Indian Journal of Pharmaceutical Chemistry and Analytical Techniques (IJPCAT)
ISSN: 3049-3765 | Vol. 02, Issue-02, March-April, 2026, pp. 55-73
https://ijpcat.com/1

Recent Trends in Chromatographic Techniques for Pharmaceutical Analysis: A Review

Perli Kranti Kumar[1], Sekar.V[1]*, Venkatesan M[2]

[1]Department of Pharmaceutical Analysis, J. K. K. Nattraja College of Pharmacy, Kumarapalayam, Namakkal, Tamil Nadu-638 183

[2]Department of Pharmaceutical Chemistry J. K. K. Nattraja College of Pharmacy, Kumarapalayam, Namakkal, Tamil Nadu-638 183

***Corresponding Author E-mail: vsekar74@gmail.com**

Abstract:

Chromatography is a critical instrument in pharmaceutical analysis, as it allows the successful separation, identification, and determination of complex drug formulations and biological matrices. Over recent years, large improvements in chromatographic technology, such as High-Performance Liquid Chromatography (HPLC), UPLC, Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC) and other hyphenated techniques, have made major improvements in the sensitivity and speed of analysis and accuracy. This review makes a concise review of the recent trends in chromatographic techniques, alongside a special focus on their use in animal studies pertaining to pharmacokinetics, toxicology and drug metabolism. The literature has been systematically evaluated to assess the methodological developments, technological innovations, and performance of analysis. The results emphasize that high-throughput, high-sensitivity, and environmentally friendly chromatographic methods have become more and more important in contemporary pharmaceutical research.

Keywords: Chromatography; Pharmaceutical Analysis; HPLC; UPLC; Animal-Based Studies; Pharmacokinetics; Drug Metabolism; Toxicology

Received: Jan. 29, 2026 **Revised: Feb. 28, 2026** **Accepted: March. 28, 2026** **Published: April 28, 2026**

DOI: https://doi.org/10.64062/IJPCAT.Vol2.Issue2.5

https://ijpcat.com/index.php/1/issue/archive

1. INTRODUCTION

The development of pharmaceutical sciences has greatly increased the requirements on analytical methods that are not only highly accurate and reliable, but also highly sensitive and

able to manage more and more complicated drug formulations and biological systems[1]. The advent of new therapeutic agents, such as biologics, nanomedicines, and combination drugs, is rapidly changing the nature of the new analysis and needs more complex and powerful methodologies. Chromatography has become in this regard one of the most effective and multifaceted analytical methods, providing extraordinary opportunities in the separation, identification and quantification of various chemical substances[2].

The chromatographic methods are central in the different drug development phases such as drug discovery, formulation design, quality control, and bioanalysis studies. They are essential in maintaining consistency, purity and safety of pharmaceutical products because they can analyze compounds with high accuracy and reproducibility. Besides this, chromatography is also extensively used in impurity profiling, stability testing and validation processes which are essential in ensuring the quality of products and ensuring they comply with regulatory requirements[3].

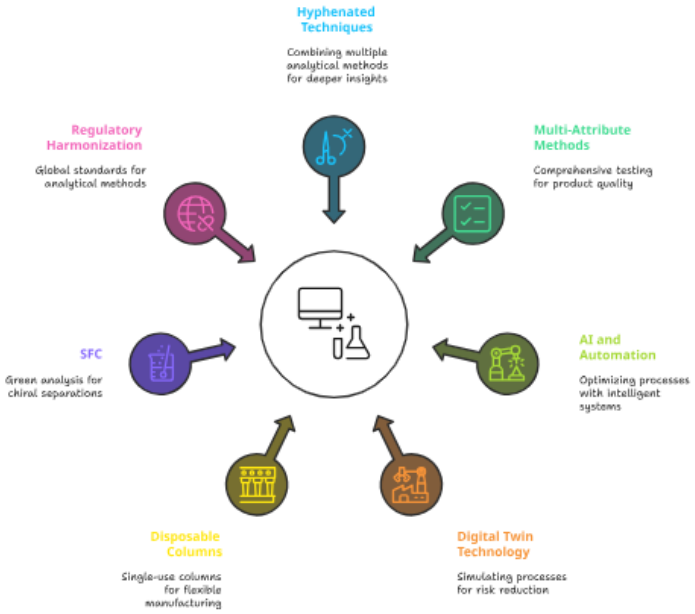

Figure 1: Chromatography in pharma

As modern therapeutics have been becoming more complex, there has been a growing demand on analytical techniques that can provide greater performance in terms of sensitivity, speed and selectivity[4]. Recent developments in chromatographic technologies, including high-efficiency columns, enhanced stationary phases, and more advanced systems of detection have greatly enhanced analytical results. These technologies have made it possible to analyze faster, resolve more closely related compounds, and detect analyte at traces and thus satisfy the changing needs of pharmaceutical studies[5].

Moreover, the strict regulatory standards, that are required by international agencies, have necessitated the application of validated and highly reliable analytical methods. Chromatographic techniques are now required to help assure that these regulations are met by giving the correct and reproducible data during the drug development process[6]. This is

especially important in bioanalytical research, where quantitative measurements of drugs and metabolites are needed.

Specifically, the use of chromatographic methods in the preclinical and animal-based research has gained prominence[7]. These tests are important in understanding the pharmacokinetics, metabolism, and toxicological characteristics of drug candidates prior to testing them in humans. Chromatology can be used to study biological fluids like plasma, tissue, and urine collected on animal models in detail, and researchers can gain insight into the behavior of drugs in complex biological systems[8]. Consequently, chromatographic methods have gained an inseparable role in filling the gap in laboratory studies and clinical usage, which ultimately leads to the realization of safe and effective pharmaceutical treatments.

1.1.Background of the study

Chromatography is a basic and widely used method of analysis in pharmaceutical sciences that is widely used in separation, identification and quantification of chemical components within a complex mixture[9]. The principle of the technique is the idea of differentiated distribution or partitioning of analytes between a stationary phase and a mobile phase. Different compounds have different migration rates, which can be determined by their physicochemical characteristics, including polarity, molecular size, and affinity, making it possible to separate them efficiently and analyze them accurately. This basic principle has rendered chromatography a very essential instrument in a vast array of applications in pharmaceutical spheres.

Chromatography has undergone numerous changes in recent years and nowadays is an essential part of various phases of pharmaceutical research and development[10]. They find application in drug discovery in screening and in the discovery of potential therapeutic compounds. Chromatography is used during the formulation development to determine the compatibility of active pharmaceutical ingredients (APIs) and excipients. Moreover, it is important in stability tests to identify degradation products that form at different environmental conditions like heat, light, and humidity[11]. Chromatographic techniques are used in quality control to assure the purity, strength and consistency of drug formulations, thereby protecting the safety of patients and assuring reliability of products[12].

Over the past few years, pharmaceutical analysis has experienced an incredible revolution due to the rapid development of analytical tools and the growing regulatory pressure[13]. Among the most notable changes is the transition to high-throughput screening methods, which enables the screening of many samples in a relatively small amount of time. This feature is especially relevant when drug development is in the initial phases, and several potential compounds have to be tested effectively[14]. Also, trace-level detection is increasingly in demand because current analytical needs require drugs and drug metabolites to be identified and quantified at extremely low concentrations, which are typically in the nanogram or even picogram range[15].

The other significant development in chromatographic analysis is the enhanced analysis of complicated biological samples particularly those which are related to animal research including plasma, serum, urine and tissue extracts. These matrices are complex in nature and have a large number of endogenous products, such as proteins, lipids and other interfering

substances, which might complicate the analysis process. To solve these problems, the technique of modern chromatographic work has been improved with increased selectivity and sensitivity. The incorporation of chromatography with state of the art detection techniques, including mass spectrometry, has further enhanced its analysis capabilities, making it capable of successful identification and quantification of even the most complex samples.

In sum, the scope of chromatographic techniques has been hugely increased by their constant development, in conjunction with technological and methodological breakthroughs, which have increased their application in pharmaceutical analysis. These advancements not only enhance the accuracy and reliability of the analysis, but also to the increasing requirements of preclinical research, especially in animal model studies, which can help in the progress of safe and effective drug development.

1.2.Objectives of the Review

The present review aims to provide a comprehensive and critical overview of recent developments in chromatographic techniques used in pharmaceutical analysis. The specific objectives are:

- To analyze recent advancements in chromatographic techniques
- To evaluate their applications in animal-based pharmaceutical studies, particularly in pharmacokinetics, toxicology, and drug metabolism
- To compare different chromatographic methodologies in terms of performance, strengths, and limitations
- To identify research gaps and propose future directions for innovation

1.3.Importance of the Topic

Chromatographic methods are critical in ensuring the safety, efficacy and quality of pharmaceutical products. They offer precise and high quality analytical information required in the approval of drugs and in regulatory compliance[16].

These methods are important in the preclinical research setting, in which animal-based research is used to assess the pharmacokinetics and toxicology of drugs. Using chromatography allows the analysis of the absorption, distribution, metabolism, and excretion (ADME) of drugs in detail, thus adding to the understanding of drug behaviour in biological systems.

Additionally, chromatography is important in achieving regulatory requirements by international bodies, whereby pharmaceutical products are safe enough to be used by humans. The ongoing development of chromatographic technologies does not only enhance the efficiency of the analysis, but it also broadens their usage in the new field of green chemistry and computerized analysis systems.

2. Advances in Chromatographic Techniques for Pharmaceutical Analysis

The high rate of development of pharmaceutical research has greatly enhanced the need to have sophisticated analytical methods that can deal with complex drug formulations and biological samples. In this respect, chromatographic methods have become an indispensable tool, with

high sensitivity, selectivity, and reproducibility. Specifically, the introduction of chromatography in animal research has helped in the understanding of pharmacokinetics, drugs metabolism and toxicological profiles in preclinical studies. Recent advances in chromatographic methods are aimed at enhancing speed of analysis, cutting solvent use, maximizing resolution, and being able to detect traces on a trace level in complex biological samples[17].

2.1. High-Performance Liquid Chromatography (HPLC)

The HPLC is among the most commonly and dependable methods of analysis in pharmaceuticals because of its strength, adaptability and high reproducibility. It is based on the principle of the differential partitioning of the analytes between a liquid mobile phase and a solid stationary phase packed in a column. The reaction between the analytes and the stationary phase-determined by the polarity, molecular size, and chemical affinity- make complex mixtures easy to separate into individual components. This is the ability that renders HPLC exceptionally useful in the analysis of compounds that have varied physicochemical characteristics.

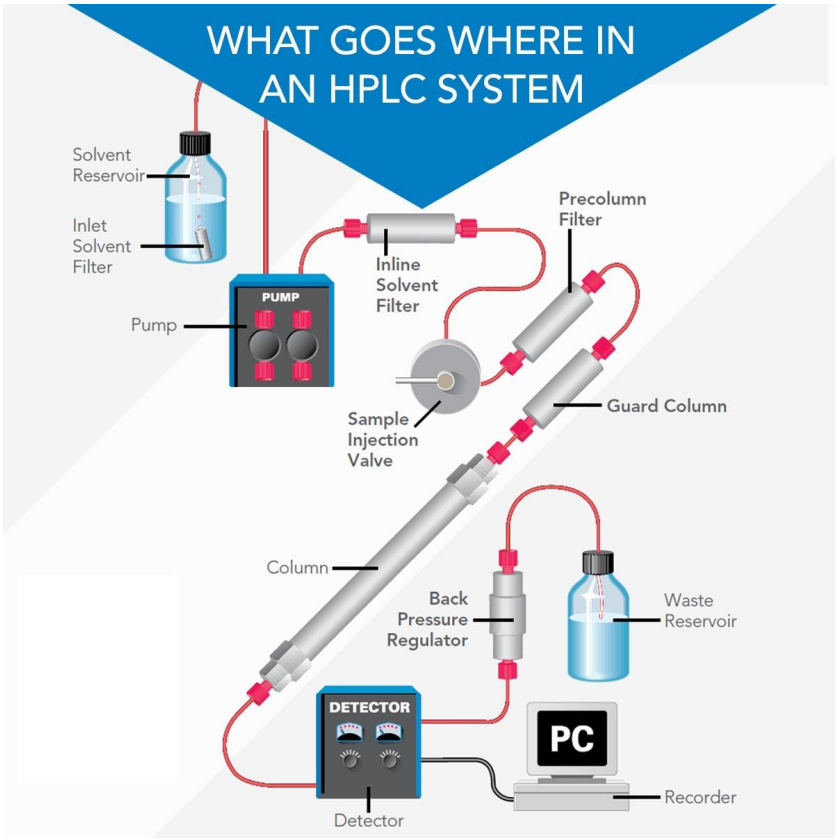

Figure 2: Schematic Diagram of a HPLC System

HPLC has become an important tool in quantitative and qualitative analysis of drugs and their metabolites in the pharmaceutical research process, particularly in complex biological samples like plasma, serum, urine and tissue samples, sampled in animal models[18]. It can be used to analyze non volatile and thermally unstable compounds compared to other chromatographic methods such as gas chromatography. Moreover, HPLC is very precise and accurate and can

be used in both routine analysis and advanced research including impurity profiling, stability testing and formulation analysis.

The significance of the HPLC is especially clear in preclinical and animal-based research, where it finds wide application to track drug behavior in the biological system. It allows investigators to produce credible pharmacokinetic information and gain insight into drug metabolism routes, which are crucial in assessing the safety and efficacy of new drug candidates prior to human testing[19].

❖ **Applications in Animal-Based Studies**

HPLC has been widely applied in various animal-based pharmaceutical studies, including:

- Pharmacokinetic studies:

HPLC is used to determine drug concentration-time profiles in animal models such as rats and mice. This helps in evaluating key pharmacokinetic parameters, including absorption, distribution, metabolism, and excretion (ADME), which are crucial for dose optimization and therapeutic effectiveness.

- Drug metabolism studies:

The technique facilitates the identification and quantification of metabolites formed in biological systems. By analyzing metabolic pathways and intermediate compounds, researchers can better understand the biotransformation of drugs in animal models.

- Bioavailability and bioequivalence studies:

HPLC is employed to assess the rate and extent of drug absorption, enabling comparisons between different formulations. This is essential for determining the effectiveness and consistency of pharmaceutical products.

❖ **Recent Trends**

The recent developments in the HPLC have greatly improved its performance in terms of analysis and its use in pharmaceutical research. Achievement of high separation efficiency, enhanced resolution, and increased applicability to a large variety of compounds is one of the most well known advances, through the usage of reverse-phase HPLC (RP-HPLC).

Also, increased focus on automation and web-based sample preparation methods has been observed, which minimize error and manual intervention, along with enhancing analytical throughput. High-throughput screening is also facilitated by automated systems, and is an advantage especially in large-scale preclinical studies[20].

The adoption of the principles of green analytical chemistry, e.g. use of environmentally friendly solvents and low solvent usage is the other significant trend. This not only limits the environmental impact but also enhances safety and sustainability of the laboratories.

Moreover, the combination of HPLC with sophisticated detection methods, e.g., UV-visible detectors, fluorescence detectors, and mass spectrometry (LC-MS) has greatly improved its sensitivity and selectivity. These hyphenated methods allow detection of analyte at trace levels

and allow in-depth structural information, which makes HPLC an even more potent instrument in the contemporary pharmaceutical analysis.

2.2. Ultra-Performance Liquid Chromatography (UPLC)

The UPLC is an important improvement of the traditional HPLC, mainly because of the sub-2 μm particle sizes in the columns used and the fact that much higher pressures can be applied in the process. These alterations allow improved chromatographic operation which leads to better resolution, sharper peaks and greatly reduced analysis time. Smaller particles have a higher surface area and thus they interact better with the stationary phase and hence result in better separation efficiency and sensitivity[21].

UPLC has emerged as a critical instrument in the analysis of pharmaceuticals today, especially when it comes to quick, high-throughput and highly sensitive pharmaceutical analysis. The fact that it can process its samples within a short time, without the loss of accuracy, renders it very effective in large-scale screening when developing drugs. In addition, UPLC needs less sample volumes and less solvent usage and is therefore cost-effective and greener than conventional chromatographic methods.

UPLC is essential in the detection and quantification of drugs and their metabolites in biological samples (plasma, serum, and tissue samples) in the preclinical, animal-based studies. This method is particularly useful with small sample volumes, which is frequently encountered with small animal models. Its great sensitivity enables it to detect trace levels of the analytes and this enables detailed pharmacokinetic and metabolic profiling.

Moreover, UPLC is often combined with other sophisticated detection methods like mass spectrometry (UPLC-MS/MS) which also increase the power of analysis. This combination allows accurate compound identification, enhanced specificity and accurate quantification even in complicated biological systems. This has made UPLC an ideal tool in pharmaceutical studies where quick, precise and repeatable analytical findings are required.

❖ **Applications**

- Rapid screening of drug compounds in animal serum and plasma
- Detection and quantification of metabolites in liver and tissue samples

❖ **Advantages**

UPLC offers several advantages over traditional methods, including reduced analysis time, higher sensitivity, improved peak resolution, and lower solvent consumption. These features make it highly suitable for high-throughput analysis in pharmaceutical research.

2.3. Gas Chromatography (GC)

GC is an effective and popular analysis method to achieve separation, identification, and quantification of volatile and semi-volatile compounds. This method is based on vaporizing the sample and pushing it through a chromatographic column with an inert carrier gas, which is usually helium or nitrogen, as the mobile phase. Separation is done depending on the difference in the volatility and interaction of the analytes with the stationary phase coated within the

column[22]. The compounds that have lower boiling points or weak interactions are eluted at a higher rate and it is therefore possible to efficiently separate complex mixtures.

Applications in pharmaceutical analysis GC is especially useful with thermally stable compounds that can be vaporized without decomposition, including volatile drugs, solvents left behind during processing, and some metabolites. It is widely used in laboratories of quality control to detect impurities and solvent residues and ensure the adherence to the regulatory requirements. GC is also characterized by high separation efficiency, excellent reproducibility, and relatively short analysis times and thus can be utilized in both routine and advanced applications[23].

In animal studies, where the analysis of volatile compounds and biological metabolites in biological matrices is needed (e.g., blood, urine, and tissue samples), the role of GC is particularly crucial. Biological samples in most instances need to be derivatized to prepare non volatile compounds to volatile forms that can be analyzed by GC[24]. This improves the limit of detection and stability of the analytes which can be quantified more accurately.

New developments in GC have only increased its use in pharmaceutical research. Incorporations of GC with mass spectrometry (GC-MS) have greatly enhanced sensitivity, selectivity and structural identification. The GC-MS finds a significant application in pharmacokinetic and toxicological research to accurately identify metabolites and trace-level substances. Also, the sophisticated sample preparation methods, like the solid-phase microextraction (SPME) and headspace analysis, have made sample processing easier and consumed less solvent[25].

❖ **Applications**

- Analysis of anesthetic agents and volatile drugs in animal studies
- Detection and identification of drug metabolites

❖ **Recent Developments**

Recent advancements in GC include its integration with mass spectrometry (GC-MS), which significantly enhances detection sensitivity and specificity. Additionally, the development of microextraction techniques has improved sample preparation efficiency and reduced solvent usage.

2.4. Supercritical Fluid Chromatography (SFC)

SFC is a relatively new and a rapidly growing method of analysis that employs supercritical carbon dioxide (CO^2) as the main mobile phase. In its supercritical form, CO 2 has some special characteristics which are between gases and liquids i.e. it has low viscosity, high diffusivity and high solvating power. These properties allow high-speed mass transfer as well as efficient contact with the stationary phase, leading to quicker separations and an enhanced chromatographic performance.

SFC is a well-performing mixture of the benefits of GC and liquid chromatography (LC) that is highly efficient, has short analysis times, and consumes less solvent. SFC can be a more

environmentally friendly alternative to traditional chromatographic methods because CO 2 as the primary mobile phase can greatly reduce the use of organic solvents[26].

SFC has received significant interest in pharmaceutical analysis because of its remarkable ability to separate chiral compounds that are a paramount necessity of drug development. A wide array of pharmaceutical compounds are available as enantiomers that exhibit varying pharmacological properties and SFC offers effective and fast separation of these stereoisomers. This renders it very useful in the research as well as quality control contexts.

Moreover, SFC is specially applicable in the study of lipophilic and non-polar molecules such as some drug molecules and metabolites found in biological matrices obtained in animal studies. It is highly sensitive and selective and therefore, can be used to quantify even complex samples. The method can also be used with other detectors, such as UV and mass spectrometry (SFC-MS), which adds to its analysis potential.

The recent changes in SFC are better instrumentation, enhanced stationary phases and improved control over the operating conditions that have increased its applicability in pharmaceutical research. Also, its conformity to the principles of green analytical chemistry because of its lesser use of solvents and lesser extent of harm to the environment have further accelerated its uptake[27].

❖ **Applications**

- Chiral separation of pharmaceutical compounds
- Analysis of lipophilic drugs in animal tissue samples

❖ **Current Trend**

SFC is widely recognized as a green analytical technique, as it reduces the use of organic solvents and minimizes environmental impact. Its growing adoption reflects the shift toward sustainable laboratory practices.

2.5. Hyphenated Techniques (LC-MS, GC-MS, LC-MS/MS)

Hyphenated techniques are an important step forward in the field of analytical science, and they combine the separation ability of chromatography with the detection and identification abilities of more sensitive spectrometric techniques. Incorporating chromatographic methods like liquid chromatography (LC) or GC with mass spectrometry (MS) these methods have high analytical capabilities and are inseparable in contemporary pharmaceutical analysis.

The key benefit of hyphenated methods is that they are capable of providing high sensitivity, selectivity, and specificity and thus being able to detect and quantify compounds at trace levels in complex biological samples. Although chromatography is useful in the separation of the elements of a mixture, mass spectrometry offers the in-depth structural details according to the mass to charge ratios that enables exact identification of analyte and its metabolites.

Hyphenated methods are widely applied in the analysis of drugs, impurities, and metabolites in pharmaceutical research, notably in preclinical animal research. LC-MS and LC-MS/MS are the most commonly used techniques in pharmacokinetic and toxicological research to determine the level of drug in the body in biological fluids including plasma, serum, and tissue

samples. Such techniques are important in learning about the absorption, distribution, metabolism, and excretion of drugs (ADME).

❖ **Applications**

- Drug metabolism and pharmacokinetic studies in animal models
- Toxicological screening for detection of harmful metabolites
- Biomarker identification and validation

❖ **Importance**

Hyphenated techniques offer unparalleled analytical capabilities, enabling detection of compounds at trace levels with high accuracy. They are indispensable tools in preclinical studies and play a crucial role in drug development and regulatory evaluation.

Table 1: Comparison of Chromatographic Techniques in Pharmaceutical Analysis

Technique	Principle	Applications (Animal Studies)	Advantages	Limitations
HPLC	Liquid-solid partitioning	Plasma drug analysis	Widely applicable, reliable	Time-consuming
UPLC	High-pressure liquid separation	Rapid metabolite detection	Fast, high sensitivity	Expensive instrumentation
GC	Gas-phase separation	Volatile compound analysis	High resolution	Limited to volatile compounds
SFC	Supercritical fluid separation	Chiral drug analysis	Eco-friendly, efficient	Specialized equipment required
LC-MS	Chromatography + Mass detection	Drug metabolism studies	Highly sensitive, selective	Complex operation

2.6. Strengths of Modern Chromatographic Techniques

The current chromatographic methods have many benefits that render them invaluable in pharmaceutical studies. They are highly sensitive and accurate and can detect the analytes at traces. The techniques can be used to analyze complex biological samples like plasma and tissue samples, which is critical in animal-based studies. Moreover, they are compatible with the sophisticated detection systems which improves the analytical performance. High reproducibility and reliability are also supported by chromatographic techniques, and thus they can be used in regulatory and quality control.

2.7. Weaknesses of Modern Chromatographic Techniques

Although they have benefits, chromatographic methods have some limitations as well. The expensive nature of advanced instrumentation and maintenance may be a major obstacle especially in resource constrained environments. Moreover, these techniques usually need

experienced staff to be operated and methods developed. Preparation of samples may be lengthy and complicated, particularly in the case of biological samples. Moreover, absence of standard protocols in various laboratories can impact result reproducibility and comparability.

Table 2: Summary of Literature on Chromatographic Techniques in Pharmaceutical Analysis

Author Name	Topic Covered	Research Study Title
D'Atri, V. et al. (2025)[28]	Role of liquid chromatography in modern pharmaceutical analysis	Trends in pharmaceutical analysis: the evolving role of liquid chromatography
D'Atri, V. et al. (2018)[29]	Advances in chromatographic techniques for drug analysis	Recent advances in chromatography for pharmaceutical analysis
Memon, N. et al. (2019)[30]	Fast liquid chromatography and high-throughput analysis	Recent trends in fast liquid chromatography for pharmaceutical analysis
Sharma, S. et al. (2021)[31]	Analytical techniques for pharmaceutical method development and validation	Modern Trends in Analytical Techniques for Method Development and Validation of Pharmaceuticals: A Review
Parys, W. et al. (2022)[32]	Importance of chromatography in pharmaceutical applications	Significance of chromatographic techniques in pharmaceutical analysis

3. METHODOLOGIES AND FINDINGS

In the pharmaceutical analysis, chromatographic methods have experienced a great development especially in the animal based studies. The methods are extensively used in the separation, identification, and quantification of drugs and their metabolites in intricate biological samples. Recent chromatographic advances have included better instrumentation, a better stationary phase, development of a better mobile phase system and have been combined with high sensitive detection systems[33]. These advances have improved the performance of the analytical process in the aspects of accuracy, sensitivity, speed and reproducible.

The approaches that have been taken in chromatographic analysis are mainly aimed at enhancing the separation efficiency, reducing the analysis time and being able to detect trace levels. Different methods, i.e. HPLC, UPLC, GC, SFC, and hyphenated have been widely used in the preclinical research on the pharmacokinetic, toxicological and metabolic assessment of pharmaceuticals in animal models.

The results of the recent literature show chromatographic methods have been significant in recent pharmaceutical studies, offering valid and accurate analysis results. Nevertheless, difficulties in the form of high cost of instrumentation, complexity of the methods, and non-standardization remain the main obstacles to their extensive use, which suggests more innovation and optimization.

3.1. Analytical Method Development and Optimization

A breakthrough in chromatographic techniques is a pivotal move when it comes to analysis of pharmaceuticals, and proper separation and quantification of the analyte. The development of methods is a critical process that entails the deliberate choice of chromatographic parameters like stationary phase, mobile phase composition, flow rate, temperature and parameters of detection.

The HPLC/UPLC techniques, which are most commonly employed in the development of methods, are highly adaptable and efficient. Normal-phase chromatography is typically used to separate polar compounds whereas reverse-phase chromatography is used to separate non-polar and moderately polar compounds. Mobile phase composition optimization, such as buffer choice and pH optimization, is important in enhancing peak resolution and retention time.

Biological matrix effects must also be taken into account in the development of methods in animal studies, where the interference of biological matrices can be reduced by strong sample preparation methods to increase the accuracy of the analytical method[34].

3.2. Detection and Quantification Techniques

The detection systems have improved the performance of the chromatographic techniques through advanced detection systems. Hyphenation of chromatography with mass spectrometry, including LC-MS and LC-MS/MS, has facilitated a very sensitive and selective detection of analytes, even in the trace level.

The methods are especially relevant when analyzing drugs in an animal model, where the concentration of drugs in biological samples can be incredibly low. The reason why LC-MS/MS has been extensively utilized in pharmacokinetic and toxicological studies is because it is very sensitive and is able to give structural data of the analytes and its metabolites.

The analysis of volatile compounds and residual solvents is usually performed using GC with mass spectrometry (GC-MS). Tandem mass spectrometry further enhances specificity of the analysis and minimizes interference of complex biological matrices.

3.3. Applications in Animal-Based Pharmaceutical Studies

In animal research, chromatographic methods have found a wide range of application to study the behavior and safety of the drug before going to human trial. The applications play a crucial role in comprehending the pharmacokinetics, metabolism, and toxicological effects.

Chemical In pharmacokinetics, chromatography is employed to measure the concentration of drugs in plasma and tissues with time, which allows calculating drug parameters including half-life, clearance, and bioavailability[35]. Chemical chromatography assists in drug metabolism research to determine the metabolism and identify intermediate compounds.

Cytotoxicological analyses also make use of chromatographic procedures to identify and measure toxic substances and biological metabolites in biological samples. These uses play a major role in the preclinical drug evaluation and approval of drugs[36].

4. CHALLENGES AND LIMITATIONS IN CHROMATOGRAPHIC ANALYSIS

Although dramatic improvements have been made, chromatographic methods have a number of shortcomings that may impact their effectiveness, availability, and relevance in the analysis of pharmaceuticals. Cost of instrumentation which includes the use of the latest systems such as UPLC and LC-MS/MS is one of the greatest constraints which not all research laboratories can afford.

Complexity of method development and validation is another important challenge. To create an efficient chromatographic system, all the parameters like mobile phase composition, column type, flow rate and the conditions used in detecting it should be optimised. This may be time consuming and needs talented individuals[37].

The problems of analyzing complex biological samples of animal research are further complicated. Endogenous compounds can interfere with accuracy and sensitivity and require the use of extensive sample preparation procedures. Although necessary, these procedures may add analysis time and variability.

Moreover, interlaboratory variability is not standardized, and as a result, the results of the analysis may vary. Differences in instrumentation, protocols, and experimental conditions can impact reproducibility and data comparability[38].

Finally, the environmental issues related to the use of organic solvents and disposal of chemical waste is another important problem. Even though green chromatography is now becoming a solution, this is not a widely used solution yet.

5. DISCUSSION

The quick development of the chromatographic methods has dramatically changed the analysis of pharmaceuticals, especially when it comes to animal experiments. The requirement of modern analysis techniques such as high sensitivity, speed and sustainability has propelled the generation of novel chromatographic techniques. The section discusses the important findings, their implications and gaps in research that exist critically with highlighting the future directions of further improvement[39].

5.1.Findings of the study

- Newer developments in chromatographic methods have shown a steep inclination towards high-performance forms of analysis, especially the introduction of UPLC which is much faster in analysis and yet remains high in resolution.

- The combination of very sensitive detection systems, particularly LC-MS/MS has made it possible to identify and quantify drugs and their metabolites in complex biological samples at trace levels.
- There has been increased interest in greener analytical methods such as SFC and greener solvents, which is in line with sustainable lab practices.
- Chromatographic methods have proven to be more applicable to animal research especially in the field of pharmacokinetics, toxicology and drug metabolism studies.
- Chromatography coupled with modern technologies has enhanced precision, reproducibility and reliability of analysis in preclinical research.

5.2.Implications and Significance

- The move to sophisticated chromatographic methods has dramatically enhanced the effectiveness and precision of pharmaceutical analysis, which is useful in making improved decisions in the development of drugs.
- Increased sensitivity in analytical sensitivity enables the detection of drug metabolites and toxic compounds at an earlier stage and this is essential in assuring drug safety during preclinical studies.
- The use of chromatography in animal studies helps in enhancing the understanding of drug behavior, such as absorption, distribution, metabolism, and excretion (ADME).
- Green chromatography methods are adopted to facilitate the sustainability of the environment through minimization of the use of hazardous solvents and wastage of chemicals.
- These innovations are important in addressing regulatory need and quality of pharmaceutical products, safety and efficacy.

5.3.Gaps and Future Research Directions

- Absence of standardized procedures across laboratories is a significant limitation, which has implication on reproducibility and comparability of findings.
- These instruments are very expensive and costly to operate, limiting access particularly in resource constrained environments.
- Minimal combination of chromatography methods with computational models and data-driven methods decreases the efficiency of method optimization and data analysis.
- Green chromatography methods should be further developed to reduce environmental impact.
- The research could be expanded and improved in the future through the incorporation of Artificial Intelligence (AI) and machine learning to interpret data automatically and optimize methods.
- Miniaturization of chromatographic systems, including microfluidic-based systems, can facilitate rapid, inexpensive and portable analysis.
- Better sample preparation methods should be developed to improve the accuracy of the analysis and decrease the time.
- High-throughput systems and more focus on automation will further simplify the pharmaceutical analysis processes.

6. CONCLUSION

Modern pharmaceutical analysis has become dependent on chromatographic methods because of the accuracy, reliability and repeatability of the results obtained of the various applications in which they are used. As new technology keeps changing, these methods have been modified to suit the increasing needs of complex drug formulations and biological matrices especially in preclinical tests involving animals[40]. The growing importance of sensitivity, speed and sustainability has also promoted innovations in chromatographic methodologies further justifying their role in drug development and regulatory procedures.

6.1. Summary of Main Insights and Conclusions

Chromatographic techniques have witnessed remarkable advancements in recent years, significantly enhancing their role in pharmaceutical analysis. Techniques such as High- HPLC, UPLC, GC, SFC, and various hyphenated methods have demonstrated improved analytical precision, sensitivity, and efficiency. These developments have enabled accurate detection and quantification of drugs and their metabolites, even at trace levels, particularly in complex biological matrices derived from animal-based studies.

The integration of advanced detection systems and automation has further strengthened the reliability and reproducibility of chromatographic methods. As a result, these techniques have become indispensable tools in preclinical research, supporting pharmacokinetic evaluation, toxicological assessment, and drug metabolism studies.

6.2. Reiteration of the Importance of the Review

- Chromatographic techniques are critically important in modern pharmaceutical analysis, particularly in animal-based studies.
- These techniques provide accurate, sensitive, and reproducible analytical data, which is essential for ensuring drug safety, efficacy, and quality.
- They play a significant role in meeting regulatory requirements and supporting the drug approval process.
- Chromatographic methods facilitate various stages of drug development, including preclinical evaluation.
- The increasing complexity of pharmaceutical compounds and biological matrices necessitates the continuous advancement of chromatographic technologies.
- Ongoing innovations in chromatography are essential for addressing emerging analytical challenges and improving preclinical research outcomes.

6.3. Recommendations

- Emphasis should be placed on the development and adoption of green chromatography techniques to reduce environmental impact and promote sustainable laboratory practices.
- Greater integration of automation and high-throughput systems is recommended to improve efficiency and reduce analysis time.
- The incorporation of Artificial Intelligence (AI) and machine learning should be encouraged for method development, optimization, and data interpretation.
- Efforts should be made to standardize chromatographic protocols across laboratories to enhance reproducibility and comparability of results.
- Future research should focus on miniaturized and cost-effective chromatographic systems to increase accessibility, particularly in resource-limited settings.

- Improved sample preparation techniques should be developed to simplify analysis of complex biological matrices and enhance accuracy.

REFERENCES

1. Khalid, M., Abubakar, M. F., & Kumar, L. (2024). Role of Chromatography in Pharmaceutical Analysis: Trends and Future Perspectives. INTERNATIONAL JOURNAL, 12(12), 1935-1940.
2. Rajput, J. M., Nandre, D. S., & Pawar, B. G. (2022). A comprehensive review on advanced chromatographic techniques and spectroscopic techniques in pharmaceutical analysis. Int J Pharm Res Appl, 7(53), 10-35629.
3. Zacharis, C. K., & Markopoulou, C. K. (2020). Recent trends in pharmaceutical analytical chemistry. Molecules, 25(16), 3560.
4. Beccaria, M., & Cabooter, D. (2020). Current developments in LC-MS for pharmaceutical analysis. Analyst, 145(4), 1129-1157.
5. Robards, K., & Ryan, D. (2021). Principles and practice of modern chromatographic methods. Academic Press.
6. Coskun, O., Akbarzadeh, S., & Günçer, B. (2025). Different chromatographic techniques and recent advancements for biomedical and pharmaceutical applications. Northern Clinics of Istanbul, 12(6), 739.

7. West, C. (2019). Recent trends in chiral supercritical fluid chromatography. TrAC Trends in Analytical Chemistry, 120, 115648.
8. Akash, M. S. H., & Rehman, K. (2020). Essentials of pharmaceutical analysis (pp. 167-174). Singapore:: Springer.
9. West, C. (2018). Current trends in supercritical fluid chromatography. Analytical and bioanalytical chemistry, 410(25), 6441-6457.
10. Badawy, M. E., El-Nouby, M. A., Kimani, P. K., Lim, L. W., & Rabea, E. I. (2022). A review of the modern principles and applications of solid-phase extraction techniques in chromatographic analysis. Analytical Sciences, 38(12), 1457-1487.
11. Adamovics, J. A. (Ed.). (2017). Chromatographic analysis of pharmaceuticals. Routledge.
12. Loos, G., Van Schepdael, A., & Cabooter, D. (2016). Quantitative mass spectrometry methods for pharmaceutical analysis. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, 374(2079), 20150366.
13. Ansari, S., & Karimi, M. (2017). Novel developments and trends of analytical methods for drug analysis in biological and environmental samples by molecularly imprinted polymers. TrAC Trends in Analytical Chemistry, 89, 146-162.
14. Yabré, M., Ferey, L., Somé, I. T., & Gaudin, K. (2018). Greening reversed-phase liquid chromatography methods using alternative solvents for pharmaceutical analysis. Molecules, 23(5), 1065.
15. Ermer, J., & Nethercote, P. W. (Eds.). (2025). Method validation in pharmaceutical analysis: A guide to best practice. John Wiley & Sons.
16. Fanali, S., Haddad, P. R., Poole, C., & Riekkola, M. L. (Eds.). (2017). Liquid chromatography: applications. Elsevier.
17. Felletti, S., Ismail, O. H., De Luca, C., Costa, V., Gasparrini, F., Pasti, L., ... & Catani, M. (2019). Recent achievements and future challenges in supercritical fluid chromatography for the enantioselective separation of chiral pharmaceuticals. Chromatographia, 82(1), 65-75.
18. Pirok, B. W., Stoll, D. R., & Schoenmakers, P. J. (2018). Recent developments in two-dimensional liquid chromatography: fundamental improvements for practical applications. Analytical Chemistry, 91(1), 240-263.
19. Cui, P., & Wang, S. (2019). Application of microfluidic chip technology in pharmaceutical analysis: A review. Journal of pharmaceutical analysis, 9(4), 238-247.
20. Tome, T., Žigart, N., Časar, Z., & Obreza, A. (2019). Development and optimization of liquid chromatography analytical methods by using AQbD principles: overview and recent advances. Organic process research & development, 23(9), 1784-1802.
21. Teixeira, J., Tiritan, M. E., Pinto, M. M., & Fernandes, C. (2019). Chiral stationary phases for liquid chromatography: recent developments. Molecules, 24(5), 865.
22. Stoll, D. R., & Carr, P. W. (2017). Two-dimensional liquid chromatography: a state of the art tutorial. Analytical chemistry, 89(1), 519-531.
23. Korany, M. A., Mahgoub, H., Haggag, R. S., Ragab, M. A., & Elmallah, O. A. (2017). Green chemistry: Analytical and chromatography. Journal of Liquid Chromatography & Related Technologies, 40(16), 839-852.

24. Patel, D. C., Wahab, M. F., Armstrong, D. W., & Breitbach, Z. S. (2016). Advances in high-throughput and high-efficiency chiral liquid chromatographic separations. Journal of Chromatography a, 1467, 2-18.
25. Zhang, H., Wang, Z., & Liu, O. (2015). Development and validation of a GC–FID method for quantitative analysis of oleic acid and related fatty acids. Journal of Pharmaceutical Analysis, 5(4), 223-230.
26. Alula, M. T., Mengesha, Z. T., & Mwenesongole, E. (2018). Advances in surface-enhanced Raman spectroscopy for analysis of pharmaceuticals: A review. Vibrational Spectroscopy, 98, 50-63.
27. Piovesana, S., Capriotti, A. L., Cavaliere, C., La Barbera, G., Montone, C. M., Zenezini Chiozzi, R., & Laganà, A. (2018). Recent trends and analytical challenges in plant bioactive peptide separation, identification and validation. Analytical and Bioanalytical Chemistry, 410(15), 3425-3444.
28. D'Atri, V., Barrientos, R. C., Losacco, G. L., Rudaz, S., Delobel, A., Regalado, E. L., & Guillarme, D. (2025). Trends in pharmaceutical analysis: the evolving role of liquid chromatography. Analytical Chemistry, 97(9), 4706-4727.
29. D'Atri, V., Fekete, S., Clarke, A., Veuthey, J. L., & Guillarme, D. (2018). Recent advances in chromatography for pharmaceutical analysis. Analytical chemistry, 91(1), 210-239.
30. Memon, N., Qureshi, T., Bhanger, M. I., & Malik, M. I. (2019). Recent trends in fast liquid chromatography for pharmaceutical analysis. Current Analytical Chemistry, 15(4), 349-372.
31. Sharma, S., Singh, N., Ankalgi, A. D., Rana, A., & Ashawat, M. S. (2021). Modern Trends in Analytical Techniques for Method Development and Validation of Pharmaceuticals: A Review. Journal of Drug Delivery & Therapeutics, 11.
32. Parys, W., Dołowy, M., & Pyka-Pająk, A. (2022). Significance of chromatographic techniques in pharmaceutical analysis. Processes, 10(1), 172.
33. Ganetsos, G., & Barker, P. E. (Eds.). (2019). Preparative and production scale chromatography. CRC Press.
34. Fekete, S., Beck, A., Veuthey, J. L., & Guillarme, D. (2015). Ion-exchange chromatography for the characterization of biopharmaceuticals. Journal of pharmaceutical and biomedical analysis, 113, 43-55.
35. de Godoi Passerine, B. F., & Breitkreitz, M. C. (2023). Recent developments in green chromatography. Braz. J. Anal. Chem, 10, 17-34.
36. Napolitano-Tabares, P. I., Negrín-Santamaría, I., Gutiérrez-Serpa, A., & Pino, V. (2021). Recent efforts to increase greenness in chromatography. Current Opinion in Green and Sustainable Chemistry, 32, 100536.
37. Dhoru, M., Shah, K., Detholia, K., & Patel, M. (2020). Green chromatography: the eco-friendly and safer concept of green analytical chemistry. International Journal of Pharmaceutical Sciences and Research, 11(3), 1022-1032.
38. Fekete, S., Veuthey, J. L., Beck, A., & Guillarme, D. (2016). Hydrophobic interaction chromatography for the characterization of monoclonal antibodies and related products. Journal of pharmaceutical and biomedical analysis, 130, 3-18.

39. Pati, S., Nie, B., Arnold, R. D., & Cummings, B. S. (2016). Extraction, chromatographic and mass spectrometric methods for lipid analysis. Biomedical Chromatography, 30(5), 695-709.
40. Dreassi, E., Ceramelli, G., & Corti, P. (2017). Thin-layer chromatography in pharmaceutical analysis. In Practical Thin-Layer Chromatography (pp. 231-247). CRC Press.