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Current trends of chromatography in pharmaceuticals:

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ABSTRACT:
In recent years, chromatography has become one of the most crucial analytical techniques employed in the medical profession for the identification and quantification of a medication and its metabolites. To distinguish between medications based on their traits and forms of interactions, numerous chromatographic approaches have been developed. Among these methods, High Performance Affinity Chromatography, cell membrane chromatography, mixed mode chromatography, and high performance liquid chromatography are utilized for pharmaceutical applications in preclinical and clinical investigations to analyze the bioactivity of pharmaceuticals. Chromatography's accomplishment in the development of quick and accurate analytical techniques offers greater specificity and sensitivity in the study of drug pharmacokinetic features in R&D. It's critical in personalized medicine to consider how individual drug dosage and effects differ. In recent years, chromatography has emerged as a viable method for examining clinical or pharmaceutical samples as well as for analyzing drug-protein binding.

Key Words: HPAC (High Performance Affinity Chromatography), CMC (cell membrane chromatography), MMC (mixed mode chromatography), HPLC (high performance liquid chromatography), GC (gas chromatography).

I. INTRODUCTION:

Chromatographic techniques are now often used in both routine and research laboratories for clinical analysis. Clinical analysis is the testing of biological materials to ascertain the validity and effectiveness of the processes involved in the diagnostic and therapeutic procedures. Chromatographic methods are indispensable, especially in the investigation of drugs, toxicity, and biomarkers. These analyses make use of biological components such whole blood, serum, plasma, urine, faeces, tissues, as well as macromolecules like lipids and proteins (Denoroy et al., 2013; Kortz et al., 2011; Van den Ouweland & Kema, 2012). Clinical toxicology, biomarker analysis for laboratory-based diagnostics, and therapeutic drug monitoring are the three general categories for clinical analysis. To distinguish between a medicine's least active concentration and minimum hazardous concentration, therapeutic drug monitoring (TDM) is frequently utilised. Biomarkers are biological substances that serve as indicators of healthy biological activities, harmful biological processes, and pharmaceutical reactions. They consist of proteins, vitamins, hormones, lipids, peptides, and low molecular weight metabolites. They are crucial for early disease diagnosis, evaluating the course of the disease, tracking the effects of medications, and therapeutic intervention. The study of clinical toxicology focuses on different toxicants that cause illnesses or other clinical symptoms following short- or long-term exposure (Kočová Vlčková et al., 2018). Clinical analysis calls for quick, highly effective, and trustworthy techniques. The reliability of these procedures declines due to low efficiency, sensitivity, and nonspecific interactions in the analyses employed in typical clinical applications. Because of this, modern approaches should be chosen over ancient ones. In recent years, chromatographic procedures have grown in popularity in both routine and research labs. An overview of the current chromatographic techniques used in clinical analysis is what this paper aims to do. This review outlines the chromatographic developments in clinical analysis, explores their benefits and drawbacks, and then presents a fresh angle for further study.

Since medical products must be both safe and effective, the pharmaceutical industry is one of the most heavily regulated sectors of the global economy. Active pharmaceutical ingredients (APIs) must closely adhere to impurity and degradation product levels regulations set forth by international authorities. Chromatography has long been the method of choice for determining the

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chemical purity of pharmacological ingredients and products, and it is frequently employed in the pharmaceutical sector, from R&D through QC labs. The best method for analyzing slightly polar to apolar chemicals, or those with an octanol-water partition coefficient (log P) between -1 and 5, is reversed-phase liquid chromatography (RPLC), which consists of a polar mobile phase and an apolar stationary phase. Due to the fact that this chromatographic mode exactly fits the physicochemical features of pharmaceuticals, RPLC is currently regarded as the gold standard in pharmaceutical analysis. Indeed, Lipinski created his renowned rule of thumb for assessing the drug-likeness of chemical compounds over 20 years ago. He listed a number of requirements, saying that an orally active medication should have a log P no more than 5 (Desfontaine et al., 2015; Feng, 2013; Lipinski et al., 2012). For the purposes of discussing applications of separation science, pharmaceutical drug development can be broken down into three different phases: drug discovery, drug development, and filing-post filing activities. This procedure is time-consuming and high-risk. Although analytical chemistry and separation science in particular are crucial to each of these stages, we concentrate on applications for the drug development stage in our article. This phase usually begins with the nomination of a drug candidate, which is then put through several stages of clinical testing where, in addition to efficacy, safety, dosage, side effects, and long-term adverse reactions are researched (Chromato- Graphic Selectivity., 1988; Lestremau et al., 2006; Pharmaceutical Research and Manufacturers of America, 2011).

1) Principle of chromatography:

Chromatography is essentially a method for fractionating mixtures between two phases while one of them is continuously moving past the other. In theory, neither the characteristics of the mobile and stationary phases nor the kinds of equilibria engaged in the partition appear to be constrained. In actuality, liquid mobile and solid or liquid stationary phases have been used in the most significant applications. A solution of the mixture to be fractionated is poured on top of an adsorbent column and gently passed through; the mixture's constituent parts are kept in zones at the top of the column according to their affinity for the adsorbent. The chromatogram is developed by separating the zones of adsorbed material throughout the column, with the more highly adsorbed chemicals being closer to the top of the column, as more solvent is passed through the column by fractional elution and re-adsorption. If the distinct zones can be quickly distinguished by colour, by ultraviolet light, or by a reagent streaking down the extruded column, they are divided by the column and eluted with the appropriate solvent. According to appropriate chemical or physical tests, solvent is pumped through the column to elute the components one at a time. The eluate is divided into arbitrary fractions and each fraction is studied separately in cases where these approaches cannot be used. In partition chromatography, the column is made out of an inert carrier on which a solvent that is soluble in the mobile solvent has been dispersed. Similar principles are used in paper chromatography, where the fractionation is accomplished by selective adsorption on the paper or partition between the mobile phase and the paper's water content, which has been equilibrated with the saturated vapours of both phases (Ketone et al., 1876)(August 27, 1949, 1949)(Lestremau et al., 2006).

Chromatography is a technique used by pharmaceutical businesses to manufacture vast volumes of chemicals that are incredibly pure as well as to check the compounds for minute impurities. Chromatography is increasingly being used in the pharmaceutical sector to separate chiral substances. These compounds have molecules with slightly different atom orientations in space. Even though the two forms—known as optical isomers, or enantiomers—are almost identical in practically all other respects, including molecule weight, element makeup, and physical properties, they can exhibit vastly distinct biological activities. For instance, the chemical thalidomide possesses two optical isomers. When women take one early in pregnancy, it results in birth abnormalities; the other isomer does not. It is critical that the benign form and the harmful isomer be entirely separated because this molecule appears promising for the treatment of several diseases that are resistant to current medications (At et al., n.d.).

2) Applications of high performance liquid chromatography:

Since it can deliver the exact results needed, HPLC is the type of liquid chromatography that is typically utilized in the pharmaceutical business. The outcomes can be used to analyze materials and final pharmaceutical products both quantitatively and qualitatively while they are being manufactured. This can be used to identify a medicine and track the success of a therapy on an illness. It is accomplished through the separation,

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measurement, and identification of components in a mixture (Braithwaite & Smith, 2018). Understanding the structure and quantifying the levels of contaminants in pharmaceutical formulations is one of the key advantages of HPLC. High molecular weight, thermally unstable, and difficult to volatilize chemicals are very well suited for HPLC. It may therefore measure a medication in both its pure and dose form. In the pharmaceutical sector, reversed-phase, denaturing, and immobilized enzyme reactor (IMER) HPLC are also used (Sciences et al., n.d.)(Papers, 2022)(Nikolin, 2004). There are several opportunities for further HPLC technique development, including developing new materials for making precise and more effective stationary phases and, in connection with that, learning new ways to combine and modify mobile phases. In the area of signal registration, new, more effective hyphenated systems, such as HPLC-MS and HPLC-NMR, have been developed as applications of the HNMR and CNMR techniques. Their use will open up a wide range of opportunities and support in medical diagnostics and in following the fate of healing substances in body fluids (Nikolin, 2004).

3) Applications of gas chromatography:

GC is frequently employed as an alternative to GC-MS/MS, HPLC-MS/MS procedures in forensic toxicology, doping analysis, and standard bio-analytical laboratories due to its high precision and broad applicability in the investigation of numerous physicochemical agents. A liquid film deposited on a column wall and open tubular capillary columns are the most common GC techniques. Targeted investigations of a wide range of volatile chemicals also use GC for lipidomics and metabolomics. The substances that are typically identified in clinical GC routines include endogenous substances like fatty acids, steroids, and other hormones, as well as narcotics like cocaine, cannabinoids, amphetamines, MeOH, and breath volatile substances, as well as anaesthetics, analgesics, antidepressants, antipsychotics, and antie-pileptics (Patel, 2019; Santos & Schug, 2017).

Based on the polarity of the chemicals, gas chromatography is a tool for analytical separation of compounds in complicated mixtures. Only volatile chemicals or those that can become volatile through the use of derivative agents are capable of being separated. Because of its efficiency, sensitivity, and general acceptance as a technique for compound separation, this one. The basis of compound separation is based on the differences in the partitioning behavior between the mobile and stationary phases. The sample is transported by a moving gas stream via a tube that is either filled with a solid that has been finely divided or may be covered with a liquid film. For the separation of various classes of compounds, various types of columns with different stationary phase compositions have been used. A mixture or sample in a suitable solvent is introduced through an injector kept at a higher temperature, which is capable of volatilizing the compound into the column (Jwaili, 2019).

4) Applications of supercritical fluid chromatography:

Supercritical fluid is a great option for the chromatographic mobile phase since it allows for quick separation with high efficiency and frequently uses enantioresolution. Supercritical fluid chromatography (SFC) is being utilized more frequently for the analytical, semi-preparative, and preparative purification of chiral substances, including the generation of enantiomers that are primarily found during the creation of pharmaceuticals. SFC is becoming more common in the pharmaceutical business as an alternative to HPLC for numerous pharmacological compounds. Great speed, low analysis times, minimal environmental effect, and high efficiency are the key benefits of SFC in chiral pharmaceutical separation. Cost, health, and safety advantages come from reducing the usage of organic solvents. These benefits allow SFC to meet all the criteria for Green Analytical Chemistry techniques (Płotka et al., 2014). Work with biological materials and pharmacological compounds has made affinity chromatography, a separation technique, more crucial. To selectively retain analytes or investigate biological interactions, this technique relies on the employment of a stationary phase that is relevant to biology. Weak affinity chromatography, step elution schemes, affinity extraction, and affinity depletion are some common formats for affinity chromatography that are taken into consideration. Lentin affinity chromatography, boronate affinity chromatography, immunoaffinity chromatography, and immobilised metal ion affinity chromatography are some of the specific separation methods that are explored. A biologically-related substance, or "affinity ligand," is used as a stationary phase in affinity chromatography to either selectively retain analytes or analyze biological interactions. A wide range of binding agents, including as proteins, enzymes, antibodies, antigens, DNA or RNA

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sequences, biomimetic dyes, enzyme substrates, inhibitors, or low mass compounds, can be used as the affinity ligand (e.g., a drug or hormone). The affinity ligand is utilized to specifically bind a specific target or set of targets inside a sample by being immobilized within a column. Because many affinity ligands are very selective, the end result is a column that may be used to test, isolate, or research specific targets even when they are present in complicated biological samples (Acta et al., 1973; Hage et al., 2012; Walters, 1985).

5) Cell membrane chromatography Applications:

He et al. introduced cell membrane chromatography (CMC) for the first time in 1996. CMC is a bio-affinity chromatography technique that has gained recognition as a critical tool for investigating drug-receptor interactions and identifying active components in herbal medicines. The CMC system offers an analytical technique with a high degree of performance, selectivity, and efficiency not only for the investigation of drug-receptor interactions but also for the discovery of active components from therapeutic herbs. The system's methodology ought to be very helpful in disciplines like pharmaceutical analysis, receptor pharmacology, and pharmacochemistry (Acta et al., 1973).

6) Applications of mixed mode chromatography:

Unique selectivity is offered by mixed-mode chromatography, particularly for polar and charged analytes. The users can modify the mobile phase/eluent settings to encourage particular interactions for specific analytes thanks to the variety of interaction mechanisms. From the basic "secondary interaction," MMC has moved to intentionally planned and managed multi-mode interactions. The latest batch of mixed-mode stationary phases is stronger and more varied. The uses of MMC have been substantially expanded by the recent commercialisation of mixed-mode stationary phases. For counter ion analysis, polar and/or charged APIs, contaminants, formulation excipients, and environmental and biological samples, MMC has been successfully used in pharmaceutics. SPEs with mixed-mode sorbents have been used successfully to clean up sample matrices and boost LC-MS sensitivity. The analysis and purification of peptides and proteins, antibody aggregation, and heterogeneity characterization all benefit from the use of MMC. In many applications, MMC can take the role of IEX, allowing the use of MS-compatible mobile phases. For some applications, MMC is preferable over RP, but mostly for the analytes that are not maintained and separated well by the conventional RP columns (Zhang & Liu, 2016).

II. CONCLUSIONS:

Chromatography will undoubtedly continue to be the preferred analytical method in the pharmaceutical sector. In fact, there have been numerous recent advancements in chromatographic modes, stationary phases, and instruments. This overview outlines a few of the issues that the pharmaceutical sector frequently faces as well as the viable remedies. We can list the need to analyze increasingly complex samples, such as API with multiple chiral centres or new protein biopharmaceuticals formats, the requirement for greener chromatographic methods, the determination of genotoxic impurities at very low concentration levels, the implementation of PAT tools to efficiently design, control, and analyze drugs, as well as the need to implement PAT tools. Numerous chromatographic solutions, including the use of method development software, fast-LC, 2D-LC, SFC, HILIC, LC-MS, GC, and GC-MS, are typically used to address all of these challenging analytical problems.

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