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The Role of HPLC and UPLC in Quality Control of Biopharmaceuticals and Biologics

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ABSTRACT

Ensuring safety, effectiveness, and regulatory compliance is important for quality control of biopharmaceuticals and biologics. Two techniques that have become indispensable analytical high-throughput approaches in biopharmaceutical development include high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC). Such chromatographic techniques have proved invaluable when characterizing, assessing purity, stability testing, and the impurity profile of complex biological products like monoclonal antibodies, peptides, and recombinant proteins. HPLC has maintained its position as a gold standard for bioanalytical applications for a long time owing to its versatility, accuracy, and reliability. But then came UPLC, which completely redefined quality control by offering superior resolutions, shorter run times, and improved sensitivity over conventional HPLC methods. In this review, we present a discussion based on the fundamental principles of high-performance liquid chromatography and ultra-performance liquid chromatography, their applications in biopharmaceutical quality control, as well as their comparative advantages related to efficiency, resolution, and compliance with regulations. Discussion extends also to challenges, recent advancements, and future perspectives with regard to chromatic techniques in assuring integrity in biologic therapeutics. The continuing integration of these technologies with emerging analytical tools keeps shaping the future of biopharmaceutical quality assurance in order to enable increasingly accurate and rapid evaluation of complex biomolecules.

Keywords: HPLC, Chromatographic Techniques, Quality Assurance.

Introduction

The sphere of biopharmaceutical and biologics free from any kind of mistrust is growing at an unprecedented rate in contemporary medicine, providing highly specified, highly effective treatments for a multiplicity of diseases, ranging from cancer to autoimmune disorders and metabolic conditions. Unlike conventional small-molecule drugs, a biopharmaceutical is a complex macromolecule that can be a monoclonal antibody, a recombinant protein, a peptide, or a nucleic- acid-based therapeutic. The very complex nature and intrinsic fragility of these molecules present unique challenges for stringent quality control to guarantee their safety, efficacy, and batch-to-batch consistency (1). The assessment of certain analytical methods is very important in determining critical quality attributes (CQAs), including the domains of purity and potency, stability, and impurity profiling. For this purpose, the chromatographic methods provide an analytical backbone, nowadays becoming increasingly important in the biopharmaceutical quality assurance process, with specific emphasis on High-Performance Liquid Chromatography (HPLC) and Ultra-High Performance Liquid Chromatography (UPLC) (2).

HPLC has forever been a pillar in pharmaceutical analysis due to its many great strengths: high precision, reproducibility, and ability to separate and quantify biomolecules from the complex matrix. The method is routinely engaged in the purity assessment of therapeutic proteins and the stability testing focused on the degradation products and assessing any post-translational modifications that may be of therapeutic relevance (3). Nevertheless, classical HPLC approaches are systematically marred by lengthy run times and large sample volumes that reduce the efficiency of HPLC for high-throughput analysis. The advancements in UPLC, with improvements in chromatographic performance due to reduced particle size and higher operational pressures, have now seen resolutions increase, sensitivities go up, and separation time shortened considerably (4). Such developments are enabling UPLC to become a preferred implement for regulatory-compliant analytical work within the biopharmaceutical sector.

The adoption of better analytical methodologies has been made essential over the past few years as a direct result of stringent regulatory requirements imposed by the FDA and EMA. The standards stress the requirement of validated high-resolution chromatographic techniques to guarantee safety and efficacy of products through the lifetime of drug development (5). For this reason, the use of HPLC and UPLC within quality control processes became essential in assessing critical product parameters, including aggregation, glycosylation, and host cell protein contamination (6).

This review aims to provide a comprehensive insight into the applications and role of HPLC and UPLC in the quality control of biopharmaceuticals and biologics. The paper will discuss the generic principles of these chromatographic techniques, their applications in compliance with regulations, and the

comparative advantages of UPLC over HPLC. Furthermore, new trends, problems encountered, and future perspectives in chromatographic analysis of biologics will be dealt with to highlight continuous evolution (7).

Fundamentals of HPLC and UPLC

Liquid chromatography (LC) forms an important toolkit in pharmaceutical and biopharmaceutical research. The two most significant advances are high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC). These are critical for the separation, identification, and quantification of complex biomolecules to assure the quality and consistency of biopharmaceutical products (8). With the high resolution, reproducibility, and versatility in methods of analysis of proteins, peptides, nucleotides, and small molecules, HPLC has been known as the gold standard for decades. However, UPLC has become an advanced alternative with enhanced efficiency, rapidity, and sensitivity due to the usage of smaller particle sizes under higher operating pressures (9).

Principles of High-Performance Liquid Chromatography (HPLC)

HPLC is an advanced separation technique that operates using the differential interaction of the sample constituents or analytes with the stationary phase and the mobile phase under high pressure. HPLC systems include basic parts: a solvent reservoir, a pump, an injector, a column, a detector, and a data acquisition system. The mobile phase is a combination of solvents such as aqueous solvents, methanol, or acetonitrile that carry analytes through the stationary phase, which is typically adhesive-based materials with different chemical modifications (10). The separation is also dependent on polarity, size of molecules, and hydrophobicity for the successful identification and quantitation of the pharmaceutical compounds.

HPLC is widely used to assess the quality of biopharmaceuticals because of its resolution and reproducibility. Reverse-phase HPLC (RP-HPLC) finds use in protein and peptide analysis, sizing exclusion chromatography (SEC) is mostly used for the determination of molecular weight, while the ion-exchange chromatography (IEX) is suitable for charge-based separations (11). In spite of its strengths, conventional HPLC is limited by long run times, high solvent consumption, and poorer separation efficiencies for highly complex biomolecules, thereby motivating the need for more advanced techniques such as UPLC (12).

Principles of Ultra-Performance Liquid Chromatography (UPLC)

Ultra-performance liquid chromatography (UPLC) is an advanced form of liquid chromatography that provides improvement against traditional HPLC with the utilization of small particle sizes (typically <2 µm) and very high operating pressure (>15,000 psi). Such characteristics enable rapid separations with enhanced resolution and sensitivity with respect to classical HPLC (13). The main principle on which UPLC operates is derived from the Van Deemter equation, which describes the relationship of particle size and a chromatographic efficiency. Smaller particles provide separation efficiency with reduced band broadening, resulting in sharper peaks and enhanced signal-to-noise ratios (14).

Major advantages in using UPLC for biopharmaceutical analysis include faster analysis, higher sensitivity, and reduced solvent consumption, making it an ideal candidate for the regulatory environment quality control (7). Areas where UPLC has found its applications in biopharmaceuticals include peptide mapping, glycan profiling, aggregation studies, and impurity identifications, thereby yielding vital information related to the stability and integrity of the biologics. Guided by its compatibility with mass spectrometry (MS), UPLC could be used further for in-depth elucidation of molecular features (15).

HPLC Versus UPLC

Although UPLC and HPLC share the same working principles, they differ owing to some key physical differences. UPLC is said to be high resolution, since its separating particle size and pressure condition are appropriate for the isolation of a wide range of complex biomolecules. UPLC reduces the analysis time significantly as a result, increasing the efficiency of laboratories and productivity. Reduced sample and solvent consumption is another major advantage of UPLC; hence analyses are cheaper and less harmful to the environment (16). However, the UPLC introduction would need a special set of instrumental design, which, in turn, can only maintain high pressure. This can be a hindering issue for some laboratories.

In conclusion, both HPLC and UPLC are essential in the biopharmaceutical industry for product safety, consistency, and regulatory compliance. While HPLC analysis is still being widely used, UPLC has opened up new horizons of analytical possibilities through enhanced speed, sensitivity, and efficiency of biopharmaceutical analysis. Given the ever-increasing demand for high-resolution characterization techniques, UPLC is poised to take center stage in the quality control of complex biologics (17).

Applications of HPLC in Quality Control of Biopharmaceuticals

High-Performance Liquid Chromatography (HPLC) would have to be one of the widely applied analytical techniques in the quality control of biopharmaceuticals due to the high resolution, reproducibility, and versatility it embodies. It serves a very crucial function in the evaluation of critical quality attributes (CQAs) like those concerning purity, potency, stability, and impurity profiling with regards to biologic drugs, including mAbs, recombinant proteins, and peptide drugs (18). Regulatory agencies such as U.S. Food and Drug Administration and European Medicines Agency insisted on using validated chromatographic methods, including HPLC, which guarantees their requirements of adherence to rigid quality standards (19).

1. Purity and Impurity Profiling

The purity of biopharmaceuticals is the crucial limiting factor with regard to their safety and efficacy. HPLC serves the most common detection and quantification methods for impurities, such as aggregates, degradation products, host cell proteins (HCPs), and residual solvents. Size-exclusion chromatography (SEC), in fact, a mode of HPLC, is frequently performed for detecting protein aggregates, thereby leaving them to immunogenic responses to the patients (20). The oxidized and deamidated species of proteins are detected with reverse-phase HPLC (RP-HPLC) according to an identification based on differentiation in the hydrophobic nature of molecules (21). Such high sensitivity and specificity possible with HPLC make it valuable for impure profiling of biopharmaceuticals.

2. Protein and Peptide Analysis

Therapeutic proteins, peptides, and glycoproteins are thoroughly analyzed with the use of HPLC. Ion-exchange chromatography (IEX-HPLC) is usually employed in separating protein variants on the basis of differing charges, which is very important in monoclonal antibody analysis and recombinant protein (22). In peptide describing, reverse-phase HPLC (RP-HPLC) is adopted for peptide mapping, which allows the identification of some post-translational modifications (PTMs) such as glycosylation, oxidation, and phosphorylation (23). Such a high resolution would allow biopharmaceutical formulation characterization in detail giving consistency analysis between batches.

3. Quantification of Active Pharmaceutical Ingredients (APIs)

Quantification of active pharmaceutical ingredients (APIs) must be accurate to ensure dose uniformity and, therefore, therapeutic efficacy. HPLC methods UV and fluorescence (FL) detection for active pharmaceutical ingredient (API) quantification have been widely adopted in biopharmaceutical applications (17). These methods allow very precise determination of proteins or peptides in drug products and ensure conformity to regulatory specifications. HPLC-MS is also suitable for the quantitation of low-abundance biomolecules due to enhanced sensitivity (6).

4. Stability Testing

Biopharmaceuticals undergo susceptibility to chemical and physical degradation that can affect their potency and safety. HPLC-based stability studies assist in observing the degradation of biologics under different environmental conditions like temperature, humidity, and pH (24). The forced degradation studies, performed with the help of RP-HPLC and SEC, help predict the long-term stability of biologics by mapping down the degradation pathways and possible degradation products (25). HPLC stability testing becomes necessary for determining shelf-life and storage conditions, thereby assuring Efficacy of biopharmaceuticals throughout their life span.

5. Bioanalytical Applications and Pharmacokinetics

HPLC is the most common means for bioanalysis and in pharmacokinetic (PK) studies for the determination of drug concentration in biological fluids such as plasma and serum. HPLC-MS/MS, or tandem mass spectrometry, is especially valuable in pharmacokinetic profiling for determining absorption, distribution, metabolism, and excretion (ADME) of the drug (26). These are necessary for determining dosage regimens, bioavailability, and bioequivalence in clinical trials and post-marketing surveillance (27).

Applications of UPLC in Quality Control of Biopharmaceuticals

This kind of Ultra Performance Liquid Chromatography is well established into the QC of biopharmaceuticals. Sufficient resolution, worked at higher sensitivity and shorter times compared with conventional HPLC, sustained by high pressures and smaller particle sizes (<2 µm) in the stationary phase, UPLC separates complex biomolecules much more efficiently, thus proving itself in pharmaceutical analysis because of regulatory compliance (8). Even the pharmaceutical industries and regulatory bodies like the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) have acknowledged UPLC as a superior analytical method that ensures the purity, stability, and potency of biopharmaceuticals (19).

1. Enhanced Purity and Impurity Profiling

Detection and quantification of impurities in biopharmaceutical formulations are one of the main applications of UPLC in QC. UPLC provides higher resolution than HPLC and, therefore, allows the precise identification of protein aggregates, degradation products, and process-related impurities. Size-exclusion chromatography (SEC-UPLC) for detection of aggregates is routinely used for most monoclonal antibodies (mAbs) and recombinant proteins; it is important in preventing immunogenicity in patients (20). Further, UPLC-MS (mass spectrometry) improves impurity profiling by allowing the specification of less abundant impurities in protein formulations at higher sensitivity and accuracy as compared with previous methods (21).

2. Mapping of Biopharmaceuticals Peptide

UPLC is especially useful in peptide mapping, which is a technique crucial for structure characterization of therapeutic proteins and monoclonal antibodies. In this sense, reverse-phase UPLC (RP-UPLC) gives even better peak resolution and shorter run times than traditional HPLC, and makes for a perfect method for analysis of PTMs such as glycosylation, oxidation, or phosphorylation (23) because these modifications determine the activity and stability of biopharmaceuticals while UPLC provides reliable characterization with minimal variability in results (22).

3. Quantification of Active Pharmaceutical Ingredients (APIs)

Active pharmaceutical ingredients (APIs) must be precisely quantified to ensure accurate dosing and therapeutic efficacy. UPLC-UV and UPLC-MS/MS are conventional techniques for biologic API quantification, which provide more sensitivity and faster analysis than HPLC (17). With improved separation efficiency of UPLC, accurate determination of protein and peptide concentrations ensures consistency in drug formulation and regulatory compliance (6).

4. Stability Studies and Forced Degradation Studies

Biopharmaceuticals undergo chemical, physical, and environmental instability; this results in reduced potency and safety. UPLC is widely used for stability tests to check for degradation of biologics under different storage conditions, such as temperature, pH, and humidity (24). Forced degradation studies are employed using RP-UPLC and SEC-UPLC, identifying degradation pathways and establishing shelf-life specifications (25). With the greater sensitivity of UPLC in detecting minor degradation products, long-term stability and reliability of biopharmaceuticals are ensured.

5. Bioanalysis and Pharmacokinetic Studies

For the examination of concentrations of biopharmaceuticals in biological matrices such as plasma and serum, UPLC is a common tool utilized in bioanalysis and pharmacokinetic (PK) studies. Quantifying therapeutic proteins, peptides, and biosimilars using UPLC-MS/MS provides greater sensitivity and specificity, allowing reliable ADME (absorption, distribution, metabolism, and excretion) assessment (26). With reduced analysis time and improved resolution, UPLC enhances the working benefits of PK and bioavailability studies performed in clinical research (27).

Comparative Analysis: HPLC vs. UPLC in Biopharmaceutical QC

Both HPLC and UPLC are techniques that find extensive application in the quality control (QC) of biopharmaceuticals. Although UPLC is gaining popularity for having better efficiency, speed, and sensitiveness over HPLC, the latter has been the gold standard for decades. Such comparisons would be needful for understanding the merits as well as the limitations of these techniques for purity, potency, and stability of biologics (8).

1. Resolution and Separation Efficiency

Specifically, column particle size affects the overall efficiency of chromatographic separation. HPLC uses column particles in the range of 3 to 5 µm and UPLC uses particles of sub-2 µm, resulting in the significant enhancement of resolution and peak capacity (28). Reduced particle size for UPLC leads to a high column efficiency and thus allows separation of complex biopharmaceutical mixtures better, especially for peptide mapping and impurity profiling (21). Furthermore, UPLC produces sharper and better-defined peaks that reduce co-elution and thus leads to more precise quantification of impurities and degradation products (23).

2. Analysis Time and Throughput

One of the biggest advantages UPLC has over HPLC is the time required for analysis. This is because at pressures as high as 15,000 psi, UPLC will be able to achieve much faster mobile phase flow rates without sacrificing separation efficiency than the previous maximum of 6,000 psi for HPLC (29). The run times are about 3-10 times shorter than HPLC, making it ideal for the high-throughput environment typical of QC (30). In addition, the faster speed of UPLC contributes greatly in the area of biopharmaceutical, allowing even more samples to be analyzed within a timeframe, which is very important in drug manufacturing (19).

3. Sensitivity and Detection Limits

UPLC is more sensitive than HPLC by virtue of exhibiting decreased peak broadening and an increased signal-to-noise ratio. This is extremely useful for impurity profiling or for stability studies in which the detection of low levels of degradation products is required (6). Studies have shown that UPLC-MS will give better detection limits than HPLC for protein modifications, post-translational modifications, and trace-level impurities in biologics (31). While it remains effective for the routine analysis it does play tend to suffer from less sensitivity due to lower column efficiency and longer run times, which lead to broader peaks and lower resolution (22).

4. Consumption and Cost-Efficiency

UPLC may, however, require expensive instruments and columns that can withstand high pressure, despite the gains made with this analytical form. Still, claim it's been more useful than it, with reductions of solvent consumption showing that up to 80% less solvent is consumed in UPLC than in HPLC (24). Less solvent leads to lower operational costs while impacting the environment for disposal purposes, thus a more sustainable option in the long run by UPLC (27).

5. Robustness and Transference of Method

Today, HPLC is often chosen because it is a robust and available method for most applications. Quality control methods approved by regulators generally include HPLC methods. Most pharmacopeial standards (e.g., USP, EP) are based on HPLC, making implementation and validation easy for routine studies (32). However, due to regulatory acceptance of UPLC, method transfer from HPLC to UPLC has been demonstrated successfully in various cases with the performance being at least comparable (33). Nevertheless, there are still some challenges such as reproducibility for complex matrices and method revalidation that must be resolved while transferring the methods from HPLC to UPLC (34).

Regulatory Aspects and Compliance

U.S. FDA, EMA, and ICH are regulatory bodies that have issued strict guidelines with an aim to maintain the quality, safety, and efficacy of biopharmaceutical products. HPLC and UPLC are analytical techniques commonly accepted in pharmaceutical quality control (QC) and are highly regulated. Compliance with these guidelines is paramount to good manufacturing practice (GMP), good laboratory practice (GLP), and validation of methods guaranteeing that the quality of the product will remain consistent (19).

1. Method Validation and Regulatory Guidelines

Method validation is an onerous requirement in regulatory terms for HPLC and UPLC applications in biopharmaceutical QC. Regulatory bodies work on the ICH Q2(R1) guidelines, which are prescriptive about parameters such as accuracy, precision, specificity, detection limit (LOD), quantification limit (LOQ), linearity, and robustness that must be considered for method validation (35). The methods (HPLC and UPLC) must, therefore, undergo extensive validation studies for reproducibility and reliability, which implies the use of methods adapted for routine quality control (36). The FDA Guidelines on Analytical Procedures and Methods Validation for Industry also affirm the aspect of method transferability from HPLC to UPLC (6).

2. Pharmacopoeial Standards and Regulatory Acceptance

Pharmacopoeias such as USP, EP, and JP give way to official monographs, which outline acceptable HPLC and UPLC methods for analysis of drug substances and drug products (37). General chapters adopted by the USP like <621> Chromatography include system suitability provisions, which cover retention time, resolution, and peak symmetry (38). HPLC is the method in dominance in pharmacopoeial monographs, but increasingly UPLC is being accepted too by the regulatory bodies, which propels the revision of methods (39).

3. GMP and Data Integrity Compliance Regulation

It is very clear that the importance of regulatory compliance in terms of biopharmaceutical QC will require adherence to guidelines for GMP to have an integrity traceability of analytical data. For the FDA, such as the EMA, the emphasizing principles related to data integrity also apply under electronic records and audit trail regulations like 21 CFR Part 11 (40). UPLC, because of advanced instrumentation and advanced software, ensures better compliance in totality with data integrity requirements, automated documentation, electronic signatures, and better audit trails (41). This has, therefore, prevented possible manipulations of data and kept analytical results used for regulatory submission credible (34).

4. Regulatory Considerations for Stability Testing and Impurity Profiling

Stability testing of biopharmaceuticals has to be conducted as per ICH Q1A(R2); these guidelines require validated chromatographic methods to be used for assessment of degradation products over time (42). Forced degradation studies, impurity profiling, and stability-indicating assays defined to shelf life and storage conditions usually employ HPLC and UPLC extensively (24). This sensitivity, mainly regarding the low detection limits of impurities, is very useful in regulatory compliance when developing a biosimilar and during comparability studies (43).

5. UPLC Adoption and Barriers to Meeting Regulatory Requirements

UPLC now has its share of challenges frustrating its regulatory acceptance despite its increased advantages. The proper example is that of method transfer and revalidation from HPLC-based pharmacopoeial methods (21). In fact, the many considerations required of HPLC standard methods would have to be adapted to those under UPLC because the HPLC to UPLC conversions are based on either equivalent or superior performance. This involves further regulatory filings plus compliance for, say, ICH Q14 (Analytical Procedure Development) (44). However, regulatory agencies support method modernization, so UPLC will gain acceptance on a much broader level in biopharmaceutical QC.

Challenges and Future Perspectives

As High-Performance Liquid Chromatography (HPLC) and Ultra-Performance Liquid Chromatography (UPLC) integrate into biopharmaceutical quality control (QC), analytical precision, sensitivity, and throughput have been developed. Nevertheless, various hurdles lie in their successful implementation, including high operating costs, regulatory requirements, difficulty in method transfer, and technological constraints. The resolution of these challenges together with the advancement of chromatographic methods will lead the way for biopharmaceuticals in the future (36).

1. Challenges in Method Development and Validation

One of the central challenges impeding the application of HPLC and UPLC to biopharmaceutical QC lies in the complexity of method development-and-validation processes. Biopharmaceuticals such as monoclonal antibodies and recombinant proteins have structural complexity, which in turn requires highly selective and sensitive analytical methods (45). Regulatory agencies require that methods be validated with great rigor under guidelines such as ICH Q2(R1), which makes the establishment of robust chromatographic methods a time-consuming and resource-demanding task (35). In addition, transferring methods from HPLC to UPLC is often challenging and frequently requires revalidation because of differences in column chemistry, flow rates, and pressure conditions (6).

2. Great Instrumentation and Maintenance Costs

On one hand, UPLC is highly advantageous from an analytical perspective; on the other hand, it has high initial investment and maintenance costs. UPLC operates at high pressures of 15,000 psi, which results in wear and tear of components like pumps, injectors, and columns (30). Moreover, special sub-2 µm particle columns are more expensive and have shorter lifespans than standard HPLC columns, contributing further to long-term running costs (24). This financial obligation, especially for some pharmaceutical companies within emerging markets, continues to be viewed as a major hindrance towards widespread implementation of UPLC (46).

3. The Regulation and Compliance Barriers

Regulatory and compliance issues often deter the migration from HPLC to UPLC. Apart from that, ever since most of the pharmacopoeial standards (USP, EP, JP) are based on HPLC methods, a very extensive justification and revalidation is required to switch to any UPLC-based methodologies (47). Initially, before switching to UPLC for routine QC, pharmaceutical companies were requested by such regulatory authorities as the FDA or EMA to demonstrate equivalency or superiority for UPLC in either checking quality or method for such use (43). Also, advanced software capabilities while meeting the complexity of compliance with data integrity regulations of electronic record-keeping, audit trails, and real-time monitoring to also report that compliance with 21 CFR Part 11 and GMP guidelines is required (34).

4. Future Perspectives: Advances in Chromatographic Technologies

This means that the next advance of chromatography in QC for biopharmaceuticals is through hyphenation, automation, and AI-based data analysis. The UPLC-MS (mass spectrometry), HPLC-MS/MS, and 2D liquid chromatography (2D-LC) systems are coming to market with greater analytical capacity for the characterization of biologics, particularly concerning their post-translational modifications, impurities, and degradation products (23). Among the topics of current research right now are the applications for artificial intelligence and machine learning optimizations in chromatography to minimize staff interaction and variability in method development (48). Furthermore, the growth of nano-LC and microfluidic chromatography technology will also provide excellent applications in ultra-sensitive biopharmaceutical analysis while consuming less sample and solvent (49).

5. Sustainability and Green Chromatography

Green chromatography is picking steam because of rising environmental concerns. It considers UPLC as it has lower solvent consumption and wastes generated by the process, thus already qualifying it as sustainable for analytical practices (50). Other innovations extend to supercritical fluid chromatography (SFC) and green solvents, which will help minimize the environmental impact of chromatographic analyses (51). As regulatory agencies continue to pressure for sustainable practices, in the next years much adoption will follow for these environmentally friendly chromatographic methods.

Conclusion

HPLC and UPLC play a prominent role in the quality control of biopharmaceuticals and biologics. These techniques ensure the safety, efficacy, and high quality of these complex therapeutic products. HPLC has built strong methodology and regulatory acceptance earning it the title "gold standard" in routine analyses for being quintessential. In contrast, UPLC utilizes higher resolution and greater sensitivity with faster analysis, thus emerging as the better tool for impurity detection, biomolecule characterization, and stability studies.

Nonetheless, the dominance of high cost instrumentation, the complexities regarding method transfer, and poor compliance with regulatory requirements have been a serious concern toward the widespread acceptance of UPLC technology in the realm of biopharmaceuticals quality control. Advances in hyphenated techniques, such as UPLC-MS or 2D-LC, combined with method optimization driven by artificial intelligence and those of green chromatography, will continue to revolutionize chromatography to be more productive, cheaper, and better for the environment.

Regulatory bodies will start redeveloping the pharmacopeial standards toward modernizing them in line with the new production techniques in chromatographic methods. Continuous method development, validation, as well as regulatory harmonization, is expected to pave ways into the establishment of cutting-edge chromatography technologies into the routine quality control process. HPLC and UPLC will still have a leading role in analytical quality assurance, thus contributing to the efforts made in the safe and effective development of biologic therapies for global healthcare needs as the biopharmaceutical industry goes forward into evolving strides.

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