Review Article

Vol: 2; Issue: 1

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY: A NEW REVOLUTION IN LIQUID CHROMATOGRAPHY

Narwate B. M[1].*, Ghule P. J[1]. , Ghule A.V[1]., Darandale A.S[1]., Wagh J.G[1]

[1]MES's College of Pharmacy Sonai, Tal: Newasa ,Dist: Ahmednagar, Maharastra, India-414105

Date Received:	Date of Accepted:	Date Published:
6TH Jan 2014	12st Jan 2014	15th Jan 2014

Abstract:
Ultra Performance Liquid Chromatography (UPLC) can be regarded as a new direction for liquid chromatography. UPLC improves three areas of Liquid chromatography namely speed, resolution, and sensitivity of analysis by using particle size less than 2 µm and the system is designed in a special way to withstand high system back-pressures. 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 and also decrease of time and solvent consumption compare to HPLC. Now a day's pharmaceutical industries as well as analytical laboratories are in search of new ways to reduce cost and time for analysis of drugs and improve quality of their product. This review focus on basic principle, instrumentation and theory of UPLC. It also summarizes various pharmaceutical applications of this technique with examples

Keywords: Ultra Performance Liquid Chromatography. UPLC Columns, High performance liquid chromatography.

Introduction

High performance liquid chromatography (HPLC) is a well known technique that has been used in laboratories worldwide from more than last 30 years. The factor responsible for the development of the technique was evolution of packing materials used to effect the separation. Ultra-high performance liquid chromatography (UPLC) has marked a radical change by opening new doors for analyst to fetch rapid analytical separation techniques without sacrificing high-quality results obtained earlier by high performance liquid chromatography (HPLC). The immaculate separation method UPLC has many advantages like robustness, ease of use, changeable sensitivity and selectivity but the main limitation is lack of efficiency in comparison to gas chromatography or capillary electrophoresis. The principles behind this evolution are governed by the van Deemter equation that describes the relationship between linear velocity and plate height. According to the van Deemter equation, decrease in particle size increases the efficiency of separations while on other hand efficiency diminishes on increased flow rates or linear velocities. But at a particle size less than 2.5 mm, not only is there a significant gain in efficiency, but the efficiency does not diminish at increased flow rates or linear velocities. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) extended to new limits, termed ultra performance liquid chromatography (UPLC).

UPLC / UHPLC stands for "Ultra High Performance Liquid Chromatography" or "Ultra High Pressure Liquid Chromatography". Pumps in conventional HPLC systems reach a pressure of max. 400 bars. Pumps in UHPLC systems can reach pressures of 1000 bar and more. This allows the use of smaller particles (< 2.0 mm) and still produce acceptable flow rates (up to 5 ml/min). The use of smaller particles allows to obtain better resolution (separation efficiency) or Perform faster chromatography or a combination of both or Increase sensitivity, due to sharper (narrower) and higher peaks.

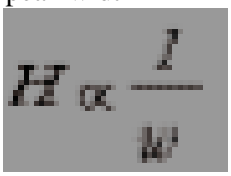
This technology takes full advantage of chromatographic principles to run separations using columns packed with smaller particles and/or higher flow rates for increased speed, with superior resolution and sensitivity.

CHEMISTRY OF SMALL PARTICLE[4-5] :

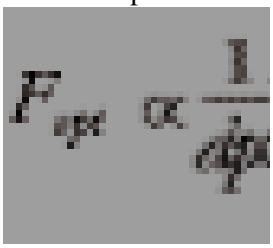
Smaller particles provide not only increased efficiency, but also the ability to work at increased linear velocity without a loss of efficiency, providing both resolution and speed. Efficiency is the primary separation parameter behind UPLC since it relies on the same selectivity and retentivity as HPLC. In the fundamental resolution (Rs) equation: resolution is proportional to the square root of *N*.

$$R = 4N^{1/2}[(\alpha - 1)/\alpha][k/(1 + k)]$$

where *N* is the efficiency, α is the separation factor, and *k* is the retention factor.But since *N* is inversely proportional to particle size (*dp*): as the particle size is lowered by a factor of three, from, for example, 5 µm (HPLCscale) to 1.7 µm (UPLC-scale), *N* is increased by three and resolution by the square root of three or 1.7. *N* is also inversely proportional to the square of the peak width



So as the particle size decreases to increase *N* and subsequently *Rs*, an increase in sensitivity is obtained, since narrower peaks are taller peaks. Narrower peaks also mean more peak capacity per unit time in gradient separations, desirable for many applications, e.g., peptide maps. Still another equation comes into play when migrating toward smaller particles



This relationship also is revealed from the van Deemter plot. As particle size decreases, the optimum flow F opt to reach maximum *N* increases. But since back pressure is proportional to flow rate, smaller particle sizes require much higher operating pressures, and a system properly designed to capitalize on the efficiency gains; A system that can both reliably deliver the requisite pressures and that can maintain the separation efficiency of the small particles with tightly managed volumes. Efficiency is proportional to column length and inversely proportional to the particle size.

The promises of the van Deemter equation cannot be fulfilled without smaller particles than those traditionally used in HPLC. The design and development of sub-2 mm particles is a significant challenge, and researchers have been active in this area for some time to capitalize on their advantages. Figure 1 shows Van Deemter plot, illustrating the evolution of particle sizes over the last three decades.

Although high efficiency, non-porous 1.5 mm particles are commercially available, they suffer from poor loading capacity and retention due to low surface area. To maintain retention and capacity similar to HPLC, UPLC must use novel porous particles that can withstand high pressures. Silica based particles have good mechanical strength, but can suffer from a number of disadvantages, which include a limited pH range and tailing of basic analytes. Polymeric columns can overcome pH

limitations, but they have their own issues, including low efficiencies and limited capacities. In 2000, a first generation hybrid chemistry that took advantage of the best of both the silica and polymeric column worlds was introduced. Producing a classical sol-gel synthesis that incorporates carbon in the form of methyl groups, these columns are mechanically strong, with high efficiency, and operate over an extended pH range. But, in order to provide the kind of enhanced mechanical stability required for UPLC, a second generation bridged ethane hybrid (BEH) technology was developed. These 1.7 mm particles derive their enhanced mechanical stability by bridging the methyl groups in the silica matrix. Packing 1.7 mm particles into reproducible and rugged columns was also a challenge that needed to be overcome. Requirements include a smoother interior surface of the column hardware, and re-designing the end fritsto 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. In addition, at high pressures; frictional heating of the mobile phase can be quite significant and must be considered. With column diameters typically used in HPLC (3.0 to 4.6 mm), a consequence of frictional heating is the loss of performance due to temperature induced non uniform flow.To minimize the effects of frictional heating, smaller diameter columns (1–2.1 mm) are typically used for UPLC.

PRINCIPLE[6-8]:

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)[6]. It was found that HETP decreases to a minimum value and then increases with increasing flow rate. However, with the 1.7 µm particles used in UPLC, HETP is lowered compared to the larger particles and does not increase at higher flow rates. This allow faster separations to be carried out on shorter columns and/or with higher flow rates, leading to column increased resolution between specific peak pairs and increased peak capacity, defined as the number of peaks that can be separated with specified resolution in a given time interval. Efficiency is three times greater with 1.7 µm particles compared to 5 µm particles and two times greater compared to 3.5 µm particles. Resolution is 70% higher than with 5 µm particles and 40% higher than with 3.5 µm particles. High speed is obtained because column length with 1.7 µm particles can be reduced by a factor of 3 compared to 5 µm particles for the same efficiency, and flow rate can be three times higher. This means separations can be nine times faster with equal resolution. Sensitivity increases because less band spreading occurs during migration through a column with smaller particles (peak width is less and peak height greater). 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 .

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

where A, B and C are constants.
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

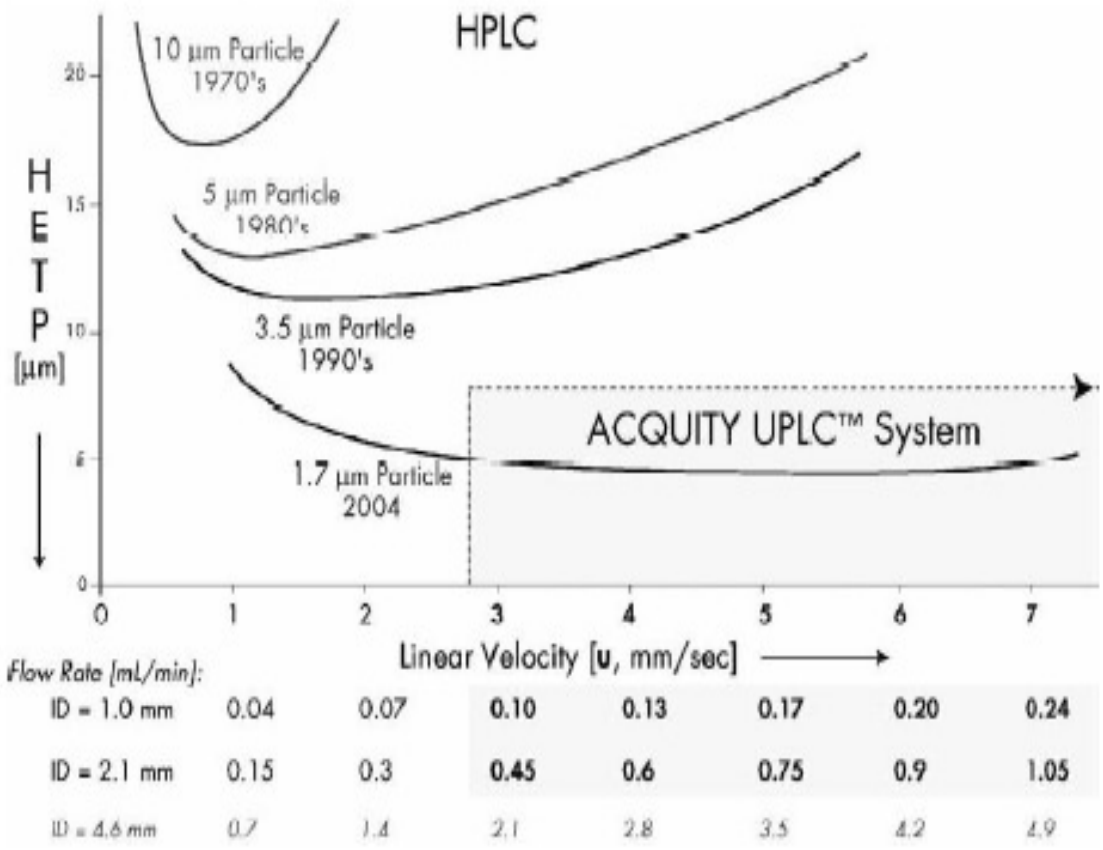


Figure 1: Van Deemter plot, illustrating the evolution of particle sizes over the last three decades.

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[5]. 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. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) extended to new limits, termed ultra performance liquid chromatography (UPLC) ,UPLC / UHPLC stands for "Ultra High Performance Liquid Chromatography" or "Ultra High Pressure Liquid Chromatography". Pumps in conventional HPLC systems reach a pressure of max. 400 bars. Pumps in UHPLC systems can reach pressures of 1000 bar and more. This allows the use of smaller particles (< 2.0 mm) and still produce acceptable flow rates (up to 5 ml/min).

This technology takes full advantage of chromatographic principles to run separations using columns packed with smaller particles and/or higher flow rates for increased speed, with superior resolution and sensitivity.

INSTRUMENTATION:[9-13]

The basic instrument in UPLC technique had to keep its tempo up in order to take advantage of the enhanced speed, superior resolution and greater sensitivity provided by small particles. A design with advanced technology in the pump, auto sampler, detector, data system, and service diagnostics was required to full fill the purpose. The basic instrumentation of UPLC is discussed below.

The schematic diagram of UPLC and various parts of the instrument are shown in Figure1, and Figure 2, respectively.

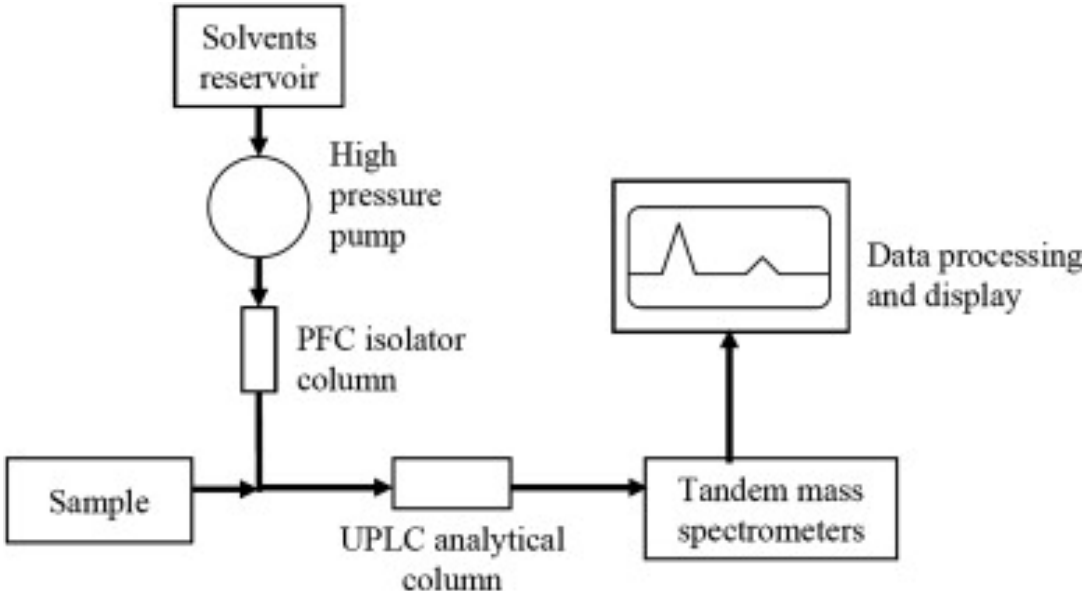


Figure 2: **Schematic diagram of UPLC**

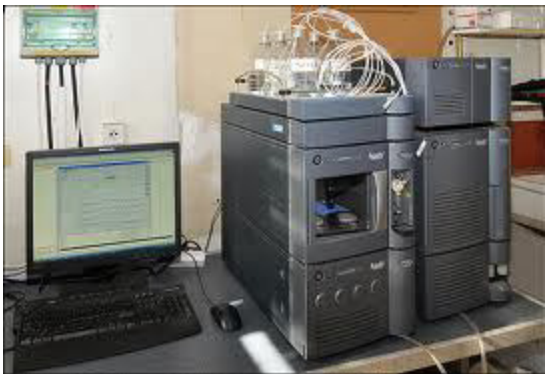


Figure 3: UPLC instrument

PUMPING DEVICE:
An ideal pump for UPLC has a capacity of delivering solvent at higher pressure around 15000 psi for the optimum flow rate with maximum efficiency across 15 cm long column packed with 1.7 µm particles. UPLC uses two serial pumps with pressure limit of 1000 bar and have inbuilt solvent selector valves, which have the capability to choose the accurate solvent ratio from up to four solvents.

SAMPLE INJECTOR MANAGER :
The UPLC system with its flow through-needle design sample manager addresses three design challenges for reliable performance: robust sealing of the needle at higher pressure, minimizing the extra column band spread for narrow peaks and performing pulse free injection process to protect column from extreme pressure fluctuations. When an injection is initiated, the inject valve diverts flow from the needle in order tocollect sample from the vial.

The needle is inserted into the vial to withdraw the exact volume of the sample required and then it returns to the injection port. The needle is pushed against the internal sealing surface of the port and the injection valve turns and the sample is pushed down to the injection port. Dispersion of the sample can be minimized by keeping less distance between the injection port and the inject valve. After injecting the sample, the needle is washed for a specified amount of time to minimize sample carryover. Beside this, there are also direct injections for biological substances mentioned in the literature.

COLUMNS :
The UPLC columns are made up of small particles having size less than 2 µm. The role played by small particle size in UPLC technique has been mentioned above. The particles are bonded in matrix as the bonded stationary phase is required for providing both retention and selectivity. Various types of columns manufactured by ACQUITY are available in the market, which can be used by UPLC technique.

BASED ON TECHNOLOGY COLUMN ARE DIFFERENTIATED AS FOLLOWS:
.
1. BEH C18 columns: This columns provide unprecedented levels of efficiency, peak symmetry and chemical stability. 1.7µm ACQUITY UPLC BEH C18 columns are universal C18 columns, with leading mobile phase pH (1-12) and temperature (80o C) compatibility. This tri functionally bonded alkyl column can impact the retention, sensitivity and selectivity of ionisable compounds.
Ex: Forced degradation studies of Glimepirade (retention time: 8.2mins); Rapid assay for Cytochrome p450 Isoenzymes.

2. BEH C8 columns: Akin to the BEH C18 columns, 1.7µm ACQUITY UPLC BEH C8 columns also provide exceptional efficiency, peak symmetry and chemical stability and are suitable for wide range of analytes. Due to its shorteralkyl chain length, these columns exhibit low hydrophobicity than C18 column, resulting in lower retention and faster elution of analyte peaks.
Ex: Analysis of banned Carcinogenic Aromatic amines: Benzidine, O-anisidine and O-toluidine and Chloroaniline exhibit retention time at 2.7mins, 2.8mins, 3mins and3.6mins respectively.

3. BEH shield RP 18 columns: This contain an embedded polar groups that combines the hydrophobicity of a straight-chain-alkyl ligand C18 with hydrophilicity of an embedded polar group(carbamate). This unique and patented bonding chemistry provides complementary selectivity to a C 18 column and enhances the peak shape for basic compounds and yielding compatibility with 100% aqueous mobile phases.
Ex: impurity profile of Doxylamine drug substance from synthetic and formulation impurities with retention time of 2.8 mins.

4. BEH phenyl columns: This column provides complementary selectivity to straight chain alkyl phases, particularly the molecule contain aromaticity due to pi-piinteractions. This employs trifunctionally-bonded phenylhexyl ligand which provides reproducibility, chemical stability and peak shape for all analyte types.
Ex: By implementing UPLC technology with TQD and a BEH phenyl column, the rapid analysis of 25polymer additives is achieved only 3.5mins; 6-10X faster than HPLC methods.

5. BEH HILIC Columns: (Hydrophilic-Interaction Chromatography) This column successfully improves the retention of very polar species as well as provides an orthogonal separation mode for mixtures of polar and ionisable compounds. This is achieved by utilising an ACNRich, low aqueous mobile phase in combination with a polar stationary phase, to elute analytes in order of increasing hydrophylicity. This column utilises an unbounded BEH particles to improve the retention of very polar basic analytes that are difficult to retain by RPLC.
Ex: separation of lipid classes.

6. BEH Amide columns: This column enable the use of a wide range of mobile phase pH (2-11) to facilitate the exceptional retention of polar analytes spanning a wide ranging polarity, structural moiety and pka. This column is ideal for the analysis of carbohydrates due to its ***exceptional chemical stability*** at high pH and high temperature to collapse reducing sugars anomers as well as ***improved quantitation accuracy*** due to the lack of Schiff-base formation.
Ex: separation of metformin with retention time at 2.4 min. UPLC-MS analysis of carbohydrates fructose, glucose, sucrose, maltose, maltotriose with retention times of 2.7 min, 2.9 min, 3.6 min, 3.8 min, 4.5 min respectively.

7. BEH 130 AND BEH 300 columns: This columns enable improved protein and peptide characterisation due to the increased resolving power. Ex: peptide mapping, conformation of genetic stability, post-translational modifications of proteins.
Ex: Analysis of Amyloid β peptides in cerebral spinal fluid by UPLC has a high degree of

selectivity, specificity and throughput can be achieved compared to the immune assays such as ELISA, Amyloid β1-38 with retention time at 5.78 min, Amyloid β1-40 with retention time at 5.95, Amyloid β1-42 with retention time at 6.17mins.

8. BEH 125, BEH 200, AND BEH 450 SEC Columns: This columns provide accurate, aggregate determinations (m.w: 1,000-80,000; 10,000-450,000 or 100,000-1,500,000 daltons, respectively), significantly faster than traditional HPLC SEC assays.
Ex: Protein calibration curves can be plotted by using these columns.

9. BEH 300 C4 Columns: This columns ideally suited for high resolution separations of proteins. These enable the differentiation of minor isoforms of either heavy or lightchains, providing enhanced assessment on the heterogeneity of a protein sample.
Ex: UPLC/MS analysis of reduced Monoclonal antibody.

10. BEH GLYCAN Columns: These columns provide exceptionally high resolution, thus improving the characterisation of glycoproteins.
Ex: UPLC/FLR analysis of 2-AB labelled Glycans from three Batches of Transtuzumab (breast cancer treatment)

11. UPLC OST C18 Columns: This are ideally suited for the characterisation of oligonucleotides by ion pair reverse phase chromatography while delivering exceptional sample resolution, peak shape and extended column life.
Ex: UPLC/MS analysis of interfering RNA oligonucleotides.

12. UPLC CSH C18 Columns: It is a universal C18 column choice, suitable for a broad range of compound classes while providing alternate selectivity to BEHC18. Built on charged surface hybrid (CSH) particle platform, the CSH C18 column provides exceptional peak shape and increase loading capacity, particularly for basic compounds under low-pH, weak-ionic-strength mobile-phase conditions.
Ex: The ACQUITY UPLC CSH C18 was used successfully characterise the peroxide degradation products of Ziprasidone in a simple 0.1% formic acid in water as mobile phase.

13. UPLC CSH PHENYL-HEXYL Columns: This columns provides complementary selectivity to straight-chain-alkyl phases, particularly for polyaromatic coumpounds. Built on the CSH particle platform, the CSH Phenyl-Hexyl column provides exceptional peak shape under low-and high- pH conditions.
Ex: Analysis of basic drugs on CSH Phenyl-Hexyl (pindolol 10µg/ml R.T at 1.18 min, Quinine 10 µg/ml R.T at 1.39 min, Labetolol 60 µg/ml R.T at 1.86 min, Diltiazem 25 µg/ml R.T at 2.3 min, Verapamil 50µg/ml R.T at 2.6 min,)

14. CSH Fluoro-Phenyl Columns: This provides exceptional selectivity for positional isomers, halogenated and polar compounds. This is due to an intricate combinations of multiple retention mechanisms including dipole-dipole, hydrogen-bonding, aromatic (π-π) and hydrophobic interactions. Due to the unique surface chemistry of CSH particle and CSH Fluoro-Phenyl Column provides enhanced retention of acidic compounds compared to traditional PFP-bounded stationary phases.
Ex: Analysis of β-lactum antibiotics.(Amoxicillin, RT at 1.5mins; Ampicillin, RT at 2mins; Pencillin-G, RT at 5.4 mins, Dicloxacillin, RT at 6.5mins and Oxacillin, RT at 6mins.) ; separation of Paroxetine Isomers 1. Cis: RT at 3.5 min 2. Trans at 3.8 min.

15. UPLC HSS T3 columns: This is ideally suited for enhanced retention of polar compounds and metabolites by RPLC. This low-ligand density C18 column enables analytes to more readily access the pore structure of the material, providing balanced retention of polar andreagents.
Ex: Analysis of powdered soy Isoflavone extracts (Glycitin at 4.9mins RT; Glycitein at 10.6mins RT; Apigenin at 13.3mins RT)

16. UPLC HSS C18 columns: These are general-purpose silica-based C18 chemistry choice with applicability to a broad range of compound classes. It provides exceptional peak shapes and low-pH stability while delivering increased retention in comparison to hybrid-based C18 columns.
Ex: Seperation of Xanthine alkaloids (Xanthine, 0.310mins RT, 7-methyl Xanthine, 0.490mins RT, Theobromine, 0.650mins RT, Paraxanthine, 0.780mins RT, caffeine 0.99mins RT)

17. UPLC HSS C18 SB and PFP columns: This column is a non-endcapped, low coverage silica based C18 chemistry that provides alternative selectivity for compounds impacted by silanophilic interactions. These results in
greater retention of basic compounds.

18. UPLC HSS Cyano columns: This provides low hydrophobicity and unique selectivity compared to straight-chain-alkyl columns. Due to the innovative monofunctional cyano bonding with protective alkyl side chains affords a ultra-stable retention. Exceptional peak shape and reproducibility under low to mid pH conditions and it is compatible to Reverse Phase and Normal Phase techniques.
Ex: separation of steroids and analgesics.

DETECTORS:
The system can be configured with a TUV, ELS, PDA and FLR detectors or a combination of them.

1. TUV (Tunable UltraViolet) detector: It is a two channel, absorbance detector. The detector. Controlled by Empower or MassLynx software for both LC/MS and LC applications.

2. PDA (Photo Diode Array) detector: It is a optical detector absorbs UV-Visible light that operate between 190-500nm.

3. ELS (Evoparative Light Scattering) detector: The detector. Controlled by Empower or
MassLynx software, which incorporates a flow-type nebuliser that is optimised for UPLC system performance.

4. FLR (Fluorescence) detector: It is a multi channel, multi wavelength detector, which has an excitation wavelength that ranges from 200 to 890 nm, an emission wavelength that ranges from 210-900 nm, offers 3D scanning capability for easier method development.

MERITS:[6,13-14]

1. The UPLC system allows shortening analysis time up to nine times comparing to the conventional system using 5 µm particle packed analytical columns.

2. Separation on UPLC is performed under very high pressures up to 100 MPa.

3. It gives increased peak capacity (number of peaks resolved per unit time) and resolution

4. UPLC dramatically improves the quality of the data, resulting in a more definitive map.

5. UPLC fulfills the promise of increased speed, resolution, sensitivity and broad range of selectivity predicted for liquid chromatography.

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

7. Expands scope of Multiresidue Methods

8. UPLC's fast resolving power quickly quantifies related and unrelated compounds

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

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

11. 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.

12. Delivers real-time analysis in step with manufacturing processes and Assures end-product quality, including final release testing.

13. Columns are withstand high back pressure system.

DEMERITS:[14]
1. Due to increased pressure requires more maintenance and reduces the life of the columns of this type.

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

4. Also detector and data collection system (CDS) may not cope with sharper peaks (data acquisition rate).
5. So far only binary pump systems (not ternary or quaternary). This may make method transfer not straightforward.

APPLICATIONS[6, 15] :

1. With UPLC increased resolution in shorter run times can generate more information faster without sacrifices. Higher sample throughput with more information per sample may decrease the time to market, an important driving force in today's pharmaceutical industry. The corresponding HPLC separation takes in excess of 12 min; UPLC accomplishes the same separation in under 30 s.
2. UPLC can also be used to significantly improve the success of the drug discovery process. Drug discovery is heavily dependent upon the early prediction of metabolic fate and interactions of drug candidate molecules.
3. Sensitivity, selectivity, and analysis time (sample throughput) are also some of the challenges analysts face when analyzing environmental samples such as soil and water. Explosives residues in soil or environmental waters are of both forensic and environmental interest. These types of assays prove challenging because of the selectivity needed to resolve positional isomers.
4. In addition, for complex samples like natural product extracts, added resolution can provide more information in the form of additional peaks. HPLC versus UPLC separation comparison of a ginger root extract sample where both speed and resolution are improved.
5. UPLC coupled with MS technology provided parent and fragment mass information of lipids in one chromatographic run, thus, providing an attractive alternative to current LC methods for targeted lipid analysis as well as lipidomic studies.
6. Applications areas of UPLC specified in Waters literature include high throughput library screening, metabolite identification and bioanalysis, peptide mapping, stability indicating analyses, and quantitative analysis.

CONCLUSION :

UPLC is a new revolution in chromatography. 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 ,due to smaller particle size. The main advantage is a reduction of analysis time, which also meant reduced solvent consumption. A negative aspect of UPLC could be the higher backpressure than in conventional HPLC. This backpressure can be reduced by increasing the column temperature. It was found that the sensitivity of UPLC was much higher than that of conventional HPLC. All categories of pharmaceutical drugs can be analyzed by UPLC method within a very short period of time and with less solvent consumption

Overall, it seems that UPLC can offer significant improvements in speed, sensitivity and resolution compared with conventional HPLC.

REFERENCES:

1. Ashok kumar, UPLC: A preeminent technique in pharmaceutical analysis. Acta poloniae pharmaceutica– drug research. 69(30), 2012, 371-380.
2. www.chromatographyonline.com.
3. Kondawar M. S., Ultra Performance Liquid Chromatography: A Faster and Sensitive Method over HPLC, phamainfo.net, 2006,4(5).
4. Joseph S., John D., Frances H.L, UPLC: ultra performance liquid chromatography, Journal of AOAC International, 2005.
5. Swartz M E. UPLC: An Introduction and Review. J of Liq Chromato & Related Techno.2005; 28:1253–1263.
6. Michael E Swartz, Ultra performance liquid chromatography UPLC: an introduction. Separation science redefined.1:2005;8-14
7. B. Srivastava, Ultra performance liquid chromatography (UPLC): A chromatography technique. International journal of pharmaceutical quality assurance. 2(1):2010;19-25
8. Preeti vinod gaikwad, Ultra performance liquid chromatography: A recent novel development in HPLC.Pharmacie globale international journal of comprehensive pharmacy, 01, 2012, 1-3.
9. www.waters.com

10. Swartz M. Murphy B J. Sievers D. UPLC: Expanding the limits of HPLC. GIT Lab J.2004; 8(5):43-45.
11. Swartz M E. UPLC: Tomorrow's HPLC technology today. Lab plus Int.2004; 18(3): 6.
12. Nguyen D T. Guillarme D. Rudaz S. Veuthey J L. Fast analysis in liquid chromatography using small particle size and high pressure. J Sep Sci. Aug 2006; 29(12):1836-48.
13. B. Srivastava, Ultra performance liquid chromatography (UPLC): A chromatography technique. International journal of pharmaceutical quality assurance. 2(1):2010;19-25
14. M.E. Swartz, *J. Liq. Chromatogr.*, in press
15. www.pharmafocusasia.com