International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 53-62 www.ijprajournal.com ISSN: 2456-4494

A Comprehensive Review on Advanced Chromatographic Techniques and Spectroscopic Techniques in Pharmaceutical Analysis

Jayesh M. Rajput[1], Dhanshri S. Nandre[2], Bhagyashri G. Pawar[3]
[1]*Department Of Pharmaceutics, Jiiu's Ali-Allana College Of Pharmacy, Akkalkuwa, Dist- Nandurbar, State- Maharashtra (India), 425415.*
[2, 3] *Department Of Quality Assurance, Jiiu's Ali-Allana College Of Pharmacy, Akkalkuwa, Dist- Nandurbar, State- Maharashtra (India), 425415.*

Submitted: 01-05-2022 Accepted: 09-05-2022

ABSTRACT: Today, chromatographic techniques prime focus is on identifying the components of a mixture and determining the present amount of each component exists within a sample. Chromatography is the backbone of separation science and is being used in all research laboratories, pharmaceutical industries, universities, etc. the process of drug development requires a suitable technique which helps the scientist to analyze the drug molecule is in accurate, precise and easiest way. Chromatography is the separation of a mixture into individual components using a stationary phase and a mobile phase. This study helps the author(s) to understand the various analytical process with a variety of separate technique available for the process of drug development which includes spectroscopy and chromatographic techniques. The purpose of this review is to present various chromatographic techniques like HPLC, TLC, UPLC, and spectroscopic techniques like UV-Visible, IR, NMR with their corresponding methods with principles and applications that have been applied in analysis of various pharmaceutical products.

KEYWORDS:
Chromatography, analytical techniques, spectroscopic techniques, nmr, ir, hplc, uplc.

I. INTRODUCTION

Pharmaceutical analysis- [1, 2, 3] Pharmaceutical analysis is defined as Analytical chemistry dealing with drugs both as bulk drug substances and as pharmaceutical products (formulation). The purpose of pharmaceutical analysis is to identification of substance, purification of substance, separation of a solution or mixture, or determination of structure of chemical compounds.

Types of Pharmaceutical Analysis-

a) Qualitative Analysis: -This method is used for the identification of the chemical compounds.

b) Quantitative Analysis: - Qualification of individual components in the mixture of the sample.

c) Semi Quantitative Analysis: - Estimate the presence of specific impurity present in sample.

Analytical Techniques- [3] The various analytical techniques which are being used in qualitative and quantitative analysis of drug substances and drug products in a pharmaceutical industry. There is variety of analytical techniques such as: Chromatographic, Electrochemical, Titrimetric, Spectroscopic, Electrophoresis and their corresponding methods have been applied for the analysis of pharmaceuticals.

II. CHROMATOGRAPHY: -

The word chromatography is derived from Greek words for colour and write. Chromatography is a separation of a mixture into individual components using stationary phase and mobile phase. It is physical method based on differential migration pattern. [4, 41] The mixture (sample) is dissolved in a fluid called mobile phase, which flow through the stationary phase. Chromatography can be analytical or preparative. The main purpose of preparative chromatography is to identify and separate the different components present in mixture, it is a form of purification. [4, 5, 6, 42] Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analyses in a mixture.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 53-62 www.ijprajournal.com ISSN: 2456-4494

History- Chromatography was first devised in Russia by the Italian-born scientist Mikhail Tsvet in 1900.[7] He produced a colourful separation of plant pigments using a column of calcium carbonate(chalk). New types of chromatography developed during the 1930s made the technique useful for many separation processes.[8] Archer John Porter Martin and Richard Laurence Millington in 1952 won Nobel Prize in Chemistry. [15, 9] They established the principles and basic techniques of partition chromatography, and their work encouraged the rapid development of chromatography methods: Paper chromatography, gas chromatography and what would become known as HPLC. Since then, the technology has advanced rapidly.

Principle of Chromatography- [10] Chromatography is a method of physical separation in which components of mixture gets separated on two phases. One of the phases is the immobile porous bed bulk liquid which is called stationary phase and other phase is the mobile fluid that flows over the stationary phase under gravity.

Classification of Chromatography-

Based on the nature of stationary phase and mobile phase: -

- Gas – Solid chromatography
- Gas – liquid chromatography
- Solid – liquid chromatography {Column chromatography, TLC, HPLC}
- Liquid – liquid chromatography {paper partition chromatography, column partition chromatography}.

Based on the principle of separation: -

- Adsorption Chromatography: - In this type of chromatography stationary phase is solid while mobile phase can be liquid/gas.
- Example: Thin layer chromatography, Column chromatography, Gas – solid chromatography and High-performance liquid chromatography.
- Partition Chromatography: - Based on the principle of partition for separation in this stationary phase is liquid over solid/gas surface, while mobile phase can be liquid/gas.
- Example: - Gas – liquid chromatography, Paper partition chromatography, Column partition chromatography

Based on the modes of chromatography: -

- Normal phase chromatography: In this the stationary phase is Polar and mobile phase is non-polar. This is not widely used in pharmacy.
- Reverse phase chromatography: - In this stationary phase is non-polar and mobile phase is polar. This is most widely used in pharmaceutical analysis.

Other types of chromatography: -

- Ion – Exchange chromatography
- Gel permeation chromatography (Gel filtration, size exclusion chromatography)
- Chiral chromatography

Types of Chromatography-

I. Column chromatography
II. Thin layer chromatography
III. Paper chromatography
IV. Gas chromatography
V. Ion – Exchange chromatography
VI. Gel Permeation chromatography
VII. Ultra-Performance Liquid chromatography

High Performance Liquid chromatography

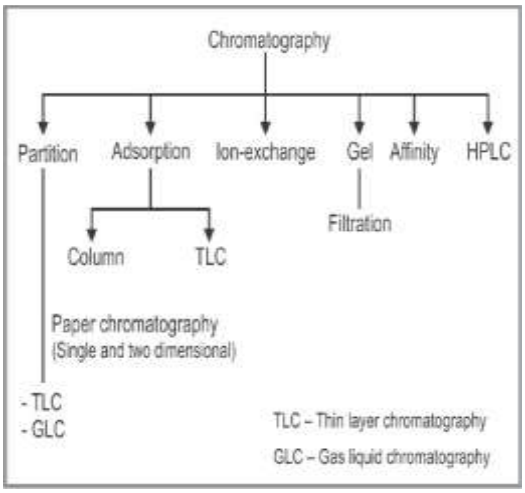

FIG 1: - Types of Chromatography

A) Column Chromatography- It is a type of absorptions chromatography in which column is used for the separation of components from mixture.

Principle: -Column chromatography is based on the principle of separation by adsorption.[8] It is a preparative technique used to purify compounds depending upon their polarity or hydrophobicity. [17] In this technique a mixture of molecules is separated based on their differential partitioning between a mobile phase and stationary phase.

[1] Column chromatography is more advanced form of separation science than paper and thin layer chromatography.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 53-62 www.ijprajournal.com ISSN: 2456-4494

Requirements:

- Stationary Phase: Solid (Silica gel), 80-100 mesh or 100-200 mesh which has a particle size of 60-200µ.
- [8] Mobile Phase: liquid (petroleum ether, Acetone, Ether, Toluene, Esters, Chloroform, etc.

Advantages of Column chromatography: -

- Any type of mixture can be separated.
- Any quantity of the mixture can be separated (µg to mg of substance).
- Wider choice of mobile phase.
- Automation is possible.
- In preparative type, the sample can be separated and reused,

Disadvantages of Column Chromatography: -

- Time consuming method.
- More amount of solvent is required.
- Automation makes the technique more complicated and Expensive.

Application: -

- Separation of mixture of compounds.
- Isolation of active constituents.
- Isolation of metabolites from biological fluids.
- Removal of impurities or purification process
- Estimation of drugs in formulation or crude extracts.

B) Thin-layer Chromatography: -Thin – layer chromatography (TLC) technique used to separate non – volatile mixtures.[13] Thin layer chromatography is performed on a sheet of an inert substrate such as glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide(alumina), or cellulose. This layer of adsorbent is known as the stationary phase and liquid as mobile phase.

Principle: -The principle of separation is adsorption chromatography. One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flow through via capillary action. The components move according to their affinity towards adsorbent. [9] The components with more affinity towards the stationary phase travels slower. The component with lesser affinity towards the stationary phase travels faster.

Advantages: -

- Simple method and cost of the equipment is low.
- Rapid technique.
- Detection is easy and not tedious.

Needs less solvent, stationary phase and time for every separation when compared to column chromatography.

Disadvantages: -

- It is time consuming process for the separation of compounds.
- It is expensive as higher quantities of solvents are required.
- The automated process becomes complicated and therefore costly.
- It has a low separation power.

Application: -

- Separation of mixtures of drugs of chemical or biological origin, plant extracts, etc.
- Separation of carbohydrates, vitamins, antibiotics, proteins, alkaloids, glycosides, etc
- Identification of related compounds in drugs.
- [43] To detect the presence of foreign substances in drugs.

C) Paper chromatography: - Paper chromatography is defined as the technique in which the separation of unknown substance is carried out mainly by the flow of solvents on specially designed filter paper.

Principle: -

[8, 43] The principle of separation is mainly partition rather than adsorption. A cellulose layer in filter paper contains moisture which acts as stationary phase. Organic solvents or buffers are used as mobile phase.

Paper used: -Choice of filter paper depends upon thickness, flow rate, purity, technique, etc.

Whatman filter papers of different grade like No.1, No.2, No.3MM and No.17 etc., are used.

Modified paper: - Acid or Base washed filter paper, glass fibre type paper.

Hydrophilic papers: - papers modified with methanol, formamide, glycol, glycerol etc.

Application: -

✓ Identification of drugs and impurities.

✓ Separation of mixture having polar and non-polar compounds.

✓ [8] It is used to control the purities of pharmaceuticals.

D) Gas Chromatography: - Gas chromatography is a chromatographic technique in which the mobile phase is gas. Gas chromatography is currently one of the most popular methods for separating and analysing compounds. This is due to its high resolution, low limits of detection, speed, accuracy

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 53-62 www.ijprajournal.com ISSN: 2456-4494

and reproducibility. [37] Gas chromatography plays an important role in the analysis of pharmaceutical drug products, and also useful to find out the impurities in pharmaceutical drug products.

Principle: -

Gas chromatography is based upon the partition of the analyte between a gaseous mobile phase and liquid phase immobilized on the surface of an inert solid. The organic compounds are separated due to differences in their partitioning behaviour between the mobile gas phase and stationary phase. [17, 18] The components are separated according to their partition coefficient which are more soluble will eluted later, which least soluble elute out first.

Carrier gas: [8, 43] These are used as mobile phase in gas chromatography in which mixture of components to be separated is mixed e.g., hydrogen, helium, nitrogen, argon.

Types of Detectors used in GC: -

a) Thermal Conductivity Detectors (TCD)
b) Thermo – Ionic Detector (TID)
c) Electron Capture Detector (ECD)
d) Flame – Ionization Detector (FID)
e) Nitrogen Phosphorus Detector (NPD)
f) Photo – Ionization Detector (PID)

Advantages: -

- High resolution power compared to other methods.
- High sensitivity.
- High accuracy and precision.
- Analysis of sample very quickly (minutes even seconds).

Disadvantages: -

- Limited to volatile sample.
- Not suitable for thermally labile sample.
- Sample be soluble and don't react with the column.
- During injection of gaseous sample proper attention is required.

Applications: -

- Purification of compound can be determined for drugs like clove oil, atropine, sulphate, stearic acid.
- Quality control and analysis of drug product like antibiotics, general anaesthetics, antivirals etc.
- [43] To determine the level of metabolites in body fluids like blood plasma, serum and urine.

E) Ion – Exchange Chromatography: -

[25, 26] It is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger. It works on almost any kind of charged molecule including large proteins, small nucleotides and amino acids. Cations and anions can be separated using this method.

Principle: -

[25, 27] The separations occur by reversible exchange of ion between the ions present in solution and those present in ion exchange resin.

Application: -

- ✓ Softening and demineralization of water.
- ✓ Separation of inorganic ions.
- ✓ Separation of sugars, amino acids and proteins.
- ✓ Purification of solution free from ionic impurities.
- ✓ For extraction of enzymes from tissues.
- ✓ Ion exchange column in HPLC.

F) Gel-Filtration Chromatography (Gel filtration, Molecular sieve): -

The basic principle of this method is to use dextran containing materials to separate macromolecules based on their differences in molecular sizes. Different gels are used for different molecular weight ranges. [23, 24] The solvent used can be of aqueous or non – aqueous type. The stationary phase consists of inert molecules with small pores. Sephadex G type is the most frequently used column material. Besides, dextran, agarose gel, polyacrylamide is also used as column materials.

G) Ultra-Performance Liquid Chromatography (UPLC): -

This is an advanced technique in liquid chromatography, which enhances mainly in three areas: Speed, Resolution and Sensitivity. [11, 14] UPLC applicable for particle less than 2µm in diameter to acquire better resolution, speed, and sensitivity compared with high performance liquid chromatography (HPLC).

H) High-Performance Liquid Chromatography: -

It is advance technique of column chromatography. It is also known as high pressure liquid chromatography (HPLC). [11, 40] It is as analytical technique used for separation, identification and quantification of each component in a mixture.

Principle: -

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 53-62 www.ijprajournal.com ISSN: 2456-4494

- The main principle of separation is adsorption. The mixture of component is mixed in the liquid solvent which administered into column under high pressure up to 400 atmospheres.
- When a mixture of components is introduced into the column. Various chemical and / or physical interactions take place between its sample molecules and the particles of the column packing.
- The components which have more affinity towards the adsorbent travels slower.
- The component which has less affinity toward the stationary phase travel faster.
- [8, 11] Hence the component of mixture moves according to their affinities towards the adsorbent.

Types of HPLC: -

✓ Normal phase HPLC: - Stationary phase is polar in nature (silica gel), while mobile phase is non-polar in nature (diethyl ether, chloroform). The non-polar sample will elute out first and polar sample are retained on column.
✓ Reverse phase HPLC: -Stationary phase is non-polar, while mobile phase polar in nature. Hence, the polar sample elute out more.
✓ Size – exclusion HPLC: - The column is filled with precisely controlled substrate. Based upon the difference in molecular sizes the separation of components of mixture occurs.
✓ Ion – exchange HPLC: - The surface of the stationary phase is ionically charged which is opposite to the charge of the sample. The mobile phase used is aqueous buffer which will control ionic strength and ph.

Instrumentation of HPLC: -

[1] HPLC consist of solvent reservoirs, solvent degasser, gradient valve, mixing vessel, high pressure pump, sample injection loop, guard column, analytical column, detectors and waste collector.

Applications: -

➢ [19, 20] In pharmaceutical application: -
- Identify active constituents in dosage forms.
- Evaluate of pharmaceutical product shelf- life.
- Dopamine in levodopa.

➢ [20, 21] In Environment application: -
- Identify diphenhydramine in deposited sample.
- Pollutant bio monitoring.

➢ [22] In Clinical application: -
- Analysis of antibiotics.
- Detection of endogenous neuropeptides in brain extracellular fluids.

➢ [16] In Food and Flavour: -
- Ensuring soft drink consistency and quality.

➢ [11, 12] Analysis of natural contamination e.g., Mercury & phenol in Sea water.
➢ Control the drug stability and quality control

III. SPECTROSCOPY: -

[2] Spectroscopy is defined as the branch of science which deals with study of interaction between sample/ matter with light or electromagnetic radiations (EMR). In other words, spectroscopy measures the changes in rotational, vibrational and/ or electronic energies.

➢ Principle: -Spectroscopy study is based on the interaction of electromagnetic radiation with matter. When a beam of electromagnetic radiation falls of sample it either adsorbed, reflected, transmitted, scattered. As a result, spectrum is obtained which is analysed.
➢ Spectrum: -A plot between the wavelength of electromagnetic radiation and frequency of light as a function of response.
➢ Spectrophotometer: -It is a standard and inexpensive technique to measure light absorption or the amount of chemicals in a solution.
➢ Spectroscopic Techniques: -

For the process of method development spectroscopic technique was the most important technique. In our pharmacopoeias this technique is based on the natural absorption of UV radiation, and other chemical reaction.[28] In pharmaceutical analysis this method was specially applied to analyse the dosage forms in pharmaceutical industries has been increased regularly.[29, 30] Also, there are some aspects for the colorimetric methods include:

- Complex formation reaction.
- A catalytic effect.
- Process of oxidation, and reduction.

A) UV-Visible Spectroscopy: - [31] UV spectroscopy or UV-Visible Spectrophotometry is based on the absorption of the electromagnetic radiation in UV/Vis region, with the wavelength ranges of 200-400 nm, called ultraviolet spectroscopy, and 400-800 nm, called visible spectroscopy. This means it uses light in the visible and adjacent ranges

Principle: - [29, 31] Basically, spectroscopy is related to the interaction of light with matter. As light is absorbed by matter, the result is an increase in the energy content of the atoms or molecules.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 53-62 www.ijprajournal.com ISSN: 2456-4494

When ultraviolet radiations are absorbed, this results in the excitation on the electrons from the ground state towards a higher energy state. Molecules containing л- electron or nonbonding electrons (n-electron) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals.

Applications: -

- It is one of the best methods for the determination of impurities in organic molecules.
- Quantitative analysis of pharmaceutical substances.
- Detection of impurities in coloured / organic sample.
- Detection of functional group presence in sample.
- UV spectrophotometer may be used as a detector for HPLC.

B) Infra-red Spectroscopy (IR): - [31, 38] Infrared spectroscopy (IR Spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum that is light with a longer wavelength and lower frequency than visible light. It covers a range of techniques, mostly based on absorption spectroscopy. Because of their characteristic's absorption, identification of functional group is easily accomplished.

Applications: -

- IR spectroscopy is widely used in industry as well as in research.
- It is a simple and reliable technique for measurement quality control and dynamic measurement.
- Identification of drug substance.
- Study of polymer.
- Ratio of cis-trans isomers in a mixture of compounds.
- It is also employed in forensic analysis in civil and criminal analysis.

C) Mass Spectroscopy: - [31, 35, 39] Mass spectroscopy is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules or/and Mass spectroscopy is an analytical technique that is used to measure the mass-to-charge ratio of ions.

Principle: - [3] It works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their masses to charge ratios.

Applications: -

- To identify molecular formula and molecular weight of a compound.
- To prepare pure isotopes, high polymer and natural products.
- To study of free radicals, determination of bond strength, evaluation of heat of sublimation.
- Environmental analysis: soil and ground water contamination.
- Clinical studies: Implementation of mass spectroscopy in clinical laboratory resulted in significant advancement.
- Structure elucidation: Mass Spectroscopy has major use in structure elucidation of compounds

D) Nuclear Magnetic resonance Spectroscopy (NMR): -

Principle: - [36] The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. When an external magnetic field is applied on the nuclei the nuclei align themselves either with or against the field of the external magnet and the nuclei spin goes to excited state. When the spin return to nits ground state level, the absorbed radiofrequency energy is emitted at the same frequency level. The emitted radiofrequency signal that gives the NMR spectrum of the concerned nucleus. [31] In radio frequency region 4-900 MHz by nuclei of the atom

Applications: -

- Employed in determination of hydrogen bonding.
- NMR is used to detect Aromaticity of the compound.
- NMR is used to distinguish between Cis and Trans isomers.
- Elucidate chemical structure of organic and inorganic compounds.
- Investigation of dynamic properties.
- Metabolite analysis: A very powerful technology for metabolite analysis.

E) Fourier-Transform Infrared Spectroscopy (FTIR): -Fourier transform infrared spectroscopy (FTIR) [33] is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. [32] The functional group and the original peak with regards the molecules were

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 53-62 www.ijprajournal.com ISSN: 2456-4494

identified by this method, and it will help scientist to develop a new method.

Applications: -

- It is used to quickly and definitively identify compounds such as compounded plastics, blends, fillers, paints, rubber, coatings, resins, and adhesives.
- Pharmaceutical research
- Forensic investigation
- Polymer analysis
- Lubricant formulation and fuel additives
- Food research.
- Quality assurance and control.

F) Fluorimetry and Phosphorimetry: -

In our pharmaceutical industries fluorimetry and Phosphorimetry technique was constantly growing for the analysis of micro sample. Fluorimetry and Phosphorimetry are analytical techniques based on the absorption of electromagnetic radiation by the processed sample, which contains or generates radiation- absorbing molecules in excited states; when the excited molecules return to the ground state, radiation is emitted, i.e., the excited molecules luminesce.[31] In the previous studies there is a constant increasing rate in the application number was observed in fluorometry or phosphometry. [34] They represent these methods for the quantitative estimation of some drugs, which available in the form of biological fluids and they were observed from past years.

Advantages: -

- Sensitivity: This technique is more sensitive since concentration as low as µg/ml or even ng/ml.
- Precision: Up to 100% can easily achieved in fluorimetry and Phosphorimetry.
- Specificity: More specific than absorption technique.
- Range of Application: Even non florescent compounds can also be converted to fluorescent compounds by chemical reaction

Applications: -

- Determination of inorganic and organic substance.
- Determination of vitamin B1 (Thiamine) and B2 (riboflavin) in food sample like meat, cereal, fish, seeds and nuts, etc.
- Extensively used in the field of nucleus research foe the determination of uranium salt.
- Qualitative and Quantitative study.
- In pharmaceutical Application.

The Common Problems of Chromatography Columns and, How to Deal with It?

Having troubles on HPLCs can be incredibly frustrating. You're trying to get some analysis done, and your research can't move forward until you figure out why your tools aren't working. There are some common problems of HPLC columns that pop up from time to time. Knowing what these are and how to fix them can save you hours of frustration. Read on to discover some of these problems and how to address them. We limited this article in basic level. We would recommend receiving consultation with the manufacturer of your HPLC system.

❖ **No Peaks**

There can be several factors that cause no peaks or very small peaks to show up on your HPLC outputs. Normal readings should have large, thin peaks that may vary somewhat in height. Small peaks or no peaks at all may mean your detector lamp is turned off, you have no mobile phase flow, your sample is missing or deteriorated, or there's a problem with your detector, integrator, Injector valve or recorder.

Start by making sure your detector is turned on, and then check all the electrical connections and cables. Make sure your autosampler vials have enough liquid and that there are no air bubbles in the sample, and recheck the system with a new standard solution. If that doesn't work, check the attenuation or gain settings status, and auto-zero if you have to.

❖ **No Flow**

If you are getting absolutely no peaks on your output, you may have no flow in your HPLC column. This may mean your pump is off or the flow is interrupted or obstructed somehow. You may also have a leak or air trapped in the pump head.

Start the pump if it's off, and check the mobile phase levels in the reservoir and flow throughout the system. Check the sample loop for any obstructions or air locks, and make sure the mobile phase components are miscible and the mobile phase is properly degassed.

From there, move on to checking the system for loose fitting and the pump for leaks or other issues. Disconnect the tubing at the guard column if you have one, and check for flow. If

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 3 May-June 2022, pp: 53-62 www.ijprajournal.com ISSN: 2456-4494

you're still having problems, purge the pump at a high flow rate, prime the system, loosen any check valves your system may have, and, if all else fails, flush the system with 100 percent methanol or isopropanol.

❖ **Pressure Issues**

If your pressure is lower than usual or you have no pressure, you may have a leak or air trapped somewhere in the system. You might also have a faulty check valve or an obstructed or interrupted mobile phase flow. If your system pressure is too high, there's likely a problem in the pump, injector, in-line filter, or tubing, or you might have obstructed guard or analytical columns. For low pressure, start by checking the whole system for leaks, loose fittings, faulty pump seals, or bad valves.

For high pressure, start by removing the guard and analytical columns from the system and replacing them with unions to reconnect the injector to the detector.

❖ **Tailing and Fronting (Leading) Peaks**

You may notice that rather than coming up and down in straight lines, your peaks start to develop a slight slant at the front or back, called fronting or tailing. This usually means your guard or analytical column may be worn out or your column may be overloaded. Tailing may be caused by a contaminated or deteriorated mobile phase or interfering mobile components in the sample, while fronting may result from problems with the sample solvent.

If you have tailing peaks, start by removing the guard column and attempting analysis, replacing it if necessary. You may also need to restore or replace the analytic column. Be sure to check on the make-up of the mobile phase and the column performance.

❖ **Negative Peaks**

If you see negative peaks start showing up, it's possible that your recorder leads are reversed. You may also have a refractive index of solute less than that of the mobile phase or a sample solvent and mobile phase that vary greatly in composition. Your mobile phase may also be more absorptive your sample components to UV wavelength.

Learn How to Resolve Problems of HPLC Columns

Running down the problems of HPLC columns can be frustrating, but remember, it's like any other problem you're trying to solve. Think critically about what might be causing the problem, start eliminating possibilities, and follow the suggested solutions here. You'll have your HPLC columns back up and running in no time.

IV. CONCLUSION

In the present study we examine the process of drug development which is based upon the analytical techniques. Now a day, chromatography is accepted as an extremely sensitive and effective separation method. In term of scientific advances, chromatography is considered to be one of the major innovations in the past few days. The practice of HPLC is restricted to analysers, but is now widely performed by students, chemists, biologists, production workers, and other research and quality control laboratories. The spectroscopy techniques for the quantitative and qualitative estimation of drugs have been includes the various methods UV-Visible spectroscopy, Mass Spectroscopy, Infrared spectroscopy, Nuclear magnetic resonance, Fluorimetry and Phosphorimetry.

The review focus on the principle, classifications, instrumentation, advantages, disadvantages and application of chromatographic and spectroscopy techniques. Chromatographic techniques improve chemical and instrumentation productivity by giving more information due to increased productivity reliability, robustness, resolution, speed and sensitivity. The time spent refining new methods can be significantly reduced.

CONFLICT OF INTEREST

The Author(s) declares "No Conflict of Interest".

REFERENCES

[1]. Manisha Bhatti, Ritika puri (2019). **An overview on Basic and Advanced chromatographic techniques: A Review**, Journal of Biomedical and Pharmaceutical Research Vol-8, Issue-3, pp.:29-33.

[2]. Imran Khan et al. (2015) **Analytical techniques (chromatography, Spectroscopy) in pharmaceutical Analysis: A Review**. International journal of research in pharmaceutical and Nano Sciences, Vol-4, Issue (1), Pp.: 19-27.

[3]. Siddiqui M R, Zeid A Alothman, Nafisur Rahman (2013). **Analytical techniques in pharmaceutical analysis: A Review**. Arabian journal of chemistry, pp. 1-13.

[4]. Hostettmann K, Marston A (1998). **Preparative chromatography techniques Application in Natural Product Isolation**

(second Ed.). Berlin, Heidelberg: Springer Berlin Heidelberg. P. 50. ISBN 9783662036310.

[5]. Gonzalez, Mirna; Mayolo- Deloisa, Karla; Rito- Palomares, Macro (2020), Matte, Allan(bed), **Chapter 5- Recent advances in antibody- based monolith chromatography for therapeutic application**, pp. 105-116, ISBN 978-0-08-103019-6.

[6]. **Alternative bio separation operation: life beyond packed- bed chromatography**. T.M. Przybycien, N.S. pujar and L.S. Steele Curr opin Biotechnol, Vol-15, Issue (5), pp.469-478 (2004).

[7]. Ettre L.S, Zlatkis A, eds. (26 August 2011).**75 years of chromatography: A Historical dialogue**. Elsevier. ISBN 978-0-08-085817-3.

[8]. **A Review on chromatography techniques by Lodha Laxminarayan**. www.ajprd.com ISSN 2320-4850.

[9]. www.wikipedia.com, Wikipedia, free encyclopaedia.

[10]. Harwood L.M., Moody C. J. **Experimental organic chemistry: Principles and Practice**. (Illustrated edition Ed.):180.

[11]. **A Review on High Performance Liquid Chromatography (HPLC)** by Mukthi Thammana on 3/10/2016 in Research and Reviews: Journal of Pharmaceutical Analysis.

[12]. Abdullah M. **A Validated stability-indicating hplc and thin layer densitometric methods for the determination of pazufloxacin: Application to pharmaceutical formulation and degradation kinetics J chromatograph separate technique**, 2014; 5:218.

[13]. Harry W. Lewis & Christopher J. Moody (13 Jun 1989), **Experimental organic chemistry; Principles and practice**. (Illustrated Ed,) Wiley Blackwell. Pp. 159-173. ISBN 978-0-632-02017-1.

[14]. Taleuzzaman M, Ali S, Gilani SJ, Imam SS and Hafeez A (2015). **Ultra-Performance Liquid Chromatography (UPLC) – A Review**, Journal of Analytical and Pharmaceutical Chemistry Vol-2, Issue-6; ISSN 2381-8913.

[15]. A Nobel Prize in chemistry 1952. Retrieved October 2021, from http://www.nobelprizes/chemistry/1952/summary/.

[16]. Nielsen, S. S. (2010). **Food analysis**. New York; Springer.

[17]. **Practical Pharmaceutical Chemistry**; Fourth edition. By A. H. Beckett and J. B. Stenlake. The Athlone Press, University of London, 2 Gower Street, London, S.C. 1, 1962.

[18]. Martin, M., & Guiochon, G. (2005). **Effect of high pressure in liquid chromatography**. Journal of Chromatography A, 1090(1-2), 16-38. http://dpoi.org/10.1016/j.chroma.2005.06.005.

[19]. Bergh, J. J., & Breytenbach, J. C. (1987). **Stability- indicating high performance liquid chromatographic analysis of trimethoprim in pharmaceuticals**. Journal of chromatography A, 387, 528-531. http://doi.org/10.1016/s0021-9673(01)94565-0.

[20]. Bounine, J. P., Tardif, B., Beltran, P., & Mazzo, D. J. (1994). **High Performance liquid chromatographic stability-indicating determination of zopiclone in tablets**. Journal of chromatography A, 677(1), 87- 93, http://doi.org/10.1016/2021-9673(94)80548-2.

[21]. Hongxia, Y. (2004). **Application of toxicity identification evaluation procedures on waste waters and sludge from a municipal sewage treatment works with industrial inputs**. Ecotoxicology and Environment Safety, 57(3), 426-430.

[22]. Shah, A. J., Adlard, M. W., & Stride, J. D. (1990). **A sensitive assay for clavulanic acid and sulbactam in biological fluids by high performance liquid chromatography and precolumn derivatization**. Journal of pharmaceutical and Biomedical Analysis, 8(5), 437-443. http://doi.org/10.1016/0731-7085(90)80072.

[23]. Helmut D. **Gel Chromatography, Gel Filtration, Gel Permeation, Molecular Sieves: A laboratory Hand book**, Springer – Verlag; 1969.

[24]. Determann H. **Gel Chromatography Gel Filtration, Gel permeations, Molecular Sieves: A Laboratory Handbook**. Chapter 2. Materials and Methods; 2012.

[25]. **Ion Exchange Chromatography Principles and Methods.** General Electric Company. 2004, pp. 11-20.

[26]. Karlsson E, Ryden L, Brewer J. **Protein purification. Principles, High Resolution Methods and Application: Ion exchange**

chromatography, 2nd edition. New York: Wiley; 1998.

[27]. Amercham Biosciences., **Ion Exchange chromatography, Principles and methods**, Amercham Pharmacia. Biotech SE. 2002; 751.

[28]. Chauhan A, Mittu B, Chauhan P. **Analytical method development and validation: A Concise Review**. J Anal Bioanal Tech. 2015; vol- 6: Issue-5.

[29]. Lacrok PM, Curran NM, Sy WW, Goreck DK, Thibault P, Blay PK. **Liquid chromatographic determination of amiodarone hydrochloride and related compounds in raw materials and tablets**. Journal of AOAC International. 1994; 77(6):1447-53.

[30]. Laidler, Keith J.; Meiser, John H. (1982). Physical Chemistry. Benjamin/Cummings.pp.488. ISBN-0-8053-5682-7." **The letters s, p, d and f relate to the descriptions "sharp", "principal", that the early spectroscopists had given to series of lines in atomic spectra".**

[31]. **Introduction to Spectroscopy** (3rd edition), by Donald L. Pavia, Gary M. Lampman, George S. Kris. Department of chemistry Western Washington University Bellingham, Washington. ISBN: 0-03-031961-7.

[32]. Singh N, Goyal K, Siddhi S, Jindal S. **Development and Characterizations of Barbaloin Gel for the safe and Effective Treatment of Psoriasis**. Journal of Drug Delivery and Therapeutics, 2020; 10(5):188-97.

[33]. Griffiths, P.; de Hasseth, J.A. (18 May 2007). **Fourier Transform Infrared Spectrometry** (2nd edition) Wiley-Blackwell, ISBN 978-0-471-19404-0.

[34]. Sethi PD, Charegaonkar D, editors. **Identification of drugs in pharmaceutical formulation by thin layer chromatography**. CBS publishers; 1999.

[35]. Downard K (2004), **Mass Spectrometry- A Foundation Course**. Royal Society of Chemistry. http://doi:10.1039/a804629h.

[36]. Harvey, David (2000). **Modern analytical chemistry**. Boston: McGraw-Hill. ISBN: 0-07-237547-7.

[37]. Sharma HK, Jain N, Jain SK. **Development of spectrophotometric method for quantitative estimation of Amlodipine besylate, Olmesartan medoxomil and hydrochlorothiazide in tablet dosage form**. Pharm Anal Acta.2010; 1:101.

[38]. **Elementary Organic Spectroscopy** (Revised Edition), By Y. R. Sharma, Published by S. Chand (2007), ISBN: 8121928842.

[39]. Subramani P, **An overview of Liquid chromatography- Mass spectroscopy Instrumentation**, Vol-5, Issue (2), 2014, pg.no. 47-54.

[40]. Anderson DJ. **High performance liquid chromatography in clinical analysis**. Analytical chemistry. 1999; 71(12): 314-27.

[41]. Kaushal C, Srivastava B. **A Process of method development: A chromatographic approach**. Journal of Biochemical and pharmaceutical Research (2010); 2(2): pg. no: 519-545.

[42]. Hostettmann K. Marston A. & Hostettmann M. (2011). **Preparative Chromatography techniques: Application in natural product isolation**. Berlin: springer.

[43]. www.rpi.edu/dept/chem-eng/biotech-environ/chromo/be-types.htm.