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A Brief Review: UHPLC

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Abstract:

In recent years, ultra-high-pressure liquid chromatography (UHPLC) become the modern standard HPLC platform. UHPLC, with its shorter analysis time and quicker column equilibration, is ideally suited to rapid method development. This article provides a critical review of the current status, the benefits and the limitations of UHPLC in method development. We use case studies to describe best practices and recent advances.

Examples include conversion of existing HPLC methods to faster analysis, rapid column/mobile-phase screening, and automated method optimization. While we focus on the development of reversed-phase methods for assay and impurity analysis of small-molecule pharmaceuticals, our insights and conclusions can be extended to other applications and sample types. Besides generating faster analysis (hen used short, small-particle column), the higher-pressure limits of UHPLC also allow the effective use of longer columns for superior routine analysis of complex samples.

Keywords: HPLC, UHPLC, UPLC, Method Development, Pharmaceutical analysis, Column/mobile-phase screening.

Introduction:

- **HPLC-** High-performance liquid chromatography (HPLC), formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column

filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

HPLC has been used for manufacturing (e.g., during the production process of pharmaceutical and biological products), legal (e.g., detecting performance enhancement drugs in urine), research (e.g., separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (e.g., detecting vitamin D levels in blood serum) purposes.

Chromatography can be described as a mass transfer process involving adsorption. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with adsorbent, leading to the separation of the sample components. The active component of the column, the adsorbent, is typically a granular material made of solid particles (e.g., silica, polymers, etc.), 2–50 μm in size. The components of the sample mixture are separated from each other due to their different degrees of interaction with the adsorbent particles. The pressurized liquid is typically a mixture of solvents (e.g., water, acetonitrile and/or methanol) and is referred to as a "mobile phase". Its composition and temperature play a major role in the separation process by influencing the interactions taking place between sample components and adsorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, most often a combination.

- **UHPLC-** By contrast, UHPLC (ultra high-performance liquid chromatography) operates in the 20,000-psi range. Comment: This is simply not true. Some UHPLC systems can go that high, but not all. In general, UPLC instruments are lower dispersion, lower volume (extra column) systems that can operate at higher pressures.? Unlike UPLC, the name isn't trademarked, and may refer to a number of manufacturers' systems. UHPLC systems were designed with a low dwell volume in mind. Comment: Dwell volume is a term that refers to gradient separations; extra column volume and dispersion are better terms to use. UPLCs have lower dwell volumes but this is because the extra-column volume is minimized. Having a low volume is not the only performance attribute of a UPLC system. They offer higher rates of efficiency than UPLC systems, though are also prone to blockage. Comment: Not sure where this comes from. If a sample isn't prepared properly, then it is possible

to clog a sub 2-micron UHPLC or UPLC column. It is the column that can become clogged, not the system.

- **UPLC-** UPLC (ultra performance liquid chromatography) systems were first introduced in 2004. By almost doubling the overall operating pressure (to 15,000 psi) in order to obtain more rapid flow rates, UPLC developers were able to achieve equal or better resolution LC separations in much shorter time frames. Comment: Rather than "doubling the overall operating pressure in order to obtain more rapid flow rates" it's better to say … "In order to take advantage of 2-micron particles higher pressures are required." The typical ID of a UPLC column is 2.1 mm and in general flow rates are lower than HPLC, but due to the efficiency increase, explained above, the overall separation time is reduced. In terms of efficiency, accuracy and productivity, this was good news for labs the world over. UPLC is a variant of HPLC, also using columns and pumps. Comment: Whether we are talking about HPLC, UPLC or UHPLC, each of these techniques employs columns and pumps.

Principle:

The basic principle of UPLC for the separation of components in a matrix is same as HPLC, the main difference is in the particle size of sorbent of the column, which is less than 2 µm. The small particles in UPLC require a high pressure (6000 psi) to work with.

This is also based on van deemeter equation which describes the relationship between flow rate and HETP or column efficiency.

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

When,

A - Eddy diffusion

B - Longitudinal diffusion

C - Equilibrium mass transfer

v - Flow rate.

Ultra-high-performance liquid-chromatography (UHPLC) covers liquid chromatography separations implementing columns enclose particles smaller than the 2.5–5 µm sizes typically used in high-performance liquid chromatography (HPLC). UHPLC work on the same assumption as that of HPLC and of which governing principle is that, as column packing particle size decrease, efficiency and thus resolution accretion. Separations using column contain smaller particles display enhance efficiency per unit time, but the efficiency cannot minimize at superior mobile phase flow rates or linear velocities. After attribute, slighter particles, rapidity, and peak resolution can be absolute to new limits.

Instrumentation:

Ultra-high-performance liquid chromatography (UHPLC) encompasses LC separations using columns containing particles smaller than the 2.5–5-µm sizes typically used in HPLC. The benefit of using columns containing smaller particles (typically sub-2 µm) is greater efficiency per unit time.

Fig. UHPLC instrument

Ultrahigh-pressure liquid chromatography (UHPLC) instruments from different manufacturers and instruments with different configurations can produce significant variations in chromatographic separation. The variety in instrument configuration increases the complexity of the method development process, which now requires a more thorough evaluation of the effect of instrument variations on the method. The studies presented here determined the typical inter instrument variations in dwell volume, extra column dispersion, and mixing efficiency as measured by mobile-phase compositional accuracy. Additionally, the dwell volume and extra column dispersion were independently and systematically varied to evaluate the resulting impact on resolution for a small-molecule test mixture during gradient elution. To account for these inter instrument variations, dwell volume and wash-out volume method translation and adjustment techniques were evaluated.

Applications of UHPLC:

1. Analysis of natural products and traditional herbal medicine.
2. Identification of metablomics.
3. Study of metabonomics/metablomics.
4. Bio analysis/bioequivalence studies.
5. Manufacturing/QA/QC.
6. Impurity profiling.
7. Forced degradation studies.
8. Dissolution testing.
9. Toxicity studies.

Advantages of UHPLC:

1. Decreases run time and increases sensitivity.
2. Reducing analysis time so that more product can be produced with existing resources.
3. Provides the selectivity, sensitivity and dynamic range of LC analysis.
4. Maintains resolution performance.
5. Fast resolving power quickly quantifies related and unrelated compounds.
6. Operation cost is reduced.
7. Less solvent consumption.
8. Very fast separations with good resolution.

9. High-resolution separations of complex samples
10. Rapid development of stability-indicating HPLC methods
11. Higher sensitivity and precision performance.

Disadvantages of UHPLC:

1. Due to increased pressure requires **more maintenance** and reduces the life of the columns of this type.
2. In addition, the phases of less than 2um are generally non-re-generable and thus have limited use.

Additional Points:

UHPLC columns - The Acuity UHPLC column involved in the front line of liquid chromatography (LC) column development by giving higher quality chromatographic data's in less time. For use in applying up to 15000 psi (1000 bar), UHPLC columns are designed, certified, and tested [9]. However, different technologies produced distinct nature of columns actuality used in UHPLC is depicted.

Charged surface hybrid - Third-generation particle technology was developed by waters, designed to recover sample load ability and peak tailing in uncertain ionic strength mobile phase composition. The charged surface hybrid retains the low-level surface charge with 1.7 µm particle size. The basic charged surface hybrid (CSH) C18 column characteristics such as peak shape and increased loading capacity, mainly for basic compounds under low-pH, weak-ionic strength mobile-phase.

The poly aromatic compound selective straight-chain-alkyl is especially used in Acquity UHPLC CSH Phenyl-Hexyl column and also it gives exceptional peak shape under different pH conditions. The Acquity UHPLC CSH Fluoro-Phenyl columns show excellent selectivity for polar compounds, positional isomer, and halogenated compounds. This is due to a dipole-dipole, hydrogen-bonding, aromatic, and hydrophobic interaction.

Ethylene-bridged hybrid - The deficiency of mechanical strength or efficacy is essential to complete the potential speed, sensitivity, and resolution capabilities for primary generation methyl hybrid particle of xTerra columns. Hence, there is need of a column with a new pressure-tolerant particle needed to create. An innovative, additional hybrid material column were set that contains an ethylene bridged hybrid material. It displayed enhanced

efficiency, pH range, and strength as compared to first-generation columns. The developed ethylene-bridged hybrid (BEH) columns fixed polar group attached to the silyl functionality with a C6 alkyl and also for UHPLC BEH phenyl columns.

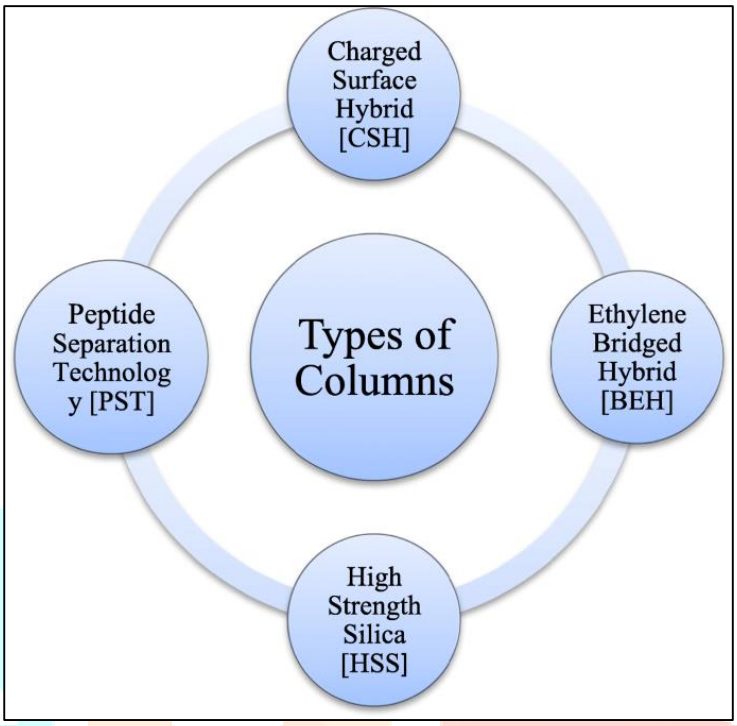

Fig. Types of UHPLC columns

High strength silica- High strength silica (HSS) is another type of column used in UHPLC. In UHPLC, high pore volume UHPLC particles do not acquire the mechanical stability necessary to hold up the high pressure innate of UHPLC separations [9]. For that, there is established a novel silica particle and appropriate morphology required to give long and lifetime efficiency UHPLC column at high pressure likely 1000 bars. HSS particle technology is the modern automation; 1.8 µm UHPLC HSS particles are designed and exclusively for separations using UHPLC. To overcome trouble during separation and retention of small water-soluble and polar organic molecules during reversed phase separation, Acquity UHPLC HSS T3 columns were developed. The Acquity UHPLC HSS C18 selectivity for bases (SB) columns is a non-end capped, low-coverage silica-based C18 chemistry that alternate selectivity for water-soluble compounds influenced by silanophilic interactions. The enhanced silanol activity of the HSS C18 SB column result in greater retention of basic compounds; due to secondary interactions with residual silanols while simultaneously reducing the retention of non-basic analyses due to the low ligand density and ionic repulsion.

Peptide separation technology - The separation or isolation of different peptides, the peptide-based peptide separation technology columns, was utilized for analysis of peptides. Developed peptide separation technology (PST) columns are C18 BEH Technology, in PST column particles sizes in the variety of 1.7 µm to 10 µm and the column dimension ranges from 75 µm to 30 mm internal diameter and column length from 50 to 250 mm. The PST columns demonstrate sharp-edged symmetrical peaks.

HPLC Vs UHPLC:

The UHPLC system is superior to HPLC system because the UHPLC system operates at high pressure up to 1000 bar or more than that, but a conventional HPLC system, compass a pressure up to 400 bars and it suffers problems like mobile phase swallowing and increases the time of analysis. But in UHPLC system, less solvent consumption and less time for analysis are required. This could unlikely outstanding use of slighter particles less than 2.0 mm and also at tolerable flow up to 5 mL/min. The use of the lesser particle size shows better resolution of peaks, perform faster analysis, sharper, and higher peaks.

Differences between HPLC and UHPLC –

These are the top 5 differences between HPLC and UHPLC:

1. Particle sizes – In HPLC particle sizes of the stationary phase are typically in the order of 3-5 µm, whilst UHPLC is characterized by particles of 2 µm or less.
2. Column dimensions – As with particle sizes there is a corresponding reduction in column dimensions with UHPLC. A typical HPLC column has an internal diameter of 4.6 mm and a length of 250 mm, whilst a UHPLC column has internal diameters of 2.1 mm or less and is much shorter, 100 mm for example.
3. Flow rates – UHPLC runs at much lower flow rates than HPLC, for example 0.2 – 0.7 ml/min against 1-2 ml/min respectively.
4. Backpressure – With the smaller particles and reduced column diameter then this manifest itself in to higher backpressures in UHPLC compared to HPLC. HPLC instruments typically operate at maximum pressures of 400-600 bar, whilst UHPLC instruments can operate at up to 1500 bar in the case of the Thermo Scientific™ Vanquish™ Horizon UHPLC System.

5. Detection parameters – Narrow peaks are produced with UHPLC, requiring a detector that can keep pace and provide the required number of data points per peak for detection. Most modern detectors, though, are capable of detection speeds of up to 250 Hz, which is sufficient for both HPLC and UHPLC.

These are the common differences between HPLC and UHPLC, but they can also be broadly separated by application area. HPLC is commonly used in routine environments, whilst UHPLC is more common in research and development, but this is not exclusive.

COMPARISON BETWEEN HPLC AND UHPLC		
Parameters	**HPLC**	**UHPLC**
Column	XTerra,C18,50 × 4.6mm	AQUITY UHPLC BEH C18,50 ×2.1mm
Particle size	4µm particles	1.7µm particles
Flow rate	3.0 ml per min	0.6 ml per min
Injection volume	20 µl	3 µl partial loop fill or 5 µl full loop fill
Total run time	10 min	1.5 min
Theoretical Plate count	2000	7500

Fig. HPLC Vs UHPLC

Why use UHPLC?

As alluded to earlier in the article, there has been a gradual shift to UHPLC from HPLC over the last decade. So why is this? There are a number of reasons:

1. **Speed and throughput –** Smaller columns and particle sizes, low system dispersion, with a pump pressure to match, means that separations can be achieved in a fraction of the time compared to HPLC thus offering a higher sample throughput. This application note provides a nice example of this: the separation took 2.5 minutes using a column with 4.0 µm particles, but when reducing this to 1.5 µm particles the separation was achieved in 1.5 minutes.

2. **Better resolution –** The use of smaller columns and particles also results in better resolved and sharper peaks to give better resolution and peak capacity.
3. **Lower costs –** As the flow rates are smaller and the separations fast, then solvent usage is reduced along with the associated disposal costs. The speed also offers the higher throughput so the instrument investment is fully utilized.

Conclusions:

In light of the benefits discussed in this review, the application of UHPLC in pharmaceutical analysis may be considered a greening pathway for liquid chromatography, which is especially significant for drug analysis in the pharmaceutical matrix. Also, UHPLC may be applied in stability studies, when the required number of determinations is especially high, with the advantage of reducing the amount of organic solvent sand the concentration of analyses.

Ultra-high-performance liquid chromatography appears to have the potential to replace the less environment-friendly analytical techniques provided that methods based on this kind of chromatography have been properly validated. Modifications of UHPLC methods will probably aim at the elimination of friction heating by looking for new solutions in the development of stationary and mobile phases.

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