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Step-up in liquid chromatography from HPLC to UPLC: A comparative and comprehensive review

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Abstract
Liquid chromatography (LC) is a powerful tool in separation science employed for clinical research as well as for therapeutic drug monitoring. It is the most widely applied technique to identify, quantify and purify the components from a mixture in which the separation process is based upon the rate of elution of components through a stationary phase typically over a mobile phase gradient using high pressures (600–6000 psi) to push the mobile phase solvents through a stationary phase column. The rate of elution depends on the affinity of the mixture components to the packing material of utilized stationary phase. A purely new system design with advanced technology has been developed, called ultra-high performance liquid chromatography (UPLC), which has been evolved from high performance liquid chromatography (HPLC). The advantages including shorter analysis time, method sensitivity, repeatability and drug specificity justify the wide use of LC techniques in almost every analytical and bio-analytical laboratory. This review focuses on the invention of new LC technique, comparative performance of UPLC and HPLC, and summarizes their application on method development and validation in pharmaceutical analysis over the last few years

Keywords: Ultra performance liquid chromatography, eddy diffusion, longitudinal diffusion, kinetic resistance, hyphenated technique, proteomics

1. Introduction
Liquid chromatography techniques have been widely used for the fast, accurate, sensitive and selective determination of trace amounts of pharmaceutical active compounds in biological samples and their dosage forms. HPLC and UPLC are the commonly used separation techniques, both of which employ the basic principles [1] of liquid chromatography but the former has been around since the 1960s [2] whereas the later one, first appeared in 2004 [3] when Waters® created their own UPLC™ systems [4]. Both can be applied for the various modes of LC, including the normal and reverse phases, and can be paired with mass spectrometry systems for better results, but the instrumentation needed and performance delivered differs significantly in both the systems.
In the LC separation technique, the underline principle is governed by van-Demeter equation [1] in which the relationship between linear velocity (flow rate) and the plate height (HETP or column efficiency) is expressed.

$$HETP = A + \frac{B}{\mu} + C\mu$$

Where, A, B and C are constants and μ is linear velocity or flow rate of mobile phase.

- The term A represents "Eddy diffusion" which is independent of flow of mobile phase. It is smallest when the column particles are small and uniform packed.
- The term B represents "Longitudinal diffusion" of molecules, and is diminished at high flow rates and so it is divided by μ.
- The term C is due to "kinetic resistance" to equilibrium in the separation process between the stationary and mobile phase. The greater the flow of mobile phase, the more an analyte on the packing material tends to lag behind the analytes molecules in the mobile phase. Thus, it is proportional to μ.

As per the above equation and various factors contributing to LC separation, it can be stated that the particle size of column packing materials is inversely proportional to the efficiency of chromatographic process. Hence, smaller particle size of packing materials can be used for gaining efficiency by considerably reducing HETP as illustrated by Fig. 1 which depicts the reduction in particles size results in significant fast analysis with resolution and peak capacity

(number of peaks resolved per unit time) to the maximum values and these values are much better than the values achieved earlier by HPLC. Amongst other parameters effecting LC separations, smaller columns, faster flow rates and elevated temperature [5] were also investigated with accuracy as a way to speed up analysis.

The results obtained from these "need for speed" investigations [6, 7] extended the limitations that were created by the particle size and pressure conventionally employed in HPLC and consequently, helped in creation of fast LC technique, known as UPLC™.

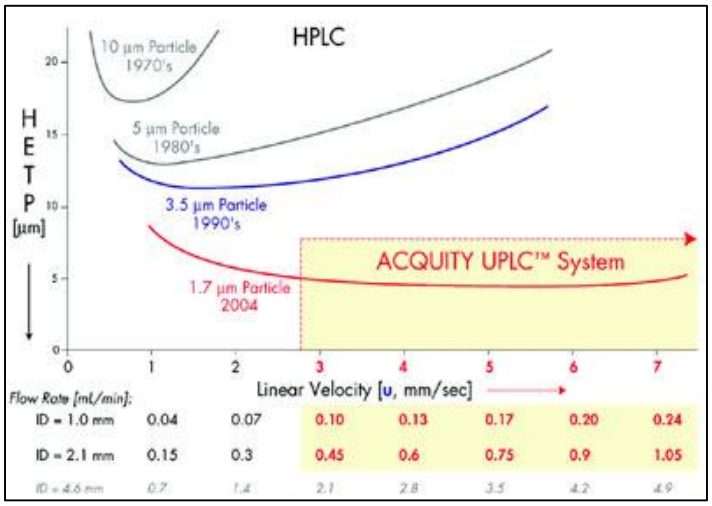

Fig 1: van-Demeter plot, illustrating the evolution of particle sizes over the last three decades

Principles of UPLC and HPLC

The basic principle of UPLC and HPLC is same and depends upon mode of separation [8] (Tab. 1), i.e., adsorption, partition, exclusion, and ion exchange, depending on the type of chromatographic sorbent.

Table 1: Main Modes of Liquid Chromatography

Types of compounds	Mode	Stationary phase	Mobile Phase
Neutral (weak acids and bases)	*Reverse phase*	C_{18}, C_8, C_4, Cyano, Amino	Aqueous or organic modifiers
Ionics, Bases, Acids	*Ion pair*	C_{18}, C_8	Aqueous or organic ion-pair reagent
Compounds insoluble in water	*Normal phase*	Silica, Amino, Cyano, Diol	Organics
Ionics or inorganic ions	*Ion exchange*	Anion or Cation exchange resin	Aqueous buffer with counter ion
High Molecular Weight Compounds, Polymers	*Size exclusion*	Polystyrene Silica	Gel Filtration Or Permeation

2. Step-up from HPLC to UPLC

a. Chemistry of Small Particles: The assumptions made on the basis of van-Demeter equation [1] cannot be fulfilled without smaller particles than those conventionally used in HPLC. The use of smaller particles provides better resolution, increased efficiency, and sensitivity due to sharper and higher peaks, and also faster analysis [9, 10] The evolution of packing materials which effect the separations was a primary cause of its development. The UPLC technique was made on the principle of using a columns consisting of particles of sub-2 µm while HPLC columns are generally filled with 3–5 µm particles. UPLC benefits from ''power of the chemistry of small particles''[11] But to maintain retention and capacity similar to HPLC, UPLC must use porous particles that can withstand high pressures; though being highly efficient, these sub-2 µm (non-porus) particles suffer from poor loading capacity and retention due to low surface area. The pores of the particles should have the proper size for the analyte [12] the easiest way to decrease plate height and analysis time is to decrease column packing particle size. This also results in the decrease of the Eddy diffusion and longitudinal diffusion coefficients hence, decreasing column packing size causes improvements in separation efficiency per unit column length and an increase in flow rate; thus, resolution also gets increased. The columns were redesigned with a uniform interior surface and smaller diameter to retain the small particles and finally, minimize effects of frictional heating and resist clogging [13-16].

b. Instrumentation Technology: To capitalize on small particle chemistry, instrument technology also should keep pace complementary with it to achieve high peak capacity UPLC separations. With the use of small particles for packing in UPLC columns, a greater pressure range than in HPLC columns was required [10]. Since, back pressure is proportional to flow rate, smaller particle causes extreme operating pressures, requiring a system that is designed well for resistance to high pressures. HPLC columns can reach a maximum pressure of 600 bar whereas UPLC systems can reach 1,200 bar and more [17]. From resolution related equations, it was found that nearly a nine-fold increase in pressure should be involved to achieve three times higher separation efficiency. As efficiency is proportional to column length and inversely proportional to particle size, UPLC combines the advantages of shorter column length, small packing material and an improved pump system. The sensitivity is also increased due to band spreading, which occurs during migration through a column with smaller particles [18].

c. Sample Introduction and Detection: Sample introduction to the chromatographic system is also critical. As common

HPLC injection valves are not capable to work at high pressures, an improved pump than can deliver the solvent smoothly and reproducibly are required. Also the injection process should be relatively pulse free to protect the column from extreme pressure fluctuations. The sample volume should also be small to reduce the band spreading [1, 19]. Sample volumes are typically 2–5 µL in UPLC systems, while they are 10–50 µL in HPLC systems. A fast injection cycle with a high sample capacity is required, with low volume injection that results in minimum carryover problems with increased sensitivity [3]. Automatic loop-style injectors have the capability to deliver very small injection volumes at pressures of 30,000–40,000 psi [20, 21].

In order to make analysis one step efficient, the detector cell should have minimal dispersion volume and have a high sampling rate for accurate, sensitive and reproducible detection of the analyte peaks. Depending on the detector type, sensitivity of UPLC can increases 2–3 times higher than HPLC [22]. Absorbance-based optical detectors, UV/Visible, florimetric and mass spectroscopy (MS) detectors, etc. are generally coupled with LC.

3. Comparison between UPLC and HPLC

Estimation of compounds by HPLC and UPLC analysis have been conducted and compared by various scientists [23]. Significant advantages of UPLC are that the speed, resolution, and sensitivity are improved. The main advantage is reduction of analysis time, which also means reduced solvent consumption. The time needed for column equilibration while using gradient elution and during method validation is also much shorter. In consequence, UPLC is cheaper than HPLC because a higher number of analyses per unit of time can be performed and consumption of eluent is much lower.

Why HPLC methods to UPLC?

- Provides faster results with better improved resolution, speed and sensitivity with no compromises
- Increased productivity: More sample analysis per system per scientist
- More accurate, sensitive and robust methods
- More quality data per unit time

Table 2: Comparison of HPLC and UPLC techniques

Characteristics	HPLC	UPLC
Particle size	3–5 µm	Sub-2 µm
Max.back pressure	35–40 MPa	103.5 MPa
Analytical column (Dimensions)	Agilent Eclipse xbd C18 (150 x 4.6 mm, 5 µm)	Acquity UPLC BEH Shield RP18 (2.1 x 50 mm, 1.7 µm)
Column temperature	25 °C	65 °C
Injection volume	10–20 µL	1–5 µL
Disadvantages	Lack of efficiency, Low diffusion coefficients	Expensive instrument, Reduced column life due to high back pressure

Ting Wu *et al.* [24] worked on developing new analytical methods for separation of 12 phthalates and the results were compared with those obtained by UPLC and HPLC. The mobile phase was a gradient prepared from methanol and water. UV detection was performed at 225 nm using PDA detector.

UPLC separation was performed with a Waters UPLC using Acquity UPLC BEH phenyl column (50 x 2.1 mm, 1.7 µm). The total run time was of 7 min with flow rate of 0.4 mL/min. While the HPLC analysis was performed with an Agilent 1100 instrument using Agilent SB-phenyl column (250 x 4.6 mm, 5µm). The total run time was of 18 min with flow rate was of 1.0 mL/min.

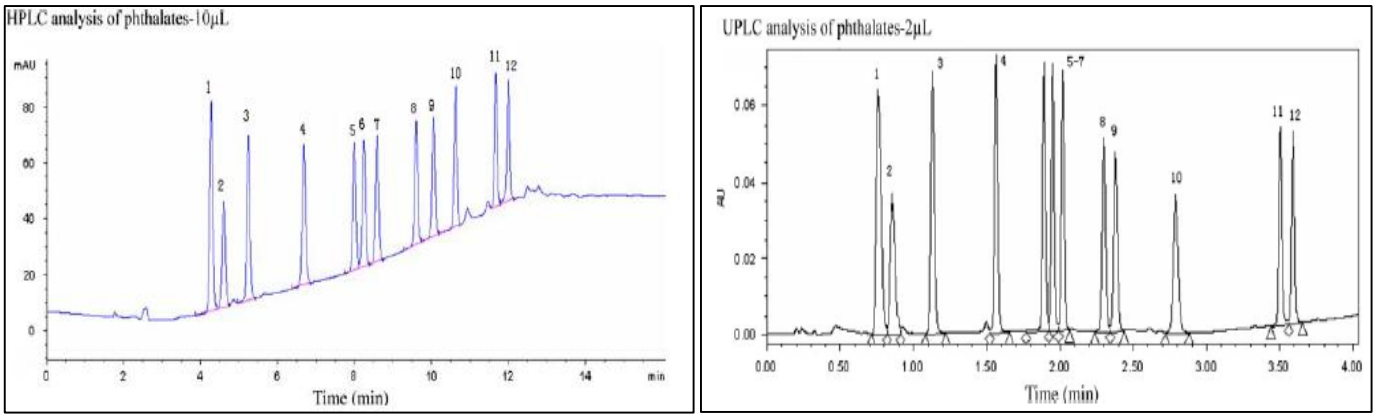

Fig 2: Comparison of HPLC and UPLC analysis of 12 phthalates

The study (Fig. 2) showed analysis time was reduced by a factor of 2.5 and solvent consumption by a factor of 6.4 in case of UPLC as compared to HPLC. Analysis time, solvent consumption, and analysis cost are very important in many analytical laboratories. The time spent in optimizing new methods was also greatly reduced.

Inga Kleczka *et al.* [25] developed and compared UPLC and HPLC methods for determination of ascorbic acid (AA) in fruit beverages and in pharmaceutical preparations. Both methods were rapid, total time of analysis was 15 and 6 min for HPLC and UPLC methods, respectively. The methods were also validated in terms of linearity, precision, LOD and LOQ, accuracy and recovery.

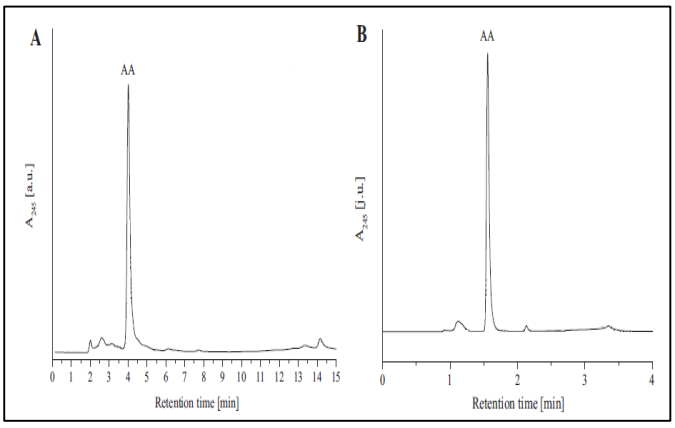

Fig 3: HPLC (A) and UPLC (B) chromatograms of AA separated from fruit juice and pharmaceutical preparation

The study (Fig. 3*)* pointed UPLC method was faster, more sensitive, consumed less eluent and it more eco-friendly than the conventional HPLC method.

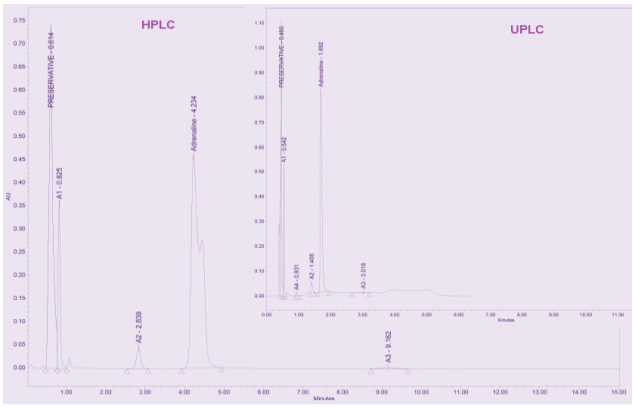

Fig 4: Comparison of BP HPLC method with proposed UPLC method for Adrenaline assay

Thurin Nicolas *et al*. [26] developed and validated an UPLC method for determination of related compounds in Adrenaline Injection which was believed superior in separation and performance than the British Pharmacopoeia HPLC method which is validated only for the determination of noradrenaline.

The proposed UPLC method was shown to provide better separation than HPLC methods *(Fig. 4)*, while reducing the run time from 15 min to 6.5 min. The method can be easily applied to quantify related compounds and degradents in adrenaline injection for final release and stability studies.

4. Applications

Application of LC methods with different coupled detectors proved to be useful for the investigation of complex media, leading to a better knowledge of the drug's Pharmacol-kinetic profile involving study of pathways of adsorption, distribution, and bio-transformation, elimination of both active and non-active metabolites and bio-availability in the system [27-29].

In pharmaceutical companies and bio-analytical laboratories, there is significant utilization of UPLC technique, because it generates valuable, reliable and precise data within short period of analysis. It is also used for developing accurate and robust methods for estimation of drugs. The UPLC/MS hyphenated technique *(Fig. 5)* has gained a widespread acceptance as an analytical tool for the qualitative and quantitative analysis of many types of compounds and their related impurities, as MS detection is significantly enhanced by UPLC [30] increased peak concentrations with reduced chromatographic dispersion at lower flow rates promotes increased source ionization efficiencies, which is particularly important for the proteomics studies. The increased resolving power made the resulting 2-dimensional data easier to interpret, since more of the MS peaks consisted of a single compound, and up to a 20-times improvement in the quality of the spectral information (vs. nano-electrospray) was obtained. Lee *et al*. [31] pointed out that, in some cases, structurally related compounds with the similar molecular masses could not be differentiated by MS, necessitating chromatographic separation on the UPLC. UPLC/MS/MS, which is a newer technique, providing 3-dimensional data covering the retention time, MS peaks and fragmentation patterns of the analytes of interest can be employed as a powerful tool for differential metabolic pathway profiling.

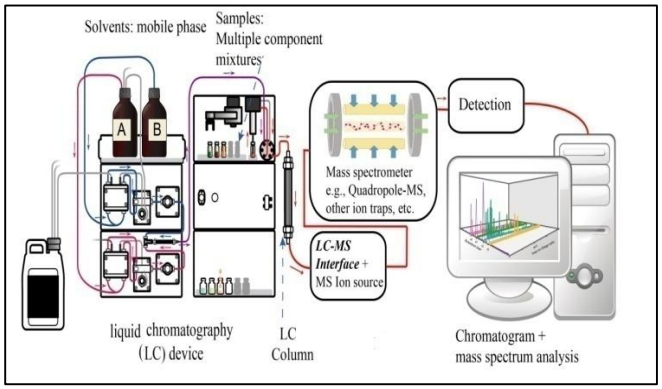

Fig 5: Diagram of liquid chromatography tandem mass spectrometry

UPLC technology (coupled as hyphenated technique) [32-34] is now a well-established and fast-growing area with a number of possible applications in the analysis of pharmaceutical and biological compounds, some of which are listed below:

1. Identification, separation and quantitative estimation of therapeutically active constituents from natural extracts and medicine of herbal origin.
2. Dissolution Testing: For quality control purposes and to demonstrate batch-to-batch uniformity of the active ingredient(s) in formulation and production processes.
3. Impurity Profiling: Detection and quantification of drug related impurities in both raw materials and final product.
4. Proteomics studies: Analysis of peptide and protein drugs.
5. Metabolomics studies: Analysis of drugs and its metabolic products in biological fluids, e.g., human plasma, urine, etc. for bio-transformation pathway profiling.
6. Pharmaco-metabonomic and related studies: Measurement of the metabolic response of the living systems to genetic modification or pathophysiological stimuli.

Because of the various advantages, easy applicability and the availability of low-cost instruments, HPLC and UPLC are the most applied methods of choice for the analysis of drugs in pharmaceutical dosage forms and body fluids.

5. Conclusions

UPLC have proved to expand the utility of separation science when the conventional HPLC have almost reached separation

barriers. Since most pharmaceutical companies try to reduce the R&D timings and cost, a faster and better UPLC separation can decrease the time and other resources for method development in analytical laboratories. The advent of UPLC has evolved *new fine particle chemistry* and *instrumentation technology* for liquid chromatography with an increase in throughput, and thus, the speed of analysis, without any decrease in chromatographic performance. With the use of smaller particles in column packing, speed and peak capacity are extended to new limits. Using the UPLC technique, it is possible to run higher resolution methods, using shorter columns, smaller size of packing particles, with higher flow rates under high pressure. It is quite obvious that increasing the peak capacity improves the quality of the data. A significant decrease in solvent consumption and column re-equilibration time is also achieved while working on UPLC systems. Injection volume is drastically reduced to nearly five-ten times in UPLC than all previously used chromatographic techniques, resulting in a better peak band and lower carryover effects related to the column diameter. The high price of the instrument is the main disadvantage of UPLC. Moreover, the increased back pressure reduces the column life. But the advantages of UPLC, such as low solvent consumption, faster separation with better selectivity and sensitivity overcome its disadvantages. It increases productivity by providing more information per unit of work, with increased resolution and speed of LC analyses.

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