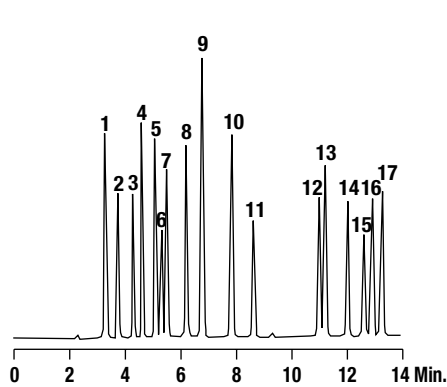


Proteins & Peptides/Reversed-Phase



VLTA ZAF OBRNUTA
www.obrnutafaza.hr
info@obrnutafaza.hr

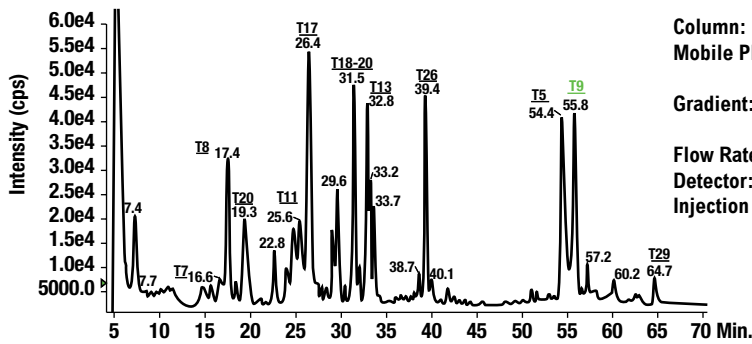
PTH Amino Acids



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

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.

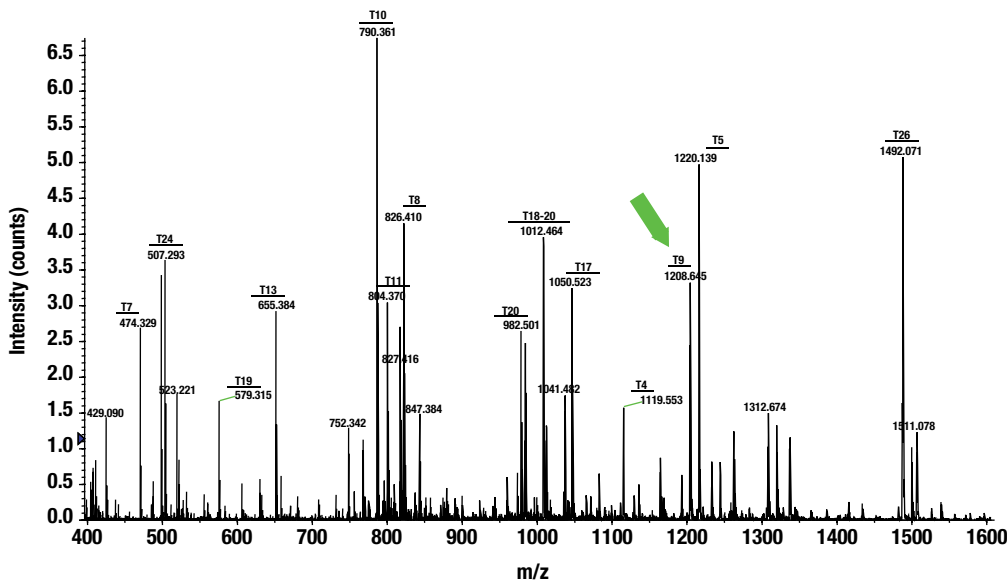


Column: Vydac®, 250 x 1mm (Part No. 238EV51)
Mobile Phase: A: Acetonitrile:0.05% Trifluoroacetic Acid (2:98)
 B: Acetonitrile:0.05% Trifluoroacetic Acid (90:10)
Gradient:

Time:	0	60
%B:	5	65

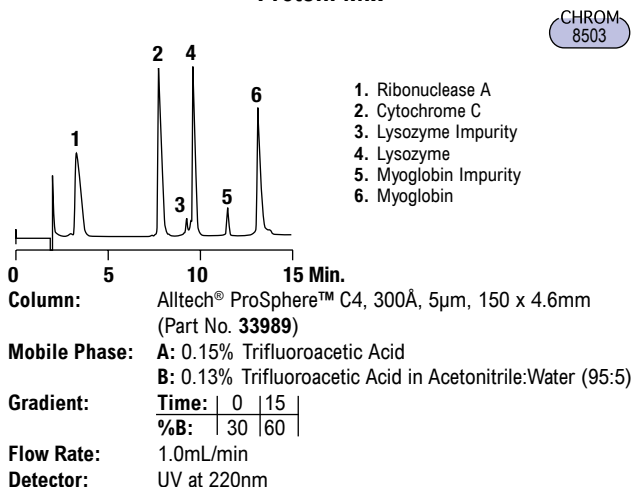
Flow Rate: 50µL/min
Detector: ms: ABI QSTAR
Injection Volume: 25µL GFP digest

Mass Spectrum of Tryptic Digest of GFP

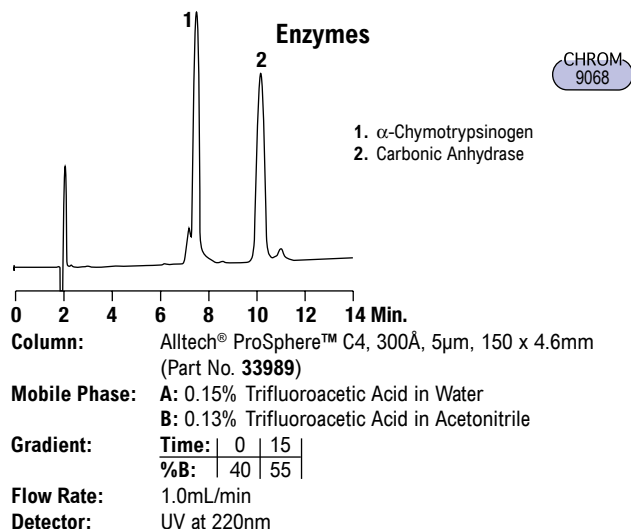


Proteins & Peptides/Reversed-Phase

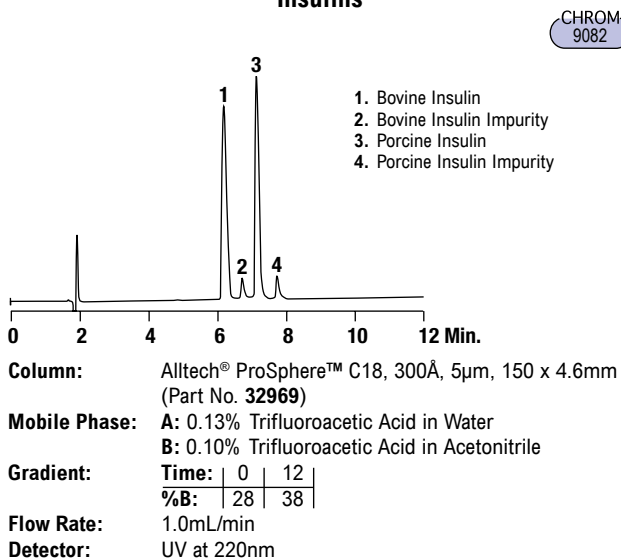
Protein Mix



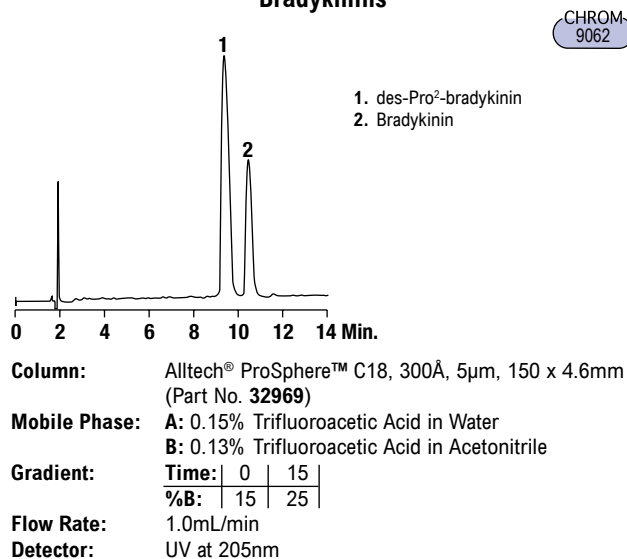
Enzymes



Insulins



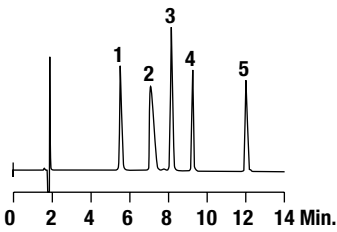
Bradykinins



Proteins & Peptides/Reversed-Phase

Bioactive Peptides

CHROM 8502



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

0 2 4 6 8 10 12 14 Min.

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:

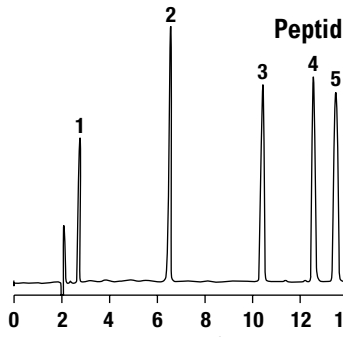
Time:	0	20
%B:	20	50

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

0 2 4 6 8 10 12 14 Min.

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:

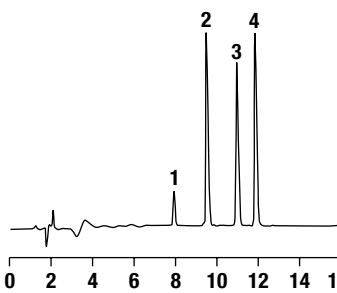
Time:	0	15
%B:	10	30

Flow Rate: 1.0mL/min

Detector: UV at 220nm

Peptide Mix

CHROM 10199



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

0 2 4 6 8 10 12 14 16 Min.

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:

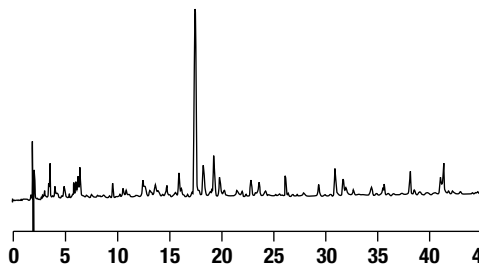
Time:	0	20
%B:	20	50

Flow Rate: 1.0mL/min

Detector: UV at 220nm

β-Lactoglobulin A Tryptic Digest

CHROM 9092



0 5 10 15 20 25 30 35 40 45 Min.

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:

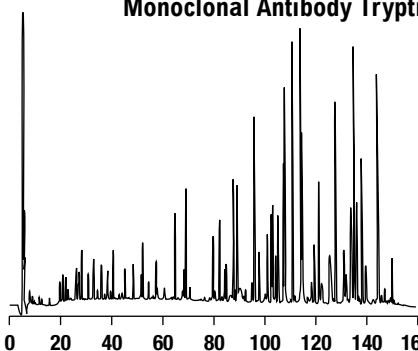
Time:	0	45
%B:	5	30

Flow Rate: 1.0mL/min

Detector: UV at 220nm

Monoclonal Antibody Tryptic Digest

CHROM 10213



0 20 40 60 80 100 120 140 160 Min.

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

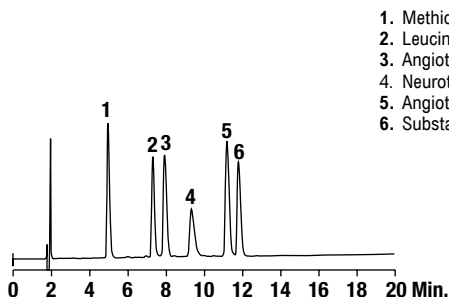
more applications

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

Proteins & Peptides/Reversed-Phase

Peptide Mix on 300Å

CHROM 10363



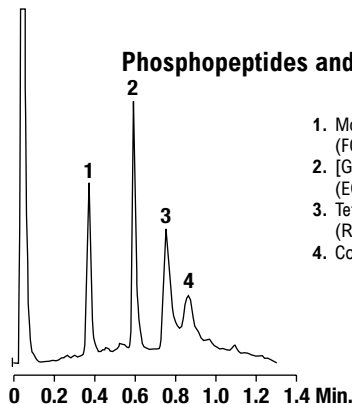
1. Methionine Enkephalin
2. Leucine Enkephalin
3. Angiotensin II
4. Neurotensin
5. Angiotensin I
6. Substance P

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 (FQpSEEQTEDELQDK)
2. [Glu¹]-Fibrinopeptide B (EGVNDNEEGFFSAR)
3. Tetraphosphopeptide (RELEELNVPGEIVeSLpSpSpSEESITR)
4. Contaminant(s)

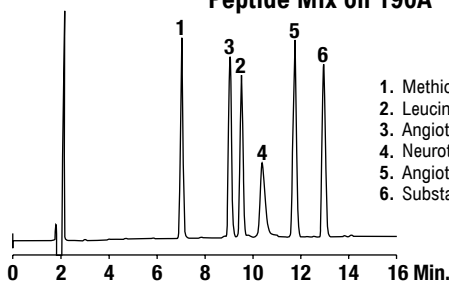
Column: Vydac® ProZap™ C18, 2.1 x 10mm
HPLC System: HPG binary pump
Mobile Phase: A: 0.1% Trifluoroacetic Acid in Water
 B: 0.085% Trifluoroacetic Acid in Acetonitrile:Water (80:20)
Gradient:

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

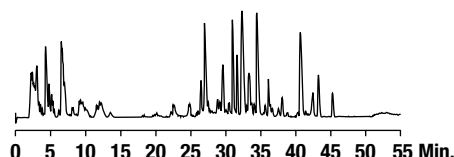
Column: Alltech® Alltima™ HP C18, 190Å, 5µm, 250 x 4.6mm (Part No. 87679)
Mobile Phase: A: 0.1% Trifluoroacetic Acid in Water
 B: 0.08% Trifluoroacetic Acid in Acetonitrile
Gradient:

Time:	0	20
%B:	20	40

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

BSA Tryptic Digest

CHROM 10582



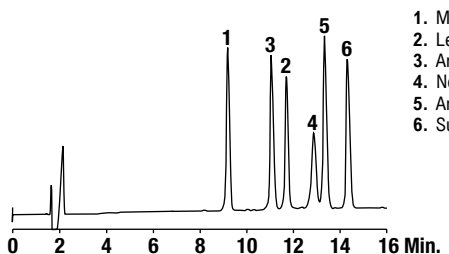
Column: Vydac® 218MS C18, 5µm, 150 x 0.075mm (Part No. 218MS5.07515)
Mobile Phase: A: Water with 0.1% Formic Acid:Acetonitrile (95:5)
 B: Water with 0.1% Formic Acid:Acetonitrile (20:80)
Gradient:

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

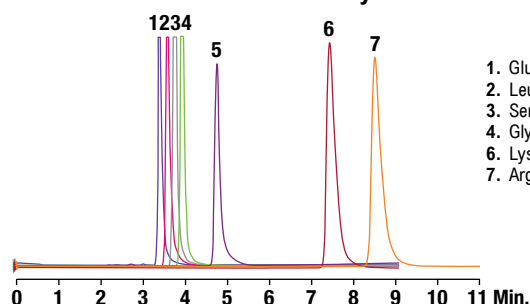
Column: Alltech® ProSphere™ C18, 100Å, 5µm, 250 x 4.6mm (Part No. 35091)
Mobile Phase: A: 0.1% Trifluoroacetic Acid in Water
 B: 0.08% Trifluoroacetic Acid in Acetonitrile
Gradient:

Time:	0	20
%B:	20	40

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

Amino Acids by HILIC

CHROM 10582

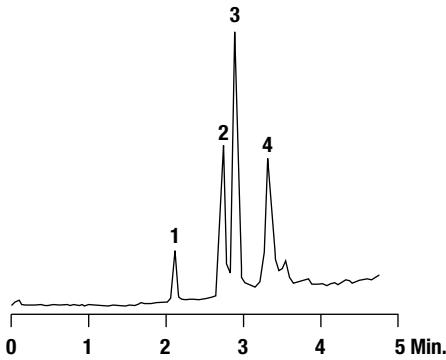


1. Glutamic Acid
2. Leucine
3. Serine
4. Glycine
6. Lysine
7. Arginine

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:

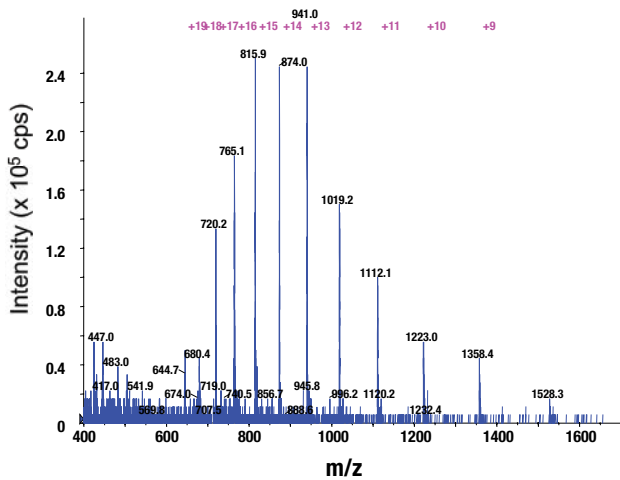
Time:	0	4.0	4.5	4.7
%B:	20	90	90	20

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.

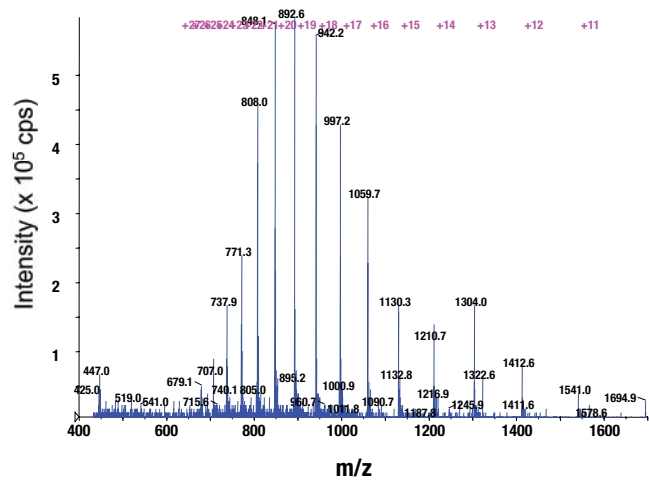
Left Chromatogram Injection Amount: 50 ng each
Right Chromatogram Injection Amount: 8µg each, except 1.5µg for growth hormone

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.)

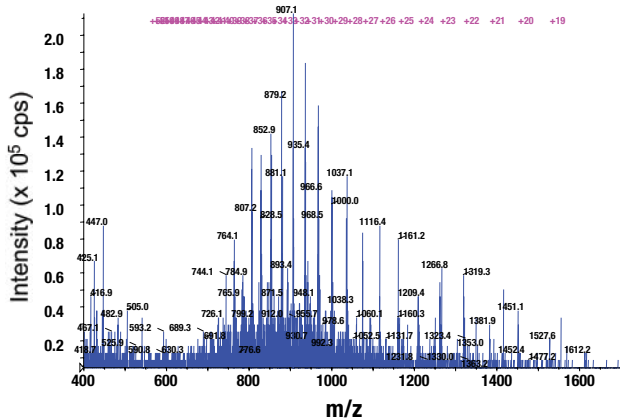
Peak 1 - Bovine Cytochrome C



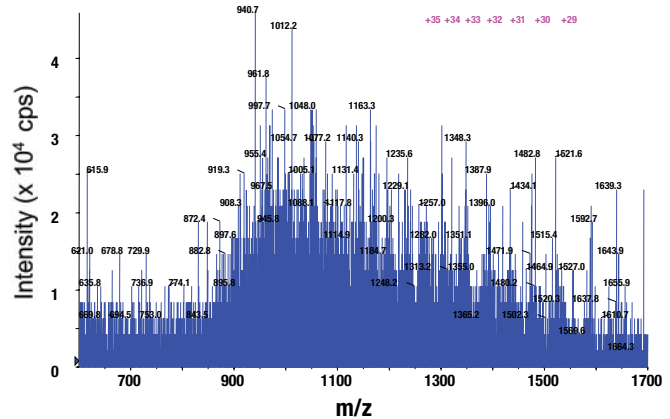
Peak 2 - Horse Apomyoglobin



Peak 3 - Bovine Carbonic Anhydrase II

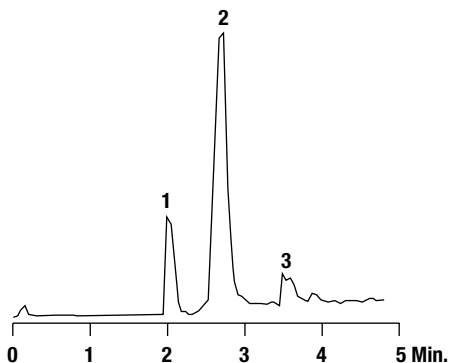


Peak 4 - Chicken Ovalbumin



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

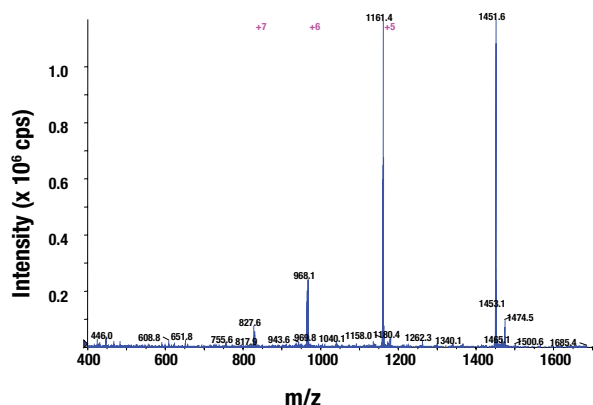
Flow Rate: 0.4mL/min
Detector: AB/MDS Sciex Q TRAP[®] MS. EMS scan 400-1700 amu;
 scan rate = 1000 amu/s; ion spray voltage = 5000 V; LIT fill time = 50 ms;
 Q3 entry barrier = 8V; declustering potential = 20; collision energy = 10 eV;
 curtain gas = 50; ion source gas 1 = 45; ion source gas 2 = 70;
 temperature (TEM) = 350°C.

Left Chromatogram Injection Amount: 50 ng each

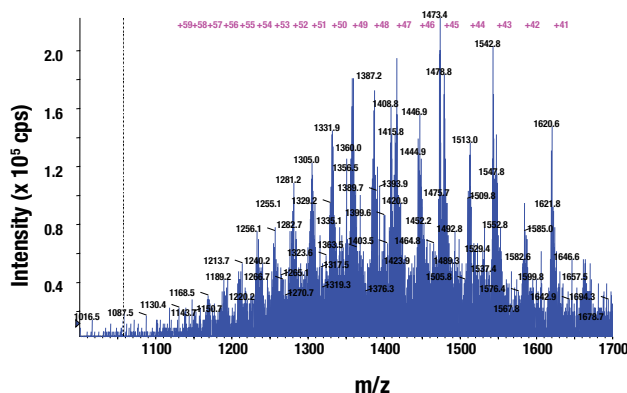
Right Chromatogram Injection Amount: 8µg each, except 1.5µg for growth hormone

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

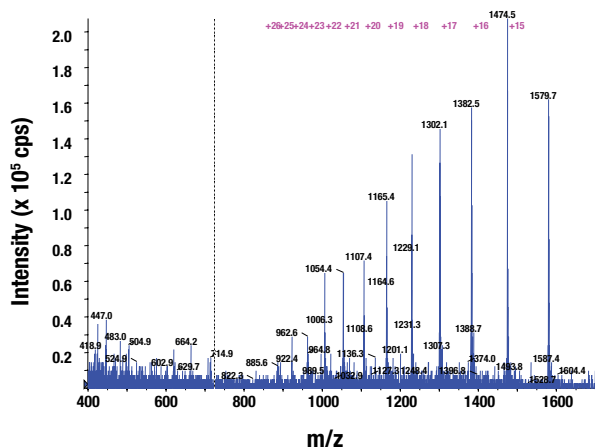
Peak 1 - Human Insulin



Peak 2 - Human Serum Albumin

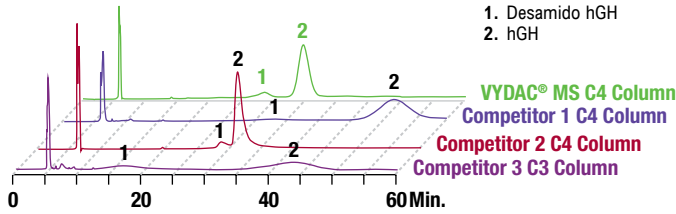


Peak 3 - Human Growth Hormone



Proteins & Peptides/Reversed-Phase

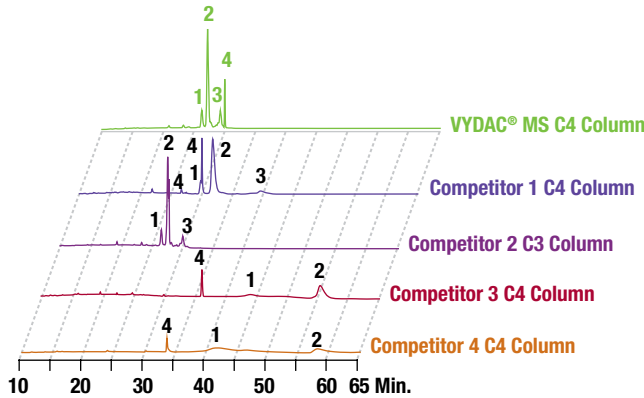
Human Growth Hormone (hGH) Assay on 300Å C4 Columns



- 1. Desamido hGH
- 2. hGH

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



- 1. 18.8 kD Protein
- 2. Non-myristoylated p14
- 3. Myristoylated p14
- 4. Triton™ X (Surfactant)

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:

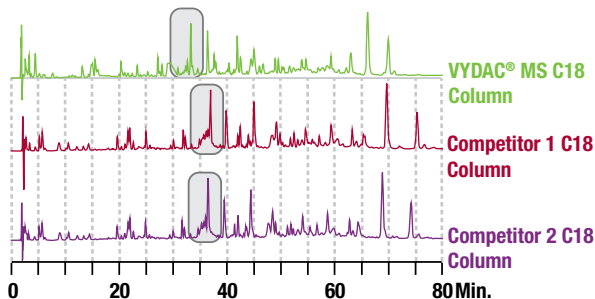
Time:	0	20	25	45
%B:	20	60	80	80

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



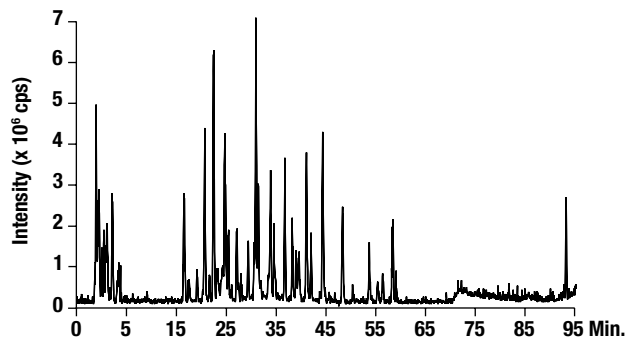
Columns: Vydac® MS C18 (Part No. 238MS5415), Competitor 1 C18, and Competitor 2 C18 (all 300Å, 5µm, 150 x 4.6mm)
Mobile Phase: A: 0.1% Trifluoroacetic Acid in Water B: 0.085% Trifluoroacetic Acid in Acetonitrile
Gradient:

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

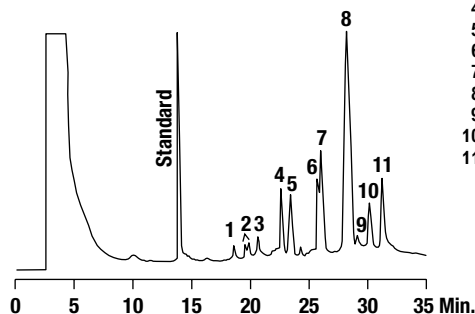


Column: Vydac® Everest® C18, 300Å, 5µm, 300µm x 150mm (Part No. 238EV5.315)
Mobile Phase: A: 0.1% Formic Acid in Water
 B: 0.1% Formic Acid in Acetonitrile
Gradient:

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

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

Human Apolipoproteins



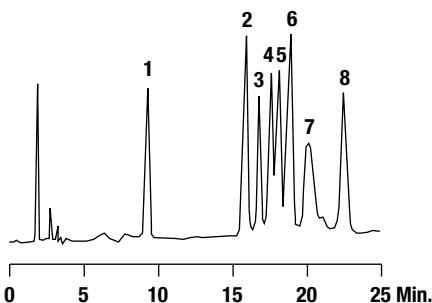
1. ApoC-IIIa
2. ApoC-IIIb
3. ApoC-IIa
4. ApoC-IIIc
5. ApoC-I
6. ApoC-IIb
7. ApoA-Ia
8. ApoA-Ib
9. ApoA-IIc
10. ApoA-IIa
11. ApoA-IIb

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



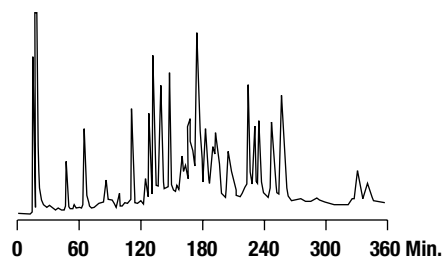
1. Chicken
2. Bovine
3. Ovine
4. Rabbit
5. Human
6. Porcine
7. Rat I
8. Rat II

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

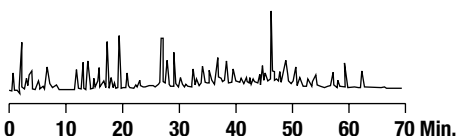


Column: Vydac® C4, 5µm, 250 x 4.6mm (Part No. 214TP54)
Mobile Phase: A: 0.1% Trifluoroacetic Acid
 B: Isopropanol
Gradient:

Time:	0	355
%B:	10	38

Detector: UV

Tryptic Digest of Bovine Serum Albumin

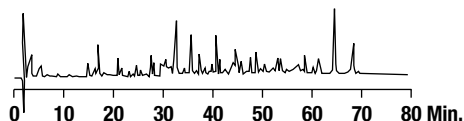


Column: Vydac® Everest® C18, 5µm, 150 x 4.6mm (Part No. 238EV5415)
Mobile Phase: A: 0.1% Trifluoroacetic Acid in Water
 B: 0.085% Trifluoroacetic Acid in Acetonitrile
Gradient:

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

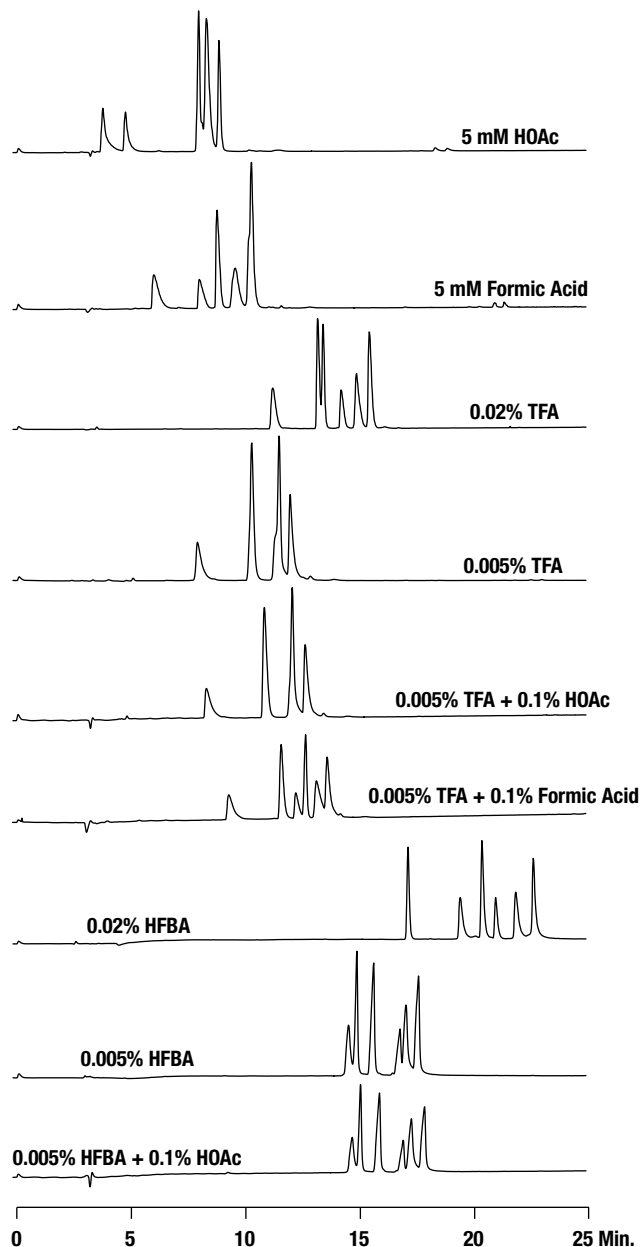
Flow Rate: 1.0mL/min
Detector: UV at 215nm

Proteins & Peptides/Reversed-Phase

Peptides on 238MS C18 Column with Various Modifiers

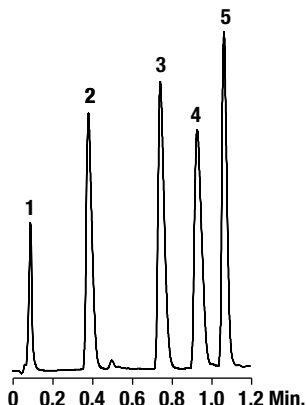
In order to demonstrate the effects of mobile phase modifiers and modifier concentrations, a mixture of six peptides was separated on a monomeric C18 MS column under identical gradient conditions with a variety of ion-pair reagents. Significant differences in selectivity arise depending on the concentrations 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



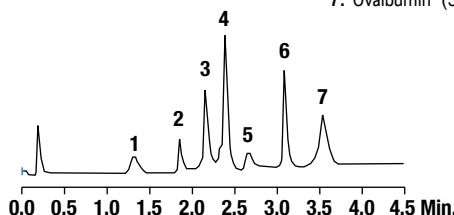
1. GY (238 Da)
2. VYV (379 Da)
3. Met Enkephalin (YGGFM, 573 Da)
4. Leu Enkephalin (YGGFL, 555 Da)
5. Angiotensin II (DRVYIHPF, 1045 Da)

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



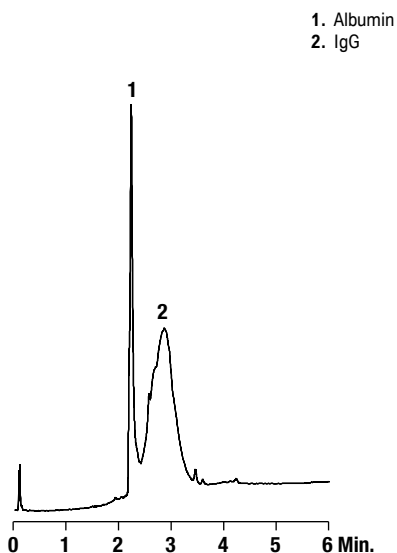
1. Ribonuclease (37ng)
2. Bovine Insulin (37ng)
3. Cytochrome C (37ng)
4. Lysozyme (4ng)
5. Bovine Serum Albumin (37ng)
6. Carbonic Anhydrase (37ng)
7. Ovalbumin (37ng)

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



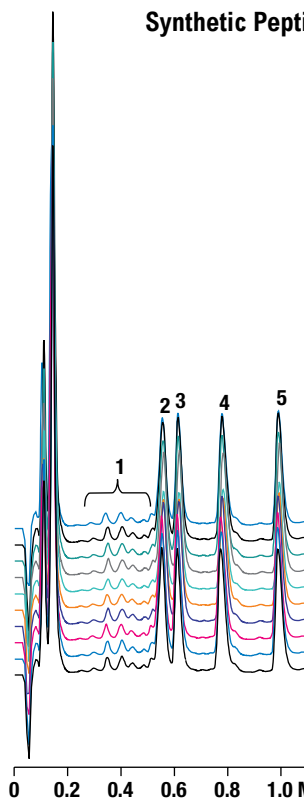
1. Albumin
2. IgG

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)

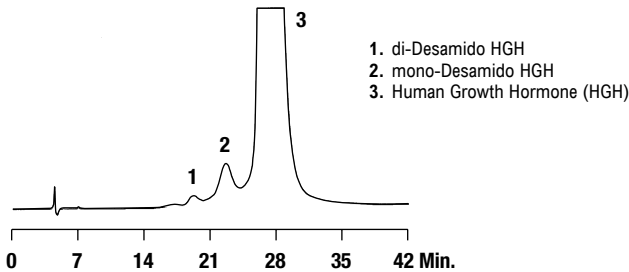
Column: Vydac® ProZap™ C18, 500Å, 1.5µm, 10 x 2.1mm
Mobile Phase: A: 0.1% Trifluoroacetic Acid in Water
 B: 0.085% Trifluoroacetic Acid:Acetonitrile (20:80)
Gradient:

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

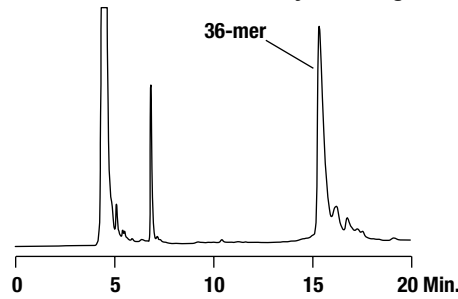
Proteins & Peptides/Reversed-Phase

Human Growth Hormone



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

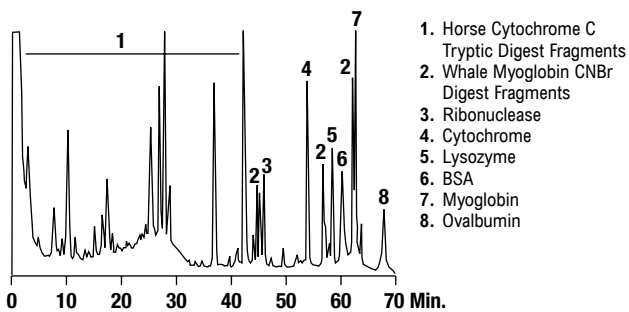


Column: Vydac® C4, 5µm, 250 x 10mm (Part No. 214TP510)
Mobile Phase: A: 0.1M Triethylammonium Acetate, pH7.0 B: Acetonitrile
Gradient:

Time:	0	14
B%:	16	23

Flow Rate: 5.0mL/min
Detector: UV

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

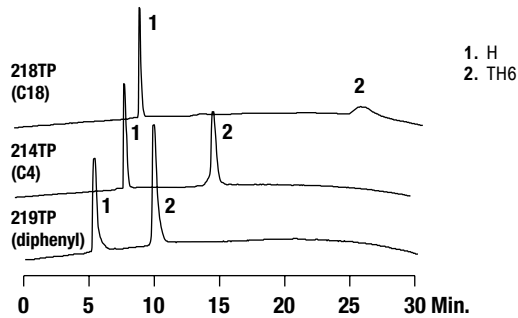


Column: Vydac® Diphenyl, 5µm, 250 x 4.6mm (Part No. 219TP54)
Mobile Phase: A: 0.1% Trifluoroacetic Acid
B: Acetonitrile
Gradient:

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

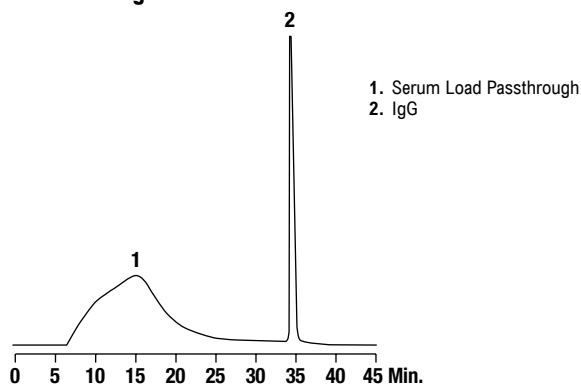


Column: Vydac® C18, C4 and Diphenyl, all 5µm, 150 x 4.6mm (Part Nos. 218TP5415, 214TP5415, 219TP5415)
Mobile Phase: A: 0.1% Trifluoroacetic Acid
B: Acetonitrile
Gradient:

Time:	0	30
B%:	10	90

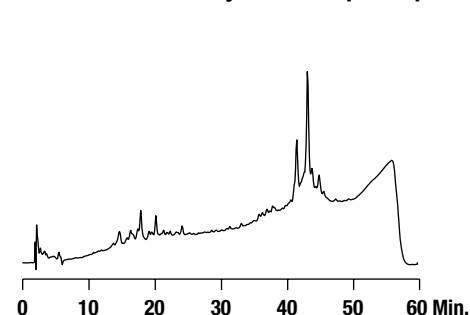
Sample: 18-Residue Helical Peptide (1) and Six-helix Template-assembled Synthetic Protein (2)
Flow Rate: 1.0mL/min
Detector: UV

IgG Purification from Bovine Serum



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

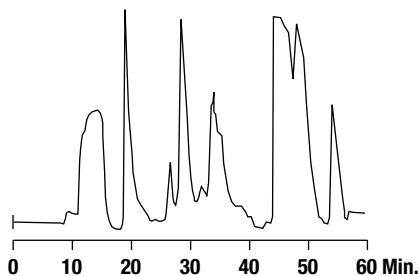
Flow Rate: 1.5mL/min
Detector: UV

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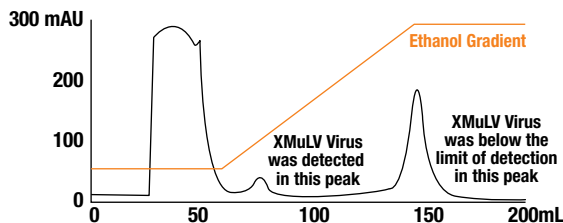
Proteins & Peptides/Reversed-Phase

Purification of Synthetic Peptide, GnRH Antagonist



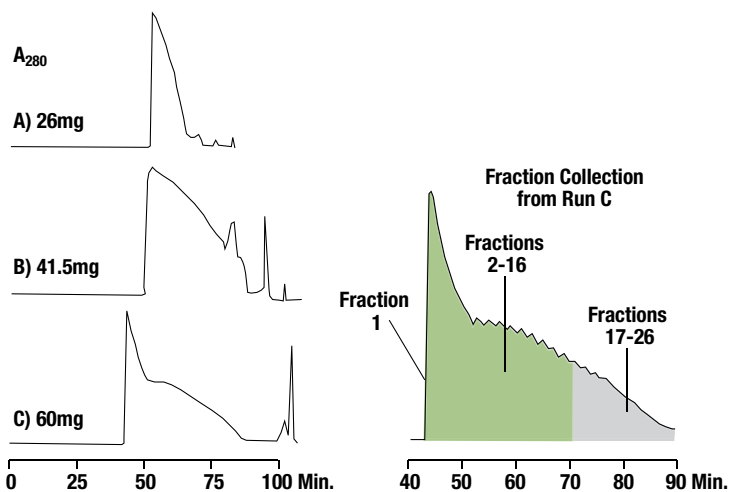
Column: Vydac® C18, 15–20µm, 50mm i.d. x 30cm (Part No. 218TP15205030)
Mobile Phase: Acetonitrile:Water/TEAP (Gradient)
Sample: 1.2g of synthesis mixture

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



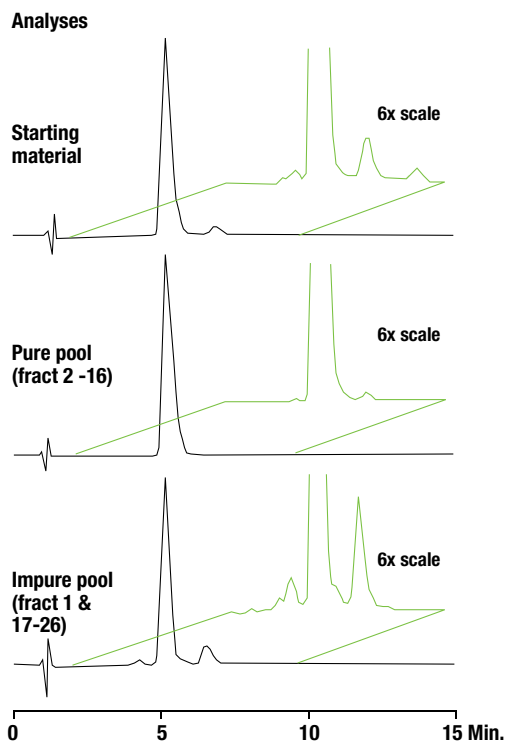
Column: Vydac® C4, 10-15µm, 10 x 250mm (Part No. 214TP101510)

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

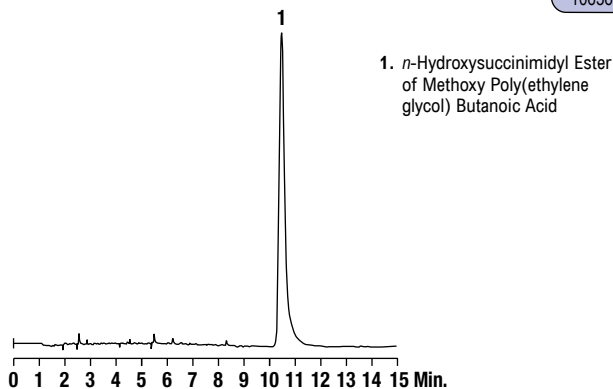
Elution: 1.5mL/min

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:

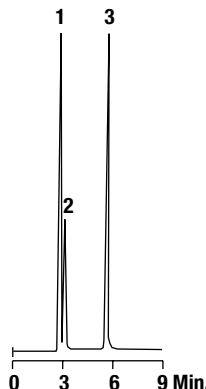
Time:	0	3	15
%B:	30	30	80

Flow Rate: 1.0mL/min
Detector: ELSD

Amino Acids

Sulfur-Containing Native Amino Acids

CHROM 9413

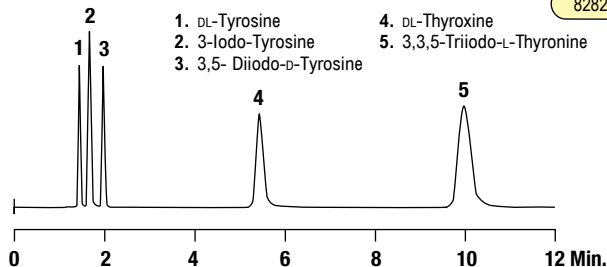


1. Cystine
 2. Cysteine
 3. Methionine

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

Thyroid Chemicals

CHROM 8282

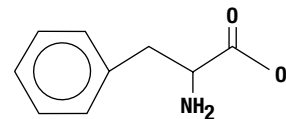
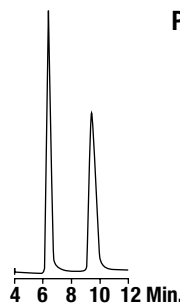


1. DL-Tyrosine
 2. 3-Iodo-Tyrosine
 3. 3,5-Diiodo-D-Tyrosine
 4. DL-Thyroxine
 5. 3,3,5-Triiodo-L-Thyronine

Column: Alltech® Platinum™ EPS C18, 5µm, 150 x 4.6mm (Part No. 32214)
Mobile Phase: 0.05M KH₂PO₄, pH3.2:Acetonitrile (65:35)
Flow Rate: 1.0mL/min
Detector: UV at 254nm

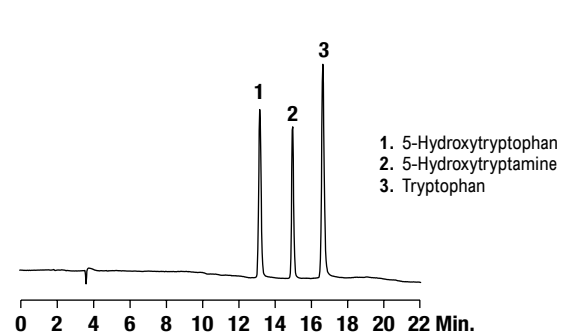
Phenylalanine Enantiomers

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

Tryptophan Derivatives



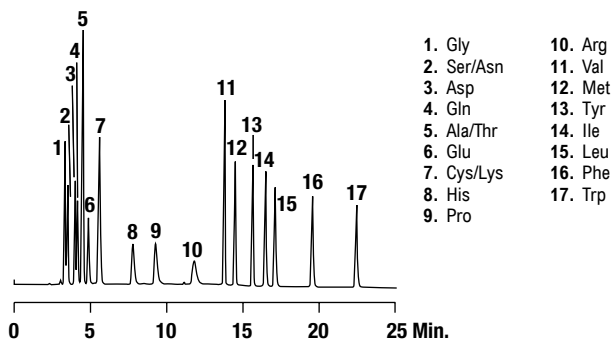
1. 5-Hydroxytryptophan
 2. 5-Hydroxytryptamine
 3. Tryptophan

Column: Vydac® C18, 5µm, 250 x 4.6mm (Part No. 201SP54)
Mobile Phase: A: 50mM KH₂PO₄, 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 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

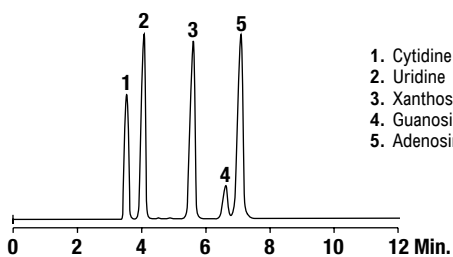
Flow Rate: 1.0mL/min
Detector: ELSD

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Nucleic Acids

Nucleosides



CHROM
10347

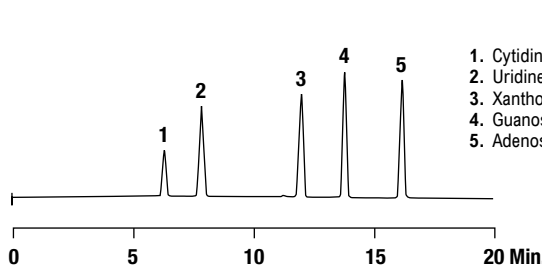
1. Cytidine
2. Uridine
3. Xanthosine
4. Guanosine
5. Adenosine

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

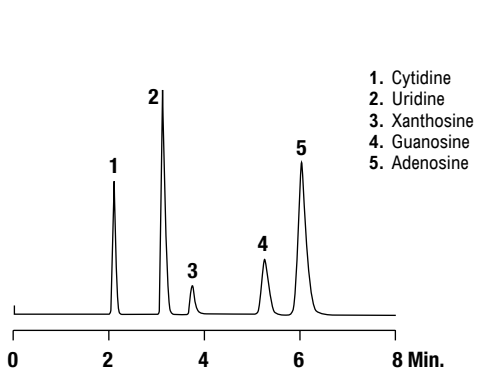
1. Cytidine
2. Uridine
3. Xanthosine
4. Guanosine
5. Adenosine

Column: Alltech® Platinum™ EPS C18, 5µm, 250 x 4.6mm (Part No. 32246)
Mobile Phase: A: 0.03M KH₂PO₄, pH3.2 B: Acetonitrile
Gradient:

Time:	0	2	20
%B:	5	5	30

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

Nucleosides

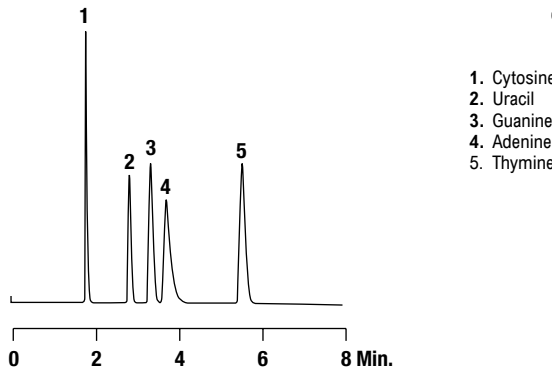


CHROM
9410

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 KH₂PO₄, pH3.0:Acetonitrile (96:4)
Flow Rate: 1mL/min
Detector: UV at 254

Nucleic Acid Bases



CHROM
9411

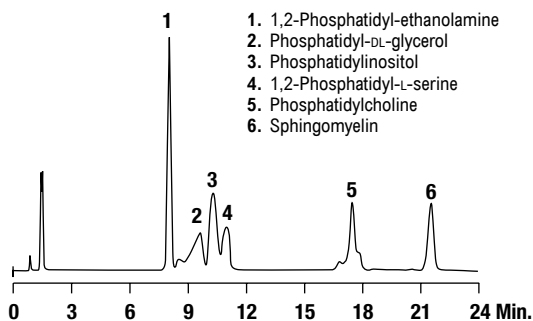
1. Cytosine
2. Uracil
3. Guanine
4. Adenine
5. Thymine

Column: Alltech® Prevail™ C18, 5µm, 150 x 4.6mm (Part No. 99208)
Mobile Phase: 0.025M KH₂PO₄, pH3.0:Acetonitrile (98:2)
Flow Rate: 1.0mL/min
Detector: UV at 254

Miscellaneous

Phospholipids

CHROM 10314



1. 1,2-Phosphatidyl-ethanolamine
2. Phosphatidyl-DL-glycerol
3. Phosphatidylinositol
4. 1,2-Phosphatidyl-L-serine
5. Phosphatidylcholine
6. Sphingomyelin

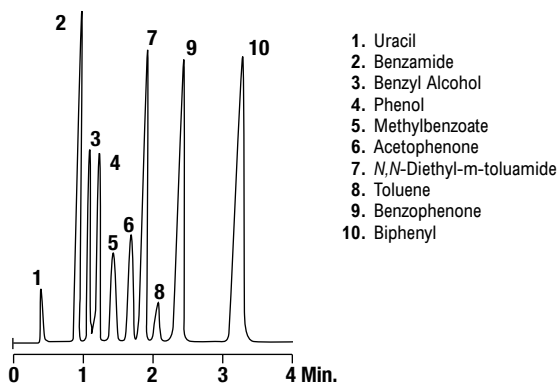
Column: Alltech® Prevail™ Silica, 3µm, 150 x 4.6mm (Part No. 99275)
Mobile Phase: A: Isopropanol B: Hexane C: Water
Gradient:

Time:	0	7	15
%B:	40	40	40
%C:	2	8	8

Flow Rate: 1.0mL/min
Detector: ELSD

Monoaromatics

CHROM 8982

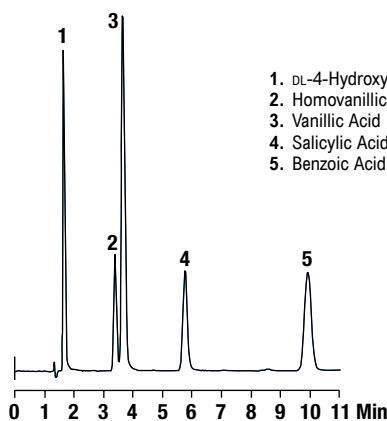


1. Uracil
2. Benzamide
3. Benzyl Alcohol
4. Phenol
5. Methylbenzoate
6. Acetophenone
7. N,N-Diethyl-m-toluamide
8. Toluene
9. Benzophenone
10. Biphenyl

Column: Alltech® Platinum™ EPS C18, 3µm, 53 x 7mm Rocket™ (Part No. 50573)
Mobile Phase: 0.05M KH₂PO₄, pH3.0:Methanol (50:50)
Flow Rate: 2.3mL/min
Detector: UV at 254nm

Aromatic Acids

CHROM 7373

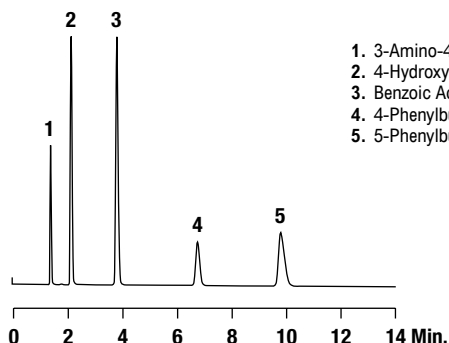


1. DL-4-Hydroxy-3-methoxy-mandelic Acid
2. Homovanillic Acid
3. Vanillic Acid
4. Salicylic Acid
5. Benzoic Acid

Column: Alltech® Alltima™ C18, 5µm, 150 x 4.6mm (Part No. 88052)
Mobile Phase: 0.05M KH₂PO₄, pH3.2:Methanol (60:40)
Flow Rate: 1.0mL/min
Detector: UV at 254nm

Benzylic Acids

CHROM 9538

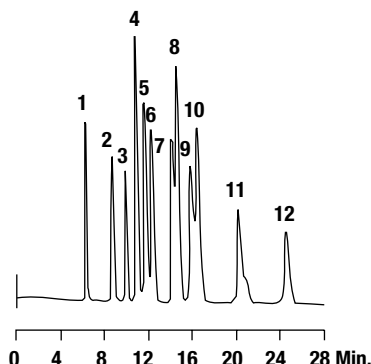


1. 3-Amino-4-hydroxybenzoic Acid
2. 4-Hydroxybenzoic Acid
3. Benzoic Acid
4. 4-Phenylbutyric Acid
5. 5-Phenylbutyric Acid

Column: Alltech® Prevail™ Organic Acid, 5µm, 150 x 4.6mm (Part No. 88640)
Mobile Phase: 0.025M KH₂PO₄, pH2.5:Acetonitrile (60:40)
Flow Rate: 1.0mL/min
Detector: UV at 254nm

Alcohols

CHROM 5440



1. Glycerol
2. Methanol
3. Ethanol
4. Isopropanol
5. tert-Butanol
6. Propanol
7. sec-Butanol
8. Isobutanol
9. tert-Pentanol
10. Butanol
11. Isopentanol
12. Pentanol

Column: Alltech® OA-1000, 9µm, 300 x 6.5mm (Part No. 9046)
Mobile Phase: 0.01N Sulfuric Acid
Flow Rate: 0.8mL/min
Column Temp: 65°C
Detector: RI

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