IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

Priyanka K. Patel[1*], Vaishali V. Karkhanis[1], Janvi P. Chauhan[1], Hina L. Bagada[1],
Department of Pharmaceutical Quality Assurance
A.R College of Pharmacy & G.H Patel Institute of Pharmacy, Vallabh Vidyanagar, Anand, Gujarat, India.

*Corresponding AuthorPriyanka K. Patel
Department of Pharmaceutical Quality Assurance
A.R College of Pharmacy & G.H Patel Institute of Pharmacy, Vallabh Vidyanagar, Anand, Gujarat,

Abstract

This Review article gives the detail information about the UPLC Method as per ICH guideline. UPLC can be regarded as latest invention for liquid chromatography. UPLC refers to ultra-performance liquid chromatography. UPLC chromatography is concerning decreased time and solvent consumption, which improves in three areas: "speed, resolution and sensitivity". Today's pharmaceutical industries are looking for new ways to cut cost and shorten time for development of drugs while at the same time improving the quality of their products and analytical laboratories are not exception in this trend. Speed allows a greater number of analyses to be performed in a shorter amount of time thereby increasing sample throughput and lab productivity. These are the benefits of ultra-performance liquid chromatography. This system uses fine particles (less than 2.5µm), so reduces length of column, saves time and reduces solvent consumption. This review introduces the theory of UPLC, and summarizes some of the most recent work in the field.

Keywords: Ultra-performance liquid chromatography, High separation efficiency, Cost effective, Highpressure.

1. 1 Introduction:

UPLC refers to Ultra Performance Liquid Chromatography. It improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption. UPLC comes from HPLC. UPLC is used in many laboratories all over the world. (1)

One of the main advantages of this technique is growth and development due to the advancement of materials used for packaging used in stimulating the separation. The Causal ideology of this advancement is governed by what is called the Van Deemeter equation. This Technology takes full benefit of Chromatographic principles to encourage separations utilizing columns chock full of tinier particles and/or superior flow rates for higher speed with exceptional resolution and excellent sensitivity. Today, because of in vivo doses or low sample size doses, new technology using narrow, high-density columns boasting higher resolution and more precise sensitivities. Speed and precision became much higher and could be expected when using UPLC. (2)

2. What is UPLC?

The term UPLC, meaning "Ultra Performance Liquid Chromatography," was introduced by Waters Corporation when they introduced their Acquit LC system. The biggest change was the use of sub-2 um particles, which were operated at higher flows and pressures than a conventional system. This concept resulted in significantly shorter analysis times. (3,4)

3. Principle

The underlying principle of UPLC is based on the van Deemeter relationship which explains the correlation between flow rate and plate height. The van Demeter equation (i) shows that the flow range with the smaller particles is much greater in comparison with larger particles for good results (6, 7)

$$H=A+\frac{B}{v}+Cv \quad \text{(i)}$$

Where H represents height equivalent to the theoretical plate (HETP), A, B & C are the constants and v is the flow rate (linear velocity) of the carrier gas. The aim is to minimize HETP to improve column efficiency. The term A does not depend on velocity and indicates eddy mixing. It is smaller if the columns are filled with small and uniform sized particles. The term B denotes the tendency of natural diffusion of the particles. At high flow rates, this effect is smaller, so this term is divided by v. The term C represents the kinetic resistance to equilibrium during the process of separation. The kinetic resistance is the time lag involved in moving from the mobile phase to the stationary phase and back again. The higher the flow rate of the mobile phase, the more a molecule on the packing material inclines to lag behind molecules in the mobile phase. Thus, this term is inversely proportional to linear velocity. Consequently, it is likely to enhance the throughput, and without affecting the chromatographic performance, the separation can be speeded up. The emergence of UPLC has necessitated the improvement of existing instrumentation facility for LC, which takes the benefit of the separation performance (by decreasing dead volumes) and consistent pressures (about 500 to 1000 bars, compared with 170 to 350 bars in HPLC). Efficiency is proportionate to the length of the column and inversely proportional to the radius of the particles (Jorgenson, et al., 1997). Consequently, the column length can be reduced by the similar factor as the particle radius without affecting the resolution. The use of UPLC has helped in the detection of drug metabolites and enhancement of the quality of separation spectra. [8, 9]

4. CHEMISTRY OF SMALL SIZE PARTICLES [10]

The chemistry of the particles used in this course of the method contributes the increased efficiency and potential to work at amplified linear velocity, thereby, providing both the speed and the resolution. Efficiency is one of the important separation parameters which plays a significant role in UPLC since it depends on the same selectivity and retentivity as HPLC. This may be understood with the help of the following basic resolution (Rs) equation (ii):

$$Rs=\frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k}{k+1}\right) \quad \text{(ii)}$$

Where α represents the selectivity factor, N is efficiency and K denotes proportionality constant. According to this, resolution increases with increase in efficiency. Since efficiency (N) is inversely proportional to particlesize (dp) equation (iii):

$$N\alpha\frac{1}{dp} \quad \text{(iii)}$$

As the size of the particle decreases by a factor of three, there is three times increase in the efficiency and in the resolution, there is an increase of the square root of three (nine times). So, there is an increase in efficiency with consequent increase in resolution and sensitivity as the particle size decreases. Similarly, efficiency (N) is directly proportional to the length of the column (L). So, the equation (iii) may be written as equation (iv) as follows:

$$N\alpha\frac{L}{dp} \quad \text{(iv)}$$

Therefore, the length of the column (L) may be reduced by the same ratio as the size of the particle without loosing the resolution.

Efficiency also has indirect relationship with the peak width (w) according to the equation (v):

$$N\alpha\frac{1}{w^2} \quad \text{(v)}$$

This means that narrower the peaks are, easier is their separation from each other. Peak height (H) also has opposite relationship with peak width (w) according to equation (vi):

$$H \alpha \frac{1}{w} \quad \text{(vi)}$$

Based on the above facts, in UPLC, by reducing the particle size (1/3rd), the column length is reduced (1/3rd), the flow rate is increased (3 times) and the separation is done faster (1/9th time) with maintaining the resolution For this reason, short columns packed with small size particles (about 2 µm) are used with these systems, to quicken the separation with higher efficiency, while maintaining a tolerable loss of load. The effect of particle size on HETP and linear velocity has been illustrated. by using van Deemter plot. Fig. shows that the particles with a smaller diameter are contributing less to band broadening compared to larger particles and are less affected by higher column flow rate.

5. Instrumentation

The Ultra Performance Liquid Chromatography have the ability to work more efficiently with higher speed, sensitivity and resolution at a much wider range of linear velocities, flow rates and backpressures to obtain superior results. The Acquit UPLC system consists of

- Binary solvent manager
- Sample manager including the column heater
- Optional sample
- Pump
- Detector

5.1 Binary Solvent Manager:

The binary solvent manager uses two individual serial flow pumps to deliver a parallel binary gradient. Thebinary solvent manager is a high-pressure pump that moves solvent through the system. It provides steady (pulse free) solvent flow at analytical flow rates. The binary solvent manager delivers solvent at flow rates of 1 ml/min at 103421 Kpa [1034 bar, 1500 psi] and up to 2 ml/min at reduced pressures to 62053 Kpa [621 bar, 9000 psF]. The solvent manager can pump two solvents immediately.

5.2 Sample Manager:

The Acuity sample manager injects the sample it draws from Micro titer plates or vials in to the chromatographic flow stream. A locating mechanism uses a probe to access sample locations and draw sample from them. The Sample manager can perform an injection in approximately 15 seconds. The sample manager also controls the column heater. Column temperatures up to 65°C can be attained. [11]

5.3 Column Heater:

The column heater is of a modular design and its foot print is identical to that of the sample manager. Thus, it attaches to the top of the sample manager and serves as that instrument's top cover. [11]

5.4 Optional Sample: [12]

Organizer The optional sample organizer stores micro miter or vial plates and transfers them to and from the sample manages, automating their processing and increasing through put.

5.5 Columns:

The design and development of sub-2µm particles is a significant challenge, and researchers have been very active in this area to capitalize on their advantages. [13]

Although high efficiency nonporous 1.5µm particles are commercially available, they suffer from low surface area, leading to poor loading capacity and retention. To maintain retention and capacity similar to HPLC, UPLC must use a novel porous particle that can withstand high pressures. Silica based particles have good mechanical strength, but suffer from a number of disadvantages. These include tailing of basic analytes and a limited Ph range. Another alternative, polymeric column can overcome pH imitations, but they have their own issues, including low efficiencies and limited capacities. In 2000, Waters introduced a first-generation hybrid chemistry, called XTerra, which combines the advantageous properties of both silica and polymeric columns - they are mechanically strong, with high efficiency, and operate over an extended

pH range. XTerra columns are produced using a classical sol-gel synthesis that incorporates carbon in the form of methyl groups. However, in order to provide the kind of enhance mechanical stability UPLC requires, a second-generation hybrid technology, was developed, called ACQUITY UPLC [16]

ACQUITY 1.7µm particles bridge the methyl groups in the silica matrix as shown in figure-1, which enhances their mechanical stability. Evolution is increased in a 1.7 µm particle packed column because efficiency is better. Separation of the components of a sample requires a bonded phase that provides both retention and selectivity. Four bonded phases are available for UPLC separation: [17]

A) ACQUITY UPLC BEH T M C18 and C8 (straight chain alkyl columns)
B) ACQUITY UPLC BEH Shield RP 18 (embedded polar group column)
C) ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionalitywith a C6 alkyl)
D) ACQUITY UPLC BEH Amide columns (trifunctionally bonded amide phase)

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

These are considered as the universal columns of choice for most UPLC separations by providing the widest pH range. They incorporate trifunctional ligand bonding chemistries which produce superior low pH stability. This low pH stability is combined with the high pH stability of the 1.7µm BEH particle to deliver the widest usable pH operating range.

(B) ACQUITY UPLC BEH SHIELD R18 columns –

These are designed to provide selectivity that complement the ACQUITY UPLC BEH T M C18 and C8 Columns.

(C) ACQUITY UPLC BEH Phenyl columns –

These utilize a trifunctional C6 alkyl ethyl between the phenyl ring and the silyl functionality.

(D) ACQUITY UPLC BEH Amide columns-

BEH particle technology, in combination with a trifunctionally bonded amide phase, provides exceptional column life time, thus improving assay robustness. BEH Amide columns facilitate the use of a wide range of phase pH [2 –11] to facilitate the exceptional retention of polar analytes spanning a wide range in polarity, structural moiety and pKa.

5.6 Pump:

The most important advantages of pumps are: higher resolution, faster analyses, and increased sample loadcapacity. Pump Module – types

- Isocratic pump -delivers content mobile phase composition; solvent must be pre-mixed
- Gradient pump – deliver variable mobile phase composition;(P Nikalje,2013)Isocratic pump - delivers constant

5.7 Detector [17, 20]

Detectors such as UV/Visible, Photodiode array (PDA), Evaporative light scattering (ELS), Refractive index (RI) and Fluorescence (FLR) are commonly used with UPLC. Beside these detectors, capability of UPLC can be greatly increased by hyphenating instrument with other technique such as mass spectrometer, ion chromatograph, nuclear magnetic resonance spectrometer, inductive coupled plasma- mass spectrometer and Infrared spectrometer.

5.7.1 Ultra-violet/visible (UV)

This detector is used for organic compound which absorb the light in the range of 190 to 800 nm This detector can be tuned to specific wavelengths in UV or Visible range for detection. It provides performance benefits for both routine and complex analyses in pharmaceutical, life science, environmental, agricultural and petrochemical applications.

5.7.2 Photodiode Array (PDA) detector

This detector offers simultaneous advanced optical detection in the range of 190 to 800 nm. It provides unprecedented trace impurity detection and quantitation with spectral analysis capabilities. Definitive

compound identification and co-elution detection with simultaneous 2D and 3 operations. This detector finds major application in drug discovery and pharmaceutical development. (Swartz ME., 2005)

5.7.3 Fluorescence (FLR) Detector

For sensitivity and selectivity to fluorescence-based applications, this detector is used. It extends the benefits of UPLC technology for the analysis of polynuclear aromatic hydrocarbons (PAHs), drugs of abuse, and vitamins – any component with chemiluminescent properties, such as fluorescence or phosphorescence. (Swartz ME., 2005)

5.7.4 Refractive index (RI) detector

RI is a universal detector which is used where chemical is having no or limited UV absorbance. These include alcohols, sugars, fatty acids, excipients, raw material and pharmaceutical drug products. Beside this characterization of low molecular weight polymers also finds application on UPLC. Main disadvantage of this detector is that it lacks sensitivity.

5.7.5 Mass detector (MS)

UPLC can be coupled with mass spectrometer (MS) and tandem mass spectrometer (MS-MS) detector which in application in various fields and is used for identification, quantitation and mass analysis of materials. Also, structural elucidation of unknown molecule can be found out by fragmentation. This detector has various mass analysers depending upon their application some of analyser are Single quadrupole, Triple quadrupoles (Tandem), Ion trap and Time of flight (TOF). These detectors provide very high sensitivity, selectivity and time resolution. Beside these detectors many other detectors can be hyphenated to UPLC such as Infrared (IR), Inductive Coupled plasma mass spectrometry (ICP-MS), nuclear magnetic resonance (NMR) and Evaporative light scattering detector (ELSD), Electrochemical detector (EC)

6. ADVANTAGES [19]

The advantages of UPLC are:

- Decreases run time and increases sensitivity.
- Provides the selectivity, sensitivity, and dynamic range of LC analysis
- Maintaining resolution performance.
- Expands scope of Multiresidue Method.
- UPLC's fast resolving power quickly quantifies related and unrelated compounds.
- Faster analysis through the use of a novel separation material of very fine particle size.
- Operation cost is reduced.
- Less solvent consumption.
- Reduces process cycle times, so that more product can be produced with existing resources.
- Increases sample throughput and enables manufacturers to produce more material that consistently meet or exceeds the product specifications, potentially eliminating variability, failed batches, or the need torework material.
- Delivers real-time analysis in step with manufacturing processes. Assures end-product quality, including final release testing (Hodges2004., R.M.; Gunjkar,2011)

7. DISADVANTAGES

- A major disadvantage of UPLC is the higher back pressures compared to conventional HPLC whichdecreases the life of the columns.
- Increasing the column temperature reduces the back pressure problem in UPLC.
- Moreover, the particles of less than 2 μm are mostly non-regenerable and, therefore, have a narrow use.

8. Draw Back

- Cost mixing
- Solvent pumping
- Lack of variety in commercial columns at 1.7 µm

9. APPLICTION

➢ **Drug Discovery**

- UPLC improves the drug discovery process by means of high throughput screening, combinational chemistry, high throughput in vitro screening to determine physiochemical and drug's pharmacokinetics. [23]

➢ **High throughput quantitative analysis**

- UPLC coupled with time-of-flight mass spectroscopy give the metabolic stability assay. [23]

➢ **Analysis of Dosage form**

- It provides high speed, accuracy and reproducible results for isocratic and gradient analysis of drugs and their related substance. Thus, method development time decrease.

➢ **Analysis of amino acids**

- UPLC used from accurate, reliable and reproducible analysis of amino acids in the areas of protein characterizations, cell culture monitoring and the nutritional analysis of foods.

➢ **Determination of Pesticides**

- UPLC couples with triple Quadra-pole tandem mass spectroscopy will help in identification of trace level of pesticides from water.
- Thus, Ultra Pressure Liquid Chromatography set a new standard in the science of chromatography. Working range with 15000 to 16000 psi pressure and column packed with less than 2 micrometres in size helped in various fields. [29]

➢ **Analysis of Natural Products and Traditional Herbal Medicine**

- UPLC is widely used for analysis of natural products and herbal medicines. The main purpose of this is to analyse drug samples arising from the complexity of the matrix and variability from sample to sample. UPLC provides high-quality separations and detection capabilities to identify active compounds in highly complex samples that results from natural products and traditional herbal medicines. [27]

➢ **Identification of Metabolite**

- UPLC/MS/MS addresses the complex analytical requirements of biomarker discovery by offering unmatched sensitivity, resolution, dynamic range, and mass accuracy.

➢ **ADME (Absorption, Distribution, Metabolism, Excretion) Screening**

- The high resolution of UPLC enables accurate detection and integration of peaks in complex matrices and extra sensitivity allows peak detection for samples generated by lower concentration incubations and sample pooling. UPLC/MS/MS provides following advantage: - UPLC can more than double throughput with no loss in method robustness. UPLC is also simpler and more robust than the staggeredseparations sometimes applied with HPLC methods.
- UPLC operating with rapid, generic gradients has been shown to increase analytical throughput and sensitivity in high throughput pharmacokinetics or bioanalysis studies, including the rapid measurement of potential p450 inhibition, induction, and drug-drug interactions. [30]

➢ **Bioanalysis / Bioequivalence Studies**

- UPLC delivers excellent chromatographic resolution and sensitivity. The sensitivity and selectivity of UPLC at low detection levels generates accurate and reliable data that can be used for a variety of different purposes, including statistical pharmacokinetics analysis. UPLC solutions are proven to increase efficiency, productivity and profitability for bio equivalence laboratories. [29]

➢ **Dissolution Testing**

- For quality control and release in drug manufacturing, dissolution testing is essential in the formulation, development and production process. UPLC provides precise and reliable automated online sample acquisition. It automates dissolution testing, from pill drop to test start, through data acquisition and analysis of sample aliquots, to the management of test result publication and distribution. [22,23]

CONCLUSION:

UPLC increases productivity in both chemistry and instrumentation by providing more information per unit of work as it gives increased resolution, speed and sensitivity for liquid chromatography. The main advantage of UPLC is a reduction time. Which also reduces solvent consumption. high sensitivity for the analysis of low concentration component. As solvent consumption for this technique is less it also reduces the cost of analysis. The method can be well developed and validated in less time on this system. UPLC finds versatile application for Drug Discovery, High throughput quantitative analysis, Analysis of amino acids, Identification of Metabolite, Dissolution Testing, Bioanalysis / Bioequivalence Studies.

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