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Ultra Performance Liquid Chromatography (Mini-Review)

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ABSTRACT

Chromatography is a widely used analytical tool for separating a mixture of compounds into individual component. High performance liquid chromatography (HPLC) is one of the most important methods used for the separation, identification and quantification of a compounds present in a mixture. It meets many criteria of analysis but its main drawbacks are it is relatively time consuming to run a chromatogram and consumes high amount of solvent compared to other analytical methods. There is a need to develop a method which can overcome these drawbacks of HPLC. Ultra performance liquid chromatography (UPLC) is the new approach which opens novel direction in the field of liquid chromatography. It works on similar principle but shows better performance than conventional HPLC. UPLC is a technique of liquid chromatography with improved runtime and sensitivity with less than 2 µm particle size. The UPLC separation process is carried out under very high pressure (up to100 MPa). Additionally, it reduces the cost of reagent with shorter run time as compared to conventional HPLC. This article updated until 2020, provides a general review on the principle, instrumentation and application of UPLC in different fields of science.

Keywords: Ultra performance liquid chromatography, Separation, Quantification, Resolution, Sensitivity, Instrumentation and applications.

INTRODUCTION

Modern analytical chemistry, playing a tremeaus role related to chemical innovation, began in the 18th century, especially in many aspects of chemistry such as chemical synthesis, qualitative and quantitative analysis[1]. Nowadays, analytical chemists are working on different instruments such as mass 4211spectroscopy (MS) Nuclear Magnetic Resonance (NMR) inductively coupled plasma, gas chromatography, HPLC and more recently UPLC. These analytical methods are not only useful for chemistry laboratories but also helpful for environmental and biological laboratories and have gained excellent benefits[2]. Amongst all the above analytical methods, HPLC has become most widely used analytical tools. In 1970s, there were various advancements in equipment and instrumentation. HPLC has started a revolution in biological, pharmaceutical chemistry and other fields of science[3]. The first commercially available UPLC system was demonstrated in 2004[4]. Today ultra-performance liquid chromatography has overtaken HPLC as the standard platform[5].

History of chromatography

Chromatography was discovered in early 20th century by M.S. Tswett who gave comprehensive details of the adsorption based separation of different compounds in complex mixtures of plant pigments. Almost 10 years later, L.S. Palmer and C. Dhere issued the similar separation processes. In 1931, Lederer purified xanthophylls on $CaCO_3$ adsorption column by using M. S.Tswett's method. Martin and Synge were awarded Nobel Prize for their discovery of partition chromatography in 1941[6]. Until 1970s, separation process exploited thin layer, paper and column chromatography. However, the main disadvantages of these techniques are the lack of accuracy for quantitative work and poor resolution for similar compounds[7,8].

RESULTS AND DISCUSSION

High performance liquid chromatography(HPLC)

It was first developed in the mid 1970's and till now it is the most used method in analytical chemistry. Following the development of column packing material and detectors, the technique rapidly improved. In 1980's HPLC was widely utilized specially for the separation of reaction mixture[9]. Some novel methods like use of computer, automation and reverse phase chromatography were developed along the time for enhanced separation methods, quantification and identification of mixtures[10]. In continuation of these advancements, by the year 2000, a tremendous development was taking place in different aspect of particle size of the stationary phase. HPLC is probably the most popular type of technique which is useful in quality control, pharmaceutical analysis, forensic analysis, clinical testing and environmental monitoring and other fields of science.

Principle

The separating principle of HPLC is derived on the difference in affinity of the compound to be separated toward the stationary and mobile phase. Detector can recognize analytes after leaving column and signals are recorded in the data system.

Instrumentation

HPLC consists of following components:

a) **Pump:** To maintain constant flow of mobile phase through the column and manage the back pressure caused by the flow resistance of the packed column.

b) **Injector:** To introduce a liquid sample into the HPLC system by injection, usually in the range of 0.1 to 100 μl of volume.

c) **Column:** It is the heart of HPLC in which separation occurs. A variety of columns are used for different substances depending on the nature of the analytes.

d) **Detector:** HPLC detector is used to detect solute present in the eluent coming out from column. There are various types of detectors such as ultraviolet detector, fluorescence detector, mass spectrometer etc[11].

Applications of HPLC

High performance liquid chromatography is widely used in many fields of science for identification, quantification and purification of a compound. These include application in the fields of pharmaceutical science, environmental science, forensic science and clinical analysis. It is widely used in quality control and dosage form. It can be employed for the determination of pharmaceutical product shelf life and also for Identification of different active metabolites. HPLC is also helpful

for the analysis of environmental material such as detection of phenolic compound in drinking water and for bio monitoring pollutant as well. Forensic applications for the quantitative analysis of the drug in blood sample and steroids identification method also require HPLC technique[12].

Ultra Performance Liquid Chromatography(UPLC)

It opened an innovative direction for liquid chromatography covering three major areas including speed, sensitivity and resolution of evaluation by means of the use of packing material with particles size less than 2 μm. The device is created to handle very high pressure experienced by the column. Ultra-performance liquid chromatography also has the advantage of reducing solvent consumption compared to conventional high-performance liquid chromatography[13].

Principle of UPLC

The ultra performance liquid chromatography is established on principle of Van Deemter equation[14].

Equation of van Demeter is:

$H=A+B/\mu +C\mu$

Where:

H= Plate height
A= Eddy diffusion
B= Longitudinal diffusion
C= Equilibrium mass transfer
µ= Flow rate

Smaller plate height value corresponds to greater peak efficiency, as more plates can occur over a fixed length of column[15,16] (Figure 1).

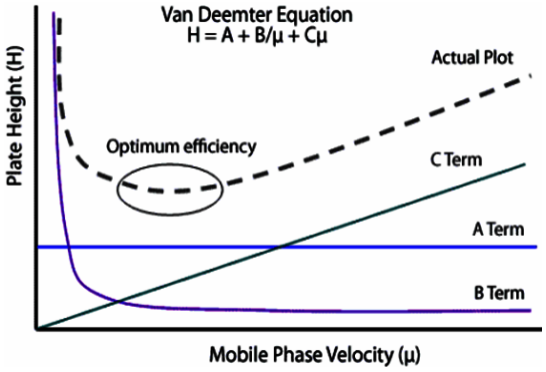

Fig. 1. Van Demeter Equation

Shorter diffusion path length of smaller particles allows a faster movement of the solute in and out of the particles. Because of this the solute/ analyte spends less time inside the particle where the peak diffusion occurs[17-18] (Figure 2).

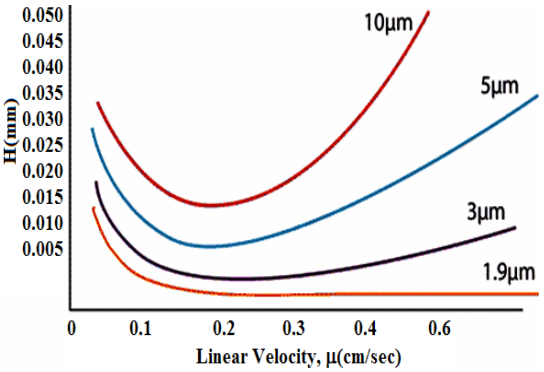

Fig. 2. Depicting the Van Deemter plots for different particle sizes

It has been noticed that using a shorter column length allows much higher sample throughput without losing chromatographic quality of the analytical method[19].

Instrument of UPLC

Ultra performance liquid chromatography instrumentation is basically similar to that of HPLC. It is designed to work under much higher pressure without disturbance and increased maintenance. For UPLC detection, new electronics and firmware are used to support the tunable UV/Visible detector at the high data rates. The tunable UV/Vis detector comprises a 10 mm flow cell path length with a volume of only half a litre.

The instrumentation of UPLC includes:

- Sample injection
- UPLC columns
- Detectors

Sample injection

The injector is used to add a small amount of solution containing the sample in the mobile phase that is precisely measured. The injection must be done consistently and precisely. Conventional injection valves can be manual or programmed, and the injection procedure must be somewhat pulse-free to protect the column from excessive pressure instabilities. To decrease the risk of band spreading, the device's swept volume should be kept to a minimum. To effectively benefit from the speed

of UPLC, a short injection cycle time is required. Low volume injections with minimum carry over are required to increase sensitivity. In UPLC, the sample volume is usually 2-5 µL. For biological samples, direct injection techniques are now commonly used. Flow chart of UPLC shown below (Figure 3).

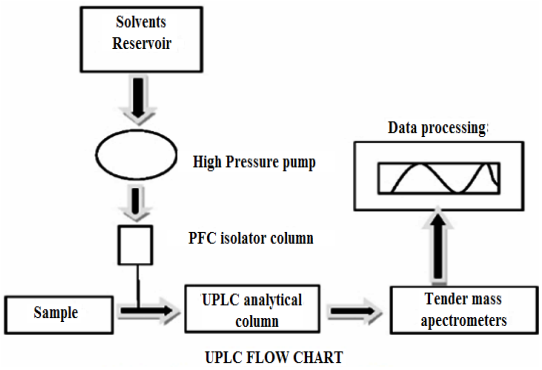

Fig. 3. UPLC flow chart

Ultra performance liquid chromatography columns are made of small particles size 2 μm. Waters associates develops and supplies most of the UPLC columns, some of which are described as follow:

Bridged Ethylene Hybrid (BEH) C18 column:

This column provides high level of column ability, symmetrical peak and stability. It is compatible with mobile phase pH 1-12 and temperature 80°C.

Example: Rapid assay for Cytochrome p450 isoenzymes degradation studies of Glimepirade (retention time: 8.2 minutes)[20].

Bridged Ethylene Hybrid (BEH) C8 column:

This column exhibits low hydrophobicity than C18 column due to its shorter alkyl chain length, thus resulting in rapid elution of analyte peaks.

Example: Analysis of different aromatic amines.

Bridged Ethylene Hybrid guard RP 18 column:

It improves the peak shape for basic compounds and also fine tunable with 100% aqueous mobile phases.

Example: Study of profiles of Doxylamine[10].

Hydrophilic interaction liquid chromatography (HILIC) Column:

This column increases the time retention of polar compound and provide separation method for mixtures of ionizable/polar compounds.

Example: Separation of different classes of lipids.

Bridged Ethylene Hybrid 130 and 300 column

This column provides an improved characterization of peptide and protein, owing to the increase in resolving power.

Example: Analysis of amyloid β peptide in cerebral spinal fluid[10].

Bridged Ethylene Hybrid phenyl column:

Provides chemical stability, improved peak shape and reproducibility for wide range of analytes.

Example: Rapid analysis of 25 polymer additives is achieved by implementing UPLC method with Tandem Quadrupole along with BEH phenyl column.

Amide column:

The column is highly suited for analysis of carbohydrates due to compatibility with wide range of pH and elevated temperatures.

Example: UPLC-MS analysis of carbohydrates and separation of metformin.

Bridged Ethylene Hybrid 300 C4 Column:

This column is suitable for the high resolution separation of protein mixtures.

Bridged Ethylene Hybrid GLYCAN Columns:

This column provides superior high resolution of glycoprotein.

Example: useful for analysis Transtuzumab, drug which is useful for breast cancer treatment.

Charged Surface Hybrid (CSH) C18 Column:

It is a universal choice for C18 column under low pH, and mobile phase of weak ionic strength.

Charged Surface Hybrid Phenyl-Hexyl Column:

Suitable particularly for polyaromatic compounds. The CSH Phenyl-hexyl column provides excellent peak shape under low and high pH conditions.

Example: Analysis of Quinine, Labetolol, Diltiazem and Verapamil.

Hollow Structural Sections (HSS) C18 Column:

This is a general purpose silica-based C18 column which is applicable at low pH (Figure 4).

Example: Separation of xanthine alkaloids (Xanthine 0.31 min retention time, 7-methyl xanthine 0.49 min retention time, Theobromine 0.65 min retention time, Paraxanthine 0.78 min retention time, Caffeine 0.99 min retention time)

Hollow Structural Sections columns Cyano Column:

This column provides low hydrophobicity.

Example: Analysis of analgesics/ steroids.

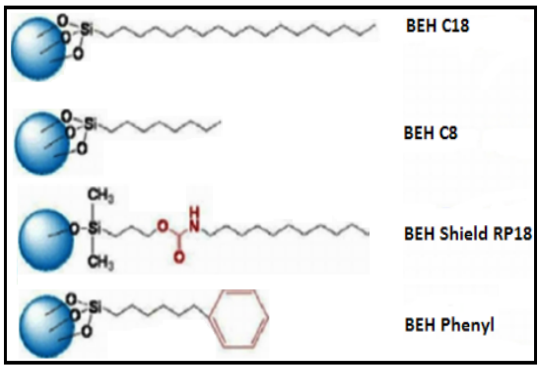

Fig. 4. UPLC Columns

Detector

The UPLC detector used should be able to provide a high sampling rate with narrow attainable peaks (1s half-height peak width) and little dispersion of the peaks so that less separated solute is wasted on the column. The UPLC methodology delivers two to three times the separation sensitivity of the previous method HPLC because of the detector method. Acquity photodiode array (PDA) and Tunable Vis-UV (TUV) detectors are utilized in the UPLC, with Teflon AF providing an internally reflecting surface that improves light transmission efficiency by removing internal absorptions. Path lengths are 10 nanometers, acquisition speeds are 20 (PDA) and 40 (TUV), and total internal capacity is 500 nanoliters. Detection by mass spectrometry has also been used with UPLC[21].

Advantages of UPLC (see Table 1) for comparison of characteristics of HPLC and UPLC)

The main advantage of UPLC is high resolution performance and rapid resolving power as well as it is more selective and sensitive. With lower operating costs and shorter run times, it also reduces process cycle time and ensures end-product quality. The use of a unique column material with very small particle size boosts sensitivity and allows for rapid examination. It reduces solvent consumption and expends the scope of multiple residue methods.

- It offers selectivity and sensitivity with minimum runtime.
- Peak resolution is enhanced in many cases
- Expands the scope of multi residue methods.
- Less solvent consumption

Disadvantages of UPLC

Ultra Performance Liquid Chromatography has many advantages but there is some drawback like high-pressure required more maintenance and shelf life of column is short[22]. When compared to standard HPLC, UPLC has greater back pressures, which reduces column life. In UPLC, increasing the column temperature lowers the problem of back pressure. Furthermore, particles smaller than 2 µm are typically non-generable and so have a limited application.

Table 1: Differences between HPLC and UPLC in a nutshell

Characteristic	HPLC	UPLC
Size of particle	3-5 μm	Less than 2μm
Back pressure	35-40 Mpa	103.5Mpa
Analytical column	C18	BEHC18
Injection volume	5 μm	2 μm
Temperature	30 C	65 C
Run time	10 min	1.5 min
Resolution	3.2	3.4
Plate count	2000	7500
Flow rate	3.0 mL/min	0.6 ml/min

New technological advancement of UPLC

The most important advantage of ultra performance liquid chromatography is speed, able for high speed resolution[23]. UPLC with sub-2-µm porous stationary particles working with high linear velocity pressures > 9000 psi, was once combined with spectrometer properly working for the rapid separation of lipids and complex compound and their metabolites[24].

Comparison between HPLC and UPLC tabulated as below

Wu *et al.,*[25] developed novel analytical methods for the separation of 12 phthalates, and the

findings were compared to UPLC and HPLC results. The mobile phase consisted of a methanol and water gradient. The PDA detector was used to detect UV at 225 nm.A Waters UPLC with an Acquity UPLC BEH phenyl column (50 x 2.1 mm, 1.7 m) was used to separate the samples. The flow rate was 0.4 mL/min and the total run time was 7 minutes. The HPLC analysis was carried out using Agilent 1100 equipment and an Agilent SB-phenyl column (250 x 4.6 mm, 5m). The flow rate was 1.0 mL/min and the total run time was 18 minutes.In comparison to HPLC, analysis time was decreased by a ratio of 2.5 and solvent usage was reduced by a factor of 6.4 (Figure 5).

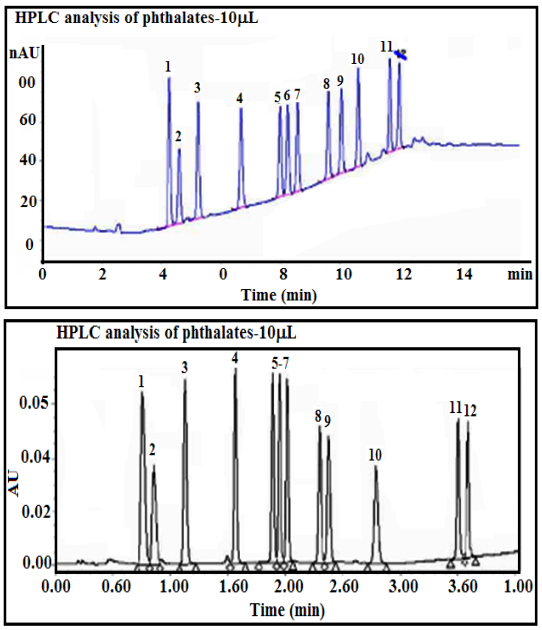

Fig. 5. Comparison of HPLC and UPLC analysis of 12 phthalates

Many analytical laboratories place a premium on analysis time, solvent use, and analysis cost. The amount of time it took to optimize new procedures was also drastically decreased. UPLC and HPLC techniques for determining ascorbic acid (AA) in fruit beverages and medicinal preparations were devised and compared by Inga Kleczka *et al.,*. Both procedures were quick, with complete analytical times of 15 and 6 min for HPLC and UPLC, respectivelyThe new UPLC method Fig. 5 was found to give superior separation than HPLC methods while lowering the run time from 18 to 7 minutes[26].

With the introduction of UPLC, new small particle chemistry and equipment technology for liquid chromatography has emerged, allowing for increased throughput and hence analysis speed without sacrificing chromatographic performance. The use of smaller particles in column packing allows for greater speed and peak capacity. It is possible to perform higher resolution procedures with the UPLC technology, using shorter columns, smaller packing particle sizes, and greater flow rates under high pressure. It goes without saying that boosting peak capacity increases data quality. Working with UPLC systems also results in a significant reduction in solvent usage and column equilibration time.In comparison to all previously employed chromatographic procedures, the injection volume in UPLC is lowered by almost five to ten times, resulting in a better peak band and lower carryover effects related to column diameter. The biggest downside of UPLC is the expensive cost of the device.

Applications of UPLC

Natural product and herbal medicine

Ultra Performance Liquid Chromatography has the ability to provide high quality of separation and detection capability of active compound which is present in mixture[27].

Examples:

a) Ultra Performance Liquid Chromatography is used for multiple components for quantitative analysis in example analysis of Hyangsapyeongwisan which is traditional medicine and used in gastric disease[28].
b) Ginseng species[29].
c) analysis of ascorbic acid and dehydroascorbic acid in liquid and solid vegetable samples[30].
d) for detection of pyrrlozidine alkaloids in herbal medicines[31].

Identification of Metabolites: UPLC/MS/MS32 offers unmatched sensitivity and accuracy in biomarker discovery[33].

Examples:

a) Fungal secondary metabolites[34].
b) UPLC-MSE was used for rapid detection and characterization of verapamil metabolites in rats[35].
c) UPLC-DAD-MS/MS was used in the metabolic of the medicinal grass Eleusine indica[36].

Drug Discovery: Useful in drug discovery process[37]. UPLC system by using acquity BEH

C18 column that method is faster and sensitive as compare to HPLC method[38].

Examples:
a) Mango leaf tea metabolites[39].
b) Analysis of fenofibrate in Human Plasma[40].
c) Determination of Mesa amine related impurities from drug sproduct by reversed phase validated UPLC method[41].

Method Development: Validation to reduce cost and improving opportunities for business success[42-44].

Examples:
a) butoconazole in active ingredient[45].
b) cefditoren pivoxil in API (active pharma ingredient)[46].
c) Glibenclamide in rat plasma[47].
d) UPLC method determination of sofosbuvir and daclatasvir in human plasma for therapeutic drug monitoring[48].

Combination study: Ultra Performance Liquid Chromatography coupled with photodiode and mass spectroscopy which can give rapid identification of compound along with sensitivity[49]. The coupling of UPLC with other devices different techniques is convenient and economical as compared to HPLC50-51.

Examples:
a) Evaluation of bisphenol[52].
b) Metabolites of Mequindox in holothurians[53].
c) UPLC-DAD-MS/MS was used in the metabolic of the medicinal grass Eleusine indica[54].

Impurity profile: Reversed phase UPLC methods are highly useful for quantitative determination of active pharmaceutical compound[55].

Examples:
a) Impurities in Maraviroc[56].
b) Determination of products and process impurities of asenapine maleate in asenapine sublingual tablets by UPLC[57].
c) Impurities of halobetasol propionate in cream[58].

Quality control: Reversed phase ultra performance provide a sensitive, rapid, and accurate result with less reagents cost and utilized in internal quality control in different dosage type[59].

Examples:
a) UPLC-QTOF/MSE a recent approach for identifying quality control analysis of fluctuation of xueshuantong lyophilized powder in clinic[60].
b) UPLC-Q-TOF/MS analysis and species differentiation for quality control of Nigella glandulifera[61].
c) UPLC method was developed for the quality control of rhubarb-based medicines.

Amino acid determination: The UPLC also suitable for analysis of different amino acids by coupling with MS technologies[62]. The methods are reliable, fast with high sensitivity and reputability[63].

Examples:
a) Method validation for amino acids[64].
b) Quantification of sulphur amino acids in aquatic invertebrates[65].
c) For quantify amines and amino acids in human disease phenotyping[65].
d) UHPLC-UV was applied for the analysis of total amino acid in infant formulas and adult nutritionals[66].

Determination of Pesticides: Combination of UPLC-MS/MS is effective for determination of pesticides. The instrument technique provides highly accurate with less matrix result[67].

Examples:
a) For pesticide analysis in different fruit and vegetable[63].
b) Analysis of residual pesticides and mycotoxins in cannabis[68].
c) Pesticides analysis of vegetables by UPLC in combination with mass spectrometry[69].

CONCLUSION

Ultra-Performance Liquid Chromatography provides much improvement over conventional HPLC. In fact, it has become the standard platform of HPLC. The main advantage is reduction of analysis time and solvent consumption. This is achieved by the use of small particle size and short column. An only drawback of UPLC could be high

back pressure which can be decreased through increasing column temperature. Throughout UPLC technique is widely acceptable and offers significant improvement of speed, sensitivity and resolution compared with conventional High Performance Liquid Chromatography.

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Conflict of interest

Authors state no conflict of interest.

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