ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY: A REVOLUTIONIZED LC TECHNIQUE

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REVIEW ARTICLE

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ABSTRACT

High Performance Liquid Chromatography (HPLC) is a major technique for qualitative and quantitative drug analysis. More than 90% of drugs prescribed in official pharmacopoeias are being analyzed HPLC. HPLC analyzes the drug content in a sample with high degree of accuracy and precision. Due to the stringent regulatory requirements the number of samples for drug content analysis has been increased significantly. Therefore, pharmaceutical industries need a fast, accurate and affordable method for drug content analysis. Here, Ultra Performance Liquid Chromatography (U-PLC) offers an advancement of HPLC which is based on the principal of use of stationary phase consisting of particles less than 2µm. By using smaller particles; speed and peak capacity can be extended to new limits and the sample can be analyzes in a shorter period of time. It provides good resolution even for congeneric compounds. The present review discusses the various aspects of UPLC in pharmaceutical analysis.

Keywords: Ultra Performance Liquid Chromatography (UPLC), HPLC, ACQUITY UPLC.

INTRODUCTION

High performance liquid chromatography (HPLC) is a major technique for qualitative and quantitative drug analysis in pharmaceutical laboratories worldwide. The underlying principles of this technique governed by the van Deemter equation. (1) The van Deemter equation is an empirical formula that describesthe relationship between linear velocity (flow rate) and plate height (HETP, or column efficiency). Since particle size is one of the variables, a van Deemter curve can be used to investigate chromatographic performance for wide range of organic compounds.

For many years, researchers have looked at "fast LC" as a way to speed up analyses. (2,3) The "need for speed" has been driven by the sheer numbers of samples in drug laboratories. Therefore, pharmaceutical industries need a fast, accurate and affordable method for drug content analysis. Ultra Performance Liquid Chromatography (U-PLC) is an advance version of HPLC which is based on the principal of use of stationary phase consisting of particles less than 2µm. Using smaller particles and therefore larger surface area, the high speed and greater resolution is achieved in UPLC. It provides good resolution even for congeneric compounds. This review traces some of the developments and technological advancements made in the first commercially available UPLC. (4)

PRINCIPLE

The UPLC is based on the principal of use of stationary phase consisting of particles less than 2 µm. The conventional HPLC columns are typically filled with particles of 3 to 5 µm. The underlying principles of this evolution are 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).(5) The van Deemter curve, governed by an equation with three components shows that the usable flow range for a good efficiency with a small diameter particle is much greater than for larger diameters.(6,7)

$H = A + B/v + Cv$

In the above equation v is the linear velocity, the carrier gas flow rate. A term is independent

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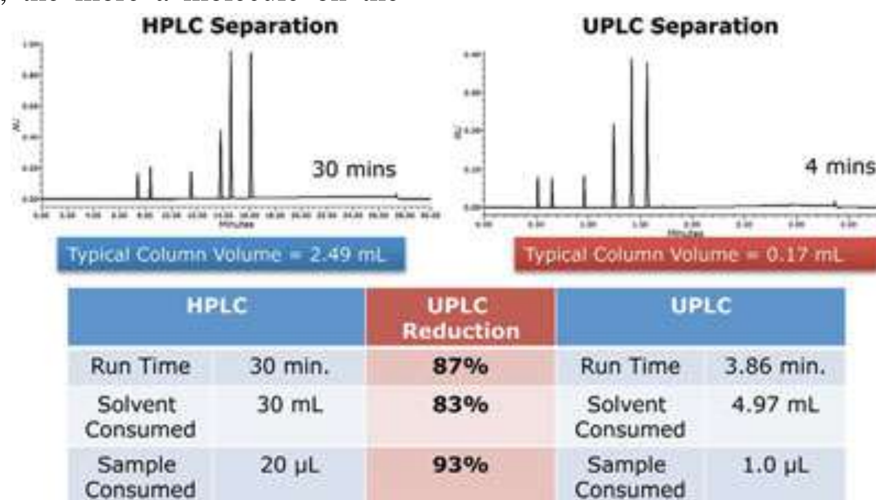
of velocity and represents "eddy" mixing. (Value of A is minimum when the packed column particles are small and uniform).

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*).

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 gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus this term is proportional to *v*.

Therefore it is possible to increase throughput, and thus the speed of analysis without affecting the chromatographic performance. Efficiency is proportional to column length and inversely proportional to the particle size. (8) Therefore, the column can be shortened by the same factor as the particle size without loss of resolution. The application of UPLC resulted in the detection of additional drug metabolites, superior separation and improved spectral quality. (9,10)



HPLC		UPLC Reduction	UPLC	
Run Time	30 min.	87%	Run Time	3.86 min.
Solvent Consumed	30 mL	83%	Solvent Consumed	4.97 mL
Sample Consumed	20 µL	93%	Sample Consumed	1.0 µL

Figure 1: Comparison-HPLC vs. UPLC

INSTRUMENTATION

1. Pump:

The most important advantages of pumps are: higher resolution, faster analyses, and increased sample load capacity.Pump Module – types

- Isocratic pump - delivers constant mobile phasecomposition; solvent must be pre-mixed
- Gradient pump - delivers variable mobile phase composition;

2. Solvent system:

A. Mobile phases-several common properties

a. Purity
b. Detector compatibility
c. Chemical inertness

B. Mobile phase reservoir

The most common type of solvent reservoir is a glass bottle. Most of the manufacturers supply these bottles with special caps, Teflon tubing and filters to connect to the pump inlet and to the purge gas (helium) used to remove dissolved air.

3. Sample injector:

In UPLC, sample introduction is critical. Conventional injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from extreme pressure fluctuations, the injection process must be relatively pulse-free and the swept volume of the device also needs to be minimal to reduce potential band spreading. A fast injection cycle time is needed to fully capitalize on the speed afforded by UPLC, which in turn requires a high sample capacity.

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Low volume injections with minimal carryover are also required to increase sensitivity. (11)

4. Column:

Resolution is increased in a 1.7 μm particle packed column because efficiency is better. Separation of the components of a sample requires a bonded phase that provides both retention and selectivity. Four bonded phases are available for UPLC separations: ACQUITY UPLC™ BEH C_{18} and C_8 (straight chain alkyl columns), ACQUITY UPLC BEH Shield RP_{18} (embedded polar group column) and ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C_6 alkyl). (12) Each column chemistry provides a different combination of hydrophobicity, silanol activity, hydrolytic stability and chemical interaction with analytes.

ACQUITY UPLC BEH C_{18} and C_8 columns are considered the universal columns of choice for most UPLC separations by providing the widest pH range. They incorporate trifunctional ligand bonding chemistries which produce superior low pH stability. This low pH stability is combined with the high pH stability of the 1.7 μm BEH particle to deliver the widest usable pH operating range. ACQUITY UPLC BEH Shield RP_{18} columns are designed to provide selectivities that complement the ACQUITY UPLC BEH C_{18} and C_8 phases. ACQUITY UPLC BEH Phenyl columns utilize a trifunctional C_6 alkyl tether between the phenyl ring and the silyl functionality. This ligand, combined with the same proprietary endcapping processes as the ACQUITY UPLC BEH C_{18} and C_8 columns, provides long column lifetimes and excellent peak shape. This unique combination of ligand and endcapping on the 1.7 μm BEH particle creates a new dimension in selectivity allowing a quick match to the existing HPLC column. (13)

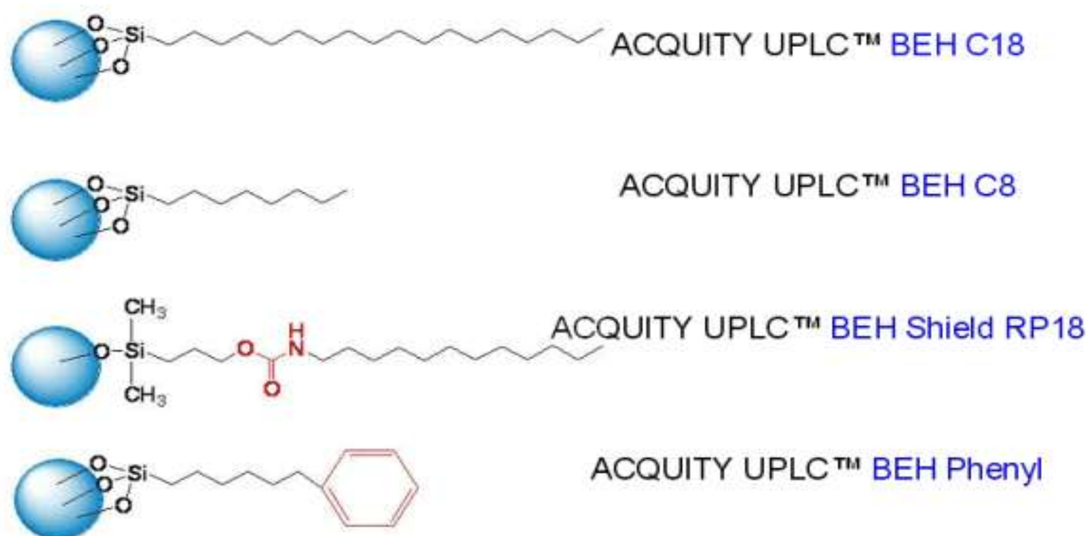

Figure 2: Columns used in UPLC

5. Detector:

For UPLC detection, the tunable UV/Visible detector is used which includes new electronics and firmware to support Ethernet communications at the high data rates. Conventional absorbance-based optical detectors are concentration sensitive detectors, and for UPLC use, the flow cell volume would have to be reduced in standard UV/Visible detectors to maintain concentration and signal. According to Beer's Law, smaller volume conventional flow cells would also reduce the path length upon which the signal strength depends. A reduction in cross-section means the light path is reduced, and transmission drops with increasing noise. Therefore, if a conventional HPLC flow cell were used, UPLC sensitivity would be compromised. The ACQUITY Tunable UV/Visible detector cell consists of a light guided flow cell equivalent to an optical fibre. Light is efficiently transferred

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down the flow cell in an internal reflectance mode that still maintains a 10mm flow cell path length with a volume of only 500mL. Tubing and connections in the system are efficiently routed to maintain low dispersion and to take advantage of leak detectors that interact with the software to alert the user to potential problems. (14)

ADVANTAGES

- Decreases run time and increases sensitivity
- Provides the selectivity, sensitivity, and dynamic range of LC analysis
- Maintaining resolution performance.
- Expands scope of Multi-residue 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
- 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 re-work material. (15,10)
- Delivers real-time analysis in step with manufacturing processes
- Assures end-product quality, including final release testing

DISADVANTAGES

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 thus have limited use. (16,17)

APPLICATION

- **Separation of multicomponent drugs**

For separation of ant tubercular drugs-Isoniazid (ISN), Pyrazinamide (PYR) and Rifampicin (RIF) were separated. Less than 2 min was necessary for the complete separation of antituberculosis drugs, while the original USP method was performed in 15 min. A large number of samples per day can be analyzed due to the short analysis times.

- **Analysis of drugs in human plasma**

For Analysis of Levofloxacin in human plasma

- Internal standard used – Niacin
- Column - BEH C18
- Mobile Phase –acetonitrile
- Buffer - 0.4% triethylamine buffer(pH3)
- Pressure – 11000 psi
- Flow rate – 0.3 mL/min

- **Identification of Metabolite**

Biotransformation of new chemical entities (NCE) is necessary for drug discovery. When a compound reaches the trial stage, metabolite identification is required and it is necessary for lab to successfully detect and identify all circulating metabolites of a contender drug.

- **In Bioanalysis / Bioequivalence Studies**

For Pharmacokinetic, Bioequivalence and toxicity studies, the quantitative analysis of a drug in biological samples is an important part of drug development process and this is carried out by UPLC**.** The drugs are generally of low molecular weight and are tested during both preclinical and clinical studies. Several biological matrices are used for quantitative bioanalysis, the most common being blood, plasma, and urine. (18)

- **In stressed degradation Studies**

The most common analytical technique for monitoring forced degradation experiments is HPLC with UV and/or MS detection for peak purity, mass balance, and identification of degradation products but these HPLC-based methodologies are time-consuming and provide only medium resolutionto ensure that all of the degradation products are accurately detected. PDA/MS (photodiode array and MS) used along with UPLC, which allows for faster and higher peak capacity separations of complex degradation product profiles.

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- **UPLC used in Impurity Profiling**

  UPLC PDA detector involves two analytical flow cells with maximum flexibility and according to application requirements, as one for maximum chromatographic resolution and a second for high sensitivity. UPLC also ensure the latest peak detection algorithms and custom calculations to optimize data processing and reporting. It also assertively detects impurities in compounds even at trace levels. To characterize impurities, it is often necessary to perform several analytical runs to obtain the necessary MS and MS/MS data.

CONCLUSION

UPLC increases productivity in both chemistry and instrumentation by providing more information per unit of work as it gives increased resolution, speed, and sensitivity for liquid chromatography. Now a day's various pharmaceutical companies are going to use HPLC and UPLC as separation techniques to increase the marketing needs. The new technology in chemistry and instrumentation provides more information per unit of work as UPLC begins to fulfill the promise of increased speed, resolution and sensitivity predicted for liquid chromatography.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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