IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC): A NOVEL APPROACH IN ANALYSIS OF PHARMACEUTICALS

Omkar G. Tekale*[1], Dr. Amol N. Khedkar[2], Vitthal B. Kaulage[3]

Department of Pharmaceutical Sciences, Saikrupa Institute of Pharmacy, Ghargaon, Shrigonda, Ahmednagar, Maharashtra, India 414728

Corresponding Authers Details: Omkar Ganesh Tekale.

Abstract:

A contemporary method called UPLC offers liquid chromatography a new path. Three aspects of liquid chromatography are enhanced by UPLC: sensitivity, resolution, and speed. A bridged ethylsiloxane/silica hybrid (BEH) structure column with fine particle size (less than 1.7µm) is used in this system. Pharmaceutical companies and analytical labs are currently looking for innovative ways to decrease the time and expense of drug analysis while enhancing product quality. Better resolution, assay sensitivity, and high sample throughput of UPLC provide cost-effective advantages over HPLC analysis and enable the performance of more analyses in less time. The UPLC separation procedure uses high pressure (up to 100 MPa). In addition, compared to traditional HPLC, it uses less reagent and has a shorter run time. The current review paper discusses the differences between HPLC and UPLC, validation of analytical methods, applications, and benefits and drawbacks of each.

Keywords: Ultra Performance Liquid Chromatography, Higher Resolution, Higher Efficiency.

Introduction:[1-5]

Chromatography is a non-destructive technique that divides a mixture of components into its constituent parts using a porous medium and solvents. Until 2004, HPLC was the preferred technique for dissecting a combination of ingredients into their individual components. But because of a number of constraints, researchers have created a brand-new, cutting-edge technique called "Ultra Performance Liquid Chromatography (UPLC)" that not only overcomes some of the drawbacks of HPLC but also works incredibly well. Ultra-Performance is the ability to achieve new heights in speed and peak capacity (number of peaks resolved per unit time) by employing smaller particles. HPLC (High Performance Liquid) is the traditional method of separation.Chromatography), which has numerous benefits such as stability, user-friendliness, high selectivity, and adaptability Sensibility. Its primary drawback is its inefficiency when compared to gas chromatography or the low diffusion coefficients in the liquid phase, which cause analytes to diffuse slowly in the stationary phase, cause capillary electrophoresis. The Van Deemter equation demonstrates that using smaller size particles increases efficiency, but doing so quickly raises back pressure, whereas the majority of HPLC system is limited to 400 bars of operation. For this reason, brief columns containing particles measuring approximately 2 µm are employed with these systems in order to quicken the analysis without sacrificing efficiency and keeping an acceptable load-loss 3. The following actions can be taken to increase HPLC separation efficiency: a) work at higher temperature b) the application of monolithic poles.

PRINCIPLE[6-12]

The Van Deemter relationship, which explains the relationship between flow rate and plate height, is the foundation of UPLC. Reference [2]. According to the van Deemter equation (i), the with the smaller particles, the flow range is much larger for good compared to larger particles outcomes [3-6].

$$H = A + \frac{B}{v} + Cv$$

Where "V" denotes the flow rate (linear velocity), "H" stands for height equivalent to the theoretical plate (HETP), and A, B, and C are the constants. Of the gas carrier. The objective is to reduce HETP to raise the efficiency of columns. The word A isn't depend on speed and show eddy mixing. Well, it is smaller if the rows are full of tiny and consistent in size particles. The letter B represents the tendency of the particles' natural diffusion. When this effect is less at high flow rates, so this term is split by v. The kinetic is represented by the term C. opposition to balance throughout the course of division. The time lag is the kinetic resistance. Necessary to transition from the mobile phase to the stationary phase followed by decreasing dead volumes) and constant pressures (roughly 500–1000 bars as opposed to HPLC's 170–350 bars). Efficiency is correlated with the column's length and inversely proportionate to the particle's radius [6]. Therefore, the The same factor can be used to shorten columns. Without changing the particle radius conclusion. Utilising UPLC has aided in the identification of medication metabolites and improvement of the separation spectra's quality [7, 8].

SMALL PARTICLE CHEMISTRY[13-21]

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 advantages4. Figure 1 shows Van Deemter plot, illustrating the evolution of particle sizes over the last three decades.

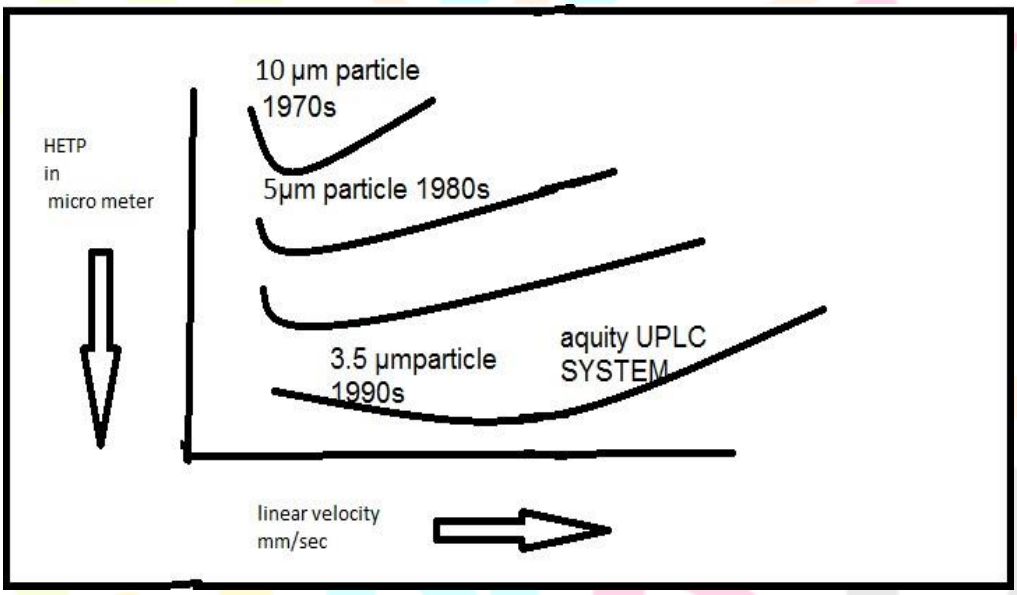


Figure 1: Van Deemter plot, illustrating the evolution of particle sizes over the last three decades.

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 basedparticles 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.

UPLC VS HPL

PARAMETER	UPLC	HPLC
Particle size	Less than 2µm	3-5µm
column	ACQUITY UPLC BEH C18 AND C8	ALLTIMA C18 ZORBAX C8
Injection volumn	2µl [std in 100% methanol]	5µl [std in 100% methanol]
Column dimension	150×2.1mm	150×3.2mm
Column temperature	65oC	30oC
Flow rate	Less	More

Even though the two techniques' principles are the same, there are still some differences that distinguish UPLC as a modern technique and HPLC as a conventional method. One easy way to shorten an analytical run is to shorten the column's length and speed up the flow. This strategy, however, may be dangerous because complex compound mixtures may not be sufficiently separated, and column efficiency may be significantly reduced [4]. Particle size of the column material is less than 2 µm, which is the primary difference. which have a significant impact on performance. This has the drawback of generating a lot of back pressure, which is unacceptable for traditional HPLC systems and analytical columns. However, it does enable high-speed analyses with high efficiency. An additional strategy to reduce analysis times is to increase the is raise the temperature due to which Mobile phase viscosity is reduced at higher temperatures [4-5]. In UPLC technique we found better resolution and separation are found as compared to HPLC also perform more sensitive analysis. Presences of monolithic columns make UPLC technique an improved version of HPLC.

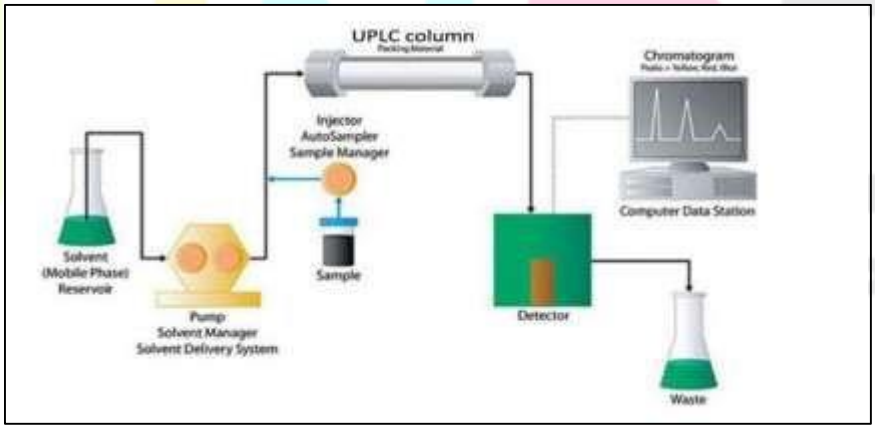
Instrumentation [22-28]

The various instruments used in the Ultra performance liquid chromatography are as follows
Pumping devices Sample injection

UPLC columns

Column manger & heater or cooler

Detectors



Figur2: Schematic diagram of UPLC

Figure 3: UPLC Instrument

Pumping devices

For maximum flow rate and efficiency, an ideal UPLC pump can transport solvent at a higher pressure of approximately 15000 psi through a 15 cm long column packed with 1.7 µm particles. The two basic classifications are
[41]

a) Constant pressure pump b) Constant flow pump

Constant pressure pump: The constant pressure is used for column packing.

Constant flow pump: This type is mostly used in all common UPLC applications.

Sample Injection

In UPLC, sample introduction is critical. Conventional injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from extreme pressure fluctuations, the injection process must be relatively pulse-free and the swept volume of the device also needs to be minimal to reduce potential band spreading. A fast injection cycle time is needed to fully capitalize on the speed afforded by UPLC, which in turn requires a high sample capacity. Low volume injections with minimal carryover are also required to increase sensitivity. There are also direct injection approaches for biological samples. The introduction of samples is important in UPLC. Whether automated or manual, conventional injection valves are not built and toughened to operate at tremendous strain. To safeguard the column against severe variations in pressure, the injection procedure must be swept at a relatively pulse-free of the gadget must also be small in order to minimize possible band dispersal. A rapid cycle of injection It takes time to fully benefit from the speed made possible by UPLC, which consequently calls for a high sample magnitude. injections at low volumes with Minimal carryover is also necessary in order to raisesen sibility. Additionally, direct injection strategies for biological specimens. A sample in the mobile phase makes up a small volume solution. Here, an accurate measurement is added using a sample injector. To ensure column safety from high pressure damage, a formal injection valve can be manually operated or programmed [10]. Injecting the sample must be done accurately. In order to maximise the speed that UPLC offers, injection cycle times should be quick in order to achieve high sample capacities. To boost sensitivity, low volume injection with little carryover is required.

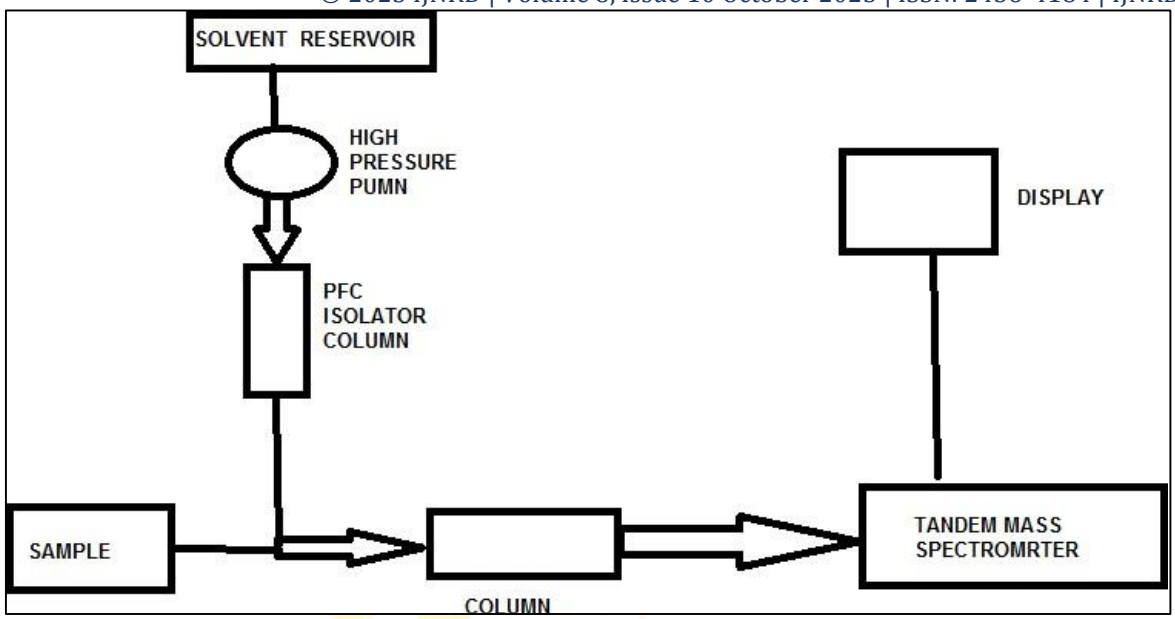


Figure 4: Schematic diagram of UPLC

UPLC columns

The 1.7 µm particle packed column has higher resolution due to its improved efficiency. A bonded phase with both retention and selectivity is necessary for the separation of a sample's individual components. Four bonded phases are available for UPLC separations**:**

1. ACQUITY UPLCTM BEH C8 (straight chain alkyl columns)
2. ACQUITY UPLCTM BEH C18 (straight chain alkyl columns),
3. ACQUITY UPLC BEH Shield RP18 (embedded polar group column) and
4. ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl).

Different combinations of hydrophobicity, silanol activity, hydrolytic stability, and chemical stability are offered by each column chemistry.participation of analytes. BEH ACQUITY UPLC Columns C8 and C18 are regarded as the universal preferred columns for the majority of UPLC separations by offering the greatest range of pH. They include tri functional chemistries that form bonds with ligands generate excellent low pH stability. Different combinations of hydrophobicity, silanol activity, hydrolytic stability, and chemical stability are offered by each column chemistry. Participation of analytes. BEH ACQUITY UPLC Columns C8 and C18 are regarded as the universal preferred columns for the majority of UPLC separations by offering the greatest range of pH. They include tri functional chemistries that form bonds with ligands generate excellent low pH stability. Such a low pH combined with the elevated pH stability of the BEH particle at 1.7µm to provide the broadest useable pH range of operation. The purpose of the ACQUITYUPLC BEHShield RP18 columns is to offer selectivity that goes well with the ACQUITYUPLC BEH C18.and phases C8. UPLC ACQUITY BEH Phenyl A tri functional C6 alkyl ether is used in columns.amid the silyl functionality and the phenyl ring.This ligand in conjunction with the identical proprietary procedures for end capping as the ACQUITY UPLC Long columns are provided by BEH C18 and C8 columns. lifetimes and superior peak condition. This distinct end capping and ligand combination on the A new dimension is created in the 1.7µm BEH particle selectivity enabling a speedy match to the current HPLC specimen. 2.1 as the internal dimension (ID) Column mm is employed. To get the highest resolution,select a length of 100 mm and, for quicker analysis, and select a 50 mm column for a higher sample throughput. Half-height peak widths of less than one second are obtained with 1.7µm particles, which gives significant challenges for the detector. In order to integrate an analyte peak accurately and reproducibly, the detector sampling rate must be high enough to capture enough data points across the peak. The detector cell must have minimal dispersion (volume) to preserve separation efficiency. Conceptually, the sensitivity increase for UPLC detection should be 2-3 times higher than HPLC separations, depending on the detection technique. MS detection is significantly enhanced by UPLC; increased peak concentrations with reduced chromatographic dispersion at lower flow rates promote increased source ionization efficiencies. The ACQUITY UPLC System consists of a binary solvent manager, sample manager including the column heater, detector, and optional sample organizer. 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. The sample manager also incorporates several technology advancements. Using pressure assisted sample introduction, low dispersion is maintained through the injection process, and a series of pressures transducers facilitate self-monitoring and diagnostics. It uses needle-in-needle sampling forimproved 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. . A column with an internal dimension (ID) of 2.1 mm is employed. Select a column with a 50 mm length for faster analysis and higher sample throughput, and a 100 mm length for maximum resolution. With 1.7µm particles, half-height peak widths of less than a second are obtained, posing serious challenges. for the sensors. To reliably and precisely integrate an analyte peak, the detector To obtain sufficient data points throughout the peak, the sampling rate needs to be high enough. The cell for detection must have a low dispersion (volume) in order to maintain separation effectiveness. In terms of concept, the sensitivity a 2-3 times greater rise for UPLC detection than HPLC separations, depending on the method of detection. UPLC greatly improves MS detection; higher peak concentrations at a lower flow with less chromatographic dispersion

Column manager & heater or cooler

The column heater heats the column compartment to any temperature from 50C to 650C.

Detectors-

After separation, the detector is the tool used to identify analytes in both qualitative and quantitative ways. One or more of the following detectors can be installed in the system: TUV, ELS, PDA, and FLR. The TUV (Tunable Ultraviolet) detector is a two-channel absorbance device. The software used to control the LC/MS and LC applications is called Empower or Mass Lynx. Detector of photo diode array (PDA): UV-visible light is absorbed by an optical detector that operates in the range of 190 to 500 nm. Evaporative light scattering, or ELS, detector: The senser driven by the Mass Lynx or Empower software, which has a flow-type nebulizer designed specifically to work with UPLC systems. Detector of fluorescence (FLR) This multi-channel, multi-wavelength detector allows for easier method development by providing 3D scanning functionality. The detectors used in UPLC analysis is UV/Visible detector. Detection of analytes is conventionally based on absorbance that is concentration sensitivity detectors. In UPLC the flow cell volume would have to be reduced to maintain concentration and signal. Based on Beer's Law, smaller volume conventional flow cells would also reduce the path length upon which the signal strength depends. A reduction in crosssection 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 unable UV/Visible detector cell consists of a light guided flow cell equivalent to an optical fiber. Light is efficiently transferred down the flow cell in an internal reflectance mode that still maintains a 10mm flow cell path length with a volume of only 500mL. 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.

Detectors used in UPLC technique are as follows

A] Tunable ultraviolet detector

B] Photo diode array detector

C] ELS Detector

TUV detector [Tunable ultraviolet detector]

The analytical cell, with a capacity of 500 neon liters and a path length of 10 mm, and Foremost sensitivity flow cell, with a capacity of 2.4 micro liters and a 25 mm path length, both Make use of the flow cell technology. The TUV detector operates at wavelengths ranging from 190 to 700 nm.

PDA [Photo diode array detector]

The PDA (photodiode array) detector is also called as ultraviolet/visible light (UV/Vis) Spectrophotometer that run between 190 and 500 nm. Detection limits are higher for pda detector therefore caution should be taken while analysis (J.R. Bertolín, Malondialdehyde determination in raw and processed meat products by UPLC-DAD and UPLC-FLD, 2019) The detector offers two flow cell options. The cell, having the volume of 500 nano liters and a path length of 10mm, 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 [13-14]

ELS detector [evaporative light scattering]

This evaporative light scattering detector designed for use in the UPLC technique. So far execution same or even better has been showed by using stationary phases of size around 2µm without the unfavorable effects of high pressure. In addition, the phases of lower than 2 µm are commonly non-regenerable and thus have restricted use [15].

Figure 5 :- UV detector

Table 1: COMPARISON BETWEEN UPLC AND HPLC.

SR. NO.	CHARACTERISTICS	HPLC	UPLC
1	**Particle size**	3 to 5µm	Less than 2 µm
2	**Maximum back pressure**	35-40 MPa	103.5 MPa
3	**Analytical column**	Alltima C18	Acquity UPLC BEH C18
4	**Column dimensions**	150 X 3.2 mm	150 X 2.1 mm
5	**Column temperature**	30°C	65°C
6	**Injection volume**	5mL (Std. In100% MeOH)	2mL (Std.In100% MeOH

Significance of UPLC [29]

A higher flow rate makes UPLC a more effective method of separation.

The viscosity of the mobile phase decreased as the temperature rose.

More precise and accurate technique in less time.

We obtain improved sensitivity, speed, and resolution.

Attached to a mass spectrometer for additional analyte analysis.

Quick drug product analysis because fewer particles smaller than 2 µm are used.

oThis method produced more resolved chromatographic peaks and increased sensitivity.

The sensitivity and specificity of ultra performance liquid chromatography are increased.

Operating expenses are decreased.

TYPES OF UPLC BY WATERS [30-32]

ACQUITY UPLC® I-Class provides the most powerful solution to the most critical need in separation science today – successfully analyzing compounds that are limited in amount or availability amid a complex matrix, more rapidly than ever before. Developed to produce the most accurate and reproducible separations, getting the most information possible and accelerate laboratory results. Complex separation challenges require LC systems designed to maximize the benefits of sub-2µm particle columns integrated in a system designed to optimize MS performance.

The ACQUITY UPLC I-Class system:
Maximizes peak capacity to enhance MS sensitivity.
Provides the lowest carryover, complementing MS sensitivity and extending MS linear dynamic range.
Has been purposefully engineered for the lowest dispersion; with an extended pressure/flow envelope, complex separations can be accelerated without compromising chromatographic fidelity.

The ACQUITY UPLC H-Class is a streamlined system that brings together the flexibility and simplicity of quaternary solvent blending and a flow-through-needle injector to deliver the advanced performance expected of UPLC type separations – high resolution, sensitivity and improved throughput – while maintaining the robustness and reliability that ACQUITY systems are known for
Choosing the ACQUITY UPLC H-Class enables to continue running existing HPLC methods on a forward-looking LC platform that allows to confidently and seamlessly transition to UPLC separations, using integrated system tools and reliable column kits for method transfer and method development that simplify migration.

The nano ACQUITY Ultra Performance LC® (UPLC®) System is designed for nano-scale, capillary, and narrow-bore separations to attain the highest chromatographic resolution, sensitivity, and reproducibility. Direct nano-flow offers significant improvements over conventional nano-flow separations technologies. It improves peak capacity and peak shape, and increase the number of components that can be detected per separation. The system's 10,000 psi operating pressure capability allows for superior high-peak capacity separations by operating longer columns packed with sub-2 micron particles. It is optimized for high-resolution identification and 2D-LC separations at precise nano-flow rates. The nano ACQUITY UPLC System provides solutions for biomarker discovery and proteomics applications, for protein identification and characterization.

The PATROL™ UPLC® Process Analyzer is a real-time Process Analytical Technology (PAT) system that detects and quantifies complex multiple component manufacturing samples and final product directly on the production floor. Designed with the same enabling technology that drives the ACQUITY UPLC® System, PATROL UPLC moves existing liquid chromatography (LC) analysis from off-line Quality Control (QC) laboratories directly to the manufacturing process, resulting in significant improvements in production efficiency:
Delivers Real-Time LC™ analysis in step with manufacturing processes
Provides the selectivity, sensitivity, and dynamic range of LC analysis
UPLC's fast resolving power quickly quantifies related and unrelated compounds
Reduces process cycle times, so that more product can be produced with existing resources
Enables manufactures to produce more material that consistently meet or exceeds the produc specifications, potentially eliminating variability, failed batches, or the need to re-work material Assures end-product quality, including final release testing.

The PATROL UPLC Process Analyzer is an ideal solution for pharmaceutical, biopharmaceutical, petrochemical, and food manufacturers that are under increased internal and external pressure to evaluate PAT programs and techniques.
Globaregulatory initiatives, such as the U.S. Food and Drug Administration and European Medicines Agency Critical Path and PAT Initiatives, and manufacturing quality-by-design programs, such as Six Sigma, are driving corporations to assess and implement novel PAT solutions such as the PATROL UPLC System.

The PATROL UPLC® Laboratory Analyzer provides real-time quantitative analysis of chemical
reactions in process development and optimization laboratories. Proven UPLC® Technology and Real- TIME LC™ analysis have been integrated to an online analyzer that provides fast and accurate quantitative results to characterize process methods. Spectroscopic technologies used in process development laboratories provide identity information about the processes; however, lack the ability to simultaneously monitor multiple components at different levels and does not provide the quantitative analysis, sensitivity, linearity/dynamic range, and resolution that UPLC provides

Development of Columns Chemistry by Different Companies. [33,34]

Waters & Company: They introduced the first-generation hybrid particle technology (HPT)-equipped XTerra line of HPLC columns. This is the result of combining organic (polymeric) and inorganic (silica) packings to create a material with a low pH range, excellent mechanical strength, and efficiency. Since Because of its speed, sensitivity, and resolution, UPLC technology

requires these characteristics. Peak capabilities are determined by these parameters. Consequently, a new wide-pH, pressurebearable A range particle was produced. A second-generation hybrid column designed to work at higher pressures with the ACQUITY UPLC system. The silica–organic hybrid chemistry is based upon bridged ethylene groups within a silica gel particle structure which gives mechanical strength and pH range from pH 1 to pH 12. As compared to the first generation methyl hybrid particle of XTerra® columns and the bridged ethyl hybrid particle of ACQUITY UPLC BEH columns exhibits improved efficiency, strength and pH range. As BEH Technology is a key enabler of the speed, sensitivity and resolution of UPLC separations. The highly efficient 1.7 µm BEH particles allow chromatographers to maximize the efficiency by increasing theoretical plates (N) of their analyte separation, when it used with the ACQUITY UPLC system, it gives improvement in the selectivity or retentivity (k) and resolution. In order to get the desired separation UPLC BEH columns were carefully chosen BEH C18, C8, Shield RP18 and Phenyl column chemistries these enable the rapid development and more robust separations.

Agilent Technologies: The Company provides regular phases with 1.8 µm version particle size. Phases include Zorbax stable Bond C8 and C18 for low-pH operation, Zorbax XDB-C8 and C18 for general-purpose separations, Zorbax Extend C18 for high-pH applications and Zorbax-SB CN which provides a different reversed-phase polarity. These involve both cartridges and standard compression fitting hardware in 2.1, 3.0 and 4.6 mm internal diameters with column lengths of 15–100 mm. In relative to other sub-2 µm columns, pressure drops are reduced by purposely widening the particle size distribution without influencing column efficiency.

Alltech Associate: The Company offers a 1.5 µm particle version for their regular columns. The company provides Platinum HPLC columns have controlled surfaces that offer dual mode separations and extend the range of polar selectivity. The C8,C18 and extended polar selectivity (EPS) phases are available in 33 and 53 mm × 7 mm columns in the Rocket form. These are silica-based columns with a 100 A0 pore size which are most enable for small-molecule separations. The Alltima HP HILIC and Pro Sphere HP ZAP C18 were used in large scale. The former non bonded, high-purity bare silica column is recommended for the hydrophilic interaction chromatography (HILIC) separations of highly polar compounds that are poorly retained or un retained on conventional reversed-phase columns. These columns are used with mobile phases consisting of mostly organic solvents with only small amounts of water in the mobile phase and are useful in LC–mass spectrometry (MS) for higher sensitivity with volatile mobile phases. For MS, a smaller 2.1 mm i.d. column is available. Columns of 10, 20 and 33 mm lengths are provided. The Pro Sphere column has a 500 A0 pore size, which makes it ideal for the high-speed reversed-phase separation of proteins. **Bischof firm:** The Company offers four columns, first three are porous (1.8 µm) and last one is a nonporous silica phase (1.5µm).The totally porous packing are based upon 300 m2/g silica. The Pronto PEARL sub-2µm, TPP-C8, ACE EPS (8% carbon loading) and C18 EPS (16% carbon) are smaller particle versions of their regular production. Their Column dimensions are 30–50 mm × 2.0 and 4.6 mm. The third phase on totally porous silica is the Pronto PEARL sub-2 TPP APS, which is a reversed phase with a polar-embedded functionality (3.5% carbon content). This packing gives higher retention for acidic compounds compared with C8 and C18 bonded phases. This 1.8 µm column was used to determine the poly phenon content. Relative to the matrix components, the poly phenol were well retained; thus, no extensive sample preparation was required except filtration through a 0.2 µm filter11

Advantages[35-37]

Various advantages of UPLC are as follows:

- Decreases run time and increases sensitivity.
- Provides the selectivity, sensitivity, and dynamic range of LC analysis.
- Maintaining resolution performance.
- Expands scope of Multi residue 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.
- Delivers real-time analysis in step with manufacturing processes.
- Assures end-product quality, including final release testing.

Disadvantages[38-39]

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-generable 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 of UPLC[40-52]

Analysis of Natural Products and Traditional Herbal Medicine :- UPLC is widely used for analysis of natural products and herbal medicines. UPLC provides high-quality separations and detection capabilities to identify active compounds in highly complex samples that results from natural products and traditional herbal medicines.

Identification of Metabolite :- Biotransformation of new chemical entities (NCE) is necessary for drug discovery. When a compound reaches the development stage, metabolite identification becomes a regulated process.UPLC/MS/MS addresses the complex analytical requirements of biomarker discovery by offering unmatched sensitivity, resolution, dynamic range, and mass accuracy.

Bio analysis / Bioequivalence Studies :- 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 analysis. UPLC/MS/MS delivers excellent chromatographic resolution and sensitivity.

ADME (Absorption, Distribution, Metabolism, Excretion) Screening:- 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.

Dissolution Testing:- In sustained-release dosage formulations, testing higher potency drugs is particularly important. The dissolution profile is used to demonstrate reliability and batch-tobatch 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.

Method Development / Validation:- UPLC help in critical laboratory function by increasing efficiency, reducing costs, and improving opportunities for business success. UPLC column chemistries can easily translate across analyticaland preparativescale 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. With UPLC, separation speed and efficiency allows for the rapid development of methodologies.

Forced DegradationStudies:- 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. 8. Impurity Profiling:-UPLC confidently detect impurities in compounds even at trace levels. UPLC combines with exact mass LCMS, 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.

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 purity of raw materials and finished products. UPLC is used for the highly regulated, quantitative analyses performed in QA/QC laboratories.

Method Development / Validation:- According to FDA, validation is defined as establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. Method development and validation is a time-consuming and complicated process: 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.

Impurity Profiling :- Impurity profiling requires high-resolution chromatography capable of reliably and reproducibly separating and detecting all of the known impurities of the active compound. 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 asecond 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. UPLC combines with exact mass LC/MS, has been successfully employed for the identification of drug and endogenous metabolites.

Manufacturing / QA / QC: In QA/QC

Laboratories, highly regulated quantitative analyses are carried out using UPLC. A supply of reliable, superior consumable product performance a crucial part of an approved analytical procedure.

Amino acid analysis:

UPLC is utilised for precise, dependable, and repeatable examination of amino acids in the protein-related areas descriptions, cell culture observation, and food nutritional analysis.

Pesticide determination:

UPLC combined with triple Quadra tandem mass spectroscopy will assist in identifying pesticides at trace levels. From liquid.

Magnolia officinalis cortex identification can be accomplished via UPLC fingerprinting.

Compound Library Maintenance:- 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

Open Access:- 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.

Conclusion

Ultra-Performance Liquid Chromatography offers significant advancements over conventional HPLC. In fact, HPLC now uses it as the standard platform. The main benefit is a reduction in the amount of time and solvent used for analysis. Short columns and small particle sizes are used to achieve this. The only disadvantage of UPLC is high back pressure, which can be reduced by raising column temperature. In general, the UPLC method is widely used and provides a significant increase in speed, sensitivity, and resolution over conventional High Performance Liquid Chromatography.

References

[1] Ashok K, Gautam S, Anroop N 2012. UPLC A Preeminent Technique in Pharmaceutical Analysis. Acta Poloniae Pharmaceutica.69(3): 376-7.

[2] Vandana P.2011. Ultra-performance liquid chromatography: a review. Int Res J Pharma. 2(6): 39-44.

[3]. Reddy SKT, Balammal G, Kumar SA.2012. Ultra-performance liquid chromatography: an introduction and review. IntJ Pharma Res Anal.2(1): 24-31.

[4] Fingerhut R, Röschinger W,2016. Markus H. A Rapid and Sensitive UPLC-MS/MS-Method for the Separation and Quantification of Branched-Chain Amino Acids from Dried Blood Samples of Patients with Maple Syrup Urine Disease (MSUD). Int J Neonatal Screen. 2(2): 2.

[5] Yuanbin Li, Sheng N, Wang L. 2016. Analysis of 2-(2-Phenylethyl) chromones by UPLC-ESI-QTOF-MS and Multivariate Statistical Methods in Wild and Cultivated Agarwood. Int J Mol Sci.17(5): 771.

[6] Nguyen, D.T.; Guillarme, D.; Rudaz, S.; Veuthey, 2006. J.L. Fast analysis in liquid chromatography using small particle sizeand high pressure. J. Sep. Sci. 29(12), 1836-1848.

[7]. Swartz, M.E.2005. Ultra performance liquid chromatography (UPLC): An introduction, separation science re-defined. LCGC Suppl, 8, 8-14.

[8]. Jerkovich, A.D.; Mellors, J.S.; Jorgenson, J.W.2003. Uplc: An Sensitive and High Throughput Analysis Over HPLC. LCGC 21(7): 600-610.

[9]. MacNair, J.E.; Lewis, K.C.; Jorgenson, J.W.1997. Ultrahigh-pressure reversed-phase liquid chromatography in packed capillary columns. Anal. Chem., : 69(6), 983-989.

[10]. MacNair, J.E.; Lewis, K.C.; Jorgenson, J.W.1997. Ultrahigh-pressure reversed-phase liquid chromatography in packed capillary columns. Anal. Chem., , 69(6), 983- 989.

[11]Beattie, K.; Joncour, J.S.; Lawson, , 2005. K. Ultra performance liquid chromatography coupledto orthogonal quadrupole TOF-MS (MS) formetabolite identification. LC GC North America, 22-30.

[12] Wang, W.; Wang, S.; Tan, S.; Wen, M.; Qian, Y.; Zeng, X.; Guo, Y.; Yu, C. 2015. Detection of urine metabolites in polycystic ovary syndrome by UPLC triple-TOFMS Clin. Chim.Acta, 448, 39-47.

[13]Nguyen, D.T.; Guillarme, D.; Rudaz, S.; Veuthey, 2006. J.L. Fast analysis in liquid chromatography using small particle size and high pressure. J. Sep. Sci. 29(12): 1836-1848.

[14]Swartz, M.E.2005. Ultra performance liquid chromatography (UPLC): An introduction, separation science re-defined. LCGC
Suppl, 8, 8-14.

[15] Jerkovich, A.D.; Mellors, J.S.; Jorgenson,J.W.2003. Uplc: An Sensitive and High Throughput Analysis Over HPLC. LCGC, 21(7): 600-610.

[16]MacNair, J.E.; Lewis, K.C.; Jorgenson,1997. J.W.Ultrahigh-pressure reversed-phase liquidchromatography in packed capillary columns. Anal. Chem., 69(6), 983-989.

[17]Unger K K. Kumar D. Grun M. Buchel G. Ludtke S. Adam T. Scumacher K. 2000. Renker S. *J. Chromatogr A.* 892: 4755.

[18] Swartz M E.2005. UPLC: An Introduction and Review. J of Liq Chromato & Related Techno. 28: 1253–1263.

[19] . Sharath C, Seelam, Priyanka G, Dhanalakshmi K, Nagarjun R.2013. Switch from HPLC to UPLC: A novel achievement in liquid chromatography technique- A review. Int J Pharm Sci Rev Res 21(1): 237-246.

[13] Swartz M E.2005. UPLC: An Introduction and Review. J of Liq Chromato & Related Techno. 28: 1253–1263.

[14] Kondawar M S**,** Patil S B, Bhise S B .2006. "Ultra Performance Liquid Chromatography:A Faster and Sensitive Method over HPLC" URL: http://www.pharmainfo.net (4,5).

[15] Gerber F, Krummen M, Potgeter H, Roth A, Siffrin C, Spoendlin C.2004. Practical aspects of fast reversed-phase highperformance liquid chromatography using 3 microm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. J Chromatogr A 1036: 127- 133.

[16] Tanaka N, Kobayashi H, Nakanishi K, Minakuchi H, Ishizuka N.2001. Monolithic LC columns. Anal Chem. 73: 420A429A.

[17] 40. www.chromatographyonline.com/

[18] Butle S R. Kalyankar T M. Chidrawar R D. Review on ultra performance liquidchromatography: an eminent, sensitive and high throughput analysis over hplc.

[19] Srivastava B. Sharma B K. Baghel U S. 2011. UPLC: a chromatographic technique. Inter J of Pharmaceu Quality Assu. 2(1): 19-25.

[20] Unger K K. Kumar D. Grun M. Buchel G. Ludtke S. Adam T. Scumacher K. Renker S. J.2000 Chromatogr *A.* 892: 4755.

[21] Swartz M E. UPLC: An Introduction and Review. J of Liq Chromato & Related Techno. 2005; 28:1253–1263.

[22] Unger K K. Kumar D. Grun M. Buchel G. Ludtke S. Adam T. Scumacher K. Renker S. *J.2000 Chromatogr A.* 892 : 4755.

[23] Swartz M E. 2005. UPLC: An Introduction and Review. J of Liq Chromato & Related Techno. 28:1253–1263.

[24] M.E. Swartz, J. Liq. Chromatogr., in press

[25] Srivastava B. Sharma B K. Baghel U S.2010. UPLC: a chromatographic technique. Inter J of Pharmaceu Quality Assu. 2(1): 19-25

[26]. www.chromatographyonline.com

[27] . www. Khanacademy. com

[28] . www.library4science.com

[29] . Nikalje Anna Pratima, 2010. Review of Ultra Performance Liquid Chromatography and Its Applications. Int J of Res in Pharm Sci,;7 (5): 1-40.

[30]Trivedi R K. Patel M C. Jadhav S B. 2011. A Rapid, Stability Indicating RP-UPLC Method forSimultaneous Determination of Ambroxol Hydrochloride, Cetirizine Hydrochloride and Antimicrobial Preservatives in Liquid Pharmaceutical Formulation. Sci Pharm. 79: 525–543.

[31] Yadava M. Rao R. Kurania H. Singhala P. Goswamia S. Shrivastav P S.2009. Application of a rapid and selective method for the simultaneous determination of protease inhibitors,lopinavir and ritonavir in human plasma by UPLC– Indian subjects. J of Pharm Biomed Anal. 49: 1115–1122.

[32] Wang D. Jiang K. Yang S. Qin F. Lu X. Li F.2011. Determination of nifedipine in human plasma by ultra performance liquid chromatography–tandem mass spectrometry and its application in a pharmacokinetic study. J of Chromato B. 879(20): 1827–1832

[33] Gaikwad P. Sawant S. Ghante M. Munot N.2010. Ultra performance liquid chromatography: Arecent novel development in HPLC. Inter J of Compre Pharm. 2 (08):1-3.

[34]. Butle S R. Kalyankar T M. Chidrawar R D. Review on ultra performance liquidchromatography: an eminent, sensitive and high throughput analysis over hplc.

[35] Srivastava B. Sharma B K. Baghel U S.2010. UPLC: a chromatographic technique. Inter J of Pharmaceu Quality Assu2(1): 19-25.

[36] Unger K K. Kumar D. Grun M. Buchel G. Ludtke S. Adam T. Scumacher K. Renker S. *J.2000. Chromatogr A.* 892 4755.

[37] Swartz M E.2005. UPLC: An Introduction and Review. J of Liq Chromato & Related Techno. 28: 1253–1263.

[38]. M.E. Swartz, J. Liq. Chromatogr., in press

[39] Srivastava B. Sharma B K. Baghel U S 2010 . UPLC: a chromatographic technique. Inter J of Pharmaceu Quality Assu 2(1): 19-25

[40] www.chromatographyonline.com

[41]. www. Khanacademy. Com

[42]. www.library4science.com

[43] Gaikwad P. Sawant S. Ghante M. Munot N.2010. Ultra performance liquid chromatography: A recent novel development in HPLC. Inter J of Compre Pharm. 2 (08): 1-3.

[44] Trivedi R K. Patel M C. Jadhav S B. 2011. A Rapid, Stability Indicating RP-UPLC Method for Simultaneous Determination of Ambroxol Hydrochloride, Cetirizine Hydrochloride and Antimicrobial Preservatives in Liquid Pharmaceutical Formulation. Sci Pharm. 79: 525–543.

[46]. www. Khanacademy. Com

[47]. www.library4science.com

[48]Srivastava B. Sharma B K. Baghel U S. 2010. UPLC: a chromatographic technique. Inter J of Pharmaceu Quality Assu. 2(1): 19-25.

[49]Unger K K. Kumar D. Grun M. Buchel G. Ludtke S. Adam T. Scumacher K. Renker S. *J.2000 Chromatogr A.* 892): 47-55.

[50] Swartz M E.2005. UPLC: An Introduction and Review. J of Liq Chromato & Related Techno. 28: 1253–1263.

[51] Kondawar M S**,** Patil S B, Bhise S B 2006. "Ultra Performance Liquid Chromatography: A Faster and Sensitive Method over HPLC": URL: http://www.pharmainfo.net (4,5)

[52] Swartz M. Murphy B J. Sievers D. 2004. UPLC: Expanding the limits of HPLC. GIT Lab J. 8(5): 43-45.