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Review Article

A Review On Ultra Performance Liquid Chromatography (UPLC)

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ABSTRACT

In recent years, significant technological advances have been made in particle chemistry performance, system optimization, detector design, and data processing and control. When brought together, the individual achievements in each discipline have created a step-function improvement in chromatographic performance. Ultra performance liquid chromatography (UPLC) is a new category of analytical separation science that retains the practicality and principles of HPLC, while increasing the overall interlaced attributes of speed, sensitivity and resolution.

UPLC can be regarded as a new direction for liquid chromatography. UPLC improves in three areas: "speed, resolution and sensitivity". In this system uses fine particles (less than 2.5 µm) so decrease the length of column, saves time and reduces solvent consumption.

Key-words: UPLC, HPLC, chromatography, sensitivity.

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INTRODUCTION TO UPLC:

UPLC, improve in three areas: ''speed, resolution, and sensitivity''. In this system uses fine particles (less than 2.5μm) so decreases the length of column, saves time and reduces solvent consumption. UPLC is comes from HPLC. HPLC has been the evolution of the packing materials used to effect the separation.

An underlying principle of HPLC dictates that as column packing particle size decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5μm, there is a significant gain in efficiency and it's doesn't diminish at increased linear velocities or flow rates according to the common Van Deemter equation,

$$H = A + B/u + Cu,$$

By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits which is known as Ultra Performance Liquid Chromatography (UPLC)

The classic separation method is of HPLC (High Performance Liquid Chromatography) with many advantages like robustness, ease of use, good selectivity and adjustable sensitivity. Its main limitation is the lack of efficiency compared to gas chromatography or the capillary electrophoresis due to low diffusion coefficients in liquid phase, involving slow diffusion of analytes in the stationary phase. [2]

The Van Deemter equation shows that efficiency increases with the use of smaller size particles but this leads to a rapid increase in back pressure, while most of the HPLC system can operate only up to 400 bars. That is why short columns filled with particles of about 2μm are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load.

This technology takes full advantages of chromatographic principles to run separations using columns packed with smaller particles and higher flow rates for increased speed, with superior resolution and sensitivity. The use of non-porous particles, however, has been limited in the pharmaceutical industry due to their low sample loading capacity. The Milford, Massachusetts based company waters corporation introduced ACQUALITY UPLC, the commercially available system that addresses the challenge of using elevated pressure and 2mm particles, which makes it a particularly attractive and promising tool for fast Liquid Chromatographic method development. Engineering challenges of operating at high pressures and the high performance expected from such columns necessitates new developed pumps, redesigned injector, reduced system volumes, an increased detector sampling rate and other improvements. To be suitable for the analysis of pharmaceutical development samples under GMP's, the UPLC instrument and columns must not only deliver on its promises for fast, high resolution separation but do so reproducibly and with the required sensitivity. In addition, to the speed at which the data can be obtained, the quality of the data is also improved. [2]

It is clear that the quality of the UPLC-MS spectra is better than that of the Capillary LC-MS spectra with much improved signal-to-noise ratio. This new category of analytical separation science retains the practicality and principles of HPLC while increasing the overall interrelated attributes of speed, sensitivity and resolution.

Today's pharmaceuticals industries are looking for new ways to cut cost and shorten time for development of drugs while at the same time improving the quality of their products and analytical laboratories are not exception in this trend. These are the benefits of faster analysis and hence the ultra-performance liquid chromatography. A typical assay was transferred and optimized for UPLC system to achieve both higher sample analysis throughout and better assay sensitivity.

PRINCIPLE OF UPLC

The UPLC is based on the principal of use of stationary phase consisting of particles less than 2 μm (while HPLC columns are typically filled with particles of 3 to 5 μm). The underlying principles of this evolution are governed by the van Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency).

The Van Deemter curve, governed by an equation with three components shows that the usable flow range for a good efficiency with a small diameter particle is much greater than for larger diameters.

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

Where *A*, *B* and *C* are constants

v = linear velocity of the carrier gas flow rate.

The *A* term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform.

The *B* term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by *v*.

The *C* term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again.

The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus this term is proportional to *v*.

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 approaches for biological samples. [3, 4, 5]

INSTRUMENTATION:

1] Sample injector

2] UPLC Columns

3] Detectors

Figure1: Ultra Performance Liquid Chromatography

1] SAMPLE INJECTION:-

In UPLC, sample introduction is critical. Conventional injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from extreme pressure fluctuations, the injection process must be relatively pulse-free and the swept volume of the device also needs to be minimal to reduce potential band spreading. A fast injection cycle time is needed to fully capitalize on the speed afforded by UPLC, which in turn requires a high sample capacity. Low volume injections with minimal carryover are also required to increase sensitivity.

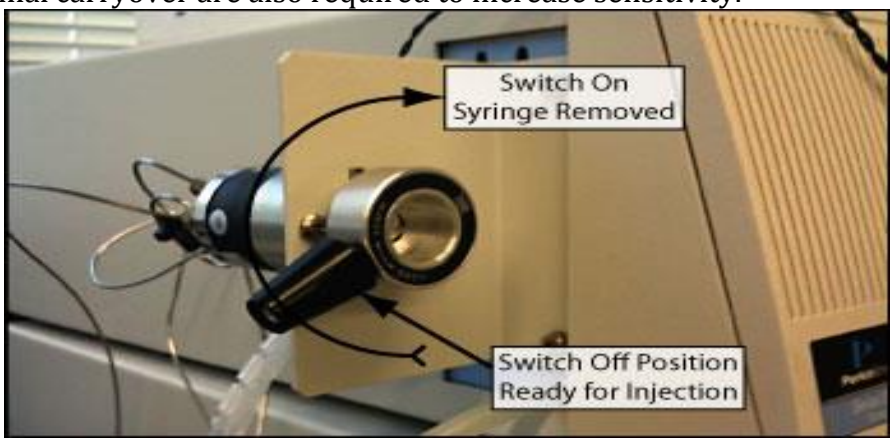

Figure2: Sample Injection

2] UPLC COLUMNS:-

Resolution is increased in a 1.7 µm particle packed column because efficiency is better. Separation of the components of a sample requires a bonded phase that provides both retention and selectivity. Four bonded phases are available for UPLC separations:

ACQUITY UPLC™ BEH C_{18} and C_8 (straight chain alkyl columns),

ACQUITY UPLC BEH Shield RP_{18} (embedded polar group columns)'

ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C_6 alkyl).

Each column chemistry provides a different combination of hydrophobicity, silanol activity, hydrolytic stability and chemical interaction with analytes.

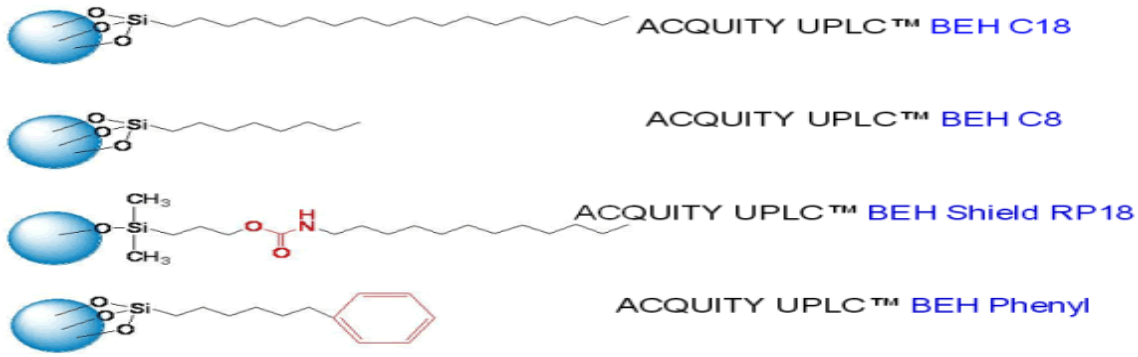

Figure3: Phases of UPLC Columns.

ACQUITY UPLC BEH C_{18} and C_8 columns are considered the universal columns of choice for most UPLC separations by providing the widest pH range. [9,10,11,12]

Detector:-

For UPLC detection, the tunable UV/Visible detector is used.

Spectrophotometric detectors in the ultraviolet (UV)-visible range for HPLC are used more frequently than any other by analysts in general, so they are relatively inexpensive and tend to be one of the first to

which lipid analysts have access. 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.

Figure4: 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.

Comparison between UPLC and HPLC

Characteristics	HPLC	UPLC
Particle size	3 to 5m	Less than 2m
Maximum backpressure	35-40 MPa	103.5 MPa
Analytical column	Alltima C_{18}	Acquity UPLC BEH C_{18}
Column dimensions	150 X 3.2 mm	150 X 2.1 mm
Column temperature	30 °C	65 °C
Injection volume	5µL (Std. In100% MeOH)	2µL (Std.In100% MeOH)

Table 1: Comparison between UPLC and HPLC

COMPARATIVE ACCOUNT OF COLUMN OF HPLC AND UPLC:-

A column tube and fittings must contain the chromatographic Packing material (stationary phase) that is used to effect a separation. It must withstand backpressure created both during manufacturing and in use. Also, it must provide a well-controlled (leak-free, minimum volume and zero-dead-volume) flow path for the sample at its inlet and analite bands at its outlet and be chemically inert relative to the separation system (sample, mobile phase and stationary phases). Most columns are constructed of stainless steel for highest pressure resistance. PEEK™ (an engineered plastic) and glass, while less pressure tolerant, may be used when inert surface are required for special chemical or biological 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	UPLU
Length	4.6 x 100 mm	2.1 x 100mm
Particle size	5 µm	1.7 µm
Pressure	1,100 psi	12 psi

Table 2: Comparison of column of HPLC and UPLC

Mechanical separation power-efficiency:-

If a column bed is stable and uniformly packed, its mechanical separation power is determined by the column length and the particle size. Mechanical separation power, also called efficiency, is often measured and compared by a plate number [symbol = N]. Smaller-particle chromatographic beds have higher efficiency and higher backpressure. For a given particle size, more mechanical separation power is gained by increasing column length. However, the trade-offs are longer chromatographic run times, greater solvent consumption, and higher backpressure. Shorter column lengths minimize all these variables but also reduce mechanical separation power.

Chemical separation power-selectivity:-

The choice of combination of particle chemistry [stationary phase] and mobile phase composition-the separation system-will determine the degree of chemical separation power [how we change the speed of each analyte]. Optimizing selectivity is the most powerful means of creating a separation; this may obviate the need for the brute force of the highest possible mechanical efficiency. To create a separation of any two specified compounds, a scientist may choose among a multiplicity of phase combinations [stationary phase and mobile phase] and retention mechanisms [modes of chromatography].

Effect of column design parameters on efficiency:-

The main difference between the HPLC and UPLC is nothing but only the column parameters from this we can imagine how important the column is. The column is the heart of the chromatographic system; and it is the only device where actual separation of the analyte mixture takes place.

Retention time:-

The distance of the peak maxima from the injection point expressed in time units is called retention time (*t*R), and it serve as an identifier for the given analyte on that particular system. Retention time is probably the most widely used descriptor of the analyte behavior and it is most easily measurable parameter. However, even though it is easily measurable, it is the least universal parameter. It was found that the retention time in UPLC is less than that of HPLC.

Retention factor:-

The analyte retention consists of two parts:

(1) The time component resides in the mobile phase actually moving through the column, and

(2) The time analyte is retained on the stationary phase.

The difference between the total retention time [*t*R] and the holdup time is called the reduced retention time [*t* _ R], and corresponding difference between the analyte retention volume and the void volume is called the reduced retention volume. The ratio of the reduced retention volume to the void volume is widely used dimensionless parameter called retention factor the retention factor of the UPLC is higher than the HPLC.

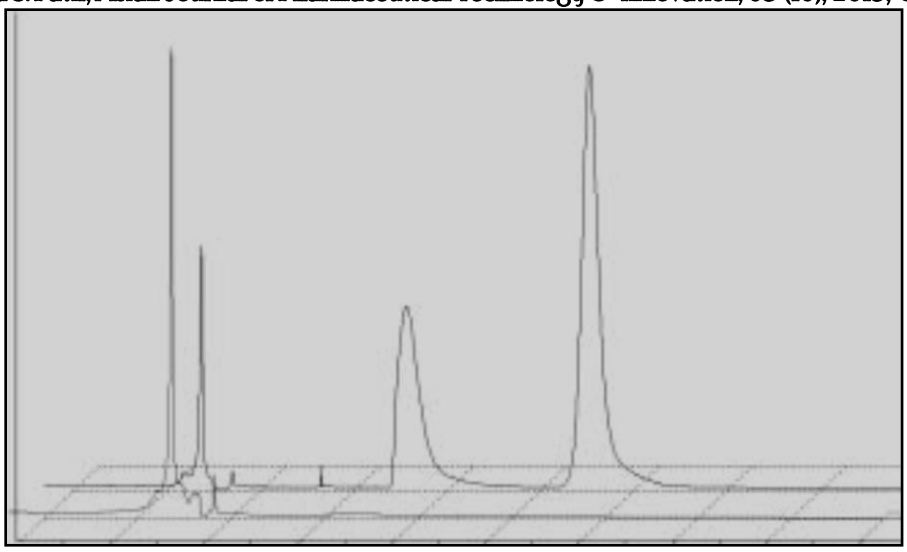

Figure5:- showing retention time

UPLC presents the possibility to extend and expand the utility of conventional HPLC, a widely used separation science. The ACQUALITY UPLC system is the first instrument of its type to incorporate Intelligent Device Management Technology.

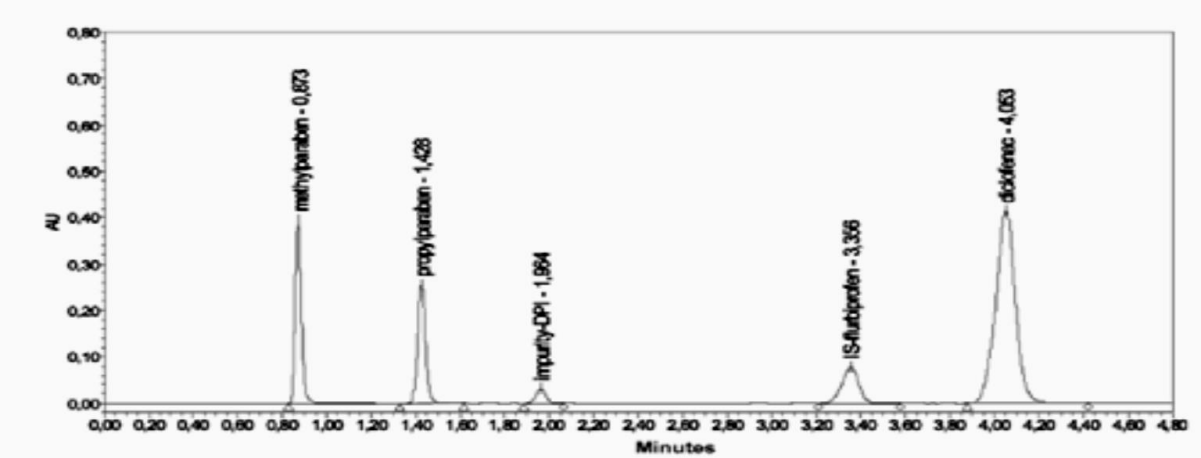

Figure6: Chromatograms of diclofenacemulgel analysis3 – Acquity UPLC 1.7 mm, 100 mm. (Graph obtained by HPLC)

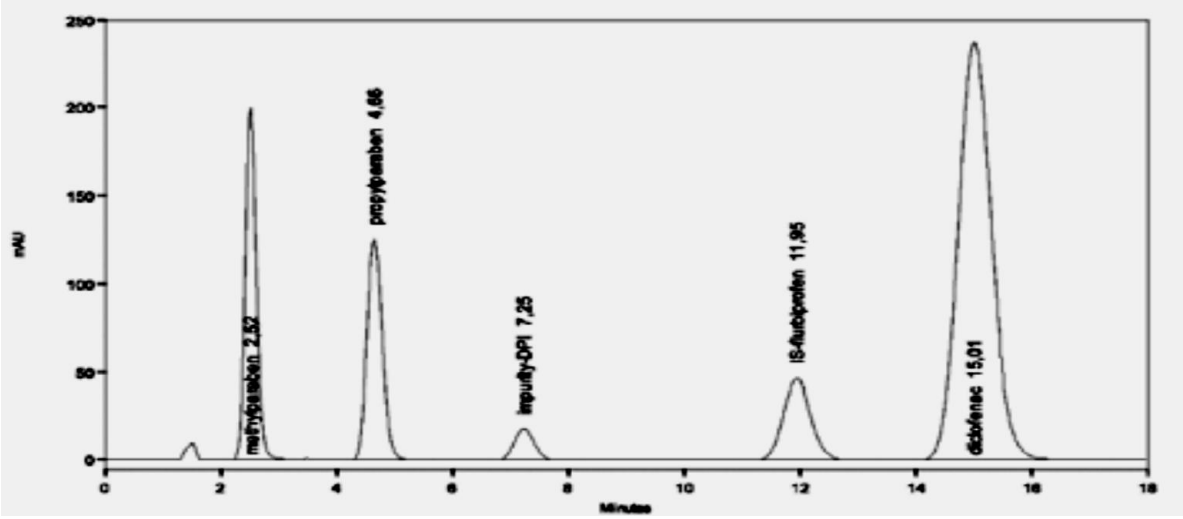

Figure7: Graph obtained by UPLC

Original HPLC verses optimized UPLC parameters:

Parameters	HPLC Assay	UPLC Assay
Column	Xterra, C18, 50 x 4.6mm, 4 µm particles	UPLC BEH C18, 50x 2.1 mm, 1.7 µm particles
Flow rate	3.0 ml / min	0.6 ml/ min
Needle wash	Methanol	Methanol
Injection volume	20 µL	3 µL partial loop fill OR 5µL full Loop fill
Gradient (time in min.) ACN:H_2O	T0 (25:75) T6.5 (25:75) T7.5 (95:5) T9 (25:75) T10(25:75)	T0(36:64) T1.1 (95:5) T1.3(36:64)
Total run time	10 min	1.5 min
Total solvent consumption(including 0.5 min of delay time in between injection)	Acetonitrile: 10.5ml, Water:21.0ml	Acetonitrile: 0.53 ml, Water: 0.66 ml
Plate count	2000	7500
USP resolution	3.2	3.4
Lower limit of quantization (LOQ)	~0.2 µg/ml	~0.054 µg/ml
Delay volume	~720 µL	~110 µL

Table 4: Original HPLC verses optimized UPLC parameters

ADVANTAGES:-

1. Decreases run time and increases sensitivity
2. Provides the selectivity, sensitivity, and dynamic range of LC analysis
3. Maintaining resolution performance
4. Expands scope of Multi residue Methods
5. UPLC's fast resolving power quickly quantifies related and unrelated compounds
6. Faster analysis through the use of a novel separation material of very fine particle size
7. Operation cost is reduced
8. Less solvent consumption
9. Reduces process cycle times, so that more product can be produced 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

DISADVANTAGES:-

1. Due to increased pressure requires more maintenance and reduces the life of the columns of this type. So far performances similar or even higher have been demonstrated by using stationary phases of size around 2 µm without the adverse effects of high pressure.
2. In addition, the phases of less than 2 µm are generally non-generable and thus have limited use. [13,14]

NEW TECHNOLOGICAL ADVANCES:-

1] Improving LC-MS sensitivity through increases in chromatographic performance:

Ultra performance liquid chromatography (UPLC), offers significant theoretical advantages in resolution, speed, and sensitivity for analytical determinations, particularly when coupled with mass spectrometers capable of high-speed acquisitions.

Overall, the improvements in LC–MS method sensitivity, speed, and resolution provided by UPLC show that further advances can be made in analytical methodology to add significant value to hypothesis-driven research.

2] Ultra-Performance LC (UPLC) utilizing sub-2-µm porous stationary phase particles operating with high linear velocities at pressures >9000 psi was coupled with orthogonal acceleration time-of-flight (oaTOF) mass spectrometry and successfully employed for the rapid separation of lipids from complex matrices. [7]

3] Advantages of application of UPLC in pharmaceutical analysis:-

The quality control analyses of four pharmaceutical formulations were transferred from HPLC to UPLC system. The results are compared for Triamcinolon cream containing trimacinoloneacetonide, methylparaben, propylparaben and triamcinolone as degradation product, for Hydrocortison cream (hydrocortisone acetate, methylparaben, propylparaben and hydrocortisone degradation product), for Indomethacin gel and for Estrogel gel.

4] UPLC-SRM/MS method to quantify urinary eicosanoids:-

A novel UPLC-SRM/MS method for simultaneous quantification of key urinary eicosanoids, including the prostaglandins (PG) tetranor PGE-M, 8-iso- and 2,3-dinor-8-iso-PGF2α; the thromboxanes (TX) 11-dehydro- and 2,3-dinor-TXB2; leukotriene (LT) E4 and 12- hydroxyeicosatetraenoic acid (HETE). [6]

APPLICATIONS OF UPLC:-

1. **Analysis of Natural Products and Traditional Herbal Medicine:-**UPLC is widely used for analysis of natural products and herbal medicines. UPLC provides high-quality separations and detection capabilities to identify active compounds in highly complex samples that results from natural products and traditional herbal medicines
2. **Identification of Metabolite:-**Biotransformation of new chemical entities (NCE) is necessary for drug discovery. When a compound reaches the development stage, metabolite identification becomes a regulated process.UPLC/MS/MS addresses the complex analytical requirements of biomarker discovery by offering unmatched sensitivity, resolution, dynamic range, and mass accuracy.
3. **Bio analysis / Bioequivalence Studies:-**The sensitivity and selectivity of UPLC/MS/MS at low detection levels generates accurate and reliable data that can be used for a variety of different purposes, including statistical pharmacokinetics analysis. UPLC/MS/MS delivers excellent chromatographic resolution and sensitivity.
4. **ADME (Absorption, Distribution, Metabolism, Excretion) Screening:-**The high resolution of UPLC enables accurate detection and integration of peaks in complex matrices and extra sensitivity allows peak detection for samples generated by lower concentration incubations and sample pooling. These are important for automated generic methods as they reduce failed sample analyses and save time.
5. **Dissolution Testing:-**In sustained-release dosage formulations, testing higher potency drugs is particularly important. The dissolution profile is used to demonstrate reliability and batch-to-batch uniformity of the active ingredient. Additionally, newer and more potent formulations require increased analytical sensitivity. UPLC provides precise and reliable automated online sample acquisition. It automates dissolution testing, from pill drop to test start.

6. **Method Development / Validation:-**UPLC help in critical laboratory function by increasing efficiency, reducing costs, and improving opportunities for business success. UPLC column chemistries can easily translate across analytical- and preparative-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.
7. **Forced Degradation Studies:-**Combining the chromatographic speed, resolution, and sensitivity of UPLC separations with the high-speed scan rates of UPLC-specific photodiode array and MS detection will give confidence for identifying degradation products and thus shortening the time required to develop stability-indicating methods.
8. **Impurity Profiling:-**UPLC confidently detect impurities in compounds even at trace levels. UPLC combines with exact mass LC/MS, 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.
9. **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.
10. **Analysis of amino acid:-**UPLC used for accurate, reliable and reproducible analysis of amino acids in the areas of protein characterizations, cell culture monitoring and nutritional analysis of foods.
11. **Determination of pesticides:-**UPLC couples with triple Quadra tandem mass spectroscopy will help in identification of trace level of pesticides from water.
12. UPLC fingerprint can be used for the identification of *magnolia officinalis* cortex. [15,16,17]

CONCLUSION:-

UPLC extends and expands the utility of chromatography. The main advantage is a reduction of analysis time, which also meant reduced solvent consumption. Analysis time, solvent consumption, and analysis cost are very important in many analytical laboratories. The time spent optimizing new methods can also be greatly reduced.

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.

Tailing factors and resolution were similar for both techniques. Peak area repeatability (as RSD) and peak retention time repeatability (RSD) were also similar for both techniques.

A negative aspect of UPLC could be the higher backpressure than in conventional HPLC. This backpressure can be reduced by increasing the column temperature.

Overall, it seems that UPLC can offer significant improvements in speed, sensitivity and resolution compared with conventional HPLC.

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