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Ultra Performance Liquid Chromatography: A New Technique in Liquid Chromatography

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ABSTRACT

Ultra Performance Liquid Chromatography (UPLC) is a modified new technique over HPLC based on the Van Deemter equation, which gives a new direction for liquid chromatography. UPLC which enhance mainly in three areas: "speed, resolution, and sensitivity. Ultra performance liquid chromatography (UPLC) applicable for the particle size of column packing is less than 2µm in diameter to acquire better resolution, speed, and sensitivity compared with high-performance liquid chromatography (HPLC). Now a day's pharmaceutical industries as well as analytical laboratories are focusing to reduce cost and time for analysis of drugs and improve the quality of their products. UPLC analysis gives a better quality of their products. The separation and identification in UPLC is done under very high pressure (up to 15000psi). As compared to HPLC, so decrease the length of the column, saves time, and reduces solvent consumption.

INTRODUCTION

Chromatography is a process of separating different parts of chemical mixtures onto an absorbent material that can then be individually analyzed because different parts are caught on the material at different rates. For many years, researchers have looked at "fast LC" as a way to speed up analysis to reduce the time of analysis. UPLC is coming from HPLC. HPLC has been the evolution of the packing materials used to effect the separation, to attain the new way in liquid chromatography Waters in 2004 launched UPLC, which contain less than 2.0µm particles in column packaging material, Van Deemter equation is the principle behind this modification which correlates the connection between linear velocity and plate height. The small particles demand a high pressure to work with UPLC *i.e.*, 6000 psi which is typically the upper limit of conventional HPLC. It was observed that when the particle size is decreased below 2.0µm, there is a remarkable increase in the effectiveness and this effectiveness does not lessen on increasing the linear speed or rate of flow. The use of speed, particles with a small radius, and a maximum number of resolvable peaks (peak capacity) understand the efficiency together with resolution. This method reduces the mobile phase volume consumption by at least 80% compared to HPLC with a shorter runtime. The smaller sized particles increase the pressure up to 15000 psi or more which can alone enhance the retention factor of the separation. Less injection volume is required for UPLC which results in better efficiency and an increase in resolution. The higher column temperature decreases the mobile phase viscosity resulting in the high diffusion coefficient and flow rate without significant loss in efficiency and increase in column backpressure.

PRINCIPLE

UPLC is based on the van Deemter relationship which explains the correlation between flow rate and plate height.

$$H = A + \frac{B}{v} + Cv \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The above van Deemter equation shows that the flow range with the smaller particles is much greater in comparison with larger particles for better results.

Where H is height equivalent to the theoretical plate (HETP), A, B & C are the constants and v is the flow rate (linear velocity) of the mobile phase. The aim is to minimize HETP to improve column efficiency. The term A denotes eddy mixing. It is smaller if the columns are

packed with small and uniform sized particles. The term B indicates the tendency of axial diffusion of the particles. At high flow rates, this effect is smaller, so this term is divided by v. The term C indicates 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. Efficiency is proportionate to the length of the column and inversely proportional to the radius of the particles. Consequently, the column length can be reduced by a similar factor as the particle radius without affecting the resolution.

ADVANTAGES OF UPLC

1] It is fast, sensitive, highly resolvable as compared to HPLC.

2] It reduces process cycle time and assures final-product quality with the reduced cost of operation and decreased run time of analysis.

3] It helps to minimize solvent consumption.

4] By employing high temperature which reduces the viscosity of mobile phase and ultimately flow rate if high.

5] It is up to 9 times faster, has up to twice the resolution and three times the sensitivity than that of HPLC.

6] Quick and ease of instrument handling

DISADVANTAGES OF UPLC

The main disadvantage of UPLC is the life of the column due to increased pressure requires more maintenance and reduces the life of the columns.

INSTRUMENTATION

The instrumentation of UPLC is modified to a superior position in chromatography analysis with an increase in speed, higher resolution, and greater sensitivity achieved by small particles. A modified technology with the advancement in the design of the pump, autosampler, detector, data system, and service diagnostics was required to full fill the purpose. The basic instrumentation of UPLC is discussed below.

1] Pumping system

2] Sample injector

3] Sample manager

4] Columns

5] Detectors

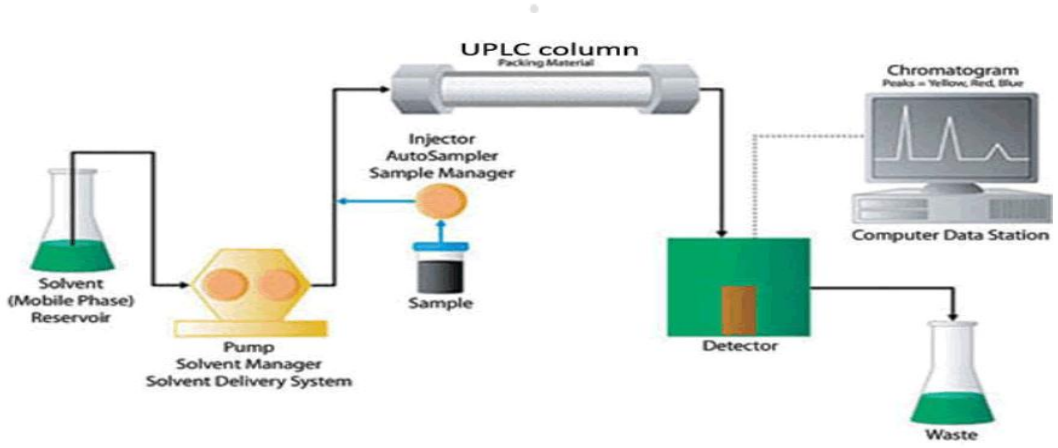

Figure No. 1: Schematic diagram of UPLC

1] Pumping system

UPLC requires higher pressure to deliver a mobile phase due to its small particle size for attainable efficiency across a 15 cm long column with 1.7µm particles is about 15,000 psi. Therefore a pump capable of delivering solvent uninterruptedly and reproducibly at these pressures, which can compensate for solvent compressibility and operate in both the gradient and isocratic separation modes, is required. Ideally, the UPLC pump has a capacity of

delivering solvent at higher pressure around 15000 psi for the optimum flow rate. There are built-in solvent proportionating valve to choose from up to four solvents.

There are two types of pumps:

a. Reciprocating pump

b. Pneumatic pump

a) Reciprocating pump:

These types of pumps operate by using a reciprocating piston or diaphragm. The solvent enters a pumping chamber through an inlet valve and is pushed out via an outlet valve by the piston. Reciprocating pumps are productive of desired effects and are suitable for very high flows. Two types of reciprocating pumps.

i) The Reciprocating piston pump

ii) The Reciprocating diaphragm pump

b) Pneumatic pump:

This type of piston was originally used for normal liquid chromatography separations but was found to be noisy and make strong flow pulses that an unstable the detector. It is now used in a limited manner for slurry packing liquid chromatography columns.

2] Sample injector

The UPLC system arranged flow through-needle design sample manager having three designs for reliable performance: robust sealing of the needle at higher pressure, minimizing the extra column bandspread for narrow peaks and performing a pulse-free injection process to protect column from overpressure fluctuations. When an injection is facilitated, the inject valve deflects flow from the needle to collect a sample from the vial. The needle is inserted into the vial to withdraw the known amount of volume of the sample required and then back to the injection port. The disunion of the sample can be minimized by keeping less distance between the injection port and the inject valve. After injecting the sample, the needle is washed for a specified amount of time to decrease sample carryover in the next run of an analysis.

3] Sample Manager

The sample manager of the UPLC system integrates several technological advancements. Injection cycle time is 60 seconds with a dual wash and 25 sec without a wash to decrease the carry-over of the sample. A variety of microtiter plate formats can also be adjusted in a thermostatically maintained environment.

4] Column

The column is often referred to as the heart of the UPLC separation process. ACQUITY UPLC columns were designed with 1.7µm particle size for better efficiency. Separation of the sample requires a bonded phase that provides retention. Four bonded phases are generally used for separation are A] ACQUITY UPLC BEH C18

B] ACQUITY UPLC BEH C8

C] ACQUITY UPLC BEH RP 18

D] ACQUITY UPLC BEH Phenyl

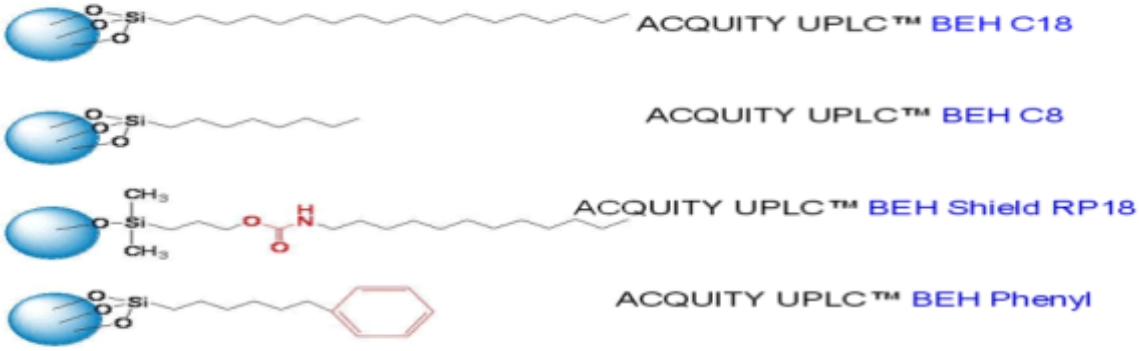

Figure No. 2: Phases of UPLC column

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

1. TUV (Tunable Ultraviolet) detector: It is a two-channel detector. Controlled by Empower or Mass Lynx software for both LC/MS and LC applications.

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

3. ELS (Evaporative Light Scattering) detector: The detector controlled by Empower or Mass Lynx software, which incorporates a flow-type nebulizer that is optimized for UPLC system performance.

4. FLR (Fluorescence) detector: Fluorescence detectors are probably the most sensitive among the existing modern HPLC detectors. Fluorescence sensitivity is 10-1000 times higher than that of the UV detector for strong UV absorbing material.

APPLICATIONS OF UPLC:

1. Analysis of Natural Products and Traditional Herbal Medicine:- UPLC is widely used.

2. The analysis of natural products and herbal medicines. UPLC provides high-quality quantification and detection capabilities to demonstrate active compounds in highly complex samples that result from natural products and traditional herbal medicines.

3. Identification of Metabolite:-The transformation of the chemical compound within a living system of new chemical entities (NCE) is necessary for drug discovery. In the stage of development, metabolite identification becomes a regulated process and it's done via UPLC for fast and better results.

4. Bioanalysis / Bioequivalence Studies:- The sensitivity and selectivity of UPLC/MS/MS at low detection levels give accurate and reliable data that can be used for a variety of different purposes, including statistical pharmacokinetics analysis. UPLC/MS/MS delivers excellent chromatographic data.

5. Determination of Phytoconstituents:- UPLC can also determine and quantify various types of compounds like Oligomers, monomers, procyanidins, phenolic compounds, isoflavones, flavonoids, alkaloids, etc.

6. Dissolution Testing:- After the development of formulations, testing higher potency drugs is necessary. The dissolution data shows reliability and batch-to-batch uniformity of the active ingredient in the drug product.

7. Method Development and Validation:-UPLC provides essential laboratory function by increasing efficiency, reducing costs, and improving opportunities for business success.

8. Forced Degradation Studies:-Combining the chromatographic speed, resolution, and sensitivity of UPLC separations with the high-speed scan rates of UPLC-specific photodiode array and LC-MS detection will give reliability for identifying degradation products and thus decreasing the time required to develop stability-indicating methods.

9. Impurity Profiling:- UPLC having higher sensitivity which can detect impurities in compounds even at trace levels.

10. Iodinated Disinfection Byproducts: In this method, IDBPs have been characterized in drinking water by using GC/MS. UPLC can also be analyzed if it joints with Electrospray ionization- triple quadruple mass spectrometer.

11. Therapeutic drug monitoring:- UPLC analyzed the drug by taking 5.5 min/ sample as compared to other samples, Carlier et al. in 2012 reported the monitoring of β-lactamase inhibitors in human plasma.

12. Determination of pesticides:- UPLC coupled with triple Quadra tandem mass spectroscopy will help in the identification of trace levels of pesticides from water.

13. Manufacturing/QA/QC:- Identification, safety, efficacy, purity, and quality is FDA drug quality regulation and it strengthening the quality of drug development, manufacturing and review are key factors for ensuring patient safety and improving clinical research.

SUMMARY

UPLC is new in chromatography techniques in liquid chromatography. Maximum efficiency is mandatory for both analytical laboratories and pharmaceutical facilities that are constantly challenged to increase throughput and deliver results to research chemists in pharmaceutical discovery. UPLC is more efficient in productivity and column chemistry and also instrumentation by providing higher resolution, speed, and sensitivity for liquid chromatography, due to modification in smaller particle size of bonded phase column packing. The main advantage is a reduction of analysis time, which also meant reduced solvent consumption about 80% as compared to HPLC.UPLC could be higher backpressure than in conventional HPLC. This backpressure can be reduced by decreasing the viscosity of the mobile phase by increasing the column temperature. It was observed that the sensitivity of UPLC was much higher than that of conventional HPLC. All categories of pharmaceutical

drugs can be analyzed by UPLC technique within a very less period and with less solvent consumption, it seems that UPLC integrated with higher speed, sensitivity, and resolution compared with conventional HPLC.

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