Comparison of UPLC with UFLC: Liquid Chromatography

Brahma Reddy Gangadasu*, Nagarjuna Reddy G., Dhanalakshmi K.
Department of pharmaceutical analysis, KLR Pharmacy college, New Paloncha, Khammam Dist, Telangana, India.
***Corresponding author's E-mail:** gbramhareddy.1989@gmail.com

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ABSTRACT

The pharmaceutical companies today are driven to create novel and more efficient to discover, develop, deliver and monitor the drugs and the development of rapid chromatographic method is crucial for analytical laboratories. Now a day's substantial technological advances have been done in enhancing particle chemistry performance, improving detector design, in optimizing the system, data processors and various controls of chromatographic techniques. By using all these together, resulted in the outstanding performance via ultra fast liquid chromatography (UFLC). It shows a great enhancement in speed, resolution as well as the sensitivity of analysis by using particle size 2.2µm and the system is designed in a special way to withstand low system back pressures (30mpa) but it has no negative influence on analytical column or other components of chromatographic system and also decrease of time and solvent consumption compare to uplc. This review focuses on the basic principle, instrumentation of UFLC and its advantages over UPLC furthermore, this article emphasizes various pharmaceutical applications of this technique and comparative analytical studies of various drugs in UFLC from UPLC.

Keywords: Uplc, Uflc, shim-pack XR columns

INTRODUCTION

Ultra-performance liquid chromatography (UPLC)[1] is a well known technique that has been used in laboratories from last 10 years. The factor responsible for the development of the technique was evolution of packing materials used to effect the separation.

Ultra fast liquid chromatography (UFLC) has marked a radical change by opening a new door for analysis to fetch rapid analytical separation techniques without sacrificing high-quality result obtained earlier by ultra performance liquid chromatography (UPLC).

The immaculate separation method has many advantages like robustness, easy to use change in sensitivity and selectivity.

UFLC is the derivative of UPLC. UFLC is ten times faster and three times better separation than UPLC. That's what prominence UFLC offers outstanding speed and separation even at normal pressure levels (35mpa).

Ultra fast liquid chromatography (UFLC)[2]. The prominence UFLC series has ultra-high-speed LC that achieves both ultra-high-speed analysis and ultra-high separation, based on high analysis precision and reliability.

In addition to shortening analysis times, thereby heightening analysis efficiency and conserving solvent, this instrument supports reliable separation and detection of trace materials in a variety of fields.

Applications include the evaluation of trace residual agricultural chemicals to ensure the safety of foods, and the evaluation to trace impurities to further improve product quality in the areas of pharmacology and chemistry.

Figure 1: Prominence UFLC system

Ultra Fast-Amazing Speed and High Separation Performance[3] in pursuit of higher speed and uncompromised separation

Ten times higher speed and three times better separation. That's what prominence UFLC offers outstanding speed and separation even at normal pressure levels, objectives were difficult for conventional systems.

By maximizing the performance of the column and the entire system, prominence UFLC minimizes deviation from the van Deemter theory.

Analyzing at even higher speeds

Experience speeds not attainable from the van Deemter theory with a stunningly auto sampler capable of injecting samples in only 10 seconds, or with a variety of automated features, such as automated feeds, such as automatic puging.

With unique and outstanding features, and by shortening the overall analysis time, prominence UFLC offers truly fast LC analysis.

Example comparing total analysis time for 3 consecutive cycles of fast analysis

(Note: example assumes an analysis time of 30 seconds per cycle)

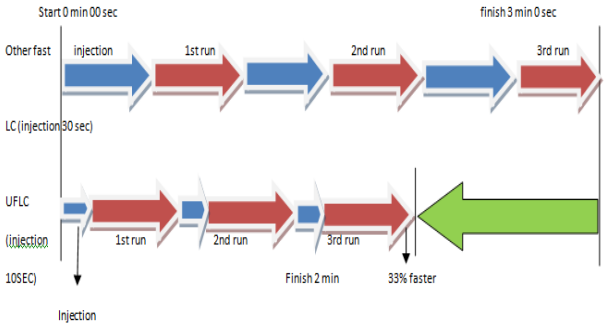


Figure 2: Comparison of total times for 3 consecutive ultrafast analyses

High Speed LC Analysis - 10 Times Faster than Conventional LC[4]

The following is a comparison of chromatograms between prominence UFLC with shim pack XR-ODS, a new reversed-phase column, and conventional LC using a conventional column (150mmL.* 4.6mm i.d., 5µm). In conventional LC, benzofluoranthene isomers (peaks 7 & 8) with separation 1.2 were eluted in 35 minutes.

Using prominence UFLC with shim pack XR-ODS, the same components were eluted in 3.5 minutes while maintaining the same excellent resolution. Retention time for benzoperylene (peak 9) is reduced from 50.4 min. to 5.07 min., while its theoretical plate number changes slightly from 10,600 to 9,100.

Prominence UFLC reduces analysis time to 1/10 of conventional LC while keeping separation efficiency.

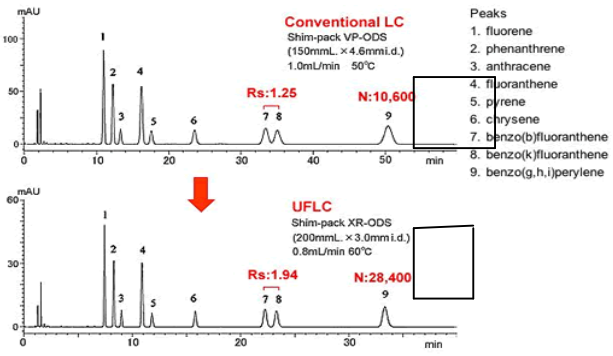


Figure 3: Analysis time reduction may vary by analytical condition

Principle

Chromatography is a technique which is a mixture sample is separated into components. Although originally intended to separated and recover the components of a sample, today, complete chromatography system are often used to both separate and quantify sample components.

Instrumentation[5]

Quantitative UFLC was performed on a binary gradient UFLC with two shimadzu prominence UFLC-20AD pumps, with a 20µl sample injection loop (manual) and a SPD M20A PDA detector. The output signal was monitored and integrated using shimadzu LC solution software.

An enable C18G column (250mm x 4.6mm i.d., 5µm) was used for separation. Chromatographic analysis was carried out at ambient temperature on the column using the Acetonitrile: 0.01M TBAHS (tetra butyl ammonium hydrogen sulfate) (50:50, v/v) as the mobile phase at a flow rate of 1.0 ml/min in isocratic mode. The .01M TBAHS solution was prepared by accurately weighing 3.395gm of TBAHS salt and dissolving it in 1000ml of HPLC grade water. Afterwards, both Acetonitrile and TBAHS were ultrasonicated up to 20 min for degassing prior to use. The PDA detection was set at 215nm.

Columns

Maximizing the reliability of data[6]

With the shim-pack XR-ODS column, high-speed and high-separation analysis is possible even at pressure below 30mpa (300kgf/cm^2).

We believe that the shim-pack XR-ODS column provides the solution for customers wanting to improve analytical efficiency by realizing both high speed and high separation using regular column pressure conditions.

The 2.2µm particle size of the shim-pack XR column packing achieves resolution equivalent to a general-purpose column with 5µm packing particle size but significantly reduces the analysis time. Ideal for use at pressure below 30mpa, it enables fast analysis to be easily performed on an existing instrument.

Shim-pack XR-ODS II/III have higher pressure resistance to achieve optimal performance by combining these columns with optimized shimadzu nexera/prominence UFLC systems.

Table 1: Comparison between UPLC and UFLC

Parameters	UPLC	UFLC
Particle size	<2µm	2.2µm
Analytical column	Acquity UPLC BEH columns	Shim-pack XR columns
Column dimension	150 x 2.1mm	75 x 3.0mm
Column temperature	65^{0}c	40^{0}c
Flow rate	0.6 ml/min	3.7 ml/min
Back pressure	103.5mpa	<35mpa
Inj. Volume	2µl	0.1-100µl

Table 2: Mobile phase, diameter, length and drugs to be analyzed in UFLC columns.

	Column	Mobile phase	Diameter (mm)	Length (mm)	Drugs to be analyzed
UFLC	Shim-pack XR-ODS	Water/Acetonitrile	2.0/3.0/4.0	30/50/75/100	Opioid antagonists and its metabolites.
	Shim-pack XR-ODS II	Water/Acetonitrile/phosphate buffer solution/methanol	2.0/3.0	75/100/150	Catechins in green tea
	Shim-pack XR-ODS III	Water/Acetonitrile	2.0	50/75/150/200	Opioid antagonists
	Shim-pack XR-C8	Methanol	2.0/3.0	30/50/75/100	Fat soluble vitamins
	Shim-pack XR-Phenyl	Phosphate buffer solution/Acetonitrile	2.0/3.0	30/50/75/100	Non-steroidal anti-inflammatory drugs
	Shim-pack XR-SIL	Hexane/ethanol	2.0/3.0	50/75/100	

Shim-pack XR-ODS column[7]

Shim-pack XR-ODS columns are analyzed opioid antagonists and its metabolites

Ex:

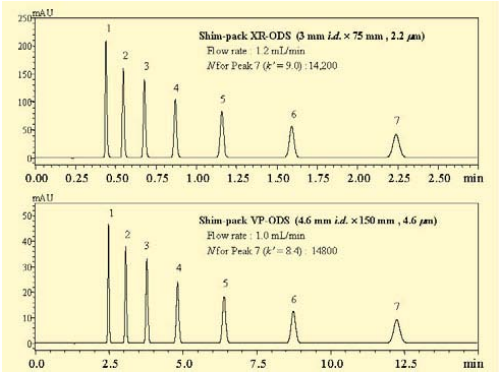


Figure 5: Column: shim-pack XR-ODS, Mobile phase: water/Acetonitrile (3/7 v/v), temp: 40°C, absorbance: 245nm, sample volume: 4µL(XR-ODS), 10µL(VP-ODS), peaks: 1. Acetophenone, 2. propiophenone, 3. butyrophenone, 4. Valerophenone, 5. hexanonephenone, 6. heptanophenone, 7. octanophenone.

Shim-pack XR-C8 analysis example[8]

This is an example of analyzing fat soluble vitamins E (tocopherol) and A (retinol). Not speed increased compared to the column with 5µm packing particle size, but in such cases of multi analyte analysis with high fat solubility, it is possible to achieve even further increases in analysis speed compared to ODS columns, due to difference in retention characteristics.

Shim-pack XR-Phenyl Analysis Example[8]

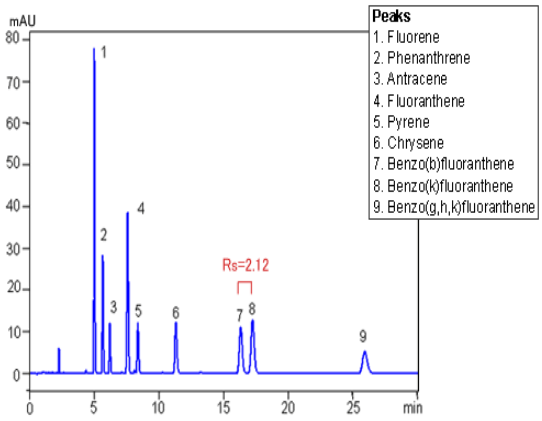
This is an example of analyzing non-steroidal anti inflammatory drugs. Since the stationary phase has a phenyl functional group, retention of aromatic compounds is relatively strong compared to ODS columns. This fact can be utilized to optimize separation. This provides an alternative when faced with separation problems using an ODS column.

Shim-pack XR-SIL analysis Example[8]

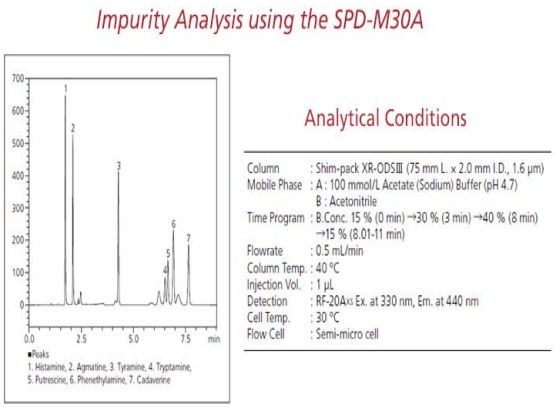
The shim-pack XR-SIL enables increasing speed and saving solvent for normal phase analyses using a silica gel column packed with 5µm particles (conventional). Since organic solvents are used for normal phase analyses, the benefit of saving solvent is greater than for reverse phase analysis.

XR-ODS II High Separation Column[9]

These are of shim-pack XR-ODS II, this column analyzed catechins in green tea.



Shim-pack XR-ODS III[10]



Method Development

Initial transfer of the UPLC assay to UFLC was accomplished by simply applying a scaling factor to the mobile phase flow rate and the sample injection volume. The scaling factor derived from the ratio of the column areas in order to retain the mobile phase liner velocity. Chromatogram for UFLC method contain very narrow peaks, the excessive resolution indicated opportunity for method improvement. The mobile phase floe rate is increased until limited by column backpressure.

Advantages over UPLC

1. Ten times faster than other conventional liquid chromatography.
2. Three times better separation than conventional LC.
3. Operation cost is reduced.
4. Less solvent consumption.
5. Reduce process cycle times, so that more product can be produced with existing resources.

Applications

Determination of iodiconazole in micro-dialysis samples[11]

Iodiconazole is a very potent antifungal agent used to treat serious fungal infections. After transdermal administration, several factors affect the exposure of iodiconazole, resulting in large variability and demanding further elucidation of drug distribution. For determination iodiconazole in dermal microdialysate, ultra-fast liquid chromatography (UFLC shimadzu) assay using UV detection at 230nm has been used. Iodiconazole was separated on a shimadzu prominence UFLC C18 column (22 micron, 50mm * 2.0 mm i.d.) using Acetonitrile 0.025% tri-ethylamine solution adjusted to pH 3.6 with phosphoric acid (65:35,v/v), at a flow rate of ml/min.

Determination of podophyllotoxin in dermal and blood micro-dialysis samples of rats[12]

The micro-dialysis samples were prepared by liquid-liquid extraction using ethyl acetate with etoposide as the internal standard (Is). Podophyllotoxin was separated with an Agilent ZORBAX XDB-C18 column (2.1 mm x 50mm, 3.5 micron). The mobile phase consisted of Acetonitrile: 10m mol/L ammonium acetate (40:60, v/v) at a flow rate of 0.3 ml/min and the analysis was performed at the ambient temperature. The UFLC-MS/MS system was operated in the mode of multiple reactions monitoring using the electro spray ionization technique in positive mode.

Simultaneous estimation of fluoroquinolones and xanthenes derivatives in serum[13]

For selective extraction of fluoroquinolones and xanthenes derivatives form human serum samples, a new molecularly imprinted polymer was synthesized using ofloxacin and theophilline as template and methacrylic acid as function monomer and it was employed as a special dispersant of matrix solid-phase dispersion. For simultaneous analysis of these derivatives in serum is done by molecularly imprinted matrix solid-phase dispersion coupled with liquid chromatography.

Analysis of isoflavones in soy[14]

Analysis of isoflavones in soy was done by using prominence UFLC. For this analysis shim-pack XR-ODS (75mm L x 3.0 mm i.d) column is used. Mobile phase used are 0.1% formic acid aqueous solution and formic acid Acetonitrile at flow rate of 1.2ml/min, and sample injection volume is 5µL at 40°C temperature.

Analysis of catechins in green tea[14]

Analysis of catechins in green tea was done by using prominence UFLC. For this analysis shim-pack XR-ODS (50mm L x 2.0mm i.d) column is used. Mobile phase used for 0.1% formic acid aqueous solution and Acetonitrile at the flow rate of 0.5mL/min, and sample injection volume is 2µL at 50°C temperatures.

Merits

1. The UFLC system allows shortening analysis time comparing to UPLC system.
2. Separation on UFLC is performed under low pressure (30mpa).
3. UFLC dramatically improves the quality of the data, resulting in a more definitive map.
4. Faster analysis through the use of a novel separation material of very fine particle size.

CONCLUSION

UFLC is a new revolution in chromatography. Due to the small particle size(2.2µm) of UFLC column leads to highly selective and chemically stable column (shim-pack XR-ODS) with high speed analysis which results in shorter retention times with reproducible result and highly robust not even more than 2min unlike UPLC. The UFLC column even can with stand the low back pressure (30mpa).

Therefore UFLC fulfils the promise of increased resolution predicted for liquid chromatography for an analyst compared to UPLC.

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