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A Review Article on Ultra Performance Liquid Chromatography (UPLC)

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ABSTRACT: The key focus of the pharmaceutical or chemical industries is to reduce the cost involved in the development of new drugs and to improve the selectivity, sensitivity, and resolution for their detection. The purpose can now be solved by the separation method called UPLC which is the modified HPLC method comprising high pressure and small sized particles (less than 2 µm) used in the column, so the length of the column decreases leading to time saving and reduction in the consumption of solvent. The underlying principle of UPLC is based on van Deemter statement which describes the connection between linear velocity with plate height. UPLC contributes to the improvement in the three areas: speed, resolution, and sensitivity. This is a new advanced category of the HPLC which has the same basic principle and methodology with improved chromatographic performance. This review is an effort to compile the principle, instrumentation, and applications of UPLC.

KEYWORDS: UPLC, HETP, HPLC

I. INTRODUCTION

Metabolomics is a powerful new technology that allows assessment of global low-molecular-weight metabolites in biological systems and holds great potential in biomarker discovery. Analysis of the key metabolites in the body fluids has become an important part of the diagnosis, prognosis, and assessment of therapeutic interventions in clinical application [1]. This review is intended to provide an overview of the main applications of ultra-performance liquid chromatography (UPLC) in metabolomics and the current utility of the UPLC-based metabolomics in the fields of oncology, metabolic, neuropsychiatric, cardiovascular, infectious, and other diseases. Especial emphasis is placed on the potential use of endogenous low-molecular-weight metabolites in clinical chemistry.

PRINCIPLE

The underlying principle of UPLC is based on the Van Deemter relationship which explains the correlation between flow rate and plate height [2]. The van Deemter equation (i) shows that the flow range with the smaller particles is much greater in comparison with larger particles for good results [3-6].

$$H = A + \frac{B}{v} + Cv$$

Where H represents height equivalent to the theoretical plate (HETP), A, B & C are the constants and "V" is the flow rate (linear velocity) of the carrier gas. The aim is to minimize HETP to improve column efficiency. The term A does not depend on velocity and indicates eddy mixing. It is smaller if the columns are filled with small and uniform sized particles. The term B denotes the tendency of natural diffusion of the particles. At high flow rates, this effect is smaller, so this term is divided by v. The term C represents the kinetic resistance to equilibrium during the process of separation. The kinetic resistance is the time lag involved in moving from the mobile phase to the stationary phase and back again. The higher the flow rate of the mobile phase, the more a molecule on the packing material inclines to lag behind molecules in the mobile phase. Thus, this term is inversely proportional to linear velocity. Consequently, it is likely to enhance the throughput, and without affecting the chromatographic performance, the separation can be speeded up. The emergence of UPLC has necessitated the improvement of existing instrumentation facility for LC, which takes the benefit of the separation performance (by decreasing dead volumes) and consistent pressures (about 500 to 1000 bars, compared with 170 to 350 bars in HPLC). Efficiency is proportionate to the length of the column and inversely proportional to the radius of the particles [6]. Consequently, the column length can be reduced by the similar factor as the particle radius without affecting the resolution. The use of UPLC has helped in the detection of drug metabolites and enhancement of the quality of separation spectra [7,8].

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II. INSTRUMENTATION

A. Sample Injection
B. UPLC Columns
C. Detectors

A. 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 pulsefree 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.

B. UPLC Columns

Resolution is increased in a 1.7µm 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:

(i) ACQUITY UPLCTM BEH C8 (straight chain alkyl columns)
(ii) ACQUITY UPLCTM BEH C18 (straight chain alkyl columns)
(iii) ACQUITY UPLC BEH Shield RP18 (embedded polar group column) and
(iv) ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl),

Each column chemistry provides a different combination of hydrophobicity, silanol activity, hydrolytic stability and chemical interaction with analytes. ACQUITY UPLC BEH C18 and C8 columns are considered the universal columns of choice for most UPLC separations by providing the widest pH range. They incorporate tri functional ligand bonding chemistries which 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. ACQUITYUPLC BEHShield RP18 columns are designed to provide selectivity that complements the ACQUITYUPLC BEH C18 and C8 phases. ACQUITY UPLC BEH Phenyl columns utilize a tri functional C6 alkyl ether between the phenyl ring and the silyl functionality. This ligand, combined with the same proprietary end capping processes as the ACQUITY UPLC BEH C18 and C8 columns, provides long column lifetimes and excellent peak shape. This unique combination of ligand and end capping on the 1.7µm 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 column. Half-height peak widths of less than one second are obtained with 1.7µm particles, which gives significant challenges for the detector. In order to integrate an analyte peak accurately and reproducibly, the detector sampling rate must be high enough to capture enough data points across the peak. The detector cell must have minimal dispersion (volume) to preserve separation efficiency. Conceptually, the sensitivity increase for UPLC detection should be 2-3 times higher than HPLC separations, depending on the detection technique. MS detection is significantly enhanced by UPLC; increased peak concentrations with reduced chromatographic dispersion at lower flow rates promote increased source ionization efficiencies. The ACQUITY UPLC System consists of a binary solvent manager, sample manager including the column heater, detector, and optional sample organizer.

The binary solvent manager uses two individual serial flow pumps to deliver a parallel binary gradient. There are built-in solvent select valves to choose from up to four solvents. There is a 15,000-psi pressure limit (about 1000 bar) to take full advantage of the sub-2µm particles. The sample manager also incorporates several technology advancements. Using pressure assisted sample introduction, low dispersion is maintained through the injection process, and a series of pressures transducers facilitate self-monitoring and diagnostics. It uses needle-in-needle sampling for improved ruggedness and needle calibration sensor increases accuracy. Injection cycle time is 25 seconds without a wash and 60 sec with a dual wash used to further decrease carry over. A variety of micro titer plate formats (deep well, mid height, or vials) can also be accommodated in a thermostatically controlled environment. Using the optional sample organizer, the sample manager can inject from up to 22 micro titer plates. The sample manager also controls the column heater. Column

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temperatures up to 65°C can be attained. To minimizesample dispersion, a "pivot out" design allows the column outlet to be placed in closer proximity to the source inlet of an MS detector.

C. Detectors

The detectors used in UPLC analysis is UV/Visible detector. Detection of analytes is conventionally based on absorbance that is concentration sensitivity detectors. In UPLC the flow cell volume would have to be reduced to maintain concentration and signal. Based on Beer's Law, smaller volume conventional flow cells would also reduce the path length upon which the signal strength depends. A reduction in cross-section means the light path is reduced, and transmission drops with increasing noise. Therefore, if a conventional HPLC flow cell were used, UPLC sensitivity would be compromised. The ACQUITY unable UV/Visible detector cell consists of a light guided flow cell equivalent to an optical fiber. Light is efficiently transferred down the flow cell in an internal reflectance mode that still maintains a 10mm flow cell path length with a volume of only 500mL. Tubing and connections in the system are efficiently routed to maintain low dispersion and to take advantage of leak detectors that interact with the software to alert the user to potential problems. [9-10]

MERITS

1. The UPLC system allows shortening analysis time up to nine times comparing to the conventional system using 5 µm particle packed analytical columns.
2. Separation on UPLC is performed under very high pressures up to 100 MPa.
3. It gives increased peak capacity (number of peaks resolved per unit time) and resolution.
4. UPLC dramatically improves the quality of the data, resulting in a more definitive map.
5. UPLC fulfilsthe promise of increased speed, resolution, sensitivity and broad range of selectivity predicted for liquid chromatography.
6. Provides the selectivity, sensitivity, and dynamic range of LC analysis.
7. Expands scope of Multiresidue Methods.
8. UPLC is fast resolving power quickly quantifies related and unrelated compounds.
9. Faster analysis through the use of a novel separation material of very fine particle size.
10. Reduces process cycle times, so that more product can be produced with existing resources.
11. 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.
12. Delivers real-time analysis in step with manufacturing processes and Assures end-product quality, including final release testing.
13. Columns are withstandhigh back pressure system. [11,12,13]

DEMERITS

1. Due to increased pressure requires more maintenance and reduces the life of the columns of this type.
2. In addition, the phases of less than 2 µm are generally non-generable and thus have limited use.
3. Higher price of instruments, spare parts and columns.
4. Also detector and data collection system (CDS) may not cope with sharper peaks (data acquisition rate).
5. So far only binary pump systems (not ternary or quaternary). This may make method transfer not straightforward.[13]

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.

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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-tobatch 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 LCMS, 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.[14,15,16]

III. CONCLUSION

UPLC increases productivity in both chemistry and instrumentation by providing more information per unit of work as it gives increased resolution, speed, and sensitivity for liquid chromatography due to smaller particle size. The main advantage is a reduction of analysis time, which also meant reduced solvent consumption. A negative aspect of UPLC could be the higher backpressure than in conventional HPLC. This backpressure can be reduced by increasing the column temperature. It was found that the sensitivity of UPLC was much higher than that of conventional HPLC. All categories of pharmaceutical drugs can be analyzed by UPLC method within a very short period of time and with less solvent consumption

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

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