Review On Active pharmaceutical ingredients and impurity profiling: Advanced Chromatographic Techniques (HPLC, UPLC, GC).

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***Abstract*—Impurity is an unwanted substance present in the active pharmaceutical ingredients. that form during the synthesis process of active pharmaceutical ingredients or any unwanted constituent that is produced besides the active ingredient during the formulation or the aging of active pharmaceutical ingredients. Even the insignificant quantity of impurity existing in the medicinal product may harm the patient life and compromises the purity and superiority of the medicinal product. According to International council for harmonization guidelines, Analytica monitoring of impurity is a prerequisite and mandatory requirement for approval of market authorization of the new drug substance. Any pharmaceutical product would be capable to serve their intended therapeutic activity when they are free from impurity. Thus, an impurity existing in an active pharmaceutical ingredient needs to be identified, and quantify with the help of modern analytical approaches. This help of modern analytical approaches. This review explores the basic information concerning impurity profiling, highlights the advantages of an analytical technique and also focuses on the limitation of different analytical methods for impurity profiling with possible wavs to overcome the limitation.**

***Index Terms*— Impurity profiling, active pharmaceutical ingredients, drug substance, hyphenated technique, chromatographic separation techniques.**

I. INTRODUCTION

Drug formulations contain Active Pharmaceutical ingredients APIs and excipient API present in the formulation contain some undesired impurities, which effects purity of the APIs (1,2). Therefore with a long percentage purity, Impurity, profiling is also needed, To be carried out of all The APIs, impurity Profiling describes the account and description of maximum possible type of identification or unidentified impurities present in the any APIs related impurities include stereochemistry, crystallization and functional group of API Various regulatory authorities such as, ICH, USFDA, etc. (3,4) The different pharmacopeias such as the British pharmacopeias united state pharmacopeia are slowly incorporating limits to allowable levels of impurities present in APIs or formulations(5,6). Various method is used to isolate, & characterize impurities in pharmaceutical, such as capillary Electrophoresis electron paramagnetic resonance gas-liquid chromatography, Gravimetric Analysis, high performance liquid chromatography, solid phase extraction method Liquid-liquid extraction, method ultraviolet spectroscopy infrared spectroscopy, supercritical fluid extraction column chromatography, mass spectroscopy, nuclear magnetic resonance Spectroscopy, and RAMAN Spectroscopy. Among all hyphenated technique the most exploited technique for impurity profiling of drug are liquid chromatography (LC) Mass spectroscopy (MS) LC-NMR, LC-NMR-MS, GC-MS & LC-MS This reveals the needed & scope of, impurity profiling of drugs in pharmaceutical research. [7,8].

The purity of the drug product is in turn determined on the basis of the percentage of the labelled amount of API found in it by A suitable analytical method (9). The presence of some impurities may deleteriously Keep that on the drug quality if they have therapeutic efficacy that is similar to the greater than the drug substance itself Never the less drug substance can

consider as compromised with respect to purity even if it contains an impurity with superior pharmacological or toxicological property (10).

II. IMPORTANCE

Board applicability (drug, peptides, Food, Environment)
High reproducibility and quantitative accuracy
Compatible with many detector (UV, DAD, FLD, MS)
Faster run times> Higher lab through output
Improved resolution and sensitivity for complex samples. (11)
A reduced solvent uses per analysis (often)
Excellent separation DPCNC for volatiles
Robust, Rapid analyses with a variety of detector (FID, ECD, TCD, MS)
High sensitivity and selectivity (especially with MS) (12)

1. Enhanced analytical precision

Advanced chromatography methods (e.g., HPMC coma UPLC, GC-MS/MS) offer;
Her resolution for separating complex mixtures.
Batter sensitivity and accuracy, even at trace levels.
Quantitative and qualitative analysis of substances. (13)

2. Pharmaceutical industry

Drug development: identifies and qualifies active pharmaceutical ingredient (APIs) And Impurities.
Quality control; Ensure the drug meet regulatory standards (14)

3. Environmental monitoring

Detects pollutants, pesticides, and contaminants in:
Air, water, soil
Supports Environmental Protection and regulatory compliance (15)

4. Food and beverage are testing

Detects food Additives, preservatives, contaminants, and toxins.
Are you sure authenticity and prevent food fraud (e.g., Verifying origin of olive oil, honey)
Supports nutritional labeling by quantifying nutrients (16)

5. Biochemical and clinical research

Biomarker discovery: Identifies Disease marker in blood, urine, tissues.
Metabolomics and Proteomic: Studies metabolites and proteins in biological system. (17)
Therapeutic drug monitoring (TDM) Major drug level inpatient samples for optimized dosing (18)

6. Chemical and Petrochemical industry

Analyze raw materials, Intermediates, And final products.
Insure product consistency and purity in the fuels, polymers, and specialty chemicals. (19)

7. Forensic science

Identifies drugs, toxins, Explosives, And other chemicals in criminal investigations.
Provide robust, legally defensible evidence. (20)

8. Industrial process control

Real time monitoring of manufacturing processes
Ensure consistent quality and efficacy In Production (21)

III. OBJECTIVE

Develop a robust, selective and precise HPLC method for target analyte.
Validate the method for linearity, accuracy, precision, specificity, LOD / LOQ and robustness. (22)
Generate reliable quantitative outputs for routine analysis.
Translate or develop HPLC methods robustness under higher pressures.
Validate UPLC method performance.
Develop GC methods for validate analytes, including derivatization when necessary.
Select suitable columns and detectors for target analytes and matrices.
Validate for sensitivity and specificity, and obtain reliable quantitation. (23)

1. Precise separation of complex mixture

Two separate compounds in complex matrix with high resolution and sensitivity, even if they are chemically similar to present trace amounts (24)

2. Accurate qualitative and quantitative analysis

To identify qualitative and major quantitative the components of a simple with high accuracy and reproducibility. (25)

3. High sensitivity and specificity

To detect very low concentrations of analytes often in the part per million (PPM) or parts per billion (PPB) ranges. To distinguish analytes from background noise or similar compounds. (26)

4. Rapid analysis and high throughput

To reduce analysis time while maintaining or improving performance. Useful in industrial settings

and research labs that handle large simple volumes. (27)

5. Automation and data integration

To allow automated sample handling, data collection, and software-based analysis, improving workflow efficiency and consistency (28).

6. Versatility across sample types

To handle a wide range of sample Types Solid, liquid, gas, biological fluids, environmental sample, pharmaceutical, etc.

7. Method development and optimization

To develop and refine analytical methods tailored for space peak analytes and matrices.

8. Ensure compliance with regulatory standards

To meet the analytical requirements of Regulatory bodies (e.g., FDA, EMA, EPA) What product approval, environmental monitoring, and quality control.

9. Supporting research and innovation

To enable the discovery of new compounds, drugs or pathway through detail molecular analysis. Important metabolomics, proteomics, and material Science.

IV. GUIDANCE

Define Analytes, Required sensitivity and sample matrix.

Choose stationary phase (C18, C8, phenyl, ion, exchange) Based on analyte chemistry.

Select Mobile phase (Aqueous buffer+ Organic modifier), Set PH for analyte stability and retention.

To prepare mobile phase use HPLC grade solvent degasses and filter.

Sample preparation Filtration, centrifugation, dilution or SPE Clean up as needed.

Set instrument parameters column temperature, flow rate, injection volume detector wavelength.

Equilibrate Column; Run System Suitability Standards (Check theoretical plates, tailing, resolution).

Run calibration standards blank and QC sample alongside unknowns.

Process data integrate peaks build calibration curve calculate concentrations.

Validate: Specificity, Linearity, accuracy, precision, LOD/ LOQ, robustness, stability.

Routine maintenance: Flush/ Condition Column, replace frits/ Filters, monitor back pressure.

Confirm analyst stability and matrix compactivity with UPLC (small injection volumes).

Select a UPLC grade column sub 2 nanometer particle suitable for analyte chemistry.

UHPLC-compatible system component low dead volume settings and high pressure rated tubing.

Prepare high purity, the degassed mobile phases; Consider lower injection volume to avoid overloading.

Optimize gradient and flow to exploit faster separation; Increase detector acquisition rate.

System suitability; Check plate count, peak shape, backpressure and reproducibility.

Run standards/QC And perform full validation (Linearity, precision, accuracy, robustness).

Monitor column wear and maintain streak sample cleanness to avoid clogging.

SS analyte validity and stability; Perform derivatization of nonvolatile/ Polar compounds (e.g., sialylation).

Choose Column Type and Dimensions (Polarity, Length, Internal Diameter, film thickness) too much analytes properties.

Select carrier gas (He, H2, or N2) And set appropriate flow or pressure.

Choose injection mode; Split, spitless, on-column; Select appropriate linear and septum.

Design temperature oven program (initial temperature, ramp rate, final temperature) for separation.

Select Detector; FID For hydrocarbons, ECD For halogenated compounds, MS for identification.

Prepare sample (clean up, internal standards), inject calibration series and check system stability.

Run samples, Process chromatograms, use MS Spectra for confirmation if available.

Validate method (Linearity, LOD/LOQ, Precision, Accuracy, specificity) (29).

V. ADVANTAGES OF CHROMATOGRAPHIC TECHNIQUE

1 High sensitivity: - Can detect analyte and very low concentration (e.g., ppm, ppb, ppt).

2 Excellent sensitivity: - Can separate and identify components even in complex mixtures.

3 High resolution: - Capable of separating compounds with very similar structure or properties

4 Quantitative accuracy: - Provide precise and reproducible measurement of analyte concentration
5 Speed and Efficiency: - Techniques like UPLC and first GC offer rapid rapids.
6 Speed and efficiency: - Technique like UHPLC And first GC offer rapid analysis with minimal sample preparation
7 Atomization capability: - Easy integrated with auto Sampler and date processing software for high throughout put analysis.
8 Versatility: - Can be used across many sample types: environmental, pharmaceutical, clinical, and food, etc.
9 Coupling with detectors: - Coupling with MS, UV, Fluorescence detector enhances identification and qualification.
10 Regulatory acceptance: - Widely accepted by regulatory bodies (FDA, EPA, EMA) Compliance and quality control
11 Versatile for many compound classes.
12 Good quantitative Accuracy and reproducibility
13 Wide choice of detector.
14 Fast run and low solvent use.
15 Lower solvent per run in many cases.
16 Excellence for volatile and semi volatile analytes high separation efficiency.
17 Improved sensitivity and resolution.
18 Much faster analyte and higher peak capacity (30.

VI. DISADVANTAGES OF CHROMATOGRAPHIC TECHNIQUES

1 High cost: - instrument like LC-MS/MS or GC-MS Are expensive to purchase and maintain.
2 Complex operations: - Required skilled person to operate the interpret result accurately.
3 Maintenance intensive: - Regular calibration maintenance, troubleshooting are essential.
4 Expensive consumables: - Column solvent commerce standard and detector gasses can be costly.
5 Time consuming setup: - Method development and validation can be timed in Intensive.
6 Sample preparation: - Some techniques require extensive and careful sample preparation (e.g., Extraction filter)
7 Data overload: - Advance detector like mass spectrometer generate large volume of complex data needing advanced software for analysis.
8 Sensitivity to contamination: - Instruments and sensitive two contamination, which can affect accuracy and reproducibility.
9 Moderate solvent consumption.
10 Slower than UHPLC for the same separation power.
11 Columns have finite lifetime and required conducting.
12 Higher capital cost and maintenance (pressure related hardware).
13 More sensitive to system dead volume and sample particulates.
14 Column costs and back pressure considered.
15 Analyte must be volatile and thermally stable or required derivatization.
16 Some detectors (MS) required complex maintenance and expertise (31)
17 Carrier gas supply and thermal control required.

VII. EXPECTED OUTPUT

Chromatograms With separated peaks (retention time, peak area).
Calibration curve (response vs Concentration)
Method Performance matrix resolution (>1.5 desired), Tailing Factor, Theoretical plates, Precision (RSD Of area commonly <2-5% Depending on method).
Sharper peaks, shorter retention time, higher resolution chromatograms.
Similar method output as HPLC but with reduced runtime and often improve LOD /LOQ.
For GC-MS, Mass Spectra for each peak enabling identification.
Method matrices, Resolution (>1.5 desirable), The retention time reproducibility, calibration curve and quantitation limits(32).

VIII. INTRODUCTION TO UPLC

UHPLC refers to ultrahigh performance liquid chromatography.
It improves in three areas, chromatographic resolution, speed, sensitivity.
UHPLC is a rising chromatographic separation technique whose packing materials have smaller particular size lesser than 2.5um.

The technology takes full advantage of chromatographic principles to run separation using column-packed with smaller particles and higher flow rates.It can withstand high system back-pressure.
Special analytical columns UHPLC BEH C18 packed with the factor responsible for development of UHPLC technique was evolution of packing material used to affect the separation.
The technology takes full advantage of chromatographic principles to run separations using columns packed with smaller particles.
It decreases analysis time and solvent consumption 1.7um particles are used in connection with system.

IX. PRINCIPLE

The Principle of UHPLC is based on van dimeter equator. which describes the relationship between flow rate and her or column efficiency.
H=A+ B/v+Cv
when, A= Eddy diffusion B= Longitudinal diffusion C= equilibrium mass transfer v= flow rate
Van dimeter equation that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency Enrollment in local colleges, 2005

Comparison between HPLC and UHPLC		
Parameters	HPLC	UHPLC
Column	Xterra, C18,50×4.6mm	AQULITY UHPLC C18,50×2.1MM
Particle size	4 nanometer particles	1.7 nanometer particle
Flow rate	3.0 ML per minute	0.6 ML per minute
Injection volume	20ML	3ML partial loop fill or 5 ml full loop fill
Total run count	10 minutes	1.5 minute
Theoretical plate count	2000	7500
Lower limit of quantization	0.2 microgram per ML	0.054 Micro ML
Total solvent consumption	Acetonitrile :10.5ml, water :20ml	A 21cetonitrile :0.53 ml, water:21ml
Delay volume	720 µ ml	110 µML
Column temperature	30°C	65 °
Maximum back pressure	35 by 40 MPA less	103.5 MPA more
Resolution	Less	High
Analysis time	More	Less
Sensitivity	Less	More

X. INSTRUMENTATION

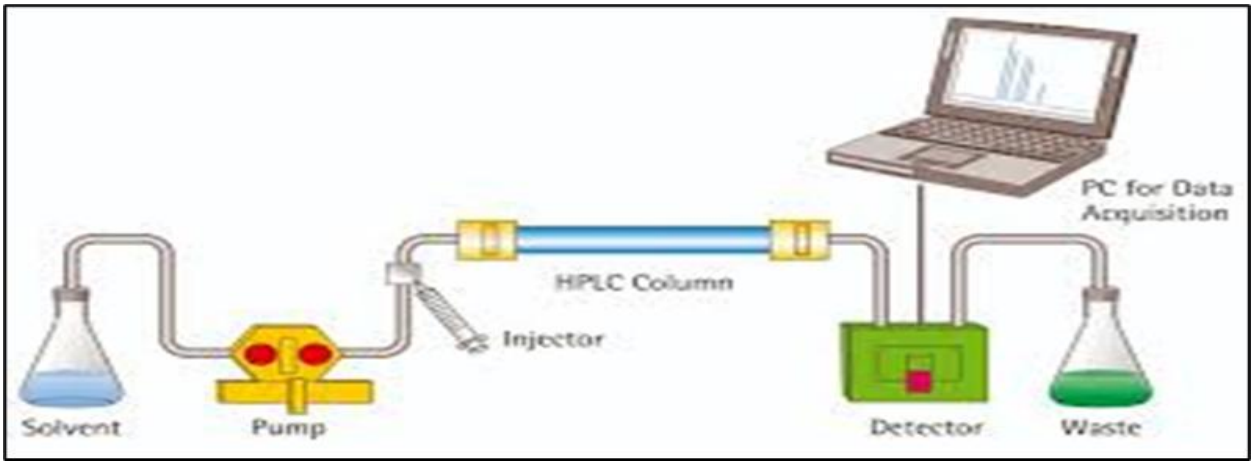

Fig No 1: UPLC

A completely new system design with advane-technology in the pump, auto sampler, detector, d. D system, and service diagnostics was required,

- The ACQUITY UHPLC system has been designed for low system and dwell volume.
- Achieving small particle, high peak capacity separations requires a greater pressure range than that achievable by HPLC System.

Solvent reservoir

The most common type of a solvent reservoir is glass bottle.

The most manufacturers supply this bottle with special cap tubing and filter to connect to the pump inlet and so Purge gas (helium) Use to remove dissolve air.

Constant pressure pump

Constant pressure is used only for column packing.

Constant flow pump

This type is Mostly used in all common UPLC application.

Reciprocating piston pump

Dual pistol pump

1 Reciprocating Piston pumps;

Consist of a small monitor drive one piston which moves rapidly back and front in hydrolytic chamber and that may vary from 35 to 400 µl in volume.

On the backstroke, the separation column wave is closed, and piston pump in solvent from the mobile phase reservoir.

On the forward stroke the pump pushes solvent out of the column from the reservoir.

2. Syringe type pump

These are the most suitable for small bore columns because these perm delivers only a finite volume of mobile phase before it has to be refiled. This pump have volume between 250 to 500Ml

The pump operates by a moisturized lead screw that that delivers mobile phase two column at constant rate.

The rate of solvent delivery is controlled by changing the voltage on the monitor.

3. Constant pressure pump

In this type of pumps the mobile phase is driven through the column with the use of pressure from the gas cylinder. A low pressure gas source is needed to generate high liquid pressure.

The waving arrangement along the rapid refill of solvent chamber whose capacity is 700 ml, These provide continuous phase flow rates.

Sample injection

In UHPLC, sample introduce is critical. conventional injection valve, Either automated or manual, and Hardened to work at extreme pressure.

To protect the column from extreme pressure fluctuations the injection process must be relatively pulse free Add a swept volume of the device also need to be minimal to reduce potential brand spreading.

Low volume injections with minimal carryover required to increase sensitivity.

UHPLC column

Reservation is increase in 1.7 µm particle packed column because 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 UHPLC separation

ACQUITY UHPLC BEH C18 AND C8 (straight chain alkyl column)

ACQUITY UHPLC BEH SHILD RP 18 (embedded polar group column)

ACQUITY UHPLC BEH (phenyl group tethered) to the silyl functionally with a C6 alkyl

AQUITY UHPLC BEH Amide columns (trifunctionally bonded amide phase)

ACQUITY UHPLC BEH C10 and C8:

These are considered as the universal columns of choice for most UHPLC separation by providing the widest pH ranges.

The low pH stability is combined with the high pH stability of the 1.7µm BEH particle to deliver the widest unstable pH operating ranges.

ACQUITY UHPLC BEH shield RP 18:

These are designed to provide selectivity that complement ACQUILITY UPLC BEN TM C18 and C8 columns. ACQUILTY UHPLC BEH phenyl columns:

These utilize a trifunctional C6 alkyl ethyl between the phenyl ring.

Enhanced mechanical stability bridging the methyl group in the silica matrix.

ACQUITY UHPLC BEH AIMDE COLOUMNS:

BEH particale technology, in combination with a trifunctionally bonded amide phase,

Provides exceptional column lifetime, thus improving assay robustness.

BEH amide columns facilities the use of wide range of phase pH (2-11).

Chemistry of small particles

As the particle size decreases to less than 2.5µm, not only there is significant gain in efficiency but the efficiency doesn't diminish at increased flow rate.
By using smaller particles speed and peak capacity number of peaks resolve per unit time in gradient separation can be extended to a new limit termed ultraPerformance Liquid Chromatography(33).

XI. DETECTORS

UV detectors
Fluorescent detector
Refractive index detector
Light scattering detector
Electrochemical detector

XII. PHOTOTUBE

Consist of the high sensitive cathode in a form of half cylinder evaluated tube.
Anode is also present along the axis of the tube, Inside layer is coated with light sensitive layer.
When light is Incident, surface coating emit electron this is attracted and collected by anode.
Current which is created between anode is regretted as a measure of radiation falling on the detector.

Photomultiplier TUI
↓
Ejected photoelectron strikes dynode
↓
Secondary electron release
↓
Voltage accelerates electron to next dynode
↓
Result in charge pack heating anode
↓
High gain and detected

Fluorescence detector
The light from an excited source faces through a filter or monochromator and strikes a sample.
A proportion of the incident light is absorbed by the sample and some of the molecules in a sample fluorescence. The fluorescent light is emitted in all directions.
Some of the fluorescent light passes through a second filter or monochromator and reaches a detector, Which is usually placed at 90 degrees Celsius to the incident light beam to minimize the risk of transmitted or reflected incident light reaching the detector(34).
Evaporative light scattering detector

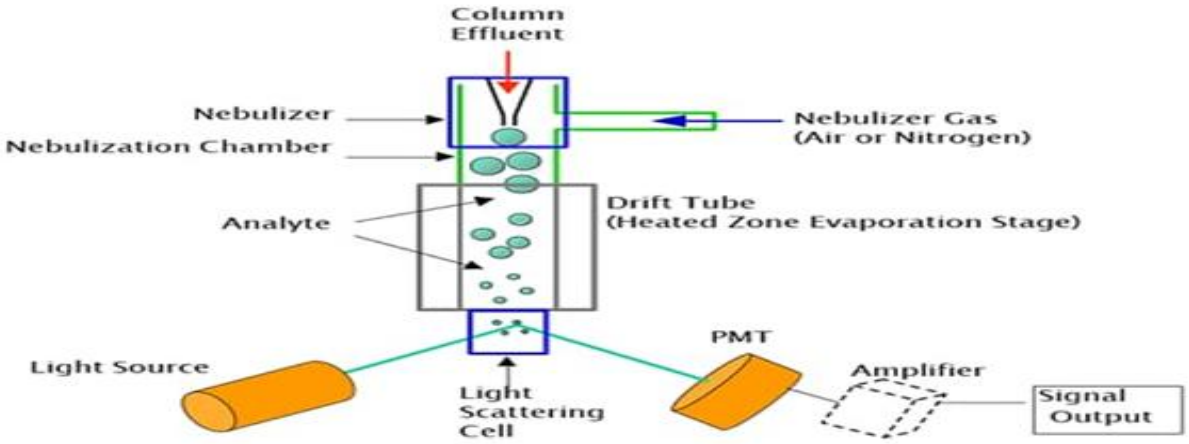

Fig .No. 2 Evaporative light scattering detector

Refractive index detector
This detector based on the deflection principle refactory when the detection of a light beam is changed when a composition in the sample flow sale changes in relation to the reference side. As sample evaluate through one side, the changing angle of refraction moves the beam. This result in the change in the photon current falling on the detector and which unbalance. The extent of unbalance is recorded on the strip chart recorder.
Advantages Of Uplc
Decreases runtime and increase sensitivity.
Reducing analysis time so that more product can be produced with exiting sources.

Provide the selectivity, selectivity and dynamic range of LC analysis.
Maintenance resolution performance.
Fast resolving power quickly qualifies related and unrelated compounds.
Operation cost is reduced.Less solvent consumption(35).

Disadvantages Of Uplc

A completely new system design with advance-technology in the pump, auto sampler, detector, d. D system, and service diagnostics was required

XIII. INTRODUCTION TO HPLC

Principle; -The principle of separation absorption when Max of compound is introduced into HPLC column they travel. According to them relative affinities. Towards the stationary phase. The compound which less affinity towards the stationary phase travel faster. Since no Two components have the same affinity towards the stationary phase (36).
Instrumentation

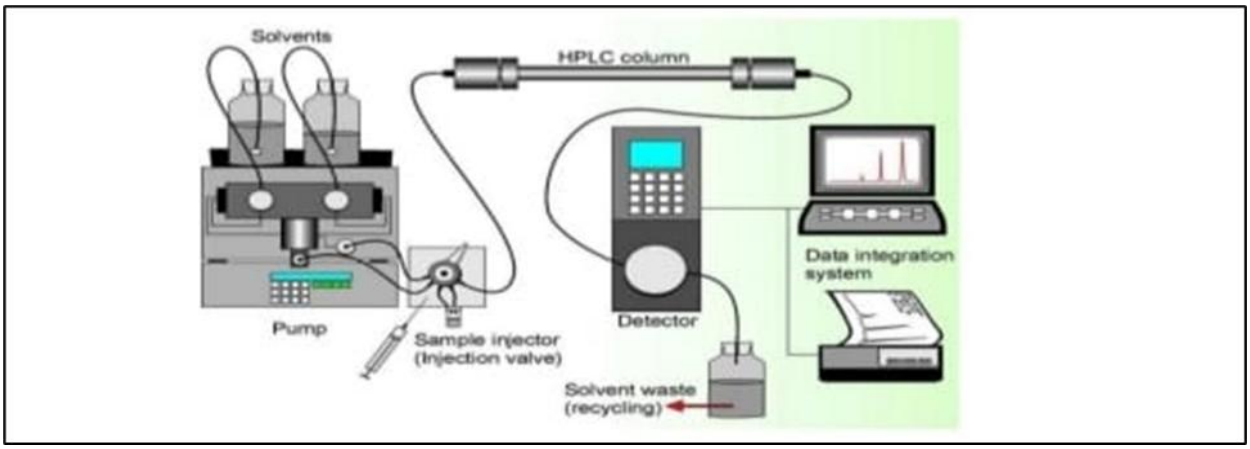

Fig No. 3 HPLC

1 Pump solvent delivery system
The solvents or mobile phase used must be passed through The column at high pressure. At about 1000 to 3000 Psi.
This is because as the political size of stationary phase is flow µ (5-10) The resistance to flow of solvent is high.
a. Mechanical pumps; Operate with constant pressure and use highly.
b. Pneumatic pumps; Operate with constant pressure and use highly compressed gas

2 Mixing unit gradient controller Insolvent degassing
a. Low pressure mixing chamber; Which use helium for degassing.
b. High pressure mixing chamber; Does not require helium for degassing solvents.

3.Injector manual or auto injector
a. Septum injector for injecting the sample through a rubber septum.
b. Stop though in which the flow of mobile phase is stopped for the file and the sample is injected through a valve Device.
c. Redone Injector it is the most popular injector and this is fixed volume loop like 20µl or more two modes.
1. Load position
2. Injection mode

4 Guards column:
Guard column has a very small quantity of absorbent and improves the life of the analytical column.
Guard column has the same material as that of analytical column does not contribute to any separation.

5. Analytical column:
Analytical column is the most important part of the HPLC technique and which decide efficiency of separation.
1. Column length varies from 5cm to 30cm
2. Column diameter ranges from 2MM to 50 MM.
3. Particle size from 1µ to 20 µ
4. Particle nature spherical, porous material.

6.Detectors.
a. UV detector;
This detector is based upon the light absorption characteristics of the sample
Two types of detectors are available.
1. When is the fixed wavelength detector operates at 245 nanometers.
2. The other variable wavelength detector which can be operated from 190nm to 600 nm.
b. Refractive index detector
This is a nonspecific or universal detector.
c. fluorometric detector; this detector Is based on the fluorescent reduction emitted by some class of compounds.
d. Conductivity detector; This detector is used when sample has conducting ions like anions and cations.
e. amperometry detector; This detector is based on reduction or oxidation of the compounds when a potential is applied.

7. Records and integrators:
Records are used to record the response obtained from the detectors after application if necessary.
They record Baseline and all the peak obtained with respect to time.
Integrators; Integrators are improved Western off record with some data processing capabilities. They can record the individual picks with retention and time height and width of peaks, peaks area, percentage of area etc.

XIV. APPLICATIONS OF HPLC

HPLC is being widely used in several fields apart from the pharmaceutical field it is used in the chemical photochemical industry.
1. This quantitative analysis; It is nothing but identification of the compound.
2.Checking the purity of compound; By comparing chromatograms of the standard and that of sample the purity of the compound can be inferred.
3.Presence of impurities
4.Quantitative analysis
a. Direct comparison method
b. Calibration curve method
c. Internal standard method
5 Isolation and identification of the drug metabolites in urine, plasma, and serum, etc.
6.Stability Studies.
7. Biopharmaceutical and Pharmacokinetic studies.

8.Isolation and identification of mixture of compounds of natural or synthetic origin.
Advantages of HPLC
1. High Resolution and sensitivity
Can separate compound with a very similar structure.
Detects very low concentration nanograms or even picograms level
2. Speed and efficiency
First analysis and compare to classical column chromatography.
Automated systems can be run many samples in short time.
3. Versatility
Can analyze a wide range of compounds polar, nonpolar, ionic, etc.
Suitable for both quantitative and qualitative analysis.
4. Precision and Accuracy
Highly reproducible results with tight control over variables.
Accurate quantification with proper calibration.
5. Atomization and data handling
Easy integrated with computer system for atomization, Data processing, and storage.
6. Nondestructive analysis
Often allow recovery of sample after analysis specially in preparative HPLC
7. Wide range of detector
UV, Fluorescence, Mass spectrometry, (LC-MS), Refractive index, Etc. Provide flexibility depending upon the analyte.
Disadvantages of HPLC
1. High cost
Instruments and maintenance are expensive.
Requires high purity solvent and consumable.
2. Complex operations
Requires train personnel for method development and maintenance.
Troubleshooting can be challenging.
3. Solvent use and waste
Use large volume of organic solvent (toxic and flammable)
Generates chemical waste that need disposal.
4. Sample preparation
Sample often required filtration and sometimes extraction, which adds the Complexity(37).
Introduction to GC

Principle:

It is a partition gas use as a mobile phase liquid which is wanted two solutions support is used as a stationary phase.(38)

The mix of the compound to be separated is convert to vapor and mixed with gaseous mobile phase.

The compound which is more Soluble in stationary phase travels slower.

The compound which is less soluble in stationary phase travel faster elute first (39).

XV. INSTRUMENTATION

1. Carrier gas

H, He, N, Argon mostly used, It should integers Hydrogen.

It has better thermal conductivity low density.

It is the use in case of thermal detectors and flame ionization detector.

2. Flow regulator and flow meter

To this plummeter used to measure flow rate of gas. They rotameter and soap bubble flowmeter.

a. Rota-meter; It has the glass tube with float head spring in gas tube calibrated, level of float is determined(40)
b. Soap Bubble meter; Per rubber is use store soap solution when bulb is Pressed A drop soap solution is converted into bubble by carrier cash and travel up.
c. Injection device; gas can be introduced into column by value Device liquid can be injected through loop septum device.
d. Columns; Made up glass / Steel.

A. Depending on its use

1. Analytical column; Length one to 1.5m outer.

Diameter of 3.6 nm.

2. preparative column; It is a big then analytical, large amount of sample has to be loaded 3 to 6 nm length outside and diameter 6.9 millimeter.

B. Depending on its nature

1. Packed column Columns are available in pack manner low polar to high polar nature are available 1 to 3 length up to 30m.
2. Stationary phase for GLC Here using a stationary phase like polyethylene glycol, high molecular weight Easter amide.
3. Open tubular column/ Capillary column/ Golay column They made up of long capillary tubing at 30 to 90M In Length and have narrow internal diameter of 0.025 to 0.075 cm These are stainless steel and in form of coil.(41)
4. Scot column This is an improved version of above capillary column
5. Temperature control device They are present along with injection device liquid sample are injected so they are converted into vapor.

The principle of separation is partition to according to solubility of in column is highly essential for efficient separation.

a. Isothermal programming

The same temperature is maintained through process of separation.

b. Linear programming

It is required when sample is has mixed as low blood sample pressure. (42)

XVI. DETECTOR USED IN GC

1. Applicability to wide range of sample
2. High Sensitivity to even small concentration
3. Rapidity of response
4. Small and easy to maintain
5. Inexpensive

The different detector uses commonly

a. Cathetometer or thermal conductivity detector
b. Flame ionization detector
c. Argon Ionization detector
d. Electron capture detector (43)

XVII. ADVANTAGES OF GAS CHROMATOGRAPHY (GC)

1. High resolution and sensitivity

Excellent separation of volatile components.

Capable of detecting compounds is very low concentration PPM to PPB levels.

2. Fast analysis

Short run times often minutes.

Suitable for high throughput environments.

3. High efficiency

Very sharp, narrow Peaks Due to the efficient interaction between analyte and stationary phase.

4. Reproducibility

Produce consistent and reproducible result with proper calibration. (44)

5. Cost effective operations

Lower solvent consumption compared to HPLC

Use gasses like helium or nitrogen

6. Powerful detection option

Flame ionization detector FID electron capture detector ECD, and coupling with mass spectroscopy (GC-MS) For identification and quantification.

7. Automated and computerized

Easily automated with sample injectors and data systems (45).

XVIII. DISADVANTAGES OF GAS CHROMATOGRAPHY (GC)

1. Limited to volatile compounds

Only suitable for compounds that are volatile and thermally stable.

Nonvolatile or thermally unstable compound cannot be analyzed directly.

2. Sample Derivatization

Polar or nonpolar compounds often required chemical modification to make them suitable for GC.

3. Destructive technique

Most detector (e.g., FID) Destroy the sample during detection.

4. Carrier gas cost and supply (46)

Required high purity gasses like helium which can be expensive and sometimes in short supply.

5. Column limitations.

Columns can degrade over time and are sensitive to contamination

Some analysts may stick to column or degrade during analysis.

6. Initial equipment cost

While operating cost may be lower than HPLC, the essential cost of GC System (especially GC-MS) is high. (47)

XIX. CONCLUSION

HPLC remains a gold standard for nonvolatile analyst due to its robustness and detector flexibility. Proper metal development and validation ensure reliable routine result.

UPLC is ideal when speed and higher separation power are priorities; Method transfer from HPLC must be done carefully to preserve sensitivity and robustness.

GC (Often GC- MS) is unrivaled for volatile analyte identification/ Quantitation. Proper sample preparation and column detector selection determine success.

REFERENCE

[1] Sunny Nagpal, Ashitosh Upadhyay, TR Bhardwaj, Arti Takk are, current pharmaceutical analysis 70-62,2011.

[2] Kavita Planica, harish k Chandrawanshi, Urmila Planica, pooja Mirchandani, Pratishtha Jain, Nitin. Journal of advanced pharmaceutical technology and and research 1(3),302-310,2010.

[3] Shreya R shah, Mayur A Patel, Miral V Naik, PK Pradhan, UM Upadhyay, International Journal of pharmaceutical sciences and research 3(10) 3603,2012.

[4] Dr.P.D. Sethi, (2001), High Performance Liquid Chromatography. First edition. New Delhi. CBS Publishers and distributor, Page no-6-8,69.

[5] Dr. Michael E. Swartz, (2005), Ultra Performance Liquid Chromatography: An introduction, Massachusetts, Separation Science Redefined, Page no:8-14.

[6] Snyder, L.R., Kirkland, J.J., Dolan, J.W. Introduction to Modern Liquid Chromatography.

[7] Skoog, D.A., Holler, F.J., Crouch, S.R. Principles of Instrumental Analysis.

[8] ICH Q2(R1) guideline — Validation of Analytical Procedures.

[9] Snyder, L.R., Kirkland, J.J., Dolan, J.W. Introduction to Modern Liquid Chromatography.

[10] Harris, D.C. Quantitative Chemical Analysis.

[11] (Manufacturer application notes on method transfer HPLC → UPLC for practical tips.)

[12] McNair, H.M., Miller, J.M. Basic Gas Chromatography.

[13] Poole, C.F. Gas Chromatography.

[14] ICH Q2(R1) — Validation of Analytical Procedures.

[15] Snyder, L. R., Kirkland, J. J., & Dolan, J. W. Introduction to Modern Liquid Chromatography (3rd ed.). Wiley.

[16] Snyder, L. R., Kirkland, J. J., & Glajch, J. L. Practical HPLC Method Development. Wiley.

[17] Giddings, J. C. Unified Separation Science. John Wiley & Sons.

[18] McNair, H. M., & Miller, J. M. Basic Gas Chromatography (2nd ed.). Wiley.

[19] Poole, C. F. Gas Chromatography (2nd ed.). Elsevier.
[20] Poole, C. F. The Essence of Chromatography. Elsevier.
[21] Poole, C. F. Essentials of Modern HPLC. Elsevier.
[22] Neue, U. D. HPLC Columns: Theory, Technology, and Practice. Wiley.
[23] Marion, D. W., & Church, J. (Eds.). Gas Chromatography. Springer.
[24] Skoog, D. A., Holler, F. J., & Crouch, S. R. Principles of Instrumental Analysis (7th ed.). Cengage.
[25] Haddad, P. R., Jackson, P. E., & Poole, C. F. Practical High Performance Liquid Chromatography. Chapman & Hall.
[26] Grob, R. L., & Barry, E. F. Modern Practice of Gas Chromatography (4th ed.). Wiley.
[27] Dittmer, D. C. Gas Chromatography and Mass Spectrometry: A Practical Guide. Academic Press.
[28] Mayer, R., & Meyer, V. R. Practical High-Performance Liquid Chromatography. Wiley.
[29] McMaster, M. C. (Ed.). Handbook of HPLC. CRC Press.
[30] Majors, R. E. LC/GC Interfaces, Instrumentation, and Methods. Chapman & Hall.
[31] Gennifer, E., & Nielson, L. U. Multidimensional Chromatography. Wiley.
[32] Berger, B. W., & Jackson, J. E. Mass Spectrometry: Principles and Applications (for coupling with LC/GC). Wiley.
[33] Poole, C. F., & Poole, S. K. Chromatography Today. Elsevier.
[34] Desty, D. H. Chromatography — Its Principles and Practice. Elsevier.
[35] 35. Principles and Applications of High-Performance Liquid Chromatography (HPLC): A Review" — Biomedical & Pharmacology Journal
[36] "Recent Advances in Liquid Chromatography–Mass Spectrometry" — PMC review
[37] "Review of the Use of Liquid Chromatography-Tandem Mass Spectrometry" — PMC
[38] "Analytical techniques in pharmaceutical analysis: A review" — ScienceDirect
[39] "High-Performance Liquid Chromatography (HPLC): Principles, Applications, Versatility, Efficiency, Innovation and Comparative Aspects" — open access review
[40] "A Review on Applications of Liquid Chromatography Coupled with Mass Spectrometry" — Wiley.
[41] . "Innovations in Liquid Chromatography: 2025 HPLC Column and Accessories Review" — Chromatography Online
[42] "Significance of Chromatographic Techniques in Pharmaceutical Analysis" — MDPI Processes
[43] "Advances in Chromatography: Contemporary Techniques and Applications" — Taylor & Francis article
[44] "Selection of the HPLC Method in Chemical Analysis" (book chapter / overview) — Elsevier / ScienceDirect
[45] "Comprehensive two-dimensional gas chromatography (GC×GC)" — Wikipedia overview, also literature pointers
[46] "Gas chromatography–vacuum ultraviolet spectroscopy (GC–VUV)" — description of GC-VUV universal detection
[47] "Direct electron ionization liquid chromatography–mass spectrometry interface" — coupling LC with EI-MS