



**IJPPR**

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

**Short Communication**


September 2020 Vol.:19, Issue:2

© All rights are reserved by V. A. Gaikwad et al.

## A Short Review on: Principle, Instrumentation and Application of Ultra Performance Liquid Chromatography



**IJPPR**  
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH  
An official Publication of Human Journals



ISSN 2349-7203

**V. A. Gaikwad\*, S. D. Varhadi, N. R. Gade, N. M. Gowekar**

*Siddhant College of Pharmacy, Sudumbare, Tal-Maval,  
Dist.-Pune, 41210, India*

**Submission:** 20 August 2020  
**Accepted:** 26 August 2020  
**Published:** 30 September 2020

**Keywords:** Chromatography, Column, HPLC, UPLC

### ABSTRACT

UPLC refers to Ultra Performance Liquid Chromatography, which improves in 3 areas; speed, resolution, and sensitivity. In UPLC principle based on the Van Deemter equation that justifies the correlation between flow rate and plate height. This technique uses fine particles (less than 2 $\mu$ m), reduces the length of the column, and saves time, and reduced solvent consumption. This review focus on the principle, instrumentation, and application of UPLC.



[www.ijppr.humanjournals.com](http://www.ijppr.humanjournals.com)

## INTRODUCTION

High-Performance Liquid Chromatography (HPLC) has proved to at least one of the foremost and predominant technology used in the analytical laboratory for the analysis of medicine word wide throughout the past 30-plus years.<sup>[1]</sup>UPLC can be regarded as a new invention for liquid chromatography. UPLC refers to Ultra Performance Liquid Chromatography. It is a method of separating a mixture of components into individual components through a porous medium under the influence of the solvent.<sup>[2]</sup>UPLC is a derivative of HPLC whose underlying principle is that as column packing particle size decreases, efficiency and thus resolution increases. If we decrease particle size less than 2µm, the efficiency shows a significant gain and it does not diminish at increased linear velocities or flow rate according to the common Van- Deemter equation.<sup>[3]</sup>UPLC brings Dramatic enhancement in sensitivity, resolution, and speed of analysis that can be calculated. Its instrumentation that operates at high pressure than that used in HPLC and this system uses fine particles (less than 2.5µm) and mobile phases at high linear velocities decreases the length of the column, reduces solvent consumption, and saves time.<sup>[4]</sup>To maintain retention and capacity similar to HPLC, UPLC must use porous particles that can withstand high pressure; though being highly efficient, this sub-2 µm (non-porous) particles suffer from poor loading capacity and retention due to low surface area.<sup>[5]</sup>This technology takes full advantage of chromatographic principles to run separations using columns packed with smaller particles and higher flow rates for increased speed, with superior resolution and sensitivity.<sup>[6]</sup>

## PRINCIPLE

The basic principle of UPLC and HPLC is the same and depends upon mode of separation, i.e. adsorption, partition, exclusion, and ion-exchange depending on the type of chromatographic sorbent.<sup>[5]</sup>The UPLC is predicated on the principle of the use of a stationary phase consisting of particles less than 2µm (while HPLC columns are typically filled with particles of 3-5 µm).<sup>[7]</sup>The underlying principle of UPLC is predicated on the Van Deemter relationship that explains the correlation between flow rate and plate height. The Van Deemter equation shows that the flow range with the smaller particles is much greater in comparison with larger particles for permanent results.<sup>[8]</sup>

$$H = A + \frac{B}{v} + Cv$$

Where,

**H** = Height Equivalent to Theoretical Plate (HETP).

**A** = Eddy's Diffusion.

**B** = Diffusion coefficient.

**C** = Resistance to mass transfer coefficient.

**v** = Linear velocity.<sup>[9]</sup>

Where A, B, and C are constants. v is the linear velocity, the carrier gas flow rate.

The A term is independent of velocity and represents "Eddy" mixing. It is the smallest when the packed column particles are small and uniform.

The B terms represent axial diffusion or the natural diffusion tendency of the molecule. This effect is diminished at high flow rates and so this term is divided by v.

The 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 chromatographic performance.<sup>[10]</sup>

## **INSTRUMENTATIONS**

The basic Instrumentation of UPLC is;

1. Sample Injection
2. UPLC Columns
3. Detectors

### **1) Sample Injection:<sup>[2]</sup>**

In UPLC, a sample introduction is essential. Conventional injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from extreme pressure fluctuation, the injection method should be comparatively pulsed free and the swept volume of the device also must 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. Low volume injections with minimal carryover are also required to increase sensitivity. There are also direct injection approaches for biological samples.

## **2) UPLC Columns:<sup>[3]</sup>**

The UPLC columns are made up of small particles having a size of less than 2 $\mu$ m. The particles are bonded in the matrix as the bonded stationary phase is needed for providing each retention and selectivity. Four bonded stationary phase columns manufactured by ACQUITY are available in the market, which can be used by the UPLC technique.

- BEH C18 and C8 columns – These are a straight alkyl chain, most preferred UPLC columns as they can be used over a wide pH range. The tri-functional ligands produce low pH stability, which is combined with high pH stability of 1.7 $\mu$ m BEH particles to produce the widest usable pH operating range.
- BEH Shield R18 Columns: They supply selectivity to UPLC as it complements C18 and C8 columns.
- BEH Phenyl Columns: They have tri-functional C6 alkyl ethyl between the phenyl- ring and the silyl functionality.
- BEH Amide Columns: The combination of the tri-functionally bonded amide phase with BEH small particles provide an exceptional column lifetime. They facilitate the use of a wide range of phase pH i.e., from pH scale 2 to 11.

## **3) Detectors:<sup>[2, 10]</sup>**

The system is often organized with a TUV, ELS, PDA, and FLR detectors or a mixture of them.

### **1. TUV (Tunable Ultra-Violet) detector:**

It is a 2 channel, absorbance detector. The detector is controlled by Empower or Mass Lynx software for each LC/MS and LC applications.

### **2. PDA (Photo Diode Array) detector:**

It is an optical detector that absorbs UV-Visible light that operates between 190-500nm.

### 3. ELS (Evaporative Light Scattering) detector:

The detector is controlled by Empower or Mass Lynx software, which incorporates a flow-type nebulizer that's optimized for UPLC system performance.

### 4. FLR (Fluorescence) detector:

It is a multi-channel, multi-wavelength detector, which has an excitation wavelength that ranges from 200 to 890 nm, an emission wavelength that ranges from 210- 900 nm, offers 3D scanning capability for easier method development.

### Applications

- UPLC Analysis can be done like:

1. Amino acid analysis.
2. Analysis of natural medicine and herbal medicine.
3. Analysis of drugs in human plasma (e.g. Levofloxacin and metabolites).
4. Study of metabonomics.<sup>[1]</sup>

- Determination of Pesticides in Groundwater:

UPLC coupled with triple quadrupole tandem mass spectrometry (UPLCTM-MS/MS) may be used to see trace level pesticides in groundwater in less time and speedy manner. The technique has increased the analysis speed, sensitivity, and resolution.

- Multi-Residue Analysis of Pharmaceuticals in Waste Water:

The water used in the pharmaceutical companies is found to have the traces of various cholesterol-lowering statin agents, anti-ulcer agents, antibiotics, beta-blockers, analgesics, anti-inflammatory agents, lipid regulating agents, psychiatric drugs, and histamine H2 receptor antagonists. UPLC coupled with Q-TOF-MS is used to confirm and screen these drugs in the samples of the wastewater treatment plant.<sup>[8]</sup>

- By UPLC technique, the analysis of creams and gels may be done with high speed, sensitivity, and improved resolution.<sup>[11]</sup>

- Analysis of Natural Products and Traditional Herbal Medicine:

The understanding of the pharmacological importance of herbs is essential for their screening, validation, and use. The active components present in these herbs can be quickly analyzed with the combined use of exact mass MS and UPLC, which is an association with Marker Lynx Software data processing for the statistical analysis of the information obtained.

- The development of the column technology makes UPLC a very practical and reliable technique for the specific analysis of Vit-D<sub>2</sub> and Vit-D<sub>3</sub>.<sup>[12]</sup>
- UPLC is used for studying hydrolysis kinetics of CL-20 and related energetic compounds.
- Simultaneous Determination of Tetracycline's and Quinolones Antibiotics in Egg may be done by Ultra-Performance Liquid Chromatography–Electrospray Tandem Mass Spectrometry.
- Determination of Coumarone in Food may also be done using Ultra-Performance Liquid Chromatography-Electro Spray-Tandem Mass Spectrometry.<sup>[13]</sup>
- determination of oleanolic acid in rat plasma and liver tissue may be done by using UPLC-MS/MS method.<sup>[14]</sup>
- B-vitamins in human milk can be determined by using the UPLC-MS/MS method. <sup>[15]</sup>
- Ginsenosides from cultivated ginseng and forest-grow wild ginseng can be separated and analyzed by using the UPLC method.<sup>[16]</sup>

## SUMMARY

From the above study, it is concluded that UPLC is an advanced Chromatographic technique that gives fast and accurate results. This special technique improves the speed, resolution, and sensitivity of the analysis. The discovery and development of UPLC have opened doors to new insights and uses of chromatography and has reduced the workload and difficulties faced by analysts and scientists.

## REFERENCES:

1. M Taleuzzaman, S Ali, SJ Galan, SS Imam, A Hafeez Ultra Performance Liquid Chromatography (UPLC) - AReview.2015; 2(6):01-05.
2. Snehal V. Chopade and Dr. V. R. Patil, Introduction to new chromatography technique- UPLC. 1.
3. Ashok Kumar, Gautam Saini, Anroop Nair, and Rishbha Sharma, UPLC: A Preeminent Technique in Pharmaceutical Analysis.2012; 69(3):371-380.
4. K. Naresh, S. Bhawani, and Maneesh Kumar, Ultra Performance Liquid Chromatography. 2014; 3: 84.

5. Sahani Kamal and Wakode Sharad, Step-up in liquid chromatography from HPLC to UPLC: A comparative and comprehensive review. 2018;7(8):343.
6. Anagha S. Patil, A Review on Ultra Performance Liquid Chromatography (UPLC).2015; 3:10: 87.
7. K.G. Sheliya and K. V. Shah Ultra Performance Liquid Chromatography (UPLC): A Modern Chromatography Technique. 2013;4: 80.
8. Gita Chawla and Chanda Rajan, Principle, Instrumentation and Applications of UPLC: A Novel Technique of Liquid Chromatograph. 2016; 3: 2.
9. [https://en.wikipedia.org/wiki/Van\\_Deemter\\_equation](https://en.wikipedia.org/wiki/Van_Deemter_equation)
10. Narwate B. M., Ghule P. J., Ghule A. V., Dharndale A. S., and Wagh J. G., Ultra Performance Liquid Chromatography: A New Revolution in Liquid Chromatography. 2014; 2:27.
11. Lucie Novakova, Ludmila Matysova, and Petr Solich, Advantages of Application of UPLC in Pharmaceutical Analysis. 2006;68(3): 908-918.
12. Rama Chandra Shekhar Reddy Pulagam and Dr. Henne Walter, UHPLC in the analysis of Drug Component .2013: 21-22.
13. <http://www.pharmatips.in/Articles/Analysis/Chromatography/Advantages-And-Application-Of-UPLC.aspx>.
14. Tian-xue Lia,b, Chao-sen Chub, Jia-yu Zhua, Tian-yi Yangc, Jie Zhangd, Yu-tao Hub, and Xing-hao Yanga\*, A rapid UPLC-MS/MS method for the determination of oleanolic acid in rat plasma and liver tissue: application to Plasma and Liver pharmacokinetic. 2016; 30(4): 520-527.
15. REN Xiang Nan, YIN Shi An, YANG Zhen Yu, YANG Xiao Guang, SHAO Bing, REN Yi Ping and ZHANG Jing, Application of UPLC-MS/MS Method for Analyzing B- vitamins in Human Milk. 2015;28(10): 738-750, 2015
16. Lanlan Zhang, wenyuan Gao, Shuiping Zhou, and Nan Cai., Application of UPLC to separation and analysis of ginsenosides from cultivated ginseng and forest-grow wild ginseng.2011; 30(05): 991-995.

