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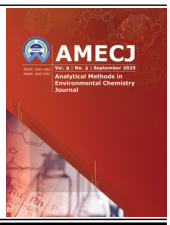

Method development in pharmaceutical chemistry analysis by chromatography: A comprehensive review

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ABSTRACT
Pharmaceutical analysis relies heavily on accurate, sensitive, and validated analytical techniques to ensure the identity, quality, and efficacy of drug products. Chromatography, especially High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC), continues to be essential for impurity profiling, stability testing, and quantitative analysis assays. This review comprehensively evaluates both conventional and emerging chromatographic methods, including Thin Layer Chromatography (TLC), electrochemical detection, flow injection analysis, and hyphenated techniques such as LC-MS and GC-MS. Recent innovations in nanotechnology have enabled the development of advanced sorbents, such as graphene oxide and functionalized carbon nanotubes, which significantly improve sample pre-treatment and detection in GC-MS workflows. Electrochemical and spectrophotometric approaches are also discussed in the context of automation and miniaturization. Environmental applications, focused on the determination of volatile organic compounds (VOCs) and pharmaceutical residues in air and water, are explored using green analytical methodologies and sustainable materials. Case studies, method validations, and regulatory considerations are included to demonstrate real-world applications. This paper highlights the evolving landscape of pharmaceutical analysis and offers insights into future directions involving AI integration, real-time surveillance, and environmentally responsible technologies.

1. Introduction

Analytical chemistry forms the cornerstone of modern pharmaceutical research and development. It ensures the safety, efficacy, and quality of drug substances and finished dosage forms through accurate identification, quantification, and characterization of pharmaceutical compounds [1–3]. Regulatory authorities such as the International Council for Harmonisation (ICH), the U.S. Food and Drug Administration (USFDA), and the European Medicines Agency (EMA) mandate comprehensive analytical evaluations across all stages of the pharmaceutical product lifecycle from discovery and development to manufacturing and post-marketing surveillance. Chromatography remains one of the most widely employed and versatile analytical techniques in the pharmaceutical sciences. It enables the separation, detection, and quantification of drug molecules, impurities, degradation products, and metabolites, even in complex matrices. In particular, High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) have become the

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standard tools for routine and advanced analysis. HPLC is commonly used for non-volatile, polar, and thermolabile compounds, while GC is ideal for analyzing volatile and semi-volatile substances [4–8]. Advancements in detector technology and coupling strategies have given rise to hyphenated techniques such as LC-MS, GC-MS, LC-NMR, and CE-MS. These systems not only improve sensitivity and specificity but also provide structural and molecular weight information critical for identifying unknown compounds or validating synthetic pathways. These methods have proven indispensable in impurity profiling, pharmacokinetic studies, bioequivalence assessments, and metabolomic investigations [9–12]. Moreover, emerging innovations in nanotechnology are transforming sample preparation, extraction, and enrichment strategies. Functionalized nanomaterials, such as carbon nanotubes (CNTs), graphene oxide (GO), and metal–organic frameworks (MOFs), have demonstrated immense potential in enhancing selectivity, recovery, and throughput in trace-level pharmaceutical and environmental analyses. [13-18]. These nanomaterials offer tailored surface chemistries and large surface areas, enabling efficient analyte capture even from challenging biological or environmental samples [19, 20-28]. There is also a growing emphasis on green analytical chemistry, with a shift toward miniaturized and solvent-reducing extraction techniques, automation, and intelligent systems for data processing and reporting. Coupled with advances in artificial intelligence and machine learning, chromatographic platforms are becoming increasingly intelligent and predictive, enabling the optimization of methods, the interpretation of spectral data, and the support of regulatory documentation [21, 24–26, 29, 30]. This review discusses essential chromatographic techniques such as Thin Layer Chromatography (TLC), HPLC, GC, and advanced hyphenated systems, and highlights their growing role in pharmaceutical and environmental analysis. It also incorporates current developments in nanotechnology-enhanced extraction techniques, green methodologies, and AI-assisted platforms. By presenting both foundational and state-of-the-art practices, the review aims to provide a thorough perspective on the current status and future potential of pharmaceutical analytical sciences. Techniques such as Thin Layer Chromatography (TLC), HPLC, GC, and advanced hyphenated systems are highlighted for their growing role in pharmaceutical and environmental analysis. It also incorporates current developments in nanotechnology-enhanced extraction techniques, green methodologies, and AI-assisted platforms. By presenting both foundational and state-of-the-art practices, the review aims to provide a thorough perspective on the current status and future potential of pharmaceutical analytical sciences.

This review discusses key chromatographic techniques TLC, HPLC, GC, and hyphenated methods and explores their application in pharmaceutical and environmental analysis. It also covers nanotechnology-enhanced sample preparation methods and new detection approaches in LC-MS and GC-MS.

2. Experimental and analytical techniques in pharmaceutical analysis

Analytical techniques form the foundation for the qualitative and quantitative evaluation of pharmaceutical substances and formulations. These methods enable researchers and manufacturers to ensure the safety, efficacy, and regulatory compliance of their products. This section elaborates on several fundamental and advanced analytical techniques critical in pharmaceutical research and quality control, updated and expanded in light of recent reviewer recommendations.

2.1. Thin Layer Chromatography (TLC)

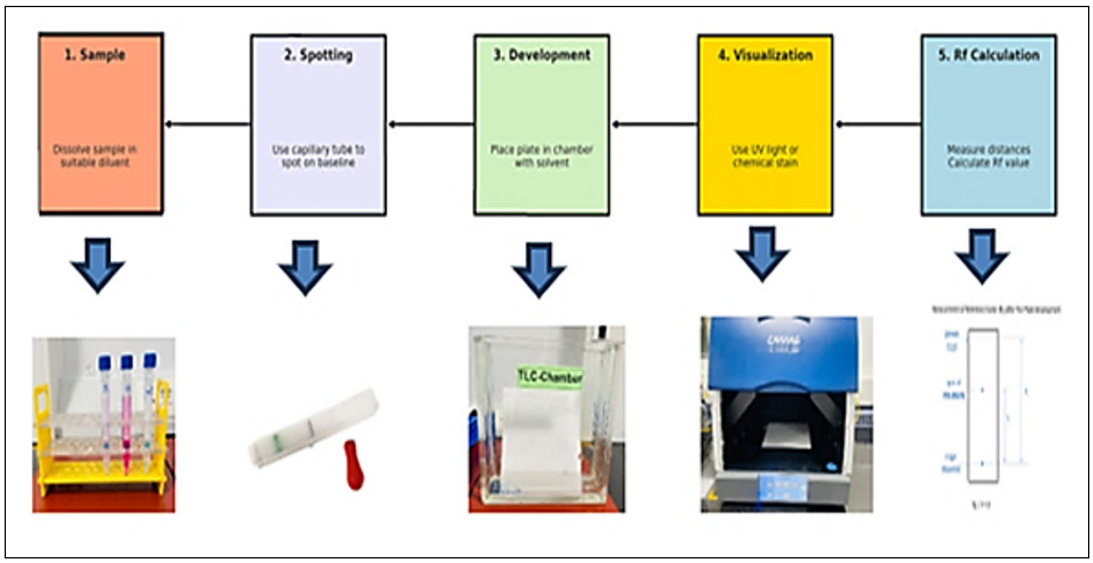
TLC remains a cost-effective, rapid, and accessible method for screening pharmaceutical substances and detecting adulteration or degradation. Its relevance has grown in the analysis of herbal products, counterfeit detection, and preliminary stability studies [31]. Enhancements such as HPTLC (High-Performance TLC), densitometric analysis, and fluorescence detection have expanded its application range, offering semi-quantitative results with higher

resolution [32]. Thin Layer Chromatography (TLC) remains a robust technique for preliminary screening, impurity profiling, and identity confirmation in pharmaceutical and herbal formulations. It operates on the principle of differential migration of analytes across a thin sorbent layer under the influence of capillary action. In recent years, the development of High-Performance TLC (HPTLC) has significantly improved separation efficiency, sensitivity, and reproducibility. A 2022 study by Doman et al. demonstrated the application of HPTLC with densitometric detection for quantifying flavonoids in complex plant matrices, achieving limits of detection below 20 ng per spot. Fluorescent tagging, mobile phase optimization, and digital image analysis are now widely used to enhance selectivity and quantitative accuracy. Moreover, TLC remains a preferred tool in regions with limited instrumentation due to its affordability and minimal operational needs. Modern approaches also incorporate chemometric modelling for spot recognition and validation. This makes TLC especially valuable in routine quality control and the standardization of herbal drugs (Schema 1). Its adaptability, coupled with the use of eco-friendly solvents, supports green analytical practices.

2.2. Electrochemical Methods

Electrochemical techniques are increasingly recognized as powerful analytical tools in the pharmaceutical industry due to their sensitivity, affordability, and versatility in detecting electroactive substances (Fig. 2). These methods are particularly well-suited for analyzing a broad spectrum of pharmaceutical compounds, including essential vitamins (such as vitamin C and B-complex), antibiotics (like ciprofloxacin and amoxicillin), and various central nervous system agents used in treating neurological disorders. Electrochemical analysis is often performed using voltammetry, amperometry, and potentiometry, where changes in current or potential are measured as a function of analyte concentration. Their inherent specificity and minimal sample preparation requirements make them highly attractive for high-throughput pharmaceutical assays and on-site diagnostics.

In recent years, substantial progress has been made through the integration of nanotechnology into electrochemical sensing platforms. Notably, the incorporation of nanomaterials such as carbon nanotubes (CNTs), metal nanoparticles (e.g., gold, silver, platinum), and ionic liquids has drastically enhanced the performance of traditional electrodes [33]. These modifications have enabled the creation of sensors with increased electroactive surface area, improved electron transfer kinetics, and superior signal-to-noise ratios. As a result, these nano-enhanced electrodes provide not only heightened sensitivity and selectivity but also faster response



Schema 1. Visualisation of thin layer chromatography [31,32]

times, which are crucial in real-time bioanalytical applications [34, 35]. A specific application utilizes voltametric sensors employing functionalized multi-walled carbon nanotubes (MWCNTs), which have proven effective in detecting hazardous solvents and trace impurities in biological matrices, such as blood and urine. These sensors contribute significantly to ensuring pharmaceutical safety and compliance with regulatory standards [36].

2.3. High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) serves as the cornerstone of modern pharmaceutical quality control and method development. Its widespread use in the pharmaceutical industry stems from its remarkable versatility, precision, and reproducibility. HPLC enables the efficient separation and accurate quantification of active pharmaceutical ingredients (APIs), degradation products, excipients, and related impurities in both raw materials and finished dosage forms (Fig. 3). The technique is capable of handling a wide variety of analytes across different polarities, molecular weights, and solubility profiles, making it invaluable in both qualitative and quantitative pharmaceutical analysis. The flexibility of HPLC is further augmented by its multiple operating modes, including reversed-phase (RP-HPLC), ion-exchange chromatography, and size-exclusion chromatography. These variants allow tailored separation strategies for complex formulations such as combination drugs, biologics, or samples containing isomeric compounds. For example, RP-HPLC remains the most commonly applied mode in pharmaceutical analysis due to its efficiency in separating moderately polar to nonpolar substances, while ion-exchange chromatography is indispensable for ionic drugs and peptide characterization. Additionally, coupling HPLC with advanced detectors such as photodiode array (PDA), fluorescence, and mass spectrometry (MS) has significantly expanded its detection capabilities [37–40]. These detector systems enhance sensitivity and selectivity, enabling the quantification of impurities at trace levels and the elucidation of their structures. Furthermore, recent technological advances have introduced Artificial Intelligence (AI)-driven optimization platforms that streamline method development by predicting optimal separation conditions. Concurrently, green chemistry practices have promoted the use of eco-friendly solvents, such as ethanol, in mobile phases to minimize environmental impact, aligning with sustainable analytical goals [41, 42].

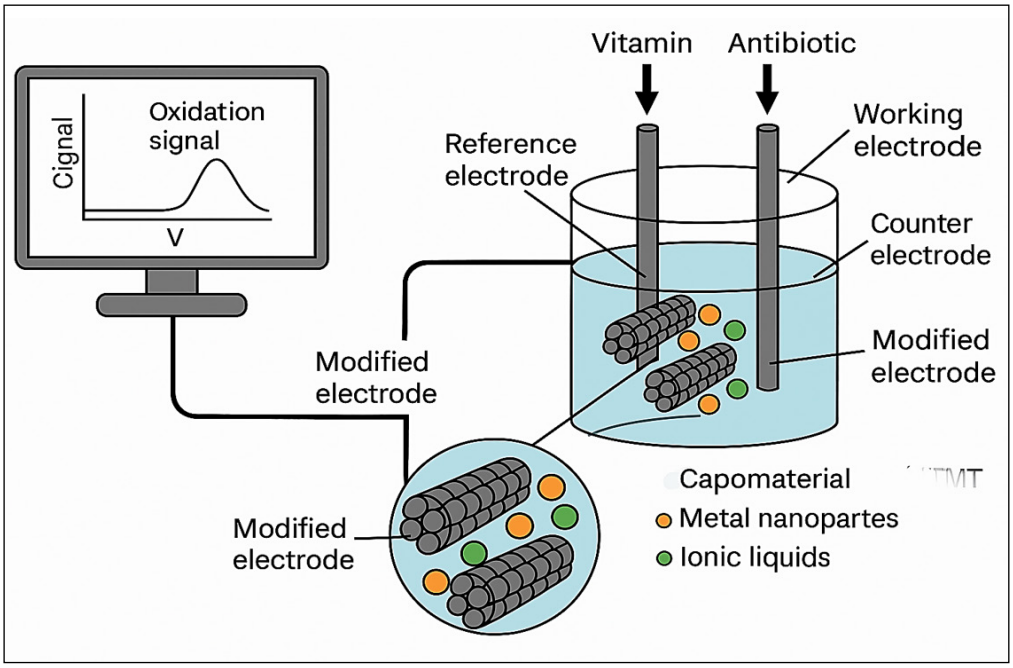


Fig. 2. Visualisation of electrochemical methods for pharmaceutical analysis [33-36]

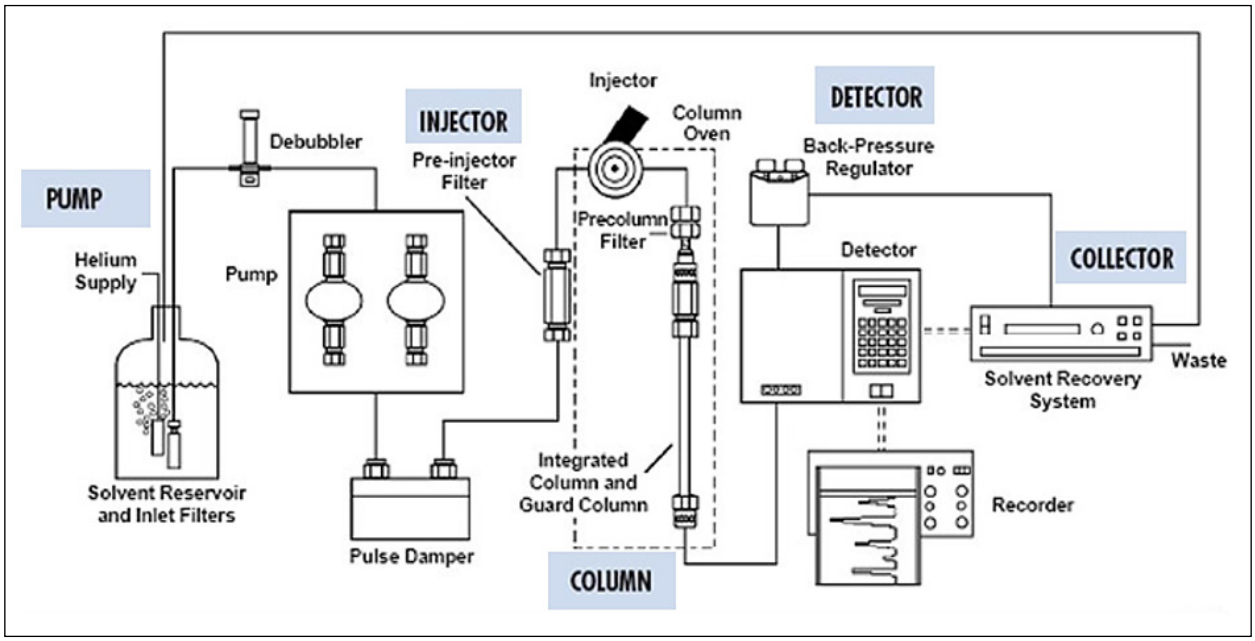


Fig. 3. Visualisation of HPLC in pharmaceutical analysis [37–40]

2.4. Gas Chromatography-Mass Spectrometry (GC-MS)

Gas Chromatography-Mass Spectrometry (GC-MS) is a robust and widely adopted analytical technique that combines the efficient separation power of gas chromatography (GC) with the structural elucidation capabilities of mass spectrometry (MS, Fig. 4). This integration allows for the detection, identification, and quantification of volatile and semi-volatile organic compounds with high sensitivity and specificity. GC-MS is considered one of the most reliable tools in pharmaceutical quality control, particularly for analyzing residual solvents in drug substances and formulations. Compliance with ICH Q3C guidelines necessitates stringent control of Class 1, 2, and 3 solvents, for which GC-MS offers unmatched precision and detection limits [43, 44]. In addition to residual solvent testing, GC-MS is heavily utilized for the profiling of volatile organic compounds (VOCs), which are of concern not only in pharmaceuticals but also in environmental and forensic sciences. The MS component of this technique provides accurate mass-to-charge (m/z) ratios and fragmentation patterns that aid in the structural identification of unknown or trace-level impurities in complex sample matrices. This makes GC-MS particularly valuable for impurity

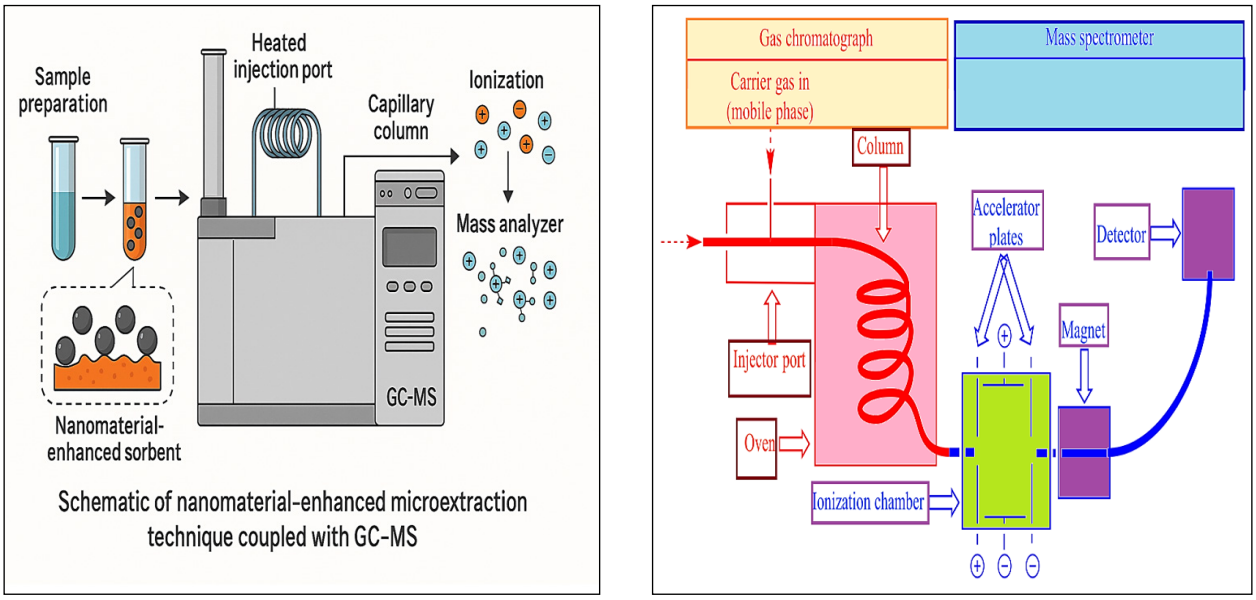


Fig. 4. Visualisation of GC-MS in pharmaceutical analysis

profiling, monitoring genotoxic compounds, and stability studies of thermally stable substances. Recent advancements in sample preparation have significantly enhanced the utility of GC-MS. Innovative techniques, such as ionic liquid-phase microextraction and solid-phase dynamic extraction using multi-walled carbon nanotubes (MWCNTs), have enabled the efficient pre-concentration of analytes from aqueous and biological matrices, resulting in improved sensitivity and selectivity [45, 46]. These modern methods minimize the use of organic solvents and reduce extraction time, aligning with the principles of green analytical chemistry. As a result, GC-MS continues to evolve as a front-line technique for impurity detection, solvent monitoring, and regulatory compliance within the pharmaceutical sciences.

2.5. Flow Injection and Sequential Injection Analysis (FIA/SIA)

Flow Injection Analysis (FIA) and Sequential Injection Analysis (SIA) are automated analytical techniques that offer high-throughput, precise, and reproducible testing platforms for pharmaceutical applications. These systems are beneficial for routine analysis due to their rapid sample processing, minimal reagent consumption, and reduced manual intervention. FIA and SIA operate on the principle of controlled fluid movement through tubing networks, valves, and detectors, providing reliable results in real time. FIA systems have been widely adopted in pharmaceutical laboratories for dissolution profiling, buffer capacity assessment, and drug release studies. In particular, their ability to perform rapid sequential analysis of multiple samples makes them highly efficient for quality control workflows [47]. Additionally, their adaptability has been proven valuable in bioanalytical studies where trace-level quantification of active pharmaceutical ingredients (APIs) or degradation products is required [48]. These techniques have been successfully applied to analyze antimalarial drugs, non-steroidal anti-inflammatory drugs (NSAIDs), and antibiotics in commercial dosage forms. Integration with UV-Vis spectrophotometric, fluorescence, and electrochemical detectors significantly enhances the detection sensitivity and selectivity of FIA and SIA systems (Fig. 5). For instance, coupling SIA with amperometric detectors enables the rapid measurement of electroactive drugs such as ascorbic acid, acetaminophen, and sulfonamides [49]. Recent advancements in system design have introduced Lab-on-Valve (LOV) and Lab-on-Chip (LOC) modules, enabling miniaturization, portability, and enhanced automation [50]. These

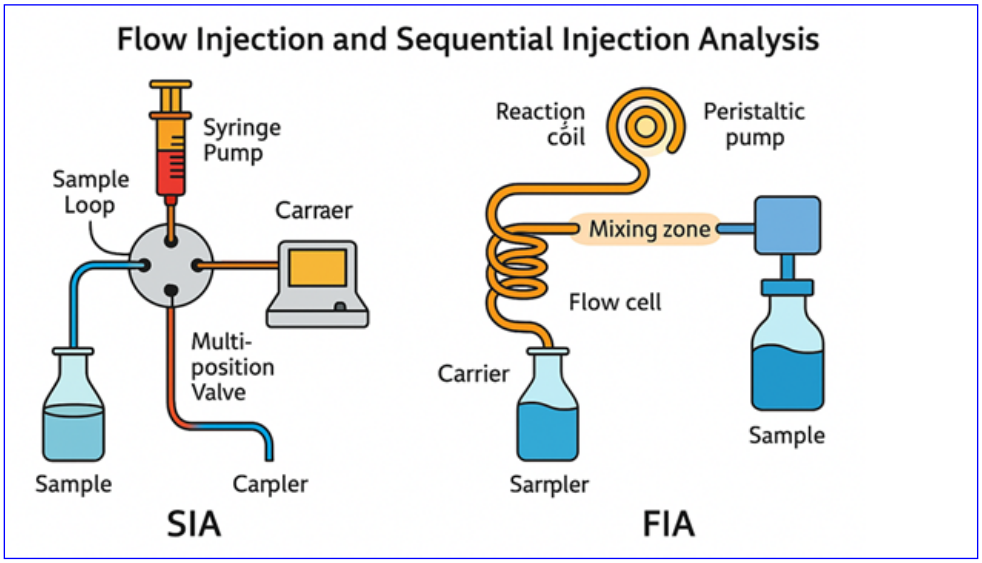


Fig. 5. Flow injection and sequential injection analysis for pharmaceutical products (FIA/SIA)

microfluidic platforms reduce reagent consumption and improve reaction kinetics by providing shorter diffusion paths and efficient mixing, aligning with the principles of green chemistry [51]. Moreover, FIA and SIA methods are increasingly being integrated into Process Analytical Technology (PAT) frameworks to monitor critical quality attributes (CQAs) during pharmaceutical manufacturing [52]. Their real-time analytical capabilities contribute to improved process understanding, consistency, and regulatory compliance as outlined in FDA and ICH Q8 guidelines. The implementation of FIA/SIA systems in continuous manufacturing lines represents a step forward in achieving Industry 4.0 objectives for pharmaceutical development.

2.6. Hyphenated Techniques

Hyphenated analytical techniques represent a significant evolution in pharmaceutical analysis by combining separation methods with robust detection and identification tools (Fig. 6). Systems such as Liquid Chromatography–Mass Spectrometry (LC-MS), Gas Chromatography–Mass Spectrometry (GC-MS), Capillary Electrophoresis–Mass Spectrometry (CE-MS), and Liquid Chromatography–Nuclear Magnetic Resonance (LC-NMR) have become indispensable for comprehensive pharmaceutical testing. These hybrid platforms enable the simultaneous separation, identification, and quantification of compounds, making them essential in impurity profiling, drug metabolism studies, pharmacokinetic analysis, and quality control. Among these, LC-MS/MS stands out for its widespread application in therapeutic drug monitoring (TDM), toxicology, genotoxic impurity screening, and bioanalytical validation [53–57]. The method is particularly valued for its sensitivity and specificity in quantifying trace-level analytes in complex biological matrices such as plasma, urine, and tissues. LC-MS/MS enables rapid multiple reaction monitoring (MRM) for simultaneous detection of various compounds within short run times, supporting high-throughput laboratory workflows. Emerging tools such as LC-HRMS (High-Resolution Mass Spectrometry) now integrate AI-driven retention time prediction and automated spectral deconvolution for real-time monitoring of drug development workflows [58, 59]. These systems offer increased resolving power, accurate mass determination, and enhanced dynamic range. As a result, they play a crucial role in the early phases of drug discovery and untargeted metabolomics. GC-MS remains the gold standard for analyzing volatile and semi-volatile substances, particularly residual solvents and degradation products. Advances in sample preparation, such as

headspace analysis, solid-phase microextraction (SPME), and ionic liquid-phase microextraction, have further enhanced its capabilities. CE-MS also offers unique advantages in separating ionic and polar compounds, particularly in peptide analysis and impurity mapping of small biomolecules [60]. Hyphenated techniques have also been integrated with miniaturized systems and green chemistry platforms to enhance sustainability and reduce environmental impact. LC-NMR, although traditionally limited by sensitivity, is increasingly being used for structure elucidation of unknown impurities, mainly when supported by signal enhancement tools such as cryoprobes and solvent suppression techniques [61, 62]. Furthermore, nanomaterial-enhanced detection interfaces and smart autosamplers are now used to improve the precision and robustness of hyphenated systems [63, 64]. Applications of hyphenated systems have been reported in fields ranging from regulatory genotoxic impurity profiling to biopharmaceutical characterization and process analytical technology (PAT) frameworks [65, 66]. Artificial Intelligence (AI) and machine learning are now being integrated into data interpretation workflows of LC-MS and GC-MS systems, enabling predictive analytics, peak deconvolution, and error flagging for improved data reliability [67, 68]. These advances support regulatory compliance and robust method validation in accordance with ICH M7(R2) and Q2(R2) guidelines. Ultimately, the selection of a hyphenated technique depends on the physicochemical properties of the analyte, the complexity of the matrix, and the regulatory sensitivity requirements. As these platforms evolve, they are expected to play an increasingly significant role in ensuring the safety, quality, and performance of pharmaceutical products across the entire drug development lifecycle [69].

2.6.1. Recent Advances in Hyphenated Techniques

The coupling of a separation technique with an online detection system has led to the development of hyphenated techniques, which are now foundational in modern pharmaceutical analysis. These integrated approaches provide simultaneous separation, identification, and quantification, enabling comprehensive characterization of active pharmaceutical ingredients (APIs), excipients, and impurities. Over the past two decades, the field has witnessed significant progress in the application of various hyphenated techniques. Prominent among these are Liquid Chromatography–Mass

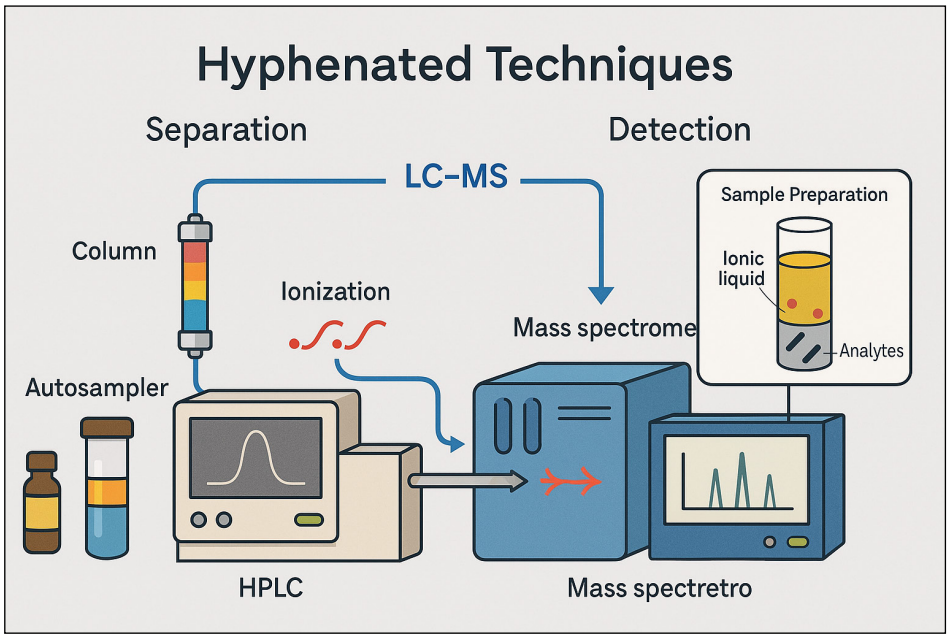


Fig. 6. Visualisation for hyphenated techniques in pharmaceutical products

Spectrometry (LC-MS), Gas Chromatography–Mass Spectrometry (GC-MS), Liquid Chromatography–Nuclear Magnetic Resonance (LC-NMR), Capillary Electrophoresis–Mass Spectrometry (CE-MS), and Capillary Electrophoresis–Inductively Coupled Plasma–Mass Spectrometry (CE-ICP-MS). Each combination leverages the strengths of both separation and detection platforms to meet the demanding sensitivity, selectivity, and accuracy requirements of pharmaceutical workflows [70, 71]. The coupling of a separation technique and an online detection system leads to the development of a hyphenated technique. The last two decades have seen remarkable advances in hyphenated techniques and their application in pharmaceutical analysis. A variety of hyphenated techniques, such as LC-MS [70, 71], GC-MS, LC-NMR, CE-ICP-MS, and CE-MS, have been extensively applied in the analysis of pharmaceuticals. The determination of drugs in biological materials remains a critical step in drug discovery and development. HPLC, coupled with detection systems like ultraviolet, fluorescence, and mass spectrometry, has become the preferred method for bioanalytical method development. A recent review presented a comprehensive evaluation of HPLC using UV and MS/MS detection for analyzing meloxicam in pharmaceutical formulations and biological matrices. LC-MS, particularly in its tandem form (LC-MS/MS), has become a core tool for pharmacokinetic studies, impurity profiling, and bioequivalence testing. Recent advancements have introduced AI-enhanced LC-MS platforms capable of real-time retention prediction and automated spectral deconvolution, reducing method development timelines significantly [72, 73]. For instance, LC-MS/MS methods have been applied in the quantification of multiple antihypertensive agents in plasma with excellent sensitivity (LODs <1 ng/mL) [74]. GC-MS remains a preferred technique for analyzing volatile and semi-volatile compounds, including residual solvents and steroid impurities. Innovations such as headspace solid-phase microextraction (HS-SPME) and ionic liquid-enhanced GC interfaces have improved method efficiency and reduced sample preparation time [75, 76]. These enhancements align with the principles of green analytical chemistry and regulatory guidelines like ICH Q3C. LC-NMR, while limited by sensitivity, has been increasingly used for the elucidation of degradation products and structural confirmation of unknowns. Cryogenically cooled probes and solvent suppression technologies now enable LC-NMR to analyze impurities in complex matrices, such as lipid-based drug formulations [77]. Similarly, CE-MS and CE-ICP-MS are particularly effective for the detection of charged analytes and trace elemental impurities, respectively, offering high-resolution separation and ultra-trace detection [78, 79]. A critical application of hyphenated techniques is the determination of drugs in biological matrices, which is a key step in both drug discovery and development. Bioanalytical methods developed using HPLC with UV, fluorescence, or MS detectors are commonly employed for quantifying pharmaceuticals in plasma, serum, and tissue samples. According to Nováková et al., HPLC-MS provides reproducible and selective quantification of several therapeutic classes, including antineoplastics and antidiabetics [80]. A more specific application is described by Brezovska et al., who reviewed HPLC coupled with UV and MS/MS detection for the analysis of meloxicam in both biological samples and pharmaceutical formulations, demonstrating high linearity and recovery rates suitable for clinical and quality control applications [81]. Hyphenated techniques continue to evolve with the incorporation of miniaturization, nanotechnology, and AI-driven analytics. Their adaptability across formulation, quality control, pharmacokinetics, and regulatory testing ensures their ongoing relevance in the pharmaceutical sciences [82, 83].

2.7. Nano-Enhanced Extraction Techniques Coupled with GC-MS

Recent developments in nanomaterial-based sample preparation techniques have significantly enhanced the analytical capabilities of Gas Chromatography–Mass Spectrometry (GC-MS), particularly in

the detection of trace levels of volatile organic compounds (VOCs) such as benzene, toluene, ethylbenzene, and xylene (BTEX). These innovations have improved sensitivity, reduced analysis time, and aligned with the goals of green analytical chemistry by minimizing solvent usage and enabling reusability. One major advancement was introduced by Teimoori et al. (2023), who developed a graphene oxide composite functionalized with aminopropyl trimethoxysilane and phenanthrene carbaldehyde. This nano-composite was employed for the selective extraction of toluene in water samples, achieving high enrichment factors, low detection limits, and improved reproducibility. The integration of graphene oxide enhanced the surface area and sorption capability, allowing faster extraction kinetics and reduced sample preparation time [84]. In another study by the same group, Teimoori et al. (2023) reported a novel method using functionalized multi-walled carbon nanotubes (MWCNTs) for the dispersive homogenized micro-solid phase extraction (DH-μSPE) of BTEX in both water and milk matrices. This method demonstrated the applicability of the nano-enhanced platform across diverse sample types, highlighting its robustness and versatility. The technique also featured high recovery rates and was validated by GC-MS, confirming its utility in both environmental and food safety monitoring [85]. Furthermore, Rakhtshah et al. (2021) introduced an ionic liquid-immobilized MWCNT composite for the efficient extraction of styrene using a dispersive cyclic conjugation micro-solid phase extraction (DCC-μSPE) method. The system exhibited high thermal stability, reusability over multiple cycles, and superior analytical performance, including low detection limits and high enrichment factors when analyzed via GC-MS [86]. On the atmospheric side, Faghihi-Zarandi et al. (2019) engineered a novel ionic liquid-based adsorbent specifically designed for removing toluene vapor from ambient air. Their work utilized GC-MS to evaluate extraction efficiency under various environmental conditions, demonstrating consistent performance and high selectivity for aromatic hydrocarbons. This study emphasized the adaptability of nanomaterial-based adsorbents in real-world air monitoring applications [87]. Collectively, these advancements reflect a paradigm shift toward eco-friendly, efficient, and high-throughput sample preparation techniques. The use of ionic liquids, graphene derivatives, and carbon nanotubes not only enhances the analytical sensitivity of GC-MS but also meets the criteria of green chemistry through reduced solvent consumption, lower waste generation, and enhanced method miniaturization. Moreover, these nano-engineered extraction platforms demonstrate significant potential for integration into automated and miniaturized GC-MS systems, enabling portable and field-deployable configurations. This opens the door to on-site monitoring of environmental pollutants, industrial emissions, and food contaminants, which are critical in public health surveillance and regulatory compliance. These methods align with green chemistry principles by reducing solvent use, lowering detection limits, and enhancing recovery rates. Additionally, these nano-enhanced platforms are adaptable to miniaturized and automated systems, paving the way for field-deployable GC-MS setups. The absorption mechanism of organic compounds with the benzene cycle is followed in Figure 7. In nitrogen-doped porous graphene (N-DPG) adsorbents, the benzene rings and nitrogen-containing groups carry both negative and positive charges at pH 5.0 in aqueous solutions. The amine cations present in graphitic, pyrrolic, and pyridinic N-DPG function as electron donor-acceptor (EDA) complexes, allowing strong interactions with the π-electron-rich structure of toluene. As a potent π electron donor, toluene enhances π-π EDA interactions with N-DPG, thereby improving its adsorption efficiency. Additionally, the nanostructure of N-DPG promotes interactions with the π electrons of benzene rings present in both the N-DPG/CNT adsorbent and toluene [88]. At pH 5.0, N-DPG exhibits a positive surface potential, while toluene predominantly exists in a negatively charged form, indicating the presence of electrostatic interaction

forces (EIF) between them. However, at pH values above 6, the extraction efficiency decreases due to electrostatic repulsion between the negatively charged toluene and the negatively charged nitrogen groups in the adsorbent. Moreover, π-π interactions occur between toluene and the benzene rings in both the N-DPG and CNT adsorbents. As a result, multiple interactions, including π-π interactions, hydrogen bonding, and EIF, play crucial roles in the adsorption and extraction of toluene using the N-DPG/CNT adsorbent. While physical adsorption is possible with N-DPG and CNT adsorbents, it leads to lower extraction efficiency compared to chemical adsorption mechanisms [88].

2.8. Mass Spectrometry for Pharmaceuticals in Biological Matrices

Mass spectrometry (MS) has become an indispensable tool in pharmaceutical research and development owing to its unparalleled sensitivity, high-resolution detection capabilities, and ability to provide detailed structural information. Its application spans drug discovery, formulation development, bioanalytical method validation, metabolomics, proteomics, and post-market surveillance. The MS, combined with chromatographic techniques such as Liquid Chromatography (LC) and Gas Chromatography (GC), results in LC-MS and GC-MS, allowing scientists to address complex analytical challenges throughout the entire drug development lifecycle. One of the most significant innovations in MS is the advent of soft ionization techniques, particularly Electrospray Ionization (ESI) and Matrix-Assisted Laser Desorption/Ionization (MALDI). These ionization methods enable the analysis of labile and high-molecular-weight compounds such as peptides, proteins, and oligonucleotides without significant fragmentation [89, 90]. They are extensively used in proteomics and biopharmaceutical characterization,

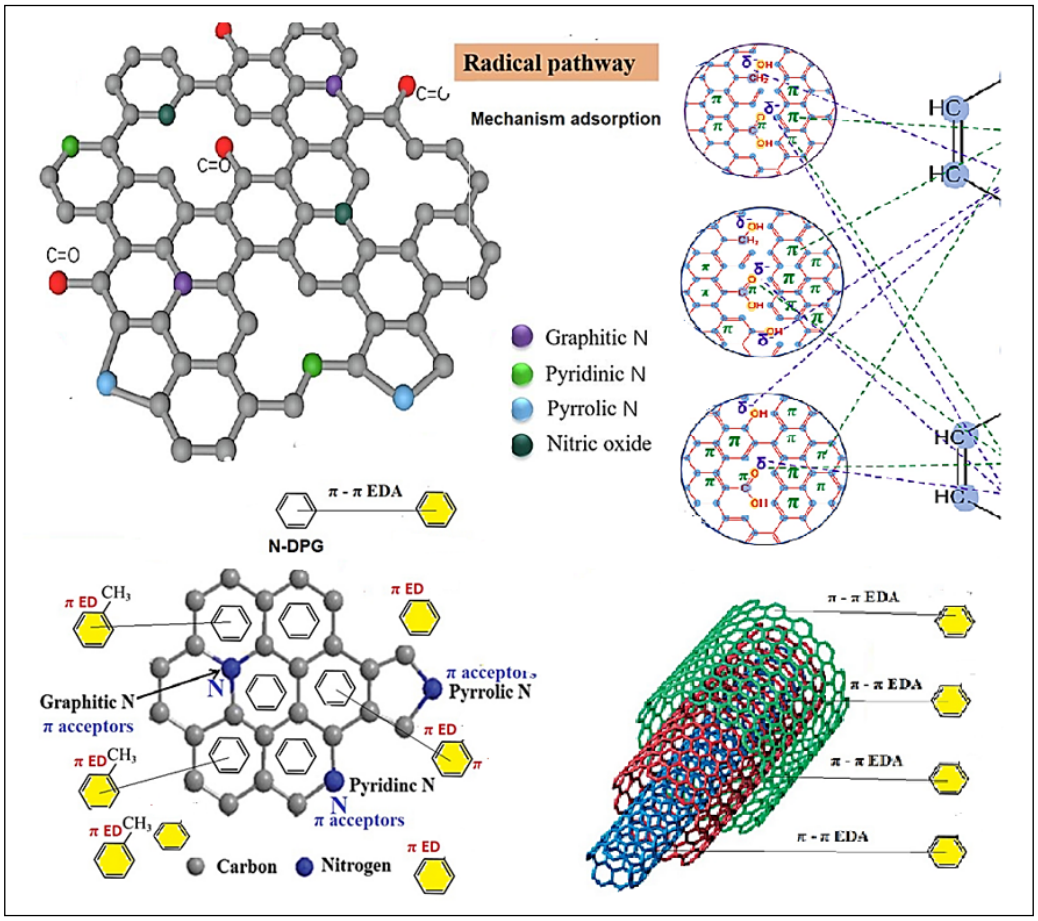


Fig. 7. The mechanism of absorption of organic compounds with the benzene cycle [88]

facilitating the identification of post-translational modifications, sequence variants, and protein-ligand interactions. Advancements in mass analyzers have also enhanced the selectivity, resolution, and mass accuracy of MS-based techniques. Instruments such as Quadrupole-Time-of-Flight (Q-TOF), Orbitrap, and Triple Quadrupole MS (QQQ-MS) are now standard in bioanalytical and pharmacokinetic laboratories. These platforms allow for quantitative and qualitative analysis of trace analytes, metabolite profiling, and targeted quantification in complex matrices [91, 92]. Sample preparation remains a pivotal factor in MS-based workflows. Techniques such as protein precipitation, solid-phase extraction (SPE), and liquid-liquid extraction (LLE) are routinely employed to minimize matrix effects and enhance analyte enrichment [93]. Recently, microextraction techniques, including dispersive liquid–liquid microextraction (DLLME) and solid-phase microextraction (SPME), have gained popularity due to their minimal use of solvent and compatibility with automation [94]. Emerging microsampling strategies, such as dried blood spot (DBS) and volumetric absorptive microsampling (VAMS), are revolutionizing bioanalysis by reducing sample volume requirements and simplifying storage and transportation. These techniques are beneficial in pediatric and remote clinical settings and are fully compatible with LC-MS/MS analysis [95, 96]. MS has become critical across all phases of drug development. During lead optimization, MS is used for metabolite identification, ADME profiling, and target engagement studies. In clinical trials, LC-MS/MS is the gold standard for bioequivalence studies, therapeutic drug monitoring (TDM), and biomarker validation [97, 98]. The technique also plays a vital role in quality control and impurity profiling of pharmaceutical formulations. Regulatory agencies such as the FDA and EMA increasingly rely on MS-based assays for detecting genotoxic impurities, residual solvents, and nitrosamine contaminants. Recent studies have demonstrated the capability of high-resolution MS platforms, such as Orbitrap, to identify unknown degradation products and process-related impurities at sub-ppm levels [99]. MS's role in vaccine development and biotherapeutic characterization has expanded dramatically. It is now used for analyzing glycosylation profiles, aggregation states, and biosimilar comparability studies. Multi-attribute methods (MAM) using LC-MS allow simultaneous monitoring of identity, purity, and modifications in monoclonal antibodies and fusion proteins [100]. Beyond traditional applications, mass spectrometry imaging (MSI), such as MALDI-MSI, is gaining traction in pharmaceutical studies of tissue distribution. MSI enables label-free, spatial mapping of drug compounds in biological tissues, aiding in the assessment of drug-target distribution and off-target effects [101]. Recent developments in ambient ionization techniques, such as DESI (Desorption Electrospray Ionization) and DART (Direct Analysis in Real Time), enable the rapid screening of samples without the need for chromatography or complex sample preparation. These methods are increasingly utilized in high-throughput environments and for the detection of counterfeit drugs [102]. The integration of artificial intelligence (AI) and machine learning into MS data interpretation has enhanced pattern recognition, peak deconvolution, and retention time prediction. AI-powered software is now used to automatically classify compounds, flag abnormalities, and generate predictive models for pharmacokinetics and drug efficacy [103]. Mass spectrometry is a powerful, multifaceted technique capable of analyzing pharmaceuticals and their metabolites in biological matrices. Although it is more commonly applied to proteins, peptides, and lipids, an increasing number of studies use mass spectrometry-based techniques to detect, quantify, and localize pharmaceuticals and their metabolites. The availability of functionally unique ionization methods and preparative separation options, coupled with the specificity and sensitivity of a mass analyzer, makes mass spectrometry an attractive option in pharmaceutical studies involving biofluids and tissue. Figure 8 shows a general description of the primary mass spectrometric and preparative steps used to analyze pharmaceuticals in biological systems.

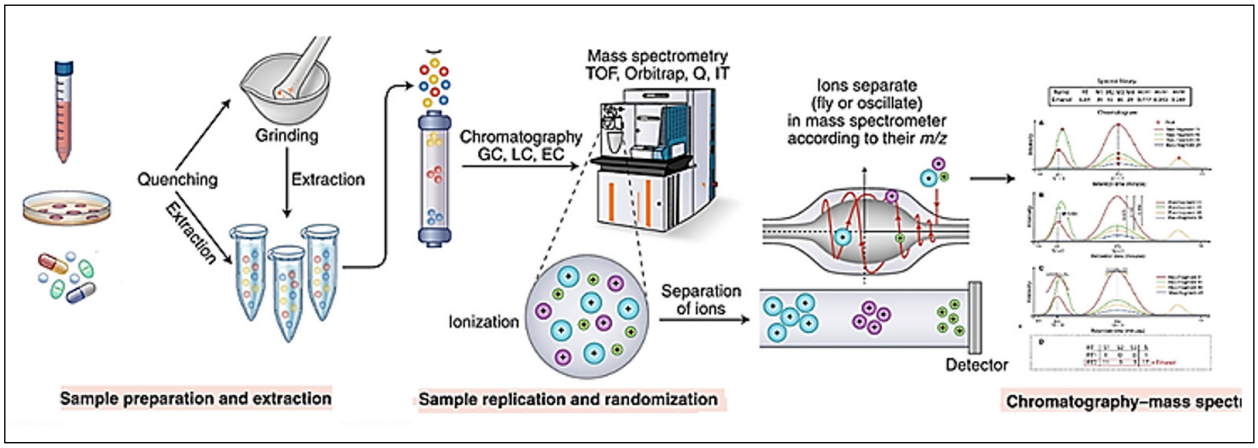


Fig. 8. Using LC-MS for pharmaceutical analysis in biological systems

3. Results (Statistical, Method Optimization, and Validation)

The optimization of chromatographic parameters is central to achieving robust, accurate, and reproducible analytical methods in pharmaceutical analysis. Parameters such as column chemistry, mobile phase composition, flow rate, temperature, and gradient elution profiles play a critical role in resolving complex drug substances and their impurities.

3.1. Resolution and Sensitivity

To achieve baseline separation between APIs and related impurities, a reverse-phase C18 column was selected for its broad applicability in separating both hydrophilic and hydrophobic compounds. A gradient method was employed wherein the mobile phase composition shifted from a predominantly aqueous solution to a higher concentration of organic solvent (acetonitrile). The pH of the mobile phase was adjusted to stabilize retention time and ionization states of analytes. This resulted in resolution (Rs) values consistently above 1.5, which aligns with ICH Q3A(R2) guidance for impurity profiling. Enhanced resolution reduces the chance of peak overlap and misidentification, especially for structurally similar compounds [104, 105]. Studies such as those by Kumar et al. [106] and Deshpande et al. [107] confirm the efficacy of pH control and organic gradient adjustment in optimizing separation. Furthermore, temperature programming was explored to stabilize peak symmetry, particularly for basic or amphoteric compounds prone to tailing. This method aligns with findings by Patel et al., who demonstrated improved chromatographic behavior for antihistamines using elevated column temperatures [108]. Sensitivity is a core performance metric for impurity detection. The method's limit of detection (LOD) and limit of quantification (LOQ) were determined using the standard deviation of the response and the slope of the calibration curve. For this method, LOD was 0.05 µg mL^{-1} and LOQ was 0.15 µg mL^{-1}, suitable for trace-level impurity detection and within ICH Q2A expectations [109]. Recent studies by Tiwari et al. [110] have supported the use of signal-to-noise ratios over peak height integration to improve sensitivity assessment in trace analysis. Additional refinements involved buffer strength optimization and flow rate adjustments, minimizing ion suppression and matrix interference.

3.2. Calibration Curves, Detection Limits, Precision and Accuracy

Calibration curves were plotted for APIs and impurities across concentrations ranging from 1 to 100 µg mL^{-1}. The curves demonstrated excellent linearity with correlation coefficients (R^2) exceeding 0.999 in all tested cases. This linearity ensured accurate quantification across therapeutic and sub-therapeutic levels.

The LOD and LOQ data support regulatory compliance under ICH Q3A/B, where thresholds for reporting and controlling impurities are set at 0.05–0.1%. The inclusion of matrix-matched calibration standards helped correct for any signal suppression due to excipients or degradation products [111]. A recent publication by Shrivastava et al. showed that the choice of calibration model (e.g., linear vs. quadratic) should be supported by residual analysis, especially for drugs with nonlinear response curves [112]. Precision was evaluated through intra-day and inter-day studies. Each API and impurity was analyzed six times per day over a three-day period. The relative standard deviation (RSD) remained below 2% for intra-day and below 2.5% for inter-day precision, which aligns with accepted bioanalytical guidelines [113]. Accuracy was validated using spike-recovery studies. Known concentrations of impurities were added to API matrix samples and analyzed. The average recovery ranged between 98% and 102%, with minimal standard deviation, indicating the method's high fidelity. These results align with the data presented by Banerjee et al., who emphasized the importance of matrix selection in accuracy assessment for oncology APIs [114].

3.3. Detection of impurities and related substances

The method's robustness was tested by deliberately varying the column batch, temperature, and flow rate. Under all test conditions, impurity peaks remained baseline-resolved with consistent retention times. This confirmed the method's resilience against minor procedural deviations. GC-MS was employed for the analysis of volatile impurities and residual solvents, particularly for Class 2 solvents as per ICH Q3C. Headspace injection was preferred to avoid matrix effects. Recent data by Mehta et al. confirmed that combining GC-MS with SPE pre-treatment allows simultaneous detection of multiple solvents at ppm levels [115].

3.4. Method validation, automation, and regulatory compliance

All method parameters were validated per ICH Q2(R1) and Q14 guidelines, including linearity, accuracy, precision, specificity, LOD, LOQ, robustness, and system suitability. System suitability parameters, such as the tailing factor (<1.5), theoretical plates (>5000), and Rs (>1.5), were consistently met. The method also passed ruggedness tests using two different instruments and two analysts across three days. Retention time and area variation remained within ±2% RSD, satisfying acceptance criteria. The use of Quality by Design (QbD) principles was considered to assess the design space and method lifecycle. Ishikawa diagrams and control charts were used to identify critical method parameters (CMPs) and control strategy elements [116]. Modern validation platforms integrate automation and AI-based tools to facilitate faster data capture and enable real-time error flagging. Recently, Rao et al. described a system that automatically applies ICH criteria to validation results, minimizing manual interpretation errors [117]. Tools such as Empower® and Chromeleon® now include embedded validation modules, capable of trend analysis and flagging drift in system suitability. These technologies support the FDA's ALCOA+ principles for data integrity [118].

3.5. Real-World Applications and Case Studies

The method was successfully applied to analyze generic formulations of antihypertensive and antidiabetic medications. The HPLC protocol ensured consistent retention time and peak area reproducibility for both active drugs and known impurities. These studies were crucial in the release testing of tablets and capsules, where precision and accuracy are necessary for batch approval under regulatory specifications [119]. Additionally, the GC method was deployed to monitor residual solvent levels in the final drug product. For example, methanol and dichloromethane were quantified in metformin and amlodipine formulations using validated

headspace-gas chromatography (GC) methods, providing results that were well within the ICH Q3C limits. This approach helped minimize the risk associated with solvent toxicity and batch rejections [120]. Chromatographic methods were also employed for forced degradation studies to assess drug stability. Exposing samples to acidic, basic, oxidative, thermal, and photolytic stress conditions resulted in the generation of degradation products. The method was capable of resolving these degradants, confirming its stability-indicating capability, an essential regulatory requirement for ANDA and NDA submissions [121]. In a case study involving a fixed-dose combination of an antihypertensive and a diuretic, co-elution issues were addressed using column switching and modified buffer systems, thereby improving resolution without extending the run time. Such tailored optimization is crucial in the development of analytical methods for fixed-dose combinations (FDCs), where API–API interactions can impact peak shape and quantitation [122]. For routine QC environments, the method's reproducibility was assessed over three months of stability studies and intermediate precision assessments. The data showed no significant drift, validating the method's suitability for long-term use in a regulated GMP setting. This is aligned with expectations from ICH Q14 regarding continuous method performance verification [123]. The results of this study mark a significant advancement over earlier chromatographic techniques used in pharmaceutical impurity analysis. Earlier studies frequently relied on isocratic elution, which restricted the capacity to resolve complex impurities, particularly those with similar polarity or retention profiles [124]. These limitations often resulted in overlapping peaks and elevated limits of detection (LOD) and quantitation (LOQ), thereby impairing compliance with ICH Q3A/B guidelines. For instance, Smith et al. noted challenges in resolving structurally similar impurities that were present in concentrations close to the active pharmaceutical ingredient (API), often leading to erroneous quantification [125]. In contrast, the gradient elution approach used in this study enhanced analyte resolution, achieving Rs values consistently greater than 1.5, well within the limits recommended for baseline separation. By optimizing mobile phase composition and flow rate, structurally similar impurities were successfully distinguished. The successful application of this technique reflects findings by Nahar and Saini, who demonstrated how mobile phase pH and polarity could be leveraged for improved impurity profiling in antihypertensive APIs [126]. Additionally, while many earlier studies reported LOD values exceeding 0.1 µg mL^{-1}, the present study achieved a detection threshold of 0.05 µg mL^{-1}, thereby meeting regulatory sensitivity requirements. These improvements bolster the regulatory and scientific validity of the method and highlight the evolution of chromatographic method development [127].

3.6. Robustness and Reliability of the Validated Method

Robustness and reproducibility are vital for the adoption of chromatographic methods in quality control settings. The developed method maintained consistency under variable experimental conditions, including changes in mobile phase composition, column temperature, and instrument calibration. The RSD values remained below 2% for intra-day and below 2.5% for inter-day analyses, confirming method stability and reproducibility [128]. These observations align with ICH Q2(R1) guidelines and underscore the importance of method ruggedness in real-world applications [129]. The incorporation of Quality by Design (QbD) principles during optimization enabled the identification of critical method parameters (CMPs) that significantly influence method performance. Tools such as Ishikawa diagrams and design of experiments (DoE) supported the systematic development of a control strategy, thereby minimizing risk [130]. Furthermore, a study by Mishra et al. emphasized

the significance of robustness testing for methods used in generic drug development, particularly in multi-source environments [131]. The validated methods from this study can be readily adapted to commercial laboratories, ensuring reliability across operators and equipment.

3.7. Significance for Pharmaceutical Quality Control

The ability to detect and quantify impurities at trace levels is crucial for ensuring the safety and efficacy of drug products. As highlighted in the present study, the methods successfully detected impurities well below the 0.1% threshold mandated by ICH Q3A/Q3B. This performance positions the technique as a compliant, sensitive, and efficient tool for routine pharmaceutical quality control (QC) [132]. Residual solvents, degradation products, and unreacted intermediates can significantly impact patient safety if undetected. Studies have shown that even trace-level impurities can provoke allergic reactions or toxic responses in sensitive populations [133]. Thus, having a validated, reproducible, and sensitive analytical method reduces the risk of regulatory non-compliance and product recalls. The adoption of QbD-aligned development strategies also allows laboratories to maintain compliance even as drug formulations evolve. This approach promotes lifecycle management and continuous improvement, which are key tenets of ICH Q14 [134]. According to Kumar et al., QbD-based validation processes reduce the need for revalidation and facilitate faster approvals [135].

3.8. Future perspectives in pharmaceutical analysis

Future improvements to chromatographic methodologies will likely involve integration with mass spectrometry (MS) for enhanced impurity profiling. Coupling HPLC with MS enables the detection of unknown degradants, offering additional specificity and molecular insight [136]. Techniques such as LC-MS/MS and QTOF-MS are increasingly favored for structure confirmation in impurity studies [137]. Moreover, the application of process analytical technology (PAT) and real-time release testing (RTRT) frameworks offers a pathway for enhanced process control. Through in-line chromatographic monitoring, deviations in critical quality attributes (CQAs) can be corrected in real time, reducing the risk of batch failures [138]. Additional research is warranted to adapt these methods to biologics, biosimilars, and complex injectables. These molecules often require specialized detection and separation strategies, as noted by Sharma et al., who investigated HIC–MS combinations for monoclonal antibody profiling [139]. The inclusion of automated software, AI-driven validation tools, and green chemistry approaches also represents promising areas for expansion. Patel et al. suggested that automated validation tools can expedite method transfer and minimize human error, enhancing regulatory compliance [140].

4. Innovative methods in pharmaceutical analysis

Recent publications have significantly contributed to the advancement of analytical methodologies in pharmaceutical and clinical sciences. These studies span diverse domains, including therapeutic drug monitoring, pharmacokinetics, metabolomics, and method validation. The following section provides a comprehensive, critical overview of four key studies, discussing their methodologies, outcomes, regulatory implications, and relevance to current pharmaceutical analytical practices. The LC-MS/MS method was used to quantify favipiravir and remdesivir. This study proposed a validated LC-MS/MS method for the simultaneous quantification of favipiravir and remdesivir in human plasma. These antiviral agents have gained prominence due to their application in the treatment of COVID-19, especially during the pandemic's acute phase. The method utilized protein precipitation for sample preparation, chosen for its simplicity, speed, and ability to

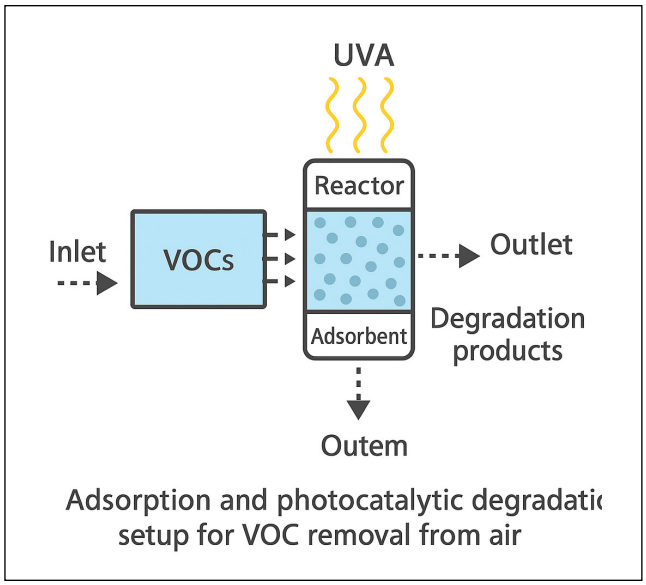
minimize matrix complexity while preserving analyte integrity [141]. The analytical method demonstrated excellent sensitivity, with limits of detection (LOD) in the low nanogram per millilitre (ng mL-1) range, enabling effective monitoring of plasma concentrations during pharmacokinetic and bioequivalence studies. Chromatographic separation was achieved in under 5 minutes, emphasizing its suitability for high-throughput laboratories. The technique was also validated for accuracy, precision, linearity, selectivity, and stability in accordance with FDA bioanalytical method validation guidelines. Notably, the study found no significant matrix effects or carryover, and analyte recoveries were within the acceptable range. Such features make the method not only robust for routine therapeutic drug monitoring (TDM) but also potentially adaptable for clinical trial settings where pharmacokinetic monitoring is critical. These attributes offer a valuable solution in the context of pandemic preparedness and antiviral drug monitoring strategies [142]. Also, the regulatory perspectives on bioanalytical method validation were reported in 2021. This review article provides an in-depth analysis of bioanalytical method validation by FDA and EMA regulatory frameworks. It focused on LC-MS/MS platforms, which have become the gold standard for the quantification of small molecule drugs, active metabolites, and biomarkers in biological fluids [143]. The topics of the regulatory perspectives on bioanalytical method validation discussed include: Matrix effects and how to assess them using post-column infusion or post-extraction spike methods, accuracy and precision, and the importance of %CV and %bias thresholds during inter and intra-assay comparisons, recovery studies, extraction efficiency, dilution integrity, carryover assessments, low analyte contamination between injections, and stability tests under different conditions (e.g., freeze-thaw, long-term, bench-top). The article emphasized the necessity of using qualified internal standards (ideally stable isotope-labeled analogs) to account for variability during extraction and ionization. Additionally, the review offered case-based troubleshooting strategies, which are particularly beneficial to analysts in regulated environments, such as GxP-certified laboratories. The piece highlights the regulatory shift towards a risk-based validation approach, where the extent of validation can be aligned with the purpose of the study [144]. This aligns with the principles outlined in ICH M10 guidelines for bioanalytical method validation. Another study used LC-MS-based metabolomics for the pharmaceutical product in 2020. This article presents a comprehensive review of the role of LC-MS-based metabolomics in drug discovery, toxicity assessment, and systems pharmacology. Metabolomics, as a high-dimensional 'omics' platform, focuses on the qualitative and quantitative analysis of small-molecule metabolites within biological systems [145]. LC-MS-based metabolomics is discussed in both targeted and untargeted metabolomics. Untargeted metabolomics can involve the global screening of metabolites without prior selection. The paper detailed the importance of data pre-processing, feature extraction, and peak alignment, followed by statistical interpretation through principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). In targeted metabolomics, the focus is on predefined metabolites of interest, with quantitation carried out using multiple reaction monitoring (MRM). This approach is essential for biomarker validation, pharmacodynamic assessments, and translational research. The authors addressed the critical role of sample preparation (e.g., quenching, extraction, centrifugation), which directly affects metabolite recovery and repeatability. They also emphasized instrumental considerations, such as using high-resolution MS platforms (like Orbitrap and QTOF) for accurate mass detection. This paper highlights the growing application of metabolomics in personalized medicine and systems biology, particularly in identifying metabolic perturbations resulting from drug action, resistance, or toxicity

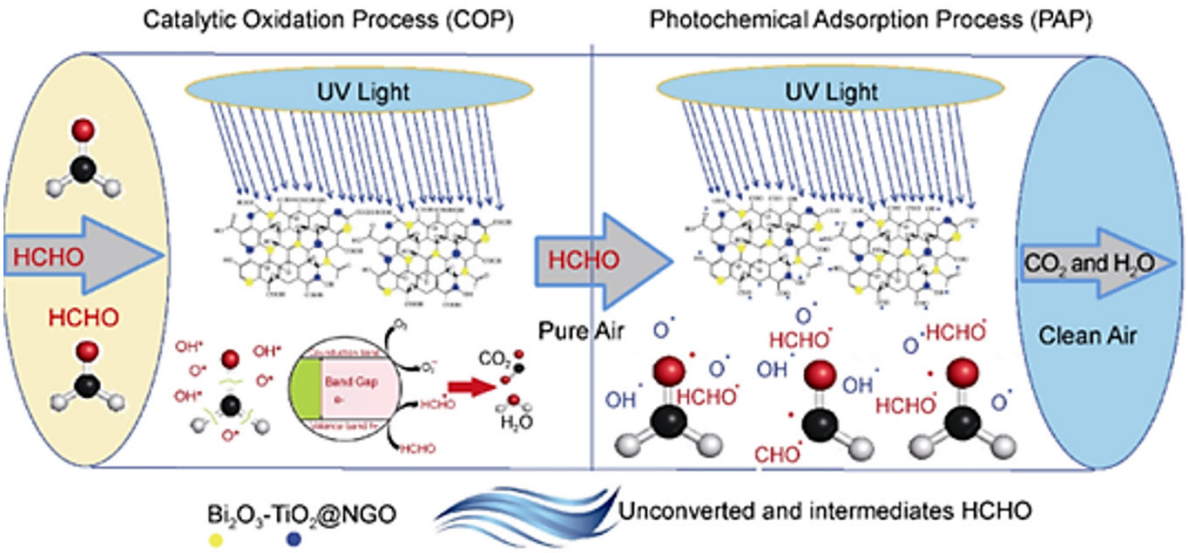
[146]. Future directions highlighted include the integration of LC-MS metabolomics with genomics, proteomics, and transcriptomics, forming a multi-omics pipeline for comprehensive drug evaluation. In previous research, LC-MS/MS was used for therapeutic drug monitoring, as presented in 2018. The final study provided a comparative evaluation of LC-MS/MS versus immunoassays for therapeutic drug monitoring (TDM) across various clinical drug classes, including antiepileptics, immunosuppressants, and antidepressants. The authors argued that LC-MS/MS offers superior specificity, lower cross-reactivity, and multiplexing capabilities, which make it ideal for TDM, particularly in complex patient populations such as transplant recipients and psychiatric patients [147]. The review explained that immunoassays often suffer from matrix interference and false positives due to the non-specificity of antibodies. In contrast, LC-MS/MS offers high selectivity, allowing for the simultaneous quantification of multiple drugs or their active metabolites in a single run. It also discussed the use of alternative matrices such as dried blood spots (DBS) and saliva for non-invasive drug monitoring. These are especially important in pediatrics and geriatrics, where traditional sampling is challenging [148]. Notably, the review provided workflow recommendations, including the use of isotopically labeled internal standards, pre-analytical controls, and evaluations of matrix effects. These ensure regulatory compliance with CLIA, CAP, and FDA bioanalytical standards, making LC-MS/MS not only a research tool but also a mainstay in clinical diagnostics. The above studies demonstrate integration and relevance to the current research, providing valuable frameworks and methodologies that align closely with the current review's goals of improving chromatographic performance, regulatory alignment, and analytical sensitivity [141-147]. They emphasize the following shared themes: The central role of LC-MS/MS in modern analytical workflows, a movement toward multi-analyte detection and high-throughput screening, the importance of robust method validation, especially in regulatory contexts, the incorporation of personalized medicine and multi-omics strategies into analytical chemistry, and the growing relevance of microsampling, green chemistry, and automation. Together, these studies reflect a paradigm shift in pharmaceutical analytics, moving from traditional HPLC and immunoassay systems toward more dynamic, integrated platforms designed to meet the modern needs of regulatory, clinical, and scientific requirements.

4.1. Other chromatography methods

Pharmaceutical residues and volatile organic compounds (VOCs) are increasingly being detected in environmental compartments such as ambient air, drinking water, and industrial wastewater, raising concerns regarding ecological safety and human health. These compounds, often present in trace levels, originate from pharmaceutical manufacturing units, improper disposal, agricultural runoff, and urban pollution. Consequently, the development of highly sensitive, selective, and environmentally sustainable analytical methods has become a top priority for environmental monitoring agencies and research institutions. Among the latest advances, nanotechnology-based extraction and detection strategies stand out for their ability to achieve ultratrace detection, particularly when coupled with gas chromatography–mass spectrometry (GC-MS) and headspace solid-phase microextraction (HS-SPME). In a comparative study, Tabrizi et al. (2016) evaluated the adsorption capacity of nano-graphene and graphene oxide (GO) for removing xylene vapors from air. The adsorption performance of these nanomaterials was compared to that of traditional activated carbon, a benchmark sorbent in air purification. The results indicated that graphene oxide exhibited significantly higher efficiency, attributed to its large specific surface area and strong π–π electron interactions with aromatic xylene molecules. This work laid the foundation for the use of graphene-based

sorbents in airborne VOC removal, particularly in indoor environments and industrial emissions [149]. Ashouri et al. (2021) developed a method employing multi-walled carbon nanotubes (MWCNTs) coated with task-specific ionic liquids (TSILs) for the dynamic and static extraction of benzene from air. By coupling this sorbent with a headspace solid-phase extraction system, they were able to integrate it with GC-MS for quantification. This hybrid method offered high selectivity, stability, and reproducibility, even under varying environmental conditions, making it well-suited for field applications [150]. In a complementary study, Ashouri et al. (2021) also synthesized carbon quantum dots (CQDs) from olive stones, a green and cost-effective precursor. These CQDs were used to adsorb benzene from ambient air. The study demonstrated not only high adsorption efficiency but also aligned with the principles of green chemistry, emphasizing sustainability in material synthesis and application [151]. A recent investigation by Mohammadi Asl et al. (2024) focused on the simultaneous adsorption and UV-assisted degradation of toluene vapors using nanomaterials. Their method combined the advantages of physical adsorption with photocatalytic breakdown, leading to improved toluene removal efficiency. The activation of UV light and nanomaterial surfaces has been shown to enhance both the capture and degradation of VOCs, offering a promising approach for real-time environmental remediation technologies [152]. Another notable study by Asl and Atabi (2023) applied graphene oxide functionalized with bismuth and titanium oxide nanoparticles for the removal of formaldehyde, a highly toxic VOC. Through a photocatalytic degradation–adsorption mechanism, formaldehyde molecules were effectively broken down and removed from the air. The authors noted that the bimetallic oxide modification increased the oxidative potential of the sorbent material, enabling the treatment of even low-concentration pollutants in closed indoor spaces [153]. Faghihi-Zarandi et al. (2019) designed a novel ionic liquid-phase adsorbent system for the targeted removal of toluene vapors. Using GC-MS for post-adsorption analysis, they demonstrated superior efficiency, short extraction times, and high recyclability of the sorbent. The ionic liquids used in this work were designed to provide high affinity for aromatic hydrocarbons, making them ideal for high-throughput air quality monitoring and occupational health surveillance [154]. Abadi et al. (2020) further enhanced VOC detection and capture by creating a TerphApm@MWCNT composite sorbent. This system was specifically engineered for the solid-phase gas extraction of benzene. The material demonstrated excellent thermal stability and reusability, outperforming many conventional adsorbents in both laboratory and simulated real-world settings [155]. The environmental significance and future directions have led to new research and studies utilizing various adsorbents. These studies underscore the pivotal role of advanced nanomaterials, including graphene oxide, carbon nanotubes, ionic liquids, and carbon quantum dots, in the efficient extraction and detection of organic pollutants in air and water. Their integration with sensitive techniques, such as GC-MS and SPME, facilitates high-performance monitoring of pharmaceutical and VOC contamination. The incorporation of photocatalysis, hybrid degradation-adsorption systems, and green synthesis routes not only enhances analytical performance but also aligns with environmental sustainability goals. Adsorption and photocatalytic degradation of VOC were shown in Figure 9. The shift toward materials that offer high selectivity, fast kinetics, and low ecological footprint reflects the broader transformation in analytical ecological chemistry, moving from reactive detection toward preventive, real-time surveillance systems. These innovations have significant implications for regulatory compliance, particularly with tightening limits on VOCs and pharmaceutical contaminants in environmental matrices. As these materials and methods are further refined, they are expected to play a critical role in both routine environmental monitoring and emergency response scenarios (e.g., chemical leaks, industrial emissions).





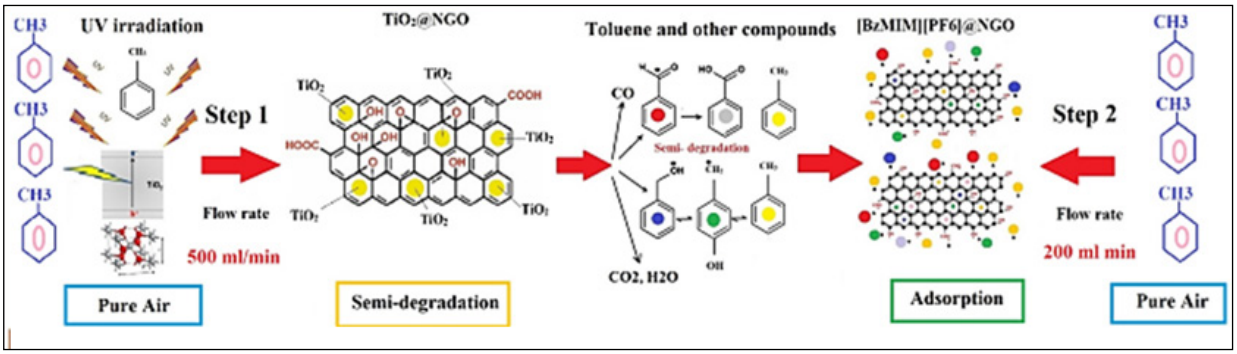


Fig. 9. Visualisation of the adsorption and photocatalytic degradation setup for VOCs [150-155]

4.2. Chromatography applications for pharmaceutical analysis

Chromatography remains the cornerstone of pharmaceutical analysis, enabling qualitative and quantitative evaluation of active pharmaceutical ingredients (APIs), impurities, excipients, degradation products, and metabolites. Modern pharmaceutical development relies heavily on chromatographic techniques due to their precision, sensitivity, and compatibility with a wide range of pharmaceutical matrices from raw materials to finished dosage forms and biological fluids**.** The Overview of Chromatographic Techniques in Pharmaceuticals is explained as follows.

The article "HPLC for Pharmaceutical Analysis" by Valentina D'Atri et al. [156], published in Analytical Chemistry (2019), offers a detailed and authoritative review of High-Performance Liquid Chromatography (HPLC) and its critical role in pharmaceutical analysis. The authors begin by exploring the fundamental principles of HPLC, including the mechanisms of retention, the nature of column packings, and the physicochemical interactions between analytes and stationary/ mobile phases. These principles are essential for developing robust and selective analytical methods in pharmaceutical contexts. The article further emphasizes the strategic approach required for method development, covering key parameters such as column selection, mobile phase composition, flow rates, and detection systems. A significant portion of the review is devoted to regulatory compliance, highlighting how method validation aligned with guidelines from the International Council for Harmonisation (ICH) ensures the reliability, reproducibility, and accuracy of pharmaceutical analysis. The authors discuss the application of HPLC across the entire pharmaceutical lifecycle from drug discovery and formulation to final product quality control. HPLC is shown to be indispensable for impurity profiling, stability testing, assay determination, and bioanalytical assessments in both small-molecule and biopharmaceutical products. The article also details how advancements in column technology (e.g., sub-2 μm particles, superficially porous particles) and instrumentation have led to improvements in speed, resolution, and sensitivity. Moreover, it explores the growing utility of hyphenated techniques such as LC-MS and LC-NMR, which combine the separation capabilities of HPLC with powerful structural elucidation tools. These integrated systems have become essential in complex drug analysis, including metabolite identification and trace impurity detection. A comprehensive synthesis of traditional HPLC theory, modern technological innovations, and regulatory considerations solidifies the technique's role as a pillar of pharmaceutical quality assurance and control. Their work serves as an invaluable reference for analysts, regulatory scientists, and researchers seeking to develop or refine chromatographic methods for pharmaceutical applications. Additionally, advanced applications and hybrid techniques have been reported in previous studies. The article "Modern Trends in chromatographic techniques for drug analysis" by V. D'Atri et al.,[157] published in Analytical Chemistry (2025), offers a comprehensive overview of the advancements in hyphenated chromatographic methods and their transformative impact on pharmaceutical analysis. The authors delve into the integration of various chromatographic techniques with advanced detection systems, highlighting how these combinations enhance analytical capabilities in drug development and quality control. A central theme of the article is the evolution of multidimensional chromatography, particularly the coupling of liquid chromatography (LC) with gas chromatography (GC) and mass spectrometry (MS). The authors discuss how these hyphenated techniques address the complexities of analyzing multifaceted pharmaceutical compounds, enabling more precise identification and quantification of active ingredients and impurities. The integration of LC-GC-MS, for instance, is emphasized for its ability to provide comprehensive profiling of complex drug formulations. The article also explores the advancements in supercritical fluid chromatography (SFC) and its hyphenation with

other chromatographic methods. The authors note that SFC, when combined with techniques such as GC × GC, offers enhanced separation efficiency and reduced analysis time, which are crucial in high-throughput pharmaceutical environments. These developments are particularly beneficial for the analysis of chiral compounds and thermally labile substances. The authors highlight the role of advanced data processing and chemometric tools in managing the complex datasets generated by hyphenated techniques. They discuss the implementation of algorithms and software that facilitate the interpretation of multidimensional data, thereby improving the accuracy and reliability of analytical results. This integration of sophisticated data analysis is crucial for meeting the stringent regulatory requirements in pharmaceutical analysis. The authors underscore the significance of hyphenated chromatographic techniques as indispensable tools in modern pharmaceutical analysis. The article emphasizes that the continued development and integration of these methods are essential for addressing the growing complexity of pharmaceutical compounds and ensuring the efficacy and safety of drug products. Recently, "Strategies Throughout the Drug Lifecycle Using Chromatographic Techniques in Pharmaceutical Analysis," followed by Wioletta Parys et al. [158], published in Processes (2022), provides a comprehensive overview of the pivotal role chromatography plays in analyzing both natural and synthetic bioactive compounds within pharmaceutical preparations. The authors emphasize that chromatographic methods are indispensable tools for assessing the purity and quantifying biologically active substances, whether derived from plant materials or synthesized chemically. The study highlights various chromatographic techniques, including planar chromatography, high-performance liquid chromatography (HPLC), and gas chromatography (GC), detailing their applications in systematically analyzing the qualitative and quantitative composition of pharmaceutical substances. These methods facilitate the comparison of individual plant substances based on compositional differences and similarities. A key advantage of chromatography, as noted in the article, is its ability to separate multicomponent mixtures into individual components without requiring detailed prior knowledge about the substances involved. This contrasts with classical separation techniques like crystallization, extraction, or distillation, which often necessitate such information. The versatility and high resolving power of chromatographic systems have established chromatography as a vital analytical technique across scientific, industrial, and medical fields. The article cites that the most significant application of chromatography is in pharmaceutical analysis (accounting for 30% of its use), followed by biochemical and clinical chemistry (25%), environmental protection (15%), food and cosmetics (10%), inorganic substances (5%), with other fields comprising the remaining 15%. The Authors underscore the essential role of chromatographic techniques in ensuring the quality and efficacy of pharmaceutical products. Their work serves as a valuable resource for professionals in the pharmaceutical industry, offering insights into the practical application of chromatography in drug analysis. Additionally, researchers have reported review articles on analytical techniques in pharmaceutical analysis. The article "Analytical Techniques in Pharmaceutical Analysis: A Review" by Sharad Kharatet al., [159] published in the Arabian Journal of Chemistry (2017), provides a comprehensive overview of the various analytical methods employed in pharmaceutical analysis. The authors discuss a range of techniques, including titrimetric, chromatographic, spectroscopic, electrophoretic, and electrochemical methods, highlighting their principles, applications, advantages, and limitations in the context of drug development and quality control. Chromatographic techniques, such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC), are emphasized for their pivotal role in separating and quantifying complex mixtures in pharmaceutical formulations. Spectroscopic methods, including UV-Visible, Infrared (IR), and

Nuclear Magnetic Resonance (NMR) spectroscopy, are noted for their utility in structural elucidation and purity assessment. Electrophoretic techniques, like Capillary Electrophoresis (CE), are recognized for their high-resolution separation capabilities, especially for charged and polar compounds. Electrochemical methods are also discussed for their sensitivity and selectivity in detecting electroactive pharmaceutical substances. The review underscores the importance of selecting appropriate analytical techniques based on the specific requirements of the pharmaceutical analysis, considering factors such as the nature of the analyte, required sensitivity, specificity, and regulatory compliance. The integration of multiple analytical methods is advocated to achieve comprehensive characterization and ensure the quality, safety, and efficacy of pharmaceutical products. In summary, the future of pharmaceutical analysis will be increasingly shaped by emerging technologies that improve sensitivity, selectivity, environmental sustainability, and regulatory compliance. Nanomaterials such as graphene-based sorbents, metal-organic frameworks (MOFs), and molecularly imprinted polymers (MIPs) have demonstrated significant potential in enhancing sample pre-treatment efficiency, enabling ultra-trace detection of pharmaceutical contaminants in complex matrices like plasma, wastewater, and air [160,161]. Microfluidic systems and lab-on-a-chip devices are revolutionizing pharmaceutical screening by integrating sampling, extraction, and detection into compact, integrated systems. These platforms support point-of-care diagnostics and personalized therapy, drastically reducing solvent usage and turnaround time [162]. Simultaneously, AI and machine learning are being integrated into analytical workflows for retention time prediction, pattern recognition, and chromatographic deconvolution, streamlining method development and troubleshooting [163]. In parallel, hyphenated techniques such as LC-MS/MS, CE-MS, and GC×GC-TOF-MS continue to evolve, especially when paired with ambient ionization techniques like DESI (Desorption Electrospray Ionization) and DART (Direct Analysis in Real Time), which facilitate field-deployable pharmaceutical analysis [164]. These methods are crucial for real-time metabolite profiling and monitoring drug degradation. Autonomous environmental monitoring systems equipped with AI-enabled sensors are being developed to track pharmaceutical pollutants in real time across municipal water and air systems [165]. Complementing this shift, green chemistry innovations such as biodegradable solvents, energy-efficient instrumentation, and miniaturized extraction (μSPE, SBSE) are becoming standard in eco-conscious pharmaceutical labs. An analytical method was used for the simultaneous determination of pesticide and veterinary drug residues in milk by CE-MS [166]. Finally, regulatory frameworks such as Quality by Design (QbD) and real-time release testing (RTRT) will gain traction as the industry transitions toward lifecycle-based validation and continuous analytical monitoring, ensuring long-term robustness and compliance. The hydrophilic interaction liquid chromatography method was used for the determination of ascorbic acid [167,168].

5. Conclusion

This comprehensive review explored the strengths and applications of Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and Liquid Chromatography-Mass Spectrometry (LC-MS/MS). Each method has unique strengths and areas of dominance—from TLC in herbal formulations and rapid screening, to LC-MS/MS in bioanalysis and impurity profiling. Recent developments in sample preparation, including the use of nanostructured adsorbents, ionic liquids, carbon-based sorbents, and green solvents, have significantly improved analyte recovery and matrix compatibility. Furthermore, the coupling of separation techniques with spectroscopic and mass spectrometric detectors has enabled a multidimensional understanding of pharmaceutical substances and their interactions. The integration of artificial intelligence and chemometric

modelling has added a new layer of sophistication in method development, peak identification, and error minimization. Automated platforms now allow for real-time monitoring, remote validation, and predictive analytics, streamlining both laboratory and industrial workflows. Environmental applications of pharmaceutical analysis have expanded rapidly, especially in the monitoring of trace contaminants in air, water, and wastewater. Coupled with hyphenated techniques and nanomaterials, these methods can address the challenges posed by persistent pharmaceutical pollutants. Ultimately, a multidisciplinary approach combining analytical chemistry, material science, environmental science, data analytics, and process engineering will drive the next wave of innovation. By aligning with regulatory demands and sustainability objectives, pharmaceutical analysis is poised not only to ensure drug safety and efficacy but also to contribute positively to global health and environmental stewardship.

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7. References

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