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# A REVIEW an Ultra High Performance Liquid Chromatography (UHPLC): Principles, Techniques and Applications

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## Abstract

Ultra-High-Performance Liquid Chromatography (UHPLC) is an advanced analytical separation technique widely used in pharmaceutical analysis, chemical research, biotechnology, food testing, and environmental monitoring. It is an improved form of High-Performance Liquid Chromatography (HPLC) that uses smaller stationary-phase particles and high-pressure-compatible instrumentation to achieve efficient separation, high resolution, and reduced analysis time.

UHPLC separates the components of a mixture based on their different interactions with a stationary phase and a liquid mobile phase. Its major components include a solvent reservoir, high-pressure pump, sample injector, analytical column, detector, and data-processing system. Various detectors, including ultraviolet (UV), photodiode array (PDA), and mass spectrometry (MS), can be used depending on the analytical requirements.

This article reviews the fundamental principles, instrumentation, working mechanism, applications, recent developments, advantages, and disadvantages of UHPLC. It highlights the importance of UHPLC as a modern analytical technique for reliable and efficient analysis of complex samples.

***Keywords:*** Ultra-High-Performance Liquid Chromatography (UHPLC); Analytical Separation; Chromatographic Instrumentation; Pharmaceutical Analysis

## 1. Introduction

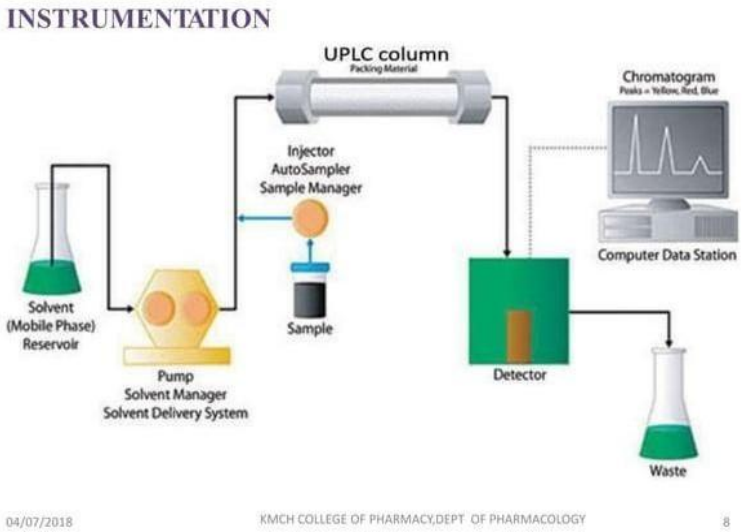
- Chromatography is an important analytical technique used for the separation, identification, and quantification of the individual components present in a mixture. It is based on the differential distribution of compounds between a stationary phase and a mobile phase.

- High-Performance Liquid Chromatography (HPLC) is widely used in pharmaceutical industries, research laboratories, and quality-control departments. It is used for drug assay, impurity determination, stability testing, and analysis of pharmaceutical formulations. However, conventional HPLC methods often require longer analysis times, and relatively high solvent consumption depending on the separation conditions.
- To improve chromatographic performance, Ultra-High-Performance Liquid Chromatography (UHPLC) was developed. It uses smaller stationary-phase particles, commonly below 2 µm, and instrumentation designed to withstand higher pressures. These features make UHPLC able to improve separation efficiency, shorten analysis time compared with appropriately selected conventional HPLC methods.
- UHPLC has become an important tool in modern analytical science due to its ability to separate complex mixtures rapidly and efficiently. It is especially useful in pharmaceutical research, as

accurate measurement of active pharmaceutical ingredients (APIs), impurities, degradation products, and drug metabolites is essential.

## 2. Principle of UHPLC

- Ultra-high-performance liquid chromatography is based on the principle of differential partitioning and interaction of the sample components with the stationary and mobile phases.
- The mobile phase transports the sample through the column, which is packed with stationary-phase particles. The interaction of each solute with the stationary and mobile phases depends on their polarity, hydrophobicity, charge, molecular properties, and chromatographic conditions. Due to the different behavior of individual components, they are separated at different retention times.




- The smaller the particle size of the stationary phase, the better are mass-transfer characteristics and, thus, the higher is column efficiency. On the other hand, reduced particle size leads to an increase in the operating pressure, which requires the use of appropriate equipment.

**Basic steps involved**

1. The mobile phase is pumped into the column at a specific flow rate.
2. A portion of the sample is introduced into the mobile-phase stream.
3. The sample components are distributed in different proportions between the mobile and stationary phases.
4. The components elute from the column with varying degrees of retardation. The detector then produces a chromatogram with peaks corresponding to the separated compounds.
5. The retention times and response of the peaks are then used to identify and quantify the substances.

Important chromatographic parameters.

1. Retention time is the time it takes for a chemical substance to pass through the column and reach the detector.
2. Resolution is the extent to which two chromatographic peaks are separated.
3. Column efficiency is a measure of the ability of a column to produce sharp peaks. The higher the resolution, the greater the number of theoretical plates.
4. Selectivity is the ability of a chromatographic system to separate two compounds.
5. Backpressure is the pressure that needs to be overcome in order to propel liquid through the column and the flow path of the pump.

## 3. Instrumentation of UHPLC

| Component | Function |
|---|---|
| Solvent reservoir | Stores the mobile-phase solvents |
| High-pressure pump | Delivers mobile phase at a controlled flow rate |
| Sample injector or autosampler | Introduces a measured sample volume |
| Analytical column | Separates sample components |
| Column oven | Maintains a controlled column temperature |
| Detector | Measures compounds as they elute |
| Data-processing system | Records chromatograms and calculates analytical results |



The detector may be UV, PDA, fluorescence, or mass spectrometry, dependent on the properties of the analytes and the purpose of the analysis.

## 5. Applications of UHPLC

UHPLC is used in several fields, due to its high separation efficiency and ability to provide rapid analysis.

### 5.1Pharmaceutical analysis

UHPLC is used for the assay of active pharmaceutical ingredients, analysis of tablets and capsules, impurity profiling, degradation-product analysis, dissolution testing, and stability studies. It may support pharmaceutical quality control when the method is appropriately developed and validated.

### 5.2Bioanalytical applications

UHPLC coupled with mass spectrometry is used to quantify drugs, metabolites, and other target compounds in biological samples, including plasma and urine. These methods may support pharmacokinetic studies and bioequivalence investigations.

### 5.3Food analysis

It may be used to measure selected food additives, preservatives, vitamins, natural products, and contaminants.



### 5.4 Environmental analysis

UHPLC supports the analysis of selected pesticides, pharmaceutical residues, and organic pollutants in environmental samples such as water.

### 5.5Biotechnology

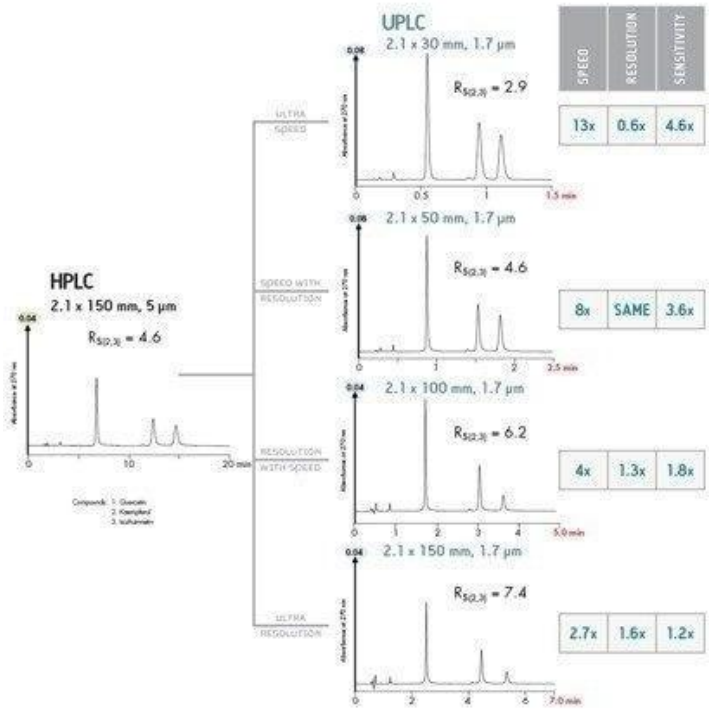
Suitable UHPLC methods may be applied to the separation and characterization of proteins, peptides, and other biomolecules.

### 5.6Forensic and toxicological analysis

When combined with suitable detection techniques, UHPLC may help to identify and quantify selected drugs, toxic compounds, and their metabolites in forensic samples.

## 6. Advantages of UHPLC

1. High separation efficiency: It provides high resolution and efficient separations using suitable columns and conditions.
2. Shorter analysis time: It allows for many methods to be performed faster than similar conventional HPLC method.
3. High resolution: It can separate closely related compounds when suitable selectivity is obtained.
4. Reduced solvent consumption: The shorter run time and optimized flow rates decrease the amount of solvent used.




5. High sample throughput: It allows more samples to be processed in a given time period.
6. Excellent quantitative performance: It can be highly quantitative and reproducible when the method is properly validated.
7. Compatibility with advanced detectors: It can be coupled with mass spectrometry to provide additional information about the analytes.
8. Useful for complex samples: It can be applied to complex samples such as those encountered in pharmaceutical analysis and many other matrixes.

## 7. Disadvantages of UHPLC

1. High instrument cost: UHPLC instrumentation usually involves a substantial initial investment compared to basic conventional HPLC instrumentation.
2. High operating pressure: UHPLC requires specialized high-pressure resistant parts such as pumps, tubing, fittings and columns.
3. Maintenance: The high-pressure parts need to be maintained and taken care of appropriately.
4. Expensive columns: Some UHPLC columns and accessories have higher replacement costs.
5. Difficult method transfer: HPLC methods often require modification when transferred to UHPLC.
6. Extra - column effects: The use of tubing, connections and detector volume can cause band broadening and loss of efficiency.

7. Sample preparation: Particulate matter in the sample or incompatible sample solvents can result in column fouling or poor peak shape.
8. Training: Operators require appropriate training to gain the necessary technical expertise to operate and troubleshoot the system.

## 8. Recent advances in UHPLC

- Recent advances have expanded opportunities for using UHPLC in pharmaceutical and analytical studies.
- The UHPLC–MS/MS approach allows more selective detection and quantification of analytes suitable for complex mixtures.
- Moreover, new stationary phases allow to increase the selectivity and efficiency of separation processes.
- Two-dimensional liquid chromatography enables to analyze of complex mixtures through two separation mechanisms.
- In addition, computer-assisted approaches allow to optimize the chromatographic methods and reduce the time of method finding.
- Methods in accordance with green chemistry principles can reduce the consumption of solvents and chemicals.
- Moreover, artificial intelligence methods allow to analyze chromatograms and optimize the analyzes – these possibilities require experimental verification.

## 9. Conclusion

- Ultra-High Performance Liquid Chromatography is a significant innovation in the field of analytical chemistry. The method uses a combination of reduced stationary-phase particle size and increased operating pressures to shorten analysis time and achieve higher resolution than traditional HPLC.
- Ultra-High Performance Liquid Chromatography is applicable in pharmaceutical analysis, biochemical, food, environmental, biotechnology, and forensic sectors of science and industry. The UHPLC method can be used for the quantitative analysis of drugs and bioactive substances, determining the identity and content of impurities, validating stability indications, and analyzing complex mixtures. The joint use of UHPLC and mass spectrometry detectors significantly extends the possibilities of chromatographic analysis.
- However, high costs of equipment, the need for qualified personnel, high pressure, and the complexity of method optimization limit the use of the technology. Moreover, it is essential to validate the analytical procedure to ensure accurate and reliable results.
- Thus, Ultra-High Performance Liquid Chromatography is a promising technique that can provide a reasonable compromise between speed and resolution in modern analytical chemistry. The method allows to increase the efficiency of the analysis, the amount of information received, shorten the time of analysis and work with complex mixtures.

## Declaration of Conflicting Interests

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