



Preparative chromatography is a powerful technique for the isolation and purification of a variety of chemicals including pharmaceutical compounds, natural products and biological molecules. Scaling from an analytical HPLC column to a preparative separation can be a challenge. It is the goal of this presentation to provide practical guidelines for developing preparative HPLC separations.

IT ALL STARTS WITH THE ANALYTICAL COLUMN!!! The use of analytical column is one of the key steps in developing any preparative HPLC separation. In order to develop and optimize a preparative HPLC separation a variety of analytical columns should be evaluated. The analytical column is essential in scaling up to a preparative separation. It is the main way of evaluating the chromatographic separation and developing a plan for the preparative HPLC separation.

In the first part of this presentation several important factors were discussed including column length, column diameter and column flow. In this second part of the presentation we will discuss particle size, stationary selection and mobile phase selection.

Particle Size

Particle size is one the key parameters for HPLC column selection. Smaller particles produce greater column efficiency, but much higher operating pressures. Generally, sub-2 micron and 3 um are not used for preparative chromatography. The pressure drop is inversely proportional to the particle diameter squared. The larger the particle the smaller the pressure drop, this allows the larger particles to be used at higher flow rates. The higher flow rates increase the throughput for preparative columns. For many preparative HPLC separations particles sizes of 7 um and 10 um perform well, however for separations of closely eluting compounds (not well separated) columns packed with 5 um particles may be required. In many cases the higher operating pressures of the 5 um may be prohibitive. Once again it all starts with the analytical column so if a separation is performed successfully on a 5 um column it would be beneficial to try the separation on a 7 um or 10 um column.

Stationary Phase

Stationary phase selection for preparative HPLC falls into several main categories:

- Normal phase chromatography
- Reversed phase chromatography
- Size exclusion
- Ion exchange chromatography
- Chiral chromatography

In this presentation we will briefly discuss normal phase chromatography, reversed phase chromatography and chiral chromatography.

Normal Phase Chromatography

For normal phase chromatography the mobile phase generally doesn't contain any water. One of the key advantages of normal phase chromatography is the lack of water in the mobile phase. Many of the solvents used in normal phase chromatography are volatile organic solvents such as hexane, ethyl acetate, methanol and ethanol to name a few. These types of solvents can be easily removed in order to recover separated compounds. Water is more difficult to remove than these organic solvents.

Preparative normal phase is well suited to small polar organic molecules and is a powerful separation/purification technique. ES Industries produces a variety of columns that are specifically designed for normal phase chromatography.

The following is a list of normal phase columns that we produce that well suited for preparative normal phase chromatography:

- Epic Silica
- Epic Amine-HD
- Chromegabond Nitro
- Chromegabond Diol

Reversed Phase Chromatography

For reversed phase chromatography the mobile generally contains water. Preparative reversed phase is well suited to ionic organic compounds, peptides, and many non-polar molecules and is a powerful separation/purification technique. ES Industries produces a variety of columns that are specifically designed for reversed phase chromatography.

The following is a list of reversed phase columns that we produce that are well suited for preparative reversed phase chromatography:

- Epic C18
- Chromegabond WR-C18
- Epic Polar
- Epic PFP (Pentafluorophenyl)
- MacroSep C18
- Epic C4-SD
- Epic TMS
- Epic C8

Chiral Chromatography

Preparative chromatography is a powerful technique for the separation/purification of chiral compounds. At ES Industries we produce several chiral stationary phases including:

RegisCell	3,5-dimethylphenylcarbamate cellulose
RegisPack	3,5-dimethylphenylcarbamate amylose
RegisPack CLA-1	5-chloro-2-methylphenylcarbamate amylose
ChromegaChiral CC2	3-chloro-4-methylphenylcarbamate cellulose
ChromegaChiral CC4	4-chloro-3-methylphenylcarbamate cellulose
ChromegaChiral CCJ	4-methylbenzoate cellulose

Mobile Phase Selection

The use of an analytical column is one of the key steps in developing any preparative HPLC separation. This statement is particularly important when selecting a mobile phase for preparative HPLC separations. Some of the factors for preparative HPLC mobile phase selection include:

- Volatility for easy removal from isolated fractions
- Viscosity for low column back pressure
- Purity for low levels of non-volatile contaminants
- Good solubility properties for maximum sample loads

The solvents used for normal phase chromatography meet most of these requirements, however many samples require reversed phase chromatography for separation. A major concern of reversed phase chromatography is column back pressure, particularly for mobile phases containing methanol. Mobile phase containing water and methanol can generate high back pressures. In addition, many reversed phase separations require the use buffers. In these cases volatile buffers salts should be employed.

The following is a list of volatile buffers that are useful for reversed phase chromatography:

<u>Volatile buffer</u>	<u>pH Range</u>
Trifluoroacetate	xx – 1.5
Ammonium formate	3.0-5.0
Ammonium acetate	3.8-5.8
Ammonium carbonate	5.5-7.5/9.3-11.3

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