GRACE
WHITEPAPER

Materials for Pharmaceutical Manufacturing

Vydac® 150HC Purification Media

Greater Loading Capacity and Resolution for Improved Productivity in Peptide Purification

Technical Development:
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M416

Background

Peptide therapeutics have been recognized as delivering the best of both worlds in terms of small molecule synthesis efficiency and biologics efficacy and reduced toxicity. However, challenges in mass production and low oral availability have historically made them less than ideal drug candidates. Recent advances in peptide synthesis technologies now enable manufacturing of complex peptides on a very large scale. In addition, emerging formulation and drug delivery technologies have improved the potency and oral delivery of peptide therapeutics.

Both of these developments have enabled new possibilities for peptide therapeutics. The process of synthesizing peptides however can produce many different side reactions and impurities. High capacity chromatographic media and purification strategies can increase loading can streamline manufacturing and increase production yields. This work demonstrates how the new Vydac® 150HC media, developed specifically for peptide purification, can improve peptide manufacturing productivity by increasing loading capacity.

Growth of Peptide Therapeutics

There has been a growing trend towards peptides as therapeutic agents in recent years. This has been driven by the many inherent benefits peptides provide over small molecule drugs and recent developments that overcome past limitations in manufacturing and delivery of peptide therapeutics.

The failure of combinatorial processes to successfully deliver viable small molecule drug candidates has renewed interest and exploration of biologic therapeutic targets. In addition, peptides often have higher potency and higher efficacy on the desired target, minimal side effects and interactions, offer greater biological and chemical diversity, and have reduced number of side effects.

Challenges of Peptide Purification

In peptide synthesis, many side reactions occur that generate impurities. These impurities often are chemically similar to the target peptide and therefore present challenges in isolation of the peptide of interest. The most powerful method for peptide purification is reversed phase chromatography. For industrial purification, important consideration and selection of particle size, pore size, and stationary phase in relation to the peptide can optimize purification. Efficient column packing and use of dynamic axial compression can further improve results.

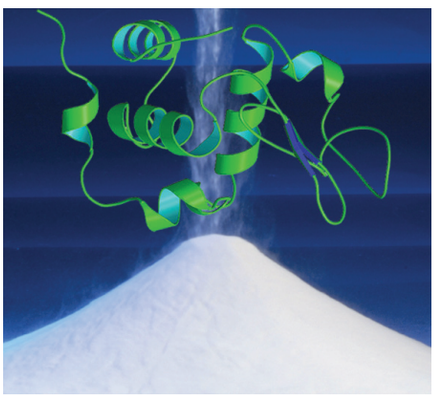
A New High Capacity Media for Peptide Purification

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.

Vydac® 150HC Media Specs	
Phase	**C18**
Pore Size	**150Å**
Pore Volume:	**1.05mL/g**
Surface Area:	**320m²/g**
Particle Size	**10µm, 20µm**
Carbon Load	**16%**

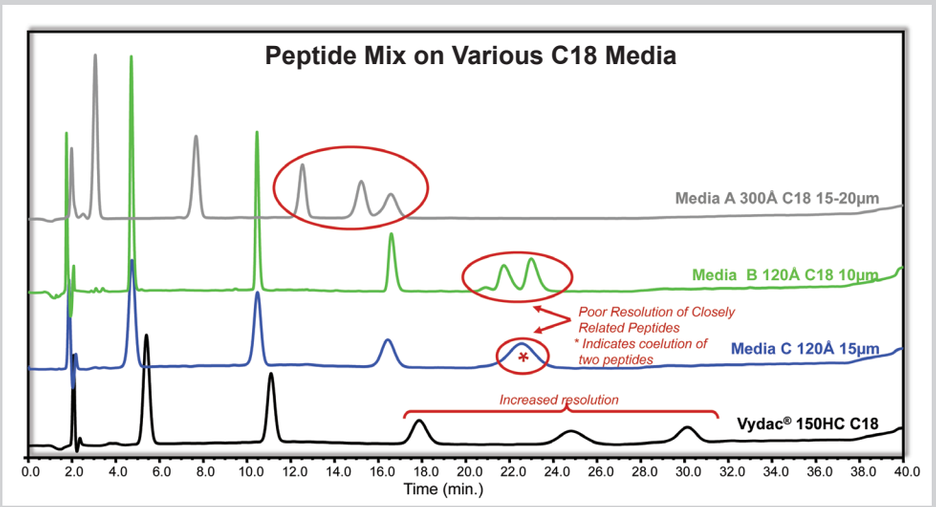
Abstract

The demand for high purity peptides is increasing. Small synthetic peptides to large cellular produced peptides are being investigated for possible therapeutic benefits. Both can be difficult to purify to high levels, >98%, because of the very similar products, many times differing by only one amino acid. Optimized purification techniques are required to meet these high purity demands in an economical manner. Reversed-phase chromatography, because of its high resolving power, has been the technique of choice for achieving the high level of purity necessary in the pharmaceutical industry. For industrial purification, important consideration and selection of particle size, pore size, and stationary phase in relation to the peptide can optimize purification. We illustrate how the new Vydac® 150Å reversed-phase media is highly effective at purifying peptides with greater loading capacity and improved productivity compared to competitive media. The media has unique selectivity that can reveal peaks masked by other C18 phases and improves resolution of closely related peptides and impurities for higher purity target peptides. The bulk media incorporates bonded phase chemistries identical to those used in analytical and prep columns, thereby assuring economical method development and reliable scale-up for preparative and process purification. Media packed in dynamic axial compression MODcol® Spring® columns demonstrate high efficiency and extended lifetime.



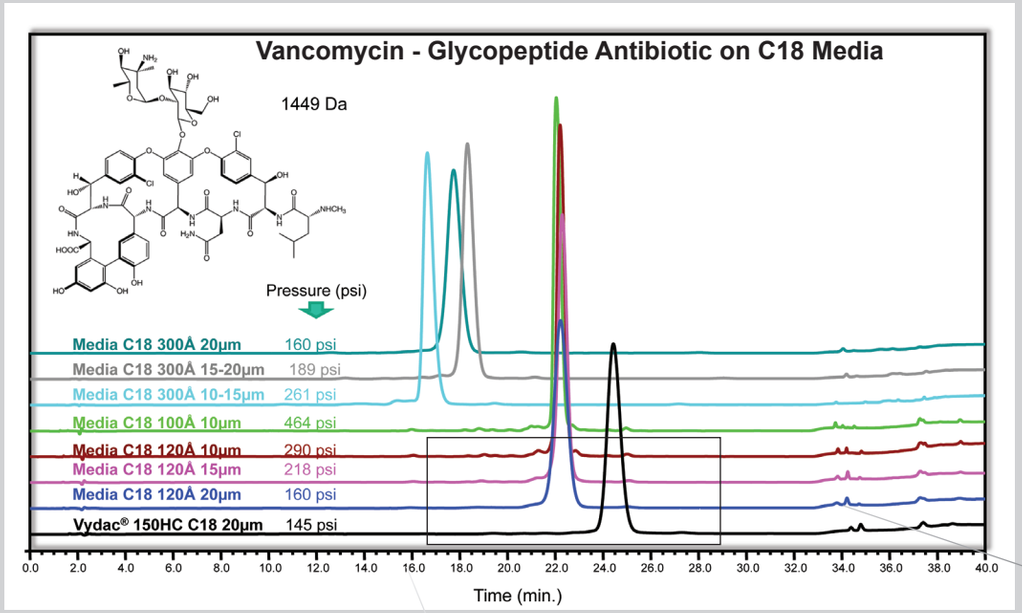
Using Selectivity as a Separation Tool

Often different reversed phase media will provide the same overall selectivity making it difficult to troubleshoot and find solutions for challenging separations. If achieving separation and purity requirements on one type of chromatographic media does not work then selecting a new media with different selectivity is critical to obtaining optimum performance. Vydac® 150HC C18 media is unique and provides different selectivity when compared to other 100Å or 300Å media.

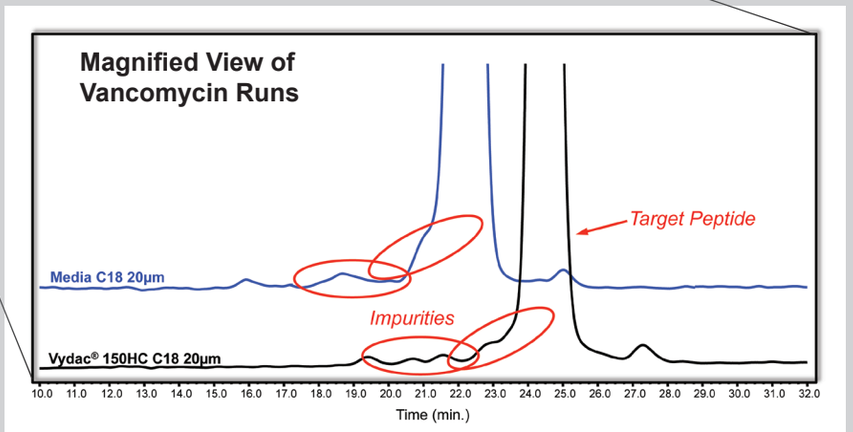


1. GY (238 Da)
2. VYV (379 Da)
3. Met Enkephalin (YGGFM, 573 Da)
4. Leu Enkephalin (YGGFL, 555 Da)
5. Angiotensin II (DRVYIHPF, 1045 Da)

Column: 4.6 x 150mm
Mobile Phase A: 0.1% TFA in Water
Mobile Phase B: 0.085% TFA in Acetonitrile
Gradient (min., %B): (0, 10), 10, 22), (25, 22), (35, 40), (40, 90), (50, 90)
Flow rate: 1.0 mL/min.



Column: 4.6x150mm
Mobile Phase A: 0.1% TFA in Water
Mobile Phase B: 0.085% TFA in Acetonitrile
Gradient (min., %B): (0.0, 5), (30, 20), (35, 80), (40, 80)
Flow Rate: 1 mL/min.
Detector: UV at 225 nm
Column Temperature: 22°C
Sample Prep: Dissolve in 0.1% TFA in Water



High Loading for Improved Productivity

Loading capacity and purity are essential to improving overall productivity in process purification of peptides. 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 on to the column while processing. Ultimately reducing the amount of purification cycles needed to process a batch. Couple this with lower overall pressure and the possibility for increased flow rates, significant time savings can be realized.

Frontal Loading Tests

For nearly 100% aqueous loading experiments, each column (2.1 x 50 mm) is tested with the following conditions: mobile phase including solvent A comprising 0.1% v/v TFA in water; and solvent B comprising 250 mg polypeptide in 5 mL acetonitrile, 2 mL 50% glacial acetic acid and 43 mL DI water. This is diluted down 1:5 with 0.1% TFA in DI water to make the 1 mg/mL polypeptide solution. A gradient process is used wherein the column is equilibrated at 100% solvent A; followed by increasing from 0% up to 100% solvent B for 1 min.; holding flow of solvent B at 100% for 100 min.; followed by increasing from 0% up to 100% solvent A (0% solvent B) for 1 min. The flow rate is 0.2 mL/min. The columns are run at a room temperature of 25ºC. UV detection at 276 nm. Loading experiments also are performed with 10% final acetonitrile concentration in solvents A and B.

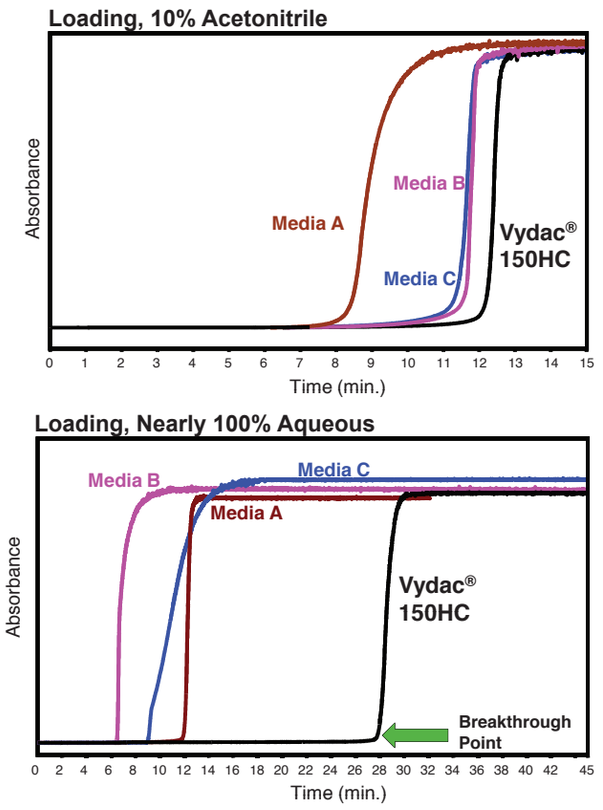
Column	Uracil Retention (min.)	10% Acetonitrile Loading Capacity (mg/mL media)	Vancomycin Loading Capacity ~100% Aqueous (mg/mL media)
Vydac® 150HC, C18 20µm	**0.65**	**49**	123
Media C, C18 15µm	0.57	33	26
Media B, C18 10µm	0.51	28	14
Media A, 15-20µm	0.67	35	53

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

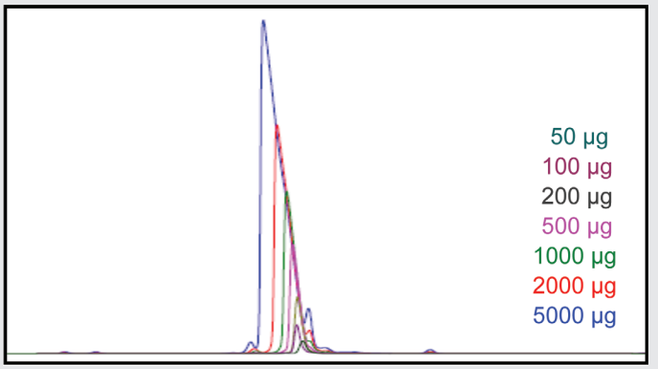
$$Q = \frac{CF_v(t_{10\%} - t_0 - t_{step} - t_e)}{V_C - F_v t_o}$$

- C = Polypeptide concentration in mobile phase B
- F = Flow rate
- $t_{10\%}$ = Frontal breakthrough time measured at 10% height
- t_0 = Column hold-up based on injection of uracil in 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.

Vancomycin Frontal Loading



Vancomycin Loading on Vydac® 150HC C18 Media

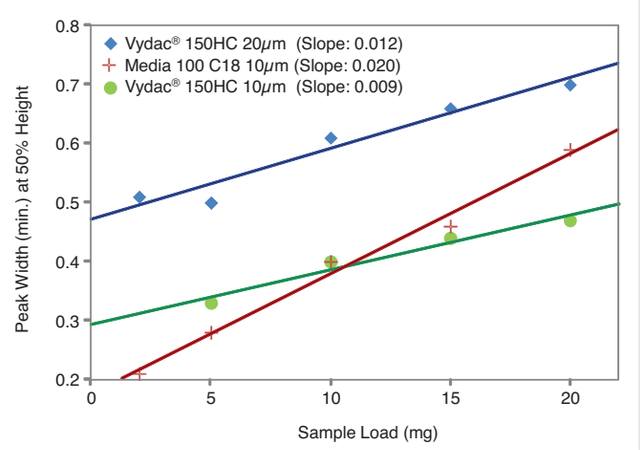


Column: 4.6 x 250mm, 20µm
Injection volume: 500 µL (fixed for all 5 samples)
Diluent: 0.1% TFA in Water
Mobile Phase A: 0.1% TFA in Water
Mobile Phase B: 0.05% TFA in Acetonitrile
Flow Rate: 1 mL/min.

Time (min)	%A	%B
0	95	5
25	60	40
27	20	80
30	20	80

Vancomycin (at 300nm)							
Sample (mg)	**0.05**	**0.1**	**0.2**	**0.5**	**1.0**	**2.0**	**5.0**
Plates/meter	35660	49904	28848	17984	10536	5328	2752
Width (at 50% ht)	0.35	0.29	0.38	0.47	0.60	0.81	1.08

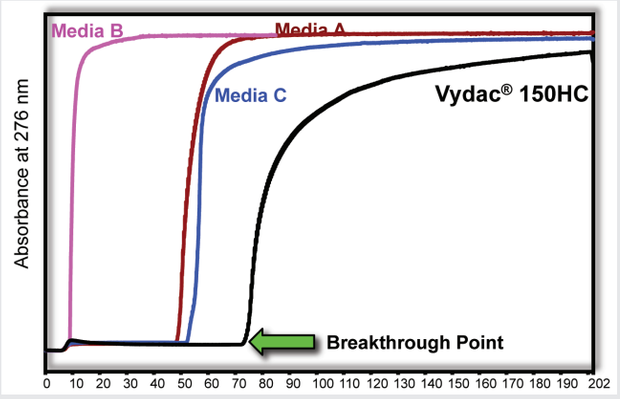
Vancomycin Loading Plot for 22 x 250mm Columns



Plot of peak width at 50% peak height vs. absolute loading

The slope of the plot indicates less variation across different sample loads for Vydac® 150HC vs. competitive media. Also, the 20µm Vydac® 150HC demonstrates comparable chromatographic performance with greater loading capacity than the competitive 10µm media and with ~3X lower backpressure. Less backpressure can help extend column lifetimes and also allows for faster flow rates which improves overall productivity.

Insulin Frontal Loading

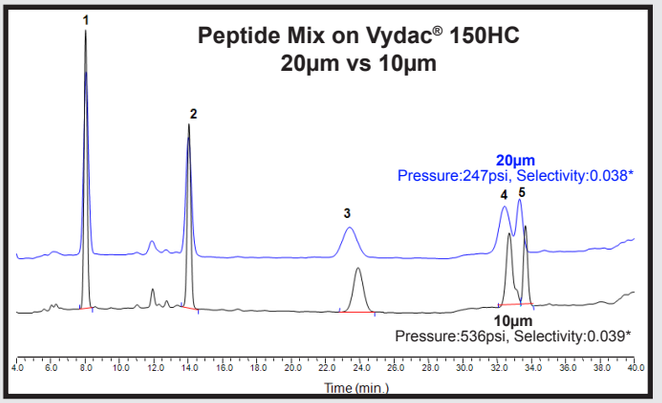


Column	Loading Capacity (mg/mL media) ~100 Aqueous
Vydac® 150HC C18, 20µm	**361**
Media C, C18 15µm	180
Media B, C18 10µm	18
Media A, C18 15-20µm	249

Available 10µm Vydac® 150HC Media

The new 10µm particle media improves performance and is well suited for smaller scale purification. The 10µm media delivers the same selectivity and increased efficiency as the 20µm Vydac® 150HC media.

Same Selectivity, Increased Efficiency for Smaller Scale

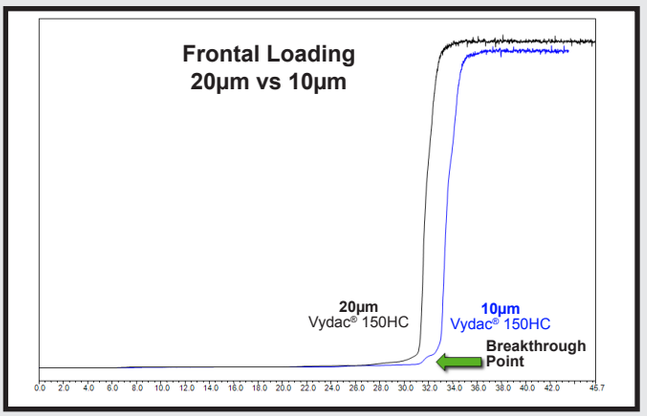


Calculation for Peptide Selectivity =
(Peak 5 retention - Peak 4 retention) / (Peak 3 retention)

1. GY (238 Da)
2. VYV (379 Da)
3. Met Enkephalin (YGGFM, 573 Da)
4. Leu Enkephalin (YGGFL, 555 Da)
5. Angiotensin II (DRVYIHPF, 1045 Da)

Column: 4.6 x 250mm
Mobile Phase A: 0.1% TFA in Water
Mobile Phase B: 0.085% TFA in Acetonitrile
Gradient (min., %B): (0, 10), 10, 22), (25, 22), (35, 40), (40, 90), (50, 90)
Flow rate: 1.0 mL/min.
Detector: UV at 225 nm
Column Temperature: 22°C

10µm Demonstrates Comparable Vancomycin Frontal Loading Capacity



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

Vancomycin (at 254 nm)				
Injection #	**1**	**2**	**3**	**4**
Column Volumes	**0**	**500**	**1000**	**1250**
Retention Time (min.)	6.43	6.47	6.54	6.52
Plates/meter	10494	10447	9823	10031
Width (at 50% height)	**0.29**	**0.29**	**0.31**	**0.30**
Pressure (bar)	19	19	20	21

Efficiency maintained over 1250 column volumes.

Further Extend Column Lifetimes

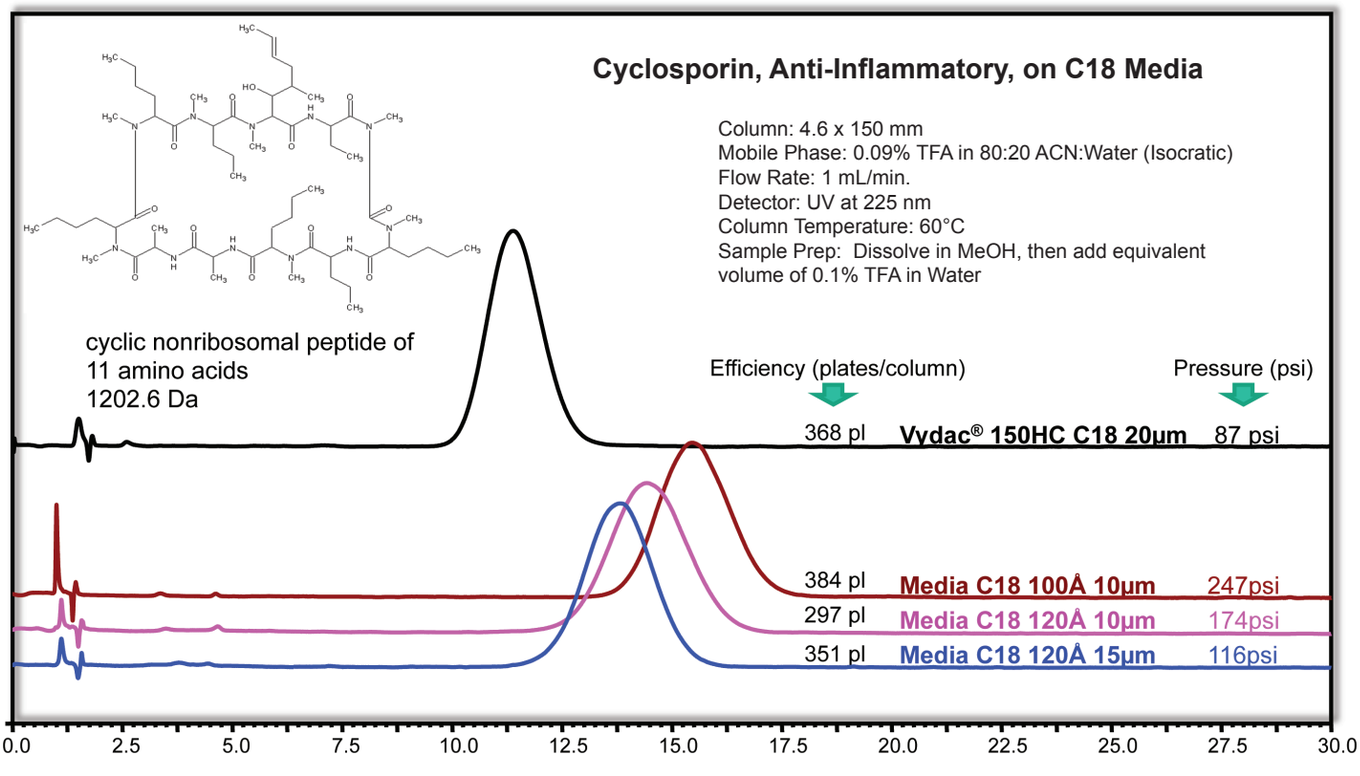
Spring® Columns

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.

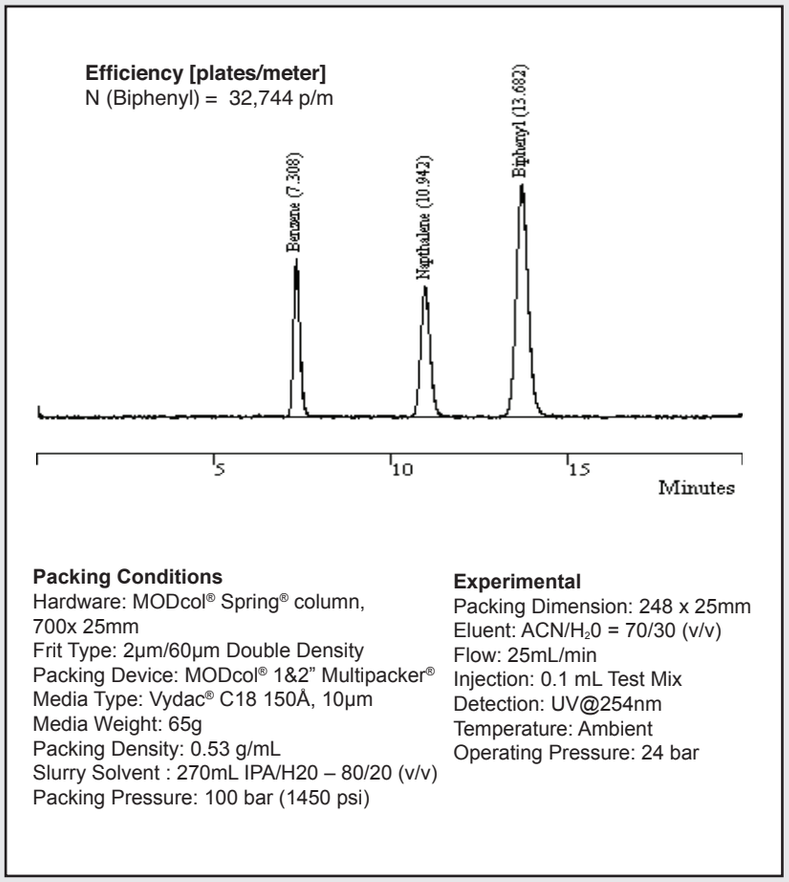
MultiPacker® Packing Station

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.

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



Vydac® 150HC 10µm Media Packed in 1" Spring® Column



Packing Conditions
Hardware: MODcol® Spring® column, 700x 25mm
Frit Type: 2µm/60µm Double Density
Packing Device: MODcol® 1&2" Multipacker®
Media Type: Vydac® C18 150Å, 10µm
Media Weight: 65g
Packing Density: 0.53 g/mL
Slurry Solvent : 270mL IPA/H20 – 80/20 (v/v)
Packing Pressure: 100 bar (1450 psi)

Experimental
Packing Dimension: 248 x 25mm
Eluent: ACN/H_20 = 70/30 (v/v)
Flow: 25mL/min
Injection: 0.1 mL Test Mix
Detection: UV@254nm
Temperature: Ambient
Operating Pressure: 24 bar

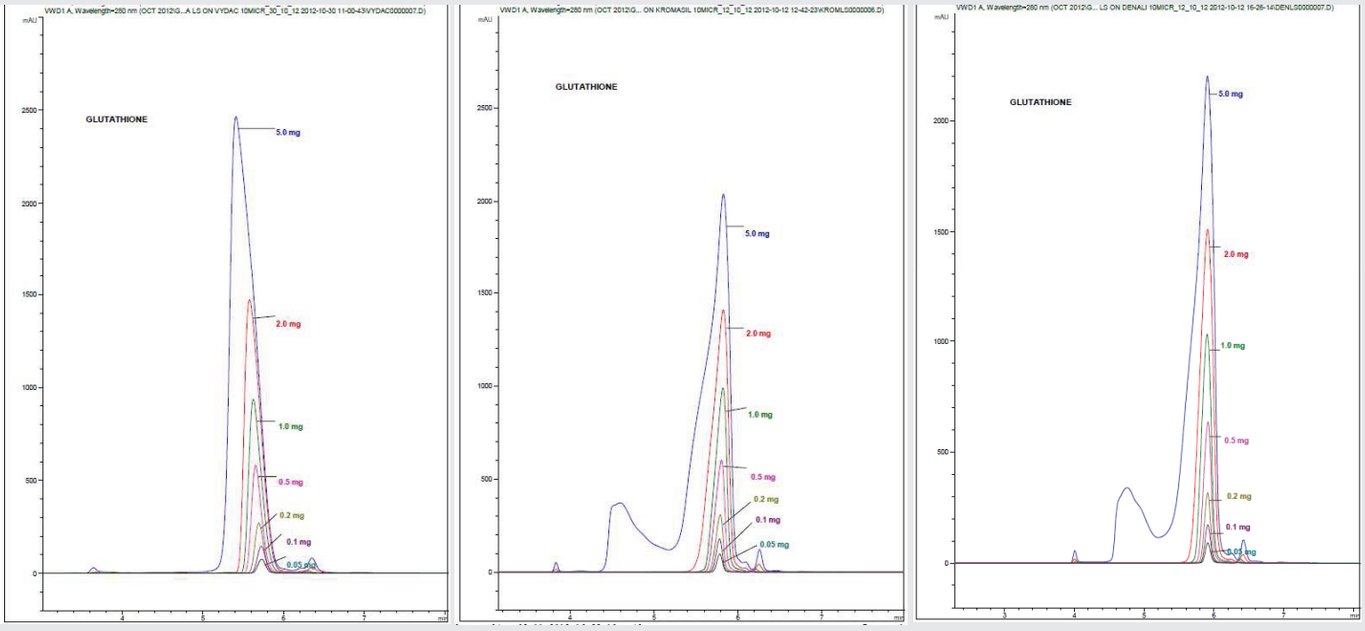
Signal	No.	Substance	Ret. Time	Plate Count	Asymmetry (10%)
UV	1	Benzene	7.31min	8167	1.11
	2	Napthalene	10.94min	8198	1.09
	3	Biphenyl	13.68min	8120	1.07

Vydac® 150HC Media Packing Efficiency

- Good peak symmetry and efficiency for 10µm and 20µm media
- Packing density: 0.49 to 0.56g/mL
- Less Vydac® media required to pack column vs. traditional media. Along with Vydac® media's higher capacity, translates to significantly lower cost.

Oxidized L-Glutathione (GSSG) Loading

At higher loading (5 mg) of GSSG at analytical scale, the Vydac® 150HC 10µm column maintained good peak shape, while peak splitting was observed on the other media columns.



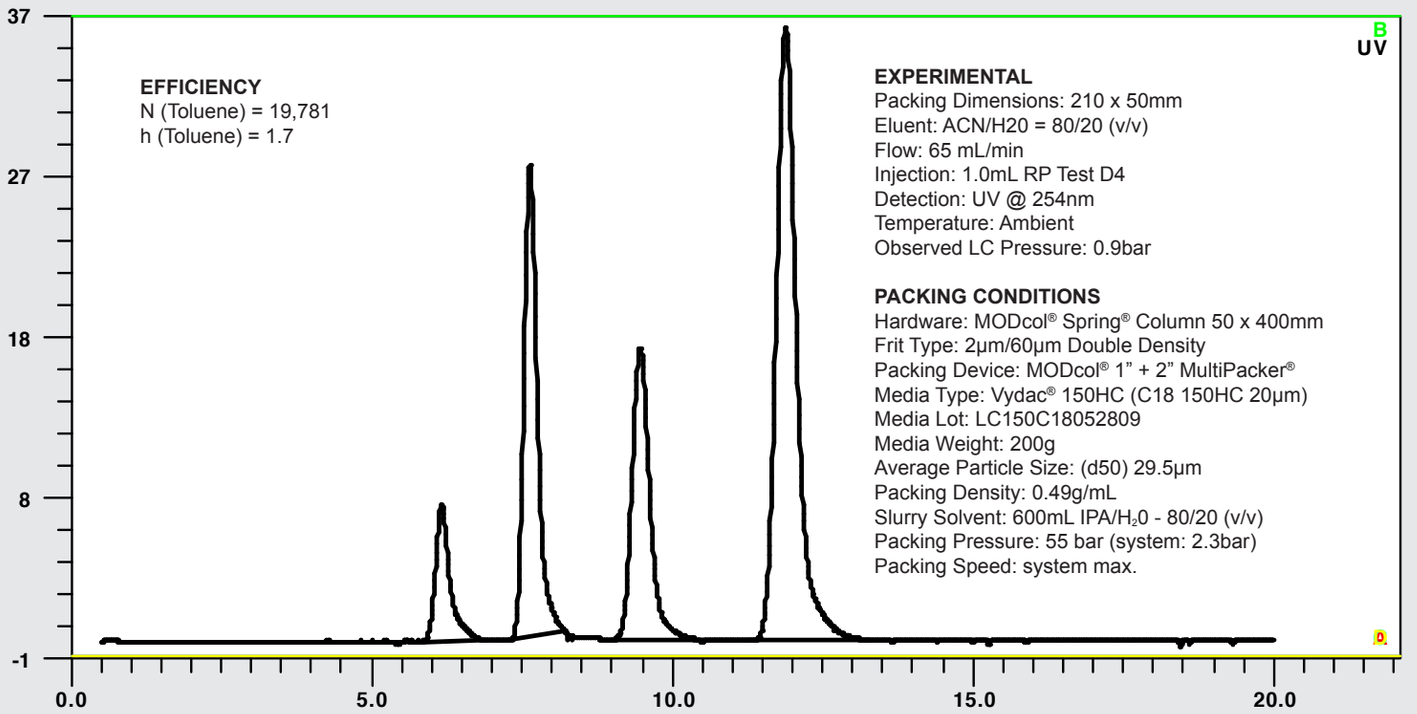
Vydac® 150HC Media 10µm, 4.6 x 250mm

Competitor C18 100Å Media 10µm, 4.6 x 300mm

Competitor C18 120Å Media 10µm, 4.6 x 300mm

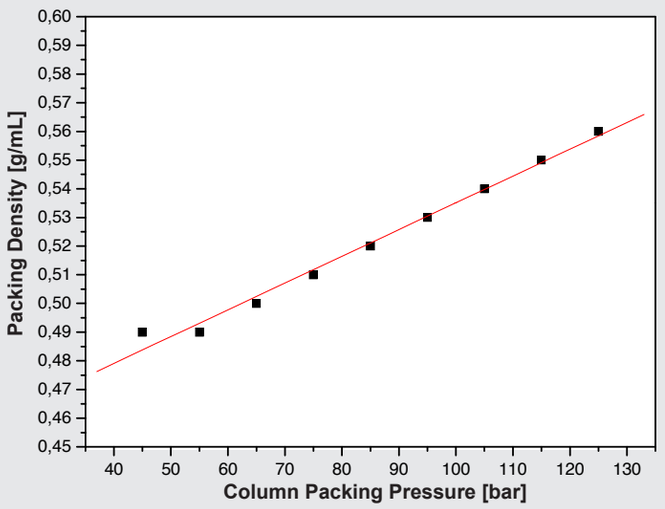
Vydac® 150HC 20µm Media Maintains Performance at 55 bar Packing Pressure

Packing Performance



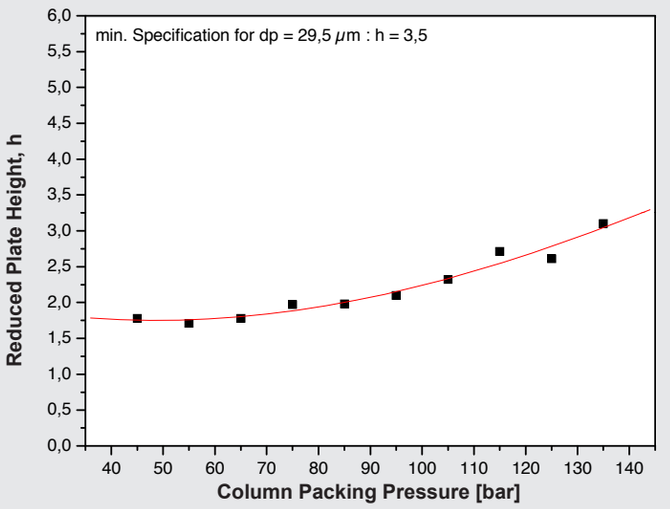
Signal	No.	Name	Retention Time	Peak Height	Peak Area	Plate Count	Asymmetry
UV	1	Uracil	4.85min	8.55	129.41	2073	1.571
	2	Phenol	5.98min	29.14	438.40	3053	1.409
	3	DEET	7.19min	219.16	331.97	3492	1.259
	4	Toluene	9.17min	38.78	822.94	4154	1.250

Stability



Vydac® 150HC Media Remains Stable in DAC Spring® Column Hardware over a Wide Range of Mechanical Packing Pressures

Efficiency



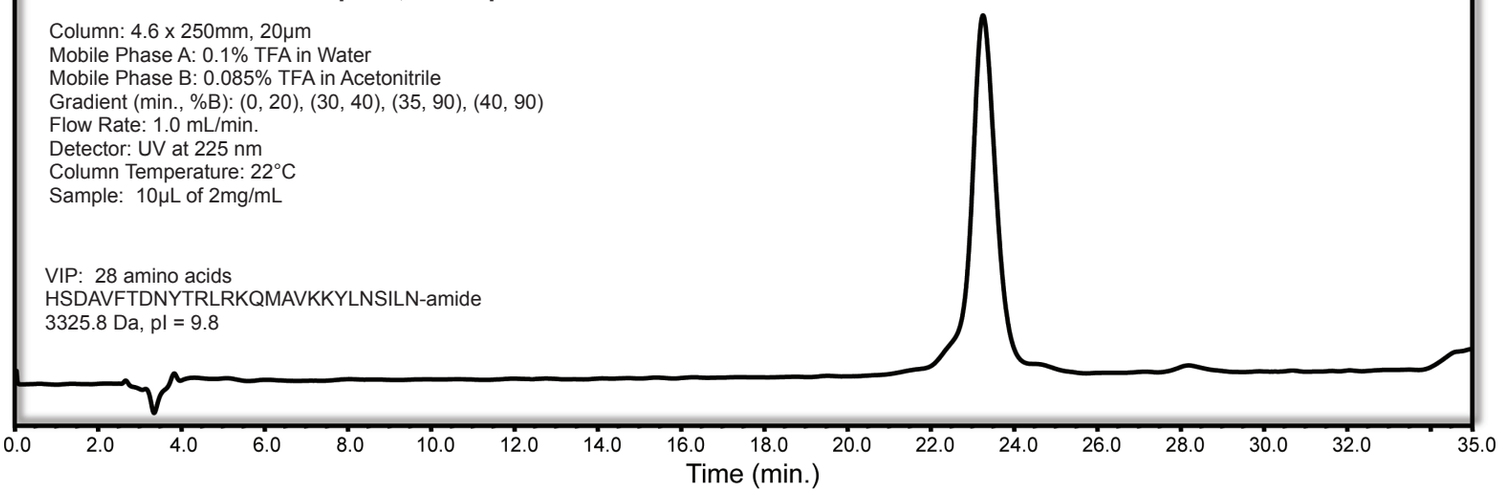
Vydac® 150HC Media is Highly Efficient in DAC Spring® Column Hardware over a Wide Range of Mechanical Packing Pressures

Other Peptides on Vydac® 150HC C18 Media

Vasoactive Intestinal Peptide, Basic pI

Column: 4.6 x 250mm, 20µm
Mobile Phase A: 0.1% TFA in Water
Mobile Phase B: 0.085% TFA in Acetonitrile
Gradient (min., %B): (0, 20), (30, 40), (35, 90), (40, 90)
Flow Rate: 1.0 mL/min.
Detector: UV at 225 nm
Column Temperature: 22°C
Sample: 10µL of 2mg/mL

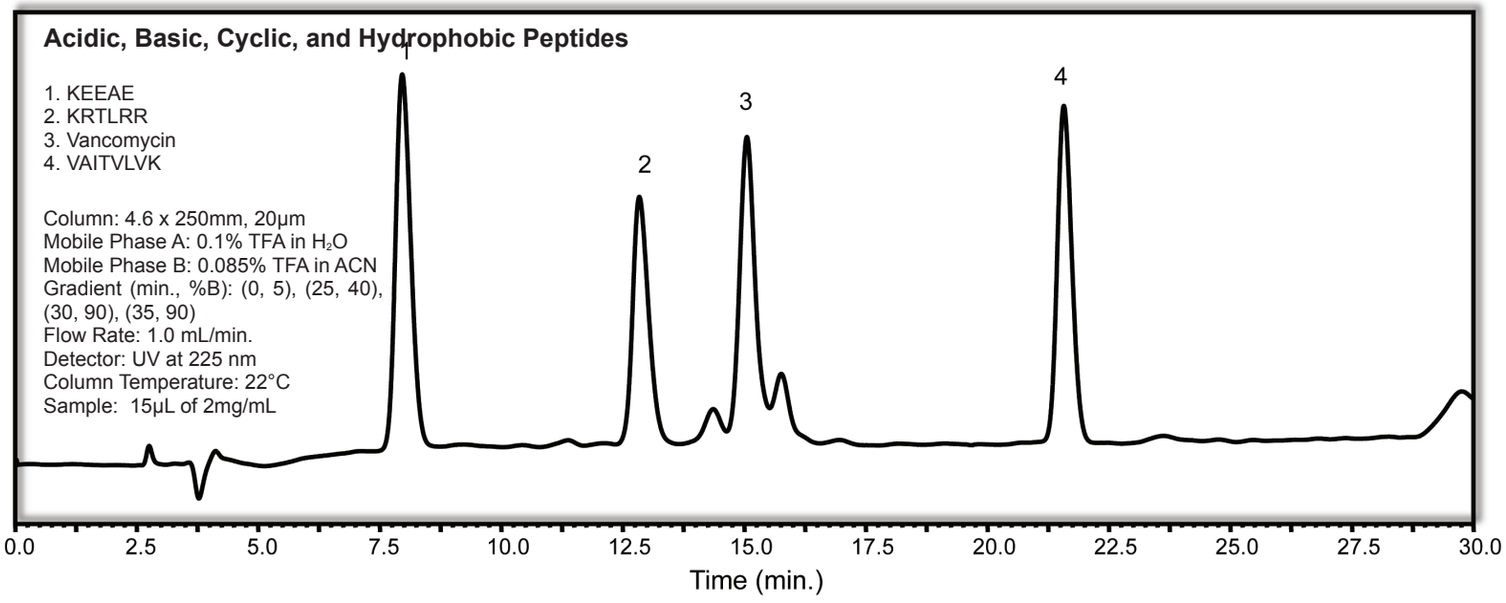
VIP: 28 amino acids
HSDAVFTDNYTRLRKQMAVKKYLNSILN-amide
3325.8 Da, pI = 9.8



Acidic, Basic, Cyclic, and Hydrophobic Peptides

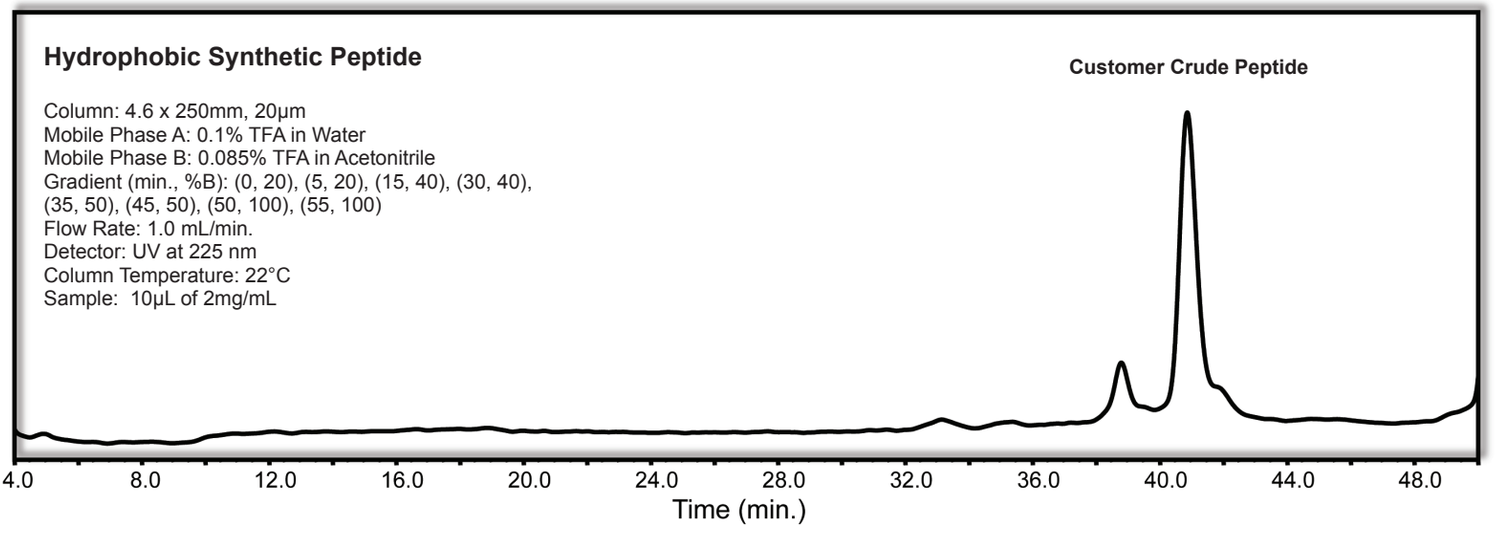
1. KEEAE
2. KRTLRR
3. Vancomycin
4. VAITVLVK

Column: 4.6 x 250mm, 20µm
Mobile Phase A: 0.1% TFA in H_2O
Mobile Phase B: 0.085% TFA in ACN
Gradient (min., %B): (0, 5), (25, 40), (30, 90), (35, 90)
Flow Rate: 1.0 mL/min.
Detector: UV at 225 nm
Column Temperature: 22°C
Sample: 15µL of 2mg/mL



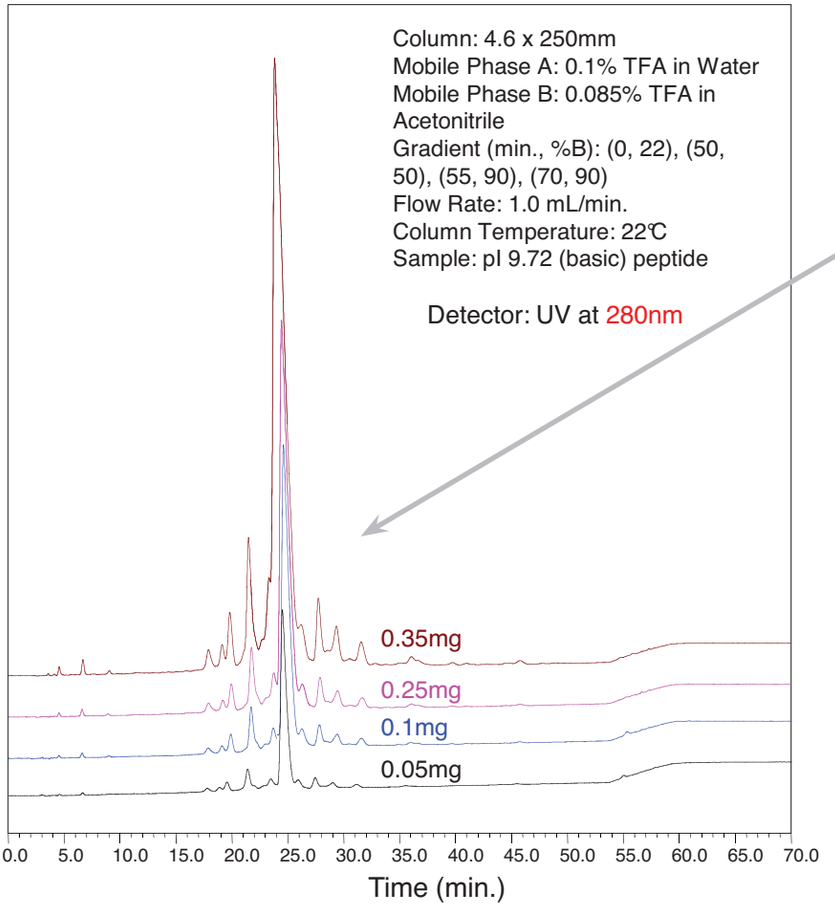
Hydrophobic Synthetic Peptide

Column: 4.6 x 250mm, 20µm
Mobile Phase A: 0.1% TFA in Water
Mobile Phase B: 0.085% TFA in Acetonitrile
Gradient (min., %B): (0, 20), (5, 20), (15, 40), (30, 40), (35, 50), (45, 50), (50, 100), (55, 100)
Flow Rate: 1.0 mL/min.
Detector: UV at 225 nm
Column Temperature: 22°C
Sample: 10µL of 2mg/mL

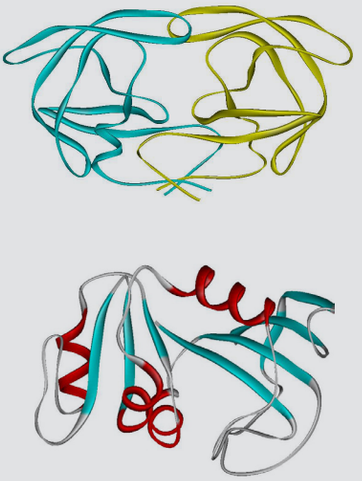


Vydac® 150HC Media: Suitable for acidic, basic, hydrophobic, and cyclic peptides.

Crude Customer Basic Peptide on Vydac® 150HC, C18 10µm Media



Vydac® 150HC media provides good resolution of impurities for crude basic peptides.



Technical Reference:
Basic Process Scale-up Calculations

Flow Rate (Constant Linear Velocity)

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

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

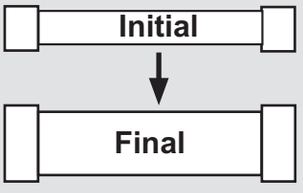
$$= 3\text{mL/min} * (22\text{mm})^2 / (10\text{mm})^2$$

$$= 14.5\text{mL/min}$$

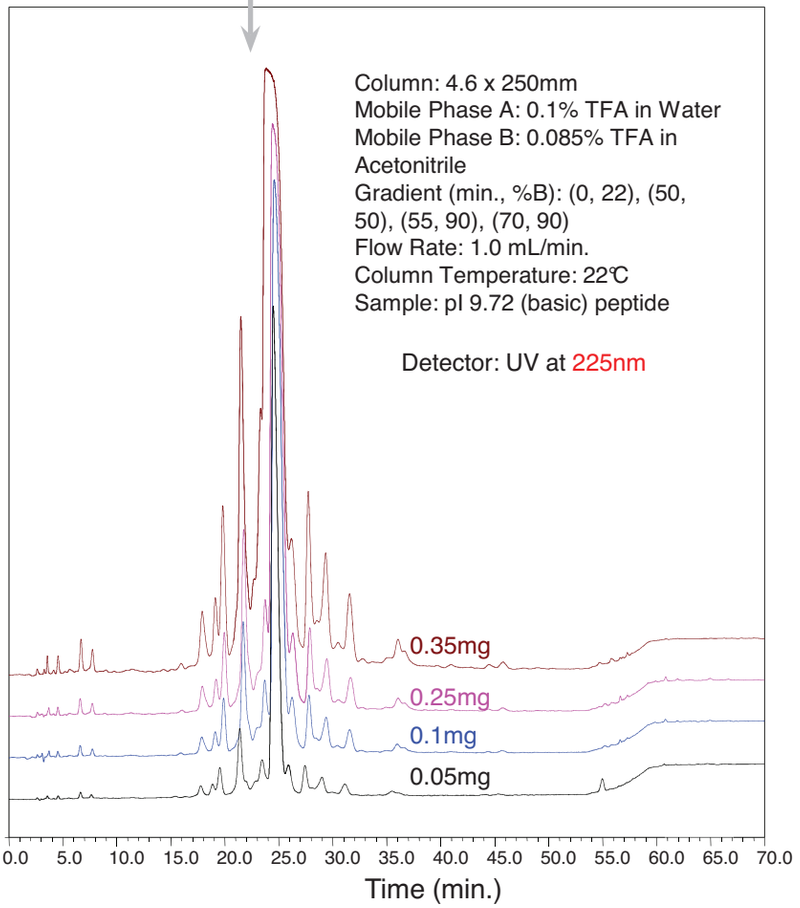
Sample Load

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

D = Diameter
L = Length



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



Conclusions

The new Vydac® 150HC (10 or 20µm) media is highly effective at purifying many small to medium-sized peptides with greater loading capacity and improved productivity compared to other chromatographic media. The unique selectivity can reveal peaks masked by other C18 media and improves resolution of closely related peptides and impurities for higher purity target peptides.

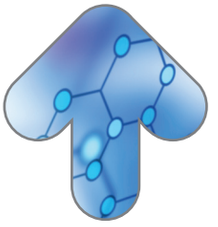
- **Higher capacity compared to other silica gels.**
- **Unique selectivity.**
- **High efficiency at high loading.**
- **Long column lifetime.**
- **Lower pressure drop with 20µm for increased number of cycles.**
- **Excellent performance in both fixed bed and DAC columns.**

Grace is the world's largest manufacturer of specialty silica gel and a leading supplier of chromatography media. We offer an extensive portfolio of products and services to support pharmaceutical manufacturing including:

synthesis intermediates

- **Chiral Building Blocks**
- **Custom Synthesis**

Grace's **Synthetech™** products and contract manufacturing services support pharmaceutical companies' synthesis needs to bring new drugs to market faster. Our in-depth understanding of chemical synthesis, expertise in complex transformations, and understanding of pharmaceutical processes form strong collaborative relationships with our customers. With grams to tons capacity, we are equipped to be your preferred one-stop source from preclinical to commercial volumes.



purification technologies

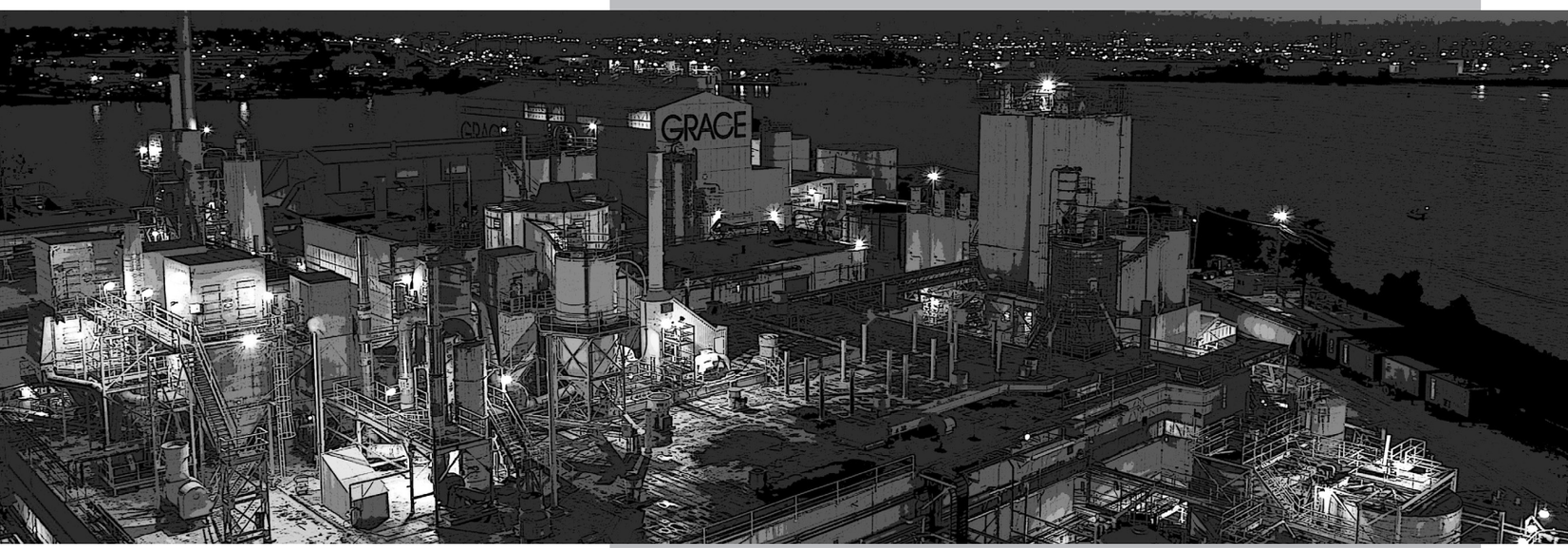
- **Bulk Chromatographic Media**
- **DAC Systems and Column Packing Expertise**

Grace has nearly 100 years experience in silica engineering technology. This experience helps deliver better chromatographic performance, improved capacity, and higher purity silica for purification needs from clinical trials through large-scale manufacturing. As one of the first media platforms for reversed-phase protein and peptide separations, Grace's **Vydac®** media remains a trusted name in biopurification. We complement our media offering with innovative column and packing solutions.

formulation and delivery

- **Multi-functional Silica Excipients**
- **Silica-Based Drug Delivery**

The advanced adsorptive properties of **Syloid®** FP silica excipients help pharmaceutical companies improve formulations and streamline manufacturing processes. Our excipients meet the high quality and regulatory standards the pharmaceutical industry demands. If your need goes beyond excipients and you require a customized silica-based drug delivery solution, Grace has formed a collaborative relationship with Formac Pharmaceuticals to develop a high-throughput screening platform to identify optimal silica-drug combinations for drug delivery of poorly water-soluble drugs.



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GRACE

Grace is the world's largest manufacturer of specialty silica gel and a leading supplier of chromatography media. We offer an extensive portfolio of products and services to support pharmaceutical manufacturing including: pharmaceutical intermediates and custom chemical synthesis; purification technologies and process optimization; and formulation excipients and silica-based drug delivery solutions.

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