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Review Article

Ultra Performance Liquid Chromatography (UPLC) -A Review

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ABSTRACT

Ultra performance liquid chromatography an improved version of conventional HPLC technique. UPLC mainly works on three areas speed, sensitivity and resolution which make this method a better process. Particle size less than 2µm is used in this method which help in separation. Using high pressure an increase in flow rate which pressurized mobile phase collision with column an increased in temperature reduces the viscosity of mobile phase all this aspect helps in drug development and UPLC help in analysis of pharmaceutical drug product. Ultra performance liquid chromatography saves our time a lesser run time and decreased in column length which directly affects separation process of UPLC. This technique enhanced sensitivity and obtained well resolved chromatographic peaks. in 21st century UPLC is a suitable technique for drug development within short period of time pharmaceutical industry are focusing towards this method.

Keywords: UPLC, High pressure, HPLC vs UPLC

INRODUCTION

Ultra performance liquid chromatography is an advanced technology of HPLC. Modern chromatographic separation techniques were developed in 1960 Era. HPLC has been most frequently used for analysis of drugs but it also has some limitation which indirectly affects the separation. While working on those drawbacks' scientist invent an improved version of HPLC [1].
One of the major factors in invention of new technique is packing material [column] a better packing material will directly affect the separation Van Demeter equation is sole of these technique HPLC and UPLC too every chromatography student is familiar with the equation. Van Demeter equation is the success mantra of ultra performance of liquid chromatography [1].

H= A+B/v+Cv

Where,
A – eddy mixing
B - diffusion
C - mass transfer
V - linear velocity

Van Demeter equation reveals the relationship between the linear velocity denoted by V and plate

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height [column efficacy, HETP] since particle size is a major factor for investigation of chromatographic performance this equation is used. The potential of UPLC to improve the analysis of the samples that are encountered during pharmaceutical development is explored. Specific significance has been placed on determining whether UPLC can decrease analysis times without compromising the quantity and quality of the analytical data generated [2-3]. Chromatographic separation was expanded and improve with respect to the stationary and mobile phase constituent, linear velocity, sample volume, and detection wavelength.

UPLC VS HPLC

PARAMETER	UPLC	HPLC
Particle size	Less than 2µm	3-5µm
column	ACQUITY UPLC BEH C18 AND C8	ALLTIMA C18 ZORBAX C8
Injection volumn	2µl [std in 100% methanol]	5µl [std in 100% methanol]
Column dimension	150×2.1mm	150×3.2mm
Column temperature	65ºC	30ºC
Flow rate	Less	More

Although the principle of both technique are same but still some difference which make HPLC a conventional method and UPLC a modern technique. The simple technique available to cut down an analytical run is to reduces the column length and increase the flow rate This approach however, could be risky, because complex mixtures of compounds will not be separated sufficiently and column efficiency will be much lower [4]. The basic difference is column material particle size which less than 2-µm. Which make a big deference in performance. This allows high- speed analyses with high efficiency, but the negative aspect is high back-pressure generation which is not acceptable for conventional HPLC systems and conventional analytical columns. The third way of shortening analysis times is raise the temperature due to which Mobile phase viscosity is reduced at higher temperatures [4-5]. In UPLC technique we found better resolution and separation are found as compared to HPLC also perform more sensitive analysis. Presences of monolithic columns make UPLC technique an improved version of HPLC.

Small particle chemistry

UPLC mainly works on three areas: ‘‘speed, resolution, and sensitivity’’. This technique uses fine particles (less than 2µm) so automatically reduces the length of column, saves time and decreases solvent consumption. (Cristiana C. Leandro a, 2005) UPLC is comes from HPLC. HPLC has been the evolution of the packing materials which effect the separation. Principle of HPLC indicate that as we reduced column packing particle size efficiency and resolution increases. As decreases in particle size to less than 2µm, there is a significant increase in effectiveness and it’s doesn’t decline at increased linear velocities or flow rates according to the common

Van Deemter equation, $H = A + B/u + Cu$

During drug discovery the focus was on “need for speed” that has came from by the choosing so many of samples in different kind of laboratories, and the use of sophisticated high maintenance instrument like UPLC with detector as mass spectrometers [6]. The property of column that is small columns and rapid flow rates (amongst other parameters) has been used. While analyzing drugs rise in temperature having the dual advantages decrease in viscosity, and raise mass transfer by increasing the diffusivity of the analytes. Some advantages in HPLC like robustness, ease of use, good selectivity and adjustable sensitivity. Along with that one disadvantage is the lack of efficiency as compared to GC or the capillary electrophoresis because of decrease in diffusion coefficients in liquid phase, include easy diffusion of analytes in the stationary phase [6-7]. Better efficiency is one of the main advantages of UPLC along with rapid analysis and this is only achieved by smaller particle size. The Van Deemter equation dictate that increase in efficacy with the use of smaller size particles size but this results in speedy increase in backpressure.

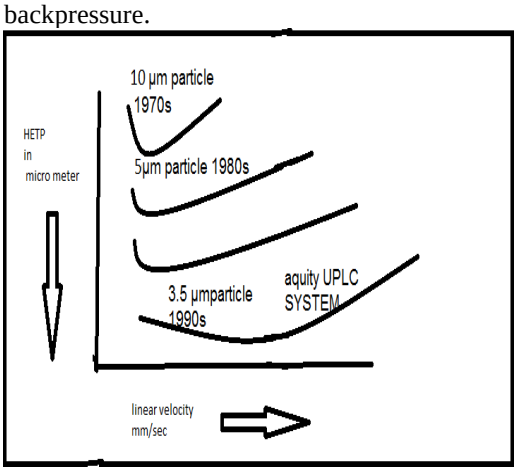

Figure 1- van Demeter plot, illustrating the evolution of particle sizes over the last three

decades while maintaining a bearable loss of load to improve the
Work at higher temperatures allows high linear velocity by Decreasing the viscosity of mobile phase which significantly Decrease back pressure.
Use of monolithic columns contains polymerize porous support structure that provides lower flow resistances than conventional particle-packed columns with the help above two parameter UPLC improved in three areas.

1. Produced Chromatogram with resolved peak.
2. Fast analysis
3. Sensitive analysis [7-9].

UPLC gives a greater range of linear velocities while keeping better chromatographic resolution and therefore can provide more rapid analysis time. The high chromatographic resolution, which results in an increased signal/noise and narrow peak width compared with conventional HPLC [9].

Instrumentation
It consists of
A] Sample injection B] UPLC Columns C] Detectors

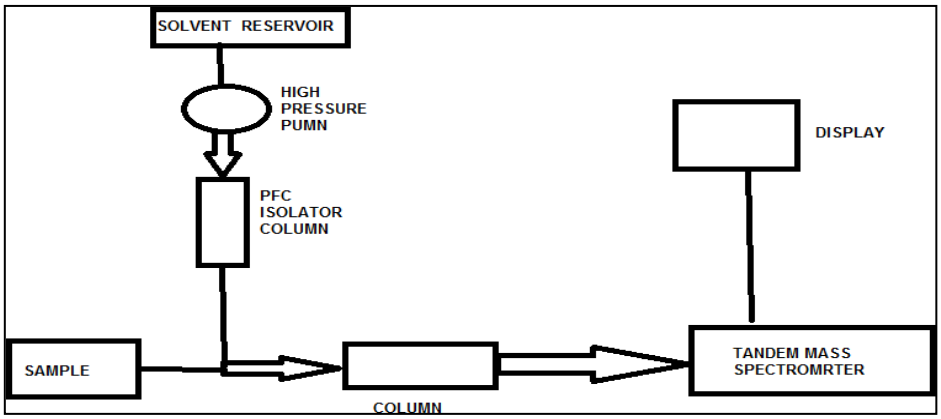

Figure 2 - schematic representation of UPLC

Sample injection

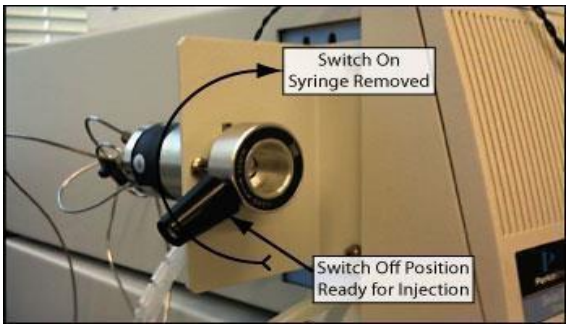

Figure 3- sample injection

A solution of small volumn consist a sample in the mobile phase. Sample injector is used here to add accurate measured. Formal injection valve may be manual or programmed to assure column safety from high pressure damaged [10]. Accurately must be done by sample injection. To gain high sample capacity injection cycle time should be fast to fully capitalized the speed afforded by UPLC Low volume injection with minimal carryover are needed to increased the sensitivity [10].

UPLC column
Efficiency of a particle packed column is much better due to increase in resolution to 1.7 µm Separation of the components of a sample requires a bonded phase that provides both retention and selectivity. We have four bonded phases for UPLC separation technique.

1. ACQUITY UPLC BEH T M C18 and C8 (alkyl columns)
2. ACQUITY UPLC BEH Shield RP 18 (polar group column)
3. ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl) [18]
4. ACQUITY UPLC BEH Amide columns (amide phase)

ACQUITY UPLC BEH T M C18 and C8 Columns – almost for all UPLC separation technique these columns are most commonly used which provide large pH range. Superior low pH stability generates by including tri-functional ligand bonding chemistries. This low pH stability is united with the high pH stability of the 1.7µm BEH particle to deliver the immense usable pH working range [11-12].

ACQUITY UPLC BEH Shield R18
Columns – These are arranged to hand over choosiness that complement the ACQUITY UPLC BEH T M C18 and C8 Columns.

ACQUITY UPLC BEH Phenyl Columns apply a tri-functional C6 alkyl ethyl among the silyl functionality and phenyl ring.

ACQUITY UPLC BEH Amide columns along with a tri-functionally bonded amide phase, hand over unusual column life time, thus enhance assay robustness. BEH Amide columns allow the use of a large range of phase pH [2 –11] to simplify the extraordinary retention of polar analytes spanning a broad range in polarity, structural moiety and Pka [12-13].

Detectors

Figure- UV detector

UV/Visible detector are used in UPLC analysis Detection of analytes is formally based on absorbance that is concentration sensitivity detectors. (Patil*, 2015) In general Than any other analysis uv-visible range for HPLC is used more frequently so they are economically low cost and tend to be one of the first which lipid analysts have permission The newly developed UPLC method for all active compounds in silymarin was found to be capable of giving shorter retention time and keeping better resolution than that with formal HPLC method. (Hong Liua, 2009) A decreased in cross-section means the light path is reduced, and transmission drops with increasing noise. Therefore, UPLC sensitivity would be compromised if a formal HPLC flow cell were used [13].

Detectors used in UPLC technique are as follows

A] Tunable ultraviolet detector
B] Photo diode array detector
C] ELS Detector

TUV detector [Tunable ultraviolet detector]

The analytical cell, with a capacity of 500 neon liters and a path length of 10 mm, and Foremost sensitivity flow cell, with a capacity of 2.4 micro liters and a 25 mm path length, both Make use of the flow cell technology. The TUV detector operates at wavelengths ranging from 190 to 700 nm.

PDA [Photo diode array detector]

The PDA (photodiode array) detector is also called as ultraviolet/visible light (UV/Vis) Spectrophotometer that run between 190 and 500 nm. Detection limits are higher for pda detector therefore caution should be taken while analysis (J.R. Bertolín, Malondialdehyde determination in raw and processed meat products by UPLC-DAD and UPLC-FLD, 2019) The detector offers two flow cell options. The cell, having the volume of 500 nano liters and a path length of 10mm, and high sensitivity flow cell, with a volume of 2.4 micro liters and a 25 mm path length, both utilize the flow cell technology [13-14].

ELS detector [evaporative light scattering]

This evaporative light scattering detector designed for use in the UPLC technique. So far execution same or even better has been showed by using stationary phases of size around 2µm without the unfavorable effects of high pressure. In addition, the phases of lower than 2 µm are commonly non-regenerable and thus have restricted use [15].

Significance of UPLC

- Increased in flow rate makes UPLC a better separation technique.
- Due to rise in temperature mobile phase viscosity decreased.
- Takes less time more précised and accurate technique.
- Better speed, sensitivity and resolution we get.
- Attached with mass spectrometer to further analysis of analyte.
- Fast analysis of drug product due to reduced in particle sized less than 2µm.
- This technique enhanced sensitivity and more resolved chromatographic peak are obtained.
- Ultra performance liquid chromatography has elevated sensitivity and specificity.
- Operation cost is reduced [16].

Disadvantages of UPLC

- UPLC analysis due to increased in the pressure the life of the column get decreased.
- Due to pressure rise this technique will need more maintenance
- Temperature rise in this technique will lead to instability need time to time maintenance.
- detector and data collection system that may not cope with fine peaks.
- Till now only binary pump systems (not ternary or quaternary). This may make method transfer not straight forward [17-18].

Applications

1. Determination of Pesticides in Ground water:To determine trace level pesticide in ground water UPLCTM-MS/MS used in specific time and fast too. The technique has intensified the analysis speed, sensitivity, and resolution.
2. Improved Resolving Power in Peptide Maps:

To characterized protein peptide mapping is necessary Due to abnormal decrease in the column dispersion and instrument itself the analyzes of tryptic digest of phosphorylase by UPLC technology provides substantial rise in resolution, peak capacity, and sensitivity compared to HPLC [19].

3. Analysis of Traditional Chinese Medicines: UPLC method is used to control the quality of traditional Chinese medicines.TCM is very complicated matrix in which all the component play a unique role for the overall success. Therefore, the analysis of all the constituents is simultaneously important for the quality control.
4. Identification of Metabolite Biomarker invention is one of the analytical requirements by providing unmatched sensitivity, resolution, dynamic range, and mass accuracy [20].
5. Multi-Residue Analysis of Pharmaceuticals in Waste Water To determine various drug present in sample this technique is used for waste water treatment plant [21-23].
6. Impurity Profiling: Impurity profiling should be well organized for ingredient detection and separation of all the impurities present in the active compound.
7. Forced Degradation Studies: To identify degradation product and to reduced time and to developed stability indicating method UPLC-specific photodiode array and MS detection is used [24].
8. Dissolution Testing: Dissolution testing an important tool in formulation, drug development and production to control the quality of drug and release of it [25].
9. Bioequivalence /Bioanalysis Studies Statistical pharmacokinetic data and several other purpose obtained by using accurate data generated at low detection of UPLC/MS/MS [26].
10. Toxicity Studies: UPLC permit accurate detection of toxicity or drug-drug interaction due to high resolution further, its sensitivity also permit the detection of peak at low concentration.
11. Therapeutic Drug Monitoring Simultaneous estimation of 2β- lactmase inhibitor and seven β-lactum antibiotic n plasma done by UPLC- MS/MS method. As compare to other technique this technique is quite faster (5.5 min /sample)[26].
12. UPLC based metabonomic applications: For drug discovery biotransformation of new drug [NCE] is necessary this technique is usefull for invention of biomarkers for the sake of several disease. When a compound touch the development stage, metabolite identification becomes a govern process [27-28].

CONCLUSION

Ultra performance liquid chromatography is one of the newest techniques we have for analysis of drugs by using this technique we can perform various analysis programmed this technique UPLC is a helping hand for our pharmaceutical sector for analysis purpose and other too. Mainly it works on three areas speed, resolution and sensitivity which makes this technique better than conventional HPLC technique. Van deemter equations is sole of this method column dimension and high flow rate is one of the reason for the success of this technique.

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