A REVIEW ON UPLC METHODS AND APPLICATIONS

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Abstract:

Ultra-Performance Liquid Chromatography (UPLC) represents a significant advancement in liquid chromatography techniques, designed to provide higher resolution, increased speed, and enhanced sensitivity for the separation of chemical compounds. By employing smaller particle sizes (typically less than 2 µm) in the stationary phase and operating at elevated pressures (up to 15,000 psi), UPLC overcomes the limitations of traditional high-performance liquid chromatography (HPLC). This technique is particularly advantageous for complex samples, enabling the efficient separation of closely related compounds in shorter run times, which is essential in high-throughput environments. UPLC allows for greater efficiency in solvent usage, leading to reduced environmental impact and lower operational costs. Moreover, UPLC can be integrated with mass spectrometry (UPLC-MS), enhancing its analytical capabilities by providing structural and quantitative information about analytes. The high resolution and sensitivity of UPLC make it a critical tool for researchers and industries requiring precise analytical methodologies in their workflows. As a result, UPLC continues to evolve, incorporating advancements in column technology and detection methods, solidifying its role in the future of analytical chemistry.

• ***Keywords***

1 Chromatography 2 Ultra-Performance 3 Liquid Chromatography 4 Separation 5 Resolution 6. Analytical Technique

Introduction: Ultra-Performance Liquid Chromatography (UPLC) can be considered a new direction in liquid chromatography. As its first producer, Waters, proclaims, UPLC stands for "speed, resolution, and sensitivity." According to the well-known Van Demeter equations, the efficiency of the chromatographic process is proportional to a decrease in particle size. Van Demeter's model, which describes band broadening and the relationship between height equivalent of theoretical plate (HETP) and linear velocity, indicates that one of the terms—specifically the path-dependent term—is influenced by the diameter of the particles packed into the analytical column.

The A term in the Van Demeter equation is independent of flow velocity and represents "eddy" mixing, which is minimized when the particles in the packed column are small and uniform. The B term accounts for axial diffusion, reflecting the natural tendency of molecules to diffuse; this effect decreases at higher flow rates, which is why it is divided by the flow velocity (v). The C term relates to kinetic resistance encountered during the separation process, specifically the time delay experienced by molecules transitioning between the gas phase and the stationary phase. As the flow of the mobile phase increases, molecules in the stationary phase tend to lag behind those in the mobile phase, making this term directly proportional to flow velocity.

The study on the separation of chiral pharmaceuticals, Including oxazepam, temazepam, and chlortalidone, using ultra-high pressure liquid chromatography (UHPLC) was conducted by Xiang et al. Capillary columns featuring C6-modified silica particles of 1.0 µm were employed in conjunction with a self-assembled UHPLC system previously described. This UHPLC approach enabled rapid chiral separations, achieving results in as little as 2 minutes with high resolution. However, for routine laboratory application of ultra-high pressure chromatography, several practical issues remained, particularly regarding sample introduction, reproducibility, and detection methods. The use of ultra-high pressure columns necessitated extremely narrow sample plugs to reduce peak broadening caused by sample volume. To address these challenges, the Acquit UPLC system was developed, as many earlier ultra-high pressure systems required in-house modifications of commercial products and often necessitated the lab's own fabrication of analytical columns, typically capillary columns.

As the efficiency and speed of analysis have become increasingly crucial in various applications of liquid chromatography, particularly in the pharmaceutical, toxicological, and clinical fields, there is a strong emphasis on enhancing throughput and lowering analysis costs. In this context, UPLC could significantly impact the future of liquid chromatography. This study aimed to compare UPLC and HPLC analyses within a pharmaceutical laboratory setting. Four complex topical formulations—Triamcinolone cream, Hydrocortisone cream, Indomethacin gel, and Estrogel gel—were evaluated, and the results were compared.

As the efficiency and speed of analysis have become increasingly important in various applications of liquid chromatography—particularly in the pharmaceutical, toxicological, and clinical fields—there is a pressing need to enhance throughput and reduce analysis costs. In this context, UPLC is poised to play a significant role in the future of liquid chromatography. This study aimed to compare UPLC and HPLC analyses within a pharmaceutical laboratory. Four complex topical formulations—Triamcinolone cream, Hydrocortisone cream, Indomethacin gel, and Estrogel gel—were evaluated, and the results were analyzed and compared.

The Initial practical applications of UPLC were implemented alongside TOF mass spectrometry detection in the areas of metabonomics and genomics. These studies demonstrated clear advantages of UPLC over HPLC, particularly in terms of peak resolution, speed, and sensitivity within these fields. Recently, the first comprehensive review on UPLC has been published, covering the theory behind UPLC and summarizing some of the latest research in this area.

History:

• History of Ultra-Performance Liquid Chromatography (UPLC)

• Origins and Development of Liquid Chromatography

1. Early Beginnings (1900s):

- Liquid chromatography (LC) emerged in the early 20th century, primarily for separating pigments in plants. In the 1930s, the concept evolved with the introduction of paper chromatography, allowing for the separation of small molecules.

2. Advancements in High-Performance Liquid Chromatography (HPLC):

- In the 1960s, the development of high-performance liquid chromatography (HPLC) marked a significant milestone. The use of smaller particles (3-5 µm) and improved pump technology led to better separation efficiency and resolution. HPLC became the dominant technique for analytical chemistry, widely adopted in pharmaceuticals, environmental analysis, and food testing.

• Emergence of UPLC

3. Introduction of UPLC (2004):

- In 2004, Waters Corporation introduced Ultra-Performance Liquid Chromatography (UPLC) as a new direction in liquid chromatography. UPLC was designed to overcome the limitations of HPLC by utilizing smaller particle sizes (≤ 2 µm) and higher operating pressures (up to 15,000 psi). This innovation provided significant improvements in resolution, sensitivity, and analysis speed.

4. Key Features and Innovations:

• Column Technology: UPLC utilized advanced column packing technology that allowed for more uniform and smaller particles, enhancing efficiency and reducing band broadening.

• Increased Speed and Resolution: By optimizing flow rates and reducing particle size, UPLC could deliver results in a fraction of the time compared to traditional HPLC while achieving higher resolution.

• Improved Sensitivity: The smaller particle sizes allowed for better interaction between analytics and the stationary phase, leading to enhanced sensitivity for detecting low-abundance compounds.

5. Adoption and Impact:

- Following its introduction, UPLC quickly gained popularity in various fields, particularly in pharmaceuticals for drug development and quality control. Its high throughput capabilities made it ideal for laboratories facing increasing demands for rapid analysis.

• Technological Advancements

6. Integration with Mass Spectrometry (UPLC-MS):

- The combination of UPLC with mass spectrometry (UPLC-MS) further expanded its capabilities, allowing for comprehensive qualitative and quantitative analysis. This integration facilitated the identification of complex mixtures and enhanced sensitivity.

• Current Trends and Future Directions

7. Continued Evolution (2010s-Present):

- UPLC technology continues to evolve with improvements in column materials, detector technologies, and software capabilities. Innovations in core-shell particle technology and new stationary phases have further enhanced performance.

8. Future Prospects:

- Ongoing research focuses on enhancing the efficiency of UPLC systems, expanding the range of analyses that can be effectively separated, and further integrating UPLC with other

analytical techniques. Developments in miniaturization and portable UPLC systems are also anticipated, aiming to bring laboratory capabilities to field applications.

• Advantages of UPLC :

THESE ARE THE ADVANTAGES OF UPLC

- Requires shorter run times while improving sensitivity.

- Offers enhanced selectivity, sensitivity, and a broad dynamic range for LC analysis.

- Produces well-resolved peaks in chromatograms.

- Supports multi-residue methodologies.

- Enables rapid analysis and accurate quantification of analytes and related compounds.

- Utilizes fine particles (2µm) for stationary phase packing, facilitating faster analyses.

- Reduces both time and costs.

- Minimizes solvent consumption.

- Allows for a greater number of products to be analyzed with existing resources.

- Increases sample throughput, helping manufacturers produce more consistent materials that meet or exceed specifications, potentially reducing variability and the need for rework.

- Provides real-time analysis aligned with manufacturing processes.

- Ensures the quality of the final product, including during release testing.

• Disadvantages :

- UPLC operates at ultra-high pressures of up to 1000 bars, which necessitates more frequent maintenance and reduces column lifespan.

- The 1.7 µm particle phases are non-regenerable, limiting their applicability.

UPLC utilizes advanced column technology designed to function under extremely high pressure, offering a faster detection rate. Since it is based on the established HPLC technique, there is optimism that UPLC will enhance the quality of pharmaceutical analyses and boost research productivity.

While it may take some time for UPLC to become a standard analytical method, there is hope that this technique will meet expectations.

- **Application of UPLC in the Pharmaceutical Industry**

In the pharmaceutical sector, the demand for UPLC (Ultra Performance Liquid Chromatography) analysis is significant due to its distinct advantages, such as high resolution in chromatograms and rapid analysis times. This efficiency enables scientists to conduct more analytical work in less time while obtaining valuable, reliable, and authentic data. UPLC facilitates the generation of highly accurate data more swiftly than traditional methods.

The technique is particularly useful for analyzing herbal products. In analytical laboratories, the need for UPLC is pronounced, as the developed methods yield precise and accurate results, thereby enhancing research capabilities at the nanoscale. This method allows for a clear distinction in the standards of analysis—be it qualitative, quantitative, or regarding sample complexity—achieving exceptionally high standards.

The UPLC coupled with mass spectrometry (UPLC/MS) system provides data that helps resolve the complexities associated with various compounds. Using mass spectrometry as a detector in conjunction with UPLC enables in-depth interpretation of analyses, making this approach especially beneficial in the bioanalytical field. The unique features of UPLC, such as high resolution and quick analysis times, are also advantageous for pharmacokinetic studies, including absorption, distribution, metabolism, and excretion (ADME). ADME studies evaluate the physical and chemical properties of compounds. The UPLC/MS/MS method significantly reduces analysis time, making it ideal for drug development and formulation processes, where the profiling, detection, and quantification of drug substances and their impurities are carried out with high accuracy.

In the pharmaceutical industry, UPLC is crucial for drug development, quality assurance, and regulatory adherence. Its capacity to quickly separate and quantify intricate drug formulations makes it an essential instrument for pharmaceutical researchers. UPLC allows for the effective analysis of active pharmaceutical ingredients (APIs), impurities, and degradation products, supporting method development, validation, and routine quality testing. Furthermore, UPLC's superior sensitivity and resolution facilitate the detection of trace-level impurities and metabolites, ensuring the safety, effectiveness, and purity of pharmaceutical products throughout their lifecycle.

- **Applications of UPLC Analysis**:

1. Analysis of amino acids.

2. Examination of natural and herbal medicines.

3. Drug analysis in human plasma (e.g., Levofloxacin and its metabolites).

4. Investigation of metabonomics.

• **INSTRUMENTATION:**

The fundamental principles of UPLC are similar to those of HPLC, with enhancements in instrumentation and hardware. A UPLC system typically includes a binary solvent system, a sample manager, a column manager, and a detector. The solvent manager employs two pumps to deliver a parallel binary gradient, which is mixed at high pressure. Additionally, a degassing system removes dissolved gases from the mobile phase, which can be selected from up to four solvents using a valve. UPLC systems can endure pressures of approximately 15,000 psi (around 1000 bar), enabling the use of sub-2-mm particles for improved performance. The sample manager features advanced technology, allowing for sample temperatures as low as 0°C, while the column manager can maintain column temperatures up to 90°C. This capability of high-temperature UPLC significantly reduces analysis time without compromising efficiency.

1.Sample injection

2. UPLC columns

3. Column manager and heater or cooler

4. Detectors

5. Softwares

6. Accessories

7. Connection insight service

1.Sample injection:

the design of injection valves and the reproducibility of separations (Wu et al., 2001). They also explored a carbon dioxide-enhanced slurry packing method on a capillary scale, applied to the separation of benzodiazepines, herbicides, and various pharmaceuticals (Wu et al., 1997). Jorgenson et al. modified a standard HPLC system to operate at 17,500 psi, utilizing 22 cm-long capillaries packed with 1.5 mm C18-modified particles for protein analysis (Tolley et al., 2001). The pressure drop at the optimal flow rate for maximum efficiency in a 15-cm column packed with 1.7 µm particles is about 15,000 psi. Thus, a pump that can deliver solvents smoothly and reliably at these pressures, while compensating for solvent compressibility in both gradient and isocratic modes, is essential. In UPLC, the sample introduction process is crucial. Traditional injection valves, whether automated or manual, are not typically designed to withstand such high pressures. To protect the column from pressure fluctuations, the injection process must be pulse-free, and the swept volume of the injection device should be minimized to limit band broadening. A rapid injection cycle is necessary to take full advantage of UPLC's speed, which in turn necessitates high sample capacity. Low-volume injections with minimal carryover are also essential to enhance sensitivity. Additionally, there are direct injection methods available for biological samples.

2. UPLC columns:

The design and development of sub-2 µm particles present a considerable challenge, and researchers have been exploring this field for some time to leverage their benefits. While high-efficiency, nonporous 1.5 µm particles are commercially available, they have limitations in loading capacity and retention due to their low surface area. Silica-based particles offer good mechanical strength but come with drawbacks, including a restricted pH range and tailing effects for basic analytes.

olymeric columns can address pH limitations, yet they face challenges such as lower efficiency, restricted loading capacities, and inadequate mechanical strength. In 2000, Waters launched XTerra®, a first-generation hybrid chemistry that combines the advantages of both silica and polymeric columns. XTerra columns are mechanically robust, highly efficient, and can function across a broad pH range. They are manufactured through a traditional sol-gel synthesis process that incorporates carbon in the form of methyl groups.

To ensure the necessary mechanical stability for UPLC, a second-generation technology known as Bridged Ethyl Hybrid (BEH) was developed, resulting in ACQUITY BEH particles. These 1.7 µm particles achieve their improved mechanical stability through the bridging of methyl groups within the silica matrix. Additionally, packing these 1.7 µm particles into reproducible and durable columns posed a significant challenge that had to be addressed.

Uniformity in packed beds is essential, particularly when shorter columns need to preserve resolution while achieving faster separations. All ACQUITY UPLC BEH columns are equipped with eCord™ microchip technology, which records manufacturing details for each column, including quality control tests and certificates of analysis.

3. Column manager and heater or cooler

The ACQUITY UPLC Column Manager features automatic column switching, designed to enhance productivity in UPLC sample processing. Its Column Heater/Cooler allows laboratories to incorporate temperature as a variable in their methods.

The ACQUITY UPLC Column Manager enables users to fully leverage the performance, variety of stationary phases, and durability of ACQUITY UPLC BEH Columns. It features temperature control ranging from 10°C to 90°C and supports automated switching between up to four columns with internal diameters of 2.1 mm and lengths of 150 mm, along with a bypass channel for flow injections.

The ACQUITY UPLC System is comprised of a binary solvent manager, a sample manager (which includes the column heater), a detector, and an optional sample organizer. The binary solvent manager employs two individual serial flow pumps to deliver a parallel binary gradient, and it has built-in solvent selection valves for up to four solvents. With a pressure limit of 15,000 psi (approximately 1000 bar), the system is optimized for sub-2 µm particle applications.

The sample manager incorporates advanced technologies, including pressure-assisted sample introduction to maintain low dispersion during the injection process. A series of pressure transducers provide self-monitoring and diagnostics. It utilizes a needle-in-needle sampling method for enhanced reliability, and a needle calibration sensor ensures improved accuracy. The injection cycle time is 25 seconds without a wash and 60 seconds with a dual wash to further minimize carryover.

dditionally, various microtiter plate formats (including deep well, mid-height, or vials) can be accommodated in a temperature-controlled setting. With the optional sample organizer, the sample manager can handle injections from up to 22 microtiter plates. It also manages the column heater, allowing for column temperatures of up to 65°C.

4. Detectors

Half-height peak widths of less than one second can be achieved using 1.7 µm particles, which presents considerable challenges for the detector. To accurately and reproducibly integrate an analyte peak, the detector's sampling rate must be sufficiently high to collect enough data points across the peak. Additionally, the detector cell should have minimal dispersion to maintain separation efficiency.

In theory, the sensitivity of UPLC detection is expected to be 2-3 times greater than that of HPLC separations, depending on the detection method used. Mass spectrometry (MS) detection benefits significantly from UPLC, as it results in increased peak concentration and reduced chromatographic dispersion at lower flow rates, leading to improved ionization efficiencies in the source.

Waters' ACQUITY UPLC detectors enhance the ability to analyze a diverse range of compounds effectively.

The Photodiode Array (PDA), Tunable UV (TUV), and Evaporative Light Scattering (ELS) detectors are specifically optimized for UPLC system technology. Their design features low dispersion characteristics and high data acquisition rates, ensuring reliable performance. Additionally, they offer cost-effective maintenance support and readily available parts, making them an efficient choice for various analytical applications.

• ACQUITY UPLC Detectors

The ACQUITY UPLC® optical and mass detectors are designed to complement and enhance the high-efficiency separations provided by ACQUITY UPLC Systems, enabling the analysis of a diverse range of compounds.

Our optical detectors are tailored for advanced UPLC® analytical techniques, offering low dispersion characteristics, user-friendly operation, and high data acquisition rates. Additionally, they come with cost-effective maintenance, support, and readily available parts.

Two tunable UV-visible photodiode array detectors, the ACQUITY PDA and ACQUITY PDA eλ, are commonly utilized for routine analysis and method development, offering the capability to detect and quantify trace impurities. These detectors achieve data rates of up to 80 Hz and feature a low noise specification of 10 Au, covering a broad range of spectral analysis—up to 500 nm for the PDA detector and 800 nm for the eλ detector. To minimize band spreading and concentration variance, low-volume light-guiding flow cells made from Teflon AF are employed. This design eliminates internal absorption by utilizing the principle of total internal reflection, thereby enhancing light transmission efficiency.

5. Softwares :

ACQUITY UPLC Systems can be efficiently controlled, diagnosed, and monitored through a graphical console interface using Empower™ and MassLynx™ software. Both Empower and MassLynx offer dynamic data processing and information management tools that transform the results produced by the ACQUITY UPLC System into actionable insights**.**

6. Accessories:

Waters is consistently enhancing the capabilities of the ACQUITY UPLC System. The eCord™ technology, now available on all ACQUITY UPLC columns, tracks column history. The Sample Organizer boosts system capacity by over tenfold, while the FlexCart system platform enhances usability, accessibility, and convenience.

7. Connection insight service :

INSIGHT™ leverages Intelligent Device Management technology to deliver diagnostic insights for ACQUITY UPLC Systems. This system establishes a virtual technical support presence in your lab, allowing Waters to offer proactive and prompt service, ensuring top-tier support and customer satisfaction.

• UPLC Method and Material:

1. Overview of UPLC: Ultra-Performance Liquid Chromatography (UPLC) is an advanced form of liquid chromatography that allows for the separation, identification, and quantification of components in complex mixtures with enhanced speed and resolution compared to traditional HPLC.

Methodology

A. Sample Preparation

• **Filtration:** Samples may be filtered through membranes (0.45 µm or 0.2 µm) to remove particulate matter, which helps prevent clogging of the UPLC column.

• **Dilution:** Samples are often diluted to ensure that analyte concentrations fall within the calibration range of the method.

Precipitation or Extraction: For complex matrices, solid-phase extraction (SPE) or liquid-liquid extraction may be employed to concentrate and purify the analytes of interest.

B. Column Selection

• **Particle Size:** UPLC columns are typically packed with smaller particle sizes (1.7 µm or less), which allows for higher efficiency and improved resolution due to increased surface area and reduced diffusion.

Column Length and Diameter: UPLC columns are generally shorter and have a smaller inner diameter than traditional HPLC columns, which reduces back pressure and enhances separation speed.

Stationary Phase: The choice of stationary phase (e.g., C18, C8, phenyl, or other specialized phases) is crucial, as it determines the interaction between the analytes and the stationary phase, influencing retention and separation.

C. Mobile Phase Selection

Composition: Mobile phases are typically mixtures of solvents such as water, acetonitrile, methanol, and various buffering agents (e.g., phosphate buffer, acetate) to maintain pH stability.

• **Additives:** Additives like formic acid or ammonium acetate may be added to improve ionization efficiency in mass spectrometry detection or to enhance peak shape.

D. Injection

• **Autosampler:** UPLC systems often feature autosamplers that enable precise and reproducible sample injection.

• **Injection Volume:** Typical injection volumes range from 1 to 10 µL, depending on the sensitivity requirements and concentration of analytes.

E. Separation Process

• **Flow Rate:** UPLC operates at higher flow rates (up to 1 mL/min or more), which helps to reduce analysis time.

• **Gradient Elution:** A gradient method can be employed, where the composition of the mobile phase changes during the run to improve separation of compounds with a wide range of polarities.

• **Temperature Control:** Some UPLC systems include column ovens to maintain a constant temperature, which can enhance reproducibility and improve resolution.

• **Types of Detectors:**

• **UV/Vis Detector:** Measures absorbance at specific wavelengths; commonly used for compounds with chromophores.

• **Fluorescence Detector:** Offers high sensitivity for fluorescent compounds.

Mass Spectrometry (MS): Coupling UPLC with MS provides detailed molecular information and is widely used for quantitative and qualitative analysis.

Conductivity and Refractive Index Detectors: These are used for specific applications, such as monitoring ionic species or non-UV active compounds.

G. Data Analysis

• **Software:** UPLC systems typically come with software that allows for data acquisition, processing, and interpretation. Common software includes Empower, ChemStation, and others that facilitate integration and quantification of peaks.

Calibration: Standard curves are generated using known concentrations of analytes, which allows for the quantification of unknown samples.

• Materials

. UPLC Columns

Material Composition: Columns are usually constructed from stainless steel or PEEK (polyether ether ketone) to withstand high pressures.

Brands and Types: Popular brands include Waters (ACQUITY UPLC), Agilent, and Thermo Fisher, each offering a variety of phases tailored to specific applications.

B. Mobile Phases

Solvents: High-purity solvents are essential for reproducible results. Common choices include:

Water: Typically deionized or distilled.

Acetonitrile: Often used for its low viscosity and excellent solvent properties

Methanol: Commonly used but can be more viscous than acetonitrile.

Buffers: Buffering agents such as phosphoric acid or ammonium acetate help maintain pH and improve peak shape.

C. Sample Vials

Types: Glass vials are preferred for volatile compounds; however, polypropylene vials are also used for certain applications.

Volume: Vials typically come in 1 mL, 2 mL, or 10 mL sizes.

D. Detector Components

Optics: UV detectors require high-quality quartz optics for accurate measurement of absorbance.

Ionization Sources: In mass spectrometry, different ionization techniques (e.g., ESI, APCI) can be used based on the analyte type.

• Conclusion :

UPLC is a powerful emerging technique that utilizes advancements in instrumentation and particle technology to enhance productivity in the pharmaceutical and other industries. This method offers improved resolution for component separation, high sensitivity for analyzing low-concentration substances, and a reduced analysis time. Additionally, its lower solvent consumption leads to cost savings in analysis. The method can be effectively developed and validated in a shorter timeframe with this system. UPLC can be easily combined with various other techniques, such as mass spectrometry, making it versatile for applications like impurity profiling, metabolite identification, dissolution testing, and process control analysis across pharmaceutical, food, environmental, forensic, and toxicological fields.

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