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A Review on Ultra High Performance Liquid Chromatography

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ABSTRACT
Ultra Performance Liquid Chromatography (UPLC) can be regarded as a new direction for liquid chromatography. UPLC improves three areas of Liquid chromatography: speed, resolution, and sensitivity. It is an analytical method commonly used in analytical chemistry and in the pharmaceutical industry. Its operation is based on column chromatography that is often used to investigate mixtures. The use of which decreases the length of column, saves time and reduces solvent consumption. The quality analyses of various pharmaceutical formulations are transferred from HPLC to UPLC system. The separation on UPLC is performed under very high pressure (up to 100MPa).Ultra-high performance liquid chromatography was used to analyse acetonitrile extractions from 210 soil samples taken from four locations across the Geelong and Surf Coast Shire areas of Victoria, Australia from March 2020 to January 2021. It is reduces the cost of reagent with shorter run time as compared to conventional HPLC. This review introduces the theory of UPLC, and summarizes some of the most recent work in the field.

KEYWORDS: Chromatography, UPLC, Resolution, HPLC.

I. INTRODUCTION:

Chromatography is a separation process used to separate components in a mixture. In Greek Chromo ‟meaning “color” and Graphic ‟meaning “writing”.

Chromatography is a physicochemical method for separation of complex mixtures was discovered at the very beginning of the twentieth century by Russian–Italian botanist
M.S. Tswett.

Chromatography is used to separate mixtures of substances into their components. The components of the mixture are dispersed in a liquid solution known as the mobile phase, which holds it through a structure containing another substance known as the stationary phase.

Component separation requires differential partitioning between the mobile and stationary phases.

Any chromatographic separation technique must contain the three main parts as follows:

1. Sample 2. Mobile Phase 3. Stationary phase.

Stationary phase: it is the solid substance at which the mixture of the components will be separated and isolated, its nature is a solid or a liquid only. Mobile phase:it is a solid or liquid substance that carries a mixture composed of a sample to be purified, isolated and separated at the surface of the stationary phase.[4,5]

The liquid surface is immobilised by a stationary phase which results in making it a stationary phase.

The mobile phase :moves from the stationary phase and components get separated. The separation depends on different partition coefficients.

Ultra High Performance Liquid Chromatography (UHPLC)

Ultra-High-Performance Liquid Chromatography is similar to HPLC, in that it is a technique used to separate different constituents of a compound. Used predominately to identify, quantify and separate components of a mixture by using high pressure to push solvents through the column.

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

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created tohandle 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 chromatograph.

INTRODUCTION OF UHPLC: [1,2,6]

- Due to some limitation a new technique has been introduced by the scientist which is highly efficient and advanced and also overcome some of the limitation of HPLC and the technique popularly known as "Ultra Performance Liquid Chromatography (UPLC)".
- UPLC is regarded as new invention for liquid chromatography.
- UPLC brings drastic changes in sensitivity, resolution and speed of analysis can be calculated.
- It has instrumentation that can perform at higher pressure as compared to that used in HPLC & in this system uses fine particles (less than 2.5 μm) and mobile phases at maximum linear velocities reduces the length of column also reduces solvent consumption and saves time.
- This review introduces working principle of UPLC along with some of the most recent work in the field. According to the van Deemter equation, as the size of particles reduces to below 2.5 μm, there is a significant gain in efficiency. Therefore, by using smaller particles, speed and peak capacity can be extended to new limits, of liquid chromatography.

PRINCIPLE OF UHPLC [2,3,7]

- The ultra performance liquid chromate graphyis established on principle of Van Deemter equation of van Demeter is**:**[49]

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

The ultra performance liquid chromate graphyis established on principle of Van Deemter 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

- **Eddy diffusion-** The mobile phase moves through the column which is packed with stationary phase.

Solute molecules will take different paths through the stationary phaseat random. This will cause band broadening.

Longitudinal diffusion-The concentration of analytes is less at the edges of the band than at the center. Analyte diffuses out from the center to the edges. This causes band broadening.

- The eddy diffusion A is smallest when the packed column particles are small and uniform.
- The greater the flow of gas, more a molecule on the packing tends to lag at the back of molecules within side the cellular phase.
- Therefore, it is possible to increase throughput, and thus the speed of analysis without affecting the chromatographic performance.
- The advent of UPLC has demanded the development of a new instrumental system for liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent with the pressures (about 8000 to 15,000 PSI, compared with 2500 to 5000 PSI in HPLC).
- The application of UPLC resulted in the detection of additional drug metabolites, superior separation and improved spectral quality.
- The advent of UPLC has demanded the development of a new instrumental system for liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent approaches for biological samples.

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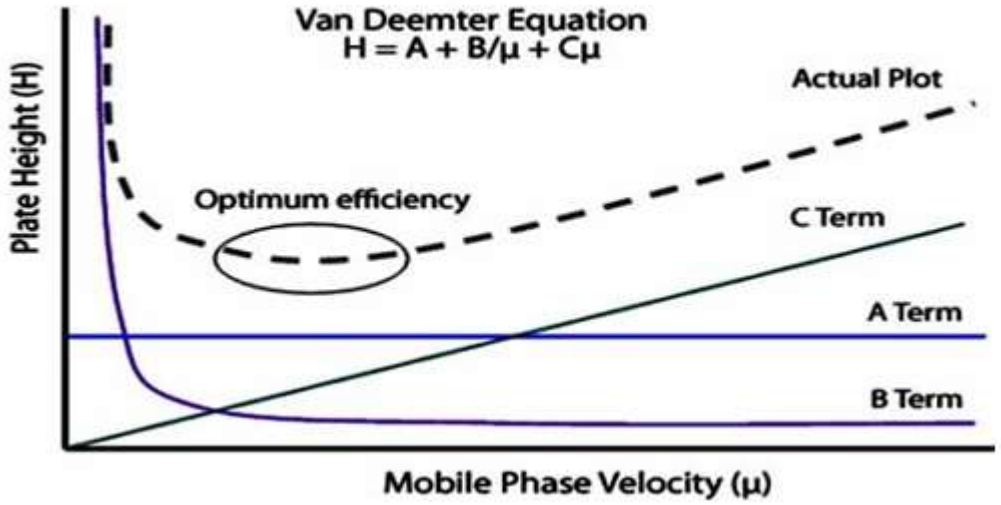
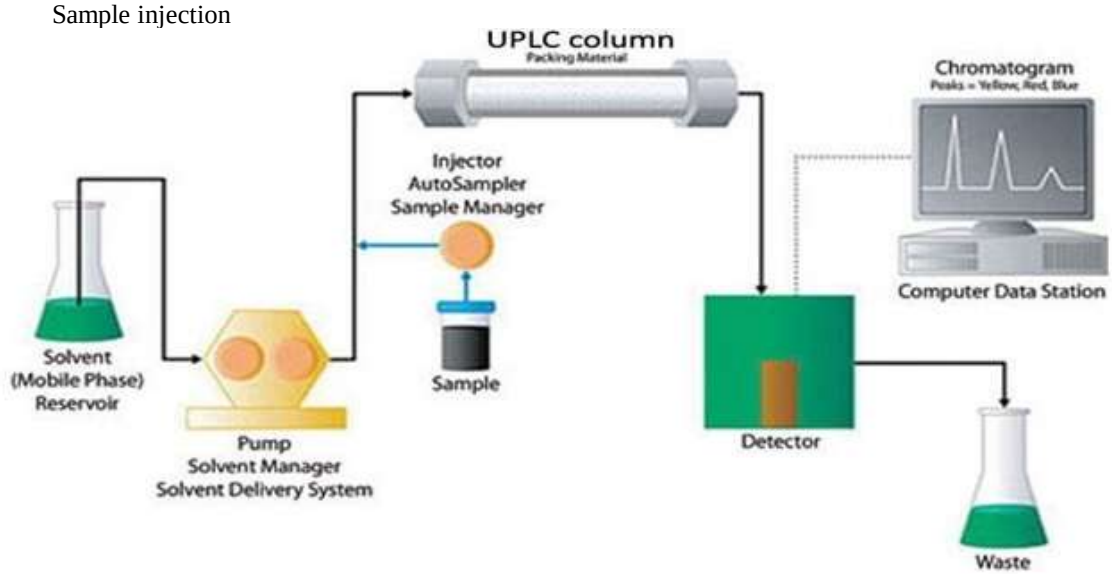


Figure 5: Van Deemter plot, illustrating the evolution of particle sizes over the last three decades.

INSTRUMENTATION: (1,2,6)

The various instruments used in the Ultra performance liquid chromatography are as follows (FIG. 6).

1. Sample injection
2. UPLC columns
3. Column manger & heater or cooler
4. Detectors



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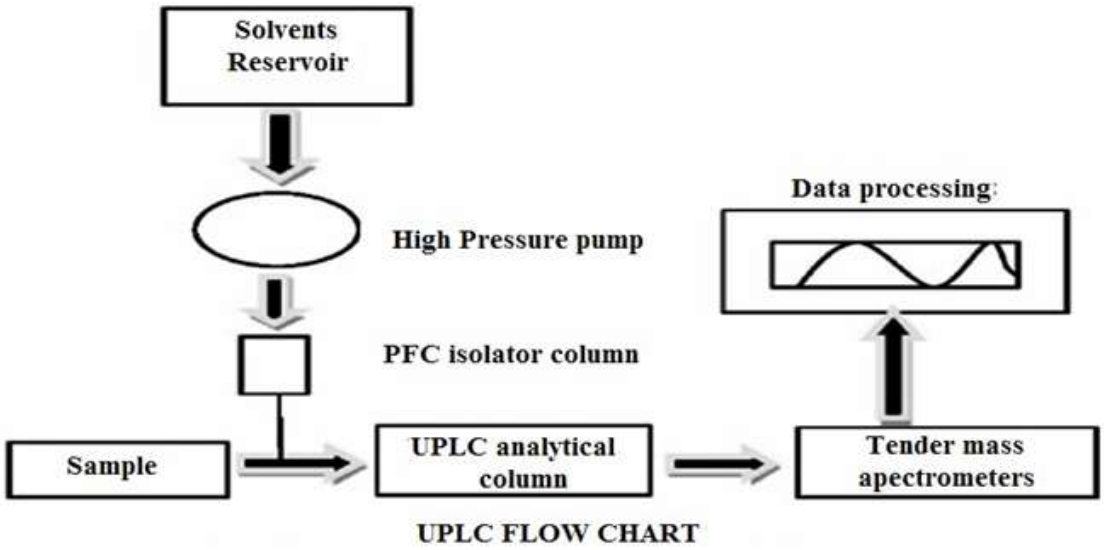

Figure 6: UPLC FLOW CHART

1) Sample System

Sample introduction is critical in UPLC. Conventional injection valves, automatic or manual, were not designed and hardened to operate under extreme pressures. To protect the column from extreme pressure fluctuations, the injection process should be relatively pulse-free and the device sweep should also be minimized to reduce the possibility of band spreading. To take full advantage of the speed offered by UPLC, short injection cycle times are required, which requires high sample volumes. Increased sensitivity also requires small injections with minimal carryover.

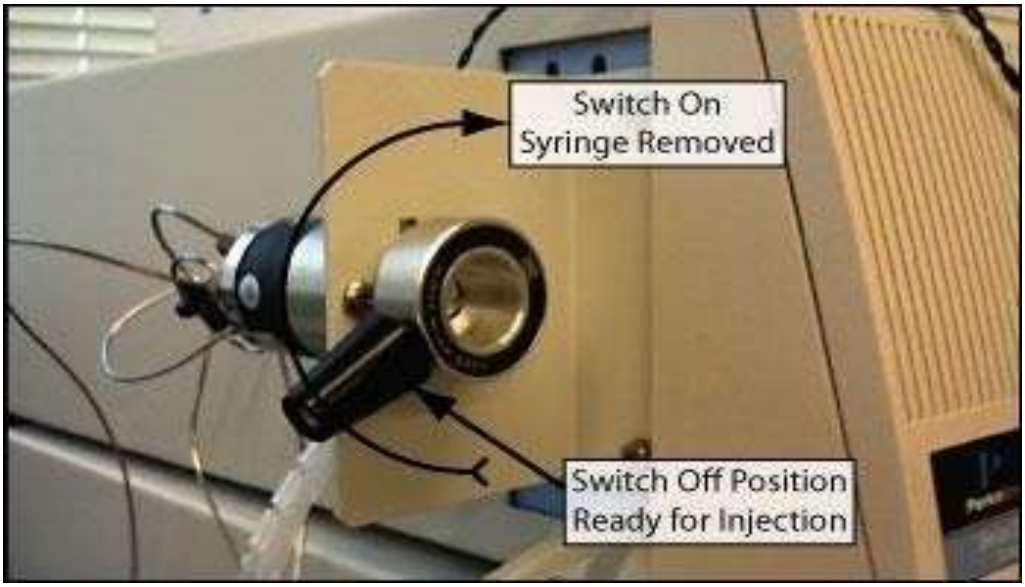

Figure 7: Sample Injection

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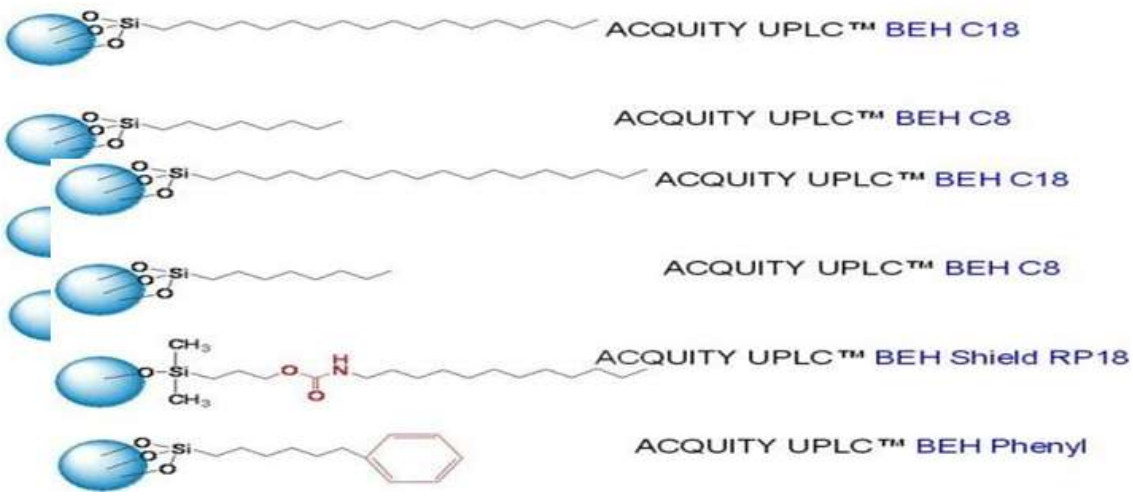
2) UPLC COLUMNS:-

Columns packed with 1.7 µm particles provide higher resolution due to better efficiency. Separating sample components requires a bonded phase that provides both retention and selectivity. Four bonded phases are available for UPLC separations. ACQUITY UPLCTM BEH C and C (linear alkyl columns), ACQUITY UPLC BEH Shield RP18 (columns-polar-groups)".

ACQUITY UPLC BEH Phenyl (phenyl group attached to silyl functionality with C6 alkyl).Each column chemistry offers a different combination of hydropho bicity, silanol activity, hydrolytic stability, and chemical interaction with analytes.

ACQUITY UPLC BEH C_{18} and C_8 columns are considered the universal columns of choice for most UPLCseparations by providing the widest pH range.



Phases of UPLC Columns

ACQUITY UPLC BEH T M C18 and C8 columns

These are considered as the universal columns of choice for most UPLC separations by providing the widest pH range.

They incorporate tri functional ligand bonding chemistrieswhich produce superior low pH stability.

This low pH stability is combined with the high pH stability of the 1.7µm BEH particle to deliver the widest usable pH operating range.

ACQUITY UPLC BEH SHIELD R18 columns

These are designed to provide selectivities that complement the ACQUITY UPLC BEH T M C18 and C8 Columns.

ACQUITY UPLC BEH Phenyl columns

These utilize a tri functional C6 alkyl ethyl between the phenyl ring and the silyl Functionality.

ACQUITY UPLC BEH Amide columns-

BEH particle technology, in combination with a tri functionally bonded amide phase, Provides excellent column lifetime and increases assay robustness.

BEH amide Columns provide a wide range of phase pH from [2–11] Excellent retention and structure of polar analytes covering a wide range of polarities Units and pKa.

Ligands combined with the same proprietary end capping process as ACQUITY UPLC BEH T M C18 and C8 columns offer long column lifetime and superior performance top form.

This unique combination creates a new dimension of selectivity, Rapid adaptation to existing HPLC columns.

Reproducible packaging of 1.7 µm particles Sturdy pillars were also a challenge to overcome.

It is column hardware required a smoother internal surface and the end frit was red signed.

Retains small particles and prevents clogging.

Packing bed uniformity also exists This is especially important when achieving this while maintaining separation on shorter columns.

The goal of rapid separation. All ACQUITY columns also include an e Cord micro tip Technology that retrieves manufacturing information for each column containing Quality control testing and certificate of analysis.

For use with Water's ACQUITYUPLC system, E-Cord database can also be updated in real time

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Information such as number of injections to B. or pressure information complete column history.

COMPARATIVE ACCOUNT OF COLUMN OF HPLC AND UPLC: [2,3]

- A column tube and fittings need to incorporate the chromatographic Packing material (stationary phase) that is used to impact a separation. It need to with stand backpressurecreatedeach during production and in use. Also, itneed to provideawell-controlled(leak-free,minimumvolumeandzero-dead- volume)flowpath for the pattern at its inlet and analite bands at its outlet and be chemically inert relative to theseparationsystem(pattern,mobilephaseandstationaryphases).Mostcolumnsareconstructedofstainlesssteelforhighestpressureresistance.PEEKTM (anengineeredplastic)andglass,whilelesspressuretolerant,maybeusedwheninertsurfacearerequiredforspecialchemicalorbiological applications.

Separation performance – Resolution:-

The degree to which two compounds are separated is called chromatographic resolution [RS]. Two principal factors that determine the overall separation power or resolution that can be achieved by an HPLC column are; mechanical separation power, created by the column length, particle size and packed- bed uniformity, and chemical separation power, created by the physiochemical competition for compounds between the packing material and mobile phase. Efficiency is measure of mechanical separation power, while selectivity is a measure of chemical separation power.

Comparison of column of HPLC and UPLC:-

Parameter	**HPLC**	**UPLC**
Length	4.6 x 100 mm	2.1 x 100mm
Particle size	5 µm	1.7 µm
Pressure	1,100 psi	12 psi
Stationary phase	XTERRA C18,Alltima C18	Acquity UPLC BEH C18
Flow rate	0.01-5 ml/ min	0.6ml/min
Pressure	>15000 psi	500-600 psi
Injection volume	5µL	2µL
Maximum	300-400 bars	1000s

3) Detector:-

A tunable UV/Visible detector is used for UPLC detection. Ultraviolet (UV)-visible spectro photometric detectors for HPLC are generally used by analysts more than any other detector, so they are relatively inexpensive and tend to be one of the first detectors. Accessibility for lipid analysts. We recommend a detector specifically designed for HPLC applications with a cuvette volume of approximately 8 microliters (as opposed to a UV spectrophotometer with an optional addition of a flow cell). Detectors constructed specifically for HPLC use with a cell volume of about 8 microlitres are recommended (as opposed to UV spectrophotometers with a flow-cell as an optional extra), and only those affording continuously variable wavelengths are of much value to lipid analysts.

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Figure 8: Detectors

❖ **UV Detector**

UV detectors can sometimes give Detectors for HPLC of lipids with special reference to evaporative light- scattering detection great selectivity and sometimes sensitivity in the analysis of specific compounds, and they are relatively insensitive to changes in ambient temperature or the flow-rate of the mobile phase. While they can be used in gradient elution applications on occasion, base-line drift can be troublesome. A detector cell can easily become contaminated in use, although this may not be immediately obvious.

Advantages of UHPLC: [1,6,7]

Various advantages of UHPLC are as follows:

- Require less run time and enhance sensitivity.
- Provides the selectivity, sensitivity, and dynamic range of LC analysis.
- In chromatogram resolved peaks are obtained.
- Multi residue methods are applied.
- Speedy analysis, quantify accurately analytes and related products.
- Uses of fine particle (2µm) for packing of stationary phase make analysis fast.
- Time and cost both are reduced.
- Consumption of solvents is less.
- More products are analyzed with existing resources.
- Increases sample throughput and helps the manufacturers to produce more material that consistently meet and exceeds the product specifications, also potentially eliminate variability, failed batches, or the need to re-work material.

Disadvantages of UHPLC:

- Due to increased pressure requires more maintenance and reduces the life of the columnsof this type.
- In addition, the phases of less than 2µm are generally non-generable thus have limiteduse.
- Also detector and data collection system (CDS) may not cope with sharper peaks (dataacquisition rate)
- So far only binary pump systems (not ternary or quaternary). This may make methodtransfer not straight forward.

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DIFFERENCECE BETWEEN HPLC VS UHPLC

Sr.No.	HPLC	UHPLC
1	Particle size of stationary phase is between 3 to 5 micron.	Particle size of stationary phase is less than 2 micron.
2	Operates at relatively lower pressure than UPLC maximum backpressure between 300-400 bars.	Operates at higher pressure due to reduced particle size maximum back pressure of 1000 bars/100 mpa.
3	Inner diameter of the column is 3-10 mm.	Inner diameter of the column is 0.75-1.8mm.
4	Dimension of column 150* 3.2 mm 2-15 cm length.	Dimension of column 150* 2.1mm 2-15 cm length
5	Less selective and sensitive	More selective and sensitive
6	Less resolving power	High resolving power
7	High cost of operation and high runtime as compared to UPLC.	Reduces process cycle time and assures end-product quality with reduced cost of operation and decreasrd run time.
8	More solvent consumption lesssample throughput	It decreases the consumption of solvent and increases sample throughput.
9	Less back pressure,more life of column.	The higher back pressure compared to conventional HPLC decreases the life of the column. Increasing the column temperature reduces the back pressure problem in UPLC.
10	Has more use	The particle of less than 2µm are mostly non-regenerable and,

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		therefore, have a narrow use.
11	Lower comparative to UPLC	Higher precision in sample Introduction.
12	Detectors uses higher flow rates and high detector volume.	Detectors uses small flow rates and low detector volume.
13	More run time	Less run time
14	Injection volume are large (generally 5 micro liter)	Injection volume are small (generally 2 micro liter)
15	Less plate count is achieved during analysis.	More plate count is achieved during analysis.
16	Less efficient is pumps are required.	System should withstands high pressure, more efficient pumps are required.
17	More extra column bands broading.	Low extra column bands broading.

APPLICATIONS: [1,2,8]

Analysis of natural products and traditional herbal medicine

These technique is popularly use for the separation of natural products and traditional herbal medicine. It has a highly advanced detection and separation capabilities to identify active compounds that are presents in the samples of natural products and herbal medicine medicines.

Study Of Metabonomic /Metabolomic

Metabonomics studies are carried out in labs to accelerate the development of new medicines. It provides a quick and robust method for detecting the changes, improves understanding of potential toxicity, and allows observing the capacity. The correct application of metabolomics and metabolomic information helps in the discovery, development, and manufacturing processes in the biotechnology and chemical industry companies.

- **Identification of metabolite**

Biotransformation of new chemical entities (NCE) is necessary for drug discovery. When a compound reaches the development stage, its identification becomes a regulated process. UPLC addresses the complex analytical requirements of new discovery by providing unmatched resolution, sensitivity, and mass accuracy

- **ADME (Absorption, Distribution, Metaboilsm, Excrection) Screening**

Pharmacokinetics studies include studies of ADME. It studies important physical and biochemical properties like absorption, distribution, metabolism, elimination, etc.where such compounds show its activity against the target disease.

- **Manufacturing / QA / QC**

Identification of purity, quality, safety and efficacy are the most important factors that need to be considered while manufacturing a drug product. For the successful production of quality pharmaceutical products, the raw materials need to meet the purity speciation. These can be achieved with the help of UPLC technique.

- **Impurity profiling**

These techniques easily detect the impurities present if it is presents in very trace levels too. UPLC combines with same mass LC/MS, which by running with different low and high collision energies, has been successfully used for the detection of drug and endogenous metabolites.

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II. CONCLUSION & SUMMARY

- Compared to conventional HPLC, ultra-performance liquid chromatography offers major improvements. In fact, it has taken the place of all platforms.
- UPLC increases and expands the significance of chromatography. The main asset is a decrease of analysis time, which also reduces consumption of solvent which plays a vital role in analytical laboratory.
- In HPLC the column length is more than that of UPLC also particle size is less this results in increase in plate number which give more accurate separation. Due increase pressure the retention time also got reduced. So by taking into consideration of all these points the UPLC is found to be more sensitive, more precise.
- An only drawback of UPLC could be high back pressure which can be decreased through increasing column temperature.
- This technology thus creates a new opportunity for business profitability in highly efficient manner and helps the product to introduced in the within short period of time.

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