T. Sunil Kumar Reddy et al. / Vol 2 / Issue 1 / 2012 / 24-31.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY: AN INTRODUCTION AND REVIEW

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ABSTRACT

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.

Keywords: UPLC, Ultra performance liquid chromatography, High pressure.

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 advantage of this technique is growth and development is due to the advancement of materials used for packaging is used in stimulating the separation. The Causal ideology of this advancement is governed by what is called the Van Deemter 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].

What is UPLC?

The term UPLC, meaning "Ultra Performance Liquid Chromatography," was introduced by Waters Corporation when they introduced their Acquity 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].

Working

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 Deemter [4], which is empirical formula that describes the relationship between linear velocity and plane height.

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

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Advantages of UPLC

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 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 [27,28]
- Delivers real-time analysis in step with manufacturing processes
- Assures end-product quality, including final release testing

Use of UPLC system

Elevated-temperature chromatography also allows for high flow rates by lowering the viscosity of the mobile phase, which significantly reduces the column backpressure 9, 10. Monolithic columns contain a polymerized porous support structure that provides lower flow resistances than conventional particle-packed columns [5-6].

Disadvantages [7-11]

The dis advantages of UPLC are

- 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.

Draw Backs

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

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

- Binary solvent manager
- Sample manager including the column heater
- Optional Sample manager
- Pumps
- Detector

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.

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.

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 [12].

Optional Sample 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 [13].

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.

The two basic classifications are

- Constant pressure pump
- Constant flow pump

Constant pressure pump

The constant pressure is used only for column packing.

Constant flow pump

This type is mostly used in all common UPLC applications

Standard UPLC pump requirements

- 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

Types of Pumps

Reciprocating Piston Pumps

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. Schematic of the reciprocating single piston pump. 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 pushes the inlet ball down and closes the path, but the outlet ball is lifted and opens the outlet valve (upper) [14-18].

Disadvantage

The main disadvantage of this type of 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 [19,21].

Schematic of a dual-head reciprocating pumps

The advantages of this pump are the unlimited solvent reservoir allowing long-term unattended use and quick changeover and clean out capability. However, unless special care has been exercised in manufacture, these pumps may have several disadvantages. There is a tendency for the incompletely compensated pulsations to be observable at high refractive index detector sensitivities, especially at low flow rates where piston cycles are widely spread. Furthermore, since each head has two check valves, pump reliability depends on the cleanliness of the mobile phase and continued sealing capability of four check valves on each cycle, with cycles normally occurring several times per minute [20].

Check valves on the reciprocating pump are the weakest part. It may be easily contaminated or clogged which leads to the pump misfunction. Most of the recent HPLC instruments use improved dual piston pumps which have three or even two check valves.

The schematic of this pump is shown above. The first piston, called low pressure, is sucks the liquid from the reservoir while the second (high pressure piston) is supplying the eluent to the system. Then the first piston refills the second piston very fast, during 1/100 of the whole pump cycle. This scheme allows the use of only 3 check valve, one of which is working under low pressure. There are no cavitation effects. Because the piston volumes are small (~100 **□**l), pressure pulastions are small and sharp and easy to damp [22].

Another type of dual piston pump uses only two check valves, but piston volumes are different. While the smaller piston dispenses an eluent in the HPLC system, the bigger piston is sucking an eluent. When pistons change their direction, the bigger piston simultaneously refills the smaller chamber and dispenses eluent into the system. This set-up allows only two check valves for the dual piston pump [23].

Detectors [21-26]

Types of Detectors:

Detectors can be classified as:

- Optical detectors
- Tunable ultra violet detectors
- Evaporative light scattering detectors
- Fluorescence detector

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 litres and 25 mm path length , both utilize the waters patented light guiding flow all technology.

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

Features

- Maximum signal-to-noise response enabled by light-guiding flow cell technology, which eliminates internal absorption, for minimal bandspreading and maintained concentration
- High sensitivity for low-level detection for simultaneous quantitation of major and minor components

Acquity Uplc Els Dectectors

The ACQUITY UPLC ELS Detector with the ACQUITY UPLC System lets you analyze 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.

The detector incorporates a flow type nebulizer that is optimized for acquity UPLC system performance

Features

1. Provides reproducible, reliable results with precise control over nebulization and desolvation processes for the measurement of temperature-sensitive molecules
2. Ensures the benefits of UPLC performance across the entire flow rate range with high data capture rates
3. One nebulizer for the entire flow rate range provides simplified set-up and the benefit of maximized performance.

Acquity Uplc Flr Detector

The ACQUITY UPLC® Fluorescence (FLR) Detector delivers sensitivity and selectivity to fluorescence-based applications. Transfer your HPLC fluorescence methods to UltraPerformance LC® for greater throughput without compromising sensitivity.

Features

1. Advanced optical design to maximize light throughput, and reduce light scatter, allowing for better signal-to-noise performance
2. Intuitive system console provides simple navigation to manage instrument parameters for easy system control
3. Intuitive software interface and diagnostic tools instill confidence that the detector is performing optimally.

Injectors of UPLC [27,28]

The injector is comprised of six miniature air actuated needle valves, plumbed to simulate the flow path of a conventional rotor/stator injector. An integral controller sends the on/off positioning signals to each valve, coordinating them to perform load, inject, and flush functions.

There are three methods for sending positioning commands to the injector:

- Manual control with the pushbuttons on the controller
- Laboratory computer via serial port communication
- Contact closure inputs

Migrating methods From HPLC to UPLC

Ultra performance liquid chromatography takes advantage of small 1.7 µm particles operated at elevated pressures to achieve un compromised separation speed , resolution, and sensitivity .

Selecting the Right column

A 1.7µm particle packed column provides significant improvements in resolution because efficiency is better. Separation of the components of a sample, however, still requires a bonded phase that provides both retention and selectivity. Four bonded phases are available for UPLC separations

ACQUITY UPLC™ BEH C_{18} and C_8 (straight chain alkyl columns), ACQUITY UPLC BEH Shield RP_{18} (embedded polar group column) and ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C_6 alkyl) as shown in Figure 1. Each column chemistry provides a different combination of hydrophobicity, silanol activity, hydrolytic stability and chemical interaction with analytes.

ACQUITY UPLC BEH C_{18} and C_8 columns were designed to be the universal columns of choice for most UPLC separations by providing the widest pH range.

Differences between UPLC and HPLC [29]

The short information between HPLC and UPLC.

HPLC

- Broader peak width provides less resolution
- Less sample throughput comparatively.
- Sample injection volume is 20 micro liters.
- Pump operates at 2000-6000 psi pressure
- Particle size in stationary phase packing material is between 5-12 micrometers.

UPLC

- Smaller peak width provides better resolution and more number of peaks getting identified.

- Higher sample throughput with more information per sample.
- Sample injection volume is as less as 3-5 micro liters.
- Pump operates at 10,000 psi pressure
- Particle size in stationary phase packing material is less than 2 micrometer
- APPLICATIONS [26,30]

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.

High throughput quantitative analysis

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

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 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.

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 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.

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.

.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.

Method Development / Validation

According to FDA, validation is defined as 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 UPLC help in critical laboratory function by increasing efficiency, reducing costs, and improving opportunities for business success. UPLC provide efficiencies in method development. Using UPLC, analysis times become as short as one minute, methods can be optimized in just one or two hours, significantly reducing the time required to develop and validate.

Fig 1. IMAGE OF ULTRA PERFORMANCE LIQUID CHROMATOGARPHY

Fig 2. ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)

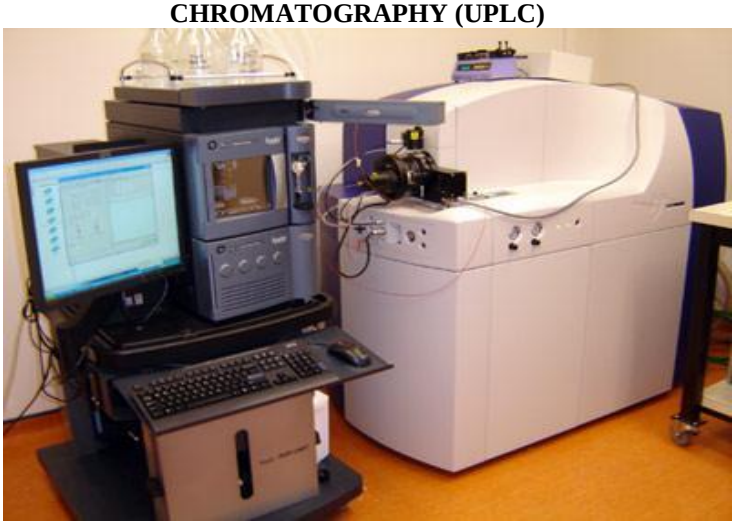


Fig 3. Sample Chromatogram of UPLC

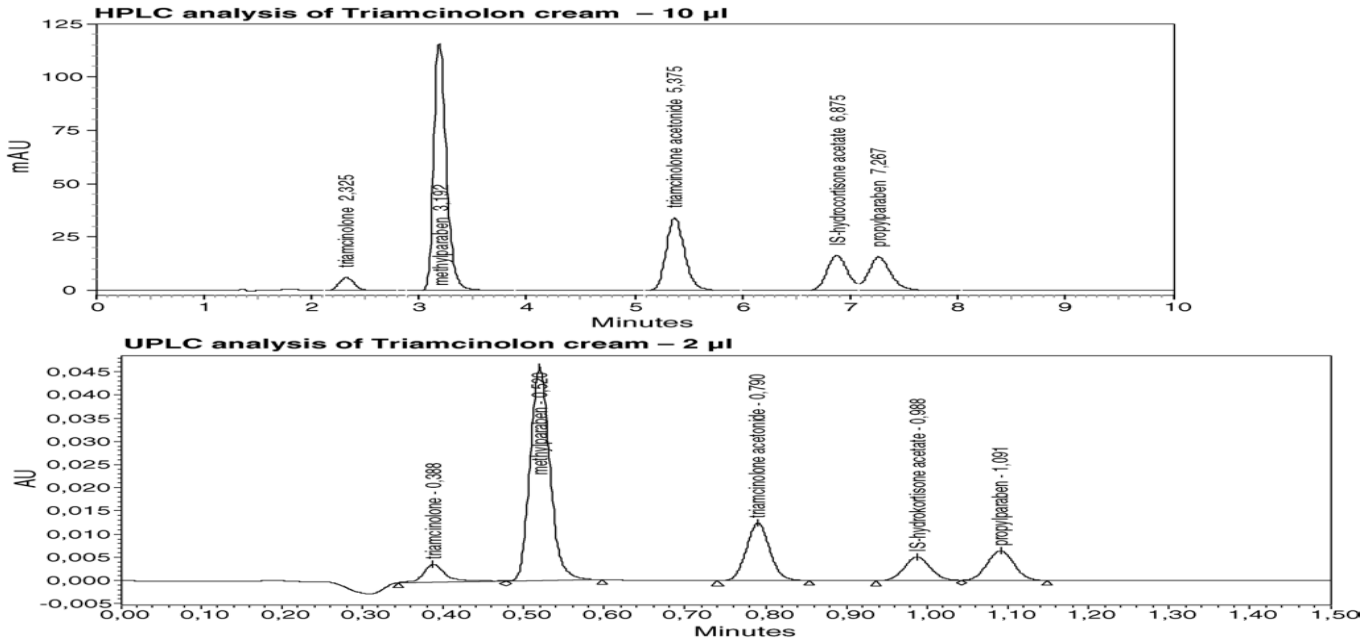


Fig 4. Reciprocating Piston Pumps

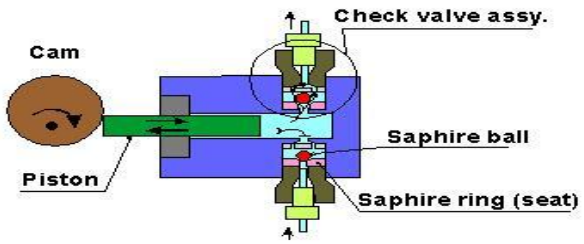


Fig 5. Dual Piston Pumps

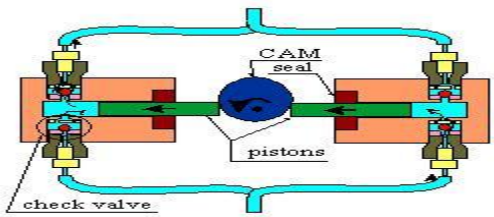


Fig 6. dual-head reciprocating pump

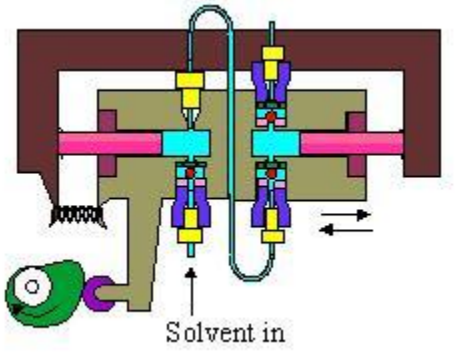
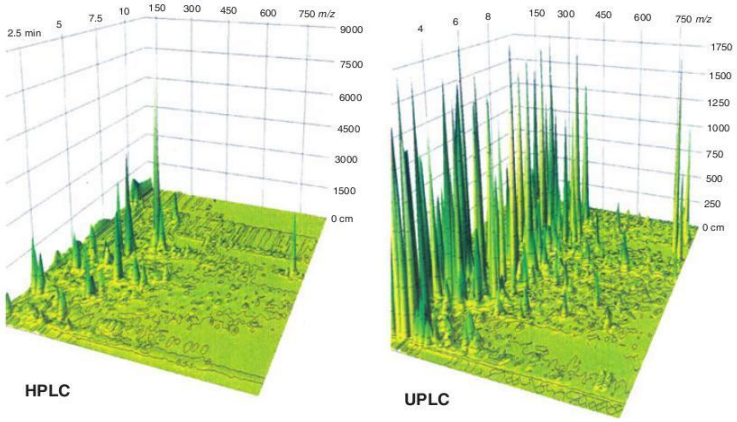


Fig 7. Differences between UPLC and HPLC



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 is a reduction of analysis time, which also meant reduced solvent consumption. UPLC can offer significant improvements in speed, sensitivity and resolution compared with conventional HPLC.

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