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# HPLC VS UPLC: THE EXTENT OF THE AREA IN ANALYTICAL CHEMISTRY

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## ABSTRACT-

Chromatography is used in various analytical as well as biochemical areas for separating a mixture of compounds into individual component. High performance liquid chromatography (HPLC) is one of the most important methods used for separation, identification and quantification of a compounds present in a mixture. HPLC has main drawbacks are it is relatively time consuming to run a chromatogram and consumes high amounts of solvents. UPLC is the new approach which opens novel directions in the field of liquid chromatography. It works on same principle like HPLC. UPLC improves runtime and sensitivity with less than 2 µm particle size. Separation process in UPLC is carried out in very high pressure up to 100 MPa. It reduces the cost of reagents with shorter run time as compared to conventional HPLC. UPLC can be regarded as a new direction for liquid chromatography. It improves in three ares speed, resolution and sensitivity, in this system fine particles are used i.e., less than 2.5µm so decrease the length of column, it saves time and reduces solvent consumption.

**KEYWORDS-** Chromatography, influence, migration, efficiency.

## INTRODUCTION-

High performance liquid chromatography is a qualitative and quantitative analysis of drugs and widely used in liquid chromatographic techniques. It is used in identifications and quantifications process of drug development. The principle behind the separation of compounds is given by van deemter equation in which the relationship between linear velocity (flow rate) and plate height (HETP, column efficacy) is explained.The efficacy of the separation, speed and resolution increases as the particle size of column material decreases.

## PRINCIPLE-

The UPLC is based on the principle that use of stationary phase containing particle size less than 2µm while in HPLC particle size is of 3 to 5 µm. The principle is totally based on the van demeetr equation which is an empirical formula and describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency). It was found that HETP decreases to a minimum value and then increases with increasing flow rate. However, with the 1.7µm particles used in UPLC, HETP is lowered compared to the larger particles and does not increase at higher flow rates. This allows faster separations to be carried out on shorter columns with higher flow rates, it increases resolution between specific peak pairs and increased peak

capacity, defined as the number of peaks that can be separated with specified resolution in given time interval. Efficacy is three times greater with 1.7 µm particles compared to 5 µm particles and 40% higher than with 3.5 µm particles. High speed is obtained because column length with 1.7 µm particles can be reduced by a factor of 3 compared to 5µm particles for the same efficiency, and flow rate can be tree times higher. This means separations can be nine times faster with equal resolution. Sensitivity increases because less bond spreading during migration through a column with smaller particles peak width is less and peak height is greater.

In ultra-performance liquid chromatography i.e., UPLC system waters changed the landscape and future of chromatography with the acquity. Chromatographers need no longer choose between the speed of short columns and the resolution of long columns. Separation's scientist can use both speed and resolution of long columns with the bonus of increased sensitivity 1. UPLC deliver more information faster without compromising data integrity.

In Acquity UPLC systems are holistically designed to dramatically improve resolution, sample throughout and sensitivity which also include,

- Small pressure tolerant particles
- High pressure fluidic modules
- Minimised system volume
- Negligible carry over
- Reduced cycle times
- Fast response detector
- Integrated system software and diagnostics

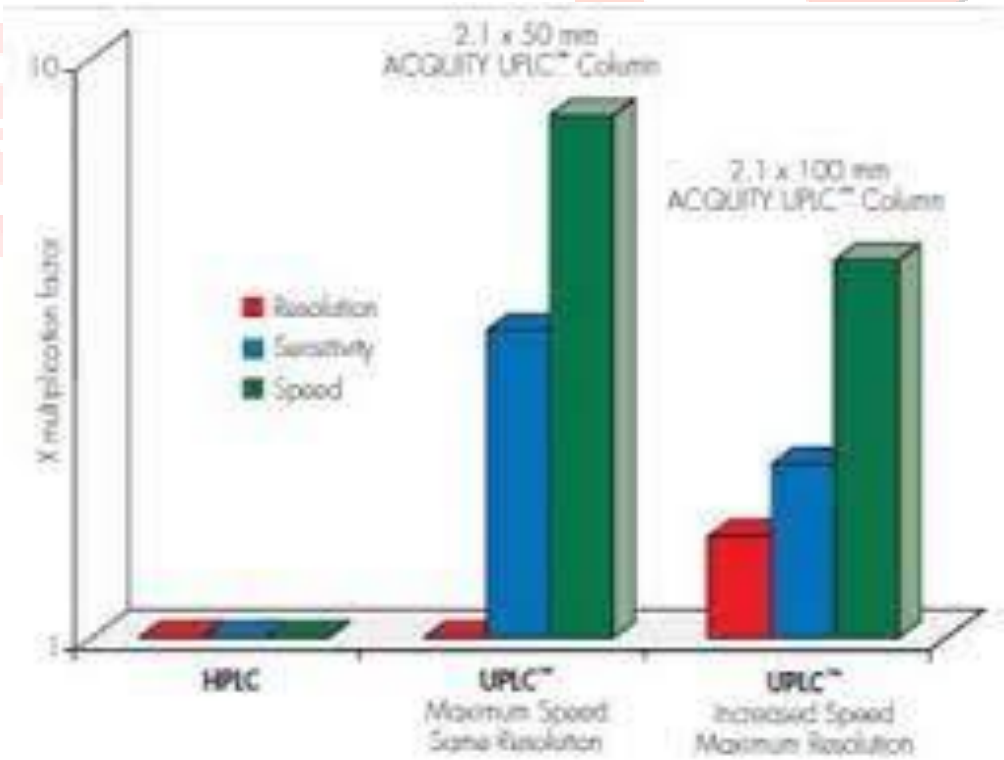


**Fig 1 Differences in the speed, sensitivity and resolution of the UPLC vs HPLC separations**

The principle of separation behind the HPLC and UPLC is based on Van demeeter relationship between plate height and flow rate.

The equation is,

**H=A+B/u + C u**

Where,

H- Height equivalent to theoretical plate (HETP)

A-eddy diffusion (particle size of packing material) dependent on how chromatographic beds are packed, it is related to the uniformity and the non-uniformity of the flow to and around a particle it is independent on velocity

B-longitudinal or axial diffusion tendency of analyte in bulk mobile phase and on the stationary phase. It decreases with increasing flow rate of mobile phase (linear velocity). This effect made smaller at high flow rates so this term is divided by u.

C-solute's mass transfer, it is related with both linear velocity of the mobile phase and the square of the particle size. It is the interaction of analyte molecules with the internal surface of the stationary phase and their distance of diffusion into and out of the pores of the packing material

u- flow rate (linear velocity) of mobile phase

**Contribution of Small particles in UPLC-**

The ideal of the van deemter equation cannot be completed without smaller particles than those traditionally used in HPLC. The van deemter equation having capacity to have an effect on the character by particles size, so the scientist focuses on the design and development of sub-2 µm particles is a significant challenge, and researchers have been active in this area for some time to take the chance to gain advantage from on their advantages

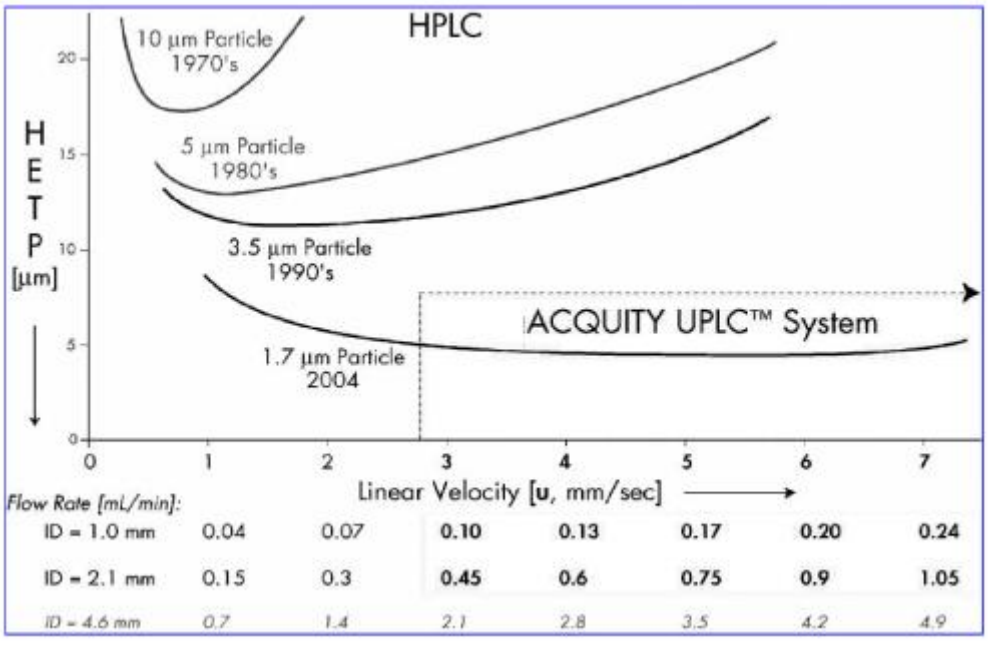


**Fig 2 Van deemter plot, illustrating the evolution of particle sizes**

"Fast LC" speed up analyses in drug discovery "need for speed" has been come from by the selecting so many of samples in different laboratories, and the ability of sophisticated instrument like UPLC with detector as mass spectrometers. The unique features of column that is smaller columns and faster flow rates

amongst other parameters have been used. During analysis higher temperature, having the dual advantages of lowering viscosity and increasing mass transfer by increasing the diffusively of the analytes, has also been investigated.However, using conventional particle sizes and pressures, limit-actions are soon reached and compromises must be made, sacrificing resolution for time.UPLC method and the classic separation method is of HPLC there are many advantages in HPLC like robustness, ease of use, good selectivity and adjustable sensitivity but it has 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. UPLC gives better efficiency with speedy analysis and this occurred only due to smaller particle size.

As per Van Deemter equation efficacy increases with the use of smaller size particles but due to this increase in efficacy rapid increase in back pressure occurs. Ae compared to HPLC it operates only up to 400 bars. Short columns filled with particles of about 2µm are used with this system, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load to improve the efficiency of HPLC separations.

- Work at higher temperature: allows high flow rates by reducing the viscosity of mobile phase which significantly reduces back pressure
- Use of monolithic columns: contains polymerize porous support structure that provides lower flow resistances than conventional particle-packed columns.

**By above tow parameter UPLC analysis improves in three areas**

1)Produced chromatogram with resolved peak.

2)fast analysis

3)Sensitive analysis

Use of fine particles save times and reduces solvent consumption. The new method makes a very big difference by retains the same analytical separation method as HPLC while other things which drastically changes are speedy analysis, sensitivity and high resolution.

To achieve new pharmaceutical drug development reduction in cost and less time for development of drugs it requires mean time in the quality of their products are not suffer analytical laboratories maintaining the whole things. Greater number of analyses to be performed in a shorter amount of time thereby increasing sample throughput and lab productivity. A typical assay was transferred and optimized for UPLC system to achieve both higher sample analysis throughput and better assay sensitivity.

Smaller particles provide not increased efficiency, but also the ability to work at increased linear velocity without a losing efficient and by providing both resolution and speed. The small particle size less than 2µm of stationary phase is the basic of UPLC techniques, UPLC required porous particles which can remains undamaged in high pressure in order to maintain their retention and capacity similar to that of HPLC. Silica particles possess good mechanical strength but their application is limited because it has narrow pH application range and shows tailing during analysis. Polymeric column did not have any pH limitations but found to have low efficiency.

To overcome this issue the first-generation hybrid chemistry utilizes the classical sol-gel synthesis. It has advantages like mechanical strength, high efficiency, operative over an extended pH range. Only the disadvantage is that they do not possess enough mechanical stability necessitated by UPLC.

Particle size has a significant impact on the analyte band as it relates to the term eddy diffusion. The path which analyte molecules take to transfer from the bulk mobile phase to the surface of the particle and around those particles takes less time as particle size is decreased. Larger particles cause analyte molecules to travel longer, more indirect path. The differences in these paths results in different migration times for the

analyte molecules within a population, resulting in broader analyte band and resulting peak. As the particle size of the packing is decreased, the paths of the analyte molecules are encouraged to be more similar in length. This results in narrower analyte bands which translate narrower peaks, higher efficiency and higher sensitivity.

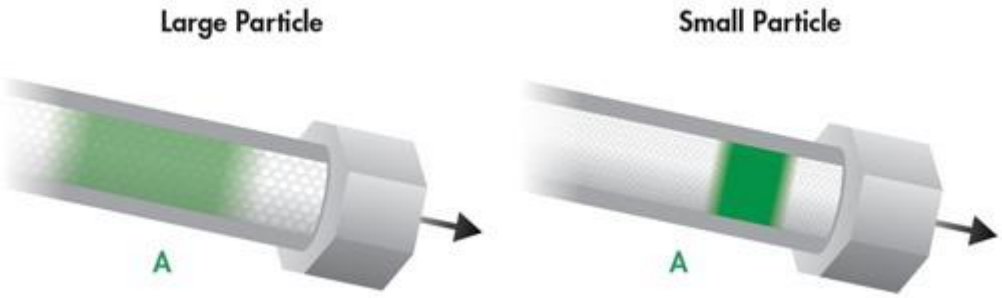


**Fig 3 The influence of particle size**

## Chemistry of small particles-

The primary separation parameter behind UPLC was its efficiency, selectivity and retentivity. In the fundamental resolution (Rs) equation-

$$R_s = \frac{k}{k+1} \times \frac{\alpha-1}{\alpha} \times \frac{\sqrt{N}}{4}$$

Retention Selectivity Efficiency

Resolution is proportional to the square root of N.

N is inversely proportional to particle size dp

N is also inversely proportional to the square of the peak width

## Some technologies in particle size chemistry-

1) **BEH technology-**

Waters launched the extra family of HPLC in 1999column featuring first generation hybrid particle technology (HPT). In this the best properties of inorganic silica and organic polymeric packings are combined to produce a material that has superior mechanical strength, efficiency, high pH stability and peak shape bases.

The first-generation methyl hybrid particles of extra columns did not possess the mechanical strength or efficiency to realize fully the potential speed, sensitivity and resolution capabilities in UPLC technology. So that, a new pressure- tolerant particle needed to be created. Hence, new second generation hybrid material was developed which utilizes a bridged ethylsiloxane and silica hybrid (BEH). Second generation hybrid technology exhibit improved efficiency, strength and pH range. This technology also has high speed, sensitivity and resolution of UPLC separations.

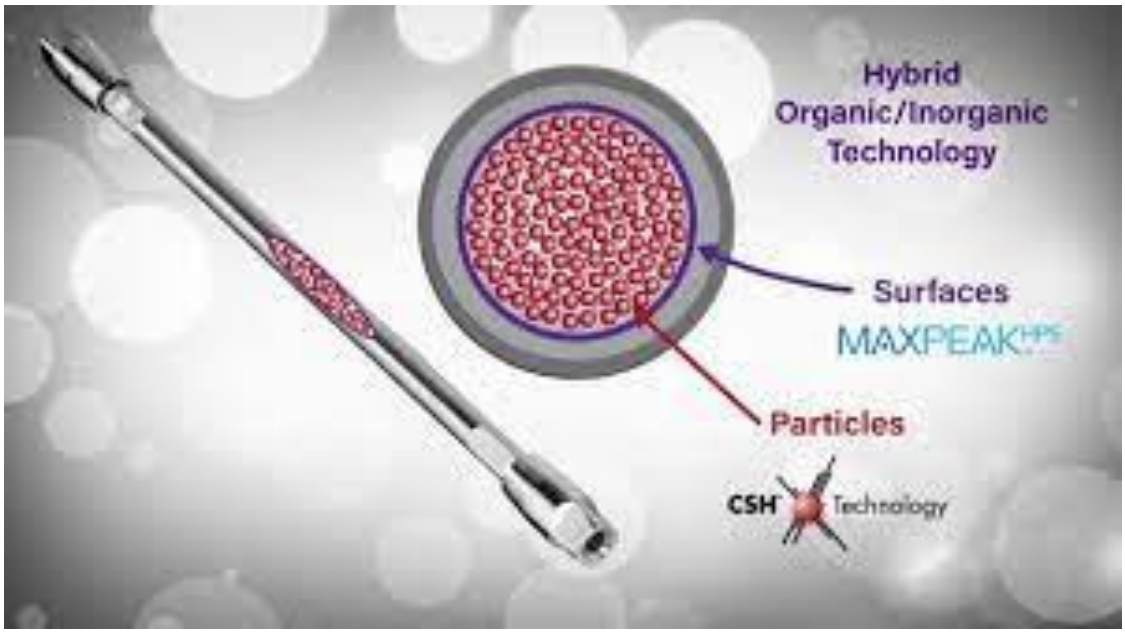


**Fig 4 Micron columns indicating particle size**

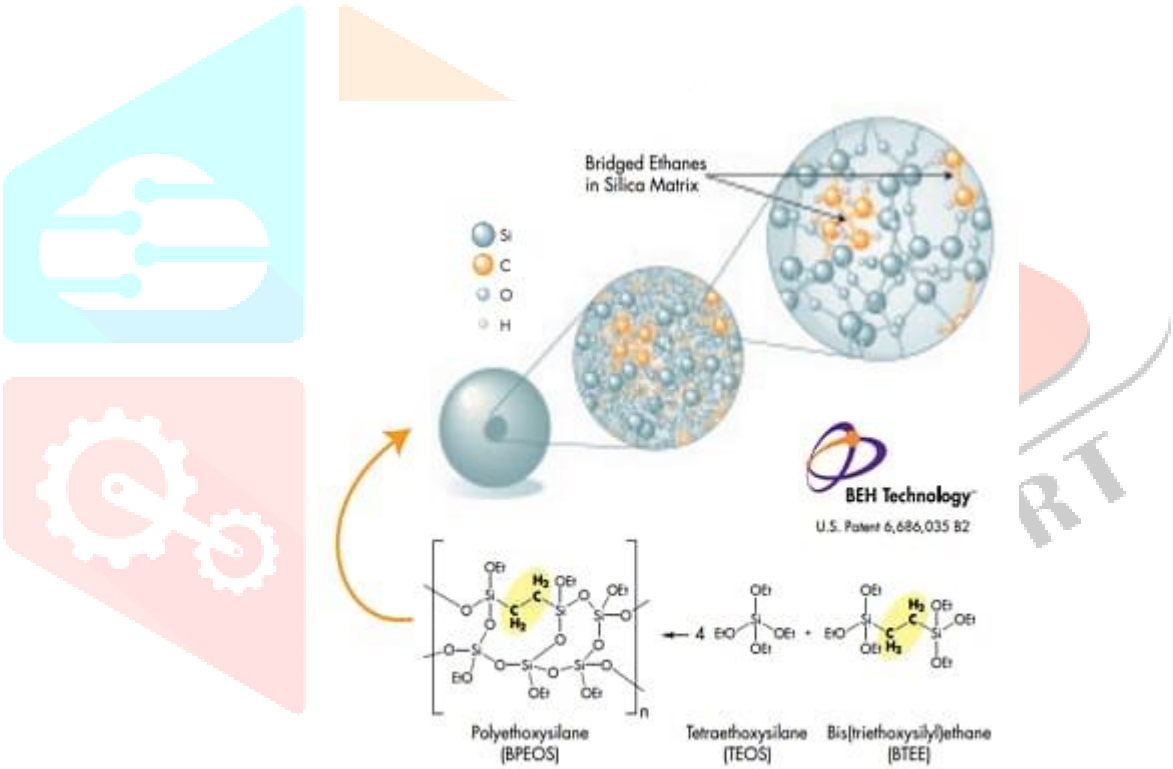


**Fig 5 Ethylene bridged hybrid**

Waters Xbridge premier columns ACQUITY UPLC Column Performance—Enabled by BEH Technology. Over the counter analgesics are designed purely for pain relief. Because of their low cost, effectiveness and safety, analgesics are recommended as a first line option by most physicians.

2) **HSS Technology (High Strength Silica)-**

A mechanically tolerated, silica-based material was designed to resists UPLC pressures. This technique increases the mechanical stability of silica and also maintains the pore volumes as like that of HPLC silica-based material. Novel technology provides increased retentivity compared to hybrid particles while serving as ideal substrate to create stationary phase that provide alternative sensitivity.

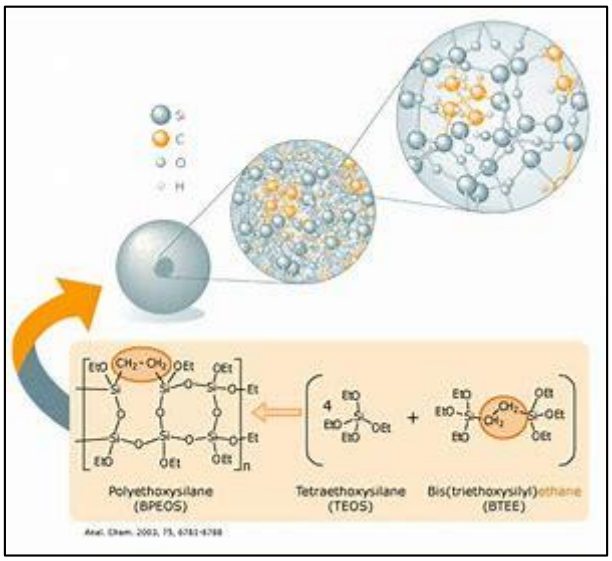


**Fig 6 High strength silica**

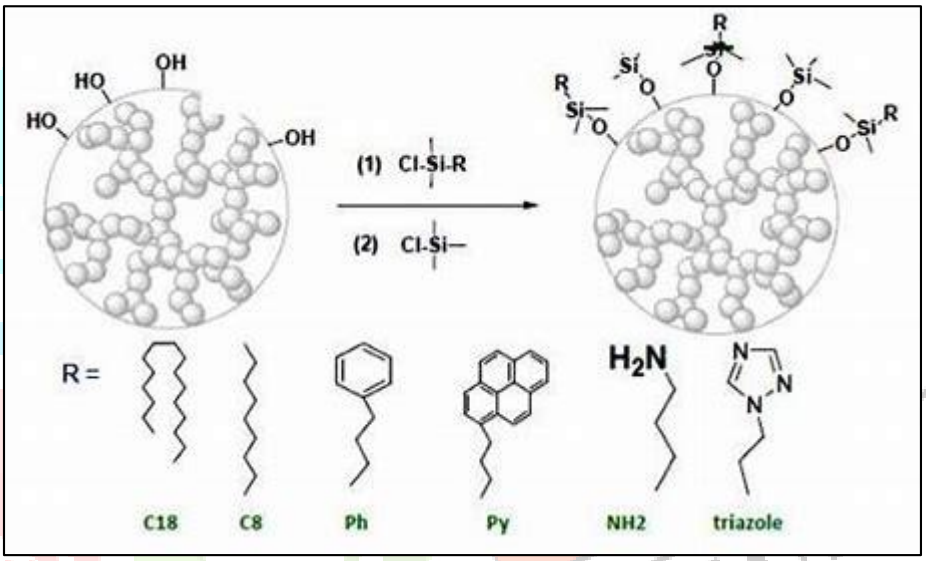


**Fig 7 Silica hybrid in HSS**

### 3) CSH Technology (Charge Surface Hybrid)-

The processes of developing and insurrection of materials science has led to significant advances in chromatographic materials, in which being hybrid-based packing materials offer exceptional peak shape, efficiency as well as leading chemical stability. This technology is the latest advancement in hybrid materials that utilises a controlled, low level surface charge to provide enhanced selectivity and exceptional peak shape, especially in low ionic strength mobile phases.

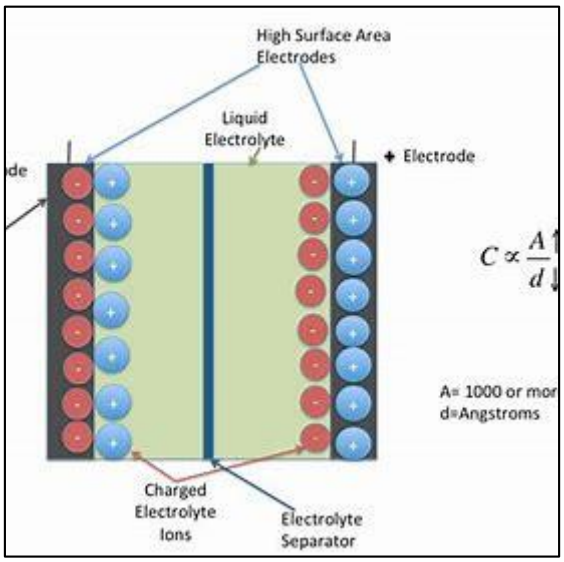


**Fig 8 CSH technology showing charged electrolyte ions**

**4) <u>UPLC 2D Technology-</u>**

With 2D one can get a range of functionality by loading more sample

i) trapping- increases sensitivity by loading more sample

ii) heart cutting- increases resolution by incorporating orthogonal chemistries

iii) at- column dissolution- enables large volume injections of sample in strong solvent

iv) parallel column regeneration- increases throughput

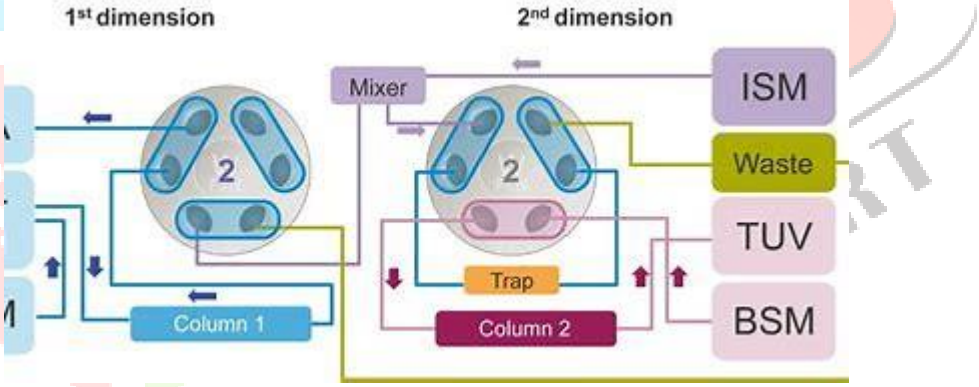


**Fig 9 UPLC 2D technology**

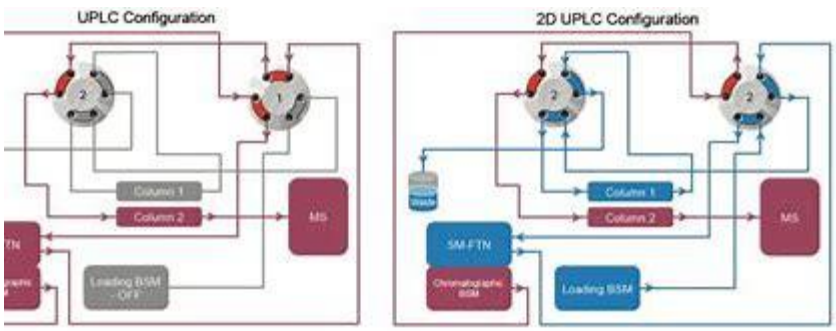


**Fig 10 Schematic representation of UPLC 2D technology**

This technology provides

-Ready to use configurations allowing foe faster 2D UPLC analysis, with less troubleshooting and more confidence

- Offer full range of sub-2-µm UPLC column chemistries for true UPLC results

- Further improve the quality and ability to eliminate unwanted interferences

- Increase peak capacity and resolution for characterizing the most complex sample

- Provide flexibility in utilization of mobile phase for, mass spectroscopy detection

-Minimize sensitivity drift in mass spectroscopy by reducing surface contamination

-Improve assay ruggedness and overall speed of analysis

**Factors influencing separation-**

1) **Parameters affecting efficiency-**
   Column length
   Particle diameter
   Particle size distribution
   Flow rate

2) **Parameter affecting retention factor**
   Eluent type
   Eluent composition
   Stationary phase type
   Analyte nature

3) **Parameters affecting sensitivity**
   Stationary phase type
   Analyte nature
   Eluent additives
   **Temperature**
   Eluent composition (ionizable analytes)

The efficiency of column is depending on column packing, the particle size and the column dimensions. When columns are long, they give narrow peaks (more efficient) but analysis time also increases so this should be inherent. Smaller particles provide more surface area and better separations giving narrower packs. Velocity of solvent is decreases by temperature reduces back pressure and inherent retention. For a variation in temperature by $10^0$c, the retention time decreases by about 20 % in isocratic chromatography. Flow rate decreases as efficiency increases and peak shape improves. Stationary phase property like polarity, nature of base of silica, physiochemical interactions with analyte, hydrophobicity affects the selectivity of separation.

**Advantages of HPLC-**

-Sensitivity and accurate quantity analysis.
-Separate nonvolatile and thermolabile compound.
-Ability to separate and identify compounds that are present in any sample that can be dissolved in a liquid in a trace concentration as low as parts per trillion.
- It has wide ranges of mobile phases for the separation of compound.

-Adsorption chromatography is an important method to separate any component that are not separated by other techniques.
- The complex sample mixture can be easily separated by this method.
- Very few types of apparatus or types of equipment required for isolation.

**Disadvantages of HPLC-**

i) Co-elution in this process whereby two or more chemical compounds elute from a chromatographic column at the same time, making separation and identification difficult.
ii) adsorbed compounds and traces are absorbed.
iii) Cost and complexity occur.

**Advantages of UPLC-**

-Decrease in consumption mobile phase volume by at least 80% compared to HPLC.
-Decreased run time and cost of operation.
-Lower injection volume is required.
-Greater signal to noise ratio (S/N) due the reduction in band broadening thereby increasing the sensitivity, because of better chromatographic peak resolution, the problem of ion suppression from co-eluting peaks isreduced.
- Faster resolving power.
-Column temperature minimizes the mobile phase velocity resulting in the high diffusion coefficient without significant loss in efficiency and increase in column back pressure.

**Disadvantages of UPLC-**

-higher back pressure compared to conventional HPLC which decreases the life of column.
- The particles of less than 2um mostly non-regenerable hence have less use.

**Analytical methods of validation-**

Validation is documented analytical procedure which is suitable for various purpose.
Following characteristics are addressed-
-Specificity
-Accuracy
-Precision (repeatability, reproducibility intermediate and precision)
-Limit of detection (LOD)/ Detection limit
-Limit of quantitation (LOQ) / Quantitation limit
-Linearity
-Range
- Robustness

For all these characteristics system suitability test was used to ensure that the chromatographic systems and procedures are sufficient foe analysis purpose. Retention time, columns efficacy, number of theoretical plates, asymmetry of chromatographic peak, tailing, resolution and reproducibility in germs of relative standard deviation of peak area are the parameters of this test.

**Applications of HPLC-**

-Stability testing
-Drug assay
-Chiral separations
-Steroid analysis
-High-throughput Screening
-Analysis of pollutant
-Analysis of food relevant compound

-Bioanalytical separations
-Forced degradation studies
-Impurity testing

**Application of UPLC**

-Raw material quality control
-In process quality control
-finished product quality control
-Method devolvement
-validation
-Forced degradation studies (FDS)
-Dissolution testing
-Bioequivalence study
-Bioanalysis study
-Toxicity studies
-Therapeutic drug monitoring
-Analysis of contaminants and foreign particles in food
-Peptide mapping
-Analysis of pesticides in groundwater
-Detection of metabolites

**Contribution of small particles in UPLC-**

The small particle size less than 2µm of stationary phase is the basis of UPLC techniques. UPLC requires porous particles, which can remain undamaged the high pressure in order to maintain their retention and capacity similar to that of HPLC.

Silica particles possess good mechanical strength but their application was limited by narrow pH application range and generally exhibit tailing during analysis of basic analysts.

**Relationship between HPLC and UPLC-**

1) Van Deemter plot comparing particle size-

When HPLC column with 3.5µm particles and UPLC column with 1.7µm provides 2-3 HETP (H) values as compared to each other, these lower H values are achieved at a higher linear velocities and lower wider range of velocities. Mass transfer is improved to a strikingly large extent with the small particle which gives better efficacy and resolution. Hence, an increased range of linear velocities can be used to increase this improved performance. Due to fast separations in fast linear velocities also increasing speed of analysis increases resolution.

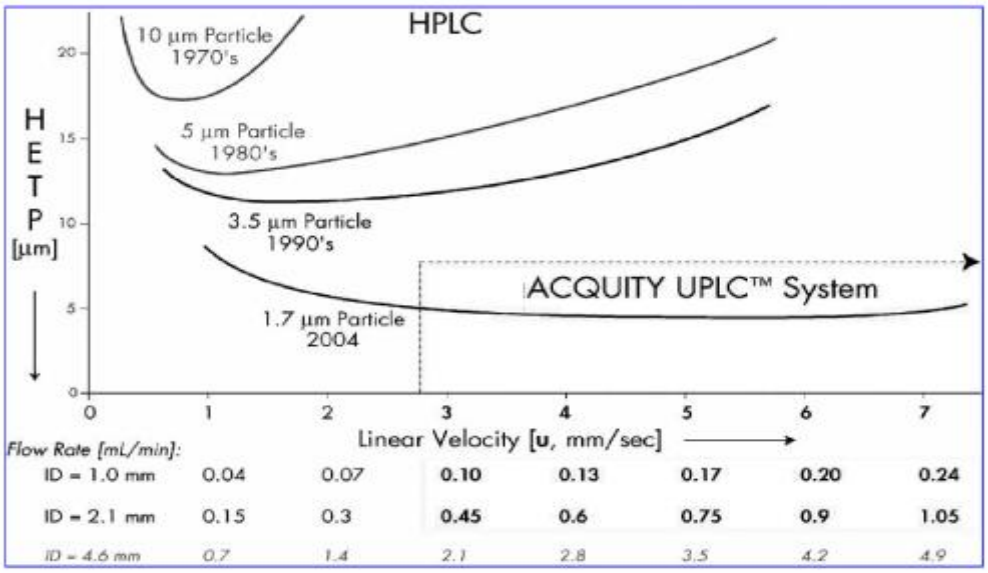


**Fig 11 Graph showing HETP vs linear velocity**

**Table 1 showing differences between HPLC and UPLC -**

| Characteristics | HPLC | UPLC |
|---|---|---|
| Plate count | 2000 | 7500 |
| Flow rate | 3.0 ml/min | 0.6 ml/min |
| Pressure | Upto 6000 psi | Upto 20,000 psi |
| Temperature of column | $30^0$ c | $65^0$ c |
| Total run time | 10 minutes | 1.5 minutes |
| USP resolution | 3.2 | 3.4 |
| Injection volume | 5 | 2 |
| Particle size | 3 to 5µm | Less than 2 µm (1.7 µm) |
| Maximum backpressure | 300-400 bars | 1000 bars |
| Analytical columns | C18 | UPLC BEH C18 |
| Column dimensions | 150×3.2 mm | 50× 2.1 mm |

**1) Band spreading effect on HPLC compared to UPLC-**

UPLC separation in the combination of instrument and column performance

Fully realize and harness of the power of substance 2µm particle columns. This occurs by minimizing band spreading within intra column and outside extra column. Also operate at the optimal linear velocities and pressure of these small particle column.

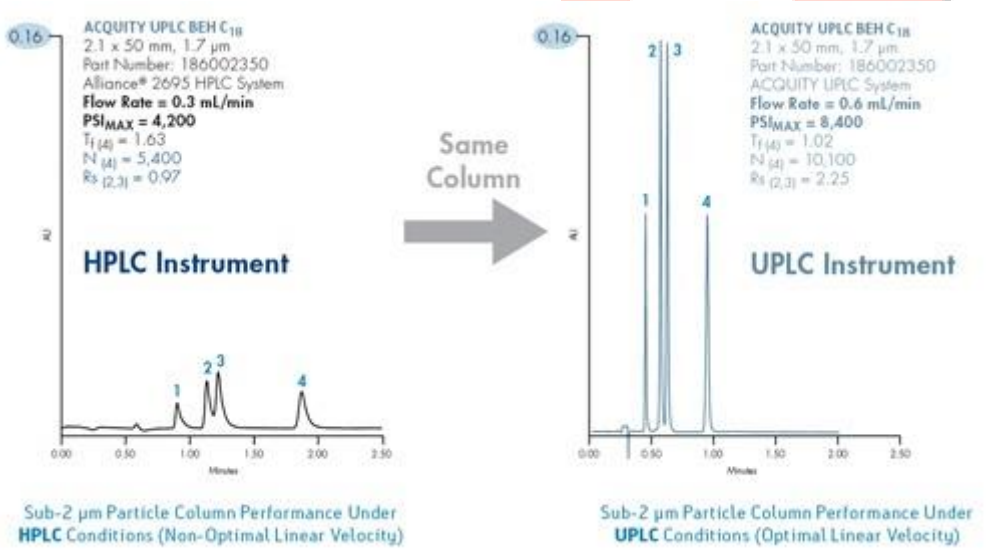


**Fig 11Column performance under HPLC and UPLC in non-optimal linear velocity and optimal linear velocity respectively**

The ability to operate in a fast, low band spread LC instrument capable of operating the optimal linear velocity is crucial to realizing the performance gains of sub-2µm particle columns. In this example four caffeine metabolites are analyzed using the same chromatographic conditions (except for flow rate as noted) on a fully optimized, microbore HPLC instrument vs standard UPLC instrument. The improvement in efficiency, resolution, peak height illustrates the benefits of UPLC technology.

To maximize separation power, one can combine the use of small particles with elevated temperature and elevated pressure to develop ultra-high efficiency separations using UPLC technology.

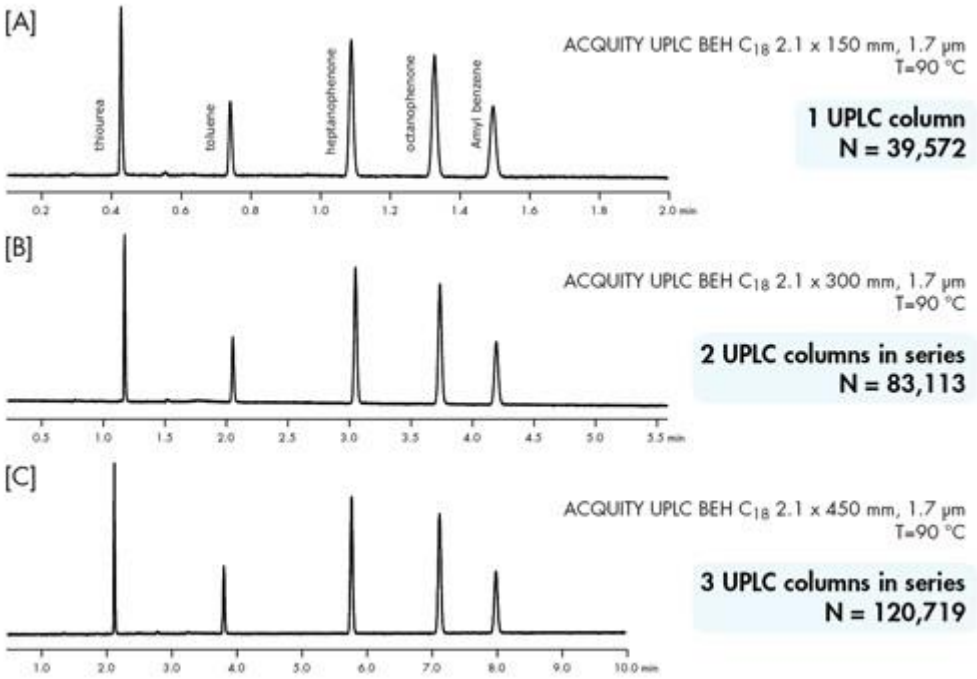


**Fig 12 Various chromatograms of UPLC using various columns**

**Combining elevated temperature with UPLC technology to maximize plate count**

In above figure single 150 mm long 1.7 µm UPLC column producing just under 40,000 plates at $90^0$C. A second column was added in series to produce a length of 300 mm, resulting in a plate count of 83,000. The full pressure range of the system is exploited by adding a third column in series, resulting in a 450 mm long UPLC column packed with 1.7 µm particles. An efficiency of 121,000 plates is achieved in only 8 minutes.

**Merits-**

-Using high temperature reduces the viscosity of mobile phase by occurring this step back pressure is reduced.

-UPLC analysis is interconnected skeletons and interconnected flow paths through-pores which can found in monolithic columns make UPLC techniques different from HPLC. In UPLC chromatogram it is found that better resolution and separation are found as compared to HPLC along with perform more sensitive analysis, reduce consumption of solvent and has high speed of analysis.

-Requires less time and enhance sensitivity

-Provides the selectivity, sensitivity and dynamic range of LC analysis

-In chromatogram resolved peaks are obtained.

-Multi residue methods are applied.

-Speedy analysis, quantify accurately analytes and related products

-Uses of fine particles (2µm) for packing of stationary phase make analysis fast

-Time and coat both are reduced

-Consumption of solvents is less.

-More products are analyzed with existing resources.

-UPLC system minimizes the time up to nine times comparing to the conventional system using 5µm particle packed analytical columns.

-Separation on UPLC is performed under very high pressure up to 100 MPa

-It gives increased peak capacity (number of peaks resolved per unit) and resolution 4.

-UPLC improves quality of the data, resulting in a more definitive map 5.

-UPLC fulfils the promise of increased speed, resolution, sensitivity and broad range of selectivity predicted for liquid chromatography.

-UPLC fast resolves power quickly quantifies related and unrelated compound.
-Faster analysis through the use of novel separation material of very fine particle size.
-Reduces process cycle times, so that more produced with existing resources.
-It increases number of products and enables manufacturers to produce more material that consistently meet the products specifications, potentially eliminating variability, failed batches, or the need to re-work material.
- Delivers real-time analysis in step with manufacturing processes and assures end products quality including final release testing.

**Conclusion-**

Liquid chromatography (LC) is a powerful tool in separation science employed for clinical research as well as for therapeutic drug monitoring. It is most widely applied technique to identify, quantify and purify the components from a mixture in which the separations process is based upon the rate of elusion of components. This revies focuses on the invention of new LC techniques, comparative performance of UPLC and HPLC with summarizing their applications on method development.

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