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An Updated Review on Ultra Performance Liquid Chromatography

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Abstract

UPLC can be regarded as latest invention for liquid chromatography. UPLC refers to Ultra Performance Liquid Chromatography. It brings drastic changes in sensitivity and speed of analysis. It has instrumentation that can be operated at higher pressure as compared to HPLC. This review includes the theories & principle of Chromatography along with the Comparison between HPLC & UPLC, advanced feature is listed here in this review. Some of the most recent applications of UPLC are also included here along with examples.

Keywords: ***UPLC; HPLC; Chromatography; Sensitivity; Time; Principle***

Introduction

Chromatography is a non-destructive procedure for separating a mixture of components in to individual components with the help of a porous medium under the influence of solvents. Before 2004, HPLC was the most frequently used technique for separating a mixture of components into individual components. But 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)" [1-5].

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 [6-10].

Brief History

Chromatography is a new technique which was first invented by Tswett, a Russian Botanist in 1906 in Warsaw. In that year, he successfully done the separation of chlorophyll, xanthophylls and several others coloured substances by percolating

vegetable extracts with the help of column of calcium carbonate. The column of calcium carbonate act as an adsorbent and the different substances get adsorbed to different extent and this gives rise to coloured bands at different position, on the column. Tswett termed this system of coloured bands as chromatogram and the technique use as chromatography after the Greek words chroma and graphos meaning colour and writing respectively [11-17].

Considerable advances have since been made and the methods is used to separate coloured along with colourless substances. The column of calcium carbonate used in Tsweet method remains stationary and is therefore named as the stationary phase. The solution of vegetable extracts moves or flows down the column and is therefore termed as mobile phase. chromatography may be regarded as a technique of separation in which separation of solutes occurs between a stationary phase and a mobile phase [18-25].

In 1930 chromatography in the form of thin layer chromatography along with ion exchange chromatography was introduced as a separation technique. In 1941, Martin and synge introduced paper chromatography and latter gas chromatography in 1952. Apart from its use in analysis it is becoming a potential technique as a method for the preparation of very pure compounds in the fields like pharmaceutical industry or in the manufacture of pure chemicals. The recent spectacular developments in the field of bioscience are entirely because of the chromatographic methods of separation of bio-molecules.

Later on, the others techniques like HPLC was introduced which has been used in Many laboratories for a long period of time then after a new technique has been introduce recently called UPLC (Ultra performance liquid chromatography).

Principle

The principle for the separation can either be adsorption or partition. Hence they can be called as adsorption chromatography or partition chromatography.

Adsorption chromatography

When adsorbate or mixture compounds is dissolved in mobile phase (eluent) travels through a column of stationary phase (adsorbent), they move according to the relative affinities towards stationary phase.

The compound which has more affinity for stationary phase travels slower and that having low affinity towards stationary phase travels faster. Hence the compounds are separated. No two compounds have the same affinity for a combination of stationary phase, mobile phase and other conditions.

Partition chromatography

The most widely used type of UPLC is partition chromatography. In the past, most of the applications have been to nonionic and polar compound of low moderate molecular mass (usually <3000.) The early forms of Partition chromatography liquid-liquid columns. These have been replaced in modern LC systems by liquid – bonded phase columns. In liquid – liquid chromatography, the liquid was held in place by physical adsorption. In bonded – phase chromatography, on the other hand, its attached by Chemical Bonding, resulting highly stable pickings insoluble in the mobile phase. Bonded Phase columns also compatible with gradient elution techniques. Therefore, our discussion focuses exclusively on bonded – phase partition Chromatography [26-35].

Instrumentation

The various instruments used in the Ultra performance liquid chromatography are as follows (FIG. 1).

- Sample injection
- UPLC columns
- Column manger & heater or cooler
- Detectors
- Software's [36-41].

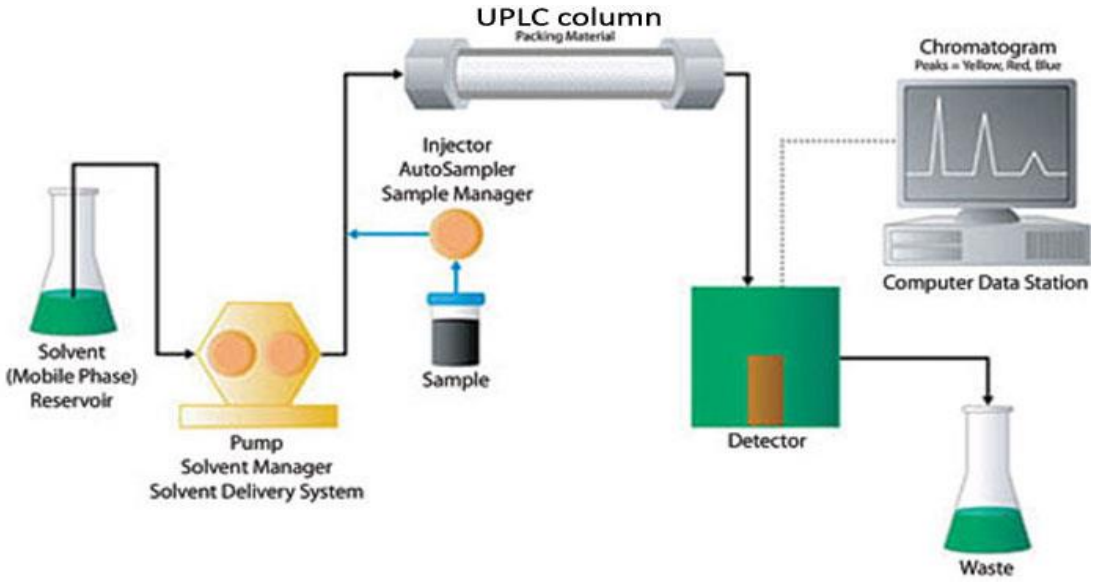

FIG. 1. **Working of UPLC.**

Advantages

- It decreases the run time and increases sensitivity.
- Provides the sensitivity, selectivity, and dynamic range of LC analysis.
- Maintains the resolution performance.
- Expands scope of Multiresidue Methods.
- Faster analysis is possible with the use of a novel separation material which are of very fine particle size [42-44].
- The cost of operation in less.
- Solvent consumption is less.
- It decreases process cycle times, which helps to produce more product with limited resources too.
- 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 [45-53].

Disadvantages

- Due to increased pressure frequent maintenance is required and reduces the life of the columns of this type.
- In addition, the phases of below than 2 μm are generally non-regenerable and thus have limited use [54-58].

Application

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 metabonomics /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 [59-63].

ADME (Absorption, Distribution, Metabolism, Excreation) 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 [64-68].

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.

UPLC fingerprint

It can be used for the identification of *Magnolia officinalis* cortex [69-75].

Conclusion

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. It gives sharp and narrow peaks all categories of pharmaceutical drugs. It also facilitates the analysis of complex mixtures in relatively short time and the peaks obtained with the help of these method provides more information which is more convenient and clear that of HPLC. 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. Overall, it seems that UPLC can offer significant improvements in speed, resolution and sensitivity compared with conventional HPLC technique [76-79].

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