



## Ultisil® Amino Acid Column Care and Use Manual

Ultisil® Amino Acid column, developed by Welch Materials, is a specialized column for amino acid analysis. It uses ultra-high purity, fully porous spherical silica gel as the matrix, and employs a unique bonding process and more thorough end-capping technology to provide excellent peak shapes for amino acid separation.

### Column Parameters:

Carbon load	Pore Size	Surface Area	pH Stability	Max. Temp.	Max. Pressure
17%	120Å	320m <sup>2</sup> /g	1.5-10.0	60 C	40MPa

### Identification of Column:

Each Welch column has a unique serial number, by which, the column can be traced back to each production procedure if any problem occurs. So when customer receives the column, please check:

1. Ensure that the packaging box is intact and that the labels match the column.
2. Verify that the box contains a CoA report with the inspector's signature.
3. Inspect the column surface for any collision marks and check that the protective plugs on both ends of the column are intact.
4. Confirm that the column has a Welch identification label. Carefully compare the model and serial numbers on the packaging box with those on the column label to ensure they match.

### The Correct Use of Column:

Chromatography columns are expensive consumables, and proper usage and maintenance are crucial to ensuring their normal operation and extending their lifespan.

#### 1. Precautions before using column:

- 1) Storage Solution in the Column: 75% methanol aqueous solution.
- 2) Equilibration of New Columns: Flush the column with 80% methanol or 80% acetonitrile at a flow rate of 0.5 mL/min for 4 hours. Then switch to a mobile phase that is miscible with the detection mobile phase and flush for 30 column volumes. Finally, stabilize the system with the mobile phase until the baseline is steady.

#### 2. Direction of column use:

The direction of the arrow on the label of high-performance liquid chromatography columns produced by Welch Materials indicates the flow direction of the mobile phase. Follow this direction during use to avoid bidirectional usage, which can lead to contamination of the packing material at both ends, making regeneration and maintenance difficult.

#### 3. Keep mobile phase and sample clean:

Small particles suspended in the sample or mobile phase can clog the frits at both ends of the chromatography column. Use chromatography-grade reagents whenever possible; reagents used in small amounts should be at least analytical grade. Water should be ultrapure or commercially available purified water. It is recommended to filter all solvents through a 0.45 µm filter before use. Filter the sample solution using a 0.45 µm syringe filter.

Impurities in the sample are one of the main causes of column contamination and decreased column efficiency. For complex samples, consider using a sample pre-treatment column (SPE column) for sample preparation. If pre-treatment is not feasible, use a matching guard column. Ensure the sample solvent is compatible with the mobile phase; samples should be miscible and not differ significantly in polarity to avoid poor peak shapes and ghost peaks. Dissolving the sample in the mobile phase can effectively prevent these issues.

#### 4. Rinse after using:

- 1) Without Buffer Salts: After analysis, reverse flush the column with 80% methanol or 80% acetonitrile for 10-20 column volumes and store the column.
- 2) With Buffer Salts: Reverse flush the column with a transition mobile phase or a low-proportion organic phase for 10-20 column volumes to remove buffer salts. Then reverse flush with 80% methanol or 80% acetonitrile for 10-20 column volumes and store the column.
- 3) With Ion-Pair Reagents: Reverse flush the column with a transition mobile phase or a low-proportion organic phase for 10-20 column volumes to remove buffer salts. Then reverse flush with 50% methanol for 10 column volumes, followed by reverse flushing with 80% methanol for 10-20 column volumes, and store the column.

### Column Regeneration:

Chromatography columns are expensive consumables in liquid chromatography. Over time and with increased sample injections, abnormal chromatographic phenomena may occur. If a column's efficiency is too low or the column pressure is too high, it is usually considered that the column has reached the end of its useful life. However, appropriate regeneration of the chromatography column is an effective way to extend its lifespan.

Appropriate Column Regeneration for Extending Lifespan:

Use each of the following solvents to flush the column for 20-30 column volumes:

100% methanol -- 100% acetonitrile -- 100% isopropanol -- 100% methanol -- 100% dichloromethane or
100% n-hexane -- 100% isopropanol -- 100% methanol

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