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UPLC in Pharmaceutical Analysis: Review

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ABSTRACT

The pharmaceutical industry's primary goal is to reduce the cost of developing new medicines while also improving the selection, sensitivity, and flexibility of their acquisition. The main objective can now be addressed separately. The UPLC method is a modified HPLC method that combines high pressure with small particles (less than 2 µm) used in the column, resulting in a shorter column length and less solvent consumption. The core policy of UPLC is based on van Demeter's statement describing the relationship between linear velocity and plate height. UPLC is involved in three areas of development: speed, resolution, and sensitivity. This is a newly developed HPLC category with the same basic goal and improved chromatographic performance as the others. This update is an attempt to integrate the UPLC's policy, instruments, and implementation. This review introduces UPLC theory and summarizes some recent works in the field.

INTRODUCTION

The introduction of ultra-high performance liquid chromatography (UPLC) marked a significant change by allowing analysts to access fast analysis techniques without sacrificing the high-quality results previously obtained at top liquid chromatography (HPLC) performance. Many laboratory experts believe that UPLC will eventually supplant all conventional HPLC technology. UPLC is based on HPLC, and its primary goal is to reduce particle size in a packing column, increasing efficiency and thus resolution. According to the Van Deemter equation, reducing particle size to less than 2 µm improves efficiency and does not decrease with increasing linear velocity or flow rates. [1] The link between linear velocity (flow rate) and plate height is defined by the van Deemter equation (HETP, or column efficiency). The van Deemter curve can also be used to examine chromatographic performance as the particle size is one of the variables. [2] According to Van Demeter's statistics, the efficiency of the chromatographic process is equivalent to particle size reduction. One of the terms (path dependent term) is dependent on particle diameter packed in the analytical column, according to his model describing the band stretch, which describes the relationship between length equal to a theoretical plate (HETP) and linear velocity. The small particle diameter can significantly reduce HETP, resulting in high performance and a Van Deemter Curve profile that is flat (Figure 1). [3]

$$H = A + B/v + Cv$$

A tested formula that describes the relationship between linear velocity (flow rate) and plate height is shown above (HETP, or column efficiency). Van Demeter's curve can also be used to evaluate chromatographic performance because the particle size is one of the variables. Carrier gas flow rate, where A, B, and C are constants and v represents the linear velocity.

A = Eddy mixing

B = Axial diffusion

C = Solute’s mass transfer

A is unaffected by velocity, indicating "eddy" mixing. When the packed column particles are homogenous and small, it is the smallest. Axial diffusion, or the natural diffusion molecule propensity, is denoted by the letter B. Although this effect vanishes at high flow rates, the

term is separated by v. In the separation process, term C is induced by kinetic resistance to equilibrium. The kinetic resistance diminishes from the gas phase through the stop and returns packing phases. The higher the gas flow, the more the molecules in the pack are typically left behind by the traveling molecules. As a result, the term equals v.

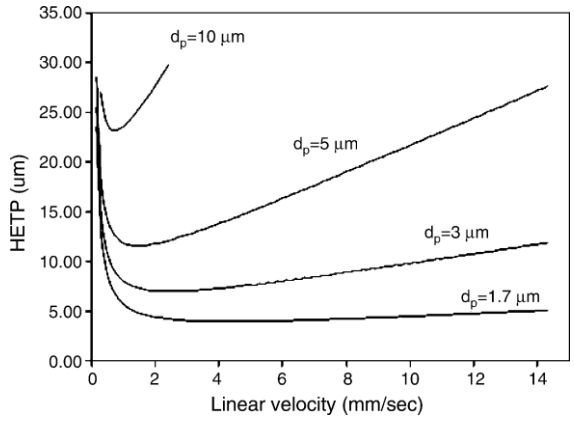

Fig. No. 1: Van Deemter curves for different particle sizes (10, 5, 3, 1.7µm).

Comparison between UPLC and HPLC

Working on the same principle but different performance.

The UPLC operates on the same principles as the HPLC, with the exception that the particle size is less than 2 m in size. Which makes a significant difference in performance once the benefits of these columns have been maximized, resulting in a powerful, robust, and reliable solution? With such a flow needle design, the Quaternary Solvent Manager (QSM) and Sample Manager (SM-FTN) of the UPLC H-class give all the flexibility and usability of your current HPLC while yet reaping the benefits of active separation that only UPLC can achieve. [4]

Table No. 1: Comparison between UPLC and HPLC

Sr. No	Characteristics	UPLC	HPLC
1.	Particle Size	Less Than 2 µg	3 to 5 µg
2.	Maximum Back Pressure	103.5 MPa	35-40MPa
3.	Analytical Column	Acquity UPLC BEH C_{18}	Altima C_{18}
4.	Column Dimension	150 X 2.1 mm	150 X 3.2 mm
5.	Column Temperature	65^0C	30 ^{0}C
6.	Injection Volume	2ml (Std in 100% MeOH)	5ml (Std in 100% MeOH)

Small Particle Chemistry

Except for smaller particles commonly employed in HPLC, promises made by the van Deemter equation cannot be fulfilled. The design and development of sub-2 mm particles is a significant challenge, but researchers have had a good start in this area. Although high in efficiency, 1.5 mm drill particles are commercially accessible, but due to their small surface area, they have poor loading and storage capacity. UPLC must use perforated new particles that can withstand high pressures to maintain retention and capacity, similar to HPLC. Silica-based particles have good performance potential, but they can endure a variety of challenges, such as a limited pH range and basic analyst tailing. Although polymeric columns can circumvent pH restrictions, they have downsides such as low efficiency and strength. The development of sub-2 mm particles is a huge task, and researchers have been working on it for some time to invest their resources. Although high-quality, non-porous 1.5 mm particles are available for trade, their low surface area results in inadequate load capacity and retention. UPLC, like HPLC, must use innovative porous-resistant particles at high pressures to maintain retention and strength. The mechanical strength of silica-based particles is good, but they may face several disadvantages, such as a pH limitation list and the tailing of basic analysts. Polymeric columns can bypass pH restrictions, but they come with their own set of problems, such as low efficiency and limiting power. [5]

Instrumentation

The UPLC system's Basic Terms and Conditions are similar to those of the HPLC system, apart from instrument and hardware development. A binary melting system, sample manager, column manager, and detector are all included in the UPLC system. Dual flow pumps are being used by Solvent Manager to produce a compatible binary gradient blended at high pressure. Dual flow pumps are used by Solvent Manager to produce a compatible binary gradient combined at high pressure. The portable phase, which is selected by the valve of up to four solvents, is harmed by the power removal system. The UPLC system can withstand pressures of up to 15,000 psi (about 1000 bar), allowing it to fully exploit particles smaller than 2 mm. The sample manager also features cutting-edge technology that allows the sample to be analyzed. Temperatures can be lowered to below 0°C., and the manager can control column temperatures up to 90°C by using extremely high temperatures. The ability of liquid chromatography (UPLC) to greatly reduce analysis time without decreasing efficiency is likely. [6]

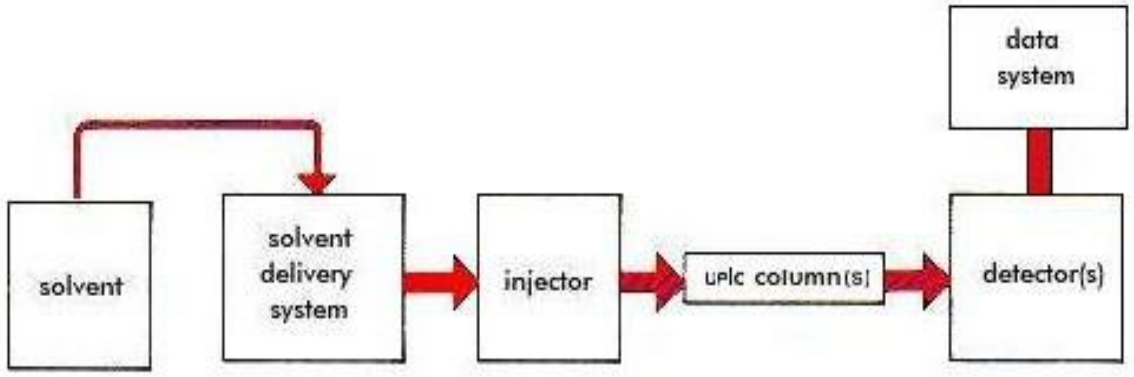

Fig. No. 2: – Schematic diagram of UPLC

Sample injector

Sample injection is very important for UPLC. Extreme pressures to be obtained from the UPLC are not carried out through regular injection valves. To prevent the hazardous effects of extreme pressure fluctuations, the process of sample injection needs to be pulse-free and the swept volume also needs to be minimal to reduce band spreading.

Pumping System

Pumping System Reducing the particle size requires a wide range of pressure. Pumps should therefore be designed in such a way that they can deliver the solvent smoothly again at such high pressures, which can work in both methods of isocratic and gradient division.

UPLC columns

- Particle design<2.0 µg is a challenging task. Columns of the UPLC must be constructed using porous or non-porous materials with high resistance to high pressures for finding storage and volume similar to HPLC. For this, silica-based particles can be used with good machinery and power, but with many evils like the tail of basic analysts, approximate pH range, etc. Polymeric columns overcome pH limitations but have lower efficiency as well as limited power. [7]

- **Waters**

- Introduced XTerra, first-generation hybrid chemistry. It possessed properties in both silica and polymeric columns, such as mechanical strength, high efficiency, and a wide pH range. Development power required by UPLC, second generation columns prepared by

bridged ethane hybrid (BEH), is unable to withstand high pressure and maintain high pH. In these BEH columns, efficiency is in direct proportion to its length and vice versa, equal to the particle size. Therefore, the use of these columns caused high divisions as well.

- Improved spectral quality.

  •ACQUITY UPLC BEH T M C18 and C8 (straight chain alkyl columns)

  • ACQUITY UPLC BEH Shield RP 18 (embedded polar group column)

- **Agilent**

- Introduced regular phases having 1.8 µg particle sizes. The phases used were:
- C8 and C18 Zorbax stable bonds for low pH range.
- Zorbax Extend C18 for a high pH range.
- Zorbax XDB-C8 and C18 for general purpose
- Zorbax-SB CN, which provides a different reversed-phase polarity.

- **Alltechs**

- It gives particle sizes of 1.5 µg. The company introduced Platinum HPLC columns to control an additional area that provides two separation methods as well as expands the range of polar selection.

• Pro sphere HP ZAP C18 for high speed reversed phase separation.

• Altima HP HILIC is a non-bonded, silica column for hydrophilic interaction chromatography separations.

- **Bischof**

Introduced three porous phases having 1.8 µg particle size and one nonporous silica phase with a particle size of 1.5 µg.

• Pronto PEARL

• TPP-C8

- ACE EPS (8 % carbon loading)
- C18 EPS (16 % carbon loading)

Detector

All standard detectors can be used on its microcolumn and capillary LC. Refractive Index (RI) adoption usually received only a little attention in Micro column and capillary LC, due to intrinsic difficulty arising from the fact that the RI difference is appropriate measured in very small volumes. However, with high power to combine lasers, nL volumes can be easily investigated. UV absorbers are the most common detector used even in microcolumn and capillary LC with column acquisition being the first method Fiber optics have been introduced to collimate the excitation light onto the flow cell and for the collection of the UV light that has passed through the flow cell, however, the sensitivity obtained was less than the column detection. The introduction of optical longitudinal flow cells a Path length of up to 3-8 mm is also tested. Photodiode array (PDA) detection was used mainly in micro-column LC. Despite the minimum optical length of the PDA flow cell, ten times lower acquisition limits were reported in capillary LC, by comparison to normal LC. [8]

Applications

Application of UPLC in the pharmaceutical industry, the need for UPLC analysis is enormous due to the unique features of the UPLC as a high resolution in the chromatogram, a short-term analysis that performs additional analysis functions in a short time with important, reliable, and accurate data. A scientist can generate more accurate data on the UPLC faster. The UPLC method is used to analyze herbal products. The need for the UPLC is very high because the method developed by them is accurate and precise, and this enhances research knowledge of analytes at the nano level.

1. Clinical chemistry

Recent developments in UPLC-based metabolomics, the approach of biomarker diagnosis of various diseases, and discuss their importance in clinical chemistry. [9]

Table No. 2: - UPLC-based metabolomics applications for discovering biomarkers of diseases in clinical chemistry

Conditions	**Application**	**Biological medium**
UPLC–QTOF/MS	HCC with liver cirrhosis	Serum
UPLC–QqQLIT/MS		
UPLC–QqQ/MS		
UPLC–MS	HCC	Serum
UPLC–QTOF/MS	HCC	Serum, Urine
GC–TOF/MS		
UPLC–QTOF/MS UPLC–	HCC	Serum
MS/MS		
UPLC–QTOF/MS	HCC	Serum
UPLC–QqQLIT/MS		
UPLC–QTOF/MS	HCC from liver cirrhosis	Serum
RRLC–QTOF/MS	HCC and CLD	Serum
UPLC–QTOF/MS	HCC	Urine
UPLC–QTOF/MS	HCC with HBV or HCV	Serum
HPLC–TQ/MS		
UPLC–QTOF/HDMS/MS^{E}	HCC	Urine
UPLC–QTOF/MS	HCC and liver cirrhosis	Feces
UPLC–LTQ Orbitrap	HCC	Liver tissue
XL-MS		
UPLC–QTOF/MS	HCC	Plasma
UPLC–TQ/MS		
GC–TOF/MS		
UPLC–QTOF/MS	Hepatitis B cirrhosis	Urine
GC–TOF/MS		
UPLC–QTOF/MS	Acute and chronic liver	Plasma

	Failure	
UPLC–QTOF/MS	Liver cirrhosis	Serum
UPLC–QTOF/HDMS/MS^{E}	HBV	Urine
UPLC–QTOF/MS	Primary biliary cirrhosis	Serum
UPLC-QTOF/MS	Liver transplantation	Bile
UPLC–QTOF/MS	Lung cancer	Plasma
UPLC–QTOF/MS	Lung cancer	Plasma
RRLC–QTOF/MS	Lung cancer	Urine
UPLC–HILIC-QTOF/MS	Lung cancer	Plasma
UPLC–Orbitrap MS	Lung cancer	Serum, Plasma
GC–MS		
UPLC–QTOF/MS	Pneumonia	Plasma, Urine
UPLC–QTOF/MS	Colorectal cancer	Urine
UPLC–QTOF/MS	Colorectal cancer	Serum
GC–TOF/MS		
UPLC–QTOF/MS	Colorectal cancer	Serum
GC–TOFMS		
UPLC–QTOF/MS	Colorectal cancer	Urine
SPE-HPLC		
UPLC–QTOF/MS	Colorectal cancer	Colon
UPLC–QTOF/MS	Intestinal fistulas	Urine
UPLC–LTQ/MS	Prostate cancer	Plasma
GC–MS		
UPLC–QTOF/MS	Ovarian cancer	Serum
UPLC–QTOF/MS	Ovarian cancer	Plasma
UPLC–QTOF/MS	Chronic renal failure	Serum
UPLC–QTOF/MS	Acute kidney injury	Urine
UPLC–QTOF/MS	Renal nephrolithiasis	Urine
UPLC–QTOF/MS	Autoimmune diabetes	Serum
GC × GC–TOF/MS		
UPLC–MS/MS	Type 1 diabetes	Plasma

1H NMR		
UPLC–QTOF/MS	Type 2 diabetes	Serum
UPLC–QTOF/MS	Type 2 diabetes	Serum
1H NMR		
UPLC–QTOF/MS	Type 2 diabetes	Serum
GC–MS		
1H NMR		
HILIC/UHPLC–MS	Alzheimer's disease	Cerebrospinal
RP/UHPLC–MS		fluid
HILIC/UHPLC–MS	Alzheimer's disease	CSF, Plasma
UPLC–QTOF/MS	Cerebral infarction	Serum
UPLC–QTOF/MS	Acute cerebral infarction	Plasma
UPLC–QTOF/MS	Schizophrenia	Serum
GC × GC–TOF/MS		
UPLC–QTOF/MS	Schizophrenia	Plasma
1H NMR		

2. Dissolution Testing

Completion tests are very important to control the quality and release of the drug. On further release, the completion of volume processing is the level-limit action, so this becomes very important in such cases. The termination profile of the drug means reliability and batch-to-batch uniformity of API (Active Pharmaceutical Ingredients) in the formulation. These days, new and more powerful methods are coming to the market that requires improved analytical sensitivity. This can be fulfilled through UPLC, which provides online sample discovery. By using UPLC, the completion test can be fully completed. It is automatic as it can perform tasks such as data detection, analysis of aliquots, management of test results, and distribution. [10]

3. Application to a bioequivalence study

The UPLC–MS/MS method allows for the determination of FAV in human plasma at a low concentration level. The accuracy and precision of the method are within satisfactory limits and are important in bioanalytical testing. Simple sample adjustment and fast FAV numbers

allow the use of this method for pharmacokinetic studies. The improved approach was successfully used in bioequivalence research in healthy Egyptian volunteers. [11]

Table No. 3: - Stability of FAV in human plasma by the proposed method

Parameters	**Bias (%)**	**RSD (%)**
(a) Short-term stability; 25 ± 4◦C, 48 h		65
Spiked concentration at QCL[a]	−14.16	3.57
Spiked concentration at QCH[a]	−5.17	3.63
(b) Post-preparative stability in autosampler; 5± 3°C, 72h		
Spiked concentration at QCL[a]	−0.23	11.75
Spiked concentration n at QCH[a]	-4.42	4.84
(c) Long-term stability of analyte in the matrix at −80±10°C, 27days		
Spiked concentration at QCL[a]	−5.87	6.24
Spiked concentration at QCH[a]	−4.72	3.88
(d)Freeze and thaw stability;−20±5°C, 4cycles		
Spiked concentration at QCL[a]	-0.92	6.97
Spiked concentration at QCH[a]	-6.75	4.96
(e)Stock solution stability (analyte); 2–8°C. 10days		
Nominal concentration (30µg/ml)[a]	-7.91	-4.14
Nominal concentration (480µg/ml)[a]	-1.23	5.74
(f) Stock solution stability (IS); 2–8°C,10days		
Nominal concentration (3.5µg/ml)[a]	-0.95	3.32

4. Analysis of Contaminants in Foodstuffs

Sudan IV dye is commonly used in petrochemical industries. Deep Sudanese color dyes attract scams to enhance the color of a few spices and food additives that can create DNA-induced DNA adduct genetic modification. UPLC combined with tandem mass spectrometry allows Sudan identification at low ppb levels in spices and peppers containing food items. Figure (3) shows the Sudan IV chromatogram and contains the fastest peaks of Sudan II, III, and IV. These quick peaks are extra peaks as they were revealed a few minutes before the main peak combinations. [12]

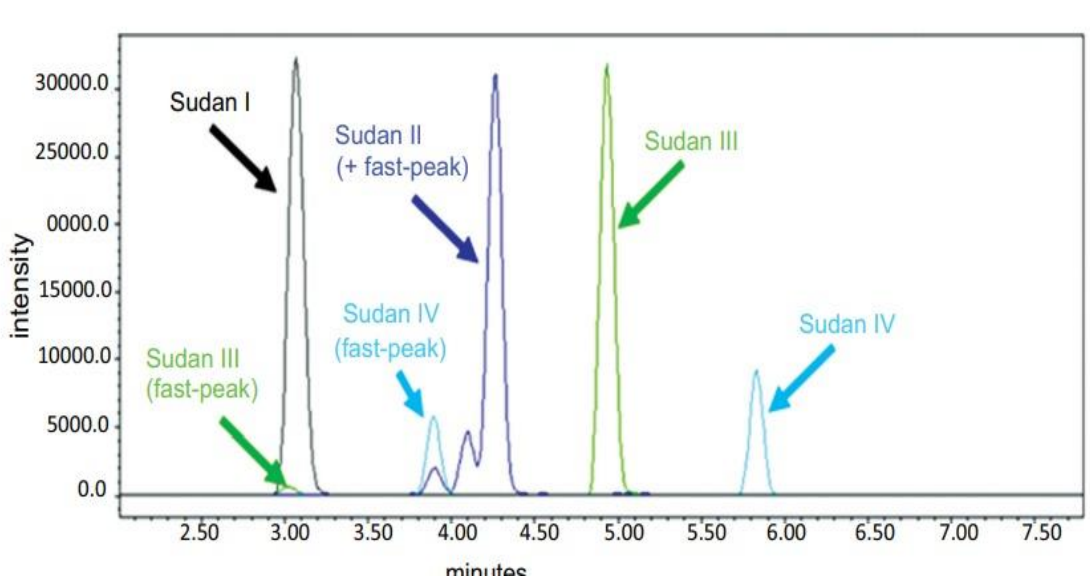

Fig. No. 3: Chromatogram of Sudan I – IV at 50 µg/L

5. Method Development / Validation

Method development and verification is a complex and time-consuming process. For the development of a robust and reliable approach, labs are required to study a wide range of combinations of parameters, e.g., cell phase, temperature, pH, column and chemical gradient, etc. UPLC is an important laboratory method that reduces costs and increases the efficiency of the required analysis to develop and validate the approach. With the UPLC, the separation speed increases as its efficiency develops, leading to the rapid development of methods. The high stability of UPLC columns provides an option for column temperature selection and pH from a wide range. [13]

CONCLUSIONS:-

UPLC is based on several proven technologies; the HPLC process also not only improves but also flours using the standard HPLC method when many scientists begin to face isolation barriers. The information provided per the unit work performed by the UPLC is much greater than the HPLC as it has high speed, resolution, and sensitivity. New chemistry and materials technology can provide more information on each unit of work as UPLC launches, fulfilling the promise of speed, resolution, and sensitivity predicted by liquid chromatography. Therefore, the transfer of the existing HPLC approach to the new UPLC approach is better and more beneficial.

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