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Review Article

A Systematic Review On UPLC

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ABSTRACT

Ultra Performance Liquid Chromatography (UPLC) can be regarded as a new direction for liquid chromatography. UPLC improves three areas of Liquid chromatography: speed, resolution, and sensitivity. In this system column containing bridged ethyl siloxane / silica hybrid (BEH) structure with fine particle size (less than 1.7µm) is utilized. The use of which decreases the length of column, saves time and reduces solvent consumption. This system is designed in a special way to withstand high system back-pressures. 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. UPLC with better resolution, assay sensitivity and high sample throughput allows a greater number of analyses to be performed in a shorter period of time and it also imparts cost effective advantage over HPLC analysis. So that conventional assay was transferred and optimized for UPLC system. This review introduces the theory of UPLC and summarizes some of its applications with examples.

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 [2]. UPLC 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 Demeter equation [3]. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which is known as Ultra Performance. The classic separation method is of HPLC (High Performance Liquid

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Chromatography) with many advantages like robustness, ease of use, good selectivity and adjustable sensitivity. Its main limitation is the lack of efficiency compared to gas chromatography or the capillary electrophoresis due to low diffusion coefficients in liquid phase, involving slow diffusion of analytes in the stationary phase. The Van Deemter equation shows that efficiency increases with the use of smaller size particles but this leads to a rapid increase in back pressure, while most of the HPLC system can operate only up to 400 bars. That is why short columns filled with particles of about 2µm are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load [4]. To improve the efficiency of HPLC separations, the following can be done: - a) work at higher temperatures b) use of monolithic columns [5] The introduction of the UPLC technique created new possibilities for analytical separation without reducing the quality of the obtained results. Many experts have argued that UPLC will replace conventional HPLC techniques. Unfortunately, one of the major disadvantages of UPLC is the financial factor. These expensive devices are not available in all laboratories, and not every researcher will be able to reproduce a given method in his laboratory. Another problem is column padding. When transferring a method from HPLC to UPLC, it is advisable to use the same type of packing. Unfortunately, many existing HPLC fillings are not available in the UPLC version. Moreover, UPLC operates at very high pressures, and the lifetime of the used columns is shortened. Another problem is some aggressive, non-polar solvents that are incompatible with these devices, making it impossible, for example, to separate inorganic ions and polysaccharides. A very important element of an efficient UPLC system is the selection of the detector. Depending on its type, the sensitivity of the method may increase two to three times in relation to HPLC [6]. Optical detectors based on absorbance, tunable UV/visible detectors, fluorimetric and mass spectroscopy (MS) detectors, etc. are generally used with HPLC. The features of UPLC (i.e., speed, resolution, and sensitivity) make it best suited for use with a mass spectrometer. For MS analyses, source ionization is more efficient with UPLC due to increased peak concentrations with reduced chromatographic dispersion at lower flow rates [7]. The profitability of using the UPLC–MS apparatus makes it a practical tool in the laboratory. This applies in particular to the possibility of working at low flows (on columns with a diameter of 1.0 mm) and the possibility of avoiding flux split, which is a very good tool for qualitative and quantitative characterization of complex mixtures using the resolving power of chromatography and the ability of mass spectrometry to identify separated compounds. The main fields of application of UPLC are chemistry, pharmacy, foodstuffs, biochemistry, and the chemistry of compounds used in the heavy metal industry [7,8,9,10,11]. The UPLC systems are also important tools in research and production. For example, they are used to detect the presence of performance-enhancing drugs in samples provided by athletes to check the purity of manufactured drugs or in the food industry to determine the concentration of important ingredients (e.g., vitamins in juices). These methods can be used to assess the number of ingredients present in a sample as well as to determine purity in the process of ensuring the quality control of test compounds. For example, many dishonest spice producers use Sudan as a red dye to improve the aesthetic value of their products. The existing UPLC method for identifying this dye in food products can give a quick and truthful answer. UPLC is also used to separate and identify amino acids, nucleic acids, proteins, hydrocarbons, pesticides, carbohydrates,

antibiotics, steroids, and many other compounds. UPLC apparatuses also prove themselves during the determination of additives used in electroplating and the analysis of explosives. In the field of ecology, the UPLC–MS method is known to determine the level of pesticides in groundwater as well as to analyze wastewater in terms of the content of medicinal substances. The UPLC method finds more and more applications in the field of drug substance analysis, especially drug identification. Many researchers attempt to modify and transfer the assay conditions from the commonly used HPLC method to the UPLC method [12]. When analyzing the available publications, it can be noticed that UPLC systems are starting to displace standard HPLC systems, especially in the pharmaceutical industry [13]. Thanks to the increased resolution, new analytical procedures are refined, in many cases based on existing methods, eliminating the need for re-analysis [14].

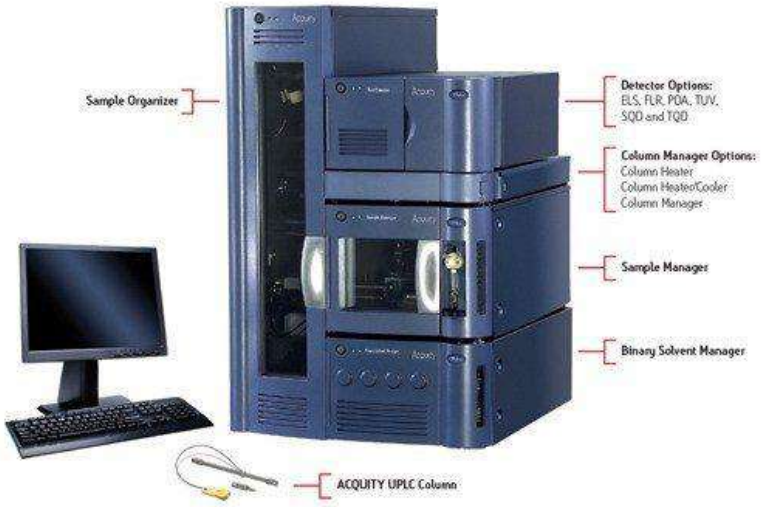

Figure 1. UPLC [1]

PRINCIPLE:

The underlying principle of UPLC is based on the Van Deemter relationship which explains the correlation between flow rate and plate height [15]. The van Deemter equation shows that the flow range with the smaller particles is much greater in comparison with larger particles for good results [16,17,18]. Where H represents height equivalent to the theoretical plate (HETP), A, B & C are the constants and "V" is the flow rate (linear velocity) of the carrier gas. The aim is to minimize HETP to improve column efficiency. The term A does not depend on velocity and indicates eddy mixing. It is smaller if the columns are filled with small and uniform sized particles. The term B denotes the tendency of natural diffusion of the particles. At high flow rates, this effect is smaller, so this term is divided by v. The term C represents the kinetic resistance to equilibrium during the process of separation. The kinetic resistance is the time lag involved in moving from the mobile phase to the stationary phase and back again. The higher the flow rate of the mobile phase, the more a molecule on the packing material inclines to lag behind molecules in the mobile phase. Thus, this term is inversely proportional to linear velocity. Consequently, it is likely to enhance the throughput, and without affecting the chromatographic performance, the separation can be speeded up. The emergence of UPLC has necessitated the improvement of existing instrumentation facility for LC, which takes the benefit of the separation performance (by decreasing dead volumes) and consistent pressures

(about 500 to 1000 bars, compared with 170 to 350 bars in HPLC). Efficiency is proportionate to the length of the column and inversely proportional to the radius of the particles [18]. Consequently, the column length can be reduced by the similar factor as the particle radius without affecting the resolution. The use of UPLC has helped in the detection of drug metabolites and enhancement of the quality of separation spectra [19].

ADVANTAGES OF UPLC [20]

1. Require less run time and enhance sensitivity.
2. Provides the selectivity, sensitivity, and dynamic range of LC analysis.
3. In chromatogram resolved peaks are obtained.
4. Multi residue methods are applied.
5. Speedy analysis, quantify accurately analytes and related products.
6. Uses of fine particle (2µm) for packing of stationary phase make analysis fast.
7. Time and cost both are reduced.
8. Consumption of solvents is less.
9. More products are analyzed 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.
12. Assures end-product quality, including final release testing.

DISADVANTAGES OF UPLC [5]

1. Due to increased pressure requires more maintenance and reduces the life of the columns of these types. So far performances similar or even higher have been demonstrated by using stationary phases of size around 2 µm without the adverse effects of high pressure. In addition, the phases of less than 2 µm are generally non-re generable and thus have limited use.

INSTRUMENTATION:

1. Sample Injection
2. UPLC Columns
3. Detectors

1. 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 [20]

2. 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 [8,21] Although high efficiency nonporous 1.5µm particles are commercially available, they suffer from low surface area, leading to poor loading capacity and retention. To maintain retention and capacity similar to HPLC, UPLC must use a novel porous particle that can withstand high pressures. Silica based particles have good mechanical strength, but suffer from a number of disadvantages. These include tailing of basic analytes and a limited pH range. Another alternative, polymeric column can overcome pH imitations, but they have their own issues, including low efficiencies and limited capacities. In 2000, Waters introduced a first generation hybrid chemistry, called X Terra, 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. X Terra columns are produced using a classical sol-gel synthesis that incorporates carbon in the form of methyl groups. However, in order to provide the kind of enhance mechanical stability UPLC requires, a second generation hybrid technology [22] was developed, called ACQUITY UPLC. ACQUITY 1.7µm particles bridge the methyl groups in the silica matrix as shown in figure-1, which enhances their mechanical stability. Evolution is increased in a 1.7 µm particle packed column because efficiency is better. Separation of the components of a sample requires a bonded phase that provides both retention and selectivity. Four bonded phases are available for UPLC separations.

a. ACQUITY UPLC BEH T M C18 and C8 (straight chain alkyl columns)
b. ACQUITY UPLC BEH Shield RP 18 (embedded polar group column)
c. ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl)[29]
d. ACQUITY UPLC BEH Amide columns (tri functionally bonded amide phase)

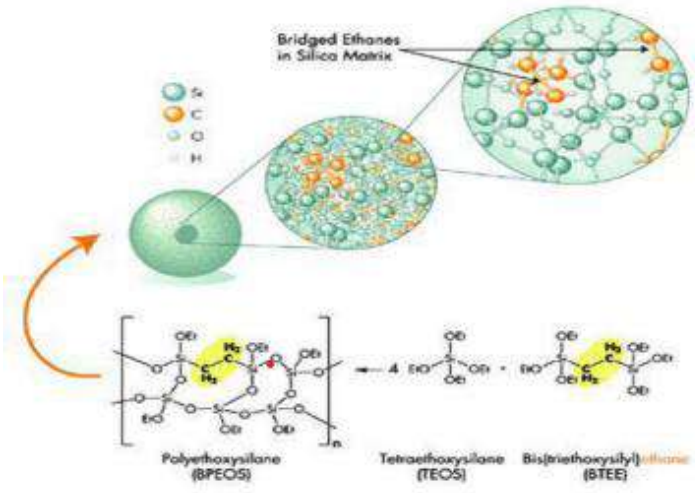

Figure 2: Synthesis and Chemistry of ACQUITY 1.7µm particles for UPLC

Each column chemistry provides a different combination and hydrophobicity, silanol activity, hydrolytic stability and chemical interaction with analytes. [figure.3]

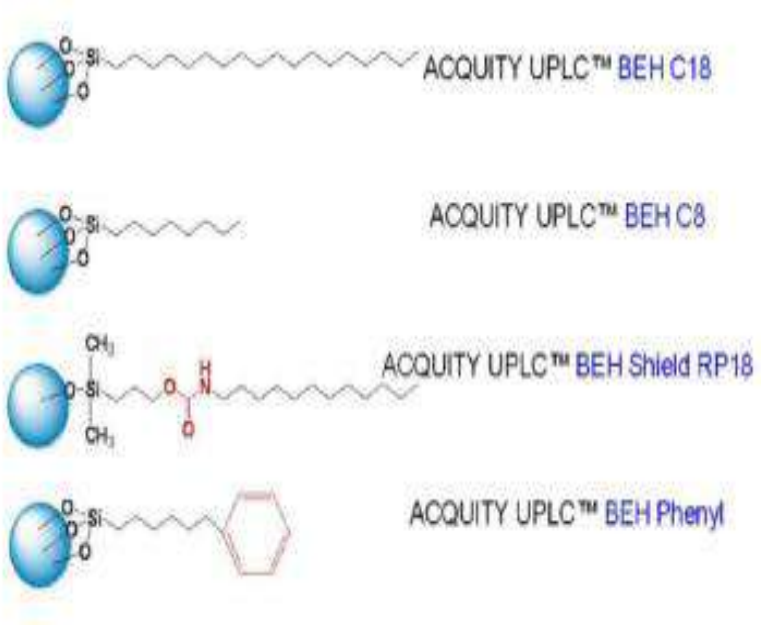

Figure 3: ACQUITY UPLC BEH Column Chemistries

A. ACQUITY UPLC BEH T M C18 and C8 columns –

These are considered as the universal columns of choice for most UPLC separations by providing the widest pH range. They incorporate tri functional 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 [23].

B. ACQUITY UPLC BEH SHIELD R18 columns

These are designed to provide selectivity's that complement the ACQUITY UPLC BEH T M C18 and C8 Columns [24].

C. ACQUITY UPLC BEH Phenyl columns

These utilize a tri functional C6 alkyl ethyl between the phenyl ring and the silyl functionality [24].

D. ACQUITY UPLC BEH Amide columns-

BEH particle technology, in combination with a tri functionally 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 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 redesigned to retain the small particles and resist clogging. Packed bed uniformity is also critical, especially if shorter columns are to maintain resolution while accomplishing the goal of faster separations. All ACQUITY columns also include the e Cord microchip technology that captures the manufacturing information for each column, including the quality control tests and certificates of analysis. When used in the Water's ACQUITY UPLC system, the e Cord 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. [24]

DETECTORS:

1. **TUV Detector (Tunable ultraviolet detector):**

The analytical cell, with a volume of 500 neon liters and a path length of 10 mm, and high sensitivity flow cell, with a volume of 2.4 micro liters and a 25 mm path length, both utilize the flow cell technology. The TUV detector operates at wavelengths ranging from 190 to 700 nm [24]

2. **PDA Detector (Photo diode array detector):**

The PDA (photodiode array) optical detector is an ultraviolet/visible light (UV/Vis) spectrophotometer that operates between 190 and 500 nm. The detector offers two flow cell options. The analytical cell, with a volume of 500 nanoliters and a path length of 10 mm, and high sensitivity flow cell, with a volume of 2.4 micro liters and a 25 mm path length, both utilize the flow cell technology [25].

3. **ELS Detector (Evaporative Light Scattering Detector):**

ELS detector is an evaporative light scattering detector designed for use in the UPLC system. So far performance similar or even higher has been demonstrated by using stationary phases of size around 2µm 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 [26].

COMPARISON BETWEEN HPLC AND UPLC

Table 1: Comparison between HPLC and UPLC [5]

Characteristics	HPLC	UPLC
Particle size	<4 µm	1.7 µm
Maximum backpressure	35-40 MPa	103.5 MPa
Analytical column	Alltima C 18	Acquity UPLC BEH C18
Column dimensions	150 X 3.2 mm	150 X 2.1 mm
Injection volume	20L	3-5 L
Pressure limit	up to 4000 psi	15000 psi
Total run time	10 min	1.5 min

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