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Review article

A REVIEW ON HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT
This article reviews about the principles and application of high performance liquid chromatography and briefly discuss about the thin layer chromatography.

Keywords: high performance liquid chromatography, thin layer chromatography.

INTRODUCTION

HPLC can separate macromolecules and ionic species, labile natural products, polymeric substances and a wide variety of high molecular weight substances. This technique is not limited due to sample volatility or thermal stability. Thin layer chromatography was first discovered by Izmailov and Shraiber in 1938. Further Stahl (1958) perfected the method and developed equipment and standardized adsorbents for the preparation of uniform layers on glass plates. And this technique, chromatography using thin layers of an adsorbent held on a glass plate or other supporting medium is called as thin layer chromatography [1].

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PRINCIPLE OF HPLC

When GLC is extremely useful for many analyses, effecting separation and quantization of components in mixtures at very low levels, it fails in the case of compounds which have low vapor pressure or are unstable at elevated temperatures. Such compounds have to be separated by liquid chromatography using solid absorbents of particle size, 5-10 um and high pressure of 300-3000psi for steady flow of solvents.This technique is called high pressure liquid chromatography or high performance liquid chromatography (HPLC) which is very convenient for non-volatile solutes.

WORKING METHODOLOGY

The solvent is taken up to the filter by pump and is fed to rotary injection valve, fitted with sample loop. The sample is added from micro syringe and then it travels the column and the detector. The output is displayed on the recorder. The solvent pressure is indicated on the gauge. The pump supplies the solvent at a constant pressure, 4500 psi and lesser flow rates of few mLmin-1.The Sample sizes is of order of 1-200L of solunum [2].

The column is made of this walled stainless steel tubing, 10 to 20 cm 1018 with particles (5-10u) which may be a)Polar solids (Al2O3 Silica gel) for use with low polarity solvents , b)Bonded Phase packing , c)Ion exchange resins, useful for the separation of amino acids or inorganic anions [3].

In G.L.C, it is the stationary phase and the temperature is key factors affecting the separation of mixtures. But in HPLC, it is the choice of solvent or solvent mixture which determines the efficiency of separations [4].

DETECTOR

The detectors used in HPLC measure the property of the solution or solutes. The solution property may be either refractive index or conductance where the solute properties may be measured by spectrophotometer or spectrofluorimeter.

APPLICATION OF HPLC

A CHROMATOGRAM OF WATER POLLUTANTS

A pre concentration step where the sample is subjected to solvent extraction to reduce the volume is often required before the analysis with HPLC. This is particularly necessary for the analysis of Poly aromatic hydrocarbons in water or pesticide residue in food. Alternatively, water samples may be absorbed on a solid absorbent (Cs or Cis bonded silica gel) followed by absorption in a solvent which could be directly fed into an HPLC column [5].

PREPARATION OF THIN LAYERS ON PLATES

Coating of glass plates with adsorbent layer, is made by spreading, pouring, spraying or dipping. The adsorbent layer may be solid or loose. For solid layers, a uniform layer of adsorbent material could be applied to a lean glass plate with an applicator. The applicator used for the preparation of 0.25 mm layer thickness film is the Stahl's original applicator. Sometimes a binder such as calcium sulphate is added to the adsorbent in small quantities before coating. Loose layers may be prepared by dipping the plates in the suspension, spraying a thin suspension or pouring of suspension on to the plate.

APPLICATION OF SAMPLE ON THE CHROMO PLATES

The application of sample on the chromo plates are similar to those used in paper chromatography. In T.L.C. 0.1-1 % solution of the sample are applied to the plates with the help of capillaries, micropipettes and micro syringes. The sample solution could be applied as single sports in a row along one side of the plate, about 2cms from the edge [6].

CHOICE OF SOLVENTS

The choice of solvent depends on the nature of the material on which the separation is preferred, because it results in better separations and a combination of two solvents gives better results. Hence the solvent mixture which is highly preferred for its efficiency is n-hexane-diethyl ether acetic acid in the ratio 90:10:1.

DEVELOPING CHAMBER

The thin layer chromatoplates are usually developed by placing them on edges in the jar containing a 0.5 - 10 cm laver of the solvent. The jar is then covered with an air tight lid. After the developing solvent ascends for about 10-12 cm above the origin, the plate is taken out of the jar.

DETECTING REAGENTS

Detecting reagents used in paper chromatography may be used in T.L.C. also. Iodine dissolved in an organic solvent could be sprayed to deduct many components. Sulphuric acid also forms complex which are colored and visible in day and all light.

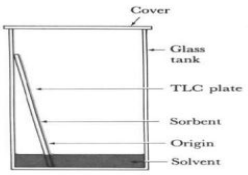
DEVELOPMENT AND DETECTION

The various development techniques available include ascending development, horizontal development, multiple development, stepwise development, gradient development, continuous development and two dimensional development. Normally ascending development is commonly used. During ascending development the sample is spotted at one end of the plate and then developed by ascending technique. The plates are usually placed vertically in a container saturated with developer vapour and solvent.

GENERAL PROCEDURE OF TLC

A plate made of glass is coated with a loose powder or with slurry of an adsorbent. This slurry adheres to the surface of the glass plate as a thin layer. The unknown substance and reference materials are dissolved in water or an organic solvent and the solution is applied in a row of spot 1-2 cm from the edge of the plate, with micropipette or micro syringe. The chromatoplate is placed in a jar containing the solvent for development and the jar is lined inside with filter paper which acts as a wick and saturates the atmosphere of the jar with the vapors of the solvent. The jar is covered with an air tight lid. As the solvent ascends by capillary action, the sample gets resolved into fractions. When the solvent front has migrated about 75% of the plate, the plate is then dried and sprayed with to iodine vapor. The position of the fractions would be indicated as brown spots [7].

Thin Layer Chromatography (TLC)



TYPES OF TLC

Adsorption TLC: Scientist Kucharezyk (1963) has used adsorption TLC for the fractionation and analysis of petroleum products. Further it has been used for analysis of waxes and fats, for analysis of essential oils, analysis of carotenoids, steroids, fat soluble vitamins and certain alkaloids by different scientists. Ion exchange TLC: This type of TLC has been used for the separation of ionic compounds from non-ionic compounds. It has been used for the fractionation of short chain carboxylic acids, sugars, amino acids, nucleotides and detergents. The other types of TLC include the Partition T.L.C. and Reverse phase partition T.L.C [8].

ADVANTAGES OF TLC

The technique helps in the separation of even microgram of the substances. The separation is very sharp. It is used to study various biological changes, to study fractionation of large number compounds, to analyze urine and blood samples [9].

CONCLUSION

Effective separation and quantization of components at low vapor pressure or are unstable at elevated temperatures can be separated by liquid chromatography. This technique is very convenient for non-volatile solutes where as thin layer chromatography helps in the separation of even microgram of the substances.

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