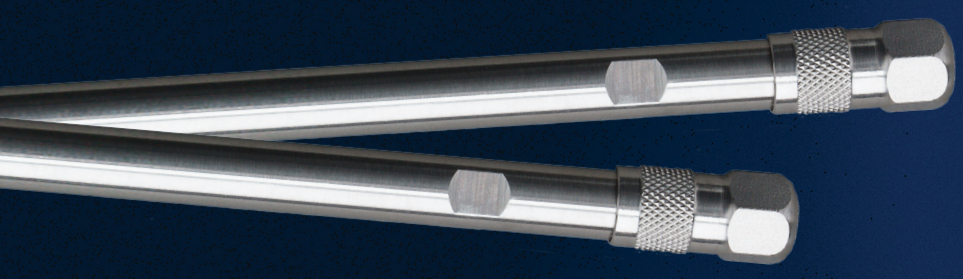
HALO®

A BETTER PATH TO SEPARATIONS
WITH FUSED-CORE®

APPLICATIONS COLLECTION



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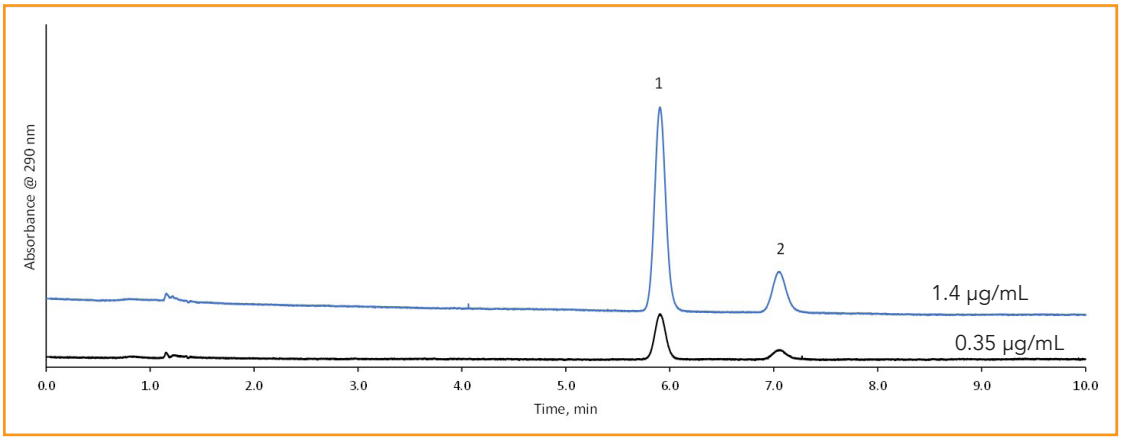
APPLICATION SEGMENTS

Sildenafil Citrate on HALO® C18, 5 µm

234-P



PEAK IDENTITIES

1. Sildenafil
2. Sildenafil N-oxide

TEST CONDITIONS:

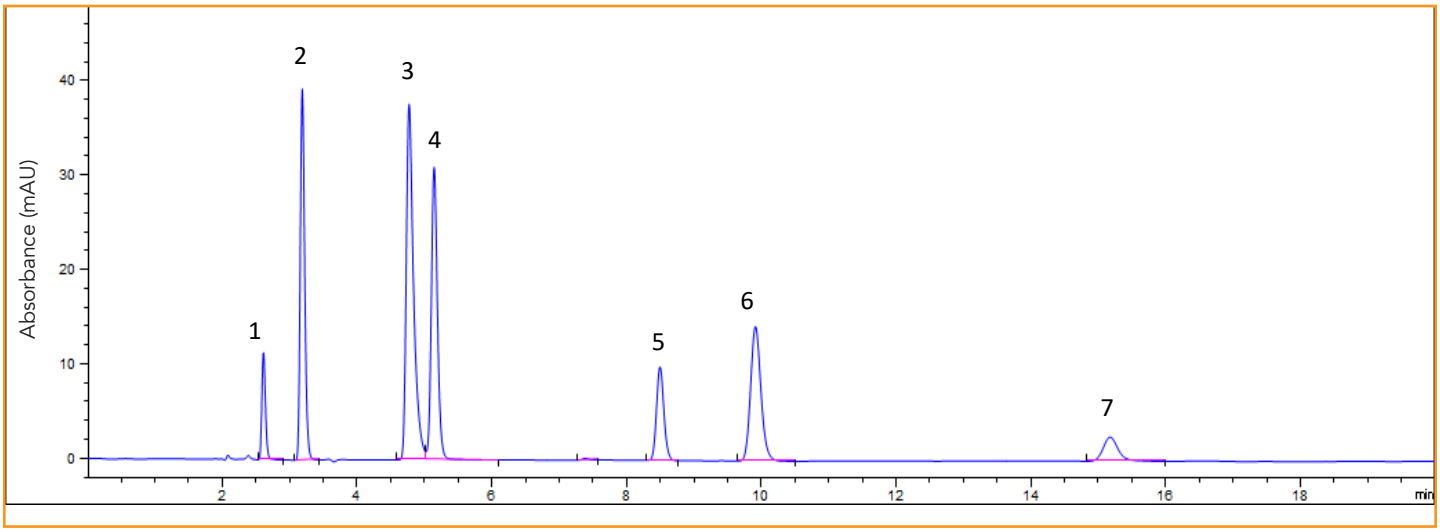
Column: HALO 90 Å C18, 5 µm, 4.6 x 150 mm
Part Number: 95814-702
Mobile Phase: 58/25/17 (v,v,v) Buffer, Methanol, Acetonitrile
Buffer: 7 mL TEA in 1 L Water, adjusted to pH: 3 w/ phosphoric acid
Flow Rate: 1.0 mL/min
Initial Back Pressure: 193 bar
Temperature: 30 °C
Detection: 290 nm
Injection Volume: 10 µL
Sample Solvent: mobile phase buffer
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Concentration	Peak	TF	R_s	S/N
1.4 µg/mL	1	1.08	5.17	100
	2	1.13		20
0.35 µg/mL	1	1.06	5.23	25
	2	1.06		5

Sildenafil (better known as Viagra) is a medication used to treat erectile dysfunction. The drug came off patent in 2019. A HALO® 5 µm C18 column is used for the HPLC methods specified within the sildenafil citrate USP Monograph. This includes the diluted sample solution (1.4 µg/mL) and the sensitivity solution (0.35 µg/mL). Tailing factor, resolution, and signal to noise ratio requirements are all met showing excellent column performance.

Chloroquine Phosphate Assay and Impurity Profiling

252-P



PEAK IDENTITIES

1. Phenol
2. Chloroquine related compound G (RCG)
3. Chloroquine related compound D (RCD)
4. Hydroxychloroquine sulfate
5. Chloroquine related compound A (RCA)
6. Chloroquine Phosphate
7. Chloroquine related compound E (RCE)

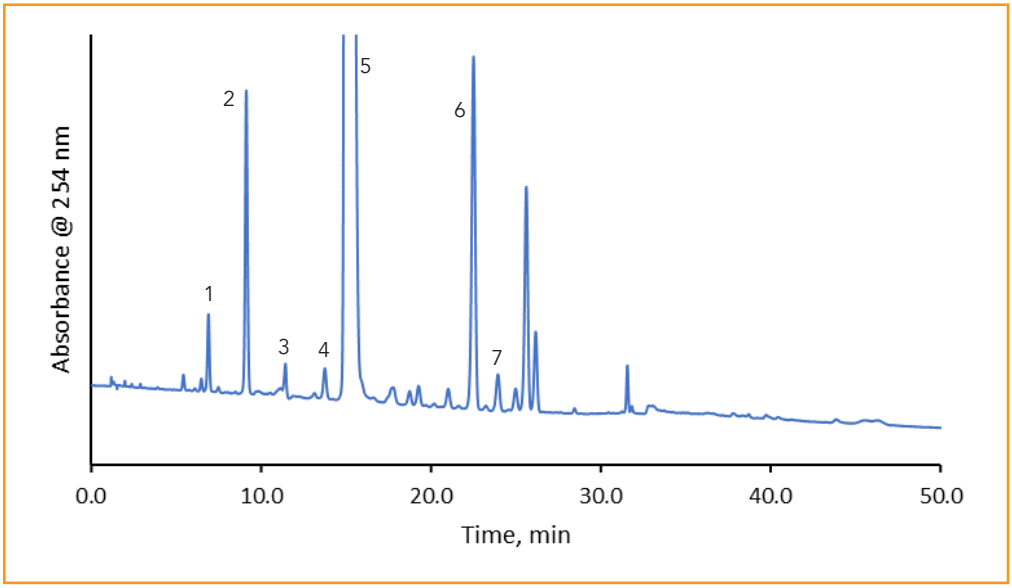
TEST CONDITIONS:

Column: HALO 90 Å C18, 5 µm, 4.6 x 250mm
Part Number: 95814-902
Mobile Phase: 70/30 Methanol/buffer/0.4% triethylamine buffer: 1.4 g K_2HPO_4 in 1000 mL, adjust to pH 3.0 using H_3PO_4
Isocratic
Flow Rate: 1 mL/min
Pressure: 237 bar
Temperature: 30 °C
Detection: UV @ 260 nm
Injection Volume: 20 µL
Sample Solvent: mobile phase
Flow Cell: 10 µL

Chloroquine Phosphate is in a class of drugs called antimalarials/ amebiasis and is used to prevent and treat malaria. A quick and easy HPLC method is used for the chromatographic purity of Chloroquine Phosphate. These conditions follow the USP43-NF38 monograph methods for Chloroquine Phosphate Assay and Impurity Profiling with minor modifications in the sample concentration. The isocratic method shows excellent resolution and peak shape using a HALO® 5 µm C18 column. A 6.0 resolution value between chloroquine phosphate and chloroquine related compound A is well over the USP requirement. (> 2.0)

Dexamethasone Sodium Phosphate (EP 10.0)

257-S



PEAK IDENTITIES

1. Impurity C
2. Impurity D
3. Impurity E
4. Impurity F
5. Dexamethasone sodium phosphate
6. Impurity A
7. Impurity G

TEST CONDITIONS:

Column: HALO 90 Å C8, 5 µm, 4.6x150 mm
Part Number: 95814-708
Mobile Phase A: 300 mL solution A, 350 mL water, 350 mL MeOH, pH: 3.8
Mobile Phase B: 300 mL solution A, pH: 4, 700 mL MeOH solution A: dissolve 7.0 g of ammonium acetate in 1000 mL water

Gradient:

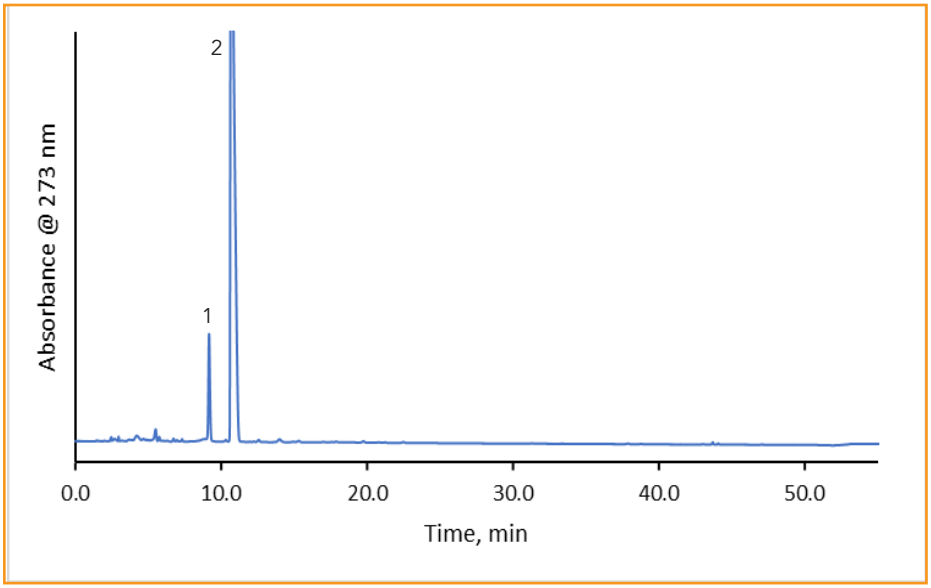
Time	%B
0.0	10
3.5	10
23.5	40
34.5	95
50.0	95

Flow Rate: 1.0 mL
Pressure: 209 bar
Temperature: 30 °C
Detection: UV 254 nm, PDA
Injection Volume: 20 µL Reference Solution B
Sample Solvent: mobile phase A
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Dexamethasone relieves inflammation and is used to treat several conditions such as arthritis, allergic reactions, and bowel disorders. A HALO 90 Å C8, 5µm column is used to separate dexamethasone and its impurities following the European Pharmacopoeia 10.0 method.

Cefuroxime Sodium According to Chinese Pharmacopoeia (CP) Method

259-CP



PEAK IDENTITIES

1. Dicarbamoyl cefuroxime
2. Cefuroxime sodium

TEST CONDITIONS:

Column: HALO 90 Å C8, 5 µm, 4.6 x 250 mm
Part Number: 95814-902
Mobile Phase A: 0.68 g sodium acetate with water (1000mL) pH 3.4 (acetic acid)
Mobile Phase B: Acetonitrile
Gradient:

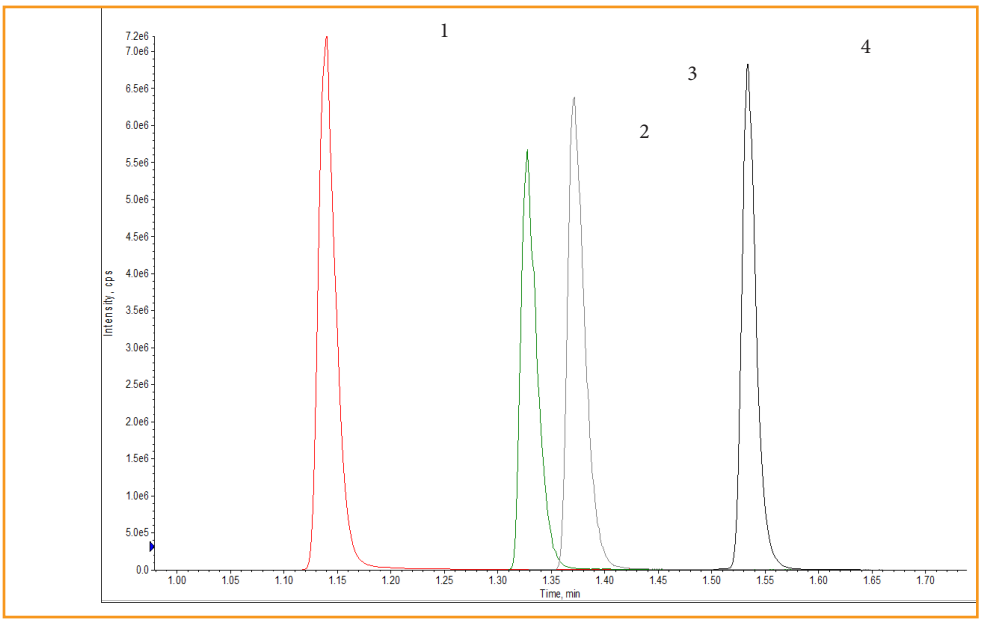
Time	%B
0.0	5
40.0	20
50.0	40
51.0	5
55.0	5

Flow Rate: 1.5 mL
Temperature: 35 °C
Detection: UV 273 nm, PDA
Injection Volume: 20 µL (0.5 mg/mL)
Initial Back Pressure: 208 bar
Sample Solvent: water
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2LC System

Cefuroxime is an antibiotic used to prevent several types of bacterial infections. A HALO 90 Å C8 column is used to separate dicarbamoyl cefuroxime from cefuroxime, achieiving high resolution. The main peak eluted in one third of the total analysis time with no peaks of interest eluting in the remainder of the specified CP assay. This illustrates the potential modernization of the assay with HALO® 5 micron particles for a 20 min assay.

LC-MS/MS Analysis of Antiviral Drugs on HALO® RP-Amide

265-AV



PEAK IDENTITIES

1. Indinavir
2. Saquinavir
3. Nelfinavir
4. Remdesivir

TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 2 µm, 2.1 x 30 mm
Part Number: 91812-307
Mobile Phase A: Water/0.01% Formic Acid
Mobile Phase B: Acetonitrile

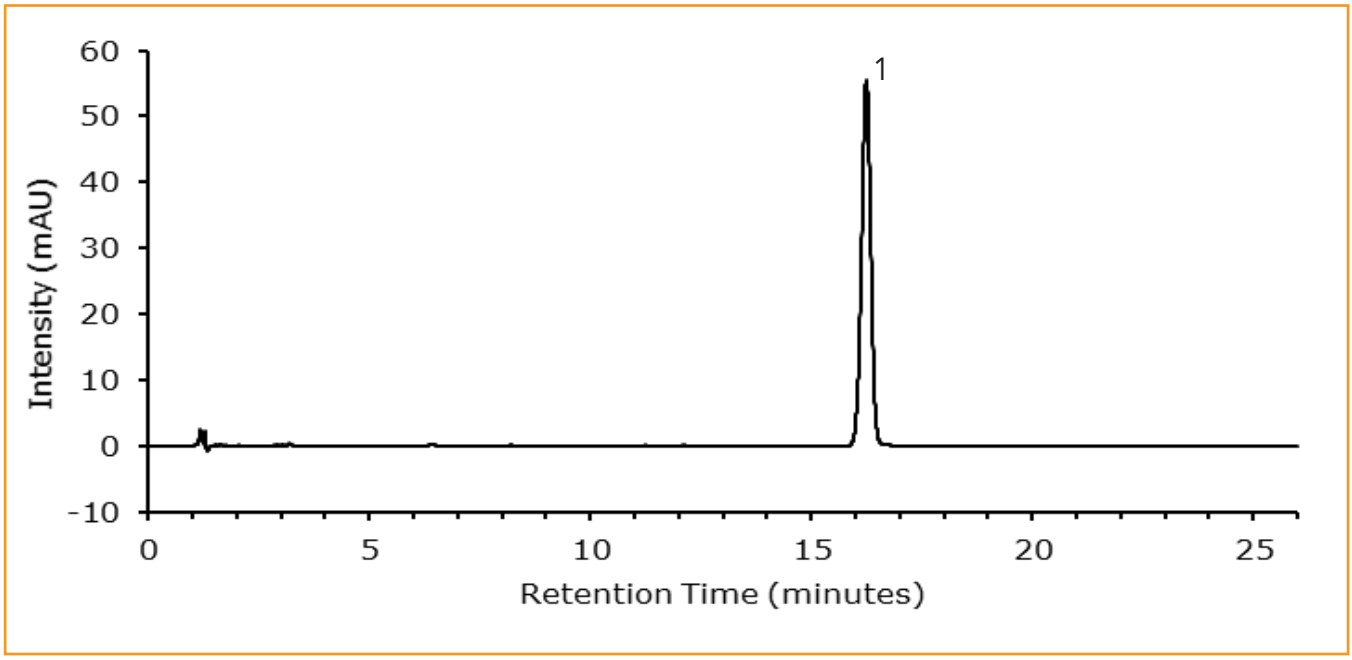
Gradient:	**Time**	**%B**
	0.0	3
	0.20	3
	1.70	34
	1.75	100
	3.00	100

Flow Rate: 0.8 mL/min
Temperature: 40 °C
Detection: LC-MS/MS ESI+
Injection Volume: 2 µL
Sample Solvent: 50/50 Acetonitrile/ Water

Remdesivir along with three structural antiviral analogues is separated on a HALO® RP-Amide column showing high speed and resolution. Remdesivir is a broad- spectrum antiviral drug that was tested in 2020 as a treatment for COVID-19.

Lopinavir Assay Method (EP 10.2)

266-L



TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-702
Mobile Phase A: Phosphate buffer pH: 6.0
0.9g dipotassium hydrogen phosphate and 2.7g potassium dihydrogen phosphate in 1000 mL water
Mobile Phase B: Acetonitrile
Isocratic: 45% B
Flow Rate: 1.0 mL/min
Initial Back Pressure: 153 bar
Temperature: 50 °C
Detection: UV: 215 nm
Injection Volume: 12 µL
Sample Solvent: 50/50 Acetonitrile/ Water

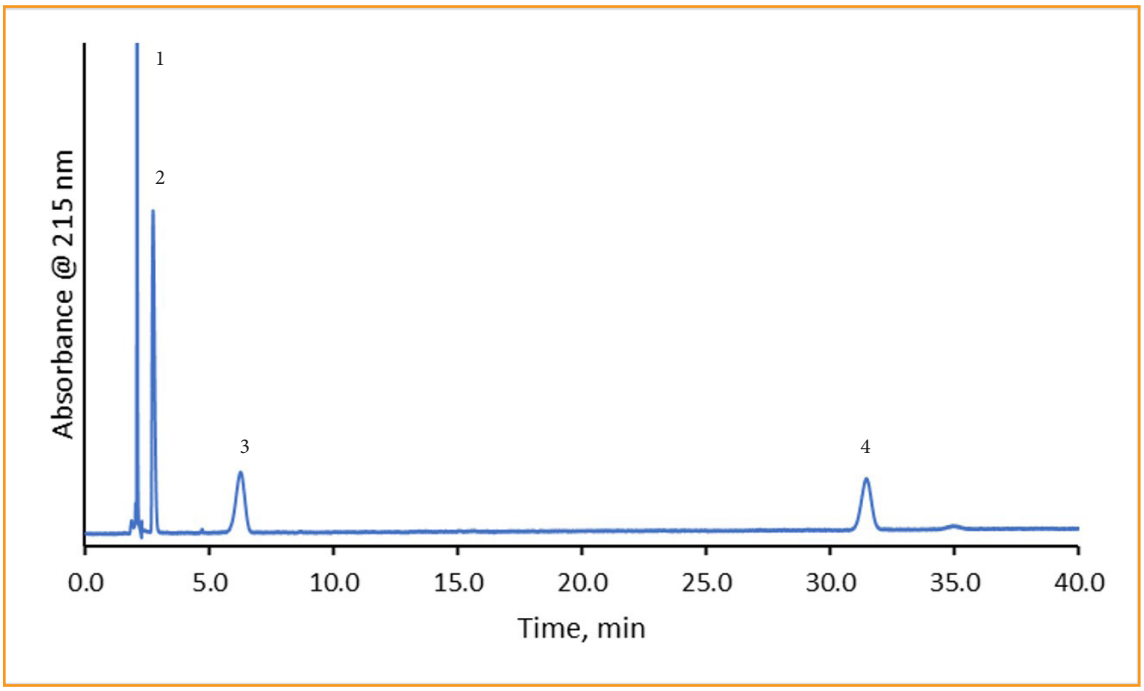
PEAK IDENTITIES

1. Lopinavir CRS

A European Pharmacopeia Method (EP 10.2) for Lopinavir has been modified to a shorter, faster alternative column saving time and mobile phase. Lopinavir is used to treat HIV infection and may slow the disease with a combination of other drugs.

Enalapril Maleate According to Chinese Pharmacopeia (CP)

267-CP



TEST CONDITIONS:

Column: HALO 90 Å C8, 5 µm, 4.6 x 250 mm
Part Number: 95814-908
Mobile Phase A: 10mM Phosphate buffer pH: 2.2
Mobile Phase B: Acetonitrile
Isocratic: 25% B
Flow Rate: 1.0 mL/min
Back Pressure: 137 bar
Temperature: 50 °C
Detection: UV: 215 nm
Injection Volume: 20 µL
Sample Solvent: mobile phase
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

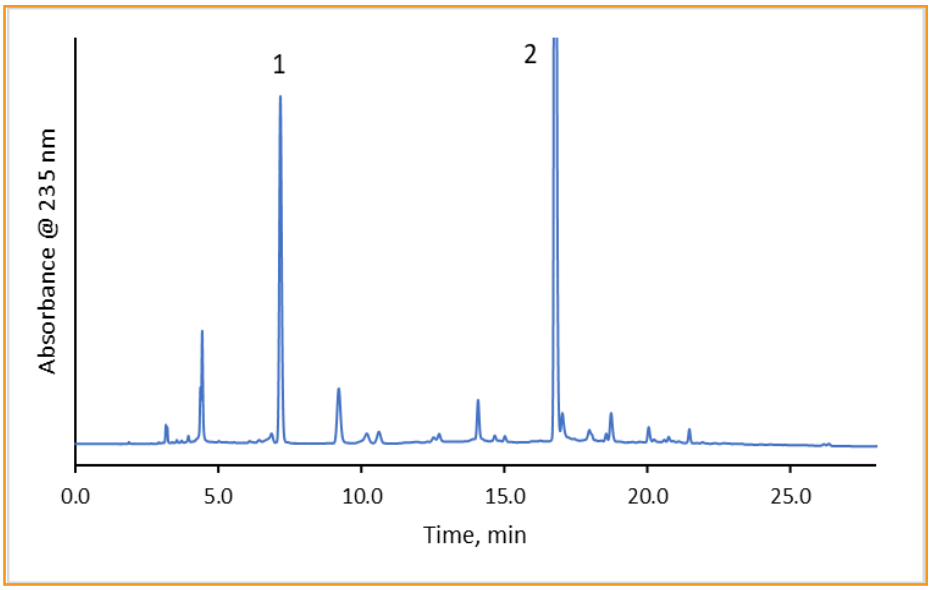
PEAK IDENTITIES

1. Maleic Acid
2. Enalaprilat (Impurity I)
3. Enalapril
4. Enalapril Diketopiperazine (Impurity II)

Enalapril is used to treat high blood pressure. A separation of enalapril along with its impurities is separated on a HALO® C8 column following the Chinese Pharmacopoeia method. High resolution along with low tailing factors are achieved.

Cefotaxime Sodium According to Chinese Pharmacopeia (CP)

268-CP



PEAK IDENTITIES

1. Impurity B
2. Cefotaxime

TEST CONDITIONS:

Column: HALO 90 Å C18, 5 µm, 4.6 x 250 mm
Part Number: 95814-902
Mobile Phase A: 86/14: 0.05 M Phosphate Buffer pH 6.25/ MeOH (7.1g anhydrous disodium hydrogen) phosphate to 1000mL)
Mobile Phase B: 60/40: 0.05 M Phosphate Buffer pH 6.25/ MeOH (7.1g anhydrous disodium hydrogen) phosphate to 1000mL)

Gradient:

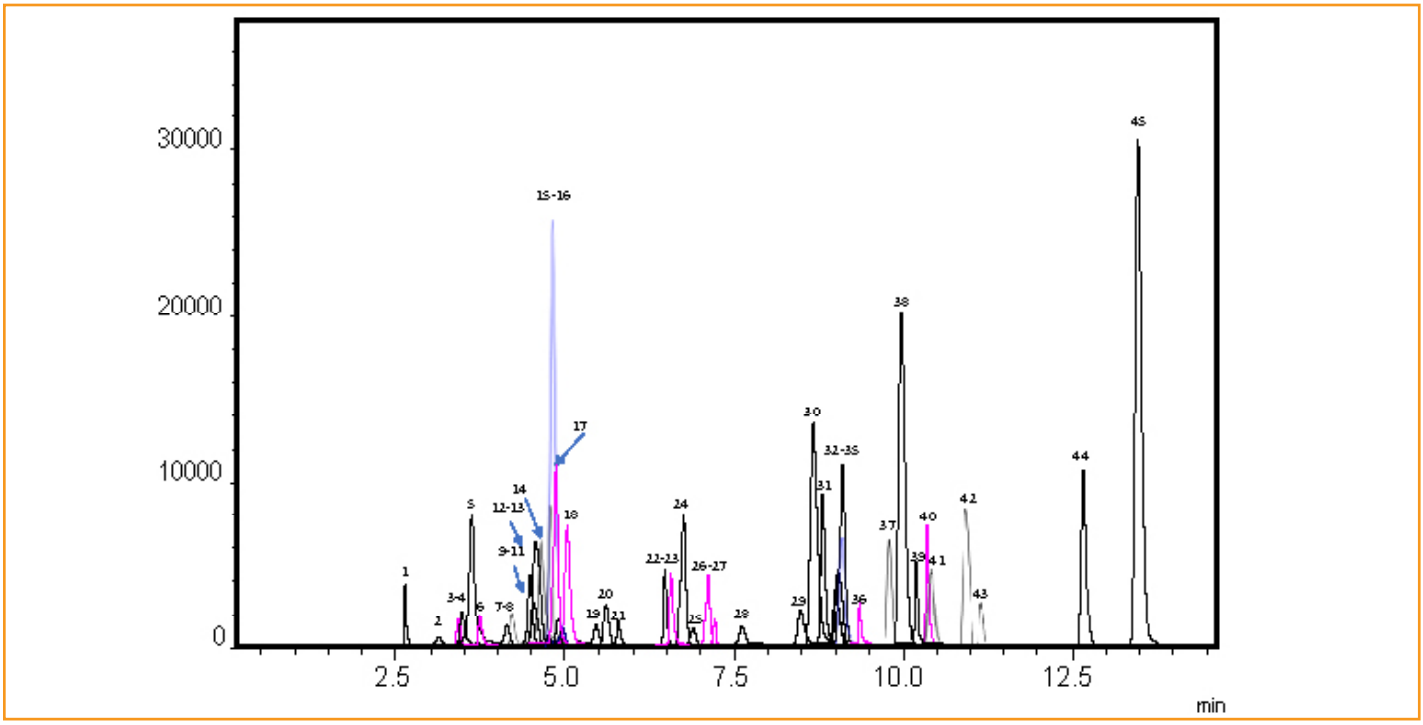
Time	%B
0.0	5
2.0	25
8.0	25
23.0	100
28.0	100
33.0	5
43.0	5

Flow Rate: 1.0 mL/min
Back Pressure: 189 bar
Temperature: 30 °C
Detection: UV: 235 nm
Injection Volume: 10 µL
Sample Solvent: Mobile Phase A
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Cefotaxime is used to treat many types of bacterial infections and can be injected or given orally. A Chinese Pharmacopeia (CP) method is used on a HALO® C18 column showing excellent resolution between peaks of interest.

LC-MS Analysis of Veterinary Drugs Using HALO® C18

276-vt



TEST CONDITIONS:

Analytical Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-602
Mobile Phase A: Water, 0.1 % Formic Acid
Mobile Phase B: ACN, 0.1% Formic Acid
Flow Rate: 0.4 mL/min
Pressure: 228 bar
Temperature: 35 °C
Injection Volume: 2.0µL
Sample Solvent: 50/50/ MEOH/H2O
Detection: +ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040
Gradient

Time	%B
0	10
14	100
16	100
16.10	10
19.0	stop

MS Source Conditions:
ESI +
Spray Voltage: 3.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 ˚C
Heat Block: 400 ˚C

Veterinary drugs are a complex group of substances that can be differentiated into different chemical classes and therapeutic areas. These compounds can further be differentiated based on their classifications, such as macrolides, quinolones, sulfonamides, benzimidazoles, tricyclines, and NSAIDs. Here we present the HALO® C18 for the separation and identification of a complex mix veterinary drugs, including macrolides, quinolones, sulfonamides, benzimidazoles, tricyclines, NSAIDs and 4 dye species which have also been used for therapeutic purposes in veterinary medicine. The high speed separation is easily accomplished and can definitely find application in high throughput environments.

Peak id	Drug	Transition	Reten. Time	Classification
1	Ciprofloxacin	332.1000>314.1000	2.515	Quinolone
2	Sulfathiazole	256.0000>92.0000	3.021	Sulfonamide
3	Lincomycin	407.2000>126.1000	3.334	Lincosamide
4	Sulfapyridine	250.1000>184.0000	3.340	Sulfonamide
5	Albendazole-2-amino	240.0000>133.1000	3.582	Benzimidazole
6	Trimethoprim	291.1000>230.0000	3.641	Quinolone
7	Ormetoprim	275.1000>123.1000	4.228	Quinolone
8	Tetracycline	445.1000>410.1000	4.234	Tetracycline
9	Enrofloxacin	360.1000>342.1000	4.520	Quinolones
10	Danofloxacin	358.1000>340.0000	4.532	Quinolones
11	Sulfaclozine	285.0000>156.0000	4.534	Sulfonamide
12	Sulfachloropyridazine	285.0100>92.0000	4.548	Sulfonamide
13	Sulfamerazine	265.0000>108.0000	4.591	Sulfonamide
14	Diclofenac	296.0000>214.0000	4.625	NSAID
15	Difloxacin	400.1000>382.1000	4.941	Quinolone
16	Amoxicillin	366.0000>113.9000	5.015	Beta-lactam
17	Chlortetracycline	479.1000>444.0000	5.027	Tetracyline
18	Sulfadoxine	311.0000>92.0000	5.280	Sulfonamide
19	Sulfaethoxypyridazine	295.0000>140.1000	5.542	Sulfonamide
20	Penicillin G	335.0000>159.9000	5.626	Beta-lactam
21	Neospiramycin	350.2000>174.2000	5.858	Macrolide
22	Spiramycin	422.4000>174.2000	6.521	Macrolide
23	Sulfadimethoxine	311.1000>108.0000	6.527	Sulfonamide
24	Albendazole Sulfoxide	282.1000>208.0000	6.638	Benzimidazole

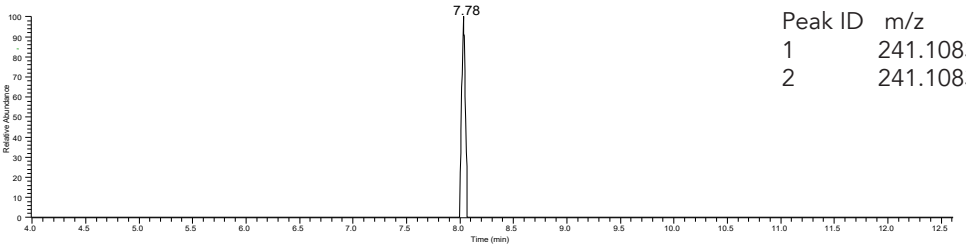
Peak id	Drug	Transition	Reten. Time	Classification
25	Albendazole Sulfone	298.0000>159.0000	6.669	Benzimidazole
26	Sulfaquinoxaline	301.1000>156.0000	7.027	Sulfonamide
27	Phenylbutazone	309.1000>120.1000	7.106	NSAID
28	Tilmicosin	435.4000>174.1000	7.527	Macrolide
29	Flumequin	262.0000>244.1000	8.508	Quinolone
30	Nalidixic Acid	233.1000>215.1000	8.542	Quinolone
31	Oxolinic Acid	261.9000>244.0000	8.646	Quinolone
32	Kitasamycin	772.3000>174.2000	9.015	Macrolide
33	Tylosin	916.5000>174.1000	9.018	Macrolide
34	Florfenicol Amine	248.0000>230.1000	9.051	Amphenicol
35	Erythromycin A	734.4000>576.4000	9.120	Macrolide
36	Malachite Green	329.2000>313.2000	9.389	Dye
37	Albendazole	266.0000>234.0000	9.829	Benzimidazole
38	Cloxacillin	436.0000>277.0000	10.030	Macrolide
39	Dicloxacillin	470.0000>160.0000	10.080	Macrolide
40	Leucocrystal Violet	374.2000>238.2000	10.360	Dye
41	Crystal Violet	372.2000>356.2000	10.450	Dye
42	Brilliant Green	385.2000>341.1000	11.000	Dye
43	Dapsone	249.0000>156.0000	11.110	Sulfone
44	Carprofen	274.0000>228.1000	12.600	NSAID
45	Ivermectin	897.6000>240.1000	13.140	Macrolide

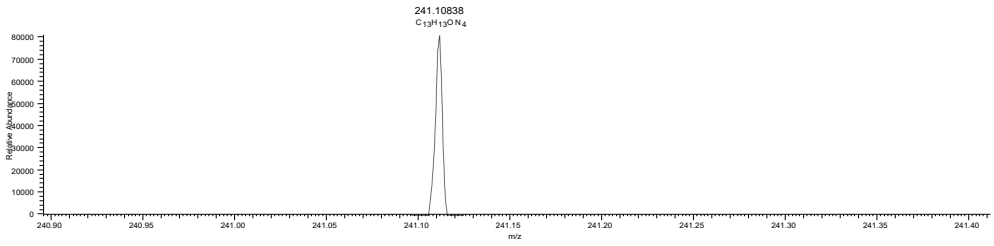
LC-MS Analysis of Varenicline NDSRI using HALO® Biphenyl

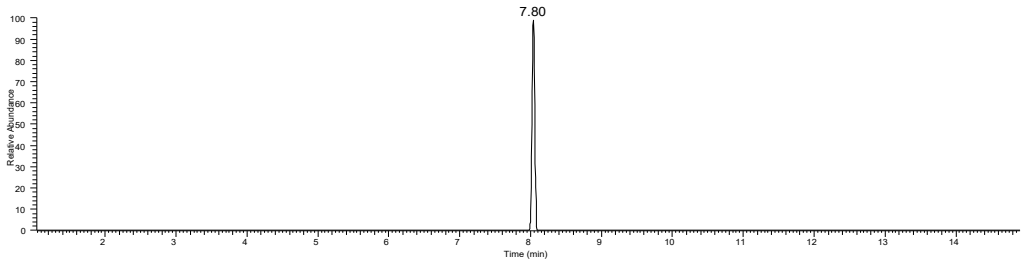
279-P

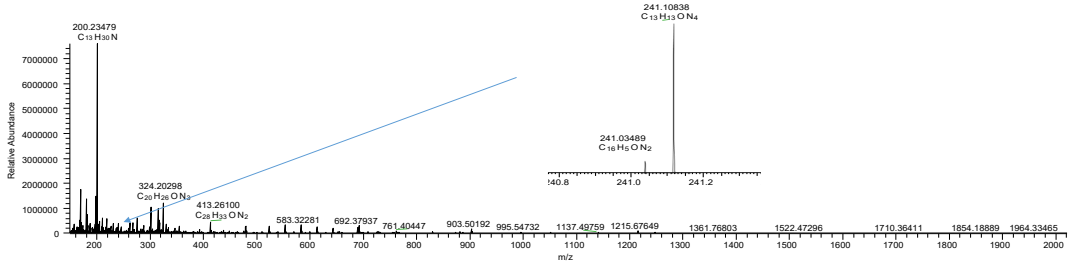
PEAK IDENTITIES:

Peak ID	m/z	Ret. time
1	241.10838	7.78
2	241.10838	7.8









Chantix, a prescription medication that is used to help people stop smoking, has recently come to attention due to a recall that was initiated by the pharmaceutical company Pfizer. This was due to N-nitroso-varenicline (the Nitroso-Drug Substance Related impurity (NDSRI)) detected above the Pfizer established Acceptable Daily Intake (ADI) level. Increased ingestion of N-nitroso-varenicline may be associated with an increased cancer risk in humans. The US Food and Drug Administration (FDA) has recently released the method "Liquid Chromatography High Resolution mass spectrometry method for the determination of Varenicline NDSRI in Chantix drug product and drug substance. In this application, the FDA method is used with the HALO® Biphenyl column to detect the impurity in a sample of the drug.

TEST CONDITIONS:

Column: HALO 90 Å Biphenyl, 2.7 µm, 3.0 x 75 mm
Part Number: 92813-511
Mobile Phase A: Water, 0.1 % Formic Acid
Mobile Phase B: MeOH, 0.1% Formic Acid
Flow Rate: 0.5 mL/min
Gradient:

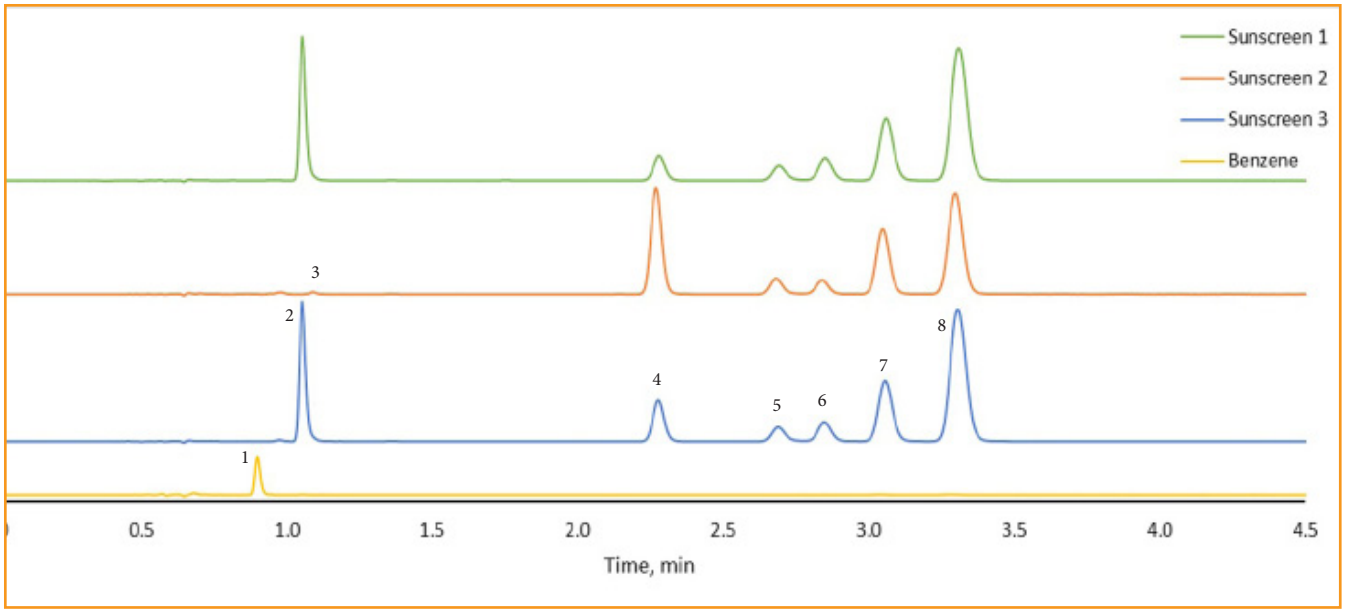
Time	%B
0.0	10
1.0	10
10.0	100
11.1	10
15.0	stop

Pressure: 175 bar
Temperature: 30 °C
Injection Volume: 5.0 µL
Sample Solvent: MeOH
Detection: +ESI
LC System: Shimadzu Nexera X2
ESI LCMS system: QExactive HF

MS Conditions:
Detection: (+) ESI
Spray Voltage: 3.5 kV
Sheath gas: 50 arbitrary units
Aux gas: 15 arbitrary units
Sweep gas: 0
Capillary temp: 250 °C
Heat temp: 400 °C
Scan Type: t-Sim
Resolution: 60,000

Benzene Screening in Aerosol Sunscreens

280-SA



TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 2.7 µm, 2.1x100 mm
Part Number: 92812-607
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Isocratic: 75% B
Flow Rate: 0.3 mL/min
Back Pressure: 122 bar
Temperature: 30 °C
Detection: UV: 210 nm
Injection Volume: 0.5 µL
Sample Solvent: Ethanol
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

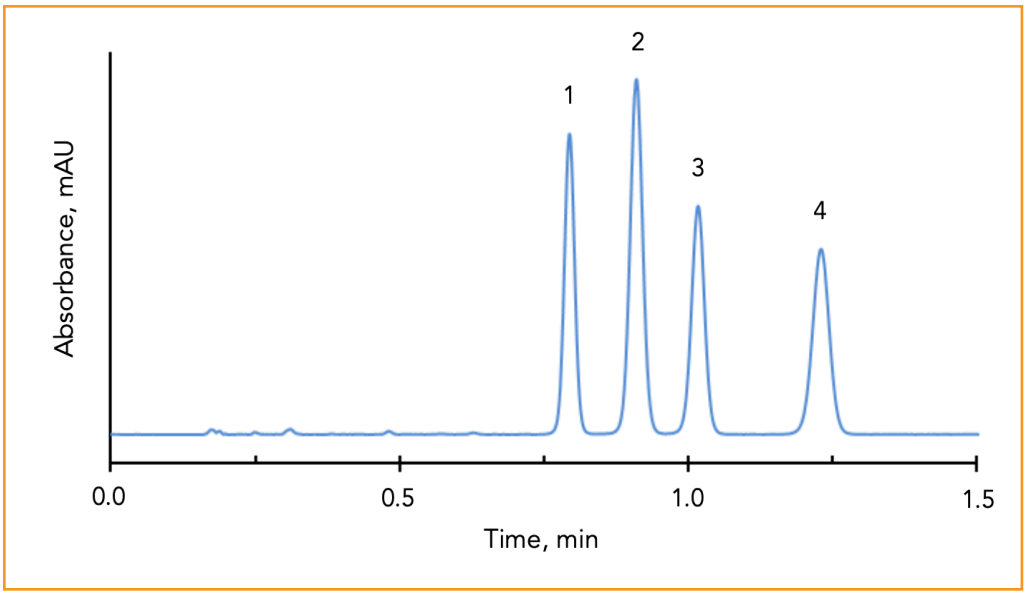
PEAK IDENTITIES

1. Benzene
2. Oxybenzone
3. Avobenzone isomer 1
4. Octocrylene
5. Avobenzone isomer 2
6. Homosalate isomer 1
7. Octisalate
8. Homosalate isomer 2

Johnson and Johnson issued a voluntary recall for specific aerosol sunscreen products due to the presence of benzene. Sunscreens are designed to reduce the risk of burning from exposure to the sun's UV rays. Overexposure to the sun increases the chances of skin cancer so it is important to use sunscreens during outdoor activities. The active contents of sunscreens can be analyzed using HPLC as shown in this application note. Approximately 200 mg of aerosol sunscreen were treated with 10 mL ethanol to dissolve the active ingredients. Aliquots of the slurries were then filtered through a Nylon 0.45 µm porosity syringe filter prior to analysis. Benzene was screened as well due to the sunscreen recall, however, no benzene was detected.

Rapid Isocratic Separation of Sulfonyl Urea Drugs on HALO® C18 Phase

Application Note 37-P



PEAK IDENTITIES:

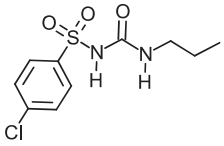
1. Chlorpropamide
2. Glipizide
3. Acetohexamide
4. Tolazamide

The sulfonyl drugs are used in the treatment of diabetes. They can be separated in about 1.3 minutes using highly efficient HALO® Fused-Core® C18 columns.

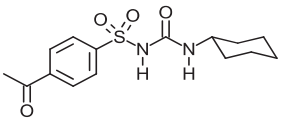
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 63/37 - A/B
A: 0.02 M phosphate buffer, pH 3.0
B: Acetonitrile
Flow Rate: 2.5 mL/min
Pressure: 260 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

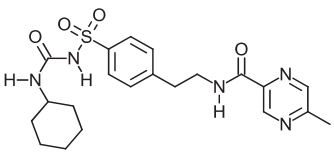
STRUCTURES:



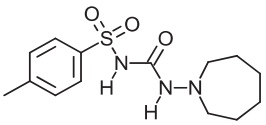
Chlorpropamide



Acetohexamide



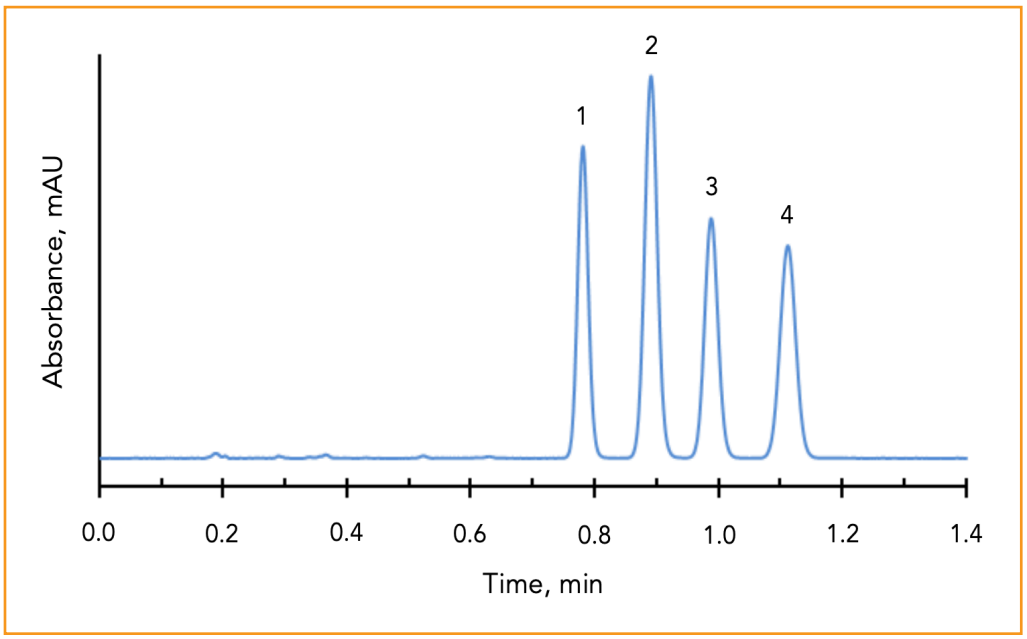
Glipizide



Tolazamide

Rapid Isocratic Separation of Sulfonyl Urea Drugs on HALO® Phenyl-Hexyl Phase

Application Note 38-P



PEAK IDENTITIES:

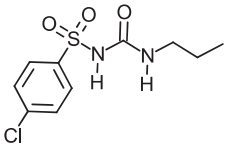
1. Chlorpropamide
2. Glipizide
3. Acetohexamide
4. Tolazamide

These sulfonyl drugs can be rapidly analyzed in less than 1.2 minutes using short, efficient HALO® Fused-Core® Phenyl-Hexyl columns.

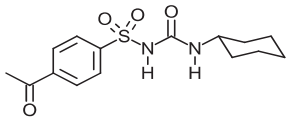
TEST CONDITIONS:

Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 62/38 - A/B
A: 0.02 M phosphate buffer, pH 3.0
B: Acetonitrile
Flow Rate: 2.5 mL/min
Pressure: 255 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

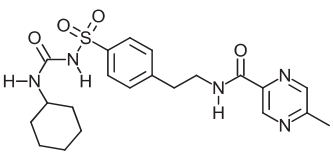
STRUCTURES:



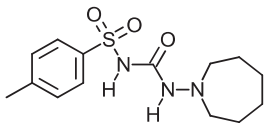
Chlorpropamide



Acetohexamide



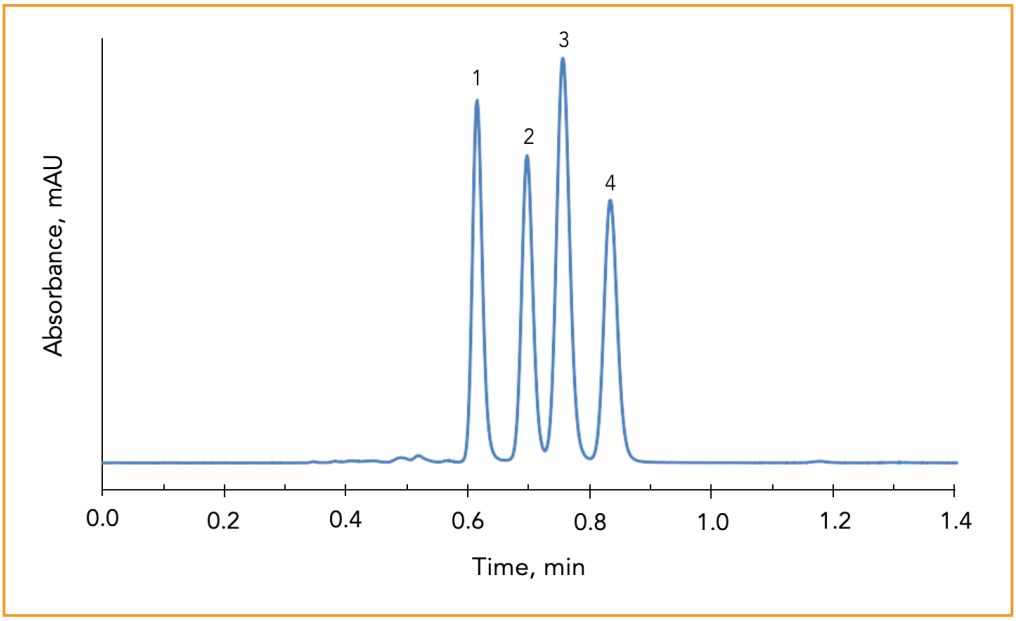
Glipizide



Tolazamide

Rapid Separation of Sulfonyl Urea Drugs on HALO® PFP Phase

Application Note 39-P



PEAK IDENTITIES:

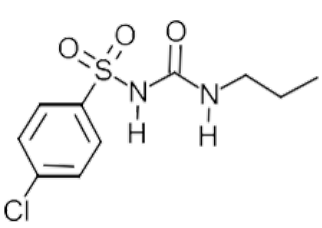
1. Chlorpropamide
2. Glipizide
3. Acetohexamide
4. Tolazamide

These sulfonyl drugs can be rapidly analyzed in less than 0.9 minutes using short, efficient HALO® Fused-Core® PFP (perfluorophenylpropyl) columns.

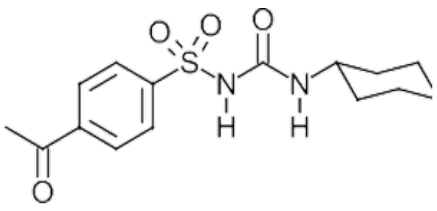
TEST CONDITIONS:

Column: HALO 90 Å PFP, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-409
Mobile Phase: 30/70 - A/B
A: 0.02 M phosphate buffer, pH 3.0
B: Methanol
Flow Rate: 1.5 mL/min
Pressure: 200 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

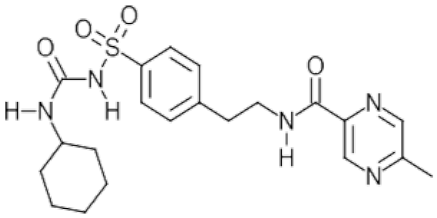
STRUCTURES:



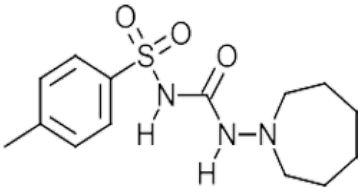
Chlorpropamide



Acetohexamide



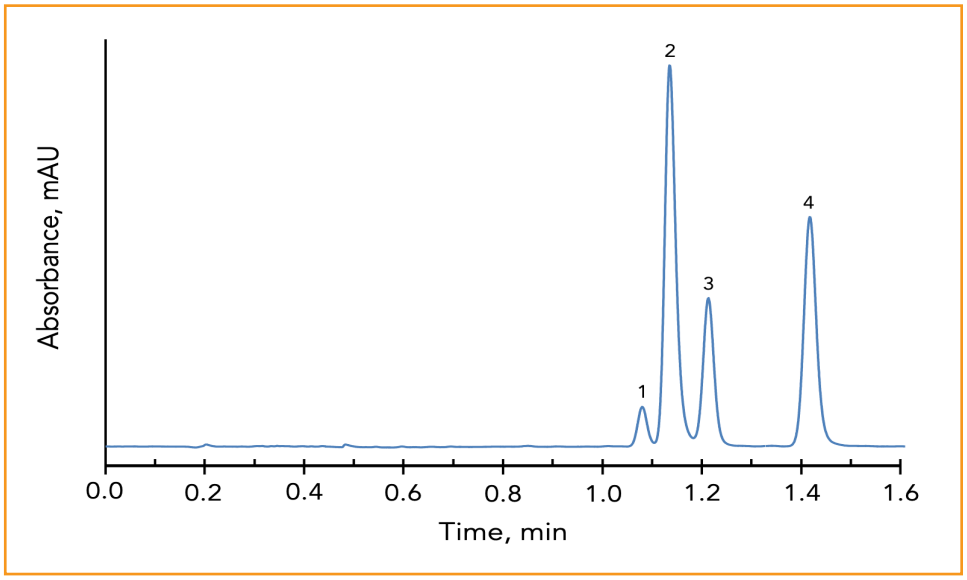
Glipizide



Tolazamide

Separation of Antiulcer Drugs on HALO® Penta-HILIC

Application Note 65-B



PEAK IDENTITIES:

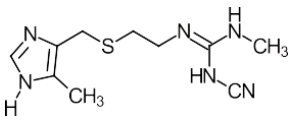
1. Cimetidine
2. Nizatidine
3. Famotidine
4. Ranitidine

The strongly basic antiulcer drugs an be rapidly separated on HALO® Penta-HILIC phase using a mobile phase that works well with a mass spectrometer detector.

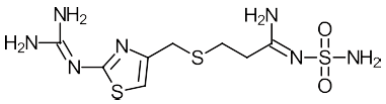
TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-605
Mobile Phase: 10/90 - A/B
A: 0.04 M ammonium formate, pH 3.0
B: Acetonitrile
Flow Rate: 3.0 mL/min
Pressure: 210 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: Mobile phase
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

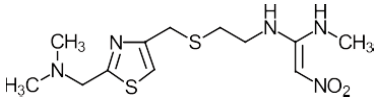
STRUCTURES:



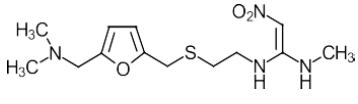
Cimetidine



Famotidine



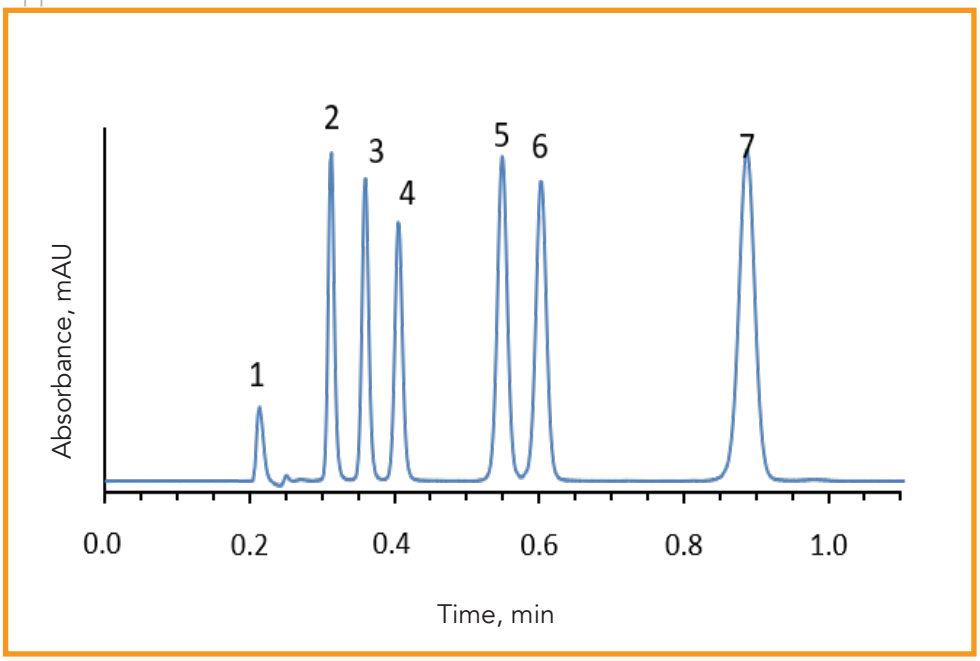
Nizatidine



Ranitidine

Separation of Sulfa Drugs on HALO® RP-Amide

Application Note 11-AB



PEAK IDENTITIES:

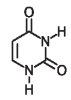
1. Uracil
2. Sulfathiazole
3. Sulfamerazine
4. Sulfamethizole
5. Sulfachloropyridazine
6. Sulfamethoxazole
7. Sulfadimethoxin

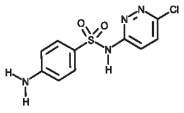
Sulfonamides, or sulfa drugs, are synthetic antibiotics used to treat bacterial infections. Six sulfa drugs are resolved in less than 1 minute on a HALO 90 Å RP-Amide column.

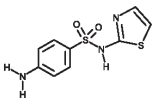
TEST CONDITIONS:

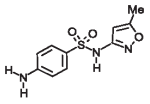
Column: HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 70/30 - A/B
A: 0.1% formic acid with 0.005 M ammonium formate, pH 3.0
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 193 bar
Temperature: 35 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

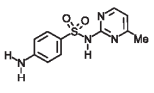
STRUCTURES:

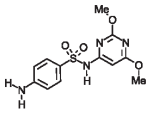

Uracil

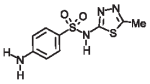

Sulfachloropyridazine


Sulfathiazole


Sulfamethoxazole

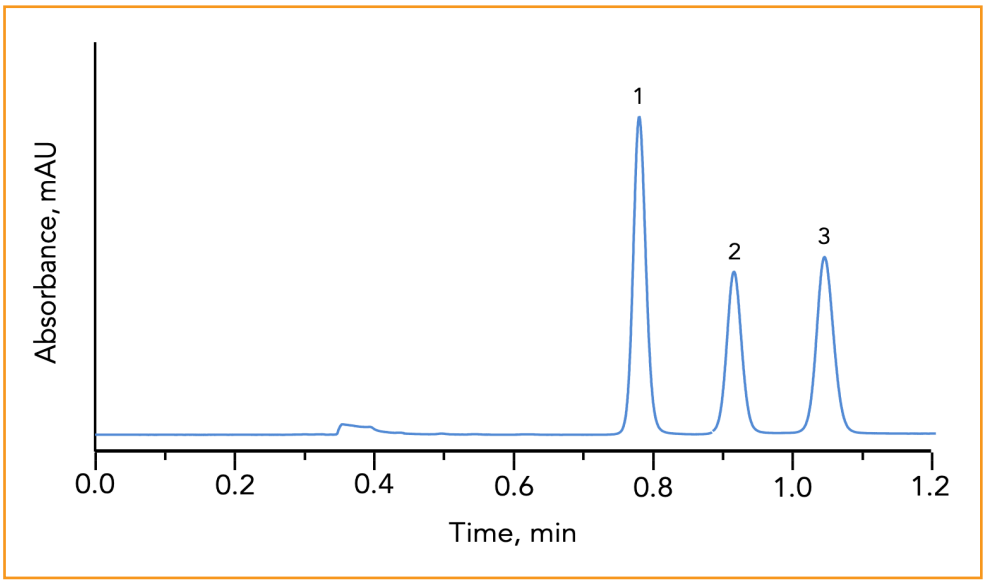

Sulfamerazine


Sulfadimethoxine


Sulfamethizole

Separation of Fluoroquinolone Drugs on HALO® Phenyl-Hexyl Phase

Application Note 66-AB



PEAK IDENTITIES:

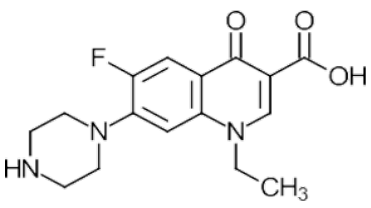
1. Norfloxacin
2. Ciprofloxacin
3. Lomefloxacin

The fluoroquinolone drugs are broad spectrum antibiotics that are used in both humans and animals. They can be quickly separated on HALO® Phenyl-Hexyl stationary phase in less than 1.2 minutes. The Fused-Core® particles allow the use of high flow rates without loss of resolution.

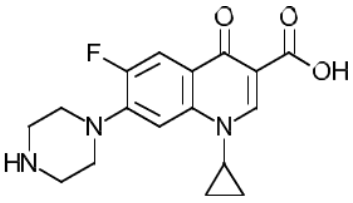
TEST CONDITIONS:

Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 82/18 - A/B
A: 0.025 M sodium phosphate, pH 2.5
B: Acetonitrile
Flow Rate: 1.5 mL/min
Pressure: 170 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.3 µL
Sample Solvent: Dimethylformamide/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

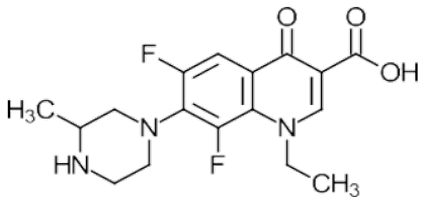
STRUCTURES:



Norfloxacin



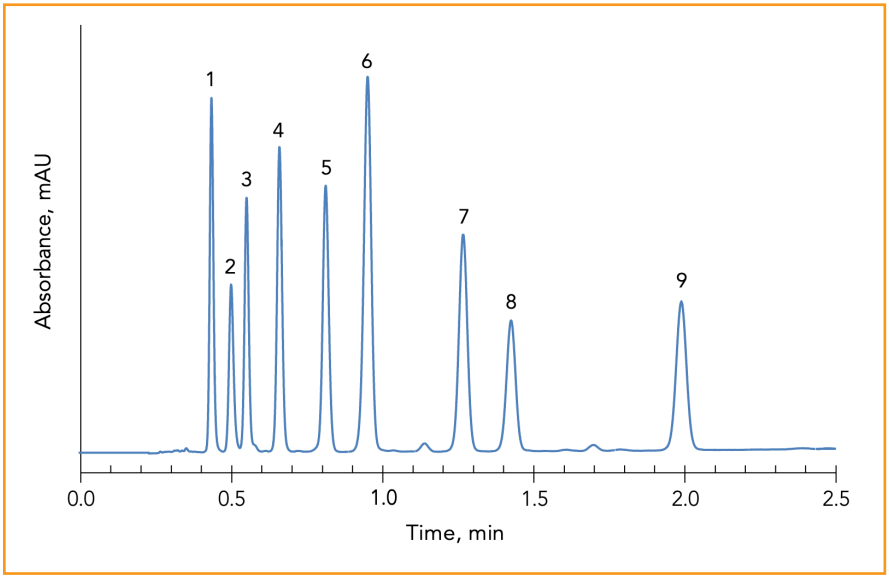
Ciprofloxacin



Lomefloxacin

Separation of Cephalosporins on HALO® ES-CN

Application Note 69-AB



PEAK IDENTITIES:

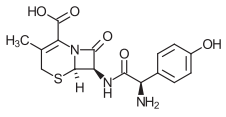
1. Cefadroxil
2. Ceftazidime
3. Cefaclor
4. Cephalexin
5. Cephradine
6. Cefotaxime
7. Cefoxitin
8. Cefazolin
9. Cephalothin

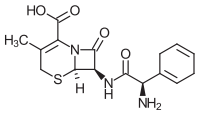
Cephalosporins are a class of α-lactam antibiotics that are used to treat staphylococcus and streptococcus infections. These nine cephalosporins can be separated in two minutes on the efficient HALO® ES-CN bonded phase column.

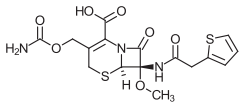
TEST CONDITIONS:

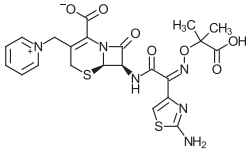
Column: HALO 90 Å ES-CN, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-404
Mobile Phase:
A: 0.02 M phosphate buffer, pH 2.7
B: Methanol
Gradient: 20% B to 40% B in 2.5 min
Flow Rate: 2.0 mL/min
Initial Pressure: 225 bar
Temperature: 40 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 70/30 water/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

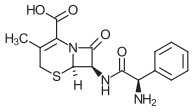
STRUCTURES:

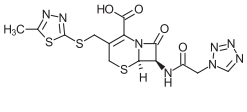

Cefadroxil

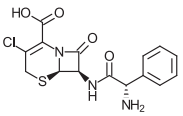

Cephalexin

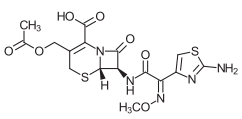

Cefoxitin

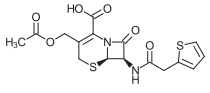

Ceftazidime


Cephradine


Cefazolin

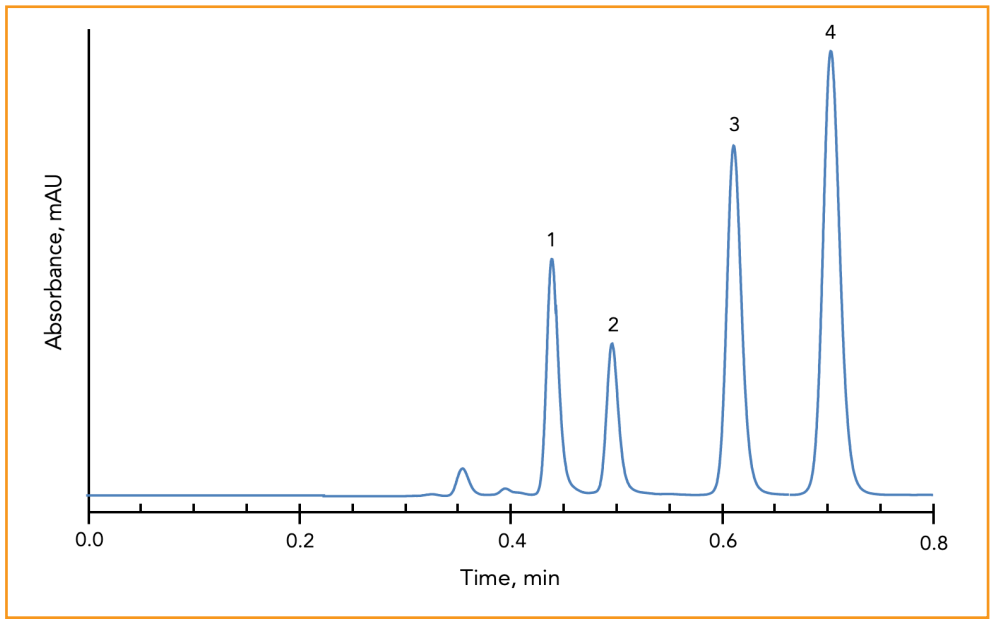

Cefaclor


Cefotaxime


Cephalothin

Separation of Penicillins on HALO® ES-CN

Application Note 71-AB



PEAK IDENTITIES:

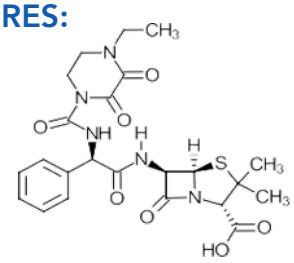
1. Piperacillin
2. Penicillin G
3. Oxacillin
4. Cloxacillin

These four penicillin drugs can be rapidly separated on HALO® Fused-Core® ES-CN bonded phase columns.

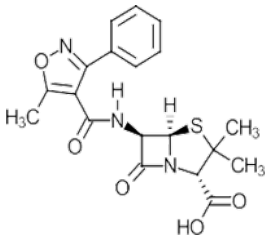
TEST CONDITIONS:

Column: HALO 90 Å ES-CN, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-404
Mobile Phase: 55/45 - A/B
A: 0.02 M Phosphate buffer, pH 3.0
B: Acetonitrile
Flow Rate: 1.5 mL/min
Pressure: 120 bar
Temperature: 40 °C
Detection: UV 230 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 water/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

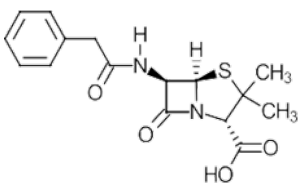
STRUCTURES:



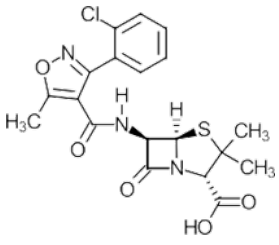
Piperacillin



Oxacillin



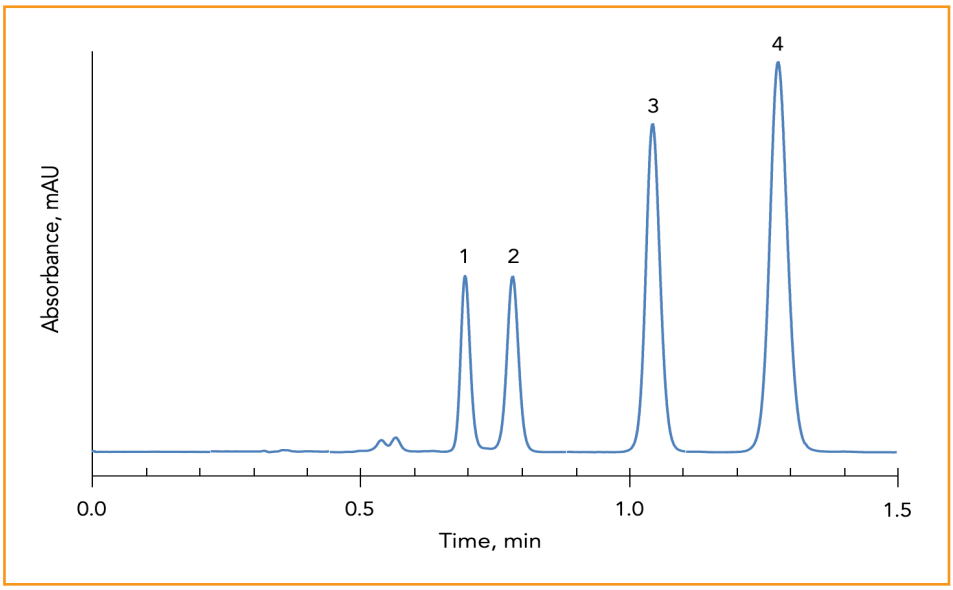
Penicillin G



Cloxacillin

Separation of Penicillins on HALO® Phenyl-Hexyl

Application Note 72-AB



PEAK IDENTITIES:

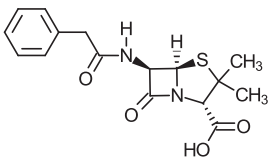
1. Penicillin G
2. Piperacillin
3. Oxacillin
4. Cloxacillin

These four penicillin drugs can be rapidly separated on HALO® Fused-Core® Phenyl- Hexyl bonded phase columns.

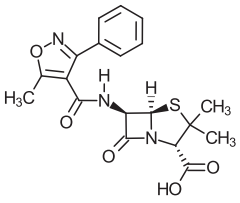
TEST CONDITIONS:

Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 40/60 - A/B
A: 0.02 M phosphate buffer, pH 3.0
B: Methanol
Flow Rate: 1.5 mL/min
Pressure: 200 bar
Temperature: 40 °C
Detection: UV 230 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 water/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

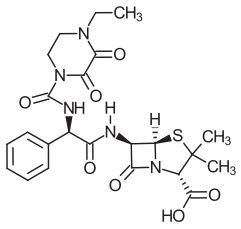
STRUCTURES:



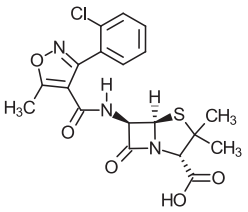
Penicillin G



Oxacillin



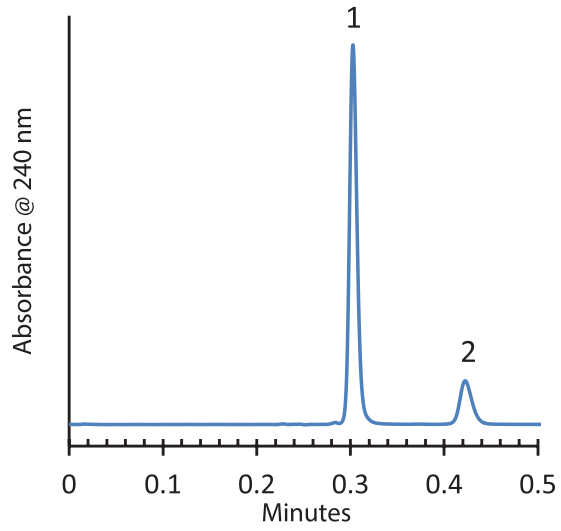
Piperacillin



Cloxacillin

Amoxicillin and Ampicillin on HALO® RP-Amide

Application Note 75-AB



PEAK IDENTITIES:

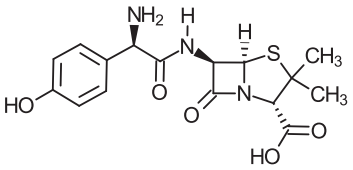
1. Amoxicillin
2. Ampicillin

Amoxicillin and ampicillin are members of the β-lactam class of antibiotics and are used to treat infections. Using a short HALO® RP-Amide column, they can be analyzed efficiently in less than one minute.

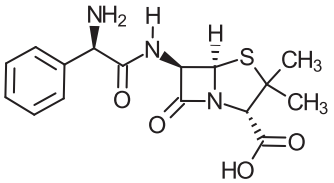
TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 82/18 - A/B
A: 0.02 M phosphate buffer, pH 2.7
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 200 bar
Temperature: 30 °C
Detection: UV 240 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 80/20 water/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

STRUCTURES:



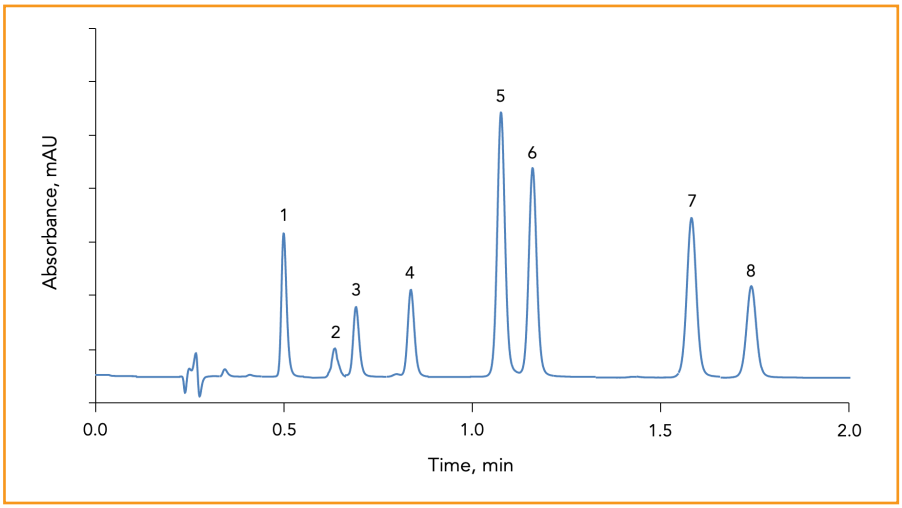
Amoxicillin



Ampicillin

Separation of Sulfonamides on HALO® Biphenyl, 2.0 µm

Application Note 194-AB



PEAK IDENTITIES:

1. Sulfacetamide
2. Sulfadiazine
3. Sulfapyridine
4. Sulfamerazine
5. Sulfamethoxazole
6. Sulfamethazine
7. Sulfamethoxypyridazine
8. Sulfachloropyridazine

A mixture of sulfonamides is separated on a HALO 90 Å Biphenyl, 2.0 µm column in less than 2 minutes. These synthetic drugs have several purposes, but are mainly used to treat bacterial infections such as urinary tract infections, eye infections, or ear infections. HALO® Biphenyl shows increased retention compared to alkyl phases due to the enhanced interactions between the aromatic moieties of the sulfonamides and the biphenyl structure. These interactions also enable more retention of polar compounds on the HALO® Biphenyl phase. When a complex mixture contains a variety of polar and non-polar compounds, use a HALO® Biphenyl column as part of the method development screening.

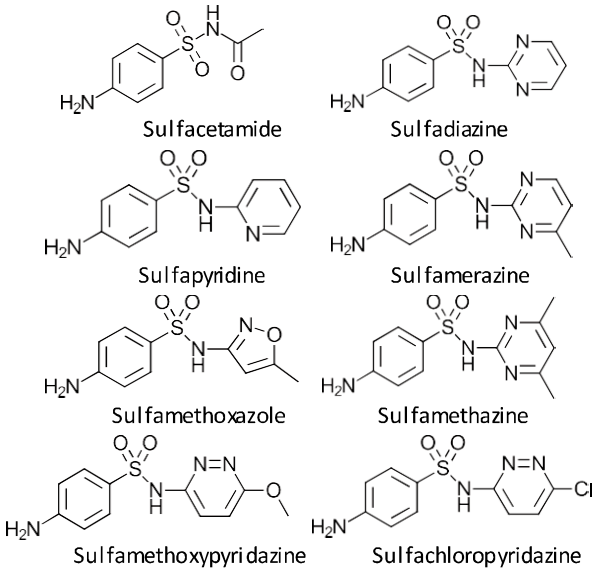
TEST CONDITIONS:

Column: HALO 90 Å Biphenyl, 2.0 µm, 2.1 x 50 mm
Part Number: 91812-411
Mobile Phase:
A: Water, 0.1% formic acid
B: Acetonitrile, 0.1% formic acid
Gradient:

Time (min)	% B
0.0	15
2.0	20

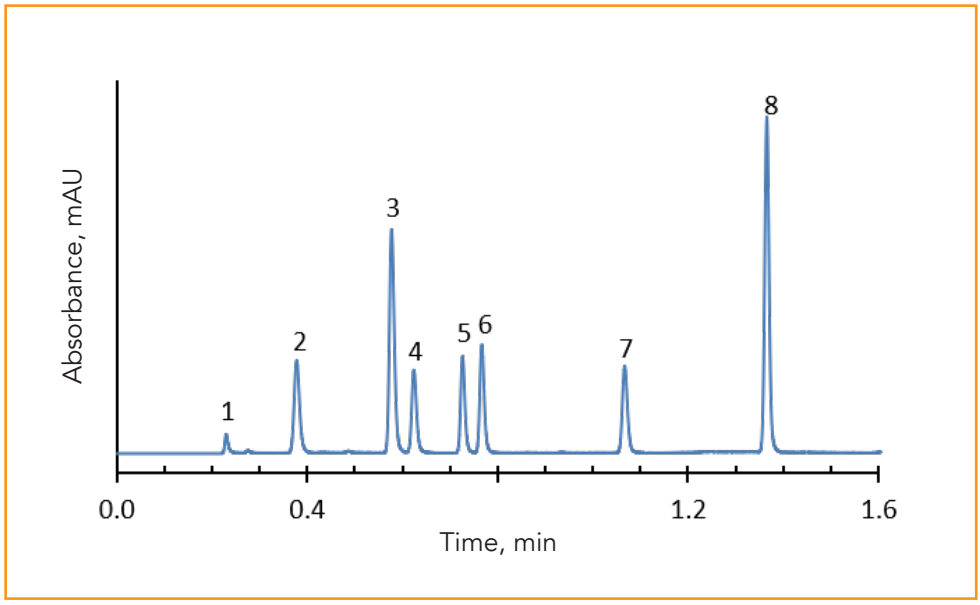
Flow Rate: 0.5 mL/min
Initial Pressure: 257 bar
Temperature: 40 °C
Detection: UV 254 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: Acetonitrile
Response Time: 0.025 sec
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

STRUCTURES:



Separation of Antibiotic and Antifungal Drugs on HALO® RP-Amide

80-AF



PEAK IDENTITIES:

1. Unknown
2. Ketoconazole
3. Naftifine
4. Clotrimazole
5. Econazole
6. Sulconazole
7. Clofazimine
8. Tolnaftate

The antimicrobial drug clofazimine and these other antifungal drugs can be rapidly analyzed using a HALO® RP-Amide column under gradient conditions with low back pressure.

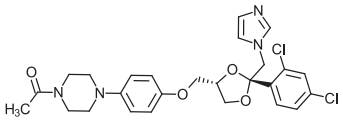
TEST CONDITIONS:

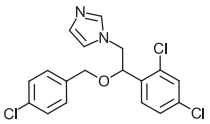
Column: HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase:
A: 0.02 M phosphate buffer, pH 3.0
B: Acetonitrile
Gradient:

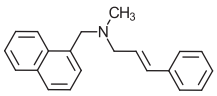
Time (min)	%B
0.0	41
1.0	80
1.6	80

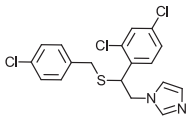
Flow Rate: 2.0 mL/min
Initial Pressure: 188 bar
Temperature: 35 °C
Detection: UV 230 nm, VWD
Injection Volume: 0.3 µL
Sample Solvent: 25/75 water/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

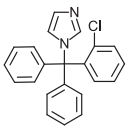
STRUCTURES:

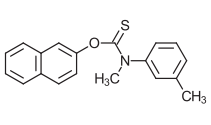

Ketoconazole

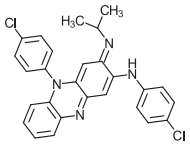

Econazole


Naftifine


Sulconazole

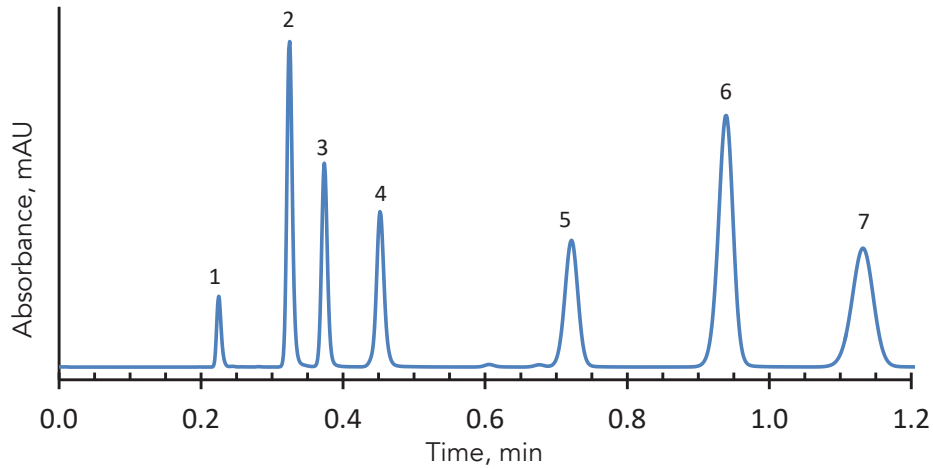

Clotrimazole


Tolnaftate


Clofazimine

Rapid HPLC Separation of Anticoagulants on HALO® Phenyl-Hexyl Phase

34-P



PEAK IDENTITIES:

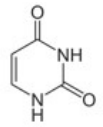
1. Uracil
2. 4-Hydroxycoumarin
3. Coumarin
4. 6-Chloro-4-hydroxycoumarin
5. Warfarin
6. Coumatetralyl
7. Coumachlor

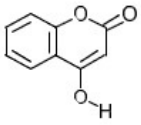
The coumarins are potent blood anticoagulants that can be used to prevent heart attacks and strokes and in large doses act as poisons for rats and mice. In this separation six coumarins are analyzed in less than two minutes on a HALO® Phenyl-Hexyl column. The high efficiency of the Fused-Core® particles at high flow rates makes this possible.

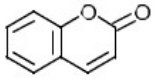
TEST CONDITIONS:

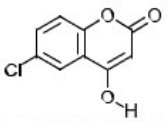
Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 40/60 - A/B
A: 0.1% formic acid in water, pH 2.66
B: 50/50 methanol/acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 215 bar
Temperature: 45 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 methanol/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

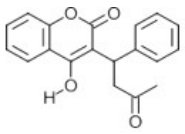
STRUCTURES:

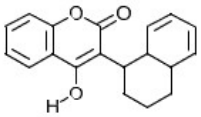

Uracil

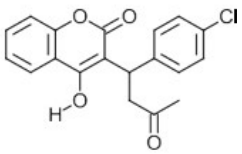

4-Hydroxycoumarin


Coumarin


6-Chloro-4-hydroxycoumarin

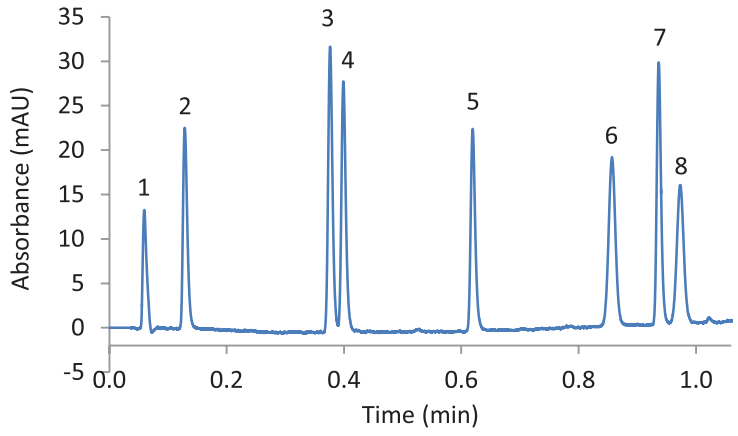

Warfarin


Coumatetralyl


Coumachlor

Separation of Anticoagulants Using HALO 90 Å C18, 2.0 µm

150-P



PEAK IDENTITIES:

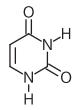
1. Uracil (t_0)
2. 6,7-Dihydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 6-Chloro-4-hydroxycoumarin
6. Warfarin
7. Coumatetralyl
8. Coumachlor

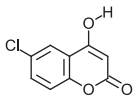
Anticoagulants are used to slow down and even prevent blood coagulation. Here, a HALO 90 Å C18, 2.0 µm column is used to separate a mixture of seven different types of anticoagulant drugs in under 1 minute.

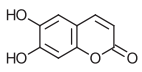
TEST CONDITIONS:

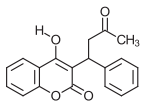
Column: HALO 90 Å C18, 2.0 µm, 2.1 x 30 mm
Part Number: 91812-302
Mobile Phase:
A: 0.02 M formic acid
B: 50/50 acetonitrile/methanol
Gradient: Hold at 20% B until 0.06 min
20-75% B from 0.06-1.06 min
Flow Rate: 1.1 mL/min
Pressure: 430 bar
Temperature: 45 °C
Detection: UV 254 nm, PDA
Injection Volume: 0.2 µL
Acquisition Rate: 200 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

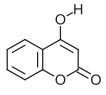
STRUCTURES:

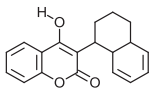

Uracil

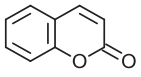

6-Chloro-4-hydroxycoumarin

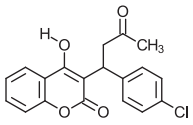

6,7-Dihydroxycoumarin


Warfarin


4-Hydroxycoumarin

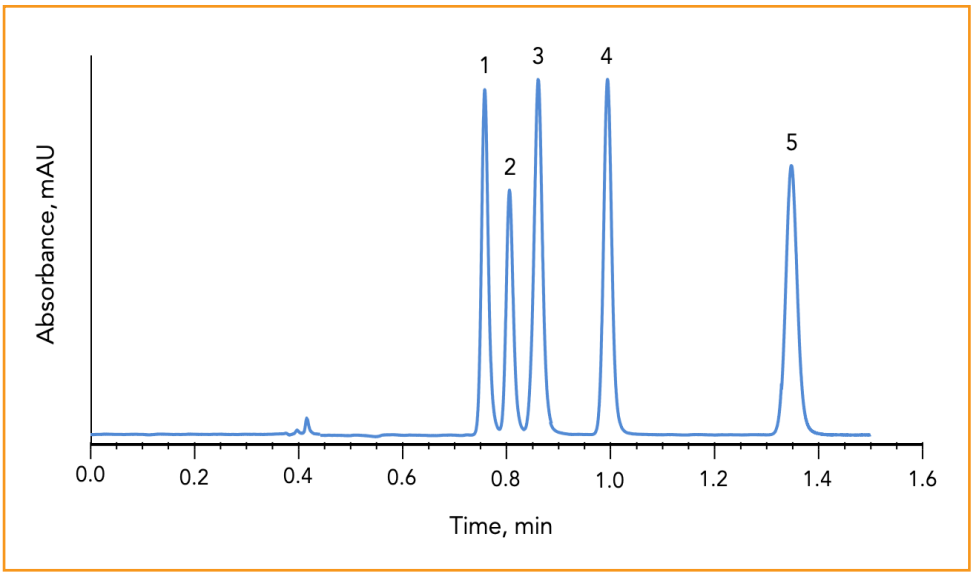

Coumatetralyl


Coumarin


Coumachlor

Separation of Antidepressants on HALO® Penta-HILIC Stationary Phase

Application Note 67-AD



PEAK IDENTITIES:

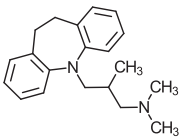
1. Trimipramine
2. Amitriptyline
3. Doxepin
4. Nortriptyline
5. Amoxapine

Basic drugs such as antidepressants can be rapidly separated under HILIC conditions with good peak shape using HALO® Penta-HILIC stationary phase.

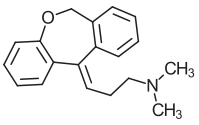
TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-605
Mobile Phase: 7/93 - A/B
A: 0.1 M ammonium formate, pH 3.5
B: Acetonitrile
Flow Rate: 2.5 mL/min
Pressure: 165 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 10/90 water/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

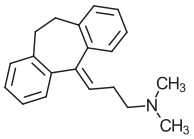
STRUCTURES:



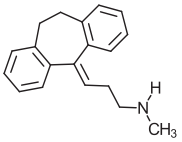
Trimipramine



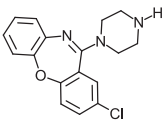
Doxepin



Amitriptyline



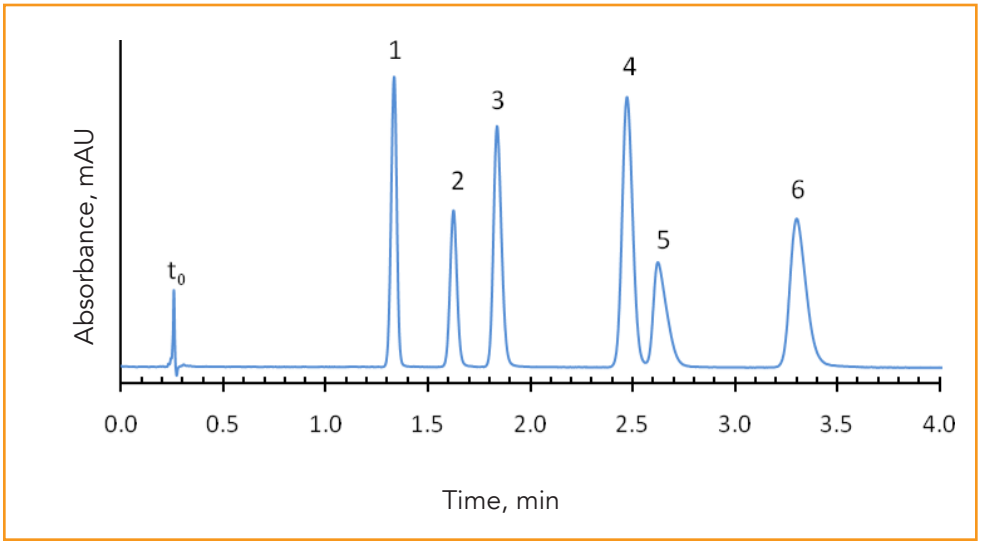
Nortriptyline



Amoxapine

Isocratic Separation of Basic Drugs on HALO® PFP

Application Note 22-B



PEAK IDENTITIES:

1. Phenylephrine
2. Trazodone
3. Procaine
4. Amoxapine
5. Propranolol
6. Desipramine

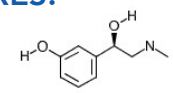
The strong retention of these basic drugs on HALO® PFP allows the use of mobile phases with high organic content which enhances sensitivity when doing LCMS.

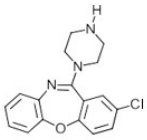
The high efficiency of HALO® Fused-Core® packings ensures that peaks will be sharp and elute in small volumes.

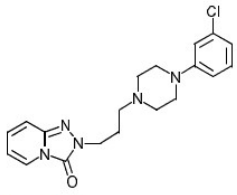
TEST CONDITIONS:

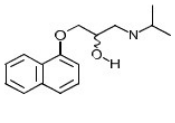
Column: HALO 90 Å PFP, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-409
Mobile Phase: 12/88 - A/B
A: 0.01 M ammonium formate buffer, pH 3.0
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 101 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 75/25 water/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

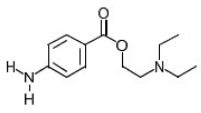
STRUCTURES:

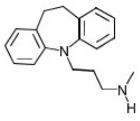

Phenylephrine


Amoxapine


Trazadone

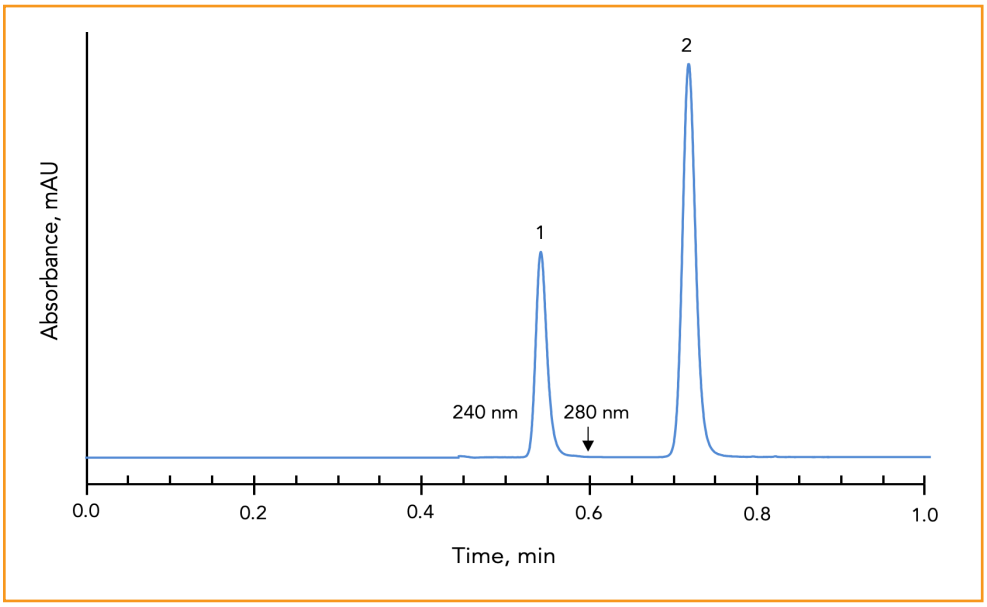

Propranolol


Procaine


Desipramine

Isocratic Separation of Amphenicols on HALO® Phenyl-Hexyl Phase

Application Note 57-AM



PEAK IDENTITIES:

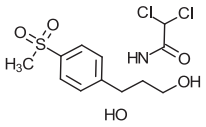
1. Thiamphenicol
2. Chloramphenicol

This separation shows a rapid HPLC method for the analysis of amphenicols on HALO® Phenyl-Hexyl stationary phase. To improve the sensitivity of detection, the first peak was monitored at 240 nm and the second at 280 nm.

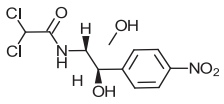
TEST CONDITIONS:

Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 55/45 - A/B
A: 0.025 M ammonium acetate buffer, pH 5.8
B: Acetonitrile
Flow Rate: 1.0 mL/min
Pressure: 94 bar
Temperature: 35 °C
Detection: UV 240/280 nm, VWD
Injection Volume: 0.3 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

STRUCTURES:



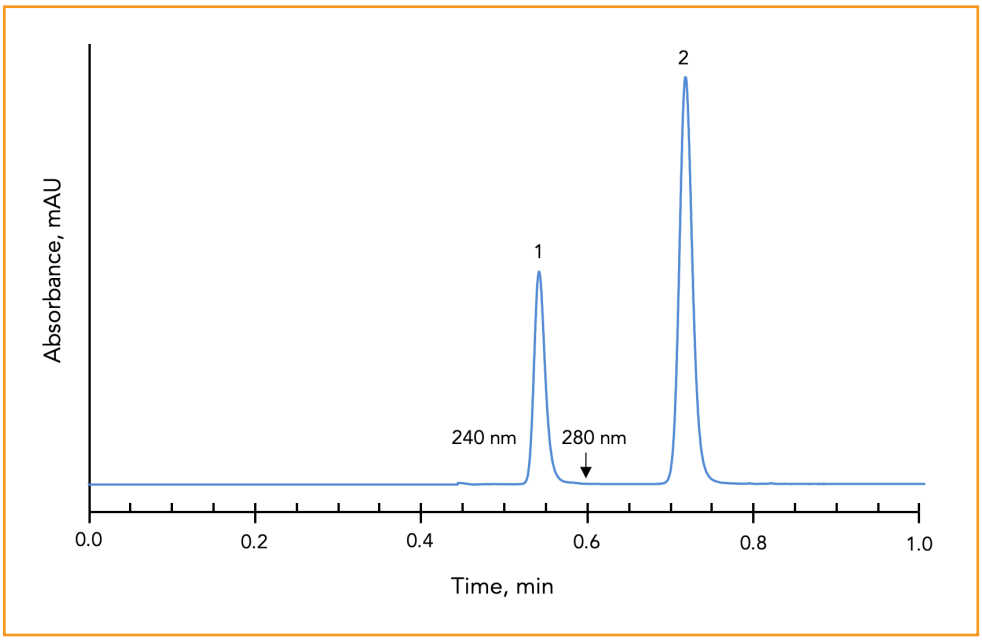
Thiamphenicol



Chloramphenicol

Isocratic Separation of Amphenicols on HALO® RP-Amide Phase

Application Note 58-AM



PEAK IDENTITIES:

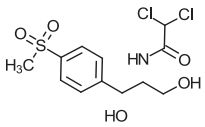
1. Thiamphenicol
2. Chloramphenicol

This separation shows a rapid HPLC method for the analysis of amphenicols using HALO® RP-Amide phase. To improve the sensitivity of detection, the first peak was monitored at 240 nm and the second at 280 nm.

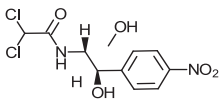
TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 55/45 - A/B
A: 0.025 M Ammonium acetate buffer, pH 5.8
B: Acetonitrile
Flow Rate: 1.0 mL/min
Pressure: 92 bar
Temperature: 35 °C
Detection: UV 240/280 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

STRUCTURES:



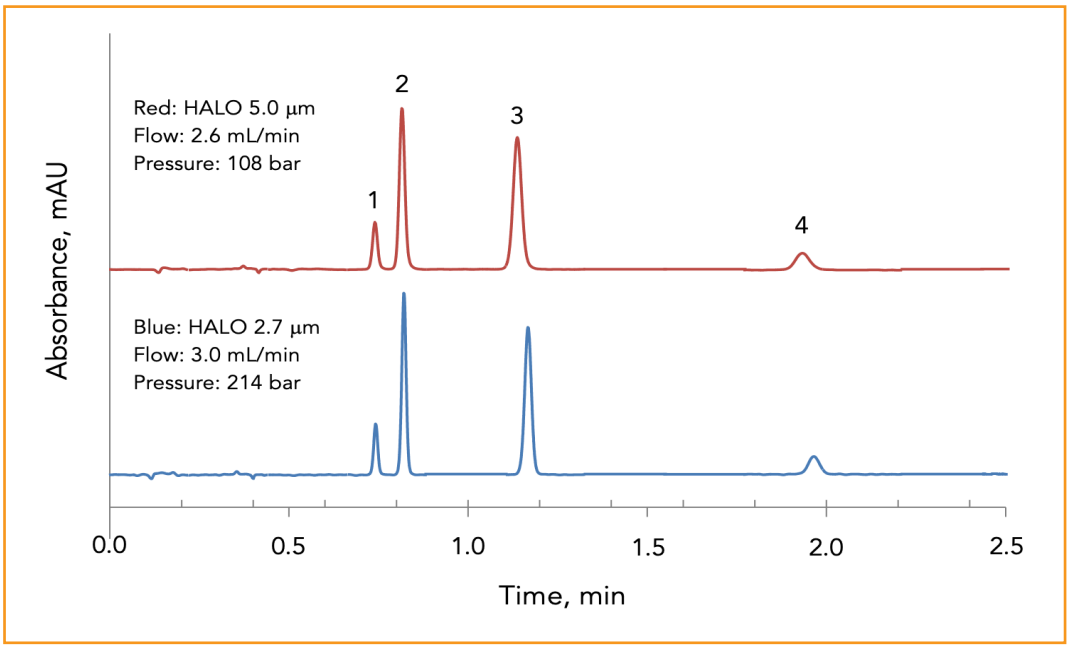
Thiamphenicol



Chloramphenicol

Comparable Selectivity Between HALO® HILIC, 5.0 µm and HALO® HILIC, 2.7 µm

Application Note 88-B



PEAK IDENTITIES:

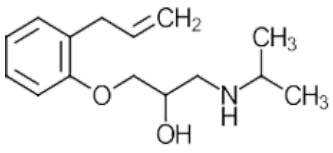
1. Alprenolol
2. Pindolol
3. Acebutolol
4. Atenolol

These drugs are β-blockers used to treat high blood pressure. This separation illustrates easy method transfer between the 5.0 µm and 2.7 µm HALO® HILIC phases after small changes in flow rate.

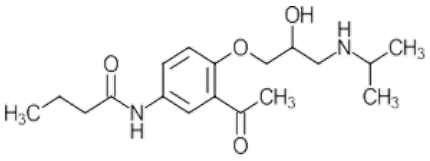
TEST CONDITIONS:

Columns:
1) HALO 90 Å HILIC, 5.0 µm, 4.6 x 100 mm
Part Number: 95814-601
2) HALO 90 Å HILIC, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-601
Mobile Phase: 11/89 - A/B
A: 0.1 M ammonium formate, pH 3.0
B: Acetonitrile
Flow Rate: See chart
Pressure: See chart
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: Mobile phase
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

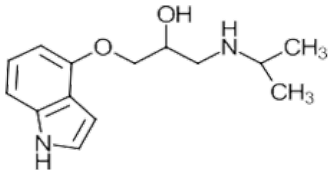
STRUCTURES:



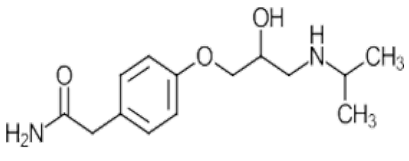
Alprenolol



Acebutolol



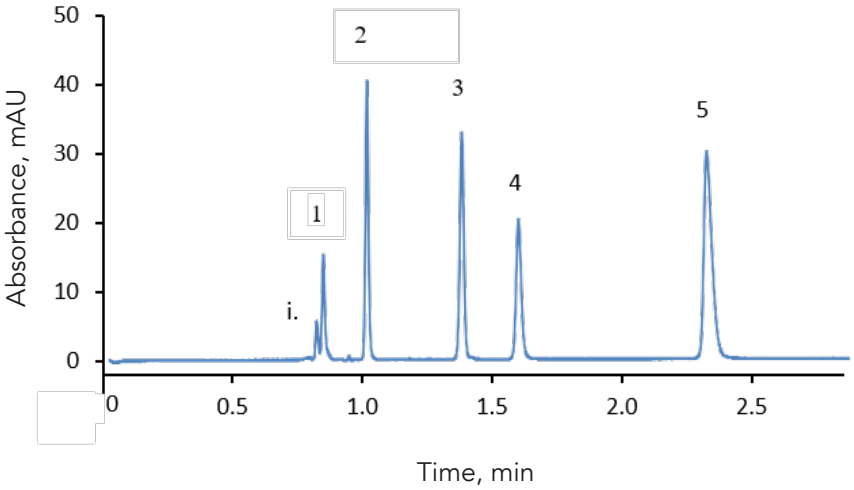
Pindolol



Atenolol

Separation of OTC Common Cold Medicinal Compounds

Application Note 152-CM



PEAK IDENTITIES:

1. Maleic acid
2. Acetaminophen
3. Guaifenesin
4. Chlorpheniramine maleate
5. Dextromethorphan HBr

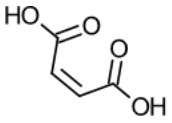
i. Impurity from Dextromethorphan HBr

Acetaminophen (analgesic), guaifenesin (expectorant), chlorpheniramine maleate (antihistamine), and dextromethorphan (cough suppressant) are common compounds found in many over-the-counter (OTC) cold medicines. A HALO 90 Å, C18 2.7 µm column is used to separate these compounds quickly and accurately under isocratic conditions.

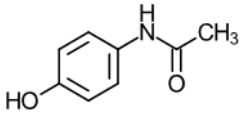
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-702
Mobile Phase:
A: 50 mM potassium phosphate buffer, pH 2.5
B: Acetonitrile
Isocratic: 30% B
Flow Rate: 1.5 mL/min
Pressure: 266 bar
Temperature: 45 °C
Detection: UV 220 nm, PDA
Injection Volume: 0.5 µL
Aquisition Rate: 40 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Agilent 1200 SL

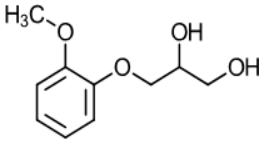
STRUCTURES:



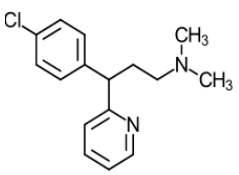
Maleic Acid



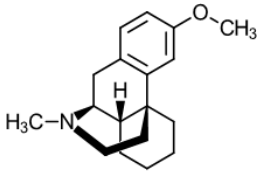
Acetaminophen



Guaifenesin



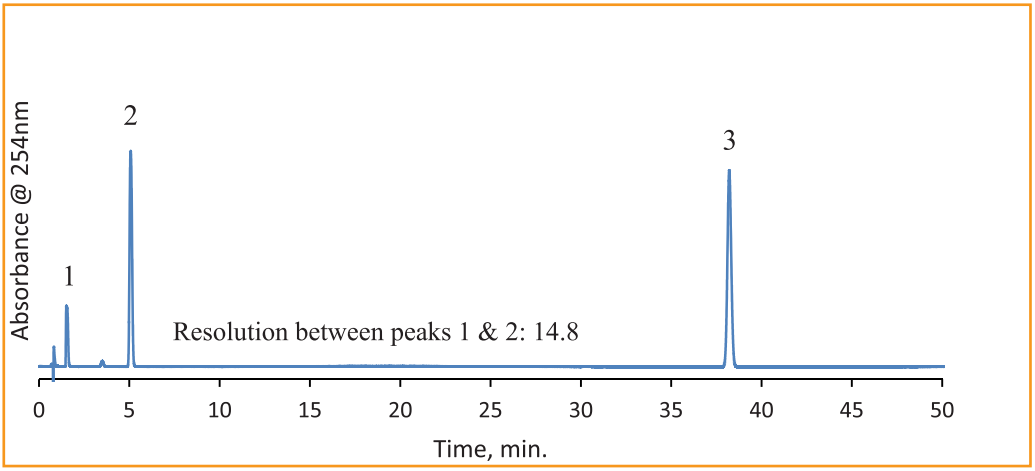
Chlorpheniramine Maleate



Dextromethorphan HBr

Separation of Paracetamol and Impurities According to EP 9.4

Application Note 171-EP



PEAK IDENTITIES:

1. 4-Aminophenol (Impurity K)
2. Paracetamol
3. N-(4-Chlorophenyl) acetamide (Impurity J)

A HALO® C18 column is used to separate paracetamol and two of its impurities following the European Pharmacopoeia 9.4 monograph for paracetamol. This method is used to examine several paracetamol impurities providing high resolution between peaks while leaving sufficient separation in the baseline for any other impurity or degradant peaks that may be present in a sample.

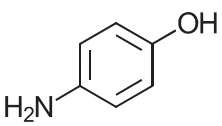
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-602
Mobile Phase:
A: 20 mM potassium phosphate buffer
B: Methanol
Gradient:

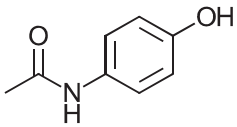
Time (min)	% B
0-1	5
1-10	5-10
10-20	10
20-40	10-34
40-50	34

Flow Rate: 0.3 mL/min
Pressure: 171 bar
Temperature: 30 °C
Detection: UV 254 nm, PDA
Injection Volume: 5.0 µL
Sample Solvent: 5/95 methanol/water
Data Rate: 40 Hz
Response Time: 0.005 sec
Flow Cell: 2.0 µL
LC System: Agilent 1200 SL

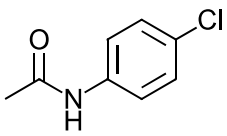
STRUCTURES:



4-aminophenol



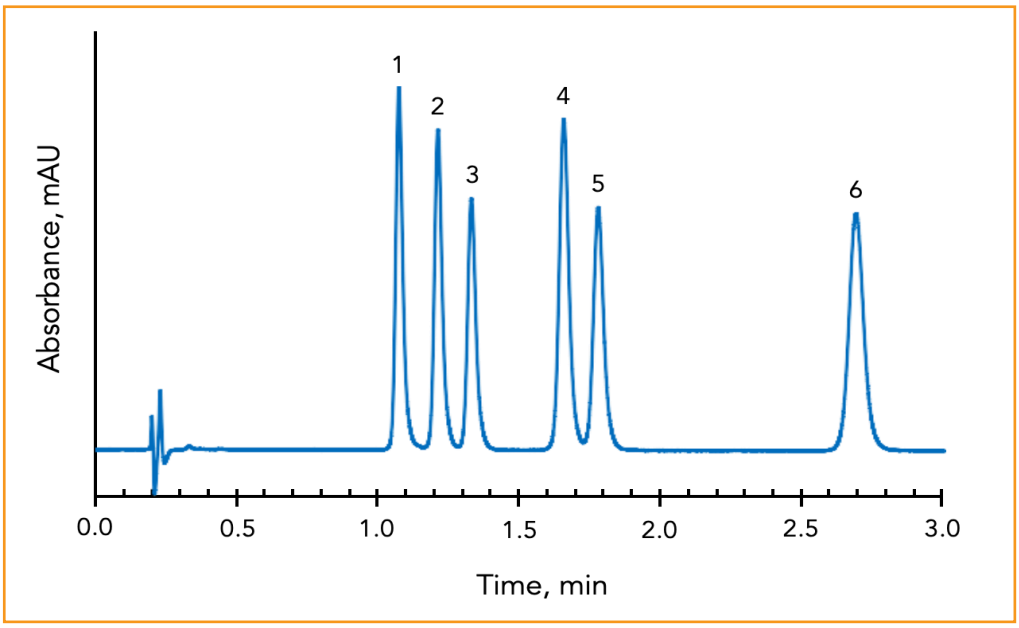
Paracetamol



N-(4-chlorophenyl) acetamide

Benzodiazepines Separation on HALO 90 Å Phenyl-Hexyl, 2.0 µm

Application Note 129-BZ



PEAK IDENTITIES:

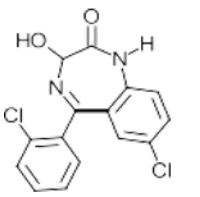
1. Lorazepam
2. Alprazolam
3. Clonazepam
4. Temazepam
5. Flunitrazepam
6. Diazepam

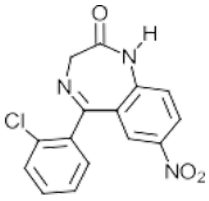
These six benzodiazepines are baseline resolved on a HALO® 2.0 µm Phenyl-Hexyl column. The π-π interactions between the Phenyl-Hexyl phase and these anti-anxiety drugs help to enhance the separation.

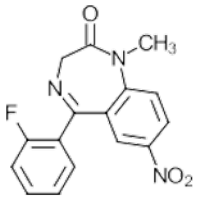
TEST CONDITIONS:

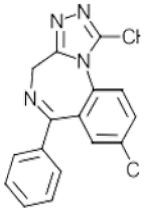
Column: HALO 90 Å Phenyl-Hexyl, 2.0 µm, 2.1 x 50 mm
Part Number: 91812-406
Mobile Phase: 62.5/37.5 - A/B
A: Water with 0.1% formic acid/0.01 M ammonium formate, pH 3.3
B: 80/20 acetonitrile/water with 0.1% formic acid/0.01 M ammonium formate
Flow Rate: 0.55 mL/min
Pressure: 311 bar
Temperature: 35 °C
Detection: UV 254 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: 30/70 water/acetonitrile
Data Rate: 80 Hz
Response Time: 0.02 sec
Flow Cell: 2.0 µL semi-micro
LC System: Agilent 1200 SL

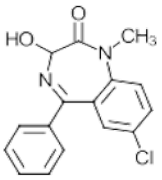
STRUCTURES:

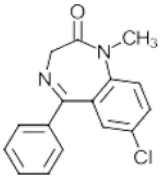

Lorazepam


Clonazepam


Flunitrazepam

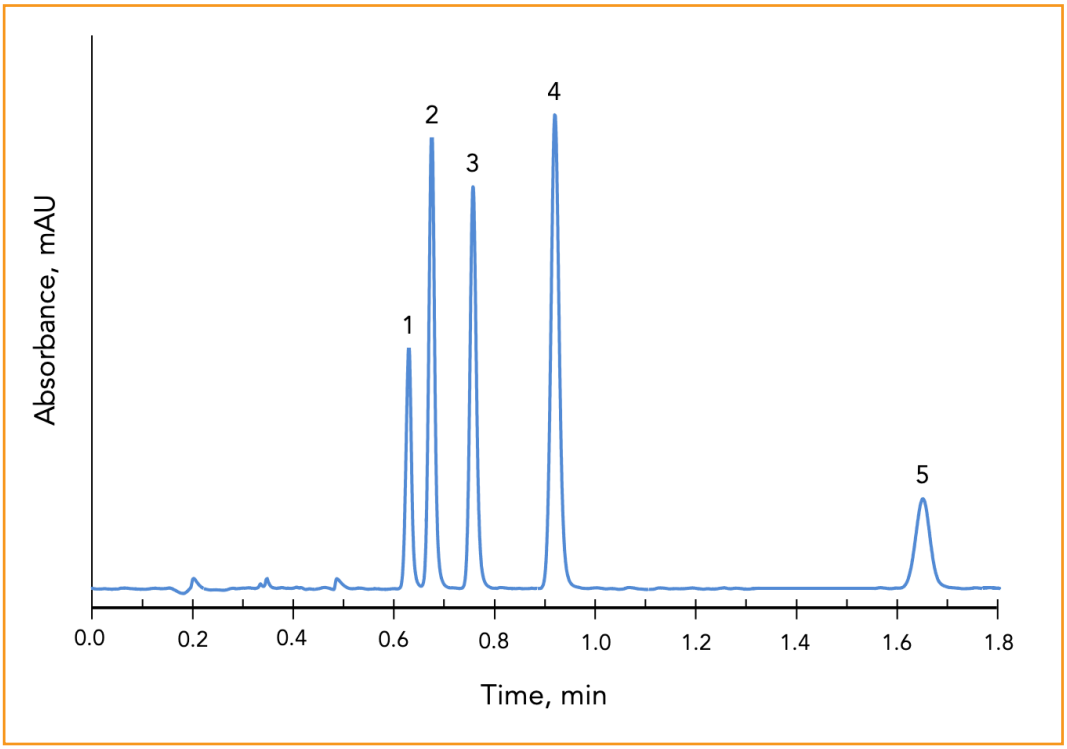

Alprazolam


Temazepam


Diazepam

Separation of Five Beta Blocker Drugs on HALO® Penta-HILIC

Application Note 64-B



PEAK IDENTITIES:

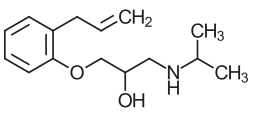
1. Alprenolol
2. Propranolol
3. Pindolol
4. Acebutolol
5. Atenolol

The HALO® Penta-HILIC stationary phase can rapidly separate highly basic compounds with good peak shapes in a mass spectrometry friendly mobile phase.

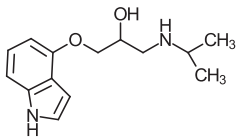
TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-605
Mobile Phase: 10/90 - A/B
A: 0.04 M ammonium formate buffer, pH 3.0
B: Acetonitrile
Flow Rate: 3.0 mL/min
Pressure: 215 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: Mobile phase
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

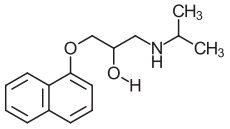
STRUCTURES:



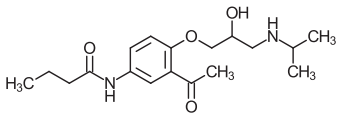
Alprenolol



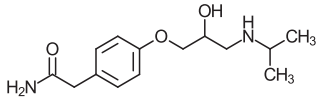
Pindolol



Propranolol



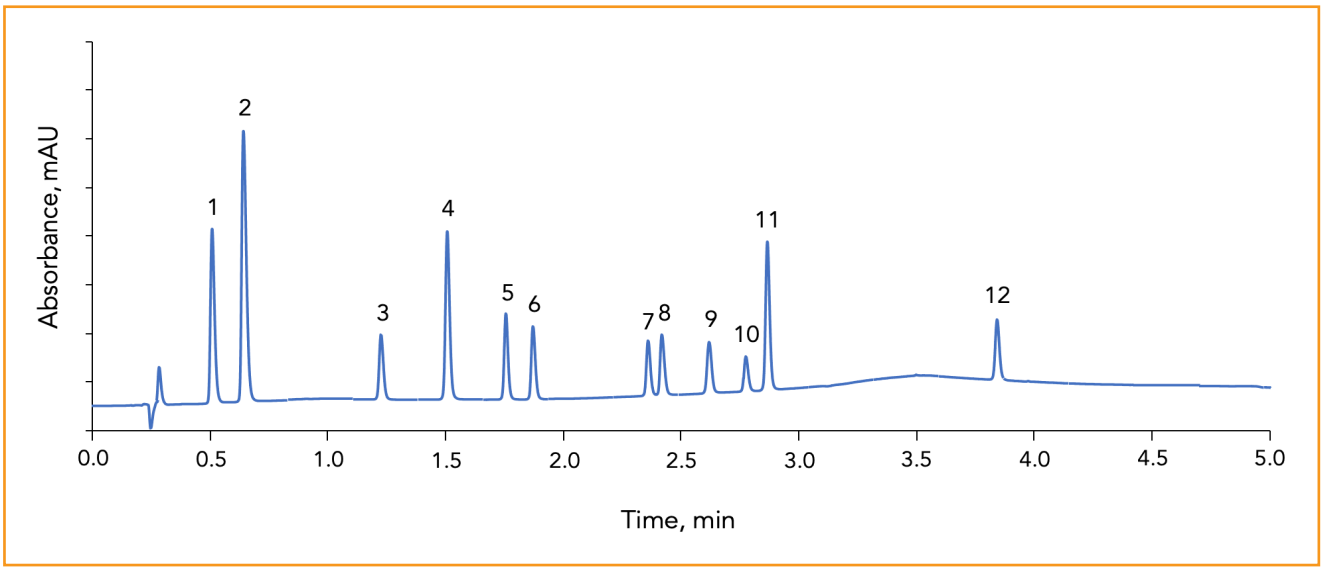
Acebutolol



Atenolol

Separation of Beta Blockers on HALO Biphenyl, 2.0 µm

Application Note 195-B



A mixture of twelve beta blockers is separated on a HALO® 2.0 µm Biphenyl column with excellent speed and resolution. Beta blockers are mainly used to treat irregular heart beats or complications with the heart such as heart attacks. Beta blockers are also known to help treat high blood pressure.

TEST CONDITIONS:

Column: HALO 90 Å Biphenyl, 2.0 µm, 2.1 x 50 mm
Part Number: 91812-411
Mobile Phase:
A: Water, 0.1% TFA
B: Acetonitrile, 0.05% TFA

Gradient:

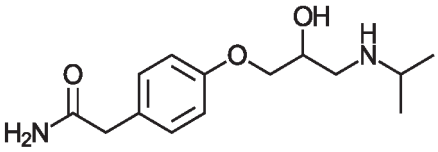
Time (min)	% B
0.0	10
5.0	50

Flow Rate: 0.5 mL/min
Initial Pressure: 272 bar
Temperature: 35 °C
Detection: UV 220 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: Water
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

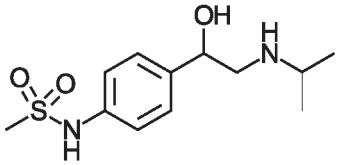
PEAK IDENTITIES:

1. Atenolol
2. Sotalol
3. Nadolol
4. Pindolol
5. Acebutolol
6. Metoprolol
7. Bisoprolol
8. Oxprenolol
9. Labetalol
10. Alprenolol
11. Propranolol
12. Carvedilol

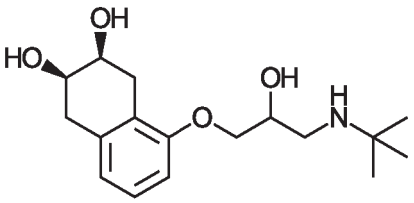
STRUCTURES:



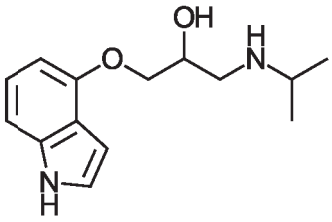
Atenolol



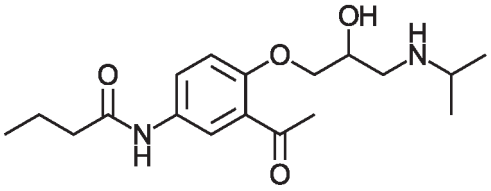
Sotalol



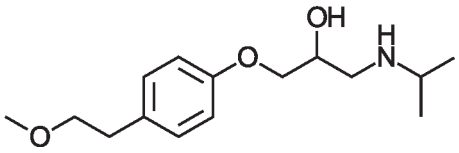
Nadolol



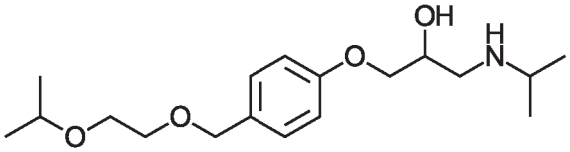
Pindolol



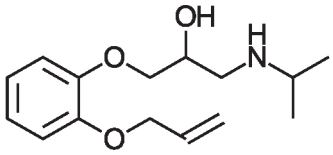
Acebutolol



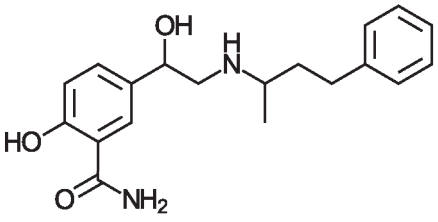
Metoprolol



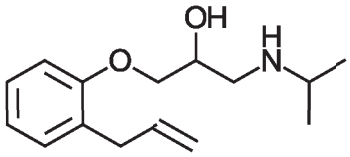
Bisoprolol



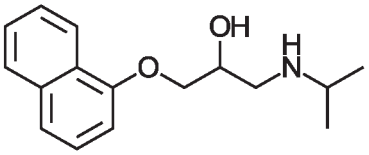
Oxprenolol



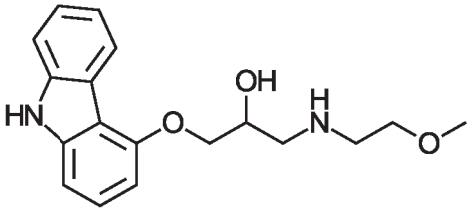
Labetalol



Alprenolol



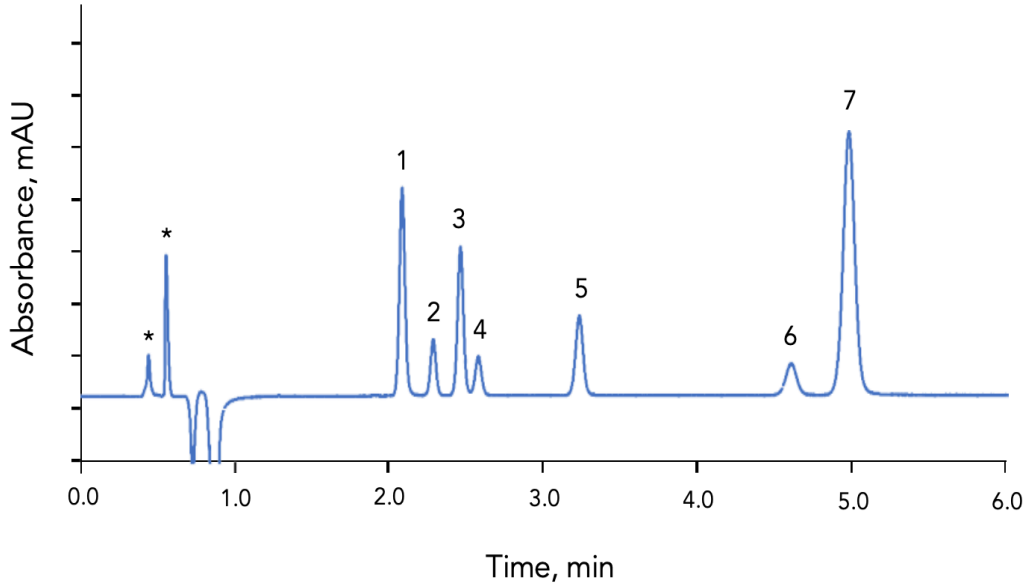
Propranolol



Carvedilol

Separation of Beta Blockers on HALO® Penta-HILIC, 2.0 µm

Application Note 196-B



PEAK IDENTITIES:

1. Carvedilol
2. Oxprenolol
3. Propranolol
4. Bisoprolol
5. Pindolol
6. Acebutolol
7. Sotalol

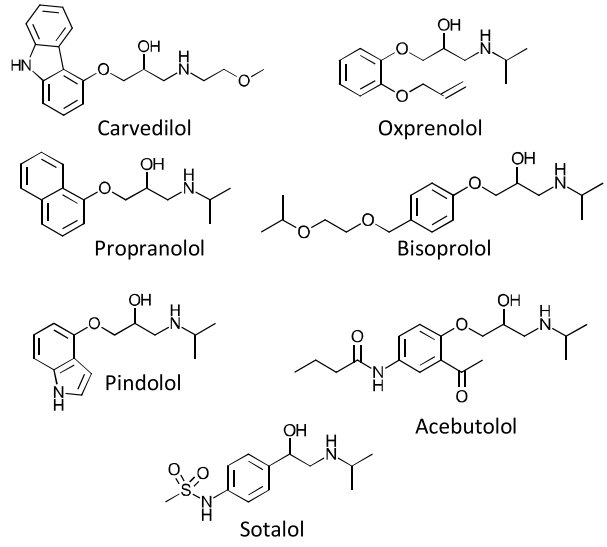
* artifact peaks from ammonium formate

A mixture of seven beta blockers is rapidly separated on a HALO® 2.0 µm Penta-HILIC column with excellent resolution. Beta blockers are mainly used to treat irregular heartbeats or complications with the heart such as heart attacks. They can also help treat high blood pressure.

TEST CONDITIONS:

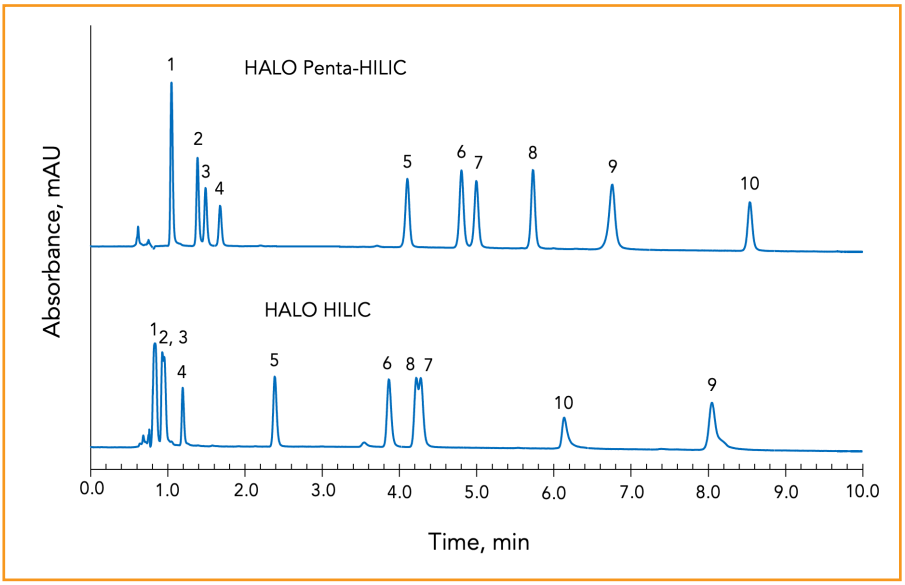
Column: HALO 90 Å Penta-HILIC, 2.0 µm, 2.1 x 100 mm
Part Number: 91812-605
Isocratic: 97/3 acetonitrile/0.1 M ammonium formate, pH 3.0
Flow Rate: 0.5 mL/min
Initial Pressure: 231 bar
Temperature: 25 °C
Detection: UV 220 nm, PDA
Injection Volume: 5.0 µL
Sample Solvent: Acetonitrile
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

STRUCTURES:



Separation of Cephalosporins on HALO® Penta-HILIC and HALO® HILIC

Application Note 68-AB



PEAK IDENTITIES:

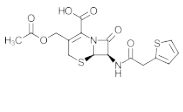
1. Cephalothin
2. Cefoxitin
3. Cefotaxime
4. Cefazolin
5. Cefaclor
6. Cephalexin
7. Cephradine
8. Cefadroxil
9. Ceftazidime
10. Cephalosporin C

The class of antibiotics called cephalosporins are β-lactam drugs that are used to treat streptococcus and staphylococcus infections. Analyzing these drugs using the HALO® Penta-HILIC phase offers an alternate selectivity to reversed-phase separations.

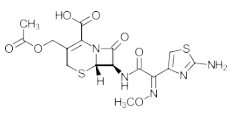
TEST CONDITIONS:

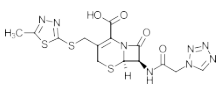
Columns:
1) HALO 90 Å Penta-HILIC, 2.7 µm, 2.1 x 150 mm
Part Number: 92812-705
2) HALO 90 Å HILIC, 2.7 µm, 2.1 x 150 mm
Part Number: 92812-701
Mobile Phase:
A: 95/5 ACN/H_2O with 5 mM NH_4formate, pH 3.0
B: 50/50 ACN/H_2O with 5 mM NH_4formate, pH 3.0 (adj.)
Gradient: 85-65% B in 10 min (Penta-HILIC)
85-70% B in 10 min (HILIC)
Flow Rate: 0.5 mL/min
Pressure: 195 bar (Penta-HILIC)
163 bar (HILIC)
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 50/50 ACN/water
Flow Cell: 5.0 µL semi-micro
LC System: Agilent 1100

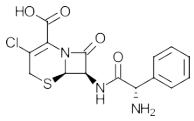
STRUCTURES:

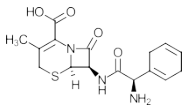

Cephalothin

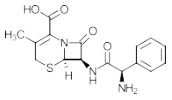

Cefoxitin

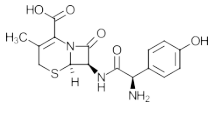

Cefotaxime

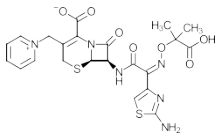

Cefazolin

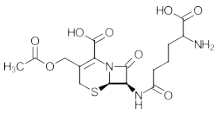

Cefaclor


Cephalexin


Cephradine

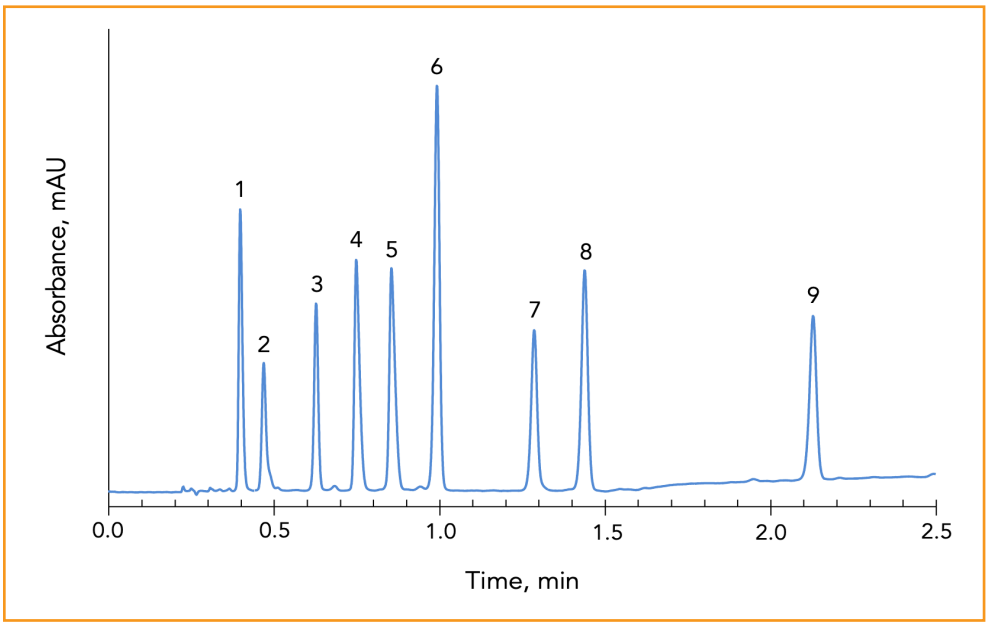

Cefadroxil


Ceftazidime


Cephalosporin C

Separation of Cephalosporins on HALO® Phenyl-Hexyl

Application Note 70-AB



PEAK IDENTITIES:

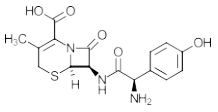
1. Cefadroxil
2. Ceftazidime
3. Cefaclor
4. Cephalexin
5. Cephradine
6. Cefotaxime
7. Cefazolin
8. Cefoxitin
9. Cephalothin

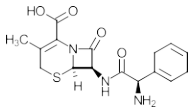
Cephalosporins are a class of β-lactam drugs. These cephalosporins can be rapidly analyzed by reversed-phase HPLC on a HALO® Fused-Core® Phenyl-Hexyl bonded phase column.

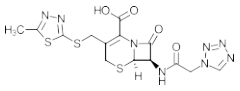
TEST CONDITIONS:

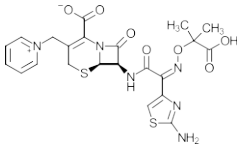
Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase:
A: 0.1% formic acid
B: 50/50 acetonitrile/methanol
Gradient: 18% B to 45% B in 2.0 min, hold for 1 min
Flow Rate: 2.0 mL/min
Initial Pressure: 225 bar
Temperature: 40 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 70/30 water/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

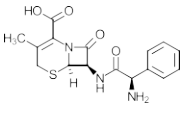
STRUCTURES:

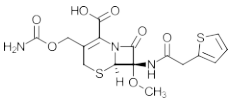

Cefadroxil

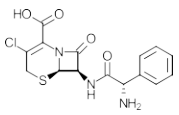

Cephalexin

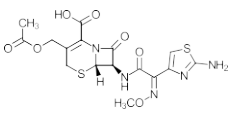

Cefazolin

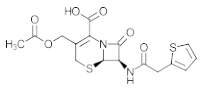

Ceftazidime


Cephradine


Cefoxitin

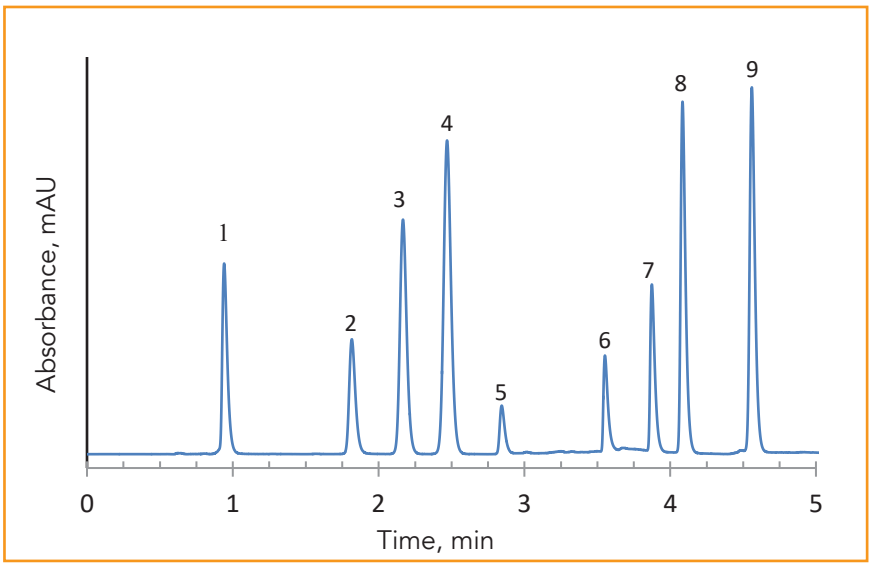

Cefaclor


Cefotaxime


Cephalothin

HPLC Separation of Diuretics on HALO® Phenyl-Hexyl

Application Note 78-DU



PEAK IDENTITIES:

1. Amiloride
2. Caffeine
3. Chlorothiazide
4. Hydrochlorothiazide
5. Triamterene
6. Torsemide
7. Furosemide
8. Indapamide
9. Bumetanide

This separation illustrates the utility of HALO® Fused-Core® Phenyl-Hexyl phase in the rapid analysis of common diuretics.

TEST CONDITIONS:

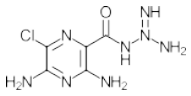
Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-606
Mobile Phase:
A: 0.02 M potassium phosphate buffer, pH 3.0
B: Acetonitrile

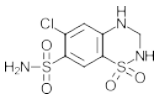
Gradient:

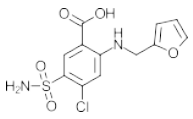
Time (min)	% B
0.0	15
1.7	15
3.0	50
7.0	60

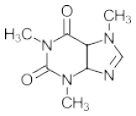
Flow Rate: 1.5 mL/min
Initial Pressure: 253 bar
Temperature: 30 °C
Detection: UV 230 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

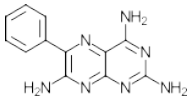
STRUCTURES:

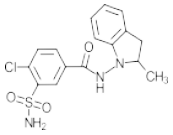

Amiloride

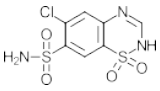

Hydrochlorothiazide

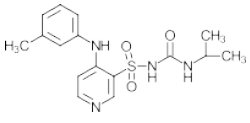

Furosemide

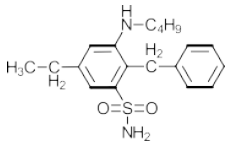

Caffeine


Triamterene


Indapamide

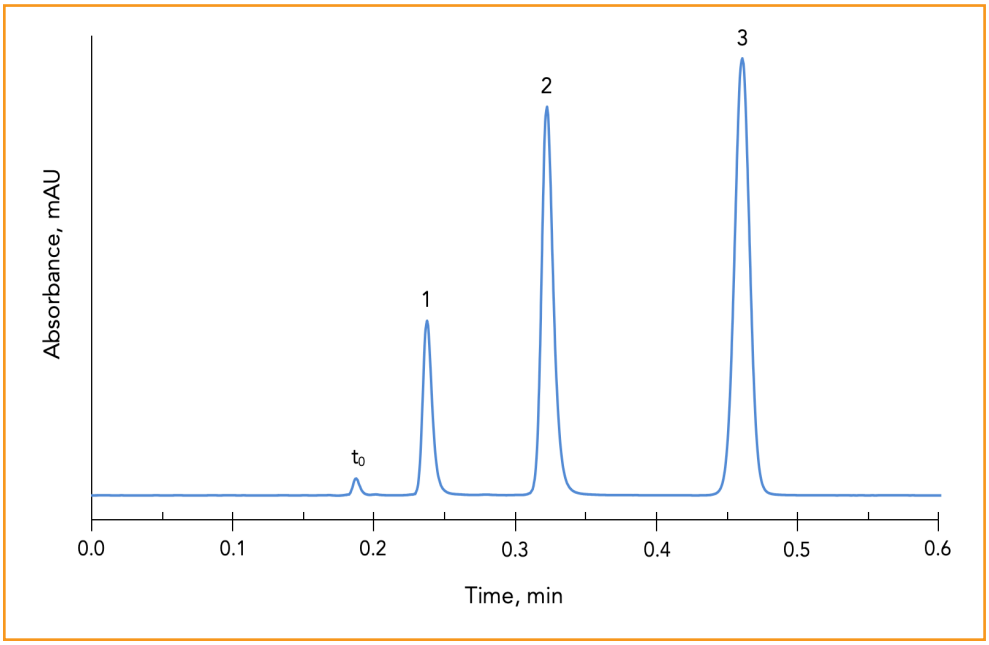

Chlorothiazide


Torsemide


Bumetanide

Rapid Isocratic Separation of Fibrates on HALO® PFP Phase

Application Note 28-P



PEAK IDENTITIES:

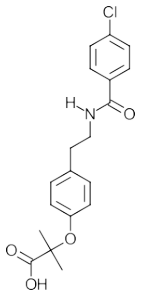
1. Bezafibrate
2. Gemfibrozil
3. Fenofibrate

Fibrates are a class of cholesterol lowering drugs that can be rapidly analyzed using HALO® PFP phase to obtain widely separated peaks in under 30 seconds.

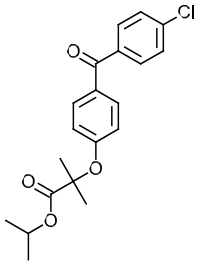
TEST CONDITIONS:

Column: HALO 90 Å PFP, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-409
Mobile Phase: 30/70 - A/B
A: 0.02 M phosphate buffer, pH 3.0
B: Acetonitrile
Flow Rate: 2.5 mL/min
Pressure: 160 bar
Temperature: 45 °C
Detection: UV 220 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 50/50 methanol/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

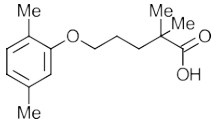
STRUCTURES:



Bezafibrate



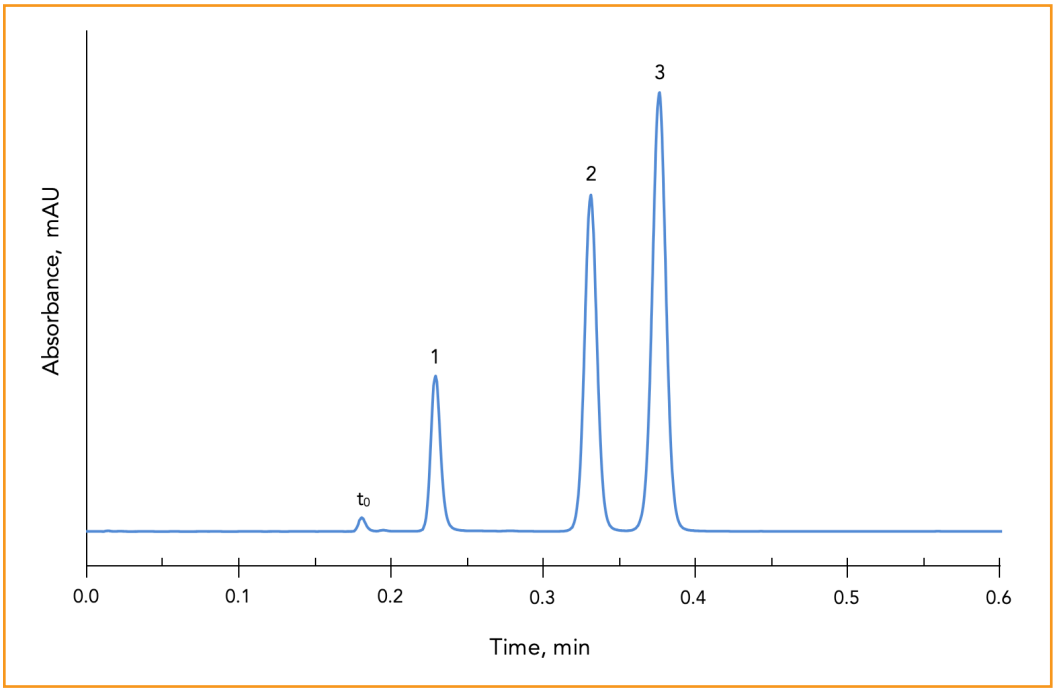
Fenofibrate



Gemfibrozil

Rapid Isocratic Separation of Fibrates on HALO® RP-Amide Phase

Application Note 29-P



PEAK IDENTITIES:

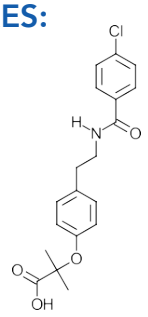
1. Bezafibrate
2. Gemfibrozil
3. Fenofibrate

Fibrates are a class of cholesterol lowering drugs that can be rapidly analyzed using HALO® RP-Amide phase to obtain well-separated peaks in under 25 seconds.

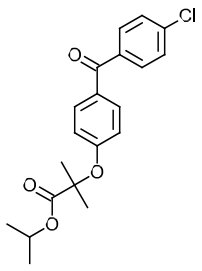
TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 20/80 - A/B
A: 0.02 M phosphate buffer, pH 3.0
B: Acetonitrile
Flow Rate: 2.5 mL/min
Pressure: 135 bar
Temperature: 45 °C
Detection: UV 220 nm, VWD
Injection Volume: 0.3 µL
Sample Solvent: 50/50 methanol/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

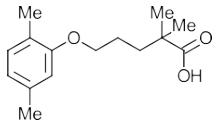
STRUCTURES:



Bezafibrate



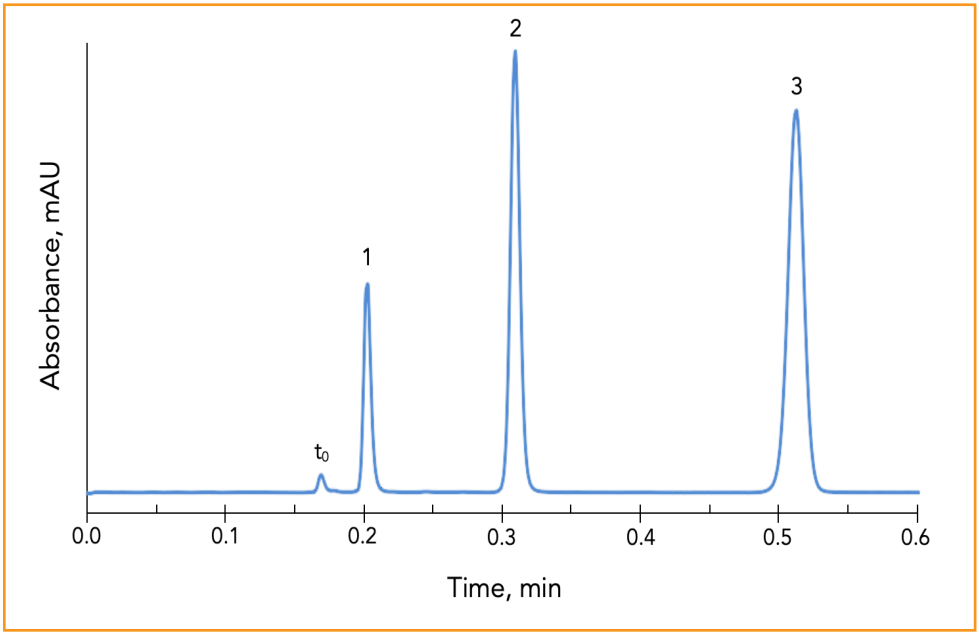
Fenofibrate



Gemfibrozil

Rapid Isocratic Separation of Fibrates on HALO® C18 Phase

Application Note 30-P



PEAK IDENTITIES:

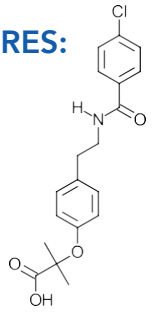
1. Bezafibrate
2. Gemfibrozil
3. Fenofibrate

Fibrates are a class of cholestrol lowering drugs that can be rapidly analyzed using HALO® C18 phase to obtain widely separated peaks in about 30 seconds.

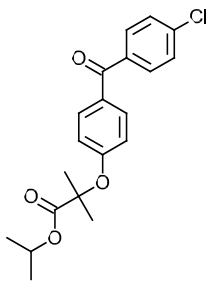
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 20/80 - A/B
A: 0.02 M phosphate buffer, pH 3.0
B: Acetonitrile
Flow Rate: 2.5 mL/min
Pressure: 150 bar
Temperature: 45 °C
Detection: UV 220 nm, VWD
Injection Volume: 0.3 µL
Sample Solvent: 50/50 methanol/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

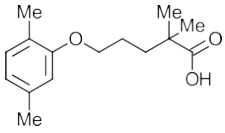
STRUCTURES:



Bezafibrate



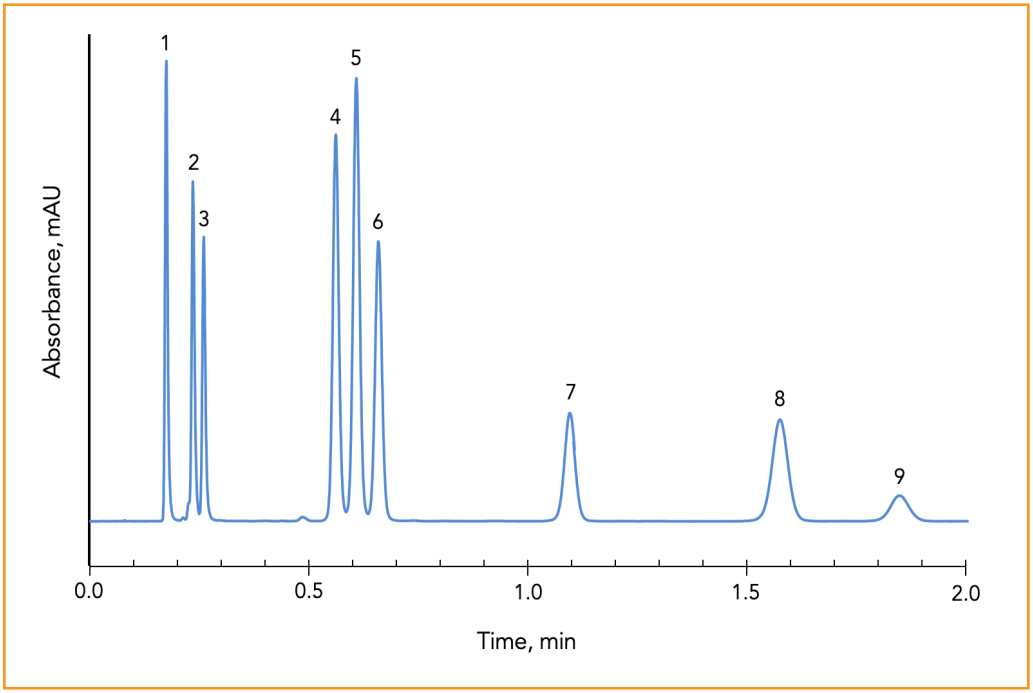
Fenofibrate



Gemfibrozil

Isocratic Separation of NSAIDs on HALO® C18

Application Note 13-NS



PEAK IDENTITIES:

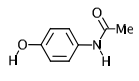
1. Acetaminophen
2. Aspirin
3. Salicylic acid
4. Tolmetin
5. Ketoprofen
6. Naproxen
7. Fenoprofen
8. Diclofenac
9. Ibuprofen

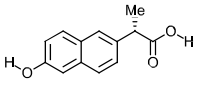
Non-steroidal antinflammatory drugs (NSAIDs) are commonly used for reduction of pain and inflammation. Here, a mixture of methanol and acetonitrile allow a better isocratic separation of this mixture than either solvent by itself as the modifier.

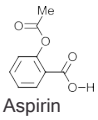
TEST CONDITIONS:

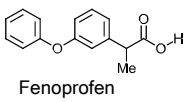
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 43/57 - A/B
A: 0.02 M sodium phosphate buffer, pH 2.5
B: 50/50 methanol/ACN
Flow Rate: 3.0 mL/min
Pressure: 338 bar
Temperature: 35 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 methanol/water
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

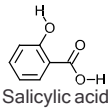
STRUCTURES:

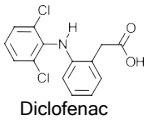

Acetaminophen

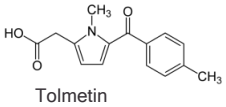

Naproxen

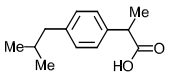

Aspirin

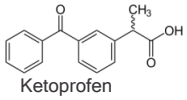

Fenoprofen


Salicylic acid


Diclofenac

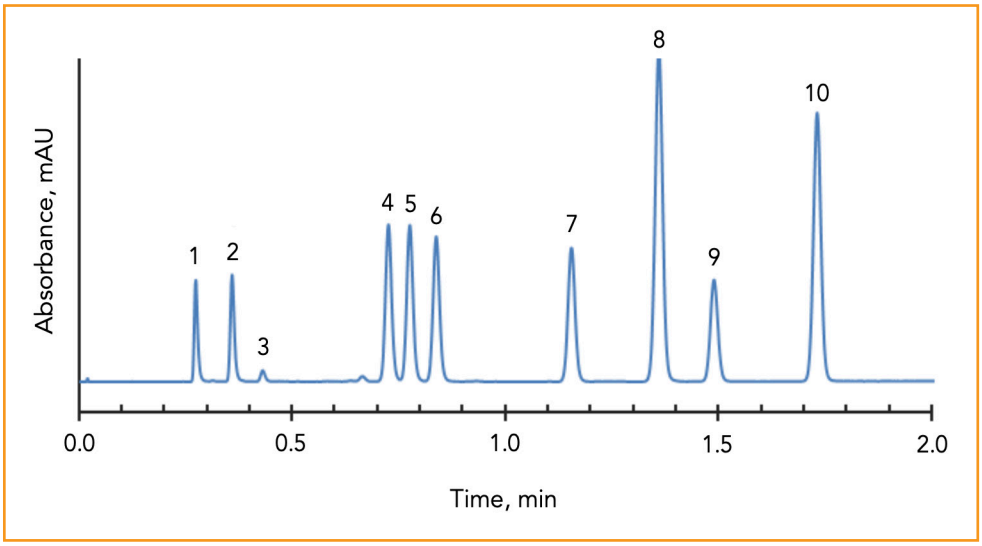

Tolmetin


Ibuprofen


Ketoprofen

Gradient Separation of NSAIDs on HALO® C8

Application Note 14-NS



PEAK IDENTITIES:

1. Acetaminophen
2. Aspirin
3. Salicylic acid
4. Tolmetin
5. Ketoprofen
6. Naproxen
7. Fenoprofen
8. Diclofenac
9. Ibuprofen
10. Mefenamic acid

Common pain and inflammation relievers are the non-steroidal anti-inflammatory drugs (NSAIDs). Using a gradient method, these popular drugs can be easily separated on the HALO® C8 phase in under two minutes.

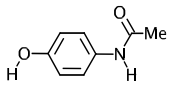
TEST CONDITIONS:

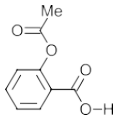
Column: HALO 90 Å C8, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-408
Mobile Phase: 38/62 - A/B (start)
A: 0.02 M sodium phosphate buffer, pH 2.5
B: Methanol
Gradient:

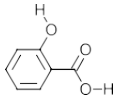
Time (min)	% B
0.0	62
0.1	62
2.0	85

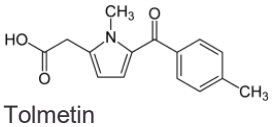
Flow Rate: 2.0 mL/min
Pressure: 286 bar
Temperature: 35 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Mobile phase
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

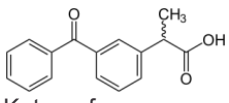
STRUCTURES:

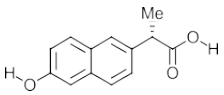

Acetaminophen

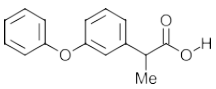

Aspirin

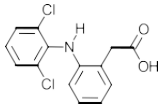

Salicylic acid

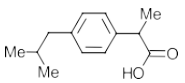

Tolmetin

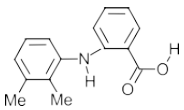

Ketoprofen


Naproxen


Fenoprofen

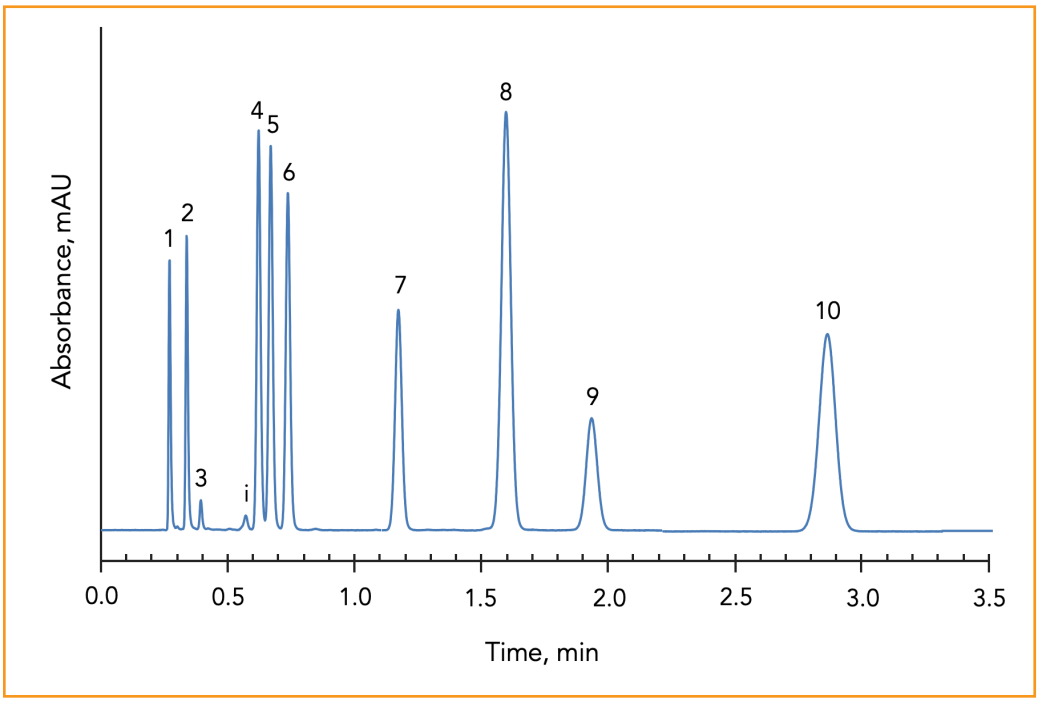

Diclofenac


Ibuprofen


Mefenamic acid

Separation of NSAIDs on HALO® C8

Application Note 15-NS



PEAK IDENTITIES:

1. Acetaminophen
2. Aspirin
3. Salicylic acid
4. Tolmetin
5. Ketoprofen
6. Naproxen
7. Fenoprofen
8. Diclofenac
9. Ibuprofen
10. Mefenamic acid

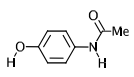
i = impurity

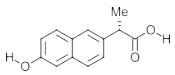
This isocratic separation of NSAIDs (non-steroidal antiinflammatory drugs) on HALO® C8 phase can be done in less than 3 minutes due to the fast flow rate and high efficiency of the Fused-Core® packing.

TEST CONDITIONS:

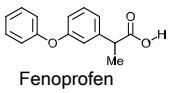
Column: HALO 90 Å C8, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-408
Mobile Phase: 35/65 - A/B
A: 0.02 M sodium phosphate buffer, pH 2.5
B: Methanol
Flow Rate: 2.0 mL/min
Pressure: 277 bar
Temperature: 35 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Mobile phase
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

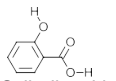
STRUCTURES:

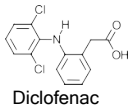

Acetaminophen

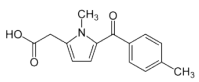

Naproxen

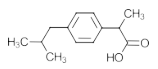

Aspirin

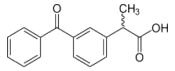

Fenoprofen

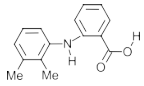

Salicylic acid


Diclofenac


Tolmetin

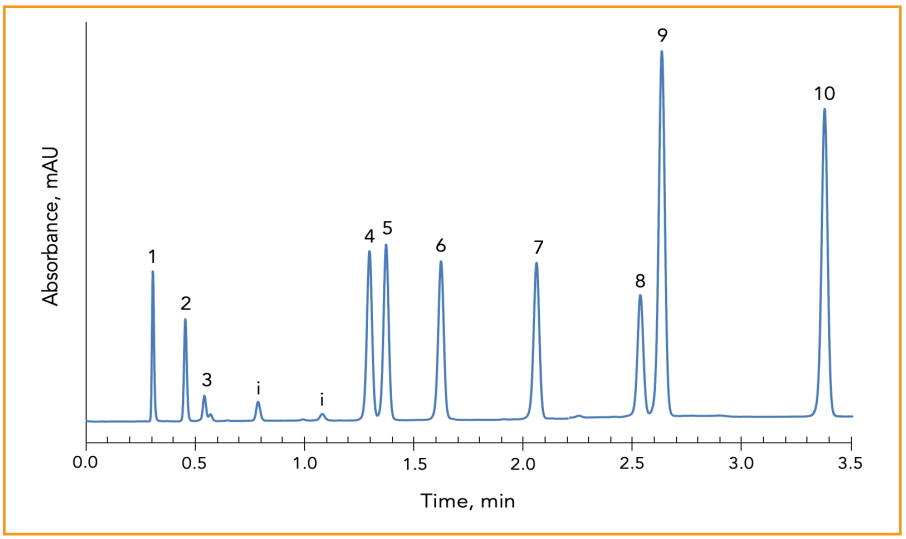

Ibuprofen


Ketoprofen


Mefenamic acid

Gradient Separation of NSAIDs on HALO® RP-Amide

Application Note 16-NS



PEAK IDENTITIES:

1. Acetaminophen
2. Aspirin
3. Salicylic acid
4. Tolmetin
5. Ketoprofen
6. Naproxen
7. Fenoprofen
8. Diclofenac
9. Ibuprofen
10. Mefenamic acid

i = impurity

Ten non-steroidal anti-inflammatory drugs (NSAIDs) can be separated in under 3.5 minutes using a short HALO® RP-Amide, 2.7 µm packed column.

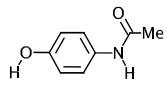
TEST CONDITIONS:

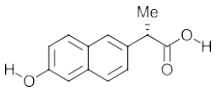
Column: HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 50/50 - A/B (start)
A: 0.02 M Sodium phosphate buffer, pH 2.5
B: Methanol
Gradient:

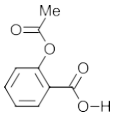
Time (min)	% B
0.0	50
0.1	50
0.5	55
3.5	80
4.0	80

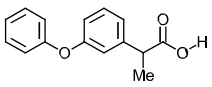
Flow Rate: 2.0 mL/min
Pressure: 289 bar
Temperature: 35 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Mobile phase
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

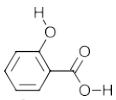
STRUCTURES:

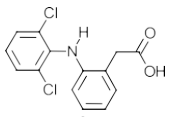

Acetaminophen

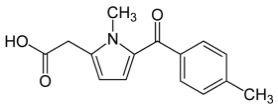

Naproxen

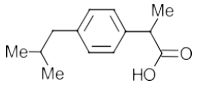

Aspirin

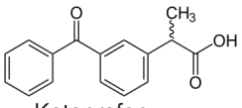

Fenoprofen

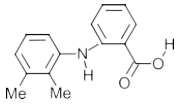

Salicylic acid


Diclofenac


Tolmetin

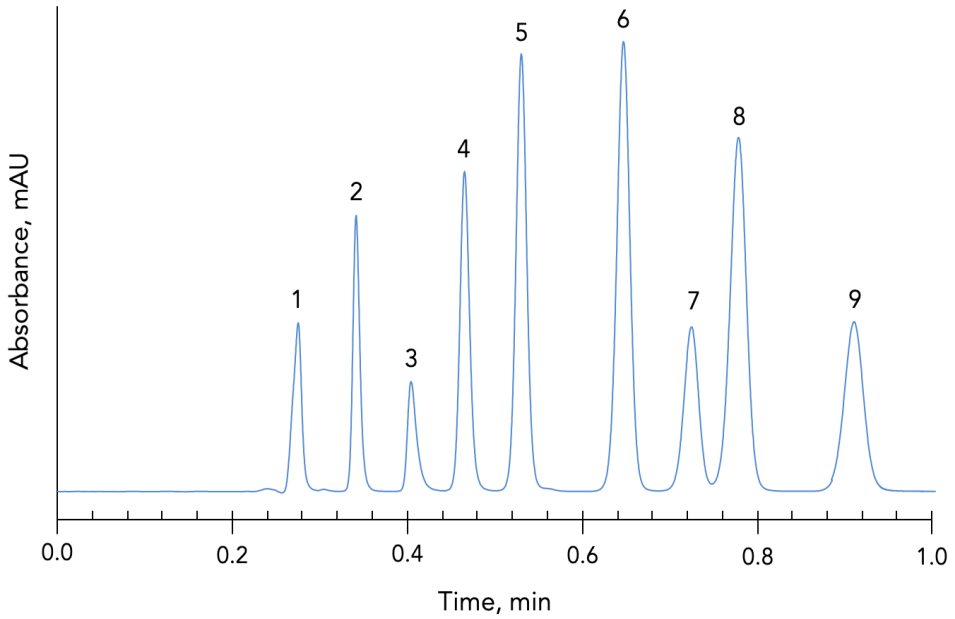

Ibuprofen


Ketoprofen


Mefenamic acid

Isocratic Separation of NSAIDs on HALO® ES-CN Phase

Application Note 56-NS



PEAK IDENTITIES:

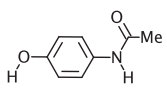
1. Acetaminophen
2. Aspirin
3. Salicylic acid
4. Tolmetin
5. Naproxen
6. Fenoprofen
7. Ibuprofen
8. Diclofenac
9. Mefenamic acid

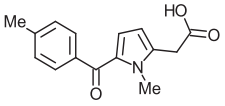
This separation illustrates the separating power of HALO® Fused-Core® stationary phases. Nine NSAID drugs are separated in under one minute on a 50 mm HALO® ES-CN column.

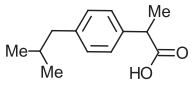
TEST CONDITIONS:

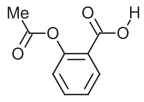
Column: HALO 90 Å ES-CN, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-404
Mobile Phase: 50/50 - A/B
A: 0.02 M potassium phosphate buffer, pH 2.5
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 165 bar
Temperature: 35 °C
Detection: UV 230 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Water/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

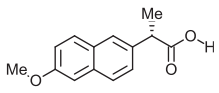
STRUCTURES:

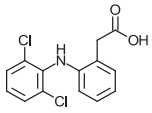

Acetaminophen

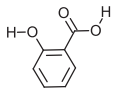

Tolmetin

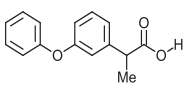

Ibuprofen

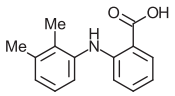

Aspirin


Naproxen


Diclofenac

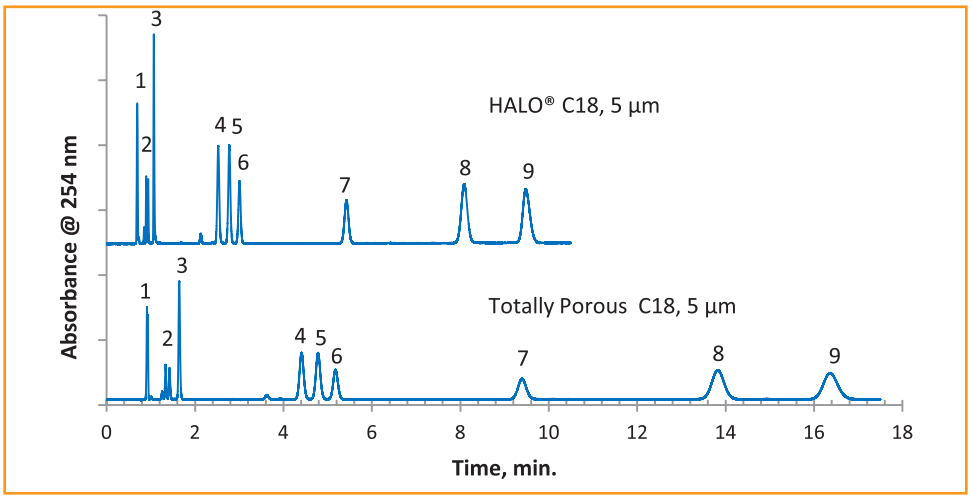

Salicylic acid


Fenoprofen


Mefenamic acid

Separation of NSAIDs on HALO® C18, 5.0 µm and Totally Porous C18, 5.0 µm

Application Note 74-NS



PEAK IDENTITIES:

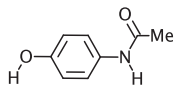
1. Acetaminophen
2. Aspirin
3. Salicylic acid
4. Tolmetin
5. Ketoprofen
6. Naproxen
7. Fenoprofen
8. Diclofenac
9. Ibuprofen

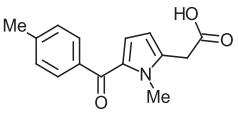
The HALO® 5.0 µm column separates this mixture of NSAIDs (non-steroidal anti-inflammatory drugs) in less than 60% of the time and with better resolution than a typical HPLC column packed with totally porous, 5-micron particles.

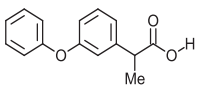
TEST CONDITIONS:

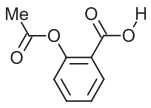
Columns:
1) HALO 90 Å C18, 5.0 µm, 4.6 x 150 mm
Part number: 95814-702
2) Totally porous C18, 5.0 µm, 4.6 x 150 mm
Mobile Phase: 48/52 - A/B
A: 20 mM potassium phosphate, pH 2.5
B: 50/50 acetonitrile/methanol
Flow Rate: 2.0 mL/min
Pressure: 240 bar (HALO)
215 bar (competitor)
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: 50/50 methanol/water
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

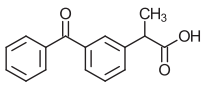
STRUCTURES:

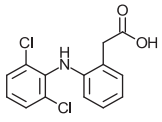

Acetaminophen

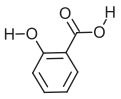

Tolmetin

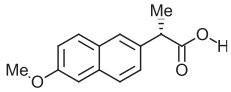

Fenoprofen

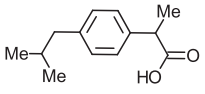

Aspirin


Ketoprofen


Diclofenac

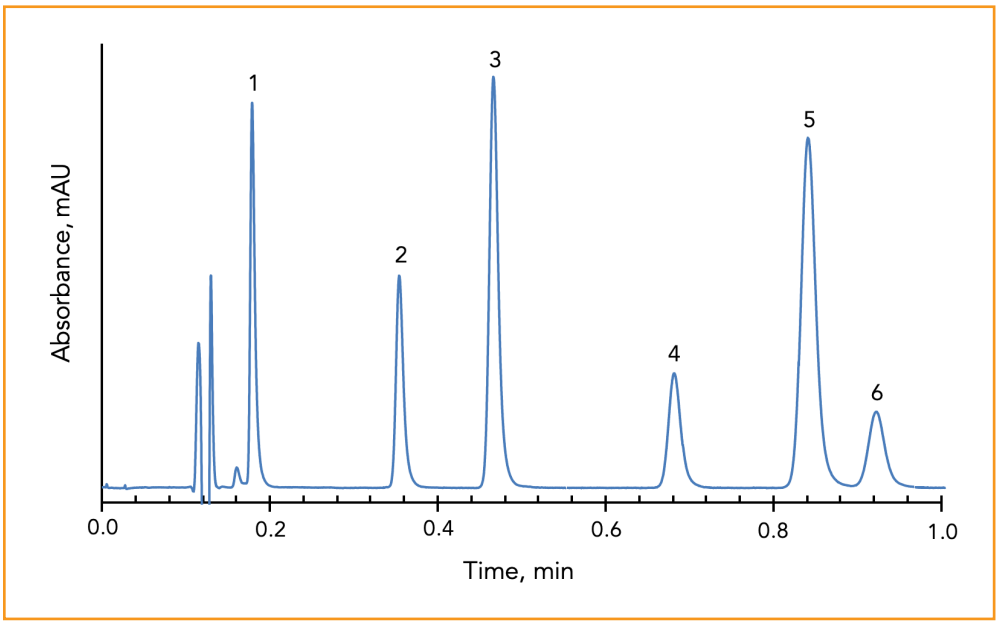

Salicylic acid


Naproxen


Ibuprofen

Separation of NSAIDS on HALO® ES-CN, 2.0 µm with MS Compatible Mobile Phase

Application Note 128-NS



PEAK IDENTITIES:

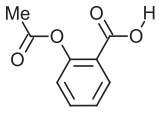
1. Aspirin
2. Tolmetin
3. Naproxen
4. Fenoprofen
5. Ibuprofen
6. Diclofenac

Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat pain and swelling. These polar drugs can be analyzed on a 2.0 µm HALO® ES-CN column in under a minute using a mass-spec friendly mobile phase.

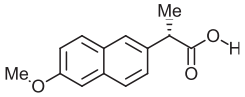
TEST CONDITIONS:

Column: HALO 90 Å ES-CN, 2.0 µm, 3.0 x 50 mm
Part Number: 91813-404
Mobile Phase: 60/40 - A/B
A: Water with 0.1% formic acid/ 10 mM ammonium formate, pH 3.3
B: 80/20 Acetonitrile/water with 0.1% formic acid/10 mM ammonium formate
Flow Rate: 2.0 mL/min
Pressure: 440 bar
Temperature: 45 °C
Detection: UV 230 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: Water/methanol
Data Rate: 80 Hz
Response Time: 0.02 sec
Flow Cell: 2.0 µL micro cell
LC System: Agilent 1200 SL

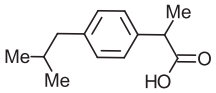
STRUCTURES:



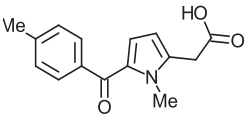
Aspirin



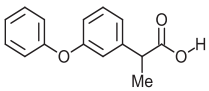
Naproxen



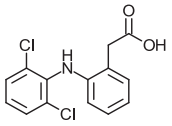
Ibuprofen



Tolmetin



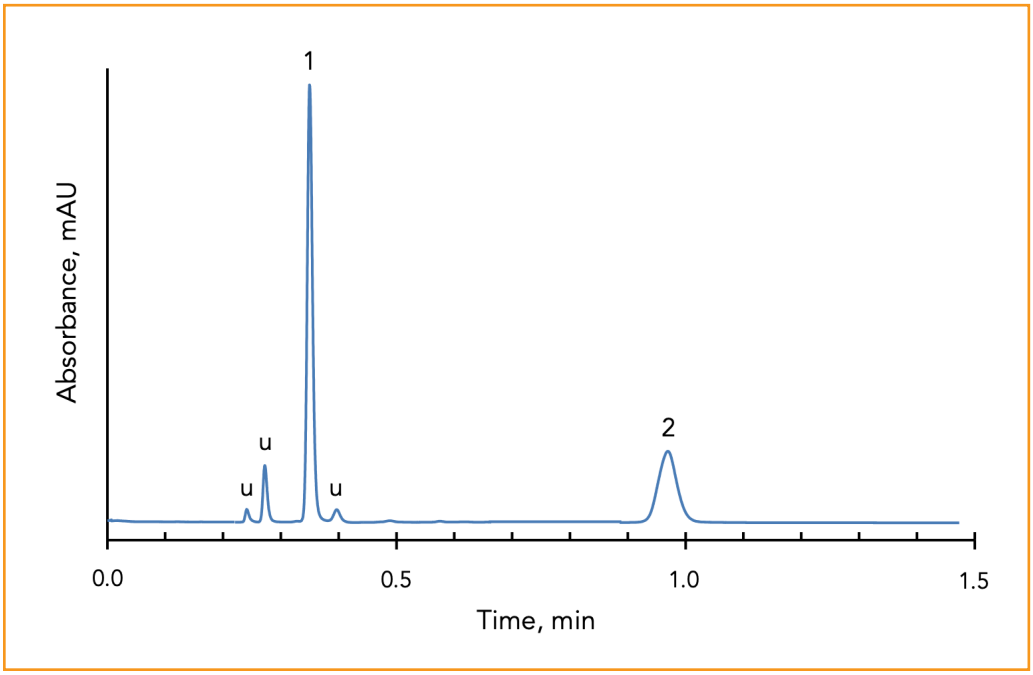
Fenoprofen



Diclofenac

Separation of Galantamine and Quetiapine on HALO® PFP

Application Note 85-PS



PEAK IDENTITIES:

1. Galantamine
2. Quetiapine

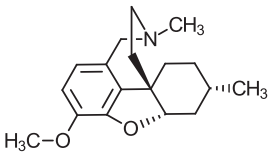
u = unknown

Galantamine and quetiapine are psychiatric drugs used to treat mental disorders. They can be rapidly separated on a HALO® PFP column in just one minute.

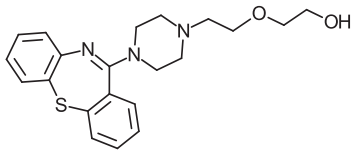
TEST CONDITIONS:

Column: HALO 90 Å PFP, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-409
Mobile Phase: 58/42 - A/B
A: 0.02 M potassium phosphate, pH 3.0
B: Acetonitrile
Flow Rate: 1.8 mL/min
Pressure: 155 bar
Temperature: 40 °C
Detection: UV 220 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

STRUCTURES:



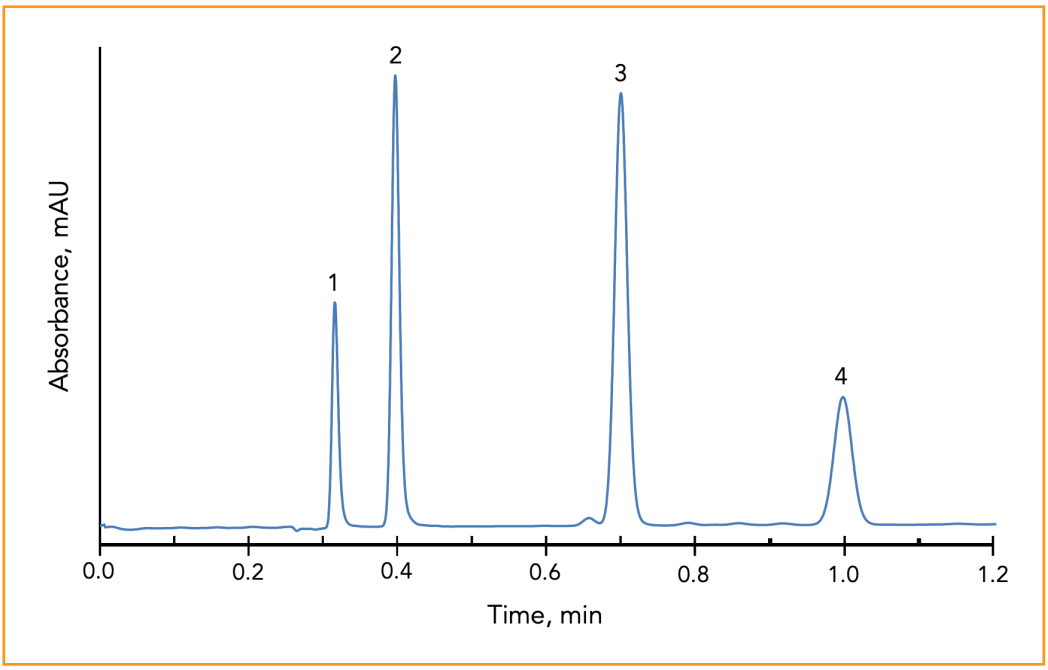
Galantamine



Quetiapine

Separation of Statin Drugs on HALO® C8

Application Note 43-ST



PEAK IDENTITIES:

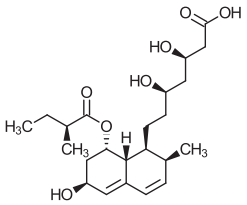
1. Pravastatin
2. Atorvastatin
3. Mevastatin
4. Simvastatin

The statin drugs are widely used to reduce the levels of cholesterol in the blood, thereby reducing the risk of cardiovascular disease and stroke. In this separation, four common statin drugs are analyzed on an efficient HALO® C8 column in about one minute.

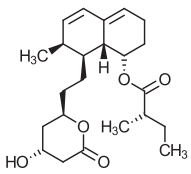
TEST CONDITIONS:

Column: HALO 90 Å C8, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-408
Mobile Phase: 20/80 - A/B
A: 0.02 M formic acid in water
B: 0.02 M formic acid in methanol
Flow Rate: 2.0 mL/min
Pressure: 240 bar
Temperature: 30 °C
Detection: UV 240 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Mobile phase
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

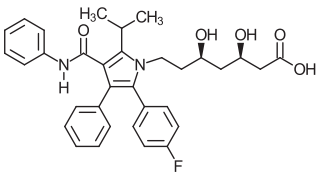
STRUCTURES:



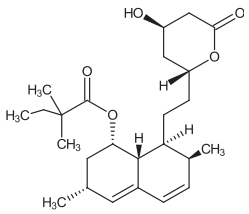
Pravastatin



Mevastatin



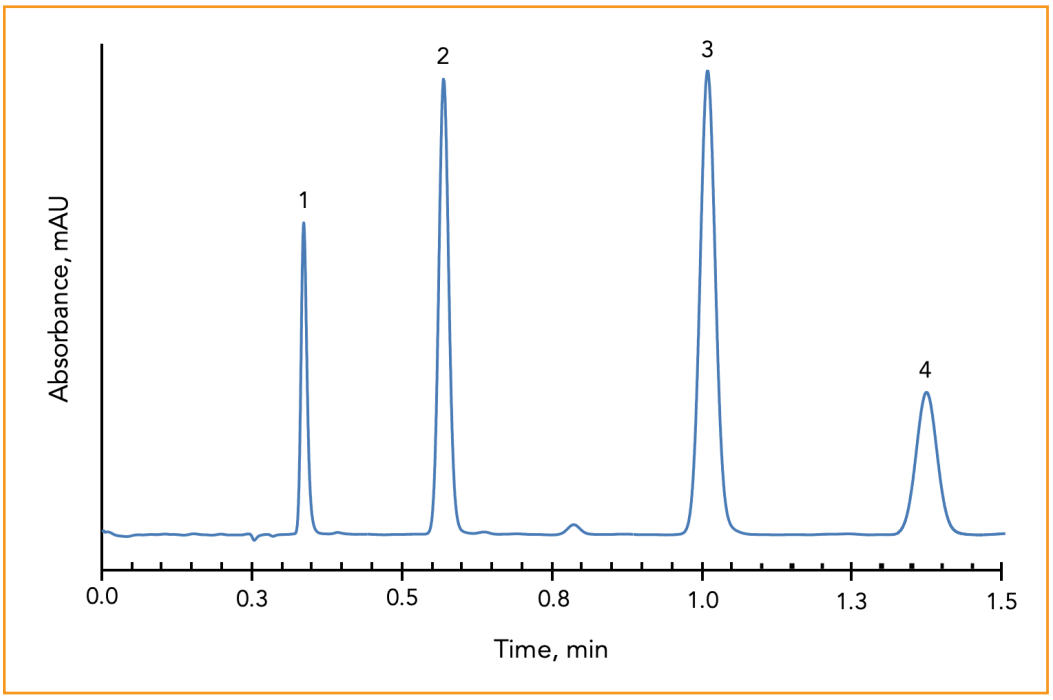
Atorvastatin



Simvastatin

Separation of Statin Drugs on HALO® Phenyl-Hexyl in Methanol

Application Note 44-ST



PEAK IDENTITIES:

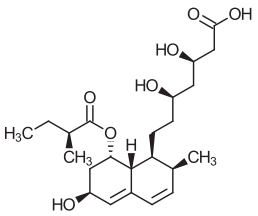
1. Pravastatin
2. Atorvastatin
3. Mevastatin
4. Simvastatin

These statin drugs can be rapidly separated using short HALO® Phenyl-Hexyl columns.

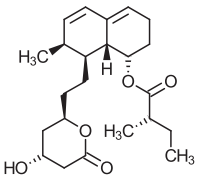
TEST CONDITIONS:

Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 20/80 - A/B
A: 0.02 M formic acid in water
B: 0.02 M formic acid in methanol
Flow Rate: 2.0 mL/min
Pressure: 250 bar
Temperature: 30 °C
Detection: UV 240 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 20/80 (water with 0.02 M formic acid)/(methanol with 0.02 M formic acid)
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

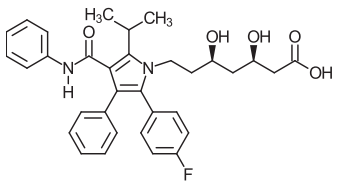
STRUCTURES:



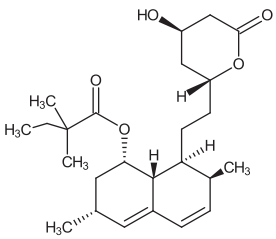
Pravastatin



Mevastatin



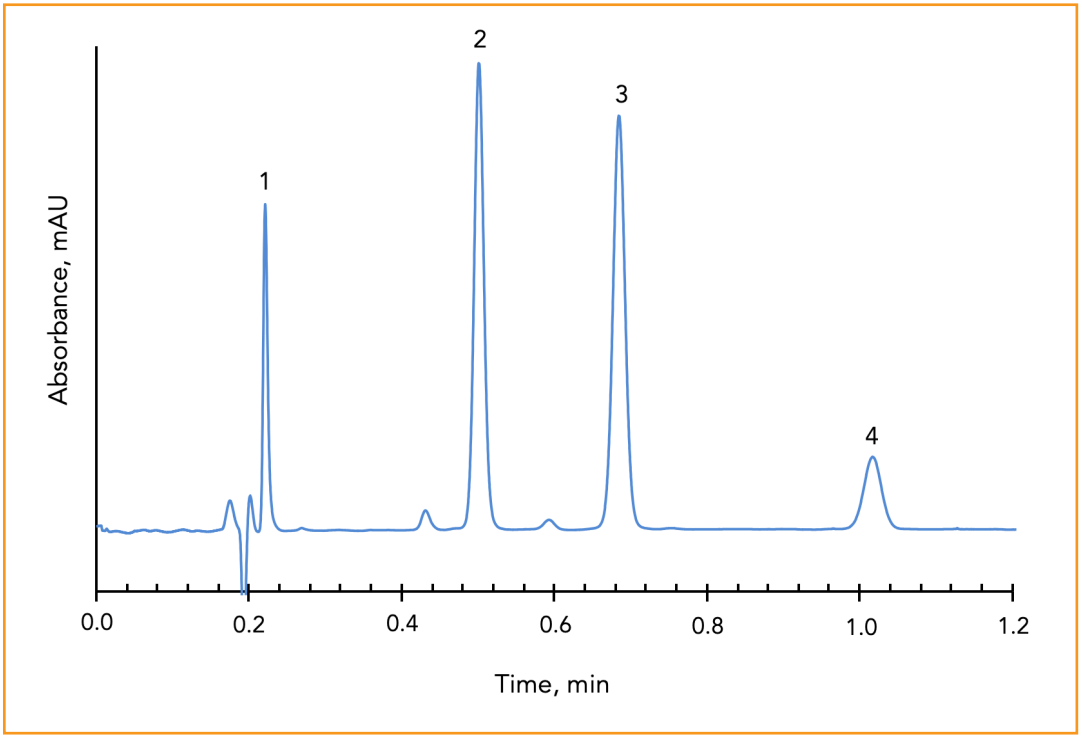
Atorvastatin



Simvastatin

Separation of Statin Drugs on HALO® Phenyl-Hexyl in Acetonitrile

Application Note 45-ST



PEAK IDENTITIES:

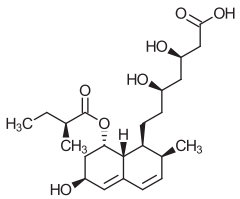
1. Pravastatin
2. Atorvastatin
3. Mevastatin
4. Simvastatin

These statin drugs can be rapidly separated using short HALO® Phenyl-Hexyl columns.

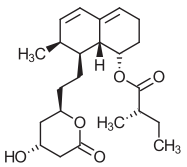
TEST CONDITIONS:

Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 43/57 - A/B
A: 0.02 M formic acid in water
B: 0.02 M formic acid in acetonitrile
Flow Rate: 2.5 mL/min
Pressure: 228 bar
Temperature: 26 °C
Detection: UV 240 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 20/80 (water with 0.02 M formic acid)/(methanol with 0.02 M formic acid)
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

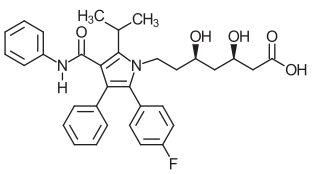
STRUCTURES:



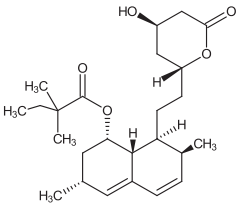
Pravastatin



Mevastatin



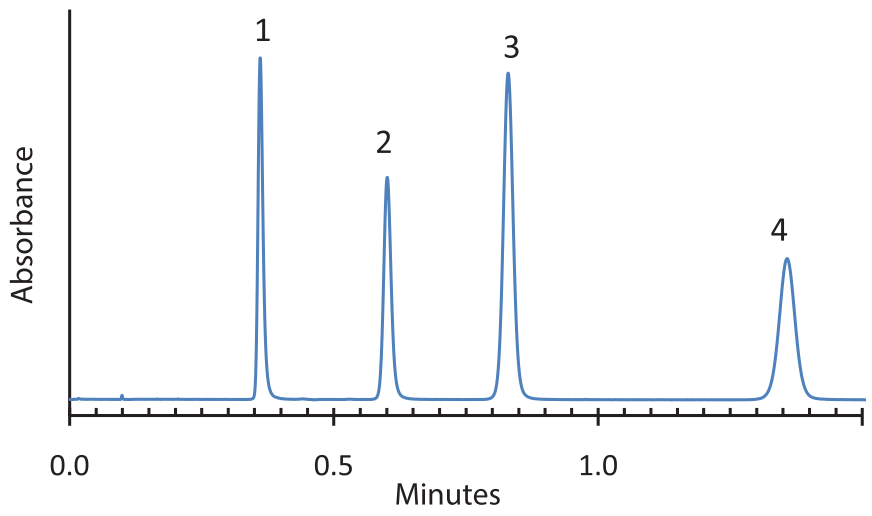
Atorvastatin



Simvastatin

Separation of Xanthines on HALO® Phenyl-Hexyl Phase

Application Note 49-XA



PEAK IDENTITIES:

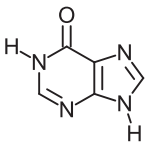
1. Hypoxanthine
2. Theobromine
3. Theophylline
4. Caffeine

These xanthines can be readily separated on a HALO® Phenyl-Hexyl column in a buffered methanolic mobile phase.

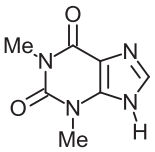
TEST CONDITIONS:

Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 70/30 - A/B
A: 0.03 M phosphate buffer, pH 3.0, in water
B: Methanol
Flow Rate: 1.5 mL/min
Pressure: 223 bar
Temperature: 35 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 30% methanol in water
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

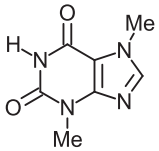
STRUCTURES:



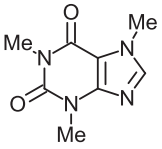
Hypoxanthine



Theophylline



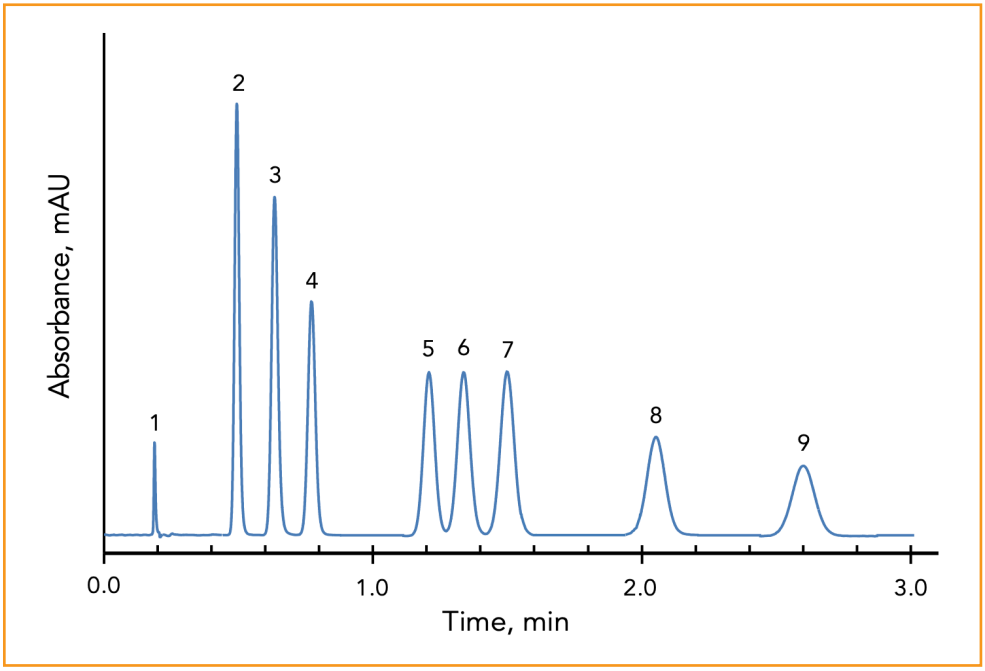
Theobromine



Caffeine

Sulfa Drugs on HALO® C18, 5 µm

Application Note 108-AB



PEAK IDENTITIES:

1. Uracil
2. Sulfadiazine
3. Sulfathiazole
4. Sulfamerazine
5. Sulfamethazine
6. Sulfamethizole
7. Sulfamethoxypyridazine
8. Sulfachloropyridazine
9. Sulfamethoxazole

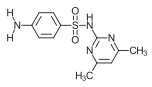
This separation shows the rapid analysis of eight sulfa drugs on the HALO® C18 (5 µm) phase. The use of mixed organic solvents improved the selectivity between compounds having similar structures.

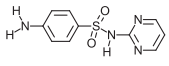
TEST CONDITIONS:

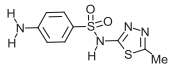
Column: HALO 90 Å C18, 5 µm, 4.6 x 50 mm
Part Number: 95814-402
Mobile Phase: 87/13 - A/B
A: 0.02 M ammonium formate, pH 3.0 (adj.)
B: 50/50 acetonitrile/methanol
Flow Rate: 2.5 mL/min
Pressure: 185 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 water/acetonitrile
Response Time: 0.02 sec
Data Rate: 50 pps
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

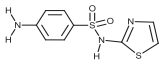
STRUCTURES:

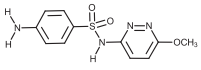

Uracil

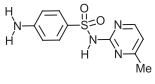

Sulfamethazine

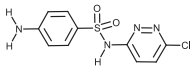

Sulfadiazine

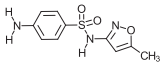

Sulfamethizole


Sulfathiazole


Sulfamethoxypyridazine

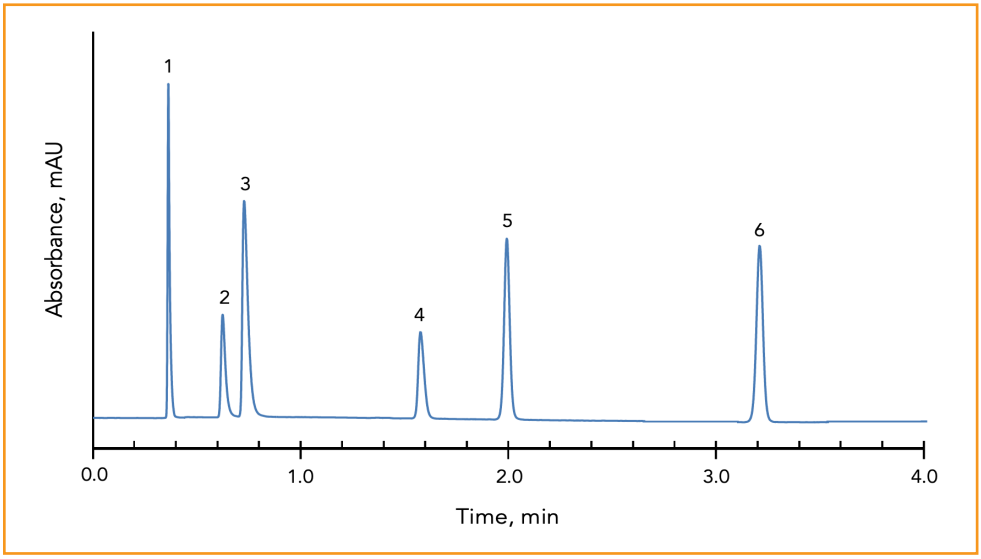

Sulfamerazine


Sulfachloropyridazine


Sulfamethoxazole

Antihistamines on HALO® C18, 5 µm

Application Note 114-AH



PEAK IDENTITIES:

1. Maleic acid
2. Pyrilamine
3. Chlorpheniramine
4. Cetirizine
5. Fexofenadine
6. Loratadine

These six antihistamines can be rapidly separated on a 5 µm HALO® Fused-Core® C18 column in under 4 minutes.

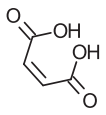
TEST CONDITIONS:

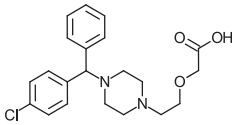
Column: HALO 90 Å C18, 5 µm, 3.0 x 100 mm
Part Number: 95813-602
Mobile Phase: 50/50 - A/B (start)
A: 0.02 M phosphate buffer, pH 2.6
B: Methanol
Gradient:

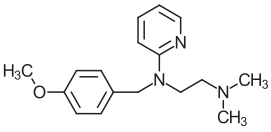
Time (min)	% B
0.0	50
0.5	50
2.5	75
4.0	75

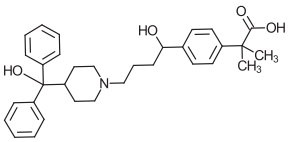
Flow Rate: 1.0 mL/min
Pressure: 191 bar
Temperature: 40 °C
Detection: UV 230 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 80% methanol in water
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

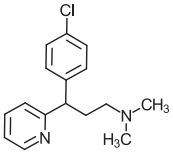
STRUCTURES:

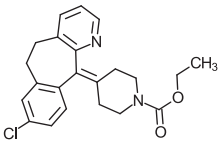

Maleic acid


Cetirizine


Pyrilamine

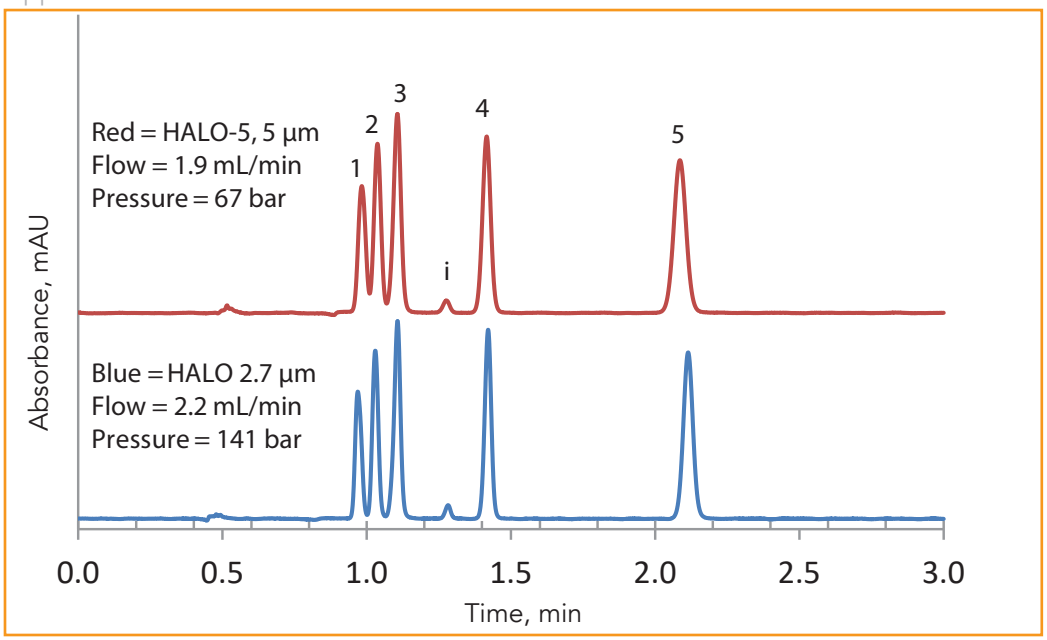

Fexofenadine


Chlorpheniramine


Loratadine

Comparable Selectivity Between HALO® Penta-HILIC 5 µm and 2.7 µm

Application Note 89-AD



PEAK IDENTITIES:

1. Trimipramine
2. Amitriptyline
3. Doxepin
4. Nortriptyline
5. Amoxapine

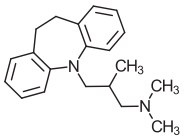
i = impurity

Similar selectivity is achieved between the 5 µm and 2.7 µm HALO® Penta-HILIC particle sizes through a slight flow rate adjustment allowing easy method transfer.

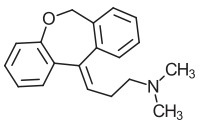
TEST CONDITIONS:

Columns:
1) HALO 90 Å Penta-HILIC, 5 µm, 4.6 x 100 mm
Part Number: 95814-605
2) HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-605
Mobile Phase: 5/95 - A/B
A: 0.1 M ammonium formate, pH 3.0 (adj.)
B: Acetonitrile
Flow Rate: See chart
Pressure: See chart
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: 10/90 water/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

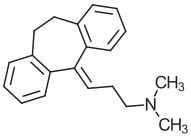
STRUCTURES:



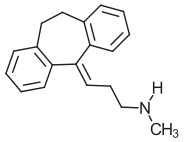
Trimipramine



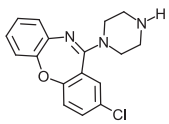
Doxepin



Amitriptyline



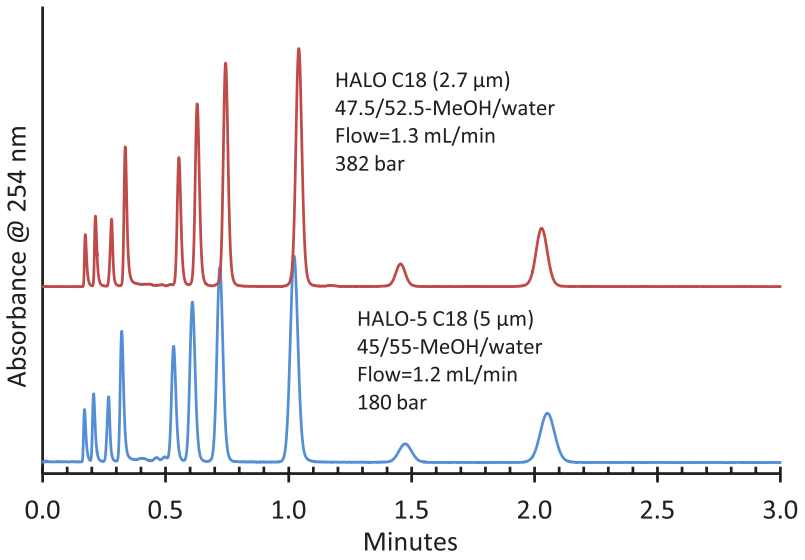
Nortriptyline



Amoxapine

Comparable Selectivity of HALO® C18, 2.7 µm and HALO® C18, 5 µm

Application Note 77-HA



PEAK IDENTITIES:

1. Uracil
2. Resorcinol
3. Aniline
4. 4-Chloroaniline
5. Acetoacetanilide
6. Dimethylphthalate
7. Cinnamyl alcohol
8. 2,6-Dinitrotoluene
9. Tolbutamide
10. 4-Chloro-3-nitroanisole

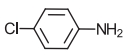
This mixture of compounds with varying functional groups and polarity show the same selectivity on both the 5 µm and 2.7 µm HALO® C18 columns with only minor adjustments in flow rate and mobile phase composition being required. This separation demonstrates the ability to change from one HALO® particle size to the other without needing to redevelop the method.

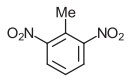
TEST CONDITIONS:

Columns:
1) HALO 90 Å C18, 2.7 µm, 3.0 x 50 mm
Part Number: 92813-402
2) HALO 90 Å C18, 5.0 µm, 3.0 x 50 mm
Part Number: 95813-402
Mobile Phase: See chart
Flow Rate: See chart
Pressure: See chart
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

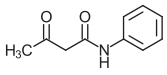
STRUCTURES:

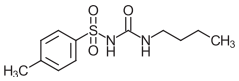

Uracil

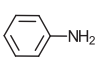

4-Chloroaniline

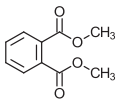

2,6-Dinitrotoluene

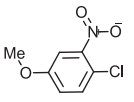

Resorcinol

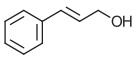

Acetoacetanilide


Tolbutamide


Aniline

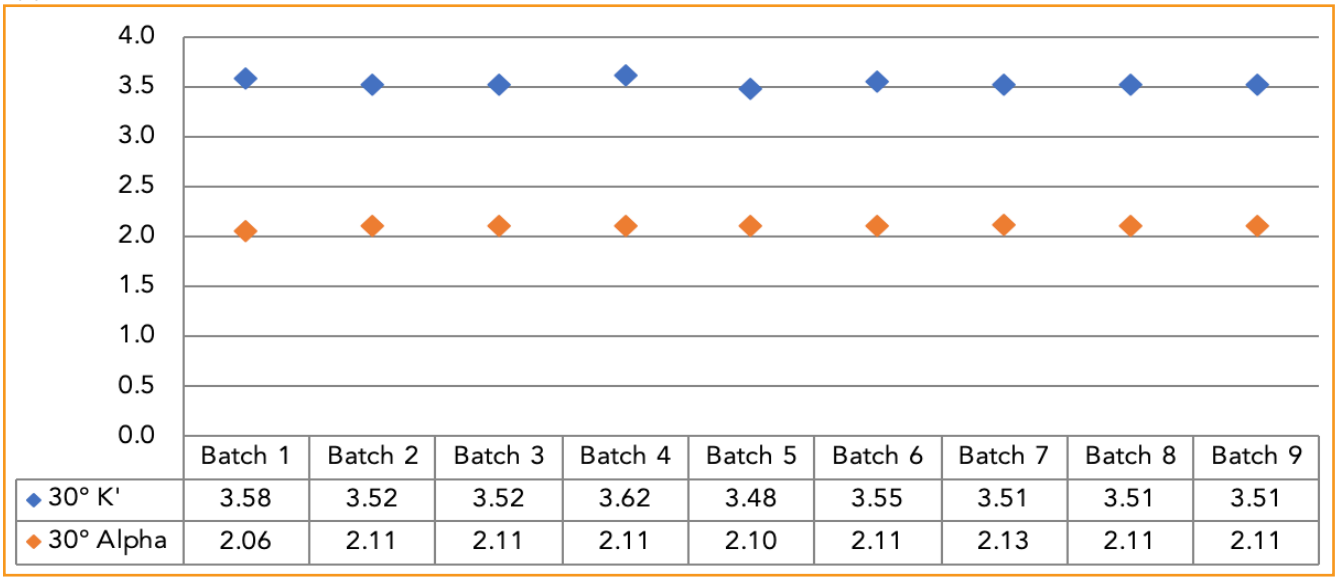

Dimethylphthalate


4-Chloro-3-nitroanisole

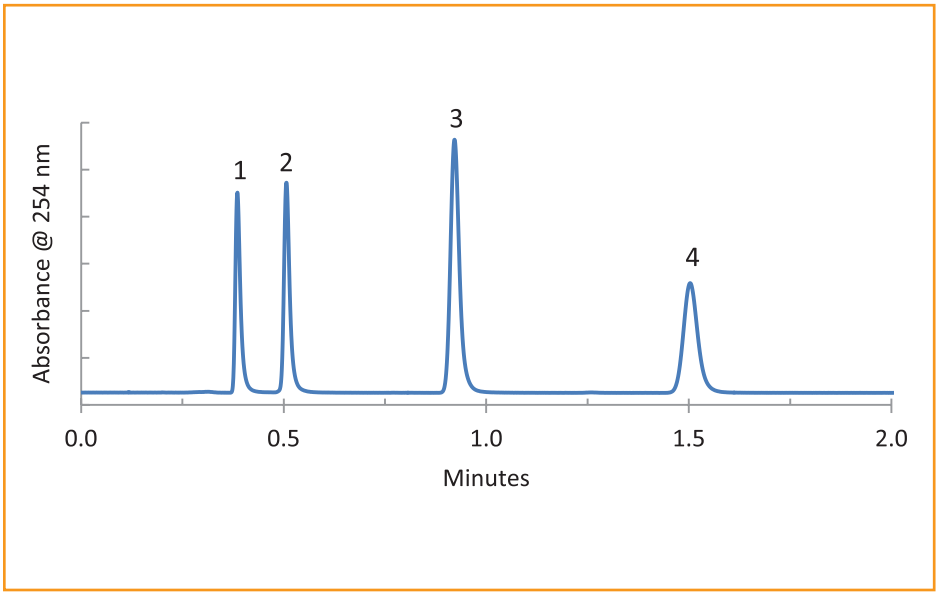

Cinnamyl alcohol

HALO® C18, 5 µm
Lot to Lot Reproducibility

Application Note 79



	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9
◆ 30° K'	3.58	3.52	3.52	3.62	3.48	3.55	3.51	3.51	3.51
◆ 30° Alpha	2.06	2.11	2.11	2.11	2.10	2.11	2.13	2.11	2.11



PEAK IDENTITIES:

1. Uracil
2. Phenol
3. 4-Cl-1-Nitrobenzene
4. Naphthalene

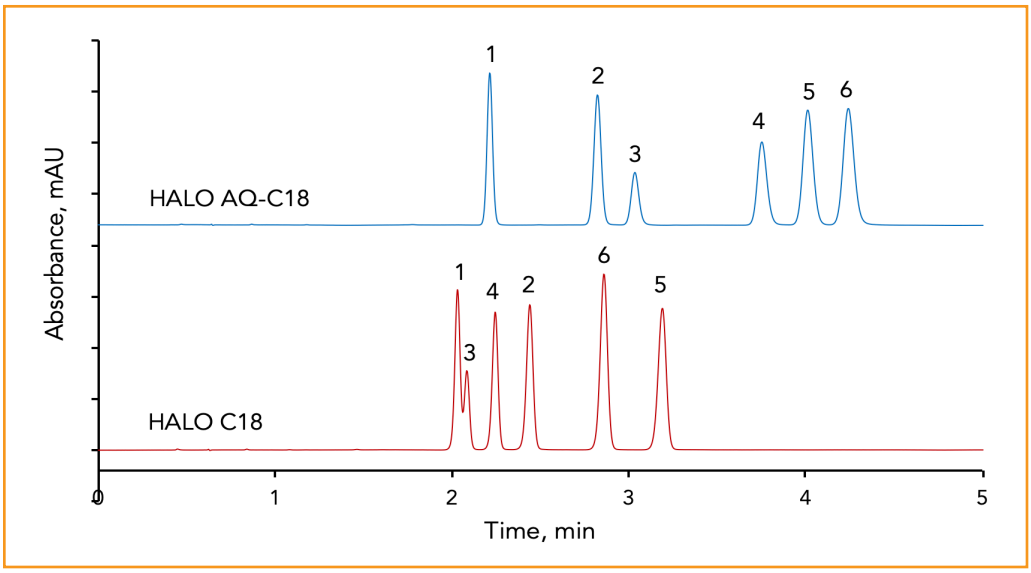
TEST CONDITIONS:

Column: HALO 90 Å C18, 5 µm, 4.6 x 50 mm
Part Number: 95814-402
Mobile Phase: 57/43 - A/B
A: Acetonitrile
B: Water
Flow Rate: 1.0 mL/min
Pressure: 39 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 ACN/water
Flow Cell: 5.0 µL semi-micro
LC System: Agilent 1100

The retention factor and selectivity calculated across several batches of HALO® 5 µm C18 show superior reproducibility. Retention factor is calculated for naphthalene while selectivity is calculated between naphthalene and 4-chloro-1-nitrobenzene.

Separation of Polar Samples on HALO® AQ-C18 and C18

Application Note 157-G



PEAK IDENTITIES:

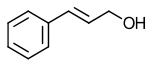
1. Cinnamyl alcohol
2. 4'-Bromoacetanilide
3. Nitrobenzene
4. Anisole
5. 3,4-Dinitrotoluene
6. 2,4-Dinitrotoluene

HALO® AQ-C18 and HALO® C18 phases have different selectivities as shown in the chromatograms above. The HALO® AQ-C18 phase delivers increased retention for polar molecules compared to C18.

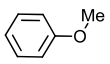
TEST CONDITIONS:

Columns:
1) HALO 90 Å C18, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-602
2) HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-622
Mobile Phase: 48/52 - A/B
A: Water
B: Methanol
Flow Rate: 1.4 mL/min
Pressure: 344 bar (C18)
329 bar (AQ-C18)
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

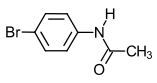
STRUCTURES:



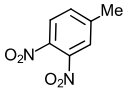
Cinnamyl alcohol



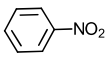
Anisole



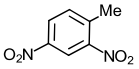
4'-Bromoacetanilide



3,4-Dinitrotoluene



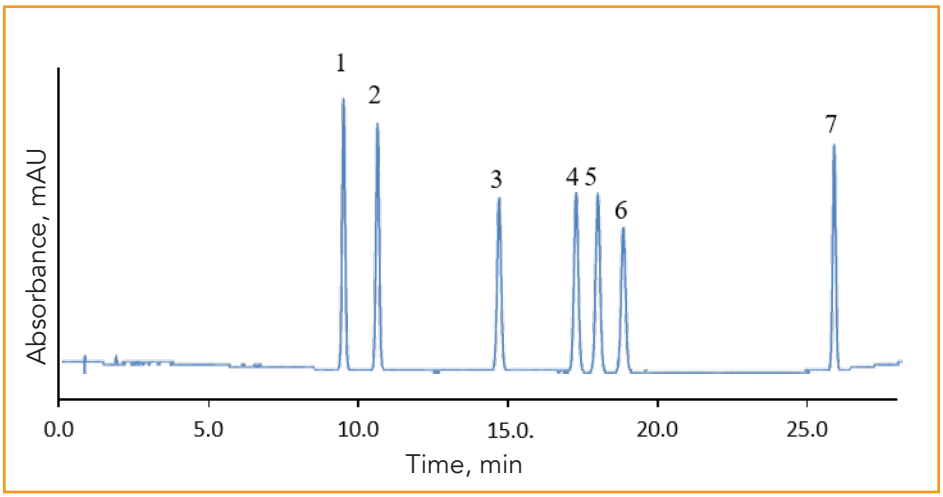
Nitrobenzene



2,4-Dinitrotoluene

Chinese Pharmacopeia Separation of Parabens on HALO® C18, 2.7 µm

Application Note 177-P



PEAK IDENTITIES:

1. Isopropyl paraben
2. Propyl paraben
3. Phenyl paraben
4. Isobutyl paraben
5. Butyl paraben
6. Benzyl paraben
7. Pentyl paraben

A separation of parabens is performed on a HALO® C18 column showing high resolution between critical pairs using a Chinese Pharmacopeia method. Parabens are esters of para-hydroxybenzoic acid and have many varieties. Parabens are widely used in a variety of cosmetics as a preservative. This can include many things such as shampoos, moisturizers, makeup, and shaving gels.

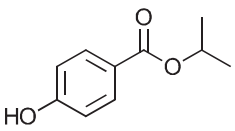
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-602
Mobile Phase:
A: Water
B: Methanol
Gradient:

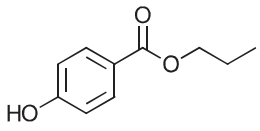
Time (min)	% B
0.0	40
23.0	55
28.0	70

Flow Rate: 1.2 mL/min
Initial Pressure: 403 bar
Temperature: 30 °C
Detection: UV 252 nm, PDA
Injection Volume: 1.5 µL
Sample Solvent: 50/50 methanol/water
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

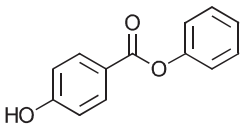
STRUCTURES:



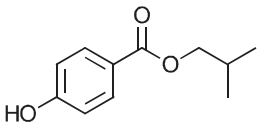
Isopropyl paraben



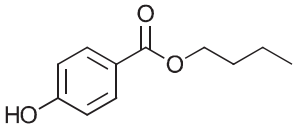
Propyl paraben



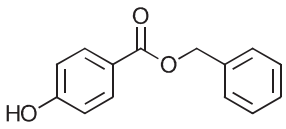
Phenyl paraben



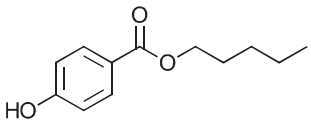
Isobutyl paraben



Butyl paraben



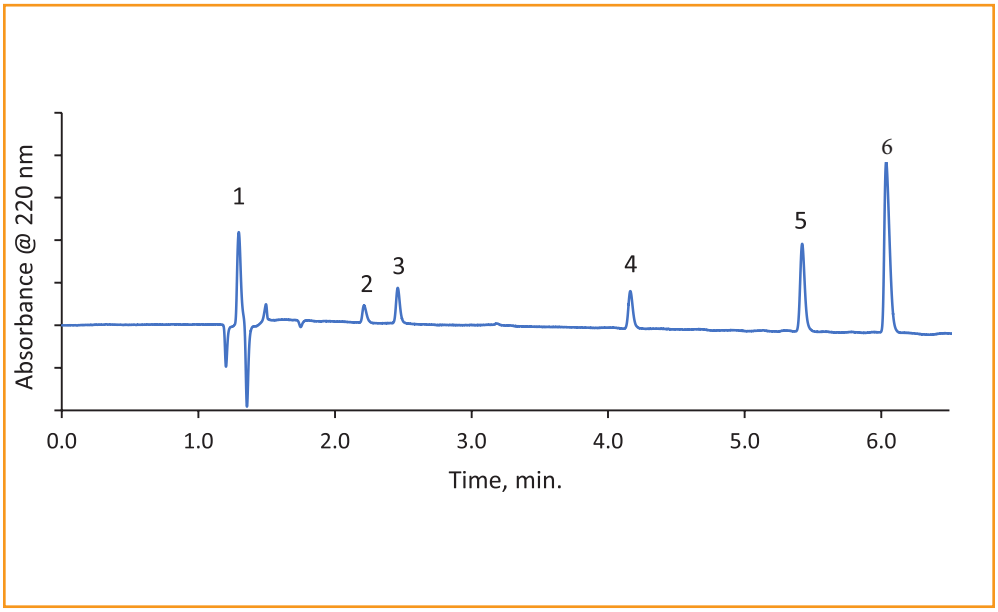
Benzyl paraben



Pentyl paraben

Amine Medications Separated Using HALO® C18, 5 µm

Application Note: 201-B



PEAK IDENTITIES:

1. Maleic Acid
2. Pseudoephedrine
3. Scopolamine
4. Doxylamine
5. Chlorpheniramine
6. Diphenhydramine

A mixture of amines including antihistamines, decongestants, and other medications is separated on a HALO® C18, 5 µm column. The column shows excellent peak shapes for basic compounds using an ammonium formate buffer at low pH.

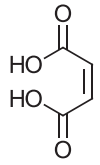
TEST CONDITIONS:

Column: HALO 90 Å C18, 5 µm, 4.6 x 150 mm
Part Number: 95814-702
Mobile Phase A: 50mM Ammonium Formate/ 0.1% Formic Acid
Mobile Phase B: 50/50 MeOH:Acetonitrile/ 0.1% Formic Acid
Gradient: Time (min.) %B

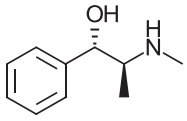
0.0	20
6.5	60

Flow Rate: 1.0 mL/min
Initial Back Pressure: 190 bar
Temperature: 30 °C
Detection: 220 nm, PDA
Injection Volume: 3 µL
Sample Solvent: 80/20 Mobile Phase A/B
Data Rate: 40 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

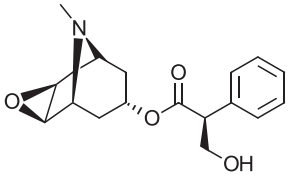
STRUCTURES:



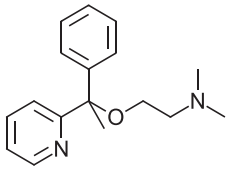
Maleic Acid



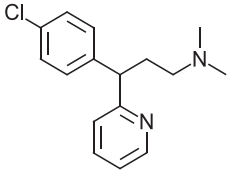
Pseudoephedrine



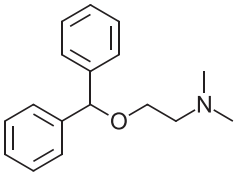
Scopolamine



Doxylamine



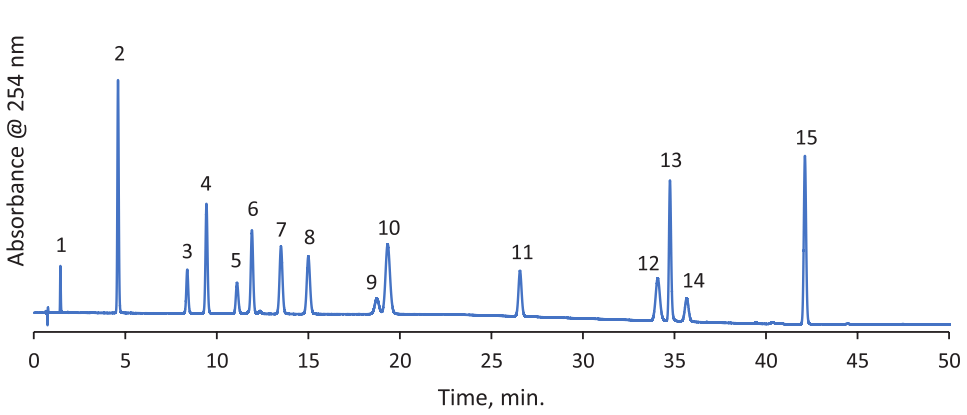
Chlorpheniramine



Diphenhydramine

Paracetamol Impurities: European Pharmacopoeia 9.4 Method

Application Note 211-EP



PEAK IDENTITIES:

1. Impurity K
2. Paracetamol
3. Impurity A
4. Impurity B
5. Impurity F
6. Impurity C
7. Impurity D
8. Impurity E
9. Impurity M
10. Impurity G
11. Impurity H
12. Impurity I
13. Impurity L
14. Impurity J
15. Impurity N

TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-602
Guard Column: HALO 90 Å C18, 2.7 µm, 2.1 x 5 mm
Part Number: 92812-102
Guard Column Holder: Part Number: 94900-001
Mobile Phase A: Phosphate Buffer (1.7g. potassium dihydrogen phosphate and 1.8g. dipotassium hydrogen in 1000mL)
Mobile Phase B: Methanol
Gradient:

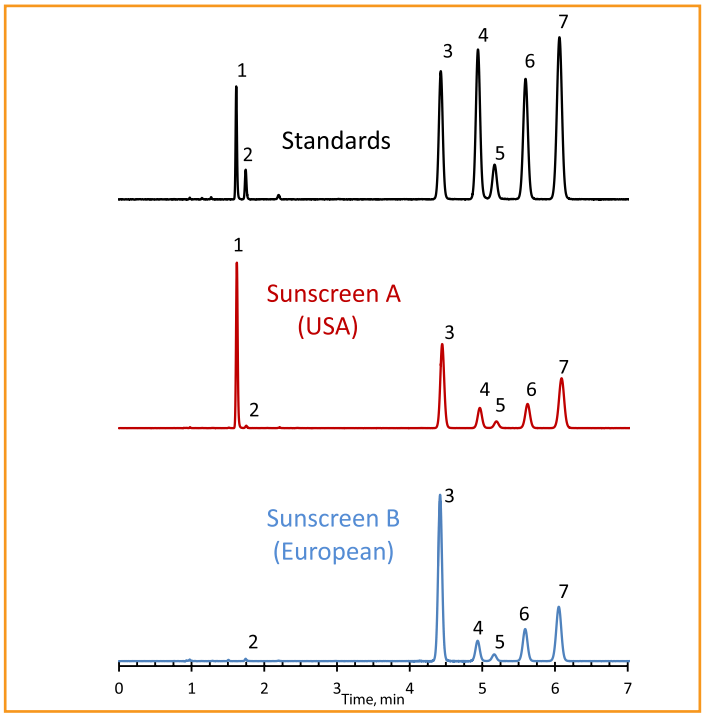
Time	% B
0.0	5
1.0	5
10.0	10
20.0	10
40.0	34
50.0	34

Flow Rate: 0.3 mL/min
Initial Pressure: 246 bar
Temperature: 30 °C
Detection: 254 nm, PDA
Injection Volume: 1 µL
Sample Solvent: 85/15 Water/ MeOH
Data Rate: 40 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Paracetamol (acetaminophen) is a common pain relief and fever medication taken individually, or in combination with other medications. An analysis of paracetamol and 14 of its impurities are separated on a HALO 90 Å C18 column following the official European Pharmacopoeia 9.4 method. Baseline resolution is obtained for all compounds including critical pairs of impurity M/G and impurities I/L/J. A HALO 90 Å C18 guard column is also used in order to provide optimum protection for your HALO® HPLC column without sacrificing the column's efficiency.

Analysis of Sunscreens using HALO® RP-Amide, 2.7 µm

Application Note: 203-SA



PEAK IDENTITIES:

1. Oxybenzone
2. Avobenzone isomer 1
3. Octocrylene
4. Avobenzone isomer 2
5. Homosalate isomer 1
6. Octisalate
7. Homosalate isomer 2

Sunscreens are designed to reduce the risk of burning from exposure to the sun's UV rays. Overexposure to the sun increases the chances of skin cancer so it is important to use sunscreens during outdoor activities. The active contents of sunscreens can be analyzed using HPLC as shown in this application note. Approximately 200 mg of sunscreen lotions were treated with 10 mL of ethanol or 1-propanol to dissolve the active ingredients and suspend insolubles. Aliquots of the slurries were centrifuged and the supernates were filtered through Nylon 0.45 µm porosity syringe filters prior to analysis.

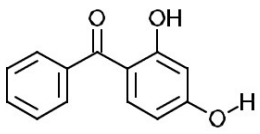
TEST CONDITIONS:

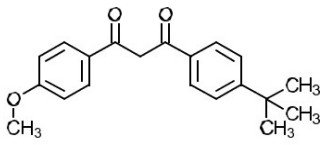
Column: HALO 90 Å RP Amide, 2.7 µm
4.6 x 150 mm
Part Number: 92814-707
Mobile Phase: A/B
A= Water
B= Acetonitrile
Gradient:

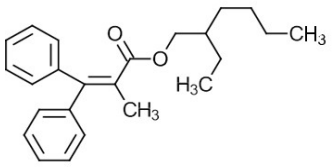
Time	% B
0.0	75
7.0	75
10	100
20	100

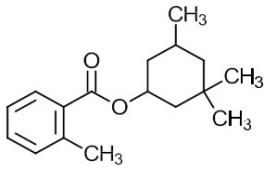
Flow Rate: 1.5 mL/min.
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

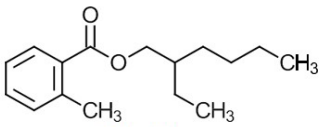
STRUCTURES:


Oxybenzone


Avobenzone

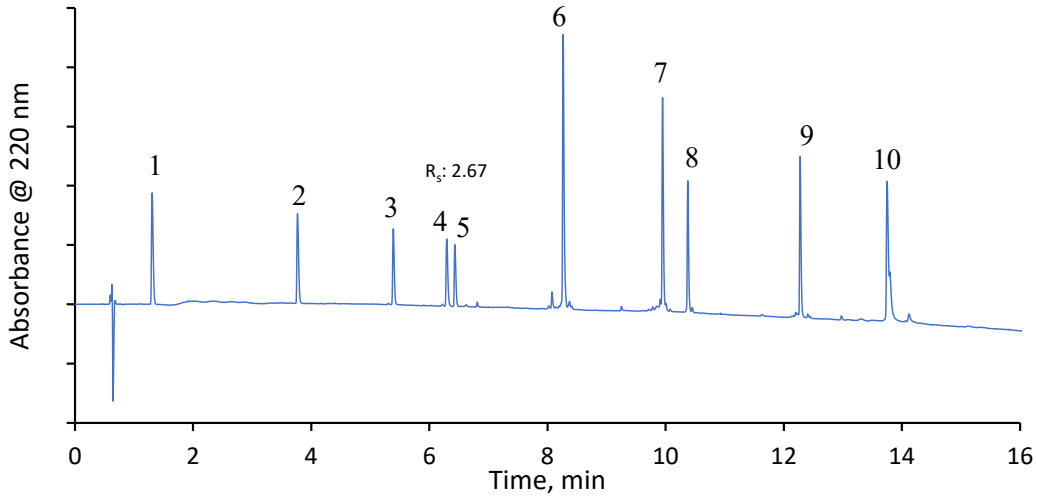

Octocrylene


Homosalate


Octisalate

Peptide and Protein Mix on HALO® 400 Å ES-C18, 3.4 µm

213-PR



TEST CONDITIONS:

Column: HALO® 400 Å ES-C18, 3.4 µm, 2.1 x 150 mm
Part Number: 93412-702
Mobile Phase A: Water + 0.1% DFA
Mobile Phase B: 80/20 Acetonitrile/Water + 0.1% DFA
Gradient:

Time	%B
0.0	**0**
15.0	**60**
16.0	**60**
16.1	**0**
20.0	**0**

Flow Rate: 0.5 mL/min
Initial Pressure: 165 bar
Temperature: 60 °C
Detection: UV 220 nm, PDA
Injection Volume: 1.5 µL
Sample Solvent: Water
Data Rate: 40 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

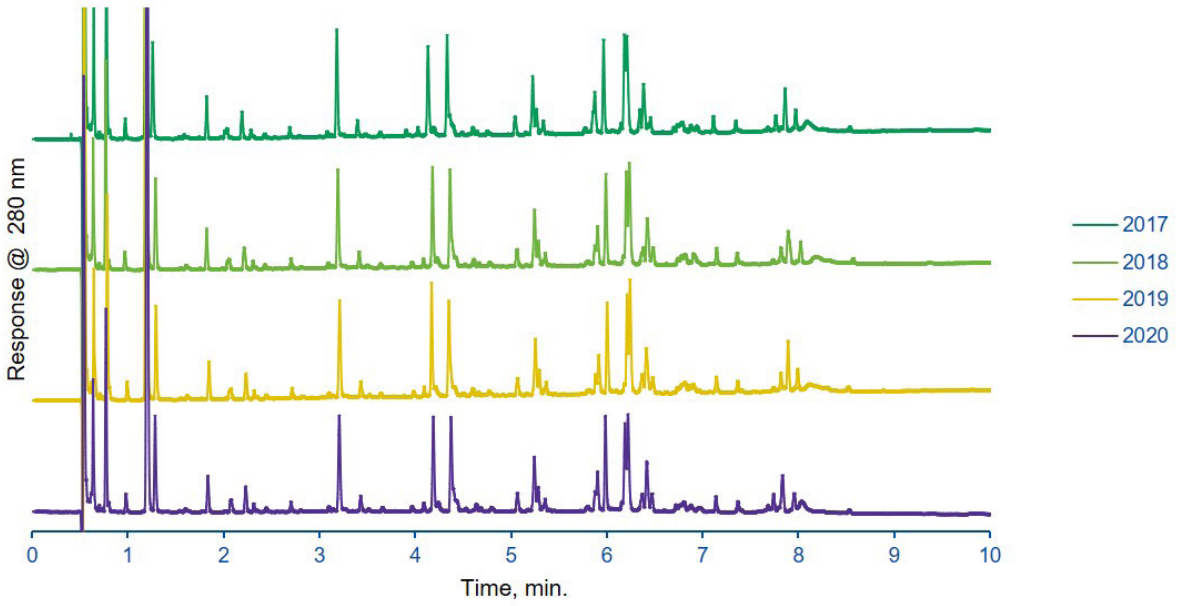
PEAK IDENTITIES:

1. Gly-Tyr
2. Val-Tyr-Val
3. Methionine Enkephalin
4. Angiotensin II
5. Leucine Enkephalin
6. RNase A
7. Cytochrome C
8. Insulin
9. Alpha-lactalbumin
10. Enolase

A mix of peptides and proteins was separated with excellent resolution and peak shape using the HALO® 400 Å ES-C18. The steric protection of this phase makes it particularly ideal for the high temperature and low pH conditions often required for peptide and protein separations. Because of its smaller pore size compared to the 1000 Å ES-C18, the 400 Å ES-C18 easily separates mixtures of peptides and smaller proteins such as cytochrome C, alpha-lactalbumin, and enolase.

Rapid Peptide Mapping of an Adalimumab (Humira®) Digest

Application Note 221-PE



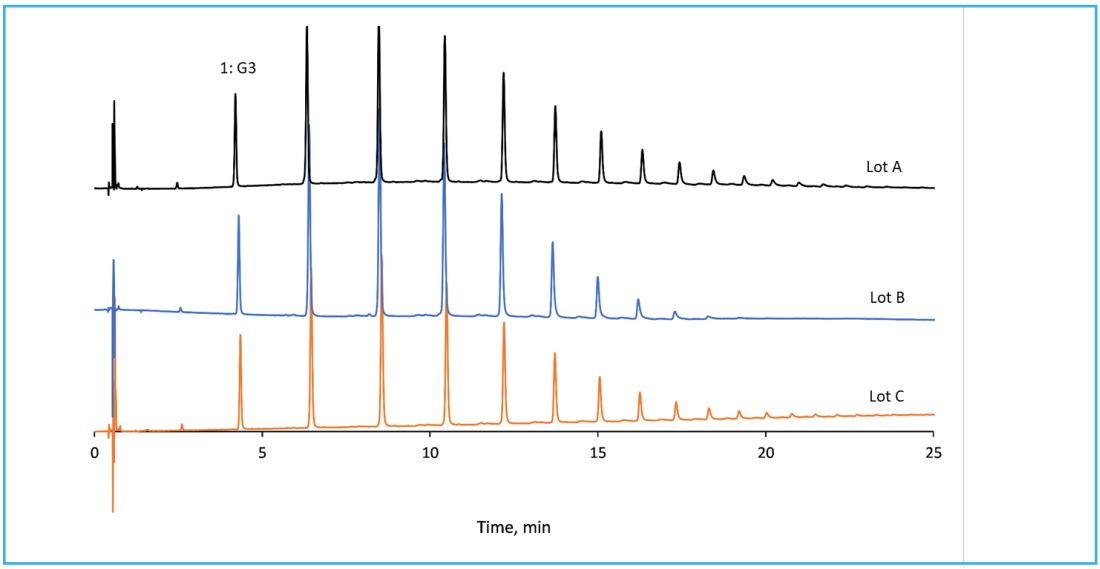
TEST CONDITIONS:

Column: HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92122-702
Mobile Phase: A: Water/0.1% DFA and B: ACN/ 0.1% DFA
Flow Rate: 600 µL/min
Pressure: 330 bar (4795 psi)
Temperature: 60 °C
Detection: 280 nm
Injection Volume: 3.0 µL
Sample Solvent: 90/10 mobile phase A/B
Response Time: 0.025 sec
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera

The outstanding reproducibility and high throughput power of the HALO 160 Å ES-C18 column is demonstrated here with the separation of an adalimumab (immunosuppressive drug) tryptic digest achieved under 10 minutes (total analysis time of 15 min). The nearly identical gradient profiles highlight the reliability and reproducibility of four different column lots, over a four-year period (2017-2020).

HALO® Glycan Lot to Lot Reproducibility

228-GL



TEST CONDITIONS:

Column: HALO 90 Å Glycan, 2.7 µm, 2.1 x 150 mm
Part Number: 92922-705
Mobile Phase A: 50 mM Ammonium Formate, pH: 4.5
B: Acetonitrile

Gradient:

Time	%B
0.0	80
25.0	55

Flow Rate: 0.6 mL/min
Pressure: 180 bar
Temperature: 60 °C
Detection: UV, 300 nm
Injection Volume: 3.0 µl
Sample Solvent: 80/20 Water/ Acetonitrile
Data Rate: 100 Hz
Response Time: 0.025 sec
LC System: Shimadzu Nexera

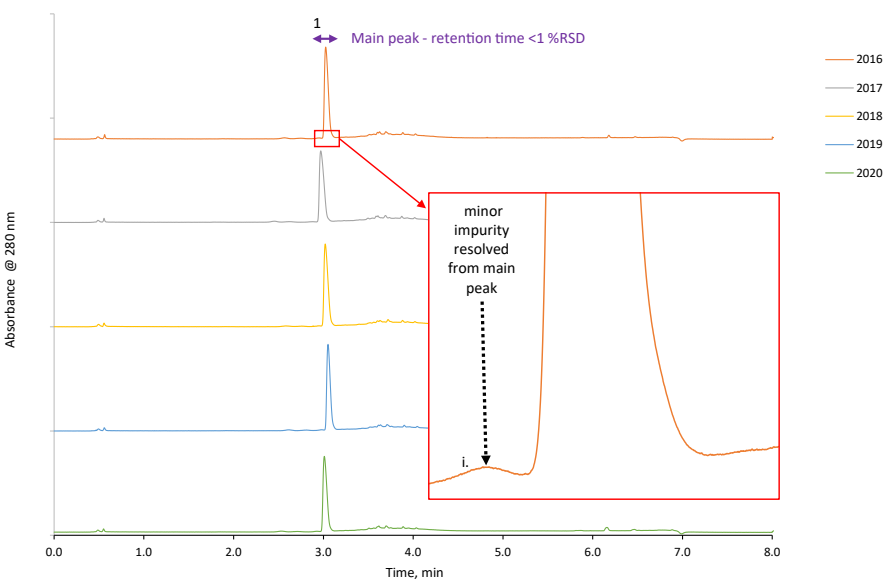
PEAK IDENTITIES

1. G3: maltotriose
   G#= DP of maltooligosaccharide

Excellent lot to lot reproducibility is observed with HALO® Glycan columns. Each chromatogram shows an efficient separation of procainamide-labeled dextran standards. Every lot of HALO® Glycan packing is tested using this sample to ensure lot to lot reproducibility and performance.

Peptide Analysis of Bee Venom Assay for Antimicrobial Properties Using HALO® Peptide

251



PEAK IDENTITIES:

1. Melittin
i. Impurity of a honey bee venom standard

TEST CONDITIONS:

Column: HALO 160 Å C18, 2.7 µm, 2.1x150mm
Part Number: 92122-702
Mobile Phase: **A:** Water/0.1% TFA
B: ACN/0.1% TFA

Gradient:

Time	%B
0.0	40
2.0	40
6.0	100
6.1	100
6.2	40
7.0	40

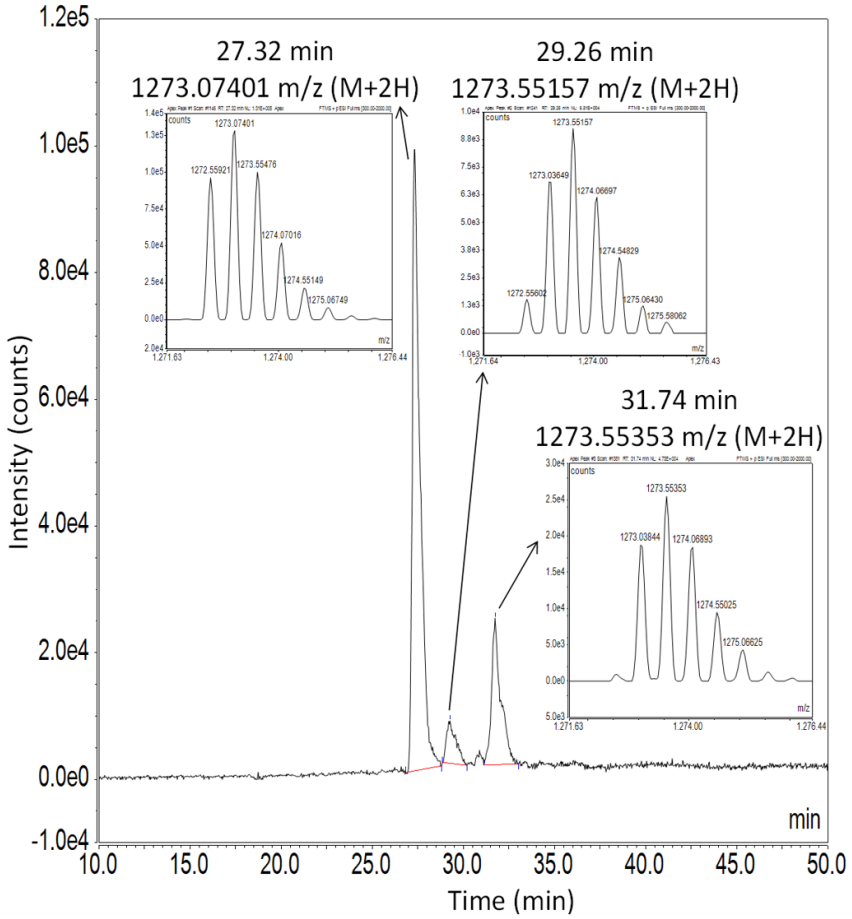
Flow Rate: 0.6 mL/min
Pressure: 408 bar
Temperature: 60 °C
Detection: 280 nm
Injection Volume: 1 µL
Sample Solvent: Water/ 0.1% TFA
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera

Antimicrobial peptides in animal venom (vAMPs) are natural antibiotics of emerging interest. As resistance over conventional antibiotics has become an area of concern, vAMPs are key alternative early drug discovery candidates. An assay of melittin from honey bee venom completed in <10 min (total analysis time) was demonstrated on five different manufactured HALO® 160 Å ES-C18 lots (2016, 2017, 2018, 2019, and 2020) illustrating the separation profile reproducibility over a five-year period.

The main active vAMP component in honey bee venom was resolved from minor related impurity peaks (unidentified) with a retention time reproducibility of <1% RSD. Furthermore, a closely related low abundant impurity peak could be separated. Critical aspects are achieved with HALO® column technology to develop reliable assays to support biomedical, and drug development research of vAMPs' physiological role in human diseases, as well as microbial and parasitic infections.

Capillary scale HILIC Separation of Deamidation Products of Trastuzumab

263-PE



PEAK IDENTITIES

Peptide fragments of GFYPSDIAVEWESNGQPENNYK

1. m/z= 1273.07401
2. m/z= 1273.55157
3. m/z= 1273.55353

The capillary HALO® Penta-HILIC column facilitated coupling of microflow LC conditions of 12 µL/min and a higher organic HILIC gradient separation. The column's high resolution capabilities resolved similar charged species required for examining peptide deamidation and isomerization products of Asn, Asp, and isoAsp forms of a peptide fragment of a trastuzumab tryptic digest.

TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 µm 0.5 x 150mm
Part Number: 98215-705
Mobile Phase A: 50 mM ammonium formate in water
Mobile Phase B: Acetonitrile/0.1% Formic acid
Gradient:

Time	%B
0.0	80
4.0	80
64.0	48

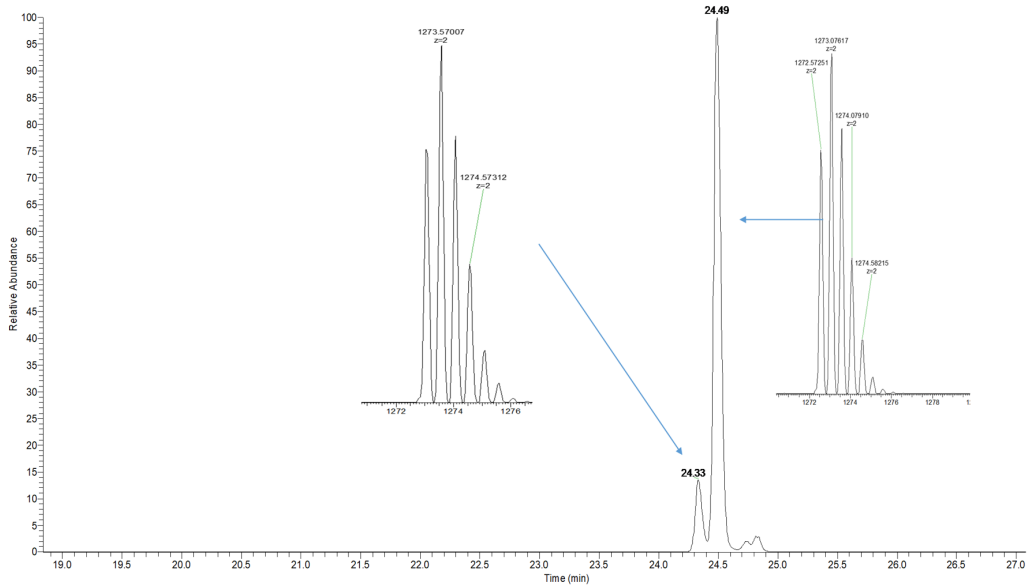
Flow Rate: 12 µL/min
Pressure: 123 bar
Temperature: 60 °C
Detection: ESI+
Injection Volume: 1 µL
Sample Solvent: 50 mM Tris-HCl /1.5M Guanidine-HCl, 0.5% formic acid
LC System: Thermo Ultimate 3000
MS System: Thermo Orbitrap Velos

MS CONDITIONS:

Spray Voltage (kV): 3.8
Capillary temperature: 300 °C
Sheath gas: 40
Aux gas: 10
RF lens: 50

Separation of Deamidation Products of the NIST mAb on HALO® ES-C18

264-PE



PEAK IDENTITIES

Peptide fragments of GFYPSDIAVEW-ESNGQPENNYK

1. m/z= 1273.57007
2. m/z= 1273.07617

Deamidation is a reaction in which an amide functional group in the side chain of the amino acids asparagine or glutamine is removed or converted to another functional group. Deamidation products are of increasing importance in proteomics because they can alter a protein's structure, or possibly its function and stability, resulting in degradation. This is especially of interest in monoclonal antibody (mAb) development as well. The HALO® ES-C18 has the high resolution nessessary to separate the deamidation products of the NIST mAb.

TEST CONDITIONS:

Column: HALO 160 Å ES-C18, 2.7 µm 2.1 x 100mm
Part Number: 95814-902
Mobile Phase A: Water/0.1% Formic acid
Mobile Phase B: Acetonitrile/0.1% Formic acid
Gradient:

Time	%B
0.0	2.0
45.0	40
45.5	80
48.0	80
48.5	2.0
55.0	End

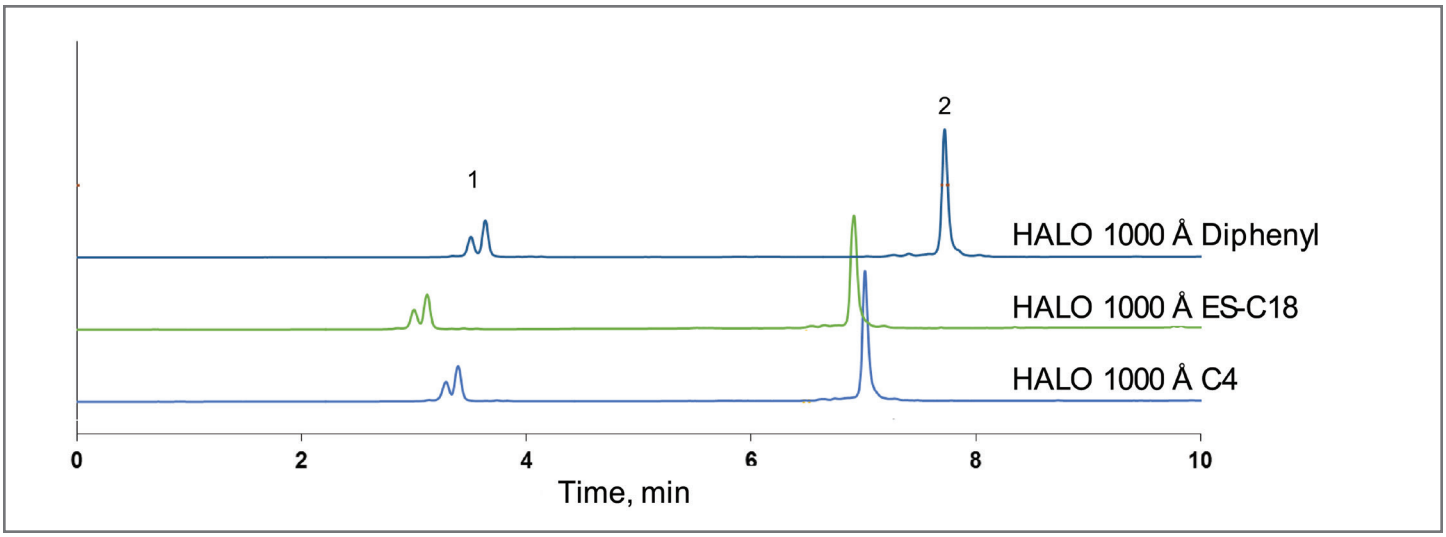
Flow Rate: 0.3 mL/min
Pressure: 124 bar
Temperature: 60 °C
Detection: ESI+
Injection Volume: 5 µL
Sample Solvent: 50 mM Tris-HCl /1.5M Guanidine-HCl, 0.5% formic acid
LC System: Shimadzu Nexera X2
MS System: Orbitrap Velos Pro

MS CONDITIONS:

Spray Voltage (kV): 4.0
Capillary temperature: 300 °C
Sheath gas: 40
Aux gas: 10
RF lens: 50

Comparison of an IdeS Digested mAb on Different HALO 1000 Å Phases

271-PR



PEAK IDENTITIES:

1. Fc/2
2. $F(ab')_2$

TEST CONDITIONS:

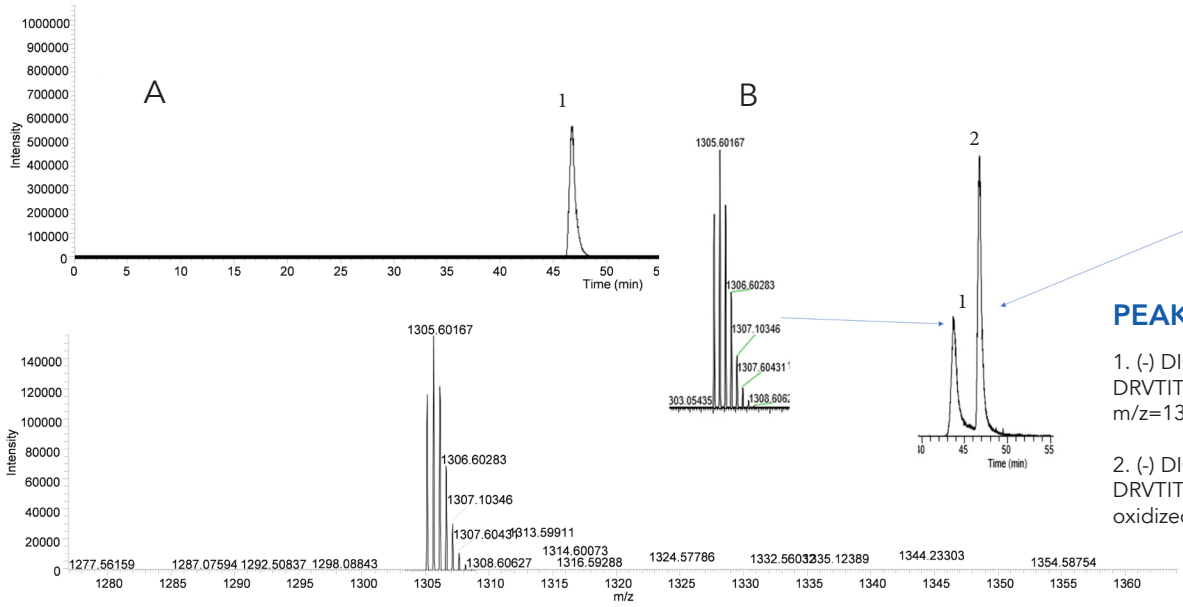
Columns:
HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-726
HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-702
HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-714

Mobile Phase A: water/0.1% TFA
Mobile Phase B: ACN/0.1% TFA
Gradient: 30-45% B in 10 min
Flow Rate: 0.4 mL/min
Temperature: 80 °C
Detection: Fluorescence (280 nm ex, 350 nm em)
Injection Volume: 0.5 µL
LC System: UPLC, I-Class

The characterization of mAbs is critically important for protein biotherapeutic drug development. Although the analysis of the heavy and light chain can provide important information, often times site specific information is more critical, and allows for a more thorough characterization of the mAb. IdeS, a cysteine protease, is often used to do a partial digestion of the mAb, and by site specific cleavage, provide heterogeneity information about the structure. Two Fc fragments (Fc/2) and one $(Fab')_2$ fragment are produced, which allows for a thorough characterization of the Fc fragment. The separation of IdeS digested Cetuximab was run on the three stationary phases that are available on the 1000 Å HALO® particle. Slightly different selectivity and retention were observed for the Diphenyl, ES-C18, and C4 with all of them providing excellent resolution and peak shape for the fragments of Cetuximab.

Oxidation of NIST mAb Fragment

274-PE



(A) Extracted ion chromatogram of (-) DIQMTQSPSSLSASVGDRVTITC(Carbamidomethyl)R(A)
(B) Extracted ion chromatogram oxidized (-) DIQMTQSPSSLSASVGDRVTITC(Carbamidomethyl)R(A) showing a mass shift of 8, as expected for an oxidized doubly charged peptide

TEST CONDITIONS:

Column: HALO® 90 Å Penta-HILIC, 2.7 µm, 0.5 x 150 mm
Part Number: 98215-705
Mobile Phase A: 50 mM Ammonium formate, pH 4.4
Mobile Phase B: 0.1% formic acid in acetonitrile
Gradient:

Time	%B
0.0	80
4.0	80
55	48
59	48
63	80
70	end

Flow Rate: 50 µL/min
Pressure: 158 bar
Temperature: 60 °C (standard)
80 °C (oxidized)
Detection: +ESI
Injection Volume: 5.0 µL
Sample Solvent: 70% ACN, 30% Water
LC System: Shimadzu Nexera X2
MS System: Thermo LTQ VELOS PRO

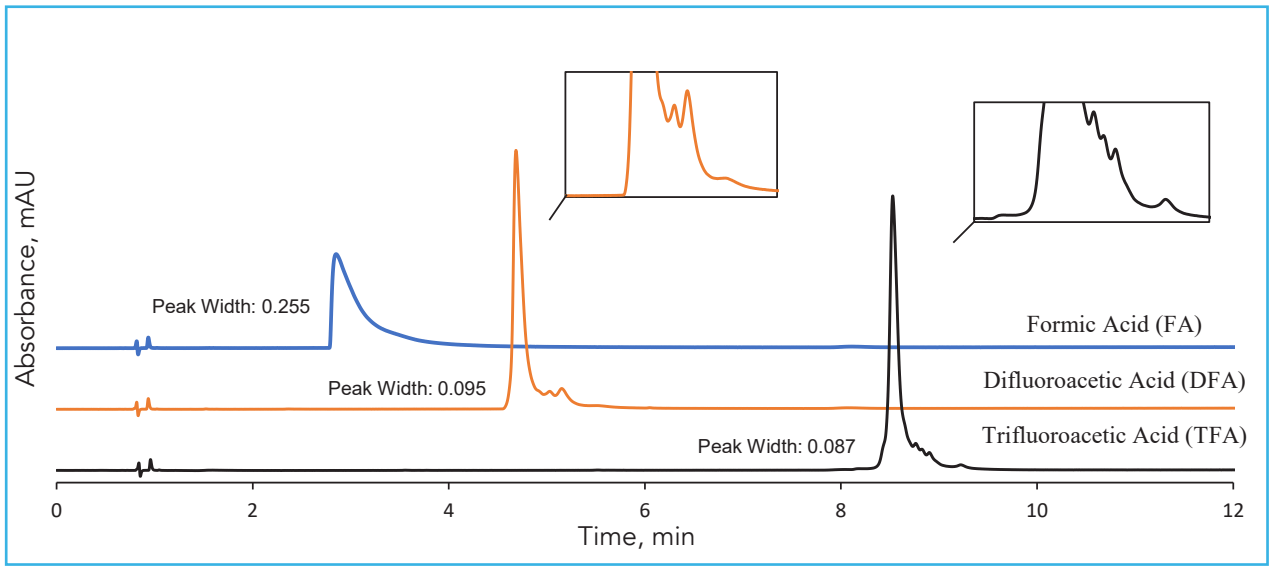
Post-translational modifications (PTMs), such as oxidation, are a critical variable that must be accounted for during protein analysis. Often times the minor mass shifts associated with these modifications are too small to be resolved during intact protein analysis, due to the charge envelope produced by large proteins, such as monoclonal antibodies (mAbs). However, chromatographically, these compounds will have a difference in retention time relative to the native, and can be separated before getting to the detector. Peptide analysis is an important method of characterization for mAbs because, in addition to revealing modifications such as oxidation, it can provide valuable insight into additional post-translational modifications, which may not be evident during intact mass analysis. In this experiment, the digested NIST mAb was exposed to high temperature in order to induce oxidation, and then analyzed using the HALO® Penta-HILIC capillary column, demonstrating it is an ideal choice for peptide oxidation analysis of mAbs.

MS CONDITIONS:

Ion mode: Positive
Aux gas: 2 arbitrary units
Sheath gas: 4 arbitrary units
Sweep gas: 0 arbitrary units
Rf lens: 55 V
Heater temp: 225°C
Ion transfer tube: 275°C
Capillary Voltage: 3.5 kV

Effect of Acid Modifiers on Intact mAb Peak Shape

Application Note 154-PR



Trastuzumab (~148 kDa) is a monoclonal antibody (mAb) used to treat breast cancer. TFA and DFA can be used as mobile phase additives instead of formic acid to provide much narrower and more symmetrical peaks, and to allow adjustments to retention and resolution among minor variants.

TEST CONDITIONS:

Column: HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-714
Mobile Phase:
A: Water with 0.1% FA, DFA, or TFA (as noted)
B: 80/20 ACN/water with 0.1% FA, DFA, or TFA (as noted)

Gradient:

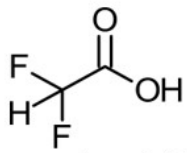
Time (min)	% B
0.0	35.0
12.0	47.5

Flow Rate: 0.4 mL/min
Pressure: 218 bar
Temperature: 80 °C
Detection: UV 280 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: 30/70 ACN/water
Response Time: 0.05 sec
Flow Cell: 1.0 µL
Data Rate: 12.5 Hz
LC System: Shimadzu Nexera X2

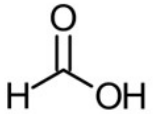
PEAK IDENTITIES:

1. Difluoroacetic acid (DFA)
2. Formic acid (FA)
3. Trifluoroacetic acid (TFA)

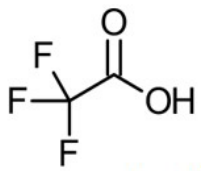
STRUCTURES:



Difluoroacetic Acid (DFA)



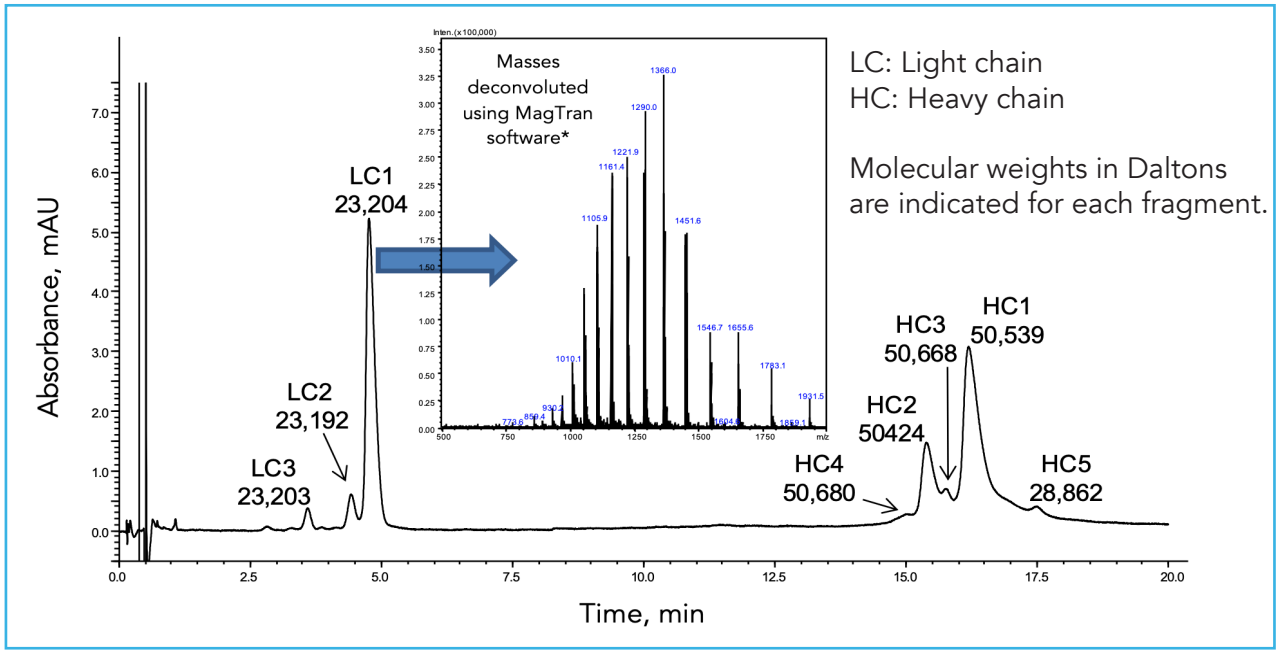
Formic Acid (FA)



Trifluoroacetic Acid (TFA)

LC-MS Analysis of Reduced IgG1 Monoclonal Antibody Fragments Using HALO 400 Å C4

Application Note 125-PR



TEST CONDITIONS:

Column: HALO 400 Å C4, 3.4 µm, 2.1 x 100 mm
Part Number: 93412-614
Mobile Phase:
A: 0.5% formic acid with 20 mM ammonium formate
B: 45% acetonitrile/45% isopropanol/0.5% formic acid/9.5% water with 20 mM ammonium formate
Gradient: 29–32% B in 20 min
Flow Rate: 0.4 mL/min
Pressure: 20 bar
Temperature: 80 °C
Detection: 280 nm and MS using 2 pps scan rate from 500 to 2000 m/z
Injection Volume: 2 µL of 2 µg/µL reduced and alkylated IgG1
Sample Solvent: 0.25% formic acid in water
MS Parameters: Positive ion mode, ESI at +4.5 kV, 400°C heat block, 225°C capillary
LC-MS System: Shimadzu Nexera and LCMS-2020 (single quadrupole MS)

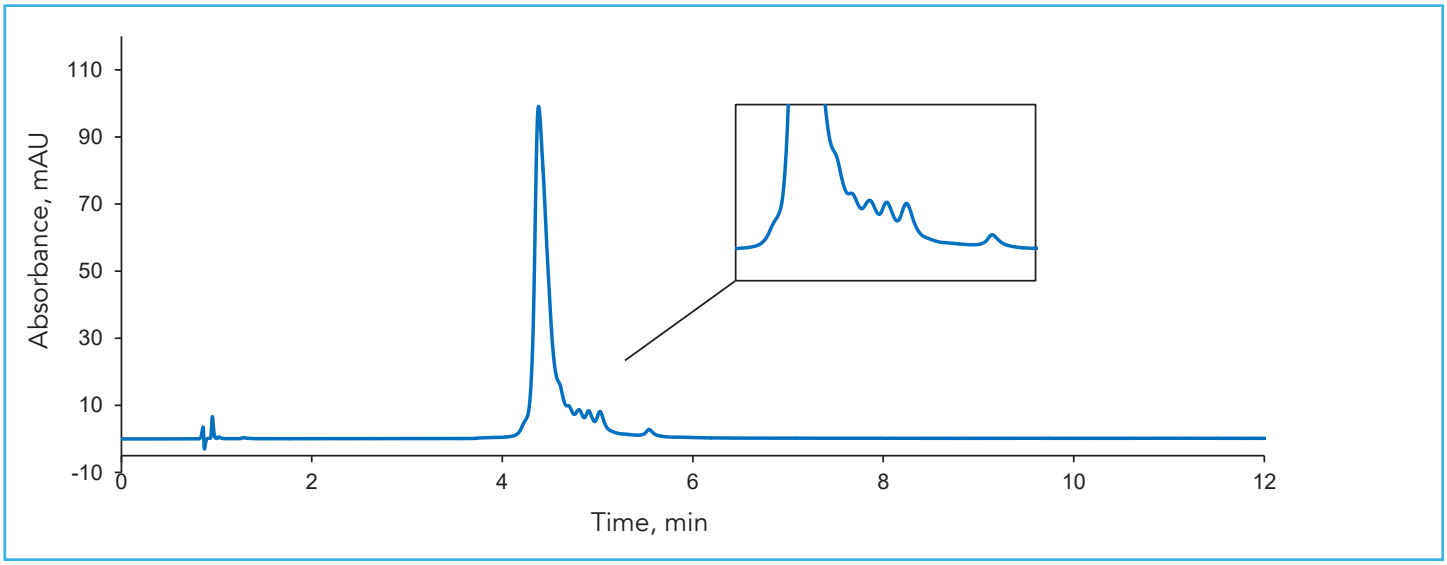
HALO 400 Å C4 has the low pH and high temperature stability that is required to analyze reduced and alkylated IgG1 using MS compatible mobile phase. The use of 80 °C enables improved peak shape while the high resolution MS allow complete analysis of the IgG1 fragments that are present.

Adapted from J. Chromatogr. A 1315 (2013) 118-126.

*Z. Zhang, A.G. Marshall, J. Am. Soc. Mass Spectrom. 9 (1998) 225.

HALO 1000 Å C4 Protein Column for High Resolution Separation of a Monoclonal Antibody

Application Note 149-PR



Trastuzumab (MW ~148 kDa) is a monoclonal antibody used to treat breast cancer. Enhanced resolution of trastuzumab and its variants is demonstrated in the chromatogram above. The pores of the HALO 1000 Å C4 Protein particles accommodate larger biomolecules enabling superior separations at high temperatures.

TEST CONDITIONS:

Column: HALO 1000 Å C4, 2.7 µm, 2.1 x 100 mm
Part Number: 92712-614
Mobile Phase:
A: Water, 0.1% TFA
B: 80/20 ACN/water, 0.085% TFA
Gradient:

Time (min)	% B
0.0	40.0
12.0	47.5

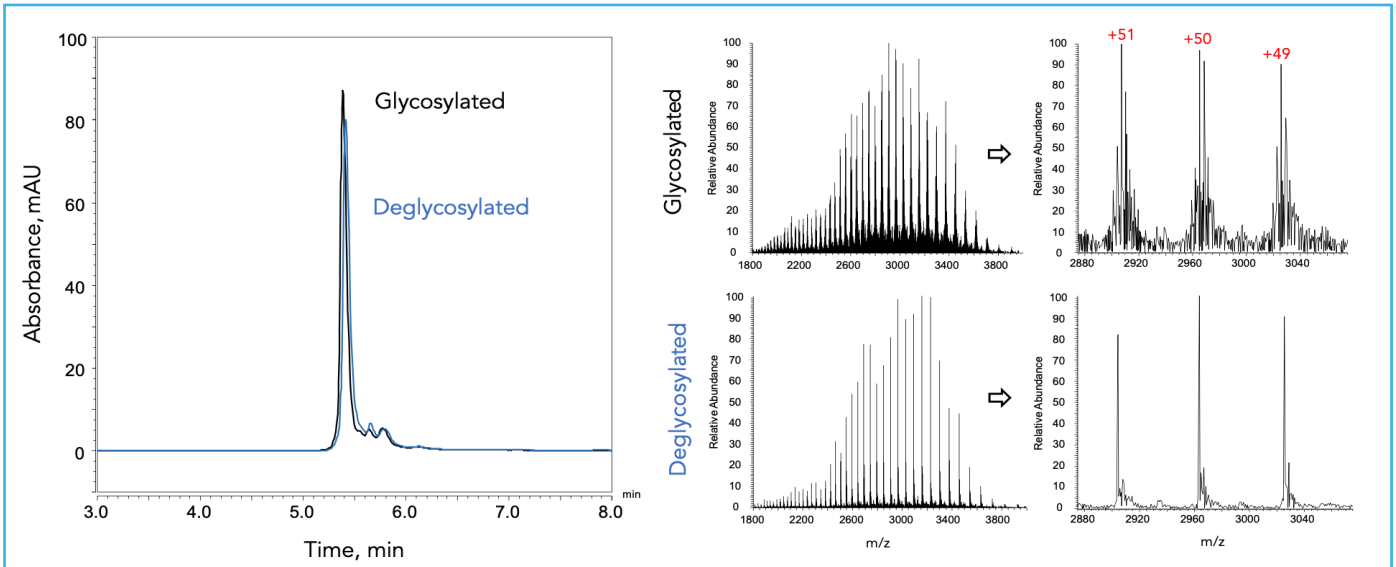
Flow Rate: 0.4 mL/min
Pressure: 210 bar
Temperature: 80 °C
Detection: UV 280 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: 70/30 water/ACN
Response Time: 0.05 sec
Data Rate: 12.5 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

Trastuzumab Structure:

Image from the RCSB PDB (www.rcsb.org) of PDB ID 1N8Z
Cho, H.-S., Mason, K., Ramyar, K.X., Stanley, A.M., Gabelli, S.B., Denney Jr., D.W., Leahy, D.J.

LC-MS Analysis of Trastuzumab Using HALO® 1000 Å C4

Application Note 151-PR



LC TEST CONDITIONS:

Column: HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-714
Mobile Phase:
A: 10 mM difluoroacetic acid (DFA) in water
B: 10 mM difluoroacetic acid in 10/90 water/acetonitrile
Gradient: 32–42% B in 10 min
Flow Rate: 0.35 mL/min
Pressure: 184 bar
Temperature: 80 °C
Detection: 280 nm
Injection Volume: 1.0 µL of 2 mg/mL trastuzumab (glycosylated/deglycosylated)
Sample Solvent: 0.1% DFA in 70/30 water/acetonitrile
LC System: Shimadzu Nexera

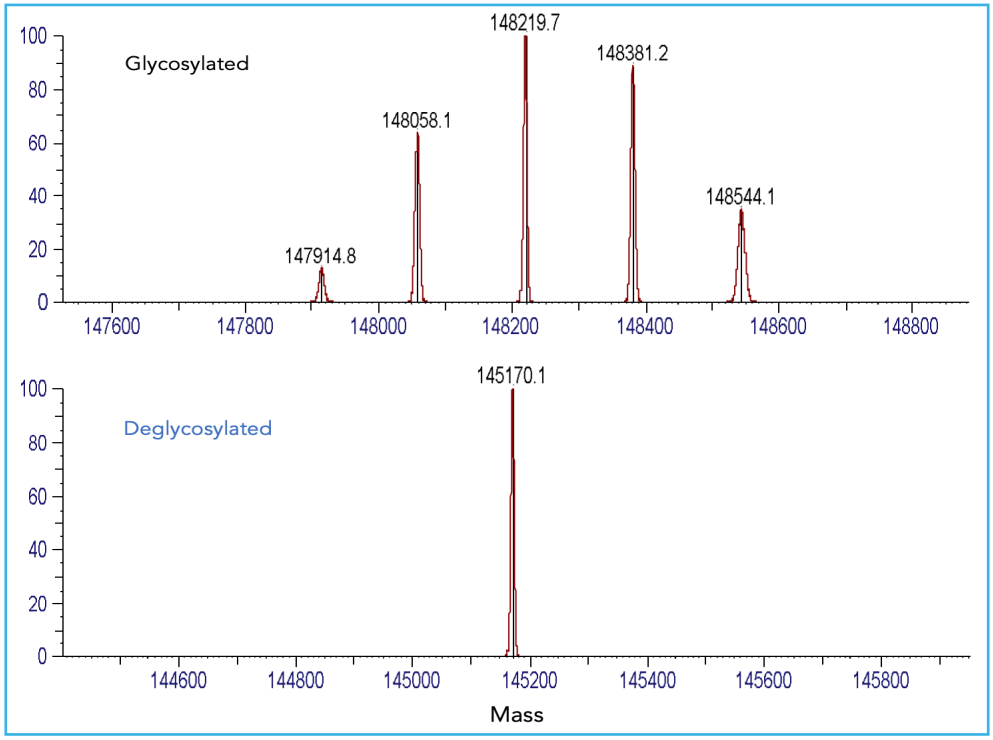
LC-MS analysis using a HALO 1000 Å C4 Protein column has been used to analyze two samples of the monoclonal antibody, trastuzumab: glycosylated and enzymatically deglycosylated. Minor variant structures are observed in both the glycosylated and deglycosylated monoclonal IgG (small peaks after main peak), indicating that the polypeptides are structure variants.

The glycosylation profile of therapeutic mAbs is an important characteristic, which must be monitored throughout the manufacturing process. Determination of the mass of the deglycosylated IgG confirms the identity and integrity of the protein.

MS TEST CONDITIONS:

MS System: Thermo Fisher Orbitrap VelosPro ETD
Scan Time: 6 µscans/250 ms max inject time
Scan Range: 1800 to 4000 m/z
MS Parameters: Positive ion mode, ESI at +4.0 kV, 225 °C capillary

Deconvoluted Spectra and Peak Information



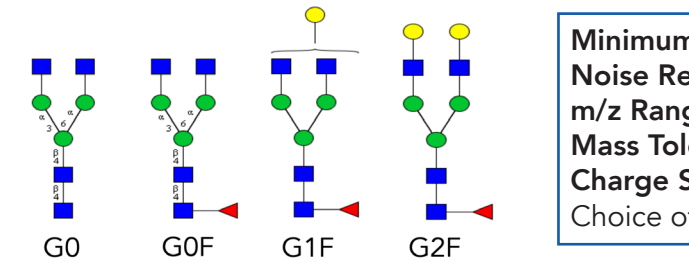
The structure of trastuzumab consists of two heavy chains and two light chains. Glycosylation occurs on the two heavy chains. One or more of the same or different carbohydrate moiety can be present on each heavy chain. The table below contains the combinations of sugars that correspond to the masses that were observed upon deconvolution of the mass spectrum on the previous page. The last column is the mass of trastuzumab upon treatment with PNGase F which cleaves the sugars.

GLYCANS:	G0/G0F		G0F/G0F		G1F/G0F		G1F/G1F, G2F/G0F		G1F/G2F		Deglycosylated Trastuzumab	
	T[1]	M[1]	T	M	T	M	T	M	T	M	T	M
Trastuzumab	147911	147915	148057	148058	148219	148220	148381	148381	148544	148544	145167	145170
ΔMass (glyc) Trastuzumab	2744	2745	2890	2888	3052	3050	3214	3211	3376	3374	---	3

T = Theoretical Mass
M = Measured Mass
[1] All masses reported in Daltons

Glycan Structures:

- Fucose
- *N*-Acetylglucosamine
- Galactose
- Mannose

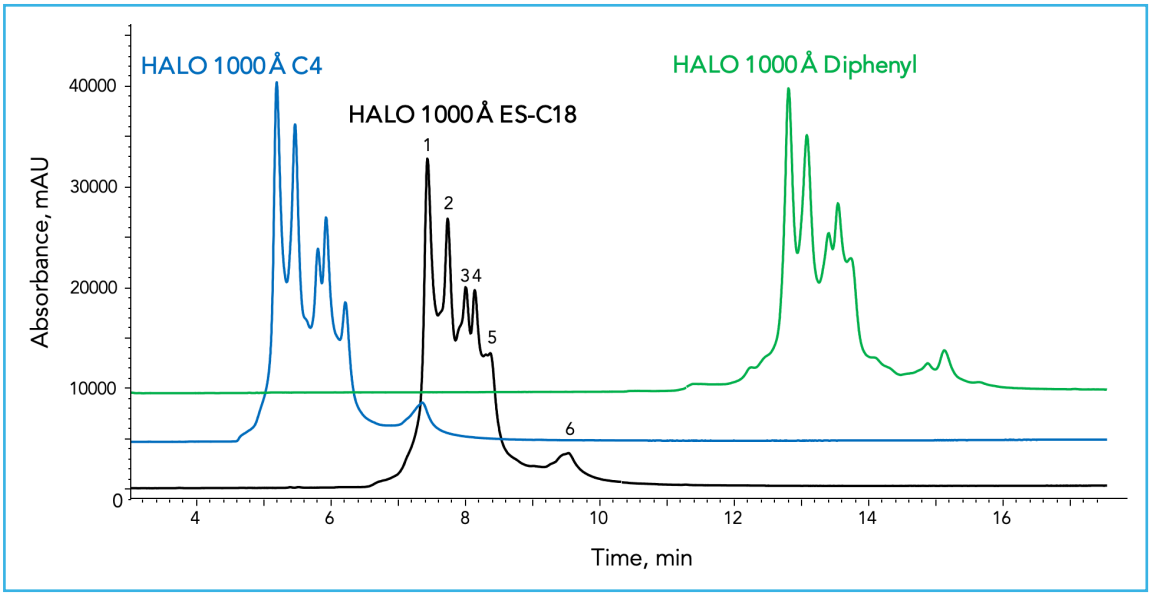


Deconvolution Parameters:

Minimum Adjacent Charges: 3 - 6
Noise Rejection: 95% Confidence
m/z Range: 1800 - 4000
Mass Tolerance: 20 ppm
Charge State Range: 40 - 120
Choice of Peak Model Intact Protein

IgG2 Comparison on HALO 1000 Å C4, ES-C18, and Diphenyl

Application Note 174-PR



There are currently three bonded phases available on HALO 1000 Å Fused-Core® particles – C4, ES-C18, and Diphenyl. Each shows unique selectivity for the separation of monoclonal antibodies. In this example, denosumab isoforms are resolved using a shallow gradient with the addition of n-propanol. Diphenyl phase is the most retentive phase, followed by ES-C18, and then C4. All three phases are recommended to be screened to determine which one yields the optimum separation for mAbs under investigation.

PEAK IDENTITIES:

1. IgG2-B
2. IgG2-B
3. IgG2-A/B
4. IgG2-A/B
5. IgG2-A
6. IgG2-A*

} Disulfide bridge isoforms of IgG2

Note: Labels on ES-C18 chromatogram also apply to C4 and Diphenyl chromatograms.

TEST CONDITIONS:

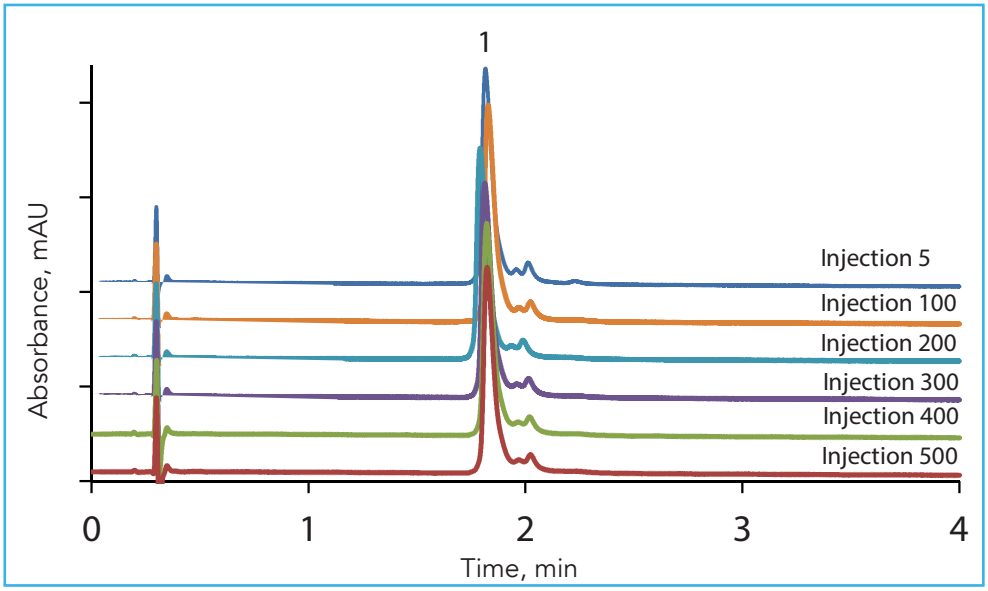
Columns:
1) HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-714
2) HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-702
3) HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-726

Mobile Phase:
A: 2/10/88 n-propanol/ACN/H_2O + 0.1% DFA
B: 70/20/10 n-propanol/ACN/H_2O + 0.1% DFA

Gradient: 16-26% B in 20 min
Flow Rate: 0.2 mL/min
Temperature: 80 °C
Detection: 280 nm, PDA; 350 nm reference
Injection Volume: 2.0 µL of 2 mg/mL denosumab
Sample Solvent: Water (0.1% TFA)
LC System: Shimadzu Nexera

High Temperature/Low pH Stability with HALO 1000 Å ES-C18, 2.7 µm

Application Note 178-PR



PEAK IDENTITIES:

1. Trastuzumab

Trastuzumab (MW ~148 kDa) is a monoclonal antibody used to treat breast cancer. A stability experiment using a HALO 1000 Å ES-C18 column shows excellent reproducibility for 500 injections of trastuzumab. The sterically protected C18 bonded phase enables rugged stability at the elevated temperature and low pH conditions that are typically used for protein analysis.

TEST CONDITIONS:

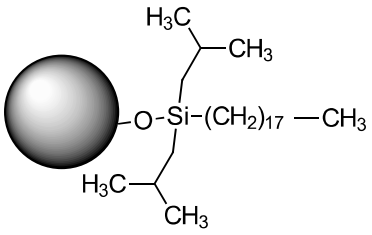
Column: HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 50 mm
Part Number: 92712-402
Mobile Phase:
A: Water/0.1% TFA
B: Acetonitrile/0.1% TFA
Gradient:

Time (min)	% B
0.0	32
4.0	38

Flow Rate: 0.4 mL/min
Pressure: 81 bar
Temperature: 80 °C
Detection: UV 280 nm, PDA
Injection Volume: 1.2 µL
Sample Solvent: Water
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

STRUCTURES:

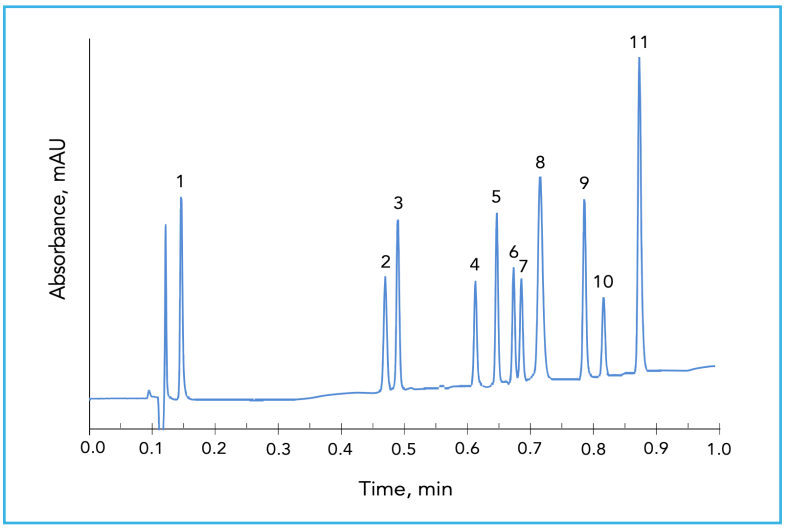
1000 Å 2.7µm particle



ES-C18 bonded phase

Separation of Peptides and Small Proteins on HALO 160 Å ES-C18

Application Note 62-PT



PEAK IDENTITIES:

1. Gly-Tyr
2. Val-Tyr-Val
3. Angiotensin (1-7) amide
4. Met-Enk
5. Angiotensin (1-8) amide
6. Angiotensin II
7. Leu-Enk
8. Ribonuclease A
9. Angiotensin (1-12) (human)
10. Angiotensin (1-12) (mouse)
11. Porcine insulin

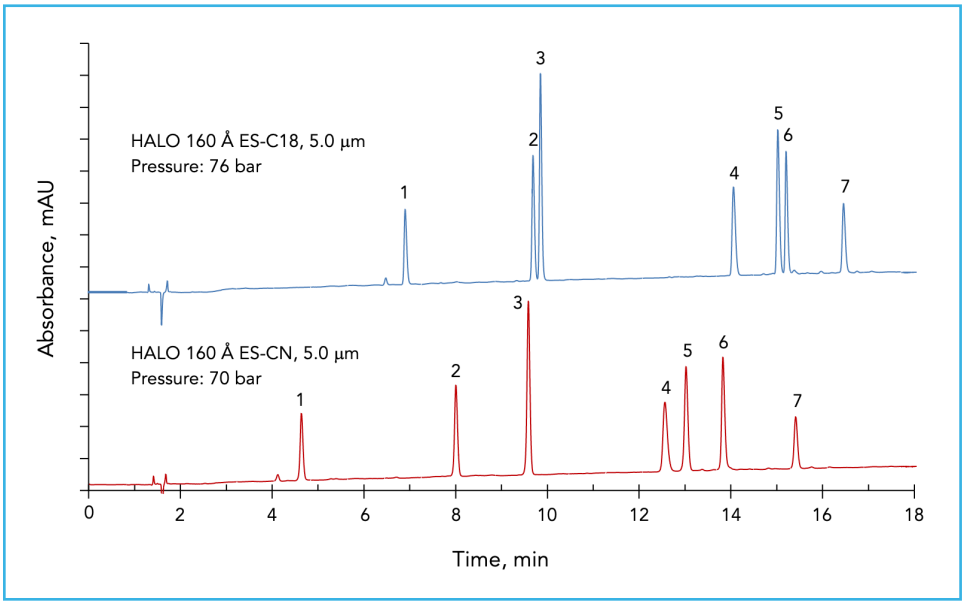
This separation shows the utility of the HALO® Fused-Core® 160 Å ES-C18 stationary phase for the separation of peptides by HPLC. An average pore size of about 160 Angstroms enhances the mass transfer of peptides and small proteins of up to a molecular weight of approximately 15 kD, depending on the molecular configuration. Also, the stationary phase is a sterically protected C18 bonded silane to increase resistance to low pH mobile phases and elevated temperatures (up to 100 °C) that are commonly used in the separation of many biological materials.

TEST CONDITIONS:

Column: HALO 160 Å ES-C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92124-402
Mobile Phase:
A: 90% (0.1% TFA in water)/10% acetonitrile
B: 30% (0.1% TFA in water)/70% acetonitrile
Gradient: 0% B to 87% B in 1 min
Flow Rate: 5.0 mL/min
Pressure: 330 bar
Temperature: 60 °C
Detection: UV 220 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Mobile phase A
Response Time: < 0.12 sec
Flow Cell: 5.0 µL semi-micro
Gradient Dwell Volume: 0.88 mL
LC System: Quaternary Agilent 1100

Separation of Seven Peptides on HALO® 5 µm 160 Å ES-C18 and ES-CN Phases

Application Note 102-PE



PEAK IDENTITIES:

1. Asp-Phe
2. Angiotensin (1-7) amide
3. Tyr-Tyr-Tyr
4. Bradykinin
5. Leu-Enk
6. Angiotensin II
7. Neurotensin

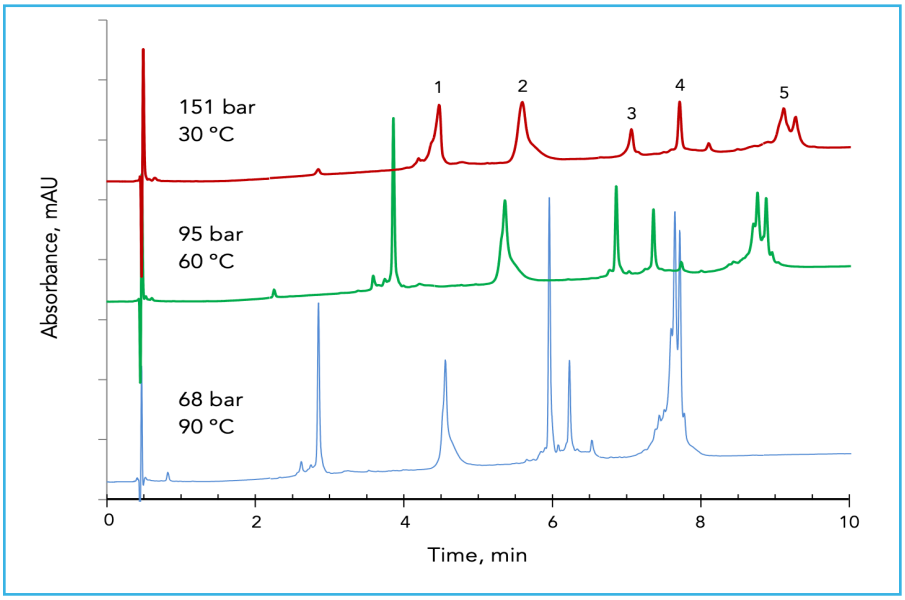
HALO® 5 µm, 160 Å pore, HPLC column phases are suitable for the separation of molecules up to about 20 kDa in size. Shown here are two different bonded phases that allow for different selectivities that can enhance separation capabilities. These two C18 and cyano bonded phases are made using sterically hindered silanes for increased stability at elevated temperatures and low pH.

TEST CONDITIONS:

Columns:
1) HALO 160 Å ES-C18, 5 µm, 4.6 x 150 mm
Part Number: 92124-702
2) HALO 160 Å ES-CN, 5 µm, 4.6 x 150 mm
Part Number: 92124-704
Mobile Phase:
A: 0.1% trifluoroacetic acid in water
B: 0.1% trifluoroacetic acid in acetonitrile
Gradient: 5% B to 50% B in 30 min
Flow Rate: 1.0 mL/min
Initial Pressure: See chart
Temperature: 40 °C
Detection: UV 215 nm, VWD
Injection Volume: 10 µL
Sample Solvent: Mobile phase A
Response Time: 0.12 sec
Flow Cell: 5.0 µL semi-micro
LC System: Agilent 1100 Quaternary

Effect of Temperature on the Separation of Proteins on HALO 400 Å C4

Application Note 103-PR



PEAK IDENTITIES:

1. Lysozyme (14.3 kDa)
2. Bovine serum albumin (66.4 kDa)
3. α-Chymotrypsinogen A (25.0 kDa
4. Enolase (46.7 kDa)
5. Ovalbumin (44.0 kDa)

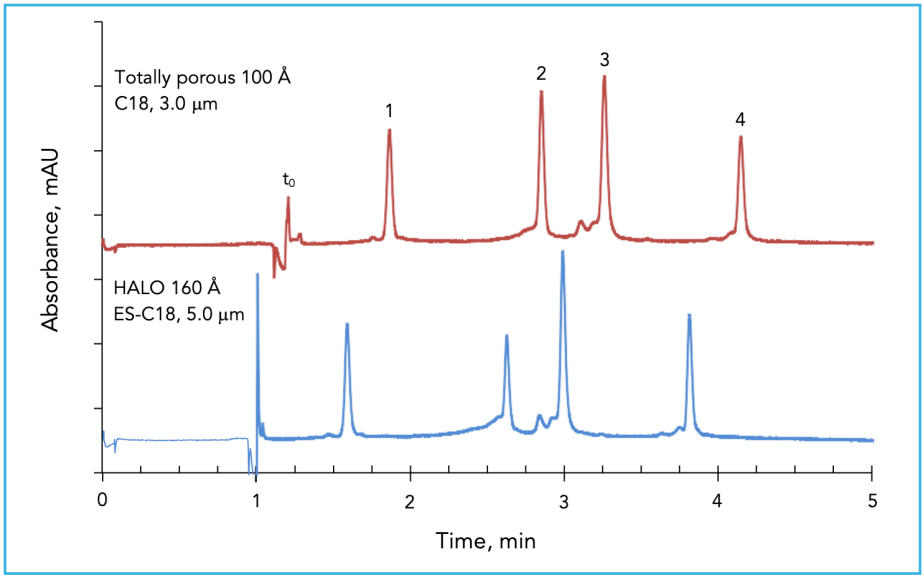
These separations demonstrate the effect of elevated temperatures on the efficiency of protein separations done under reversed-phase conditions on a HALO 400 Å C4, 3.4 µm, column. One observes larger and narrower peaks as the temperature increases. The HALO® C4 phase has been shown to be very stable even at these elevated temperatures.

TEST CONDITIONS:

Column: HALO 400 Å C4, 3.4 µm, 2.1 x 100 mm
Part Number: 93412-614
Mobile Phase: 72/28 - A/B
A: 0.1% trifluoroacetic acid in water
B: 0.1% trifluoroacetic acid in acetonitrile
Gradient: 28% B to 58% B in 10 min
Gradient Delay Volume: ~250 µL
Flow Rate: 0.45 mL/min
Pressure: See chart
Temperature: See chart
Detection: UV 215 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: Mobile phase A
Response Time: 1.0 sec
Flow Cell: 2.0 µL micro cell
LC System: Agilent 1200 SL

Separation of Four Small Proteins on HALO® 160 Å ES-C18, 5 µm vs. Totally Porous C18, 3.0 µm

Application Note 104-PR



PEAK IDENTITIES:

1. Ribonuclease A (13.7 KDa)
2. Cytochrome c (12.4 KDa)
3. Lysozyme (14.3 KDa)
4. α-Lactalbumin (14.2 KDa)

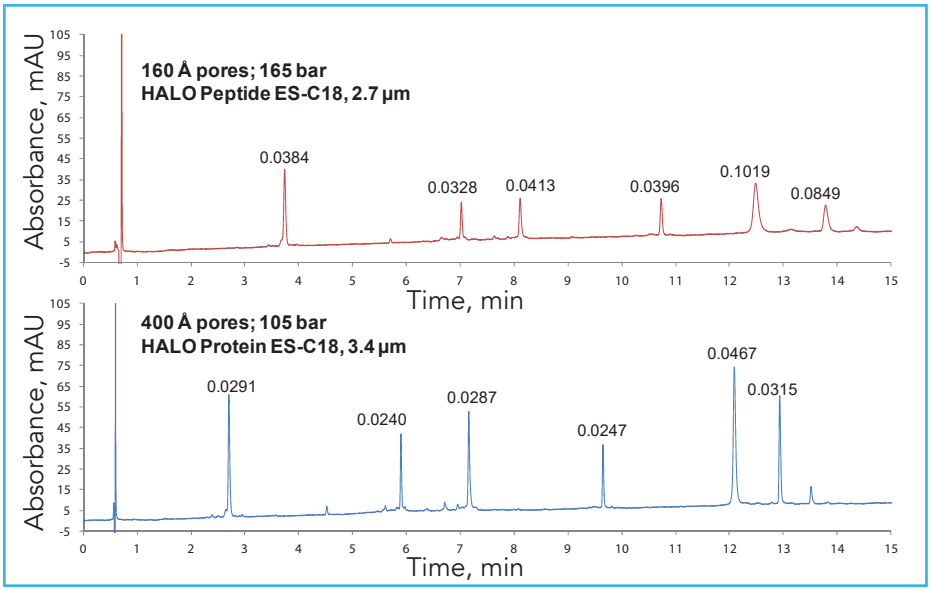
These chromatograms show the separation of four low MW proteins on HALO 160 Å ES-C18, 5 µm column vs. a totally porous C18, 3.0 µm column. The separations are similar with the benefit of the HALO® 5 µm column having lower back pressure and similar resolution. The HALO® 5 µm ES-C18 phase is made with sterically hindered silanes during manufacture, enhancing the stability-even at temperatures up to 90 °C. The stability of the totally porous C18 column was not evaluated.

TEST CONDITIONS:

Columns:
1) HALO 160 Å ES-C18, 5 µm, 4.6 x 150 mm
Part Number: 95124-702
2) 100 Å totally porous C18, 3.0 µm, 4.6 x 150 mm
Mobile Phase: 72/28 - A/B (start)
A: Water with 0.1% trifluoroacetic acid
B: Acetonitrile with 0.1% trifluoroacetic acid
Gradient: 28% B to 55% B in 5 min
Flow Rate: 1.5 mL/min
Pressure: 95 bar (HALO®)
170 bar (competitor)
Temperature: 60 °C
Detection: UV 280 nm, PDA
Injection Volume: 15 µL
Sample Solvent: Mobile phase A
Response Time: 0.1 sec
Flow Cell: 2.0 µL micro cell
LC System: Agilent 1200 SL

Effect of Silica Pore Size on Protein Separations

Application Note 130-PR



PEAK IDENTITIES:

1. Ribonuclease A (13.7 kDa)
2. Cytochrome C (12.4 kDa)
3. Lysozyme (14.3 kDa)
4. α-Lactalbumin (14.2 kDa)
5. Catalase (tetramer of ~60 kDa each)
6. Enolase (46.7 kDa)

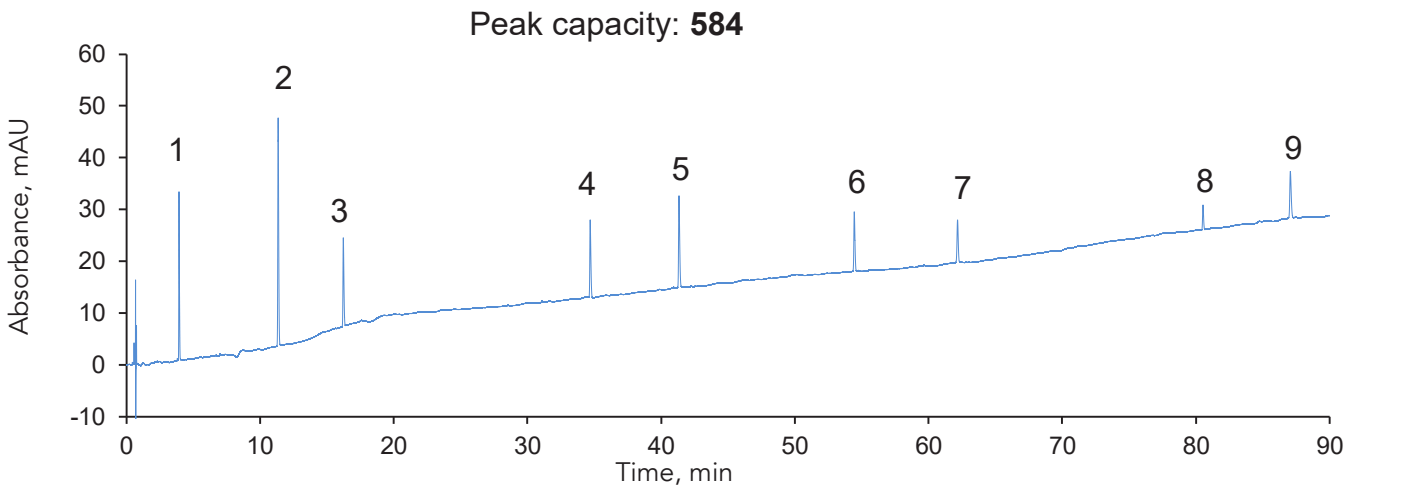
Sharper, taller peaks are observed using the HALO 400 Å ES-C18 column because the larger pore size allows unrestricted diffusion for these biomolecules into and out of the porous shell. The half height peak widths above each protein peak are significantly smaller with the HALO 400 Å column despite the larger particle size of the packing material, emphasizing the importance of larger pores when separating proteins.

TEST CONDITIONS:

Columns:
1) HALO 160 Å ES-C18, 2.7 µm, 4.6 x 100 mm
Part Number: 92124-602
2) HALO 400 Å ES-C18, 3.4 µm, 4.6 x 100 mm
Part Number: 93414-602
Mobile Phase:
A: 0.1% trifluoroacetic acid in water
B: 0.1% trifluoroacetic acid in acetonitrile
Gradient: 23% B to 50% B in 15 min
Flow Rate: 1.5 mL/min
Initial Pressure: See chart
Temperature: 60 °C
Detection: UV 215 nm, VWD
Injection Volume: 5.0 µL
Sample Solvent: Mobile phase A
Response Time: 0.12 sec
Flow Cell: 5.0 µL semi-micro
Data Rate: 14 Hz
LC System: Agilent 1100 Quaternary

Very High Peak Capacity with HALO 160 Å ES-C18, 2.0 µm

Application Note 136-PE



With a HALO® 2.0 µm 160 Å ES-C18 column, very high peak capacity values can be obtained within 90 minutes. The sharp, narrow peaks facilitate separations of complex, challenging samples, such as tryptic digests.

TEST CONDITIONS:

Column: HALO 160 Å ES-C18, 2.0 µm, 2.1 x 150 mm
Part Number: 91122-702
Mobile Phase:
A: 0.1% Trifluoroacetic acid in water
B: 0.1% Trifluoroacetic acid in 80/20 acetonitrile/water
Gradient: 5% B to 50% B in 90 min
Flow Rate: 0.5 mL/min
Max. Pressure: 577 bar
Temperature: 60 °C
Detection: UV 215 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: Mobile phase A
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

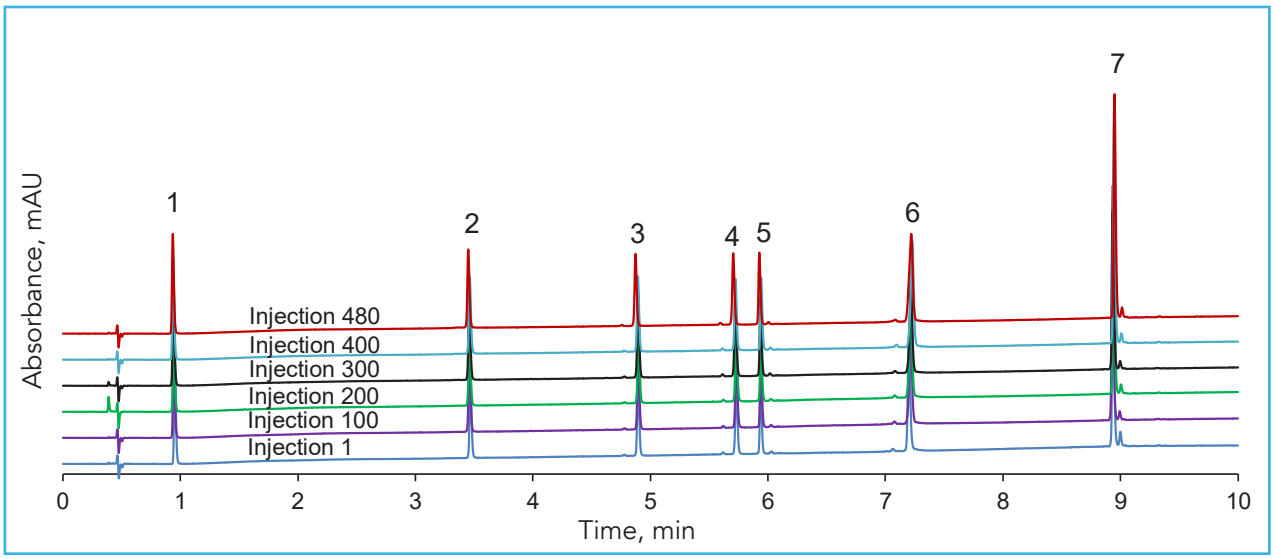
PEAK IDENTITIES:	MW (g/mol):
1. Asp-Phe	280
2. Tyr-Tyr-Tyr	508
3. Angiotensin (1-7) amide	898
4. Angiotensin II	1046
5. Angiotensin (1-12) human	1509
6. Neurotensin	1673
7. ß-endorphin	3465
8. Sauvagine	4599
9. Mellitin	2847

Peak Capacity: $n_{pc} = \frac{(t_f - t_i)}{W_{4\sigma}}$

where t_i is the time for initial measurable peak in the gradient, t_f is the time for final peak and $W_{4\sigma}$ is the average four-sigma width in time for the peaks in the chromatogram

High Temperature/Low pH Stability with HALO 160 Å ES-C18, 2.0 µm

Application Note 137-PE



The sterically-protected C18 phase on the HALO® 2.0 µm 160 Å column enables high temperature stability with low pH mobile phases. The replicate injections were stopped at injection 480 (15,500 column volumes). The column is expected to have a lifetime of ~1000 injections, depending on the type of sample and conditions used.

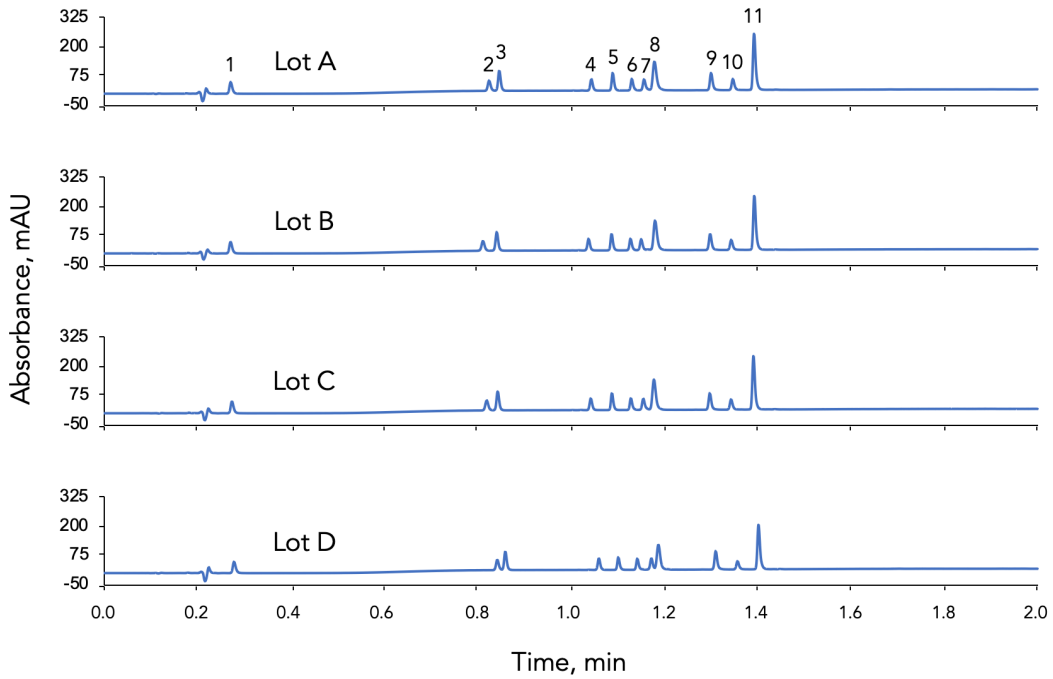
PEAK IDENTITIES:	MW (g/mol):
1. Gly-Tyr	238
2. Val-Tyr-Val	380
3. Met-enkephalin	574
4. Angiotensin II	1046
5. Leu-enkephalin	556
6. Ribonuclease A	13,700
7. Bovine insulin	5733

TEST CONDITIONS:

Column: HALO 160 Å ES-C18, 2.0 µm, 2.1 x 100 mm
Part Number: 91122-602
Mobile Phase:
A: 0.1% trifluoroacetic acid in water
B: 0.1% trifluoroacetic acid in 80/20 acetonitrile/water
Gradient: 6% B to 54% B in 10 min
Flow Rate: 0.5 mL/min
Initial Pressure: 395 bar
Maximum Pressure: 417 bar
Temperature: 60 °C
Detection: UV 215 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: Mobile phase A
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

HALO 160 Å ES-C18, 2.0 µm
Lot Reproducibility

Application Note 138-PE



The lot-to-lot reproducibility of HALO® 2.0 µm 160 Å ES-C18 is maintained by tightly controlled manufacturing practices and quality assurance testing. This ensures the reliability of the product over its lifetime.

TEST CONDITIONS:

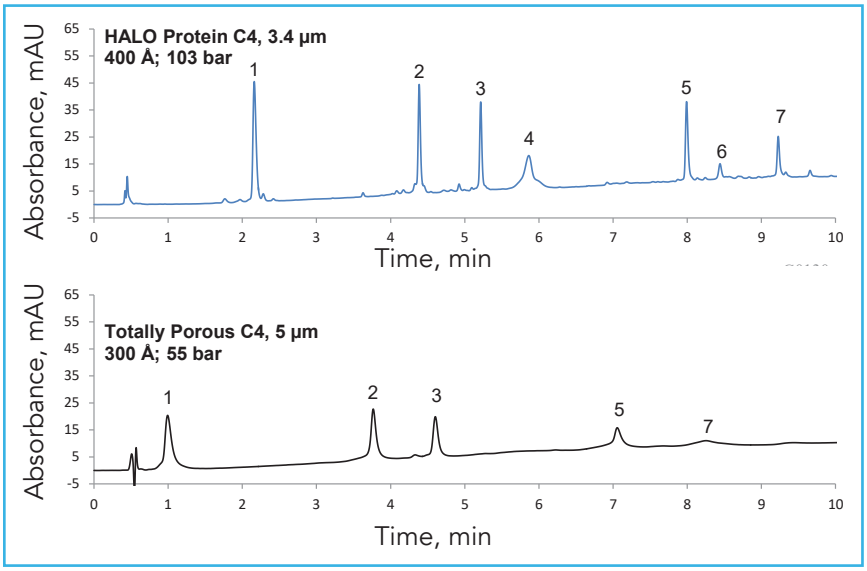
Column: HALO 160 Å ES-C18, 2.0 µm, 3.0 x 50 mm
Part Number: 91123-402
Mobile Phase:
A: 0.1% trifluoroacetic acid in water
B: 0.1% trifluoroacetic acid in 80/20 acetonitrile/water
Gradient: Hold at 12.5% B for 0.1 min; 12.5% B to 93% B from 0.1 – 2.0 min
Flow Rate: 1.1 mL/min
Initial Pressure: 278 bar
Temperature: 60 °C
Detection: UV 215 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: Mobile phase A
Response Time: 0.025 sec
Flow Cell: 1.0 µL
Data Rate: 200 Hz
LC System: Shimadzu Nexera X2

PEAK IDENTITIES:

	MW (g/mol)	% RSD (retention times)
1. Gly-Tyr	238	1.21
2. Val-Tyr-Val	380	1.59
3. Angiotensin 1/2 (1-7) amide	898	0.95
4. Met-enkephalin	574	0.92
5. Angiotensin 1/2 (1-8) amide	1045	0.60
6. Angiotensin II	1046	0.61
7. Leu-enkephalin	556	0.82
8. Ribonuclease A	13,700	0.35
9. Angiotensin (1-12) (mouse)	1573	0.46
10. Bovine Insulin	5733	0.49
11. Angiotensin (1-12) (human)	1509	0.36

Improved Separations with HALO 400 Å C4 Compared to Totally Porous C4

Application Note 141-PR



PEAK IDENTITIES:

1. Ribonuclease A (13.7 kDa)
2. Cytochrome C (12.4 kDa)
3. Lysozyme (14.3 kDa)
4. Holotransferrin (77 kDa)
5. Apomyoglobin (17 kDa)
6. Catalase (tetramer of ~60 kDa each)
7. Enolase (46.7 kDa)

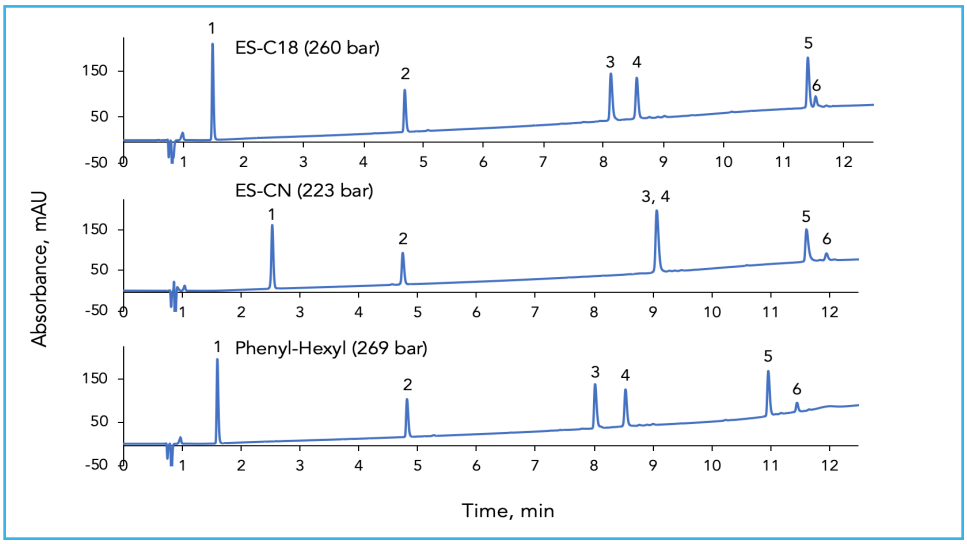
Sharper, taller peaks are observed using the HALO 400 Å C4 column compared to a conventional totally porous C4 column. Additionally, the HALO 400 Å C4 column provides improved recoveries for holotransferrin, apomyoglobin, catalase, and enolase.

TEST CONDITIONS:

Columns:
1) HALO 400 Å C4, 3.4 µm, 2.1 x 100 mm
Part Number: 93412-614
2) Totally Porous C4, 5 µm, 2.1 x 100 mm
Mobile Phase:
A: Water/0.1% TFA
B: Acetonitrile/0.1% TFA
Gradient: 25% B to 52% B in 10 min
Flow Rate: 0.5 mL/min
Initial Pressure: See chart
Temperature: 60 °C
Detection: UV 215 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: Mobile phase A
Response Time: 1.0 sec
Data Rate: 5 Hz
Flow Cell: 2.0 µL micro cell
LC System: Agilent 1200 SL

Enhanced Selectivity for the Separation of Peptides Comparing HALO 160 Å with Three Different Bonded Phases

Application Note 159-PE



PEAK IDENTITIES:

1. Tyr-Tyr-Tyr
2. Angiotensin II
3. Angiotensin 1-12
4. Melittin
5. Sauvagine
6. β-Endorphin

The initial separation using a HALO 160 Å ES-C18 column showed inadequate resolution of peaks 5 and 6. The same separation was attempted on a 160 Å ES-CN column which provided improved resolution of peaks 5 and 6, but resulted in coelution of peaks 3 and 4. The HALO 160 Å Phenyl-Hexyl column delivered excellent resolution between both peak pairs.

TEST CONDITIONS:

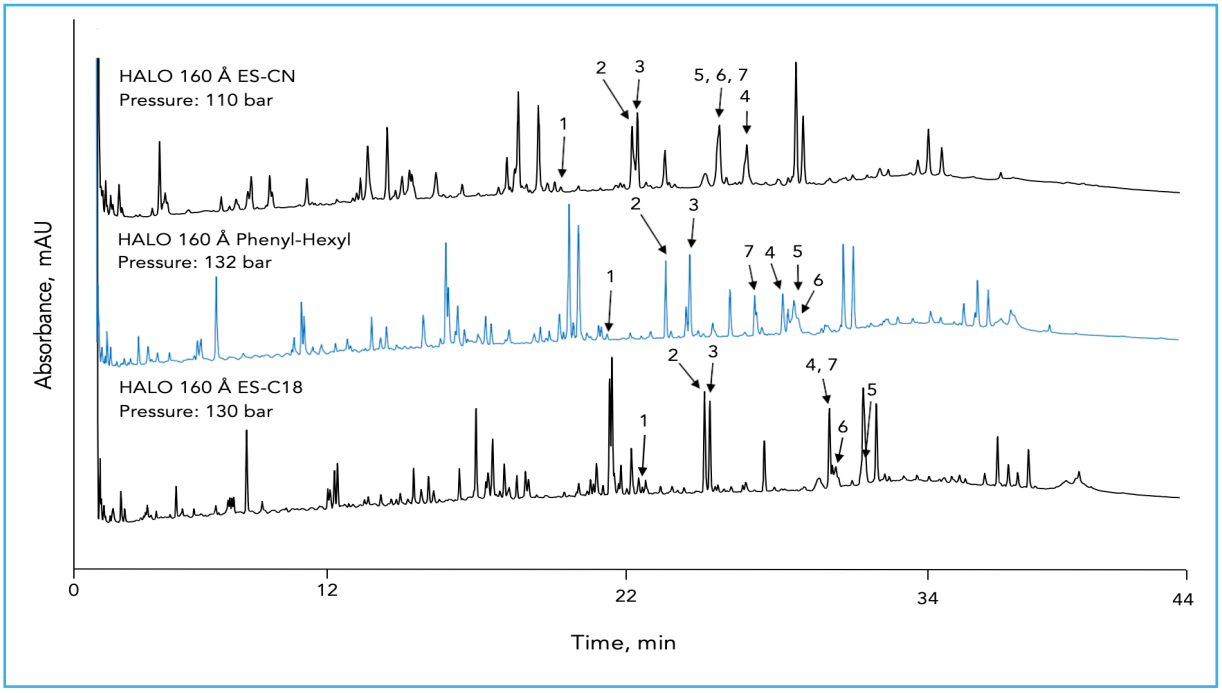
Columns:
1) HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92122-702
2) HALO 160 Å ES-CN, 2.7 µm, 2.1 x 150 mm
Part Number: 92122-704
3) HALO 160 Å Phenyl-Hexyl, 2.7 µm, 2.1 x 150 mm
Part Number: 92112-706

Mobile Phase:
A: 0.1% formic acid in water + 10mM ammonium formate
B: 50/50 n-propanol/water + 0.1% formic acid + 10mM ammonium formate, pH 3.45

Gradient: 10-60% B in 15 min
Flow Rate: 0.4 mL/min
Temperature: 60 °C
Detection: UV 220 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: Water, 0.1% TFA
Response Time: 0.24 sec
Data Rate: 12.5 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera

Enhanced Selectivity with HALO 160 Å Phenyl-Hexyl for a Tryptic Digest using LC-MS

Application Note 166-PE



TEST CONDITIONS:

Column:
1) HALO 160 Å ES-CN, 2.7 µm, 2.1 x 100 mm
Part Number: 92122-604
2) HALO 160 Å Phenyl-Hexyl, 2.7 µm, 2.1 x 100 mm
Part Number: 92112-606
3) HALO 160 Å ES-C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92122-602

Mobile Phase:
A: Water + 10 mM difluoroacetic acid (DFA)
B: ACN + 10 mM difluoroacetic acid

Gradient: 2 to 50% B in 60 min
Flow Rate: 0.3 mL/min
Temperature: 60 °C
Detection: UV 220 nm, VWD
Injection Volume: 5.0 µL of 0.2 mg/mL digest
Sample Solvent: 50 mM Tris-HCl/1.5 M Guanidine-HCl with 0.25% formic acid
Response Time: 0.15 sec
Data Rate: 10 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Nexera

PEAK IDENTITIES: (using one-letter amino acid abbreviations):

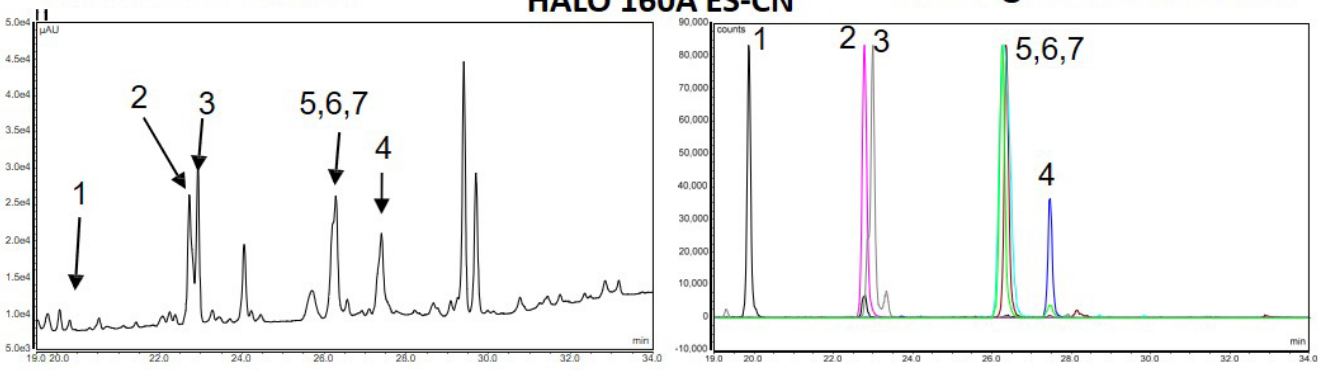
1. FTISADTSKNTAYLQMNSLR (754 m/z)
2. LScAASGFNIKDTYIHWVR (747 m/z)
3. GFYPSDIAVEWESNGQPENNYK (849 m/z)
4. LLIYSASFLYSGVPSR (592 m/z)
5. SGTASVVcLLNNFYPR (899 m/z)
6. ScDKTHTcPPcPAPELLGGPSVFLFPPKPK (834 m/z)
7. VVSVLTVLHQDWLNGKEYK (1115 m/z)

The HALO 160 Å Phenyl-Hexyl column provided improved resolution between tryptic digest fragments 2 and 3 compared to the 160 Å ES-CN column and the 160 Å ES-C18 column. Peptide identification was accomplished by using MS-MS fragmentation spectra.

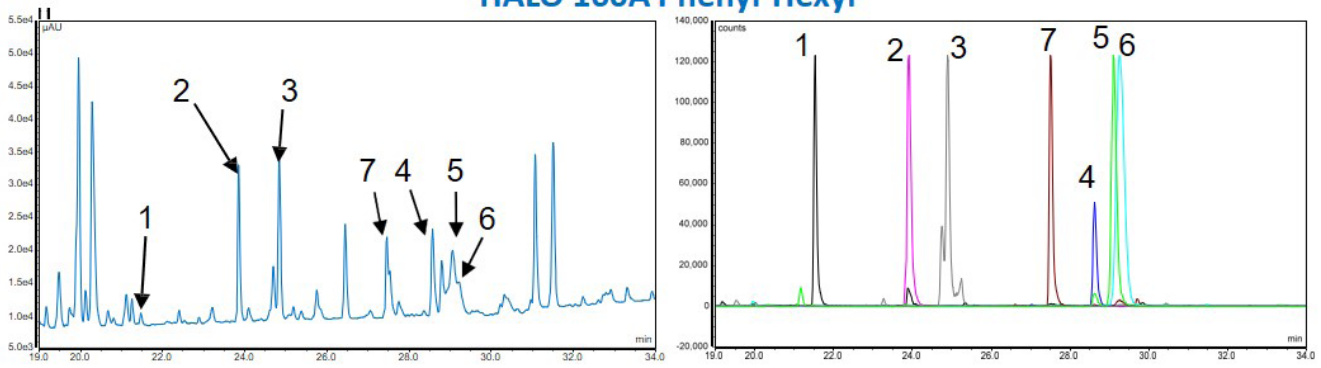
UV 220 nm traces

HALO 160Å ES-CN

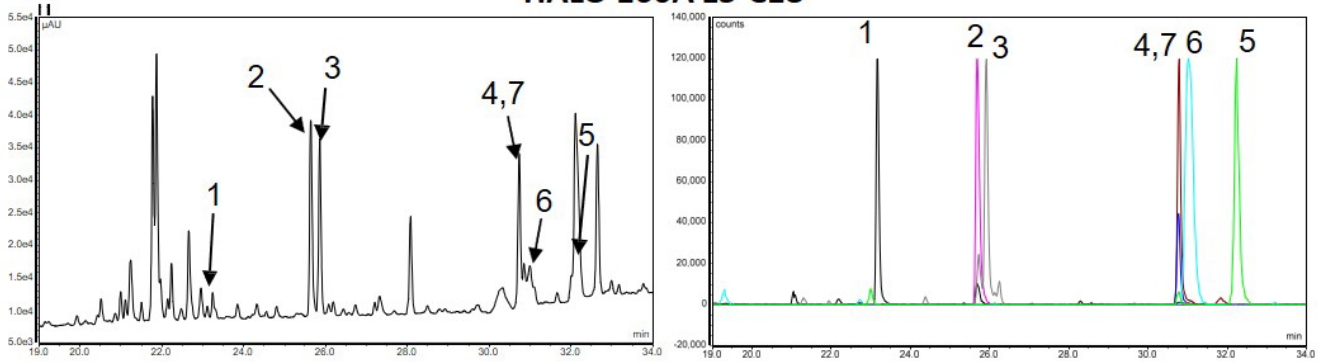
EIC signals normalized



HALO 160Å Phenyl-Hexyl



HALO 160Å ES-C18

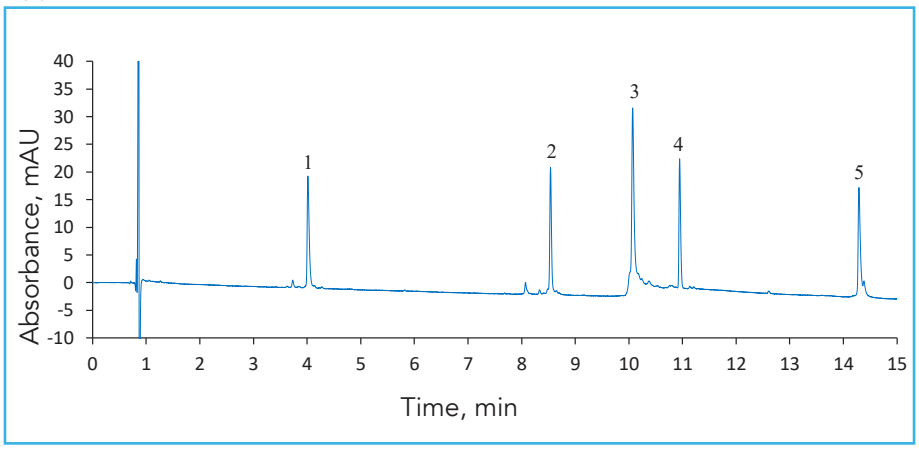


The HALO 160 Å Phenyl-Hexyl column also provided improved resolution between tryptic digest fragments 4 and 7 compared to the 160 Å ES-C18 column. The extracted ion current chromatogram (EIC) and the mass spectrum,corresponding to each peptide fragment, are shown. The use of difluoroacetic acid (DFA) in the mobile phase facilitates symmetrical peak shape and good retention, while enabling good ionization efficiency and sensitivity.

MS System: Thermo Fisher Orbitrap VelosPro ETD
ESI: +3.5 kV
Scan Range: 50-2000 m/z
Scan Rate: 2 pps
Capillary: 225 °C
Sheath Gas: 35
Auxiliary Gas: 10
Scan Time: 2 µscans/200 ms max inject time

Protein Separation on HALO 1000 Å ES-C18, 2.7 µm

Application Note 167-PR



PEAK IDENTITIES:

1. Ribonuclease A	13.7 kDa
2. Lysozyme	14.3 kDa
3. SigmaMAb	~150 kDa
4. α-Lactalbumin	14.2 kDa
5. Enolase	46.0 kDa monomer

This mix of proteins with a wide range of molecular weights is separated with high efficiency on a HALO 1000 Å ES-C18 column. With improved access to the particle surface, the 1000 Å pore size enables large biomolecule analysis with excellent peak shape and high resolution.

TEST CONDITIONS:

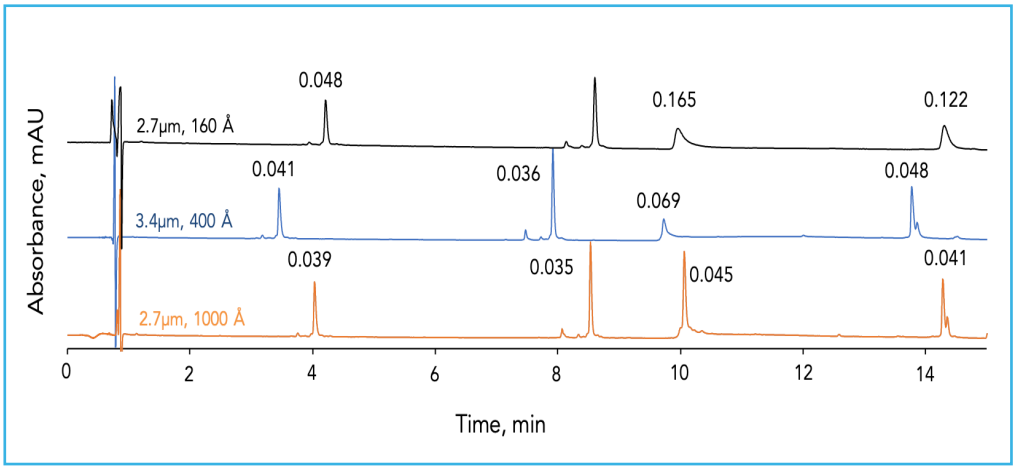
Column: HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-702
Mobile Phase:
A: Water, 0.1% TFA
B: 80/20 ACN/water, 0.085% TFA
Gradient:

Time (min)	% B
0.0	27
15.0	60

Flow Rate: 0.4 mL/min
Pressure: 268 bar
Temperature: 60 °C
Detection: UV 280 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: Water/0.1% TFA
Response Time: 0.05 sec
Data Rate: 12.5 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

Effect of HALO® ES-C18 Pore Size on Protein Peak Shape and Width

Application Note 170-PR



PEAK IDENTITIES:

1. Ribonuclease A (13.8 kDa)
2. Lysozyme (14.4 kDa)
3. SILu™ Lite SigmaMAb Antibody (~150 kDa)
4. Enolase (46.7 kDa)

Pore size can play an important part in HPLC separations. A range of proteins and a monoclonal antibody are separated on HALO® ES-C18 160 Å, 400 Å, and 1000 Å columns. Peak widths decrease as the column's pore size becomes larger, especially for the monoclonal antibody. The 160 Å pore size is recommended for molecules in the range of 100 Da to 15kDa. The 400 Å pore size is recommended for molecules between 2kDa to 500 kDa. The 1000 Å pore size is used for molecules over 50 kDa.

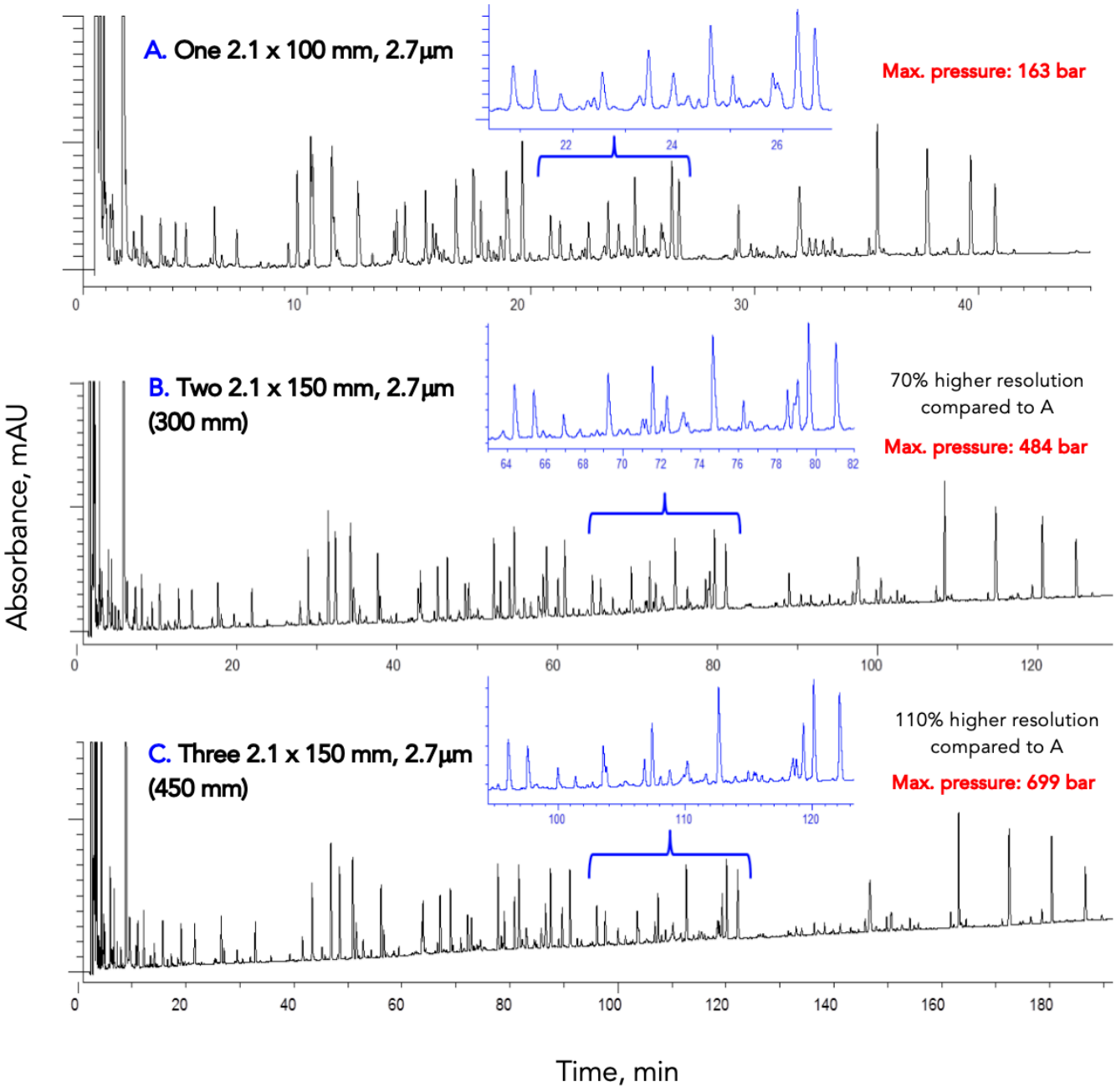
TEST CONDITIONS:

Columns:
1) HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92122-702
2) HALO 400 Å ES-C18, 3.4 µm, 2.1 x 150 mm
Part Number: 93412-702
3) HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-702
Mobile Phase:
A: Water (0.1% TFA)
B: 80/20 acetonitrile/water (0.085% TFA)
Gradient: 27–60% B in 15 min
Flow Rate: 0.4 mL/min
Temperature: 60 °C
Detection: UV 280 nm, PDA
Injection Volume: 4.0 µL
Sample Solvent: Water (0.1% TFA)
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

STRUCTURES:

Analysis of Apotransferrin Tryptic Digest on HALO® 160 Å Columns

Application Note 179-PE



TEST CONDITIONS:

Columns:
1) HALO 160 Å ES-C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92122-602
2) HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92122-702
Mobile Phase:
A: Water with 0.1% TFA
B: 80/20 acetonitrile/water with 0.1% TFA
Flow Rate: 0.4 mL/min
Temperature: 60 °C
Detection: UV 215 nm, PDA
Injection Volume: 10 µL
Sample Solvent: Water
Response Time: 0.05 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

Gradient A:	Time (min)	% B
	0.0	5
	60	60

Gradient B:	Time (min)	% B
	0.0	5
	180	60

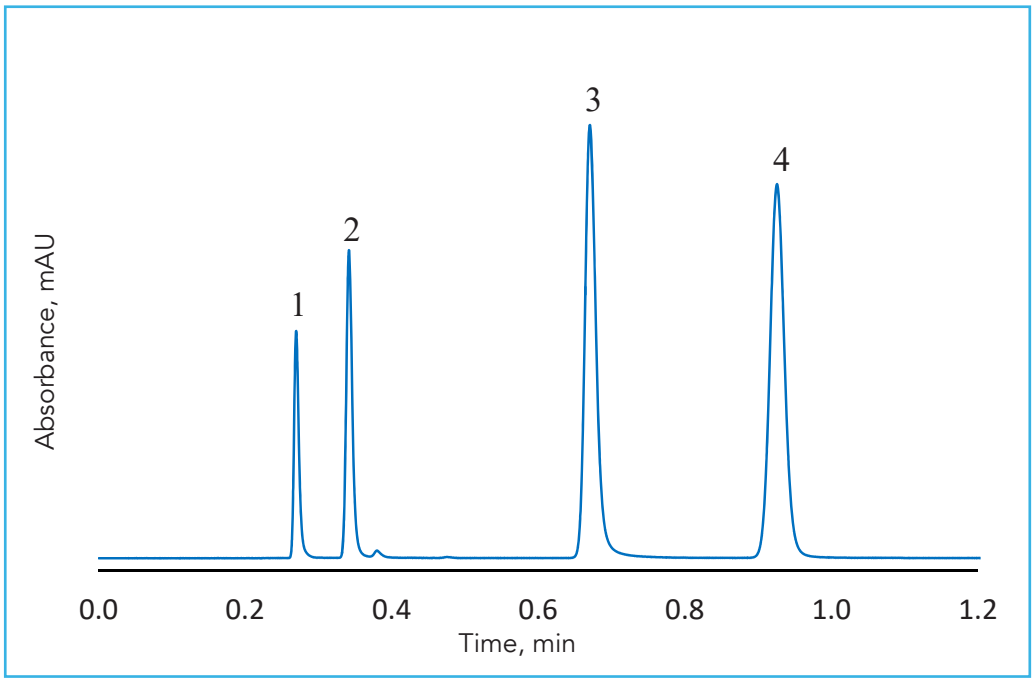
Gradient C:	Time (min)	% B
	0.0	5
	270	60

The chromatograms on the preceding page show a comparison of an apotransferrin tryptic digest sample analyzed on three different lengths of HALO® 160 Å ES-C18 columns: a single 2.1 x 100 mm, two 2.1 x 150 mm columns in series, and three 2.1 x 150 mm columns in series. The insets show examples of the improved performance obtained using longer column lengths along with longer gradient times for demanding samples. Resolution increases of approximately 70% and 110% are achieved by increasing column length by 3-fold and 4.5-fold respectively. Gradient times of 60, 180 and 270 minutes were used for the top, middle and bottom chromatograms, respectively.

Lower pressures afforded by both 2.7 and 5 µm HALO® Peptide particles allow two or more columns to be used in series for additional resolution and peak capacity for challenging peptide mapping analyses. HALO® 160 Å ES-C18 is also available in 2.0 µm particle sizes in 2.1 and 3 mm IDs up to 150 mm length for additional options in run time and peak capacity.

HALO® AQ-C18 Separation of Nucleobases

Application Note 158-NU



PEAK IDENTITIES:

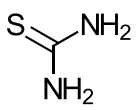
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine

This separation of nucleobases on a HALO® AQ-C18 column shows excellent peak shape and efficiency using 100% aqueous mobile phase conditions.

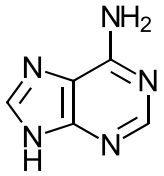
TEST CONDITIONS:

Column: HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-422
Isocratic: Water, 0.1% TFA
Flow Rate: 2.0 mL/min
Pressure: 290 bar
Temperature: 30 °C
Detection: UV 254 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: Water, 0.1% TFA
Response Time: 0.05 sec
Flow Cell: 1.0 µL
Aquisition Rate: 100 Hz
LC System: Shimadzu Nexera X2

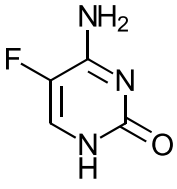
STRUCTURES:



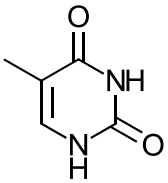
Thiourea



Adenine



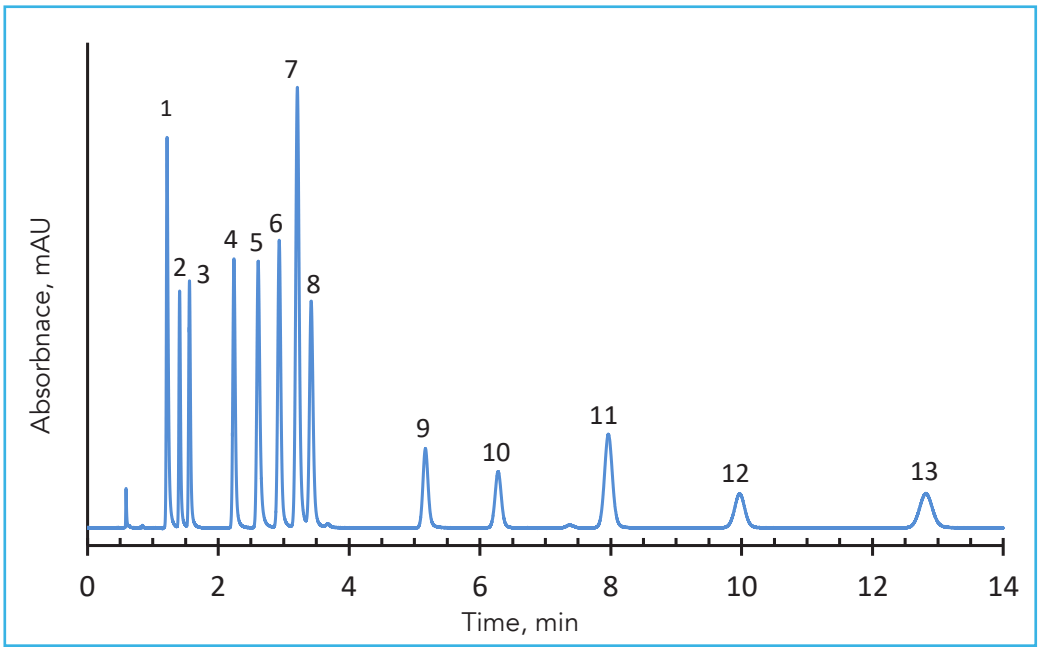
5-Fluorocytosine



Thymine

Separation of Nucleosides and Nucleobases on 2.7 µm HALO® Penta-HILIC

Application Note 76-NU



PEAK IDENTITIES:

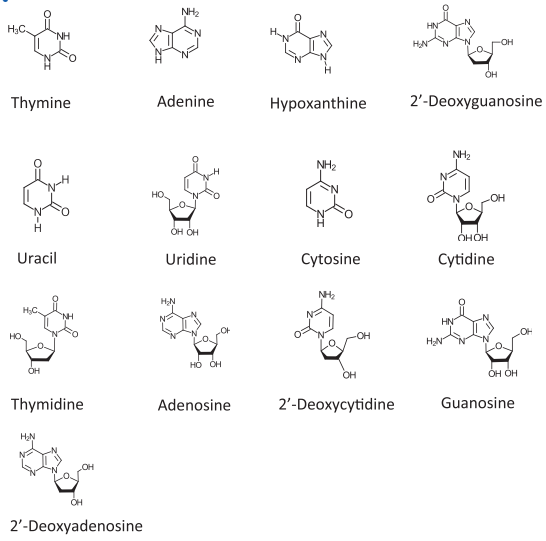
1. Thymine
2. Uracil
3. Thymidine
4. 2-Deoxyadenosine
5. Adenine
6. Uridine
7. Adenosine
8. Hypoxanthine
9. Cytosine
10. 2-Deoxycytidine
11. 2-Deoxyguanosine
12. Cytidine
13. Guanosine

The new HALO® Penta-HILIC stationary phase is an HPLC phase having a hydroxylrich surface for performing separations in the hydrophilic interaction chromatography mode. Here, a mixture of 13 nucleosides and nucleobases are separated isocratically in a short time with excellent resolution. These bonded superficially porous 2.7 µm HALO® particles allow high resolution with modest back pressure.

TEST CONDITIONS:

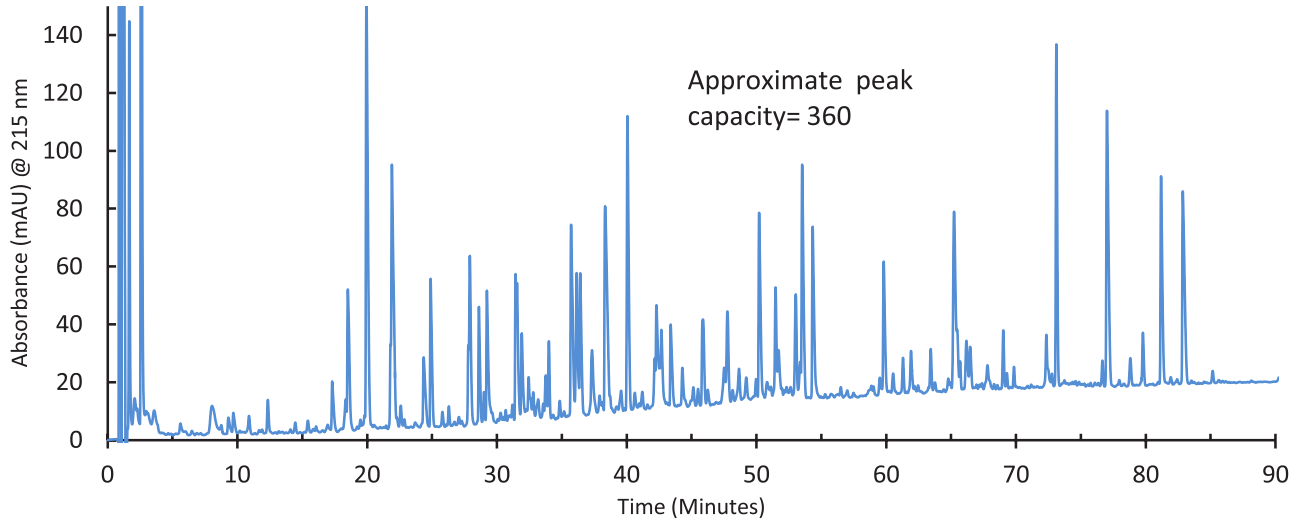
Column: HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-605
Mobile Phase: 8/92 - A/B
A: Water
B: Acetonitrile with 0.01 M ammonium formate, pH 6.0 (adj.)
Flow Rate: 1.5 mL/min
Pressure: 99 bar
Temperature: 35 °C
Detection: UV 260 nm, DAD
Injection Volume: 2.0 µL
Sample Solvent: Mobile phase
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Nexera

STRUCTURES:



Analysis of Apotransferrin Tryptic Digest on HALO 160 Å ES-C18

Application Note 100-PE



This separation shows the separation of the products from a tryptic digest of apotransferrin on coupled 2.7 µm HALO 160 Å ES-C18 columns in less than 90 minutes. Two columns were coupled to increase the peak capacity.

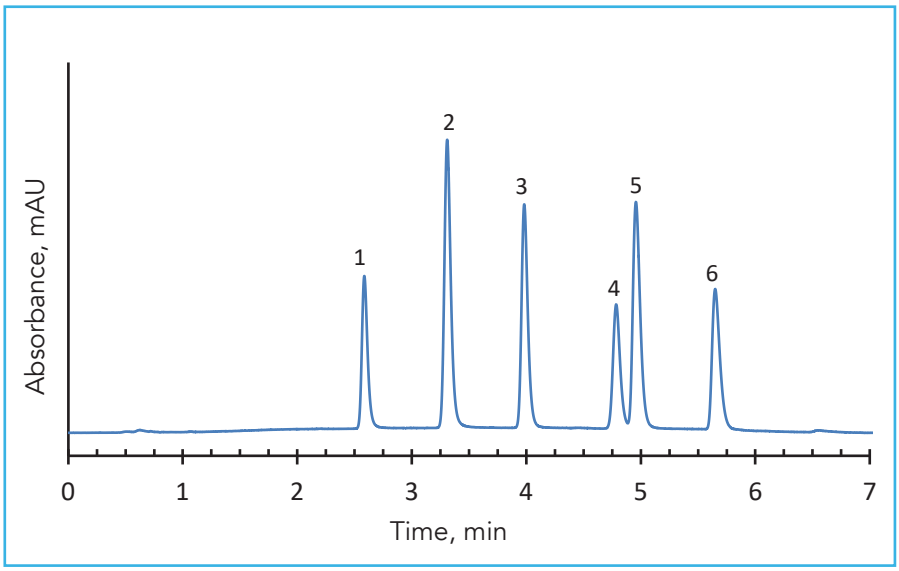
The use of elevated temperature improves the peak sharpness and aids in resolution. The excellent stability of this phase at elevated temperature is a result of the use of a sterically protected silane in the stationary phase synthesis.

TEST CONDITIONS:

Column: 2-Coupled HALO 160 Å ES-C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92122-602
Mobile Phase: 95/5 - A/B (start)
A: Water with 0.1% trifluoroacetic acid (TFA)
B: 80/20 water/acetonitrile with 0.1% TFA
Gradient: 5% B to 60% B in 120 min
Flow Rate: 0.5 mL/min
Max. Pressure: 380 bar
Temperature: 60 °C
Detection: UV 215 nm, PDA
Injection Volume: 35 µL
Sample Solvent: Mobile phase A
Response Time: 0.1 sec
Data Rate: 40 Hz
Flow Cell: 2.0 µL micro cell
LC System: Agilent 1200 SL

Separation of Nucleotides on HALO® Penta-HILIC, 2.7 µm

Application Note 101-B



PEAK IDENTITIES:

1. Adenosine monophosphate (AMP)
2. Guanosine monophosphate (GMP)
3. Adenosine diphosphate (ADP)
4. Guanosine diphosphate (GDP)
5. Adenosine triphosphate (ATP)
6. Guanosine triphosphate (GTP)

This separation demonstrates the utility of the HALO® Penta-HILIC phase for analysis of nucleotides. Fused-Core® technology gives high resolution separations at moderate pressures without the difficulties of using sub two-micron-particle columns.

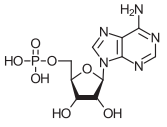
TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-605
Mobile Phase:
A: 50/50 acetonitrile/0.025 M ammonium phosphate, pH 6.0
B: 75/25 acetonitrile/0.025 M ammonium phosphate, pH 6.0
Gradient:

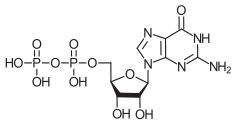
Time (min)	% B
0.0	90
8.0	40

Flow Rate: 0.3 mL/min
Pressure: 76 bar
Temperature: 50 °C
Detection: UV 260 nm, DAD
Injection Volume: 1.0 µL
Sample Solvent: Mobile phase B
Response Time: 0.02 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL micro cell
LC System: Shimadzu Nexera

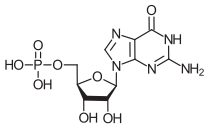
STRUCTURES:



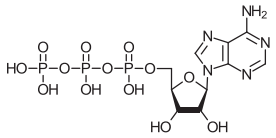
Adenosine Monophosphate



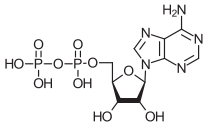
Guanosine Diphosphate



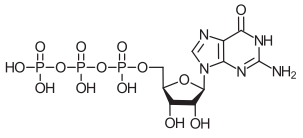
Guanosine Monophosphate



Adenosine Triphosphate



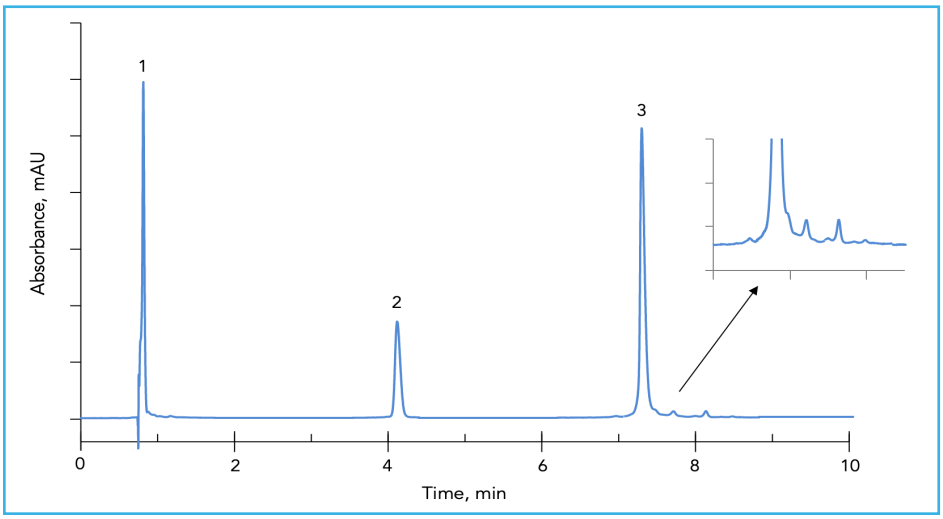
Adenosine Diphosphate



Guanosine Triphosphate

HPLC Separation of IgG2-B Monoclonal Antibody on HALO 400 Å C4, 3.4 µm

Application Note 105-PR



PEAK IDENTITIES:

1. t_0
2. Light chains, (~25 kDa)
3. Heavy chains (~50 kDa)

The HALO® Fused-Core® 400 Å C4, 3.4 µm stationary phase is useful for the separation of proteins up to 500 kDa in size. Shown here is the separation of light and heavy chains from a reduced IgG2-B antibody. Note the resolution of small peaks at the end of the chromatogram.

Special endcapping procedures ensure that the columns will be stable at elevated temperatures, even with aggressive mobile phases.

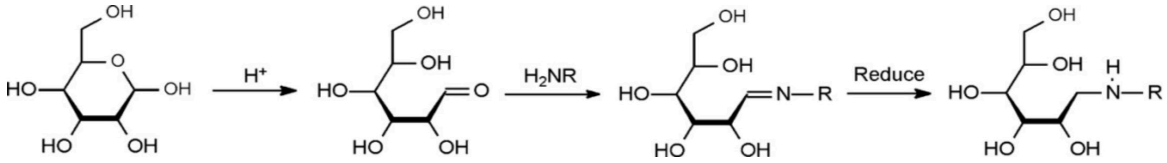
TEST CONDITIONS:

Column: HALO 400 Å C4, 3.4 µm, 2.1 x 100 mm
Part Number: 93412-614
Mobile Phase: 67/33 - A/B (start)
A: Water with 0.1% trifluoroacetic acid (TFA)
B: 80/20 (acetonitrile/water)/0.1% TFA
Gradient: 33% B to 40% B in 10 min
Flow Rate: 0.25 mL/min
Initial Pressure: 42 bar
Temperature: 80 °C
Detection: UV 280 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: 0.5 mg/mL IgG2-B treated with 100 mM DTT in 8 M guanidine-HCl @ 50 °C for 35 min
Response Time: 0.08 sec
Flow Cell: 1.0 µL micro cell
LC System: Shimadzu Nexera
Gradient Delay Volume: ~115 µL

Separation of PNGase-Released and Labeled N-Glycans by HILIC Using HALO® Glycan Column

Application Note 121-GL

Digestion of N-linked proteoglycans using PNGase F releases oligosaccharides, which can be reacted with an amine via Schiff base formation. The Schiff's base derivatives (imines) can be easily reduced to form stable amine derivatives for analysis.



Many amines have been applied for labeling glycans (Harvey, 2011, J. Chromatogr. B, 879, 1196-1225). In this application brief, procainamide was chosen because of reported improvements in ESI-MS detection (Klapoetke, et. al., 2010, J. Pharm. Biomed. Anal., 53, 315-324).

Typical Labeling Conditions:

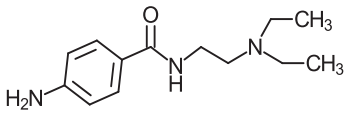
1) Glycan in water (up to 10% volume)
2) 90+% volume of:
   - 0.4 M procainamide
   - 1M sodium cyanoborohydride in 30% glacial acetic acid/70% DMSO

12-16 hr reaction at 37°C
SEC cleanup on Sephadex G-10 minicolumn
Absorbance Detection @300 nm or Fluorescence with Ex 330/Em 380 nm

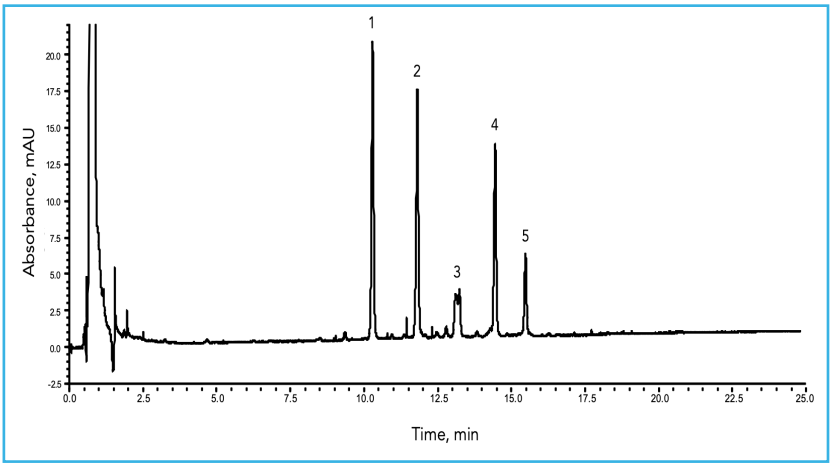
TEST CONDITIONS:

Column: HALO 90 Å Glycan, 2.7 µm, 2.1 x 150 mm
Part Number: 92922-705
Mobile Phase:
A: 50 mM Ammonium formate, pH 4.45
B: Acetonitrile
Gradient: 80% B to 55% B in 25 min
Flow Rate: 0.6 mL/min
Pressure: 190 bar
Temperature: 60 °C
Detection: UV 300 nm
Injection Volume: 3.0 µL
Sample Solvent: 70/30 ACN/water
Response Time: 0.5 sec
Data Rate: 3.3 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Nexera

STRUCTURE:



Procainamide (PAm)

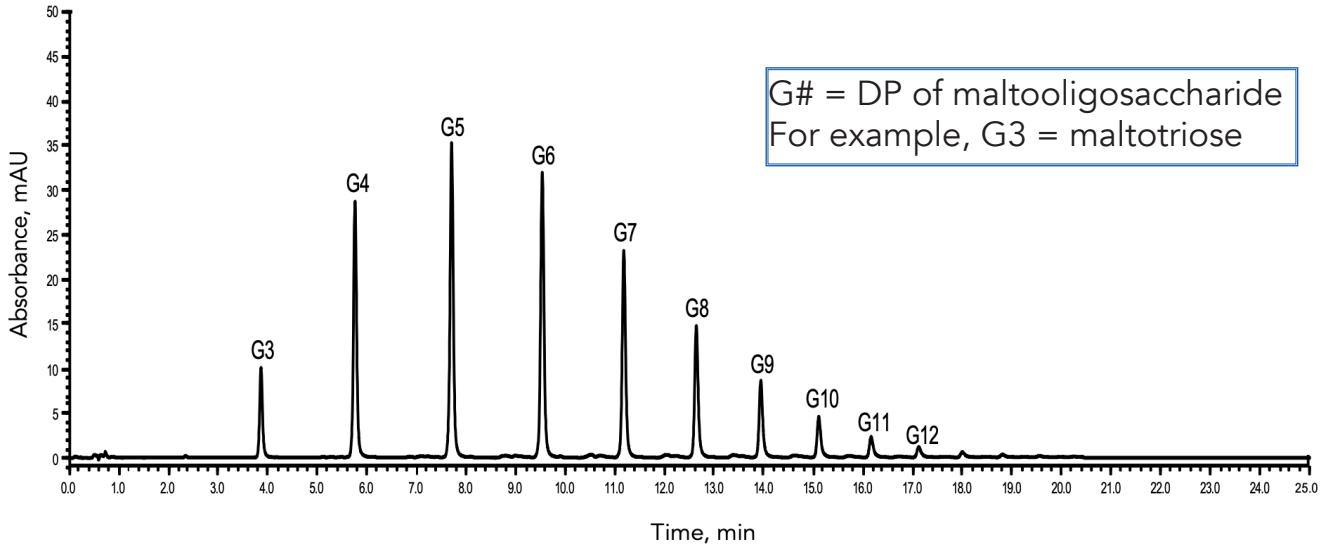


PEAK IDENTITIES:
1. PAm-GlcNAc$_2$Man$_5$
2. PAm-GlcNAc$_2$Man$_6$
3. PAm-GlcNAc$_2$Man$_7$
4. PAm-GlcNAc$_2$Man$_8$
5. PAm-GlcNAc$_2$Man$_9$

A fast separation of PNGase-released and procainamide-labeled N-Glycans from Ribonuclease B is accomplished with a HALO 90 Å Glycan column.

Separation of Procainamide-Labeled Dextran Standards on HALO® Glycan

Application Note 122-GL



A HALO® Glycan column shows an efficient separation of procainamide-labeled dextran standards (Sigma-Aldrich 1:1 (w/w) of part numbers 00268 and 00269) at 0.5 µg/µL in 70% ACN/30% water. Each lot of HALO® Glycan packing is tested using this sample to assure lot-to-lot reproducibility and performance.

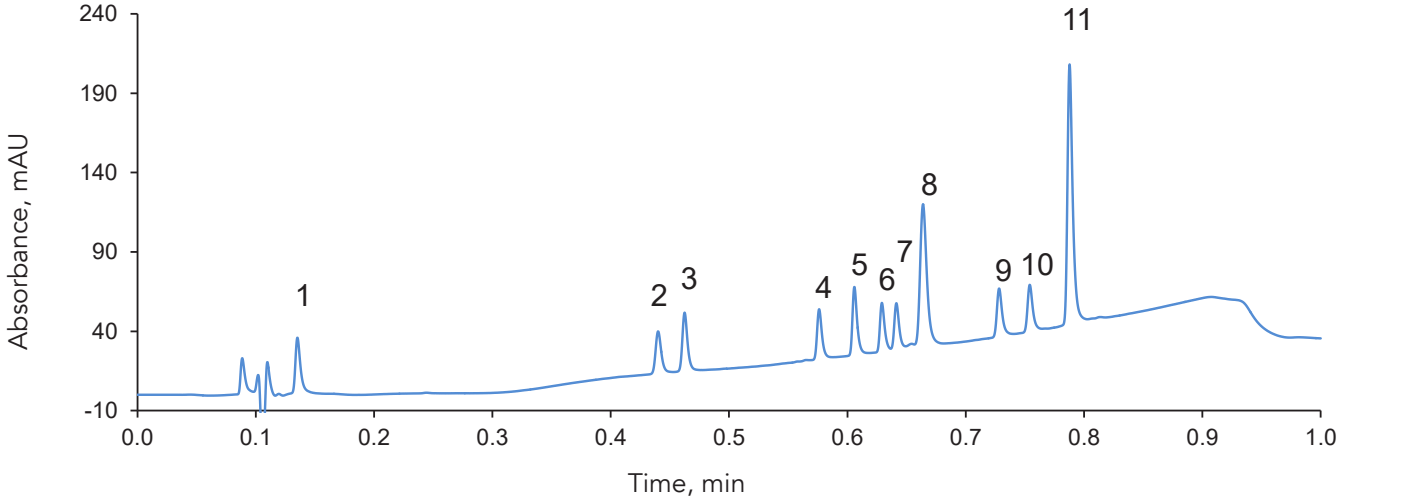
TEST CONDITIONS:

Column: HALO 90 Å Glycan, 2.7 µm, 2.1 x 150 mm
Part Number: 92922-705
Mobile Phase:
A: 50 mM ammonium formate, pH 4.45
B: Acetonitrile
Gradient: 80-55% B in 25 min
Flow Rate: 0.6 mL/min
Pressure: 190 bar
Temperature: 60 °C

Detection: UV 300 nm
Injection Volume: 3.0 µL
Sample Solvent: 70/30 ACN/water
Response Time: 0.5 sec
Data Rate: 3.3 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Nexera

Fast Peptide Separation with HALO 160 Å ES-C18, 2.0 µm

Application Note 135-PE



A one-minute separation of a mixture of peptides and small proteins is demonstrated on a HALO 160 Å ES-C18, 2.0 µm column. Separations can be run at high flow rate in order to maximize sample throughout.

TEST CONDITIONS:

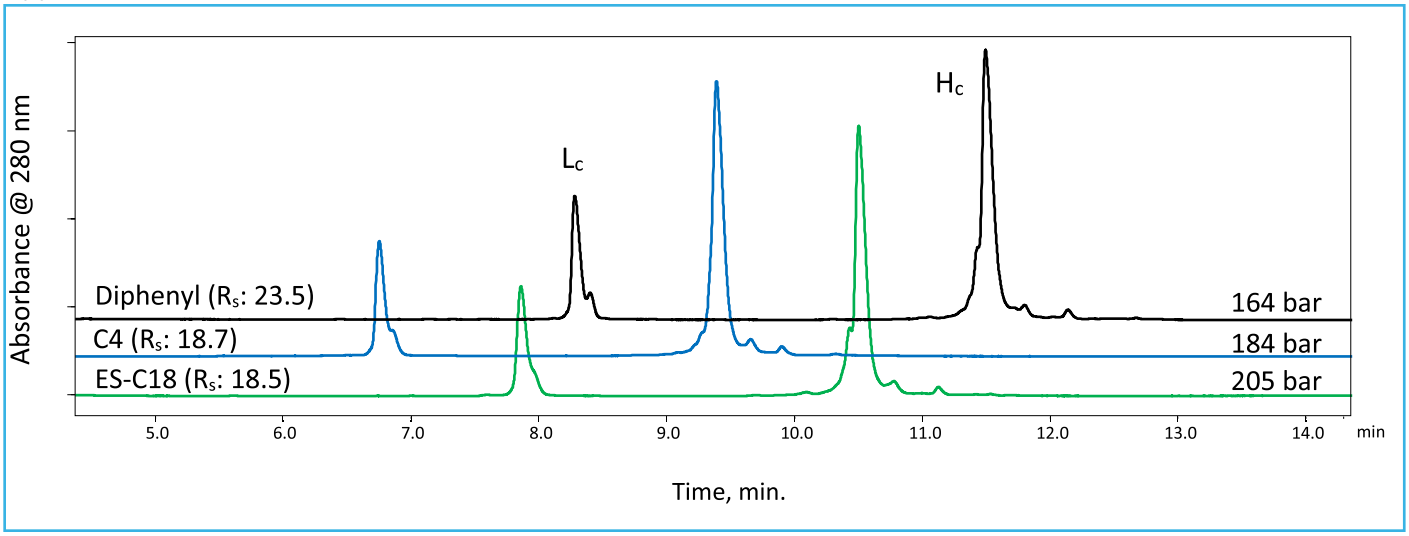
Column: HALO 160 Å ES-C18, 2.0 µm, 3.0 x 50 mm
Part Number: 91123-402
Mobile Phase:
A: 0.1% Trifluoroacetic acid in water
B: 0.1% Trifluoroacetic acid in 80/20 acetonitrile/water
Gradient: Hold at 12.5% B for 0.1 min; 12.5% B to 63% B from 0.1-1.0 min
Flow Rate: 2.2 mL/min
Initial Pressure: 556 bar
Temperature: 60 °C
Detection: UV 215 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: Mobile phase A
Response Time: 0.025 sec
Data Rate: 200 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

PEAK IDENTITIES:

	MW (g/mol):
1. Gly-Tyr	238
2. Val-Tyr-Val	380
3. Angiotensin 1/2 (1-7) amide	898
4. Met-enkephalin	574
5. Angiotensin 1/2 (1-8) amide	1045
6. Angiotensin II	1046
7. Leu-enkephalin	556
8. Ribonuclease A	13,700
9. Angiotensin (1-12) (mouse)	1573
10. Bovine insulin	5733
11. Angiotensin (1-12) (human)	1509

Reduced IgG1 (Trastuzumab) Retention Comparison on Three HALO® 1000 Å Phases

Application Note 199-PR



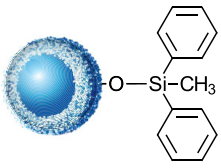
Trastuzumab is a monoclonal antibody used to treat breast cancer. Enhanced resolution of trastuzumab's heavy and light chains is demonstrated in the chromatograms above using three different HALO® bonded phases. The 1000 Å pores of the HALO® Protein columns readily accommodate large biomolecules, and allow unrestricted pore assess, narrower peaks and superior separations at high temperatures.

TEST CONDITIONS:

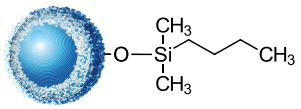
Columns:
HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-726
HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-714
HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-702

Mobile Phase A: Water/ 0.1% TFA
Mobile Phase B: Acetonitrile/ 0.1% TFA
Gradient: Time (min.) %B
0.0 30
14.0 40
Flow Rate: 0.4 mL/min
Temperature: 80 °C
Detection: 280 nm, PDA
Injection Volume: 2 µL
Sample Solvent: Water
Data Rate: 12.5 Hz
Response Time: 0.25 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

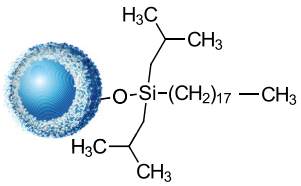
STRUCTURES:



HALO 1000 Å Diphenyl



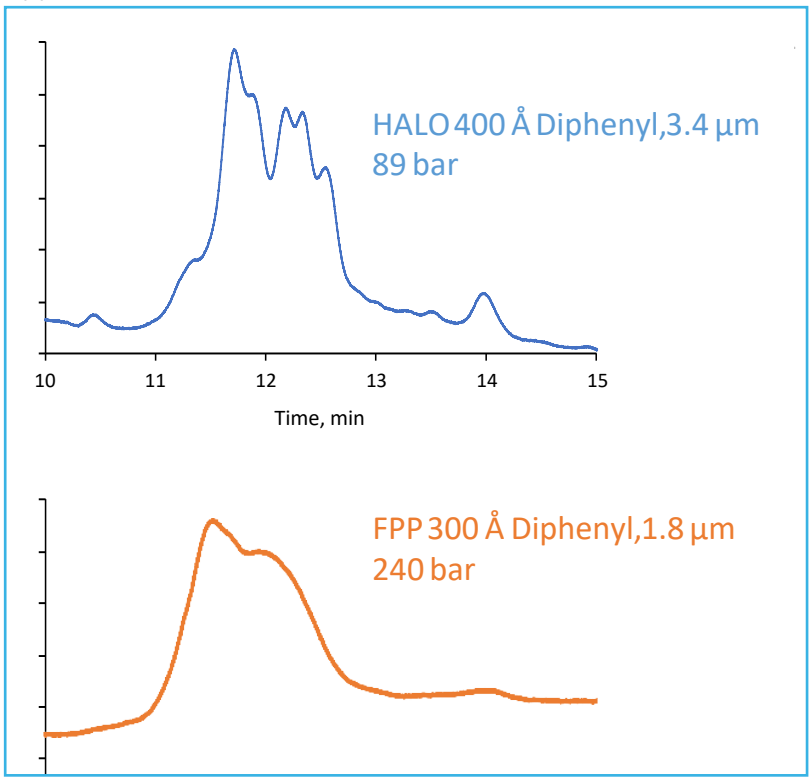
HALO 1000 Å C4

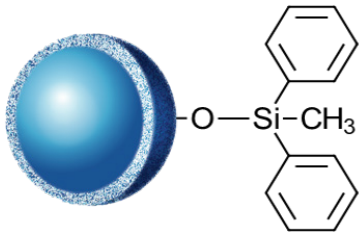


HALO 1000 Å ES-C18

Increased Resolution with HALO 400 Å Diphenyl Compared to FPP 300 Å Diphenyl

Application Note: 207-PR





HALO 400 Å Diphenyl, 3.4 µm Particle Shell with 400 Å pores

Denosumab, a human IgG2 monoclonal antibody that is used to treat cancer in the bones was analyzed on two different types of HPLC columns. The HALO 400 Å column outperformed the 300 Å fully porous diphenyl column by providing much better resolution at 2.5-fold lower back pressure along with a quicker run time.

TEST CONDITIONS:

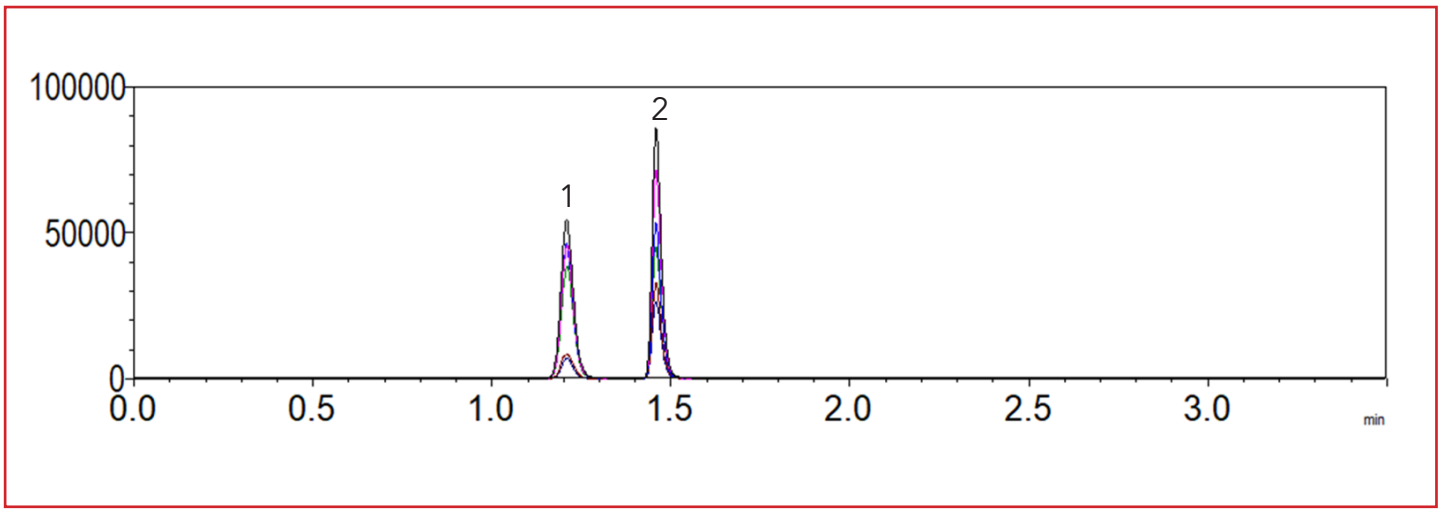
Columns: HALO 400 Å Diphenyl, 3.4 µm, 2.1x150 mm
Part Number: 93412-726
FPP 300 Å Diphenyl, 1.8 µm, 2.1x150 mm
Mobile Phase A: 88/10/2: Water/Acetonitrile/**n-Prop/ 0.1% *DFA
Mobile Phase B: 70/20/10: **nProp/Acetonitrile/Water/ 0.1% *DFA

Gradient:	**Time (min.)**	**%B**
	0.0	18
	20.0	28

Flow Rate: 0.2 mL/min.
HALO® SPP Initial Back Pressure: 89 bar
FPP Initial Back Pressure: 240 bar
Temperature: 60 °C
Detection: 220 nm, PDA
Injection Volume: 2 µL
Sample Solvent: Water/ 0.1% DFA
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2
*DFA = difluoroacetic acid
**nProp = n- propanol

LCMS Separation of T3/rT3

224-HM



PEAK IDENTITIES

1. T3
2. rT3

Species	Precursor	Product	Collision Energy
T3	652.07	606.1	35 CE
	652.07	508.1	36 CE
rT3	652.07	606.1	35 CE
	652.07	508.1	36 CE

TEST CONDITIONS:

Column: 90 Å C18, 2.7µm 3.0 x 30 mm
Mobile Phase A: Water/ 0.1% Formic Acid
B: Methanol/ 0.1% Formic Acid
Gradient:

Time	%B
0.0	55
0.45	55
1.50	100
2.50	100
2.51	55
3.5	55

Flow Rate: 0.4 ml/min
Injection: 1.0 µl (20µg/mL, in SigMatrix Serum Diluent, w/ 0.1% Formic acid)
Temperature: 40 °C
Instrument: Shimadzu 8040 LCMS

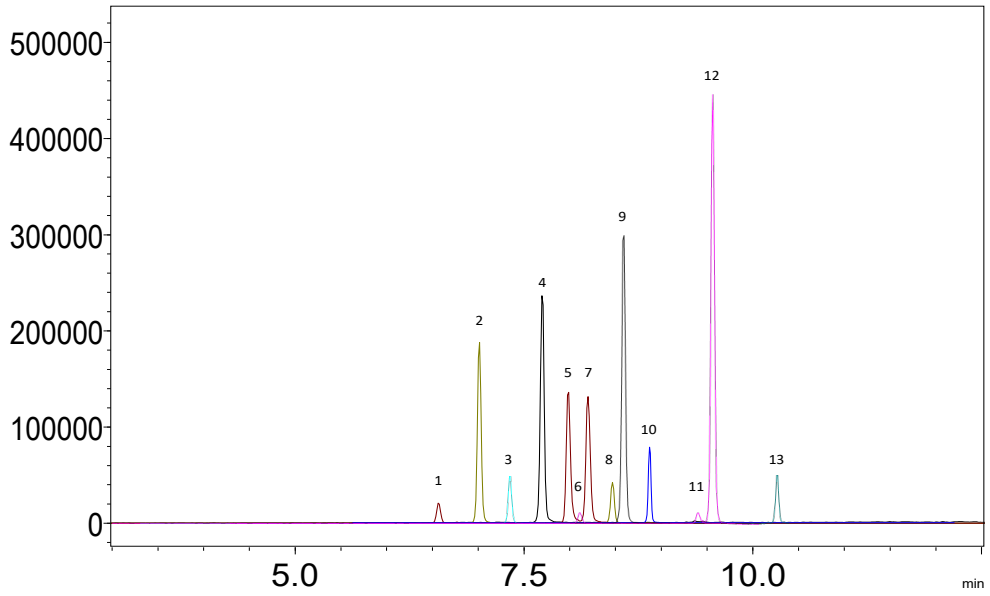
Triiodothyronine (T3), produced from thyroxine (T4), is thyroid hormone that affects many physiological processes in the body, including growth, metabolism, body temperature, and heart rate.

Reverse triiodothyronine (rT3), an isomer of T3 and also produced from T4, if found in high levels in the thyroid, can be indicative of hypothyroidism. The high rT3 level generally means that most of the T4 is being converted to rT3 and a deleterious effect of rT3 is that it will bind to T3 receptors, but it has no activity. Increased rT3 levels have been attributed to illness, starvation and excessive cortisol (stress).

The separation of T3 and rT3 is challenging due to the isomeric nature of the compounds, and therefore good chromatography is imperative not only for separation, but also for identification.

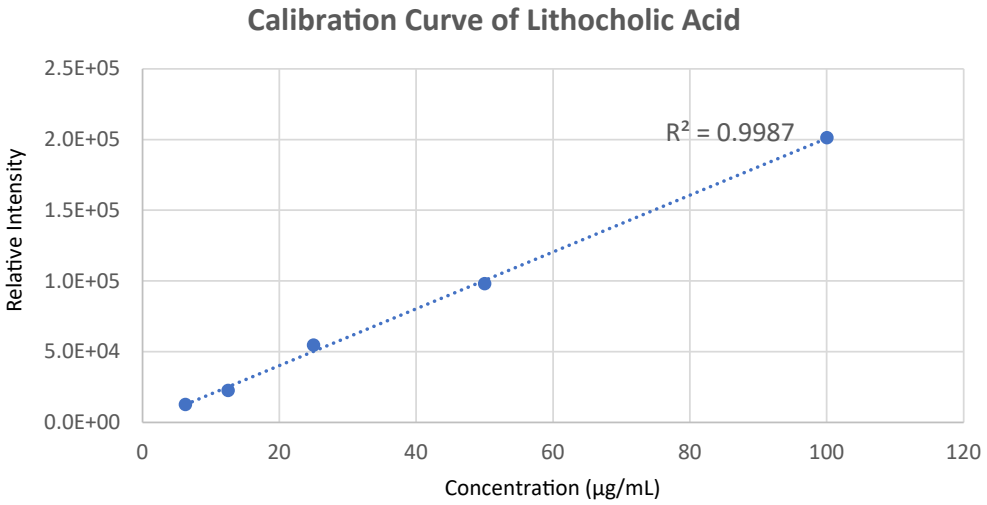
LCMS Separation of Bile Acids Using HALO® C18

232-A



PEAK IDENTITIES, MRM TRANSITIONS, COLLISION ENERGIES, AND LINEARITIES

Peak Number	Analyte	MH^-	Transition	CE	R^2
1	Sodium-tauroursodeoxycholate (TUDC)	498.7	498.7>124.1	51	0.9998
2	Glycoursodeoxycholic acid (GDC)	448.2	448.2>74.1	34	0.9988
3	Taurocholic acid sodium salt hydrate (TCA)	514.3	514.3>80.0	35	0.9986
4	Glycocholic acid hydrate (GCA)	464.2	464.3>402.3	34	0.9978
5	Sodium taurochenodeoxycholate (TCDC)	498.7	498.7>124.1	52	0.9982
6	Ursodeoxycholic acid (UDC)	391.5	391.5>391.5	8	0.9993
7	Sodium-taurodeoxycholate hydrate (TDC)	498.2	498.7>124.1	52	0.9971
8	Sodium glycochenodeoxycholate (GCDC)	448.2	448.2>74.1	30	0.9981
9	Cholic acid (CA)	407.5	407.5>407.5	8	0.9957
10	Sodium-taurolithocholate (TLC)	482.2	482.2>124.1	50	0.9973
11	Chenodeoxycholic acid (CDC)	391.5	391.5>391.5	8	0.9955
12	Deoxycholic acid (DC)	391.5	391.5>391.5	8	0.9986
13	Lithocholic acid (LC)	375.5	375.5>375.5	8	0.9987



TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 2.1 × 150 mm 92812-702
Mobile phase A: 5 mM ammonium formate and 0.012% formic acid in water
Mobile Phase B: 5 mM ammonium formate and 0.012% formic acid in methanol

Gradient:

Time	%B
0.00	30
10.00	95
15.00	95
15.10	30
18.00	30
18.00	End

Flow Rate: 0.4 mL/min
Pressure: 185 bar
Temperature: 40 °C
Injection: 1.0 µL (12.5 µg/mL, in SigMatrix Serum Diluent)
Instrument: Shimadzu Nexera

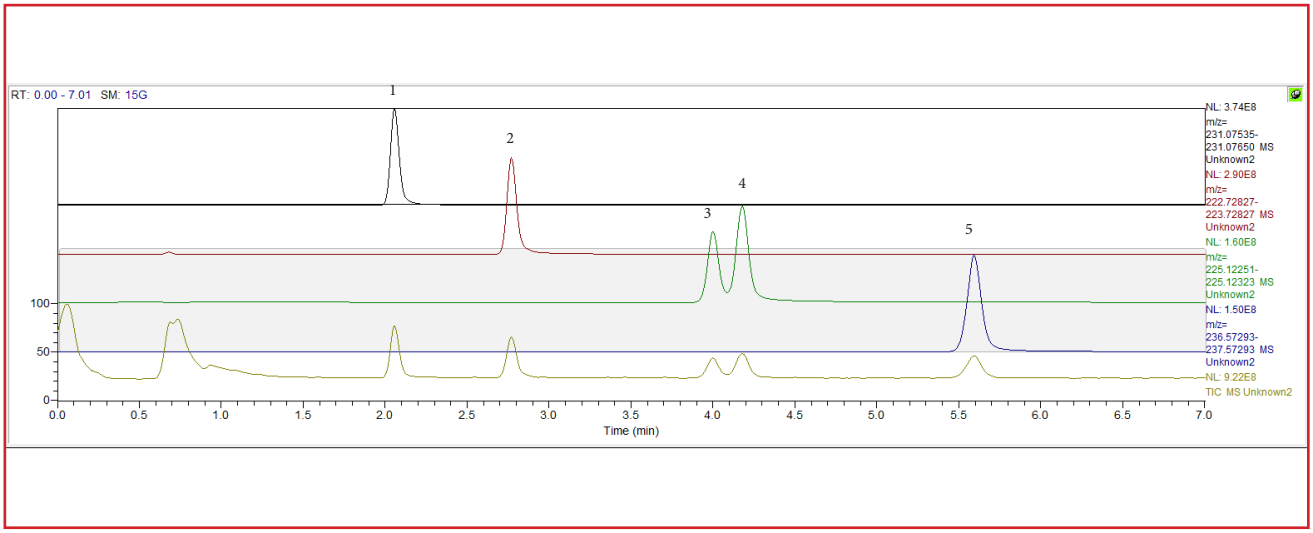
MS TEST CONDITIONS:

Mass Spectrometer: Shimadzu 8040
Ion mode: Negative Electrospray
Heat Block Temperature: 400 °C
Drying line: 300 °C
Nebulizing Gas Flow: 3 L/min
Drying Gas Flow: 18 L/min
Spray Voltage: -4000 V
Q1/Q2 Resolution: High

An LC MS/MS method was developed for the analysis of bile acids on a HALO® C18 column. The column demonstrated excellent performance in the separation of multiple isobaric compounds and rugged reliability with excellent linearity, enabling clinically relevant concentrations to be analyzed. The main limitation with identification by MSMS is associated to indistinguishable transitions, so the chromatographic separation is paramount for identification. The resolution, precision and narrow peak widths provided by the HALO® C18 column allows for these acids to be clearly separated and identified, and the linearity shows that these acids can be detected and quantitated at clinically relevant levels.

LCMS Separation of Barbiturates

241-TOX



PEAK IDENTITIES

	Barbiturate	Precursor Ion (m/z)	Product Ion (m/z)
1	Phenobarbital	231.1	188
2	Butalbital	223	180
3	Pentobarbital	225.1	182
4	Amobarbital	225.1	182
5	Secobarbital	237.1	194.1

Barbiturates are central nervous system depressants. These drugs are commonly prescribed to treat headaches, insomnia, and seizures. An LCMS separation of barbiturates is demonstrated on a HALO® C18 column, resolving all peaks including the isomers. The mix of barbiturates was diluted with a negative urine standard and detected using an LCMS.

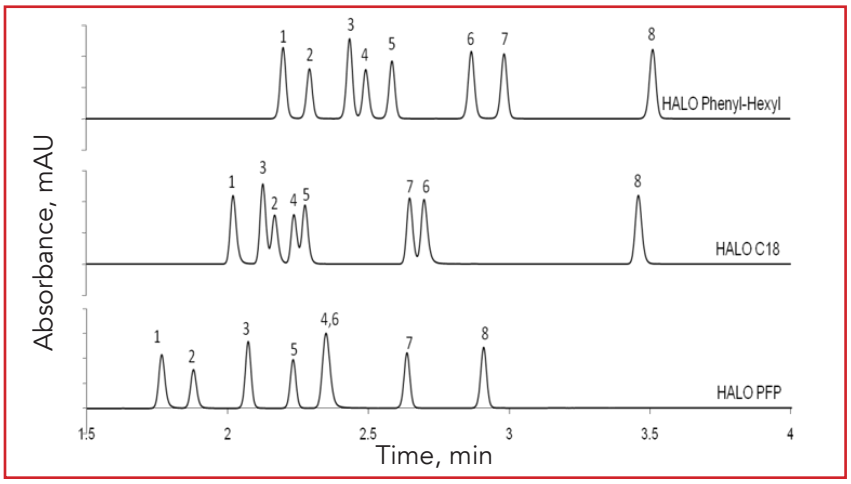
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92812-702
Mobile Phase A: Water/ 0.1% Formic Acid
Mobile Phase B: Acetonitrile
Isocratic: 30 %B
Flow Rate: 0.4 mL/min
Temperature: 30 °C
Detection: -ESI
Injection Volume: 0.5 µL

Sample Solvent: Surine negative urine standard
LC System: Shimadzu Nexera X2
MS System: QExactive HF
ESI voltage: 2.5 kV
Heater Temp: 425 °C
Sheath gas: 50 (arbitrary units)
Aux gas: 13 (arbitrary units)
Tube lens voltage: 50 V

Separation of Benzodiazepines on HALO® Phenyl-Hexyl, C18, and PFP Phases

Application Note 51-BZ



PEAK IDENTITIES:

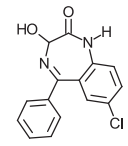
1. Oxazepam
2. Lorazepam
3. Nitrazepam
4. Alprazolam
5. Clonazepam
6. Temazepam
7. Flunitrazepam
8. Diazepam

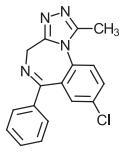
These separations of benzodiazepines on three different HALO® Fused-Core® HPLC stationary phases show the utility of having a variety of phases to optimize selectivity and/or to shorten analysis time.

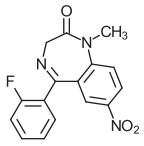
TEST CONDITIONS:

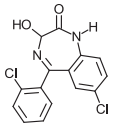
Columns:
1) HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
2) HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
3) HALO 90 Å PFP, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-409
Mobile Phase:
A: 25 mM Ammonium acetate in water, pH 5.8 (not adjusted)
B: Acetonitrile
Gradient: 34-63% B in 3.5 min
Gradient Dwell Volume: 0.88 mL
Flow Rate: 1.5 mL/min
Pressure: 200 bar
Temperature: 35 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Standard diluted with acetonitrile and buffer
Response Time: < 0.12 sec
Flow Cell: 5.0 µL semi-micro
LC System: Agilent 1100

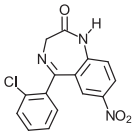
STRUCTURES:

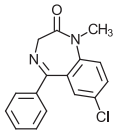

Oxazepam

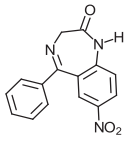

Alprazolam

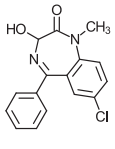

Flunitrazepam


Lorazepam


Clonazepam

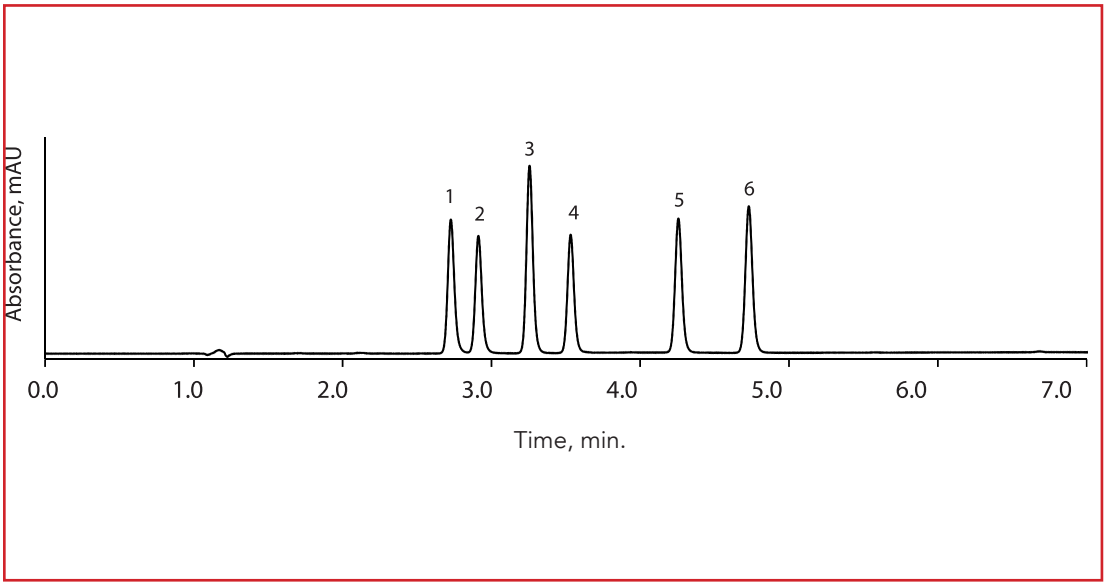

Diazepam


Nitrazepam


Temazepam

Separation of Benzodiazepines on HALO® PFP, 5 µm

Application Note 186-BZ



PEAK IDENTITIES:

1. Oxazepam
2. Lorazepam
3. Nitrazepam
4. Clonazepam
5. Flunitrazepam
6. Diazepam

Benzodiazepines are a class of compounds known to be minor tranquilizers, which are mainly used to treat anxiety, insomnia, and seizures in people, as well as animals. A separation of six benzodiazepines is performed on a HALO® 5.0 µm PFP column.

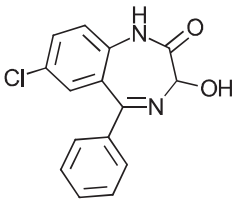
TEST CONDITIONS:

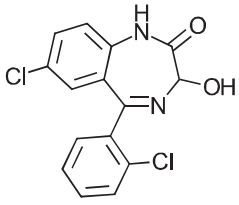
Column: HALO 90 Å PFP, 5 µm, 4.6 x 100 mm
Part Number: 95814-609
Mobile Phase:
A: 25 mM Ammonium acetate, pH 5.5
B: Acetonitrile
Gradient:

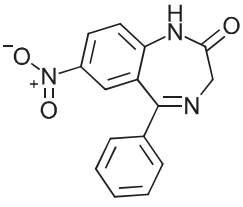
Time (min)	% B
0.0	36
7.0	65

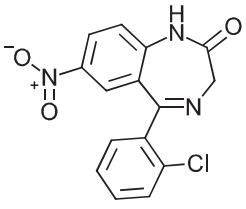
Flow Rate: 0.75 mL/min
Pressure: 46 bar
Temperature: 35 °C
Detection: UV 254 nm
Injection Volume: 1.0 µL
Response Time: < 0.12 sec
Flow Cell: 5.0 µL semi-micro
LC System: Agilent 1100

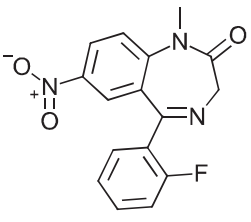
STRUCTURES:

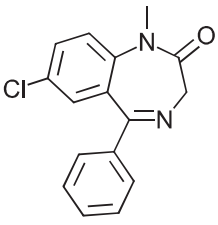

Oxazepam


Lorazepam


Nitrazepam

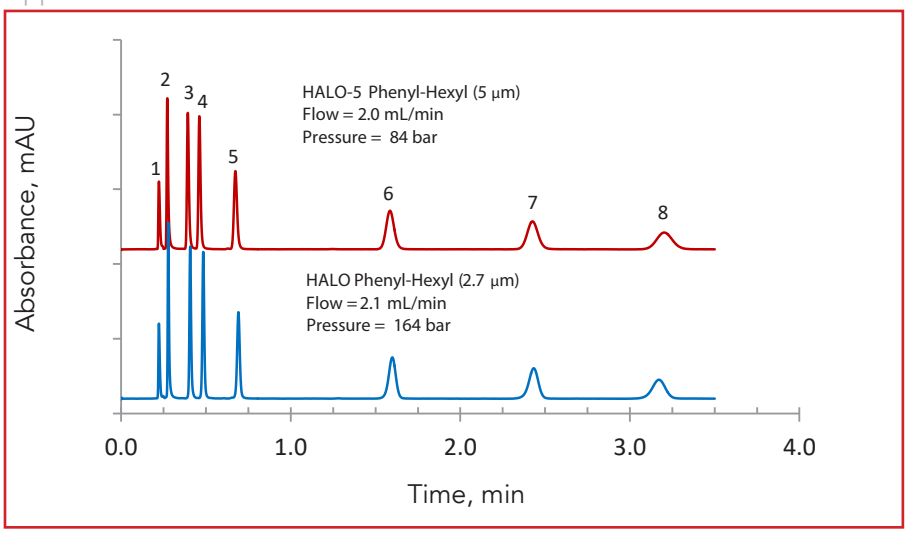

Clonazepam


Flunitrazepam


Diazepam

Comparable Selectivity Between HALO® 5 µm and HALO® 2.7 µm Phenyl-Hexyl Phases

Application Note 82-HA



PEAK IDENTITIES:

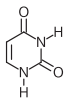
1. Uracil (t_0)
2. 6,7-Dihydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 6-Chloro-4-hydroxycoumarin
6. Warfarin
7. Coumatetralyl
8. Coumachlor

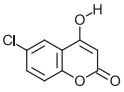
These chromatograms show the similarity in selectivity between the 5 µm and the 2.7 µm HALO® Phenyl-Hexyl phases which allows the easy transfer of methods from one particle size to another.

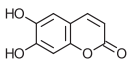
TEST CONDITIONS:

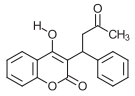
Columns:
1) HALO 90 Å Phenyl-Hexyl, 5 µm, 4.6 x 50 mm
Part Number: 95814-406
2) HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 55/45 - A/B
A: 0.1% formic acid in water
B: 50/50 methanol/acetonitrile
Flow Rate: See chart
Pressure: See chart
Temperature: 45 °C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: 30/70 water (0.1% formic acid)/ methanol
Response Time: 0.12 sec
Flow Cell: 5.0 µL
LC System: Agilent 1100

STRUCTURES:

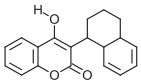

Uracil

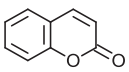

6-Chloro-4-hydroxycoumarin

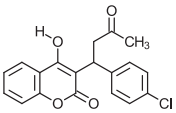

6,7-Dihydroxycoumarin


Warfarin


4-Hydroxycoumarin

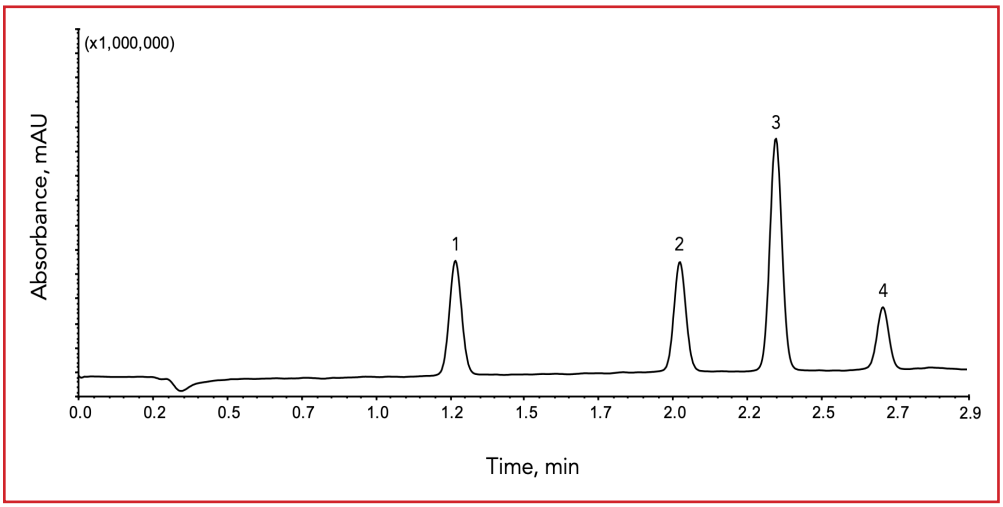

Coumatetralyl


Coumarin


Coumachlor

LC-MS Separation of Fentanyl and Analogues in Synthetic Urine

Application Note 172-OP



PEAK IDENTITIES:

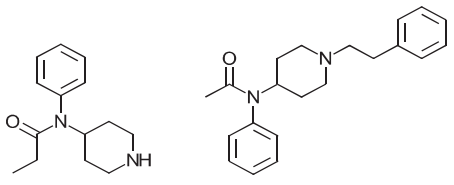
1. Norfentanyl	TIC/233
2. Acetyl Fentanyl	TIC/323
3. Fentanyl	TIC/337
4. Sufentanil	TIC/387

A mixture of fentanyl and some of its analogues spiked into synthetic urine are separated on a HALO® Biphenyl column using LC-MS detection. These opioids are known to be much more potent than heroin and have become a significant contributor towards the opiate crisis in America.

TEST CONDITIONS:

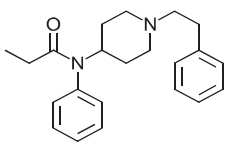
Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 50 mm
Part Number: 92812-411
Mobile Phase:
A: Water/0.1% formic acid/10mM ammonium formate
B: Methanol/0.1% formic acid/10mM ammonium formate
Gradient: 40-90% B in 3 min
Flow Rate: 0.8 mL/min
Initial Pressure: 380 bar
Temperature: 30 °C
Injection Volume: 0.5 µL
Sample Solvent: Surine Negative Urine
LC System: Shimadzu Nexera
MS System: Shimadzu LCMS 2020 (single quadrupole)
ESI: 4.5 kV
Heat Block: 300 °C
Nebulizing Gas Flow: 1.3 L/min

STRUCTURES:

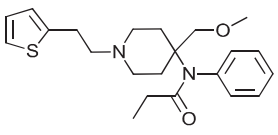


Norfentanyl

Acetyl Fentanyl



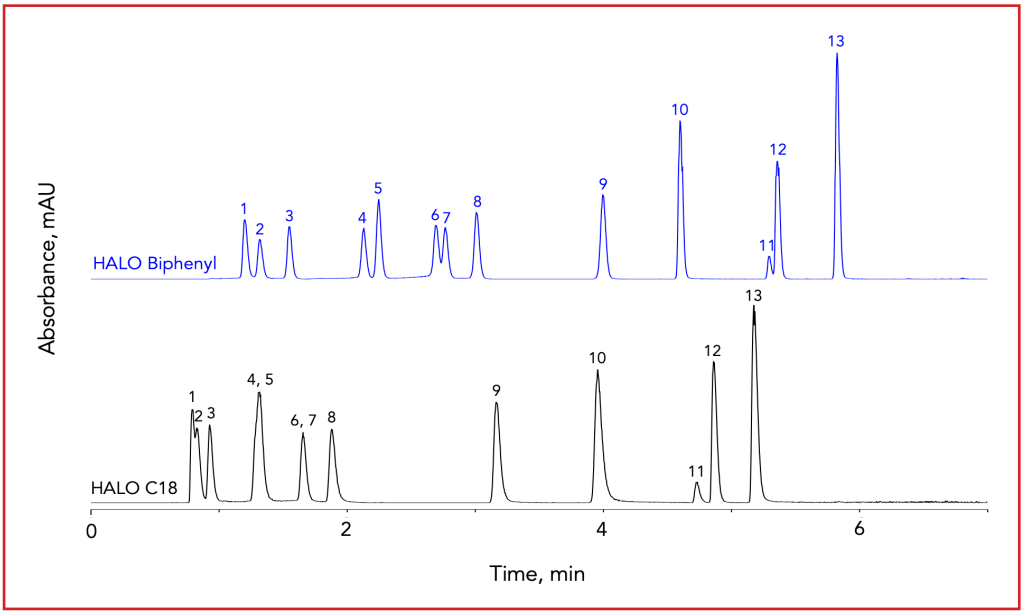
Fentanyl



Sufentanil

Pain Management Panel Comparison on HALO® Biphenyl and C18

Application Note 173-OP



PEAK IDENTITIES:

1. Morphine
2. Oxymorphone
3. Hydromorphone
4. Naloxone
5. Codeine
6. Naltrexone
7. Oxycodone
8. Hydrocodone
9. cis-Tramadol HCl
10. Meperidine
11. Fentanyl
12. Buprenorphine
13. (±)-Methadone

The HALO® Biphenyl phase provides greater retention and improved resolution for the polar analytes in this mixture of pain management drugs. Compound pairs 1/2 and 4/5 are baseline separated using the HALO® Biphenyl column, but co-elute on the HALO® C18 column. Analytes 6 and 7 are partially resolved on the HALO® Biphenyl column, but they co-elute using the HALO® C18 column. These bonded-phase selectivity differences are very useful for method development, and provide a basis for LC-MS analyses of large pain medicine panels.

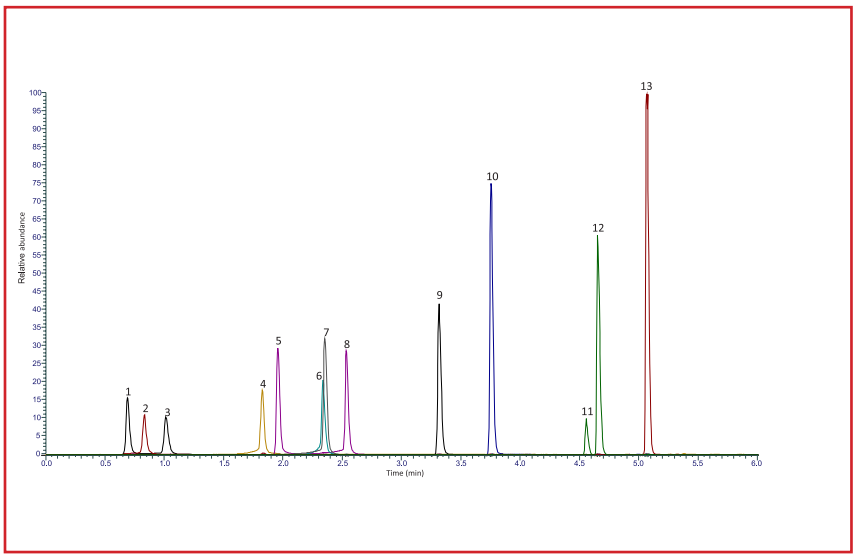
TEST CONDITIONS:

Columns:
1) HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-611
2) HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-602
Mobile Phase:
A: Water/0.1% formic acid
B: ACN/0.1% formic acid
Gradient: 0-3 min 10-20% B
3-3.5 min 20-100% B
3.5-6 min hold at 100% B
Flow Rate: 0.3 mL/min
Temperature: 30 °C
Injection Volume: 2.0 µL
Sample Solvent: 99/1 water/methanol
Dwell Volume: 0.19 mL
LC System: Agilent 1290

MS System: Agilent 6210 TOF
ESI: +4 kV
Gas Temperature: 360 °C
Gas Flow: 12 L/min
Nebulizer: 50 psi
Scan Rate: 5 spectra/s
Fragmentor: 175 V
Skimmer: 65 V
Octopole RF: 250 V

LC-MS Separation of Pain Management Opiates on HALO® Biphenyl, 2.0 µm

Application Note 192-OP



PEAK IDENTITIES:	m/z
1. Morphine	286
2. Oxymorphone	302
3. Hydromorphone	286
4. Naloxone	328
5. Codeine	300
6. Naltrexone	342
7. Oxycodone	316
8. Hydrocodone	300
9. cis-Tramadol	264
10. Meperidine	248
11. Fentanyl	337
12. Buprenorphine	468
13. (±)-Methadone	310

The 2.0 µm HALO® Biphenyl is an ideal choice for high throughput analysis of drug panels, in which isobaric species separation is needed. Note the resolution between codeine and hydrocodone, (peaks 1 and 3, respectively) and morphine and hydromorphone (peaks 5 and 8, respectively).

TEST CONDITIONS:

Column: HALO 90 Å Biphenyl, 2.0 µm, 2.1 x 100 mm
Part Number: 91812-611
Mobile Phase:
A: Water/0.1% formic acid
B: Acetonitrile/0.1% formic acid

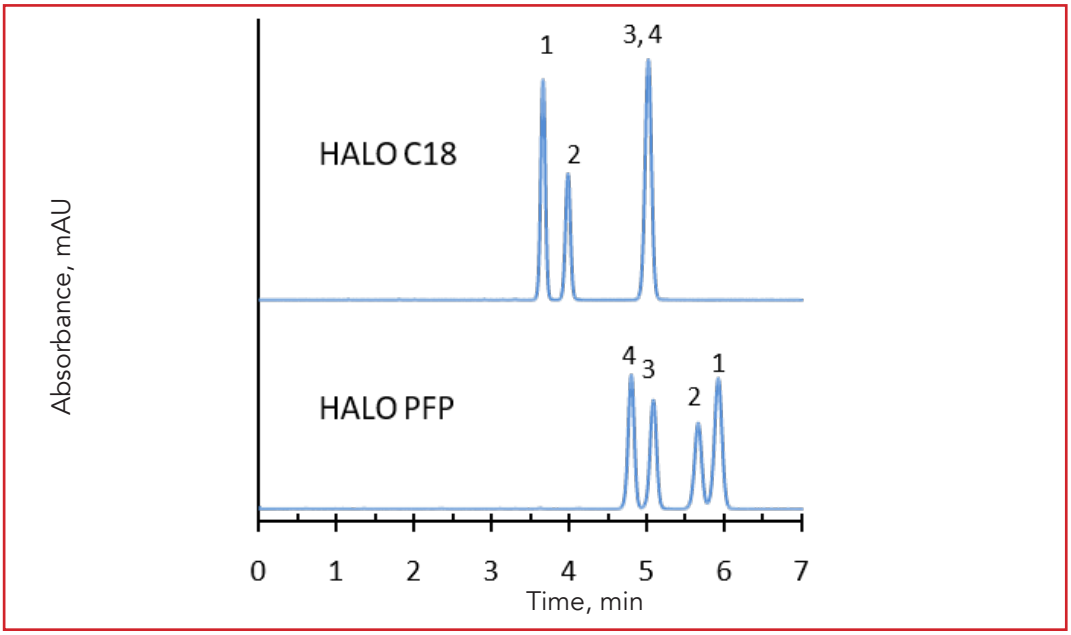
Gradient:

Time (min)	% B
0.00	10
2.22	20
5.00	60
5.50	60
5.51	10
6.50	END

Flow Rate: 0.4 mL/min
Initial Pressure: 325 bar
Temperature: 40 °C
Detection: +ESI MS
Injection Volume: 1.0 µL
Sample Solvent: 95/5 water/acetonitrile
LC System: Shimadzu Nexera X2

Separation of Structurally Similar Steroids on HALO® C18 and PFP

Application Note 47-STR



PEAK IDENTITIES:

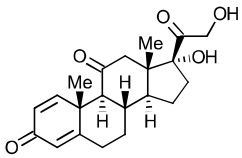
1. Prednisone
2. Cortisone
3. Prednisolone
4. Hydrocortisone

The unique selectivity of HALO® PFP is useful in the separation of the closely related steroids prednisolone and hydrocortisone. The electron-deficient ring structure of the perfluorophenyl group aids in separating compounds through pi-pi interactions with the sample.

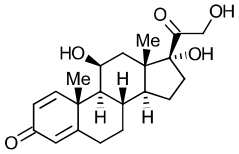
TEST CONDITIONS:

Columns:
1) HALO 90 Å C18, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-602
2) HALO 90 Å PFP, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-609
Mobile Phase: 50/50 - A/B
A: Water
B: Methanol
Flow Rate: 1.0 mL/min
Pressure: ~230 bar
Temperature: 35 °C
Detection: UV 240 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 80% methanol in water
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

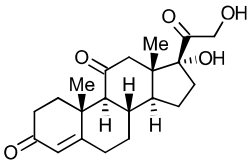
STRUCTURES:



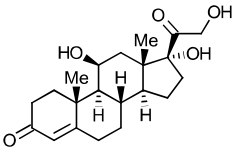
Prednisone



Prednisolone



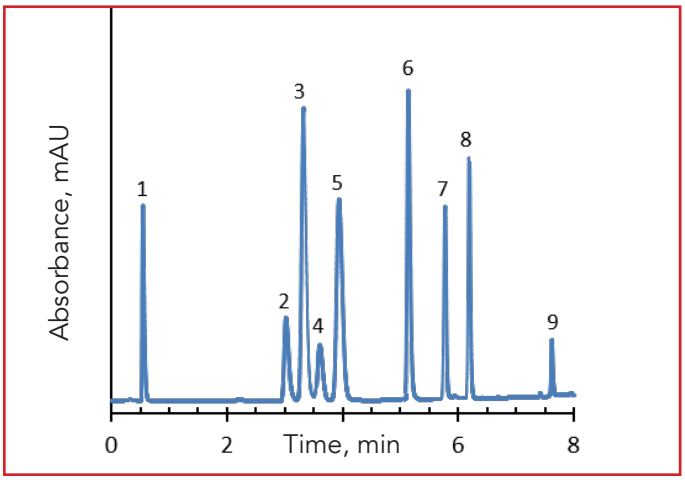
Cortisone



Hydrocortisone

Separation of Steroids on HALO® PFP, 2.0 µm

Application Note 116-STR



PEAK IDENTITIES:

1. Uracil
2. Hydrocortisone
3. Prednisolone
4. Cortisone
5. Prednisone
6. Dexamethasone
7. β-Estradiol
8. Estrone
9. Halcinonide

HALO® PFP, 2.0 µm is useful in the separation of closely related steroids. Even though this separation was run on a system with 14 µL of extra column volume, there is sufficient efficiency with a HALO® 2.0 µm column to separate the first four steroids during the isocratic hold at the beginning of the run.

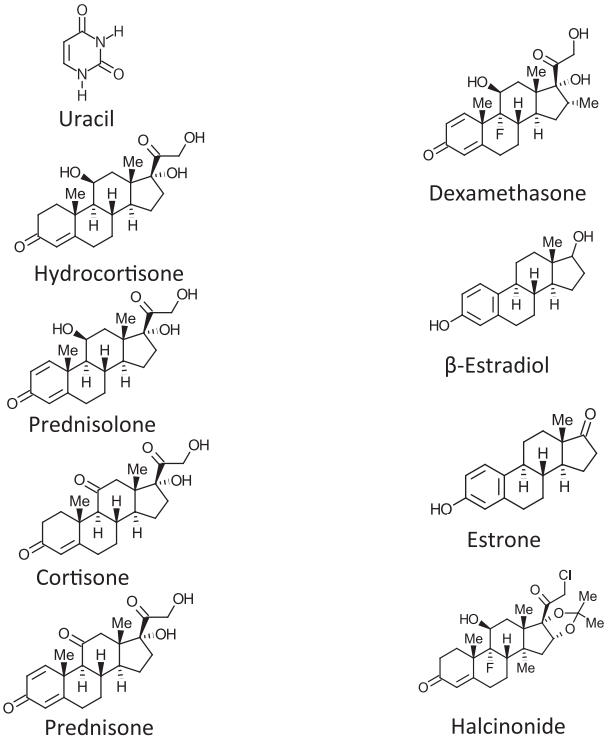
TEST CONDITIONS:

Column: HALO 90 Å PFP, 2.0 µm, 3.0 x 50 mm
Part Number: 91813-409
Mobile Phase:
A: Water
B: Methanol
Gradient:

Time (min)	% B
0.0	47
3.0	47
8.0	88

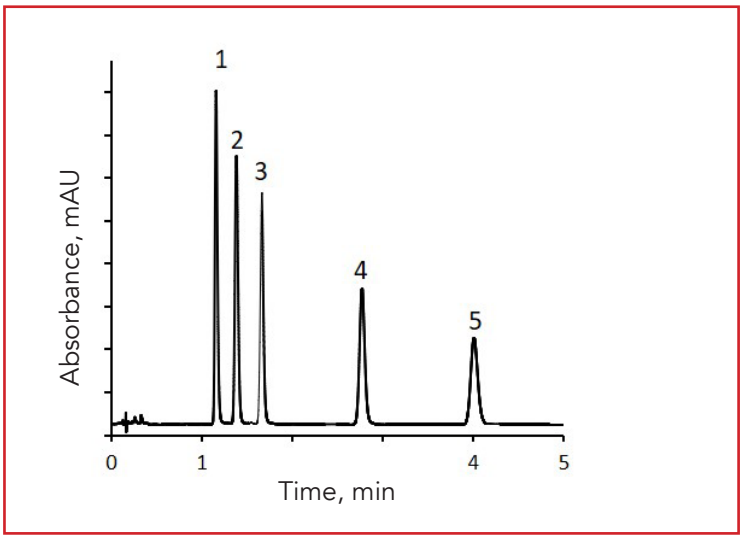
Flow Rate: 0.4 mL/min
Pressure: 180 bar
Temperature: 35 °C
Detection: UV 280 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

STRUCTURES:



Separation of Anabolic Steroids on HALO® C18, 2.0 µm

Application Note 139-STR



PEAK IDENTITIES:

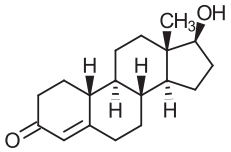
1. Nandrolone
2. Methandienone
3. Testosterone
4. Epitestosterone
5. Norethandrolone

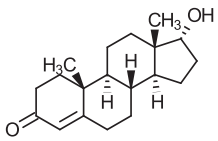
Screening for steroid use is common in both sports and medicine. These five anabolic steroids are separated in less than 5 minutes using a 2-micron HALO® C18 column.

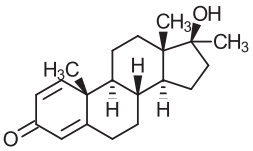
TEST CONDITIONS:

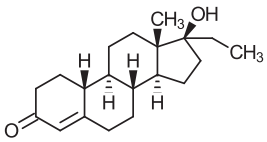
Column: HALO 90 Å C18, 2.0 µm, 2.1 x 50 mm
Part Number: 91812-402
Mobile Phase: 70/30 - A/B
A: Water
B: Acetonitrile
Flow Rate: 0.8 mL/min
Pressure: 476 bar
Temperature: 40 °C
Detection: UV 254 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: 37.5/62.5 water/organic solvent (acetonitrile, methanol, and 1,2-dimethoxyethane)
Response Time: 0.02 sec
Flow Cell: 2.0 µL micro cell
Data Rate: 80 Hz
LC System: Agilent 1200 SL

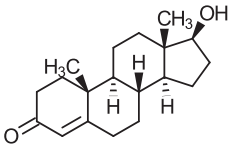
STRUCTURES:


Nandrolone


Epitestosterone

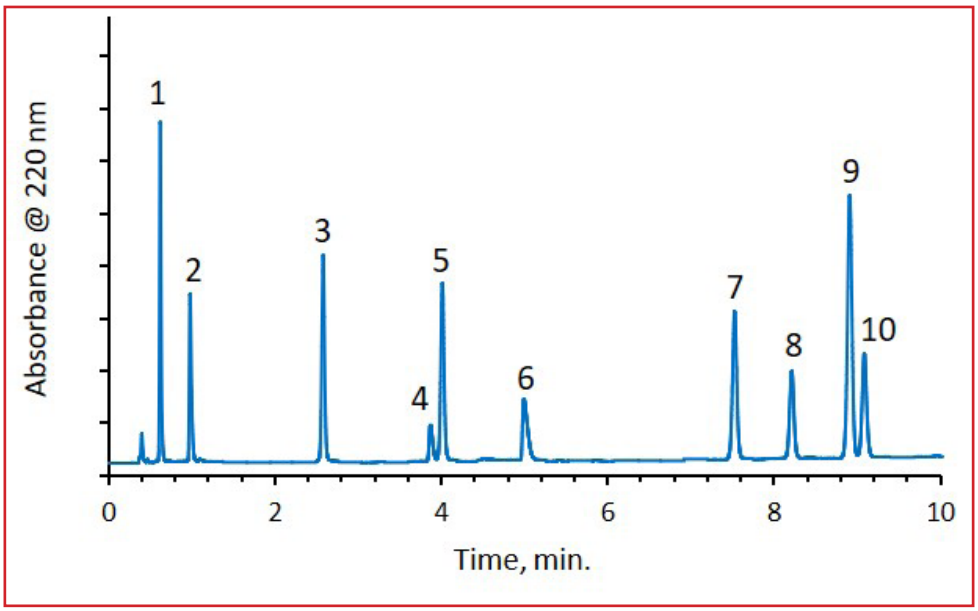

Methandienone


Norethandrolone


Testosterone

Separation of Steroid Hormones and Hormone Conjugates on HALO® C18

Application Note 142-STR



PEAK IDENTITIES:

1. Estriol-3-(β-D-glucuronide)
2. Estriol-3-Sulfate
3. Estrone-3-(β-D-glucuronide)
4. β-Estradiol-3-Sulfate
5. Estriol
6. Estrone-3-Sulfate
7. β-Estradiol
8. α-Estradiol
9. Androstenedione
10. Estrone

Steroid hormones and hormone conjugates are monitored for a variety of medical reasons. This fast separation of ten estrogens and estrogen-related compounds was accomplished with a HALO® C18 column.

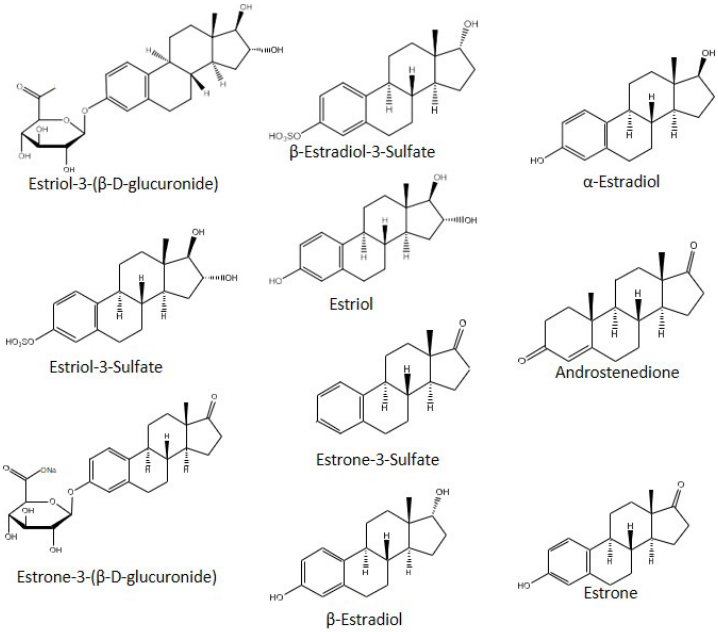
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-602
Mobile Phase:
A: 10 mM phosphate buffer, pH 7.0
B: Acetonitrile
Gradient:

Time (min)	% B
0.0	20
10.0	43

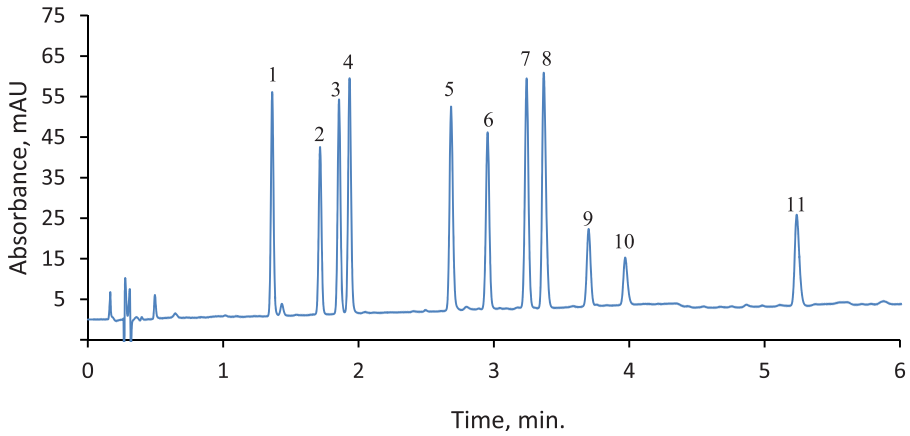
Flow Rate: 0.5 mL/min
Pressure: 366 bar
Temperature: 25 °C
Detection: UV 220 nm, PDA
Injection Volume: 4.0 µL
Sample Solvent: 84/16 water/acetonitrile
Response Time: 0.05 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

STRUCTURES:



Separation of Steroids on HALO 90 Å Biphenyl

Application Note 169-STR



PEAK IDENTITIES:

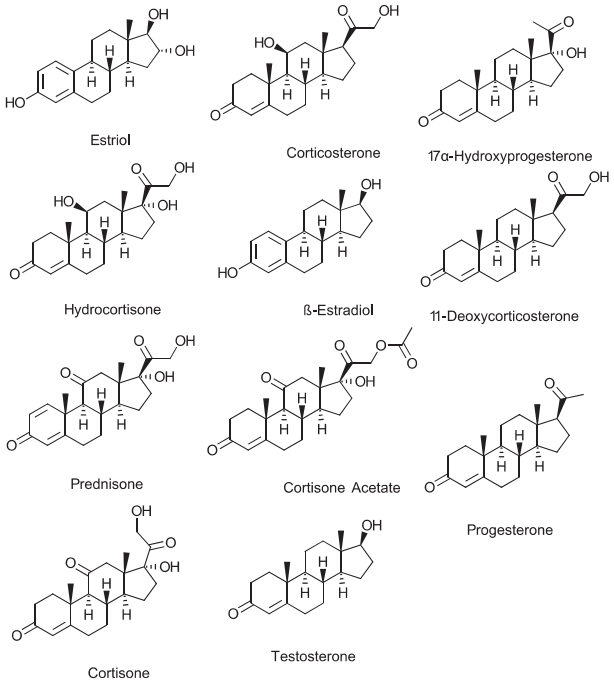
1. Estriol
2. Hydrocortisone
3. Prednisone
4. Cortisone
5. Corticosterone
6. β-Estradiol
7. Cortisone Acetate
8. Testosterone
9. 17-α-Hydroxyprogesterone
10. 11-Deoxycorticosterone
11. Progesterone

A mixture of eleven steroids is separated using a 6-minute gradient on a HALO 90 Å Biphenyl column. The chromatogram shows very good resolution between all peak pairs with excellent peak shape and high efficiency.

TEST CONDITIONS:

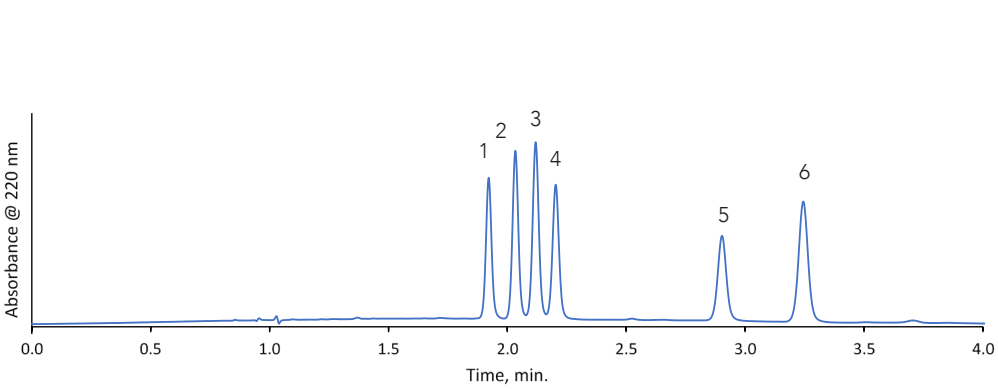
Column: HALO 90 Å Biphenyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-411
Mobile Phase:
A: Water
B: Acetonitrile
Gradient: 20-60% B in 6 min
Flow Rate: 1.85 mL/min
Pressure: 344 bar
Temperature: 30 °C
Detection: UV 215 nm, PDA
Injection Volume: 4.0 µL
Sample Solvent: 37.5/62.5 acetonitrile/water
Response Time: 0.025 sec
Data Rate: 100 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

STRUCTURES:



Separation of Glucocorticoids on HALO® C30

Application Note 184-STR



PEAK IDENTITIES:

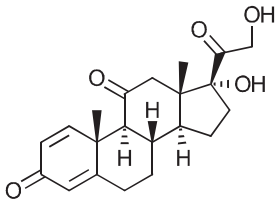
1. Prednisone
2. Cortisone
3. Prednisolone
4. Hydrocortisone
5. Dexamethasone
6. Corticosterone

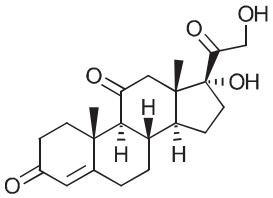
Glucocorticoids are a class of steroid drugs that have anti-inflammatory and anti-allergy benefits, as well as antilymphatic cancer uses. This mixture of six glucocorticoids is separated with high resolution in less than four minutes on a HALO® C30 column.

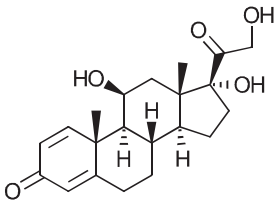
TEST CONDITIONS:

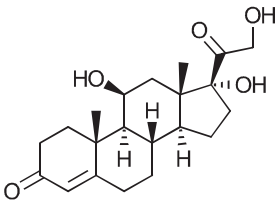
Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Part Number: 92114-730
Mobile Phase:
A: Water
B: 50/50 acetonitrile/methanol
Isocratic: 50% B
Flow Rate: 1.5 mL/min
Pressure: 355 bar
Temperature: 50 °C
Detection: UV 220 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: Acetonitrile
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

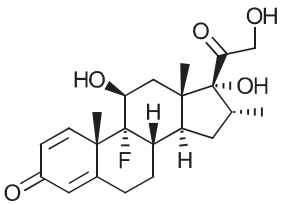
STRUCTURES:

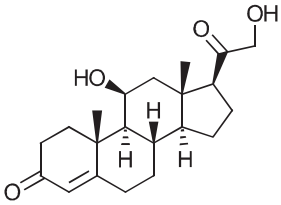

Prednisone


Cortisone


Prednisolone

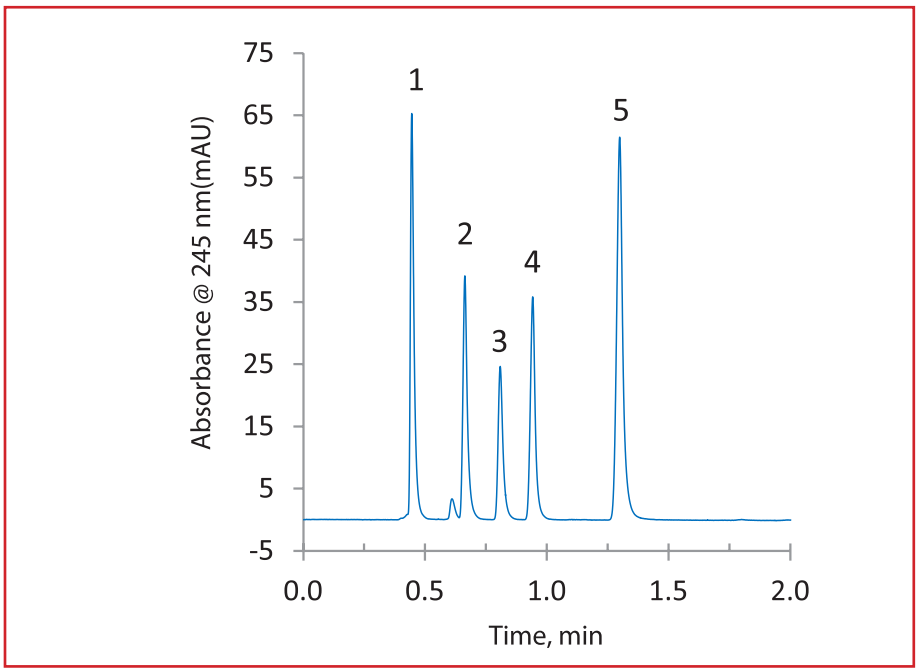

Hydrocortisone


Dexamethasone


Corticosterone

Separation of Local Anesthetics on HALO® Penta-HILIC, 2.0 µm

Application Note 119-B



PEAK IDENTITIES:

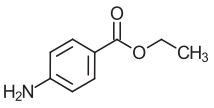
1. Benzocaine
2. Lidocaine
3. Tetracaine
4. Procaine
5. Procainamide

The separation of these basic anesthetics shows the utility of the 2.0 µm HALO® Penta-HILIC phase for basic compounds. The highly efficient Fused-Core® particles allow complete separation of these compounds in less than 1.5 minutes.

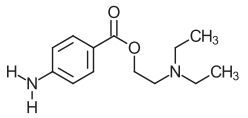
TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.0 µm, 2.1 x 100 mm
Part Number: 91812-605
Isocratic: 92/8 ACN/water with 5 mM ammonium formate buffer, pH 3.0
Flow Rate: 0.5 mL/min
Pressure: 229 bar
Temperature: 30 °C
Detection: UV 245 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: 90/10 ACN/0.1 M ammonium formate buffer, pH 3.0
Response Time: 0.1 sec
Data Rate: 40 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Agilent 1200 SL

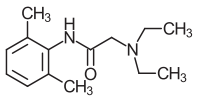
STRUCTURES:



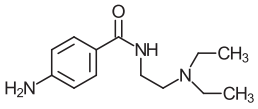
Benzocaine



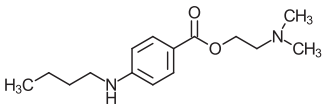
Procaine



Lidocaine



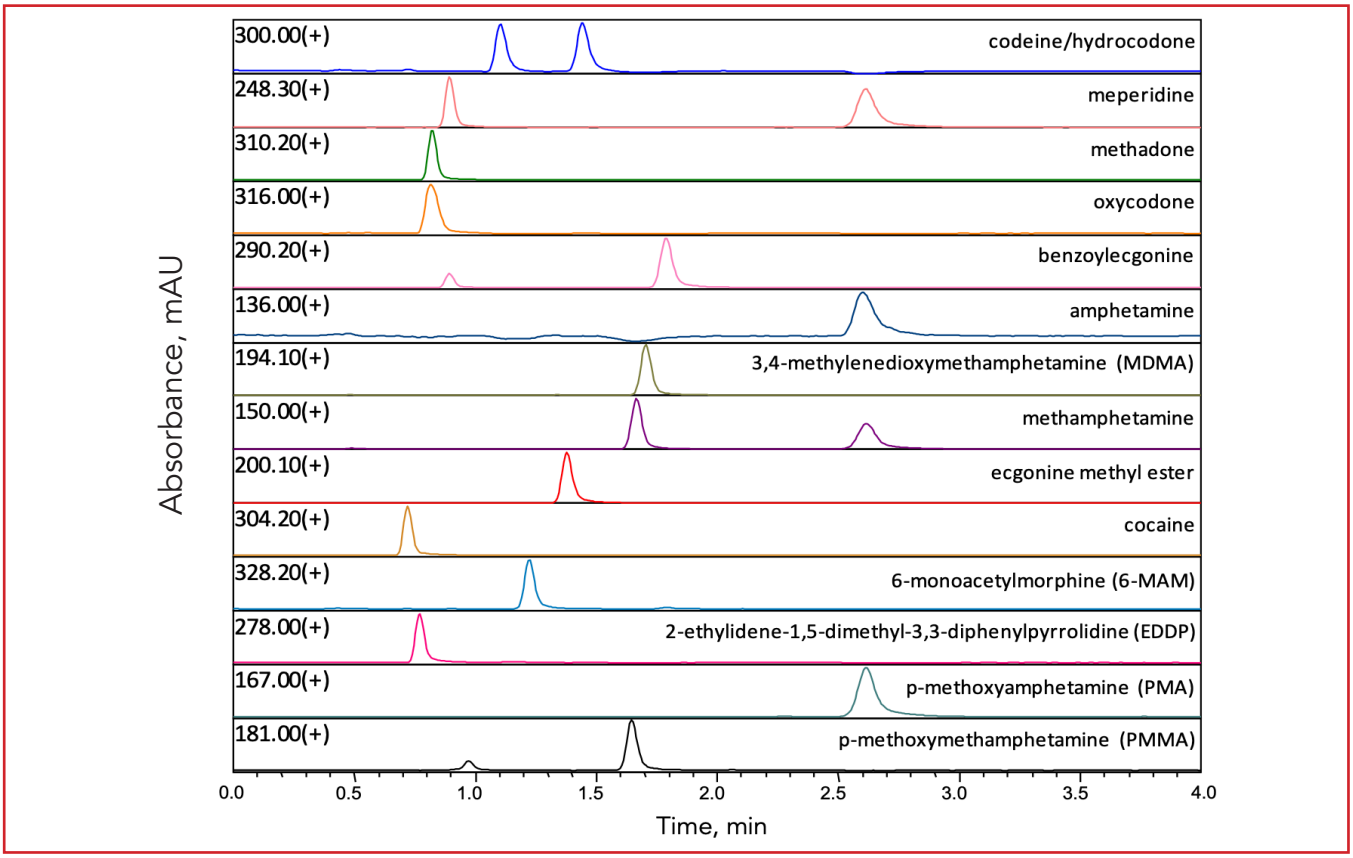
Procainamide



Tetracaine

LC-MS Separation of Drugs of Abuse and Metabolites on HALO® Penta-HILIC

Application Note 123-DA



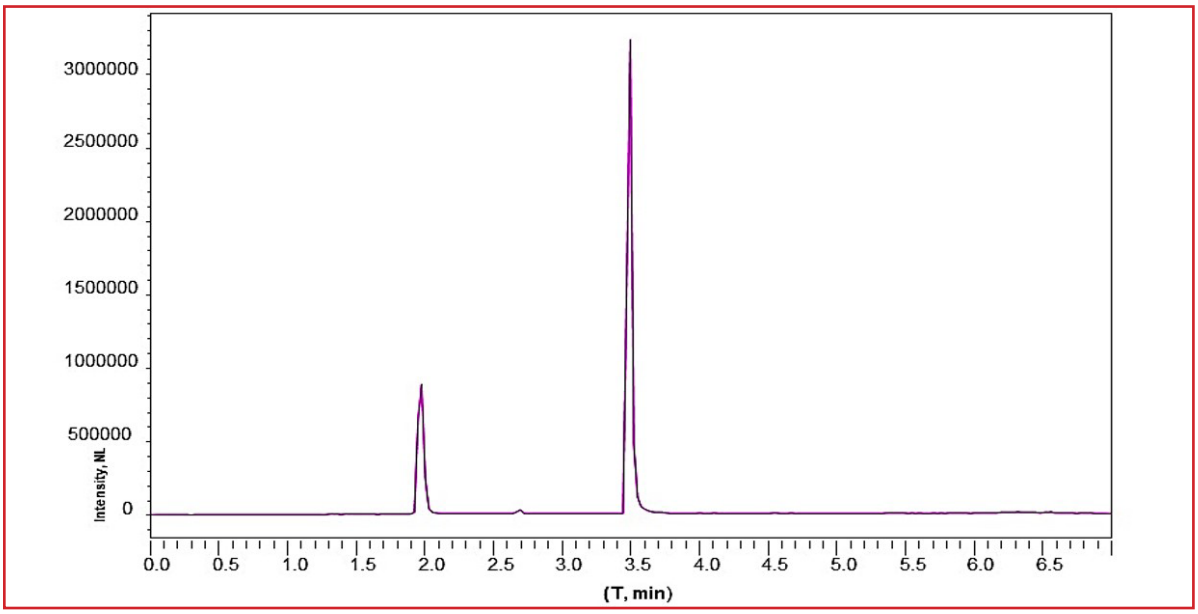
TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-605
Mobile Phase:
A: 5 mM Ammonium formate, pH 3.0
B: Acetonitrile
Isocratic: Pre-mixed 5/95 - A/B
Flow Rate: 0.5 mL/min
Pressure: 149 bar
Temperature: 60 °C
Detection: Selected Ion Monitoring as indicated
Injection Volume: 1.0 µL
Sample Solvent: 90/10 ACN/water
MS Parameters: Positive ion mode, 2 kV, 400 °C heat block 225 °C capillary
LC-MS System: Shimadzu Nexera and LCMS-2020 (single quadrupole MS)

This mixture of drugs of abuse and metabolites is quickly identified using a HALO® Penta-HILIC column and selected ion monitoring (SIM) for improved sensitivity. Adapted from J. Pharm. Anal. 2013; 3 (5): 303-311.

LC-MS Separation of Kratom and its Metabolite on HALO® C18, 2 µm

Application Note: 204-TOX



The 2 µm HALO® C18 is an ideal choice for analysis of kratom and its metabolite. Kratom is an herbal extract that comes from the leaves of an evergreen tree (Mitragyna speciosa) grown in Southeast Asia. Believed to act on opioid receptors, kratom has been used by people to mitigate the symptoms of opioid withdraw. However, studies on the effects of kratom have identified many safety concerns and no clear benefits, and kratom is not currently regulated by the United States.

TEST CONDITIONS:

Column: HALO 90 Å C18, 2 µm, 2.1 x 50 mm
Part Number: 91812-402
Mobile Phase A: Water/0.1% Formic acid
Mobile Phase B: ACN/0.1% Formic acid
Gradient:

Time	%N=B
0.0	10
4.00	95
5.00	95
5.01	95
7.00	END

Flow Rate: 0.4 mL/min
Initial Pressure: 315 bar
Temperature: ambient
Injection Volume: 2 µL
Sample Solvent: 95/5 ACN/Water

MS CONDITIONS:

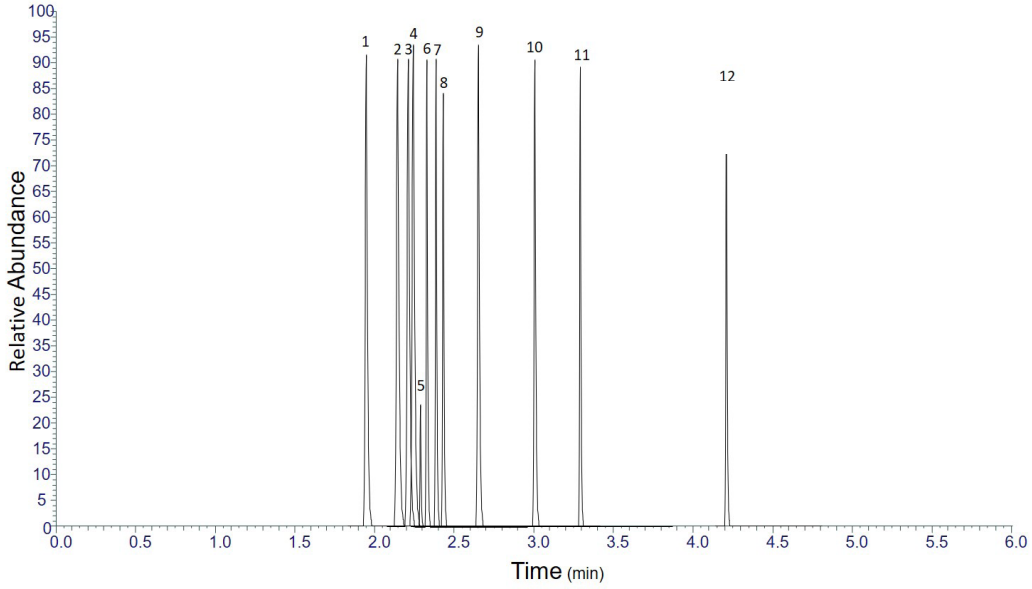
LCMS system: Shimadzu LCMS-2020
Detection: +ESI MS
Spray voltage: 4.50 kV
Drying line temp: 300 °C
Heat Block: 450 °C

PEAK IDENTITIES:

1. 7-OH Mitragynine (MH+=415.502 g/mol)
2. Mitragynine (MH+=399.453 g/mol)

LC-MS Separation SAMHSA 5 Panel on HALO® Biphenyl 2 µm

Application Note: 205-TOX



The 2 µm HALO® Biphenyl is an ideal choice for high throughput analysis of drug panels, in which isobaric species separation is needed. Note the resolution between methamphetamine and phentermine, (peaks 3 and 5, respectively). The SAMHSA 5 panel consists of amphetamines, cocaine, marijuana, opiates, and phencyclidine (PCP).

TEST CONDITIONS:

Column: HALO 90 Å Biphenyl, 2 µm, 2.1 x 100
Part Number: 91812-611
Mobile Phase A: Water/0.1% Formic acid
Mobile Phase B: Methanol/0.1% Formic acid
Gradient:

Time	%B
0.0	5
4.00	98
5.00	98
5.01	5
7.00	END

Flow Rate: 0.4 mL/min
Initial Pressure: 325 bar
Temperature: 40 °C
Injection Volume: 2 µL
Sample Solvent: 95/5 MeOH/Water
LC System: Shimadzu Nexera X2

MS CONDITIONS:

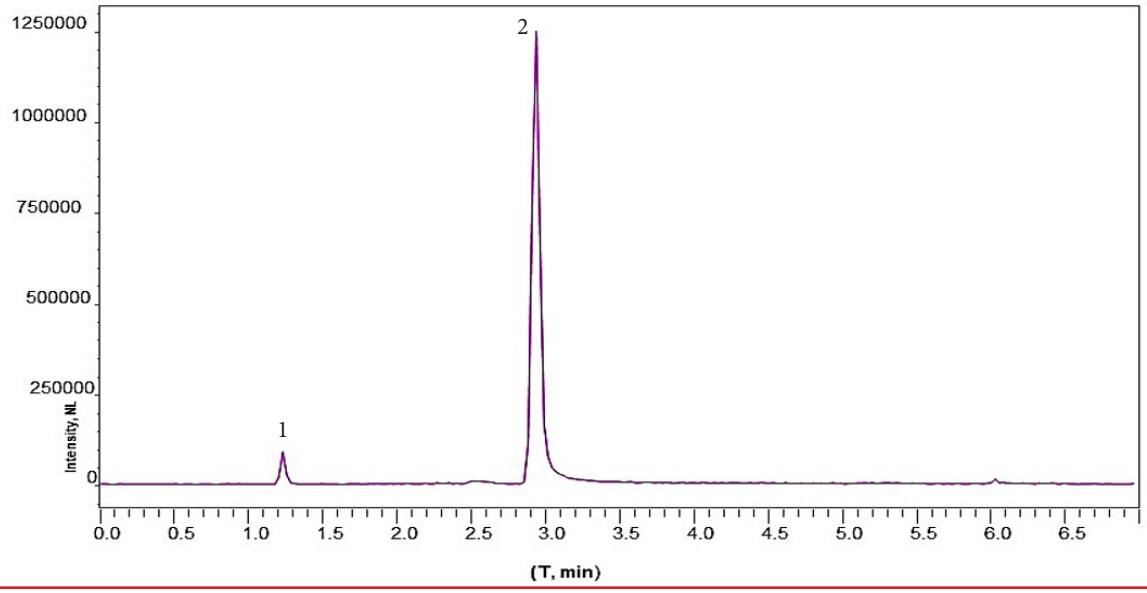
Detection:: +ESI MS
Mass Spectrometer: Thermo Exactive HF
Sheath gas flow rate: 50 (arbitrary units)
Aux gas flow rate: 13 (arbitrary units)
Sweep gas flow rate: 0 (arbitrary units)
Spray voltage: 3.50 k V
Cap temp: 263 °C
S-lens RF level: 70 V
Aux gas heater temperature: 425 °C

PEAK IDENTITIES:

1. Morphine (MH^+= 286.341 g/mol)
2. Amphetamine (MH^+= 136.206 g/mol)
3. Methamphetamine (MH^+= 150.237 g/mol)
4. MDA (MH^+= 180.221 g/mol)
5. Phentermine (MH^+= 150.233 g/mol)
6. Codeine (MH^+= 300.364 g/mol)
7. 6-MAM (MH^+= 328.380 g/mol)
8. MDMA (MH^+= 194.246 g/mol)
9. MDEA (MH^+= 208.271 g/mol)
10. Benzoylecgonine (MH^+= 290.331 g/mol)
11. PCP (MH^+= 244.387 g/mol)
12. THC-COOH (MH^+= 345.415 g/mol)

LC-MS Separation of EtG/EtS from urine on HALO® Penta-HILIC, 2 µm

Application Note: 206-TOX



Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are metabolites of ethanol that are found in urine. The presence of these can be used to determine if an alcoholic beverage was ingested. Zero tolerance programs often use this test.

TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2 µm
2.1 x 100mm
Part Number: 91812-605
Mobile Phase A: 5 mM ammonium formate/ 0.1% formic acid in 95:5 ACN/water
Mobile Phase B: 5mM ammonium formate/ 0.1% formic acid in 80:20 ACN/water

Gradient:	**Time**	**%B**
	0.00	0
	1.00	100
	5.00	100
	5.01	0
	7.00	END

Flow Rate: 0.4 mL/min
Initial Pressure: 325 bar
Temperature: 40 °C
Injection Volume: 2 µL
Sample prep: 5ng/mL EtG/EtS in 20 uL of synthetic urine. 10 fold dilution with mobile phase A.

PEAK IDENTITIES:

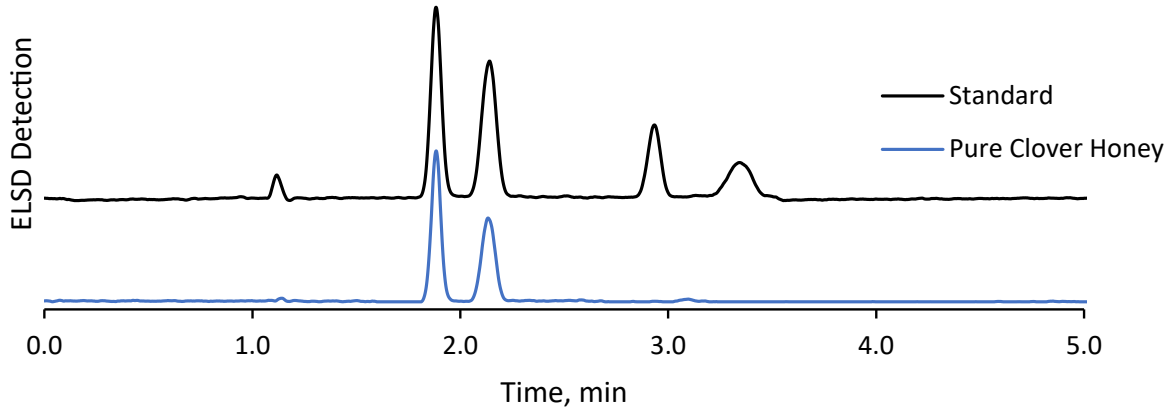
1. EtS (MH-=125.120 g/mol)
2. EtG (MH-=221.193 g/mol)

MS CONDITIONS:

LCMS system: Shimadzu LCMS-2020
Detection: -ESI MS
Spray voltage: 4.50 kV
Drying line temp: 300 °C
Heat Block: 450 °C

Analysis of Sugars in Pure Honey Using HALO® Penta-HILIC

215-SU



PEAK IDENTITIES:

1. D-(-) Fructose
2. D-(+) Glucose
3. Sucrose
4. D-(+) Maltose

TEST CONDITIONS:

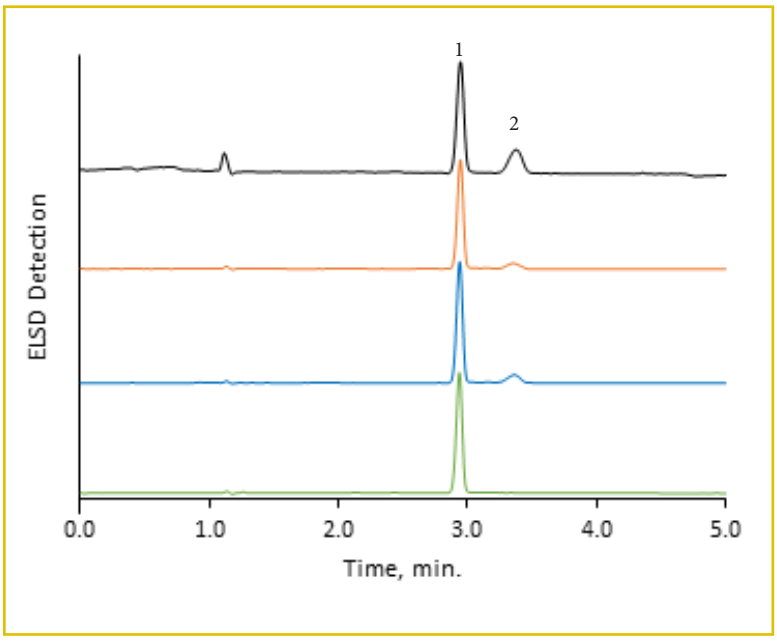
Column: HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-705
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Isocratic: 80% B
Flow Rate: 1.4 mL/min
Initial Pressure: 213 bar
Temperature: 65 °C
Detection: ELSD, 40 °C, 3.3 bar
Injection Volume: 15 µL
Sample Solvent: 80/20 ACN/ Water
Data Rate: 10 Hz
Response Time: 0.10 sec
LC System: Shimadzu Nexera X2

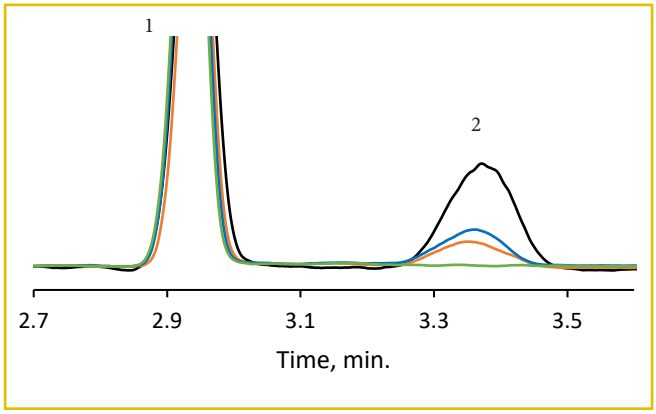
Honey can significantly range in quality depending on its purity and levels of sucrose and maltose. Natural honey primarily consists of fructose and glucose, while adulterated honey can contain high levels of sucrose and maltose.

A HALO® Penta-HILIC column separates the primary monosaccharides in pure honey clover showing no signs of adulteration.

Analysis of Sucrose and Lactose in Chocolate Using HALO® 90 Å Penta-HILIC

216-SU





PEAK IDENTITIES:

1. Sucrose
2. D-(+) Lactose monohydrate

TEST CONDITIONS:

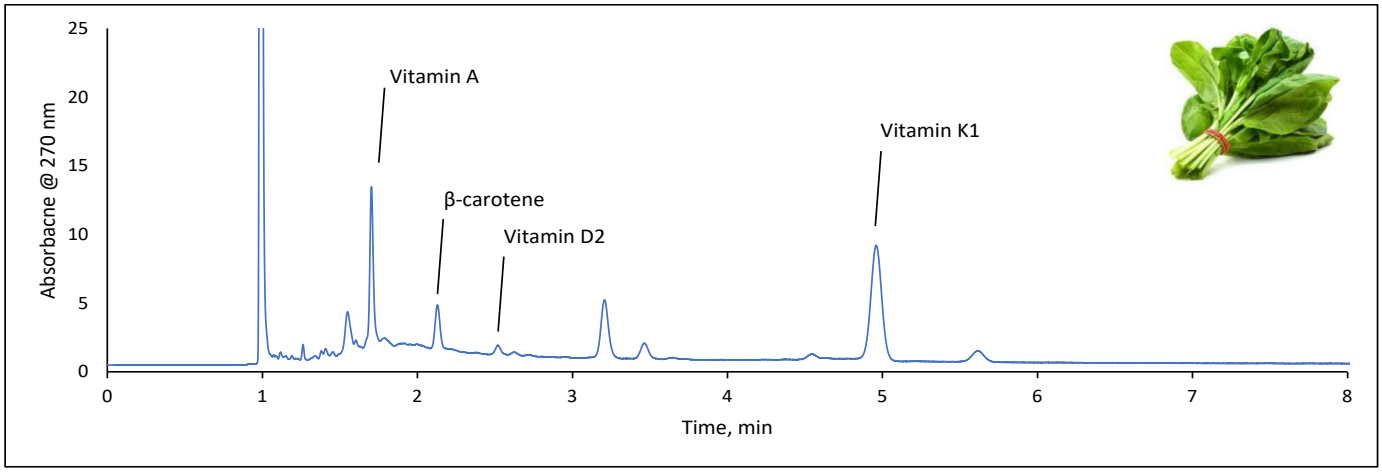
Column: HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-705
Mobile Phase A: Water
Mobile Phase A: Acetonitrile
Flow Rate: 1.4 mL/min
Pressure: 213 bar
Temperature: 65 °C
Detection: ELSD, 40 °C, 3.3 bar
Injection Volume: 15 µL
Sample Solvent: 80/20 ACN/ Water
Response Time: 0.10 sec
DataRate: 10 Hz
LC System: Shimadzu NexeraX2

Chocolate is a very well-known, popular, food type worldwide. It is used for all occasions and can even have some health benefits as well, which include improved blood flow and brain function. There are four main types of chocolate to choose from- milk, white, dark, and raw.

Analysis of three different types of chocolate (milk, white, and dark) was carried out (or performed) in HILIC mode using an ELSD detector. The compounds of interest were sucrose and lactose. The HALO® Penta-HILIC column was used, which has a polar ligand with 5 hydroxyl groups tethered via novel proprietary linkage chemistry to Fused-Core® silica particles.

Analysis of Spinach

223-V



TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Part Number: 92114-730
Mobile Phase: Methanol
Flow Rate: 1.5 mL/min
Pressure: 265 bar
Temperature: 30 °C
Detection: UV 270 nm
Injection Volume: 1.0 µL
Sample Solvent: Hexane
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

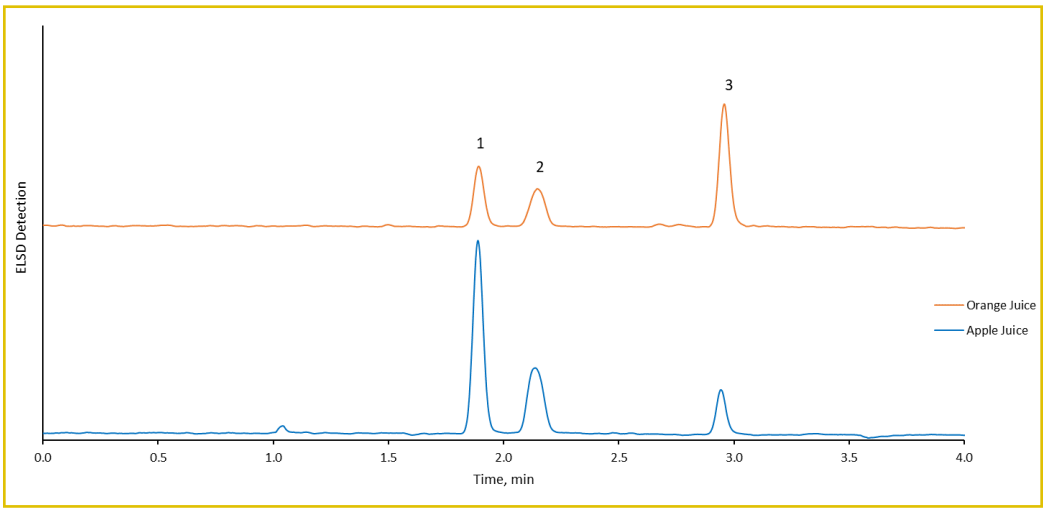
PEAK IDENTITIES:

1. Retinyl acetate (Vitamin A)
2. β-carotene
3. Ergocalciferol (Vitamin D2)
4. 2, 3 - trans-phylloquinone (Vitamin K1)

Spinach is common leafy green vegetable found all over the world and is one of the most nutritious to consume. A sample of spinach is dissolved in hexane and analyzed using a HALO® C30 column. Several fat soluble vitamins are found in the sample using isocratic HPLC conditions.

Analysis of Sugars in Juice using HALO® Penta HILIC

227-F



PEAK IDENTITIES

1. Sucrose
2. Glucose
3. Fructose

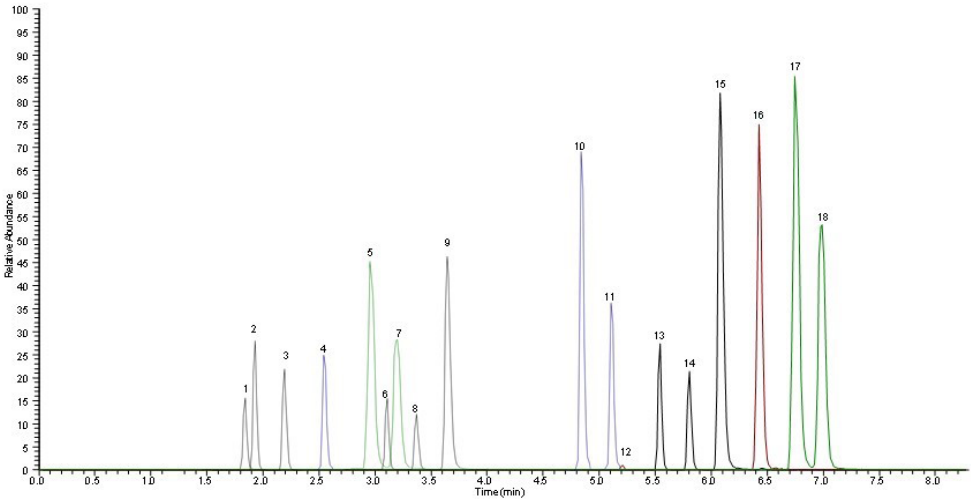
TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 µm 4.6 x 150 mm
Part Number: 92814-705
Mobile Phase **A:** Water
B: Acetonitrile
Isocratic: 80 %B
Flow Rate: 1.4 mL/min
Pressure: 213 bar
Temperature: 65 °C
Detection: ELSD, 40°C, 3.3 bar
Injection Volume: 0.2 µL
Sample Solvent: Water
Data Rate: 10 Hz
Response Time: 0.10 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera

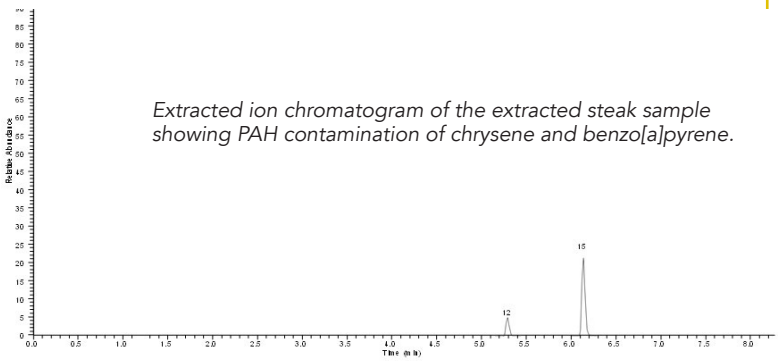
The main sugars in natural fruit juice are fructose, glucose, and sucrose. Each type of juice will contain different ratios of these sugars. Juices obtained from concentrate can also be found to have various amounts of artificial sweeteners. Analysis of sugars is performed on a HALO® Penta-HILIC column with excellent speed and resolution. A comparison of the different sugars in apple juice and orange juice is observed using an ELSD detector.

LCMS of PAHs in Grilled Meat using HALO® PAH

237-P



TIC of a high resolution separation of 18 PAHs in under 7.5 minutes



Extracted ion chromatogram of the extracted steak sample showing PAH contamination of chrysene and benzo[a]pyrene.

Peak #	Compound	Precursor Ion	Fragment 1	Fragment 2
1	Naphthalene	128	78	102
2	Acenaphthylene	152	126	151
3	1-Methylnaphthalene	142	89	115
4	2-Methylnaphthalene	142	115	141
5	Acenaphthene	154	126	153
6	Fluorene	166	115	165
7	Phenanthrene	178	151	176
8	Anthracene	178	152	176
9	Fluoranthene	202	150	200

PEAK IDENTITIES AND ELUTION ORDER

Peak #	Compound	Precursor Ion	Fragment 1	Fragment 2
10	Pyrene	202	150	200
11	Benzo[a]anthracene	228	150	226
12	Chrysene	228	200	226
13	Benzo[b]fluoranthene	252	224	250
14	Benzo[k]fluoranthene	252	224	250
15	Benzo[a]pyrene	252	224	250
16	Dibenzo[a,h]anthracene	278	248	276
17	Benzo[ghi]perylene	276	248	274
18	Indeno[1,2,3-cd]pyrene	276	246	274

The HALO® PAH column continues in the tradition of HALO® products by offering high resolution separations, in high throughput time frames. 18 PAH compounds with 6 sets of isomeric compounds were able to be quickly and efficiently resolved in under 8 minutes. In addition, the high resolution separation of the HALO® PAH column, enabled chrysene and benzo[a]pyrene to be resolved from a complex meat matrix, enabling quantitation of PAH contamination present in barbequed steak. The concentration of PAHs in the sample, were below those established by the EU, and demonstrates that not only can the HALO® PAH column be used in the stringent regulatory testing of current established methods, but also be relied upon as future regulations dictate the establishment of new methods, requiring lower limits of detection. The HALO® PAH column offers a rugged and reproducible particle design meeting the needs of complex matrix testing. Fused-Core® technology is ideal for PAH analysis in particular, enabling customers to achieve analytical goals of speed, accuracy, and precision LC separations.

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 2.1 x 100 mm
Part Number: 92842-612
Flow Rate: 0.4 mL/min
Pressure: 289 bar
Column Temperature: 30 °C
Injection Volume: 1 µL
Sample Solvent: Methanol
LC System: Shimadzu Nexera
Mobile Phase **A:** Water/0.1% formic acid
B: Acetonitrile/0.1% formic acid

Gradient:

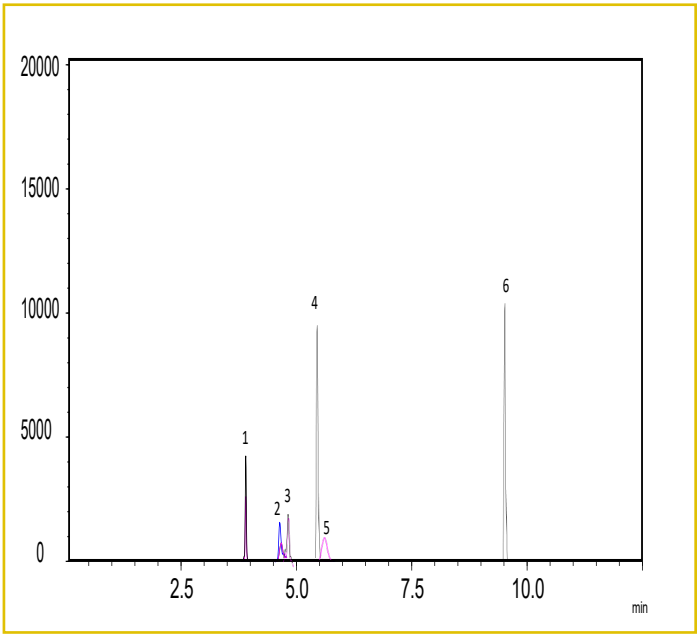
Time	%B
0.0	40
5.0	100
8.0	100
8.01	40

MASS SPECTROMETRY CONDITIONS:

MS System: Thermo Scientific™ Q Exactive™ HF
ESI voltage: 5.5 kV
Heater Temp: 400 °C
Sheath gas: 35 (arbitrary units)
Aux gas: 8 (arbitrary units)
Tube lens voltage: 40 V

LCMS Screening Comparison of Mycotoxins in Craft and Home Brewed Beers

256-M

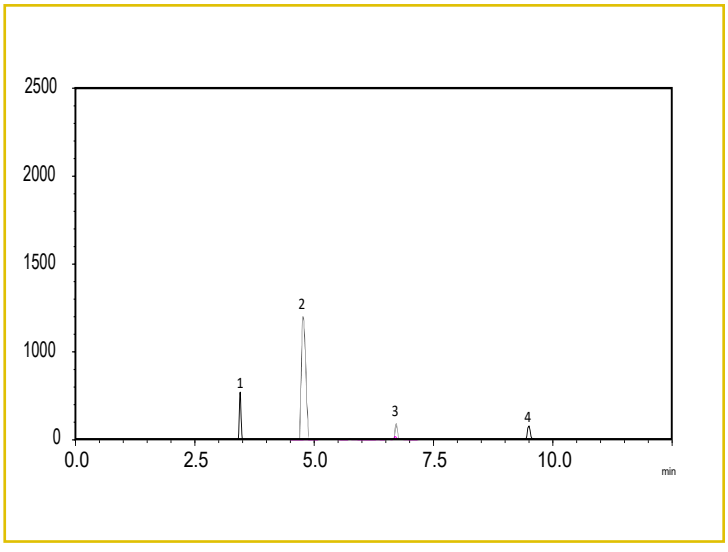


Craft Brewed Beer

Peak Id	Mycotoxin	Retention Time (min)	Precursor Ion	Product Ion
1	T-2 Toxin	3.95	489.2	245.1
2	Aflatoxin G2	4.65	331.1	189.2
3	15-acetylde-oxynivalenol	4.88	339.1	321.1
4	Aflatoxin B2	5.52	315.1	287.1
5	Aflatoxin M1	5.75	329.1	273.3
6	Zearalenone	9.55	319.1	283.2

Home Brewed Beer

Peak Id	Mycotoxin	Retention Time (min)	Precursor Ion	Product Ion
1	T-2 Toxin	3.95	489.2	245.1
2	15-acetylde-oxynivalenol	4.88	339.1	321.1
3	Aflatoxin M1	5.75	329.1	273.3
4	Zearalenone	9.55	319.1	283 .2



TEST CONDITIONS:

Analytical Column: HALO 90 Å PFP, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-609
Mobile Phase A: Water, 5 mM Ammonium Formate, 0.1 % Formic Acid
Mobile Phase B: Methanol, 0.1% Formic Acid
Gradient:

TIME	%B
0	0
0.5	14
2	14
3	60
3.5	60
8	100
10	100
10.5	0
12.5	End

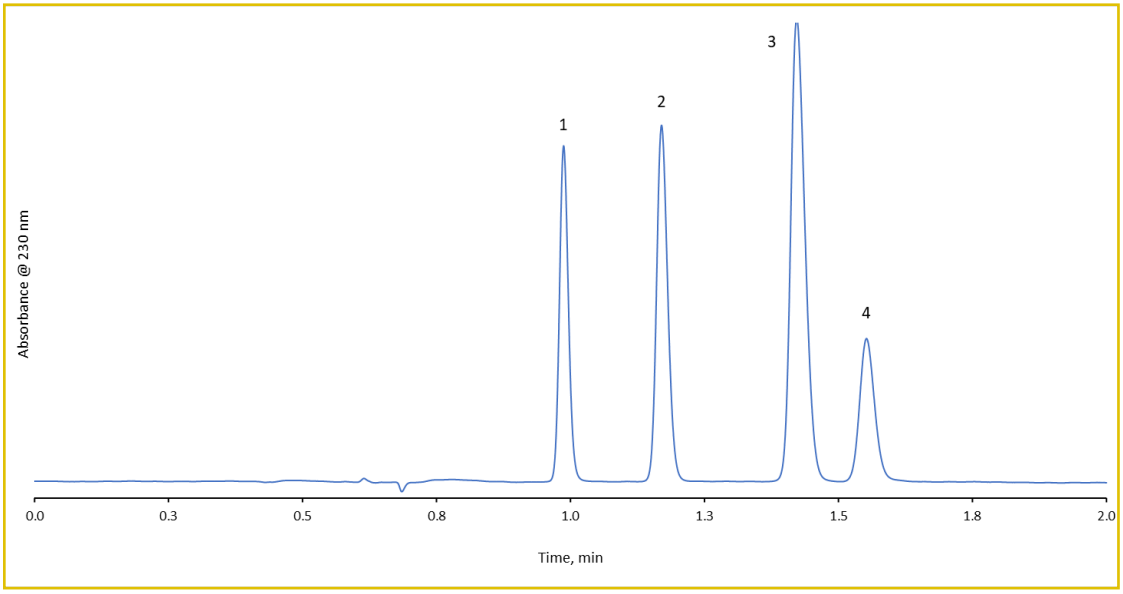
Flow Rate: 0.4 mL/min
Pressure: 290 bar
Temperature: 40 °C
Injection Volume: 7.0 µL
Sample Solvent: 49/50/1 ACN/H_2O/Acetic acid
Detection: +ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS System: Shimadzu LCMS-8040

Mycotoxin contamination can have serious health implications. Although there are no set regulatory limits for mycotoxins in beer, most governments have clear levels for mycotoxins in various types of grain and animal feed. For example, in the United States, most levels are in the mid to high ppb range. Despite relatively low levels of mycotoxin activity in the beer, given the propensity for people to indulge in excessive drinking, and the cumulative effects of the toxicity of these compounds, excessive consumption would lead to a cumulative toxic effect, which warrants further analysis and regulation.

Beer analysis can be challenging due to matrix effects and interference, often resulting in low sensitivity and ambiguous results; therefore, it is critical to have a column that has superior performance. The HALO 90 Å PFP can not only meet these challenges, but exceed them by demonstrating superior performance and sensitivity, making it an ideal column to be used in environmental, and, specifically, mycotoxin analysis.

Food Additives Assay using HALO® AQ-C18, 5µm

260-P



PEAK IDENTITIES:

1. Acesulfame
2. Benzoic acid
3. Sorbic acid
4. Saccharin sodium

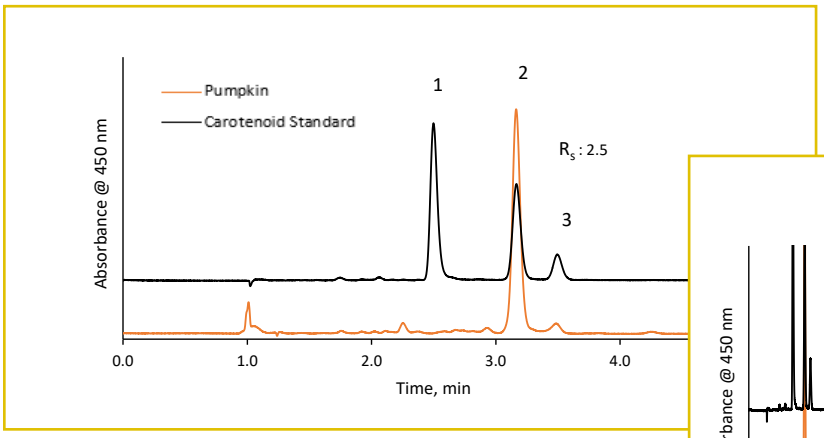
TEST CONDITIONS:

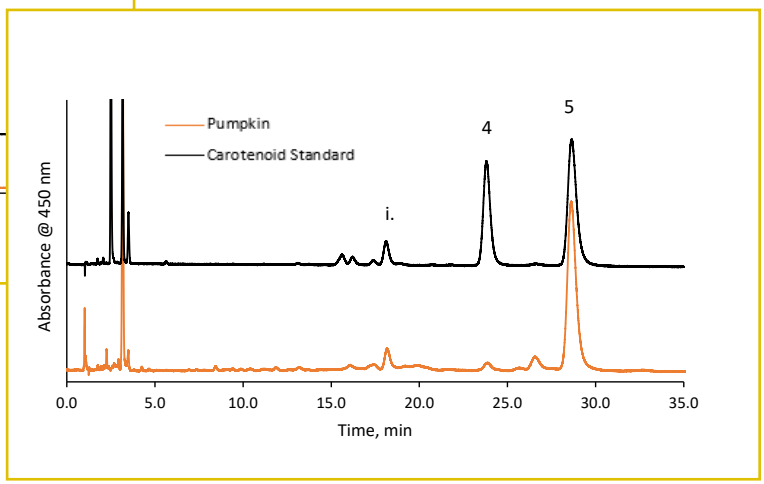
Column: HALO 90 Å AQ-C18 5 µm, 4.6 × 150 mm
Part Number: 95814-722
Mobile Phase A: 20 mM ammonium acetate
Mobile Phase B: Methanol
Isocractic: 90/10 A/B
Flow Rate: 2 mL/min
Pressure: 336 bar
Temperature: 30°C
Detection wavelength: 230 nm
Injection Volume: 10 µL
Sample Solvent: mobile phase
Data Rate: 100 hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

A rapid and highly efficient assay <400 bar for food security and safety measurements is demonstrated with a HALO 90 Å AQ-C18 5 µm, 4.6 × 150 mm column. Determination of acesulfame, benzoic acid, sorbic acid and saccharin sodium food additives are specified in China's national standard regulation methods GB 5009.28-2016 and GB 5009.140-2016. These compounds are used as anti-septic/anti-microbial agents to prevent spoilage of food products by micro-organisms. A baseline resolution separation is completed <1.7 min; modernization of this method is as easy as exploiting the 5 micron HALO® column - compatible with HPLC and UHPLC instruments.

Carotenoid Analysis in Pumpkin

250-V





PEAK IDENTITIES

1. Astaxanthin
2. Lutein
3. Zeaxanthin
4. α- Carotene
5. β-Carotene

   i. unidentified isomers

TEST CONDITIONS:

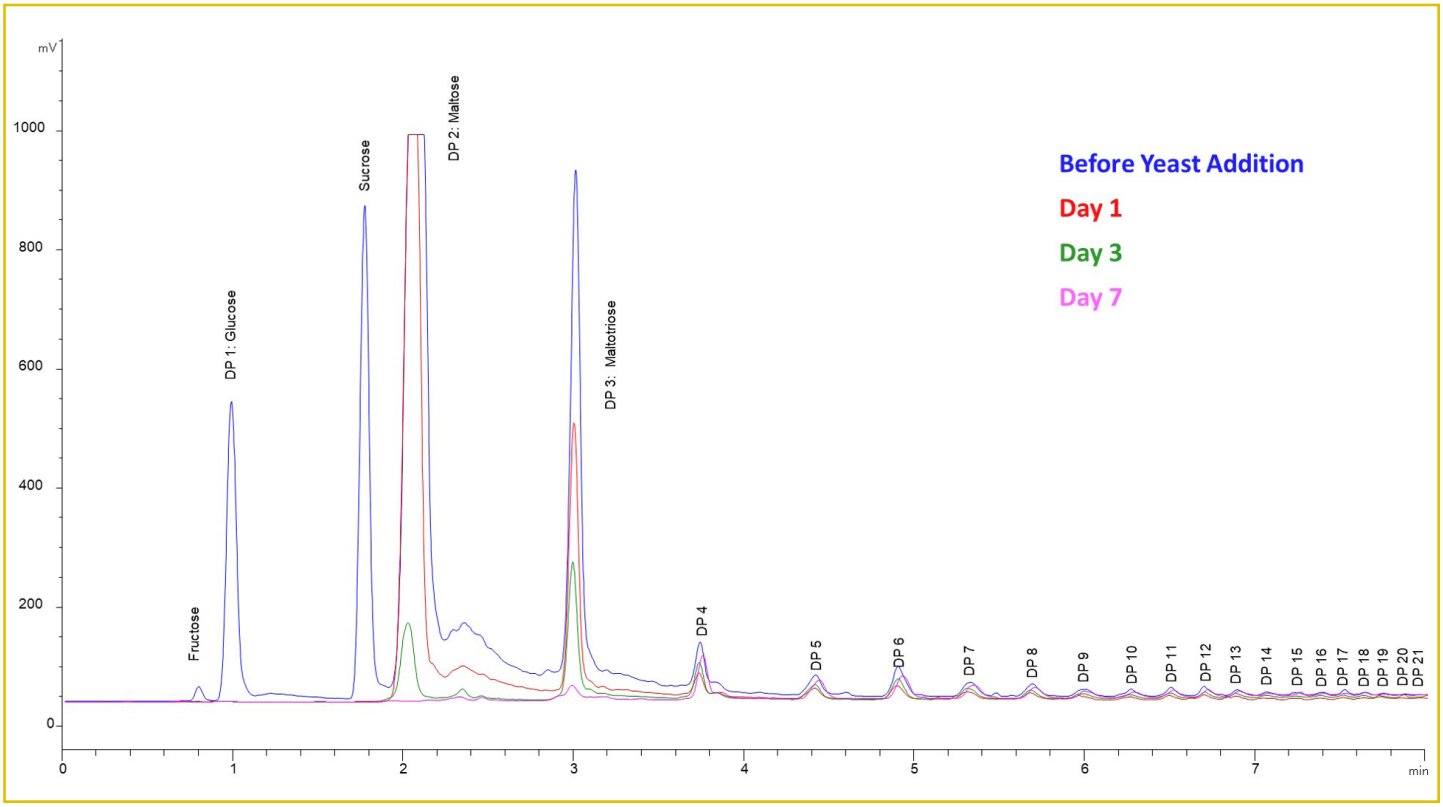
Column: HALO® C30, 2.7 µm, 4.6 x 150 mm
Part Number: 92114-730
Isocratic: 100% Methanol
Flow Rate: 1.5 mL/min
Initial HALO® Pressure: 277 bar
Temperature: 15 °C
Detection: 450 nm,
Injection Volume: 20.0 µL

Sample Solvent: Methanol
Data Rate: 14 Hz
Response Time: 0.12 sec.
Flow Cell: 5 µL semi-micro
LC System: LC System: Agilent 1100

Pumpkins contain high amounts of carotenoids, especially beta carotene. Carotenoids are fat-soluble compounds that can be split into two main groups called xanthophylls and carotenes. These compounds both contain anti-oxidant properties and some can be converted into vitamin A when released into the body. A liquid-liquid extraction is performed with 0.2g of pumpkin pulp. Carotenoids are extracted from the pumpkin and analyzed on a HALO® C30 column. The HPLC oven set at sub-ambient temperature enables optimum resolution of early eluting peaks.

Beer Fermentation Analysis using HALO® Penta-HILIC

269-SU



TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 µm, 3.0 x 50 mm
Part Number: 92813-405
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Gradient:

Time	%B
0.0	92
8.0	52

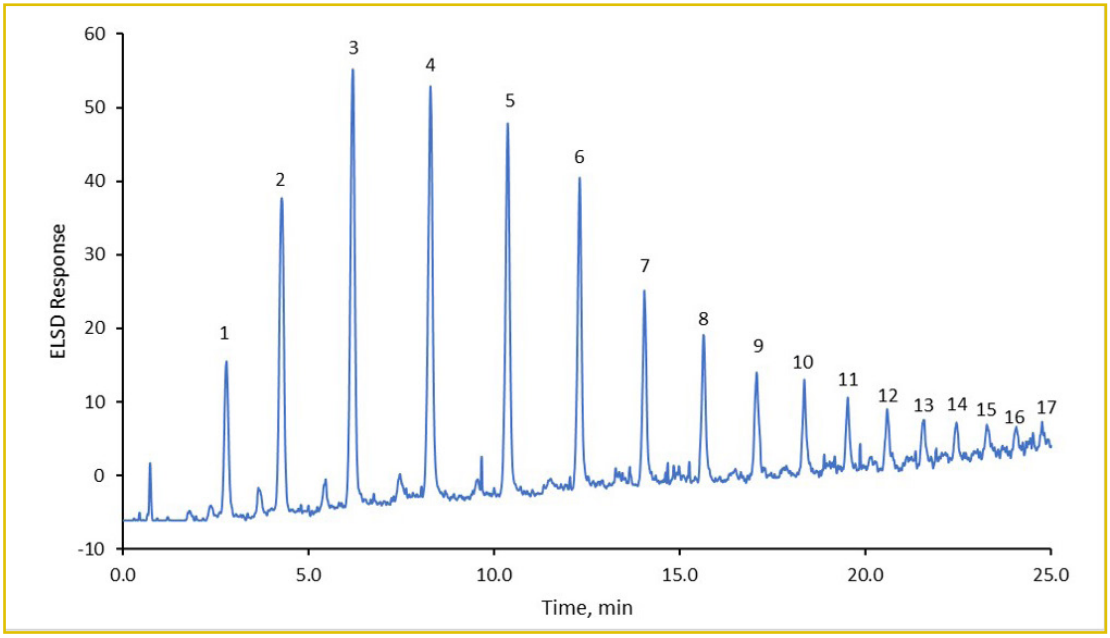
Flow Rate: 0.75 mL/min
Temperature: 65 °C
Detection: ELSD, 40°C, 45 psi
Injection Volume: 2 µL
Data Rate: 10 Hz, 2 sec filter

Data Courtesy of Merlin K. L. Bicking, Ph. D. (ACCTA, Inc.)

A Belgian ale is analyzed with a HALO® Penta-HILIC column using an evaporative light scattering detector (ELSD). Sugars, oligosaccharides, and polysaccharide levels are monitored throughout the fermentation process in order to track yeast behavior. These levels will decrease over time as the yeast converts the sugars to ethanol.The Penta-HILIC/ ELSD combination is a great way to perform rapid sugar analysis providing high resolution and good peak shape at elevated temperatures.

High Resolution Separation of Oligosaccharides on HALO 90 Å Penta-HILIC

272-SU



PEAK IDENTITIES:

1. DP 3
2. DP 4
3. DP 5
4. DP 6
5. DP 7
6. DP 8
7. DP 9
8. DP 10
9. DP 11
10. DP 12
11. DP 13
12. DP 14
13. DP 15
14. DP 16
15. DP 17
16. DP 18
17. DP 19

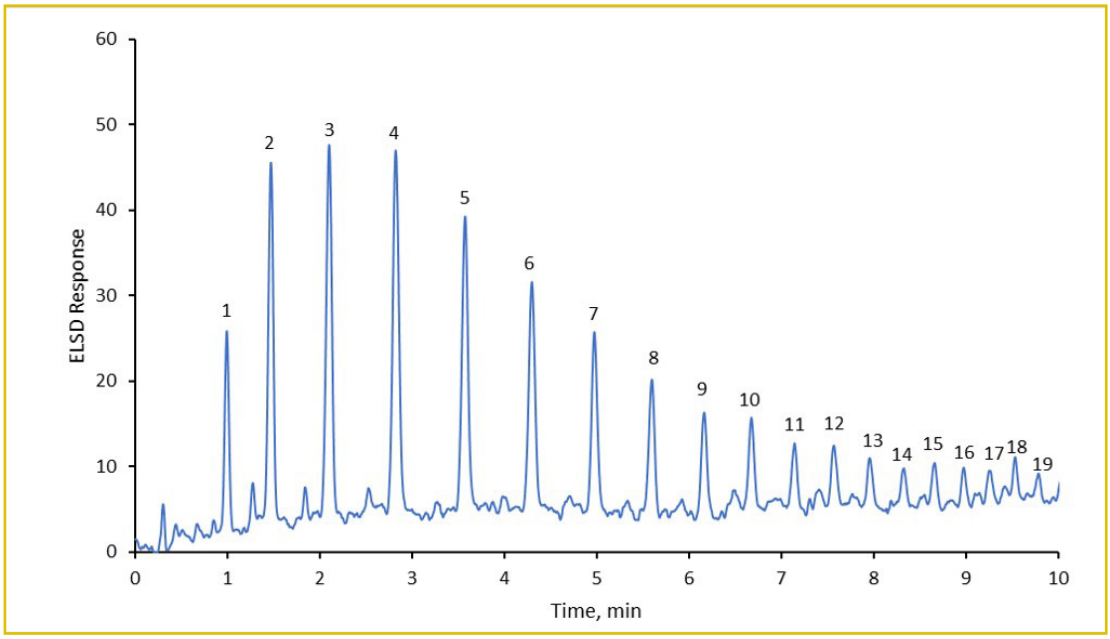
TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 µm, 2.1 x 150 mm
Part Number: 92812-705
Mobile Phase A: Water
Mobile Phase B: ACN
Gradient: 75-55% B in 25 min
Flow Rate: 0.5 mL/min
Pressure: 168 bar
Temperature: 65 °C
Detection: ELSD, 40 °C, 3.3 bar
Injection Volume: 20 µL
Sample Solvent: 70/30 ACN/Water
Data Rate: 10 Hz
Response Time: 0.10 sec
LC System: Shimadzu Nexera X2

High resolution of oligosaccharides is demonstrated using a dextran ladder on a HALO® Penta-HILIC column with the simple mobile phases of acetonitrile and water. The use of the evaporative light scattering detector (ELSD) eliminates the need to label the sugars with either a UV or fluorescent tag, reducing the time required for sample preparation. Peak identities are labeled by degree of polymerization (DP).

Fast Separation of Oligosaccharides using HALO 90 Å Penta-HILIC

273-SU



PEAK IDENTITIES:

1. DP 3
2. DP 4
3. DP 5
4. DP 6
5. DP 7
6. DP 8
7. DP 9
8. DP 10
9. DP 11
10. DP 12
11. DP 13
12. DP 14
13. DP 15
14. DP 16
15. DP 17
16. DP 18
17. DP 19
18. DP 20
19. DP 21

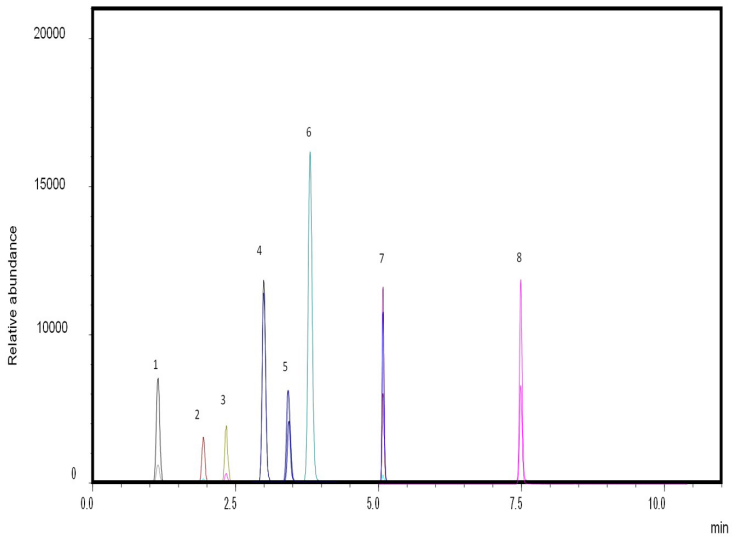
TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-405
Mobile Phase A: Water
Mobile Phase B: ACN
Gradient: 75-55% B in 10 min
Flow Rate: 2.0 mL/min
Pressure: 105 bar
Temperature: 65 °C
Detection: ELSD, 40 °C, 3.3 bar
Injection Volume: 20 µL
Sample Solvent: 70/30 ACN/Water
Data Rate: 10 Hz
Response Time: 0.10 sec
LC System: Shimadzu Nexera X2

The combination of evaporative light scattering detection (ELSD) and a short 50 mm HALO® Penta-HILIC column enables a fast analysis of oligosaccharides in under 10 minutes whereas traditional columns could have analysis times as long as 30 minutes to more than an hour. Using ELSD eliminates the need to label the sugar with either a UV or fluorescent tag, which simplifies the analysis. Peak identities are labeled by degree of polymerization (DP).

Steroids spiked in ground beef on HALO 90 Å C18

275-F



Peak id	Compound	Transition	RT (Min)
1	ALDOSTERONE	361.0000>343.1000	1.154
2	CORTICOSTERONE	347.6000>109.0000	1.965
3	ZERANOL	321.0000>277.0000	2.355
4	MGA	395.0000> 325.1000	3.100
5	TESTOSTERONE	289.0000>109.0000	3.366
6	17A-METHYLTESTOSTERONE	303.1000>97.0000	3.839
7	PROGESTERONE	315.0000>109.1000	5.085
8	ESTRADIOL 17ß	272.4000>159.1000	7.501

TEST CONDITIONS:

Analytical Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-602
Mobile Phase A: Water, 5 mM Ammonium Formate, 0.1 % Formic Acid pH 4.0
Mobile Phase B: Methanol
Flow Rate: 0.3 mL/min
Pressure: 190 bar
Temperature: 50 °C
Injection Volume: 2.0 µL
Sample Solvent: 45/55/ MEOH/H_2O
Detection: +ESI/ -ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040

Gradient:

Time	%B
2.0	14
3.0	60
3.5	60
8.0	100
10.0	100
10.5	0
12.5	stop

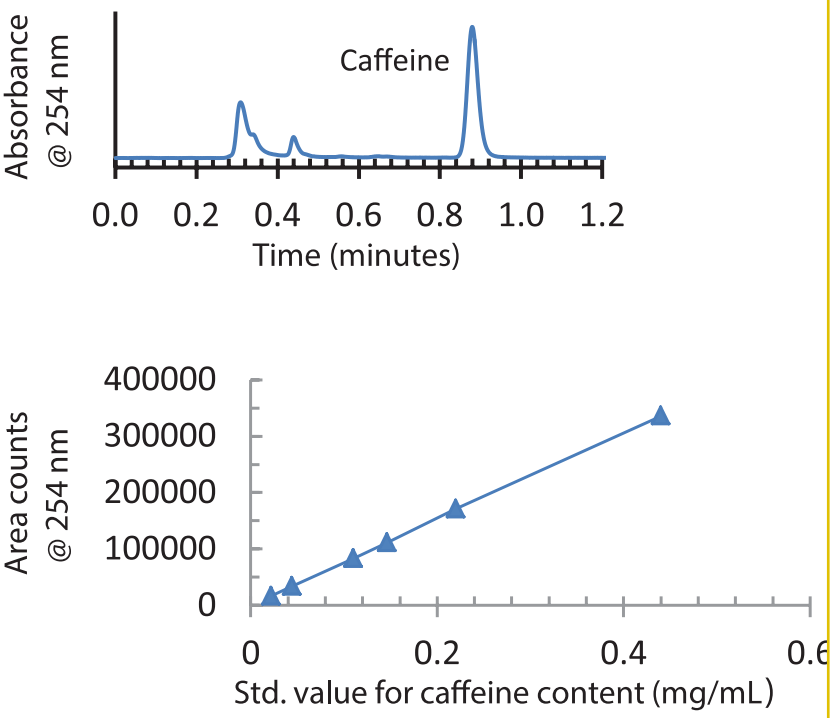
MS Source Conditions:

Spray Voltage: 3.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 ˚C
Heat Block: 400 ˚C

For over fifty years, the Food and Drug Administration (FDA) has approved the use of a number of steroids in beef cattle, including natural estrogen, progesterone, testosterone, and their synthetic versions such as trenbolone acetate (TBA). The function of these drugs is to increase growth rate and the efficiency by which the animals convert the feed they eat into muscle/meat. The drugs are usually administered as implants (dosing of 100-200 days), which are placed under the skin on the back side of the animal's ear. The implants dissolve slowly under the skin and are not removed. Although cooking the meat does have some effect on the stability of the steroids in beef, it does not eliminate the exposure, as many steroids are stable at elevated temperatures. A standard panel of steroids spiked into ground beef, and then run on the HALO 90 Å C18, shows a highly resolved separation of all compounds. The panel consisted of common growth promotors and those used for therapeutic purposes, and was chosen to represent the most common steroids that can be expected to be found in beef, through therapeutic or growth promotion utilization.

Determination of Caffeine in Soda Using HALO® C18, 5 µm

Application Note 145-F



Sample	Caffeine tested mg/(355 mL)	Can value mg/(355 mL)
Store brand cola 1	12	N/A
Cola 2	53	54
Cola 3	43	43
Cola 4	36	38
Cola 5	38	38
Store brand diet cola 1	12	N/A
Diet cola 2	45	46
Diet cola 3	34	34
Diet cola 4	36	35
Energy drink 1*	160	160
Energy drink 2**	79	80
Diet Energy drink**	79	80
Non-cola drink 1	53.3	54
Non-cola drink 2	22	22
Diet non-cola drink	43	41
Diet cola 1 non caffeinated	0	N/A
Diet cola 2 non-caffeinated	0	N/A
Diet cola 3 non-caffeinated	0	N/A

355 mL = 12 oz.
*amount in 16 oz. (473 mL) cans
**amount in 8.4 oz (248 mL) cans

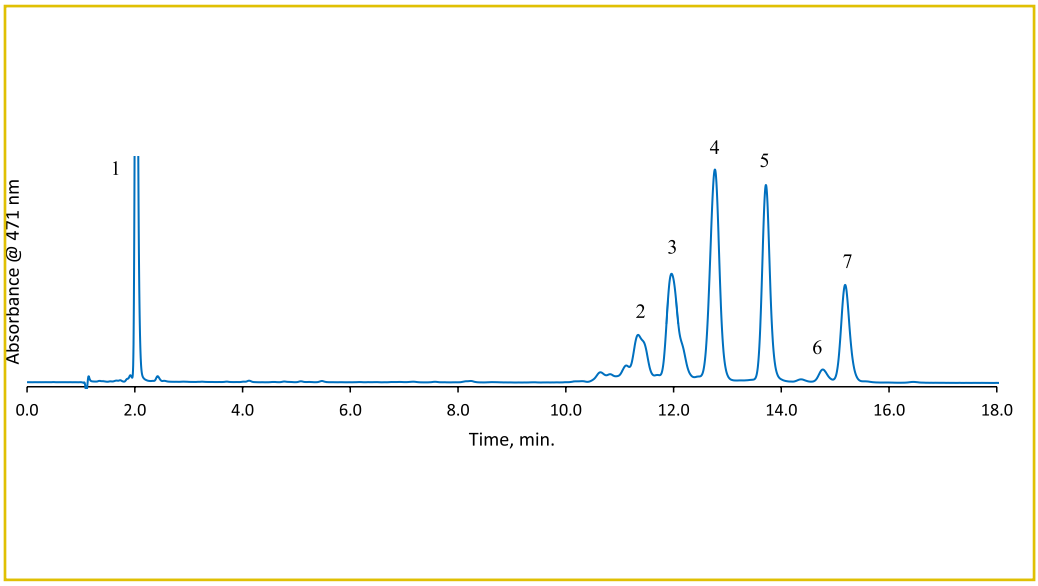
TEST CONDITIONS:

Column: HALO 90 Å C18, 5 µm, 3.0 x 50 mm, HALO 5 µm guard column
Part Numbers: 95813-402, 95813-102
Mobile Phase: 75/25 - A/B
A: 0.1% formic acid in water
B: Methanol
Flow Rate: 0.8 mL/min
Pressure: 120 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: (Caffeine std.) mobile phase
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

Caffeine is a stimulant found at various levels in coffee, colas, and energy drinks. HPLC is a convenient way to determine the amount of caffeine present. Here, sodas were analyzed by direct injection onto a 5 µm HALO® C18 column after decarbonation. A guard column should be used in this application.

Separation of Carotenoids on HALO® C30

Application Note 191-V



PEAK IDENTITIES:

1. Lutein
2. cis-carotenoid 1
3. cis-carotenoid 2
4. α-Carotene
5. β-Carotene
6. cis-Lycopene
7. Lycopene

Carotenoids can be split into two main classes called xanthophylls and carotenes. They are responsible for absorbing light for photosynthesis and protecting chlorophyll from photodamage. A separation done by Nature's Sunshine Products shows excellent resolution of carotenoids on a HALO® C30 column.

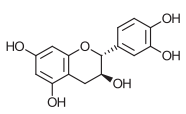
TEST CONDITIONS:

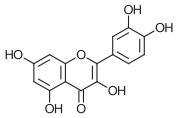
Column: HALO 160 Å C30, 2.7 µm, 3.0 x 150 mm
Part Number: 92113-730
Mobile Phase:
A: Methanol
B: Ethanol
Gradient:

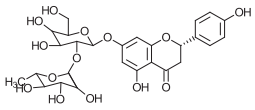
Time (min)	% B
0.0	0
20.0	40

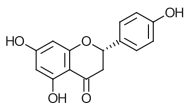
Flow Rate: 0.65 mL/min
Temperature: 38 °C
Detection: UV 471 nm, PDA
Injection Volume: 0.6 µL
Response Time: 2.0 sec
Data Rate: 2.5 Hz
Flow Cell: 13 µL
LC System: Agilent 1100
Data Courtesy of Nature's Sunshine Products

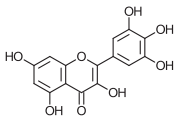
STRUCTURES:

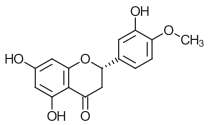

Catechin


Quercetin


Naringin

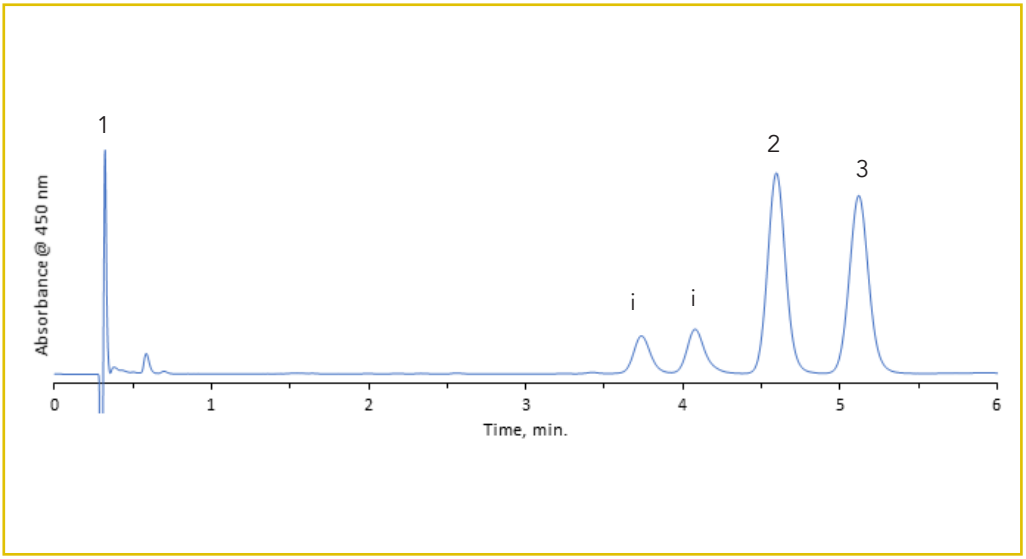

Naringenin


Myricetin


Hesperetin

Carotenoids Extracted from Carrot Juice Analyzed Using HALO® C30

Application Note 183-V



PEAK IDENTITIES:

1. Lutein
2. α-carotene
3. β-carotene

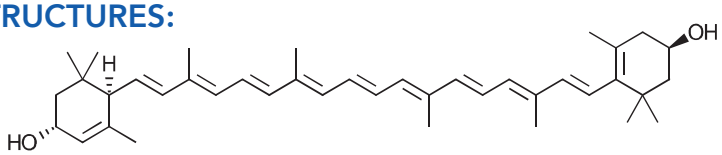
i = Unidentified isomers

The carotenoids lutein, α-carotene, and β-carotene were isolated from a commercially available carrot juice using liquid liquid extraction. Carotenes are responsible for the orange color in vegetables such as carrots and are considered antioxidants. The separation was performed on a HALO® C30 column with high resolution between the α- and β-carotene peaks.

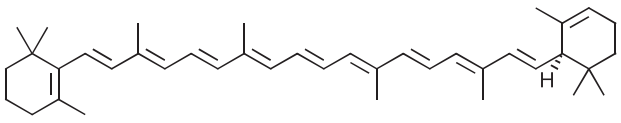
TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 2.1 x 50 mm
Part Number: 92112-430
Isocratic: 100% Methanol
Flow Rate: 0.4 mL/min
Pressure: 100 bar
Temperature: 30 °C
Detection: UV 450 nm, PDA
Injection Volume: 2.5 µL
Sample Solvent: Methanol/isopropyl alcohol
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

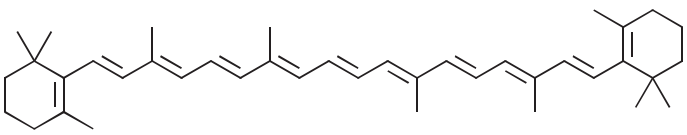
STRUCTURES:



Lutein



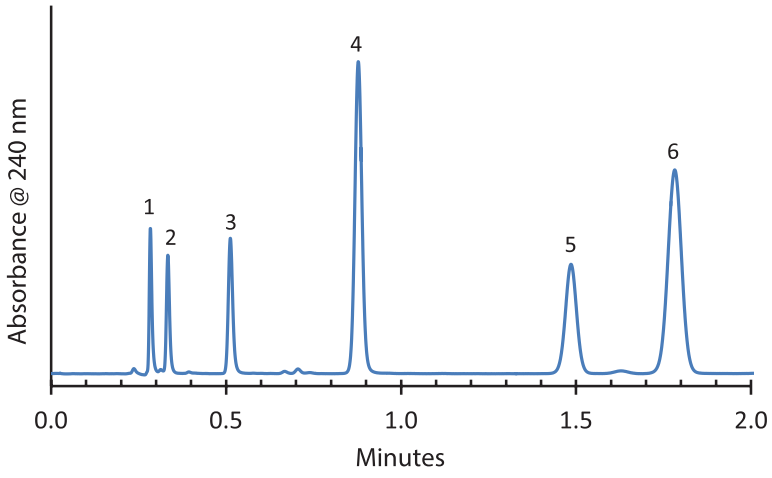
Alpha carotene



Beta carotene

Separation of Six Flavonoids on HALO® C18, 2.7 µm

Application Note 96-FL



PEAK IDENTITIES:

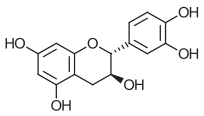
1. Catechin
2. Naringin
3. Myricetin
4. Quercetin
5. Naringenin
6. Hesperetin

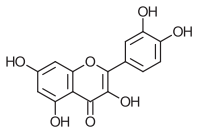
Flavonoids are naturally occurring polyphenols that are found in plant leaves, flowers and seeds. They have beneficial health effects and are often taken as dietary supplements. Analysis of this flavonoids mixture can be carried out in less than 2 minutes using a short HALO® Fused-Core® C18 column.

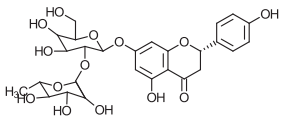
TEST CONDITIONS:

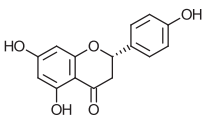
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 70/30 - A/B
A: 0.02 M phosphate buffer, pH 2.9, (adj.)
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 224 bar
Temperature: 30 °C
Detection: UV 240 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

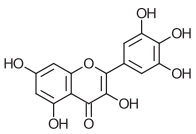
STRUCTURES:

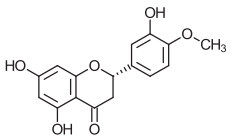

Catechin


Quercetin


Naringin

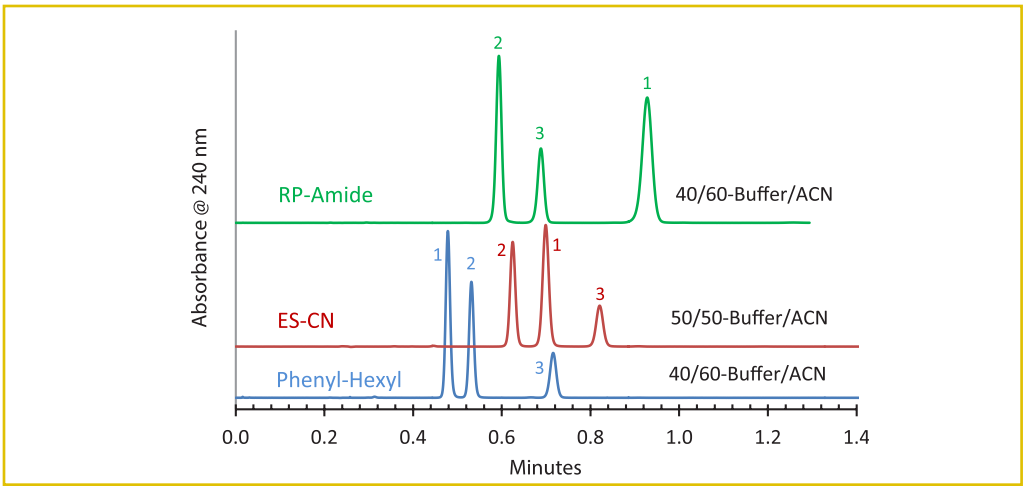

Naringenin


Myricetin


Hesperetin

Separation of Three Flavonoids on HALO® RP-Amide, ES-CN and Phenyl-Hexyl, 2.7 µm

Application Note 97-FL



PEAK IDENTITIES:

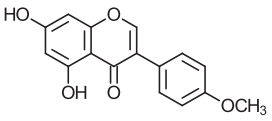
1. Biochanin A
2. Flavone
3. Flavanone

These separations illustrate different selectivities for three flavonoids on three HALO® Fused-Core® (2.7 µm) columns. These phase choices allow flexibility during method development and optimization. Note the short separation time and modest back pressure.

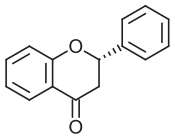
TEST CONDITIONS:

Columns:
1) HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
2) HALO 90 Å ES-CN, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-404
3) HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: A/B - See chart
A: 0.02 M Potassium phosphate buffer, pH 2.9
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: ~170 bar
Temperature: 30 °C
Detection: UV 240 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 water/acetonitrile
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

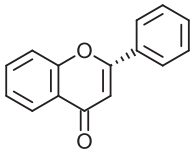
STRUCTURES:



Biochanin A



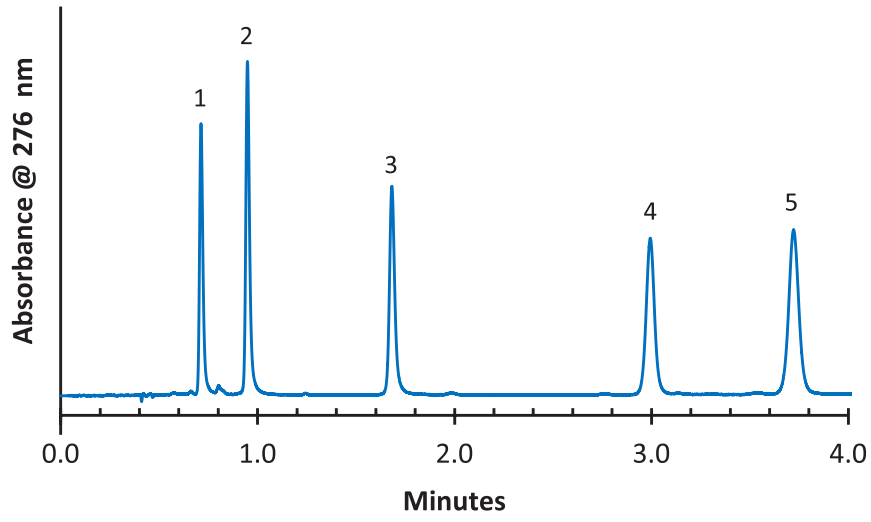
Flavanone



Flavone

Separation of Five Flavonoids on HALO® C8, 2.0 µm

Application Note 127-FL



PEAK IDENTITIES:

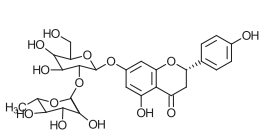
1. Naringin
2. Myricetin
3. Quercetin
4. Naringenin
5. Hesperetin

Flavonoids are colored compounds found in many plants and may have beneficial effects for anti-inflammatory and cardiovascular health. Five of these compounds are shown separated on a 2.0 µm HALO® C8 column in under four minutes.

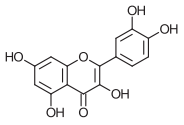
TEST CONDITIONS:

Column: HALO 90 Å C8, 2.0 µm, 2.1 x 100 mm
Part Number: 91812-608
Mobile Phase: 75/25 - A/B
A: 0.025 M ammonium formate, pH 3.0
B: Acetonitrile
Flow Rate: 0.5 mL/min
Pressure: 473 bar
Temperature: 40 °C
Detection: UV 276 nm, PDA
Injection Volume: 0.1 µL
Sample Solvent: Methanol
Response Time: 0.025 sec
Data Rate: 100 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera
Extra Column Volume: ~7 µL

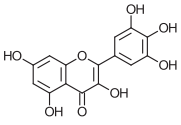
STRUCTURES:



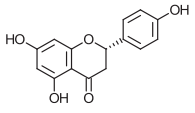
Naringin



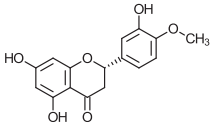
Quercetin



Myricetin



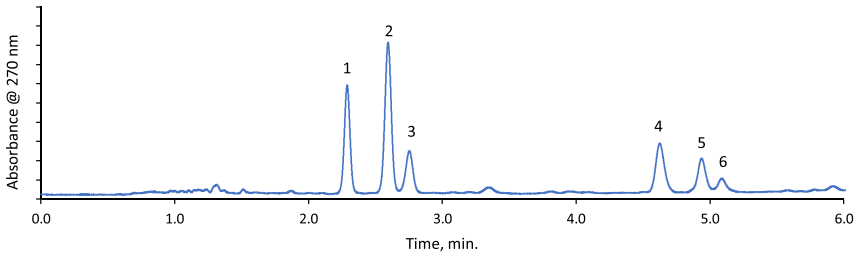
Naringenin



Hesperetin

Separation of Hop Acids on HALO® 5 µm Biphenyl

Application Note 193-OA



PEAK IDENTITIES:

Alpha Acids	Beta Acids
1. Cohumulone	4. Colupulone
2. Humulone	5. Lupulone
3. Adhumulone	6. Adlupulone

Hops are primarily made up of essential oils and alpha and beta acids. They have many benefits in the beer brewing process, including their antiseptic nature and bitterness flavor they give to the beer. Alpha and beta acids from the International Calibration Standard Extract (ICE-3) are separated on a HALO® Biphenyl column.

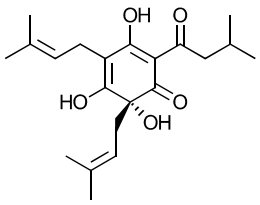
TEST CONDITIONS:

Column: HALO 90 Å Biphenyl, 5 µm, 4.6 x 150 mm
Part Number: 95812-611
Mobile Phase:
A: Water, 0.1% formic acid
B: Acetonitrile, 0.1% formic acid
Gradient:

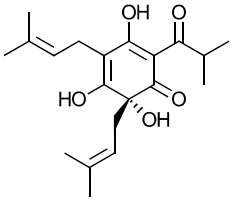
Time (min)	% B
0.0	60
3.0	60
6.0	80

Flow Rate: 2.0 mL/min
Initial Pressure: 236 bar
Temperature: 30 °C
Detection: 270 nm, PDA
Injection Volume: 5.0 µL
Sample Solvent: Acetonitrile
Response Time: 0.025 sec
Data Rate: 100 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

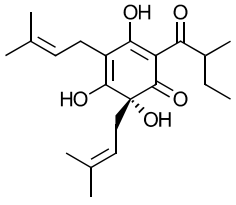
STRUCTURES:



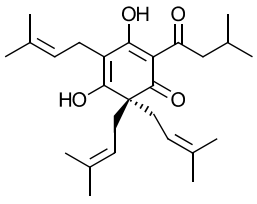
Cohumulone



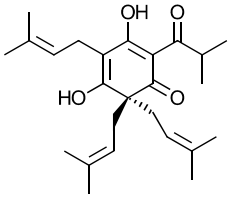
Humulone



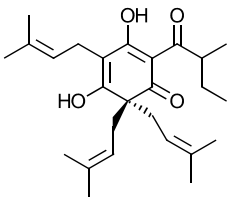
Adhumulone



Colupulone



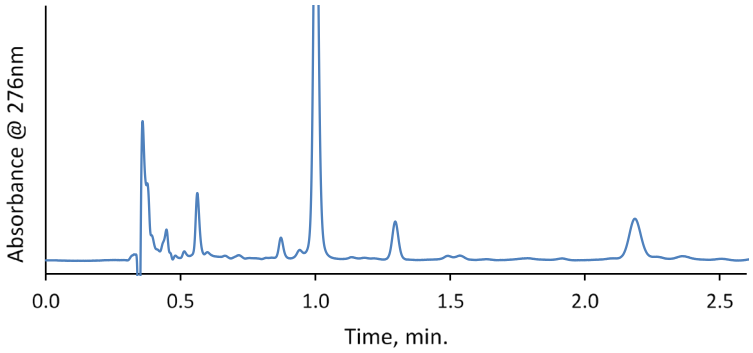
Lupulone



Adlupulone

Separation of Patulin and HMF on HALO 90 Å Biphenyl

Application Note 175-M



PEAK IDENTITIES:

1. 5-(Hydroxymethyl) furfural
2. Patulin

In the United States, the FDA maintains different limits for mycotoxins in many foods and beverages. Patulin, a mycotoxin that is produced from mold on a variety of fruits has a limit of 50 µg/kg. For analysis, patulin was spiked into apple juice and the sample was cleaned up using solid phase extraction. Interfering analytes such as 5-(Hydroxymethyl) furfural (HMF) can make analysis more challenging. This separation shows the two compounds separated on a HALO® Biphenyl column with enough resolution to easily check for sample recovery.

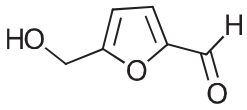
TEST CONDITIONS:

Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-611
Mobile Phase:
A: Water with 0.1% acetic acid
B: Acetonitrile with 0.1% acetic acid
Gradient:

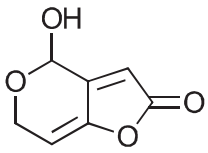
Time (min)	%B
0.0	5
2.6	90

Flow Rate: 0.6 mL/min
Initial Pressure: 285 bar
Temperature: 40 °C
Detection: UV 276 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: Apple juice spiked with HMF and 50 ng/mL Patulin
Response Time: 0.025 sec
Data Rate: 100 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

STRUCTURES:



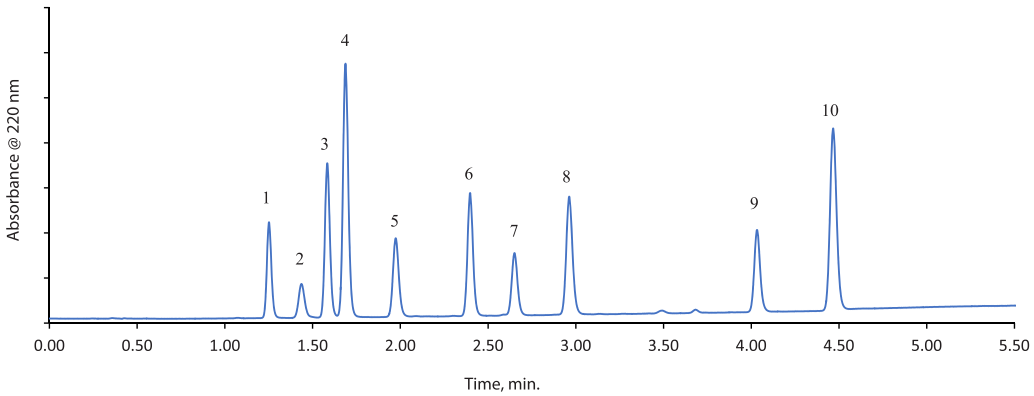
5-(Hydroxymethyl) furfural



Patulin

Separation of Phenolic Acids on HALO 90 Å RP-Amide, 2.7 µm

Application Note 188-P



PEAK IDENTITIES:

1. Homovanillic acid
2. Caffeic acid
3. Syringic acid
4. Vanillic acid
5. Chlorogenic acid
6. Sinapic acid
7. Ferulic acid
8. p-Coumaric acid
9. trans-Cinnamic acid
10. Resveratrol

Phenolic acids can be found in many plant-based foods and beverages. Fruits, vegetables, and even olive oils all contain different varieties of these acids. For example, sinapic acid can be found in wine and caffeic acid can be found in coffee, cabbage, and apples. These compounds have antioxidant, anti-inflammatory, and antimicrobial properties so they can be effective against skin disorders. They also affect the flavors of the food or oil. A separation of ten phenolic acids is completed on a HALO 90 Å RP-Amide, 2.7 µm column with excellent speed and resolution.

TEST CONDITIONS:

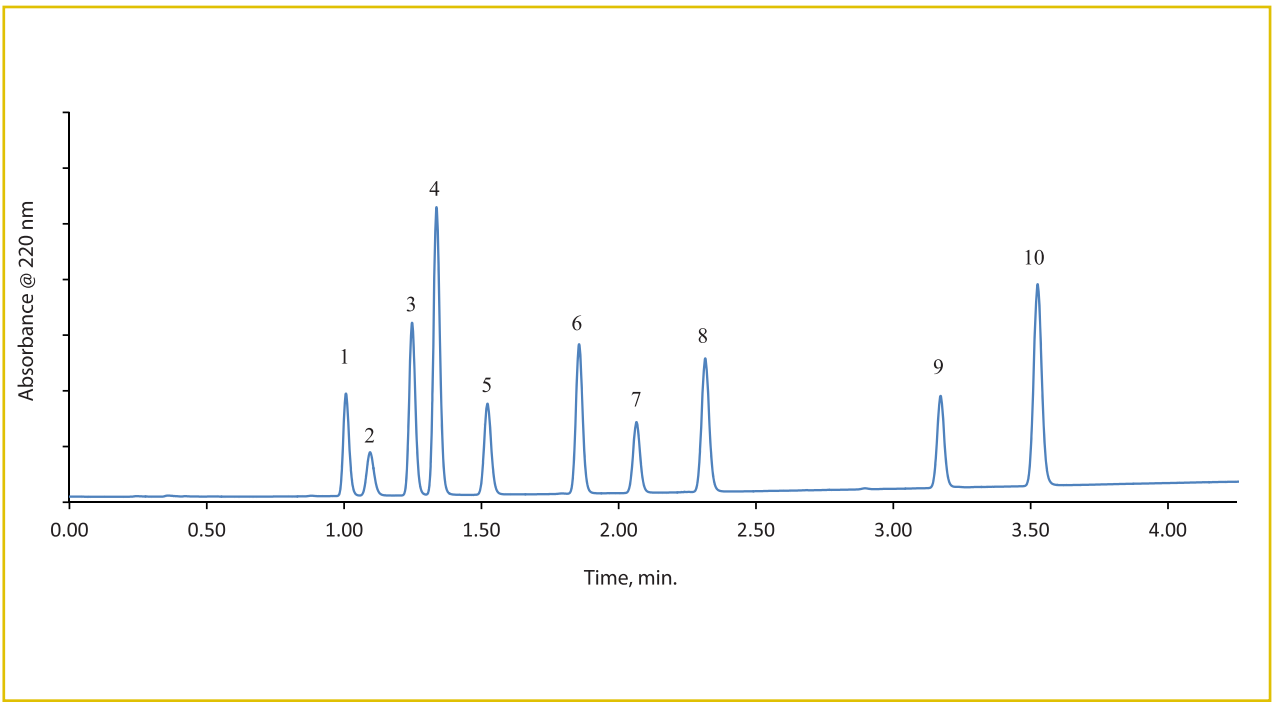
Column: HALO 90 Å RP-Amide, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-607
Mobile Phase:
A: 20mM phosphoric acid
B: Methanol
Gradient:

Time (min)	% B
0.00	25
5.00	60
5.50	60

Flow Rate: 0.5 mL/min
Initial Pressure: 345 bar
Temperature: 35 °C
Detection: UV 220 nm, PDA
Injection Volume: 0.7 µL
Sample Solvent: Methanol
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

Separation of Phenolic Acids on HALO® 90 Å RP-Amide, 2.0 µm

Application Note 190-P



PEAK IDENTITIES:

1. Homovanillic acid
2. Caffeic acid
3. Syringic acid
4. Vanillic acid
5. Chlorogenic acid
6. Sinapic acid
7. Ferulic acid
8. p-Coumaric acid
9. Trans-cinnamic acid
10. Resveratrol

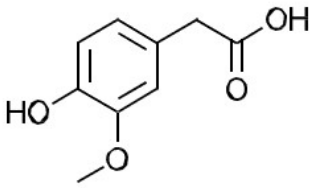
TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 2.0 µm, 2.1 x 100 mm
Part Number: 91812-607
Mobile Phase:
A: 20mM phosphoric acid
B: Methanol
Gradient:

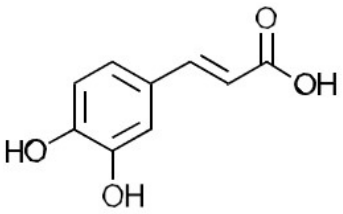
Time (min)	% B
0.00	30
3.75	60
4.25	60

Flow Rate: 0.5 mL/min
Initial Pressure: 716 bar
Temperature: 35 °C
Detection: UV 220 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: Methanol
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

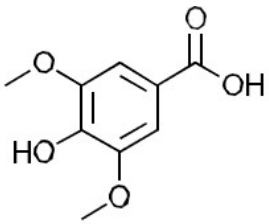
STRUCTURES:



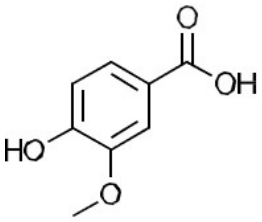
Homovanillic acid



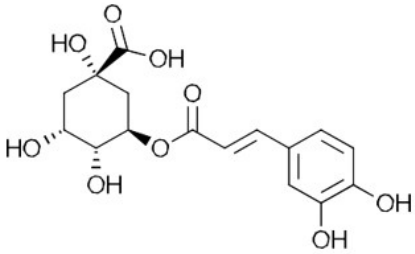
Caffeic acid



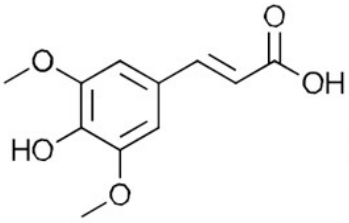
Syringic acid



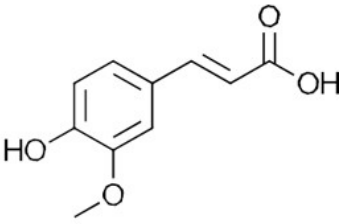
Vanillic acid



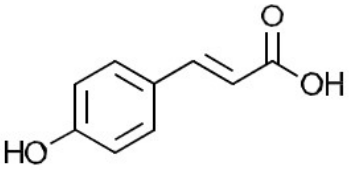
Chlorogenic acid



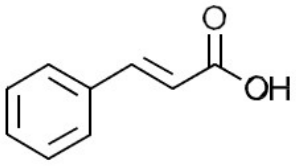
Sinapic acid



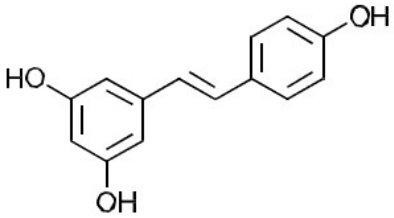
Ferulic acid



p- Coumaric acid



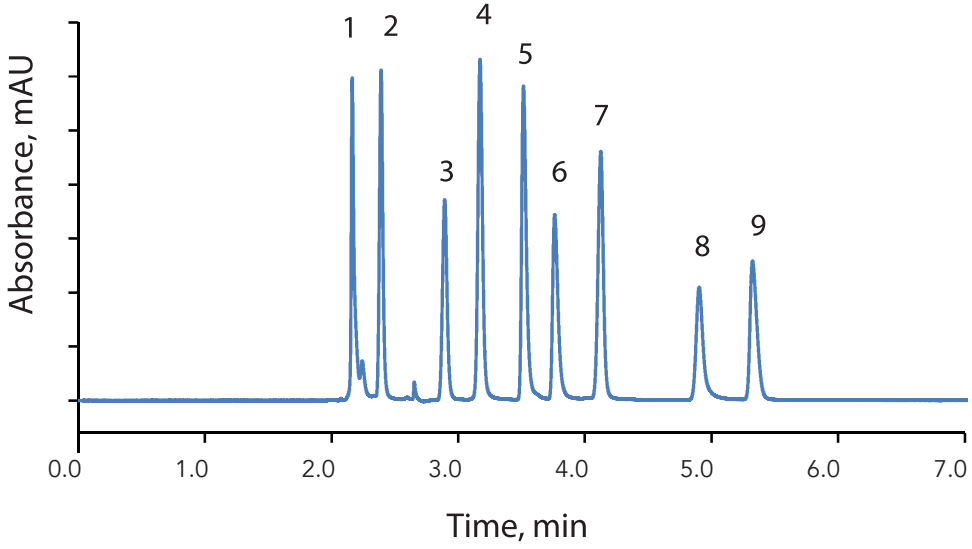
trans- Cinnamic acid



Resveratrol

Separation of Polar Organic Acids on HALO® AQ-C18

Application Note 160-OA



PEAK IDENTITIES:

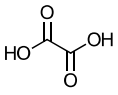
1. Oxalic acid
2. Tartaric acid
3. Malic acid
4. Ascorbic acid
5. L-Lactic acid
6. Acetic acid
7. Citric acid
8. Succinic acid
9. Fumaric acid

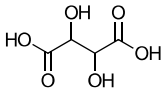
Organic acids are common in the food and beverage industry and can be found in many sample types such as fruits, vegetables, and wines. This separation of nine polar organic acids is performed on a HALO® AQ-C18 column using 100% aqueous mobile phase at low pH. The 250 mm column length was chosen to provide excellent resolution with reasonable run time for this polar mixture.

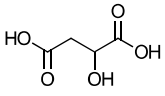
TEST CONDITIONS:

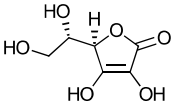
Column: HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 250 mm
Part Number: 92814-922
Isocratic: 20 mM potassium phosphate buffer, pH 2.7
Flow Rate: 1.0 mL/min
Pressure: 307 bar
Temperature: 40 °C
Detection: UV 214 nm, PDA
Injection Volume: 20 µL
Sample Solvent: Mobile phase
Response Time: 0.025 sec
Data Rate: 100 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

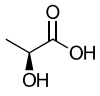
STRUCTURES:


Oxalic acid

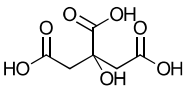

Tartaric acid

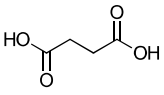

Malic acid

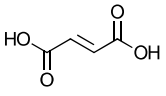

Ascorbic acid


L-Lactic acid


Acetic acid

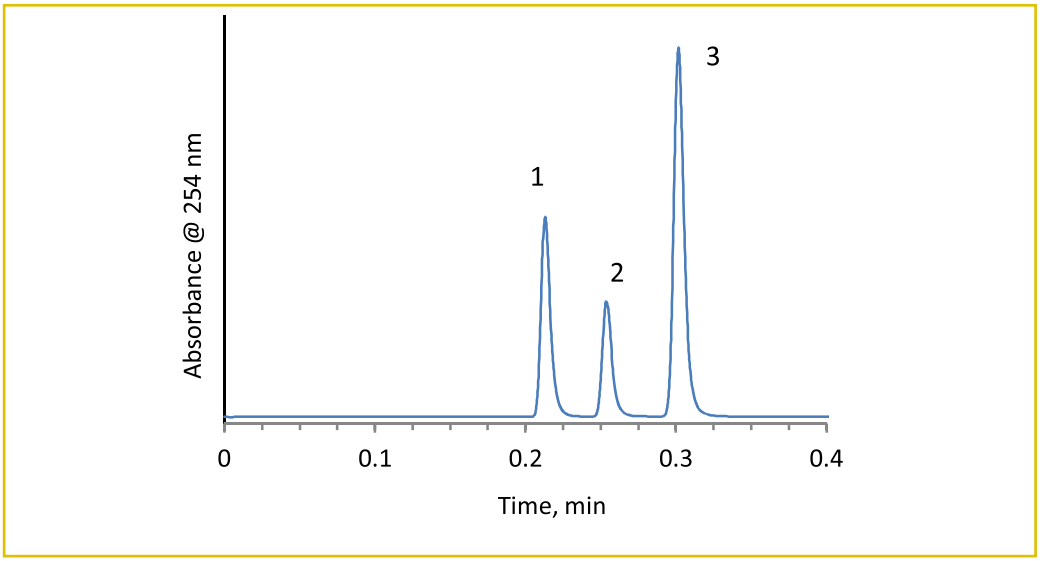

Citric acid


Succinic acid


Fumaric acid

Separation of Vanillins on HALO® C18

Application Note 18-P



PEAK IDENTITIES:

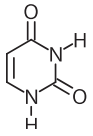
1. Uracil
2. Vanillin
3. o-Vanillin

Vanilla is a popular flavor in many kinds of food including ice cream, baked goods, and others. The vanillins are components of vanilla extract from vanilla beans and synthetic vanilla flavoring. This separation shows the baseline resolution of two of the main flavor components.

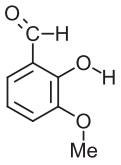
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 35/65 - A/B
A: Water
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 166 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

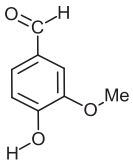
STRUCTURES:



Uracil



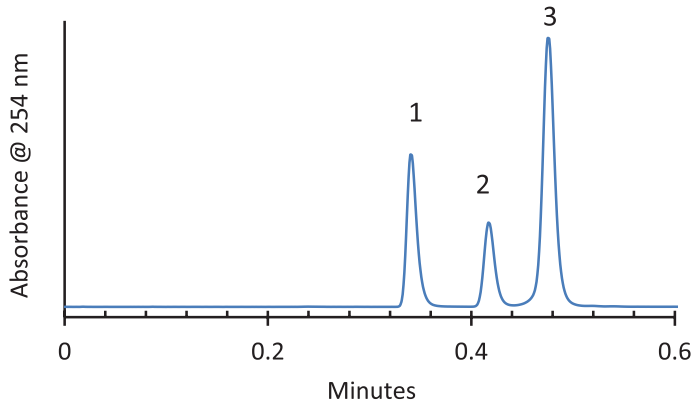
o-Vanillin



Vanillin

Separation of Vanillins on HALO® Phenyl-Hexyl Phase

Application Note 19-P



PEAK IDENTITIES:

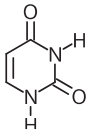
1. Uracil
2. Vanillin
3. o-Vanillin

Vanillins are flavor components found in the extract from vanilla beans or in synethic vanilla flavoring. Vanilla is a very popular flavor for ice cream and in the baking trade. HALO® Phenyl-Hexyl phase easily separates these two flavoring agents.

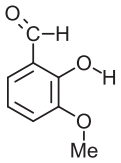
TEST CONDITIONS:

Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 25/75 - A/B
A: Water
B: Methanol
Flow Rate: 1.5 mL/min
Pressure: 196 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

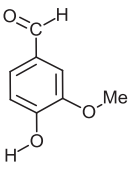
STRUCTURES:



Uracil



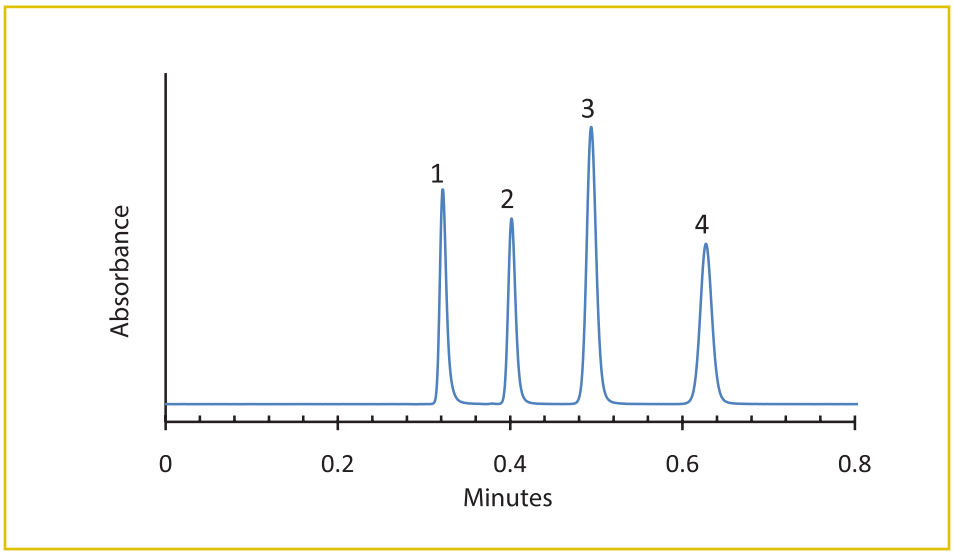
o-Vanillin



Vanillin

Separation of Xanthines on HALO® RP-Amide Phase

Application Note 48-XA



PEAK IDENTITIES:

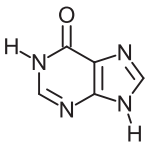
1. Hypoxanthine
2. Theobromine
3. Theophylline
4. Caffeine

Xanthines are stimulants that can be found in coffee, chocolate, and other foods and are often used in medications. These materials can be rapidly analyzed on a HALO® RP-Amide column in less one minute.

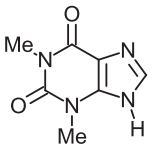
TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 85/15 - A/B
A: 0.03 M phosphate buffer, pH 3.0, in water
B: Acetonitrile
Flow Rate: 1.5 mL/min
Pressure: 150 bar
Temperature: 35 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 30% methanol in water
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

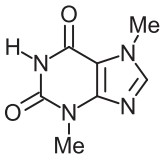
STRUCTURES:



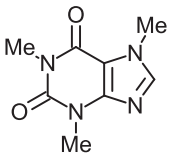
Hypoxanthine



Theophylline



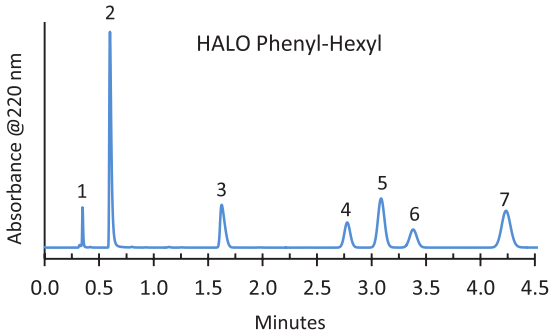
Theobromine



Caffeine

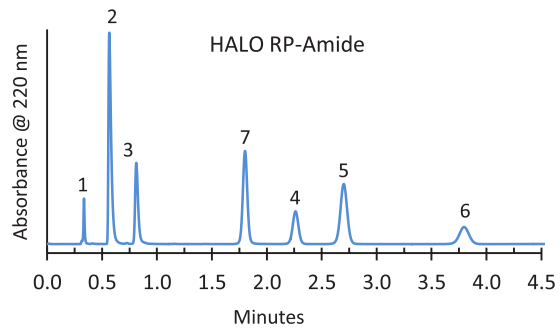
Separation of Food Additives on HALO® Phenyl-Hexyl and RP-Amide Phases

Application Note 95-P



PEAK IDENTITIES:

1. Ascorbic acid
2. Saccharin
3. Aspartame
4. Sorbic acid
5. Benzoic acid
6. Methyl paraben
7. Dehydroacetic acid

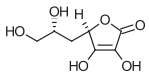


These compounds are often added to foods to sweeten or preserve them. They can be rapidly analyzed using HALO® Phenyl-Hexyl or RP-Amide phases. Note the difference in retention and selectivity of the two phases when run under the same conditions. This allows for flexibility in method development and optimization of the separation.

TEST CONDITIONS:

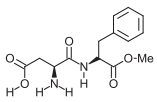
Columns:
1) HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
2) HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 70/30 - A/B
A: 0.025 M phosphate buffer, pH 2.5
B: Methanol
Flow Rate: 1.5 mL/min
Pressure: ~220 bar
Temperature: 40 °C
Detection: UV 220 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: 50/50 water/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

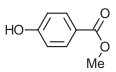
STRUCTURES:

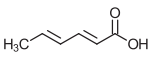

Ascorbic acid

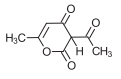

Saccharin


Benzoic acid


Aspartame

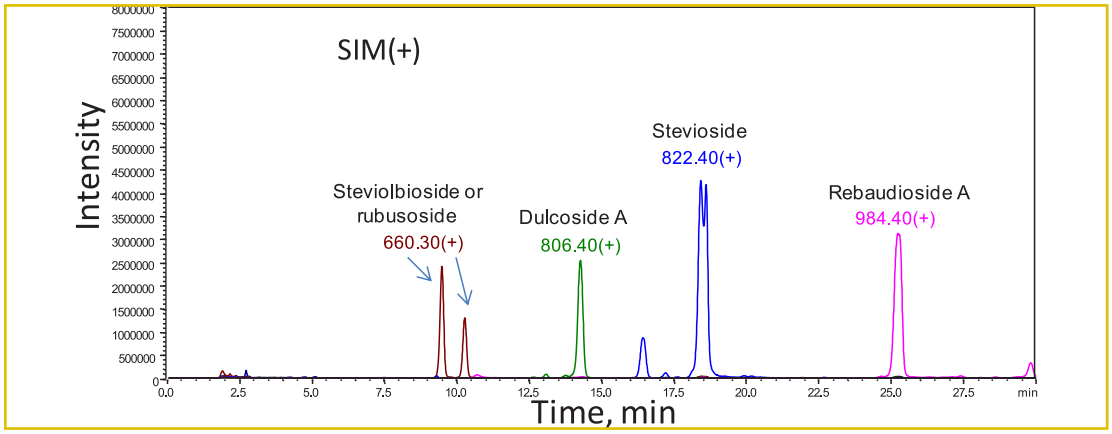

Methyl paraben


Sorbic acid


Dehydroacetic acid

LC-MS Analysis of Stevia Extract on HALO® Penta-HILIC, 5 µm

Application Note 124-F



Stevia is a natural sweetener and is used as a substitute for sugar. LC/MS analysis of Stevia glycosides from a Stevia extract is easily accomplished using a HALO® Penta-HILIC, 5 µm column due to its unique bonded phase containing five OH groups and the high efficiency of the 5-micron Fused-Core® particles.

TEST CONDITIONS:

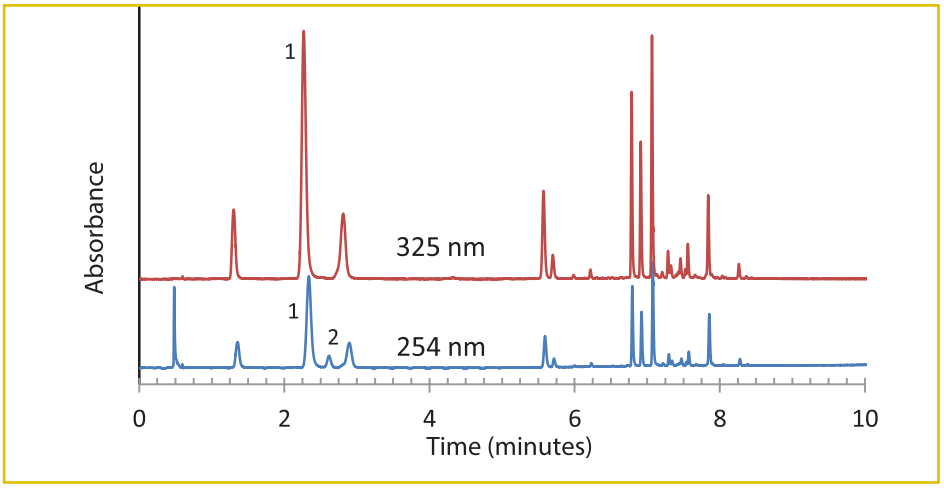
Column: HALO 90 Å Penta-HILIC, 5 µm, 3.0 x 250 mm
Part Number: 95813-905
Mobile Phase:
A: 50/50 water/acetonitrile with 5 mM ammonium formate, pH 3.0
B: 5/95 water/acetonitrile with 5 mM ammonium formate, pH 3.0
Gradient: 90% B to 67% B in 30 min
Flow Rate: 0.5 mL/min
Pressure: 60 bar
Temperature: Ambient
Injection Volume: 5.0 µL
Sample Solvent: 80/20 acetonitrile/water
LC System: Shimadzu Nexera
MS: Shimadzu LCMS 2020 (single quadrupole)
ESI: +4.5 kV
Scan Range: 200-1200 m/z
Scan Rate: 2 pps
Capillary: 250 °C
Heat Block: 350 °C
Nebulizing Gas Flow: 1.5 L/min
Drying Gas Flow: 15 L/min

EXTRACTION PROCEDURE:

1. Weigh 400 mg of Stevia rebaudiana leaves (Sigma S5381)
2. Crush leaves with mortar and pestle and transfer to vial
3. Add 8.0 mL of 50/50 (v/v) acetonitrile/water
4. Sonicate vial contents for 15 minutes
5. Filter sample using 25 mm syringe filter having 0.2 µm PTFE membrane (VWR 28145-495)
6. Centrifuge @ 10K rpm (5 min) and collect supernate
7. Dilute 400 µL of extract in 600 µL of acetonitrile for overall concentration of 80/20 acetonitrile/water
8. Centrifuge diluted sample @ 10K (5 min.) rpm and inject the supernate

HPLC Analysis of Chlorogenic Acid in Green Coffee Extract on HALO® C18, 2.7 µm

Application Note 134-F



PEAK IDENTITIES:

1. Chlorogenic acid
2. Caffeine

Green coffee extract is a dietary supplement to aid in weight loss. Chlorogenic acid is its active ingredient. Here, a commercial dry extract was extracted with a solvent and analyzed on a HALO® C18, 2.7 µm column.

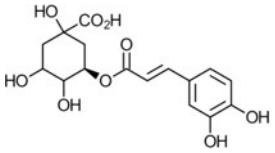
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 3.0 x 100 mm
Part Number: 92813-602
Mobile Phase: A/B
A: Water with 0.1% formic acid
B: Acetonitrile with 0.1% formic acid

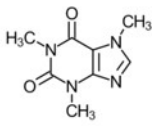
Gradient: Time (min)	**% B**
0.0	10
4.0	10
9.0	50
11.0	100
13.0	100

Flow Rate: 0.75 mL/min
Initial Pressure: 250 bar
Temperature: 30 °C
Detection: UV 254, 325 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 water/acetonitrile
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

STRUCTURES:



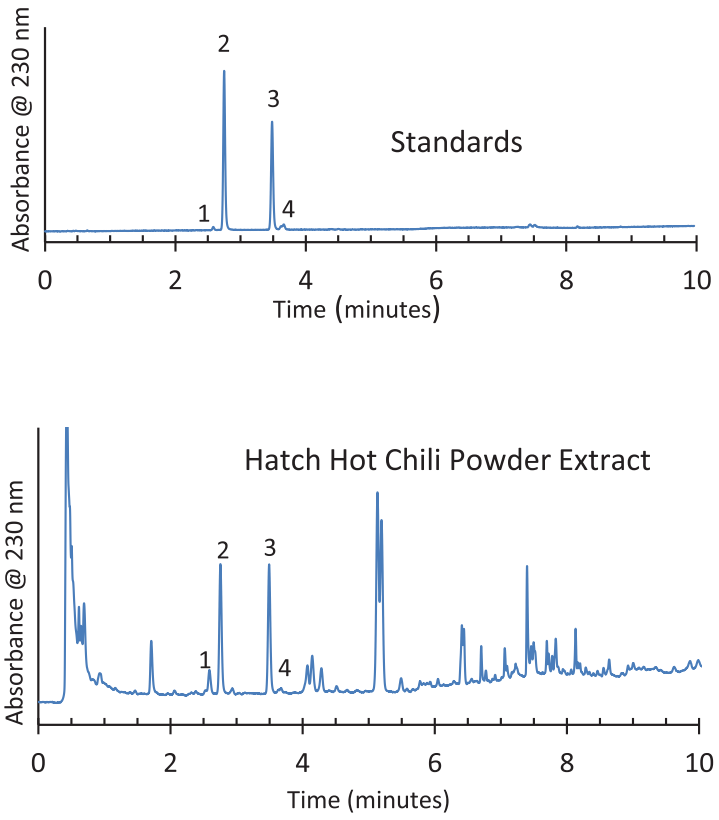
Chlorogenic Acid



Caffeine

Separation of Capsaicins in Chili Powder on HALO® C18, 2.7 µm

Application Note 209



PEAK IDENTITIES:

1. Capsaicin 1
2. Capsaicin 2
3. Dihydrocapsaicin 1
4. Dihydrocapsaicin 2

Capsaicin and dihydrocapsaicin are two of the main components of chili powder that give it the "heat" when making a batch of "chili". The amount of heat is often measured by a subjective test and then rated in terms of Scoville units that are a dilution factor beyond which the capsaicins and other hot compounds cannot be detected. One can also use HPLC to measure these compounds more objectively. Here these two ingredients are separated from an acetonitrile extract using a HALO® C18 column.

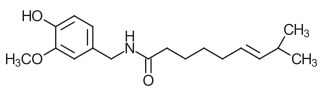
TEST CONDITIONS:

Column: HALO 90 Å, C18, 2.7 µm, 3.0 x 100 mm
Part Number: 92813-602
Mobile Phase: A/B
A= water
B= acetonitrile Gradient:

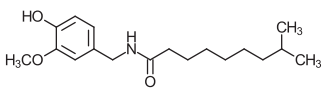
Time (min)	% B
0.0	40
5.0	60
7.0	100
20.0	100

Flow Rate: 0.8 mL/min.
Pressure: 223 bar starting pressure
Temperature: 40 °C
Injection Volume: 1.0 µL
Sample Solvent: acetonitrile Detection: UV 230 nm, VWD
Response Time: 0.02 sec. Data rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR ECV: ~14 µL

STRUCTURES:



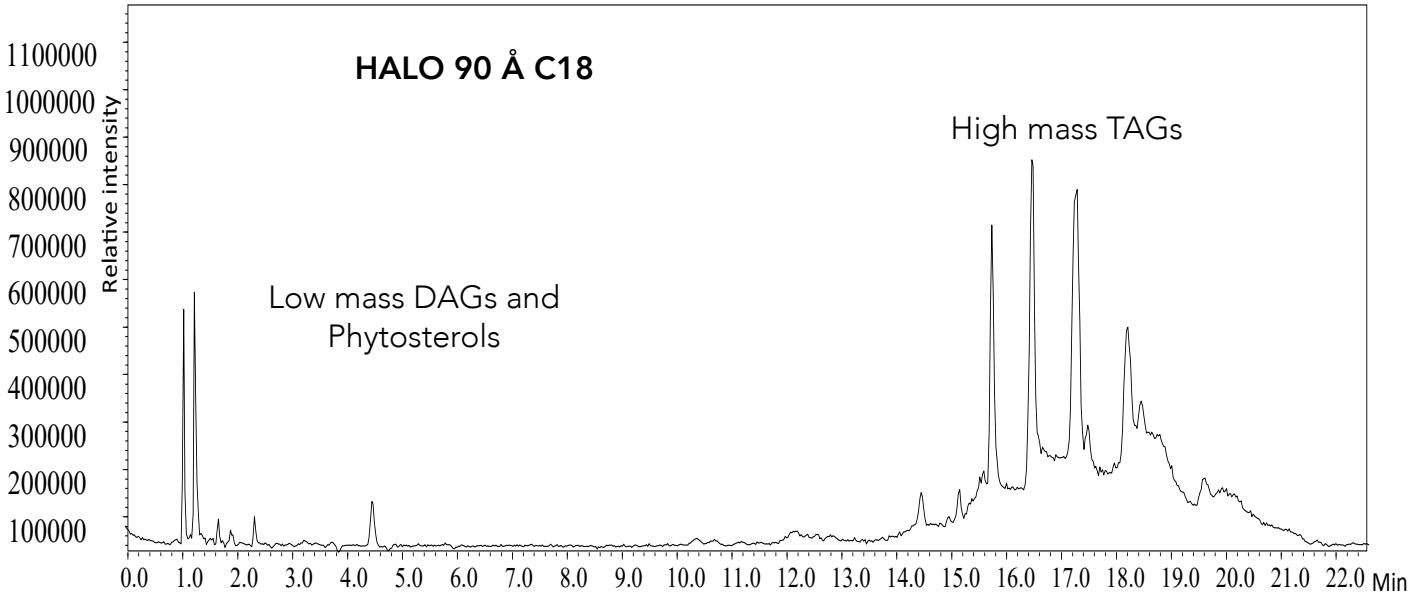
Capsaicin

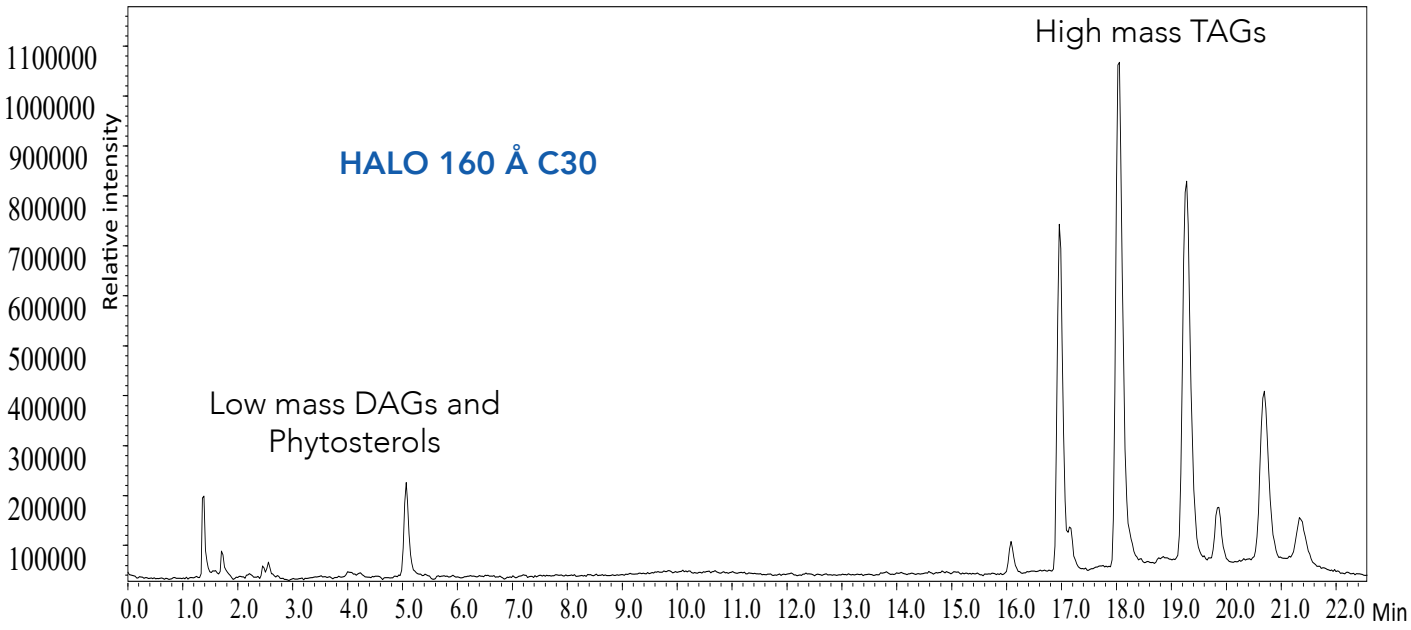


Dihydrocapsaicin

LC-MS Separation of Corn Oil on HALO® C30 Compared to HALO® C18

Application Note: 208-LI





DAGs = diacylglycerols or diglycerides
TAGs = triacylglycerols or triglycerides

TEST CONDITIONS:

Columns: HALO 90 Å C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92812-702
Columns: HALO 160 Å C30, 2.7 µm, 2.1 x 150 mm
Part Number: 92112-730
Mobile Phase A: Methanol
Mobile Phase B: IPA/0.1% Formic acid

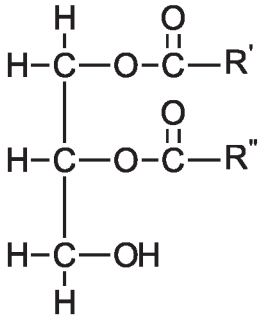
Gradient:	**Time**	**% B**
	0.00	10
	10.00	10
	14.00	40
	22.00	40
	22.01	10
	24.00	END

Flow Rate: 0.3 mL/min
Initial Pressure: 325 bar
Temperature: Ambient
Injection Volume: 2 µL
Sample Solvent: MeOH
LC System: Shimadzu Nexera X2

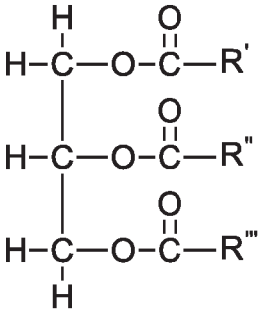
MS TEST CONDITIONS:

MS system: Shimadzu LCMS-2020
Ionization: +ESI
Spray voltage: 4.50 kV
Drying line temp: 300 °C
Heat Block: 450 °C

STRUCTURES:



DAGs

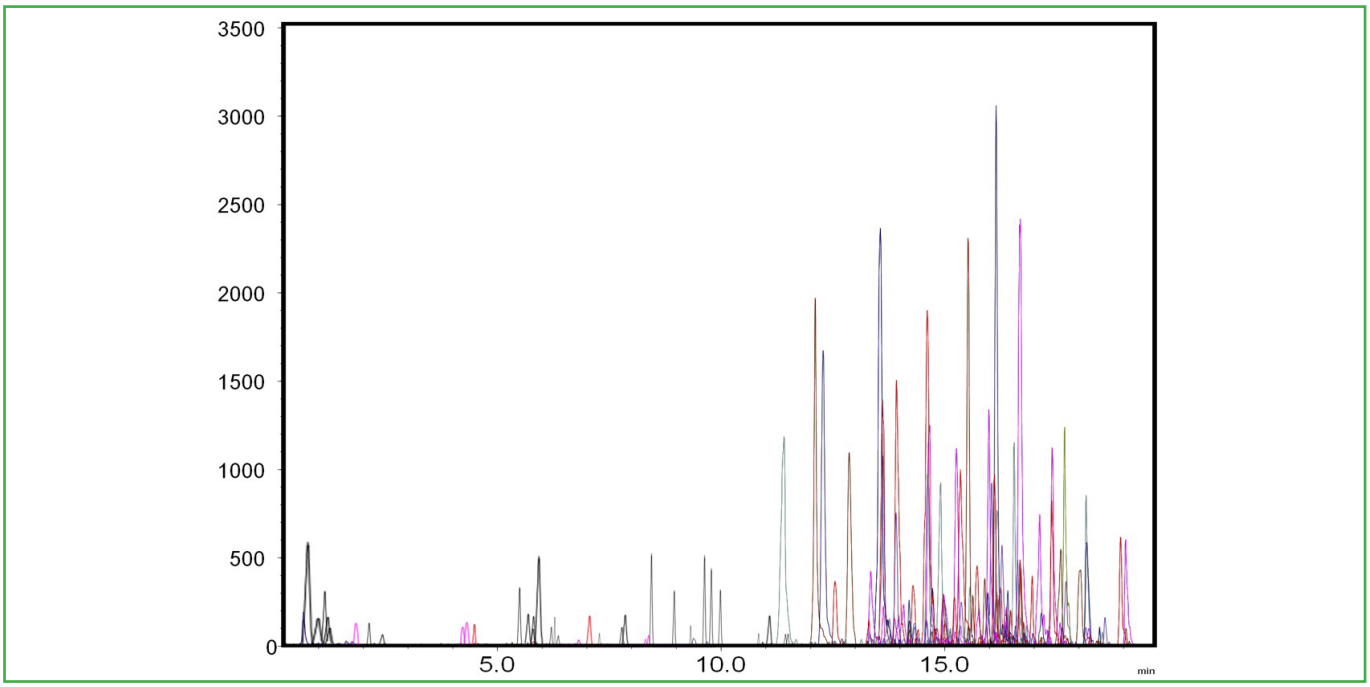


TAGs

Corn oil, composed mainly of long chain fatty acids and esters, is an edible oil which comprises approximately 5-10% of edible oil consumption. In recent years, corn oil has been used in biodiesel, pharmaceutical, and cosmetic applications as well. The use of a C18 column for the analysis of edible oils is difficult due to the high concentration of hydrophobic triglycerides (TAGs); therefore, the C30 phase has seen increased application in this area. Here we show a comparison between the C18 and C30 phase, and demonstrate that the 2.7 µm HALO® C30 is an ideal choice for the separation and resolution of high mass triglycerides found in edible oils such as corn oil. C30 offers superior specificity compared to C18 columns by exhibiting higher shape selectivity, enabling better separation of hydrophobic, long-chain, structures.

LC-MS Analysis of Pesticides and Environmental Contaminants Spiked in Eggs using HALO® Biphenyl

282-P



TEST CONDITIONS:

Analytical Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-611
Mobile Phase A: Water, 5 mM Ammonium Formate, 0.1 % Formic Acid
Mobile Phase B: ACN, 0.1 % Formic Acid
Flow Rate: 0.3 mL/min
Pressure: 144 bar
Temperature: 30 °C
Injection Volume: 1.0 µL
Sample Solvent: 50/50/ ACN/H2O
Detection: +ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040

Gradient:

Time	%B
0.0	5
20.0	100
22.0	100
22.10	5
25.0	End

MS Source Conditions:
ESI +
Spray Voltage: 2.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 ˚C
Heat Block: 400 ˚C

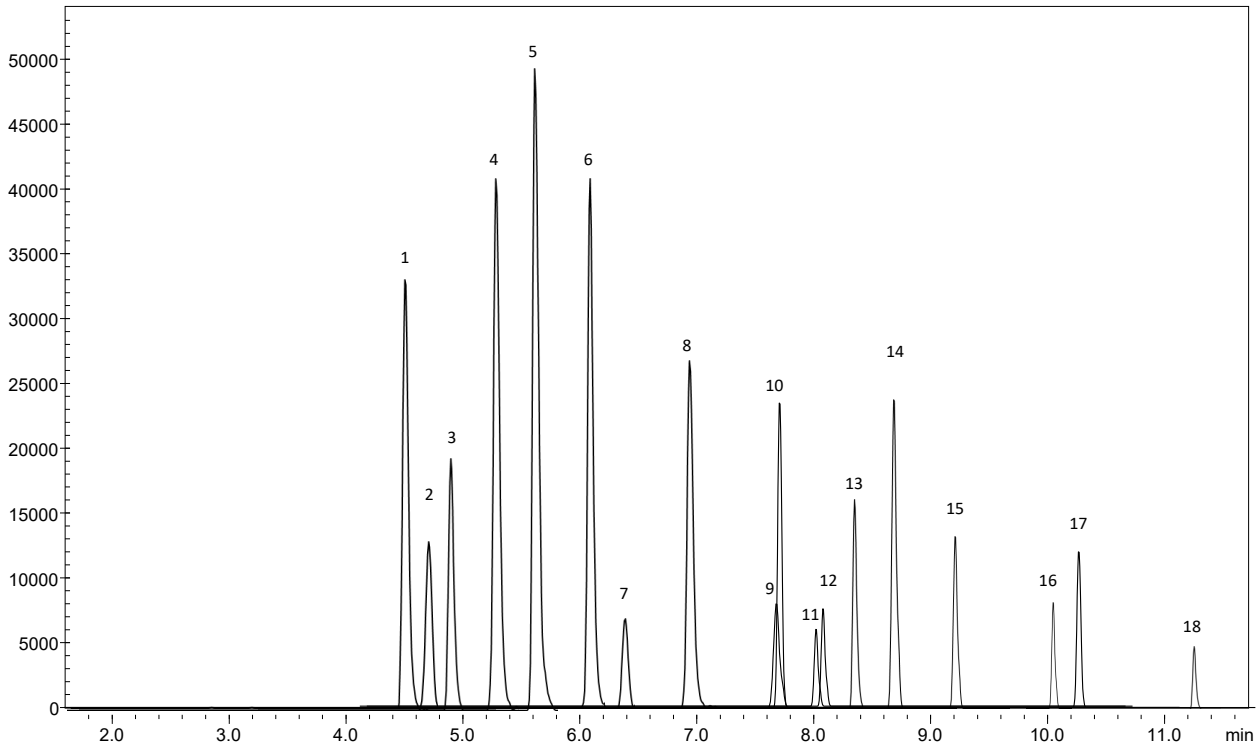
Many challenges exist in environmental and food safety analysis, but possibly the most difficult one is presented by emerging contaminants, and as further research is carried out, the toxicity of these compounds will be further defined. Pesticides are examples of compounds that are commonly found in the environment and food supply, with increasing frequency. Although subjected to regulation, maximum allowable limits are decreasing.

It is critical in an evolving situation such as food safety analysis, to not only meet the demands and regulations of today, but also be able to address the future regulations that will be imposed. Here we present the HALO® Biphenyl for the separation and identification of a mixture of 161 pesticides and environmental contaminants spiked into egg samples at a concentration on 0.045ng/mL, in under 20 minutes. The high-speed separation is easily accomplished and can definitely find application in high throughput environments.

ID#	Name	m/z	Ret. Time
1	MercaptoMethylimidazole	114.8800>57.1000	0.449
2	Dimetridazole hydroxy	158.0000>140.1000	0.544
3	Diuron	232.9400>72.0000	0.557
4	Daminozide	161.0100>143.0000	0.669
5	Ketoprofen	255.1000>77.0000	0.729
6	Propanil	218.0000>162.1000	0.921
7	NalidixicAcid	233.1000>215.1000	1.095
8	Methamidophos	141.9000>94.0000	1.739
9	Methomyl	163.0200>106.0000	2.163
10	NiflumicAcid	283.0000>265.0000	2.441
11	Acephate	184.0000>143.1000	2.515
12	Aldicarb sulfoxide	207.0200>132.1000	4.269
13	Dinotefuran	203.0600>129.1000	4.376
14	Omethoate	214.0000>182.9000	4.552
15	Quinclorac	241.9000>224.0000	5.051
16	Flonicamid	230.0200>203.1000	5.758
17	Aldicarb sulfone	223.0200>86.1000	5.812
18	Salbutamol	240.2000>148.1000	5.842
19	Ipronidazole hydroxy	186.0000>168.0000	6.089
20	Pymetrozine	217.9900>104.9000	6.104
21	Carbendazim	192.0000>160.1000	6.219
22	Flunixin	297.0000>279.0000	6.641
23	Nitenpyran	271.0000>126.0000	6.969
24	OxamylNH4	237.0100>72.0000	7.051
25	OxydemetonMethyl	246.9300>169.1000	7.348
26	Clothianidin	250.0000>169.2000	7.705
27	AldicarbNH4	208.1000>116.1000	7.821
28	Ciprofloxacin	332.1000>314.1000	8.318
29	Dicrotophos	238.0000>112.0000	8.371
30	Thiamethoxam	292.0100>211.1000	8.426
31	Dimethoate	229.9500>199.1000	8.843
32	Cymoxanil	199.0000>128.1000	9.295
33	SulfoxaflorNH4	294.9700>174.1000	9.396
34	Atrazine	216.0300>174.1000	9.398
35	MeclofenamicAcid	296.0000>278.0000	9.681
36	Imidacloprid	255.9400>209.0000	9.987
37	Xylazine	221.0000>164.0000	10.047
38	Mercaptobenzimidazole	150.9600>93.0000	10.399
39	Dichlorvos	220.9000>109.0000	10.762
40	Acetamiprid	223.0100>126.0000	10.948
41	Cyprodinil	226.0500>93.1000	11.113
42	Tebuthiuron	229.0000>172.3000	11.389
43	Morantel	221.1000>123.0000	11.395
44	Imazethapyr	290.0200>245.1000	11.445
45	Trimethoprim	291.1000>230.0000	11.467
46	Diflufenzopyr	335.0000>206.2000	11.574
47	Metalaxyl	280.0100>220.2000	11.796
48	Carbofuran	222.0000>123.0000	12.106
49	Thiacloprid	252.9800>126.2000	12.282
50	Imazalil	296.9700>159.1000	12.551
51	AlbendazoleSulfone	298.0000>159.0000	12.561
52	Fenbufen	255.1000>181.1000	12.675
53	Flunixin-d3	300.0000>282.0000	12.785
54	ThiophanateMethyl	343.0200>151.0000	12.871
55	Clencyclohexerol	319.1000>301.0000	12.986
56	Propyphenazone	231.1000>189.1000	13.135
57	Linuron	248.9000>160.1000	13.304
58	Flubendazole2amino	256.0000>123.0000	13.347
59	Fenobucarb	208.0500>95.0000	13.355
60	Fosthiazate	283.9800>228.0000	13.513
61	Dodemorph	282.2000>116.1000	13.556
62	Azamethiphos	324.9000>183.0000	13.613
63	Azamethiphos	324.9000>183.0000	13.619
64	Ethiprole	398.9000>352.9000	13.626
65	EthiproleNH4	413.9000>351.0000	13.641
66	Pronamide	256.0000>190.0000	13.716
67	Pyrimethanil	200.1000>107.2000	13.739
68	Paclobutrazol	294.0300>70.0000	13.914
69	Norflurazon	303.9000>284.0000	13.936
70	Cyantraniliprole	475.1000>286.0000	13.956
71	Triadimenol	296.0000>70.1000	14.074
72	Methiocarb	226.0100>169.0000	14.209
73	Etoxazole	360.1700>141.0000	14.222
74	Chlorsulfuron	357.9000>167.1000	14.234
75	Triasulfuron	401.9800>167.0000	14.299
76	FenthionSulfone	311.0000>125.0000	14.328
77	Mabuterol	311.1000>237.0000	14.576
78	Fluxapyroxad	382.0000>362.1000	14.591
79	Iprovalicarb	321.1000>119.0000	14.611
80	Fluopyram	396.9800>208.0000	14.619
81	Flutolanil	324.0000>242.1000	14.652
82	Chlorantraniliprole	484.0000>452.9000	14.715
83	Fenhexamid	302.0600>96.9000	14.732
84	Myclobuthanil	289.0100>70.0000	14.903
85	Penthiopyrad	360.0400>276.1000	14.961
86	Tetraconazole	372.0000>159.1000	14.987
87	Fenamidone	312.0000>236.2000	15.003
88	Saflufenacil	501.0000>349.0000	15.014
89	Boscalid	343.0000>307.1000	15.092
90	Clethodim	360.0000>164.0000	15.123
91	Ethoprophos	243.0600>172.9000	15.222
92	Methidathion	302.8800>144.9000	15.233
93	Methoxyfenozide	369.1000>149.1000	15.266
94	Fenarimol	330.9000>268.1000	15.309
95	Hexaconazole	315.9900>69.9000	15.353
96	Thiodicarb	354.9600>88.0000	15.361
97	Tebuconazole	308.0200>70.0000	15.375
98	Fenoterol	304.1000>107.2000	15.491
99	Fenamiphos	304.0000>217.1000	15.524
100	Diflubenzuron	310.9600>158.0000	15.536
101	Penconazole	285.9500>70.0000	15.568
102	Flufenacet	363.9500>194.1000	15.582
103	Bifenazate	301.1000>198.1000	15.726
104	Penoxsulam	484.0000>195.2000	15.758
105	Benzovindiflupyr	398.0500>342.0000	15.897
106	Flusilazole	315.9900>247.0000	15.965
107	Epoxiconazole	330.0000>121.1000	15.991
108	Dimethomorph	388.0600>301.0000	16.048
109	Phosmet	318.0100>160.0000	16.114
110	Fenoxycarb	302.0000>116.0000	16.145
111	Triazophos	313.9200>162.0000	16.152
112	Spirotetramat	374.1200>302.1000	16.161
113	Diazinon	305.0000>169.1000	16.176
114	Spiromesifen	388.1100>273.2000	16.237
115	Fenbuconazole	337.0200>125.0000	16.238
116	Bitertanol	338.1100>269.1000	16.367
117	Cyazofamid	324.9000>107.9000	16.372
118	Tolyfluanid	347.0000>137.0000	16.377
119	Novaluron	493.0100>158.2000	16.396
120	Tetrachlorvinphos	364.9000>127.0000	16.414
121	Triflumizole	346.0500>277.9000	16.473
122	Chlorfenvinphos	358.9000>155.1000	16.476
123	Isofenphos	346.0100>245.1000	16.531
124	Phorate	260.9300>74.9000	16.554
125	Picoxystrobin	368.0000>145.1000	16.556
126	Propiconazole	342.0700>159.1000	16.564
127	PyraflufenEthyl	412.9000>339.0000	16.633
128	PirimiphosMethyl	305.9000>164.2000	16.686
129	Azoxystrobin	404.0400>372.1000	16.686
130	ChlorimuronEthyl	414.9680>186.0000	16.699
131	Disulfoton	274.9500>88.9000	16.834
132	Fenthion	279.0000>247.0000	16.838
133	Tebufenpyrad	334.0900>145.1000	16.954
134	Prallethrin	301.0500>123.1000	16.975
135	Spinetoram	748.4000>142.2000	17.118
136	Prochloraz	375.9000>308.2000	17.16
137	Profenofos	372.9000>302.8000	17.162
138	ChlorpyriphosMethyl	321.9000>125.0000	17.206
139	Clofentezine	303.0000>138.0000	17.322
140	Fluoxastrobin	459.0000>427.0000	17.396
141	Trifloxystrobin	409.1000>186.1000	17.411
142	MalichiteGreen leuco	331.2000>239.1000	17.577
143	Difenoconazole	406.0000>250.9000	17.584
144	Phosalone	367.9000>182.1000	17.631
145	PiperonylButoxide	356.1100>177.2000	17.687
146	Pyraclostrobin	388.1000>194.2000	17.699
147	FenoxapropEthyl	361.9800>288.0000	17.716
148	Indoxacarb	527.9000>248.8000	17.751
149	QuizalofopEthyl	373.0000>299.1000	18.024
150	CrystalViolet leuco	374.2000>238.2000	18.168
151	Pyriproxyfen	322.0600>95.9000	18.172
152	Pyrazophos	374.0100>222.0000	18.186
153	Coumaphos	362.8000>227.0000	18.217
154	PropargiteNH4	368.1000>231.2000	18.245
155	Hexythiazox	353.0100>228.1000	18.431
156	Spirodiclofen	411.1000>313.0000	18.463
157	Acequinocyl	357.2000>329.3000	18.521
158	FenpropathrinNH4	367.1100>125.0000	18.586
159	Fenpyroximate	422.2000>366.1000	18.934
160	Phenothrin	351.0800>183.0000	19.048
161	Pyridaben	365.0500>309.0000	19.054

PFAS Analysis According to EPA 537.1 Using HALO® 90 Å C18, 2.0 µm

218-PF



Per-and polyfluoroalkyl substances (PFASs) are a toxic group of chemicals that have found wide ranging application across numerous industries due to their chemical structure, which includes both a hydrophobic fluorocarbon section, and a hydrophilic carboxylate section. PFAS exposure in humans has been linked to a variety of diseases, including cancer, ulcerative colitis, thyroid disease, and hypercholesterolemia. EPA Method 537.1 can be used for the quantitation of 18 PFAS in drinking water, using solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC/MS/MS). The method stipulates two columns be used for chromatography, one to be used as a delay column to mitigate PFAS contamination from the HPLC, and the other to be used as the analytical column and perform the separation.

PEAK IDENTITIES

Peak Number	PFAS Species	Observed Transition	Retention Time
1	PFHxA	313.0000>269.0000	4.502
2	PFBS	299.0000>80.0000	4.618
3	HFPO-DA	285.0000>169.0000	4.812
4	PFHpA	363.0000>319.0000	5.341
5	ADONA	377.0000>250.9000	5.637
6	PFOA	413.0000>369.0000	6.145
7	PFHxS	399.0000>80.0000	6.451
8	PFNA	463.0000>419.0000	6.925
9	N-MeFOSAA	570.0000>419.0000	7.681
10	PFDA	513.0000>469.0000	7.696
11	N-EtFOSAA	584.0000>419.0000	8.022
12	PFOS	499.0000>80.0000	8.102
13	PFUnA	563.0000>519.0000	8.498
14	9Cl-PF3ONS	530.9000>351.0000	8.739
15	PFDoA	613.0000>569.0000	9.333
16	PFTriA	663.0000>619.0000	10.179
17	11Cl-PF3OUdS	630.7000>451.0000	10.475
18	PFTreA	713.0000>669.0000	11.053

TEST CONDITIONS:

Delay Column: HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm
Part Number: 92812-402
Analytical Column: HALO 90 Å C18, 2.0 µm, 2.1 x 100 mm
Part Number: 91812-602
Mobile Phase A: (95/5) H_2O/ACN 0.1% acetic acid
Mobile Phase B: (95/5) ACN/H_2O 10 mM ammonium formate/ 0.1% acetic acid
Flow Rate: 0.3 mL/min
Sample Solvent: (95/5) MeOH/ H_2O

Gradient:

Time	%B
0.0	0
6.0	50
13.0	85
14.0	100
17.0	100
18.0	0
21.0	stop

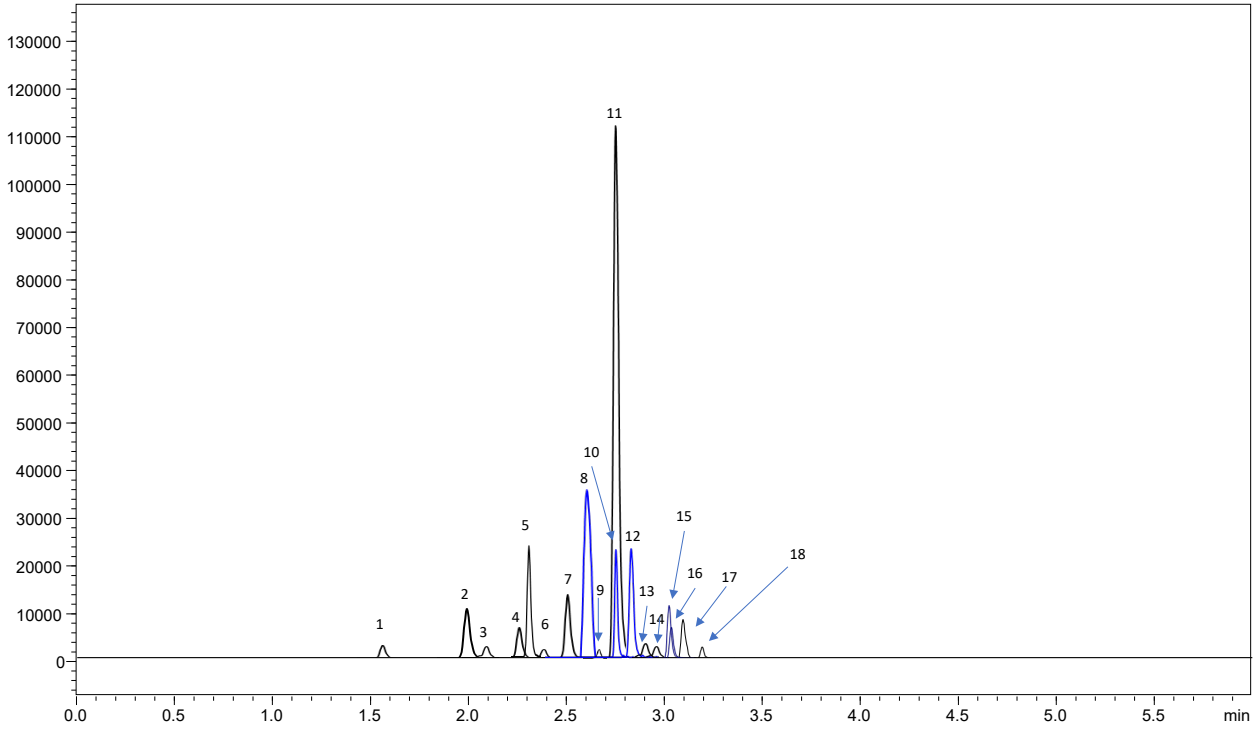
Initial Pressure: 315 bar
Temperature: 40 °C

MS CONDITIONS:

Detection: -ESI MS
LC System: Shimadzu NexeraX2 ESI
LCMS system: Shimadzu LCMS-8050
Spray Voltage: -2.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 °C
Heat Block: 400 °C

Rapid PFAS Analysis According to EPA 537.1 Using HALO® 90 Å Phenyl-Hexyl, 2.7 µm Peak

219-PF



As technological advancements continue to progress, mass spectrometers will continue to be improved in regards to the level of sensitivity, mass resolution, and scanning speed. This will undoubtedly change the requirements of EPA 537.1, and column performance must be able to handle these advancements. With this in mind, we developed a method for separation at maximum speed to test the suitability of the column for use in these advanced conditions.

PEAK IDENTITIES

Peak Number	PFAS Species	Observed Transition	Retention Time
1	PFBS	299.0000>80.0000	2.008
2	PFHxA	313.0000>269.0000	2.325
3	HFPO-DA	285.0000>169.0000	2.339
4	PFHpA	363.0000>319.0000	2.595
5	PFHxS	399.0000>80.0000	2.630
6	ADONA	377.0000>250.9000	2.631
7	PFOA	413.0000>369.0000	2.771
8	PFNA	463.0000>419.0000	2.901
9	PFOS	499.0000>80.0000	2.917
10	9Cl-PF3ONS	530.9000>351.0000	3.009
11	PFDA	513.0000>469.0000	3.011
12	PFUnA	563.0000>519.0000	3.099
13	N-MeFOSAA	570.0000>419.0000	3.106
14	N-EtFOSAA	584.0000>419.0000	3.166
15	11Cl-PF3OUdS	630.7000>451.0000	3.176
16	PFDoA	613.0000>569.0000	3.177
17	PFTriA	663.0000>619.0000	3.244
18	PFTreA	713.0000>669.0000	3.311

TEST CONDITIONS:

Delay Column: HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm
Part Number: 92812-702
Analytical Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 2.1 x 100 mm
Part Number: 92112-730
Mobile Phase A: H_2O 10mM ammonium formate/ 0.1% formic acid
Mobile Phase B: Methanol
Flow Rate: 0.4mL/min
Sample Solvent: (95/5) MeOH/ H_2O

Gradient:

Time	%B
0.00	30
3.00	90
6.00	90
6.01	30
9.00	stop

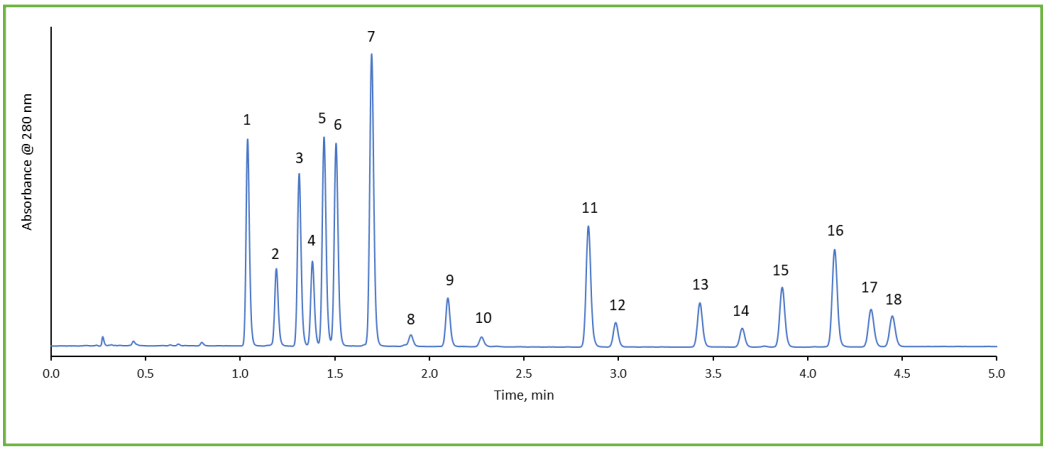
Initial Pressure: 325 bar
Temperature: 40 °C

MS CONDITIONS:

Detection: -ESI MS
LC System: Shimadzu NexeraX2 ESI
LCMS system: Shimadzu LCMS-8040
Spray Voltage: -2.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 °C
Heat Block: 400 °C

Separation of 16 PAH Compounds Specified in EPA 610 + 2 additional PAH Compounds using HALO® PAH

229-P



PEAK IDENTITIES

1. Naphthalene
2. Acenaphthylene
3. 1-methylnaphthalene
4. 2-methylnaphthalene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo(a)anthracene
12. Chrysene
13. Benzo[b]fluoranthene
14. Benzo[k]fluoranthene
15. Benzo[a]pyrene
16. Dibenzo[a,h]anthracene
17. Benzo[g,h,i]perylene
18. Indeno[1,2,3-cd]pyrene

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 4.6 x 50 mm
Part Number: 92844-412
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:

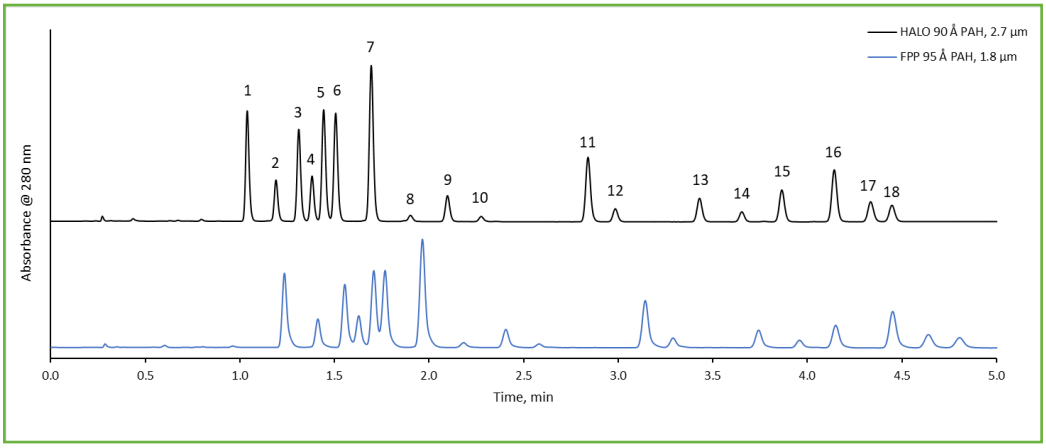
Time	%B
0.0	50
4.0	100
5.0	100
5.01	50

Flow Rate: 1.8 mL/min
Pressure: 256 bar
Temperature: 30 °C
Detection: 280 nm
Injection Volume: 2 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of more than 100 chemicals released from the combustion of coal, oil, gasoline, tobacco, and wood. They can also be found in cooked food. PAHs are persistent chemicals and must be closely regulated for early detection/monitoring to minimize hazardous exposure in the environment and/or use of contaminated raw materials in different industries. A rapid separation of the 16 compounds specified in EPA 610 and an additional 2 PAH compounds that are regularly analyzed is demonstrated on the HALO® PAH column showing excellent speed and resolution.

Comparison of HALO® PAH vs. FPP column for 18 PAH Compounds

230-P



PEAK IDENTITIES

1. Naphthalene
2. Acenaphthylene
3. 1-methylnaphthalene
4. 2-methylnaphthalene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo(a)anthracene
12. Chrysene
13. Benzo[b]fluoranthene
14. Benzo[k]fluoranthene
15. Benzo[a]pyrene
16. Dibenzo[a,h]anthracene
17. Benzo[g,h,i]perylene
18. Indeno[1,2,3-cd]pyrene

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 4.6 x 50 mm
Competitor Column: FPP 95 Å PAH, 1.8 µm, 4.6 x 50 mm
Part Number: 92844-412
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:

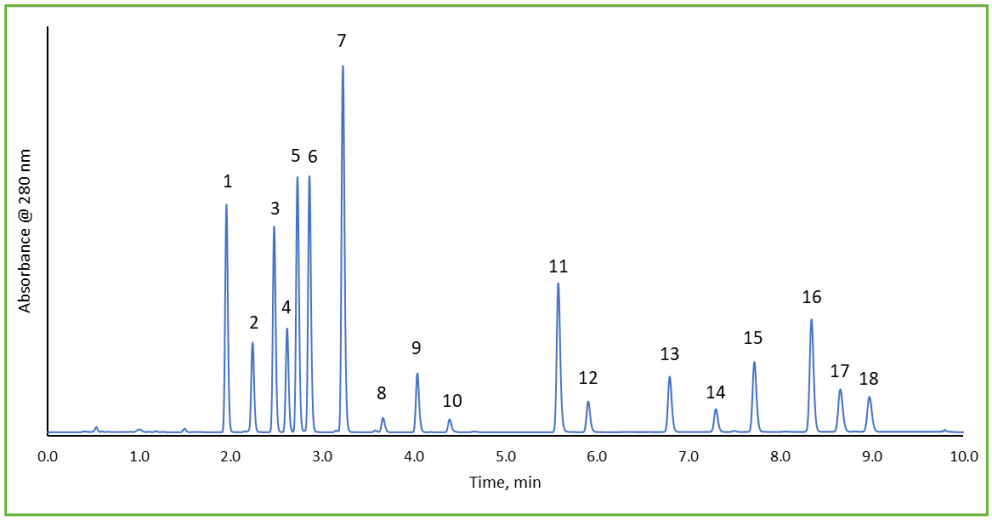
Time	%B
0.0	50
4.0	100
5.0	100
5.01	50

Flow Rate: 1.8 mL/min
HALO® Back Pressure: 256 bar
Competitor Back Pressure: 344 bar
Temperature: 30 °C
Detection: 280 nm
Injection Volume: 2 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of more than 100 chemicals released from the combustion of coal, oil, gasoline, tobacco, and wood. They can also be found in cooked food. PAHs are persistent chemicals and must be closely regulated for early detection/monitoring to minimize hazardous exposure in the environment and/or use of contaminated raw materials in different industries. A separation of eighteen PAH compounds is performed on a HALO® PAH column and a FPP PAH competitor column. The HALO® column shows excellent peak resolution, along with a lower overall back pressure compared to the competitor's unresolved peaks and peak tailing.

Separation of 18 PAH Compounds using HALO® PAH

231-P



PEAK IDENTITIES

1. Naphthalene
2. Acenaphthylene
3. 1-methylnaphthalene
4. 2-methylnaphthalene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo(a)anthracene
12. Chrysene
13. Benzo[b]fluoranthene
14. Benzo[k]fluoranthene
15. Benzo[a]pyrene
16. Dibenzo[a,h]anthracene
17. Benzo[g,h,i]perylene
18. Indeno[1,2,3-cd]pyrene

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 3.0 x 100 mm
Part Number: 92843-612
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:

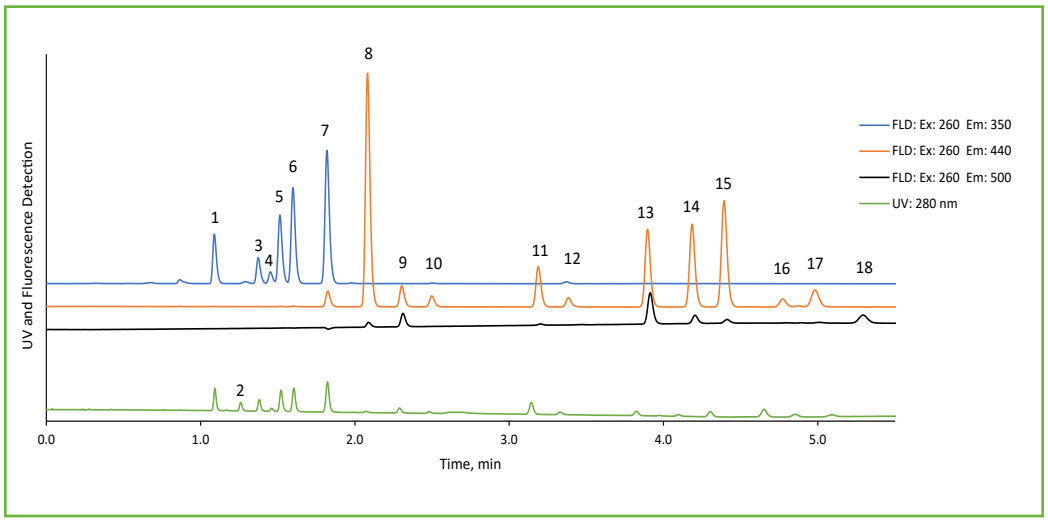
Time	%B
0.0	50
8.0	100
10.0	100

Flow Rate: 0.77 mL/min
Initial Back Pressure: 263 bar
Temperature: 30 °C
Detection: 280 nm
Injection Volume: 2 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of more than 100 chemicals released from the combustion of coal, oil, gasoline, tobacco, and wood. They can also be found in cooked food. PAHs are persistent chemicals and must be closely regulated for early detection/monitoring to minimize hazardous exposure in the environment and/or use of contaminated raw materials in different industries. A rapid separation of the 16 compounds specified in EPA 610 and an additional 2 PAH compounds that are regularly analyzed is demonstrated on the HALO® PAH column showing excellent speed and resolution.

Separation of PAH Compounds using UV and Fluorescence Detection

233-P



PEAK IDENTITIES

1. Naphthalene
2. Acenaphthylene
3. 1-methylnaphthalene
4. 2-methylnaphthalene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo[a]anthracene
12. Chrysene
13. Benzo[b]fluoranthene
14. Benzo[k]fluoranthene
15. Benzo[a]pyrene
16. Dibenzo[a,h]anthracene
17. Benzo[g,h,i]perylene
18. Indeno[1,2,3-cd]pyrene

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 4.6 x 50 mm
Part Number: 92844-412
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:

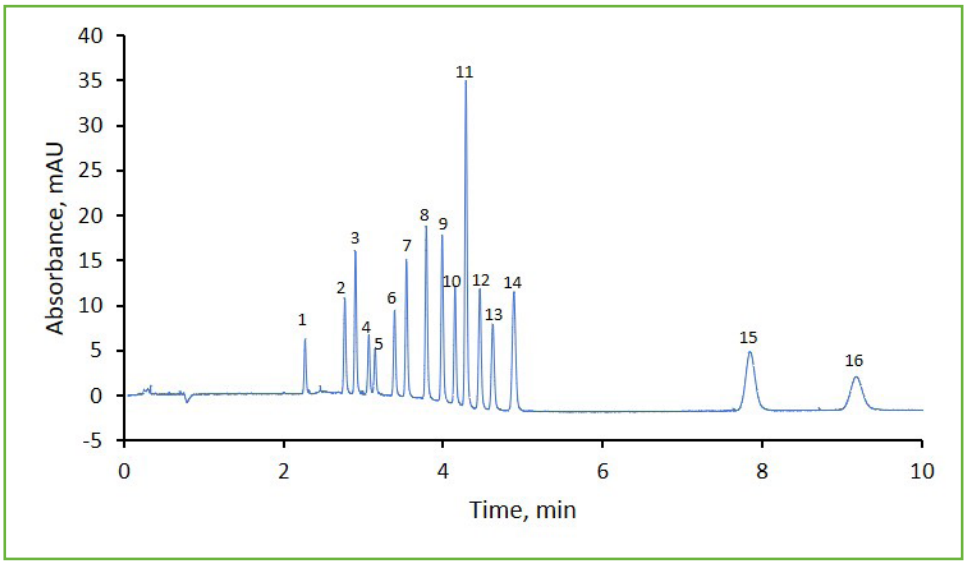
Time	%B
0.0	50
4.0	100
5.0	100
6.0	100

Flow Rate: 1.8 mL/min
Initial Back Pressure: 256 bar
Temperature: Ambient
Detection: FLD: Ex: 260/ Em: 350/440/500
UV: 280 nm
Injection Volume: 0.3 µL
Sample Solvent: Methanol
LC System: Shimadzu Nexera X2

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of more than 100 chemicals released from the combustion of coal, oil, gasoline, tobacco, and wood. They can also be found in cooked food. PAHs are persistent chemicals and must be closely regulated for early detection/monitoring to minimize hazardous exposure in the environment and/or use of contaminated raw materials in different industries. These compounds can be detected several ways including a UV and/or a fluorescence detector (FLD). A rapid separation of the 16 compounds specified in EPA 610 and an additional 2 PAH compounds that are regularly analyzed is demonstrated using a UV and fluorescence detector. The FLD gain in sensitivity compared to the UV is associated to the advantage of no background for FLD and the ability to select both an excitation and emission wavelength; which can be optimized further with systematically testing the S/N as a function of the detector's gain parameter. Slight retention time and peak width increases for the FLD response are due to the greater tubing volume of this detector.

Separation of EU 15 + 1 using HALO® PAH

235-P



PEAK IDENTITIES

1. Benzo[c]fluorene
2. Cyclopenta[cd]pyrene
3. Benzo[a]anthracene
4. Chrysene
5. 5-Methylchrysene
6. Benzo[j]fluoranthene
7. Benzo[b]fluoranthene
8. Benzo[k]fluoranthene
9. Benzo[a]pyrene
10. Dibenzo[a,l]pyrene
11. Dibenz[a,h]anthracene
12. Benzo[ghi]perylene
13. Indeno[1,2,3-cd]pyrene
14. Dibenzo[a,e]pyrene
15. Dibenzo[a,i]pyrene
16. Dibenzo[a,h]pyrene

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 4.6 x 50 mm
Part Number: 92844-412
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:

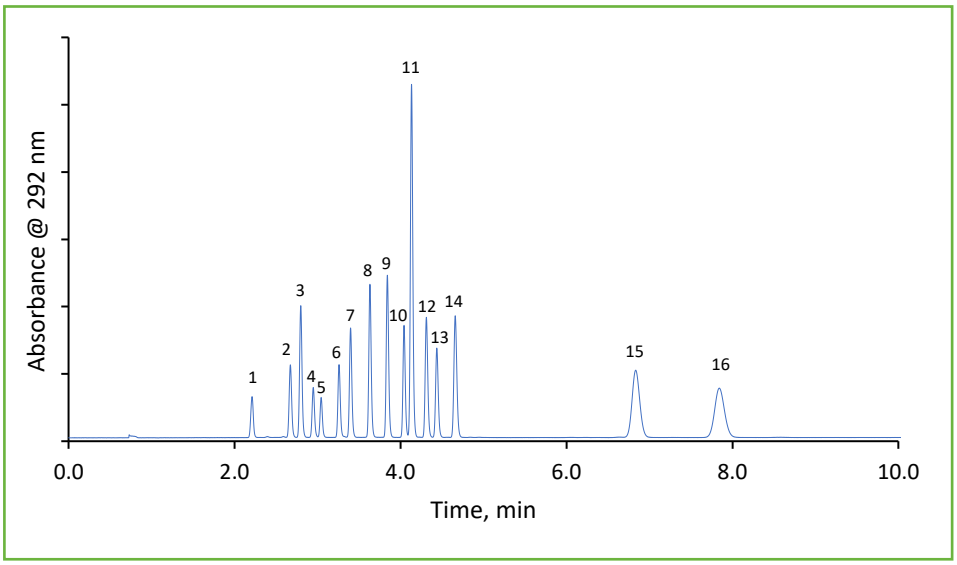
Time	%B
0.00	50
4.00	100
15.00	100
15.01	50

Flow Rate: 1.8 mL/min
Temperature: 30 °C
Detection: 292 nm
Injection Volume: 10 µL
Data Rate: 20 Hz
LC System: Acquity UPLC I-Class
Data Courtesy of Hall Analytical Laboratories, Ltd.

The EU 15 + 1 list of PAH compounds was established by the European Commission in 2005 specifically for food analysis. The list contains eight of the EPA's priority PAHs along with eight other compounds that are known carcinogens. The separation is completed on a 4.6 x 50 mm HALO® PAH column in less than ten minutes with excellent resolution between the critical pairs 4 and 5 which only differ by the presence of a methyl group.

Separation of EU 15 + 1 using HALO® PAH

236-P



PEAK IDENTITIES

1. Benzo[c]fluorene
2. Cyclopenta[cd]pyrene
3. Benzo[a]anthracene
4. Chrysene
5. 5-Methylchrysene
6. Benzo[j]fluoranthene
7. Benzo[b]fluoranthene
8. Benzo[k]fluoranthene
9. Benzo[a]pyrene
10. Dibenzo[a,l]pyrene
11. Dibenz[a,h]anthracene
12. Benzo[ghi]perylene
13. Indeno[1,2,3-cd]pyrene
14. Dibenzo[a,e]pyrene
15. Dibenzo[a,i]pyrene
16. Dibenzo[a,h]pyrene

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 4.6 x 50 mm
Part Number: 92844-412
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:

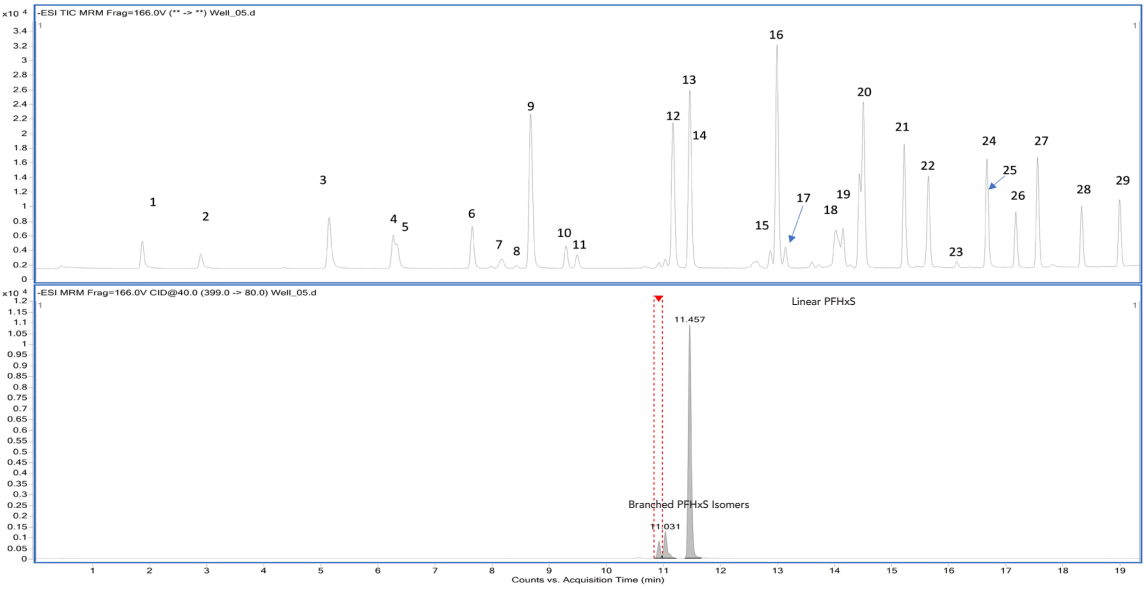
Time	%B
0.00	50
4.00	100
15.00	100
15.01	50

Flow Rate: 1.8 mL/min
Temperature: 30 °C
Detection: 292 nm
Injection Volume: 10 µL
Data Rate: 100 Hz
LC System: Shimadzu Nexera X2

The EU 15 + 1 list of PAH compounds was established by the European Commission in 2005 specifically for food analysis. The list contains eight of the EPA's priority PAHs along with eight other compounds that are known carcinogens. The separation is completed on a 4.6 x 50 mm HALO® PAH column in less than ten minutes with excellent resolution between the critical pairs 4 and 5 which only differ by the presence of a methyl group.

Analysis of PFAS in Well Water Spiked with Standards

243-PF



Peak #	Compound	t_R (min)	Transition	Peak #	Compound	t_R (min)	Transition
1	PFBA	1.88	213.0>169.0	16	PFOA	12.99	413.0>369.0
2	PFMPA	2.90	229.0>85.0	17	PFHpS	13.14	449.0>80.0
3	PFPeA	5.15	263.0>219.0	18	PFNA	14.43	463.0>419.0
4	PFBS	6.27	299.0>80.0	19	PFOS	14.50	499.0>80.0
5	PFMBA	6.34	279.0>85.0	20	9Cl-PF3ONS	15.22	531.0>351.0
6	PFEESA	7.66	315.0>135.0	21	8:2FTS	15.59	527.0>507.0
7	NFDHA	8.18	295.0>201.0	22	PFDA	15.64	513.0>469.0
8	4:2FTS	8.43	327.0>307.0	23	NMeFOSAA	16.13	570.0>419.0
9	PFHxA	8.67	313.0>269.0	24	NEtFOSAA	16.66	584.0>419.0
10	PFPeS	9.29	349.0>80.0	25	PFUnA	16.67	563.0>519.0
11	HFPO-DA	9.49	285.0>169.0	26	11Cl-PF3OUdS	17.17	631.0>451.0
12	PFHpA	11.17	363.0>319.0	27	PFDoA	17.55	613.0>569.0
13	PFHxS	11.46	399.0>80.0	28	PFTrA	18.32	663.0>619.0
14	ADONA	11.47	377.0>251.0	29	PFTA	18.99	713.0>669.0
15	6:2FTS	12.87	427.0>407.0				

TEST CONDITIONS:

Analytical Column: HALO® PFAS, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-613
Delay Column: HALO® PFAS Delay, 3.0 x 50 mm
Part Number: 92113-415
Mobile Phase A: 20 mM Ammonium Acetete
B: Methanol
Gradient:

Time	%B
0.0	20
15	90
20	90

Flow Rate: 0.4 mL/min
Pressure: 505 bar
Temperature: 44 °C
Detection: -ESI MRM
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)
LC System: Agilent Triple Quadrupole LC/MS 6400

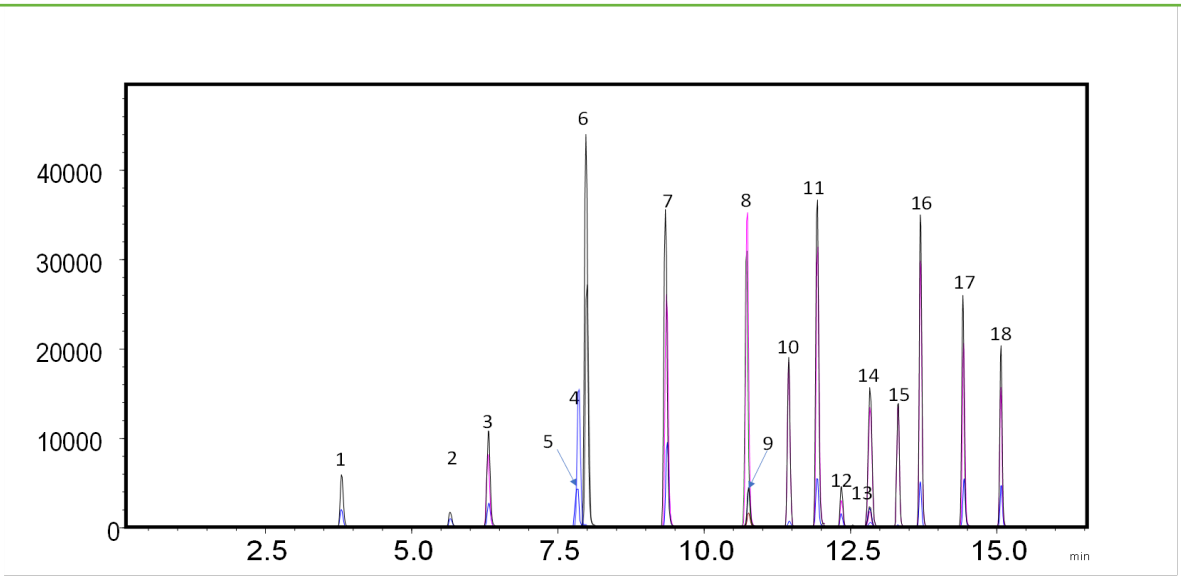
MS Conditions:

Gas Temp: 130 °C
Nebulizer: 25 psi
Gas Flow: 11 L/min
Sheath Gas Heater: 250 °C
Capillary: 3500 V
Data courtesy of STRIDE Center for PFAS Solutions

In 2019 EPA method 533 was introduced and focused on "short chain" PFAS, those PFAS with carbon chain lengths of four to 12. Method 533 complements EPA Method 537.1 and can be used to test for 11 additional PFAS species. PFAS analysis, however, is an evolving area of study, and with nearly 5,000 different types of PFAS, undoubtedly more methods will be developed to include additional compounds. As PFAS science progresses, Advanced Materials Technology offers both PFAS delay and analytical columns, to further mitigate the effects of PFAS contamination from instrumentation, and provide a more accurate analysis. Here we show a clear separation of the branched and linear isomers of PFAS species PFHxS, found in a well water sample spiked with standards.

PFAS Analysis According to EPA 537.1

244-PF



Peak #	Compound	Transition	t_R (min)	Peak #	Compound	Transition	t_R (min)
1	PFBS	299.0000>80.0000	3.789	10	9Cl-PF3ONS	530.9000>351.0000	11.439
2	PFHxA	313.0000>269.0000	5.639	11	PFDA	513.0000>469.0000	11.857
3	HFPO-DA	285.0000>169.0000	6.307	12	N-MeFOSAA	570.0000>419.0000	12.336
4	PFHpA	363.0000>319.0000	7.723	13	PFUnA	563.0000>519.0000	12.822
5	PFHxS	399.0000>80.0000	7.936	14	N-EtFOSAA	584.0000>419.0000	12.827
6	ADONA	377.0000>250.9000	7.978	15	11Cl-PF3OUdS	630.7000>451.0000	13.311
7	PFOA	413.0000>369.0000	9.368	16	PFDoA	613.0000>569.0000	13.690
8	PFNA	463.0000>419.0000	10.715	17	PFTrDA	663.0000>619.0000	14.435
9	PFOS	499.0000>80.0000	10.762	18	PFTeDA	713.0000>669.0000	15.083

TEST CONDITIONS:

Analytical Column: HALO® PFAS, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-613
Delay Column: HALO® PFAS Delay, 3.0 x 50 mm
Part Number: 92113-415
Mobile Phase A: 10 mM Ammonium Acetete
Mobile Phase B: Methanol
Gradient:

Time	%B
0.0	33
18	98
18.1	100
21.0	100
21.1	33
26.0	End

Flow Rate: 0.4 mL/min
Initial Back Pressure: 485 bar
Temperature: 35 °C
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)

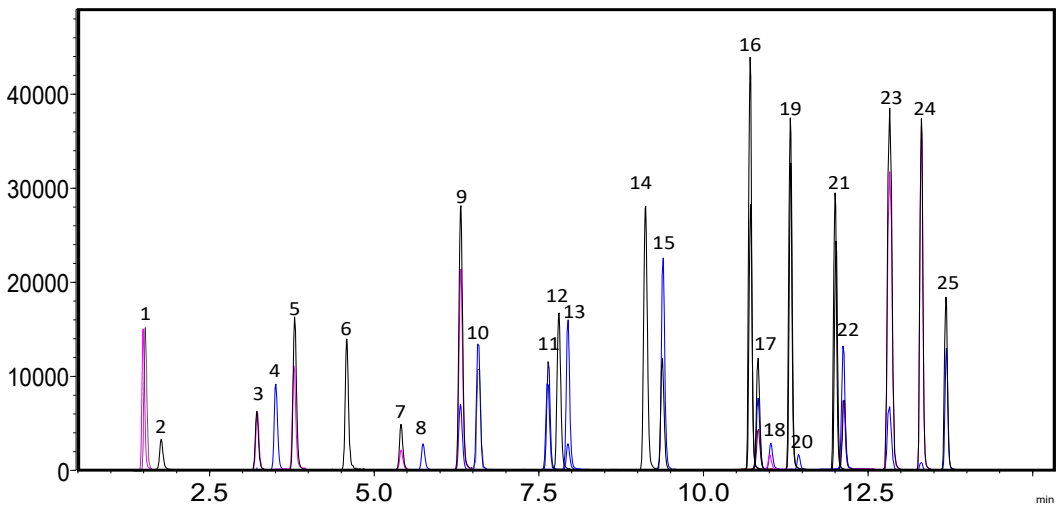
EPA Method 537.1 is used for the quantitation of 18 PFAS in drinking water, using solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC/MS/MS). The method stipulates two columns be used for chromatography, one to be used as a delay column to mitigate PFAS contamination from the HPLC, and the other to be used as the analytical column and perform the separation. Here we present this high resolution separation on the HALO® PFAS delay column and the HALO® PFAS analytical column.

MS Conditions:

Detection: -ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS System: Shimadzu LCMS-8040
Spray Voltage: -2.0 kV
Nebulizing Gas: 2 L/min
Drying Gas: 15 L/min
DL Temperature: 250 ˚C
Heat Block: 400 ˚C

PFAS Analysis According to EPA 533

245-PF



Peak #	Compound	Transition	t_R (min)
1	PFBA	213.0000>169.0000	1.358
2	4:2FTS	229.0000>85.0000	1.890
3	PFPeA	263.0000>219.0000	3.219
4	PFBS	299.0000>80.0000	3.810
5	PFHpS	279.0000>85.0000	3.967
6	PFPeS	315.0000>135.0000	4.791
7	PFMPA	327.0000>307.0000	5.431
8	PFHxA	313.0000>269.0000	5.684
9	PFEESA	349.0000>80.0000	6.099
10	HFPO-DA	285.0000>169.0000	6.335
11	PFHpA	363.0000>319.0000	7.763
12	PFHxS	399.0000>80.0000	7.985
13	ADONA	377.0000>250.9000	8.012

Peak #	Compound	Transition	t_R (min)
14	PFOA	413.0000>369.0000	9.398
15	PFMBA	449.0000>80.0000	9.512
16	PFNA	463.0000>419.0000	10.751
17	PFOS	499.0000>80.0000	10.793
18	9Cl-PF3ONS	530.9000>351.0000	11.459
19	PFDA	513.0000>469.0000	11.885
20	8:2FTS	549.0000>80.0000	11.897
21	6:2FTS	498.0000>78.0000	12.680
22	NFDHA	599.0000>80.0000	12.847
23	PFUnA	563.0000>519.0000	12.862
24	11Cl-PF3OUdS	630.7000>451.0000	13.329
25	PFDoA	613.0000>569.0000	13.708

TEST CONDITIONS:

Analytical Column: HALO® PFAS, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-613
Delay Column: HALO® PFAS Delay, 3.0 x 50 mm
Part Number: 92113-415
Mobile Phase A: 10 mM Ammonium Acetete
Mobile Phase B: Methanol

Gradient:

Time	%B
0.0	33
18	98
18.1	100
21.0	100
21.1	33
26.0	End

Flow Rate: 0.4 mL/min
Initial Back Pressure: 485 bar
Temperature: 35 °C
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)

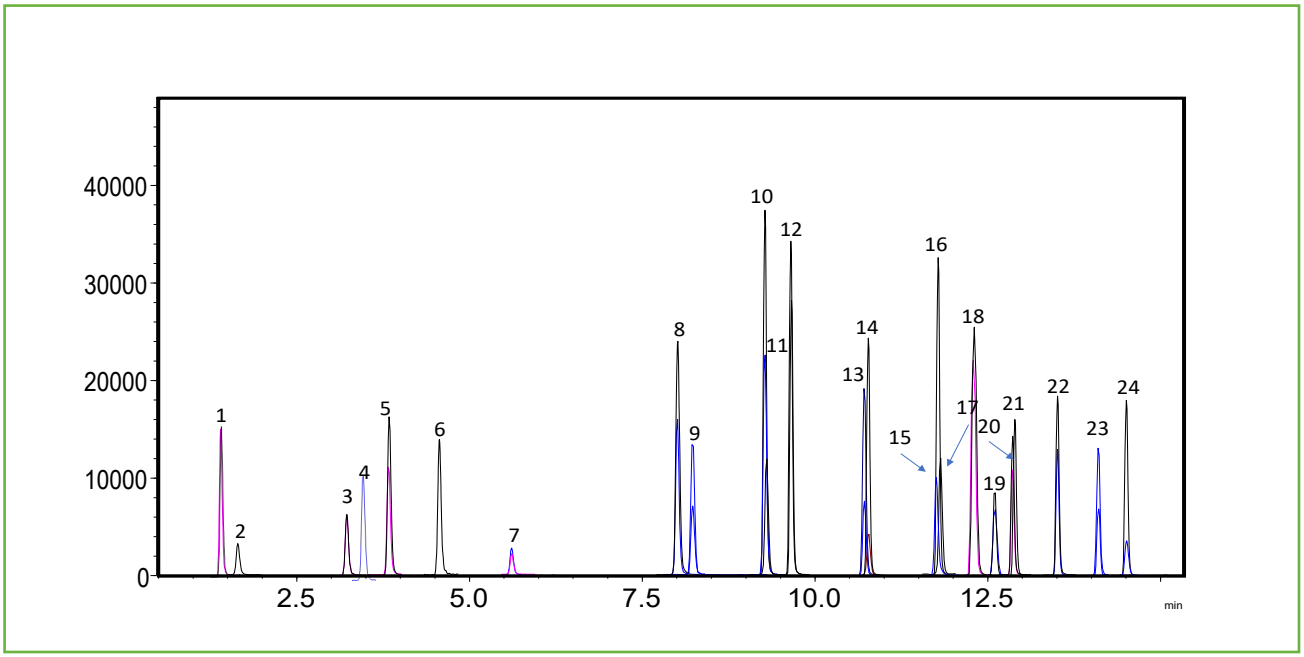
MS Conditions:

Detection: -ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS System: Shimadzu LCMS-8040
Spray Voltage: -2.0 kV
Nebulizing Gas: 2 L/min
Drying Gas: 15 L/min
DL Temperature: 250 ˚C
Heat Block: 400 ˚C

In 2019 EPA method 533 was introduced and focused on "short chain" PFAS, those PFAS with carbon chain lengths of four to 12. Method 533 complements EPA Method 537.1 and can be used to test for 11 additional PFAS species. Here we present this high resolution separation on the HALO® PFAS delay column and the HALO® PFAS analytical column.

PFAS Analysis According to EPA 8327

246-PF



Peak #	Compound	Transition	t_R (min)	Peak #	Compound	Transition	t_R (min)
1	PFBA	213.0000>169.0000	1.358	13	PFNA	463.0000>419.0000	10.751
2	4:2FTS	229.0000>85.0000	1.890	14	PFOS	499.0000>80.0000	10.793
3	PFPeA	263.0000>219.0000	3.219	15	PFNS	527.0000>507.0000	11.843
4	PFBS	299.0000>80.0000	3.810	16	PFDA	513.0000>469.0000	11.885
5	PFHpS	279.0000>85.0000	3.967	17	8:2FTS	549.0000>80.0000	11.897
6	PFPeS	315.0000>135.0000	4.791	18	N-MeFOSAA	570.0000>419.0000	12.366
7	PFHxA	313.0000>269.0000	5.684	19	6:2FTS	498.0000>78.0000	12.680
8	PFHpA	363.0000>319.0000	7.763	20	PFUnA	563.0000>519.0000	12.862
9	PFHxS	399.0000>80.0000	7.985	21	N-EtFOSAA	584.0000>419.0000	12.865
10	FOSA	427.0000>407.0000	9.304	22	PFDoA	613.0000>569.0000	13.708
11	PFOA	413.0000>369.0000	9.398	23	PFTrDA	663.0000>619.0000	14.446
12	PFDS	295.0000>201.0000	9.695	24	PFTeDA	713.0000>669.0000	15.103

TEST CONDITIONS:

Analytical Column: HALO® PFAS, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-613
Delay Column: HALO® PFAS Delay, 3.0 x 50 mm
Part Number: 92113-415
Mobile Phase A: 10 mM Ammonium Acetete
Mobile Phase B: Methanol

Gradient:

Time	%B
0.0	33
18	98
18.1	100
21.0	100
21.1	33
26.0	End

Flow Rate: 0.4 mL/min
Initial Back Pressure: 485 bar
Temperature: 35 °C
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)

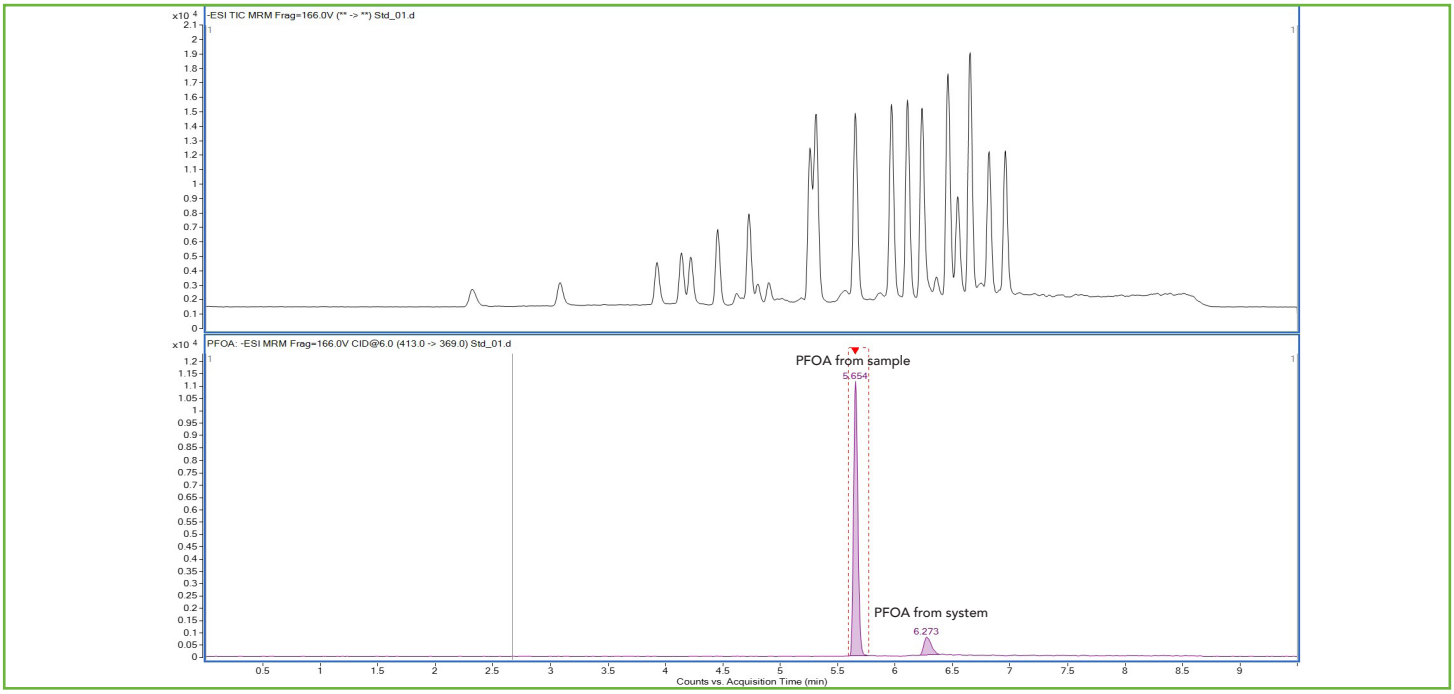
MS Conditions:

Detection: -ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS System: Shimadzu LCMS-8040
Spray Voltage: -2.0 kV
Nebulizing Gas: 2 L/min
Drying Gas: 15 L/min
DL Temperature: 250 ˚C
Heat Block: 400 ˚C

In 2019, the EPA validated method 8327 for non-potable water testing, which includes the analysis of 24 total PFAS compounds in a variety of aquatic matrices with 14 compounds being common across this method and EPA 537.1. Here we present this high resolution separation on the HALO® PFAS delay column coupled with the HALO® PFAS analytical column.

Demonstration of the HALO® PFAS Delay Column

247-PF



TEST CONDITIONS:

Analytical Column: HALO® PFAS, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-613
Delay Column: HALO® PFAS Delay, 3.0 x 50 mm
Part Number: 92113-415
Mobile Phase A: 20 mM Ammonium Acetete
B: Methanol
Gradient:

Time	%B
0.0	20
6	90
8	90
8.10	20
10.00	End

Flow Rate: 0.4 mL/min
Pressure: 505 bar
Temperature: 44 °C
Detection: -ESI MRM
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)
LC System: Agilent Triple Quadrupole LC/MS 6400

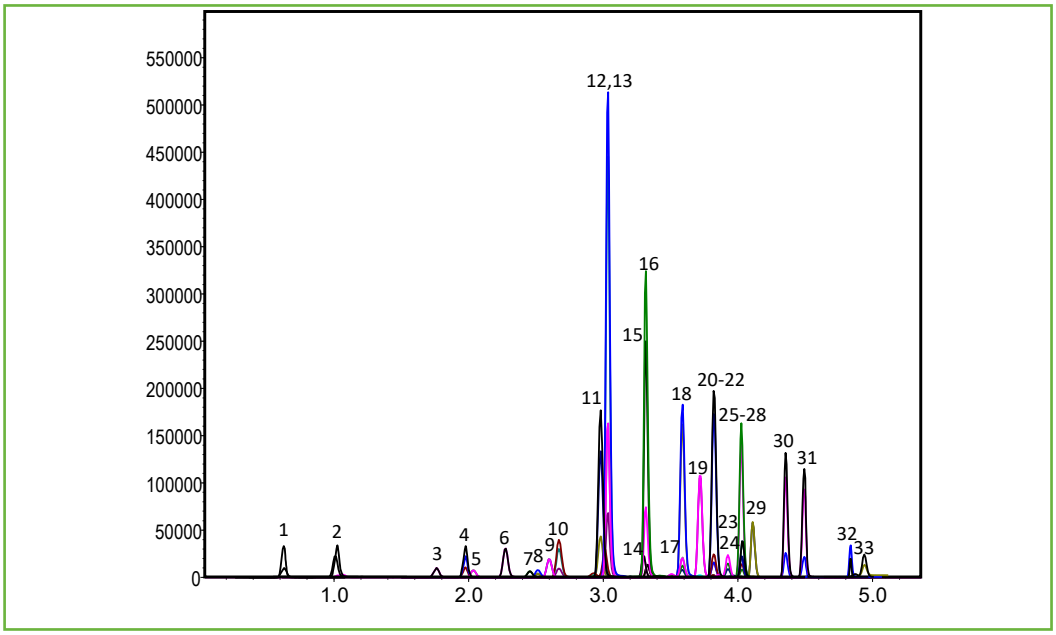
MS Conditions:

Gas Temp: 130 °C
Nebulizer: 25 psi
Gas Flow: 11 L/min
Sheath Gas Heater: 250 °C
Capillary: 3500 V
Data courtesy of STRIDE Center for PFAS Solutions

Advanced Materials Technology offers both HALO® PFAS delay and analytical columns to further mitigate the effects of PFAS contamination from instrumentation, and provide a more accurate analysis. Here we show the functionality of the delay column by showing PFAS species PFOA, separated from the PFOA originating from the instrument components.

Rapid Analysis of 33 PFAS Compounds in Under 5 Minutes

248-PF



Peak #	Compound	Transition	t_R (min)
1	PFBA	213.0000>169.0000	0.755
2	4:2FTS	229.0000>85.0000	1.031
3	PFPeA	263.0000>219.0000	1.762
4	PFBS	299.0000>80.0000	1.979
5	PFHpS	279.0000>85.0000	2.035
6	PFPeS	315.0000>135.0000	2.273
7	PFMPA	327.0000>307.0000	2.454
8	PFHxA	313.0000>269.0000	2.514
9	PFEESA	349.0000>80.0000	2.599
10	HFPO-DA	285.0000>169.0000	2.670
11	PFHxS	399.0000>80.0000	3.013
12	NaDONA	377.0000>251.0000	3.033
13	ADONA	377.0000>250.9000	3.034
14	FOSA	427.0000>407.0000	3.299
15	PFOA	413.0000>369.0000	3.316
16	PFMBA	449.0000>80.0000	3.328
17	PFHpA	363.0000>319.0000	3.388

Peak #	Compound	Transition	t_R (min)
18	PFOS	499.0000>80.0000	3.588
19	9Cl-PF3ONS	530.9000>351.0000	3.719
20	8:2FTS	549.0000>80.0000	3.816
21	PFNS	527.0000>507.0000	3.820
22	PFDA	513.0000>469.0000	3.822
23	N-MeFOSAA	570.0000>419.0000	3.925
24	PFNA	463.0000>419.0000	3.942
25	NFDHA	599.0000>80.0000	4.015
26	PFUnA	563.0000>519.0000	4.025
27	N-EtFOSAA	584.0000>419.0000	4.029
28	6:2FTS	498.0000>78.0000	4.033
29	11Cl-PF3OUdS	630.7000>451.0000	4.110
30	PFTrDA	663.0000>619.0000	4.355
31	PFDoA	613.0000>569.0000	4.496
32	PFTeDA	713.0000>669.0000	4.745
33	PFDS	295.0000>201.0000	4.921

TEST CONDITIONS:

Analytical Column: HALO® PFAS, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-613
Delay Column: HALO® PFAS Delay, 3.0 x 50 mm
Part Number: 92113-415
Mobile Phase A: 10 mM Ammonium Acetete
B: Methanol
Gradient:

Time	%B
0.0	33
4.0	98
4.10	100
6.00	100
6.10	33
7.50	End

Flow Rate: 0.4 mL/min
Pressure: 479 bar
Temperature: 35 °C
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)

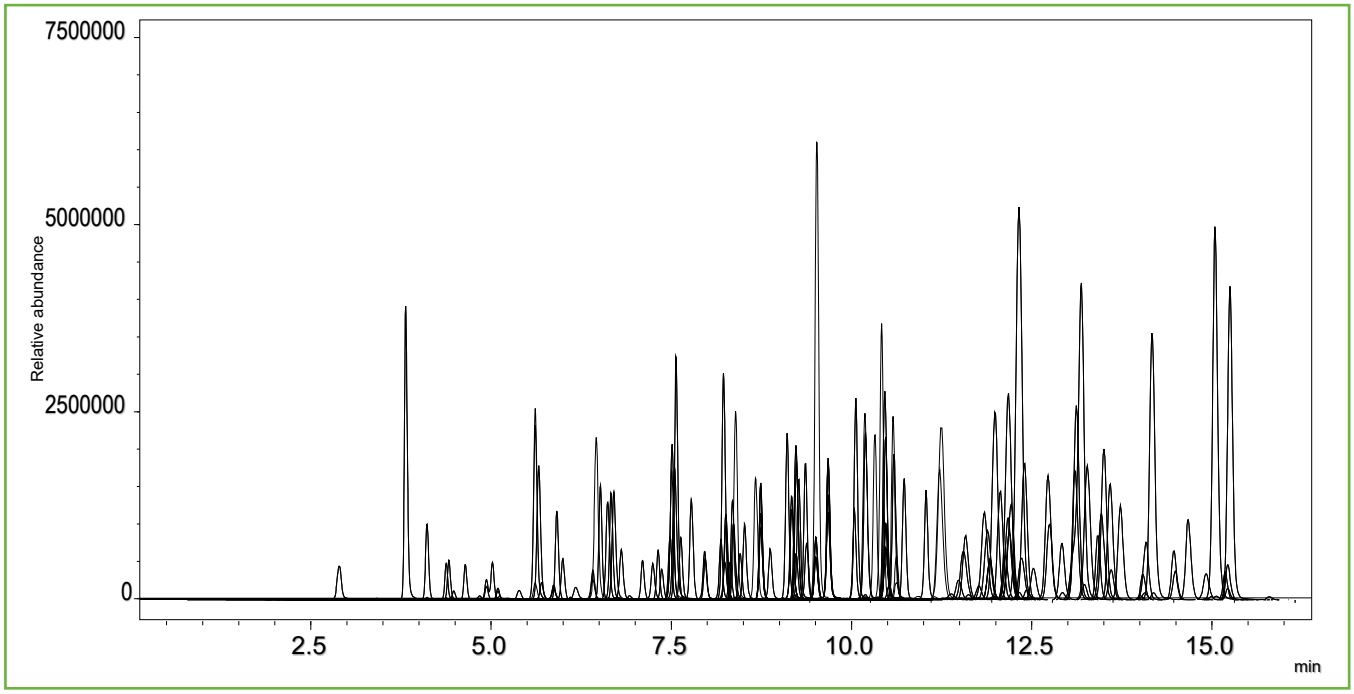
MS Conditions:

Detection: -ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS System: Shimadzu LCMS-8040
Spray Voltage: -2.0 kV
Nebulizing Gas: 2 L/min
Drying Gas: 15 L/min
DL Temperature: 250 ˚C
Heat Block: 400 ˚C

As technological advancements continue to progress, mass spectrometers will continue to be improved in regards to the level of sensitivity, mass resolution, and scanning speed. This will undoubtedly impact future developments in PFAS analysis, and column performance must be able to handle these advancements. With this in mind, we developed a method for separation at maximum speed to test the suitability of the columns for use in these advanced conditions. The higher scanning speed of the MS instruments will lead to faster analysis time and higher flow rates, but a deleterious effect however, is often times an increase in the speed of analysis will lead to a decrease in the resolution therefore causing coelutions. Here we present this high resolution separation on the HALO® PFAS delay column and the HALO® PFAS analytical column for the separation of 33 PFAS species found in EPA 537.1, EPA 533, and EPA 8327, completed in under 5 minutes.

Large Panel Pesticide Screening on HALO® Biphenyl

249-PS



TEST CONDITIONS:

Column: HALO 90 Å Biphenyl, 2.7 µm 2.1 x 100 mm
Part Number: 92812-611
Mobile Phase A: Water/0.1% formic acid, 5 mM ammonium acetate
B: Methanol/0.1% formic acid

Gradient:

Time	%B
0.0	0
12	100
16	100

Flow Rate: 0.4 mL/min
Pressure: 225 bar
Temperature: 30 °C
Detection: +ESI
Injection Volume: 1.0 µL
Sample Solvent: Methanol
LC System: Shimadzu 8040 LCMS

With its combination of hydrophobic, aromatic, and polar selectivities, the HALO® Biphenyl column is ideal for screening 191 pesticides in less than 16 minutes. Both polar and non-polar pesticides are well retained, enabling the HALO® Biphenyl column to be an excellent choice for high throughput screening for environmental applications.

Peak #	Name	Retention Time (min)	m/z transition
1	Cyromazine	2.894	167.0000>85.1000
2	Terbufos sulfone	3.427	321.1000>171.0000
3	Metoxuron	4.135	229.1000>72.0500
4	Propamocarb	4.530	189.0000>102.1000
5	Omethoate	4.840	214.0000>125.1000
6	Monolinuron	4.841	215.1000>126.1000
7	Simetryn	4.841	214.0000>124.0000
8	Butocarboxim sulfoxide	5.117	207.1000>75.1000
9	Butocarboxim	5.118	208.1000>75.1000
10	Aldicarb sulfoxide	5.119	207.1000>89.2000
11	Dinotefuran	5.157	203.2500>129.0500
12	Butoxycarboxim	5.700	223.0000>106.2000
13	Aldoxycarb	5.787	240.2000>86.1000
14	Flonicamid	5.867	230.1000>203.0000
15	Sebuthylazine	5.868	229.9000>174.0500
16	Atrazine-desisopropyl	6.174	173.8000>68.1000
17	Carbendazim	6.408	192.1000>160.1000
18	Pymetrozine	6.459	218.1000>105.1000
19	Oxamyl	6.721	237.1000>72.1000
20	Nitenpyram	6.747	271.0000>56.1500
21	Methomyl	6.807	162.8000>106.0000
22	Oxydemeton-methyl	6.920	247.0000>169.0000
23	Clothianidin	7.243	250.1000>132.0000
24	Demeton-s-methyl sulfone	7.352	262.8000>169.0000
25	Fuberidazole	7.458	185.0000>157.0500
26	Fenuron	7.505	164.9000>72.0500
27	Thiabendazole	7.547	202.1000>175.0000
28	Cyproconazole Isomer	7.708	292.0000>70.0000
29	3-hydroxycarbofuran	7.963	255.1000>163.1000
30	Ethidimuron	8.188	264.9000>114.0000
31	Chloridazon	8.241	222.1000>104.1000
32	Ethirimol	8.363	210.1000>98.0500
33	Dioxacarb	8.392	224.0000>123.1000
34	Methiocarb	8.395	226.1000>169.1000
35	Vamidothion	8.447	288.0000>146.0500
36	Cymoxanil	8.517	199.1000>128.0000
37	Ametryn	8.669	242.1000>122.1000
38	Mesurol sulfoxide	8.669	242.1000>122.1000
39	Terbutryn	8.671	242.1000>186.1000
40	Imidacloprid	8.871	256.1000>175.0000
41	Oxycarboxin	9.137	268.1000>175.0000
42	Monuron	9.170	199.1000>72.0500
43	Cycluron	9.171	199.1000>72.0500
44	Methiocarb-sulfone	9.230	258.1000>122.1000
45	Metolcarb	9.267	166.1000>109.0000
46	Thidiazuron	9.378	221.1000>102.1000
47	Diuron	9.675	232.8000>72.1000
48	Fluometuron	9.677	233.1000>72.1000
49	Propoxur	10.059	210.1000>111.0000
50	Fenthion sulfoxide	10.169	295.1000>280.0000
51	Imazamethabenz-methyl	10.186	289.1000>144.0000

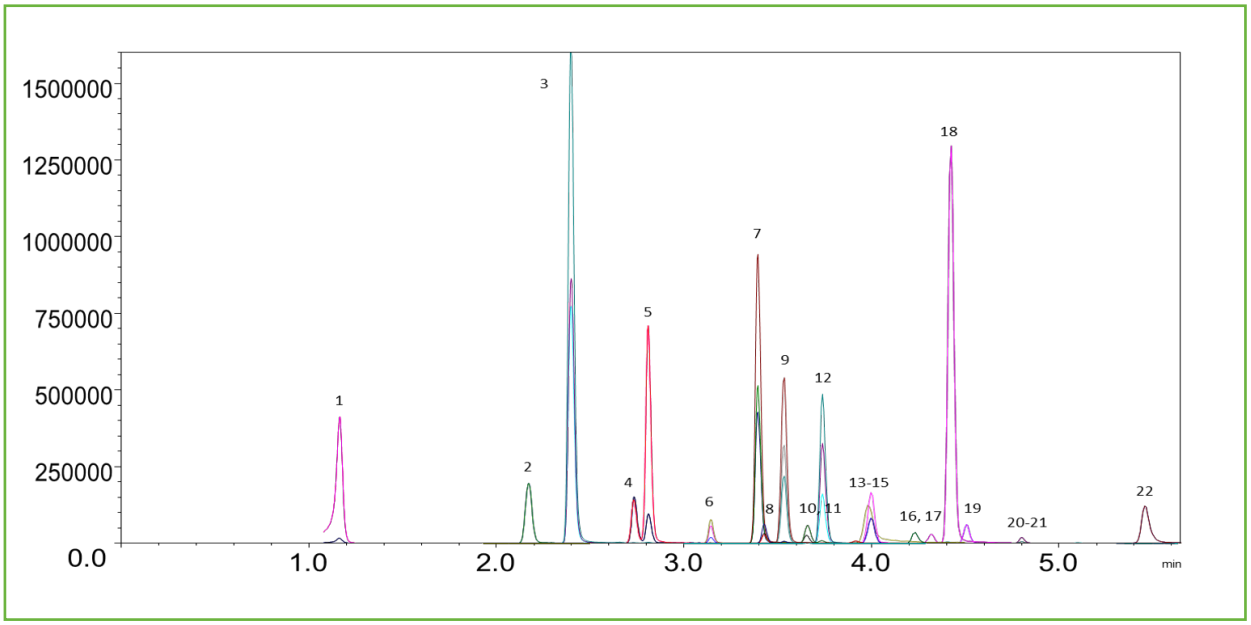
Peak #	Name	Retention Time (min)	m/z transition
52	Myclobutanil	10.194	289.0000>70.0000
53	Bendiocarb	10.194	224.1000>109.1000
54	Chlorotoluron	10.325	212.9000>72.0000
55	Terbumeton	10.355	226.1000>170.1000
56	Propargite	10.358	368.3000>231.2000
57	Pyracarbolid	10.415	218.1000>125.1000
58	Thiacloprid	10.462	253.0000>126.0500
59	Forchlorfenuron	10.473	248.1000>129.0000
60	Methabenzthiazuron	10.488	222.1000>165.2000
61	Carbofuran	10.489	222.2000>165.1000
62	Quinoclamine	10.515	208.0000>89.0000
63	Isoprocarb	10.576	194.1000>95.0000
64	Carbaryl	10.589	202.1000>145.1000
65	Metobromuron	10.623	259.0000>148.0500
66	Benoxacor	10.630	260.0000>149.1000
67	Buturon	10.731	237.1000>84.1000
68	Isoproturon	10.747	207.0000>72.1500
69	Sulfentrazone	10.789	387.0000>309.0000
70	Ethiofencarb	10.831	226.0000>107.1000
71	Naptalam	10.875	292.1000>144.1000
72	Thiobencarb	10.894	258.0000>125.0000
73	Tepraloxydim	10.963	342.2000>250.1500
74	Spiroxamine	11.083	298.0000>144.1500
75	Carboxin	11.087	236.0000>143.0500
76	Tebuthiuron	11.090	229.1000>172.4000
77	Fenpropimorph	11.266	304.2000>147.1000
78	Linuron	11.276	249.0000>159.9500
79	Fenobucarb	11.304	208.0000>95.1000
80	Siduron	11.377	233.3000>94.0000
81	Penconazole	11.393	284.1000>70.0000
82	Ethiprole	11.402	396.9500>350.8500
83	Ethoxyquin	11.452	218.0000>174.0500
84	Desmedipham	11.496	318.0000>182.5000
85	1-Dodecylguanidine	11.517	228.1000>71.1000
86	Phenmedipham	11.602	318.1000>168.0000
87	Disulfoton sulfoxide	11.612	291.0000>213.0000
88	Halofenozide	11.636	331.1000>105.0000
89	Azamethiphos	11.636	325.0000>183.0000
90	Promecarb	11.753	208.1000>109.0000
91	Thifensulfuron-methyl	11.798	388.1000>167.1000
92	Diethofencarb	11.802	268.2000>226.1000
93	Tridemorph	11.814	298.1000>130.1000
94	Flurtamone	11.950	334.1000>247.0500
95	Tebufenpyrad	11.950	334.0000>145.0000
96	Fenthion sulfone	11.956	311.0000>109.0000
97	Cyprodinil	11.960	226.0000>93.0000
98	Pencycuron	11.961	329.2000>125.1000
99	Fomesafen	12.044	456.1000>344.0000
100	Iprovalicarb	12.131	321.2000>119.2000
101	Flutolanil	12.154	324.1000>242.0000
102	Chlorantriniliprole	12.251	484.1000>452.9000

Peak #	Name	Retention Time (min)	m/z transition
103	Trinexapac-ethyl	12.252	253.2000>69.0000
104	Neburon	12.257	275.1000>88.1000
105	Isoxaflutole	12.308	360.1000>251.1000
106	Benalaxyl	12.316	326.2000>294.1000
107	Chloroxuron	12.407	291.1000>72.1000
108	Dimethametryn	12.409	256.1000>186.0500
109	Fenazaquin	12.439	307.1000>161.0000
110	Terbufos-sulfoxide	12.444	305.1000>186.8000
111	Ethofumesate	12.449	287.1000>258.9000
112	Fenamidone	12.493	312.1000>92.1000
113	Clethodim	12.528	360.0000>164.0500
114	Piperonyl butoxide	12.554	356.2000>177.2000
115	Boscalid	12.568	343.0000>307.0000
116	Methoxyfenozide	12.585	369.2000>149.0000
117	Bioresmethrin	12.619	339.2000>171.0500
118	Hydramethylnon	12.632	495.2000>323.2000
119	Rimsulfuron	12.698	432.1000>182.0000
120	Fenchlorphos oxon	12.699	304.9000>109.0000
121	Tralkoxydim	12.720	330.2000>284.1500
122	Epoxiconazole	12.721	330.1000>121.1000
123	Ipconazole Isomer	12.827	334.2000>70.0000
124	Thiofanox	12.834	219.2000>57.2000
125	Fenbuconazole	12.909	337.0000>124.9000
126	Zoxamide	12.910	336.1000>187.0000
127	Benthiazole	12.922	239.0000>179.9500
128	Isoxaben	13.019	333.2000>165.0000
129	Metconazole	13.032	320.2000>70.0500
130	Triflumuron	13.057	359.1000>156.0000
131	Mandipropamid	13.071	412.2000>328.1500
132	Isoprothiolane	13.084	291.0000>230.9500
133	Acibenzolar-s-methyl	13.166	210.9000>136.0000
134	Cyflufenamid	13.247	413.2000>295.1000
135	Dimethomorph	13.266	388.2000>301.1000
136	Flutriafol	13.278	302.1000>70.1000
137	Fenoxycarb	13.284	302.2000>116.0000
138	Spirotetramat	13.301	374.3000>302.1500
139	Novaluron	13.308	491.1000>471.1000
140	Fluquinconazole	13.393	376.1000>349.0500
141	Spinosad (Spinosyn A)	13.430	732.5000>142.2000
142	Bensulfuron-methyl	13.439	411.2000>149.1000
143	Cyazofamid	13.485	325.1000>108.0000
144	Carfentrazone-ethyl	13.515	412.1000>346.0000
145	Pinoxaden	13.527	401.2000>317.2000
146	Picoxystrobin	13.570	368.1000>145.0000
147	Pyraflufen-ethyl	13.610	413.1000>339.0000
148	Phoxim	13.632	299.0000>77.1000
149	Fenothiocarb	13.634	254.1000>72.1000

Peak #	Name	Retention Time (min)	m/z transition
150	Mefenacet	13.636	298.9000>148.0500
151	Triflusulfuron-methyl	13.659	493.2000>264.1000
152	Azoxystrobin	13.724	404.2000>372.1000
153	Hexaflumuron	13.726	462.8000>158.1000
154	Chlorimuron-ethyl	13.746	415.1000>186.0000
155	Haloxyfop-methyl	13.769	376.0500>316.0000
156	Lufenuron	13.782	509.0000>339.0000
157	Metaflumizone	13.788	507.2000>178.0000
158	Kresoxim-methyl	13.844	313.9500>267.3000
159	Anilofos	13.963	368.2000>125.0000
160	Tetraconazole	13.964	372.1000>159.0000
161	Sethoxydim	14.062	328.1000>296.3000
162	Famoxadone	14.078	392.0000>331.1000
163	Teflubenzuron	14.082	381.1000>141.2000
164	Clofentezine	14.122	303.0000>138.0000
165	Haloxyfop-etotyl	14.186	434.0500>315.9000
166	Trifloxystrobin	14.197	409.1000>186.1000
167	Pretilachlor	14.237	312.0000>252.1000
168	Diflubenzuron	14.265	328.0000>141.0000
169	Diclobutrazol	14.266	328.2000>70.2000
170	Fluoxastrobin	14.287	459.1000>427.1000
171	Flufenoxuron	14.374	489.1000>158.1000
172	Metrafenone	14.403	409.1000>209.0500
173	Piperophos	14.425	354.1000>170.9000
174	Fenoxaprop-ethyl	14.475	362.1000>288.1000
175	Pyraclostrobin	14.483	390.1000>194.1000
176	Benzoximate	14.560	364.0000>199.0000
177	Diniconazole	14.633	326.2000>70.2000
178	Isocarbophos	14.721	307.0000>121.1000
179	Spiromesifen	14.724	371.3000>273.2000
180	Chlorfluazuron	14.744	540.1000>383.0000
181	Chlorthiophos	14.761	360.7500>304.9000
182	Furathiocarb	14.772	383.2000>195.1000
183	Pyriproxyfen	14.821	322.0000>96.0000
184	Chinomethionate	14.833	235.0000>207.0500
185	Spirodiclofen	15.001	411.2000>71.1000
186	Propaquizafop	15.117	444.2000>100.1000
187	Avermectin B1a.	15.265	890.5000>567.5000
188	Rotenone	15.267	395.2000>213.1000
189	Fenpyroximate	15.339	422.3000>366.2000
190	Cyphenothrin	15.351	376.2000>181.0000
191	Phenothrin	15.423	351.2000>183.0000

255-M

LC-MS Separation of Mycotoxins on HALO® PFP, 2.7 µm



Peak ID	Mycotoxin	RT (min)	Precursor	Product	Peak ID	Mycotoxin	RT (min)	Precursor	Product
1	Nivalenol	1.166	313.2	175.1	12	Aflatoxin B1	3.738	313.1	241.8
2	Fusarenone X	2.172	355.1	175.1	13	Ochratoxin B	3.916	370.1	324.1
3	Neosolaniol	2.397	399.9	185.2	14	Citrinin	3.981	251.1	233.3
4	15- acetyldeoxyniva-lenol	2.732	339.1	321.3	15	T2 Toxin	3.998	489.3	245.2
5	3- acetyldeoxyniva-lenol	2.733	339.1	231.4	16	Ochratoxin A	4.231	405.1	239.2
6	Aflatoxin M1	3.143	329.1	273.6	17	Zearalenone	4.423	319.2	283.1
7	Diacetoxyscripenol	3.394	383.9	247.5	18	Sterigmatocystin	4.506	324.3	310.2
8	Aflatoxin G2	3.427	331.1	198.1	19	Fumonisin B2	4.801	706.8	336.1
9	Aflatoxin G1	3.534	329.1	243.3	20	Fumonisin B3	4.801	706.4	336.1
10	HT2 Toxin	3.653	447.2	345.6	21	Fumonisin B1	5.102	722.4	334.2
11	Aflatoxin B2	3.661	315.1	287.2	22	Beauvericin	5.459	783.9	244.1

TEST CONDITIONS:

Column: HALO 90 Å PFP, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-609
Mobile Phase A: Water, 2 mM Ammonium Formate, 0.1% Formic Acid
Mobile Phase B: Methanol, 2 mM Ammonium Formate, 0.1% Formic Acid
Gradient:

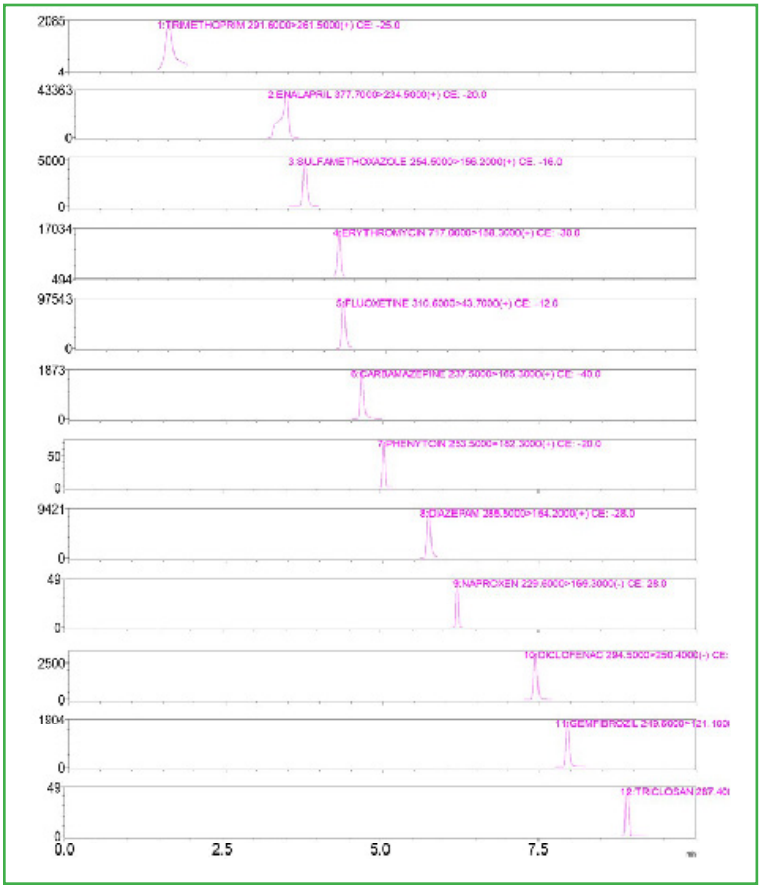
Time	%B
0.0	15
4.5	100
10.0	100

Flow Rate: 0.4 mL/min
Pressure: 280 bar
Temperature: 40 °C
Injection Volume: 7.0 µL
Sample Solvent: Methanol
Detection: +ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040

Mycotoxin contamination can have serious implications, including devastating economic losses, and human and animal death. It is imperative to successfully screen for these toxins to ensure the integrity of the food supply. Environmental analysis can be challenging due to matrix effects and interference, often resulting in low sensitivity and ambiguous results; therefore, it is critical to have a column that has superior performance. The HALO 90 Å PFP can not only meet these challenges, but exceed them by demonstrating high speed and sensitivity. The HALO 90 Å PFP is an ideal column to be used in environmental, and mycotoxin analysis.

LCMS of Pharmaceutical and Personal Care Products based on EPA 542

277-P



PEAK IDENTITIES:

Peak #	Compound	Transition	ESI Mode	CE
1	Trimethoprim	291.60>261.50	ESI +	25
2	Enalapril	377.70>234.50	ESI +	20
3	Sulfamethoxazole	254.50>156.20	ESI +	16
4	Erythromycin	717.00>158.30	ESI +	30
5	Fluoxetine	310.60>43.70	ESI +	12
6	Carbamazepine	237.50>165.30	ESI +	40
7	Phenytoin	253.50>182.30	ESI +	20
8	Diazepam	285.50>154.20	ESI +	28
9	Naproxen	229.30>169.30	ESI -	28
10	Diclofenac	294.50>250.40	ESI -	10
11	Gemfibrozil	249.60>121.10	ESI -	10
12	Triclosan	287.40>34.40	ESI -	8

Pharmaceutical and personal care products (PPCPs) have been a growing concern to our environment. These products, which include over-the-counter medications, veterinary prescriptions, soaps, lotions, and even insect repellents have entered the environment through various sources including municipal wastewater, polluting ground water, and even drinking water.

Validated LC-MS methods have been completed in order to screen for these wide range of chemical compounds, however, the methods can further be optimized in order to achieve better resolution and selectivity. LC-MS method development was performed based on the EPA 542 PPCP method in order to achieve an improved chromatographic separation using a HALO® RP-Amide column.

TEST CONDITIONS:

Column: HALO® RP-Amide, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-607
Mobile Phase A: Water, 0.1% formic acid
Mobile Phase B: Acetonitrile, 0.1% formic acid
Gradient:

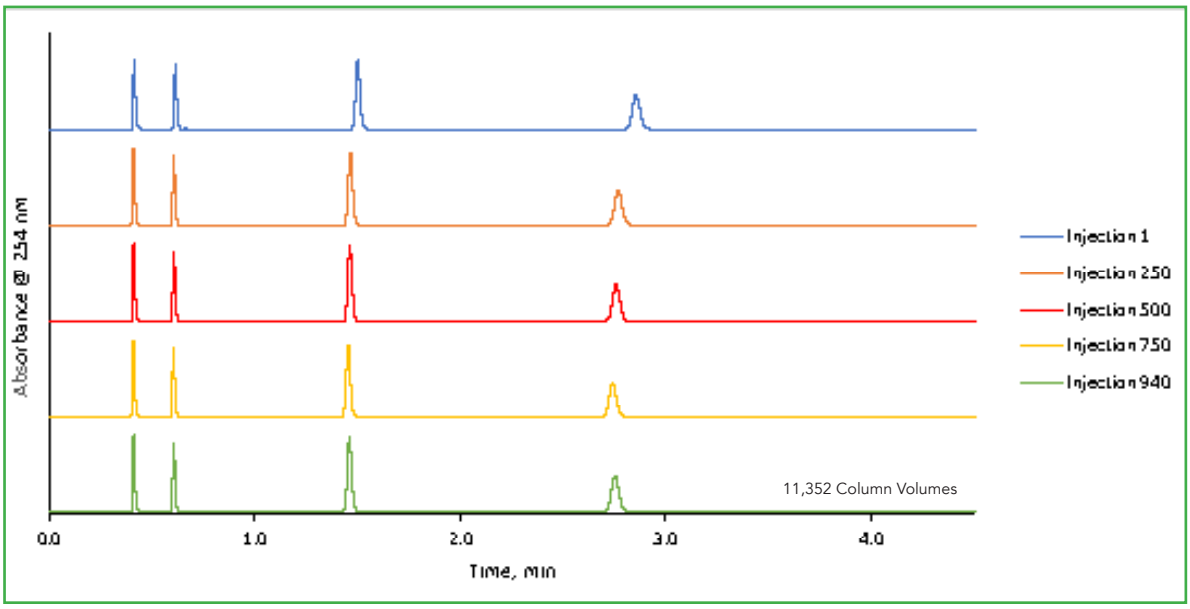
Time	%B
0.0	10
0.5	10
10.0	100
11.0	100

Flow Rate: 0.3 mL/min
Pressure: 213 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 Water/ Methanol

MS Conditions:
Detection: (+/-) ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040
Spray Voltage: 2.5 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 ˚C
Heat Block: 400 ˚C

HALO® PAH Stability at 600 bar

278-P



PEAK IDENTITIES:

1. Uracil
2. Phenol
3. 1-Cl-4-Nitrobenzene
4. Naphthalene

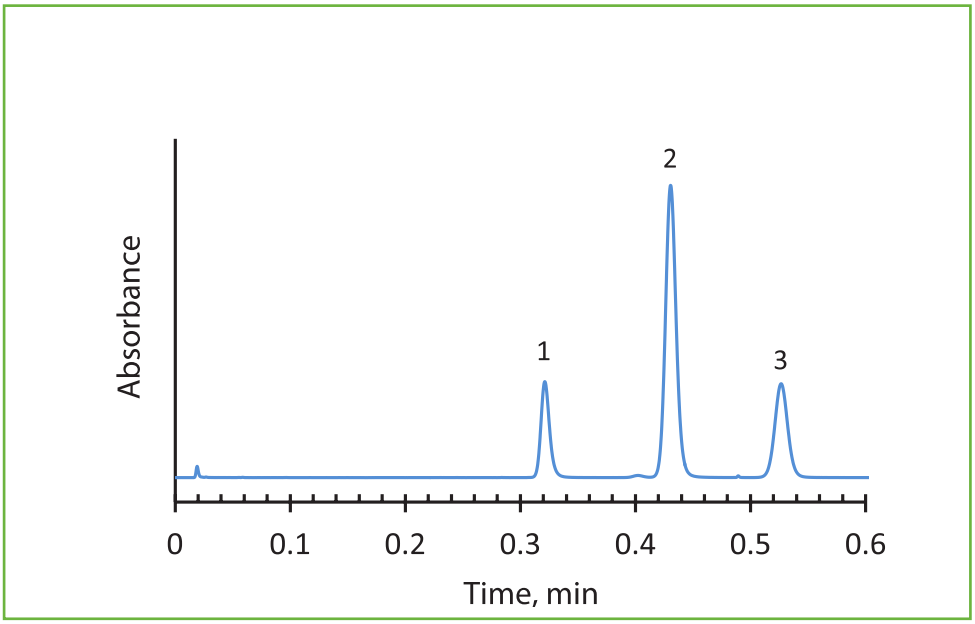
TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 2.1 x 150 mm
Part Number: 92842-712
Mobile Phase A: Water
B: Acetonitrile
Isocratic: 50% B
Flow Rate: 0.6 mL/min
Back Pressure: 597 bar
Temperature: 30 °C
Detection: 254 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: 60/40 ACN/ Water
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µl
LC System: Shimadzu Nexera X2

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of more than 100 chemicals released from the combustion of coal, oil, gasoline, tobacco, and wood. They can also be found in cooked food. PAHs are persistent chemicals and must be closely regulated for early detection/monitoring to minimize hazardous exposure in the environment and/or use of contaminated raw materials in different industries. The HALO® PAH column shows excellent stability at elevated back pressure making it an excellent choice for polycyclic aromatic hydrocarbon analysis.

Separation of Carbamate Pesticides on HALO® ES-CN Phase

Application Note 60-CB



PEAK IDENTITIES:

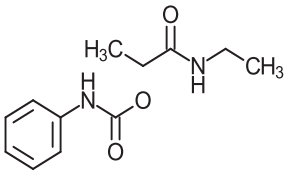
1. Carbetamide
2. Propham
3. Chlorpropham

This separation illustrates a rapid HPLC determination of three carbamate pesticides on the HALO® ES-CN phase in just over half of a minute. The unique Fused-Core® technology allows the use of high flow rates at moderate pressures while retaining high efficiency.

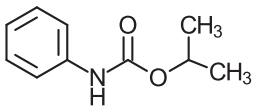
TEST CONDITIONS:

Column: HALO 90 Å ES-CN, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-404
Mobile Phase: 40/60 - A/B
A: Water
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 165 bar
Temperature: 30 °C
Detection: UV 240 nm, VWD
Injection Volume: 0.2 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

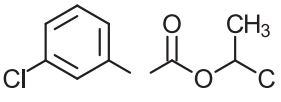
STRUCTURES:



Carbetamide



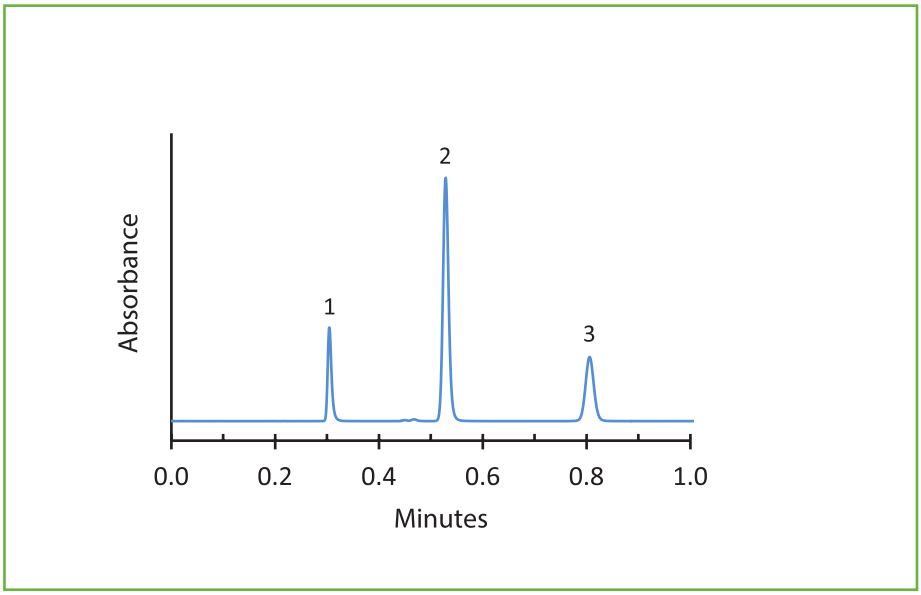
Propham



Chlorpropham

Separation of Carbamate Pesticides on HALO® C18 Phase

Application Note 61-CB



PEAK IDENTITIES:

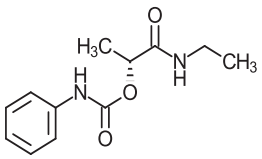
1. Carbetamide
2. Propham
3. Chlorpropham

This separation illustrates a rapid HPLC determination of three carbamate pesticides on the HALO® C18 phase in just under a minute. The Fused-Core® technology allows the use of high flow rates at moderate pressures while retaining high efficiency.

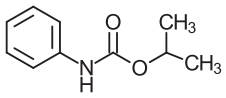
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 40/60 - A/B
A: Water
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 130 bar
Temperature: 30 °C
Detection: UV 240 nm, VWD
Injection Volume: 0.2 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

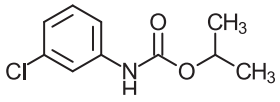
STRUCTURES:



Carbetamide



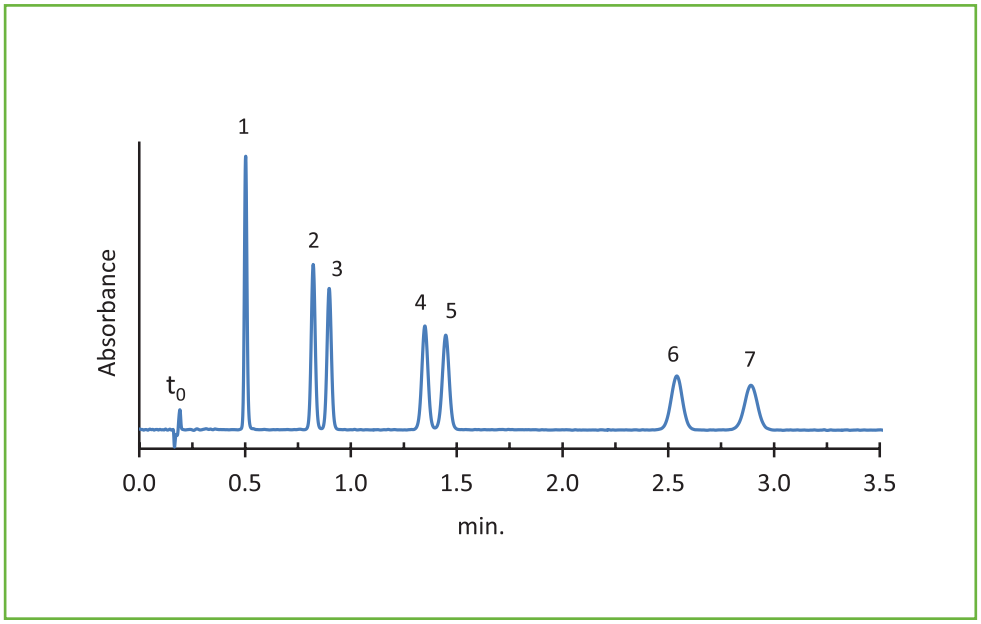
Propham



Chlorpropham

Rapid Separation of Triazine Pesticides on HALO® C18 Phase

Application Note 41-TR



PEAK IDENTITIES:

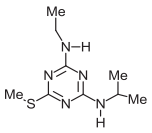
1. Simazine
2. Atrazine
3. Prometon
4. Ametryn
5. Propazine
6. Prometryn
7. Terbutryn

This triazine pesticides mixture can be rapidly separated on a HALO® Fused-Core® C18 column while retaining good peak shape and high column efficiency.

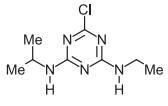
TEST CONDITIONS:

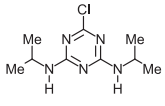
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 50/50 - A/B
A: 0.02 M Ammonium formate, adj. to pH 6.0
B: Acetonitrile
Flow Rate: 2.5 mL/min
Pressure: 270 bar
Temperature: 30 °C
Detection: UV 220 nm, VWD
Injection Volume: 0.3 µL
Sample: Supelco Triazine Pesticides Mix-48392
Sample Solvent: Methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

STRUCTURES:

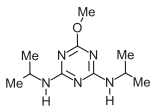


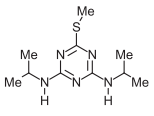
Ametryn



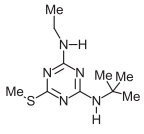


Propazine





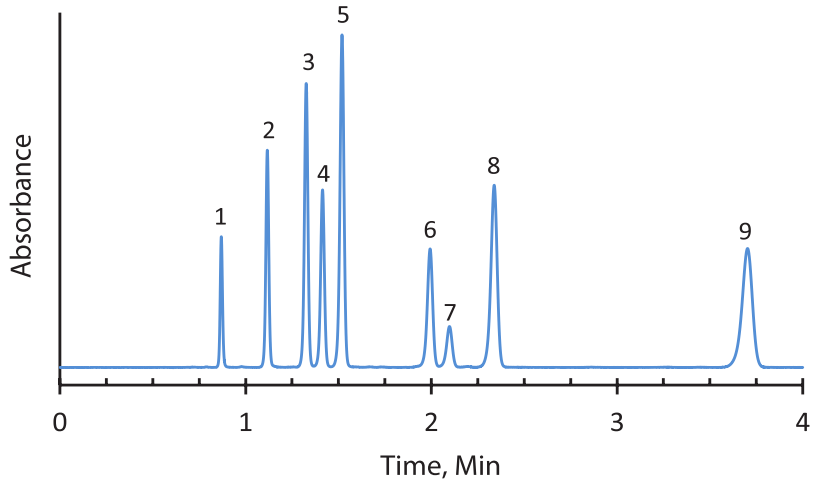
Prometryn



Terbutryn

Separation of Phenyl Urea Pesticides on HALO® Phenyl-Hexyl Phase

Application Note 55-PU



PEAK IDENTITIES:

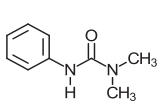
1. Fenuron
2. Monuron
3. Fluomethuron
4. Isoproturon
5. Diuron
6. Siduron A
7. Siduron B
8. Linuron
9. Neburon

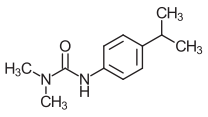
This separation illustrates the use of the highly efficient HALO® Fused-Core® Phenyl- Hexyl stationary phase in the analysis of common herbicides. The short run times allow analyses using isocratic conditions so that column equilibration time is not required between runs.

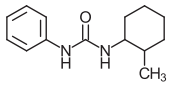
TEST CONDITIONS:

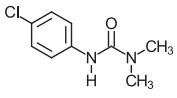
Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-606
Mobile Phase: 50/50 - A/B
A: 0.025 M Potassium phosphate buffer, adj. to pH 2.5
B: Acetonitrile
Flow Rate: 1.5 mL/min
Pressure: 220 bar
Temperature: 30 °C
Detection: UV 245 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

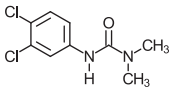
STRUCTURES:

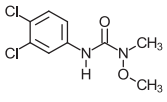

Fenuron

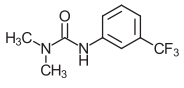

Isoproturon

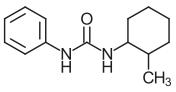

Siduron B

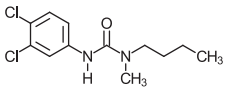

Monuron


Diuron


Linuron

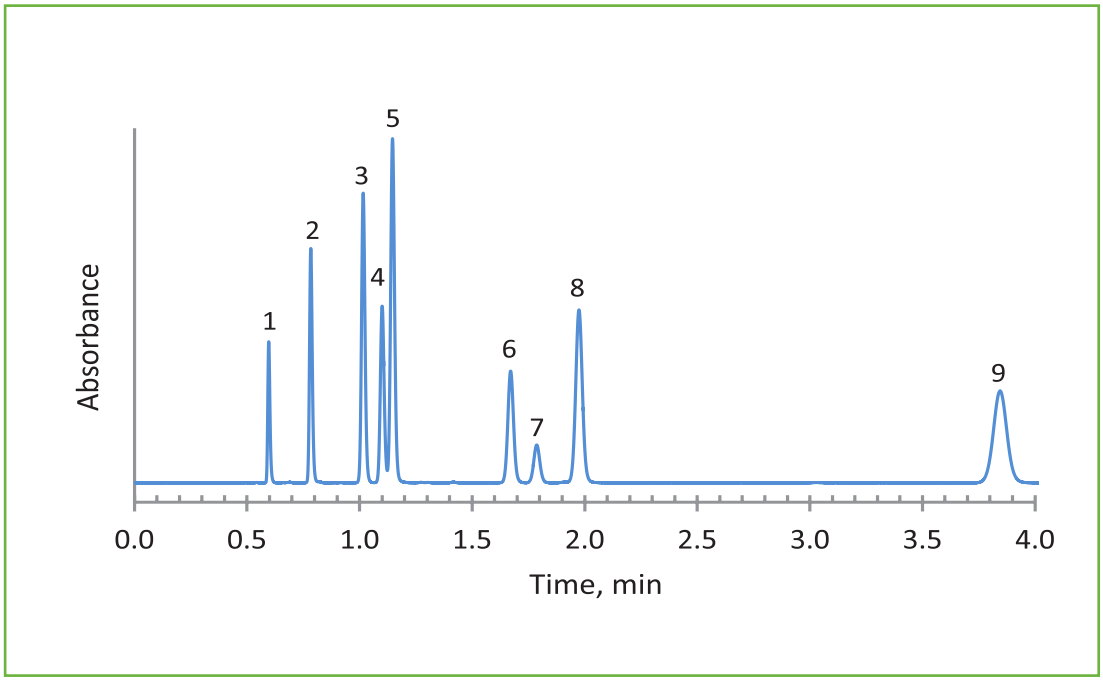

Fluomethuron


Siduron A


Neburon

Separation of Phenyl Urea Pesticides on HALO® C18 Phase

Application Note 59-PU



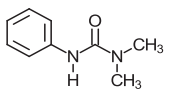
PEAK IDENTITIES:

1. Fenuron
2. Monuron
3. Fluomethuron
4. Isoproturon
5. Diuron
6. Siduron A
7. Siduron B
8. Linuron
9. Neburon

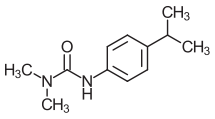
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-602
Mobile Phase: 50/50 - A/B
A: 0.025 M potassium phosphate buffer, adj. to pH 2.5
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 300 bar
Temperature: 30 °C
Detection: UV 245 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

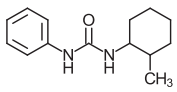
STRUCTURES:



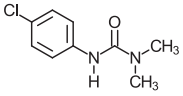
Fenuron



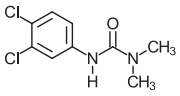
Isoproturon



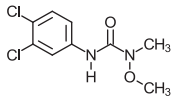
Siduron B



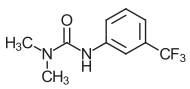
Monuron



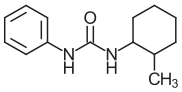
Diuron



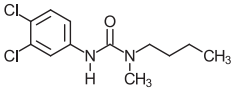
Linuron



Fluomethu ron



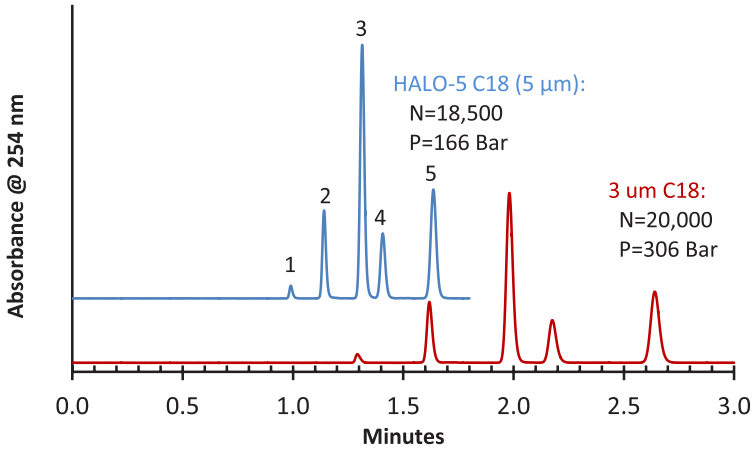
Siduron A



Neburon

Comparison of Separations on HALO® 5 µm Fused-Core® C18 and a Competitive 3.0 µm Totally Porous C18 Phase

Application Note 73-PS



PEAK IDENTITIES:

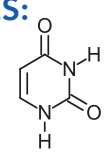
1. Uracil (t_0)
2. Fenuron
3. Monuron
4. Fluometuron
5. Diuron

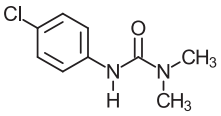
The chromatograms pictured show similar column efficiencies between the two packings but with much lower back pressure in the case of the HALO® 5 µm, allowing users with lower pressure HPLC instruments to get 3.0 µm particle performance with the lower pressure requirement of a 5 µm particle.

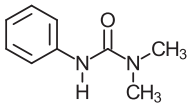
TEST CONDITIONS:

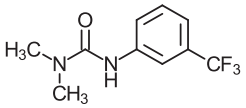
Columns:
1) HALO 90 Å C18, 5 µm, 4.6 x 150 mm
Part Number: 95814-702
2) Totally porous C18, 3.0 µm, 4.6 x 150 mm
Mobile Phase: 25/75 - A/B
A: 0.02 M potassium phosphate buffer, adj. to pH 3.0
B: Methanol
Flow Rate: 1.3 mL/min
Pressure: 166 bar (HALO®)
306 bar (competitor)
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 50/50 water/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

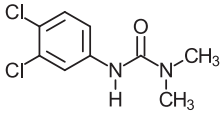
STRUCTURES:


Uracil


Monuron

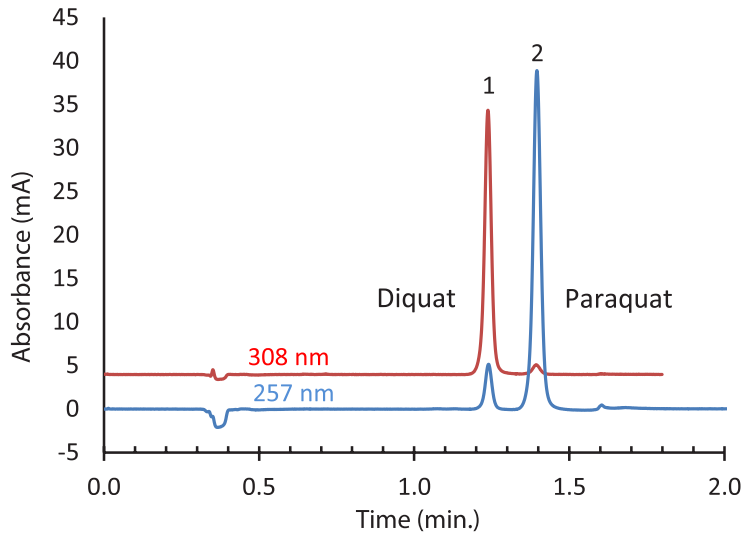

Fenuron


Fluometuron


Diuron

Separation of Nonselective Herbicides on HALO® Phenyl-Hexyl, 5 µm

Application Note 131-P



PEAK IDENTITIES:

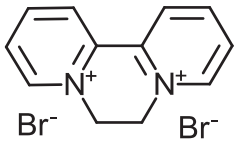
1. Diquat dibromide
2. Paraquat dichloride

The herbicides paraquat and diquat may be separated rapidly in under 2 minutes using a HALO® 5 µm Phenyl-Hexyl HPLC column. Large injection volumes are required to achieve the desired sensitivity. The separation conditions are based on the EPA method 549.2.

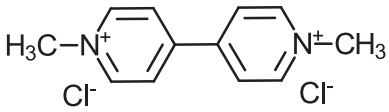
TEST CONDITIONS:

Column: HALO 90 Å Phenyl-Hexyl, 5 µm
3.0 x 100 mm
Part Number: 95813-606
Mobile Phase: 13.5 mL orthophosphoric acid, 10.3 mL diethylamine and 3.0 g of hexane-sulfonic acid, sodium salt in 1 L of water
Flow Rate: 1.0 mL/min
Pressure: 156 bar
Temperature: 30 °C
Detection: UV 257, 308 nm, VWD
Injection Volume: 40 µL
Sample Solvent: Water
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

STRUCTURES:



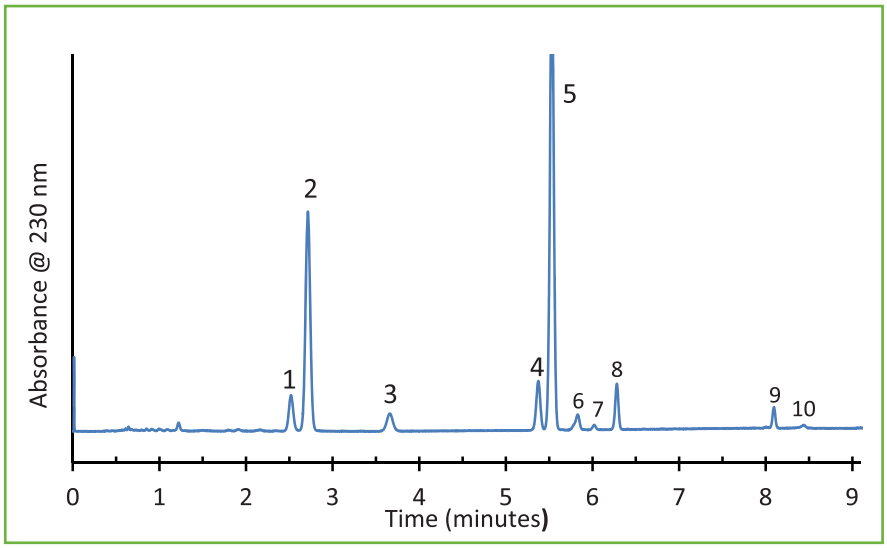
Diquat Dibromide



Paraquat Dichloride

Separation of Six Pyrethrins on HALO® C18, 5 µm

Application Note 161-PS



PEAK IDENTITIES:

1. Cinerin II
2. Pyrethrin II
3. Jasmolin II
4. Cinerin I
5. Pyrethrin I
6. Unknown
7. Unknown
8. Jasmolin I
9. Unknown
10. Unknown

Pyrethrins are potent insecticides that affect the nervous systems of insects. These six pyrethrin isomers can be separated rapidly using a HALO® 5 µm C18 column with low back pressure and good resolution.

TEST CONDITIONS:

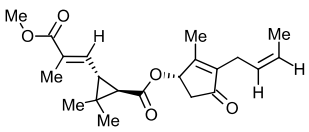
Column: HALO 90 Å C18, 5 µm, 3.0 x 150 mm
Part Number: 95813-702
Mobile Phase:
A: Water
B: Acetonitrile

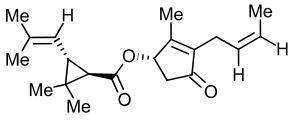
Gradient:

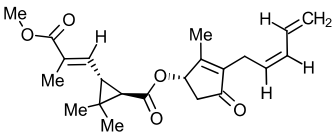
Time (min)	% B
0.0	60
3.0	60
5.0	72
7.0	90
9.0	90

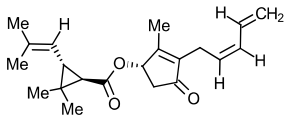
Flow Rate: 1.1 mL/min
Pressure: 170 bar
Temperature: 30 °C
Detection: UV 230 nm, VWD
Injection Volume: 3.0 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Data Rate: 17 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

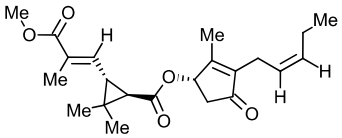
STRUCTURES:

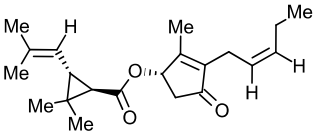

Cinerin II


Cinerin I


Pyrethrin II

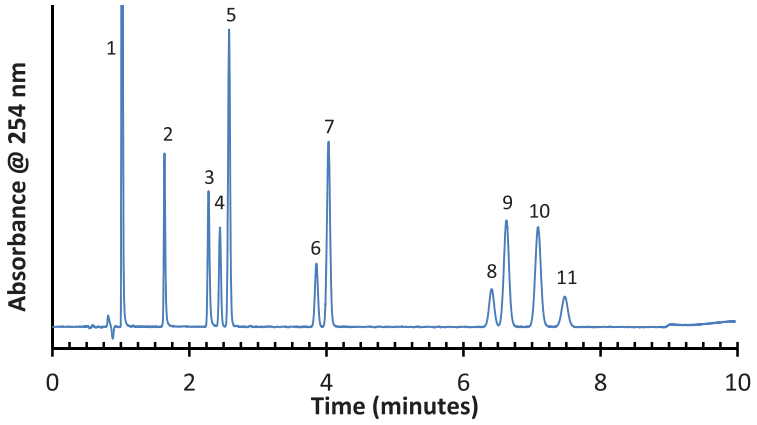

Pyrethrin I


Jasmolin II


Jasmolin I

Separation of Triazine Pesticides on HALO® AQ-C18, 2.7 µm

Application Note 163-PS



PEAK IDENTITIES:

1. Acetone (solvent)
2. Atraton
3. Prometon
4. Simazine
5. Simetryn
6. Atrazine
7. Ametryn
8. Propazine
9. Prometryn
10. Terbutryn
11. Terbuthylazine

Triazianes are a class of common herbicides that reduce weeds and increase crop yields. The wide use of these chemicals has created concern about the levels in soil and water. They can be analyzed using a HALO® AQ-C18 column in a fast gradient mode.

TEST CONDITIONS:

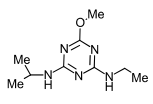
Column: HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-722
Mobile Phase:
A: 0.02 M sodium phosphate buffer, pH 3.0
B: Acetonitrile

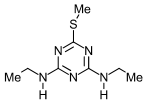
Gradient:

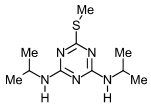
Time (min)	% B
0.0	40
8.0	40
10.0	75

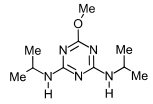
Flow Rate: 1.6 mL/min
Initial Pressure: 310 bar
Temperature: 35 °C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: 25/75 acetone/acetonitrile
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

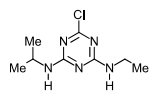
STRUCTURES:

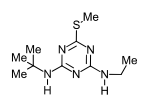

Atraton

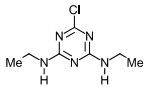

Simetryn

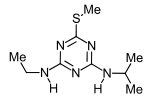

Prometryn

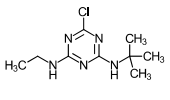

Prometon

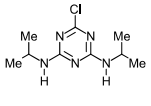

Atrazine


Terbutryn


Simazine

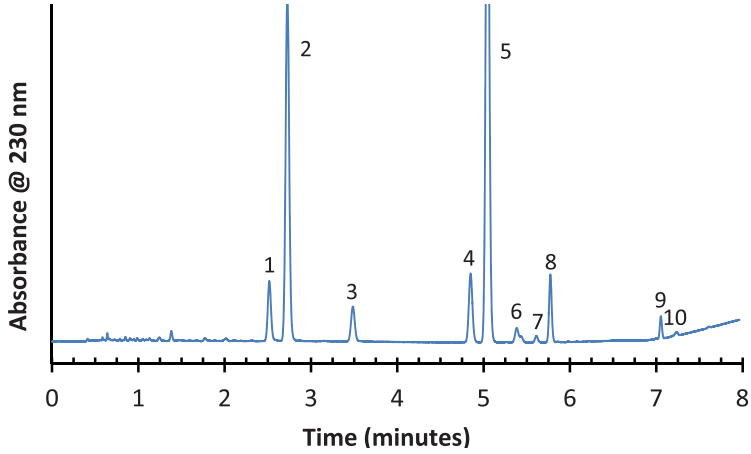

Ametryn


Terbuthylazine


Propazine

Separation of Six Pyrethrins on HALO® AQ-C18, 2.7 µm

Application Note 164-PS



PEAK IDENTITIES:

1. Cinerin II
2. Pyrethrin II
3. Jasmolin II
4. Cinerin I
5. Pyrethrin I
6. Unknown
7. Unknown
8. Jasmolin I
9. Unknown
10. Unknown

Pyrethrins are insecticides derived from chrysanthemum flowers. The extracted chemicals can paralyze the nervous systems of insects and lead to death. These naturally occurring pyrethrin isomers can be separated rapidly with good resolution using a HALO® AQ-C18 column.

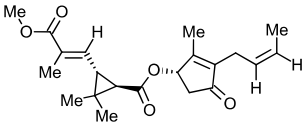
TEST CONDITIONS:

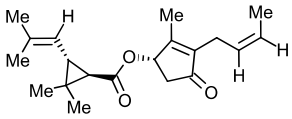
Column: HALO 90 Å AQ-C18, 2.7 µm, 3.0 x 100 mm
Part Number: 92813-622
Mobile Phase:
A: 0.02 M sodium phosphate buffer, pH 3.0
B: Acetonitrile
Gradient:

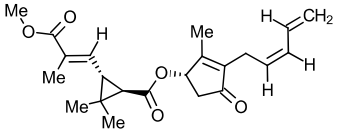
Time (min)	% B
0.0	65
2.5	65
5.0	75
6.0	90
8.0	90

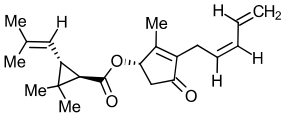
Flow Rate: 2.2 mL/min
Pressure: 245 bar
Temperature: 30 °C
Detection: UV 230 nm, VWD
Injection Volume: 4.0 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
Data Rate: 25 Hz
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

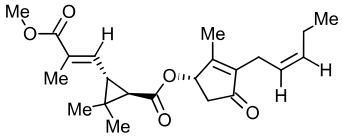
STRUCTURES:

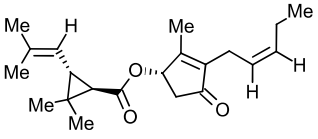

Cinerin II


Cinerin I


Pyrethrin II

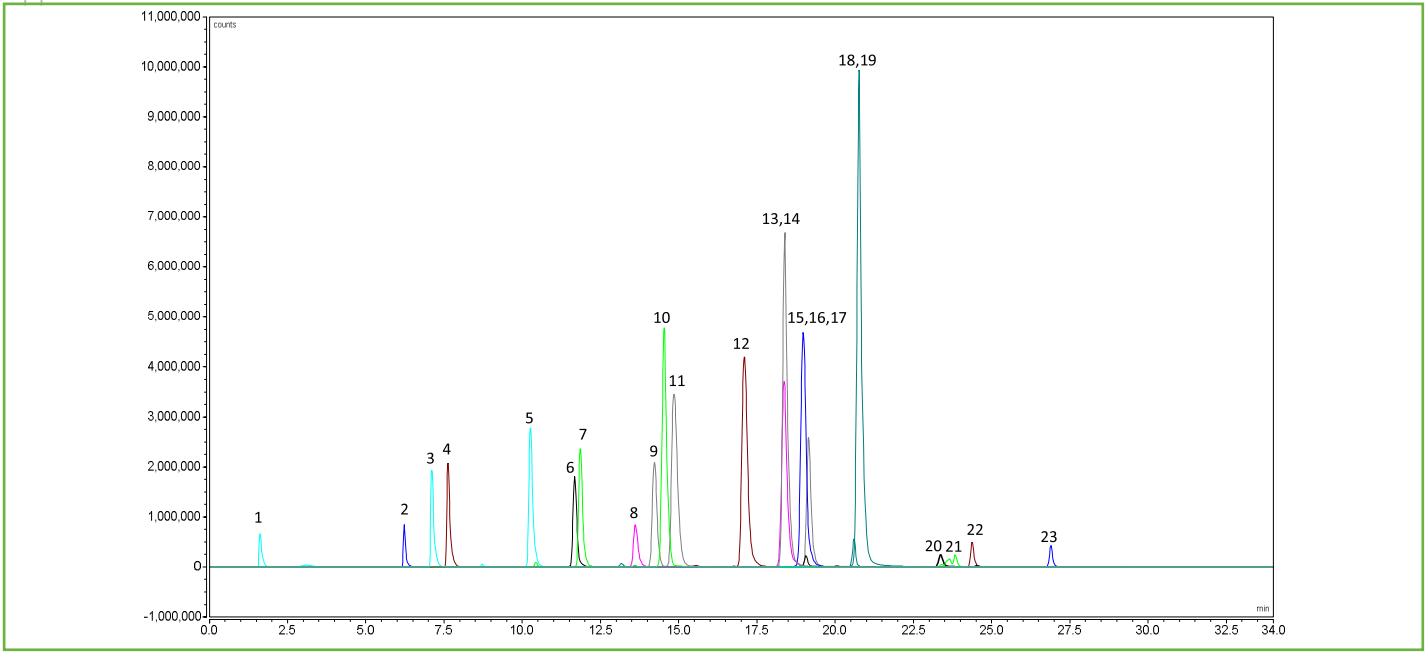

Pyrethrin I


Jasmolin II


Jasmolin I

Pesticides Separation on HALO 90 Å Biphenyl

Application Note 168-PS



TEST CONDITIONS:

Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-611
Mobile Phase:
A: Water/0.1% formic acid/4 mM ammonium formate
B: Acetonitrile/0.1% formic acid/4 mM ammonium formate

Gradient:

Time (min)	%B
0.00	0
1.01	15
4.00	35
5.00	62
30.00	100
34.00	100

Flow Rate: 0.2 mL/min
Initial Pressure: 89 bar
Temperature: 40 °C
Detection: UV 254 nm
Injection Volume: 1.0 µL
Sample Solvent: Acetonitrile
Data Rate: 10 Hz
LC System: Shimadzu Nexera X2
MS System: Thermo Fisher Orbitrap VelosPro ETD
ESI: +3.8 kV
Scan range: 150-1000 m/z
Scan Rate: 1.33 pps
Capillary: 350 ˚C
Sheath Gas: 35
Auxiliary Gas: 10
Scan Time: 2 µscans/50 ms max inject time
Heater Temperature: 150 ˚C

A mixture of pesticides with a wide range of polarities is separated with high efficiency using a HALO 90 Å Biphenyl column. Closely-eluting and co-eluting compounds are easily identified using mass spectrometry detection, and quantified using extracted-ion chromatograms (see page 2 for peak identities). Pesticides, such as these, are commonly screened for in medical marijuana samples.

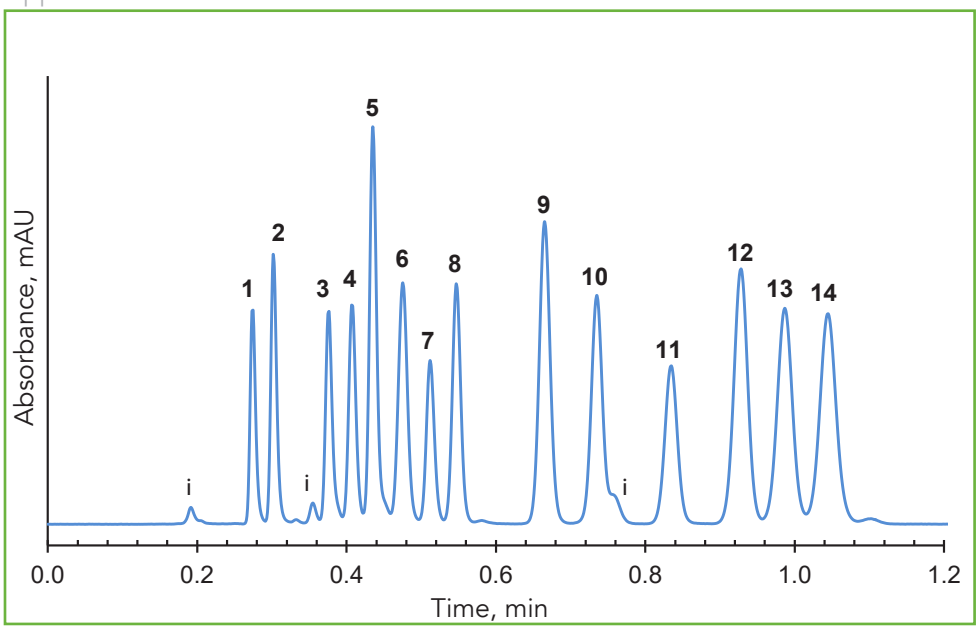
PEAK IDENTITIES:

	Compound	m/z	Retention (min)
1	Daminozide	161.096	1.616
2	Flonicamid	230.000	6.224
3	Thiamethoxam	292.000	7.109
4	Imidacloprid	256.050	7.631
5	Paclobutrazol	294.130	10.256
6	Fenhexamid	302.079	11.678
7	Myclobutanil	289.129	11.849
8	Bifenazate	301.150	13.610
9	Dimethomorph Isomer 1	388.130	14.226
10	Spirotetramat	374.190	14.535
11	Dimethomorph Isomer 2	388.130	14.846
12	Spinosad A	732.480	17.089
13	Spinosad D	746.490	18.363
14	Trifloxystrobin	409.100	18.391
15	Spinetoram	748.520	18.970
16	Pyrethrin II	373.200	19.068
17	Piperonyl butoxide	356.240	19.151
18	Pyrethrin I	329.210	20.594
19	Etoxazole	360.180	20.759
20	Abamectin A	895.500	23.370
21	Cypermethrin	433.110	23.610
22	Bifenthrin	440.160	24.370
23	Acequinocyl	407.230	26.890
observed in negative ion mode	Fludioxonil	247.048	9.763

An important advantage of the HALO 90 Å Biphenyl column is that it can be used with 100% aqueous mobile phase without pore dewetting and loss of retention. This is especially useful for very polar pesticides, which are sometimes unretained or poorly retained on other column phases.

Rapid HPLC Separation of Aromatic Compounds on HALO® Phenyl-Hexyl

Application Note 86



PEAK IDENTITIES:

1. Uracil
2. Benzamide
3. Benzonitrile
4. Propyl paraben
5. Benzylbenzoate
6. Diethylphthalate
7. Toluene
8. 1-Chloro-4-nitrobenzene
9. Di-n-Propylphthalate
10. n-Propylbenzene
11. n-Butylbenzene
12. Biphenyl
13. Acenaphthene
14. Phenanthrene

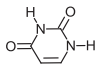
i = Unknown compound

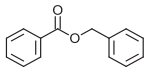
The high efficiency of the HALO® Fused-Core® Phenyl-Hexyl stationary phase allows the rapid separation of 14 compounds in under 1.2 minutes. This feature will speed up method development and also result in shorter analysis times.

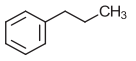
TEST CONDITIONS:

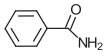
Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 23/77 - A/B
A: Water
B: Methanol
Flow Rate: 1.8 mL/min
Pressure: 400 bar
Temperature: 40 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Flow Cell: 5.0 µL low-volume
LC System: Agilent 1100

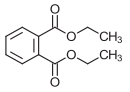
STRUCTURES:

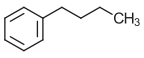

Uracil


Benzylbenzoate


n-Propylbenzene

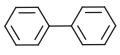

Benzamide

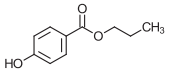

Diethylphthalate

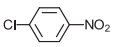

n-Butylbenzene


Benzonitrile

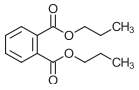

Toluene

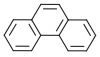

Biphenyl


Propylparaben


1-Chloro -4-nitrobenzene

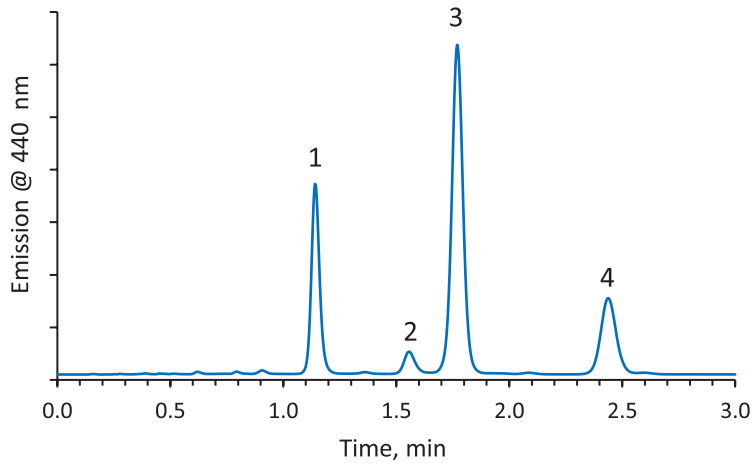

Acenaphthene


Di-n-P ropylphthalate


Phenanthrene

Isocratic Separation of Aflatoxins on HALO® C18

Application Note 144-M



PEAK IDENTITIES:

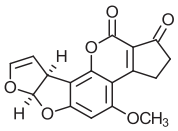
1. Aflatoxin B1
2. Aflatoxin B2
3. Aflatoxin G1
4. Aflatoxin G2

Aflatoxins are classified as mycotoxins, which are secondary metabolites produced by fungi. Under certain conditions, the fungi can grow on corn, peanuts, or tree nuts resulting in the production of aflatoxins, which are extremely toxic. A fast and sensitive method for separating four aflatoxins is demonstrated using a short HALO® C18 column.

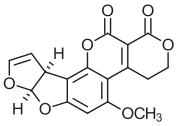
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm
Part Number: 92812-402
Mobile Phase:
A: Water
B: 50/50 acetonitrile/methanol
Isocratic: 74/26 - A/B
Flow Rate: 0.8 mL/min
Pressure: 365 bar
Temperature: 30 °C
Detection: Fluorescence Excitation - 360 nm; Emission - 440 nm
Injection Volume: 5.0 µL
Sample Solvent: 70/30 water/methanol
Response Time: 0.05 sec
Data Rate: 5 Hz
Flow Cell: 3.0 µL
LC System: Shimadzu Nexera X2

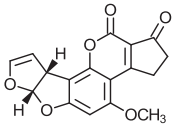
STRUCTURES:



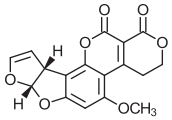
Aflatoxin B1



Aflatoxin G1



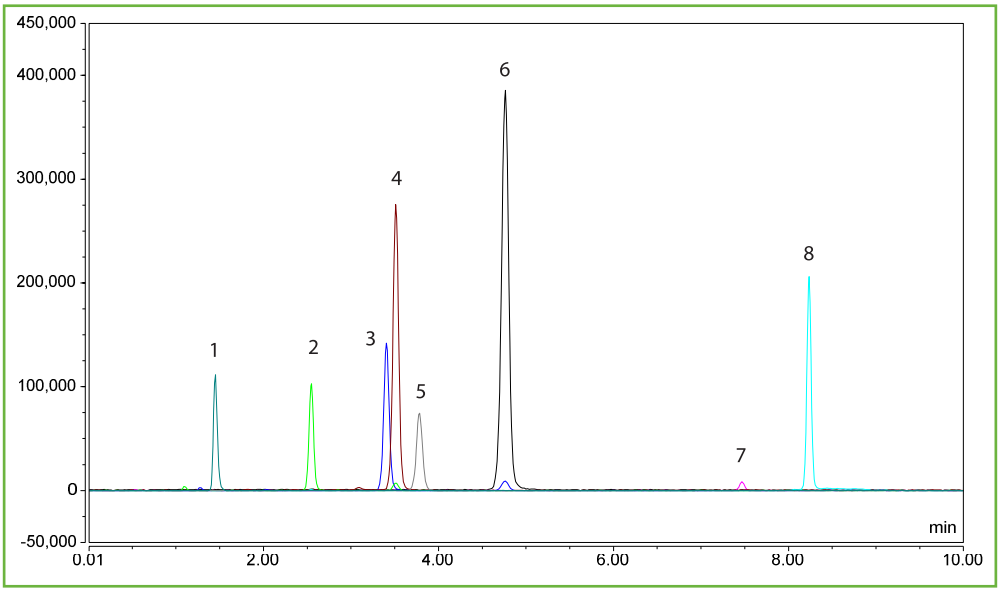
Aflatoxin B2



Aflatoxin G2

LC-MS Analysis of Multiple Mycotoxins on HALO 90 Å Biphenyl

Application Note 176-M



PEAK IDENTITIES:

1. Fumonisin B1 (m/z: 722.8)
2. Aflatoxin G2 (m/z: 331.3)
3. Aflatoxin B2 (m/z: 315.3)
4. Aflatoxin G1 (m/z: 329.3)
5. Fumonisin B2 (m/z: 706.8)
6. Aflatoxin B1 (m/z: 313.3)
7. Zearalenone (m/z: 319.4)
8. Ochratoxin A (m/z: 404.8)

Mycotoxins are a broad range of compounds that are metabolites of various types of fungi. The can be very toxic when eaten by humans or animals. Many foods and feeds, especially nuts are analyzed for this reason. Here, a HALO® Biphenyl column is used with a mass spectrometer detector to analyze a variety of these toxic compounds.

TEST CONDITIONS:

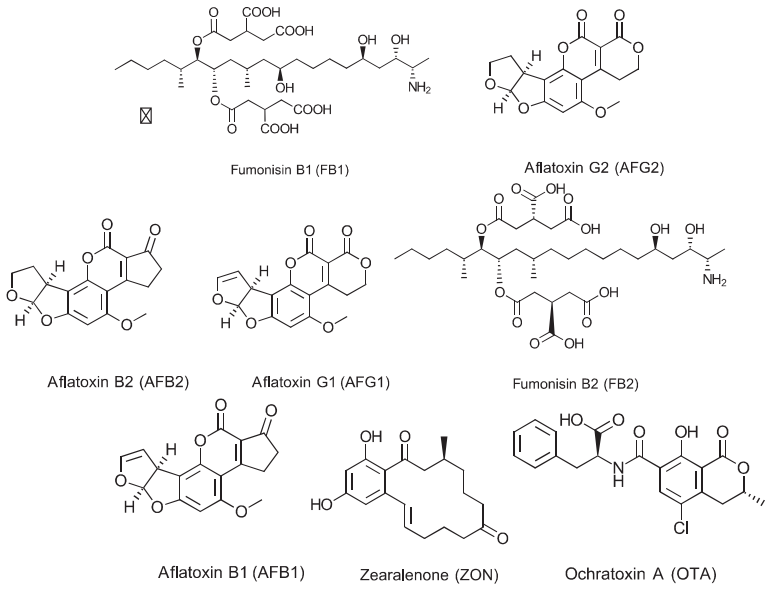
Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-611
Mobile Phase:
A: Water with 0.1% formic acid/ 5mM ammonium formate
B: Acetonitrile with 0.1% formic acid/ 5mM ammonium formate

Gradient:

Time (min)	%B
0.0	32
5.0	34
10.0	60

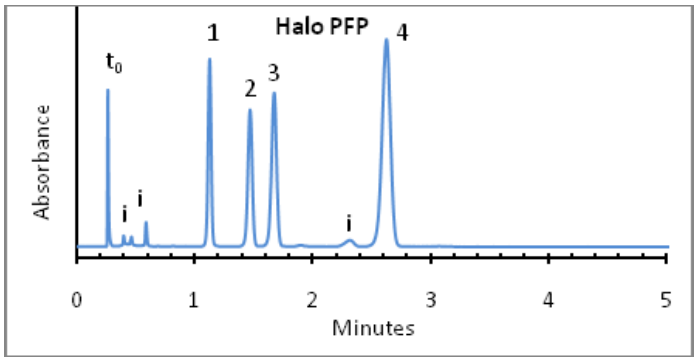
Flow Rate: 0.4 mL/min
Initial Pressure: 182 bar
Temperature: 40 °C
Detection: LC-MS
Injection Volume: 2.0 µL
MS System: Thermo Fisher Orbitrap VelosPro ETD
ESI: +4
Heat Block: 350 °C
Sheath Gas Flow: 34.88
Aux Gas Flow: 10.00

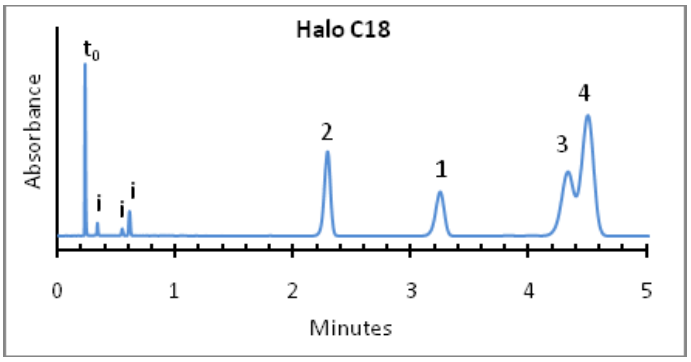
STRUCTURES:

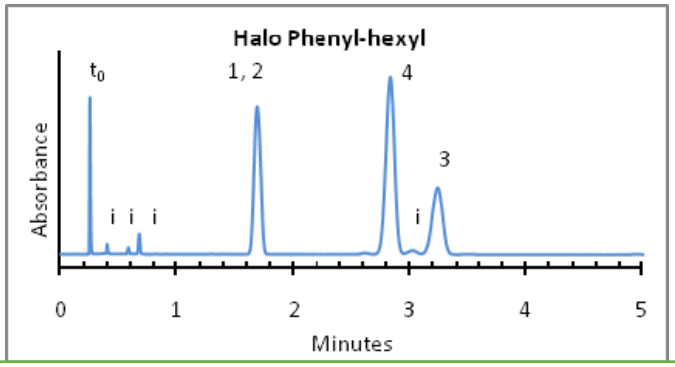


Separation of Neutral Aromatics on HALO® PFP, C18 and Phenyl-Hexyl

Application Note 23-N







TEST CONDITIONS:

Columns:
1) HALO 90 Å PFP, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-409
2) HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
3) HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 30/70 - A/B
A: Water
B: Methanol
Flow Rate: 2.0 mL/min
Pressure: ~250 bar
Temperature: 40 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

PEAK IDENTITIES:

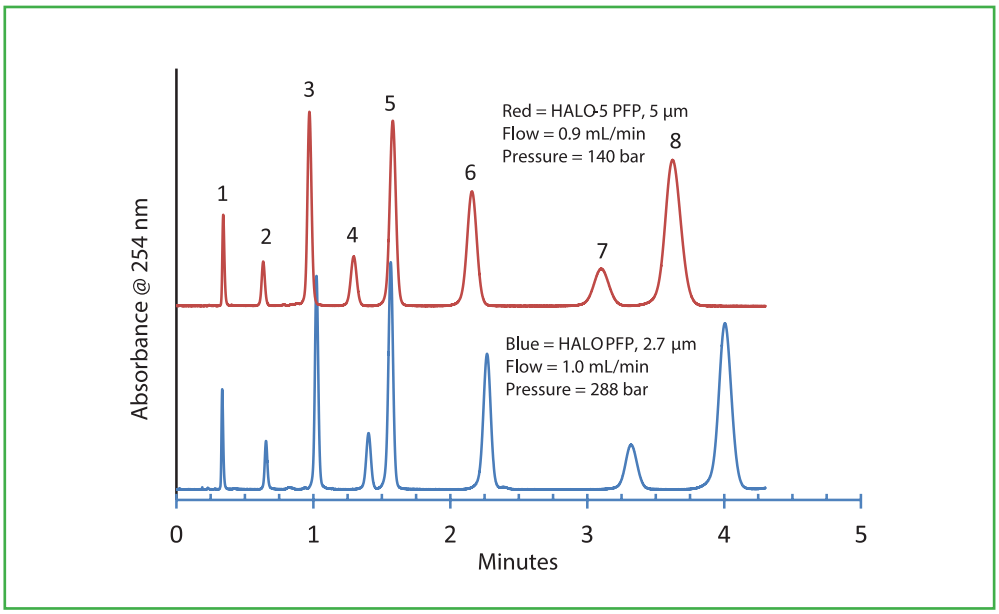
1. Butylbenzene
2. Acenaphthene
3. 1-Phenylnaphthalene
4. Pyrene

i = impurities

The separation of nonpolar aromatic compounds on these three HALO® bonded phases under the same conditions show differences in selectivity that can be utilized in optimizing difficult separations.

Comparable Selectivity Between HALO® 5 µm and HALO® 2.7 µm PFP Phases

Application Note 81-HA



PEAK IDENTITIES:

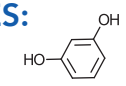
1. Resorcinol
2. Vanillin
3. Benzonitrile
4. Benzoin
5. Nitrobenzene
6. Benzanilide
7. Bisphenol A
8. Diethylphthalate

The similar selectivity between the 2.7 µm and the 5 µm HALO® PFP allows easy method transfer between these two particle size phases. Note the slight adjustment in flow to compensate for differences in void volume.

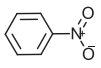
TEST CONDITIONS:

Columns:
1) HALO 90 Å PFP, 5 µm, 3.0 x 50 mm
Part Number: 95813-409
2) HALO 90 Å PFP, 2.7 µm, 3.0 x 50 mm
Part Number: 92813-409
Mobile Phase: 55/45 - A/B
A: 0.02 M KH_2PO_4 buffer, pH 3.0
B: Methanol
Flow Rate: See chart
Pressure: See chart
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

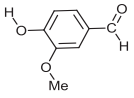
STRUCTURES:



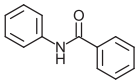
Resorcinol



Nitrobenzene

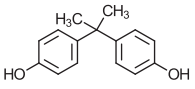


Vanillin

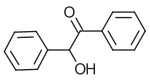


Benzanilide

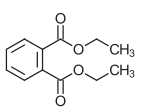
Benzonitrile



Bisphenol A



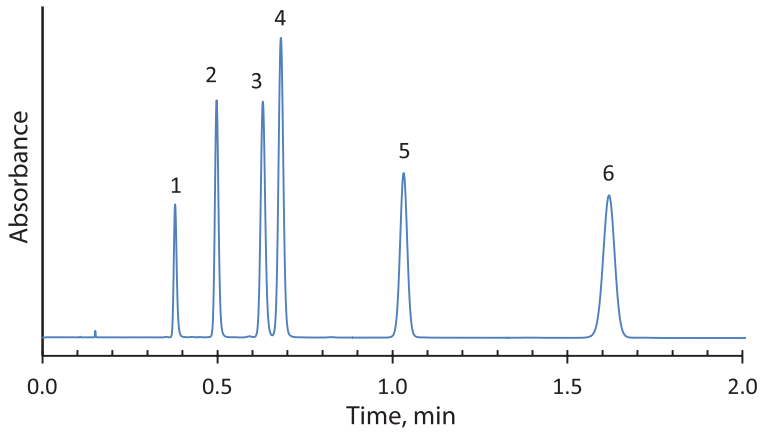
Benzoin



Diethylphthalate

Isocratic Separation of Phenyl Ureas on HALO® ES-CN

Application Note 54-P



PEAK IDENTITIES:

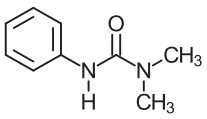
1. Fenuron
2. Monuron
3. Fluomethuron
4. Diuron
5. Linuron
6. Neburon

Phenyl urea compounds are common herbicides. Due to concern about these chemicals being in ground and drinking water, HPLC can be used to determine the levels present. In this separation, six phenyl ureas are analyzed on a HALO® RP-Amide column in under two minutes.

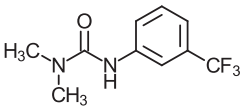
TEST CONDITIONS:

Column: HALO 90 Å ES-CN, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-404
Mobile Phase: 50/50 - A/B
A: 0.02 M phosphate buffer, adj. to pH 2.5
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 200 bar
Temperature: 20 °C
Detection: UV 245 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Acetonitrile/water
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

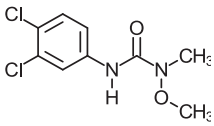
STRUCTURES:



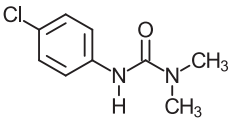
Fenuron



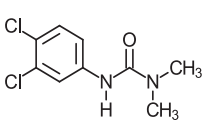
Fluomethuron



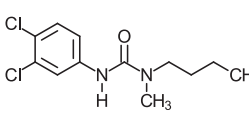
Linuron



Monuron



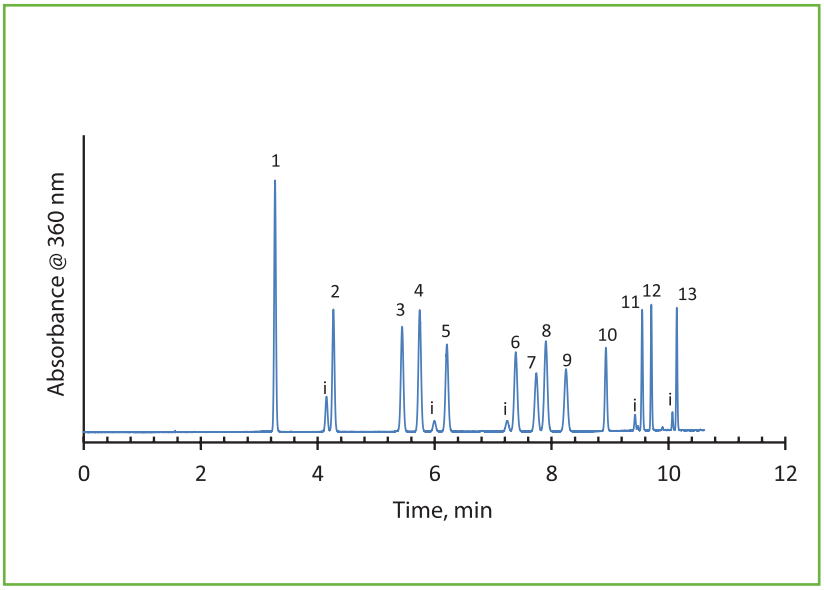
Diuron



Neburon

Separation of Carbonyl Compounds as Dinitrophenylhydrazone Derivatives on HALO® C18, 2.7 µm

Application Note 90-DNPH



PEAK IDENTITIES:

1. Formaldehyde-2,4-DNPH
2. Acetaldehyde-2,4-DNPH
3. Acetone-2,4-DNPH
4. Acrolein-2,4-DNPH
5. Propionaldehyde-2,4-DNPH
6. Crotonaldehyde-2,4-DNPH
7. 2-Butanone-2,4-DNPH
8. Methacrolein-2,4-DNPH
9. Butyraldehyde-2,4-DNPH
10. Benzaldehyde-2,4-DNPH
11. Valeraldehyde-2,4-DNPH
12. m-Tolualdehyde-2,4-DNPH
13. Hexaldehyde-2,4-DNPH

2,4-DNPH = 2,4-Dinitrophenylhydrazone
i = anti, syn, isomers of the respective DPNH derivatives

TEST CONDITIONS:

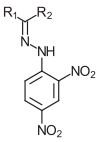
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-702
Mobile Phase: 55/45 - A/B
A: Water
B: Acetonitrile/THF (80/20)
Gradient:

Time (min)	% B
0.0	45
7.5	58
9.0	80
12.0	80

Flow Rate: 1.5 mL/min
Pressure: 355 bar
Temperature: 30 °C
Detection: UV 360 nm, VWD
Injection Volume: 0.3 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

This separation is based on modified EPA methods 8315 and 554 and achieves baseline resolution of the sample components by the use of a small particle size packing and a mobile phase containing both acetonitrile and tetrahydrofuran (THF). The addition of THF is necessary to achieve this resolution. As a result, peak elution order is also changed.

STRUCTURES:

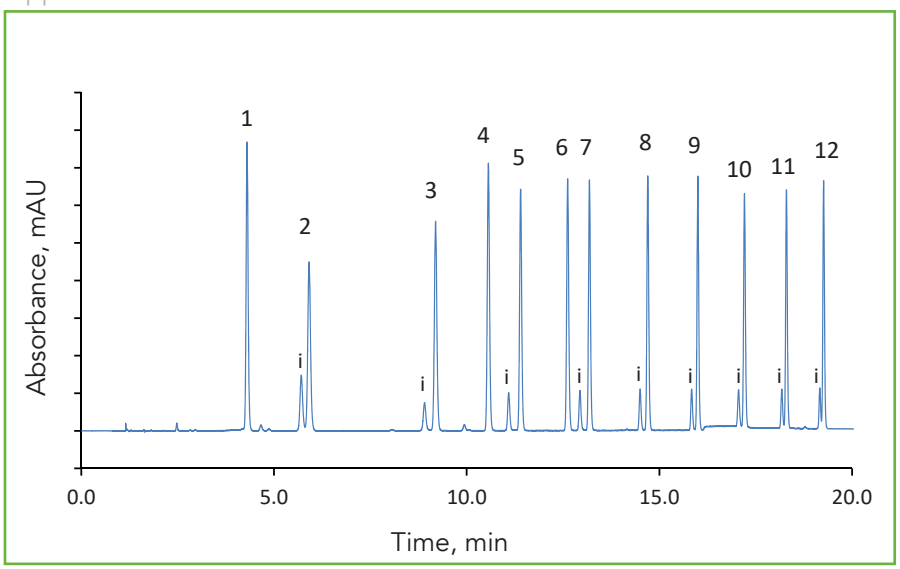


General -2,4-DNPH structure

Peak	R 1	R 2
1	-H	-H
2	-H	—CH_3
3	—CH_3	—CH_3
4	-H	CH_2 H
5	-H	CH_3
6	-H	H CH_3 H
7	—CH_3	CH_3
8	-H	CH_2 CH_3
9	-H	CH_3
10	-H	
11	-H	CH_3
12	-H	CH_3
13	-H	$(CH_2)_4$ CH_3

Separation of Carbonyl Compound DNPH Derivatives on HALO® C18, 5 µm

Application Note 156-DNPH



PEAK IDENTITIES:

1. Formaldehyde-2,4-DNPH
2. Acetaldehyde-2,4-DNPH
3. Propionaldehyde-2,4-DNPH
4. Crotonaldehyde-2,4-DNPH
5. Butyraldehyde-2,4-DNPH
6. Cyclohexanone-2,4-DNPH
7. Valeraldehyde-2,4-DNPH
8. Hexaldehyde-2,4-DNPH
9. Heptaldehyde-2,4-DNPH
10. Octylaldehyde-2,4-DNPH
11. Nonaldehyde-2,4-DNPH
12. Decaldehyde-2,4-DNPH

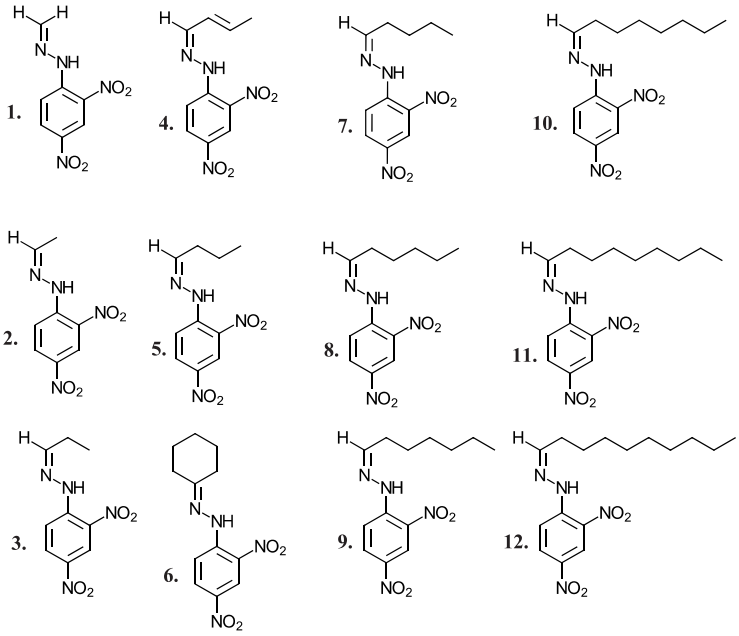
*DNPH = Dinitrophenylhydrazone
i = anti, syn, isomers of the respective DNPH derivatives

A fast, high resolution separation of carbonyl-DNPH derivatives is performed on a HALO® C18, 5 µm column. DNPH, or 2,4-Dinitrophenylhydrazine is used to derivatize these highly volatile and reactive carbonyl compounds. It is important to monitor the levels of these reactive compounds in the environment because they are combustion byproducts found in air, water and soil.

TEST CONDITIONS:

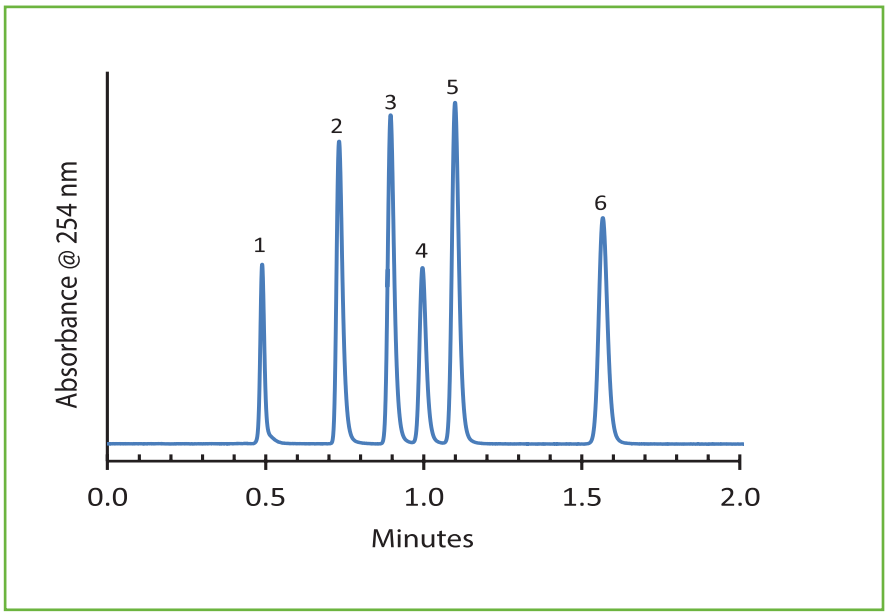
Column: HALO 90 Å C18, 5 µm, 4.6 x 250 mm
Part Number: 95814-902
Mobile Phase:
A: Water
B: 80/20 ACN/THF
Gradient: Hold at 45% B for 5 min
45-95% B from 5-20 min
Flow Rate: 1.5 mL/min
Pressure: 223 bar
Temperature: 30 °C
Detection: UV 360 nm
Injection Volume: 2.0 µL
Sample Solvent: 50/50 ACN/water
Response Time: 0.12 sec
Flow Cell: 5.0 µL semi-micro, bypassed
LC System: Agilent 1100 Series Quaternary

STRUCTURES:



Separation of Neonicotinoids on HALO® C18, 2.7 µm

Application Note 92-PS



PEAK IDENTITIES:

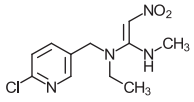
1. Nitenpyram
2. Thiamethoxam
3. Clothianidin
4. Imidacloprid
5. Acetamiprid
6. Thiacloprid

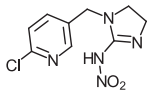
Neonicotinoids are systemic insect neurotoxins that have recently been in the news, since this class of pesticides may have negative effects on bees. This application note shows a rapid separation of six neonicotinoids using a Fused-Core®, 2.7 µm, HALO® C18 column. This superficially porous packing allows high resolution at moderate back pressures.

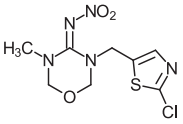
TEST CONDITIONS:

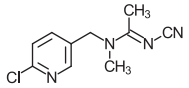
Column: HALO 90 Å C18, 2.7 µm, 3.0 x 100 mm
Part Number: 92813-602
Mobile Phase: 70/30 - A/B
A: 0.1% formic acid in water
B: Acetonitrile
Flow Rate: 0.8 mL/min
Pressure: 252 bar
Temperature: 35 °C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: 50/50 water/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

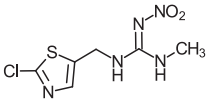
STRUCTURES:

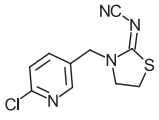

Nitenpyram


Imidacloprid


Thiamethoxam

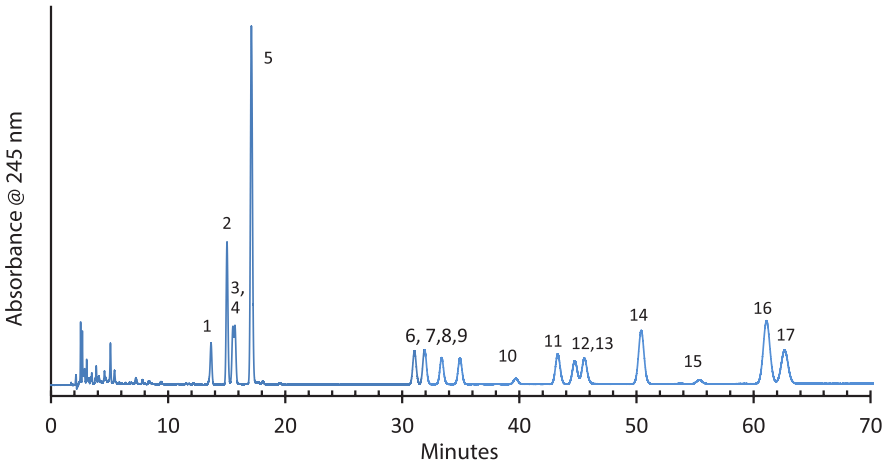

Acetamiprid


Clothianidin


Thiacloprid

Separation of Pyrethrins/Pyrethroids on HALO® C18, 2.7 µm

Application Note 99-PS



PEAK IDENTITIES:

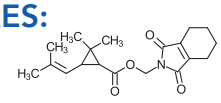
1. Tetramethrin: 1, 2
2. Allethrin: 3, 4, 5
3. Cyfluthrin: 6, 7, 8, 9
4. Resmethrin: 10, 11
5. Fenvalerate: 12, 13
6. Permethrin: 14, 17
7. Phenothrin: 15, 16

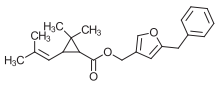
This separation of pyrethrins/pyrethroids was adapted from EPA method 1660 which describes the use of coupled 5 µm C18 columns. The tandem high performance Fused-Core®, 2.7 µm HALO® C18 columns achieve better resolution of the various isomers of these compounds with a slightly longer run time.

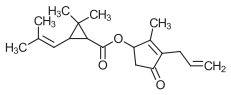
TEST CONDITIONS:

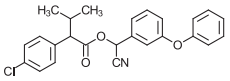
Column: HALO 90 Å C18, 2.7 µm,
4.6 x 150 mm and 4.6 x 100 mm
Part Numbers: 92814-702, 92814-602
Mobile Phase: 25/75 - A/B
A: Water
B: 50/50 acetonitrile/methanol
Flow Rate: 1.0 mL/min
Pressure: 317 bar
Temperature: 30 °C
Detection: UV 245 nm, VWD
Injection Volume: 10 µL
Sample Solvent: 50/50 acetonitrile/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

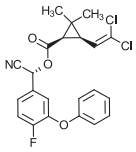
STRUCTURES:

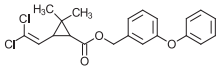

Tetramethrin

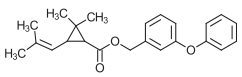

Resmethrin


Allethrin


Fenvalerate

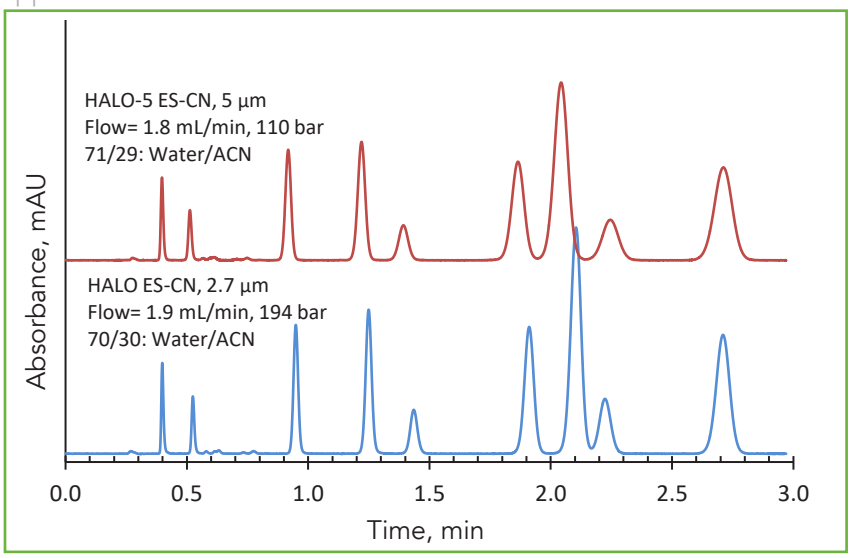

Cyfluthrin


Permethrin


Phenothrin

Comparison of Selectivity of HALO® ES-CN, 5 µm and 2.7 µm Phases

Application Note 87-HA



PEAK IDENTITIES:

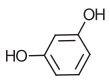
1. Resorcinol
2. Vanillin
3. Benzonitrile
4. Benzoin
5. Nitrobenzene
6. Benzanilide
7. Bisphenol A
8. Diethylphthalate
9. 3,4-Dinitrotoluene

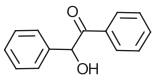
These chromatograms show the similarity in selectivity between the 5 µm and the 2.7 µm HALO® ES-CN phases which allows the easy transfer of methods from one particle size packing to another.

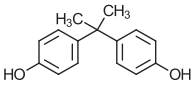
TEST CONDITIONS:

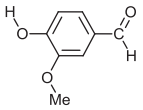
Columns:
1) HALO 90 Å ES-CN, 5 µm, 4.6 x 50 mm
Part Number: 95814-404
2) HALO 90 Å ES-CN, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-404
Mobile Phase: A/B - See chart for ratios
A: Water
B: Acetonitrile
Flow Rate: See chart
Pressure: See chart
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

STRUCTURES:

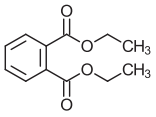

Resorcinol

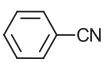

Benzoin

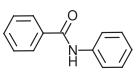

Bisphenol A

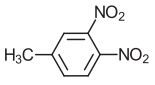

Vanillin


Nitrobenzene


Diethylphthalate

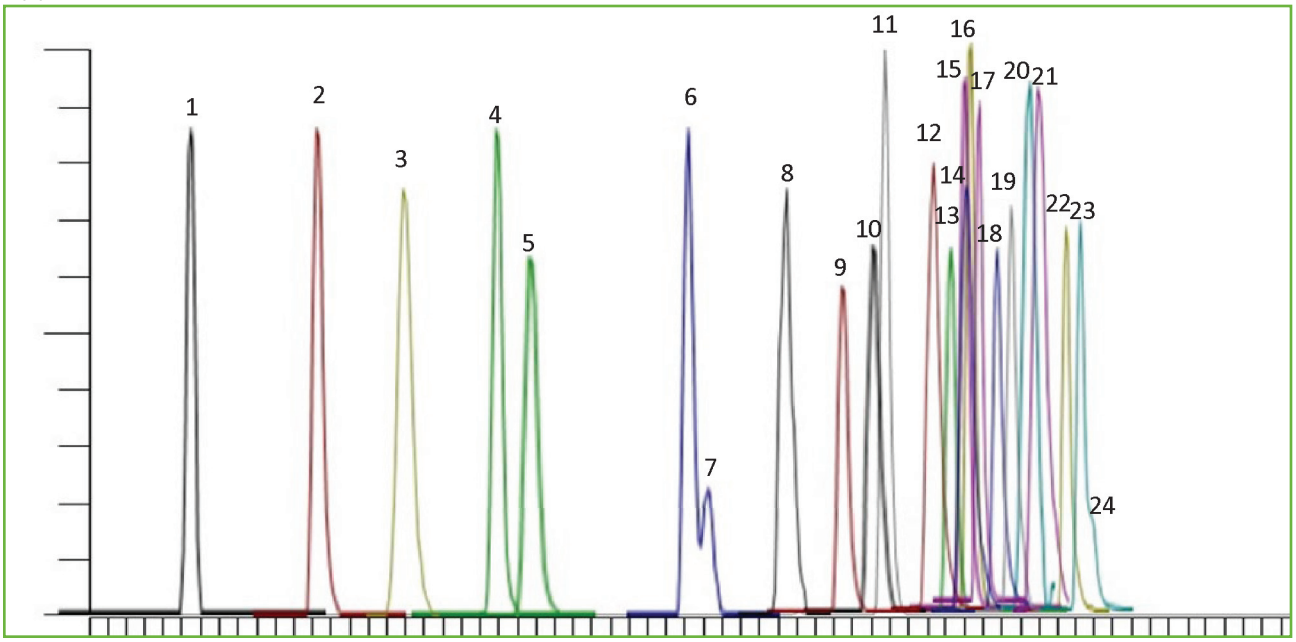

Benzonitrile


Benzanilide


3,4-Dinitrotoluene

High Throughput, High speed LC-MS/MS Separation of Mycotoxins on HALO® PFP, 2 µm

Application Note 198



The 2 µm HALO® PFP is an ideal choice for high throughput LCMS analysis of mycotoxins, in which multiple isobaric species separation is needed. Note the separation of 24 compounds in 5.5 minutes.

TEST CONDITIONS:

Column: HALO 90 Å PFP, 2 µm, 2.1 x 50 mm
Part Number: 91812-409
Mobile Phase A: Water/2mM ammonium formate/0.1% Formic acid
Mobile Phase B: Methanol/2mM ammonium formate/0.1% Formic acid

Gradient:

Time	% B
0.01	15
1.0	25
2.0	40
2.50	41
4.50	100
5.50	100
5.51	15
6.50	Finished

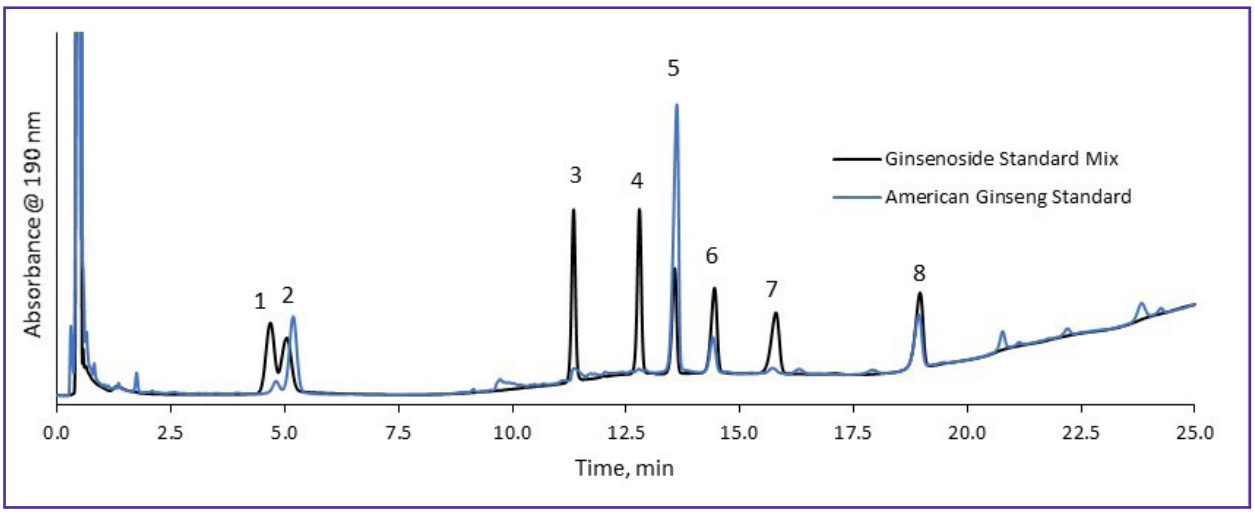
Flow Rate: 0.4 mL/min
Initial Pressure: 485 bar
Temperature: 40 °C
Injection Volume: 1 µL
Sample Solvent: 95/5 water/methanol
LC System: Shimadzu Nexera X2
Detection: +ESI MS/MS

PEAK IDENTITIES:

Peak Number	Compound	Retention Time	Precursor Ion	Product Ion
1	Nivalenol	0.71	313.1235	175.10
2	Deoxynivalenol	1.38	297.1335	249.09
3	Deoxynivalenol-3-glu-coside	1.70	459.1850	193.10
4	Fusarenon X	2.37	355.1387	247.10
5	Neosolaniol	2.87	383.1702	365.16
6	15-Acetyldeoxyniva-lenol	3.33	339.1378	321.15
7	3-Acetyldeoxyniva-lenol	3.36	339.1378	231.15
8	Gliotoxin	3.97	327.0436	196.08
9	Aflatoxin G2	4.27	331.0759	312.97
10	Aflatoxin M1	4.39	329.0604	273.12
11	Aflatoxin G1	4.40	329.0601	242.90
12	Aflatoxin B2	4.44	315.0820	284.87
13	HT-2 + Na	4.47	447.1934	345.10
14	Diacetoxyscirpenol	4.49	367.2637	307.15
15	Aflatoxin B1	4.52	313.0662	286.99
16	Ochratoxin A	4.67	404.0855	238.99
17	T-2 +Na	4.72	489.2049	245.09
18	Ochratoxin B	4.88	370.1321	324.15
19	Citrinin	4.96	251.0860	233.09
20	Zearalenone	5.11	319.1491	283.08
21	Patulin +MEOH	5.11	187.0723	98.95
22	Fumonisin B1	5.24	722.3868	334.25
23	Fumonisin B3	5.41	706.3901	336.25
24	Fumonisin B2	5.44	704.3901	336.25

Ginseng Analysis using 5 µm HALO® C18

225-F



TEST CONDITIONS:

Column: HALO 90 Å C18, 5 µm 3.0 x 50mm
Part Number: 95813-402
Mobile Phase A: Water
B: Acetonitrile

Gradient:

Time	%B
0.0	19
5.6	19
11.6	29
17.0	29
25.0	40

Flow Rate: 0.425 mL/min
Pressure: 60 bar
Temperature: 30 °C
Detection: 190 nm
Injection Volume: 4 µl
Sample Solvent: Methanol
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera

PEAK IDENTITIES:

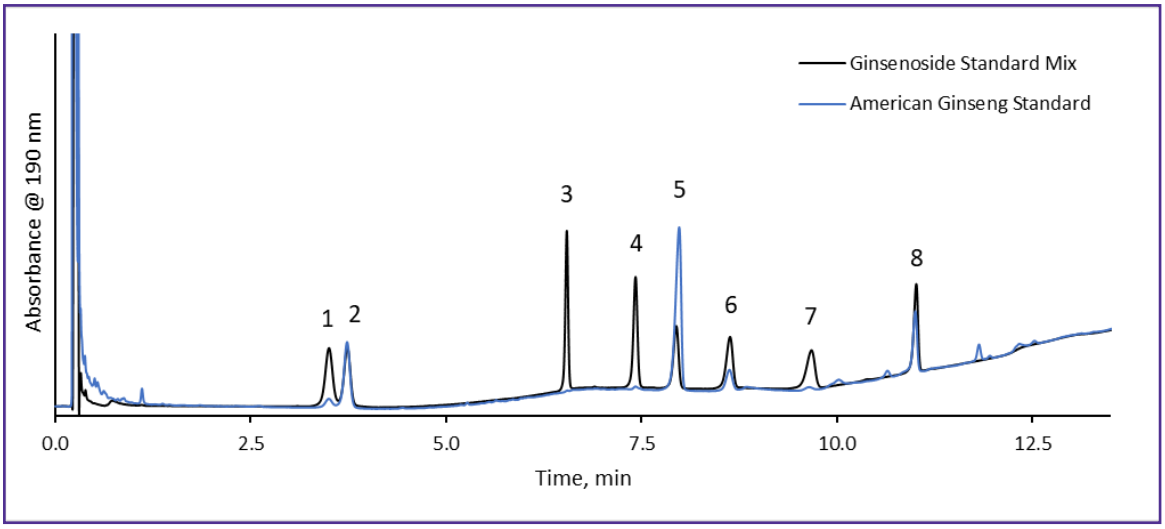
1. Ginsenoside Rg1
2. Ginsenoside Re
3. Ginsenoside Rf
4. Ginsenoside Rg2
5. Ginsenoside Rb1
6. Ginsenoside Rc
7. Ginsenoside Rb2
8. Ginsenoside Rd

Ginseng root has been used as a traditional medicine for centuries. It is believed to benefit the immune system, brain function, and act as an antioxidant that may reduce inflammation. Ginseng can be prepared as a dietary supplement, an herbal tea, or even used in cooking.

Ginsenosides are a class of natural product steroid saponins primarily found in ginseng root. Ginseng root from Panax quinquefolium (American ginseng) is overlayed with a standard mixture of eight ginsenosides on a 5 µm HALO® C18 column showing excellent resolution at low back pressures.

Ginseng Analysis using 2.7 µm HALO® C18

226-F



TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm 3.0 x 50 mm
Part Number: 92813-402
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:

Time	%B
0.0	19
3.024	19
6.264	29
9.18	29
13.5	40

Flow Rate: 0.788 mL/min
Pressure: 298 bar
Temperature: 30 °C
Detection: 190 nm
Injection Volume: 2.8 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera

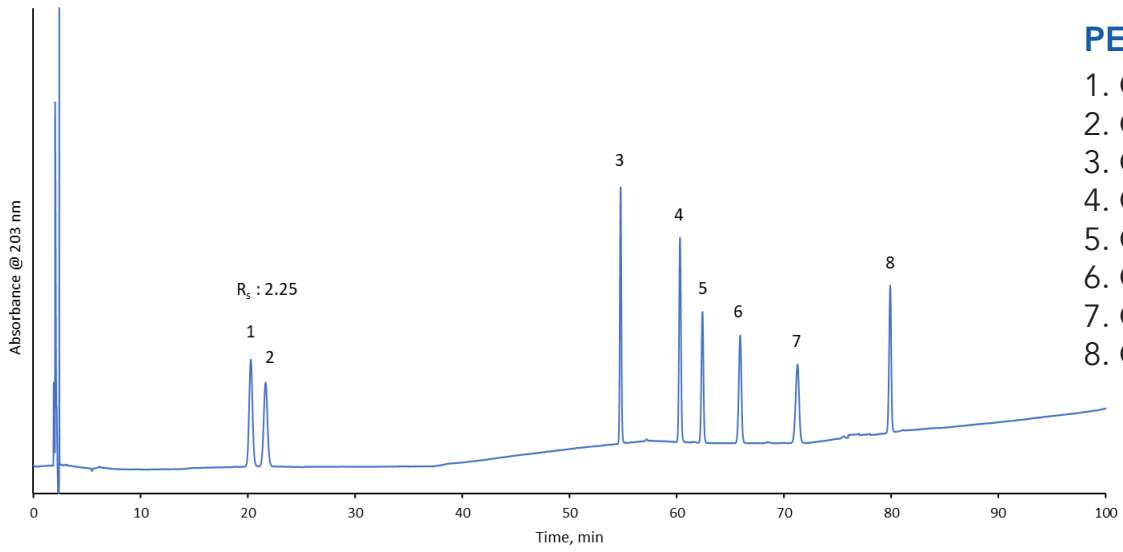
PEAK IDENTITIES

1. Ginsenoside Rg1
2. Ginsenoside Re
3. Ginsenoside Rf
4. Ginsenoside Rg2
5. Ginsenoside Rb1
6. Ginsenoside Rc
7. Ginsenoside Rb2
8. Ginsenoside Rd

Ginseng root has been used as a traditional medicine for centuries. It is believed to benefit the immune system, brain function, and act as an antioxidant that may reduce inflammation. Ginseng can be prepared as a dietary supplement, an herbal tea, or even used in cooking. Ginsenosides are a class of natural product steroid saponins primarily found in ginseng root. Ginseng root from Panax quinquefolium (American ginseng) is overlayed with a standard mixture of eight ginsenosides on a 2.7 µm HALO® C18 column showing excellent resolution between critical pairs.

Ginseng Analysis According to Chinese Pharmacopoeia (CP) Method using 5 µm HALO® C18

261-F



PEAK IDENTITIES:

1. Ginsenoside Rg1
2. Ginsenoside Re
3. Ginsenoside Rf
4. Ginsenoside Rg2
5. Ginsenoside Rb1
6. Ginsenoside Rc
7. Ginsenoside Rb2
8. Ginsenoside Rdm

TEST CONDITIONS:

Column: HALO 90 Å C18, 5 µm 4.6 x 250mm
Part Number: 95814-902
Mobile Phase A: Water
Mobile Phase B: Acetonitrile

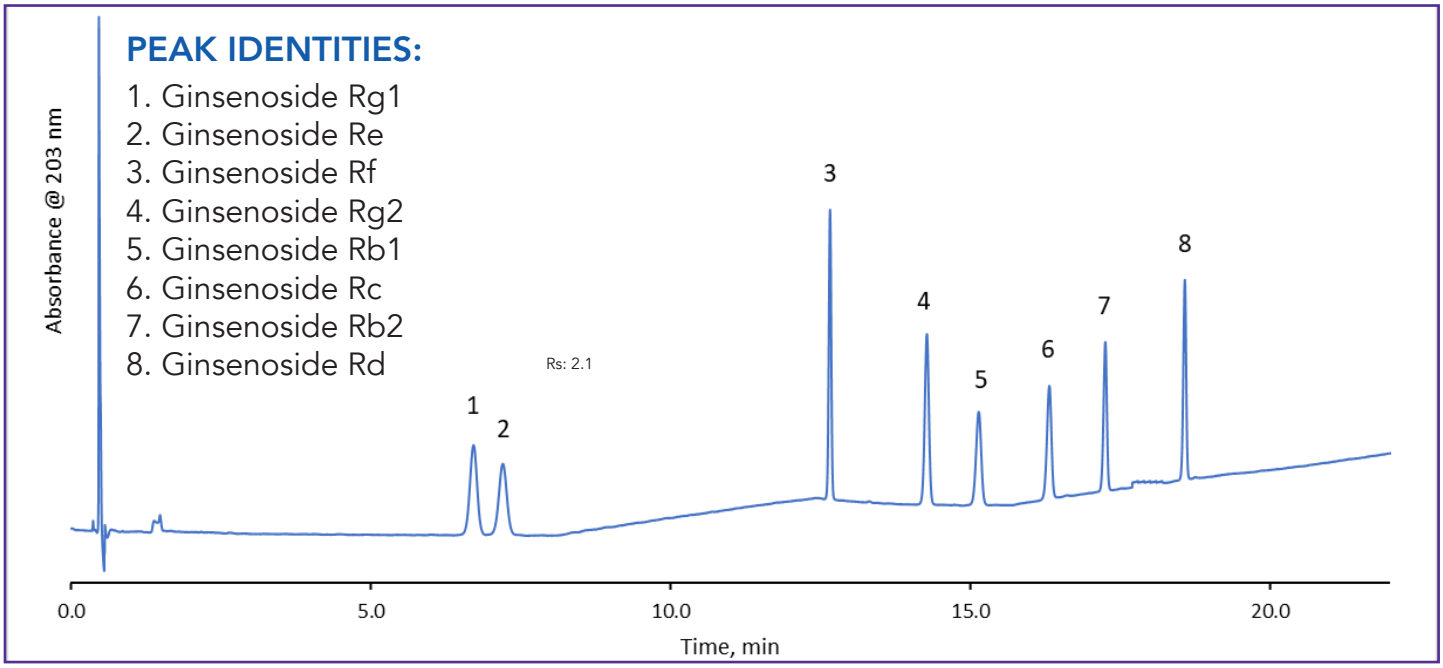
Gradient:	**Time**	**%B**
	0.0	19
	35.0	19
	55.0	29
	70.0	29
	100.0	40

Flow Rate: 1.0 mL/min
Pressure: 185 bar
Temperature: 30 °C
Detection: 203 nm
Injection Volume: 5 µL
Sample Solvent: Acetonitrile
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Ginseng root has been used as a traditional medicine for centuries. It is believed to benefit the immune system, brain function, and act as an antioxidant that may reduce inflammation. Ginseng can be prepared as a dietary supplement, an herbal tea, or even used in cooking. Ginsenosides are a class of natural product steroid saponins primarily found in ginseng root. A separation of eight ginsenosides is achieved on a 5 µm HALO® C18 column following the Chinese Pharmacopoeia (CP) Method.

Modified Ginseng Analysis According to Chinese Pharmacopoeia (CP) Method using HALO® C18, 2.7 µm

262-F



TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm 4.6 x 100 mm
Part Number: 92814-602
Mobile Phase A: Water
Mobile Phase B: Acetonitrile

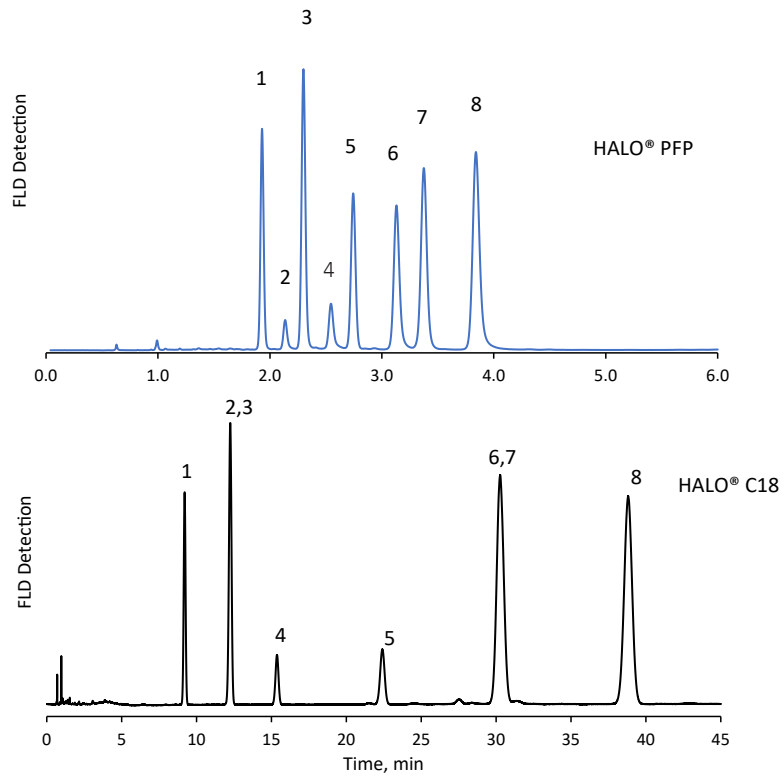
Gradient:	**Time**	**%B**
	0.00	19
	7.56	19
	11.88	29
	15.12	29
	21.60	40

Flow Rate: 1.85 mL/min
Pressure: 403 bar
Temperature: 30 °C
Detection: 203 nm
Injection Volume: 2.3 µL
Sample Solvent: Acetonitrile
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Ginseng root has been used as a traditional medicine for centuries. It is believed to benefit the immune system, brain function, and act as an antioxidant that may reduce inflammation. Ginseng can be prepared as a dietary supplement, an herbal tea, or even used in cooking. Ginsenosides are a class of natural product steroid saponins primarily found in ginseng root. A separation of eight ginsenosides is achieved on a 2.7 µm HALO® C18 column following a modified Chinese Pharmacopoeia (CP) Method.

Phase Comparison for Tocopherols and Tocotrienols

242-V



PEAK IDENTITIES

1. δ-tocotrienol
2. β-tocotrienol
3. γ-tocotrienol
4. α-tocotrienol
5. δ-tocopherol
6. β-tocopherol
7. γ-tocopherol
8. α-tocopherol

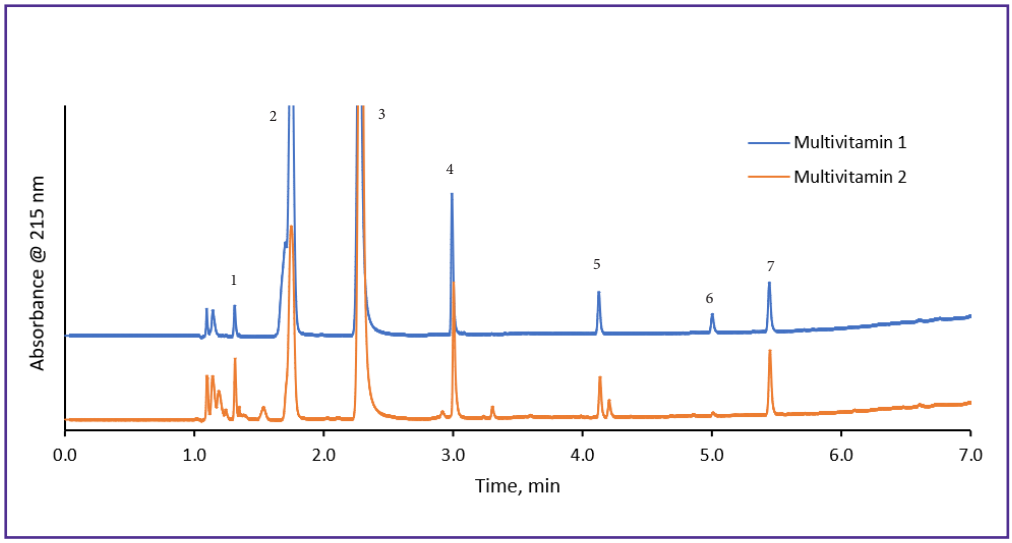
TEST CONDITIONS:

Column: HALO 90 Å PFP, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-709
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-702
Mobile Phase A: Water
B: Methanol
Isocratic: 90 %B
Flow Rate: 1.5 mL/min
Initial Back Pressure: 383 bar
Temperature: 25 °C
Detection: FLD: Ex: 296/ Em: 325
Injection Volume: 1.0 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
LC System: Shimadzu Nexera X2

Tocopherols and tocotrienols are a form of Vitamin E (fat-soluble) that have antioxidant properties in both the body and in food. They are also used for cosmetics and many personal care products. A separation of tocopherols and tocotrienols is performed on a HALO® PFP and C18 column. The PFP column shows 10x faster run times along with baseline resolution compared to the C18 column under the same testing conditions.

Separation of Water Soluble Vitamins Found in Multivitamins

253-V



PEAK IDENTITIES

1. Thiamine (B1)
2. Ascorbic acid (C)
3. Nicotinamide (B3)
4. Pyridoxine (B6)
5. Pantothenic acid (B5)
6. Folic acid (B9)
7. Riboflavin (B2)

TEST CONDITIONS:

Column: HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-722
Mobile Phase A: 25mM Potassium Phosphate, pH: 2.5
Mobile Phase B: Methanol
Gradient:

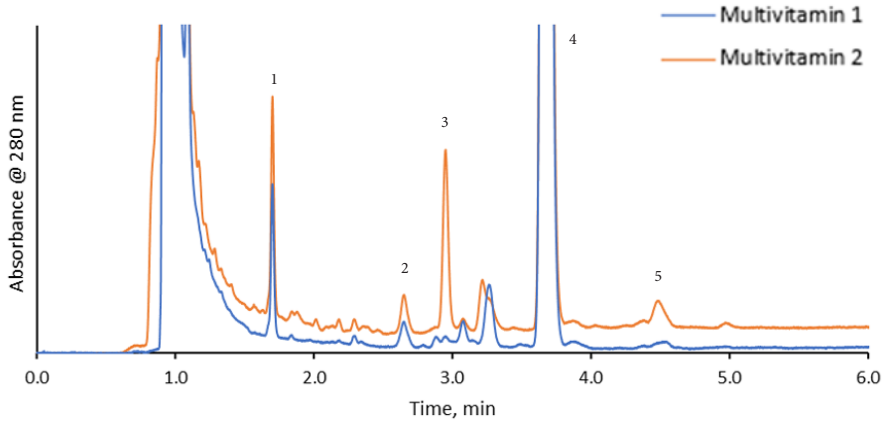
Time (min)	%B
0.0	0
1.0	0
6.0	70
10.0	70

Flow Rate: 1.2 mL/min
Initial Back Pressure: 243 bar
Temperature: 30 °C
Detection: UV 215 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: Water
Data Rate: 100 Hz
LC System: Shimadzu Nexera X2

HALO® AQ-C18 columns can be used with high or completely aqueous mobile phases making the column an ideal candidate for separating water-soluble vitamins. Seven water-soluble multivitamins are well-separated from multivitamin tablets in under six minutes using a 100% aqueous isocratic hold. Minor differences are seen between the two samples, varying in each component's concentration.

Separation of Fat Soluble Vitamins Found in Multivitamins

254-V



PEAK IDENTITIES

1. Retinyl acetate (A)
2. Cholecalciferol (D3)
3. Alpha tocopherol (E)
4. DL-alpha tocopherol acetate (E)
5. 2,3-trans-phylloquinone (K)

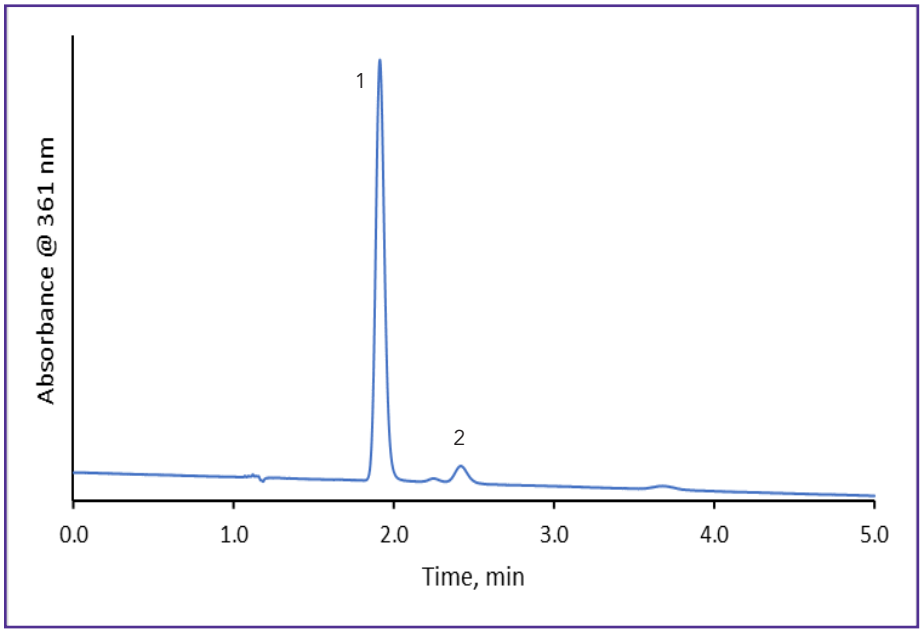
TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Part Number: 92114-730
Isocratic: Methanol
Flow Rate: 1.5 mL/min
Initial Back Pressure: 262 bar
Temperature: 30 °C
Detection: UV 280 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
LC System: Shimadzu Nexera X2

Fat soluble vitamins are stored in the liver and fatty tissue. These vitamins are essential to good health and contribute to several physiological functions, including bone growth, immune system regulation, cell division, and blood clotting. HALO® C30 enables a fast, efficient separation of fat soluble vitamins in two different multivitamin tablets. The column is capable of identifying differences between the two tablets, which at first glance may seem similar due to the solvent front and the high abundance of DL-alpha tocopherol acetate (E). Upon closer inspection, differences in the concentrations of the relatively minor peaks, particularly for alpha-tocopherol are clearly evident. Such capabilities are vital to confirm the food label content information. Also, in some extreme cases, it could be crucial to verify the identity of a multi-vitamin e.g. fradulent re-labelling of cheaper tablets as higher priced products.

Vitamin B12 Analysis According to Chinese Pharmacopoeia (CP) Method

258-V



PEAK IDENTITIES

1. Cobalamin (B12)
2. Impurity

TEST CONDITIONS:

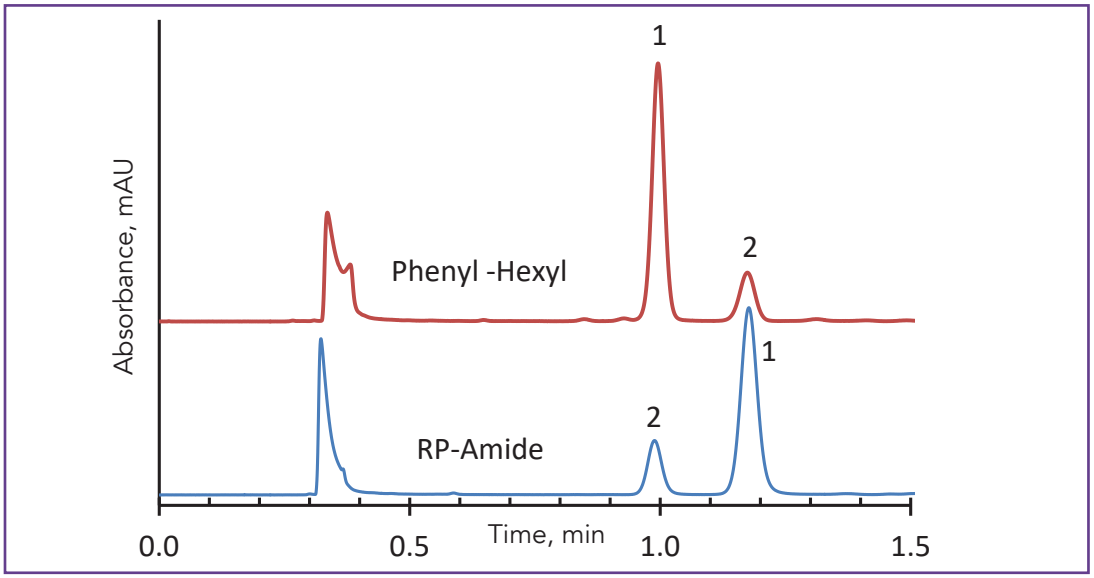
Column: HALO 90 Å C18, 5 µm, 4.6 x150 mm
Part Number: 95814-702
Isocratic: 26/74 MeOH/ 28 mM Na_2HPO_4 pH: 3.5
Flow Rate: 1.0 mL
Pressure: 209 bar
Temperature: 30 °C
Detection: UV 361 nm, PDA
Injection Volume: 10 µL System Suitability Solution

Back Pressure: 205 bar
Sample Solvent: mobile phase
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Cobalamin, better known as vitamin B12, is one of the eight water soluble vitamins. It is the largest and most complex vitamin. A separation of cobalamin is achieved using a HALO 90 Å C18, 5 µm column following the Chinese Pharmacopoeia method. A resolution value of 4.35 is observed, well above the specification required in the method (>2.5).

Separation of Diosmin and Hesperidin on HALO® Phenyl-Hexyl and HALO® RP-Amide

Application Note 83-FL



PEAK IDENTITIES:

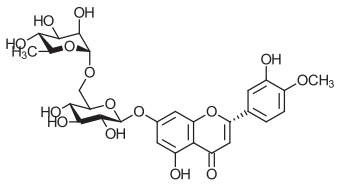
1. Diosmin
2. Hesperidin

These two semi-synthetic flavonoids are often taken to enhance vascular health. The two compounds may be easily separated using either HALO® RP-Amide or HALO® Phenyl-Hexyl phases. Note the difference in elution order on the two phases.

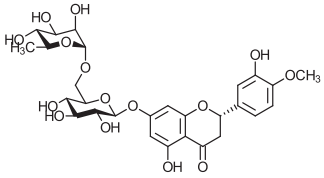
TEST CONDITIONS:

Columns:
1) HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
2) HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 78/22 - A/B
A: Water
B: Acetonitrile
Flow Rate: 1.5 mL/min
Pressure: 145 bar
Temperature: 40 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Dimethylformamide (needed for solubility reasons)
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

STRUCTURES:



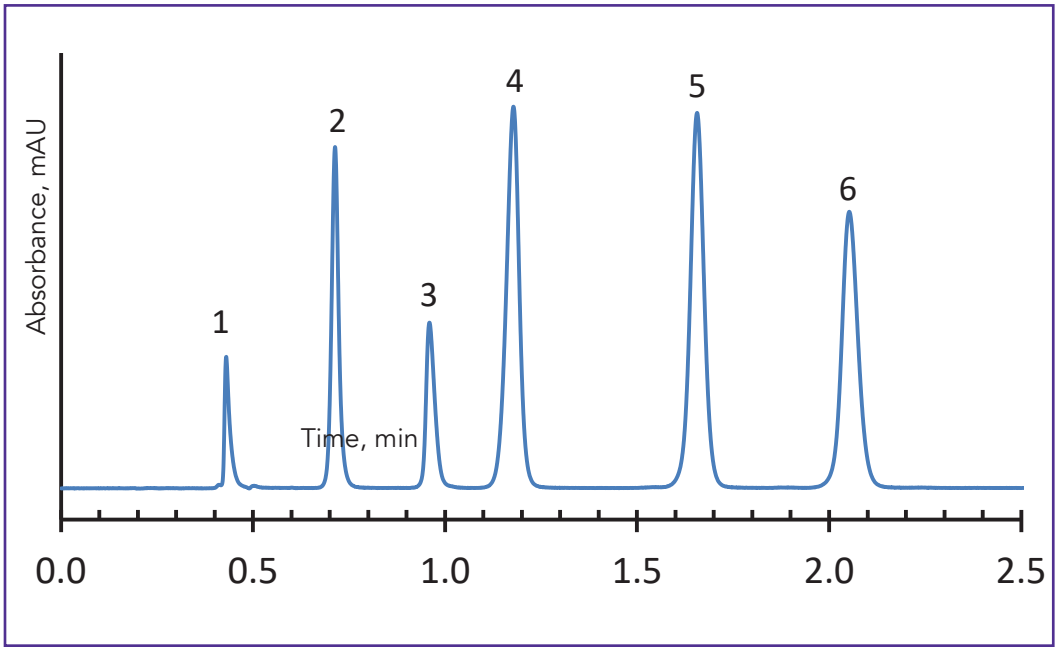
Diosmin



Hesperidin

Separation of Biogenic Amines on HALO® Phenyl-Hexyl 5 µm by Ion-Pairing

140-B



PEAK IDENTITIES:

1. System peak, t_0
2. L-Tyrosine
3. Octopamine
4. ± Synephrine
5. Tyramine
6. Hordenine

TEST CONDITIONS:

Column: HALO 90 Å Phenyl-Hexyl, 5 µm, 3.0 x 100 mm
Part Number: 95813-606
Mobile Phase: 78/22 - A/B
A: 0.05 M Phosphate buffer, (pH 3.0) with 2.7 g/L of sodium hexanesulfonate
B: Methanol

Gradient:

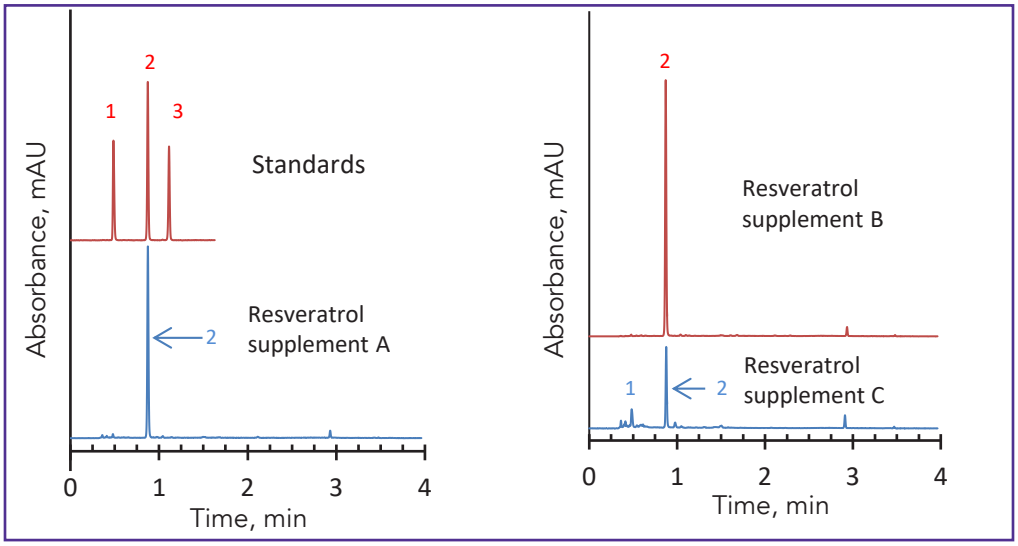
Time (min)	% B
0.0	22
4.0	30

Flow Rate: 0.8 mL/min
Pressure: 170 bar
Temperature: 30 °C
Detection: UV 280 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: 90/10 water/methanol
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

These five biogenic amines can be rapidly separated with excellent peak shape on a HALO® Phenyl-Hexyl 5 µm column using a methanol/phosphate buffer mobile phase containing an ion-pairing reagent.

Separation of Resveratrols on HALO® C18, 2.7 µm

Application Note 132-P



PEAK IDENTITIES:

1. Polydatin
2. trans-Resveratrol
3. cis-Resveratrol

Resveratrols are polyhydroxy compounds and have been reported to have antioxidant and anti-aging properties and are available as food supplements. These food supplements can be analyzed rapidly using short HALO® Fused-Core® C18 columns.

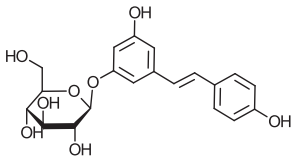
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 75 mm
Part Number: 92814-502
Mobile Phase:
A: Water
B: Acetonitrile
Gradient:

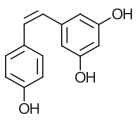
Time (min)	% B
0.0	30
2.0	50
3.0	90
4.0	90

Flow Rate: 1.8 mL/min
Pressure: 240 bar
Temperature: 35 °C
Detection: UV 290 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 acetonitrile/methanol
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

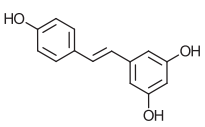
STRUCTURES:



Polydatin



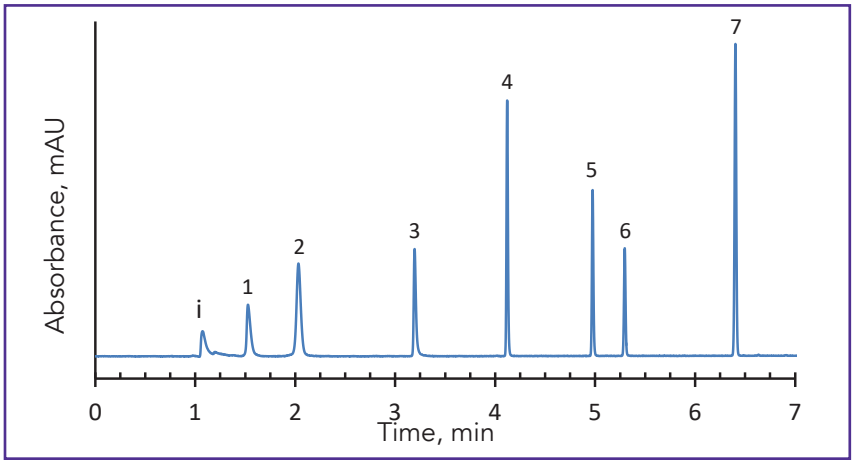
cis-Resveratrol



trans-Resveratrol

Separation of Melatonin and Related Compounds on HALO® RP-Amide

Application Note 143-B



PEAK IDENTITIES:

i. Impurity
1. Serotonin
2. 5-hydroxy-L-tryptophan
3. L-Tryptophan
4. N-Acetyl-5-hydroxytryptamine
5. Melatonin
6. 3-Indoleacetic acid
7. Indole

Serotonin and melatonin are bioactive amines and are found in plant and animal tissues. In this application a mixture containing serotonin, melatonin and related amine compounds is well separated in less than 10 minutes using a HALO® RP-Amide column. The gradient may be adjusted to accommodate possible interfering peaks from sample matrices.

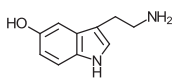
TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-707
Mobile Phase: A/B
A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile
Gradient:

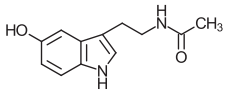
Time (min)	% B
0.0	5
1.5	5
7.0	70
8.5	95

Flow Rate: 1.5 mL/min
Pressure: 273 bar
Temperature: 35 °C
Detection: UV 280 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

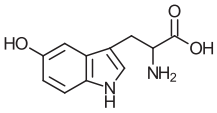
STRUCTURES:



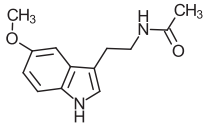
Serotonin



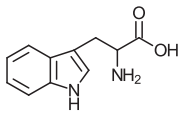
N-Acetyl-5-hydroxytryptamine



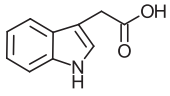
5-Hydroxy-L-tryptophan



Melatonin



L-Tryptophan

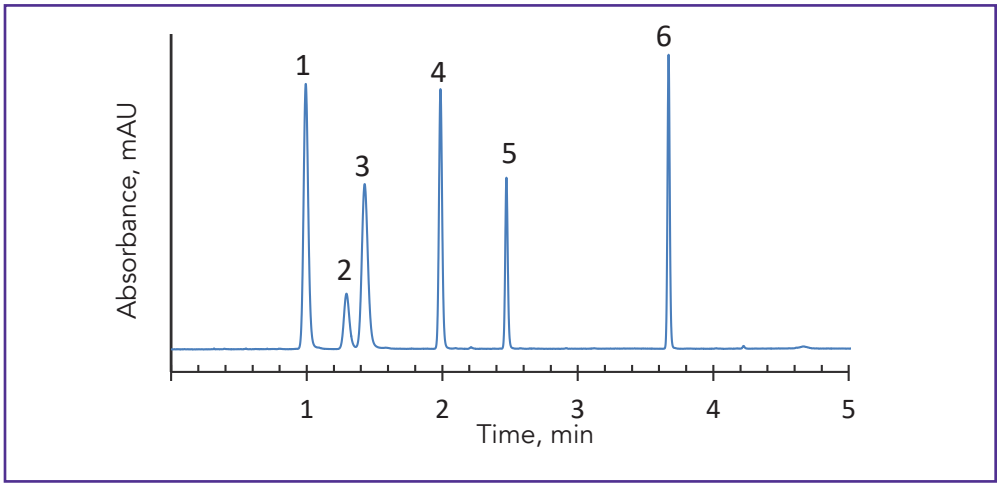


3-Indoleacetic acid

Indole

Separation of Resveratrols and Related Compounds on HALO® C18, 5 µm

Application Note 133-P



PEAK IDENTITIES:

1. trans-Polydatin
2. Piceatannol
3. trans-Oxyresveratrol
4. trans-Resveratrol
5. cis-Resveratrol
6. Pterostilbene

These naturally occurring compounds can be found in grapes and grape vines and other plants and are claimed to have health benefits. Resveratrol and these related compounds can be analyzed in less than 5 minutes using a HALO® C18, 5 µm column.

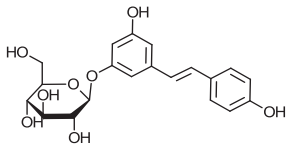
TEST CONDITIONS:

Column: HALO 90 Å C18, 5.0 µm, 3.0 x 100 mm
Part Number: 95813-602
Mobile Phase:
A: Water
B: Methanol
Gradient:

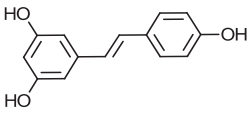
Time (min)	% B
0.0	32
1.0	32
4.0	90
5.0	90

Flow Rate: 1.2 mL/min
Pressure: 245 bar
Temperature: 35 °C
Detection: UV 290 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 acetonitrile/water
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

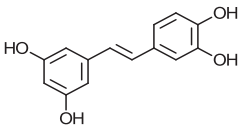
STRUCTURES:



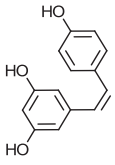
trans-Polydatin



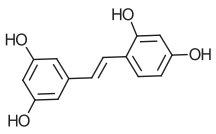
trans-Resveratrol



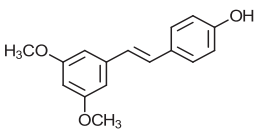
Piceatannol



cis-Resveratrol



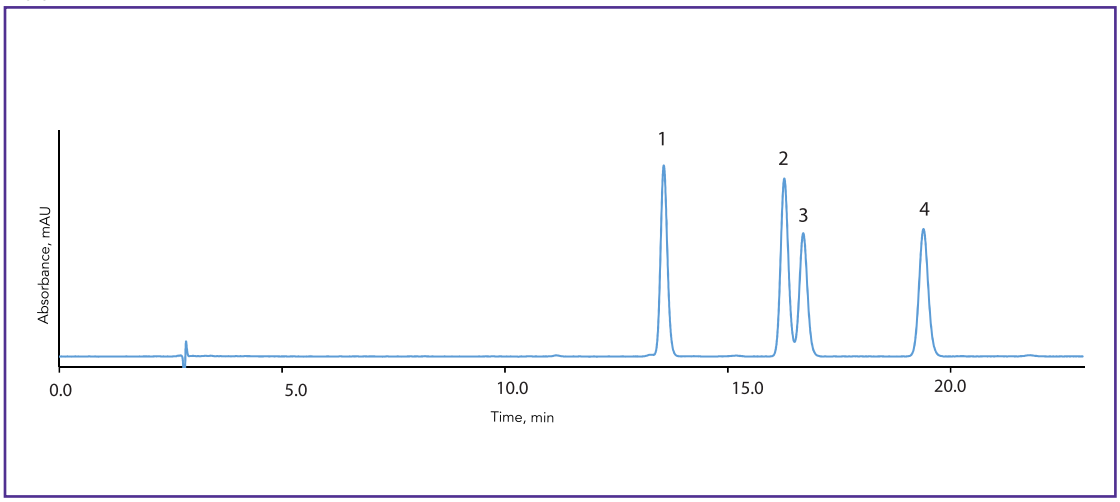
trans-Oxyresveratrol



Pterostilbene

Separation of Tocopherols on HALO® C30 based on GB (Chinese Standards)

Application Note 189-V



PEAK IDENTITIES:

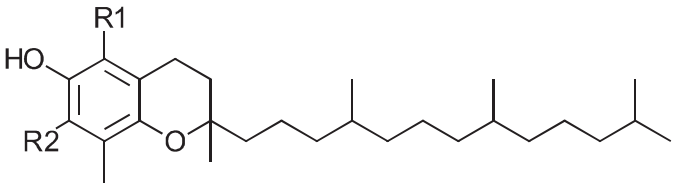
1. δ-tocopherol
2. γ- tocopherol
3. β- tocopherol
4. α- tocopherol

Tocopherols are forms of vitamin E (fat-soluble) that have antioxidant properties in both the human body and in food. They are also used for cosmetics and many personal care products. Here, tocopherols are separated on a 250 mm 160 Å pore size HALO® C30 column using a GB (Chinese standard) method. Due to the shape selectivity of the C30 phase, separation of the four isomers is achieved.

TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 250 mm
Part Number: 92114-930
Mobile Phase:
A: Water
B: Methanol
Isocratic: 95% B
Flow Rate: 0.9 mL/min
Initital Pressure: 240 bar
Temperature: 30 °C
Detection: UV 294 nm, PDA
Injection Volume: 20 µL
Sample Solvent: Methanol
Response Time: 2.0 sec
Data Rate: 20 Hz
Flow Cell: 13 µL
LC System: Agilent 1100
Data Courtesy of Beijing Institute for Drug Control

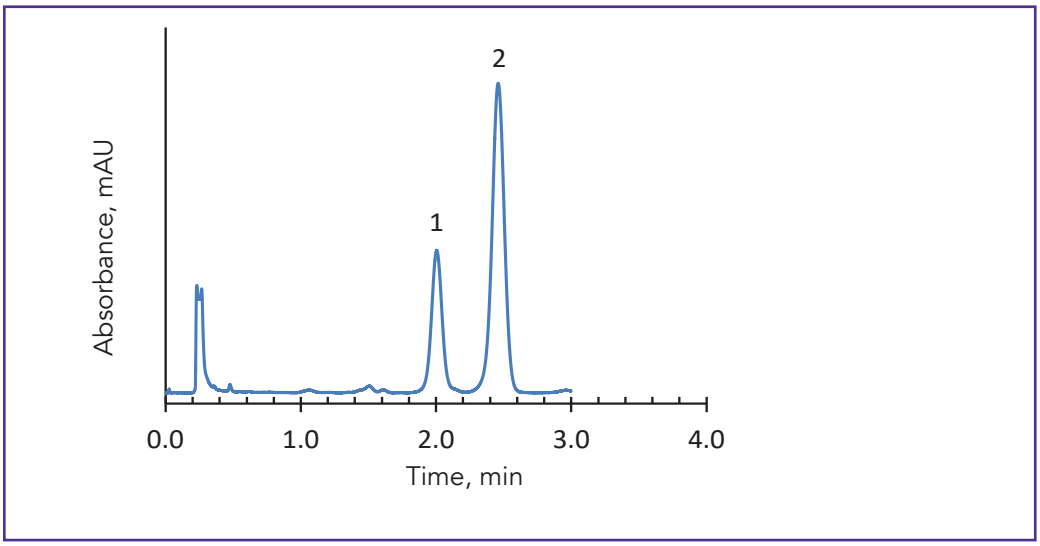
STRUCTURE:



Tocopherol	R1	R2
Alpha (α)	CH_3	CH_3
Beta (β)	CH_3	H
Gamma (γ)	H	CH_3
Delta (δ)	H	H

HPLC Separation of Hesperidin and Diosmin on HALO® PFP, 5 µm

84-FL



PEAK IDENTITIES:

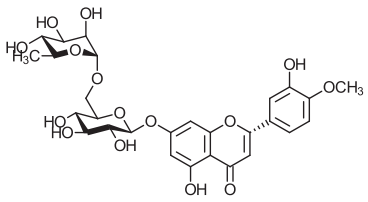
1. Hesperidin
2. Diosmin

These two semisynthetic flavonoids can be rapidly separated using HALO® PFP (pentafluorophenyl) 5 µm stationary phase at a low pressure. Note that just the addition of a double bond results in a difference that allows these two very similar compounds to be separated.

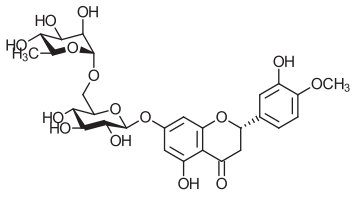
TEST CONDITIONS:

Column: HALO 90 Å PFP, 5 µm, 3.0 x 50 mm
Part Number: 95813-409
Mobile Phase: 85/15 - A/B
A: 0.02 M Potassium phosphate buffer, pH 3.0
B: Acetonitrile
Flow Rate: 1.0 mL/min
Pressure: 92 bar
Temperature: 30 °C
Detection: UV 260 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Dimethylformamide (needed for solubility reasons)
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

STRUCTURES:



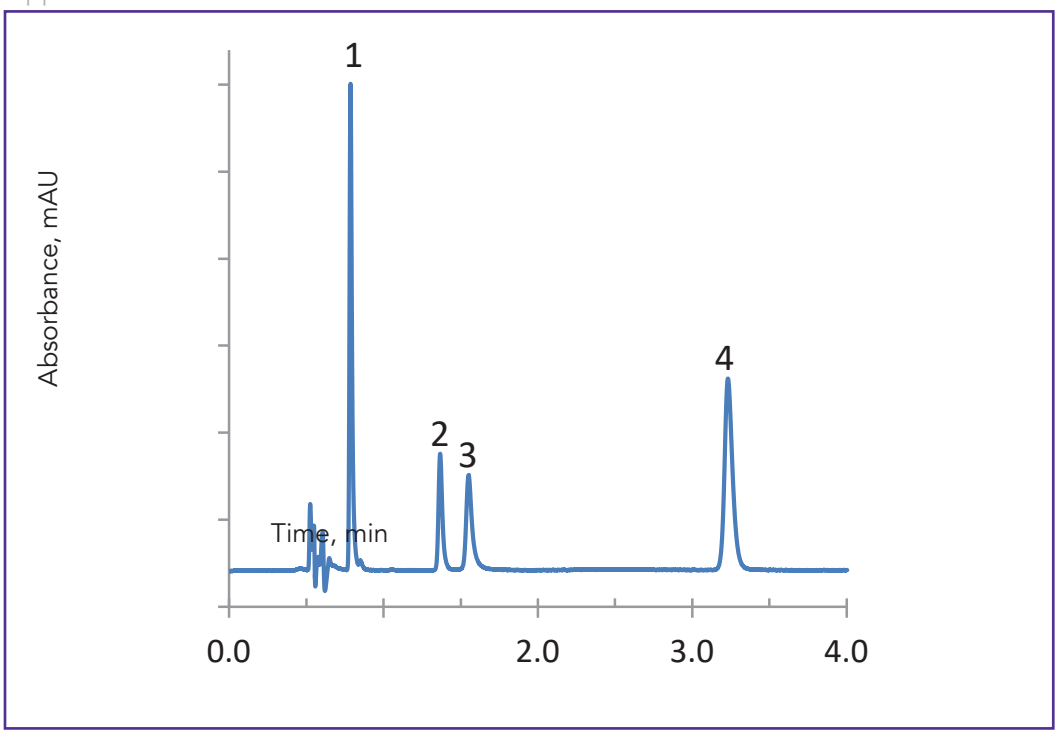
Diosmin



Hesperidin

Separation of Water Soluble Vitamins on HALO® HILIC, 2.0 µm

Application Note 120-F



PEAK IDENTITIES:

1. Nicotinamide
2. Riboflavin
3. Ascorbic acid
4. Nicotinic acid

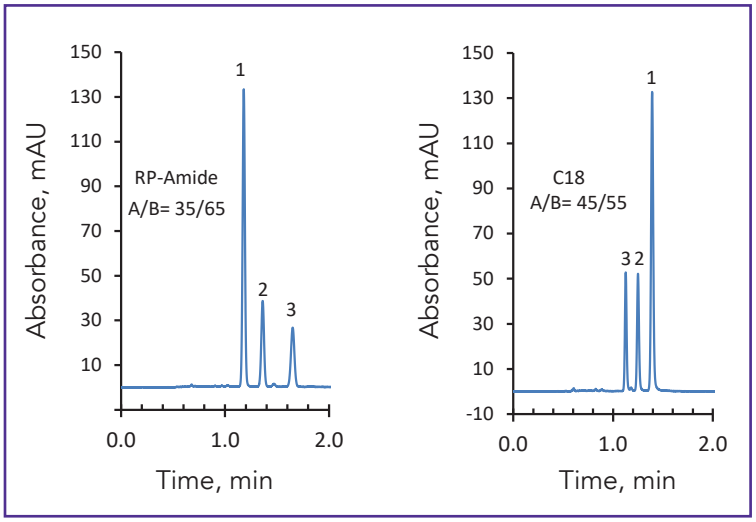
TEST CONDITIONS:

Column: HALO 90 Å HILIC, 2.0 µm, 2.1 x 100 mm
Part Number: 91812-601
Isocratic: 92/8 ACN/water with 5 mM ammonium formate, pH 3.0
Flow Rate: 0.5 mL/min
Pressure: 220 bar
Temperature: 30 °C
Detection: UV 265 nm, PDA
Injection Volume: 0.3 µL
Sample Solvent: 75/25 ACN/methanol with 2% formic acid
Response Time: 0.1 sec
Data Rate: 40 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Agilent 1200 SL

A fast separation of four water soluble vitamins is accomplished on a 2.0 µm HALO® HILIC column.

Analysis of Curcumins on HALO® RP-Amide and HALO® C18

Application Note 148-F



PEAK IDENTITIES:

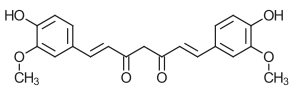
1. Curcumin
2. Desmethoxycurcumin
3. bis-Desmethoxycurcumin

Turmeric spice contains circumins that are used as dietary supplements. A methanolic extract of turmeric powder was filtered and analyzed on both HALO® C18 and RP-Amide columns, showing the different selectivity for circumin and two derivatives.

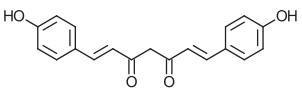
TEST CONDITIONS:

Columns:
1) HALO 90 Å C18, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-602
2) HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-607
Mobile Phase: A/B - See chart for ratios
A: 0.025 M phosphate buffer in water, pH 3.0
B: Acetonitrile
Flow Rate: 1.8 mL/min
Pressure: 215 bar
Temperature: 35 °C
Detection: UV 420 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Methanol
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

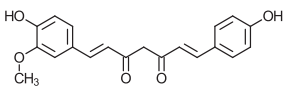
STRUCTURES:



Curcumin



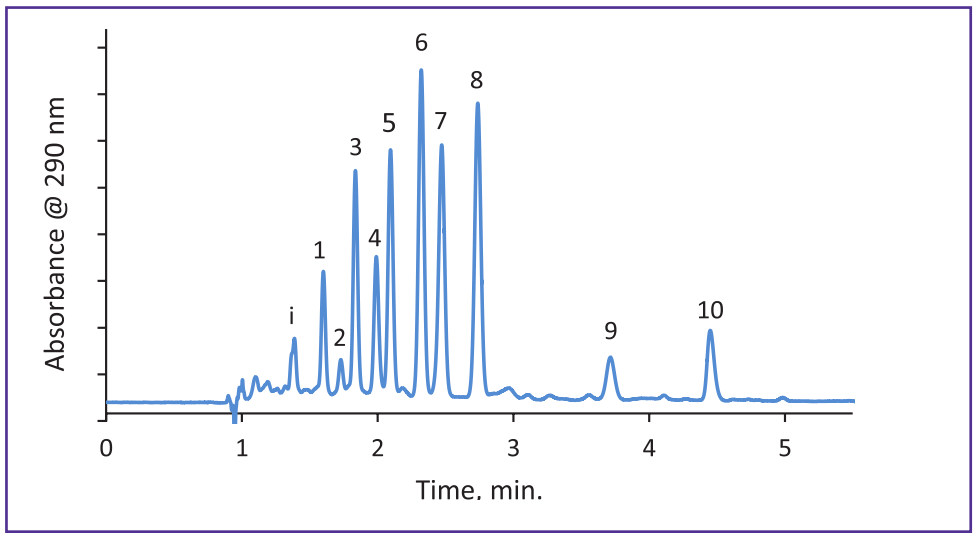
bis-Desmethoxycurcumin



Desmethoxycurcumin

Rapid Separation of Vitamin E Congeners on HALO® PFP

Application Note 146-V



PEAK IDENTITIES:

i = impurity
1. δ-Tocotrienol
2. β-Tocotrienol
3. γ-Tocotrienol
4. α-Tocotrienol
5. δ-Tocopherol
6. β-Tocopherol
7. γ-Tocopherol
8. α-Tocopherol
9. α-Tocopherol acetate
10. α-Tocopherol nicotinate

TEST CONDITIONS:

Column: HALO 90 Å PFP, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-709
Mobile Phase:
A: Water
B: Methanol

Gradient:

Time (min)	%B
0.00	92
2.75	92
3.00	95
5.00	95

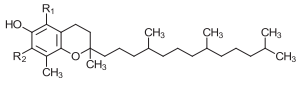
Flow Rate: 1.5 mL/min
Pressure: 380 bar
Temperature: 25 °C
Detection: UV 290 nm, PDA
Injection Volume: 5.0 µL
Sample Solvent: Ethanol
Response Time: 0.05 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

Vitamin E capsules can contain up to eight related, but different constituents, including up to four tocopherols and four tocotrienols. Ester derivatives of vitamin E are made to increase the stability of the compound. Vitamin E is important due to its antioxidant properties in both the body and in food and cosmetics.

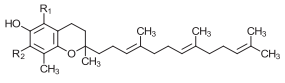
The sample used for analysis was combination of standards and a vitamin supplement purchased locally. The soft gel vitamin supplement contained the four tocotrienols and α-tocopherol. Only the liquid in the soft gel was used for the analysis. The four tocopherols, α-tocopherol acetate, and α-tocopherol nicotinate were standards obtained from SigmaAldrich. The small, unidentified peaks are unknown materials from the soft gel capsule.

STRUCTURES:

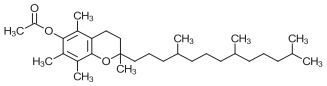
Tocopherol/Tocotrienol	R1	R2
Alpha (α)	CH_3	CH_3
Beta (β)	CH_3	H
Gamma (γ)	H	CH_3
Delta (δ)	H	H



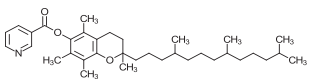
Tocopherol



Tocotrienol



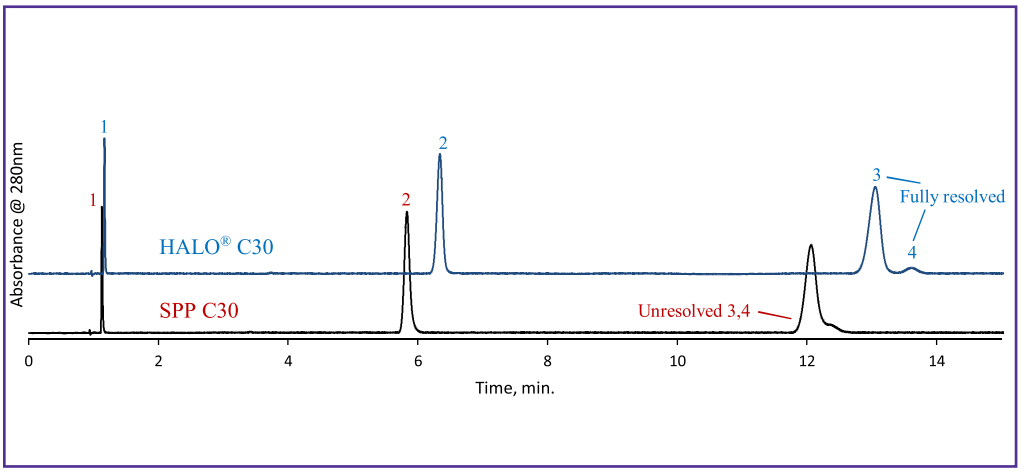
α-Tocopherol acetate



α-Tocopherol nicotinate

Vitamin K1 Isomer Analysis on HALO® C30

Application Note 180-V



PEAK IDENTITIES:

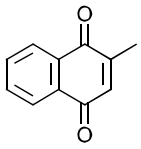
1. Menadione (K3)
2. Menaquinone 4 (K2)
3. 2,3-trans-phylloquinone (K1)
4. cis-phylloquinone (K1)

Vitamin K, a fat-soluble vitamin, is beneficial for blood clotting and bone health. Vitamin K1 is produced from plants and can be found in high amounts in green vegetables. It can also be converted into K2 within the body, while K3 is a synthetic form of vitamin K. The cis form of K1 is bio inactive so it is important to monitor how much is present in vitamin supplements. Baseline resolution of K1 isomers is obtained on a HALO® C30 column compared to a coelution on a competitor SPP C30 column.

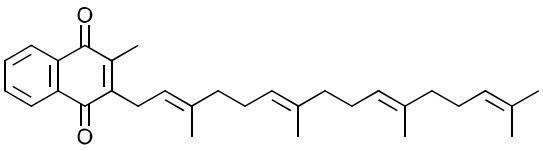
TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Part Number: 92114-730
Mobile Phase:
A: Water
B: Methanol
Isocratic: 95% B
Flow Rate: 1.5 mL/min
Initial Pressure: 341 bar (HALO®)
371 bar (competitor)
Temperature: 25 °C
Detection: UV 280 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: Methanol
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

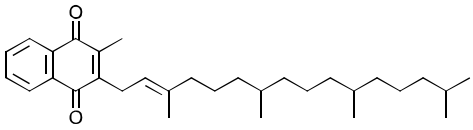
STRUCTURES:



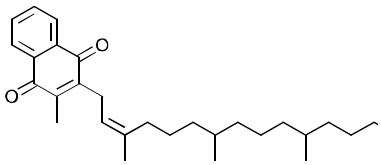
Vitamin K3: Menadione



Vitamin K2: Menaquinone 4



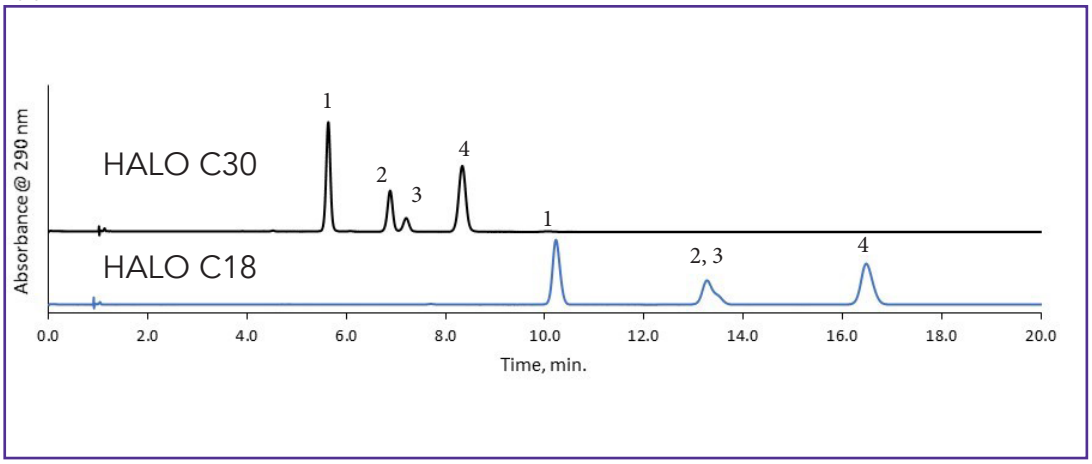
Vitamin K1: 2,3-*trans*-phylloquinone



Vitamin K1: *cis*-phylloquinone

Separation of Tocopherols on HALO® C30

Application Note 185-V



PEAK IDENTITIES:

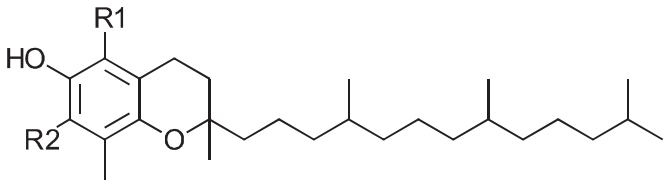
1. δ-tocopherol
2. γ- tocopherol
3. β- tocopherol
4. α- tocopherol

Tocopherols are a form of vitamin E (fat-soluble) that have antioxidant properties in both the body and in food. They are also used for cosmetics and many personal care products. Here, tocopherols are separated on a 160 Å C30 column with baseline resolution between the beta and gamma isomers compared to a 90 Å C18 column. While the HALO® C18 has more surface area (135 m^2/g vs. 90 m^2/g) and exhibits twice the retention, it produces a coelution of the isomers. Due to the C30's shape selectivity, complete separation of the isomers is achieved.

TEST CONDITIONS:

Columns:
1) HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Part Number: 92114-730
2) HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-702
Mobile Phase:
A: Water
B: Methanol
Isocratic: 95% B
Flow Rate: 1.5 mL/min
Pressure: 337 bar for C30
348 bar for C18
Temperature: 10 °C
Detection: UV 290 nm, PDA
Injection Volume: 1.5 µL
Sample Solvent: Ethanol/methanol
Response Time: 0.02 sec
Data Rate: 80 Hz
Flow Cell: 2.0 µL
LC System: Agilent 1200 SL

STRUCTURES:

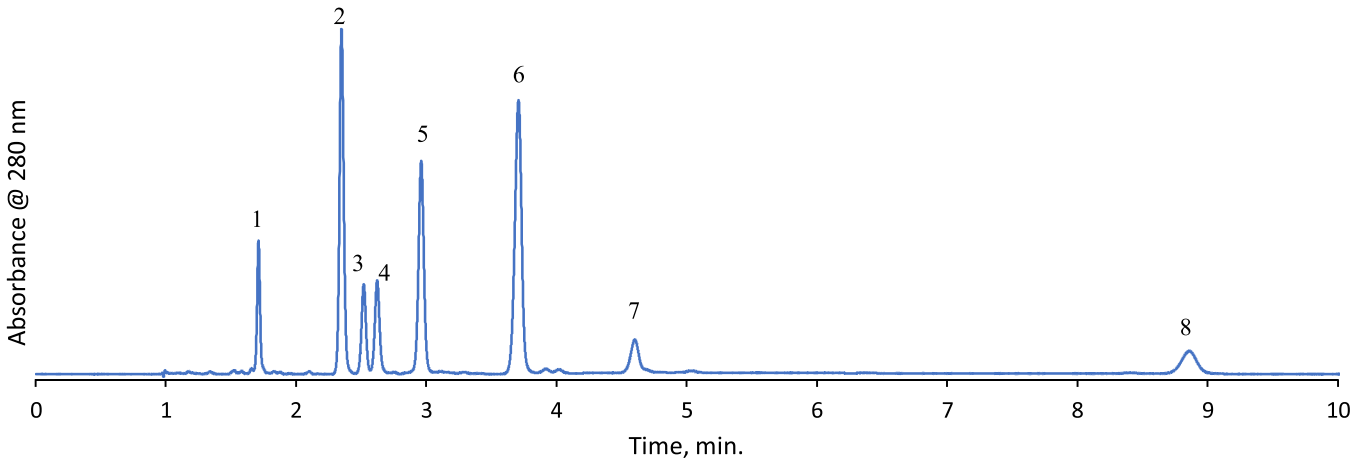


Tocopherol

Tocopherol	R1	R2
Alpha (α)	CH_3	CH_3
Beta (β)	CH_3	H
Gamma (γ)	H	CH_3
Delta (δ)	H	H

Separation of Fat Soluble Vitamins on HALO® C30

Application Note 182-V



Fat soluble vitamins are stored in the liver and fatty tissue. These vitamins are essential to good health and contribute to several physiological functions, including bone growth, immune system regulation, cell division, and blood clotting. Vitamin E acts as an antioxidant. HALO® C30 enables a fast, efficient separation of a typical fat soluble vitamin panel in less than 9 minutes, while maintaining baseline resolution between vitamins D2 and D3.

PEAK IDENTITIES:

1. Retinyl acetate (A)
2. Delta tocopherol (E)
3. Ergocalciferol (D2)
4. Cholecalciferol (D3)
5. Alpha tocopherol (E)
6. DL-alpha-tocopherol acetate (E)
7. 2,3-trans-phylloquinone (K)
8. Retinyl palmitate (A)

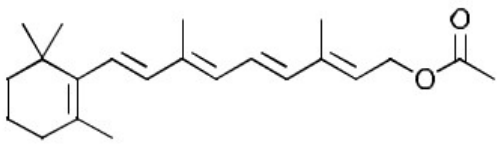
CONCENTRATION:

0.15 mg/mL
0.08 mg/mL
0.08 mg/mL
0.08 mg/mL
0.08 mg/mL
0.08 mg/mL
0.31 mg/mL
0.15 mg/mL

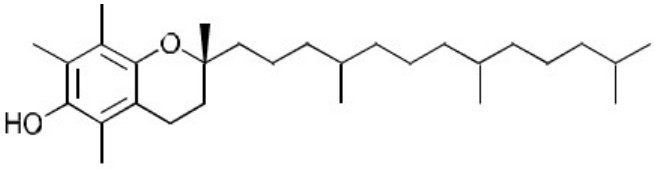
TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Part Number: 92114-730
Isocratic: 100% methanol
Flow Rate: 1.5 mL/min
Pressure: 262 bar
Temperature: 30 °C
Detection: UV 280 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: Methanol
Response Time: 0.025 sec
Data Rate: 40 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

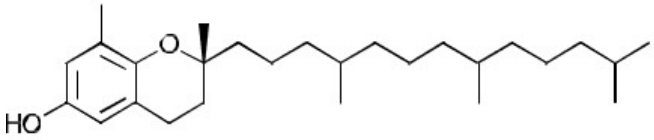
STRUCTURES:



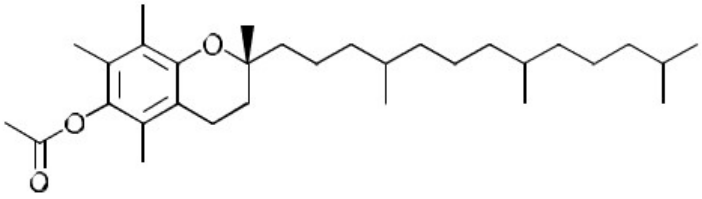
Retinyl acetate (A)



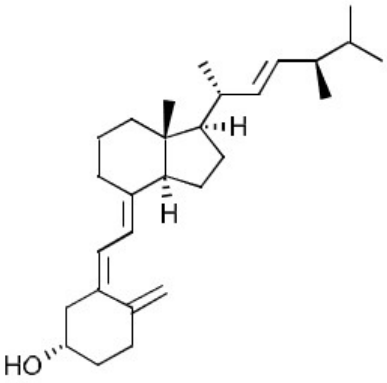
Alpha tocopherol (E)



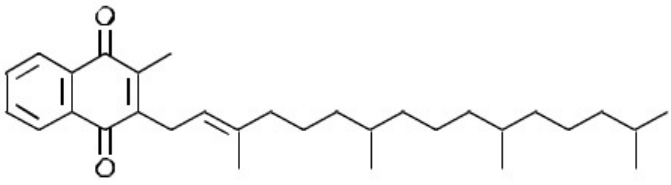
Delta tocopherol (E)



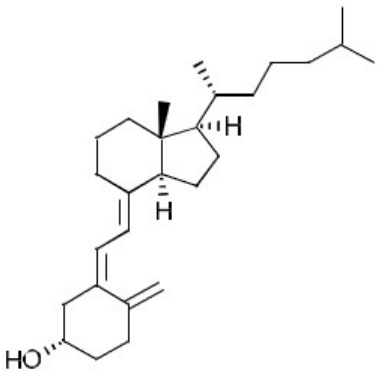
DL-alpha-tocopherol acetate (E)



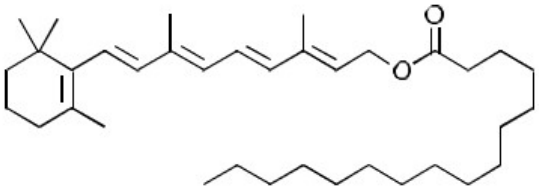
Ergocalciferol (D2)



2,3-*trans*-phylloquinone (K)



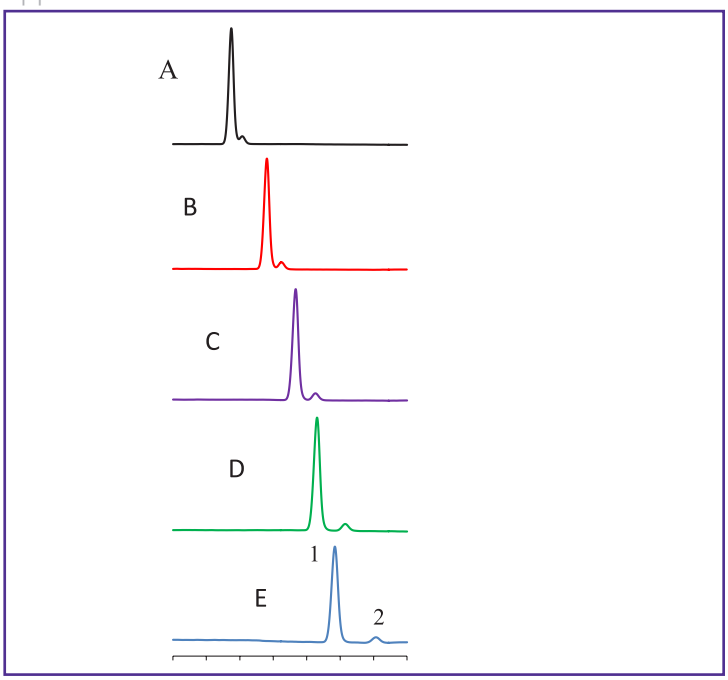
Cholecalciferol (D3)



Retinyl palmitate (A)

Vitamin K1 Analysis: Temperature vs. Resolution

Application Note 197-V



PEAK IDENTITIES:

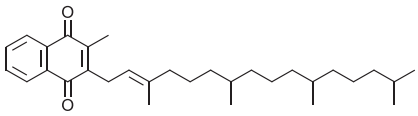
1. 2,3-trans-phylloquinone (K1)
2. cis-phylloquinone (K1)

Vitamin K, a fat-soluble vitamin, is beneficial for blood clotting and bone health.
Vitamin K1 is produced from plants and can be found in high amounts in green vegetables. Baseline resolution of the vitamin K1 isomers is increased as the temperature of the column decreases.

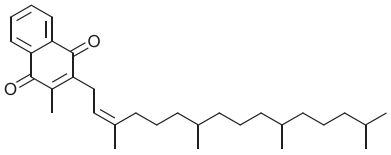
TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm
4.6 x 150 mm
Part Number: 92114-730
Mobile Phase A: Water
Mobile Phase B: Methanol
Isocratic: 95% B
Flow Rate: 1.5 mL/min
Back Pressure: 341 bar
Detection: 280 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: Methanol
Response Time: 0.12 sec.
Flow Cell: 5 µL Semi-Micro
LC System: Agilent 1100 Series

STRUCTURES:



Vitamin K1: 2,3-*trans*-phylloquinone

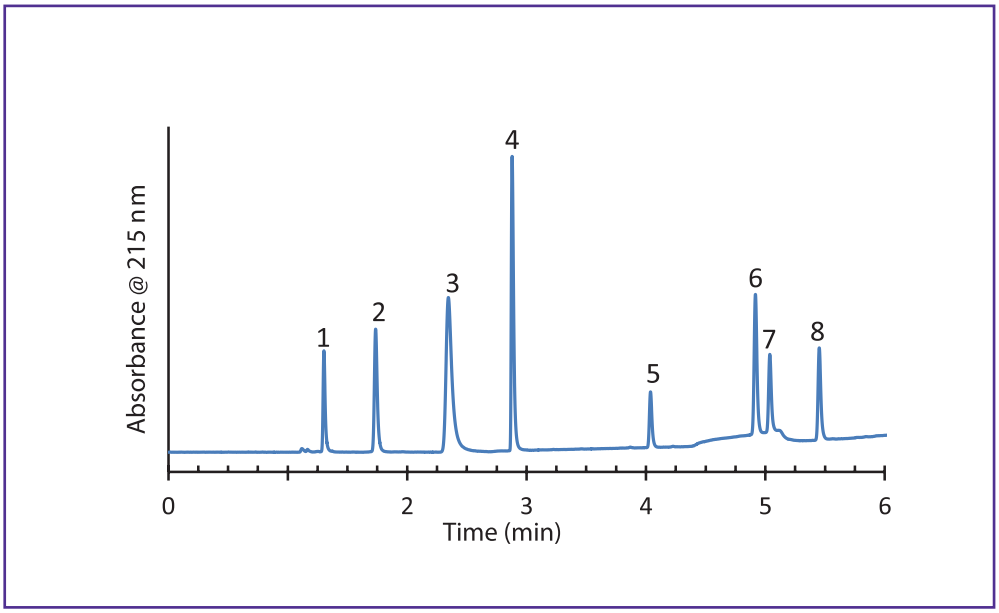


Vitamin K1: *cis*-phylloquinone

	Resolution	Temperature
A	1.53	35 ºC
B	1.58	30 ºC
C	1.78	25 ºC
D	2.2	20 ºC
E	3.03	15 ºC

Separation of Water-Soluble Vitamins on HALO® AQ-C18

Application Note: 200-V



PEAK IDENTITIES:

1.Thiamine (B1)
2. Ascorbic acid (C)
3. Nicotinamide (B3)
4. Pyridoxine (B6)
5. Pantothenic acid (B5)
6. Cyanocobalamin (B12)
7. Folic acid (B9)
8. Riboflavin (B2)

HALO® AQ-C18 columns can be used with totally or mostly aqueous mobile phases. In this application, eight water-soluble vitamins are well-separated using this phase in under six minutes using a gradient from 0-70% methanol, with a 1-minute initial hold.

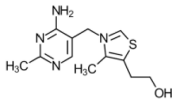
TEST CONDITIONS:

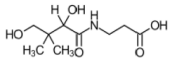
Column: HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-722
Mobile Phase: A/B
A= 0.025 M, potassium phosphate in water, pH=2.5
B= Methanol
Gradient:

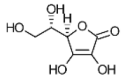
Time (min.)	%B
0.0	0
1.0	0
6.0	70
10.0	70

Flow Rate: 1.2 mL/min.
Initial Pressure: 243 bar
Temperature: 30°C
Injection Volume: 2.0 µL
Sample Solvent: water
Detection: 215 nm, VWD Response Time: 0.02 sec.
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

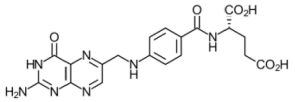
STRUCTURES:

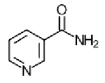

Thiamine

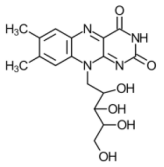

Pantothenic acid

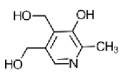

Ascorbic acid

Cyanocobalamin
(structure not included to space constraints)


Folic Acid

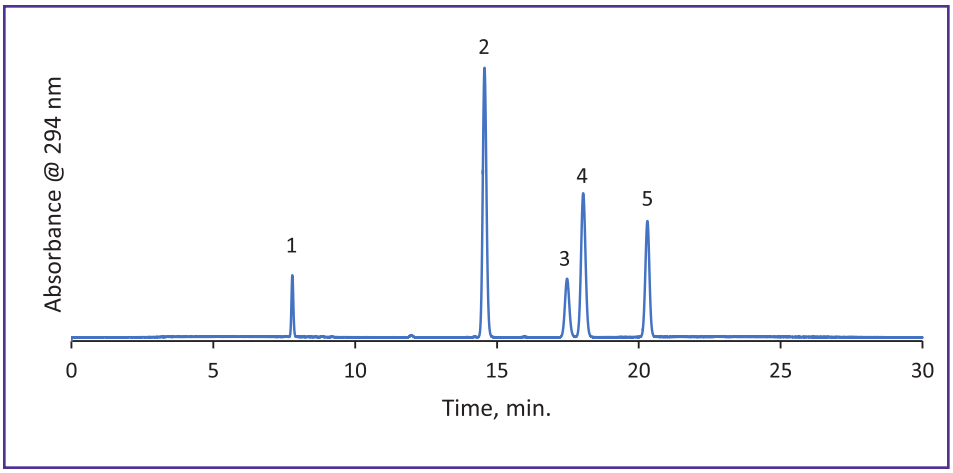

Nicotinamide


Riboflavin


Pyridoxine

Analysis of Vitamin A and Vitamin E Isomers using GB Method

Application Note 210-V



PEAK IDENTITIES:

1. Retinyl Acetate
2. δ- tocopherol
3. γ- tocopherol
4. β- tocopherol
5. α- tocopherol

The 2.7 µm HALO® C30 is an ideal choice for the separation of vitamin A and the isomers of vitamin E using the official GB method. The shape selectivity of C30 allows for baseline resolution of gamma and beta tocopherol, which typically coelute on other bonded phases.

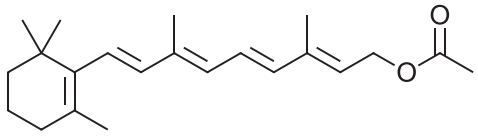
TEST CONDITIONS:

Column: HALO 160 Å C30, 2.7 µm 4.6 x 250 mm
Part Number: 92114-930
Mobile Phase A: Water
Mobile Phase B: Methanol
Gradient:

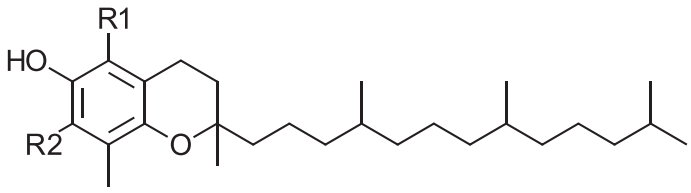
Time	%B
0.0	96
13.0	96
20.0	100
24.0	100
24.5	96
30.0	96

Flow Rate: 0.8 mL/min
Initial Pressure: 237 bar
Temperature: 20 °C
Detection: 294 nm, PDA
Injection Volume: 10 µL
Sample Solvent: Methanol/ Ethanol
Data Rate: 14 Hz
Response Time: 0.12 sec.
Flow Cell: 5 µL semi-micro
LC System: Agilent 1100

STRUCTURES:



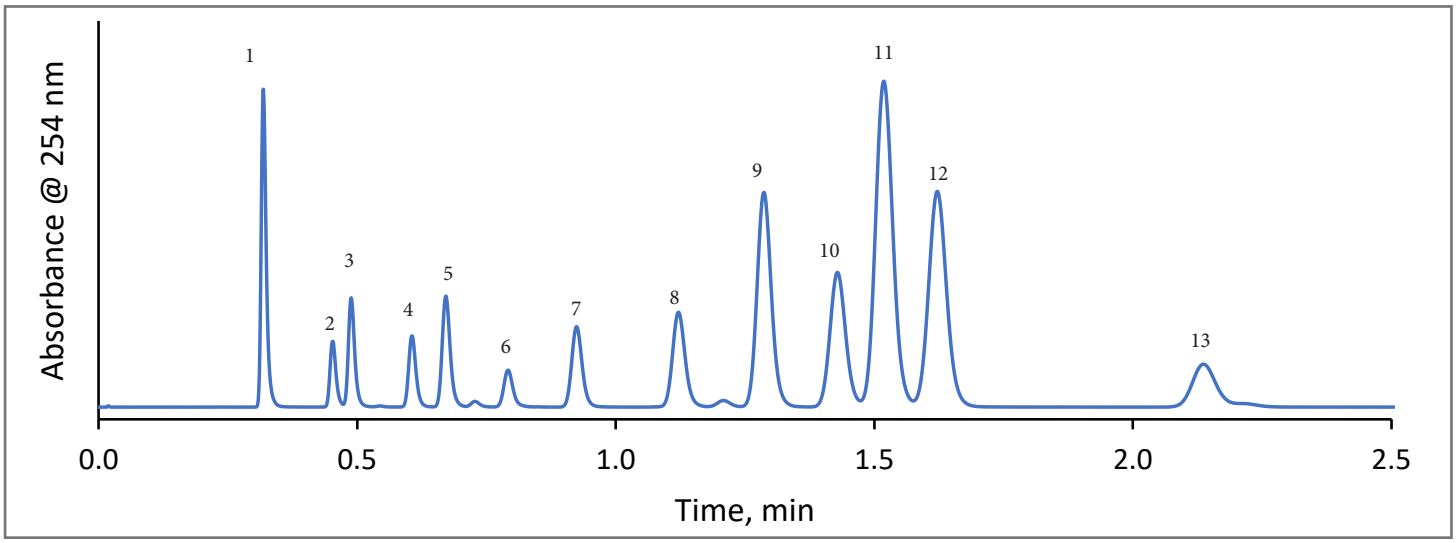
Retinyl acetate



Tocopherol	R1	R2
Alpha (α)	CH_3	CH_3
Beta (β)	CH_3	H
Gamma (γ)	H	CH_3
Delta (δ)	H	H

Separation of Phthalates and Neutral Compounds on HALO® C8

212



PEAK IDENTITIES:

1. Uracil
2. 1-Indanol
3. Dimethyl phthalate
4. Anisole
5. Diethyl phthalate
6. Benzophenone
7. Naphthalene
8. Dipropyl phthalate
9. Hexanophenone
10. Phenanthrene
11. Anthracene
12. 3-phenyltoluene
13. Dibutyl phthalate

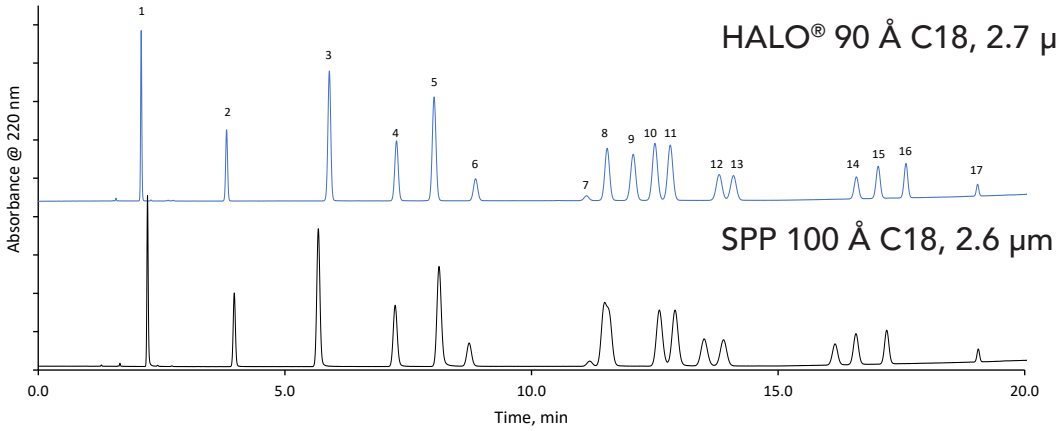
TEST CONDITIONS:

Column: HALO 90 Å C 8, 2.7 µm, 4.6 x 50mm
Part Number: 92814-408
Mobile Phase: 63/37 - A/B
A: Water
B: 30 Acetonitrile/ Methanol
Isocratic:
Flow Rate: 1.5 mL/min
Pressure: 136 bar
Temperature: 27 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

A separation of phthalates and neutral compounds are separated on a HALO® C8 column with excellent speed and resolution. Phthalates are commonly used as plasticizers and added to plastics in order to increase their durability and physical properties.

HPLC Separation of Explosives: Comparison of HALO® to a Competitor SPP Column

238-EX



PEAK IDENTITIES

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. 3,5-Dinitroaniline
6. Nitrobenzene
7. Nitroglycerin
8. Tetryl
9. 2,4,6-Trinitrotoluene
10. 2-Amino-4,6-dinitrotoluene
11. 4-Amino-2,6-dinitrotoluene
12. 2,4-Dinitrotoluene
13. 2,6-Dinitrotoluene
14. 2-Nitrotoluene
15. 4-Nitrotoluene
16. 3-Nitrotoluene
17. PETN (pentaerythritol tetranitrate)

TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-702
Competitor Column: SPP 100 Å C18, 2.6 µm, 4.6 x 150 mm
Mobile Phase A: Water
Mobile Phase B: Methanol
Gradient:

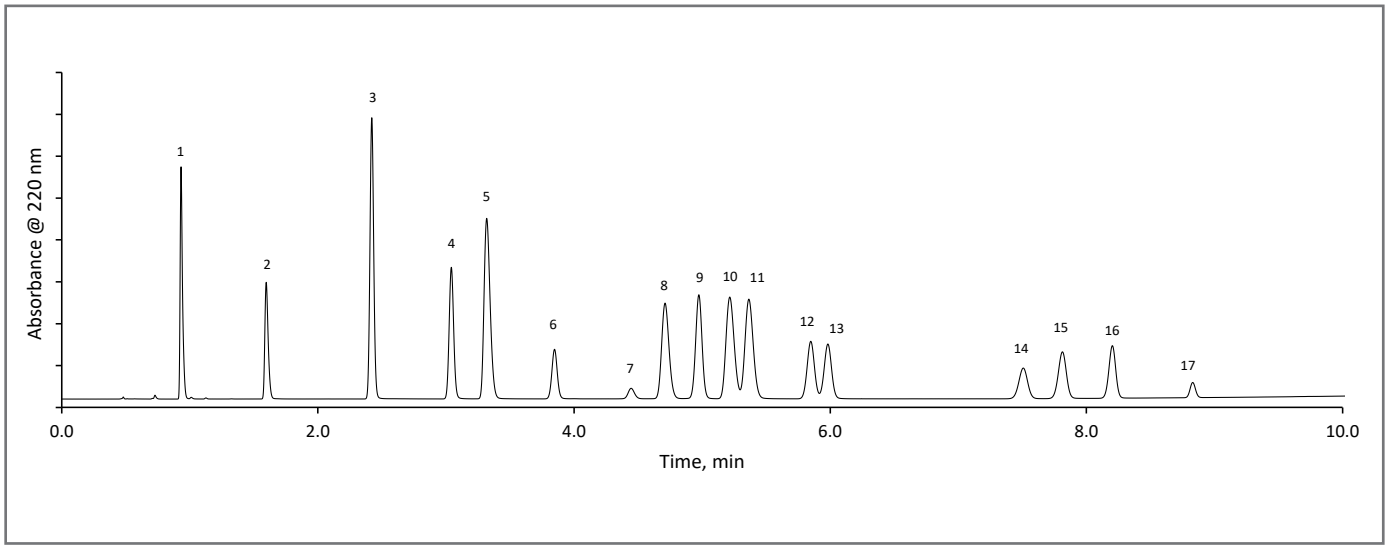
Time	%B
0.0	25
14.0	35
20.0	62

Flow Rate: 1.5 mL/min
Initial HALO® Back Pressure: 441 bar
Initial Competitor Back Pressure: 490 bar
Temperature: 43°C
Detection: 220 nm
Injection Volume: 0.2 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
LC System: Shimadzu Nexera X2

The determination of explosives in the environment is outlined in EPA method 8330B. 17 explosive compounds are separated on a HALO 90 Å C18 column in less than 20 minutes using a water/methanol gradient. These compounds are either used in the manufacture of explosives or propellants.The impurities or degradation of these compounds could be found in water, soil, or sediment samples. Baseline resolution is maintained on the HALO® column while there are peak coelutions with a similar superficially porous particle column.

UHPLC Separation of Explosives on 2 µm HALO® C18

239-EX



PEAK IDENTITIES

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. 3,5-Dinitroaniline
6. Nitrobenzene
7. Nitroglycerin
8. Tetryl
9. 2,4,6-Trinitrotoluene
10. 2-Amino-4,6-dinitrotoluene
11. 4-Amino-2,6-dinitrotoluene
12. 2,4-Dinitrotoluene
13. 2,6-Dinitrotoluene
14. 2-Nitrotoluene
15. 4-Nitrotoluene
16. 3-Nitrotoluene
17. PETN (pentaerythritol tetranitrate)

TEST CONDITIONS:

Column: HALO 90 Å C18, 2 µm, 3.0 x 100 mm
Part Number: 91813-602
Mobile Phase A: Water
Mobile Phase B: Methanol
Gradient:

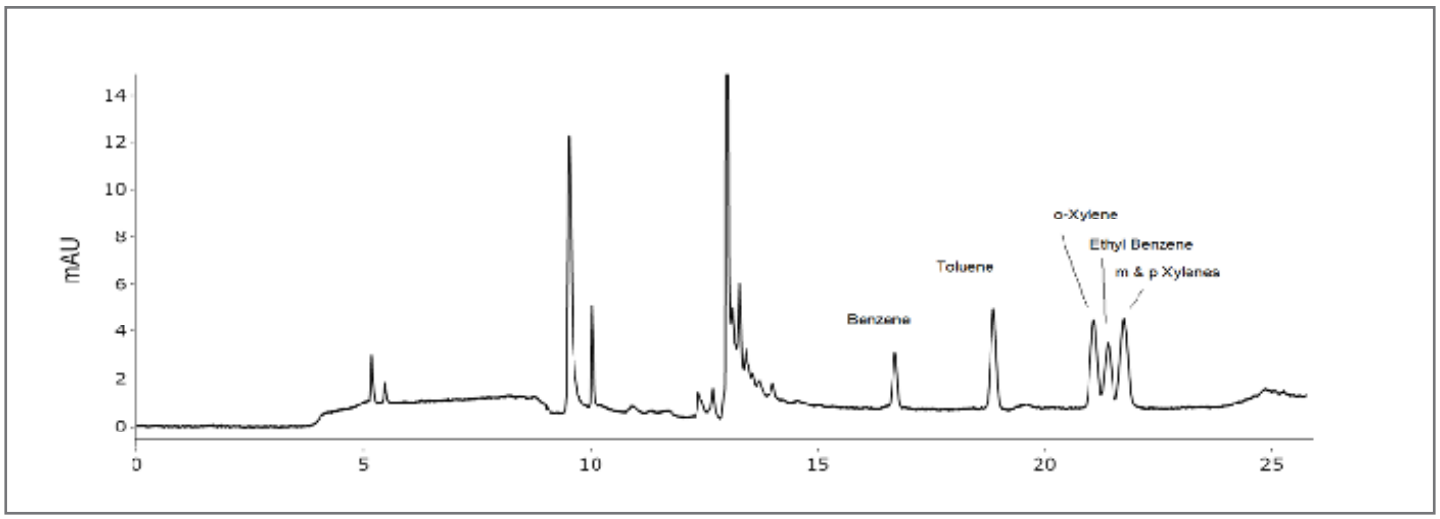
Time	%B
0.0	25
6.9	35
9.9	62

Flow Rate: 0.85 mL/min
Initial Back Pressure: 571 bar
Temperature: 43°C
Detection: 220 nm
Injection Volume: 0.2 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
LC System: Shimadzu Nexera X2

The determination of explosives in the environment is outlined in EPA method 8330B. 17 explosive compounds are separated on a HALO 90 Å 2 µm C18 column *in less than 10 minutes* using a water/methanol gradient. These compounds are either used in the manufacture of explosives or propellants. The impurities or degradation of these compounds could be found in water, soil, or sediment samples.

HPLC Separation of BTEX in Process Water

281-PT



PEAK IDENTITIES:

1. Benzene
2. Toluene
3. o-Xylene
4. Ethylbenzene
5. m-, p-Xylenes

TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 0.25 x 170mm
Mobile Phase:
A: 94.5/5.0/0.5 Water/ Acetonitrile/ formic acid
B: Acetonitrile

Gradient:	Time	%B
	0.0	3
	9.0	5
	10.0	50
	22.0	62
	30.0	90

Flow Rate: 1.5 µL/min
Pressure: 276 bar
Temperature: Ambient
Detection: 255 nm, UV
Delay Volume: 1 µL
LC System: Axcend Focus LC
Data Courtesy of: Axcend

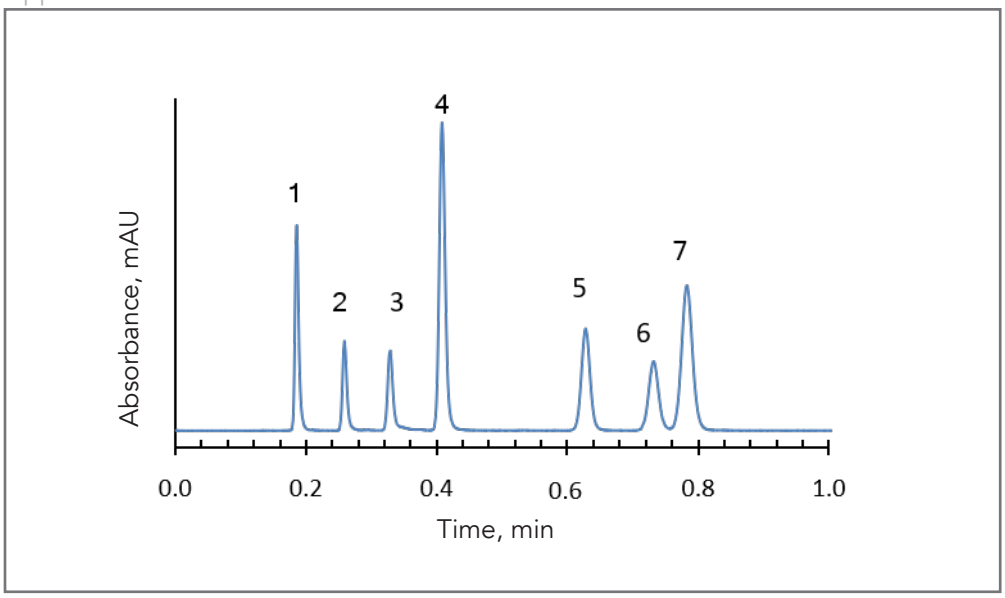
Benzene, toluene, ethylbenzene, and xylene (BTEX) are compounds that occur naturally in crude oil and can be found in sea water close to natural gas and petroleum deposits where drilling occurs.

BTEX compounds are created and used during the processing of petroleum products. In this application, a separation of BTEX at 6 ppm is performed using a HALO® C18 capillary column on an Axcend Focus portable LC.

Liquid chromatography is an alternative method to gas chromatography, which eliminates the use of a flame and hydrogen gas which is ideal for off- shore oil rigs. Smaller column internal diameters, like capillaries, allow for higher sensitivity which can benefit those who are working with very small sample concentrations.

Isocratic Separation of Anilines on HALO® RP-Amide

Application Note 21-B



PEAK IDENTITIES:

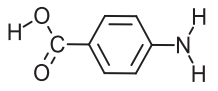
1. p-Aminobenzoic acid
2. 1, 2-Phenylenediamine
3. p-Anisidine
4. Aniline
5. 3-Nitroaniline
6 4-Chloroaniline
7. 2-Nitroaniline

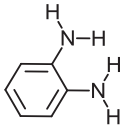
In this separation on the HALO® RP-Amide phase, aniline and six derivatives can be separated isocratically in less than one minute. These and similar compounds are often used in the dyes industry.

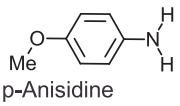
TEST CONDITIONS:

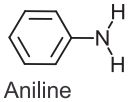
Column: HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 60/40 - A/B
A: 0.02 M sodium phosphate buffer, pH 7.0
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 180 bar
Temperature: 25 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 ACN/water
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

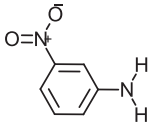
STRUCTURES:

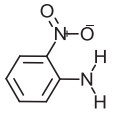

p-Aminobenzoic acid

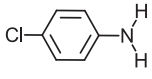

1,2-phenylenediamine


p-Anisidine


Aniline

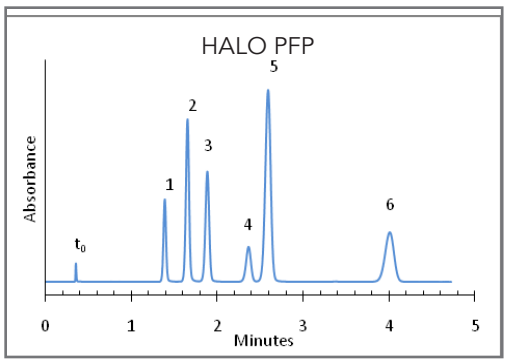

3-Nitroaniline

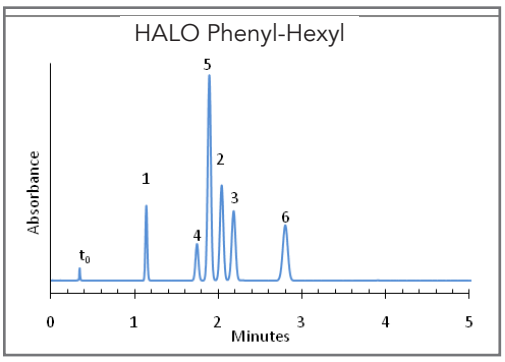

2-Nitroaniline


4-Chloroaniline

Separation of Aromatic Nitro Compounds on HALO® PFP and Phenyl-Hexyl

Application Note 26-P





PEAK IDENTITIES:

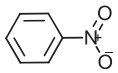
1. Nitrobenzene
2. 1-Cl-4-Nitrobenzene
3. 2,6-Dinitrotoluene
4. 4-Nitrotoluene
5. 3-Nitrotoluene
6. 4-Cl-3-Nitroanisole

Differences in the interaction of the phenyl rings 3-Nitrotoluene on the bonded phases with the pi electron systems of the nitro aromatic compounds result in significantly different selectivities that can be used to optimize these separations.

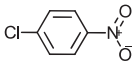
TEST CONDITIONS:

Columns:
1) HALO 90 Å PFP, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-409
2) HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-406
Mobile Phase: 45/55 - A/B
A: Water
B: Methanol
Flow Rate: 1.5 mL/min
Pressure: ~200 bar
Temperature: 40 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: ~20/80 water/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

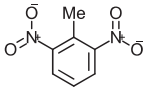
STRUCTURES:



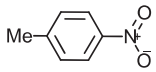
Nitrobenzene



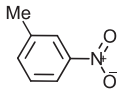
1-Chloro-4-Nitrobenzene



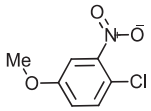
2, 6-Dinitrotoluene



4-Nitrotoluene



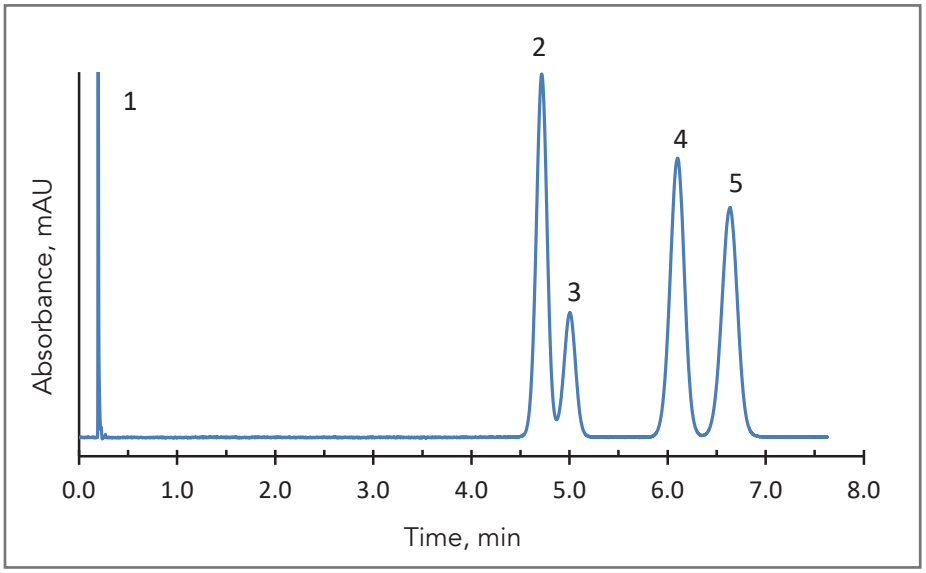
3-Nitrotoluene



4-Chloro-3-Nitroanisole

Isocratic Separation of Dinitrotoluenes on HALO® RP-Amide Phase

Application Note 35-EX



PEAK IDENTITIES:

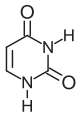
1. Uracil
2. 2,4-Dinitrotoluene
3. 2,6-Dinitrotoluene
4. 3,4-Dinitrotoluene
5. 2,3-Dinitrotoluene

These dinitrotoluenes are difficult to separate, but can be separated with almost baseline resolution in under 7 minutes using a 50 mm long HALO® Fused-Core® RP-Amide column.

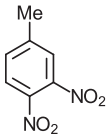
TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 80/20 - A/B
A: Water
B: Acetonitrile
Flow Rate: 2.5 mL/min
Pressure: 257 bar
Temperature: 27 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 acetonitrile/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

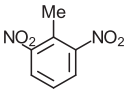
STRUCTURES:



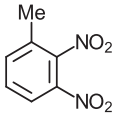
Uracil



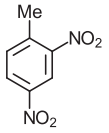
3,4-Dinitrotoluene



2,6-Dinitrotoluene



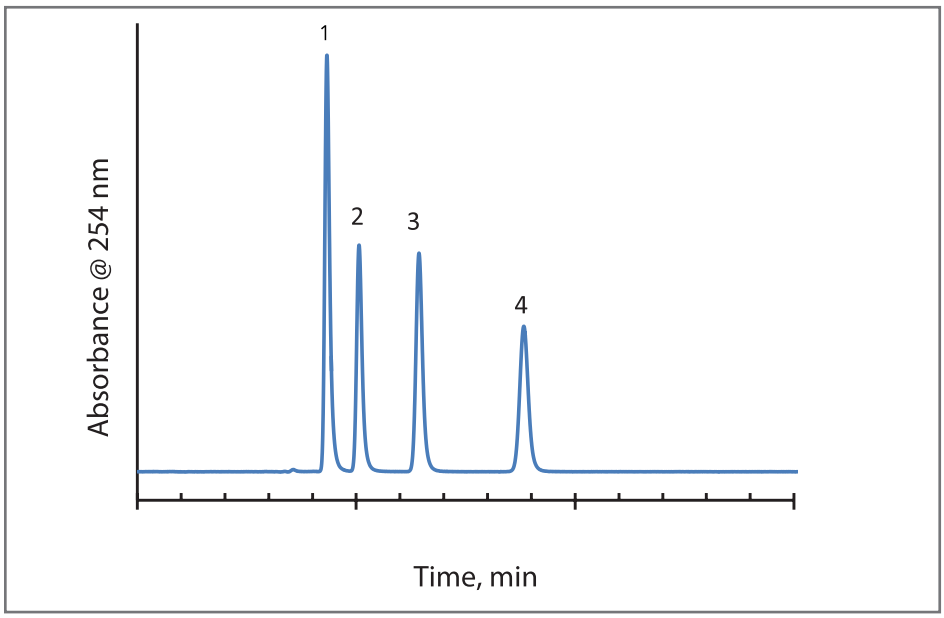
2,3-Dinitrotoluene



2,4-Dinitrotoluene

Separation of p-Hydroxybenzoic Acid Esters (Parabens) on HALO® C18, 2.7 µm

Application Note 94-P



PEAK IDENTITIES:

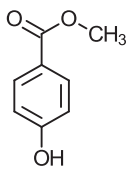
1. Methyl paraben
2. Ethyl paraben
3. Propyl paraben
4. Butyl paraben

The parabens are used as preservatives in many cosmetics, shampoos, medications and food. They are considered to be safe but recent studies have indicated a possible connection with breast cancer. Four common parabens can be rapidly determined using a short HALO® C18, 2.7 µm column at a relatively low pressure.

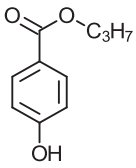
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 30/70 - A/B
A: Water
B: Methanol
Flow Rate: 1.5 mL/min
Pressure: 196 bar
Temperature: 40 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 50/50 water/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

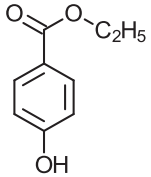
STRUCTURES:



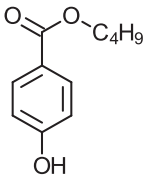
Methyl Paraben



Propyl Paraben



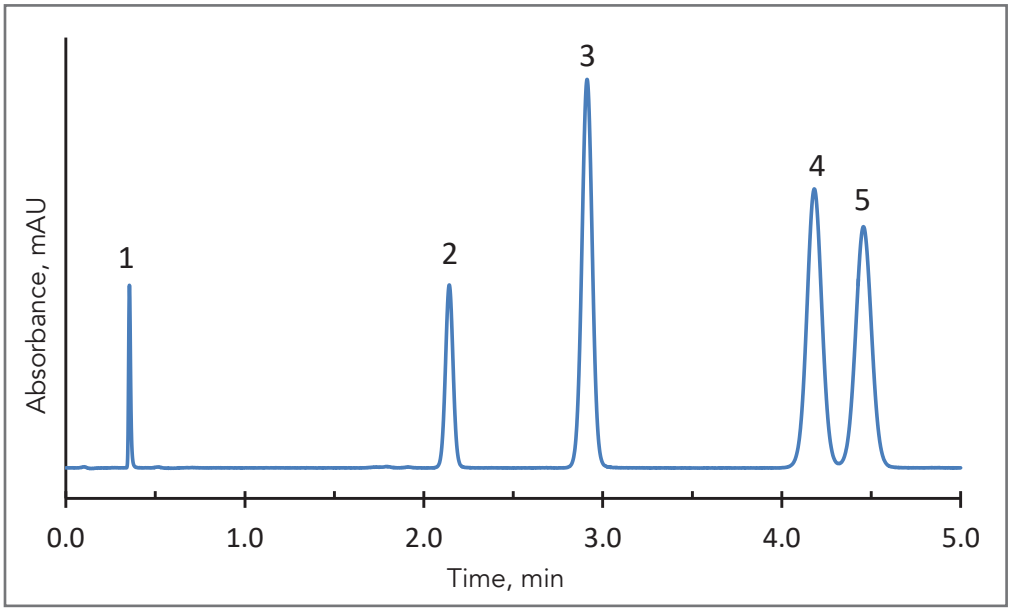
Ethyl Paraben



Butyl Paraben

Isocratic Separation of Dinitrotoluenes on HALO® PFP Phase

Application Note 36-EX



PEAK IDENTITIES:

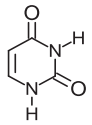
1. Uracil
2. 2,6-Dinitrotoluene
3. 2,4-Dinitrotoluene
4. 3,4-Dinitrotoluene
5. 2,3-Dinitrotoluene

These dinitrotoluenes are difficult to separate, but can be separated with baseline resolution in under 5 minutes using a HALO® Fused-Core® PFP (perfluorophenylpropyl) column.

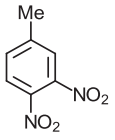
TEST CONDITIONS:

Column: HALO 90 Å PFP, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-409
Mobile Phase: 45/55 - A/B
A: Water
B: Methanol
Flow Rate: 1.5 mL/min
Pressure: 225 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 acetonitrile/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

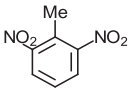
STRUCTURES:



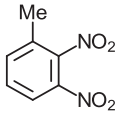
Uracil



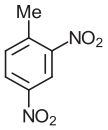
3,4-Dinitrotoluene



2,6-Dinitrotoluene



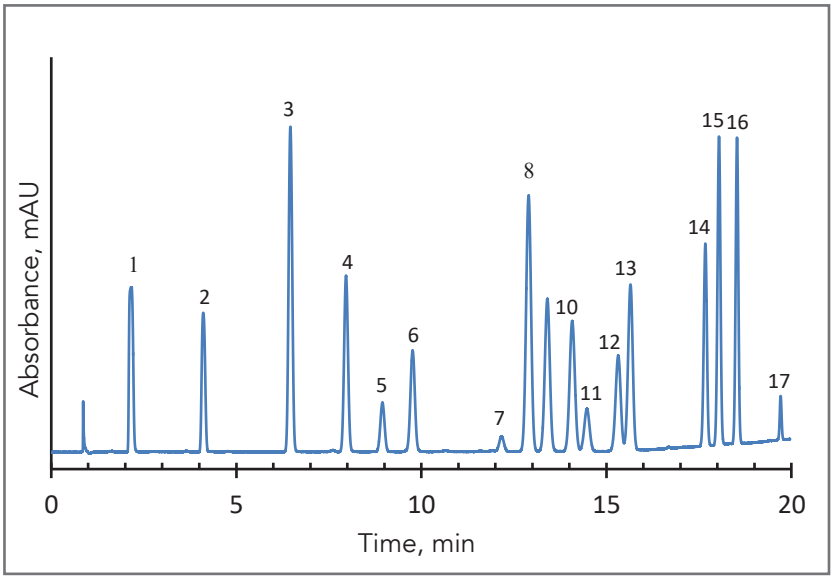
2,3-Dinitrotoluene



2,4-Dinitrotoluene

Separation of 17 Explosives on HALO® C18, 2.7 µm

Application Note 31-EX



PEAK IDENTITIES:

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. 3,5-Dinitroaniline
6. Nitrobenzene
7. Nitroglycerin
8. Tetryl
9. 2,4,6-Trinitrotoluene
10. 2-Amino-4,6-Dinitrotoluene
11. 4-Amino-2,6-Dinitrotoluene
12. 2,4-Dinitrotoluene
13. 2,6-Dinitrotoluene
14. 2-Nitrotoluene
15. 4-Nitrotoluene
16. 3-Nitrotoluene
17. PETN (pentaerythritol tetranitrate)

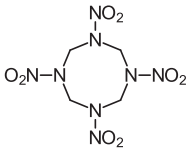
TEST CONDITIONS:

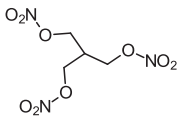
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-702
Mobile Phase:
A: Water
B: Methanol
Gradient:

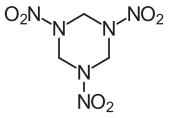
Time (min)	% B
0.0	25
14.0	35
20.0	62

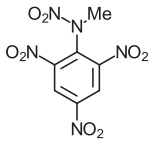
Flow Rate: 1.5 mL/min
Pressure: 366 bar to start, max. 405 bar
Temperature: 43 °C
Detection: UV 220 nm, VWD
Injection Volume: 40 µL
Sample Solvent: 50/50 water/methanol
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

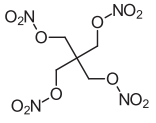
STRUCTURES:


HMX


Nitroglycerin


RDX

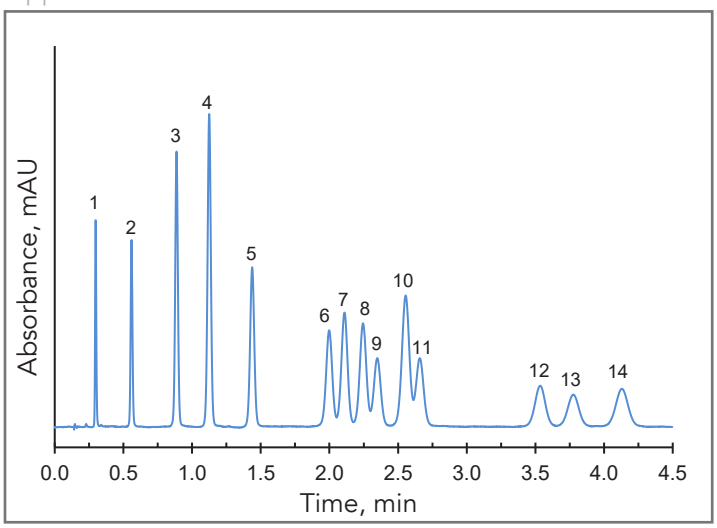

Tetryl


Pentaerythritol Tetranitrate

The determination of explosives in the environment is outlined in EPA method 8330B and under the conditions recommended, requires two column phases to determine 17 compounds. However, all 17 explosive compounds can be separated on a HALO® C18, 2.7 µm column in less than 20 minutes using a water/methanol gradient.

Separation of Explosives on HALO® C18

Application Note 50-EX



PEAK IDENTITIES:

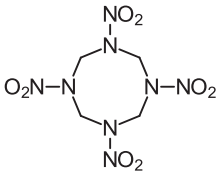
1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. Tetryl
7. 2, 4, 6-Trinitrotoluene
8. 2-Amino-4,6-dinitrotoluene
9. 4-Amino-2,6-dinitrotoluene
10. 2,6-Dinitrotoluene
11. 2,4-Dinitrotoluene
12. 2-Nitrotoluene
13. 4-Nitrotoluene
14. 3-Nitrotoluene

Fourteen explosive materials can be rapidly separated on the highly efficient HALO® C18 phase in under 5 minutes at a relatively high flow rate and moderate pressure.

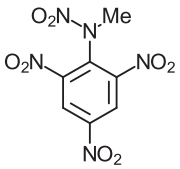
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 73/27 - A/B
A: Water
B: Methanol
Flow Rate: 3.3 mL/min
Pressure: 343 bar
Temperature: 40 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample: Standards diluted with methanol/water
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

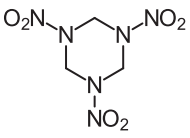
STRUCTURES:



HMX



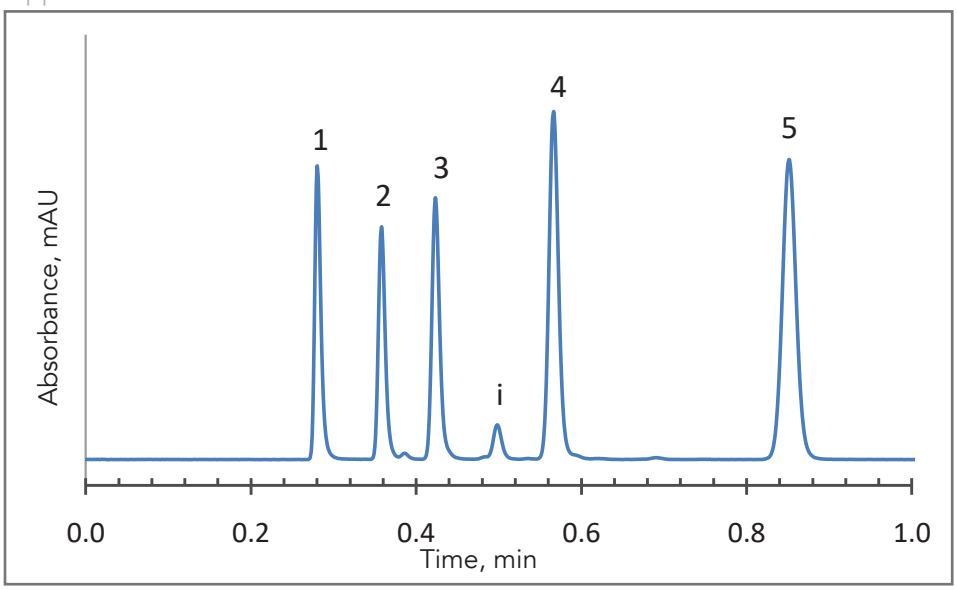
Tetryl



RDX

Isocratic Separation of Phthalate Esters on HALO® C18

Application Note 24-P



PEAK IDENTITIES:

1. Uracil
2. Dimethylphthalate
3. Diethylphthalate
   i = impurity
4. Di-n-propylphthalate
5. Di-n-butylphthalate

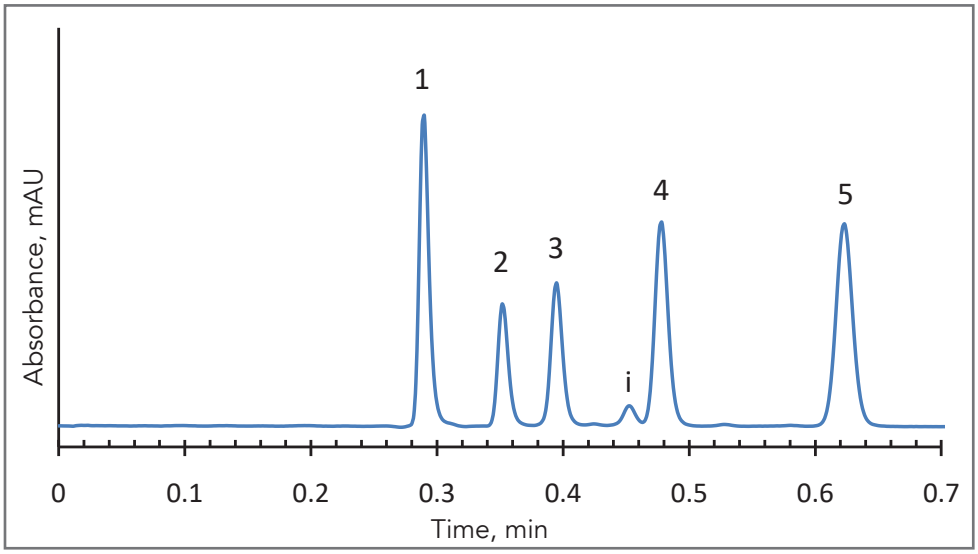
Plasticiizers are used widely as additives in plastics to increase flexibility, durability and other desirable properties. Lower molecular weight phthalates can be volatile and are suspected of causing health problems. Here several of these are easily analyzed on a HALO® C18 column in under one minute.

TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 20/80 - A/B
A: Water
B: Acetonitrile
Flow Rate: 1.5 mL/min
Pressure: 97 bar
Temperature: 27 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

Isocratic Separation of Phthalate Esters on HALO® RP-Amide

Application Note 25-P



PEAK IDENTITIES:

1. Uracil
2. Dimethylphthalate
3. Diethylphthalate
   i = impurity
4. Di-n-propylphthalate
5. Di-n-butylphthalate

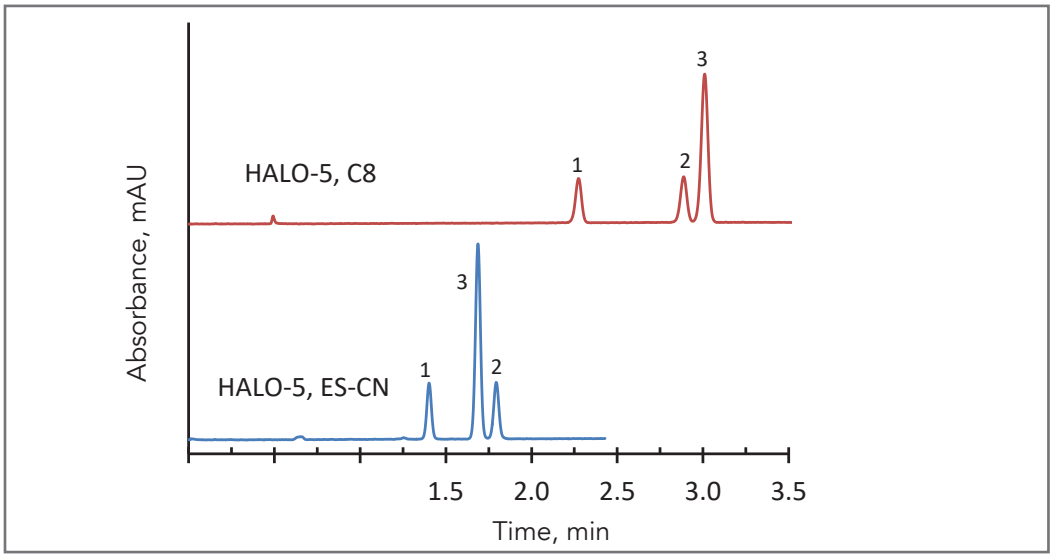
In this separation four common plasticizers are analyzed on a HALO® RP-Amide column in a fraction of a minute. These compounds are used in the plastics industry to add desirable properties such as flexibility and durability. However, due to their volatility these lower molecular weight phthalates are suspected of causing health issuses.

TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-407
Mobile Phase: 20/80 - A/B
A: Water
B: Acetonitrile
Flow Rate: 1.5 mL/min
Pressure: 88 bar
Temperature: 27 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra column volume: ~14 µL

Separation of Stilbenes on HALO® C8 and ES-CN, 5 µm

Application Note 115



PEAK IDENTITIES:

1. trans-Stilbene oxide
2. trans-Stilbene
3. cis-Stilbene

These two HALO® 5 µm phases illustrate the difference in selectivity for the cis- and trans-isomers of these stilbene compounds and the utility of different bonded phases.

TEST CONDITIONS:

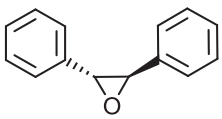
Columns:
1) HALO 90 Å C8, 5 µm, 4.6 x 50 mm
Part Number: 95814-408
2) HALO 90 Å ES-CN, 5.0 µm, 4.6 x 50 mm
Part Number: 95814-404
Mobile Phase:
A: Water
B: Acetonitrile

Gradient:

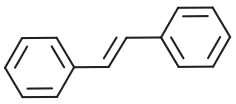
Time (min)	% B
0.0	40
3.0	60
4.0	60

Flow Rate: 2.0 mL/min
Initial Pressure: 120 bar
Temperature: 30 °C
Detection: UV 230 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 water/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

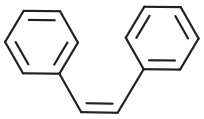
STRUCTURES:



trans-Stilbene Oxide



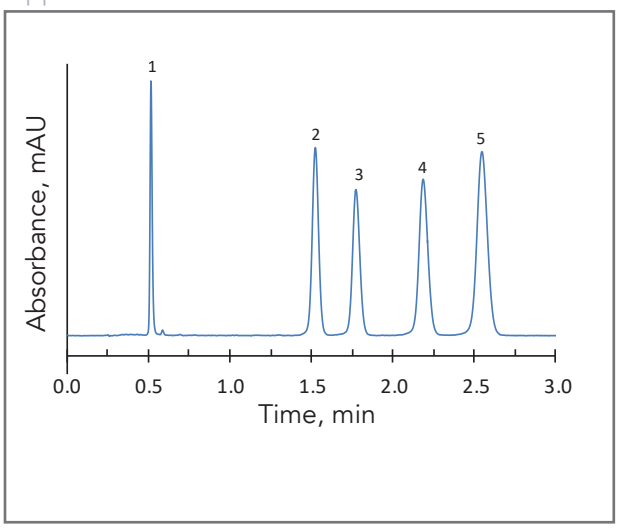
trans-Stilbene



cis-Stilbene

Separation of Iodonium Salts on HALO® Phenyl-Hexyl

Application Note 126-IP



PEAK IDENTITIES:

1. Diphenyliodonium chloride
2. (4-Nitrophenyl)(2,4,6-Trimethylphenyl) Iodonium triflate
3. (3-Bromophenyl)(2,4,6-Trimethylphenyl) Iodonium triflate
4. Bis(2,4,6-Trimethylphenyl) Iodonium Triflate
5. (4-Iodophenyl)(2,4,6-Trimethylphenyl) Iodonium Triflate

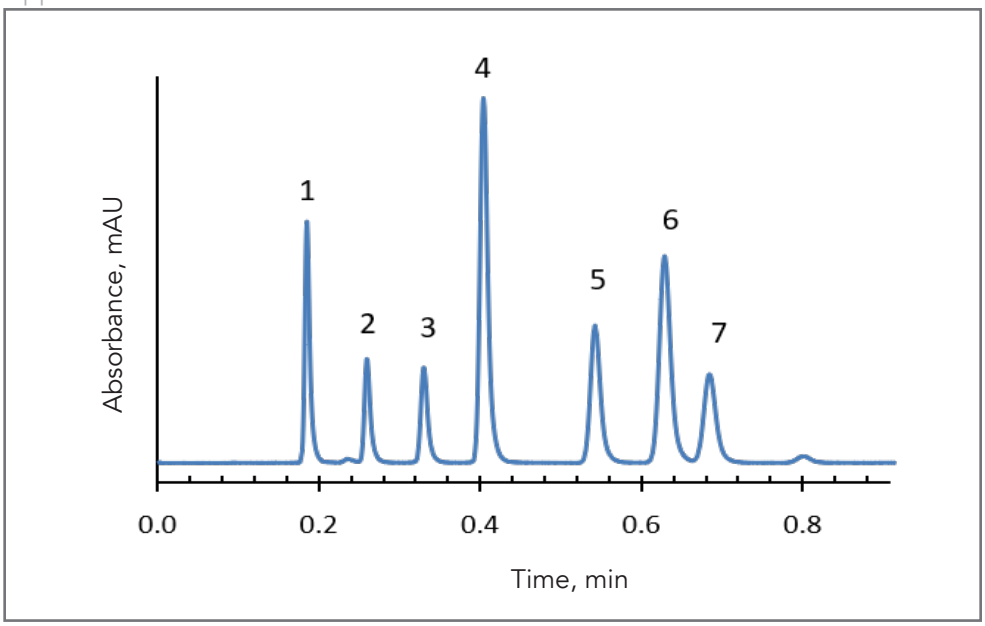
TEST CONDITIONS:

Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-405
Mobile Phase: 30/70 - A/B
A: Water
B: Methanol with 50 mM sodium heptane sulfonate
Flow Rate: 1.8 mL/min
Pressure: 276 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: Mobile phase
Response Time: 0.02 sec
Data Rate: 25 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

Iodonium salts have gained favor as reagents for organic synthesis. They can be rapidly analyzed by HPLC using a HALO® Fused-Core® Phenyl-Hexyl column in an ion pairing separation mode.

Isocratic Separation of Anilines on HALO® C18

Application Note 20-B



PEAK IDENTITIES:

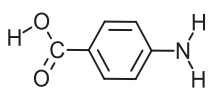
1. p-Aminobenzoic acid
2. 1, 2-Phenylenediamine
3. p-Anisidine
4. Aniline
5. 3-Nitroaniline
6. 2-Nitroaniline
7. 4-Chloroaniline

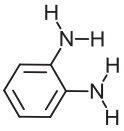
Aniline and its derivatives are often used in the dyes industry. Here, aniline and some derivatives can be separated on the highly efficient HALO® C18 phase in less than one minute.

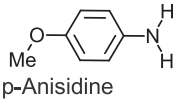
TEST CONDITIONS:

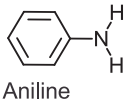
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 60/40 - A/B
A: 0.02 M sodium phosphate buffer, pH 7.0
B: Acetonitrile
Flow Rate: 2.0 mL/min
Pressure: 211 bar
Temperature: 25 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 ACN/water
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

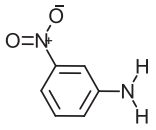
STRUCTURES:

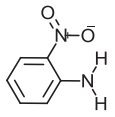

p-Aminobenzoic acid

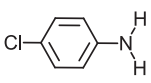

1,2-phenylenediamine


p-Anisidine


Aniline

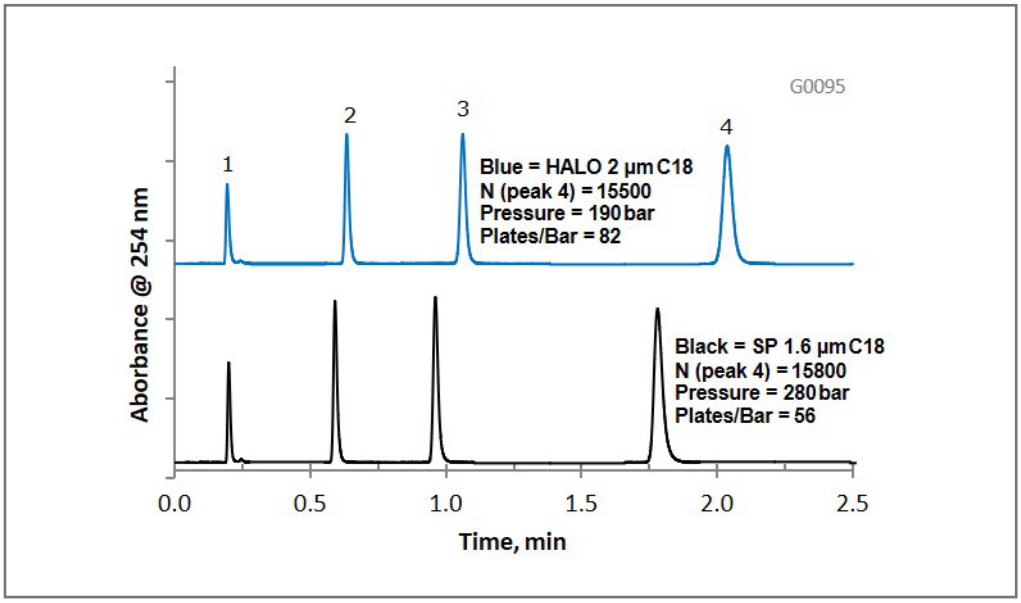

3-Nitroaniline


2-Nitroaniline


4-Chloroaniline

Comparable Efficiency of HALO® Fused-Core® C18, 2.0 µm and Superficially Porous (SP) C18, 1.6 µm Columns

Application Note 111



PEAK IDENTITIES:

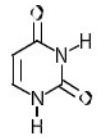
1. Uracil
2. Pyrene
3. Decanophenone
4. Dodecanophenone

With a HALO® 2.0 µm C18 column, one can achieve the same performance at only 68% of the back pressure of a competitor's superficially porous 1.6 µm C18 column.

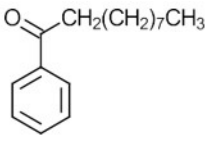
TEST CONDITIONS:

Columns:
1) HALO 90 Å C18, 2.0 µm, 2.1 x 50 mm
Part Number: 91812-402
2) Superficially porous C18, 1.6 µm, 2.1 x 50 mm
Mobile Phase: 15/85 - A/B
A: Water
B: Acetonitrile
Flow Rate: 0.5 mL/min
Pressure: See chart
Temperature: 25 °C
Detection: UV 254 nm, PDA
Injection Volume: 0.2 µL
Sample Solvent: 20/80 water/acetonitrile
Response Time: 0.16 sec
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera
Extra Column Volume: ~7 µL

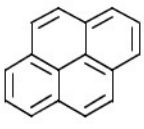
STRUCTURES:



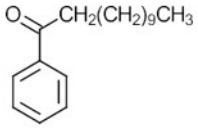
Uracil



Decanophenone



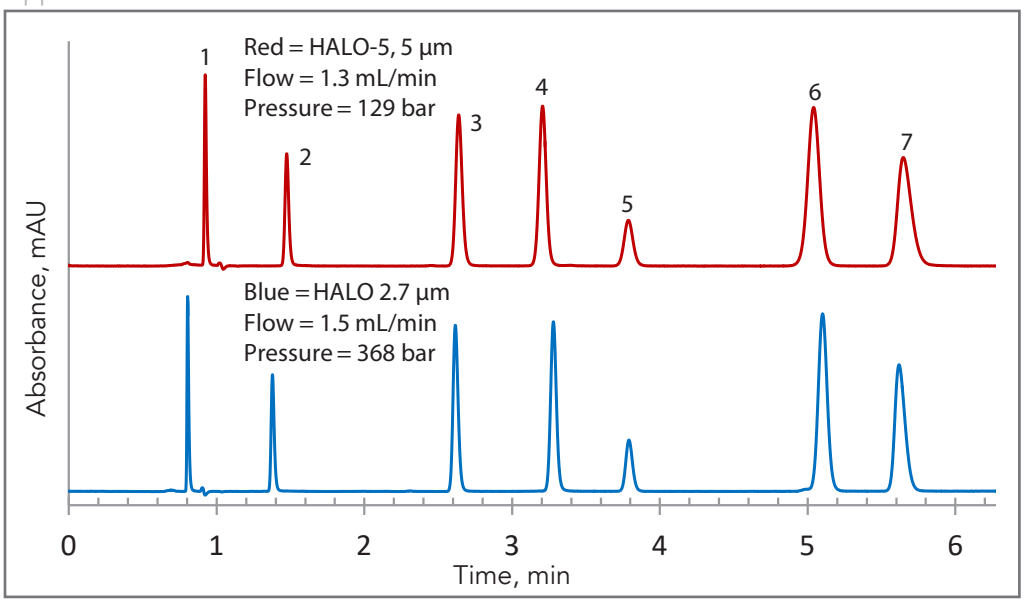
Pyrene



Dodecanophenone

Comparable Selectivity Between HALO® 5 µm and HALO® 2.7 µm RP-Amide Phases

Application Note 106



PEAK IDENTITIES:

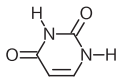
1. Uracil
2. p-Aminobenzoic acid
3. Acetylsalicylic acid
4. Dehydroacetic acid
5. Benzoic acid
6. Methyl paraben
7. 3-Fluorobenzoic acid

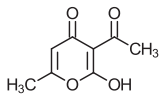
Similar selectivity is achieved between the 5 µm and 2.7 µm HALO® RP-Amide particle sizes through a slight flow rate adjustment allowing easy method transfer.

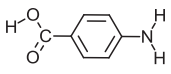
TEST CONDITIONS:

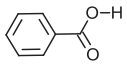
Columns:
1) HALO 90 Å RP-Amide, 5 µm, 4.6 x 150 mm
Part Number: 95814-707
2) HALO 90 Å RP-Amide, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-707
Mobile Phase: 70/30 - A/B
A: Water/0.1% formic acid
B: Acetonitrile
Flow Rate: See chart
Pressure: See chart
Temperature: 25 °C
Detection: UV 254 nm, VWD
Injection Volume: 5.0 µL
Sample Solvent: 50/50 water/acetonitrile
Response Time: 0.12 sec
Flow Cell: 5.0 µL semi-micro
LC System: Agilent 1100

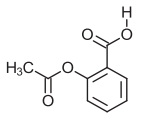
STRUCTURES:

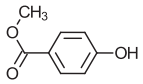

Uracil

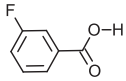

Dehydroacetic Acid


p-Aminobenzoic Acid


Benzoic Acid

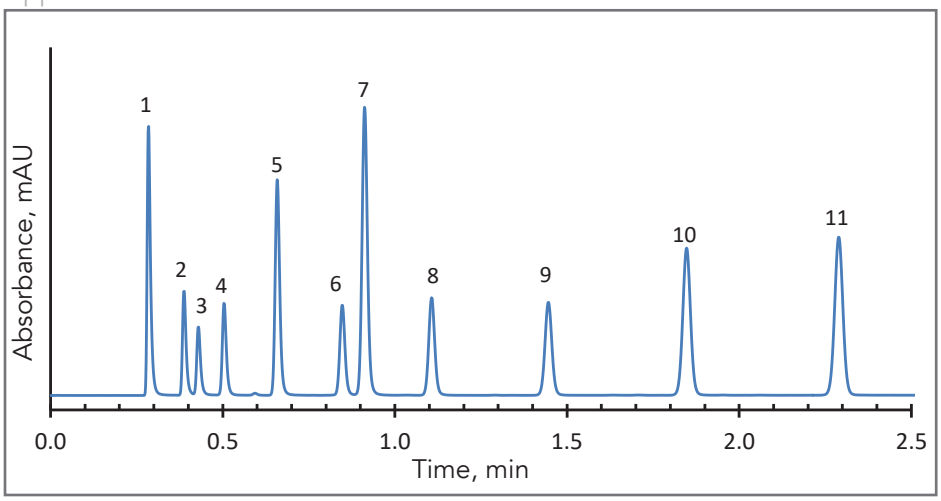

Acetylsalicylic Acid


Methyl Paraben


3-Fluorobenzoic Acid

Rapid HPLC Separation of Phenones on HALO® C18 Phase

Application Note 27-P



PEAK IDENTITIES:

1. Uracil
2. 2',4'-Dihydroxyacetophenone
3. 2',6'-Dihydroxyacetophenone
4. Acetophenone
5. Propiophenone
6. Butyrophenone
7. Benzophenone
8. Valerophenone
9. Hexanophenone
10. Heptanophenone
11. Octanophenone

Phenones are often used in synthetic organic chemistry as starting materials. The purity or concentration or purity of these materials can be determined as shown in this short separation on a HALO® C18 column.

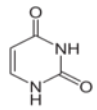
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 92814-402
Mobile Phase: 40/60 - A/B
A: Water
B: Acetonitrile
Gradient:

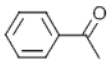
Time (min)	% B
0.0	60
2.0	80
2.5	80

Flow Rate: 1.5 mL/min
Pressure: 126 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50 methanol/acetonitrile
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

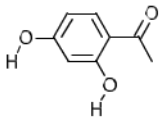
STRUCTURES:



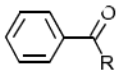
Uracil



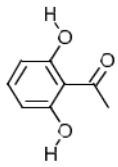
Acetophenone



2',4'-Dihydroxyacetophenone



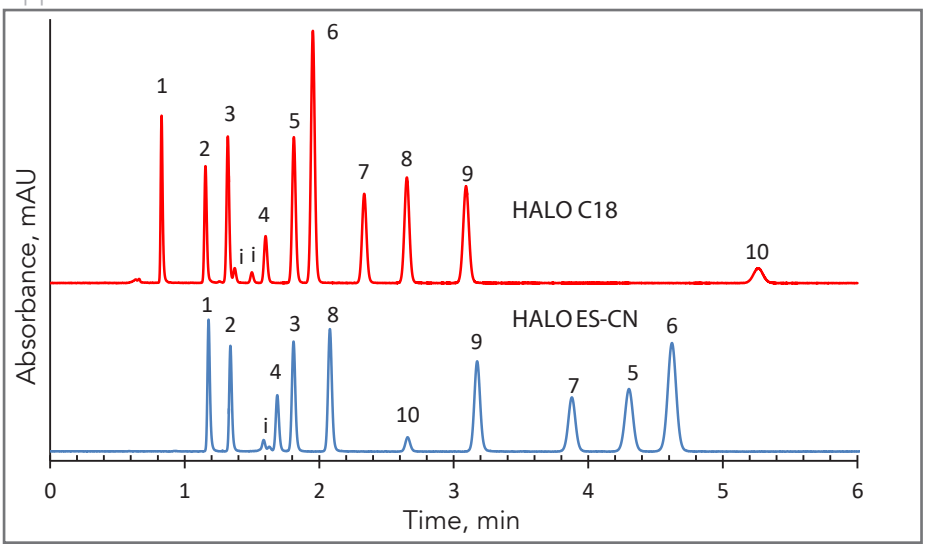
Substituted Phenones



2',6'-Dihydroxyacetophenone

Separation of Mixed Polarity Compounds on HALO® C18 and ES-CN

Application Note 53-G



PEAK IDENTITIES:

1. Resorcinol
2. Benzyl alcohol
3. Phenylacetonitrile
4. 1-Indanol
5. 3,4-DNT
6. 2,3-DNT
7. 2,4-DNT
8. Anisole
9. 1-Chloro-4-nitrobenzene
10. Toluene

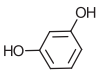
DNT = dinitrotoluene
i = impurity

These separations of polar and non-polar compounds show significant differences in selectivity between HALO® C18 and ES-CN stationary phases. Note the increased retention of nitro compounds and reduced retention of non-polar compounds on the HALO® ES-CN phase.

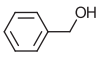
TEST CONDITIONS:

Columns:
1) HALO 90 Å C18, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-402
2) HALO 90 Å ES-CN, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-404
Mobile Phase: 40/60 - A/B for C18
50/50 - A/B for ES-CN
A: Water
B: Methanol
Flow Rate: 1.25 mL/min
Pressure: ~300 bar
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: Water/methanol
Response Time: 0.02 sec
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Extra Column Volume: ~14 µL

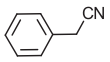
STRUCTURES:



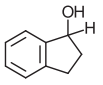
Resorcinol



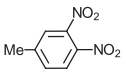
Benzyl alcohol



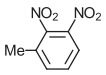
Phenylacetonitrile



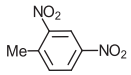
1-Indanol



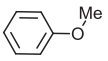
3,4-DNT



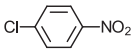
2,3-DNT



2,4-DNT



Anisole

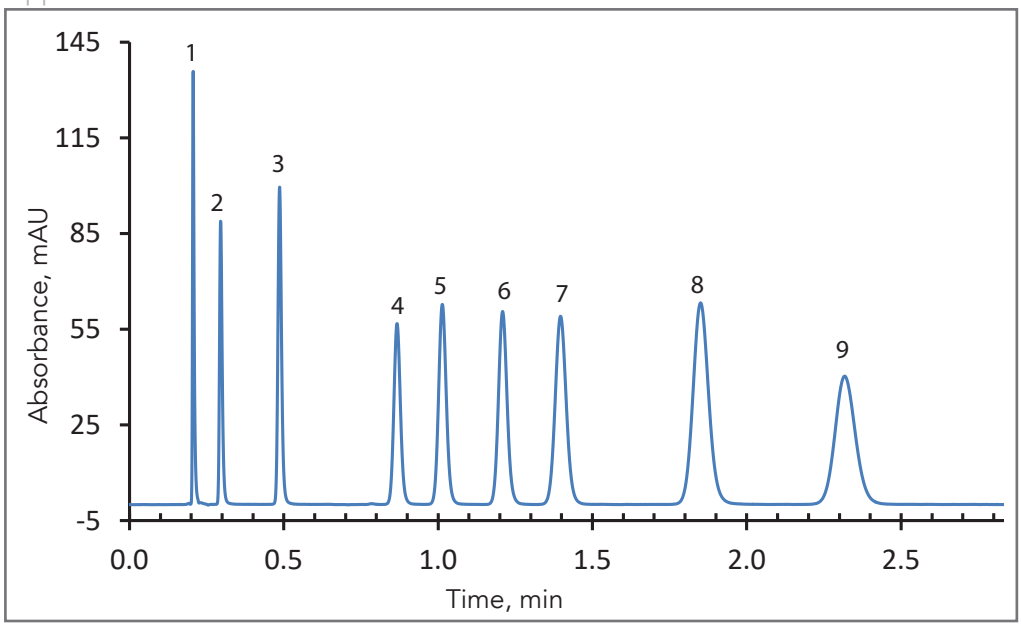


1-Chloro-4-nitrobenzene

Toluene

Polar Compounds Separated by HALO® RP-Amide, 5 µm

Application Note 107-P



PEAK IDENTITIES:

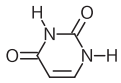
1. Uracil
2. Benzamide
3. Aniline
4. Cinnamyl Alcohol
5. Dimethyl Phthalate
6. 2-Nitroaniline
7. 4′-Bromoacetanilide
8. 2,2′-Biphenol
9. 4,4′-Biphenol

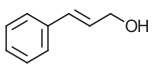
Nine polar compounds can be separated in less than 2.5 minutes on this 5 µm HALO® RP-Amide column. This is possible due to the high efficiency of the Fused-Core® particles, even at very high flow rates.

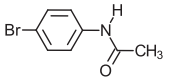
TEST CONDITIONS:

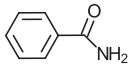
Column: HALO 90 Å RP-Amide, 5 µm, 4.6 x 100 mm
Part Number: 95814-607
Mobile Phase: 70/30 - A/B
A: 20 mM potassium phosphate, pH 7.0
B: Acetonitrile
Flow Rate: 4.0 mL/min
Pressure: 308 bar
Temperature: 26 °C
Detection: UV 254 nm, VWD
Injection Volume: 5.0 µL
Sample Solvent: 50/50 water/acetonitrile
Response Time: 0.12 sec
Flow Cell: 5.0 µL semi-micro
LC System: Agilent 1100

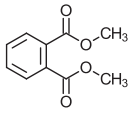
STRUCTURES:

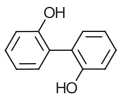

Uracil

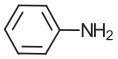

Cinnamyl Alcohol

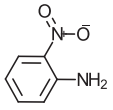

4′-Bromoacetanilide

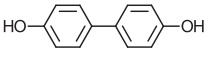

Benzamide


Dimethyl Phthalate


2,2′-Biphenol

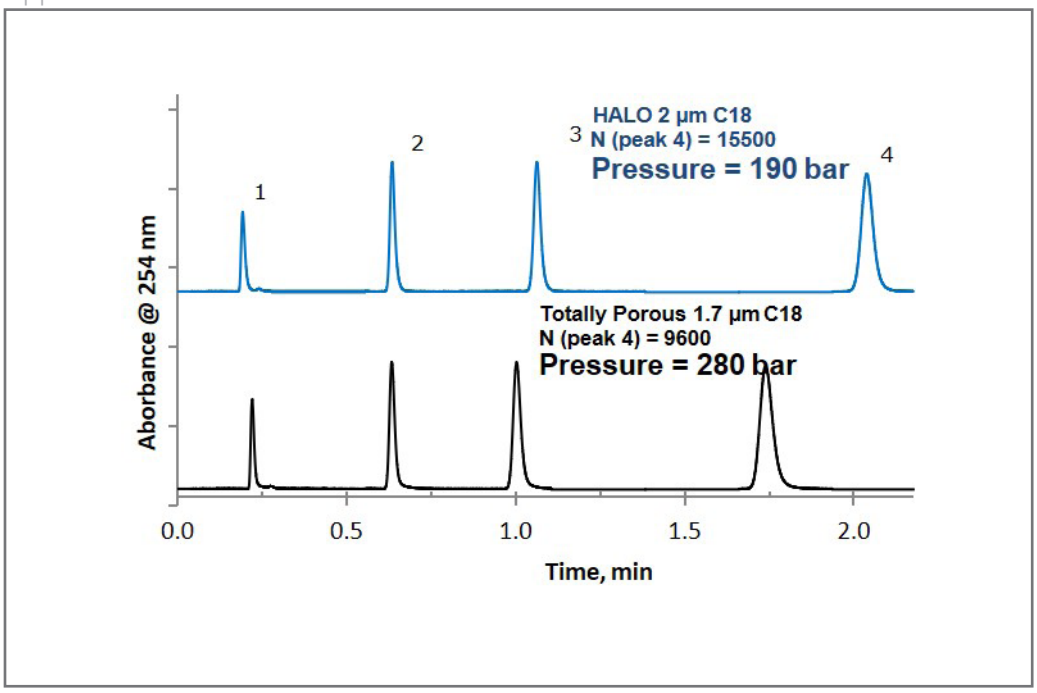

Aniline


2-Nitroaniline


4,4′-Biphenol

Higher Efficiency of HALO® C18 (2.0 µm Fused-Core®) Compared to a 1.7 µm Totally Porous C18 Column

Application Note 113



PEAK IDENTITIES:

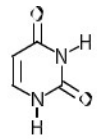
1. Uracil
2. Pyrene
3. Decanophenone
4. Dodecanophenone

With a HALO® 2.0 µm C18 column, one can achieve a higher separation efficiency at less pressure than with a competitor's totally porous C18, 1.7 µm column.

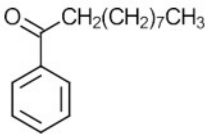
TEST CONDITIONS:

Columns:
1) HALO 90 Å C18, 2.0 µm, 2.1 x 50 mm
Part Number: 91812-402
2) Totally porous C18, 1.7 µm, 2.1 x 50 mm
Mobile Phase: 15/85 - A/B
A: Water
B: Acetonitrile
Flow Rate: 0.5 mL/min
Pressure: See chart
Temperature: 25 °C
Detection: UV 254 nm, PDA
Injection Volume: 0.2 µL
Sample Solvent: 20/80 water/acetonitrile
Response Time: 0.16 sec
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera
Extra Column Volume: ~7 µL

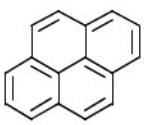
STRUCTURES:



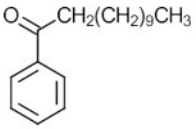
Uracil



Decanophenone



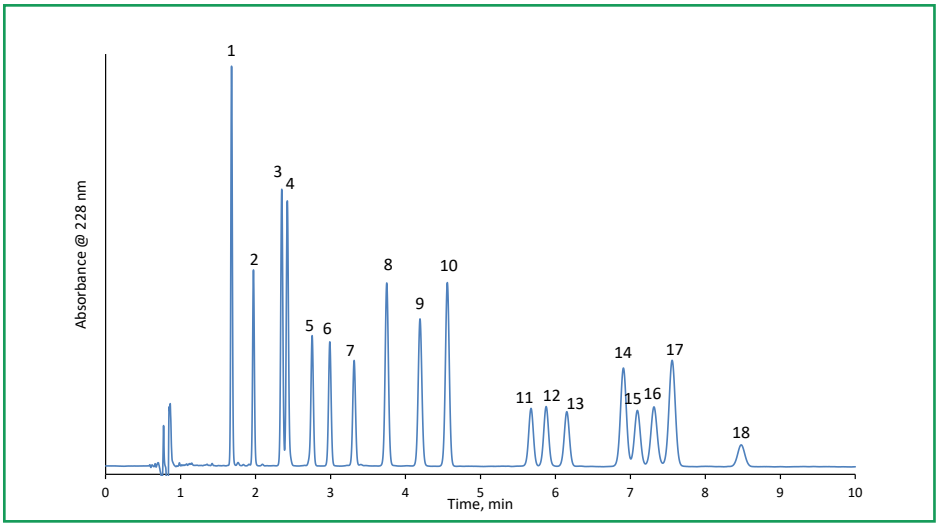
Pyrene



Dodecanophenone

Isocratic Separation of 18 Cannabinoids

222-CN



PEAK IDENTITIES

1. Cannabidivarinic acid (CBDVA)
2. Cannabidivarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerolic acid (CBGA)
5. Cannabigerol (CBG)
6. Cannabidiol (CBD)
7. Tetrahydrocannabivarin (THCV)
8. Tetrahydrocannabivarinic acid (THCVA)
9. Cannabinolic acid (CBNA)
10. Cannabinol (CBN)
11. Exo-tetrahydrocannabinol (EXO-THC)
12. delta 9- Tetrahydrocannabinol (D9-THC)
13. delta 8- Tetrahydrocannabinol (D8-THC)
14. Tetrahydrocannabinolic acid A (THCA-A)
15. Cannabichromenic acid (CBCA)
16. Cannabicycol (CBL)
17. Cannabichromene (CBC)
18. Cannabicyclolic acid (CBLA)

TEST CONDITIONS:

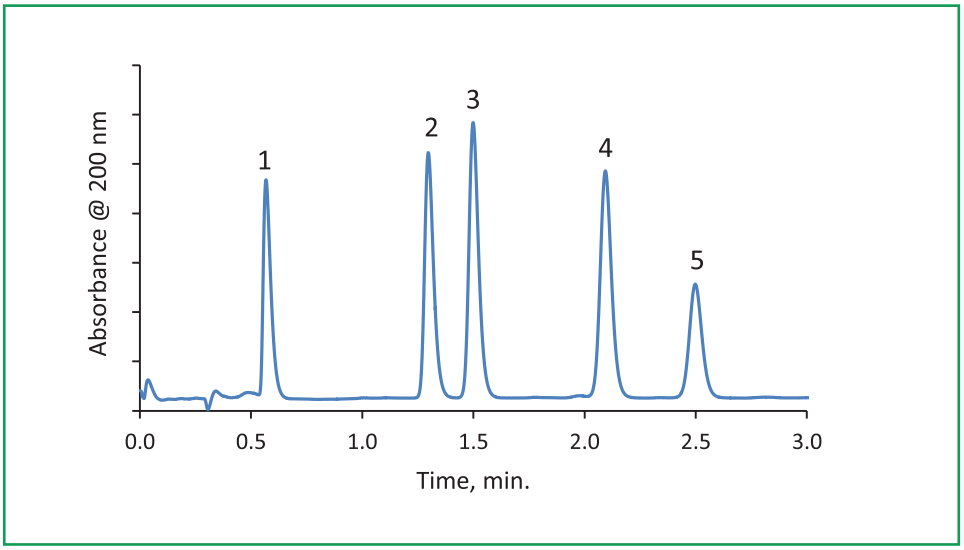
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 150mm
Part Number: 92814-702
Mobile Phase:
A: 20 mM Ammonium Formate, pH 2.9
B: Acetonitrile
Isocratic: 76% B
Flow Rate: 1.5 mL/min
Pressure: 231 bar

Temperature: 35 °C
Detection: UV 228 nm
Injection Volume: 4.0 µL
Sample Solvent: Methanol
Response Time: 0.025 sec
Flow Cell: 1.0 µL
System: Shimadzu Nexera X2

A HALO® C18 column is used to separate a mixture of eighteen cannabinoids, showing fast results and high resolution within critical pairs. Cannabinoids are a class of chemical compounds primarily found in the marijuana plant. Many of these compounds have been found to provide medicinal benefits such as reduction in pain and inflammation.

Isocratic Separation of Synthetic Cannabinoids on HALO® C18

Application Note 147-SC



PEAK IDENTITIES:

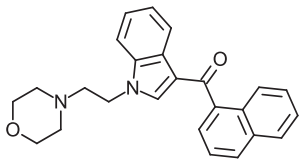
1. JWH-200
2. (±)-CP 47, 497
3. (±)-CP 47, 497 C8 Homologue
4. JWH-250
5. HU-211

Synthetic cannabinoids are man-made compounds that act like the chemicals found in the marijuana plant. The five compounds in this mixture are illegal and represent only a small number of the variations that exist. Just as one compound is made illegal, another variation will be made to take its place. This represents a growing challenge for law enforcement agencies. Using a HALO C18 column gives a fast, efficient separation of these illegal drugs with ample resolution for the next generation of illegal species.

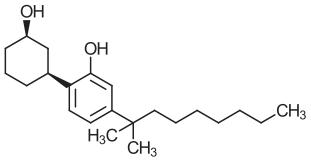
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-602
Mobile Phase: 25/75 - A/B
A: 5 mM ammonium formate, pH unadjusted
B: 95/5 acetonitrile/water with 5 mM ammonium formate
Flow Rate: 0.6 mL/min
Pressure: 247 bar
Temperature: 30 °C
Detection: UV 200 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 50/50 water/acetonitrile
Data Rate: 50 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

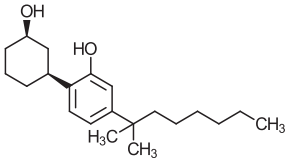
STRUCTURES:



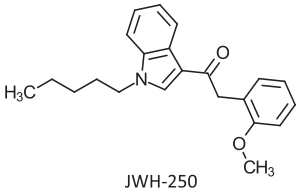
JWH-200



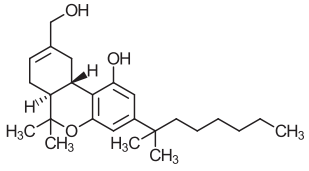
(±)-CP 47, 497 C8 Homologue



(±)-CP 47, 497



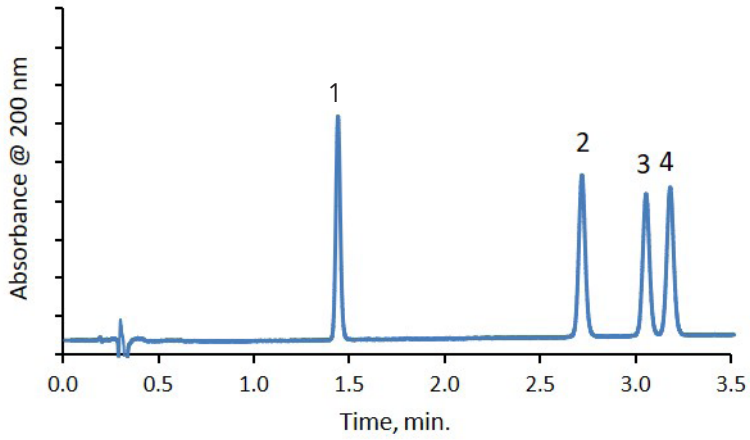
JWH-250



HU-211

Isocratic Separation of Synthetic Cannabinoids Using MS Confirmation

Application Note 153-SC



PEAK IDENTITIES:

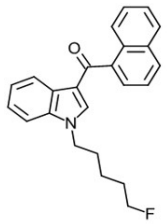
1. AM2201 (359.44 g/mol)
2. JWH-081 (371.47 g/mol)
3. JWH-122 (355.47 g/mol)
4. JWH-019 (355.47 g/mol)

The four compounds in this mixture are separated using a HALO® 90 Å C18 column. This column gives a fast, efficient separation of these cannabinoids with ample resolution.

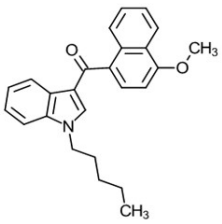
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-602
Mobile Phase: 25/75 - A/B
A: 5 mM ammonium formate
B: 95/5 acetonitrile/water with 5 mM ammonium formate
Flow Rate: 0.6 mL/min
Pressure: 279 bar
Temperature: 30 °C
Detection: UV 200 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 50/50 water/acetonitrile
Data Rate: 100 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

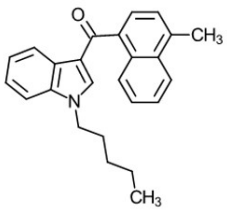
STRUCTURES:



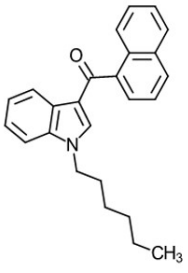
AM2201



JWH-081



JWH-122

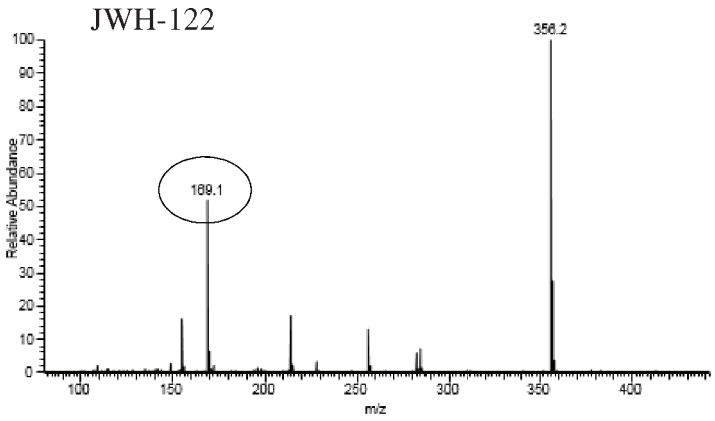


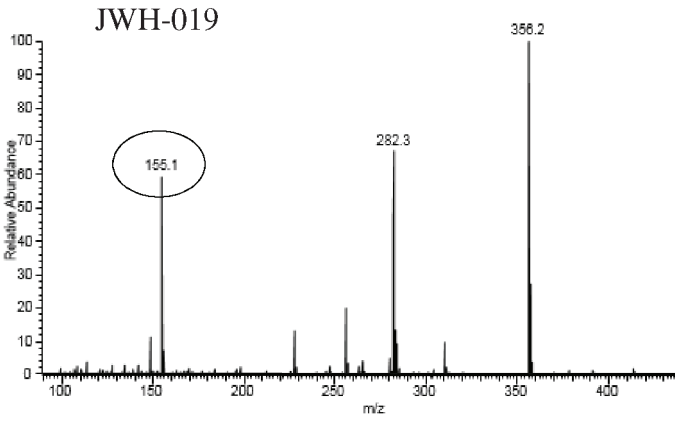
JWH-019

MS TEST CONDITIONS:

MS System: Thermo Fisher Orbitrap VelosPro ETD
Scan Time: 6 µscans/250 ms max inject time
Scan Range: 50-2000 m/z
MS Parameters: Positive ion mode, ESI at +4.0 kV, 225 °C capillary

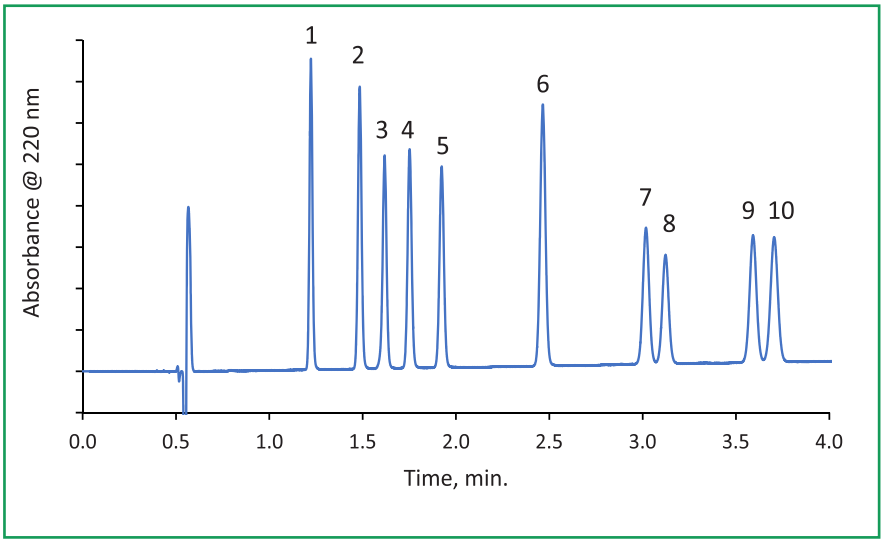
Synthetic cannabinoids can be very similar in their chemical structure. In fact, many of these cannabinoids are analogs or isomers of each other and can be difficult to distinguish. Two homologues in this particular sample were fraction collected and then identified using an orbital ion trap MS system. The Orbitrap allows us to see signature fragmentations of a particular compound, allowing positive identification of each isomer.





Fast Separation of Ten Cannabinoids on HALO® C18

Application Note 155-CN



PEAK IDENTITIES:

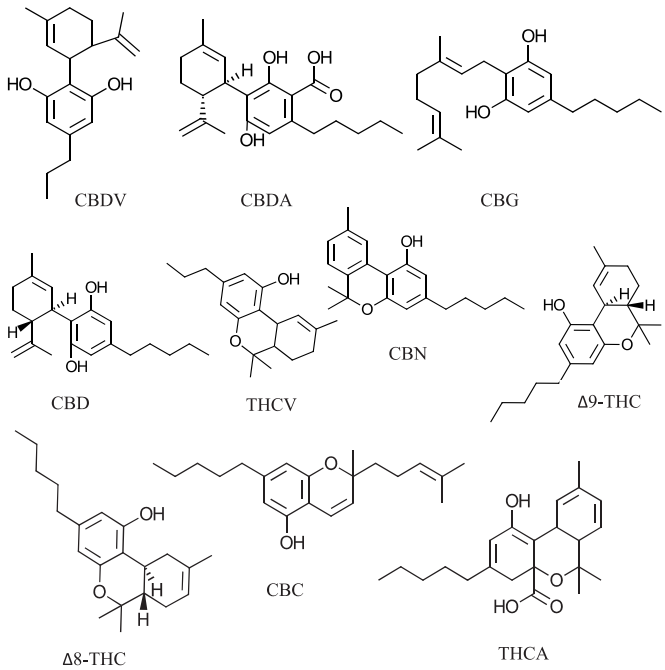
1. Cannabidivarin (CBDV)
2. Cannabidiolic acid (CBDA)
3. Cannabigerol (CBG)
4. Cannabidiol (CBD)
5. Tetrahydrocannabivarin (THCV)
6. Cannabinol (CBN)
7. delta-9-Tetrahydrocannabinol (Δ9-THC)
8. delta-8-Tetrahydrocannabinol (Δ8-THC)
9. Cannabichromene (CBC)
10. delta-9-Tetrahydrocannabinolic acid A (THCA)

A HALO® C18 column is used to separate a mixture of ten cannabinoids, showing fast results and high resolution within critical pairs. Cannabinoids are a class of chemical compounds primarily found in the marijuana plant. Many of these compounds have been found to provide medicinal benefits such as reduction in pain and inflammation.

TEST CONDITIONS:

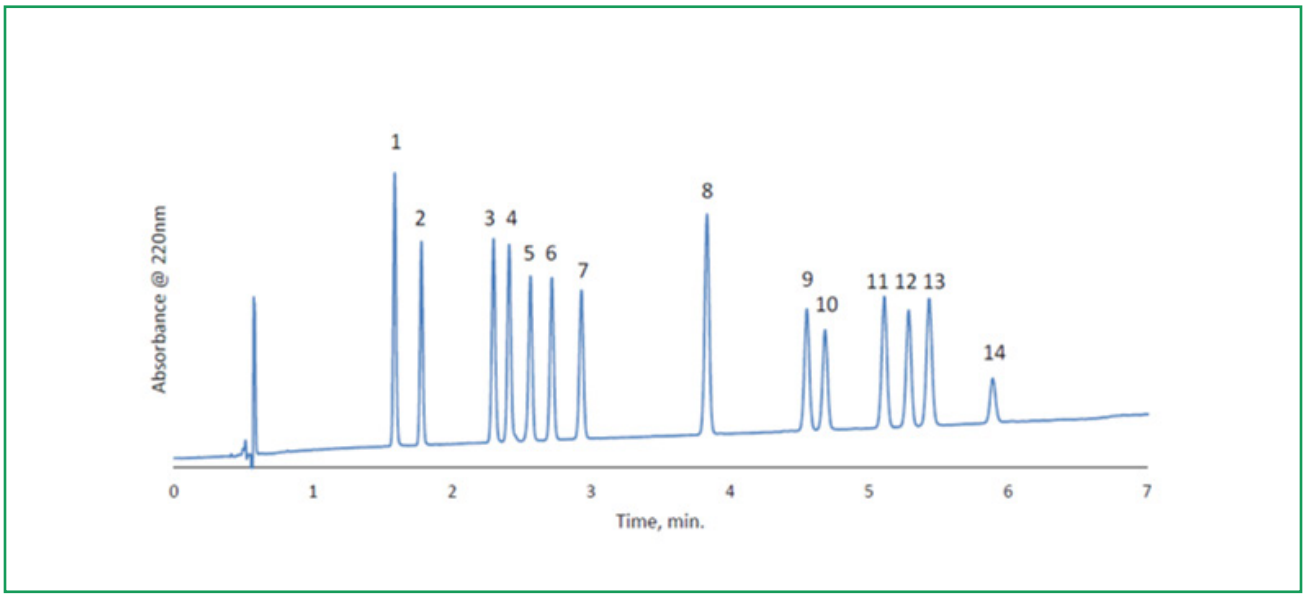
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 100 mm
Part Number: 92814-602
Mobile Phase:
A: Water/0.1% formic acid
B: Acetonitrile/0.085% formic acid
Gradient: 77-85% B in 4 min
Flow Rate: 1.5 mL/min
Initial Pressure: 197 bar
Temperature: 38 °C
Detection: UV 220 nm, PDA
Injection Volume: 1.3 µL
Dwell Volume: 0.471 mL
Sample Solvent: 75/25 methanol/water
Response Time: 0.025 sec
Data Rate: 100 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

STRUCTURES:



Separation of 14 Cannabinoids on HALO® C18

Application Note 162-CN



PEAK IDENTITIES:

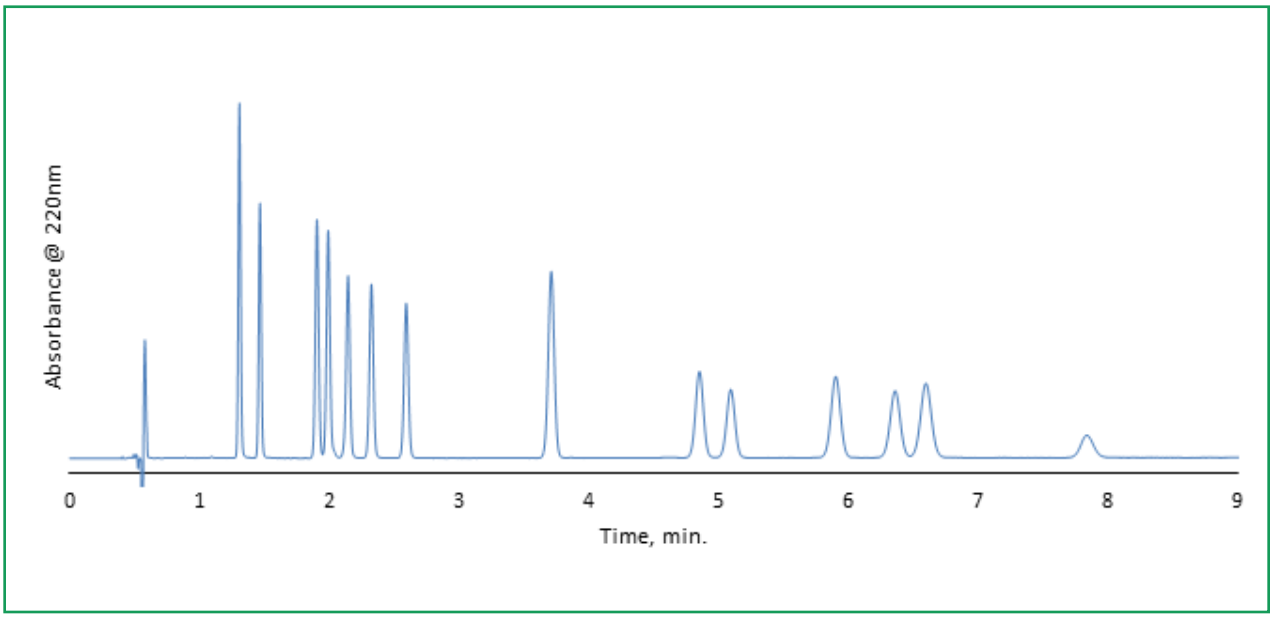
1. Cannabidivarinic acid (CBDVA)
2. Cannabidvarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerolic acid (CBGA)
5. Cannabigerol (CBG)
6. Cannabidiol (CBD)
7. Tetrahydrocannabivarin (THCV)
8. Cannabinol (CBN)
9. delta-9- Tetrahydrocannabinol (Δ9-THC)
10. delta-8-Tetrahydrocannabinol (Δ8-THC)
11. Cannabicyclol (CBL)
12. Cannabichromene (CBC)
13. delta-9-Tetrahydrocannabinolic acid A (THCA)
14. Cannabichromenic acid (CBCA)

TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 3.0 x 150 mm
Part Number: 92813-702
Mobile Phase:
A: Water/0.1% formic acid
B: Acetonitrile/0.085% formic acid
Gradient: 70-88% B in 6 min
Flow Rate: 1.0 mL/min
Initial Pressure: 350 bar
Temperature: 30 °C
Detection: UV 220 nm, PDA
Injection Volume: 0.6 µL
Dwell Volume: 0.471 mL
Sample Solvent: 75/25 methanol/water
Response Time: 0.025 sec
Data Rate: 100 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

Isocratic Separation of 14 Cannabinoids on HALO® C18

Application Note 165-CN



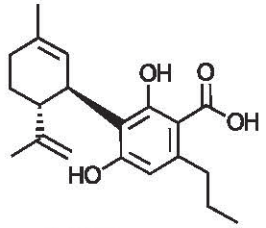
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 3.0 x 150 mm
Part Number: 92813-702
Mobile Phase:
A: Water/0.1% formic acid
B: Acetonitrile/0.085% formic acid
Isocratic: 75% B
Flow Rate: 1.0 mL/min
Initial Pressure: 350 bar
Temperature: 30 °C
Detection: UV 220 nm, PDA
Injection Volume: 0.6 µL
Dwell Volume: 0.471 mL
Sample Solvent: 75/25 methanol/water
Response Time: 0.025 sec
Data Rate: 100 Hz
Flow Cell: 1.0 µL
LC System: Shimadzu Nexera X2

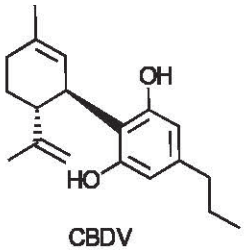
PEAK IDENTITIES:

1. Cannabidivarinic acid (CBDVA)
2. Cannabidvarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerolic acid (CBGA)
5. Cannabigerol (CBG)
6. Cannabidiol (CBD)
7. Tetrahydrocannabivarin (THCV)
8. Cannabinol (CBN)
9. delta-9- Tetrahydrocannabinol (Δ9-THC)
10. delta-8-Tetrahydrocannabinol (Δ8-THC)
11. Cannabicyclol (CBL)
12. Cannabichromene (CBC)
13. delta-9-Tetrahydrocannabinolic acid A (THCA)
14. Cannabichromenic acid (CBCA)

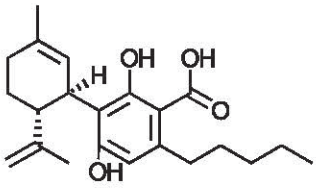
STRUCTURES:



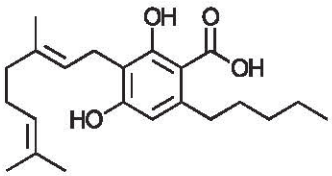
CBDVA



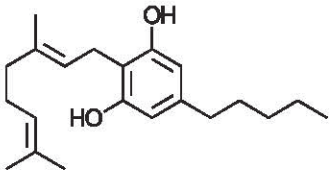
CBDV



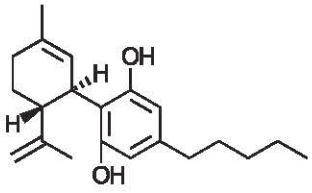
CBDA



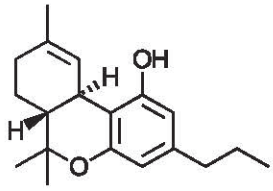
CBGA



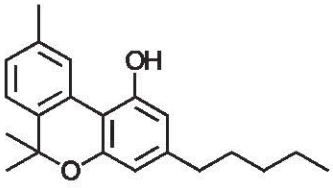
CBG



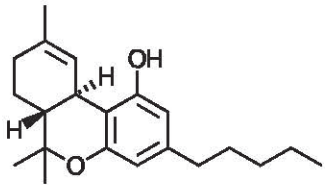
CBD



THCV

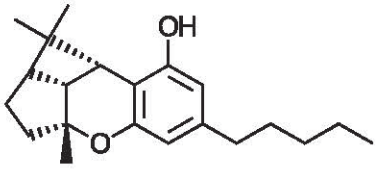


CBN

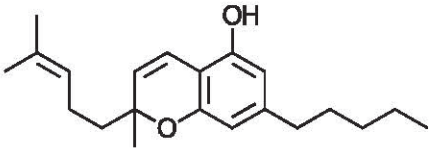


Δ9-THC

Δ 8-THC

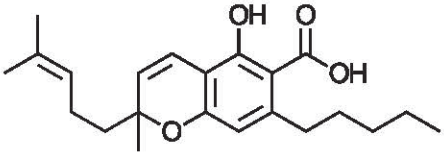


CBL



CBC

THCA



CBCA