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AI IN ANALYTICAL CHEMISTRY TRANSFORMING CHROMATOGRAPHY FOR HEALTH DIAGNOSTICS

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ABSTRACT:
Chromatography can be used in the diagnosis of diseases to separate and examine complicated biological samples like blood, urine, or the presence of particular substances like biomarkers, metabolites, or proteins that may indicate the presence of disease. The sensitivity, precision, and speed of chromatography technologies have improved throughout time, enabling the detection and quantification of biomolecules linked to a variety of disorders. Chromatography can be merged with other techniques like NMR and Mass spectroscopy in order to overcome various drawbacks that prevent it from being used alone for illness detection[19], such as time-consuming skill requirements, sensitivity restrictions, sample interference, etc. Our goal is to improve the accuracy and efficiency of chromatography in illness diagnosis for a future that is healthier by merging it with current Artificial Intelligence (AI) technologies [12]. It aids in the discovery of biomarkers linked to particular diseases. Analyzing the changes in the early and late stages of the disease is also beneficial. Chromatography can be used to examine malignancies, neurological illnesses, immunological disorders, metabolic disorders, and to track the effectiveness of treatment when combined with AI. The incorporation of chromatography will facilitate the diagnosis of diseases and offer precise, timely, and comprehensive insights into the molecular and chemical constituents of biological materials.

Index Terms - **Chromatography, Disease Diagnosis, Hemoglobinopathies, Integration, Artificial intelligence, Immunoassays, Crystallography, Electrophoresis.**

INTRODUCTION:

Chromatography [1] is a flexible separation method that may be used to separate and analyze complex mixtures into their constituent parts in a variety of scientific and industrial sectors. It is predicated on the idea that various components in a mixture will migrate differently as a result of having distinct affinities for a stationary phase and a mobile phase, leading to separation.

The primary elements in chromatography are:

The sample is transported through the chromatographic system by the mobile phase, which might be a gas or a solvent. Depending on the type of chromatography being performed, a mobile phase should be selected. The stationary phase is a substance that remains anchored in the chromatography column, either as a solid or a liquid. Differential migration and separation are caused by the interaction of the mixture's constituent parts with the stationary phase.

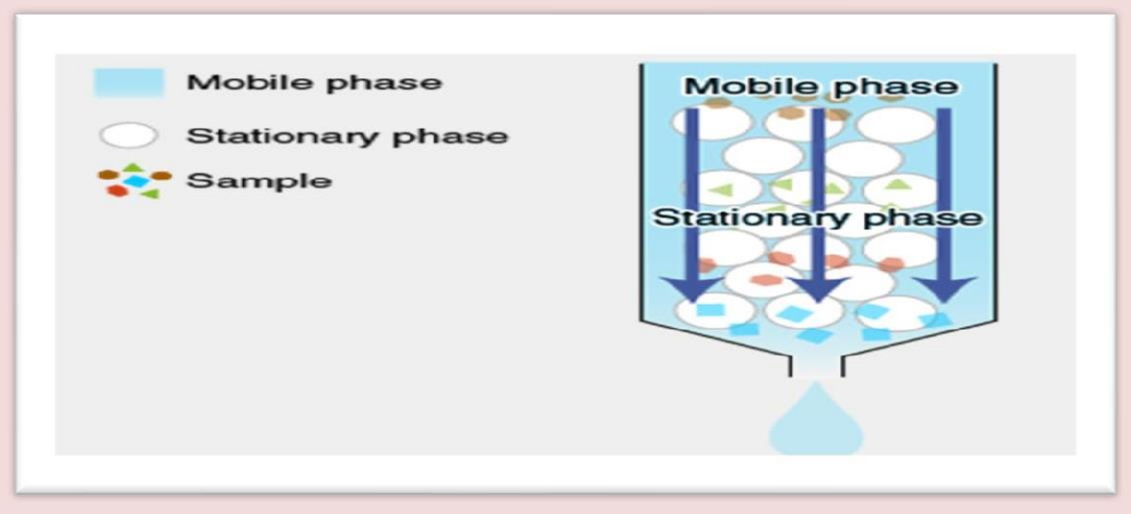

Figure-1: Representing the components of chromatography

DIFFERENT KINDS OF CHROMATOGRAPHY TECHNIQUES EXIST, INCLUDING:

1. **Liquid chromatography (LC):** Employs a liquid as the mobile phase and a solid, liquid, or gel as the stationary phase. High-performance liquid chromatography (HPLC), ion exchange chromatography, size exclusion chromatography, and other techniques fall within the category of LC.
2. **High-performance liquid chromatography (HPLC):** Achieves high-resolution separations by using a liquid mobile phase and a stationary phase filled with tiny particles.
3. **Low-pressure liquid chromatography (LPLC):** Comparable to high-pressure liquid chromatography (HPLC), except it operates at lower pressures and is often employed for simpler separations.
4. **Gas-Liquid Chromatography (GLC):** A gaseous mobile phase passes through a liquid-coated stationary phase to separate components based on their volatility and interaction with the stationary phase.
5. **Ion exchange chromatography (IEC):** Separates ions based on their charge by using a stationary phase with charged functional groups that attract and retain oppositely charged ions.
6. **Size-exclusion chromatography (SEC):** Separates molecules based on their size and molecular weight. Larger molecules elute faster, while smaller molecules are retained longer.
7. **Affinity chromatography (AC):** This technique selectively binds and purifies the target molecule by using a particular affinity [2] between an interesting molecule and a ligand immobilized on the stationary phase.
8. **Chiral chromatography:** utilizes the interactions of enantiomers (mirror-image isomers) with a chiral stationary phase to distinguish between them.
9. **Partition chromatography:** uses liquid-liquid chromatography, for example, to differentially partition components between two immiscible phases.
10. **Thin-layer chromatography (TLC):** A quick and easy method using a small layer of adsorbent material deposited on a flat plate or glass slide as the stationary phase.
11. **Paper chromatography:** A traditional chromatographic procedure in which paper serves as the stationary phase and the mobile phase passes through it to separate the components according to their affinities for the stationary phase and the solvent.
12. **Supercritical fluid chromatography (SFC):** This method, which offers several advantages over conventional liquid or gas chromatography, uses supercritical fluids as the mobile phase.

Figure -2: various chromatographic Equipments

Fig-2a: Gas chromatography

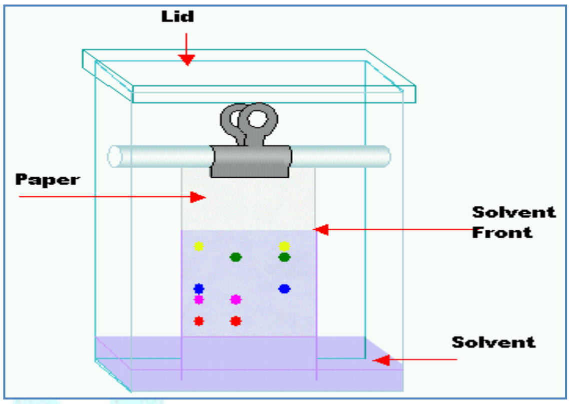

Fig-2b: paper chromatography

Fig -2c: Ion-exchange chromatography

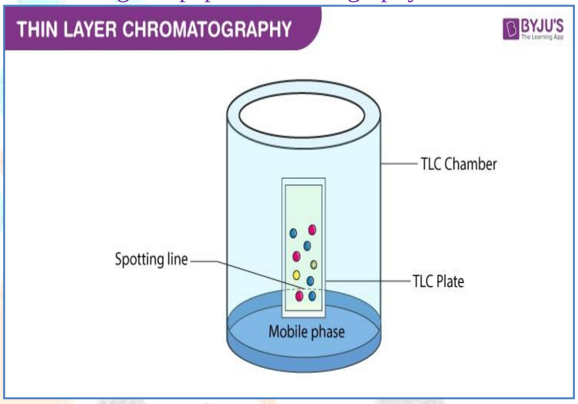

Fig-2d: TLC

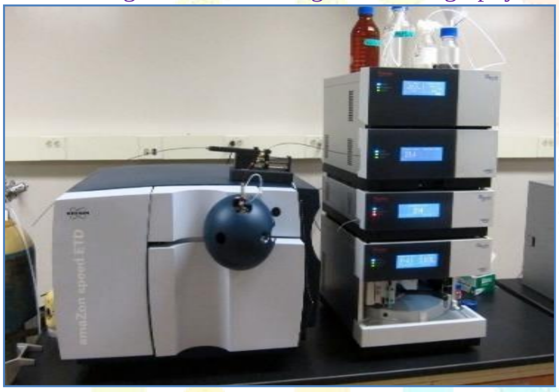

Fig-2e: LC-MS chromatography

Fig-2f: CE-MS

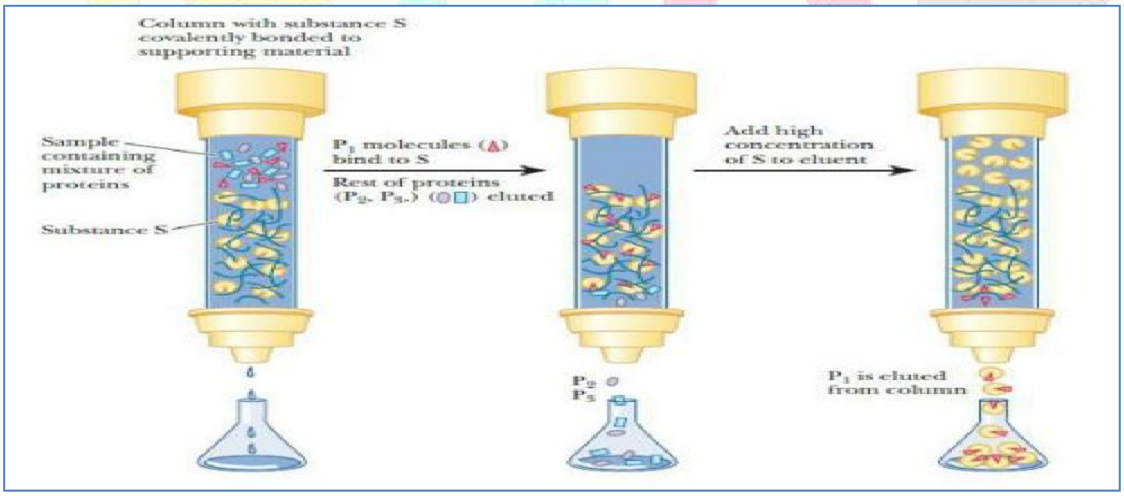

Fig-2g: Affinity Chromatography

Each chromatography method has its own distinct advantages and applications, making them essential instruments in a variety of scientific disciplines like chemistry, biochemistry, pharmacology, and environmental research.

CHROMATOGRAPHIC TECHNIQUES FOR DISEASE DIAGNOSIS

Chromatographic methods are frequently employed in clinical laboratories and research settings and are important in the diagnosis of disease. These methods make it possible to separate, identify, and quantify the different chemicals found in biological samples such tissues, blood, urine, and saliva. Healthcare experts can find anomalies linked to various diseases by examining the makeup of these samples. Among the frequently employed chromatographic methods [17] for illness diagnoses are:

High-Performance Liquid Chromatography (HPLC):

It is used for the examination of tiny molecules, including as medicines, hormones, vitamins, and metabolites, in the diagnosis of diseases. Diabetes, metabolic abnormalities, and several endocrine ailments are among the conditions that HPLC [4] is particularly effective at diagnosing. diagnoses thalassemia and other hemoglobinopathies by analyzing different hemoglobin variations.

Gas chromatography (GC):

GC is frequently used in the diagnosis of diseases in order to identify drugs of abuse, analyze fatty acid profiles, and measure the concentration of volatile organic compounds (VOCs) in breath samples in order to identify lung diseases and some cancers, which can be indicative of particular diseases or infections. utilized to identify metabolic diseases like phenylketonuria.

Thin-Layer Chromatography (TLC):

TLC [3] is a technique that can be used to analyze body fluids to identify medicines and their metabolites, detect lipid diseases, and determine lipid profiles. It can also be used to evaluate the purity of chemicals used in pharmaceutical formulations.

Liquid chromatography-mass spectrometry (LC-MS):

LC-MS [5] combines the ability to separate with mass spectrometry's sensitivity and selectivity of detection. It is widely used in clinical laboratories for targeted and untargeted analysis of biomolecules, such as medications, peptides, lipids, and metabolites, in order to diagnose and track the progression of diseases. used to identify inborn metabolic disorders like maple syrup urine sickness.

Capillary Electrophoresis (CE):

Based on their electrophoretic mobility, charged molecules can be separated using the effective method known as CE. Hemoglobinopathies, hereditary diseases, and other ailments with aberrant protein or nucleic acid profiles can all be diagnosed with this technique.

Ion chromatography (IC): IC is used to analyze ionic species, such as inorganic ions and organic acids, which can be useful in identifying specific intoxications as well as metabolic disorders, kidney diseases, and ions and electrolytes in urine and serum.

Affinity chromatography:

It is used to isolate and purify biomolecules of interest, such as antibodies, enzymes, or receptors, which are crucial for diagnosing a variety of diseases, including autoimmune disorders and some cancers. Affinity chromatography is used to detect anions and cations in various clinical samples.

Size exclusion chromatography:

By examining protein structures, size exclusion chromatography is used to diagnose some hereditary illnesses, such as alpha1 antitrypsin deficiency. These chromatographic technologies are essential for early disease identification, disease monitoring, and tailored medicine strategies when used in conjunction with other analytical techniques. They offer insightful information about patients' biochemical profiles, enabling better patient outcomes in terms of diagnosis and therapy choices.

Figure-3:

Fig-3a: TLC in diagnosis

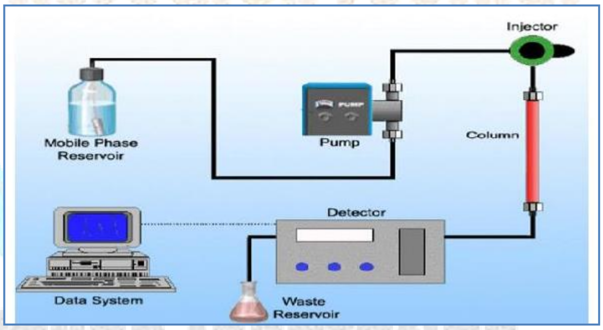

Fig -3b: HPLC in diagnosis

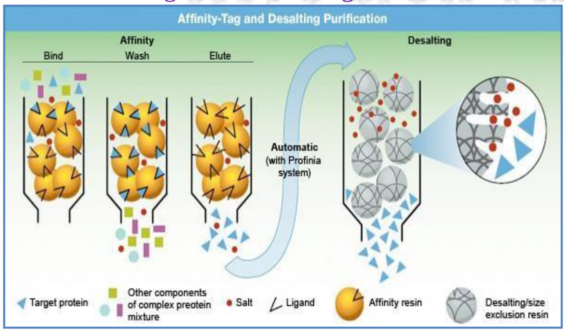

Fig-3c: affinity chromatography

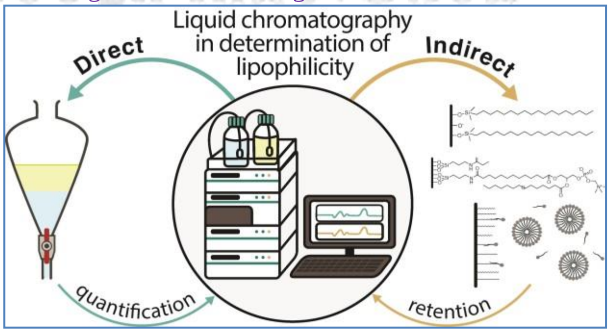

Fig-3d: liquid chromatography

STEPS INVOLVED IN CHROMATOGRAPHY-BASED DIAGNOSIS

In order to gather knowledge about the disease's underlying causes, biomarkers, or therapy alternatives, chromatography is used to separate and identify numerous substances found in biological samples like blood, urine, or tissue. In order to examine diseases and the biochemical changes they cause; chromatography is a potent technology that is frequently employed in clinical and research contexts. The usual procedure for using chromatography in illness analysis is as follows:

Reference collection: A biological sample should be taken from the patient, which could be blood, urine, saliva, or any other pertinent physiological fluid or tissue.

Preparation sample: Before analysis, the collected sample must be properly prepared. In order to separate the particular components of interest, this may include dilution, filtration, or extraction.

Selection of chromatographic techniques: Depending on the kind of sample and the substances you want to investigate, pick the right chromatographic procedure. Typical chromatographic methods for diagnosing diseases include:

a. High-Performance Liquid Chromatography (HPLC) is a technique for identifying and measuring individual components in mixtures.

b. Gas chromatography (GC) [14] is a particularly effective method for evaluating volatile substances.

c. Thin-Layer Chromatography (TLC): Efficient and affordable, appropriate for some applications.

Calibration standards: Prepare calibration standards containing the target chemicals in amounts that are known to exist. The calibration curve and chemical concentration in the patient's sample will be calculated using these standards.

Chromatographic analysis: Run the analysis by injecting the prepared sample into the chromatograph. The sample will move along the chromatographic column, where different components are separated according to their physical and chemical characteristics.

Detection and data analysis: A detector, such as UV-Vis, fluorescence, or mass spectrometry, will be used to find the separated components. The detector's data will be examined to determine the compounds of interest and to measure them.

Interpretation: To ascertain the existence and concentration of particular disease indicators or analytes, compare the chromatographic results of the patient's sample with the calibration standards and reference information.

Disease correlation and diagnosis: Once the chemicals have been identified, they can be connected to certain illness signs, the development of the disease, or the patient's response to treatment. It is possible to find disease-related biomarkers that could be employed in the diagnosis, monitoring, or evaluation of treatment effectiveness.

In order to get a thorough diagnosis, chromatography is frequently employed in conjunction with other analytical methods and clinical [9] evaluations. Additionally, accurate calibration, sample preparation, and careful interpretation of chromatographic results are necessary for the diagnosis to be successful. Therefore, for precise illness diagnosis utilizing chromatography, competent personnel and defined methods are essential.

CHROMATOGRAPHY APPLICATIONS FOR DIAGNOSIS:

Numerous businesses, including the food and beverage industry, forensics, biochemistry, environmental analysis, and the pharmaceutical industry, use chromatography. It is a crucial instrument for both qualitative and quantitative investigation of complicated mixtures, chemical identification, and the extraction of particular components from mixtures. Particularly in the areas of analytical chemistry and medicine, chromatography is essential for many diagnostic applications. It is a method for disentangling, identifying, and quantifying elements of a mixture, which makes it crucial for diagnosing diseases. Gas chromatography (GC), liquid chromatography (LC), high-performance liquid chromatography (HPLC) [7], and other chromatographic methods are used in diagnostics. Some applications of chromatography in diagnosis are Drug Testing: Chromatographic methods are frequently used to examine biological samples, such as blood or urine, in order to find out whether drugs or their metabolites are present. This is crucial for drug testing programs and for keeping track of patients who are taking medicine. Clinical toxicology**:** Chromatography is used to locate and quantify toxic compounds in blood or urine, assisting in the diagnosis of poisoning or drug overdose cases. Clinical Chemistry: Chromatography is used to measure the concentration of different substances, including proteins, glucose, cholesterol, and other biomarkers in biological fluids, assisting in the diagnosis and treatment of diseases like diabetes, hyperlipidemia, and kidney ailments. Hemoglobinopathies: In individuals with diseases like sickle cell anemia or thalassemia, chromatography can be used to detect aberrant hemoglobin variations. Chromatographic methods are used to evaluate the amounts of hormones in blood or urine, which is useful in identifying endocrine diseases. A contagious illness Chromatography can be used to locate and measure particular infectious agents or biomarkers in biological samples, aiding in the diagnosis of a variety of infectious disorders. Metabolomics: Chromatography is used in metabolomics [10] research to evaluate and identify metabolites in biological samples. This information can be utilized to gain knowledge about metabolic pathways and how they are affected by various disorders. Chromatographic techniques are used to identify allergens in food or environmental samples, assisting in the diagnosis and treatment of allergies. DNA sequencing uses chromatography to ascertain the nucleotide sequence of genetic material. Sanger sequencing is one such approach. This is crucial for determining specific genetic mutations and diagnosing genetic illnesses. Chromatography is a technique used in proteomics research to separate and identify proteins in biological samples, advancing our understanding of disease causes and possibilities for diagnostic biomarkers.

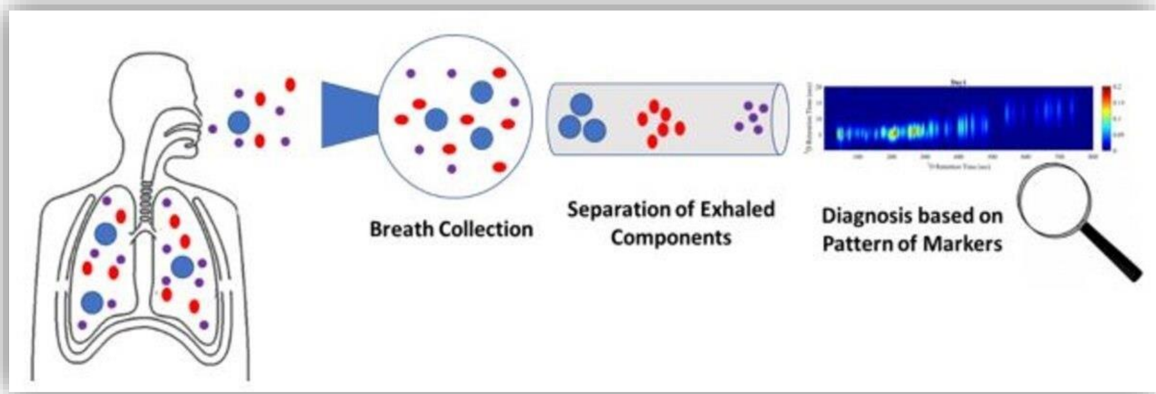

Figure-4: showing disease diagnosis based on biomarker pattern using chromatography

CHROMATOGRAPHY IN DISEASE DIAGNOSIS: UNDERSTANDING THE DISADVANTAGES

Chromatography has some drawbacks [11] while being a useful analytical method utilized in many scientific disciplines, including the diagnosis of diseases. The following are a few drawbacks of using chromatography in disease diagnosis*:*

1. *Time consuming*: Chromatographic analysis can take a long time, especially if complicated sample preparation is necessary. Delay in diagnosis could have serious consequences in some medical disorders when quick action is required.
2. *Skill-intensive:* Proper operation and interpretation of chromatographic data require employees who have received the appropriate training and are knowledgeable about chromatography methods. Data that has been improperly trained may be erroneous or misconstrued.
3. *Sample interference*: Samples used to diagnose diseases may have complicated matrices, making it difficult for chromatographic separation to always successfully isolate the desired chemicals. False positives or false negatives could be the consequence of interference from other compounds in the sample.
4. *Limitations on sensitivity*: Chromatography is generally a sensitive technology, although some disease markers may be present in biological materials at very low quantities, making their detection difficult.
5. *Insufficient details*: Information regarding the presence and concentration of particular chemicals is provided via chromatography. It might not, however, provide information about the underlying illness mechanisms or more extensive physiological changes connected to the condition.
6. *Sample preparation:* A successful chromatographic analysis depends on a well prepared sample. Sample preparation can take a lot of time, and poor preparation can produce false results.
7. Reproducibility problems: Chromatographic procedures can occasionally experience reproducibility problems, which can cause discrepancies in the results obtained from various laboratories.
8. *Limited specificity:* Some chromatographic techniques may be unable to distinguish between chemically similar molecules due to a lack of specificity. If similar substances are found in the sample, this could make disease diagnosis difficult.
9. Despite these disadvantages, chromatography remains a valuable tool in disease diagnosis when used appropriately and in conjunction with other diagnostic methods. It is essential to consider the specific requirements of each disease and patient population to determine the most suitable diagnostic approach.

PROCEDURE FOR AI-INTEGRATED CHROMATOGRAPHY IN DISEASE DIAGNOSIS

1. Here is a detailed procedure for integrating AI into chromatography for disease diagnosis:
Sample Collection and Preparation Sample Collection: Obtain biological samples (e.g., blood, urine, tissue) from patients. Sample Preparation: Prepare samples for chromatography, including steps like filtration, centrifugation, or chemical treatment to isolate desired analytes.
2. Chromatographic Separation Selection of Chromatographic Method: Select an appropriate chromatography technique (e.g., liquid chromatography, gas chromatography, ion exchange chromatography) based on the type of analytes and the sample matrix.
Data Acquisition Detection: To identify and measure the separated components, use detectors (such as UV-Vis, MS, and FID).
Data gathering: Compile unprocessed chromatographic data, such as peak heights, peak regions, and retention times.
3.Preprocessing Data
Noise reduction: To reduce background noise and enhance signal quality, apply filters.
Baseline Correction: To improve peak identification and account for drift, chromatograms' baselines should be adjusted. Normalization: Data should be standardized to take into consideration differences in injection volume and sample concentration.
4.Extraction of Features
Peak detection involves finding the peaks in the chromatograms and analyzing their properties, such as height, area, and retention time.
Feature Selection: Gather pertinent features (such as particular peak patterns or intensity ratios) that may be signs of illness biomarkers.
6. AI Model Development
Dataset Compilation: Compile a dataset of chromatographic features from both diseased and healthy samples.

Training Data Preparation: Split the dataset into training and validation sets.
Model Selection: Choose suitable AI algorithms (e.g., machine learning classifiers, deep learning networks) for pattern recognition and disease classification.
Training: Train the AI model using the training dataset, optimizing parameters to improve accuracy.

7. Model Validation and Testing

Validation: Validate the trained model using the validation dataset to assess performance metrics such as sensitivity, specificity, accuracy, and precision.
Testing: Test the model with an independent dataset to ensure robustness and generalizability.

8. Integration into Diagnostic Workflow

Software Integration: Develop or integrate software that combines chromatographic data acquisition with AI analysis.
Automation: Implement automated data processing and analysis pipelines to streamline workflow.
User Interface: Create a user-friendly interface for clinicians and laboratory personnel to input samples and obtain diagnostic results.

9. Clinical Application

Clinical Trials: Conduct clinical trials to validate the diagnostic tool in real-world settings.
Regulatory Approval: Obtain necessary regulatory approvals (e.g., FDA, CE marking) for clinical use.
Deployment: Deploy the integrated AI-chromatography diagnostic tool in healthcare facilities.

10. Continuous Improvement

Monitoring: Continuously monitor the performance of the AI model in clinical practice.
Feedback Loop: Collect feedback from users and update the model periodically to incorporate new data and improve accuracy.
Research and Development: Invest in ongoing R&D to enhance chromatographic techniques and AI algorithms.

AI INTEGRATED CHROMATOGRAPHY APPLICATIONS IN DISEASE DIAGNOSIS:

By improving the precision, speed, and efficiency of the analytical process, AI-integrated chromatography has the potential to transform illness diagnosis. When paired with AI, chromatography has a number of uses for illness diagnosis:

- AI can aid in the discovery of novel biomarkers connected to certain diseases by examining intricate chromatographic data. These biomarkers can be used to diagnose diseases early, predict outcomes, and track therapeutic outcomes.
- AI can examine chromatographic patterns in patient samples to identify small alterations suggestive of early-stage illnesses. This could be especially helpful for illnesses with hazy or nonexistent early signs.
- Precision [15] medicine: Clinicians can customize treatment regimens based on a patient's particular molecular profile by combining patient-specific data with AI-driven chromatographic analysis. This may result in therapies that are more individualized and effective.
- Drug development: To guarantee the caliber, purity, and consistency of pharmaceutical goods, AI can be used to refine chromatographic techniques during drug [16] development. This can speed up the process of developing new drugs and increase the security of pharmaceuticals.
- AI-integrated chromatography can help in the diagnosis of infectious diseases by identifying particular pathogens or infectious agents that are present in clinical samples. This is especially beneficial for the quick and precise detection of infections.
- Chromatography can be used in the diagnosis of cancer to examine the metabolomic and proteome profiles of cancer patients. These profiles contain complex patterns that AI can analyze to help with cancer diagnosis, subtype classification, and treatment choice.
- AI can examine the chromatographic information in blood or cerebrospinal fluid to find indicators linked to neurodegenerative diseases like Alzheimer's, Parkinson's, and multiple sclerosis.
- Monitoring treatment efficacy: AI can assist in monitoring the efficacy of treatments and interventions by observing changes in chromatographic profiles over time. This can help inform any necessary modifications to treatment regimens.
- Forensic medicine: AI-driven chromatography can help detect poisons, narcotics, and other substances in forensic samples, aiding in precise cause-of-death findings and criminal investigations.
- Environmental health monitoring: Chromatographic data from environmental samples can be analyzed by AI to identify pollutants, poisons, and other contaminants, which helps to determine the hazards to the environment's health.
- Autoimmune disorders: Chromatography and artificial intelligence (AI) can be used to detect autoantibodies and other immune-related indicators linked to autoimmune disorders, enabling early diagnosis and tracking the course of the condition.
- Metabolic illnesses: AI-integrated chromatography is able to examine the metabolic profiles of patient samples, assisting in the identification of inherited metabolic illnesses that affect certain biochemical pathways.

Overall, by delivering more precise, quick, and in-depth insights into the molecular and chemical components of biological materials, the integration of AI with chromatography has the potential to greatly improve illness detection. Nevertheless, it's crucial to keep in mind that even though these apps show potential, more study, validation, and clinical studies are required to guarantee their efficacy and dependability in actual healthcare settings.

ADDITIONAL TECHNOLOGIES TO CHROMATOGRAPHY:

As chromatography has some drawbacks, it can be combined with other methods or technologies to improve it and produce more thorough data on a range of illnesses. The following are some methods used in conjunction with chromatography to diagnose diseases:

Mass spectrometry and chromatography are frequently combined to precisely and sensitively identify and measure compounds. In metabolomics and proteomics research for disease biomarker discovery and diagnostics, gas chromatography-mass spectrometry (GC-MS) [8] and liquid chromatography-mass spectrometry (LC-MS) are frequently utilized. To clarify the structures of complex compounds, such as proteins and metabolites, Nuclear Magnetic Resonance [20] (NMR) spectroscopy is used in conjunction with chromatography. NMR has a lot to say about the dynamics and interactions of molecules in relation to disease pathways. To identify and measure certain antibodies or antigens in biological materials, chromatography methods such as high-performance liquid chromatography (HPLC) [18] or liquid chromatography-mass spectrometry (LC-MS) [6] can be combined with immunoassays. This combination is used to diagnose a variety of immunological conditions, including autoimmune disorders and infectious diseases. Electrochemical detection can be combined with chromatography to add to the information available about redox-active compounds. In the study of neurotransmitters, medications, and biomarkers connected to oxidative stress in neurodegenerative illnesses, this combination is very helpful. To separate and study charged molecules, such as proteins, nucleic acids, and ions, capillary electrophoresis can be used in conjunction with chromatography. In proteomics and genomics research, mass spectrometry coupled with CE (CE-MS) is used to find disease biomarkers. Infrared spectroscopy can be used to examine the chemical bonds and functional groups that are present in a sample. It is occasionally combined with chromatography to provide supplementary structural data regarding substances that are associated to diseases. Though X-ray crystallography and chromatography are not directly integrated, they can be used together to map the three-dimensional structures of proteins and other biomolecules connected to disease pathways.

These linkages give academics and medical professionals strong tools for locating illness biomarkers, researching disease causes, and creating more precise diagnostic procedures for a range of medical disorders. A deeper knowledge of diseases and the underlying biochemical processes that underlie them is possible thanks to the integration of chromatography and other techniques, which enables a multidimensional examination of complicated biological data.

FUTURE OUT LOOK:

Enhanced Data Analytics: As AI technology develops, more complicated and large-scale datasets will probably be analyzed by more advanced models, which will increase the precision of disease diagnosis.

Real-time Diagnostics: By enabling rapid diagnostic results through prompt interventions, the development of real-time AI-powered chromatographic systems could greatly improve patient outcomes.

Personalized Medicine: AI can help tailor chromatographic analyses to the unique patient profiles, resulting in more individualized and efficient treatment regimens. This is known as personalized medicine.

Integration with Other Technologies: AI-integrated chromatography in conjunction with other diagnostic technologies, such proteomics and genomics, may offer a more thorough knowledge of diseases and improve diagnostic performance.

Remote Diagnostics: AI-enabled portable chromatographic instruments may be utilized in resource-constrained or remote environments, increasing access to sophisticated diagnostic tools on a worldwide scale.

Automated Workflows: More automation in sample preparation, data processing, and reporting could be achieved by future technology, which would lower the risk of human mistake and boost throughput in clinical laboratories

Regulatory clearances and Standards: To guarantee the broad acceptance and dependability of AI-integrated chromatographic systems in clinical practice, standardizing protocols and securing regulatory clearances will be essential as these systems become more common.

Educational Programs: To equip the upcoming generation of academics and healthcare professionals, training programs emphasizing the application of AI in chromatography will be crucial.

To be concise, AI-integrated chromatography has a bright future in disease diagnosis, with the potential to greatly improve accessibility, speed, and accuracy of diagnosis. To reach its full potential in education and technology, sustained research and development as well as thoughtful investments will be essential in medical field.

CONCLUSION:

In conclusion, AI-integrated chromatography is a significant development in the diagnostics of health field. Many of the conventional drawbacks of chromatography, including long analysis times, difficult data interpretation, and the requirement for highly qualified operators, are addressed by this hybrid approach, which combines the sensitivity and precision of chromatographic techniques with the analytical power of artificial intelligence. Artificial intelligence algorithms have the potential to optimize peak detection, boost resolution, and expedite the precise and timely analysis of intricate chromatographic data. In addition to streamlining the diagnostic procedure, this integration boosts its dependability, which may result in earlier and more precise illness diagnosis. But there are issues that need to be resolved, like data protection, the requirement for large training datasets, and integrating AI systems into current laboratory operations.

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