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Review of Ultra Performance Liquid Chromatography and Its Applications

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ABSTRACT

Ultra Performance Liquid Chromatography (UPLC) can be regarded as a new direction for liquid chromatography. UPLC improves three areas of Liquid chromatography: speed, resolution, and sensitivity. In this system column containing bridged ethylsiloxane / silica hybrid (BEH) structure with fine particle size (less than 1.7 m) is utilized. The use of which decreases the length of column, saves time and reduces solvent consumption. This system is designed in a special way to withstand high system back-pressures. Now a day's pharmaceutical industries as well as analytical laboratories are in search of new ways to reduce cost and time for analysis of drugs and improve quality of their product. UPLC with better resolution, assay sensitivity and high sample throughput allows a greater number of analyses to be performed in a shorter period of time and it also imparts cost effective advantage over HPLC analysis. So that conventional assay was transferred and optimized for UPLC system. This review introduces the theory of UPLC and summarizes some of its applications with examples.

KEY WORDS: Ultra performance liquid Chromatography, High performance liquid chromatography

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1. INTRODUCTION

UPLC refers to Ultra Performance Liquid Chromatography. It improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption[1]. UPLC 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 2.5 m, 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 Deemterequation[2]. 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. 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. 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 2 m are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load[3].

To improve the efficiency of HPLC separations, the following can be done: - a) work at higher temperatures b) use of monolithic columns.

1.1 SMALL PARTICLE CHEMISTRY

The promises of the van Deemter equation cannot be fulfilled without smaller particles than those traditionally used in HPLC. The design and development of sub-2 mm particles is a significant challenge, and researchers have been active in this area for some time to capitalize on their advantages[4]. Figure 1 shows Van Deemter plot, illustrating the evolution of particle sizes over the last three decades.

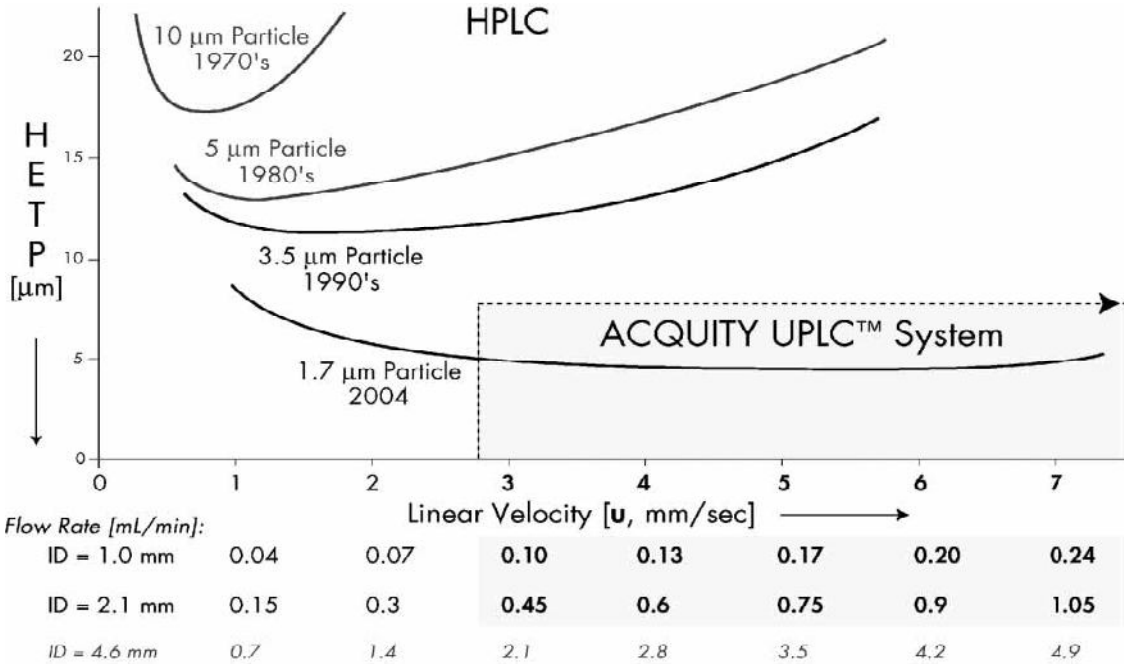

Figure 1: Van Deemter plot, illustrating the evolution of particle sizes over the last three decades.

Although high efficiency, non-porous 1.5 mm particles are commercially available, they suffer from poor loading capacity and retention due to low surface area. To maintain retention and capacity similar to HPLC, UPLC must use novel porous particles that can withstand high pressures. Silica based

particles have good mechanical strength, but can suffer from a number of disadvantages, which include a limited pH range and tailing of basic analytes. Polymeric columns can overcome pH limitations, but they have their own issues, including low efficiencies and limited capacities. In 2000, a first generation hybrid chemistry that took advantage of the best of both the silica and polymeric column worlds was introduced. Producing a classical sol-gel synthesis that incorporates carbon in the form of methyl groups, these columns are mechanically strong, with high efficiency, and operate over an extended pH range. But, in order to provide the kind of enhanced mechanical stability required for UPLC, a second generation bridged ethane hybrid (BEH) technology was developed. These 1.7 mm particles derive their enhanced mechanical stability by bridging the methyl groups in the silica matrix. Packing 1.7 mm particles into reproducible and rugged columns was also a challenge that needed to be overcome. Requirements include a smoother interior surface of the column hardware, and re-designing the end fritsto retain the small particles and resist clogging. Packed bed uniformity is also critical, especially if shorter columns are to maintain resolution while accomplishing the goal of faster separations. In addition, at high pressures; frictional heating of the mobile phase can be quite significant and must be considered. With column diameters typically used in HPLC (3.0 to 4.6 mm), a consequence of frictional heating is the loss of performance due to temperature induced non uniform flow.To minimize the effects of frictional heating, smaller diameter columns (1–2.1 mm) are typically used for UPLC[5].

2. INSTRUMENTATION

The schematic diagramme of UPLC and various parts of the instrument are shown in Figure1, and Figure 2, respectively.

2.1 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. There are also direct injection approaches for biological samples.

2.2 UPLC COLUMNS

Resolution is increased in a 1.7 m 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 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 tri functional ligand bonding chemistries which produce superior low pH stability. This low pH stability is combined with the high pH stability of the 1.7 m BEH particle to deliver the widest usable pH operating range. ACQUITY UPLC BEH Shield RP_{18} columns are designed to provide selectivity that complements the ACQUITY UPLC BEH C_{18} and C_8 phases. ACQUITY UPLC BEH Phenyl columns utilize a tri functional C_6 alkyl tether between the phenyl ring and the silyl functionality. 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 m 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 1.7 m 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-2 m 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 temperatures up to 65°C can be attained. To minimize sample dispersion, a "pivot out" design allows the column outlet to be placed in closer proximity to the source inlet of an MS detector.

2.3 DETECTORS

For UPLC detection, the tunable UV/Visible detector is used which includes new electronics and firmware to support Ethernet communications at the high data rates. Conventional absorbance-based optical detectors are concentration sensitive detectors, and for UPLC use, the flow cell volume would have to be reduced in standard UV/Visible detectors to maintain concentration and signal. According to 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 Tunable 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[5-9].

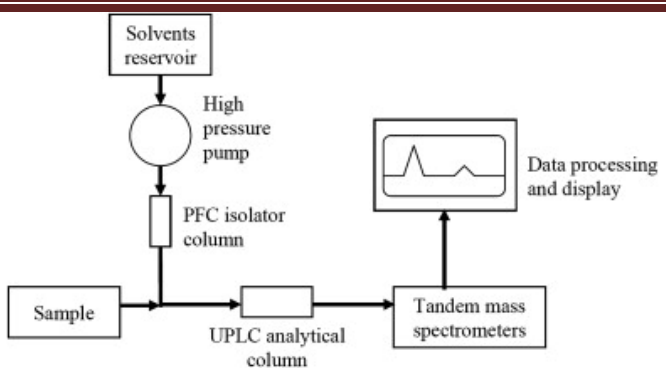

Figure 2: **Schematic diagram of UPLC**

Figure 3: **UPLC instrument**

3. COMPARISON BETWEEN HPLC AND UPLC

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

Table 1: Comparison between HPLC and UPLC

CHARACTERISTICS	HPLC	UPLC
Particle size	<4 µm	1.7 µm
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
Injection volume	20 L	3-5 L
Pressure limit	up to 4000 psi	15000 psi
Total run time	10 min	1.5 min

4. Development of Columns Chemistry by Different Companies

Waters and company: They launched the XTerra family of HPLC columns featuring first generation hybrid particle technology (HPT). This involves inorganic (silica) and organic (polymeric) packings combined to produce a material that has superior mechanical strength, efficiency and low pH range. As these attributes are necessary for UPLC technology because speed, sensitivity and resolution capabilities of peak depends on these parameter. Therefore, a new pressure bearable and wide pH range particle had been created.

A second-generation hybrid column designed to work at higher pressures with the ACQUITY UPLC system. The silica–organic hybrid chemistry is based upon bridged ethylene groups within a silica gel particle structure which gives mechanical strength and pH range from pH 1 to pH 12. As compared to the first generation methyl hybrid particle of XTerra® columns and the bridged ethyl hybrid particle of ACQUITY UPLC BEH columns exhibits improved efficiency, strength and pH range. As BEH Technology is a key enabler of the speed, sensitivity and resolution of UPLC separations. The highly efficient 1.7 m BEH particles allow chromatographers to maximize the efficiency by increasing theoretical plates (N) of their analyte separation, when it used with the ACQUITY UPLC system, it gives improvement in the selectivity or retentivity (k) and resolution. In order to get the desired separation UPLC BEH columns were carefully chosen BEH C18, C8, Shield RP18 and Phenyl column chemistries these enable the rapid development and more robust separations.

Agilent Technologies: The Company provides regular phases with 1.8 m version particle size. Phases include Zorbax stable Bond C8 and C18 for low-pH operation, Zorbax XDB-C8 and C18 for general-purpose separations, Zorbax Extend C18 for high-pH applications and Zorbax-SB CN which provides a different reversed-phase polarity. These involve both cartridges and standard compression fitting hardware in 2.1, 3.0 and 4.6 mm internal diameters with column lengths of 15–100 mm. In relative to other sub-2 m columns, pressure drops are reduced by purposely widening the particle size distribution without influencing column efficiency.

Alltech Associate: The Company offers a 1.5 m particle version for their regular columns. The company provides Platinum HPLC columns have controlled surfaces that offer dual mode separations and extend the range of polar selectivity. The C8, C18 and extended polar selectivity (EPS) phases are available in 33 and 53 mm × 7 mm columns in the Rocket form. These are silica-based columns with a 100 A^0 pore size which are most enable for small-molecule separations. The Alltima HP HILIC and

Pro Sphere HP ZAP C18 were used in large scale. The former non bonded, high-purity bare silica column is recommended for the hydrophilic interaction chromatography (HILIC) separations of highly polar compounds that are poorly retained or un retained on conventional reversed-phase columns. These columns are used with mobile phases consisting of mostly organic solvents with only small amounts of water in the mobile phase and are useful in LC–mass spectrometry (MS) for higher sensitivity with volatile mobile phases. For MS, a smaller 2.1 mm i.d. column is available. Columns of 10, 20 and 33 mm lengths are provided. The Pro Sphere column has a 500 A^0 pore size, which makes it ideal for the high-speed reversed-phase separation of proteins.

Bischof firm: The Company offers four columns, first three are porous (1.8 m) and last one is a nonporous silica phase (1.5 m).The totally porous packing are based upon 300 m^2/g silica. The Pronto PEARL sub-2µm, TPP-C8, ACE EPS (8% carbon loading) and C18 EPS (16% carbon) are smaller particle versions of their regular production. Their Column dimensions are 30–50 mm × 2.0 and 4.6 mm. The third phase on totally porous silica is the Pronto PEARL sub-2 TPP APS, which is a reversed phase with a polar-embedded functionality (3.5% carbon content). This packing gives higher retention for acidic compounds compared with C8 and C18 bonded phases. This 1.8 m column was used to determine the poly phenol content. Relative to the matrix components, the poly phenol were well retained; thus, no extensive sample preparation was required except filtration through a 0.2 m filter[11].

5. TYPES OF UPLC BY WATERS

1) ACQUITY UPLC® I-Class provides the most powerful solution to the most critical need in separation science today – successfully analyzing compounds that are limited in amount or availability amid a complex matrix, more rapidly than ever before. Developed to produce the most accurate and reproducible separations, getting the most information possible and accelerate laboratory results. Complex separation challenges require LC systems designed to maximize the benefits of sub- 2-µm particle columns integrated in a system designed to optimize MS performance.

The ACQUITY UPLC I-Class system:

- Maximizes peak capacity to enhance MS sensitivity.
- Provides the lowest carryover, complementing MS sensitivity and extending MS linear dynamic range.

- Has been purposefully engineered for the lowest dispersion; with an extended pressure/flow envelope, complex separations can be accelerated without compromising chromatographic fidelity.

2) The ACQUITY UPLC H-Class is a streamlined system that brings together the flexibility and simplicity of quaternary solvent blending and a flow-through-needle injector to deliver the advanced performance expected of UPLC type separations – high resolution, sensitivity and improved throughput – while maintaining the robustness and reliability that ACQUITY systems are known for.

Choosing the ACQUITY UPLC H-Class enables to continue running existing HPLC methods on a forward-looking LC platform that allows to confidently and seamlessly transition to UPLC separations, using integrated system tools and reliable column kits for method transfer and method development that simplify migration.

3) The nano ACQUITY Ultra Performance LC® (UPLC®) System is designed for nano-scale, capillary, and narrow-bore separations to attain the highest chromatographic resolution, sensitivity, and reproducibility. Direct nano-flow offers significant improvements over conventional nano-flow separations technologies. It improves peak capacity and peak shape, and increase the number of components that can be detected per separation. The system's 10,000 psi operating pressure capability allows for superior high-peak capacity separations by operating longer columns packed with sub-2 micron particles. It is optimized for high-resolution identification and 2D-LC separations at precise nano-flow rates. The nano ACQUITY UPLC System provides solutions for biomarker discovery and proteomics applications, for protein identification and characterization.

4) The PATROL™ UPLC® Process Analyzer is a real-time Process Analytical Technology (PAT) system that detects and quantifies complex multiple component manufacturing samples and final product directly on the production floor. Designed with the same enabling technology that drives the ACQUITY UPLC® System, PATROL UPLC moves existing liquid chromatography (LC) analysis from off-line Quality Control (QC) laboratories directly to the manufacturing process, resulting in significant improvements in production efficiency:

- Delivers Real-Time LC™ analysis in step with manufacturing processes
- Provides the selectivity, sensitivity, and dynamic range of LC analysis
- UPLC's fast resolving power quickly quantifies related and unrelated compounds

- Reduces process cycle times, so that more product can be produced with existing resources
- Enables manufactures to produce more material that consistently meet or exceeds the product specifications, potentially eliminating variability, failed batches, or the need to re-work material
- Assures end-product quality, including final release testing

The PATROL UPLC Process Analyzer is an ideal solution for pharmaceutical, biopharmaceutical, petrochemical, and food manufacturers that are under increased internal and external pressure to evaluate PAT programs and techniques. Global regulatory initiatives, such as the U.S. Food and Drug Administration and European Medicines Agency Critical Path and PAT Initiatives, and manufacturing quality-by-design programs, such as Six Sigma, are driving corporations to assess and implement novel PAT solutions such as the PATROL UPLC System.

5) The PATROL UPLC® Laboratory Analyzer provides real-time quantitative analysis of chemical reactions in process development and optimization laboratories. Proven UPLC® Technology and Real-TIME LC™ analysis have been integrated to an online analyzer that provides fast and accurate quantitative results to characterize process methods. Spectroscopic technologies used in process development laboratories provide identity information about the processes; however, lack the ability to simultaneously monitor multiple components at different levels and does not provide the quantitative analysis, sensitivity, linearity/dynamic range, and resolution that UPLC provides.

6. ADVANTAGES OF UPLC

Various advantages of UPLC are as follows:

• 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 re-work material.

• Delivers real-time analysis in step with manufacturing processes.

• Assures end-product quality, including final release testing[3-5].

7. DISADVANTAGES OF UPLC:

Due to increased pressure requires more maintenance and reduces the life of the columns of these types. So far performances similar or even higher have been demonstrated by using stationary phases of size around 2 m without the adverse effects of high pressure. In addition, the phases of less than 2 m are generally non-re generable and thus have limited use[3].

8. APPLICATIONS OF UPLC:

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.

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. It is of the utmost importance for lab to successfully detect and identify all circulating metabolites of a candidate drug. UPLC/MS/MS addresses the complex analytical requirements of biomarker discovery by offering unmatched sensitivity, resolution, dynamic range, and mass accuracy.

Study of Metabonomics / Metabolomics

Metabonomics studies are carried out in labs to accelerate the development of new medicines. The ability to compare and contrast large sample groups provides insight into the biochemical changes that occur when a biological system is exposed to a new chemical entity (NCE). Metabonomics provides a rapid and robust method for detecting these changes, improves understanding of potential toxicity, and

allows monitoring the efficacy. The correct implementation of metabonomic and metabolomic information helps similar discovery, development, and manufacturing processes in the biotechnology and chemical industry companies. UPLC analysis rapidly generates and interprets information-rich data, allowing rapid and informed decisions to be made.

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.

Bio analysis / Bioequivalence Studies

Applications of UPLC/MS/MS in bioequivalence and bio analysis are: - In UPLC/MS/MS, LC and MS instruments and software combine in a sophisticated and integrated system for bio analysis and bioequivalence studies, providing unprecedented performance and compliance support. UPLC/MS/MS delivers excellent chromatographic resolution and sensitivity. Increase the sensitivity of analyses, quality of data including lower limits of quantitation (LLOQ), and productivity of laboratory by coupling the UPLC System's efficient separations with fast acquisition rates of tandem quadrupole MS systems. Easily acquire, quantify and report full system data in a compliant environment using security-based data collection software. Ensure the highest quality results and reliable system operation in regulated environment.

Dissolution Testing

For quality control and release in drug manufacturing, dissolution testing is essential in the formulation, development and production process. UPLC provides precise and reliable automated online sample acquisition. It automates dissolution testing, from pill drop to test start, through data acquisition and analysis of sample aliquots, to the management of test result publication and distribution.

Forced Degradation Studies

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. UPLC combined with specific

Photodiode array detector and MS detection will give confidence for identifying degradation products and thus shortening the time required to develop stability-indicating methods.

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 pharmaceutical products requires purity of raw materials and finished products. UPLC is used for the highly regulated, quantitative analyses performed in QA/QC laboratories.

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.

The following parts of UPLC are important to give the required information:-

UPLC columns: High stability allows for a wide range of column temperatures and pHs to be explored. UPLC Column Manager: Easily evaluate column temperatures from 10 °C below room temperature to 90 °C; enables to use HPLC methods on the UPLC before scaling to UPLC.UPLC Calculator: Put information at fingertips about how to transition existing chromatographic analyses to faster UPLC methods.

Impurity Profiling

Impurity profiling requires high-resolution chromatography capable of reliably and reproducibly separating and detecting all of the known impurities of the active compound. UPLC System and Columns specifically address high-throughput analysis requirements while maintaining high peak resolution. UPLC- PDA detector involves two analytical flow cells are available for maximum flexibility according to application requirements, one for maximum chromatographic resolution and a

second for high sensitivity. UPLC also involves the latest peak detection algorithms and custom calculations to optimize data processing and reporting. It also confidently detects impurities in compounds even at trace levels. UPLC combines with exact mass LC/MS, has been successfully employed for the identification of drug and endogenous metabolites.

Compound Library Maintenance

The use of the fast-scanning MS along with the throughput of the UPLC System's remote status monitoring software allows chemists to obtain high-quality comprehensive data about their compounds in the shortest possible timeframes. This, combined with intelligent open access software, allows making informed decisions faster, and better supporting the needs of the modern drug discovery process.

Open Access

UPLC and UPLC/MS systems and software enable versatile and open operation for medicinal chemistry labs, with easy-to-use instruments, a user-friendly software interface, and fast, robust analyses using UV or MS for nominal and exact mass measurements[3, 10].

9. LITERATURE SURVEY OF UPLC ANALYSIS

Trivedi R K. et al have developed a stability indicating RP-UPLC method for simultaneous determination of ambroxol hydrochloride, cetirizine hydrochloride, methyl paraben and propyl paraben in liquid pharmaceutical formulation. The separation was achieved on an Agilent Eclipse plus C18, 1.8 m (50 x 2.1 mm) column using a mixture of 0.01 M phosphate buffer, 0.1 % triethylamine and acetonitrile within 3.5 min[12].

Yadav M. et al have reported UPLC–ESI-MS/MS method for determination of protease inhibitors, lopinavir and ritonavir in human plasma using 10mM ammonium formate (pH 4) and the run time was 1.2 min[13].

Wang D. et al worked for UPLC–MS/MS method development for the determination of nifedipine in human plasma using acetonitrile–10 mmol/L, ammonium acetate (75:25, v/v) with a flow rate 0.20 ml/min and run time 1.4 min per sample[14].

Berg T. et al determined opiates, cocaine and benzoylecgonine in urine by UPLC–MS/MS by using 5 mM ammonium bicarbonate (pH 10.2) and methanol and total run time, including injection and equilibration time was 5.7 min[15].

Arthur L D. et al developed a multiplex UPLC-MS/MS method requiring 100 µl of plasma for simultaneous quantification within 7 min of Fluconazole, Itraconazole, Hydroxyitraconazole, Posaconazole, Voriconazole, Voriconazole-*N*-Oxide, Caspofungin, and Anidulafungin[16].

Stolker A M. et al have used UPLC–ToF-MS method for screening and quantification of more than 100 veterinary drugs in milk including benzimidazoles, macrolides, penicillins, quinolones, sulphonamides, pyrimidines, tetracylines, nitroimidazoles, tranquillizers, ionophores, amphenicols and non-steroidal anti-inflammatory agents (NSAIDs). After protein precipitation, centrifugation and solid-phase extraction (SPE), the extracts were analyzed by UPLC–ToF-MS[17].

Verplaetse R. et al determined 9 narcotic analgesics and metabolites (buprenorphine, O-desmethyltramadol, fentanyl, nor buprenorphine, nor fentanyl, pethidine, piritramide, tilidine and tramadol) in urine and whole blood using gradient elution with 10 mM ammonium bicarbonate (pH 9) and methanol[18].

Tylova T. et al have developed UPLC method with UV detection for determination of tetracycline, oxytetracycline, chlortetracycline and doxycycline including their epimers in the liquid hog manure. The antibiotics were extracted with ethyl acetate and separated on UPLC BEH Shield RP18 column[19].

Sesherdi R. et al estimated Metoprolol (MT), Atorvastatin (AT)and Ramipril (RM) from capsule dosage form by UPLC method using Zorbax® XDB-C18 (4.6 mm x 50 mm, 1.8 m) column with a mobilephase consisting of ratio of 0.0045 M Sodium lauryl sulphate buffer : Acetonitrile (50:50 v/v), and retention times were about 1.3, 2.1 and 2.6 min for them respectively[20].

Chen L. et al, developed UPLC–MS/MS method for the quantitative determination of azithromycin in human plasma and its application in a pharmacokinetic study with roxithromycin as internal standard using ACQUITY UPLC™ BEH C_{18} column (50 mm × 2.1 mm, i.d., 1.7 m) with gradient elution at flow rate of 0.35 ml/min[21].

UPLC/MS/MS method was developed by Cai S. et al, for the quantitative determination of mitiglinide in human plasma with nateglinide as internal standard and the mobile phase used was methanol and 10 mmol/L ammonium acetate (65:35, v/v) at a flow rate of 0.25 ml/min[22].

Loos W J. et al have reported UPLC–MS/MS method for the simultaneous quantitative determination of dextromethorphan (DM) and its metabolites dextrorphan (DX), 3-methoxymorphinan (3MM) and 3-hydroxymorphinan (3HM), in human lithium heparinized plasma at a flow-rate of 0.25 ml/min on a gradient of acetonitrile. The overall cycle time of the method was 7 min, with elution times of 1.3 min for DX and 3HM, 2.8 min for 3MM and 2.9 min for DM[23].

A new stability indicating assay method was developed for the estimation of trandolapril and its degraded products by isocratic reversed phase chromatographic technique using ultra performance liquid chromatography by Sahu K., et al. Separation was achieved on a BEH (bridged ethylene hybrid) C18 column utilizing water-acetonitrile in the ratio of 20:80, at the flow rate of 0.2 ml/min[24].

Huang J. et al developed method of UPLC–MS/MS for quantification of nano formulated ritonavir, indinavir, atazanavir, and efavirenz in mouse serum and tissue Chromatographic separation was achieved using a gradient mobile phase (5% acetonitrile in methanol and 7.5 mM ammonium acetate (pH 4.0)) on an ACQUITY UPLC BEH Shield RP 18 column. All compounds eluted within a 7 min run time[25].

Narasimham L**.** et al worked on rapid, precise and specific stability indicating UPLC method for simultaneous determination of beta-blockers and diuretic drugs in pharmaceutical formulations. The Chromatographic separations of all the drugs were achieved on a Waters Acquity BEH C18, 50×2.1 mm, 1.7 m UPLC column within a short runtime of 3.3 min[26].

Huang W. et al developed a simple, rapid and sensitive UPLC method for simultaneous analysis of five compounds (narirutin, hesperidin, naringin, neohesperidin and meranzin hydrate) in *Fructusaurantii*-type preparations in less than 12 min using a C18 column with gradient elution using acetonitrile, water and acetic acid at a flow rate of 0.3 ml/min and with a PDA detector[27].

Proenca P. et al determined Velproic acid in blood by UPLC-MS/MS method with electrospray ionization source in negative ion mode. The chromatographic separation was achieved using an Acquity UPLC BEH (2.1 × 50 mm id, 1.7 µm) column and a mobile phase containing ammonium acetate and acetonitrile, at a 0.5 ml/min flow rate[28].

Wang L. et al established UPLC fingerprint for the identification of *Magnoliae officinalis* cortex processed. It was extracted by methanol using an ultrasonic extractor. Column was BEH C_{18} and a DAD detector and flow rate was 0.3 ml/min. The method was rapid and efficient[29].

Yang Z. et al developed a sensitive and reproducible UPLC–MS/MS method to analyze matrine, an anticancer compound, and to use it to investigate its biopharmaceutical and pharmacokinetic behaviors in rats. A sensitive and fast UPLC–MS/MS method was successfully applied to determine matrine in rat plasma, intestinal perfusate, bile, microsomes, and cell incubation media[30].

Lor E. et al quantified and confirmed 47 pharmaceuticals in environmental and wastewater samples by UHPLC–MS method They determined analgesic and anti-inflammatories, cholesterol lowering statin drugs and lipid regulators, antidepressants, anti-ulcer agents, psychiatric drugs, cardio vasculars and a high number (26) of antibiotics from different chemical groups with a run time of only 10 min[31].

Trivedi R. et al determined quetiapine in pharmaceutical dosage form by RP-UPLC method using 0.1% aqueous triethylamine (pH 7.2) and 80:20 v/v mixtures of acetonitrile and methanol within a run time of 5 min[32].

Ma Y. et al developed UPLC–MS/MS method for the determination and pharmacokinetic study of amlodipine in human plasma using an ACQUITY UPLC™ BEH C18 column (50mm×2.1 mm, i.d., 1.7m) with gradient elution at a flow-rate of 0.35 ml/min and the mobile phase was water and acetonitrile both was containing 0.3% formic acid[33].

Avery L. et al carried out validation for quantitation of protein free efavirenz as well as total concentrations of efavirenz in human blood and seminal plasma by UPLC–MS/MS technique using racemic fluorinated analog of efavirenz as the internal standard and they were detected via negative ion multiple reaction monitoring in 8.0 min[34].

Dongre V. et al developed a new isocratic reverse phase chromatographic method using UPLC for primaquine phosphate bulk drug. The chromatographic separation was achieved on a Waters Acquity BEH C18 column within a short runtime of 5 min[35].

Nikalje AG. et al developed a novel, simple, rapid and stability-indicating reversed-phase ultra performance liquid chromatographic method and subsequently validated for quantization of Emtricitabine from drug substance matrix on Waters ACQUITY UPLC BEH C_{18} (50 x 2.1) mm, 1.7µm column in isocratic mode with flow rate 0.25 ml/min. Mobile phase used was 0.015 M potassium dihydrogen phosphate buffer pH 2.2 and acetonitrile in ratio 75:25 v/v. The retention time of emtricitabine was 1.2 minutes[36].

10. CONCLUSION

UPLC by using 1.7µm particle size 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.

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