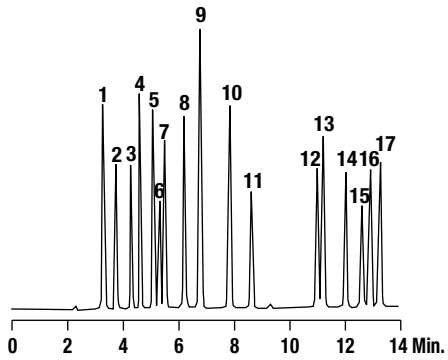
Proteins & Peptides/Reversed-Phase

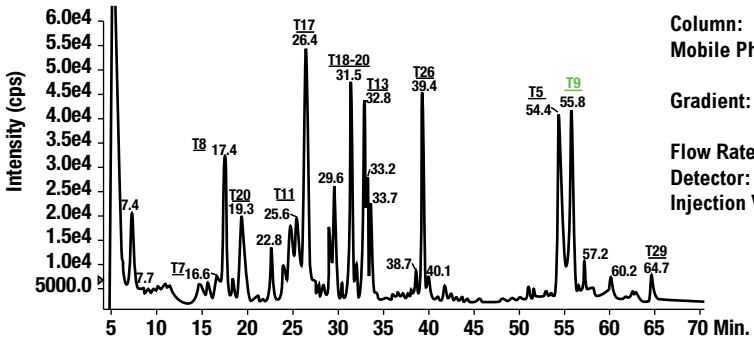
PTH Amino Acids



1. His
2. Cys Acid
3. Arg
4. Asp
5. Ser
6. Gln
7. Thr
8. Gly
9. Cys
10. Ala
11. Tyr
12. Met
13. Pro
14. Trp
15. Phe
16. Ile
17. Leu

Column: Vydac® Denali®, 5µm, 150 x 4.6mm (Part No. **238DE5415**)
Mobile Phase: **A:** Acetonitrile:25mM Potassium Phosphate, pH3.3 (20:80)
B: Acetonitrile:25mM Potassium Phosphate, pH3.3 (80:20)
Flow Rate: 1.0mL/min
Detector: UV at 254nm

TIC of Tryptic Digest of Green Fluorescent Protein (GFP): 150pmol Load on 1mm i.d.

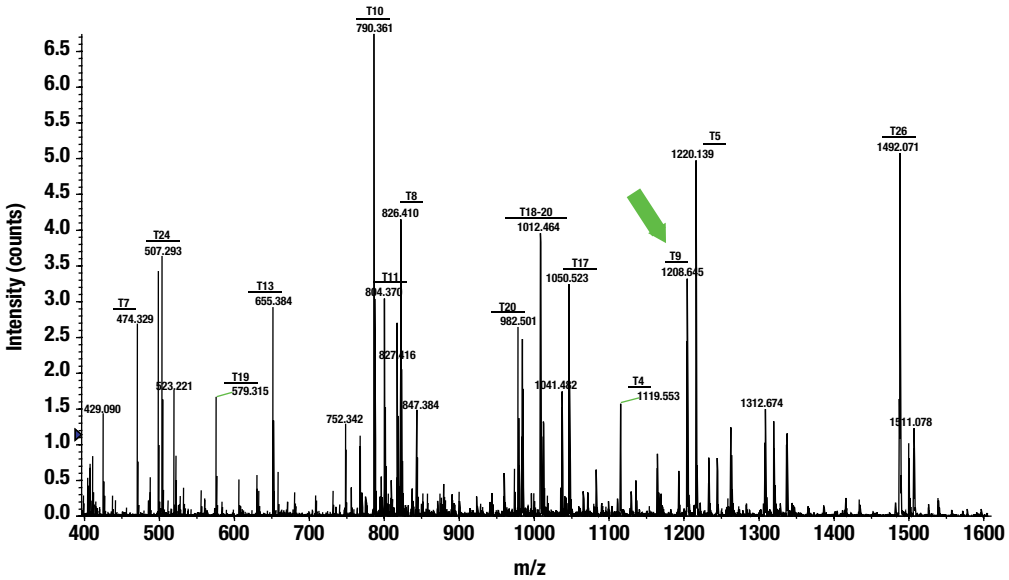


Column: Vydac®, 250 x 1mm (Part No. **238EV51**)
Mobile Phase: **A:** Acetonitrile:0.05% Trifluoroacetic Acid (2:98)
B: Acetonitrile:0.05% Trifluoroacetic Acid (90:10)
Gradient:

Time:	0	60
%B:	5	65

Flow Rate: 50µL/min
Detector: ms: ABI QSTAR
Injection Volume: 25µL GFP digest

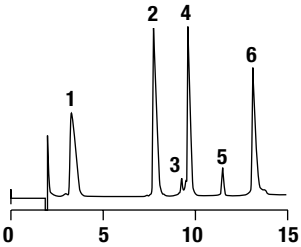
Mass Spectrum of Tryptic Digest of GFP



Proteins & Peptides/Reversed-Phase

Protein Mix

CHROM 8503



1. Ribonuclease A
2. Cytochrome C
3. Lysozyme Impurity
4. Lysozyme
5. Myoglobin Impurity
6. Myoglobin

Column: Alltech® ProSphere™ C4, 300Å, 5µm, 150 x 4.6mm (Part No. **33989**)

Mobile Phase: **A:** 0.15% Trifluoroacetic Acid
B: 0.13% Trifluoroacetic Acid in Acetonitrile:Water (95:5)

Gradient:

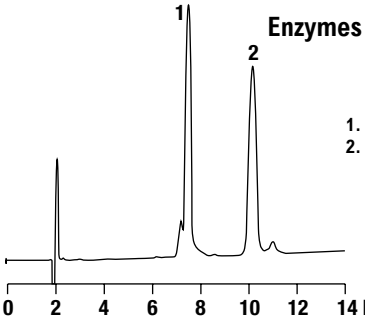
Time:	0	15
%B:	30	60

Flow Rate: 1.0mL/min

Detector: UV at 220nm

Enzymes

CHROM 9068



1. α-Chymotrypsinogen
2. Carbonic Anhydrase

Column: Alltech® ProSphere™ C4, 300Å, 5µm, 150 x 4.6mm (Part No. **33989**)

Mobile Phase: **A:** 0.15% Trifluoroacetic Acid in Water
B: 0.13% Trifluoroacetic Acid in Acetonitrile

Gradient:

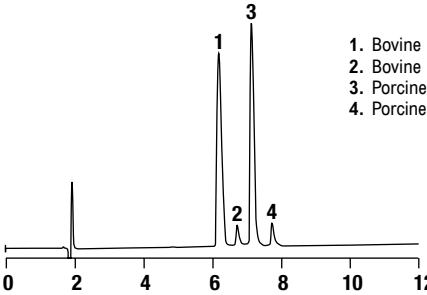
Time:	0	15
%B:	40	55

Flow Rate: 1.0mL/min

Detector: UV at 220nm

Insulins

CHROM 9082



1. Bovine Insulin
2. Bovine Insulin Impurity
3. Porcine Insulin
4. Porcine Insulin Impurity

Column: Alltech® ProSphere™ C18, 300Å, 5µm, 150 x 4.6mm (Part No. **32969**)

Mobile Phase: **A:** 0.13% Trifluoroacetic Acid in Water
B: 0.10% Trifluoroacetic Acid in Acetonitrile

Gradient:

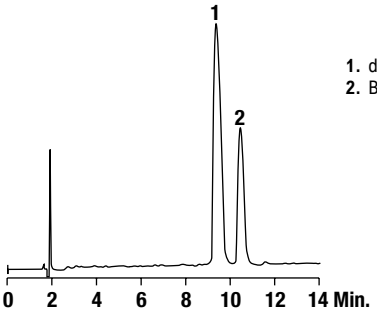
Time:	0	12
%B:	28	38

Flow Rate: 1.0mL/min

Detector: UV at 220nm

Bradykinins

CHROM 9062



1. des-Pro^2-bradykinin
2. Bradykinin

Column: Alltech® ProSphere™ C18, 300Å, 5µm, 150 x 4.6mm (Part No. **32969**)

Mobile Phase: **A:** 0.15% Trifluoroacetic Acid in Water
B: 0.13% Trifluoroacetic Acid in Acetonitrile

Gradient:

Time:	0	15
%B:	15	25

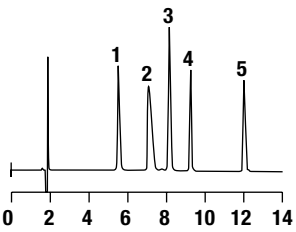
Flow Rate: 1.0mL/min

Detector: UV at 205nm

Proteins & Peptides/Reversed-Phase

Bioactive Peptides

CHROM 8502



1. Methionine Enkephalin
2. Bradykinin
3. Leucine Enkephalin
4. Physalaemin
5. Substance P

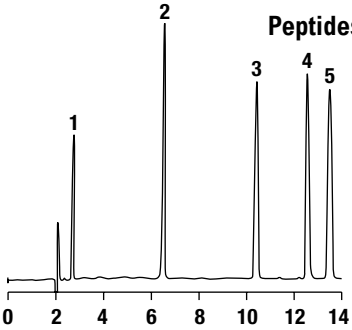
Column: Alltech® ProSphere™ C18, 300Å, 5µm, 150 x 4.6mm (Part No. **32969**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.085% Trifluoroacetic Acid in Acetonitrile:Water (95:5)
Gradient:

Time:	0	20
%B:	20	50

Flow Rate: 1.0mL/min
Detector: UV at 220nm

Peptides

CHROM 9066



1. GLY-TYR
2. VAL-TYR-VAL
3. Methionine Enkephalin
4. Leucine Enkephalin
5. Angiotensin II

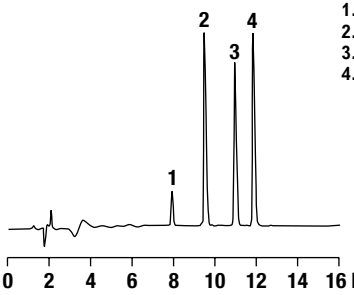
Column: Alltech® ProSphere™ C18, 300Å, 5µm, 150 x 4.6mm (Part No. **32969**)
Mobile Phase: **A:** 0.15% Trifluoroacetic Acid in Water
B: 0.13% Trifluoroacetic Acid in Acetonitrile
Gradient:

Time:	0	15
%B:	10	30

Flow Rate: 1.0mL/min
Detector: UV at 220nm

Peptide Mix

CHROM 10199



1. Oxytocin
2. Angiotensin II
3. Angiotensin I
4. Eledosin

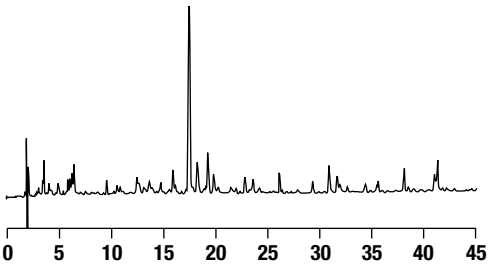
Column: Alltech® ProSphere™ C18, 100Å, 5µm, 250 x 4.6mm (Part No. **35087**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.1% Trifluoroacetic Acid in Acetonitrile:Water (90:10)
Gradient:

Time:	0	20
%B:	20	50

Flow Rate: 1.0mL/min
Detector: UV at 220nm

β-Lactoglobulin A Tryptic Digest

CHROM 9092



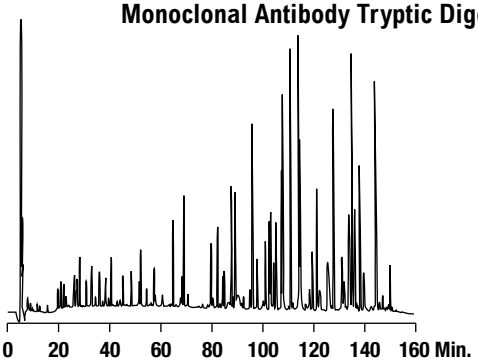
Column: Alltech® ProSphere™ C18, 300Å, 5µm, 150 x 4.6mm (Part No. **32969**)
Mobile Phase: **A:** 0.15% Trifluoroacetic Acid in Water
B: 0.13% Trifluoroacetic Acid in Acetonitrile
Gradient:

Time:	0	45
%B:	5	30

Flow Rate: 1.0mL/min
Detector: UV at 220nm

Monoclonal Antibody Tryptic Digest

CHROM 10213



Column: Alltech® ProSphere™ C18 100Å, 5µm, 250 x 4.6mm (Part No. **35087**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.08% Trifluoroacetic Acid in Acetonitrile
Gradient:

Time:	0	160
%B:	0	45

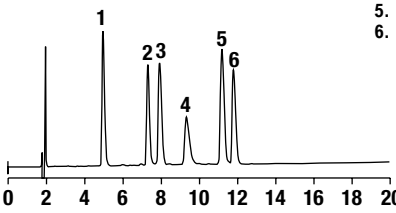
Flow Rate: 0.5mL/min
Detector: UV at 220nm

more applications
View our complete searchable chromatogram database at www.discoverysciences.com/chromdb/

Proteins & Peptides/Reversed-Phase

Peptide Mix on 300Å

CHROM 10363



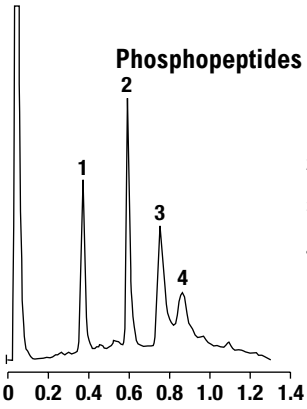
1. Methionine Enkephalin
2. Leucine Enkephalin
3. Angiotensin II
4. Neurotensin
5. Angiotensin I
6. Substance P

Column: Alltech® ProSphere™ C18, 300Å, 5µm, 150 x 4.6mm (Part No. **32969**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.08% Trifluoroacetic Acid in Acetonitrile
Gradient:

Time:	0	20
%B:	20	40

Flow Rate: 1.0mL/min
Detector: UV at 220nm

Phosphopeptides and Fibrinopeptide



1. Monophosphopeptide (FQpSEEQQTEDELQDK)
2. [Glu¹]-Fibrinopeptide B (EGVNDNEEGFFSAR)
3. Tetraphosphopeptide (RELEELNVPGEIVEpSLpSpSpSEESITR)
4. Contaminant(s)

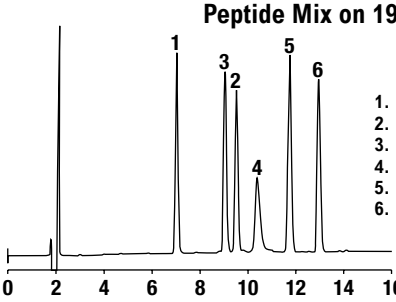
Column: Vydac® ProZap™ C18, 2.1 x 10mm
HPLC System: HPG binary pump
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.085% Trifluoroacetic Acid in Acetonitrile:Water (80:20)
Gradient:

Time:	0.0	1.0	1.1	1.3	1.4
%B:	12	60	80	80	12

Flow Rate: 0.8mL/min
Detector: UV at 215nm

Peptide Mix on 190Å

CHROM 10369



1. Methionine Enkephalin
2. Leucine Enkephalin
3. Angiotensin II
4. Neurotensin
5. Angiotensin I
6. Substance P

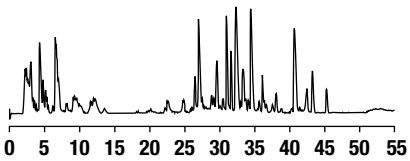
Column: Alltech® Alltima™ HP C18, 190Å, 5µm, 250 x 4.6mm (Part No. **87679**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.08% Trifluoroacetic Acid in Acetonitrile
Gradient:

Time:	0	20
%B:	20	40

Flow Rate: 1.0mL/min
Detector: UV at 220nm

BSA Tryptic Digest

CHROM 10582



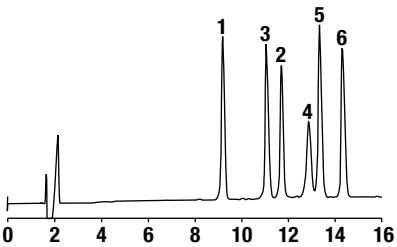
Column: Vydac® 218MS C18, 5µm, 150 x 0.075mm (Part No. **218MS5.07515**)
Mobile Phase: **A:** Water with 0.1% Formic Acid:Acetonitrile (95:5)
B: Water with 0.1% Formic Acid:Acetonitrile (20:80)
Gradient:

Time:	0	5	50	60
%B:	0	0	50	100

Flow Rate: 0.25µL/min

Peptide Mix on 100Å

CHROM 10370



1. Methionine Enkephalin
2. Leucine Enkephalin
3. Angiotensin II
4. Neurotensin
5. Angiotensin I
6. Substance P

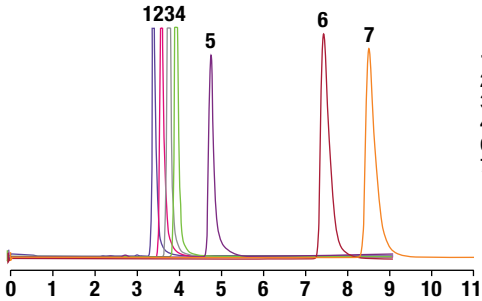
Column: Alltech® ProSphere™ C18, 100Å, 5µm, 250 x 4.6mm (Part No. **35091**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.08% Trifluoroacetic Acid in Acetonitrile
Gradient:

Time:	0	20
%B:	20	40

Flow Rate: 1.0mL/min
Detector: UV at 220nm

Amino Acids by HILIC

CHROM 10582

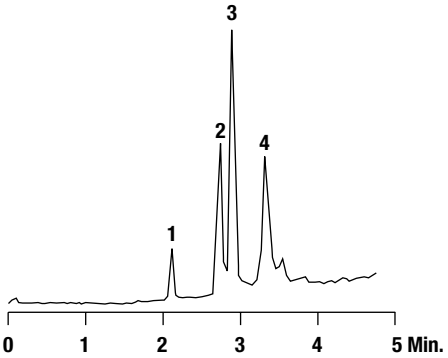


1. Glutamic Acid
2. Leucine
3. Serine
4. Glycine
6. Lysine
7. Arginine

Column: Alltech® Alltima™ HP HILIC, 150 x 4.6mm
Mobile Phase: Acetonitrile:10mM Ammonium Acetate with 0.2% Acetic Acid (70:30)
Flow Rate: 0.75mL/min
Detector: ELSD

Proteins & Peptides/Reversed-Phase

LC-MS of Proteins



Column: Vydac® ProZap™ C18, 2.1 x 10mm
Mobile Phase: **A:** 0.2% Formic Acid, 0.01% Trifluoroacetic Acid in Acetonitrile:Water (5:95)
B: 0.2% Formic Acid, 0.01% Trifluoroacetic Acid in Acetonitrile:Water (80:20)

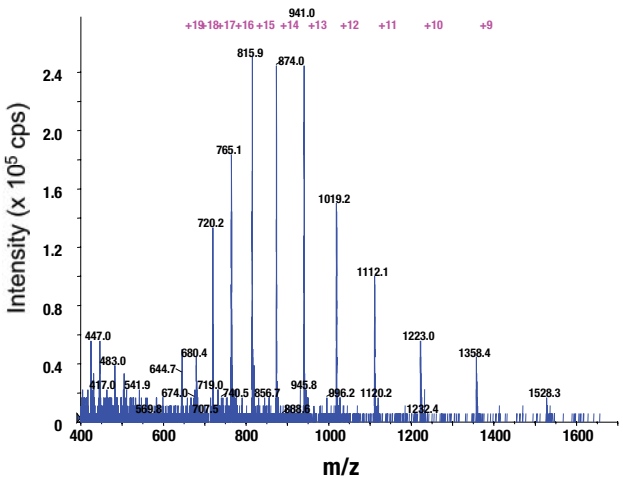
Gradient:

Time:	0	4.0	4.5	4.7
%B:	20	90	90	20

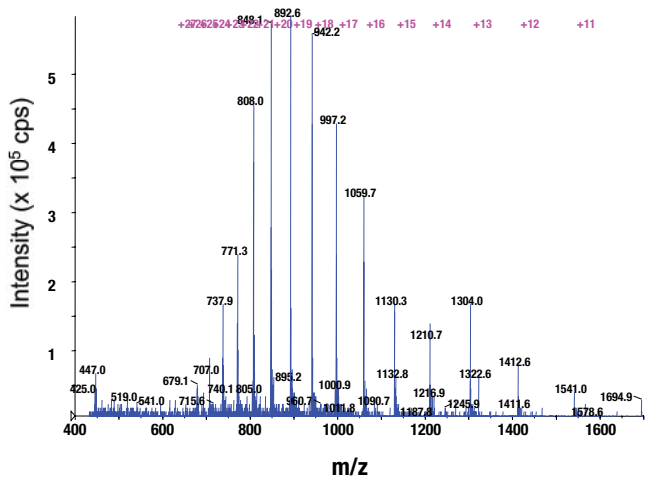
Flow Rate: 0.4mL/min
Detector: AB/MDS Sciex Q TRAP® MS. EMS scan 400-1700 amu; scan rate = 1000 amu/s; ion spray voltage = 5000 V; LIT fill time = 50 ms; Q3 entry barrier = 8V; declustering potential = 20; collision energy = 10 eV; curtain gas = 50; ion source gas 1 = 45; ion source gas 2 = 70; temperature (TEM) = 350°C.
Left Chromatogram Injection Amount: 50 ng each
Right Chromatogram Injection Amount: 8µg each, except 1.5µg for growth hormone

1. Bovine Cytochrome C, M =12222 (ave.)
2. Horse Apomyoglobin, M = 16941 (ave.)
3. Bovine Carbonic Anhydrase II, M = 28998 (ave.)
4. Chicken Ovalbumin, M = 44455 (ave.)

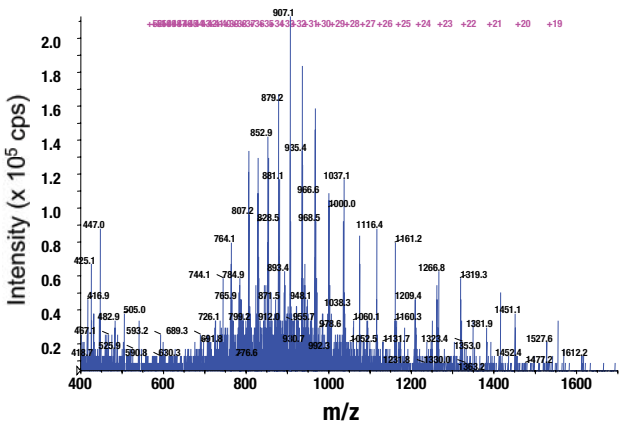
Peak 1 - Bovine Cytochrome C



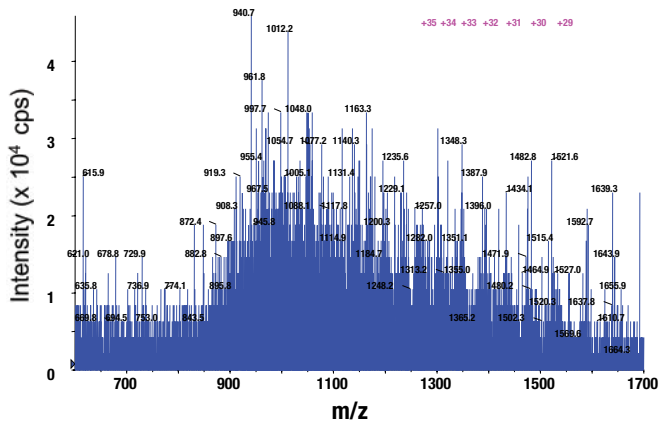
Peak 2 - Horse Apomyoglobin



Peak 3 - Bovine Carbonic Anhydrase II

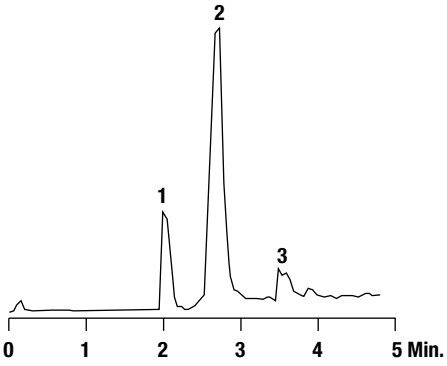


Peak 4 - Chicken Ovalbumin



Proteins & Peptides/Reversed-Phase

LC-MS of Proteins



Column: Vydac.® ProZap™ C18, 2.1 x 10mm
Mobile Phase: **A:** 0.2% Formic Acid, 0.01% Trifluoroacetic Acid in Acetonitrile:Water (5:95)
B: 0.2% Formic Acid, 0.01% Trifluoroacetic Acid in Acetonitrile:Water (80:20)

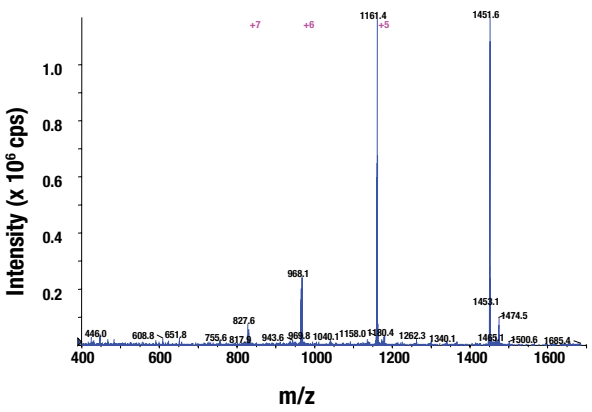
Gradient:

Time:	0	4.0	4.5	4.7
%B:	20	90	90	20

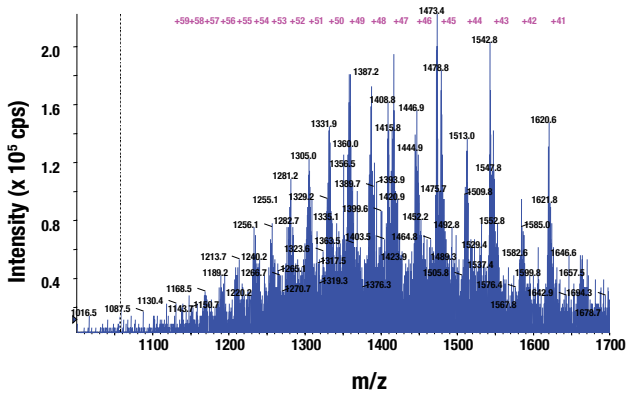
Flow Rate: 0.4mL/min
Detector: AB/MDS Sciex Q TRAP® MS. EMS scan 400-1700 amu; scan rate = 1000 amu/s; ion spray voltage = 5000 V; LIT fill time = 50 ms; Q3 entry barrier = 8V; declustering potential = 20; collision energy = 10 eV; curtain gas = 50; ion source gas 1 = 45; ion source gas 2 = 70; temperature (TEM) = 350°C.
Left Chromatogram Injection Amount: 50 ng each
Right Chromatogram Injection Amount: 8µg each, except 1.5µg for growth hormone

1. Human Insulin, M = 5803 (ave.)
2. Human Serum Albumin, M = 66520 (ave.)
3. Human Growth Hormone, M = 22123 (ave.)

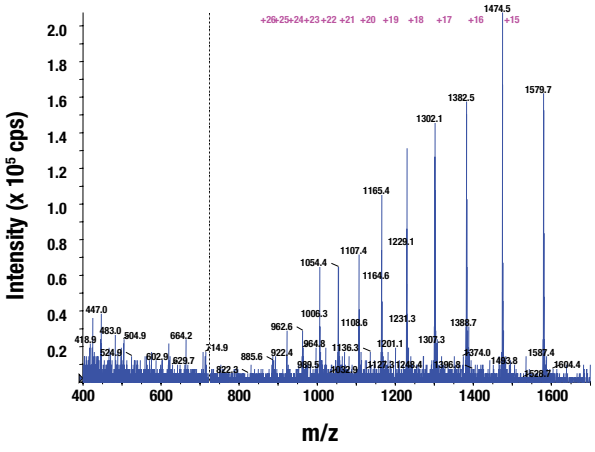
Peak 1 -Human Insulin



Peak 2 - Human Serum Albumin

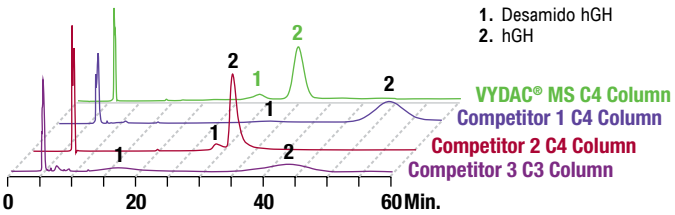


Peak 3 - Human Growth Hormone



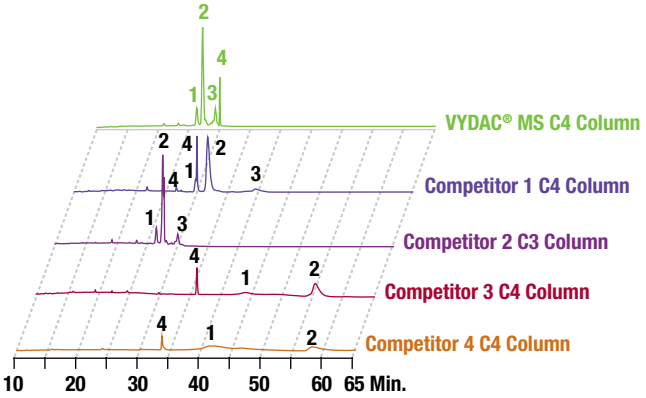
Proteins & Peptides/Reversed-Phase

Human Growth Hormone (hGH) Assay on 300Å C4 Columns



Columns: Vydac® MS C4 (Part No. 214MS54), Competitor 1 C4, Competitor 2 C4, and Competitor 3 C3 (all 300Å, 250 x 4.6mm packed with 5µm except 4µm for Competitor 1)
Mobile Phase: 50mM Tris, pH7.5:*n*-Propanol (71:29)
Flow Rate: 0.5mL/min
Detector: UV at 220nm

Transmembrane Protein p14 on 300Å C4 Columns



Columns: Vydac® MS C4 (Part No. 214MS54), Competitor 1 C4, Competitor 2 C3, Competitor 3 C4, and Competitor 4 C4 (all 300Å, 250 x 4.6mm packed with 5µm except 4µm for Competitor 1)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water **B:** 0.085% Trifluoroacetic Acid in Acetonitrile
Gradient Vydac®, Comp. 1, & Comp. 2:

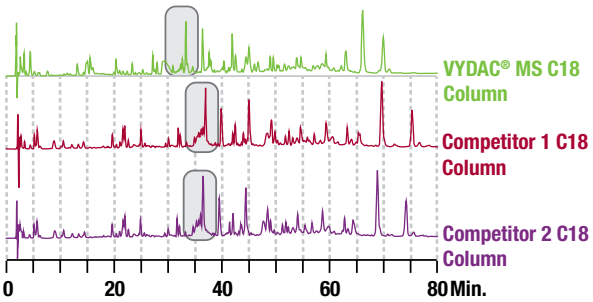
Time:	0	20	25	45
%B:	20	60	80	80

Gradient Comp. 3 & Comp. 4:

Time:	0	20	25	45	50	80
%B:	20	60	80	80	90	90

Flow Rate: 1.0mL/min
Detector: UV at 215nm

Tryptic Digest of Fetuin on 300Å C18 Columns



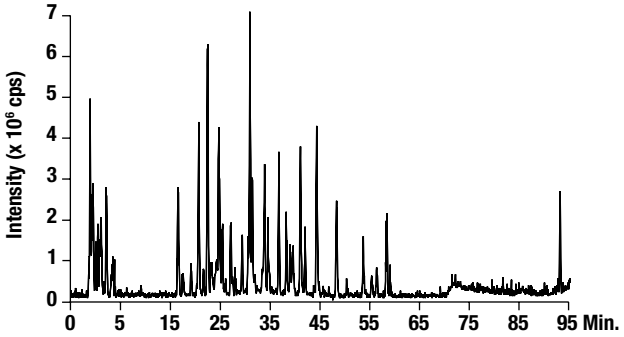
Columns: Vydac® MS C18 (Part No. **238MS5415**), Competitor 1 C18, and Competitor 2 C18 (all 300Å, 5µm, 150 x 4.6mm)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water **B:** 0.085% Trifluoroacetic Acid in Acetonitrile
Gradient:

Time:	0	5	80	90	100
%B:	4	4	40	90	90

Flow Rate: 1.0mL/min
Detector: UV at 215nm

Proteins & Peptides/Reversed-Phase

Base Peak Chromatogram of Tryptic Digest of BSA



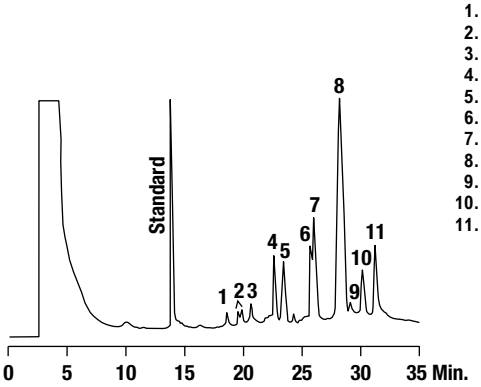
Column: Vydac® Everest® C18, 300Å, 5µm, 300µm x 150mm (Part No. **238EV5.315**)
Mobile Phase: **A:** 0.1% Formic Acid in Water
B: 0.1% Formic Acid in Acetonitrile

Gradient:

Time:	0	5	75	85	95
%B:	4	4	40	90	90

Flow Rate: 5µL/min
Column Temp: 22ºC
Peptide Load: 0.1µL injection of 5.8µg/µL (580ng or 9pmol)
MS Detector: AB/MDS Sciex Q-Trap.

Human Apolipoproteins



1. ApoC-IIIa
2. ApoC-IIIb
3. ApoC-IIa
4. ApoC-IIIc
5. ApoC-I
6. ApoC-IIb
7. ApoA-Ia
8. ApoA-Ib
9. ApoA-IIc
10. ApoA-IIa
11. ApoA-IIb

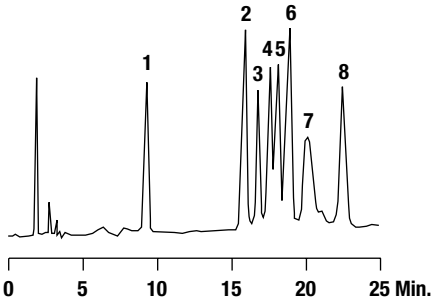
Column: Vydac® C18, 5µm, 250 x 4.6mm (Part No. **218TP54**)
Mobile Phase: **A:** Acetonitrile:0.1% Trifluoroacetic Acid (25:75)
B: Acetonitrile:0.1% Trifluoroacetic Acid (58:42)

Gradient:

Time:	0	33
%B:	0	100

Flow Rate: 1.2mL/min
Detector: UV

Insulin Variants



1. Chicken
2. Bovine
3. Ovine
4. Rabbit
5. Human
6. Porcine
7. Rat I
8. Rat II

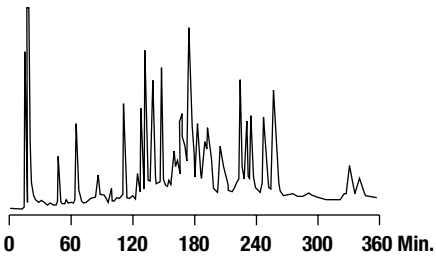
Column: Vydac® C4, 5µm, 250 x 4.6mm (Part No. **214TP54**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid
B: Acetonitrile

Gradient:

Time:	0	25
%B:	27	30

Detector: UV

Ribosomal Proteins



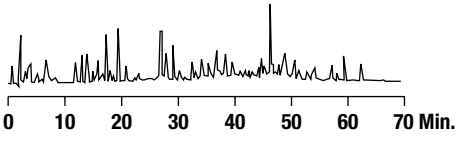
Column: Vydac® C4, 5µm, 250 x 4.6mm (Part No. 214TP54)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid
B: Isopropanol

Gradient:

Time:	0	355
%B:	10	38

Detector: UV

Tryptic Digest of Bovine Serum Albumin



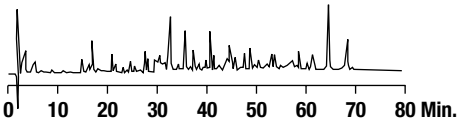
Column: Vydac® Everest® C18, 5µm, 150 x 4.6mm (Part No. **238EV5415**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.085% Trifluoroacetic Acid in Acetonitrile

Gradient:

Time:	0	5	80	90	100
%B:	4	4	40	90	90

Flow Rate: 1.0mL/min
Detector: UV at 215nm

Tryptic Digest of Fetuin (a Glycoprotein)



Column: Vydac® Everest® C18, 5µm, 150 x 4.6mm (Part No. **238EV5415**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.085% Trifluoroacetic Acid in Acetonitrile

Gradient:

Time:	0	5	80	90	100
%B:	4	4	40	90	90

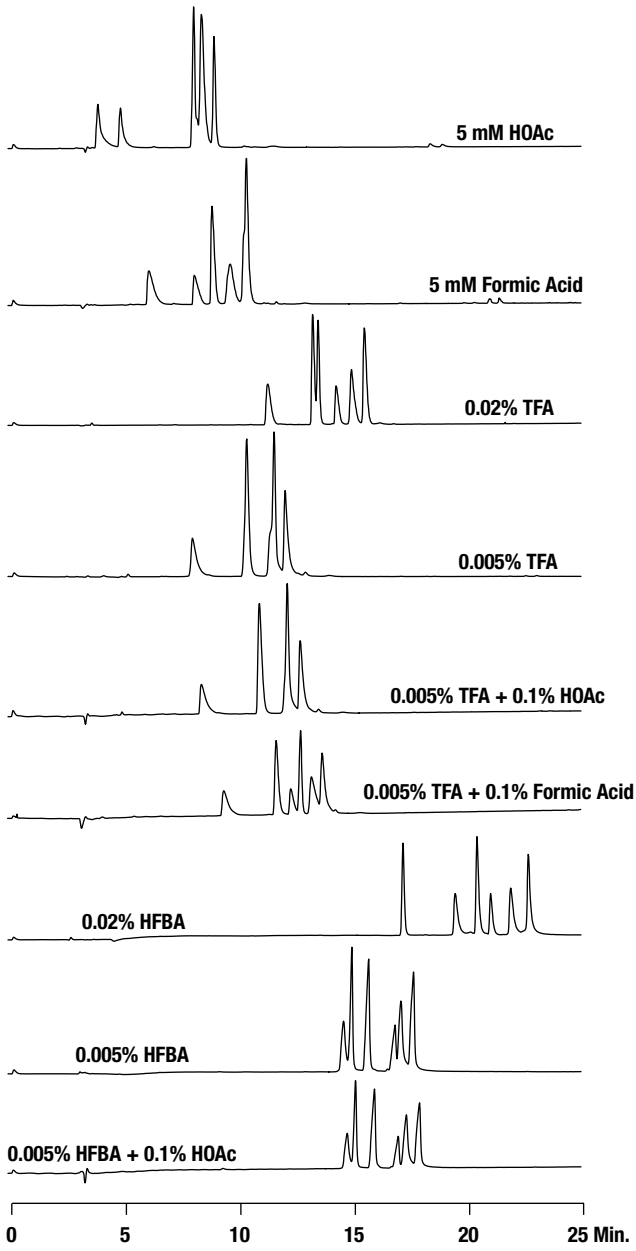
Flow Rate: 1.0mL/min
Detector: UV at 215nm

Proteins & Peptides/Reversed-Phase

Peptides on 238MS C18 Column with Various Modifiers

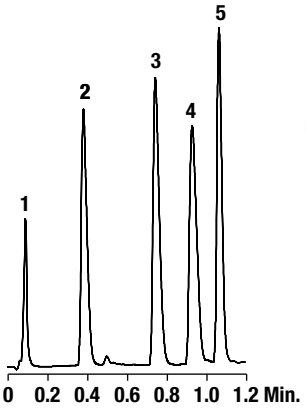
In order to demonstrate the effects of mobile phase modifiers and modifier concentrations, a mixture of six peptides was separated on a monomeric C18 MS column under identical gradient conditions with a variety of ion-pair reagents. Significant differences in selectivity arise depending on the concentrations and identities of the ions present in the mobile phase. Perfluorinated Acidic modifiers even at very low levels (0.005% w/v) produce increased retention. For this particular mixture, the best spread of peaks was obtained with heptafluorobutyric Acid (HFBA) at 0.02% (w/v). However, this might be expected to differ for other peptides.

Column: Vydac® Monomeric C18, 5µm, 250 x 4.6mm (Part No. **238MS54**)
Gradient: 10 to 40% Acetonitrile over 30 min for all chromatograms with constant concentration of modifier as indicated (w/v)
Flow Rate: 1.0mL/min
Detector: UV at 220nm
Sample: Oxytocin, Bradykinin, Angiotensin II, Eledoisin-related Peptide, Neurotensin, and Angiotensin I



Proteins & Peptides/Reversed-Phase

Peptides



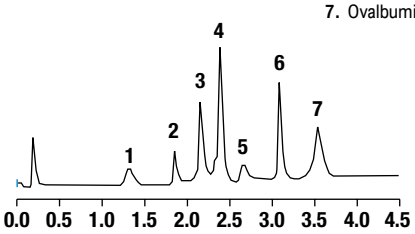
1. GY (238 Da)
2. VYV (379 Da)
3. Met Enkephalin (YGGFM, 573 Da)
4. Leu Enkephalin (YGGFL, 555 Da)
5. Angiotensin II (DRVYIHPF, 1045 Da)

Column: Vydac® ProZap™ C18, 500Å, 1.5µm, 10 x 2.1mm
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.085% Trifluoroacetic Acid:Acetonitrile (20:80)
Gradient:

Time:	0	0.1	0.7	1.1	1.2
%B:	4	15	20	50	4

Flow Rate: 0.8mL/min
Detector: UV at 215nm

Fast Protein Analysis



1. Ribonuclease (37ng)
2. Bovine Insulin (37ng)
3. Cytochrome C (37ng)
4. Lysozyme (4ng)
5. Bovine Serum Albumin (37ng)
6. Carbonic Anhydrase (37ng)
7. Ovalbumin (37ng)

Column: Vydac® ProZAP™ C18, 1.5µm, 10 x 2.1mm
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.085% Trifluoroacetic Acid in Acetonitrile
Gradient:

Time:	0	4.0	4.5	4.7
%B:	23	75	75	23

Flow Rate: 0.2mL/min
Detector: UV at 280nm

Antibodies in Sheep Serum on ProZap

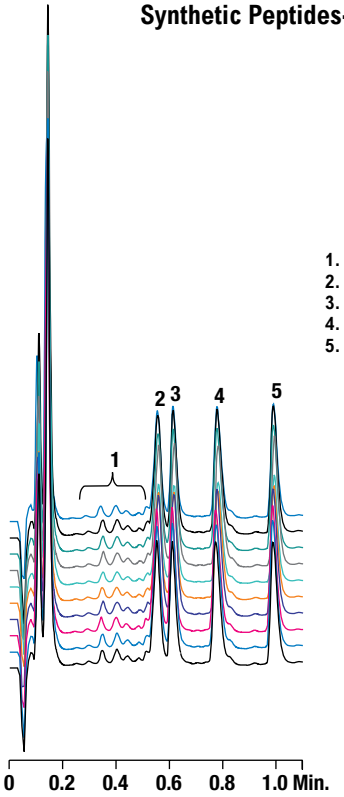
1. Albumin
2. IgG

Column: Vydac® ProZap™ C18, 500Å, 1.5µm, 10 x 2.1mm
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.085% Trifluoroacetic Acid:*n*-Propanol (10:90)
Gradient:

Time:	0	6.0	6.5	7.0
%B:	5	75	75	5

Flow Rate: 0.5mL/min
Detector: UV at 280nm

Synthetic Peptides-10 Injections



1. Contaminant Peptides/Impurities
2. RGAGGLGLGK-amide (883 Da)
3. Ac-RGAGGLGLGK-amide (926 Da)
4. Ac-RGVGGLGLGK-amide (954 Da)
5. Ac-RGVVGLGLGK-amide (996 Da)

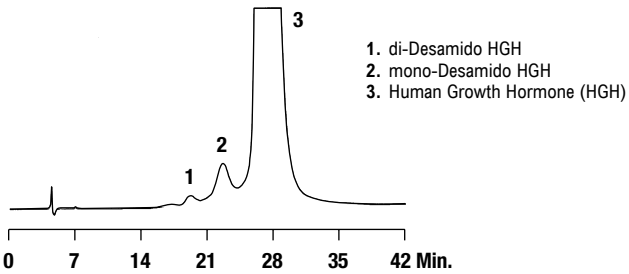
Column: Vydac® ProZap™ C18, 500Å, 1.5µm, 10 x 2.1mm
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid in Water
B: 0.085% Trifluoroacetic Acid:Acetonitrile (20:80)
Gradient:

Time:	0	0.1	0.7	1.1	1.2
%B:	4	15	20	50	4

Flow Rate: 0.8mL/min
Detector: UV at 215nm

Proteins & Peptides/Reversed-Phase

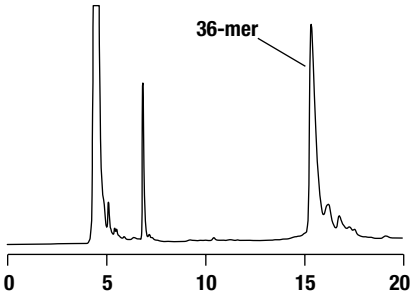
Human Growth Hormone



1. di-Desamido HGH
2. mono-Desamido HGH
3. Human Growth Hormone (HGH)

Column: Vydac® C4, 5µm, 250 x 4.6mm (Part No. **214TP54**)
Mobile Phase: Isopropanol:0.01M Tris buffer, pH7.5 (29:71)
Flow Rate: 1.0mL/min
Detector: UV

Purification of Trityl-On Oligonucleotides

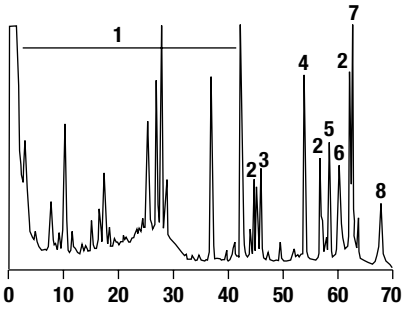


Column: Vydac® C4, 5µm, 250 x 10mm (Part No. **214TP510**)
Mobile Phase: **A:** 0.1M Triethylammonium Acetate, pH7.0 **B:** Acetonitrile
Gradient:

Time:	0	14
B%:	16	23

Flow Rate: 5.0mL/min
Detector: UV

Separation of Protein / Peptide Mixture on Vydac® 219TP Diphenyl Column



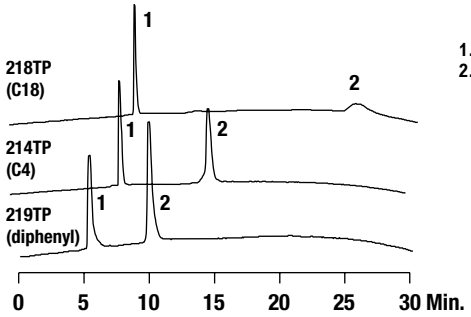
1. Horse Cytochrome C Tryptic Digest Fragments
2. Whale Myoglobin CNBr Digest Fragments
3. Ribonuclease
4. Cytochrome
5. Lysozyme
6. BSA
7. Myoglobin
8. Ovalbumin

Column: Vydac® Diphenyl, 5µm, 250 x 4.6mm (Part No. **219TP54**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid
B: Acetonitrile
Gradient:

Time:	0	67.5
B%:	0	70

Flow Rate: 1.0mL/min
Detector: UV

Comparison of Polypeptide Separations on C18, C4 and Diphenyl Reversed-Phase Columns



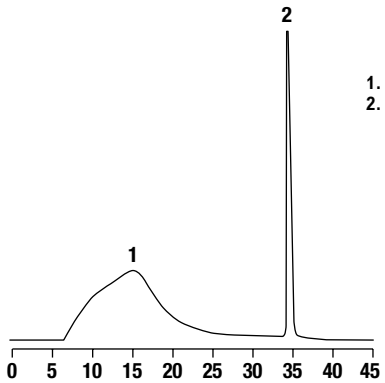
1. H
2. TH6

Column: Vydac® C18, C4 and Diphenyl, all 5µm, 150 x 4.6mm (Part Nos. **218TP5415, 214TP5415, 219TP5415**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid
B: Acetonitrile
Gradient:

Time:	0	30
B%:	10	90

Sample: 18-Residue Helical Peptide (1) and Six-helix Template-assembled Synthetic Protein (2)
Flow Rate: 1.0mL/min
Detector: UV

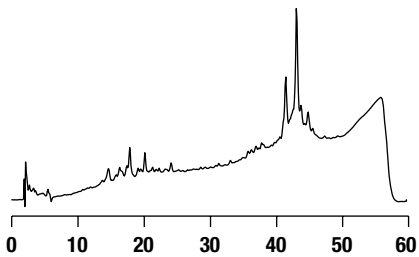
IgG Purification from Bovine Serum



1. Serum Load Passthrough
2. IgG

Column: Vydac® Venture® A RAVE Recombinant Protein A, 15-20µm, 50 x 4.6mm (Part No. **1520450**)
Mobile Phase: **A:** 20mM Phosphate, pH7.4, 150mM Sodium Chloride
B: 0.1 M Sodium Citrate, pH3.2
Gradient: Equilibrate with A for 3 min, load sample for 9 min, wash with A for 12 min, elute with B for 3 min, re-equilibrate with A for 6 min
Flow Rate: 1.38mL/min (linear velocity = 500cm/hr)
Detector: UV at 280nm

Analysis of a Lipid Peptide



Column: Vydac® Diphenyl, 5µm, 250 x 4.6mm (Part No. **219TP54**)
Mobile Phase: **A:** 0.1% Trifluoroacetic Acid
B: *n*-Propanol:Acetonitrile:0.1% Trifluoroacetic Acid (50:50)
Gradient:

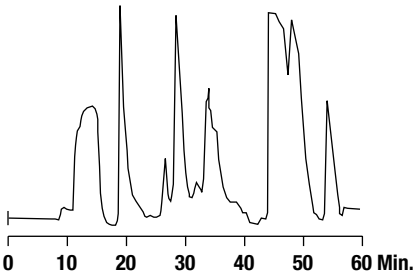
Time:	0	45
%B:	25	70

Flow Rate: 1.5mL/min
Detector: UV

more applications
View our complete searchable chromatogram database at www.discoverysciences.com/chromdb/

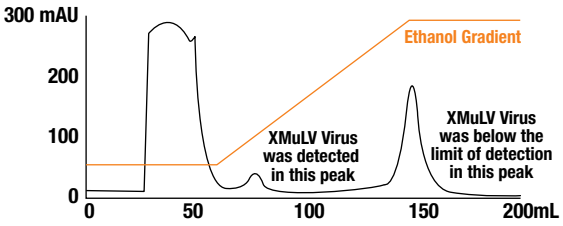
Proteins & Peptides/Reversed-Phase

Purification of Synthetic Peptide, GnRH Antagonist



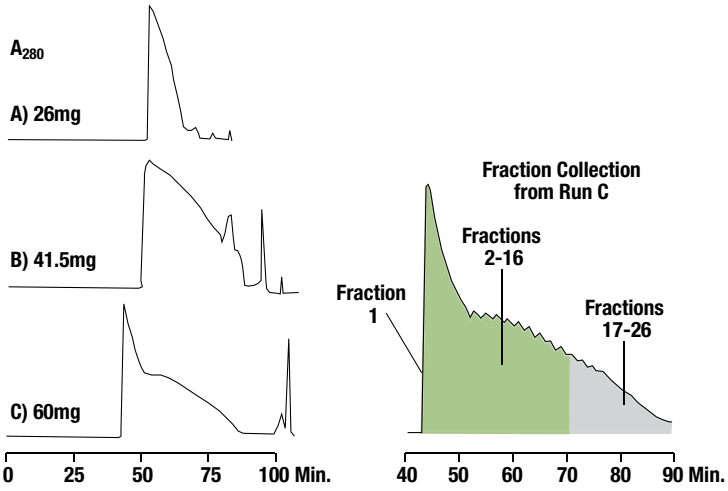
Column: Vydac® C18 , 15–20µm, 50mm i.d. x 30cm (Part No. **218TP15205030**)
Mobile Phase: Acetonitrile:Water/TEAP (Gradient)
Sample: 1.2g of synthesis mixture

Separation of Xenotropic Murine Leukemia Virus from Target Protein



Column: Vydac® C4 , 20-30µm (Part No. **214TPB2030**)
Mobile Phase: Ethanol gradient

Preparative Chromatography of Porcine Insulin



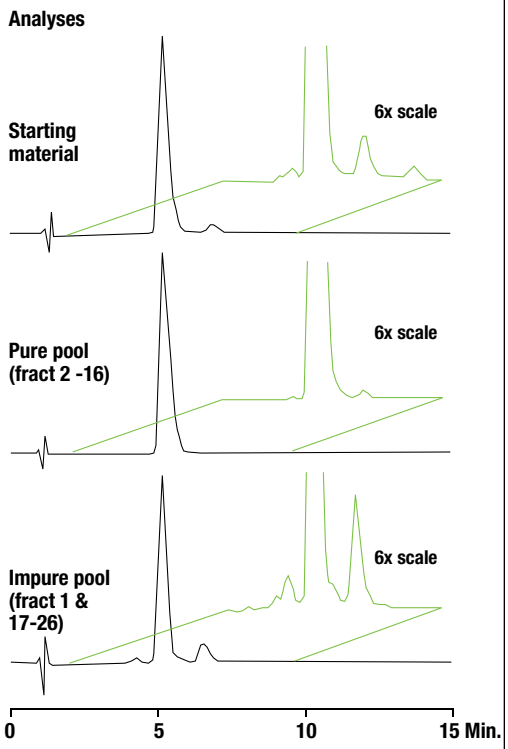
Column: Vydac® C4, 10-15µm, 10 x 250mm (Part No. **214TP101510**)

A) Sample: 26mg partially purified porcine insulin in 30mL of 20% Acetonitrile/0.1% Trifluoroacetic acid, pumped on at 8mL/min
Elution: 4mL/min
Gradient: 20-35% Acetonitrile in 0.1% Trifluoroacetic acid over 90 min

B) Sample: 41.5mg in 42mL of 20% Acetonitrile/0.1% Trifluoroacetic acid, pumped on at 8mL/min
Elution: 4mL/min
Gradient: 20-27% Acetonitrile in 35 min, hold 27% for 10 min followed by 27-28% in 20 min, 28%-35% in 10 min, and 35-95% in 5 min, all in 0.1% Trifluoroacetic acid.

C) Sample: 60mg in 60mL of 20% Acetonitrile/0.1% Trifluoroacetic acid, pumped on at 8mL/min
Elution: 4mL/min
Gradient: 20-27% Acetonitrile in 35 min, hold 27% for 10 min followed by 27-28.5% in 30 min, hold 28.5% for 20 min

Analyses of Starting Material and Pooled Fractions from Preparative Column

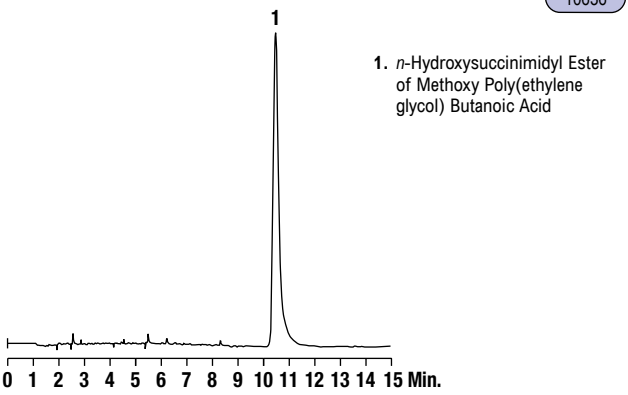


Column: Vydac® C4, 5µm, 4.6 x 150mm (Part No. **214TP5415**)
Samples: Starting material and pooled fractions of preparative run C, as indicated.
Detection: UV at 215nm
Elution: 1.5mL/min
Gradient: 30-33% Acetonitrile in 0.1% Trifluoroacetic acid over 15 min

Proteins & Peptides/Reversed-Phase

mPEG-SBA

CHROM 10636



1. *n*-Hydroxysuccinimidyl Ester of Methoxy Poly(ethylene glycol) Butanoic Acid

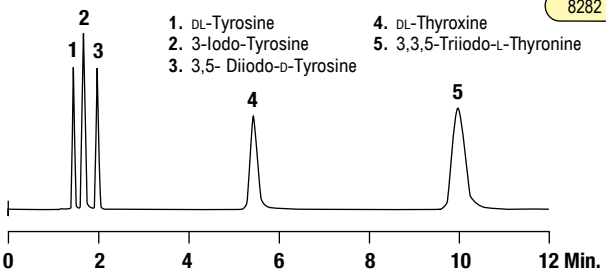
Column: Alltech® ProSphere™ 300, 5µm, 150 x 4.6mm (Part No. **35560**)
Mobile Phase: **A:** Water **B:** Acetonitrile
Gradient:

Time:	0	3	15
%B:	30	30	80

Flow Rate: 1.0mL/min
Detector: ELSD

Thyroid Chemicals

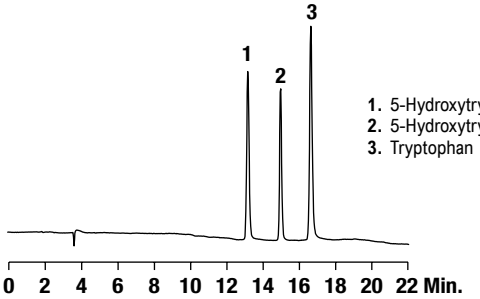
CHROM 8282



1. DL-Tyrosine
2. 3-Iodo-Tyrosine
3. 3,5- Diiodo-D-Tyrosine
4. DL-Thyroxine
5. 3,3,5-Triiodo-L-Thyronine

Column: Alltech® Platinum™ EPS C18, 5µm, 150 x 4.6mm (Part No. **32214**)
Mobile Phase: 0.05M KH_2PO_4, pH3.2:Acetonitrile (65:35)
Flow Rate: 1.0mL/min
Detector: UV at 254nm

Tryptophan Derivatives



1. 5-Hydroxytryptophan
2. 5-Hydroxytryptamine
3. Tryptophan

Column: Vydac® C18, 5µm, 250 x 4.6mm (Part No. **201SP54**)
Mobile Phase: **A:** 50mM KH_2PO_4, pH4.59
B: 90% Acetonitrile
Gradient:

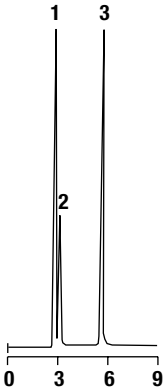
Time:	0	3	10	15
%B:	1	1	15	35

Flow Rate: 1.5mL/min
Detector: UV at 254nm

Amino Acids

Sulfur-Containing Native Amino Acids

CHROM 9413

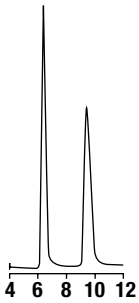


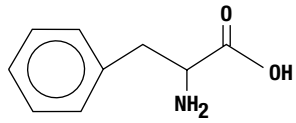
1. Cystine
2. Cysteine
3. Methionine

Column: Grom™ Sil 120 ODS-4 HE, 5µm, 250 x 4mm (Part No. **5113626**)
Mobile Phase: Water
Flow Rate: 0.8mL/min
Detector: UV at 210nm

Phenylalanine Enantiomers

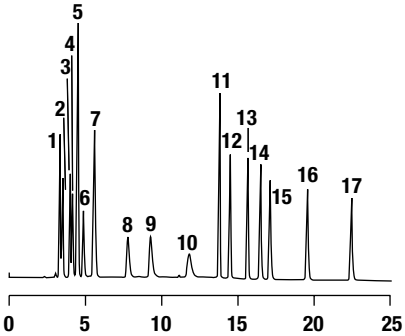
CHROM 8770





Column: Astec™ Chirobiotic® T™, 250 x 4.6mm (Part No. **12053**)
Mobile Phase: Ethanol:Water (80:20)
Flow Rate: 1.0mL/min
Detector: UV at 210nm

Amino Acids without Derivatization



1. Gly
2. Ser/Asn
3. Asp
4. Gln
5. Ala/Thr
6. Glu
7. Cys/Lys
8. His
9. Pro
10. Arg
11. Val
12. Met
13. Tyr
14. Ile
15. Leu
16. Phe
17. Trp

Column: Alltech® Prevail™ C18, 5µm, 250 x 4.6mm (Part No. **99210**)
Mobile Phase: **A:** 5mM Heptafluorobutyric Acid, 0.7% Trifluoroacetic Acid
B: Acetonitrile
Gradient:

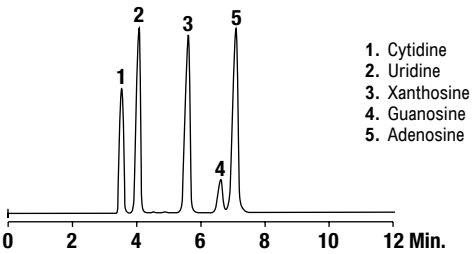
Time:	0	6	8	25
%B:	0	0	15	35

Flow Rate: 1.0mL/min
Detector: ELSD

Nucleic Acids

Nucleosides

CHROM 10347



1. Cytidine
2. Uridine
3. Xanthosine
4. Guanosine
5. Adenosine

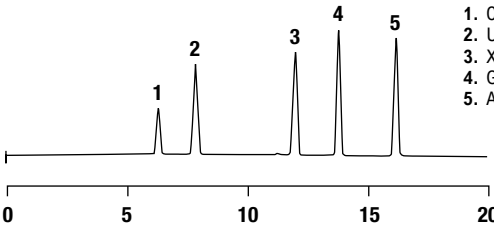
Column: Alltech® Alltima™ HP EPS C18, 5µm, 150 x 4.6mm (Part No. **87715**)
Mobile Phase: **A:** 0.03M Potassium Phosphate, pH3.2 **B:** Acetonitrile
Gradient:

Time:	0	2	20
%B:	5	5	30

Flow Rate: 1.0mL/min
Detector: UV at 260nm

Nucleosides

CHROM 8245



1. Cytidine
2. Uridine
3. Xanthosine
4. Guanosine
5. Adenosine

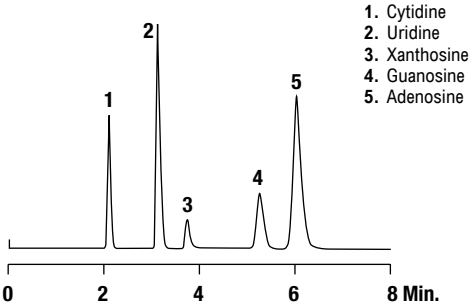
Column: Alltech® Platinum™ EPS C18, 5µm, 250 x 4.6mm (Part No. **32246**)
Mobile Phase: **A:** 0.03M KH_2PO_4, pH3.2 **B:** Acetonitrile
Gradient:

Time:	0	2	20
%B:	5	5	30

Flow Rate: 0.7mL/min
Detector: UV at 260nm

Nucleosides

CHROM 9410

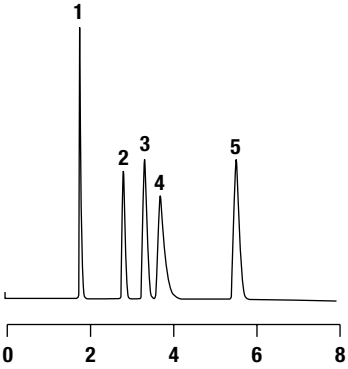


1. Cytidine
2. Uridine
3. Xanthosine
4. Guanosine
5. Adenosine

Column: Alltech® Prevail™ C18, 5µm, 150 x 4.6mm (Part No. **99208**)
Mobile Phase: 0.025M KH_2PO_4, pH3.0:Acetonitrile (96:4)
Flow Rate: 1mL/min
Detector: UV at 254

Nucleic Acid Bases

CHROM 9411



1. Cytosine
2. Uracil
3. Guanine
4. Adenine
5. Thymine

Column: Alltech® Prevail™ C18, 5µm, 150 x 4.6mm (Part No. **99208**)
Mobile Phase: 0.025M KH_2PO_4, pH3.0:Acetonitrile (98:2)
Flow Rate: 1.0mL/min
Detector: UV at 254

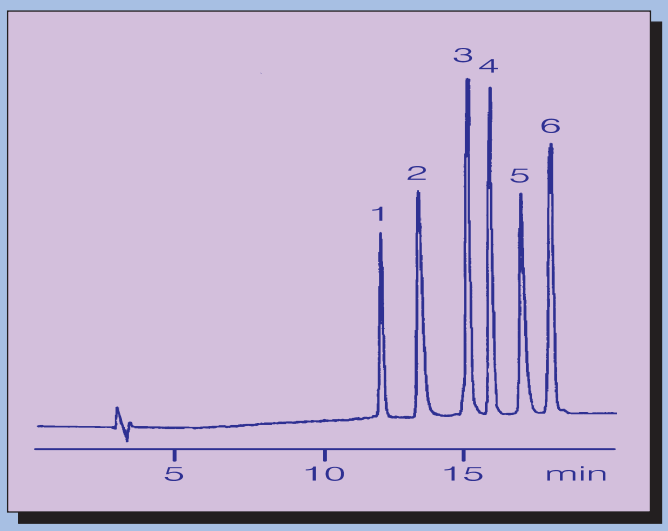
Tailor-made, customized columns for particular applications

In order to ensure the validity of HPLC results over an extended period of time, not only is the reproducibility of filling the column important but also to a high degree the batch-to-batch reproducibility of the packing material itself, whereby excellent resolution, respectively high efficiency and long stand times must be taken for granted. This is particularly true for the analysis of complex mixtures such as pesticides, polyaromatic hydrocarbons, amino acids, etc. For a range of selected applications, the time and effort of optimisation of the separation (selection of stationary phase, eluent composition, flow rate, temperature, etc.) may be eliminated simply by choosing the appropriate ***GROM*** special column. These columns are packed with stationary phases especially developed in our R&D laboratories for a particular application. The stationary phases are subjected to stringent quality control in order to guarantee the absolute reproducibility of their selectivity for the separation in question. Each special column is accompanied by a detailed protocol of the chromatographic conditions and, where applicable, the conditions of derivatization.
GROM special columns are available in both the standard ***NovoGROM*** hardware or as ***NovoGROM*** cartridges for use with quick connectors in the sizes 2, 3 and 4 mm internal diameter. (Please enquire for other dimensions, e.g., ***NovoGROM*** Microbore or ***NovoGROM*** capillary columns.)

I. Biochemical applications

Proteins and peptides

10 129 Peptide Separation



1) Oxytocin
2) Bradykinin
3) Angiotensin
4) Eledoisin
5) Neurotensin
6) Angiotensin I

Column phase:		GROM-Bio RP-1 (C18), 5 µm
Column size:		250 x 2 mm
Eluent	**A:**	0.1% TFA in H_2O
	B:	0.1% TFA in H_2O /ACN = 30 / 70
Gradient:		5 - 100% B (0-30 min)
Flow rate:		0.3 ml/min
Pressure:		16 MPa
Temperature:		RT
Detection (UV):		210 nm
Injection:		10 µl (= 10 pMol)

Stationary phase	Field of application	Order number
GROM-Bio Reversed Phase I widepore C18	small polypeptides, nucleotides	GS BR 1 0530 K 2502 250 x 2 mm cartridge
GROM-Bio Reversed Phase II widepore C8	peptides, medium sized proteins	GS BR 2 0530 K 2502 250 x 2 mm cartridge
GROM Bio Reversed Phase III widepore C4	proteins (MW 3 000 Dalton)	GS BR 3 0530 K 2502 250 x 2 mm cartridge

Amino acids

For detailed information see pages 53-67.

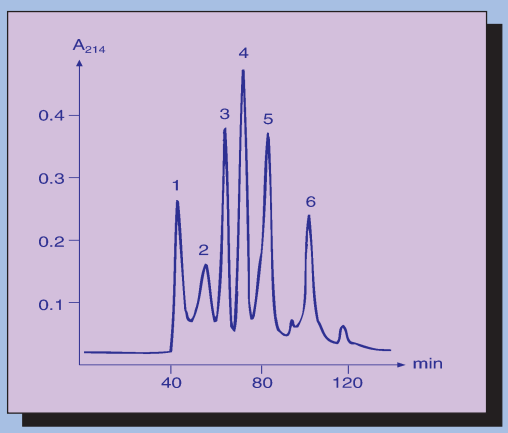
High performance prepacked size exclusion chromatography column

Novarose™ SE - 100/17 for Size Exclusion Chromatography

Superior performance in purification, molecular weight determination and fast desalting of peptides and proteins

A new emulsification and crosslinking method has made it possible to modify highly purified agarose and to produce small, spherical beads, thereby decreasing pore size and particle size while keeping the matrix volume low. Thus, outstanding results are obtained employing ***Novarose SE*** in high performance size exclusion chromatography. *Novarose ™ SE* - 100 / 17 columns are designed for analytical and semi-preparative separations of biopolymers such as proteins in the 10,000 to 100,000 Daltons molecular weight range. Due to the low diffusion coefficients involved, protein separation requires comparatively ***low flow rates*** to give ***high resolution*** in size exclusion chromatography. This is especially true when highly heterogeneous samples are applied. Nevertheless, impurities, which are always of interest but hidden at higher flow rates, can easily be detected at relative low flow rates.

10 184 Analytical separation of protein mixture



Experimentals conditions: **column:** Novarose ™ SE - 100 / 17–300 x 8 mm; **eluent:** 0.05 M Na-phosphate pH 6.7, 0.15 M NaCl; **flow rate:** 0.25 ml / min; **temperature:** ambient; **detection (UV):** 214 nm; **sample injected:** mixture of 1) 24.6 µg, thyroglobulin, 2) 24.6 µg aldolase, 3) 12.3 µg ovalbumin, 4) 12.4 µg carbonic anhydrase, 5) 24.0 µg ribonuclease and 25.0 µg insulin dissolved in 80 µl eluent buffer.

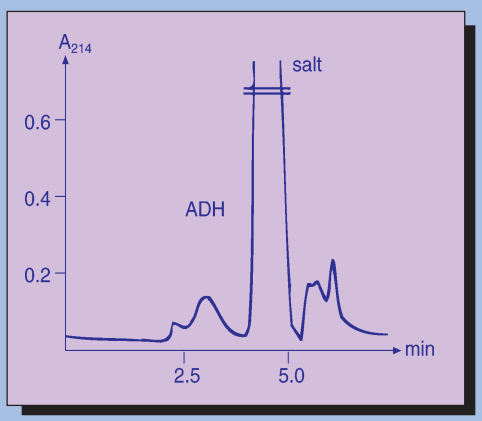
The following table summarizes the separation efficiency for carbonic anhydrase (peak 4) at different flow rates. Decreasing the flow rate clearly demonstrates the slower diffusion rate of larger molecules. The efficiency nearly doubles when reducing the flow rate by half.

Data of resolution of proteins

flow rate [ml / min]	resolution between two successive peaks 2 / 3	3 / 4	4 / 5	5 / 6	efficiency* N / m
1.00	0.44	0.56	0.71	1.08	1420
0.50	0.54	0.79	0.91	1.16	2900
0.25	0.77	0.96	1.11	0.89	4267
0.10	0.96	1.30	1.46	1.88	8113
0.05	1.11	1.34	1.57	2.00	8317

* calculated on carbonic anhydrase, peak 4 (same experimental conditions as in Applic. 10 184)

10 186 Desalting of alcohol dehydrogenase

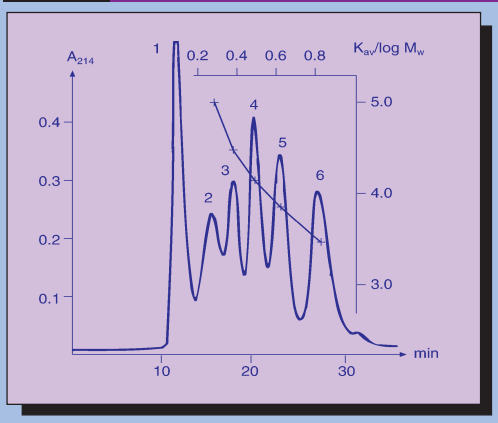


However, often speed is also considered as an important parameter in chromatographic procedures especially for less complex separations, such as the removal of salt from a biopolymer preparation or buffer exchange. A quick and simple desalting step is often required prior to enzymatic digestion. The rigidity and chemical stability of the ***Novarose SE*** also make it ideal for high flow rates or use with extreme pH values. Thus, the figure „desalting of alcohol-dehydrgenase" shows the facile removal of 6 M guanidinum hydrochloride and dithiotreitol from alcohol dehydrogenase within a few minutes (flow rate 4,0 ml/min).

10 185 Protein molecular weight determination

This figure represents an easy, quick method for the determination of molecular weights of globular proteins. The K_{av} values for the different proteins in the mixture are calculated and plotted against the logarithm of their molecular weight. Good linearity is shown in the recommended separation range.

Experimental conditions:
s. above, except 0.5 ml/min flow rate



II. *GROM* Gel Permeation Chromatography (GPC)

... to meet all the needs of highly sophisticated Polymer Analysis

NovoGROM-GPC columns are packed with novel **styrol divinylbenzene copolymer** packings. The spherical, macroporous particles show excellent, narrow particle and pore size distributions, therefore guaranteeing:

- **Extraordinary performance, i.e., superior resolution**
- **Excellent reproducibility**
- **Unique mechanical and chemical strength/stability**
- **High solvent compatibility**
- **Long column durability/life time**

These features are, as always additionally enhanced by ***GROM***'s quick delivery and prompt, cost-saving refill service. Specially designed, easy-to-handle column hardware enables the optimal flow diversion needed for high resolution. These individually tested columns are normally packed and shipped in tetrahydrofuran (THF). However, upon request, they can be packed in other solvents as well. **Note!** Water or alcohols must never be used as eluents.

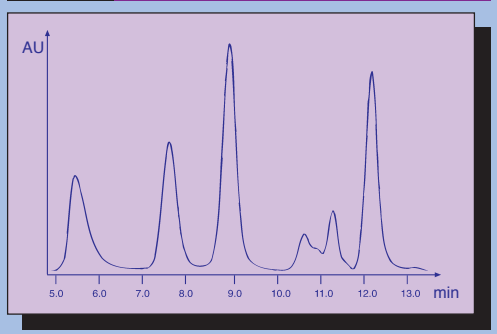
Description

Product	Particle Size [µm]	Mol. Weight [Dalton]
NovoGROM- GPC - 100 Å	5 / 10	< 3 x 10^3
NovoGROM- GPC - 500 Å	5 / 10	< 2 x 10^4
NovoGROM- GPC - 1,000 Å	5 / 10	1 x 10^3–4 x 10^4
NovoGROM- GPC - 10,000 Å	5 / 10	4 x 10^3–5 x 10^5
NovoGROM- GPC - 100,000 Å	5 / 10	1 x 10^4–2 x 10^6
NovoGROM- GPC - 1,000,000 Å	5 / 10	2 x 10^5–1 x 10^7

Specifications

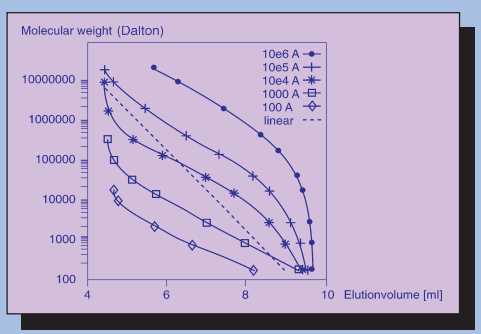
Parameters	Particle Size [5 µm]	[10 µm]
Efficiency [N/m], min.	55 000	35 000
typically	> 65 000	> 40 000
Maximum Pressure.	160	160
typical pressure drop / 30 cm	25	15
Maximum Lin. Flow. [cm/min]	3	6
Temperature [°C]	< 100°	< 140°

10 187 Polystyrol separation



Column phase: Linear, 5 µm
Column size: 300 x 8 mm
Sample: mixture of polystyrols
Eluent: THF
Flow rate: 1 ml / min
Detection: RI
Temperature: 25°C

Calibration curves of polystyrols
(column: 300 x 8 mm)



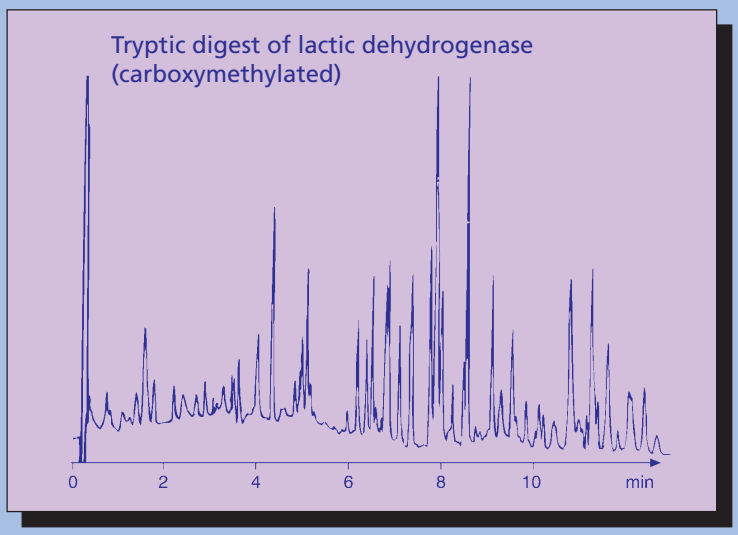
Field of application

Solvents commonly used	Applications
Tetrahydrofuran	styrols, (meth-)acrylates, dienes, PVC, PIB, PC, phenol resins, cellulose esters, tricarbanilates, epoxide resins, polysilanes, PEG
Chloroalkanes	styrols, (meth-)acrylates, dienes, PVC, PIB, PC, polyphenylene oxide etc.
Dioxane	Cl-butadiene, vinyl acetate
Methylethyl ketone	styrols, etc.
Ethyl acetate	styrols, etc.
Cyclohexane	dienes, etc.
Benzene	styrols, dienes, PIB
Toluene	styrols, dienes, PIB, phenylene oxides, silicones
Xylene	styrols, dienes, PIB, phenylene oxides, silicones
o-Dichlorbenzene	styrols, ethylenes, propylenes, EPR, PIB, asphalt, wax
Trichlorbenzene	styrols, ethylenes, propylenes, EPR, PIB, asphalt, wax
m-Kresol	styrols, polyamides, polyesters, photoresists
Chinoline	styrols
Dimethylformamide	APS, PAN, PEO, polysulfones, polyvinyl pyrrolidones, polyurethanes, melamine and phenolic resins
Dimethylacetamide	APS, PAN, PEO, polysulfones, polyviny lpyrrolidones, polyurethanes, melamine and phenolic resins
N-Methylpyrrolidone	polyimide, poly-THF, polyvinyl pyrrolidone, saccharides
Dimethylsulfoxide	gelatin, lignin, starch, polysaccharides
Hexafluoro-iso-propanol	melamine resins, polyamides, polyesters, polyketones

III. Novel High Performance High Speed

either by uniformly spherical, porous 1.5 µm ODS particles or by fully spherical, non-porous 1.5 µm ODS particles packed in the unique, out-standing NovoGROM 33 x 2.0 mm or resp. 33 x 4.6 mm column hardware

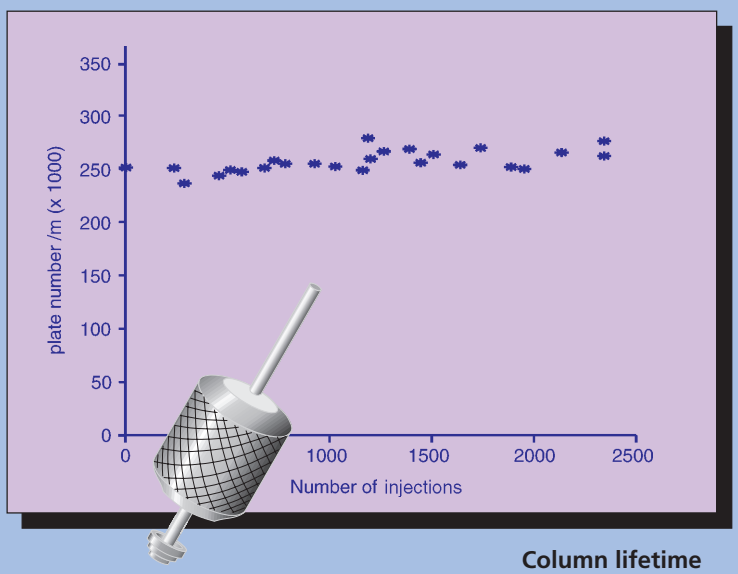
For high-speed, high-resolution HPLC two types of uniformly spherical **1,5 µm *GROM*-Sil ODS-2 FE** particles are available, either fully porous micro-spheres (pore size 100 Å / particle size 1.5 ± 0.3 µm, specific surface area of 200 m^2/g) or monodisperse **non-porous** beads (particle size 1.5 ± 0.05 µm, specific surface area of 2.1 m^2/g). Both are fully end-capped C18 phases meeting all the requirements of the most advanced high performance liquid chromatography applications. For instance, in a minimum of analysis time, they perform excellent separations of nucleotides, peptides, proteins, pharmaceuticals, etc.



10 179 Peptides, tryptic digested

Column phase:	GROM-SIL 100 ODS-2 FE, 1.5 µm
Column size:	33 x 4.6 mm
Eluent A:	H_2O, 0.1% TFA
Eluent B:	ACN, 0.1% TFA
Gradient:	0–15 min, 5–60% B
Flow rate:	1ml/min
Detection (UV):	215 nm
Temperature:	RT
Injection:	2 µl

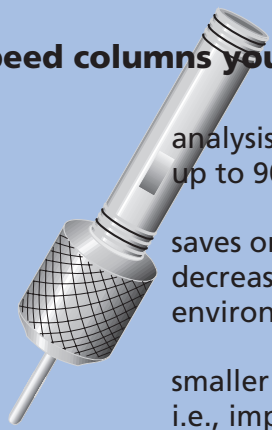
Even after more than 2 500 injections, both types of columns packed with either 1.5 µm porous- or 1.5 µm non-porous particles still show excellent physical and chemical stability (even in 0.1% TFA containing eluents). There is no change in retention time and practically no increase in pressure drop and peak asymmetry over the life of the column. These ***NovoGROM*** high speed columns of only 33 mm length provide up to 10 000 plates, and thus in most cases have a resolution nearly identical to that which is typically found for standard analytical HPLC columns.



Column lifetime

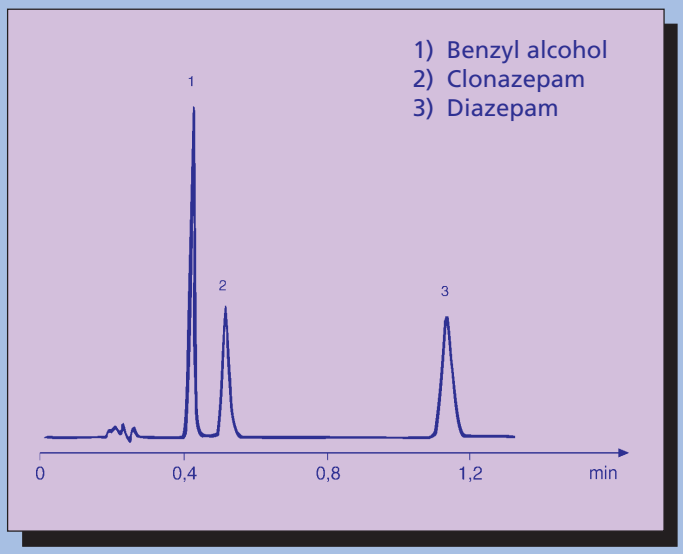
Employing *NovoGROM* high speed columns you gain tremendously in

- **Speed:** analysis time in seconds or minutes, with up to 90% increase in sample throughput
- **Low solvents consumption:** saves on purchase and disposal costs, decreasing solvent consumption brings improved environmental compatibility
- **Decreased diffusion:** smaller peak volume and less band broadening, i.e., improved sensitivity



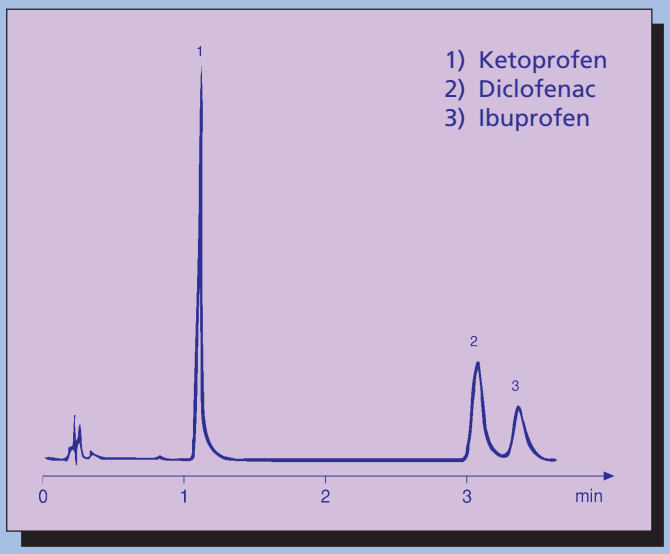
Liquid Chromatography

10 030 b Sedatives -Benzodiazepins



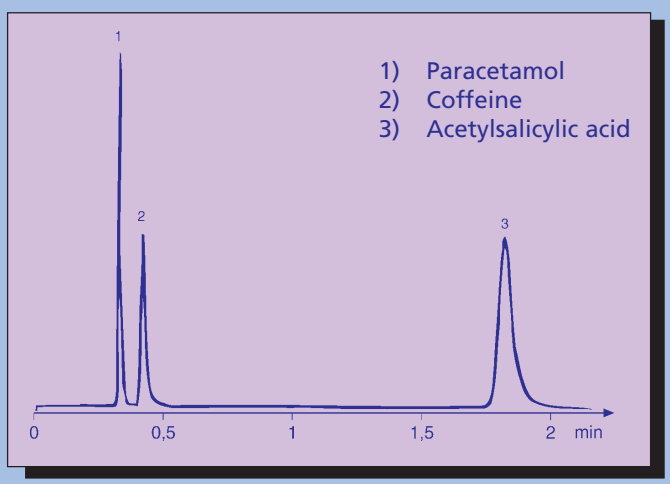
Column phase: GROM-SIL 100 ODS-2 FE, 1.5 µm
Column size: 33 x 4.6 mm
Eluent: 35% v/v Acetonitrile, 65% v/v 0.1% TFA
Flow rate: 1.5 ml/min
Pressure: 26.2 MPa
Temperature: RT
Detection: 254 nm
Sample: 1 µl

10 031 b Nonsteroid anti-inflammatory drugs



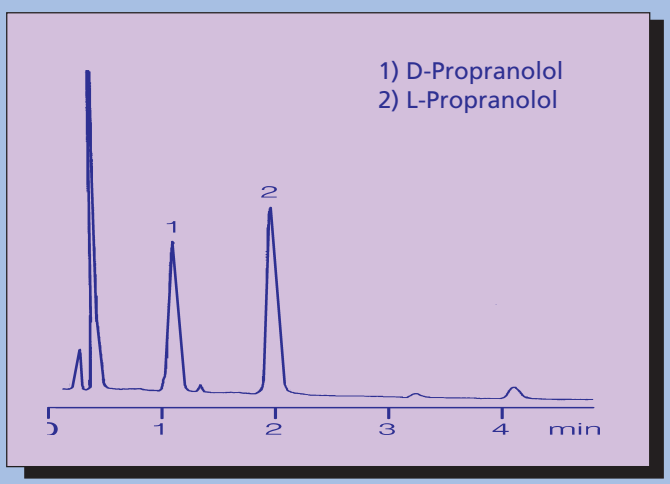
Column phase: GROM-SIL 100 ODS-2 FE, 1.5 µm
Column size: 33 x 4.6 mm
Eluent: 40% v/v Acetonitrile, 60% v/v 0.1% TFA
Flow rate: 1.5 ml/min
Pressure: 26 MPa
Temperature: RT
Detection: 254 nm
Sample: 1 µl (100 µg/ml, Ibuprofen 1000 µg/ml)

10 032 c Analgesic, antipyretic Thomapyrin pill



Column phase: GROM-SIL 100 ODS-2 FE, 1.5 µm
Column size: 33 x 4.6 mm
Eluent: 15% v/v Acetonitrile, 85% v/v 0.1% TFA
Flow rate: 1.5 ml/min
Pressure: 29.2 MPa
Temperature: RT
Detection: 274 nm; after 1 min, 227 nm
Sample: 10 µl (Paracetamol 2 mg/m², Coffeine 0.5 mg/ml, ASA 2 mg/ml)

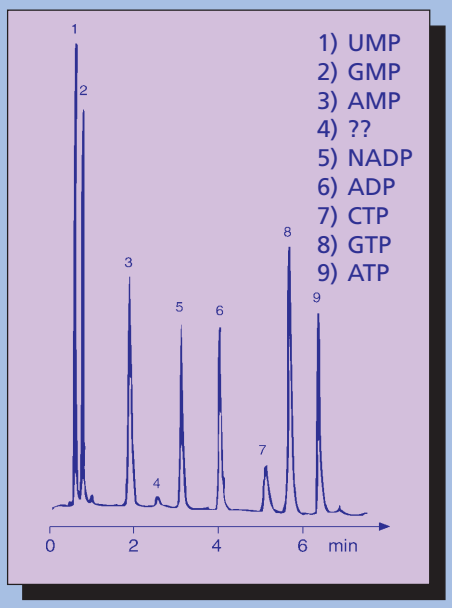
10 053 b High Speed HPLC Analysis -Determination of Optical Antipodes of a β-Blocker



Column phase: GROM-SIL 100 ODS-2 FE, 1.5 µm
Column size: 33 x 4.6 mm
Eluent: water / acetonitrile = 30 / 70
Flow rate: 1.0 ml/min
Pressure: 18 MPa
Temperature: RT
Detection (Fluor.): 263 nm (exc.), 313 nm (em.)
Injection: 1µl (= 500 fmol)

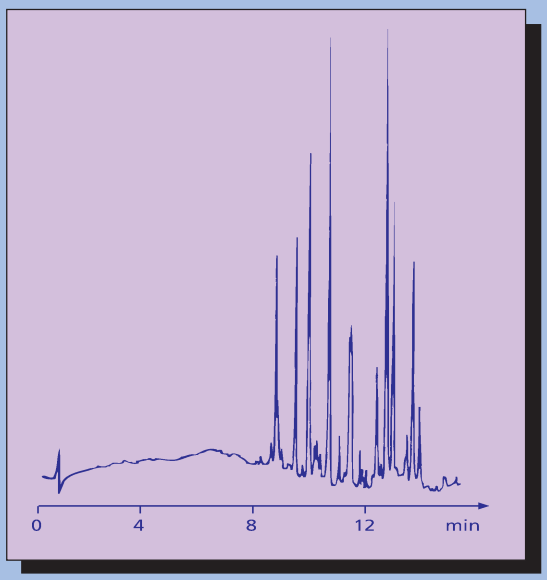
High Speed Liquid Chromatography

10 180 Nucleotides



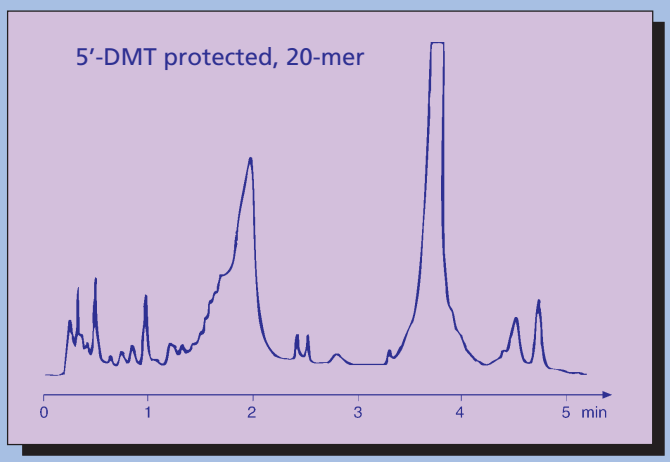
Column phase: GROM-SIL 100 ODS-2 FE, 1.5 µm
Column size: 33 x 4.6 mm
Eluent: A: 30 mM KH_2PO_4 / K_2HPO_4, 0.5 MTBA, pH 6.0
B: 60% A + 40% ACN
Gradient: 0–40% B (0–8 min)
Flow rate: 1.5 ml/min
Temperature: RT
Detection (UV): 254 nm
Sample: 10 µl (10 µg each)

10 182 Protein Digest



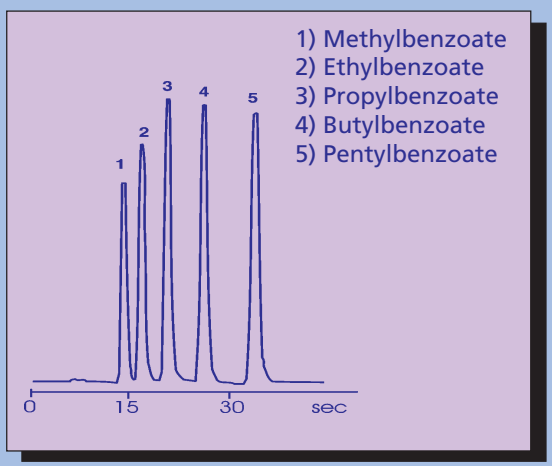
Column phase: GROM-SIL 100 ODS-2 FE, 1.5 µm
Column size: 33 x 2.0 mm
Eluent: A: 0.1% TFA in H_2O
B: 0.1% TFA in ACN
Gradient: 5–50% B (0–12 min)
Flow rate: 0.2 ml/min
Pressure: 10–27 MPa
Temperature: RT
Detection (UV): 214 nm
Sample: 1 µl (5 pMol)

10 181 Antisense-Oligonucleotides



Column phase: GROM-SIL 100 ODS-2 FE, 1.5 µm
Column size: 33 x 4.6 mm
Eluent: A: 0.05 M TEAA, pH 7.0
B: 30% A + 70% ACN
Gradient: 0–70% B (0–6 min)
Flow rate: 1.0 ml/min
Temperature: RT
Detection (UV): 260 nm
Sample: 10 µl (10 µg each)

10 162 High Speed Separation of Benzoates



Column phase: GROM-SIL-ODS 0 AB, 1.5 µm
Column size: 33 x 4 mm
Eluent: H_2O / ACN = 30 / 70
Flow rate: 2 ml/min
Pressure: 23,8 MPa
Temperature: RT
Detection (UV): 254 nm
Sample: 1 µl (0,5-1,5 mg/ml of each)

Fast Protein and Peptide Analyses with Vydac 3μm 300Å Reversed-Phase Columns

Decreasing the particle size of an HPLC packing facilitates rapid chromatography by speeding diffusion between the mobile phase and adsorptive surfaces of the stationary phase. For small molecule separations this leads to

- **sharper peaks**
- **resolution on shorter columns**
- **reduced analysis time**
- **reduced solvent consumption.**

In fact, much of the performance gain of modern HPLC over earlier chromatographic techniques is the result of improvements in the manufacture and use of small-particle adsorbents.

Unlike small molecules, peptides and proteins typically do not partition by repeatedly adsorbing and desorbing from the stationary-phase as they move through a chromatography column. Instead, separation is based on mobile-phase gradients. Strong adsorption is followed by selective release when the concentration of organic component reaches a specific value for each analyte. Retention of proteins and peptides is more an all-or-nothing phenomenon.

The main effect of reducing adsorbent particle size for this type of separation is to allow mobile phase molecules to move rapidly in and out of the packing. This permits a quick and uniform release of each adsorbed analyte as the critical mobile-phase composition is attained. Performance advantages are somewhat different from those for small molecules. Much protein and peptide chromatography is already run on short columns with 5μm to 10μm packings. The 3μm particle size speeds exchange between the mobile phase and packing, and allows you to

- **increase the mobile phase flow rate**
- **run a shallower gradient on a per-volume basis during the same run time.**

This results in the fast, high resolution separations shown here with Vydac's 3μm 300Å reversed phases.

Figure 1 shows a comparison of separations of five standard proteins run on a 100-mm-long 3μm reversed-phase column (Vydac 238TP3410) with a flow rate of 2.5 mL/minute, and using the same gradient timing on a 50-mm-long column with the same packing (Vydac 238TP3405) at a higher flow rate of 4.0 mL/minute. The higher flow rate is made possible by the lower back pressure of the shorter column. Note the faster analysis time together with improved resolution on the shorter column due to the shallower elution gradient.

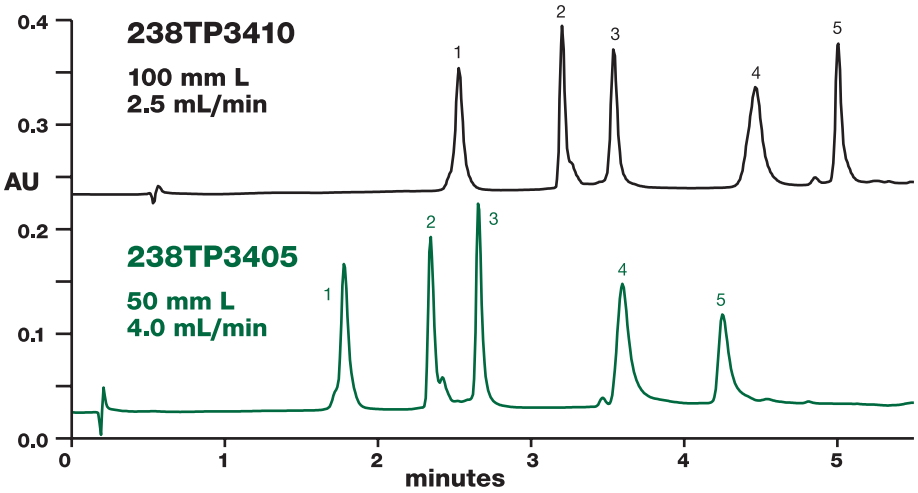


Figure 1. Comparison of protein separations on 3μm 300Å reversed-phase columns of 100 mm and 50 mm lengths. Note the higher flow rate on the 50 mm column which produces a shallower mobile-phase composition gradient on a per-volume basis. Columns: Vydac 238TP3410 "monomeric" C18 reversed-phase, 3μm particle size, 300Å pore size, 4.6mmID x 100mmL, and Vydac 238TP3405, identical packing in 4.6mmID x 50mmL column. Flow rates: 2.5 mL/min and 4.0 mL/min, respectively. Mobile phase: A = 20% acetonitrile (v/v) in water with 0.1% TFA (w/v). B = 45% acetonitrile (v/v) in water with 0.1% TFA (w/v). Gradient: 0% to 100% B in 4 minutes for both columns. Detection: 215nm. Sample: Standard protein mixture. Peaks: (1) ribonuclease, (2) insulin, (3) cytochrome C, (4) BSA, and (5) myoglobin.

The Nest Group • 45 Valley Road • Southborough, MA 01772.1306 • USA
Toll-Free: 800.347.6378 • Tel: 508.481.6223 • Fax: 508.485.5736 • EMail: info@nestgrp.com

3µm 300Å Reversed-Phase Columns for Proteins and Peptides

The same five proteins (Figure 2) and also a mixture of six standard peptides (Figure 3) were run under the fast separation conditions on 3µm 50-mm-long columns with three different reversed-phase chemistries for a comparison of selectivities. The 218TP and 238TP packings are both octadecyl (C18) phases on the same 300Å silica base, but with polymeric and monomeric bonding chemistries, respectively. The 214TP packing is a polymerically bonded butyl (C4) phase.

Good separations of the five proteins were obtained on all three columns, with the C4 column providing the best peak shape and resolution.

In the case of the peptide mixture, the C4 column (separation not shown) failed to resolve peaks 2 and 3. Both C18 columns were effective in resolving all components, with the monomeric bonding chemistry (238TP) providing the best resolution and peak shapes.

These results suggest that Vydac's 238TP monomeric C18 may be the best all-around choice for both peptide and protein analyses. Still, for mixtures of uncertain composition the ability to screen on a variety of reversed-phase chemistries with varying selectivity provides added assurance that all components will be seen.

Vydac's 3µm columns make rapid analysis possible and are ideal for:

- **in-process testing**
- **combinatorial library screening**
- **screening of recombinant clones**
- **studies of reaction kinetics**

Ordering Information Call 800.347.6378

Cat. No.	Description
	50 mm long columns:
238TP3405	Column, Octadecyl (C18), Monomeric, 3µm, 300Å, 4.6mm ID x 50mm L
218TP3405	Column, Octadecyl (C18), Polymeric, 3µm, 300Å, 4.6mm ID x 50mm L
214TP3405	Column, Butyl (C4), Polymeric, 3µm, 300Å, 4.6mm ID x 50mm L
	100 mm long columns:
238TP3410	Column, Octadecyl (C18), Monomeric, 3µm, 300Å, 4.6mm ID x 100mm L
218TP3410	Column, Octadecyl (C18), Polymeric, 3µm, 300Å, 4.6mm ID x 100mm L
214TP3410	Column, Butyl (C4), Polymeric, 3µm, 300Å, 4.6mm ID x 100mm L

Proteins

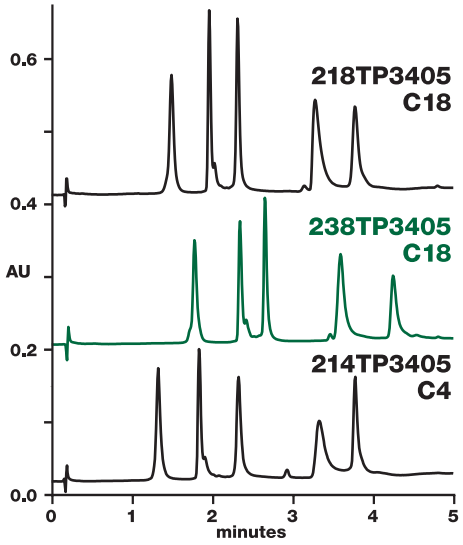


Figure 2. Comparison of protein separation on three 3µm 300Å reversed-phase columns.
Flow rate: 4.0 mL/min. Columns: Vydac 218TP3405 "polymeric" C18, 238TP3405 "monomeric" C18, and 214TP3405 C4, all 4.6mmID x 50mmL. Conditions: As described for Figure 1. Sample: Standard protein mixture. Peak order: Same as Figure 1.

Peptides

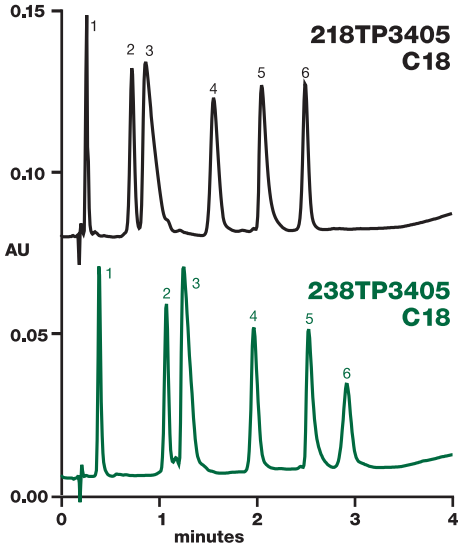


Figure 3. Comparison of peptide separation on two 3µm 300Å reversed-phase columns.
Flow rate: 4.0 mL/min. Columns: Same as Figure 2. Mobile phase: A = 15% acetonitrile (v/v) in water with 0.1% TFA (w/v). B = 25% acetonitrile (v/v) in water with 0.1% TFA (w/v). Gradient: 0% to 100% B in 3 minutes. Detection: 215nm. Sample: Peptide mixture. Peaks: (1) neurotensin fragment 1-8, (2) oxytocin, (3) neurotensin fragment 8-13, (4) angiotensin II, (5) neurotensin, and (6) angiotensin I.

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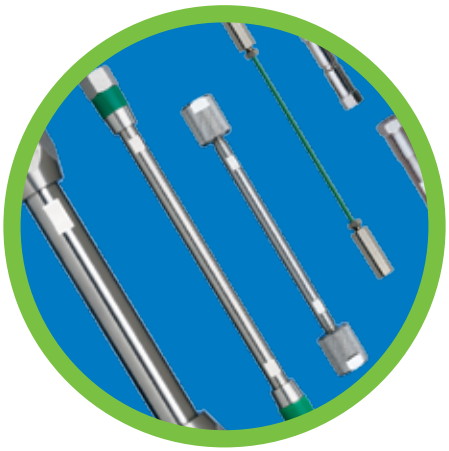
High Polar Biotech Products

with VisionHT™ Media Platform Columns

- **Speed and resolution on any system - LC, UHPLC, or Semi-Prep**
- **1.5-10µm orthogonal chemistries simplify separation strategies**
- **Single particle technology improves laboratory and organizational efficiency.**



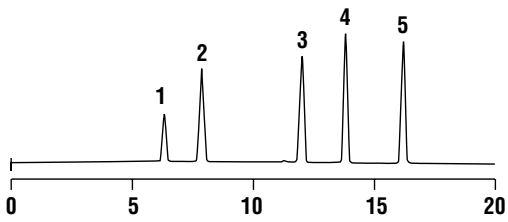
VisionHT™ dual-mode reversed-phase/silanophilic retention mechanism is a perfect match for biotechnology applications. Biopolymers and their building blocks all have structures with polar and non-polar regions. VisionHT™'s bonded phase interacts with the non-polar regions, while VisionHT™'s silanols interact with the polar regions. This powerful combination is a versatile tool for biotechnology applications.

VisionHT™ Media Platform Specifiations

Particle Size:	1.5, 3.0, 5.0,10µm
Base Silica:	High Purity Type B
Pore Size:	100Å for C18 Classic & C18 Polar 120Å for C18 Basic & C18 High Load
Surface Area:	200m² C18 Classic & C18 Polar 220m² C18 Basic & C18 High Load
pH Stability:	1-10
Pressure Rating:	16,000psig (UHPLC formats)

Nucleosides

CHROM 8245



1. Cytidine
2. Uridine
3. Xanthine
4. Guanosine
5. Adenosine

Column: VisionHT™ C18 Polar, 5µm, 150 x 4.6mm (Part No. **5152061**)
Mobile Phase: **A:** 0.03M KH_2PO_4, pH 3.2 **B:** Acetonitrile
Gradient:

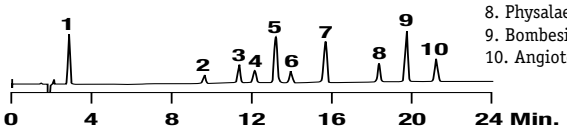
Time:	0	2	20
%B:	5	5	30

Flowrate: 0.7mL/min **Detector:** UV at 260nm

Peptides (≥5 Amino Acid Residues)

CHROM 8723

1. Peptide T
2. (Arg-8)-Vasotocin
3. (Lys-8)-Vasopressin
4. (Arg-8)-Vasopressin
5. Methionine Enkephalin
6. Oxytocin
7. Leucine Enkephalin
8. Physalaemin
9. Bombesin
10. Angiotensin



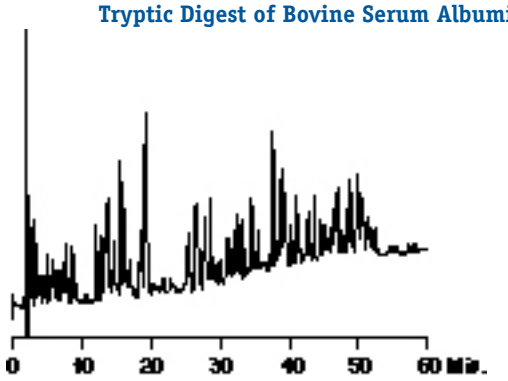
Column: VisionHT™ C18 Polar, 5µm, 150 x 4.6mm (Part No. **5152061**)
Mobile Phase: **A:** 0.15% v/v TFA in Water
B: 0.13% v/v TFA in 95:5 Acetonitrile:Water
Gradient:

Time:	0	2	30
%B:	15	15	55

Flowrate: 1.0mL/min
Detector: UV at 216nm

Tryptic Digest of Bovine Serum Albumin

CHROM 8852



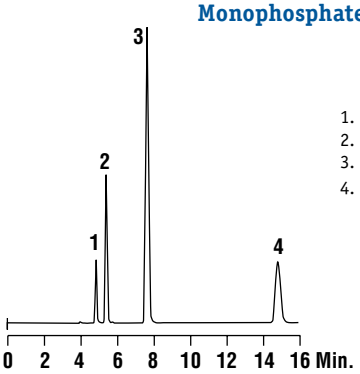
Column: VisionHT™ C18 Classic 5µm 150 x 4.6mm (Part No. **5152017**)
Mobile Phase: **A:** 0.15% TFA in Water
B: 0.13% TFA in 95:5 Acetonitrile:Water
Gradient:

Time:	0	60
%B	5	35

Flowrate: 1.0mL/min
Detector: UV at 216nm

Monophosphate Nucleotides

CHROM 8296



1. Cytidine- 5'-Monophosphate
2. Uridine-5'-Monophosphate
3. Guanosine-5'-Monophosphate
4. Adenosine-5'-Monophosphate

Column: VisionHT™ C18 Polar, 5µm, 150 x 4.6mm (Part No. **5152061**)
Mobile Phase: Methanol:0.05M Ammonium Acetate pH 5.5 (5:95)
Flowrate: 10mL/min
Detector: UV at 254nm

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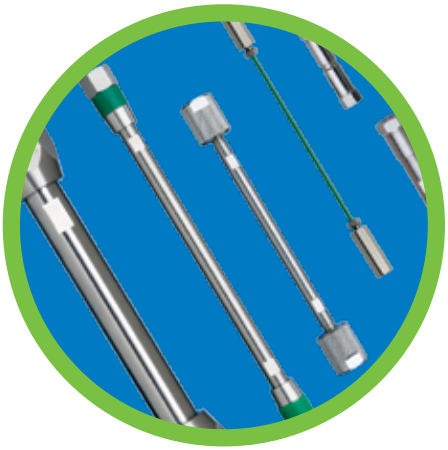
Fast peptide Separations with increased resolution

with VisionHT™ Media Platform Columns

- **Speed and resolution on any system - LC, UHPLC, or Semi-Prep**
- **1.5-10µm orthogonal chemistries simplify separation strategies**
- **Single particle technology improves laboratory and organizational efficiency**.



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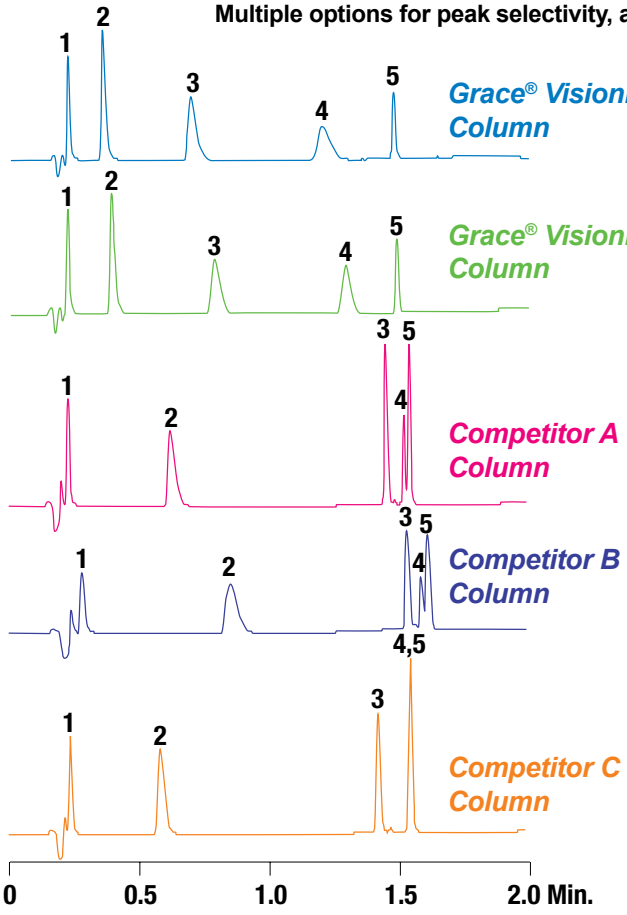
Surface Area:	200m² C18 Classic & C18 Polar 220m² C18 Basic & C18 High Load
pH Stability:	1-10
Pressure Rating:	16,000psig (UHPLC formats)

Multiple options for peak selectivity, and increased resolution for complex samples.



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)

Columns: Sub 2µm, 2.0 x 50mm
HPLC System: Agilent 1200 RRLC
Mobile Phase A: 0.1% TFA in Water
Mobile Phase B: 0.085% TFA in 80:20 Acetonitrile:Water
Gradient (min,%B): (0.0, 5), (1.0,100), (2,100)
Flow Rate: 0.8mL/min.
Detector: UV at 215nm
Injection Volume: 10µL
Injection Amount: 1000ng each
Column Temperature: 45°C
Column Backpressure: 508bar

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