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# Review on Automation and Digitalization in HPLC for Method Development and Data Process

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## 1. ABSTRACT :

In many fields today, including biotechnology, pharmaceuticals, environmental food, and polymer industries, high-performance liquid chromatography (HPLC) is a commonly used instrumentation technique for separation and purification. Introduction to this Review of High-Performance Liquid Chromatography Chromatographic terms, types, and various HPLC classes. Additionally, it includes brief overview of the HPLC principle, pumps, and equipment Detailed inclusion with their effectiveness. The purpose of this article is to review various HPLC-related subjects such as principles, separation technique, instrumentation, features, important parameters, and numerous applications across a range of industries.

**Keywords:** biotechnology, pharmaceuticals, environmental food, polymer, Pump, principle, equipment.

## 2.INTRODUCTION :

The development of liquid chromatography (HPLC) took place in the late 1960s and early 1970s. A unit's separation between the liquid mobile phase and the stationary phase determines how much of it stays in the column. The interface phase interaction between the solute/stimulus and the solute/residual phase in HPLC influences this separation. Therefore, in contrast to GC, modifications in Your split may be significantly impacted by the composition of your mobile phase because The compounds exit the column at different times due to their varying mobilities periods. Stated differently, the retention periods vary [1].

Additionally considered is the way in which these substances interact with the column's stationary state. The equipment required to perform high performance liquid The components of chromatography are a pump, a stationary phase, and a detector that transports the mobile phase and analyte throughout the column. The analyte's retention time is also provided by the detector. The length of the retention period varies based on how strongly the analyte and the stationary state. [2, 3]

**Fig.no.1. HPLC Instrument**

**# DIFFERENT CHROMATOGRAPHY TECHNIQUES:**

**1) Normal phase chromatography:** This type of column chromatography is the most fundamental. HPLC is comparatively easy to use. Silica molecules fill the column, and the The solvent isn't polar. The mixture's polar components passing stick more firmly to the polar silica particles than the nonpolar ones throughout the column. As a result, the column's non-polar compounds travel more quickly [4,5].

2**) Reverse phase chromatography:** The most widely used kind of HPLC is reverse phase HPLC. Long hydrocarbon chains are attached to the surface in this mode, which reduces silica. The use of the polar mobile phase [6] As a result, the hydrocarbon chains bonded to the silica and the polar molecules in the solution are weaker than the bond between the polar metal and the polar molecules in the mixture [7]. Van der Waals dispersion forces cause hydrocarbon groups to be drawn to non-polar compounds. In metals, it dissolves less readily [8,9].

**3) Size exclusion chromatography:** Gel permeation chromatography, another name for size exclusion chromatography. It can also be used to determine the semi- and tertiary structures of amino acids and proteins. This technique is employed to ascertain the polysaccharide molecular weight.

4**) Ion exchange chromatography:** Ion exchange chromatography preserves the attraction between the dissolved ions and the ion sites connected to the stationary phase. This kind of chromatography is frequently employed in high pH anion exchange, protein ion exchange chromatography, ligand exchange chromatography, and water purification. oligosaccharide and carbohydrate chromatography, among other uses [10,11].

**5) Bioaffinity:** The basis for separation is a particular, reversible interaction between ligands and proteins. It shields the proteins that are bonded to the column's ligand. Common molecular forces like hydrogen bonds, van der Waals interactions, electrostatic interactions, dipole-dipole interactions, and hydrophobic interactions all play a part in the formation of these complexes.

**6) Separation Mode:** In HPLC, there are two different kinds of separation depending on the characteristics of the eluent:

**I) Isocratic:** The eluent composition remains constant in the isocratic separation mode. This indicates that the velocity of the compounds passing through the column and the equilibrium conditions within it are the same.

**II) Gradient:** The mode of gradient separation includes various combinations of detergents. This technique significantly boosts the system's resolution power. because of the increased intensity. The peak's width is determined by the speed of blending the detergent.

**3.PRINCIPLE OF HPLC :**

The principle of HPLC is that the sample solution is injected into a column of porous materials(Stationary phase) and the liquid phase (mobile phase) is pumped into higher pressure. The principle of phase separation is the introduction of solutes into the stationary phase based on its affinity for the stationary phase [12].

**4.INSTRUMENTATION:**

1. Solvent reservoir

2. Pump

3. Sample injector

4. Column

5. Detector

6. Data collection device or integrator

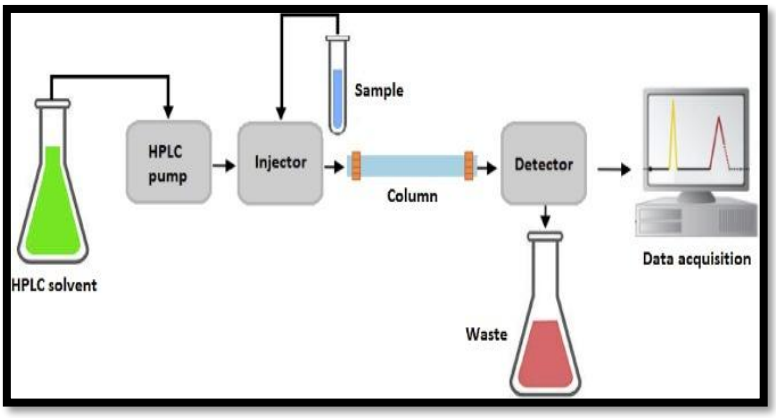


**Fig.no.2. HPLC Instrumentation**

**4.1.Solvent Reservoir:-** The solvent is stored in the solvent reservoir (mobile phase). Here are containers crafted from glass or stainless steel that stay free from discoloration. Glass bottles are considered the most common type of solvent reservoir. In addition to handling the mobile phase, the pump must meticulously and precisely blend solvents together. Low pressure mixing and high pressure mixing are the two types of mixing units. The degassing mechanism eliminates any air bubbles that may have been trapped in the solution. [13]

**Fig.no.3. Solvent Reservoir**

**4.2. Pump:-** The pump is an important part in liquid chromatography. The mobile phase is Drawn from the metal tank to the column using a pump at high pressure. The Pump pressure range is 4000 – 6000 psi. Mobile phase composition, particle Size, mobile phase flow and column size are factors that depend on the proper Operation of the pumps. The best features of pumps are metal [14,15].

**Fig. no.4. Pump**

**4.3. Sample injector :-** Septum machines are available for injection of sample Fluid. The ability to introduce [objective] the sample into a continuous flow Mobile phase stream which transports the sample to the HPLC column is Delivered by an injector (sampler or autosampler). The combination of the ring Injector and the new rotary valve can produce repeatable results. Sample Volumes range from 5 to 20 microliters (liters).Septum injections are available To inject sample fluid. Sample injection can be done when the mobile phase is Running or stopped. The combination of the ring injector and the new rotary Valve can produce repeatable results [16, 17].

**Fig.no.5. Sample injector**

**4.4. Columns:-** Columns are typically manufactured from clean stainless steel, Measuring approximately 50 to 300 millimetres in length, with an inner Diameter ranging from 2 to 5 millimeters. They are typically filled with a Stationary component having a molecular size ranging from 3 to10 millimeter [18-25].


**Fig.no.6.Columns**

**4.5. Detector :-**Detectors in HPLC are placed at the end of the analytical column. The function of the converter ( Detector) is to check the solution that came from column. The electrical signal is proportional to the concentration of the individual units in the filter.

**a) UV- visible Detector:-** This detector is the most commonly used in HPLC. Most organic compounds absorb light in the UV range (190-400 nm) and the visible range (400-750 nm). This is based on the Beer-Lambert law. Deuterium and high pressure xenon lamps are sources of UV radiation. It has many advantages and disadvantages [26].

**b) Refractive index Detector :-** Refractive index is an important property of column eluent. In this detector, the detection of the solute change depends on the total refractive index of the mobile phase. Mass spectrometers have a low sensitivity. Refractive index is very useful for detecting non-ionic compounds, and non-ions do not absorb ultraviolet range and fluorescence [27].

**The different types of RI players are as follows [28].**

i. Christiansen effect detector

ii. Interferometer detector

iii. Thermal lens detector

iv. Dielectric constant detector

**• Advantages:**

1.Reacts with all solvent

2. Does not affect flow rate

**• Disadvantages:**

1. Not as sensitive as most other types of detectors

2. Cannot be used with gradient washes.

**c) Photodiode detector (PAD), diode array detector :-**Photodiode array (radioactive devices) are used in the detection module. DAD detects absorption in the UV to VIS region. Although there is only one light-receiving unit on the sampling side, DAD has multiple photodiode arrays (1024 for L-2455/2455U) to acquire information over a wide range of wavelengths simultaneously. is one of the benefits of DAD [29].

**d) Fluorescence Detector:-** This detector stands as the most sensitive and specific among all the existing High-Performance Liquid Chromatography (HPLC) detectors. It is capable of identifying the presence of a single analyte molecule within the flow cell. The sensitivity of this detector is measured to be 10 - 1000 times greater than that of a UV detector [30].

• There are several types of fluorescence detectors available, including:

1. Single Wavelength Excitation Fluorescence Detector

2. Multi-Wavelength Fluorescence Detector

3. Laser-Induced Fluorescence Detector

**e) Electrical Conductivity Detector: -** This device offers a universal, reproducible, and highly sensitive method for the detection of various charged species, including anions, cations, metals, and organic acids. It measures the conductivity of the total mobile phase, thereby classifying it as a bulk density detector. The electrodes of this detector are typically composed of platinum, stainless steel, or other noble metals [31].

**Features of Detectors used in HPLC :-**

1) It must generate a reliable and reproducible signal

2) It should be non destructive

3) The peaks should not be expanded

4) It must to be unaffected by the gradient or eluent composition

5) The response must not be impacted by temperature changes [32].

6) Data collection Device:-The data collection device gracefully captures signals from the detector, which are then recorded on graph recorders or electronic integrators. These devices vary in their multifaceted capabilities, including processing, storing, and reprocessing chromatographic information. The interconnected PC monitors the health of the components, coordinating their responses and transferring data to a readable chromatograph. The output is typically captured as a sequence of peaks, with each peak corresponding to a specific compound within the mixture as it flows through the detector and absorbs UV light.

5.**Automation and digitalization in HPLC for method development and data process :-**

Automation and digitalization in HPLC method development and data processing have significantly enhanced efficiency, accuracy, and throughput. Modern HPLC systems leverage artificial intelligence (AI), machine learning, and advanced software automation to optimize chromatographic parameters, perform real-time data evaluation, and reduce human intervention in method development and analysis.

**## Automation in HPLC Method Development**

Automated HPLC method development uses AI-driven systems to plan, execute, and optimize chromatographic methods in real-time. For example, the LabExpert system applies artificial intelligence to control experimental parameters like column choice, mobile phase composition, and flow rates, evaluating output data instantaneously to guide decision-making. This closed-loop automation reduces the need for expert manual input at each step and accelerates optimization processes compared to traditional trial-and-error approaches. Bayesian optimization algorithms have also been employed to adjust key variables automatically, optimizing chromatogram quality within a limited number of experiments, thus enabling operator-free method development.

**## Digitalization in Data Processing**

Digitalization in HPLC encompasses integration with advanced data processing software that automates tasks like peak detection, resolution evaluation, and statistical analysis, often employing machine learning models such as Support Vector Machines and genetic algorithms. These tools streamline data interpretation, predict optimal chromatographic conditions based on molecular properties, and facilitate robustness testing by handling multiple performance criteria simultaneously.

## Benefits and Applications

- Increased throughput and faster development times due to automated, real-time method optimization.

- Improved reproducibility and precision through consistent instrument control and data evaluation.

- Reduction in operator dependency and human error.

- Enabling high-throughput screening of columns and mobile phases.

- Enhanced capability to handle complex mixtures, supporting pharmaceutical development, bioanalysis, and clinical testing.

Overall, the integration of automation and digitalization in HPLC method development and data processing represents a transformative shift toward more intelligent, efficient, and operator-independent analytical workflows in pharmaceutical and chemical analysis.

These insights are supported by recent literature describing AI-based systems like LabExpert and Bayesian optimization for fully automated, real-time HPLC method optimization, as well as reviews on software-assisted method development, modern autosamplers, and machine learning applications in chromatographic data analysis.

**6.Components of method validation:**

The following are typical analytical Performance characteristics which may be tested during methods validation:

1. Accuracy
2. Precision
3. Linearity

4. Detection limit

5. Quantitation limit

6. Specificity

7. Range

8. Robustness

**6.1.Accuracy:-** Accuracy is defined as the degree to which a measured value Approaches the true or accepted value. Accuracy refers to the difference Between the observed mean value and the actual value. To determine lt , apply The procedure to samples with known amounts of analyte added. Analyze these Against standard and blank solutions to guarantee no interference. The Accuracy of the test results is calculated as a percentage of the analyte Recovered by the assay. Recovery can be represented as the assay of known Levels of analyte [33].

6.2.Precision :- The degree of agreement (scatter) between measurements Acquired from multiple samplings of a homogenous sample under specified Conditions. Precision measures the reproducibility of the entire analytical Technique [34].

It has two components: repeatability and intermediate Precision.

Repeatability refers to the variation encountered by a single analyst on the Same instrument. The method does not distinguish between variations caused By the instrument or system and those caused by sample processing. Validation involves testing numerous replicates of an assay composite sample Using a specific analytical procedure. The recovery value is calculated. Intermediate precision refers to variations within a laboratory, including Between days, instruments, and analysts [35,36].

**6.3. Linearity:-** Linearity is an analytical process that produces a result. Proportional to the concentration of analytes in the sample. If the process is Linear, the test results are proportional to the concentration of analyte in. Samples from a specific range. Linearity is commonly defined as the confidence.[37].

**6.4. Quantitative and detection limits:** The limit of detection (LOD) is the Minimal amount of an analyte in a sample that is detectable but not Measured. A concentration at a particular signal-to-noise ratio, usually 3:1, is referred to as LOD. The lowest value is known as the limit of quantitation (LOQ).Concentration of an analyte in a sample that can be ascertained using the specified operational parameters of the method with a satisfactory level of precision and accuracy [38, 39].

**6.5.Specificity:-** Specificity is the ability to accurately assess an analyte. In the presence of predicted components: impurities, degradation agents, matrix, and Other components are typical examples. Analytic procedures with limited Additional procedures can be used to increase specificity.

The following implications apply: Identification ensures an analyte's identity. Purity tests ensure the accuracy of an analyte's impurities, including related An assay measures compounds, heavy metals, residual solvents, and other factors. The concentration or potency of an analyte in a sample, which yields precise results [40].

**6.6. Range:-** The upper and lower levels of an analyte are referred to as the method's range. Determined with linearity, accuracy, and precision that are acceptable. Usually, it is Stated using the same units as the test results, and it may be derived from a linear or Nonlinear response curve [41].

**6.7. Robustness:-** An analytical procedure's robustness is determined by its capacity to tolerate small but intentional changes in method parameters, demonstrating its dependability during regular use [42].

**7.Future trends:**

Emerging technologies.

Additional advances in HPLC technology:

• Nano-HPLC provides increased sensitivity while using fewer samples.

• Microfabricated columns: Miniaturization results in faster and more efficient Separation.

• Advanced detection methods: enhanced sensitivity and selectivity through Detector advancements. Integration of Other Techniques. HPLC and Other Analytical techniques are increasingly being combined to produce better outcomes.

• HPLC-MS: combines HPLC with mass spectrometry to provide molecular Information

• HPLC-NMR: Combines high-performance liquid chromatography and nuclear magnetic resonance to reveal structural details.

• HPLC-FTIR: combines HPLC and Fourier-transform infrared spectroscopy. For comprehensive compound identification.

**8. Advantages:**

1.It has contributed significantly to the advancement of analytical science and Its wide range of uses in food, forensics, pharmaceuticals, and the environment. Clinical applications, polymers and plastics, etc.

2.HPLC offers a relatively quick, highly specific, and reasonably precise Analytical technique for a variety of complex samples.

3.HPLC is capable of handling macromolecules.

4. It works extremely well with the majority of "pharmaceutical drug substances."

5. It provides a productive way to analyze "labile natural products."

6. HPLC makes sample preparation and introduction quick and easy.

7. Compound resolution and separation speed are high.

8.Results from HPLC software can be reported with accuracy and precision.

9. The detectors' sensitivity is high.

**9. Disadvantages:**

1.HPLC is regarded as one of the most crucial methods of the past The twentieth century's decade. Despite all of the benefits there Are some restrictions as well. The cost of columns and solvents are among the limitations. And the column's proprietary nature, which prevents long-term reproducibility Packing.

2.The difficulty of separating some protein-specific antibodies.

3.The price of creating an HPLC device for an assay or technique of the degree of component separation is enormous.

4.Certain compounds have low sensitivity to the stationary phase in the Columns is challenging.

5.Some substances are absorbed or interact with the substances in the Packing supplies for the column.

6. Occasionally, the pressure may fluctuate so much that the column is unable to Resist or separation might not occur.

7. If HPLC is not interfaced with mass, qualitative analysis may be restricted. Spectroscopy.

8. Very complex samples have limited resolution.

9. Newer trends with better efficacy have been established.

**10.APPLICATION :-**

- HPLC is used in the food business as well as in the pharmacy, environmental, clinical, and forensic domains. Resolution, identification, and quantification of a molecule are among the data that HPLC may provide. It also helps with purification of mixtures of substances, molecular weight measurement, and chemical separation.

Other applications includes:-

**• Pharmaceutical application:-**

1) Research on the dissolution of tablets used

to administer medicinal dosages. stability research and shelf-life calculations.

2) Determining the pharmacological active components in dose formulations.

3) Pharmaceutical formulation assay and impurity analysis.

4) Quality assurance.

5) Development and research.

**• Environmental application:-** include the identification of phenolic chemicals in potable water. Diphenhydramine detection in sedimented samples. Pollutant biomonitoring.

**• Forensic:-** Drug quantification in biological samples is part of forensics. Anabolic steroid detection in sweat, hair, urine, and serum. identifying whether cocaine and its metabolites are present in the blood. textile industry forensic analysis.

**• Clinical:-** Quantification of ions in human urine. Antibiotic analysis in blood plasma. Estimation of bilirubin and bilivirdin in blood plasma in the presence of hepatic diseases. Endogenous neuropeptides can be detected in extracellular fluids.

**• Food and Flavor:-** Maintaining the standard of drinking water and soft drinks. evaluation of alcohol and its byproducts.analysis of fruit liquids for sugar. Polycyclic chemicals in vegetables are analyzed. trace examination of agricultural produce containing military high explosives. checking fruits for pesticides and insecticides.

**11.USES OF HPLC:**

1) This method is employed in chemistry and biochemistry Research to analyze complex mixtures, purify chemical compounds, create Synthesis processes for chemical compounds, isolate natural products, or Forecast physical characteristics.

2) It is also employed in quality control to monitor degradation, quantify tests Of finished products, manage and enhance process yields, and guarantee the Purity of raw materials.

3) It is also employed for the analysis of water and air contaminants.

4) Food and pharmaceuticals goods are surveyed by federal and state Regulatory bodies using HPLC.

**12.Conclusion :-**

The HPLC has mostly used an analytical technique. It can be used in both Laboratory and clinical science. Reverse phase elution is advantageous over the normal phase. Since isocratic elution decreases the loading capacity, Gradient elution is more useful. C8 and C18 columns are generally used. UV Detectors are widely used. The typical average pH of reversed-phase on silicabased packing is 5.0. Adequate buffer concentration is 10-50 mM. Different Applications are in the field of pharmaceutical analysis, environment, forensic, Food, and clinical. Other applications include preparation, chemical Separation, purification, and identification. The only disadvantage of HPLC is The high cost.

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