14 and Q2(R2) guidelines further formalize the lifecycle approach to analytical procedures and explicitly recognize multivariate procedures and enhanced approaches to development [2], [3].

III. Methodology

This paper adopts a qualitative, descriptive review design. It synthesizes foundational and widely cited peer-reviewed literature, standard reference texts, and regulatory guidance on chemometrics and chromatographic method development. The tools were compared across four dimensions: (i) the purpose in the development cycle (screening, optimization, data resolution, or validation); (ii) the data requirements; (iii) the main advantages; and (iv) the principal limitations. From this comparison, the paper synthesizes a generic chemometric workflow for chromatographic method optimization, which is described below.

Step 1: Define the analytical target profile. The analyst specifies the analytes, the matrix, the required range, and the performance criteria, for example minimum resolution of 2.0 between the critical pair, tailing factor below 1.5, and a run time under a stated limit [2], [38].

Step 2: Preliminary scouting. A few exploratory runs on different columns and mobile-phase systems establish a feasible region. Knowledge of analyte properties (pKa, log P, UV spectrum, stability) guides the choice of initial conditions [1].
Step 3: Risk assessment and factor selection. Candidate factors are ranked, using tools such as Ishikawa diagrams and risk matrices, to reduce the list to those most likely to affect critical responses [38], [39].
Step 4: Screening design. A fractional factorial or Plackett–Burman design identifies the statistically significant factors [9], [15].
Step 5: Response surface design. For the two to four significant factors, a CCD or BBD is executed. Responses such as retention factors, resolution of critical pairs, peak asymmetry, and analysis time are recorded for each run [11], [13].
Step 6: Modeling and diagnostics. Quadratic models are fitted by multiple linear regression. Analysis of variance, lack-of-fit tests, residual plots, and cross-validated predictive ability (Q^2) are used to judge model adequacy [13], [14].
Step 7: Multi-response optimization. Desirability functions or Pareto-based methods are used to find compromise conditions [17], [18]. Surface and contour plots help visualize the region of acceptable performance.
Step 8: Confirmation and robustness. Predicted optimum conditions are verified experimentally. The MODR is defined, and robustness is tested around the set point with a small design [39], [42].
Step 9: Data-analytical support. Where peaks overlap or multichannel detectors are used, preprocessing and MCR or PARAFAC are applied to verify peak purity and resolve interferences [27], [33], [35].
Step 10: Validation and lifecycle management. The final method is validated according to current guidelines and monitored over its lifecycle [2], [3].

IV. Results and Discussion

4.1 Mapping Chemometric Tools to Development Tasks

Table 1 summarizes how the principal chemometric tools map onto the tasks encountered in chromatographic method development for drug analysis.

Table 1. Chemometric Tools and Their Roles in Chromatographic Method Development

Tool	Primary Role	Typical Output	Key Limitation
Plackett–Burman / fractional factorial	Factor screening	Ranking of main effects	Interactions confounded
Central composite / Box–Behnken	Response surface modeling	Quadratic model; optimum region	Needs few (2–4) factors
Desirability function	Multi-response compromise	Single overall desirability	Subjective weights
NSGA-II (evolutionary)	Multi-objective search	Pareto front	Needs a predictive model or many evaluations
Simplex / Bayesian optimization	Sequential, model-light search	Improved conditions in few runs	Local optima (simplex); modeling choices (Bayesian)
PCA	Exploratory analysis	Scores/loadings plots	Unsupervised; interpretation needed
PLS	Calibration/regression	Prediction models	Requires representative calibration set
MCR-ALS / PARAFAC	Peak resolution	Pure profiles and spectra	Rotational ambiguity; trilinearity assumption

4.2 Screening and Modeling in Practice

In a typical stability-indicating HPLC method for a drug and its degradation products, the number of candidate factors is large, but experience shows that

a few usually dominate selectivity: mobile-phase pH, organic-solvent proportion or gradient slope, and column temperature. Screening designs confirm this quickly and inexpensively. A Plackett–Burman design with 12 runs, for example, can screen up to 11 factors, which would require many more experiments by OFAT [9]. The follow-up Box–Behnken design for three factors needs only 15 runs including center-point replicates [11], yet it supports a full quadratic model with interaction and curvature terms. This economy is the principal practical argument for chemometric optimization. Fewer experiments mean less solvent consumption, less instrument time, and faster development.

The quality of the resulting model determines the reliability of the optimum. Diagnostic checks are indispensable. A significant lack of fit indicates that a quadratic model is inadequate, and the region may need to be narrowed or a transformation applied. Replicated center points give an estimate of pure error, which makes lack-of-fit testing possible [13], [14]. Over-reliance on a single high R^2 is a common pitfall. Predictive ability assessed by cross-validation, or by additional confirmation experiments, gives a more honest picture.

4.3 Handling Competing Objectives

When the goals conflict, the desirability approach offers a transparent way to document the analyst's priorities. For instance, a method for a fixed-dose combination might set the critical-pair resolution as most important, run time as moderately important, and tailing as a constraint. The optimizer then returns conditions that balance them [17]. The weights, however, are subjective, and different weighting schemes can lead to different optima. Reporting the sensitivity of the optimum to the weights, or presenting the Pareto front when NSGA-II is used, lets the decision-maker see the trade-offs explicitly rather than hiding them inside a single number [18]. Because a Pareto front from an evolutionary search needs many model evaluations, it is normally applied to fitted surrogate models rather than to physical experiments.

4.4 Interaction with Retention Modeling

Chemometric optimization is often combined with mechanistic retention models. In gradient HPLC, retention can be predicted from a small number of scouting runs at different gradient times or temperatures, and software can then simulate chromatograms across many conditions and find the most favorable ones [1], [21]. These approaches rely on well-established theory of solute retention, which makes them efficient, but they work best for compounds that follow the underlying model. For ionizable analytes, models that incorporate pH-dependent retention are needed. Statistical RSM, by contrast, requires no mechanistic assumptions and applies equally to the less well-behaved cases, such as ion-pair systems or mixed-mode columns. In practice, the two perspectives complement each other: mechanistic modeling narrows the search space and DoE verifies and refines the result with real data.

4.5 Data-Analytical Tools in Peak Purity and Co-elution

Even after optimization, residual overlap may occur, particularly in complex matrices such as biological fluids, herbal formulations, or forced-degradation samples. HPLC-DAD data combined with MCR-ALS can resolve a co-eluting interferent and quantify the analyte without requiring complete physical separation [33], [34]. This offers two benefits: shorter run times and a built-in peak-purity check, since the number of significant components in a peak region can be estimated from the data. The method's reliability, however, depends on the structure of the data. If the interferent's spectrum is highly similar to the analyte's, or if the profiles are strongly rank deficient, the solution suffers from rotational ambiguity [34]. PARAFAC offers unique solutions when trilinearity holds, but retention-time shifts between runs violate this assumption and must be corrected, for example by alignment, before modeling [28], [35].

4.6 Regulatory and Lifecycle Considerations

The chemometric workflow aligns closely with the lifecycle approach in ICH Q14. The ATP defines the performance criteria, DoE generates the knowledge for the development report, and the MODR provides a documented operating space [2], [38], [39]. ICH Q2(R2) additionally accepts that some analytical procedures, such as those based on multivariate calibration, require specific validation strategies [3]. For analysts, this means that the models and the data used to build them are part of the method's documentation and must be maintained, version-controlled, and transferable.

Several practical lessons emerge from the comparison:

1. **Use the right design for the right stage.** Screening designs should not be used to claim an optimum, and response surface designs are wasteful with too many factors [15].
2. **Verify, don't trust.** Predicted optima always need experimental confirmation under the intended conditions [13].
3. **Mind the factor ranges.** Models are valid only within the studied domain. Extrapolation can fail, particularly for pH near analyte pKa values [1].
4. **Document the decisions.** Weights, constraints, and acceptance criteria must be justified in terms of the ATP and not chosen to produce a desired outcome [38].
5. **Combine physical and mathematical selectivity thoughtfully.** Mathematical resolution complements, but should not replace, adequate chromatographic

separation for stability-indicating methods [34].

V. Challenges and Future Directions

Despite its maturity, chemometric-assisted optimization faces several challenges, and the literature points to a number of directions for further development.

Increasing automation. Modern HPLC systems with automated mobile-phase blending, column switching, and software-controlled sequencing permit closed-loop optimization, in which an algorithm proposes experiments, the instrument runs them, and the results feed back into the model. Sequential optimization methods such as the simplex and Bayesian optimization are natural candidates for this setting because they select each experiment on the basis of all previous results [23], [25].

Machine learning and hybrid models. Methods beyond quadratic polynomial models, such as Gaussian processes, random forests, and neural networks, can capture strongly nonlinear behavior. They require care to avoid overfitting with the small datasets typical of chromatography. Hybrid approaches that embed mechanistic knowledge in data-driven models are a promising route, since they can reach good accuracy with fewer experiments [25].

Method transfer and column variability. A MODR established on one instrument and column batch may shift on another because of differences in dwell volume, extra-column dispersion, and column lot-to-lot selectivity. Including such noise factors in the robustness design, and characterizing columns with standardized test protocols, remains an open practical problem [1], [42].

Multi-way data and hyphenated techniques. As LC-MS, two-dimensional LC, and comprehensive detectors become routine, the data volume grows, and multi-way methods such as PARAFAC and MCR-ALS become more relevant. Improving their reliability, for example by handling retention-time shifts and nonlinearity, and integrating them into validated workflows, is an active field [33], [35].

Training and software accessibility. Many practitioners still lack formal training in DoE and multivariate statistics. User-friendly software and clear guidance on interpreting diagnostics are important to prevent misuse, for example fitting models without checking lack of fit or extrapolating beyond the design space [15].

Sustainability. DoE reduces experiment counts, but chemometrics can go further by explicitly including environmental criteria, such as solvent toxicity and waste volume, as responses in multi-objective optimization, which supports greener analytical methods without sacrificing performance [18].

VI. Conclusion

Chromatographic method development for drug analysis is inherently multivariate, and chemometric tools provide the means to address it rigorously. Screening designs reduce the factor space economically, response surface designs model the relationships between factors and critical attributes, desirability functions and evolutionary algorithms reconcile competing objectives, and multivariate data-analysis methods such as PCA, PLS, MCR-ALS, and PARAFAC extract information from complex or incompletely resolved signals. Embedded in an AQbD framework, these techniques yield methods that are better understood, more robust, and easier to defend and maintain over their lifecycle. The principal cautions are that models are valid only within the studied domain, that subjective choices such as weighting must be justified against the analytical target profile, and that mathematical resolution should complement rather than replace physical separation. Future progress will likely come from automated closed-loop optimization, hybrid mechanistic and machine-learning models, better handling of method transfer variability, and the inclusion of sustainability criteria in optimization. As regulatory guidance increasingly endorses enhanced, knowledge-based approaches to analytical procedures, chemometric-assisted optimization is likely to become the standard rather than the exception in pharmaceutical analysis.

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