International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 1 Jan-Feb 2022, pp: 31-36 www.ijprajournal.com ISSN: 2249-7781

Ultra High Performance Liquid Chromatography (Uplc): A New Look In Analysis-A Review

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Submitted: 01-01-2022 Accepted: 10-01-2022

ABSTRACT
UPLC is an advance technique of liquid chromatography where it takes advantage of innovation in various technologies such as instrumentation and particle size to achieve dramatic increases in resolution, speed and sensitivity of the liquid chromatography. It operates at higher pressure than that used in HPLC and uses fine particles (less than 2.5µm) & mobile phases at high linear velocities. UPLC Technology is now applied throughout the world produce quality data with reproducible and robust methods as compared to the conventional HPLC. UPLC can be hyphenated with other techniques such as Mass spectrometer (MS), Ion chromatograph (IC), Nuclear magnetic resonance spectrometer (NMR) and Infrared spectrometer (IR) etc. This technique provides unique end-to-end solutions for all industries and has found application in various fields such as pharmaceutical, food, environmental, forensic, toxicology and pesticide. Separation efficiency remains maintained or is even improved by UPLC.

KEYWORD: UPLC, Theory, Detectors, 2.5µm, Advantage, Applications.

I. INTRODUCTION

From past 30 year HPLC is the predominant technique to use for analysis in Laboratory but due to significant advances and innovation in instrumentation, detector design, data processing and particle size technology, leads to the development of Ultra Performance Liquid Chromatography (UPLC). Principle of UPLC basically remains same only with the help of technology; it achieves dramatic increases in resolution, speed and sensitivity of the liquid chromatography. UPLC Technology is applied throughout the world for providing advantage of time, cost and quality. Beside this it is also reproducible result and robust method as compared to conventional HPLC.

THEORY

Separation in chromatography can be explained by Van Deemter equation which gives relation of resolving power of chromatographic column with various flows and Kinetic mass transfer.

HETP = A + B/u + Cu,

Where, HETP is height equivalent to theoretical plate A is eddy diffusion B is longitudinal diffusion C is mass transfer and u is linear velocity (flow rate) Eddy diffusion (A) is caused by a turbulence in the solute flow path and is mainly unaffected by flow rate, but changes with particle size. Smaller the particle size less is the eddy diffusion. Longitudinal diffusion (B) is related the movement of an analyte molecule outward from the center to the edges of its band. Thus higher the lineary velocities will limit the outward distribution, keeping the band tighter. Mass transfer (C) is the movement of analyte, or transfer of its mass, between the mobile and stationary phases. Through this type of diffusion, increased flows have been observed to widen analyte bands or lower peak efficiencies.

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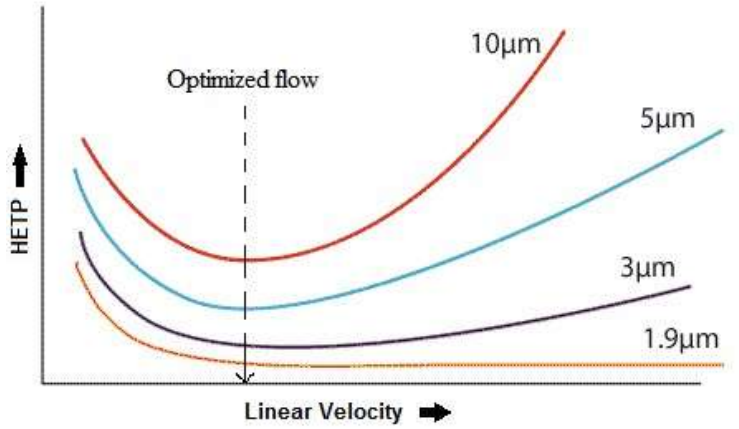

Fig. 1 - Van Deemter plots for various particle sizes

Fig-1 indicates that the decrease in particle size results in an increase in efficiency of column and on the other hand increase in linear velocity (flow rate) increase the efficiency for the column for particle size less than 1.9 µm and after the optimized flow it remains same, while for column with particle size greater than 1.9µm, efficiency again decrease after certain optimized flow.

INSTRUMENTATION

Pumping System

Achieving small particle, high peak capacity separations requires a greater pressure range than that achievable by today's HPLC instrumentation. The calculated pressure drop at the optimum flow rate for maximum efficiency across a 15 cm long column packed with 1.7 µm particles is about 15,000 psi. Therefore a pump capable of delivering solvent smoothly and reproducibly at these pressures, which can compensate for solvent compressibility and operate in both the gradient and isocratic separation modes, is required. The binary solvent manager uses two individual serial flow pumps to deliver a parallel binary gradient. There are built-in solvent select valves to choose from up to four solvents. There is a 15,000-psi pressure limit (about 1000 bar) to take full advantage of the sub-2µm particles.

There are two types of pumps:

1. Reciprocating pump
2. Pneumatic pump

1) Reciprocating pump These types of pump operate by using a reciprocating piston or diaphragm. The liquid enters a pumping chamber via an inlet valve and is pushed out via a outlet valve by piston Reciprocating pumps are generally very efficient and are suitable for very high flows.

There are two general types of reciprocating pumps.

A) The piston pump

B) The diaphragm pump.

There are two types of diaphragm pumps. The hydraulically operated diaphragm metering pumps.This type of pump can be used for pumping toxic and explosive fluids. The pump can deliver heads of up to 700 bar and transfer flows of up 20 m 3 /hr.

The air actuated type: The pump capacity is limited by the air pressure available (generally 7 bar) and the design of the diaphragm. A flow rate of about 40 m3 /hr is a reasonable maximum achievable flow with a larger pump.

2) Pneumatic pump;

This type of piston was originally used for normal liquid chromatography separations but was found to be noisy and produced strong flow pulses that destabilized the detector. It is now used almost exclusively for slurry packing liquid chromatography columns. It is the simplest type of pump that can be designed to provide exceedingly high pressures.

Sample Injector

In UPLC, sample introduction is critical. Conventional injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from extreme pressure fluctuations, the injection process must be relatively pulse free and the swept volume of the device also needs to be minimal to reduce potential band spreading. A fast injection cycle time is needed to fully capitalize on the speed afforded by UPLC, which in turn requires a high sample capacity. Low volume injections with minimal carryover are also required to increase

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sensitivity. There are also direct injection approaches for biological samples.

Sample Manager

The sample manager also incorporates several technology advancements. Using pressure assisted sample introduction, low dispersion is maintained through the injection n process, and a series of pressures transducers facilitate self-monitoring and diagnostics. It uses needle in-needle sampling for improved ruggedness and needle calibration sensor increases accuracy. Injection cycle time is 25 seconds without a wash and 60 sec with a dual wash used to further decrease carry over. A variety of micro liter plate formats (deep well, mid height, or vials) can also be accommodated in a thermostatically controlled environment. Using the optional sample organizer, the sample manager can inject from up to 22 micro liter plates. The sample manager also controls the column heater.Column temperatures up to 65°C can be attained. To minimize sample dispersion, a "pivot out "design allows the column outlet to be placed in closer proximity to the source inlet of an MS detector.

There are two types of columns:

1.Analytical column

2.Guard column

1. Analytical column

Different columns used in UPLC are Pro C18, Pro C8, Hydrosphere C18, YMC30, YMC basic.

2. Guard column

A guard column and retention gap is the same thing, but they serve different purposes. Both are 1-10 meters of deactivated fused silica tubing attached to the front of the column. Deactivated fused silica tubing does not contain any stationary phase; however, the surface is deactivated to minimize solute interactions. A suitable union is used to attach the tubing to the column. In most cases, the diameter of the retention gap or guard column should be the same as the column. If the tubing sizes are different, it is better to have a larger diameter guard column or retention gap than a smaller one. Guard columns are used when samples contain non-volatile residues that may contaminate a column. The non-volatile residues deposit in the guard column and not in the column. This greatly reduces the interaction between the residues and the sample since the guard column does not retain the solutes (because it contains no stationary phase). Also, the residues do not coat the stationary phase which often results in poor peak shapes. Periodic cutting or trimming of the guard column is usually required upon a build-up of residues. Guard columns are often 5-10 meters in length to allow substantial trimming before the entire guard column has to replace. The onset of peak shape problems is the usual indicator that the guard column needs trimming or changing.

Advantages

• 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
• Delivers real-time analysis in step with manufacturing processes
• Assures end-product quality, including final release testing

Disadvantages:

Due to increased pressure requires more maintenance and reduces the life of the columns of this type.

Column Heater

The column heater heats the column compartment to any temperature from 50C to 650C.

Detectors

TUV Detector (Tunableultraviolet detector)

The analytical cell, with a volume of 500 neon liters and a path length of 10 mm, and high sensitivity flow cell, with a volume of 2.4 micro liters and a 25 mm path length, both utilize the flow cell technology. The TUV detector operates at wavelengths ranging from 190 to 700 nm.

PDA Detector (Photo diode array detector)

The PDA (photodiode array) optical detector is an ultraviolet/visible light (UV/Vis) spectrophotometer that operates between 190 and

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500 nm. The detector offers two flow cell options. The analytical cell, with a volume of 500 nanoliters and a path length of 10 mm, and high sensitivity flow cell, with a volume of 2.4 micro liters and a 25 mm path length, both utilize the flow cell technology.

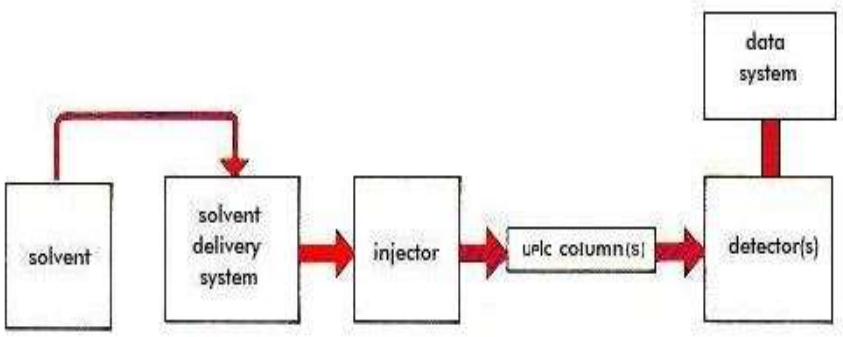

Fig. 2 – Schematic diagram of UPLC

Comparison between UPLC and HPLC

The sensitivity and flexibility of exact mass time-of-flight mass spectrometry with alternating collision cell energies, combined with the high resolving power of the UPLC system, allows for the rapid profiling and identification of impurities.

Characteristics	HPLC	UPLC
Particle size	to 5m	Less than 2m
Maximum backpressure	35-40 MPa	103.5 MPa
Analytical column	Alltima C	Acquity UPLC BEH C
Column dimensions	X 3.2 mm	X 2.1 mm
Column temperature	°C	°C

ADVANTAGE OF UPLC OVER HPLC

UPLC has many advantages over conventional HPLC of which major advantages are speed, quality and cost of analysis. Due to development of particle size technology and the use of sub micron particle size in column packing, significantly reduced in the analysis run time which result in the development of faster methods and faster analysis of samples. Compound separated on UPLC are more resolved as compared to conventional HPLC also peak capacity is also increased (number of peaks resolved per unit time). Sensitivity of the method is increased up to 3- 5 fold. Band spreading is reduced due to the population of analyte molecule is more concentrated in UPLC resulting in a higher plate count.

Following figure-3 show the comparison of UPLC and HPLC chromatogram.

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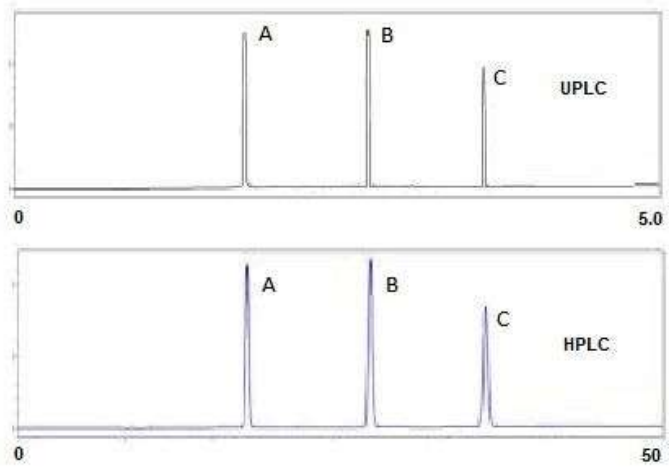

Fig. 3 – Chromatographic separation comparison on UPLC and HPLC

The time spent in optimizing and validating new methods is greatly reduced with the use of UPLC. Mobile phase solvent consumption for the analysis is greatly reduced due to low flow rate and short run time. Reduces process cycle times, so that more product can be produced with existing resources. Real time analysis by UPLC reduced the cost of failure of product and process control. UPLC system can be hyphenated with various techniques for which it finds application in vast areas. Conventional HPLC are nowadays are replaced with UPLC considering greater commercial benefit, superior sensitivity, resolution, speed and sample throughput.

APPLICATIONS OF UPLC

Pharmaceutical analysis

UPLC finds major application in pharmaceutical analysis. Methods use in drug substances and drug product analysis should be well developed and validated and these processes are much time consuming on conventional HPLC. UPLC gives scope for development and validation of analytical methods in less time. In the drug development impurity profiling is major activity where detecting and quantifying impurities in drug substance and drug product can be done by UPLC as it has good resolving power, reproducibility, efficiency and short time. As UPLC provides faster analysis of samples, this advantage can be use to monitor the inprocess and real time samples of reaction monitoring where very short time is required to control the processes thus saving the cost of failure. UPLC also finds application in dissolution testing, which is one of very important test in the formulation of drug product. This test requires uniformity reliability and batch to batch reproducibility. Metabolites studies are required in the new drug development process where main metabolite is determined and identified as rapidly as possible in the drug discovery phase. UPLC is capable of determining and identifying metabolite and biomarker structure elucidation.

Environmental analysis

Environmental sample requires innovative techniques to detect and identify the chemical contamination. UPLC provides the analysis of these samples with less time, cost and more information about sample content. Some applications of UPLC are analysis of organic component in soil, air, hazardous wastes, drinking water, wastewater, pesticide residue and perfluorinated compounds (PFCs) analysis.

Food analysis

Food manufacturer are always in search of a compressive solution for food testing thus UPLC decrease operation cost, increase productivity and provide identification of diverse chemicals in a food sample, thus providing public safety. Beside this it also offers quality and consistency of the product. UPLC is also applied for food profiling, identification of natural toxins, vitamins and pesticide residue in food product.

Forensic and Toxicological

UPLC finds good application in the identification of drug of abuse from blood, urine and oral samples. Several cannabinoids, opioids, barbiturates can be identified and analysed by UPLC. The combination of UPLC with various instruments gives the unique benefit of drug screening with excellent sensitivity and accuracy at trace level.

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II. CONCLUSION

UPLC is a powerful emerging technique which by use of advances in instrumentation and particle technology increases the productivity for pharmaceutical and other industries. This technique provides the better resolution for separating component, high sensitivity for the analysis of low concentration component and reduces time of analysis. 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. This technique can be easily hyphenated with various other techniques such as mass spectrometry, UPLC finds versatile application for impurity profiling, metabolite identification, dissolution testing and process control analysis in pharmaceutical, food, environment, forensic and Toxicological areas.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.

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