Vydac® 150HC Media for Insulin Purification

purification technologies

Introduction

Insulin is a peptide hormone, produced by beta cells of the pancreas, and regulates carbohydrate and fat metabolism in the body. Insulin causes cells in the liver, skeletal muscles, and fat tissue to absorb glucose from the blood. Biosynthetic insulin is manufactured for widespread clinical use using recombinant DNA technology in order to treat diabetes.

It is estimated that growth of insulin production will continue at a brisk 9%[1] year over year as the incidence of diabetes continues to grow and new technologies allow greater transport and access to insulin globally. Currently, there are nearly 6,000 new insulin drugs in clinical trial studies[2].

Biopharmaceuticals, are much more likely to require purification chromatography due to the complexity of sample matrixes. One of the earliest classes of biopharmaceuticals to use chromatography in its production was human insulin, specifically reversed phase chromatography. This powerful purification technique continues to be the method of choice due to the degree of resolution and purity it provides.

Vydac® 150 HC Media - Ideally Suited for the Demands of Insulin Purification

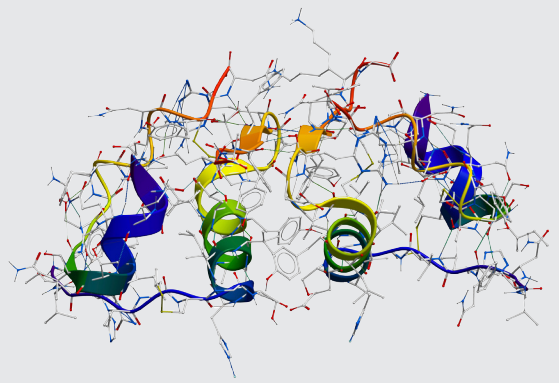
To address the growing demand for effective peptide purification, Grace has developed the new Vydac® 150HC purification media. Vydac® media has been a trusted name in biopurification for over 30 years. The new Vydac® 150HC media is highly effective at purifying many small to medium-sized peptides with greater loading capacity and improved productivity compared to competitive media. It has unique selectivity that can reveal peaks masked by other C18 media and improves resolution of closely related peptides and impurities for higher purity target peptides.

The bar is continually being raised for process development teams to improve production methods and increase productivity and yield. The new Vydac® 150HC reversed phase chromatography media is designed for increased loading and capacity which improves overall production and throughput. The added capacity of Vydac® 150HC C18 provides additional resolution needed to obtain high purities, allowing greater sample loading for increased throughput.

Vydac® 150HC is available in bulk quantities of 10 or 20µm particles and is designed for use in preparative and process scale purification. Scout and preparative columns are also available.

[1] EvaluatePharma® - World Preview 2018 Beyond the Patent Cliff. June 2012
[2] U.S. NIH Clinical Trials Website - http://www.clinicaltrials.gov/ct2/results?term=insulin&Search=Search. As of July 2013

Molecular Structure of Insulin - Human insulin is composed of 51 amino acids and has a molecular weight of 5808 Da.



Vydac® 150HC Media Specifications	
10µm Part No.:	5167350
20µm Part No.:	5149682
Phase	C18
Pore Size	150Å
Pore Volume:	1.05mL/g
Surface Area:	320m²/g
Carbon Load	16%

www.discoverysciences.com

Materials for Pharmaceutical Manufacturing

synthesis intermediates

purification technologies

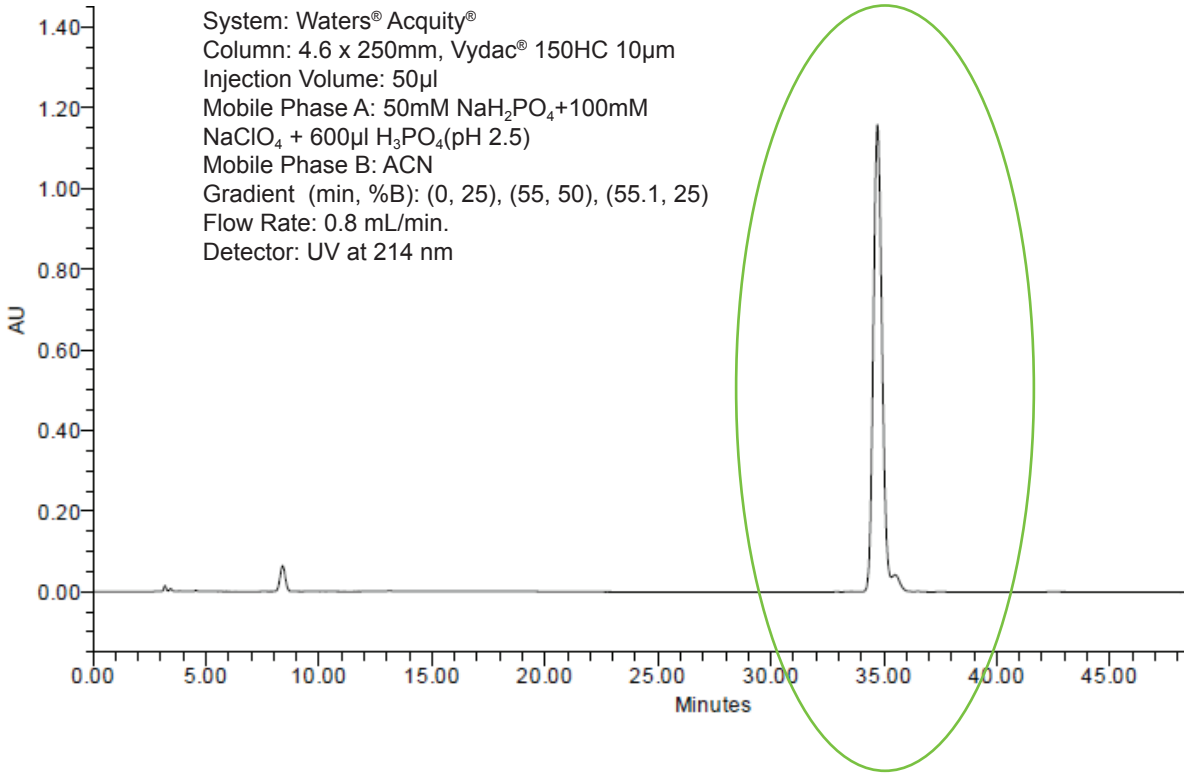
formulation and delivery

GRACE
Materials Technologies

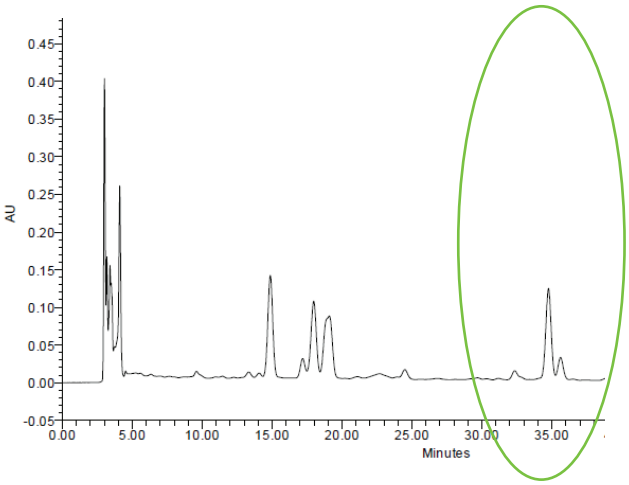
Vydac® 150HC Media - Chromatographic Results on Crude Insulin Samples

A variety of processes are used to purify insulin. Reversed phase HPLC is often chosen to remove the insulin-like components due to its high resolution capabilities. Some insulin variants differ by as little as a single amino acid.

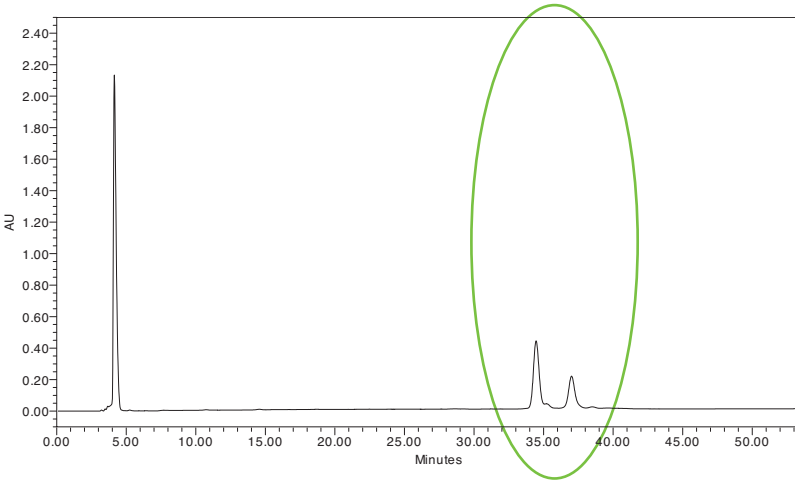
Insulin Standard



Crude Insulin Sample 1



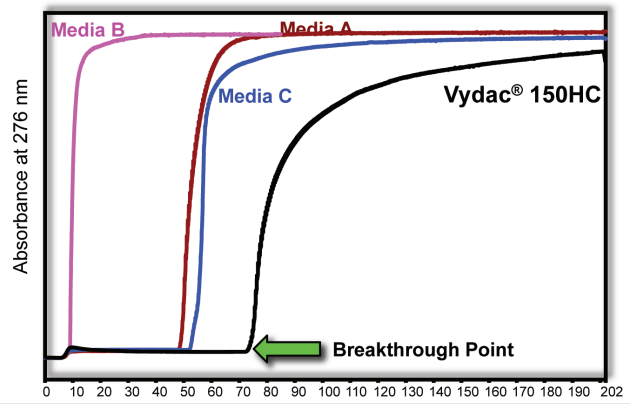
Crude Insulin Sample 2



High Loading for Improved Productivity

Loading capacity and purity are essential to improving overall productivity in process purification. By increasing loading capacity while maintaining or improving purity, more sample can be purified in the same amount of time. The unique physical properties of the Vydac® 150HC C18 media increase loading capacity by 2-3X, allowing more sample to be loaded onto the column while processing. Ultimately, this reduces the amount of purification cycles needed to process a batch. Couple this with lower overall pressure and the possibility for increased flow rates, and significant time savings can be realized.

Insulin Frontal Loading



Column	Loading Capacity (mg/mL media) ~100% Aqueous
Vydac® 150HC C18, 20µm	**361**
Media C, C18 15µm	180
Media B, C18 10µm	18
Media A, C18 15-20µm	249

The capacity, Q, of each material is calculated from the following equation:

$$Q = \frac{CF_v(t_{10\%} - t_0 - t_{step} - t_e)}{V_C - F_v t_o}$$

C = Polypeptide concentration in mobile phase B

F = Flow rate

$t_{10\%}$ = Frontal breakthrough time measured at 10% height

t_0 = Column hold-up is determined from an injection of uracil in 90:10 Acetonitrile/Water

t_e = Extra-column time, or transit time between pump mixer and column inlet, and between column outlet and detector cell

V_c = Column volume.

Scaling-up the Purification Process

After optimizing the insulin purification method at laboratory scale, you can calculate scale-up parameters for use in pilot and process scale. Using Vydac® 150HC media from laboratory through process scale will help minimize scale-up experiments required and give predictable results at larger scale with similar recoveries and elution patterns.

The proportions of Vydac® media and insulin load need to be adjusted based on the internal volume of the column. In order to maintain the same elution pattern when scaling a separation between columns of the same phase but different inner diameters, gradient volume must also be scaled in proportion to column volume.

Technical Reference:
Basic Process Scale-up Calculations

Flow Rate (Constant Linear Velocity)

for $L_{final} = L_{initial}$

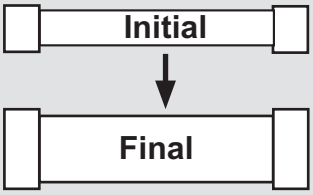
$$\text{Flow Rate}_{final} = \text{Flow Rate}_{initial} * \frac{(D_{final})^2}{(D_{initial})^2}$$

Sample Load

$$\text{Load}_{final} = \text{Load}_{initial} * \frac{(D_{final})^2 L_{final}}{(D_{initial})^2 L_{initial}}$$

D = Diameter

L = Length



Gradient Volume

The volume of a gradient is given by:

$V_g = T_g F$

where:

T_g = gradient time

F = mobile-phase flow rate

Column Volume

The bed volume of a cylindrical column is given by the formula:

$V_c = (0.25)\ (\pi)\ (D^2)\ (L)$

where:

D = column bed diameter

L = column bed length

Note that for columns of equal length, column volume is proportional to the square of the diameter.

Need more process scale-up information? Request application notes AN123, AN127, AN131. Or, contact your Grace representative.

Vydac® Media Lifetime and Stability

Selecting a media that is robust and withstands loading of peptide mixtures is critical for long term productivity and consistency. Compared to competitive media the Vydac® 150HC 20µm media exhibits a lower pressure drop and maintains column performance after multiple purification cycles. Starting with a low pressure drop media enables multiple peptide mixtures be processed before column pressures build.

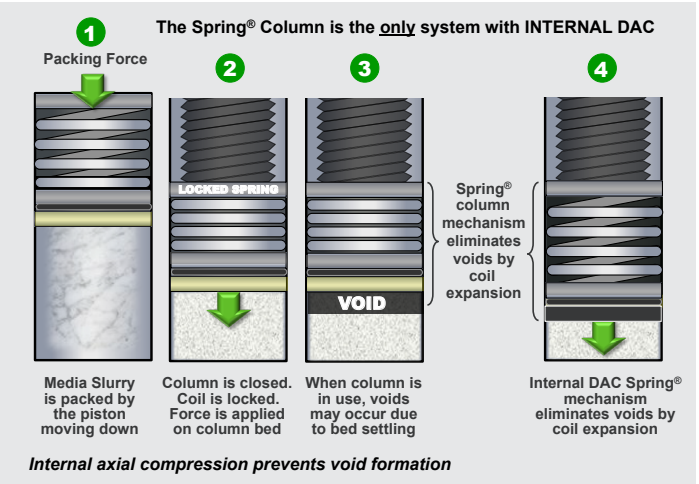
QC Test Mix (Values for Toluene) (at 254 nm)						
Injection #	**1**	**2**	**3**	**4**	**5**	**6**
Column Volumes	**0**	**250**	**500**	**750**	**1000**	**1250**
Retention Time (min.)	12.68	13.1	11.86	11.53	11.41	12.40
Plates/meter	**11137**	**10890**	**10999**	**11866**	**10458**	**10898**
Width (at 50% height)	0.56	0.59	0.53	0.49	0.52	0.55
Pressure (bar)	14	14	15	15	15	16

Efficiency maintained over 1250 column volumes for prep column (25mm i.d. x 25.5cm bed length Spring® column).

Further Extend Column Lifetimes with Dynamic Axial Compression

Spring® Columns

The Spring® Column is the DAC system of choice for chromatographers who need high performance, robust, and long lasting columns. This highly versatile Dynamic Axial Compression (DAC) technology is self-contained in a small and portable package, providing highly advanced and flexible technology for preparative and chromatography columns.



MultiPacker® Packing Station

With the MultiPacker® packing station, the DAC mechanism remains contained within the Spring® column. This allows continued DAC benefits once the column is removed from the packing station and the ability to pack multiple columns with a single unit. The MultiPacker® system incorporates several safety features to give users maximum confidence and peace of mind to perform in-house packing.

Pack multiple DAC columns and use independently of packing device!

Need more information about Spring® Columns or the Multipacker® Instrument? Request Tech Note M301. Or, contact your Grace representative.

W. R. Grace & Co.-Conn.
7500 Grace Drive, Columbia, MD 21044

IN THE AMERICAS:
2051 Waukegan Road
Deerfield, IL 60015-1899
Tel: 1 847 948 8600
Fax: 1 847.948.1078
Email: discoverysciences@grace.com

IN EUROPE:
Brandstraat 12
B-9160 Lokeren, Belgium
Tel: +32 (0)9-340-65 65
Fax: +32 (0)9 340 65 60
Email: discoverysciences.EU@grace.com

IN ASIA:
19th Floor, K.Wah Center
1010 Huai Hai Zhong Road
Shanghai, 200031 PRC
Tel: 86 21 5467 4678
Fax: 86 21 5405 1500
Email: dsbiz.asia@grace.com

IN INDIA:
17 Commerce Center
Opposite Krishna Hospital, Paud Road
Pune, 411038 India
Tel: +91 20 6644 9900
Fax: +91 20 2544 1740
Email: ananth.sc@grace.com

IN AUSTRALIA/NEW ZEALAND:
2 Kerr Court
Rowville, 3178
Victoria, Australia
Tel: +61 3 9237 6100
Fax: +61 3 9237 6101
Email: discoverysciences.AU@grace.com

08/2013 M452