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Review Article on HPLC & Gas Chromatography

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Abstract: *Two of the most potent and popular analytical separation methods in contemporary pharmaceutical, biomedical, environmental, and food-quality testing are high-performance liquid chromatography (HPLC) and gas chromatography (GC). For the qualitative and quantitative assessment of complicated mixes, both methods offer excellent sensitivity, accuracy, and repeatability. For the examination of thermally labile, non-volatile, polar, and high-molecular-weight chemicals, HPLC has developed into a vital instrument. Different stationary phases, detectors, and elution techniques increase its adaptability and allow for high-resolution separation of multi-component systems. Speed, selectivity, and detection limits have been greatly enhanced by innovations like UHPLC, monolithic columns, core-shell particles, and hyphenated techniques (LC–MS, LC–NMR).On the other hand, gas chromatography is very effective for volatile and semi-volatile substances because it uses inert carrier gases, exact temperature control, and sophisticated detectors like FID, ECD, and MS. Its use in toxicology, forensics, petroleum analysis, and environmental monitoring has increased thanks to innovations like GC–MS/MS, rapid GC, multidimensional GC (GC×GC), and enhanced micro-injection systems.The principles, instrumentation, sample preparation requirements, applications, and recent developments of both approaches are thoroughly compared in this review article. Their analytical performance factors, method development considerations, validation aspects, and applicability for particular analyte classes are highlighted.*

Keywords: High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Chromatographic Separation, Analytical Techniques, Method Development, Validation, Detection Systems, Hyphenated Techniques, UHPLC, GC–MS

I. INTRODUCTION

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

History of Chromatography-

- The Russian botanist Mikhail Tswett coined the term chromatography in 1906 to describe his experiments in separating different colored constituents of leaves through a column.
- ”Chromatography”; Khroma (colour) and graphic (writing)

One of the most potent, adaptable, and popular analytical methods for the separation, identification, and measurement of chemical substances is High-Performance Liquid Chromatography (HPLC). Pharmaceutical, biological, environmental, food, chemical, and forensic analysis have all been transformed by HPLC, which was first introduced in the late 1960s as a development of traditional column chromatography. HPLC can analyse a variety of polar, non-polar, thermolabile, and high-molecular-weight molecules, in contrast to gas chromatography, which needs volatile and thermally stable analytes[1-2].

In order to achieve high-resolution separation in less time, the method uses high pressure to force the mobile phase through a densely packed stationary phase. HPLC may be customised for a variety of analytical requirements because of its versatility in column chemistry, mobile phase composition, and detection devices. Reversed-phase materials, monolithic silica, core-shell particles, and ultra-high-pressure LC (UHPLC) are examples of innovative column

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technologies that have greatly improved sensitivity, selectivity, and analytical speed. Pharmaceutical quality control, method validation, impurity profiling, bioanalytical tests, and natural product research all depend on HPLC these days[3-4].

Principle of HPLC

Differential partitioning between a stationary phase (solid) and a mobile phase (liquid) is the basis for HPLC's operation. When a sample is injected, its constituent parts disperse between the flowing solvent and stationary phase according to their molecular interactions, polarity, and affinity, such as:

- Interactions between hydrophobic and hydrophilic
- Adsorption
- Exchange of ions
- Exclusion of size
- Hydrogen bonding. Each compound elutes at a characteristic retention time. Better separation requires optimization of flow rate, mobile phase composition, column chemistry, and temperature[5-6].

Instrumentation[7]-

The experimental set-up of HPLC mainly involves:

1. Solvent reservoir
2. Pump
3. Damping device
4. Sampling device
5. Column
6. Detector
7. Fraction collector
8. Recorder

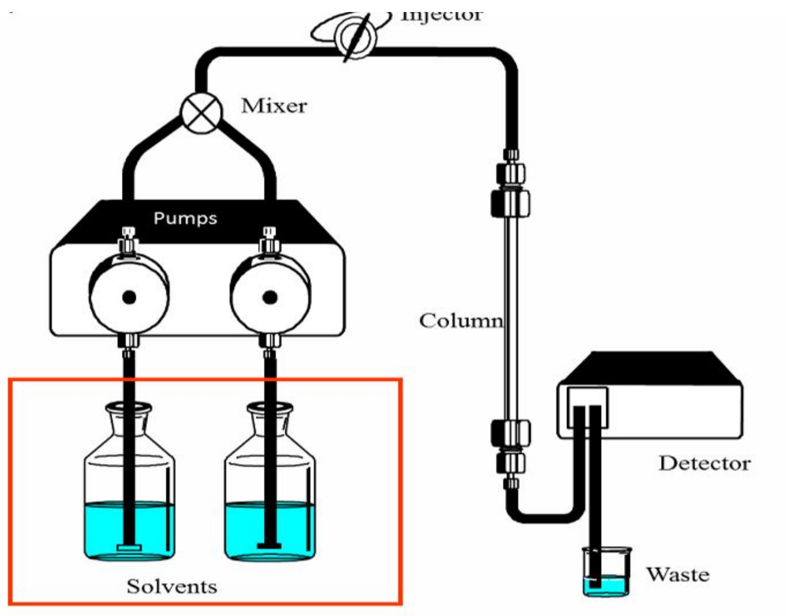

Fig.1: HPLC

HPLC Instrumentation and Techniques[8]-

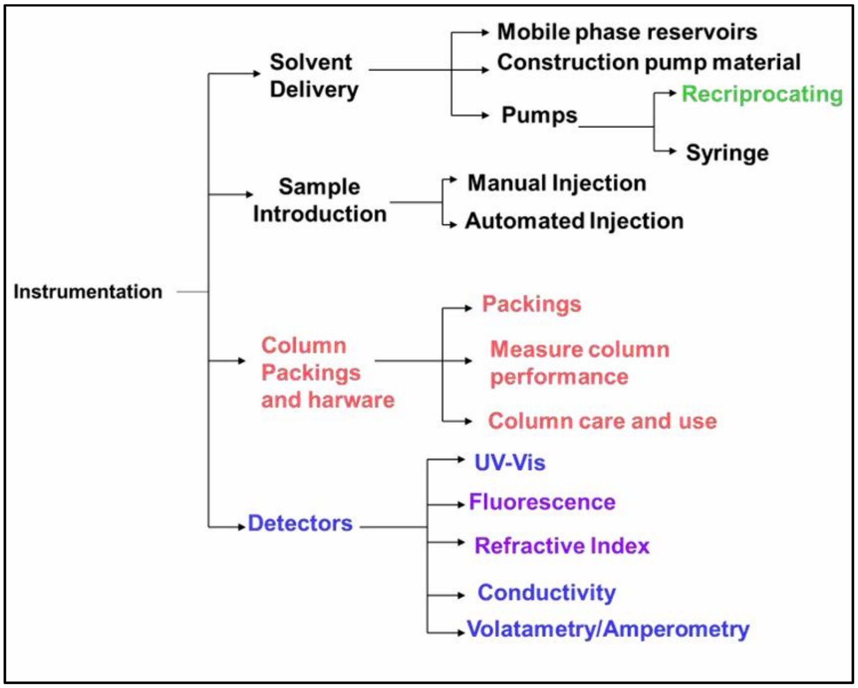

Fig.2: HPLC Instrumentation and Techniques

Types of HPLC-

Depending on how analytes interact with the stationary or mobile phase, High-Performance Liquid Chromatography has evolved into several modes. The main categories are:

NP-HPLC, or normal phase HPLC[9-10]

Principle: Polarity is the basis for separation.

Stationary phase: Alumina or polar silica

Non-polar mobile phase (isopropanol, hexane, and chloroform)

Mechanism:

Strong interactions between polar analytes and the stationary phase result in extended retention.

Non-polar analytes elute faster

Applications:

Separation of geometric isomers

Lipids and fatty acids

Low-polarity natural compounds

Method development[11-15]-

Table.1: Method development

Step No.	Method Development Parameter	Description / Purpose	Important Considerations
1	Understanding Physicochemical Properties of Analyte	Study analyte structure, pKa, polarity, solubility, UV absorption	• pKa affects ionization • Solubility determines solvent choice • Chromophore needed for UV detection
2	Selection of Chromatographic Mode	Choose RP, NP, Ion Exchange, SEC depending on analyte nature	• RP-HPLC for most drugs and natural compounds • NP-HPLC for non-polar analytes

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			• IE-HPLC for charged species
3	Column Selection	Choose appropriate stationary phase	• C18 is universal • Chiral columns for enantiomers • Particle size (1.7–5 µm) affects efficiency
4	Mobile Phase Selection	Choose appropriate solvents (Water, MeOH, ACN, buffers)	• Polarity and pH influence retention • Buffer pH ideally 2–7 • Compatibility with detector
5	Mobile Phase Optimization	Adjust ratio of aqueous/organic phases	• Gradient for complex mixtures • Isocratic for simple mixtures • Additives (TEA, FA) to improve peak shape
6	pH and Buffer Optimization	Stabilizes analyte ionization state	• pH should be ±1 of analyte pKa • Buffer concentration 5–20 mM
7	Flow Rate Selection	Controls retention time and resolution	• Typical 0.5–1.5 mL/min • Higher flow increases pressure
8	Temperature Optimization	Improves peak shape and reproducibility	• Typical range 25–40°C • Higher temperature reduces viscosity
9	Detection Parameter Selection	Choose appropriate wavelength and detector type	• PDA for multi-component detection • Select λmax for UV sensitivity • MS for high selectivity
10	Injection Volume Optimization	Prevents peak broadening or overload	• Typically 5–20 µL • Too large volume distorts peaks
11	System Suitability Testing (SST)	Ensures system is performing correctly	• Parameters: RT, Rs≥2, Tailing≤2, Plates (N), %RSD ≤2
12	Trial Runs & Optimization	Fine-tune parameters for best separation	• Adjust mobile phase ratio, flow rate, pH, column
13	Method Validation (ICH Q2 Guidelines)	Confirm reliability and reproducibility	• Accuracy, precision, linearity, LOD, LOQ, robustness
14	Documentation & Reporting	Record final conditions and chromatograms	• Include chromatograms, tables, SST, validation results

Applications in HPLC[16-20]-

- Analysis of Pharmaceutical Drugs -HPLC is widely used for Active Pharmaceutical Ingredients (APIs) assay, purity testing, and quality control. It offers precise bulk and formulation drug quantification. Even trace contaminants can be reliably detected thanks to its great sensitivity.
- Stability studies and impurity profiling - During stress investigations, HPLC detects and measures degradation products. It aids in determining the stability of drugs in various environments, including heat, light, oxidation, and humidity. This guarantees shelf-life prediction and safe formulation development.
- Pharmacokinetic and Bioavailability Research-Drug concentrations in biological fluids including urine and plasma are measured using HPLC. It encourages ADME research, which is crucial for the creation of new drugs. Its accuracy aids in assessing dosage schedules and treatment efficacy.
- Monitoring of Therapeutic Drugs (TDM)-Used in hospitals to determine the medication levels in blood samples from patients. In order to prevent toxicity or underdosing, it guarantees that medications stay within the therapeutic range. Common for immunosuppressants, antibiotics, and antiepileptics.
- Herbal Drug Standardization-HPLC quantifies marker compounds and phytochemicals in herbal extracts. It ensures batch-to-batch consistency and quality of herbal medicines. Helps detect adulteration, substitution, or degradation of botanicals.

- Control of Food and Drink Quality -Sugars, amino acids, food colours, vitamins, preservatives, and poisons are all detected using HPLC. Dairy products, juices, drinks, and packaged foods all frequently contain it. guarantees adherence to food safety laws.
- Monitoring of the Environment - used to find organic contaminants, pollutants, and pesticides in soil, water, and the air. Hazardous substances can be found in trace amounts using HPLC. supports initiatives aimed at reducing pollution and promoting environmental safety.
- Investigations in Toxicology and Forensics- Toxins, poisons, drugs of abuse, and narcotics can all be found in biological samples using HPLC. Both post-mortem study and legal investigations benefit from it. guarantees forensic evidence detection that is precise and repeatable.
- Protein Analysis and Biotechnology- HPLC separates proteins, peptides, nucleotides, and enzymes for research and diagnostic purposes. Specialized modes like ion-exchange and size-exclusion are extensively used. It guarantees biomolecule purity and structural analysis.
- Analysis of Cosmetic and Personal Care Products- used to measure antioxidants, active substances, aroma compounds, and preservatives in cosmetics. Guarantees the uniformity and safety of the product. Aids in upholding quality standards and legal compliance.

GAS CHROMATOGRAPHY

Carrier Gas Supply System[21]

- Mobile phase: Helium, Nitrogen, Argon, or Hydrogen
- Must be highly pure (99.999%)
- Flow is controlled by pressure regulators or mass flow controllers

Function: Transports vaporized sample through the system.

Sample Injection System

Types:

- Split Injection → for high-concentration samples
- Splitless Injection → for trace analysis
- On-column Injection → for thermally sensitive compounds

Function: Introduces a liquid/gas sample into a vaporization chamber (injector port) at 250–300°C.

Column and Oven[22]

Column Types:

- Packed Columns (2–4 mm ID) → low resolution, GC for gases
- Capillary Columns (0.1–0.53 mm ID) → high resolution, GC–MS

Oven:

- Maintains constant temperature (isothermal)
- Can run temperature programs (gradual increase)
- Function: Controls separation efficiency through temperature.

Detectors

Common GC detectors include:

Flame Ionization Detector (FID)

- Most widely used
- Sensitive to hydrocarbons
- High sensitivity, wide linear range

Electron Capture Detector (ECD)

- Sensitive to halogenated compounds
- Used for pesticides and environmental pollutants

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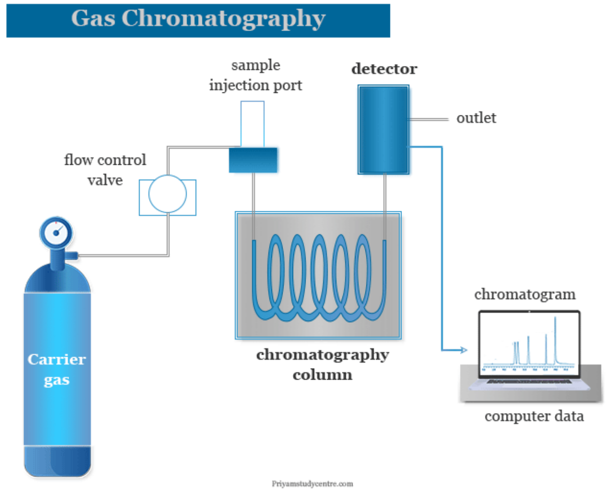

Fig.3: Gas Chromatography

Thermal Conductivity Detector (TCD)

- Universal detector
- Non-destructive

Mass Spectrometer (MS)

- Provides structural information
- Excellent sensitivity

Applications of GC[23-24]:

1. Analysis of Volatile Organic Compounds (VOCs)-Used to separate volatile substances in air, solvents, and industrial emissions. Helps monitor environmental and workplace contamination.
2. Pharmaceutical Analysis-Used for residual solvent analysis, drug purity, and impurity profiling. Ensures compliance with ICH guidelines for solvent limits.
3. Environmental Monitoring-Detects pesticides, herbicides, halogenated pollutants, and toxic gases. Essential for water, soil, and air quality assessment.
4. Petrochemical and Oil Industry-Used in petroleum refining to analyze hydrocarbons, gasoline composition, and natural gas. Ensures accurate fuel formulation.
5. Food and Flavor Analysis-Detects essential oils, flavoring agents, alcohols, preservatives, and aroma compounds. Ensures authenticity and detects adulteration.
6. Forensic Science and Toxicology-Analyzes drugs of abuse, poisons, accelerants, and blood alcohol levels. Widely used for criminal investigations and legal cases.
7. Clinical and Biomedical Analysis-Determines fatty acids, hormones, alcohols, and metabolic biomarkers. Supports diagnosis of metabolic diseases.
8. Chemical and Polymer Industry-Used for monomer purity, polymer additives, and residual solvents. Ensures quality control in chemical manufacturing.
9. Perfume and Cosmetic Industry- Separates and identifies fragrance ingredients and volatile oils. Helps in formulation stability and quality testing.
10. Natural Product Analysis- Analyses essential oils, terpenes, and plant volatile components. Widely used in herbal and Ayurvedic product development.

Future Scope of Study[25]:

As one of the most sensitive and selective analytical methods, gas chromatography (GC) is still developing and has a very bright future in the scientific, industrial, and regulatory domains. GC is anticipated to play a major role in analytical laboratories because to the growing emphasis on quality control, environmental monitoring, and quick contamination detection on a global scale. By enabling on-site and real-time analysis, innovations like micro fabricated columns, portable GC–MS devices, and miniaturized GC systems will lessen reliance on centralized facilities. These developments greatly expand the technique's use in domains like industrial hygiene, forensic science, homeland security, and occupational safety.

Furthermore, GC's sensitivity and compound identification capabilities will be further enhanced by its integration with advanced detectors such as time-of-flight mass spectrometry (TOF-MS), electron capture detectors (ECD), and flame photometric detectors (FPD).

By automating peak discovery, lowering errors, and facilitating predictive technique optimization, the use of Chemometrics and artificial intelligence (AI) in chromatographic data interpretation is anticipated to transform GC. Growing environmental awareness is also shown in the creation of green carrier gases and environmentally friendly stationary phases.

GC will remain essential in the pharmaceutical industry for the analysis of derivative biomolecules, residual solvents, and volatile contaminants. Stricter regulations will increase demand for GC-based ultra-trace detection in food and environmental sciences. All things considered, the future of gas chromatography is characterized by increased multidisciplinary applications, automation, sustainability, and technological sophistication.

II. CONCLUSION

The two most potent and popular analytical separation methods in contemporary scientific research and quality control are still high-performance liquid chromatography (HPLC) and gas chromatography (GC). While GC offers remarkable efficiency for volatile, semi-volatile, and thermally stable chemicals, HPLC is best suited for thermally unstable, non-volatile, and big biomolecules. When combined, these methods provide a thorough analytical platform that can handle a variety of industrial, chemical, medicinal, and environmental problems. With developments like UHPLC, monolithic columns, biocompatible systems, and enhanced detection technologies including PDA, fluorescence, MS, and electrochemical detectors, HPLC is still evolving. It is essential for pharmaceutical analysis, stability studies, drug development, clinical diagnostics, and nutraceutical research because to its high sensitivity, repeatable quantification, and robust separation.In contrast, complex combinations of volatile substances can be effectively separated using gas chromatography, which offers high-speed analysis and greater resolution. Selectivity and structural elucidation are greatly improved by contemporary hyphenated techniques like GC–MS, GC–FID, and GC–ECD. To guarantee accuracy and adherence to legal requirements, both approaches require methodical method development, meticulous optimization, and strict validation. The capabilities of both HPLC and GC are being further strengthened by the integration of automation, miniaturization, AI-driven data analysis, and green analytical chemistry as technology develops. HPLC and GC together form the foundation of analytical chemistry, offering unparalleled dependability, adaptability, and scientific significance in a variety of domains. A growing role in upcoming analytical and research applications is ensured by their ongoing innovation.

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