UniPS® 10-300

Reversed Phase Chromatography Resin

PRODUCT INSTRUCTION MANUAL

Document No.: NM-M-DF-0401-1

Version No.: A0

纳 微
NanoMicro

UniPS® 10-300

Reversed Phase Polymeric Chromatography Resin

NanoMicro Technology provides monodisperse reversed phase polymeric chromatography resin of various particle sizes and pore sizes, which can be classified into UniPS®, Uni®PMM, Uni®PSN, Uni®PSA and NM depending upon the matrices and properties, and mainly used for laboratory analysis and separation & purification of organic compounds, natural products, proteins, insulin, peptides, nucleic acids, etc. Benefited from its significant advantages of good alkali resistance, long life and eliminating tailing of alkaline compounds, the reversed-phase polymeric chromatography resin complements the reversed phase silica gel resin in separation performance and solvent compatibility.

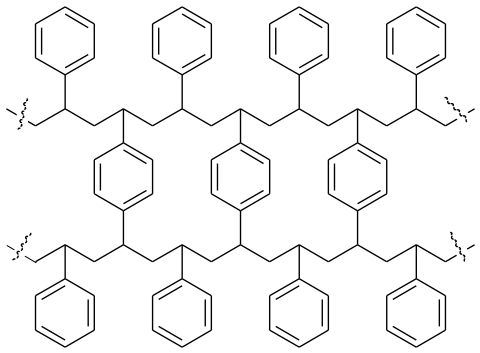

Figure 1. Chemical structure of UniPS®

Highly cross-linked polystyrene/divinylbenzene (PS/DVB) is selected as the matrix of UniPS® 10-300 monodisperse reversed-phase polymeric resin (Figure 1). Because there are abundant phenyl groups in the substrate, the resin is highly hydrophobic with strong reversed-phase retention, allowing for unique surface modifications and versatile selectivity. Its high mechanical strength and chemical stability due to the highly cross-linked matrix make it more resistant to pressure, allowing for medium and high-pressure DAC packing and mixture and regeneration of extremely acidic or alkaline organic solvents.

Compared to similar products in the market, UniPS®10-300 has the following advantages:

- Narrow particle size distribution: CV ⩽ 3%;
- High mechanical strength: more resistant to pressure due to highly cross-linked PS/DVB base matrix; and not easily broken during repeated packing for DAC under high pressure;
- Long lifetime: corrosion resistance, pollution resistance, easy to regenerate, low cost;
- High chemical stability: suitable for separation and purification at pH 1 - 14.

The specific technical parameters of UniPS® 10-300 are listed as follows:

Table 1. Specifications of UniPS® 10-300

Separation principle	Re versed phase chromatography
Matrix	Polystyrene/divinylbenzene (PS/DVB)
Particle size	10 μm
Pore size	300 Å
Particle size CV	⩽3%
Max. pressure	<40 bar
Pore volume	~0.7 cm^3/g
Specific surface area	~470 m^2/g
Range of pH	1~14

Application Cases of Peptide

UniPS® 10-300 preparative resin has been widely used in the purification of plant extracts, peptides, contrast agents, insulin, etc. especially, the purification of peptides. It has a similar purification performance as C18 silica gel media and has a long lifetime, resistant to strong regeneration conditions e.g., acidic and alkaline conditions. In the purification of eptifibatide, it is characterized by high loading capacity, high purity, and high yield.

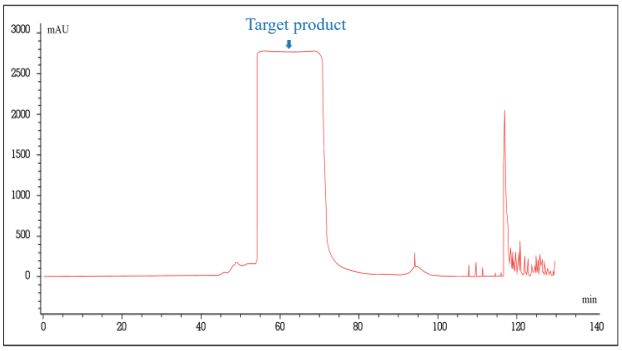

Figure 2. HPLC purification and preparation of eptifibatide

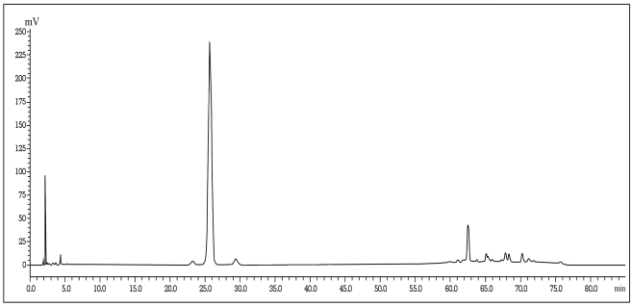

Figure 3. HPLC analysis of crude product (purity 64.4%)

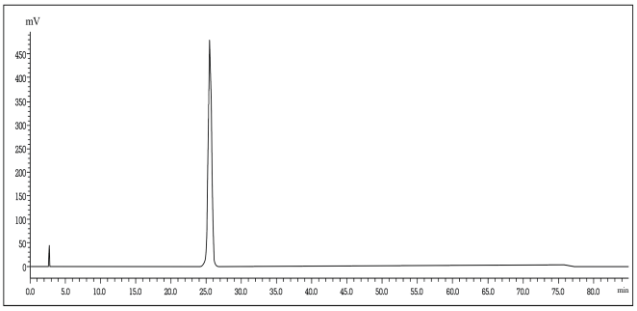

Figure 4. HPLC analysis of the purified product (purity 99.2%)

Operation Guide

Packing

(Dynamic axial compression method is recommended)

1). Calculation of resin volume: calculate the column volume Vc according to the formula below:

$$Vc = h \times \pi r^2$$

**Vc: column volume (mL); h: column height (cm); r: inner diameter of column (cm).*

** In order to obtain a tight column bed, it is recommended to use slightly excessive resin, e.g. about 1.1 times the volume V_C calculated.*

2). Prepare the chromatography column with a sufficient volume to allow for pouring of the homogenate at one time. Wet the plate at the bottom of the column with homogenate solvent by washing or backflow, leaving liquid with the height of 1-2 cm at the bottom of the column. Then close the column outlet valve.

3). Preparation of resin homogenate: prepare 20% ethanol homogenate solvent and degas in advance. Pour the resin (homogenized with a volume concentration of about 65%) into the prepared homogenizing solvent and dilute to about 50%. Stir (at no more than 50 rpm/min) while pouring until complete dispersion. Conditional sonication can be used for 30 min for homogenization. Magnetic stirring is not recommended.

4). Slowly pour the homogenate into the column and wash the inner wall of the column using 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 resin with particle sizes of 10 to 20 μm, a pressure of 20 to 40 bar is recommended. For resin with particle sizes of 20 to 40 μm, a pressure of 5 to 20 bar is recommended.

Performing Column Qualification Test

Generally, the column performance should be tested before using the column, and the test results should be saved to provide an important reference for evaluating the changes in column performance in the future. The recommended mobile phase and test substance for column efficiency test of this resin are acetonitrile and acetone, respectively. Prior to test, 3 times the column volume of mobile phase should be used to equilibrate the column. Refer to Table 2 for detailed test conditions.

Table 2. Recommended test conditions for UniPS® 10-300 column qualification.

Probe	10 % (v/v) acetone in acetonitrile solution
Probe volume	0.1% - 0.5% column volume
Mobile phase	Acetonitrile
Linear velocity	150 cm/h
Detector	UV@254 nm
Recommended qualification specifications	As:0.8-1.5; Plates(N/m): >30,000

Recommended Mobile Phase

UniPS®10-300 allows isocratic or gradient elution. Generally, water is used as the base solvent, and a certain amount of polar solvent miscible with water is added, e.g. methanol, acetonitrile, ethanol, isopropanol, etc. In general, the methanol/water system meets the separation requirements of most samples and has a low viscosity and cost when used as mobile phase. The acetonitrile/water system can also be used for initial experiments because of the higher eluting strength and lower viscosity of acetonitrile than methanol, meeting the requirements of UV detection at 185 nm-205 nm. In addition, the ethanol/water, isopropanol/water, and tetrahydrofuran/water systems can also be used.

Cleaning

The column can be cleaned with a series of solvents with increasing non-polarity. For contaminants such as proteins and pigments, ethanol/methanol/acetonitrile-water solution with increasing organic solvent content until pure ethanol/methanol/acetonitrile could be used. Then flush the column with the original mobile phase.

Regeneration

After long-term use of column, the column efficiency (number of theoretical plates) tends to decrease. In this case, the column can be regenerated. It is recommended to wash 3-5 BV with methanol/0.1-0.5 M NaOH (60%:40%) solution, followed by 3-5 BV with methanol/0.1-0.5 M HCl (60%:40%) or methanol/acetic acid (60%:40%) solution. Then, the mobile phase could be used for equilibrium. Protein aggregates and other contaminants could be easily destroyed and eluted in the acidic or alkaline solution. Regeneration by backflush is preferred whenever possible as it saves the volume of mobile phase required for regeneration.

Storage

The resin or column used should be cleaned or regenerated (with the column equilibrated with 3-5 times the column volume of 20 % ethanol or 70 % acetonitrile) before being sealed and stored in a cool and dry place. The unopened resin can be stored in a cool and dry place, with the recommended storage temperature of 4-25°C.

In addition, it is also recommended to periodically check the adequacy of the preservation solution and confirm the tightness of the column to avoid drying out, including regularly replacing the preservation solution with fresh one to prevent the resin from bacterial growth.

Note:

1. The shelf life of unopened resin is 5 years.

2. When used, the sample and mobile phase must be filtered by a filter membrane with pore size of 0.45 μm.

3. Under no circumstances should the products be washed with nitric acid.

Trouble Shooting Guide

Problems that occasionally occur during UniPS® 10-300 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 resin.
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 additives.
	inappropriate pH of sample	Adjust pH to increase binding strength.
	The column is contaminated	Wash the column or remove strong contaminants in the sample.
	Too high organic solvent ratio in sample solution	Decrease the organic solvent ratio in 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 mobile phase 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 eluents A and B at the same UV wavelength.	Detect at different wavelengths or run a blank gradient.
	Contaminated eluent	Use a new or high purity solvent.
Presence of unknown impurity peaks	Incomplete elution of the previous sample	Adjust the regeneration process during purification for regeneration.
	Impure eluent	Run a blank control or use a high-purity chromatography grade reagent.

Order Information

Product	Size	Catalog #
UniPS® 10-300	30 mL	02000-010030--2030
	50 mL	02000-010030--2050
	100 mL	02000-010030--2100
	300 mL	02000-010030-2300
	500 mL	02000-010030-2500
	1 L	02000-010030-1001
	5 L	02000-010030-1005
	10 L	02000-010030-1010
	50 L	02000-010030-1050
	100 L	02000-010030-1100

Note: We can also provide you with a preparation column 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.

纳 微
NanoMicro

Suzhou NanoMicro Technology Co., Ltd.

National Hotline: 400-828-1622

Email: info@nanomicrotech.com

Chinese website: www.nanomicrotech.com

English website: en.nanomicrotech.com

Headquarters address: No. 2 Baichuan Street, Suzhou Industrial Park 215123

Nov 2022 Version I