IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, June 2024

A Review on UPLC: A Prominent Analytical Technique for Pharmaceuticals.

Ms. Rohini B. Chougale[1], Mr. Chaitanya V. Pawar[2], Mr. Satyam G. Apare[3], Ms. Wale R. R.[4], Ms. Ghavane. A. R.[5]
Department of Pharmaceutical Chemistry
Channabasweshwar Pharmacy College, Latur, SRTM University, Nanded, India[1-5]

Abstract: *Today's pharmaceutical industry and analytical laboratories are constantly looking for innovative solutions to save costs, shorten drug analysis and improve product quality. Ultra-performance liquid chromatography is one innovative method of using liquid chromatography. By using particles smaller than 2 µm, UPLC improves three characteristics of liquid chromatography, namely speed, sensitivity and analytical resolution. The mechanism can withstand high back pressure. UPLC separation consumes up to 100 MPa, but does not affect the analytical column or other components of the chromatography system. UPLC requires less time and less solvent thanHPLC. Using high-performance liquid chromatography, improved resolution and sensitivity can be achieved through particle chemistry, system optimization and data processing. Using particles smaller than 2 mm at higher linear velocities and equipment operating at higher pressures than HPLC can greatly improve resolution, sensitivity and speed of analysis. This new branch of analytical and separation science preserves the value and concepts of HPLC by providing a step-by-step function of chromatographic efficiency. This review focuses on the basic theory, instruments and principles of UPLC. This review explains UPLC chromatography technology along with the latest research in the field.*

Keywords: HPLC, high resolution, resolution, sensitivity, UPLC

I. INTRODUCTION

Chromatography is a method for separating the components of a mixture, or solutes, based on how equally each solute is distributed among the mobile phase, or solid phase, adjacent to the moving liquid stream. While the stationary phase can be either a solid or a liquid, the mobile phase can be either a liquid or a gas. Factors affecting this separation are molecular properties related to adsorption, affinity and partitioning, as well as variations in their molecular weights . As a result, certain components of the mixture pass quickly through the mobile phase and leave the chromatographic system, while others pass slowly through the stationary phase and spend more time there. Based on this methodology, the chromatography technique consists of three elements.

- Solid phase: In this phase, there is always a solid phase or liquid layer that is absorbedon the surface of the solid support.
- Separated molecules.
- Mobile phase: This phase usually contains a liquid or gas component .

An important liquid chromatography (LC) method to separate the different components of mixtures is high-performance liquid chromatography. It has been used for years to quantify and identify compounds in the drug process worldwide. Column technology (column size and column particle size) and instrumentation have advanced significantly to further improve the sensitivity, resolution, and speed of LC. To achieve the above goals, Waters developed and patented Ultra Performance Liquid Chromatography (UPLC) in 2004. UPLC is based on small porous particles (sub2 micron particles). The theory behind this development that relates the relationship between plate height and linear velocity is the Van Deemter equation. Since conventional HPLCs have an upper pressure limit of about 6,000 psi, small particles require high pressure to work with UPLC. It has been shown that the efficiency increases significantly when the particle size is reduced below 2.5 µm, and that this effect does not decrease when the flow rate or linear velocity is increased. Using speed, small radius particles, and the highest number of resolvable peaks (peak power) combine

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, June 2024

efficiency and resolution. Compared toHPLC, this approach uses less mobile phase volume and has a run time of about 1.5 minutes. The separation retention factor can only be increased with smaller particles by increasing the pressure to 1000 bar or more. UPLC requires a smaller injection volume, which increases both efficiency and resolution. The lower mobile phase viscosity resulting from the higher column temperature results in higher flow rates and higher diffusion coefficients without suffering significant efficiency losses and column back pressure increases. Recently developed techniques in system optimization, particle chemical efficiency, data processing, detector control and design can be reflected in UPLC, a variant of HPLC . This development greatly improves resolution, sensitivity and efficiency, and speeds up.

Principle

The basic principle of UPLC is based on the van Deemeter relationship, which explains the relationship between flow rate and plate height. The Van Demeter equation (i) shows that the flow area with smaller particles is much larger for good results compared to larger particles.

$$H = A\ B/V + CV$$

If H represents the height corresponding to the theoretical plate (HETP), A, B and C are constants and v is the flow rate (linear velocity) of the carrier gas. The goal is to minimize HETP to improve column efficiency. The term A is independent of velocity and represents eddy mixing. It is less if the columns are packed with small and uniform items. The expression B refers to the natural diffusion tendency of the particles. At high flow rates this effect is less, so that expression is divided by v. The term C represents the kinetic resistance to equilibrium during the separation process. Kinetic resistance is the time delay associated with the transition from the mobile phase to the stationary phase and back. The higher the flow rate of the mobile phase, the more the molecules on the packing material tend to lag behind the molecules of the mobile phase. Thus, this term is inversely proportional to the linear velocity. Thus, it is likely to improve the throughput and the separation can be accelerated without affecting the chromatographic efficiency. The advent of UPLC necessitated improvements to existing LC equipment that utilize resolution (reducing dead volume) and constant pressure (around 500-1000 bars compared to 170-350 bars in HPLC). Efficiency is proportional to column length and inversely proportional to particle radius (Jorgenson et al., 1997). Thus, the column length can be shortened by the same factor as the particle radius without affecting the resolution. The use of UPLC helped identify drug metabolites and improve the quality of separation spectra.

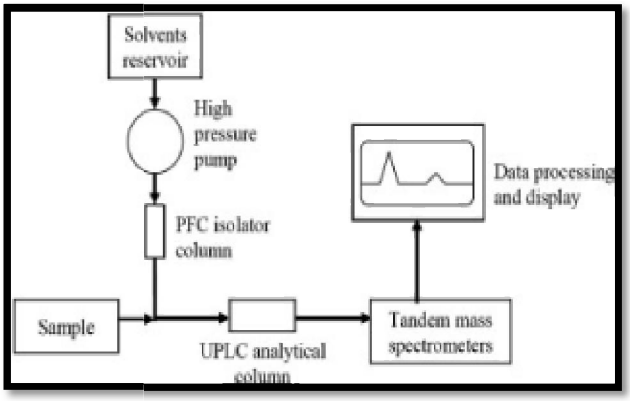
Equipment

Ultra Performance Liquid Chromatography has the ability to operate more efficiently at higher speed, sensitivity and resolution in a much wider range of linear velocity, flow rate and back pressure to achieve excellent results. The Acquit UPLC system consists of:

- Binary Solvent Manager
- Sample Manager containing a column heater
- Optional sampler
- Pump

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, June 2024

- Detector



1. Binary Solvent Manager: Binary Solvent Manager uses two individual series flow pumps for binary gradient to create parallel. The binary solvent control is a high-pressure pump that moves the solvent through the system. This provides a steady (pulseless) solvent flow at analytical flow rates. The binary solvent controller delivers solvent at a flow rate of 1 mL/min at 103421 Kpa [1034 bar, 1500 psi] and up to 2 mL/min at reduced pressures up to 621 bar, 9000 psF. The solvent manager can pump two solvents simultaneously.

2. Sample management: Akuity sample management injects a sample taken from microtiter plates or vials into the chromatography flow stream. The positioning mechanism uses a probe to access and sample the sample sites. The sample manager can complete the injection in about 15 seconds. The sample control also controls the column heater. The temperature of the column can reach up to 65 °C.

3. Column Heater: The column heater has a modular design and footprint identical to the sample controller. So it attaches to the top of the sample guide and acts as the top cover for that instrument.

4. Optional Sample: Organizer The optional sample organizer stores microwells or vials and transfers them to and from sample management, automating their handling and increasing production.

5. Columns : Submodule Design and Development 2 µm particles is a significant challenge, and researchers have been very active in this area, not exploiting its advantages. Although high-performance non-porous 1.5 µm particles are commercially available, they suffer from low surface area, resulting in poor loading capacity and storage time. To maintain retention and capacity similar to HPLC, UPLC must use a new porous particle that can withstand high pressure. Silica-based particles have good mechanical strength, but have several disadvantages. These include the exclusion of basic analytes and a limited pH range. Another option, the polymer column, can outperform pH mimics but has its own problems, including low efficiency and limited capacity. In 2000, Waters introduced a first-generation hybrid chemistry called XTerra, which combines the favorable characteristics of both silica and polymer columns - they are mechanically strong, efficient and long-lasting pH range. XTerra columns are manufactured using the classic sol-gel synthesis, which contains carbon in the form of methyl groups. However, to improve the mechanical stability required by UPLC, a second generation hybrid technology was developed called ACQUITY UPLC.ACQUITY 1.7 µm particles bridge the methyl groups of the silica matrix improving their mechanical stability. Due to better efficiency, development is increased in a column packed with 1.7 µm particles. Separation of sample components requires a binding phase that provides both retention and selectivity. Four bonded phases are available forUPLC separations:

A) ACQUITY UPLC BEH T M C18 and C8 (straight chain alkyl columns)

B) ACQUITY UPLC BEH Shield RP 18 (immersion polar column)

C) ACQUITY UPLC UPLC PHENYL- coupled silyl functionality C6 - with alkyl)

D) ACQUITY UPLC BEH Amide Columns (Trifunctionally Bonded Amide Phase)

(A) ACQUITY UPLC BEH T M C18 and C8 Columns –:

These are considered the most common columns for most UPLC separations, providing the widest pH range. They have a trifunctional bonding chemistry that provides excellent low pH stability. This low pH stability is combined with the high pH stability of the 1.7 µm BEH particle to provide the widest usable pH range.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, June 2024

(B) ACQUITY UPLC BEH SHIELD R18 Columns -These are designed to provide selectivity that complements the ACQUITY UPLC BEH. T M C18 and C8 columns.
(C) ACQUITY UPLC BEH Phenyl Columns -These use a trifunctional C6 alkylethyl between the phenyl ring and silyl function.
(D) ACQUITY UPLC BEH Amide ColumnsBEH particle technology combined with trifunctional particle columns. bound amide phase provides exceptional column lifedum improving the endurance of the analysis. BEH amide columns allow the use of a wide phase-pH range [2-11] tofacilitate\unexceptional retention of polar analytes covering a wide range of polarity, structural part and pKa.

Pump:
The main advantages of pumps are: higher resolution, faster analyzes and higher sample loading capacity. PumpModule – types

- Isocratic pump - gives the content of the mobile phase composition; the solvent must be premixed
- Gradient pump - deliver variable mobile phase composition UPLC typically uses scattering (ELS), refractive index (RI) and fluorescence (FLR). In addition to these detectors, UPLC capabilities can be greatly increased by coupling the instrument with other techniques such as a mass spectrometer, ion chromatograph, nuclear magnetic resonance spectrometer, inductively coupled plasma mass spectrometer, and infrared spectrometer.

Detectors:
Ultraviolet/Visible (UV)
This detector is used for organic compounds that absorb light at wavelengths 190-800 nm. This detector can be configured to detect specific wavelengths in the UV or visible region. It offers performance advantages for both routine and complex analyzes in pharmaceutical, life science, environmental, agricultural and petrochemical applications.
PDA Detector
This detector offers simultaneous advanced optical detection in the 190-800 nm range. It offers unprecedented trace/impurity detection and quantification with spectrum analysis capabilities.
Fluorescence detector (FLR)
This detector is used to determine the sensitivity and selectivity of fluorescence applications. This extends the benefits of UPLC technology to the analysis of polynuclear aromatic hydrocarbons (PAHs), drugs of abuse, and vitamins - any component with chemiluminescent properties, such as fluorescence or phosphorescence.
Refractive Index Indicator (RI)
RI is a general indicator used when a chemical has no or limited UV absorption. These include alcohols, sugars, fatty acids, excipients, raw materials and drugs. In addition to characterizing these low molecular weight polymers, UPLC is also used. The main disadvantage of this detector is its sensitivity.
Mass Detector (MS)
UPLC can be combined with mass spectrometer (MS) and tandem mass spectrometer (MS-MS) detector, which are used in many fields and for material identification, quantification and mass analysis. Structural elucidation of an unknown molecule can also be determined by fragmentation. This detector has different mass analyzers depending on their application, some analyzers are single quadrupole, triple quadrupole (Tandem), ion trap and time of flight (TOF). These detectors offer very high sensitivity, selectivity and time resolution. In addition to these detectors, many other detectors such as Infrared (IR), Inductive Coupled Plasma Mass Spectrometry (ICP-MS), Nuclear Magnetic Resonance (NMR), and Evaporative Light Scattering Detector (ELSD), an electrochemical detector, can be connectedto the UPLC.

ADVANTAGES
The advantages of UPLC are:

- Reduces run time and increases sensitivity.
- Provides selectivity, sensitivity and dynamic range for LC analysis.
- Maintains resolution.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, June 2024

- Extends the multiresidue method.
- UPLC rapid resolution quickly identifies bound and unbound compounds.
- Faster analysis using new ultra-fine separation material.
- Lower operating costs.
- Less solvent consumption.
- Shortens process cycle times so more products can be done with available resources.
- Increases sampling capacity and enables manufacturers to produce more materials that consistently meet or exceed product specifications, potentially eliminating variations, failed batches, or the need for material rework.
- Provide real-time analysis in terms of production processes. Ensure final product quality, including final release testing.

Disadvantages

- The major disadvantage of UPLC is the higher back pressure compared to conventional HPLC, which shortens the life of the columns.
- Increasing the column temperature reduces the problem of UPLC back pressure.
- In addition, the particles are more small above 2 µm are largely non-recoverable and thus have a narrow target.
- Mixing cost
- Solvent pumping
- Lack of choice of commercial columns 1.7 µm9.

APPLICATION

- Drug discovery.
- UPLC improves the drug discovery process by using high-throughput screening, combinatorial chemistry, and high-throughput in vitro screening to determine the physicochemistry and pharmacokinetics of drugs.
- High-throughput quantitative analysis.
- UPLC combined with time-of-flight mass spectroscopy enables the determination of metabolic stability.
- Dosage form analysis.
- It provides high speed, accuracy, and reproducible results for isocratic and gradient analysis of drugs and related substances. Thus, the method development time is reduced.
- Analysis of amino acids.
- UPLC is used for accurate, reliable, and reproducible analysis of amino acids in the fields of protein characterization, cell culture monitoring, and nutritional analysis of foods.
- Determination of Pesticides.
- UPLC combines triple quadrupole and tandem mass spectroscopy to help detect residual pesticides\in water.
- Ultra-pressure liquid chromatography thus sets a new standard in chromatographic science. An operating range of 15,000 to 16,000 psi pressure and a column size of less than 2 micrometers helped in various areas.
- Analysis of Natural Products and Traditional Herbal Medicine.
- UPLC is widely used for the analysis of natural products and herbal medicines. Its main purpose is to analyze drug samples due to matrix complexity and sample variation. UPLC provides high-quality separation and detection capabilities for the identification of active compounds in highly complex samples derived from natural products and traditional herbal medicines.
- Metabolite Identification.
- UPLC/MS/MS meets the complex analytical requirements of biomarker discovery by providing unparalleled sensitivity, resolution, dynamic range and mass accuracy.
- ADME (Absorption, Distribution, Metabolism, Excretion) screening.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, June 2024

- UPLC high resolution allows accurate detection and integration of peaks in complex matrices, and additional sensitivity allows peak detection in samples produced by lower concentration incubations and sample pooling.
- UPLC/MS/MS offers the following advantage: - UPLC can more than double yield. without losing method robustness. UPLC is also simpler and more robust than the step separation sometimes used with HPLC methods.
- UPLC with rapid universal gradients increases analytical efficiency and sensitivity in high-throughput pharmacokinetic or bioanalytical studies, including rapid potency measurement. p450 inhibition, induction and drug-drug interactions.
- Bioanalytical / Bioequivalence studies
- UPLC offers excellent chromatographic resolution and sensitivity. The sensitivity and selectivity of UPLC at low detection levels provides accurate and reliable data that can be used for a variety of purposes, includingstatistical pharmacokinetic analysis. UPLC solutions are proven to increase efficiency, productivity and profitability in bioequivalence laboratories.
- Dissolution Testing
- Regarding quality control and drug release manufacturing, dissolution testing is essential in the formulation, development and manufacturing. UPLC offers accurate and reliable automated network sampling. It automates dissolution testing from pill play to test initiation, collecting data and analyzing sample sets to manage the publication and distribution of testresults.

Table 1: Comparison of UPLC and HPLC

CHARACTERICTICS	HPLC	UPLC
Particle Size	3 to 5µm	Less than 2µm
Maximum Back Pressure	35-40 Mpa	103.5MPa
Analytical Column	Alltima C18	Acquity UPLC BEH C18
Column Dimensions	150X3.2mm	150 X 2.1mm
Column Temperature	30ºC	65ºC
Injection Volume	5µl(Std in 100% MeOH)	2µl (Std in 100% MeOH)

Table 2: METHOD DEVELOPMENT FASTER WITH UPLC (Time Saving)

	UPLC Gradient Conditions				Equivalent HPLC Gradient Conditions			
Column Conditions	2.1X50mm				2.1X50mm			
Particle Size	1.7µm				5µm			
Flow Rate	0.5ml/min				1.0ml/min			
Gradient	Time (min)	Profile %A	Profile %B	Profile	Time (min)	Profile %A	Profile %B	Profile
	0.0	95	5	6	0.0	95	5	6
	5.0	10	90	6	35.0	10	90	6
Peak Capacity	150				150			

II. SUMMARY

UPLC increases productivity in both chemistry and instrumentation by providing more 6information per unit of work because it improves the resolution, speed, and sensitivity of liquid chromatography. The main advantage of UPLC is the reduction time. Which also reduces solvent consumption high sensitivity for low-concentration component analysis. Since the solvent consumption of this technique is lower, it also reduces the analysis cost. In this system, the method can be well developed and validated in less time. UPLC finds diverse applications in pharmaceutical research, high-throughput quantitative analysis, amino acid analysis, metabolite detection, dissolution testing, and bioanalysis/bioequivalence studies.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 1, June 2024

One of the most useful techniques in analytical chemistry is ultra-performance liquid chromatography (UPLC), which improves chromatographic analysis in terms of speed, resolution, and sensitivity while reducing the amount of time and solvent needed. UPLC generates peaks with lower background noise and a higher signal-to-noise ratio. The pharmaceutical business can benefit much from the UPLC technique, and it is also the preferred method. This instrument will show to be indispensable in boosting both the precision of pharmaceutical analysis and the efficiency of scientists conducting such analysis. It's a crucial technique for creating and evaluating methods in the lab, as it saves money and maximizes efficiency. With UPLC, the separation can be completed more quickly and more efficiently, leading to the rapid development of techniques that open up a new window of opportunity for business profitability and facilitate the product's quicker introduction to the market.

REFERENCES

[1]. Cuatrecasas P, Wilchek M, Anfinsen CB. Selective enzyme purification by affinity chromatography. Proceedings of the National Academy of Sciences. 1968 Oct;61(2):636-43.
[2]. Porath J. From gel filtration to adsorptive size exclusion. Journal of protein chemistry. 1997 Jul;16(5):463-8.
[3]. Harris DC. Exploring chemical analysis. 3rd ed,WH. Freeman&Co 2004
[4]. Khan H, Ali J. UHPLC: Applications in pharmaceutical analysis. Asian J. Pharm. Ana. 2017 May;7(2):124-31.
[5]. Wu N, Lippert JA and Lee ML. J. Chromatogr., A, 2001, 1-12,911.
[6]. Unger KK, Kumar D, Grun M, Buchel G, Ludtke S, Adam Th, Scumacher K and Renker S. J. Chromatogr., A, 892(47),2000, 56-80.
[7]. LCGC: Solution for Seperation Scientist.
[8]. Nguyen, D.T.; Guillarme, D.; Rudaz, S.; Veuthey, J.L. Fast analysis in liquid chromatography using small particle sizeand high pressure. J. Sep. Sci., 2006, 29(12), 1836-1848.
[9]. Swartz, M.E. Ultra performance liquid chromatography (UPLC): An introduction, separation science re-defined. LCGCSuppl, 2005, 8, 8-14.
[10]. Jerkovich, A.D.; Mellors, J.S.; Jorgenson, J.W. Uplc: An Sensitive and High Throughput Analysis Over HPLC. LCGC,2003, 21(7), 600-610.
[11]. MacNair, J.E.; Lewis, K.C.; Jorgenson, J.W. Ultrahigh-pressure reversed-phase liquid chromatography in packedcapillary columns. Anal. Chem., 1997, 69(6), 983-989.
[12]. Beattie, K.; Joncour, J.S.; Lawson, , K. Ultra-performance liquid chromatography coupled to orthogonal quadrupoleTOF-MS (MS) for metabolite identification. LC GC North America, 2005, 22- 30.
[13]. Wang, W.; Wang, S.; Tan, S.; Wen, M.; Qian, Y.; Zeng, X.; Guo, Y.; Yu, C. Detection of urine metabolites in polycysticovary syndrome by UPLC triple-TOF-MS Clin. Chim.Acta, 2015, 448, 39-47
[14]. McLoughlin DA, Olah TV, Gilbert JD: J. Pharm. Biomed. Anal, 1997; 15: 1893- 1901.
[15]. Tanaka N. et al. Anal. Chem., 73, 2001, 420A-429A13. Wu N. et al. Anal. Chim. Acta., 523, 2004, 149-156.
[16]. Jerkovitch AD, Mellors JS, Jorgenson JW: LCGC 2003; 21(7): 600-610.
[17]. Dao T. Nguyen, Davy Guillarme, Serge Rudaz, Jean-Luc Veuthey: Fast analysis in liquid chromatography using smallparticle size and high pressure. Journal of Separation Science 2006; 29(12): 1836–1848.
[18]. Wyndham KD, et al.: Anal. chem. 2003; 75: 6781-6788.
[19]. Swartz ME.: Ultra Performance Liquid Chromatography (UPLC): An Introduction, Seperation Science Re-Defined,LCGC Supplement, 2005: 11.