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# UniSil® 10-300 C18

Reversed Phase Silica Gel Chromatography Media

## PRODUCT INSTRUCTION MANUAL

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## Reversed Phase Silica Gel Chromatography Media

UniSil® products are monodisperse silica gel chromatography media for HPLC analysis and purification. Developed by NanoMicro with its unique patented technology, it has distinct characteristics, such as uniform particle size (Figure 1), perfect spherical shape, and excellent mechanical strength. In addition, its strict manufacturing processes ensure excellent batch-to-batch consistency for precise pore size and particle size, meeting a variety of application demands.

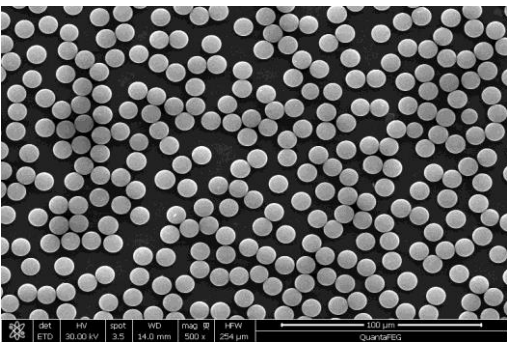


Figure 1. SEM image of NanoMicro monodisperse silica gel

UniSil® 10-300 C18 is an important member of the NanoMicro UniSil® product line. This reversed phase chromatography media configures functional groups by surface modification of UniSil® silica gel matrix (Figure 2), thus having characteristics of uniform particle size, high mechanical strength, high column efficiency, and high resolution. Its pore structure and chemical bonding are particularly suitable for the separation and purification of small molecules such as antibiotics, peptides, and natural products.

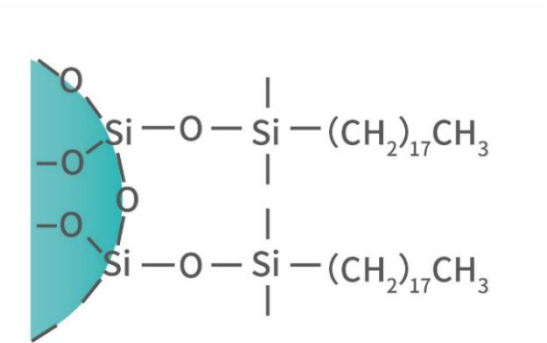


Figure 2. UniSil® 10-300 C18 chemical structure

UniSil® 10-300 C18 takes advantage of advanced surface bonding and end-capping technology that ensures excellent chemical stability. Quality control tests of multiple batches of media stored at room temperature for 5 years showed no significant change from the initial results of quality control test.

Compared with similar products in the market, UniSil® 10-300 C18 has the following unique advantages:

- Monodisperse silica gel media have a very narrow particle size distribution with coefficient of variation (CV) of ≤ 5%.
- It precisely controls pore size, pore size distribution, effective specific surface area and pore volume, resulting in high loading capacity.
- It has unique end-capping technology that can effectively control the residual silanol groups on the particle surface to meet different application demands.
- It has good chemical stability suitable for separation and purification at pH 2-8.

Specific performance specifications for UniSil® 10-300 C18 are listed in the table below:

Table 1. Specifications of UniSil® 10-300 C18

| Separation principle  | Reversed-phase chromatography |
|-----------------------|-------------------------------|
| Matrix                | Monodisperse silica gel       |
| Particle size         | 10 µm                         |
| Pore size             | 300 Å                         |
| Particle size CV      | ≤5%                           |
| Max. pressure         | < 600 bar                     |
| Pore volume           | ~1.00 cm <sup>3</sup> /g      |
| Specific surface area | ~100 m <sup>2</sup> /g        |
| Bulk density          | ~0.45g /mL                    |
| Carbon content        | ~7%                           |
| Range of pH           | 2~8                           |

## High Uniformity of Particle Size

UniSil®10-300 C18 reversed phase silica gel has very narrow particle size distribution with  $CV \leq 5\%$  and highly uniform particle size (Figure 3).

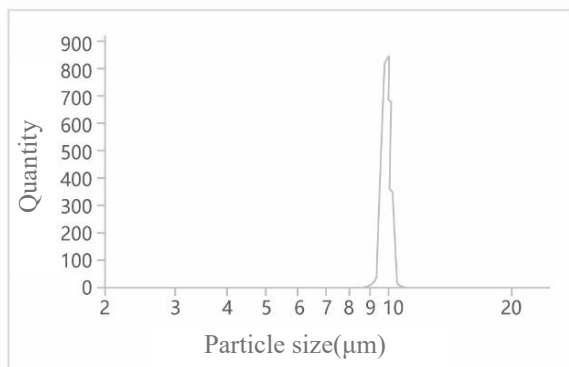


Figure 3. UniSil® 10-300 C18 particle size distribution comparison

## Superior Mechanical Strength

After 20 repeated column packings with UniSil® 10-300 C18 monodisperse silica gel on DAC, no column pressure changes and no fragments were observed, indicating excellent mechanical strength and lifetime (Figure 4).

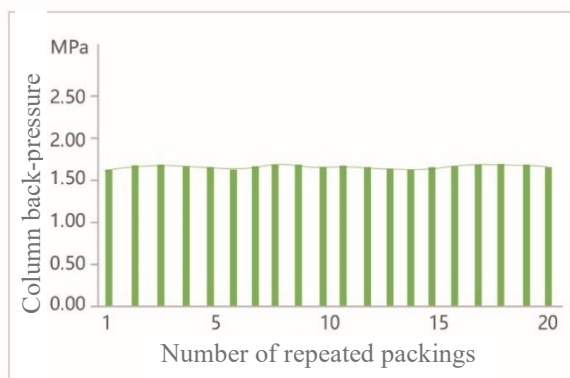


Figure 4-1. The column pressure changes of UniSil® 10-300 C18 after repeated packing 20 times

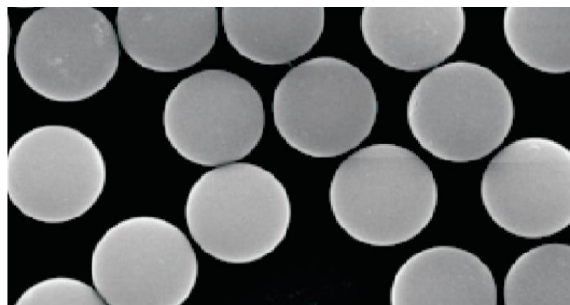


Figure 4-2. SEM micrograph of particles after 20 repeated packings for UniSil® 10-300 C18

## Good Batch Stability

Precise manufacturing technology, strict quality assurance system and advanced detection methods ensure the stability of the product and batch-to-batch reproducibility (Figure 5). Each batch of products will be subject to strict quality inspection and a series of application tests before delivery to ensure the stable application performance of products.

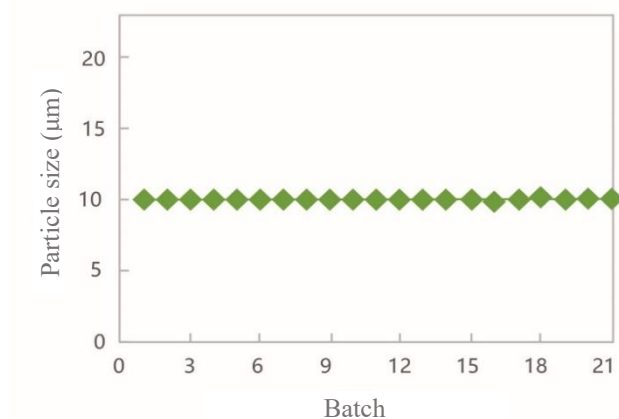


Figure 5-1. UniSil® 10-300 C18 batch-to-batch particle size testing

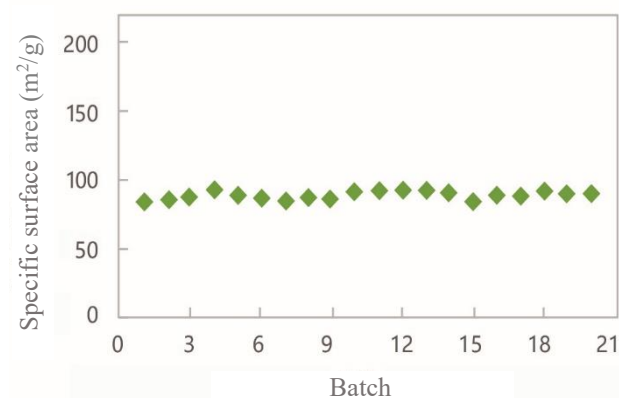


Figure 5-2. UniSil® 10-300 C18 batch-to-batch specific surface area testing

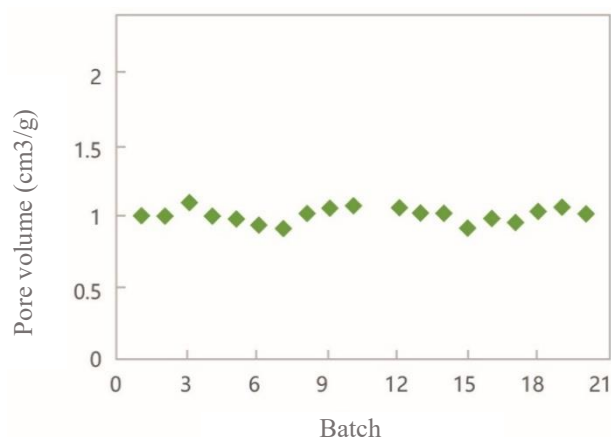


Figure 5-3. UniSil® 10-300 C18 batch-to-batch pore volume testing

## Excellent Chemical Stability

UniSil® 10-300 C18 chromatography media has excellent alkaline stability. The product has been compared with competitor products in terms of retention time of toluene peak, column efficiency and symmetry factor over a period of washing with alkaline solution at pH 10, and the results indicate that UniSil® has better chemical stability (Figure 6).

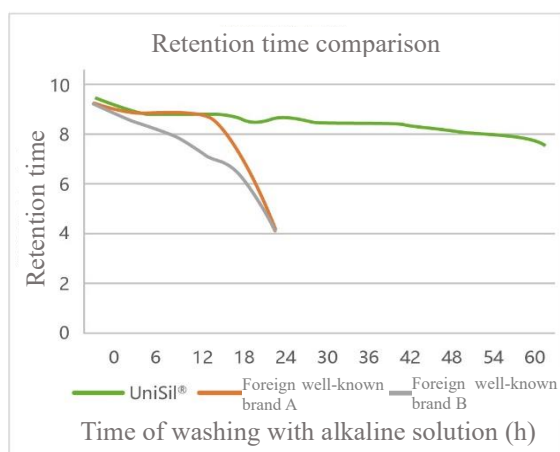


Figure 6-1. Comparison of impact from washing with alkaline solution at pH 10 on retention time of toluene peak

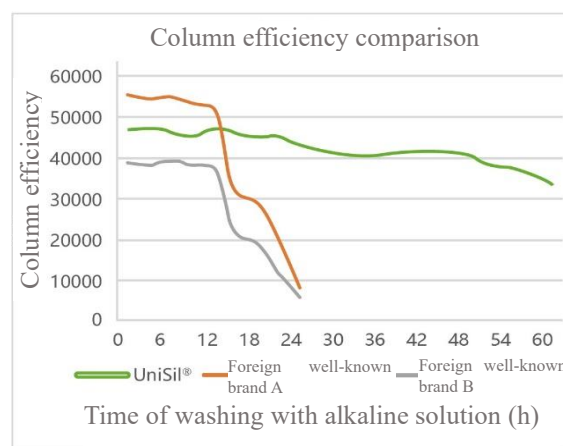


Figure 6-2. Comparison of impact from washing with alkaline solution at pH 10 on column efficiency of toluene peak

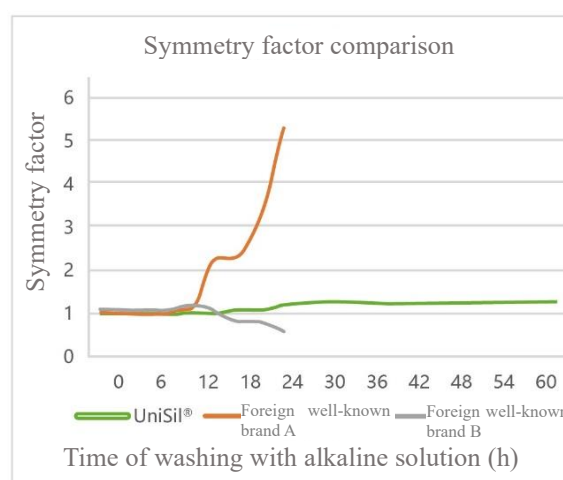


Figure 6-3. Comparison of impact from washing with alkaline solution at pH 10 on symmetry factor of toluene peak

## Test Conditions

Alkaline solution: Triethylamine solution at pH 10

Mobile phase B: 60% acetonitrile for column efficiency testing

Flow rate: Wash at flow rate of 1 mL/min until baseline stabilized and then wash for 3 hours at lower flow rate

Column temperature: 50°C

Sample: Toluene

## Operation Guidelines

Packing (dynamic axial compression method is recommended)

- 1) Calculate the chromatography column volume  $V_c$  and mass ( $m$ ) of media required to fill the column volume according to the formula below:

$$\text{Eq. 1} \quad V_c = h \times \pi r^2$$

$$\text{Eq. 2} \quad m = \rho \times V_c$$

\* $V_c$ : chromatography column volume;  $h$ : chromatography column height;  $r$ : chromatography column radius;  $\rho$ : media density.

\* In order to obtain a tight column bed, it is recommended to use slight excess amount of media, for example, 1.05 to 1.10 times the mass of the media required from calculation.

- 2) Prepare the chromatography column with total volume sufficient to fill in the homogenate at one time. Wet the sieving plate at the bottom of the chromatography column with homogenate solvent using wash bottle or by backflow, retaining liquid with the height of 1~2 cm at the bottom of the column, and then close the column outlet valve.
- 3) Preparation of the media homogenate: Prepare 2.5 to 3 mL of the homogenate solvent, such as isopropanol, for 1 g of media. Pour the media into the prepared homogenate solvent while stirring (at no more than 50 rpm/min) until evenly dispersed. If it is possible, mix them by ultrasound rather than magnetic stirring for 30 minutes.
- 4) Slowly pour the homogenate into the column, and then wash the inner wall of the column with a wash bottle containing homogenate solvent to prevent mixing with gas.
- 5) Open the outlet at the bottom of the column while packing the column using dynamic axial compression. For silica gel media with particle sizes of 8  $\mu\text{m}$  and 10  $\mu\text{m}$ , a pressure of 80 to 100 bar is recommended; For silica gel media with particle sizes of 20 to 40  $\mu\text{m}$ , a pressure of 40 to 70 bar is recommended.

## Performing Column Qualification Test

Generally, the chromatography column performance should be tested before use, and the test results should be saved to provide an important reference for evaluating the changes in chromatography column performance in the future. The recommended mobile phase and test substance for column efficiency test of this media are methanol and toluene, respectively. Prior to test, 2~3 column volumes of mobile phase should be used to equilibrate silica gel

chromatography column. Refer to Table 3 for detailed test conditions.

**Table 2.** Recommended test conditions for UniSil® 10-300 C18 column qualification

| Probe                                    | 2% (v/v) toluene in methanol solution |
|------------------------------------------|---------------------------------------|
| Probe volume                             | 0.1% column volume                    |
| Mobile phase                             | Methanol                              |
| Linear velocity                          | 100~250 cm/h                          |
| Detector                                 | UV@254 nm                             |
| Recommended qualification specifications | As: 0.8-1.5; Plates (N/m): >30,000    |

## Recommended Mobile Phase

When using UniSil® 10-300 C18 media, the organic solvents such as isopropanol, methanol, ethanol, acetonitrile, and tetrahydrofuran can be used as mobile phases, or ion-pairing reagents such as TFA for improving separation. This media is not suitable for running in 100% aqueous phase nor for long-term running in mobile phase with organic phase ratio lower than 10%.

## Cleaning

If chromatography media should be cleaned, it is recommended to be repeatedly washed with a mobile phase of lower polarity, such as pure acetonitrile, methanol, or a 95%:5% mixture of dichloromethane and methanol. If the above cleaning method is not effective, the extreme method, i.e., dimethyl sulfoxide or dimethylformamide at low flow rates can be used.

Note: When washing with solvents of different polarities, the liquid environment of the media should be replaced by washing with a solvent miscible with the previous washing solvent, such as isopropanol. Washing with mobile phase with organic/aqueous phase ratio greater than 50% is necessary for cleaning and storage after use.

## Regeneration

After long-term use of chromatographic column, the column efficiency (number of theoretical plates) tends to decrease. In this case, the column can be regenerated. Generally, a washing procedure is defined as more than 2 column volumes (the linear flow rate should be controlled at 150 ~ 200 cm/h). The column may also be washed with a series of solvents with increasing

non-polarity, such as acetonitrile (methanol) → isopropanol → dichloromethane, and then changed back to the original mobile phase in reverse order.

Note: The shelf life of unopened media is 5 years.

\*Note: In the process of use, the used sample and mobile phase must be filtered by a filter membrane with pore size of 0.45 µm.

## Storage

Used media should be washed clean for salts and then sealed with a high organic phase (pure ethanol or pure isopropanol) in a cool and dry place. The unopened media can be directly stored in a cool and dry place. Recommended storage temperature is 4 ~ 35°C.

## Trouble Shooting Guide

Problems that occasionally occur during If you have any problems when using UniSil® 10-300 C18 reversed phase chromatography and suggested solutions are listed below. We also have an experienced and dedicated application team to provide full technical support, from method development, scale-up design, to commercial production.

| Problem                                                     | Possible Cause                                                                  | Recommended solution                                                                                                                           |
|-------------------------------------------------------------|---------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| Increase of column pressure                                 | On-line filter of instrument clogged                                            | Remove and wash filter or replace the filter if possible; Filter sample and eluent prior to use.                                               |
|                                                             | Contaminated chromatography column after long-term use                          | Replace the chromatography column or replace media.                                                                                            |
| Most of the sample flowing through the column after loading | Too low ion strength or no salt in ionizable sample solution                    | Appropriately increase ionic strength by adding NaCl or other measures.                                                                        |
|                                                             | Inappropriate pH of sample                                                      | Adjust pH to increase binding strength.                                                                                                        |
|                                                             | The column is contaminated                                                      | Wash the column or remove strong contaminants remaining in the sample.                                                                         |
|                                                             | Too high organic solvent ratio in sample solution                               | Decrease the organic phase ratio of sample solution close to that of the mobile phase.                                                         |
| Failure to elute sample during the elution process          | Too weak elution strength of the eluent                                         | Increase the organic solvent ratio in sample solution or replace the eluent with another eluent with stronger elution strength (less polarity) |
|                                                             |                                                                                 | Adjust pH of the eluent                                                                                                                        |
| Baseline drift                                              | Bubbles after mixing of mobile phase                                            | Try to elute as early as possible after mixing the mobile phase                                                                                |
|                                                             | Chromatography column not well-equilibrated                                     | Increase the equilibration time.                                                                                                               |
|                                                             | Different absorption coefficients for eluent A and B at the same UV wavelength. | Detect at different wavelengths or run a blank gradient.                                                                                       |
|                                                             | Contaminated eluent                                                             | Use a new solvent or high purity solvents.                                                                                                     |

## Order Information

| Product            | Size   | Catalog #         |
|--------------------|--------|-------------------|
| UniSil® 10-300 C18 | 30 g   | 19001-100030-4030 |
|                    | 50 g   | 19001-100030-4050 |
|                    | 100 g  | 19001-100030-4100 |
|                    | 300 g  | 19001-100030-4300 |
|                    | 500 g  | 19001-100030-4500 |
|                    | 1 kg   | 19001-100030-3001 |
|                    | 5 kg   | 19001-100030-3005 |
|                    | 10 kg  | 19001-100030-3010 |
|                    | 50 kg  | 19001-100030-3050 |
|                    | 100 kg | 19001-100030-3100 |

Note: We can also provide you with preparation columns with an inner diameter of 10/21.2/30/50 mm and a length of 100/150/250 mm. Please contact us for further details.



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