

Column Care and Use Instructions

YMC-UltraHT Series

1. Introduction

Thank you for purchasing a YMC-UltraHT series column. YMC-UltraHT is an ODS column based on ultra-high purity silica. The column is designed for ultra-fast analysis. YMC-UltraHT series can reduce analysis time with excellent separation capacity. Two (2) micron YMC-UltraHT series has high separation capacity across broad flow and pressure ranges compared to 3 μm and 5 μm materials.

YMC-UltraHT series, which are manufactured under highly controlled conditions, must pass a series of stringent tests before being accepted for shipment. (Please refer to the column inspection report). To ensure optimal performance and durability of the column, please read these instructions carefully before using this column.

2. Recommendations for column connections, detector settings, and data processing considerations

- The "WT" at the end of the product code indicates the style of column endfittings. WT=Waters style.
- Tubing must have flat ends and must bottom out in the column endfitting. Tubing must be connected to the column correctly to avoid creating a void between the column frit and tubing, which can cause a leak and result in poor column performance (e.g. peak tailing, loss of theoretical plate number).
- The shortest possible length of tubing with narrow inner diameters (tubing less than 0.15 mm, 0.006 inch I.D. is recommended) should be used for the connections from the injector to the column and from the column to detector. Make sure not to have a void in the connection.
- Use a detector equipped with low-volume flow cell designed for the narrow bore column.
- Use an injector for the narrow bore column and a low-volume sample loop.
- A sampling rate and a detector response (time constant) should be optimized to detect the earliest eluting sharp peak properly. We recommend a sampling rate of about 10 points per second or higher and a detector response of 0.1 s or faster.

3. Mobile phase and sample solution

- The shipping solvent is 60% acetonitrile aqueous solution. Replace with this solvent for storage.
- The correct direction of the solvent flow is indicated by an arrow on the column identification label.
- Aqueous or non-aqueous solvent can be used as a mobile phase. Repetitive replacement among solvents with large difference in polarities might degrade the column performance. In general, acetonitrile, methanol and tetrahydrofuran (THF) are recommended for regular use. When using THF as a mobile phase, be mindful of the solvent resistance of your system or tubing (PEEK parts are especially unsuitable for use with THF).
- Recommended pH ranges of the column are between 2.0 – 8.0. When using the column at pH near the upper or lower limit, a mobile phase containing 10% concentration of organic solvent should be used. The column lifetime will shorten under certain conditions by temperature and mobile phase composition.
- When replacing mobile phases, make sure of the miscibility among the organic solvents and take care to prevent the precipitation of buffer salts/additives to avoid overpressuring the column. Sample should be injected after carefully checking the miscibility with the mobile phase to avoid precipitation of solutes or salts contained in the sample solvents.
- When possible, the sample should be dissolved in a solvent that is of the same composition as the initial mobile phase. Using a stronger solvent than the initial mobile phase for sample dissolution might result in distorted peak symmetry and degraded resolution.
- To prevent exposure of the column to excessive pressure, the mobile phase and sample solution should be filtered through a 0.2 μm membrane or smaller to remove particulates.

4. Column cleaning (general method)

[After using mobile phase not containing buffer salts/additives]

- Flush the column with solution containing a higher ratio of organic solvent for washing out the compounds that have a great capacity for retention in the column.
- Usable concentration of organic solvent is up to 100%. A cleaning solution containing THF might be effective when removing highly hydrophobic (lipid-soluble) substances that are adsorbed onto the gel.

[After using mobile phase containing buffer salts/additives]

- First replace with a water/organic solution containing no buffer salts or additives (A ratio of water to organic solvent should be set at the same proportions as a mobile phase). Then flush the column in accordance with the method described above.
- Mobile phase containing about 50 mM or less in buffer salts and additives can be replaced directly with about 60% acetonitrile aqueous solution.

[General proposals]

- Flushing with 100% water after using the column around the pH limit might shorten column lifetime. Flush the column with water/organic solution as described above, such as 60% acetonitrile aqueous solution.
- Once macromolecules such as proteins or polysaccharides are adsorbed onto the gel, they are hardly removed, even if solvents with high eluting capability are used. To avoid contamination of the column by them, conduct sample pretreatment carefully before introduction into the column. Alternatively, use a guard column.

5. Other environments

- The operating pressure should be kept under 50 MPa (7250 psi) for more than 50 mm length column, under 40 MPa (5800 psi) for 30 mm length column, under 20 MPa (2900 psi) for 4.6 mm I.D. column.
- Avoid using a column repeatedly near the pressure limit or abrupt change in pressure to prevent shortening of the column life.
- Adjust the flow rate appropriately because the pressure changes depending on the column length, temperature, types of organic solvent etc.
- The upper limit of column temperature is 50 °C. However, we recommend using the column at 20 – 40 °C, because column lifetime varies depending on conditions such as pH.