CARE AND USE MANUAL

# Boltimate EXT-C18 HPLC Column Care and Use Manual

Welch

Distributed by AKRIBIS



# Boltimate EXT-C18 HPLC Column
## Care and Use Manual

**Dear User:**

Thank you for choosing our liquid chromatography column products! This manual is designed to help you use our products under optimal conditions and maximize column lifespan. Please read the usage instructions carefully before operation.

A Boltimate core-shell column is constructed with a 1.7 µm solid core and a 0.5 µm porous layer. This core-shell particle design delivers high column efficiency (~200,000 plates/m) and exceptional separation performance, comparable to sub-2 µm particles, while generating only 50% of the backpressure of sub-2 µm columns. The combination of high efficiency and low backpressure makes it easily compatible with existing LC systems.

# Boltimate EXT-C18 HPLC Column
## Care and Use Manual

By simply optimizing the flow paths and detector flow cell of your instrument, the core-shell column can achieve outstanding performance under your current experimental conditions without the need for costly equipment upgrades.

As a versatile C18 column, Boltimate EXT-C18 features an organic-inorganic hybrid porous layer, significantly enhancing the pH tolerance of the silica.

# Preparation Before Use

## Column Identification

To ensure the quality of every column, each column sold by Welch Materials is assigned a unique identification code. This code allows us to trace quality-related issues. Upon receiving your column, please follow these steps:

1. Verify that the packaging is intact and matches the ordered column model.
2. Ensure the package contains a COA report with the inspector's signature.
3. Check the column surface for any damage and confirm that the protective plugs on both ends are intact.
4. Verify that the column body bears a Welch Materials identification label. Confirm that the model and serial number on the box match those on the column label.

Note:

The packing material inside the column is composed of fine particles. Do not attempt to open the column arbitrarily, as the material may become airborne and pose a health risk. If opening is necessary, use appropriate protective measures.

## System Optimization:

The Boltimate core-shell chromatography column operates effectively within the pressure limits of traditional LC instruments and achieves particle performance comparable to sub-2 μm particles on UHPLC systems. To maximize the performance advantages of the core-shell column, it is recommended to optimize your LC system as outlined below. These optimizations are especially important when using small-volume columns (e.g., column ID 1.0 mm, 2.1 mm, 3.0 mm, or length shorter than 100 mm):

**Detector:**

1. Use semi-micro flow cells compatible with your instrument (e.g., flow cell volume ＜3 μL for general use; flow cells with volume ＜2 μL for ID 2.1 mm columns).
2. Adjust the scan rate and response time to the fastest settings to maintain an optimal signal-to-noise ratio and improve detector performance.

**Injector:**

When using a manual injector, select a low-dead-volume semi-micro injector (e.g Rheodyne Model 8125).

**Connection Tubing:**

1. Minimize the length of tubing between the injector, column, and detector to reduce extra-column volume.
2. Use tubing with an internal diameter (ID) of 0.1 mm (0.004 inches) whenever possible, or 0.12 mm (0.005 inches) and 0.17 mm (0.007 inches) as alternatives. Avoid tubing with an ID of 0.25 mm or larger, as thicker tubing can degrade column performance.
3. Ensure proper and stable connections by using compatible fittings, and verify that all tubing connections are secure.

**Sample Dissolution and Injection Volume – Minimize Pre-column Sample Dispersion:**

1. Isocratic Methods: Dissolve the sample in the mobile phase or a solvent weaker than the mobile phase.
2. Gradient Methods: Dissolve the sample in the solvent used at the beginning of the gradient or a solvent weaker than that at the end of the gradient.
3. Use a lower injection volume (typically 2 µL or less) to maintain optimal chromatographic performance.

## Installation

To achieve optimal column efficiency, use narrow-diameter tubing for connections. The connections to the column inlet and outlet must be tightly sealed, with the tubing ends polished and free of burrs or bevels. Use professional tools to cut and trim tubing.

1. Remove the column from its packaging. Check the direction of the eluent flow indicated by the arrow on the column body. Unscrew the protective plugs at both ends of the column. Position the column so that the eluent flow aligns with the direction indicated, as shown in Figure 1.

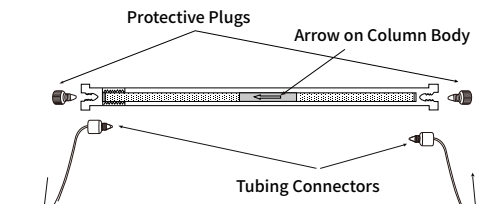



Figure 1. Column Installation

2. Carefully screw stainless steel or other compatible material fittings into both ends of the column. Ensure tight, zero-dead-volume connections to prevent leakage during operation.

## Product Specifications

| | |
|---|---|
| Column | Boltimate EXT-C18 |
| USP Code | L1 |
| Pore Size | 90 Å |
| Particle Size | 2.7 μm |
| Specific Surface Area | 120 $m^2/g$ |
| Carbon Loading | 8 % |
| pH Range | 1.5-12.0 |
| Max. Temperature | 60 °C (mobile phase pH ⩽6.5) |
| | 40 °C (mobile phase pH >6.5) |
| Max. Pressure Tolerance | 60 MPa |

**Note:**

When used at pH ＞9, use buffers with concentrations not exceeding 20 mM and temperatures not exceeding 40 ℃ to maximize column lifespan. Extreme pH or temperature conditions can significantly reduce column durability.

## Column Activation

(Recommended for new columns or those unused for over a month)

Boltimate EXT-C18 columns are shipped in a solution of 60% acetonitrile in water. Activation should follow these steps:

**Step 1: Activation**

| Activation Procedure | | |
|---|---|---|
| Specification | ID≤3 mm | ID>3 mm |
| Flow Rate | 0.1 mL/min | 0.3 mL/min |
| Mobile Phase | 80% Methanol (or Acetonitrile) | |
| Time | 4 hours | |
| Temperature | 30 °C | |

**Step 2: Transition**

If subsequent experiments involve buffer salts in the mobile phase, perform a transition procedure after Step 1.

| Transition Procedure | | |
|---|---|---|
| Specification | ID≤3 mm | ID>3 mm |
| Flow Rate | 0.2 mL/min | 1 mL/min |
| Mobile Phase | 10% Methanol (or Acetonitrile) | |
| Time | 1 hour | |
| Temperature | 30 °C | |

Step 2 is skipped if no buffer salts are involved.

AKRIBIS · www.akribis.info · contacto@akribis.info

## Routine Column Washing (Backflush is Recommended):

| Method Mobile Phase | Without acids, bases, salts | Containing acids, bases, salts | Containing ion-pairing reagents |
|---|---|---|---|
| Washing Mobile Phase | 80% Methanol | 10% → 80% Methanol | 10% → 50% → 80% Methanol |
| Flow Rate | Method Flow Rate | | |
| Time | 30 mins each (Column Length ⩽100mm) | | |
| | 40 mins each (Column Length ＞100mm) | | |
| Storage | Store the column in the final washing solvent, with protective plugs tightly sealed. Place in a cool, dry place. | | |
| Note | Methanol in the mobile phase can be substituted with acetonitrile. | | |

## Column Regeneration:

If the column exhibits increased pressure, abnormal peak shapes, reduced efficiency, or decreased resolution, first flush with a transition mobile phase (10% methanol or 10% acetonitrile) to remove salts. Then wash as follows:

| | |
|---|---|
| Flow Rate | 1/4 of Method Flow Rate |
| Mobile Phase | 100% Methanol<br>→ 100% Acetonitrile<br>→ 100% Isopropanol<br>→ 100% Acetonitrile |
| Time | 100 mins each (Column Length ⩽100mm) |
| | 120 mins each (Column Length ＞100mm) |
| Note | Isopropanol's high viscosity increases pressure; adjust flow rate within range. |

## Glossary of Terms:

**pH Range:** The pH range tolerable for the column's mobile phase and sample solutions.

**Specific Surface Area:** The surface area per gram of silica.

**Pore Size:** The diameter of pores within silica particles, selected based on analyte molecular weight.

**Carbon Loading:** The percentage of carbon content in bonded functional groups relative to the total silica mass.

**USP Code:** Universal code for bonded phases in the USP chromatography column database.

**Transition Mobile Phase:** A solvent used to gradually adjust the type and ratio of mobile phase, typically free of buffers, acids, and bases.

**Transition Procedure:** A procedure to ensure miscibility between activation mobile phase and method mobile phases using transition mobile phase.

**Maximum Pressure Tolerance:** The upper pressure limit the column can withstand. Long-term use is recommended at pressures below 60 MPa. Monitor the instrument's pressure for safe operation.

**Storage Solvent:** Solvent used during storage or transport to maintain the activity of packing material. Handle with appropriate protective measures.