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Review Article

HPLC & It's Utilization in Disease Diagnosis

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A B S T R A C T

High Performance Liquid Chromatography is a technique in analytical chemistry used to separate, identify and quantify each component in a mixture. The HPLC is suitable for a variety of clinical applications, including pharmaceutical development, legal application such as detecting the Presence of illicit drugs in urine & blood. High-performance liquid chromatography is a technique introduced for the accurate diagnosis of hemoglobinopathies, thalassemias & estimation of glycosylated hemoglobin. The advantage of the HPLC system is the excellent resolution, reproducibility & quantification of several normal & abnormal hemoglobin resulting in accurate diagnosis of thalassemia syndromes. HPLC technique which is used to separate the component in across the two equivalents phases i.e. stationary phases is solid or liuqid supported on a solid which is packed into a column while sample moves in along with mobile phase is liuqid or gas. The purpose of this study is to evaluate the HPLC technique in diagnosis of thalassemia syndromes and also correlate it with clinicohematological profile in these cases.

Key words : Hemoglobinopathies, Thalassemias, Glycosylated, Resolution, Clinicohematological.

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INTRODUCTION

High Performance Liquid Chromatography is a technique in analytical chemistry used to separate, identify and quantify each component in a mixture. The analytical technique of High Performance Liquid Chromatography is used extensively throughout the pharmaceutical industry. Liquid chromatography was initially discovered as an analytical technique in the early twentieth century and was first used as a method of separating colored compounds. It is used to provide information on the composition of drug related samples. HPLC is used at all the different stages in the creation of new drug. The aim of the analysis will depend on both nature of sample & stage of development. HPLC, scientists used standard liquid chromatographic techniques. HPLC is a chromatographic technique that basically a highly improved form of column chromatography. [1,2]

High-performance liquid chromatography (HPLC) is a technique introduced for the accurate diagnosis of hemoglobinopathies and thalassemias. The advantage of the HPLC system is the excellent resolution, reproducibility & quantification of several normal and abnormal hemoglobin resulting in accurate diagnosis of thalassemia syndromes.[15,17] The purpose of this study is to evaluate the HPLC technique in diagnosis of thalassemia syndromes and also correlate it with clinicohematological profile in these cases. The use of cation-exchange high-performance liquid chromatography (CE-HPLC) to separate and quantify various normal and abnormal hemoglobin (Hb) fractions has been increasing 1,2,3,4. This method has also been proposed for screening Hbs of clinical significance. The use of the Bio-Rad Variant analyzer with the β Thalassemia & the Bio-Rad Variant Hemoglobin Testing System, a totally automated CE-HPLC instrument, has been used in our laboratory for routine quantification of HbA_2.[5]

WORKING OF HPLC

A reservoir holds the solvent called the mobile phase, because it moves. A high-pressure pump solvent delivery system or solvent manager is used to generate and meter a specified flow rate of mobile phase, typically milliliters per minute. An injector autosampler is able to introduce the sample into the continuously flowing mobile phase

stream that carries the sample into the HPLC column. The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to see the separated compound bands as they elute from the HPLC column most compounds have no color, so we cannot see them with our eyes. The mobile phase exits the detector and can be sent to waste.

The detector is wired to the computer data station, the HPLC system component that records the electrical signal needed to generate the chromatogram on its display and to identify and quantitate the concentration of the sample constituents. [6, 10]

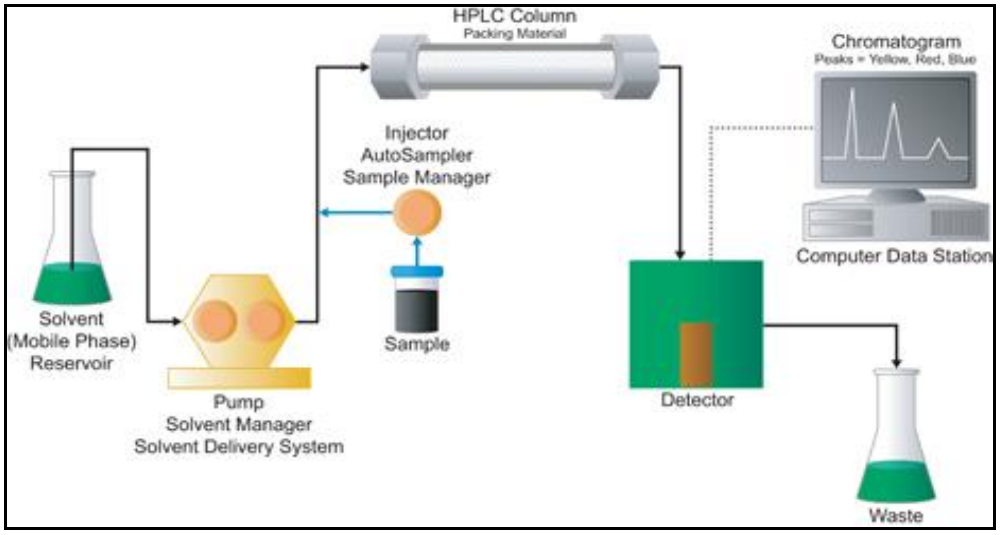

Figure: 1 Working of HPLC

HPLC OPERATION

Mobile phase enters the column from the left, passes through the particle bed, and exits at the right. It represents the column at time zero the moment of injection], when the sample enters the column and begins to form a band. The sample shown here, a mixture of yellow, red, and blue dyes, appears at the inlet of the column as a single black band. After a few minutes lower image, during which mobile phase flows continuously and steadily past the packing material particles, we can see that the individual dyes have moved in separate bands at different speeds. This is because there is a competition between the mobile phase and the stationary phase for attracting each of the dyes or analytes. Notice that the yellow dye band moves the fastest and is about to exit the column. The yellow dye likes [is attracted to] the mobile phase more than the other dyes. Therefore, it moves at a faster speed, closer to that of the mobile phase. The blue dye band likes the packing material more than the mobile phase. Its stronger attraction to the particles causes it to move significantly slower. The red dye band has an intermediate attraction for the mobile phase & separate it chromatographically.[5, 9, 10]

HPLC ANALYSIS

The Bio-Rad Variant, a fully automated HPLC system, uses double-wavelength detection (415 and 690 nm). Several elution methods, including specific columns, buffers and softwares, are available from the manufacturer. The β Thalassemia Short program, the most widely used Variant program, has been designed to separate and determine in 5–6 min the area percentages for HbA & to provide qualitative determinations of abnormal Hbs. The β-Thalassemia, a 3 × 0.46 cm nonporous cation-exchange column is eluted at a flow rate of 2 mL/min by a gradient of two phosphate buffers that differ in pH and ionic strength. The various Hb components give slight differences in elution time from one column to another. The elution time for a Hb component also varies slightly according to its concentration in the sample. Bio-Rad Variant HPLC system highly specific and reproducible HPLC method to simplify the process of acquiring fast and accurate Hb result. [8,9,10,11,15,17]

HPLC UTILIZATIONS IN DISEASE DIAGNOSIS:-

High-performance liquid chromatography (HPLC) is a technique introduced for the accurate diagnosis of disease. Many disorders related to body metabolism, those related to endocrine and exocrine gland secretion, alteration in body fluids are diagnosed by HPLC analysis of concerned fluids. For Example :-

- Estimation of metabolites of purines, pyrimidines or other metabolites from plasma, cerebrospinal fluid and urine samples in patients.
- Estimation of corticoids from plasma in disorders of the adrenal gland which secretes an endocrine hormone.

HPLC technique introduced for the accurate diagnosis of hemoglobinopathies and thalassemias. The advantage of the HPLC system is the excellent resolution, reproducibility & quantification of several normal & abnormal hemoglobin resulting in accurate diagnosis of thalassemia syndromes. The purpose of this study is to evaluate the HPLC technique in diagnosis of thalassemia syndromes and also correlate it with clinicohematological profile in these cases. The identity of hemoglobin variants is generally inferred from electrophoretic mobility, its quantity, and the patient's ethnic background. Family studies can be of considerable importance in elucidating the nature of disorders of hemoglobin synthesis, but the definite identification can be achieved only by DNA

analysis or amino acid sequencing. Hemoglobin fraction analysis by cation exchange HPLC has the advantage of quantifying Hb value along with hemoglobin variant screening in a single, highly reproducible system, making it an excellent technology to screen for hemoglobin variant and hemoglobinopathies along with thalassemias. The simplicity of the automated system with internal sample preparation, superior resolution, rapid assay time, and accurate quantification of hemoglobin fraction makes this an ideal methodology for the routine clinical laboratory. The thalassemias and hemoglobinopathies diagnosed by Bio- Rad variant II HPLC system The aim of the present study was to evaluate the role of cation exchange HPLC (CE-HPLC) in the diagnosis of thalassaemia syndromes/hemoglobinopathies and to correlate Hb profile in such cases with clinico-hematological features.[12,13,15,16,17]

CONCLUSION

HPLC is an excellent powerful diagnostic tool for direct identification of hemoglobinopathies and thalassemias however use of other complimentary techniques may help in arriving at the final conclusions in certain situations.

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