Vol 2 | Issue 1 | 2012 | 61-68.

International Journal
of
Pharmacy Review & Research
www.ijprr.com

e-ISSN: 2248 – 9207
Print ISSN: 2248 – 9193

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY: A STEP AHEAD TO HPLC

Tanvi K Desai*, Anand A Mahajan, Anil Thaker

*Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management,
NMIMS, Mumbai, Maharashtra, India.

ABSTRACT

UPLC can be regarded as new direction for liquid chromatography. UPLC refers to ultra performance liquid chromatography, improves in three areas: "speed, resolution and sensitivity". In this system uses fine particles (less than 2.5 µm).So reduces length of column, saves time and reduces solvent consumption. UPLC chromatographic system is designed in a special way to withstand high system back-pressures. 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.

Keywords: UPLC, High pressure, Cost effective.

INTRODUCTION

UPLC refers to Ultra Performance Liquid Chromatography. It improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption [1-5]. 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 column packing particle size 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 [6]. 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.

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 [7, 8] 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 bar. That is why short columns filled with particles of about 2µm 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
b. use of monolithic columns

PRINCIPLE

The UPLC is based on the principal of use of stationary phase consisting of particles less than 2 µm

Corresponding Author **Tanvi K Desai** **E mail:** tanvidesai612@gmail.com

(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 a small diameter particles is much greater than for larger diameters [15, 16, 17].

$H=A+B/v+Cv$

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*.

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 [18]. 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 [19, 20].

INSTRUMENTATION

Pumping System [32, 33]:

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

There are two types of pumps:

1. Reciprocating pump

2. Pneumatic pump

1) Reciprocating pump

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

There are two general types of reciprocating pumps.

A) The piston pump

B) The diaphragm pump.

There are two types of diaphragm pumps.

The hydraulically operated diaphragm metering pumps [35]: This type of pump can be used for pumping toxic and explosive fluids. The pump can deliver heads of up to 700 bar and transfer flows of up 20 m 3 /hr.

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

2) Pneumatic pump [36]: This type of piston was originally used for normal liquid chromatography separations but was found to be noisy and produced strong flow pulses that destabilized the detector. It is now used almost exclusively for slurry packing liquid chromatography columns. It is the simplest type of pump that can be designed to provide exceedingly high pressures.

Sample Injector

In UPLC, sample introduction is critical. Conventional injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from extreme pressure fluctuations, the injection process must be relatively pulse-free and the swept volume of the device also needs to be minimal to reduce potential band spreading. A fast injection cycle time is needed to fully capitalize on the speed afforded by UPLC, which in turn requires a high sample capacity. Low volume injections with minimal carryover are also required to increase sensitivity. There are also direct injection approaches for biological samples [38, 39].

Sample Manager

The sample manager also incorporates several technology advancements. Using pressure assisted sample introduction, low dispersion is maintained through the injection n process, and a series of pressures transducers facilitate self-monitoring and diagnostics. It uses needle-in-needle sampling for improved ruggedness and needle calibration sensor increases accuracy. Injection cycle time is 25 seconds without a wash and 60 sec with a dual wash used to further decrease carry over. A variety of micro 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.

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 [37].

Columns [40]:
There are two types of columns :
1.Analytical column
2.Guard column

1. Analytical column [41]
Different columns used in UPLC are
Pro C18, Pro C8, Hydrosphere C18, YMC30 , YMC basic

2. Guard column [42]

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

Advantages
• Decreases run time and increases sensitivity
• Provides the selectivity, sensitivity, and dynamic range of LC analysis
• Maintaining resolution performance.
• Expands scope of Multiresidue Methods
• UPLC's fast resolving power quickly quantifies related and unrelated compounds
• Faster analysis through the use of a novel separation material of very fine particle size
• Operation cost is reduced
• Less solvent consumption
•Reduces process cycle times, so that more product can be produced with existing resources
• Increases sample throughput and enables manufacturers to produce more material that consistently meet or exceeds the product specifications, potentially eliminating variability, failed batches, or the need to re-work material [27,28]
• Delivers real-time analysis in step with manufacturing processes
• Assures end-product quality, including final release testing

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

Column Heater
The column heater heats the column compartment to any temperature from 5^0C to 65^0C.

Detectors
TUV Detector (Tunable ultraviolet detector) [43, 44]: The analytical cell, with a volume of 500 neon liters and a path length of 10 mm, and high sensitivity flow cell, with a volume of 2.4 micro liters and a 25 mm path length, both utilize the flow cell technology. The TUV detector operates at wavelengths ranging from 190 to 700 nm.
PDA Detector (Photo diode array detector) [45]: 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
ELS Detector [45]: 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.
In addition, the phases of less than 2 μm are generally non-regenerable and thus have limited use [29, 30].

Applications of UPLC
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.

Metabonomics-based analysis, using UPLC, exact mass MS, and MarkerLynx Software data processing for

multivariate statistical analysis, can help quickly and accurately characterize these medicines and also their effect on human metabolism.

Preparative-scale fractionation and purification is used along with classic quantitative bioanalytical tools used in drug development.

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.

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.

ADME (Absorption, Distribution, Metabolism, Excreation) Screening :

Pharmacokinetics studies include studies of ADME (Absorption, Distribution, Metabolism and Excreation). 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 to properly identify 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:-

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.

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.

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.

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.

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.

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
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 throughput
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
Ensure the highest quality results and reliable system operation in regulated environment

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.

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, photostability, and temperature to understand resulting byproducts 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), 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.

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. Continued monitoring of material stability is also a component of quality assurance and control.
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.

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 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
The following parts of UPLC are important to give the required information:-
UPLC columns: High stability allows for a wide range of column temperatures and pHs to be explored.
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
UPLC Calculator: Put information at fingertips about how to transition existing chromatographic analyses to faster UPLC methods.

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.

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.
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.
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.

Compound Library Maintenance

Confirming the identity and purity of a candidate 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.

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.

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 to make informed decisions faster, and better support the needs of the modern drug discovery process.

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.
System management is just as simple. Online, the central administrator can remotely define system users and their privileges for operating instruments across the network.

Comparison between UPLC and HPLC

Characteristics	HPLC	UPLC
Particle size	3 to 5m	Less than 2m
Maximum backpressure	35-40 MPa	103.5 MPa
Analytical column	Alltima C_{18}	Acquity UPLC BEH C_{18}
Column dimensions	150 X 3.2 mm	150 X 2.1 mm
Column temperature	30 °C	65 °C
Injection volume	5µL (Std. In100% MeOH)	2µL (Std.In100% MeOH)

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. When many scientists experience separation barriers with conventional HPLC, UPLC extends and expands the utility of chromatography. The main advantage is a reduction of analysis time, which also meant reduced solvent consumption. 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. 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.

REFERENCES

1. Jerkovich AD, Mellors JS and Jorgenson JW. *LCGC*, 21(7), 2003, 660-611.
2. Wu N, Lippert JA and Lee ML. *J. Chromotogr*., 911(1), 2001, 55-60.
3. Unger KK, Kumar D, Grun M, Buchel G, Ludtke S, Adam Th, Scumacher K and Renker S. *J. Chromatogr*. *A*, 892(47), 2000, 155-172.
4. Swartz ME and Murphy B. *Lab Plus Int.*, 18(6), 2004, 85-102.
5. Swartz ME and Murphy B, *Pharm. Formulation Quality*, 6(5), 2004, 40.
6. Van Deemter JJ, Zuiderweg EJ, Klinkenberg A. Longitudinal diffusion and resistance to mass transfer as causes of non-ideality in chromatography. *Chem. Eng. Sci*., 5, 1956, 271-289.
7. Zhang YH, Gong XY, Zhang HM, Larock RC and Yeung ES. *J. Comb. Chem*., 2, 2000, 450-452.
8. Zhou C. Jin Y, Kenseth JR, Stella M, Wehmeyer KR and Heineman WR. *J. Pharmac. Sci*., 94, 2005, 576-589.
9. Zhu J, Goodall DM and Wren SAC. *LCGC*, 23(1), 2005, 54-72.
10. Greibrokk T and Andersen T. *J. Chromatogr. A*, 1000, 2003, 743-755.
11. Gerber F, Krummen M, Potgeter H, Roth A, Siffrin C and Spoendlin C. *J. Chromatogr., A*,1036, 2004, 127-133.
12. Tanaka N, Kobayashi H, Nakanishi K, Minakuchi H and Ishizuka N. *Anal. Chem*., 73, 2001, 420A-429A.
13. Wu N, Dempsey J, Yehl PM, Dovletoglu A, Ellison A and Wyvratt J. *Anal. Chim. Acta*, 523, 2004, 149-156.
14. Swartz ME. Ultra Performance Liquid Chromatography (UPLC): An Introduction, Separation Science Re-Defined, *LCGC Supplement*, 2005, 8.
15. Jerkovich AD, Mellors JS and Jorgenson JW. *LCGC*, 21(7), 2003, 600-610.
16. MacNair JE, Lewis KC and Jorgenson JW. *Anal. Chem*., 69, 1997, 983-989.
17. Swartz ME. Ultra Performance Liquid Chromatography (UPLC): An Introduction, Separation Science Re-Defined, *LCGC Supplement*, 2005, 10.
18. MacNair JE, Patel KD, Aqnd Jorgenson JW. *Anal. Chem*., 71, 1999, 700-708.
19. Wu N, Lippert JA and Lee ML. *J. Chromatogr., A*, 911, 2001, 1–12.
20. Swartz ME. Ultra Performance Liquid Chromatography (UPLC): An Introduction, Separation Science Re-Defined, *LCGC Supplement*, 2005, 12.
21. Lars Y and Honore HS. *J. Chromatogr., A*, 1020, 2003, 59-67.
22. McLoughlin DA, Olah TV and Gilbert JD. *J. Pharm. Biomed. Anal*., 15, 1997, 1893-1901.
23. Swartz ME. Ultra Performance Liquid Chromatography (UPLC): An Introduction, Separation Science Re-Defined, *LCGC Supplement*, 2005, 11.
24. Lippert JA, Xin B, Wu N and Lee ML. J. *Microcolumn Sep*., 11, 1997, 631-643.
25. Swartz ME. Ultra Performance Liquid Chromatography (UPLC): An Introduction, Separation Science Re-Defined, *LCGC Supplement*, 2005, 13.
26. Jerkovitch AD, Mellors JS and Jorgenson JW. *LCGC*, 21(7), 2003.
27. Swartz M. *LCGC*, 23(1), 2005, 46-53.
28. Broske A, et al. Agilent Technologies application note, 2004, 5988-9251EN.
29. Goodwin L, White SA, Spooner N. Evaluation of ultra-performance liquid chromatography in the bioanalysis of small molecule drug candidates in plasma. *J. Chromatogr. Sci.*, 45(6): 2007, 298-304.
30. www.waters.com
31. httimages.google.com
32. Said AS. *J. Chemical Engineering*, 2, 1956, 477.
33. Martin AJ and Synge RLM. *J. Biochem*, 35, 1941, 1358.
34. James AT and Martin AJ. *J. Biochem*, 50, 1952, 579.
35. Lippert JA, Xin B, Wu N and Lee M.L. *J. Microcolumn*., 1997, 11631-643.
36. Lars Y and Honore HS. *J. Chromatogr., A*, 1020, 2003, 59-67.
37. McLoughlin DA, Olah TV, Gilbert JD. *J. Pharm. Biomed. Anl., 15*,1997, 1893-1901.
38. Knox JH and Kaliszan RJ. *J. Chromatogr*., 349, 1985, 211.

39. Smith RJ and CS Nieass. *J. Liq. Chromatogr*., 3, 1986, 1387.
40. Scott RPW and Kucera P. *J. Chromatogr*., 15, 1987, 69.
41. Alhedai A, Martire DE and Scott RP. *J. Analyst*., 43, 1989, 898.
42. Scott RPW and CE Reese. *J. Chromatogr*., 114, 1989, 869.
43. Purnell JH and Bohemen J. *J. Chem. Soc*., 2, 1961, 2030.
44. Desty DH and Goldup A. *J. Gas Chromatography*, 3, 1960, 162.