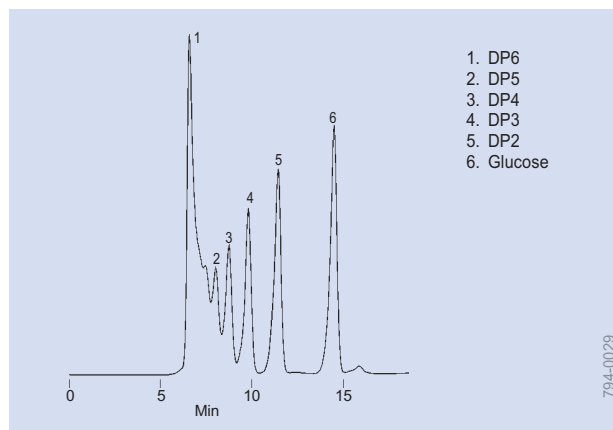
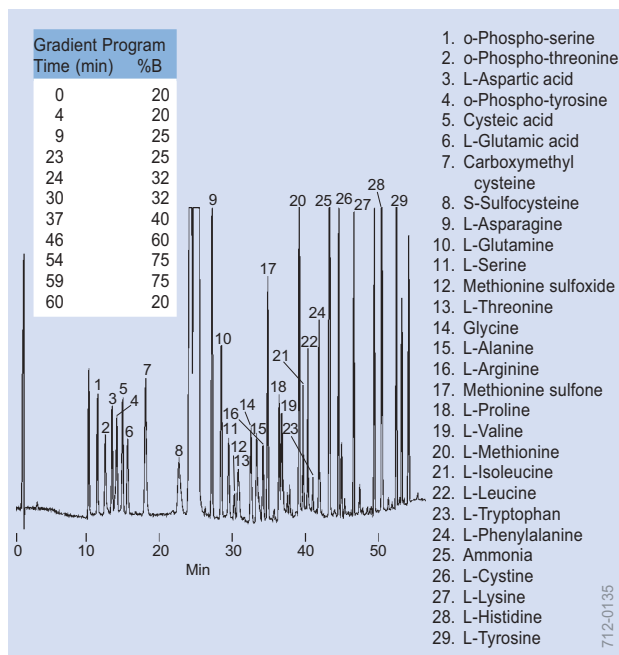


HPLC Applications

Amino Acids, Carbohydrates

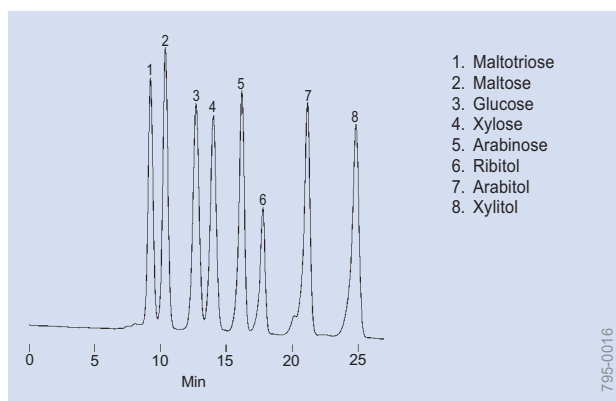


Dark Corn Syrup (HPLC)

Column: SUPELCOGEL Ag, 30cm x 7.8mm ID
 Cat. No.: 59315
 Mobile Phase: water
 Flow Rate: 0.5mL/min
 Temp.: 85°C
 Det.: RI
 Inj.: 10µL of 1g syrup/10mL DI water, filtered (0.20µm filter)

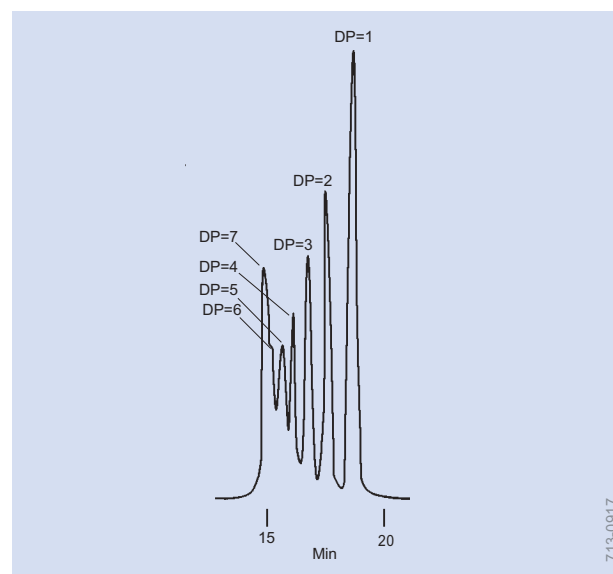
Amino Acids, Dabsyl (HPLC)

Column: SUPELCOSIL LC-DABS, 15cm x 4.6mm ID, 3µm particles
 Cat. No.: 59137
 Mobile Phase: A = 0.025M KH_2PO_4 , pH 7.0 with potassium hydroxide
 B = acetonitrile:methanol (70:30)
 Flow Rate: 1.5mL/min
 Det.: VIS, 436nm
 Inj.: 5µL, 50pmole each amino acid on-column



Carbohydrates (HPLC)

Column: SUPELCOGEL Ca, 30cm x 7.8mm ID
 Cat. No.: 59305-U
 Mobile Phase: water
 Flow Rate: 1.5mL/min
 Temp.: 80°C
 Det.: RI
 Inj.: 10µL, 2.5mg/mL each analyte in water

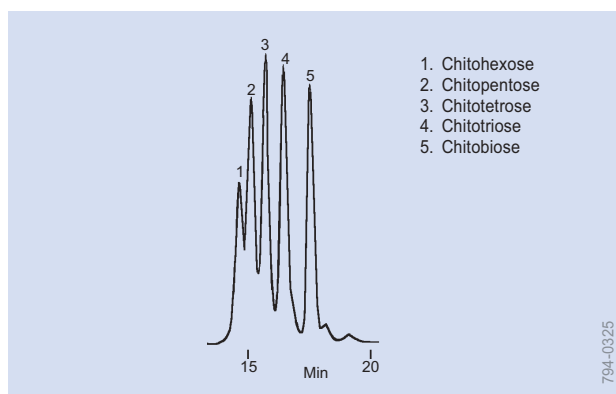


β-Cyclodextrin, Hydrolyzed (HPLC)

Column: Two TSKgel G-Oligo-PW, 30cm x 7.8mm ID, 6µm particles
 Cat. No.: 808031
 Mobile Phase: distilled water
 Flow Rate: 1mL/min
 Temp.: 60°C
 Det.: refractive index

HPLC Applications

Carbohydrates, Drugs

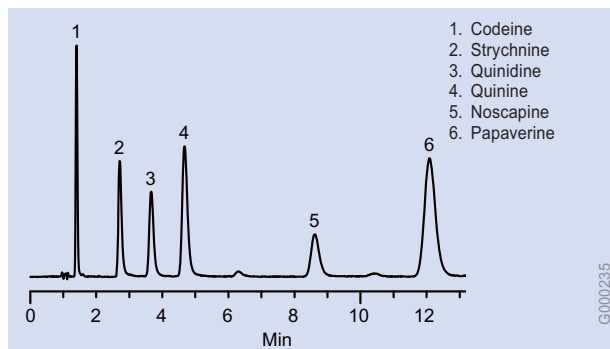


1. Chitohexose
2. Chitopentose
3. Chitetetrose
4. Chitotriose
5. Chitobiose

794-0325

Chito Oligosaccharides (HPLC)

Column: TSKgel G-Oligo-PW, 30cm x 7.8mm ID, 6 μ m particles
 Cat. No.: 808031
 Eluent: distilled water
 Flow Rate: 1.0mL/min
 Temp.: 60°C
 Det.: RI

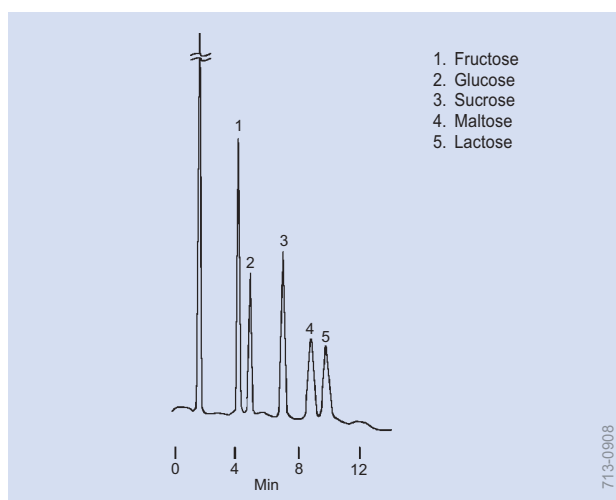


1. Codeine
2. Strychnine
3. Quinidine
4. Quinine
5. Noscapine
6. Papaverine

G000235

Alkaloids (HPLC)

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 505013
 Mobile Phase: methanol:25mM KH₂PO₄, pH 3.0 (20:80)
 Flow Rate: 2mL/min
 Temp.: 35°C
 Det.: UV, 254nm
 Inj.: 10 μ L

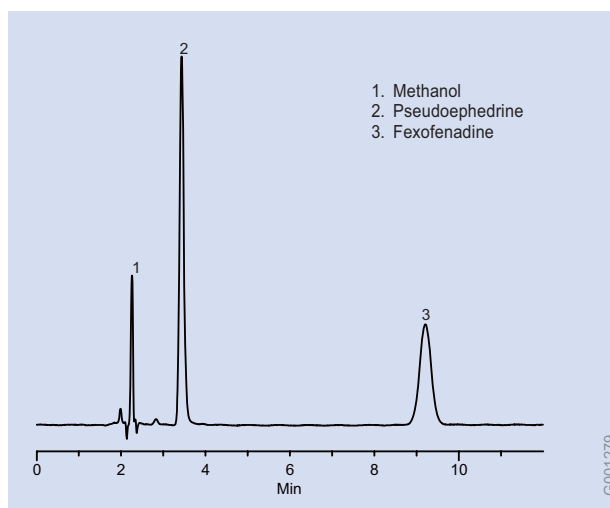


1. Fructose
2. Glucose
3. Sucrose
4. Maltose
5. Lactose

713-0908

Carbohydrates (HPLC)

Column: SUPELCOSIL LC-NH₂, 25cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 58338
 Mobile Phase: acetonitrile:water (75:25)
 Flow Rate: 2mL/min
 Det.: refractive index
 Inj.: 10 μ L, 150 μ g each sugar



1. Methanol
2. Pseudoephedrine
3. Fexofenadine

G001279

Allegra Ingredients (HPLC)

Column: Discovery Cyano, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 59356-U
 Mobile Phase: acetonitrile:25mM KH₂PO₄, pH 7.0 (15:85)
 Flow Rate: 1mL/min
 Temp.: 25°C
 Det.: UV, 254nm

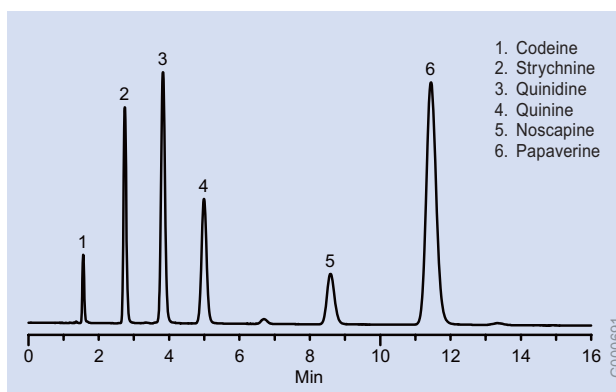
Order: 1.800.325.3010 Technical Service: 1.800.359.3041 Web: www.sigma-aldrich.com/supelco

Liquid
Chromatography

SUPELCO

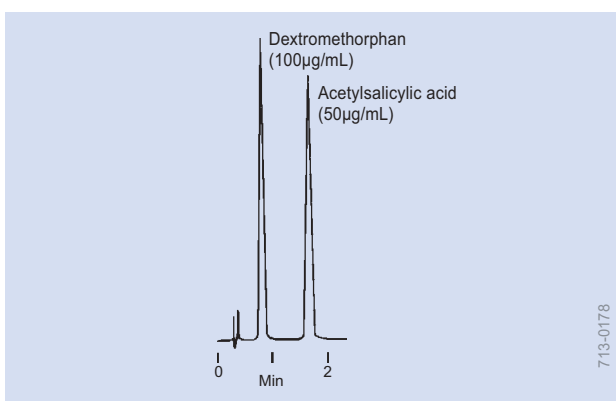
HPLC Applications

Drugs



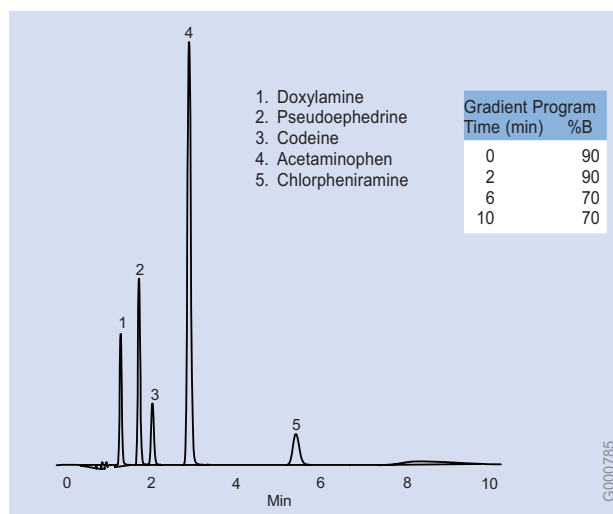
Amides (HPLC)

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 505013
Mobile Phase: 80:20 25mM Potassium Phosphate (pH 3.0):MeOH
Flow Rate: 2.0mL/min
Temp.: 35°C
Det.: UV at 254nm
Inj.: 10µL



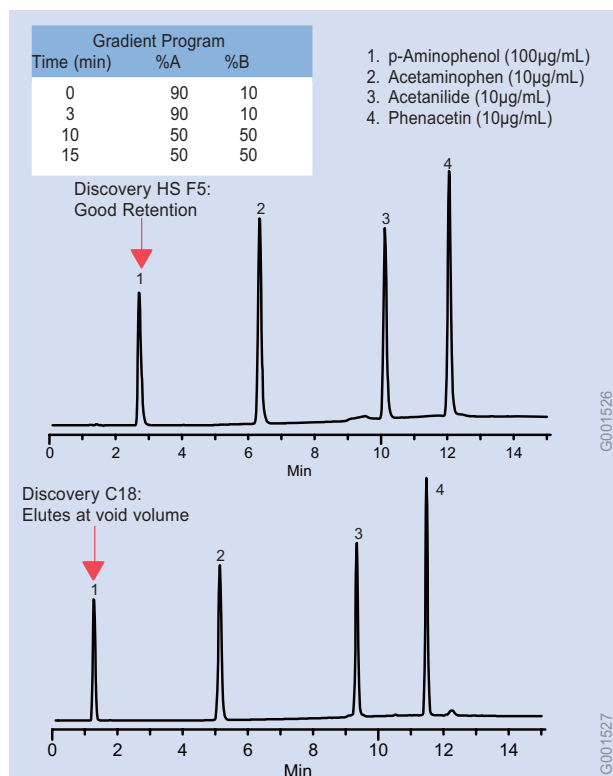
Analgesics (HPLC)

Column: SUPELCO SIL LC-ABZ, 5cm x 4.6mm ID, 5µm particles
Cat. No.: 59141
Mobile Phase: acetonitrile:25mM KH₂PO₄, pH 2.3 with phosphoric acid (20:80)
Flow Rate: 2mL/min
Det.: UV, 230nm
Inj.: 5µL mobile phase, analyte quantities shown



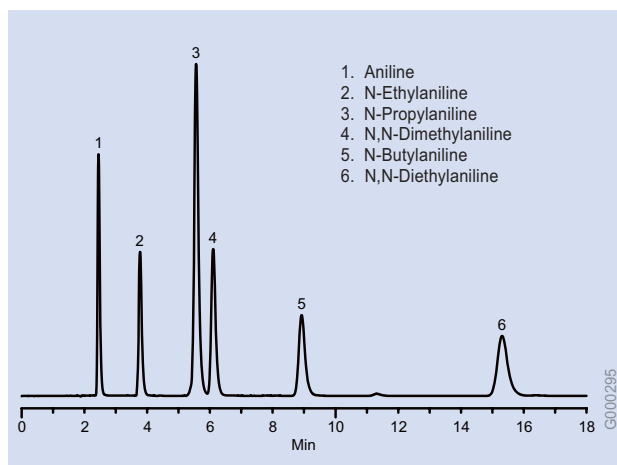
Analgesics and Antihistamines in Dosage Form (HPLC)

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 505013
Mobile Phase: A = acetonitrile
B = 25mM KH₂PO₄, pH 2.3
Flow Rate: 2mL/min
Temp.: ambient
Det.: UV, 214nm
Inj.: 10µL



Analgesic Drugs (HPLC)

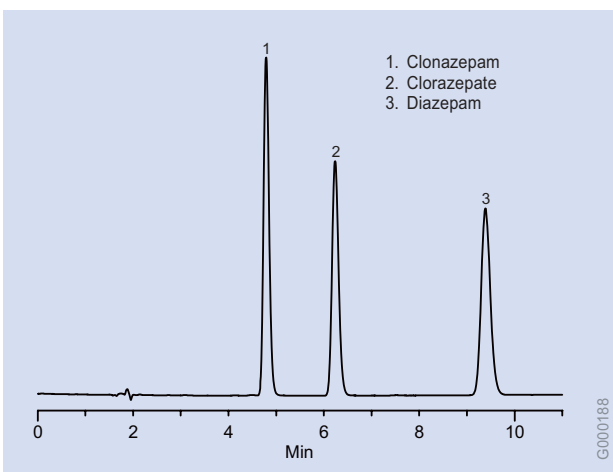
Columns: 15cm x 4.6mm ID, 5µm particles
Cat. Nos.: 567516-U (HS F5), 504955 (C18)
Mobile Phase: (A) 10mM ammonium acetate, 0.1% formic acid; (B) MeOH
Flow Rate: 1.5mL/min
Temp.: 35°C
Det.: UV at 254nm
Inj.: 10µL



1. Aniline
2. N-Ethylaniline
3. N-Propylaniline
4. N,N-Dimethylaniline
5. N-Butylaniline
6. N,N-Diethylaniline

Aniline Homologs (HPLC)

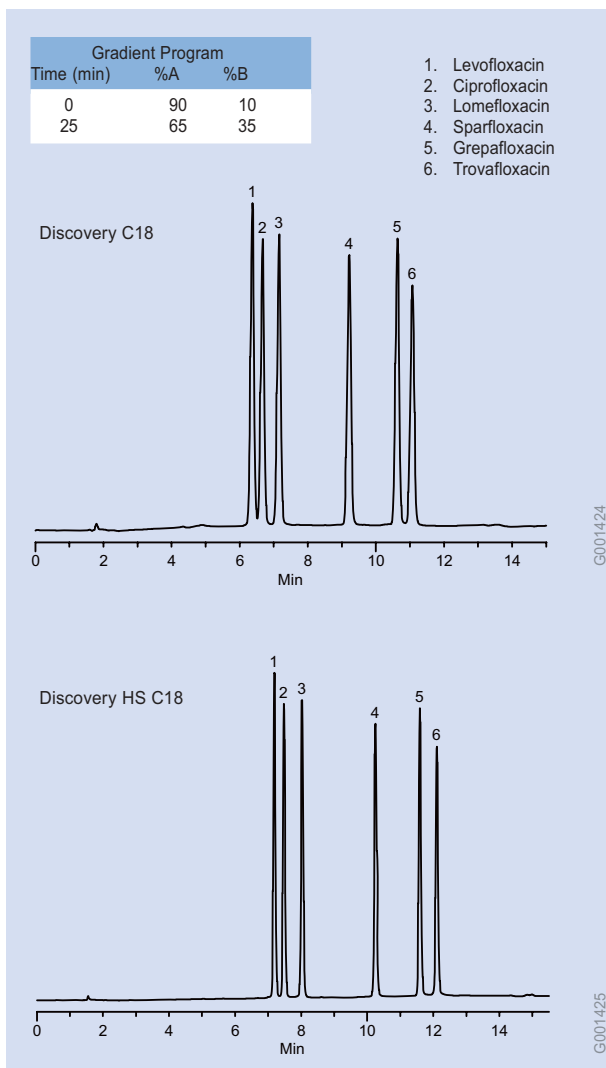
Column: Discovery C18, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 504955
 Mobile Phase: MeOH:H₂O (60:40)
 Flow Rate: 1mL/min
 Temp.: 30°C
 Det.: UV, 254nm



1. Clonazepam
2. Clorazepate
3. Diazepam

Anticonvulsants (HPLC)

Column: Discovery C18, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 504955
 Mobile Phase: acetonitrile:25mM KH₂PO₄, pH7.0 (40:60)
 Flow Rate: 1mL/min
 Temp.: 40°C
 Det.: UV, 254nm
 Inj.: 10µL



1. Levofloxacin
2. Ciprofloxacin
3. Lomefloxacin
4. Sparfloxacin
5. Grepafloxacin
6. Trovafloxacin

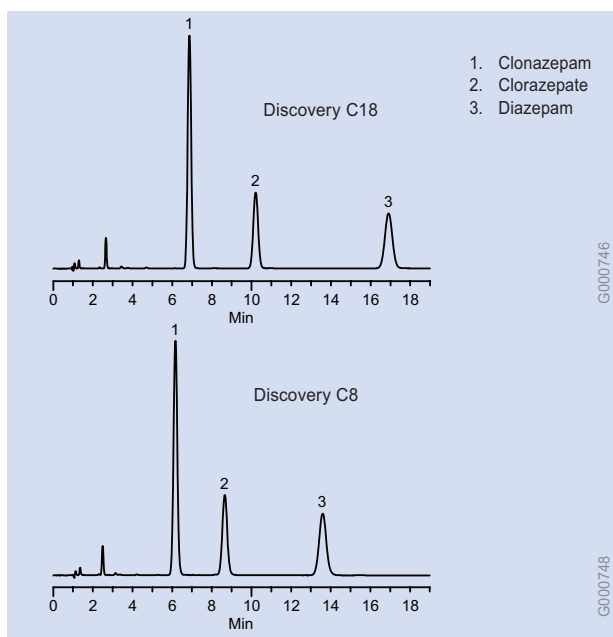
Antibiotics (Fluoroquinolones from Tablets) (HPLC)

Columns: 15cm x 4.6mm ID, 5µm particles
 Cat. Nos.: 504955 (C18), 568522-U (HS C18)
 Mobile Phase: (A) 25 mM potassium phosphate (pH 3.0)
 (B) CH₃CN
 Flow Rate: 1.0mL/min
 Temp.: 35°C
 Det.: UV, 220nm
 Inj.: 10µL

Order: 1.800.325.3010 Technical Service: 1.800.359.3041 Web: www.sigma-aldrich.com/supelco

HPLC Applications

Drugs



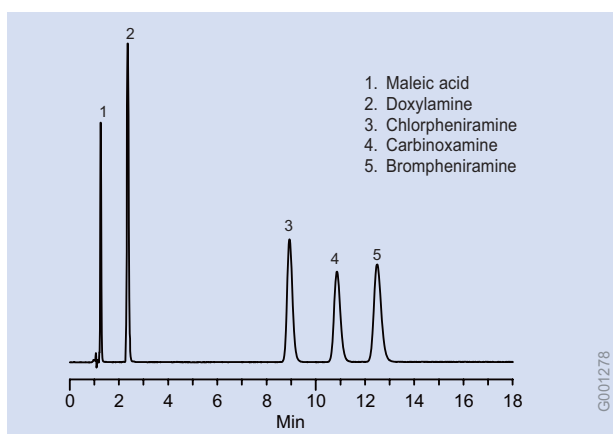
1. Enalapril
2. Lisinopril
3. Captopril

Antihypertensive ACE Inhibitors (HPLC)

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 505013
 Mobile Phase: acetonitrile:25mM KH_2PO_4 , pH 2.3 (33:67)
 Flow Rate: 0.6mL/min
 Temp.: 35°C
 Det.: UV, 214nm
 Inj.: 3µL

Anticonvulsants (HPLC)

Columns: 15cm x 4.6mm ID, 5µm particles
 Cat. Nos.: 504955 (C18), 59353-U (C8)
 Mobile Phase: 70:30 water: CH_3CN
 Flow Rate: 2.0mL/min
 Det.: UV, 254nm
 Temp.: 20°C
 Inj.: 10µL



1. Atenolol
2. Amiloride
3. Hydrochlorothiazide
4. Caffeine

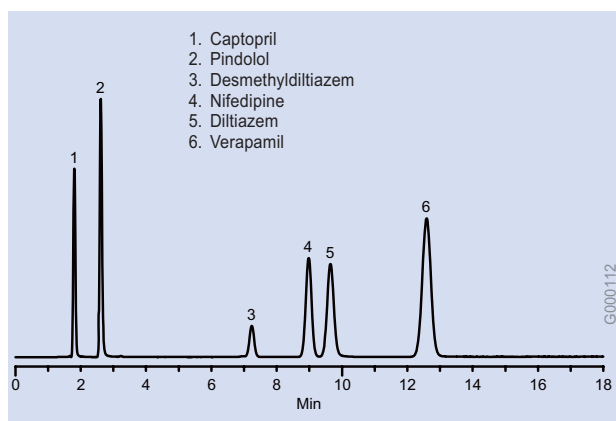
Antihypertensive-Diuretic Combination (HPLC)

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 505013
 Mobile Phase: methanol: 25mM K_2HPO_4 , pH 7.0 (22:78)
 Flow Rate: 1mL/min
 Temp.: 30°C
 Det.: UV, 254nm
 Inj.: 10µL

Antihistamines (HPLC)

Column: Discovery C8, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 59353-U
 Mobile Phase: acetonitrile:25mM KH_2PO_4 , pH 2.3 (8:92)
 Flow Rate: 2mL/min
 Temp.: 25°C
 Det.: UV, 254nm
 Inj.: 2µL

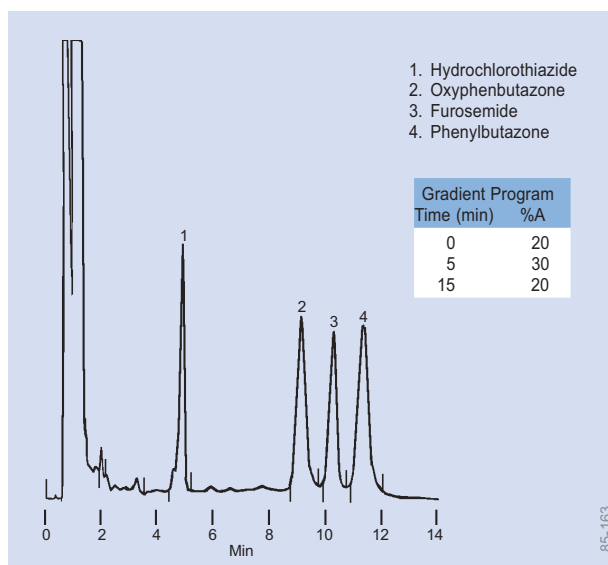
HPLC Applications Drugs



Antihypertensive Drugs (HPLC)

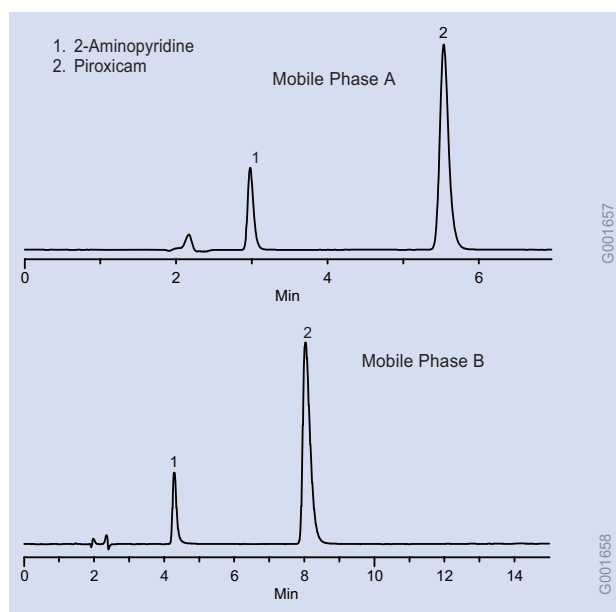
(Beta/Calcium Channel Blockers and ACE Inhibitor)

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 505013
 Mobile Phase: methanol:25mM KH_2PO_4 , pH 7.0 (40:60)
 Flow Rate: 1mL/min
 Temp.: 35°C
 Det.: UV, 214nm
 Inj.: 5µL



Anti-Inflammatory Drugs and Diuretics in Serum (HPLC)

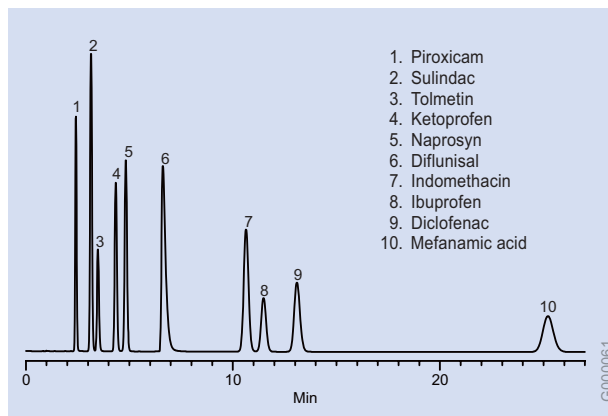
Column: Hisep, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 58935
 Mobile Phase: A = acetonitrile, B = 180mM ammonium acetate, pH 5.4
 Flow Rate: 2mL/min
 Temp.: 30°C
 Det.: UV, 267nm
 Inj.: 10µL horse serum containing 5µg/mL hydrochlorothiazide, 10µg/mL each other drug



Anti-Inflammatory Drugs

(Aminopyridine and Piroxicam) (HPLC)

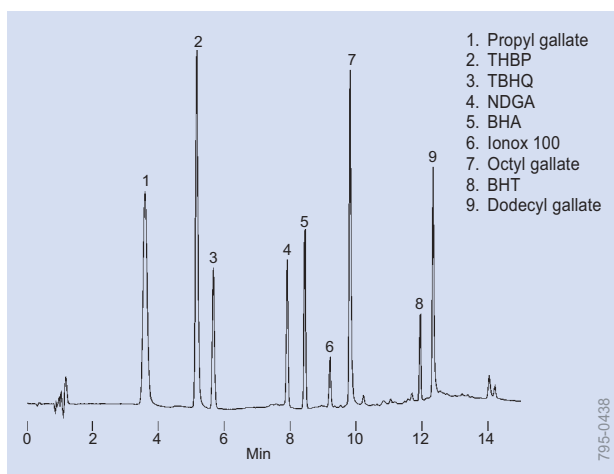
Columns: Discovery C18, 15cm x 4.6mm ID, 5µm particles
 Cat. Nos.: 504955
 Mobile Phase: A = 85:15, 10 mM potassium phosphate (pH 2.5): CH_3CN
 B = 85:15, 10 mM potassium phosphate (pH 6.8): CH_3CN
 Flow Rate: 1.0mL/min
 Det.: UV, 220nm
 Inj.: 5µL



Anti-Inflammatory Drugs, Non-Steroidal (HPLC)

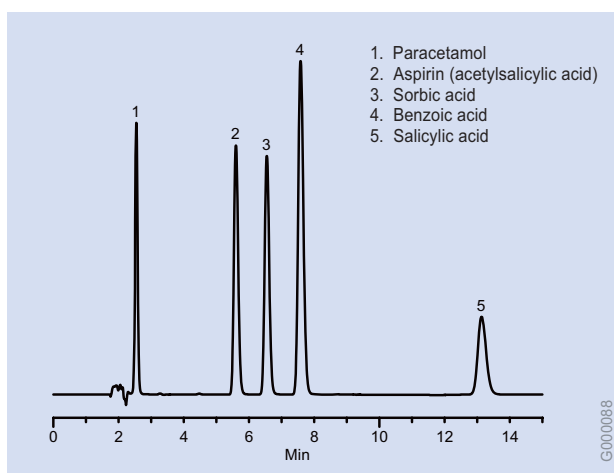
Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 505013
 Mobile Phase: acetonitrile:25mM KH_2PO_4 , pH 3.0 (40:60)
 Flow Rate: 1mL/min
 Temp.: 30°C
 Det.: UV, 230nm
 Inj.: 10µL, 1µg/mL each analyte

HPLC Applications Drugs



Antioxidants (HPLC)

Column: SUPELCO SIL LC-18, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 58230-U
 Mobile Phase: A = 5% acetic acid in deionized water
 B = acetonitrile:methanol (1:1)
 70% A/30% B to 100% B, linear gradient over 10 min, hold 10 min
 Flow Rate: 2.0mL/min
 Det.: UV, 280nm
 Inj.: 10µL, 20µg/mL each antioxidant
 (Cat. No. 47192)

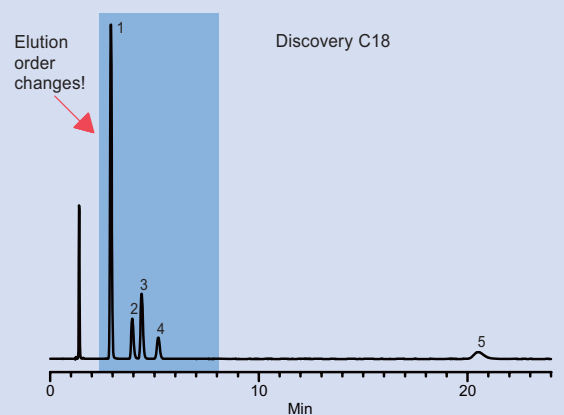
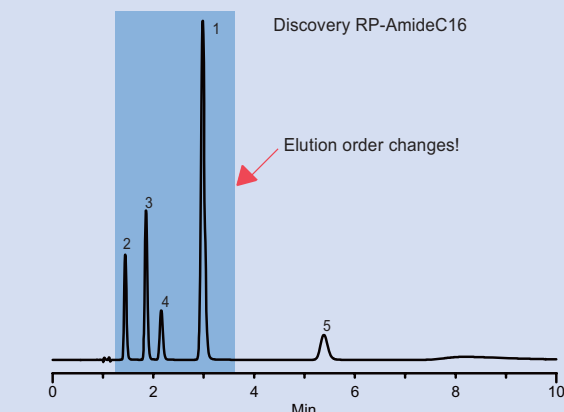


Antipyretics/Analgesics/Antifungals (HPLC)

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 505013
 Mobile Phase: 0.1% TFA in (25:75) acetonitrile:water
 Flow Rate: 1mL/min
 Temp.: 30°C
 Det.: UV, 254nm
 Inj.: 10µL

Gradient Program		
Time (min)	%A	%B
0	90	10
2	90	10
4	70	30
8	70	30
10	50	50

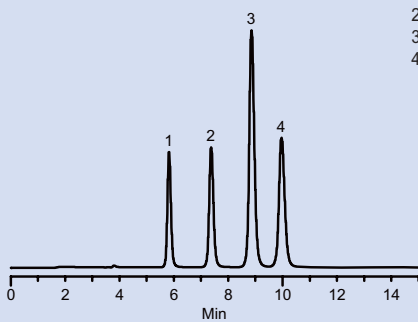
1. Acetaminophen
2. Doxylamine
3. Pseudoephedrine
4. Codeine
5. Chlorpheniramine



Antitussive/Antihistamine/Antipyretic Mix (HPLC)

Columns: 15cm x 4.6mm ID, 5µm particles
 Cat. Nos.: 505013 (RP-AmideC16), 504955 (C18)
 Mobile Phase: (A) 25 mM Potassium Phosphate (pH 2.3)
 (B) CH₃CN
 Flow Rate: 2.0mL/min
 Temp.: ambient
 Det.: UV, 214nm
 Inj.: 10µL

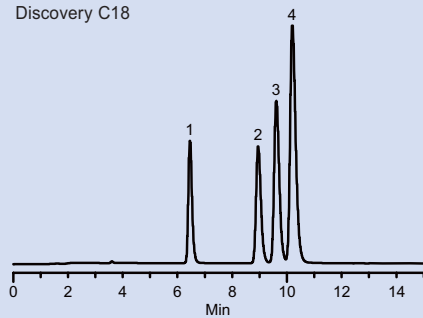
Discovery RP-AmideC16



1. Nizatidine
2. Ranitidine
3. Famotidine
4. Cimetidine

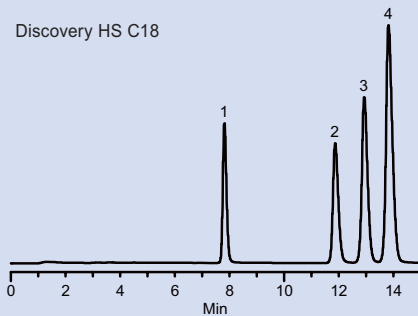
G001427

Discovery C18



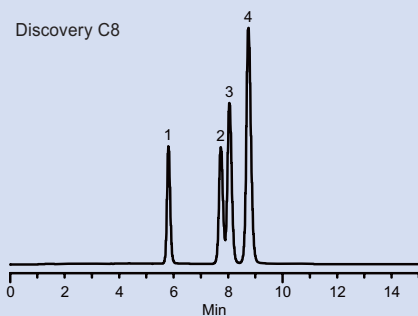
G001428

Discovery HS C18



G001430

Discovery C8

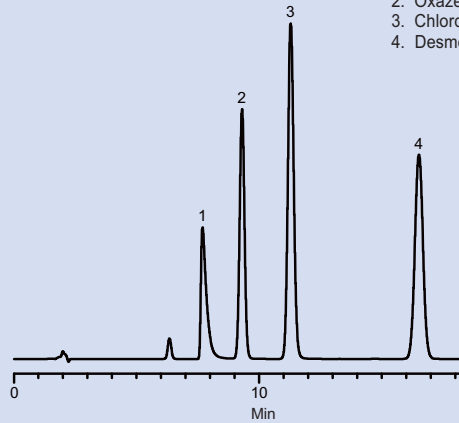


G001429

Ant ulcer Compounds (HPLC)

Columns: 15cm x 4.6mm ID, 5µm particles
 Cat. Nos.: 505013 (RP-AmideC16), 504955 (C18),
 569252-U (HS C18), 59353-U (C8)
 Mobile Phase: MeOH:25mM H₃PO₄/KOH, pH 3.0 (15:85)
 Flow Rate: 0.5mL/min
 Temp.: ambient
 Det.: UV, 215nm
 Inj.: 3µL

Nordoxepin is notoriously difficult to chromatograph, and the slight tailing is a good peak shape for this drug.

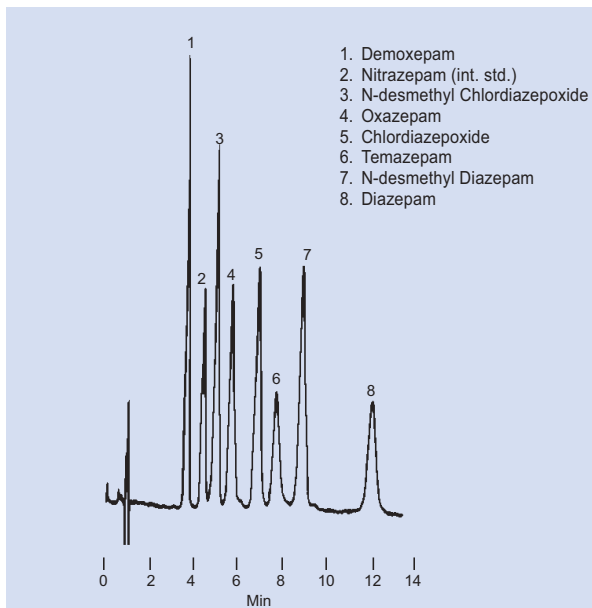


1. Nordoxepin
2. Oxazepam
3. Chlordiazepoxide
4. Desmethyldiazepam

797-0662

Anxiolytics (HPLC)

Column: Discovery C18, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 504955
 Mobile Phase: acetonitrile:25mM KH₂PO₄, pH 7.0 (30:70)
 Flow Rate: 1mL/min
 Temp: 20°C
 Det.: UV, 254nm
 Inj.: 10µL



1. Demoxepam
2. Nitrazepam (int. std.)
3. N-desmethyl Chlordiazepoxide
4. Oxazepam
5. Chlordiazepoxide
6. Temazepam
7. N-desmethyl Diazepam
8. Diazepam

713-1390

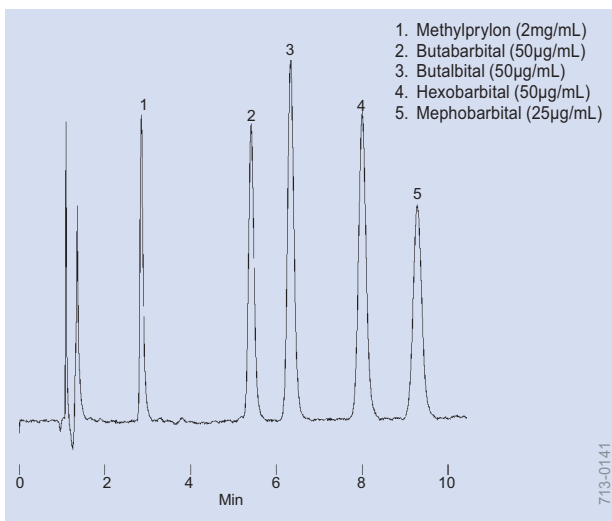
Anxiolytics (HPLC)

Column: SUPELCOSIL LC-8, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 58220-U
 Mobile Phase: methanol:acetonitrile:0.005M KH₂PO₄ and 0.1M ammonium acetate buffer (pH 6.0 with glacial acetic acid), 26.5:16.5:57 (v/v/v)
 Flow Rate: 2mL/min
 Temp.: 30°C
 Det.: UV, 245nm
 Sample: 10µL

Order: 1.800.325.3010 Technical Service: 1.800.359.3041 Web: www.sigma-aldrich.com/supelco

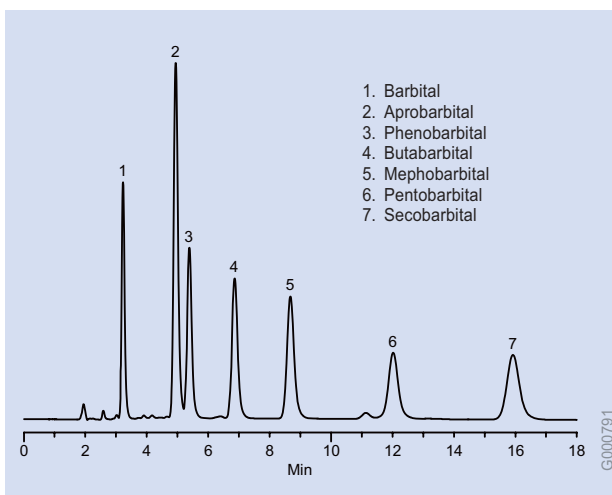
HPLC Applications

Drugs



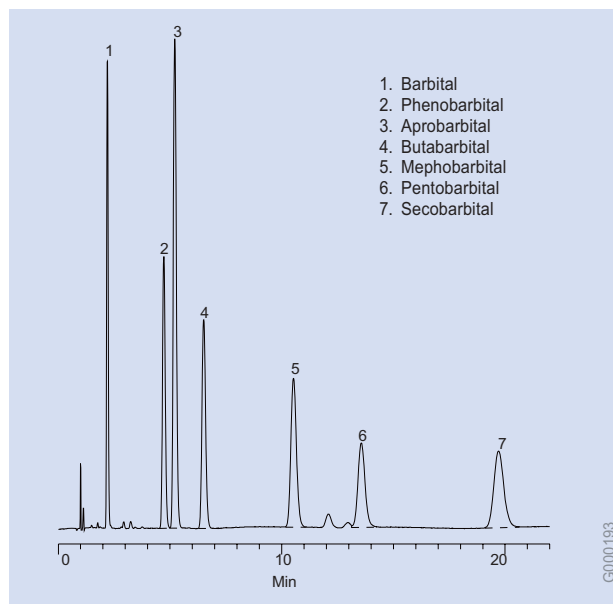
Barbiturates (HPLC)

Column: SUPELCOSIL LC-ABZ, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 59140-U
Mobile Phase: acetonitrile:25mM potassium phosphate buffer, pH 6.9 (25:75)
Flow Rate: 1.5mL/min
Det.: UV, 254nm
Inj.: 25µL



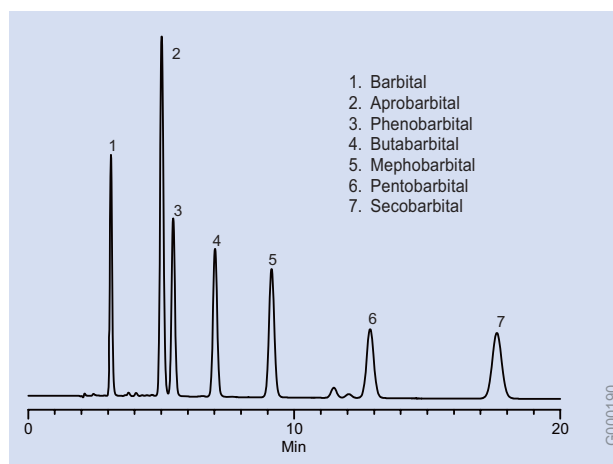
Barbiturates (HPLC)

Column: Discovery C8, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 59353-U
Mobile Phase: 55:45 water:MeOH
Flow Rate: 1.0mL/min
Det.: UV at 214nm
Temp.: ambient
Inj.: 5µL



Barbiturates (HPLC)

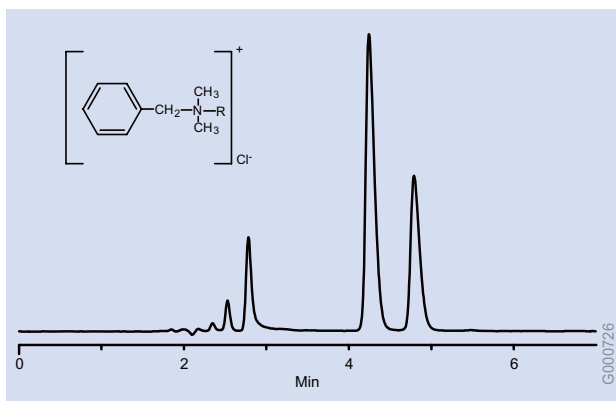
Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 505013
Mobile Phase: methanol:acetonitrile, 60:40:water, 25:75
Flow Rate: 2mL/min
Temp.: 30°C
Det.: UV, 214nm
Inj.: 2µL



Barbiturates (HPLC)

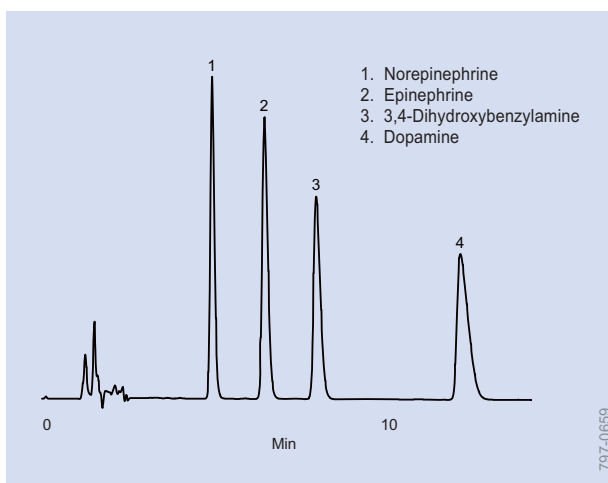
Column: Discovery C18, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 504955
Mobile Phase: 55:45 water:MeOH
Flow Rate: 1.0mL/min
Temp.: ambient
Det.: UV, 214nm
Inj.: 10µL

HPLC Applications Drugs



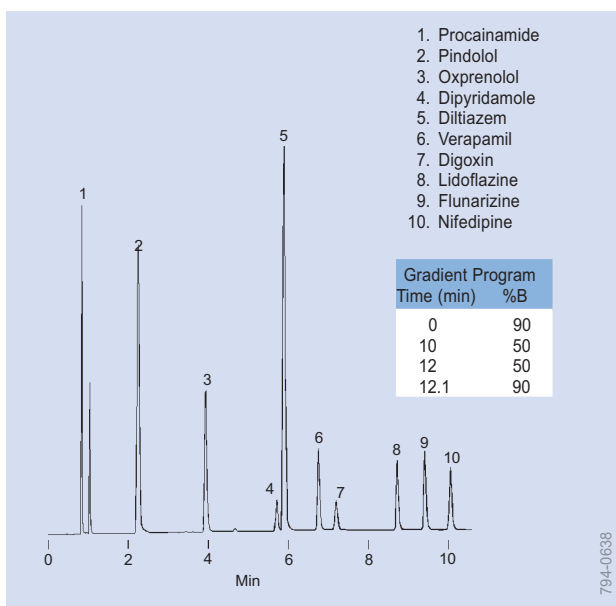
Benzalkonium Chlorides (HPLC)

Column: Discovery Cyano, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 59356-U
 Mobile Phase: CH₃CN:acetate buffer, pH 4.5, (60:40)
 Flow Rate: 1mL/min
 Temp.: ambient temp.
 Det.: UV, 254nm
 Sample: Undefined C8 to C18 Benzalkonium Chlorides



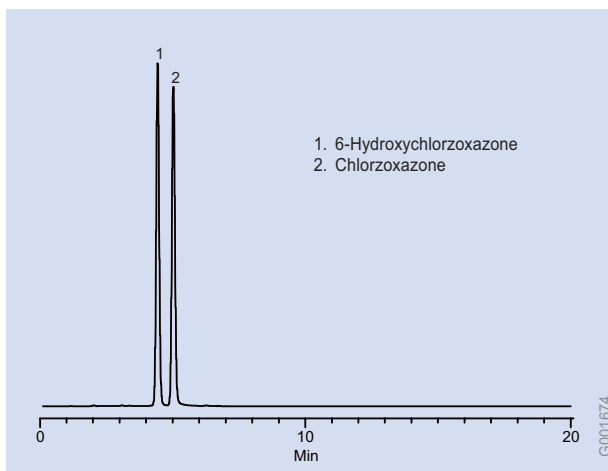
Catecholamines (HPLC)

Column: Discovery C18, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 504955
 Mobile Phase: acetonitrile:50mM KH₂PO₄, pH 3.0 with H₃PO₄, 100mg/L EDTA, 200mg/L 1-octane-sulfonic acid (5:95)
 Flow Rate: 1mL/min
 Temp.: 20°C
 Det.: UV, 254nm
 Inj.: 10µL



Cardiac Drugs (HPLC)

Column: SUPELCOSIL ABZPlus, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 59196
 Mobile Phase: A = acetonitrile
 B = 25mM KH₂PO₄ (pH 3.0)
 Flow Rate: 2mL/min
 Det.: UV, 220nm
 Inj.: 20µL methanol:water, 50:50 (30µg/µL pindolol and diltiazem, 100µg/mL dipyridamole, 50µg/mL other analytes)

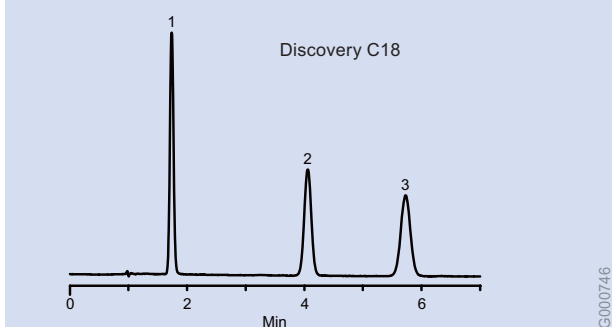
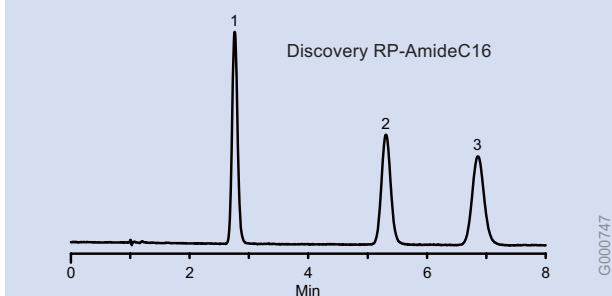
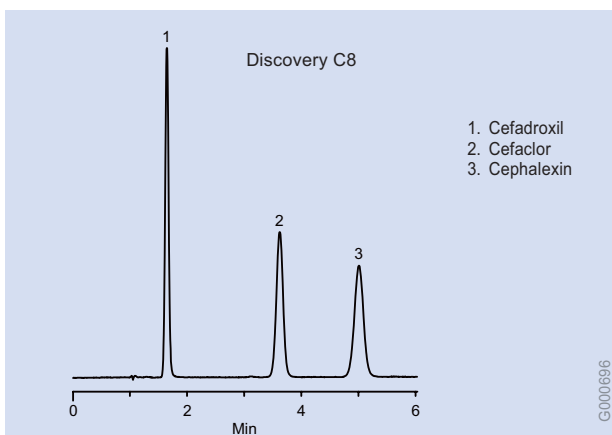


Chlorzoxazone (HPLC)

Column: Discovery HS PEG, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 567416-U
 Mobile Phase: 70:30 20mM acetic acid in water (pH 4.5 with ammonium hydroxide):CH₃CN
 Flow Rate: 1.0mL/min
 Temp.: 30°C
 Det.: UV, 285nm
 Inj.: 10µL

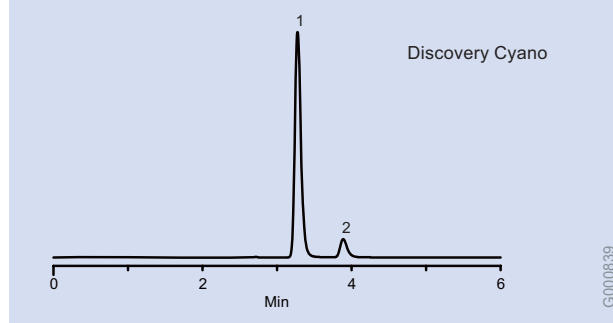
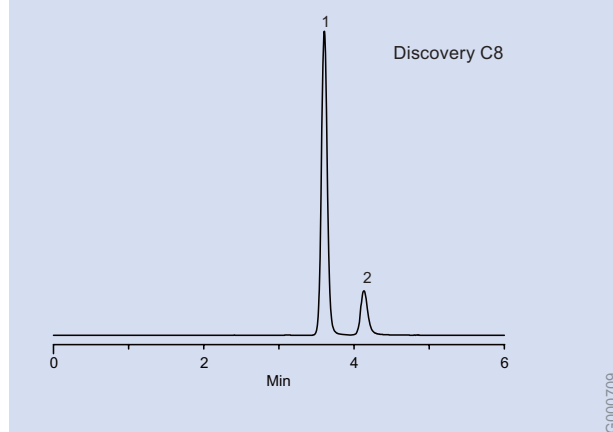
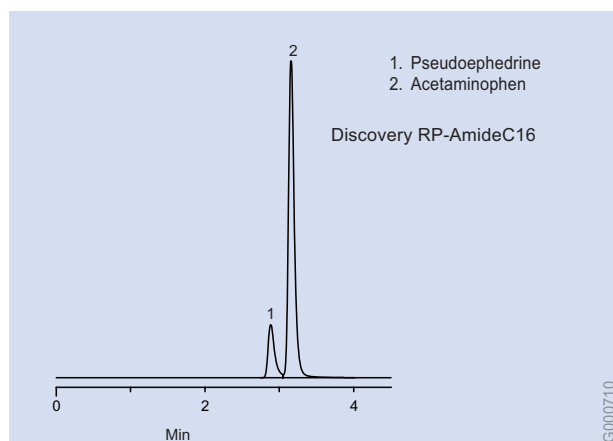
HPLC Applications

Drugs



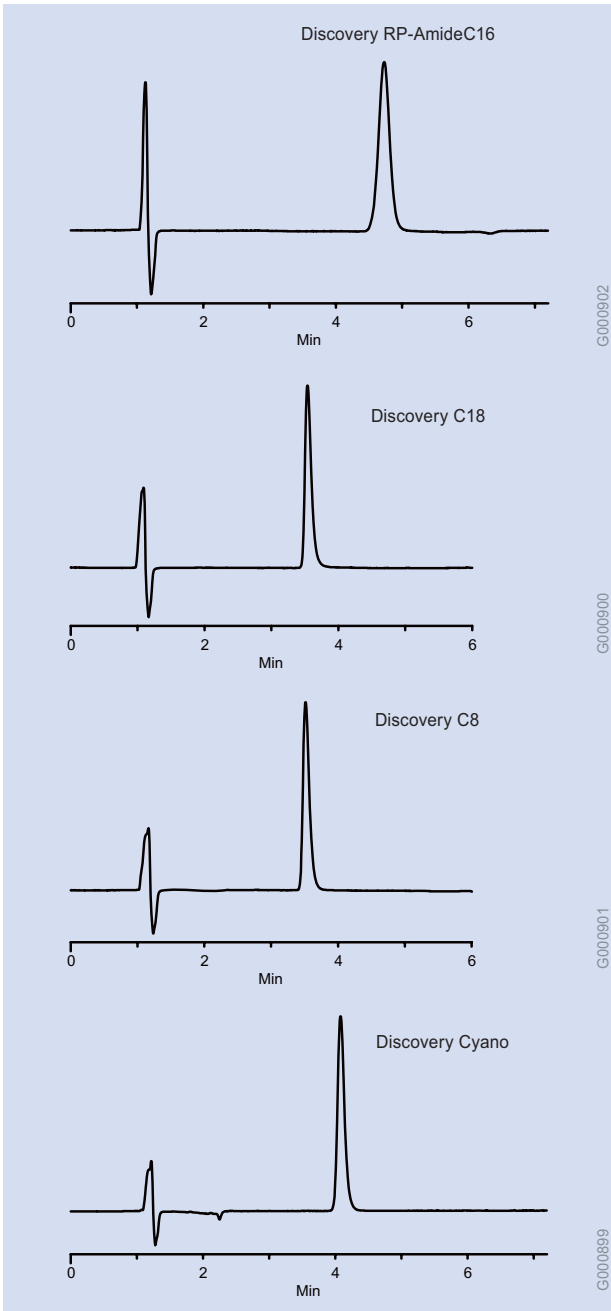
Cephalosporins (HPLC)

Column: 15cm x 4.6mm ID, 5 μ m particles
 Cat. Nos.: 59353-U (C8), 505013 (RP-AmideC16), 504955 (C18)
 Mobile Phase: methanol:25mM KH_2PO_4 , pH 3.0, (20:80) for C18 and C8,
 (10:90) for RP-Amide C16
 Flow Rate: 2mL/min
 Temp.: 20°C
 Det.: UV, 254nm
 Inj.: 1 μ L



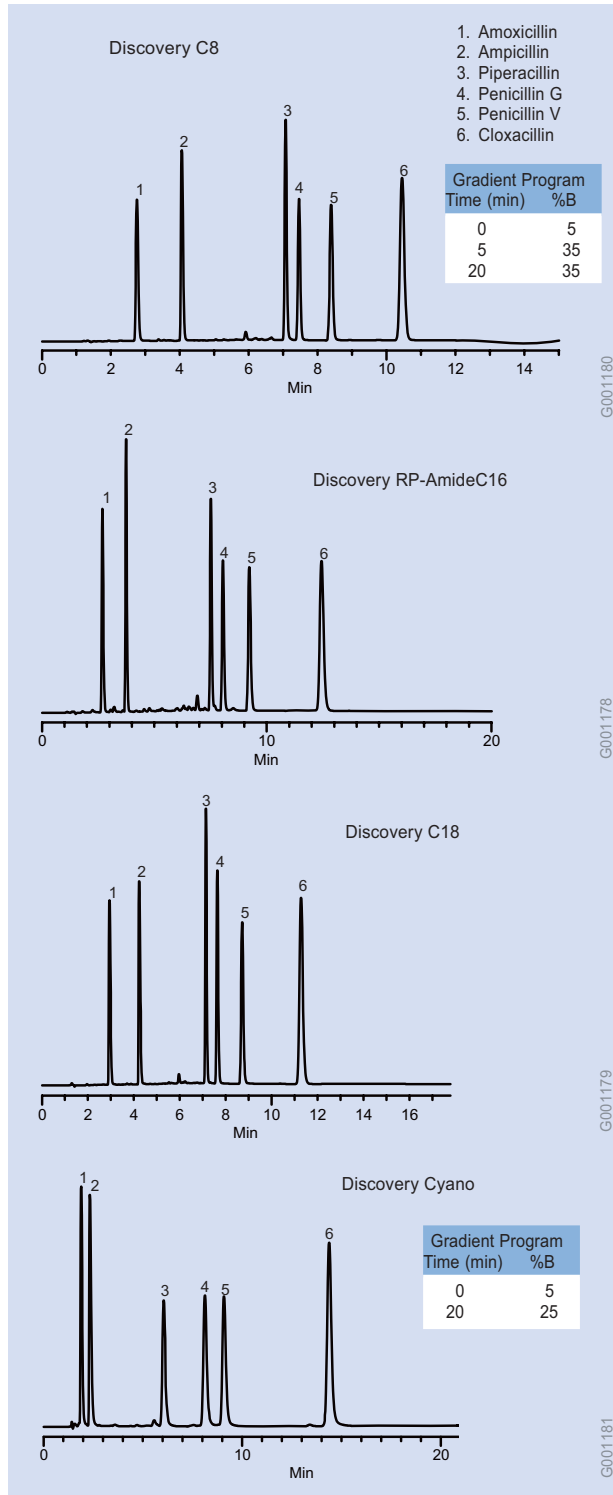
Cold Remedy Ingredients/Antihistamines (HPLC)

Columns: 15cm x 4.6mm ID, 5 μ m particles
 Cat. Nos.: 505013 (RP-AmideC16), 59353-U (C8), 59356-U (Cyano)
 Mobile Phase: acetonitrile:25mM KH_2PO_4 , pH 7.0 (15:85)
 Flow Rate: 1mL/min
 Temp.: 20°C
 Det.: UV, 214nm
 Inj.: 1 μ L



Fluoxetine (Prozac) (HPLC)

Columns: 15cm x 4.6mm ID, 5µm particles
 Cat. Nos.: 505013 (RP-AmideC16), 504955 (C18),
 59353-U (C8), 59356-U (Cyano)
 Mobile Phase: CH₃CN:25mM KH₂PO₄, pH 7.0 (40:60)
 Flow Rate: 2mL/min
 Temp.: 30°C
 Det.: UV, 214nm
 Inj.: 1µL



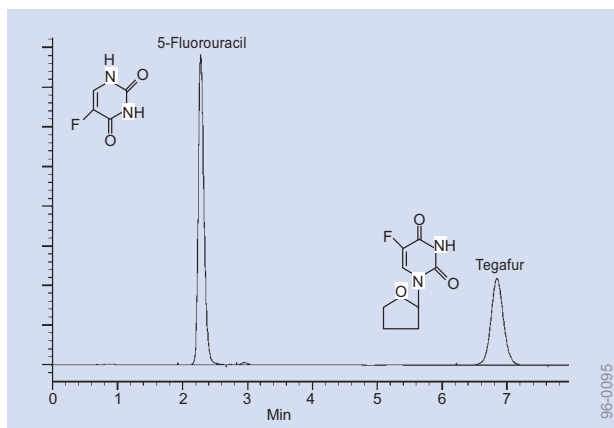
β-Lactam Antibiotics (HPLC)

Column: 15cm x 4.6mm ID, 5µm particles
 Cat. Nos.: 59353-U (C8), 505013 (RP-AmideC16),
 504955 (C18), 59356-U (Cyano)
 Mobile Phase: A = 0.01% TFA in water;
 B = 0.01% TFA in CH₃CN
 Flow Rate: 1.5mL/min
 Temp.: 30°C
 Det.: UV, 254nm

Order: 1.800.325.3010 Technical Service: 1.800.359.3041 Web: www.sigma-aldrich.com/supelco

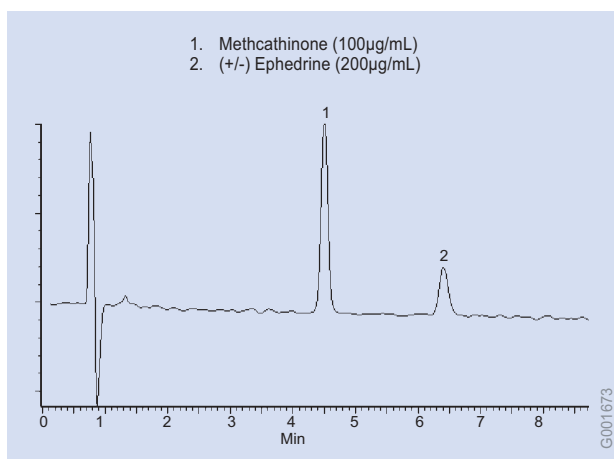
HPLC Applications

Drugs



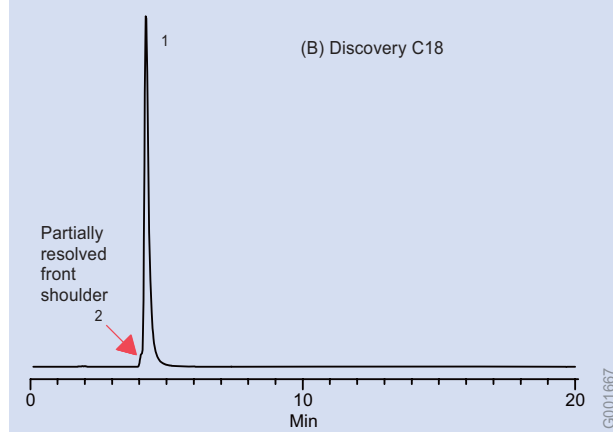
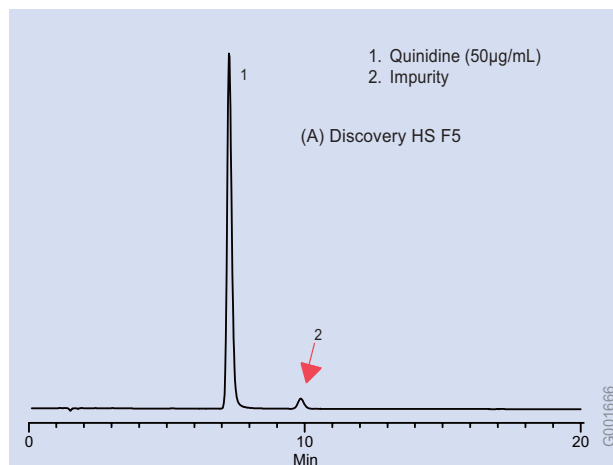
5-Fluorouracil and Tegafur (HPLC)

Column: SUPELCOGEL TPR-100, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 59154
 Mobile Phase: acetonitrile:water (10:90)
 Flow Rate: 1.0mL/min
 Temp.: 30°C
 Det.: UV, 270nm



Methcathinone and Ephedrine (HPLC)

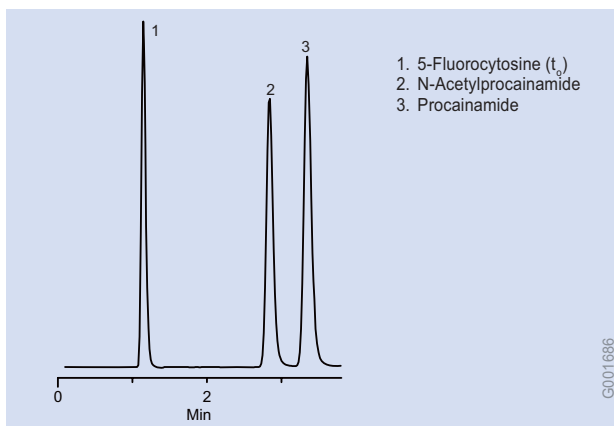
Column: Discovery HS F5, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 567516-U
 Mobile Phase: (30:70) 10mM Ammonium Acetate (pH 6.98): CH₃CN
 Flow Rate: 2.0mL/min
 Temp.: 35°C
 Det.: Photodiode Array
 Inj.: 5 μ L



Quinidine and Trace Impurity (HPLC)

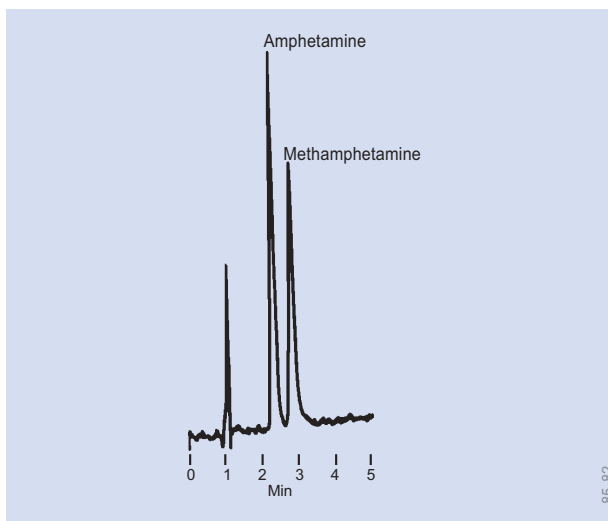
Columns: 15cm x 4.6mm ID, 5 μ m particles
 Cat. Nos.: 567516-U (HS F5), 504955 (C18)
 Mobile Phase: 25 mM ammonium phosphate (pH 7.0):CH₃CN
 varying ratios: (A) 35:65, (B) 70:30
 Flow Rate: 1.0mL/min
 Temp.: 30°C
 Det.: UV, 235nm
 Inj.: 10 μ L

HPLC Applications Drugs



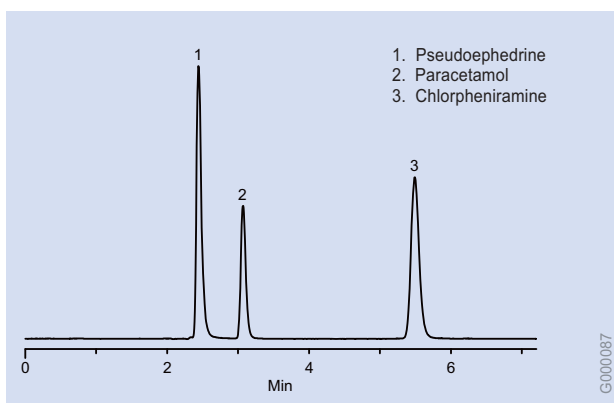
Procainamides (HPLC)

Column: Discovery HS F5, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 567516-U
 Mobile Phase: (65:35) 25 mM ammonium phosphate (pH 7):CH₃CN
 Flow Rate: 1.0mL/min
 Temp.: 30°C
 Det.: UV, 280nm
 Inj.: 5 μ L
 Sample: 50 μ g/mL of each



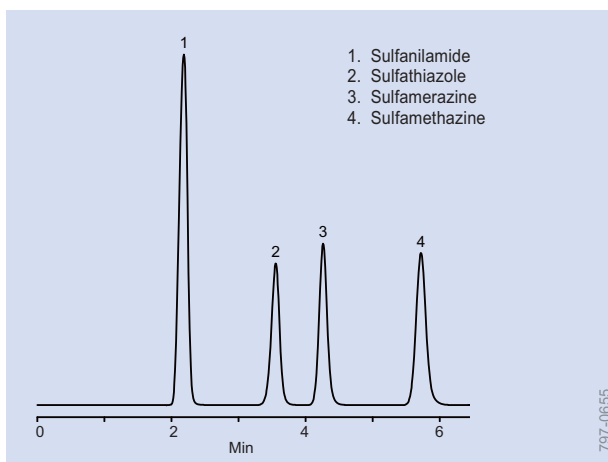
Stimulants, Amphetamine (HPLC)

Column: Suplex pKb-100, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 58932
 Mobile Phase: acetonitrile:25mM K₂HPO₄, pH 7.0 with phosphoric acid (15:85)
 Flow Rate: 2mL/min
 Det.: UV, 254nm
 Inj.: 10 μ L mobile phase (50 μ g/mL each analyte)



Sinus Medications (HPLC)

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 505013
 Mobile Phase: acetonitrile:50mM KH₂PO₄, pH 3.0 (25:75)
 Flow Rate: 1mL/min
 Temp.: 30°C
 Det.: UV, 220nm
 Inj.: 10 μ L

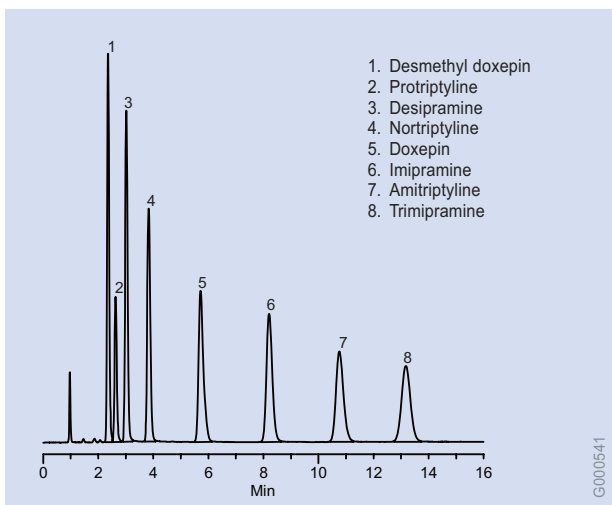


Sulfa Drugs (HPLC)

Column: Discovery C18, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 504955
 Mobile Phase: methanol:water with 1% CH₃COOH (20:80)
 Flow Rate: 1mL/min
 Temp.: 20°C
 Det.: UV, 254nm
 Inj.: 10 μ L

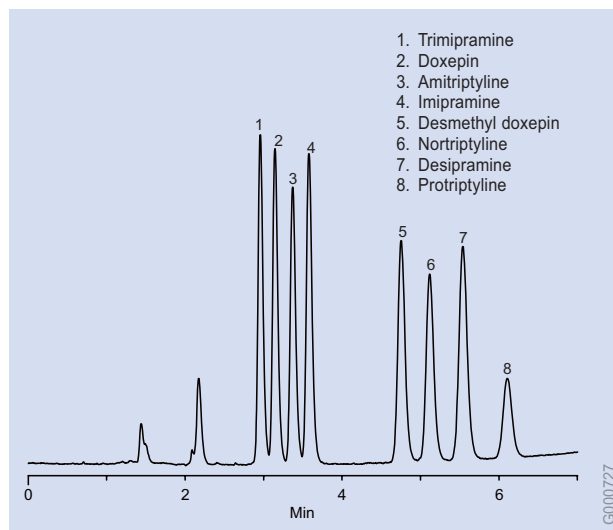
HPLC Applications

Drugs



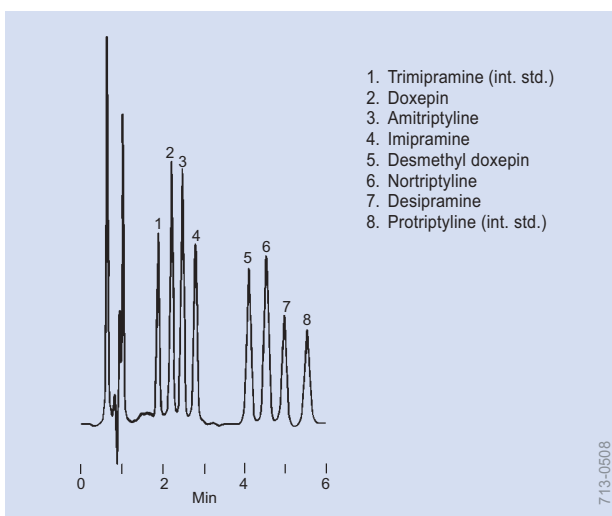
Tricyclic Antidepressants (HPLC)

Column: Discovery C8, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 59353-U
Mobile Phase: methanol:10mM K₂HPO₄, pH 7.0
Flow Rate: 2mL/min
Temp.: 40°C
Det.: UV, 254nm



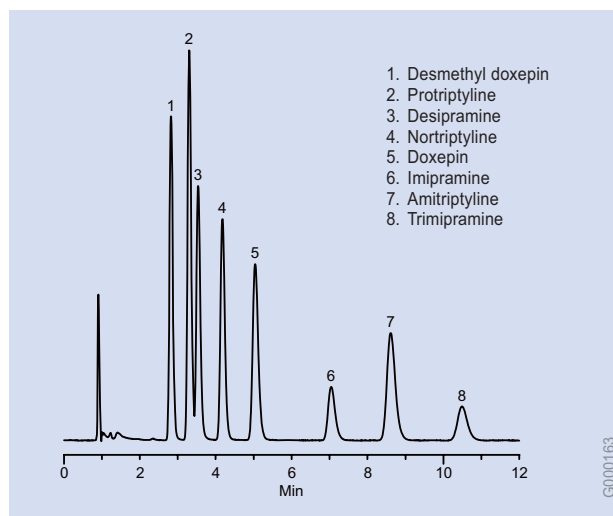
Tricyclic Antidepressants (HPLC)

Column: Discovery Cyano, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 59356-U
Mobile Phase: acetonitrile:methanol:10mM K₂HPO₄, pH 7.0, 60:20:20
Flow Rate: 1mL/min
Temp.: 35°C
Det.: UV, 254nm



Tricyclic Antidepressants (HPLC)

Column: SUPELCOSIL LC-PCN, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 58377
Mobile Phase: acetonitrile:methanol:0.01M dipotassium hydrogen phosphate, pH 7.0 with phosphoric acid (60:15:25)
Flow Rate: 2mL/min
Temp.: 30°C
Det.: UV, 215nm
Inj.: 50ng each drug

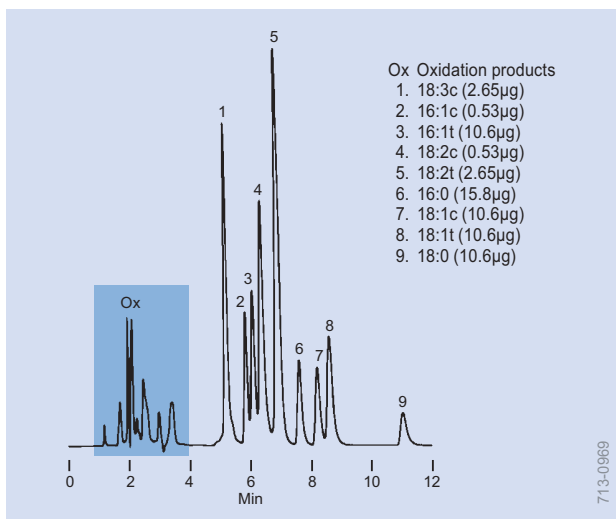


Tricyclic Antidepressants (HPLC)

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 505013
Mobile Phase: methanol:25mM KH₂PO₄, pH 7, 60:40
Flow Rate: 2mL/min
Temp.: 40°C
Det.: UV, 230nm
Inj.: 10µL

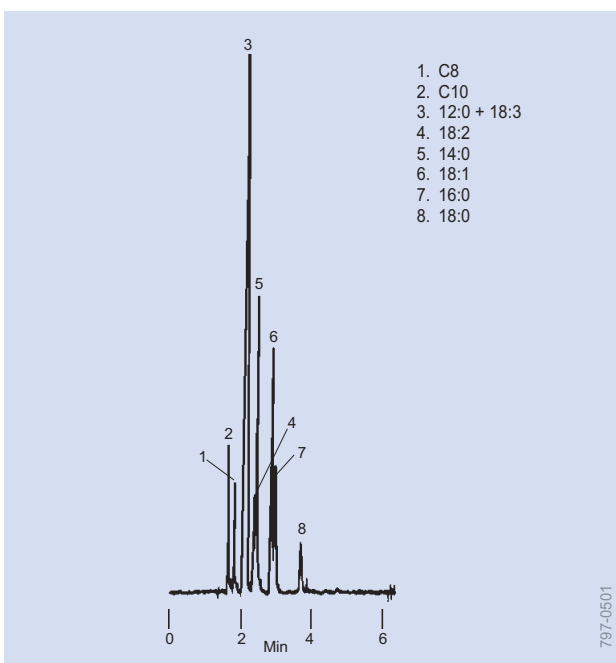
HPLC Applications

Fatty Acids, Foods



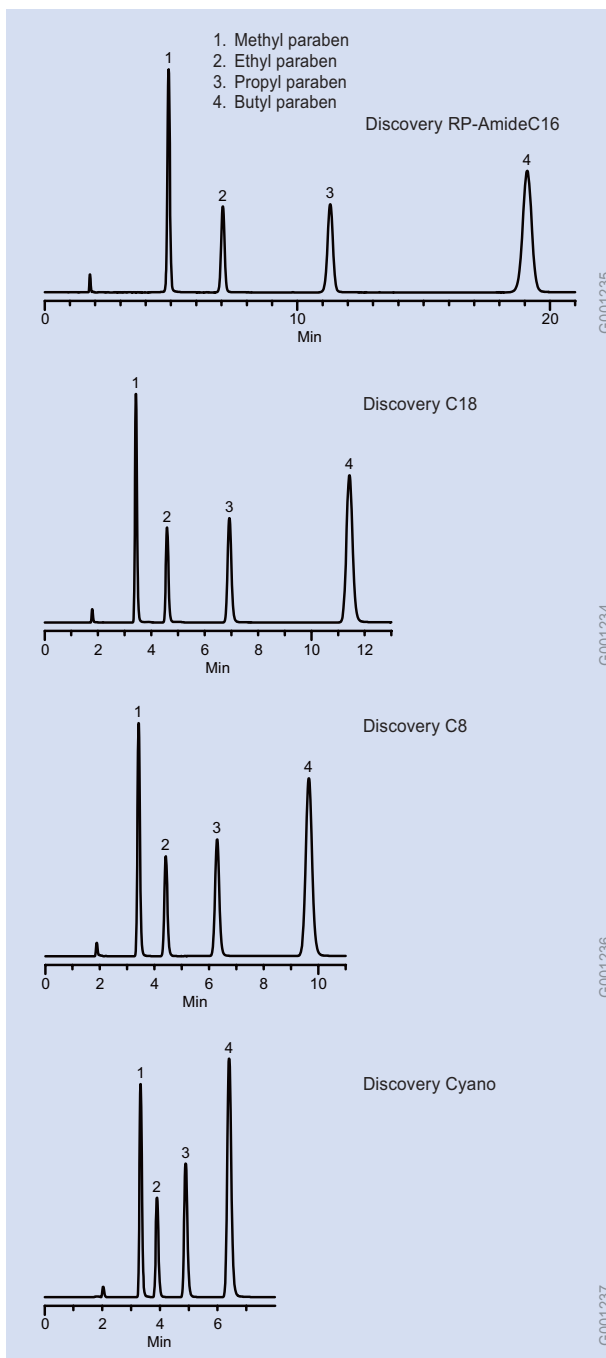
Fatty Acids, Free (HPLC)

Column: SUPELCOSIL LC-8, 15cm x 4.6mm ID, 3µm particles
 Cat. No.: 58983
 Mobile Phase: acetonitrile:tetrahydrofuran: 0.1% phosphoric acid (50.4:21.6:28)
 Flow Rate: 1mL/min
 Temp.: 35°C
 Det.: UV, 215nm
 Inj.: 10µL



Fatty Acid Methyl Esters (HPLC)

Column: SUPELCOSIL LC-18, 25cm x 4.6mm ID, 5µm particles
 Cat. No.: 58298
 Mobile Phase: acetonitrile:acetone (59.0:41.0, v/v)
 Flow Rate: 1.0mL/min
 Det.: RI
 Sample: 10µL of 9% C8 to C18 saturated and unsaturated FAMES in mobile phase

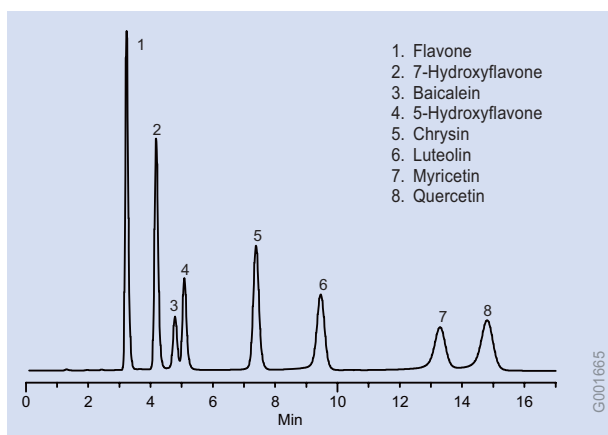


Paraben Preservatives (HPLC)

Columns: 15cm x 4.6mm ID, 5µm particles
 conditions for RP-AmideC16, C18, and C8
 Cat. Nos.: 505013 (RP-AmideC16), 504955 (C18),
 59353-U (C8)
 Mobile Phase: CH₃CN:H₂O, (40:60)
 Flow Rate: 1mL/min
 Temp.: 20°C
 Det.: UV, 254nm
 Inj.: 10µL
 conditions for Cyano
 Cat. No.: 59356-U (Cyano)
 Mobile Phase: CH₃CN:H₂O, (30:70)
 Flow Rate: 1mL/min
 Temp.: 20°C
 Det.: UV, 254nm
 Inj.: 10µL

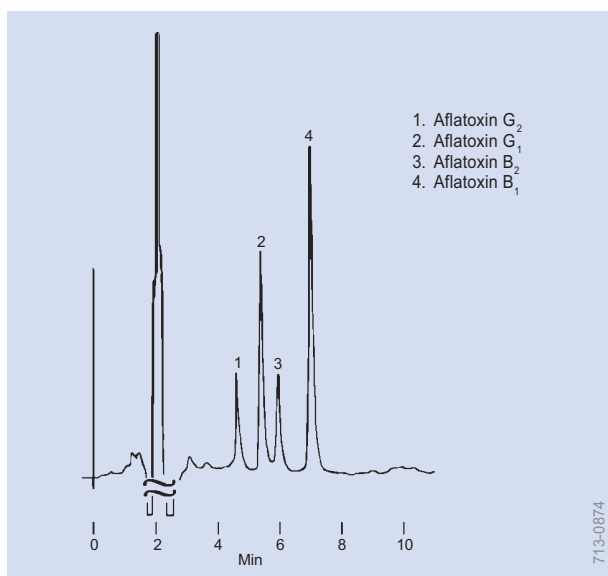
HPLC Applications

Flavor, Mycotoxins, Nucleosides, Nucleotides



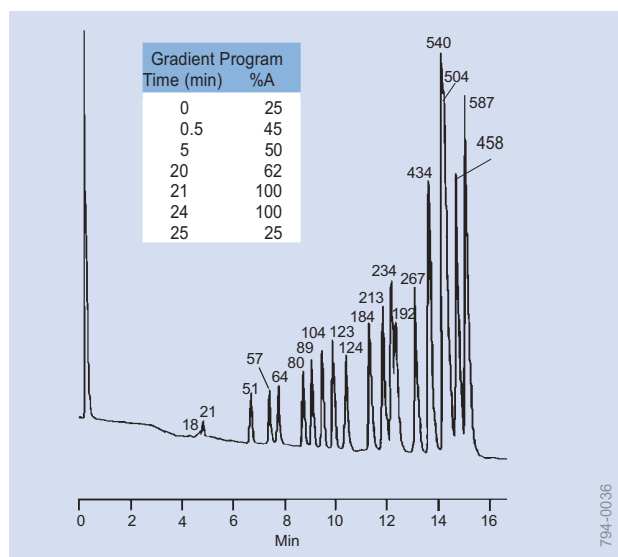
Flavones (HPLC)

Column: Discovery HS PEG, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 567416-U
 Mobile Phase: 45:55 0.1% formic acid in water :
 0.1% formic acid in MeOH
 Flow Rate: 1.0mL/min
 Temp.: 30°C,
 Det.: UV, 254nm
 Inj.: 10µL



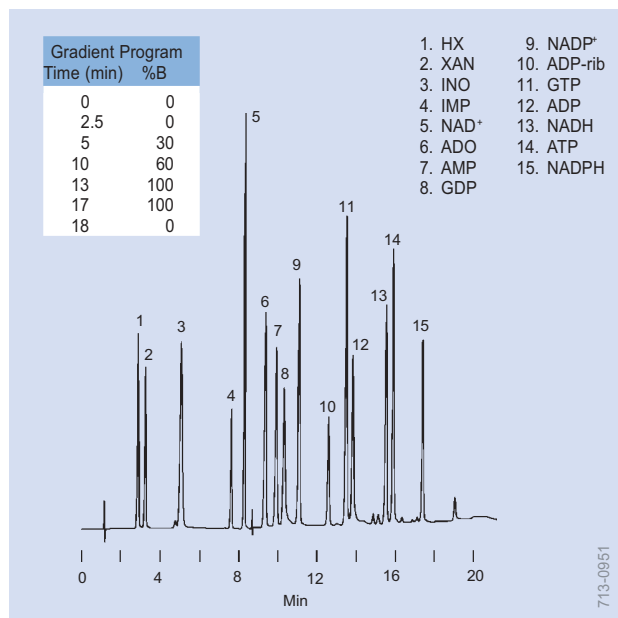
Aflatoxins (SPE/HPLC)

Sample: cornmeal spiked with aflatoxins (30ppb G₂ and B₂, 100ppb G₁ and B₁) Blend 50g sample for 1 min in 100mL methanol:water (8:2) and filter.
 Extraction Tube: Supelclean LC-CN, 3mL, 0.5g packing
 Cat. No.: 57013
 Conditioning: 2mL 0.5% aqueous acetic acid
 Sample Addition: 1mL filtered extract + 4mL 0.5% aqueous acetic acid
 Washing: 500µL 20% THF in 0.5% aqueous acetic acid, then 2mL hexane, Dry packing under nitrogen. 3mL 25% THF in hexane, Dry packing 1 min under nitrogen.
 Elution: 2 x 2mL 1% THF in methylene chloride
 Evaporate eluate to dryness under nitrogen. Reconstitute with 100µL of methanol, then dilute with 100µL of 0.5% aqueous acetic acid.
 Column: SUPELCOSIL LC-18, 25cm x 4.6mm ID, 5µm particles (with guard column)
 Cat. No.: 58298
 Mobile Phase: methanol:acetonitrile:water (22.5:22.5:55)
 Flow Rate: 1.5mL/min
 Det.: VIS, 365nm
 Inj.: 100µL



pBR322 DNA-Hae III Digest (HPLC)

Column: TSKgel DEAE-NPR, 3.5cm x 4.6mm ID,
 2.5µm particles (with 5mm DEAE-NPR guard column)
 Cat. No.: 813075
 Mobile Phase: A = 1M NaCl/25mM Tris-HCl, pH 9.0
 B = 25mM Tris-HCl, pH 9.0
 Flow Rate: 1mL/min
 Temp.: ambient
 Det.: UV, 260nm
 Inj.: 20µL of 50µg/mL HAE III digest of pBR322 DNA

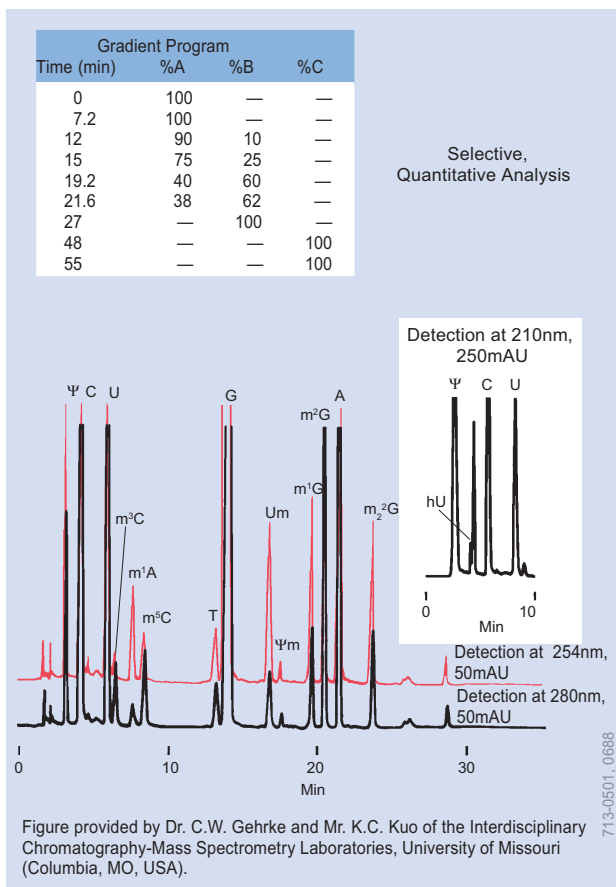


Nucleotides (HPLC)

Column: SUPELCOSIL LC-18-T, 15cm x 4.6mm ID, 3µm particles
 Cat. No.: 58970-U
 Mobile Phase: A = 0.1M potassium phosphate buffer/4mM tetrabutylammonium hydrogen sulfate, pH 6.0
 B = A:methanol (70:30), pH 7.2
 Flow Rate: 1.5mL/min
 Det.: UV, 254nm
 Inj.: 20µL deionized water (25-530µM each analyte)

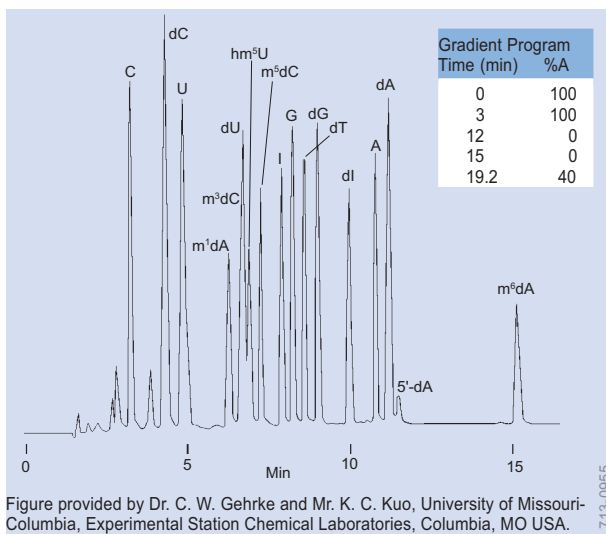
HPLC Applications

Nucleosides, Nucleotides, Organic Acids



Single Species tRNAs (HPLC)

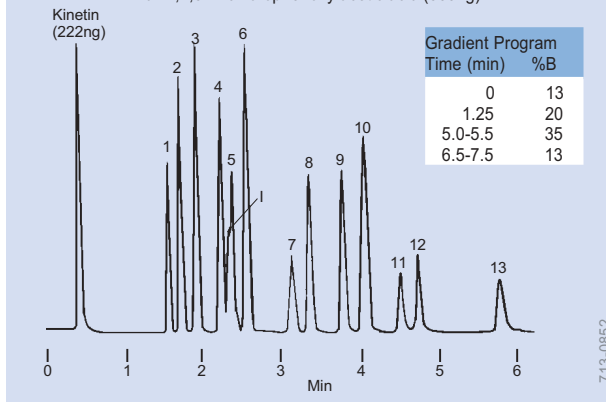
Columns: SUPELCOSIL LC-18-S, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 58931
 Mobile Phase: A = 0.01M KH₂PO₄ (pH 5.3):methanol, 97.5:2.5
 B = 0.01M KH₂PO₄ (pH 5.1):methanol, 80:20
 C = 0.01M KH₂PO₄ (pH 4.9):acetonitrile, 65:35
 Equilibration: solvent A, 15 min at 1mL/min
 Flow Rate: 1mL/min
 Temp.: 26°C
 Det.: UV, 210nm, 254nm, 280nm
 Inj.: 50µL hydrolysate buffer (pH 7.8) containing 5µg bovine tRNA^{Leu} hydrolysate



Deoxyribonucleosides and Ribonucleosides (HPLC)

Column: SUPELCOSIL LC-18-S, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 58931
 Mobile Phase: 0.05M K₂HPO₄/KH₂PO₄, pH 4.0:methanol
 A = 97.5:2.5, B = 80:20
 Flow Rate: 1.0mL/min
 Temp.: 30°C
 Det.: UV, 254nm
 Inj.: nucleoside standards in water

- 6-Benzylaminopurine riboside (445ng)
- Indole-3-acetic acid (222ng)
- Abscisic acid (222ng)
- α-Naphthalene acetamide (222ng)
- Abscisic acid impurity
- Colchicine (555ng)
- Indole-3-propanoic acid (222ng)
- p-Chlorophenoxy acetic acid (333.5ng)
- Indole-3-butyric acid (333.5ng)
- α-Naphthalene acetic acid (333.5ng)
- β-Naphthalene acetic acid (333.5ng)
- 2,4-Dichlorophenoxy acetic acid (555ng)
- Indole-3-acetic acid ethyl ester (445ng)
- 2,4,5-Trichlorophenoxy acetic acid (555ng)

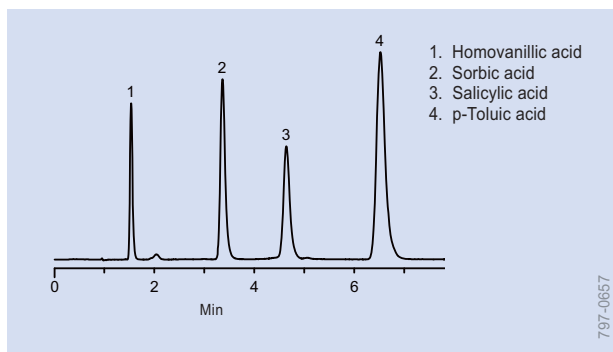


Plant Growth Regulators (HPLC)

Column: SUPELCOSIL LC-18-DB, 3.3cm x 4.6mm ID, 3µm particles
 Cat. No.: 58978
 Mobile Phase: A = 1% acetic acid/1mM tetrabutylammonium phosphate in water, pH 2.8
 B = acetonitrile
 Flow Rate: 2mL/min
 Temp.: 35°C
 Det.: UV, 280nm
 Inj.: compounds in 13:87 A:B

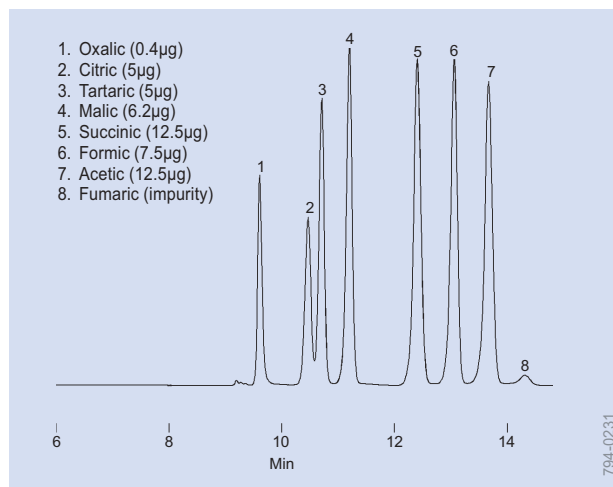
HPLC Applications

Organic Acids



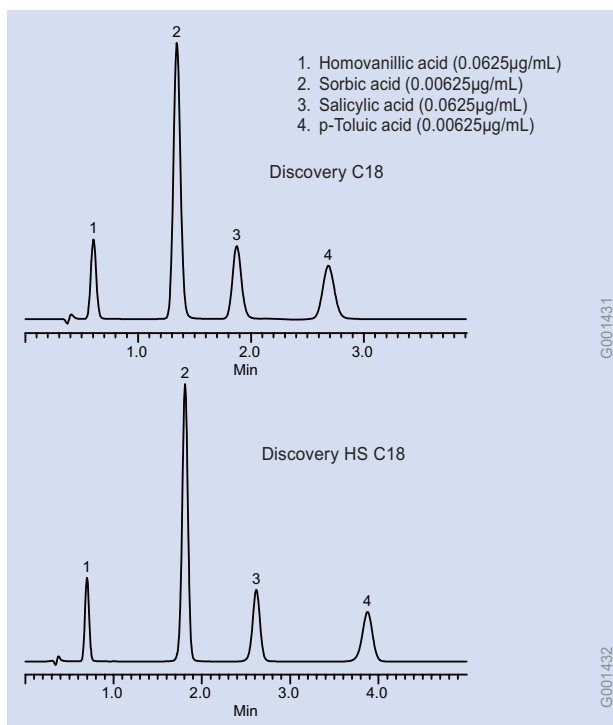
Organic Acids (HPLC)

Column: Discovery C18, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 504955
 Mobile Phase: methanol:water, 0.1% TFA (40:60)
 Flow Rate: 1mL/min
 Temp.: 20°C
 Det.: UV, 254nm
 Inj.: 10 μ L, 1mg/mL each analyte



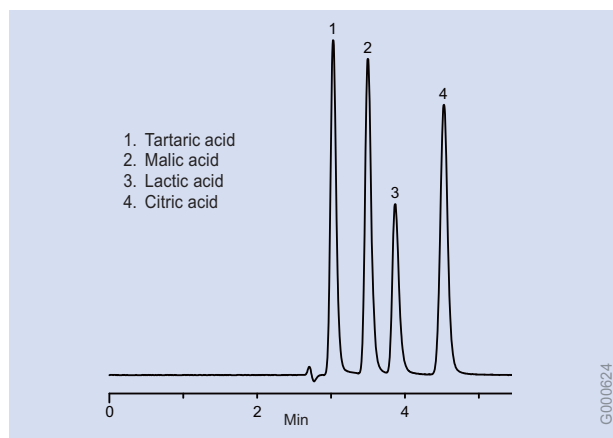
Organic Acids (HPLC)

Column: SUPELCOGEL C-610H, 30cm x 7.8mm ID
 Cat. No.: 59320-U
 Mobile Phase: 0.1% phosphoric acid
 Flow Rate: 0.5mL/min
 Det.: UV, 210nm
 Inj.: 5 μ L



Organic Acids (HPLC)

Columns: 5cm x 4.6mm ID, 5 μ m particles
 Cat. Nos.: 504947 (C18), 568520-U (HS C18)
 Mobile Phase: 60:40 0.1%TFA in water:MeOH
 Flow Rate: 2.0mL/min
 Temp.: 20°C
 Det.: UV, 254nm
 Inj.: 10 μ L

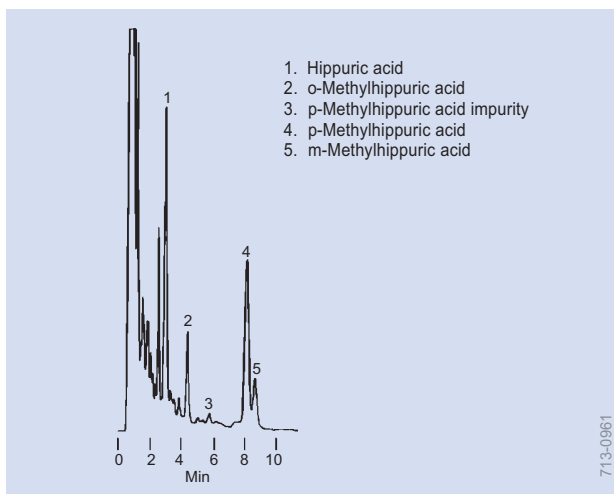


α -Hydroxy Aliphatic Acids (HPLC)

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 505013
 Mobile Phase: 25mM KH_2PO_4 , pH 3.0
 Flow Rate: 1mL/min
 Temp.: 30°C
 Det.: UV, 220nm
 Inj.: 10 μ L

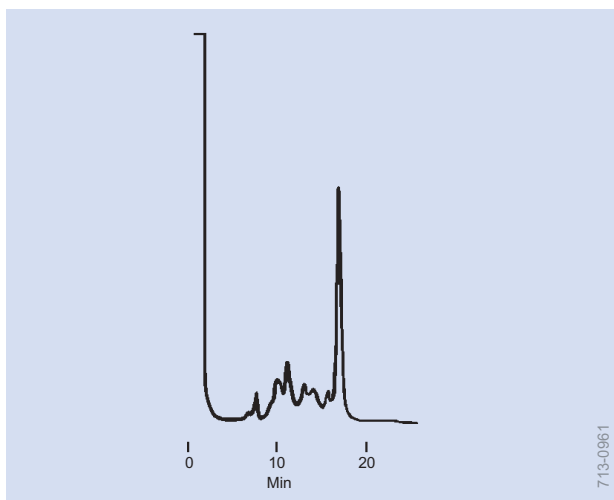
HPLC Applications

Organic Acids, Peptides and Proteins



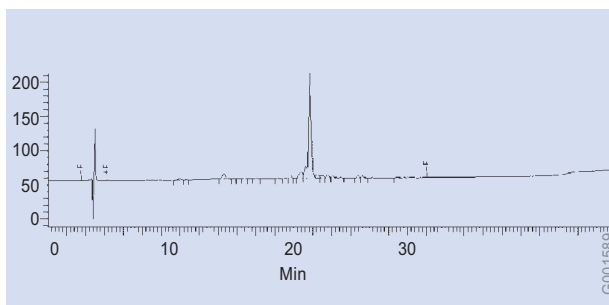
Hippuric and Methylhippuric Acids (HPLC)

Column: SUPELCOSIL LC-18, 7.5cm x 4.6mm ID, 3 μ m particles
Cat. No.: 58984
Mobile Phase: tetrahydrofuran:10mM KH₂PO₄, pH 3.0 with phosphoric acid (3:97)
Flow Rate: 1.5mL/min
Det.: UV, 260nm
Inj.: 10 μ L spiked urine (300 μ g/mL HA, o-MHA; 150 μ g/mL m-MHA, p-MHA)



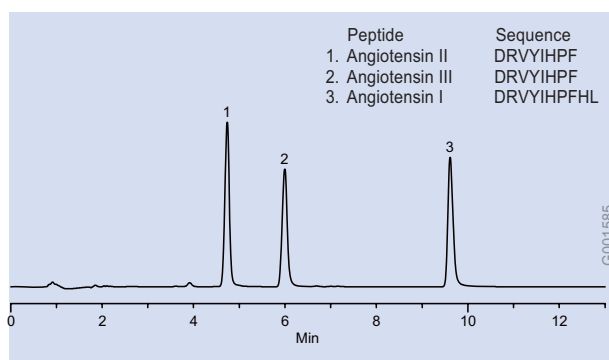
α -Amylase, Crude (HPLC)

Column: TSKgel Ether-5PW, 7.5cm x 7.5mm ID, 10 μ m particles
Cat. No.: 808641
Mobile Phase: A = 0.1M phosphate buffer + 1.1M Na₂SO₄, pH 7.0
B = 0.1M phosphate buffer
A to B over 60 min
Flow Rate: 1.0mL/min
Det.: UV, 280nm



Amyloid β -Protein Fragment 10-35 (HPLC)

Columns: Discovery BIO Wide Pore C18, 25cm x 4.6mm ID, 5 μ m particles
Cat. No.: 568223-U
Mobile Phase: (A) 0.1% TFA in water
(B) 25:75, (0.1% TFA in water): (0.1% TFA in CH₃CN)
Flow Rate: 1.3mL/min
Temp: 30°C
Det.: UV, 215nm
Inj.: 10 μ L, 1mg/mL in water
Gradient: 35-55%B in 20 min, 55%B for 5 min, 35%B for 5 min

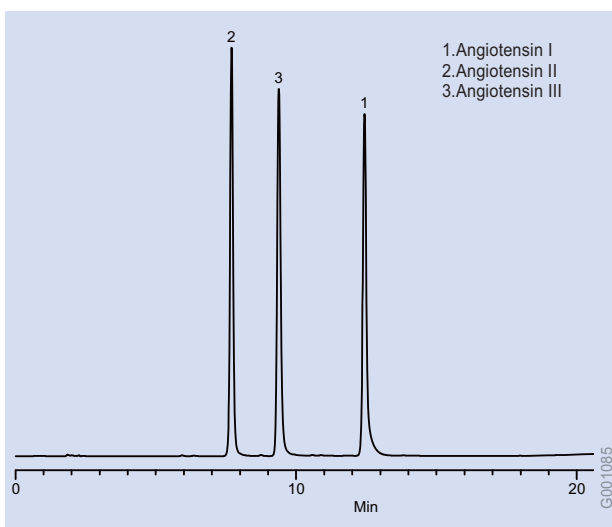


Angiotensins (HPLC)

Column: Discovery BIO Wide Pore C18, 15cm x 4.6mm ID, 5 μ m particles
Cat. No.: 568222-U
Mobile Phase: (A) 65:35, (10mM NH₄OAc, pH 7):(50% CH₃CN in 20mM NH₄OAc, pH 7)
(B) 25:75, (10mM NH₄OAc, pH 7):(50% CH₃CN in 20mM NH₄OAc, pH 7)
Flow Rate: 1.0mL/min
Temp: ambient
Det.: UV, 215nm
Inj.: 6 μ L (10 μ g each) in water
Gradient: 0-100%B in 12.5 min

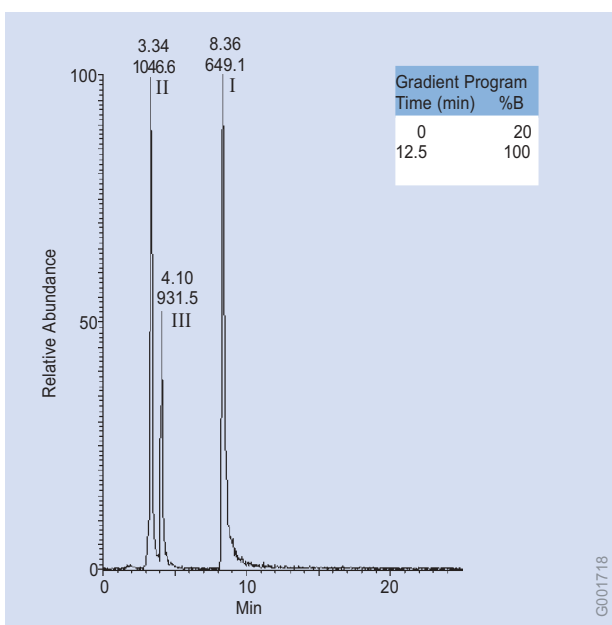
HPLC Applications

Peptides and Proteins



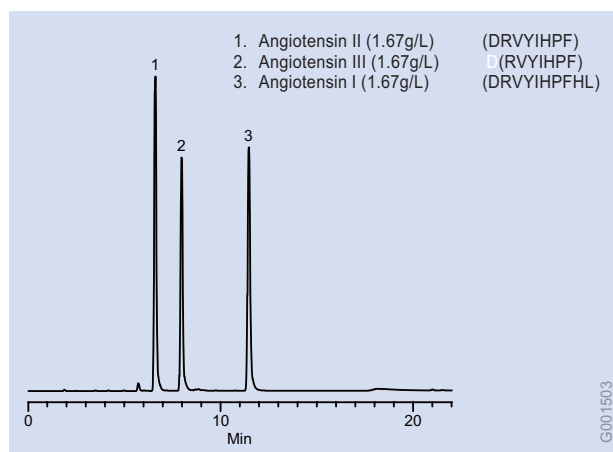
Angiotensins (HPLC)

Column: Discovery C18, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 504955
Mobile Phase: (A) 5mM (NH₄)H₂PO₄/NH₄OH, pH 7.0
(B) 5mM (NH₄)H₂PO₄/NH₄OH, pH 7.0: CH₃CN (50:50)
Flow Rate: 1mL/min
Temp.: 35°C
Det.: UV, 220nm
Gradient: 30-60% B in 15 min



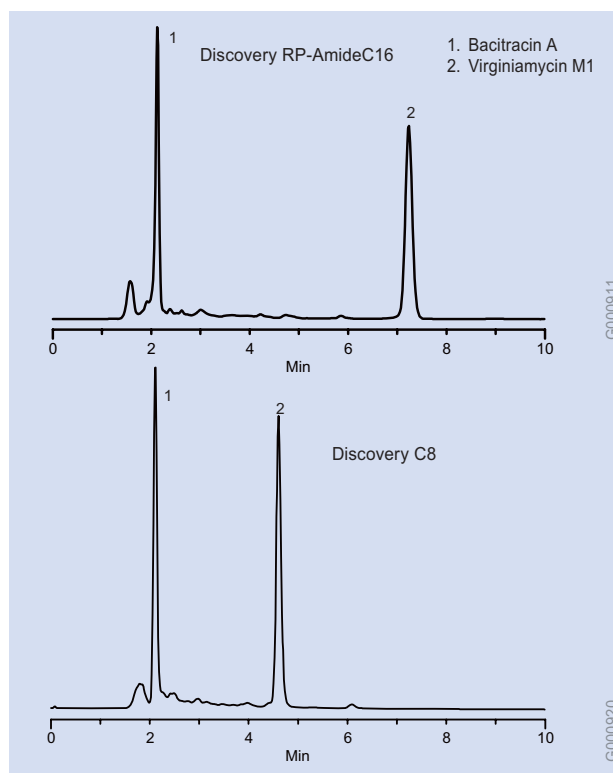
Angiotensins Capillary Separation (HPLC)

Column: Discovery BIO Wide Pore C18, 10cm x 0.32mm ID, 3µm particles
Cat. No.: 65527-U
Solution x: 10mM NH₄OAc/NH₄OH, pH 7.0
Solution y: 50:50 (20mM NH₄OAc/NH₄OH, pH 7.0) : CH₃CN
Mobile Phase A: x:y, 65:35
Mobile Phase B: x:y, 25:75
Flow Rate: 6µL/min
Det.: +ES, source 2.5kV, capillary 12V, 130°C
Temp.: ambient
Inj.: 50pL



Angiotensins (HPLC)

Column: Discovery BIO Wide Pore C8, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 568322-U
Mobile Phase: (A) 10mM NH₄H₂PO₄/NH₄OH, pH 7
(B) 50:50, (20mM NH₄H₂PO₄/NH₄OH, pH 7):CH₃CN
Flow Rate: 1mL/min
Temp: 30°C
Det.: UV, 215nm
Inj.: 6µL in water
Gradient: 30-60% B in 15 min

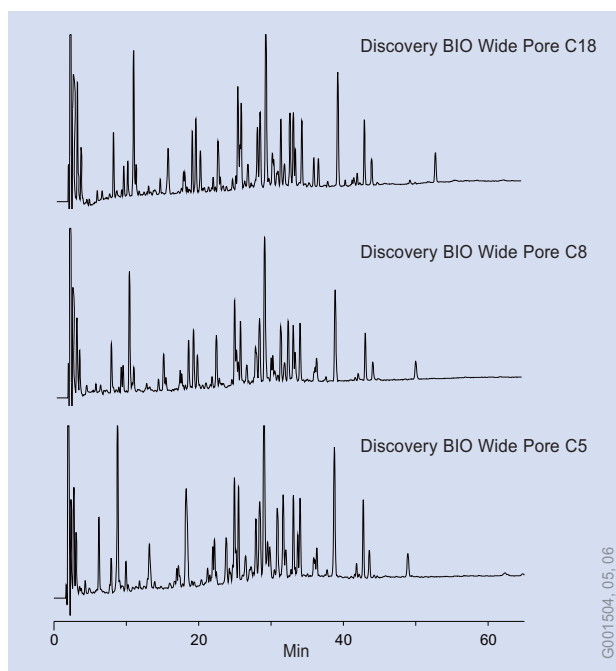


Antibiotics, Peptide (HPLC)

Columns: 15cm x 4.6mm ID, 5µm particles
Cat. Nos.: 505013 (RP-AmideC16), 59353-U (C8)
Mobile Phase: 25mM KH₂PO₄, pH 3:acetonitrile, 60:40
Flow Rate: 1mL/min
Temp.: 35°C
Det.: UV, 220nm
Inj.: 10µL 25mM KH₂PO₄, pH 3 containing 50µg/mL each analyte

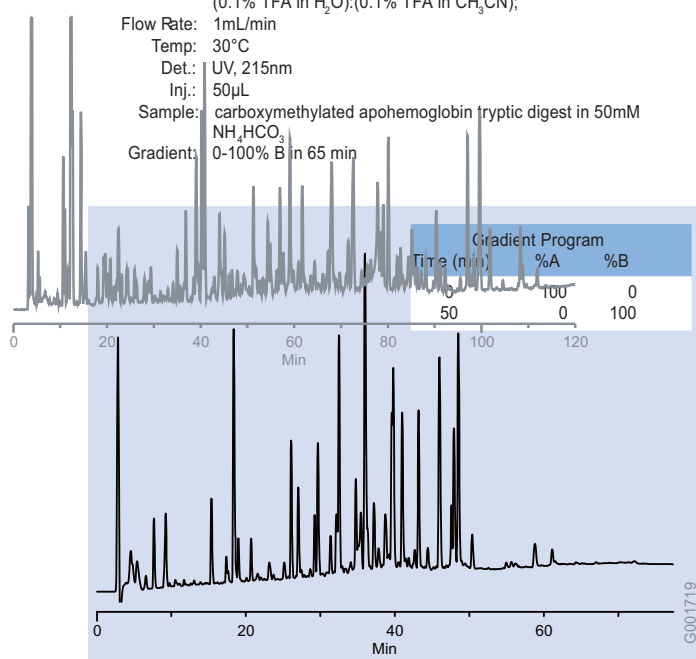
Order: 1.800.325.3010 Technical Service: 1.800.359.3041 Web: www.sigma-aldrich.com/supelco

HPLC Applications Peptides and Proteins



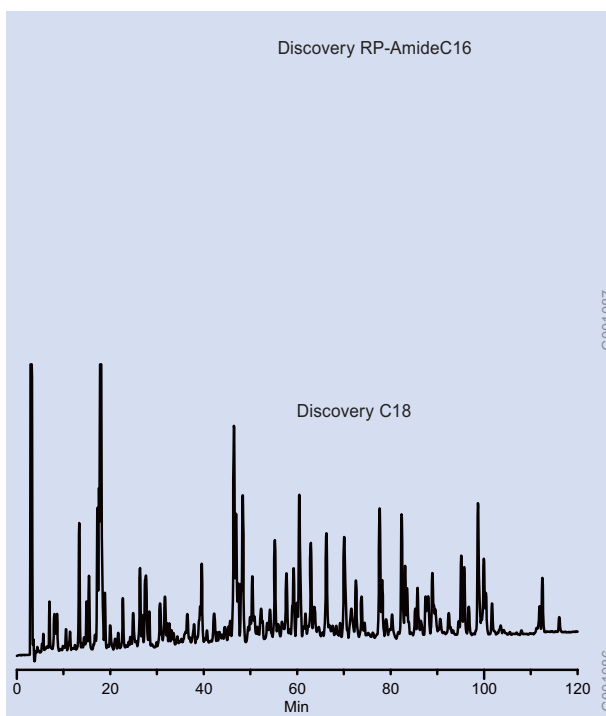
Apo-hemoglobin, Carboxymethylated, Tryptic Digest (HPLC)

Columns: 15cm x 4.6mm ID, 5 μ m particles
 Cat. Nos.: 568222-U (C18), 568322-U (C8), 568422-U (C5)
 Mobile Phase: (A) 95:5, (0.1% TFA in H₂O):(0.1% TFA in CH₃CN); (B) 50:50, (0.1% TFA in H₂O):(0.1% TFA in CH₃CN);
 Flow Rate: 1mL/min
 Temp.: 30°C
 Det.: UV, 215nm
 Inj.: 50 μ L
 Sample: carboxymethylated apo-hemoglobin tryptic digest in 50mM NH₄HCO₃
 Gradient: 0-100% B in 65 min



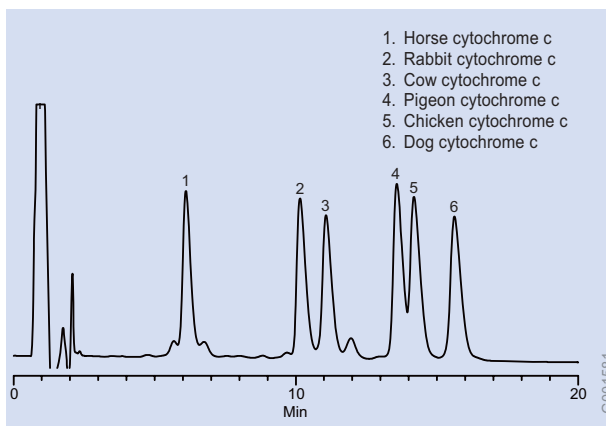
Apo-hemoglobin (Horse), Carboxymethylated, Tryptic Digest (HPLC)

Column: Discovery BIO Wide Pore C18, 15cm x 2.1mm ID, 3 μ m particles
 Cat. No.: 567202-U
 Mobile Phase A: water/0.1% TFA
 Mobile Phase B: 50:50, (water/0.1% TFA) : (CH₃CN/0.1% TFA)
 Flow Rate: 0.208mL/min
 Temp.: 35°C
 Det.: UV, 215nm
 Inj.: 5 μ L
 Sample: digest



Carboxyamido-methylated BSA Tryptic Digest (HPLC)

Columns: 25cm x 4.6mm ID, 5 μ m particles
 Cat. Nos.: 505013 (RP-AmideC16), 504955 (C18)
 Mobile Phase: (A) 0.1% TFA, 1% CH₃CN in H₂O
 (B) 0.1% TFA, 40% CH₃CN
 Flow Rate: 1 mL/min
 Det.: UV, 215nm
 Temp.: 35°C
 Gradient: 0-100% B in 130 min

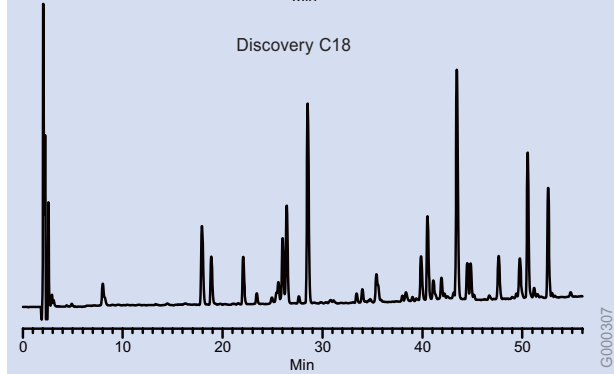
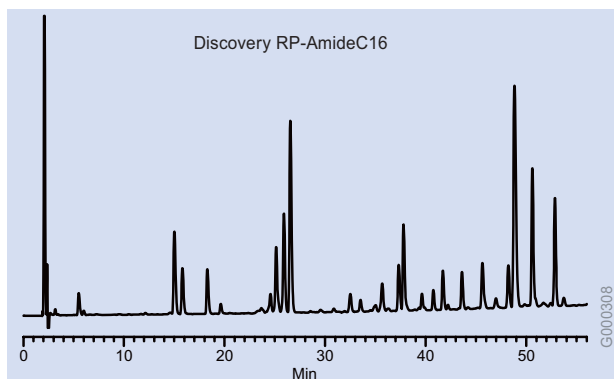


Cytochrome c (HPLC)

Column: Discovery BIO Wide Pore C18, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 568222-U
 Mobile Phase: (A) 70:30, (0.1% TFA in water):(0.1% TFA in CH₃CN);
 (B) 64:36, (0.1% TFA in water):(0.1% TFA in CH₃CN)
 Flow Rate: 1.0mL/min
 Temp.: ambient
 Det.: UV, 220nm
 Inj.: 12 μ L each at 0.8mg/mL in 0.1%TFA
 Gradient: 0-100%B in 30 min

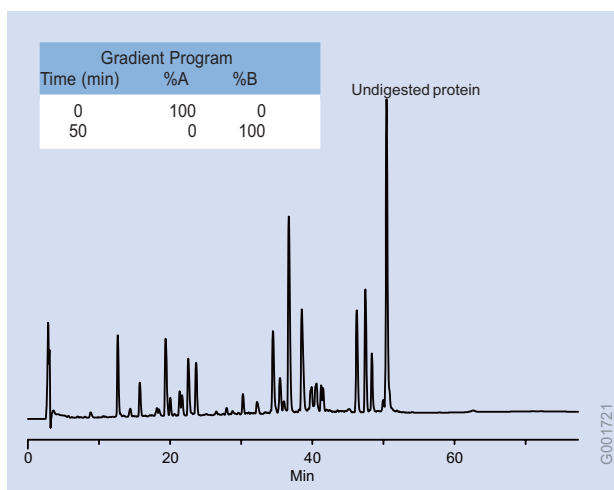
HPLC Applications

Peptides and Proteins



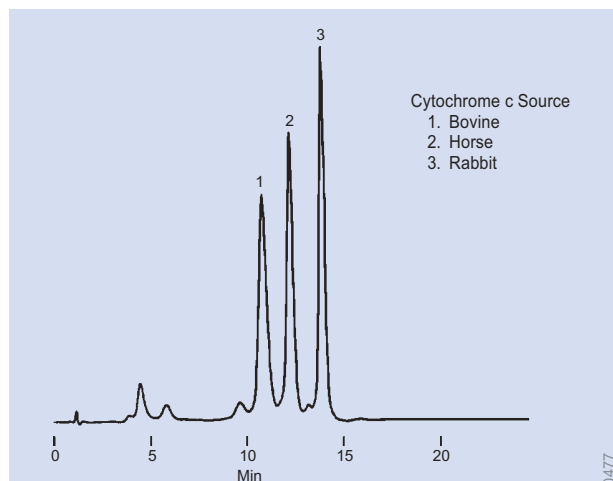
Cytochrome c Tryptic Digest (HPLC)

Columns: 15cm x 4.6mm ID, 5 μ m particles
 Cat. Nos.: 505013 (RP-AmideC16), 504955 (C18)
 Mobile Phase: (A) 0.1% TFA, 3% n-propanol in H₂O
 (B) 0.1% TFA, 3% n-propanol in CH₃CN
 Flow Rate: 1mL/min
 Temp.: 35°C
 Gradient: 1 - 37% B in 72 min



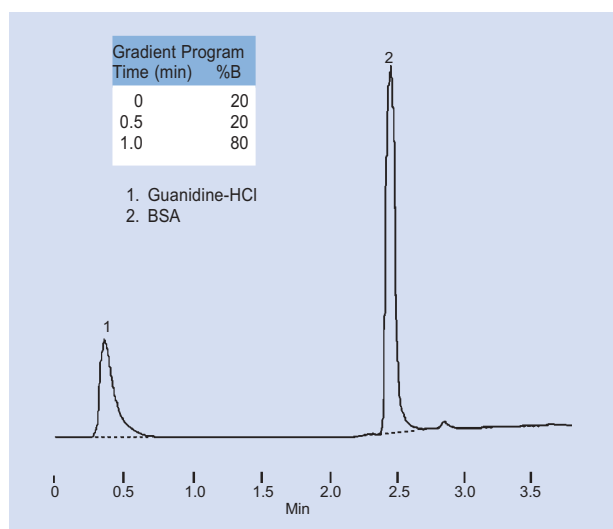
Carboxymethylated Cytochrome c (Chicken) Tryptic Digest (HPLC)

Column: Discovery BIO Wide Pore C18, 15cm x 2.1mm ID, 3 μ m particles
 Cat. No.: 567202-U
 Mobile Phase A: water/0.1% TFA
 Mobile Phase B: 50:50, (water/0.1% TFA) : (MeCN/0.1% TFA)
 Flow Rate: 0.208mL/min
 Temp: 35°C
 Det.: 215nm
 Inj.: 5 μ L
 Sample: digest



Cytochrome c Species Variants (HPLC)

Column: ProtEx-SP, 5cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 54740-U
 Mobile Phase: A = 20mM Bis-Tris HCl, pH 7.0
 B = A + 0.5M NaCl
 24-69% B over 20 min
 Flow Rate: 0.5mL/min
 Det.: UV, 280nm
 Inj.: 10 μ g each variant

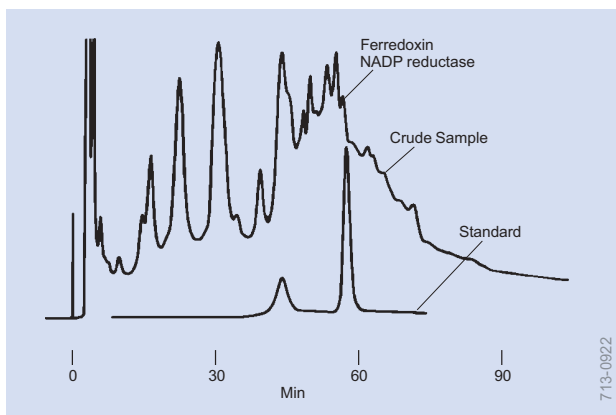


Desalting of Proteins (HPLC)

Column: Supelguard LC-308, 2cm x 4.6mm ID, 5 μ m particles, 300Å pores
 Cat. No.: 59511-U
 Mobile Phase: A = 0.1% TFA in water
 B = 0.09% TFA in acetonitrile
 Flow Rate: 2mL/min
 Det.: UV, 220nm
 Inj.: 40 μ L of 200 μ g BSA in 3M guanidine-HCl

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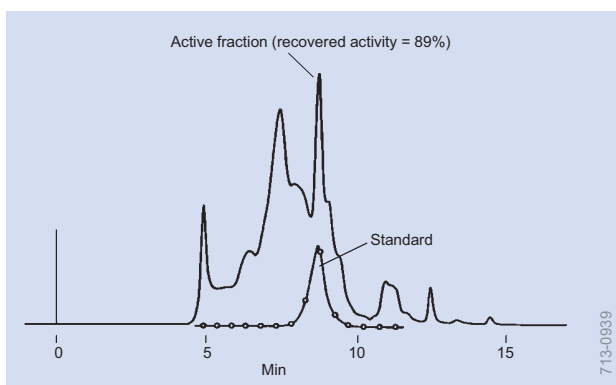
HPLC Applications Peptides and Proteins



713-0922

Ferredoxin NADP Reductase (HPLC)

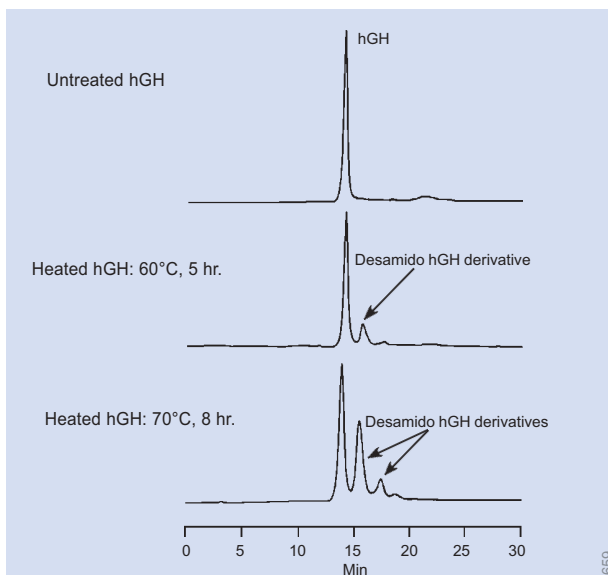
Column: TSKgel Phenyl-5PW column, 7.5cm x 7.5mm ID, 10 μ m particles
 Cat. No.: 807573
 Mobile Phase: 60 min decreasing linear gradient from 1.5M to 0M ammonium sulfate in 0.1M sodium phosphate buffer, pH 7
 Flow Rate: 0.5mL/min
 Temp.: 25°C
 Det.: UV, 280nm
 Inj.: 3mg (crude) or 0.2mg (standard)



713-0939

Glutathione S-Transferase (HPLC)

Column: TSKgel G3000SW_{XL}, 30cm x 7.8mm ID, 5 μ m particles
 Cat. No.: 808541
 Mobile Phase: 0.3M NaCl/0.05M NaH₂PO₄, pH 7.0
 Flow Rate: 1mL/min
 Temp.: 25°C
 Det.: UV, 220nm
 Inj.: 100 μ L crude glutathione S-transferase preparation from guinea pig liver (0.7mg)

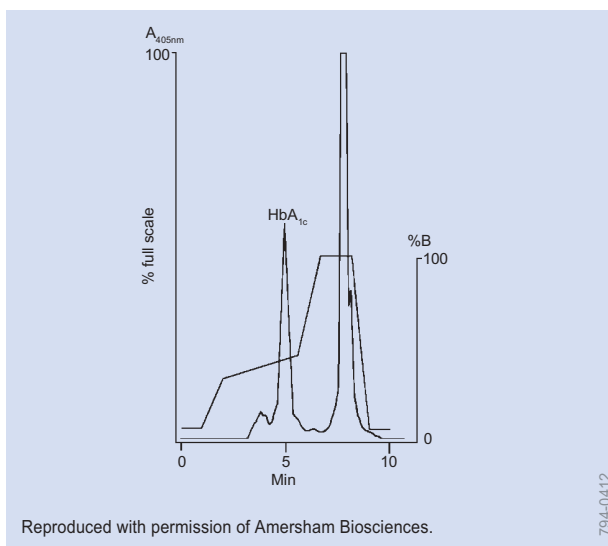


796-0659

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Purity Evaluation of Human Growth Hormone (hGH) (HPLC)

Column: ProtEx-DEAE, 5cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 54742
 Mobile Phase: A = 20mM Tris-HCl, pH 8.0
 B = A + 0.5M NaCl
 5-70% B over 30 min
 Flow Rate: 0.5mL/min
 Inj.: 10mg hGH



794-0412

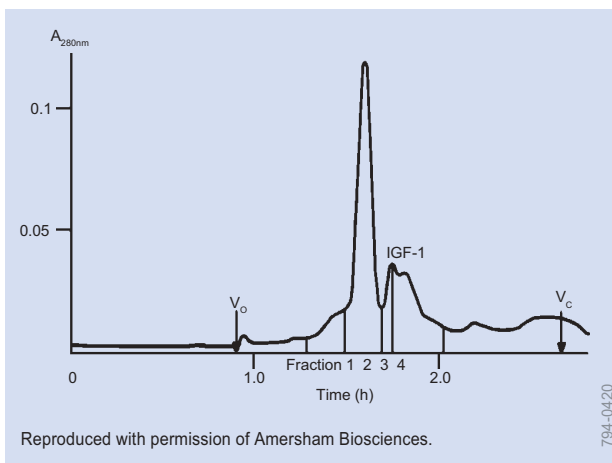
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Hemoglobin HbA_{1c} (HPLC)

Column: Mono S HR5/5, 10 μ m particles
 Cat. No.: 54808
 Mobile Phase: A = 0.01M malonate-NaOH, pH 5.7
 B = 0.3M LiCl in mobile phase A
 Flow Rate: 2.0mL/min
 Det.: VIS, 405nm
 Inj.: 50 μ L hemolysate diluted 1:5

HPLC Applications

Peptides and Proteins

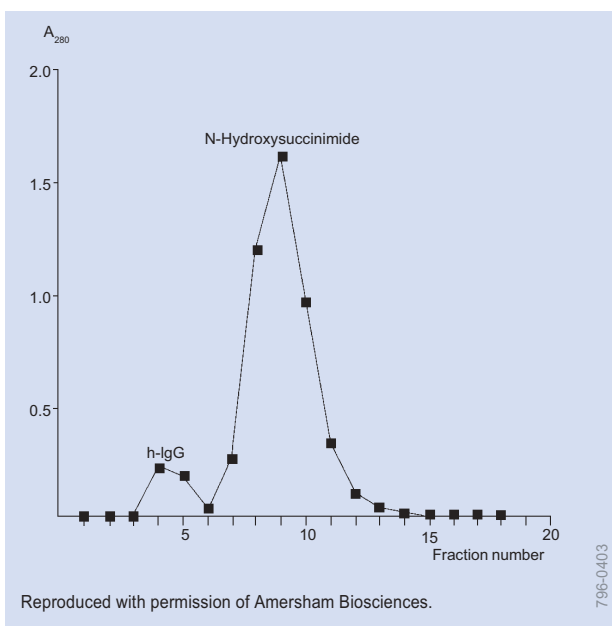


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794-0420

IGF-1, Insulin-like Growth Factor-1 (HPLC)

Column: HiLoad 16/60 Superdex 75 prep grade, 34 μ m particles
 Cat. No.: 54802-U
 Mobile Phase: 0.15M ammonium acetate, pH 6.0
 Flow Rate: 0.75mL/min
 Det.: UV, 280nm
 Inj.: IGF-1, ZZ fusion protein and uncleaved material

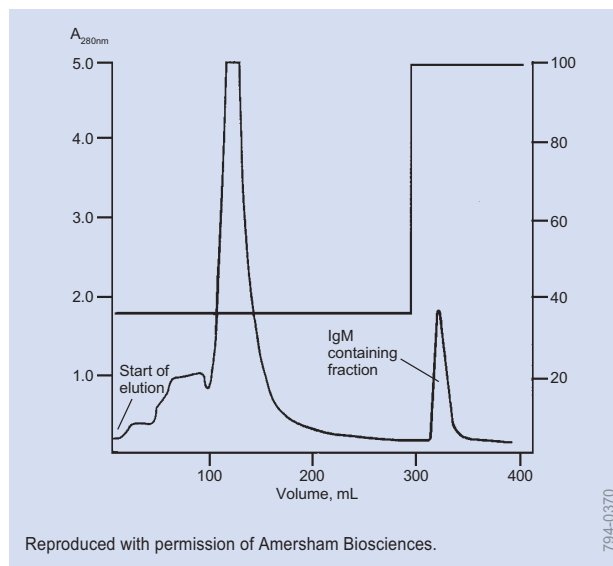


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796-0403

Immunoglobulins, IgG, Human (HPLC)

Column: HiTrap Desalting, 5mL
 Cat. No.: 54822
 Mobile Phase: 0.2M sodium carbonate buffer, pH 8.5
 Flow Rate: syringe use
 Det.: UV, 280nm
 Inj.: 0.5mL unbound human IgG in 0.2M sodium carbonate buffer after coupling to a HiTrap NHS 1mL column. Fraction volume 0.5mL

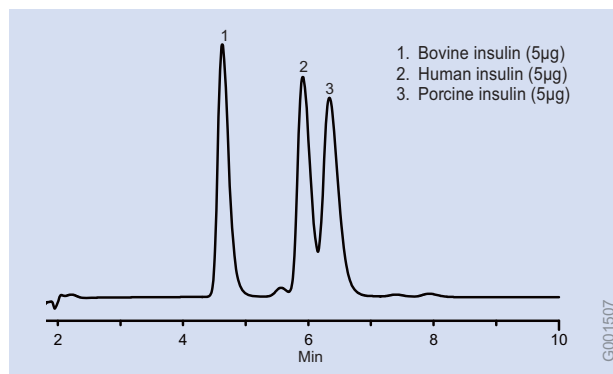


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794-0370

Immunoglobulins, IgM (HPLC)

Medium: Q Sepharose Fast Flow
 Cat. No.: Q1126-100ML
 Column: 30cm x 50mm ID
 Mobile Phase: A = 0.02M NaH₂PO₄, pH 6.5
 B = A + 0.5M NaCl, pH 6.5
 Flow Rate: 25cm/hr
 Det.: UV, 280nm
 Inj.: culture supernatant



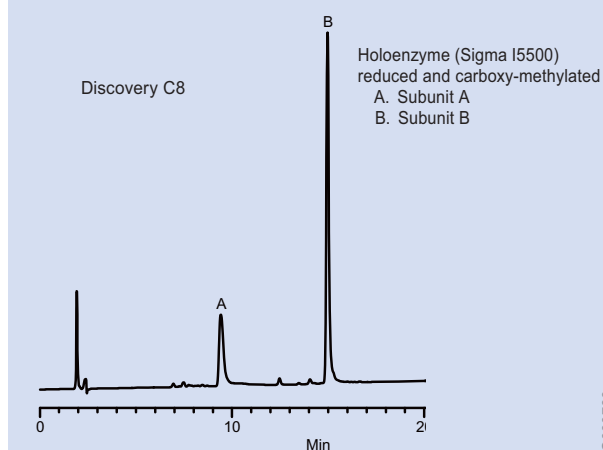
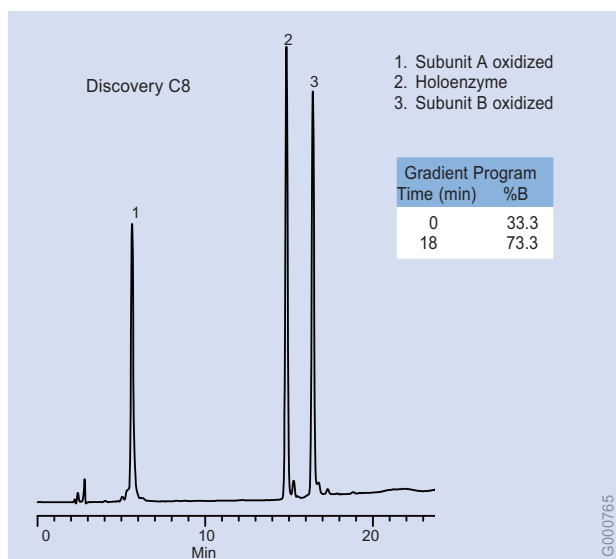
Insulin (HPLC)

Column: Discovery BIO Wide Pore C8, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 568322-U
 Mobile Phase: (A) 71:29, (0.1% TFA in H₂O):(0.1% TFA in CH₃CN);
 (B) 68:32, (0.1% TFA in H₂O):(0.1% TFA in CH₃CN)
 Flow Rate: 1mL/min
 Temp: 30°C
 Det.: UV, 215nm
 Inj.: 50 μ L in 0.1% TFA
 Gradient: 0-100% B in 30min

G001507

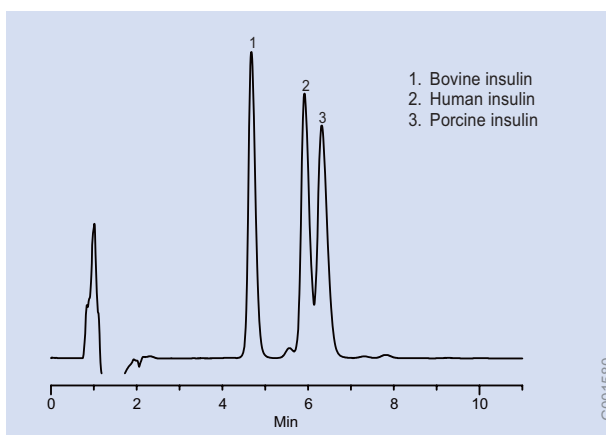
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HPLC Applications Peptides and Proteins



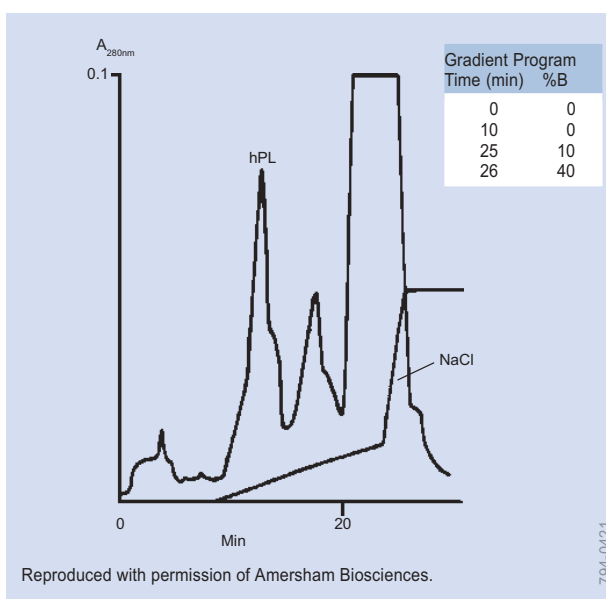
Insulin, Bovine (HPLC)

Columns: Discovery C8, 5cm x 4.6mm ID, 5µm particles
Cat. Nos.: 59353-U
Mobile Phase: 25mM KH_2PO_4 , pH 3:acetonitrile, 60:40
Mobile Phase: A = 50mM H_2NaPO_4 , 50mM Na_2SO_4 , pH 3.0 (w/ H_3PO_4), 5% acetonitrile
B = 50mM H_2NaPO_4 , 50mM Na_2SO_4 , pH 3.0 (w/ H_3PO_4), 50% acetonitrile
Flow Rate: 1mL/min
Temp: 35°C
Det.: UV, 220nm



Insulin (HPLC)

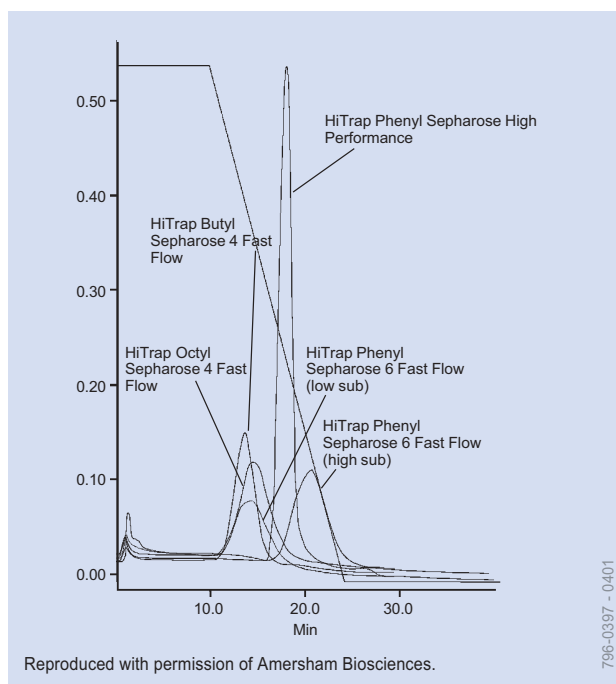
Column: Discovery BIO Wide Pore C5, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 568422-U
Mobile Phase: (A) 71:29, (0.1% TFA in water):(0.1% TFA in CH_3CN);
(B) 68:32, (0.1% TFA in water):(0.1% TFA in CH_3CN)
Flow Rate: 1.0mL/min
Temp: ambient
Det.: UV, 215nm
Inj.: 5µL each at 1mg/mL in 0.1%TFA
Gradient: 0-100%B in 30 min



Lactogen, Human Placental (HPLC)

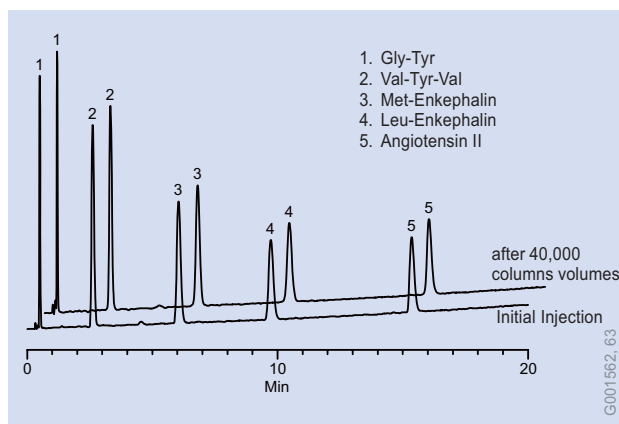
Column: Mono Q HR5/5, 10µm particles
Cat. No.: 54807
Mobile Phase: A = 20mM bis-Tris, pH 6.0
B = A + 1M NaCl
Flow Rate: 1.0mL/min
Det.: UV, 280nm
Inj.: 2.5mL hPL fraction from size exclusion medium

HPLC Applications Peptides and Proteins



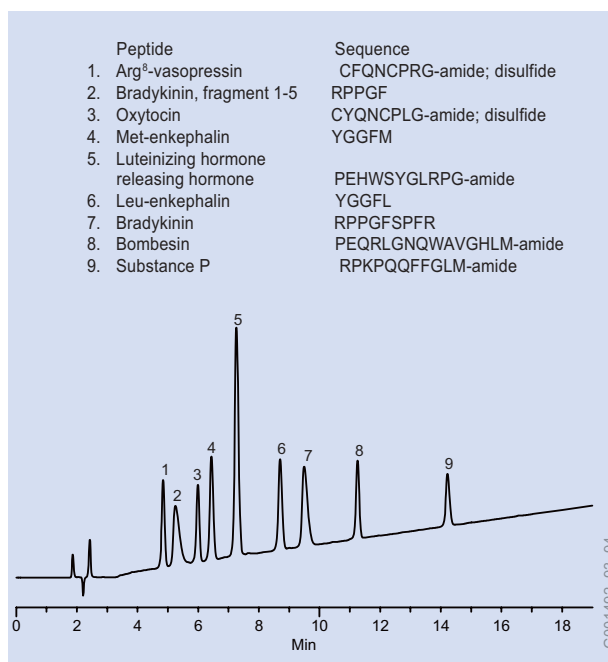
Monoclonal Antibody (HPLC)

Column: HiTrap HIC cartridges
 Cat. No.: 54814
 Mobile Phase: A = 50mM sodium phosphate, 1.0M ammonium phosphate, pH 7.0
 B = 50mM sodium phosphate, pH 7.0
 Flow Rate: 1.0mL/min
 Det.: UV, 280nm
 Inj.: 100 μ L anti-lac cl 507, 8.8mg/mL



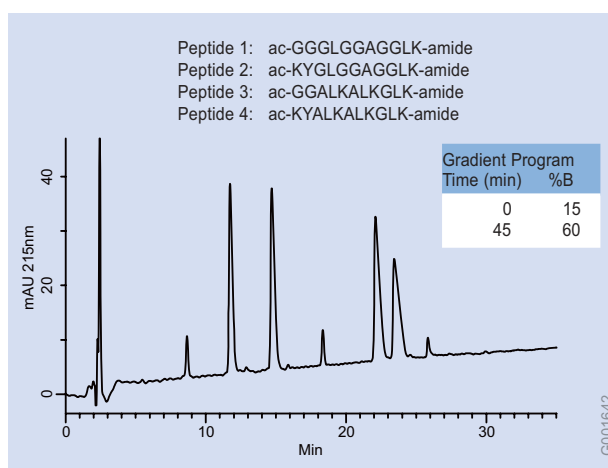
Peptides (HPLC)

Column: Discovery BIO Wide Pore C18, 5cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 568220-U
 Mobile Phase: (A) 5:95, (0.5% TFA in water):(0.5% TFA in CH₃CN);
 (B) 25:75, (0.5% TFA in water):(0.5% TFA in CH₃CN)
 Flow Rate: 2.0mL/min
 Temp: 70°C
 Det.: UV, 220nm
 Inj.: 5 μ L, 2.5 μ g each peptide (Sigma Peptide Mix, Cat. No. H 2016) in mobile phase A
 Gradient: 2-24%B in 22 min, 8 min at 100%A



Peptides (HPLC)

Column: Discovery BIO Wide Pore C5, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 568422-U
 Mobile Phase: (A) 0.1% PFPA (pentafluoropropionic acid) in water:CH₃CN (81:19);
 (B) 0.1% PFPA in water:CH₃CN (62:38)
 Flow Rate: 1.0mL/min
 Temp: 30°C
 Det.: UV, 215nm
 Inj.: 10 μ L, ~0.25 μ g each peptide (Sigma Peptide Mix, Cat. No. P 2693) in mobile phase A
 Gradient: 0-100%B in 19 min

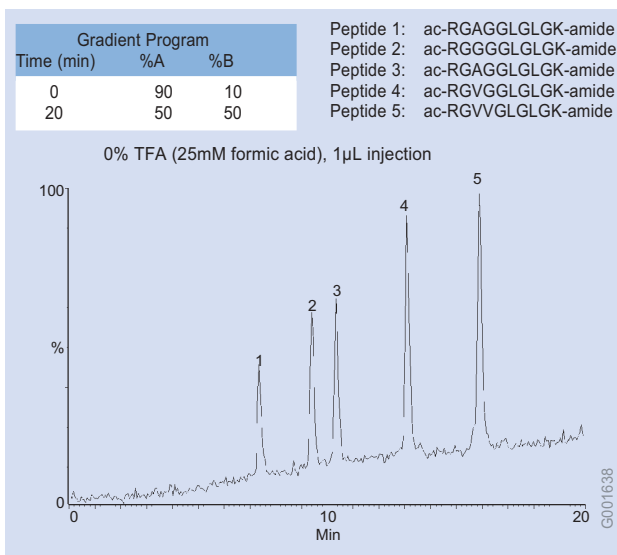


Peptides, Basic, No TFA (HPLC)

Columns: Discovery BIO Wide Pore C18, 15cm x 2.1mm ID, 5 μ m particles
 Cat. No.: 568202-U
 Mobile Phase A: 25mM formic acid in water
 Mobile Phase B: 50:50 (25mM formic acid in water) : (20mM formic acid in CH₃CN)
 Flow Rate: 0.208mL/min
 Det.: UV, 215nm
 Temp.: 35°C
 Inj.: 0.5 μ L (~0.25 μ g ea peptide)
 Sample: RP Peptide Ionic Interactions Standard, p/n RPS-I0020 (Alberta Peptide Institute)

HPLC Applications

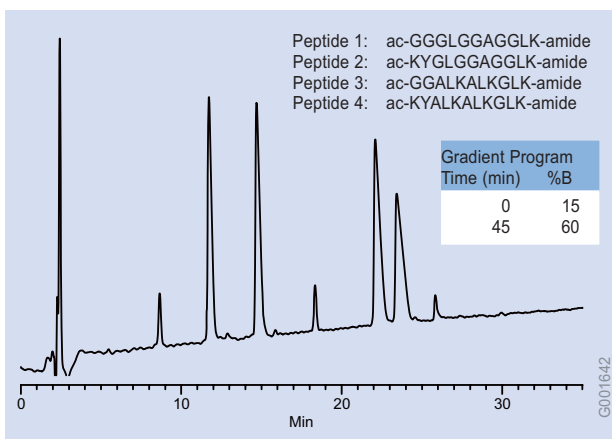
Peptides and Proteins



Peptides, Alberta Mix, No TFA (HPLC)

Column: Discovery BIO Wide Pore C18, 15cm x 2.1mm ID, 3 μ m particles
 Cat. No.: 567202-U
 Mobile Phase A: 25mM formic acid in water,
 Mobile Phase B: 50:50 (25mM formic acid in water) : (20mM formic acid in CH₃CN)^a,
 Flow Rate: 0.208mL/min^b
 Det.: +ES
 Temp.: ambient
 Inj.: 1 μ L
 Sample: RP Peptide Performance Standard, p/n RPS-P0010 (Alberta Peptide Institute)

- a) molarity of formic acid adjusted to provide minimum baseline drift
 b) linear velocity equal to 1mL/min on 4.6mm ID columns



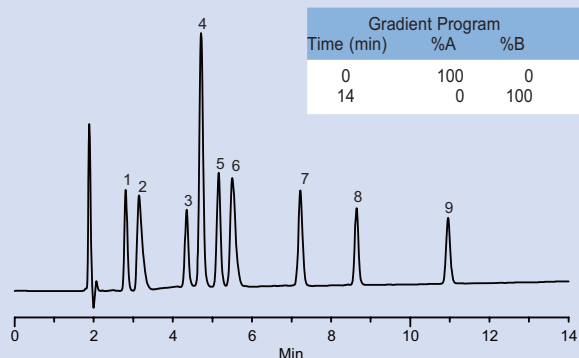
Peptides, Synthetic (HPLC)

Columns: Discovery BIO Wide Pore C18, 15cm x 2.1mm ID, 5 μ m particles
 Cat. No.: 568202-U
 Mobile Phase A: 25mM formic acid in water
 Mobile Phase B: 50:50 (25mM formic acid in water):(20mM formic acid in CH₃CN)
 Flow Rate: 0.208 mL/min
 Det.: UV, 215nm
 Temp.: 35°C
 Inj.: 0.5 μ L (~0.25 μ g ea peptide)
 Sample: RP Peptide Ionic Interactions Standard, p/n RPS-I0020 (Alberta Peptide Institute)

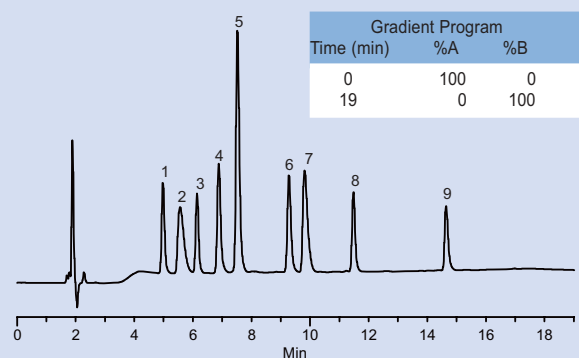
TFA mobile phase:

Peak	Peptide	Amino Acid Sequence
1.	Arg ⁸ -vassopressin	CYFQNCPRG-amide; disulfide
2.	Bradykinin, fragment 1-5	RPPGF
3.	Oxytocin	CYIQNCPLG-amide; disulfide
4.	LHRH*	pEHWSYGLRPG-amide **
5.	Met-enkephalin	YGGFM
6.	Bradykinin	RPPGFSPFR
7.	Leu-enkephalin	YGGFL
8.	Bombesin	pEQRLGNQWAVGHLM-amide **
9.	Substance P	RPKPQQFFGLM-amide

* Luteinizing Hormone Releasing Hormone
 ** pE is pyroglutamate



PFFA (penta-fluoropropionic acid) mobile phase:

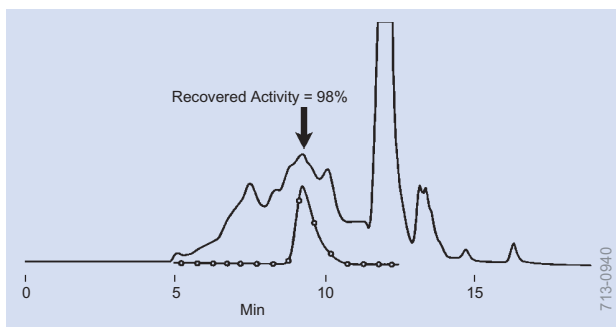


Peptides, Synthetic (HPLC)

TFA:
 Column: Discovery BIO Wide Pore C18, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 568222-U
 Mobile Phase A: 80:20, (water/0.1%TFA) : (CH₃CN/0.1%TFA)
 Mobile Phase B: 66:34, (water/0.1%TFA) : (CH₃CN/0.1%TFA)
 Flow Rate: 1.0mL/min;
 Det.: UV, 215nm
 Temp.: 30°C
 Inj.: 10 μ L Sigma Peptide Mix P2693 (~25 μ g ea. Peptide)

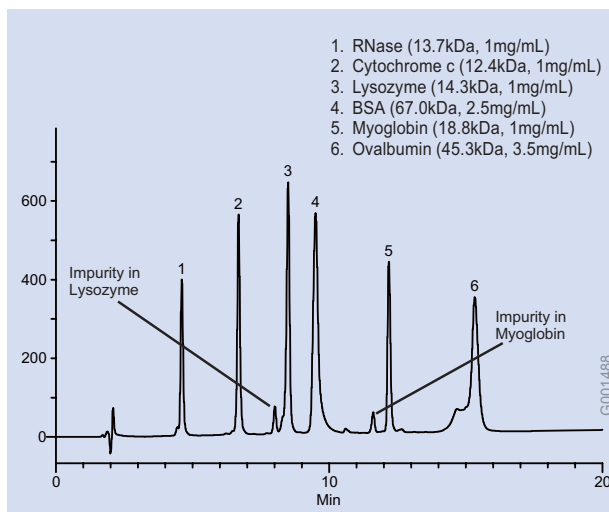
PFFA:
 Column: Discovery BIO Wide Pore C18, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 568222-U
 Mobile Phase A: 81:19, (water/0.1%PFFA) : (CH₃CN/0.1%PFFA)
 Mobile Phase B: 62:38, (water/0.1%PFFA) : (CH₃CN/0.1%PFFA)
 Flow Rate: 1.0mL/min
 Det.: UV, 215nm
 Temp.: 30°C
 Inj.: 10 μ L Sigma Peptide Mix P2693 (~25 μ g ea. Peptide)

HPLC Applications Peptides and Proteins



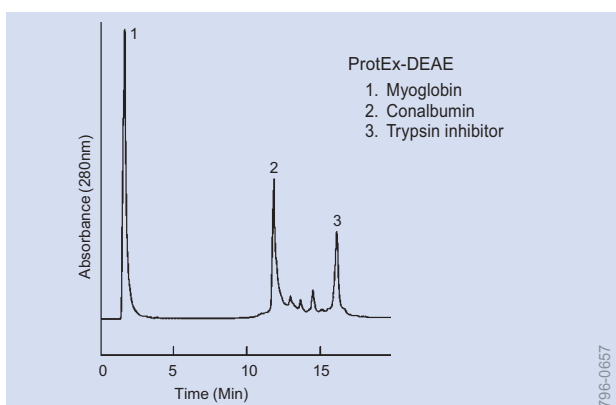
Peroxidase (HPLC)

Column: TSKgel G3000SW_{XL}, 30cm x 7.8mm ID, 5 μ m particles
 Cat. No.: 808541
 Mobile Phase: 0.3M NaCl/0.05M phosphate buffer, pH 7.0
 Flow Rate: 1mL/min
 Temp.: 25°C
 Det.: UV, 220nm
 Inj.: 100 μ L crude peroxidase preparation from Japanese horseradish (0.15mg)

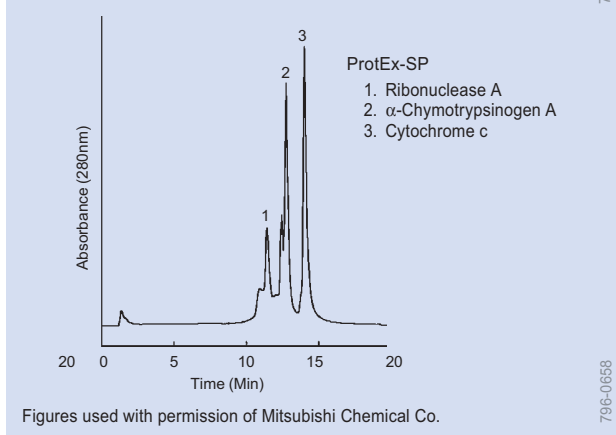


Proteins (HPLC)

Columns: Discovery BIO Wide Pore C5, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 568422-U
 Mobile Phase: 75:25, (0.1% TFA in water):(0.1% TFA in CH₃CN);
 Flow Rate: 1.0mL/min
 Temp: ambient
 Det.: UV, 220nm
 Inj.: 12 μ L in 0.1%TFA
 Gradient: 0-100%B in 25 min



ProtEx-DEAE
 1. Myoglobin
 2. Conalbumin
 3. Trypsin inhibitor

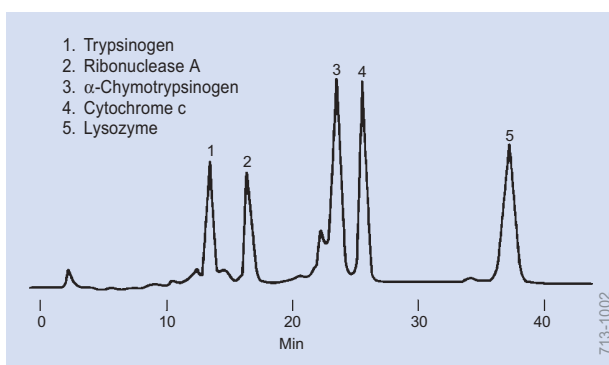


ProtEx-SP
 1. Ribonuclease A
 2. α -Chymotrypsinogen A
 3. Cytochrome c

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Proteins (HPLC)

Column: ProtEx-DEAE or ProtEx-SP, 5cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 54742 (ProtEx-DEAE) or 54740-U (ProtEx-SP)
 Mobile Phase: Left: A = 20mM Tris-HCl, pH 8.0, B = A + 0.5M NaCl
 5-100% B over 15 min
 Right: A = 20mM sodium phosphate, pH 7.0,
 B = A + 0.5M NaCl
 5-100% B over 15 min
 Flow Rate: 0.5mL/min
 Det.: UV, 280nm
 Inj.: 10 μ g trypsin inhibitor, 5 μ g conalbumin, myoglobin or
 10 μ g ribonuclease A, 5 μ g cytochrome c, α -chymotrypsinogen A



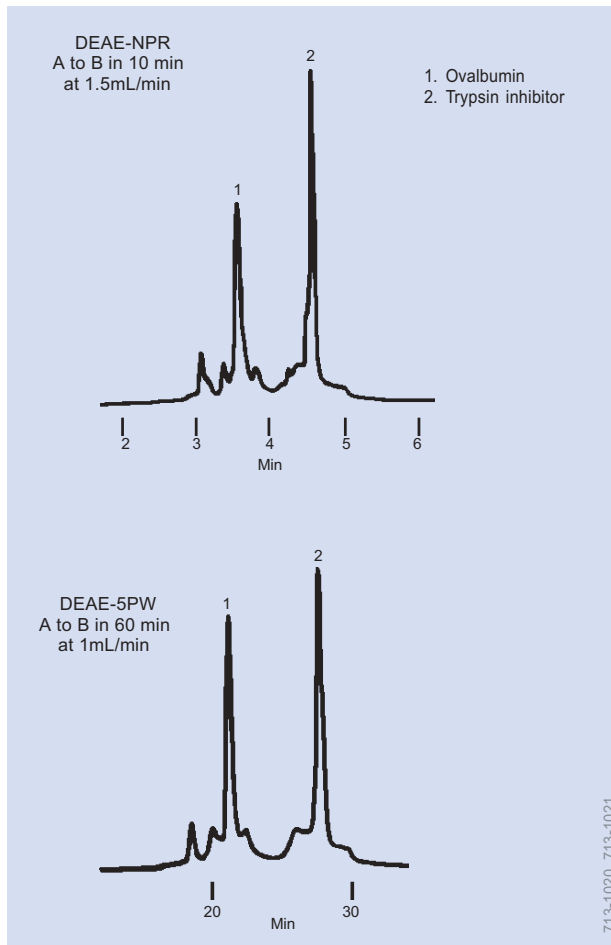
1. Trypsinogen
 2. Ribonuclease A
 3. α -Chymotrypsinogen
 4. Cytochrome c
 5. Lysozyme

Proteins (HPLC)

Column: TSKgel CM-5PW, 7.5cm x 7.5mm ID, 10 μ m particles
 Cat. No.: 813068
 Mobile Phase: A = 0.1M NaH₂PO₄ buffer, pH 7.0
 B = 0.5M NaCl, A to B in 60 min
 Flow Rate: 1.0mL/min
 Det.: UV, 280nm

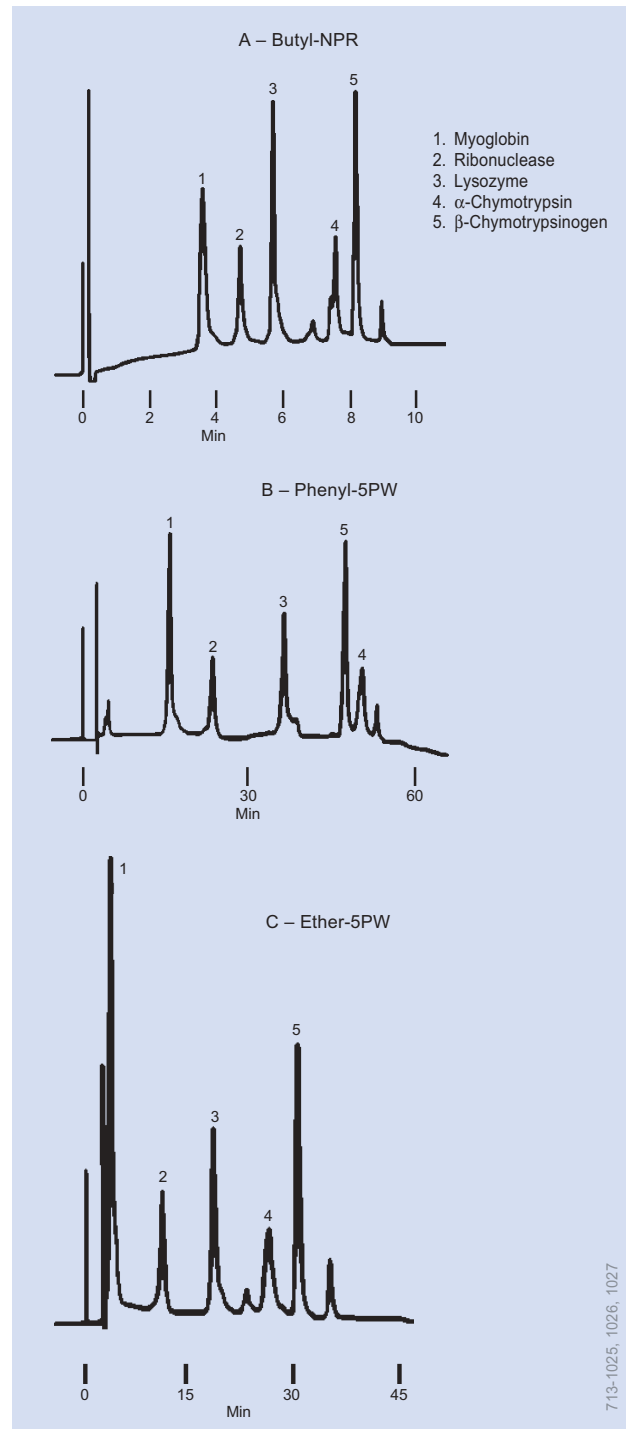
HPLC Applications

Peptides and Proteins



Proteins (HPLC)

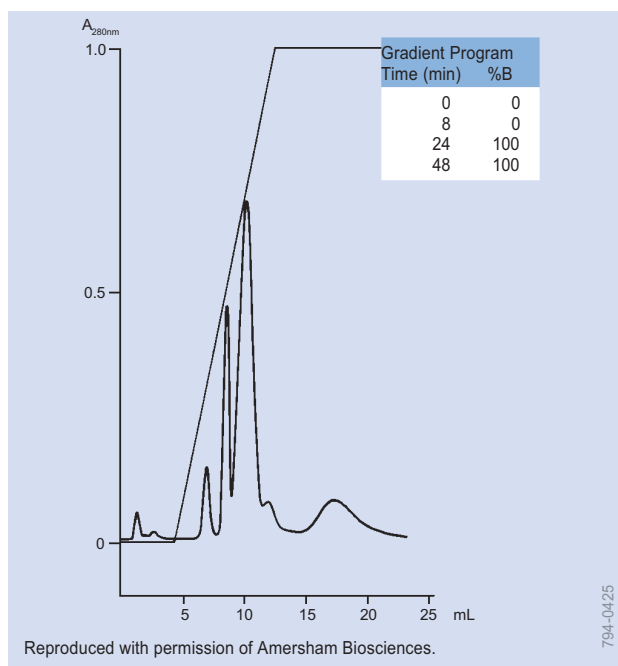
Column A: TSKgel DEAE-NPR, 3.5cm x 4.6mm ID, 2.5 μ m particles
Cat. No.: 813075
Column B: TSKgel DEAE-5PW, 7.5cm x 7.5mm ID, 10 μ m particles
Cat. No.: 807164
Mobile Phase: A = 20mM Tris-HCl buffer, pH 8.0
B = 0.5M NaCl in A
Det.: UV, 280nm



Proteins (HPLC)

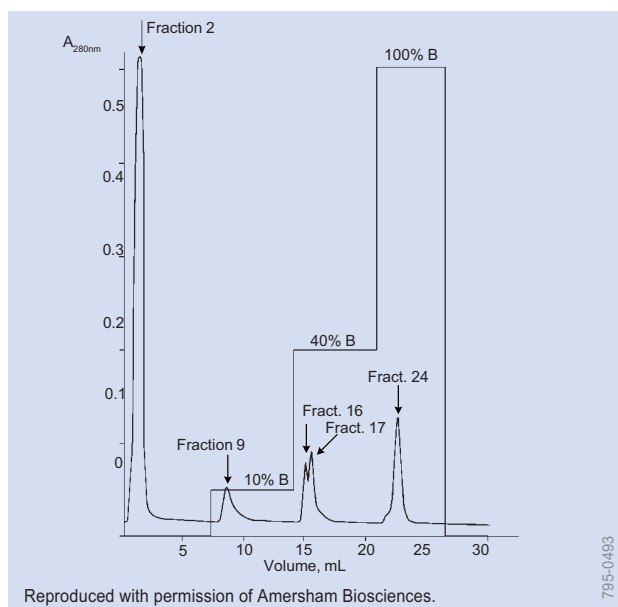
Column A: TSKgel Butyl-NPR, 3.5cm x 4.6mm ID, 2.5 μ m particles
Cat. No.: 814947
Column B: TSKgel Phenyl-5PW, 7.5cm x 7.5mm ID, 10 μ m particles
Cat. No.: 807573
Column C: TSKgel Ether-5PW, 7.5cm x 7.5mm ID, 10 μ m particles
Cat. No.: 808641
Mobile Phase: A = 0.1M phosphate buffer, pH 7.0 plus $(\text{NH}_4)_2\text{SO}_4$, 2.3M to 0M in 12 min
B & C = 0.1M phosphate buffer, pH 7.0 plus $(\text{NH}_4)_2\text{SO}_4$, 1.8M to 0M in 60 min
Flow Rate: 1mL/min
Det.: UV, 280nm

HPLC Applications Peptides and Proteins



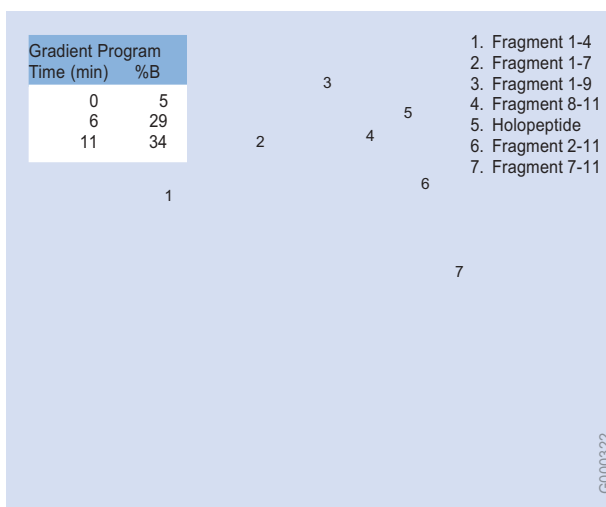
Proteins, Egg White (HPLC)

Column: HiTrap Chelating, 1mL
 Cat. No.: 54834
 Mobile Phase: A = 0.2M NaH₂PO₄, 1M NaCl, pH 7.2
 B = 0.02M NaH₂PO₄, 1M NH₄Cl, pH 7.2
 Flow Rate: 0.5mL/min
 Temp.: 40°C
 Det.: UV, 280nm
 Inj.: 200µL egg white



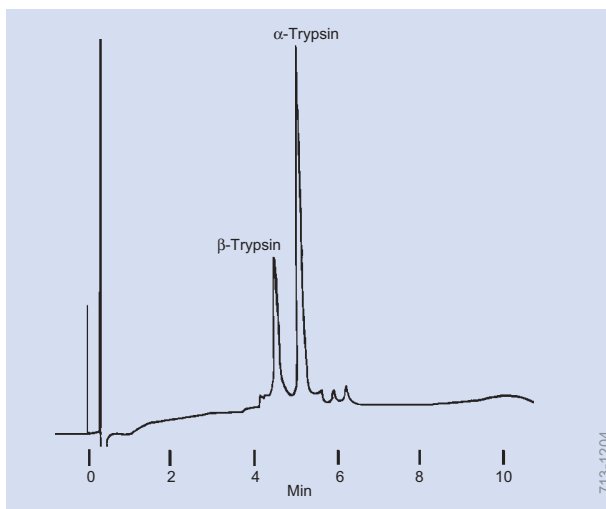
Proteins, Milk (HPLC)

Column: HiTrap SP, 1mL
 Cat. No.: 54817
 Mobile Phase: A = 50mM Na acetate, pH 6.0
 B = 50mM Na acetate/1.0M NaCl, pH 6.0
 Flow Rate: 1mL/min
 Det.: UV, 280nm
 Inj.: 0.5mL filtered casein-precipitated human milk (buffer exchanged to mobile phase A)



Substance P and Its Fragments (HPLC)

Column: Discovery C18, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 504955
 Mobile Phase: A = water + 0.1% TFA
 B = acetonitrile + 0.1% TFA
 Flow Rate: 1mL/min
 Temp.: 35°C
 Det.: UV, 220nm
 Inj.: Substance P in initial mobile phase

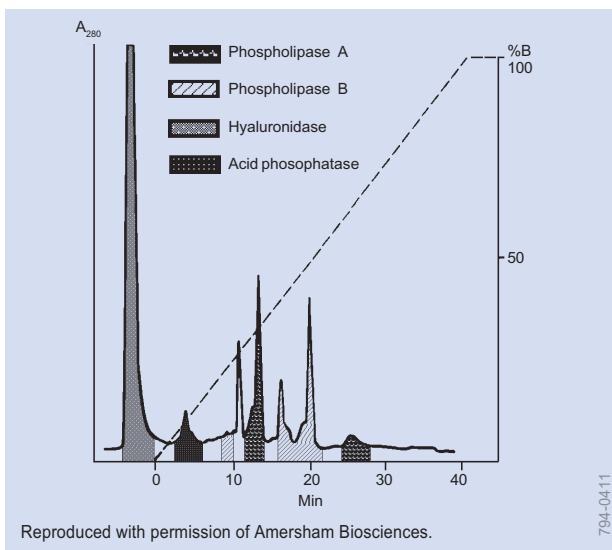


Trypsin (HPLC)

Column: TSKgel Butyl-NPR, 3.5cm x 4.6mm ID, 2.5µm particles
 Cat. No.: 814947
 Mobile Phase: 2M to 0M ammonium sulfate in 20mM Tris-HCl buffer, pH 7.5 in 10 min
 Flow Rate: 1.0mL/min
 Det.: UV, 280nm
 Inj.: commercial trypsin sample

HPLC Applications

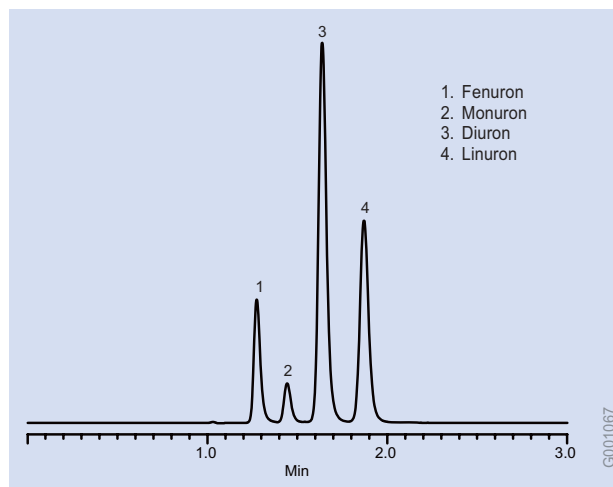
Peptides, Proteins, Pesticides



794-0411

Venom, White-Faced Hornet (HPLC)

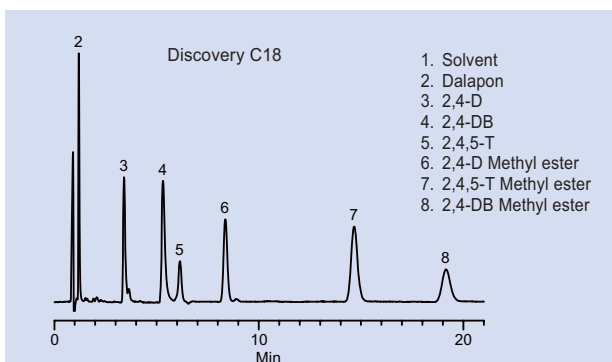
Column: Mono S HR5/5, 10µm particles
 Cat. No.: 54808
 Mobile Phase: A = 50mM BICINE, pH 8.4
 B = A + 0.35M NaCl
 Flow Rate: 1.0mL/min
 Det.: UV, 280nm
 Inj.: 7mg venom in mobile phase A



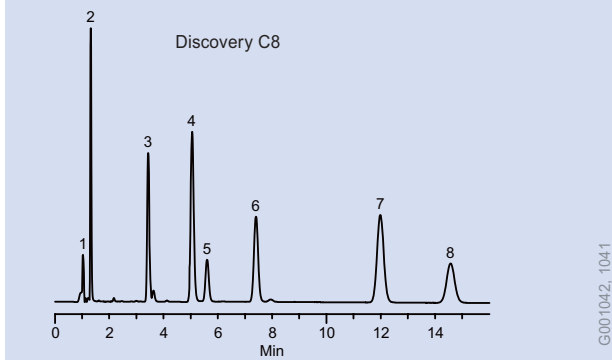
GC001067

Urea Pesticides (HPLC)

Column: Discovery Cyano, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 59356-U
 Mobile Phase: 60:40 water:CH₃CN
 Flow Rate: 2.0mL/min
 Temp.: 20°C
 Det.: UV, 214nm
 Inj.: 1µL

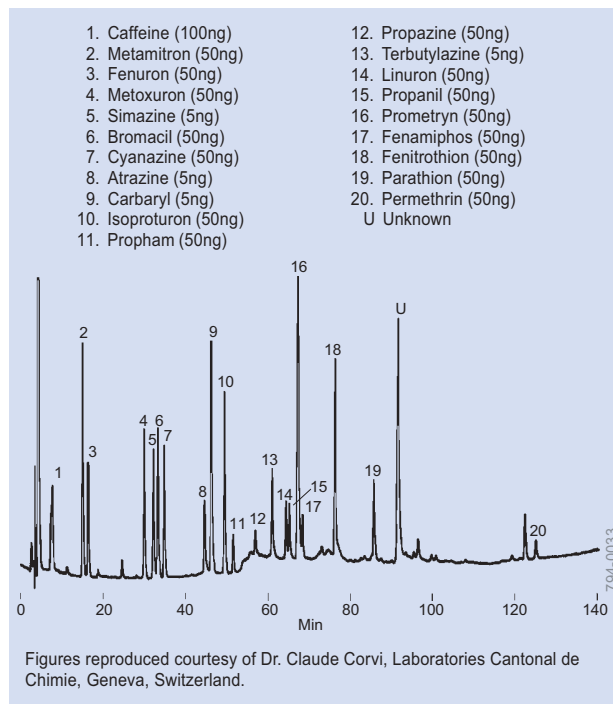


GC001042, 1041



Organo-Acid Pesticides (HPLC)

Columns: 15cm x 4.6mm ID, 5µm particles
 Cat. Nos.: 504955 (C18), 59353-U (C8)
 Mobile Phase: 60:40 25mM potassium phosphate, (pH 2.3):CH₃CN
 Flow Rate: 2.0mL/min
 Det.: UV, 214nm
 Temp.: 20°C
 Inj.: 1µL



794-0033

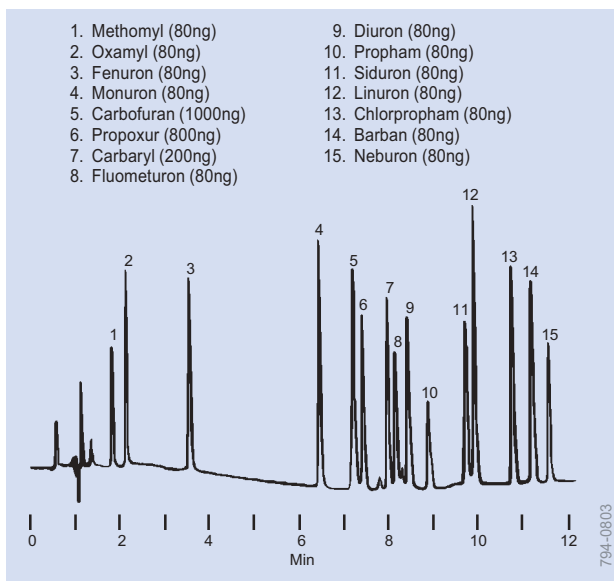
Figures reproduced courtesy of Dr. Claude Corvi, Laboratories Cantonal de Chimie, Geneva, Switzerland.

Pesticides and Herbicides (HPLC)

Column: SUPELCOSIL LC-ABZ, 25cm x 4.6mm ID, 5µm particles
 Cat. No.: 59142
 Mobile Phase: 10% to 90% acetonitrile in water at 0.5%/min
 Flow Rate: 1mL/min
 Temp.: 40°C
 Det.: UV, 225nm
 Inj.: 50µL water, spiked with analytes

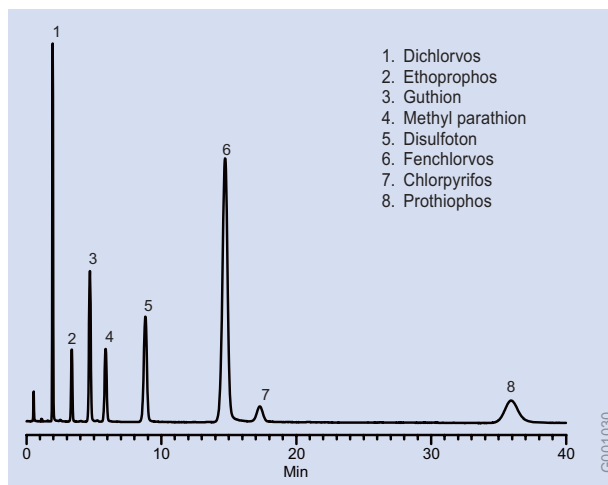
Order: 1.800.325.3010 Technical Service: 1.800.359.3041 Web: www.sigma-aldrich.com/supelco

HPLC Applications Pesticides



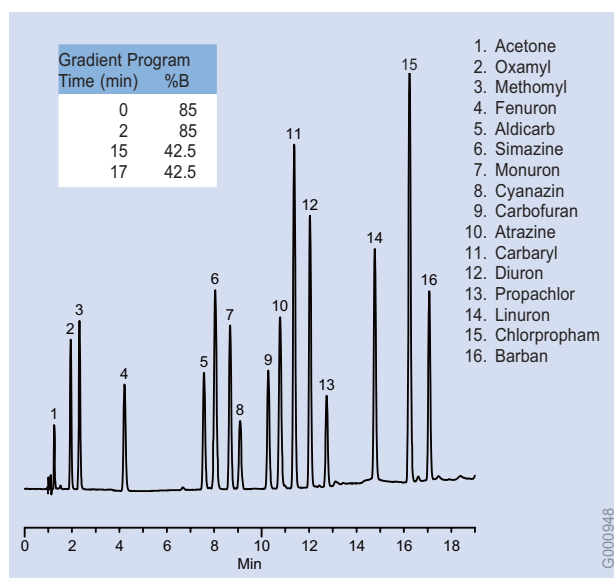
Carbamate and Urea Pesticides (HPLC)

Column: SUPELCO SIL LC-8, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 58220-U
 Mobile Phase: acetonitrile:water gradient, 18:82 to 65:35 in 9 min, hold 3 min
 Flow Rate: 2.0mL/min
 Temp.: 35°C
 Det.: UV, 240nm
 Inj.: 10µL



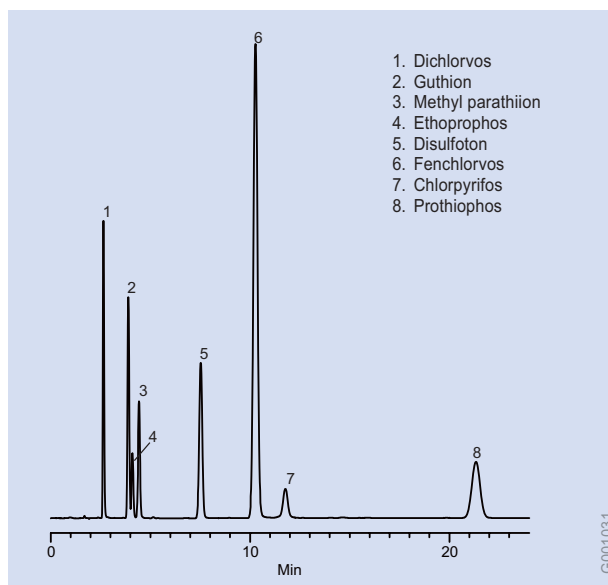
Organophosphorus Pesticides (HPLC)

Column: Discovery Cyano, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 59356-U
 Mobile Phase: 75:25 water:CH₃CN
 Flow Rate: 2.0mL/min
 Temp.: 20°C
 Det.: UV, 214nm
 Inj.: 1µL



Urea/Carbamate/Triazine/Azine Pesticides (HPLC)

Column: Discovery C18, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 504955
 Mobile Phase: A = acetonitrile
 B = water
 Flow Rate: 2mL/min
 Temp.: 20°C
 Det.: UV, 214nm
 Inj.: 1µL

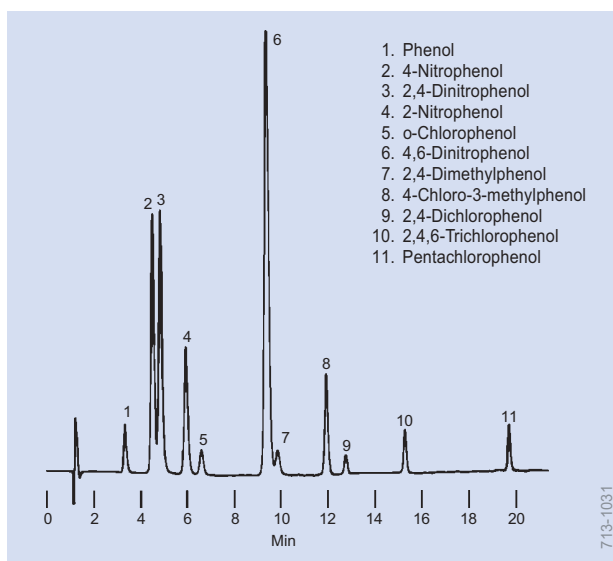


Organophosphorus Pesticides (HPLC)

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 505013
 Mobile Phase: acetonitrile:water, 60:40
 Flow Rate: 1mL/min
 Temp.: 20°C
 Det.: UV, 214nm
 Inj.: 1µL

HPLC Applications

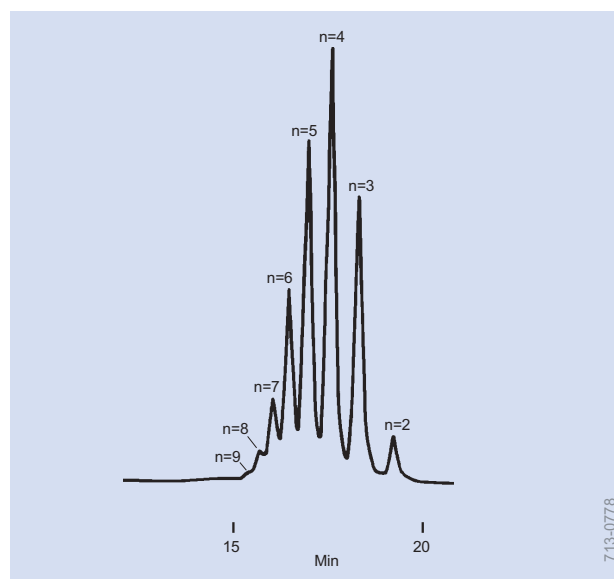
Phenols, Polymers, Oligomers



713-1031

Phenols (HPLC)

Column: SUPELCO SIL LC-8, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 58220-U
 Mobile Phase: 1% acetic acid/methanol:1% acetic acid/water, 35:65 to 100:0
 (20 min), return to 35:65 (5 min)
 Flow Rate: 1.5mL/min
 Temp.: 30°C
 Det.: UV, 280nm

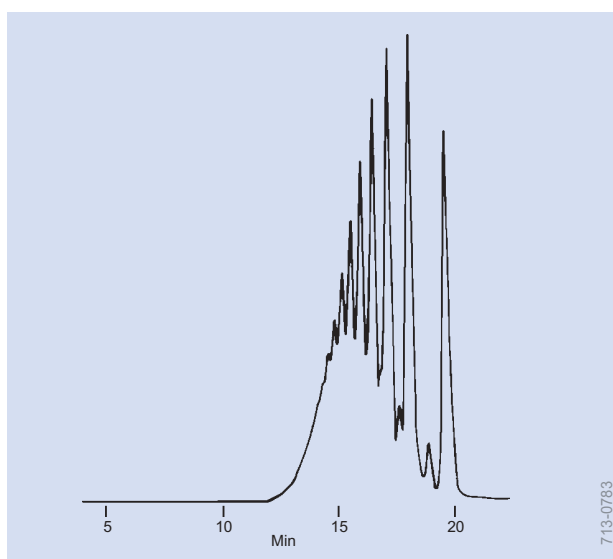


713-0778

Polyethylene Glycol 200 (HPLC)

Column: TSKgel G-OLIGO-PW, 30cm x 7.8mm ID, 6µm particles*
 Cat. No.: 808031
 Mobile Phase: distilled water
 Flow Rate: 1mL/min
 Det.: refractive index

*Two columns in series.

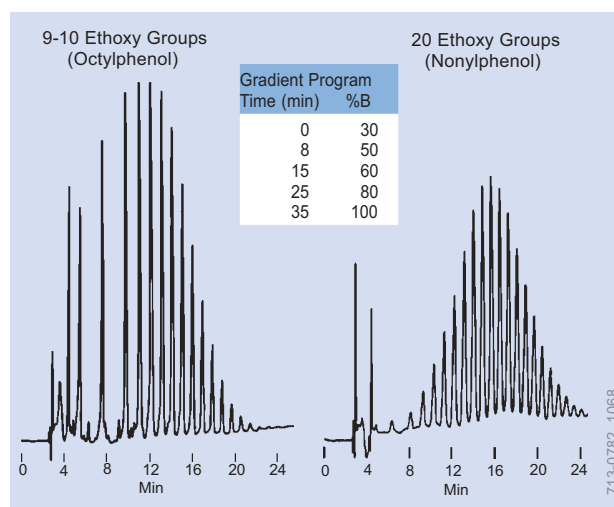


713-0783

Epoxy Resin, Epikote 1001 (HPLC)

Column: TSKgel G3000H_{XL}, 30cm x 7.8mm ID, 6µm particles*
 Cat. No.: 816136
 Mobile Phase: tetrahydrofuran
 Flow Rate: 1mL/min
 Det.: UV, 254nm

*Two columns in series.



713-0782, 1068

Nonionic Surfactants (HPLC)

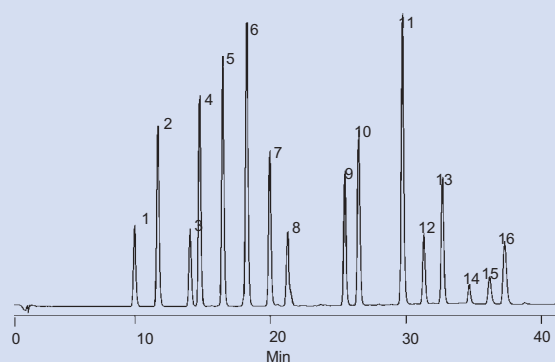
Column: SUPELCO SIL LC-Diol, 25cm x 4.6mm ID, 5µm particles
 Cat. No.: 58201
 Mobile Phase: A = hexane:methylene chloride (95:5)
 B = hexane:methylene chloride:methanol (50:40:10)
 Flow Rate: 1mL/min
 Temp.: 35°C
 Det.: UV, 280nm
 Inj.: 10µL methylene chloride containing 20µg surfactant

HPLC Applications Polynuclear Aromatic Hydrocarbons

1. Naphthalene 1000ng/mL
2. Acenaphthylene 2000ng/mL
3. Acenaphthene 1000ng/mL
4. Fluorene 200ng/mL
5. Phenanthrene 100ng/mL
6. Anthracene 100ng/mL
7. Fluoranthene 200ng/mL
8. Pyrene 100ng/mL
9. Benzo(a)anthracene 100ng/mL
10. Chrysene 100ng/mL
11. Benzo(b)fluoranthene 200ng/mL
12. Benzo(k)fluoranthene 100ng/mL
13. Benzo(a)pyrene 100ng/mL
14. Dibenzo(a,h)anthracene 200ng/mL
15. Benzo(ghi)perylene 200ng/mL
16. Indeno(1,2,3-cd)pyrene 100ng/mL

Gradient Program	
Time (min)	% ACN
0	50
5	50
30	100
45	100

Flow increased at 2.0 min



796-0086

PAHs in Water (SPME/HPLC)

Sample: 5µL PAH mix (Cat. No. 4-8743) in 5mL water
 SPME Fiber: polydimethylsiloxane, 100µm film
 Cat. No.: 57301
 Extraction: immersion, 30 min (rapid stirring)
 Desorption*: static, 200µL acetonitrile:water, 40:60, 2 min
 Column: SUPELCOSIL LC-PAH, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 58318
 Mobile Phase: acetonitrile:water gradient (see program)
 Flow Rate: 0-2 min: 0.2mL/min 2-45 min: 1.0mL/min
 Det.: UV, 254nm

*Requires SPME/HPLC interface.

1. 2-OH-DMBA(t-diol)
 2. 7-OHM-12-MBA-(t-10,11-diol)
 3. 7-M-12-OHMBA-(t-8,9-diol)
 4. 7-OHM-12-MBA-(t-8,9-diol)
 5. 7-OHM-(t-3,4-diol)
 6. Mixed diols, incl. DMBA-(t-8,9-diol & t-10,11-diol)
 7. Position of 7,12-bis-OHMBA
 8. 7-OHM-12-MBA-2-ol
 9. 7-OHM-12-MBA-(3-ol)
 10. 7-M-12-OHMNA-4-ol
 11. 7-OHM-12-MBA-4-ol
 12. 7-M-12-OHMBA-(3-ol)
 13. 7-M-12-OHMBA-2-ol
 14. 7-OHM-12-MBA
 15. 7-M-12-OHMBA
 16. DMBA-2-ol
 17. DMBA-3-ol
 18. DMBA-4-ol
 19. DMBA
- (parentheses = tentative identifications)

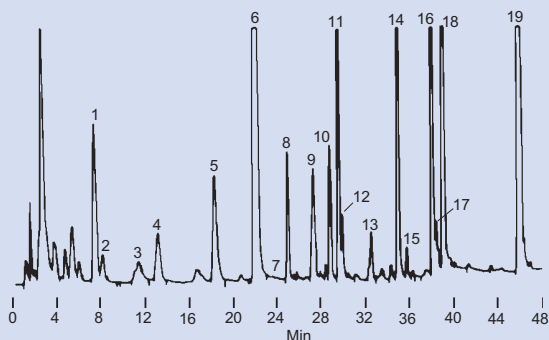


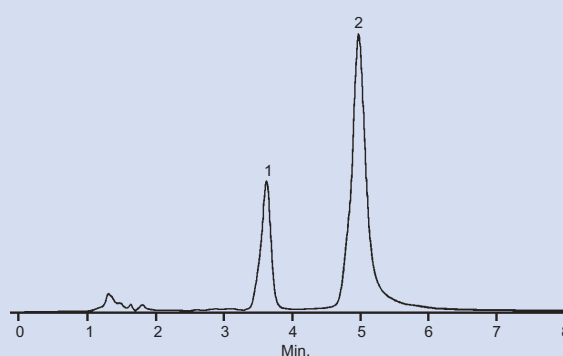
Figure provided by Drs. J. Milner and J. Grunau, University of Illinois, Urbana, Illinois, USA.

713-1043

Metabolites of 7,12-Dimethylbenzo(a)anthracene (HPLC)

Column: SUPELCOSIL LC-18, 25cm x 4.6mm ID, 5µm particles
 Cat. No.: 58298
 Mobile Phase: methanol:water, 50:50 (10 min) to 100:0 at 2.5% /min
 Flow Rate: 0.8mL/min
 Det.: fluorescence
 Inj.: rat liver, 9000 x g supernatant fraction from Aroclor-treated animals

1. 2,7-Dihydroxynaphthalene (300µg/mL)
2. 2,3-Dihydroxynaphthalene (1000µg/mL)



796-0042

Chelating Agent, DERT Test (HPLC)

Column: SUPELCOGEL TPR-100, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 59154
 Mobile Phase: ammonium acetate (25mM, pH 7):acetonitrile (55:45)
 Flow Rate: 1.0mL/min
 Temp: ambient
 Det.: UV, 254nm
 Inj.: 10µL

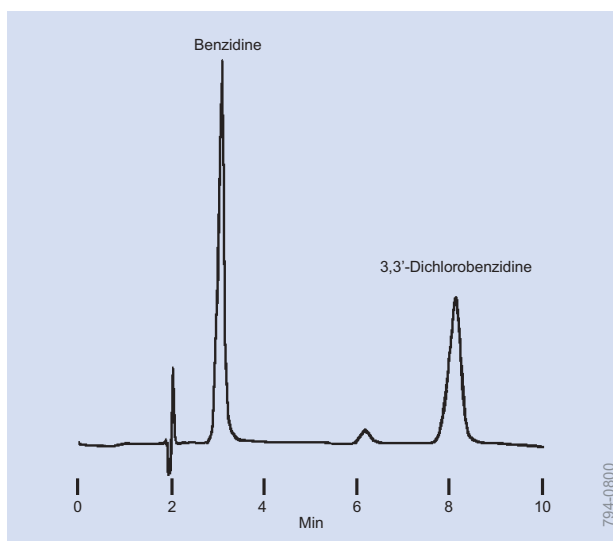
Order: 1.800.325.3010 Technical Service: 1.800.359.3041 Web: www.sigma-aldrich.com/supelco

Liquid
Chromatography

SUPELCO

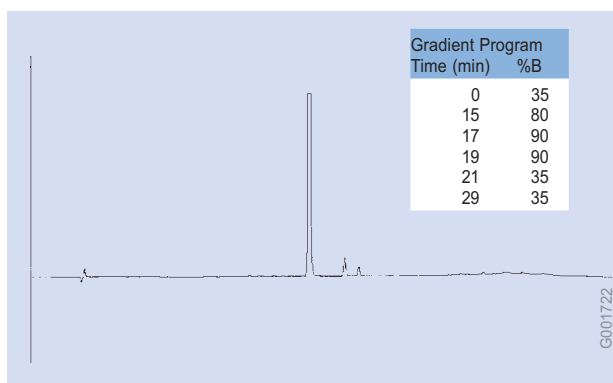
HPLC Applications

Semivolatiles, Toxins, Vitamins



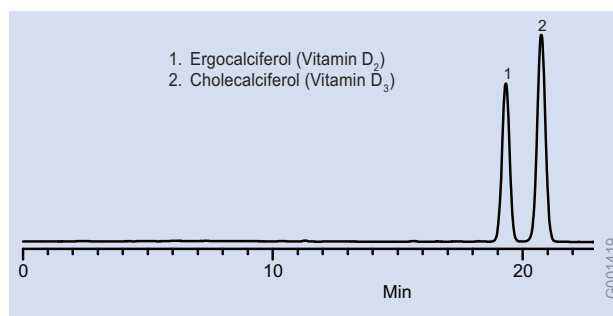
Benzidines by US EPA Method 605 (HPLC)

Column: SUPELCOSIL LC-1, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 58210-U
 Mobile Phase: 0.1M acetate buffer (pH 4.7):acetonitrile, 80:20
 Flow Rate: 1mL/min
 Det.: UV, 254nm
 Inj.: 10 μ L (0.5 μ g each analyte)



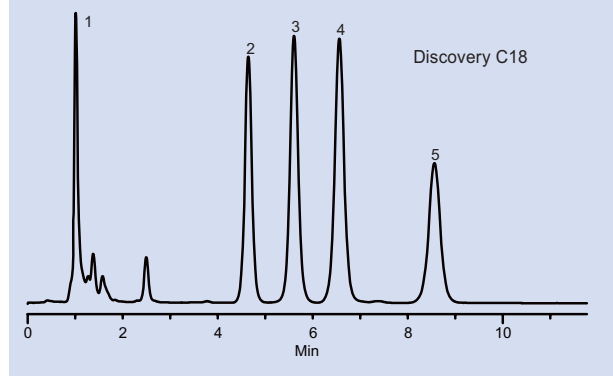
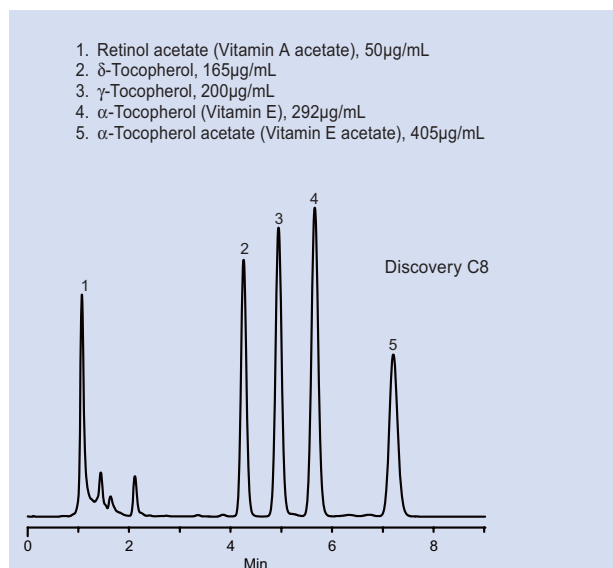
Bufalin Toxin (HPLC)

Column: Discovery BIO Wide Pore C18, 25cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 568223-U
 Mobile Phase: A = 0.1% TFA in water
 B = 0.1% TFA in acetonitrile:water (3:1)
 Flow Rate: 1.3mL/min
 Temp: ambient
 Det.: UV, 298nm
 Inj.: 10 μ L bufalin, 0.5mg/mL in acetonitrile



Vitamins, Fat Soluble (D₂ and D₃) (HPLC)

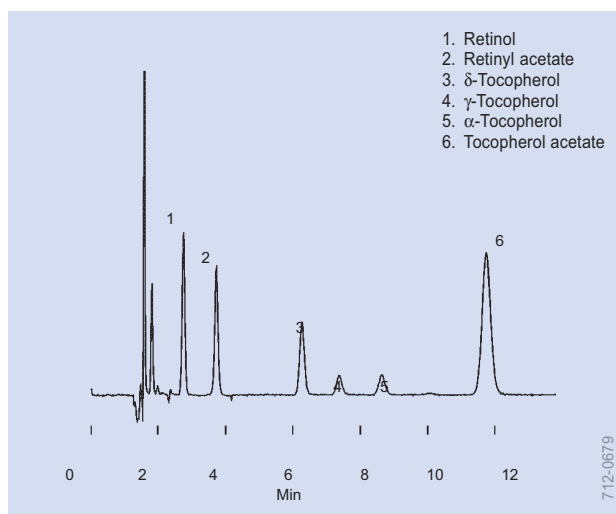
Column: Discovery HS C18, 15cm x 4.6mm ID, 3 μ m particles
 Cat. No.: 569252-U
 Mobile Phase: 100% CH₃CN
 Flow Rate: 0.8mL/min
 Temp: 30°C
 Det.: UV, 290nm
 Inj.: 10 μ L, 50 μ g/mL of each analyte



Vitamins, Fat Soluble (A and E) (HPLC)

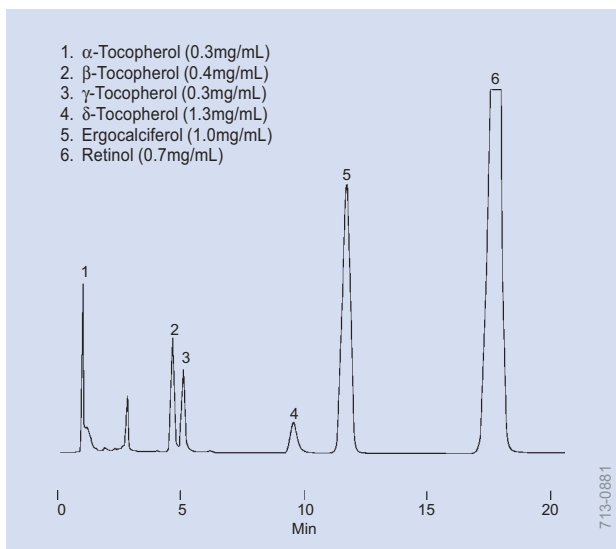
Column: 15cm x 4.6mm ID, 5 μ m particles
 Cat. Nos.: 59353-U (C8), 504955 (C18)
 Mobile Phase: Discovery C18, MeOH:H₂O (95:5),
 Discovery C8 CH₃CN:H₂O (90:10)
 Flow Rate: 2mL/min
 Temp: 30°C
 Det.: UV, 290nm
 Inj.: 10 μ L

HPLC Applications Vitamins



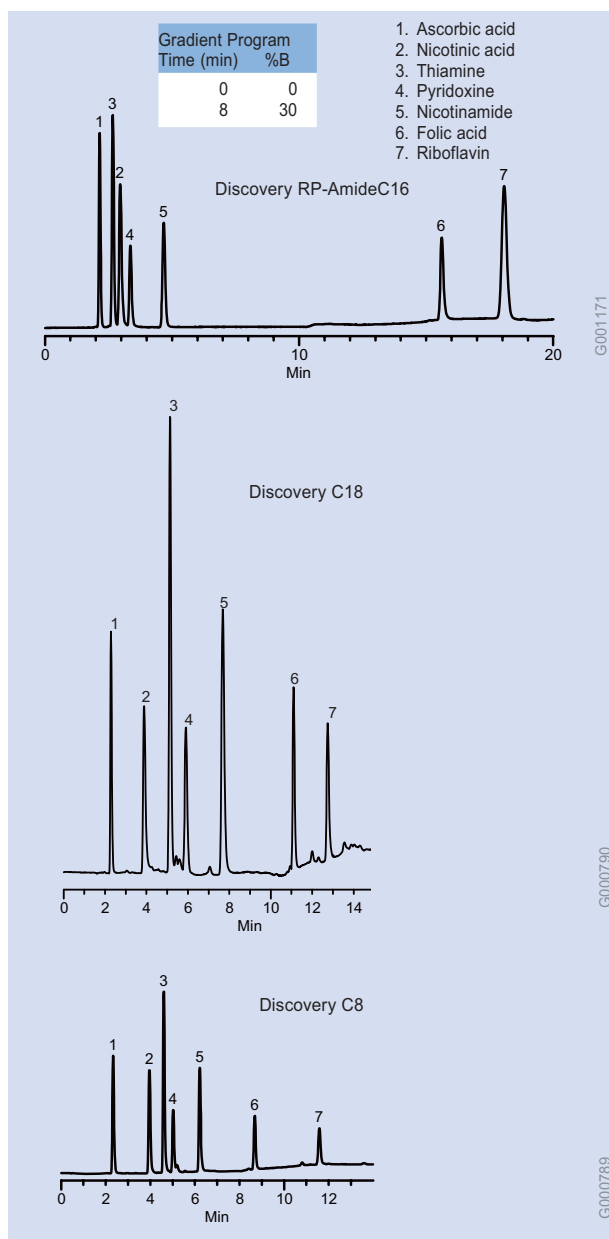
Vitamins, Fat Soluble (A and E) (HPLC)

Column: SUPELCO SİL C-18, 25cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 58298
 Mobile Phase: methanol:deionized water (98:2)
 Flow Rate: 2mL/min
 Det.: UV, 325nm (retinol, retinyl acetate) or 290nm (tocopherols, tocopherol acetate)
 Inj.: 20 μ L methanol (0.2-1mg/mL each analyte)



Vitamins, Fat Soluble (A and E) (HPLC)

Column: SUPELCO SİL LC-Si, 15cm x 4.6mm ID, 5 μ m particles
 Cat. No.: 58200-U
 Mobile Phase: hexane:amyl alcohol (99.65:0.35)
 Flow Rate: 2mL/min
 Det.: UV, 280nm
 Inj.: 20 μ L



Vitamins, Water Soluble (HPLC)

Column: Discovery RP-Amide, 15cm x 4.6mm ID, 5 μ m particles
 Cat. Nos.: 505013 (RP-AmideC16),
 Mobile Phase: MeOH:50mM KH_2PO_4 , pH 4.5 (30:70)
 Temp.: 35°C
 Det.: UV, 220nm
 Inj.: 10 μ L

Column: Discovery C18 or C8, 15cm x 4.6mm ID, 5 μ m particles
 Cat. Nos.: 504955 (C18), 59353-U (C8)
 Mobile Phase: 50mM KH_2PO_4 , pH 4.5 to MeOH:50mM KH_2PO_4 , pH 4.5 (30:70) in 12 min (Discovery C8) or 8 min (Discovery C18)
 Flow Rate: 1mL/min
 Temp.: ambient temp.
 Det.: UV, 254nm
 Inj.: 1 μ L