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ADVANCES IN HPLC TECHNOLOGY FOR DETERMINATION OF DRUG IMPURITIES

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ABSTRACT

For the purpose of ensuring the quality, efficacy, and safety of pharmaceutical products, it is very necessary to precisely detect and quantify drug contaminants. In the field of impurity profiling, High-Performance Liquid Chromatography (HPLC) has emerged as an indispensable analytical instrument due to its exceptional resolution, sensitivity, and reproducibility. The ability to detect impurities at trace levels, such as degradation products and contaminants related to the process, has been significantly improved thanks to the development of modern high-performance liquid chromatography (UHPLC), hybrid detection systems (for example, HPLC-MS/MS), new stationary phases, and improved gradient elution methods. Aside from reducing the amount of solvent that is used and the amount of time that is spent on analysis, these technological developments have made it feasible to detect many contaminants simultaneously with greater precision. In addition, the pharmaceutical industry has been able to standardise and verify procedures in a more efficient manner as a result of the integration of high-performance liquid chromatography (HPLC) with chemometric equipment and regulatory criteria such as ICH Q3A and Q3B. The purpose of this research is to address significant technological advancements in high-performance liquid chromatography (HPLC) and the ways in which these advancements have impacted impurity profiling. It provides a comprehensive perspective on the current trends and future directions in pharmaceutical analysis.

Keywords: HPLC, Drug, Technology, Impurities, HPLC-MS/MS

INTRODUCTION

Stringent rules guarantee the quality, safety, and efficacy of pharmaceutical goods. Any point of a pharmaceutical's life cycle—including production, formulation, storage, or degradation—impurity introduction compromises its therapeutic effectiveness and patient safety. Quality control and regulatory compliance in the pharmaceutical sector increasingly much depend on the capacity to find, identify, and measure drug impurities. High-performance liquid chromatography, or HPLC, is among the most often used analytical techniques for identifying pharmaceutical contaminants. Its remarkable sensitivity, selectivity, and flexibility let it find trace-level pollutants and separate complex combinations. HPLC has long been recognised for its ability to follow ICH-like entities' regulatory criteria. This is particularly true in relation to policies Q3A and Q3B, which address contaminants in innovative pharmaceutical products and materials, respectively.

Though conventional HPLC systems have proven beneficial, alternative technologies have developed to satisfy the rising demands for faster analysis, improved resolution, and higher sensitivity. To find genotoxic pollutants and pollutants in complicated formulations like biologicals and nano-formulations, analytical techniques have to be enhanced and reinforced. Consequently, advances in detection methods, column

chemistry, and HPLC devices have moved at an extraordinary pace. Many advances in HPLC technology have helped impurity profiling to be a much more sophisticated field. Ultra-High-Performance Liquid Chromatography (UHPLC) is among the most significant developments as it allows faster and more efficient research by employing smaller particle-sized columns and operating at higher pressures. Ultimately, there is more throughput, faster run times, and sharper resolution. Hyphenated techniques have also helped to identify and define undiscovered pollutants with great precision and structural clarity. One example is the pairing of contemporary detectors like mass spectrometry (LC-MS/MS) with high-performance liquid chromatography (HPLC).

Further developments in column technology—such as the use of core-shell particles and creativestationary phases—in accordance with green chemistry ideas have led to greater separation efficiency and decreased solvent use. The utilisation of automation and modern software technologies has greatly simplified data processing, peak tracking, and method development. These digital developments result in enhanced accuracy, reproducibility, and reliability in impurity detection and quantification. The evolution of HPLC technology has changed impurity identification in medicines. This innovation has made faster and more thorough analytical techniques possible as well as adherence to strict quality standards. Apart from improvements in the quality and safety of medication, these developments also facilitate the manufacture of drugs and their regulatory approval.

OBJECTIVES

1. To examine new developments in HPLC for drug impurity analysis and detection.
2. To evaluate how new HPLC technologies affect the accuracy and efficiency of impurity profiling.

MATERIAL AND METHOD

In the course of the investigation, cutting-edge high-performance liquid chromatography (HPLC) apparatus was used in order to evaluate the efficacy of impurity profiling via the application of modern chromatographic techniques. The use of a variety of columns made it possible to achieve separations that were both more expedient and effective. These comprised monolithic columns, which had a low back pressure and a high permeability, as well as small-particle columns, which were able to tolerate high pressure and temperatures. The incorporation of stationary phases, such as core-shell and polar-embedded phases, resulted in an increase in both the selectivity and peak resolution of the system. In order to facilitate the selection of an organised procedure, columns were arranged in a methodical manner, with consideration given to the particle size, phase chemistry, and application suitability of each column. In order to further enhance analytical approaches, automated method development methods were used. These methods guaranteed repeatability while simultaneously reducing the need for human participation. Mass spectrometry (LC-MS) and diode array detectors (DAD) are two examples of the most cutting-edge detector technologies that were used in the impurity detection procedure. These technologies allowed for increased sensitivity as well as the ability to shed light on the structure of certain substances. In order to ensure that the impurity analysis is accurate, precise, and reliable, all of the chromatographic techniques were checked to ensure that they adhered to the guidelines of the International Council for Harmonisation (ICH).

RESULT

Use of Monolithic Columns for Enhanced Efficiency

Since 2001, monolithic reversed-phase (RP) silica columns have garnered attention for their faster analytical times and higher flow rates than packed columns. Monomers polymerise into a continuous porous rod, which is utilised to make monolithic columns instead of packed particles. This interlaced structure provides mesopores (13 nm) for high-efficiency chromatography and macropores (2 lm) for low-flow resistance. Tanaka et al. described multiple monolithic column preparation and characterisation processes. Miyabe and Guiochon studied RP-LC monolithic column chromatography and fundamental work. Cabrera covered the history, characteristics, and diverse applications of silica monolithic columns. Most authors found that monolithic columns' plate height efficiency remained flat as linear velocity increased, whereas 5-lm-particle columns' efficiency declined. Even at 9 mL/min flow rates, monolithic column pressure increases were minimal, within standard pump operating range. Reduced back pressures allow for more flow rate settings and column pairing. Figure 1 exhibits shorter run lengths at higher flow rates maintaining resolution. For this separation, the flow rate was fourfold increased, reducing run time.

Changing the chromatogram's time scale to a similar size plot allows for visual performance comparison. A tiny impurity before the second major peak and three surrounding peaks maintained equal separation. The unique nature of monolithic column manufacture and the polymer covering bonding process have raised concerns regarding repeatability and durability. Several authors have examined monolithic column robustness, repeatability, and considerations. Most studies have compared 5-lm or 3.5-lm RP packed columns to Chromolith columns (E. Merck). To retain like packed columns, Wu et al. found monoliths required weaker solvents. Although certain sample components had more tailing, the columns were repeatable. Pharmacopeial techniques may be safely transferred to Chromolith columns, according to Nederkassel et al. Converting all methods to monolithic columns and switching packed column brands were unsuccessful. Transferred methods were resilient and shortened run lengths, however intermediate flow rates were suggested to minimise fluctuation. After 22,000 column volumes, the monoliths exhibited no ageing. Gerber et al. reported stable columns, quicker analytical times, and successful separations. Mass transfer effects reduce efficiency of larger molecules at high flow rates. Several more monolithic column applications have been researched. Capillary, ion, hydrophilic contact, chiral, and two-dimensional chromatography are examples. Application development will likely continue when monolithic column technology is mastered.

Advanced Small-Particle Columns for High-Pressure and High-Temperature Applications

1. Sub-2-lm particle columns

Smaller, porous silica particles (<2 lm mean diameter) may improve LC separation resolution and speed, a significant advancement in drug substance impurity detection. Pharmaceutical HPLC separations have utilised three- to five-micrometre particles over the last decade. According to theoretical plates (N), resolution and separation efficiency are directly connected to the square root of the number of plates (N1/2) and stationary phase particle size (dp). Thus, lowering particle size from 5 to 1.7 lm increases N by 2.9, improving resolution by 1.7. Since column diameter, flow rate, and length affect column efficiency and

back-pressure, the method's goals should be considered while choosing conditions. Due to particle sizes of 3.5-5 lm and a flow rate of 1 mL/min, several impurity profile approaches need 45-60 min gradient separations. The time it takes samples to separate makes the analysis longer due to injections for blanks, standards, and system suitability testing.

Thus, reducing test separation time may boost lab productivity. Smaller particles boost chromatographic efficiency, allowing shorter columns to reduce separation times while maintaining resolution. Run times comparable to larger particle columns may be obtained while improving resolution. Sub-2 lm-particle columns have been tested for pharmaceutical research at pressures within current instrument range. Many vendors provide sub-2-lm particle columns with different qualities, but the ones utilised in that study were built with a greater particle size dispersion to minimise back pressures. The 50 mm lengths of certain sub-2 lm particle columns make them appropriate for typical LC instruments with lower back pressures. Shorter columns are less efficient in the long run, but smaller particles more than make up for the lower plate number. As demonstrated in Figure 2, a 3.5-lm particle, 4.6 • 150 mm column separated drug contaminants, whereas a 1.8-lm particle, 4.6 • 50 mm column did the same. Using the 1.8-lm particle column halved analysis time while maintaining separation.

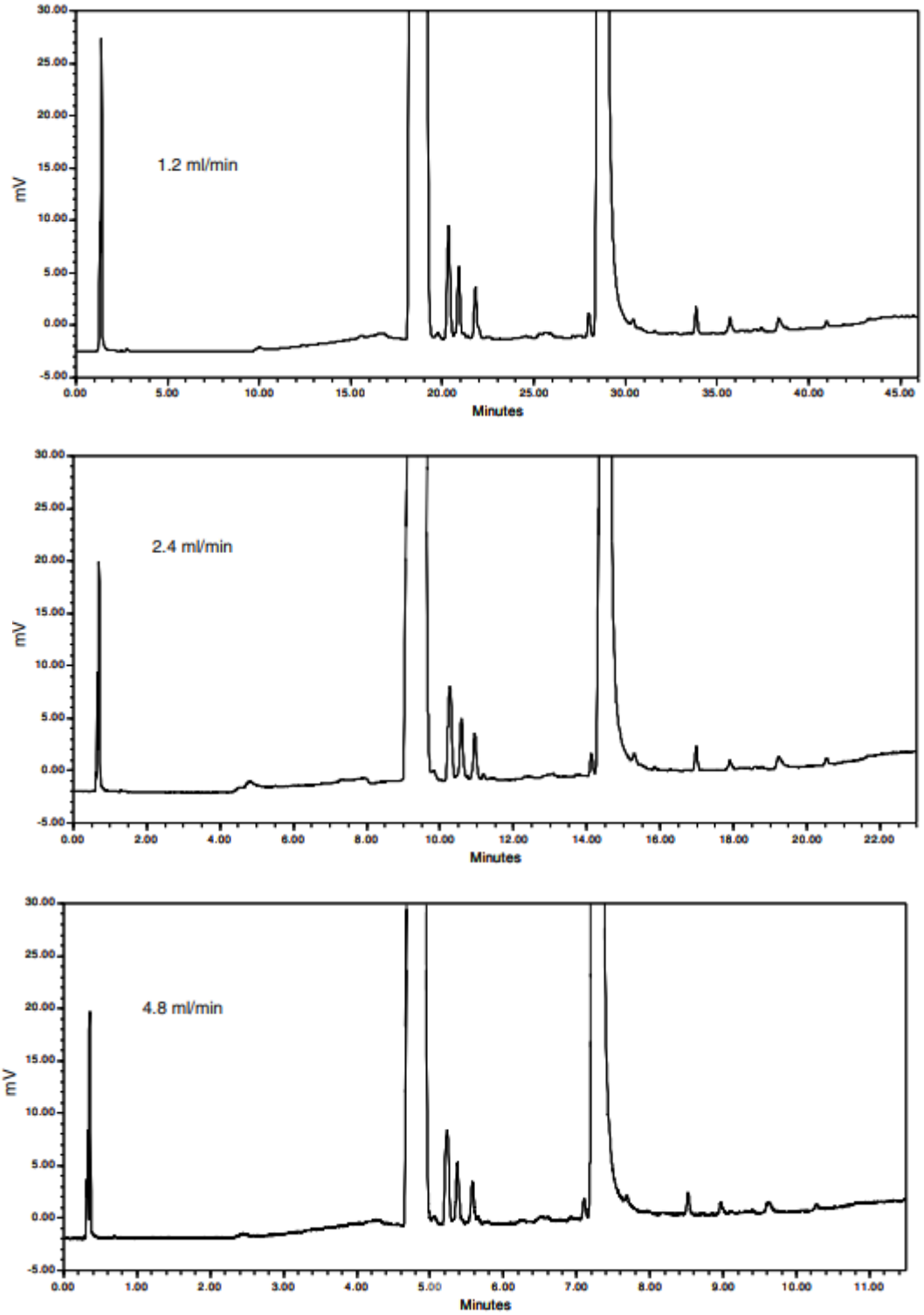


Figure 1. Monolithic column chromatograms at varying flow rates. Column: Chromolith RP-18e (Merck, KgaA), 100 • 4.6 mm i.d., mobile phases A and B = buffer:methanol, 80:20 and 10:90, gradient elution profile for 1.2 mL/min.: 100% A for 5 min., linear ramp to 100% B in 35 min, hold 6 min. Changes in flow rate affect gradient hold and ramp.

2. High-pressure operation

The back pressure generated by packed column HPLC is directly proportional to the column length and the inverse of the square of the particle diameter, as shown in Eq. (1).

$$\Delta P = \phi \eta L \mu / d_p^2 \quad (1)$$

where ϕ is the flow resistance factor, g is the viscosity of the solvent, L is the length of the column, and dp is the diameter of the particles.

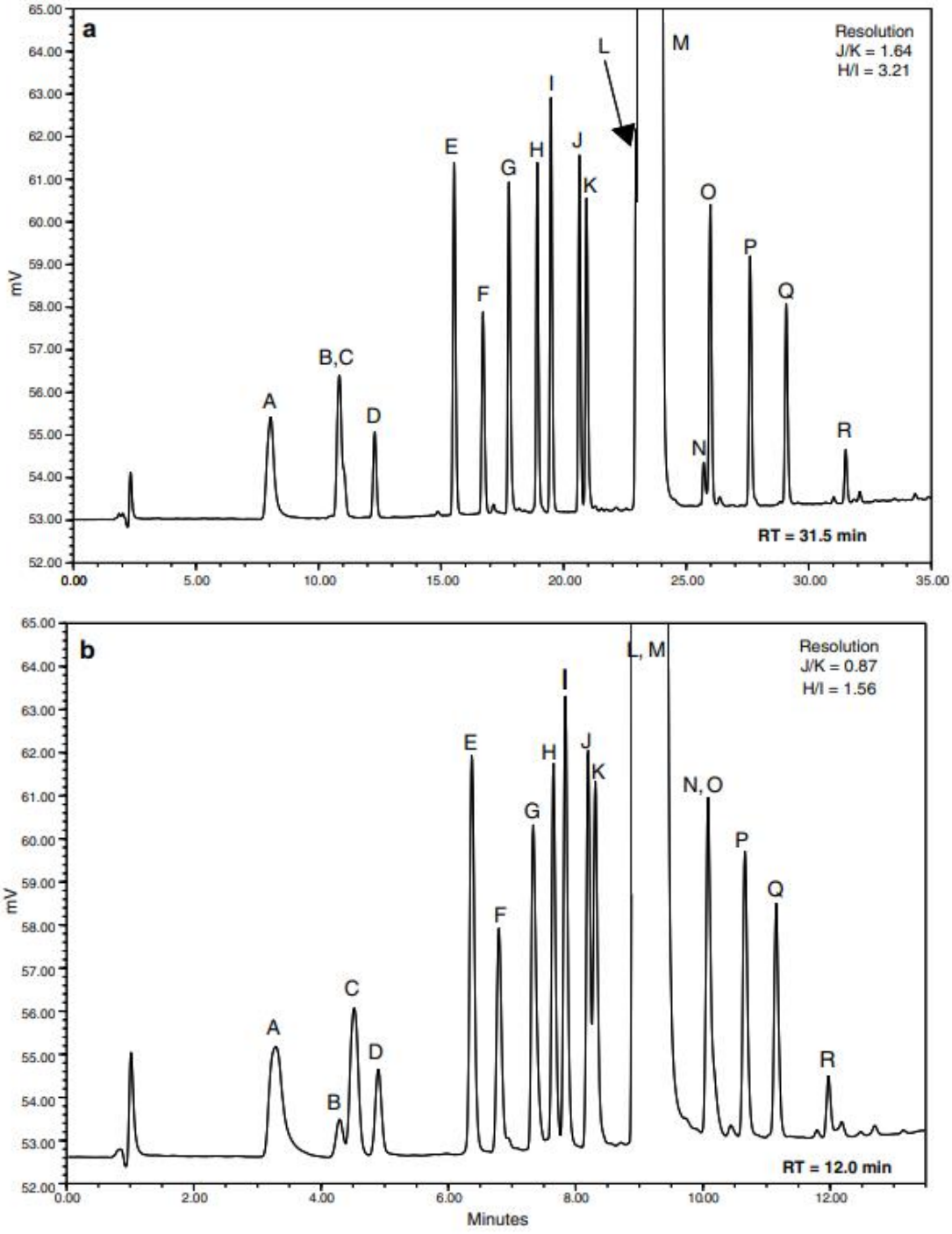


Figure 2. Chromatograms for an HPLC impurity technique employing (a) a 4.6 • 150 mm column with 3.5-lm particles (1.0 mL/min.) at typical pressures (2900 psi) and (b) a 4.6 • 50 mm column with 1.8-lm particles (4350 psi). Impurities may be present in each drug sample chromatogram. Gradient times and sample injection were scaled to column dimensions.

Sub-2-lm particle columns create pressures near to or more than 15,000 psi when used with the same flow rates (1-2 mL/min.) and column lengths (100-150 mm) as larger particle columns. Liquid chromatography (LC) apparatus and columns often reached 400 bar, or 5800 psi, until recently. Waters and Jasco make 15,000-psi LC systems. With sub-2-lm particle columns up to 150 mm long, these devices improve chromatographic separation efficiency. Many companies produce sub-2 lm particle columns, however not all can operate at these high pressures. Waters' 1.7-lm particle columns may run at 15,000 pressure and have various stationary phase properties. Jorgensen et al. studied Waterss's first microscopic "bridged ethyl hybrid" (BEH) particles. Some have tried utilising these columns at higher pressures for pharmacological analysis. Longer columns and/or greater linear velocities with high pressure devices increase efficiency. The combination of parameters determines the proportional resolution or analysis time gains over larger particle columns. A

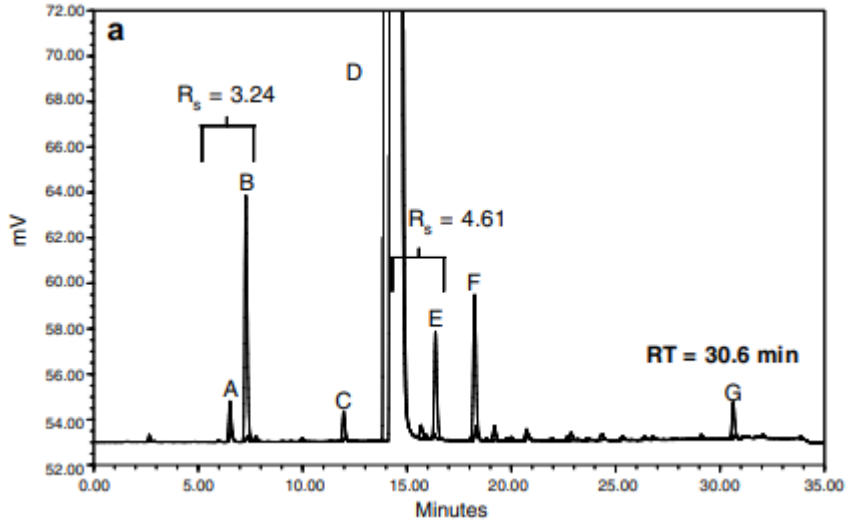
n impurity separation utilising conventional pressure and a 4.6 • 150 mm column with 3.5-lm particles is shown in Figure 3. Using a 2.1 • 100 mm column with 1.7-lm particles on a Waters Acquity high pressure LC system, two flow rates produced pressures of 5190 and 14,400 psi, respectively. We reduced run time by 1.5-fold and improved resolution by lowering the flow rate to 5190 psi. The 14,400-pressure flow rate quadrupled run length, but resolution was equivalent to the 3.5-lm column separation. Preliminary findings in the literature and the authors' lab suggest that these novel technologies should increase laboratory efficiency by shortening the time it takes to complete expensive impurity assays or by improving complex separation resolution.

3. High-temperature liquid chromatography

High-temperature liquid chromatography occurs over 100C. The main advantage of HTLC is dramatically reducing chromatographic run times. Eluent viscosity declines 5-10 times and analyte diffusivity rises at temperatures up to 200C, which is the major reason. Lowering eluent viscosity lowers column back pressure, allowing higher flow rates. Historically, interphase mass transfer broadened with higher flow rates and temperatures. However, increasing analyte diffusivity reduces this effect. Changes in column temperature may shorten analytical run time by 5–20. HTLC's unstable analyte population, temperature gradients between the eluent and stationary phase, and stationary phase stability up to 200C are issues. Developments in recent years have mitigated these barriers. Many thermally stable stationary phases are accessible. Traditional silica-based phases or those based on metal oxides (like zirconia), porous graphitic carbon, or organic polymers may be used. Plot widening may occur when the eluent and stationary phase are greater than 5 degrees Celsius apart. To reduce this disparity, numerous air-based and water/oil bath designs have been explored. Selerity Technologies sells a forced-air column heater that pre-heats the mobile phase to 200C and cools it after the column. Finally, analytes only spend a brief time exposed to high temperatures, therefore degradation may not occur often. Additionally, HTLC may combine temperature programming with chromatographic separation. Some find this helpful in solving complicated analytes. HTLC is still in its early stages of general usage, although it shows promise as an efficient approach in several applications.

Innovations in Stationary Phase Technology

Silica-bound stationary phases, especially C8 and C18 alkyl phases, dominate. New phases like polarembedded, fluorinated, zirconia, and high-pH stable will be described. A polar-embedded phase's alkyl chain possesses an amide, carbamate, urea, or ether polar group. Silanol interactions with basic analytes inspired these phases. Nowadays, these stable phases maintain polar analytes and perform excellent aqueous separations. Several studies categorise and compare commercially available polar-embedded phases. Compared to alkyl phases (C8 or C18), polar-embedded phases provide alternative selectivity. Fig. 4 shows that a standard C8 phase and a polar-embedded phase retain impurity mixture components differently, demonstrating selectivity. Grumbach et al. found that densely-bonded phases such Atlantis dC18 and Waters held polar analytes better than polarembedded phases.



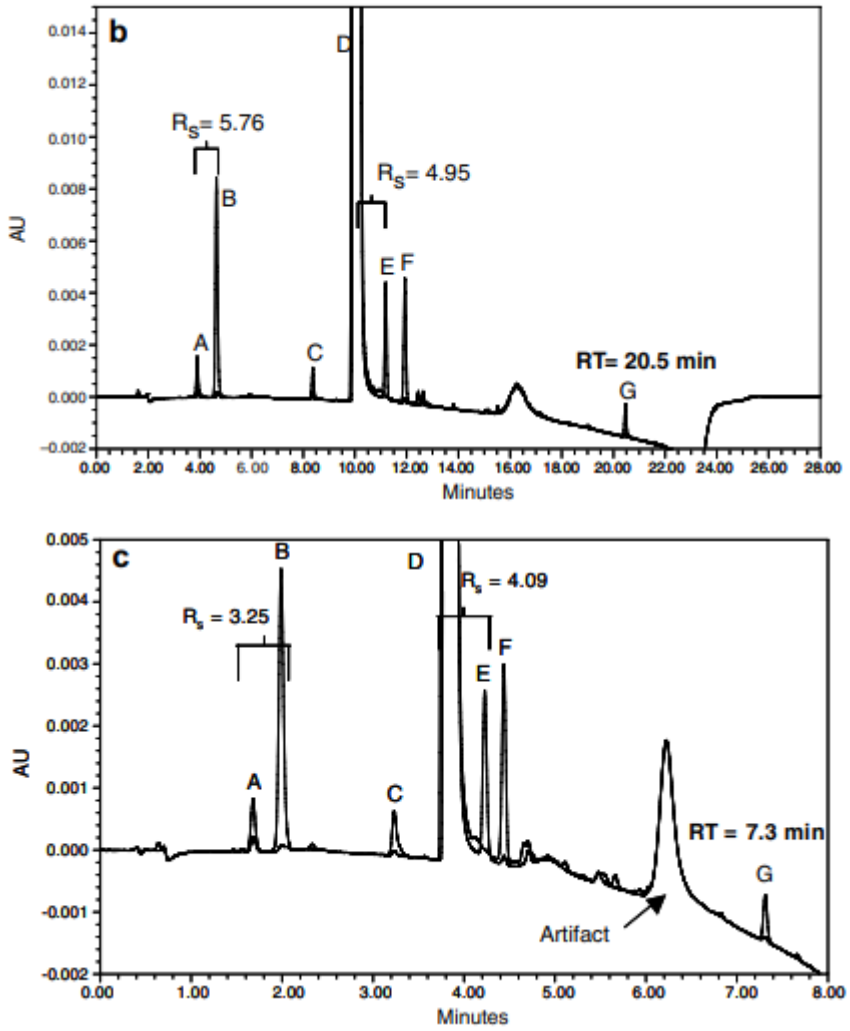


Figure 3. Chromatograms for HPLC impurity separation using (a) 3.5-lm particle, 4.6 • 150 mm column, flow rate 1.0 mL/min., at conventional pressures (4350 psi), (b) 1.7-lm particle, 2.1 • 100 mm column, flow rate 0.2 mL/min., at 5200 psi, and (c) the same column as (b), flow rate 0.6 mL/min., at 14,400 psi.

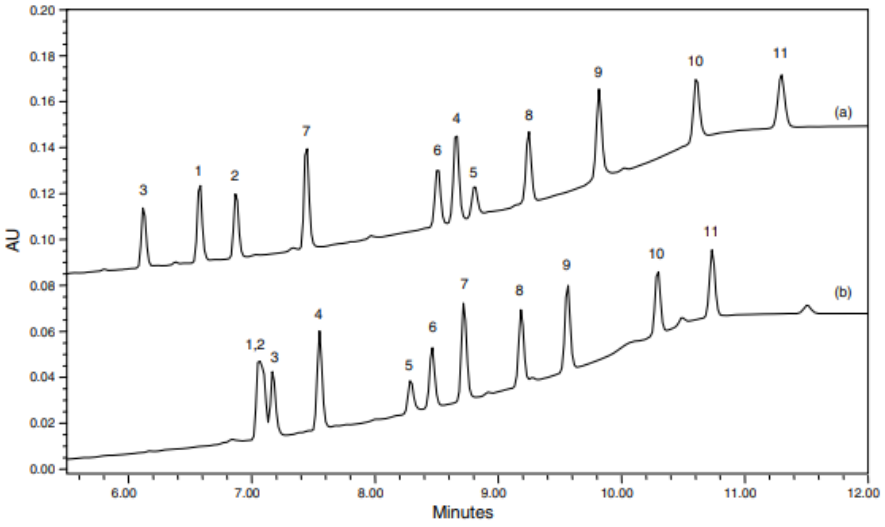


Figure 4. HPLC chromatograms of an impurity mixture (basic analytes) on (a) a Zorbax Bonus RP column and (b) an SB-C8 column. This sample shows varied selectivity due to the Zorbax Bonus RP phase's polar-embedded group.

In recent years, fluorinated phenyl and alkyl phases have gained popularity. Some categorisation evaluations and a report are here. Selectivity distinguishes them from C8 and C18 phases. These phases have been studied mechanistically, although the primary chromatographic interaction is yet unknown. These procedures work well for fluorinated analytes but may be used for many. Many basic analytes retain more than neutral ones. Recently, zirconia stationary phases have grown increasingly prominent. These phases are stable throughout pH and 200C. RP-HPLC may use polystyrene, polybutadiene, carbon-coated, and C18 grafted to carbon-coated zirconia (ZirChrom) phases. The analyte and zirconia substrate will interact, however Lewis acid-base interactions from silica phases may be leveraged to one's advantage. Kucera et al. found that the polystyrene-bonded zirconia phase removed ibuprofen and its impurities more selectively than the silica-based C18 phase. Mobile phase pH may considerably alter basic and acidic analyte retention. Zirconia-based and polymeric phases (e.g., apHera, Astec) may operate up to pH 12, however silica particles dissolve above pH 7.5, making their use with mobile phases unfeasible. Recent advances enable silica stationary phases to be used up to pH 12. These phases include Zorbax Extend (Agilent) and XTerra (Waters), which employ bidentate bonding and double end-capping to provide stability up to pH 11.5 and a hybrid particle of silica and organic (methylsilane) components to ensure stability up to pH 12. In their work, Dong et al. suggested high pH for impurity analysis. High-pH separations create problems including column longevity and gradient/system artefacts, but they also increased base retention.

Systematic Classification of Columns for Optimal Application

New approaches for comparing RP-HPLC columns have developed recently, however categorisation has been attempted for a long. Column classification methods are used in two situations: when the nominal column is unavailable, an equivalent column must be used for separation, and when developing semi-orthogonal conditions, columns with as varied selectivity as possible are preferred. Compared to trial-and-error procedures, systematic column characterisation and comparison algorithms identify columns faster. Five terms have been used to quantify column selectivity in many works, as illustrated in Eq. (2). Selectivity parameters for solutes (g, r, b, a, j) or columns (H, S, A, B, C). K is the retention factor for any solute, whereas kref is the reference solute's k.

$$\log(k/k_{ref}) \equiv \log \alpha = \eta' H + \sigma' S + \beta' A + \alpha' B + \kappa' C \quad \textbf{(2)}$$

Column parameters have these traits: A through C reflect a column's hydrophobicity, steric resistance into the stationary phase, hydrogen bond acidity or basicity, and cation-exchange activity. Retention measurements of 18 solutes (10 if type-B alkylsilica columns are only addressed) provide selectivity term characteristic values. These parameters may be used to derive the matching value, Fs, which shows column selectivity similarity (Eq. (3)).

$$F_s^* = \{[12.5(H_2 - H_1)]^2 + [100(S_2^* - S_1^*)]^2 + [30(A_2 - A_1)]^2 + [143x_B(B_2 - B_1)]^2 + [83x_C(C_2 - C_1)]^2\}^{1/2} \quad \textbf{(3)}$$

If Fs = 6.3, then it's likely that the columns will provide similar selectivity. Depending on the separation's critical resolution requirements, larger numbers could potentially be appropriate. One might modify the weighting factors for the B and C components in Eq. (3) in accordance with the acids and bases found in the samples under consideration. The results showed that column similarity predictions were typically accurate across a number of practical situations. A different team has created a column categorisation method that uses PCA of 36 measurements. They went on to prove that a mere quartet of parameters was all that was needed for proper discriminating of distinct column selectivities.

Three repeatable test methods were able to get these characteristics, which served as markers of hydrophobicity, silanol activity, steric selectivity, and metallic impurities. By comparing the selectivities achieved on various columns for many separations reported in pharmacopoeial monographs, the classification system was evaluated on pharmaceutical systems via comparison. For the separations that were considered, the findings were generally consistent across columns that were deemed comparable. Additionally, it was observed that the system-suitability criteria provided in the techniques of the monograph did not necessarily reflect the column's selectivity for separating related chemicals. Using principal component analysis (PCA), Euerby et al. have characterised 135 RP columns, some of which include bonded phases of various kinds.

Streamlined Method Development with Automation

A pharmaceutical impurity technique may require time and effort to develop. The first systematic HPLC technique development focused on mobile phase and column organic content. In the 1990s, higher-purity silica allowed researchers to study a wider pH range without affecting peak tailing. Dolan et al. examined temperature's influence in optimising approaches. Researchers use automated technologies to build solid approaches fast. One LC system can assess pH (2–9), organic solvents (methanol and acetonitrile), orthogonal columns, and detectors (ELSD, MS, and PDA). Commercial and in-house solutions automate mobile phase and column changes and improve separations using scouting runs and retention estimates to create HPLC techniques. Use the previously stated column classification methods to screen for orthogonal selectivity.

An analyse structure and column attribute model predicts retention. IRIS Technologies' Chromsword is one. After the scouting runs, the system uses experimental and predicted data to find the best settings. Structure and column chemistry may predict chromatographic separations in ACD/LC Simulator. Several instrument vendors provide automated method generation. These capabilities provide several experimental design, instrument control, data collection, and interpretation choices. AMDS by Waters allows customers pick which columns to screen and then utilises DryLab (Rheodyne) LC-modeling software to automatically optimise separation on a single column for isocratic and gradient conditions. Due to its automated data gathering and interpretation across multiple experimental conditions, HPLC system overnight or weekend runs speed up technique development. Separation complexity and analyte parameters determine automated

method development effectiveness. Knowing the path of future method improvement is often achievable without knowing the final circumstances.

Advanced Detection Techniques for Improved Impurity Profiling

UV/Vis is still the pharmaceutical lab's major spectroscopic detection tool. Usually relevant, easy to use, and affordable. The most typical reason for alternative detection techniques is when target analytes lack a strong UV/Vis chromophore. This field has made promising development employing charged aerosol detection. Nebulising column effluent creates droplets of analyte. The procedure is drying. When charged nitrogen vapour touches analytes, they become charged. When charged analytes send their charges to a collector, a signal is proportional to their concentration. Most analytes have similar response factors when eluted under the same conditions. Gramache et al. described this detection method for nonvolatile analytes. Despite its promise, this detection approach has certain drawbacks: The analyte's reaction will vary as the eluent's composition varies (like gradient elution), but this is predictable and can be normalised.

It needs volatile buffers and nonvolatile analytes. Gramache et al. discovered that Corona CAD from ESA, a commercial CAD detector, beat ELSD, another typical approach for non-chromophoric analytes, in sensitivity and accuracy. Recently employed detection methods include online nuclear magnetic resonance spectroscopy. HPLC coupled with nuclear magnetic resonance has created several operating modes, including continuousflow, stop-flow, and peak-collection, during the previous two decades. Recent LC-NMR improvements have focused on sensitivity. Solid-phase extraction (SPE) following chromatographic separation (HPLC-SPENMR) may increase NMR peak concentration. Using numerous trappings on the SPE cartridge may increase the approach's sensitivity, according to Sandvoss et al. Using a cryogenic probe increases sensitivity. Lewis et al. found that cryogenic probes may double sensitivity. Last but not least, LC, NMR, and MS integration allows the most unknown identification. Spraul et al. examined urine paracetamol metabolites using LC-NMR-MS. They observed that NMR and MS data were needed to identify certain metabolites. NMR will likely be utilised as an LC detector when qualitative structural information about a component is needed instead of quantitative sample levels.

CONCLUSION

The capacity to identify and quantify drug contaminants with increased sensitivity, precision, and efficiency has been considerably improved as a result of advancements in High-Performance Liquid Chromatography (HPLC) technology. The development of ultra-high-performance liquid chromatography (UHPLC), improved column chemistries, and advanced detection systems such as mass spectrometry (MS) and diode array detectors (DAD) has allowed for the precise separation and identification of impurities at trace levels, which is critical for ensuring drug safety and efficacy. As a result of these technical advancements, analysis times have been shortened, resolution has been improved, and the amount of solvent used has decreased. This is in accordance with both the expectations of regulatory agencies and the practices of sustainable laboratories. Additionally, the integration of software and automation have simplified the process of data analysis and technique validation, which has resulted in a reduction in human error and an increase in throughput. For the purpose of providing a strong platform for comprehensive impurity profiling throughout the whole process of drug development and production, high-performance liquid chromatography (HPLC) continues to play an increasingly important role as pharmaceutical formulations become more complicated.

In general, the developments that have been made in high-performance liquid chromatography (HPLC) technology play a significant part in ensuring that pharmaceutical goods continue to fulfil the severe criteria that have been established by worldwide regulatory bodies.

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