Available Online at www.jddt.in

ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics 2 (20) 2014, 32-37

REVIEW ARTICLE

Ultra performance liquid chromatography: an introduction

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Received 20 July 2014; Accepted 30 July 2014

ABSTRACT

Ultra-performance liquid chromatography (UPLC) has been investigated as an alternative to HPLC for the analysis of pharmaceutical development compounds. Ultra Performance Liquid Chromatography (UPLC) is a relatively new technique giving new possibilities in liquid chromatography, especially concerning decrease of time and solvent consumption. UPLC chromatographic system is designed in a special way to withstand high system back-pressures. The quality control analyses of various pharmaceutical formulations are transferred from HPLC to UPLC system. The separation on UPLC is performed under very high pressures (up to 100 MPa) but it has no negative influence on analytical column or other components of chromatographic system. Separation efficiency remains maintained or is even improved by UPLC.This review introduces the theory of UPLC, and summarizes some of the most recent work in the field.

Key Words: UPLC, HPLC, Chromatography.

INTRODUCTION:

High performance liquid chromatography (HPLC) is proven technique that has been used in laboratories worldwide over the past 30-plus years. One of the primary drivers for the growth in this technique has been the evolution of packing materials used to effect separation. An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. The classic separation method is of HPLC (High Performance Liquid Chromatography) with many advantages like robustness, ease of use, good selectivity and adjustable sensitivity. Its main limitation is the lack of efficiency compared to gas chromatography or the capillary electrophoresis due to low diffusion coefficients in liquid phase, involving slow diffusion of analytes in the stationary *p*hase. The Van Demeter equation shows that efficiency increases with the use of smaller size particles but this leads to a rapid increase in back pressure, while most of the HPLC system can operate only up to 400 bar. That is columns filled with particles of about 2µm are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load**.** As particle size decreases to less than 2.5µm, there is a significant gain in efficiency and it's 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 describes the relationship between linear velocity (flow rate) is known as Ultra Performance. The UPLC is a registered trademark of Waters corporation designates certain of its systems for liquid chromatography high pressure. Introduced in 2004 and using the same principles as the HPLC , UPLC HPLC improves in three areas: chromatographic resolution, sensitivity and speed of analysis. [1]

UPLC refers to Ultra Performance Liquid Chromatography, which improves in three areas:

1) Chromatographic resolution
2) Speed
3) Sensitive analysis

It uses fine particles and saves time and reduces solvent consumption. This new category of analytical separation science retains the practicality and principles of HPLC while increasing the overall interrelated attributes of speed, sensitivity and resolution. 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 faster analysis and hence the ultra performance liquid chromatography. A typical assay was transferred and optimized for UPLC system to achieve

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both higher sample analysis throughput and better assay sensitivity. Analysis of operation cost and sample throughput found UPLC cost advantageous over HPLC. [2]

PRINCIPLE:

The UPLC is based on the principal 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 underlying principles of this evolution are governed by the van Demeter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency). The Van Demeter curve, governed by an equation with three components shows that the usable flow range for a good efficiency with small diameter particles are much greater than for larger diameters. [3]

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.

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 Acquity UPLC system consists of

1. Binary solvent manager
2. Sample manager including the column heater
3. Optional Sample manager
4. Pumps
5. Detector

1. Binary Solvent Manager:

The binary solvent manager uses two individual serial flow pumps to deliver a parallel binary gradient. The binary 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. [4]

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. [5]

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. [6]

3. Optional Sample Organizer:

The optional sample organizer stores micro meter or vial plates and transfers them to and from the sample manages, automating their processing and increasing through put. [7]

4. Pumps:

The UPLC pump is considered to be one of the most important components in a liquid chromatography system which has to provide a continuous constant flow of the eluent through the UPLC injector, column, and detector. [8-10]

The two basic classifications are

a) Constant pressure pump
b) Constant flow pump

Constant pressure pump: The constant pressure is used for column packing. [11]

Constant flow pump: This type is mostly used in all common UPLC applications. [12]

Standard UPLC pump requirements: [13-14]

- ✓ Sample injection volume is as less as 3 – 5 micro liters
- ✓ Pump operates at 10000 psi pressure
- ✓ Particle size in stationary phase packing material is less than 2 micro meter

5. Detectors:

For UPLC detection, the tunable UV/Visible detector is used which includes new electronics and firmware to support Ethernet communications at the high data rates. Conventional absorbance-based optical detectors are concentration sensitive detectors, and for UPLC use, the flow cell volume would have to be reduced in standard UV/Visible detectors to maintain concentration and signal. According to Beer's Law, smaller volume conventional flow cells would also reduce the path length upon which the signal strength depends. A reduction in

cross-section means the light path is reduced, and transmission drops with increasing noise. Therefore, if a conventional HPLC flow cell were used, UPLC sensitivity would be compromised. The ACQUITY Tunable UV/Visible detector cell consists of a light guided flow cell equivalent to an optical fibre. Light is efficiently transferred down the flow cell in an internal reflectance mode that still maintains a 10mm flow cell path length with a volume of only 500nL. Tubing and connections in the system are efficiently routed to maintain low dispersion and to take advantage of leak detectors that interact with the software to alert the user to potential problems. [15-18]

UPLC CHROMATOGRAPHIC CONDITIONS:

Columns: ACQUITY UPLC BEM C18, BEH Shield RP18, BEH C8 OR BEH

Phenyl Column Dimensions: 2.1X50mm 1.7µm
Mobile Phase A1: 20mM NH4COOH in H_2O, pH 3.0
Mobile Phase A2: 20mM NH4HCOO3 in H_2O, pH 10.0
Mobile Phase B1: Acetonitrile
Mobile Phase B2: Methanol
Flow rate: 0.5ml/min
Injection Volume: 10.0µl
Week needle wash: 3% methanol
Strong needle wash: 90% acetonitrile
Temperature: 30ºC
Detection: UV @ 254 nm
Sampling rate: 20pts/sec
Time constant: 0.1
Instrument: ACQUITY UPLC TM with 2996 ACQUITY PDA Detector.

Table 1: METHOD DEVELOPMENT FASTER WITH UPLC (Time Saving)

	UPLC GRADIENT CONDITIONS				EQUIVALENT HPLC GRADIENT CONDITIONS			
Column conditions	2.1x50mm				4.6x150mm			
Particle size	1.7 µm				5 µm			
Flow rate	0.5 ml/min				1.0ml/min			
Gradient	Time (min)	Profile %A	Profile %B	curve	Time (min)	Profile %A	Profile %B	curve
	0.0	95	5	6	0.0	95	5	6
	5.0	10	90	6	35.0	10	90	6
PEAK CAPACITY	150				150			

P = 1+ tg/w

UPLC screening method is 7X faster than directly scaled HPLC method

ADVANTAGES: [19]

- Decreases run time and increases sensitivity.
- Provides the selectivity, sensitivity, and dynamic range of LC analysis
- Maintaining resolution performance.
- Expands scope of Multi residue Methods
- 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 to re-work material.
- Delivers real-time analysis in step with manufacturing processes.
- Assures end-product quality, including final release testing.

DISADVANTAGES: [20]

- Due to increased pressure requires more maintenance and reduces the life of the columns of this type. So far performance similar or even higher has been demonstrated by using stationary phases of size around 2 µm without the adverse effects of high pressure.
- In addition, the phases of less than 2 µm are generally non-regenerable and thus have limited use.

USE OF THE UPLC SYSTEM: [21-22]

Elevated-temperature chromatography also allows for high flow rates by lowering the viscosity of the mobile phase, which significantly reduces the column backpressure. Monolithic columns contain a polymerized porous support structure that provides lower flow resistances than conventional particle-packed columns.

➢ **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.

➢ **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 micrometer in size helped in various fields.

➢ **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 analyze drug samples arise 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.

➢ **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 advantages:-

- UPLC can more than double throughput with no loss in method robustness. UPLC is also simpler and more robust than the staggered separations 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.

➢ **Method Development / Validation:**

According to FDA, validation is defined as an establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes .Method development and validation is a time-consuming and complicated process: labs need to evaluate multiple combinations of mobile phase, pH, temperature, column chemistries, and gradient profiles to arrive at a robust, reliable separation for every activity.
UPLC help in critical laboratory function by increasing efficiency, reducing costs, and improving opportunities for business success. UPLC column chemistries can easily translate across analytical- and preparative-scale separation tasks.
UPLC provide efficiencies in method development: Using UPLC, analysis times becomes as short as one minute, methods can be optimized in just one or two hours, significantly reducing the time required to develop and validate
With UPLC, separation speed and efficiency allows for the rapid development of methodologies
The following parts of UPLC are important to give the required information:-

- UPLC columns: High stability allows for a wide range of column temperatures and p^Hs to be explored
- UPLC Column Manager: Easily evaluate column temperatures from 10 °C below room temperature to 90 °C; enables to use HPLC methods on the UPLC before scaling to UPLC
- UPLC Calculator: Put information at fingertips about how to transition existing chromatographic analyses to faster UPLC methods.

➢ **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.

➢ **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.

➢ **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.

Table 2: COMPARISION OF UPLC AND HPLC: [21]

CHARACTERISTICS	HPLC ASSAY	UPLC ASSAY
Column	150x3.2mm	150x2.1 mm
Particle size	3 to 5 µm	Less than 2 µm
Flow rate	3.0 ml/min	0.6 ml/min
Needle wash	methanol	methanol
Injection volume	5 µL (Std in 100% methanol)	2 µL (Std in 100% methanol)
Column temperature	30^0c	65^0c
Total run time	10 min	1.5 min
Maximum back pressure	35-40 MPa	103.5 MPa
Plate count	2000	7500
USP Resolution	3.2	3.4
Delay volume	750 µl	110 µl
Gradient(time in min) ACN:H_2O	T0 (25:75), T6.5 (25:75), T7.5 (95:5), T9(25:75), T10 (25:75)	T0 (36:64), T1.1 (95:5), T1.3 (36:64)
Total solvent composition (including 0.5 min delay time in between injections)	Acetonitrile:10.5 ml, water:21.0ml	Acetonitrile:0.5ml, water:0.66ml
Analytical column	Alltima C_{18}	Acquity UPLC BEH C_{18}

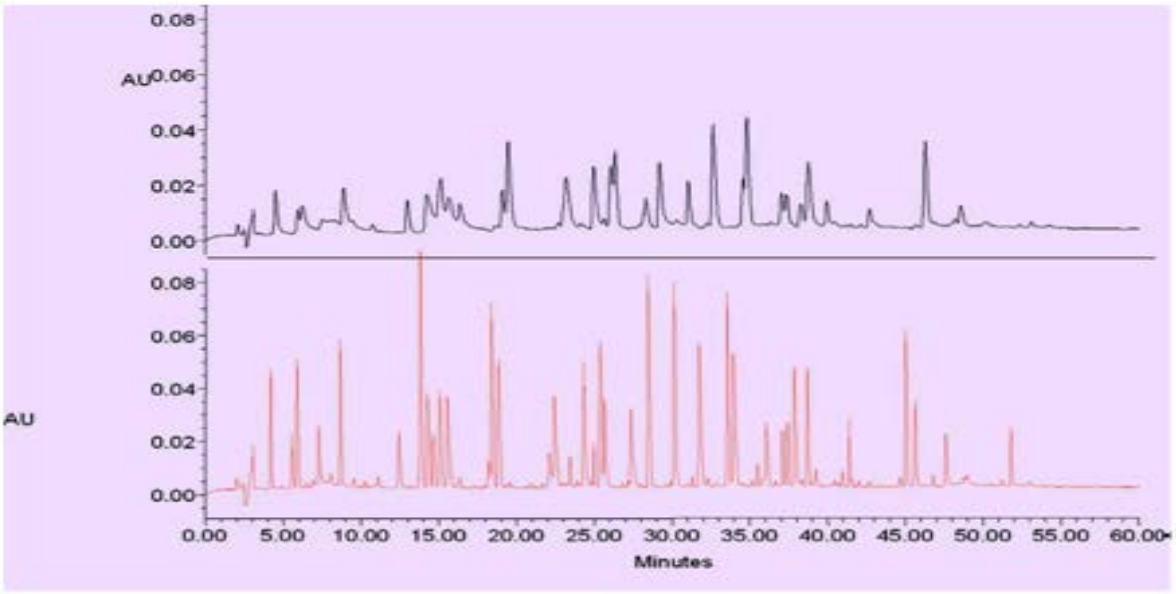

Figure 1: HPLC VS UPLC peak capacity. In this gradient peptide map separation, the HPLC (top) separation (on a 5µm Col-18µmn) yields 70 peaks, or a peak capacity of 143, while the UPLC separation (bottom) run under identical conditions yields 168 peaks, or a peak capacity of 360, a 2.5 x increase.

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. When many scientists experience separation barriers with conventional HPLC, UPLC extends and expands the utility of chromatography. The main advantage is a reduction of analysis time, which also meant reduced solvent consumption. The time spent optimizing new methods can also be greatly reduced. The time needed for column equilibration while using gradient elution and during method validation is much shorter. Sensitivity can be compared by studying the peak width at half height. It was found that the sensitivity of UPLC was much higher than that of conventional HPLC. Tailing factors and

resolution were similar for both techniques. Peak area repeatability (as RSD) and peak retention time repeatability (RSD) were also similar for both techniques.
A negative aspect of UPLC could be the higher backpressure than in conventional HPLC.
This backpressure can be reduced by increasing the column temperature. Overall,it seems that UPLC can offer significant improvements in speed, sensitivity and resolution compared with conventional HPLC.

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