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The Evolving Role of Chromatography in Pharmaceutical Drug Discovery & Development

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Abstract:

Chromatography serves as the analytical backbone of the modern pharmaceutical industry. It enables the progression of chemicals from early discovery to market-ready products. This narrative review is based on a systematic evaluation of peer-reviewed literatures. Mostly those published b/w 2015 to 2025. It includes authoritative regulatory documents (FDA, EMA, ISO, ICH Q2(R2), WHO and IUPAC). Key market analyses projected that growth of the chromatography market from USD 13.30 billion in 2025 to USD 19.80 billion by 2030 (CAGR 8.4%). Seminal contributions from leading researchers and high-impact journals were collected. It including Journal of Chromatography A. They were critically analyzed to synthesize current trends. The review examines chromatographic techniques across the drug development lifecycle. I highlighted affinity chromatography and high-throughput LC-MS in early discovery. Reverse Phase and Chiral HPLC in lead optimization. Protein A/G and IMAC in biologics purification. Advanced platforms such as Supercritical Fluid Chromatography and Simulated Moving Bed chromatography are discussed in the context of green chromatography & continuous manufacturing. Regulatory-driven validation and stability-indicating applications have been discussed. It includes HPTLC, GC, and TLC-densitometry are emphasized. Collectively, the findings underscore chromatography as a central safeguard for drug safety, efficacy and quality in an era of increasingly complex and personalized medicine.

Key Words: *Pharmaceutical Chromatography; Drug Development; High-Throughput LC–MS; Reverse-Phase HPLC; Biologics Purification; Method Validation*

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VOLUME. 5 ISSUE 1 (2026)

1. Background:

Chromatography in drug development stands as one of the most powerful & versatile analytical techniques in modern pharmaceutical science. In fact, this separation method has become increasingly important. Useful in pharmaceutical science, biochemistry, biotechnology and environmental science in recent years. As we explore the landscape of drug discovery and development. We cannot overlook how chromatographic techniques form the backbone of numerous critical techniques. Affinity chromatography is particularly recognized as the most effective technique for protein purification. It dates back to 1910 when Starkenstein first used an insoluble starch substrate to isolate α-amylase. Furthermore, in today's pharmaceutical sector various chromatographic methods serve multiple essential functions. It includes the detection, analysis and quantification of chemical substances in samples as well as the production of highly pure compounds. These techniques enable the separation of chiral molecules, assessment of mixture purity, identification of unknown compounds even synthesis of new drugs .Throughout this article, we will examine how High-Performance Liquid Chromatography (HPLC) and High-Performance Thin Layer Chromatography (HPTLC). Howit is essential analytical tools in pharmaceutical research. Along with other advanced chromatographic techniques contribute to every stage of drug development from early discovery through regulatory approval and manufacturing. By understanding these methodologies, we can better appreciate their critical role in bringing safe, effective medications to patients worldwide.

2. Chromatography in Early-Stage Drug Discovery

The initial phases of drug discovery rely heavily on chromatographic techniques to identify potential therapeutic targets and screen compound libraries. These methods provide critical insights that shape the trajectory of drug development from its earliest stages.

2.1 Target Identification Using Affinity Chromatography

Affinity chromatography remains the most widely used method for identifying molecular targets of bioactive compounds [1]. This technique involves immobilizing a small

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Molecule on a solid support to create an affinity probe that can capture target proteins from complex biological samples. Modern approaches couple this traditional method with powerful mass spectrometry techniques to improve sensitivity and specificity. One notable advancement is the development of quantitative proteomics approaches like SILAC (Stable Isotope Labelling by Amino Acids in Cell Culture), which has become the in target identification since its introduction in 2002 most frequently used method [2]. This technique eliminates the need to visualize target proteins by gel staining, instead relying on mass spectrometry for identification. Chemical proteomics, essentially a postgenomic version of classical drug affinity chromatography, combines high-resolution MS with bioinformatic analyzes to identify protein targets with unprecedented precision [2]. Meanwhile, affinity-based probes (AfBPs) that bind through reversible non-covalent interactions minimize impact on the natural biological functions of proteins [1].

2.2 High-Throughput Screening with HPLC and LC-MS

High-throughput screening (HTS) using liquid chromatography-mass spectrometry has revolutionized the ability to rapidly evaluate large compound libraries. The most commonly used MS-based HTS method is affinity selection-MS screening (ASMS), which can screen over 100,000 compounds per day[3]. Notably, a typical 1 million compound screen with a pooling strategy can take merely 5-7 days, with follow-up experiments ranging 1-3 weeks to confirm and characterize compound binding [4]. Modern LC-MS systems have been optimized to achieve remarkable efficiency—some configurations can achieve a 72-second injection-to-injection cycle time [5]. Another innovative MS-based HTS method, High-Affinity Mass Spectrometry screening (HAMS), uniquely avoids false positive hits while screening over 10,000 compounds per day with minimal protein consumption (nanogram quantities per compound) [3]. The calculated Z' factor is >0.6, indicating excellent suitability for high-throughput screening of strong binders [3].

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PRINT ISSN
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VOLUME. 5 ISSUE 1 (2026)

2.3 Fragment-Based Drug Discovery via SEC and IEC

Fragment-based drug discovery (FBDD) utilizes libraries of small molecule fragments with molecular weights typically ≤300 Da as an innovative approach to hit identification [6]. Size-exclusion chromatography (SEC) coupled with MS has emerged as a powerful technique in this area. For instance, SEC-RPC-MS (size-exclusion chromatography coupled with reverse phase chromatography-MS) allows more compounds to be interrogated simultaneously [3]. Similarly, ion exchange chromatography (IEC) helps in analysing the binding characteristics of these fragments. The FBDD approach has led to significant breakthroughs, such as the discovery of vemurafenib, a selective inhibitor of the oncogenic target B-RAF [4]. Consequently, fragment libraries designed with at least one point of diversity amenable to further derivatization enable rapid assessment of structure-activity relationships around each core fragment [6].

3. Chromatographic Techniques for Lead Optimization

During lead optimization, pharmaceutical scientists rely on advanced chromatographic techniques to refine promising drug candidates. This critical stage transforms initial hits into compounds with improved potency, selectivity, and physicochemical properties.

3.1 Reverse Phase HPLC for Compound Purity

(RP-HPLC) serves as an indispensable tool for purity assessment during lead optimization. This technique enables precise quantification of both active pharmaceutical ingredients (APIs) and their impurities or degradation products, offering excellent resolving power, accuracy, and reliability Reverse Phase High-Performance Liquid Chromatography[7]. RP-HPLC aligns particularly well with small-molecule drug development due to its compatibility with the hydrophobic nature of many candidates. Ultraviolet detection—whether variable wavelength or photodiode array—provides exceptional precision (RSD < 0.2%) for quality control applications [7]. The five-order magnitude linear UV response allows convenient single-point calibration in stability-

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VOLUME. 5 ISSUE 1 (2026)

Indicating assays, making it ideal for monitoring both the API and related substances that maintain similar chromospheres [7].

3.2 Enantiomer Separation, Chiral HPLC:

Chiral HPLC has become the gold standard for separating and purifying enantiomers during lead optimization [8]. This method's importance stems from the wide availability of commercial chiral stationary phases (CSPs) and operational modes, offering remarkable versatility for evaluating enantiomeric purity—a fundamental requirement for regulatory purposes [8]. Recent developments have focused on creating CSPs suitable for ultrafast separations, thereby facilitating high-throughput screening of chiral bioactive compounds [8]. The method offers three primary approaches: direct separation using chiral stationary phases, forming adducts with chiral mobile-phase additives, or separating diastereomers produced through pre-column derivatization [9].

3.3 Hydrophobic Interaction Chromatography in Protein Profiling

Hydrophobic Interaction Chromatography (HIC) complements other techniques by separating proteins based on surface hydrophobicity under non-denaturing conditions [10]. Unlike Reverse-Phase Chromatography, HIC works in a more polar environment that preserves protein structure and function [11].

The technique employs stationary phases with low-density, moderately hydrophobic ligands attached to a hydrophilic underlayer [10]. Therefore, HIC interacts only with specific hydrophobic residues on the protein's surface, causing elution in order of increasing surface hydrophobicity with high recovery and exceptional sensitivity to conformational variations [10].

Recent innovations include the development of more hydrophobic HIC materials that balance retention and denaturation with MS-compatible salt concentrations [10]. The salt type—particularly chloride ions—has proven crucial for enhancing chromatographic selectivity, attributed to the ion-specific spatial distribution at protein surfaces [2].

ONLINE ISSN
3006-9726

PRINT ISSN
3006-9718

VOLUME. 5 ISSUE 1 (2026)

4. Role of Affinity Chromatography in Biologics Development

Affinity chromatography stands at the core of biologics development, offering powerful purification solutions for complex therapeutic proteins. This highly selective technique enables the separation of target molecules from complex mixtures through specific binding interactions between biological molecules and immobilized affinity ligands [1].

4.1 Protein A and G Columns for Antibody Purification

Protein A and G chromatography media represent the gold standard in antibody purification due to their exceptional binding affinity and specificity for the Fc region of immunoglobulins [12]. The binding of antibodies to protein A occurs through hydrophobic interactions at neutral or alkaline pH values, involving a highly conserved histidine residue in the IgG binding site [12]. for human IgG1, IgG2, and IgG4, but binds poorly to IgG3 Protein A demonstrates high affinity[13]. In contrast, Protein G exhibits broader binding specificity across IgG subclasses but requires harsher elution conditions—pH 3.0 or lower—potentially affecting antibody activity [12].

Recently, manufacturers have developed more robust Protein A resins that withstand high pH washes to prevent biocontamination, with some versions tolerating up to 1 molar sodium hydroxide [1]. Moreover, engineered antibody affinity resins enabling mild-pH elution have emerged, allowing purification without traditional low-pH buffers—a significant advantage for pH-labile biologics [1].

4.2 Lectin Affinity Chromatography for Glycoproteins

an essential tool for purifying glycoproteins based on specific sugar-lectin interactions. This technique not only facilitates glycoprotein identification but also resolves microheterogeneity in these molecules—an otherwise impracticable challenge Lectin affinity chromatography (LAC) provides[3]. Serial LAC (SLAC) offers even greater resolution by rechromatographing breakthrough fractions on different lectin columns in sequence [3]. The procedure typically involves binding glycoproteins to immobilized lectins, washing away unbound proteins, then eluting with simple sugars that resemble

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PRINT ISSN
3006-9718

VOLUME. 5 ISSUE 1 (2026)

the bound sugar ligands [4]. Beyond purification, LAC has proven valuable for glycosylation pattern analysis in disease diagnosis and potential treatment design [3].

4.3 Immobilized Metal Affinity Chromatography (IMAC)

IMAC utilizes metal ions immobilized on a stationary phase to selectively capture proteins through coordination with specific amino acid residues. Although widely used for laboratory-scale His-tagged protein purification from E. coli, its application in mammalian-expressed proteins has been limited by compatibility issues with cell culture media [5]. Recent advances have overcome these limitations, with specialized resins like Ni Sepharose excel demonstrating resistance to EDTA and reducing agents [5]. Optimized IMAC protocols incorporate detergents or low-concentration alcohols in washing buffers to significantly improve host cell protein removal while maintaining high yields [5]. Under optimal conditions, these resins can maintain acceptable yields and stable product quality attributes across more than 50 purification cycles [5]. Overall, these affinity techniques form the cornerstone of modern biologics purification strategies, enabling the development of highly pure therapeutic proteins with maintained biological activity.

5.Advanced Chromatographic Methods in Drug Formulation

Modern pharmaceutical formulations demand sophisticated analytical and purification approaches that go beyond conventional chromatography methods. Advanced techniques have emerged to address specific challenges in drug development, especially for complex or sensitive compounds.

5.1 Supercritical Fluid Chromatography for Heat-Sensitive Compounds

Supercritical Fluid Chromatography (SFC) combines features of both gas and liquid chromatography by using supercritical CO_2 as the mobile phase. This technique offers exceptional benefits for that might degrade under high GC temperatures thermally labile pharmaceutical compounds[14]. Beyond its thermal advantages, SFC delivers faster separations with higher resolution and dramatically reduced organic solvent usage [15].

ONLINE ISSN
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PRINT ISSN
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VOLUME. 5 ISSUE 1 (2026)

Importantly, SFC has become the preferred technique for enantiomeric separations, offering exceptional enantioselectivity with faster results than traditional normal-phase HPLC methods [14]. The mobile phase—typically supercritical CO_2 with an organic modifier like ethanol—ensures effective separation while supporting green chemistry principles through minimal solvent consumption [16].

5.2 Two-Dimensional LC for Complex Mixture Analysis

Two-dimensional liquid chromatography (2D-LC) has earned recognition for its superior separation capabilities compared to one-dimensional techniques [6]. By exploiting two different separation mechanisms, 2D-LC increases peak capacity and achieves optimal resolution of complex pharmaceutical mixtures [6]. The technique operates in several modes: heart-cutting for targeted analysis, comprehensive for total sample evaluation, or selective comprehensive for quantitative purposes [6]. Even more beneficial, 2D-LC hyphenated with mass spectrometry has proven valuable for analyzing monoclonal antibodies and biopharmaceuticals, eliminating the need for protein precipitation while providing information on multiple variants in a single analysis [17].

5.3 Simulated Moving Bed Chromatography for Continuous Purification

Simulated Moving Bed (SMB) chromatography represents a continuous counter-current separation process that surpasses traditional batch chromatography in efficiency [18]. This technique is exceptionally well-suited for binary separations such as enantiomers, diastereomers, or closely related impurities [19]. Through continuous operation, SMB achieves higher productivity, improved purity and yield, and substantially reduced solvent consumption—up to 90% less than comparable methods [20]. A real-world example demonstrated SMB's remarkable capabilities when producing , while recovering 99.6% of the process solvent through advanced recycling 1,635 kg of API weekly with 99.3% chiral purity[19].

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6. Regulatory and Analytical Applications in Drug Development

Beyond the technical applications, chromatography plays a pivotal role in regulatory compliance throughout drug development. Pharmaceutical manufacturers must adhere to strict guidelines ensuring product safety, efficacy, and consistency.

6.1 ICH Q2(R2) Guidelines for Chromatographic Method Validation

Regulatory agencies worldwide follow the International Council for Harmonization (ICH) Q2(R2) guidelines for chromatographic method validation. These guidelines outline essential validation parameters:

- Specificity - Ability to measure the analyte without interference from excipients or impurities
- Accuracy - Closeness of measured value to true value, typically assessed at 80%, 100%, and 120% levels
- Precision - Measured as repeatability (intra-day) and intermediate precision (inter-day)
- Linearity - Demonstrates proportional detector response to analyte concentration
- Range - Interval where the method shows acceptable linearity, accuracy, and precision [21]

The ICH emphasizes lifecycle management of analytical procedures, including continual method monitoring through Analytical Quality by Design (AQbD) approaches [21].

6.2 Stability Testing Using HPTLC and GC

Stability-indicating chromatographic methods verify a drug's stability over time under various conditions. High-Performance Thin Layer Chromatography (HPTLC) offers advantages for stability studies, including high sample throughput and minimal sample preparation. Regarding validation, HPTLC methods must demonstrate linearity () and acceptable limits of detection (LOD) and quantification (LOQ) $r \geq 0.9997$[22]. Gas

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VOLUME. 5 ISSUE 1 (2026)

chromatography (GC) remains essential for volatile compound analysis during stability testing, helping assess degradation pathways under different storage conditions [23].

6.3 Impurity Profiling in Final Drug Products

Impurity profiling represents a critical application of chromatographic techniques in finished pharmaceuticals. According to ICH guidelines, identification of impurities is not typically required unless they are unusually potent or toxic below 0.1% level[24]. Reverse-phase HPLC with UV detection stands as the most commonly submitted method for impurity analysis, allowing separation, quantification, and identification of components in pharmaceutical mixtures [25]. Furthermore, this technique excels at elucidating impurity structures and determining their quantities in pharmaceutical formulations [26].

Conclusion

Chromatography offers unmatched versatility & precision from early target identification to final regulatory approval. In drug discovery techniques such as affinity chromatography, LC-MS, reverse-phase HPLC and chiral chromatography are used. They enable rapid, accurate molecular characterization. Biologic drug development benefits substantially from advanced affinity methods. It Includes Protein A/G & IMAC. It ensures efficient purification while preserving biological activity. Emerging platforms such as supercritical fluid chromatography, two-dimensional LC or simulated moving bed systems further expand analytical and manufacturing capabilities. Chromatography also provide regulatory compliance through stability testing, impurity profiling and validated methods beyond innovation. With continued advances in automation, sensitivity and sustainability chromatography will remain central to pharmaceutical innovation. The delivery of safer more effective medicines.

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