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Current challenges and future prospects in chromatographic method development for pharmaceutical research

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ABSTRACT

A survey of different strategies for chromatographic method development in pharmaceutical research and development is presented. Owing to the widespread utilization of chromatography within diverse areas of pharmaceutical research, a variety of strategies for method development have arisen. We survey the current state of the art, discuss recent trends and approaches and highlight future prospects and capability gaps.

1. Introduction

The technique of High Performance Liquid Chromatography (HPLC) has been a favored analytical tool within the pharmaceutical industry for decades, and with the increasing adoption of techniques employing mass spectrometry (MS), HPLC shows every indication of continuing to dominate the field of pharmaceutical analysis. Instrumentation, software and modernized workflows enable the rapid application of known HPLC methods to the routine analytical characterization essential to pharmaceutical research. From the support of early investigations into discovery synthesis, metabolism, and process development to final product release, dissolution and content uniformity testing, HPLC is embedded throughout pharmaceutical science and the drug development continuum. Automation-enhanced workflows and laboratory data management systems allow workers with minimal training in chromatographic theory or practice to carry out efficient analytical testing using existing methods. However, the creation of new chromatographic methods is typically less streamlined and automated, often representing a significant stumbling block and source of delay for novice and intermediate users alike. While such delays are undesirable in late stage pharmaceutical development or commercial pharmaceutical production, they are fundamentally incompatible with the fast paced challenge of modern drug discovery and early development, where flexible and rapid problem solving is of paramount importance. As HPLC is the preferred frontline tool for analysis in these faster paced areas of pharmaceutical research, there has been a longstanding effort to streamline the task of chromatographic method development [1–4].

Given the overwhelming choices of chromatographic stationary phases, mobile phases, detection techniques, operating temperature, column dimensions, instrumentation types and general approaches to chromatographic separation, the challenge of rapidly developing a new method for chromatographic analysis can be daunting. Technical solutions that improve the throughput and success of method development through automated screening or the use of separation modeling software have been a focus of active research within the pharma industry for many years. While each of these strategies has been effective in delivering value, further improvements are still needed. In this review, we survey the state of the art in chromatographic method development for pharmaceutical analysis, reporting what has been accomplished to date, what is currently feasible but unrealized and what needs to be put in place to enable the next generation of streamlined chromatographic method development tools.

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2. Customized chromatographic methods for specific workflows

A wide range of different analytical challenges within the pharmaceutical industry are addressed using chromatographic methods. Method development is therefore often customized to particular workflows or areas of research, each with different needs and constraints. The challenges span a broad range of complexity, from the single component separations used for dissolution testing, to the two component separations used for enantiomers, to the analysis of a single biomarker in the presence of hundreds of other components in bioanalytical research. Similarly, method requirements may differ dramatically between different areas of pharmaceutical research. As a case in point, the most important criteria for an analytical release method for supporting quality control of pharmaceutical manufacturing may be sensitivity, ruggedness, selectivity, accuracy and precision, whereas a method for investigational reaction monitoring by synthetic chemists might emphasize speed, simplicity and generality. Fig. 1 summarizes some of the different types of chromatographic methods commonly encountered in pharmaceutical research.

While there is no one-solution-fits-all workflow for chromatographic method development, many of the same instrument setups and experimental approaches can be assembled to provide customized workflows for different classes of chromatographic method development problems. Chromatographic method development involves the systematic exploration of the experimental variables surrounding the chromatographic experiment to rapidly identify and optimize those conditions and settings that most impact the separation. The most important parameters that are commonly considered in chromatographic method development are illustrated in Fig. 2.

Even with just a few choices for each of these factors, the possible combinations range into the millions, thus strategies for systematically surveying the experimental landscape are needed to arrive at a workable method within a reasonable time. Strategies and techniques for navigating the complexities of chromatographic method development are continually changing and evolving, reflecting the ongoing need for researchers in pharmaceutical sciences to quickly develop suitable analytical methods.

3. Progress in chromatographic instrumentation enables method development screening

In the early days of HPLC, manual sample injection, strip chart recorders and manual measurement of results meant that carrying out systematic surveys of chromatographic conditions was a labor intensive, full time effort that required the experimenter to be present for every single injection [5]. As automated autosamplers and computerized integrating recorders became widespread in the 1980s, the benefits of setting up automated sequences of chromatographic runs that would take place over several hours or overnight was immediately grasped as a valuable addition to the toolbox of researchers interested in chromatographic method development [6]. In the 1990s, the development of automated column switching allowed researchers to investigate multiple columns under a variety of different conditions, and the modern era of automated chromatographic method development screening was born [7]. Continued innovations in instrumentation, parallel screening capabilities and the introduction of software that facilitates organization and control of method development screening has allowed chromatographic method development to continue to evolve at a rapid pace.

In addition to ongoing developments in chromatographic instrumentation, a significant evolution in chromatographic stationary phase media has occurred over the last half century (Fig. 3), with ongoing innovations in new stationary phase particles that improve efficiency and bonding chemistries that enhance selectivity and durability [9]. For example, the standard particle size used in HPLC continually decreased from the 30–100 μm irregular

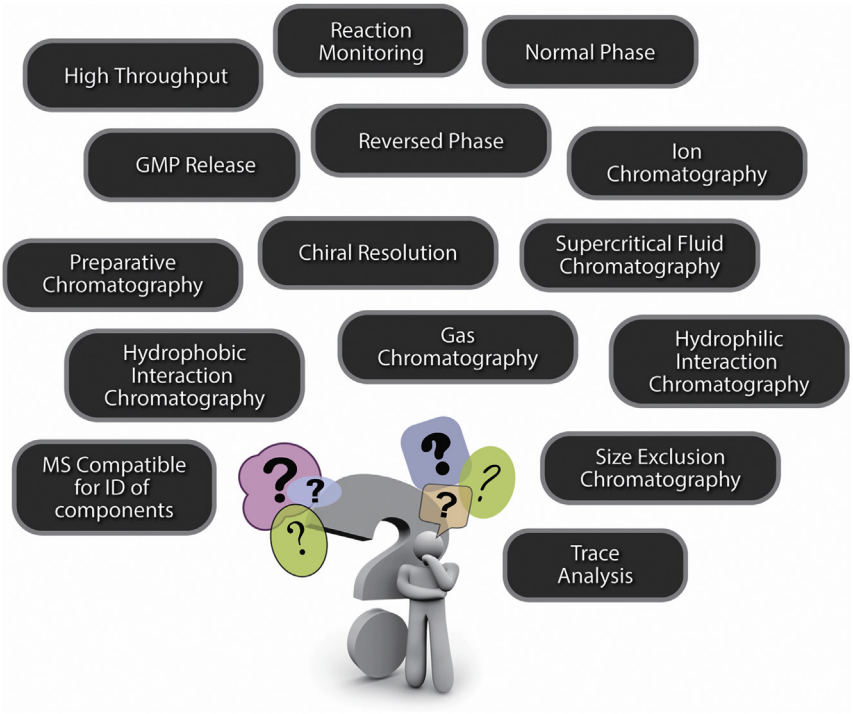


Fig. 1. Different types of chromatographic methods used in pharmaceutical research.

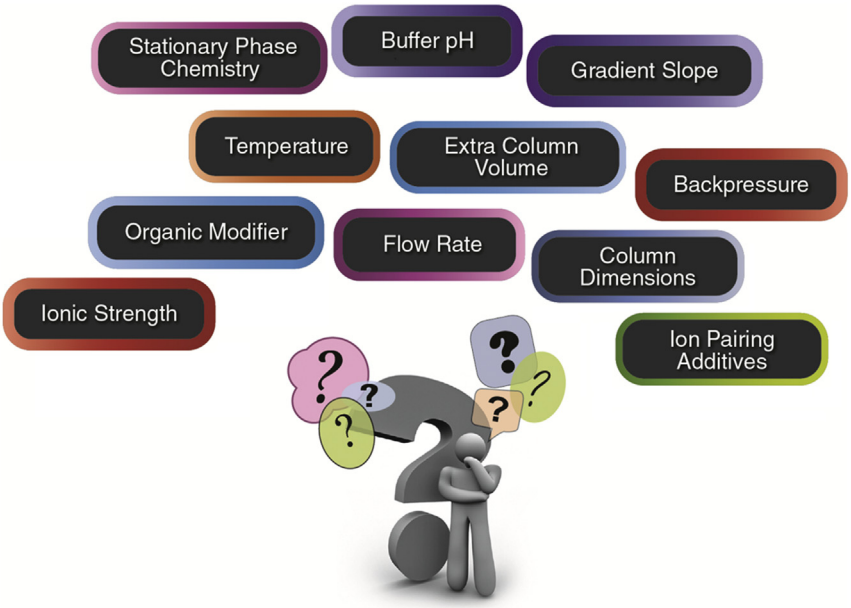


Fig. 2. Experimental parameters that are commonly adjusted in chromatographic method development.

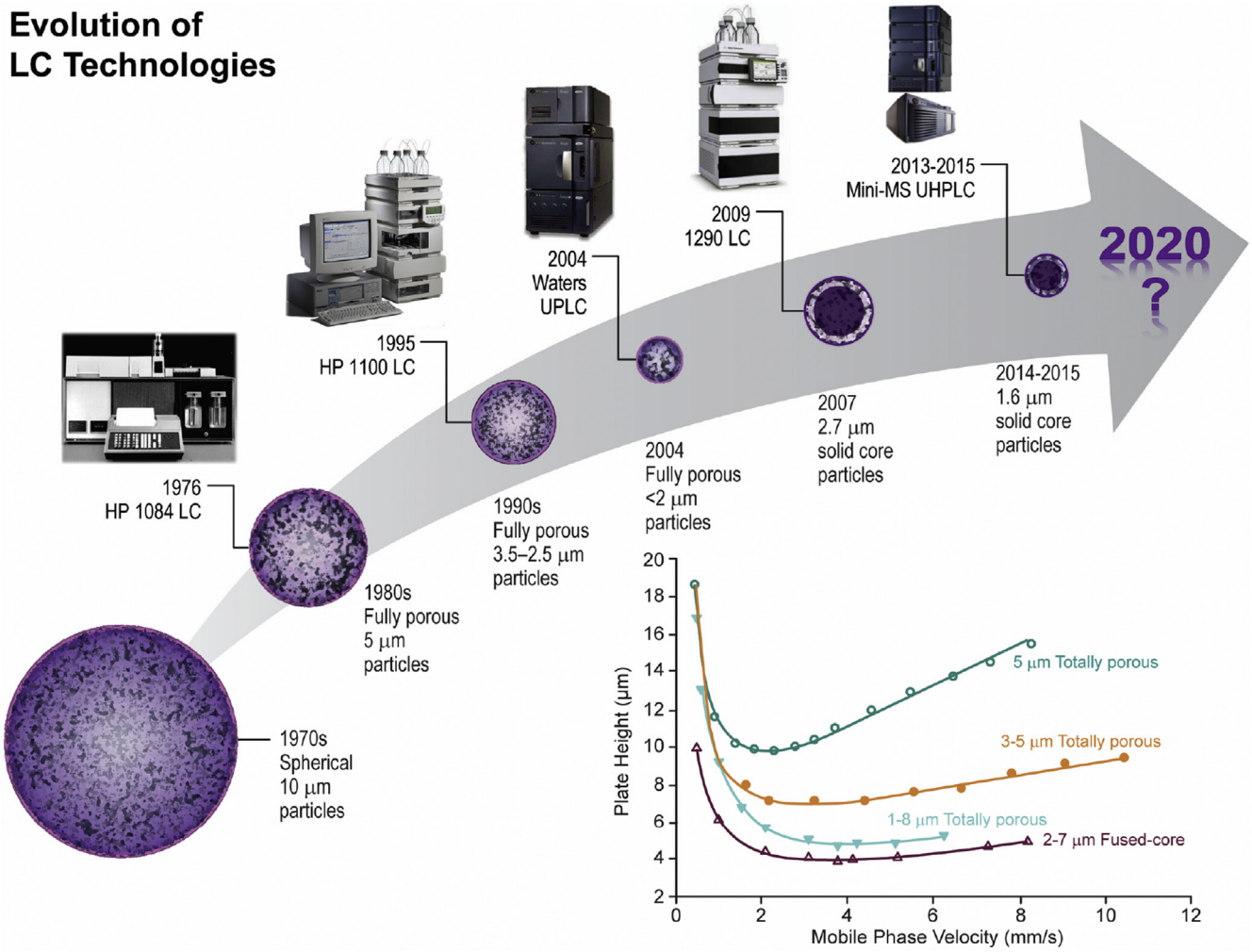


Fig. 3. Evolution of instrumentation and particle technologies enables modern chromatographic method development. Van Deemter plot extracted from Ref. [8].

particles used in the 1970s to the sub-2-μm spherical particles that are commonly used for today's industrial applications [10]. Improvements in column stationary phases have translated into improvements in efficiency and peak capacity, permitting the use of shorter columns and faster methods. Currently, fast separations with exceptional performance can be obtained using a variety of columns packed with sub-2-μm or sub-3-μm fully porous or core-shell particles [11] or with silica monolith columns [12]. Importantly, the ongoing reduction in stationary phase particle size over time has led to significant drawbacks in terms of increased column backpressure, necessitating complementary innovations in instrumentation design that allow performance at these elevated pressures. The pressure drop across a column, ΔP, necessary to obtain a mobile-phase linear velocity, u, is given by Equation (1):

$$\Delta P = \frac{\varphi \times \eta \times L \times u}{d\mathrm{p}^2} \quad (1)$$

where φ is the flow resistance factor, η is the viscosity of the solvent, L is the length of the column and *d*p is the particle diameter [13]. Note that ΔP is inversely proportional to the square of the particle diameter, which means that reducing particle size from 10 μm to 2 μm for Equation (1) will increase the column backpressure by a factor of 25. Conventional HPLC instruments have pressure limitations of ~400 bar (~6000 psi) or less, which preclude the effective use of sub-2-μm particle size column technologies. The evolution of particles to sub-2-μm size led to the development of LC instruments that can use significantly higher inlet pressures [14]. Currently, common commercially available U-HPLC instruments can operate up to or over 1500 bar.

4. Method screening for enantiomer separations

Method development for the chromatographic separation of enantiomers was one of the first chromatographic workflows in pharmaceutical research to be systematized and automated, and many innovations from this area have been applied to subsequent method development challenges. The need for a manageable chromatographic development workflow arose from the rapid proliferation of different chiral stationary phase (CSP) columns during the 1980s [15,16]. Method development is a relatively straightforward task when only one or two CSPs are available, but as the number of CSPs increased, the complexity of the task of method development rapidly escalated. Researchers initially followed rules of thumb dictating which column would be most likely to separate the enantiomers of which racemate. However, as the number of CSP choices grew, it soon became clear that the rules of thumb concerning structure-selectivity were not very accurate. In addition, it became fairly common for chiral method development investigations to become bogged down, taking days or even weeks to sequentially investigate different CSP possibilities. It is important to note that a significant problem at this time was also a tendency for marginal success to draw researchers into efforts to optimize separations before a thorough assessment of all CSP options was complete. With the advent of automated column switching in the 1990's, researchers began to adopt a fully automated column screening approach where rote execution of a script specifying elution on a number of different CSPs was carried out, oftentimes running unattended overnight [17,18]. Having full access on the next day to all of the results allowed researchers to quickly select the most promising options for optimization, avoiding the temptation to waste time in attempted optimization of early, suboptimal results.

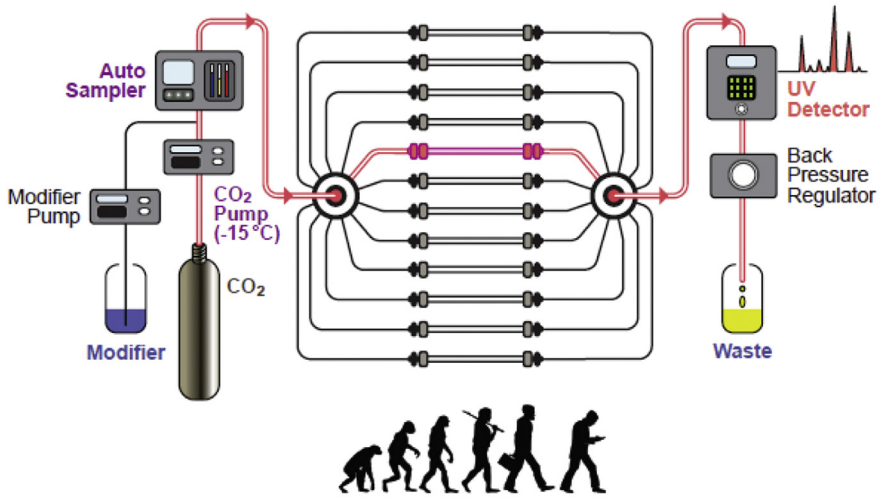
At this time, chiral chromatography was very much a normal phase technique, and not well suited to the use of gradient elution, with slow equilibration, rolling, uneven baselines and column bleeding being the norm. Consequently, early screening methods were often comprised of a set of isocratic elution conditions, with the correct polarity estimated by the researcher, discovered through trial and error or suggested by thin layer chromatography (TLC) experiments [19,20].

Supercritical fluid chromatography (SFC) has quickly evolved to become the preferred method for the analysis of enantiomeric mixtures in pharmaceutical research and development, owing to performance advantages stemming from the lower viscosity and higher diffusivity of liquefied or supercritical CO_2-containing eluents. This conveys a considerable speed and performance advantage relative to normal phase separations, and also allows for rapid equilibration in gradient elutions. While the marginal signal to noise afforded by early SFC instruments limited applicability to certain workflows [21], these shortcomings are addressed in modern instrumentation, and SFC is now becoming widely used even in regulatory release assays [22]. Fig. 4 summarizes the evolution of automated chiral SFC method development over the years, with run times of more than a half hour in the 20th century giving way to current speeds of just a few minutes as new instrumentation, particles and columns became available [23–25]. In addition to a compelling speed advantage, the success rate and labor savings provided by automated chiral SFC screening are substantial, leading to the widespread deployment of such method development systems throughout the pharmaceutical industry and in many academic laboratories. Improved peak shape, resolution and run time have been a significant driver underlying this expansion of SFC, with sub-minute analysis times now becoming commonplace in discovery and early development. These advantages lead to more rapid method validation and considerable cost savings in addition to the green chemistry advantages of decreasing the consumption of organic solvents [26].

5. Method screening for reversed phase separations

Automated method development for reversed phase separations began with the introduction of automated sample injectors and rapidly progressed as integrated column and solvent switching valves became available. Although choice of stationary phase is important, other factors such as pH, organic modifier and ionic additives are often of comparable importance when developing reversed phase chromatographic methods [27,28]. Even a cursory survey of pH, organic modifiers, ionic additives and stationary phases leads to a large number of experimental conditions, making rapid method development a challenge. A staged workflow can be used to reduce the number of initial screening experiments while providing an acceptably high success rate, with automated reversed phase method development screening often employing a straightforward instrument setup with relatively few columns and eluent combinations.

Often a dedicated screening instrument with a generic reversed phase gradient is used to identify those factors most likely to influence chromatographic performance and selectivity, with follow up method optimization taking place on a separate instrument. This approach allows a number of different users to benefit from initial leads unearthed by the shared method development screening instrument. Alternatively, several individual instruments can be dedicated to distinct types of chromatography within a specialized method development laboratory, allowing for rapid simultaneous screening of, for example, the influence of stationary phase, pH, polar modifiers, additives and detection type. Clearly, such an approach is best suited to large groups of researchers,



Conditions	1990s →	2005 →	Current
Column size (mm)	250 x 4.6	250 x 4.6	150 x 4.6
Particle size (µm)	10	5	3
Flow rate (mL/min)	1.5	2	3
Starting % MeOH	4	4	1
Approx. run time (min)	30	16	6

Fig. 4. Continuously improving chiral SFC screening for analytical method development.

where the costs of instrumentation and specialized labor can be shared and where the equipment and personnel can be kept fully occupied. It is also important that chromatographic method development workflows be devised with an eye to the particular needs of the projects where the methods will be used. For example, workflows for developing methods for trace impurity monitoring for analytical release will be quite distinct from workflows for developing methods for high throughput catalyst screening.

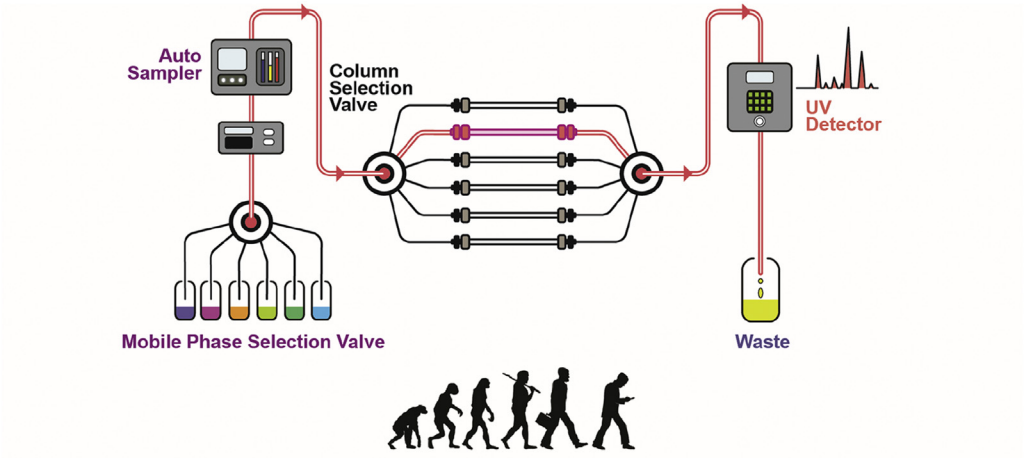
As a rule of thumb, reversed phase stationary phases with significantly different chemistries will yield different selectivity, however there are numerous reports where the use of principal component analysis or determination of hydrophobicity can be applied to assess stationary phase orthogonality [29,30]. Including several columns with orthogonal selectivity in primary method development screening is a useful means of ensuring the maximum probability of achieving a suitable separation of a mixture of unknown components within a reasonable timeframe.

Walk-up screening system design needs to be adaptable and flexible to accommodate the incorporation of recently emerging column technologies while ensuring that performance is generally robust, reproducible and dependable. The recent introduction of porous sub-2-µm particles and fused core column technologies has led to remarkable advances in separation efficiency [31]. However, the need for column ruggedness and acceptable lot-to-lot variation requires the selection of commercial columns with proven dependability for line-of-sight transfer from the method development stage to the end user. As instruments within a given organization often range from recently introduced, cutting-edge, high performance systems to older instruments approaching the end of their useful life, it is vitally important that method development screening results are appropriate and easily transferrable. The introduction of superficially porous particle columns has enabled high efficiency with relatively low backpressure, and selecting dimensions that bridge the gap between U-HPLC and HPLC helps to ensure that 'hits' can easily be transferred to a variety of instruments. However, it is important to note that the larger system volumes of older instruments may result in sub-optimal performance when methods are transferred directly [32]. Optimization of method development screening hits often involves optimization of the flow rate, gradient time and column dimensions to best suit the available instrumentation. Computational tools are available to assist with method translation between instruments [33], and are often provided by column and instrument vendors. A typical screening configuration is shown in Fig. 5, which helps to illustrate how column and instrument technology in walk-up screening has evolved over time.

Since the peak order often changes when screening different columns, pH and eluents, understanding the identity of peaks eluting in the various chromatographic experiments may also be important to consider while developing a method for a complex mixture. Integrated mass spectrometry and comparison of characteristic compound-specific ultraviolet (UV) spectral features are commonly employed for this task, as described by Xue and coworkers in the description of a Comprehensive Orthogonal Method Evaluation Technology (COMET) system [34]. This strategy has since been commercialized with software packages capable of interfacing with off-the-shelf screening configured HPLC's [35].

6. Method screening for other types of chromatography

Many researchers in the pharmaceutical industry employ dedicated screening systems to quickly evaluate whether or not a particular type of chromatography will be suitable for a given separation problem. A variety of method development strategies for HILIC [36], normal phase [37], polar organic [38], achiral SFC [39], size exclusion [40], ion exchange [41], ion chromatography [42], etc. have been described. These screening systems can be



Conditions	1990s		2005			Current
Column size (mm)	250 x 4.6	150 x 4.6	100 x 4.6	100 x 2.1	100 x 3.0	100 x 2.1
Particle size (µm)	10	5	3.5	<2	2.7*	<2*
Approx. run time (min)	60	40	35	15	20	10
Max system pressure (bar)	200	400	400	1000	600	1000
Available pH range	2-7	2-9	2-11	2-11	2-11	2-11

* Superficially porous particles

Fig. 5. Continuously improving reversed phase screening for analytical method development.

particularly valuable for challenging separation problems that are not solved using more conventional, first line chromatographic screening approaches, giving researchers an opportunity to rapidly and simultaneously evaluate a recalcitrant separation problem using a variety of less commonly used techniques. Each system is set up with automated selection of columns and eluent combinations that have previously proven to be generally useful.

7. A quandary: dedicated screening systems vs. 'on-again-off-again' systems?

Ideally, dedicated instruments for different method development screening, optimization and routine analysis tasks would allow each to be run concurrently, resulting in minimal delays in experimentation and problem solving. However, maintaining dedicated chromatography screening instruments for use at a moment's notice is a labor, cost and space intensive proposition that is simply not possible in smaller companies or academic groups. Not surprisingly, scientists have frequently experimented with approaches where a single instrument can sometimes function as a method development screening tool and sometimes be used for method optimization and routine analysis. In recent years, computer–assisted HPLC method development tools that facilitate the coordination of screening runs, method optimization and validation, while allowing for the tradeoff with conventional instrument utilization, have become available. Commercial software like ChromSword® Auto [43], ACD/Autochrom™, and Fusion AE™ [44] automate a single conventional HPLC or U-HPLC instrument into an efficient, automated method development platform. These software packages enable screening design, results evaluation and data visualization, while interfacing with the Chromatographic Data System (CDS) to perform conventional optimization and analysis studies.

While software innovations have made it easier to operate in 'on-again-off-again' mode, this approach can lead to frustrations and problems, especially when multiple users share a single instrument. For example, a minor change in instrument settings or failure to correctly replace a column or eluent can lead to incorrect screening results and can even damage columns and instrumentation. Protocols for ensuring the success of on-again-off-again screening become increasingly important as the number of users increases.

8. Parallel method development screening

While significant progress has been made in the development of new and improved chromatographic stationary phases, no single stationary phase provides a universal ability to resolve most mixtures. Thus, column screening is still an integral part of many method development workflows. In order to achieve speed advantages over sequential screening, several attempts have been made to utilize parallel screening for method development. The development of a microfluidic 8 channel HPLC instrument with 16 pumps and solvent reservoirs was reported in 2006 [45]. Use of this tool for simultaneous parallel evaluation of 8 different chiral stationary phases in either normal phase or reversed phase mode was subsequently reported, allowing a complete screen of 8 columns to be completed in only 20 min [46].

Similar parallel screening approaches employing shared flow have been developed within pharmaceutical laboratories and elsewhere. This approach is attractive from a cost and complexity standpoint, as it eliminates the need for separate pumps for each column. However, shared flow parallel chromatography instruments are vulnerable to uneven and variable flows within the different columns arising from differences in backpressure, particularly in the SFC mode. Sepiatech has introduced a strategy to

address this problem using intelligent post column backpressure regulators on each channel [47].

9. Fast chromatographic methods

Screening for fast separations is an important aspect of chromatographic method development within the pharmaceutical industry, and one that has been growing considerably in recent years. Faster separations are important in all aspects of chromatographic research, and the recent adoption of U-HPLC technology has generally led to speed increases across most areas of chromatographic analysis [48,49]. The need for speed is particularly important in the analytical support of high throughput experimentation within pharmaceutical discovery and development. In these areas, high throughput investigations of catalysts, enzymes or reaction conditions can generate dozens or even hundreds of samples in a single day, leading to an analysis bottleneck when chromatographic analysis times are long [50].

The systematic development of fast chromatographic separations is still a growing area of research, but recent studies have shown that in many cases an assessment of the degree of 'superfluous resolution' in a given separation can lead to a fairly accurate estimate of the fastest resolution speed [51]. Using this approach, a variety of ultrafast separations can be obtained, often using very short columns of 1 cm or less [52–54]. Specifically in the area of chiral separations, truly remarkable separation times have been obtained in recent years, with most racemates being completely resolved in less than a minute, and some in less than 5 s [55]. Interestingly, with such fast separations, narrow peaks and short columns, it is critically important to minimize extra-column volume and to use suitably fast detector sampling rates [56]. It is equally important to note that faster injections cycles are required to realize these performance gains.

10. Method development for preparative chromatographic separations

In addition to the requirements common to all analytical method development workflows, larger scale preparative chromatographic methods also require optimization of chromatographic productivity (the amount of the desired compound that can be purified with a given amount of stationary phase in a given time). It should be noted that smaller scale preparative separations to support medicinal chemistry are often carried out without method optimization, utilizing fit for purpose methods [57], while preparative method development for larger scale separations frequently follows a two tier strategy of first identifying appropriate columns and conditions that provide adequate resolution *vs.* time, and then carrying out subsequent loading studies to provide an estimate of productivity [58]. As loading studies can consume significant amounts of precious sample mixtures, there is growing interest in the use of miniaturized screening approaches to estimate productivity. A recent study showed that loading studies carried out with a 300 μm i.d. column packed with 20 μm diameter particles afforded perfect prediction of chromatographic performance when scaled up by a factor of 1 million to a 30 cm i.d. column containing the same particles, (i.e. injection of 42 μg on the microcolumn at a flow rate of 6 μL/min afforded identical chromatography with injection of 42 g at a flow rate of 6 L/min on the larger column) [59]. Several high throughput methods for the *ex-column* selection of promising adsorbents for preparative chromatography have been reported, with the reasoning that highly selective adsorbents identified in these screens will also generally afford productive separations with chromatography under flow conditions [60]. Similarly, automated methods using partial elution through small parallel filter beds are often employed in the development of preparative chromatographic methods for biomolecules [61,62].

11. Fit-for-purpose and universal chromatographic methods

Optimization of individual chromatographic methods is essential in some areas of research and development, but in other areas where the pace of research is rapid, fit for purpose or universal chromatographic methods are often employed as a time-saving expedient. For example, practicing medicinal chemists engaged in the fast-paced synthesis of new drug candidates may perform dozens of chromatographic separations each day using only a single reversed phase gradient method with MS detection. The development of such universal methods is a method development challenge in its own right, with robustness, speed, performance and generality (universality) being the key desirable qualities.

Developing filing-ready HPLC methods for regulatory release testing is a complex and time-consuming process that often involves a combination of column and eluent screening, gradient and temperature optimization, the use of stationary phase selectivity kits [63], chromatographic modeling packages [64] and the analysis of numerous representative and stressed samples. Method optimization is often carried out by a skilled chromatographic scientist in studies that may take several months. Although this work is critical for late-stage programs, intensive method optimization is often deferred for early-stage projects, where attrition is high.

The chromatogram in Fig. 6 shows a recent example from our hepatitis C drug development program where a fit-for-purpose method has been used in development to address the need for simple and effective assessments of reaction conversion. Similarly, this same method has been used for a variety of other programs and also applied during the investigation of the purity of starting materials, intermediates and final products where a tailored method has not yet been developed. Such a generic method requires significant resolving power in order to separate a broad range of different molecules without additional method customization, but must be simple enough to allow for routine use by chemists and chemical engineers with little experience in chromatographic method development. The advantages and disadvantages listed in Fig. 6 illustrate that such methods must be adjusted as needed for the particular application as development continues. Additionally, it is important to note that an appropriate fit-for-purpose method may need to be adapted depending on the target development area. For example, this method utilizes a relatively slow gradient with a phosphoric acid-containing eluent that affords very low background signal and excellent UV sensitivity, desirable properties when the ability to resolve and detect low-level impurities is critical, but less desirable when high throughput analysis or MS detection is needed. Consequently, fit for purpose methods may vary between research areas depending on needs and preferences. Within an organization, harmonizing on one broadly applicable and robust analytical method ensures consistency, facilitates instrument maintenance, enables collaboration and team problem solving.

12. Chromatography simulation and modeling in method development

Chromatography simulation and modeling software has become an integral part of the method development toolkit, particularly for reversed phase separations [65]. In many cases, use of simulation and modeling in conjunction with a few range-finding experiments can be used to guide the rapid convergence on optimal chromatographic performance without the need for rote exploration of all the possible combinations of column, eluent, temperature,

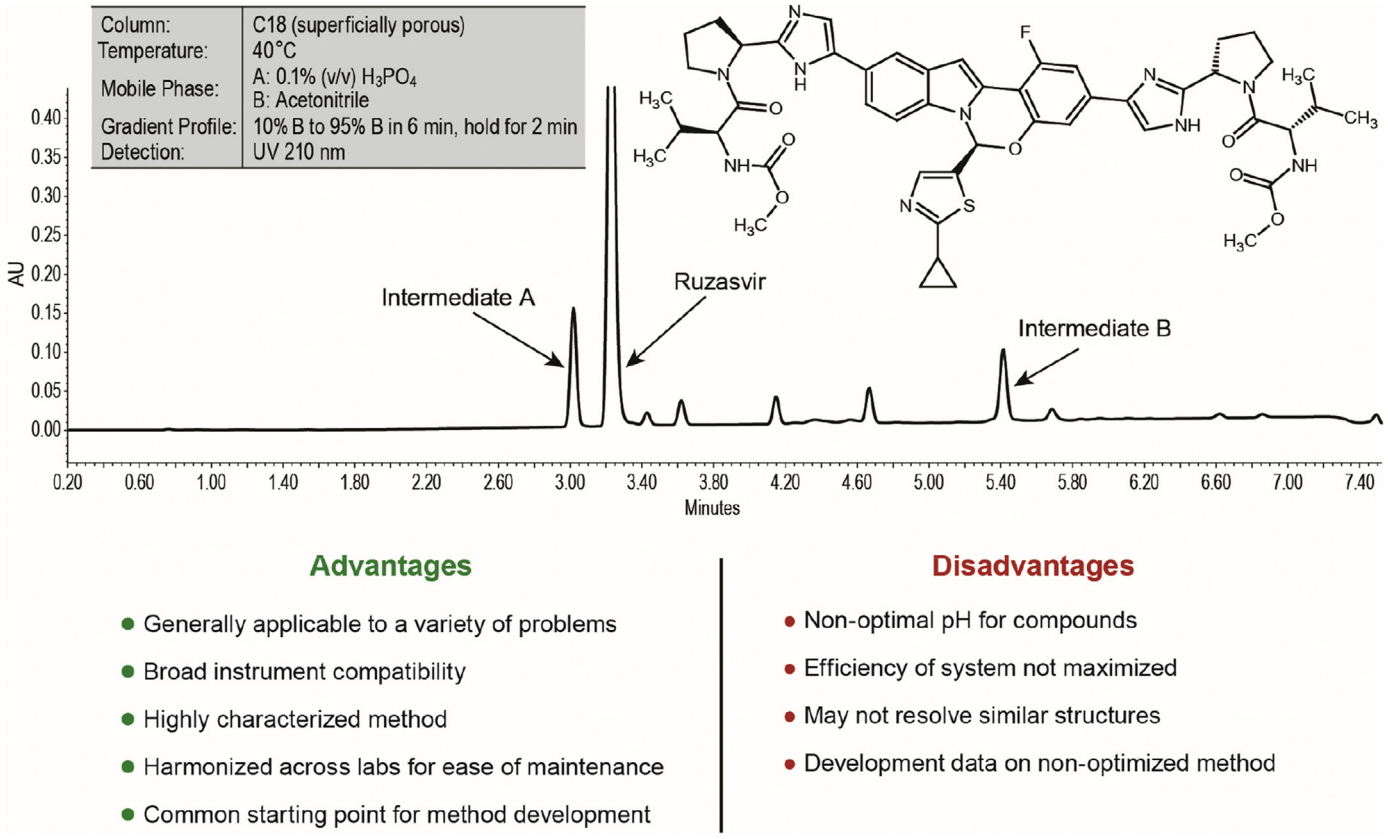


Fig. 6. Example of fit-for-purpose method for pharmaceutical development – advantages and disadvantages.

gradient, pH, additives, etc. The first stage of reversed phase chromatographic method development is often to identify the most suitable column, mobile phase pH, aqueous phase buffer and organic solvent to resolve the active pharmaceutical ingredient (API) from its impurities in a particular sample. After initial starting conditions have been identified, the next stage is to determine optimal and robust separation conditions, a task that becomes more complicated as the number of operating variables increases. To simplify and accelerate the optimization process, a number of different computer simulation software packages have been employed [66]. DryLab®, developed by Snyder et al. in the late 1980's [67], was the first software for the modeling and simulation of reversed phase HPLC separations. The basic principle for the modeling of retention factor *vs.* solvent strength is based on Horváth's solvophobic theory [68]. This was later expanded to include thermodynamic effects and other factors such as pH, buffer concentration etc., with curve fitting by polynomial regression. This approach enables the use of a small set of well-defined experimental data acquired on a particular stationary phase to predict the effects of changes in mobile phase composition or temperature, thereby improving the throughput of method optimization. ACD/Labs LC-Simulator™ (Advanced Chemistry Development, Toronto) [69], and Chromsword® (Merck KGaA, Darmstadt) [70] both perform similar functions, albeit with additional features. ChromSword® and ACD/Labs ChromGenius [71] offer the additional option of quantitative structure-retention relationship (QSRR) from structure fragments to afford an approximate prediction of retention behavior. Once an optimal column, organic modifier and buffer pH have been determined, the next step in method development is often the adjustment of gradient and temperature to obtain optimal resolution of the critical pairs. Shallow and steep gradients are typically run with a range of temperatures appropriate for the method and the results are transferred to a method optimization tool such as DryLab® or LC Simulator™ to construct a resolution map. These predicted resolution maps provide a graphical tool that allows the user to accurately model a separation at a given set of conditions. An example using a predicted resolution map for method optimization is shown in Fig. 7. Once optimal gradient and temperature conditions have been determined on the basis of the desired resolution achieved for the critical pairs, the best method can be further optimized for speed using chromatography simulation software.

ChromSword® Auto [43] and ACD/Autochrom™ [65] are both examples of integrated method screening and optimization software that are capable of controlling an HPLC instrument and optimizing separations automatically. The software includes a workflow manager in addition to the predictive tools to generate retention models based on experimental data. Furthermore, they include tools for tracking components across experiments, although in most cases, human interpretation is required to ensure correct peak tracking. ACD/Labs® software can also predict physical chemical properties of the analytes including pKa, LogD, logP, solubility, etc. with good accuracy, which can be helpful in guiding the selection of method development strategies such as separation mode and buffer pH [72]. Although significant investment is required both in terms of capital and training, there have been many success stories from utilizing such software tools to achieve optimal separations, and it is fair to say that this approach has now become firmly established in pharmaceutical research and development work.

Fusion AE™, developed by Verseput at S-Matrix Corporation, is another software package for HPLC method development that is

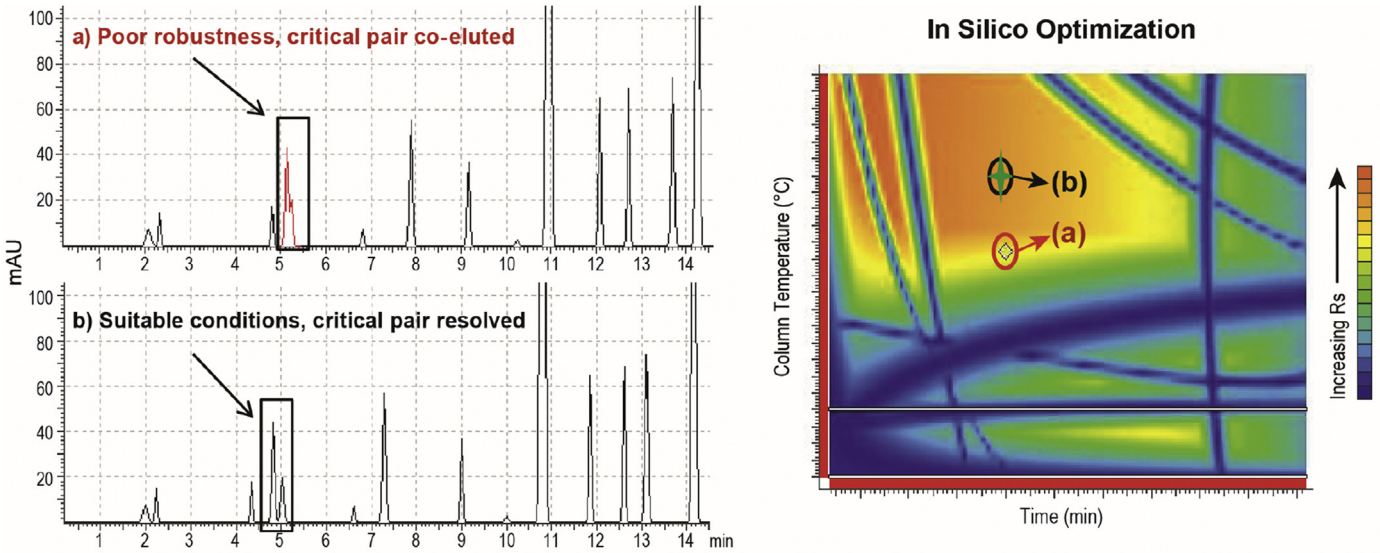


Fig. 7. Using predicted resolution maps in method optimization.

based on statistical analysis principles [73]. The software combines statistical design of experiment and automated generation of methods in a chromatography data system, such as Empower, which significantly improves the efficiency of method development. Parameters such as column, buffer pH, additive concentration, ionic strength, organic solvent, gradient slope and temperature, etc. can be thoroughly evaluated, albeit with a large and potentially cumbersome data set acquired using design of experiment (DOE)-based strategies. Statistical analysis on responses of interest such as resolution, tailing factor, retention factor, etc., can be performed to aid in the selection of the most promising combination of HPLC parameters for further optimization, which can be performed using Fusion AE™ or the aforementioned simulation software.

Guidance from regulatory agencies states that the issue of method robustness should be addressed during formal method development. Once the optimal conditions are defined, computer software can help to simulate method robustness under deliberate variation of HPLC parameters. For instance, DryLab® has a built-in function to calculate the method performance for the permutation of major parameters such as temperature, gradient time, flow rate, solvent strength etc., and to map out the method robustness range. Integrating quality by design (QbD) principles into method development, Fusion AE™ software can also be used to perform multifactorial evaluation of method robustness for all factors with potential impact, including temperature, gradient time, flow rate, solvent strength, solvent ratio, additive concentration, pH, dwell volume, etc. Based on the results, the software can recommend appropriate system suitability requirements and propose design spaces.

13. Future outlook: algorithms and intelligent systems for chromatographic method development

As the use of computational modeling and simulation continues in chromatography method development, and as machine learning, big data and autonomous robotic systems become a feature of our 21st century world, the question of a 'self-driving' chromatography method development station becomes a matter of 'when' rather than 'if'. The idea of an autonomous method development robot has captivated the imagination of chromatographers for some time, and prototype components, algorithms and subroutines that could be incorporated into such a system have been described [74–77]. Ideally, such a system could query the structures of the components of the separation mixture to make intelligent choices about which column-eluent combinations would be most likely to lead to success, giving first priority to certain lines within the screening protocol and deprioritizing others deemed less likely to succeed. An ability to perform real time assessment and scoring of the screening runs would provide information for feedback control that could be used to influence subsequent screening steps. In cases where all components within the chromatogram have eluted, individual runs could be terminated, leading to improvements in the speed to successful result. Similarly, in cases where chromatograms are obtained that afford a suitable quality score, the screening run could be brought to an early conclusion, allowing subsequent samples to be run or perhaps a next level method optimization study to be carried out. Several studies using ChromSword® to drive screening protocols have been reported. While results to date have been somewhat modest, increasing activity in this area suggests further progress will be forthcoming. Perhaps one of the most interesting aspects of this approach is that, as with design challenges for autonomous vehicles, competing algorithms or strategies can be pitted against one another, hybridized and evolved to speed the pace of improvement.

A starting point for many of these approaches is to use computational prediction based on the chemical structures of the mixture components to afford an intelligent guess of the best place to begin the screening process. In some cases, the mixtures are too complex or not sufficiently well known for this approach to be useful; however, detailed chemical information is often available for samples in pharmaceutical research and development. An early example of this approach is the EluEx, which can suggest initial experimental conditions for reversed phase chromatography based on chemical structures [78]. Similarly, we have recently reported a quantitative structure activity relationship (QSAR) model derived from >100,000 literature-reported enantioseparations that has some elementary ability to predict the most likely stationary phase for a particular analyte based solely on chemical structure [79].

Simplified user interfaces will be an ongoing area of need as computer-enhanced chromatography method development systems continue to be developed. Considerable progress has been made in recent years in the development of chromatography instrument control software that helps to keep the business of high

throughput chromatographic method development streamlined, well organized and easy to understand.

14. Unleashing the power of data: the use of intelligent databases and predictive tools to inform method development and enable knowledge capture.

The ability to search existing data and learn from prior experiences is crucial to efficiently developing new chromatographic methods. Furthermore, the ability to mine this knowledge and to build useful prediction models for chromatographic retention and selectivity is a long-standing goal that has recently become increasingly attainable. A key impediment that still exists to fully realizing these predictive analytics is the inaccessibility of structured, complete laboratory data. Data are often required from multiple instrumentation types and vendors to build robust models; however, each data set is presented in a separate proprietary vendor data format. This raw and processed instrument data must also be aggregated or placed in context with sample and instrument parameters, “meta-data,” to be useful for data mining and modeling applications. The lack of structure and precise semantic taxonomies and ontologies prevents computers from automating the data aggregation process, requiring manual formatting of the data, which is resource intensive and susceptible to transcription errors. Also problematic is that this data is restricted or unavailable to data mining in the sense that access to the data is only available within the data-generating application.

The semantic web has been developed as a general solution to address these data interoperability issues, allowing context to be attached to raw data files. The Open Biomedical Ontologies (OBO) Foundry [80] vets and governs interoperable science ontologies (data indexing system) such as Chemical Methods (CHMO) and Chemical Entities of Biological Interest (ChEBI). Taxonomies and ontologies for analytical chemistry and a vendor application-agnostic file format for raw and processed data are being developed by the Allotrope Foundation [81]. The same technique can also be used internally within an enterprise with confidential information to provide true knowledge management in a cloud type environment. Such an infrastructure also allows testing of new hypotheses through previously unknown or new data sources that are automatically linked as they become available.

Once widely implemented, convenient and comprehensive access to relevant scientific data from diverse sources can be expected to lead to significant improvements in the generation of useful predictive models that aid and simplify the process of chromatographic method development. Indeed, it is likely that for certain areas of chromatographic method development, the use of predictive modeling studies will become an integral first step, followed by targeted laboratory verification experiments.

15. Conclusion

Chromatographic method development is fundamentally important to modern pharmaceutical discovery and development research. While the diverse needs of different research areas require a variety of method development solutions, some common strategies for the use of instrumentation, automation, databases, predictive tools and screening approaches can be found. The science of automated chromatographic method development is fast evolving toward an envisioned future where the use of predictive models, artificial intelligence and machine learning optimization will enable the rapid development of chromatographic methods for use by the non-expert, thereby significantly increasing the power and scope of chromatography.

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