



ES Industries strives to produce HPLC columns that provide the chromatographer with the longest possible column lifetime. However, columns do inevitably fail at some point. There are many causes for the failure of HPLC columns. It's the goal of this series to look at the possible causes of column failures and to offer solutions.

Mobile Phase

The mobile phases used for HPLC analysis may lead to column failures through a number of ways.

1. Generally, HPLC grade solvents are filtered thru 0.2um porosity filters by solvent suppliers. This level of filtration is more than adequate to protect the HPLC column from plugging or clogging. In addition most HPLC water purification systems used in laboratories contain 0.2 um filter as a final polishing step. However, many solvents (including minor modifiers additives) non-HPLC grade and these chemical must be filter thru 0.2um filters. Solid chemicals including buffer salts maybe chemically pure and even HPLC grade, but unfortunately these chemicals may contain physical contaminants. These contaminants may come from various sources and can clog or plug an HPLC column after a period of use. In these cases it is very useful to filter prepared mobile phases, especially when they contain buffer salts or ion pairing reagents.
2. A major cause of column failure is related to buffer solubility in various mobile solvents such as acetonitrile. Phosphate buffers are particularly problematic when used with acetonitrile. When acetonitrile/water composition is about 50/50 v/v and the phosphate buffer is 50 mM. At this composition a precipitate will form, may be not immediately but slowly as a recrystallization process. These crystals will coat the pumping system and clog the column. Recrystallization of buffer salts can occur with pre-mixed mobile phases as well as gradient mixtures. In the case of pre-mix mobile phases the concentration of buffer salts can be critical and if there is a possibility of recrystallization then the solvent should be prepared a day in advance to observe the formation of any crystals. The chromatographer should be careful with the concentration of buffer used for gradient mixing. If there is a question of precipitation or recrystallization occurring with gradient mixing, then a pre-mix mobile phase mixture should be tested using the highest level of organic solvent used in the gradient profile to check for crystal formation. In addition, care must be exercised when cooling columns below sub-ambient because crystals may form at these reduced temperatures. Also when salt buffers are used it is critical that the salt buffer solvent be removed from column before storage. If a column is stored containing salt buffers there is a very high probability that the column will clog.

Sample

The injected sample may lead to column failures through several ways.

1. Particulate matter from injected samples may lead to column clogging. To prevent sample particulate clogging samples should be pre-filtered. However, for small volume sample the hold-up of the filter maybe too great in these cases centrifugation of the sample can be used.
2. Unfortunately, many samples can't be filtered. For these samples guard columns are most useful. A guard column is basically a small HPLC column packed with a material similar to the analytical column. At ES Industries we produce guard column cartridges that are easy to change and protect the analytical column. Additional, information about ES Industries guard columns can be found on our website: www.esind.com.

In next month's newsletter we will discuss other reasons for column failures and how to prevent them.

ES INDUSTRIES
701 South Route 73
West Berlin NJ 08091

Toll-Free: 1-800-356-6140
Telephone: 856-753-8400
Fax: 856-753-8484

email: esindustries@msn.com website: www.esind.com