

UHPLC Column

YMC-Triart C18 1.9 μm
Column Care and Use Instructions**1. Introduction**

Thank you for purchasing a YMC-Triart C18 1.9 μm column for ultra high-performance liquid chromatography (UHPLC).

YMC-Triart C18 1.9 μm is an ODS column, based on 1.9 μm hybrid silica gel, designed to use in UHPLC analysis. YMC-Triart C18 1.9 μm exhibits higher efficiency over a wide range of flow rate and pressure compared to conventional 5 μm /3 μm columns, and therefore could reduce the analysis time without compromising resolution. Additionally, YMC-Triart C18 1.9 μm column offers easy method transfer between UHPLC and HPLC since it shows identical selectivity to 5 μm and 3 μm of YMC-Triart C18.

YMC-Triart C18 1.9 μm is manufactured under highly controlled conditions and must pass a series of strict tests before being accepted for shipment (Please refer to the column inspection report). In order 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

- When connecting YMC-Triart C18 1.9 μm column to the LC system, please make sure the fitting is tightened and seated at the exact port depth of the column endfitting (3.3 mm, 0.130 inch). Tubing must have flat ends and must bottom out in the column endfitting. In case tubing is not properly connected to the column, a void appears between the bottom of the column endfitting and tubing. And it will create a leak and/or result in poor column performance (e. g. peak tailing, loss of theoretical plate number).
- Extra column volume has great impact on band spreading. In order to minimize influence of band spreading on chromatographic performance, LC system should be optimized as follows;
 - 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 connection from the injector to the column and from the column to the detector. Make sure not to have void in connection.
 - Use a detector equipped with low-volume flow cell designed for narrow bore column.
 - Use an injector for narrow bore column and low-volume sample loop.
- YMC-Triart C18 1.9 μm gives high operating pressure compared to conventional 5 μm /3 μm columns. Pay attention to the usable maximum pressure of tubing and LC system.
- A sampling rate and a detector response (time constant) should be optimized to detect earliest eluting sharp peak properly. We would recommend using a sampling rate of about 10 points per second or higher and a detector response of 0.1 seconds or lower.
- Generally YMC-Triart C18 1.9 μm is designed for use with UHPLC system, which has 60 MPa or higher pressure tolerance and reduced system volume.

3. Shipping solvent

100% Acetonitrile. Replace with this solvent for storage. When replacing an eluent containing buffer or salts, take extra care to prevent precipitation of salt.

4. Precautions for use

- The correct direction of solvent flow is indicated by an arrow on the column identification label.
 - Do not disconnect a column from LC system before the pressure drops to zero. Note that abrupt pressure change or continuous use under extreme pressure may result in shorter column lifetime.
 - The maximum operating pressure for YMC-Triart C18 1.9 μm column is 100 MPa (14500 psi.). Recommended flow rates for usage are 0.3 ~ 0.8 ml/min for 50 X 2.0 mm I.D. and 0.6 ~ 1.8 ml/min for 50 X 3.0 mm I.D. when using with acetonitrile/aqueous as an eluent. The column pressure differs depending on column length, column temperature, eluent composition and so on. So adjust a flow rate that can provide optimal performance and practical pressure in use.
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- Recommendations of pH and temperature for column use

pH range	Temperature range	
	Regular use (recommended)	Upper limit
1 ~ 12	20 ~ 40°C	pH 1 ~ 7 : 70°C pH 7 ~ 12 : 50°C

- * Column lifetime varies depending on conditions of use such as pH, temperature and eluent composition. In general, using under higher temperature, higher concentration of buffer salts/additives and/or lower concentration of organic solvents may result in shorter column lifetime.
- * When using the column under alkaline conditions for a long term, a column should be used with a low concentration (about 1 to 10 mM) of organic buffer, like triethylamine, glycine, etc., at a lower temperature (less than 30°C). The recommended organic solvent is methanol.
- Aqueous or non-aqueous solvents can be used as an eluent. Repetitive replacement among solvents with large difference in polarities may result in degradation of column performance. In general, acetonitrile, methanol and tetrahydrofuran (THF) are recommended for regular use (Please also see above recommendation under alkaline eluent conditions). When using THF as an eluent, mind the solvent resistance of your system or tubing (Especially PEEK parts are not suitable for use with THF.).
- Make sure of miscibility among the organic solvents and take care to prevent the precipitation of buffer salts/additives to avoid over-pressuring the column.
- Use a sample diluent that has the same composition as the initial eluent conditions. Injecting samples in a stronger solvent than the initial eluent can result in peak broadening and/or split peaks.
- An eluent and a sample must be filtered out with a 0.2 µm membrane to remove insoluble substances before use or injection.

5. Column cleaning (general method)

[After using eluent not containing buffer salts/additives]

- Flush the column with solution containing higher ratio of organic solvent for washing out the highly retained compounds from the column.
- Usable concentration of organic solvent is up to 100 %. A cleaning solution containing THF may be effective when removing highly hydrophobic (lipid-soluble) substances adsorbed onto the gel.

[After using eluent containing buffer salts/additives]

- Firstly replace with water/organic solution that does not contain buffer salts/additives (A ratio of water to organic solvent should be set at the same proportions as an eluent). Then flush the column in accordance with the method mentioned above.
- The eluent containing about 50 mM or less buffer salts/additives could be replaced directly with about 60% acetonitrile aqueous solution.

[General proposals]

- Flushing with 100% water after using the column around the pH limit may shorten column lifetime. Flush the column with water/organic solution, described above or 60% acetonitrile aqueous solution, taking care not to precipitate buffer salts/additives.
- Once macromolecules like proteins or polysaccharides adsorbed onto the gel, they are hardly removed, even if solvents with high eluting ability are used. In order to avoid contamination of the column by them, conduct sample pretreatment carefully prior to introducing to the column.

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