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# RECENT TRENDS IN HPLC TECHNIQUES FOR DRUG DEVELOPMENT REVIEW

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## ABSTRACT

Today chromatography is the backbone for analysis the compound and separation of the compound utilized worldwide in pharmaceutical companies and research labs. One type of chromatography is HPLC. techniques used for analysis Compound, safety, efficacy and impurities. Current trends towards the achievement of higher separation efficiency and shorter analysis time. In this review, a information about HPLC along with principles, introduction. HPLC have some disadvantages because of that have to use a fresh developments in HPLC. as Nano LC, RRLC, UPLC, and UFLC. Our primary focus in this review study is on RRLC, UPLC, UFLC, and Nano LC.

**KEYWORDS:** HPLC, RRLC, UPLC, UFLC, and Nano LC.

## INTRODUCTION

Drugs play important roles for curing disease. Drugs origin by synthetic semisynthetic and natural. They are used in pharmaceutical products or pharmaceutical formulations to show therapeutic effects and increase the pharmacokinetics action of drug. The pharmaceutical dose from are biological and chemical substances formulated into conventional doses forms such as solid are tablets and capsules, liquid are suspension and injectable, semi-solids are ointments. Hplc is used by analytical laboratories of pharmaceutical companies for analysis of bulk material drug materials, Product, impurities and degradation.[1] The pharmaceutical formulation are delivered of drug substance are should be stable, nontoxic and appropriate form, guaranteeing its therapeutic efficacy and bioavailability. Pharmaceutical items' efficacy, safety, and quality should all be considered. Pharmacological, toxicological, and adverse effects— which are primarily brought on by contaminants in bulk and dose forms—

are what determine a drug's safety. Unwanted pharmacological or toxicological consequences caused by drug contaminants may outweigh any potential benefits of administering the medication.[2] The qualities and safety are onitoring of pharmaceutical products and controlling impurities. The analytical activity can reduce impurities drugs and solve used in modern pharmaceutical analysis. This efficient technique is used for analysis procedures of pharmaceutical products.[3]

High-Performance Liquid Chromatography (HPLC) is an analytical method that has revolutionized chemical analysis and scientific research thanks to its accuracy and adaptability. HPLC is a highly accurate and efficient technique for detecting, measuring, and separating chemical components inside complicated mixtures. With its introduction, analytical chemistry underwent a dramatic change, giving scientists a powerful instrument to explore the molecular intricacies of diverse substances in a variety of domains and applications.[4-6]

Today, Chromatography is a backbone of the separation of compounds and analysis of the pharmaceutical products and used in research research laboratories and pharmaceutical industries of the world. This method used first time performed by Russian botanists tswett in (1903).[7] Compounds contained in a solution can be separated using HPLC, a type of liquid chromatography. The hallmark of HPLC is the use of high pressure to force a mobile phase solution through a stationary phase column, enabling high- resolution separations of complicated mixtures. The components of an HPLC instrument include a separation column, an injector pump, a mobile phase reservoir, as well as a decorator. A sample combination is injected into a column to separate the compounds. Because of the way they split between the stationary phase and the mobile phase, the various components in the mixture move through the column at differentiation. To stop air bubbles from forming, the mobile phase needs to be gassed. When compared to traditional column chromatography, the HPLC technology is known as better performance. Unlike traditional column chromatography, HPLC uses high pressure. The evolution of decreasing particle sizes is responsible for the development of HPLC from classical column chromatography. An HPLC instrument is made up of a detector, a column, a pump, and an injector. HPLC have a some disadvantage they are costly and complex to operate and they do not analysis the drug and accurate molecule and are not successful small molecule Because of that have use recent techniques analysis a sample.

**Principles**

the adsorption principle of separation in both normal and reverse phase modes. The way a mixture of components moves through an HPLC column depends on how well they bind to the stationary phase. The van Deemter equation is an empirical formula that describes the link between a chromatographic column's efficiency, which is commonly represented by the height equivalent to a theoretical plate (HETP), and the linear velocity (or flow rate) of the mobile phase. This equation helps in understanding how various factors contribute to the performance of a chromatographic separation.

The equation is usually written as.

$HETP=A+uB+C\cdot u$

Where

- The column's efficiency is indicated by its HETP (Height Equivalent to a Theoretical Plate). • The variables A, B, and C are unique to the system (mobile phase, stationary phase, and column design) that is being used. • u is the mobile phase's linear velocity.

**Core Concepts**

1. **A**: Represents the effects of eddy diffusion, which arises due to irregularities in the packing of the column, leading to variations in the flow path.
2. **B**: Reflects longitudinal diffusion of the solute in the mobile phase. At higher velocities, this term becomes less significant because the solute has less time to diffuse.
3. **C**: Corresponds to the mass transfer resistance between the mobile and stationary phases. As the flow rate increases, this resistance grows due to insufficient time for the solute to equilibrate between the two phases.

4. U:linear velocity[8,9]

The equation shows that there is an optimal flow rate that minimises HETP and maximises column efficiency. Both very slow and very fast flow rates lead to higher HETP values, causing decreased separation efficiency. Understanding this relationship is crucial for optimising flow rates in chromatography to achieve the best balance between speed and resolution.

**New modifications techniques in hplc**

Rapid Resolution Liquid Chromatography, Ultra Performance Liquid Chromatography, Ultra

Fast Liquid Chromatography, and Nano Liquid Chromatography are the hallmarks of HPLC's classical procedures.[10]

**RRLC (Rapid Resolution Liquid Chromatography)**

In order to minimize analysis time and maximize analysis speed, resolution, and pressure, the Rapid Resolution Liquid Chromatography (RRLC) system was created.[11] Because it provides advantages such superior peak forms, improved reproducibility, high sensitivity, quick detection, and reduced analytical costs, it has become a standard and crucial technology in the pharmaceutical business. This makes it very helpful for herbal medication quality control.[12]

In High Performance Liquid Chromatography (HPLC), separation efficiency and analysis time reduction have steadily improved. Further advancements have resulted from the growing adoption of HPLC with lower particle sizes.[12] Our current high-throughput, high-resolution HPLC systems are a result of research done in the 1970s that examined the link between separation efficiency, mobile phase velocity, and particle size.

Reducing column length helps shorten analysis time, but it can also result in a loss of theoretical plates, which in turn can reduce chromatographic resolution, especially for complex mixtures. Smaller particle sizes are employed to increase column efficiency in order to combat this. Higher efficiency and resolution are provided by longer columns filled with smaller particles. The most recent RRLC technology ensures high-quality findings even for complicated samples by drastically cutting down on analysis time without sacrificing chromatographic resolution.[8]

**Advantage over HPLC**

- The increase in throughput is the main high-throughput RRLC.
- The decrease in the cost of analysis.
- The adoption of a shorter column length with high resolution separation capability is what caused the reduction in analysis time.
- The fastest and most accurate analysis speed.
- The sensitivity is greatly enhanced.
- It provides genuinely quick LC analysis; on some columns, a high temperature of up to 100°C permits greater selectivity flexibility.[13]

**Applications of RRLC**

**Paeonlifiorin was quickly determined from paeonia sinjiang by K.Y. Pan[14]**

In traditional Chinese medicine, the root of Paeonia sinjiang K. Yaphank is used for its blood-cooling, blood-clearing, and blood-activating qualities. It is also used to cure carbuncles and encourage the absorption of abscesses. A quick and effective binary reverse-phase rapid resolution liquid chromatography (RRLC) technique has been created to measure the main active ingredient in Paeonia sinjiang K. Yaphank extracts, paeoniflorin. This technique allows for a quick analysis time by achieving separation utilizing an Agilent Zorbax XDB-C18 column (4.6 mm x 50 mm, 1.8 µm) with a mobile phase made up of methanol and 0.05 mol/L potassium phosphate mono-basic.

**Rhodiola rosea roots and commercially standardized products are subjected to rapid analysis for quality control.[15]**

In order to analyze the six bioactive compounds—salidroside, tyrosol, rosarin, rosavin, rosin, and rosiridin—found in Rhodiola rosea L. root and powder extracts, a straightforward, accurate, and trustworthy reversed-phase Rapid Resolution Liquid Chromatography (RRLC) method was created and validated. This technique employs a gradient mobile phase of acetonitrile and water in a Phenomenex C18HST column that is kept at 40°C. Dual UV detection is carried out at 205 and 254 nm, with the flow rate set at 1.0 mL/min. In less than eight minutes, the six compounds were successfully isolated with baseline resolution. The technique also made it possible to measure the amount of rosavins (rosarin, rosavin, and rosin) in real R. rosea roots and extracts, giving the roots a unique RRLC profile. The technique has been shown to be extremely sensitive and accurate.

**UPLC(Ultra performance liquid chromatography)**

The fundamental distinction between UPLC and HPLC is the design of the column material particle size, which is less than 2 µm 16.The following adjustments increase UPLCC's efficiency: By using high temperatures, the mobile phase's viscosity is decreased, which eventually results in a high flow rate. Back pressure is significantly lower.[16] The interconnecting skeletons and flow routes (through-pores) found in its monolithic columns are what make UPLC (Ultra Performance Liquid Chromatography) unique. which sets it apart from HPLC (High Performance Liquid Chromatography). These design elements contribute to superior resolution and separation in UPLC chromatograms compared to HPLC. Additionally, UPLC enables more sensitive analyses, lowers solvent consumption, and

provides faster analysis times 17-19.The particles size of UPLC less than 2µm and maximum pack pressure are 103.5Mpa and anyaltical column are should be Acquity UPLC BEH C18, column dimensions are 150 X 2.1mm,column temperature are 65c,injected volume are 2ml(std.in100%MeOH).[16]

**Advantage over HPLC**

- Use for particles smaller than 2 µm.
- Cost and time are decreased.
- Reduce run time and improve sensitivity.
- Less solvent is consumed. • Resolved peaks are found in the chromatogram.[16]
- Application of UPLC

**UPLC anylsis for cardiovascular drugs**

Cardiovascular medications and substances are used to treat heart and blood vessel diseases, blood clots, high and low blood pressure, heart failure, stroke, circulation problems, and other heart-related conditions. The patient will choose which of the numerous medications for different forms of cardiovascular disease to take.[20-23] Examples of medications used most frequently in cardiovascular medicine include anticoagulants (e.g., warfarin, heparin, etc.), antiplatelet medications (e.g., lopidogrel and clopidogrel), angiotensin receptor blockers (ARBs and sartan), beta-blockers (e.g., bisoprolol and sotalol), calcium channel blockers (e.g., amlodipine and diltiazem), diuretics (e.g., chlorothiazide and furosemide), vasodilators (e.g., isosorbide and hydralazine), digoxin used to slow the heart rate, and other medications used to control abnormal heart rhythms, such as quinidine, lidocaine, amiodarone, and adenosine. Valsartan Hydrochlorothiazyd is one of the active ingredients in some cardiovascular medications that are analyzed using the UPLC technique. The column is Kromasil Eternity C-18 (50 × 2.1 mm, 3.5 µm), and the mobile phase is A-methanol; B-0.1% triethylamine pH3; A:B (75:25, v/v); the flow rate is 0.6 mL/min, and the detection wavelength is uv235 nm.[24] Lodenafil and BEH C18 (50 × 2.1 mm, 1.7 µm) are the additional active ingredients. The mobile phase consists of A-methanol; B-0.1% formic acid pH4; A:B (55:45, v/ v); the flow rate is 0.4 mL/min; and the detection is done in MS.[25]

**UPLC anylsis for NSAIDs**

Because of their wide availability and variety of effects, NSAIDs are utilized all over the world. They are typically administered to manage inflammation, fever, and pain. They are frequently used to alleviate the symptoms of a variety of chronic pain conditions, including

headaches, toothaches, painful periods, sprains, colds, flu, and arthritis.[26-29] The uplc approach uses a column called BEH C18 (50 × 2.1 mm, 1.7 µm) and active ingredients like diclofenac in various NSAID medications. A-methanol, B-phosphoric acid, pH2.5 A:B (65:35, v/v), and BEH C18 (100 × 2.1 mm, 1.7 µm) are the mobile phase and flow rate, respectively, of 0.4 or 0.45 mL/min at UV 254 nm[30] A-0.1% triethylamine buffer pH3.2 with phosphoric acid, acetonitrile (80:20, v/v) and B-0.1% triethylamine buffer pH3.2 with phosphoric acid, acetonitrile (50:50, v/ v) are the mobile phases; gradients are 0/0, 7.5/50, 17/50, 17.5/0, and 20/0; flow rate is 0.4 mL/min; and the UV detection is 220 nm.[31] A few other medications include Ibuprofen diphenhydramine as their active ingredients.

**UFLC(ULTRA FAST LIQUID CHROMATOGRAPHY)**

A Shimadzu Model CBM-20A/20 Alite UFLC system (Shimadzu Co., Kyoto, Japan) fitted with an SPD M20A prominence photodiode array detector was used to validate a novel RP-UFLC technique for the quantification of flucytosine in its capsule formulation. A C8 (2) 100A (Luna) column (250 mm × 4.60 mm i.d., 5 µm particle size) kept at room temperature was used for the analysis. To determine flucytosine, a mobile phase comprising 50:50 (v/v) tetrabutylammonium hydrogen sulfate and acetonitrile was employed at a flow rate of 1 mL/min with UV detection at 215 nm. With a regression equation of y = 107100x – 5776.2 (r2 = 0.9999), flucytosine demonstrated linearity across the concentration range of 0.1–120 µg/mL, and the system satisfied all validation requirements. The limit of detection (LOD) was found to be 0.1786 µg/mL, and the limit of quantification (LOQ) was 0.5419 µg/mL.[32]

**Advantage over hplc**

- Compared to a standard LC system, cut the analysis time by 75%.
- Better performance in terms of separation.[32]

**Application of UFLC**

**Iodiconazole determination in microdialysis samples[33]**

Iodiconazole is a highly effective anti fungal agent employed for the treatment of severe fungal infections. Upon transdermal application, its exposure is influenced by various factors, leading to considerable variability and necessitating further investigation into its distribution. Using an ultra-fast liquid chromatography (UFLC) approach with UV detection at 230 nm, iodiconazole in cutaneous microdialysate has been measured. A Shimadzu Prominence UFLC C18 column (2.2 micron, 50 mm x 2.0 mm i.d.) was used for the iodiconazole separation. A mobile phase consisting of acetonitrile and 0.025% triethylamine solution was used, with

phosphoric acid adjusted to pH 3.6 in a 65:35 (v/v) ratio, and the flow rate was set at 0.5 ml/min.

- **Rats' skin and blood microdialysis samples were examined for podophyllotoxin**[34]

Ethyl acetate was used for liquid-liquid extraction of micro-dialysis samples, with etoposide acting as the internal standard (IS). On an Agilent ZORBAX XDB-C18 column (2.1 mm x 50 mm, 3.5 micron), podophyllotoxin was successfully separated. Acetonitrile and 10 mM ammonium acetate (40:60, v/v) made up the mobile phase. The analysis was carried out at room temperature with a flow rate of 0.3 ml/min. Using positive ion electrospray ionization (ESI) for detection, the UFLC-MS/MS system was run in multiple reaction monitoring (MRM) mode.

**Nano liquid chromatography**

The concept of stereoisomerism and its impact on the biological activity of compounds in living organisms was first introduced in 1948 through Louis Pasteur's discovery of the optical activity of tartaric acid. Enantiomers, despite having identical physico-chemical properties in an achiral environment, can cause differing biological effects due to their distinct optical activities. This has led to growing interest in the study of enantiomers across various fields, including pharmaceuticals, biomedical sciences, agrochemicals, and food industries. In recent times, the focus has shifted from the challenging process of stereospecific synthesis for the production of a single enantiomer to the more complex task of separating racemic mixtures using advanced analytical techniques. This review aims to provide a current overview of the methods used for separating chiral compounds via nano and capillary liquid chromatography (LC). The article is divided into two sections: the first outlines the fundamentals of nano-LC, including its theoretical aspects and experimental setup, and also discusses the separation of enantiomers, chiral selectors, and chiral stationary phases. The second section highlights recent applications, demonstrating the significant potential of nano-LC in enantiomeric separation.[35]

**Advantage over hplc**

- significantly lowers the amount of mobile phase used and the waste that is produced as a result.
- reduces internal diameter, which increases sensitivity and/or requires less sample.
- is significantly faster and less expensive than its conventional counterpart.
- and improves detection sensitivity in MS due to lower flow rates in smaller columns.[36]

**Applications of Nano LC Separation of sulfonamides**[37]

Using a capillary column with an inner diameter of 100 µm and a Kinetex® C18 core-shell stationary phase, 18 sulfonamides were determined by nano- liquid chromatography. At a low flow rate of 190 nL/min, a gradient mode binary mobile phase comprising acetonitrile and water with 0.1% (v/v) formic acid was employed.

**Separation of peptide**[38]

Using an immobilized enzymatic reactor, a multidimensional nano-flow liquid chromatography platform was created that combined online digestion with protein and peptide separation. Proteome analysis was successfully conducted using this technique.

## CONCLUSION

When compared to conventional HPLC techniques, RRLC provides improved sensitivity and quicker run times. It offers great consistency, wide applicability, low detection limits, high sensitivity, and an easy-to-use setup. Since many researchers find that traditional HPLC has limitations in terms of separation, UPLC provides a means of expanding and expanding the capabilities of chromatography. High-speed separation in UPLC may be impacted by extra-column band broadening, which is more likely to occur in columns with shorter lengths or smaller internal diameters. Compared to traditional HPLC, ultra-fast analysis significantly increases productivity and sample throughput (5–10 times). The most recent development in separation technology, nano LC, enables detection at nanogram or even lower levels.

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