A REVIEW ON ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

UPLC is an advanced liquid chromatography technology that uses advances in a variety of technologies, including apparatus and particle size, to obtain significantly higher liquid chromatography sensitivity, speed, and resolution.Pharmaceutical companies are under pressure to develop more effective instruments for medication analysis and estimate in the current environment. Therefore, the creation of a quick chromatography technique has become crucial for analytical labs.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 processing.

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 products.Ultra performance liquid chromatography is a better approach than traditional HPLC. Compared to the conventional system, the UPLC system can reduce analysis times by up to nine times and three times, respectively, by using 5 µm and 3 µm particle-packed analytical columns.This review presents the UPLC theory and provides an overview of some of the most current research in the area.

Keywords : High separation efficacy,UPLC,High pressure,HPLC ,Sensitivity ,Quantification ,Resolution,Cost effective.

Introduction

From past 30 year HPLC is the predominant technique to use for analysis in-laboratory but due to significant advances and innovation in instrumentation, detector design, data processing and particle size technology, leads to the development of Ultra Performance Liquid Chromatography (UPLC). Principle of UPLC basically remains same only with the help of technology; it achieves dramatic increases in resolution, speed and sensitivity of the liquid chromatography. UPLC Technology is applied throughout the world for providing Over the last three decades, HPLC has been the most widely used technique for laboratory analysis. However, substantial advancements and innovations in apparatus, detector design, data processing, and particle size technology have led to the creation of Ultra Performance UPLC, or liquid chromatography. (1)The UPLC principle essentially stays the same, but with the With the aid of technology, it is possible to attain significantly higher resolution, speed, and sensitivity of the chromatography in liquid form. Around the world, UPLC technology is used to provide advantage of time ,Cost and Quality.UPLC outperforms HPLC in terms of chromatography resolution, sensitivity, and cost effectiveness. It has a low solvent usage and a high analysis velocity.(2)

Fig 1: UPLC instrument

High Performance Liquid Chromatography (HPLC) is a traditional separation technique that has several benefits, including robustness, usability, superior selectivity, and customization sensitivity. Its primary drawback is that it is less effective than gas chromatography. (4,5) A

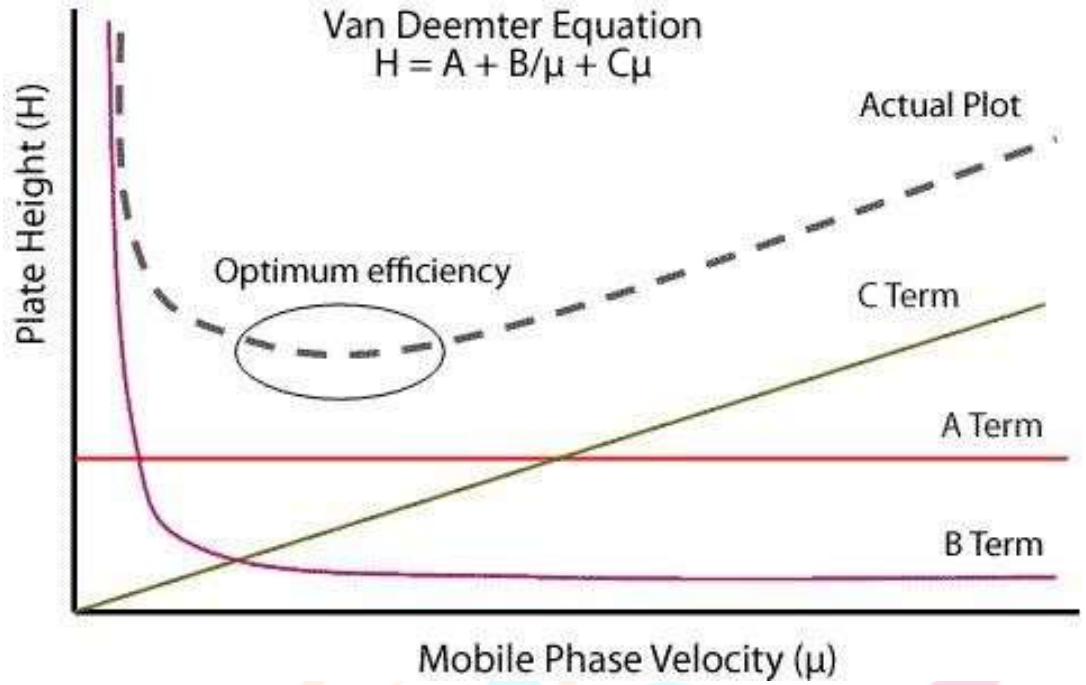
fundamental tenet of HPLC states that efficiency and, hence, resolution rise with decreasing column packing particle size. Efficiency increases significantly as particle size falls to less than 2.5µm, and it doesn't decline at higher flow rates or linear velocities in accordance with the standard Van Demeter formula. By employing smaller particles, peak capacity (amount of peaks resolved per unit of time) can be expanded to novel boundaries, a process referred to as Ultra Execution.(3) The Van Demeter equation is the key of liquid chromatography extreme performance .

H = A + B / v + C v Whereas

A: Eddy mixing Diffusion (B)

C stands for mass transfer. V stands for linear velocity.

This equation gives relationship between linear velocity and plate height.(6) The pharmaceutical businesses of today are searching for innovative approaches to reduce costs and accelerate the time needed to create new treatments while also enhancing the quality of analytical labs and their products are not an exception. in this pattern. The advantages of quicker analysis and The ultra performance liquid chromatography was thus created. UPLC offers the chance to increase and broaden the use of traditional HPLC.



Brief history

The novel method of chromatography was created in Warsaw in 1906 by Russian botanist T swett. That year, he was able to successfully separate various colored compounds, including xanthophylls and chlorophyll, by percolating vegetable extracts using a calcium

carbonate column. The calcium carbonate column functions as an adsorbent, allowing various compounds to be adsorbed to varying degrees. This results in the formation of colored bands at various positions on the column. T swett used the Greek words "chromatogram" and "chromatography" to refer to this system of colored bands and the method of using them. Graph's and chroma, which represent writing and color, respectively. (7-13)

Since then, significant progress has been made, and the technique is now utilized to differentiate compounds that are colored and those that are colorless. The stationary phase in the Tsweet method refers to the calcium carbonate column that stays in place.

The vegetable extract solution is referred to as the mobile phase since it travels or flows down the column. chromatography could be thought of as a separation technique where solutes are separated between a stationary phase and a mobile phase.(14-16.)The techniques of thin layer chromatography and ion exchange chromatography were first introduced in 1930.as a method of division. Paper chromatography was first introduced by Martin and Synge in 1941, and gas chromatography followed in 1952.

In addition to being used in analysis, this technology is starting to show promise as a way to prepare extremely pure molecules for use in the pharmaceutical business or in the production of pure chemicals. The chromatography techniques for bio-molecule separation are solely responsible for the recent remarkable advancements in the field of bioscience Later, alternative methods were developed, including as HPLC, which has long been a mainstay in many laboratories.subsequently with the recent introduction of a brand-new method known as UPLC (Ultra performance liquid chromatography).

Principle

The Van Demeter relationship serves as the foundation for UPLC and explains the relationship between plate height and flow rate (20) According to the van Demeter equation (i), the With the smaller particles, the flow range is substantially larger for good compared to larger particles outcomes (17-19)

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

Where "V" denotes the flow rate (linear velocity), "H" stands for height equal to the theoretical plate (HETP),

and A, B, and C are the constants. of the gas carrier. The objective is to reduce HETP to bolster the effectiveness of columns. The phrase A doesn't depend on speed and show the presence of eddy mixing. Well, it is smaller if the rows are full of tiny and homogeneous particle size. The letter B represents the tendency of the particles' natural diffusion. At This effect is less at high flow rates, hence this word is split by v. The kinetic is represented by the word C. opposition to-balance throughout the course of disconnection.(21) The time lag required to transition from the gas phase to the packing stationary phase and return is known as the kinetic resistance. A molecule on the packing tends to lag behind molecules in the mobile phase more with increased gas flow.

Consequently This phrase has a relationship to v.(22) Consequently, it is feasible to boost throughput and analytical speed without compromising chromatography performance.

Since the introduction of UPLC, liquid chromatography has required the creation of a new instrument system that can take benefit of the separation performance (via the decrease of dead volumes) and in line with the pressures, which are roughly 8000–15,000 PSI as opposed to 2500–5000 PSI in HPLC.Efficiency is inversely correlated with particle size and proportional to column length.Consequently, by the same factor as the particle size, the column can be shortened without resolution loss. The use of UPLC led to the discovery of further medicines metabolites, enhanced spectral quality, and greater separation.(22) **Instrumentation**

The only differences between the UPLC and HPLC systems' basic principles and instrumentation are in the updated hardware and instrumentation. The sample manager, column manager, detector, and binary solvent system make up the UPLC system. The manager of solvents delivers a parallel binary gradient that is combined at high pressure using two flow pumps.(23)] The mobile phase, which is chosen by a valve among up to four solvents, is degassed by the degassing system. The UPLC system can tolerate pressures up to 15,000 psi, or 1000 bar, in order to take full benefit of the particles less than 2 µm(24) Additionally, the sample manager has improved technology that allows for sample temperatures as low as 0°C but column Managers can use high temperatures to control column temperature up to 90°C.

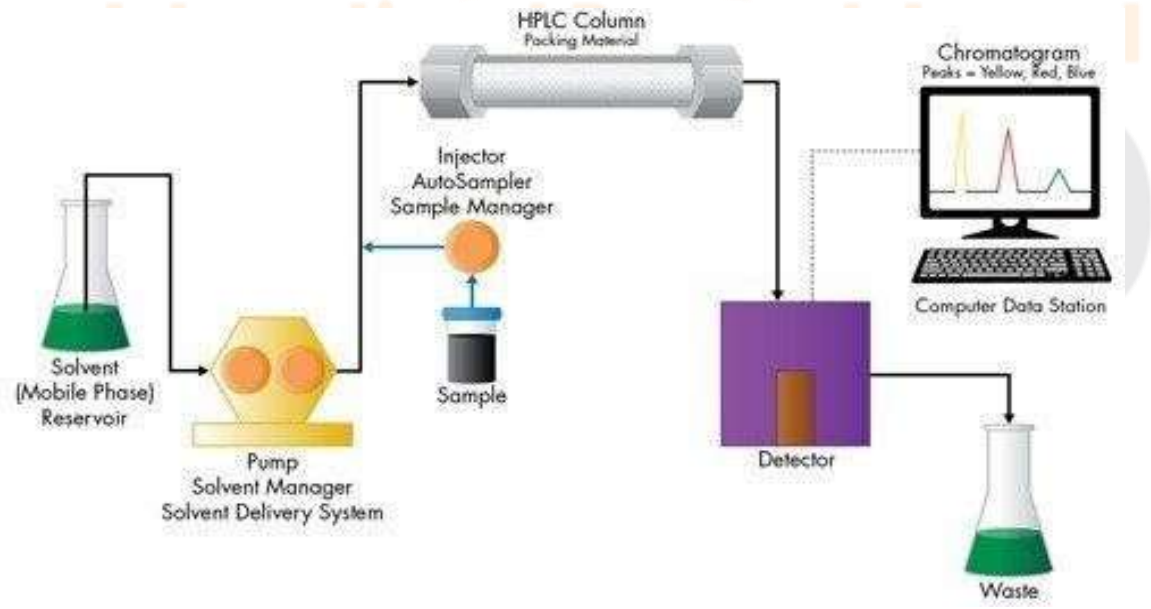


Fig 2:Schematic representation of UPLC

The basic instrumentation of UPLC are as follows:

1. Pumping System
2. Sample injection
3. UPLC columns

4.Column manger & heater or cooler 5.Detectors
6.Software's6 [36-41].

1. Pumping System

A wider pressure range is needed to achieve small particle, high peak capacity separations than what is currently possible with HPLC apparatus. The estimated pressure drop across a 15 cm long column filled with at the ideal flow rate for maximum efficiency 15,000 psi is equivalent to 1.7 μm particles. Consequently, a solvent-delivery pump at these pressures, consistently and smoothly, which can offset solvent compressibility and function in isocratic and gradient separation modes, respectively, is necessary. Two separate serial flow pumps are used by the binary solvent management to provide a binary gradient in parallel. There are up to four integrated solvent choose valves available.solvents. To fully benefit from this, the pressure must not exceed 15,000 psi, or around 1000 bar. particles less than 2 μm.(25-26)

There are two types of pumps:

A. Reciprocating pump
B. Pneumatic pump

A. Reciprocating pump

These pumps work by means of a diaphragm or reciprocating piston. Via an entrance valve, the liquid enters a pumping chamber, where it is forced out by a piston via an outlet valve. Reciprocating pumps work well at very high flows and are typically quite efficient.

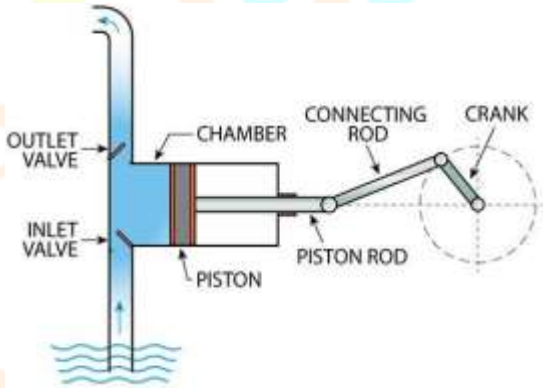


Fig 3 :Reciprocating pump

There are two types of reciprocating pumps:

a) The piston pump
b) The diaphragm pump

There are two types of diaphragm pumps:
-The hydraulically operated diaphragm metering pumps
-The air actuated type
-The hydraulically operated diaphragm metering pumps:Toxic and explosive fluids can be pumped with this kind of pump. The pump has a maximum head of 700 bars and a maximum transfer flow of 20 m3 per hour.
-Air actuated pump:The diaphragm's design and the available air pressure, which is typically 7 bar, limit the pump's capability. The greatest flow rate that is reasonable is 40 m3 per hour. attainable flow by using a bigger pump.

B. Pneumatic pump

Originally intended for standard liquid chromatographic separations, this kind of piston was shown to be loud and to produce intense flow pulses that could destabilize the detector. These days, liquid chromatography column slurry packing is its nearly sole application. It's the the most basic kind of pump that is capable of producing extremely high pressures.

Fig 4 : Pneumatic pump

2. Sample injection : A sample in the mobile phase is contained in a tiny volume solution.

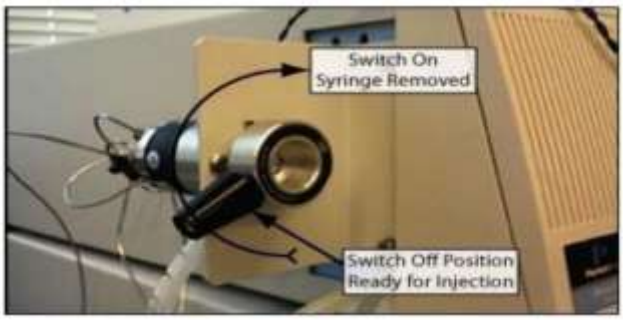


Fig:Sample injection

Here, an accurate measurement is added using a sample injector. A formal injection valve could automated or manually operated to ensure column safety due to damage from high

pressure . Precisely needed be carried out by injecting a sample. to obtain a large sample cycle time for capacity injection should be quick to fully profited from the speed that UPLC Low offered injection of volume with little carryover is required to raise the level of sensitivity .(27)

3. UPLC column

The improved efficiency of a particle-packed column can be attributed to the resolution enhancement to 1.7 µm.When a sample's components are separated, a bonded phase that offers both retention and selectivity. For UPLC, we have four bonded phases. technique of separation.(28)

However, a second generation hybrid technology17 known as ACQUITY was created in order to offer the kind of enhanced mechanical stability UPLC demands.1.7µm UPLC.ACQUITY particles connect the methyl groups. as illustrated in Figure 1 in the silica matrix, which improves their mechanical steadiness. A 1.7 µm increase in resolution particle-packed column due to its higher efficiency. For the components of a sample to be separated, a bonded.phase that offers selectivity and retention. Four There are bonded stages available for separations using UPLC:

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 These large pH range columns are most frequently utilized for practically all UPLC separation techniques. superior stability at low pH produces by utilizing ligated bonding with three functions. chemical reactions. This unstable pH is combined with the excellent pH stability of the BEH particle, measuring

1.7µm provide a vast acceptable pH working range [11–]QUITY UPLC BEH Amide columns (amide phase)

ACQUITY UPLC BEH Shield R18 Columns:These are set up to provide a complementary handover to the ACQUITY UPLC BEH T M C18 and C8 Column

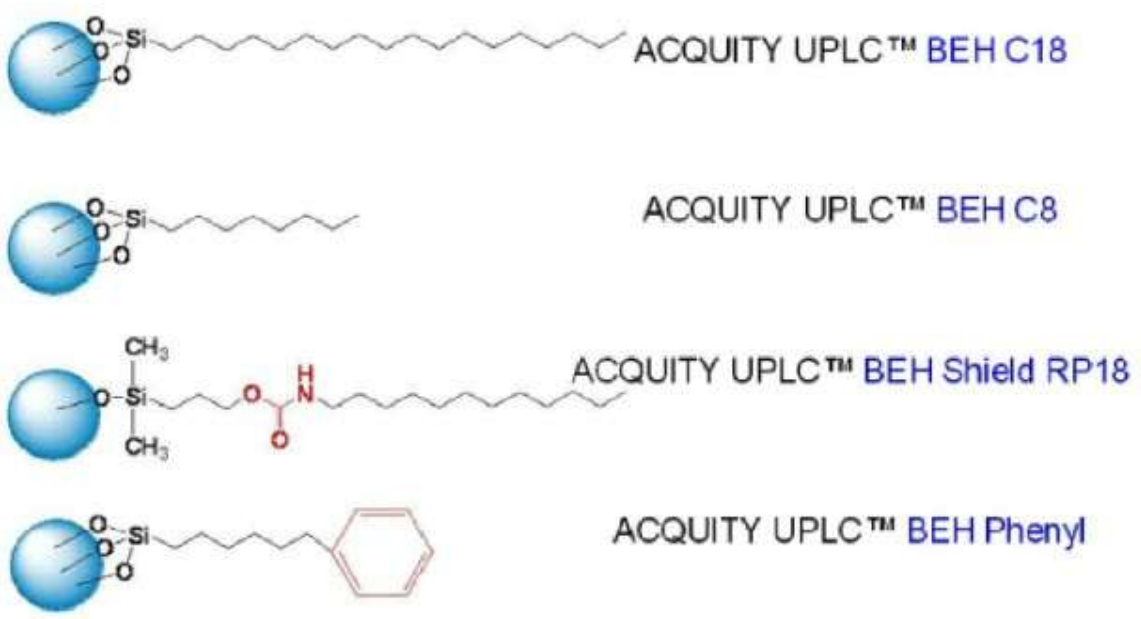


fig 6 : UPLC BEH Column Chemistries

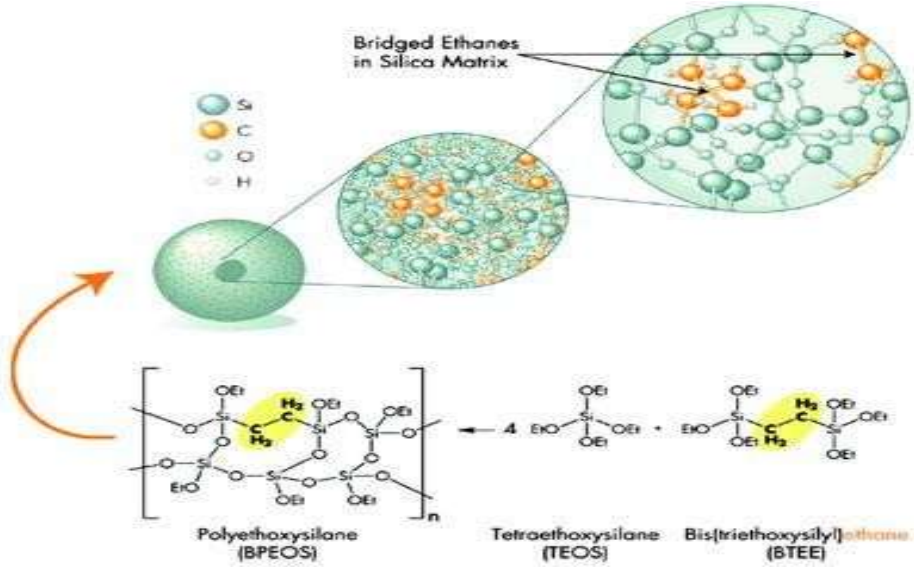


Figure 7 : Synthesis and Chemistry of ACQUITY 1.7µm particles for UPLC

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

ACQUITY UPLC BEH Amide columns in addition to a trifunctionally linked amide phase, transfer peculiar column life duration, so improving assay resilience. Amide columns from BEH permit the use of a broad phase pH range [2–11] to make the exceptional polar analyte retention over a wide variation in Pka, structural moiety, and polarity12-13

Detector

For UPLC detection, the tun-able UV/Visible detector is used which includes new electronics and firmware to support Ethernet communications at the high data rates. Conventional absorbance-based optical detectors are concentration sensitive detectors, and for UPLC use, the flow cell volume would have to be reduced in standard UV/Visible detectors to maintain concentration and signal. According to Beer's Law, smaller volume conventional flow cells would also reduce the path length upon which the signal strength depends. A reduction in cross-section means the light path is reduced, and transmission drops with increasing noise. Therefore, if a conventional HPLC flow cell were used, UPLC sensitivity would be compromised. The ACQUITY Tun-able UV/Visible detector cell consists of a light guided flow cell equivalent to an optical fiber. (29-33)

TUV detector

Fig:Tunable ultra violet detector(TUV)

The tun-able UV/Visible detector, which has new hardware and firmware to handle Ethernet connections at the high data rates, is utilized for UPLC detection. Because traditional optical detectors based on absorbance are concentration-sensitive, the flow cell volume would must be decreased in order to preserve signal and concentration in conventional UV/visible detectors. As stated Smaller volume conventional flow cells would likewise shorten the path length upon which Beer's Law would apply. The strength of the transmission varies. A smaller cross-section indicates a shorter light path, and As noise level rises, transmission decreases. Thus, in the event that a traditional HPLC flow cell were
employed, The sensitivity of the UPLC would be affected. The UV/Visible ACQUITY Tun-able detector cell is composed of an optical fiber-like light-guided flow cell Potential.l

PDA [Photo diode array detector]

Another name for the PDA (photo-diode array) detector is an ultraviolet/visible light (UV/Vis) spectrophotometer, which has a range of 190 to 500 nm.

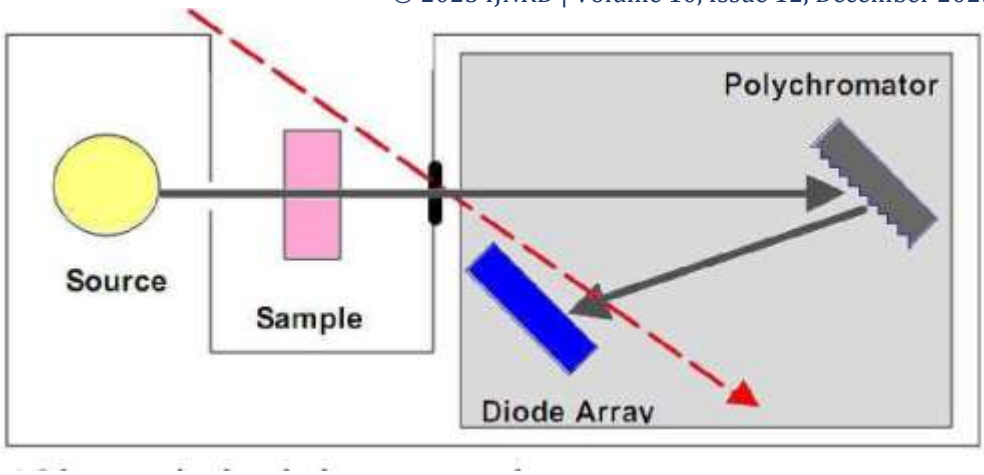


Fig:PDA detector

The high sensitivity flow cell has a volume of 2.4 and a route length of 10 mm. The cell has a volume of 500 nanoliters.Microliters and a route length of 25 mm both make use of the technique of flow cells.(34-36)

ELS detector [evaporative light scattering]

This evaporation 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 nonregenerable and thus have restricted use This light scattering detector is evaporative and is intended to be used with the UPLC method. So far, it has been demonstrated that utilizing the same or even superior execution stationary phases around 2 µm in size without the adverse consequences of elevated pressure. In addition, Phases smaller than 2 µm are typically nonregenerable, which limits their application .

Advantages

1. It raises sensitivity and shortens run time.
2. Offers the dynamic range, sensitivity, and selectivity of LC analysis. 3.Preserves the performance of the resolution.
4. Broadens the application of multi residue methods.
5. Utilizing a unique separation material with extremely tiny particle sizes allows for faster analysis(37-39)
6. Lower operating costs.
7. There is reduced solvent consumption.
8. It also helps to create more with less resources by reducing process cycle duration's.
9. .Boosts sample throughput and assists producers in producing more material that reliably meets and surpasses the product requirements, possibly removing failures in batches, unpredictability, or the requirement for material rework.

Disadvantages

1. This sort of column's lifespan is shortened by the need for frequent maintenance brought on by the increased pressure.
2. Furthermore, phases smaller than 2 µm are typically non-regenerate, which limits their utility.(40-41)

Table 1: HPLC vs UPLC

Sr. no.	Validation parameters	HPLC method	UPLC method
1	Accuracy (% Recovery)	99.16% – 100.42%	98.31% – 100.30%
2	Linearity (Concentration range)	20 – 80 µg/ml	10-40 µg/ml
3	Regression equation	y = 21577 x - 42557	y = 30742 x - 6515.2
4	Co-relation coefficient (R2)	0.9998	0.9997
5	Intermediate precision (% RSD)	0.12	0.07
6	Method precision (% RSD)	0.16	0.18
7	Robustness (% RSD)	0.21	0.25
8	Specificity (% RSD)	0.32	0.41
9	Limit of detection (µg/ml)	0.08 µg/ml	0.075 µg/ml
10	Limit of quantitation (µg/ml)	0.175µg/ml	0.150 µg/ml
11	Total analysis time (Minute)	15 minute	5 minute

Applications of UPLC

1. Analysis of Natural Products and Traditional Herbal Medicine

Herbal and natural product analyses are common uses for UPLC.UPLC offers superior separations and the ability to detect and identify active ingredients With incredibly intricate samples that originate from natural goods as well as conventional herbal remedies.

2.Identification of Metabolite

Drug discovery requires the bio-transformation of novel chemical entities (NCE). Once a substance reaches the stage of development, discovery of metabolites turns into a controlled process.UPLC/MS/MS provides unparalleled sensitivity, resolution, dynamic range, and mass spectrometry to meet the demanding analytical needs of biomarker identification,precision.

3.Bio analysis / Bio-equivalence Studies

Accurate and trustworthy data are produced by the low detection level sensitivity and selectivity of UPLC/MS/MS. that is versatile and may be applied to many different situations, encompassing an investigation of statistical pharmacokinetics.The UPLC/MS/MS method provides superior chromatographic sensitivity and resolution.

4.ADME (Absorption, Distribution, Metabolism, Excretion) Screening

Precise peak identification and integration in intricate matrices and additional data are made possible by the high resolution of UPLC.For samples, sensitivity enables peak detection.produced by incubation s at lower concentrations and pooling of samples. These are crucial for automated generic techniques since they lower failing sample analyses and avoid wasting time.

5 Manufacturing / QA / QC

UPLC is used for the highly regulated, quantitative analyses performed in QA/QC laboratories. The supply of consistent, high quality consumable products plays an important role in a registered analytical method.In QA/QC laboratories, highly regulated quantitative analyses are carried out using UPLC. The availability of reliable, superior consumable product performance a crucial part of an approved analytical procedure.

6. Analysis of amino acid

UPLC is utilized in the fields of protein analysis to provide precise, dependable, and repeatable amino acid analysis. descriptions, cell culture observation, and food analysis based on nutrition.

7. .Determination of pesticides

Triple Quadra tandem mass spectroscopy in conjunction with UPLC will aid in the identification of pesticide traces from the water.

8. Dissolution testing

It's crucial to test medications with increasing potencies when using sustained-release dose forms. The profile of dissolution is utilized to exhibit dependability and batch-to-batch homogeneity of the active component. Furthermore, More recent and effective formulas are needed. the sensitivity of the analysis.UPLC offers accurate and trustworthy automatic web sample procurement. Dissolution testing is automated by it, from pill drop to initiate the test.

9. Method development and validation

UPLC help in critical laboratory function by increasing efficiency, reducing costs, and improving opportunities for business success. UPLC column chemistry can easily translate across analytical-and preparation-scale separation tasks. UPLC provide efficiencies in method development: Using UPLC, analysis times becomes as short as one minute, methods can be optimized in just one or two hours. With UPLC, separation speed and efficiency allows for the rapid development of methodologies.

10. forced degradation Studies

Combining high-speed scan rates with the chromatography speed, resolution, and sensitivity of UPLC separations of photo-diode arrays tailored to UPLC and MS detection will boost identification confidence. breakdown products, hence reducing the duration necessary to create ways for indicating stability.

11. Impurity profiling

UPLC confidently detect impurities in compounds even at trace levels. UPLC combine with exact mass LCMS, which by operating with alternating low- and high-collision energies, known as MS, has been successfully employed for the identification of drug and endogenous metabolites.Even at minuscule quantities, contaminants in compounds can be reliably detected using UPLC.When UPLC and precise mass LCMS are combined, which by using a switch between low- and high-collision MS, also known as energies, has been effectively utilized for drug identification and natural metabolites.

12. **UPLC fingerprint**

The Magnolia officials cortex can be identified using a UPLC fingerprint .(42,43,44,45)

Conclusion

Chromatography. UPLC boosts efficiency in instrumentation and chemistry by offering more data per work unit as it delivers enhanced sensitivity, speed, and resolution for lower particle size in liquid chromatography. The primary benefit is a decrease in analysis time.which also equated to less solvent being used. An One drawback of UPL can be the increased back pressure compared to traditional HPLC. This Reducing back pressure can be achieved by raising the temperature of the column. It was discovered that the The UPLC's sensitivity was significantly higher than traditional HPLC. Every class of Pharmaceutical medicines are amenable to UPLC analysis. technique in a really brief amount of time and with reduced use of solvents In general, it appears that UPLC can provide substantial enhancements to resolution, sensitivity, and speed compared with Conventional HPLC.

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