



# Recent Advances in Liquid Chromatography and Its Uses in A Variety of Domains

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**Abstract:** The most common activities involved in the analytical development of a way are separation and characterization of impurities as well as degraded products, analytical investigations, studies for identification, and finally fixing of parameters optimization to specific requirements. Chemical compound production and analysis depend heavily on compound separation. There are several ways to get effective separation results. One of the most popular methods for separating chemicals across length scales, from bigger biomacromolecules to tiny organic compounds, is liquid chromatography. Additionally, liquid chromatography enables simple modification, the combination of suitable mobile and stationary phases, the performance of qualitative and quantitative tests, and the concentration of materials. Notably, the stationary phase is the primary component of a liquid chromatography arrangement. Depending on affinities, sizes, and electrostatic interactions, the stationary phase directly interacts with the samples through a variety of fundamental kinds of interactions.

Compound separation will eventually result from a variety of interactions between the compounds and the stationary phase. Recently, stationary phases have been created to improve binding selectivity, tunability, and reuse. This article describes the development of stationary phases for separating macromolecule proteins and microscopic chemical compounds, such as small chiral molecules and polycyclic aromatic hydrocarbons, in order to illustrate the use of liquid chromatography across length scales of target molecules. Many plant-specific chemicals, including polyphenols, may be found using liquid chromatography. These compounds can be found using light absorption or fluorescence, the latter of which can be used with or without sample derivatization. Although they are blind for many of the substances that MS can identify, these detection methods are often sensitive and selective. Such bioactivity detectors might supplement LC analyses because it is widely known for LC procedures to couple numerous detectors in-line to acquire, for example, absorbance and MS detection concurrently. However, these detection techniques need reliable biosensors that are readily available and suited to LC conditions. This opens the door to further investigation into cutting-edge chromatographic separation detection techniques.

**Index Terms** - Analytical Method, Liquid Chromatography, Absorption, Fluorescence, Biosensors.

**Introduction:** The main purpose of development of analytical methods are for identification, purification and eventually to quantification any required drug etc., the most activities involved within the analytical development of a way are separation and characterization of impurities also as degraded products, analytical investigations, studies for identification and eventually fixing of parameters optimization to specific requirements. Column Chromatography is divided into two types, adsorptions column chromatography and partition column chromatography. adsorptions column chromatography works on the principle of differences in affinity between the two components towards the stationary phase, thus various components of a sample mixture get separated. (i) Selection of Column: The preferred ratio of diameter to the length of the column is 1:20 to 1:100, with the latter having high efficiency of separation. The length of the column used for separation is selected based on: the type of adsorbent used for packing; the number of components that are to be separated. (ii) Selection of Adsorbents: silica gel for column, activated alumina, starch, charcoal, magnesia etc. (iii) Selection of solvent system: different mobile phases used are petroleum ether, carbon tetra chloride,

ether, toluene etc. (iv) Packing of column: Packing can be performed by two methods: wet packing and dry packing (v) Elution: isocratic elution and fractional elution (vi) Detection: coloured components can be identified as well-defined zones in the column. Colourless compounds can be detected using UV-visible detectors (compound which absorb UV-visible light), fluoresce compounds through fluorescence detector, flame ionization detector or refractive index detector etc. used for detection of the compounds. The stationary phase and the compounds interact in a variety of ways, which eventually results in compound separation. In order to improve binding selectivity, tunability, and reusability, stationary phases have recently been designed. This study discusses the development of stationary phases for separating macromolecule proteins and minute chemical compounds, like small chiral molecules and polycyclic aromatic hydrocarbons, in order to show how liquid chromatography can be used to separate target molecules across a range of length scales.

Notably, the stationary phase serves as the main building block of a liquid chromatography setup. The stationary phase directly interacts with the samples through a number of fundamental forms of interactions depending on affinities, sizes, and electrostatic interactions. Various interactions between the compounds and the stationary phase will ultimately lead to compound separation. Stationary phases have been developed recently in order to enhance binding selectivity, tunability, and reusability.

In order to demonstrate the application of liquid chromatography across length scales of target molecules, this study examines the current advancement of stationary phases for separating macromolecule proteins and tiny chemical compounds, such as small chiral molecules and polycyclic aromatic hydrocarbons (PAHs). Until recently, the use of liquid chromatography (LC) in the examination of pesticides was often limited to groups of chemicals or single compounds for which there were no adequate circumstances for gas chromatography analysis (GC). However, recent advancements in detection and column material science demonstrate that LC's application range in this area of investigation has greatly increased. Of course, the most prominent example is the relatively quick shift in the employment of LC-MS in pesticide trace analysis from an experimental and scientifically in-vogue technology to one that is robust, sensitive, and selective in its detection methods. New LC column packing materials, such as immuno-affinity sorbents, materials for restricted access mediums, and molecularly imprinted polymers, have also recently been developed, and these materials considerably enhance the screening of polar pesticides using reversed-phase LC with UV detection.

This chapter emphasises the advantages and perspectives of these important LC findings as well as its ramifications for current and future applications in pesticide trace investigation. High-Performance Liquid Chromatography (HPLC) is one among the foremost used analytical techniques, it is advance technique of column chromatography. It is used for separation, identification and quantification of each component in a mixture.

A separation technique including mass-transfer between stationary and mobile phase is what is meant by the term "chromatographic process." A liquid mobile phase is used in HPLC to separate the mixture's constituent parts. A liquid or a solid phase is frequently the stationary phase. These components are first made to dissolve in a solvent before being made to flow quickly down a chromatographic column. The mixture separates into its component parts in the Column.

The degree of resolution is crucial and relies on how much the solute components interact with the stationary phase. The packing that is motionless within the column is referred to as the stationary phase. The relationship between the solute and moving and stationary Phases can frequently be changed by selecting various stationary phases and solvents. HPLC consist of solvent reservoirs, solvent degasser, gradient valve, mixing vessel, high pressure pump, sample injection loop, guard column, analytical column, detectors and waste collector. HPLC may be a Highly improved sort of chromatography [1-3].

Instrumentation and Components of the HPLC system:

- A. Solvent reservoir, mixing system and degassing system
- B. High pressure pump
- C. Sample injector
- D. Column
- E. Detector
- F. Data recording system

1. **Solvent reservoir, mixing system and degassing system:** Solvent reservoir stores the solvent (mobile phase). These are glass or stain- less-steel containers. The most prevalent type of solvent reservoir is a glass container. In addition to distributing mobile phase, the pump must precisely and properly blend solvents. There are two distinct types of mixing units: low pressure and high pressure. The removal of air bubbles that have accumulated in the solution is accomplished using a degassing apparatus. Filtration and ultrasonication are employed as degassing techniques.

2. A high-pressure pump is used to pressurize liquids and deliver a certain flow rate. The unit used to measure flow rate is milliliters per minute (ml/min). Normal flow rates range from 1-2 ml/min. Between 400 and 600 bar, or 6000 to 9000 psi, the pump functions. The three most common types of pumps are reciprocating piston pumps, constant pressure pumps, and syringe pumps.

3. **Sample injector:** A liquid sample is injected into the mobile phase using a sample injector. A sample valve exists between the pump and the column (Jena AK, 2011). An injector can inject the sample into the constantly flowing mobile phase stream that carries it to the HPLC column (auto sampler). Typically, samples have a volume of 5 to 20 microliters (l). Injectors come in two varieties: manual and automatic.

4. **Column:** The column is where the actual separation of components takes place. The column is made of stainless steel. It is between 5 and 25 cm long, with an internal diameter of 2.46 cm.

5. **Detector:** By recognizing each component that elutes from the column, the detector may convert the data into an electrical signal. Examples of specific detectors are the UV-VIS detector, photo diode array detector, fluorescence detector, and mass spectrometric detector. Bulk property detectors include electrochemical detectors, light scattering detectors, and refractive index detectors.

6. **Data recording system:** The output is saved as a series of peaks, and the computer linked to the display can calculate the area below each peak automatically.

The process of developing an analytical technique include choosing a precise assay plan to identify the formulation's ingredients. An analytical technique development approach is carried out to demonstrate that an analytical method is suitable for usage in a laboratory. According to the ICH guidelines Q2 (R1), analytical methods must be created in compliance with the stated protocols and approval criteria and employed in GMP and GLP settings.

The following criteria must be satisfied before a technique may be developed:

Certified analysts, qualified and calibrated instruments, documented methodologies, reliable reference standards, carefully chosen and reliable samples, and change control are only a few of the requirements. The creation of analytical techniques can help with novel molecule development, new process and reaction development, active components (macro analysis), residues (micro analysis), impurity profiles, degradation studies, and herbal products, among other things.

The upgraded liquid chromatography method known as Ultra-Performance Liquid Chromatography enhances speed, resolution, and sensitivity (UPLC). For particles less than 2 m in diameter, HPLC is superior than UPLC in terms of resolution, speed, and sensitivity.

UPLC, a relatively recent technique, is expanding the potential applications of liquid chromatography, especially in terms of time and solvent savings. The UPLC chromatographic system can withstand substantial system back pressure thanks to a special design feature. Specialized analytical columns UPLC BEH C18 filled with 1.7 m particles are used in combination with this equipment. The quality control assessments of various pharmaceutical formulations are converted using the HPLC to UPLC technology. The UPLC system offers a nine-fold and three-fold decrease in analysis time when compared to the traditional system, which employs analytical columns with particle packing of 5 m and 3 m, respectively. Particle size reduction causes back-pressure to rise by around nine times (when compared to 5 m) or three times (when compared to 3 m). The analytical column and other chromatography system components are not adversely affected despite the separation on a UPLC system being performed at very high pressures (up to 100 MPa). The UPLC method maintains or maybe improves separation efficiency. The UPLC equipment and columns must not only fulfil their claims of quick, high-resolution separations but also do so with repeatability in order to be appropriate for the analysis of pharmaceutical research materials under GMPs. The quality of the data is enhanced in addition to the data retrieval process being sped up. Due to their significantly better signal-to-noise ratio, it is evident that UPLC-MS spectra are of higher quality than Capillary LC-MS spectra. This new field of analytical separation science enhances the overall related properties of speed, sensitivity, and resolution while maintaining the value and HPLC principles. Analytical laboratories are not the only place where the pharmaceutical industry is now searching for creative methods to reduce costs, expedite the discovery of new medications, and maintain product quality. These are the

advantages of UPLC in addition to faster analysis. With the aid of UPLC, the utility of traditional HPLC may be improved and enhanced [4-7].

**Instrumentation:** A fully new system design must incorporate contemporary parts for the pump, auto sampler, detector, data system, and service diagnostics. The resolution of the ACQUITY UPLC system is not impaired when operating at low system and dwell volumes. Thanks to the use of UPLC, which also improved separation and spectral 6-7, more drug metabolites were found. There are several methods to go about developing a rapid LC method. Software for method development simulation, such as ACDTM 8, Dry LabTM 9, or Chromsword TM 10, can be used to optimise and simplify processes. By using such software, it is feasible to anticipate the ideal separation circumstances and enhance the knowledge gained from a small number of tests.

The ACQUITY UPLC BEH T M C18 and C18 Columns are recognised as the standard columns of preference for the majority of UPLC separations since they provide Dissolution testing. Impurity profiling in manufacturing, no. 7 maintaining computer libraries, The analysis of the food, nutrition, and cell culture media as well as the assessment of the protein's amino acid composition, Purification and analysis of oligonucleotides using C18 columns examining the circumstances of cell culture, food, and protein's amino acid composition, The UPLC-ELS detector was created for use in situations where analytes show weak or no UV and VISIBLE response or do not ionise by MS. UPLC method is commonly employed in the mapping of peptide, Biomolecules, natural products, antibiotics, and antivirals.

The ACQUITY UPLC BEH Shield R18 Columns' main function is to provide selectivity's that are compatible with the ACQUITY UPLC BEH T M C18 and C8 Columns. Between the silyl functionality and the phenyl ring, a trifunctional C6 alkyl ethyl is used in the phenyl columns from the ACQUITY UPLC BEH. Assay robustness is improved by using an amide phase that is trifunctionally bound and ACQUITY UPLC BEH Amide columns with extended column lifetimes. The use of BEH Amide columns in a wide range of phase pH [2 -11] enables the remarkable retention of polar analytes with a wide range of polarity, structural moiety, and Pka.[8-10]

**DETECTORS:** A tunable UV/Visible detector is used for UPLC detection. It makes use of cutting-edge hardware and firmware to handle Ethernet connections at high data speeds. Because they are concentration-sensitive detectors and must maintain concentration and signal, conventional absorbance-based optical detectors would require a smaller flow cell capacity for UPLC application. According to Beer's Law, conventional flow cells that are smaller will have a shorter route and a stronger signal.

As noise levels rise, transmission falls as a result of a shorter light path from a smaller cross-section. As a result, using a conventional HPLC flow cell would limit UPLC sensitivity.

The Precision The light-guided flow cell used in the tunable UV/Visible detector cell resembles an optical wire. Internal reflectance mode efficiently transmits light down the flow cell while maintaining a 10mm flow cell route length with a volume of only 500nL. Leak detectors are employed, which communicate with the software to alert users of any problems, while tubing and connectors are strategically distributed throughout the system to ensure minimal dispersion [11-14].

#### **UPLC BENEFITS:**

1. Shortens runtime while boosting sensitivity.
2. Offers the LC analysis's selectivity, sensitivity, and dynamic range.
3. Preserving resolution effectiveness.
4. Widens the use of multi-residue methods
5. Related and unrelated chemicals can be quantified rapidly because to UPLC's quick resolving power.
6. Use of a unique, very-fine-particle separation material allows for quicker analysis.
7. Operating expenses are decreased.
8. Less use of solvents
9. Shortens process cycle times, enabling the production of more products using already available resources.
10. Enables manufacturers to generate more material that consistently meets or exceeds the product requirements and boosts sample throughput, perhaps removing variability, failed batches, or the need to modify material.
11. Offers real-time analysis that is coordinated with manufacturing techniques. guarantees the ultimate product's quality, including testing before release.

**DISADVANTAGES:**

1. This type of column's lifespan is shortened due to greater pressure, which also necessitates additional maintenance. Using stationary phases with a size of around 2  $\mu$ m, performance comparable to or even greater has been shown so far without the negative consequences of high pressure.
2. In addition, the utilisation of phases smaller than 2  $\mu$ m is constrained since they are often non-regenerable.

**UPLC APPLICATIONS:**

1. Evaluation of natural goods and conventional herbal remedies
  2. Determination of the metabolite
  3. research into metabolomics and metabonomics.
  4. research on bioanalysis and bioequivalence
  5. Testing for dissolution.
  6. Manufacturing/QA/QC
  7. Impurity profiling, no.
  8. Upkeep of computer libraries
  9. The examination of cell culture medium, diet, and nutrition, as well as the determination of the amino acid content of proteins.
  10. Using C18 columns to purify and analyse oligonucleotides
  11. The examination of diet and nutrition, cell culture conditions, and amino acid content of proteins.
  12. Applications where analytes exhibit weak or no UV and VISIBLE response, or don't ionise by MS, have led to the development of the UPLC-ELS detector.
- Example: Biomolecules, natural products, antibiotics, and antivirals.
13. Peptide mapping frequently uses the UPLC method.
  14. Use of this method for separation and purification of polar analytes.

**An evaluation of UHPLC and HPLC techniques:**

Since both UHPLC and HPLC procedures use the same mechanism of separation, the principles guiding their optimization are the same. It is required to prove the equivalence of such procedures due to the use of smaller particle size fills, high mobile phase pressure values, and velocity. The differences in the test parameters used by the HPLC and UHPLC procedures to identify a heterocyclic drug in organic solvent extracts are shown in **Table 1**.

Table 1: Differences in the test parameters used by the HPLC and UHPLC

S.No	Parameters of Set	HPLC Method	UHPLC Method
1	Column	XTerra C18, 50 $\times$ 9.4 mm, 4 $\mu$ m particles	ACQUITY UPLC BEH C18, 50 $\times$ 9.2 mm, 1.7 $\mu$ m particles
2	Flow rate	3.0	0.6 mL/min
3	Needle wash	Methanol	Strong needle wash: 200IL Methanol. Weak Needle wash 600IL ACN:H <sub>2</sub> O (10:90)
4	Injection volume	20IL	3IL partial loop fill
5	Gradient (time in min) (CAN:H <sub>2</sub> O)	T0(25:75), T6.5 (25:75), T7.5(95:5), T9(25:75)	T0(36:64), T1.1(95:5), T1.3(36:64)
6	Total run time	10 min.	1.5 min.
7	Total solvent Consumption	Acetonitrile: 10.5ML, Water 21.0mL	Acetonitrile: 0.53ML, Water: 0.66ML
8	Plate count for API	2,000	7,500

A technique was used to go from a 10-min HPLC test to a 1-min UHPLC assay. UHPLC and HPLC have been used in several studies to examine pharmacological and biological matrixes. J. Jastreborca et al. looked studied the separation of folic acid derivatives using UHPLC and HPLC methods employing BEHC18/HSST3 and X bridge C18/Atlantis C18 columns, respectively. It was demonstrated that the UHPLC method had enhanced sensitivity, increased linearity, and adequate separation efficiency. Lower LOD values were reached, and the analysis's run-time was reduced by four times. Both methods required the use of gradient elution in order to detect a common antineoplastic agent in the presence of 14 contaminants in a pharmaceutical matrix, and equivalent validation parameters were reached. The UHPLC analysis took 34 minutes longer than HPLC. The Usefulness of UHPLC appears to be beneficial for drug determination in pharmaceutical, biological, and biopharmaceutical matrixes based on a review of the technology's analytical applications. **Table 2** provides illustrations of UHPLC detection techniques for pharmaceutical compounds in biological and pharmaceutical matrixes. The majority (73%) of publications published between 2008 and 2012 concentrated on using UHPLC techniques to research medicinal compounds in their dosage forms.

**Table 2: Illustrations of UHPLC detection techniques for pharmaceutical compounds in biological and pharmaceutical matrixes:**

S.No.	The aim of studies/material	Conditions of separations
1	<i>Pharmaceutical matrix, Analysis of active pharmaceutical ingredients in bulk substances, Simultaneously determination of drugs: Determination of b-blockers: Atenolol, pindolol, propranolol, alprenolol</i>	Stationary phase:BEH C18 (100mm 92.1mm, 1.71m); Mobile Phase: A solvent: 0.1%trifluoroacetic acid in water; B Solvent: 0.1%trifluoroacetic acid in acetonitrile. Flow rate: 0.5 MI/min. Tem. Column: ambient Injection volume: 5IL
2	The Determination of drug in the presence of its impurities: Determination of primaquine phosphate in presence of two impurities	Stationary phase:BEH C18 (50mm 92.1mm, 1.71m); Mobile Phase: 0.01%trifluoroacetic acid in acetonitrile. Flow rate: 1 MI/min. Tem. Column: 35°C Detector:UV Injection volume: 10IL

Due to its high sensitivity and resolution, the UHPLC method has been successful in identifying analytes in both pharmaceutical dosage forms and bulk compounds. Testing chemicals with similar chemical structures, such as analogues from the same therapeutic class, was one of the applications of UHPLC procedures. When related compounds, such as pollutants and degradation products, are present, these approaches can also be utilised. The following problems have been the centre of the most prevalent UHPLC applications in pharmaceutical analysis in recent years:

- Research on the stability of API in both its pharmaceutical and bulk forms, particularly when developing stability-indicating analytical techniques.
- Impurity profiles
- Studies on dissolution.

A broad adoption of UHPLC techniques might assist to address the issue of excessive time and solvent consumption while keeping adequate resolution and sensitivity since those fields of study require a substantial number of API measurements. The identification of metabolites and bioequivalence studies in biological fluids are two applications of the UHPLC techniques for API analysis in biological matrixes. In the end, it could be conceivable to quantify API at the lowest attainable concentration levels by combining a mass spectrometer with a UHPLC [15-25].

## Conclusions

The use of UHPLC in pharmaceutical analysis may be viewed as a greening pathway for liquid chromatography in light of the advantages highlighted in this study, which are essential for drug analysis in the pharmaceutical matrix. Because it uses less organic solvent and has lower analyte concentrations, UHPLC can also be used in stability studies when several determinations are needed. If operations using this kind of chromatography have been properly investigated, UPLC looks to have the potential to replace the less environmentally friendly analytical methods. By adding stationary and mobile phases, modifications to UHPLC technology are likely made to get rid of friction heating. While reducing run times and enhancing sensitivity for the analysis of diverse chemicals, a system that is properly developed holistically may provide considerably better resolution. Utilizing 1.7  $\mu$ m particles, UPLC is a tried-and-true technique. With UPLC, chromatography's value might be elevated and expanded.

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