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Ultra Performance Liquid Chromatography (UPLC) - A Review

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Abstract

UPLC is a modern technique which gives a new direction for liquid chromatography. Ultra performance liquid chromatography (UPLC) is a technique that improves in three key areas: "speed, resolution, and sensitivity.". When compared to high-performance liquid chromatography, ultra performance liquid chromatography (UPLC) is suited for particles with a diameter of less than 2 µm and can achieve superior resolution, speed, and sensitivity (HPLC). High pressure is used during the UPLC separation process (up to100 MPa). Additionally, it reduces the cost of reagent with shorter run time as compared to conventional HPLC. The present review paper differentiates HPLC and UPLC, analytical method validation, applications, advantages and disadvantages of HPLC and UPLC.

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

Introduction

Chromatography is a non-destructive process that uses a porous material and solvents to separate a mixture of components into their individual components. Prior to 2004, HPLC was the method of choice for decomposing a mixture of components into their constituent parts. However due to various restrictions, scientists have developed a new technology known as "Ultra Performance Liquid Chromatography (UPLC)" that is very effective and cutting-edge while also overcoming some of HPLC's limitations.[1-5]

Mobile Phase- That is the phase that advances in a clear manner. Based on the polarity of the stationary phase, the type of the sample, and the chromatographic procedure, the mobile phase is chosen.. Example- Acetone.

Stationary phase- In chromatographic separation, it is a stationary phase. It is the substance that has been firmly positioned for the chromatography process**.** Example- silica.

Ultra Performance Liquid Chromatography is referred to as UPLC. Chromatographic resolution, speed, and sensitivity analysis all see advances. It uses tiny particles, saves time, and requires less solvent. [6-7]. This overview introduces the UPLC's operating principle and some of the most recent research in the area. The van Deemter equation predicts a large increase in efficiency as particle size decreases to below 2.5 µm. Hence, by utilising smaller particles, liquid chromatography's speed and peak capacity can be increased to previously unimaginable levels. [8-12].

Brief History

Chromatography is a novel technology that was invented by Russian botanist Tswett in Warsaw in 1906. He was successful in separating chlorophyll, xanthophylls, and a number of other coloured compounds from vegetable extracts using a calcium carbonate column during that year. The calcium carbonate column serves as an adsorbent, and the various compounds are adsorbed to varying degrees, resulting in coloured bands at various positions on the column. The Greek terms chroma and graphos, which imply colour and writing, respectively, inspired Tswett to use the term "chromatogram" to describe this system of coloured bands and the technique used to generate it[13-19]. Since then, significant progress has been made, and the techniques are now employed to differentiate coloured and colourless compounds. The stationary phase is the calcium carbonate column included in the Tsweet method, which remains stationary throughout. Vegetable extract solution is referred to as mobile phase because it flows or flows down the column.

The separation of solutes between a stationary phase and a mobile phase occurs during the separation process of chromatography[20-26]. Thin layer chromatography and ion exchange chromatography were both first introduced as a method of separation in 1930. Paper chromatography was first introduced by Martin and Synge in 1941, and gas chromatography followed in 1952. It is becoming a prospective technique for the preparation of extremely pure substances in industries like the pharmaceutical industry or in the manufacturing of pure chemicals, in addition to its usage in analysis. The chromatographic methods of biomolecule separation are totally responsible for the recent outstanding developments in the field of bioscience. Subsequently, other techniques such as HPLC were established, which have been utilised in many laboratories for a long time. More recently, a new technology known as UPLC was introduced (Ultra performance Liquid Chromatography)

The UPLC is based on the principal of use of stationary phase consisting of particles less than 2 μm.[37]

The underlying principles of this evolution are governed by the Van Deemter equation, which is an empirical formula that describes the relationship between, linear (flow rate) and plate height (HETP or column efficiency).[38,39]

The equation is as follows:

H = A + B/V + CV

Where,

A, B and C are constant

H= HETP A = Eddy diffusion

B = Longitudinal diffusion

C = Equilibrium mass transfer

V = flow rate Eddy diffusion

Through the heavily packed column of stationary phase, the mobile phase flows. Randomly, various paths will be taken by solute molecules through the stationary phase. The band will widen as a result. The concentration of analytes is lower near the band's margins than it is in the middle due to longitudinal diffusion. From the centre to the edges, the analyte diffuses. The band widens as a result.

Instrumentation

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
- Software's

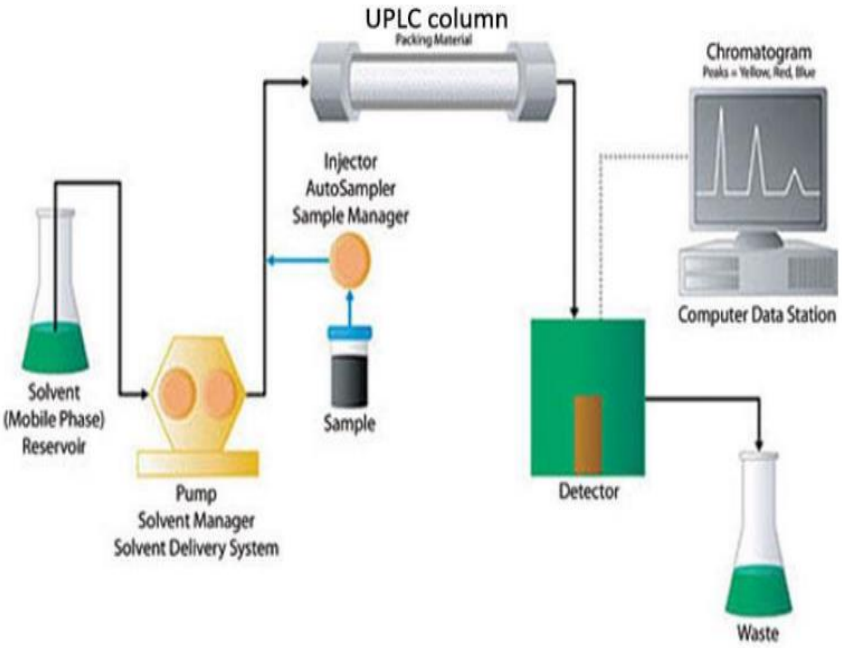

Figure 1: Schematic diagram of UPLC

Figure 2: UPLC instrument

❖ **Pumping devices**

An ideal UPLC pump can carry solvent through a 15 cm long, packed with 1.7 µm particles column at a higher pressure of roughly 15000 psi for the maximum flow rate and efficiency.

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, An excellent introduction is essential. Automated or manual, conventional injection valves are not built and hardened to operate at extreme pressure. The injection process must be largely pulse-free in order to protect the column from large pressure fluctuations, and the device's swept volume must be as small as possible in order to prevent band spreading. To fully advantage from the speed provided by UPLC, a quick injection cycle time is necessary, which in turn requires a large sample volume. Moreover, to improve sensitivity, low volume injections with little carryover are needed. For biological material, there are other direct injection methods

❖ **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).

❖ **Column manager & heater or cooler**

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

❖ **Detectors-[40]**

The detector is the device used to detect analytes after separation in both qualitative and quantitative ways. The system can be set up with one or more of the following detectors: TUV, ELS, PDA, and FLR. It is a two channel absorbance detector called a TUV (Tunable Ultraviolet) detector. Empower or Mass Lynx software is used to control the LC/MS and LC applications. PDA (Photo Diode Array) detector: An optical detector that operates between 190 and 500 nm absorbs UV-visible light. ELS detector (Evaporative Light Scattering): The detector Powered by Empower or Mass Lynx software, which includes a flow-type nebulizer optimized for the operation of UPLC systems. Fluorescence (FLR) detector It is a multi-channel, multi-wavelength detector that gives 3D scanning functionality for simpler method development. Its excitation wavelength ranges from 200 to 890 nm and its emission wavelength goes from 210 to 900 nm.

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

Applications

- This technique has been successfully used to analyse a variety of medicinal substances, including Aspirin, Metoprolol (MT), Ramipril (RM), Diclofenac, and Fluconazole.
- Analyses of Natural Products and Conventional Herbal Medicine.
- Identification of Metabolites.
- investigation of metabolics or metabonomics
- ADME (Absorption, Distribution, Metabolism, Excretion) Screening
- Bio analysis / Bioequivalence Studies
- Dissolution Testing
- Study of Forced Degradation
- Manufacturing / QA / QC
- Method Development / Validation
- Impurity Profiling
- Compound Library Maintenance
- Open Access

Advantages Of UPLC

- Using multiple residue techniques
- Additional items are analyzed using the existing resources.
- Decreases run time and increases sensitivity.
- Maintaining resolution performance constant.
- Operation cost is reduced
- Process cycle times are shortened, allowing more product to be produced using the same amount of resources.
- Less solvent consumption.

Disadvantages Of UPLC

- Due to increased pressure, these columns' life are shortened and require additional maintenance.
- The use of stationary phases with a size of roughly 2 µm has so far been successful in demonstrating performances that are comparable to or even higher than those without the adverse effects of high pressure. Additionally, the utilisation of the phases with a size of less than 2 µm is limited because they are typically non-regenerable.

Why use UHPLC?

UHPLC has gradually replaced HPLC over the past ten years, as was mentioned before in the article. Now, why is that? There are several factors.:

1**. Speed and throughput** – Smaller columns and particle sizes, reduced system dispersion, and a matching pump pressure allow separations to be completed more quickly than with HPLC, increasing sample throughput. This application note is a good illustration of this: When utilising a column with 4.0 µm particles, the separation took 2.5 minutes, however when using 1.5 µm particles, the separation only took 1.5 minutes.

2**. Better resolution** – The use of smaller columns and particles also produces sharper, better-resolved peaks, improving peak capacity.

3**. Lower costs** – The use of solvents and related disposal costs are decreased because the flow rates are lower and the separations occur quickly. In order to effectively utilise the instrument investment, speed also provides increased throughput.

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.

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