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Review Article

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ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY: A RECENT NOVEL DEVELOPMENT IN HPLC

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ABSTRACT

Ultra Performance Liquid Chromatography (UPLC) is a relatively new technique giving new possibilities in liquid chromatography, especially concerning decrease of time and solvent consumption. UPLC chromatographic system is designed in a special way to withstand high system back-pressures. Special analytical columns UPLC BEH C_{18} packed with 1.7 µm particles are used in connection with this system. The quality control analyses of various pharmaceutical formulations are transferred from HPLC to UPLC system. The UPLC system allows shortening analysis time up to nine times and three times comparing to the conventional system using 5 µm and 3 µm particle packed analytical columns respectively. The negative effect of particle size decrease is back-pressure increase about nine times (versus 5 µm) or three times (versus 3 µm), respectively. The separation on UPLC is performed under very high pressures (up to 100 MPa) but it has no negative influence on analytical column or other components of chromatographic system. Separation efficiency remains maintained or is even improved by UPLC.

Keywords: UPLC, High separation efficiency, Cost effective, Pharmaceutical analysis, High pressure.

INTRODUCTION

Ultra performance liquid chromatography systems take advantage of technological pace in particle chemistry performance, system optimization, detector design and data processing. When taken together, these achievements have created an improvement in chromatographic performance. UPLC retains the practicality and principles of HPLC and along with that increases the overall interrelated attributes of speed, sensitivity and resolution. Speed allows a greater number of analyses to be performed in a shorter amount of time thereby increasing sample throughput and lab productivity. Faster analysis and hence called as ultra performance liquid chromatography, achieves both higher sample analysis throughput and better assay sensitivity. Analysis of operation cost and sample throughput UPLC cost advantageous over HPLC.[1]

The factor responsible for the development of UPLC technique was evolution of packing materials used to effect the separation. The principles behind this evolution are governed by the van Dee meter equation that describes the relationship between linear velocity and plate height. According to the van Dee meter equation, decrease in particle size increases the efficiency of separations while on other hand efficiency diminishes on increased flow rates or linear velocities. At a particle size less than 2.5 mm, there is a significant gain in efficiency and the efficiency does not diminish at increased flow rates or linear velocities. By using smaller particles, speed and peak capacity can be extended to new limits, termed ultra performance liquid chromatography (UPLC).

This technology takes full advantage of chromatographic principles to run separations using columns packed with smaller particles and/or higher flow rates for increased speed, with superior resolution and sensitivity.[1] The use of non-porous particles, however, has been limited in the pharmaceutical industry due to their low sample loading capacity. The Milford, Massachusetts based company Waters Corporation introduced ACQUITY UPLC, the commercially available system that addresses the challenge of using elevated pressure and 2 mm particles, which makes it a particularly attractive and promising tool for fast Liquid Chromatographic method development. [2]Engineering challenges of operating at high pressures and the high performance expected from such columns necessitates new developed pumps, redesigned injector, reduced system volumes, an increased detector sampling rate, and other improvements. To be suitable for the analysis of pharmaceutical development samples under GMPs, the UPLC instrument and columns must not only deliver on its promises for fast, high resolution separations but do so reproducibly and with the required sensitivity.[2] In addition to the speed at which the data can be obtained, the quality of the data is also improved. It is clear that the quality of the UPLC-MS spectra is better than that of the Capillary LC-MS spectra with much improved signal-to-noise ratio.[3] This new category of analytical separation science retains the practicality and principles

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of HPLC while increasing the overall interrelated attributes of speed, sensitivity and resolution. Today's pharmaceutical industries are looking for new ways to cut cost and shorten time for development of drugs while at the same time improving the quality of their products and analytical laboratories are not exception in this trend. These are the benefits of faster analysis and hence the ultra performance liquid chromatography. A typical assay was transferred and optimized for UPLC system to achieve both higher sample analysis throughput and better assay sensitivity (Table **1**).

UPLC presents the possibility to extend and expand the utility of conventional HPLC, a widely used separation science. The ACQUITY UPLC System is the first instrument of its type to incorporate Intelligent Device Management technology (Figure **1**).

Figure 1. Chromatograms of diclofenac emulgel analysis[3] – Acquity UPLC 1.7 mm, 100 mm

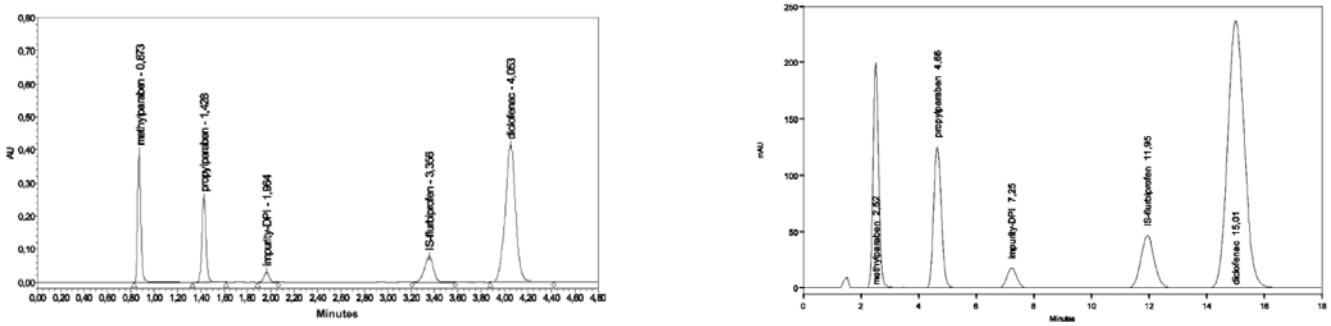

Table1. Original HPLC verses optimized UPLC parameters.

Parameters	HPLC Assay	UPLC Assay
Column	Xterra, C18, 50 x 4.6 mm, 4 µm particles	AQUITY UPLC BEH C18, 50 x 2.1 mm, 1.7 µm particles
Flow rate	3.0 ml / min	0.6 ml / min
Needle wash	Methanol	Methanol
Injection volume	20 µl	3 µl partial loop fill OR 5 µl full loop fill.
Gradient (time in min) ACN:H2O	T0 (25:75), T6.5 (25:75), T7.5 (95:5), T9 (25:75), T10 (25:75)	T0 (36:64), T1.1 (95:5), T1.3 (36:64)
Total run time	10min	1.5min
Total solvent consumption (including 0.5 min of delay time in between injections)	Acetonitrile: 10.5 ml, Water: 21.0 ml	Acetonitrile: 0.53 ml, Water: 0.66 ml
Plate count	2000	7500
USP resolution	3.2	3.4
Lower limit of quantization (LOQ)	~ 0.2 µg/ml	~ 0.054 µg/ml
Delay volume	~ 720 µl	~ 110 µl

ADVANTAGES OF UPLC

1. The UPLC system allows shortening analysis time up to nine times comparing to the conventional system using 5 µm particle packed analytical columns.
2. Separation on UPLC is performed under very high pressures up to 100 MPa.
3. It gives increased peak capacity(number of peaks resolved per unit time) and resolution
4. UPLC dramatically improves the quality of the data, resulting in a more definitive map.
5. UPLC fulfills the promise of increased speed, resolution, sensitivity and broad range of selectivity predicted for liquid chromatography. [4,5]

APPLICATIONS OF UPLC

Drug Discovery: UPLC improves the drug discovery process by means of high throughput screening, combinational chemistry, high throughput in vitro screening to determine physiochemical and drug's pharmacokinetics.

High throughput quantitative analysis: UPLC coupled with time of flight mass spectroscopy give the metabolic stability assay.

Analysis of Dosage form: It provides high speed, accuracy and reproducible results for isocratic and gradient analysis of drugs and their related substance. Thus method development time decrease.

Analysis of amino acids: UPLC used from accurate, reliable and reproducible analysis of amino acids in the areas of protein characterizations, cell culture monitoring and the nutritional analysis of foods.

Determination of Pesticides: UPLC couples with triple Quadra-pole tandem mass spectroscopy will help in identification of trace level of pesticides from water.

Ultra Performance Liquid Chromatograph (UPLC) fingerprint can be used for the identification of *Magnoliae officinalis* cortex.[6]

CONCLUSION

New materials and smaller particles are now available which give improved separations, mostly following expected trends. For UPLC, some reduction in sample size, significantly show reductions in flow rate. As we go smaller we need matching of particle size, chemistry, analytes, and separation method. Alternate materials/coatings are possible in smaller format e.g. proteins, porous monoliths, chiral materials, nanoparticles. Thus Ultra Pressure Liquid Chromatography set a new standard in the science of chromatography. Working range with 15000 to 16000 psi pressure and column packed with less than 2 micrometer in size helped in various fields. This system is not only useful because of these properties but it also reduces the

noise and improve signal-to-noise ratio. Due to very narrow and sharp peaks, more number of peaks may appear in less time which may facilitate in analysis of complex mixtures and it may give more information regarding sample to be analyzed. Very low dead volume in the system (due to smaller column, narrower internal diameter etc.) makes this an excellent HPLC system with significant advantage over conventional HPLC system of having high pressure capacity. Hence use of such UPLC systems will become the option of choice for the development of fast LC methods in pharmaceutical development in the near future.

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