



AN INTRODUCTION TO INSTRUMENTATION OF ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

Devika Tripathi*

Department of Pharmacy, Shri Ram Murti Smarak College of Engineering and Technology, Bareilly.

***Author for Correspondence: Prof. Devika Tripathi**

Department of Pharmacy, Shri Ram Murti Smarak College of Engineering and Technology, Bareilly.

Article Received on 16/06/2017

Article Revised on 06/07/2017

Article Accepted on 27/07/2017

ABSTRACT

Ultra performance liquid chromatography (UPLC) takes advantage of technological pace made in particle chemistry performance, system optimization, detector design and data processing and control. This new category of analytical separation science retains the practicality and principles of HPLC while creating a step function improvement in chromatographic performance. It uses fine particles and saves time and reduces solvent consumption. The separation on UPLC is performed under very high pressures (up to 100 MPa) without negative influence on analytical column or other components of chromatographic system. It shows a great enhancement in speed, resolution as well as the sensitivity of analysis by using particle size less than 2 μm and the system is designed in a peculiar way to withstand high system back- pressures. This review is basically emphasizes on the basic technology and instrumentation of UPLC.

KEYWORDS: UPLC, HPLC, Sensitivity, Resolution.

INTRODUCTION

UPLC refers to Ultra Performance Liquid Chromatography. UPLC is comes from HPLC. HPLC has been the evolution of the packing materials used to effect the separation. An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5 μm , there is a significant gain in efficiency and it doesn't diminish at increased linear velocities or flow rates according to the common Van Demeter equation. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which is known as Ultra Performance. UPLC is a derivative of HPLC whose underlying principle is that as column packing particle size decreases, efficiency and resolution increases. If we decrease particle size less than 2 μm , the efficiency shows a significant gain¹ by making use of the smaller particles, the speed of analysis and peak capacity i.e., number of peaks resolved per unit time, can be prolonged to the maximum values and these values are much better than the values achieved earlier by HPLC.^[1,2]

Working Principle

The UPLC is based on the principle of use of stationary phase consisting of particles less than 2 μm (while HPLC

columns are typically filled with particles of 3 to 5 μm). The under lying principles of this evolution are governed by the Van Demeter, which is empirical formula that describes the relationship between linear velocity (FLOW RATE) and plate height (HETP or column efficiency).

$$H=A+B/v+Cv$$

Where A, B and C are constants and v is the linear velocity, the carrier gas flow rate. The A term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform. The B term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by v. The C term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus this term is proportional to v. Therefore it is possible to increase throughput and thus the speed of analysis without affecting the chromatographic performance.^[2, 3, 4]

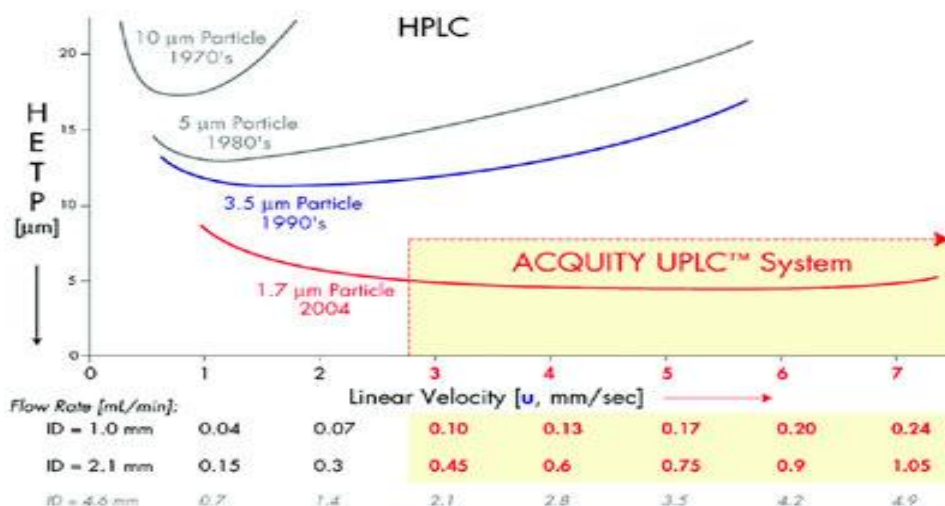


Fig 1: Van De Meter plot, illustrating the evolution of particle sizes over the last three decades.

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. It consists of following.

Sample system manager

The UPLC system with its flow through needle design sample manager addresses three design challenges for reliable performance: robust sealing of the needle at higher pressure, minimizing the extra column band spread for narrow peaks and performing pulse free injection process to protect column from extreme pressure fluctuations. 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. After injecting the sample, the needle is washed for a specified amount of time to minimize sample carryover. Beside this, there are also direct injections for biological substances are also reported.^[3, 4]

UPLC Columns

The UPLC columns are usually made up of small particles having size less than 2 μm . The particles are bonded in matrix as the bonded stationary phase is required for providing both retention and selectivity. Four bonded phases are available for UPLC separations.

ACQUITY UPLC M BEH C18 and C8: Straight chain alkyl columns.

They are considered 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 1.7 μm BEH particle to deliver the widest usable pH operating range. For e.g.: Analysis of banned

Carcinogenic Aromatic amines: Benzidine, O-anisidine and O-toluidine and Chloroaniline exhibit retention time at 2.7mins, 2.8mins, 3mins and 3.6mins respectively.

ACQUITY UPLC BEH Shield RP18: Embedded polar group column.

These are designed to provide selectivities that complement the ACQUITY UPLC BEH C18 and C8 phases. This contains an embedded polar group that combines the hydrophobicity of a straight-chain-alkyl ligand C18 with hydrophilicity of an embedded polar group (carbamate). This unique and patented bonding chemistry provides complementary selectivity to a C18 column and enhances the peak shape for basic compounds and yielding compatibility with 100% aqueous mobile phases. For instance, impurity profile of Doxylamine drug substance from synthetic and formulation impurities with retention time of 2.8 mins can be obtained.

ACQUITY UPLC BEH Phenyl columns: Phenyl group tethered to the silyl functionality with a C6 alkyl.

These columns utilize a trifunctional C6 alkyl tether between the phenyl ring and the silyl functionality. This ligand, combined with the same proprietary end capping processes as the ACQUITY UPLC BEH C18 and C8 columns, provides long column lifetimes and excellent peak shape. This unique combination of ligand and end capping on the 1.7 μm BEH particle creates a new dimension in selectivity allowing a quick match to the existing HPLC column. For instance, by implementing UPLC technology with TQD and a BEH phenyl column, the rapid analysis of 25 polymer additives is achieved only 3.5mins; 6-10X faster than HPLC methods.

Pumps

The UPLC pumps play an important role in liquid chromatography and have considered being one of the important components. It is used to provide a continuous constant flow of the eluent through the UPLC injector,

column, and detector. Standard UPLC pumps required usually sample injection volume as less as 3–5 micro liters, operating pressure at 10000 psi and particle size in stationary phase packing material less than 2 micro meters.

Types of pumps

Reciprocating piston pump

The basic principle of the reciprocating single piston pumps is that it expels liquid through a one-way valve (check valve). The pumping rate is usually adjusted by controlling the distance the piston retracts, thus limiting the amount of liquid pushed out by each stroke, or by the cam rotating speed. CAM is pushing a sapphire piston back and force. When the piston is moving backwards it sucks the eluent through the inlet check valve (on the bottom). The sapphire ball is lifted and opens the path for the eluent. When the piston moves forward, the liquid

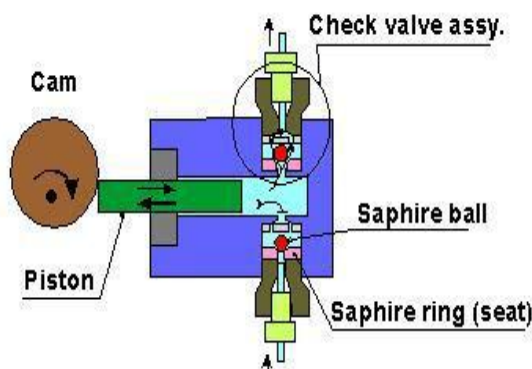


Fig 2: Reciprocating piston pump.

Detectors

The system can be equipped with following types of detectors. These are.

Optical Detectors

Optical Detectors are used in ultra-performance liquid chromatography analytical techniques, featuring low dispersion characteristics, simple operation, and high data acquisition rates as well as cost effective maintenance, support and parts. The system can be configured with a TUV, PDA or ELS optical detector or any combination of the three.

Tunable Ultra Violet detector

For UPLC detection, the TUV (tunable ultra violet) detector is used which includes new electronics and firm ware to support. The TUV optical detector is a two channel ultra violet / visible absorbance detector designed for the use of in the acquity UPLC system. The detector offers two flow cell options. The analytical cell flow, with a volume of 500 nano liters and a path length of 10 nm and the high sensitivity flow cell with a volume of 2.4 micro liters and 25 mm path length, both utilize the waters patented light guiding flow all technology.

pushes the inlet ball down and closes the path, but the outlet ball is lifted and opens the outlet valve (upper). The main drawback of this pump is sinusoidal pressure pulsations which lead to the necessity of using pulse dampers.

Dual piston pumps

A more efficient way to provide a constant and almost pulse free flow is the use of dual-headed reciprocating pumps. Both pump chambers are driven by the same motor through a common eccentric cam; this common drive allows one piston to pump while the other is refilling. As a result, the two flow-profiles overlap each other significantly reducing the pulsation downstream of the pump; this is visualized below. Since the acceleration/deceleration profile is somewhat non-linear, the more efficient types of these pumps use eccentricity-shaped cams to obtain the best overlapping of the pressure curves and to obtain smooth flow.

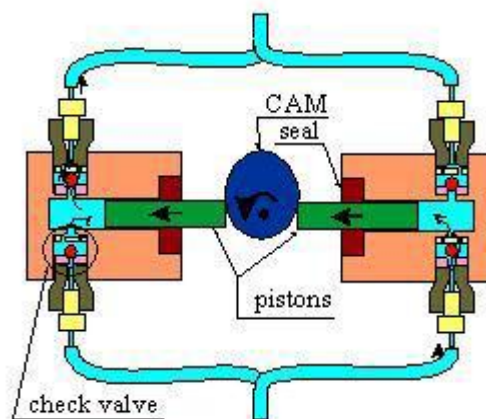


Fig 3: Dual piston pump .

The TUV detector operates at wave length ranging from 190 to 700 nm.

PDA (Photo Diode Array) detector

It is an optical detector absorbs UV-Visible light that operate between 190-500nm.

ELS (Evaporative Light Scattering) detector

The detector incorporates a flow type nebulizer that is optimized for acquity UPLC system performance. Analyzes more molecules (including sugars, triglycerides, phospholipids, antibiotics, and natural products) in a single analytical run. Anywhere large numbers of compounds are screened rapidly, the detector offers a convenient stackable design, easy maintenance, and long lamp lifetimes.

FLR (Fluorescence) detector

It is a multichannel, multi wavelength detector, which has an excitation wavelength that ranges from 200 to 890 nm, an emission wavelength that ranges from 210-900 nm, offers 3D scanning capability for easier method development.

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.

HPLC v/s UPLC

In HPLC particle size in stationary phase packing material is between 5-12 micrometers whereas in UPLC

is less than 2 micrometers. Higher sample throughput with more information per sample can be obtained in UPLC which was comparatively less in case of HPLC. Usually pump operates at 2000-6000 psi pressure in HPLC whereas 10,000 psi pressure is required for UPLC. Broader peak width provides less resolution in HPLC whereas smaller peak width provides better resolution and more number of peaks getting identified in UPLC.



Fig 3: Image of instrumentation of Ultra Performance Liquid Chromatography (UPLC).

Advantages of UPLC

1. Expands scope of Multiresidue Methods.
2. Faster analysis through the use of a novel separation material of very fine particle size.
3. Provides the selectivity, sensitivity, and dynamic range of LC analysis.
4. Increases sample throughput and enables manufacturers to produce more material that consistently meet or exceeds the product specifications.
5. Less solvent consumption.

Disadvantages of UPLC

1. Due to increased pressure requires more maintenance and reduces the life of the columns.
2. The phases of less than 2 μm are generally non-generable.

Applications

1. **Drug Discovery:** UPLC improves the drug discovery process by means of high throughput screening, combinatorial chemistry, high throughput in vitro screening to determine physiochemical and drug's pharmacokinetics.
2. **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.
3. **Determination of Pesticides:** UPLC couples with triple Quadra-pole tandem mass spectroscopy will

help in identification of trace level of pesticides from water.

4. **Analysis of Natural Products and Traditional Herbal Medicine:** 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.
5. **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.
6. **Identification of Metabolite:** UPLC operating with rapid, generic gradients has been shown to increase analytical throughput and sensitivity in high throughput pharmacokinetics or bio-analysis studies, including the rapid measurement of potential p450 inhibition, induction, and drug-drug interactions.

CONCLUSION

Due to reduced analysis time and reduced solvent consumption UPLC dramatically improves the quality of data and resulting into more reproducible results as conventional HPLC. It also increase more productivity due to smaller particle size of UPLC column which 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 to re-work material.

REFERENCES

1. Reddy SKT, Balammal G, Kumar AS; International Journal of Pharmaceutical Research & Analysis, 2012; 2(1): 24-31.
2. Chandra S, Seelam, Priyanka G, Dhanalakshmi K, Reddy N. Switch from HPLC to UPLC: A Novel Achievement in Liquid Chromatography Technique - A Review, Int. J. Pharm. Sci. Rev. Res, 2013; 21(1): 237-246.
3. Swartz ME. Ultra Performance Liquid Chromatography (UPLC): An Introduction: Separation Science Re-Defined, LCGC Supplement, 2005; 8.
4. B. Srivastava, Ultra Performance Liquid Chromatography (UPLC): A Chromatography Technique, International Journal of Pharmaceutical Quality Assurance, 2010; 2(1): 19-25.
5. Jerkovich AD. et al. *LCGC*, 2003; 21(7): 600-610.
6. Mac Nair J.E. et.al. *Anal. Chem*, 1997; 69: 983-989.