ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC): A MODERN CHROMATOGRAPHY TECHNIQUE

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ABSTRACT
UPLC can be regarded as a new direction for liquid chromatography. UPLC refers to ultra performance liquid chromatography, which improves in three areas: “speed, resolution and sensitivity”. Today’s pharmaceutical industries are looking for new ways to cut cost and shorten time for development of drugs while at the same time improving the quality of their products and analytical laboratories are not exception in this trend. Speed allows a greater number of analyses to be performed in a shorter amount of time thereby increasing sample throughput and lab productivity. These are the benefits of ultra performance liquid chromatography. This system uses fine particles (less than 2.5 m), so reduces length of column, saves time and reduces solvent consumption. This review introduces the theory of UPLC, and summarizes some of the most recent work in the field.

Keywords: Ultra performance liquid chromatography, High separation efficiency, Cost effective, High pressure.

INTRODUCTION
UPLC refers to Ultra Performance Liquid Chromatography. UPLC is new category of analytical separation science works on the similar principles of HPLC while increasing the overall interlaced attributes of speed, sensitivity & resolution. It uses fine particles and saves time and reduces solvent consumption.

For many years, researchers have looked at “fast LC” as a way to speed up analyses. The “need for speed” has been driven by the sheer numbers of samples in some laboratories (particularly in drug discovery) and the availability of affordable, easy to use mass spectrometers. Smaller columns and faster flow rates (amongst other parameters) have been used. Elevated temperature, having the dual advantages of lowering viscosity, and increasing mass transfer by increasing the diffusivity of the analytes, has also been investigated. However, using conventional particle sizes and pressures, limit-actions are soon reached and compromises must be made, sacrificing resolution for time.
UPLC is comes from HPLC. HPLC has been the evolution of the packing materials used to effect the separation. An underlying principle of HPLC dictates that as particle size of column packing decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5µm, there is a significant gain in efficiency and it’s doesn’t diminish at increased linear velocities or flow rates according to the common Van Deemter equation.\(^\text{[3]}\) By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which is known as Ultra Performance.\(^\text{[4]}\)

The classic separation method is of HPLC (High Performance Liquid Chromatography) with many advantages like robustness, ease of use, good selectivity and adjustable sensitivity. Its main limitation is the lack of efficiency compared to gas chromatography or the capillary electrophoresis \(^\text{[5,6]}\) 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. That is why short columns filled with particles of about 2 µm are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load To improve the efficiency of HPLC separations, the following can be done :-

A. Work at higher temperatures- allows high flow rates by reducing the viscosity of mobile phase which significantly reduces back pressure\(^\text{[7,8]}\)

B. Use of monolithic columns- contains polymerized porous support structure that provide lower flow resistances than conventional particle-packed columns.\(^\text{[9,10,11]}\)

UPLC improves in three areas\(^\text{[12-16]}\)

1. Chromatographic resolution
2. Speed
3. Sensitive analysis

It uses fine particles and saves time and reduces solvent consumption. This new category of analytical separation science retains the practicality and principles of HPLC while increasing the overall interrelated attributes of speed, sensitivity and resolution. Today’s pharmaceutical industries are looking for new ways to cut cost and shorten
time for development of drugs while at the same time improving the quality of their products and analytical laboratories are not exception in this trend. Speed allows a greater number of analyses to be performed in a shorter amount of time thereby increasing sample throughput and lab productivity. These are the benefits of faster analysis and hence the ultra performance liquid chromatography. A typical assay was transferred and optimized for UPLC system to achieve both higher sample analysis throughput and better assay sensitivity. Analysis of operation cost and sample throughput found, UPLC cost advantageous over HPLC.[14,15]

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. Monolithic columns contain a polymerized porous support structure that provide lower flow resistances than conventional particle-packed columns.[10,17,18]

Principle:

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 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.[19]

\[ H = A + B/v + Cv \]

Where A, B and C are constants and \( v \) is the linear velocity, the carrier gas flow rate. The A term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform. The B term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by \( v \). The C term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus this term is proportional to \( v \).[20]
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 is 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.\textsuperscript{[20]}

Instrumentation:
To truly take advantage of the increased speed, superior resolution and sensitivity afforded by small particles, instrument technology also had to keep pace. A completely new system design with advanced technology in the pump, auto sampler, detector, data system, and service diagnostics was required.\textsuperscript{[21]}

Pumping System\textsuperscript{[22,23]}
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-2 μm 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 bars and transfer flows of up to 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 m$^3$/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 injection

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.$^{[24,25]}$

Sample Manager

The sample manager also incorporates several technology advancements. Using pressure assisted sample introduction, low dispersion is maintained through the injection process,
and a series of pressures transducers facilitate self-monitoring and diagnostics. 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 titer 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 titer plates. The sample manager also controls the column heater.\[^{26}\]

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.\[^{26}\]

**UPLC columns**

The design and development of sub-2µm particles is a significant challenge, and researchers have been very active in this area to capitalize on their advantages.\[^{13,27}\]

Although high efficiency nonporous 1.5µm particles are commercially available, they suffer from low surface area, leading to poor loading capacity and retention. To maintain retention and capacity similar to HPLC, UPLC must use a novel porous particle that can withstand high pressures. Silica based particles have good mechanical strength, but suffer from a number of disadvantages. These include tailing of basic analytes and a limited pH range. Another alternative, polymeric column can overcome pH imitations, but they have their own issues, including low efficiencies and limited capacities. In 2000, Waters introduced a first generation hybrid chemistry, called XTerra, which combines the advantageous properties of both silica and polymeric columns - they are mechanically strong, with high efficiency, and operate over an extended pH range. XTerra columns are produced using a classical sol-gel synthesis that incorporates carbon in the form of methyl groups. However, in order to provide the kind of enhance mechanical stability UPLC requires, a second generation hybrid technology,\[^{28}\] was developed, called ACQUITY UPLC.

ACQUITY 1.7µm particles bridge the methyl groups in the silica matrix as shown in figure-1, which enhances their mechanical stability. Evolution 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:\[^{29}\]
A) ACQUITY UPLC BEH T M C18 and C8 (straight chain alkyl columns)
B) ACQUITY UPLC BEH Shield RP 18 (embedded polar group column)
C) ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl)[29]
D) ACQUITY UPLC BEH Amide columns (trifunctionally bonded amide phase)

Each column chemistry provides a different combination and hydrophobicity, silanol activity, hydrolytic stability and chemical interaction with analytes. [figure.2]
ACQUITY UPLC BEH T M C18 and C8 columns -
These are considered as 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 1.7µm BEH particle to deliver the widest usable pH operating range.\[29\]

ACQUITY UPLC BEH SHIELD R18 columns -
These are designed to provide selectivities that complement the ACQUITY UPLC BEH T M C18 and C8 Columns.\[30\]

ACQUITY UPLC BEH Phenyl columns -
These utilize a trifunctional C6 alkyl ethyl between the phenyl ring and the silyl functionality.\[30\]

ACQUITY UPLC BEH Amide columns -
BEH particle technology, in combination with a trifunctionally bonded amide phase, provides exceptional column life time, thus improving assay robustness. BEH Amide columns facilitate the use of a wide range of phase pH [2 –11] to facilitate the exceptional retention of polar analytes spanning a wide range in polarity, structural moiety and pKa.\[30\]

Ligand combined with the same proprietary end capping processes as the ACQUITY UPLC BEH T M C18 and C8 columns provides long column lifetimes and excellent peak shape. This unique combination creates a new dimension in selectivity allowing a quick match to the existing HPLC column. Packing a 1.7µm particle in reproducible and rugged columns was also a challenge that needed to be overcome.\[30\]

The column hardware required a smoother interior surface and the end frits were redesigned to retain the small particles and resist clogging. Packed bed uniformity is also critical, especially if shorter columns are to maintain resolution while accomplishing the goal of faster separations. All ACQUITY columns also include the eCord microchip technology that captures the manufacturing information for each column, including the quality control tests and certificates of analysis. When used in the Water’s ACQUITY UPLC system, the eCord database can also be updated with real time method information, such as the number of injections, or pressure information, to maintain a
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. 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 promotes increased source ionization efficiencies.

Column: 2.1 by 30 mm 1.7 µm ACQUITY UPLC C at 35°C. A 9-45% B linear gradient over 0.8 minutes, at a flow rate of 0.86 mL/min was used. Mobile phase A was 0.1% formic acid, B was acetonitrile. UV detection at 273nm. Peaks are in order: acetazolamide, hydrochlorothiazide, impurity, hydroflumethiazide, clopamide, trichlormethiazide, indapamide, bendroflumethiazide, and spironolactone, 0.1mg/ml of each in water [figure.3].

Figure 3 shows comparison between HPLC and UPLC peak capacity. In this gradient peptide map separation, the HPLC (top) separation (on a 5µm C18um column) yields 70 peaks, or a peak capacity of 143, while the UPLC separation (bottom) run under identical conditions yields 168 peaks, or a peak capacity of 360, (2.5 x increase in peak capacity).
Column Heater

The column heater heats the column compartment to any temperature from 5°C to 65°C.\[19\]

Detectors

TUV Detector (Tunable ultraviolet 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.\[30\]

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 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.\[31\]

ELS Detector

ELS detector is an evaporative light scattering detector designed for use in the UPLC
system. 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.\textsuperscript{[32]}

In addition, the phases of less than 2 μm are generally non-regenerable and thus have limited use.

\textbf{Advantages:}\textsuperscript{[32,33]}

1) Decreases run time and increases sensitivity.

2) Provides the selectivity, sensitivity, and dynamic range of LC analysis.

3) Maintaining resolution performance.

4) Expands scope of Multiresidue Methods.

5) UPLC’s fast resolving power quickly quantifies related and unrelated compounds.

6) Faster analysis through the use of a novel separation material of very fine particle size.

7) Operation cost is reduced.

8) Less solvent consumption.

9) Reduces process cycle times, so that more product can be produced with existing resources.

10) 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.\textsuperscript{[32]}

11) Delivers real-time analysis in step with manufacturing processes.

12) Assures end-product quality, including final release testing.\textsuperscript{[33]}

\textbf{Disadvantages:}

Due to increased pressure requires more maintenance and reduces the life of the columns of this type. So far performances similar or even higher have been demonstrated by using stationary phases of size around 2 μm without the adverse effects of high pressure. In addition, the phases of less than 2 μm are generally non-regenerable and thus have limited use.\textsuperscript{[33]}

\textbf{Applications of UPLC:}

\textbf{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.\cite{4}

**Study of Metabonomics / Metabolomics**

Metabonomics studies are carried out in labs to accelerate the development of new medicines. The ability to compare and contrast large sample groups provides insight into the biochemical changes that occur when a biological system is exposed to a new chemical entity (NCE). Metabonomics provides a rapid and robust method for detecting these changes, improves understanding of potential toxicity, and allows monitoring the efficacy. The correct implementation of metabonomic and metabolomic information helps similar discovery, development, and manufacturing processes in the biotechnology and chemical industry companies. With these studies, scientists are better able to visualize and identify fundamental differences in sample sets. The UPLC/MS System combines the benefits of UPLC analyses, high resolution exact mass MS, and specialized application managers to rapidly generate and interpret information-rich data, allowing rapid and informed decisions to be made.\cite{34}

**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. 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.\textsuperscript{[4]}

**ADME (Absorption, Distribution, Metabolism, Excretion) 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. Failure in properly identifying these potential toxic events can cause a compound to be withdrawn from the market. 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. These are important for automated generic methods as they reduce failed sample analyses and save time.

UPLC/MS/MS provides following advantages:\textsuperscript{[19]}

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. Tandem quadrupole MS provides sensitivity and selectivity for samples in matrix using multiple reaction monitoring (MRM) for detection and automated compound optimization.\textsuperscript{[19]}

UPLC/MS/MS 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. As well, since this UPLC-based approach can help labs pre-emptively 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.\textsuperscript{[19]}

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.\cite{19}

Bioanalysis / 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.\cite{19}

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.\cite{21}

Developing a robust and compliant LC/MS/MS assay has traditionally been the domain of very experienced analysts. UPLC/MS/MS helps in the processes of method development for bioanalysis into logical steps for MS, LC, and sample preparation. Quantitative bioanalysis is also an integral part of bioequivalence studies, which are used to determine if 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 solutions are proven to increase efficiency, productivity and profitability for bioequivalence laboratories.

Applications of UPLC/MS/MS in bioequivalence and bioanalysis are;
In UPLC/MS/MS, LC and MS instruments and software combine in a sophisticated and integrated system for bioanalysis and bioequivalence studies, providing unprecedented performance and compliance support. UPLC/MS/MS delivers excellent chromatographic resolution and sensitivity.\cite{21}

MS delivers simultaneous full-scan MS and multiple reaction monitoring (MRM) MS data with high sensitivity to address matrix monitoring.
UPLC Sample Organizer maximizes efficiency by accommodating large numbers of samples in a temperature-controlled environment, ensuring maximum throughout increase the sensitivity of analyses, quality of data including lower limits of quantitation (LLOQ), and productivity of laboratory by coupling the UPLC System's efficient
separations with fast acquisition rates of tandem quadrupole MS systems easily acquire, quantify and report full system data in a compliant environment using a security-based data collection software.\textsuperscript{[1,9]}

Ensure the highest quality results and reliable system operation in regulated environment.\textsuperscript{[1,9]}

**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 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.\textsuperscript{[4]}

**Forced Degradation Studies**

One of the most important factors 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 is important for defining storage conditions, shelf life, selection of proper formulations, protective packaging and for regulatory documentation. Forced degradation, or stress testing, is carried out under even harsher conditions than those used for accelerated stability testing.\textsuperscript{[3,4]}

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.\textsuperscript{[4]}

PDA/MS (photodiode array and MS), allows for faster and higher peak capacity
separations, for complex degradation product profiles also. Combining the chromatographic speed, resolution, and sensitivity of UPLC separations with the high-speed scan rates of UPLC-specific photodiode array and MS detection will give confidence for identifying degradation products and thus shortening the time required to develop stability-indicating methods.\textsuperscript{[4]}

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 raw materials with proper quality and purity. That manufacturing processes proceed as designed. Those final pharmaceutical products meet, and hopefully exceed, defined release specifications. Continued monitoring of material stability is also a component of quality assurance and control.\textsuperscript{[21]}

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. The need for consistency over the lifetime of a drug product which could be in excess of 30 years is essential in order to avoid method revalidation and associated production delays.\textsuperscript{[21]}

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. Method development and validation is a time-consuming and complicated process that 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.\textsuperscript{[19]}

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.\textsuperscript{[19]}

UPLC provide efficiencies in method development: Using UPLC, analysis times becomes as short as one minute, methods can be optimized in just one or two hours, significantly reducing the time required to develop and validate with UPLC, separation
speed and efficiency allows for the rapid development of methodologies.\textsuperscript{[21]}

The following parts of UPLC are important to give the required information:

1) UPLC columns: High stability allows for a wide range of column temperatures and pH to be explored.

2) UPLC Column Manager: Easily evaluate column temperatures from 10°C below room temperature to 90°C; enables to use HPLC methods on the UPLC before scaling to UPLC.

3) UPLC Calculator: Put information at fingertips about how to transit from existing chromatographic analyses to faster UPLC methods.\textsuperscript{[19]}

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. Impurity profiling requires high-resolution chromatography, capable of reliably and reproducibly separating and detecting all of the known impurities of the active compound.\textsuperscript{[4]}

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.

UPLC System and Columns specifically address high-throughput analysis requirements while maintaining high peak resolution. UPLC PDA Detector involves two analytical flow cells are available for maximum flexibility according to application requirements, one for maximum chromatographic resolution and a second for high sensitivity.\textsuperscript{[4]}

UPLC also involves the latest peak detection algorithms and custom calculations to optimize data processing and reporting. It also confidently 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 mass LC/MS, which by operating with alternating low- and high-collision energies, known as MS, 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.[21]

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.[21]

Compound Library Maintenance

Confirming the identity and purity of a pharmaceutical is critical to effectively screening chemical libraries that contain vast types of small molecules across a range of biological targets. Chemists need to be sure they have synthesized the expected compound. In this high-throughput screening environment, the ability to obtain information in multiple MS and UV detection modes in a single injection is invaluable.[19]

LC/MS analysis helps excludes false positives and maintain high product quality, but can be time-consuming in moving a drug through the discovery process. Achieving high sample throughput is key to moving compounds from hit to lead status.

Combining fast analysis with open-access software delivers the power of LC/MS to chemists who are not analytical instrumentation specialists. A single complete system enables them thoroughly screen a compound, from sample introduction to end results. It allows them to quickly and easily know what they’ve made, and allows the experts to work on the difficult analytical problems.[21]

The use of the fast-scanning MS along with the throughput of the UPLC System’s remote status monitoring software allows chemists to obtain high-quality comprehensive data about their compounds in the shortest possible timeframes. This, combined with intelligent open access software, allows making informed decisions faster, and better supporting the needs of the modern drug discovery process.[19]

Open Access

Maximum efficiency is essential for analytical laboratories that are constantly challenged to increase throughput and deliver results to research chemists in pharmaceutical discovery. UPLC and UPLC/MS systems and software enable versatile and open operation for medicinal chemistry labs, with easy-to-use instruments, a user-friendly software interface, and fast, robust analyses using UV or MS for nominal and exact mass measurements.[34]
System management is just as simple. Online, the central administrator can remotely define system users and their privileges for operating instruments across the network.\textsuperscript{[34]}

**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 of UPLC is a reduction of time, which also reduces solvent consumption. The time spent optimizing new methods can also be greatly reduced. The time needed for column equilibration for 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.

**Comparison between HPLC and UPLC\textsuperscript{[34]}**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HPLC Assay</th>
<th>UPLC Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>150x3.2 mm</td>
<td>150x2.1 mm</td>
</tr>
<tr>
<td>Particle size</td>
<td>3 to 5 µm</td>
<td>Less than 2 µm</td>
</tr>
<tr>
<td>Flow rate</td>
<td>3.0 ml/min.</td>
<td>0.6 ml/min.</td>
</tr>
<tr>
<td>Needle wash</td>
<td>Methanol</td>
<td>Methanol</td>
</tr>
<tr>
<td>Injection volume</td>
<td>5 µL (std. in 100% MeOH)</td>
<td>2 µL (std. in 100% MeOH)</td>
</tr>
<tr>
<td>Column temperature</td>
<td>30ºC</td>
<td>65ºC</td>
</tr>
<tr>
<td>Maximum backpressure</td>
<td>5-40 Mpa</td>
<td>103.5 Mpa</td>
</tr>
<tr>
<td>Gradient (Time in min.)</td>
<td>T0 (25:75), T6.5(25:75), T7.5(95:5), T9(25:75), T10(25:75)</td>
<td>T0 (36:64), T1.1(95:5), T1.3(36:64)</td>
</tr>
<tr>
<td>Total run time</td>
<td>10 min.</td>
<td>1.5 min.</td>
</tr>
<tr>
<td>Total solvent consumption</td>
<td>Acetonitrile : 10.5ml Water : 21.0 ml</td>
<td>Acetonitrile : 0.53 ml Water : 0.66 ml</td>
</tr>
<tr>
<td>Plate count</td>
<td>2000</td>
<td>7500</td>
</tr>
<tr>
<td>USP resolution</td>
<td>3.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Delay volume</td>
<td>750 µl</td>
<td>110 µl</td>
</tr>
</tbody>
</table>

**REFERENCES**


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