



ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY AN ADVANCED ANALYTICAL TOOL

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Abstract

Ultra Performance Liquid Chromatography (UPLC) takes the advantage of technological strides made in particle chemistry performance, system optimization, detector design and data processing and control. Using sub 2 mm particles and mobile phases at higher linear velocities and instrumentation that operates at higher pressures than those used in HPLC, dramatic increases in resolution, sensitivity and speed of analysis can be obtained. This new category of analytical separation science retains the practicality and principles of HPLC while creating a step function improvement in chromatographic performance. This review introduces the theory of UPLC and summarizes some of the most recent work in the field

Key words: Modern analytical techniques, UPLC, sensitivity

INTRODUCTION

UPLC refers to Ultra Performance Liquid Chromatography. UPLC is new category of analytical separation science works on the similar principles of HPLC while increasing the overall interlaced attributes of speed, sensitivity & resolution.[1] It uses fine particles and saves time and reduces solvent consumption.[2] For many years, researchers have looked at “fast LC” as a way to speed up analyses. The “need for speed” has been driven by the sheer numbers of samples in some laboratories (particularly in drug discovery) and the availability of affordable, easy to use mass spectrometers. Smaller columns and faster flow rates (amongst other parameters) have been used. Elevated temperature, having the dual advantages of lowering viscosity, and increasing mass transfer by increasing the diffusivity of the analytes, has also been investigated. However, using conventional particle sizes and pressures, limit-actions are soon reached and compromises must be made, sacrificing resolution for time.[2] UPLC is 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 particle size of column packing decreases, efficiency and thus resolution also increases. As particle size decreases to less than 2.5 μm , 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 Deemter equation.[3] 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.[4]

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 [5,6] 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 μm are used with these systems, to accelerate the analysis without loss of efficiency, while maintaining an acceptable loss of load To improve the efficiency of HPLC separations, the following can be done :-

A. Work at higher temperatures- allows high flow rates by reducing the viscosity of mobile phase which significantly reduces back pressure [7,8]

B. Use of monolithic columns- contains polymerized porous support structure that provide lower flow resistances than conventional particle-packed columns.[9,10,11]

UPLC improves in three areas [12-16]

1. Chromatographic resolution
2. Speed
3. Sensitive analysis

It uses fine particles and saves time and reduces solvent consumption. This new category of analytical separation science retains the practicality and principles of HPLC while increasing the overall interrelated attributes of speed, sensitivity and resolution. Today's pharmaceutical industries are looking for new ways to cut cost and shorten time for development of drugs while at the same time improving the quality of their products and analytical laboratories are not exception in this trend. Speed allows a greater number of analyses to be performed in a shorter amount of time thereby increasing sample throughput and lab productivity. These are the benefits of faster analysis and hence the ultra performance liquid chromatography. A typical assay was transferred and optimized for UPLC system to achieve both higher sample analysis throughput and better assay sensitivity. Analysis of operation cost and sample throughput found, UPLC cost advantageous over HPLC.[14,15]

Instrumentation:

To truly take advantage of the increased speed, superior resolution and sensitivity afforded by small particles, instrument technology also had to keep pace. A completely new system design with advanced technology in the pump, auto sampler, detector, data system, and service diagnostics was required.[16]

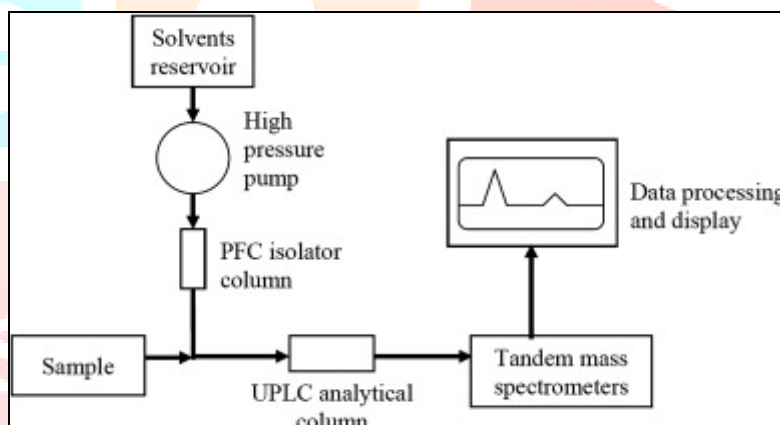


Fig. No. 01:Instrumentation of UPLC

Pumping System [17]

Achieving small particle, high peak capacity separations requires a greater pressure range than that achievable by today's HPLC instrumentation. The calculated pressure drop at the optimum flow rate for maximum efficiency across a 15 cm long column packed with 1.7 μm particles is about 15,000 psi. Therefore a pump capable of delivering solvent smoothly and reproducibly at these pressures, which can compensate for solvent compressibility and operate in both the gradient and isocratic separation modes, is required. 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 μm particles.

There are two types of pumps:

1. Reciprocating pump
 2. Pneumatic pump
- 1) Reciprocating pump

These types of pump operate by using a reciprocating piston or diaphragm. The liquid enters a pumping chamber via an inlet valve and is pushed out via a outlet valve by piston. Reciprocating pumps are generally very efficient and are suitable for very high flows.

There are two general types of reciprocating pumps.

- A) The piston pump
- B)The diaphragm pump.

There are two types of diaphragm pumps.

The hydraulically operated diaphragm metering pumps:

This type of pump can be used for pumping toxic and explosive fluids. The pump can deliver heads of up to 700 bars and transfer flows of up to 20 m³ /hr.

The air actuated type:

The pump capacity is limited by the air pressure available (generally 7 bar) and the design of the diaphragm. A flow rate of about 40 m³ /hr is a reasonable maximum achievable flow with a larger pump.

2) Pneumatic pump:

This type of piston was originally used for normal liquid chromatography separations but was found to be noisy and produced strong flow pulses that destabilized the detector. It is now used almost exclusively for slurry packing liquid chromatography columns. It is the simplest type of pump that can be designed to provide exceedingly high pressures.

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.[18]

Sample Manager

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. 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.[19] 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.

UPLC columns

The design and development of sub-2µm particles is a significant challenge, and researchers have been very active in this area to capitalize on their advantages.[20] Although high efficiency nonporous 1.5µm particles are commercially available, they suffer from low surface area, leading to poor loading capacity and retention. To maintain retention and capacity similar to HPLC, UPLC must use a novel porous particle that can withstand high pressures. Silica based particles have good mechanical strength, but suffer from a number of disadvantages. These include tailing of basic analytes and a limited pH range. Another alternative, polymeric column can overcome pH limitations, but they have their own issues, including low efficiencies and limited capacities. However, in order to provide the kind of enhanced mechanical stability UPLC requires, a second generation hybrid technology,[21] was developed, called ACQUITY UPLC. ACQUITY 1.7µm particles bridge the methyl groups in the silica matrix, enhancing their mechanical stability. Evolution is increased in a 1.7 µm 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 [22]

- A) ACQUITY UPLC BEH T M C18 and C8 (straight chain alkyl columns)
- B) ACQUITY UPLC BEH Shield RP 18 (embedded polar group column)
- C) ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl)
- D) ACQUITY UPLC BEH Amide columns (trifunctionally bonded amide phase)

Detectors

TUV Detector (Tunable ultraviolet detector)

The analytical cell, with a volume of 500 neon liters and a path length of 10 mm, and high sensitivity flow cell, with a volume of 2.4 micro liters and a 25 mm path length, both utilize the flow cell technology. The TUV detector operates at wavelengths ranging from 190 to 700 nm.[23]

PDA Detector (Photo diode array detector)

The PDA (photodiode array) optical detector is an ultraviolet/visible light (UV/Vis) spectrophotometer that operates between 190 and 500 nm. The detector offers two flow cell options. The analytical cell, with a volume of 500 nanoliters and a path length of 10 mm, and high sensitivity flow cell, with a volume of 2.4 micro liters and a 25 mm path length, both utilize the flow cell technology.[24]

ELS Detector

ELS detector is an evaporative light scattering detector designed for use in the UPLC system. 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.[25] In addition, the phases of less than 2 μm are generally non-regenerable and thus have limited use.

Advantages of UPLC

Various advantages of UPLC are as follows:

- Require less run time and enhance sensitivity.
- Provides the selectivity, sensitivity, and dynamic range of LC analysis.
- In chromatogram resolved peaks are obtained.
- Multi residue methods are applied.
- Speedy analysis, quantify accurately analytes and related products.
- Uses of fine particle (2 μm) for packing of stationary phase make analysis fast.
- Time and cost both are reduced.
- Consumption of solvents is less
- More products are analyzed 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.

Disadvantages of UPLC

In UPLC analysis the main disadvantage occurs are life of columns, during analysis high pressure developed because the particle size. Increase pressure reduces the life of the columns. Due to increased pressure requires more maintenance and reduces the life of the columns of these types. Using stationary phase of particle size 2 μm perform better analysis without the adverse effects of high pressure.

APPLICATIONS OF UPLC

1. Determination of Pesticides in Groundwater [26]

UPLC coupled with triple quadrupole tandem mass spectrometry (UPLCTM-MS/MS) can be utilized to determine the trace level pesticides in groundwater in less time and speedy manner. The technique has enhanced the analysis speed, sensitivity, and resolution.

2. Improved Resolving Power in Peptide Maps [27]

Peptide mapping is an essential technique for the characterization of proteins. Due to exceptionally reduced instrument and column dispersion, the analyzes of tryptic digest of phosphorylase by UPLC technology provides significantly improved resolution, peak capacity, and sensitivity compared to HPLC, allowing the detailed characterization of the protein

3. Analysis of Traditional Chinese Medicines (TCM) [28]

The identification and quantification of components of TCM by chromatographic analysis is one of the major challenges. TCM is a complex matrix in which all the constituents play a specific role for the overall

efficacy. Therefore, the analysis of all the constituents is synchronously necessary for the quality control. The new technique UPLC is used for the quality control of the TCM

4. Multi-Residue Analysis of Pharmaceuticals in Waste Water [29]

The water used in the pharmaceutical companies is found to have the traces of various cholesterol-lowering statin agents, anti-ulcer agents, antibiotics, beta-blockers, analgesics, anti-inflammatory agents, lipid regulating agents, psychiatric drugs, and histamine H₂ receptor antagonists. UPLC coupled with Q-TOF-MS is used to confirm and screen these drugs in the samples of waste water treatment plant.

5. Identification of Metabolites [30]

The identification and detection of all the possible metabolites of the candidate drugs for the discovery of new chemical entities is a very important step. For the identification of the metabolites, a high sample throughput is required to be maintained by the analysts to provide quick results to the medicinal chemists. UPLC-MS/MS is helpful in biomarker discovery as it meets tough analytical requirements and provides sensitivity, mass accuracy, dynamic range, and resolution.

6. In Manufacturing / Quality Assurance (QA) / Quality Control (QC) [31]

Identification, quantification, purification, efficacy and safety are key parameters to be evaluated during manufacturing of a drug product and pharmaceutical dosage form. Material stability is also observed as a component of QA and QC. UPLC is used as an important tool in QA/QC laboratories for the quantitative and extremely regulated analysis.

7. Impurity Profiling [32]

Impurity profiling should be efficient for consistent detection and separation of all the impurities present in the active compound. The drug development and formulation process demand accurate measurement/testing of low-level impurities present with the active pharmaceutical ingredients or the excipients or the raw materials used in the preparation of the final product. Thus, the presence of excipients in the sample makes the profiling difficult and with HPLC method, it takes longer time for analysis to achieve sufficient resolution. Thus, the combination of UPLC with mass spectrometry has been useful for the documentation of drug and endogenous metabolites in the final product.

8. Method Development / Validation [33]

Method development and validation is a complex process and consumes a lot of time. For the development of a robust and reliable method, the labs are required to study many combinations of different parameters e.g. mobile phase, temperature, pH, column and gradient chemistry etc. UPLC is an important method used in the laboratory which reduces the cost and increases the efficiency of analysis required for developing and validating the method. With UPLC, the speed of the separation increases and efficiency improves, which results in the fast development of methodologies. High stability of the UPLC columns provides the possibility of selection of column temperature and pH from a wide range.

9. Forced Degradation Studies (FDS) [34]

This study is done to access the chemical stability of the candidate compound in the pharmaceuticals. Usually, it is performed at the preliminary stage in the process of drug development. Forced degradation/stress testing is performed under accelerated environment. The experimental conditions cause the candidate compound to degrade under extreme conditions like acid and base hydrolysis, peroxide oxidation, photo-oxidation and thermal stability to identify the resultant degradation products. This helps to establish degradation pathways and thus intrinsic stability of a drug substance. The stability of product describes shelf life and storage conditions and helps in the selection of appropriate formulations and their suitable packaging.

10. Dissolution Testing [35]

Dissolution testing is one of the most important step carried out during formulation and manufacturing process to test the drug release. The dissolution data provides understanding to validate consistency and uniformity of the active ingredient in every batch. Testing of potent drugs in sustained release dosage form is very important as their dissolution studies data can affect the delivery of the medicine. Moreover, new and potent formulations require higher separation sensitivity. UPLC method provides accurate and consistent automated online sample acquirement.

11. Bioequivalence / Bioanalysis Studies [36]

Bioequivalence studies are pharmacokinetic studies needed for the quantitation of drugs in biological samples. This is an important step to compare the rate and exposure level of newly developed formulations of prevailing drugs with that of the original formulation. The selectivity and sensitivity of UPLC-MS/MS produce reliable and precise data. UPLC- MS/MS solutions have increased efficacy, output, and profitability for the bioequivalence laboratories. UPLC sample manager enhances the effectiveness by considering a huge number of samples in a temperature controlled atmosphere, confirming maximum throughput which increases the sensitivity and quality of data acquisition rates of tandem quadrupole MS systems.

12. Toxicity Studies [37]

During the drug development process, toxicity issue causes a fall out of drug candidates and this causes monetary loss to the organization. It is a complicated task to estimate candidate drugs for possible inhibition or initiation of metabolizing enzymes, toxicity or drug-drug interactions in the body. UPLC allows precise detection due to its high resolution. Further, its sensitivity also allows the detection of the peaks at low concentrations. These factors lessen the time for analysis and decrease failure of sample analysis.

13. Iodinated Disinfection Byproducts (IDBPs) [38]

Till date, a few numbers of IDBPs have been characterized in drinking water by using GC/MS. But with the help of coupling UPLC to the electrospray ionization-triple quadrupole mass spectrometer (ESI-tqMS), pictures of IDBPs in samples of water, treated with chlorine and chlorine- ammonia have been collected and 17 IDBPs structures were provisionally projected.

14. Therapeutic Drug Monitoring [39]

The monitoring of β -lactam antibiotic concentration in plasma of patients with different pharmacokinetics. In their studies, they tried to validate a UPLC-MS/MS method for the simultaneous estimation of two β -lactamase inhibitors and seven β -lactam antibiotics in human plasma. The main benefit of the technique is the faster speed of analysis (5.5 min /sample) compared to other approaches used for this type of multiple analytes.

15. Analysis of Explosives [40]

UPLC proved to be an enhanced procedure to analyze various explosives. In addition, analysis of explosive remains from hand swipes can also be detected by UPLC with minimal sample preparation requirement (less than 9 min), thus enhancing the lab output as well as freeing up valuable MS time for further analysis.

16. Analysis of Contaminants in Foodstuffs [41]

Sudan I-IV is a red colored dye which is commonly used in petrochemical industries. The intensive color of Sudan dyes lured frauds for improving the color of several spices and food stuffs which can form DNA adducts causing mutations. UPLC coupled to tandem mass spectrometry allows the identification of Sudan at low ppb levels in spices and chilli containing food stuffs. These fast peaks are additional peaks as these eluted a few minutes before the main peak of the compounds.

17. Dendrimers Characterization [42]

Dendrimers are highly branched symmetric polymers having a compact round structure (diameter 1.1nm to 9 nm) and unique behavior. They are normally synthesized from a central polyfunctional core by repetitive addition of polymers. Dendrimers surfaces provide a brilliant stage for the attachment and appearance of cell specific targeting groups, solubility modernizers and stalth moieties that decrease immunological interactions. Polyamidoamine (PAMAM) dendrimers are one of the widely used dendrimers. HPLC has been utilized to isolate and to check the purity of many PAMAM dendrimer generation or conjugates. This technique also helps to study the solubility of multi functionalized dendrimers and the interactions between them and biomolecules. UPLC reduces the retention time of analytes with an improvement of the resolution proficiency during dendrimers studies

18. Determination of Phytoconstituents [43]

UPLC can be used to identify and quantify procyanadines, phenolic compounds, monomers, oligomers, isoflavones, flavonoids, coumarins and alkaloids such as caffeine and theobromine. It is clear from this figure that UPLC method completes the process in less time and sharp peaks are obtained.

19. Identifying Static and Kinetic Lipid Phenotype [44]

High resolution UPLC-MS is employed to study the concentration of lipids and their endogenous production. Therefore, this technique was found to be useful in determining the contribution of different pathways and synthesis that could affect lipid biology.

20. Analysis of Free Amino Acids (FAA) in Wines [45]

The production of FAA in the less aged white wines can be determined by the UPLC. The UPLCTM method is an established method for the analysis of amino acids using 6-aminoquinolyl. This new UPLCTM method has made the separations quick and reliable for 24 amino acids within 23 minutes. This method proved to be superior compared to original HPLC method due to much improvement in resolution with reduced run time.

21. Identification of Metabolic Biomarkers to Diagnose Epithelial Ovarian Cancer (EOC) [46]

Currently available tests are insufficient to distinguish patients with EOC from normal individuals. Plasma specimens of EOC patients and normal individuals were analyzed using UPLC/QTOF/MS. Eight biomarkers were identified which may serve as novel biomarkers for diagnosis.

22. ADME [47]

ADME studies include absorption, digestion, metabolism, and elimination. Yan developed and validated the sensitive UPLC-MS/MS method for the pharmacokinetic studies of HZ08 liposome injection in plasma and tissues of rats to study plasma kinetic and tissue distribution respectively.

23. Drug Abuse [48]

UPLC-MS/MS method can be used to develop and evaluate a fast, robust and specific screening platform for the determination and quantification of a variety of commonly used drugs of abuse (opioids, benzodiazepines etc) in urine.

CONCLUSION

UPLC is one of the most important tools in analytical chemistry which increases the speed, resolution, and sensitivity of the chromatographic analysis and decreases the time, solvent consumption and cost involved. The peaks obtained through UPLC have decreased noise and better signal to noise ratio. It gives sharp and narrow peaks of more or less all categories of pharmaceutical drugs. It also facilitates the analysis of complex mixtures in less time and the peaks obtained through this method depicts more information which is more clearer in comparison to the peak obtained through HPLC. This method is widely used for the analysis of different pharmaceuticals such as amino acids, peptide mapping, glycans analysis, phenotyping, drug discovery, metabolomics etc. This technology thus creates a new opportunity for business profitability in highly efficient manner and allows the product to be introduced to the market in less time.

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