e-ISSN: 2320-1215
p-ISSN: 2322-0112

Research and Reviews: Journal of Pharmacy and Pharmaceutical Sciences

High Performance Liquid Chromatography in analysis

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Commentary

Received: 15/02/2014
Revised: 10/03/2014
Accepted: 16/03/2014

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Keywords: Chromatography, HPLC, Analysis, Column

INTRODUCTION

High Performance Liquid Chromatography, also known as high pressure liquid chromatography is the most commonly used analytical methods in the pharmaceutical industry. High Performance Liquid Chromatography (HPLC) is mainly used for the separation, identification and qualitative & quantitative analysis of the components in a sample mixture. Usually it is used in the case of semi-volatile or non-volatile organic compounds [1,2].

It is a type of column chromatography but is highly sensitive and extremely automated compared to the column chromatography. Unlike the solvent allowed under gravity through a column in column chromatography, the HPLC technique utilizes high pressure up to 400 atm [3-5]. It allows faster and better separation than the column chromatography due to the pressure and the greater surface area of the packing material in the column which is due to the smaller particle size accommodated. This greater surface area facilitates better interactions between the packing material which is the stationary phase and the molecules to be separated which flow through the stationary phase [6-9].

Operation of the HPLC technique is a little confusing. It involves a sampler, pump and a detector. HPLC uses the concept of affinity between the mobile phase which is the solvent and stationary phase [10,11]. A pump is used to inject a continuous flow of solvent. Into this solvent a dissolved sample is injected. Once the sample is in the solvent, it passes through the column [12-14]. The components in the sample are separated based on their affinity to the molecules in the column. After the compounds in the sample are separated, they pass the detectors. User software is utilized in the HPLC technique for the data analysis [15-18].

The HPLC technique can be used in two forms [19];

Normal phase HPLC

This is the basic column chromatography. It is less frequently used form of HPLC
The column is filled with silica molecules, and the solvent used is non-polar.
Polar components in the mixture being passed through the column will adhere longer to the polar silica particles than non-polar compounds. The non-polar compounds will therefore pass more quickly through the column [20,21].

Reversed phase HPLC

Reversed phase HPLC is the most frequently used form of HPLC.

In this case, the silica is made non-polar by attaching long hydrocarbon chains to its surface. A polar mobile phase is used [22].

Therefore, there will be a strong adherence between the polar solvent and polar molecules in the mixture being passed than between the hydrocarbon chains attached to the silica and the polar molecules in the solution [23].

Non-polar compounds will form attractions with the hydrocarbon groups because of van der Waals dispersion forces. They are less soluble in the solvent. Therefore this will slow them down on their way through the column. Now, the polar molecules will pass through the column more quickly [24,25].

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