Analytica Chimica Acta 1293 (2024) 342178

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Computer-assisted multifactorial method development for the streamlined separation and analysis of multicomponent mixtures in (Bio) pharmaceutical settings

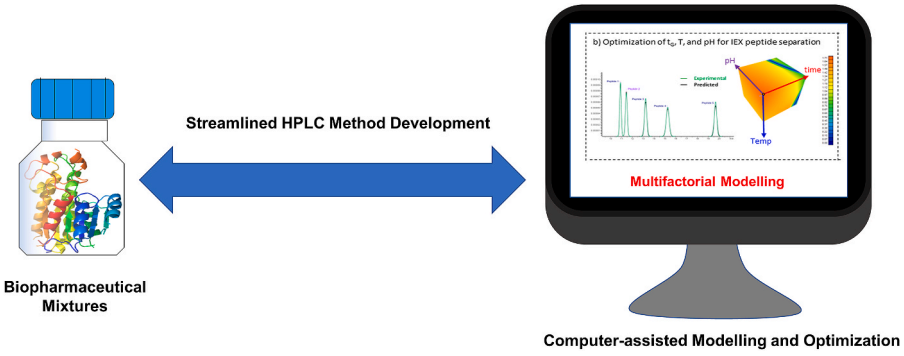
Mohamed Hemida *, Imad A. Haidar Ahmad **, Rodell C. Barrientos, Erik L. Regalado

Analytical Research and Development, MRL, Merck & Co., Inc., 126 E. Lincoln Avenue, Rahway, NJ, 07065, United States

HIGHLIGHTS

- Computer-Assisted multifactorial chromatographic strategies were addressed.
- Using proper linear and non-linear models were discussed.
- Applications in various chromatographic techniques are critically reviewed.
- Important parameters for retention mechanism modelling are demonstrated.
- Computer-Assisted optimization strategies are highlighted for generic methods.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Computer-assisted
Optimization
Liquid chromatography
Method development
Complex mixtures
(Bio)pharmaceuticals

ABSTRACT

The (bio)pharmaceutical industry is rapidly moving towards complex drug modalities that require a commensurate level of analytical enabling technologies that can be deployed at a fast pace. Unsystematic method development and unnecessary manual intervention remain a major barrier towards a more efficient deployment of meaningful analytical assay across emerging modalities. Digitalization and automation are key to streamline method development and enable rapid assay deployment. This review discusses the use of computer-assisted multifactorial chromatographic method development strategies for fast-paced downstream characterization and purification of biopharmaceuticals. Various chromatographic techniques such as reversed-phase liquid chromatography (RPLC), hydrophilic interaction liquid chromatography (HILIC), ion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC), and supercritical fluid chromatography (SFC) are addressed and critically reviewed. The most significant parameters for retention mechanism modelling, as well as mapping the separation landscape for optimal chromatographic selectivity and resolution are also discussed. Furthermore, several computer-assisted approaches for optimization and development of chromatographic methods of therapeutics, including linear, nonlinear, and multifactorial modelling are outlined. Finally, the potential of the chromatographic modelling and computer-assisted optimization strategies are also illustrated,

* Corresponding author. Analytical Research and Development, MRL, Merck & Co., Inc., 126 E. Lincoln Avenue, Rahway, NJ, 07065, United States.
** Corresponding author. Analytical Research and Development, MRL, Merck & Co., Inc., 126 E. Lincoln Avenue, Rahway, NJ, 07065, United States.
E-mail addresses: mohdhamdy_84108@hotmail.com (M. Hemida), iahmad.umn@gmail.com (I.A. Haidar Ahmad).

https://doi.org/10.1016/j.aca.2023.342178
Received 29 October 2023; Received in revised form 13 December 2023; Accepted 23 December 2023
Available online 27 December 2023

highlighting substantial productivity improvements, and cost savings while accelerating method development, deployment and transfer processes for therapeutic analysis in industrial settings.

1. Introduction

Recent trends in drug discovery and development point towards tremendous increase in new drug modalities changing the way complex diseases are treated. This can range from multi-active ingredients to challenging co-formulations and multicomponent pharmaceutical mixtures bringing challenges from both small and large molecule worlds [1–3]. The number of therapeutic large molecules, such as monoclonal antibodies (mAbs), fusion proteins, bioconjugates, and biosimilars, is constantly evolving [3]. As such, there is a great need to introduce new and reliable analytical techniques and methods to enable their development at a tremendous speed [4–6]. A variety of tools have been introduced in recent years to facilitate simpler and robust method development strategies [1,4,7–10]. Because of their physico-chemical complexity, method development for these modalities becomes extremely challenging. Chromatography has proved to be a workhorse and the gold-standard analytical technique for pharmaceutical analysis throughout the various drug development phases from discovery to commercialization [1–10]. Because of the increasing complexity of biopharmaceuticals entering the market, the need for automation and streamlined method optimization is quickly becoming a bottleneck [1,6,11,12].

Recent efforts have mainly focused on developing innovative strategies to facilitate high throughput chromatographic method development. First, the use of generic or scouting methods to identify the best column and mobile phase combination has contributed to simplifying method development [8,13–18]. Second, automation of method scouting workflow with the use of solvent and column selector valves aids to streamline identification of the initial conditions for further optimization [10,16,19–21]. These recent advances do not fully address all inherent challenges encountered throughout the method development life cycle. Apart from widely used RPLC approaches, optimization of other chromatographic modes such as HILIC, IEX and SFC is mainly based on inefficient trial-and-error approaches and unnecessary manual intervention [1,6,11,12].

The use of computer-assisted simulation tools to accurately generate retention models has demonstrated a dramatic impact in accelerating chromatographic method development [19–22]. Numerous research projects have focused on this subject for target molecules across both academic and industrial sectors [22–31]. Initially, computer-assisted models were available exclusively for RPLC due to its straightforward and well-understood separation mechanism [22–37]. However, it has been extensively demonstrated that more complex models and equations can also successfully predict the retention of a wide spectrum of compounds using more complex chromatographic techniques (*e.g.* HILIC, HIC, IEX, SFC, chiral, etc.) [24,30,32–34]. Typically, coupling initial method screening with the use of predictive tools was fundamental to obtain effective separation of mixtures containing closely related compounds [31–38] (See Table 1).

In this review, we aim to highlight and critically analyze the latest trends in the use of computer-assisted approaches towards more efficient and streamlined chromatographic method development for challenging (bio)pharmaceutical samples. A discussion on different optimization and prediction models is covered that tackles the challenge of predicting the retention behavior of small and large molecules across different chromatographic techniques. Furthermore, the usefulness of computer-assisted multifactorial simulations to address and predict the influence of multiple chromatographic parameters onto the separation outcome is also discussed. In addition, a brief description of the latest applications currently illustrated in the literature involving computer-assisted modelling is provided.

2. Tools for computer-assisted method development

Chromatography method development often requires tremendous amount of work to obtain reliable and deployable assays. It involves optimizing various parameters, including, but not limited to the (1) stationary phase type and chemistry; (2) column temperature; (3) gradient slope or steepness; (4) mobile phase pH; and (5) the concentration and type of mobile phase additives. Computer-assisted method development streamlines this optimization process by constructing retention models. Retention models are mathematical relationships of the analyte retention time as a function of various parameters that can be reliably controlled such as those previously mentioned. These retention models are then used to simulate the separation using a computer, thus, reducing the burden of having to run multiple trial-and-error experiments. In general, retention modelling can facilitate efficient LC method optimization, development and transfer [37,39]. There are five basic retention models that can be used to describe the retention of analytes, *i.e.* the linear-solvent-strength model (LSS), the Neue–Kuss model, the quadratic model, the adsorption model, and the mixed-mode model [39]. Alternatively, empirical models can be applied, using a few number of experiments, and correlating analyte with measurement abstract parameters to predict retention mechanisms. The detailed mathematical descriptions of these models are beyond the scope of this present review, but the reader is referred to a comprehensive review of the subject published previously [39].

In recent years, various commercial software packages have been introduced that incorporated the ability for retention modelling, chromatographic simulation, and automation of method development such as DryLab (Molnar- Institute, Germany), ACD/LC Simulator and ACD/AutoChrom (ACD/Laboratories, Canada), ChromSword Software packages (ChromSword, Latvia), Fusion (S-Matrix, USA), Osiris (Datalys, France) and EluEx software by CompuDrug Chemistry Ltd (Budapest, Hungary) (Table 2).

DryLab and ACD/LC Simulator are considered the most common tools, they employ empirical mathematical models for simulation, and streamlined method development by mapping the separation landscape of multicomponent mixtures and generating multifactorial resolution maps containing optimal resolution spaces [37–41]. DryLab can predict retention time for optimization of chromatographic resolution under a wide range of parameters from a few experimental runs (2–12). DryLab can also operate systematically with the chromatographic data system (CDS) to acquire data, build models and visualize resolution maps (critical resolution $R_{s,crit}$) as a function of multiple variables [41]. The latest version of DryLab 'Peak Tracking', and 'Column Match' can deliver peak identification, peak assignment, and columns comparison from a large library [41]. Alternatively, ACD/LC simulator offers automated screening capabilities, multifactorial optimization including multiple factors (gradient, temperature, pH, buffer concentration, salt concentration, solvent ratio), retention prediction and optimal resolution under various experimental parameters, with minimized experimental input [42,43]. This system also can run with CDS to acquire data, build models and visualize resolution maps ($Rs_{,crit}$). The latest version from ACD software (ACD/AutoChrom) delivers peak tracking, peak assignment and peak selection capabilities using a searchable column library.

On the other hand, ChromSword is a powerful software package that delivers acceptable retention prediction derived from the molecular structure [16]. The latest version of ChromSword enables automated method development including screening (column, solvents, buffers), optimization and method robustness in RPLC, NPLC, IEX modes using both LSS and polynomial models, with the capability to run as a CDS

Table 1
List of abbreviations.

Abbreviations	Definition
ADCs	Antibody drug conjugates
AQbD	Analytical Quality by Design
API	Active Pharmaceutical Ingredient
Fab	Antigenbinding fraction of mAbs
BAs	Biogenic amines
bsAbs	Bispecific antibodies
CAD	Charged Aerosol Detector
CDS	Computer Data Systems
CMPs	Critical method parameters
DAR	Drug-to antibody ratio
DGUC	Dual-gradient unifiedchromatography
Fc	The crystallizable fraction of mAbs
HC	Heavy chain (HC) variants of a recombinant mAb
HIC	Hydrophobic Interaction Chromatography
HILIC	Hydrophilic interaction Liquid chromatography
IC	Ion Chromatography
IEX	Ion-exchange chromatography (IEX),
LSS	Linear solvent strength
LogD	Logarithm of distribution coefficient between 1-octanol and water
log P	Logarithm of partition coefficient between1-octanol and water
mAbs	Monoclonal antibodies
mD-LC	Multi-dimensional chromatographic techniques
MPC	Multifactorial peak crossover
MS	Mass Spectrometry
NPLC	Normal phase liquid chromatography
PR&D	Pharmaceutical Research and Development
PDA	Photo diode array detector
Pd	Palladium
pKa	Negative algorithm of the acid dissociation constant
QSRR	Quantitative Structure-Retention Relationships
RPLC	Reversed phase liquid chromatography
R_t	Retention time
Rs	Resolution
$R_{s,crit}$	Critical resolution
SDM-R	Stochiometric Displacement Model for Retention
SEC	Size exclusion chromatography
SFC	Supercritical fluid chromatography
t_G	Gradient Time
UHPLC	Ultra-high pressure liquid chromatography

Table 2
Tools and software packages for computer-assisted method development.

Name	Developer	Comments	Ref
ACD/LC Simulator and AutoChrom	ACD/Laboratories (Canada)	Screening, R_t prediction, generates resolution maps to visualize optimal R_s v ($Rs_{,crit}$) in RPLC, HILIC, HIC, NPLC and IEX, works with CDS to acquire data, build models, enables selection of higher-degree polynomial fits for modeled parameters, ACD/AutoChrom version provides peak tracking, peak assignment and column selection.	[40, 42]
ChromSword	ChromSword (Latvia)	It provides approximate R_t prediction, screening, Rs optimization in RPLC, HILIC, HIC, NPLC, IEX modes using LSS and polynomial models, it operates as a CDS system, utilizes asmart algorithm that requires no analyst input for optimizing the gradient profile.	[16]
DryLab	Molnar-Institute (Germany)	Screening, R_t prediction and optimal Rs ($R_{s,crit}$) from a 3D cube module in RPLC, HILIC and IEX, works with CDS, enables peak identification, peak assignment, and columns selection.	[41]
Fusion	S-Matrix (USA)	Screening, optimization, development, and validation. It supports LC (RPLC, NPC, IEX, SEC, HILIC, HIC, Chiral) and SFC techniques and peak tracking.	[46]
EluEx	CompuDrug Chemistry Ltd (Hungary)	Uses chemical structure to initially calculate logD, logP and pKa values to find mobile phase compositions and pH range for simulating the chromatogram and optimum Rs in RPLC mode.	[44]
Osiris	Datalys (France)	Supports R_t prediction, R_s optimization and method validation for LC	[45]

system to automatically control LC instrumentation. EluEx software uses chemical structure to calculate logD, logP and pKa values to predict mobile phase compositions and pH range to assist in stationary phase scouting and resolution (Rs) optimization in RPLC mode [44]. Whereas Osiris software supports retention prediction, peak tracking, and optimization for LC by employing empirical mathematical models [45]. Fusion is a quality by design software which automates method development optimization by employing statistical Design of Experiments. This software also delivers screening and optimization options for the entire analytical workflow including sample preparation phase, and support LC, and SFC techniques often use in combination with Empower software for automation of method robustness studies [46]. Other strategies were also reported for the optimization of chromatographic conditions using MATLAB and Microsoft Excel software, which are cheap and available for education purposes [37].

3. Challenges in chromatographic method development for biopharmaceuticals

Biopharmaceuticals are drugs derived from living cells or organisms through a plethora of biotechnological techniques [3]. These molecules range from small, for example, penicillin to large molecules like monoclonal antibodies (mAbs), recombinant proteins, oligonucleotides, and virus like particles (VLPs) [3,7,33]. Hybrid molecules have also been introduced such as bioconjugates including antibody-drug conjugates (ADCs), lipidated peptides, PEGylated proteins, and many others [3,7,37]. Another type of hybrid molecules, fusion proteins, are formed by two or more different proteins chemically linked together. Examples include Fc-fusion proteins and multi-specific antibodies [7,37]. All these large molecules are complex, having a wide range of sizes (from <1 kDa to over 15 MDa), hydrophobicity, solubility in aqueous solutions, and isoelectric points (pI) [3,37]. Recent trends suggest that there is a mounting number of these modalities in the drug discovery space and various stages of clinical development. As such, new analytical approaches are greatly needed to accelerate assay development and deployment across modalities [37].

Method development in chromatography aims to explore the optimum conditions to achieve the best separation of target components. These parameters include column temperature, gradient steepness, pH, and mobile phase composition. Consequently, method development can often be tedious, laborious and time consuming. It also requires expert knowledge, trial-and-error, and substantial quantity of chemical reagents while also coping with a rapidly evolving complexity in molecular structure across the portfolio. Alongside, analytical strategies have to evolve over the years by introducing digital and automation tools that undoubtedly accelerated chromatographic assay development and deployment [37–47].

Chromatographic method development for large biopharmaceuticals remains challenging due to their chemical nature that dictates their on-column complex behavior. The complexity of these molecules stems from: 1) size, 2) structure and chemical composition, 3) pH sensitivity, and 4) reduction-oxidation potential. All these factors contribute to the

observed behavior of these molecules in chromatography. In most chromatographic modes, large biopharmaceuticals demonstrate retention mechanisms fundamentally different to what is observed for small molecules, such as (1) precipitation–redissolution, i.e., solubility-based retention mechanism rather than analyte-stationary phase interaction; (2) attachment to multi-point of the stationary phase surface; (3) an on/off retention at the column inlet until getting desorbed by the mobile phase gradient without further analyte-stationary phase interaction [5,37]. These are very sensitive to small changes in chromatographic parameters, such as column temperature, mobile phase composition and pH, to name a few.

For instance, mAbs show significant deviation in RPLC mode from the common linear temperature dependence of the retention (van't Hoff behavior), which can be attributed to its conformational change. Therefore, column temperature should be kept within a very narrow range to achieve acceptable separation and peak shape [33,37]. Similar situation was reported for the deviation of protein retention mechanism with small changes in mobile phase pH, organic modifier and ion-pairing reagent concentrations [37]. Hence in most cases, chromatographic parameters must be optimized in a very narrow range and limited design space. Not only can simple linear models describe retention mechanisms, but also polynomial, quadratic, and logarithmic models are applicable options. In this scenario, method optimization and development for the separation of large molecules and biopharmaceuticals have considerable challenges. Hence, method development for such molecules requires slow and long gradients, which is tedious and time-consuming. Besides this, column temperature can change retention behaviour, introducing post-translational modifications, denaturation, or thermal degradation [37,48–50]. This could result in retention time shift, peak broadening, and poor resolution. Thus, column temperature and mobile phase elution strength are critical optimization factors for those compounds.

Method screening or method scouting is also important in every stage of drug discovery and development to address chromatography challenges for large biopharmaceuticals, Fig. 1 [33]. It is often limited to the screening of different column chemistries, mobile phase additives and, to a limited extent, pH. Hence, generic methods can be quickly established as a viable option to rapidly analyze many biopharmaceutical samples [1]. After screening, optimization is often required to achieve sufficient chromatographic separation. In this scenario, computer-assisted approaches can potentially enable quick and automated method development for increasingly complex mixtures [8,23,28–32]. Improved selectivity, efficiency and excellent separation can be achieved with the fine-tuning of secondary parameters *via* computer-assisted strategies [17,23,28–32,40–47].

4. The role of computer-assisted approaches in method development and method transfer

Computer-assisted method development is highly valuable for chromatographic method development of large biomolecules that are sensitive to changes in mobile phase strength and column temperature [37,48]. Over the last decades, numerous publications demonstrated the value of computer-assisted strategies to speed up and automate method development by reducing the number of experiments needed compared to conventional approaches [8,35,37,47]. This enables a faster turnaround to obtain robust separation methods for the analysis of complex compounds, such as nucleotides [8], phytocannabinoids [38], palladium scavengers [47], closely related pharmaceutical intermediates [35] and proteins [37]. The rationale behind this is quite straightforward:

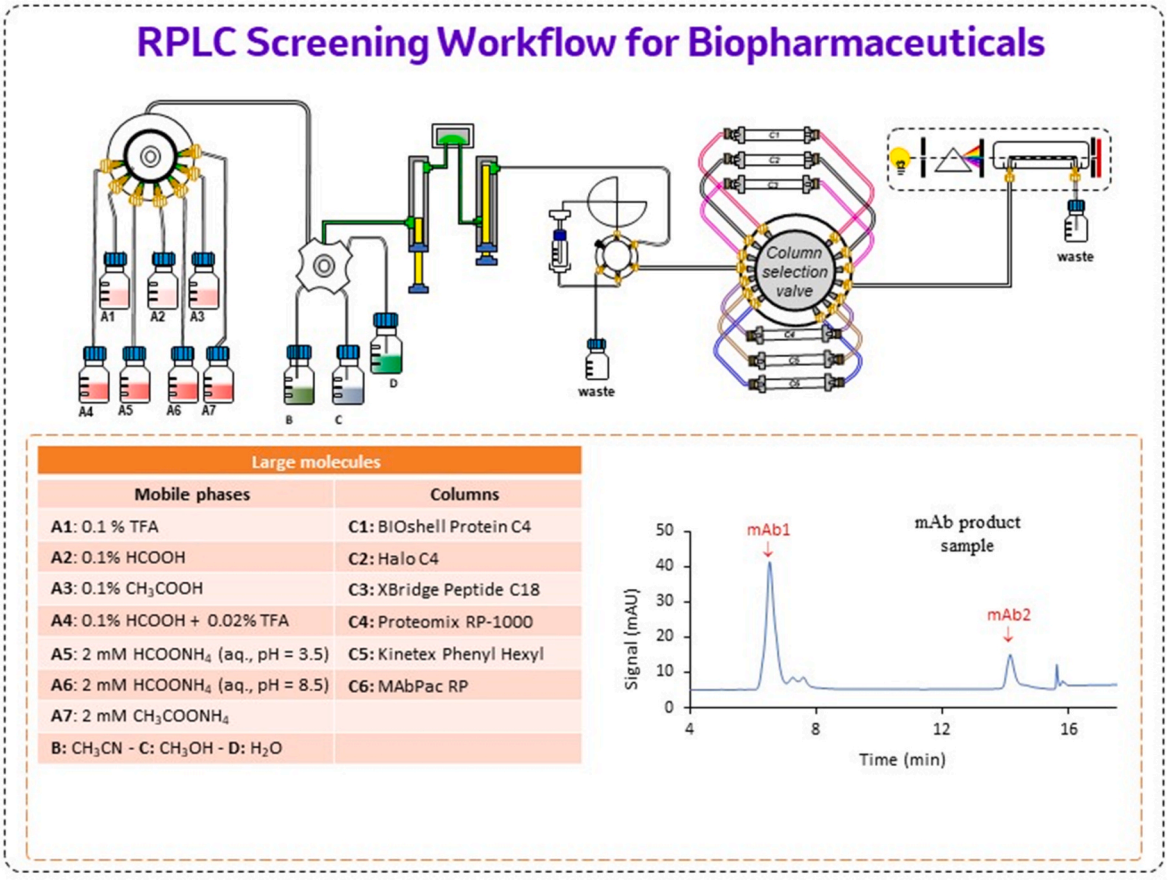


Fig. 1. Automated screening and method development workflow for purity profile of monoclonal antibodies (mAbs) mixture under RPLC conditions, (adapted with permission from American Chemical Society [33].

retention models can quickly identify a full set of chromatographic conditions where optimum baseline resolution of mixture components can be achieved in a cost-effective manner from fewer experimental runs. This approach can become quite useful to better understand the impact of numerous secondary variables on the quality of separation, especially for critical pairs in complex multicomponent mixtures [25,35,36]. For example, the influence of gradient time, column temperature and mobile phase pH has been quickly scouted to generate 3D resolution maps capturing the best separation of 24 neoplastic agents [35,36]. Due to the high number of ionizable species, the combined optimization of pH, temperature and gradient time resulted in a robust method for baseline resolution of all components with excellent correlation between predicted and experimental results. Similar approaches have led to the same optimal results [35,36], leading to the identification of robust separation conditions for facile method transfer.

It is worth noting that computer-assisted techniques not only deliver advantages in the method screening space, but also enable fast and robust method transfer between laboratories [51]. A clear trend in the use of computer-assisted strategies is to define critical method parameters (CMPs) and, consequently, design a space in which CMPs variability does not impact the overall quality of the analytical method by mapping the robustness range. This approach, called by many as "Analytical Quality by Design" (or AQbD), has been thoroughly investigated in different applications. For example, in understanding robustness of different columns [37,50,51], in developing efficient chromatographic methods for analytes present in complex matrices [51,52], and many other applications. As demonstrated by the authors, the use of simulations and modelling software identified conditions rapidly to ensure superior performance across different laboratories and easier method transfer. For more details on this topic, a comprehensive review is available [60].

Generic approaches to computer-assisted method development usually focus on understanding and subsequently predicting the interactions of a restricted number of combinations between different chromatographic parameters (*e.g.*, organic solvent ratio *vs* retention time; column temperature *vs* retention time, *etc.*) [37,53–60]. This strategy, however, does not always translate into a successful reduction in experiments, particularly when analyzing complex and challenging multicomponent mixtures. In these cases, the possibility to model combinations between more than two parameters simultaneously can represent a huge improvement, bringing down the time spent in developing and optimizing the chromatographic method while also identifying a space where the conditions obtained through modelling provide robust results.

The typical process of method development requires elevated level of knowledge and expertise. It also involves scouting and numerous trials-and-errors which could take up to several weeks of work with large amount of LC solvent consumption [37,60]. This is laborious, requires long analysis time and high cost. Alternatively, computer-based method development offers dramatic reduction in the solvent use and waste generation by minimizing the required number of experiments to maintain robust and short separation methods. For example, if column temperature (T) and gradient (t_G) are being optimized, four experiments (2 (T) × 2 (t_G)) are needed to establish a reliable model to identify optimum chromatographic conditions instead of running multiple experiments. Moreover, computer-assisted strategies have the potential to significantly speed up the process of method development, this is particularly important for pharmaceutical industries [37,38].

Analytical strategies often applied to obtain a baseline resolution of all components for complex mixtures require multidimensional chromatography. Multi-dimensional chromatographic techniques (mD-LC) are becoming routinely used for complex biopharmaceutical mixtures. Method development for mD-LC can be challenging to scientists who are not fully accustomed to the complex instrumentation. There has been a growing interest in applying modelling for mD-LC method development. The example in Fig. 2 [58] illustrates how the use of multifactorial

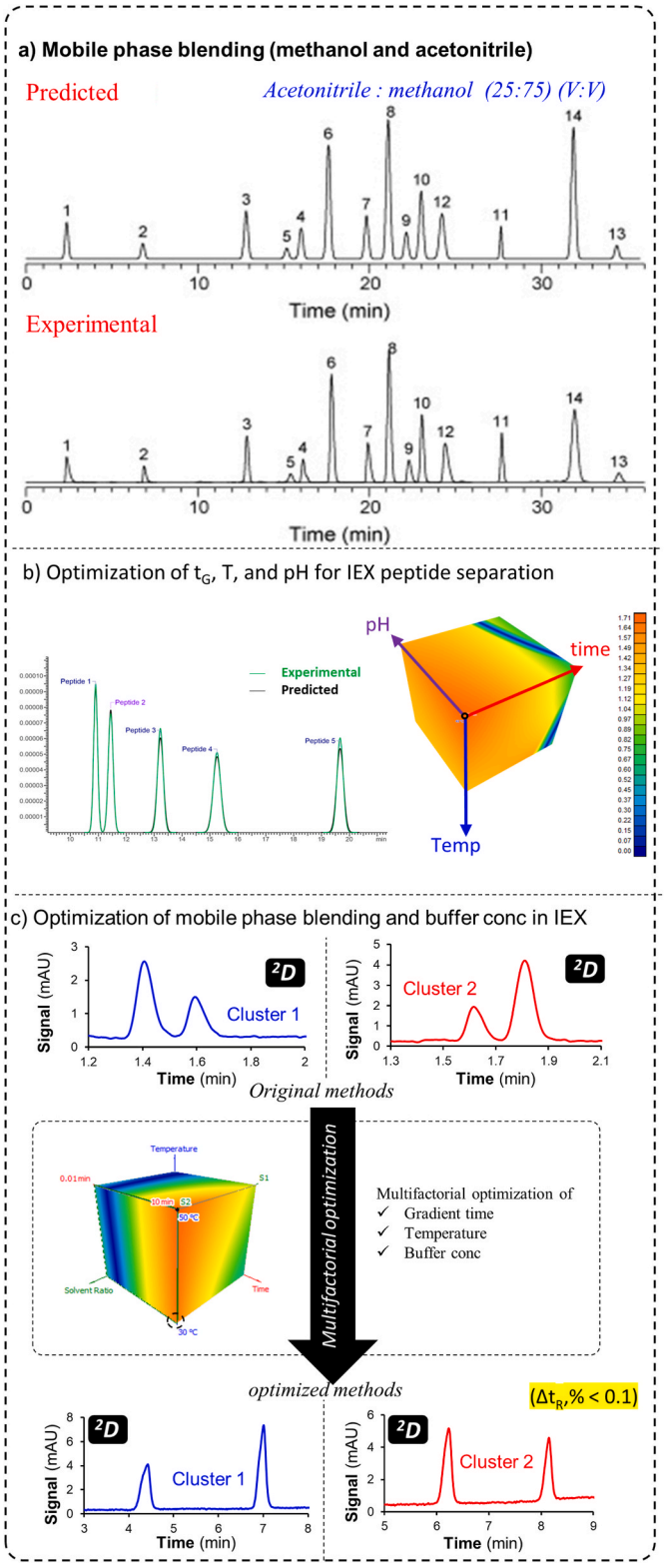


Fig. 2. Examples of multi-factorial optimization applied for challenging separations. (a) Optimizing methanol-acetonitrile blending in RPLC for development of robust methods, adapted with permission from Elsevier, [59]. (b) Modeling IEX separation of peptide mixture through optimizing pH, T, and t_G simultaneously, (c) *In Silico* optimization of 2D IEX method in two-dimensional LC separation by optimizing T, t_G, and buffer concentration (adapted with permission from American Chemical Society [58].

modelling serves to reduce the number of experiments required to establish a new analytical assay This has been demonstrated to unlock the separation of all unresolved components in the first dimension [40,56–58]. Multifactorial models enabled the simultaneous optimization of three different parameters (column temperature *vs* pH *vs* gradient time) to obtain a robust and fast method with baseline separation of all mixture components [40,58,60]. Three-dimensional resolution plots aid to assess the impact of more than two parameters on the final separation [40,47,58]. In this work [61], authors have demonstrated how a computer-assisted modelling strategy can be incredibly beneficial to predict and precisely choose the proper ratio of loop filling, with the aim to develop efficient methods in the second dimension while minimizing the amount of sample loss in the loops.

In general, there are different models which can be employed for understanding and optimizing separation mechanisms. The selection of the relevant model relies on the sample composition and employed chromatographic technique. For biopharmaceutical samples containing ionizable analytes (acidic or basic), pH could be an important variable for the chromatographic model. Alternatively, in the case of samples containing polar-neutral compounds, these compounds contain functional groups which can form H-bonding with the eluent and/or stationary phase. In this case, the organic modifier and concentration can be a crucial factor. Other variables should be considered in the model including additive or buffer concentration, isocratic composition, t_G, T, and ionic strength. Mathematical transformations can be used to linearize the impact of some variables (e.g., isocratic %B), and therefore they can be studied at two-level model. Other variables which have nonlinear retention mechanisms (e.g., pH, ternary composition, ionic strength), they may require three levels (or more) measurements.

5. Computer-assisted approaches in liquid chromatography

5.1. Computer-assisted approaches for RPLC

In general, RPLC offers superior efficiency, resolution, and robustness for hydrophobic (bio)pharmaceuticals and macromolecules [33,37,59,60]. Here, mobile phases consist of water, acetonitrile, or methanol-based eluents with a myriad of additives to enhance separation and detection. In many cases, volatile additives make RPLC highly suitable for routine use particularly with mass spectrometric and charged aerosol detector analyses [5,37,60]. However, there are some inherent challenges associated with RPLC such as slow mass transfer and low diffusivity, post-translational, and isomer modifications of biopharmaceuticals [5,48–50]. Moreover, protein variants are overly sensitive to changes in temperature, mobile phase composition, salt type and salt concentration [5,48,50,60].

In RPLC, optimization variables typically interrogated include gradient profile, mobile phase temperature, and mobile phase ternary composition. In general, there are different retention mechanisms reported for biopharmaceutical separation in RPLC mode, which are fundamentally different from retention mechanisms reported for small molecules [60,61]. The traditional approach to model retention behavior in RPLC follows linear solvent strength (LSS) model [62]. A simple linear relationship between the retention factor (logarithmic form) and the mobile phase elution strength (expressed as amounts of organic solvent mixed to mobile phase) was previously reported [62]. Authors in Ref. [63] discussed the validity of the LSS model with RPLC mode, as it provided minimal differences between experimental and predicted values for retention time. This was possible thanks to the narrow elution window demonstrated by the class of drug proteins, a behavior that has been witnessed in other studies as well [64,65].

Later, in the case of complex biomolecules such as peptides and proteins, there was a need to investigate both linear and non-linear models. This topic was discussed by different scientists who brought critical points on the use of LSS with RPLC retention prediction [32,39]. In this work [32,37,60], it has been demonstrated how the LSS model might not be the best prediction model for retention mechanism under RPLC conditions. The authors observed significant shifts between predicted and experimental retention factors for poorly and strongly retained compounds, regardless of their nature, and the stationary and mobile phases. Meanwhile, non-linear modelling functions provide better accuracy in the prediction of chromatographic behavior for proteins, depending on the nature of the additive used in the mobile phase (i.e., chaotropic agents). Hence, in Ref. [32] it was suggested to limit the use of LSS model for analytes within the retention factor range of 1–30. Meanwhile, in a different work [37], authors have reached a similar conclusion to that of the later report [32], using once again a series of small molecules in RPLC. This remark is particularly interesting as small molecules have been considered to fit best with the LSS model [66,67]. Therefore, for those molecules that are either not well or too strongly retained by the column under RPLC mode, different models have been suggested to yield more robust results, such as the quadratic approach [60] and the Neue-Kuss model for capillary-scale LC [68]. Modifications of the original LSS model have also been proposed to improve the reliability of the simulations [69]. This suggests that the chromatographic behavior of biomolecules, particularly therapeutic proteins, can be influenced by the conformation changes inherently present with complex structures, and by other factors such as the chemical composition and concentration of mobile phase additives or column temperature. This can affect the denaturation of biomolecules, causing induced unfolding and accordingly altering the retention mechanism [5,60,67]. Fortunately, the recent improvement in computational capabilities allowed the creation of robust linear and non-linear model in an automated manner [37,38,60].

In this scenario, the prediction model can be plotted in the two-dimensional resolution map to visualize the optimum conditions. The resolution maps translate into a facile selection of optimum conditions to maximize separation, with minimum number of experiments providing the critical resolution ($R_{s,crit}$) values for method development (Fig. 3). This strategic framework has delivered remarkable success with RPLC for the separations of a complex mixture of nucleotides, illustrating how retention modelling can be conveniently applied to streamline method development in industrial settings.

Recently, an innovative approach combining tandem-column with computer-assisted modelling strategies was established for complex chromatographic separations [31]. In this work, the authors have successfully demonstrated effective combinations (chiral and achiral) of serially coupled stationary phases in RPLC mode together with computer-assisted simulations. The set-up was used for complex samples containing multiple impurities with closely related structures. Attempts to use this strategy in more complex scenarios, such as in the context of chiral separations performed using tandem coupling of columns in supercritical fluid chromatography, have also been recently reported [34].

5.2. Computer-assisted approaches for HILIC

HILIC is a common complementary chromatographic mode to RPLC employed for the analysis of small polar compounds and therapeutic proteins [26,60]. It is typically compatible with various detectors including mass spectrometry and other detection techniques. HILIC could deliver more benefits for large molecules analysis, for example, it can employ moderate temperature to the mobile phase [60]. This enhances mass transfer, lowers the viscosity of the mobile phase and backpressure, allowing the use of different columns in tandem to improve the overall resolution for poorly recovered analytes [27,60]. HILIC has demonstrated enormous potential in proteomics, membrane protein, intact protein, therapeutic protein, and saccharides analysis [70]. The recent introduction of short columns with wide-pore silica stationary phases enabled the diffusion and separation of large molecules, particularly biological samples, and proteins using HILIC systems [70,71]. Meanwhile hydrophilic proteins, such as mAb glycoforms, showed much better separation in HILIC than other techniques [37,60].

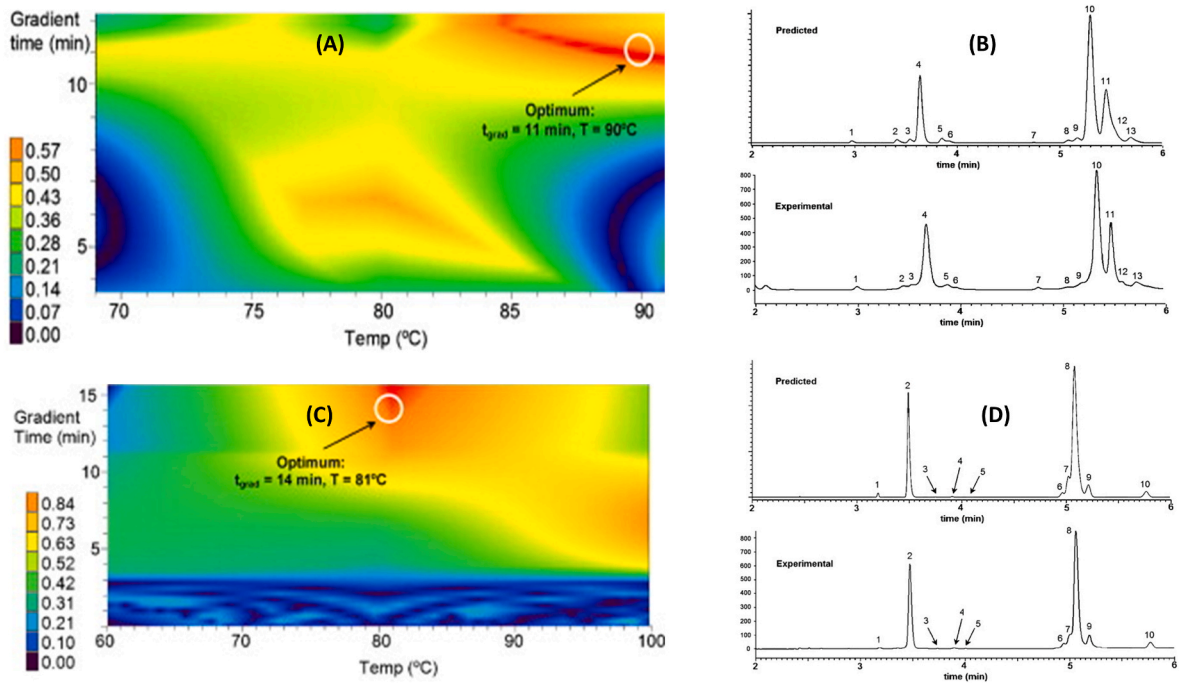


Fig. 3. Examples of computer-assisted RPLC optimization (A) two-dimensional resolution map of the gradient (tG) and column temperature (T (°C)) for the separation of Bevacizumab Fc and Fab fragments; (B) Predicted and experimental chromatograms of Bevacizumab Fc and Fab fragments using quadratic model; (C) Two-dimensional resolution map of the gradient (tG) and column temperature (T (°C)) for the separation for the separation of Rituximab LC and HC fragments; (D) predicted and experimental chromatograms of Bevacizumab Fc and Fab fragments using quadratic model, adapted with permission from Elsevier [112].

HILIC retention mechanism combines both hydrophilic partitioning and adsorption with hydrogen bonds, electrostatic and ionic interactions [32,60]. The limitations of a simple linear model, such as the LSS, become more evident when considering chromatographic techniques, such as HILIC [72] or even in the case of chiral separations [19]. In HILIC, sophisticated models are needed for accurate prediction of retention mechanisms and robust method development of large molecules (Fig. 4) [71]. Non-linear, polynomial, and mixed models have proved to be valid in predicting accurate retention times for proteins and biotherapeutics under HILIC conditions, with robust conditions quickly identified [37,73,74]. Further, multifactorial models (for gradient and temperature) were also utilized for the analysis of mAbs in mixed mode HILIC/RPLC mode [75].

Quantitative structure-retention relationship (QSRR) was reported for retention prediction and optimization in HILIC method development [32]. For a variety of proteins under HILIC conditions, a linear relationship was demonstrated between the logarithm of retention factors and the logarithm of water content in the mobile phase [32,73,74]. These results were obtained with a retention model called "Stochiometric Displacement Model for Retention" (SDM-R), correlating the complex retention-elution mechanism displayed in HILIC mode to the concentration of water molecules (acting as displacing agents). The proposed approach, which has been also validated for small molecules in HILIC mode, helps bringing a simple model of potential retention behaviors for many compounds, with the potential to facilitate method development thru a chromatographic technique that is known for its complex retention mechanism. In conclusion, the gradient time or gradient steepness with mobile phase temperature was shown to be the most vital variables for resolution and method development, meanwhile organic modifier is considered a non-important variable.

5.3. Computer-assisted approaches for IEX

IEX is a common non-denaturing technique for the analysis, purification and characterization of ionized molecules and biopharmaceuticals (i.e., charged proteins and peptides) [76]. In IEX systems, cation-exchange chromatography (CEX) is the most common mode for therapeutic protein, while anion exchange chromatography (AEX) is widely used for mAbs analysis [76,77]. Typically, column type, mobile phase pH, salt concentrations and salt gradient are considered as the most influential variables for chromatographic optimization of selectivity and resolution in IEX [76,77]. Retention mechanisms and prediction models for IEX can be categorized as stoichiometric or non-stoichiometric, which are based on the molecular structure of the ionized variants [60,78]. Numerous stoichiometric and non-stoichiometric retention mechanisms were reported for IEX techniques [32,37,60]. However, these approaches require thorough chemical characterization and time-consuming molecular structure calculation.

For example, CEX was reported for the separation and characterization of 10 charge mAbs, applying both pH and salt gradient models (Fig. 5) [79,80]. The salt gradient delivered higher resolution and higher peak capacity, with relative error <1.0 % for retention time prediction. The salt gradient IEX models were applied with linear elution at constant pH and temperature. Gradient steepness, and mobile phase pH showed significant importance to chromatographic selectivity and resolution, while mobile phase temperature did not appear to be a significant variable for the model. The applied mobile phase pH defined the net charge and elution order of the protein in the gradient program. This suggests that salt gradient could be of higher accuracy than pH gradient for the characterization of intact and fragments mAbs. On the other hand, pH gradient is a powerful strategy for the separation of protein isoforms, by applying wide range of pH, using different mobile phase buffers. There are few publications discussing the application of pH gradient mode in IEX separation of mAbs [79,80].

In general, the retention mechanism of intact mAbs demonstrated a linear behavior, with high significance for both variables, pH gradient slope and mobile phase temperature, providing high accuracy for retention time prediction for mAbs. Most recently in 2022, our research group developed a new screening workflow using both CEX and AEX

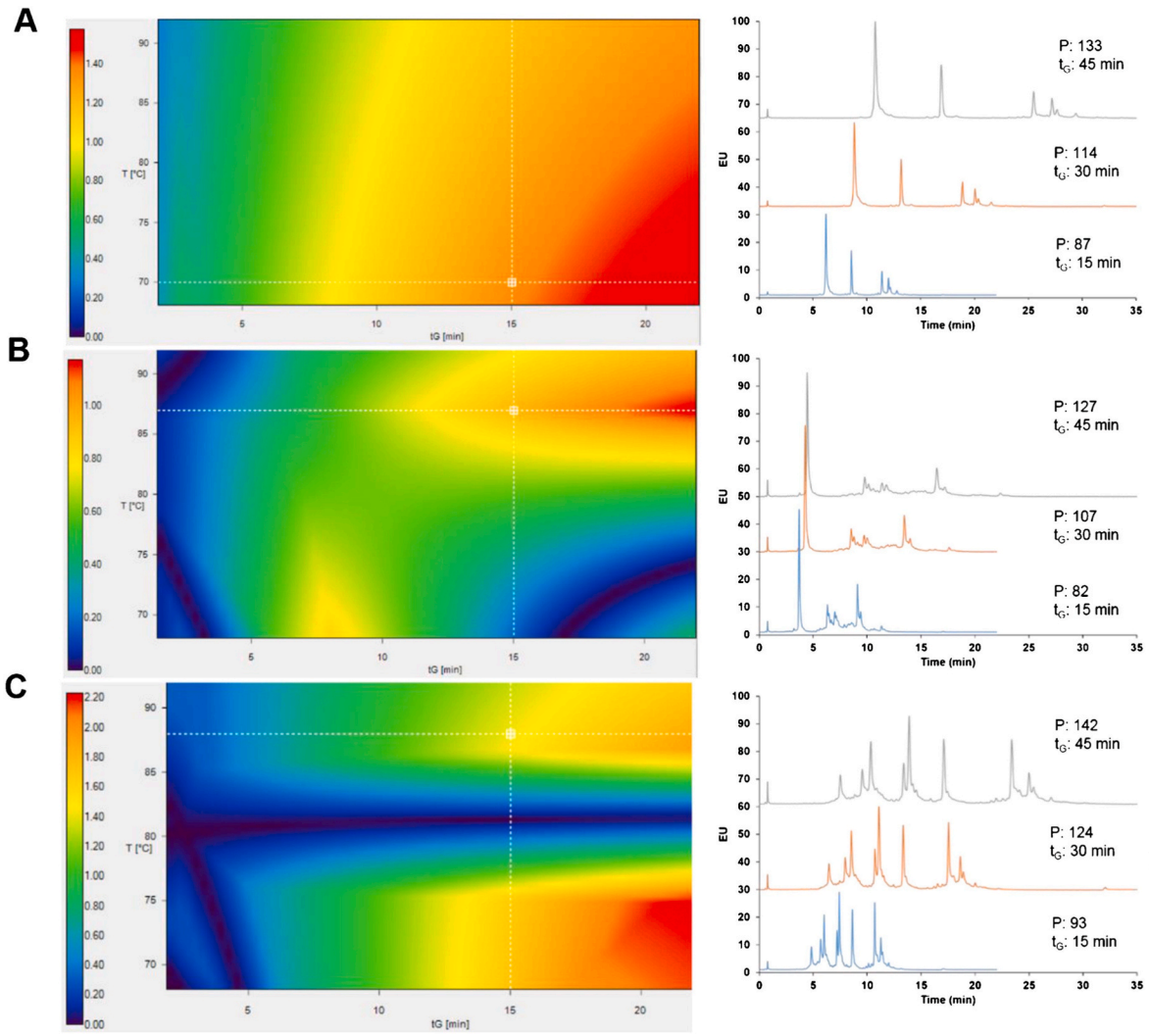


Fig. 4. Resolution maps with peak capacities in HILIC, optimized at different gradient times (tG) and Temperature (T (°C)) of (A) NISTmAb, (B) cetuximab and (C) brentuximab vedotin, adapted with permission from Elsevier [71].

technique and computer-assisted simulation for characterization and purification of nucleotides and peptides mixture in biopharmaceutical industrial settings [81]. In this work, the IEX screening columns were used for first dimension (^{1}D) with RPLC conditions in the second dimension as two-dimensional liquid chromatography configuration. For final method optimization, multifactorial model of chromatographic variables including mobile phase pH, column temperature, and gradient time were studied for the optimal separation of peptides. The multifactorial model demonstrated high accuracy (<0.5 %) for the studied compounds.

Computer-assisted simulations have facilitated the proliferation of innovative strategies for long-standing analytical challenges. For example, a recent methodology was recently reported to resolve closely related and co-eluted peaks of pharmaceutical mixtures that are highly challenging to be separated and purified in analytical and preparative scales in IEX and RPLC [29]. In this work, chromatographic modelling as a function of column temperature, mobile phase gradient, or a multifactorial combination, enabled the development of a new technique called multifactorial peak crossover (MPC) [29]. Using MPC, authors were able to map the separation landscape of studied mixtures and quickly identify peak coelution crossings and selectively switch the elution order of target peaks away from undesired coeluting peaks. This approach demonstrated an immense potential to facilitate efficient purification, and characterization of complex therapeutic substances and their impurities.

In further work, computer-assisted method development was used to establish a 2D-IEX-IEX method that otherwise would have normally meant a laborious amount of work [69]. Computer-assisted model was built for the system, and 3D-plots for the resolution of the second dimension IEX analysis, of nucleotides mixtures partially resolved *via* IEX analysis in the first dimension are given [69]. In this scenario, computer-assisted simulations minimized the number of experimental runs needed and were successful in employing multi-dimensional liquid chromatography (mD-LC) across high-throughput screening laboratories. This shows a disruptive combination for the developments of robust mD-LC making the technology more accessible.

5.4. Computer-assisted approaches for HIC

HIC is a non-denaturating mode for the analysis and purification of a wide range of biotherapeutics, including mAbs, ADCs and bsAbs [82–88]. This technique is based on an inverse salt gradient, as analyte's hydrophobic interaction with a stationary phase is altered using a kosmotropic salt, allowing the analyte to be retained by weakly hydrophobic stationary phases *via* the "salting-out effect". In contrast to RPLC, HIC uses minimal to no organic solvent for the elution of the analytes. There are different suggested retention mechanisms for HIC mode in the literature, which have been reviewed in a previous work [82,83].

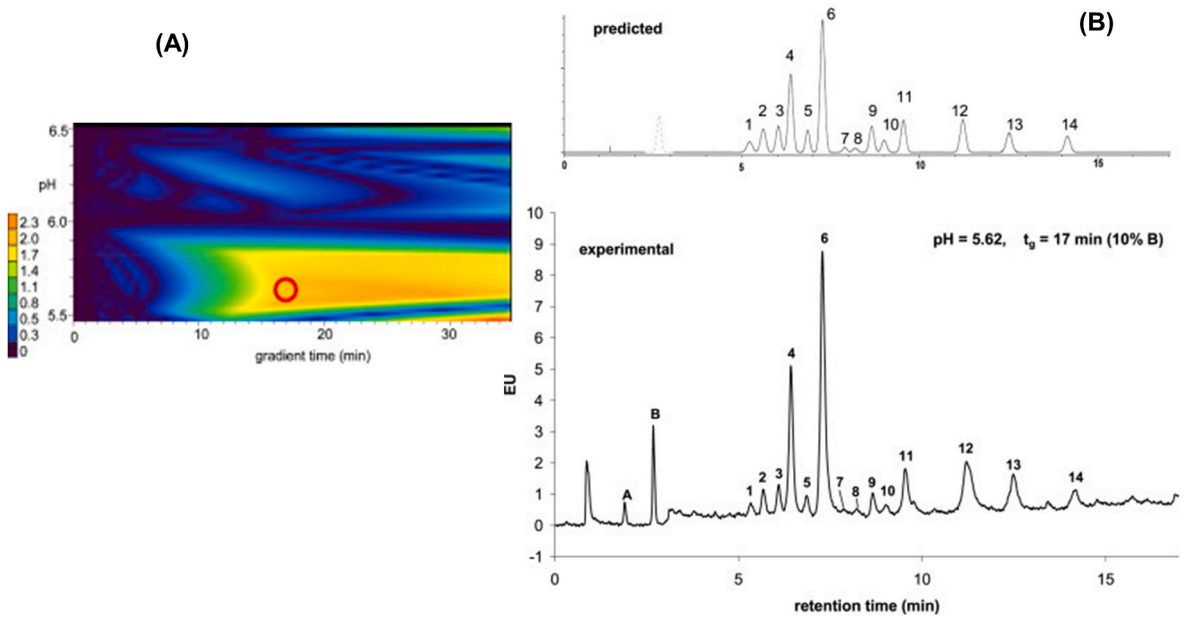


Fig. 5. (A) Resolution map for gradient (t_G−pH) IEX model of cetuximab sample, (B) chromatograms for predicted and experimental conditions, showing successful IEX model with good, adapted with permission from Elsevier [79].

Typically, HIC method development includes the selection of stationary phase, salt type and salt concentration, column temperature and pH of the mobile phase [83].

In a previous paper, it was demonstrated that the amount of organic modifier in the mobile phase has a crucial influence on HIC retention mechanism, and it is an important parameter in optimization of highly hydrophobic proteins (i.e., ADCs) [84]. Later, the same group demonstrated that mobile phase temperature and gradient steepness also influence HIC retention mechanisms following a linear retention model [83,84]. This result is consistent with Karger and Szepesy earlier work showing that the retention mechanisms of proteins follow the LSS model in HIC gradient systems [85,86]. In addition to the above, mobile phase temperature and gradient steepness were also studied and showed an LSS behavior for mAbs separation, with high prediction accuracy (error ~ 1.0 %) for retention time model [83]. The same research group also published the application of non-linear models in HIC gradient retention mechanisms, and its potential to enhance the separation between different Drug-Antibody-Ratio (DAR) species [83]. It is worth noting that the resolution for the separated compounds was evaluated using three different gradient models, namely power function, linear, and logarithmic. The logarithmic gradient profile provided the highest overall resolution and prediction accuracy, and most peak focusing with equidistant retention distribution for the DAR species, against the linear model for HIC [87,88].

Most recently in 2022, Barrientos et al., [88] introduced an automated multi-column and multi-eluent HIC platform coupled with an

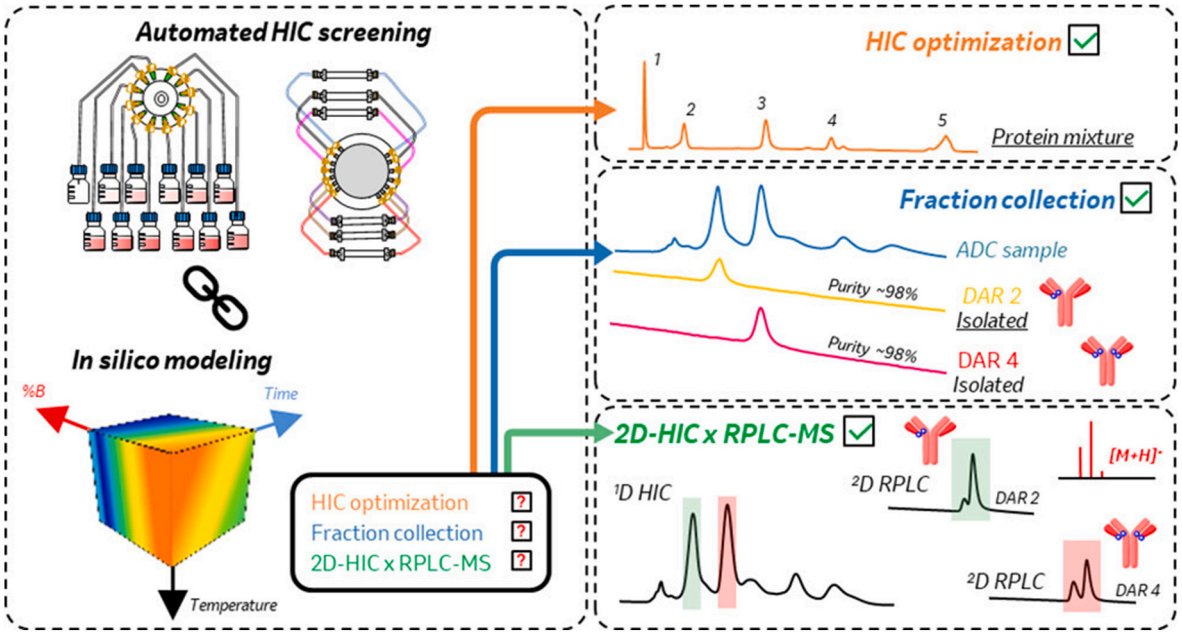


Fig. 6. Examples of using computer assisted modeling for automated screening, multifactorial modeling and optimization for the separation and purification of biopharmaceutical targets including protein, ADCs and mAbs in HIC mode (adapted with permission from American Chemical Society [88].

integrated fraction collection and computer-assisted multifactorial simulation (Fig. 6). This work streamlined automated method screening, optimization, and purification workflow for biopharmaceuticals including proteins, mAbs, ADCs, and oxidation variants. 3D multifactorial model was built from gradient steepness, column temperature, and salt blending variables to obtain the optimum resolution and selectivity. The retention models were built with the commercially available LC simulator software, deliveing less than 5 % error across all (bio)pharamceutical mixtures.

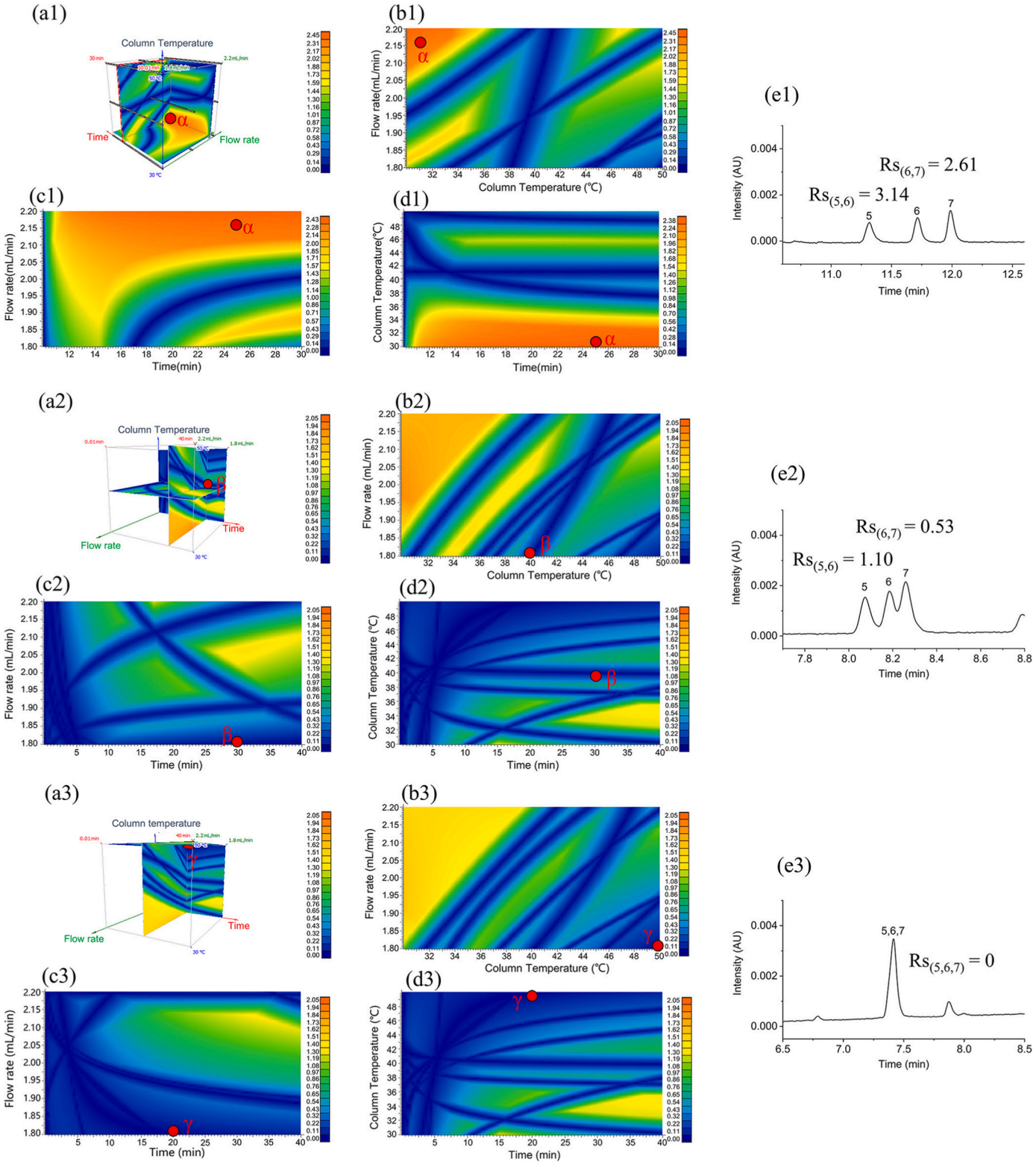


Fig. 7. SFC sperations of biogencic amines using in silco modelling, (a1–a3) 3D resolution maps for multifactorial optimization at different column temperatures, gradients, and flow rates; (b1–b3) 2D resolution map for column temperature and flow rate, (adapted with permission from American Chemical Society [94].

5.5. Computer-assisted approaches for SFC

SFC is performed using sub/supercritical fluid mobile phases containing pressurized carbon dioxide blended with a cosolvent. In general, supercritical fluid chromatography (SFC) can deliver unique advantages such as high peak capacity, reduced backpressure, solvent requirements, and shorter run time. This technique has been historically viewed as challenging to model due to the nature of its mobile phase, its compressibility, and its multimode retention mechanisms [89–91]. Recently, there has been a mounting interest in using computer-assisted strategies with less common chromatographic techniques such as SFC [89,91–95]. For example, Tyteca et al. [89], explored the possibility of modelling retention mechanism and method development in SFC using computer assisted method, MATLAB-Software. In this work, both isocratic and gradient techniques were applied for the analysis of atorvastatin and its related impurities, using different column chemistries. The later work successfully demonstrated that isocratic retention mechanism can be used accurately with non-linear retention models. Further, it showed that pressure had a noteworthy influence on the interconversion between isocratic and gradient retention mechanisms in SFC. Furthermore, the same authors investigated retention predictions after gradient scouting runs to estimate the critical retention parameters, providing retention predictions with high accuracy (<5 %) for applied gradients programs, only with the same starting conditions.

Later in 2019, Akchich et al., highlighted enantioseparation of dihydropyridone stereoisomers using SFC serially coupled with two polysaccharide-based chiral columns [43]. Due to the impact of pressure increase, this tandem configuration yielded enhanced chiral resolution through increased column efficiency and selectivity of two paired stationary phases. The same group further employed two empirical quantitative pressure-retention relationships reported earlier by Wang et al. [96], to predict the retention times on a tandem column at any pressure value. This enabled mathematical modelling of retention times with tandem column configurations at different pressure settings, which showed to be a highly valuable tool for stereoisomers and therapeutics assay development.

In another work, a novel approach called Isomolar Plot was introduced to model the common retention shifts occurring with pressure drops in SFC techniques [97]. Using Isomolar Plot, an interesting application was reported for retention time shifts for efficient method transfer from SFC to ultra-high performance SFC. The efficient calculation of the apparent retention factor at any column pressure change, enabled streamlining the transfer of chromatographic method performed using different instruments. Recently in 2022, Duan et al., reported a new strategy for effective simulation and separation of a challenging mixture of closely related biogenic amines (BAs) by using ultrahigh-performance supercritical fluid chromatography (UHPSFC) and PDA detector on a BEH column [94]. The separation landscape of the 10 amines was mapped using ACD/LC simulator software (Fig. 7). To achieve optimum resolution, a multi-factorial model was applied by selecting the influential variables including gradient elution, column temperature, and various flow rates. The latter application was successful in rapidly separating all studied compounds, with high resolution (~2.6), and accuracy (84.1–117.1 %) while also delivering higher detection sensitivity (LOD = 1.2, 10 ng/mL) and detection dynamic range (10–2500 ng/mL). This enabled efficient separation and fast method development of complex mixtures of Bas with massive reduction of cost, time and solvent consumption compared to traditional SFC approaches.

More recently in 2022, our group developed a new hybrid separation workflow using SFC instrumentation, named dual-gradient unified chromatography (DGUC) [98]. This new platform was built upon an automated dynamic modulation of CO2, organic modifier, and water blends. A DGUC automated screening system using multiple columns and mobile phases delivered simultaneous multicomponent analysis of both small and large molecules (ADCs), synthetic intermediates, nucleosides, cyclic and linear peptides) across a wide polarity range in single experimental runs. DGUC would benefit from the use of computer-assisted multifactorial simulations to streamline method optimization.

6. Computer-assisted chromatographic simulations in the development of generic analytical assays

Generic or more universal chromatographic methods can separate a wide number of compound classes in a single experimental run [1,8,47]. Such generic analytical conditions are very convenient to accelerate the deployment of reliable analytical assays across various stages of (bio) pharmaceutical development at a faster pace. Recent advances in analytical instrumentation including pumping systems, column technologies, and detection systems are extremely useful in the development of generic chromatographic methods [4,11,99–105]. Computer-assisted chromatographic modelling is becoming a powerful tool for efficient development of robust generic chromatographic methods [106–112]. Additionally, chromatographic simulations can help minimize tedious optimization endeavors when dealing with new reactions and complex samples.

Fekete's group introduced a generic RPLC method for mAbs tackling critical challenges including chromatographic resolution, and the potential thermal degradation [112]. Using computer-assisted modelling, it was demonstrated that temperature and gradient steepness were not following typical van't Hoff type linear models, and LSS model was not able to predict the retention time with acceptable accuracy. Meanwhile, nonlinear quadratic models provided high prediction accuracy for the retention (0.5–1% relative error) using only 6–9 number of experiments to usefully separate all targeted mAbs in the mixture.

In the example shown in Fig. 8, a new generic IEC method for the separation of 20 nucleotides and closely related synthetic intermediates in therapeutic substances was introduced from computer-assisted modelling [1,8]. This generic method offered the capacity to streamline the separation of nucleotide-based drugs, with minimum number of experiments, which was applied in both analytical and preparative scale. The method showed an excellent accuracy, with less than 0.26 % ΔtR difference between predicted and experimental outcomes. In another example, the analysis of palladium-scavengers in complex palladium-catalyzed reaction mixtures was facilitated with a generic ion chromatography–conductivity detection (IC–CD) assay [47]. The new approach was enabled *via* software-assisted simulations delivering excellent resolving power, linearity, recovery, repeatability, and sensitivity for baseline separation and analysis of more than 10 Pd scavenger species plus salt counterions commonly used in PR&D laboratories.

7. Conclusions

Multifactorial computer-assisted separation approaches are an important addition to existing analytical toolbox towards a more streamlined deployment of meaningful and reliable assays across (bio) pharmaceutical laboratories. In this review, the importance of computer-assisted strategies across different chromatographic modes was discussed showcasing current challenges and limitations including the use of classic and linear computer-assisted models. Further, the beneficial uses of polynomial and non-linear regression models were also outlined. Multifactorial strategies from several reports were also discussed, illustrating a higher correlation and prediction accuracy for more complex stationary phase-analyte interactions as typically occurs with large molecules. These provided deeper understanding of retention mechanisms in various chromatographic techniques and more insights on how to enhance the accuracy of retention prediction. Finally, different unconventional applications have emerged in recent years showing how computer-assisted strategies can be successfully used in many creative ways to overcome extremely challenging separations involving complex reaction mixtures and modern separation techniques.

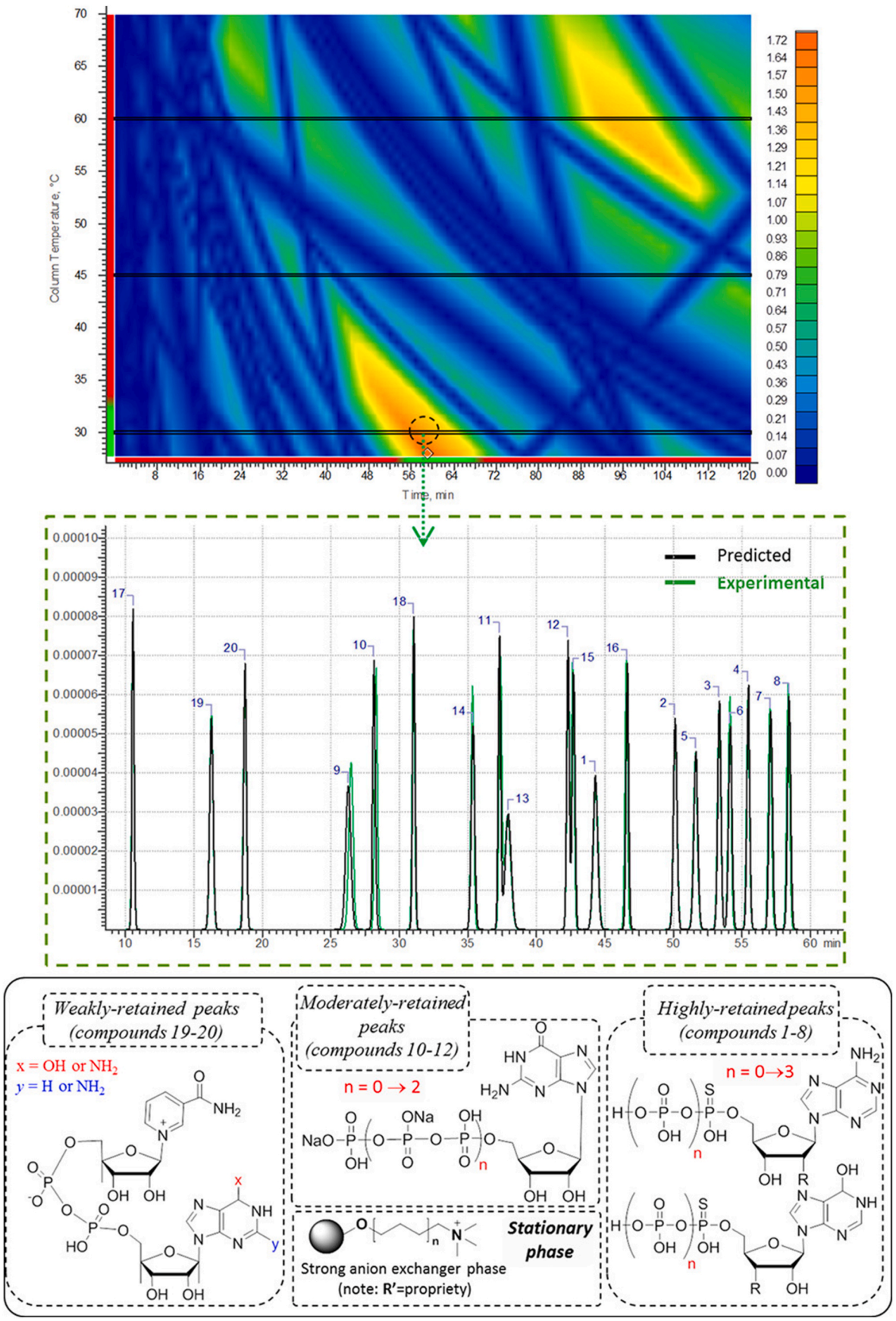


Fig. 8. Generic IEC method for the analysis and separation of 20 nucleotides from computer-assisted modelling to experimental run, Adapted with permission from Elsevier [1,8].

Overall, computer-assisted strategies prove undeniable potential for method development and optimization, playing a crucial role in enabling rapidly growing (bio)pharmaceutical targets. With the emergence of artificial intelligence and robotics, it is anticipated that computer-assisted strategies will dominate method optimization and development across both industrial and academic settings.

CRediT authorship contribution statement

Mohamed Hemida: Writing – original draft, Writing – review & editing, Conceptualization, Visualization. **Imad A. Haidar Ahmad:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Rodell C. Barrientos:** Writing – review & editing. **Erik L. Regalado:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to inappropriately influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

[1] E.L. Regalado, I.A. Haidar Ahmad, R. Bennett, V. D'Atri, A.A. Makarov, G. R. Humphrey, I. Mangion, D. Guillarme, The emergence of universal chromatographic methods in the research and development of new drug substances, Acc. Chem. Res. 52 (2019) 1990–2002.

[2] S. Lin, S. Dikler, W.D. Blincoe, R.D. Ferguson, R.P. Sheridan, Z. Peng, D. V. Conway, K. Zawatzky, H. Wang, T. Cernak, Mapping the dark space of chemical reactions with extended nanomole synthesis and MALDI-TOF MS, Science 361 (2018) 6236.

[3] G. Walsh, Biopharmaceutical benchmarks 2018, Nat. Biotechnol. 36 (2018) 1136–1145.

[4] M. Hemida, P.R. Haddad, S.C. Lam, L.J. Coates, F. Riley, A. Diaz, A.A. Gooley, H.-J. Wirth, S. Guinness, S. Sekulic, Small footprint liquid chromatography-mass spectrometry for pharmaceutical reaction monitoring and automated process analysis, J. Chromatogr. A 1656 (2021), 462545.

[5] V. D'Atri, A. Murisier, S. Fekete, J.-L. Veuthey, D. Guillarme, Current and future trends in reversed-phase liquid chromatography-mass spectrometry of therapeutic proteins, TrAC, Trends Anal. Chem. 130 (2020), 115962.

[6] F.T. Mattrey, A.A. Makarov, E.L. Regalado, F. Bernardoni, M. Figus, M.B. Hicks, J. Zheng, L. Wang, W. Schafer, V. Antonucci, Current challenges and future prospects in chromatographic method development for pharmaceutical research, TrAC, Trends Anal. Chem. 95 (2017) 36–46.

[7] B.L. Duivelshof, A. Murisier, J. Camperi, S. Fekete, A. Beck, D. Guillarme, V. D'Atri, Therapeutic Fc-fusion proteins: current analytical strategies, J. Separ. Sci. 44 (2021) 35–62.

[8] F.-R. Tsay, I.A.H. Ahmad, D. Henderson, N. Schiavone, Z. Liu, A.A. Makarov, I. Mangion, E.L. Regalado, Generic anion-exchange chromatography method for analytical and preparative separation of nucleotides in the development and manufacture of drug substances, J. Chromatogr. A 1587 (2019) 129–135.

[9] M. Hemida, A. Ghiasvand, M. Macka, V. Gupta, P.R. Haddad, B. Paull, Recent advances in miniaturization of portable liquid chromatography with emphasis on detection, J. Separ. Sci. (2023), 2300283.

[10] K. Plachká, F. Švec, L. Nováková, Ultra-high performance supercritical fluid chromatography in impurity control: searching for generic screening approach, Anal. Chim. Acta 1039 (2018) 149–161.

[11] M. Hemida, A. Ghiasvand, V. Gupta, L.J. Coates, A.A. Gooley, H.-J. Wirth, P. R. Haddad, B. Paull, Small-footprint field-deployable LC/MS system for on-site analysis of per-and polyfluoroalkyl substances in soil, Anal. Chem. 93 (2021) 12032–12040.

[12] F. Bernardoni, H.M. Halsey, R. Hartman, T. Nowak, E.L. Regalado, Generic gas chromatography flame ionization detection method using hydrogen as the carrier gas for the analysis of solvents in pharmaceuticals, J. Pharm. Biomed. Anal. 165 (2019) 366–373.

[13] J. Zhan, X.-z. Shi, Y. Ding, N. Qian, J. Zhou, S.-d. Xie, G.-z. Cao, X.-f. Chen, A generic and rapid analytical method for comprehensive determination of veterinary drugs and other contaminants in raw honey, J. Chromatogr. A 1665 (2022), 462828.

[14] Y. Shi, M. Liu, X. Li, N. Xu, S. Yuan, Z. Yu, P. Xiang, H. Wu, Simultaneous screening of 239 synthetic cannabinoids and metabolites in blood and urine samples using liquid chromatography–high resolution mass spectrometry, J. Chromatogr. A 1663 (2022), 462743.

[15] S.C. Lam, L.J. Coates, M. Hemida, V. Gupta, P.R. Haddad, M. Macka, B. Paull, Miniature and fully portable gradient capillary liquid chromatograph, Anal. Chim. Acta 1101 (2020) 199–210.

[16] https://www.chromsword.com/, accessed 12 September 2023.

[17] G.L. Losacco, H. Wang, I.A. Haidar Ahmad, J. DaSilva, A.A. Makarov, I. Mangion, F. Gasparrini, M. Lämmerhofer, D.W. Armstrong, E.L. Regalado, Enantioselective UHPLC screening combined with in silico modeling for streamlined development of ultrafast enantiopurity assays, Anal. Chem. 94 (2021) 1804–1812.

[18] R. Bennett, G.F. Pirrone, T. Nowak, D. Mukherjee, V. Shchurik, C. Mapelli, J. L. Hickey, E.L. Regalado, I. Mangion, A.A. Makarov, Ultra-high-throughput SPE-MALDI workflow: blueprint for efficient purification and screening of peptide libraries, Anal. Chim. Acta 1142 (2021) 10–18.

[19] H. Wang, H.R. Herderschee, R. Bennett, M. Potapenko, C.J. Pickens, B.F. Mann, I. A.H. Ahmad, E.L. Regalado, Introducing online multicolumn two-dimensional liquid chromatography screening for facile selection of stationary and mobile phase conditions in both dimensions, J. Chromatogr. A 1622 (2020), 460895.

[20] A.L. Capriotti, C. Cavaliere, A. Cavazzini, F. Gasparrini, G. Pierri, S. Piovesana, A. Laganà, A multidimensional liquid chromatography–tandem mass spectrometry platform to improve protein identification in high-throughput shotgun proteomics, J. Chromatogr. A 1498 (2017) 176–182.

[21] M. Pérez-Cova, J. Jaumot, R. Tauler, Untangling comprehensive two-dimensional liquid chromatography data sets using regions of interest and multivariate curve resolution approaches, TrAC, Trends Anal. Chem. 137 (2021), 116207.

[22] N. Abdulhussain, S. Nawada, P. Schoenmakers, Latest trends on the future of three-dimensional separations in chromatography, Chem. Rev. 121 (2021) 12016–12034.

[23] S. Han, Y. Song, X. Jiang, J. Qiao, A. Kang, H. Deng, D. Zhu, R. Liu, H. Lian, Novel computer-assisted approach to quick prediction and optimization of gradient separation for online enrichment-reversed phase liquid chromatography tandem system, Chin. Chem. Lett. (2023), 108139.

[24] R. Karongo, T. Ikegami, D.R. Stoll, M. Lämmerhofer, A selective comprehensive reversed-phase× reversed-phase 2D-liquid chromatography approach with multiple complementary detectors as advanced generic method for the quality control of synthetic and therapeutic peptides, J. Chromatogr. A 1627 (2020), 461430.

[25] I.A.H. Ahmad, G.L. Losacco, V. Shchurik, X. Wang, R.D. Cohen, A.N. Herron, S. Aiken, D. Fiorito, H. Wang, M. Reibarkh, Trapping-Enrichment multi-dimensional liquid chromatography with on-line deuterated solvent exchange for streamlined structure elucidation at the microgram scale, Angew. Chem. 134 (2022), e202117655.

[26] A. Periat, B. Debrus, S. Rudaz, D. Guillarme, Screening of the most relevant parameters for method development in ultra-high performance hydrophilic interaction chromatography, J. Chromatogr. A 1282 (2013) 72–83.

[27] G. Pirrone, R. Mathew, A. Makarov, F. Bernardoni, A. Klapars, R. Hartman, J. 401 Limanto, E.L. Regalado, Supercritical fluid chromatography-photodiode array detection-402 electrospray ionization mass spectrometry as a framework for impurity fate mapping in the 403 development and manufacture of drug substances, J. Chromatogr. B 1080 (2018) 42–49.

[28] J. Samuelsson, F.F. Eiriksson, D. Åsberg, M. Thorsteinsdóttir, T. Fornstedt, Determining gradient conditions for peptide purification in RPLC with machine-learning-based retention time predictions, J. Chromatogr. A 1598 (2019) 92–100.

[29] I.A. Haidar Ahmad, V. Shchurik, T. Nowak, B.F. Mann, E.L. Regalado, Introducing multifactorial peak crossover in analytical and preparative chromatography via computer-assisted modeling, Anal. Chem. 92 (2020) 13443–13451.

[30] N. Guichard, S. Fekete, D. Guillarme, P. Bonnabry, S. Fleury-Souverain, Computer-assisted UHPLC–MS method development and optimization for the determination of 24 antineoplastic drugs used in hospital pharmacy, J. Pharm. Biomed. Anal. 164 (2019) 395–401.

[31] R. Bennett, I.A. Haidar Ahmad, J. DaSilva, M. Figus, K. Hullen, F.-R. Tsay, A. A. Makarov, B.F. Mann, E.L. Regalado, Mapping the separation landscape of pharmaceuticals: rapid and efficient scale-up of preparative purifications enabled by computer-assisted chromatographic method development, Org. Process Res. Dev. 23 (2019) 2678–2684.

[32] M. Taraji, P.R. Haddad, R.I. Amos, M. Talebi, R. Szucs, J.W. Dolan, C.A. Pohl, Prediction of retention in hydrophilic interaction liquid chromatography using solute molecular descriptors based on chemical structures, J. Chromatogr. A 1486 (2017) 59–67.

[33] I.A. Haidar Ahmad, Automated column screening and computer-assisted modeling for analysis of complex drug samples in pharmaceutical laboratories, Chromatographia 85 (2022) 977–984.

[34] L.S. Roca, S.E. Schoemaker, B.W. Pirok, A.F. Gargano, P.J. Schoenmakers, Accurate modelling of the retention behaviour of peptides in gradient-elution hydrophilic interaction liquid chromatography, J. Chromatogr. A 1614 (2020), 460650.

[35] I.A.H. Ahmad, W. Chen, H.M. Halsey, A. Klapars, J. Limanto, G.F. Pirrone, T. Nowak, R. Bennett, R. Hartman, A.A. Makarov, Multi-column ultra-high performance liquid chromatography screening with chaotropic agents and computer-assisted separation modeling enables process development of new drug substances, Analyst 144 (2019) 2872–2880.

[36] R.K. Lindsey, B.L. Eggimann, D.R. Stoll, P.W. Carr, M.R. Schure, J.I. Siepmann, Column selection for comprehensive two-dimensional liquid chromatography using the hydrophobic subtraction model, J. Chromatogr. A 1589 (2019) 47–55.

[37] S. Fekete, I. Molnar, Software-Assisted-Method-Development-In-high-performance-Liquid-Chromatography, World Scientific Publishing Europe Ltd, 2019.
[38] S. Fekete, V. Sadat-Noorbakhsh, C. Schelling, I. Molnár, D. Guillarme, S. Rudaz, J.-L. Veuthey, Implementation of a generic liquid chromatographic method development workflow: application to the analysis of phytocannabinoids and Cannabis sativa extracts, J. Pharm. Biomed. Anal. 155 (2018) 116–124.
[39] M.J. den Uijl, P.J. Schoenmakers, B.W. Pirok, M.R. van Bommel, Recent applications of retention modelling in liquid chromatography, J. Separ. Sci. 44 (2021) 88–114.
[40] I.A. Haidar Ahmad, A. Kiffer, R.C. Barrientos, G.L. Losacco, A. Singh, V. Shchurik, H. Wang, I. Mangion, E.L. Regalado, In silico method development of achiral and chiral tandem column reversed-phase liquid chromatography for multicomponent pharmaceutical mixtures, Anal. Chem. 94 (2022) 4065–4071.
[41] https://molnar-institute.com/drylab/, accessed 12th September 2023.
[42] https://www.acdlabs.com/, accessed 12th September 2023.
[43] A. Akchich, J. Charton, E. Lipka, Application of tandem coupling of columns in supercritical fluid chromatography for stereoisomeric separation: optimization and simulation, J. Chromatogr. A 1588 (2019) 115–126.
[44] P.P. Csokan, F. Darvas, F. Csizmadia, K. Valko, HPLC method development through retention prediction using structural data, LC GC 12 (1994) 40–48.
[45] S. Goga-Remont, S. Heinisch, E. Lesellier, J. Rocca, A. Tchapla, Use of optimization software for comparing stationary phases in order to find HPLC conditions suitable for separating 16 PAHs, Chromatographia 51 (2000) 536–544.
[46] http://www.smatrix.com/, accessed 12th September 2023.
[47] L. Zou, R. Bennett, I.A. Haidar Ahmad, B.M. Jocher, L. Zhang, X. Bu, I. Mangion, E.L. Regalado, Generic ion chromatography–conductivity detection method for analysis of palladium scavengers in new drug substances, Org. Process Res. Dev. 23 (2019) 1060–1068.
[48] J.M. Davis, J.C. Giddings, Statistical theory of component overlap in multicomponent chromatograms, Anal. Chem. 55 (1983) 418–424.
[49] K. Cohen, K. Schellenberg, K. Benedek, B. Karger, B. Grego, M. Hearn, Mobile-phase and temperature effects in the reversed phase chromatographic separation of proteins, Anal. Biochem. 140 (1984) 223–235.
[50] C. Lafossas, F. Benoit-Marquié, J.C. Garrigues, Analysis of the retention of tetracyclines on reversed-phase columns: chemometrics, design of experiments and quantitative structure-property relationship (QSPR) study for interpretation and optimization, Talanta 198 (2019) 550–559.
[51] L. Renai, C.V.A. Scordo, A. El Ghadraoui, S. Santana-Viera, J.J.S. Rodriguez, S. Orlandini, S. Furlanetto, D. Fibbi, D. Lambropoulou, M. Del Bubba, Quality by design optimization of a liquid chromatographic-tandem mass spectrometric method for the simultaneous analysis of structurally heterogeneous pharmaceutical compounds and its application to the rapid screening in wastewater and surface water samples by large volume direct injection, J. Chromatogr. A 1649 (2021), 462225.
[52] G. Magdy, A.F. Abdel Hakiem, F. Belal, A.M. Abdel-Megied, A novel quality by design approach for development and validation of a green reversed-phase HPLC method with fluorescence detection for the simultaneous determination of lesinurad, febuxostat, and diflunisal: application to human plasma, J. Separ. Sci. 44 (2021) 2177–2188.
[53] E. Tyteca, A. Liekens, D. Clicq, A. Fanigliulo, B. Debrus, S. Rudaz, D. Guillarme, G. Desmet, Predictive elution window stretching and shifting as a generic search strategy for automated method development for liquid chromatography, Anal. Chem. 84 (2012) 7823–7830.
[54] J. Torres-Lapasió, M. García-Álvarez-Coque, Levels in the interpretive optimisation of selectivity in high-performance liquid chromatography: a magical mystery tour, J. Chromatogr. A 1120 (2006) 308–321.
[55] M. De Beer, F. Lynen, K. Chen, P. Ferguson, M. Hanna-Brown, P. Sandra, Stationary-phase optimized selectivity liquid chromatography: development of a linear gradient prediction algorithm, Anal. Chem. 82 (2010) 1733–1743.
[56] C. Chamseddin, T. Jira, Multifactorial design principles applied for the simultaneous separation of local anesthetics using chromatography modeling software, Anal. Methods 6 (2014) 6702–6710.
[57] M. Otto, W. Wegscheider, Multifactor model for the optimization of selectivity in reversed-phase chromatography, J. Chromatogr. A 258 (1983) 11–22.
[58] I.A. Haidar Ahmad, D.M. Makey, H. Wang, V. Shchurik, A.N. Singh, D.R. Stoll, I. Mangion, E.L. Regalado, In silico multifactorial modeling for streamlined development and optimization of two-dimensional liquid chromatography, Anal. Chem. 93 (2021) 11532–11539.
[59] I. Molnár, H.-J. Rieger, K. Monks, Aspects of the "Design Space" in high pressure liquid chromatography method development, J. Chromatogr. A 1217 (2010) 3193–3200.
[60] E. Tyteca, J.-L. Veuthey, G. Desmet, D. Guillarme, S. Fekete, Computer assisted liquid chromatographic method development for the separation of therapeutic proteins, Analyst 141 (2016) 5488–5501.
[61] A. Moussa, T. Lauer, D. Stoll, G. Desmet, K. Broeckhoven, Numerical and experimental investigation of analyte breakthrough from sampling loops used for multi-dimensional liquid chromatography, J. Chromatogr. A 1626 (2020), 461283.
[62] P. Jandera, Z. Kučerová, J. Urban, Retention times and bandwidths in reversed-phase gradient liquid chromatography of peptides and proteins, J. Chromatogr. A 1218 (2011) 8874–8889.
[63] L.R. Snyder, J.W. Dolan, High Performance Gradient Elution. The Practical Application of the Linear-Solvent-Strength Model, John Wiley & Sons, 2007.
[64] E. Tyteca, J. De Vos, N. Vankova, P. Cesla, G. Desmet, S. Eeltink, Applicability of linear and nonlinear retention-time models for reversed-phase liquid chromatography separations of small molecules, peptides, and intact proteins, J. Separ. Sci. 39 (2016) 1249–1257.
[65] S. Fekete, A. Murisier, J.M. Nguyen, M.A. Lauber, D. Guillarme, Negative gradient slope methods to improve the separation of closely eluting proteins, J. Chromatogr. A 1635 (2021), 461743.
[66] G.M. Randazzo, D. Tonoli, P. Strajhar, I. Xenarios, A. Odermatt, J. Boccard, S. Rudaz, Enhanced metabolite annotation via dynamic retention time prediction: steroidogenesis alterations as a case study, J. Chromatogr. B 1071 (2017) 11–18.
[67] R. Græsbøll, H.G. Janssen, J.H. Christensen, N.J. Nielsen, Optimizing gradient conditions in online comprehensive two-dimensional reversed-phase liquid chromatography by use of the linear solvent strength model, J. Separ. Sci. 40 (2017) 3612–3620.
[68] A. Kensert, G. Collaerts, K. Efthymiadis, G. Desmet, D. Cabooter, Deep Q-learning for the selection of optimal isocratic scouting runs in liquid chromatography, J. Chromatogr. B 1638 (2021), 461900.
[69] D.M. Makey, V. Shchurik, H. Wang, H.R. Lhotka, D.R. Stoll, A. Vazhentsev, I. Mangion, E.L. Regalado, I.A.H. Ahmad, Mapping the separation landscape in two-dimensional liquid chromatography: blueprints for efficient analysis and purification of pharmaceuticals enabled by computer-assisted modeling, Anal. Chem. 93 (2020) 964–972.
[70] Q. Sheng, M. Liu, M. Lan, G. Qing, Hydrophilic interaction liquid chromatography promotes the development of bio-separation and bio-analytical chemistry, TrAC, Trends Anal. Chem. (2023), 117148.
[71] B. Bobály, V. D'Atri, A. Beck, D. Guillarme, S. Fekete, Analysis of recombinant monoclonal antibodies in hydrophilic interaction chromatography: a generic method development approach, J. Pharm. Biomed. Anal. 145 (2017) 24–32.
[72] J. Baeza-Baeza, M. García-Alvarez-Coque, Extension of the linear solvent strength retention model including a parameter that describes the elution strength changes in liquid chromatography, J. Chromatogr. A 1615 (2020), 460757.
[73] F. Wang, F. Yang, J. Liu, Q. Bai, Studies on the retention mechanism of solutes in hydrophilic interaction chromatography using stoichiometric displacement theory II, in: HILIC/RPLC Dual-Retention Mechanism of Solutes in Hydrophilic Interaction Chromatography over the Entire Range of Water Concentration in Mobile Phase, Talanta, 2023, 124858.
[74] B.W. Pirok, S.R. Molenaar, R.E. van Outersterp, P.J. Schoenmakers, Applicability of retention modelling in hydrophilic-interaction liquid chromatography for algorithmic optimization programs with gradient-scanning techniques, J. Chromatogr. A 1530 (2017) 104–111.
[75] D. Obradović, Ł. Komsta, D. Agbaba, Novel computational approaches to retention modeling in dual hydrophilic interactions/reversed phase chromatography, J. Chromatogr. A 1619 (2020), 460951.
[76] S. Fekete, A. Beck, J.-L. Veuthey, D. Guillarme, Ion-exchange chromatography for the characterization of biopharmaceuticals, J. Pharm. Biomed. Anal. 113 (2015) 43–55.
[77] J. Svasti, C. Milstein, The disulphide bridges of a mouse immunoglobulin G1 protein, Biochem. J. 126 (1972) 837–850.
[78] J. Ståhlberg, Retention models for ions in chromatography, J. Chromatogr. A 855 (1999) 3–55.
[79] S. Fekete, A. Beck, J. Fekete, D. Guillarme, Method development for the separation of monoclonal antibody charge variants in cation exchange chromatography, Part I: salt gradient approach, J. Pharm. Biomed. Anal. 102 (2015) 33–44.
[80] S. Fekete, A. Beck, J. Fekete, D. Guillarme, Method development for the separation of monoclonal antibody charge variants in cation exchange chromatography, Part II: pH gradient approach, J. Pharm. Biomed. Anal. 102 (2015) 282–289.
[81] G.L. Losacco, M.B. Hicks, J.O. DaSilva, H. Wang, M. Potapenko, F.-R. Tsay, I.A. H. Ahmad, I. Mangion, D. Guillarme, E.L. Regalado, Automated ion exchange chromatography screening combined with in silico multifactorial simulation for efficient method development and purification of biopharmaceutical targets, Anal. Bioanal. Chem. 414 (2022) 3581–3591.
[82] N. Rácz, R. Kormány, Retention modeling in an extended knowledge space, Chromatographia 81 (2018) 585–594.
[83] S. Fekete, J.-L. Veuthey, A. Beck, D. Guillarme, Hydrophobic interaction chromatography for the characterization of monoclonal antibodies and related products, J. Pharm. Biomed. Anal. 130 (2016) 3–18.
[84] A. Cusumano, D. Guillarme, A. Beck, S. Fekete, Practical method development for the separation of monoclonal antibodies and antibody-drug-conjugate species in hydrophobic interaction chromatoraphy, part 2: optimization of the phase system, J. Pharm. Biomed. Anal. 121 (2016) 161–173.
[85] N. Miller, B. Karger, High-performance hydrophobic-interaction chromatography on ether-bonded phases: chromatographic characteristics and gradient optimization, J. Chromatogr. A 326 (1985) 45–61.
[86] G.z. Rippel, A.g. Bede, L.s. Szepesy, Systematic method development in hydrophobic interaction chromatography I. Characterization of the phase system and modelling retention, J. Chromatogr. A 697 (1995) 17–29.
[87] B. Bobály, G.M. Randazzo, S. Rudaz, D. Guillarme, S. Fekete, Optimization of non-linear gradient in hydrophobic interaction chromatography for the analytical characterization of antibody-drug conjugates, J. Chromatogr. A 1481 (2017) 82–91.
[88] R.C. Barrientos, G.L. Losacco, M. Azizi, H. Wang, A.N. Nguyen, V. Shchurik, A. Singh, D. Richardson, I. Mangion, D. Guillarme, Automated hydrophobic interaction chromatography screening combined with in silico optimization as a

framework for nondenaturing analysis and purification of biopharmaceuticals, Anal. Chem. 94 (2022) 17131–17141.
[89] E. Tyteca, V. Desfontaine, G. Desmet, D. Guillarme, Possibilities of retention modeling and computer assisted method development in supercritical fluid chromatography, J. Chromatogr. A 1381 (2015) 219–228.
[90] Z. La, J. Charton, L. Etienne, J. Bourey, E. Lipka, Supercritical fluid chromatography and liquid chromatography for isomeric separation of a multiple chiral centers analyte, J. Chromatogr. A 1651 (2021), 462270.
[91] L. Si-Hung, T. Bamba, Current state and future perspectives of supercritical fluid chromatography, TrAC, Trends Anal. Chem. 149 (2022), 116550.
[92] J.O. DaSilva, D. Lehnherr, J. Liu, R. Bennett, I.A. Haidar Ahmad, M. Hicks, B. F. Mann, D.A. DiRocco, E.L. Regalado, Generic enhanced sub/supercritical fluid chromatography: blueprint for highly productive and sustainable separation of primary hindered amines, ACS Sustain. Chem. Eng. 8 (2020) 6011–6021.
[93] G.L. Losacco, S. Fekete, J.-L. Veuthey, D. Guillarme, Investigating the use of unconventional temperatures in supercritical fluid chromatography, Anal. Chim. Acta 1134 (2020) 84–95.
[94] Q.-L. Duan, H.-W. Shi, L. Tan, Z. Liu, Q. Huang, W. Shen, L. Cao, H.K. Lee, S. Tang, Ultrahigh-performance supercritical fluid chromatography and detection of multiple biogenic amines in gentamicin sulfate: method development using computer-assisted modeling, Anal. Chem. 94 (2022) 7229–7237.
[95] S. Delahaye, F. Lynen, Implementing stationary-phase optimized selectivity in supercritical fluid chromatography, Anal. Chem. 86 (2014) 12220–12228.
[96] C. Wang, A.A. Tymiak, Y. Zhang, Optimization and simulation of tandem column supercritical fluid chromatography separations using column back pressure as a unique parameter, Anal. Chem. 86 (2014) 4033–4040.
[97] M. Enmark, J.r. Samuelsson, T. Fornstedt, A retention-matching strategy for method transfer in supercritical fluid chromatography: introducing the isomolar plot approach, Anal. Chem. 93 (2021) 6385–6393.
[98] G.L. Losacco, R. Bennett, I.A.H. Ahmad, R.C. Barrientos, J.O. DaSilva, Y. Dong, A. W. Schuppe, Z. Wang, S. Aiken, I. Mangion, Dual-gradient unified chromatography: a new paradigm for versatility in simultaneous multicomponent analysis, Angew. Chem. 134 (2022), e202208854.
[99] L.R. Snyder, M. Stadalius, M.A. Quarry, Gradient elution in reversed-phase HPLC-separation of macromolecules, Anal. Chem. 55 (1983) 1412A–1430A.
[100] A. Goyon, C. Masui, L.E. Sirois, C. Han, P. Yehl, F. Gosselin, K. Zhang, Achiral–chiral two-dimensional liquid chromatography platform to support automated high-throughput experimentation in the field of drug development, Anal. Chem. 92 (2020) 15187–15193.
[101] I.A.H. Ahmad, G.L. Losacco, M. Figus, D. Korani, M.J. Gunsch, H.R. Lhotka, K. Hullen, R. Hartman, S. Lohani, S. Hamilton, Generic reversed-phase ultra-high-pressure liquid chromatography methodology developed by using computer-assisted modeling for streamlined performance evaluation of a wide range of stationary phase columns, Sep. Sci. Plus 5 (2022) 138–145.
[102] M. Gilar, J. Hill, T.S. McDonald, F. Gritti, Utility of linear and nonlinear models for retention prediction in liquid chromatography, J. Chromatogr. A 1613 (2020), 460690.
[103] L. Zhang, S. Parasnavis, Z. Li, J. Chen, S. Cramer, Mechanistic modeling based process development for monoclonal antibody monomer-aggregate separations in multimodal cation exchange chromatography, J. Chromatogr. A 1602 (2019) 317–325.
[104] C. De Luca, S. Felletti, G. Lievore, T. Chenet, M. Morbidelli, M. Sponchioni, A. Cavazzini, M. Catani, Modern trends in downstream processing of biotherapeutics through continuous chromatography: the potential of Multicolumn Countercurrent Solvent Gradient Purification, TrAC, Trends Anal. Chem. 132 (2020), 116051.
[105] E. Ferencz, É.-K. Kelemen, M. Obreja, E. Sipos, S. Vida, M. Urkon, Z.-I. Szabó, Computer-assisted UHPLC method development and optimization for the determination of albendazole and its related substances, J. Pharm. Biomed. Anal. 203 (2021), 114203.
[106] J. Kopp, F.B. Zauner, A. Pell, J. Hausjell, D. Humer, J. Ebner, C. Herwig, O. Spadiut, C. Slouka, R. Pell, Development of a generic reversed-phase liquid chromatography method for protein quantification using analytical quality-by-design principles, J. Pharm. Biomed. Anal. 188 (2020), 113412.
[107] T. Sasaki, K. Todoroki, T. Toyo'oka, Simultaneous optimization of pH and binary organic composition by grid form modeling of the retention behavior in reversed-phase ultra high-performance liquid chromatography, J. Pharm. Biomed. Anal. 146 (2017) 251–260.
[108] J. Parinet, Y. Makni, T. Diallo, T. Guérin, Liquid chromatographic retention time prediction models to secure and improve the feature annotation process in high-resolution mass spectrometry, Talanta 267 (2023), 125214.
[109] A. Periat, S. Fekete, A. Cusumano, J.-L. Veuthey, A. Beck, M. Lauber, D. Guillarme, Potential of hydrophilic interaction chromatography for the analytical characterization of protein biopharmaceuticals, J. Separ. Sci. 448 (2016) 81–92.
[110] M.A. Lauber, S.M. Koza, Mapping IgG Subunit Glycoforms Using HILIC and a Wide-Pore Amide Stationary Phase, 2015. Waters application note 720005385EN.
[111] G. Greco, S. Grosse, T. Letzel, Study of the retention behavior in zwitterionic hydrophilic interaction chromatography of isomeric hydroxy-and aminobenzoic acids, J. Chromatogr. A 1235 (2012) 60–67.
[112] S. Fekete, S. Rudaz, J. Fekete, D. Guillarme, Analysis of recombinant monoclonal antibodies by RPLC: toward a generic method development approach, J. Pharm. Biomed. Anal. 70 (2012) 158–168.

Mohamed received his Ph.D.in Analytical Chemistry from the University of Tasmania, under the supervision of Prof Paul Haddad and Prof Brett Paull, he is currently a postdoctoral research fellow in the Analytical Research & Development department at Merck Research Laboratories mentored by Dr. Erik Regalado.

Imad received his Ph.D. from Florida State University under the mentorship of Dr. André Striegel. Following that, he completed his postdoctoral research with Prof. Peter Carr at University of Minnesota. Imad held various scientific roles at both Novartis and Merck. He is currently a Research Director of Synthetics Separations at Amgen, Thousand Oaks, CA.

Rodell received his Ph.D. from University of North Carolina at Greensboro, and completed a postdoctoral fellowship at the Walter Reed Army Institute of Research and US Military HIV Research Program. He is currently a senior scientist in the Analytical Research & Development department at Merck Research Laboratories.

Erik is currently the scientific leader of the Assay Development & Screening laboratories in the Analytical Research & Development department at Merck Research Laboratories, where he previously completed his postdoctoral research mentored by Dr. Christopher J. Welch. He obtained his Ph.D. working with Prof. Olivier P. Thomas at the University of Nice Sophia Antipolis, France, and Prof. Clara Nogueiras at the University of Havana.