

LC columns

Smart tips

How to condition amino columns before equilibration



Conditioning of amino columns for use in HILIC mode

Thermo Scientific™ Hypersil GOLD™ amino columns;
Thermo Scientific™ Synchronis™ amino columns

- The column is shipped in ethanol. This allows the column to be conditioned more rapidly into HILIC/reversed-phase conditions than if shipped in its test solvent
- To condition into HILIC mode, first equilibrate with at least 20 column volumes of acetonitrile followed by re-equilibration with a 70/30 (v/v) acetonitrile/water mix for 20-30 column volumes. (The high concentration of water introduced here is an essential step for wetting the polar surface and allowing water enriched layer to be formed and stabilized)
- Once the column has stabilized, it is possible to now adjust to your mobile phase of between 70%-90% acetonitrile/water mobile phase. Re-equilibrate and inject standards to check retention stability
- When injecting samples, make sure that the matrix has been adequately filtered and be aware that matrix impurities can modify the column surface over time

If you intend to use this column for the analysis of polar compounds such as sugars, you will need to condition the column before equilibration with your aqueous mobile phase.



- Washing/re-equilibration: The wash procedure you have used is for using the amino columns in organic mobile phases. When using in HILIC mode, the washing needs to avoid water immiscible solvents. An effective wash to remove buffer and matrix is to use the 70/30 v/v acetonitrile/water as a regular conditioning step at the end of an analytical run
- Do not run the column in 100% aqueous or 100% organic as part of any gradient
- Do not run the column with an increase in temperature, preferably less than 40 °C



Read further detail on the use of Thermo Scientific columns in HILIC mode

Conditioning of amino columns for use in HILIC mode

Thermo Scientific™ Hypersil™ APS-2 columns

- The column is shipped in the test solvent of 85% iso-octane/15% isopropanol
- To allow the use of this column in HILIC mode, the immiscible shipping solvent must be displaced completely before equilibrating with the mobile phase
- As the mobile phase to be used is immiscible, this flush of the column with a co-solvent requires at least 100 column volumes. This step takes on average 3-5 hours and may be run overnight at a reduced volumetric flow rate. Propan-2-ol and ethanol are the usual co-solvents since they are miscible with non-polar normal phase solvents and also polar reverse-phase solvents
- Having ensured that the solvent in the column is miscible with the mobile phase, the column should be equilibrated for at least 20 column volumes of mobile phase. It is recommended that for HILIC mode this mobile phase is initially 70/30 (v/v) acetonitrile/water for 20 column volumes and then is changed to the user's mobile phase of up to 90/10 (v/v) acetonitrile/water. Any buffer required should only be added at this stage. This requires at least 20 column volumes and is done using the appropriate flow rate compatible with the diameter of the column

If you intend to use this column for the analysis of polar compounds such as sugars, you will need to condition the column before equilibration with your aqueous mobile phase.



- For most applications this equilibration will take 2-3 hours to complete. Inject standards to check retention stability
- When injecting samples make sure that the matrix has been adequately filtered. Be aware that matrix impurities can modify the column surface over time
- Washing/re-equilibration: The wash procedure quoted in the Thermo Scientific Hypersil technical guide and column care sheet is for using the amino columns in organic mobile phases. When using in HILIC mode, the washing needs to avoid water immiscible solvents and an effective wash to remove buffer and matrix is to use the 70/30 (v/v) acetonitrile/water as a regular conditioning step at the end of an analytical run
- Do not run the column in 100% aqueous or 100% organic as part of any gradient



Read further detail on the use of Thermo Scientific columns in HILIC mode

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