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An Overview of chromatographic techniques : Principle, types and applications

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ABSTRACT

The name "chromatography" comes from the Greek words "graphien," which means to write, and "chroma," which means color. One method of separating components in a mixture is chromatography. The mixture's constituent parts are distributed throughout a liquid solution known as the mobile phase, which retains them inside a

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framework made of a different material known as the stationary phase. Differential partitioning between the mobile and stationary phases is necessary for component separation. Chromatography is mostly used to extract and purify one or more sample components. Its analytical objective is to ascertain the qualitative and quantitative chemical composition of a sample. It is the fastest, most trustworthy, safest, and most adaptable technology for ensuring the quality of medicinal ingredients.While basic chromatography still prevails as the most important analytical tools in molecular chemistry. This paper will discuss overview, principle, types and applications of different chromatography techniques.

KEY-WORDS

Chromatography, Component, Stationary phase, Mobile phase, Ion-Exchange

INTRODUCTION

The work of Archers John Porter Martin and Richard Laurence Millington Synge, who were awarded the 1952 Nobel Prize in Chemistry for their contributions throughout the 1940s and 1950s, greatly advanced the method of chromatography.[1] Their work stimulated the quick development of various chromatographic techniques, including paper chromatography, gas chromatography, and what would later be known as high-performance liquid chromatography. They also pioneered the fundamentals and procedures of partition chromatography. Since then, technological advancement has been swift. Researchers discovered that the fundamental ideas of Tsvet's chromatography could be used in a variety of ways, leading to the many chromatographies that are discussed below. Technological developments in chromatography are continuously enhancing its performance, making it possible to separate more identical molecules.

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation. [2] Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is

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stationary (stationary phase) while the other (the mobile phase) moves in a definite direction. [3] Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive. [4]

PRINCIPLE OF CHROMATOGRAPHY

The foundation of chromatography is the idea that mixtures of molecules applied to surfaces or solids, as well as fluid stationary phases (stable phases), separate from one another while moving with the help of a mobile phase. The molecular properties associated with adsorption (liquid-solid), partition (liquid-solid), affinity, or variations in their molecular weights are the factors that have an impact on this separation process. These variations lead certain mixture components to enter quickly into the mobile phase and exit the chromatographic system more quickly than others. certain mixture components also linger longer in the stationary phase and move more slowly through the system.

Thus, the chromatography process is based on three components.

- Stationary phase: This phase is always composed of a “solid” phase or “a layer of a liquid adsorbed on the surface a solid support”.
- Mobile phase: This phase is always composed of “liquid” or a “gaseous component”.
- Separated molecules.

The type of interaction between the stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other. [5]

DIFFERENT CHROMATOGRAPHIC TECHNIQUES

The commonly used chromatographic techniques among those include [6-12]

➢ **Planar Chromatography**

- Paper chromatography
- Thin layer chromatography (TLC)

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- High performance TLC

➢ **Column Chromatography**

- High Performance Liquid Chromatography (HPLC)
- Gas chromatography (GC)
- Ion-Exchange chromatography (IEC)

Paper Chromatography[13,14]

In this method of separation the mixture of compounds by using specially designed chromatographic paper as stationary phase into individual compounds.

Components:-

- ❖ Stationary phase and papers – filter paper of different grades, paper impregnated with silica or alumina.
- ❖ Mobile phase – mixture of solvents, pure solvents
- ❖ Sample applicator.
- ❖ Chromatographic chamber.

Principle:-

In paper chromatography, partitioning and absorption occur both. However, the primary one is partition chromatography in which the compounds are divided in two liquid phases. The movement of mobile phase, due to the capillary action of pores in the paper, separates the mixture compounds.

Procedure:-

The sample mixture is placed on the piece of chromatography paper which is later place in a container solvent. Individual components travel to a varying degree of distances based on the various in their adsorbent and solvent affinity. Polar molecules are adsorbed onto the filter paper and transported to smaller distances while non-polar molecules migrate further. The extent of movement of components is measured by calculating the “Rf value”.

Rf value is defined as the distance travelled by the component from application point divided by distance travelled by solvent from application point. Rx value is the ratio of distance travelled by the sample and the distance travelled by the standard. Rx value is always closer to one. Rf value is always less than one but Rx can be greater than one. The factors affecting the Rf value are the solvent system and

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its composition, temperature, pH of the solution, quality of paper and adsorbents and distance through which the solvent runs.

Advantages:-

- Simple and easily available equipment.
- Better efficacy of separation.
- Closely related homologous, isomers, isotopes and very labile, reactive substances can be separated.

Applications:- Specially used for isolation of polar and non-polar compounds from mixtures. It also use for separation of amino acid, pigments, dyes and inks. To recognize organic and other biochemical compounds in urine, for hormones and medicine determination, evaluation of inorganic compounds like complexes and salts.

Thin Layer Chromatography[15-18]

TLC exists mainly as a complementary technique to other column based liquid chromatographic methods to provide additional knowledge in separations (multi-modal separation techniques). TLC plays a crucial role in the early phase of drug development when there is insufficient information on impurities and degradation products in drug substance and drug product.

Principle:-

TLC operates upon the absorption principle. Nonetheless there is normally adsorption and partition or a mixture of both. Elements with more affinity fly slower and vice versa.

Components:-

- TLC plates – stable and chemically inert plates used as a support for stationary phase (glass, plastic, or aluminium support)
- TLC chambers – used for the development of TLC plate, maintenance of uniform environment.
- Stationary phase (solid phase) – thin layer (0.25 mm thick) of adsorbent coated on a TLC plates.
- Filter paper – prevents the evaporation of solvents.
- Mobile phase – comprises of a solvent or solvent.

Procedure:-

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The sample mixture spots are placed, near the bottom of the thin layer plate. Solvents are allowed to percolate up the plate by capillary action. The chamber is saturated with solvent vapor so as to prevent the solvent evaporating from the plate surface and also controlling the retention mechanism by surface deactivation. The plate is then placed in the chamber without allowing dipping of sample spot. A constituent that is strongly adsorbed will move slower. Results are represented by Rf value same as in paper chromatography.

Advantages:-

- Quick, simple, inexpensive high sample throughput technique.
- Wide choice of the mobile phases.
- Sample preparation is minimum.
- Several samples can be run simultaneously using mobile phase in small quantity.
- Used in analytical laboratories for limited resources.

Applications:-

- It is used for separation of all types of natural products. E.g., acids, alcohols, amines, amino acids and proteins, etc.
- Mostly used for identification and purification.
- To check the performance of other separation processes.
- To measure the reaction process by assessment of intermediates, reaction course, etc.
- For separation of Inorganic Ions – Used for separating cationic & anionic substances.
- Separation of vitamins – Vitamin E, Vitamin D3, vitamin A.
- Quantitative analysis

High performance thin layer chromatography [19-25]

It's a more advanced type of TLC with a quick separation approach that can separate a wide range of samples and just takes a few minutes to analyse the complex or crude sample clean-up.

Procedure

With the help of an applicator machine, prepared sample and standards are applied to the pre-washed and pre-conditioned chromatographic layer. The sample is then placed

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in a chromatographic chamber with mobile phase to separate. Detectors or scanners are used to read the produced chromatogram.

Precondition

The equilibrium of the solvent vapour and the plate will be different from the equilibrium of the solvent and the plate. The developing solvent is present in a separate reservoir, comparable to normal development. The plate is placed in the enclosure for a few minutes to achieve equilibrium with the solvent vapour. The plate is then placed in the developing solvent, with the separation being processed in the developing solvent. The plate is placed in the enclosure for a few minutes to achieve equilibrium with the solvent vapour. The plate is then inserted in this manner, dipping its end into the developing solvent, and the separation is carried out as usual. The apparatus for pre-equilibrium of a thin layer plate is shown in Figure, as well as the differences between the two TLCs. Pre-saturation of the TLC plate increases the velocity of the solvent front compared to the unsaturated plate, and separated components are much closer to the solvent front in the unsaturated plate

Advantages

- Simultaneous sample and standard processing improve analytical precision and accuracy, reducing the need for an internal standard.
- Reduced analysis time and cost per analysis, as well as lower maintenance costs.
- Simple sample preparation - work with a variety of samples.
- There is no preceding solvent treatment, such as filtration or degassing. V. Low persample mobile phase usage.
- No contamination due to contamination from prior analyses — each analysis uses fresh stationary and mobile phases.

Applications

- An established for determining and purifying various medicines in the pharmaceutical business.
- Food and drug analysis includes herbal medicine quantification, vitamin analysis, water soluble food dye analysis, and pesticide analysis in fruits, vegetables, and other foods.

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- Fingerprint analysis, detection of misuse substances, poisons, adulterations, chemical weapons, and illicit drugs are all forensic services. IV. Environmental analysis and cosmetology

High performance liquid chromatography (HPLC)[26-29]

HPLC is a superior liquid chromatography method and one of the most commonly used analytical techniques. Due to the physical properties of HPLC column high pressure should be applied to have an eluent flow through the column. Because of this earlier the method was termed as high pressure chromatography.

Principle:-

Separation is depended on the relative solubility between two liquid phases of the analyte. HPLC utilizes various types of stationary phase (typically, hydrophobic saturated carbon chains), a pump that pushes the mobile phase and analytes through the column, and a detector that provides a characteristic retention time for the analyte. The retention time of analyte varies depending on the column temperature, the ratio/composition of solvent used, and the mobile phase flow rate. For HPLC, a pump (rather than gravity) provides the higher pressure needed to propel the mobile phase through the densely packed column and analyte.

Chromatographic analysis:-

The number of peaks recorded determines the number of components in a sample. The amount of a given component in a sample is determined by the region under the peaks. The retention time help within components identification. A stetionary phase of small particle size which increases the efficiency of separation forms the basis for HPLC. This is because the solute can balance between the two phases more quickly. Larger particle size stationary phase forms the basis of low-pressure liquid chromatography in which flow of the eluent through the column is either gravity-fed or pumped by a low pressure pump. It is cheaper to run but lacks the high resolution.[8] As the particles size of stationary phase decreases, surface area increases and indirectly number of plates are increases and hence resolution increased. Yet resistance to the mobile phase flow increases because increased capillary action with small particle sizes. Such resistance produce a backpressure that reduces the flow rate and make it more difficult to drain the column under gravity. To overcome this high pressure system has to be applied to maintain the flow of solvent.

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Mobile phases:-

Up to four different eluents can be handled by HPLC instruments. High purity eluents should be used. Single eluent or two or more eluents premixed in defined proportions may be made with a single pump whereas in gradient elution to produce two eluent in proportions predetermined by a gradient programmer. For reverse phase HPLC is eluent, which is strong for regular phase HPLC, is weak and the opposite is true too.

Buffer systems Composition:-

The composition of elution buffer shouldn't impact resolution.

Column elution:-

Appropriate eluent stored in a buffer reservoir is passed through the column that a consistent uniform rate for sample separation containing the components. The sample is uniformly applied to the top of the stationary phase at the end.

Filters and degassers:-

Membrane filter must eliminate dust particles. Degassing by heating, vigorously stirring with a magnetic stirrer, applying a vaccume, ultrasonic, and budding through the eluent reservoir of helium gas. As the size of the particles of stationary phase decreases, surface area increases and indirectly number of plates increases and hence increased resolution.

Mobile phases:- HPLC instruments can accommodated up to four different eluent. It is appropriate to use high purity eluents. In isocratic elution, single eluent or two or more eluents premixed in defined proportions may be made with a single pump whereas in gradient elution separate pumps are used to deliver two eluents in proportions predetermined by a gradient programmer. Eluent, which is strong in normal phase and weak in reversed phase HPLC and the opposite is also true.

Pumping system:-

Special pumps are available for the transport of eluent and are one of the most important features of HPLC systems. The function of the pump is to force a mobile phase at a specific flow rate.

Sample introducer:-

The method of sample introduction is most commonly used by a loop injector. It consists of a small volume metal loop that can be filled with the sample [5 to 25microliters (μL)]. By changing the position of the valve, the eluent is channelled through the loop and the sample is flushed onto the column. HPLC injectors can be

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manual ones but in case of high number of samples an auto sampler is more convenient to apply.

Columns:-

The column is often called the “heart of chromatography” in separation process, and the availability of stable, high performance stationary phases and columns is critical to the development of reproducible and robust methods. Silica is the most commonly used column packing material for its reliable strength and rigidity, relative inertness and ability to be modified chemically.

Types of columns in HPLC depending on the use

- Analytical [internal diameter 1.0 – 4.5-mm; lengths 25 – 250 mm]
- Preparative [i.d. > 4.6 mm; lengths 50 – 250 mm]
- Capillary [i.d. 0.1 – 1.0 mm; various lengths] Tubing materials
- Stainless steel (the most popular; gives high pressure capabilities)
- Glass (mostly for biomolecules)

Reduced eluent consumption, ideal for interfacing with a mass spectrometer due to the slower flow rates and increased sensitivity due to the higher concentration of analytes that can be used are the advantages of open tubular columns over conventional. New columns are made of stainless steel with highly polished interior walls, plastic material, glass-lined inner surfaces and outer column surfaces made from the rigid polymer, soft polymeric material for the outer surface and by compressing the column radially.

HPLC is divided into normal phase (NP) HPLC and reversed phase (RP) HPLC methods based on the polarity of the stationary and the mobile phase.

Applications:-

HPLC is suitable for the separation of the non-volatile and thermally unstable chemical and biological compounds.

- Pharmaceuticals like aspirin, ibuprofen, or acetaminophen.
- Potassium phosphate, sodium chloride and other salts.
- Proteins like blood protein, egg white.
- Organic chemicals like polymers (e.g., polystyrene, polyethylene).
- Motor oil and other hydrocarbons.
- Many natural products such as ginseng, herbal medicines, plant extracts.
- Thermally unstable compounds such as trinitrotoluene (TNT), enzymes.

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HPLC instruments are everywhere in drug research and development, pharmaceutical manufacturing, quality assurance, diagnostics, toxicology, research and other laboratories.

GAS CHROMATOGRAPHY: [30-34]

Gas chromatography differs from other forms of chromatography in that the mobile phase is a gas and the components are separated as vapors.It is thus used to separate and detect small molecular weight compounds in the gas phase.The sample is either a gas or a liquid that is vaporized in the injection port. The mobile phase for gas chromatography is a carrier gas, typically helium because of its low molecular weight and being chemically inert.The pressure is applied and the mobile phase moves the analyte through the column. The separation is accomplished using a column coated with a stationary phase.The equilibrium for gas chromatography is partitioning, and the components of the sample will partition (i.e. distribute) between the two phases: the stationary phase and the mobile phase.Compounds that have a greater affinity for the stationary phase spend more time in the column and thus elute later and have a longer retention time (Rt) than samples that have a higher affinity for the mobile phase.Affinity for the stationary phase is mainly due to intermolecular interactions and Stationary phase polarity can be chosen to maximize interactions, Separation. Due to their random nature, ideal peaks are Gaussian and symmetrical. Interaction of analyte with column. Separation is therefore done by splitting the sample between the gases a thin layer of non-volatile liquid held on a solid support. A sample containing solute is injected into the heated block in which it is placed Vaporizes quickly and is carried away as a vapor slag by the carrier gas stream column entrance.

Solutes are adsorbed to the stationary phase and desorbed to the New stationary phase carrier gas. This process is repeated for each plate while the samples are moving to the outlet. Each solute moves through the column at its own speed.Your band is divided into different zones according to division Modulus and Bandwidth Spread. Solutesare eluted sequentially in ascending order of kd, It enters a detector attached to the outlet end of the column. Now record a series of signals resulting from changes in concentration, Recorder elution rate as a plot of carrier composition versus time gas flow. You can measure the appearance time, height, width and area of these peaks Provides quantitative data.

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Ion-Exchange chromatography [35-44]

One of the most popular types of column chromatography is ion-exchange chromatography. It is used in research, analysis, and process-scale purification of proteins. The first observations recorded in the literature that refer to ion exchange were made by way and Thompson in 1850.

Application of ion exchange chromatography:

Ion exchange chromatography can be used to separate and purify a variety of charged or ionizable compounds from both natural and synthetic sources, including proteins, peptides, enzymes, nucleotides, DNA, antibiotics, vitamins, and more. Example of some application ion exchange chromatography as follow:

- **Separation of Actinides**. The actinide series' transplutonium elements were first discovered using the ion exchange chromatographic method. Since some of these elements could only be created in atom quantities, the actinide series also exhibits elution in the opposite order of the atomic number due to actinide contraction. This
- proved to be the sole method for identifying these elements. One could argue that any other method of separation would not have allowed for their seclusion.
- **Purification of Organic Compounds**. Many natural products extracted in water have been found to be contaminated with ions originally present in water. Ion-exchange procedures can be used to get rid of such ions.
- **Sugar separation**. The approach was created in 1951 by Khym and Zill. Borate complexes are first created from these sugars.
- **Preparation of Pure Reagents**. Carbonate is invariably present in solutions of sodium hydroxide used for volumetric determinations. This gives rise to errors in acid-base titrations. The simplest method for removing carbonate involves running the solutions through a column of a strongly basic anion exchange resin in the hydroxide form. As the carbonate is absorbed, an equivalent concentration of hydroxide is released.

APPLICATION OF CHROMATOGRAPHY IN CHEMISTRY [45]

Following are some of the uses of chromatography in chemistry.

- Chromatography is a technique used to determine the relationship between various mixes.

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- This method of determining the sample's purity is highly successful.
- Chromatography may be used to determine the quantity of mixture contained in a sample.
- Chromatography may be used to separate chimicals, which are remarkably identical in terms of molecular weight, elemental makeup, and physical characteristics, differing only in optical isomers.
- It is employed in the process of separating mixtures of chemicals. In particular, paper chromatography works incredibly well for identifying and separating mixtures of chemicals.

APPLICATION OF CHROMATOGRAPHY IN MEDICINE[45]

- Chromatography is used in pharmaceutical businesses to manufacture huge quantities of pure ingredients needed to make more medications.
- Using paper chromatography, the different inks or dyes are extracted from the mixture.
- By employing gas chromatography, the presence of alcohol or certain other substances in blood or urine may be found.
- Chromatography is a crucial analytical tool in pharmacy used to determine the accuracy of medicine produced.
- By identifying combustible substances and burn residue left in body parts after fires or explosions, forensic science may solve several cases.

CONCLUSION

Amino acids, biomolecules, crude drugs, medications, oil, hydrocarbons, enzymes, organic polymers, biological resin, pesticides, herbicides, surfactant polymers, fossil fuels, acids, alcohols, amines, vitamins, and so on are among the different component mixtures that can be separated using chromatography techniques. Chromatography is capable of quantifying, purifying, separating, and measuring components qualitatively. A successful separation may be achieved by choosing the appropriate absorbing substance because various mixtures might be liquid, gas, volatile, insoluble, etc. As was already established, there are many various kinds of analytical methodologies; nonetheless, each pharmaceutical requires a unique approach to determine which is best. Researchers aim for the most precise or "gold standard" method for a pharmacological analyte even in the earliest phases of drug development. Therefore, innovations in current procedures together with the development of new bio-analytical

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techniques (such as innovative chromatographic techniques) are essential for the effective development of a medicine.

REFERENCES

1. “The Nobel Prize in Chemistry 1952”. Nobelprize.org. Retrieved 25 August 2016.
2.McMurry, John (2011). Organic chemistry: with biological applications (2nd ed.). Belmont, CA: Brooks/Cole.
3.IUPAC. Compendium of Chemical Terminology, 2nd ed. (the “Gold Book”). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997).
4. Hostettmann, K; Marston, A; Hostettmann, M (1998). Preparative Chromatography Techniques Applications in Natural Product Isolation (Second ed.). Berlin, Heidelberg: Springer Berlin Heidelberg.
5. https://microbenotes.com/chromatography-principle-types-and-applications/
6. www.rpi.edu/dept/chem-eng/biotechenviron/chromo/be_types.htm
7. www.chem.neu.edu/courses/1221/PAM/chromatogr/ index.htm
8. www.wikipedia.com,wikipedia, free encyclopedia
9. Balston, J.N. and Talbot, B.E., 1952. A guide to filter paper and cellulose powder chromatography. A guide to filter paper and cellulose powder chromatography.
10. Practical Pharmaceutical Chemistry. By. A. H. Beckett and J. B. Stenlake. The Athlone Press, University of London, 2 Gower Street, London, S.C. 1, 1962. VIII + 378PP. 15.5 × 25cm. Price $10.10. (1963). Journal of Pharmaceutical Sciences, 52(5), 511. Http://doi.org/10.1002/jps.2600520537
11. DE ZEEUW, R.A., 1969. Paper and thin layer chromatographic techniques for separation and identification of barbiturates and related hypnotics. In Progress in Chemical Toxicology (Vol. 4, pp. 59-142). Elsevier.
12. DE ZEEUW, R.A., 1969. Paper and thin layer chromatographic techniques for separation and identification of barbiturates and related hypnotics. In Progress in Chemical Toxicology (Vol. 4, pp. 59-142). Elsevier
13. Sliepcevich A, Gelosa D. Gas and liquid chromatography. In: Sergio Carra, editor. Fundamentals of Chemistry. Italy: UNESCOEOLLS., 2009.

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 01, 2024

14. Justus G. Kirchner, Thin-layer chromatographic quantitative analysis, Journal of Chromatography A ,1 August 1973, Volume 82, Issue 1, 101-115.

15. Skoog D. A., Holler F.J. and Nieman T.A., "Principles of instrumental analysis, Saunders college publishing, 5th edition, 2006 ,761-766.

16. Chittum, J. W. (1957). Chromatography: A review of principles and applications. Second Edition, revised (Lederer, Edgar, and Lederer, Michael). *Journal of Chemical Education*, *34*(12), 628. http://doi.org/10.1021/ed034p628.2

17. Vidya Sagar, Instrumental methods of drug analysis, Pharma Med Press, First edition 2009,263

18. Reich, E., & Schibli, A. (2007). *High-performance thin-layer chromatography for the analysis of medicinal plants*. New York: Thieme.

19. Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. Arab J Chem., 2013; 6: 124-36.

20. Chakravarti B, Mallik B, Chakravarti DN. Column Chromatography. Curr Protoc Essent Lab Tech., 2008; 6–2.

21. Wilson K, Walker JM. Principles and techniques of biochemistry and molecular biology. 7th ed. New York: Cambridge University Press., 2010.

22 Bak I. Modern analytical techniques in the pharmaceutical and bioanalysis. Medical and Health Science Center, University of Debrecen., 2011 Oct 31.

23. Edward Lau (Deceased), in Separation Science and Technology, 2001

24. Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. Arab J Chem., 2013; 6: 124-36

25.https://www.intechopen.com/books/column-chromatography/ion-exchange chromatography-and-its-applications

26. Lauback, R. G., Rice, J. J., Bleiberg, B., Muhammad, N., & Hanna, S. A. (1984). Specific high-performance liquid chromatographic determinatioin of ampicillin in bulks, injectables, capsules, and oral suspensions by reverse-phase ion-pair chromatography. *Journal of Liquid Chromatography*, *7*(6), 1243–1265. http://doi.org/10.1080/01483918408074041

27. Eriksson Wiklund, A.-K., & Dag Broman, B. S. (2005). Toxicity evaluation by using intact sediments and sediment extracts. *Marine Pollution Bulletin*, *50*(6), 660–667. http://doi.org/10.1016/j.marpolbul.2005.02.030

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 01, 2024

28. Kwok, Y. C., Hsieh, D. P. H., & Wong, P. K. (2005). Toxicity identification evaluation (tie) of pore water of contaminated marine sediments collected from Hong Kong waters. *Marine Pollution Bulletin*, *51*(8-12), 1085–1091. http://doi.org/10.1016/j.marpolbul.2005.06.009

29. Hongxia, Y. (2004). Application of toxicity identification evaluation procedures on wastewaters and sludge from a municipal sewage treatment works with industrial inputs. *Ecotoxicology and Environmental Safety*, *57*(3), 426–430. http://doi.org/10.1016/j.ecoenv.2003.08.024

30. Lee DC, Webb ML. Pharmaceutical analysis. Canada: Blackwell Publishing Ltd., 2003.

31. Grob RL, Barry EF. Modern Practice of Gas Chromatography. 4th ed. New Jersey: John Wiley & Sons Inc., 2004.

32. Sliepcevich A, Gelosa D. Gas and liquid chromatography. In: Sergio Carra, editor. Fundamentals of Chemistry. Italy: UNESCOEOLLS., 2009.

33. Ballance R. Advanced instrumental analysis. In: Bartram J, Ballance R, editors. Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. Geneva: UNEP/WHO., 1996.

34. Chromatography-Modern Chemical techniques. London: The Royal Society of Chemistry, Unilever., 116-59

35. Moura, Alexandre Varão, José Domingos Santos da Silva, and Priscila Gubert. "Ion Chromatography: Principles and Instrumentation." Orbital: The Electronic Journal of Chemistry (2022): 110-115.

36. Acikara, Özlem Bahadir. "Ion-exchange chromatography and its applications." Column chromatography 10 (2013): 55744

37. Kumar, Sanjeev, and Sapna Jain. "History, introduction, and kinetics of ion exchange materials." Journal of chemistry 2013 (2013).

38. Cummins, Philip M., Oonagh Dowling, and Brendan F. O'Connor. "Ion-exchange chromatography: basic principles and application to the partial purification of soluble mammalian prolyl oligopeptidase." Protein Chromatography: Methods and Protocols (2011): 215-228.

39. Williams, Alan, and Verna Frasca. "Ion-exchange chromatography." Current protocols in molecular biology 44.1 (1998): 10-10.

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 01, 2024

40. Healthcare, G. E. "Ion Exchange Chromatography: Principles and Methods." General Electric Company: GE Healthcare (2016).

41. Jungbauer, Alois, and Rainer Hahn. "Ion-exchange chromatography." Methods in enzymology 463 (2009): 349-371.

42. Rossomando, Edward F. "[24] Ion-exchange chromatography." Methods in enzymology. Vol. 182. Academic Press, 1990. 309-317.

43. Fekete, Szabolcs, et al. "Ion-exchange chromatography for the characterization of biopharmaceuticals." Journal of pharmaceutical and biomedical analysis 113 (2015): 43-55.

44. Ladiwala, Asif, et al. "A priori prediction of adsorption isotherm parameters and chromatographic behavior in ion-exchange systems." Proceedings of the National Academy of Sciences 102.33 (2005): 11710-11715.

45. https://chemistry.tutorvista.com/analytical-chemistry/uses-ofchromatography.html