Flash chromatography and Preparative HPLC (Review)

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Abstract

HPLC (High performance liquid chromatography) is an analytical instrument to analysis of various compounds like synthetic drugs, natural products, Bio molecules. Another important application of HPLC is isolation of mixture of compounds. In most of the cases industries are using Preparative HPLC than Preparative column Chromatography due to more advantages. But the cost of Preparative HPLC column is too expensive. With the help of Flash chromatography we can obtain same advantages at low maintenance. This review explicates the applications, differences, advantages of Flash chromatography and Preparative HPLC.

Keywords: Flash chromatography, Preparative HPLC

Introduction

The term preparative HPLC is usually associated with large columns and high flow rates. However, it is not the size of the instrumentation or the volume of mobile phase pumped through the system that determines a preparative HPLC experiment, but rather the objective of the separation. The objective of an analytical HPLC run is the qualitative and quantitative determination and estimation of a compound. For a preparative HPLC run it is the isolation and purification of a valuable product. Since preparative HPLC is a rather expensive technique, compared to traditional purification methods such as distillation, crystallization or extraction, it had been used only for rare or expensive products. With increasing demand for production of highly pure compounds in varying amounts for activity, toxicology and pharmaceutical screenings the field of operation for preparative HPLC is changing. Flash Chromatography is a rapid form of preparative column chromatography based on optimized pre-packed columns through which are pumped solvent at a high flow rate. It is also called as medium pressure chromatography. It is a simple and economical approach to Preparative LC Orochem's polypropylene. They are safer than glass columns, with no risk of breakage. Cartridges are packed with symmetrical sorbent compression to ensure improved separation and reproducibility.

Preparative HPLC:

Important chromatographic parameters to achieve reliable and accurate results are resolution, peak width and peak symmetry. If more and more sample amount is applied to the column, the peak height and peak

area increases but the peak symmetry and the capacity factor remain unchanged. In analytical HPLC the optimal peak shape resembles a Gaussian curve. . If more than a certain amount of sample is injected onto the column the adsorption isotherm becomes non-linear. This means the peak becomes unsymmetrical, shows strong tailing and the capacity factor decreases. In preparative HPLC this effect is called concentration overloading. In some cases, depending on the compound, the capacity factor increases with increasing overloading, which leads to a strongly fronting peak. Since the adsorption isotherm is dependent on the compounds the chromatographic system column loadability has to be determined for each preparative HPLC experiment.

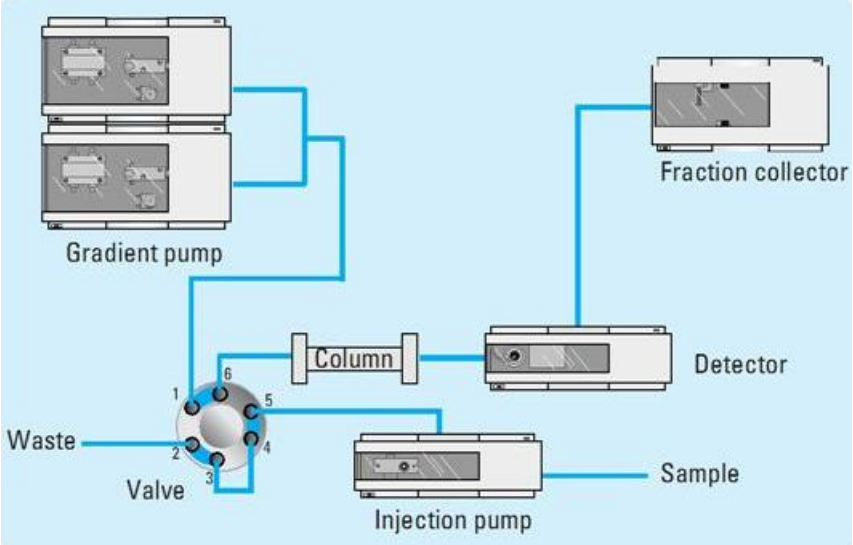

Figure.1 Preparative HPLC Configuration

For purification of large sample amounts two methods are possible: Scale-up of the analytical system or column overloading. Scale-up of the analytical system would mean. using larger column diameter, higher flow rate and increasing the sample volume with the column length and sample concentration remaining constant. The peaks would then remain sharp and symmetrical. The disadvantage of this method is that large columns and high solvent volumes are required to separate rather small amounts of compound, hence, the method would not be economical .Therefore, column overloading, that is, increasing the applied sample amount under the same analytical conditions, is usually the method of choice. Using column overloading allows to separate samples in the milligram range even on analytical columns.

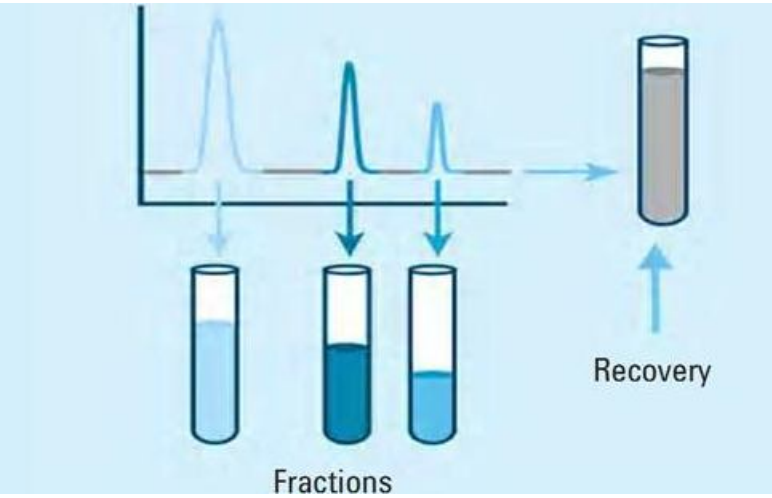

Figure.2 Recovery collection

For larger amounts of sample an additional scale-up of the system is necessary. Column overloading can be done in two ways – concentration or volume overloading. In concentration overloading the concentration of the sample is increased but the sample volume injected remains the same. The capacity factor, k' ,decreases and the peak shape changes from a Gaussian curve to a triangle. Concentration overloading is only possible when the sample compound has good solubility in the mobile phase. If the compound has poor solubility, concentration overloading cannot be used and more sample volume must be injected. This technique is called volume overloading. Beyond a certain injection volume the peak height does not increase and the peaks become broader and rectangular. In preparative HPLC concentration overloading is favored over volume overloading because the sample amount, which can be separated, is higher. Since the solubility of compounds is usually a limiting factor both overloading techniques are used in combination.

Figure.3 Preparative HPLC

Flash chromatography (medium pressure chromatography):

Flash chromatography is a fast and inexpensive separation technique for the purification of organic syntheses products e.g. in drug discovery or from natural extracts. It is a popular alternative when other separation techniques cannot be used or are too difficult.

Figure.4 Flash Chromatography

In flash chromatography Columns are disposable plastic cartridges, advantage of cartridges are time save and reproducibility. Based on sample volume we may select different size of cartridges. Now a days readily prepared cartridges are available based on particle size and stationary phase volume.

Figure.5 Different of Sizes of Cartridges

Sampling loading is by solid sample module and injection valve. Pressure of pump is nearly 100 psi. Due to this much pressure apply we can separate compounds rapidly. With narrow particle size distribution we may reduce back pressure and we may get higher efficiency separated compound. The columns links with detectors. These detectors advanced with fraction collectors. By using suitable software we may collect target isolated compound based on purity. Flash chromatography is not expected to provide the resolution or reproducibility of HPLC, it is a technique that can quickly improve the purity of samples to an acceptable level. Compound must have TLC Rf of 0.15 to 0.20 in the solvent system. DCM (Di Chloro Methane)/ Ether/Ethyl acetate, Hexane are common solvents for Flash Chromatography. We may use high polar solvents to increase elution of compounds rapidly.

Table.1 Applications and differences of FLASH and Preparative chromatography

Application	Preparative Chromatography	Flash Chromatography
1	Maximum Quantities of the sample can be separated (0.1-2.0g)	Maximum Quantities of the sample can be separated (0.5-2.0 g)
2	Separation time is Based on number of compounds present. (10 min to 1 Hour)	Separation time is 10-15 min
3	Elaborate equipment and the purchase of expensive equipment is necessary	Elaborate equipment and the purchase of expensive equipment is not necessary
4	Column is highly Expensive	Cartridges are reuse full, Nearly 8 times cheaper than Preparative columns.
5	Sample should soluble in mobile phase	No need to solubility of sample in Mobile phase
6	More useful in Agro chemistry, Synthetic chemistry, Natural products separation.	More useful in separation of various antibiotics, Impurities, Peptides.
7	This technique saves time.	This technique saves time and solvents
8	Reliable	Reliable and cost effective

Conclusion

Preparative HPLC and Flash chromatography both are efficient and advance techniques for separation of various chemical compounds. Flash chromatography is cost effective and low maintenance. In the case of the target molecule or compound is in high concentration, flash Chromatography is preferable. Then we may isolate the compound with high purity. In the case of sample have more chemical constituents, without information of concentrations of that chemical constituents, preparative chromatography is preferable.

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