

A SYNOPSIS OF ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)

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ABSTRACT

UPLC is refers to Ultra Performance Liquid Chromatography, which improves in three area that are speed, resolution and sensitivity. It is works on the principle of van deemter equation. It is used for fine particles (less than 2 µm), which can reduce the length of column, save time and reduce solvent consumption. UPLC plays a crucial role in separation and quantification of API, Excipients and Impurities in pharmaceutical product .UPLC is used for analysis of biological sample fluids, dosage form and determines the accurate concentration of plant extracts and Pharmacokinetic parameters. It is reducing the analysis time up to 9 times as compared to other chromatographic system. It has been under high pressure (100mpa).In 21st century uplc is suitable for the drug development within short period of time.

KEYWORDS: UPLC, Resolution and Drug.

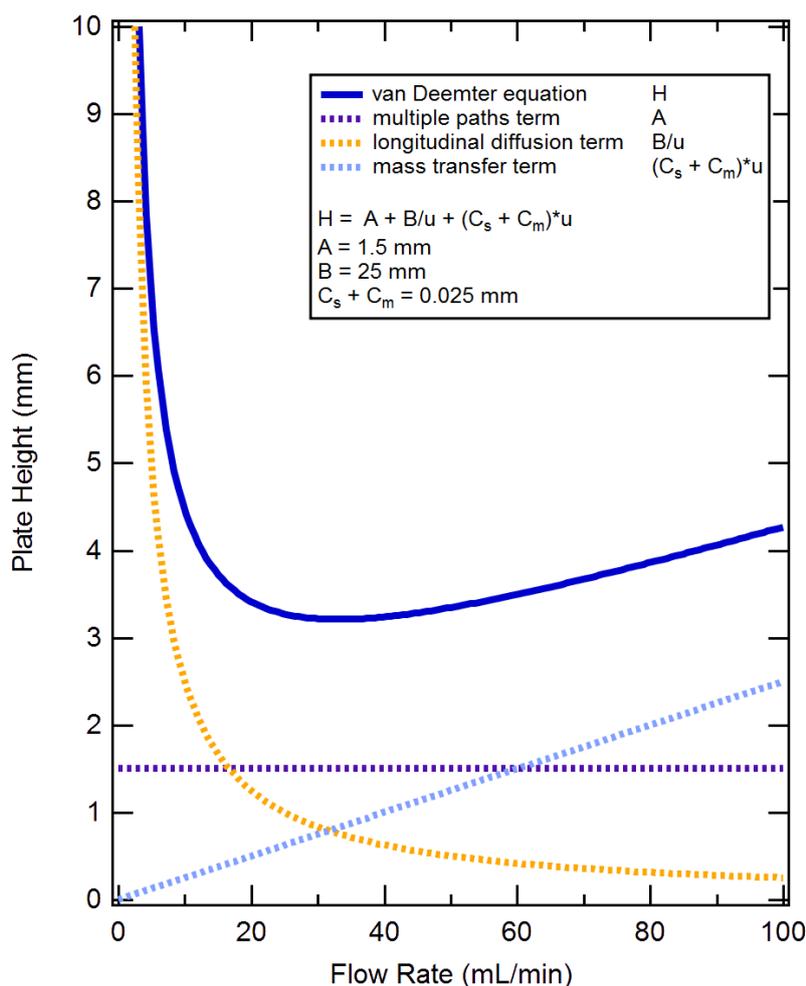
INTRODUCTION

Chromatographic technique is used in the analytical laboratory for both the qualitative and quantitative analysis of medicine in worldwide throughout 30 plus year. It is a method of separating a mixture of compound into individual component using porous material. This system use very fine particle (less than 2.5µm), mobile phase at higher linear velocities, reduces the length of column, decrease solvent consumption and time consumption is also less.^[1] It is widely used for drug development, QC, regulatory compliance and analytical testing. UPLC is particularly effective for separation, identification and quantification of complex mixture of API, excipient and impurities done at high pressure (100 mpa).^[3] It is advanced technology of HPLC, Which is important part of Diagnosis, prognosis and assessment of

therapeutic intervention in clinical application.^[10] The current utility of the Uplc is in the field of oncology, metabolic, neuropsychiatric, cardiovascular and other disease related drugs.^[9]

Principle

The van deemter equation describes column efficiency in chromatography which depends upon mobile phase velocity (speed of solvent moves through column). Uplc column are too shorter than Hplc column, ranges from 30mm to 150mm. The shorter length of column allows UPLC to achieve faster separation, high resolution, better sensitivity as compared to HPLC. The basic principle of UPLC and HPLC is same and depends on the mode of separation [i.e., Adsorption, Partition, Exclusion and ion-exchange] and depending on type of chromatographic sorbent. The stationary phase consist of less than 2 μ m in diameter, which predicts by van deemter equation that explains correlation between flow rate and plate height. It shows the ranges of smaller particle is greater than larger particle for the resulting^[1,2,13,7]



$$H = A + B/V + CV$$

H = Height Equivalent to Theoretical Plate [HETP]

A = Eddy's diffusion

B = Diffusion co-efficient

C = Resistance to mass transfer co - efficient

V = Linear velocity

FACTORS AFFECTING SEPARATION

Separation of chromatography is depends upon efficiency, retention factor and selectivity.

PARAMETERS AFFECTING EFFICACY

1. Column length

Larger column has more efficacies, than more interaction between analyte and stationary phase which has increase back pressure. So that selection of column is optimum for the separation.

2. Particle size

Small particle has high surface area and improve analyte-stationary phase interaction which reduce the broadening of peak and increased efficiency and resolution. It is used only for very small particle (2 μm).

3. Particle size distribution

Uniform packing of the particle shows even distribution and reducing the void space in column. Band broadening is minimized which leads to sharpen the peak and improves efficiency.

4. Flow rate

Flow rate controlling the time of interaction. It is followed by van deemter equation;

Low flow rate - Broadening of peak

High flow rate - Reduce resolution

Optimum flow rate - Maximum efficacy^[4,5]

PARAMETERS OF RETENTION FACTOR

1. Eluent type

Polarity of solvent may affects the solubility of analyte. Different solvent can change the migration of analyte, so retentive factor is altered.

Strong eluents - Reduces retention time

Weak eluents - Increases retention time

2. Eluent composition

The ratio of solvent may affects strength of elution. Retention time is depends upon mobile phase

Higher organic content - Reduces retention time

Lower organic content - Increases retention time

3. Stationary phase type

Different stationary phase has different polarities, analyte interaction may differ from each phase. So the retention time variation is occurs;

Strong interaction - Increases retention time

Weak interaction - Reduces retention time

4. Nature of analyte

Retention factor is controlling by nature of analyte are polarity, molecular size, ionization, P^H and Hydrophobic compounds.^[4,5]

PARAMETERS OF SELECTIVITY

1. Stationary phase

The different stationary phase shows different selectivity, chemical reaction variation is happened between the analyte may influence relative retention that may cause impact on selectivity

Eg: Inertsil C18, C8, phenyl phase separate differently.

2. Analyte nature

The interaction gets affected due to the structure of analyte. The functional group presents in the molecule influence the selectivity. Polarity difference plays major role in the chromatographic separation. Analyte chemistry will be helps to determine the selectivity.

3. Eluent addition

Buffers are used to control the ph of the mobile phase. Ion-pair reagent modifying the behavior of the ionic analyte. Peak tailing is decreased by the unwanted reaction occurs between stationary and mobile phase.

4. Temperature

Higher temperature influence viscosity of the mobile phase. Viscosity is improved by the mass transfer of phase. Retention time of the analyte is decreased, selectivity between compounds can change with temperature.

5. PH

Ionization of analyte is controlled by ph, small changes in ph can affect the selectivity;

Ionised compound - Elute fast

Neutral compound - Retain long

Basic compound - Elute slow^[4,5]

ANALYTICAL METHOD VALIDATION

Analytical method is suitable to demonstrate the analytical procedure for its intended use.

- Specificity
- Accuracy
- Precision
- Limit of Detection [LOD]
- Limit of Quantification [LOQ]
- Linearity
- Range
- Robustness^[4]

AS PER ICH Q2 [R1]

COMPARISON OF HPLC VS UPLC^[4, 2,10,7]

S.NO	PARAMETER	HPLC	UPLC
1.	Abbreviation	High performance liquid chromatography	Ultra performance liquid chromatography
2.	Particle size	3 - 5 μm	< 2 μm
3.	Operating pressure	6000 Psi	up to 15,000 Psi

4.	Flow rate	High	Low
5.	Running time	High	Low
6.	Resolution	Less resolution power is required	High resolution power is required
7.	Sensitivity	Moderate	Low
8.	Consumption of mobile phase	More	Less than 50- 80%
9.	Sample volume	Larger injection is required	Smaller injection is required
10.	Life time of column	More life span	Less life span
11.	Cost	Less expensive	Expensive
12.	Pump	Binary /quaternary pump system are used	Only binary pump system is used
13.	Maintenance	Low maintenance	High maintenance
14.	Suitability	Routine analysis	Fast, high speed
15.	Type of chromatography	Column chromatography	Column chromatography
16.	Stationary phase	Filled into column	Filled into column
17.	Chromatography phase	Reversed phase	Reversed phase
18.	Usage of solvent	20ml/run	6ml/run
19.	Column temperature	Up to 80 °C with exception	Up to 90 °C or higher

INSTRUMENTATION AND METHODOLOGY OF UPLC

In 2004 WATER CORPORATION introduced UPLC as new direction in liquid chromatography to overcome the difficulties in HPLC. Both the HPLC and UPLC were working as same Principle and Instrumentation.

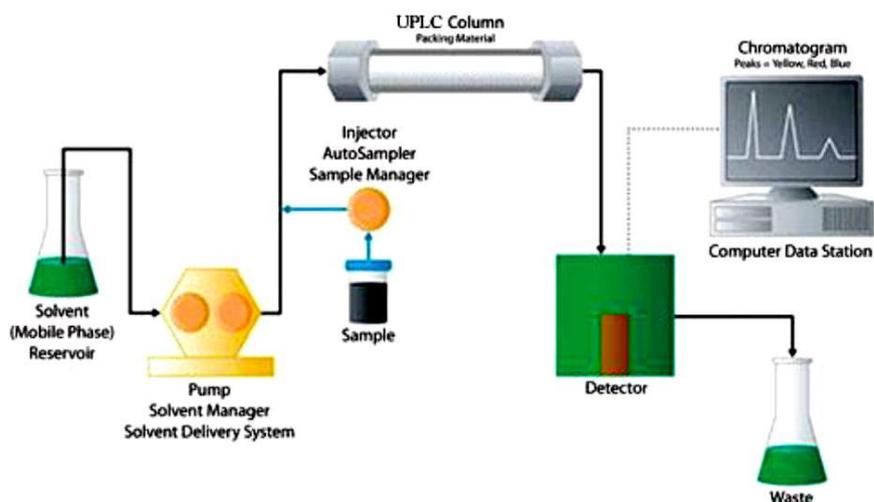


Fig. 1: Block Diagram of HPLC.

Components of UPLC are

- ✓ Solvent Reservoir (Mobile Phase)
- ✓ Pump device
- ✓ Solvent delivery system
- ✓ Sample injector
- ✓ Sample manager
- ✓ Stationary Phase/UPLC columns
- ✓ Column heater
- ✓ Chromatographic conditions

- ✓ Detectors
- ✓ Software
- ✓ Waste collector

1. Mobile Phase/Solvent Reservoir

Mobile phase is mixed with many solvent such as water, acetonitrile, Methanol and various buffering agent phosphate buffer, acetate], which helps to maintain pH stability. Mobile phase has been stored in the either glass bottle or plastic bottle.

2. Pump Device

UPLC pump is one of important component of this system, it delivers continuous and constant flow of mobile phase through injector, column and detector. Operating pressure of pump is about 15,000Psi to support stationary phase. It should deliver solvent smoothly, reproducibly to achieve efficiency. Only the Binary pump is used in this system.^[14]

3. Solvent delivery system

The solvent delivery system in the UPLC will provide a smooth, constant and reproducible flow of mobile phase at high pressure. Operating ranges from 8,000-15,000psi. The system should be compatible with Isocratic as well as linear and non-linear gradient elution at high pressure. This system ensures pulse free solvent flows up to 1-2mL/min.^[14]

4. Sample injector

In Uplc, sample injection is a major step requiring accurate and reproducible injection of a small volume of sample into mobile phase. Needle injector is used to withstand high pressure and ensure sealing. Injection is designed pulse free for protecting fluctuation of column.^[14]

- **Filtration:** Sample is filter through the membrane (0.45µm - 0.2µm) to remove particulate matter which protects the column from clogging.
- **Dilution:** Sample is diluted to ensure concentration of the analyte within the calibration range of method.
- **Autoanalyser:** In this system autoanalyser allows precise and reproducible sample injection.
- **Injection volume:** The volume of injection is about 1-10 µl depending upon its concentration and requirements.

5. Sample manager

ACQUITY UPLC sample manager is responsible for injecting the sample into chromatographic flow. The sample is stored in the vials and microtiter plate which delivers accurate and reproducibly. System performing fast injection ranges from 15 seconds to support quick analysis. Column temperature can be maintained up to 65°C for improving separation efficiency and reproducibility.^[14]

6. Stationary phase /Uplc column

Column is the heart of the UPLC System. Sample particle are small is essential for achieving faster and high resolution separation in uplc. Convectional silica particle offers good mechanical strength to the column but suffer from limited P^H range and tailing. Polymeric column overcome the limitation of P^H but show low efficiency and mechanical strength. Hybrid column such as XTerra combine with silica and polymeric materials to provide stability and efficiency.^[11] Second generation BEH [Bridged Ethyl Hybrid] particles to improves mechanical strength, pH tolerance and reduce viscosity.^[11]

- **Particle size:** Uplc column is packed with small particle (1.7 μ m), which allows high efficiency and resolution due higher surface area and diffusion is reduced.
- **Column length and diameter:** UPLC column is shorter and have small diameter than HPLC, which reduces back pressure and enhance speed of separation. This system has more choice of stationary phase are C18,C8, Phenyl or other specialized phase, which determine the interaction between analyte and stationary phase influencing retention and separation.

7. Column heater

The column heater is attached to the top of the sample manager and located beneath the instrument top cover. It maintains the temperature of column from 5 °C - 65°C to improve reproducibility, efficiency and reduces solvent viscosity.^[14]

8. Chromatographic conditions

- **Flow rate:** Uplc operating at high flow range from 1ml/min or more, which helps to reducing time of analysis.
- **Isocratic elution:** Only one mobile phase has been used to analyze the sample throughout the analysis.
- **Gradient elution:** Composition of mobile phase is changes during the time of run to improving the process separation of various compounds.
- **Temperature control:** In this system temperature should maintain constant to enhance reproducibility and resolution.

9. Detector

Detector is widely used for detection system due to their versatility and reliability.

Modern detector offers sensitivity and reduce noise.^[7] Commonly used detector include Photodiode Array (PDA), Tunable Ultraviolet (TUV), Evaporative Light Scattering (ELS), FLR and Mass spectroscopy detector.^[10]

- **Uv/vis detector:** It can measure the absorbance at specific wavelength, which is common for the chromophore.
- **Photodiode Array detector:** It is an optical detector that absorb UV- visible light that operate between 190-500nm.^[6]
- **Tunable ultra violet detector:** It has 2 channels, controlled by Empower and Mass Lynx software for each LC/MS and LC application. Wavelength from 190-700nm^[6]
- **Evaporative light scattering:** The detector is controlled by Empower or Mass Layrx software, which incorporates a flow type nebulizer that optimizes uplc system.^[1]
- **Fluorescence detector:** It offers for the fluorescence containing compound. It has multi channel, multiwavelength detector which an excitation wavelength ranges from 200nm-890nm.3D scanning capability is offered by this system.^[1]
- **Mass spectroscopy:** It combines with uplc for providing detail about molecular information, used for both qualitative and quantitative analysis.

9. Software

Uplc system is monitor, diagnosed and controlled by software through graphical console interface. Software such as EmpowerTM, Empower, Chemstation and MassLynxTM allows the data acquisition, processing and interpretation. It enables data acquisition, converts chromatographic data into analytical result.^[14]

10. Waste collector

Waste collector is a component design to collect the waste after the detection of mobile phase and sample. It should be maintain clean and safe instrument operation, which is disposed according to the Gudiline of the disposing of Waste. Waste collector is made up of **Glass bottle, High-density polyethylene (HDPE) container, PTFE tubing, and Safety cap with vent/filter.**

ADVANTAGES OF UPLC

1. Consumption of mobile phase is less than 80% as compared to HPLC.
2. The volume of injection is lower.
3. Time of running is low.
4. Enhance sensitivity.
5. The time of analysis is reduced.
6. Higher resolution performance.
7. Fast analysis due to high pressure.
8. Multi-residue method are applied which is used in pharmaceutical, bio-analytic and environmetal.^[4,6,9]

DISADVANTAGES OF UPLC

1. Very high back pressure as compared to conventional HPLC.
2. It has shorter half life.
3. Column are mostly non regenerative.
4. Instrumentation and column is expensive.
5. Binary pump system only used not tertiary (or) quaternary.
6. It requires special instrumentation.
7. The cost of maintenance is greater than HPLC.
8. High operating pressure (up to 15,000 psi).
9. AI and environmental analysis.^[6, 11, 14]

APPLICATION

- **Preformulation:** UPLC is used to study physical and chemical properties of drug samples including solubility, stability and purity.^[3]
- **Pharmacokinetics and bio analysis:** UPLC is used in bio-analytical laboratories to measure concentration of drug in Plasma, Urine, Traditional medicines and other biological fluids such as Sweat, Serum, Bile, Saliva, CSF, Synovial fluid, Semen and ADME screening.^[3]
- **Metabolic profiling:** It is used in drug development, primary drug is tested in animal and human to understand the metabolic pathways.^[3]
- **GMP:** GMP guidelines are issued by WHO, are instrumental in maintain GMP compliance during drug development.^[3]
- **Determination of pesticide:** It is used determine the pesticide present in the water by using hyphenated technique UPLC-MS.

- **Therapeutic Drug Monitoring:** TDM is precise measurement of drug level in patient samples for the optimal therapeutic outcomes and avoid adverse reaction, modern chromatographic method offer rapid and accurate analyzing of drug samples.^[8]
- **Dissolution test:** Dissolution test is an important tool in formulation, drug development for controlling the quality of drug and to release of it.
- **Toxicity studies:** UPLC allows accurate detection of toxicity (or) drug-drug interaction due to high resolution.^[8]

CASE STUDIES IN PHARMACEUTICAL APPLICATION

CASE STUDY 1

Determination of residual solvent in drug formulation: Uplc has been used extensively to measure concentration of residual solvent in drug products which quantify the trace amount of solvent such as methanol, ethanol, (or) acetone in final drug formulation.

CASE STUDY 2

Analysis of drug impurities in generic drug products: It is used for testing the quality of generic drug, so that easily demonstrate the trace amount of impurities in the sample drugs.^[3]

FUTURE TRENDS AND DIRECTION IN UPLC FOR PHARMACEUTICAL

1. **Integration with data analytics:** Integration with AI and advance data analytic is helps to reduce human error and improves the decision making in quality control.
2. **Miniaturization of UPLC system:** UPLC is more accessible in the laboratories including in QC labs, development of smaller, compact and portable instrument which has faster analysis time and the operating cost is low.
3. **Green chemistry application:** It is focused on eco - friendly analytical techniques such as using less solvent, less waste and less energy usage, which is align with global sustainability goals in pharmaceutical industries.^[3]

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