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# ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) - A REVIEW

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**ABSTRACT**

UPLC is a modern technique which gives a new direction for liquid chromatography. UPLC refers to ultra performance liquid chromatography, which enhance mainly in three areas: "speed, resolution and sensitivity. Ultra performance liquid chromatography (UPLC) applicable for particle less than 2µm in diameter to acquire better resolution, speed, and sensitivity compared with high-performance liquid chromatography (HPLC). In twenty first centenary pharmaceutical industries are focusing for new ways to in economy and shorten time for development of drugs. UPLC analysis at the mean time gives the better quality of their products and analytical laboratories are not exception in this trend. The separation and quantification in UPLC is done under very high pressure (up to 100M Pa). As compare to HPLC, under high pressure it is observed that not any negative influence on analytical column and also other components like time and solvent consumption is less in UPLC.

## INTRODUCTION

UPLC is regarded as new invention for liquid chromatography. UPLC brings drastic changes in sensitivity, resolution and speed of analysis can be calculated. It has instrumentation that can perform at higher pressure as compared to that used in HPLC & in this system uses fine particles (less than 2.5 µm) and mobile phases at maximum linear velocities reduces the length of column also reduces solvent consumption and saves time. This review introduces working principle of UPLC along with some of the most recent work in the field. According to the van Deemter equation, as the size of particles reduces to below 2.5 µm, there is a significant gain in efficiency. Therefore, by using smaller particles, speed and peak capacity can be extended to new limits, of liquid chromatography [(1-5).] High performance liquid chromatography (HPLC) has proven to one of the most and predominant technology used in analytical laboratories for the analysis of drugs worldwide during the past 30- plus years [(6,7)]. One of the basic concerns for the growth of this technique is the packing material which effects the separations. In this separation mechanism the principal apply is Van Deemeter equation, with which any student of chromatography is intimately familiar. H=A+B/v +Cv. The above equation is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP, or column efficiency). And, since particle size is one of the variables, a Van Deemter curve can be used to investigate chromatographic performance. Where A, B and C are constants and v is the linear velocity, the carrier gas flow rate.

A= Eddy mixing

B =Axial diffusion

C=Solute's mass transfer

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 [8-10].

**PRINCIPLE**

The UPLC is based on the principle of adsorption. It involves stationary phase consisting of particles sub 2 µm, as HPLC columns are typically filled with particles of 3 to 5 µm. According to Van Demeter equation, smaller particles provide not only increased efficiency, but also the ability to do work at increased linear velocity without a loss of efficiency, providing both resolution and speed. Efficiency is the primary separation parameter behind UPLC since it relies on the same selectivity and retentivity as HPLC. In the fundamental resolution equation resolution is proportional to the square root of *N*.

$$Rs = \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{\kappa}{\kappa+1}\right)$$

Where *N* is number of theoretical plates, $\alpha$ is Selectivity factor and *k* is mean retention factor. But since *N* is inversely proportional to particle size (*dp*);

$$N \propto \frac{1}{dp}$$

As the particle size is lowered by thrice i.e. from 5 mm to 1.7 mm, *N* is increased by three and the resolution by square root of three i.e. 1.7. *N* is also inversely proportional to the square of the peak width.

$$N \propto \frac{1}{\omega^2}$$

This illustrates that the narrower the peaks they are easy to separate from each other. Also peak height is inversely proportional to the peak width (*w*):

$$H \propto \frac{1}{\omega}$$

So as the particle size decreases to increase *N* and subsequently *Rs*, an increase in sensitivity is obtained, since Narrower peaks also mean more peak capacity per unit time in gradient separations, desirable for many applications such as natural extracts, peptide maps etc. Still another equation comes in to force from the Van Demeter plot when moving toward smaller particles:

$$Fopt \propto \frac{1}{dp}$$

As particle size decreases, the optimum flow rate ($F_{opt}$) reaches to maximum *N*. But since back pressure is proportional to flow rate, smaller particle sizes require much higher operating pressure and a system properly designed for the same. Higher resolution and efficiency can be level further when analysis speed is the primary objective. Efficiency is proportional to column length and inversely proportional to the particle size:

$$N \propto \frac{L}{dp}$$

Therefore the column can be shortened by the same factor as the particle size without loss of resolution. Using a flow rate three times higher due to smaller particles and shortening the column by one third, the separation is completed in 1/9$^{th}$ the time while maintaining resolution.

**COLUMNS OF UPLC**: Column researchers and manufacturers have encountered need with the development of more efficient and more reliable packing materials. One of the areas in which improvements have been made is the particle size reduction. According to chromatographic theory, column efficiency (N) is inversely proportional to particle size (d p). Thus, smaller particles provide higher resolution and lower retention time. Resolution is increased in a 1.7µm 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 separations.

(i) ACQUITY UPLCTM BEH C8 (straight chain alkyl columns),

(ii) ACQUITY UPLCTM BEH C18 (straight chain alkyl columns),

(iii) ACQUITY UPLC BEH Shield RP18 (embedded polar group column) and

(iv) ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl), ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl).

**Types of capillary columns**

**1. Packed capillary columns**

Packed columns are made by padding the capillary with silica-modified particles of 3–5 μm particles of even smaller sizes 1.5–1.8 μm were successfully employed in UPLC. Such a small particle size provides chromatography systems with higher efficiency, resolution, selectivity, and shorter analysis time; but does not increase the back-pressure. Many research labs pack the columns "in-house". But it is a difficult and skill-demanding process. To ascertain the particles of the same diameter and to avoid undesirable void volumes. The application of packed capillary columns is the most explored option in UPLC.

**2. Monolithic Capillary Column:** Monoliths are a block of continuous materials made of highly porous rods with two types of pore structures (macropores and mesopores of different sizes), which allow the use of higher flow rates and thus reduces the analysis time. Monolithic polymer columns were first used in the late 1980s, but not available until 2000. Presently four types of monolithic capillary columns can be found: particle fixed, silica based, polymer based, and molecular imprinted monolith. Now a day there is not much research information on application of monolithic capillary in UPLC.

**3. Open tubular capillary columns:** In open tubular liquid chromatography column, the capillary wall is coated with highly permeable porous material that serves as the stationary phase. The OT capillary has lower sample loading capacity of the column, because only a small surface area is available for analytes interaction that can result in column overloading causing peak asymmetry and poor efficiency.

## APPLICATION OF UPLC

**1. UPLC with MS**

UPLC coupled to quadrupole tandem mass spectrometry which operates with rapid, generic gradients and shows increase in analytical throughput and also shows sensitivity in high throughput pharmacokinetics or bioanalysis studies, the rapid measurement of potential p450 inhibition, induction, and drug-drug interactions had been studied by UPLC/MS/MS. As UPLC based approach can helps to the labs pre-emotively and determine candidate toxicity and drug-drug interactions, it enables organizations to be more confident in the viability of candidate medicines that do progress to late-stage clinical trials. The UPLC/ TM technology is based on the use of columns packed with 1.7μm porous particles enabled to improve in peak resolution, sensitivity and speed of analysis. UPLC TM chromatograms shows a very sharp peaks with less than 2 s wide at the base, This enhanced efficiency resulted in an increased separation speed of the whole UPLC TM-MS/MS procedure that required less than 5 min.

**2. UPLC in Pharmaceutical Development**

Now a day's UPLC is a very attractive tool for the pharmaceutical development laboratory because of high resolution obtained in extremely short period of analysis times, as UPLC provides high throughput, high productivity and high resolution. These categories have specific pharmaceutical applications and distinct separation goals. Now these goals have been achieved by utilizing conventional UPLC with typical column dimensions and particle sizes. The recent introduction of UPLC has provided a new potential for method development and analysis. Pharmaceutical chemists determine the impact of this emerging technology, using sub2 μm particle size column along with increased linear velocities. As mobile phase viscosity must be minimized or the chromatography system must be redesigned to withstand an increased backpressure. Today, there are many commercially available UPLC systems capable of exceeding conventional pressure limits of

400 bars. The advantage of UPLC over conventional HPLC is the capability to increase the speed without sacrificing efficiency. In comparison to traditional HPLC, The research showed that UPLC can decrease run times up to 7×. In addition, for high resolution applications, UPLC achieved significant efficiency advantages over traditional HPLC.

**3. UPLC used in Identification of Metabolite:** Biotransformation of new chemical entities (NCE) is necessary for drug discovery. When a compound reaches the trial stage, metabolite identification is required and it is necessary for lab to successfully detect and identify all circulating metabolites of a contender drug. Discovery studies are generally carried out *in vitro* to identify major metabolites so that metabolic weak spots on the drug contender molecule can be recognized and protected by changing the compound structure. The key for analysts in metabolite identification is maintaining high sample throughput and providing results to medicinal chemists as soon as they are available. UPLC/MS/MS addresses the complex analytical requirements of biomarker discovery by offering unmatched sensitivity, resolution, dynamic range, and mass accuracy. Tandem quadrupole MS combines with UPLC in ADME screening for sensitivity and selectivity with fast analyses of samples in matrix to be achieved with minimal cleanup, using MRM (multiple reaction monitoring) for detection and automated compound optimization.

**4. UPLC used in Bio analysis / Bioequivalence Studies**

For Pharmacokinetic, Bioequivalence and toxicity studies, the quantitative analysis of a drug in biological samples is an important part of drug development process and this is carried out by UPLC. The drugs having low molecular weight are tested during both preclinical and clinical studies, as several biological matrices are used for quantitative bioanalysis, the most common being used are the blood, plasma, and urine. The primary technique for quantitative bioanalysis of new entity is LC/MS and the sensitivity and selectivity of UPLC/MS/MS at low detection levels generates accurate and reliable data that can be used for a variety of different purposes, including statistical pharmacokinetics (PK) analysis. UPLC/MS/MS develop a robust and compliant assay which has been traditionally the domain of very experienced analysts. UPLC/MS/MS helps in the method development for bioanalytical products. It helps to determine whether new formulations of existing drugs allow the compound to reach the bloodstream at a similar rate and exposure level as the original formulation.UPLC/MS/MS has proven to increase efficiency, productivity, and profitability at bioequivalence laboratories. An application of UPLC/MS/MS in bioequivalence and bioanalysis gives efficient separations with fast acquisition rates using tandem quadrupole MS systems, which easily acquire report and quantify full system data in a compliant environment. And ensure the highest quality results and reliable system operation in regulated environment. UPLC is sophisticated and integrated system for bioanalysis and bioequivalence studies, providing excellent chromatographic resolution and sensitivity along with multiple reactions monitoring.

**5. UPLC used in stressed degradation Studies**

One of the most important factors that impacts the quality and safety of pharmaceuticals is chemical stability. The FDA and ICH Guideline provide stability testing data to understand how the quality of an API (active pharmaceutical ingredient) or a drug product changes with time under the influence of forced degradation factors such as heat, light, pressure and moisture or humidity. Knowledge of these stability characteristics helps in storage conditions and shelf life, the selection of proper formulations and protective packaging is required for regulatory documentation. Forced degradation, is carried out under even harsher conditions than those used for accelerated stability testing. The test generally performed early in the drug development process, as laboratories cause the potential drug to degrade under a variety of conditions like peroxide oxidation, acid and base hydrolysis, photo stability, and temperature to understand resulting by products and pathways that are necessary to develop stability indicating methods. The most common analytical technique for monitoring forced degradation experiments is HPLC with UV and/or MS detection for peak purity, mass

balance, and identification of degradation products but these HPLC-based methodologies are time-consuming and provide only medium resolution to ensure that all of the degradation products are accurately detected. PDA/MS (photodiode array and MS) used along with UPLC, which allows for faster and higher peak capacity separations of complex degradation product profiles.

**6. UPLC used in Impurity Profiling;** Impurity profiling is an important task for the drug development and formulation process, which helps in detecting, profiling and quantifying drug substances and their impurities in raw materials and final product. As impurity profiling requires high-resolution chromatography which is capable of reliably and reproducibly separating and detecting all of the known and unknown impurities of the active compound. It is critical assignment to accurately measure low-level impurities at the same time as the higher concentration active pharmaceutical component. UPLC system and columns specifically gives high-throughput analysis along with high peak resolution. UPLC PDA detector involves two analytical flow cells with maximum flexibility and according to application requirements, as one for maximum chromatographic resolution and a second for high sensitivity. UPLC also ensure the latest peak detection algorithms and custom calculations to optimize data processing and reporting. It also assertively detects impurities in compounds even at trace levels. To characterize impurities, it is often necessary to perform several analytical runs to obtain the necessary MS and MS/MS data. UPLC combines with exact MS, which operate with alternating low and high collision energies, known as UPLC/MS/MS, which has been successfully employed for the identification of drug and endogenous metabolites. The rapid switching of the collision cell energy produces both precursor and product ions of all of the analytes in the sample while maintaining a sufficient number of data points across the peak for reliable quantification.

**7. UPLC used in Dissolution Testing**

Dissolution testing is essential in the formulation and development along with production process in association with quality control. In sustained-release and metered release dosage formulations, testing of higher potent drugs is particularly important where dissolution can be the rate-limiting step in medicine delivery. The dissolution profile is used to demonstrate reliability and batch-to-batch uniformity of the active ingredient and dosage form. Additionally, newer and more potent formulations require increased analytical sensitivity. UPLC provides precise and reliable automated online sample acquisition.

**8. UPLC fingerprint:** It can be used for the identification of *Magnolia officinalis* cortex [11-17].

**ADVANTAGES OF UPLC**

UPLC allows faster laboratory method development with maintaining resolution, selectivity, sensitivity, and dynamic range of LC analysis. As it reduces process cycle times, so that more product can be analyzed with existing resources. It assures quality results with decrease in run time, cost and increases sensitivity of sample analysis along with less solvent consumption. UPLC increases sample through the use of a novel separation material of very fine particle size 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. It's fast resolving power quickly quantifies related and unrelated compounds.

**DISADVANTAGES OF UPLC**

The performance of UPLC may be similar or even higher that have been demonstrated by using stationary phases of size around 2 µm without the adverse effects of high pressure. The phases of less than 2 µm are generally non-regenerable and thus have limited use as increased pressure it requires more maintenance and reduces the life of the columns.

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