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Recent Advancements in High-Performance Liquid Chromatography: A Comparative Approach

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Abstract

Modern separation science relies heavily on chromatography, which is used in all research institutes and pharmaceutical industries all around the globe. Of all the chromatography approaches, HPLC i.e. high performance liquid chromatography is the one that is most frequently used analytically. Recent improvements in chromatographic supports and liquid chromatography (LC) equipment have made rapid and incredibly effective separations possible. This summary emphasizes the key milestones and foundational advances in HPLC instrumentation during the last few decades. It provides an overview of recent advancements in HPLC, including as UFLC, RRLC, UPLC, and Nano LC, along with their uses. This article mainly contrasts recent developments in HPLC with regard to instrumental operational parameters such injection volume application, flow rate, and column temperature, as well as benefits of each approach over HPLC. Pharmaceutical and biological materials are assessed using HPLC technology, which is important for both quantitative and qualitative analysis. It is the most rapid, versatile, and secures chromatographic analysis technique known for drug component quality control. It is anticipated that the capabilities of High-Performance Liquid Chromatography (HPLC) would be further expanded by forthcoming improvements. Two significant advancements are the equipment's miniaturization, which will enable more efficient and compact systems, and its integration with mass spectrometry, which will increase the analytical capabilities of HPLC. Automation will streamline procedures, increasing speed and consistency, while improvements in stationary phase chemistry will boost separation performance. This review will help the readers to understand the role and significance of this pivotal analytical technique in biologicals and pharmaceutical quality control.

Keywords: Chromatography, HPLC, Applications, Advancements, RRLC, UPLC, UFLC, Nano Liquid Chromatography.

INTRODUCTION

Chromatography is a broad category of compound separation techniques. Column chromatography & liquid chromatography are two specialized techniques used in high-pressure liquid chromatography (HPLC), commonly referred to as high-performance liquid chromatography, to separate, characterize, and investigate the mixture's active ingredients. The current evaluation centers on HPLC technology, including a comprehensive analysis of its types, applications, measurements, structure, and improvements.

HPLC is essential for quantitative and qualitative analysis, playing an important role in the assessmentof chemical and pharmaceutical specimens. It is the fastest, most flexible, and safest chromatographic analytical technique, particularly when it comes to pharmaceutical ingredients quality control. The authors of this paper also hope

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to give a brief summary of current developments in the application of HPLC to analysis. The significance of HPLC in pharmaceutical & biological product quality control is examined in detail in this article. The groundbreaking research conducted in 1903 by Russian botanist Mikhail Tswett is credited with the development of chromatography. Mass exchange among a stationary phase & a mobile phase is a part of the separation process used in chromatography [1–5].

A simple idea to illustrate this process is determined by the Van Deemter equation, a formula that shows the connection between flow rate i.e. linear velocity & height of the plate (HETP or 1/column efficiency).

Van Deemter equation = A + B/μ+ C μ

Where,
A-Eddy diffusion
B-Longitudinal diffusion
C-Concentration
μ-Linear velocity [4].

Innovations in Technology

HPLC has the following differences compared to traditional methods, refer Figure 1.

- Rapid separation liquid chromatography (RRLC)
- Ultra-performance liquid chromatography (UPLC)
- Ultra-fast liquid chromatography Analysis (UFLC)
- Nano Liquid Chromatography (NANO LC) [2].

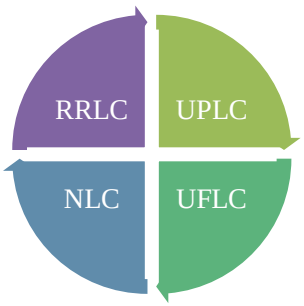

Figure 1. Recent Advancements in HPLC.

RAPID RESOLUTION LIQUID CHROMATOGRAPHY (RRLC)-

The RRLC system is intended to provide evaluation of the speed, resolution and pressure while reducing these problems. It's regarded as the fastest, most efficient and flexible liquid chromatography system in the world. Over time, it has shown to be a successful strategy. For achieving higher results, improving understanding and reducing costs [5].

In the pharmaceutical sector, this approach has been standard analytical methodology. That provides the best results, increases reproducibility, tolerance, and rapid detection and reduces measurement costs. It is especially beneficial for maintaining the therapeutic plants' quality. Since its inception in the early 1970s, HPLC using small particles has become popular. In order to further improve the conduct of the system, the connection between separation efficiency, phase's cell linear velocity & size has been studied in detail. These qualitative studies paved the way for the high-throughput, high-resolution HPLC systems known today [6]. However, this will result in a loss of theoretical plate count and therefore reduce the chromatographic resolution, especially for mixed products. To solve this problem, more columns have been loaded using small particles. Extended columns brimming with tiny particles provide better performance and resolution. With the emergence of RRLC technology, the analysis time

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can be shortened without affecting the chromatographic resolution, while keeping the same solution [7].

This is achieved using sub-2 micron column particle chemistry and high flow rates. Increased temperatures are frequently employed to reduce system back pressure. With the widespread use of RRLC, problems have arisen regarding its compatibility with HPLC detectors. The application of aerosol products with traditional HPLC & RRLC systems is covered in this review, along with time analysis costs and performance precision improvements. Refer Table 1 [8].

RRLC Application

- Wastewater is used as irrigation for RRLC-tandem mass spectrometry, which is used onshore to determine the presence of endocrine disrupting chemicals (EDCs) in items such silicone, salicylic acid, bendason, and pharmaceuticals as well as cleaning products (PPCP). It is employed in product standards and Rhodiola rosea root quality control.
- RRLC in addition to Triethylamine, is also suitable for estimation of acetyl aniline, acetylpropiophenone, octophenone, phenylhydrazine and other compounds.
- High-resolution reversed-phase HPLC separation of licorice extract on HT 1.8-μm chromatography column.
- MS determination of benzodiazepines in oral fluids is easily done.
- Use of liquid chromatography & fluorescence detection to achieve faster, higher and lower LOD bycomparing 1100 Series LC to 1200 Series of Rapid Release System [9].

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) –

UHPLC, which Jorgenson first used in 1997, is an acronym for ultra-high-pressure liquid chromatography. The usage of nano-columns filled with non-porous 1.0–1.5 μm silica-based particles on a prototype system capable of operating at very high pressures—up to 4100 bar in 1997 and 7200 bar in 2003—was first introduced by Jorgenson's group. The possibility of UHPLC for pressures up to 3600 bar was also confirmed by Lee et al. Expanding upon these ideas, Waters launched the first chromatographic system suitable for 1000 bar of pressure commercially in 2004 under the name ultra-performance liquid chromatography (UPLC). This technique used completely porous particles (FPPs) with a 1.7-μm diameter, packed into narrow-bore columns. The technology is better known by its chosen acronym, UHPLC, which stands for "ultra-high-performance liquid chromatography" or "ultra-high-pressure liquid chromatography." As an alternative, a few publications have made reference to very high-pressure liquid chromatography (VHPLC).

When UHPLC was first introduced to the market, it was mostly used to accomplish quick separations. For example, a nine-fold reduction during the analysis phase. With equal kinetic performance is possible when utilizing a UHPLC column of 50 x 2.1 mm I.D., 1.7 μm instead of a standard HPLC column of 150 x 4.6 mm I.D., 5 μm. Due to the massive number of samples, this high-throughput capability is attractive in the environmental, food, and chemical analysis fields where productivity needs to be improved. The pharmaceutical industry, particularly throughout the phases of drug research and development, such as quality control, pharmacokinetics, and drug metabolism, is another factor that propels quick separations. In addition to rapid separations, UHPLC has several other benefits [10].

Initially, during its commercialization, UHPLC was primarily employed for achieving rapid separations. For instance, using a UHPLC column of 50 x 2.1 mm I.D., 1.7 μm instead of a regular HPLC column of 150 x 4.6 mm Internal Diameter, 5 μm, allows for a ninefold reduction in analysis time with equivalent kinetic performance. This high-throughput capability is appealing in environmental, food, and chemical analysis where productivity needs enhancement due to an abundance of examples. Another motivating factor for swift separations is in the pharmaceutical field, especially during drug discovery and development processes, such as in quality control, pharmacokinetics, and drug metabolism. Beyond high-speed separations, UHPLC offers various other advantages [10]. Refer Table 1.

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UPLC has the following benefits over HPLC:

- Increases sensitivity while cutting down on analysis time.
- Maintains resolution performance while providing LC analysis's selectivity, sensitivity, and variability.
- Broadens the variety of applications for multi-residue techniques.
- Uses cutting-edge separation materials with extremely tiny particle sizes to achieve speedier analysis; Rapid resolving capability of UPLC facilitates speedy quantification of related and unrelated chemicals.
- Lowers solvent usage and operating costs.
- Reduces cycle durations in processes, enabling more production using current resources.
- Increases sample throughput, making it possible for producers to regularly produce more material that satisfies or exceeds product specifications. This could lead to the elimination of variability, failed batches, and the requirement for material rework.
- Offers real-time analysis in line with production procedures.
- Guarantees end-product quality, including testing for the final release [11].

UPLC applications

- *Drug Discovery:* Using combinatorial chemistry, high-throughput in vitro evaluation to ascertain drug physiochemical and pharmacokinetics, and high-throughput screening, UPLC enhances the drug discovery process.
- *High-throughput quantitative analysis:* metabolic stability experiments are performed using time-of-flight. Mass. spectrometry in conjunction with UPLC.
- *Analysis of Dosage Form:* By providing fast, precise, and repeatable findings during isocratic and gradient analyses of pharmaceuticals and their related chemicals, UPLC shortens the time needed to create new methods.
- *Amino Acid Analysis:* In the domains of protein analysis, cell culture evaluation, and nutritional evaluation of foods, UPLC is utilized for accurate, trustworthy, and repeatable amino acid analysis [12–15].
- *Pesticide Determination:* Triple Quadra-pole tandem mass spectroscopic technique in conjunction with pesticide traces in water can be identified more easily with the use of UPLC.
- *Analysis of Traditional Herbal Medicine and Natural Products:* UPLC is extensively employed in the examination of herbal medicines and natural products [16].
- *Dissolution testing:* During the manufacturing, research and development, and production phases of the drug-making process, dissolution testing is essential to quality control & release. From pill drop to the test start, UPLC provides accurate and dependable automated online sample gathering. It also manages test findings publication and distribution additionally to data collection and sample aliquot analysis.
- *Metabolite Identification:* By offering unparalleled sensitivity, resolution, variable range, and peak accuracy, UPLC/MS/MS meets the complex analytical needs of biomarker development.
- *ADME (Absorption, Distribution, Metabolism, & Excretion) Evaluation:* Precise peak identification and integration in intricate matrices are made possible by UPLC's high resolution. Peak identification for samples produced by lesser concentration incubation & specimen pooling is made possible by its increased sensitivity [17–18].
- *Bio-analysis and bioequivalency studies:* UPLC provides exceptional sensitivity and chromatographic resolution. The moderate detection level sensitivity and selectivity of this method yield accurate and reliable data that may be applied to a range of tasks, including statistical pharmacokinetics analysis. For bioequivalency laboratories, UPLC systems have been shown to increase production, profitability, and efficiency.
- The main goal is to assess drug samples that originate from the intricacy and sample-to-sample variability of the matrix. Excellent quality separates and detection abilities are provided by UPLC, making it possible to recognize active ingredients in incredibly complicated samples

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made from conventional herbal remedies and natural goods [19].

ULTRA FAST LIQUID. CHROMATOGRAPHY (UFLC)

With its unmatched speed and improved separation efficiency, UFLC is a revolutionary development in chromatographic procedures. The field of analytical chemistry has completely changed because to this ground-breaking method, which operates at over ten times faster rates and offers threefold improved separation compared to conventional LC techniques. UFLC minimizes departures from chromatographic theories by providing exceptional speed & separation even at typical pressure levels, making it a potent tool for a variety of applications. We examine the fundamentals, uses, and revolutionary effects of UFLC on the area of theliquid chromatography in this investigation. Refer Table 1.

Comparing Ultra-Fast Liquid Chromatography (UFLC) to standard High-Performance Liquid Chromatography (HPLC), there are a number of benefits:

1. *Enhanced Speed:* UFLC functions at a much faster rate than HPLC, enabling quicker analysis & lower run times. The efficiency and throughput of the laboratory are improved by this faster pace.
2. *Greater Resolution and Peak forms:* UFLC achieves greater resolution and peak forms by providing superior separation efficiency. As a result, the data for intricate sample analysis is more precise and trustworthy [20–22].
3. *Shorter Analysis Time:* UFLC's quicker operating speed results in a significant shortening of analysis time. This is especially helpful for labs that handle large volumes of samples, as prompt and effective analysis are essential.
4. *Improved Sensitivity:* UFLC frequently demonstrates enhanced sensitivity, making it possible to identify trace chemicals at reduced concentrations. This is useful in applications where it's critical to identify low-abundance analytes.
5. *Less Solvent Consumption:* Because UFLC has shorter run durations and higher efficiency than HPLC, it usually uses less solvent. As a result, expenses are reduced and a greener strategy is used.
6. *Enhanced Throughput:* In UFLC, increased effectiveness of separation and faster speed work together to enhance sample throughput. A greater number of samples may be processed by laboratories in a given amount of time.
7. *Reduced Variation from Chromatographic Principles:* UFLC reduces variations from chromatographic principles like the van Deemter equation by optimizing column and system performance.
8. As a result, the outcomes are more dependable and predictable. Compatibility with Current HPLC Instruments such as UFLC technologies are frequently made to work with current HPLC equipment, making it simpler to incorporate this cutting-edge technology into well-established laboratory setups [23, 24].

Uses for UFLC include

(a) *Iodiconazole determination in microdialysis:* Examples, after transdermal delivery, the strong antifungal drug Iodiconazole exhibits variable exposure and is employed to manage grave fungal infections. To provide a better understanding of medication distribution, Iodiconazole in cutaneous microdialysate is assessed through an assay by UFLC of Shimadzu with UV detection at 230 nm. The separation is carried out using a Shimadzu Prominence UFLC C18 column (2.2 micron, 50 mm x 2.0 mm i.d.). The solution is modified at a rate of 0.5 milliliters per minute using a pH 3.6 phosphoric acid (65:35, v/v) solution that contains acetonitrile and 0.025% tri-ethylamine [25].

(b) *Podophyllotoxin within Dermal & Blood Measurements Dialysis on a microscale Rat Samples:* To measure podophyllotoxin, micro-dialysis samples are put together by liquid-liquid. extraction with ethyl acetate and etoposide as an internal standard. The separation process uses the Agilent ZORBAX XDB-C18 column (2.1 mm x 50 mm, 3.5 micron) using an acetonitrile: ammonium acetate (10 mmol/L, 40:60, v/v) mobile phase flowed at 0.3 ml/min. Employing the electron spray

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ionization in positive mode, the UFLC-.MS/MS system functions in various reactions [26].

(c) *Analyzing Fluoroquinolones as well as the Xanthenes Derivatives in the serum simultaneously:* This method involves employing a molecularly etched polymer that was created using theophylline and ofloxacin as templates to selectively extract fluoroquinolones as well as xanthenes derivatives via human serum. Liquid chromatography and solid-phase dispersion of a matrix with molecular imprinting are used to perform simultaneous analysis.

(d) *Isoflavone Analysis in Soy:* Prominence UFLC is used in conjunction with the Shim-pack XR-ODS (75 mm L × 3.0 mm i.d) columns to analyze isoflavones in soy. The volume of the sample injection is 5 μL at 40°C, moreover, the mobile phase is made upby an aqueous solution of 0.1% formic acid & acetonitrile, it flows at a rate of 1.2 mL/min.

(e) *Examining Green Tea's Catechin Content:* A Shim-pack column XR-ODS having (50 mm L × 2.0 mm i.d) and Prominence UFLC are used to analyze the catechins inthe green tea. At 50°C, the sample's volume needed for injection is 2 μL, and mobile phase is made up by an aqueous solution of 0.1% formic acid and acetonitrile, pumped at a rate of 0.5 mL/min [27].

NLC-NANO LIQUID CHROMATOGRAPHY

Often known as nano LC, it is very sensitive and compact type, which is utilized for separation. and analysis of minute amounts of various chemicals.

Key information on Nano Liquid Chromatography is provided here. See Table 1.

1. *Reduced Scale:* Functions at a significantly smaller magnitude in contrast to traditional liquid chromatography. Usually employs column sizes in the nanoscale range, permitting the test of little sample volumes.
2. *Great Sensitivity:* It's very helpful for assessing sample having a tiny amount of target compounds because it provides excellent sensitivity & resolution in separation of complicated mixtures [28].
3. *Sample Volume:* Needs incredibly tiny sample quantities, frequently in the span of microliters or even nanoliters. Perfect for uses when sample availability is constrained.
4. *Column Technology:* Particle sizes and column dimensions are tuned for better chromatographic performance. Nano-scale columns filled with tiny particles are used to increase separation efficiency.
5. *Flow Rates:* Generally employs capillary columns with lower mobile phase flow rates; works at lower flow rates than conventional liquid chromatography.
6. *High Throughput:* Due of its efficiency and speed, nano LC can deliver high-throughput analysis even in small-scale operations.
7. Used in diverse range of life sciences applications, such as proteomics and metabolomics; useful for evaluating biological samples that are complicated and have an extensive variety of molecular weights [29, 30].
8. *Coupling with Mass Spectrometry:* This technique improves component identification and quantification by combining with mass spectroscopy (nano LC-MS). It also enhances detection, sensitivity and specificity.
9. *Mass Spectrometry Coupling:* This technique improves component identification and quantification by enhancing both specificity, & sensitivity in detection. It is frequently combined with mass. spectroscopy (nano LC-MS).
10. *Benefits:* Greater peak performance and resolution due to the smaller column size. Improved trace-level analytical sensitivity and detection limits.
11. *Difficulties:* Necessitates specific equipment and knowledge. Sensitive to changes in the experimental setup, requiring meticulous adjustment.
12. *Evolution:* Constantly changing in response to technological breakthroughs, viz. creation of novel column materials and enhanced instrumentation [31–33].

Uses for Nano LC

(a) *Sulfonamide Separation:* Eighteen sulfonamides were simultaneously determined by employing

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a combination of mass spectrometry (MS) and nano-liquid chromatography (NLC). For this, a 100 μm I.D. capillary column fitted with the Kinetex® carbon 18 stationary phase was utilized. Water as well as acetonitrile, with 0.1% (v/v).formic acid, was used as the binary. mobile phase in the gradient mode during the separation process, with a rate of flow of 190 nL/min [34].

(b) *Peptide Separation:* An immobilized, enzymatic reactor was used to create an integrated multivariate nano-flow LC system that included the online digestion of proteins and peptides. The proteome analysis demonstrated the efficacy of this technology [35].

(c) *Investigating Glycomics for Biomarker research Identification using Nano-LC:* Nano-LC, sometimes referred to as nanoflow LC, provides a very quantitative and sensitive method for separating and naming glycans. This skill is crucial in the search for putative biomarkers.

(d) *Glycobioanalysis using Nano-LC:* The structural characteristics of glycoconjugates & glycans inside biological matrices were investigated by means of Nano-LC. Stationary phases were designed for on-chip configurations and nanoflow conditions, including C18, graphitized carbon, including amide-based materials. This modification greatly increased the sensitiveness of structural analysis and produced a deeper level of data on complex glycan and glycoconjugate mixtures [36].

(e) Additional uses:

- Analyzing biologic and ecological specimens-mainly useful for managing tiny sample volumes, including newborn blood, CSF fluid, hormonal substances, digestive enzymes, & xenobiotics at nanoscale levels.
- When obtaining incredibly low limits of detection is critical, Nano-LC greatly helps high-throughput screening (HTS) & the discovery of drugs.
- Proteomic and genomic studies.
- Determining the drug residues that have collected in the human body [37].

Table 1. Comparing and contrasting NLC, UPLC, UFLC, RRLC, and HPLC [2].

Characteristics	HPLC	RRLC	UPLC	UFLC	Nano LC
Particle size	3 to 10 μ	1.8 μ	Less than 2u	1.7–2.2 μ	1.7–3 μ
Analytical column	XTerraC18, Alltima C18	ZORBAX Eclipse XDB-C18 RRHT	Acquity UPLCbeh C18, C8.rp	Shim-pack XR-ODS column	Capillary HPLC, Micro HPLC
Column dimensions (length × I.D)	150 × 2.1 mm	2.1-4.6 mm	150 × 3.2 mm	75 mm × 3.0 mm	125 mm × 0.05 mm–4.6 mm
Column temperature	300 C	Up to 1000 C	650 C	400 C	25–350 C
Injection volume	5 μL	1.5 μL	2 μL	0.1–100 μL	10 nL–100 μL
Flow rate	0.01–5 mL/min	0.2–20 μL/min	0.6 mL/min	3.7 nL/min	20–200 nL/min

SUMMARY AND DISCUSSION

Compared to traditional HPLC techniques, Rapid Resolution Liquid Chromatographic technique (RRLC) offers increased sensitivity, faster run times, and increased analytical efficiency. High sensitivity is where RRLC shines. It has an excellent repeatability and a low detection limit, a wide range of applications, and user-friendly features that make setup simple.

- As many scientists now find that traditional HPLC separations have limitations, UPLC presents a chance to increase and broaden the chromatography's range of uses.
- When performing high-speed separations in UPLC, columns with shorter lengths and/or smaller internal diameters are more likely to experience extra band-broadening outside the column.
- When compared to conventional HPLC methods, ultra-fast analysis yields a significant increase in productivity and sample throughput (5–10 times).
- The most recent development in separation science, nano LC allows for detections at the nanogram. or smaller levels.
- High-Performance Liquid Chromatography (HPLC) is set to undergo significant progress in the future. The downsizing of HPLC systems is one significant advancement that will lead to

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equipment that are more portable, efficient, and small. Furthermore, mass spectrometry's analytical capabilities will be expanded by combining HPLC, allowing for more thorough and accurate examinations. Improvements in stationary phase chemistry will improve the performance of separation, giving complex mixtures improved selectivity and resolution. Further streamlining HPLC procedures by automation will increase throughput, maintain uniformity, and minimize manual intervention. Last but not least, artificial intelligence (AI) is set to play a major role in driving HPLC to new heights of performance and utility in the future by optimizing chromatographic settings and enabling more intelligent, efficient data analysis and interpretation.

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