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A COMPREHENSIVE REVIEW ON HPLC ANALYTICAL METHOD

Maithili Dhond*, Punam Kelkar, Ashlesha Bhogate, Suyash Mandavkar, Sushant Kadam and Amankumar Chavan

Shree Saraswati Institute of Pharmacy, Tondavali.

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***Corresponding Author**
Maithili Dhond
Shree Saraswati Institute of Pharmacy, Tondavali.

ABSTRACT

HPLC (High Performance Liquid Chromatography) is a type of column chromatography. It is analytical technique which is used to examine solution that is eluting from column. It is also one of the most powerful technique for separation and quantitative determination of component in mixture. Most of the drug that present in multicomponent dosage for can be analyze by HPLC method. It is work on principle that involve two phases: “Stationary Phase” and “Mobile Phase”. According to ICH Guidelines, validation of HPLC process includes testing for system appropriateness, accuracy, precision, specificity, linearity, range, limit of detection and quantification, robustness and other factors.

KEYWORDS: HPLC.

INTRODUCTION

General Introduction

High-performance liquid chromatography (HPLC), previously known as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify particular components in mixtures. The acronym for high performance liquid chromatography is HPLC. “Chromatography” refers to a method of separation, “chromatogram” to the outcome of the process, and “chromatograph” to the tool employed in the process. Chromatography (from Greek: Chroma "color" and graph in "to write") is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's

partition coefficient result in differential retention on the stationary phase and thus changing the separation.

HPLC is a very accurate analytical technology used for both quantitative and qualitative pharmacological product examination, including stability determination. In HPLC, there are two phases: mobile phase and stationary phase. The HPLC principle involves injecting a sample solution into a porous column (stationary phase) and pumping the liquid phase (mobile phase) at high pressure through it. HPLC is frequently used in manufacturing.

HPLC can separate and detect each chemical based on the difference in speed through the column. The liquid used to dissolve the target compound is referred to as the mobile phase. The stationary phase is the section of a column that interacts with the desired substance. In the 1960s, LC with low-pressure glass columns evolved into high-pressure chromatography (HPLC) using metal columns. HPLC primarily uses a column to retain packing material (stationary phase), a pump to transport the mobile phase(s) through the column, and a detector to display the retention periods of the molecules. Retention time varies based on the retention time varies based on the interactions between the stationary phase, the molecules under investigation, and the solvent(s) utilized.

History of Chromatography

The history of chromatography begins during the mid-19th century. Chromatography, literally "color writing", was used and named in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll. New types of chromatography developed during the 1930s and 1940s made the technique useful for many types of the separation process. Chromatography became developed substantially as a result of the work of Archer John Porter Martin and Richard Laurence Millington synge during the 1940s and 1950s. They established the principles and basic techniques of partition chromatography, and their work encouraged the rapid development of several types of chromatography methods: Paper chromatography, gas chromatography, and what would become known as high-performance. Since then, technology has advanced rapidly. Researchers found that the main principles of Teat's chromatography could be applied in many different ways, resulting in different varieties of chromatography. Simultaneously, advances continually improved the technical performance of chromatography, allowing the separation of increasingly similar molecules.

TYPES OF HPLC

HPLC can be classified as follows:

- **Based on a scale of operation**

Preparative HPLC and analytical HPLC 7.

- **Based on the principle of separation**

Affinity chromatography, adsorption chromatography, size exclusion chromatography, ion-exchange chromatography, chiral phase chromatography.

- **Based on the elution technique**

Gradient separation and isocratic separation.

- **Based on modes of operation**

Normal phase chromatography and reverse-phase chromatography.

A. Normal phase chromatography

Normal phase chromatography divides analyte according to polarity. The mobile phase in normal phase chromatography is nonpolar, whereas the stationary phase is polar. As an outcome, the polar analyte is retained by the station phase. The increased polarity of solute molecules improves adsorption capacity, resulting in a longer elution time. In this chromatography, a stationary phase of chemically modified silica (cyanopropyl, aminopropyl, and Diol) is used. As an example, A typical column has an interior diameter of around 4.6 mm and a length ranging from 150 to 250 mm. Polar compounds in the mixture that are passed through the column will stick to the polar silica for a longer period than non-polar compounds. As a result, the non-polar ones will go quickly through column.

B. RP-HPLC (Reversed-phase HPLC)

It is also called as “Switched phases”. The stationary phase of RP-HPLC is non-polar, and the mobile phase is polar or moderately polar. As a result of repulsive forces between a polar eluent, the comparatively non-polar analyte, and the non-polar stationary phase, RPC works on the theory Of hydrophobic interactions. The non-polar stationary phase will hold analytes that are comparatively less polar in a combination of components for a longer period than those that are substantially more polar. As a result, the most polar component elutes the first. Reversed phase chromatography can separate Molecules that are somewhat hydrophobic with great recovery and resolution.

C. Ion exchange Chromatography

The attraction between solute ions and charged sites bound to the stationary phase drives retention in ion-exchange chromatography. Same-charge ions are not included. This type of chromatography is frequently employed in the ion-exchange chromatography of proteins, the ligand-exchange chromatography of proteins, the high-pH anion-exchange chromatography of carbohydrates and oligosaccharides, and other processes for the purification of water. The attraction between solute ions and charged sites bound to the stationary phase drives retention in ion-exchange chromatography. Ionic or ionizable samples are virtually often employed with this approach. The higher the charge on the sample, the stronger it will be attracted to the ionic surface and hence, the longer it will take to elute.

D. Size exclusion Chromatography

SEC, also known as gel permeation chromatography or gel filtration chromatography, is a type of chromatography that primarily uses size to separate particles. It is helpful for figuring out the quaternary and tertiary structures of proteins and amino acids. The molecular weight of polysaccharides can be determined using this technique. Particles are mostly separated based on size using SEC, also known as gel permeation chromatography or gel filtration chromatography. The sample is simply screened or filtered, and the column is loaded with material with carefully regulated pore diameters. Larger molecules quickly pass through the column while smaller molecules enter the packing particles' pores and elute later. This method is frequently used to determine the molecular weight of polysaccharides.

E. Bioaffinity chromatography

Separation based on a particular, reversible interaction between ligands and proteins. A bio-affinity matrix has ligands covalently bonded to a solid support that holds onto proteins that interact with the ligands connected to the column.

A Bioaffinity column can elute proteins bound to it in one of two ways

- Incorporating a free ligand in the elution solution to compete with column-bound ligands in biospecific elution.
- Aspecific elution: alteration in pH, salt content, etc. that reduces protein-substrate interaction.

Bioaffinity chromatography can produce very high purity in a single stage (10–1000-fold) due to the selectivity of the contact.

PRINCIPLE

High-Performance Liquid Chromatography (HPLC) is a separation technique utilizing differences in the distribution of compounds to two phases, called stationary phase and mobile phase. The stationary phase designates a thin layer created on the surface of fine particles and the mobile phase designates the liquid flowing over the particles. Under a certain dynamic condition, each component in a sample has different distribution equilibrium depending on solubility in the phases and/or molecular size. As a result, the components move at different speeds over the stationary phase and are thereby separated from each other. This is the principle behind HPLC. The column is a stainless steel (or resin) tube that is packed with spherical solid particles. The mobile phase is constantly fed into the column inlet at a constant rate by a liquid pump. A sample is injected from a sample injector near the column inlet. The injected sample enters the column with the mobile phase and the components in the sample migrate through it, passing between the stationary and mobile phases. Compounds move in the column only when are in the mobile phase. Compounds that tend to be distributed in the mobile phase, therefore, migrate faster through the column while compounds that tend to be distributed in the stationary phase migrate slower. In this way, each component is separated on the column and sequentially elutes from the outlet. Each compound eluting from the column is detected by a detector connected to the outlet of the column. When the separation process is monitored by the recorder starting at the time the sample is injected, a graph is obtained. This graph is called a chromatogram. The time required for a compound to elute (called retention time) and the relationship between compound concentration (amount) and peak area depend on the characteristics of the compound. Retention time is therefore used as an index for qualitative determination and peak surface area (or height) as an index for quantitative determination. The retention time of the target compounds and the concentration for each unit of peak area is based on data obtained in advance by analyzing a sample with known quantities of the reference standards. Normally, the reference standards are highly purified target compounds (shown in following fig.)

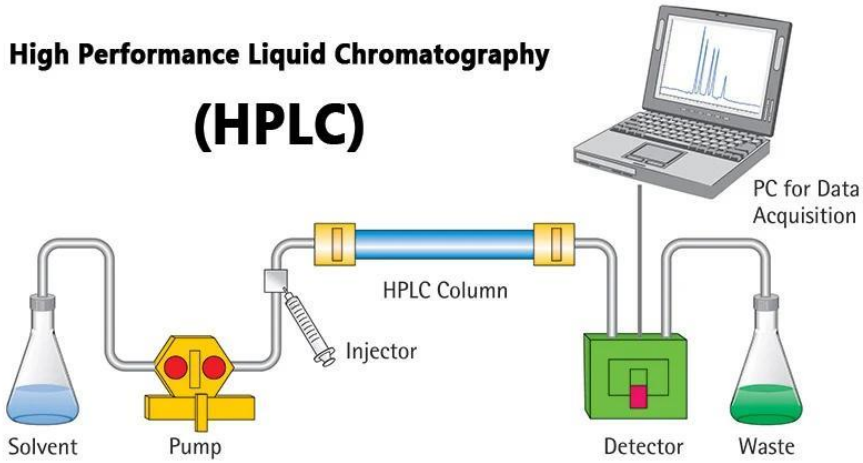

Fig. no. 1.

INSTRUMENTATION

A. Pump

The HPLC pump, often called a solvent delivery system, provides a precise flow of mobile phases of a specified composition to the column. The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from the solvent reservoir into the system. High-pressure generation is a "standard" requirement of pumps besides which, it should also to be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate. Most pumps used in current LC systems generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces "pulses".

Their performance, pressure ratings, and reliability have increased dramatically in the last two decades to accommodate the demands from the use of narrow columns packed with small particles in ultrahigh-pressure liquid chromatography (UHPLC).

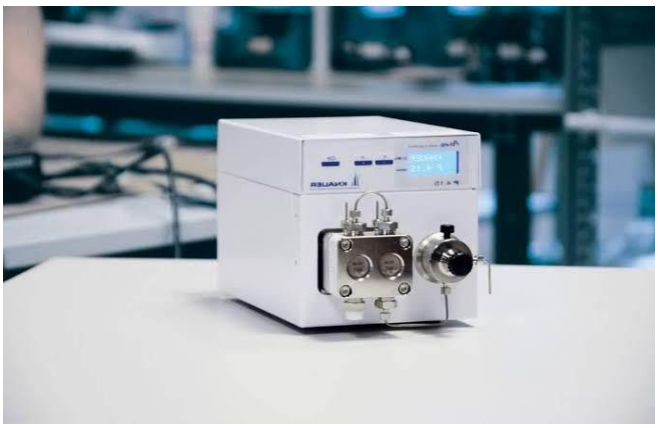

Fig. no. 2.

Key terms related to HPLC pump

- **Piston**

A rod made of inert materials like sapphire, ceramics, steel, or alloy. The piston is reciprocating within a piston chamber or cylinder in the pump head.

- **Drive**

A mechanical unit that actuates a piston's back-and-forth motion. The drive train is typically powered by a motor, and includes a mechanism like a circular cam or screw drive to transform rotations into linear motion.

- **Pump head**

A metal body in which one or multiple cylinders or chambers are milled or drilled to accommodate the piston(s).

- **Check valve**

A device that allows liquid flow in one direction (typically using a passive gravity–based ball-and-seat design). It prevents any backflow and pressure drops, such as those that occur during the piston's recharge cycle. Some check valves are active or electronically actuated (examples include Agilent 1100, 1200, and 1260).

- **High-pressure pump unit**

A subunit of an HPLC pump delivering a high-pressure flow for a single or mixed solvent. Connecting the outlets of two pump units makes a binary pump. A pump unit may be a core of an isocratic pump, or a quaternary pump by attaching a four-port proportioning valve at the inlet side.

- **Proportioning valve**

A block with typically four solenoid valves, each connected to a separate solvent line to allow blending of a mixed mobile phase from up to four solvents using a single pump unit. The opening of the solenoid valves is electrically driven, and the solvent mixing ratio is determined by the timings of the opening of the solenoid valves.

- **Dwell volume**

The volume in the HPLC system from the point of mixing of two or more solvents to the head of the column. Dwell volumes translate into gradient delay times, which become

important for high-throughput analysis and low-flow gradient applications.

- **Mixer**

Mixers are required to ensure adequate mixing of blended mobile phases. The volume of the mixer should be large enough for applications sensitive to composition fluctuations, such as UV-detection with UV-absorbing additives in the mobile phases. Mixer volumes range from a few µL to 2 mL in analytical HPLC systems.

- **Pulse damper**

An elastic chamber located in the high-pressure flow path of the pump that functions to reduce residual flow pulsations from the reciprocating pump unit(s).

- **Degasser**

An in-line device to reduce the gas content of the solvent before entering the pump unit. The most common design uses tubes of semi-permeable membrane located inside an evacuated chamber to eliminate dissolved oxygen and nitrogen.

B. Injector

The autosampler, an automated sample injector, introduces a precise aliquot of a sample solution from a sample container to the high performance liquid chromatography (HPLC) column under high-pressure flow conditions. It is a sophisticated automation device, capable of great precision and long-term reliability. An injector is placed next to the pump. The simplest method is to use a syringe, and the sample is introduced to the flow of eluent. The most widely used injection method is based on sampling loops. Their performance, pressure ratings, and reliability have increased dramatically in the last two decades to accommodate demands from the use of narrower columns in ultrahigh-pressure liquid chromatography (UHPLC).

Fig. no. 3.

Fig. no. 4.

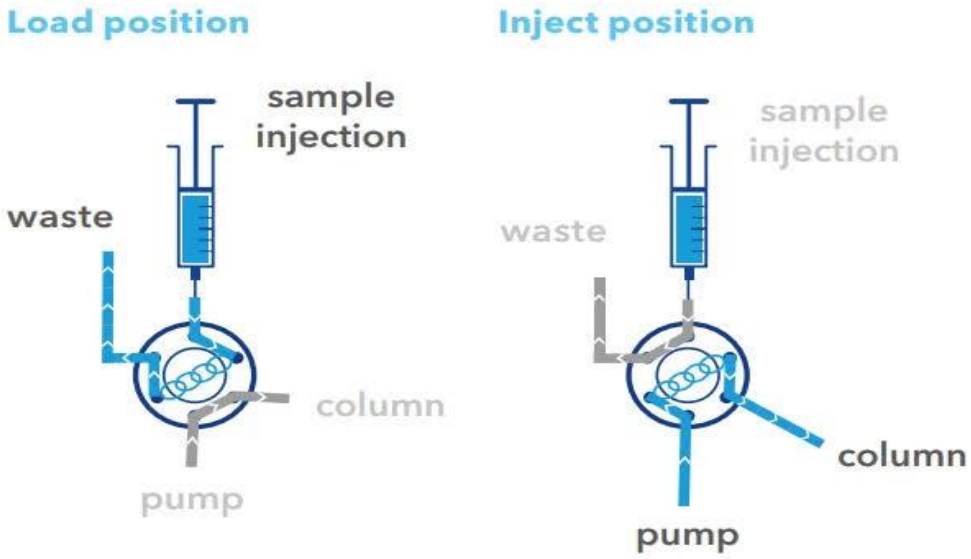

Fig. no. 5.

Key terms related to injector

- **Autosampler**

A device that automates the introduction of an aliquot of sample solution to the HPLC column. An HPLC autosampler typically comprises a sample storage compartment with an injector, consisting of a valve, a sample dosing or metering device, and a moving sampling needle**.**

- **Injection Valve**

A device such as a rotary valve that allows the introduction of a sample solution in the HPLC column under high-pressure and flow conditions. An injection valve can be a standalone manual valve, or part of an autosampler. It typically consists of a needle port, a rotor and stator combination, and an exchangeable sample loop.

- **Pulled-loop, pushed-loop, or split-loop design**

These are the three common modes of sample introduction designs. A split-loop design includes the sampling needle as part of the sample loop, and aspires an exact sample aliquot to be introduced. A pulled-loop design aspires the sample by vacuum from an external sampling syringe into the loop. A pushed-loop design fills a loop with pressure.

- **Full-loop or partial-loop mode**

Two modes of injection are used with pulled- or pushed-loop autosamplers. In a full-loop (or fixed-loop) mode, the entire sample loop is filled with the sample solution. In a partial-loop or variable-loop mode, only a fraction of the loop (10%–50%) is filled. The full-loop mode is more precise, particularly for small-volume injections, but requires more sample solution to overfill the loop (three times the sample loop volume to ensure that the entire loop is filled with the sample solution). The partial-loop mode offers more flexibility than the full-loop mode by its ability to inject different sample volumes without a physical change of the sample loop.

- **Sampling syringe or metering device**

A precise volume dosing unit required for sample aspiration. Two types are commonly used. One is a flow-through metering device, which is a part of the high-pressure flow path of a split-loop autosampler; the other is a low-pressure dead-end sampling syringe.

- **Sample container**

Typically, a 2-mL glass vial containing the sample dissolved in a diluent (final sample solution). Vials are placed in an autosampler-compatible vial holder, typically rectangular vial trays (vial racks), or vial carousels. Other configurations are microplates, commonly used in high-throughput screening (HTS) applications.

- **Sampling needle**

Typically, a 22-gauge blunt-tip needle of a manual sample syringe connected to the end of a movable stainless steel or polyether ether ketone (PEEK) sampling capillary, typically used with pushed-loop type. Split-loop autosamplers use highly specific sampling needles that match the needle-port, and must be capable of operating at high pressure.

- **Needle wash port**

A needle port connected to waste for parking the sampling needle and rinsing the outside of

the sampling needle with a solvent that is supplied by a peristaltic pump (or by the sampling syringe flushing through the inside and outside of the needle). The needle wash is used to reduce carryover from the previous sample, which adheres to the outside of the needle.

- **Needle seat/port**

For pushed-loop autosamplers, an orifice through which the sampling needle can fill the sample loop; for split-loop autosamplers, a counterpart port for the sample needle that must seal to the maximum system pressure level. This port is not required for pulled-loop autosamplers.

- **Carryover**

The amount of the main component from the prior injected sample, which is observed in a subsequent injection of the sample diluent (blank) without analyte. Carryover performance is highly analyte-dependent, and is particularly problematic for very basic compounds and proteins.

- **Injection volume precision**

Precision reflects the variation (scatter) of the delivered sample volumes during repetitive injections as a relative standard deviation (RSD) of peak area or peak height.

- **Injection volume accuracy**

Accuracy describes the deviation or systematic error of the absolute injection volume from the intended volume. The volume accuracy requirement is less relevant in practice, since method calibration (standardization with a reference compound) is performed in quantitative HPLC analysis.

- **Injection cycle time**

The time required to accomplish a sample injection that includes device movements to bring the sampling needle to the sample, sample volume aspiration, and needle wash or loop cleaning. The injection cycle can be reduced by overlapping with the ongoing separation cycle time (if allowed by the instrument and control software).

- **Pressure transducer**

The pressure sensor in the autosampler flow path used in some autosamplers to monitor the

injection sequence. The pressure sensor can be used to optimize the injection routine timing, or warn the customer in case of any irregular behavior.

C. Column

In HPLC chromatography, the column is where the separation of the sample takes place. This is where the sample is passed through the stationary phase with the mobile phase, resulting in the separation of the sample components. As the sample being analyzed is likely to vary in terms of its chemical and physical properties, therefore so should the method used to analyze it. Hence why there are multiple types of columns. This also goes for the stationary and mobile phases being used. In HPLC chromatography, the column is where the separation of the sample takes place. As columns are so important to the chromatography process, it's important you use the right type of column for the correct use, otherwise, it can lead to inaccurate results or even failed separation.

Four Types of HPLC Columns

1. Normal Phase Column
2. Reverse Phase Column
3. Ion-exchange Column
4. Size Exclusion Column

1. Normal Phase Column

The normal phase column is packed with extra molar material than the mobile phase. In normal phase columns, the mobile phase should be methanol, ethanol, acetone, acetonitrile, chloroform, etc. The use of water as a mobile phase is not recommended in normal phase columns because water has a higher polarity in nature. The normal phase column is packed with extra molar material than the mobile phase. The eluent is dissolved in less polar material like methanol, when passes through the column and get in interacts with the high polar stationary phase, thus good separation happened. Such columns are very popular in pharmaceutical product testing. Columns containing silica are very efficient for separating non-polar and moderately polar isometric compounds.

Fig. no. 6.

2. Reverse Phase Column

In reverse phase columns as its name states, it is the reverse of the normal phase columns. It has a non-polar or less polar stationary phase than the more polar mobile phase. Bonded hydrocarbons like C8 and C18 and other non-polar hydrocarbons are used as stationary phase in reverse phase columns while aqueous organic solution like water-methanol or water-acetonitrile mixture is used as mobile phase. In reverse phase columns as its name states, it is the reverse of the normal phase columns. Separation of sample components in reverse phase columns also occurs on the basis of the polarity of the sample components but it happens just opposite of the normal phase HPLC columns, therefore, this type of chromatography is known as Reversed Phase Chromatography.

Fig. no.7.

3. Ion exchange HPLC columns

Ion exchange HPLC columns have charged packing. An ion-exchange column can be either cationic or anionic. This type of HPLC column separates polar molecules based on their charge. The mobile phase is an aqueous buffer. Ion exchange HPLC columns can be used to separate many types of analytes and are commonly used for the separations of carbohydrates, amino acids, and proteins. Ion exchange and ligand exchange chromatography may be

combined in a column. Ion exchange and ligand exchange chromatography may be combined in a column. In these combined-mode columns, ion exchange is usually via metal ions, and the ligands are electron-donor molecules such as hydroxyl groups or amines. This type of HPLC column is frequently used for the separation of monosaccharides.

Fig. no. 8.

4. Size Exclusion Columns

Size exclusion chromatography separates the sample using particle size. It uses a porous stationary phase that only allows small particles into the pores, leaving the larger molecules to pass through the column faster. The pore size in the stationary phase determines the retention time and elution profile of each sample component, as each molecule diffuses into the pores to a different extent. Size exclusion chromatography separates the sample using particle size. The stationary phase needs to have porous particles for size exclusion chromatography so molecular sieves (such as zeolites), polysaccharides, and polymers (like a typical silica column) are most commonly used. This technique is used to separate proteins and carbohydrates.

Fig. no. 9.

D. Detector

Separation of analytes is performed inside the column, whereas a detector is used to observe the obtained separation. The composition of the eluent is consistent when no analyte is present. While the presence of analyte changes the composition of the eluent. What detector does is to measure these differences. This difference is monitored as a form of an electronic signal. There are different types of detectors available.

Detectors for HPLC are designed to take advantage of some physical or chemical attribute of either the solute or mobile phase in the chromatographic process in one of four ways:

- A bulk property or differential measurement
- Analyte specific properties
- Mobile phase modification
- Hyphenated techniques

Types of Detectors

1. UV-Visible Detector

The UV–vis absorbance detector is the most common HPLC detector in use today because many compounds of interest absorb in the UV (or visible) region (from 190 to 600 nm). Sample concentration, output as absorbance, is determined by the fraction of light transmitted through the detector cell by Beer's law:

$$A = \varepsilon lc$$

Where A is absorbance, ε is the molar extinction coefficient (which depends on the nature of the chemical and the wavelength of the light used), l is the length of the path light must travel in the solution in centimetres, and c is the concentration of a given solution.

UV absorbance occurs as a result of the transition of electrons from $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$, or $n \rightarrow \sigma^*$ molecular orbitals; most aromatic compounds absorb strongly at or below 260 nm, compounds with one or more double bonds (for example. Carbonyls and olefins) at ~215 nm, and aliphatic compounds at ~205 nm.

There are three different types of UV detectors:

1. Fixed-wavelength detectors
2. Variable-wavelength
3. Photodiode-array detectors

2. Fluorescence Detectors

Fluorescence detectors measure the optical emission of light by solute molecules after they have been excited at a higher energy wavelength, and they can be very sensitive for compounds that have native fluorescence or that can be made to fluoresce through derivatization. Schematically, they resemble UV detectors, except that the grating is replaced by a filter or monochromator at a right angle to the incident light to simplify the optics and reduce background noise. The light source is usually a broad spectrum deuterium or xenon flash lamp. The excitation wavelength (often close to the UV λmax) is selected by a filter or monochromator between the lamp and the flow cell, always at a higher energy (lower wavelength) than the emission wavelength. Laser-induced fluorescence (LIF) detectors using lasers as the excitation source are sometimes used in micro- or capillary-LC systems in which the higher energy of the laser provides better sensitivity in the small diameter flow cells necessary to limit dispersion.

Fluorescence detectors can be as much as 100× more sensitive than a UV detector, making them particularl useful for trace analyses, or in sample-limited or low-concentration sample situations.

3. Electrochemical Detectors

For compounds that can be oxidized or reduced, the electrochemical (EC) detector is one of the most sensitive and selective HPLC detectors available (21,22). EC detectors require the use of electrically conductive HPLC mobile phases (buffers suffice), and when properly used and maintained are the standard bearer when it comes to response levels for the HPLC analysis of compounds such as catecholamines and neurotransmitters. EC detectors for HPLC usually contain three separate electrodes: a working, a counter (auxiliary), and a reference electrode. Common electrode materials are carbon, gold, silver, or platinum. A fixed-potential difference is applied between the working electrode and the reference electrode to drive an electrochemical reaction at the working electrode's surface. Current produced from the electrochemical reaction as compounds are oxidized or reduced at the working electrode is balanced by a current flowing in the opposite direction at the counter electrode. The EC detector response output is the amplified current resulting from the electrochemical reaction at the working electrode.

4. Radioactivity Detectors

Radioactivity detectors (sometimes referred to as radiometric or radio-flow detectors) are

used to measure radioactive analytes as they are eluted from the HPLC column. Most radioactivity detectors are based on liquid scintillation technology to detect phosphors caused by the radioactive nuclides, such as low-energy β-emitters (for example, 35S, 14C, 3H, and 32P) or stronger α-, β-, and γ-emitters (for example, 131I, 210Po, and 125Sb); tritium and 14C being the most common. A liquid scintillator can be added postcolumn (called homogeneous operation) or the flow cell can be packed with beads of a permanent, solid-state scintillator (heterogeneous operation). The radioactivity detector can be very sensitive, and is extremely useful for the detection of radiolabeled compounds in toxicological, metabolism, or degradation studies. Large flow-cell volumes typically are used in radioactivity detectors to increase analyte residence time, which increases the number of radioactive decays that can be detected. Peak tailing and broadening caused by the larger cell volume can be minimized by using larger volume columns assuming that sufficient sample is available for larger mass injections to compensate for on column dilution. The use of large cell, column, and injection volumes generally limits radiochemical detector use in UHPLC.

5. Conductivity Detection

The conductivity detector is a bulk property detector that measures the conductivity of the mobile phase. Conductivity detectors are the detector of choice for ion chromatography or ion-exchange separations when the analyte does not have a UV chromophore (23,24). In a conductivity detector, the resistance (or strictly the impedance) between two electrodes in the flow cell is measured. For many applications, particularly ion chromatography, in which conductive buffers are required in the mobile phase, a suppressor column is used postanalytical column (before the detector) to reduce the background conductance of the mobile phase.

E. Recorders

The change in eluent detected by a detector is in the form of an electronic signal, and thus it is still not visible to our eyes. In older days, the pen (paper)-chart recorder was popularly used. Nowadays, a computer-based data processor (integrator) is more common. There are various types of data processors; from a simple system consisting of the in-built printer and word processor while those with software that are specifically designed for an LC system which not only data acquisition but features like peak-fitting, baseline correction, automatic concentration calculation, molecular weight determination, etc.

Signals from the detector may be collected on chart recorders or electronic integrators that

vary in complexity and in their ability to process, store and re-process chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret. Frequently called the data system, the computer not only controls all the modules of the HPLC Instrument but it takes the signal from the detector and uses it to determine the time of elution (retention time) of the sample components (qualitative analysis) and the amount of sample (quantitative analysis)

Advantages of HPLC

- Speed
- Efficiency
- Accuracy
- Versatile and extremely precise when it comes to identifying and quantifying chemical components.

Limitations of HPLC

- Cost: Despite its advantages, HPLC can be costly, requiring large quantities of expensive organics.
- Complexity
- HPLC does have low sensitivity for certain compounds, and some cannot be detected as they are irreversibly adsorbed.
- Volatile substances are better separated by gas chromatography

Applications of HPLC

- Analysis of drugs
- Analysis of synthetic polymers
- Analysis of pollutants in environmental analytics
- Determination of drugs in biological matrices
- Isolation of valuable products
- Product purity and quality control of industrial
- Products and fine chemicals
- Separation and purification of biopolymers such as enzymes or nucleic acids
- Water purification
- Pre-concentration of trace components
- Ligand-exchange chromatography

- Ion-exchange chromatography of proteins
- High-pH anion-exchange chromatography of carbohydrates and oligosaccharides.

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