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Review Article **Pharmaceutical Analysis**

A Review on Ultra Performance Liquid Chromatography

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Abstract

UPLC is a modern technique which gives a new direction for liquid Chromatography. UPLC refers to ultra performance liquid chromatography, which enhance mainly in three areas: "speed, resolution, and sensitivity. Ultra Performance liquid chromatography (UPLC) applicable for particles less than 2µm in diameter to acquire better resolution, speed, and sensitivity compared with high-performance liquid chromatography (HPLC). In twenty first centenary pharmaceutical industries are focusing for new ways to in economy and shorten Time for development of drugs. UPLC analysis at the meantime gives the better Quality of their products and analytical laboratories are not exception in this trend. The separation and quantification in UPLC are done under very high pressure (up to 100M Pa). As compared to HPLC, under high pressure it is observed that not any negative influence on analytical columns and other components like Time and solvent consumption is less in UPLC.

Keywords: Ultra Performance Liquid Chromatography, High Separation Efficiency, Cost Effective, High Pressure, Speed.

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INTRODUCTION

Chromatography is a method used to separate a mixture into its individual components using a porous medium and solvents. Before 2004, High-Performance Liquid Chromatography (HPLC) was the most common technique for this purpose. However, HPLC had some limitations, leading to the development of a more advanced and efficient technique called Ultra Performance Liquid Chromatography (UPLC). UPLC offers better resolution, faster analysis, and improved sensitivity compared to HPLC, making it a superior choice for modern analytical applications.

Ultra Performance Liquid Chromatography (UPLC) enhances chromatographic resolution, speed, and sensitivity by using fine particles, which saves time and reduces solvent consumption. UPLC evolved from High Performance Liquid Chromatography (HPLC), which improves efficiency and resolution as particle size decreases. However, smaller particles increase back pressure, which HPLC systems can only handle up to 400 bars. UPLC can operate at higher pressures, allowing for faster and more efficient separations. It is widely used in various fields, including chemistry, pharmacy, food industry, and environmental analysis [1].

Chromatography is physical method of separation of the mixture into its individual Components. It is used as an analytical technique to get information about what is present in the mixture, how much the individual compound is in mixture. It is also used as a purification method to separate and collect the components of mixture. Chromatography is Greek word where chromates mean colour and graphic means writing. So, basically chromatography is colour writing process.

The separation of components of mixture is achieved on the simple fact that different component of mixture having different affinity towards mobile phase and stationary phase the least affianced component emerges first; the most strongly affianced compound (retained) elutes last. The concept of chromatography is first time put forwarded in 1906 by Great Russian botanist, Michael Tsweet.

Chromatography is a method of separation of components of mixture in which a sample is introduced into a mobile phase which is carried along with a column and solid support called stationary phase. Generally, the stationary phase is either solid or liquid and mobile phase is liquid or gas. Depending upon the

stationary phase and mobile the chromatography is classified in different forms.

Principle of Chromatography

Different components of mixture having different affinity towards the stationary phase and mobile phase are the basic principle of chromatography. The components of the mixture are having different affinities or distribution coefficient kd towards the stationary md mobile phase. The basis of all types of chromatography is the partition or distribution coefficient which describes the way in which compound distributes itself in two immiscible phases.

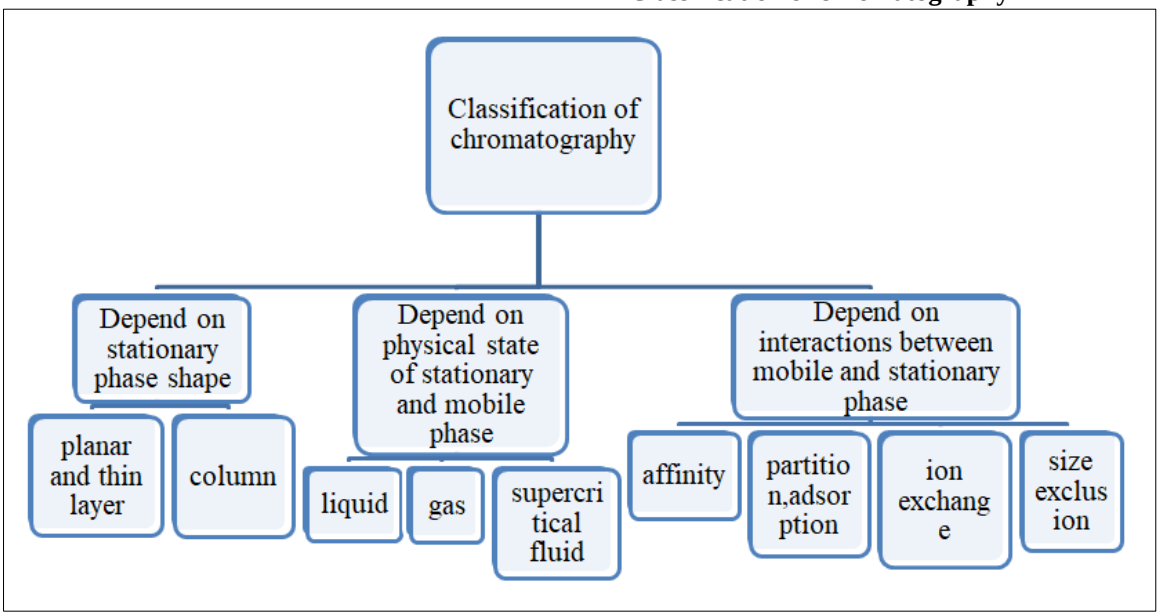
Based on the Approach Three Components from the Basis of the Chromatography Technique:

- **Stationary Phase**: The solid or liquid phase of a chromatography system on which the materials are to be separated or selectively absorbed. Stationary phase in chromatography is the one which does not move with the sample. The fluid (liquid or gas) that flows through a chromatography system, moving the materials to be separated at different rates over the stationary phase.
- **Mobile Phase**: This phase is always composed of liquid or a gaseous component. Separated molecules The individual components are isolated based on their interactions with both phases·

Importance of Chromatography

1. It is a very rapid, accurate, sensitive, gentle method.
2. A very minute amount of sample is required for the whole analysis.
3. Decomposition of compounds can be avoided in this method. This is useful especially for biological products.
4. It is a non-destructive method of analysis which means we can recover samples after the analysis.
5. Chromatography is useful in all fields such as biology, chemistry, dyes, medicine, forensics, and preclinical studies etc.

Classification of chromatography



Column Chromatography

Column chromatography is used extensively in nucleic acid chemistry to purify or characterize products from chemical reactions. This appendix focuses on separations using silica gel or alumina. The procedures described include loading the column, assembling the apparatus, separating compounds, and collecting fractions, and optimizing chromatography conditions [2].

Types of Column Chromatography:

1. Adsorption column chromatography
2. Partition chromatography
3. Gel chromatography

Planar Chromatography

A laboratory technique used to separate, identify, and quantify the components of a mixture by applying a sample to a stationary phase, typically a thin layer of silica gel or alumina, and then developing the plate with a mobile phase. Also known as Thin-Layer Chromatography (TLC), planar chromatography is a simple, inexpensive, and powerful tool for analyzing complex mixtures [3].

Examples Include:

- Paper chromatography
- Thin layer chromatography

High Pressure Liquid Chromatography

Using this chromatography technique, it is possible to perform structural, and functional analysis,

and purification of many molecules within a short time, this technique yields perfect results in the separation, and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules, In HPLC, mobile phase passes through columns under 10–400 atmospheric pressure, and with a high (0.1–5 cm//sec) flow rate. In this technique, use of small particles, and application of high pressure on the rate of solvent flow increases separation power, of HPLC and the analysis is completed within a short time. Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled with the aid of a computerized system, and material is accrued [4].

Gas Chromatography

Gas chromatography is the process of separating compounds in a mixture by injecting a gaseous or liquid sample into a mobile phase. Gas Chromatography is a normally utilized analytic technique as a part of numerous research and industrial research facilities for quality control and in addition identification and quantity of components in a mixture. This technique is well-recognized for its ability in unknown compound analysis.

The stationary phase is either a solid adsorbent, called Gas-Solid Chromatography (GSC), or a liquid on an inert support, called Gas-Liquid Chromatography (GLC). GC are quite similar in a way that it involves gaseous compounds and high temperature. GC is used for both qualitative identification and quantitative measurement of individual compounds which are presented in complex mixtures. Gas chromatography is an instrumental technique used forensically in drug analysis, arson, and toxicology analyses of other organic compounds [5].

Affinity Chromatography

This chromatography technique is used for the purification of enzymes, hormones, antibodies, nucleic acids, and specific proteins. A ligand which can make a complex with specific protein (dextran, polyacrylamide, cellulose etc.) binds the filling material of the column. The specific protein which makes a complex with the ligand is attached to the solid support (matrix), and retained in the column, while free proteins leave the column. Then the bound protein leaves the column by means of changing its ionic strength through alteration of pH or addition of a salt solution [6, 7].

Ion- Exchange Chromatography

Ion- exchange chromatography is based on electrostatic interactions between charged protein groups, and solid support material (matrix). Matrix has an ion load opposite that of the protein to be separated, and the affinity of the protein to the column is achieved with ionic ties. Proteins are separated from the column either by changing pH, concentration of ion salts or ionic strength of the buffer solution. Positively charged ion- exchange matrices are called anion-exchange matrices and adsorb negatively charged proteins. While matrices bound with negatively charged groups are known as cation-exchange matrices, and absorb positively charged proteins [8, 9].

Size Exclusion Chromatography (SEC)

Size exclusion chromatography (SEC) is a powerful tool for analyzing size variants of proteins in academic and biopharmaceutical laboratories. Recent advancements in SEC methodology have improved size separation range, resolution, and sensitivity for analytes of interest. SEC analysis augmented various detectors including light scattering, fluorescence, refractive index (RI), viscometer, and mass spectrometry, in-line with the classical UV-Vis detector has dramatically expanded its capabilities for characterizing primary and higher order structures, post translational modifications (PTMs), and other physicochemical attributes of biologics in a single analytical method [10].

Why UPLC is More Superior than Other Chromatography?

UPLC (Ultra-Performance Liquid Chromatography) is superior to other chromatography techniques due to its higher efficiency, faster analysis, and improved resolution. It uses smaller particle sizes (<2 µm) and operates at higher pressures (up to 15,000 psi), enabling faster run times and sharper peaks. UPLC offers greater sensitivity with lower detection limits, making it ideal for trace-level analysis. It also reduces solvent consumption, making it more cost-effective and environmentally friendly. Its enhanced reproducibility, better accuracy, and compatibility with mass spectrometry (MS) make it widely used in pharmaceutical, food safety, and bioanalytical applications.

Table 1: Comparison between HPLC and UPLC Systems [11]

Parameters	HPLC	UPLC
Particle size	3-5µm	< 2µm
Operating pressure	Up to 6,000 psi	Up to 15,000 psi
Column length	150-250 mm	50-150 mm
Internal diameter	4.6 mm	2.1 mm
Flow rate	1-5 mL/min	0.2-1 mL/min
Injection volume	10-100 µL	1-10µL

UPLC refers to Ultra Performance Liquid Chromatography. It improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and

reduces solvent consumption. UPLC comes from HPLC. HPLC has been the evolution of the packing materials used to effect separation. An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5µm, there is a significant gain in efficiency, and it don't diminish at increased linear velocities or flow rates according to the common Van Demeter equation. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which are known as Ultra Performance.

UPLC represents a milestone in liquid chromatography by drastically improving efficiency, resolution, and speed of analysis. It has overcome many limitations of HPLC, making it the preferred technique for laboratories requiring high-throughput, high-precision analysis. Due to its advantages, UPLC has revolutionized chromatography and continues to be a valuable tool in modern analytical science.

While HPLC became a standard analytical technique, limitations such as long analysis times and reduced resolution prompted further advancements. In 2004, the breakthrough in liquid chromatography came with the introduction of UPLC by Waters Corporation. This technique is widely used across various fields, including pharmaceutical analysis, environmental monitoring, clinical diagnostics, and food safety testing. It plays a crucial role in drug purity testing, biomarker discovery, and metabolomics, making it an indispensable tool in modern analytical laboratories.

With its superior performance, UPLC has set new standards in chromatography and is increasingly replacing traditional HPLC with many applications. Despite its higher operational costs, the benefits of speed, sensitivity, and resolution make it a valuable investment for high-throughput analytical environments. In pharmaceutical analysis, UPLC is widely used for drug development, stability testing, and impurity profiling, ensuring regulatory compliance and quality control. In clinical and biomedical research, it plays a key role in biomarker discovery, metabolomics, and proteomics. Moreover, in food and environmental sciences.

UPLC is a technique which comprises the above-mentioned features and stands better than HPLC in many ways as it shows better chromatographic resolution, performs more sensitive analysis, consumes less time, reduces solvent consumption.

Underlying Principle of UPLC [13]

Ultra-Performance Liquid Chromatography (UPLC) operates based on the Van-Deemter equation, which describes the relationship between flow rate and plate height. This equation demonstrates that smaller particle sizes enable a broader optimal flow range compared to larger particles, leading to improved chromatographic efficiency.

Equation of van Demeter is: $H=A+B/\mu+C\mu$

Where:
H= Plate height
A= Eddy diffusion
B= Longitudinal diffusion
C= Equilibrium mass transfer
M= Flow rate

Smaller plate height value corresponds to Greater peak efficiency, as more plates can occur Over a fixed length of column (Figure 1).

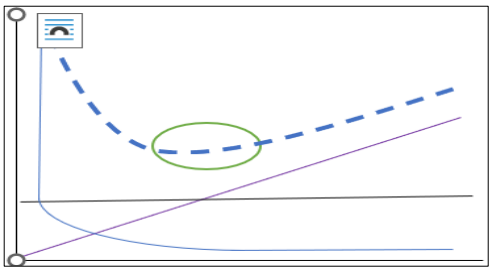


Figure 1: Van Demeter Equation

Shorter diffusion path length of smaller particles allows a faster movement of the solute in and out of the particles. Because of this the solute/ analyte spends less time inside the particle where the peak diffusion occurs 17-18 (Figure 2).

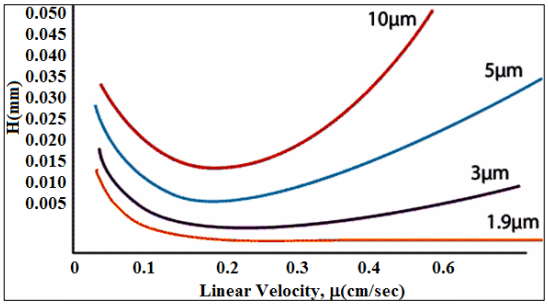


Figure 2: Depicting the Van Deemter plots for different sizes

Instrumentation of UPLC

UPLC instrumentation is like HPLC but designed to handle much higher pressures without disruption or excessive maintenance. It uses advanced electronics and firmware to support tunable UV/Visible (UV/Vis) detectors that operate at high data rates. The detector features a 10 mm flow cell path length with a small volume of 0.5 mL, ensuring precise and efficient detection [14]

The instrumentation of UPLC includes:

- Sample injection
- UPLC columns
- Detectors

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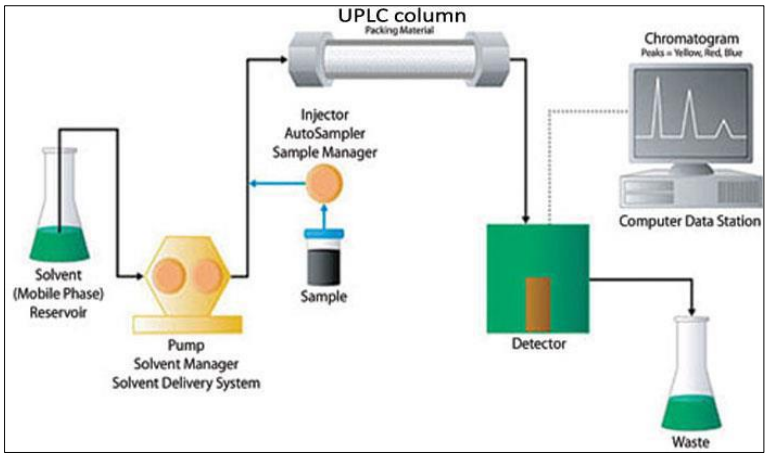

Figure 3: Working of UPLC

Sample Injector in UPLC

The Sample Injector Manager in a UPLC system is designed with a flow-through needle to ensure accurate and efficient sample injection under high pressure. It addresses three key challenges:

1. Robust Needle Sealing – Ensures a tight seal at high pressures to prevent leaks.
2. Minimized Extra-Column Band Spread – Reduces peak broadening for sharper chromatographic peaks.
3. Pulse-Free Injection – Maintains a stable flow to protect the column from extreme pressure fluctuations.

When an injection starts, the inject valve diverts flow from the needle to collect the sample from the vial. The needle enters the vial, withdraws the exact sample volume, and returns to the injection port. The needle seals against the injection port, and the valve switches to push the sample into the system. To minimize sample dispersion, the distance between the injection port and the valve is kept short. After injection, the needle is washed to prevent sample carryover. Additionally, direct injections for biological samples are also referenced in literature.

Columns

The earliest commercial columns made for UHPLC were 2.1 x 50 mm and 2.1 x 100 mm in size, and they were packed with 1.7-µm particles. The particles were composed of an ethylene-bridged organic/inorganic hybrid derivatized with C18 groups, and they were totally porous with an average pore size of 130 Å. For RP separations of analytes with molecular weights under around 5 kDa, the columns were designed. After accounting for system dispersion, these columns have been claimed to have maximum efficiency of 280,000 plates/m. Numerous new surface chemistries have been applied to hybrid and silica sub-2-µm particles throughout the last ten years.

UHPLC columns have been developed for size-exclusion, ion-exchange, hydrophilic interaction, and normal phase chromatography in addition to the RP mode. Analytes up to about 2 MDa, such as industrial and biopolymers, can currently be separated using UHPLC columns. Commercially, UHPLC columns come with internal diameters (i.e.) ranging from 0.075 to 4.6 mm. Selectivity and application diversity have significantly increased, but UHPLC column efficiency per unit length—a key indicator of the column's capacity to generate narrow peaks—has not increased as much. However, with the recent introduction of columns containing sub-2-µm solid core particles (SCPs), this has started to change. Acuity UHPLC columns have played a key role in advancing liquid chromatography (LC) by delivering high-quality chromatographic data in a shorter time. Designed to withstand pressures of up to 15,000 psi (1,000 bar), these columns undergo rigorous testing to ensure optimal performance.

Different Types of Columns used in UPLC Based on Particle Size:

a) Charged Surface Hybrid (CSH)
b) Ethylene-Bridged Hybrid (BEH)
c) High Strength Silica (HSS)
d) Peptide Separation Technology (PST)

a) Charged Surface Hybrid (CSH)

Waters developed a third-generation particle technology designed to enhance sample lo audibility and minimize peak tailing in mobile phases with uncertain ionic strengths. Featuring a 1.7 µm particle size, the charged surface hybrid (CSH) maintains a low surface charge while ensuring superior peak shape and improved loading capacity, particularly for basic compounds in low-pH, weak ionic strength mobile phases. The Acuity UHPLC CSH Phenyl-Hexyl column is particularly effective for polyaromatic compounds, delivering excellent peak shape across different pH conditions. Similarly, the Acuity UHPLC CSH Fluorophenyl column exhibits exceptional selectivity for polar compounds, positional isomers, and halogenated compounds due to its combination of dipole-dipole, hydrogen-bonding, aromatic, and hydrophobic interactions.

b) Ethylene-Bridged Hybrid (BEH)

To maximize the speed, sensitivity, and resolution of first-generation methyl hybrid particles, a more pressure-tolerant particle was needed. This led to the development of ethylene-bridged hybrid (BEH) columns, which improved efficiency, strength, and pH range compared to their predecessors. These BEH columns are particularly useful in UHPLC BEH Phenyl applications, where they integrate a C6 alkyl polar group connected to a silyl functionality. This advancement allows for greater mechanical strength and more effective separations under high-pressure conditions.

c) High Strength Silica (HSS)

High Strength Silica (HSS) columns are designed to address the mechanical stability issues associated with high-pore-volume UHPLC particles, which often struggle under the extreme pressures of UHPLC separations. To overcome this limitation, a novel silica particle with an optimized morphology was introduced, allowing for durable, high-efficiency UHPLC columns that withstand pressures up to 1,000 bar. Modern HSS particle technology, featuring 1.8 µm UHPLC HSS particles, is tailored for complex separations. The Acuity UHPLC HSS T3 columns were specifically developed to handle the retention challenges posed by small water-soluble and polar organic molecules in reversed-phase separations. Additionally, the Acuity UHPLC HSS C18 SB columns, which utilize a non-end capped, low-coverage silica-based C18 chemistry, offer unique selectivity for water-soluble compounds influenced by silanophilic interactions. This enhanced silanol activity results in greater retention of basic compounds due to secondary interactions with residual Silano's, while simultaneously reducing non-basic analyte retention due to lower ligand density and ionic repulsion.

d) Peptide Separation Technology (PST)

For the effective separation and analysis of peptides, peptide separation technology (PST) columns were developed. Utilizing C18 BEH technology, these columns come in particle sizes ranging from 1.7 µm to 10 µm, with internal diameters spanning 75 µm to 30 mm and column lengths between 50 mm and 250 mm. PST columns are specifically designed to provide sharp, symmetrical peak shapes, ensuring high-resolution peptide analysis [15].

Detector

The UPLC detector used should be able to provide a high sampling rate with narrow attainable peaks (1s half-height peak width) and little dispersion of the peaks so that less separated solute is wasted on the column. The UPLC methodology delivers two to three times the separation sensitivity of the previous method HPLC because of the detector method. Acuity photodiode array (PDA) and Tunable Vis-UV (TUV) detectors are utilized in the UPLC, with Teflon AF providing an internally reflecting surface that improves light transmission efficiency by removing internal absorptions. Path lengths are 10 nanometers, acquisition speeds are 20 (PDA) and 40 (TUV), and total internal capacity is 500 nanoliters. Detection by mass spectrometry has also been used with UPLC21.

A) TUV Detector (Tunable Ultraviolet Detector)

It functions within a wavelength range of 190 to 700 nm. It employs flow cell technology and offers two flow cell configurations: an analytical cell with a 500-nanoliter volume and a 10 mm path length, as well as a high-sensitivity flow cell with a 2.4-microliter volume and a 25 mm path length.

PDA Detector (Photodiode Array Detector)

It is a UV/Vis spectrophotometer capable of detecting wavelengths between 190 and 500 nm. Like the TUV detector, it features two flow cell options: an analytical cell with a 500-nanoliter volume and a 10 mm path length, and a high-sensitivity flow cell with a 2.4-microliter volume and a 25 mm path length, both utilizing flow cell technology.

ELS Detector (Evaporative Light Scattering Detector)

It is specifically designed for use in UPLC systems. It provides performance comparable to or exceeding that of traditional detectors when using stationary phases around 2 µm in size, without the drawbacks associated with high pressure. However, stationary phases below 2 µm are typically non-regenerable, limiting their usability [16].

Advantages and Disadvantages of UPLC

Advantages [17]

1. Faster Analysis – Runs tests quicker than HPLC.
2. Better Sensitivity & Resolution – Detects even small differences in compounds.
3. Lower Solvent Use – Saves money and is eco-friendly.
4. More Samples in Less Time – Increases lab efficiency.
5. Sharper Peaks – Makes results more accurate.
6. Better Detectors – Works well with advanced detection methods like UV, PDA, and MS.
7. Uses Less Sample – Ideal for limited or expensive samples.

Disadvantages [18]

1. Expensive Equipment – Costs more than HPLC.
2. More Maintenance – Requires frequent system checks.
3. Shorter Column Life – High pressure wears out columns faster.
4. Not Always Compatible – Some HPLC methods need adjustments.

Applications

A. Natural Product and Herbal Medicine:

Ultra Performance Liquid Chromatography Has the ability to provide high quality of separation and detection capability of active compound which Is present in mixture.

Examples: Ultra Performance Liquid Chromatography Is used for multiple components for Quantitative analysis in example analysis of Hyangsapyeongwisan which is traditional Medicine and used in gastric disease [19].

B. Identification of Metabolites*:*

UPLC/MS/MS32 offers unmatched sensitivity and accuracy in Biomarker discovery.

Example: UPLC-MSE was used for rapid detection and Characterization of verapamil metabolites in Rats1 [20]

C. Drug Discovery:

Useful in drug discovery Process. UPLC system by using acuity BEHC 18 column that method is faster and sensitive as compare to HPLC method.

Example: Determination of Mesa amine related Impurities from drug products by reversed Phase validated UPLC method [21].

D. Method Development:

Validation to Reduce cost and improving opportunities for business Success.

Example: UPLC method determination of sofosbuvir and daclatasvir in human plasma for therapeutic drug monitoring [22].

E. Combination Study:

Ultra Performance Liquid Chromatography coupled with photodiode and mass spectroscopy which can give rapid Identification of compound along with sensitivity. The coupling of UPLC with other devices different Techniques is convenient and economical as Compared to HPLC.

Example: UPLC-DAD-MS/MS was used in the metabolic of the medicinal grass Eleusine indica [23].

F. Impurity Profile:

Reversed phase UPLC methods are highly useful for quantitative Determination of active pharmaceutical compound.

Example: Determination of products and process Impurities of asenapine maleate in asenapine Sublingual tablets by UPLC [24].

G. Quality Control:

Reversed phase ultra-Performance provide a sensitive, rapid, and accurate Result with less reagents cost and utilized in internal Quality control in different dosage types.

Examples: UPLC-QTOF/MSE a recent approach for identifying quality control analysis of Fluctuation of xueshuantong lyophilized Powder in clinic [25].

H. Amino acid Determination:

The UPLC Also suitable for analysis of different amino acids by coupling with MS technologies. The methods are Reliable, fast with high sensitivity and reputability.

Example: Quantification of Sulphur amino acids in Aquatic invertebrates [26].

I. Determination of Pesticides:

Combination of UPLC-MS/MS is effective for determination of Pesticides. The instrument technique provides highly Accurate with less matrix result.

Example: Pesticides analysis of vegetables by UPLC In combination with mass spectrometry.

CONCLUSION

Ultra-Performance Liquid Chromatography (UPLC) has revolutionized chromatographic analysis by offering superior resolution, faster analysis times, and improved sensitivity compared to High-Performance Liquid Chromatography (HPLC). By utilizing smaller particle sizes and higher operating pressures, UPLC enhances chromatographic efficiency, reducing solvent consumption and increasing sample throughput. This technique has found widespread applications in pharmaceutical analysis, clinical diagnostics, food safety, and environmental monitoring, making it an essential tool in modern analytical science.

Despite its higher operational costs and maintenance requirements, UPLC remains the preferred choice for laboratories requiring high-precision, high-throughput analysis. Its integration with advanced detection methods such as mass spectrometry (UPLC-MS) further strengthens its role in complex compound identification and biomolecular studies. As chromatography technology continues to evolve, UPLC is expected to set new benchmarks in analytical performance, solidifying its place as a vital advancement in liquid chromatography.

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