International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Review Article **CODEN: IJPNL6**

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY: A CHROMATOGRAPHY TECHNIQUE

G. Madhava Prathap* and Amreen Nishat

Montessori Siva Sivani Institute of Science & Technology-College of Pharmacy, Gurrajupalem, Mylavaram (M), Krishna Dist, Andhra Pradesh, INDIA

***Corresponding author e-mail:** madhavaprathap@gmail.com

ABSTRACT

Ultra performance liquid chromatography systems take advantage of technological pace in particle chemistry performance, system optimization, detector design and data processing. When taken together, these achievements have created an improvement in chromatographic performance. 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.

Keywords: Ultra Performance Liquid Chromatography, High separation efficiency, Cost effective, Pharmaceutical analysis, High pressure

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. It's 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***- allows high flow rates by reducing the viscosity of mobile phase which significantly reduces back pressure.[9-10]

B***. use of monolithic columns***- contains polymerized porous support structure that provide lower flow resistances than conventional particle-packed columns.[11-13]

UPLC refers to Ultra Performance Liquid Chromatography, which improves in three areas: [14-18]

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.

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 [9,10]. Monolithic columns contain a polymerized porous support structure that provide lower flow resistances than conventional particle-packed columns [19,20,21].

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) [22]. 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 [23,24,25].

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

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 Uttam Singh Baghel et al. / UPLC: A Review 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[26]. 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[27,28].

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. The ACQUITY UPLC system has been designed for low system and dwell volume to take full advantage of low dispersion and small particle technology. The lines of attack for fast LC method development are varied. Method development simulation softwares such as ACD™[29] , DryLab ™[30] or Chromsword ™[31] are valuable tools for optimizing and streamlining methods. Such softwares allow to increase the information obtained from a limited number of runs and to predict the best possible separation conditions.[Table No.1]

Pumping systems: 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 15,000psi. 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.

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[32]. There are also direct injection approaches for biological samples.[33-34]

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 organiser, 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 the sample dispersion a "pivot out "design allows the column outlet to be placed in closer proximity to the source inlet of an MS detector. [35]

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 [36-37]. 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 columns,can over come 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 produces 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[38],was developed, called ACQUITY UPLC.ACQUITY 1.7µm particles bridge the methyl groups in the silica matrix as shown in fig1, which enhances their mechanical stability.[figure.1]
esolution 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:

i) ACQUITY UPLC BEH T M C18 and C8 (straight chain alkyl columns)
ii) ACQUITY UPLC BEH Shield RP 18(embedded polar group column)
iii) ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl)[39]
iv) 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 c18 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.

• acquity uplc beh shield r18 columns –

These are designed to provide selectivities that complement the ACQUITY UPLC BEH T M C18 and C8 Columns.

• ***acquity uplc beh phenyl columns –***

These utilize a trifunctional C6 alkyl ethyl between the phenyl ring and the silyl functionality.

• ***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.

• 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.

The column hardware required a smoother interior surface and the end frits were re-designed to retain the small particles and resist clogging. Packed bed uni-formity 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 Waters 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 complete column history.

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.[figure3]

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. UVdetection at 273nm. Peaks are in order: acetazolamide ,hydrochlorothiazide, impurity , hydroflumethiazide, clopamide, trichlormethiazide, indapamide ,bendroflumethiazide, and spironolactone, 0.1mg/ml of each in water[figure.4]

In Figure 4. HPLC vs. UPLC peak capacity. In this gradient peptide map separation, the HPLC (top) separation (on a 5µm Col-18umn) yields 70 peaks, or a peak capacity of 143, while the UPLC separation (bottom) run under identical conditions yields 168peaks, or a peak capacity of 360, a 2.5 x increase.

Detectors: For UPLC detection, the tunable UV/Visible detector is used which includes new electronics and firmware to support Ethernet communications at the high data rates. Conventional absorbance-based optical detectors are concentration sensitive detectors, and for UPLC use, the flow cell with a volume would have to be reduced in standard UV/Visible detectors to maintain concentration and signal. According to Beer's Law, smaller volume conventional flow cells would also reduce the path length upon which the signal strength depends. A reduction in cross-section means the light path is reduced, and transmission drops with increasing noise.

Therefore, if a conventional HPLC flow cell were used, UPLC sensitivity would be compromised. The ACQUITY Tunable UV/Visible detector cell consists of a light guided flow cell equivalent to an optical fibre. Light is efficiently transferred down the flow cell in an internal reflectance mode that still maintains a 10mm flow cell path length with a volume of only 500nL. 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 [40].

UPLC CHROMATOGRAPHIC CONDITIONS:

Columns: ACQUITY UPLC BEM C18, BEH Shield RP18, BEH C8 OR BEH Phenyl Column Dimensions: **2.1X50mm 1.7µm**

Mobile Phase A1: 20mM NH4COOH in H2O, pH 3.0.
Mobile Phase A2: 20mM NH4HCOO3 in H2O, pH 10.0
Mobile Phase B1: Acetonitrile
Mobile Phase B2: Methanol
Flow rate: 0.5ml/min
Injection Volume: 10.0µl
Week needle wash: 3% methanol
Strong needle wash: 90% acetonitrile
Temperature: 30ºC
Detection: UV @ 254 nm
Sampling rate: 20pts/sec
Time constant: 0.1
Instrument: ACQUITY UPLC TM with 2996 ACQUITY PDA Detector. [Table.2]

$$P = 1+ tg/w$$

UPLC screening method is 7X faster than directly scaled HPLC method.

ADVANTAGES

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

DISADVANTAGES

-Due to increased pressure requires more maintenance and reduces the life of the columns of this type. 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.[41-42]

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 to provide evidence-based validation of their effectiveness as medicines and to establish safety parameters for their production.. 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 Marker Lynx 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 bio analytical tools used in drug development. [Figure.5]

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.

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.

Bio analysis/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 bio analysis, the most common being blood, plasma, and urine. The primary technique for quantitative bio analysis is studies, scientists are better able to visualize and identify 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 harmacokinetics (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 bio analysis into logical steps for MS, LC, and sample preparation. Quantitative bio analysis is also an (integral part of bioequivalence studies, which are used to d 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 bio analysis are:-

-In UPLC/MS/MS, LC and MS instruments and software combine in a sophisticated and integrated system for bio analysis and bioequivalence studies, providing unprecedented performance and compliance support.

- UPLC/MS/MSdelivers 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 quadrapole MS systems.

-Easily acquire, quantify and report full system data in a compliant environment using 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 conformity 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.

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 and manufacturing processes proceed as designed. 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 reduction delays.

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 recipients in the sample, often resulting in long HPLC analysis times to achieve sufficient resolution. 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 analyses 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.

Computer 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. 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.

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. 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.

Table 1: Comparison of UPLC and HPLC

CHARACTERICTICS	HPLC	UPLC
Particle Size	3 to 5µm	Less than 2µm
Maximum Back Pressure	35-40 Mpa	103.5MPa
Analytical Column	Alltima C18	Acquity UPLC BEH C18
Column Dimensions	150X3.2mm	150 X 2.1mm
Column Temperature	30ºC	65ºC
Injection Volume	5µl(Std in 100% MeOH)	2µl (Std in 100% MeOH)

Table 2: METHOD DEVELOPMENT FASTER WITH UPLC (Time Saving)

	UPLC Gradient Conditions				Equivalent HPLC Gradient Conditions			
Column Conditions	2.1X50mm				2.1X50mm			
Particle Size	1.7µm				5µm			
Flow Rate	0.5ml/min				1.0ml/min			
Gradient	Time (min)	Profile		Profile	Time (min)	Profile		Profile
		%A	%B			%A	%B	
	0.0	95	5	6	0.0	95	5	6
	5.0	10	90	6	35.0	10	90	6
Peak Capacity	150				150			

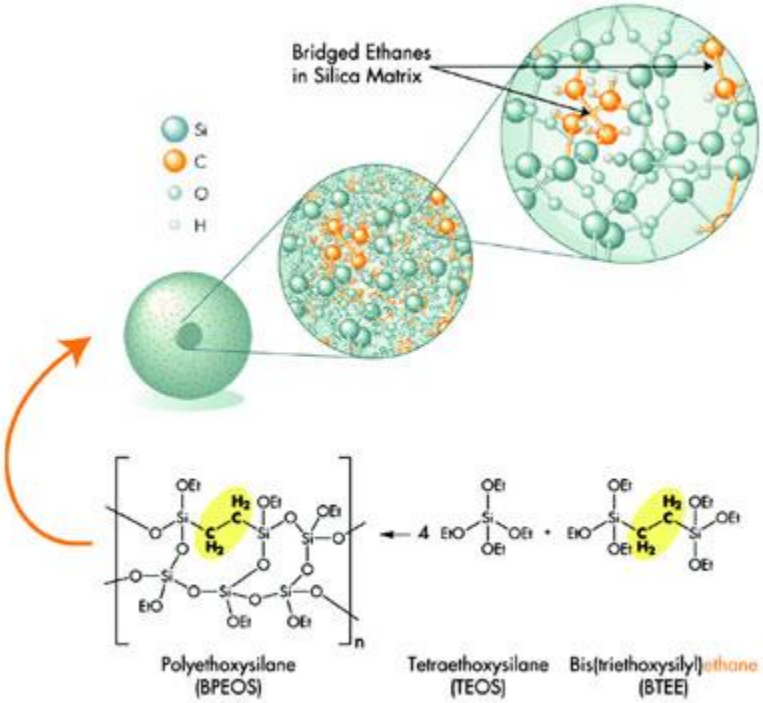

Figure 1: Synthesis and Chemistry of ACQUITY 1.7µm particles for UPLC

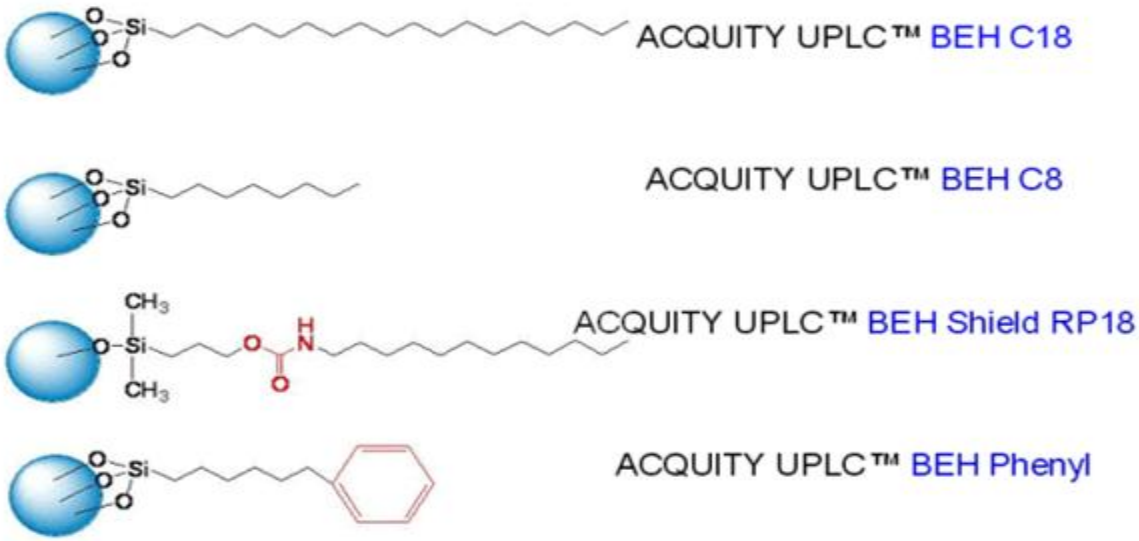

Figure 2: ACQUITY UPLC BEH Column Chemistries

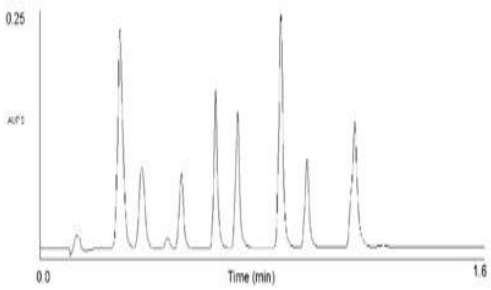

Figure 3: UPLC separation of eight diuretics.

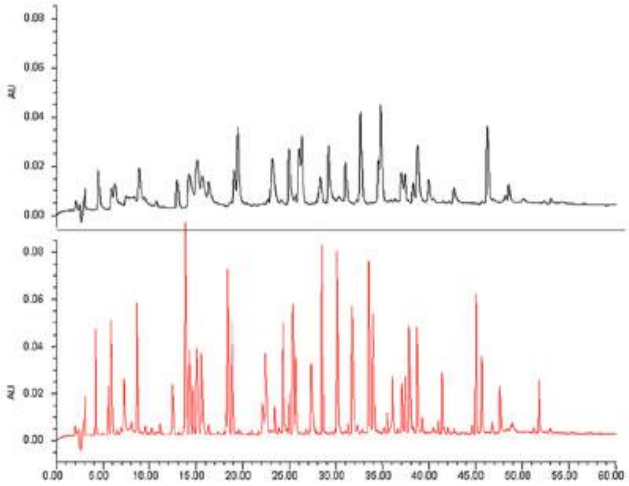

Figure 4: HPLC vs. UPLC

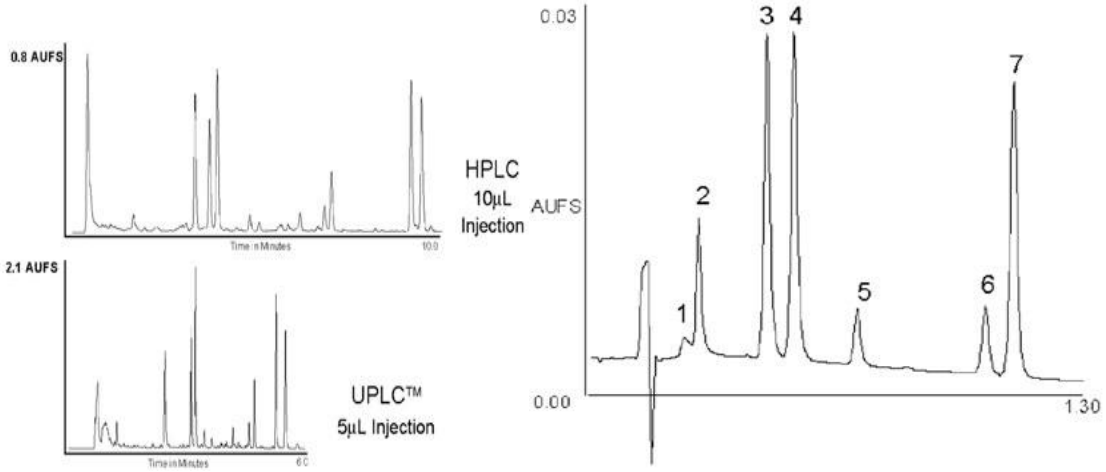

Figure 5: Comparison of Natural Product Extraction with UPLC and HPLC

REFERENCES

1. Jerkovich AD, Mellors JS, Jorgenson JW. LCGC, 2003; 21(7):660-611.
2. Wu N, Lippert JA, Lee ML. J Chromatogr, 2001; 911(1).
3. Unger KK, Kumar D, Grun M, Buchel G, Ludtke S, Adam Th, Scumacher K, Renker S. J Chromatogr A, 2000;892(47).
4. Swartz ME, Murphy B. Lab Plus Int, 2004; 18(6).
5. Swartz ME, Murphy B. Pharm Formulation Quality, 2004; 6(5):40.
6. Van Deemter JJ, Zuiderweg EJ,Klinkenberg A. Chem. Eng. Sci,1956; 5:271-289.
7. Zhang YH, Gong XY, Zhang HM, Larock RC, Yeung ES. J Comb. Chem. 2000; 2:450-452.
8. Zhou C, Jin Y, Kenseth JR, Stella M,Wehmever KR, Heineman WR. J Pharmac. Sci. 2005; 94:576-589.
9. Zhu J, Goodall DM, Wren SAC. LCGC, 2005; 23(1):54-72.
10. Greibrokk T, Andersen T. J Chromatogr, 2003; A 1000:743-755.
11. Gerber F, Krummen M, Potgeter H, Roth A, Siffrin C, Ppoendlin C. J Chromatogr, 2004; A 1036:127-133.
12. Tanaka N, Kobayashi H, Nakanishi K, Minakuchi H, Ishizuka N. Anl. Chem, 2001; 73:420A-429A.
13. Wu N, Dempsey J, Yehl PM, Dovletoglu A, Ellison A. Wyvratt J,2004.
14. Jerkovich AD,Mellors JS, Jorgenson JW, LCGC,2003; 21(7):660-611.
15. Wu N, Lippert JA, Lee ML. J Chromatogr,2001;911(1).
16. Unger KK, Kumar D, Grun M, m Buchel G, Ludtke S, Adam Th. J Chromatogr,2000;A 892(47).
17. Swartz ME, Murohy B. Lab Plus Int, 2004; 18(6). B Haritha Reddy et al/JITPS,2010; Vol.1 (3):115-132.

18. Swartz ME, Murohy B, Pharm Formulation Quality, 2004;6(5):40.
19. MacNair JE, Patel KD, Jorgenson JW. Anal Chem , 1999;71:700-708.
20. Wu N, Lippert JA, Lee ML. J Chromatogr, 2001;A 911:1-12.
21. Swaartz ME. Ultra Performance Liquid Chromatography (UPLC): An Introduction, Seperation Science Re-Defined, LCGC Supplement, 2005;12.
22. Lars Y, Honore HS. J Chromatogr, 2003; A 1020:59-67.
23. McLoughlin DA, Olah TV, Gilbert JD. J Pharm. Biomed. Anal, 1997; 15:1893-1901.
24. Swartz ME. Ultra Performance Liquid Chromatography (UPLC): An Introduction, Seperation Science Re-Defined, LCGC Supplement, 2005;11.
25. Lippert JA, Xin B,Wu N, Lee Ml. J Microcolumn, LCGC 21, Sep 11,1997; 631-643.
26. Swartz ME. Ultra Performance Liquid Chromatography (UPLC): An Introduction, Seperation Science Re-Defined, LCGC Supplement, 2005;13.
27. Jerkovitch AD, Mellors JS, Jorgenson JW. LCGC,2003;21(7).
28. Wu N, Lippert JA, Lee ML. J Chromatogr,2000;911(1).
29. www.acdlabs.com/products/chrom_lab.
30. www.rheodyne.com/chromatography/drylab.
31. www.iristechnologies.net/ChromSword/ChromSwordAuto.htm.
32. Swaartz ME. Ultra Performance Liquid Chromatography(UPLC): An Introduction, Seperation Science Re-Defined, LCGC Supplement, 2005;12.
33. Lars Y, Honore HS. J Chromatogr, 2003; A 1020:59-67.
34. McLoughlin DA, Olah TV, Gilbert JD. J Pharm. Biomed. Anal, 1997; 15:1893-1901.
35. Lippert JA, Xin B,Wu N, Lee Ml. J Microcolumn, LCGC 21, Sep 11,1997; 631-643.
36. Jerkovitch AD, Mellors JS, Jorgenson JW. LCGC, 2003;21(7),21:7.
37. Wu N, Lippert JA, Lee ML. J Chromatogr, 2001;911(8).
38. yndham KD, et al. Anal. chem., 2003; 75:6781-6788.
39. Swartz ME. Ultra Performance Liquid Chromatography (UPLC): An Introduction, Seperation Science Re-Defined, LCGC Supplement,2005;11.
40. Swartz ME. Ultra Performance Liquid Chromatography (UPLC): An Introduction, Seperation Science Re-Defined, LCGC Supplement, 2005; 13.
41. Swartz M. LCGC, 2005; 23(1):46-53.
42. Broske AD,et al, Agilent technologies application note,2004; 5988-9251EN.