

A Comprehensive Review on UHPLC and UPLC: Advancements, Comparison, and Applications

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ABSTRACT

Ultra-High-Performance Liquid Chromatography (UHPLC) and Ultra Performance Liquid Chromatography (UPLC) are two of the most advanced liquid chromatographic techniques, offering improved separation, sensitivity, and speed over traditional High-Performance Liquid Chromatography (HPLC). While both technologies share similar principles, they differ in system configurations, column technologies, and applications. UHPLC generally refers to any liquid chromatography system capable of using sub-2 μm particles, while UPLC is a proprietary technology developed by Waters Corporation that optimizes chromatographic performance specifically with sub-2 μm particles. This review explores the principles, advancements, differences, and applications of UHPLC and UPLC, discussing their unique advantages and limitations, and providing insights into their roles in industries such as pharmaceuticals, food safety, environmental analysis, clinical diagnostics, and forensics.

Keywords: UHPLC, UPLC, Pharmaceutical Analysis.

INTRODUCTION

Liquid chromatography (LC) has become a cornerstone technique in analytical chemistry for separating, identifying, and quantifying compounds in complex mixtures. Over the past few decades, significant improvements in chromatographic technology have led to the development of Ultra-High-Performance Liquid Chromatography (UHPLC) and Ultra Performance Liquid Chromatography (UPLC). These technologies, which provide higher resolution, speed, and sensitivity compared to traditional HPLC, have revolutionized various fields of analysis. While both UHPLC and UPLC utilize sub-2 μm particle columns to achieve high-resolution separations, there are key differences in their system design, operating conditions, and specific applications. UHPLC is a more generalized term for advanced liquid chromatography systems, whereas UPLC, developed by Waters Corporation, is a specific brand optimized for ultra-fast and high-resolution separations using sub-2 μm particles. Understanding the advantages, limitations, and differences between these two technologies is essential for selecting the appropriate system for various analytical applications. This review aims to compare and contrast UHPLC and UPLC, focusing on their technological advancements, system

configurations, performance characteristics, and practical applications across diverse industries.

Principles of UHPLC and UPLC

UPLC: UPLC (ultra performance liquid chromatography) systems were first introduced in 2004. By almost doubling the overall operating pressure (to 15,000 psi) in order to obtain more rapid flow rates, UPLC developers were able to achieve equal or better resolution LC separations in much shorter time frames. Comment: Rather than “doubling the overall operating pressure in order to obtain more rapid flow rates” it’s better to say ... “In order to take advantage of 2-micron particles higher pressures are required.” The typical ID of a UPLC column is 2.1 mm and in general flow rates are lower than HPLC, but due to the efficiency increase, explained above, the overall separation time is reduced. In terms of efficiency, accuracy and productivity, this was good news for labs the world over. UPLC is a variant of HPLC, also using columns and pumps. Comment: Whether we are talking about HPLC, UPLC or UHPLC, each of these techniques employs columns and pumps.

Principle: The basic principle of UPLC for the separation of components in a matrix is same as HPLC, the main difference is in the particle size of sorbent of the column, which is less than 2 μm . The small particles in UPLC require a high pressure (6000

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psi) to work with. This is also based on van Deemter equation which describes the relationship between flow rate and HETP or column efficiency. This review explores the underlying principles, development, key differences, and practical applications of UHPLC and UPLC. By analyzing their capabilities and limitations, the article aims to provide a detailed comparative overview of these advanced chromatographic techniques.

$$H = A + B/v + Cv$$

A - Eddy diffusion

B - Longitudinal diffusion

C - Equilibrium mass transfer

v - Flow rate

UHPLC

Ultra-High-Performance Liquid Chromatography (UHPLC) is a high-resolution separation technique that typically uses columns packed with smaller particles (ranging from 1.7 μm to 2.5 μm). The reduction in particle size leads to improved separation efficiency and reduced analysis times. UHPLC systems can typically operate at higher pressures, up to 15,000 psi or 1034 bar, allowing the mobile phase to pass through tightly packed columns.

Key Features:

- **Column Chemistry:** Uses columns packed with small (1.7–2.5 μm) particles.
- **Pressure:** Operates at high pressures (up to 15,000 psi or 1034 bar).
- **Flexibility:** Capable of using a wide variety of column chemistries and mobile phase compositions.

Differences between UHPLC and UPLC

Although UHPLC and UPLC share many similarities in terms of their ability to use sub-2 μm particles and operate at high pressures, several differences set these two techniques apart. The primary differences lie in the **system design, column technology, and flexibility**.

Column Technology and Particle Size

- **UHPLC:** The use of 1.7 μm to 2.5 μm particle sizes in columns allows for higher efficiency and faster separations compared to traditional HPLC (which uses 3–5 μm particles). However, UHPLC systems offer more flexibility in column chemistries, enabling the use of a wide range of stationary phases.
- **UPLC:** UPLC columns typically use **sub-2 μm** particles (1.7 μm), which are specifically

designed to achieve the highest resolution and speed. The smaller particle sizes lead to reduced peak broadening and faster separations, but UPLC systems are optimized for these specific columns, limiting their flexibility compared to UHPLC.

System Configuration and Optimization

- **UHPLC:** UHPLC systems offer a broad range of configurations and are compatible with various manufacturers' components. This flexibility makes UHPLC a suitable option for labs with diverse needs or those transitioning from traditional HPLC.
- **UPLC:** UPLC systems, on the other hand, are more proprietary. Developed by Waters Corporation, UPLC systems are optimized for use with sub-2 μm particles and designed to operate under very high pressures (up to 15,000 psi). This specific system configuration ensures maximum efficiency and reproducibility, but limits flexibility in terms of hardware and column selection.

Speed and Resolution

- **UHPLC:** Although UHPLC improves speed and resolution compared to traditional HPLC, the separations are typically slower than those achieved with UPLC, primarily due to differences in system optimization and particle size. UHPLC offers separations 3–5 times faster than traditional HPLC, but it does not always achieve the ultra-fast separations of UPLC.
- **UPLC:** UPLC is specifically designed to provide faster separations and higher resolution than UHPLC, particularly in high-throughput environments. UPLC systems can achieve separations **up to 10 times faster** than HPLC, making it ideal for applications requiring high sample throughput.

Advancements in UHPLC and UPLC:

Ultra-High-Performance Liquid Chromatography (UHPLC) and Ultra Performance Liquid Chromatography (UPLC) have undergone significant advancements over the past decade. These improvements have contributed to their widespread adoption in various fields, such as pharmaceuticals, environmental analysis, clinical diagnostics, and food safety. The advancements in both techniques are primarily driven by innovations in **instrumentation, column technology, data processing, and method**

development. Below is a detailed overview of the key advancements in UHPLC and UPLC.

1. Column Technology Advancements

Sub-2 μm Particles

- **Smaller particle size columns** (typically sub-2 μm) are a hallmark of both UHPLC and UPLC technologies. The reduction in particle size leads to better resolution and faster separations. While UHPLC typically uses **1.7–2.5 μm particles**, UPLC is optimized for **1.7 μm particles**.
- **Improved packing efficiency** of columns allows for higher pressure and faster flow rates, contributing to reduced analysis times and enhanced sensitivity.

Novel Stationary Phases

- The development of new **stationary phases** (such as **superficially porous particles**, **core-shell particles**, and **graphitized carbon**) has been crucial for improving separation efficiency and retention times.
 - **Core-shell particles**, also known as **pellicular particles**, provide a combination of high surface area and low resistance to flow, reducing band broadening and providing faster separations.
 - **Porous-layer** and **monolithic columns** have improved mass transfer, reducing backpressure and allowing for even faster analysis while maintaining resolution.

Column Selectivity

- There has been an improvement in the **selectivity** of stationary phases, allowing the separation of difficult-to-resolve compounds (e.g., polar compounds, proteins, and peptides). This is particularly important in complex samples like **biological fluids**, **foods**, and **environmental samples**.

2. High Pressure and Flow Optimization

Enhanced Pressure Capabilities

- Both UHPLC and UPLC systems can now operate at pressures of up to **15,000 psi** (1034 bar) or higher. This enables the use of smaller particles (sub-2 μm) without compromising separation efficiency or resolution. Higher pressures improve the interaction between the mobile phase and stationary phase, leading to faster, more efficient separations.

Improved Flow Control

- Recent developments in **pressure pulse damping** and **high-pressure pumps** provide more **stable flow rates**, reducing the chance of flow instability and improving reproducibility in chromatographic separations. These advancements have made high-throughput analysis possible in **clinical diagnostics** and **pharmaceutical analysis**, where sample throughput is critical.

3. Miniaturization and High-Throughput Capabilities

Microfluidic Systems

- The integration of **microfluidic technologies** into chromatographic systems has been a significant advancement. These systems enable **smaller sample volumes** and faster separations, increasing sample throughput without sacrificing resolution. This is especially beneficial in industries like **biotechnology** and **clinical diagnostics**, where small sample sizes are often analyzed.

High-Throughput Screening

- The development of automated **sample handling systems** and **multi-dimensional chromatography setups** has made **high-throughput screening a reality**. Systems like **UPLC coupled with mass spectrometry (MS/MS)** allow laboratories to process thousands of samples in a day, improving productivity and efficiency.

4. Coupling with Advanced Detectors

Mass Spectrometry (MS) Coupling

- The coupling of UHPLC and UPLC with **mass spectrometers (MS)** has significantly enhanced sensitivity, quantitative accuracy, and compound identification. Modern MS detectors, such as **triple quadrupole MS (MS/MS)**, provide higher selectivity and sensitivity, making it possible to detect low-abundance compounds in complex samples.
- Recent advances in **ionization techniques** (e.g., **electrospray ionization (ESI)** and **matrix-assisted laser desorption/ionization (MALDI)**) have further improved the sensitivity and resolution of **LC-MS/MS** systems, facilitating **pharmacokinetic** and **biomarker discovery** studies.

Diode-Array Detectors (DAD) and Fluorescence Detectors

- **Diode-array detectors (DAD) and fluorescence detectors** have been improved for use with UHPLC and UPLC systems, offering more sensitive and precise detection of compounds across a wide range of wavelengths.
- **Multi-wavelength** detection capabilities have also advanced, allowing for more accurate identification and quantification in complex matrices.

5. Improved Software and Data Analysis

Advanced Data Processing Algorithms

- The development of **advanced software platforms** for chromatographic analysis has significantly improved data processing and interpretation. Algorithms for **peak deconvolution, quantification, and data smoothing** have made it easier to analyze complex datasets, resulting in faster decision-making.
- **Real-time data processing** now enables more immediate results, which is crucial in applications like **clinical diagnostics** and **pharmaceutical quality control**.

Automation and Integration

- Automation technologies have enabled the **integration of UHPLC/UPLC systems with other laboratory instruments**, such as **mass spectrometers, spectrometers, and fraction collectors**. This integration has reduced human error, improved reproducibility, and increased throughput.
- Automated **sample preparation systems** and **data integration** with laboratory information management systems (LIMS) have streamlined the workflow in large-scale analyses.

6. Green Chemistry and Sustainability

Solvent and Mobile Phase Innovations

- **Green chromatography** has emerged as a trend, with improvements in **solvent selection** and the development of **eco-friendly mobile phases**. The use of **supercritical fluids** or **alternative solvents** like **ionic liquids** can reduce the environmental impact of chromatographic separations.
- **Reduced solvent consumption** and **enhanced column lifetimes** due to improvements in stationary phase materials and pressure optimization contribute to a more sustainable approach in analytical chemistry.

Applications of UHPLC and UPLC

1. Pharmaceutical Industry

Both UHPLC and UPLC are extensively used in pharmaceutical analysis for quality control, method development, and pharmacokinetic studies. The high resolution and sensitivity provided by both techniques are essential for the detection of impurities and the analysis of complex formulations.

UHPLC: Used for method development, impurity profiling, stability testing, and bioanalytical applications.

UPLC: Ideal for high-throughput analysis, routine quality control, and the analysis of small molecules, proteins, and peptides.

2. Environmental Analysis

In environmental monitoring, UHPLC and UPLC are used for the detection of contaminants such as pesticides, herbicides, heavy metals, and other pollutants in water, soil, and air.

UHPLC: Suitable for detecting low concentrations of a wide range of environmental pollutants.

UPLC: Preferred for fast and sensitive analysis, especially in high-throughput environmental screening.

3. Food Safety and Quality Control

In food analysis, UHPLC and UPLC are used to detect contaminants, such as pesticides, preservatives, and toxins, and to verify the authenticity of food products.

UHPLC: Preferred for method development in complex food matrices.

UPLC: Useful for rapid, high-throughput testing of food products for contaminants.

4. Clinical Diagnostics

In clinical applications, both UHPLC and UPLC are used for the analysis of biomarkers, drugs, and metabolites in biological fluids such as blood and urine.

UHPLC: Applied for detailed clinical assays and the analysis of complex biological samples.

UPLC: Ideal for high-throughput clinical testing, providing faster and more sensitive results.

5. Forensic Toxicology

Both UHPLC and UPLC are used in forensic toxicology for the analysis of drugs, alcohol, and poisons in biological samples.

UHPLC: Suitable for more complex forensic applications that require method development.

UPLC: Used for rapid analysis and high-throughput toxicology screening in forensic laboratories.

CONCLUSION

Both **Ultra-High-Performance Liquid Chromatography (UHPLC)** and **Ultra Performance Liquid Chromatography (UPLC)** represent the forefront of liquid chromatography technology, offering significant improvements over traditional HPLC in terms of resolution, speed, and sensitivity. While **UHPLC** is more versatile and flexible in terms of column chemistries and system configurations, **UPLC** is optimized for high-speed, high-resolution separations with sub-2 μm columns and specific hardware configurations. Each technique has its strengths, and the choice between UHPLC and UPLC depends on the specific application and requirements of the analysis. For high-throughput, routine testing and ultra-fast separations, **UPLC** offers unmatched performance, particularly in industries such as pharmaceuticals, food safety, and clinical diagnostics. **UHPLC**, on the other hand, offers broader flexibility and is suitable for more diverse applications, making it an ideal choice for method development and complex analyses. Overall, both technologies represent the evolution of liquid chromatography and continue to shape the future of analytical chemistry across multiple industries.

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