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A Review on Ultra High Performance liquid Chromatography Method

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Abstract: ***UPLC is a modern technique which gives a new direction for liquid Chromatography. UPLC refers to ultra performance liquid chromatography, Which enhance mainly in three areas: "speed, resolution and sensitivity. Ultra Performance liquid chromatography (UPLC) applicable for particle less than 2µm in diameter to acquire better resolution, speed, and sensitivity compared With high-performance liquid chromatography (HPLC). In twenty first centenary Pharmaceutical industries are focusing for new ways to in economy and shorten Time for development of drugs. UPLC analysis at the mean time gives the better Quality of their products and analytical laboratories are not exception in this trend. The separation and quantification in UPLC is done under very high pressure (up To 100M Pa). As compare to HPLC, under high pressure it is observed that not Any negative influence on analytical column and also other components like Time and solvent consumption is less in UPLC.***

Keywords: Ultra performance liquid chromatography, High separation Efficiency, Cost effective, High pressure, Speed .

I. INTRODUCTION

Beginning in the 18th century, modern analytical chemistry has played a significant role in chemical innovation, particularly in a number of areas such chemical synthesis and qualitative and quantitative analysis[1]. Today, analytical chemists use a variety of instruments, including gas chromatography, HPLC, and more recently, UPLC. Other equipment include nuclear magnetic resonance (NMR), inductively coupled plasma, mass 4211 spectroscopy (MS), and gas chromatography. These analytical techniques have excellent advantages and are beneficial for environmental and biological laboratories in addition to chemistry labs.[2] . The most popular analytical tool among the aforementioned analytical techniques is HPLC. There were many technological advances in equipment and instrumentation throughout the 1970s. In the disciplines of biological, pharmacological, and other sciences, HPLC has sparked a revolution[3]. The first UPLC system that was marketed for use was exhibited in 2004. HPLC is no longer the standard platform; instead, ultra-Performance liquid chromatography has taken its place.

High performance liquid chromatography (HPLC) has Proven to one of the major analytical technique used in The qualitative and quantitative analysis of drugs worldwide. The packing material of the column is the Basic feature for the growth of this technique which Directly responsible for the chromatographic separations. The principle of separation of compounds is given by Van Deemter equation, which is an empirical formula That describes the relationship between linear velocity (flow rate) and plate height (HETP, column efficiency). According to the principle of separation of HPLC, as the Particle size of column material decreases, the efficiency Of the chromatographic separation, speed and resolution also increases. The HPLC is the most simple, economic, reliable and worldwide used technique in the Pharmaceutical analysis. [4]

However certain analytical requirements cannot be fulfilled by HPLC technique. Such as determination of complex samples such as formulation excipients, Biological samples, drug metabolites, degradation products, impurities, and drug isomers by HPLC, several problem arises related to determination of analytes at Low level (0.1%), speed of analysis and resolution per Unit time. For the need of high resolution separation, the researchers have been involved in the designing of sub- 2µm particles. The non-porous silica, porous silica and Polymeric particles of sub-2µm sizes were developed possesses their own characteristics of separation. Porous Packing materials can tolerate higher pressure and give much higher sample capacity than non-porous packings. UHPLC is now becoming an advance and modern Technique which gives a new direction for liquid Chromatography. UHPLC refers to ultra-high performance liquid chromatography, which enhance Mainly in three areas: speed, resolution and sensitivity. UHPLC applicable for particle less than 2µm in diameter to acquire better resolution, speed, and sensitivity compared HPLC. In twenty first centenary Pharmaceutical industries are focusing for new ways to In economy and shorten time for development of drugs. [5]

The separation and quantification In UHPLC is done under very high pressure (up to 100M Pa). as compare to HPLC, under high pressure it is observed that not any Negative influence on analytical column and also other components like time and solvent consumption is less in UHPLC.

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First time in the year 1999, Waters developed the hybrid particle technology (HPT) column for HPLC. the HPT is the combination of inorganic silica and Organic polymeric packings which has high mechanical Strength, efficiency, pH stability and peak shape for Basic compounds. The second generation hybrid material particle composed with bridged ethylsiloxane /silica Hybrid (BEH) structure was developed which provides Improved efficiency, strength and pH range. High Strength silica (HSS) particle technology has also been Used which increases the mechanical stability of silica which provides increased retention time and selectivity of compounds compared to hybrid particles. Charge Surface Hybrid (CSH) Technology was the latest Advancement in hybrid materials which contains surface charge within the packing materials to provide enhanced selectivity and better peak shape for entire range of ionic mobile phases . [6] From the above development in column packing material and particle size, waters company was given the trade name of UHPLC, which was known as UPLC.

A. 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 CaCO3 Adsorption Column by using M. S.T swett's method. Martin and Synge were awarded Nobel Prize for their discovery Of partition chromatography in 1941 [7] . 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 .

B. Principle

The difference between the compound's affinities for the stationary and mobile phases is the basis for HPLC's separation principle. After analytes have left a column, the detector can still identify them, and signals are stored in the data system.

C. Instrumentation

HPLC consists of following components:

1) *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.
2) *Injector:* To introduce a liquid sample into The HPLC system by injection, usually in the Range of 0.1 to 100 µl of volume.
3) *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.
4) *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 [8] .

D. Applications of HPLC

In several scientific disciplines, high performance liquid chromatography is frequently employed for the identification, quantification, and purification of a substance. Forensic science, environmental science, pharmaceutical science, and clinical analysis are a few of these applications. It is frequently utilised in dose form and quality control. It can be used to determine the shelf life of medicinal products and to identify various active metabolites. The examination of environmental materials, such as the finding of phenolic compounds in drinking water and contaminant biomonitoring, can also benefit from HPLC. HPLC technique [9] is also required for forensic applications such as the quantitative analysis of drugs in blood samples and the method for identifying steroids.

E. Ultra Performance Liquid Chromatography(UPLC)

By using packing material with particles smaller than 2 m, it created a unique route for liquid chromatography that addressed three key areas: speed, sensitivity, and resolution of assessment. The device is designed to withstand the extremely high pressure that the column experiences. Another benefit of ultra-performance liquid chromatography is that it uses less solvent. In contrast to customary high-performance liquid chromatography, [10].

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F. Principle of UPLC :

The ultra performance liquid chromatography is established on principle of Van Deemter Equation [11].

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

M= Flow rate

Smaller plate height value corresponds to Greater peak efficiency, as more plates can occur Over a fixed length of column (Figure 1).

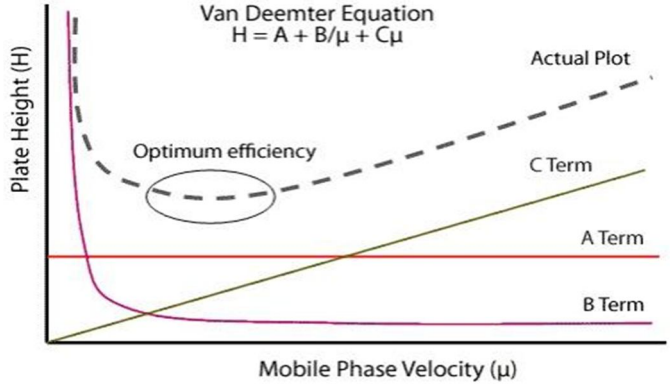


Fig. 1. Van Demeter Equation

G. Instrument of UPLC

The equipment used in ultra-performance liquid chromatography is essentially the same as that used in HPLC. It is intended to operate under significantly greater strain with little disruption and more frequent maintenance. New hardware and firmware are employed to support the tunable UV/Visible detector for UPLC detection. When data rates are high. The volume of the tunable UV/Vis detector, which has a 10 mm flow cell path length, is only 0.5 liters.

The instrumentation of UPLC includes:

1) Sample Injection

A little amount of solution containing the precisely measured sample in the mobile phase is added using the injector. Consistent and accurate injection technique is required. The injection method must be substantially pulse-free to prevent the column from excessive pressure instabilities. Conventional injection valves might be manual or programmed. The device's swept volume should be kept to a minimum to reduce the chance of band spreading. A brief injection cycle time is needed to fully capitalise on UPLC's speed. To maximise sensitivity, low volume injections with less carryover are necessary. The sample volume for UPLC is typically 2 to 5 L. Direct injection techniques are increasingly widely employed for biological material. Below is a flowchart of the UPLC (Figure 2).

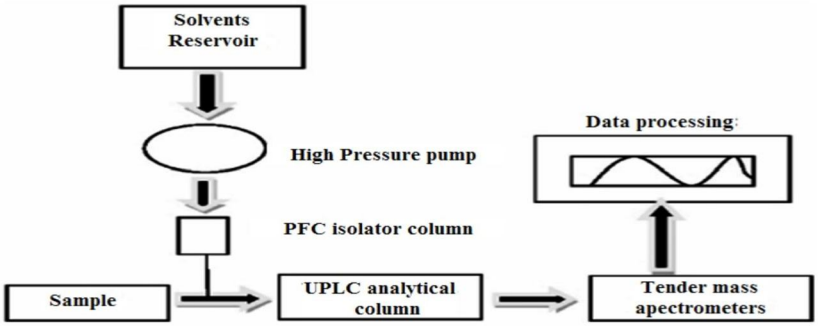


Fig. 2. UHPLC flow chart

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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:

2) UHPLC Columns

By providing higher quality chromatographic data in less time, the Acquity UHPLC column has been at the forefront of liquid chromatography (LC) column development. UHPLC columns are created, approved, and tested for usage in applying up to 15000 psi (1000 bar). However, FFig.3 shows how various technologies produced distinct types of columns that were actually used in UHPLC. [12]

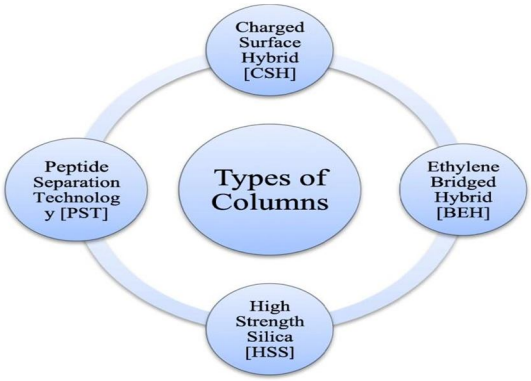


Fig .3. Types of column

a) Charged Surface Hybrid

Waters created a third-generation particle technology that is intended to recover sample loadability and peak tailing in mobile phase compositions with unknown ionic strengths. With 1.7 m particle size, the charged surface hybrid maintains the low level surface charge. Basic properties of the charged surface hybrid (CSH) C18 column, such as peak form and improved loading capacity, are particularly relevant for basic chemicals in a mobile phase with a low pH and weak ionic strength. The polyaromatic compound selective straight-chain-alkyl is especially used in Acquity UHPLC CSH Phenyl-Hexyl column and also it gives exceptional peak shape under different pH conditions . The Acquity UHPLC CSH Fluorophenyl columns show excellent selectivity for polar compounds, positional isomer, and halogenated compounds. This is due to a dipole-dipole, hydrogen-bonding, aromatic, and hydrophobic interaction .[12]

b) Ethylene-bridged Hybrid

To fully realise the potential speed, sensitivity, and resolution capabilities for first-generation methyl hybrid particles of x Terra columns, a mechanical strength or efficacy deficiency is required. Therefore, it is necessary to create a column with a new pressure-tolerant particle. Innovative columns made of extra hybrid materials that are ethylene-bridged were placed. In comparison to first-generation columns, it demonstrated improved efficiency, pH range, and strength. In addition to being used for UHPLC BEH. Phenyl columns, the developed ethylene-bridged hybrid (BEH) columns fixed the polar group connected to the silyl functionality with a C6 alkyl.[12]

c) High Strength Silica

High strength silica (HSS) is another type of column used in UHPLC. In UHPLC, high pore volume UHPLC Particles do not acquire the mechanical stability necessary to hold up the high pressure innate of UHPLC separations . For that, there is established a novel silica particle and appropriate morphology required to give long and lifetime efficiency UHPLC column at high pressure likely 1000 bars. HSS particle technology is the modern automation; 1.8 µm UHPLC HSS particles are designed and exclusively for separations using UHPLC . To overcome trouble during separation and retention of small water-soluble and polar organic molecules during reversed phase separation, Acquity UHPLC HSS T3 columns were developed. The Acquity UHPLC HSS C18 selectivity for bases (SB) columns is a non-endcapped, low-coverage silica-based C18 chemistry that alternate selectivity for water-soluble compounds influenced by silanophilic interactions. The enhanced silanol activity of the HSS C18 SB column result in greater retention of basic compounds; due to secondary interactions with residual silanols while simultaneously reducing the retention of non-basic analytes due to the low ligand density and ionic repulsion. [12]

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d) Peptide Separation Technology

The separation or isolation of different peptides, the peptide-based peptide separation technology columns, was utilized for analysis of peptides. Developed peptide separation technology (PST) columns are C18 BEH technology, in PST column particles sizes in the variety of 1.7 µm to 10 µm and the column dimension ranges from 75 µm to 30 mm internal diameter and column length from 50 to 250 mm. The PST columns demonstrate sharp-edged symmetrical peaks .[12]

3) Detector

In order to reduce the amount of separated solute that is wasted on the column, the employed UPLC detector should be capable of providing a high sampling rate with small achievable Peaks (1s half-height peak width) and little peak dispersion. Because of the detector technology, the UPLC approach provides two to three times the separation sensitivity of the prior HPLC method. The UPLC uses Acquity photodiode array (PDA) and Tunable Vis-UV (TUV) detectors, and Teflon AF offers an internally Reflecting surface that boosts light transmission efficiency by reducing internal absorptions. The total internal capacity is 500 nanoliters, the path lengths are 10 nanometers, and the acquisition speeds are 20 (PDA) and 40 (TUV). Mass spectrometry detection has also been employed in conjunction with UPLC[13] .

H. Advantages of UPLC

Various advantages of UPLC are as follows:

1) Require less run time and enhance sensitivity.
2) Provides the selectivity, sensitivity, and dynamic range of LC analysis.
3) In chromatogram resolved peaks are obtained.
4) Multi residue methods are applied.
5) Speedy analysis, quantify accurately analytes and related Products.
6) Uses of fine particle (2µm) for packing of stationary phase Make analysis fast.
7) Time and cost both are reduced.
8) Consumption of solvents is less.
9) More products are analyzed with existing resources.
10) Increases sample throughput and enables manufacturers To produce more material that consistently meet or Exceeds the product specifications, potentially eliminating Variability, failed batches, or the need to re-work material.
11) Delivers real-time analysis in step with manufacturing Processes.
12) Assures end-product quality, including final release Testing.

I. Disadvantages of UPLC

In UPLC analysis the main disadvantage occurs are life of Columns, during analysis high pressure developed because the Particle size. Increase pressure reduces the life of the columns. Due To increased pressure requires more maintenance and reduces the Life of the columns of these types. Using stationary phase of particle Size 2µm perform better analysis without the adverse effects of high Pressure. [14]

II. DIFFERENCES BETWEEN HPLC AND UPLC :

Characteristics	**HPLC**	**UHPLC**
Size of particle	3-5 µm	Less than 2µm
Back pressure	35-40 Mpa	103.5Mpa
Analytical Column	C18	BEHC18
Injection value	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

Table 1: Differences between HPLC and UPLC in a nutshell [15]

III. UHPLC METHOD OF VARIOUS DRUG AND THEIR RESEARCH OUTCOME

DRUG	CATEGORY	MOBILE PHASE	RESEARCH OUTCOME	AUTHOR
Teriflunomide	anti-inflammatory	Mobile phase of Acetonitrile : Water in the ratio of 60:40 v/v.	In this study, a unique and reliable method for teriflunomide quantification was developed. The developed RP-UHPLC approach was proven to be simple, economical, precise, linear, sensitive, and accurate for estimating Teriflunomide API and its marketed preparation.	Arun Maruti Kashid , Pranali Prakash, …..et.al (2022)[16]
Apixaban	Anticoagulant	A (MP-A) was prepared with buffer and acetonitrile 90:10 v/v, while (MP-B) contained water and acetonitrile 10:90 v/v	In this work, a new UHPLC method has been developed for the in-process control analysis of apixaban. Method development was supported by state-of-the-art chromatographic modeling software. The quality of the separation (resolution map) was studied in an extended knowledge space by combining three complementary design spaces.	Robert Kormany , Norbert Racz ,…..et.al (2021)[17]
Ropinirole Hydrochloride	Antidepressant	A and 70/30% (v/v) solution of acetonitrile/methanol as mobile phase B with UV detection at 250 nm within 75 min.	This work presented the development of a new UHPLC method for determining nine process-related impurities of ropinirole hydrochloride applying the AQbD approach. The current pharmacopeial method was not able to efficiently separate impurities E and H and impurities B and G, and therefore the accurate quantitative determination of these four impurities was impossible.	Tim tom , ales obreza ,……et.al (2020)[18]
Tedizolid and linezolid	Antibiotics Agent	(A) 0.1% formic acid in water and (B) Acetonitrile 0–0.3 min, 20–95% (B); 0.3–1.5 min,95–95% (B); 1.5–1.6 min, 95–20% (B);1.6–3.0 min, 20–20% (B)	In the present study, we developed a method for the quantification of linezolid and tedizolid in human plasma using LC-MS/MS. Sample preparation was conducted by a simple operation. Cost considerations are also required for routine TDM measurement in addition to method simplicity and the present method is considered useful .	Yu HC, pan CW,……et.al (2016)[19]
Daclatasvir	Antiviral Agent	10 mm Ammonium formate, pH 3.5and acetonitrile (50:50% v/v)	A new, simple, rapid, useful and cost-effective spectrophotometric methods have been developed for determination of DAC in pure form and tablets using cerium (IV) as oxidizing agent and validated as per the current ICH guidelines.	Rezk MR , Bendas ER, …… et.al (2016)[20]
Dendrobine	Anti-cancer Agent	(A) 0.1% formic acid and (B)acetonitrile. 0–1.0 min, 20–40 (B); 2min, 40–95% (B), 0.5 min, 95% (B);then decreased to 20% (B) within 0.1 min, maintained at 20% (B) for 0.4 min	Dendrobine, considered as the major active alkaloid compound, has been used for the quality control and discrimination of Dendrobium which is documented in the Chinese Pharmacopoeia .	Wang S, Wu H,……. Et.al (2016)[21]
Ibuprofen	Anti – Inflammatory (NSAID)	Water: methanol (35:65% v/v) both containing 10 mm ammonium acetate and 0.1% formic acid. 0–12 min, 35% (A) and 65% (B); 12.01–14 min 100% (B); 15.1 min, 35% (A) and 65% (B)	An enantioselective modified UPLC-MS/MS method was developed and validated for the quantitation of (R) and (S) ibuprofen enantiomers in human plasma .	He X, Zhang Y, ……. Et.al (2016)[22]
Oleanolic acid	Antiviral Agent	10 mm Ammonium Acetate with 0.1% formic acid in water: acetonitrile (10: 90% v/v)	A reliable high-throughput (UPLC-MS/MS) method was developed and validated for oleanolic acid (OA) determination in rat plasma and liver tissue using glycyrrhetic acid as the internal standard (IS). Plasma and liver homogenate samples were prepared using solid-phase extraction .	Li TX , Chu CS , …….. et.al (2016)[23]
TM-2	Anti-cancer Agent	(A) Acetonitrile and (B) 2 m mol/Lammonium acetate in water. 1.5 min, 60–90% (A); 2.8 min, 90% (A); 3.5 min, 90–60% (A)	Describes a rapid, sensitive and specific UHPLC-MS/MS method developed 219 for the quantification of paliperidone and its validation for the analysis of paliperidone in 220 beagle plasma. The UHPLC–MS/MS method has significant advantages over other 221 techniques including the simplicity of sample preparation .	Lin H, Zhao Y, ……. Et.al (2015)[24]
Berberrubine	Anti-cancer Agent	(A) 0.1% formic acid and (B)acetonitrile. 1.5 min, 30–60% (B);within 0.5 min, 95% (B); then decreased to 30% (B) within in 0.1 min, maintained 30% for 0.4 min	BBR, as a multi-target, multi-path plant extract, can interfere with the development of PCOS and related pathological process from many aspects, with less adverse reactions than conventional drugs. It is mentioned in this review that BBR can alleviate IR, reduce the level of serum androgen, alleviate abnormal lipid metabolism and chronic inflammation.	Wang X, Wang S,….. et.al (2015)[25]
Oxcarbazepine	Antiepileptic Agent	Acetonitrile–10 mm ammonium acetate (85:15% v/v)	A rapid and sensitive ultra performance liquid chromatography tandem mass spectrometry assay was developed for the simultaneous analysis of oxcarbazepine and its main metabolite in human plasma. The assay involves a simple solid-phase extraction procedure .	Bhatt M, …. Et.al (2015)[26]
Letrozole	Anti-cancer Agent	(A) Acetonitrile and 0.1% formic acid in water (B). 0 min, 20% (A): 80% (B);0.3 min, 20% (A): 80% (B); 2 min, 95% (A): 5% (B); 2.5 min, 95% (A): 5% (B); 2.6 min, 20 % (A): 80% (B)	A sensitive and selective UPLC-MS/MS method for determination of letrozole in rat plasma was developed. After addition of midazolam as internal standard (IS), protein precipitation by acetonitrile-methanol (9: 1, v/v) was used as sample preparation .	Cao G, Zhang Q , …….. et.al (2015)[27]
Solasonine	Anti-cancer Agent	(A) 0.1% formic acid and (B)acetonitrile. 0–0.5 min, 15% (B); 0.5–3.5 min, 15–75% (B); 3.5–4.0 min, 75–85% (B); 4.0–5.0 min, 85% (B)	In this work, a simple, sensitive and fast ultra performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) method was developed and validated for the quantitative determination of solasonine in rat plasma. Plasma samples were processed with a protein precipitation.	Chen Y, Zhang S,….. et.al (2015)[28]
Cephalomannine	Antibiotics Agent	0.1% formic acid in water (A) and acetonitrile (B)0–0.6 min, 20–85% (B); 0.6–1.8 min,85% (B); 1.8–2.0 min, 80–30% (B)	To the best of our knowledge, this is the first report of the determination of cephalomannine level in rat plasma using an UPLC–MS/MS method. The method offered sample preparation with a simple one-step precipitation of plasma protein by perchloric acid–methanol (1:9, v/v) and shorter run time of 2.0 min.	Wang XS, Sun JC,…….et.al (2014)[29]
Amphotericin - B	Antifungal Agent	(A) Methanol: acetonitrile (50 : 50% v/v) containing 0.1% formic acid; and (B) 10 mM ammonium formate (pH 3 ± 0.2), containing 0.2% formic acid and 1% acetonitrile 0.0–2.0 min, 35–90% (A); 2.0–2.7 min, 90–35% (A)	Amphotericin B (AmB) is the first-line agent for the treatment of life-threatening invasive fungal infection . A selective, sensitive and precise UPLC MS/MS method was developed to measure Amphotericin B concentrations in these patients .	Al – Quadeid BT , Radwan MA , ……. Et.al (2014)[30]

Table 2: U HPLC Method Of Various Drug And Their Research Outcome

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IV. APPLICATIONS OF UPLC

A. 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 .

Examples: 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 [11].

B. Identification of Metabolites

UPLC/MS/MS32 offers unmatched sensitivity and accuracy in Biomarker discovery .

Example: UPLC-MSE was used for rapid detection and Characterization of verapamil metabolites in Rats[12].

C. Drug Discovery

Useful in drug discovery Process. UPLC system by using acquity BEHC 18 column that method is faster and sensitive as compare to HPLC method .

Example: Determination of Mesa amine related Impurities from drug products by reversed Phase validated UPLC method [13].

D. Method Development

Validation to Reduce cost and improving opportunities for business Success .

Example: UPLC method determination of sofosbuvir and daclatasvir in human plasma for therapeutic drug monitoring [31].

E. Combination Study

Ultra Performance Liquid Chromatography coupled with photodiode And mass spectroscopy which can give rapid Identification of compound along with sensitivity. The coupling of UPLC with other devices different Techniques is convenient and economical as Compared to HPLC .

Example: UPLC-DAD-MS/MS was used in the metabolic Of the medicinal grass Eleusine indica [32].

F. Impurity Profile

Reversed phase UPLC methods are highly useful for quantitative Determination of active pharmaceutical compound .

Example: Determination of products and process Impurities of asenapine maleate in asenapine Sublingual tablets by UPLC [33].

G. 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 .

Examples: UPLC-QTOF/MSE a recent approach For identifying quality control analysis of Fluctuation of xueshuantong lyophilized Powder in clinic [34].

H. Amino acid Determination

The UPLC Also suitable for analysis of different amino acids by Coupling with MS technologies. The methods are Reliable, fast with high sensitivity and reputability .

Example: Quantification of sulphur amino acids in Aquatic invertebrates [35].

I. Determination of Pesticides

Combination Of UPLC-MS/MS is effective for determination of Pesticides. The instrument technique provides highly Accurate with less matrix result .

Example: Pesticides analysis of vegetables by UPLC In combination with mass spectrometry [36].

V. 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.

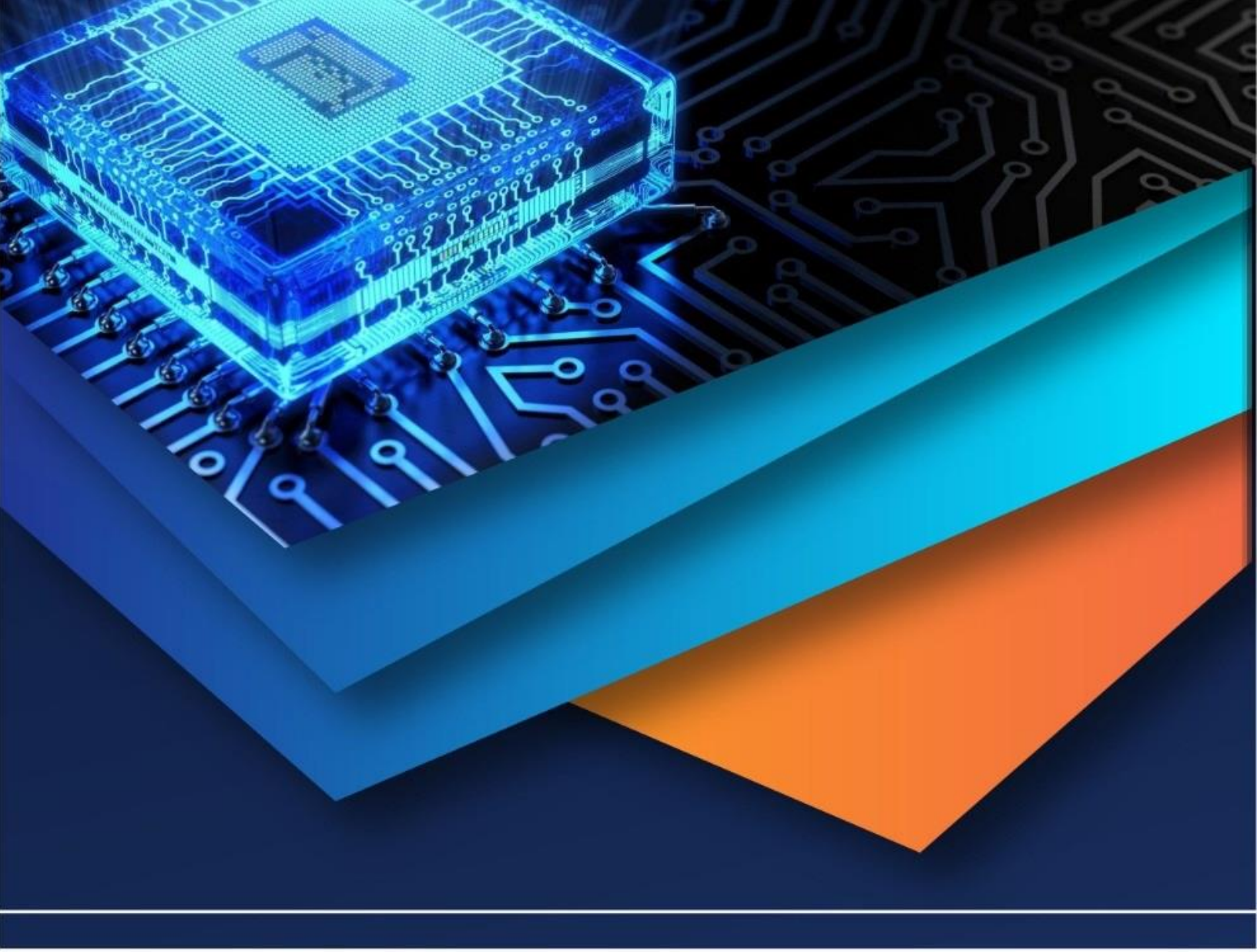
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An only drawback of UPLC could be high Back pressure which can be decreased through Increasing column temperature. Throughout UPLC Technique is widely acceptable and offers significant Improvement of speed, sensitivity and resolution Compared with conventional High Performance Liquid Chromatography.

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