UniSil® 8-120 C18

Reversed Phase Silica Gel Chromatography Media

PRODUCT INSTRUCTION MANUAL

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NanoMicro

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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.

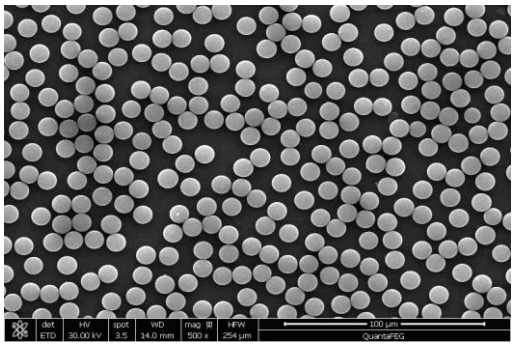

Figure 1. SEM image of NanoMicro monodisperse silica gel

UniSil® 8-120 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.

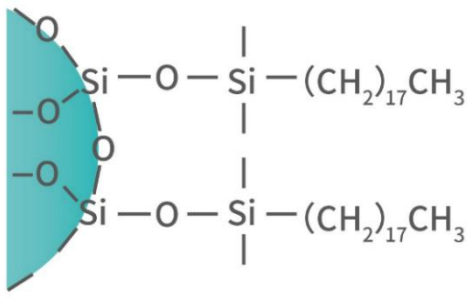

Figure 2. UniSil® 8-120 C18 chemical structure

UniSil® 8-120 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® 8-120 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® 8-120 C18 are listed in the table below:

Table 1. Specifications of UniSil® 8-120 C18

Separation principle	Reversed-phase chromatography
Matrix	Monodisperse silica gel
Particle size	8 μm
Pore size	120 Å
Particle size CV	⩽5%
Max. pressure	＜600 bar
Pore volume	~1.00 cm^3/g
Specific surface area	~350 m^2/g
Bulk density	~0.55g /mL
Carbon content	~15%
Range of pH	2~8

Operation Guidelines

Packing (dynamic axial compression method is recommended)

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

Eq. 1 $Vc = h \times \pi r^2$

Eq. 2 $m = \rho \times Vc$

*Vc: chromatography column volume; h: chromatography column height; r: chromatography column radius; ρ: 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 μm and 10 μm, a pressure of 80 to 100 bar is recommended; For silica gel media with particle sizes of 20 to 40 μm, 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® 8-120 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): >40,000

Recommended Mobile Phase

When using UniSil® 8-120 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.

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.

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.

Trouble Shooting Guide

Problems that occasionally occur during If you have any problems when using UniSil® 8-120 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®8-120 C18	30 g	19001-080012-4030
	50 g	19001-080012-4050
	100 g	19001-080012-4100
	300 g	19001-080012-4300
	500 g	19001-080012-4500
	1 kg	19001-080012-3001
	5 kg	19001-080012-3005
	10 kg	19001-080012-3010
	50 kg	19001-080012-3050
	100 kg	19001-080012-3100

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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