

8. CLEANING INSTRUCTIONS FOR PACKED COLUMNS (CIP)

The cleaning of a chromatography column involves the following three stages: regeneration of the column packing, sterilisation and depyrogenation.

Regeneration removes chemical and organic contamination that becomes non-specifically attached to the chromatography material, considerably reducing the capacity and resolution of the column. This kind of contamination is usually caused by lipids and pyrogens, protein aggregates, pigments, polyphenols and metal complexes.

Sterilisation is the removal and/or denaturing of micro-organisms and spores, which could contaminate the purified product, by chemical treatment. The most frequently used sterilisation method is treatment with sodium hydroxide, acetic acid or ethanol solutions containing sodium hydroxide or acetic acid.

Depyrogenation includes the breaking-down of endotoxins that have become attached to the chromatography material or the column hardware (frits, tubing etc.) and can soil the target compounds in question by being washed gradually through the column. Often the methods used to sterilise equipment will also break down pyrogens.

Chromatography columns can be purified and sterilised by taking the following steps:

The column is dismantled and the individual parts (column body, pistons, end fittings, frits) are washed in a dilute solution of caustic soda or sodium hypochloride (0.5 N NaOH or dilute NaOCl); the frits should be left in the same solution for 30-60 minutes. Before the column is re-assembled, all parts should be washed in a sterile, pyrogen-free solution.

The column must be packed in a sterile environment. All solvents and solutions used for the column must be sterile and pyrogen-free. We recommend in-line filtration through a 0.22 µm filter.



PLEASE NOTE: Check carefully that all moistened parts of the column are stable with all reagents used. If in doubt, contact Essential Life.