Normal Phase HPLC Column Cleaning and Regeneration

While it is best to take pre-emptive steps to minimize potential contamination of the column, the following column cleaning recommendations can be used to extend column lifetimes, and might even be utilized to aid in troubleshooting diagnostics when the logic behind each step is considered.

General

1. At each step, begin with a low linear velocity (flow rate), monitoring the backpressure carefully. Increase only when the backpressure stabilizes at working levels.

2. Reverse flush with 10 column volumes of the modifier-free weak solvent, such as hexane or chloroform.

3. Flush with 20 column volumes of the strong solvent used in the mobile phase, such as methylene chloride or isopropanol.

4. 100% isopropanol is polar enough to have sufficient normal phase elution strength to elute anything that is retaining on the column in a normal phase mechanism. If this does not suffice to restore chromatography, the column may have a void, which will not be possible to restore. The chromatographic symptom associated with the failure and subsequent chromatography after regeneration steps should give clues as to whether this is the case, which often signals the end of the column's natural lifetime.

Water Removal

Some chromatographic symptoms, such as retention time shifts or other indications of selectivity changes can point to a change in the column stationary phase, possibly with the packing integrity still intact. In normal phase chromatography, the moisture content on the stationary phase will often be a critical parameter affecting selectivity. And considering most solvents have at least a small degree of water solubility (water = 0.0111% w/w solubility in hexane at 20 degC), the subsequent moisture content of the column is the most common factor in normal phase chromatography that leads to selectivity changes over time.

Flushing the column with 30 column volumes of 2.5% dimethoxypropane + 2.5% glacial Acetic Acid in hexane can be utilized to remove moisture from the column and subsequently restore the original/desired selectivity.

Considering the difficulty in completely eliminating moisture from reaching the mobile phases and column, a common and arguably simpler approach could be to utilize mobile phases with a controlled moisture content (such as half saturated with water) in order to maintain the initial selectivity of the method throughout without the need for lengthy water removal steps.

In either case, the most important parameter is to maintain consistency; and once a normal phase column is exposed to moisture, it is recommended to dedicate that column permanently for such to ensure reproducibility when transferred to a new column.

Key words: HPLC, column, cleaning, regeneration, normal phase, half-saturation, moisture

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