North Clin Istanb 2025;12(6):739–752
doi: 10.14744/nci.2025.42966

REVIEW BIOPHYSICS

Different chromatographic techniques and recent advancements for biomedical and pharmaceutical applications

Ozlem Coskun,[1] Sama Akbarzadeh,[2,3] Basak Guncer[2]

[1]Department of Biophysics, Canakkale Onsekiz Mart University Faculty of Medicine, Canakkale, Turkiye
[2]Department of Biophysics, Istanbul University Istanbul Faculty of Medicine, Istanbul, Turkiye
[3]Istanbul University, Institute of Graduate Studies in Health Sciences, Istanbul, Turkiye

ABSTRACT

Chromatography remains a cornerstone analytical technique in pharmaceutical and biomedical sciences, with recent innovations significantly expanding its capabilities. Advances such as fast chromatography, two-dimensional liquid chromatography (2D-LC), supercritical fluid chromatography (SFC), and hyphenated techniques, including liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS), have broadened the scope of its applications. These developments enhance resolution, sensitivity, and efficiency, enabling more robust analysis of complex biological and pharmaceutical samples. These methods address complex analytical challenges, improving precision, speed, and efficiency in separating and analyzing biomolecules. Emerging technologies, including miniaturized liquid chromatography, shear flow chromatography, column arrays, and microfluidic chip-based systems, present exciting opportunities for the future. These developments enhance the capability of chromatography to analyze trace compounds, optimize drug formulations, and ensure the quality control of pharmaceuticals. Chromatography is also increasingly integrated with cutting-edge techniques like metabolomics and proteomics, furthering its impact on biomarker discovery and personalized medicine. This paper reviews recent advancements in chromatographic methods and their practical applications in the pharmaceutical and biomedical fields. It highlights the critical role of chromatography in drug discovery, purification of therapeutic compounds, and metabolite profiling.

Keywords: Biomedical analysis; biomarker discovery; chromatography; pharmaceutical applications; protein.

Cite this article as: *Coskun O, Akbarzadeh S, Guncer B. Different chromatographic techniques and recent advancements for biomedical and pharmaceutical applications. North Clin Istanb 2025;12(6):739–752.*

Chromatography is a versatile biophysical technique used for the separation, identification, and purification of compounds from complex mixtures, enabling both qualitative and quantitative analysis. In this technique, the separation of a compound into the desired shape, size, charge, and groups according to the binding specificity [1].

Chromatography distinguishes itself from classical separation techniques, such as crystallization, extraction, or distillation, by its ability to be effectively applied even when the exact composition of a mixture is unknown. However, selecting an appropriate chromatographic method necessitates a comprehensive understanding of the physicochemical properties of the compounds involved. The versatility

Received: October 30, 2025 *Accepted:* November 17, 2025 *Online:* December 11, 2025

Correspondence: Ozlem COSKUN, MD. Canakkale Onsekiz Mart Universitesi Tip Fakultesi, Biyofizik Anabilim Dali, Canakkale, Turkiye.
Tel: +90 532 581 54 41 e-mail: ozlemcoskun@comu.edu.tr

Istanbul Provincial Directorate of Health - Available online at www.northclinist.com

and superior resolving power of chromatographic systems have solidified its status as a premier analytical technique, extensively utilized in scientific, industrial, and medical contexts. It plays an integral role in the methodologies of numerous scientific studies and serves as a vital tool for environmental monitoring and the production of high-purity compounds in the pharmaceutical and chemical industries. Moreover, beyond its critical function in protein analysis, chromatography is essential for the separation and analysis of small-molecule drugs, which constitute a significant class of pharmaceutical compounds [2, 3].

The advantages of new chromatography methods are high efficiency, faster separation speed, high flow rate, increased detection sensitivity, and cost savings. Chromatography has many medical applications, such as pharmaceutical analysis, the food and beverage industry, the chemical industry, forensic medicine, and environmental science [4].

Chromatography's versatility and high resolving power have established it as a premier analytical technique, widely employed across scientific, industrial, and medical domains. It serves as a cornerstone in the methodologies of numerous scientific studies and is instrumental in environmental monitoring and the production of high-purity compounds in the pharmaceutical and chemical industries. In pharmaceutical applications, particularly during preclinical and clinical studies, various chromatographic methods are utilized to evaluate the bioactivity of pharmaceuticals—defined as the effect of a substance on living tissues or biological systems, encompassing efficacy, receptor binding, and metabolic impact. These methods include high-performance affinity chromatography (HPAC), cell membrane chromatography (CMC), mixed-mode chromatography (MMC), and high-performance liquid chromatography (HPLC), each offering unique advantages in analyzing bioactive compounds [5, 6].

The success of chromatography in developing fast and accurate analytical techniques offers greater specificity and sensitivity in drug research. In recent years, chromatography has attracted attention as a valid method for examining clinical or pharmaceutical samples and analyzing drug-protein binding [3, 5].

In recent years, chromatography has become one of the most important techniques used in clinical analysis to separate drugs according to their properties and interaction patterns, identify their metabolites, and determine their amount. Chromatographic methods are essential for early diagnosis of disease, evaluation of the course of the disease, monitoring of drug effects, and therapeutic intervention, where biological materials such as whole blood, serum, plasma, urine, stool, and tissue are tested during the diagnosis and treatment process in research and routine settings [7].

Highlight key points

- Chromatography, electrophoresis, and microfluidic chips enable the separation of specific analytes and the analysis of chemical compounds with high purification sensitivity.
- Techniques such as fast LC, 2D-LC, SFC, LC-MS, and GC-MS provide more information with increased productivity, reliability, robustness, resolution, speed, and sensitivity.
- Chromatographic methods play a fundamental role in biotechnology and biopharmaceutical analysis by in-depth investigations of protein structure and function, enabling the design of separation methods tailored to the target protein.
- Microfluidic chips offer a new research platform for the miniaturization, integration, and portability of analytical devices, simplifying analysis processes.
- Three-dimensional chromatographic separation has the potential to revolutionize various industries by offering high efficiency and resolution for the separation of complex samples.

The adverse effects of environmental chemical pollutants on quality of life and their contribution to the incidence and development of life-threatening chronic diseases are well established. However, the routes of exposure to these chemicals and their roles in chronic diseases are still unclear. Environmental pollutants enter the human body through various routes and are transported to target organs through blood. The risks of human exposure to these substances and the relationship between chemical pollutants and chronic diseases are extensively studied by monitoring them with biomarkers in plasma or serum samples. Chromatography is a widely utilized technique in clinical laboratories for diagnosing inborn errors of carbohydrate, protein, and lipid metabolism. It facilitates the measurement of key parameters, including vitamins, hormones, metabolites, tumor markers, and drugs in biological fluids. Additionally, chromatography enables the analysis of environmental samples for drugs, toxins, and pollutants, contributing to the discovery of novel biomolecules and providing valuable insights into disease mechanisms and biomarker identification [8, 9].

In this review, we aim to provide information about the basic principles of chromatography and emphasize the importance of new chromatographic techniques applied in pharmaceutical and clinical analysis based on current literature.

Table 1. Classification of chromatography

Classification basis	Type	Subtype/description
Based on the interaction of solute to the stationary phase	Adsorption Chromatography	Based on surface adsorption (e.g., silica gel in TLC)
	Ion Exchange Chromatography (IEC)	Ion-exchange chromatography facilitates the separation of analytes by leveraging their electrostatic interactions with a charged stationary phase.
	Partition Chromatography	Separation via solute partitioning between two phases
	Size Exclusion Chromatography (SEC)	SEC separates molecules, such as proteins and polymers, by size using porous beads as the stationary phase.
Based on chromatographic bed shape	Two Dimensional	Thin Layer Chromatography (TLC), Paper Chromatography
	Three Dimensional	Column Chromatography (used in most HPLC applications)
Chromatographic methods are classified by the mobile phase's physical state	Liquid Chromatography (LC)	Widely used in HPLC and bio-separations
	Super Critical Fluid Chromatography (SFC)	Combines properties of gas and liquid phases for efficient separations
	Gas Chromatography (GC)	Used for volatile compounds and environmental samples

CLASSIFICATION OF CHROMATOGRAPHY

The separation of proteins, which are fundamental to vital biological processes, from biomaterials is critical for investigating their structures and functions. Biochemical transitions from a normal to a pathological state in living systems are often associated with health issues, particularly through molecular, structural, and functional alterations in proteins that drive the onset and progression of various diseases. Owing to these alterations, proteins represent valuable biomarkers for disease screening, early diagnosis, and the determination of disease type and stage, as well as for elucidating the molecular mechanisms underlying the transition from physiological to pathological states. Protein separation and purification are generally accomplished through one or more chromatographic steps. These processes rely on the differential retention times of proteins within the chromatographic column, which result from distinct interactions between proteins and the column's stationary phase materials. Chromatographic techniques are classified based on various principles, including the nature of solute-stationary phase interactions, the physical state of the mobile phase, and the configuration of the chromatographic bed (Table 1) [10, 11].

Specifically, chromatography methods can be classified into several modes based on their separation mechanisms. HPLC has various subtypes, such as Ion Exchange Chromatography (IEC), Hydrophobic Interaction Chromatography (HIC), Affinity Chromatography, Size Exclusion Chromatography (SEC), and Reverse Phase Chromatography (RP-HPLC), each employing distinct physicochemical interactions between the analyte and stationary phase. Additionally, non-HPLC-based techniques such as Gel Chromatography, Hydroxyapatite Chromatography (HAC), and Supercritical Fluid Chromatography (SFC) are used in specific contexts. Beyond these established chromatographic techniques, modern and specialized chromatography methods such as Paper Chromatography Hybridization Assay (PACHA) [12], Optical force chromatography (OFC) [13], High-performance and Immunoaffinity chromatography [14], Mixed mode chromatography [15], and Dye ligand chromatography [16] are gaining importance. With the further development of biotechnology and in-depth studies of the structure and function of proteins, protein separation and purification technology is also developing rapidly.

HIGH-PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is the most accurate analytical method and is widely used for both quantitative and qualitative analysis of drug products and for determining drug product stability. HPLC method is important for separating and measuring the parent drug and reaction impurities [17].

HPLC is highly practical for both laboratory and clinical applications, delivering accurate and precise results with enhanced specificity. Extensive research and numerous patents have validated the versatility of HPLC across various domains within the healthcare sector, opening up numerous promising opportunities for this analytical technique [18].

The use of biological samples such as serum, plasma, cerebrospinal fluid (CSF), breast aspirate, saliva, tear samples, tissue homogenates, and cellular lysates in the HPLC-protein separation method provides a great opportunity for comparison and correlation of markers observed in these different systems for a single disease. It also helps provide additional information about various biochemical processes in the biological system. Using the "Reverse Phase" HPLC technique is generally advantageous in separating minimal amounts of proteins. In any abnormal condition, there is a change in the expression levels of proteins depending on the ongoing biochemical processes [19].

In the field of medicine, HPLC has various applications, such as the analysis of antibiotics, endogenous neuropeptide detection in brain extracellular fluids, drug stability studies, quality control, and the evaluation of pharmaceutical products' shelf life [20].

HPLC analysis of pharmaceutical products is used to determine the amount of drug in biological samples, confirm the drug's identity, and provide quantitative results [21].

Various chromatographic methods, notably reverse-phase (RP) chromatography, are under investigation for the separation of neurotransmitters (NTs) and their metabolites. High-Performance Liquid Chromatography (HPLC) coupled with intracerebral microdialysis and electrochemical detection (ECD) is widely employed to study brain serotonergic and dopaminergic systems. As a prominent separation technique in NT analysis, HPLC, particularly under reverse-phase conditions, delivers rapid and selective results. The integration of HPLC with ECD offers a robust approach for elucidating the dynamics of NTs and their metabolites, advancing neuroscience research and facilitating potential clinical applications. Ongoing research into HPLC-ECD systems provides promising opportunities for enhancing the sensitivity and specificity of neurotransmitter detection [22].

Significant advancements in classical High-Performance Liquid Chromatography (HPLC) have led to the development of innovative techniques, including Rapid Resolution Liquid Chromatography (RRLC), Ultra Performance Liquid Chromatography (UHPLC), Ultra Fast Liquid Chromatography (UFLC), and Nano Liquid Chromatography (Nano LC). These modern approaches enhance analytical performance by improving resolution, speed, and sensitivity, thereby expanding the applicability of HPLC in various scientific and industrial contexts.

Rapid Resolution Liquid Chromatography (RRLC)

RRLC is not a distinct type of chromatography but rather a refinement of the classical HPLC technique. It employs shorter chromatographic columns and stationary phases packed with smaller particle sizes (typically 1.8–3 μm) to allow for faster separation without compromising resolution. These modifications improve efficiency, reproducibility, and peak capacity while significantly reducing analysis time and solvent consumption [23].

This system has become widely adopted in the pharmaceutical industry to provide maximum resolution and rapidity of testing. Increased reproducibility and sensitivity are achieved during analysis with the RRLC method. High detection speed and reduced analysis cost make this method valuable in the quality control of herbal medicines. RRLC has the advantage of being faster chromatography and having high resolving power compared to HPLC [24].

Nano Liquid Chromatography (NLC)

Nano-liquid chromatography can be defined in various ways depending on the flow rate of the mobile phase and the column diameter. Here, the flow rate of the mobile phase is nano ml/min. NLC is a faster and cheaper technique than HPLC, producing less waste due to reduced mobile phase consumption. It helps reduce the sample requirement and increase the sensitivity. It has been found that the resolution power increases when analyzing complex samples. It also has higher efficiency [25]. Although NLC is particularly useful for small molecules due to its high sensitivity and low sample volume, it is also widely applied in the separation and quantification of peptides and proteins, especially in proteomics and biomarker research [26].

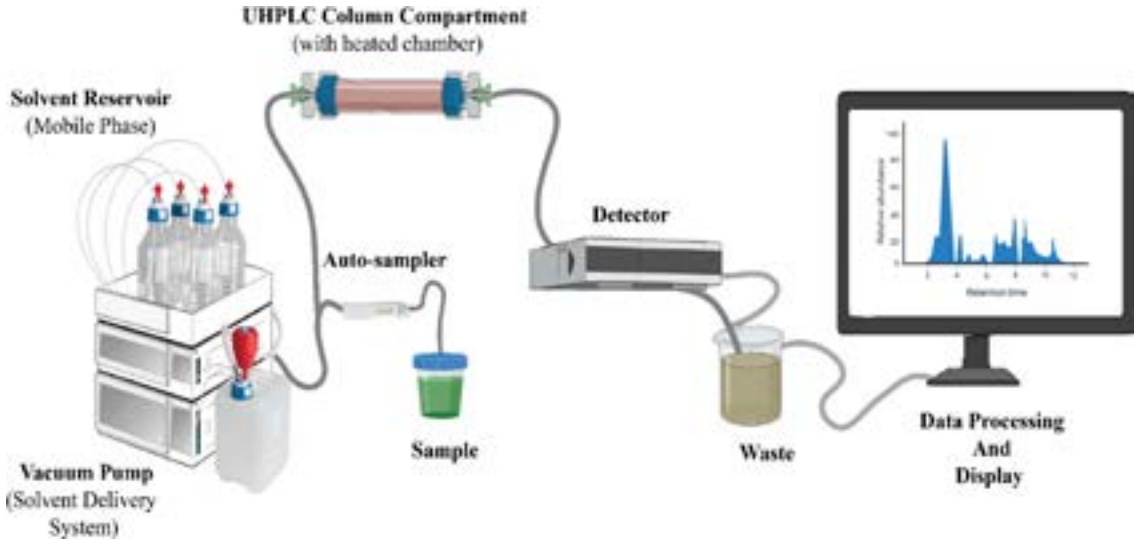

FIGURE 1. Schematic representation of UHPLC [31].

UHPLC: Ultra performance liquid chromatography.

Ultra-High Performance Liquid Chromatography (UHPLC)

Ultra-High-Performance Liquid Chromatography (UHPLC) has gained significant traction in recent years due to its ability to achieve rapid separation of small molecules with high resolution. This technique is widely employed for the analytical separation of components in multicomponent mixtures, finding applications across diverse fields, including chemistry, pharmaceuticals, food science, and biochemistry. UHPLC serves as a critical tool in both research and industrial settings, enabling high-quality separations without compromising analytical performance. Its superior resolution has facilitated the development of novel analytical procedures, building on existing methods and reducing the need for reanalysis, thereby marking a significant advancement in liquid chromatography. In the pharmaceutical domain, UHPLC is particularly vital for the analysis of drugs, which play a central role in medical practice. Rational prescribing, grounded in clinical pharmacology, is essential to ensure that the appropriate therapeutic agents are administered to patients at the optimal time, enhancing treatment efficacy and safety [27]. Recent advancements in analytical tools have significantly enhanced the discovery and monitoring of new pharmaceuticals. The development of rapid chromatographic methods has become increasingly vital for analytical laboratories. Notable technological progress has been achieved through improvements in detector design, optimization of data processing systems, and refinements in chromatographic techniques. Ultra-High-Performance Liquid Chromatography (UHPLC), which builds upon the foundational principles of High-Performance Liquid Chromatography (HPLC), delivers exceptional performance, offering enhanced resolution, speed, and sensitivity for pharmaceutical analysis (Fig. 1). While UHPLC shows a dramatic increase in speed, resolution, and analysis sensitivity as well as analysis sensitivity by having a particle size of less than 2 pm and operating the system at higher pressure, the mobile phase can operate at higher linear speeds compared to HPLC. In addition, the analysis time and solvent consumed in UHPLC are less than those of all previously used chromatographic techniques. This technique is considered a new focus in liquid chromatographic studies. Therefore, it has been shown in the literature that an existing HPLC method can be developed and replaced by the UHPLC method in the pharmaceutical industry. It has been demonstrated that UHPLC is an important and meaningful tool for enhancing the quality of pharmaceutical analyses and the efficiency of researchers [28–30].

The only disadvantage of UHPLC may be the high back pressure, which can be reduced by increasing the column temperature. The UHPLC technique is generally acceptable and significantly improves speed, sensitivity, and resolution compared to conventional HPLC [32]. In conclusion, the UHPLC technique is a technique that provides ultra-fast analysis without affecting analytical reliability and sensitivity, with a speed approximately ten times higher than conventional liquid chromatographic methods applied in the analysis of pharmaceutical substances [33].

The literature reports a wide range of applications for Ultra-High-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UHPLC-MS/MS) in the analysis of various drug classes, including antidiabetic, anticancer, antibiotic, cardiovascular, antiviral, nonsteroidal anti-inflammatory drugs (NSAIDs), and others. Recent studies demonstrate that UHPLC-MS/MS effectively separates drugs and their metabolites from impurities and degradation products. This technique provides a robust platform for the isolation, characterization, and identification of degradation products and impurities, yielding critical insights into drug properties. Such information supports optimized storage conditions, enhances quality control methodologies, and contributes to the development of safer therapeutic treatments [34].

SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC)

SFC provides a cost-effective and rapid approach for the purification of small proteins and pharmaceutical compounds. Thavendran Govender and colleagues developed an SFC-based method for purifying biosynthesized human insulin, thereby establishing a scalable process suitable for large-scale production. Their findings demonstrated that insulin samples retained full biological activity following SFC purification, confirming the method's effectiveness in producing high-purity, biologically active compounds [35–37]. SFC offers distinct advantages in kinetic performance and serves as a complementary technique to liquid chromatography (LC), making it highly effective for pharmaceutical analysis. Preparative chromatography represents a pivotal separation approach in the pharmaceutical industry, particularly during the early stages of drug development when larger quantities of material are required. Selecting the most cost-efficient method to obtain sufficient amounts of target compounds is therefore essential. In this context, SFC has emerged as a promising strategy for the analysis of drugs within formulations and biological matrices [38, 39]. Modern SFC instruments, complementary to HPLC, are a widely applicable technique with robust and high-resolution properties [40, 41]. Recent technological advances have resolved the known limitations of SFC, such as poor UV sensitivity, limited reliability, and poor quantitative performance, with the introduction of new generation instruments and columns from major chromatographic instrumentation providers. Ultra-high Performance Supercritical Fluid Chromatography (UHPSFC) has emerged as an advanced form of SFC, offering enhanced resolution, faster analysis, and improved reproducibility due to the use of smaller particle columns under higher pressures [42].

HYDROPHOBIC INTERACTION CHROMATOGRAPHY (HIC)

HIC is a separation technique employed to isolate proteins and polypeptides based on differences in surface hydrophobicity. This method enables the purification of biologically active proteins without compromising their native conformation. In HIC, the sample is applied to a hydrophobic stationary phase in the presence of a high-concentration salt solution, promoting adsorption through hydrophobic interactions under conditions of elevated ionic strength. Subsequently, selective desorption is achieved by gradually or linearly decreasing the ionic strength of the mobile phase. Weakly hydrophobic molecules are eluted at higher ionic strengths, whereas strongly hydrophobic molecules are eluted as the ionic strength diminishes [43, 44].

TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY (2D-LC)

Comprehensive two-dimensional liquid chromatography (LC×LC) is a highly effective technique for the separation of complex samples, offering significant advantages over traditional one-dimensional liquid chromatography (1D-LC). By leveraging orthogonal selectivities in two separation dimensions, LC×LC, particularly when coupled with mass spectrometry (LC×LC-MS), achieves high throughput and enhanced resolution. The increasing popularity of two-dimensional liquid chromatography (2D-LC) stems from its successful applications, such as the discovery of novel biopharmaceuticals. LC×LC is particularly valuable for analyzing complex samples containing non-volatile analytes or matrix compounds, providing superior capacity and selectivity compared to 1D-LC. When the two separation dimensions are carefully selected, LC×LC delivers reliable and reproducible results, enabling rapid and efficient comparative analysis, for instance, in the characterization of natural drugs. Despite its complexity, ongoing improvements in robustness and reliability are expected to further enhance the utility of 2D-LC in analytical applications [45, 46].

TABLE 2. Applications of multidimensional chromatography in pharmaceutical and clinical fields

Field	Sample types	Applications	Techniques	Ref
Clinical pharmacology	Human plasma, serum, CSF	Quantification of drugs and metabolites; therapeutic drug monitoring	LC-MS/MS, 2D-LC	[51]
Pharmaceutical research	Cell lysates, tissues	Drug discovery; identification of active metabolites	LC-MS, MMC, 3D-LC	[52]
Toxicology	Plasma, urine	Detection of toxins and pharmaceuticals in overdose or exposure	LC-GC, LC-MS/MS	[53]
Endocrinology	Serum, plasma	Analysis of hormones and hormone-like drugs	LC-MS/MS, 2D-HPLC	[54]
Biomarker discovery	Biological fluids, tissues	Identification and quantification of disease-specific markers	2D-LC, LC-MS, GC-MS	[55, 56]
Oncology	Tumor tissue, blood,	Targeted therapy development; pharmacodynamics and proteomics	LC-MS/MS, 3D-LC	[57]

LC-MS/MS: Liquid chromatography–mass spectrometry/mass spectrometry; 2D-LC: 2-Dimensional liquid chromatography; 3D-LC: 3-Dimensional liquid chromatography; LC-GC: Liquid chromatography–gas chromatography; MMC: Mixed-mode chromatography; 2D-HPLC: 2-Dimensional high-performance liquid chromatography; GC-MS: Gas chromatography–mass spectrometry.

MULTIDIMENSIONAL CHROMATOGRAPHY

Given the complexity of samples comprising diverse components and the inherent limitations in the separation efficiency of single-column chromatography, multidimensional chromatography—integrating multiple techniques with orthogonal separation principles and varying affinities—has garnered substantial interest as a solution. This approach synergistically combines chromatographic methods, thereby reducing analysis time while enhancing overall efficiency, sensitivity, and accuracy. However, solvent system incompatibility remains a primary challenge in the implementation of two-dimensional (2D) chromatography. Due to the intricate nature of analytes and their low concentrations within biological matrices, multidimensional chromatography finds extensive application in the biomedical and pharmaceutical industries (Table 2). The analysis of complex compounds requires analytical systems that are highly reliable, selective, and precise. Recent studies indicate that mixed-mode chromatography (MMC), which provides superior separation efficiency for proteins compared to single-mode techniques and alleviates mobile phase compatibility issues, holds substantial promise for improving analytical performance and achieving more robust separation outcomes [47–49].

RECENT DEVELOPMENTS IN THE QUANTITATIVE ANALYSIS OF THERAPEUTIC PROTEINS IN BIOLOGICAL MATRICES

The inherent complexity of therapeutic proteins, characterized by their intricate molecular structures and the presence of diverse active substances within complex biological matrices, presents substantial challenges to the development of analytical methods that achieve high specificity, sensitivity, accuracy, and robustness. The creation of reliable and precise quantitative assays for these sophisticated protein-based therapeutics in biological fluids, such as plasma or serum, continues to be a pivotal and formidable obstacle in advancing biopharmaceutical research and development [50].

Pharmaceutical applications of therapeutic proteins in treating many diseases are continuously being developed. Although various analytical methods are available for quantifying biofluid drugs, no ideal or universal method can be applied to various therapeutic proteins or all conditions. Efficient and reliable bioanalytical methods are required to accelerate therapeutic protein identification and successful clinical development. Selective quantitative analyses, especially in high-throughput formats, are critical to meet regulatory requirements for pharmacokinetic and pharmacodynamic evaluation

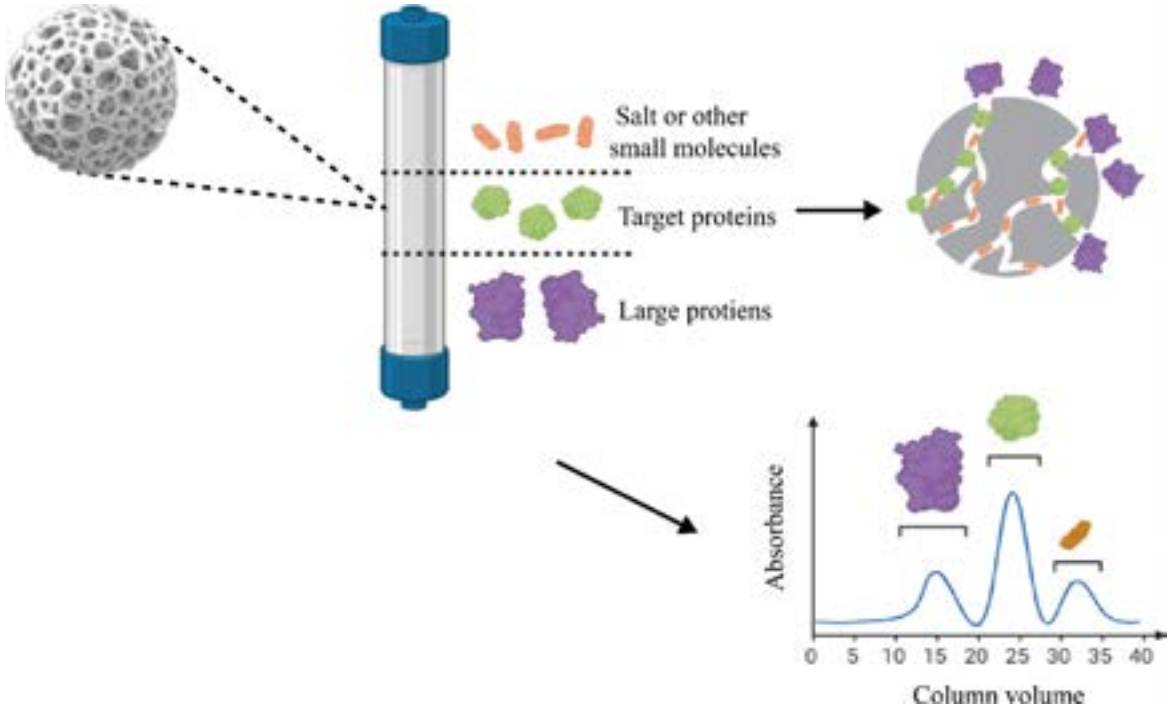

FIGURE 2. Size exclusion chromatography (SEC) process [63].

of drugs for new drug approval. However, although it is not a standard approach, Liquid Chromatography-Mass Spectrometry (LC-MS/MS) is often the preferred method for identifying and quantitatively analyzing therapeutic proteins in complex biological samples due to its high sensitivity, specificity, and efficiency. In recent years, the devices have become highly developed, automated, and equipped with high-level information technology, allowing high throughput, automatic sample analysis, data processing, and storage [58].

Routine measurement of cancer biomarkers contributes significantly to better clinical outcomes for patients regarding early diagnosis, risk stratification, and treatment monitoring, among other applications. Protein tumor markers are important because proteins carry out most of the biological processes and thus dynamically reflect changes in cancer pathophysiology. Mass spectrometry-based targeted proteomics is a powerful tool for absolute peptide and protein quantification in biological matrices and has numerous advantages in clinical applications in oncology. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based methodologies allows laboratories to overcome the difficulties associated with more commonly used immunoassays for tumor marker measurement [59, 60].

SIZE EXCLUSION CHROMATOGRAPHY (SEC)

Size Exclusion Chromatography (SEC) is widely used in the qualitative and quantitative characterization of therapeutic proteins (Fig. 2). The primary advantage of this technique lies in the suitability of its mobile phase conditions for characterizing proteins. Due to their distinct physicochemical properties, therapeutic proteins are susceptible to various modifications—such as oxidation, deamidation, glycosylation, aggregation, misfolding, and adsorption—during preparation, formulation, or storage. The clinical application of therapeutic proteins has enabled the treatment of numerous life-threatening conditions. Hundreds of therapeutic proteins are currently under clinical evaluation for the treatment of various diseases, including genetic disorders, cancer, and infectious diseases [61].

In this context, the characterization of protein modifications requires a range of analytical methods because a single technique does not allow the evaluation of all necessary parameters. Protein aggregates are usually analyzed by light scattering-based methods such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), size exclusion liquid chromatography (SEC-HPLC), asymmetric area flow fractionation (AF4), fluorescence spectroscopy, circu-

lar dichroism (CD) or multi-angle laser light scattering (MALLS). Among these techniques, SEC-HPLC is currently used as the standard separation technique for quantifying protein dimers, trimers, and oligomers. The main advantage of this approach is the mild elution conditions that allow the characterization of the protein by identifying the conformational structure. As a result, significant advances have been made through SEC approaches that provide a new level of chromatographic performance or efficiency [62].

AFFINITY CHROMATOGRAPHY (AC)

Affinity Chromatography (AC) is a specialized form of liquid chromatography that exploits the specific and reversible binding interactions between biological macromolecules and their corresponding ligands. In this technique, a ligand is immobilized on a stationary matrix, enabling the selective separation of a target protein based on the specificity of its interaction with the ligand. For instance, Immobilized Metal Affinity Chromatography (IMAC) has been effectively utilized to purify angiotensin-converting enzyme (ACE) inhibitor peptides from casein hydrolysates. AC offers several advantages, including high selectivity, robust stability, cost-effectiveness, and excellent reproducibility. However, protein isolation methods, which rely on subtle differences in the physicochemical properties of macromolecules, often face challenges such as prolonged processing times and low yields, resulting in difficulties achieving high-purity proteins [64–66].

AC is an increasingly vital separation technique in the analysis of biological samples and pharmaceutical agents. By employing a biologically relevant stationary phase, AC enables the selective retention of analytes and the investigation of biomolecular interactions. This method can be applied independently or in combination with complementary techniques, such as reversed-phase High-Performance Liquid Chromatography (HPLC) or mass spectrometry. Given its numerous recent applications, AC is poised to gain further prominence as a critical tool for the separation and analysis of biological and pharmaceutical agents in complex matrices [67, 68]. AC is a versatile analytical technique widely applied across diverse fields, from investigating drug–protein binding interactions to improving the detection and quantification of low-abundance proteins. It plays a pivotal role in elucidating the kinetic properties of drug–protein interactions and in identifying specific drug-binding sites, as reported in numerous studies. Moreover, AC has been extensively utilized in omics research—including proteomics, metabolomics, and genomics—where it contributes to the advancement of high-throughput screening platforms for potential therapeutic agents, particularly when integrated with complementary analytical methodologies [69, 70].

MICROFLUIDICS-BASED CHROMATOGRAPHY

Liquid chromatographic chips are increasingly gaining prominence in research and applications due to their substantial advantages over conventional, larger-scale analytical systems. These advantages include minimal requirements for sample and reagent volumes, rapid and cost-efficient analysis, elimination of dead volume in connections, and the capacity for multiplex measurements, making them a highly effective tool for advanced analytical applications (Fig. 3). Liquid chromatographic (LC) chips and conventional chromatographic systems, such as High-Performance Liquid Chromatography (HPLC) and Ultra-High-Performance Liquid Chromatography (UHPLC), share theoretical applicability across similar domains. However, as LC chip technology continues to evolve, research primarily focuses on validating methodological concepts, including particle design, packaging processes, chip architecture, pumping systems, hyphenation techniques, and detection methods. The advent of microfluidic chip-based LC-MS systems has significantly advanced proteomics research by enabling high-throughput analysis of biological macromolecules, such as peptides, proteins, and glycans, in diverse biological matrices, as well as small molecules, including pharmaceutical compounds, drugs, and their metabolites, in applications such as tumor and disease analysis. LC chip-MS systems are poised for widespread adoption due to their efficiency in analyzing small-volume samples. Ongoing development efforts focus on critical areas such as micropackaging preparation, sample injection performance, detection sensitivity, parallelization, and hyphenation with other analytical platforms. Despite the diverse developmental trajectories of LC chip technology, it is evident that future efforts will prioritize significant miniaturization of analytical devices, further enhancing their utility in analytical sciences [71–73].

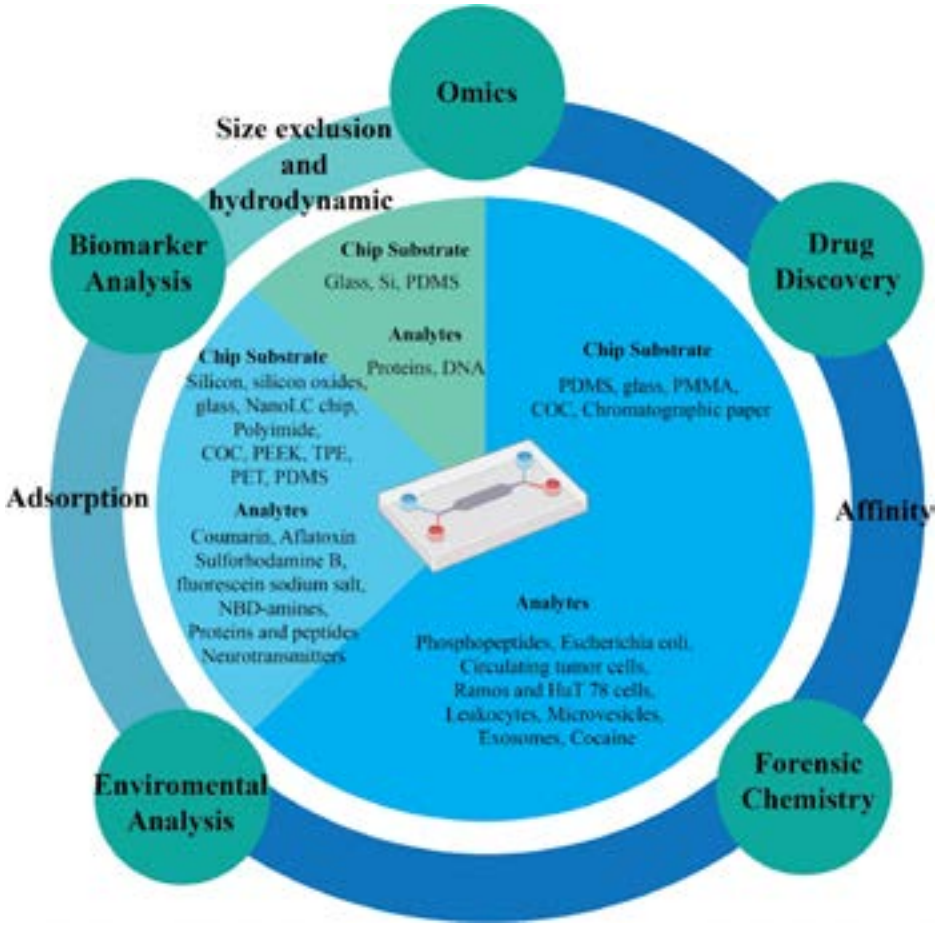

FIGURE 3. The applications and separation mechanisms of microfluidics-based liquid chromatography [74].

PDMS: Polydimethylsiloxane; PMMA: Polymethyl methacrylate; COC: Cyclic olefin copolymer; PEEK: Polyether ether ketone; PET: Polyethylene terephthalate; TPE: Thermoplastic Elastomer; PDMS: Polydimethylsiloxane; Si: Silicon.

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC)

High-performance thin-layer chromatography (HPTLC) is one of the most widely applied methods for analysis in pharmaceutical industries, clinical chemistry, forensic chemistry, biochemistry, cosmetology, food and drug analysis, environmental analysis, etc. Due to its many advantages, it is the only chromatographic method that offers the option of presenting the results as images, for example. HPTLC is one of the advanced instrumental techniques based on all the features of thin-layer chromatography (TLC). Its advantages, such as automation, screening, complete optimization, selective detection principle, minimum sample preparation, etc., make it a powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples, foodstuffs, and other substances. Other advantages include simplicity, low cost, parallel analysis of samples, high sample capacity, fast results, and multiple detection. Many reports on clinical medicine studies have already been published in many journals. The use of HPTLC is now highly recommended for the analysis of drugs in serum and other tissues [75–77].

IMMOBILIZED ARTIFICIAL MEMBRANE CHROMATOGRAPHY (IAM)

The development of immobilized artificial membrane (IAM) chromatography has opened up new perspectives for using chromatographic techniques in drug discovery by combining the simulation of the cell membrane environment with rapid measurements. Studies are being conducted on the potential of IAM chromatography to model permeation through significant physiological barriers and drug-membrane interactions. Other applications are also

being investigated to calculate complex pharmacokinetic properties related to tissue binding and to predict cell accumulation/retention. The unstable nature of IAM chromatography as a boundary condition between passive diffusion and binding defines its potential applications. However, despite its successful performance in many drug-membrane interaction studies, IAM is still used as a supporting technique rather than a stand-alone technique. To have a more focused and consistent application in drug discovery, further studies considering different biological processes are still needed to examine IAM chromatography efficiency [78]. The primary goal is to purify and extract one or more components of a sample for the analytical purpose of chromatography to determine the sample's qualitative and quantitative chemical structure [79].

MAGNETO CHROMATOGRAPHY (MC)

The advent and progress of nanotechnology have driven remarkable advancements across various scientific disciplines. In contrast to conventional magnetic separation techniques, magnetic or magneto-chromatography (MC) systems have emerged as a powerful method, garnering significant attention. MC employs an external magnetic field to separate particles based on their size or to distinguish cations, anions, and various compounds with differing magnetic susceptibilities. Additionally, MC can be adapted to separate diamagnetic compounds through strategies such as complexation with metal ions, reactions with metal complexes, or binding to magnetic or magnetized particles. The external magnetic field is critical to the separation process. MC systems offer distinct advantages, enabling the effective separation of nanoparticles and diverse compounds with varying magnetic susceptibilities [80, 81].

CHROMATOGRAPHIC TECHNIQUES USED SIMULTANEOUSLY IN DIAGNOSIS

The application of chromatographic techniques in clinical diagnosis is highly successful. Bioanalytical techniques such as GC-MS and gas chromatography-thermal conductivity are widely used to diagnose diseases. All these techniques are noninvasive and have great potential in the medical field and the development of medical biomedical analytical methods. Bioanalytical tools such as chromatography, spectroscopy, and immunological analysis are used in the diagnosis of diseases such as AIDS, hepatitis, and acute respiratory disorders. Furthermore, hybrid techniques such as LC-NMR, LC-MS/MS, GC-MS/MS, etc., can be used in medicine and forensic sciences in the near future [82, 83]. Due to new tools and improved bioinformatics, screening complex biological samples such as exhaled air, tissue, blood, and urine has become increasingly popular in identifying biomarkers in different types of cancer. However, despite some progress, identifying biomarkers has proven to be challenging due to the small number of new biomarkers (except for recent genetic markers) considered for introduction into clinical analysis. Recent developments in gas chromatographic methods for identifying biomarkers in cancer detection, diagnosis, and treatment offer scope for the Gas Chromatography (GC) method. Future studies may focus on different types of body fluids, such as ascitic fluid in ovarian cancer, secretions in pancreatic cancer, or pleural fluid in lung cancer. So far, two multidimensional analytical tools, GC-MS, have been predominantly used in cancer studies. Advancements in high-resolution gas chromatography (HRGC), coupled with developments in mass spectrometry (MS), have enabled the application of advanced techniques such as GC-MS-MS (tandem mass spectrometry), MDGC-MS (multidimensional gas chromatography with mass spectrometry), and GC×GC-ToFMS (comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry) for monitoring metabolite changes in complex biochemical processes associated with cancer cells. These multidimensional gas chromatography (MDGC) separation techniques, leveraging enhanced separation capabilities through coupled GC column methods, facilitate improved detection and identification of metabolites, thereby contributing to the discovery of novel cancer biomarkers [84, 85].

CONCLUSION

Chromatographic methods are indispensable for researchers, enabling the selective separation, identification, and analysis of specific analytes, including elements and chemical compounds. Techniques such as chromatography, electrophoresis, and microfluidic chips exhibit exceptional purification sensitivity. Advances in biotechnology, coupled with in-depth studies of protein structure and function, have driven rapid progress in separation and purification technologies. Chromatography is widely recognized as a highly sensitive and effective separation method, enhancing analytical efficiency through improved productivity, reliability, robustness, resolution, speed, and sensitivity. Its continued prominence in bio-

medical and biopharmaceutical analysis is assured, yet achieving optimal purification often necessitates the integration of multiple techniques, as a single method is typically insufficient. A variety of chromatographic techniques—including method development software, fast liquid chromatography (LC), two-dimensional liquid chromatography (2D-LC), supercritical fluid chromatography (SFC), liquid chromatography–mass spectrometry (LC–MS), gas chromatography (GC), and gas chromatography–mass spectrometry (GC–MS)—are routinely employed to overcome analytical challenges associated with complex biomolecules. Moreover, the design of customized separation strategies based on the physicochemical characteristics of the target protein is of critical importance. Microfluidic chip technology has recently emerged as a promising platform, offering substantial potential for the miniaturization, integration, and portability of advanced analytical systems.

The new chromatographic approaches have high efficiency and sensitivity, increased productivity and resolution speed, and less time consumption. In the future, three-dimensional chromatographic separation will be possible, which will be helpful in various industries. Therefore, multidimensional chromatography is quite interesting for separating complex samples.

Furthermore, in the field of medical sciences, there has been increasing interest in molecular-level identification to improve rapid and sensitive detection in early diagnosis. The gradual application of chromatography technology in clinical applications has led to remarkable advances in health and medicine, a vital part of systems biology.

To achieve tremendous success in the pharmaceutical industry and biomedical sciences, advances in these techniques are necessary to drive major and modern developments in impurity analysis.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: The authors declare that no AI-assisted tools, such as large language models (e.g., ChatGPT), were used in the preparation of this article on advancements and applications of chromatographic techniques.

Authorship Contributions: Concept – OC; Design – OC, SA; Supervision – OC, SA, BG; Data collection and/or processing – OC, SA, BG; Analysis and/or interpretation –OC, SA, BG; Literature search – OC, SA; Writing – OC, SA; Critical review – OC, SA, BG.

Peer-review: Externally peer-reviewed.

REFERENCES

1. Kumari VC, Patil SM, Ramu R, Shirahatti PS, Kumar N, Sowmya BP, et al. Chromatographic techniques: types, principles, and applications. In: Egbuna C, Patrick Iwuanyanwu KC, Shah MA, Ifemeje JC, Rasul A, editors. Analytical Techniques in Biosciences. 5th ed. Holland: Elsevier; 2022. p. 73-101. [Crossref]
2. Debnath S, Das M, Mondal S, Sarkar BK, Babu G. Advances in chromatography: contemporary techniques and applications. Essential Chem 2025;2:1-27. [Crossref]
3. D'Atri V, Fekete S, Clarke A, Veuthey JL, Guillarme D. Recent Advances in Chromatography for Pharmaceutical Analysis. Anal Chem 2019;91:210-39. [Crossref]
4. Gulhane CA, Fuladi OA, Bakal RL, Manwar JV. Recent advances in various chromatographic techniques used for analysis of drugs in pharmaceutical products: A review. GSC Biol Pharm Sci 2022; 19:288-95. [Crossref]
5. Parys W, Dołowy M, Pyka-Pająk A. Significance of chromatographic techniques in pharmaceutical analysis. Processes 2022;10:172. [Crossref]
6. Ma W, Wang C, Liu R, Wang N, Lv Y, Dai B, He L. Advances in cell membrane chromatography. J Chromatogr A 2021;1639:461916. [Crossref]
7. Rajput JM, Nandre DS Pawar BGA. Comprehensive review on advanced chromatographic techniques and spectroscopic techniques in pharmaceutical analysis. Int J Pharm Res Appl 2022;7:53-62.
8. Zhang Y, Gao Y, Liu QS, Zhou Q, Jiang G. Chemical contaminants in blood and their implications in chronic diseases. J Hazard Mater 2024;466:133511. [Crossref]
9. Hajnajafi K, Iqbal MA. Mass-spectrometry based metabolomics: an overview of workflows, strategies, data analysis and applications. Proteome Sci 2025;23:5. [Crossref]
10. Liu S, Li Z, Yu B, Wang S, Shen Y, Cong H. Recent advances on protein separation and purification methods. Adv Colloid Interface Sci 2020;284:102254. [Crossref]
11. Kumar Barik A, Mathew C, Sanoop PM, John RV, Adigal SS, Bhat S, et al. Protein profile pattern analysis: A multifarious, in vitro diagnosis technique for universal screening. J Chromatogr B Analyt Technol Biomed Life Sci 2024;1232:123944. [Crossref]
12. Dhull P, Dunuweera S, Bietsch J, Bandu R, Wannere C, Achanta S, Senanayake C. Recent advances and application of liquid chromatography in pharmaceutical industry. J Liq Chromatogr Relat Technol 2025;48:1-20. [Crossref]
13. Tsopelas F, Stergiopoulos C, Danias P, Tsantili-Kakoulidou A. Biomimetic separations in chemistry and life sciences. Microchimica Acta 2025;192:133. [Crossref]
14. Akash MSH, Rehman K. Comprehensive Insights Into Chromatography and Chromatographic Techniques. Essentials of Pharmaceutical Analysis. Singapore: Springer Nature Singapore; 2025. p. 533-75. [Crossref]
15. Wan QH. Mixed-Mode Chromatography: Principles, Methods, and Applications. 1st. ed. Singapore: Springer Singapore; 2021. p. 423-82. [Crossref]
16. Siddiqui MR, Othman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. Arab J Chem 2017;10:S1409-21. [Crossref]
17. Ali AH. High-performance liquid chromatography (HPLC): A review. Ann Adv Chem 2022;6:10-20. [Crossref]
18. Bhati C, Minocha N, Purohit D, Kumar S, Makhija M, Saini S. High performance liquid chromatography: recent patents and advancement. Biomed Pharmacol J 2022;15:729-46. [Crossref]

19. Thomas SL, Thacker JB, Schug KA, Maráková, K. Sample preparation and fractionation techniques for intact proteins for mass spectrometric analysis. J Sep Sci 2021;44:211-46. [Crossref]
20. Elnekaib MA, Musa KA, Almdaaf MA. The role of High-Performance Liquid Chromatography in the pharmaceutical analysis. Med J Pharm Pharm Sci 2025;5:57-62.
21. Pawar SV, Gaware VM. A Review on: Bioanayltical Method Development and Validation. Asian J Pharm Res Dev 2025;13:167-79.
22. Guiard BP, Gotti G. The high-precision liquid chromatography with electrochemical detection (HPLC-ECD) for monoamines neurotransmitters and their metabolites: a review. Molecules 2024;29: 496. [Crossref]
23. Bansal AA, Patel S. Recent Advancements in High-Performance Liquid Chromatography: A Comparative Approach. J Polym Compos 2025;13:2.
24. Shan L, Jones BR, Nano-LC. An updated review. Biomed Chromatogr 2022;36:e5317. [Crossref]
25. Aydoğan C. New Advances in Nano-Liquid Chromatography for Proteomics Analysis. In: Grinberg N, Carr WP, editors. 1st ed. Advances in Chromatography: CRC Press; 2024 p. 79-115. [Crossref]
26. Gumułka P, Żandarek J, Dąbrowska M, Starek M. UPLC technique in pharmacy-an important tool of the modern analyst. Processes 2022;10:2498. [Crossref]
27. Yang X, Duan X, Xia Z, Huang R, He K, Xiang G. The Regulation Network of Glycerolipid Metabolism as Coregulators of Immunotherapy-Related Myocarditis. Cardiov Ther 2023;2023: 8774971. [Crossref]
28. Kotadiya R. Enhancing Pharmaceutical Analysis with Analytical Quality by Design in UHPLC: A Review of Methodological Innovations (2014-2025). Crit Rev Anal Chem 2025;12:1-21. [Crossref]
29. Gorja A, Medala P, Madhavi M, Meghana MA. Review on Ultra Performance Liquid Chromatography. Saudi J Med Pharm Sci 2025;11:279-86. [Crossref]
30. Kiran G, Madankar V. Ultra Performance Liquid Chromatography (Uplc): A New Trend in Analysis. Int J Sci Res Technol 2025;2:458-67.
31. Dutt M, Arigo A, Famiglini G, Zappia G, Palma P, Cappiello A. Exploring Negative Chemical Ionization of Per-and Polyfluoroalkyl Substances via a Liquid Electron Ionization LC-MS Interface. J Am Soc for Mass Spectrom 2024;35:890-901. [Crossref]
32. Basharat R, Kotra V, Lean YL, Mathews A, Kanakal M, Devi CBP, et al. Ultra Performance Liquid Chromatography (Mini-Review). Orient J Chem 2021;37:847-57. [Crossref]
33. Bhattacharya K, Chanu NR, Bhattacharjee A, Sahariah BJ, Devi CM, Bordoloi R. Ultra-Performance Liquid Chromatography-An Updated Review. Res J Pharm Technol 2022;15:5849-53. [Crossref]
34. Rathod RH, Chaudhari SR, Patil AS, Shirkhedkar AA. Ultra-high performance liquid chromatography-MS/MS (UHPLC-MS/MS) in practice: analysis of drugs and pharmaceutical formulations. Future J Pharm Sci 2019;5:1-26. [Crossref]
35. Schiavone NM, Bennett R, Hicks MB, Pirrone GF, Regalado EL, Mangion I, et al. Evaluation of global conformational changes in peptides and proteins following purification by supercritical fluid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2019 Mar 15;1110-1111:94-100. [Crossref]
36. Govender K, Naicker T, Baijnath S, Chuturgoon AA, Abdul NS, Docrat T, et al. Sub/supercritical fluid chromatography employing water-rich modifier enables the purification of biosynthesized human insulin. J Chromatogr B Analyt Technol Biomed Life Sci 2020;1155:122126. [Crossref]
37. Yan X, He B, Wang D, Hu L, Liu L, Liao C, Jiang G. Two-dimensional (weak anion exchange chromatography-gel electrophoresis) separations coupling to inductively coupled plasma mass spectrometry strategy for analysis of metalloproteins. Talanta 2018;184:404-10. [Crossref]
38. Chouhan B. Supercritical Fluid Chromatography (SFC): A Review on Key Technique of Green Analytical Chemistry in Advanced Pharmaceutical Spectral Analysis. TMP Univ J Adv Pharm Scie 2025;1:1-10. [Crossref]
39. Brunelli, C. Supercritical fluid chromatography in the pharmaceutical industry: implementation in development and quality control. LCGC Supplements 2018;31:40-46.
40. Kingston J, Leek H, Buica A, Öhlén K, Proctor K, Raubo J, et al. Application of preparative SFC in the pharmaceutical industry. Sep Sci Technol 2022;14:133-65. [Crossref]
41. Si-Hung L, Bamba T. Current state and future perspectives of supercritical fluid chromatography. TrAC Trends Anal Chemi 2022;149:116550. [Crossref]
42. Ismail Y, Seelam J. Ultra-Performance Liquid Chromatography (UPLC) in Analytical Method Development and Validation Following In-vitro Studies. J Pharm Insights Rese 2025;3:082-9. [Crossref]
43. King C, Patel R, Ponniah G, Nowak C, Neill A, Gu Z, et al. Characterization of recombinant monoclonal antibody variants detected by hydrophobic interaction chromatography and imaged capillary isoelectric focusing electrophoresis. J Chromatogr B Analyt Technol Biomed Life Sci 2018;1085:96-103. [Crossref]
44. Hjertén, S. Hydrophobic interaction chromatography. Advances in chromatography. 1st ed. Florida: CRC Press; 2021. p. 111-23. [Crossref]
45. Pirok BWJ, Stoll DR, Schoenmakers PJ. Recent Developments in Two-Dimensional Liquid Chromatography: Fundamental Improvements for Practical Applications. Anal Chem 2019;91:240-63. [Crossref]
46. Pirok BWJ, Gargano AFG, Schoenmakers PJ. Optimizing separations in online comprehensive two-dimensional liquid chromatography. J Sep Sci 2018;41:68-98. [Crossref]
47. Amin R, Alam F, Dey BK, Mandhadi JR, Bin Emran T, Khandaker MU, et al. Multidimensional chromatography and its applications in food products, biological samples and toxin products: a comprehensive review. Separations 2022;9:326. [Crossref]
48. Chu S, Letcher RJ. A mixed-mode chromatographic separation method for the analysis of dialkyl phosphates. J Chromatogr A 2018;1535:63-71. [Crossref]
49. Ren X, Zhang K, Gao D, Fu Q, Zeng J, Zhou D, et al. Mixed-mode liquid chromatography with a stationary phase co-functionalized with ionic liquid embedded C18 and an aryl sulfonate group. J Chromatogr A 2018;1564:137-44. [Crossref]
50. Mu R, Yuan J, Huang Y, Meissen JK, Mou S, Liang M, et al. Bioanalytical Methods and Strategic Perspectives Addressing the Rising Complexity of Novel Bioconjugates and Delivery Routes for Biotherapeutics. BioDrugs 2022;36:181-96. [Crossref]
51. Li W, Wen C, Ye B, Gujarathi P, Suryawanshi M, Vinchurkar K, et al. Targeted drug monitoring in oncology for personalized treatment with use of next generation analytics. Discov Oncol 2025;16:1523. [Crossref]
52. Beccaria M, Cabooter D. Current developments in LC-MS for pharmaceutical analysis. Analyst 2020;145:1129-57. [Crossref]
53. Escrivá L, Manyes L, Font G, Berrada H. Mycotoxin Analysis of Human Urine by LC-MS/MS: A Comparative Extraction Study. Toxins (Basel) 2017;9:330. [Crossref]
54. Li ZM, Kannan K. Determination of 19 Steroid Hormones in Human Serum and Urine Using Liquid Chromatography-Tandem Mass Spectrometry. Toxics 2022;10:687. [Crossref]

55. Rischke S, Hahnefeld L, Burla B, Behrens F, Gurke R, Garrett TJ. Small molecule biomarker discovery: Proposed workflow for LC-MS-based clinical research projects. J Mass Spectrom Adv Clin Lab 2023;28:47-55. [Crossref]
56. Gowda GA, Djukovic D. Overview of mass spectrometry-based metabolomics: opportunities and challenges. Methods Mol Biol 2014;1198:3-12. [Crossref]
57. Wong AL, Xiang X, Ong PS, Mitchell EQY, Syn N, Wee I, et al. A Review on Liquid Chromatography-Tandem Mass Spectrometry Methods for Rapid Quantification of Oncology Drugs. Pharmaceutics 2018;10:221. [Crossref]
58. Song JG, Baral KC, Kim GL, Park JW, Seo SH, Kim DH, Jung DH, Ifekpolugo NL, Han HK. Quantitative analysis of therapeutic proteins in biological fluids: recent advancement in analytical techniques. Drug Deliv 2023;30:2183816. [Crossref]
59. Macklin A, Khan S, Kislinger T. Recent advances in mass spectrometry based clinical proteomics: applications to cancer research. Clin Proteomics 2020;17:17. [Crossref]
60. Wenk D, Zuo C, Kislinger T, Sepiashvili L. Recent developments in mass-spectrometry-based targeted proteomics of clinical cancer biomarkers. Clin Proteomics 2024;21:6. [Crossref]
61. Melby JA, Roberts DS, Larson EJ, Brown KA, Bayne EF, Jin S, Ge Y. Novel Strategies to Address the Challenges in Top-Down Proteomics. J Am Soc Mass Spectrom 2021;32:1278-94. [Crossref]
62. Sánchez-Cachero A, López-Gutiérrez A, Fariñas NR, Bernardo FJG, Ríos Á, Martín-Doimeadios RCR. Electrical asymmetrical flow field-flow fractionation: Fundamentals, evolution, applications, and prospects. J Chromatogr A 2025;1739:465522. [Crossref]
63. Hall, M. Size exclusion chromatography (SEC). In: Jagschies G, Lindskog E, Łącki K, Galliher P, editors. Biopharmaceutical Processing. Florida:Elsevier; 2018. p. 421-32. [Crossref]
64. Khodabandehloo A, Chen DD. Particle sizing methods for the detection of protein aggregates in biopharmaceuticals. Bioanalysis 2017;9:313-26. [Crossref]
65. Bolje A, Gobec S. Analytical Techniques for Structural Characterization of Proteins in Solid Pharmaceutical Forms: An Overview. Pharmaceutics 2021;13:534. [Crossref]
66. Tao P, Poddar S, Sun Z, Hage DS, Chen J. Analysis of solute-protein interactions and solute-solute competition by zonal elution affinity chromatography. Methods 2018;146:3-11. [Crossref]
67. Grom M, Kozorog M, Caserman S, Pohar A, Likozar B. Protein A affinity chromatography of Chinese hamster ovary (CHO) cell culture broths containing biopharmaceutical monoclonal antibody (mAb): Experiments and mechanistic transport, binding and equilibrium modeling. J Chromatogr B Analyt Technol Biomed Life Sci 2018;1083:44-56. [Crossref]
68. Liu P, Lan X, Yaseen M, Chai K, Zhou L, Sun J, et al. Immobilized metal affinity chromatography matrix modified by poly (ethylene glycol) methyl ether for purification of angiotensin I-converting enzyme inhibitory peptide from casein hydrolysate. J Chromatogr B Analyt Technol Biomed Life Sci 2020;1143:122042. [Crossref]
69. Chamrád I, Simerský R, Lenobel R, Novák O. Exploring affinity chromatography in proteomics: A comprehensive review. Anal Chim Acta 2024;1306:342513. [Crossref]
70. Valko KL. Biomimetic chromatography-A novel application of the chromatographic principles. Anal Sci Adv 2022;3:146-53. [Crossref]
71. Kaur J, Saxena A. Chromatography. In: Verma C, Verma DK, editors. Handbook of Biomolecules. 1st ed. Florida: Elsevier; 2023 p 217-25. [Crossref]
72. Kecskemeti A, Gaspar A. Particle-based liquid chromatographic separations in microfluidic devices - A review. Anal Chim Acta 2018;1021:1-19. [Crossref]
73. Sonia K, Lakshmi KS. HPTLC method development and validation: An overview. J Pharm Sci Res 2017;9:652.
74. Bao B, Wang Z, Thushara D, Liyanage A, Gunawardena S, Yang Z, et al. Recent advances in microfluidics-based chromatography-A mini review. Separations 2020;8:3. [Crossref]
75. Kulkarni RN, Pandhare RB, Deshmukh VK, Mohite PB, Pawar AR. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. J Pharm Biol Sci 2021;9:7-14. [Crossref]
76. Panchal DMK, Patel D, Patel K, Dalwadi M, Upadhyay U. A Review on "High Performance Thin Layer Chromatography". World J Pharm Sci 2022;11:282-97.
77. Barnali, D. High performance thin layer chromatography (HPTLC). J Appl Biochem Lab Med 2022;3:33-4. [Crossref]
78. Pattanayak P, Singh SK, Gulati M, Vishwas S, Kapoor B, Chellappan DK, et al. Microfluidic chips: recent advances, critical strategies in design, applications and future perspectives. Microfluid Nanofluidics 2021;25:99. [Crossref]
79. Tsopelas F, Vallianatou T, Tsantili-Kakoulidou A. Advances in immobilized artificial membrane (IAM) chromatography for novel drug discovery. Expert Opin Drug Discov 2016;11:473-88. [Crossref]
80. Jain S, Jain AA. Brief Review on Different Chromatography Techniques. J Pharm Insights Rese 2024;2:072-7.
81. Manouchehri M, Seidi S, Abdullah FO. Application of magnetic nanomaterials in magnetic-chromatography: A review. Talanta 2021;229:122273. [Crossref]
82. Semysim FA, Hussain BK, Hussien MA, Azooz EA, Snigur D. Assessing the Greenness and Environmental Friendliness of Analytical Methods: Modern Approaches and Recent Computational Programs. Crit Rev Anal Chem 2025;55:670-83. [Crossref]
83. Ali HH. Gas Chromatography: Principles, Medicine and Pharmaceutical. Energy, Fuel Applications and Environmental Analysis. SAR J Med Biochem 2025;6:94-105. [Crossref]
84. Zafar S, Hafeez A, Shah H, Mutiullah I, Ali A, Khan K, et al. Emerging biomarkers for early cancer detection and diagnosis: challenges, innovations, and clinical perspectives. Eur J Med Res 2025;30:760. [Crossref]
85. Aleixandre M, Horrillo MC. Recent Advances in SAW Sensors for Detection of Cancer Biomarkers. Biosensors (Basel) 2025;15:88. [Crossref]