ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC): A REVIEW OF METHODOLOGY, APPLICATIONS, AND ADVANTAGES

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ABSTRACT

The pharmaceutical and chemical industries aim to enhance drug development efficiency while improving selectivity, sensitivity, and resolution in detection. This can be achieved through the utilization of UPLC, a modified HPLC method employing high pressure and small-sized particles in the column (less than 2 µm). UPLC offers advantages such as time and solvent consumption reduction, attributed to its basis on the van Deemter statement relating linear velocity with plate height. This review highlights the principle, instrumentation, and applications of UPLC, representing an advanced category of HPLC with improved chromatographic performance.

Keywords: *UPLC, HETP, HPLC*

INTRODUCTION

Metabolomics, a cutting-edge technology, enables the comprehensive analysis of low-molecular-weight metabolites in biological systems and shows promising potential in biomarker discovery. The analysis of key metabolites in bodily fluids plays a crucial role in clinical applications, aiding in diagnosis, prognosis, and evaluation of therapeutic interventions [1]. This review presents an overview of the primary applications of ultra-performance liquid chromatography (UPLC) in metabolomics, highlighting its current utility in various disease fields such as oncology, metabolic, neuropsychiatric, cardiovascular, infectious diseases, and others. Particular attention is given to the significance of endogenous low-molecular-weight metabolites in clinical chemistry.

PRINCIPLE:

The fundamental principle of UPLC is based on the Van Deemter relationship, which correlates flow rate with plate height [2]. The objective is to minimize the height equivalent to the theoretical plate (HETP) to enhance column efficiency. The three constants, A, B, and C, in the Van Deemter equation play essential roles: A represents eddy mixing, B denotes natural particle diffusion, and C indicates kinetic resistance to equilibrium during separation [3-6].

To achieve improved throughput without compromising chromatographic performance, UPLC employs smaller and uniform-sized particles, reducing the column length in proportion to particle size. The instrumentation required for UPLC includes facilities for maintaining consistent high pressures (about 500 to 1000 bars) compared to traditional HPLC (170 to 350 bars) [6]. The use of UPLC has been instrumental in detecting drug metabolites and enhancing separation spectra quality [7,8].

II. INSTRUMENTATION

A. Sample Injection

B. UPLC Columns

C. Detectors

a.Sample injection

Sample injection is a critical step in UPLC, demanding careful consideration. Conventional injection valves, whether automated or manual, are not designed to withstand extreme pressures. To safeguard the column from pressure fluctuations, it is essential to ensure a pulse-free injection process with minimal swept volume to reduce band spreading. Achieving a fast injection cycle time is vital to fully exploit the speed advantages of UPLC, necessitating a high sample capacity. Moreover, low volume injections with minimal carryover are necessary to enhance sensitivity. In certain cases, direct injection approaches are used for biological samples.

b.UPLC columns

In UPLC, achieving higher resolution is possible by using columns packed with 1.7µm particles, as they offer improved efficiency. For successful separation of sample components, a bonded phase that provides both retention and selectivity is required. UPLC offers four main bonded phase options for effective separations:

(i) ACQUITY UPLCTM BEH C8: Utilizes straight-chain alkyl columns. (ii) ACQUITY UPLCTM BEH C18: Also employs straight-chain alkyl columns. (iii) ACQUITY UPLC BEH Shield RP18: Consists of an embedded polar group column. (iv) ACQUITY UPLC BEH Phenyl: Features a phenyl group tethered to the silyl functionality with a C6 alkyl.

The choice of UPLC column chemistry provides different characteristics such as hydrophobicity, silanol activity, hydrolytic stability, and chemical interaction with analytes. ACQUITY UPLC BEH C18 and C8 columns are commonly preferred due to their broad pH range and superior low pH stability, making them suitable for various separations. ACQUITY UPLC BEH Shield RP18 complements C18 and C8 phases, offering specific selectivity. ACQUITY UPLC BEH Phenyl columns use a unique ligand and end-capping process, ensuring long column lifetimes and excellent peak shape.

To optimize resolution, a 100 mm column length is preferred, while a 50 mm column enables faster analysis and higher sample throughput. The detector in UPLC faces challenges due to peak widths of less than one second, requiring a high sampling rate and minimal dispersion to maintain efficiency. UPLC enhances sensitivity, especially with MS detection, resulting in increased source ionization efficiencies and improved peak concentrations.

The ACQUITY UPLC System consists of a binary solvent manager, sample manager with pressure-assisted sample introduction, and an optional sample organizer. The binary solvent manager uses two flow pumps for a parallel binary gradient, and the sample manager employs technology advancements for self-monitoring and diagnostics during sample injection. The system accommodates various microtiter plate formats and allows for precise column temperature control to minimize sample dispersion and improve MS detector performance.

C. Detectors in UPLC

In UPLC analysis, the most commonly used detector is the UV/Visible detector, which relies on absorbance for detecting analytes, making it a concentration-sensitive detector. However, in UPLC, certain adjustments are required to maintain sensitivity and signal strength. The flow cell volume needs to be reduced to compensate for the increased speed of UPLC separations, and the path length for signal detection also needs to be minimized. Smaller volume conventional flow cells lead to reduced light path, resulting in decreased transmission and increased noise, potentially compromising UPLC sensitivity if standard HPLC flow cells were used.

To address this issue, the ACQUITY UPLC UV/Visible detector utilizes a specialized flow cell designed as an optical fiber, efficiently guiding light down the flow cell in an internal reflectance mode. Despite a reduced volume of only 500µL, the detector maintains a 10mm flow cell path length, preserving sensitivity. The system is designed with efficient routing of tubing and connections to minimize dispersion and utilize leak detectors that interact with the software to alert users to potential issues [9-10].

Merits of UPLC:

- UPLC enables significantly shorter analysis times, up to nine times faster compared to conventional systems using 5 µm particle packed columns.
- Separations are achieved under very high pressures, up to 100 MPa.
- It provides increased peak capacity and resolution, enhancing the quality and definitiveness of data.
- UPLC fulfills the promises of increased speed, resolution, sensitivity, and a broad range of selectivity predicted for liquid chromatography.
- The system offers the selectivity, sensitivity, and dynamic range of LC analysis.
- UPLC expands the scope of Multiresidue Methods and allows faster quantification of related and unrelated compounds.
- It reduces process cycle times, leading to increased production with existing resources.
- Sample throughput is enhanced, ensuring consistent production meeting or exceeding product specifications, potentially eliminating variability, failed batches, or the need for rework.
- UPLC provides real-time analysis in line with manufacturing processes, ensuring end-product quality and final release testing.
- UPLC columns are designed to withstand high back pressure, ensuring durability and stability [11,12,13].

Demerits of UPLC:

- The increased pressure requirement may lead to more frequent maintenance and reduced column lifespan.
- Phases with particle sizes less than 2 µm are generally non-regenerable, limiting their usability.
- UPLC instruments, spare parts, and columns can be more expensive compared to traditional HPLC.
- Some detectors and data collection systems may struggle to handle sharper peaks due to data acquisition rate limitations.
- Currently, only binary pump systems are widely available, making method transfer less straightforward [13].

APPLICATIONS OF UPLC:

- Analysis of Natural Products and Traditional Herbal Medicine: UPLC is widely used to analyze natural products and herbal medicines, providing high-quality separations and detection capabilities to identify active compounds in complex samples.
- Metabolite Identification: UPLC/MS/MS is crucial for metabolite identification during drug discovery, offering unmatched sensitivity, resolution, dynamic range, and mass accuracy for biomarker discovery.
- Bioanalysis/Bioequivalence Studies: UPLC/MS/MS with its high sensitivity and selectivity generates accurate and reliable data, used for various purposes, including pharmacokinetics analysis.
- ADME (Absorption, Distribution, Metabolism, Excretion) Screening: UPLC's high resolution and sensitivity enable accurate detection and integration of peaks in complex matrices, reducing failed sample analyses and saving time.
- Dissolution Testing: UPLC is valuable in dissolution testing, especially for sustained-release dosage formulations and higher potency drugs, providing precise and reliable automated online sample acquisition.
- Method Development/Validation: UPLC increases efficiency and reduces costs in method development and validation, with the ability to optimize analysis times and rapidly develop methodologies.
- Forced Degradation Studies: UPLC's speed, resolution, and sensitivity, combined with high-speed scan rates of photodiode array and MS detection, aid in identifying degradation products and developing stability-indicating methods.
- Impurity Profiling: UPLC confidently detects impurities in compounds even at trace levels, especially when coupled with exact mass LCMS for identifying drug and metabolites.
- Manufacturing/QA/QC: UPLC is extensively used for highly regulated, quantitative analyses in QA/QC laboratories, ensuring consistent, high-quality consumable products in registered analytical methods.

- Amino Acid Analysis: UPLC is employed for accurate and reproducible analysis of amino acids in protein characterizations, cell culture monitoring, and nutritional analysis of foods.
- Determination of Pesticides: UPLC coupled with triple Quadra tandem mass spectrometry aids in identifying trace levels of pesticides in water.
- UPLC fingerprinting can be used for identifying magnolia officinalis cortex.

[14, 15, 16]

CONCLUSION

In conclusion, UPLC represents a groundbreaking advancement in chromatography. It enhances productivity in both chemistry and instrumentation by providing more information per unit of work, thanks to its increased resolution, speed, and sensitivity attributed to smaller particle size. The significant reduction in analysis time and solvent consumption is a major advantage of UPLC. However, the higher backpressure compared to conventional HPLC could be a limitation, which can be mitigated by increasing the column temperature.

UPLC exhibits much higher sensitivity compared to conventional HPLC, allowing for the analysis of various pharmaceutical drugs within a short timeframe and with reduced solvent usage. Overall, UPLC appears to offer substantial improvements in speed, sensitivity, and resolution over traditional HPLC methods. Its application has brought about a new era in liquid chromatography, enabling researchers to achieve more efficient and precise separations in various fields of analysis and contributing to advancements in scientific research and quality control processes.

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