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微纯生物科技

WEPURE BIOTECH

PrePulite® XP Hybrid Silica Packing Media and its application in the Semaglutide purification process

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About WePure Biotech

WePure Biotech is committed to “Becoming an excellent supplier of Global Analysis,Testing,Separation & Purification Solution.” It is one of the few manufacturers in the world that fully masters the preparation technology of liquid chromatography silica gel and resin material.With more than 20 years of industry experience,the team has accumulated rich product application and method development experience.The company possesses a complete industrial chain that covers the production of raw material monomers for silica gel and resin microspheres,internationally leading surface bonding modification technology,analytical column,semi-prep column,and preparation packing media.It provides high-quality,cost-effective,stable supply and rapid delivery of products and services to industries,such as pharmaceutical,biotechnology,food safety,chemical and environment

I,WePure Biotech's three technology platforms

1,Stable porous microsphere syntheses technology

WePure can produce microsphere in a stable and large scale,includes 1.7μm-100μm high-purity silica,organic-inorganic structure hybrid silica（XP） and high-strength silica（HSS）,inorganic-inorganic structure hybridized SiZ microspheres and so on.

2,Advanced surface modification technology

WePure provides triple bond C18/C8,double bond C18/C8,single bond C18/C8,NH_2,Amide,hexyl-phenyl,Fluoro-phenyl（PFP）,Diol,RP18/18 Plus,PHS charged modification technology,unique T3 bond technology,mix mode bond technology,meet the needs of analysis,separation and purification.

3,High efficient and stable columns packing platform

WePure products cover UPLC,UHPLC,HPLC and semi-preparative columns,with stable production technology and strict testing,ensure excellent stability and reproducibility of column to column,batch to batch.

II. Micro-purity Quality Control

Under the strict quality control system of ISO 9001, we have carried out strict and detailed quality control over every link in the production process, from the quality control of raw materials， the synthesis of silica , precise screening of particle sizes, fine modification of bonded phases, and even the final forming of the packing media. In the entire production process, we have carefully designed and implemented more than 30 different types of testing items, covering more than 200 detailed and critical indicators. Through such comprehensive and strict quality inspection system, we ensure that the quality of each product can accurately meet the original design requirements, while also effectively ensuring the stability of the product during long-term use, allowing customers to use with confidence.

III. PrePulite® XP Hybrid Silica packing media Overview

The XP series of hybrid silica gel Packing Media adopt a special hybrid technology, which combines the characteristics of organic and inorganic Packing Media. It not only retains the properties of pure silica g but also has the characteristics of polymer materials, such as stability in wide pH range and low activity of silanol groups. This ensures both the separation performance and service life. There are various specifications of the Packing Media available for selection, which can be linearly scaled up from analytical columns to preparative columns, ensuring the continuation and transfer of the method, improving work efficiency.

1. Advantages of PrePulite® XP Hybrid Silica Packing Media

Ordinary silica gel is composed of a Si-O-Si structure. In an acidic system, the bonded phase of this structure is prone to detachment, and in an alkaline system, the silicon - oxygen bonds are liable to dissolve. XP hybrid silica introduces organic groups into the silica skeleton, significantly delaying the dissolution of silica and hydrolysis of the bond phase, so the pH tolerance range expand to 2-12.

1.1 excellent Chemical Stability and pH Tolerance

Wide pH Range: Ordinary pure silica is prone to bonded phase detachment under strong acid (pH < 2) or strong alkali (pH > 8) conditions, while XP hybrid silica significantly delays the dissolution of silica and hydrolysis of bonded phases by introducing organic groups into the silica skeleton, extending the pH tolerance range to 2-12.

Hydrolysis Resistance: Organic groups enhance the chemical inertness of the skeleton structure, especially show more stability in alkaline mobile phases, and extend the service life of columns.

1.2 Separation Performance

Reduced Silanol Effect: XP hybrid silica gel effectively reduces the ion - exchange interaction between basic compounds and silanol groups by decreasing the density of silanol groups. XP series can improve peak tailing and enhances the separation resolution.

1.3 Mechanical Strength and Structural Stability

High Rigid Skeleton: The XP packing media possesses the high strength of silica and the ability to withstand high pressure. Meanwhile, it also has the flexibility of polymers, which can reduce the risk of the packing media breaking.

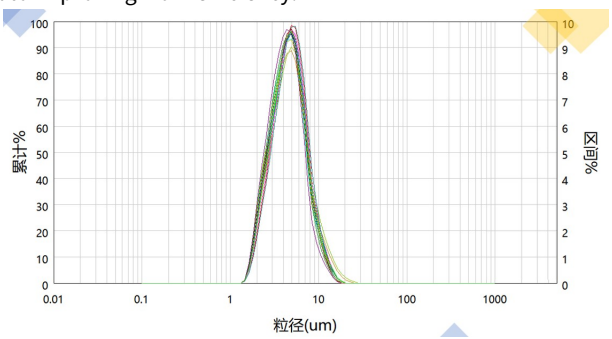
1.4 Expanded Application Range

Industrial Preparation Scaling-up: The stable structure and consistency between batches support seamless scaling-up from laboratory-scale to industrial production, it is beneficial to improving work efficiency.

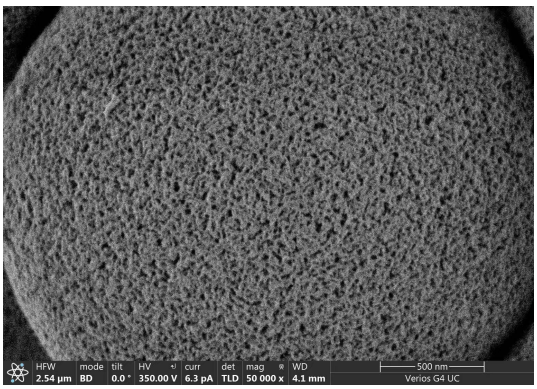
2. Product Performance

2.1 Excellent Particle Size ,Pore Size Distribution and Batch Stability

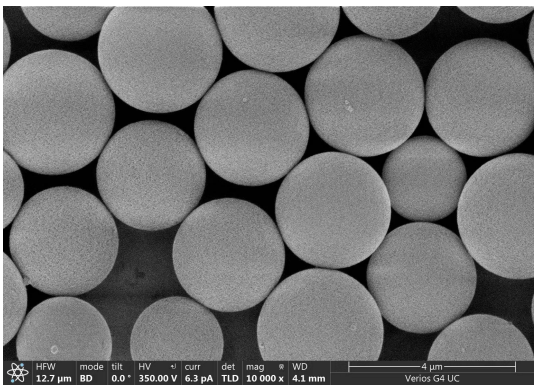
» The batch-to-batch stability of silica synthesis particle , ensures the consistency of subsequent products.



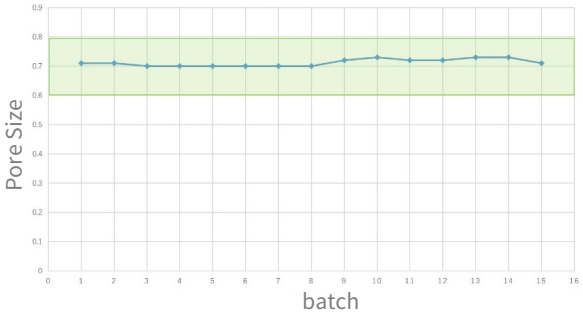
» Homogeneous, penetrating pore structure is a good guarantee of chromatographic performance

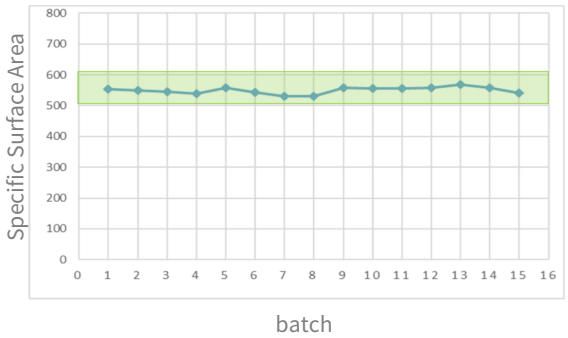


» The control of particle size uniformity is a good guarantee of column efficiency



The particle size of silica gel is stable from batch to batch, ensure products consistency



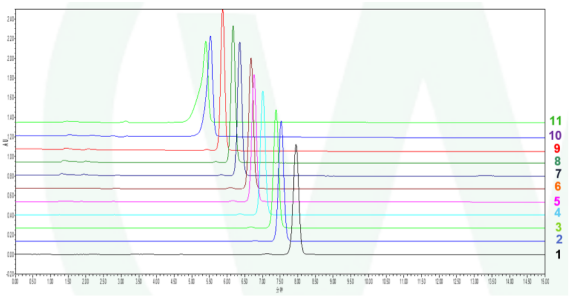


2.2 Alkaline resistance performance

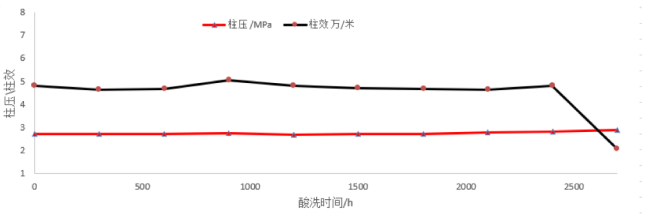
In the pharmaceutical field, strong alkaline conditions (such as NaOH solution) are commonly used for in-situ cleaning (CIP) to remove protein adsorption impurities on the surface of packing media, especially widely used in the purification process of biopharmaceuticals， such as insulin，GLP-1 and so on . Traditional packing media is prone to chemical degradation (such as hydrolysis or bonded phase detachment) under strong alkaline condition, resulting in a shortened service life of packing media. The XP series of hybrid packing media, through innovative organic-inorganic hybrid technology, constructs an alkali-resistant three-dimensional skeleton structure, which can withstand extreme pH conditions of 2-12. Experimental data shows that the packing media still maintains stable performance (column efficiency ⩾ 95%, back pressure ⩽ 110% initial pressure) after 40 hours of CIP cleaning in 0.1M NaOH solution, significantly superior to traditional silica. This outstanding alkaline resistance makes it an ideal choice for processes requiring frequent alkaline cleaning, effectively reducing the cost of consumables and downtime of production process.

Alkaline Wash Test Conditions：
Packing Media： PrePulite® XP C8 10μm
Column： 4.6*250mm
Mobile Phase: ACN: 0.1mol NaOH Solution = 7:3
Flow Rate： 1mL/min
Column Temperature： 30°C
Alkaline Wash Time: 300 min/ time

Column Efficiency Test Conditions：
Mobile Phase： ACN:Water=7:3
Flow Rate： 1mL/min
Column Temperature： 30°C
Standard Sample： 1ml/mL Naphthalene 5μL



Column Efficiency Test After Alkaline Wash of WePure XP packing media



Column Efficiency and Pressure Changes After Alkaline Wash of WePure XP packing media

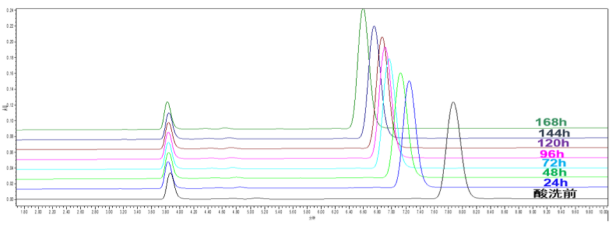
Conclusion: Under the condition of pH=13, the continuous alkaline washing of XP C8 packing media results in a lifespan of 2400 minutes.

2.3 Acid resistance performance

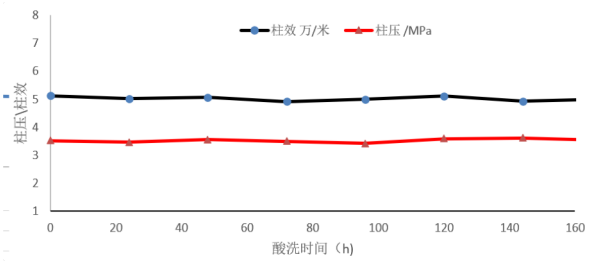
In reverse-phase purification processes, 0.1% TFA acidic mobile phase is commonly used elution system. However, traditional silica matrix packing media is prone to bonded phase breakage under long-term acidic environment. XP hybrid packing media show outstanding acid resistance in 0.1% TFA mobile phase.

Acid Wash Test Conditions：
Packing Media： PrePulite® XP C8 10μm
Column： 4.6*250mm
Mobile Phase： ACN:0.1TFA Solution =7:3
Flow Rate： 1mL/min
Column Temperature： 60°C
Acid Wash Time： 24h/time

Column Efficiency Test Conditions：
Mobile Phase： ACN: Water =7:3
Flow Rate： 1mL/min
Column Temperature： 30°C
Standard Sample： 1ml/mL Naphthalene 5μL



Column Efficiency Test After Acid Wash of WePure XP packing media

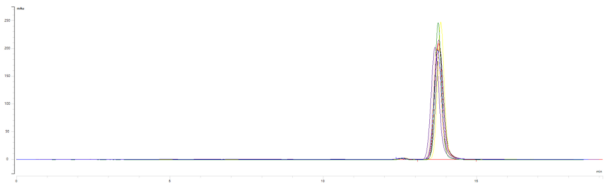


Column efficiency and pressure changes after acid wash of WePure XP hybrid packing media

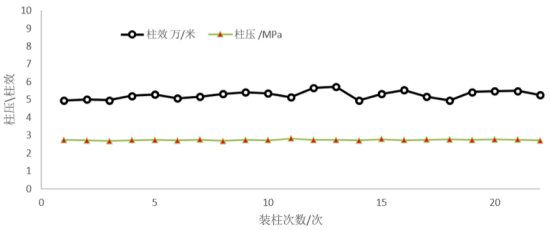
Conclusion: The XP packing is continuously flush at condition of 60°C and pH 1,the packing life is more than 160 hours.

2.4 Mechanical Strength

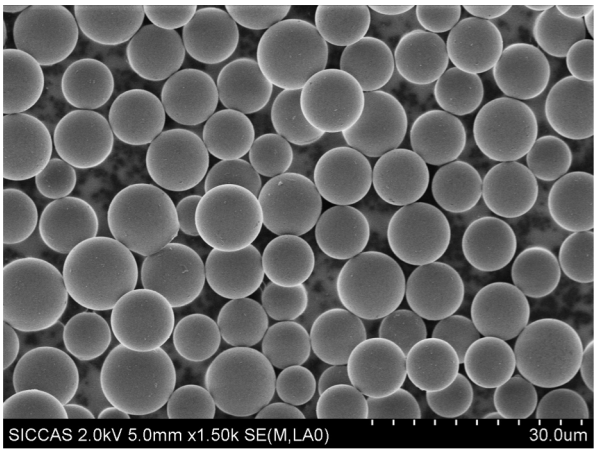
In industrial chromatography purification, the high-frequency loading and unloading operations of the dynamic axial compression (DAC) system put forward strict requirements for the mechanical properties of the packing media. The rigid skeleton design of the XP hybrid packing media (organic-inorganic hybrid) can effectively resist mechanical stress, avoid the production of fragments due to particle breakage, thereby maintaining the stability of the column bed porosity and preventing a sudden rise of column pressure. PrePulite® XP C8 10μm packing media is still intact after being loaded and unloaded 22 times in the DAC.



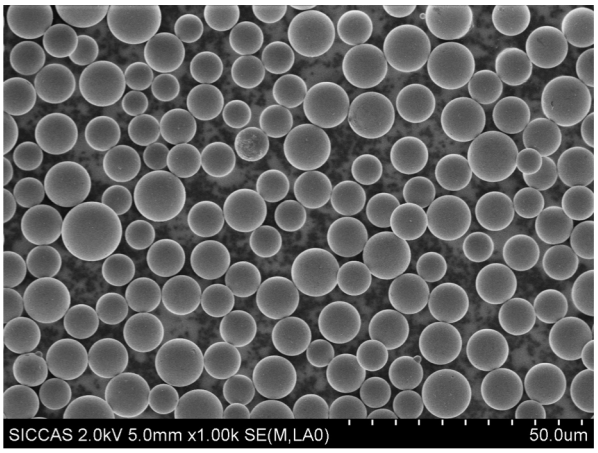
Column Efficiency after Repeated Packing Test of WePure XP Hybrid packing media



Column Efficiency and Pressure Changes After Repeated Packing of WePure XP Hybrid packing media



Before Packing the material



After Packing the material 20 Times

IV. Preparative Column and DAC Packing Method

1. To meet the needs for pilot-scale and semi-industrial scale production, in addition to the conventional columns(4.6*250mm), we also provide the following 15 specifications of pre-packed columns:

Column Length (mm)	Diameter (mm)				
150	10	20	21.2	30	50
250					
300					

2. DAC Column Packing Method

2.1 Main Equipment and Reagents

Table 1: List of Main Equipments and Reagents

Item	Specification	Quantity	Main Purpose	Remarks
Preparation System	As required	1 set	Column packing and column efficiency testing	
Ultrasonic Device	As required by the preparation system	1 unit	Cleaning sieves, homogenizing packing media	Consult the equipment supplier for the ultrasonic device size required for ultrasonic sieves
Air Compressor/Air Source	As required by the preparation system	1 set	DAC loading and operation	Pressure and airflow need to be communicated with the equipment supplier in advance
Homogenization Tank/Bottle	Refer to Table 2	1 set	Used for packing material homogenization	It is recommended to configure a homogenization tank for DAC with a size of 300mm or above
Balance	Accuracy 0.1 gram	1 unit	Weight packing material	
Measuring Cylinder	1L	1 piece	Measuring solvent	
Ruler	Over 30cm	1 piece	Measuring column height	
Packing Media	As required by the process	Refer to Table 2	DAC packing media	
Isopropanol	Chromatographic grade/preparative grade	As needed	Homogenization solution and cleaning reagent	
Acetonitrile	Chromatographic grade/preparative grade	As needed	Mobile phase for column efficiency testing	
Water	Purified water recommended	As needed	Column efficiency testing	
Sodium Hydroxide	Analytical grade	As needed	Cleaning	
Acetic Acid	Analytical grade	As needed	Cleaning	
Acenaphthene	Analytical grade	As needed	Column efficiency test sample	

2.2 Weight of the packing media

The required weight of packing media is calculated according to the following formula:

Weight of packing media (g) = DAC cross-sectional area (cm^2) X Packing height (cm) X Packing density (g/cm^3)

For example, packing a 150mm DAC with XP C8 10-micron packing media to a height of 25 cm, the packing media to be prepared is:

Weight of packing media=0.6 X3.14X7.5X 7.5X 25 =2649g

Table 2. Packing Data for Different Specifications of DAC

Column Inner Diameter (mm)	Packing Media Usage (kg)	Homogenization Solution Volume (L)	Solvent Volume (L)
50	0.3	0.9	0.7
100	1.2	3.6	2.8
150	2.7	7.8	6
300	10.6	30	24
450	23.8	67	54
600	42.0	120	95

Note: The packing media density is assumed to be 0.6g/mL; the column length is calculated as 25cm.

Remarks:
a. The density is different because packing media type is different.When calculating, the actual density of packing media should be used instead of 0.6 g/cm^3 in this case.
b. There will be losses of material during the processes of cyclic packing and disassembly. It is recommended to purchase 10% to 20% more than the theoretical quantity.

2.3 Homogenization Solvent

Choose an appropriate homogenization solvent according to the type of packing media. Isopropanol is recommended for reverse-phase materials, and the homogenization concentration is about 35%. The classic operation is as follows: the volume of the homogenization solvent : the weight of packing media = 2 mL : 1 g.

Taking the 150mm DAC as an example, considering the losses, approximately 2.7 kg of material is actually packed each time, and the volume of the solvent required is approximately 6 L.

2.4 Column Packing

2.4.1 Cleaning

According to the requirements of the DAC manufacturer, clean the frit and other components successively with alkaline solution, hydrophilic solvent, and organic solvent. Clean the inside of the column tube, and finally flush it with the column packing reagent.

2.4.2 Leak Test

It is recommended to use pure water to test whether the system will leak. The column pressure of test should be slightly higher than the actual packing pressure.

2.4.3 Homogenization

Pour the material into the homogenized solution while stirring. In order to remove the bubbles and disperse the material better, it is advisable to ultrasonicate for about 10 minutes. And keep stirring until it is transferred into the column tube.

2.4.4 Packing

Pour quickly the homogenized solution into the DAC column tube, clean the residual packing media on the column head, quickly pack the column, and let it stand for more than 30 minutes after packing, then measure the height of column bed.

2.5. Column Efficiency Test

First, flush the column with the homogenizing solution at a low flow rate for 1 - 2 column volumes, and then replace the homogenizing solution with the mobile phase for column efficiency testing. The flow rate should be increase gradually from low to high.To prevent the baseline from becoming unstable due to heat absorption during solvent mixing, pre - mix the organic phase and water in proportion in advance, stir the mixture, and wait until the temperature returns to room temperature before column efficiency testing.

After packing, the column bed is not yet very stable. If the column efficiency is not satisfactory, you can repeat the column efficiency test several times. If it still fails after 3-5 times testing, it is recommended to test overnight or repack the column.

Column Efficiency Test：
Mobile Phase: Acetonitrile/Water （70/30，v/v）
Flow Rate: 60%-80% of the normal preparation flow rate
Detection Wavelength： 254 nm
Sample: Naphthalene（1mg/mL）

V . Ordering Information

Packing Media Type	Bonded Phase	Particle Size (μm)	Pore Size (Å)	Pore Volume (mL/g)	Specific Surface Area (m^2/g)	End-capping	Carbon Loading	Packaging Specification	Part Number
XP	C8	10	130	0.7	185	Yes	14%	50 100 500 1000	XPC18-10-50G XPC18-10-100G XPC18-10-500G XPC18-10-1000G
XP	C18	10	130	0.7	185	Yes	19%	50 100 500 1000	XPC18-10-50G XPC18-10-100G XPC18-10-500G XPC18-10-1000G

VI Application Case - Semaglutide Purification

Fermentation-Synthesized Semaglutide Purification Process Flow

1. Sample Pretreatment

The crude product is dissolved using a 0.4% ammonium phosphate buffer (pH 8.0–8.5) via ultrasonic dissolution, with a dissolution concentration of 10–30 mg/mL.

2. Crude Product Testing

Testing conditions:

Column：Phenomenex Kinetex C18 2.6μm 4.6mm×150mm

Flow Rate：0.7mL/min

Wavelength：210nm

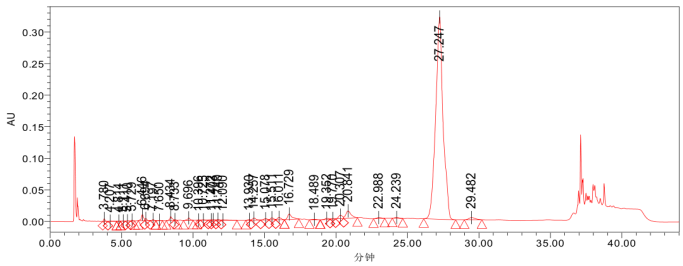
Column Temperature：30°C

Sample concentration: Diluted to 2 mg/mL

Injection volume: 15 μL

Mobile Phase：A Phase： 0.08 mol/L ammonium dihydrogen phosphate buffer (pH 3.6) : acetonitrile = 9:1 (v:v)； B Phase：Acetonitrile : isopropanol : water =3:1:1（v:v:v）

time/min	A%	B%
0	54%	46%
7	47%	53%
37	47%	53%
49	10%	90%
52	10%	90%



峰结果

	名称	保留时间（分钟）	面积（微伏*秒）	高度（微伏）	含量	单位	% 面积
14		10.396	2247	333			0.02
15		10.725	9365	619			0.07
16		11.272	6543	772			0.05
17		11.403	14795	1077			0.11
18		11.740	19154	1703			0.15
19		12.090	15691	646			0.12
20		13.930	15118	1297			0.12
21		14.257	61260	3548			0.47
22		15.078	42117	2059			0.32
23		15.511	56194	3419			0.43
24		16.011	69221	3847			0.53
25		16.729	183007	8760			1.40
26		18.489	19950	899			0.15
27		19.352	34599	1935			0.26
28		19.770	18777	1038			0.14
29		20.307	106099	5528			0.81
30		20.841	288473	11634			2.21
31		22.988	36617	1609			0.28
32		24.239	28723	1373			0.22
33		27.247	11563343	320329			88.55
34		29.482	116616	3504			0.89

Figure 1: Typical chromatogram of the crude product （88.55%）

3. The first step of purification

3.1 The first step is purification conditions：

Column：PrePulite® XP- C8 10μm，4.6mm×250mm

Flow Rate： 1mL/min

Wavelength: 210/280nm

Load capacity: 10g/L (1.7%) for crude product calculation

Mobile Phase：A Phase： 0.1% TFA；B Phase： acetonitrile

time/min	A	B
0	95%	5%
5	72%	28%
85	57%	43%

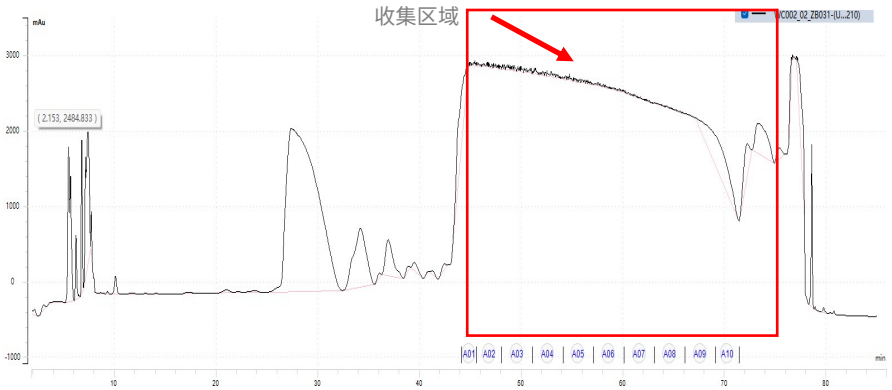


Figure 2: Typical first step purification chromatogram

3.2 Collection of fractions

Collect the main peak in Figure 2 and detect it using the crude peptide detection method.

3.3 Control conditions for qualified fractions in the first step

Purity greater than 99%, with single impurities less than 0.3%. (Yield greater than 90%)

4. Step 2 Purification

4.1 Step 2 Purification Conditions

Column：PrePulite® XP- C8 10μm，4.6mm×250mm

Flow Rate：1mL/min

Wavelength：210/280nm

Sample processing: The first step is to dilute the qualified fraction with water by one volume.

Load capacity: Calculated at 8g/L (1.3%) based on semaglutide.

Mobile phase: Phase A: 0.4% sodium dihydrogen phosphate, pH 2.4; Phase B: Acetonitrile

Time/min	A	B
0	95%	5%
5	69%	31%
125	59%	41%

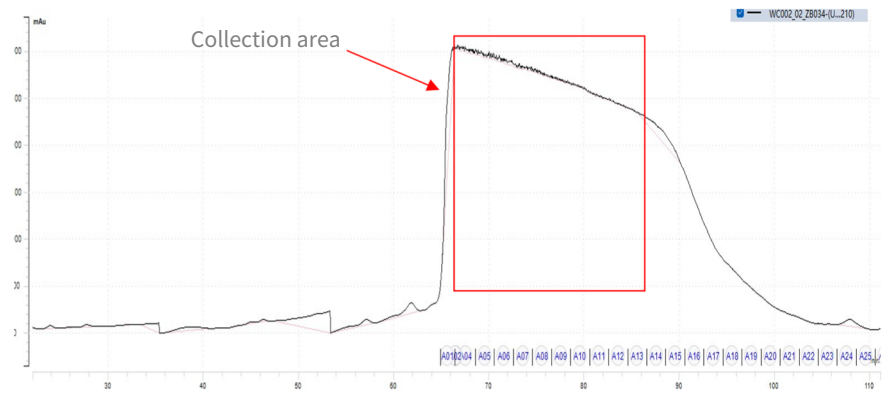


Figure 3: Typical second step purification chromatogram

4.2 Collection of fractions

Collect the main peaks in Figure 3 and detect them using the crude peptide detection method.

4.3 Control conditions for the second qualified fraction

Combine fractions with a purity greater than 99.5% and a single impurity less than 0.1%; Although some impurities exceed 0.1% and only appear in a small segment, after merging all fractions, the impurities are still less than 0.1% and can also be merged into qualified fractions. (The yield of the second step is greater than 85%)

5. Purification results and summary

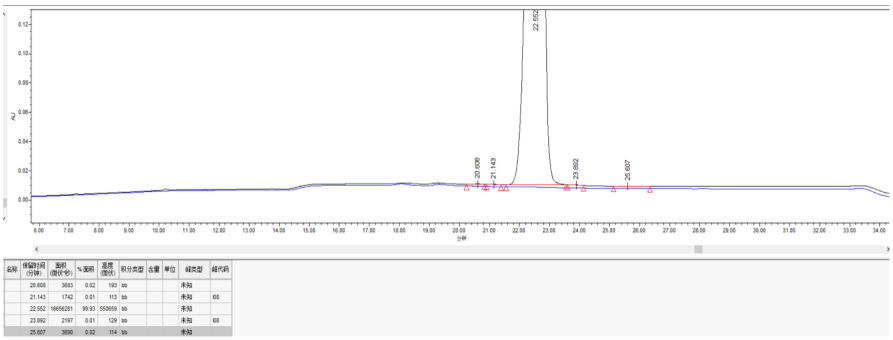


Figure 4: Chromatogram of a typical second step purified qualified fraction

Purification result: The total purification yield after two-step purification is greater than 70%.

Step	Carrying Capacity	Purity	Yield	Total yield
Step 1	10g/L(1.7%)	99.3%	90%	77%
Step 2	8g/L(1.3%)	99.9%	85%	

Purification Process Flow for Fully Chemically Synthesized Semaglutide

1. Sample pre-treatment

The crude product is dissolved in 0.4% ammonium phosphate pH 8.0-8.5 buffer salt by ultrasound, with a dissolution concentration of 10-30mg/mL.

2. Crude product testing

Testing conditions:

Column: Phenomenex Kinetex C18 2.6μm 4.6mm × 150mm

Flow rate: 0.7mL/min

Wavelength: 210nm

Column temperature: 30°C

Sample concentration: Dilute to 2mg/mL

Injection volume: 15μL

Mobile phase: Phase A: 0.08 mol/L ammonium dihydrogen phosphate buffer (pH 3.6): acetonitrile=9:1 (v: v);
Phase B: Acetonitrile: Isopropanol: Water=3:1:1 (v: v: v)

time/min	A	B
0	54%	46%
7	47%	53%
37	47%	53%
49	10%	90%
52	10%	90%

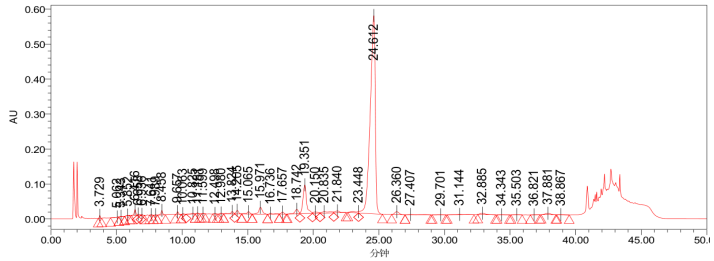


Figure 1: Typical crude chromatogram (purity 77.09%)

3. The first step of purification

3.1 Purification conditions for the first step

Column: PrePulite® XP- C8 10μm，4.6mm×250mm

Flow rate: 1mL/min

Wavelength: 210/280nm

Load capacity: Calculated as 8g/L (1.3%) for crude product

Mobile phase: Phase A: 0.08 mol/L ammonium dihydrogen phosphate buffer (pH 3.6): acetonitrile=9:1 (v: v);
Phase B: Acetonitrile: Isopropanol: Water=3:1:1 (v: v: v)

time/min	A	B
0	95%	5%
5	52%	48%
85	44%	56%

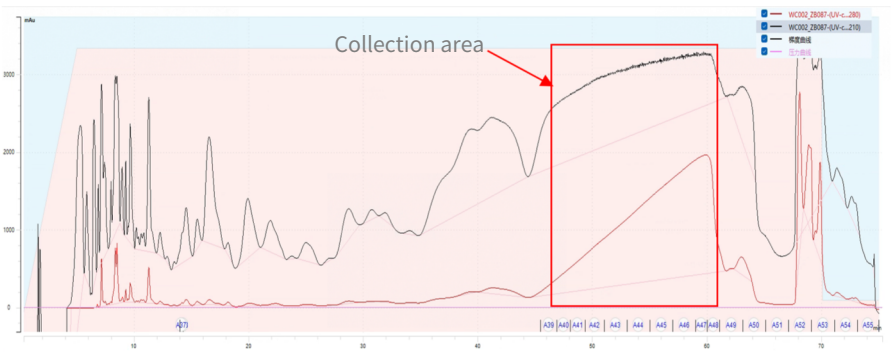


Figure 2: Typical first step purification chromatogram

3.2 Collection of fractions

Collect the main peak in Figure 2 and detect it using the crude peptide detection method.

3.3 Control conditions for qualified fractions in the first step

Purity greater than 95%, RRT=0.88, single impurity less than 0.05%, remaining single impurities less than 0.3%, RRT0.66 less than 0.1%, RRT1.08 less than 1.5%. (Yield greater than 80%)

4.Step 2 Purification

4.1 Purification conditions for the second step:

Chromatography column: PrePulite® XP- C8 10μm，4.6mm×250mm

Flow rate: 1mL/min

Wavelength: 210/280nm

Sample treatment: in the first step, dilute the qualified fraction with 0.4% ammonium phosphate pH8.0-8.5 buffer salt by one volume or remove half of the volume of acetonitrile by spinning, or dilute it with water and adjust the pH between 6.5 and 8.0.

Load capacity: 8g/L (1.3%) calculated based on semaglutide.

Mobile phase: Phase A: 0.2% sodium dihydrogen phosphate, pH 6.5; Phase B: Acetonitrile

Time/min	A	B
0	95%	5%
5	76%	24%
89	64%	36%

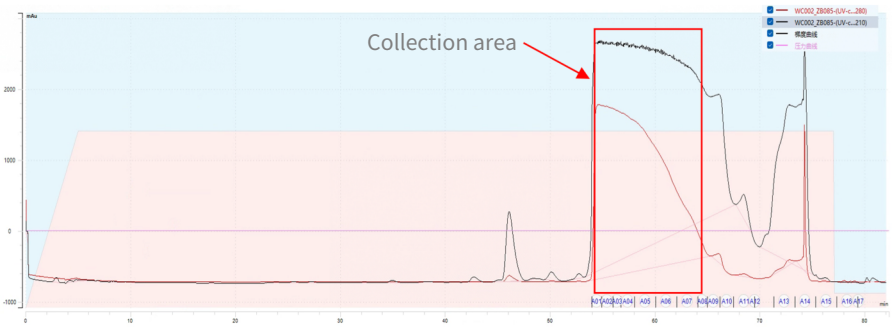


Figure 3: Typical second step purification chromatogram

4.2 Collection of fractions

Collect the main peaks in Figure 3 and detect them using the crude peptide detection method.

4.3 Control conditions for qualified fractions in the second step

Combine fractions with a purity greater than 98.5% and a single impurity less than 0.1%; Although some impurities exceed 0.1% and only appear in a certain segment, after merging all fractions, the impurities are still less than 0.1% and can also be merged into qualified fractions. (The yield of the second step is greater than 80%)

5. Purification results

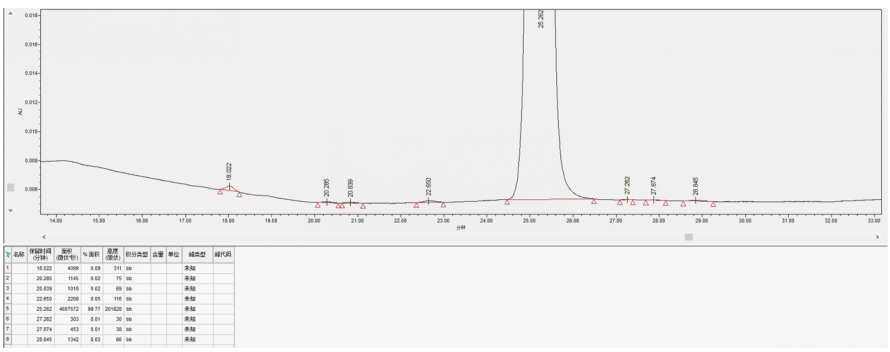


Figure 4: Chromatogram of a typical second step purified qualified fraction

Preparation results: The yield of both purification steps is greater than 80%, and the total purification yield is greater than 65%.

Step	Carrying Capacity	Purity	Yield	Total yield
Step 1	8g/L(1.3%)	98.5%	80%	65%
Step 2	8g/L(1.3%)	99.7%	80%	

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