UPLC

The term UPLC, meaning “Ultra Performance Liquid Chromatography,” was introduced by Waters Corporation when they introduced their Acquity LC system.

Types of Liquid Chromatography

(TLC) Paper Chrom.

Gravity Chrom. Tsvett, 1903

Flash Chrom. 1978

HPLC 1952

UPLC 2004

UltraPerformance Liquid Chromatography (UPLC) Technology

- In 2004, further advances in instrumentation and column technology were made to achieve very significant increase in:
  - RESOLUTION
  - SPEED
  - SENSITIVITY
  - Increase separation EFFICIENCY
- Columns with smaller particles [<1.7um]
- Mobile phase delivery is done at >15,000psi

WHAT CHANGES MADE THE DIFFERENCE?

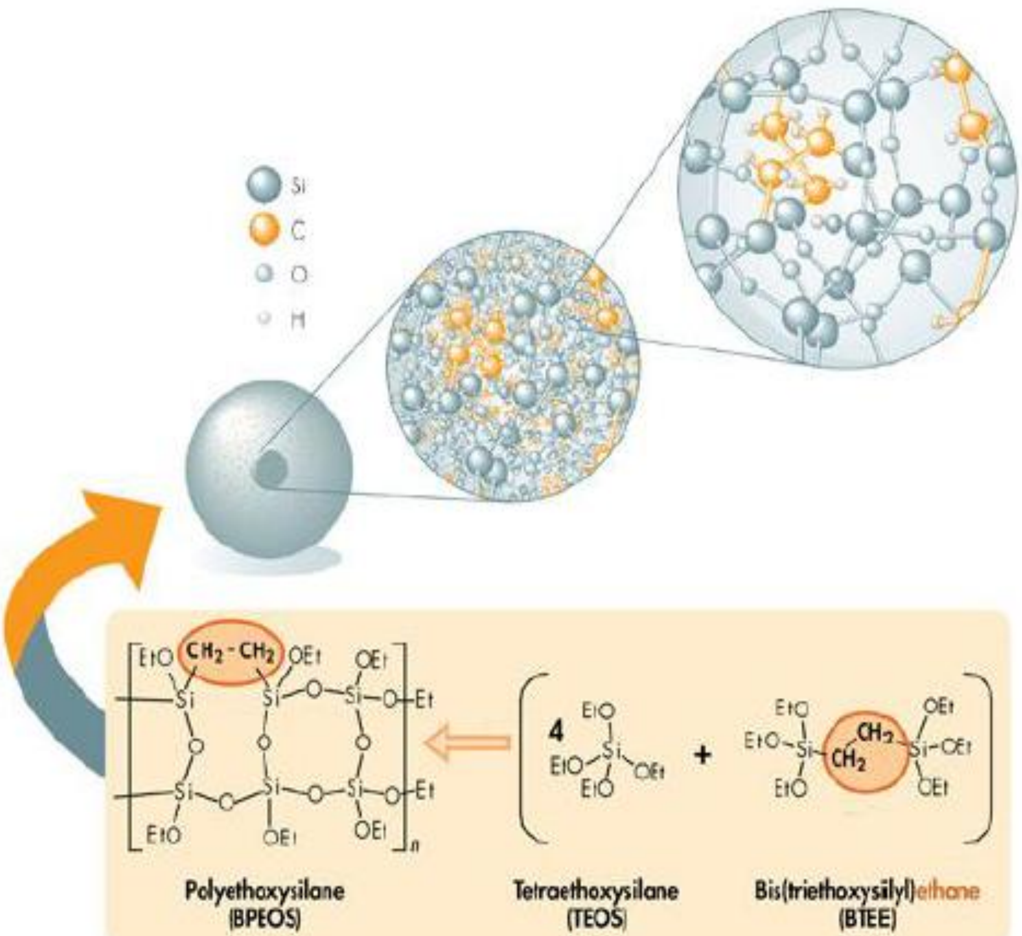
The Major change: use of sub-2 um particles.

Key benefits:

- System can be operated at higher flows and pressures than a conventional system.
- This concept resulted in significantly shorter analysis times.

UPLC columns

an ethylene bridged hybrid (BEH) structure



Anal. Chem. 2003, 75, 6781-6788

- Superior mechanical strength
- Efficiency
- High pH stability and peak shape for bases
- C8; C18;Phenyl
- pH range 1-12
- Max pressure 15,000psi
- Particle size 1.7um
- Pore diameter/volume 130A 0.7 mL/g
- Surface Area 185 m^2/g

- **Peptides**
- **Proteins**
- **Oligonucleotides DNA/RNA**
- **Amino acids**

UPLC- A NEED BASED INVENTION/MODIFICATION OF HPLC

- High performance liquid chromatography (HPLC) is approved technique as it has been used in laboratories worldwide over the past 30-plus years.
- For many years, researchers have looked at “fast LC” as a way to speed up analysis.
- HPLC technology simply doesn’t have the capability to take full advantages of sub-2µm particles.
- using conventional particle sizes and pressures, limitations are soon reached and compromises must be made, sacrificing resolution.

UPLC - SUPERIORITY

The Ultra Performance Liquid Chromatography have the ability to work more efficiently with higher speed, sensitivity and resolution at a much wider range of linear velocities, flow rates and backpressures to obtain superior results.

UPLC- BASICS

- The UPLC is based on use of stationary phase consisting of particles less than 2.5 µm (while HPLC columns are typically filled with particles of 3 to 5 µm).
- The underlying principles of this evolution is governed by the Van Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency)

Van Deemter model

$$H = A + B/u + u\,[C_M + C_S]$$

A: random movement through stationary phase

B: diffusion in mobile phase

C: interaction with stationary phase

H: plate height

u: average linear velocity $u = L/\,t_M$

Where;

A, *B* and *C* are constants

VAN DEEMTER MODEL

$$H = A + B/v + Cv$$

Where;

A, *B* and *C* are constants

v is the linear velocity, the carrier flow rate.

*The *A* term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform.

* The *B* term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by *v*.

- The *C* term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the mobile phase to the packing stationary phase and back again.
- The greater the flow rate, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus term is proportional to *v.*

Chromatographic resolution is described by

$$Rs = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right) \qquad (1)$$

Where,

Rs = Resolution

N = Separation efficiency (theoretical plate)

α = Selectivity factor

k = Retention factor (Capacity factor)

29-Oct-15

Separation efficiency (N)

$$N = \frac{L}{H} = \frac{L}{hd_p} \qquad (2)$$

Where,

L = Column length

H = Height of theoretical plate

h = Reduced plate height

dp = Particle diameter

Therefore,

$$Rs \propto N \propto \frac{1}{d_p}$$

Why is UPLC more efficient

- Peak capacity (P) is the number of peaks that can be resolved in a specific amount of time.
- P is proportional to the inverse of the square root of the Number of theoretical plates (N): $N = L/H$
- Lower plate heights generate a smaller number of plates
- Plate heights are correlated through the Van Deemter equation

Theory of separations using small particles

The fundamental resolution equation

$$Rs = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right)$$

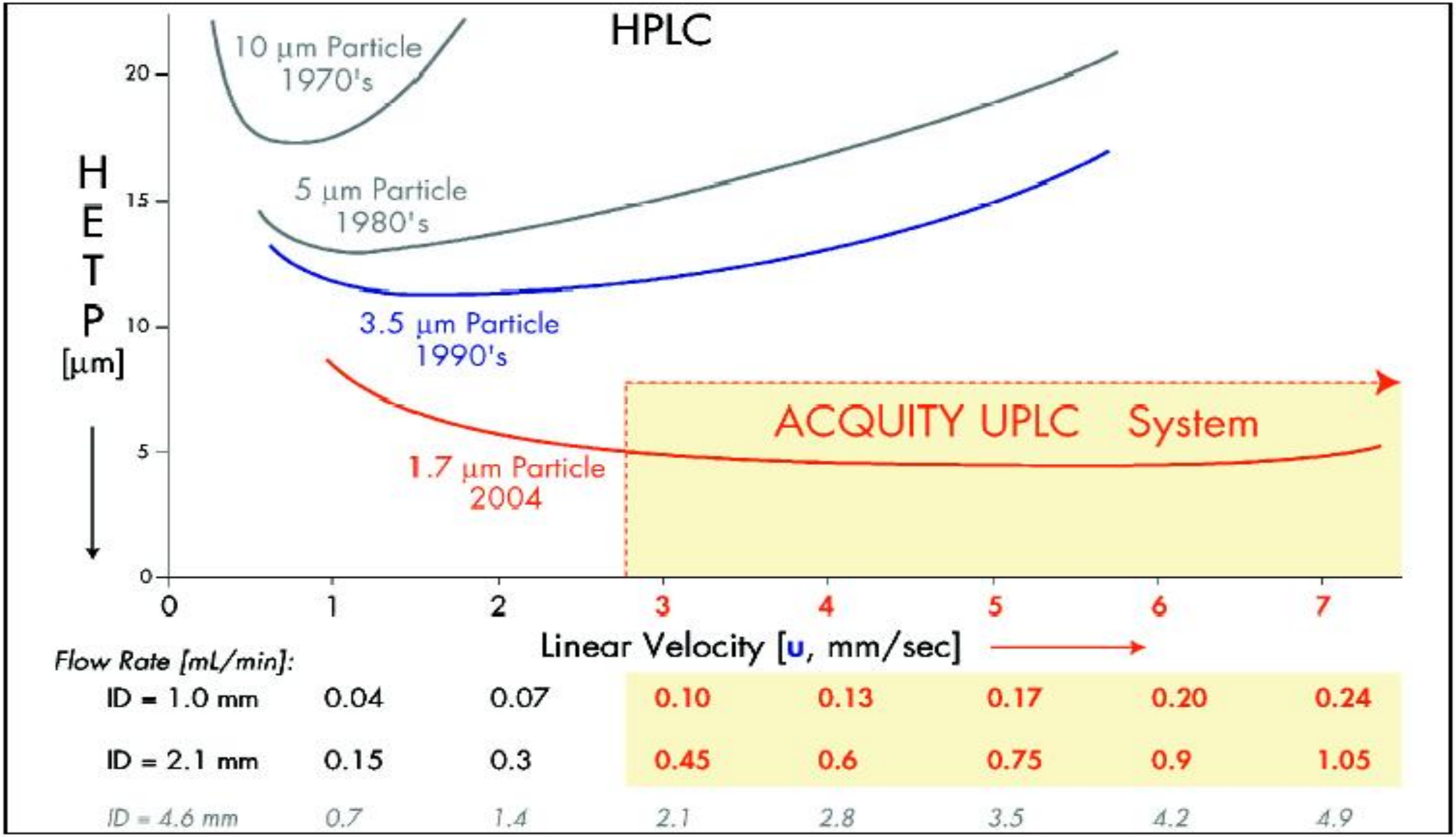
$$N \propto \frac{1}{dp}$$

$$N \propto \frac{1}{\omega^{2}}$$

$$H \propto \frac{1}{\omega}$$

$$F_{opt} \propto \frac{1}{dp}$$

Efficiency is proportional to column length and inversely proportional to the particle size: $N \propto \frac{L}{dp}$



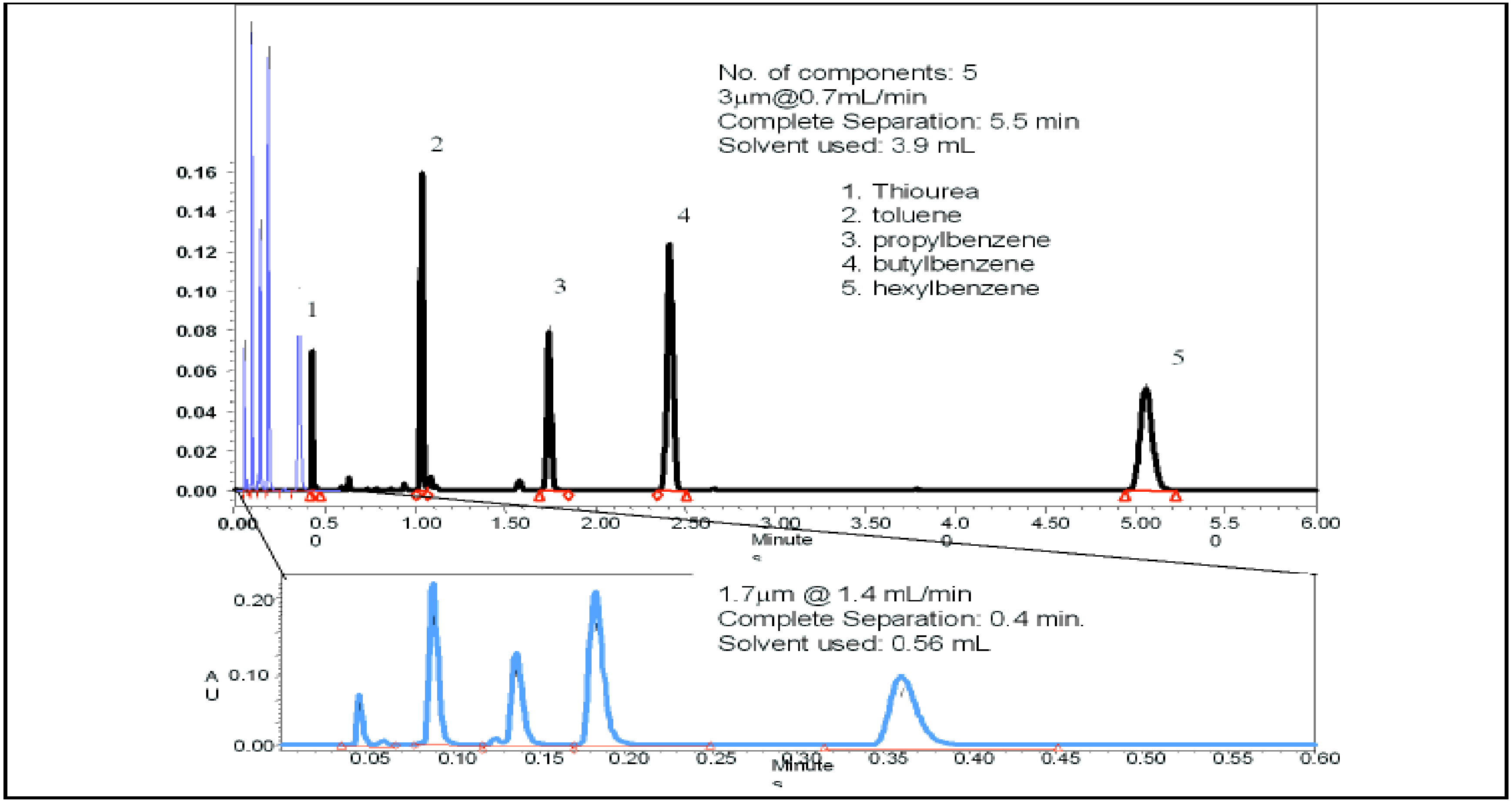
Van Deemter plot illustrating the evolution of particle sizes

As the particle size decreases to less than 2.5µm, not only there is significant gain in efficiency, but the efficiency doesn't diminish at increased flow rates.

By using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separation) can be extended to new limits, termed ultra performance liquid chromatography.

HPLC TO UPLC

- Therefore it is possible to increase throughput, and thus the speed of analysis without affecting the chromatographic performance.
- The advent of UPLC has demanded the development of a new instrumental system for liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent with the pressures (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC).
- Efficiency is proportional to column length and inversely proportional to the particle size.
- The smaller particle size provide increased efficiency as well as the ability to work at increased linear velocity without a loss of efficiency, providing both resolution and speed.
- Efficiency is the primary separation parameter behind UPLC since it relies on the same selectivity and retentivity as HPLC.

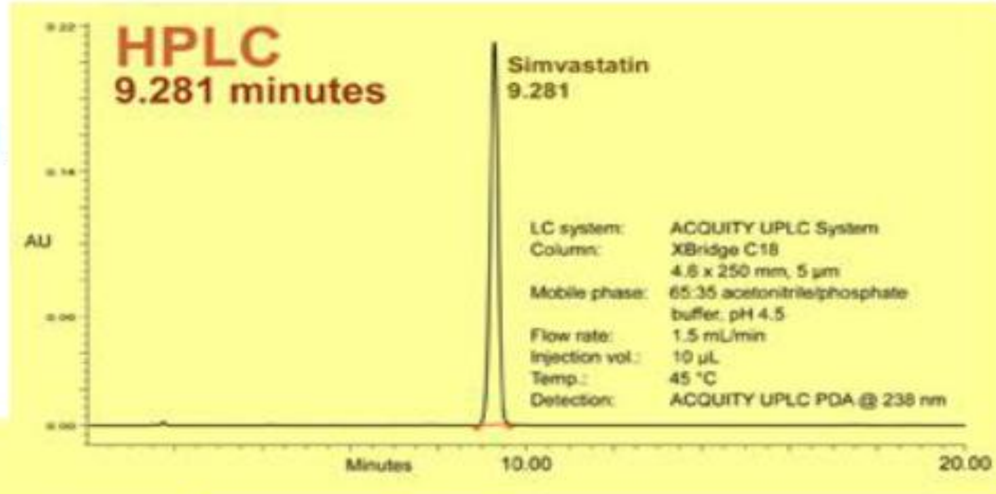


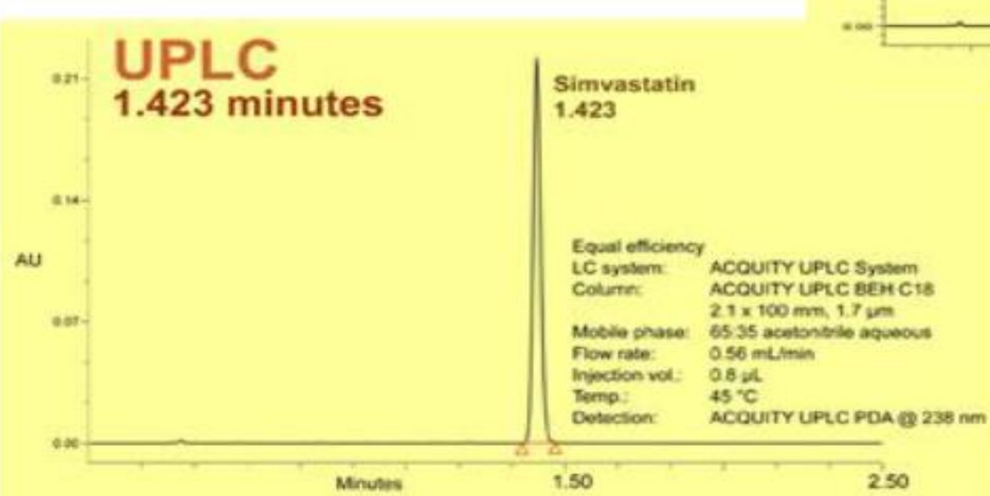
I:It is an overlay of both conventional (3 µm) HPLCand 1.7 µm UPLC for a five component sample mixture.

II:It is an expansion of the first 0.6 minutes of the overlay to show the increased speed of UPLC, while resolution is still maintained.

Chromatograms of simvastatin

- **hypolipidemic drug**
- **control elevated cholesterol**





FACTORS AFFECTING PRFORMANCE OF UPLC

- Pressure
- Column
- Particle size of packing
- Temperature

ADVANTAGES OF UPLC:

- Decreases run time and increases sensitivity
- Provides the selectivity, sensitivity, and dynamic range of LC analysis
- Maintaining resolution performance.
- Expands scope of Multiresidue Methods
- UPLC's fast resolving power quickly quantifies related and unrelated compounds
- Faster analysis through the use of a novel separation material of very fine particle size
- Operation cost is reduced
- Less solvent consumption

ADVANTAGES OF UPLC:

- Elevated-temperature chromatography also allows for high flow rates by lowering the viscosity of the mobile phase, which significantly reduces the column backpressure.
- Monolithic columns contain a polymerized porous support structure that provides lower flow resistances than conventional particle-packed columns.
- Reduces process cycle times, so that more product can be produced with existing resources
- 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 rework material
- Delivers real-time analysis in step with manufacturing processes
- Assures end-product quality, including final release testing

LIMITATIONS/DISADVANTAGES/DRABACKS-

The disadvantages of UPLC are:

- ✓ Due to increased pressure requires more maintenance and reduces the life of the columns of this type.
- ✓ In addition, the phases of less than 2 μm are generally non-regenerable.
- ✓ Cost
- ✓ Solvent pumping
- ✓ Lack of variety in commericial columns at 1.7 μm

Peptides

Amino acids

Nucleic acids

Organic acids

Carbohydrates

Why we need UPLC Technology?

Vitamins

Polyphenols

Alkaloids

Inorganic species

- **Metabolomics** is the comprehensive assessment of endogenous metabolites of low-molecular weight (<1,000 Da)of a biological system.

- These small molecules, including peptides , amino acids , nucleic acids , carbohydrates , organic acids, vitamins , polyphenols , alkaloids and inorganic species act as small-molecule biomarkers that represent the functional phe in a cell , tissue or organism.

- Applications: drug discovery, toxicology, nutrition, cancer, natural product discovery, etc.

- These large-scale analyses of metabolites are intimately bound to advancements in ultra-performance liquid chromatography–electrospray (UPLC) technologies and have emerged in parallel with the development of novel mass analyzers an hyphenated techniques.

Contrasting HPLC and UPLC

- UPLC gives faster results with better resolution
- UPLC uses less of valuable solvents like acetonitrile which lowers cost
- The reduction of solvent use is more environmentally friendly

HPLC X UPLC

PARAMETRS	HPLC	UPLC
1. Peak pattern and their resolution	➢Broader peak width provides less resolution	➢Smaller peak width provides better resolution and more number of peaks getting identified.
2. sample throughput	➢ Less sample throughput comparatively.	➢ Higher sample throughput with more information per sample.
3. Sample injection volume	➢ Sample injection volume is 20 micro liters.	➢Sample injection volume is as less as 3-5 micro liters.
4. Pump operational pressure	➢ Pump operates at 2000-6000 psi pressure	➢ Pump operates at 10,000 psi pressure
5. Particle size in stationary phase	➢ Particle size in stationary phase packing material is between 5-12 micrometers.	➢ Particle size in stationary phase packing material is less than 2 micrometer