CHROMATOGRAPHIC TECHNIQUES FOR PHARMACEUTICAL ANALYSIS

Abstract

This chapter serves as a comprehensive review of various chromatographic techniques employed in pharmaceutical analysis. It delves into the principles, instrumentation, and factors influencing separation for essential chromatographic methods, including High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Thin Layer Chromatography (TLC), and other significant techniques like Size Exclusion Chromatography, Ion Exchange Chromatography, Supercritical Fluid Chromatography, and Chiral Chromatography. Emphasizing the importance of HPLC and GC, the chapter explores their extensive applications in drug analysis, impurity profiling, assay determination, and drug stability studies, ensuring the quality and safety of pharmaceutical products. The simplicity and cost-effectiveness of TLC find prominence in qualitative analysis, compound identification, and purity checks. Method validation, a critical aspect in chromatographic analysis, is meticulously addressed to highlight its role in ensuring accuracy, precision, specificity, and robustness in pharmaceutical research and quality control. As an essential resource in the book, this chapter offers valuable insights into cutting-edge advancements and best practices in chromatographic techniques for pharmaceutical analysis, aiding researchers and analysts in staying at the forefront of the field.

Keywords: Chromatographic Techniques; Gas Chromatography; High Performance Liquid Chromatography; Method Validation; Pharmaceutical Analysis; Thin Layer Chromatography

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I. INTRODUCTION

In the rapidly evolving landscape of pharmacy and nursing, chromatographic techniques play a pivotal role in pharmaceutical analysis [1]. Chromatography, as a powerful separation and identification tool, is indispensable in the pharmaceutical industry for ensuring the quality, safety, and efficacy of drugs and formulations [2], [3]. The chapter introduces the fundamental principles of chromatography, highlighting its differential interaction between the stationary and mobile phases, leading to precise separation of complex mixtures. Subsequently, the instrumentation and key parameters affecting separation in prominent techniques like High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Thin Layer Chromatography (TLC) are explored. Additionally, the chapter discusses other important chromatographic techniques, including Size Exclusion Chromatography, Ion Exchange Chromatography, Supercritical Fluid Chromatography, and Chiral Chromatography, each offering unique advantages in pharmaceutical analysis. The significance of method validation to ensure the accuracy and reliability of results is emphasized throughout the chapter. By providing valuable insights into cutting-edge advancements and best practices, this chapter equips researchers and analysts with the knowledge needed to leverage chromatographic techniques effectively in pharmaceutical research and quality control.

II. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1. **HPLC:** HPLC is the most widely used chromatographic technique for analysis of drugs and pharmaceutical formulations. It offers high resolving power, sensitivity, accuracy and reproducibility [4]. HPLC has advantages like high selectivity, sensitivity and speed of analysis which make it an invaluable technique for pharmaceutical analysis and quality control applications. Developing robust and optimized HPLC methods is an essential part of drug development and product lifecycle management [5].

2. **Principle of HPLC:** HPLC separates components based on their differential partitioning between a stationary phase and a mobile phase. In reverse phase HPLC, nonpolar stationary phase and polar mobile phase are used [6].

3. **Instrumentation of HPLC System :** A basic HPLC system consists of high pressure pumps to deliver mobile phase, injection system to inject sample, chromatographic column packed with stationary phase, a detector like Ultraviolet (UV), Photo Diode Array (PDA), Evaporative light scattering detector (ELSD), etc., and data acquisition and processing system [7]. Table 1 compares Ultraviolet-Visible (UV/Vis), Evaporative light scattering detector (ELSD), Mass spectrometry (MS) and fluorescence detection based on parameters like sensitivity, selectivity, compatibility with mobile phase, suitable applications, etc.

Table 1: Comparison of different Detectors Employed in HPLC

Parameter	Detection technique			
	UV/Vis	**ELSD**	**MS**	**Fluorescence**
Sensitivity [7]	Moderate	High	High	High
Selectivity [4]	Low	Low	High	High

Compatible mobile phases [5]	All	All	Limited	Limited
Typical applications [5]	Determination of compounds with UV chromophores	Determination of non-UV compounds like sugars, lipids	Determination of molecular weight; identification of compounds	Based on intrinsic fluorescence of compounds
Suitable applications[5], [5]	Analysis of drugs and impurities in pharmaceuticals	Analysis of excipients in formulations	Metabolic stability studies; identification of unknown compounds	Analysis of biological samples; trace impurity analysis

4. Parameters affecting HPLC separation

Following are the parameters [6] that affect HPLC separation:

- Nature of stationary and mobile phases
- pH, ionic strength and temperature of mobile phase
- Flow rate of mobile phase
- Column dimensions

5. Applications of HPLC in pharmaceutical analysis

The applications of HPLC in pharmaceutical analysis [7] are:

- Determination of drug content and impurities in samples
- Simultaneous determination of multiple drug components
- Determination of drugs in biological samples like plasma and urine
- Separation of enantiomers for chiral drugs
- Stability-indicating assays for drug degradation products

III. GAS CHROMATOGRAPHY (GC)

GC offers advantages like high sensitivity, selectivity and speed of analysis. It is particularly suitable for analysis of volatile and semi-volatile compounds like small molecule drugs [8]. Developing robust GC methods with proper temperature programming and choice of stationary phase can help resolve complex mixtures commonly encountered in the pharmaceutical industry [9].

1. **Principle of GC:** GC separates components based on their volatility and differential partitioning between a mobile gas phase and a stationary phase coated on the inner surface of the GC column [10].

2. **Instrumentation of GC System:** A typical GC system consists of: Gas cylinders to supply carrier and makeup gases, gas flow controllers to regulate flow rate, inlet system to volatilize and inject sample, GC column packed with stationary phase, oven to control

column temperature, and detectors like Flame ionization detection (FID), The thermal conductivity detector (TCD), Electron capture detector (ECD), etc [11], [12].

3. **Parameters Affecting GC Separation:** The parameters affecting GC separation are: nature and flow rate of carrier gas, temperature and pressure of column, nature of stationary and coated phases, column dimensions[13]

4. **Applications of GC in Pharmaceutical Analysis**
   - Analysis of thermal stability and degradation products of drugs
   - Analysis of organic volatile impurities in drugs and formulations
   - Determination of residual solvents and pesticide residues
   - Chiral separation of drug enantiomers
   - Detection of counterfeit and substandard drugs

IV. THIN LAYER CHROMATOGRAPHY (TLC)

TLC is a simple, cost-effective and rapid technique for preliminary separation and analysis of drugs. Though it lacks sensitivity and resolution compared to HPLC and GC, TLC finds practical applications as an initial step in method development and quality control testing.

1. **Principle of TLC:** TLC works on the same principle as column chromatography. A small quantity of sample is applied as a spot on a thin layer of adsorbent coated on a flat support plate. As the mobile phase travels up the plate by capillary action, different compounds separate based on their partition coefficients.

2. **Instrumentation of TLC:** The basic components of a TLC system are: TLC plates coated with adsorbent like silica gel, mobile phase solvents for development, developing chamber, and UV lamp or staining reagents for visualization.

3. **Applications of TLC in pharmaceutical analysis**
   - Quick screening and identification of compounds in complex mixtures
   - Determination of Rf values for standardization and method development
   - Purity testing of drug substances and formulations
   - Separation of isomers and enantiomers
   - Identification of degradation products

The characteristic differences between HPLC, GC, and LC are listed out in Table 2.

Table 2: Comparison of HPLC, GC, and TLC

Parameter/ Technique	HPLC	GC	TLC
Separation mechanism [13]	Differential partitioning between mobile and stationary phases	Volatility based partitioning between mobile gas and coated stationary phase	Differential adsorption based on partition coefficient

Resolving power [14]	High	High-Moderate	Low
Sensitivity [15]	High-Moderate	High	Low
Speed of Analysis [8], [9]	Moderate-Fast	Fast	Fast
Cost [12]	Moderate-High	High	Low
Suitable applications [13]	Drug identification and quantification, impurity profiling, chiral separations	Volatile compound analysis, thermal stability studies, residual solvent determination Initial screening,	Method development, purity testing, isomer separation

V. OTHER CHROMATOGRAPHIC TECHNIQUES

1. **Size Exclusion Chromatography:** Also known as gel permeation chromatography. Separation is based on size rather than interactions. Large molecules are excluded and elute first while smaller molecules penetrate into pores and elute later [16]–[18]. It is used for:
   - Determining molecular weight distribution of polymers
   - Purification of macromolecules

2. **Ion Exchange Chromatography:** Separation based on ionic interactions between sample ions and oppositely charged stationary phase[19], [20]. This technique is used for:
   - Determination of ionic impurities
   - Purification of chiral drugs by changing buffer pH and salt concentration

3. **Super Critical Fluid Chromatography**: This technique uses supercritical carbon dioxide as mobile phase instead of liquids [21], [22]. The advantages of this method are:
   - Higher diffusivity and lower viscosity for better separation
   - Greens chemistry technique
   - Used for analysis of thermally labile compounds

4. **Chiral Chromatography**: This chromatographic technique uses Enantioselective separation of chiral drugs using chiral stationary phases or chiral mobile phases [23], [24]. It is used for:

   - Enantiomeric purity testing of chiral drugs
   - Separation of enantiomers for development of single enantiomer drugs

These chromatographic techniques offer various separation mechanisms and advantages that complement HPLC and GC. They find applications in specific areas like determination of impurities, purification of samples and chiral separations which are important tasks in pharmaceutical analysis. The differences in various chiral chromatography techniques based on factors like resolving ability, type of stationary phase, range of applicable compounds, cost, etc. are described in Table 3.

Table 3: Comparision of chiral chromatography techniques

Chromatography Method	Resolving Ability	Type of Stationary Phase	Range of Applicable Compounds	Cost
High-Performance Liquid Chromatography (HPLC) [2]	High	Chiral selectors, polysaccharides, cyclodextrins	Small to large molecules	Moderate to high
Gas Chromatography (GC) [10]	Moderate	Cyclodextrin derivatives, Cyclodextrin-based phases	Volatile compounds	Moderate
Supercritical Fluid Chromatography (SFC) [22]	Moderate to High	Chiral stationary phases, Polysaccharides, Protein-based phases	Non-volatile and semi-volatile compounds	High
Thin Layer Chromatography (TLC) [1], [2]	Low to Moderate	Chiral plates coated with chiral selectors	Limited range, mostly for small molecules	Low to moderate
Capillary Electrophoresis (CE) [25]	Moderate	Chiral selectors, Cyclodextrins	Small to medium-sized molecules	Low to moderate
Immobilized Metal Affinity Chromatography (IMAC) [15]	Low to Moderate	Immobilized metal ions on stationary phase	Peptides, proteins	Low to moderate

The recent advances in latest column technologies with their key features and applications are summarized in Table 4.

Table 4: Recent advances in column technologies

Column Technology	Key features	Applications
HPLC		
Core-Shell Columns [8], [25]	These columns have a solid core and porous shell, leading to higher efficiency, faster separations, and lower backpressure	They find applications in pharmaceutical analysis, environmental monitoring, & food and beverage testing
Monolithic Columns [12]	Single continuous stationary phase results in reduced band broadening, high permeability, and fast separations	Suitable for peptide mapping, protein analysis, and oligonucleotide separation
Ultra-high-performance liquid	UHPLC columns offer smaller particle size and higher pressure capabilities improved resolution and	Drug discovery, metabolomics, and biochemical analysis

chromatography (UHPLC) Columns [14]	speed	
GC		
Micro- and Nano-columns [13]	These miniaturized columns with smaller internal diameters reduce analysis time and improve sensitivity	Ideal for environmental analysis, petrochemical analysis, and forensic toxicology
Chiral GC Columns [24]	Designed with chiral selectors, they offer enhanced enantioselectivity and improved resolution of chiral compounds	Finds applications in pharmaceutical analysis, flavor and fragrance analysis, and environmental monitoring
High-Temperature GC Columns [25]	These columns are stable at elevated temperatures and enable the analysis of high-boiling compounds	Suitable for petrochemical analysis, polymer characterization, and food safety analysis
SFC		
Sub-2 µm Particle Columns [21]	Featuring sub-2 µm particle size, they offer higher efficiency, improved peak capacity, and speed	Useful for chiral separation, natural product isolation, and drug purification
Chiral SFC Columns [22]	These columns have chiral selectors for enantioselective separations, complementing chiral HPLC and GC	Pharmaceutical analysis, agrochemical analysis, and chiral compound isolation
Hybrid Columns [22]	Combining SFC with other techniques like HPLC or GC, provide enhanced selectivity and separation power	Useful for complex sample analysis, natural product analysis, and biomolecule separation

VI. METHOD VALIDATION FOR CHROMATOGRAPHIC TECHNIQUES

1. **Specificity:** It is defined as the ability of method to measure analyte response in the presence of interferences. Specificity is assessed by comparing chromatograms of blank, standard and sample.[26]

2. **Accuracy:** Accuracy is closeness of test results to the true value. It is determined by recovery studies at multiple concentration levels.[26]

3. **Precision:** Precision is the degree of reproducibility of test results under normal operation. It is assessed by repeatability and intermediate precision.[26]

4. **Limit of Detection (LOD) and Quantification (LOQ):** The smallest concentration that can be reliably detected is called as LOD and while that can be quantified is called LOQ by the method. It is determined from the calibration curve.[26]

5. **Linearity and Range:** Method's ability to obtain test results proportional to concentration within a given range. It is established by analyzing standards at multiple concentration levels.[26]

6. **Robustness:** Robustness is the method's capacity to remain steady with small alterations in parameters. It is assessed by deliberately changing conditions and analyzing the impact on method performance.[26]

Thorough method validation as per regulatory guidelines is essential to demonstrate that a chromatographic technique will consistently provide reliable results for its intended use. The various parameters ensure the method is specific, accurate, precise and rugged enough for quantitative analysis of drugs and impurities [26]. Table 5 list outs various method validation parameters as per Food and Drug Administration (FDA), International Council for Harmonisation (ICH) and other regulatory guidelines and the extent of validation required for different applications.

Table 5: Regulatory requirements for method validation

Validation parameters	**FDA, ICH, and other regulatory guidelines**
Accuracy	All regulatory guidelines, including FDA and ICH, mandate accuracy validation for quantitative analysis of drug substances and products, bioanalytical methods for pharmacokinetic studies, and stability-indicating methods [27]
Precision	Precision validation is required by FDA, ICH, and other regulatory guidelines for quantitative analysis of drug substances and products, bioanalytical methods for pharmacokinetic studies, and stability studies [28]
Specificity/Selectivity	FDA, ICH, and other regulatory guidelines necessitate specificity/selectivity validation for stability-indicating methods, assay of drug substances and products, and cleaning validation assays [6]
Sensitivity	Sensitivity validation is required by FDA, ICH, and other regulatory guidelines for impurity testing, bioanalytical methods, and cleaning validation assays [9]
Linearity	FDA and ICH mandate linearity validation for calibration curve preparation in assay and impurity methods, as well as for quantitative analysis of drug substances and products. Other regulatory guidelines recommend it [5]
Range	FDA and ICH require range validation for calibration range in assay and impurity methods, and linearity range for quantitative analysis. Other regulatory guidelines recommend it.[26]
LOD and LOQ	FDA, ICH, and other regulatory guidelines recommend LOD and LOQ validation for impurity testing, bio analytical methods, and cleaning validation assays [26]
Robustness	FDA and ICH require robustness validation for changes in pH, temperature, and mobile phase composition, as well as variations in sample preparation. Other regulatory guidelines recommend it [2]
Ruggedness	While not specified by FDA and ICH, other regulatory guidelines recommend demonstrating method consistency across different laboratories, analysts, and instruments [14]
System Suitability	While not specified by FDA and ICH, other regulatory guidelines recommend using system suitability tests to monitor column

	performance before sample analysis and to ensure the system is suitable for the intended analysis [18]
Forced Degradation Studies	While not specified by FDA and ICH, other regulatory guidelines recommend conducting forced degradation studies to evaluate the stability-indicating capability of the method and identify degradation products [24]
Repeatability and Intermediate Precision	While not specified by FDA and ICH, other regulatory guidelines recommend assessing method precision under repeatability conditions and across different days, analysts, and instruments. [25]

VII. CONCLUSION

In conclusion, this chapter highlights the vital role of chromatographic techniques in pharmaceutical analysis. The review of various methods, including HPLC, GC, TLC, and others, underscores their indispensable applications in drug analysis and quality control. Emphasizing precision through method validation ensures reliable results and safe pharmaceutical products. As the field evolves, staying informed about cutting-edge advancements and best practices will shape the future of pharmacy and nursing, elevating standards in research and development. Chromatography remains a powerful tool that continues to shape the pharmaceutical landscape, contributing to safer and more efficacious medications.

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