
UniSil® Revo 10-100 C8

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.

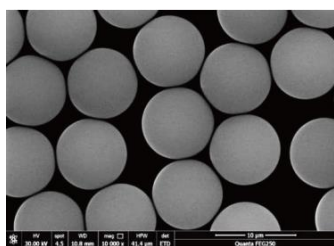


Figure 1. SEM image of NanoMicro monodisperse silica gel

UniSil® Revo 10-100 C8 is an important addition to NanoMicro UniSil® Revo 10-120 product line. Utilizing revolutionary PCS (Precisely Controlled Silica) technology enables **more open and interconnected through pores and thicker skeleton among holes**, resulting in superior mechanical strength, selectivity and pH tolerance. This C8 reversed phase chromatography media configures functional groups by surface modification of UniSil® silica gel matrix (Figure 2). Its pore structure and chemical bonding are particularly suitable for the separation and purification of small molecules such as GLP-1, insulin, peptides etc.

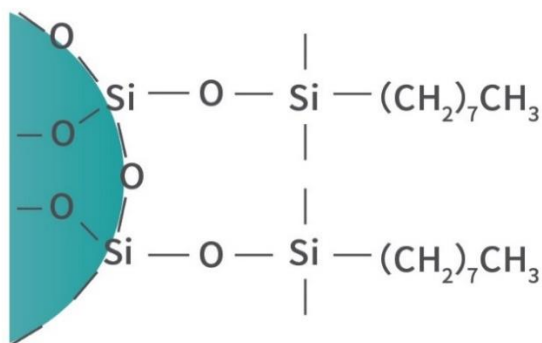


Figure 2. UniSil® Revo 10-100 C8 chemical structure

UniSil® Revo 10-100 C8 takes advantage of advanced surface bonding and end-capping technology that ensures excellent chemical

stability.

Compared with similar products in the market, UniSil® Revo 10-100 C8 has the following unique advantages:

- Monodisperse silica gel media have a very narrow particle size distribution with coefficient of variation (CV) of $\leq 5\%$;
- It precisely controls pore size, pore size distribution, effective specific surface area and pore volume, resulting in high loading capacity;
- There are three advantages of UniSil Revo 10-100 over another silica:
 - Similar 100Å pore size and similar retention for direct replacement of Kromasil 100;
 - Surface has an inert treatment to further improve chemical lifetime at high pH by 3x;
 - C18/C8/C4 phases are further optimized for better yield and purity of insulin and GLP-1.

Specific performance specifications for UniSil® Revo 10-100 C8 are listed in the table below:

Table 1. Specifications of UniSil® Revo 10-120 C8.

Separation mode	Reversed-phase chromatography
Matrix	Monodisperse silica gel
Particle size	10 µm
Pore size	100 Å
Particle size CV	$\leq 5\%$
Max. pressure	<600 bar
Pore volume	~1.00 cm ³ /g
Specific surface area	~320 m ² /g
Bulk density	~0.60 g/mL
Carbon content	~12%
Range of pH	2~10

High pH Stability Test

Under continuous 0.1M NaOH (pH 13) flash condition, UniSil® Revo 10-100 C8 still maintained good efficiency up to 22 hours, while silica based C8 lasted only 6 hours and pressure is over limit and plates dropped more than 30%.

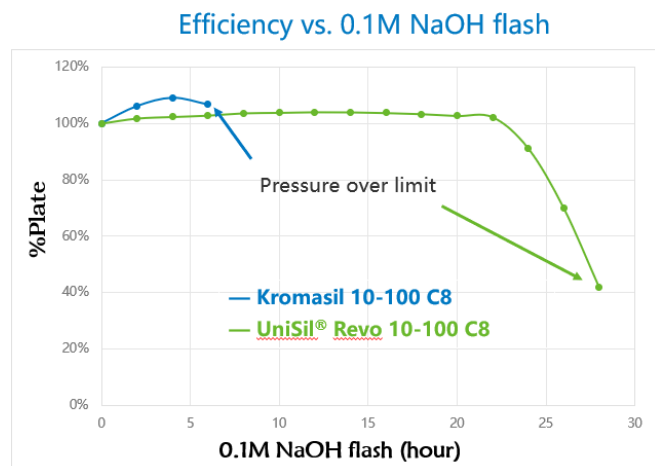


Figure 3-1: Plates comparison of UniSil® Revo 10-100 C8 and other competitor's silica 10-100 C8 in pH 13 condition.

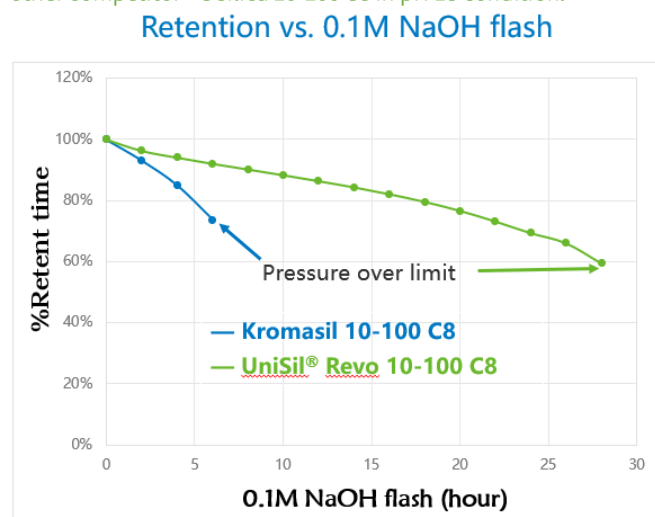


Figure 3-2: Retention comparison of UniSil® Revo 10-100 C8 and other competitor's silica 10-100 C8 in pH 13 condition.

Application-Synthetic Semaglutide Purification

For the purification of fully synthetic semaglutide, the same resin UniSil® Revo 10-100 C8 is used for both steps. The yield and purity can meet customer requirements by this simpler procedure. The results are as follows:

Purification preparation conditions

Column: UniSil® Revo 10-100 C8, SS 4.6mm×250mm

Sample: 10 g, purity 87%

Mobile A 1st Step pH 2.5, 2nd Step pH 7; Mobile B ACN

Flow: 0.75 mL/min

Experimental Results

Purification steps	Purity	Yield	Total yield
1 st Step	98.4%	95.4%	79.4%
2 nd Step	99.6%	83.2%	

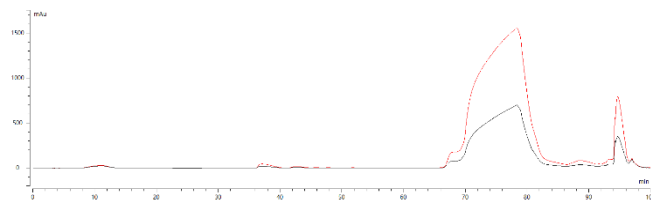


Figure 4-1. Fully synthetic semaglutide 1st Step purification.

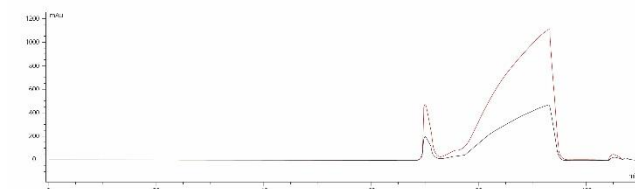


Figure 4-2. Fully synthetic semaglutide 2nd Step purification.

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; 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 slurry: Prepare 2.5 to 3 mL of the slurry solvent, such as isopropanol, for 1 g of media. Pour the media into the prepared slurry 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 slurry into the column, and then wash the inner wall of the column with a wash bottle containing slurry 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 parameter for evaluating the changes in chromatography column performance in the future. The recommended mobile phase for column efficiency test is methanol, and the test sample is toluene or naphthalene. Prior to test, 2~3 column volumes of mobile phase should be used to equilibrate the column. Refer to Table 2 for detailed test conditions.

Table 2. Recommended test conditions for UniSil® Revo 10-100 C8 column qualification.

Probe	2% (v/v) toluene in methanol solution or naphthalene of 1 g/L
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® Revo 10-100 C8 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 suitable for running in 100% aqueous mobile phase condition without exhibiting phase collapse problem.

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 from 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® Revo 10-120 C8 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.
	Column contamination after long-term use	Replace the chromatography column or replace media.
Most of the sample passing 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	Package size	Catalog #
UniSil® Revo 10-120 C8	30g、50g、100g、300g、500g、1kg、 5kg、10kg、50kg、100kg	19702-100010

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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