ISSN: 0975-766X
CODEN: IJPTFI
Review Article

Available through Online
www.ijptonline.com

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY: A RECENT DEVELOPMENT IN HPLC

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Received on 18-11-2011 *Accepted on 10-12-2011*

Abstract

UPLC is a new liquid chromatographic technique. It is latest version of HPLC. UPLC Stand for "Ultra Performance Liquid Chromatography". It improves speed, resolution, and sensitivity. In this system uses fine particles (less than 2.5μm) so decreases the length of column, saves time and reduces solvent consumption.

UPLC which involves with very high pressure and column having very small particle size. The factor responsible for the development of the technique was advance of packing materials used to effect the separation. The principles behind this progress are governed by the van Demeter equation that describes the relationship between linear velocity and plate height.

Special analytical columns UPLC BEH C18 packed with 1.7 μm particles are used in connection with this system. The quality control analyses of various pharmaceutical formulations are transferred from HPLC to UPLC system. The UPLC system allows shortening analysis time up to nine times and three times comparing to the conventional system using 5 μm and 3 μm particle packed analytical columns respectively. The negative effect of particle size decrease is back-pressure increase about nine times (versus 5 μm) or three times (versus 3 μm), respectively. 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.

Keyword: Ultra Performance Liquid Chromatography UPLC, High separation efficiency, Cost effective, High pressure UPLC, Calibration of UPLC.

Introduction

UPLC refers to Ultra Performance Liquid Chromatography. It improves chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption.[1]

UPLC comes from HPLC. HPLC has been the progress of the packing materials used to effect the separation. An underlying principle of HPLC dictate 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. It is 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 phase. The Van Deemter 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 bars. [1, 2]

This technology takes full benefit of chromatographic principles to run separations using columns packed with smaller particles and/or higher flow rates for increased speed, with superior resolution and sensitivity.[1] The use of non-porous particles, however, has been limited in the pharmaceutical industry due to their low sample loading capacity. The Milford, Massachusetts based company Waters Corporation introduced ACQUITY UPLC, the commercially available system that addresses the challenge of using elevated pressure and 2 µm particles, which makes it a particularly attractive and promising tool for fast Liquid Chromatographic method development.[2]Engineering challenges of operating at high pressures and the high performance expected from such columns necessitates new developed pumps, redesigned injector, reduced system volumes, an increased detector sampling rate, and other improvements. To be suitable for the analysis of pharmaceutical development samples under GMPs, the UPLC instrument and columns must not only deliver on its promises for fast, high resolution separations but do so reproducibly and with the required sensitivity.[2]

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. These are the benefits of faster analysis and hence the ultra performance liquid chromatography.[3]

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 Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency). The Van Deemter curve, governed by an equation with three components shows that the usable flow range for a good efficiency with small diameter particles is much greater than for larger diameters.[7]

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

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 diffusion". It is smallest when the packed column particles are small and uniform. The *B* term represents longitudinal diffusion, refers to the diffusion of individual analyte molecules in the mobile phase along the longitudinal direction of a column. Longitudinal diffusion contributes to peak broadening only at very low flow rates below the minimum plate height. 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. The advent of UPLC has demanded the development of a new instrumental system for

liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent with the pressures (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC). Efficiency of column is directly proportional to column length and inversely proportional to the particle size. Therefore, the column can be shortened by the same factor as the particle size without loss of resolution. The application of UPLC resulted in the detection of additional drug metabolites, superior separation and improved spectral quality.[9]

Small particle chemistry:

The promises of the van Deemter equation cannot be fulfilled without smaller particles than those traditionally used in HPLC. The design and development of sub-2 mm particles is a significant challenge, and researchers have been active in this area for some time to capitalize on their advantages. Although high efficiency, non-porous 1.5 mm particles are commercially available, they suffer from poor loading capacity and retention due to low surface area. To maintain retention and capacity similar to HPLC, UPLC must use novel porous particles that can withstand high pressures. Silica based particles have good mechanical strength, but can suffer from a number of disadvantages, which include a limited pH range and tailing of basic analytes. Polymeric columns can overcome pH limitations, but they have their own issues, including low efficiencies and limited capacitie.[7]

In 2000, Waters introduced XTerra®, a first generation hybrid chemistry that took advantage of the best of both the silica and polymeric column worlds. XTerra columns are mechanically strong, with high efficiency, and operate over an extended pH range. They are produced using a classical sol-gel synthesis that incorporates carbon in the form of methyl groups. But in order to provide the necessary mechanical stability for UPLC, a second generation bridged ethyl hybrid (BEH) technology was developed. Called ACQUITY BEH, these 1.7 µm particles derive their enhanced mechanical stability by bridging the methyl groups in the silica matrix.In addition, at high pressures, frictional heating of the mobile phase can be quite significant and must be considered. With column diameters typically used in HPLC (3.0 to 4.6 mm), a consequence of frictional heating is the loss of performance due to temperature induced non uniform flow. To minimize the effects of frictional heating, smaller diameter columns (1–2.1 mm) are typically used for UPLC.[6, 14]

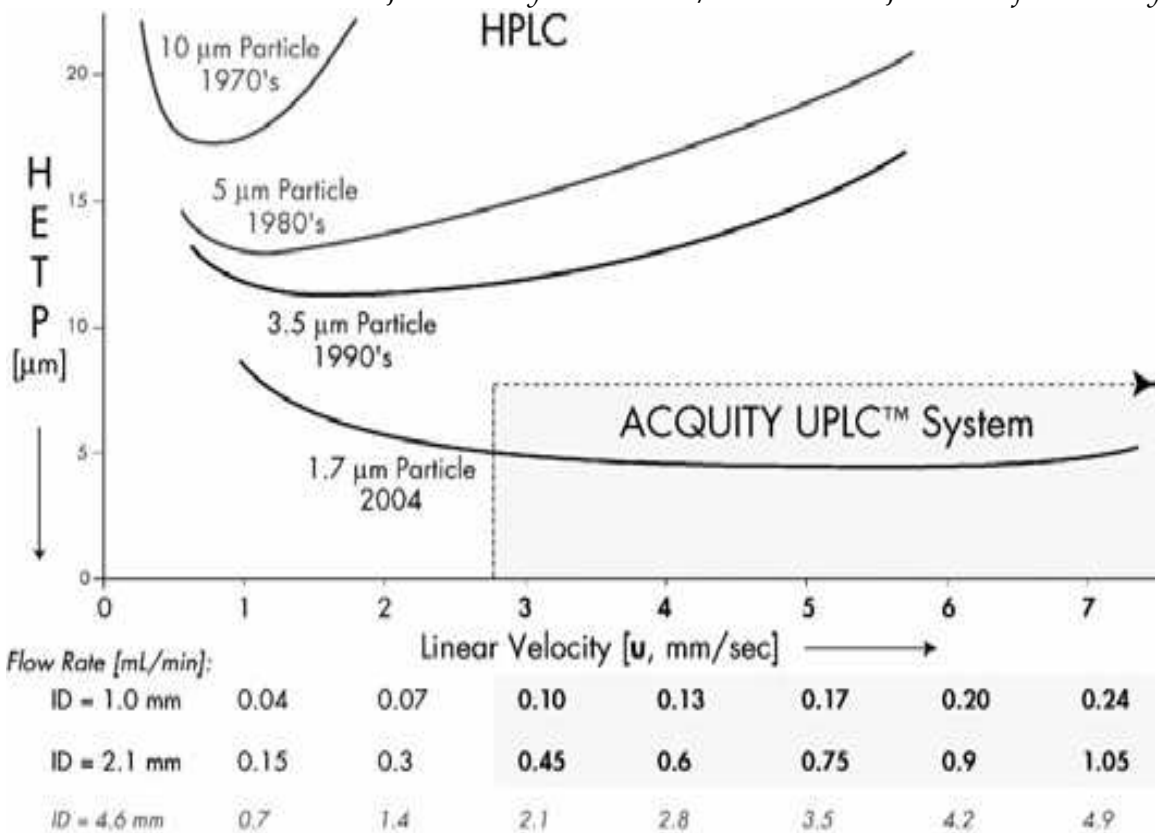

Figure2: Van Deemter plot, illustrating the evolution of particle sizes over the last three decades.[6,14].

According to van Deemter 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 [13]; resolution is proportional to the square root of *N*.

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

Where *N* is number of theoretical plates, α 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 [13].

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

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

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

So as the particle size decreases to increase *N* and subsequently *Rs*, an increase in sensitivity is obtained, since narrower peaks are taller peaks. 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 Deemter plot when moving toward smaller particles:

$$F_{opt} \propto \frac{1}{dp}$$

As particle size decreases, the optimum flow rate (F_{opt}) to reach maximum *N* increases. 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.[7,14]

CAPITALIZING ON SMALLER PARTICLES:

Small particles alone do not make it possible to fulfill the promises of the van Deemter equation. Instrument technology also had increased speed, superior resolution, and sensitivity afforded by smaller particles. Standard HPLC technology (pumps, injectors, and detectors) simply doesn't have the horsepower to take full advantage of sub-2 mm particles. [7]

Jorgenson et al. modified a commercially available HPLC system to operate at 17,500 psi and used 22 cm long capillaries packed with 1.5 mm C18-modified particles for the analysis of proteins 15. These reports illustrated that, to take full benefit of low dispersion and small particle technology to achieve high peak capacity UPLC separations, a greater pressure range than that realizable by HPLC instrumentation was required. The calculated pressure drop at the optimal flow rate for maximum efficiency across a 15 cm long column packed with 1.7 mm particles is about 15,000 psi. Therefore, a pump capable of delivering solvent smoothly and reproducibly at these pressures, that can reimburse for solvent compressibility, and can operate in both the gradient and isocratic separation modes, was required. Sample introduction is also critical. Conventional injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from experiencing extreme pressure fluctuations, the injection process must be relatively pulse-free. 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 realize the increased sensitivity benefits. With 1.7 mm particles, half-height peak widths of less than one second are obtained, posing significant challenges for the detector. In order to accurately and reproducibly integrate an analyte peak, the detector sampling rate must be high enough to capture enough data points across the peak. In addition, the detector cell must have minimal dispersion (volume) to preserve separation efficiency. Conceptually, the sensitivity increase for UPLC detection should be 2-3 times higher than with HPLC separations, depending on the detection technique that is used. [14]

In early 2004, the first commercially available UPLC system that embodied these requirements was described for the separation of various pharmaceutical related small organic molecules, proteins, and peptides; it is called the ACQUITY UPLC System. Using UPLC, it is now possible to take full benefit of chromatographic principles to run separations using shorter columns, and/or higher flow rates for increased speed, with superior resolution and sensitivity.

USE OF THE 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.[29,30] Monolithic columns contain a polymerized porous support structure that provide lower flow resistances than conventional particle-packed columns.[19,20,31]

INSTRUMENTATION: [9, 10]

The ACQUITY UPLC System consists of

1. A Binary solvent Manager.
2. Sample Manager (including the column heater).
3. Detector and Optional Sample Organizer.

A Binary solvent Manager:

It uses two individual serial flow pumps to deliver a parallel binary gradient mixed under high pressure. There are built in solvent degassing as well as 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- 2-µm particles.

The Sample Manager:

The injection device should be pulse free and have the following characteristics: a small swept volume to reduce possible band spreading, fast injection cycle, high sample capacity, automated operation over long periods, low injection volumes with minimal carryover, and temperature control. Sample volumes of 2-5 µL are typical in UPLC. The Acquity Sample Manager has the following features: use of two SBS (Society for Bimolecular Screening)-compliant microliter plates or vial holders in any combination, temperature control from 4- 40 0C, needle-in-needle sample probe for injection of 1 µL out of 4 µL for sample-limited applications, and pressure-assisted injection of 0.1-50 µL volumes. The optional Sample Organizer extends sample capacity to 22 standard microliter plates, 14 intermediate plates and vial holders, or 8 deep-well plates or vial holders, with random access of any combination for transport into the injection compartment for processing. Overall system dead volume (tubing and

fittings) must be minimized as much as possible and leak-free valves and connections are needed for successful UPLC. Empower or Mass Lynx software provides instrument control, monitoring, and diagnostic capabilities.

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

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

PDA Detector:

It includes new electronics and firmware to support Ethernet communications at the high data rates necessary for UPLC detection. Conventional absorbance-based optical detectors are concentration-sensitive detectors, and for UPLC, the flow cell volume would have to be reduced in standard UV-visible detectors to maintain concentration and signal. The ACQUITY UPLC System detector cell consists of a light guided flow cell equivalent to an optical fiber. Light is efficiently transferred down the flow cell in an internal reflectance mode that still maintains a 10-mm flow cell path length with a volume of only 500 mL. 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.

The detector must have a high sampling rate for sensitive detection and reliable quantification of the narrow peaks produced and have minimal dispersion (volume) so the separation achieved on the column is not lost. The potential sensitivity increase of UPLC is estimated at 2-3 times that obtained by HPLC, depending on the detection method. Acquity photodiode array (PDA) and tunable Vis-UV (TUV) detectors utilize fiber optic flow cell designs that incorporate Teflon AF, an internally reflective surface to improve light transmission efficiency by eliminating internal absorptions.

UPLC COLUMNS: [20, 30, 33]

Although columns with high efficiency, non-porous 1.5 mm particles are commercially available, they suffer from poor loading capacity and retention due to low surface area. Silica-based particles have good mechanical strength but can suffer from a number of disadvantages, which include a limited pH range and tailing of basic analytes. Polymeric columns can overcome pH limitation, but they have their own issues including low efficiency, limited loading capacity and poor mechanical strength.

In 2000, XTerra® columns were introduced, a first generation hybrid chemistry that took advantage of the best of both the silica and polymeric column. XTerra columns are mechanically strong, with high efficiency, and operate over an extended pH range but in order to provide the necessary mechanical stability for UPLC; a second generation bridged ethyl hybrid (BEH) technology was developed. Resolution is increased in a 1.7 µm 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: ACQUITY UPLCTM BEH C18 and C8 (straight chain alkyl columns), ACQUITY UPLC BEH Shield RP18 (embedded polar group column) and ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl). Each column chemistry provides a different combination of hydrophobicity, silanol activity, hydrolytic stability and chemical interaction with analytes. ACQUITY UPLC BEH C18 and C8 columns 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. ACQUITY UPLC BEH Shield RP18 columns are designed to provide selectivities that complement the ACQUITY UPLC BEH C18 and C8 phases. ACQUITY UPLC BEH Phenyl 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.

An internal dimension (ID) of 2.1 mm column is used. For maximum resolution, choose a 100 mm length and for faster analysis, and higher sample throughput, choose 50 mm column.

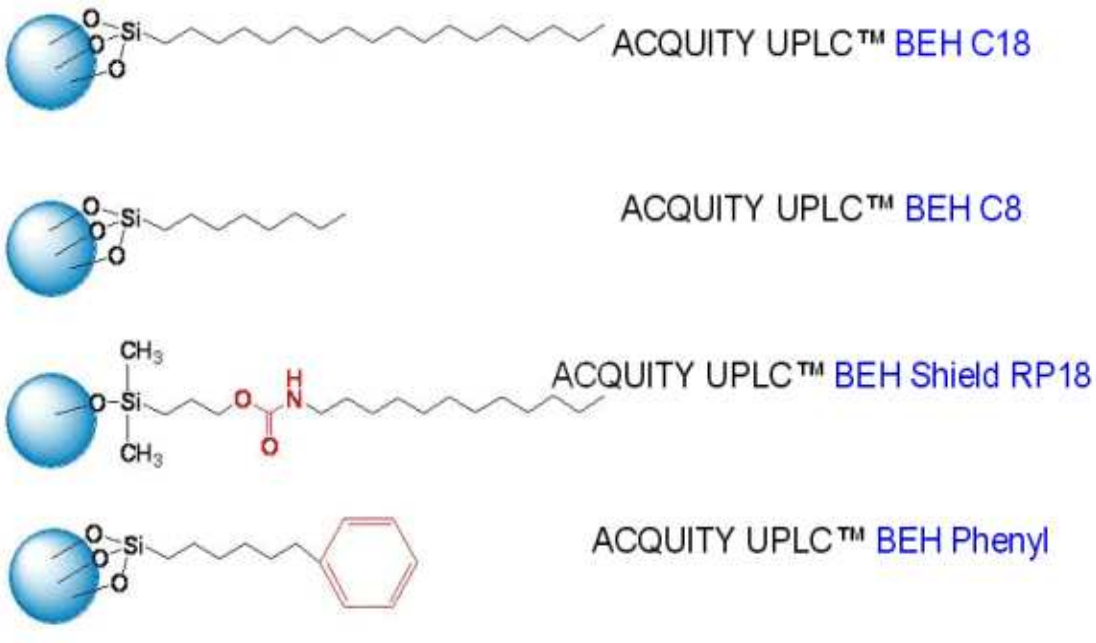

Figure4: ACQUITY UPLC BEH Column chemistries.

Half-height peak widths of less than one second are obtained with 1.7µm particles, which gives significant challenges for the detector. In order to integrate an analyte peak accurately and reproducibly, the detector sampling

rate must be high enough to capture enough data points across the peak. The detector cell must have minimal dispersion (volume) to preserve separation efficiency. Conceptually, the sensitivity increase for UPLC detection should be 2-3 times higher than HPLC separations, depending on the detection technique. MS detection is significantly enhanced by UPLC; increased peak concentrations with reduced chromatographic dispersion at lower flow rates promote increased source ionization efficiencies.[22]

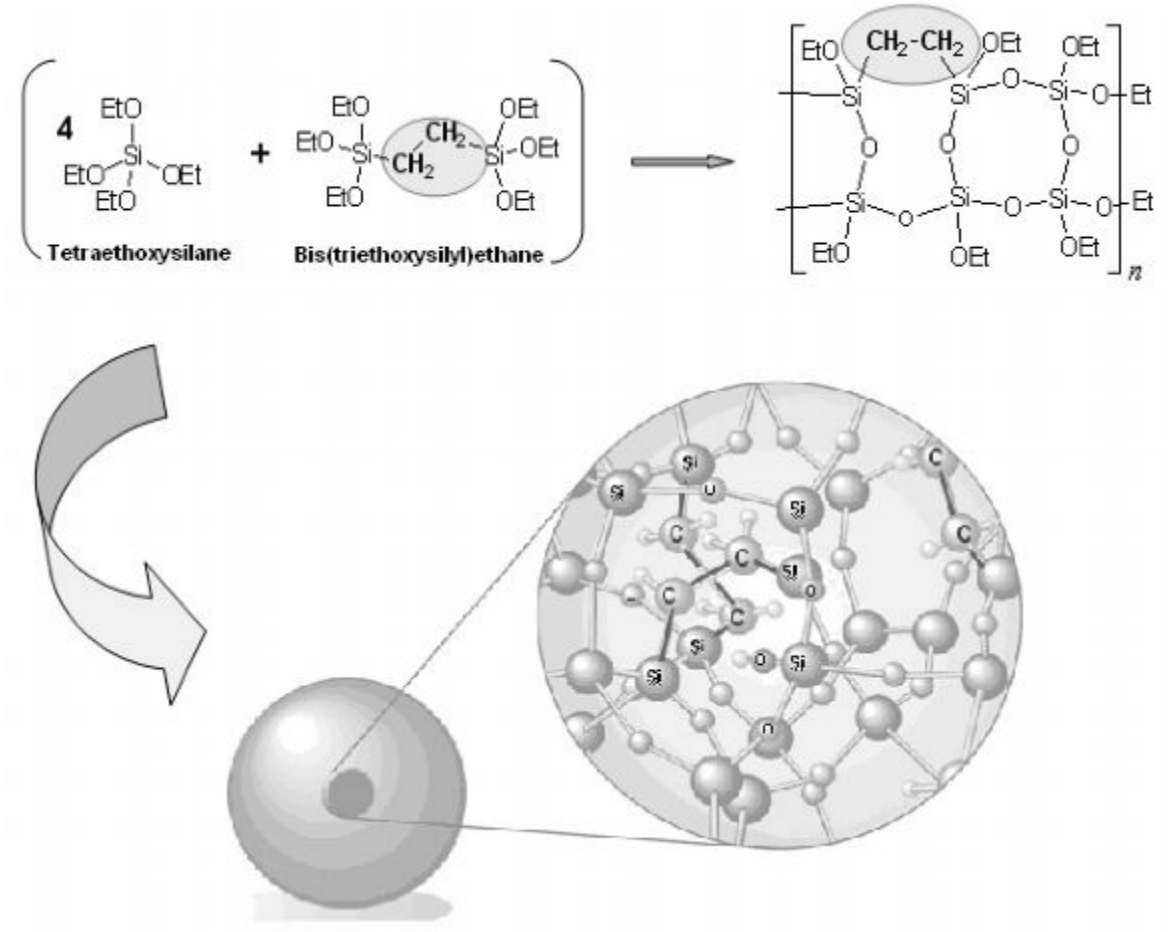

Figure5: Structure of second generation X-Terra particles.

Table-2: Difference Between HPLC and UPLC: [24, 26]

Sr. No.	Parameter	HPLC	UPLC
1	Pump	Quardinary pump	Binary Pump
2	Pressure	Up to 5000psi	Up to 15000psi
3	Auto injector	Normal speed	High speed

4	Run time	Long Run time	Short Run time
5	Solvent Consumption	More	Less
6	Sensitivity	Normal	High
7	Dwell Volume	High	Low
8	Particle size	3 to 5 µm	Less than 2 µm
9	Analytical column	Alltima C_{18}	Acquity UPLC BEH C_{18}
10	Column dimensions	150 X 3.2 mm	150 X 2.1 mm
11	Column temperature	30 °C	65 °C

Advantages: [4, 5]

1. Decreases run time and increases sensitivity
2. It increase sensitivity, selectivity and dynamic range of LC analysis
3. Expands scope of Multiresidue Methods
4. Faster analysis through the use of a novel separation material of very fine particle size
5. Operation cost is reduced
6. 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.
7. Better resolution (separation efficiency)
8. Less solvents consumption

Disadvantages:

1. Due to increased pressure requires more maintenance and reduces the life of the columns of this type.
2. 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.

3. In addition, the phases of less than 2 μm are generally non-regenerable and thus have limited use.
4. Higher price of instruments, spare parts and Columns.[5]

CALIBRATION OF UPLC: [8,9,10]

Calibration of Acquity UPLC involves following parameter

1. Flow Rate Accuracy
2. Temperature Accuracy
3. System Precision
4. Wavelength Accuracy Test
5. Detector Linearity and Detector Sensitivity Test
6. Injector Linearity and Injector Accuracy
7. Detector Noise Test
8. Flow Rate Linearity
9. Carry Over Test
10. Gradient Performance Test

Reagent: Calibration of Acquity UPLC requires following reagent.

1. Caffeine
2. Thiourea
3. Acetophenone
4. 2-acetyl furan
5. Acetanilide
6. Propiophenone
7. Benzophenone
8. Valerophenone
9. Butyl paraben

APPLICATIONS OF UPLC: [4, 5, 20, 22, 24]

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. Ultra Performance Liquid Chromatography (UPLC) fingerprint can be used for the identification of Magnoliae officinalis cortex.[4]

Analysis of Natural Products and Traditional Herbal Medicine:

UPLC is widely used for analysis of natural products and herbal medicines. For traditional herbal medicines, also known as natural products or traditional Chinese medicines, analytical laboratories need to expand their understanding of their pharmacology to provide evidence-based validation of their effectiveness as medicines and to establish safety parameters for their production.

The main purpose of this is to analyze drug samples arise from the complexity of the matrix and variability from sample to sample. Purification and qualitative and quantitative chromatography and mass spectrometry are being applied to determine active drug candidates and to characterize the efficacy of their candidate remedies.

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:

Biotransformation of new chemical entities (NCE) is necessary for drug discovery. When a compound reaches the development stage, metabolite identification becomes a regulated process. It is of the utmost importance for lab to successfully detect and identify all circulating metabolites of a candidate drug. Discovery studies are generally carried out in vitro to identify major metabolites so that metabolic weak spots on the drug candidate molecule can be recognized and protected by changing the compound structure.

ADME Screening:

Pharmacokinetics studies include studies of ADME (Absorption, Distribution, Metabolism and Excretion). ADME studies measure physical and biochemical properties absorption, distribution, metabolism, elimination, and toxicity of drugs where such compounds exhibit activity against the target disease. A significant number of candidate medicines fall out of the development process due to toxicity. If toxic reactions or any side effect occurs in the discovery/development process, then it becomes more costly. It is difficult to evaluate candidate drugs for possible toxicity, drug-drug interactions, inhibition, and/or induction of metabolizing enzymes in the body.

Bioanalysis or Bioequivalence Studies:

For pharmacokinetic, toxicity, and bioequivalence studies, quantitation of a drug in biological samples is an important part of development programs. The drugs are generally of low molecular weight and are tested during both preclinical and clinical studies. Several biological matrices are used for quantitative bioanalysis, the most common being blood, plasma, and urine.

The primary technique for quantitative bioanalysis is LC/MS/MS. 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.

Dissolution Testing:

For quality control and release in drug manufacturing, dissolution testing is essential in the formulation, development and production process. In sustained-release dosage formulations, testing higher potency 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. Additionally, newer and more potent formulations require increased analytical sensitivity. UPLC provides precise and reliable automated online sample acquisition. It automate 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.[21]

Forced Degradation Studies:

One of the most important factor that impacts the quality and safety of pharmaceuticals is chemical stability. The FDA and ICH require 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 environmental factors such as heat, light, pressure and moisture or humidity. Knowledge of these stability characteristics defines storage conditions and shelf life, the selection of proper formulations and protective packaging, and is required for regulatory documentation. Forced degradation, or stress testing, is carried out under even harsher conditions than those used for accelerated stability testing. Generally performed early in the drug development process, 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 byproducts and pathways that are necessary to develop stability indicating methods.[24]

Manufacturing / QA / QC:

Identity, purity, quality, safety and efficacy are the important factors to be considered while manufacturing a drug product. The successful production of quality pharmaceutical products requires that raw materials meet purity specifications. That manufacturing processes proceed as designed. Those final pharmaceutical products meet, and hopefully exceed, defined release specifications. UPLC is used for the highly regulated, quantitative analyses

performed in QA/QC laboratories. The supply of consistent, high quality consumable products plays an important role in a registered analytical method.

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 become as short as one minute, methods can be optimized in just one or two hours, significantly reducing the time required developing and validating.

Impurity Profiling:

For the drug development and formulation process, profiling, detecting, and quantifying drug substances and their impurities in raw materials and final product testing is an essential part. It's requires high-resolution chromatography capable of reliably and reproducibly separating and detecting all known impurities of the active compound. Also critical is the ability to accurately measure low-level impurities at the same time as the higher concentration active pharmaceutical component. This activity, however, can be complicated by the presence of excipients in the sample, often resulting in long HPLC analysis times to achieve sufficient resolution.

Conclusion:

Analysis time, solvent consumption, and analysis cost are very important in many analytical laboratories. 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.

The AQUITY UPLC system improves high speed, less time consumption and better resolution and sensitivity. This system is also reduces the noise and improve signal-to-noise ratio. Due to very narrow and sharp peaks, more number of peaks may appear in less time which may facilitate in analysis of complex mixtures and it may give more information regarding sample to be analyze.

UPLC removed the barrier of traditional chromatographic packing material by development of new, highly efficient, mechanically strong, 1.7 mm bridge hybrid particles that are stable over a broad pH operating range. This achievement enables method development to be more efficient, allowing products to be brought to market faster. Hence use of UPLC systems will become the option of choice for the development of fast LC methods in pharmaceutical development in the near future.

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