



Reversed-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 95% water 5% acetonitrile - It is generally acceptable to reverse flush most Phenomenex HPLC columns, but check the specific column manufacturer to confirm before performing.
 - a. This is intended to remove the mobile phase buffers from the column, and potentially remove particulates or ionic precipitates that may be fouling the front of the column.
 - b. It is recommended to disconnect from the detector and elute to waste during this step to protect the detector from potential damage.
 - c. Guard column systems and sample filtration are good ways to avoid these types of blockages.
3. Initiate a gradient from 95% water 5% acetonitrile up to 5% water 95% acetonitrile over 3-5 column volumes.
 - a. This is intended to increase the elution strength of the mobile phase to elute hydrophobic compounds that possibly have retained on the column.
 - b. A gradient is recommended to aid in the elution of said contaminants before they precipitate (particularly protein type contaminants).
 - c. 5% organic is suggested initially to prevent hydrophobic phase collapse, but may not be necessary for some reversed phase columns containing some polar functionalities (enabling 100% aqueous phase stability).
4. Continue flushing with 95% acetonitrile for 30 column volumes.
 - a. 100% organic is ok at this step.
 - b. If chromatography is restored at this step (particularly for isocratic methods), a routine cleaning gradient or periodic organic flush may be a sufficient long-term solution.
 - c. Increasing the strength of the organic modifier may be useful, especially for methods where the gradient is routinely run up to a high organic percentage.

Hydrophobic Component Removal

Contaminants that are strongly retained on the column through hydrophobic interactions should be eluted with a strong reversed phase solvent. Use the same organic modifier as in the method conditions to start with, increasing the solvent strength as necessary (if it is suspected that there are more strongly retained components leading to the chromatography degradation).

1. Methanol (acetonitrile should be substituted for methanol in the initial general steps if methanol is instead the primary organic modifier of the method)
2. Acetonitrile

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3. Isopropanol
4. Tetrahydrofuran
5. Dichloromethane
6. Hexane

Because dichloromethane and hexane are not water miscible, they are typically not used in reversed phase HPLC, but can be useful for removing extremely hydrophobic contaminants in some cases. The column must first be flushed with 10-15 column volumes of an intermediate solvent that is miscible with both hexane and water, such as Isopropanol. And flush again with isopropanol before re-introducing an aqueous-based mobile phase.

If this type of extensive cleaning procedure is required routinely, it may be more efficient to employ a better sample preparation technique, such as solid phase extraction to remove the indicted components prior to injection.

Upcoming cleaning topics:

Protein removal, HILIC, and normal phase