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AN OVERVIEW OF ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC): PRINCIPLES, INSTRUMENTATION AND APPLICATIONS

Sudipta Saha†[1], B. C. Nandy[2]

[1]Department of Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, U.P., India

[2] Department of Pharmaceutical Sciences, Jayoti Vidya Peeth Women's University, Jaipur, Rajasthan, India

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Corresponding Author:

†Sudipta Saha

Department of Pharmaceutical Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, U.P., India.

Email: sudiptapharm@gmail.com

ABSTRACT

The term UPLC, meaning "Ultra Performance Liquid Chromatography," was introduced by Waters Corporation when they introduced their Acquity LC system. The underlying principles of this evolution are governed by the van Demeeter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency). It was found that HETP decreases to a minimum value and then increases with increasing flow rate. According to FDA, validation is defined as establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. UPLC can accommodate more number of samples than HPLC so sample throughput is enhanced. It gives not only high quality results but also ensures the safety of data by using security based data collection software.

Keywords: UPLC, HPLC, principle, instrumentation, applications.

INTRODUCTION

What is UPLC?

The term UPLC, meaning "Ultra Performance Liquid Chromatography," was introduced by Waters Corporation when they introduced their Acquity LC system [1-3].

Advantages of UPLC

The advantages of UPLC are [2-5]:

- Decreases run time and increases sensitivity.
- Provides the selectivity, sensitivity, and dynamic range of LC analysis.
- Maintaining resolution performance.
- Expands scope of Multi-residue Methods.
- UPLC's fast resolving power quickly quantifies related and unrelated compounds.
- Faster analysis through the use of a novel separation material of very fine particle size.
- Operation cost is reduced less solvent consumption.
- Reduces process cycle times, so that more product can be produced with existing resources.
- 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 rework material.
- Delivers real-time analysis in step with manufacturing processes.
- Assures end-product quality, including final release testing.

Disadvantages

The disadvantages of UPLC are [5-7]**:**

- Due to increased pressure requires more maintenance and reduces the life of the columns of this type.
- So far performance similar or even higher has been demonstrated by using stationary phases of size around 2µm without the adverse effects of high pressure.
- In addition, the phases of less than 2 µm are generally non-regenerable.

Draw Backs

The draw backs of UPLC are [5-7]:

- Cost mixing,
- Solvent pumping, and
- Lack of variety in commercial columns at 1.7 µm.

Use of UPLC system

Elevated-temperature chromatography also allows for high flow rates by lowering the viscosity of the mobile phase, which significantly reduces the column backpressure. Monolithic columns contain a polymerized porous support structure that provides lower flow resistances than conventional particle-packed columns [5-6].

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PRINCIPLE

The UPLC is based on the principle [4-7] 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). It was found that HETP decreases to a minimum value and then increases with increasing flow rate. However, with the 1.7 µm particles used in UPLC, HETP is lowered compared to the larger particles and does not increase at higher flow rates. This allow faster separations to be carried out on shorter columns and/or with higher flow rates, leading to column increased resolution between specific peak pairs and increased peak capacity, defined as the number of peaks that can be separated with specified resolution in a given time interval. Efficiency is three times greater with 1.7 µm particles compared to 5 µm particles and two times greater compared to 3.5 µm particles. Resolution is 70% higher than with 5 µm particles and 40% higher than with 3.5 µm particles. High speed is obtained because column length with 1.7 µm particles can be reduced by a factor of 3 compared to 5 µm particles for the same efficiency, and flow rate can be three times higher. This means separations can be nine times faster with equal resolution. Sensitivity increases because less band spreading occurs during migration through a column with smaller particles (peak width is less and peak height greater).

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 and it is much greater than for larger diameters [4-5].

$H=A+B/v+Cv$

Where; *A*, *B* and *C* are constants and *v* is the linear velocity, 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*.

Differences between UPLC and HPLC

The short information between HPLC and UPLC are [6-9]-

HPLC

- Broader peak width provides less resolution less sample throughput comparatively.
- Sample injection volume is 20 micro liters. Pump operates at 2000-6000 psi pressure.
- Particle size in stationary phase packing material is between 5-12 micrometers.

UPLC

- Smaller peak width provides better resolution and more number of peaks getting identified.
- Higher sample throughput with more information per sample.
- Sample injection volume is as less as 3-5 micro liters. Pump operates at 10,000 psi pressure.
- Particle size in stationary phase packing material is less than 2 micrometer.

The characteristics of HPLC and UPLC and advantages of UPLC over HPLC are summarized in Table 1 & Figure 1.

Table 1: Comparison between UPLC and HPLC

Characteristics	HPLC	UPLC
Particle size	3 to 5m	Less than 2m
Maximum Back pressure	35-40 MPa	103.5 MPa
Analytical column	Alltima C_{18}	Acquity UPLC BEH
Column dimensions	150 X 3.2 mm	C18; 150 X 2.1 mm
Column temperature	30 °C	65 °C
Injection volume	5-20µL	2-5µL
Pressure limit	up to 4000 psi	15000 psi
Total run time	10 min	1.5 min

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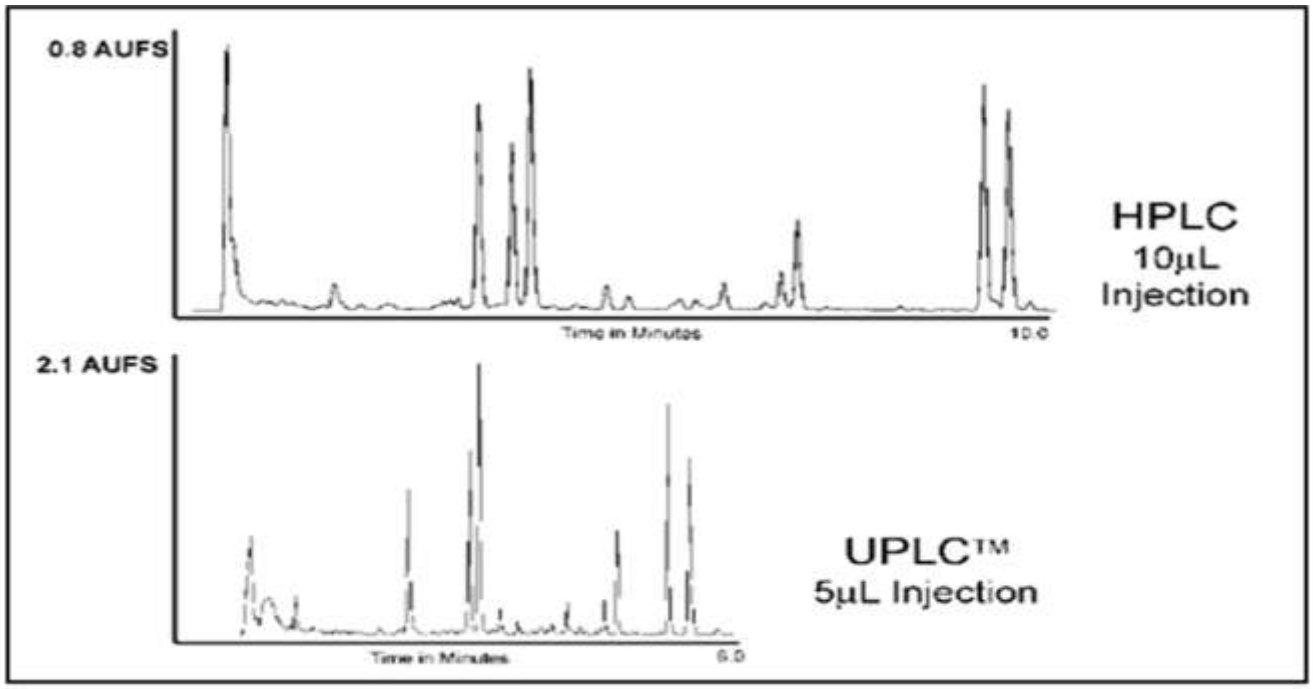

Figure 1: Comparison between HPLC and UPLC graph

INSTRUMENTATION

Instrumentation:

The UPLC System (Figures 2 & 3) has been holistically designed to match the performance needs of innovative column chemistries with robust hardware, easy-to-use software and specialized support services [8-16].

It consists of:

- Small, pressure-tolerant particles
- High-pressure fluidic modules
- Minimized system volume
- Negligible carryover
- Reduced cycle times
- Last response detectors
- Integrated system software and diagnostics

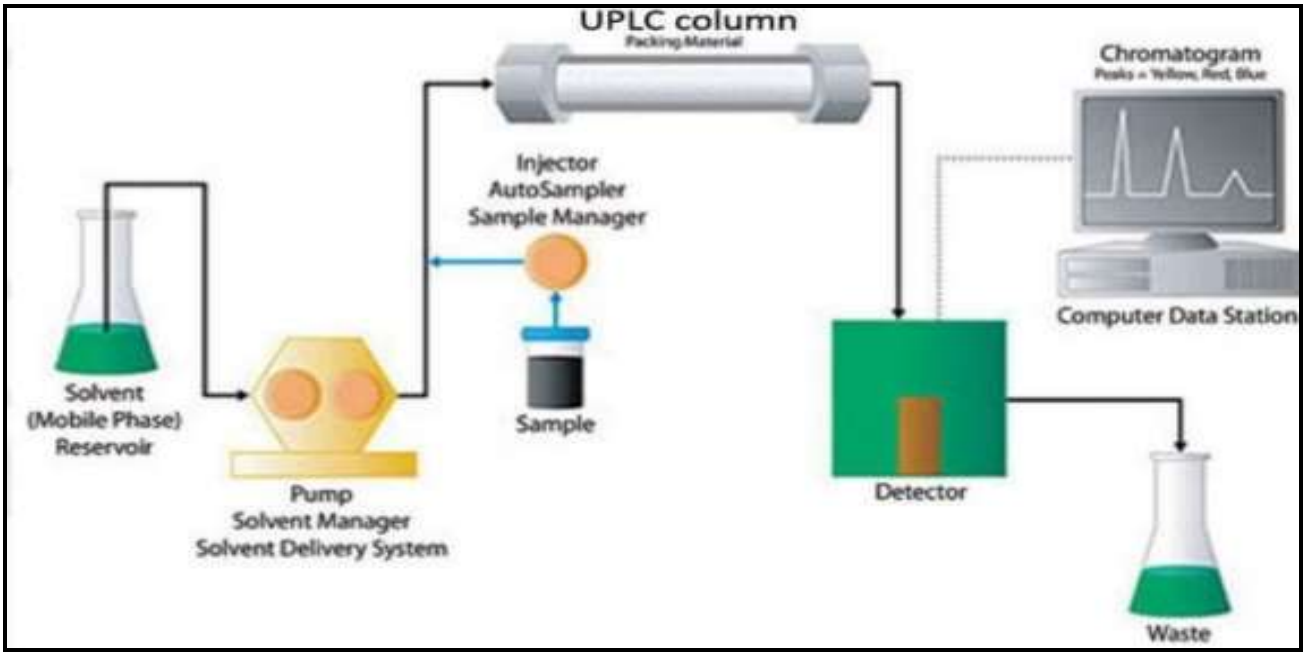

Figure 2: Schematic diagram of UPLC

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Figure 3: **UPLC instrument**

ACQUITY UPLC

These systems are easily controlled, diagnosed, and monitored via a graphical system console interface.

The console offers:

- Quick and easy access to critical instrument parameters.
- Simple system start-up, elegant system status monitoring and predictive performance indicators to ensure maximum productivity.
- Data management capabilities that are supported by both MassLynx™ and Empower™ software.

The ACQUITY UPLC [17-22] System is also supported by Intelligent Device Management technology with our Connections INSIGHT™ service, providing instrument diagnostics.

1. Sample injection
2. UPLC columns
3. Column manager & heater or cooler
4. Detectors
5. Soft wares
6. Accessories
7. Connection insight service (if provided by manufacturing. company water provided it)

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. There are also direct injection approaches for biological samples.

UPLC Columns

Resolution is increased in a 1.7 µm particle packed column (Figure 4) 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 column) and 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.

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. They incorporate trifunctional ligand bonding chemistries which produce superior low pH stability. This low pH stability is combined with the high pH stability of the 1.7 µm BEH particle to deliver the widest usable pH operating range. ACQUITY UPLC BEH Shield RP_{18} columns are designed to provide selectivities that complement the ACQUITY UPLC BEH C_{18} and C_8 phases. ACQUITY UPLC BEH Phenyl columns utilize a trifunctional C_6 alkyl tether between the phenyl ring and the silly functionality.

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This ligand, combined with the same proprietary end capping processes as the ACQUITY UPLC BEH C_{18} and C_8 columns, provides long column lifetimes and excellent peak shape. This unique combination of ligand and end capping on the 1.7 µm BEH particle creates a new dimension in selectivity allowing a quick match to the existing HPLC column. An internal dimension (ID) of 2.1 mm column is used. For maximum resolution, choose a 100 mm length and for faster analysis, and higher sample throughput, choose 50 mm.

UPLC system with 2D technology adds a new magnitude of orthogonal separations, providing the superior sensitivity and selectivity required for complex sample analysis.

This technology [20-24]:

- Provides ready-to-use configurations allowing for faster 2D UPLC analysis, with less troubleshooting, and more confidence.
- Offer a full range of sub-2-µm UPLC column chemistries for true UPLC results.
- Enhance the ability to eliminate unwanted interferences.
- Increase peak capacity and resolution for characterising the most complex samples
- Provide flexibility in utilization of mobile phase for mass spectroscopy detection.
- Minimise sensitivity drift in mass spectroscopy by reducing source contamination.
- Improve assay ruggedness and overall speed of analysis.

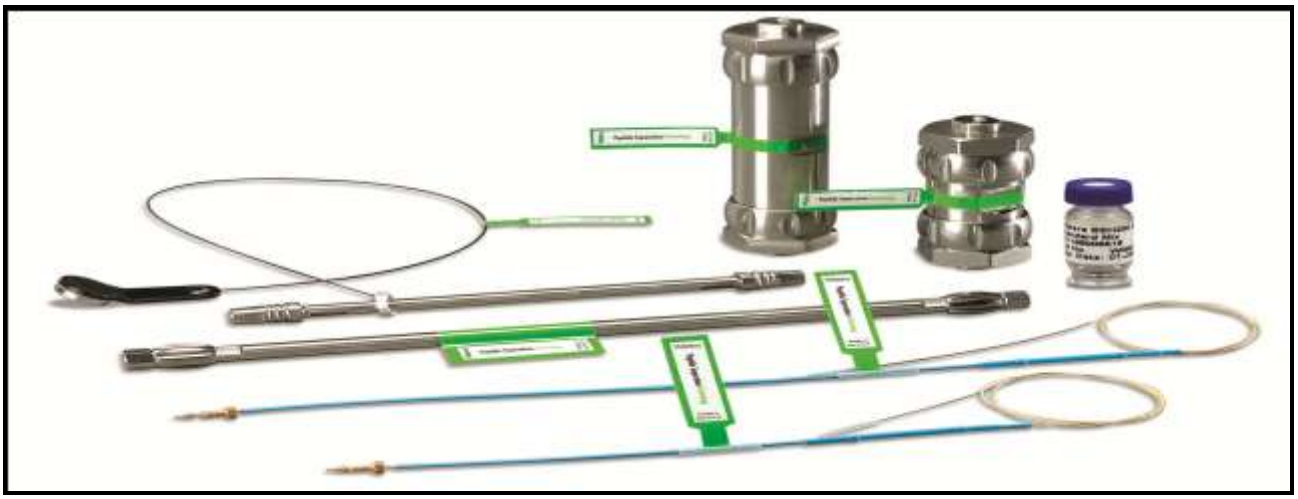

Figure 4: UPLC Columns are designed for peptide mapping

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.

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.

APPLICATIONS of UPLC

Rapid analysis of products

UPLC can be extensively used in the analysis of natural products and herbal medicines. In 2004, the first commercially available UPLC system was the ACQUITY UPLC that fulfilled all the requirements needed for the separation of various pharmaceutical products [24-26].

Analysis of Natural Products and Traditional Herbal Medicine

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. Metabonomics-based analysis, using UPLC, exact mass MS, and Marker Lynx Software data processing for multivariate statistical analysis, can help quickly and accurately characterize these medicines and also their effect on human metabolism [25-28].

ADME (Absorption, Distribution, Metabolism, Excretion) Screening

ADME studies measure physical and biochemical properties – absorption, distribution, metabolism, elimination, and toxicity of drugs where such compounds exhibit activity against the target disease. Tandem quadrupole MS combines with UPLC in ADME screening for sensitivity and selectivity with fast analyses of samples in matrix to be achieved with minimal cleanup, using MRM (multiple reaction monitoring) for detection and automated compound optimization [28-29].

Method Development / Validation

According to FDA, validation is defined as establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. Method development and validation is a time-consuming and complicated process: labs need to evaluate multiple combinations of mobile phase, pH, temperature, column chemistries, and gradient profiles to arrive at a robust, reliable separation for every activity [29-31].

Dissolution testing

The dissolution profile of a drug signifies reliability and batch to batch uniformity of API (Active Pharmaceutical Ingredients) in the formulations.

Nowadays, newer and relatively high potent formulations are coming in the market which requires enhanced analytical sensitivity. By using UPLC, dissolution testing can be fully automated as it can perform functions like data acquisition, analysis of samples aliquots, management of test results and distribution [29-32].

Drug Discovery

UPLC improves the drug discovery process by means of high throughput screening, combinational chemistry, high throughput in vitro screening to determine physiochemical and drug's pharmacokinetics [32].

High throughput quantitative analysis

UPLC coupled with time of flight mass spectroscopy give the metabolic stability assay [32].

Analysis of Dosage form

It provides high speed, accuracy and reproducible results for isocratic and gradient analysis of drugs and their related substance. Thus method development time decrease [33].

Analysis of amino acids

UPLC used from accurate, reliable and reproducible analysis of amino acids in the areas of protein characterizations, cell culture monitoring and the nutritional analysis of foods [34].

Determination of Pesticides

UPLC couples with triple Quadra-pole tandem mass spectroscopy will help in identification of trace level of pesticides from water.

Identification of metabolites

When a new chemical entity (NCE) reaches the development stage, identification of its metabolites becomes a perpetual process. The detection of all circulating metabolites of a candidate drug is necessary. The identification of major metabolites is done by performing *in vitro* discovery studies. The weak spots of metabolites of drug candidate molecules are recognized and hence protected by altering the com-pound structure. Plumb et al. have explored the application of UPLC-MS for analysis of metabolites of candidate drug. They also examined the application of UPLC-MS in profiling metabolic pathways for functional genomics studies. Their studies revealed that higher resolution of UPLC in terms of specificity and spectral quality diminishes the risk of missing any detection of potentially important metabolites [32-35].

Bioequivalence studies

The detection of drug in biological sample is very important to study the pharmacokinetics, toxicity and bioequivalence of drug. Blood, plasma and urine can be used as biological matrices which consist of sample drug in them. Previously LC-MS was used for detecting drug in these biological matrices. But nowadays UPLC-MS is used as it has some advantages over LC-MS such as:

UPLC-MS provides unprecedented performance and compliance support. It has excellent chromatographic resolution and sensitivity [33-36].

Forced Degradation Studies

One of the most important factors that impacts the quality and safety of pharmaceuticals is chemical stability. The FDA and ICH require stability testing data to understand how the quality of an API (active pharmaceutical ingredient) or a drug product changes with time under the influence of environmental factors such as heat, light, pressure and moisture or humidity.

Knowledge of these stability characteristics defines storage conditions and shelf life, the selection of proper formulations and protective packaging, and is required for regulatory documentation. Forced degradation, or stress testing, is carried out under even harsher conditions than those used for accelerated stability testing. Generally performed early in the drug development process, laboratories cause the potential drug to degrade under a variety of conditions like peroxide oxidation, acid and base hydrolysis, photostability, and temperature to understand resulting by products and pathways that are necessary to develop stability indicating methods [35-37].

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Manufacturing / QA / QC

Identity, purity, quality, safety and efficacy are the important factors to be considered while manufacturing a drug product .The successful production of quality [33-35].

Detection of impurities

The detection of impurities in raw material as well as in final product is the most vital phase of the drug development process. Earlier studies have accounted for excellent detection of impurities by HPLC as it has sufficient resolution for the determination of the lowest level of impurities with good reproducibility results, but due to the presence of excipients, there is prolonged HPLC analysis so it becomes necessary to perform several analytical runs to get the required data. This curb can be over-come by using UPLC technique as it offers exact required data and is operational at alternate low and high collision energies. The fast change of collision energy produces both precursor and product ions of all analytes present in the sample, which allows rapid identification and profiling of impurities [36].

Toxicity Studies

A significant number of candidate drugs falls out of the development process due to toxicity and these results in great loss to the manufacturers. It is difficult to evaluate candidate drugs for possible toxicity, drug-drug interactions, inhibition, and/or induction of metabolizing enzymes in the body.

Failure to properly identify these potential toxic events causes a compound to be withdrawn from the market. 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 analysis and saves time [36-37].

CONCLUSION

UPLC by using 1.7 µm particle sizes gives increased resolution, speed and sensitivity for liquid chromatography. The main advantage of UPLC is a reduction of analysis time, along with reduced solvent consumption, high throughput analysis and reduction in cost of analysis. From the literature survey it can be concluded that all categories of pharmaceutical drugs can be analyzed by UPLC method within a very short period of time and with less solvent consumption. UPLC Technology is transforming lives and laboratories, creating new opportunities for business profitability, and bringing new meaning to quality. The literature survey shows that research on UPLC analysis, both, at national and international level have been successfully done on all categories of drugs.

According to FDA, validation is defined as establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes. UPLC can accommodate more number of samples than HPLC so sample throughput is enhanced.

It gives not only high quality results but also ensures the safety of data by using security based data collection software.

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