International Journal of Engineering and Allied Sciences (IJEAS)
||Volume 2|| ||Issue 1|| ||2016||
ISSN: 2455-2054

Ultra Performance Liquid Chromatography: High Throughput Analysis over High Performance Liquid Chromatography

Smrita Singh[a*], Lalit Prasad[b*], Vimal Kumar[c], Susanta Roy[d], Santosh Kumar Patnaik[e]

[a]*Bioinformatics Infrastructure Facility, Gargi College (University of Delhi), New Delhi, India*
[b]*Department of Chemistry, School of Basic andApplied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India*
[c]*Science Laboratory, Archaeological Survey of India, Dehradun-248001, Uttrakhand, India*
[d]*Bioexplore, JSSATE-Science and Technology Entrepreneurs Park, C-20/1, Sec-62, Noida,India*
[e]*Jubilant Generics Ltd. Noida, UP, India*

*Email: smrita1@gmail.com, lalit.prasad@galgotiasuniversity.edu.in

----------ABSTRACT----------

Ultra Performance Liquid Chromatography (UPLC) can be regarded as new invention for liquid chromatography. These days not only pharmaceutical industries but also several chemical industries are trying to develop new cost effective technique to reduce analysis time for development of new product in order to improve quality of their products. Analytical laboratories are no exception to this new trend. In recent years, significant advancement has been made in liquid chromatographic separation technique. Before development of UPLC, HPLC was an approved liquid separation technique used for more than 30 years in laboratories and Industries. Significant advancement has been made in terms of particle chemistry in the last few years. UPLC comes from HPLC. This new and advanced technique uses fine particles probably less than 2.5µm.UPLC takes full advantage of all basic principle of chromatography, using advanced column with smaller particle size and higher flow rates for increase in speed along with better, superior sensitivity and resolution. Use of fine particles in UPLC system decreases column length, running time and solvent consumption. This new and improved category of liquid chromatographic separation technique has similar practicality and principles of HPLC with additional improved step function. Elevated temperature chromatography has advantages of lowering mobile phase viscosity that reduces the column backpressure. UPLC technology has advantage in particle chemistry performance, system optimization, detector design, data processing and data control. UPLC is cost effective over HPLC. This study explains the UPLC theory and its applications in the diverse field.

Keywords - UPLC, HPLC, Cost effective, Particle chemistry, Liquid chromatography.

I. INTRODUCTION

Ultra Performance Liquid Chromatography (UPLC) is one of the new technologies in analytical separation science. It works on the similar principles of High performance Liquid Chromatography (HPLC). It improves chromatographic resolution, speed and sensitivity [1]. It uses fine particles and saves time and reduces solvent consumption. Ultra Performance Liquid Chromatography was introduced by Waters Corporation in 2004 and trademarked it by UPLC. UPLC is a one step ahead technology of HPLC that has underlying principle as co lumn packing particle size decreases, efficiency and resolution increases. The use of porous sub2 micron particles, operated at higher flows and pressures was the greatest change in the UPLC than a conventional system [2]. If the particle size was made lesser than 2 µm, a significant increase in effectiveness was observed [3].This concept shortened sample analysis time and mobile phase consumption by 80% significantly compared to

HPLC. UPLC has technology advantages in particle chemistry performance, system optimization, detector design, data processing and control [4].UPLC advancement is governed by the Van Deemter equation.

II. PRINCIPLE & DISCUSSION

The UPLC principal is based on use of stationary phase consisting of particles less than 2 μm while HPLC columns particle sizes are typically 3 to 5 μm. The underlying principles of UPLC are governed by the van Deemter equation, an empirical formula which describes the relationship among flow rate and plate height (HETP or column efficiency). HETP increases with increasing flow rate after going down to a minimum value. According to van deemter equation (i) the flow range with the smaller particles is much greater in comparison with larger particles for good results [5].

$$H=A+B/v+Cv \quad \text{(i)}$$

Where, H represents height equivalent to the theoretical plate (HETP). A, B & C are the constants and v is the flow rate of the carrier gas. The "A" term is independent of velocity. It is small when the packed column particles are small and uniform. The "B" term is axial diffusion of molecules. The axial diffusion is smaller at high flow rates therefore this term is divided by v. The term "C" is kinetic resistance to equilibrium in the separation process. The time interval taken in moving from the gas phase to the packing stationary phase and back again is termed as kinetic resistance. This term is inversely proportional to v (linear velocity).Therefore it is possible to increase throughput and speed of separation without affecting the chromatographic performance. UPLC efficiency is proportional to column length and inversely proportional to the radius of the [6]. The application of UPLC resulted in the detection of additional drug metabolites, superior separation and improved spectral quality [7, 8]. The comparison between UPLC and HPLC is given in the Table 1.

Table 1: Comparison between UPLC and HPLC

Characteristics	HPLC Assay	UPLC Assay
Column	150 X 3.2 mm	150 X 2.1 mm
Particle size	3 to 5mm	Less than 2mm
Flow rate	3.0 ml / min	0.6 ml / min
Needle wash	Methanol	Methanol
Injection volume	5μL (Std.In 100 % MeOH)	2μL(Std.In 100 % MeOH)
Column temperature	30 °C	65 °C
Maximum backpressure	35-40 MPa	103.5 MPa
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
Delay volume	750 μl	110 μl

Chemistry of small size particles

Small particles in UPLC increases efficiency, working ability at high linear velocity without a loss of efficiency and provide both good resolution and speed. The basic resolution (Rs) equation (ii) explains efficiency, selectivity and proportionality.

$$Rs = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{K}{K+1}\right)$$

System efficiency Selectivity Proportionality (ii)

In equation (ii), N is efficiency factor, α is selectivity factor and K symbolizes proportionality constant. According to equation (ii), resolution increases with increase in efficiency. Efficiency (N) is inversely proportional to particle size (dp) equation (iii)

$$N \propto \frac{1}{dp} \quad \text{(iii)}$$

Efficiency increases with increase in resolution and sensitivity as t he particle size decreases. Efficiency (N) is directly proportional to the length of the column (L), therefore equation (iii) can be written as equation (iv)

$$N \propto \frac{L}{dp} \quad \text{(iv)}$$

Efficiency is also indirectly proportional to peak width (w) denoted as equation (v). This explains that lesser the peak width or narrower the peak helps in easy separation from each other.

$$N \propto \frac{1}{w^2} \quad \text{(v)}$$

Peak height (H) is inversely proportional with peak width (w) according to equation (vi)

$$H \propto \frac{1}{w} \quad \text{(vi)}$$

Above mentioned facts in UPLC, explains that by reducing particle size, the column length is reduced and flow rate is increased also the separation becomes faster without loosing resolution loss [1]. The effect of particle size on HETP and linear velocity in Fig.1 Shows that smaller diameter particles overcomes band broadening in comparison to larger particles also they are less affected by higher column flow rate [9, 10].

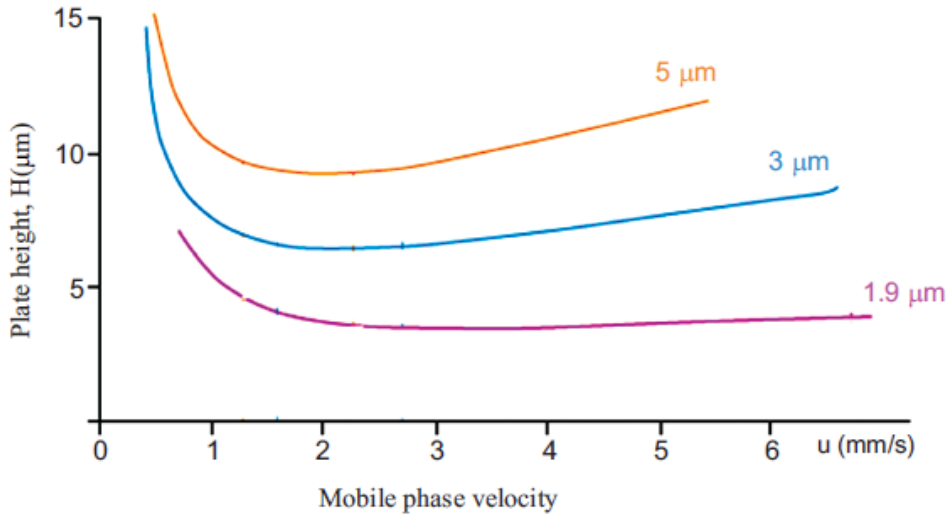

Figure 1. Van Deemter Plot: The effect of particle size (in µm) on plate height (H). Smaller particle size provides higher overall peak efficiencies and wide range of flow rates [9, 10]

Instrumentation

UPLC instrument has three parts (Sample injection, UPLC columns and Detectors).

Sample Injection

Sample injection part adds precisely measured sample, a small volume in the mobile phase. Sample injection must be reproducible and accurately. UPLC takes care of quick injection cycle time, sensitivity and low volume injections with minimal carryover [11]. Sample volume in UPLC is 2-5 µL.

UPLC Column

UPLC columns are packed with small particles having size less than 2µm. There are different types of columns based on di fferent technologies [12] like Charged Surface Hybrid [CSH] particle technology, Ethylene Bridged Hybrid [BEH] particle technology, High Strength Silica [HSS] particle technology and Peptide Separation Technology (PST). UPLC columns have been manufactured and developed by Waters: Acquity UPLC columns and Vanguard Pre-columns [12]. Agilgent technology: Poroshell 120 columns, ZORBAX Rapid Resolution High definition columns, ZORBAX Eclipse pluscolumns and ZORBAX Rapid Reduction High Throughput columns [13], Altech Associate [14], Phenomenex: Kinetex® Coreshell HPLC/UHPLC columns of high efficiency and performance[15].

Solvent Delivery System

The solvent delivery system of UPLC works at 8000-15000 psi for isocratic, linear and non-linear gradient elution. UPLC binary solvent manager has two solvent delivery modules that operates for high pressure merges two solvents in <140 µL internal system volume.

The Detector

The detector in UPLC is Acquity photodiode array (PDA) and Tunable Vis –UV (TUV) in which Teflon AF is used. This gives an internal reflective surface and increases the light transmission efficiency. These have path lengths 10 nun, a cquisition rates 20 (PDA) and 40 (TUV) points, and total internal volume 500 nL [16, 17 and 18].

Advantages and Disadvantages

UPLC has advantages over selectivity, sensitivity and high resolution performance through the use of a novel column packed with small particle size. It decreases run time and reduces operation cost. It reduces solvent consumption and increases sample throughput [19, 20 and 21]. UPLC disadvantage is the high back pressure that decreases column life [1, 22 and 23].

Applications of UPLC

There are many applications of UPLC in diverse fields like analysis of natural products, metabolite, bio analysis, ADME screening and impurity profiling etc.

Analysis of Natural Products and Traditional Chinese medicines

Natural products and Traditional Chinese Medicines are complex constituent matrix having specific importance for each constituent for the overall efficacy. It is essential to analyse all the constituents for quality control and is accomplished by UPLC [24].

Identification of Metabolite

Identification and detection of metabolites for the discovery of new chemical entities is required to further explore its medicinal properties by medicinal chemists. UPLC-MS/MS is useful in biomarker discovery [25, 26 and 27].

Bio analysis Studies

Bio analysis studies include pharmacokinetic studies of biological samples for the drug quantification. Comparison of the rate and exposure level of new drug formulations with its original formulation is important part. The use of UPLC-MS/MS provides reliable and precise data [28, 29].

ADME Screening

ADME stands for absorption, distribution, metabolism and excretion. These are pharmacokinetic studies that measure physical and biochemical properties of drugs where compounds show activity against the target disease.

Impurity Profiling

Impurity profiling is important while drug development and formulation process. UPLC coupled with mass spectrometry has been useful for profiling impurities and identifying endogenous metabolites in the final product [30, 31 and 32].

III. CONCLUSION

UPLC is important analytical instrument that increases speed, resolution and sensitivity of the chromatographic analysis. It decreases analysis time, operating cost and solvent consumption. UPLC columns have capacity of withstand high back pressure. The applications of UPLC are in wide

spectrum of pharmaceuticals like drug discovery, metabolomics, peptide mapping etc. This technology provides efficient product launch in the market within less span of time.

REFERENCES

[1] N. Wu, J. A. Lippert, M. L. Lee, *J. Chromatogr. A., 911*(2001) 1-12.

[2] A. kumar, *Acta poloniae pharmaceutica–drug research*, 69 (2012) 371-380.

[3] S. M. Chesnut, J. J. Salisbury, *J. Sep. Sci.,* 30 (2007) 1183-1190.

[4] M. M. Fallas, U. D. Neue, M. R. Hadley, D. V. McCalley, *J. Chromatogr., A 1217* (2010) 276-284.

[5] J. E. Mac Nair, K. D. Patel, J.W. Jorgenson, *Anal. Chem.,* 71 (1999) 700-708.

[6] J. E. Mac Nair, K. C. Lewis, J. W. Jorgenson, *Anal. Chem.,* 69 (1997) 983-989.

[7] K. N. Patel, International journal of biomedical research and analysis. 1(2010) 1-4.

[8] K. Beattie, J. S. Joncour, K. Lawson, *LC GC North America,* (2005) 22-30.

[9] J. J. V. Deemter, E. J. Zuiderweg, A. Klinkenberg, *Chem. Eng. Sci.,* 5 (1956)271-289.

[10] G. W. David, *Pharmaceutical analysis. Sec edition; Elsevier Churchill Livingstone: UK*, (2005) 224-225.

[11] G. M. Prathap, A. Nishat, *Int. J. Pharmacy,* 3 (2013) 251-260.

[12] Acquity UPLC column, *Available at:* https://www.waters.com/webassets/cms/library/docs/720001140en.pdf

[13] Agilent Technologies, Available at: https://www.agilent.com/cs/library/catalogs/public/5991-1059EN%20LC_Columns.pdf,

[14] http://http://www.mfgpages.com/

[15] http://http://www.phenomenex.com/

[16] Y. Lars, H.S. Honore, *J. Chromatogr.,* 1020 (2003) 59-67.

[17] D. A. McLoughlin, T. V. Olah, J. D. Gilbert, *J. Pharmaceut. Biomed.,* 15 (1997) 1893-1901.

[18] A. D. Broske, *Agilent technologies application, note* 2004, Available at: https://www.agilent.com/cs/library/primers/Public/5991-3326EN

[19] A. B. Roge, S. N. Firke, R. M. Dhane, V. J. Gunjkar, S. M. Vadvalkar, *Int. J. Pharm. Tech. Res.,* 3 (2011) 1423-1429.

[20] Y. Yang, C.C. Hodges, *LC GC Chromatography,* 2005, 31-35.

[21] L. Nováková, D. Solichová, and P. Solich, *J. Sep. Sci.,* 29 (2006) 2433-2443.

[22] S. Sridhar, S. Divya, S. Sridhar, R. Madhuri, M. Sudhakar, *Int. J. Pharm. Chem. Bio.Sci.,* 3 (2013) 1139-1152.

[23] P.V. Gaikwad, *Inter. J. of Compre. Pharm.,* 2 (2010) 1-3.

[24] G. L. Yang, L. W. Yang, Y. X. Li, H. Cao, W. L. Zhou, , Z. J. Fang, H. B. Zhou, J. L. Mo, S. X. Xiao and H. R. Lin, *J. Chromatogr. Sci.,* 48 *(*2010) 18-21.

[25] J. Mao, Y. Xu, B. Lu, J. Liu, G. Hong, Q. Zhang, S. Sun, J. Zhang, *Anal. Bioanal. Chem.,* 407 (2015)4101-4109.

[26] Y. N. Tang, Y. X. Pang, X. C. He, Y. Z. Zhang, J. Y. Zhang, Z. Z. Zhao, T. Yi, H. B. Chen, *J. Ethnopharmacol.,* 165 (2015) 127-140.

[27] W. Zeng, H. Han, Y. Tao, L. Yang, Z. Wang, K. Chen, *Biomed. Chromatogr.,* 27 (2013) 1129-1136.

[28] M. Yadav, R. Rao, H. Kurani, P. Singhal, S. Goswami, P. S. Shrivastav, *J. Pharm. Biomed. Anal.,* 49 (2009) 1115-1122.

[29] Y. V. Pidpruzhnykov, V. E. Sabko, V. V. Iurchenko, I.A. Zupanets, *Biomed. Chromatogr.,* 26 (2012) 599-605.

[30] B. Srivastava, B.K. Sharma, U. S. Baghel, S. N. Yashwant, *Int. J. Pharm. Qua. Assur.,* 2 (2010) 19-25.

[31] H. M. Lotfy, S. S. Saleh, N. Y. Hassan, H. Salem, *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.,* 1006 (2015) 121-129.

[32] L. Prakash, H. Malipeddi, B. V. Subbaiah, N. S. Lakka, *J. Chromatogr. Sci.,* 53, 2015, 112-121.