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Boltimate® Core-Shell Column Care and Use Manual

The Boltimate® column is a fast and efficient liquid chromatography column launched by Welch Materials. It utilizes a core-shell silica as the matrix, modified through Welch 's carefully designed bonding process. The silica particles are of a core-shell type, consisting of a thin layer of high-purity porous silica enveloping a solid silica core. This design reduces the diffusion path of the sample, allowing the chromatography column to exhibit excellent separation performance. The 2.7μm core shell silica features a solid core with a diameter of 1.7μm and a porous layer with a thickness of 0.5μm.

Phase Characteristics:

Phase	Particle size μm	Pore size Å	S.A. m^2/g	Carbon load%	Endcap	pH range
C18	2.7	90	120	9	Yes	2-8.5
Phenyl-Hexyl	2.7	90	120	7	Yes	2-8.5
EXT-C18	2.7	90	120	8	Yes	1.5-12
EXT-PFP	2.7	90	120	5	Yes	1.5-10
HILIC	2.7	90	120	-	-	2-8.5
LP-C18	2.7	90	120	7	No	1-8.5
C8	2.7	90	120	5	Yes	2-8.5
Phenyl	2.7	90	120	5	Yes	2-8.5

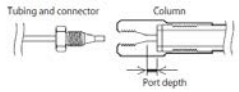
Each Boltimate® chromatography column undergoes rigorous performance testing before leaving the factory and is accompanied by a test certificate(-COA). Each chromatography column is assigned a unique serial number for traceability throughout the production process. Upon unsealing the chromatography column, please verify the information against the COA. If you have any questions about the performance of the chromatography column, follow the instructions in this manual to optimize your instrument system or contact Welch Materials at any time.

Operating Guide:

Thank you for choosing Welch Materials' Boltimate® core-shell chromatography column. To ensure correct usage, please read the relevant content in this manual before use. Ensure usage within the correct and reasonable range to avoid damage to the chromatography column.

1. Column Installation:

1. Please use appropriate fitting to connect with the chromatography column to prevent leaks or the generation of additional dead volume due to mismatches, which may affect usage and column performance.



Port style of Endfitting	Port depth
Parker	2mm

2. For the initial unpacking and use, please replace the mobile phase system of the chromatograph with the mobile phase indicated in the chromatographic column COA or a mobile phase with a higher organic phase ratio as shown in the certificate.

3. Remove the white plugs at both ends of the chromatography column and correctly install the column onto the chromatograph. Use a testing flow rate of 1/4 to 1/2, and flush the chromatography column with an elution volume approximately 5 times the column volume.

4. After the flushing is complete (with minimal pressure fluctuations), proceed with the normal experimental operation.

2. Notice:

- Use the chromatography column following the direction of the arrow on the column body; avoid using it upside down, as this may contaminate the packing material at both ends of the column head, leading to a decrease in the column's lifespan.
- The chromatography column contains solvent for storage, and unless otherwise specified, it is the mobile phase stated in the COA. Please pay attention to using compatible solvents before activation to avoid damaging the chromatography column.
- Use secondary purification deionized water and high-quality chromatography-grade organic solvents. All the solutions should be filtered through a 0.22μm membrane filter before using. The sample solution should be dissolved and filtered before injection.
- Please use the chromatography column within the pH tolerance range of the stationary phase. Excessively high or low pH of the mobile phase can lead to the dissolution of silica gel or detachment of the bonded phase. This damage is irreversible and may render the chromatography column unusable.
- The maximum operating temperature for the chromatography column is 60°C under low pH conditions and 40°C under high pH conditions.
- The maximum allowable operating pressure for the chromatography column is 60MPa (approximately 9000psi). Avoid sudden and significant fluctuations in pressure.

3. System optimization:

Boltimate® 2.7μm core-shell chromatography column performs well under the pressure of traditional LC instruments and exhibits column performance equivalent to sub-2μm particles on UHPLC instruments. To maximize the advantages of core-shell chromatography columns, it is recommended to optimize your liquid phase system, especially when using small-volume chromatography columns (such as ID 1.0mm, 2.1mm, 3.0mm, or columns with lengths <100mm). This optimization is particularly crucial in the following aspects:

- Detector:

  1. Utilize a semi-microflow cell that is instrument-specific (normally the flow cell volume should less than 3μL, but for ID 2.1mm column, the flow cell volume should less than <2μL).

  2. Optimize detector settings by adjusting the scan rate and/or response time to the fastest possible settings such that signal-to-noise (s/n) is not adversely affected.

- Injector:

  If a manual injector is employed, use a semi-micro injector with a low dead volume design (e.g., Rheodyne Model 8125).

- Connection pipelines:

  1. Minimize the length of the connection lines between the injector, chromatography column, and detector to reduce extra-column volume.

  2. Use 0.1mm ID (0.004 inches) lines or 0.12mm (0.005 inches) and 0.17mm (0.007 inches) lines, avoid to the use of lines with IDs ≥ 0.25mm, as thicker lines can compromise column performance.

  3. Use matching connectors and ensure that all connections are correct and stable.

- Sample Dissolution and Injection Volume - Minimizing Pre-column

Sample Diffusion:

1. Isocratic Method: Dissolve the sample in the mobile phase or a solvent weaker than the mobile phase.

2. Gradient Method: Dissolve the sample in the mobile phase which is the same ratio of beginning of the gradient method or a solvent weaker than the mobile phase which is the same ratio of the end of the gradient method.

3. Employ a lower injection volume (2μL or smaller).

4. Care and Storage:

Boltimate® chromatography columns use 2μm frits at both ends. Due to the extremely narrow particle distribution and excellent uniformity of the core-shell silica, 2μm frits can be used. In comparison to other sub-2μm UHPLC columns that use 0.5μm frits, Boltimate® chromatography columns avoid the disadvantage of easily clogging frits in sub-2μm UHPLC columns.

Please use only high-purity reagents and high-quality chromatography-grade solvents to prepare the mobile phase. Traces of impurities can significantly reduce column life. All mobile phases must be filtered and degassed before use. Ensure that samples and matrices are completely dissolved in the mobile phase. Insoluble solvents or precipitates from buffer salts can permanently damage the chromatography column.
It is recommended to utilize a high-pressure online filter and a guard column system to maximize the lifespan of the chromatography column.

➢ Cleaning and Regeneration:

In the case of blockages caused by solid particulate matter or the accumulation of strongly retained substances, leading to an increase in column pressure or a decline in column performance, reverse flushing at a low flow rate with an appropriate mobile phase can be employed to remove contaminants. This process helps, to a certain extent, restore the efficiency of the chromatography column.

It is recommended to use the following flow rates and solvents for reverse flushing the chromatography column:

Column Internal Diameter (mm)	Flow Rate (mL/min)
2.1	2.1
3.0	3.0
4.6	4.6

Empty Column Tube Volume (only for reference)

Dimensions ID x L (mm)	1.0x50	2.1x50	3.0x50	4.6x50
Empty Column Tube Volume (mL)	0.04	0.17	0.35	0.83

Boltimate® reversed-phase bonded phases (C18, Phenyl-Hexyl, EXT-C18, EXT-PFP, LP-C18) are sequentially flushed with the following solvents at 10-20 times the column volume:

1)5:95 acetonitrile/water (or methanol/water) to remove buffer salts

2)95:5 acetonitrile/water (or methanol/water)

3)100% isopropanol transition

4)95:5 dichloromethane/methanol to remove strongly retained substances

5)100% isopropanol transition

6)95:5 acetonitrile/water (or methanol/water)

7)Mobile phase equilibration for forward usage and testing

Boltimate® HILIC chromatography columns are recommended to be flushed sequentially with the following solvents at 10 to 20 times the column volume:

1)80:20 water/acetonitrile to remove buffer salts

2)80:20 ammonium acetate (100mM, pH 5.8)/acetonitrile

3)80:20 water/acetonitrile

4)Mobile phase equilibration

5)Forward usage and testing

➢ Storage

Reversed-phase (C18, Phenyl-Hexyl, EXT-C18, EXT-PFP, LP-C18) chromatography columns:

When a chromatography column is not in use for several days, it should be stored in a solution of >50% (v/v) acetonitrile/water or methanol/water. If the mobile phase contains buffer salts, prior to storage, flush the chromatography column with a high proportion water/acetonitrile or water/methanol solution at 10 to 20 times the column volume to remove the buffer salts. Subsequently, store the column in a solution with a high organic phase ratio. After flushing the chromatography column, tightly seal both ends with column end caps to prevent solvent evaporation and drying of the column bed.

HILIC

For HILIC chromatography columns, when not in use for several days, they should be stored in a solution of 90% (v/v) acetonitrile/water. If the mobile phase contains buffer salts, prior to storage, flush the chromatography column with acetonitrile/water (90:10) at 10 to 20 times the column volume to remove the buffer salts. After flushing the chromatography column, tightly seal both ends with column end caps to prevent solvent evaporation and drying of the column bed.

5. Safety Considerations:

- Operators should be mindful to avoid contact with toxic and flammable organic solvents used in the mobile phase and take necessary protective measures in advance.
- Chromatography columns should be used in well-ventilated environments to minimize the health risks associated with solvent evaporation.
- The operational pressure limit for Boltimate® core-shell chromatography columns is 600 bar (9000 psi). The safety pressure for the column is 1000 bar (16000 psi). If, during use, the pressure in the chromatography column exceeds the operational limit of 600 bar, please replace the column.
- Due to the micron-sized particles filled inside the chromatography column, there is a risk of inhalation, causing harm to the human body. If it is necessary to open the chromatography column head, perform the operation in a well-ventilated area and use respiratory protection. Opening the chromatography column head can damage the performance of the column.

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