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Review Article

REVIEW ON COMPARISON BETWEEN HPLC AND UPLC METHOD IN PHARMACEUTICAL ANALYSIS

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ABSTRACT
Pharmaceutical analysis plays a crucial role in ensuring the safety, efficacy, and quality of drug products. High-performance liquid chromatography (HPLC) has been the cornerstone technique for many years. However, in recent years, ultrahigh-performance liquid chromatography (UPLC) has emerged as a promising alternative. This review aims to provide a comprehensive comparison between HPLC and UPLC methods in pharmaceutical analysis, highlighting their advantages, limitations, and application considerations. The review begins by discussing the fundamental principles and operating parameters of both HPLC and UPLC systems. It elaborates on the differences in column particle size, column dimensions, and system pressures, which significantly impact the separation efficiency and analysis time. Various practical aspects, such as instrument cost, method development, and compatibility with existing HPLC methods, are also addressed. Next, the review evaluates the analytical performance of HPLC and UPLC methods, including resolution, sensitivity, linearity, and precision. The enhanced resolution and sensitivity offered by UPLC are explored, along with its ability to achieve shorter analysis times and improved peak shapes. Additionally, the impact of different sample matrices and analyte properties on method selection is discussed, highlighting scenarios where HPLC or UPLC may be more suitable.The limitations and challenges associated with both techniques are critically analyzed, covering aspects such as column lifetime, system robustness, and method transferability. Practical guidelines for method development and optimization are provided to assist analysts in selecting the most appropriate technique based on their specific requirements. In summary, this review offers a comprehensive comparison between HPLC and UPLC methods in pharmaceutical analysis. It provides valuable insights into the advantages and limitations of each technique, assisting researchers and analysts in making informed decisions regarding method selection, development, and optimization in the field of pharmaceutical analysis.

Keywords: Chromatography, HPLC, UPLC, Method development, Resolution.

INTRODUCTION

Chromatography is a physical method of separation that is widely used in analytical chemistry, biochemistry, and other scientific disciplines. It is based on the principle that different components of a mixture will interact differently with a stationary phase and a mobile phase. The stationary phase is a solid or liquid that is held in place, while the mobile phase is a liquid or gas that flows over the stationary phase. The components of the mixture will distribute themselves between the stationary and mobile phases in different ways, depending on their chemical properties. This difference in distribution leads to the separation of the components of the mixture.

In the realm of analytical chemistry, high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) have become essential techniques for separating, identifying, and quantifying a wide range of compounds. HPLC has long been the go-to method for liquid chromatography, but with advancements in technology, UPLC has emerged as a promising alternative. This article provides a comprehensive comparison between HPLC and UPLC, exploring their principles, advantages, and applications.

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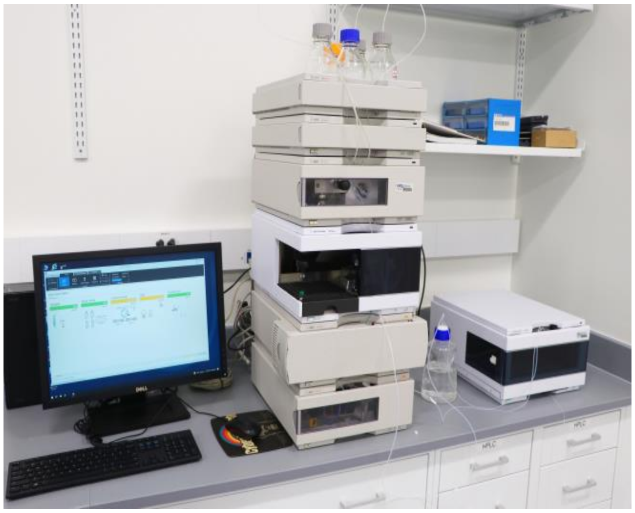
UNDERSTANDING THE PRINCIPLES

High-performance liquid chromatography (HPLC) is a type of liquid chromatography that uses high pressures to force a mobile phase through a column packed with a stationary phase. The mobile phase is a liquid that carries the sample compounds through the column, while the stationary phase is a solid that interacts with the sample compounds in different ways, depending on their chemical properties. This interaction causes the sample compounds to travel through the column at different rates, which results in their separation.



HPLC

Ultra-performance liquid chromatography (UPLC) is a type of liquid chromatography that uses smaller particles and higher pressures than traditional HPLC. This allows for faster separations with better resolution and sensitivity. UPLC is often used in pharmaceutical, biomedical, and food science research.

UPLC

TYPES OF HPLC

NORMAL PHASE HPLC: In this method the separation is based on polarity. The stationary phase is polar, mostly silica is used and non-polar phase used is hexane, chloroform and diethyl ether. The polar samples are retained on column.

REVERSE PHASE HPLC: It is reverse to normal phase HPLC. The mobile phase is polar and the stationary phase is non-polar or hydrophobic. The more is the non-polar nature the more it will be retained.

SIZE EXCLUSION HPLC: The column will be incorporating with precisely controlled substrate molecules. Based on the difference in molecular sizes the separation of constituents will occur.

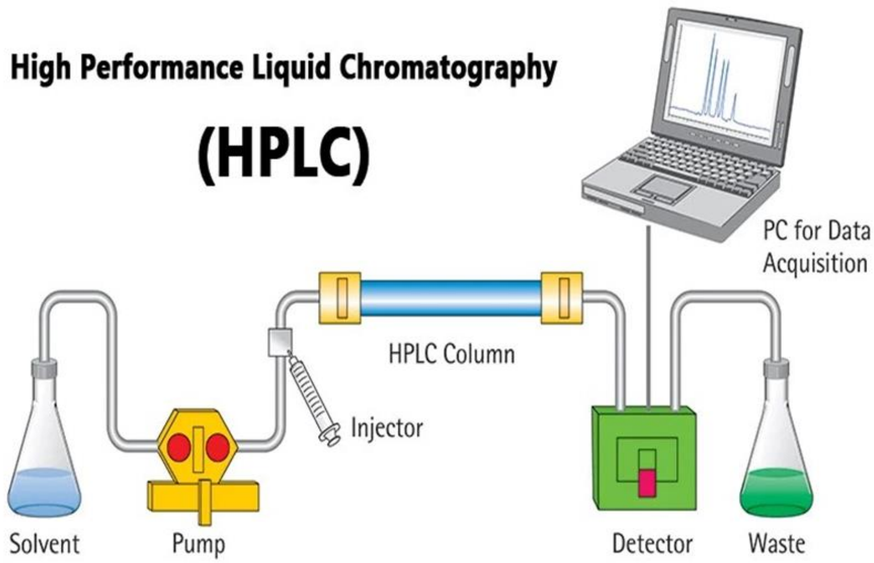
ION EXCHANGE HPLC: The stationary phase has a surface that is electrically charged in the opposite direction of the sample charge. Aqueous buffer is utilized as the mobile phase and will regulate the PH and ionic strength.

Sr.No	Mode	Solvent type used	Type of compound used
1	Reversed phase	Water / buffer, methanol	Neutral or non ionized compound which can be dissolved in water
2	Ion pair	Water / buffer, methanol	Ionic or Ionizable compound
3	Normal phase	Organic solvent	A Mixture of isomer & compound not soluble in organic / water mixture
4	Ion exchange	Water / buffer	Inorganic ions, protein , nucleic acid , organic acid
5	Size exclusion	Water chloroform	High molecular weight compound

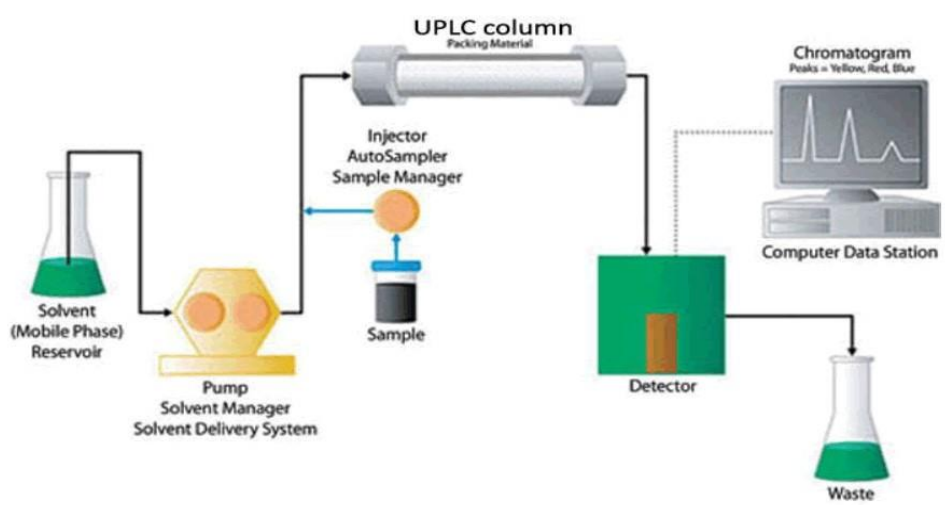
TYPES OF UPLC

Acquity UPLC: This is the original UPLC technology developed by Waters Corporation. Acquity UPLC systems use columns packed with 1.7-2.5 µm particles and operate at pressures of up to 15,000 psi.

UPLC-X: This is a newer UPLC technology developed by Thermo Fisher Scientific. UPLC-X systems use columns packed with 1.3-1.7 µm particles and operate at pressures of up to 20,000 psi.



S.NO	Instruments	Process
1	Solvent Delivery System	Delivering the mobile phase (solvent or solvent mixture) to the HPLC column at a constant flow rate. It typically consists of one or more solvent reservoirs, a high-pressure pump, and a degasser to remove any dissolved gases from the solvent.
2	Injection system	To introduce the sample into the HPLC system. It consists of an injection valve or autosampler. Autosamplers are commonly used in modern HPLC systems for automated and precise sample introduction.
3	HPLC column	It is a crucial component where the separation of analytes takes place. It is packed with a stationary phase that interacts with the sample components differently, leading to their separation based on their physicochemical properties.
4	Column Oven	To control the temperature of the HPLC column. Temperature control can improve separation efficiency, reduce retention time variability, and enhance method reproducibility, particularly for thermally sensitive compounds.



S.NO	Instruments	Process
1	Pumps	UPLC pumps are designed to operate at pressures of up to 15,000 psi. This is necessary to force the mobile phase through the small particles in the column.
2	Column	UPLC columns are packed with small particles, typically less than 2 µm in diameter. This allows for faster separations and improved resolution.
3	Autosamplers	Autosamplers are used to automatically inject samples into the system. This can save time and improve the accuracy of the analysis. Fraction collectors: Fraction collectors are used to collect fractions of the eluent from the column. This can be useful for further analysis, such as mass spectrometry.
4	Fraction collectors	Fraction collectors are used to collect fractions of the eluent from the column. This can be useful for further analysis, such as mass spectrometry.
5	Data systems	Data systems are used to collect and store data from the detector. This data can be used to create chromatograms and identify analytes

OPERATION TECHNIQUES

HPLC

High performance liquid chromatography (HPLC) is a powerful analytical technique that can be used to separate and identify a wide variety of compounds. The basic operation of an HPLC system is as follows:

1. The sample is dissolved in a mobile phase, which is a solvent or mixture of solvents.
2. The mobile phase is pumped through a column packed with a stationary phase, which is a solid material that has been modified to interact with the solutes in the mobile phase.
3. The solutes in the mobile phase interact with the stationary phase to different extents, depending on their chemical properties.
4. The different solutes elute from the column at different times, depending on their retention times.
5. The eluted solutes are detected by a detector, which measures their concentration or some other property.

UPLC

The basic operation of UPLC is similar to that of HPLC. The sample is dissolved in a mobile phase, which is pumped through a column packed with a stationary phase. The solutes in the mobile phase interact with the stationary phase to different extents, depending on their chemical properties. The different solutes elute from the column at different times, depending on their retention times. The eluted solutes are detected by a detector, which measures their concentration or some other property.

The following are some of the key steps involved in operating an HPLC & UPLC system:

- **Prepare the mobile phase.** The mobile phase must be carefully selected to ensure that the desired separation is achieved. The mobile phase must also be filtered to remove any particles that could damage the column.
- **Prepare the column.** The column must be conditioned by running a solvent through it before the sample is injected. This helps to remove any residual solvents or chemicals from the column that could interfere with the analysis.
- **Inject the sample.** The sample is injected into the mobile phase using a syringe. The volume of the injected sample is typically very small, on the order of a few microliters.
- **Run the analysis.** The pump is turned on and the mobile phase is pumped through the column. The detector is turned on and the eluted solutes are detected and recorded.
- **Analyze the results.** The retention times of the eluted solutes are used to identify the compounds in the sample. The concentrations of the compounds can be determined by comparing the peak areas to the peak areas of standards.

HPLC and UPLC both are powerful and versatile analytical technique that can be used to analyze a wide variety of samples. However, it is important to note that HPLC and UPLC area complex techniques and requires careful attention to detail in order to obtain accurate and reproducible results.

Here are some additional tips for operating an HPLC and UPLC system:

- Always use clean and dry glassware and solvents.
- Purge the system with a solvent before injecting the sample.
- Use a guard column to protect the analytical column from contaminants.
- Monitor the system pressure and flow rate to ensure that they are within the specified ranges.
- Calibrate the detector regularly.
- Keep a log of all experiments conducted on the HPLC or UPLC system.

Resolution

HPLC and UPLC are two liquid chromatography techniques that are used to separate and analyze complex mixtures. HPLC is a mature technology that has been used for decades, while UPLC is a newer technology that offers several advantages over HPLC, including improved resolution.

Resolution is a measure of how well two different components in a mixture can be separated. It is calculated as the difference in retention times between the two components divided by the average width of the two peaks. Higher resolution means that the two components can be separated more clearly.

UPLC offers several advantages over HPLC that can lead to improved resolution. First, UPLC uses smaller particle sizes in the column packing material. Smaller particle sizes lead to increased efficiency, which means that the components of the mixture spend more time in the column and are separated more completely. Second, UPLC uses higher pressures. Higher pressures allow for the use of smaller particle sizes, which further increases efficiency and resolution.

As a result of these advantages, UPLC can achieve significantly better resolution than HPLC. In some cases, UPLC can achieve a tenfold improvement in resolution. This can be important for the analysis of complex mixtures, where it is necessary to be able to separate even closely related components.

Here is a table that summarizes the comparison between HPLC and UPLC in terms of resolution:

Characteristic	**HPLC**	**UPLC**
Particle size	3-5 µm	<2 µm
Operating pressure	<6000 psi	10,000-15,000 psi
Efficiency	Low	High
Resolution	Low	High
Speed	Slow	Fast

As you can see, UPLC offers several advantages over HPLC in terms of resolution. This can be important for the analysis of complex mixtures, where it is necessary to be able to separate even closely related components.

Speed

UPLC can achieve significantly faster separations than HPLC for a few reasons. First, UPLC uses smaller particle sizes in the column packing material. Smaller particle sizes lead to increased efficiency, which means that the components of the mixture spend less time in the column and are separated more quickly. Second, UPLC uses higher pressures. Higher pressures allow for the use of smaller particle sizes, which further increases efficiency and speed.

As a result of these advantages, UPLC can achieve separations that are up to 10 times faster than HPLC. This can be important for high-throughput applications, where it is necessary to be able to analyze large numbers of samples quickly.

Overall, UPLC is a powerful analytical technique that offers several advantages over HPLC, including improved resolution, increased sensitivity, and faster separations. UPLC should be considered for applications where these advantages are important, such as the analysis of complex mixtures, high-throughput applications, and environmental analysis.

Efficiency and sample throughput

Efficiency and sample throughput are important factors to consider when comparing HPLC (High-Performance Liquid Chromatography) and UPLC (Ultra-Performance Liquid Chromatography). Here is a comparison between the two techniques in terms of efficiency and sample throughput:

Efficiency: Particle Size: UPLC utilizes smaller particle sizes (typically sub-2 µm) compared to HPLC (typically 3-5 µm). The smaller particle size in UPLC leads to increased column efficiency, resulting in sharper peaks and improved resolution. HPLC, with its larger particle size, generally has lower efficiency compared to UPLC.

Plate Number: The number of theoretical plates (N) is a measure of column efficiency. UPLC columns, due to their smaller particle size and increased packing density, offer significantly higher theoretical plate numbers compared to HPLC columns. This higher efficiency in UPLC allows for better separation and improved resolution of analytes.

Band Broadening: UPLC systems exhibit reduced band broadening effects, such as extra-column dispersion, compared to HPLC. The minimized band broadening in UPLC contributes to higher peak capacity and improved separation efficiency.

Sample Throughput:

Analysis Time: UPLC typically offers faster analysis times compared to HPLC due to its smaller particle size and higher column efficiency. The improved resolution and peak shape obtained in UPLC allow for shorter separation times while maintaining good separation quality. This shorter analysis time in UPLC translates to increased sample throughput.

Flow Rate: UPLC systems operate at higher flow rates compared to HPLC systems. The smaller particle size and reduced column dimensions in UPLC allow for higher flow rates without sacrificing separation efficiency. The increased flow rate in UPLC contributes to faster analysis and higher sample throughput.

Gradient Performance: Both HPLC and UPLC can employ gradient elution for optimized separations. UPLC benefits from its higher efficiency, enabling the use of steeper gradients without significant loss of resolution. This feature allows for faster method development and shorter analysis times, further enhancing sample throughput in UPLC.

Injection Systems: UPLC systems often incorporate advanced injection systems such as autosamplers, which enable automated and precise sample introduction. These systems facilitate rapid sample loading, reduce sample preparation time, and enhance overall sample throughput. It is important to note that the actual efficiency and sample throughput achieved in both HPLC and UPLC depend on various factors, including the analyte characteristics, column chemistry, mobile phase composition, instrument configuration, and method optimization. While UPLC generally offers higher efficiency and faster analysis times, there are instances where HPLC may still be suitable, especially when dealing with complex samples, analytes that require specific column chemistries, or when compatibility with existing HPLC methods and instruments is desired.

In conclusion, UPLC offers higher efficiency, sharper peak resolution, and faster analysis times compared to HPLC, resulting in increased sample throughput. However, the selection between HPLC and UPLC should be based on the specific analytical requirements, considering factors such as sample complexity, target analytes, available resources, and desired analysis speed.

Column Particle Size:

HPLC columns typically use larger particle sizes ranging from 3 to 5 µm. These particles provide sufficient surface area for analyte interactions and separation. HPLC columns with larger particle sizes are generally more commonly available and less expensive.

UPLC columns utilize smaller particle sizes, usually below 2 µm and commonly in the sub-2 µm range. The reduced particle size provides higher efficiency and improved separation capabilities. UPLC columns with smaller particle sizes offer increased surface area, leading to enhanced chromatographic resolution.

Column Pressure:

HPLC operates at relatively lower pressures compared to UPLC. The typical operating pressure ranges from a few hundred to several thousand psi (pounds per square inch). HPLC systems are designed to handle these lower pressures and are compatible with standard laboratory instruments.

UPLC systems operate at higher pressures due to the smaller particle size of the columns and the need for increased flow rates. UPLC systems commonly operate in the range of 6,000 to 18,000 psi. The higher-pressure capabilities of UPLC systems require specialized instrumentation designed to withstand and manage these elevated pressures.

Implications:

Efficiency: The smaller particle size in UPLC columns leads to higher efficiency, resulting in improved peak resolution and separation. The increased efficiency allows for better resolution of closely eluting peaks compared to HPLC.

Back pressure: The use of smaller particles in UPLC columns leads to increased back pressure due to the higher resistance to the flow of the mobile phase. UPLC systems require robust pumps and high-pressure-rated components to handle the increased back pressure.

Flow Rate: UPLC systems operate at higher flow rates compared to HPLC due to the smaller particle size and the need to generate sufficient pressure to drive the mobile phase through the column. The increased flow rates contribute to faster separations and reduced analysis times in UPLC.

When comparing HPLC (High-Performance Liquid Chromatography) and UPLC (Ultra-Performance Liquid Chromatography) in terms of method development and adaptability, several factors come into play. Here's a comparison between the two techniques in these aspects:

Instrumentation Cost:

When considering the cost of HPLC (High-Performance Liquid Chromatography) and UPLC (Ultra-Performance Liquid Chromatography), several factors come into play. Here's a comparison between the two techniques in terms of cost considerations:

HPLC systems are generally less expensive compared to UPLC systems. HPLC instruments have been widely used for many years, resulting in a mature market with a variety of options available at different price points. The cost of HPLC instruments can vary depending on the brand, features, and specifications.

UPLC systems tend to be more expensive than HPLC systems. The advanced technology, higher pressure capabilities, and specialized components required for UPLC contribute to the increased cost. UPLC instruments are often marketed as high-end, cutting-edge systems, which can reflect in their pricing.

Column Cost:

HPLC columns, with their larger particle sizes, are generally less expensive compared to UPLC columns. The wider availability of HPLC columns from different manufacturers and the longer-established market for HPLC contribute to competitive pricing.

UPLC columns, with their smaller particle sizes and higher efficiency, tend to be more expensive than HPLC columns. The smaller particle sizes require more precise manufacturing processes, which can increase production costs. UPLC columns are often proprietary and designed specifically for UPLC systems, which can limit the options and increase the cost.

Maintenance and Repairs:

HPLC systems have been in use for a long time and have established service networks and readily available spare parts, which can contribute to lower maintenance and repair costs. There is also a larger pool of experienced technicians and engineers familiar with HPLC systems.

UPLC systems, being relatively newer and more advanced, may have higher maintenance and repair costs. The specialized components and higher-pressure capabilities may require specialized service and support, which can be more expensive. However, the maintenance costs can vary depending on the manufacturer and the service contracts available.

Analyte characteristics between the two techniques:

Analyte Size and Molecular Weight:

HPLC is suitable for a wide range of analyte sizes and molecular weights. It can accommodate both small molecules and larger biomolecules, such as peptides and proteins. HPLC columns with larger particle sizes can handle higher molecular weight compounds effectively.

UPLC is particularly advantageous for small molecules due to the use of smaller particle sizes in columns. The increased efficiency and reduced band broadening in UPLC make it well-suited for the separation of small molecules, especially in complex mixtures.

Analyte Polarities:

HPLC is versatile in separating analytes with a wide range of polarities. It offers various column chemistries and mobile phase compositions that can be tailored to suit the specific polarity requirements of the analyte.

UPLC can efficiently separate both polar and non-polar compounds. The smaller particle sizes and improved resolution make UPLC especially useful for the separation of highly polar compounds or closely eluting peaks.

Analyte Stability:

HPLC is generally well-suited for the analysis of analytes with stability concerns. The lower pressure and longer analysis times in HPLC can be advantageous when dealing with labile compounds that may degrade under higher pressures or prolonged exposure to the mobile phase.

UPLC's shorter analysis times and higher pressures can pose challenges for the analysis of unstable analytes. The increased speed and pressure in UPLC may require careful method development to balance the need for separation and the analyte's stability.

Analyte Sensitivity:

HPLC can provide excellent sensitivity for a wide range of analytes. With appropriate sample preparation and optimized detection methods, HPLC can achieve low limits of detection for both UV-Visible and mass spectrometric detection.

UPLC offers increased sensitivity compared to HPLC due to its improved efficiency and reduced band broadening. The enhanced peak capacity and narrower peaks generated by UPLC can lead to better signal-to-noise ratios and lower limits of detection.

Analyte Complexity and Sample Throughput:

HPLC is suitable for the analysis of complex mixtures and samples with a high number of components. HPLC methods are often employed in routine analysis and high-throughput applications where large sample batches need to be processed.

UPLC's higher efficiency and faster separations make it ideal for complex samples requiring high-resolution separations within shorter analysis times. UPLC's increased sample throughput is advantageous for time-sensitive applications and high-throughput laboratories.

It's important to note that while there are general trends regarding analyte characteristics in HPLC and UPLC, the specific behavior of analytes can vary depending on the column chemistry, mobile phase composition, and other method parameters. It is recommended to consider the unique properties of the analyte and the specific analytical requirements when selecting between HPLC and UPLC for a given analysis.

Advanced Method Development: UPLC may require more sophisticated method development compared to HPLC due to its higher pressures, smaller particle sizes, and faster separations. The goal is to optimize method parameters to achieve the desired separation efficiency, resolution, and sensitivity.

Both HPLC and UPLC share common analytical goals, including separation, identification, quantification, and method development. However, UPLC's emphasis on higher efficiency, faster analysis, increased sensitivity, and advanced separations distinguishes it as a more advanced and powerful technique for specific applications that require enhanced performance.

APPLICATION:

Pharmaceutical applications

There is a wide variety of applications throughout the process of creating a new drug from drug discovery to the manufacture of formulated products that will be administered to patients.

This Process to create a new drug can be divided into 3 main stages

1) Drug discovery
2) Drug development
3) Drug manufacturing

1. Tablet dissolution study of the pharmaceutical dosage form.
2. To control drug stability, Shelf-life determination.
3. Identification of active ingredients.
4. Pharmaceutical quality control.
5. Tablet dissolution of pharmaceutical dosage forms.

Food and Flavor analysis

1. Rapid screening and analysis of components in non-alcoholic drinks.
2. Measurement of quality of soft drugs and water.
3. preservative analysis.
4. Multi residue analysis of lots of pesticides in food samples by LC triple quadrupole MS.

Environmental applications

1. Detection of phenol compounds in drinking water.
2. Bio-monitoring of pollutant.
3. Rapid separation and identification of carbonyl compounds by HPLC.
4. LC/MS/MS solution for pharmaceuticals and personal care products in water, sediment, soil and biosolids by HPLC/MS/MS.
5. Determination of 3-mercaptopropionic acid by HPLC

Forensics applications

1. Quantification of the drug biological samples.
2. Identification of anabolic steroids in serum, urine, sweat & hair.
3. Forensic analysis of textile dyes.

Clinical applications

1. Catecholamines such as epinephrine and dopamine are highly important for many biological functions. Analyzing their precursors and metabolites can provide diagnosis of diseases such as Parkinson’s disease, heart disease, and muscular dystrophy.
2. Quantification of ions in human urine analysis of antibiotics in blood plasma.
3. Estimation of bilirubin & biliviridin in blood plasma in case of hepatic disorders.
4. Detection of endogenous neuropeptides in extracellular fluids of the brain.

CONCLUSION:

One of the most popular analytical techniques is HPLC. It has several benefits over traditional chromatographic methods. HPLC makes precise and quick identification and determination of a variety of natural and synthetic substances possible because of its ease of use and effectiveness. In terms of quantitative and qualitative estimation, it has several applications in a variety of sectors, including pharmaceutical, environmental, forensic, food and flavor, clinical, and many others. It can be used in both laboratory and clinical science. Essentially, it is a greatly enhanced kind of column chromatography. Solvent is externally driven through a column at high pressures of up to 400 atm rather than dripping through it while only being affected by gravity. This greatly speeds up the chromatographic procedure. Additionally, it permits the use of column packing material with very fine particle size, providing a lot more surface area for interactions between the stationary phase and the molecules passing through it. As a result, it makes it possible to separate the mixture's components better. The cost of HPLC is its lone drawback.

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