UPLC: A Review

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***Abstract*- Chromatography is a non-destructive method that uses a porous material and solvents to separate a mixture of components into their individual components. UPLC is a modern analytical technique. UPLC refers to ultra-performance liquid chromatography. This technique improves three areas of liquid chromatography: Speed, resolution, and sensitivity. In this system, columns contain fine particle sizes (less than 1.7 m). Due to the small partical size, it decreases the length of the column, saves time, and reduces solvent consumption. The design of this system is such that it can withstand high system backpressures. In the twenty-first century, pharmaceutical industries and analytical laboratories mainly focused on reducing time and also decreasing cost in new drug development The Van Deemter relationship, which explains the link between flow rate and plate height (HETP), is the foundation of the UPLC method. This review shows the theories which are used in separation technology related with UPLC and also tells about its applications.**

***Keywords*: UPLC, HPLC, Separation, Quantification, Resolution, Sensitivity, etc.**

INTRODUCTION:

UPLC can be say that it is a new invention for liquid chromatography. UPLC refers to Ultra Performance Liquid Chromatography. It is a method of separating a mixture of components into individual components through a porous medium under the influence of the solvent. Ultra-performance liquid chromatography (UPLC) has most prominently use technology in analytical laboratories for the analysis of drugs worldwide during the past 20-25 years. Growth of this analytical technique due to effective separations which is possible because of packing material –its size, withstand in high pressure, high temperature. In this separation mechanism the principle apply is Van Deemter equation, which explains the correlation between flow rate and plate height (HETP). [1]

The principle of UPLC is the difference in affinity of the compound to be separated toward the stationary and mobile phase. [2] Detector can recognize analytes after leaving column and signals are recorded in the data system. Separation can either be adsorption or partition. Hence they can be called as adsorption chromatography or partition chromatography [3] The van Deemter equation stated that the flow range with the smaller particles is much greater in comparison with larger particles which is use for good results. [4]

$$H = A + B/V + CV$$

With the above equation: we have suggestion that it should describe the relationship between linear velocity (flow rate) and plate height (HETP).

In which A, B and C are constants in nature and v is variable and it also known as the linear velocity, the carrier gas flow rate

A: Eddy diffusion: The term A is not depend of velocity and it also known as"Eddy"diffusion. When the packed column particles are small and uniform. Then value of A is small

B: Longitudinal diffusion: The term B also known as longitudinal diffusion or axial diffusion the effect of longitudinal may be diminished at max flow rates and so longitudinal may divided by v.

C: Resistance to mass transfer: The term C resistance to mass transfer It is due to kinetic resistance in the separation process. The kinetic resistance to mass is the time delay in gas phase running toward the stationary phase and reverse again. The larger the flow of gas, the more a molecule on the packing favor to delay behind molecules in the mobile phase. Therefore this term is proportional to v. so it is possible to increase work rate and thus also the speed of analysis without affecting chromatographic performance. [5]

v : Represent Average mobile phase velocity

H- Height equivalent to the theoretical plate (HETP)

The development of UPLC system has been done in the year 2004. [6]

Instrumentation:

The various instruments used in the Ultra performance liquid chromatography are as follows (Figure 1).

- Sample injection
- UPLC columns
- Column manger & heater or cooler
- Detectors
- Software's

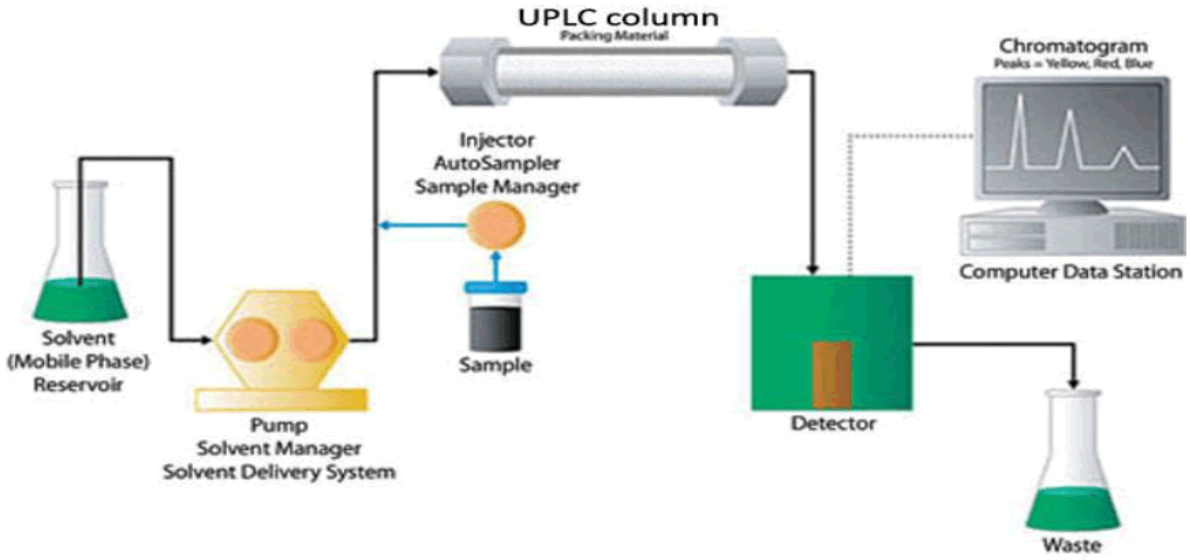


Figure1: Instrumentation of UPLC. [7]

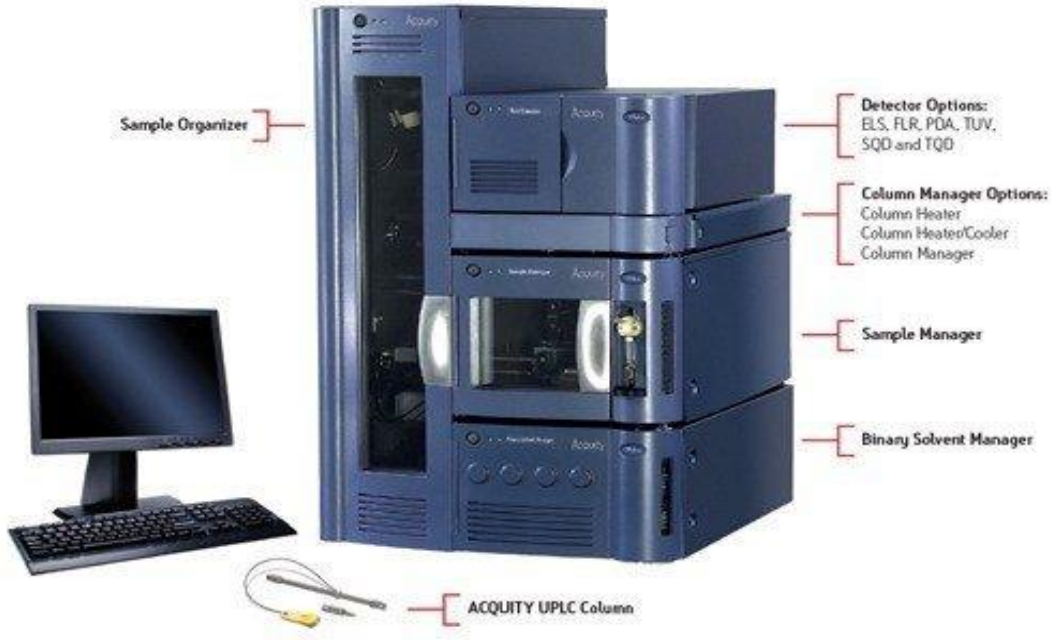


Figure2: The Acquity UPLC System.[8]

Sample injection

In UPLC, sample addition is done by Manual system or automatic system. Extreme pressure fluctuations where seen in column and To protect the column from these high pressure change, the injection process was to be moderately pulse free and volume of sample must be low. In volume introduction are also necessary to improve sensitivity. A rapid injection cycle is required.

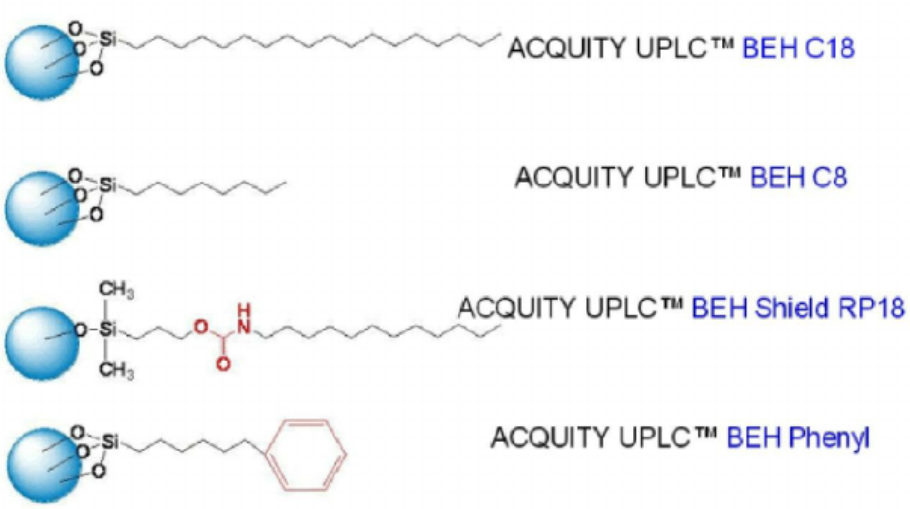
Injector: To introduce a liquid sample into the UPLC column by injection, commonly in the range of 2 µL .

Injection cycle It is a time of introduction of sample in column first time 25 seconds required to inject sample without a wash and then next injection time is 60 sec with a dual wash .it is used to reduce carry over. [9]

UPLC Columns

In UPLC Column 1.7 µm particle packed due to small particle size Resolution is increase because of efficiency is better. For the separation of components in a sample requires bonded phase that provides both retention and selectivity. With a wide range of column options and formats available from Waters, including HPLC, UPLC, UHPLC, GPC, SFC, and SEC, and all of them based on dependable quality production, we can be sure that the techniques we use today will produce the same reproducible results tomorrow.

There are total Four stationary phases are available for UPLC separation technique:



(I) Acquity UPLCTM Beh C8 (Straight Chain Alkyl Columns)

- Trifunctionally Bonded C
- Wide pH range

These are a straight alkyl chain, most preferred UPLC columns as they can be used over a wide pH range

(II) ACQUITY UPLCTM BEH C18 (Straight Chain Alkyl Columns)

- Trifunctionally Bonded C18
- First UPLC column choice
- Superior peak shape & efficiencies

The tri-functional molecule shows pH stability at low pH, which is formed with high pH stability of 1.7µm BEH particles to produce the large pH operating level

(III) ACQUITY UPLC BEH Shield RP18 (Embedded Polar Group Column)

- Monofunctionally bonded
- Embedded carbamate group
- Alternate selectivities

They supply selectivity to UPLC as it correlative with C18 and C8 columns.

(Iv) Acquity UPLC Beh Phenyl

(phenyl functional group tie to the silyl functional group also contain a C6 alkyl),They should contain tri-functional C6 alkyl ethyl between the phenyl- ring and the silyl functional group.

Different levels of hydrophobicity, silanol activity, hydrolytic stability, and chemical interactions with analytes must be provided by the column employed in UPLC. As the default columns for UPLC, BEH - C18 and C8 columns are expressed. These columns' separations provide a wide range at various pH levels. It also provides long column life span and also better peak shape It contain the 1.7µm BEH particle size. And internal dimension of 2.1 mm size is required in column. For maximum resolution, 100 mm length use. [10]

Column Manger & Heater or Cooler

A binary solvent management system, sample manager, column heater, detector, and optional sample organizer make up the UPLC System. Additionally, the solvent manager uses two distinct serial flow pumps to generate a binary gradient. These are also present solvent selection valves to choose from up to four solvents. There is a 15,000-psi pressure limit. The column manager controls the column temperatures upto 65 It also consist needle-in-needle sampling for improved ruggedness and needle calibration sensor increases accuracy. the optional sample organizer,and the sample manager also inject up to range 22 micro titer plates. In a thermostatically regulated environment, a range of micro titer plate forms (deep well, mid height, or vials) can also be supported. The column heater is likewise managed by the sample manager. To overcome sample dispersion, a “pivot out” design is used the column outlet to be placed in closer proximity to the source inlet of an MS detector. [11]

Pumps

The UPLC pump, one of the most important components of a liquid chromatography system, is in charge of ensuring a steady flow of eluent through the UPLC injector, column, and detector .The two fundamental categories are:

- Pump with constant flow
- Pump with constant pressure

Only column packing makes use of the continuous pressure.

perpetual flow pump

The majority of UPLC applications employ this type of pump.

Regular UPLC pump specifications

Sample injection volumes of up to 3 to 5 microliters should be used.

The stationary phase packing material's particle size is less than 2 micrometres, and the pump operates at a pressure of 10,000 psi. [12]

Types of Pumps

Reciprocating Piston Pumps

The fundamental idea behind reciprocating single piston pumps is that liquid is sent through a check valve (a one-way valve). The distance the piston retracts, hence regulating the volume of liquid pushed out by each stroke, or the cam's rotational speed are two common ways to modify the pumping rate. The reciprocating single piston pump is shown schematically. A sapphire piston is being forced back and forth by CAM. Eluent is sucked through the inlet check valve (on the bottom) as the piston moves backward. The sapphire ball is raised, allowing the eluent to pass through. The liquid pushes the inlet ball downward and shuts the passage when the piston advances, whereas the outlet ball is lifted and opens the outlet valve (upper) when the piston goes backward. [13]

Disadvantage The main problem with this kind of pump is sinusoidal pressure pulsations, which necessitate the installation of pulse dampers.

Dual Piston Pumps

Using dual-headed reciprocating pumps to generate a steady, nearly pulse-free flow is a more effective method. One piston can pump while the other is filling because both pump chambers are driven by the same motor via an eccentric cam. As a result, the pulsation downstream of the pump is greatly reduced by the two flow-profiles crossing each other; this is shown below. The more effective varieties of these pumps use eccentricity-shaped cams to provide the best overlapping of the pressure curves and smooth flow because the acceleration/deceleration profile is somewhat non-linear. [14]

Dual-head reciprocating pump schematic

The advantages of this pump are its quick changeover and clean out capabilities, unlimited solvent reservoir, and long-term unsupervised use. These pumps could have a number of drawbacks, though, unless extra care was taken during production. When piston cycles are widely dispersed at low flow rates, there is a propensity for the incompletely compensated pulsations to be detectable at high refractive index detector sensitivities. Additionally, since each head has two check valves, the cleanliness of the mobile phase and the continued sealing ability of four check valves during each cycle—cycles that typically happen several times per minute—determine the reliability of the pump. [15]

The weakest component of the reciprocating pump is the check valve. It might become quickly polluted or clogged, which would cause the pump to malfunction. The majority of current HPLC devices include upgraded dual piston pumps with three or even two check valves.

Above is a schematic for this pump. While the second piston (high pressure piston) is providing the system with eluent, the first piston, known as low pressure, is sucking the liquid out of the reservoir. The second piston is then quickly filled by the first piston during the last 1/100 of the pump cycle. Only three check valves are permitted to be used in this arrangement, one of which operates at low pressure. No cavitation effects are present. The piston volumes are modest (100), resulting in pressure pulsations that are small, acute, and simple to damp. [16]

However, the piston volumes are different. Another form of dual piston pump employs simply two check valves. In the HPLC system, the larger piston is sucking an eluent while the smaller piston is dispensing an eluent. When the orientation of the pistons changes, the larger piston simultaneously discharges eluent into the system and fills the smaller chamber. The dual piston pump can only use two check valves with this configuration. [17]

Detectors:

Concentration-sensitive absorbance detectors are used for analyte detection. UV/Visible detectors are the detectors used in UPLC analysis. Using a light-guided flow, the ACQUITY Tunable UV/Visible detector cell is composed. Internal reflectance mode, which maintains a 10mm flow cell path length with a capacity of only 500mL, is used to transport light down the flow cell. [18]

1) Acquity Uplc Els Detector

This detector is made exclusively for the ACQUITY UPLC PLUS line of systems and can be utilised for a variety of sample sizes, especially for high volume or open access situations and huge numbers of compounds that need to be quickly screened. These detectors are simple to develop and require little upkeep.[19]

2) Acquity Uplc Refractive Index Detector

The ACQUITY UPLC Refractive Index (RI) Detector used for the isocratic UHPLC/UPLC analysis of analytes. It should work without a UV chromophore. Its low-dispersion amplifier are designed to match the performance needed for narrow UPLC peaks while still delivering stable baseline performance, low noise, and a wide, linear dynamic range.[20]

3) Acquity Uplc And Acquity Premier Flr Detectors

The ACQUITY UPLC and ACQUITY Premier Fluorescence (FLR) detectors are high-sensitivity, multi-channel fluorescence detectors.. These detectors possess features like innovative flow cell design, low-noise electronics and support for high-speed data rates that gives better sensitivity and selectivity to UPLC/UHPLC separations. [21]

4) Acquity Uplc And Acquity Premier Tunable Uv Detectors

Today's labs require higher sensitivity to detect compounds at lower levels. ACQUITY UPLC and ACQUITY Premier Tunable UV (TUV) Detectors have low-noise, high-speed detection for productivity, sensitivity, and resolution.[22]

5) Acquity Uplc Pda Detector

It is the perfect detector for any laboratory application, including technique development and compound identification.The ACQUITY UPLC PDA Detector is a trustworthy, simple device. [23]

Softwares and Accessories

With the help of the Empower™ and MassLynx™ software, ACQUITY UPLC Systems may be easily controlled, diagnosed, and monitored via a graphical system console interface. The dynamic data processing and information management capabilities needed to turn the output of the ACQUITY UPLC System into useful knowledge are offered by both Empower and MassLynx.

Waters is consistently increasing the ACQUITY UPLC System's capabilities: All ACQUITY UPLC columns have the eCordTMtechnology, which keeps track of column history. A sample organizer that more than ten times the system's capacity. The FlexCart platform may increase usability, accessibility, and practicality. [24]

Connection INSIGHTTM Service

Connections INSIGHTTM provides diagnostic data for the ACQUITY UPLC Systems using Intelligent Device Management technology. By establishing a virtual technical support presence in your lab with Connections INSIGHT, Waters is able to offer you prompt, proactive assistance with the highest level of support and satisfaction. [25]

Advantages of UPLC

- The following are some of the UPLC's many benefits:
- It improves sensitivity and needs less running time.
- It offers sensitivity and selectivity.
- Resolved peaks are produced in chromatograms.
- Multiple residue techniques are used.
- rapid analysis, precise quantification
- The packing of the stationary phase with small (2 m) particles speeds up analysis.
- Both time and money are saved.
- Less solvents are consumed.
- Additional products are examined using the current resources. [26]

Disadvantages of UPLC

- The main drawback of UPLC analysis is the short column life, which is caused by the small particle size. High pressure also develops during the analysis. Increased pressure shortens the columns' useful lives. Increased pressure shortens the life of the columns and necessitates greater maintenance.
- Additionally, the utilisation of the phases of fewer than 2 is often limited because they are non-regenerable. [27]

Applications of UPLC:

1) Multi-Residue Analysis of Pharmaceuticals in Waste Water:
2) It was discovered that the water used by pharmaceutical companies contained traces of several drugs, including histamine H2 receptor antagonists, antibiotics, beta-blockers, analgesics, anti-inflammatory agents, and cholesterol-lowering statin agents. These medicines are confirmed and screened in the wastewater treatment plant sample using UPLC and Q-TOF-MS.[28]
3) Use of UPLC in the Analysis of Natural Products and Traditional Herbal Medicine:
4) UPLC is primarily employed in the analysis of natural products and herbal remedies. In order to identify active chemicals in complicated samples resulting from natural products and conventional herbal remedies, UPLC offers high-quality separations and detection capabilities.
5) Metabolite Identification:
6) A step in the process of discovering new chemical entities (NCE) for drugs, metabolite identification. UPLC is a sophisticated analytical method for finding new biomarkers.
7) Manufacturing, QA, and QC:
8) In QA/QC laboratories, highly regulated quantitative analyses are carried out using UPLC. A recognised analytical technique depends significantly on the availability of reliable, high-quality consumable items.
9) Amino acid analysis:
10) UPLC is utilized for precise, dependable, and repeatable amino acid analysis in protein characterization, cell culture monitoring, and nutrient analysis of foods.
11) Determining pesticides:
12) Triple Quadra Tandem Mass Spectrometry in combination with UPLC allows for the identification of pesticide traces in water. Magnolia officinalis should be identified using the UPLC fingerprint.
13) UPLC may detect contaminants using impurity profiling.[29]

CONCLUSION:

From the above study, it is concluded that UPLC is an advanced Chromatographic technique that gives fast and accurate results. UPLC is a new inovation in chromatography. UPLC increases productivity in both chemistry and instrumentation by giving increase resolution, speed, and sensitivity for liquid chromatography due to smaller particle size. The main advantage this technique is a reduction of analysis time, also reduced solvent consumption. The column temperature can be raised to lower backpressure. It was discovered that UPLC has a substantially higher sensitivity than traditional HPLC. All types of pharmaceutical drugs can be analyzed by UPLC method within short period of time and with less solvent consumption The UPLC can improve speed,sensitivity and resolution.

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