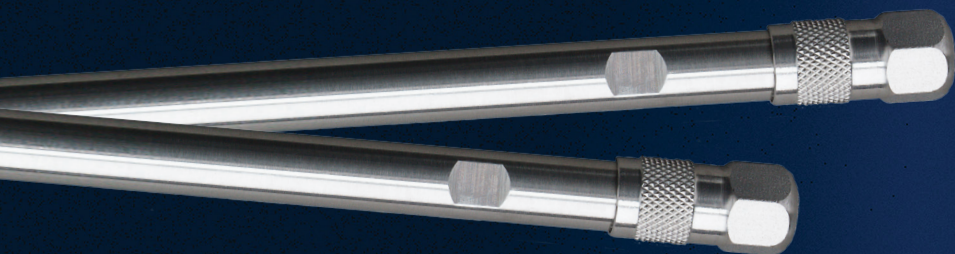


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**APPLICATIONS COLLECTION**





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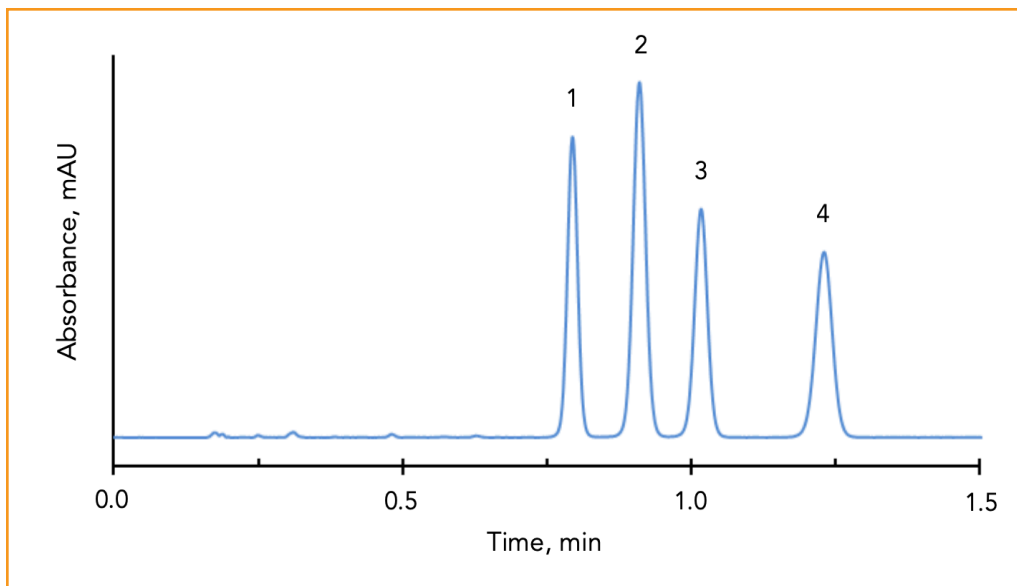
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## Rapid Isocratic Separation of Sulfonyl Urea Drugs on HALO® C18 Phase

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

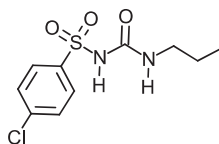
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

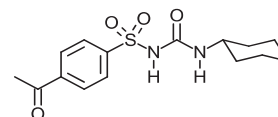
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

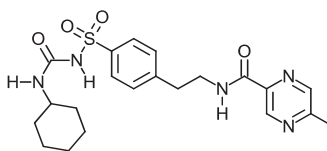
### STRUCTURES:



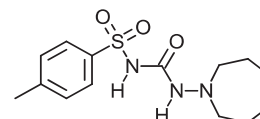
Chlorpropamide



Acetohexamide



Glipizide



Tolazamide



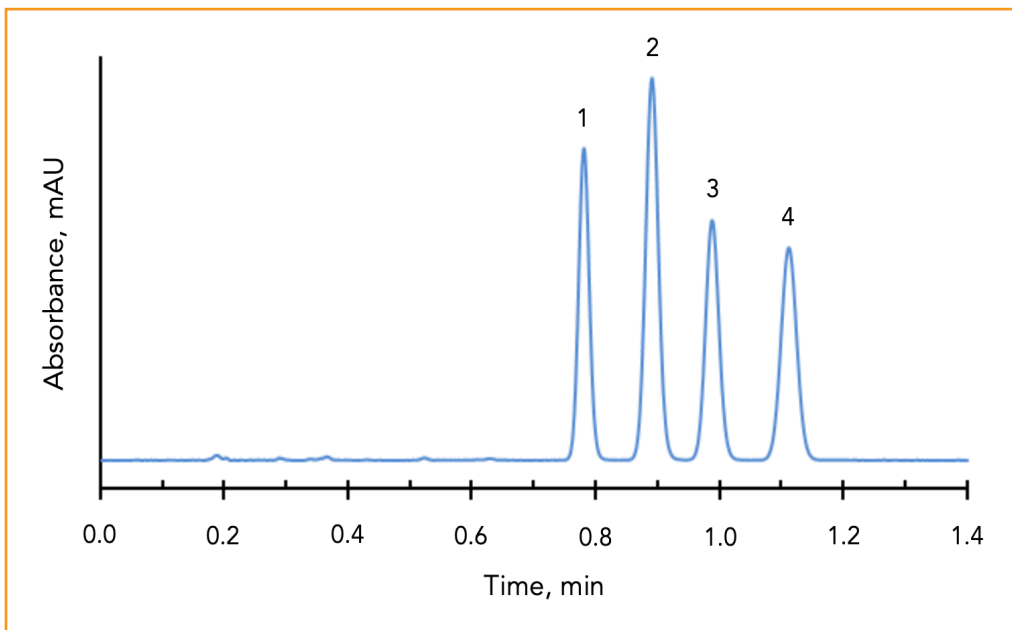
2





## 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

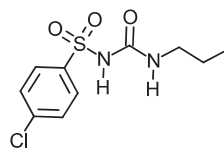
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

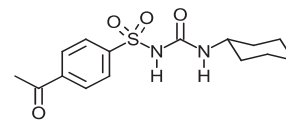
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

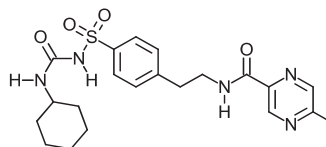
### STRUCTURES:



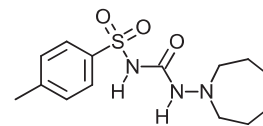
Chlorpropamide



Acetohexamide



Glipizide



Tolazamide

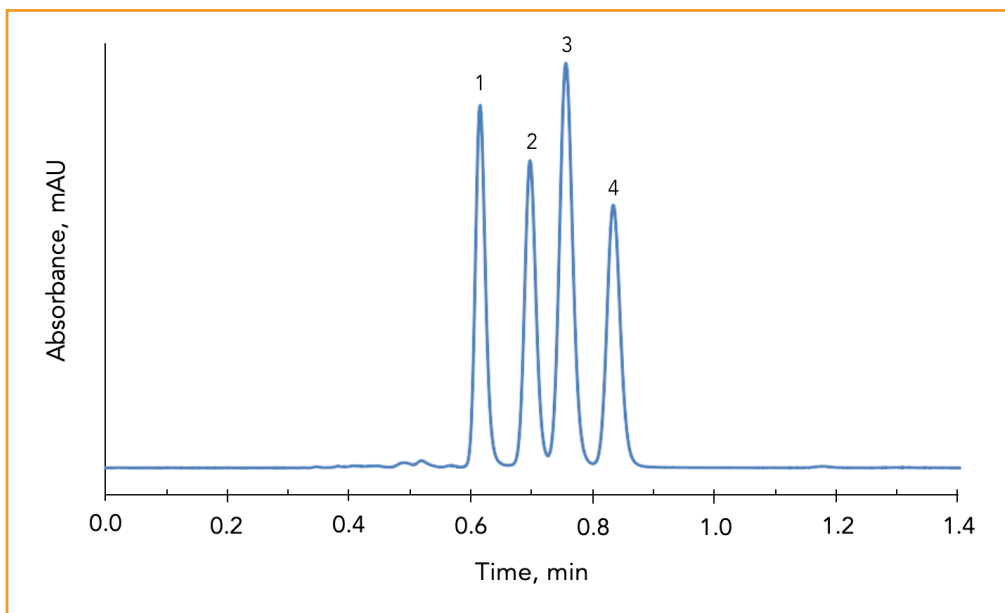






## Rapid Separation of Sulfonyl Urea Drugs on HALO® PFP Phase

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

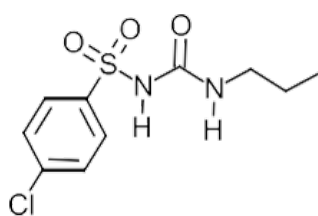
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

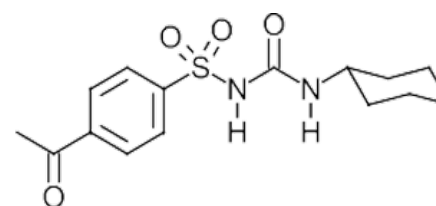
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

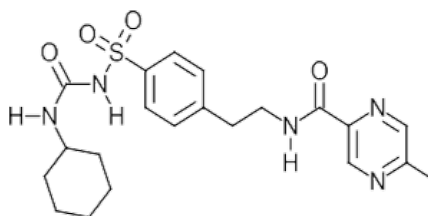
### STRUCTURES:



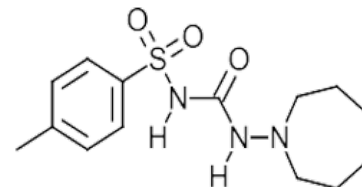
Chlorpropamide



Acetohexamide



Glipizide



Tolazamide

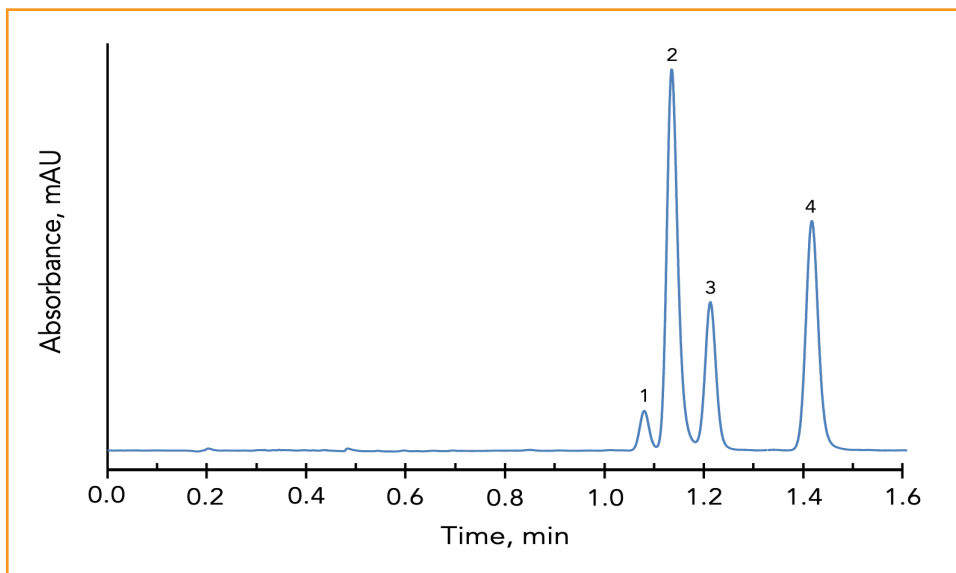






## Separation of Antiulcer Drugs on HALO® Penta-HILIC

Application Note 65-B



### PEAK IDENTITIES:

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

The strongly basic antiulcer drugs can 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  $\mu\text{m}$ ,  
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  $\mu\text{L}$

**Sample Solvent:** Mobile phase

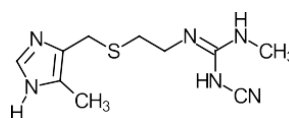
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu\text{L}$  semi-micro

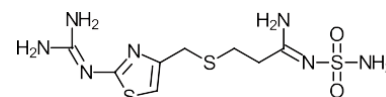
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu\text{L}$

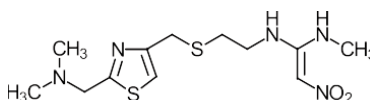
### STRUCTURES:



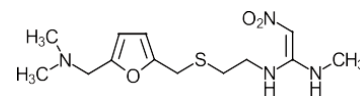
Cimetidine



Famotidine



Nizatidine



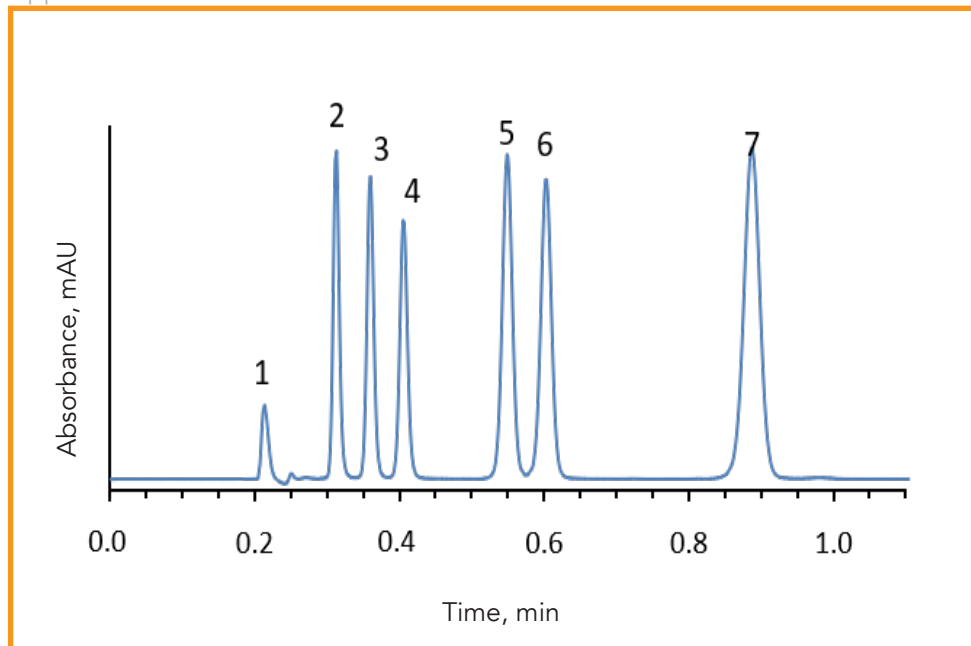
Ranitidine





## 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

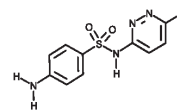
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

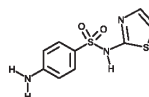
### STRUCTURES:



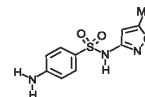
Uracil



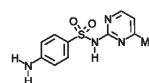
Sulfachloropyridazine



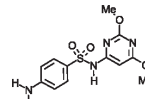
Sulfathiazole



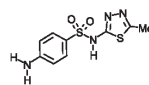
Sulfamethoxazole



Sulfamerazine



Sulfadimethoxine



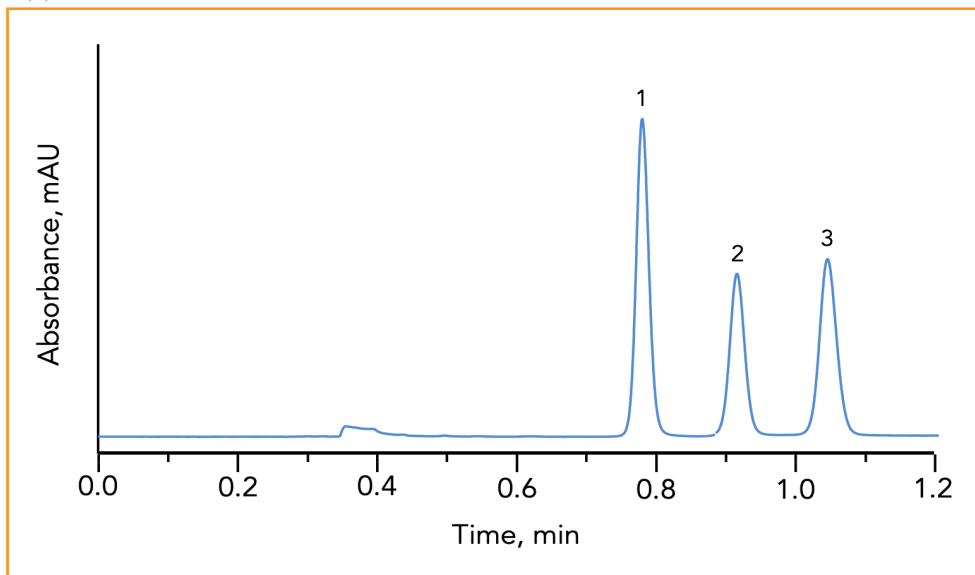
Sulfamethizole





## 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

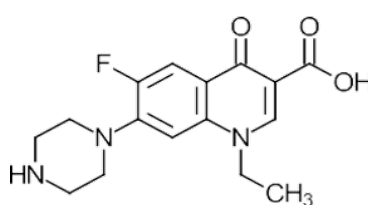
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

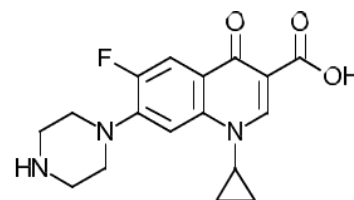
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

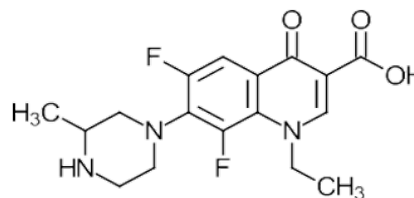
### STRUCTURES:



Norfloxacin



Ciprofloxacin



Lomefloxacin

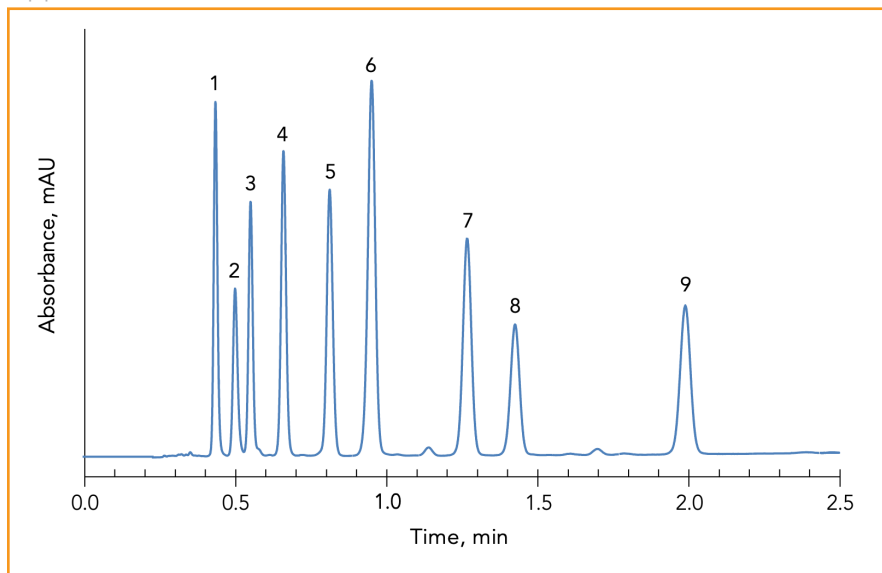






## Separation of Cephalosporins on HALO<sup>®</sup> ES-CN

Application Note 69-AB



### PEAK IDENTITIES:

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

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

### TEST CONDITIONS:

**Column:** HALO 90 Å ES-CN, 2.7  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** 70/30 water/methanol

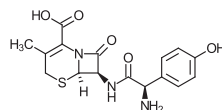
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

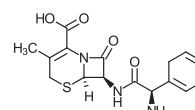
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu$ L

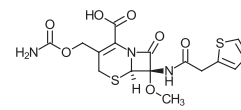
### STRUCTURES:



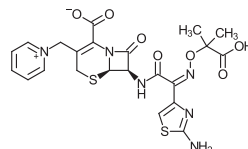
Cefadroxil



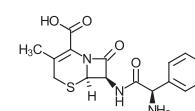
Cephalixin



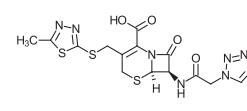
Cefoxitin



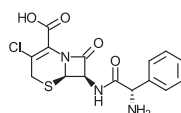
Ceftazidime



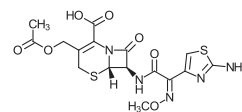
Cephadrine



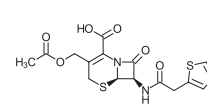
Cefazolin



Cefaclor



Cefotaxime



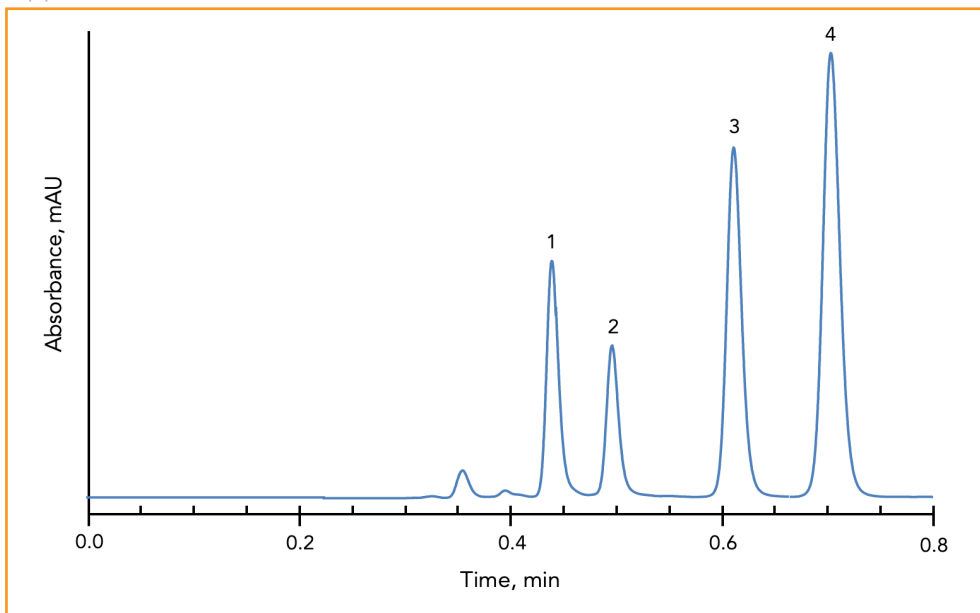
Cephalothin





## Separation of Penicillins on HALO<sup>®</sup> 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<sup>®</sup> Fused-Core<sup>®</sup> ES-CN bonded phase columns.

### TEST CONDITIONS:

**Column:** HALO 90 Å ES-CN, 2.7  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** 50/50 water/acetonitrile

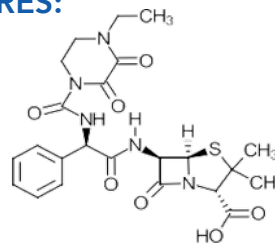
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

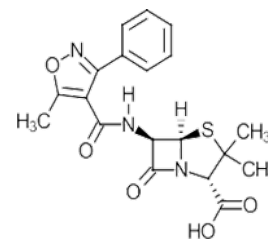
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu$ L

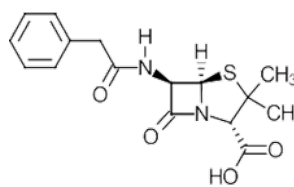
### STRUCTURES:



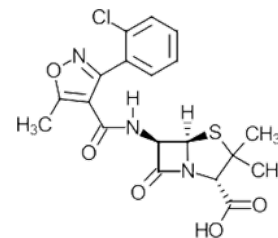
Piperacillin



Oxacillin



Penicillin G



Cloxacillin

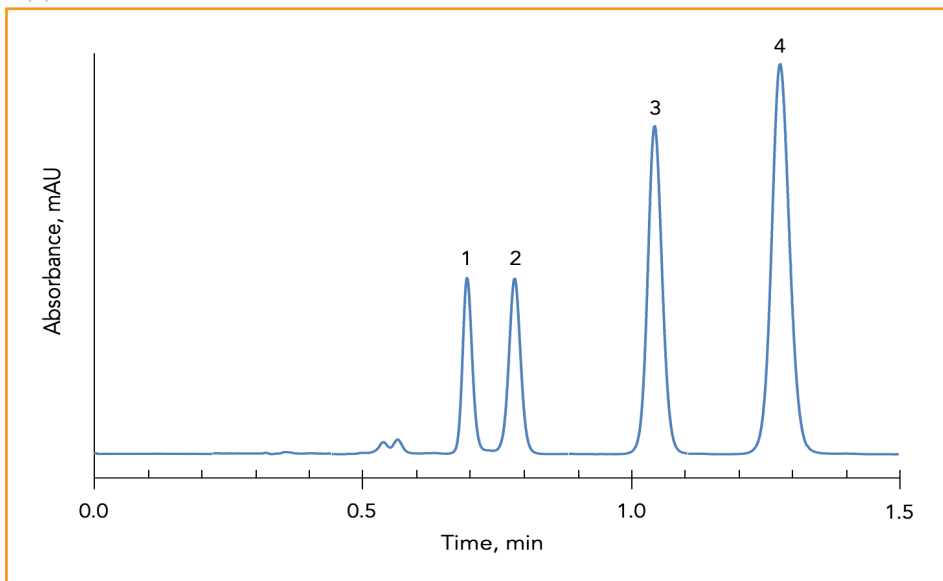






## Separation of Penicillins on HALO® Phenyl-Hexyl

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

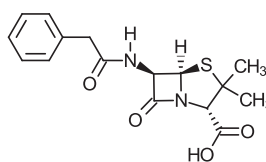
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

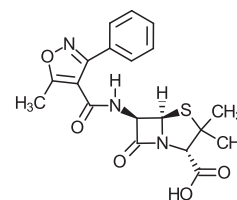
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

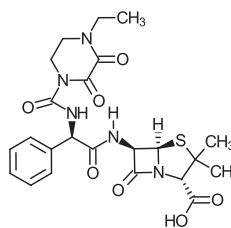
### STRUCTURES:



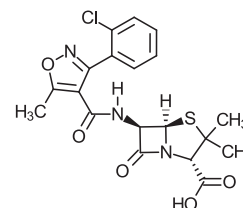
Penicillin G



Oxacillin



Piperacillin



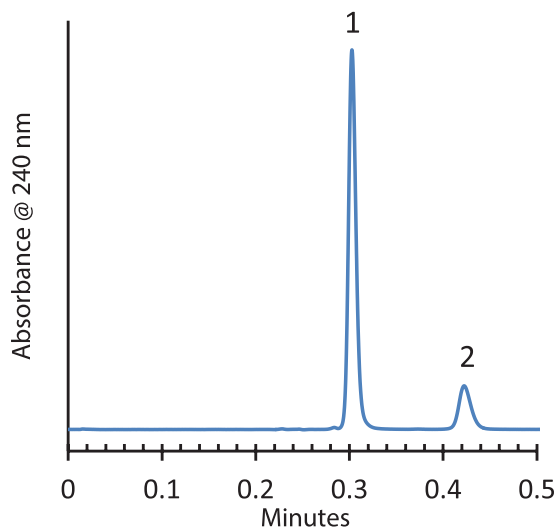
Cloxacillin





## Amoxicillin and Ampicillin on HALO® RP-Amide

Application Note 75-AB



### PEAK IDENTITIES:

1. Amoxicillin
2. Ampicillin

Amoxicillin and ampicillin are members of the  $\beta$ -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  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** 80/20 water/acetonitrile

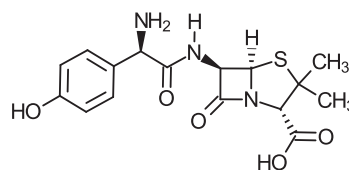
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

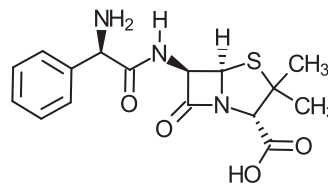
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu$ L

### STRUCTURES:



Amoxicillin



Ampicillin

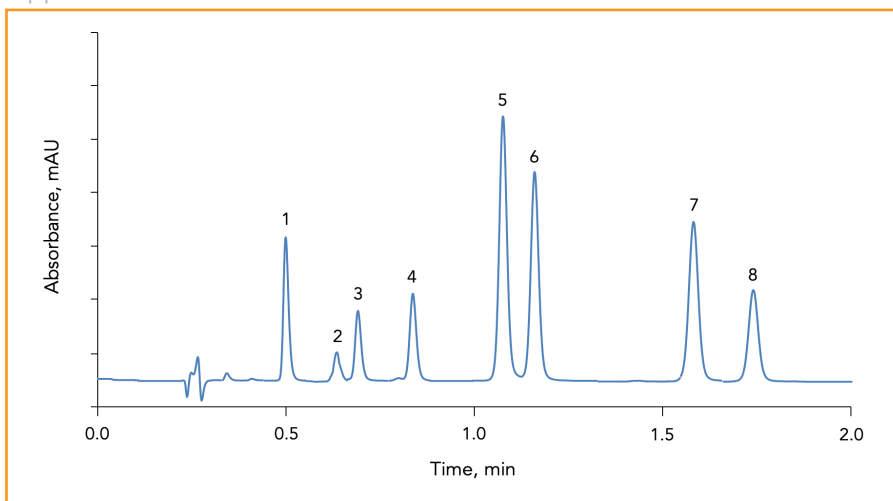






## Separation of Sulfonamides on HALO® Biphenyl, 2.0 µm

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

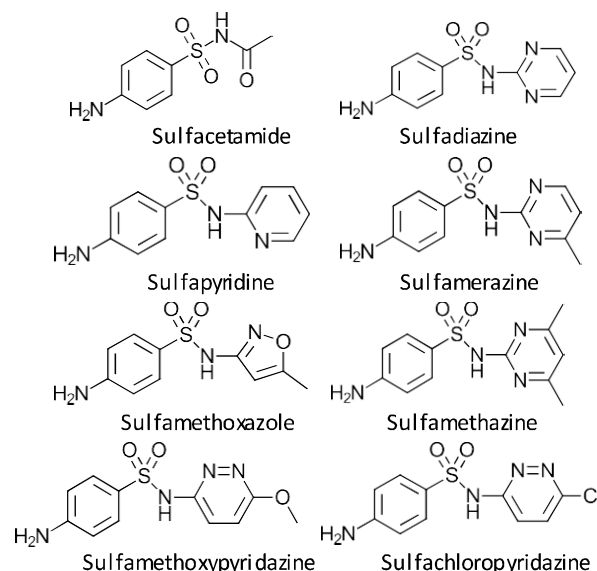
**Sample Solvent:** Acetonitrile

**Response Time:** 0.025 sec

**Flow Cell:** 1.0 µL

**LC System:** Shimadzu Nexera X2

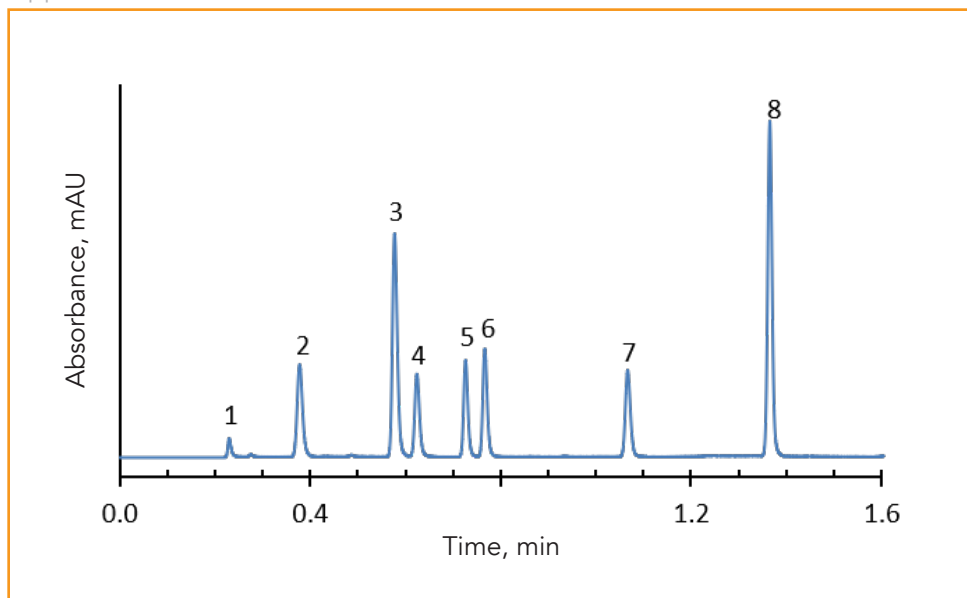
### STRUCTURES:





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

Application Note 80-AF



### PEAK IDENTITIES:

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

The antimicrobial drug clotrimazine 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

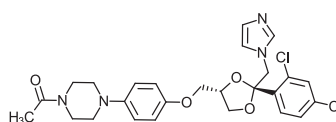
**Sample Solvent:** 25/75 water/acetonitrile

**Response Time:** 0.02 sec

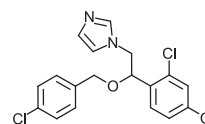
**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Prominence UFLC XR

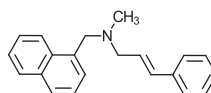
### STRUCTURES:



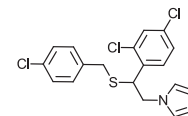
Ketoconazole



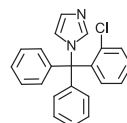
Econazole



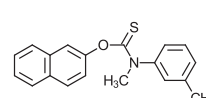
Naftifine



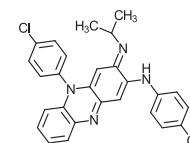
Sulconazole



Clotrimazole



Tolnaftate



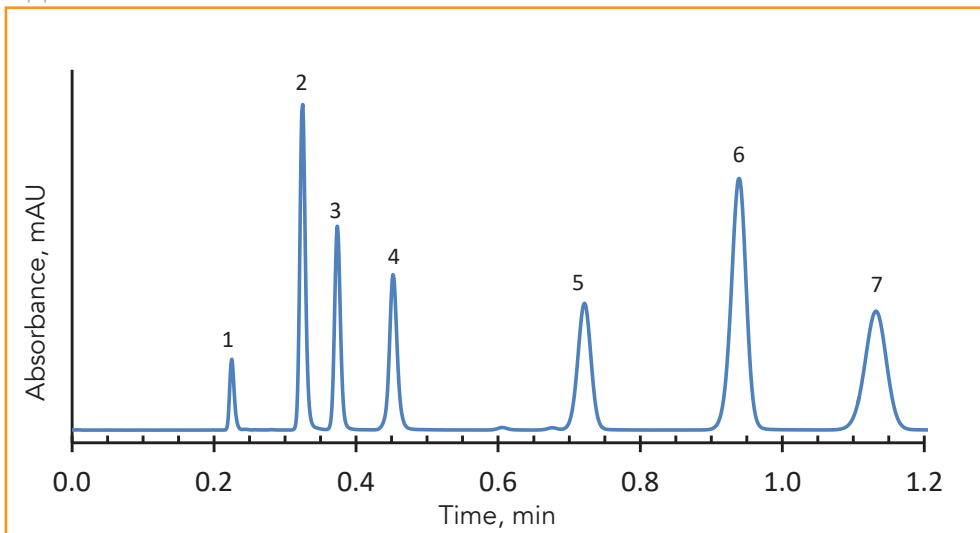
Clotrimazine





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

Application Note 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

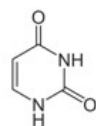
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

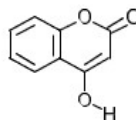
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

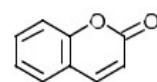
### STRUCTURES:



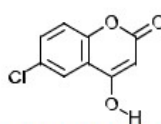
Uracil



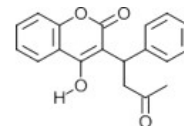
4-Hydroxycoumarin



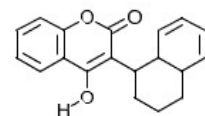
Coumarin



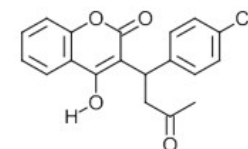
6-Chloro-4-hydroxycoumarin



Warfarin



Coumatetralyl



Coumachlor

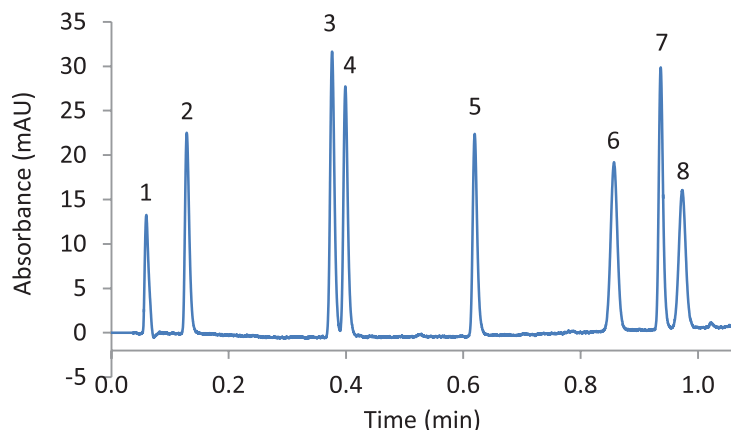






## Separation of Anticoagulants Using HALO 90 Å C18, 2.0 µm

Application Note 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

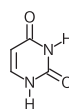
**Injection Volume:** 0.2 µL

**Acquisition Rate:** 200 Hz

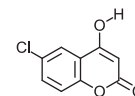
**Flow Cell:** 1.0 µL

**LC System:** Shimadzu Nexera X2

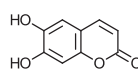
### STRUCTURES:



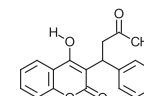
Uracil



6-Chloro-4-hydroxycoumarin



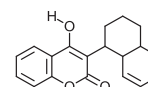
6,7-Dihydroxycoumarin



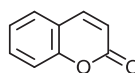
Warfarin



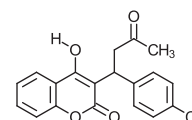
4-Hydroxycoumarin



Coumatetralyl



Coumarin



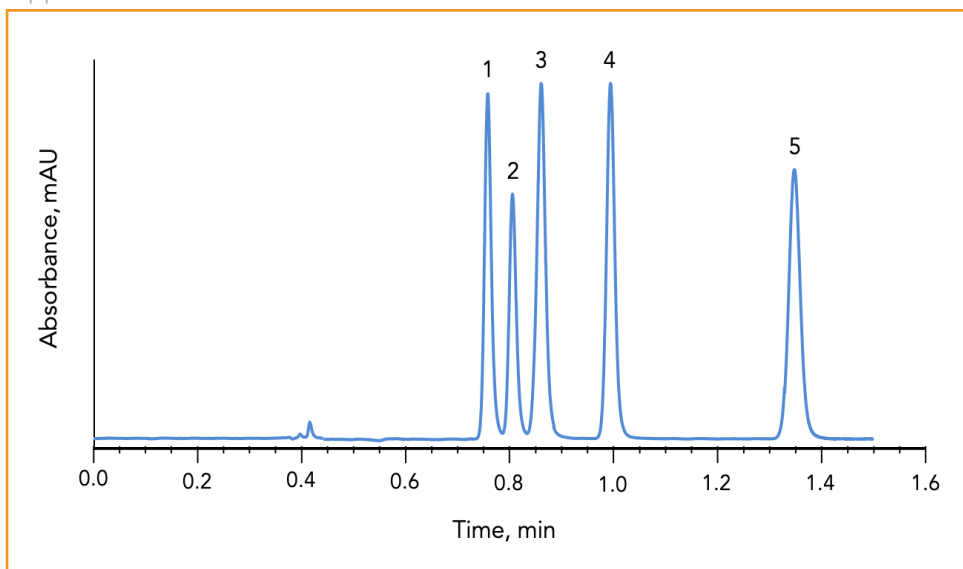
Coumachlor





## Separation of Antidepressants on HALO® Penta-HILIC Stationary Phase

Application Note 67-AD



### PEAK IDENTITIES:

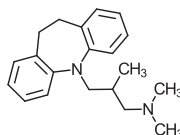
1. Trimipramine
2. Amitriptyline
3. Doxepin
4. Nortriptyline
5. Amoxapine

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

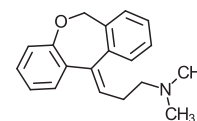
### TEST CONDITIONS:

**Column:** HALO 90 Å Penta-HILIC, 2.7  $\mu$ m,  
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  $\mu$ L  
**Sample Solvent:** 10/90 water/acetonitrile  
**Response Time:** 0.02 sec  
**Flow Cell:** 2.5  $\mu$ L semi-micro  
**LC System:** Shimadzu Prominence UFLC XR  
**Extra column volume:** ~14  $\mu$ L

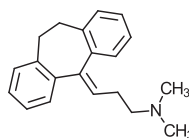
### STRUCTURES:



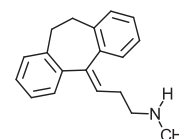
Trimipramine



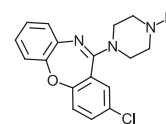
Doxepin



Amitriptyline



Nortriptyline



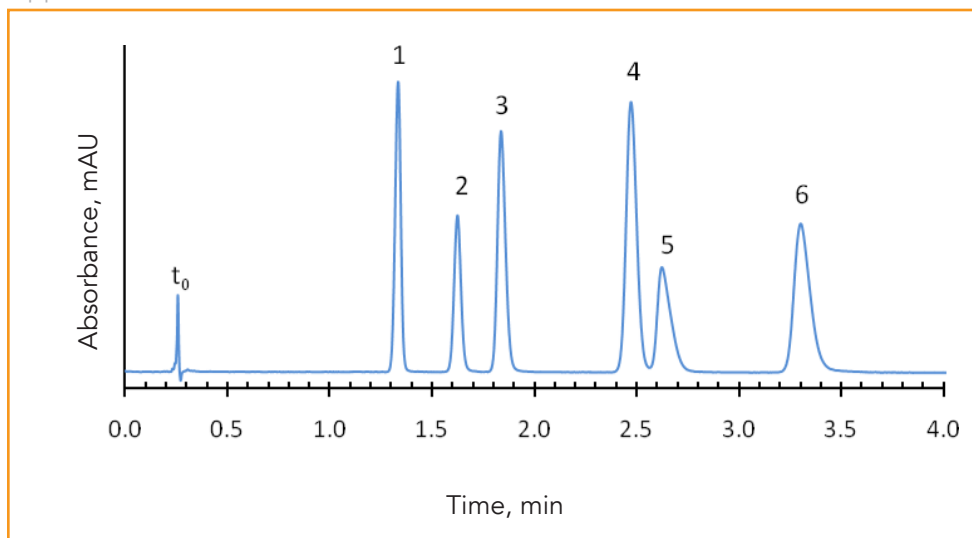
Amoxapine





## 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  $\mu\text{m}$ ,  
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  $\mu\text{L}$

**Sample Solvent:** 75/25 water/methanol

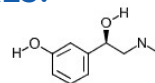
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu\text{L}$  semi-micro

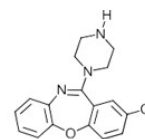
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu\text{L}$

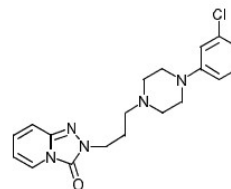
### STRUCTURES:



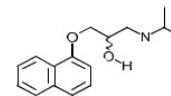
Phenylephrine



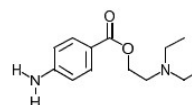
Amoxapine



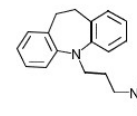
Trazodone



Propranolol



Procaine



Desipramine

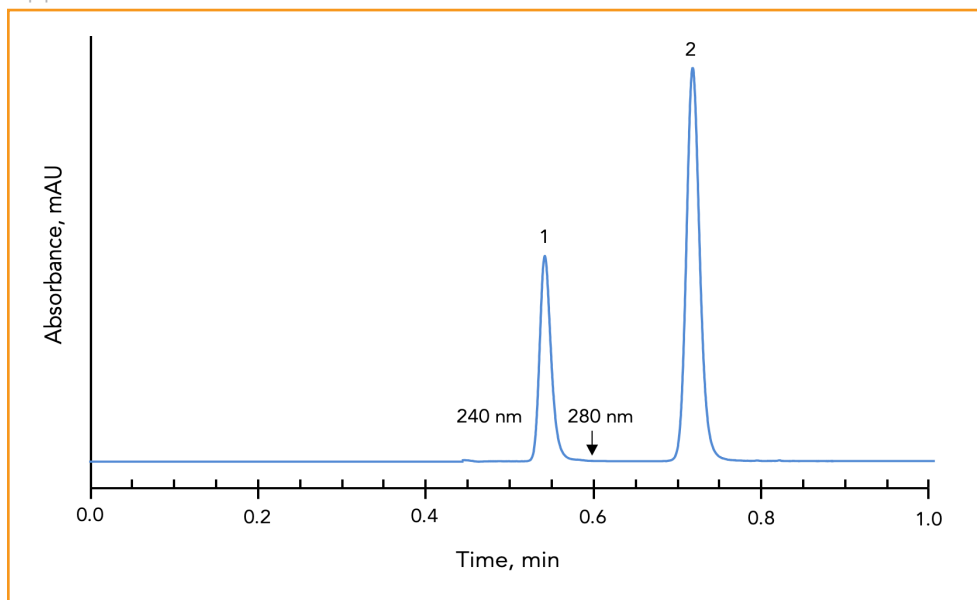






## Isocratic Separation of Amphenicols on HALO® Phenyl-Hexyl Phase

Application Note 57-AM



### PEAK IDENTITIES:

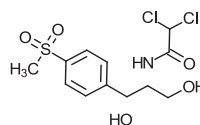
1. Thiamphenicol
2. Chloramphenicol

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.

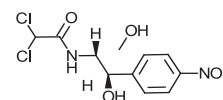
### TEST CONDITIONS:

**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

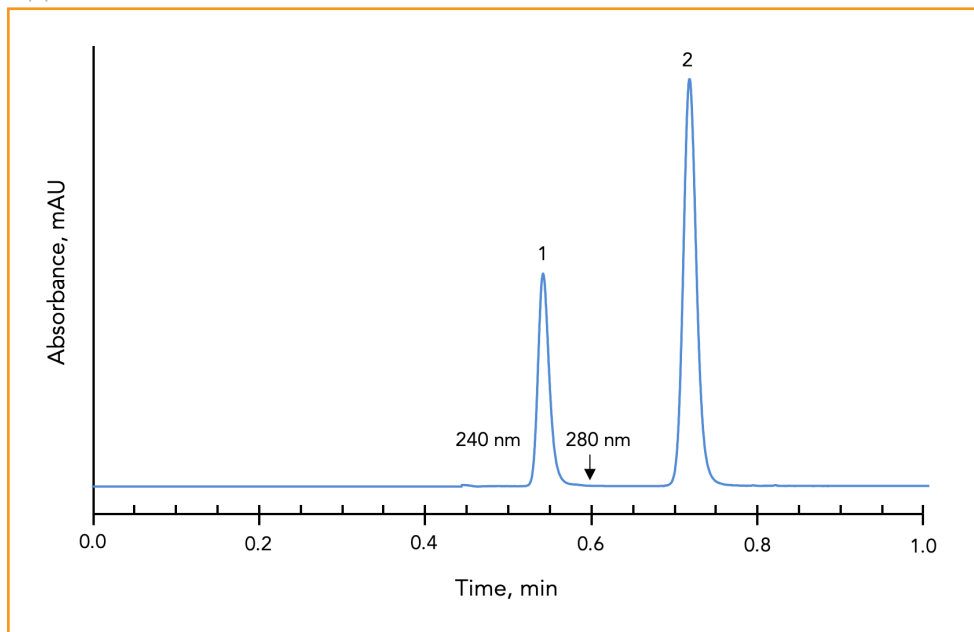


18



## Isocratic Separation of Amphenicols on HALO® RP-Amide Phase

Application Note 58-AM



### PEAK IDENTITIES:

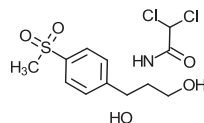
1. Thiamphenicol
2. Chloramphenicol

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.

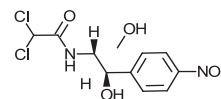
### TEST CONDITIONS:

**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



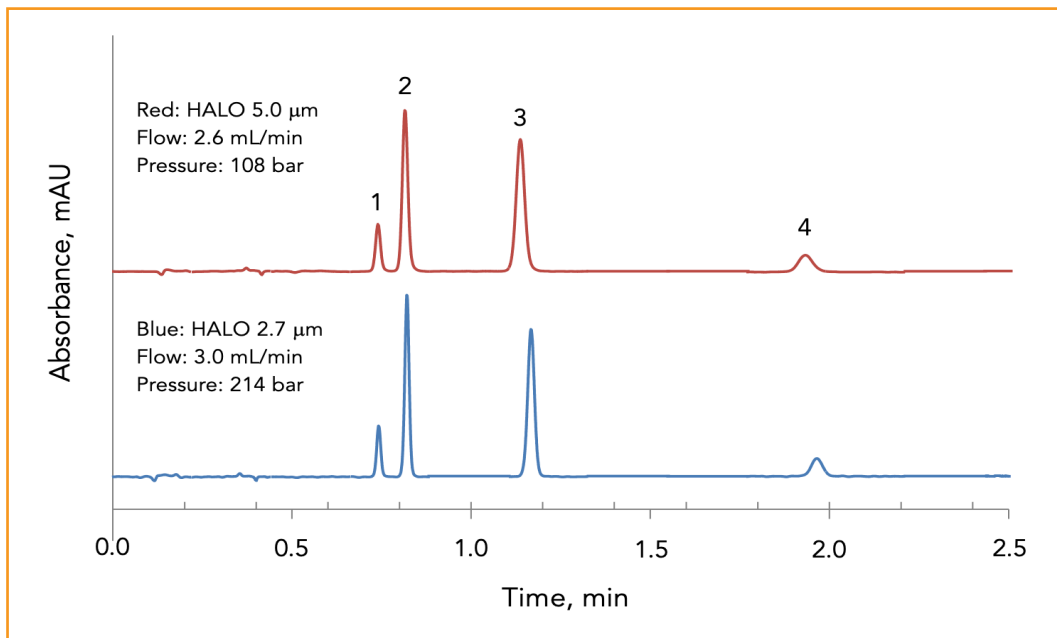
Chloramphenicol





## Comparable Selectivity Between HALO<sup>®</sup> HILIC, 5.0 $\mu\text{m}$ and HALO<sup>®</sup> HILIC, 2.7 $\mu\text{m}$

Application Note 88-B



### PEAK IDENTITIES:

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

These drugs are  $\beta$ -blockers used to treat high blood pressure. This separation illustrates easy method transfer between the 5.0  $\mu\text{m}$  and 2.7  $\mu\text{m}$  HALO<sup>®</sup> HILIC phases after small changes in flow rate.

### TEST CONDITIONS:

#### Columns:

- 1) HALO 90 Å HILIC, 5.0  $\mu\text{m}$ , 4.6 x 100 mm  
Part Number: 95814-601
- 2) HALO 90 Å HILIC, 2.7  $\mu\text{m}$ , 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  $\mu\text{L}$

**Sample Solvent:** Mobile phase

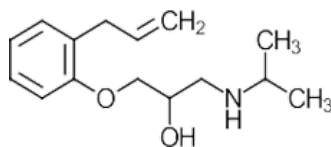
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu\text{L}$  semi-micro

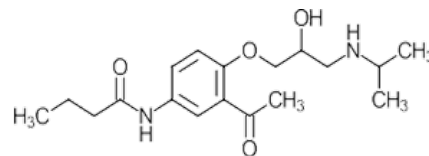
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu\text{L}$

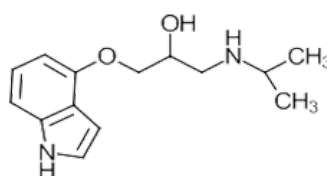
### STRUCTURES:



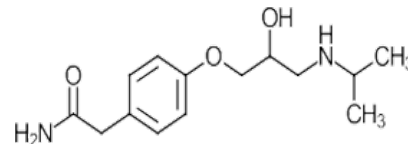
Alprenolol



Acebutolol



Pindolol



Atenolol

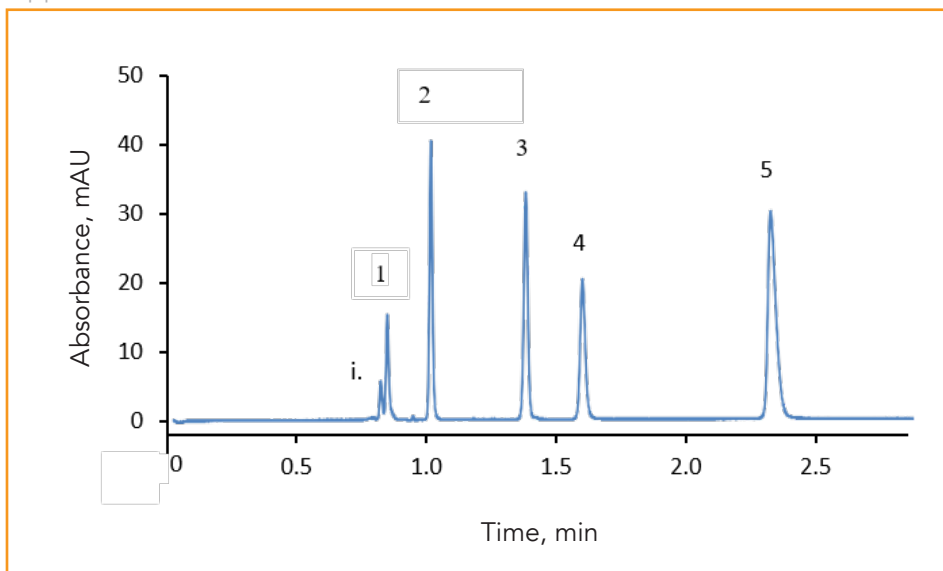






## Separation of OTC Common Cold Medicinal Compounds

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

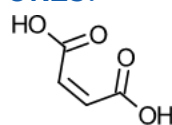
**Injection Volume:** 0.5 µL

**Acquisition Rate:** 40 Hz

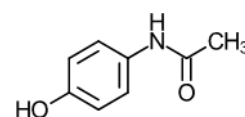
**Flow Cell:** 2.5 µL semi-micro

**LC System:** Agilent 1200 SL

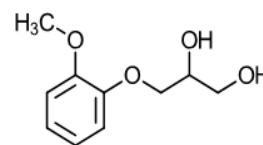
### STRUCTURES:



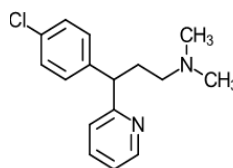
Maleic Acid



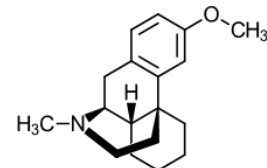
Acetaminophen



Guaifenesin



Chlorpheniramine Maleate



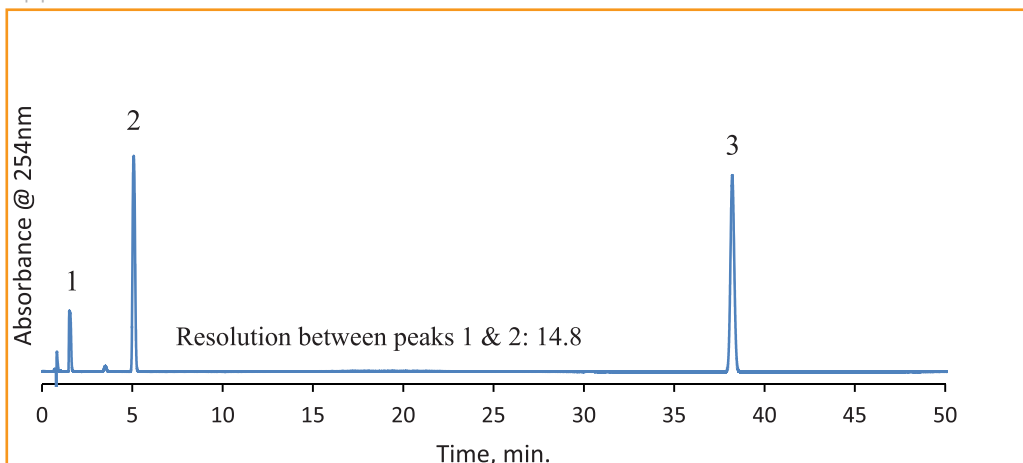
Dextromethorphan HBr





## 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

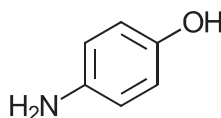
**Data Rate:** 40 Hz

**Response Time:** 0.005 sec

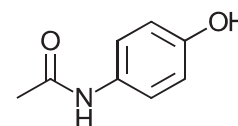
**Flow Cell:** 2.0 µL

**LC System:** Agilent 1200 SL

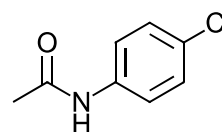
### STRUCTURES:



4-aminophenol



Paracetamol



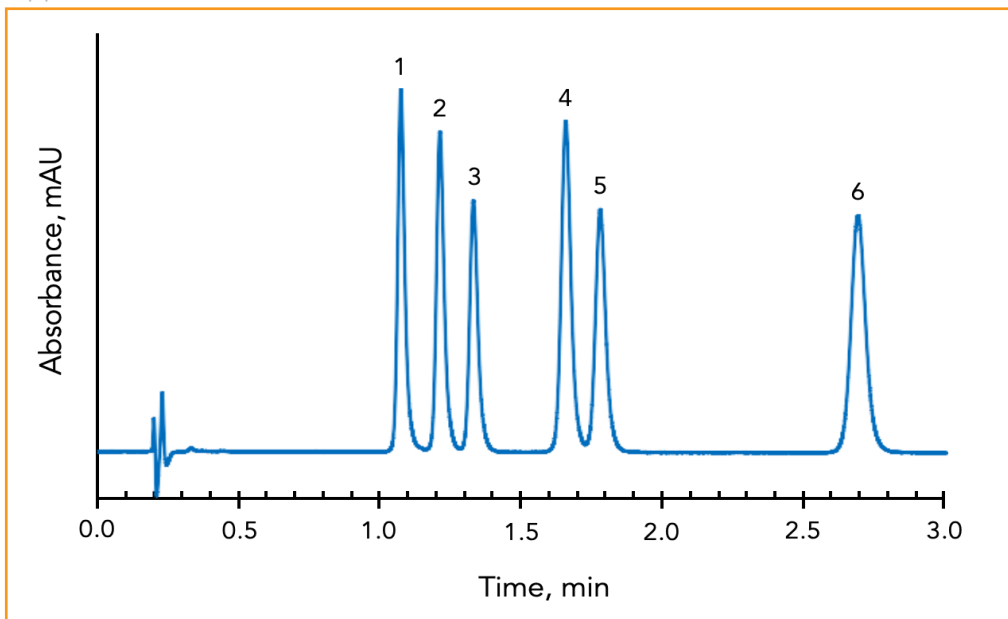
N-(4-chlorophenyl) acetamide





## 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  $\pi$ - $\pi$  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

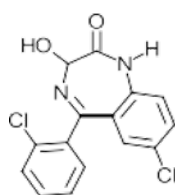
**Data Rate:** 80 Hz

**Response Time:** 0.02 sec

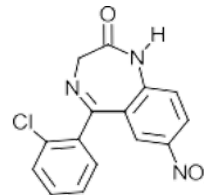
**Flow Cell:** 2.0 µL semi-micro

**LC System:** Agilent 1200 SL

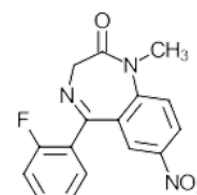
### STRUCTURES:



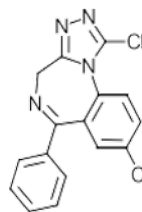
Lorazepam



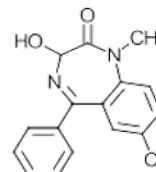
Clonazepam



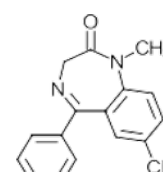
Flunitrazepam



Alprazolam



Temazepam



Diazepam

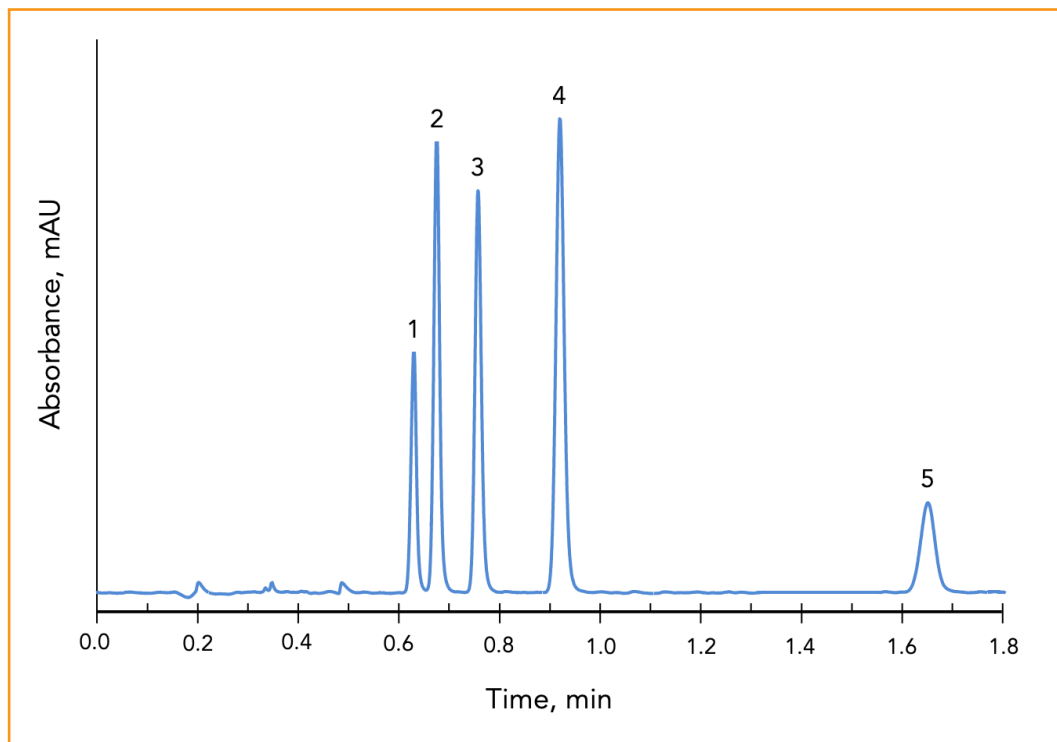






## Separation of Five Beta Blocker Drugs on HALO® Penta-HILIC

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  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** Mobile phase

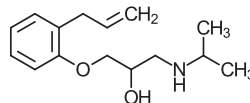
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

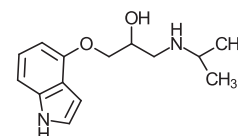
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu$ L

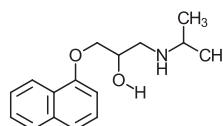
### STRUCTURES:



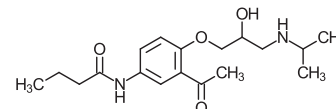
Alprenolol



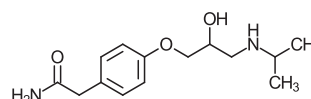
Pindolol



Propranolol



Acebutolol



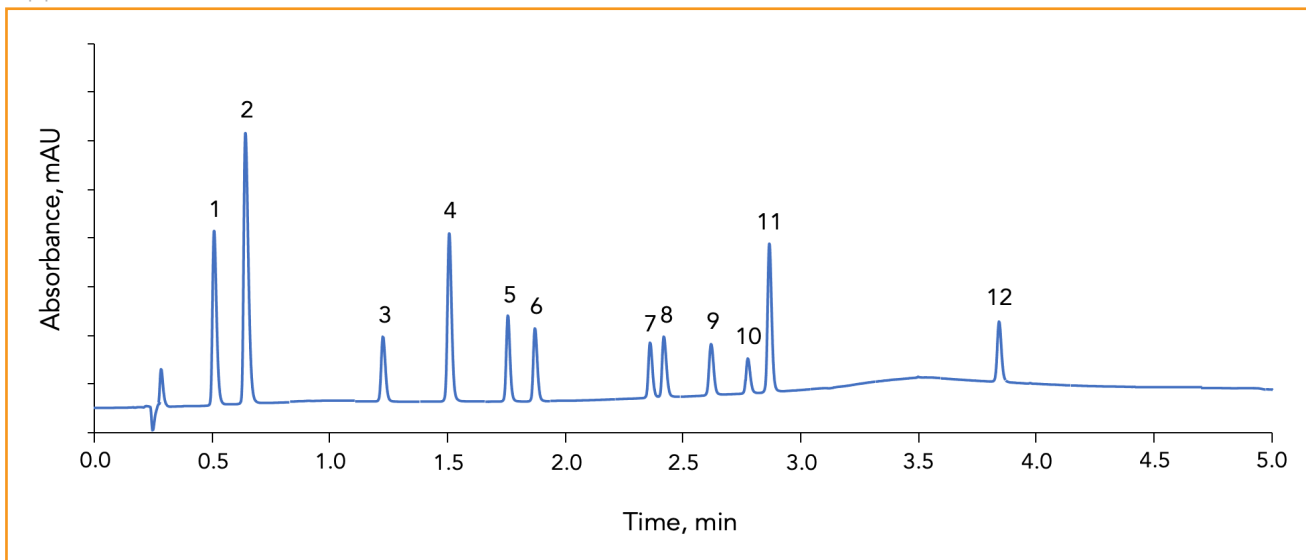
Atenolol





## Separation of Beta Blockers on HALO Biphenyl, 2.0 $\mu$ m

Application Note 195-B



A mixture of twelve beta blockers is separated on a HALO® 2.0  $\mu$ m 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  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** Water

**Response Time:** 0.025 sec

**Data Rate:** 40 Hz

**Flow Cell:** 1.0  $\mu$ L

**LC System:** Shimadzu Nexera X2

### PEAK IDENTITIES:

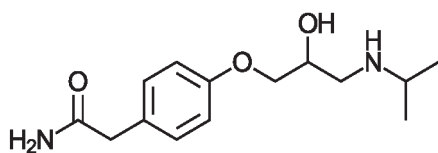
1. Atenolol
2. Sotalol
3. Nadolol
4. Pindolol
5. Acebutolol
6. Metoprolol
7. Bisoprolol
8. Oxprenolol
9. Labetalol
10. Alprenolol
11. Propranolol
12. Carvedilol



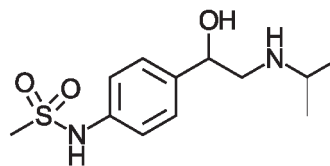


Application Note 195-B

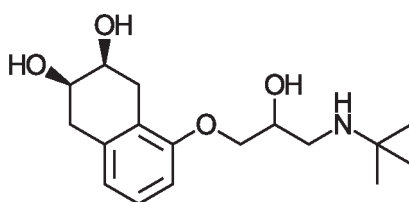
## STRUCTURES:



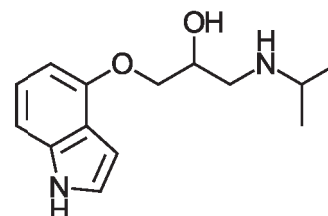
Atenolol



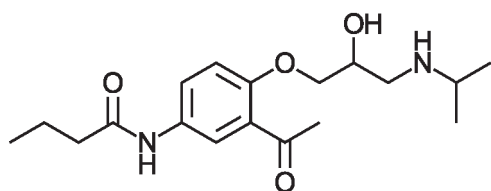
Sotalol



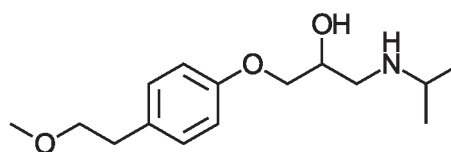
Nadolol



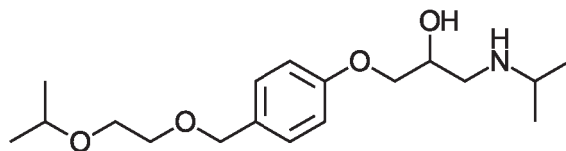
Pindolol



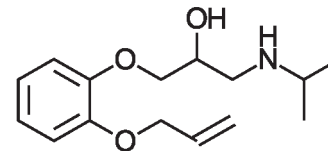
Acebutolol



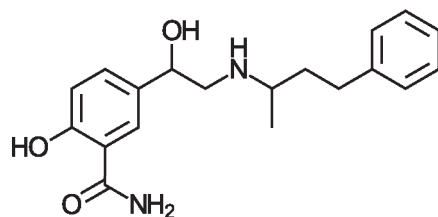
Metoprolol



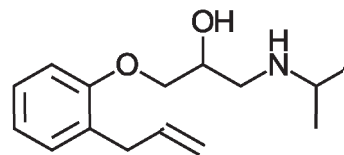
Bisoprolol



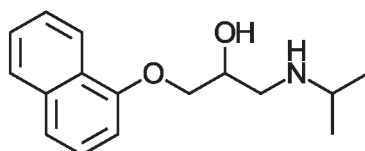
Oxprenolol



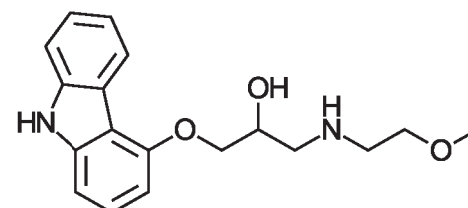
Labetalol



Alprenolol



Propranolol

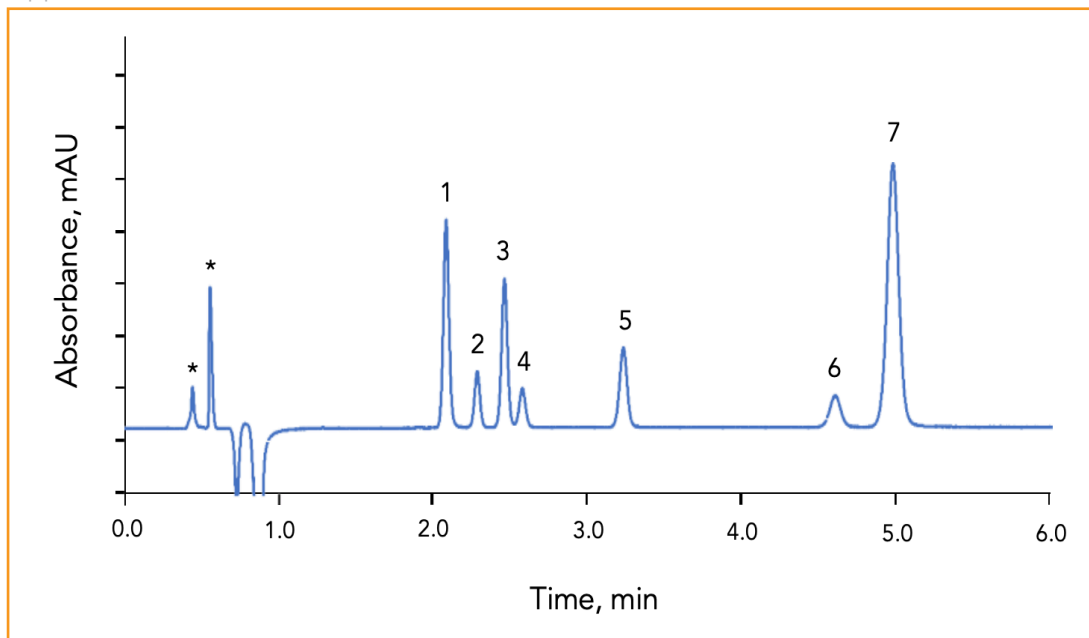


Carvedilol



## Separation of Beta Blockers on HALO<sup>®</sup> Penta-HILIC, 2.0 $\mu$ m

Application Note 196-B



### PEAK IDENTITIES:

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

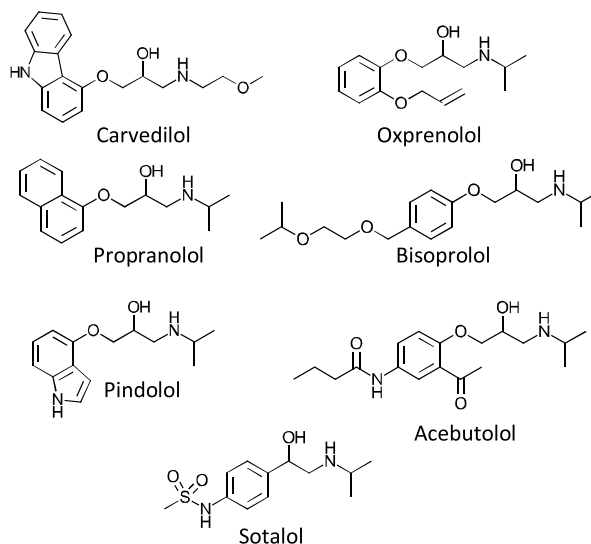
\* artifact peaks from ammonium formate

A mixture of seven beta blockers is rapidly separated on a HALO<sup>®</sup> 2.0  $\mu$ m 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  $\mu$ m, 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  $\mu$ L  
**Sample Solvent:** Acetonitrile  
**Response Time:** 0.025 sec  
**Data Rate:** 40 Hz  
**Flow Cell:** 1.0  $\mu$ L  
**LC System:** Shimadzu Nexera X2

### STRUCTURES:

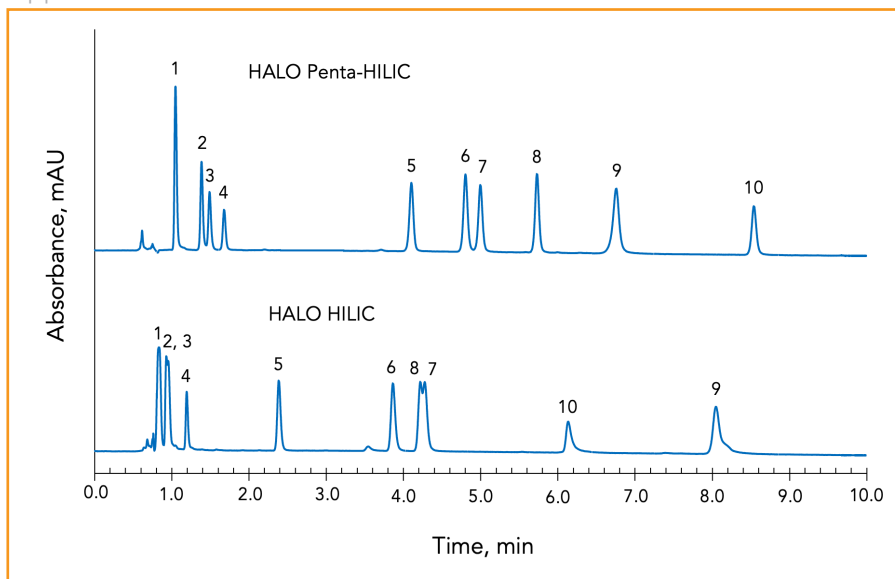






### Separation of Cephalosporins on HALO® Penta-HILIC and HALO® HILIC

Application Note 68-AB



#### PEAK IDENTITIES:

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

The class of antibiotics called cephalosporins are  $\beta$ -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  $\mu$ m, 2.1 x 150 mm

**Part Number:** 92812-705

2) HALO 90 Å HILIC, 2.7  $\mu$ m, 2.1 x 150 mm

**Part Number:** 92812-701

##### Mobile Phase:

A: 95/5 ACN/H<sub>2</sub>O with 5 mM NH<sub>4</sub> formate, pH 3.0

B: 50/50 ACN/H<sub>2</sub>O with 5 mM NH<sub>4</sub> formate, 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

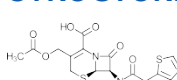
**Injection Volume:** 0.5  $\mu$ L

**Sample Solvent:** 50/50 ACN/water

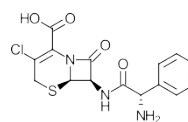
**Flow Cell:** 5.0  $\mu$ L semi-micro

**LC System:** Agilent 1100

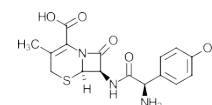
#### STRUCTURES:



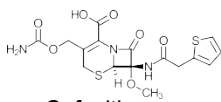
Cephalothin



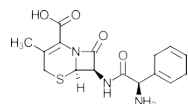
Cefaclor



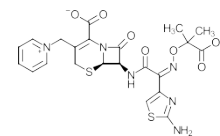
Cefadroxil



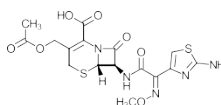
Cefoxitin



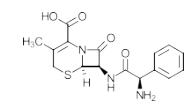
Cephalexin



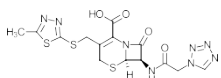
Ceftazidime



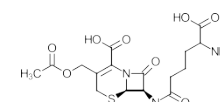
Cefotaxime



Cephadrine



Cefazolin



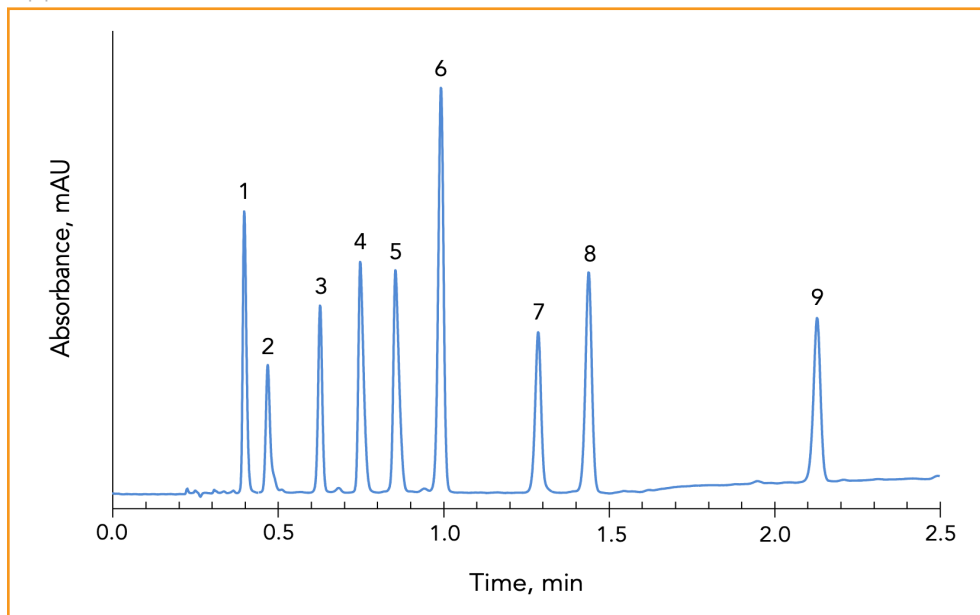
Cephalosporin C





## Separation of Cephalosporins on HALO® Phenyl-Hexyl

Application Note 70-AB



### PEAK IDENTITIES:

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

Cephalosporins are a class of  $\beta$ -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  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** 70/30 water/methanol

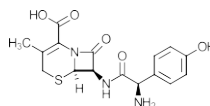
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

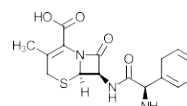
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu$ L

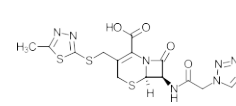
### STRUCTURES:



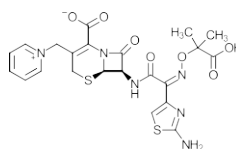
Cefadroxil



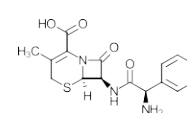
Cephalexin



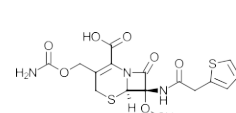
Cefazolin



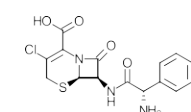
Ceftazidime



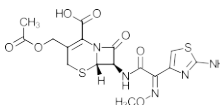
Cephadrine



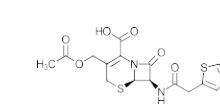
Cefoxitin



Cefaclor



Cefotaxime



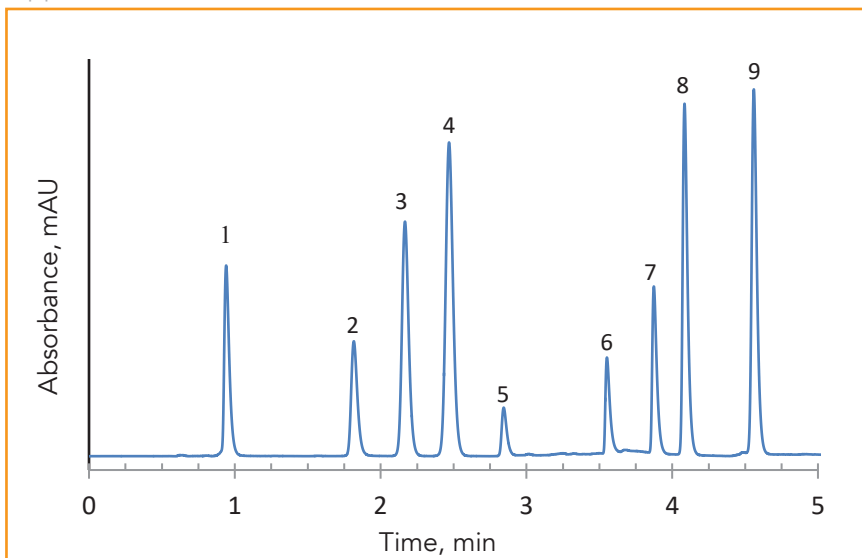
Cephalothin





## 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

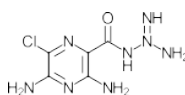
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

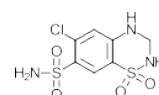
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

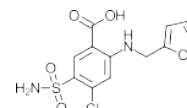
### STRUCTURES:



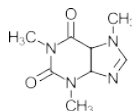
Amiloride



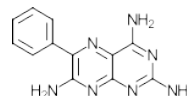
Hydrochlorothiazide



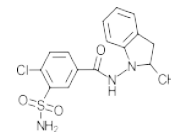
Furosemide



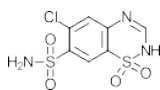
Caffeine



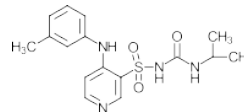
Triamterene



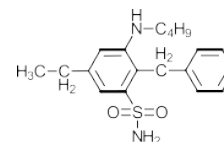
Indapamide



Chlorothiazide



Torsemide



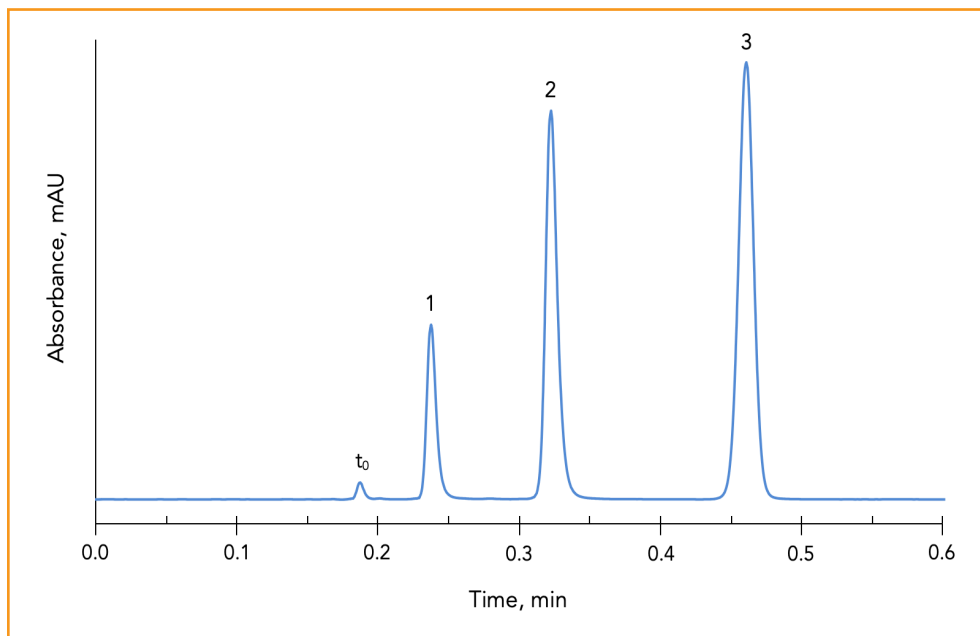
Bumetanide





## 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  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** 50/50 methanol/acetonitrile

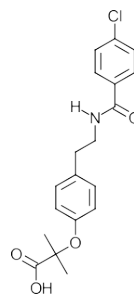
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

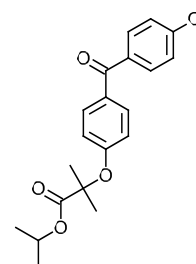
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu$ L

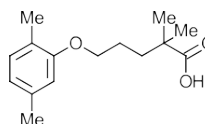
### STRUCTURES:



Bezafibrate



Fenofibrate



Gemfibrozil

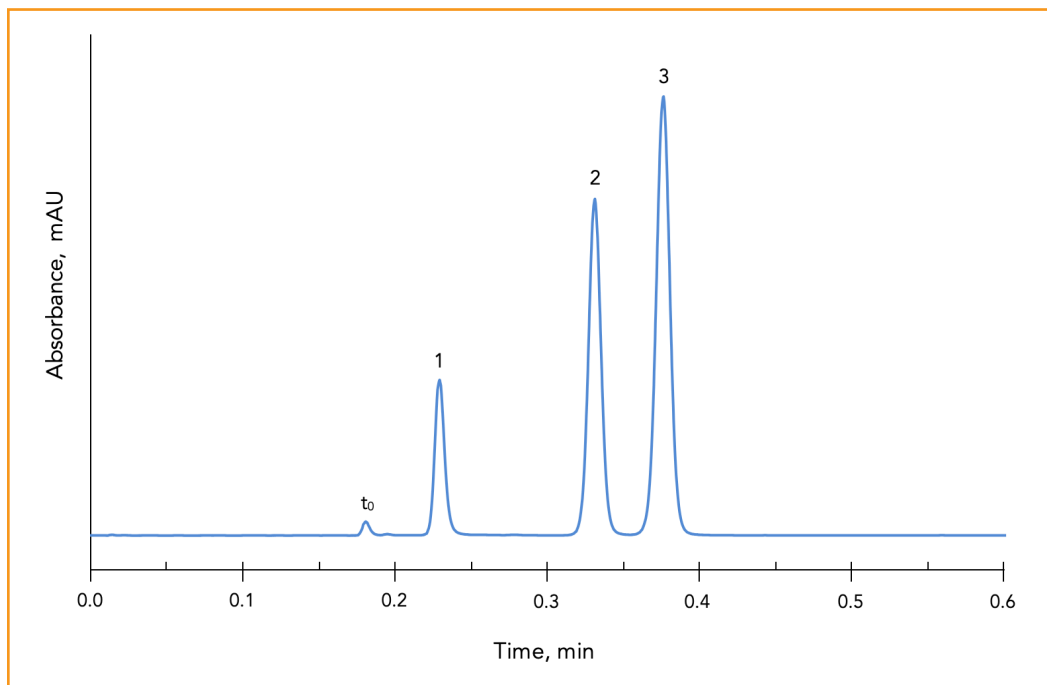






## Rapid Isocratic Separation of Fibrates on HALO® RP-Amide Phase

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

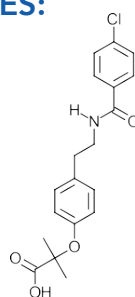
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

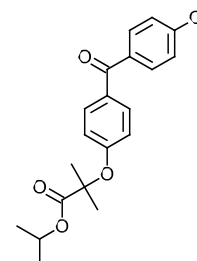
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

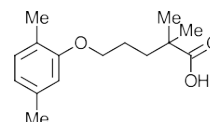
### STRUCTURES:



Bezafibrate



Fenofibrate



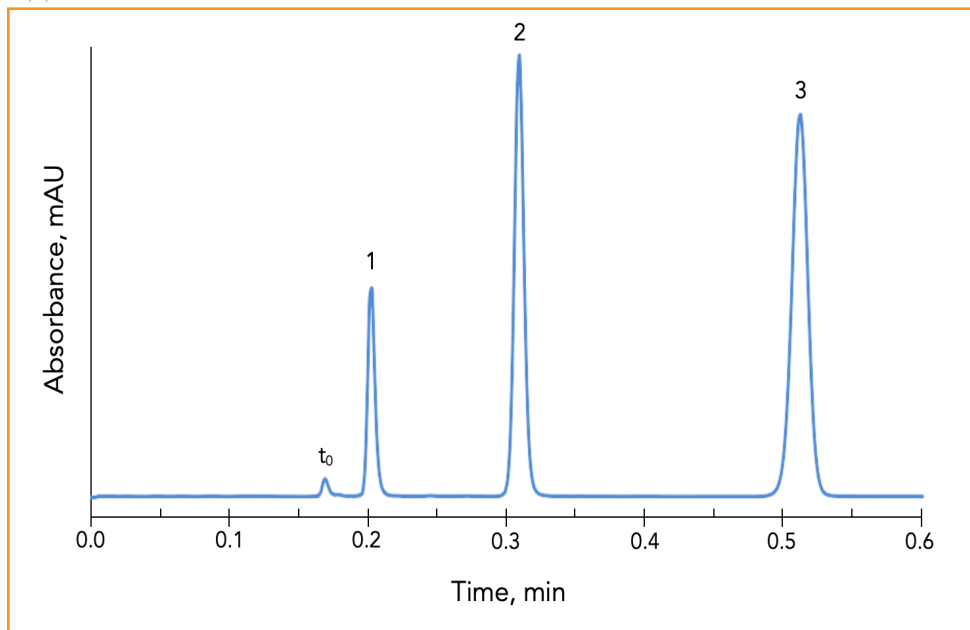
Gemfibrozil





## 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 cholesterol 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

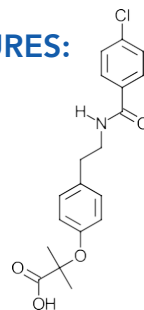
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

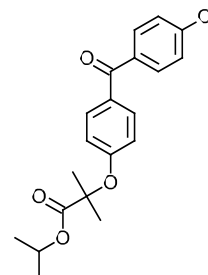
**LC System** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

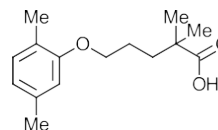
### STRUCTURES:



Bezafibrate



Fenofibrate



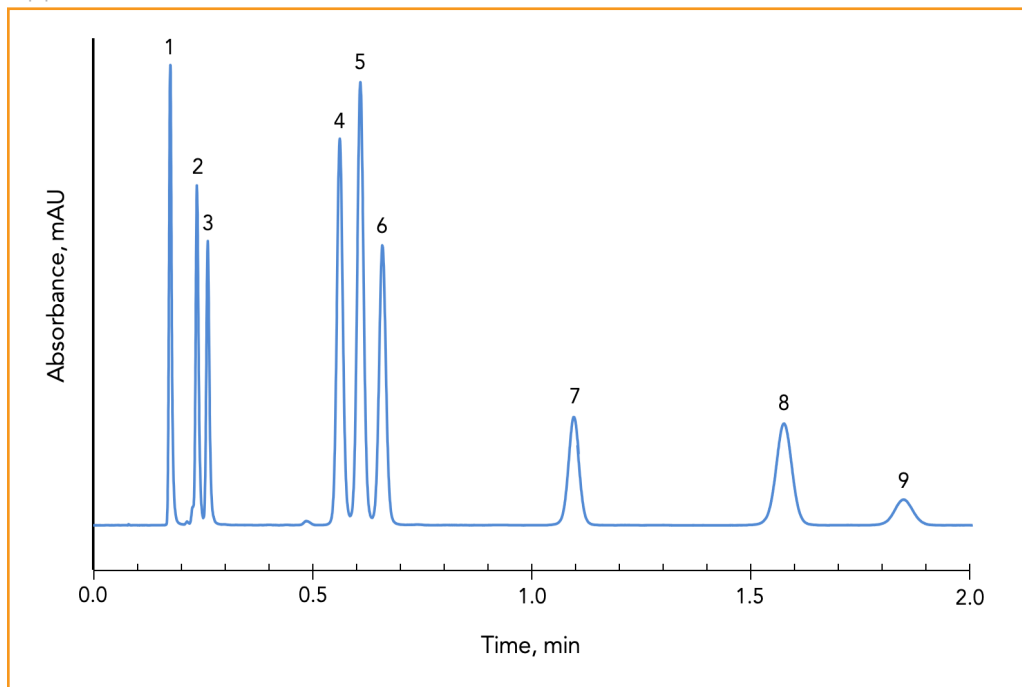
Gemfibrozil





## 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. Fenopropfen
8. Diclofenac
9. Ibuprofen

Non-steroidal antiinflammatory 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  $\mu\text{m}$ ,  
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  $\mu\text{L}$

**Sample Solvent:** 50/50 methanol/water

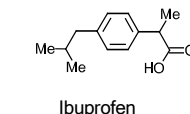
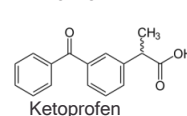
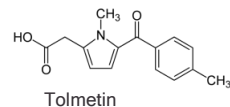
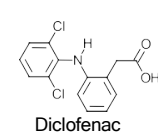
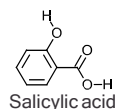
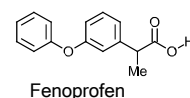
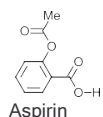
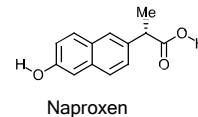
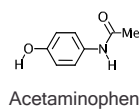
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu\text{L}$  semi-micro

**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu\text{L}$

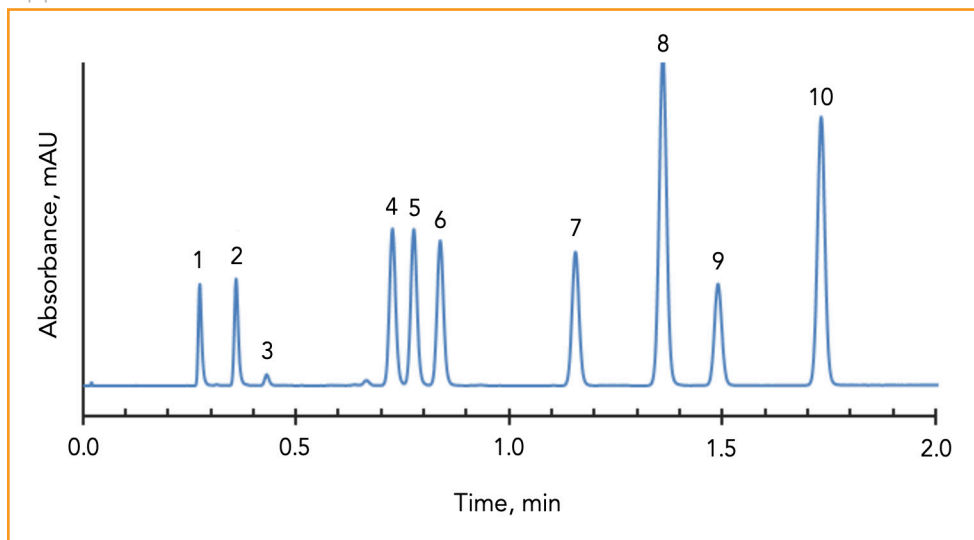
### STRUCTURES:





## 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. Fenopropfen
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

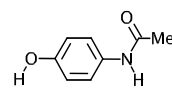
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

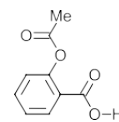
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

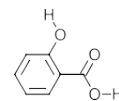
### STRUCTURES:



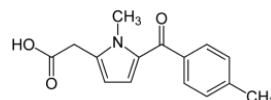
Acetaminophen



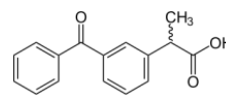
Aspirin



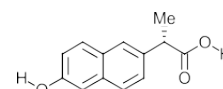
Salicylic acid



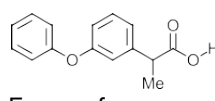
Tolmetin



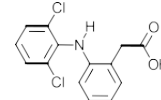
Ketoprofen



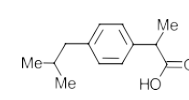
Naproxen



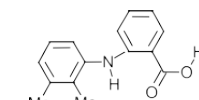
Fenopropfen



Diclofenac



Ibuprofen



Mefenamic acid

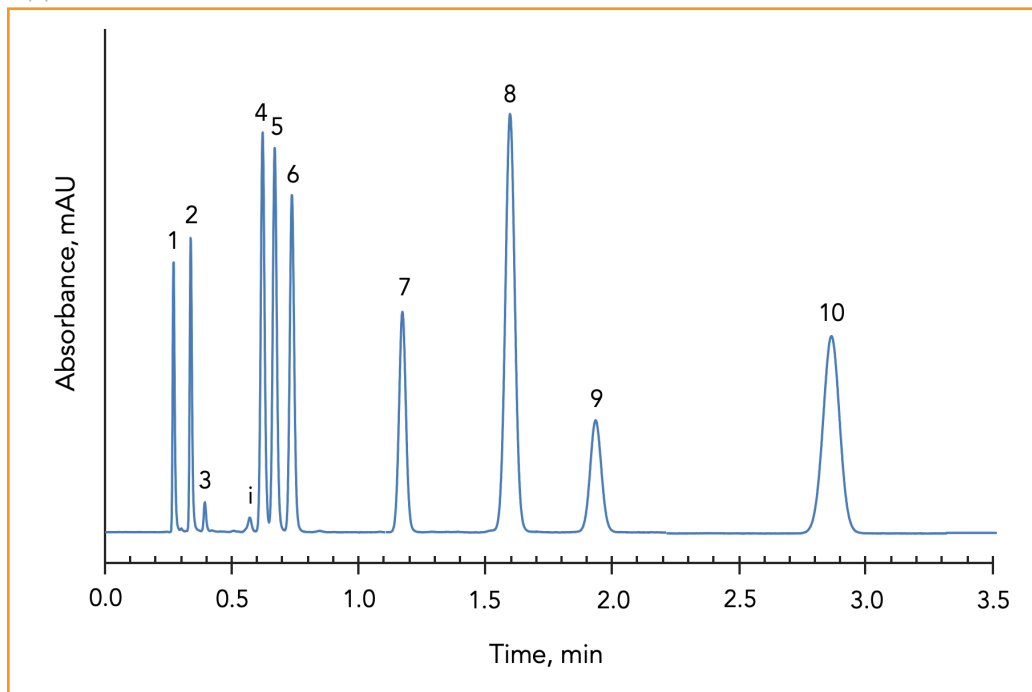






## Separation of NSAIDs on HALO® C8

Application Note 15-NS



### PEAK IDENTITIES:

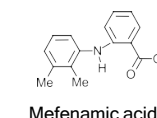
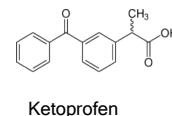
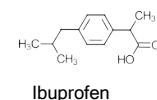
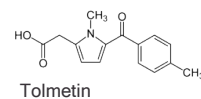
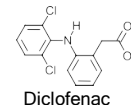
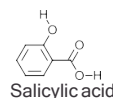
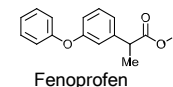
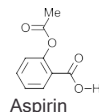
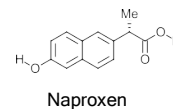
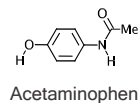
1. Acetaminophen
  2. Aspirin
  3. Salicylic acid
  4. Tolmetin
  5. Ketoprofen
  6. Naproxen
  7. Fenopufen
  8. Diclofenac
  9. Ibuprofen
  10. Mefenamic acid
- i = impurity

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

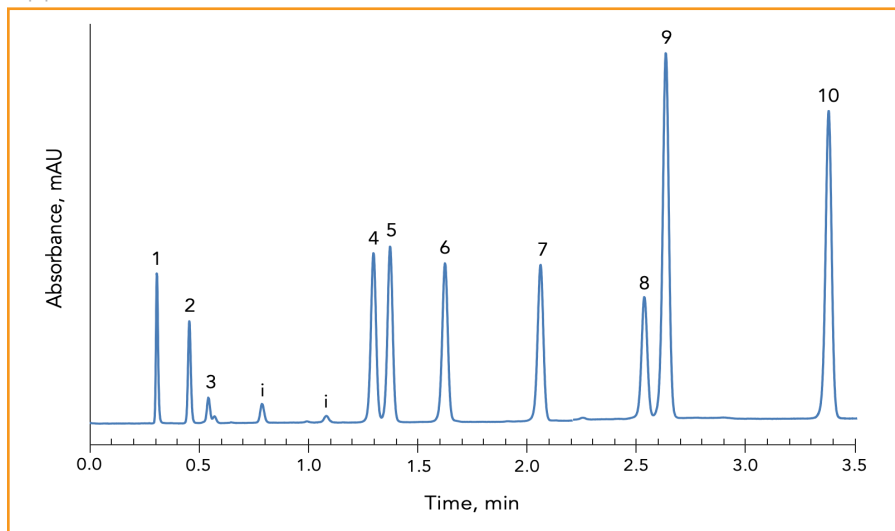
### STRUCTURES:





## 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. Fenopropfen
  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  $\mu$ m packed column.

### TEST CONDITIONS:

**Column:** HALO 90 Å RP-Amide, 2.7  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** Mobile phase

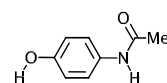
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

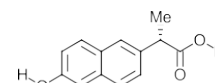
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu$ L

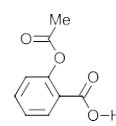
### STRUCTURES:



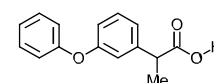
Acetaminophen



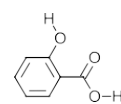
Naproxen



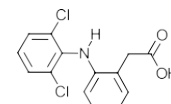
Aspirin



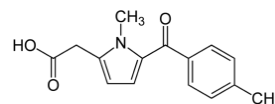
Fenopropfen



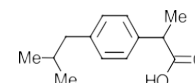
Salicylic acid



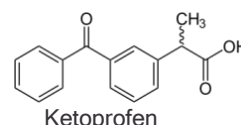
Diclofenac



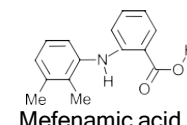
Tolmetin



Ibuprofen



Ketoprofen



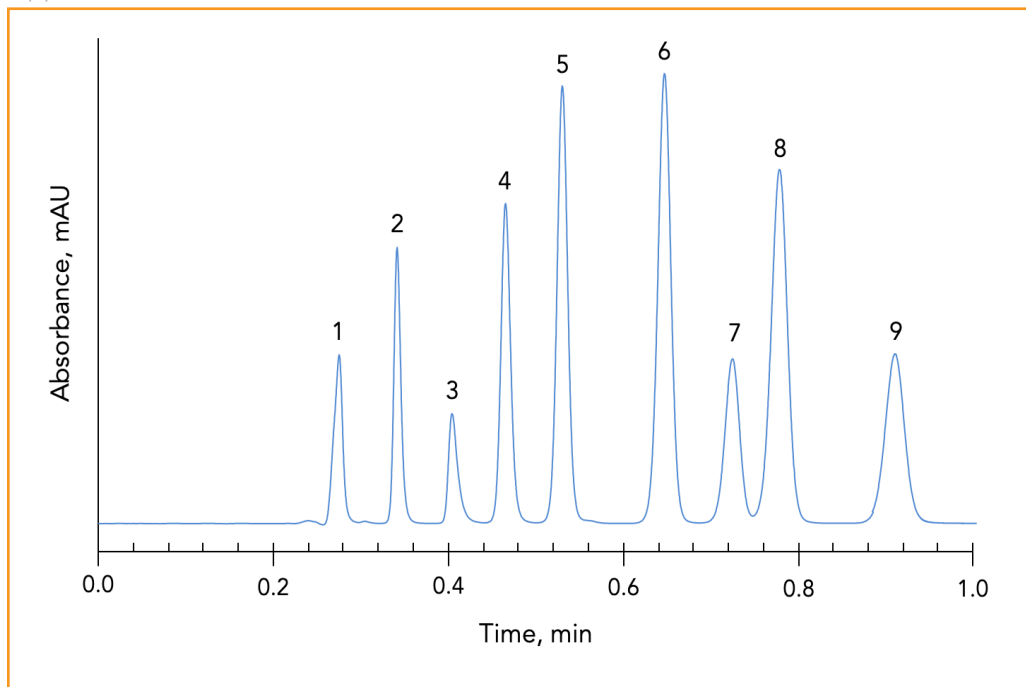
Mefenamic acid





## Isocratic Separation of NSAIDs on HALO<sup>®</sup> ES-CN Phase

Application Note 56-NS



### PEAK IDENTITIES:

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

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

### TEST CONDITIONS:

**Column:** HALO 90 Å ES-CN, 2.7  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** Water/methanol

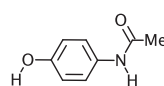
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

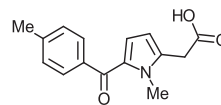
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu$ L

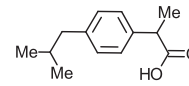
### STRUCTURES:



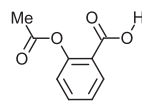
Acetaminophen



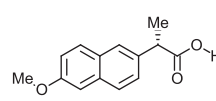
Tolmetin



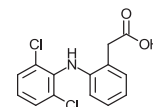
Ibuprofen



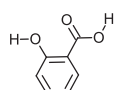
Aspirin



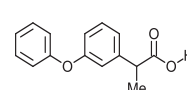
Naproxen



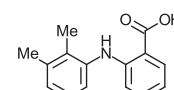
Diclofenac



Salicylic acid



Fenopropfen



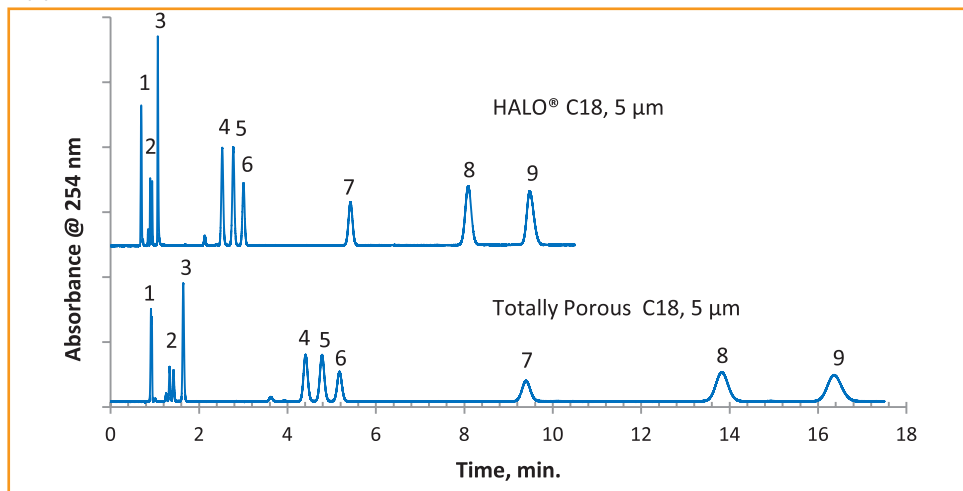
Mefenamic acid





## Separation of NSAIDs on HALO<sup>®</sup> C18, 5.0 $\mu$ m and Totally Porous C18, 5.0 $\mu$ m

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<sup>®</sup> 5.0  $\mu$ m 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  $\mu$ m, 4.6 x 150 mm

Part number: 95814-702

2) Totally porous C18, 5.0  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** 50/50 methanol/water

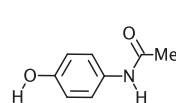
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

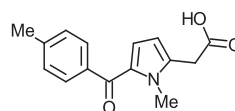
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu$ L

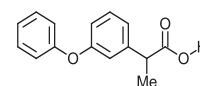
### STRUCTURES:



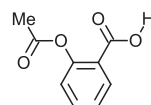
Acetaminophen



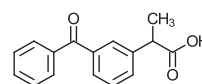
Tolmetin



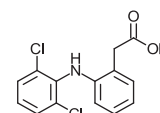
Fenoprofen



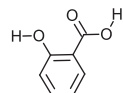
Aspirin



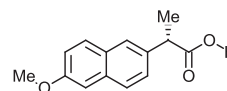
Ketoprofen



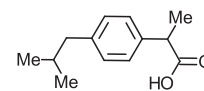
Diclofenac



Salicylic acid



Naproxen



Ibuprofen

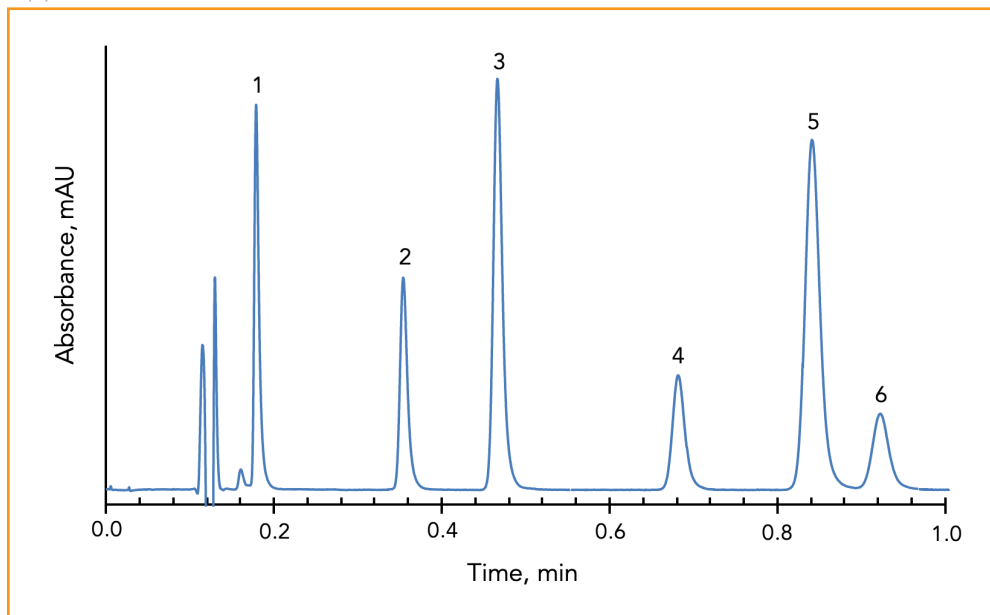






## Separation of NSAIDs on HALO® ES-CN, 2.0 µm with MS Compatible Mobile Phase

Application Note 128-NS



### PEAK IDENTITIES:

1. Aspirin
2. Tolmetin
3. Naproxen
4. Fenopropfen
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

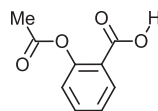
**Data Rate:** 80 Hz

**Response Time:** 0.02 sec

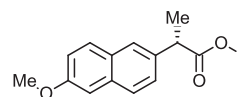
**Flow Cell:** 2.0 µL micro cell

**LC System:** Agilent 1200 SL

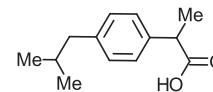
### STRUCTURES:



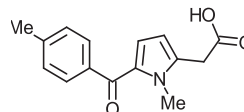
Aspirin



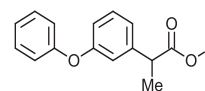
Naproxen



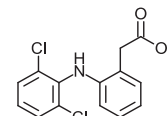
Ibuprofen



Tolmetin



Fenopropfen



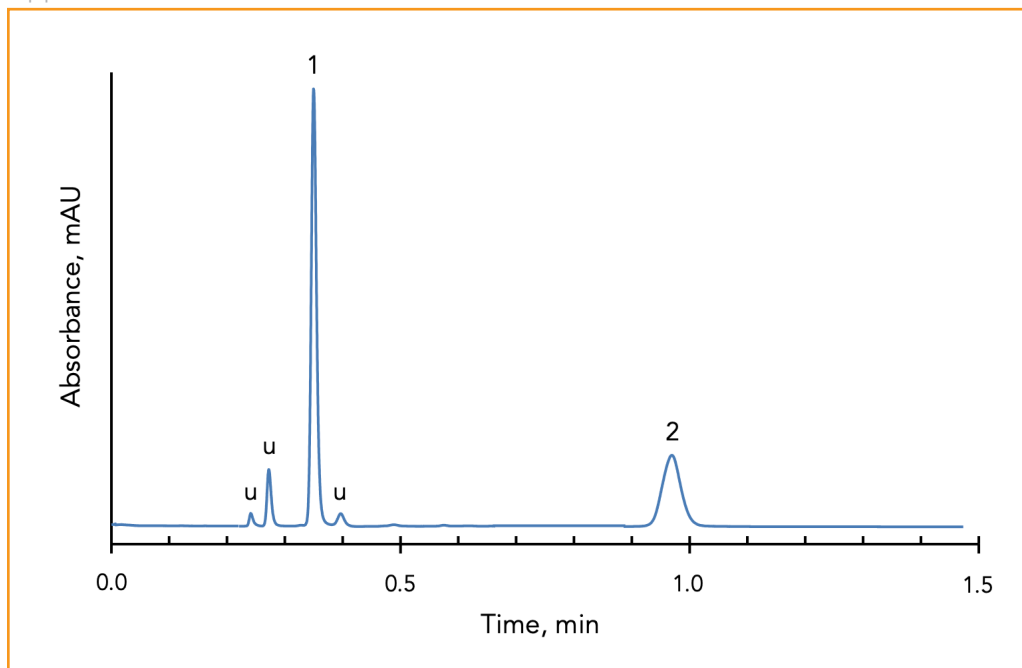
Diclofenac





## 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

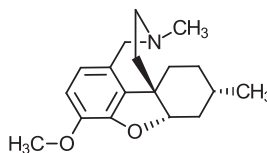
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

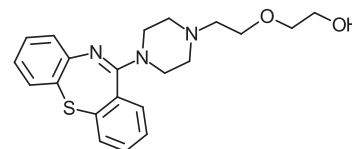
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

### STRUCTURES:



Galantamine



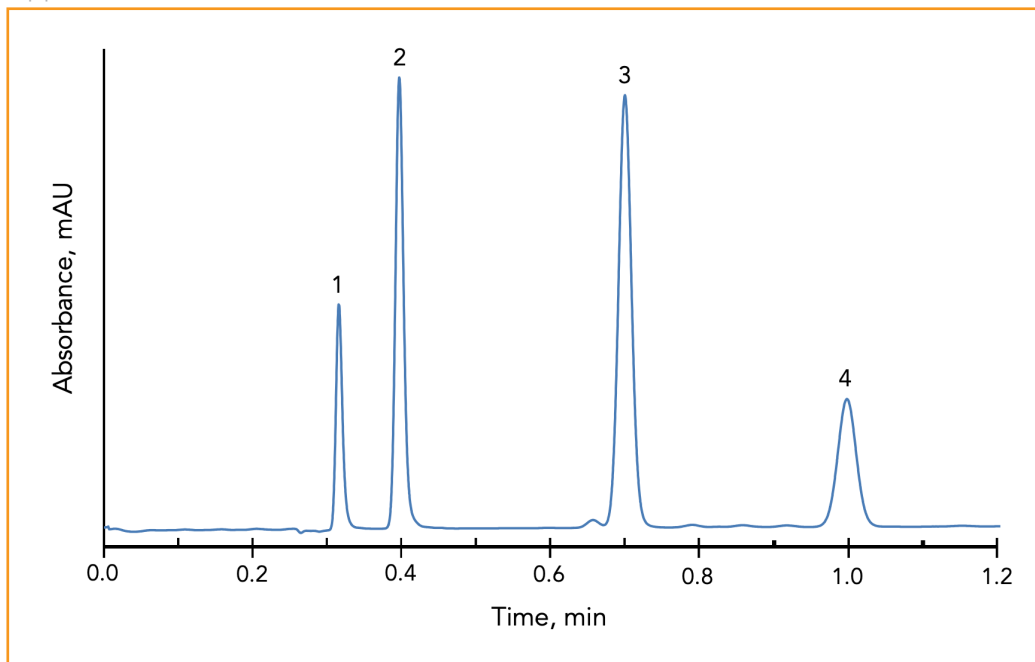
Quetiapine





## 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  $\mu\text{m}$ ,  
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  $\mu\text{L}$

**Sample Solvent:** Mobile phase

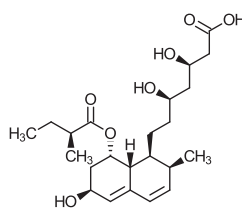
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu\text{L}$  semi-micro

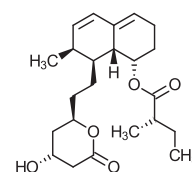
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu\text{L}$

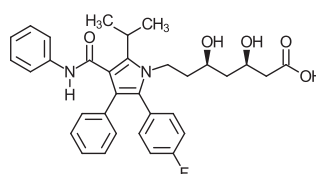
### STRUCTURES:



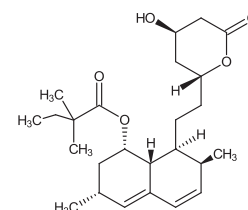
Pravastatin



Mevastatin



Atorvastatin



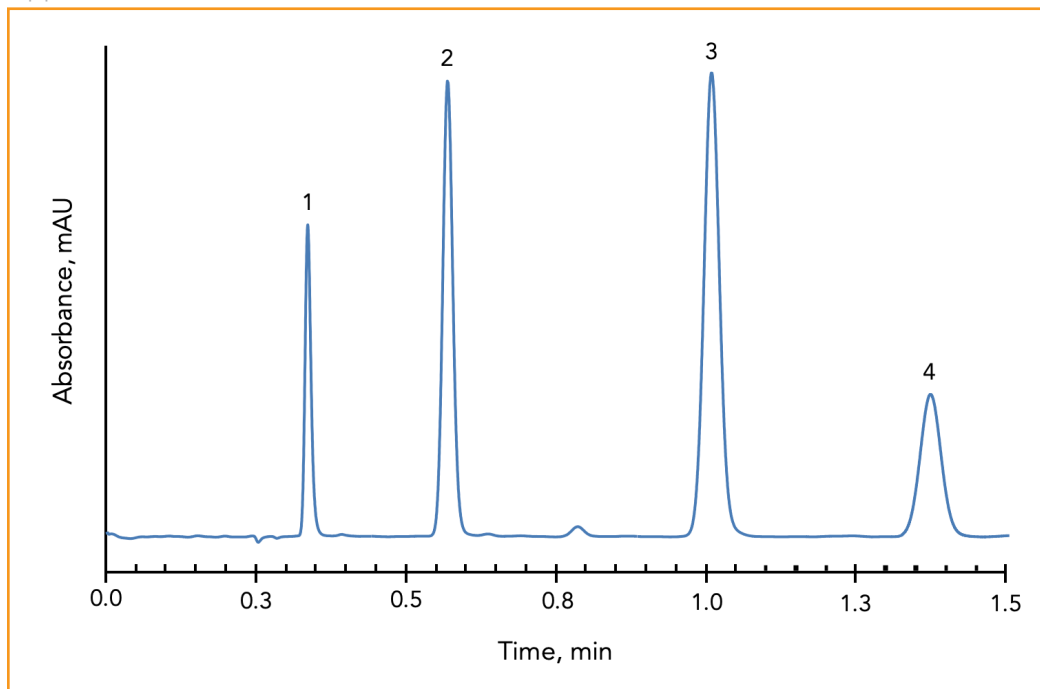
Simvastatin





## 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)

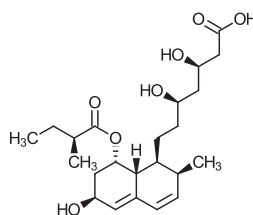
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

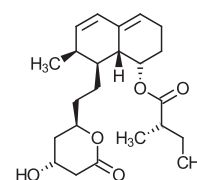
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

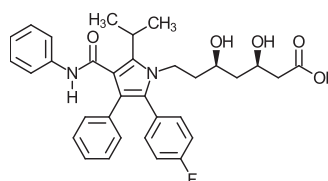
### STRUCTURES:



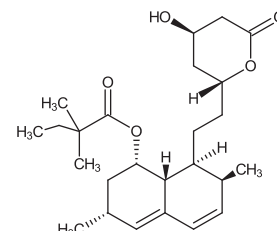
Pravastatin



Mevastatin



Atorvastatin



Simvastatin

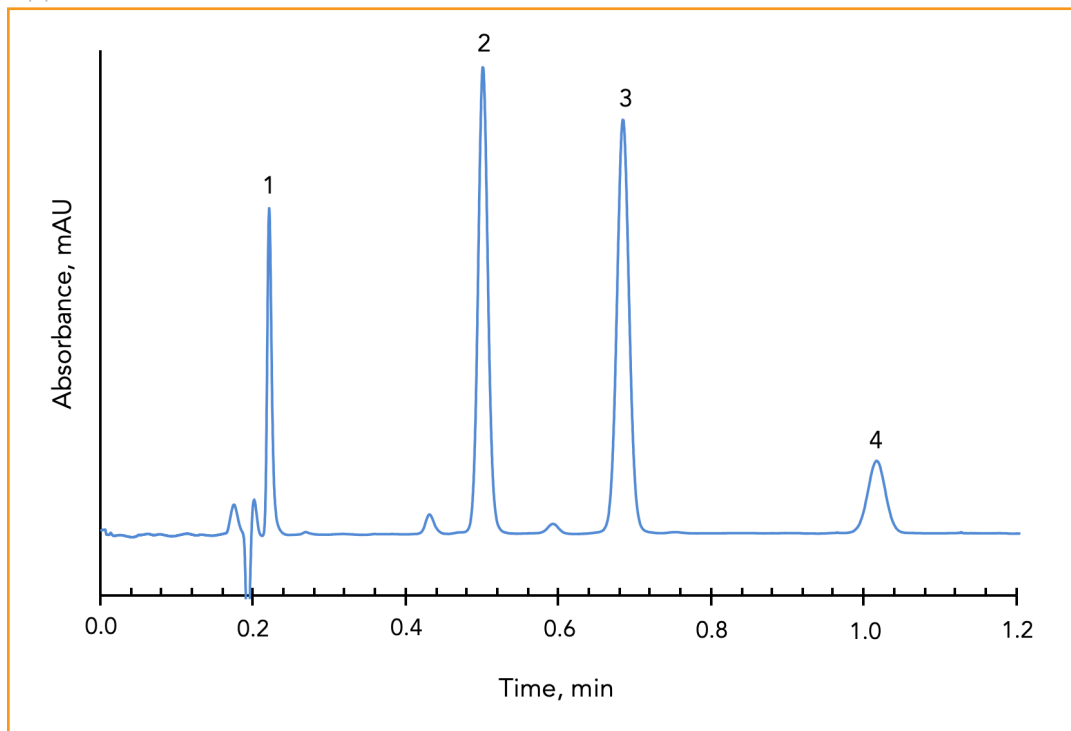






## Separation of Statin Drugs on HALO® Phenyl-Hexyl in Acetonitrile

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)

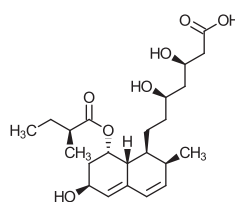
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

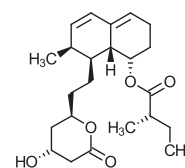
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

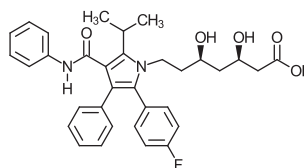
### STRUCTURES:



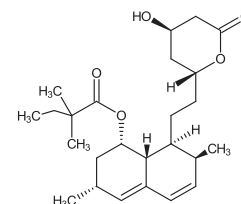
Pravastatin



Mevastatin



Atorvastatin



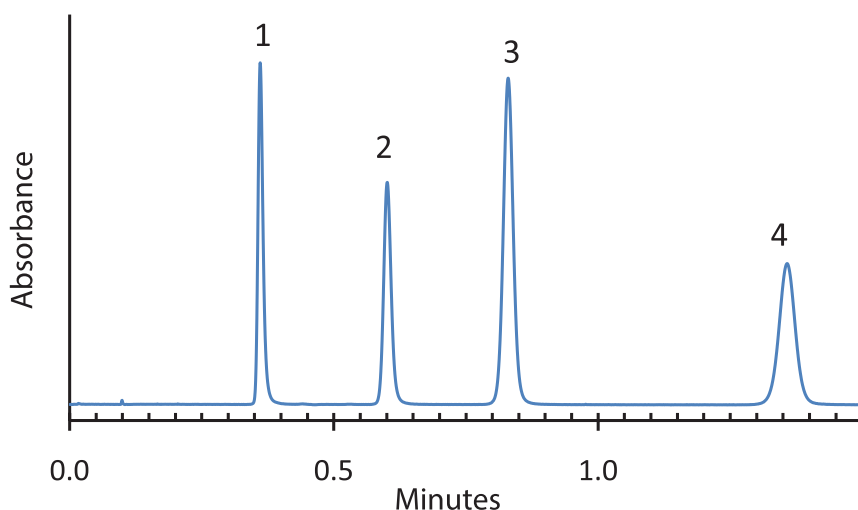
Simvastatin





## Separation of Xanthines on HALO<sup>®</sup> 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<sup>®</sup> 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

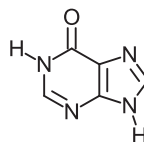
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

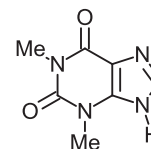
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

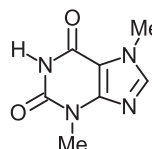
### STRUCTURES:



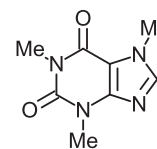
Hypoxanthine



Theophylline



Theobromine



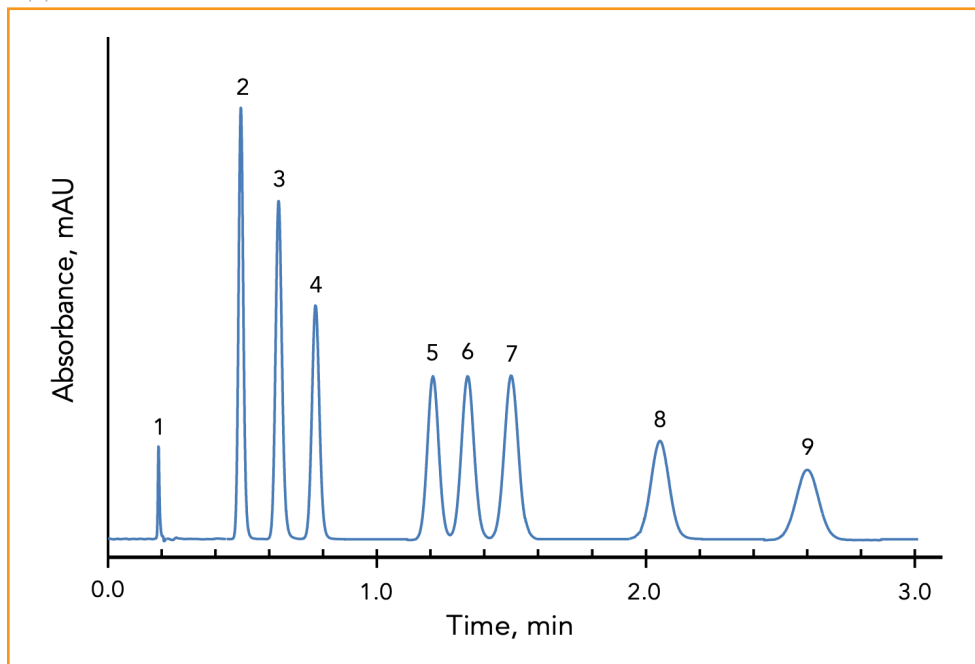
Caffeine





## 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

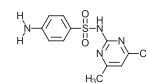
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

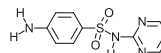
### STRUCTURES:



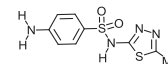
Uracil



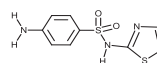
Sulfamethazine



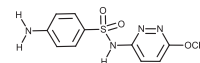
Sulfadiazine



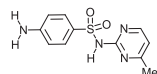
Sulfamethizole



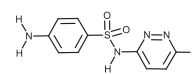
Sulfathiazole



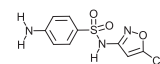
Sulfamethoxypyridazine



Sulfamerazine



Sulfachloropyridazine



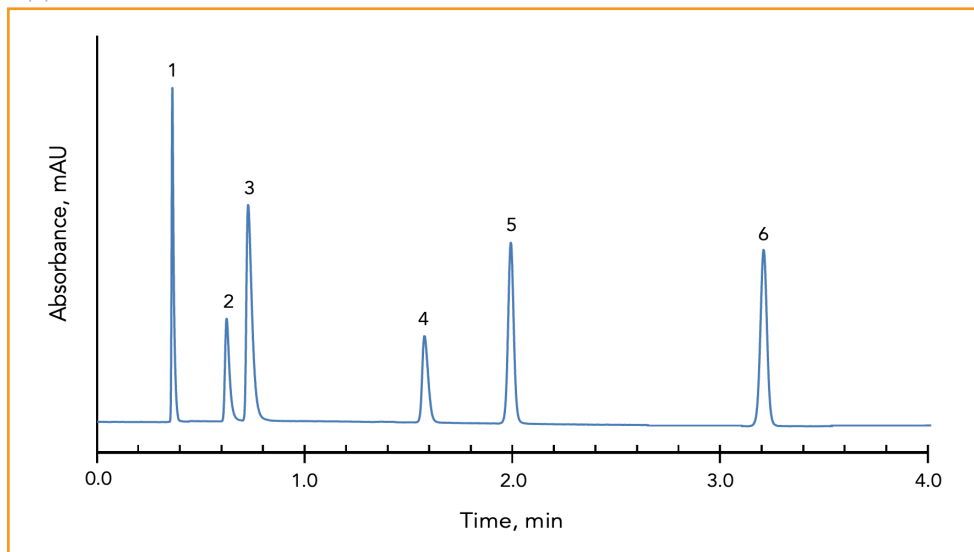
Sulfamethoxazole





## 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

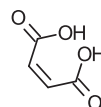
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

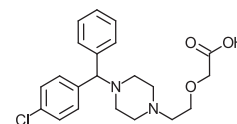
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14 µL

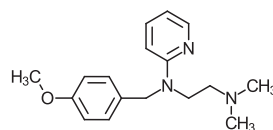
### STRUCTURES:



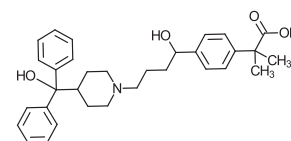
Maleic acid



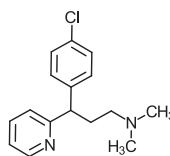
Cetirizine



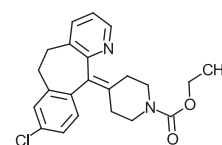
Pyrilamine



Fexofenadine



Chlorpheniramine



Loratadine

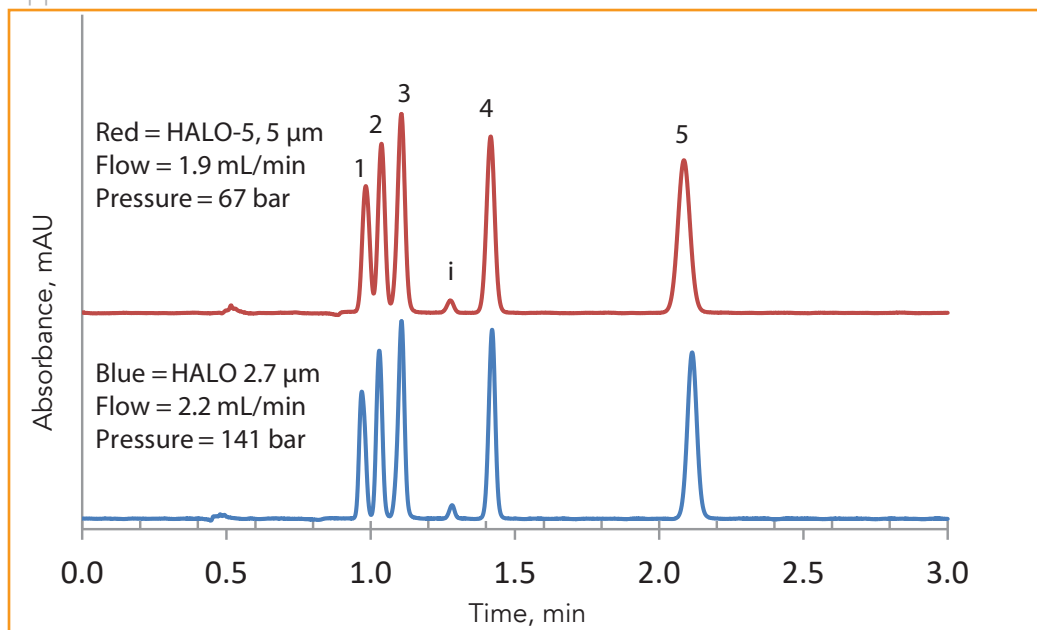






## Comparable Selectivity Between HALO® Penta-HILIC 5 $\mu\text{m}$ and 2.7 $\mu\text{m}$

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  $\mu\text{m}$  and 2.7  $\mu\text{m}$  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  $\mu\text{m}$ , 4.6 x 100 mm  
Part Number: 95814-605
- 2) HALO 90 Å Penta-HILIC, 2.7  $\mu\text{m}$ , 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  $\mu\text{L}$

**Sample Solvent:** 10/90 water/acetonitrile

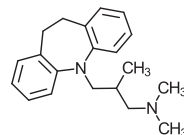
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu\text{L}$  semi-micro

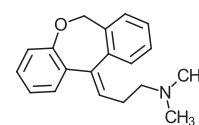
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu\text{L}$

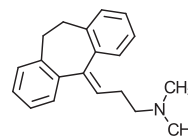
### STRUCTURES:



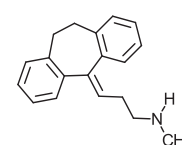
Trimipramine



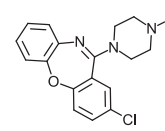
Doxepin



Amitriptyline



Nortriptyline



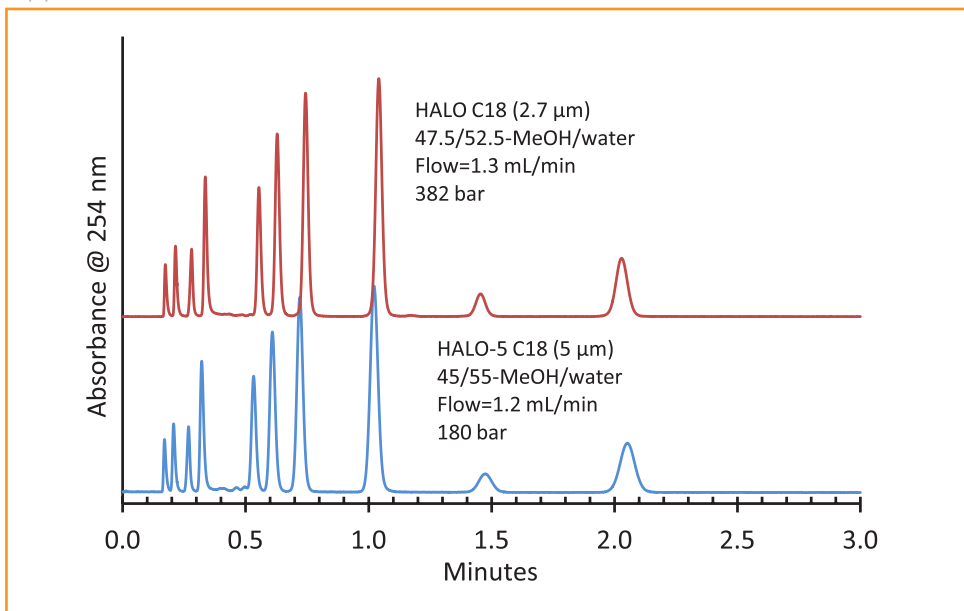
Amoxapine





## 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

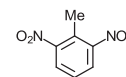
### STRUCTURES:



Uracil



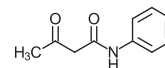
4-Chloroaniline



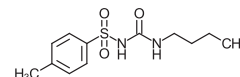
2,6-Dinitrotoluene



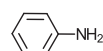
Resorcinol



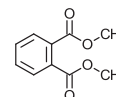
Acetoacetanilide



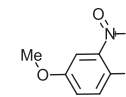
Tolbutamide



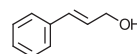
Aniline



Dimethylphthalate



4-Chloro-3-nitroanisole



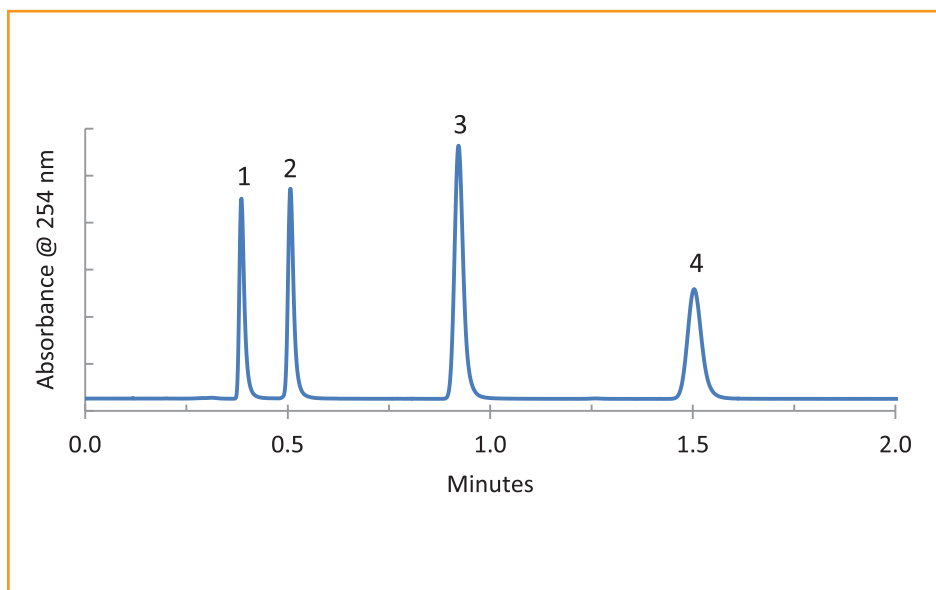
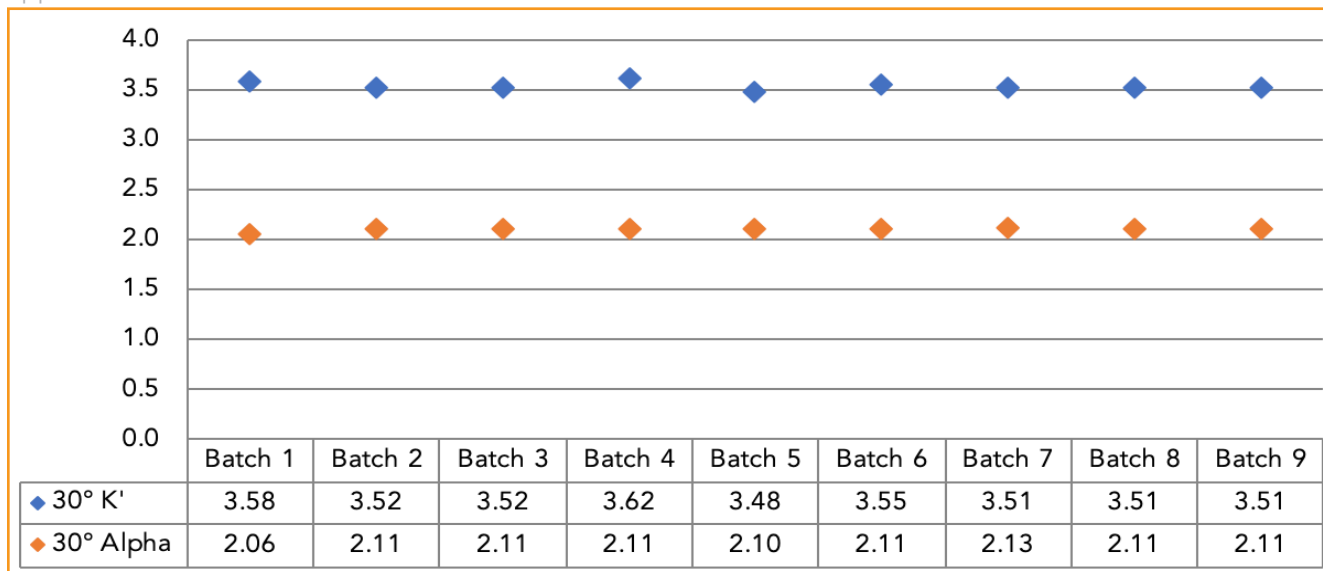
Cinnamyl alcohol





## HALO® C18, 5 µm Lot to Lot Reproducibility

Application Note 79



### 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.

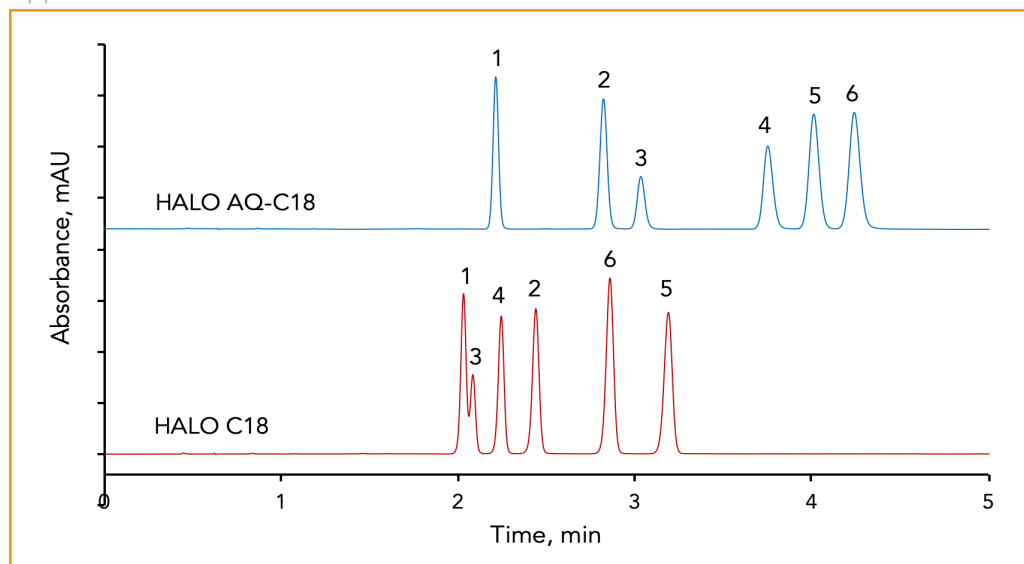


50



## Separation of Polar Samples on HALO<sup>®</sup> 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<sup>®</sup> AQ-C18 and HALO<sup>®</sup> C18 phases have different selectivities as shown in the chromatograms above. The HALO<sup>®</sup> AQ-C18 phase delivers increased retention for polar molecules compared to C18.

### TEST CONDITIONS:

#### Columns:

1) HALO 90 Å C18, 2.7  $\mu$ m, 4.6 x 100 mm

**Part Number:** 92814-602

2) HALO 90 Å AQ-C18, 2.7  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** Methanol

**Response Time:** 0.02 sec

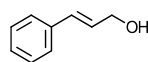
**Data Rate:** 25 Hz

**Flow Cell:** 2.5  $\mu$ L semi-micro

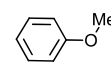
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu$ L

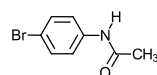
### STRUCTURES:



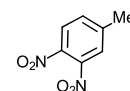
Cinnamyl alcohol



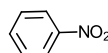
Anisole



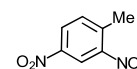
4'-Bromoacetanilide



3,4-Dinitrotoluene



Nitrobenzene



2,4-Dinitrotoluene

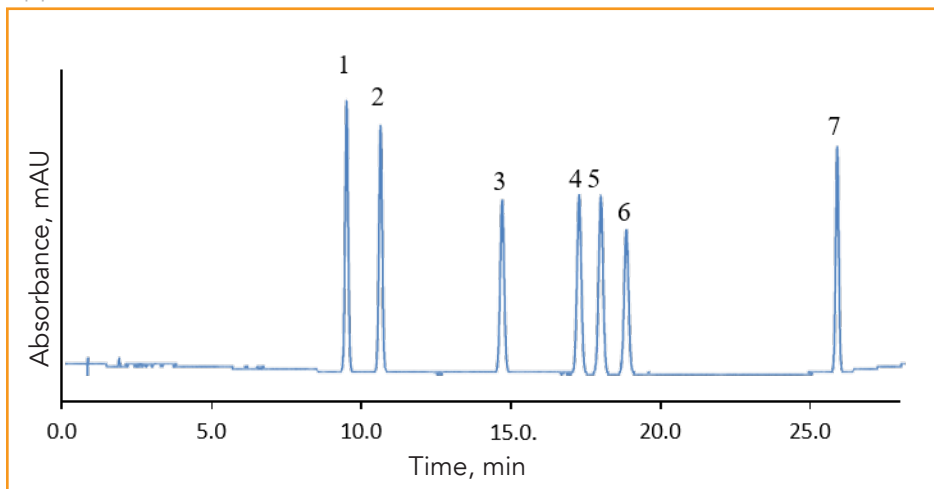






## Chinese Pharmacopeia Separation of Parabens on HALO® C18, 2.7 µm

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

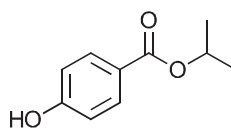
**Response Time:** 0.025 sec

**Data Rate:** 40 Hz

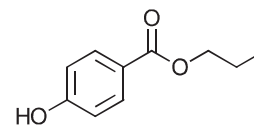
**Flow Cell:** 1.0 µL

**LC System:** Shimadzu Nexera X2

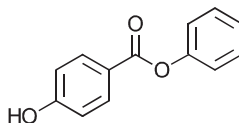
### STRUCTURES:



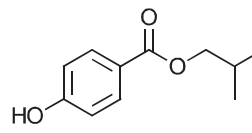
Isopropyl paraben



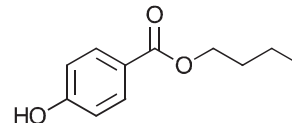
Propyl paraben



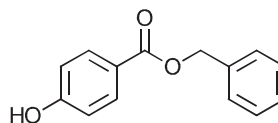
Phenyl paraben



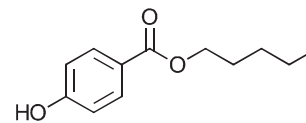
Isobutyl paraben



Butyl paraben



Benzyl paraben



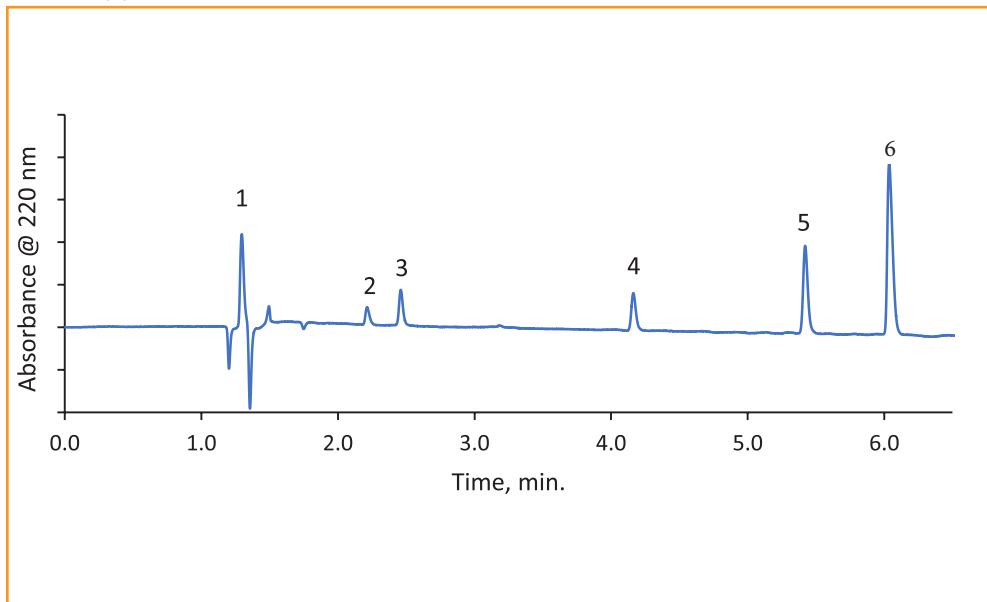
Pentyl paraben





## 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

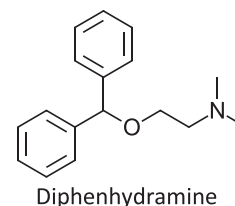
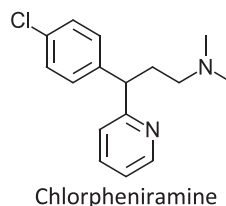
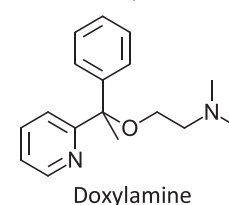
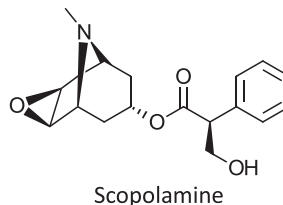
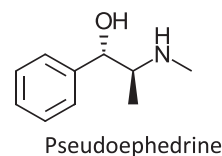
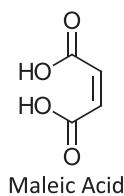
**Data Rate:** 40 Hz

**Response Time:** 0.025 sec.

**Flow Cell:** 1 µL

**LC System:** Shimadzu Nexera X2

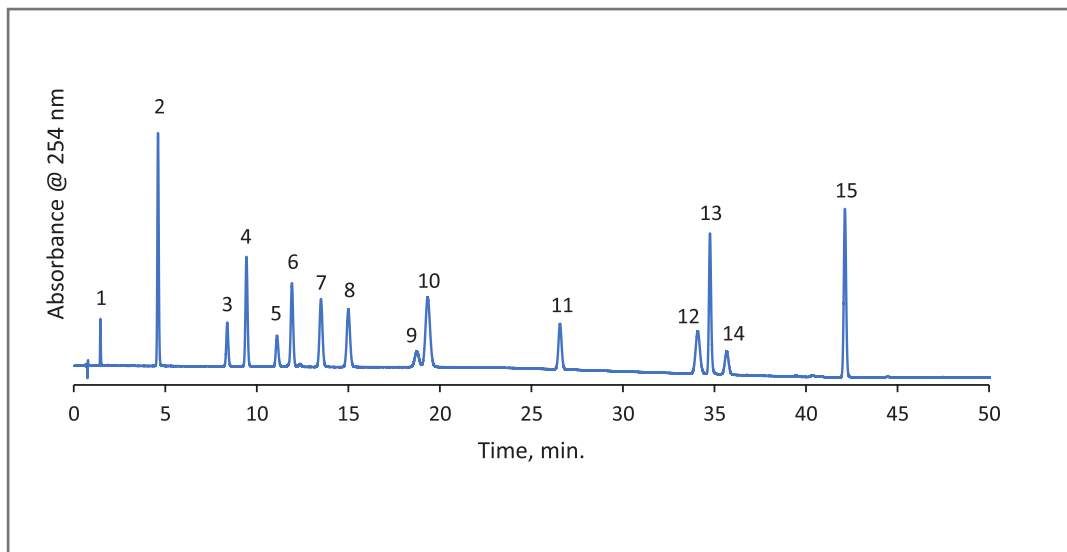
### STRUCTURES:





## 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

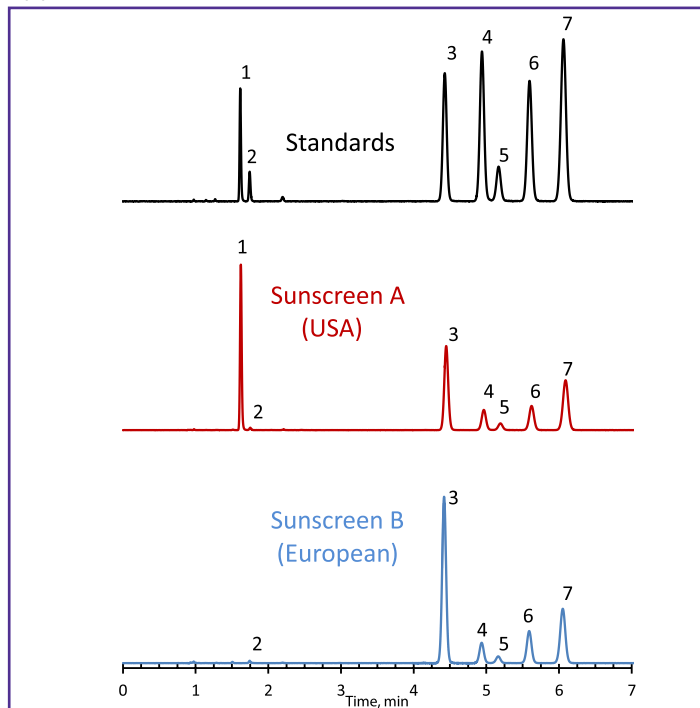
Paracetamol (acetaminophen) is a common pain relief and fever medication taken individually, or in combination with other medications. An analysis of paracetamol and 14 of its impurities are separated on a HALO 90 Å C18 column following the official European Pharmacopoeia 9.4 method. Baseline resolution 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<sup>®</sup> RP-Amide, 2.7 $\mu$ m

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  $\mu$ m porosity syringe filters prior to analysis.

### TEST CONDITIONS:

**Column:** HALO 90 Å RP Amide, 2.7  $\mu$ m  
4.6 x 150 mm

**Part Number:** 92814-707

**Mobile Phase:** A/B

A= Water

B= Acetonitrile

**Gradient:**

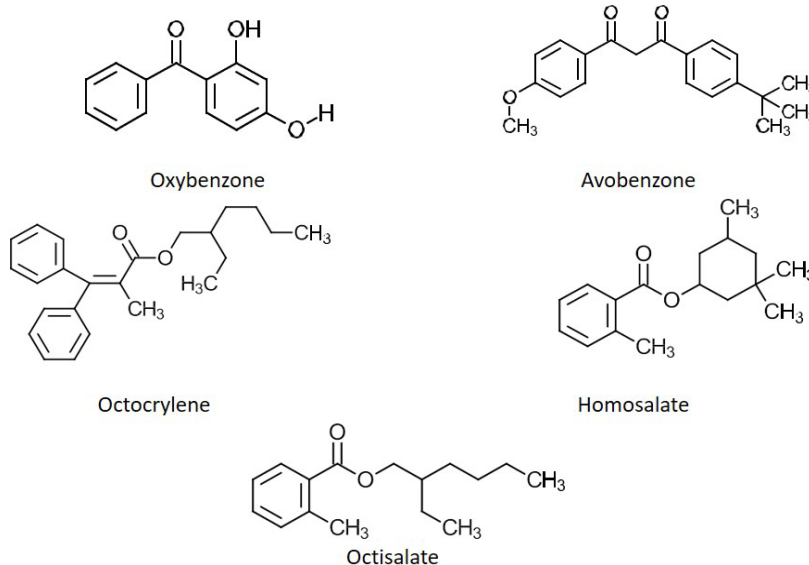
Time	% B
0.0	75
7.0	75
10	100
20	100

**Flow Rate:** 1.5 mL/min.

**LC System:** Shimadzu Prominence UFLC XR

**ECV:** ~14  $\mu$ L

### STRUCTURES:

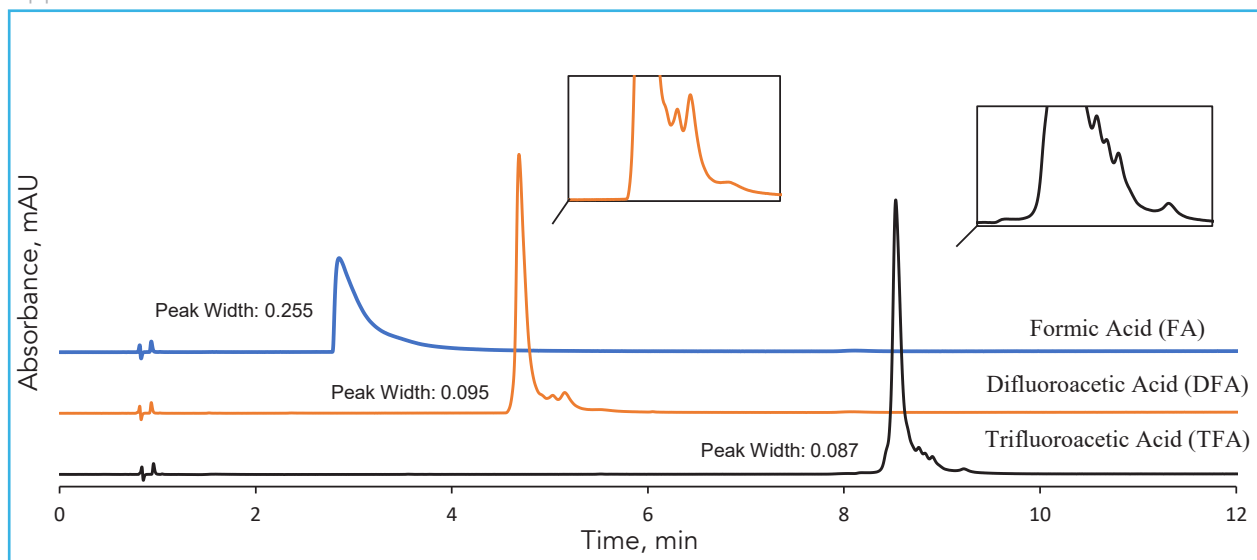






## Effect of Acid Modifiers on Intact mAb Peak Shape

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

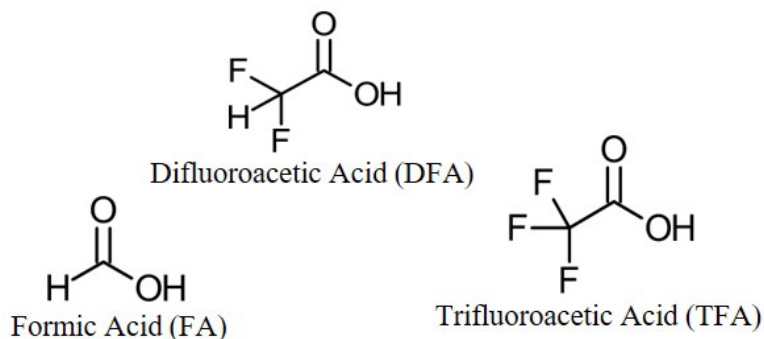
**Data Rate:** 12.5 Hz

**LC System:** Shimadzu Nexera X2

### PEAK IDENTITIES:

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

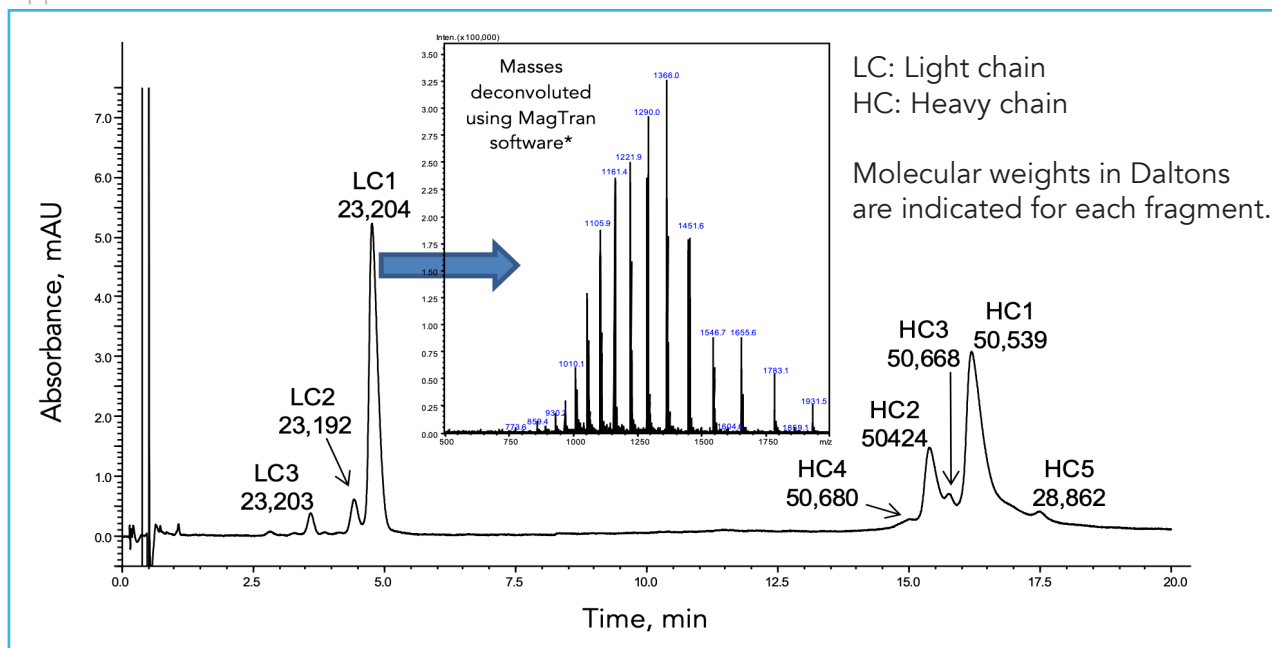
### STRUCTURES:





### 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.

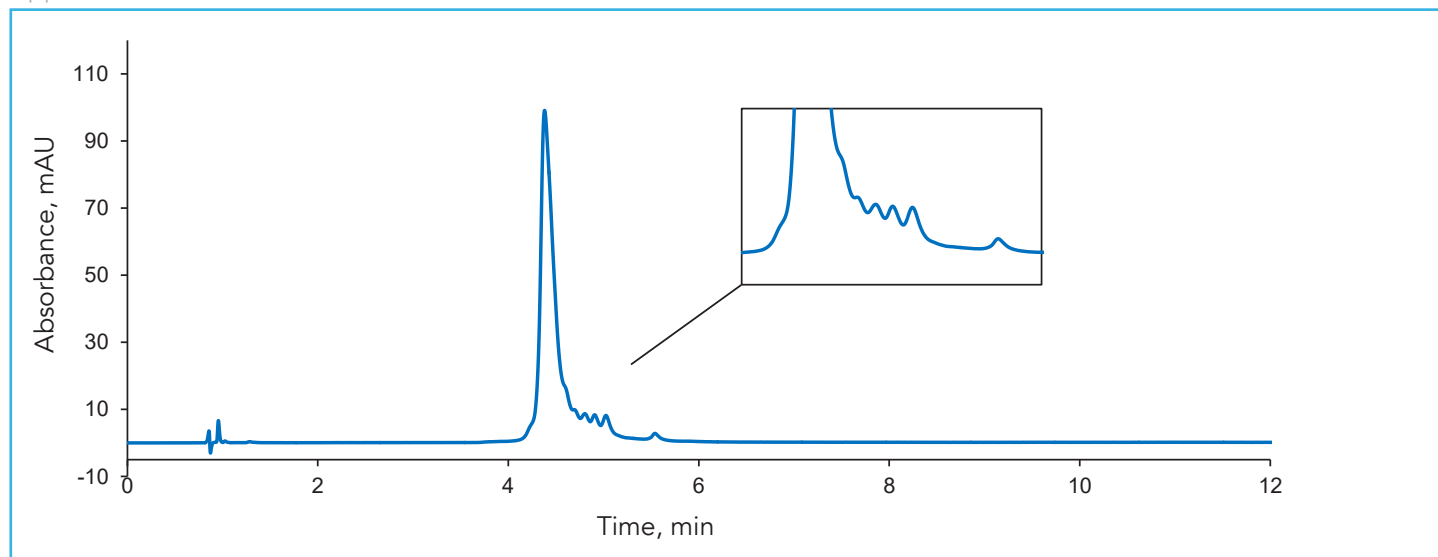
\*Z. Zhang, A.G. Marshall, J. Am. Soc. Mass Spectrom. 9 (1998) 225.





## 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:

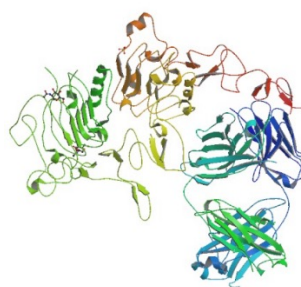


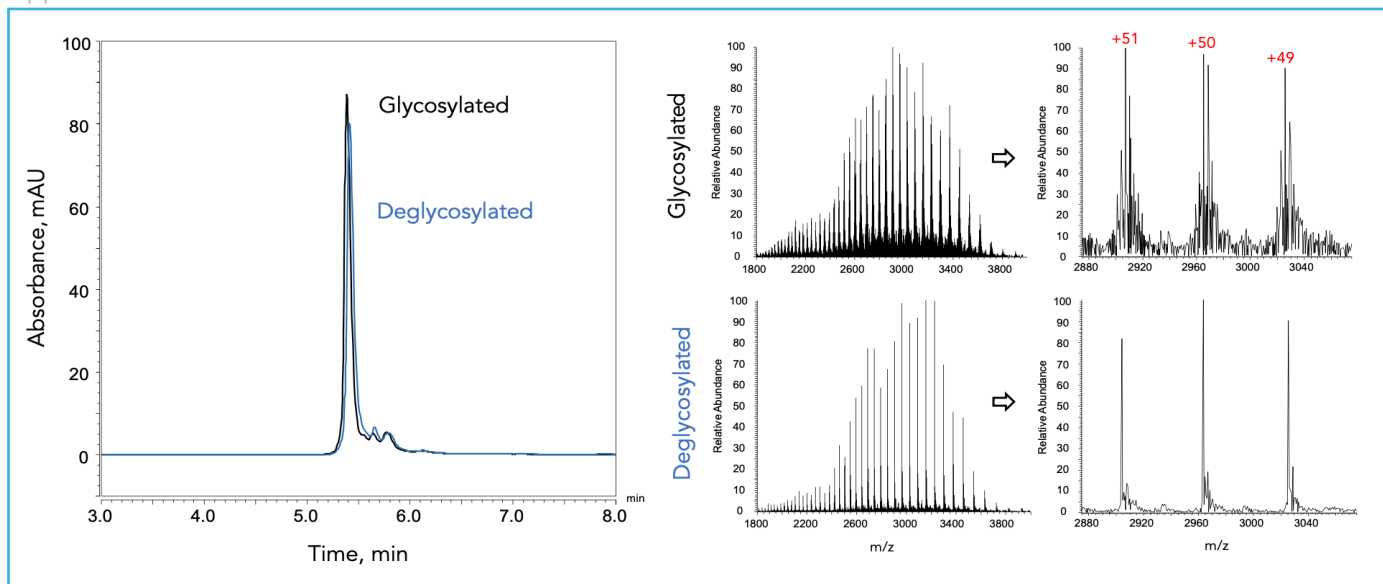
Image from the RCSB PDB ([www.rcsb.org](http://www.rcsb.org)) of PDB ID 1N8Z  
Cho, H.-S., Mason, K., Ramyar, K.X., Stanley, A.M., Gabelli, S.B., Denney Jr, D.W., Leahy, D.J.





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

Application Note 151-PR



### LC TEST CONDITIONS:

**Column:** HALO 1000 Å C4, 2.7  $\mu$ m,  
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  $\mu$ L 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  $\mu$ scans/250 ms max inject time

**Scan Range:** 1800 to 4000 m/z

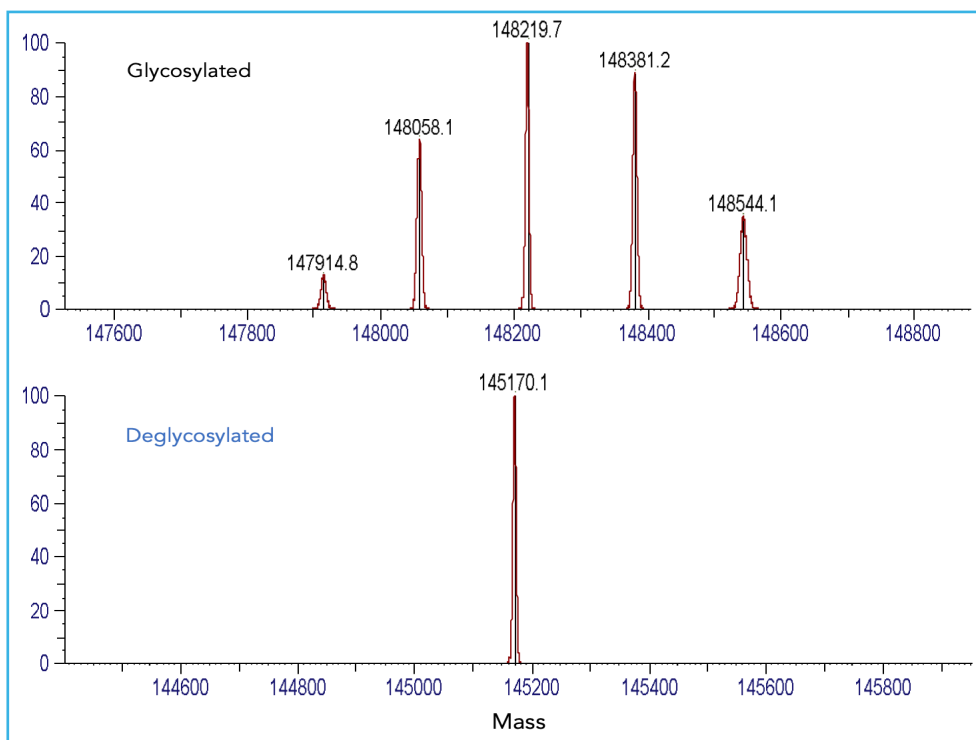
**MS Parameters:** Positive ion mode, ESI at +4.0 kV, 225 °C capillary







### 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 <sup>1</sup>	M <sup>1</sup>	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

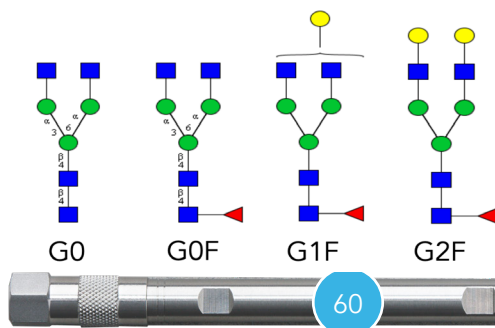
T = Theoretical Mass

M = Measured Mass

<sup>1</sup> All masses reported in Daltons

#### Glycan Structures:

-  Fucose
-  N-Acetylglucosamine
-  Galactose
-  Mannose



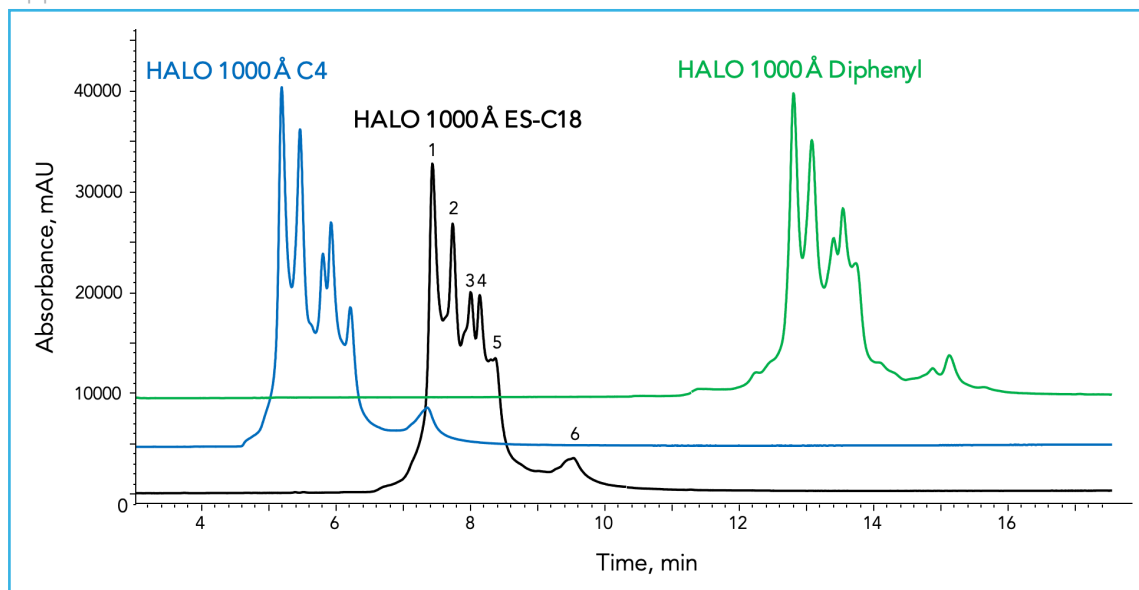
#### Deconvolution Parameters:

**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<sup>®</sup> 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  $\mu$ m, 2.1 x 150 mm  
**Part Number:** 92712-714
- 2) HALO 1000 Å ES-C18, 2.7  $\mu$ m, 2.1 x 150 mm  
**Part Number:** 92712-702
- 3) HALO 1000 Å Diphenyl, 2.7  $\mu$ m, 2.1 x 150 mm  
**Part Number:** 92712-726

#### Mobile Phase:

- A: 2/10/88 n-propanol/ACN/H<sub>2</sub>O + 0.1% DFA
- B: 70/20/10 n-propanol/ACN/H<sub>2</sub>O + 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  $\mu$ L of 2 mg/mL denosumab

**Sample Solvent:** Water (0.1% TFA)

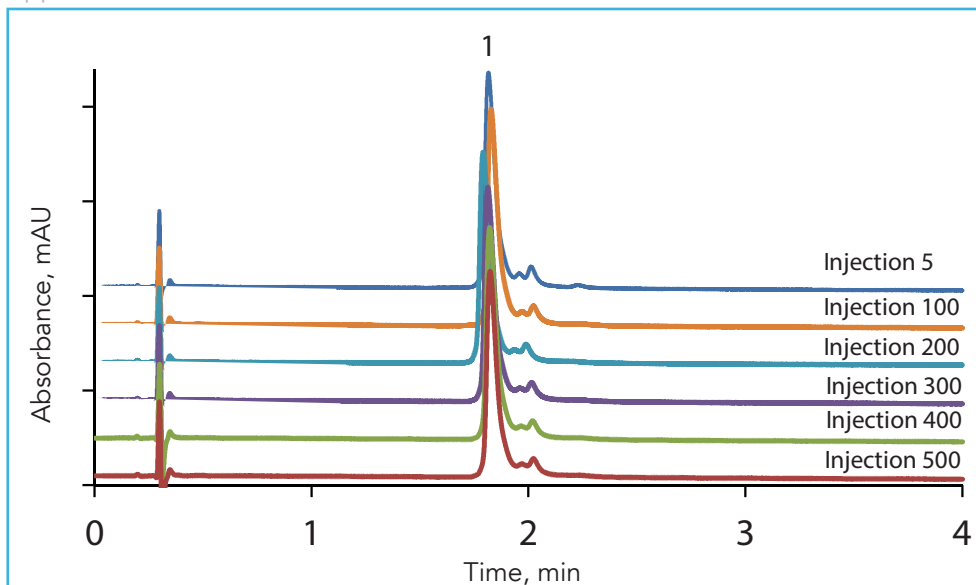
**LC System:** Shimadzu Nexera





## 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

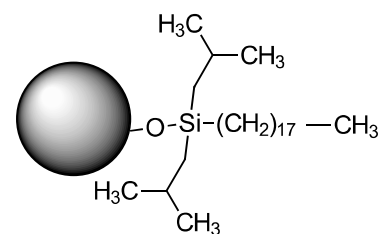
**Flow Cell:** 1.0 µL

**LC System:** Shimadzu Nexera X2

### STRUCTURES:



1000 Å 2.7µm particle



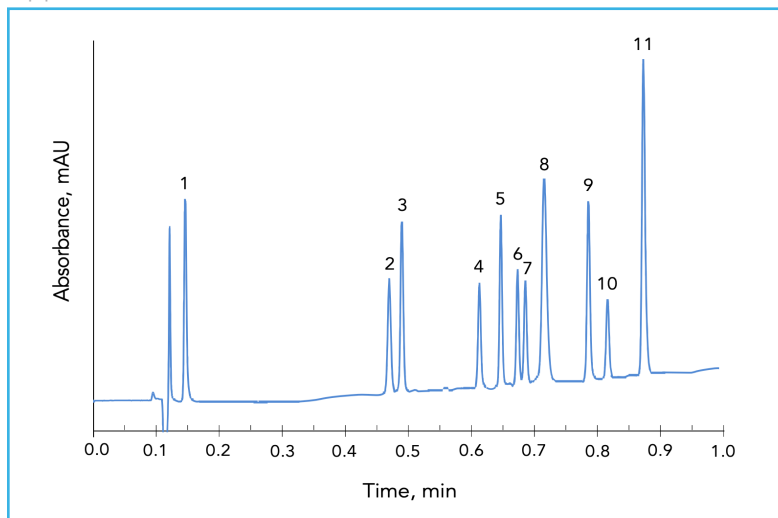
ES-C18 bonded phase





## 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

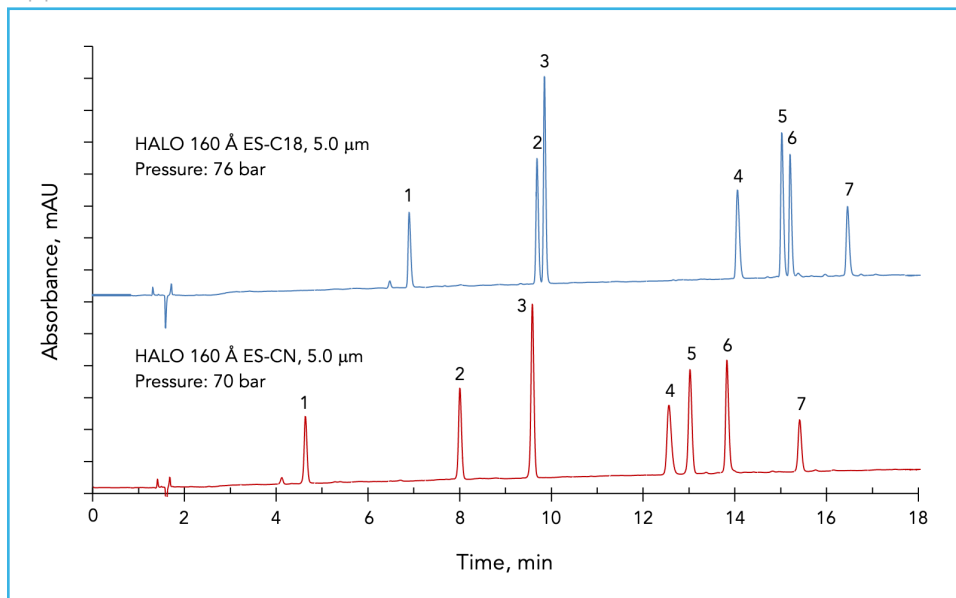






## Separation of Seven Peptides on HALO® 5 µm 160 Å ES-C18 and ES-CN Phases

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

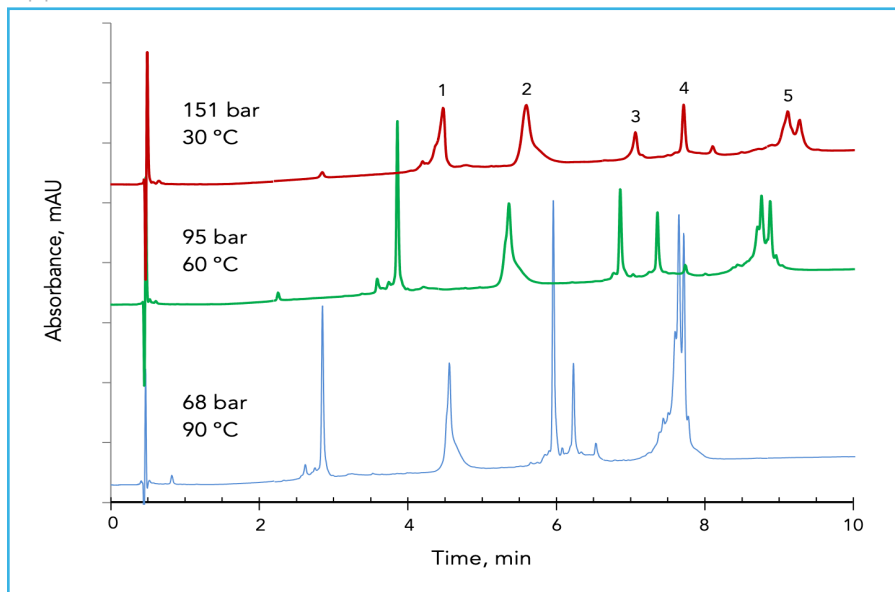
**LC System:** Agilent 1100 Quaternary





## 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.  $\alpha$ -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  $\mu$ m, 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  $\mu$ m,  
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  $\mu$ L

**Flow Rate:** 0.45 mL/min

**Pressure:** See chart

**Temperature:** See chart

**Detection:** UV 215 nm, PDA

**Injection Volume:** 2.0  $\mu$ L

**Sample Solvent:** Mobile phase A

**Response Time:** 1.0 sec

**Flow Cell:** 2.0  $\mu$ L micro cell

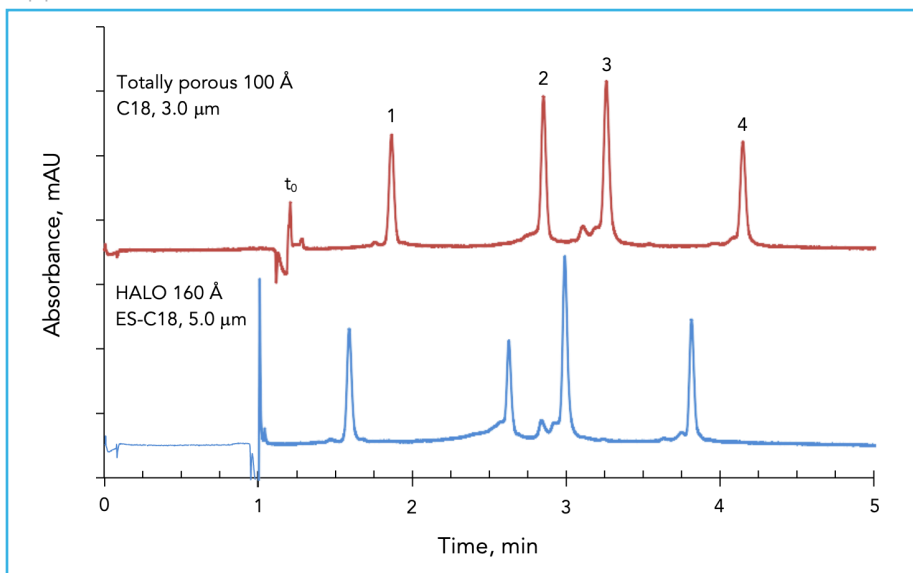
**LC System:** Agilent 1200 SL





## 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.  $\alpha$ -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

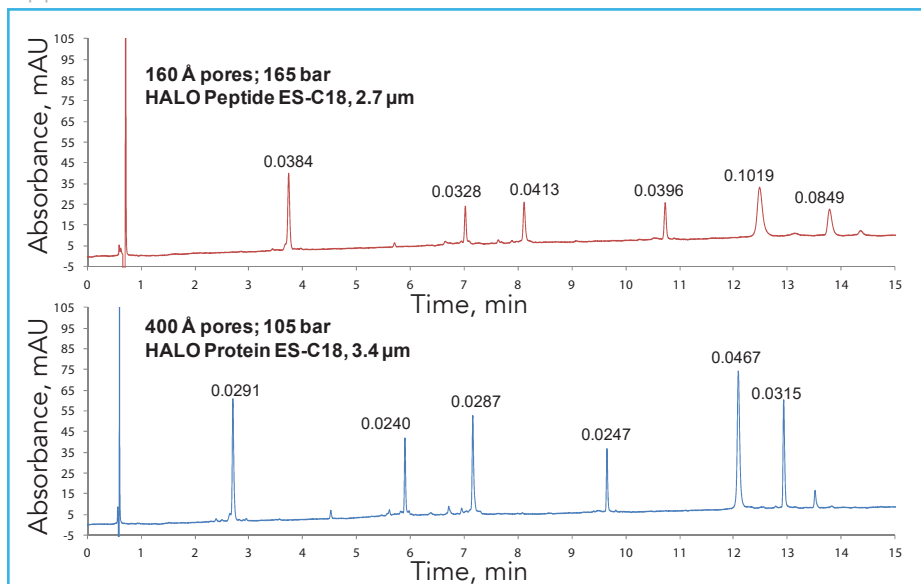
**LC System:** Agilent 1200 SL





## 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.  $\alpha$ -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  $\mu$ m, 4.6 x 100 mm  
**Part Number:** 92124-602
- 2) HALO 400 Å ES-C18, 3.4  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** Mobile phase A

**Response Time:** 0.12 sec

**Flow Cell:** 5.0  $\mu$ L semi-micro

**Data Rate:** 14 Hz

**LC System:** Agilent 1100 Quaternary

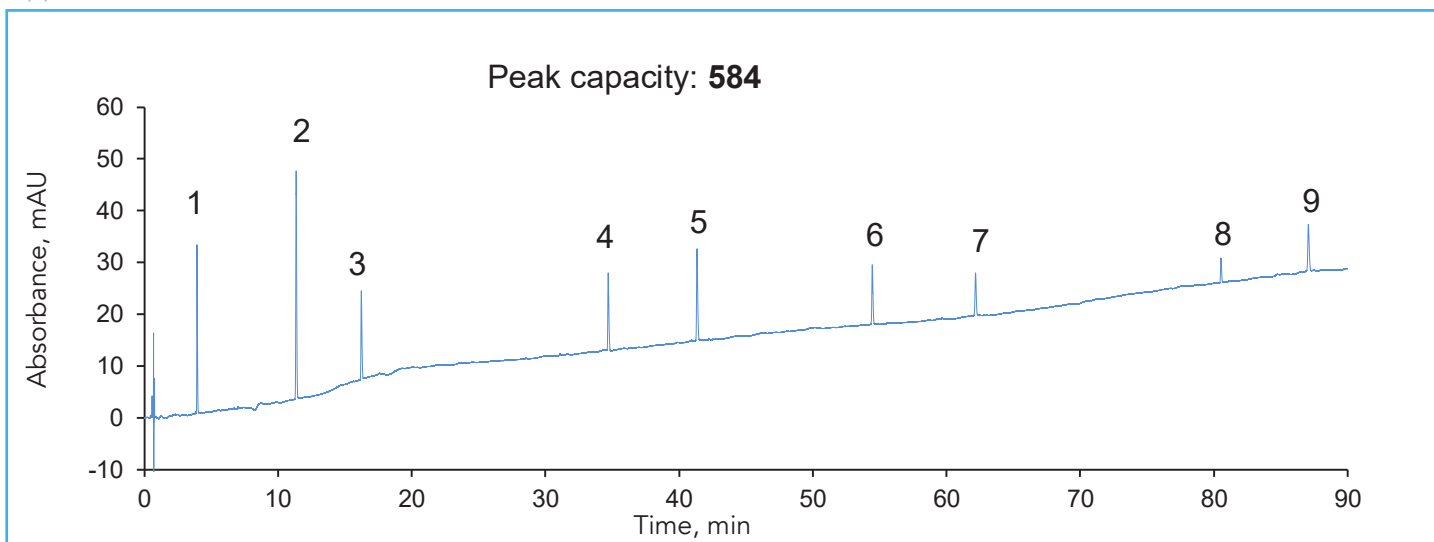






## Very High Peak Capacity with HALO 160 Å ES-C18, 2.0 µm

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. β-endorphin	3465
8. Sauvagine	4599
9. Mellitin	2847

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

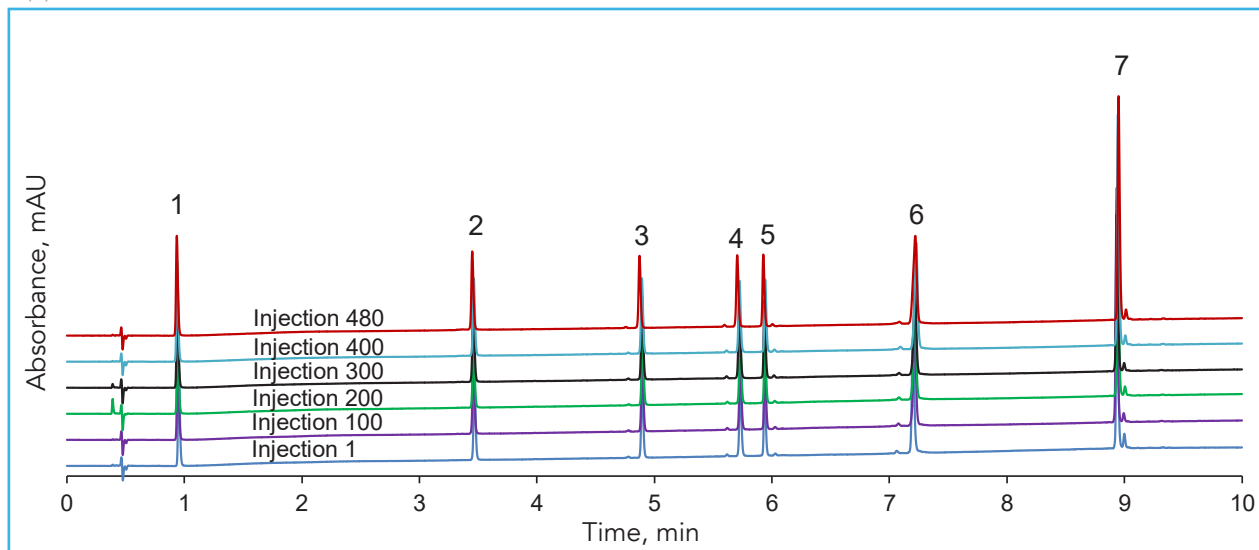
where  $t_i$  is the time for initial measurable peak in the gradient,  $t_f$  is the time for final peak and  $W_{4\sigma}$  is the average four-sigma width in time for the peaks in the chromatogram





## 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

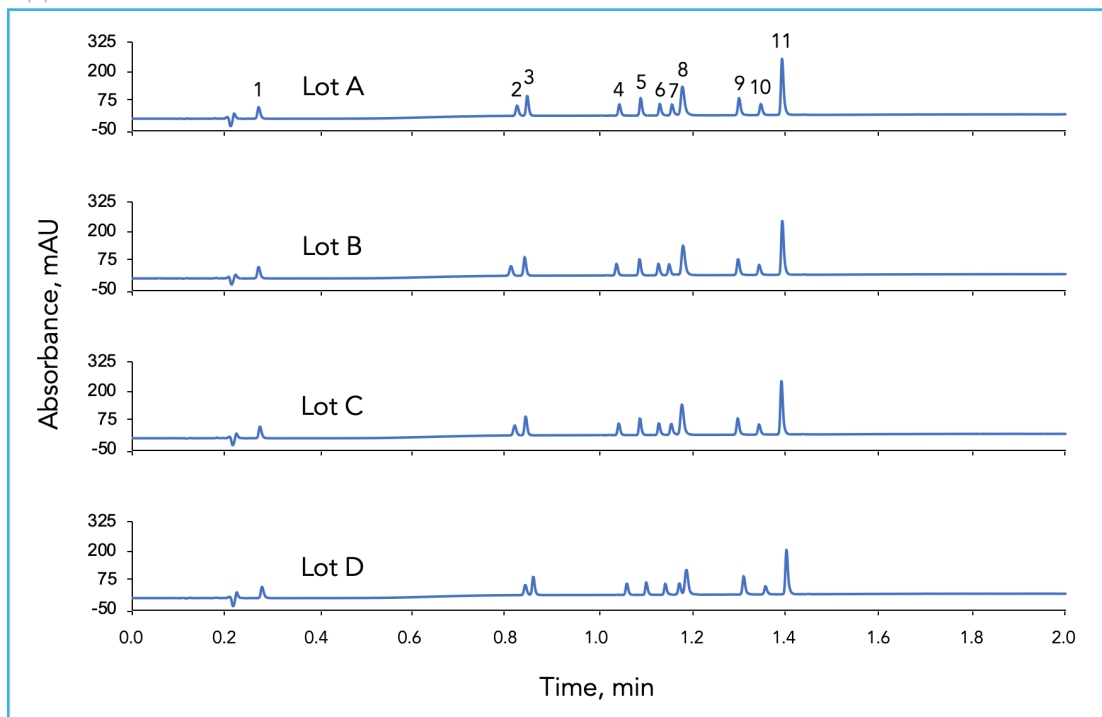
**LC System:** Shimadzu Nexera X2





### 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:

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

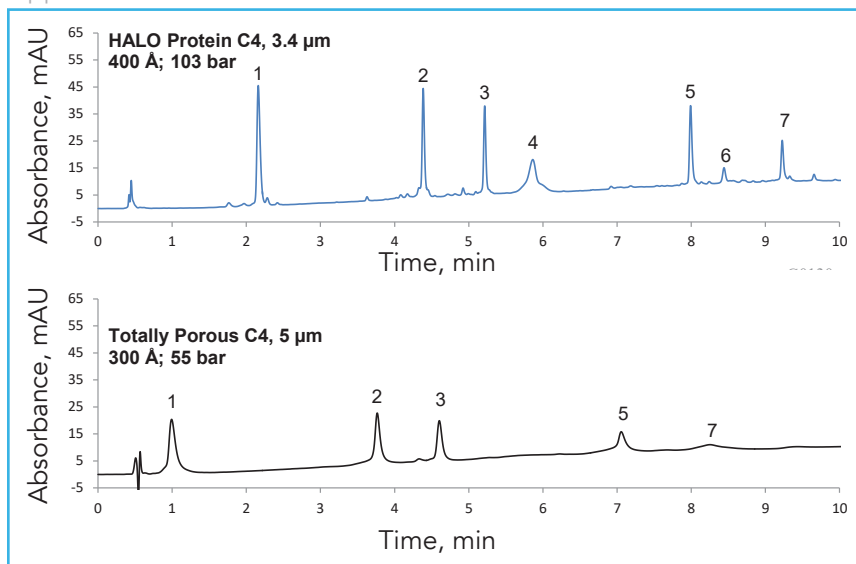
MW (g/mol)	% RSD (retention times)
238	1.21
380	1.59
898	0.95
574	0.92
1045	0.60
1046	0.61
556	0.82
13,700	0.35
1573	0.46
5733	0.49
1509	0.36





## 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

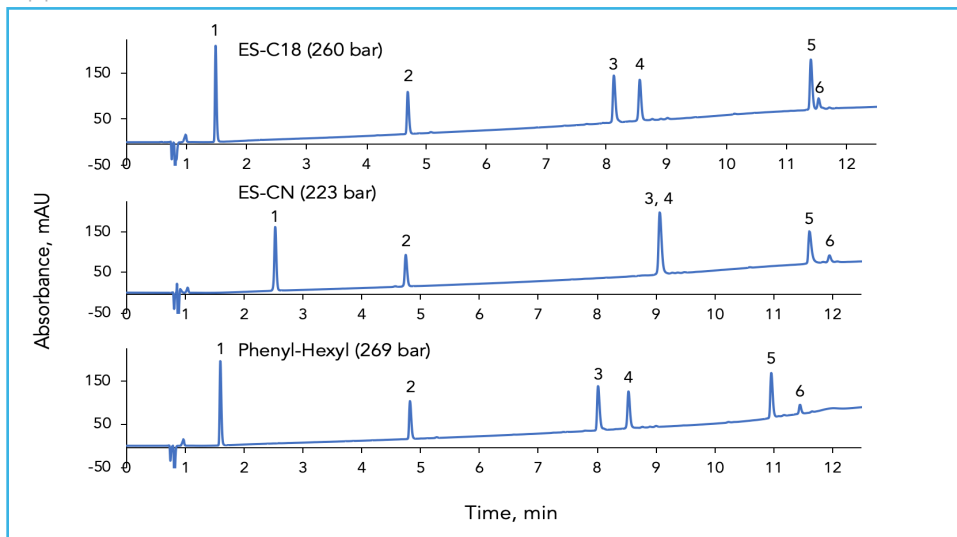






## Enhanced Selectivity for the Separation of Peptides Comparing HALO 160 Å with Three Different Bonded Phases

Application Note 159-PE



### PEAK IDENTITIES:

1. Tyr-Tyr-Tyr
2. Angiotensin II
3. Angiotensin 1-12
4. Melittin
5. Sauvagine
6.  $\beta$ -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  $\mu$ m, 2.1 x 150 mm  
**Part Number:** 92122-702
- 2) HALO 160 Å ES-CN, 2.7  $\mu$ m, 2.1 x 150 mm  
**Part Number:** 92122-704
- 3) HALO 160 Å Phenyl-Hexyl, 2.7  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** Water, 0.1% TFA

**Response Time:** 0.24 sec

**Data Rate:** 12.5 Hz

**Flow Cell:** 1.0  $\mu$ L

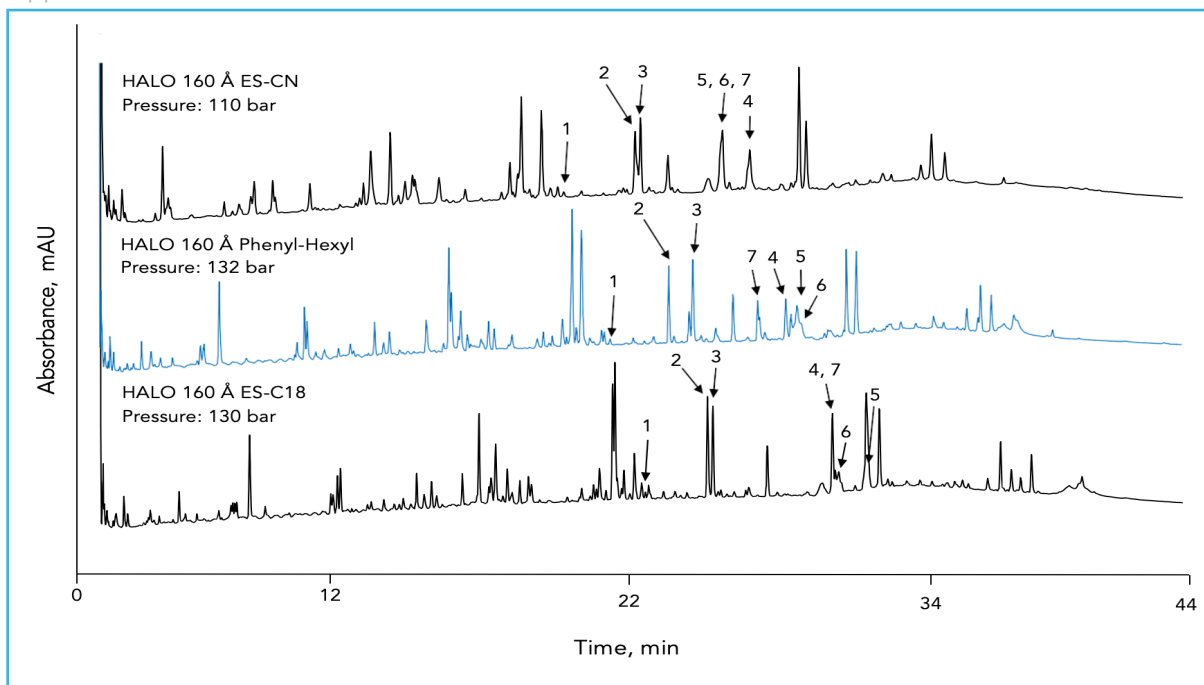
**LC System:** Shimadzu Nexera





## 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  $\mu$ m, 2.1 x 100 mm  
**Part Number:** 92122-604
- 2) HALO 160 Å Phenyl-Hexyl, 2.7  $\mu$ m, 2.1 x 100 mm  
**Part Number:** 92112-606
- 3) HALO 160 Å ES-C18, 2.7  $\mu$ m, 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  $\mu$ L 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  $\mu$ L semi-micro

**LC System:** Shimadzu Nexera

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

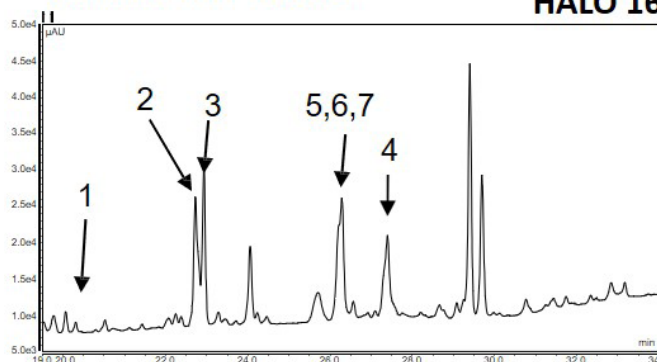
1. FTISADTSKNTAYLQMNSLR (754 m/z)
2. LScAASGFNIKDTYIHWVR (747 m/z)
3. GFYPSDIAVEWESNGQPENNYK (849 m/z)
4. LLIYSASFLYSGVPSR (592 m/z)
5. SGTASVcLLNNFYPR (899 m/z)
6. ScDKTHTcPPcPAPELLGGPSVFLFPPKPK (834 m/z)
7. VSVLTVLHQDWLNGKEYK (1115 m/z)

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.



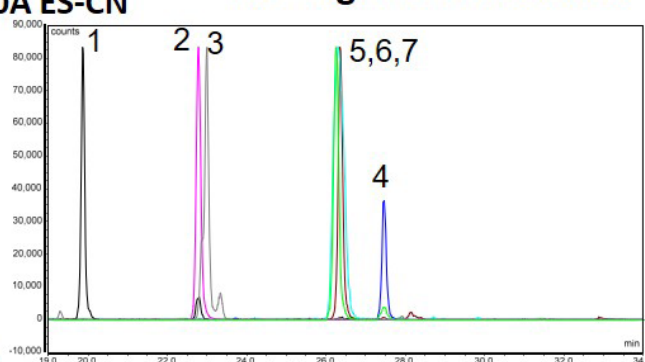


### UV 220 nm traces

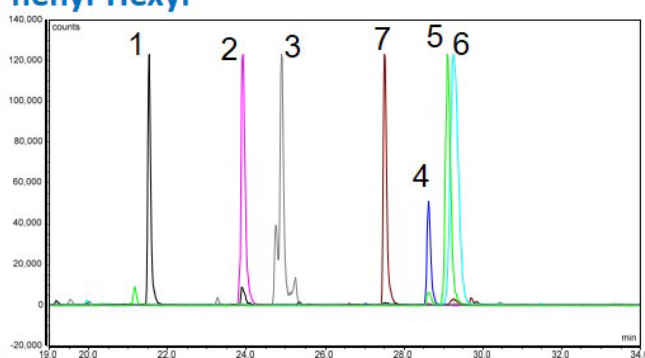
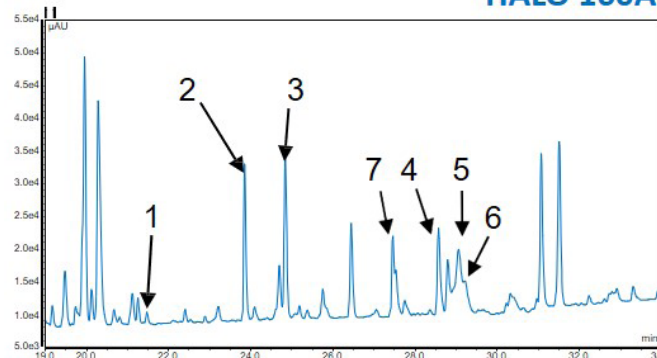


### HALO 160Å ES-CN

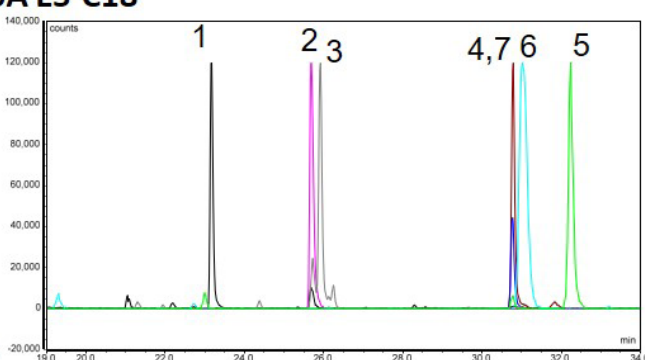
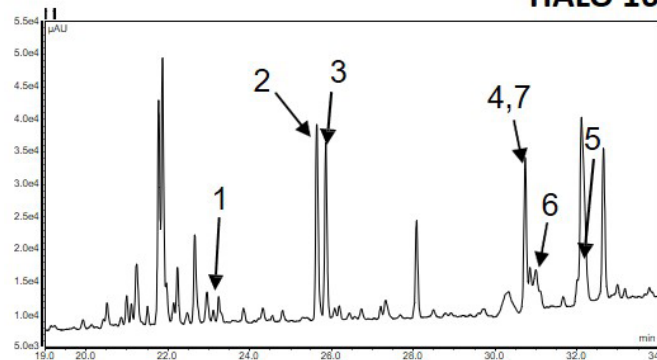
### EIC signals normalized



### 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.

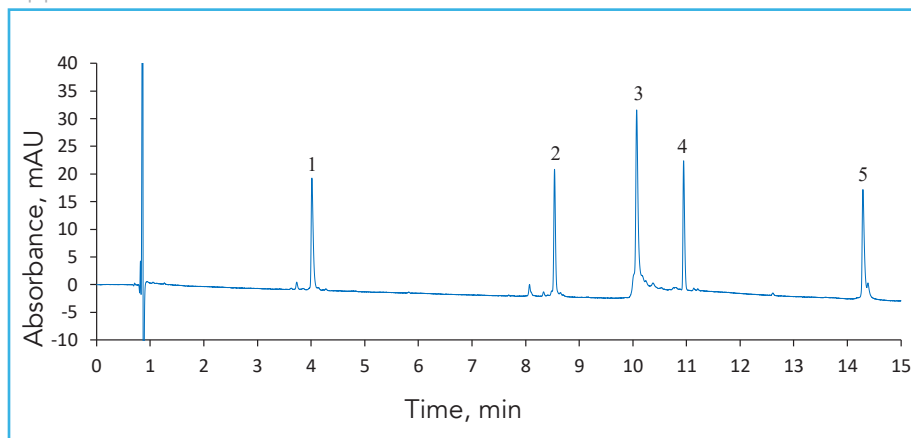
**MS System:** Thermo Fisher Orbitrap VelosPro ETD  
**ESI:** +3.5 kV  
**Scan Range:** 50-2000 m/z  
**Scan Rate:** 2 pps  
**Capillary:** 225 °C  
**Sheath Gas:** 35  
**Auxiliary Gas:** 10  
**Scan Time:** 2 µscans/200 ms max inject time





## 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

<b>Gradient:</b>	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



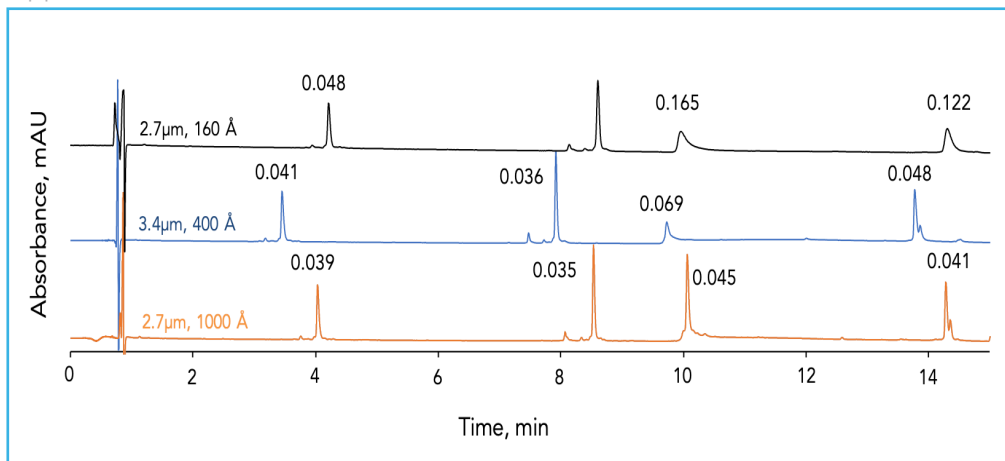
75





## 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 15 kDa. The 400 Å pore size is recommended for molecules between 2 kDa 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)

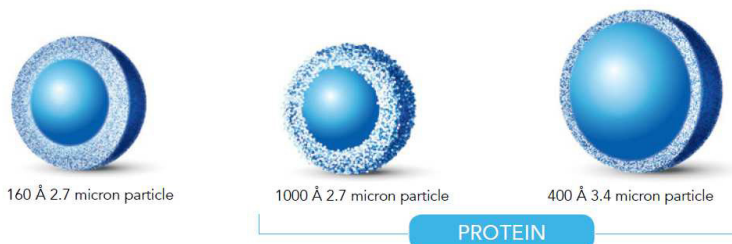
**Response Time:** 0.025 sec

**Data Rate:** 40 Hz

**Flow Cell:** 1.0 μL

**LC System:** Shimadzu Nexera X2

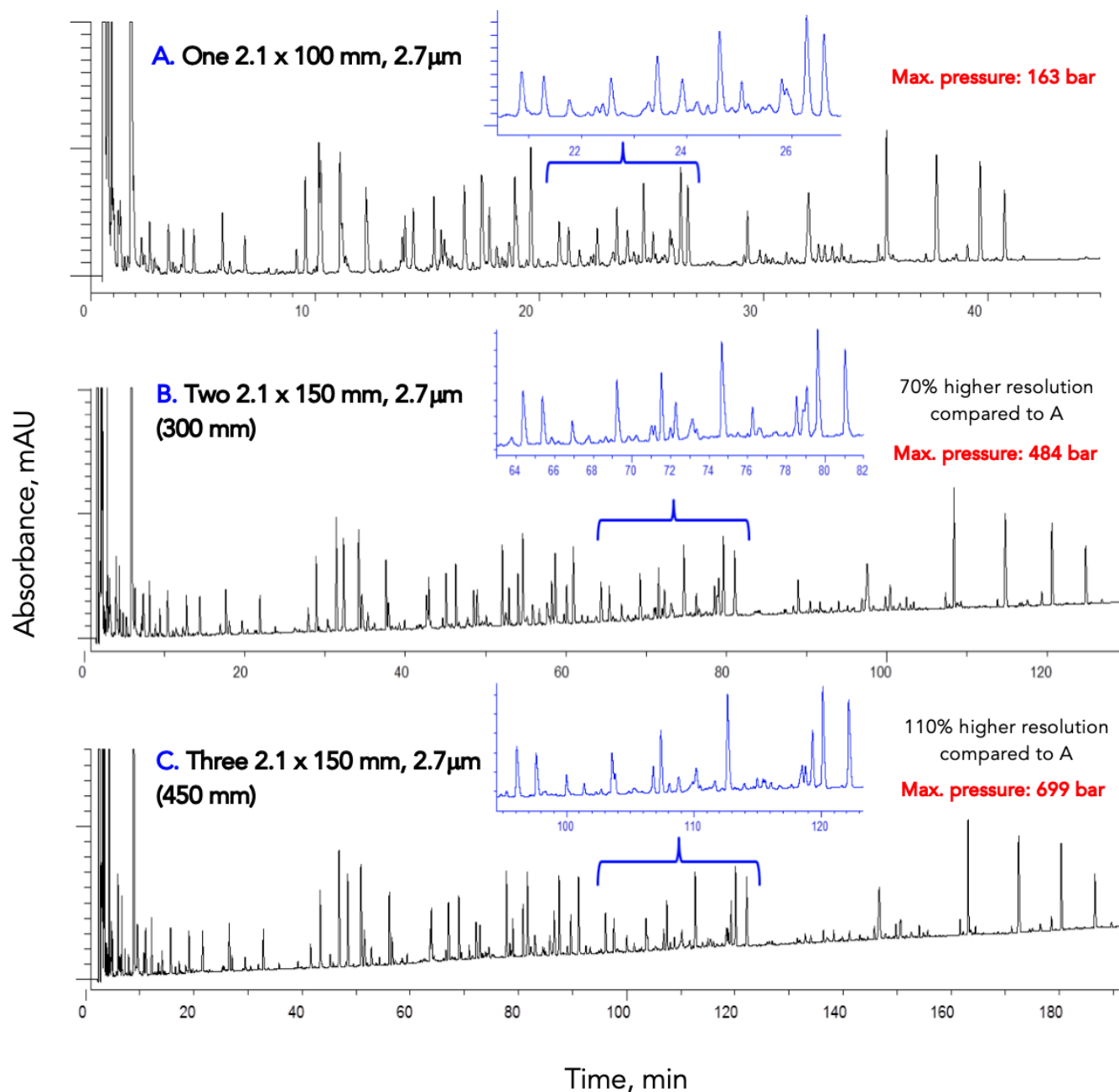
### STRUCTURES:





## Analysis of Apotransferrin Tryptic Digest on HALO® 160 Å Columns

Application Note 179-PE



77



### 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

<b>Gradient A:</b>	Time (min)	% B
	0.0	5
	60	60

<b>Gradient B:</b>	Time (min)	% B
	0.0	5
	180	60

<b>Gradient C:</b>	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.

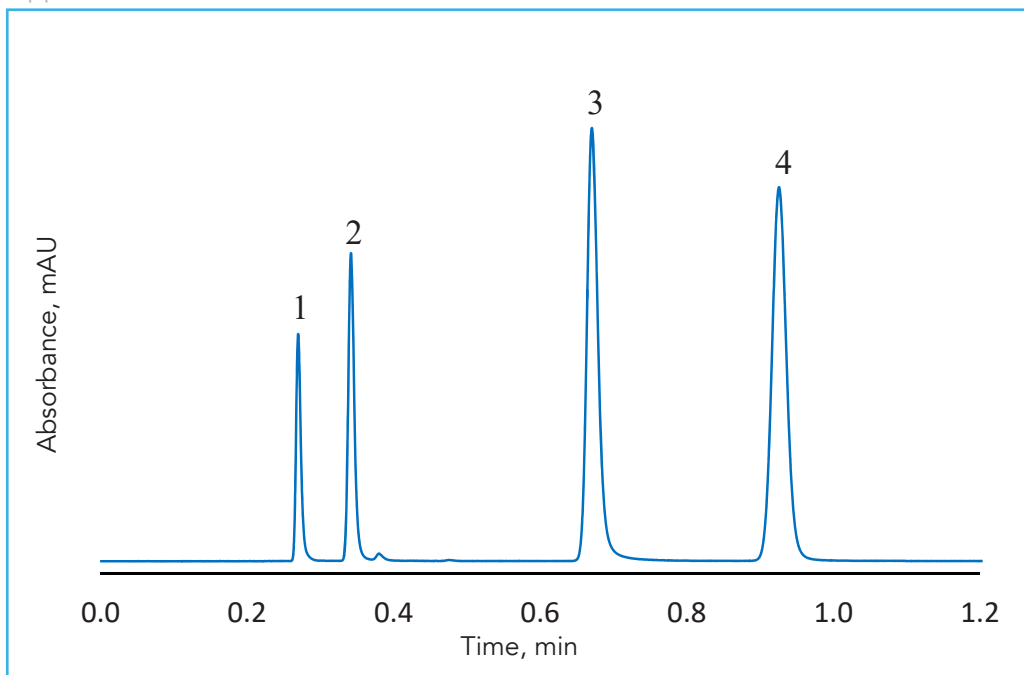
Lower pressures afforded by both 2.7 and 5 µm HALO® Peptide particles allow two or more columns to be used in series for additional resolution and peak capacity for challenging peptide mapping analyses. HALO® 160 Å ES-C18 is also available in 2.0 µm particle sizes in 2.1 and 3 mm IDs up to 150 mm length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

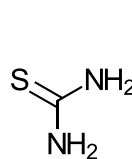
**Response Time:** 0.05 sec

**Flow Cell:** 1.0 µL

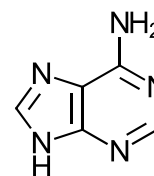
**Acquisition Rate:** 100 Hz

**LC System:** Shimadzu Nexera X2

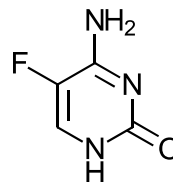
## STRUCTURES:



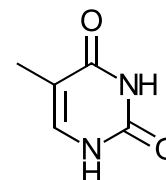
Thiourea



Adenine



5-Fluorocytosine



Thymine

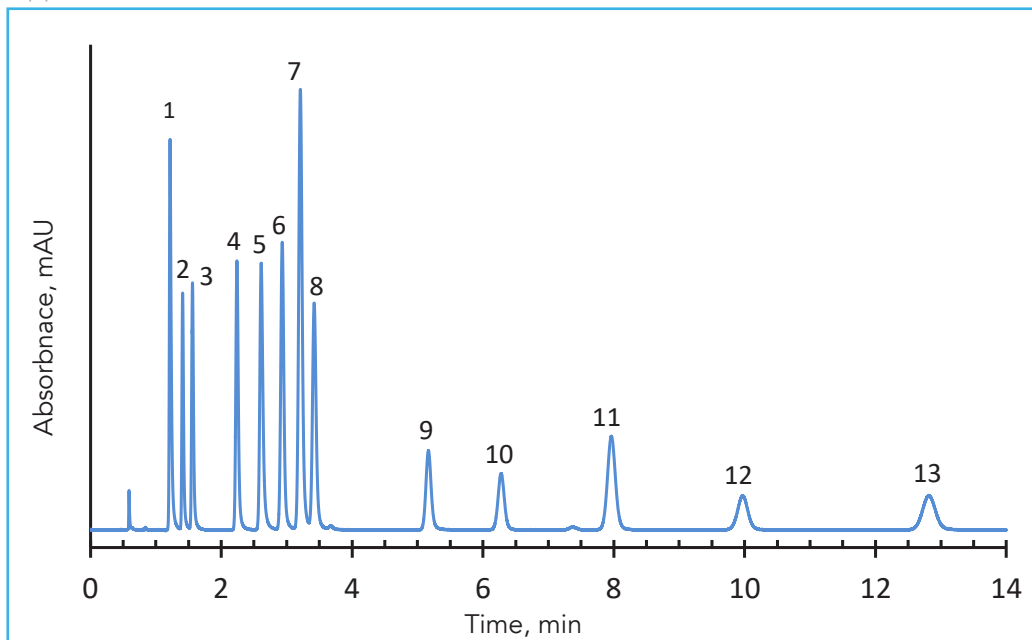






## Separation of Nucleosides and Nucleobases on 2.7 µm HALO® Penta-HILIC

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 hydroxyl-rich 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:



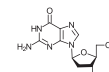
Thymine



Adenine



Hypoxanthine



2'-Deoxyguanosine



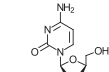
Uracil



Uridine



Cytosine



Cytidine



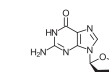
Thymidine



Adenosine



2'-Deoxycytidine



Guanosine



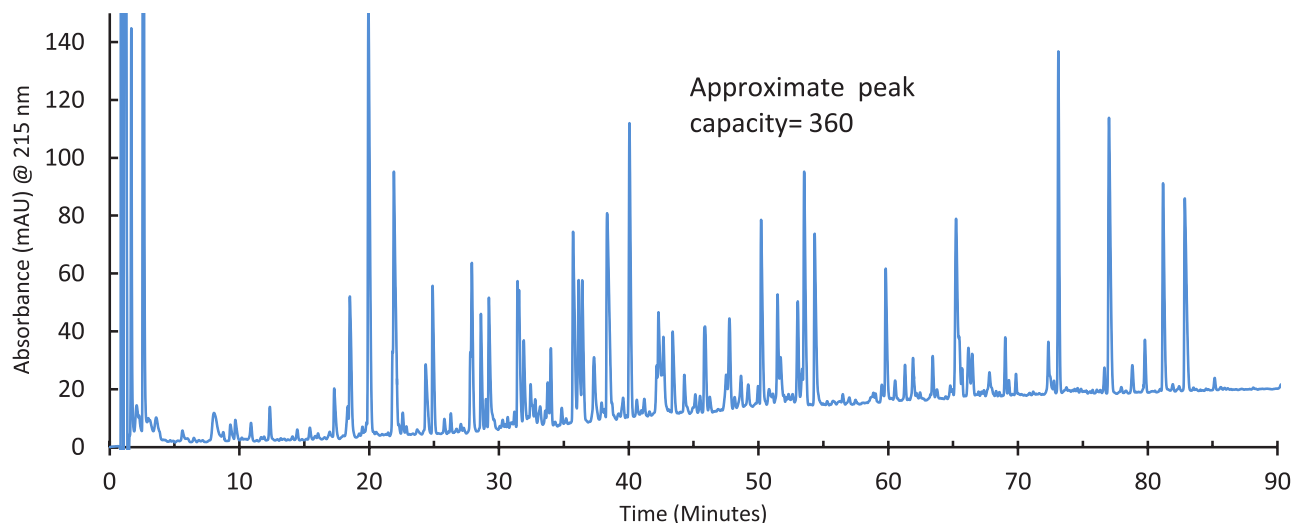
2'-Deoxyadenosine





## 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  $\mu\text{m}$  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  $\mu\text{m}$ ,  
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  $\mu\text{L}$

**Sample Solvent:** Mobile phase A

**Response Time:** 0.1 sec

**Data Rate:** 40 Hz

**Flow Cell:** 2.0  $\mu\text{L}$  micro cell

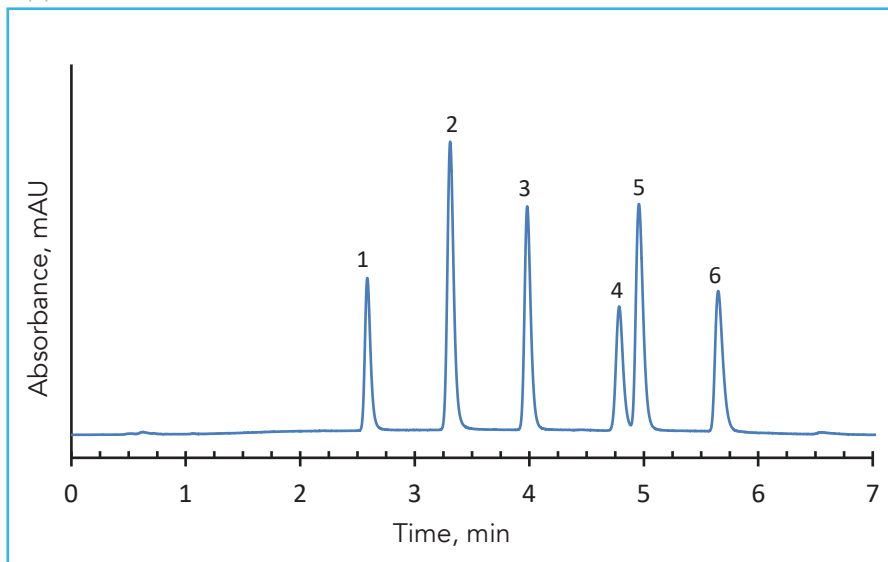
**LC System:** Agilent 1200 SL





## 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

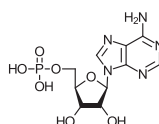
**Response Time:** 0.02 sec

**Data Rate:** 40 Hz

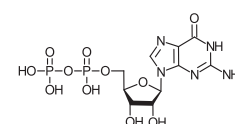
**Flow Cell:** 1.0 µL micro cell

**LC System:** Shimadzu Nexera

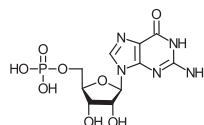
### STRUCTURES:



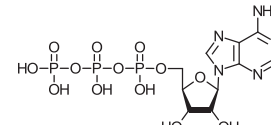
Adenosine Monophosphate



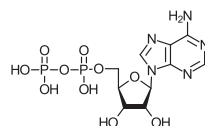
Guanosine Diphosphate



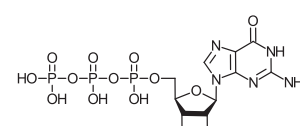
Guanosine Monophosphate



Adenosine Triphosphate



Adenosine Diphosphate



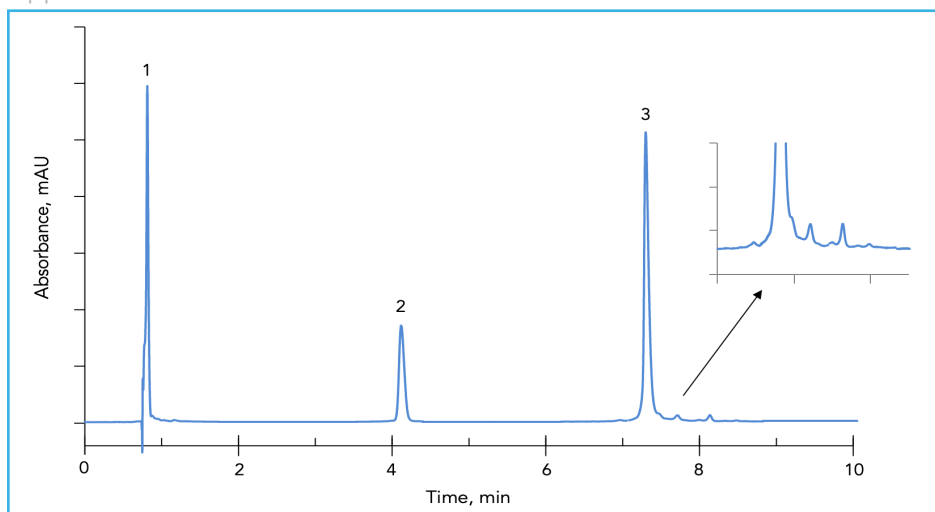
Guanosine Triphosphate





## 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



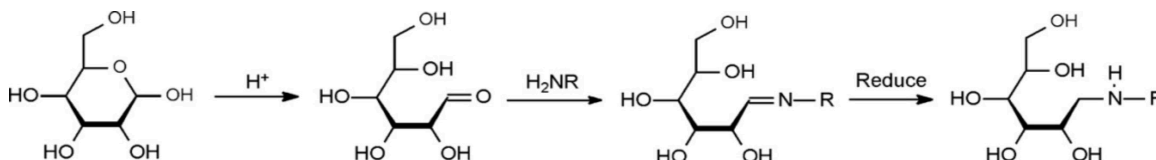




## Separation of PNGase-Released and Labeled N-Glycans by HILIC Using HALO® Glycan Column

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

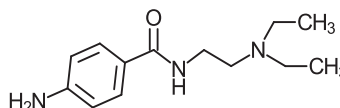
**Response Time:** 0.5 sec

**Data Rate:** 3.3 Hz

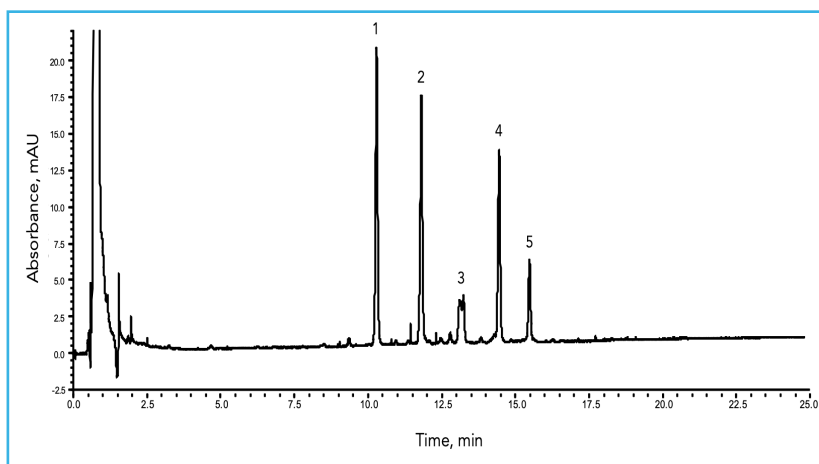
**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Nexera

### STRUCTURE:



Procainamide (PAm)



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

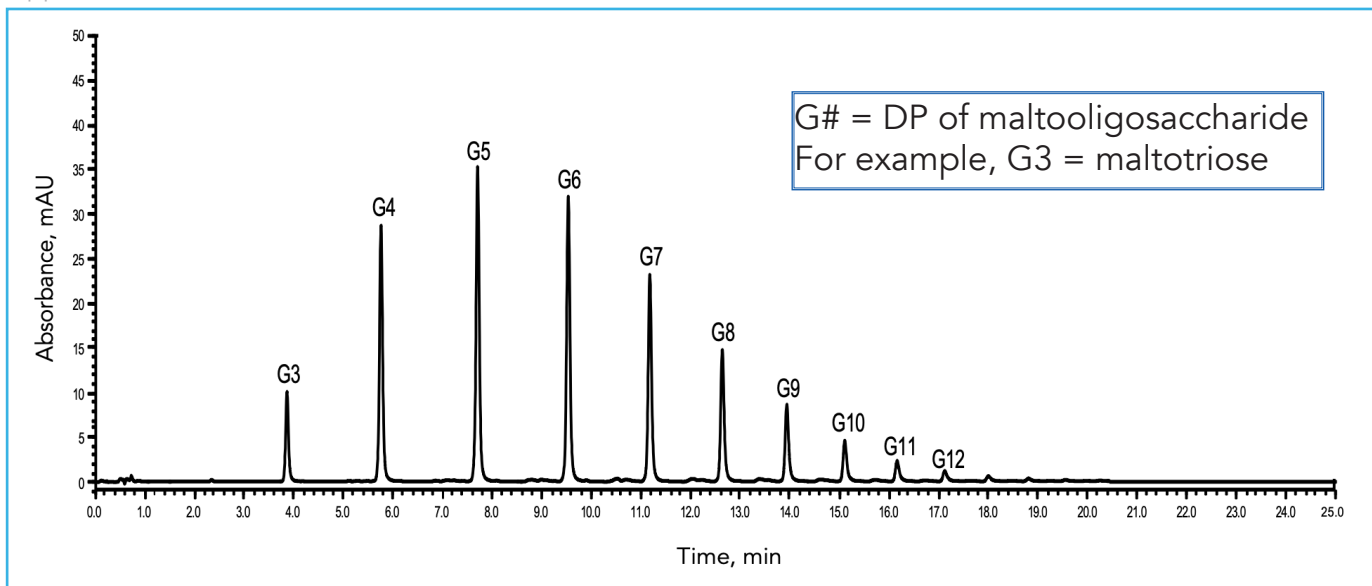
- PEAK IDENTITIES:**
1. PAm-GlcNAc<sub>2</sub>Man<sub>5</sub>
  2. PAm-GlcNAc<sub>2</sub>Man<sub>6</sub>
  3. PAm-GlcNAc<sub>2</sub>Man<sub>7</sub>
  4. PAm-GlcNAc<sub>2</sub>Man<sub>8</sub>
  5. PAm-GlcNAc<sub>2</sub>Man<sub>9</sub>





## 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

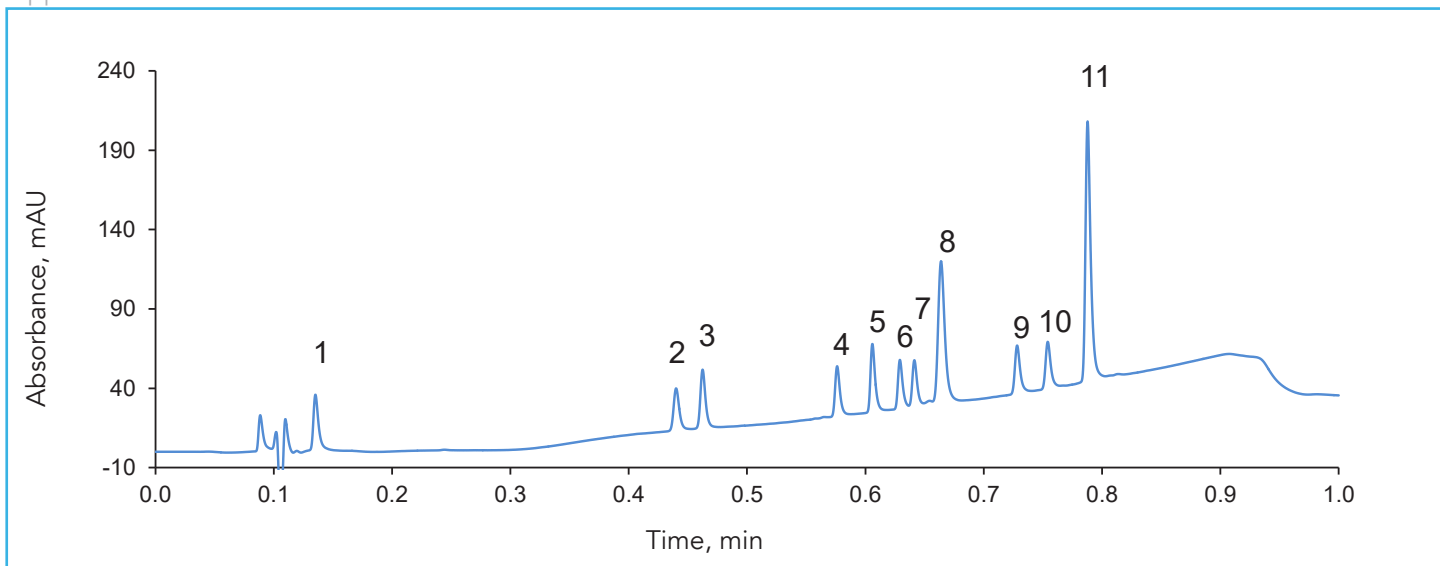
**LC System:** Shimadzu Nexera





## 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 throughput.

### 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:

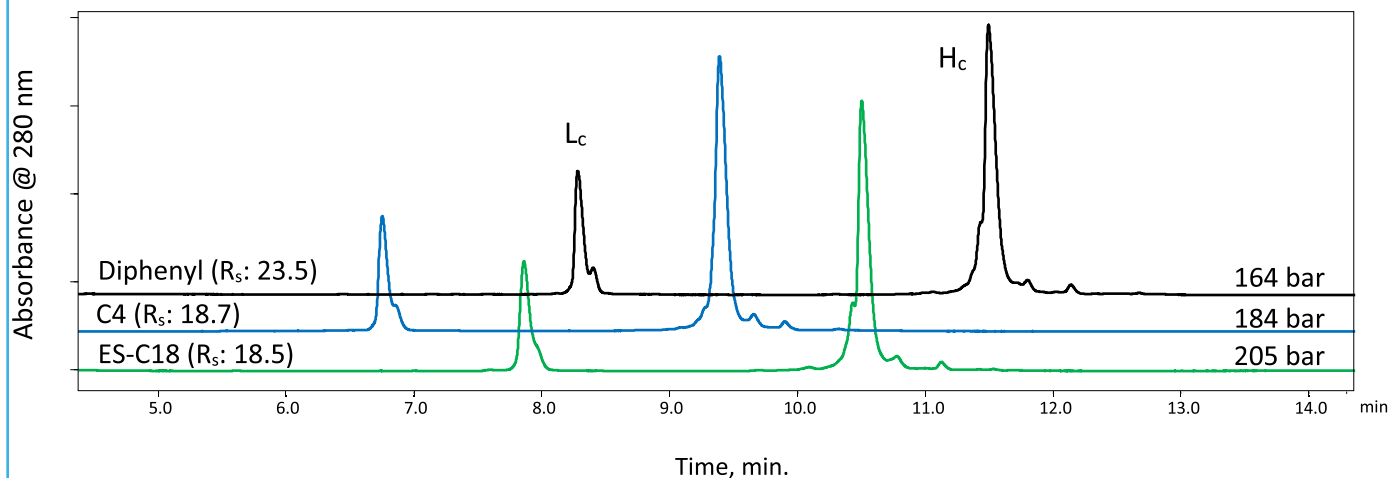
	MW (g/mol):
1. Gly-Tyr	238
2. Val-Tyr-Val	380
3. Angiotensin 1/2 (1-7) amide	898
4. Met-enkephalin	574
5. Angiotensin 1/2 (1-8) amide	1045
6. Angiotensin II	1046
7. Leu-enkephalin	556
8. Ribonuclease A	13,700
9. Angiotensin (1-12) (mouse)	1573
10. Bovine insulin	5733
11. Angiotensin (1-12) (human)	1509





## 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 access, 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

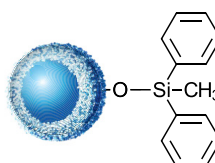
**Data Rate:** 12.5 Hz

**Response Time:** 0.25 sec.

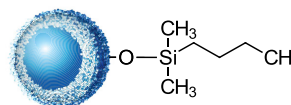
**Flow Cell:** 1 µL

**LC System:** Shimadzu Nexera X2

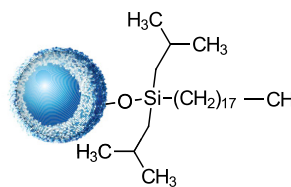
### STRUCTURES:



HALO 1000 Å Diphenyl



HALO 1000 Å C4



HALO 1000 Å ES-C18

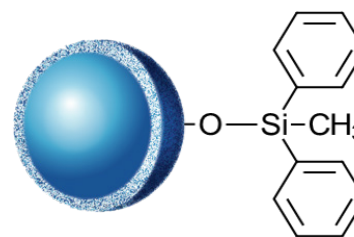
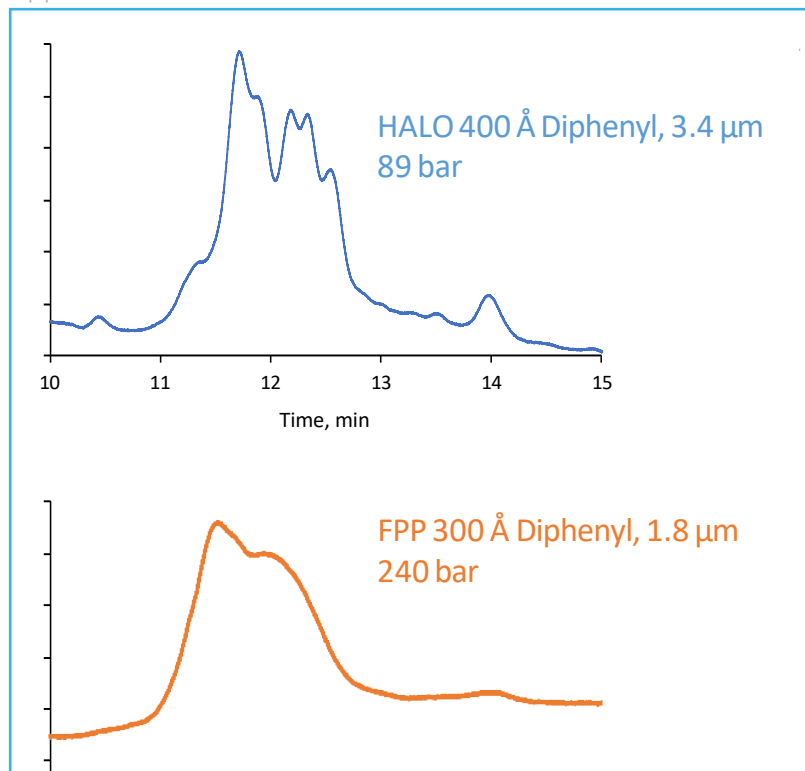






## Increased Resolution with HALO 400 Å Diphenyl Compared to FPP 300 Å Diphenyl

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

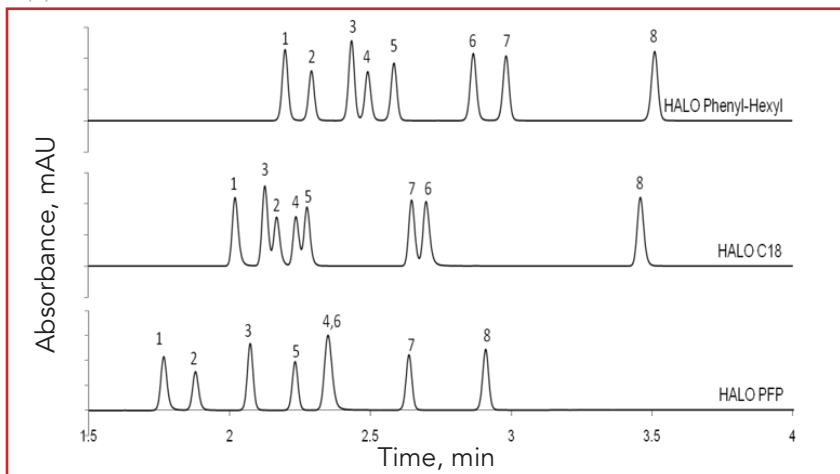
\*\*nProp = n- propanol





## 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  $\mu$ m, 4.6 x 50 mm

**Part Number:** 92814-406

- 2) HALO 90 Å C18, 2.7  $\mu$ m, 4.6 x 50 mm

**Part Number:** 92814-402

- 3) HALO 90 Å PFP, 2.7  $\mu$ m, 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  $\mu$ L

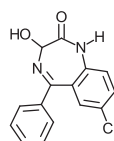
**Sample Solvent:** Standard diluted with acetonitrile and buffer

**Response Time:** < 0.12 sec

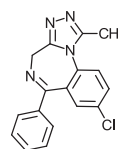
**Flow Cell:** 5.0  $\mu$ L semi-micro

**LC System:** Agilent 1100

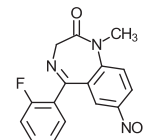
### STRUCTURES:



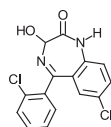
Oxazepam



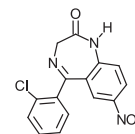
Alprazolam



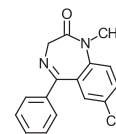
Flunitrazepam



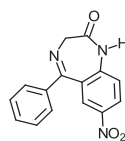
Lorazepam



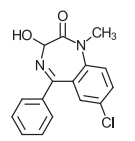
Clonazepam



Diazepam



Nitrazepam



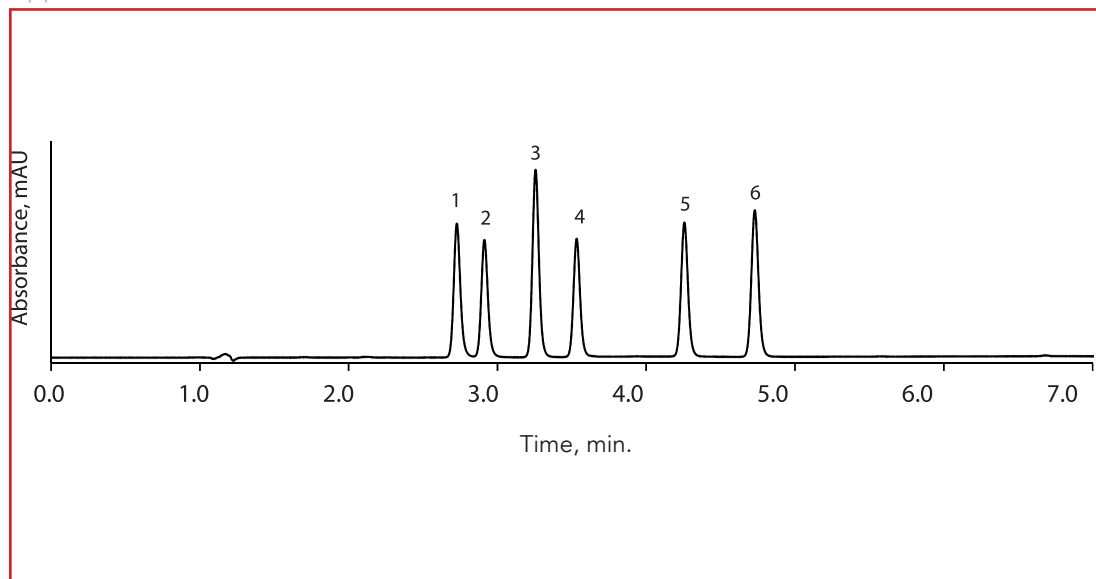
Temazepam





## 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

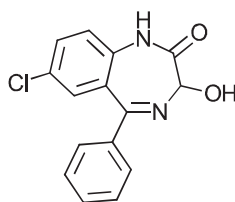
**Injection Volume:** 1.0 µL

**Response Time:** < 0.12 sec

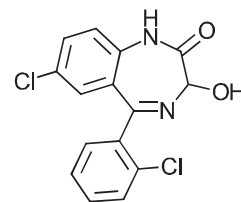
**Flow Cell:** 5.0 µL semi-micro

**LC System:** Agilent 1100

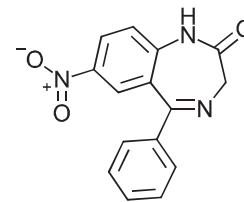
### STRUCTURES:



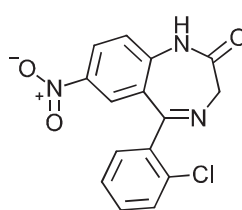
Oxazepam



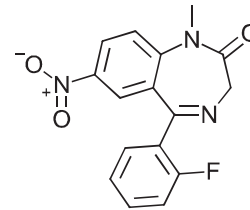
Lorazepam



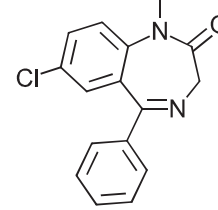
Nitrazepam



Clonazepam



Flunitrazepam



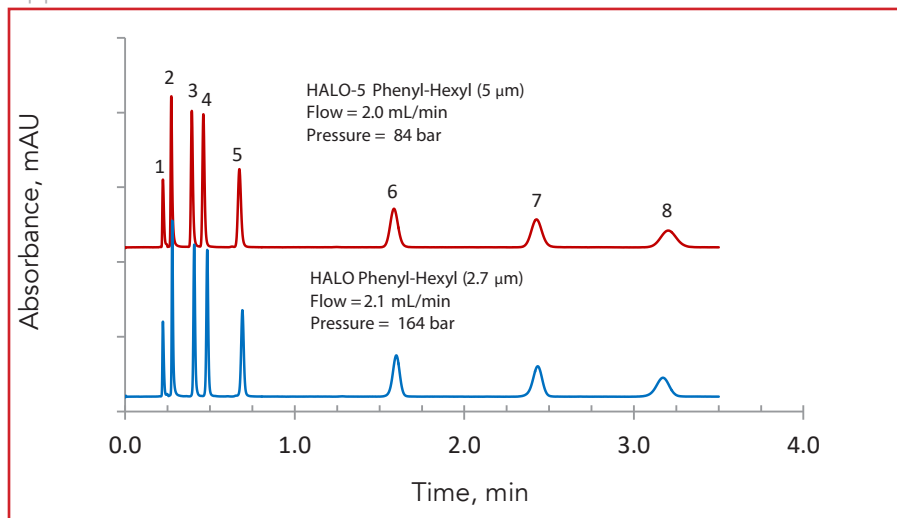
Diazepam





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

Application Note 82-HA



### PEAK IDENTITIES:

1. Uracil ( $t_R$ )
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

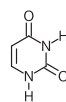
#### Sample Solvent: 30/70 water (0.1% formic acid)/ methanol

#### Response Time: 0.12 sec

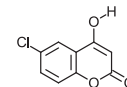
#### Flow Cell: 5.0 µL

#### LC System: Agilent 1100

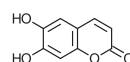
### STRUCTURES:



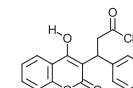
Uracil



6-Chloro-4-hydroxycoumarin



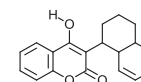
6,7-Dihydroxycoumarin



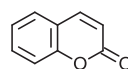
Warfarin



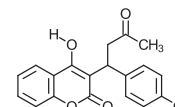
4-Hydroxycoumarin



Coumatetralyl



Coumarin



Coumachlor

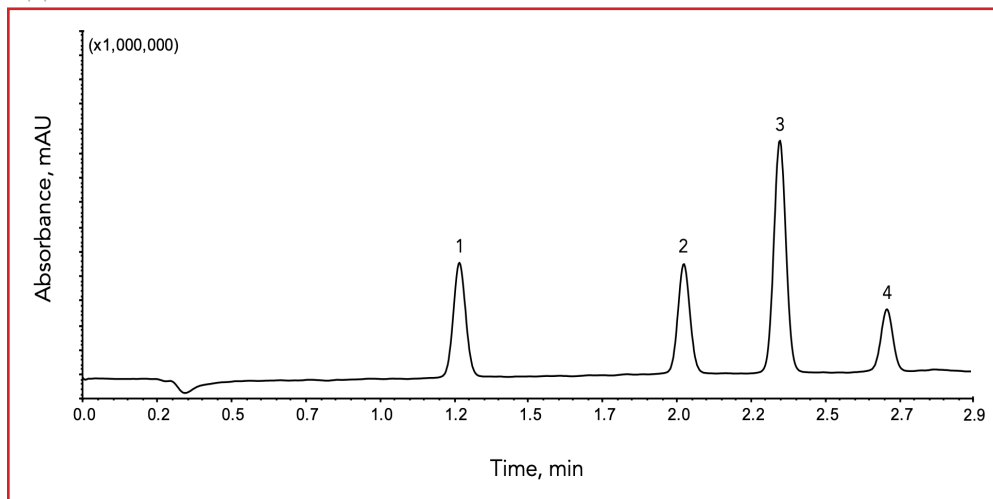






## LC-MS Separation of Fentanyl and Analogues in Synthetic Urine

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

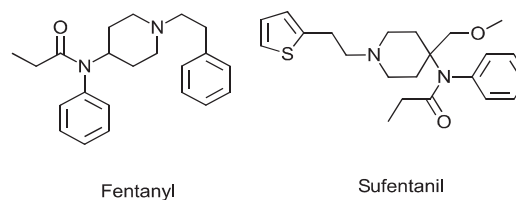
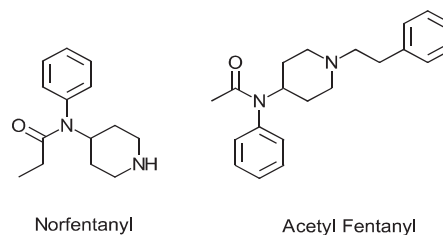
**MS System:** Shimadzu LCMS 2020 (single quadrupole)

**ESI:** 4.5 kV

**Heat Block:** 300 °C

**Nebulizing Gas Flow:** 1.3 L/min

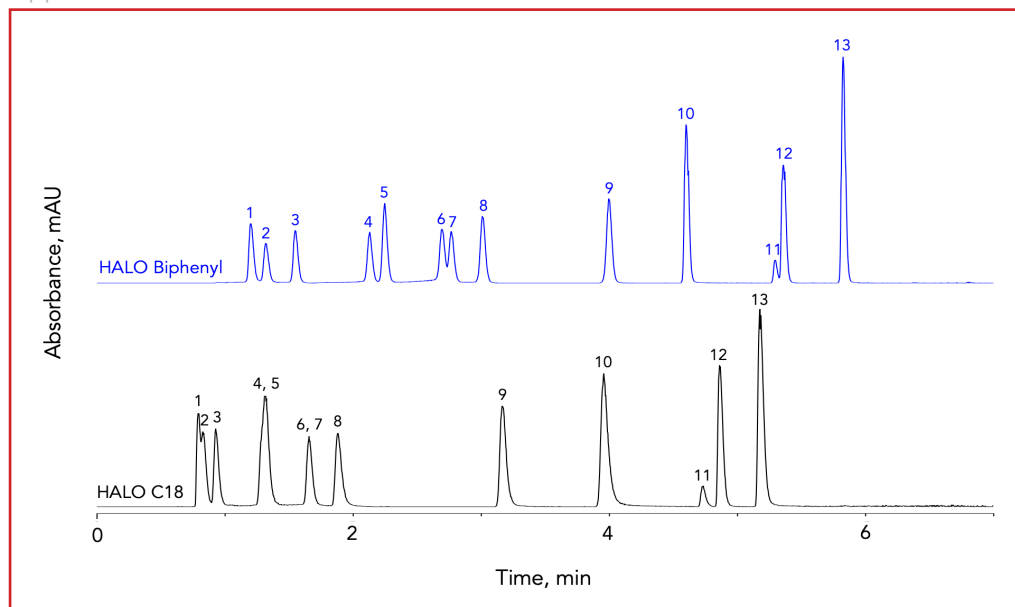
### STRUCTURES:





## 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  $\mu$ m, 2.1 x 100 mm

**Part Number:** 92812-611

2) HALO 90 Å C18, 2.7  $\mu$ m, 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  $\mu$ L

**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

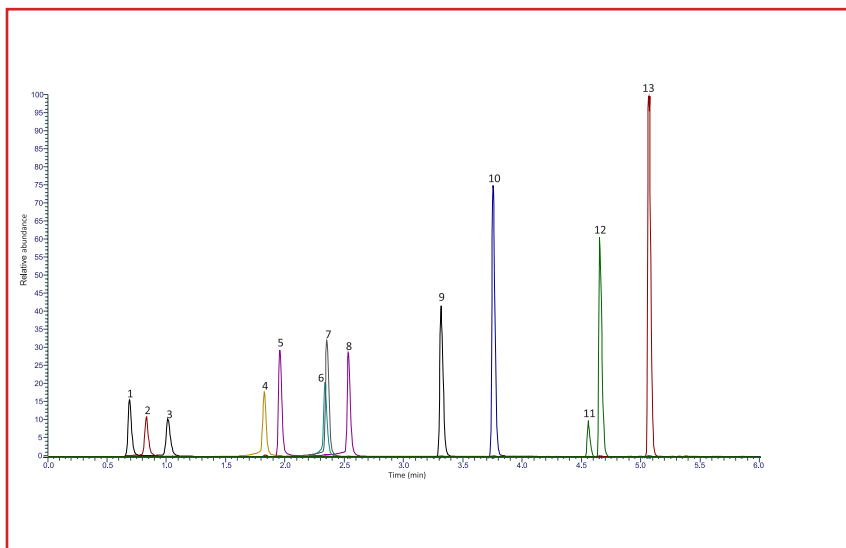
**Octopole RF:** 250 V





## 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

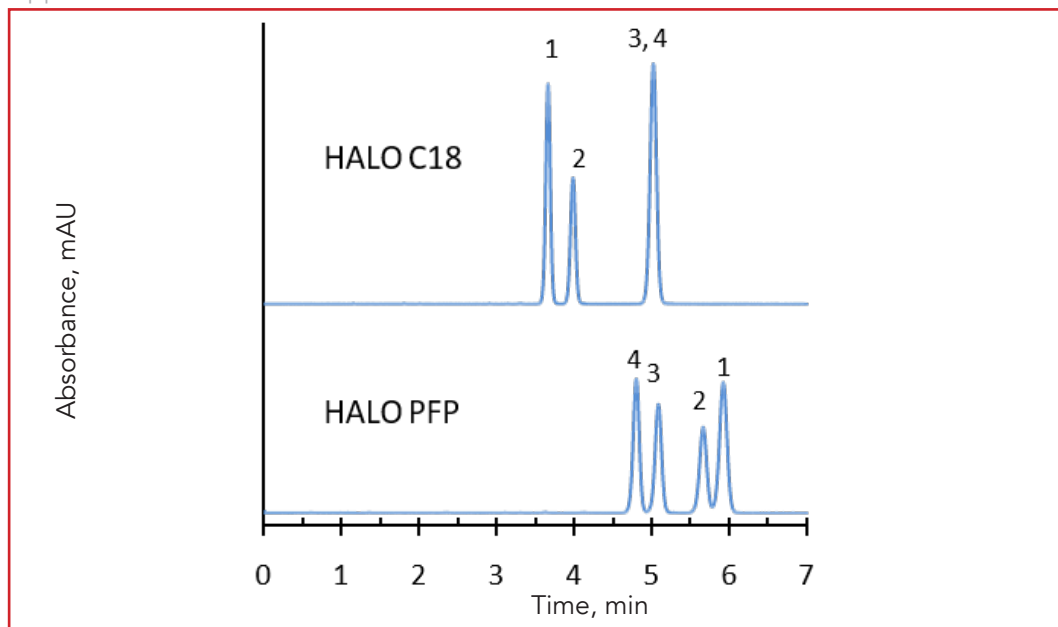
**LC System:** Shimadzu Nexera X2





## 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  $\mu$ m, 4.6 x 100 mm

**Part Number:** 92814-602

2) HALO 90 Å PFP, 2.7  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** 80% methanol in water

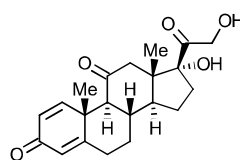
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

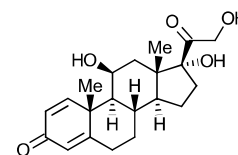
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu$ L

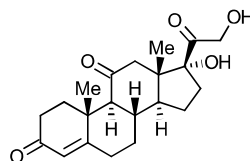
### STRUCTURES:



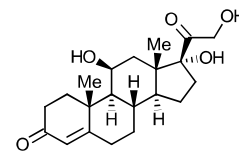
Prednisone



Prednisolone



Cortisone



Hydrocortisone

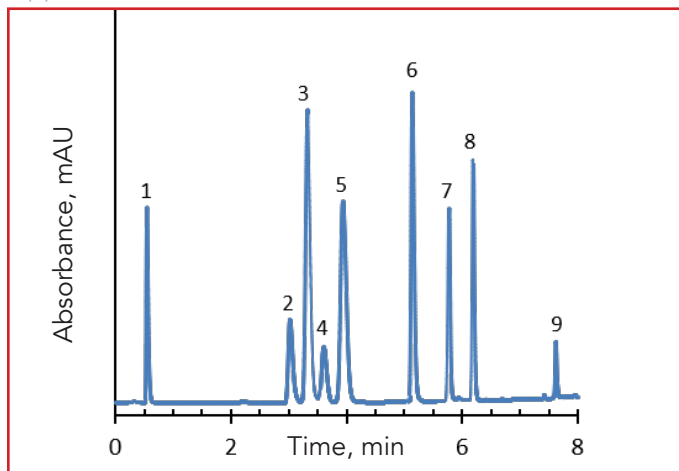






### Separation of Steroids on HALO® PFP, 2.0 µm

Application Note 116-STR



#### PEAK IDENTITIES:

1. Uracil
2. Hydrocortisone
3. Prednisolone
4. Cortisone
5. Prednisone
6. Dexamethasone
7.  $\beta$ -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

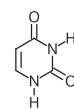
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

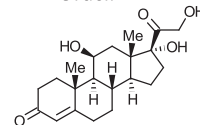
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

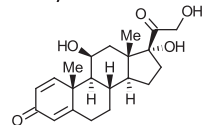
#### STRUCTURES:



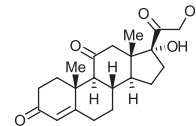
Uracil



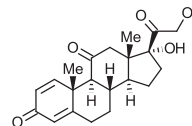
Hydrocortisone



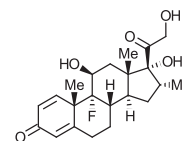
Prednisolone



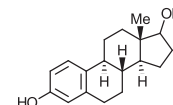
Cortisone



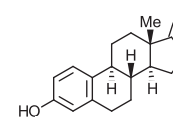
Prednisone



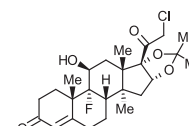
Dexamethasone



$\beta$ -Estradiol



Estrone



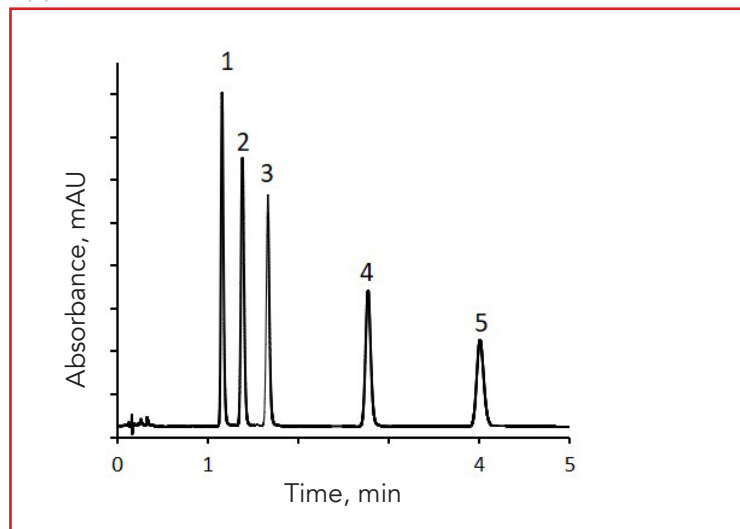
Halcinonide





## 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)

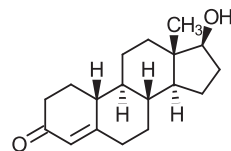
**Response Time:** 0.02 sec

**Flow Cell:** 2.0 µL micro cell

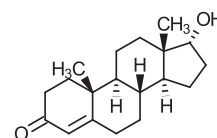
**Data Rate:** 80 Hz

**LC System:** Agilent 1200 SL

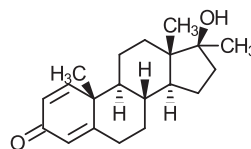
### STRUCTURES:



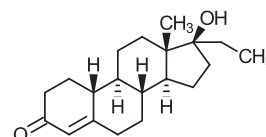
Nandrolone



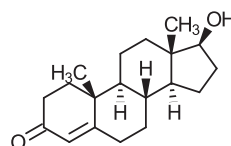
Epitestosterone



Methandienone



Norethandrolone



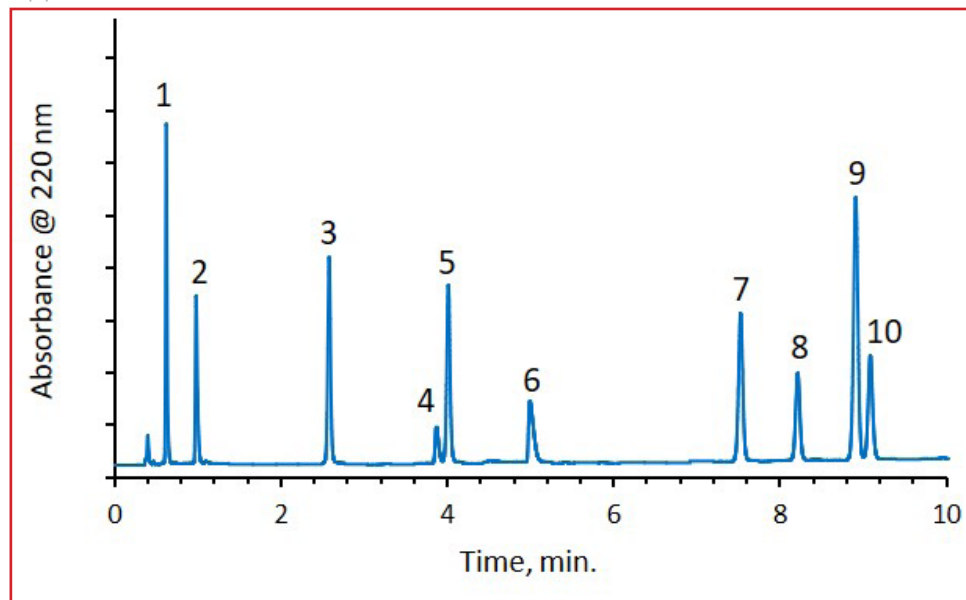
Testosterone





### Separation of Steroid Hormones and Hormone Conjugates on HALO® C18

Application Note 142-STR



#### PEAK IDENTITIES:

1. Estriol-3-( $\beta$ -D-glucuronide)
2. Estriol-3-Sulfate
3. Estrone-3-( $\beta$ -D-glucuronide)
4.  $\beta$ -Estradiol-3-Sulfate
5. Estriol
6. Estrone-3-Sulfate
7.  $\beta$ -Estradiol
8.  $\alpha$ -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  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** 84/16 water/acetonitrile

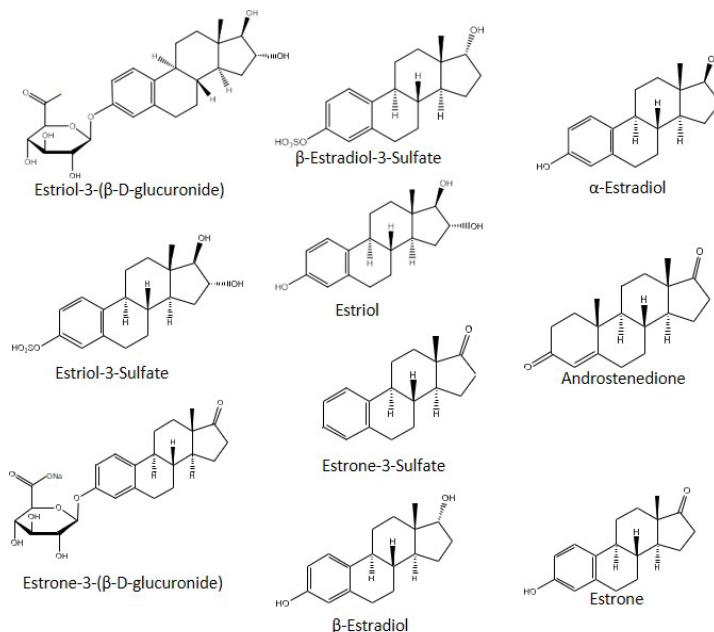
**Response Time:** 0.05 sec

**Data Rate:** 40 Hz

**Flow Cell:** 1.0  $\mu$ L

**LC System:** Shimadzu Nexera X2

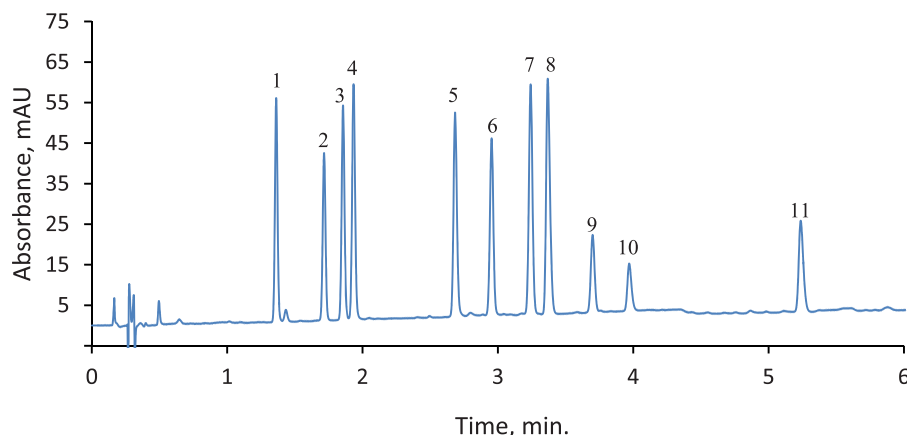
#### STRUCTURES:





## 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.  $\beta$ -Estradiol
7. Cortisone Acetate
8. Testosterone
9. 17- $\alpha$ -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  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** 37.5/62.5 acetonitrile/water

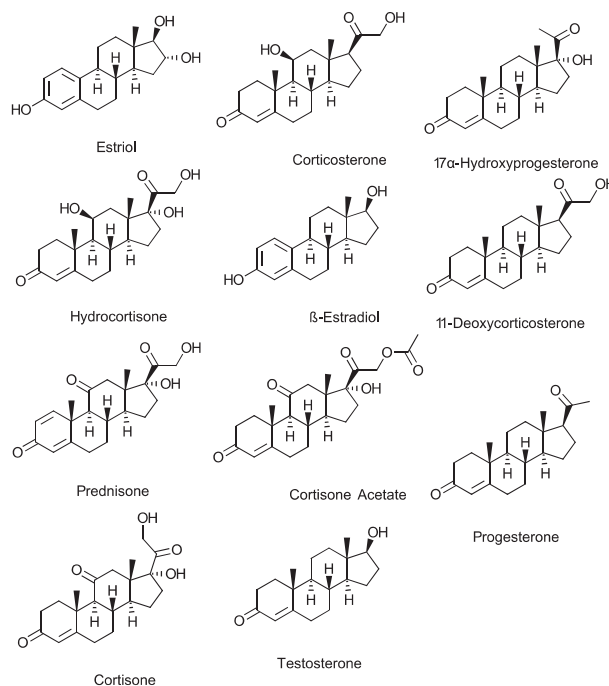
**Response Time:** 0.025 sec

**Data Rate:** 100 Hz

**Flow Cell:** 1.0  $\mu$ L

**LC System:** Shimadzu Nexera X2

### STRUCTURES:

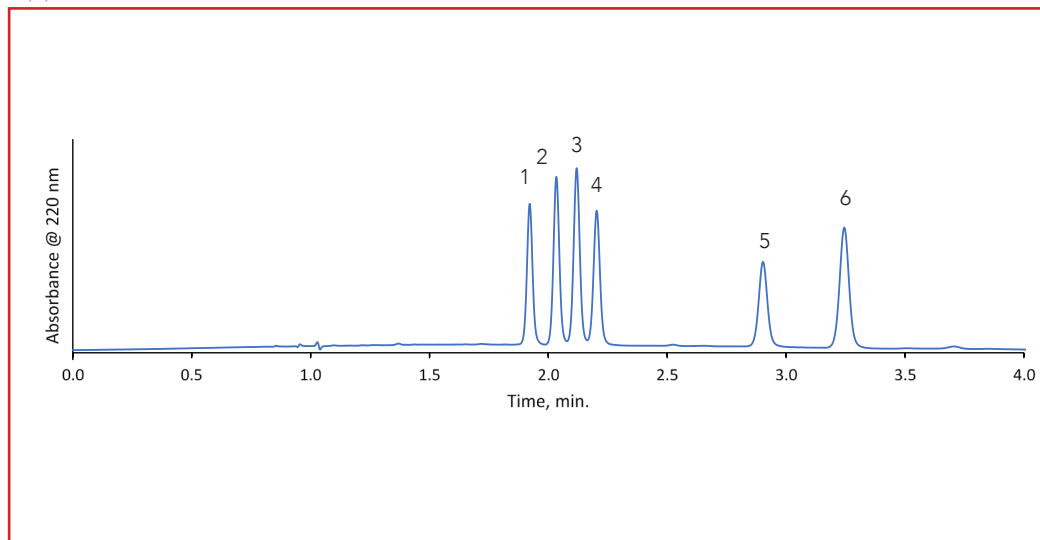






## Separation of Glucocorticoids on HALO® C30

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

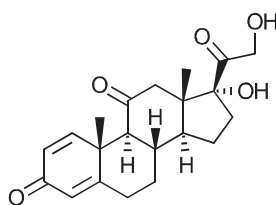
**Response Time:** 0.025 sec

**Data Rate:** 40 Hz

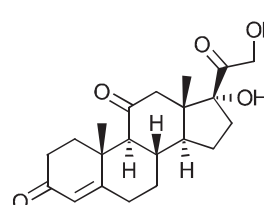
**Flow Cell:** 1.0 µL

**LC System:** Shimadzu Nexera X2

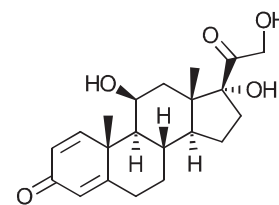
### STRUCTURES:



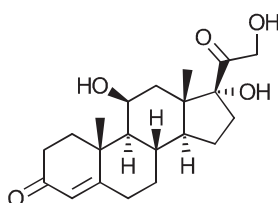
Prednisone



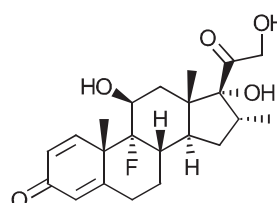
Cortisone



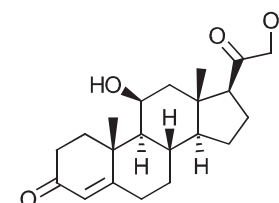
Prednisolone



Hydrocortisone



Dexamethasone



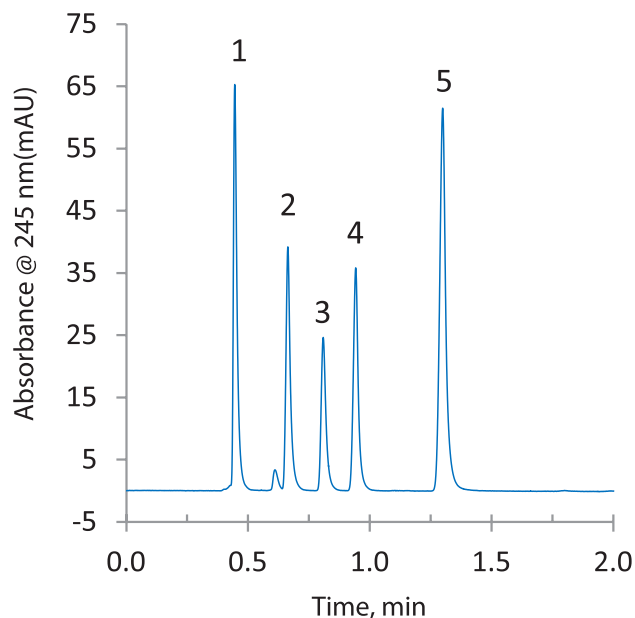
Corticosterone





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

Application Note 119-B



### PEAK IDENTITIES:

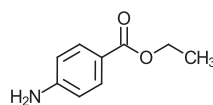
1. Benzocaine
2. Lidocaine
3. Tetracaine
4. Procaine
5. Procainamide

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.

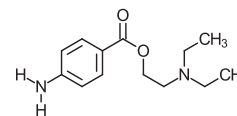
### TEST CONDITIONS:

**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

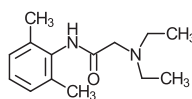
### STRUCTURES:



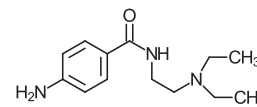
Benzocaine



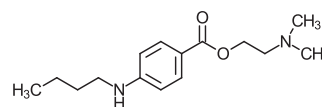
Procaine



Lidocaine



Procainamide



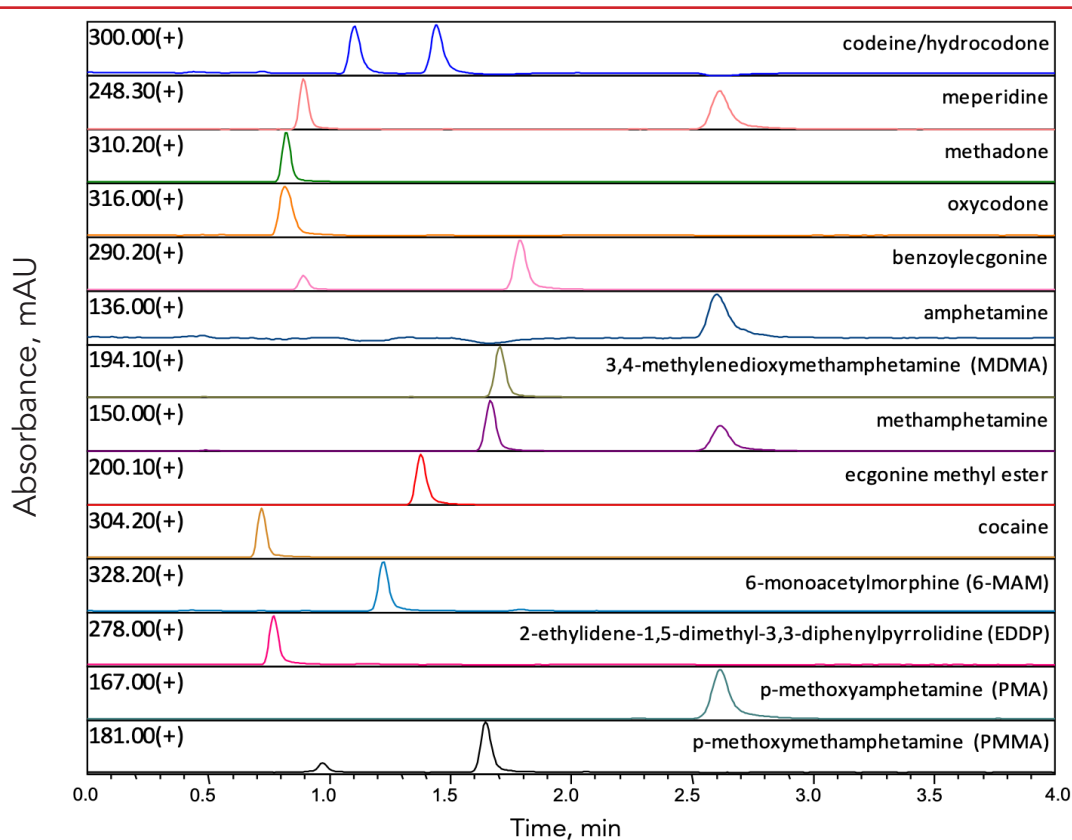
Tetracaine





## 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)

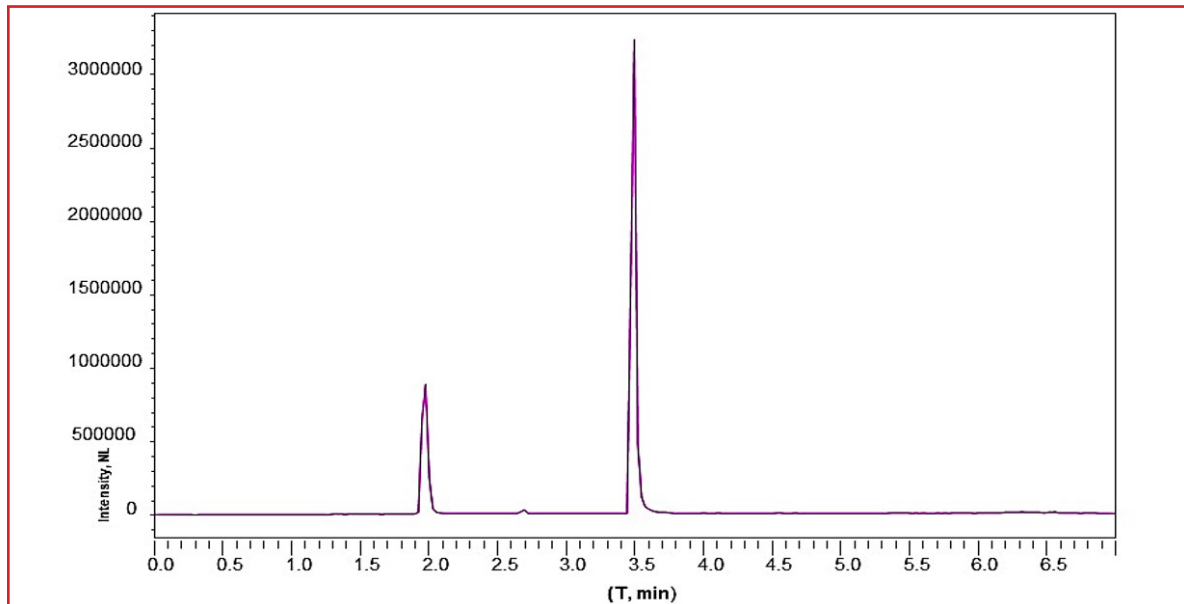
This mixture of drugs of abuse and metabolites is quickly identified using a HALO® Penta-HILIC column and selected ion monitoring (SIM) for improved sensitivity. Adapted from J. Pharm. Anal. 2013; 3 (5): 303-311.





## 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

<b>Gradient:</b>	<b>Time</b>	<b>%N=B</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)

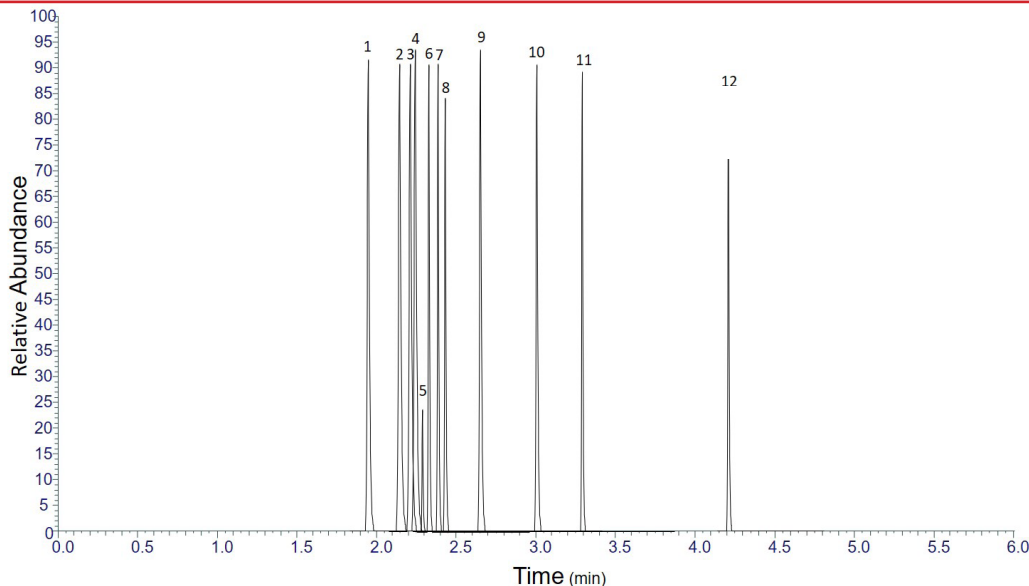






### LC-MS Separation SAMHSA 5 Panel on HALO® Biphenyl 2 µm

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 kV  
Cap temp: 263 °C  
S-lens RF level: 70 V  
Aux gas heater temperature: 425 °C

#### PEAK IDENTITIES:

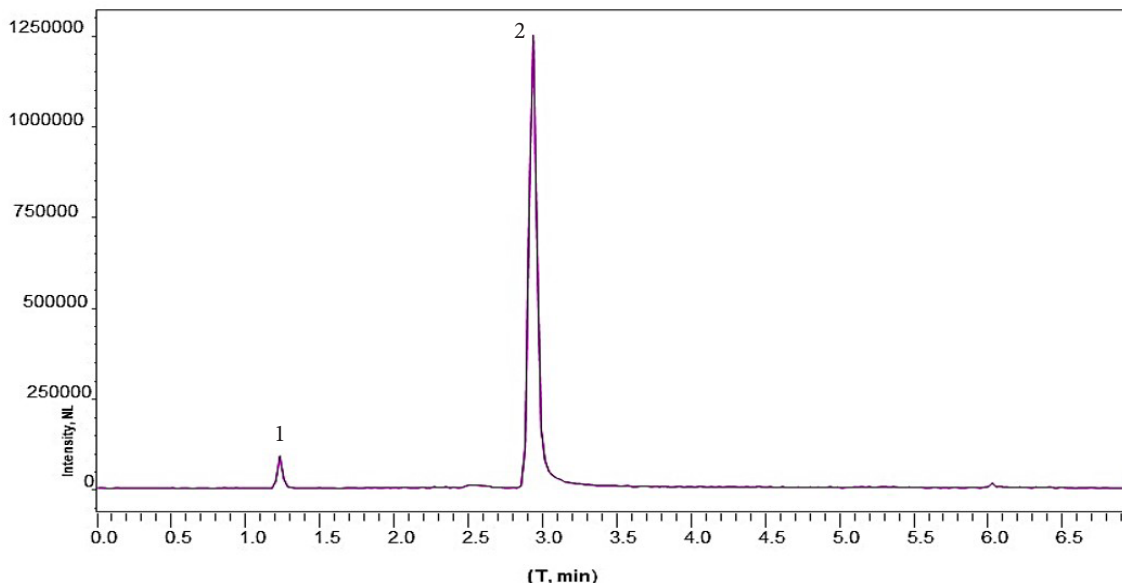
1. Morphine (MH<sup>+</sup>= 286.341 g/mol)
2. Amphetamine (MH<sup>+</sup>= 136.206 g/mol)
3. Methamphetamine (MH<sup>+</sup>= 150.237 g/mol)
4. MDA (MH<sup>+</sup>= 180.221 g/mol)
5. Phentermine (MH<sup>+</sup>= 150.233 g/mol)
6. Codeine (MH<sup>+</sup>= 300.364 g/mol)
7. 6-MAM (MH<sup>+</sup>= 328.380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





## LC-MS Separation of EtG/EtS from urine on HALO<sup>®</sup> Penta-HILIC, 2 $\mu$ m

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  $\mu$ m  
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  $\mu$ L

**Sample prep:** 5ng/mL EtG/EtS in 20  $\mu$ L of synthetic urine. 10 fold dilution with mobile phase A.

### PEAK IDENTITIES:

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

### MS CONDITIONS:

LCMS system: Shimadzu LCMS-2020

Detection: -ESI MS

Spray voltage: 4.50 kV

Drying line temp: 300 °C

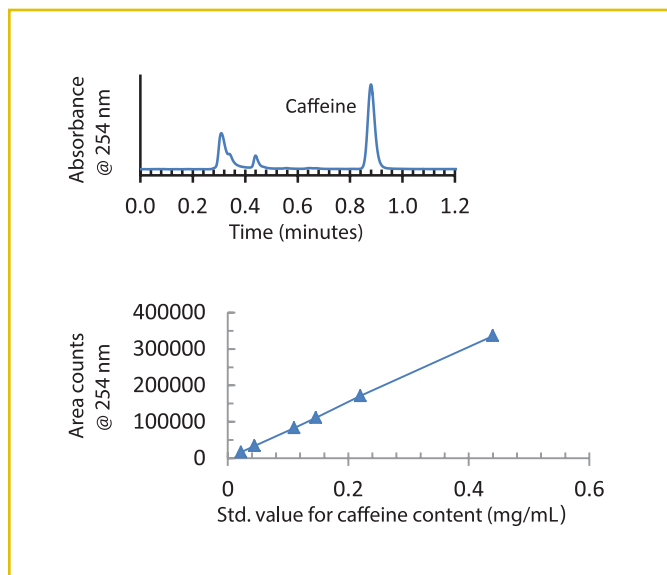
Heat Block: 450 °C





### 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

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.

#### 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

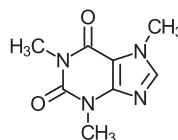
**Data Rate:** 25 Hz

**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

#### STRUCTURE:



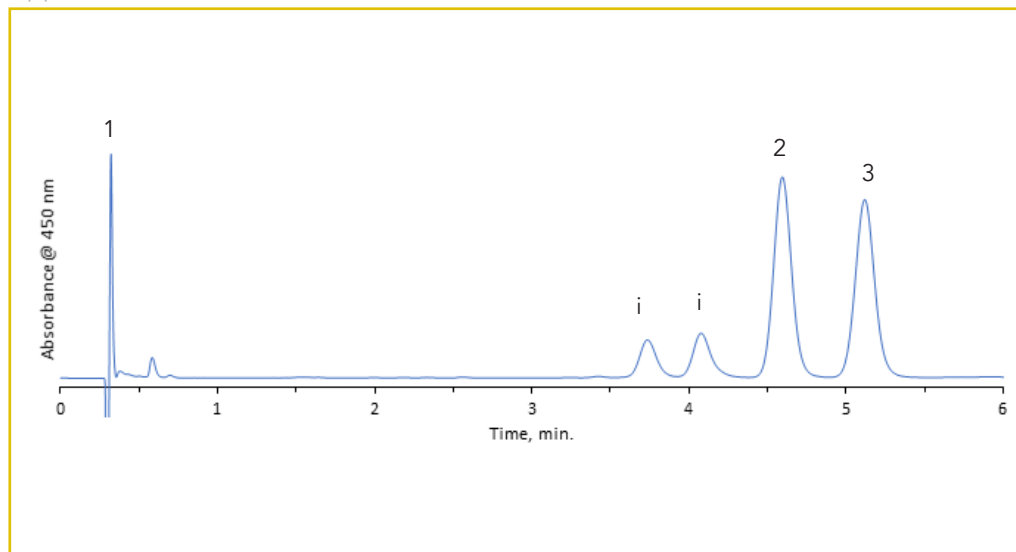
Caffeine





## Carotenoids Extracted from Carrot Juice Analyzed Using HALO® C30

Application Note 183-V



### PEAK IDENTITIES:

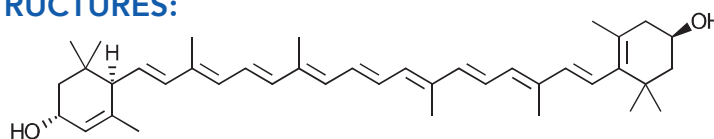
1. Lutein
  2.  $\alpha$ -carotene
  3.  $\beta$ -carotene
- i = Unidentified isomers

The carotenoids lutein,  $\alpha$ -carotene, and  $\beta$ -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  $\alpha$ - and  $\beta$ -carotene peaks.

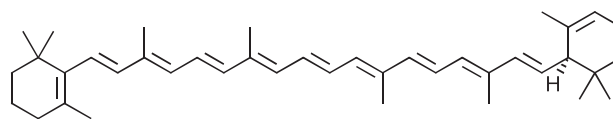
### TEST CONDITIONS:

**Column:** HALO 160 Å C30, 2.7  $\mu\text{m}$ ,  
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  $\mu\text{L}$   
**Sample Solvent:** Methanol/isopropyl alcohol  
**Response Time:** 0.025 sec  
**Data Rate:** 40 Hz  
**Flow Cell:** 1.0  $\mu\text{L}$   
**LC System:** Shimadzu Nexera X2

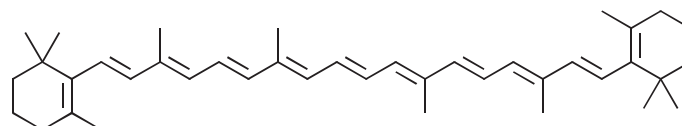
### STRUCTURES:



Lutein



Alpha carotene



Beta carotene

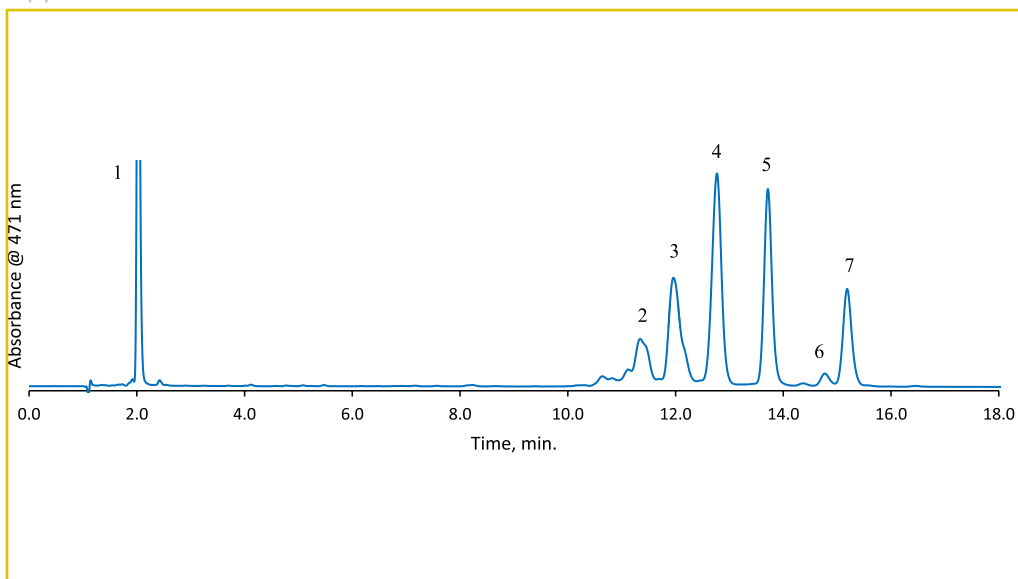






## Separation of Carotenoids on HALO® C30

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

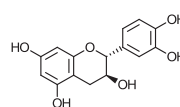
**Data Rate:** 2.5 Hz

**Flow Cell:** 13 μL

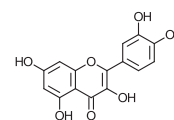
**LC System:** Agilent 1100

Data Courtesy of Nature's Sunshine Products

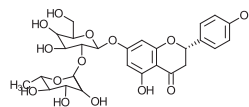
### STRUCTURES:



Catechin



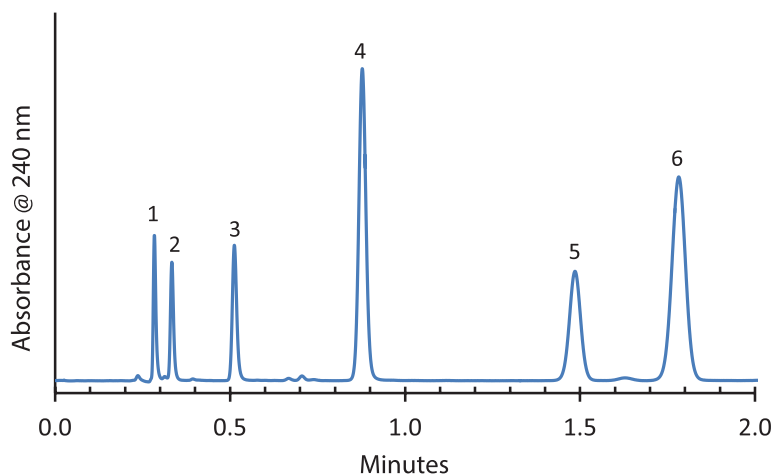
Quercetin





## 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, WVD

**Injection Volume:** 1.0 µL

**Sample Solvent:** Methanol

**Response Time:** 0.02 sec

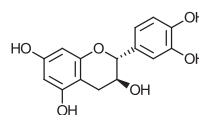
**Data Rate:** 25 Hz

**Flow Cell:** 2.5 µL semi-micro

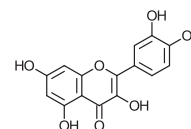
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

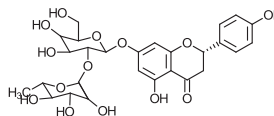
### STRUCTURES:



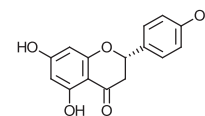
Catechin



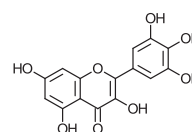
Quercetin



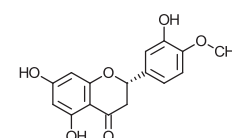
Naringin



Naringenin



Myricetin



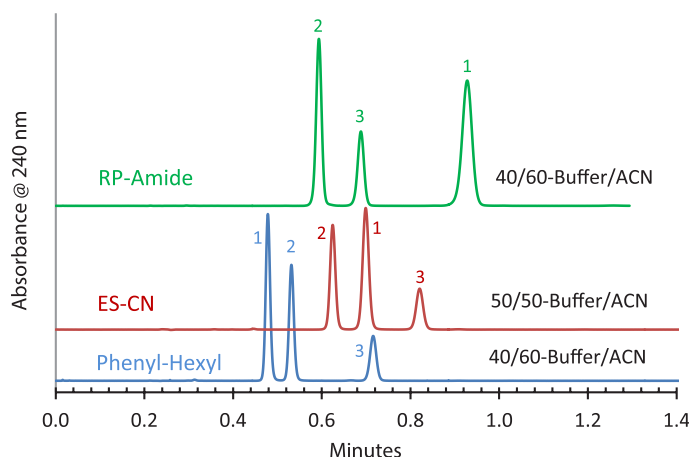
Hesperetin





## 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

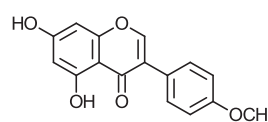
**Data Rate:** 25 Hz

**Flow Cell:** 2.5 µL semi-micro

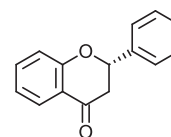
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

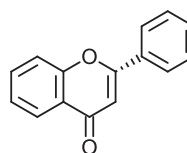
### STRUCTURES:



Biochanin A



Flavanone



Flavone

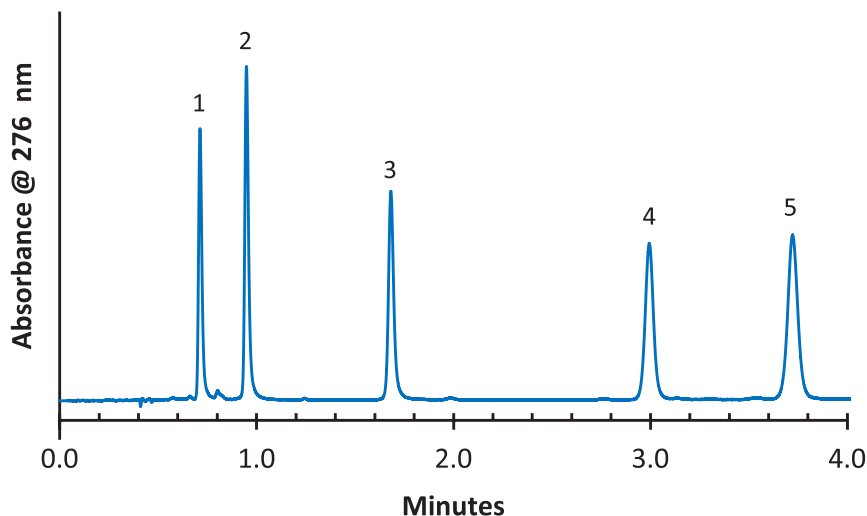


110



## Separation of Five Flavonoids on HALO® C8, 2.0 µm

Application Note 127-FL



### PEAK IDENTITIES:

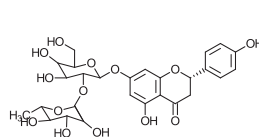
1. Naringin
2. Myricetin
3. Quercetin
4. Naringenin
5. Hesperetin

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.

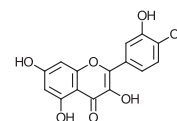
### TEST CONDITIONS:

**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

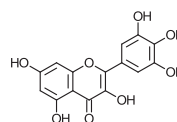
### STRUCTURES:



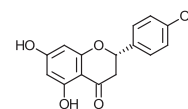
Naringin



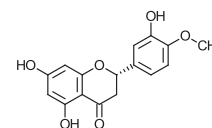
Quercetin



Myricetin



Naringenin



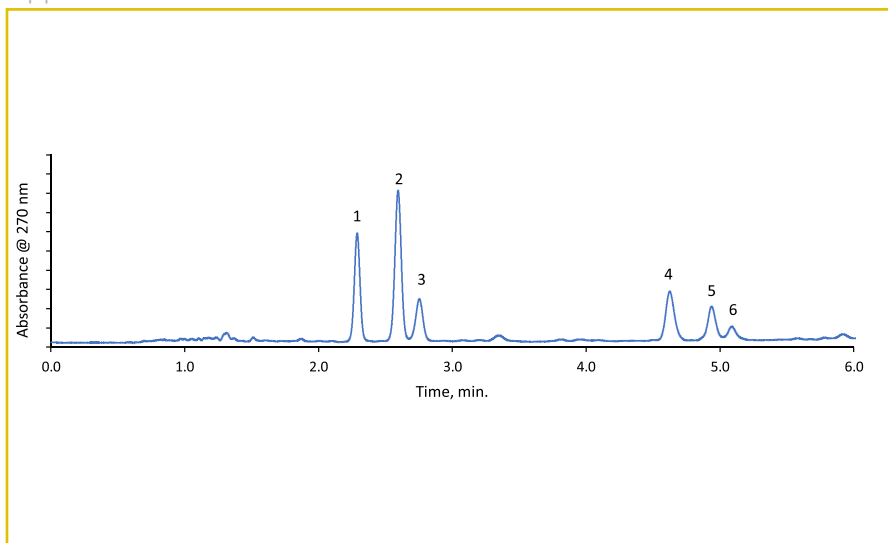
Hesperetin





## 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

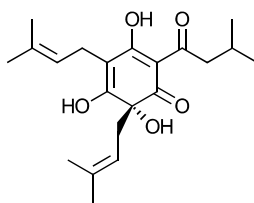
**Response Time:** 0.025 sec

**Data Rate:** 100 Hz

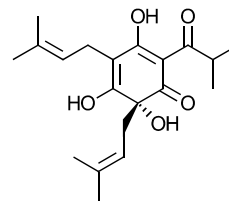
**Flow Cell:** 1.0 µL

**LC System:** Shimadzu Nexera X2

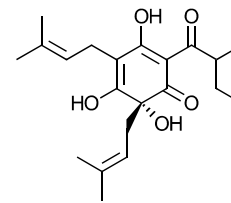
### STRUCTURES:



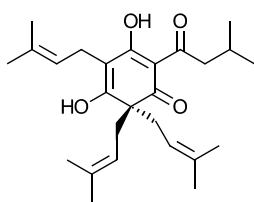
Cohumulone



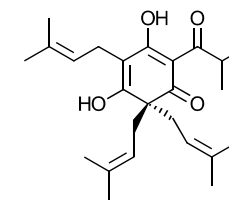
Humulone



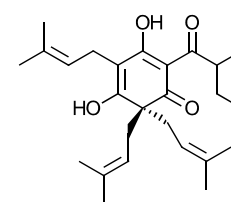
Adhumulone



Colupulone



Lupulone



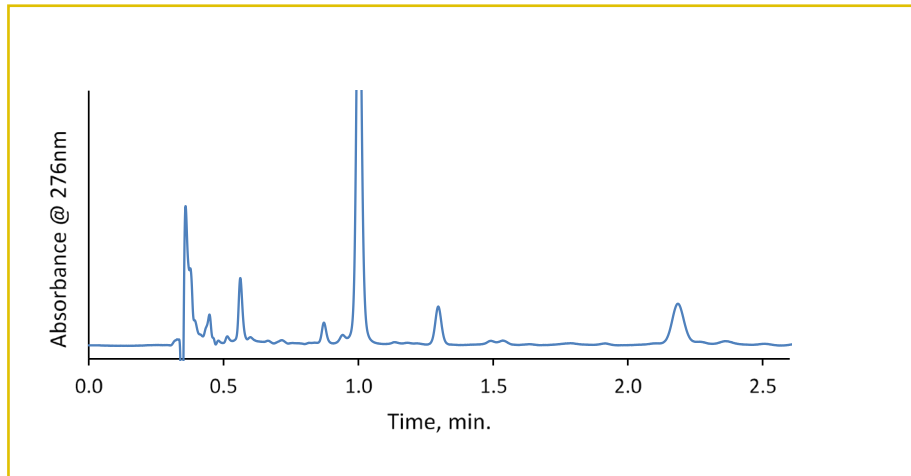
Adlupulone





## 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

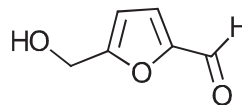
**Response Time:** 0.025 sec

**Data Rate:** 100 Hz

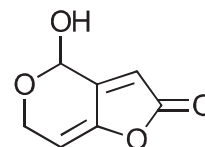
**Flow Cell:** 1.0 µL

**LC System:** Shimadzu Nexera X2

### STRUCTURES:



5-(Hydroxymethyl) furfural



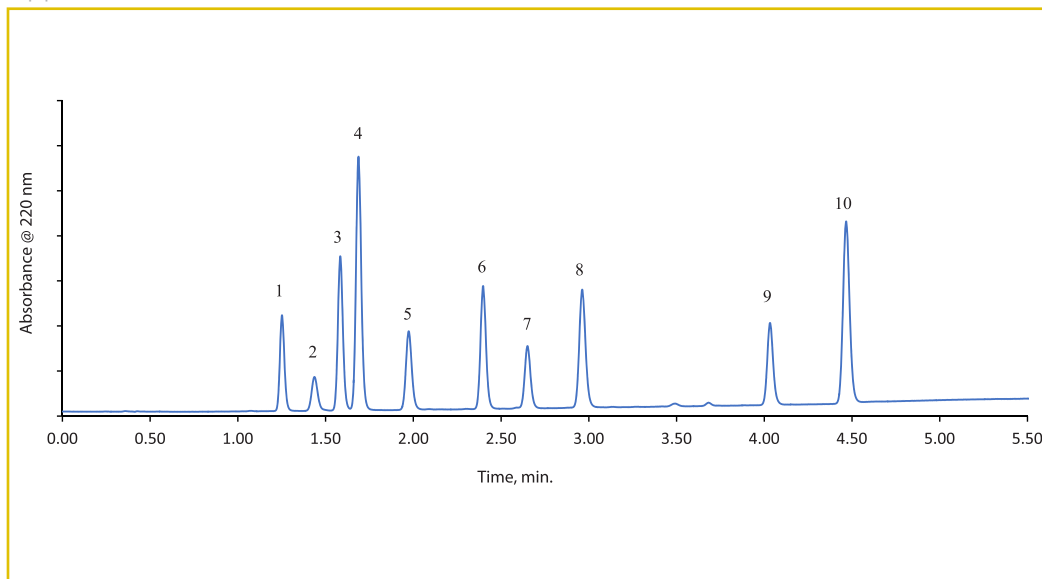
Patulin





## 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

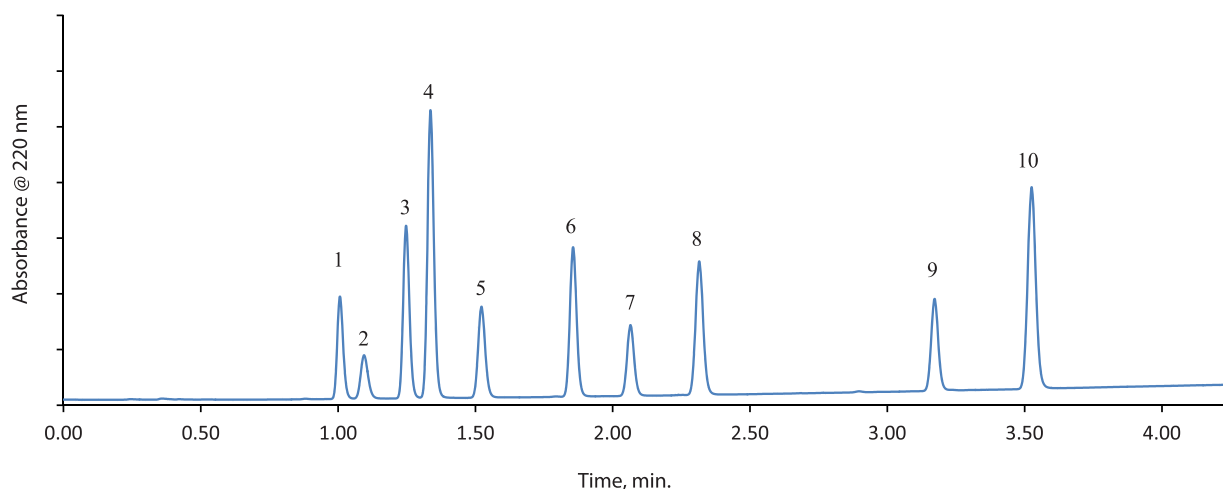
**LC System:** Shimadzu Nexera X2





## 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

<b>Gradient:</b>	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

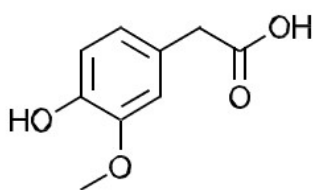
**LC System:** Shimadzu Nexera X2



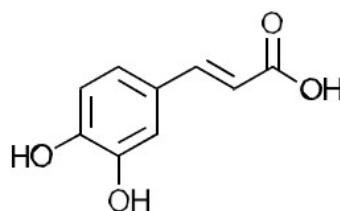




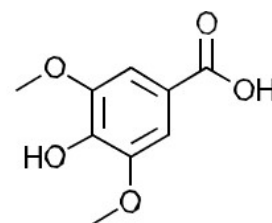
## STRUCTURES:



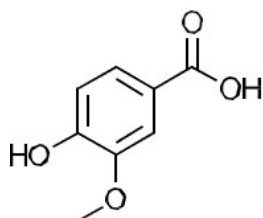
Homovanillic acid



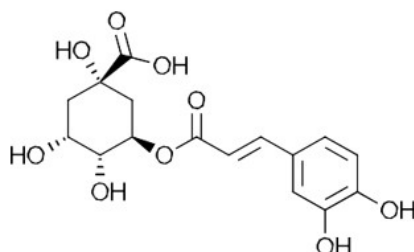
Caffeic acid



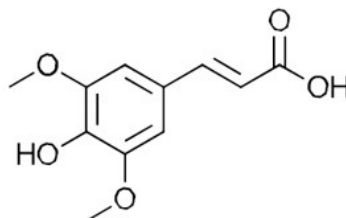
Syringic acid



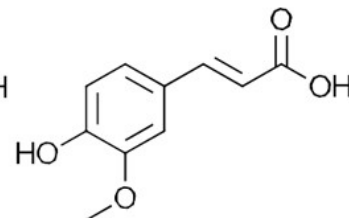
Vanillic acid



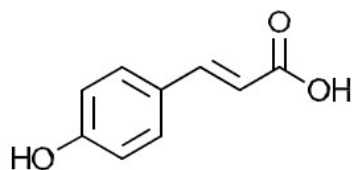
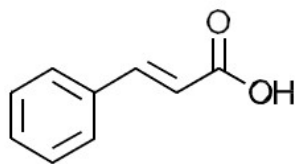
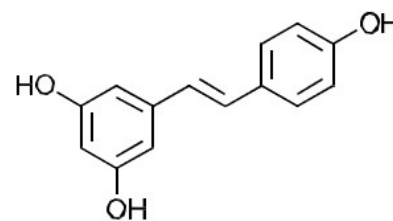
Chlorogenic acid



Sinapic acid



Ferulic acid

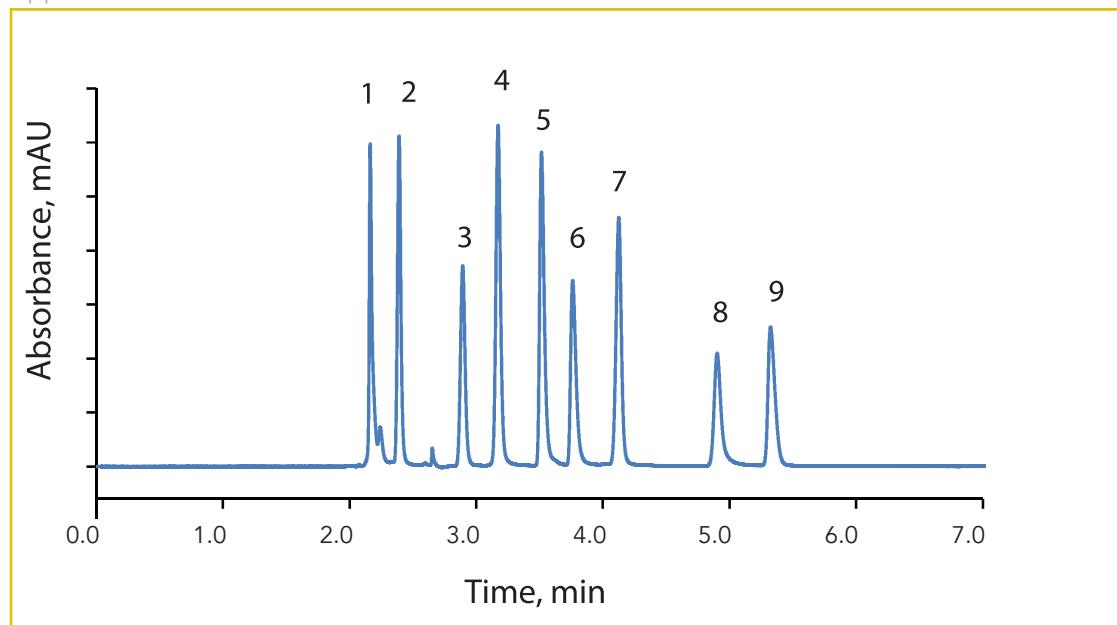
*p*- Coumaric acid*trans*- Cinnamic acid

Resveratrol



## Separation of Polar Organic Acids on HALO® AQ-C18

Application Note 160-OA



### PEAK IDENTITIES:

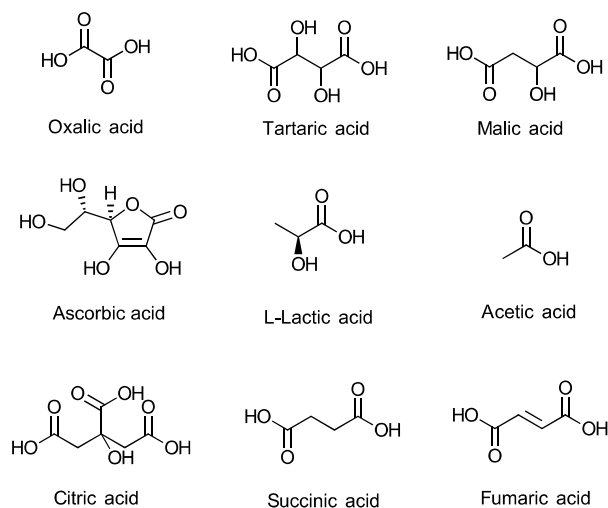
1. Oxalic acid
2. Tartaric acid
3. Malic acid
4. Ascorbic acid
5. L-Lactic acid
6. Acetic acid
7. Citric acid
8. Succinic acid
9. Fumaric acid

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  $\mu$ m,  
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  $\mu$ L  
**Sample Solvent:** Mobile phase  
**Response Time:** 0.025 sec  
**Data Rate:** 100 Hz  
**Flow Cell:** 1.0  $\mu$ L  
**LC System:** Shimadzu Nexera X2

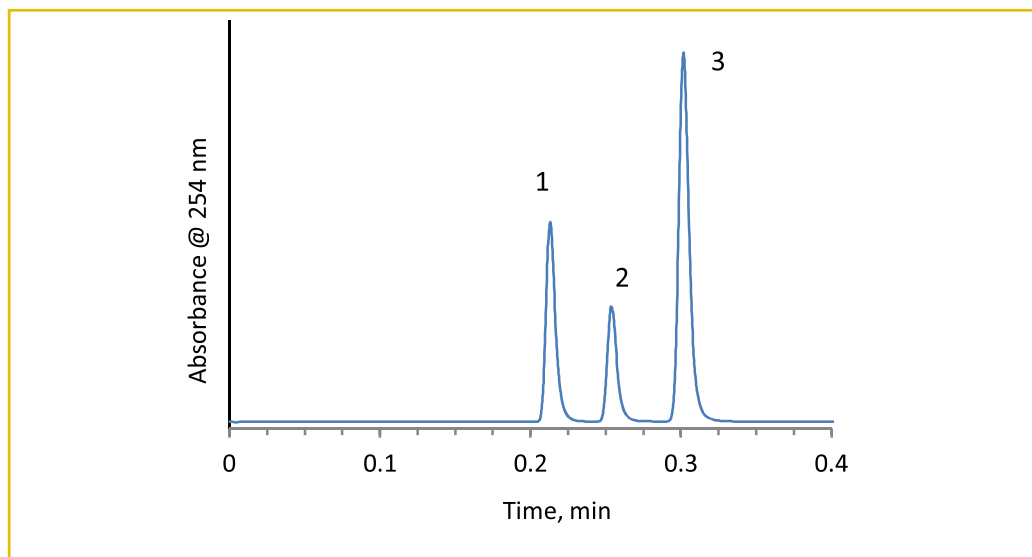
### STRUCTURES:





## 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  $\mu\text{m}$ ,  
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  $\mu\text{L}$

**Sample Solvent:** Methanol

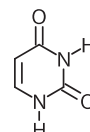
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu\text{L}$  semi-micro

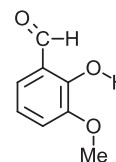
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu\text{L}$

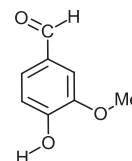
### STRUCTURES:



Uracil



O-Vanillin



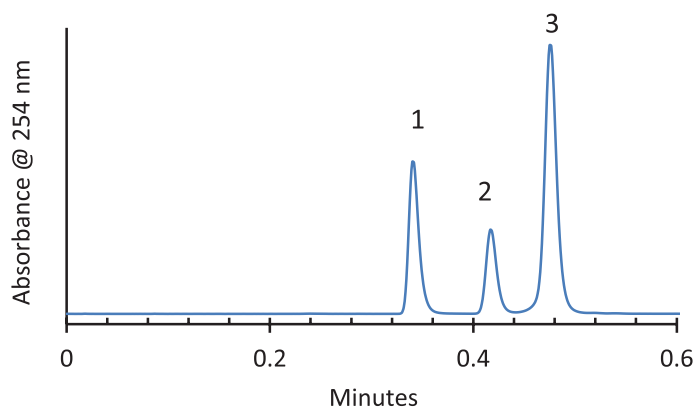
Vanillin





## 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 synthetic 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

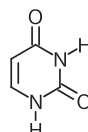
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

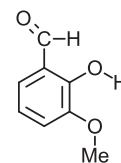
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

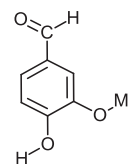
### STRUCTURES:



Uracil



O-Vanillin



Vanillin



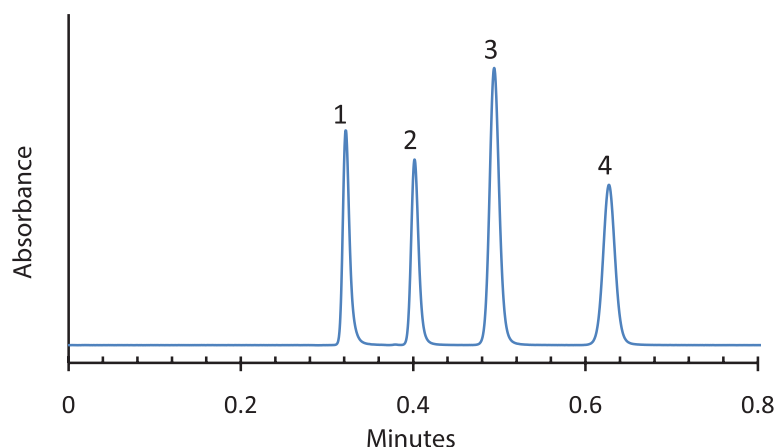
119





## 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

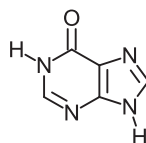
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

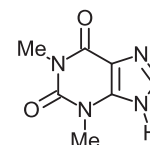
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

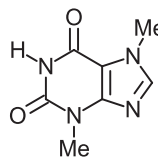
### STRUCTURES:



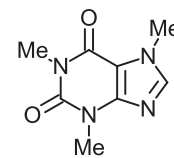
Hypoxanthine



Theophylline



Theobromine



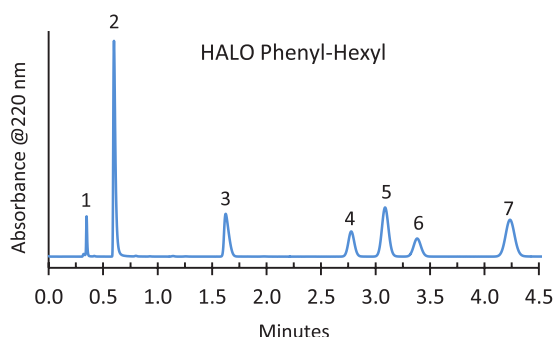
Caffeine





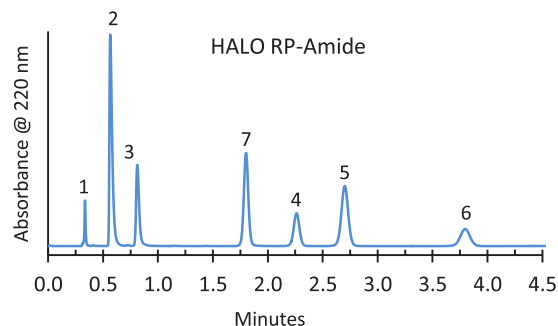
## Separation of Food Additives on HALO® Phenyl-Hexyl and RP-Amide Phases

Application Note 95-P



### PEAK IDENTITIES:

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

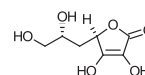
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

### STRUCTURES:



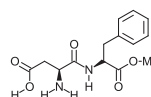
Ascorbic acid



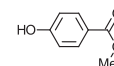
Saccharin



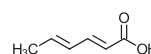
Benzoic acid



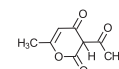
Aspartame



Methyl paraben



Sorbic acid

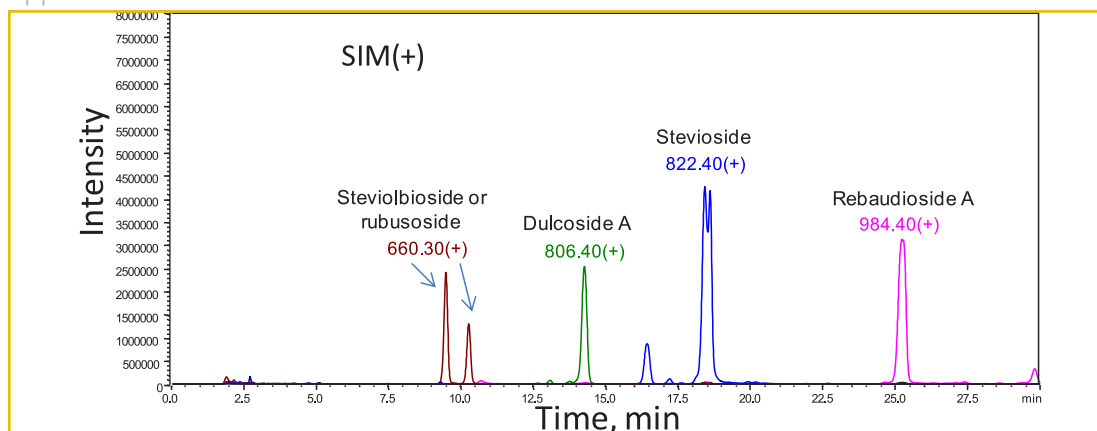


Dehydroacetic acid



## 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:

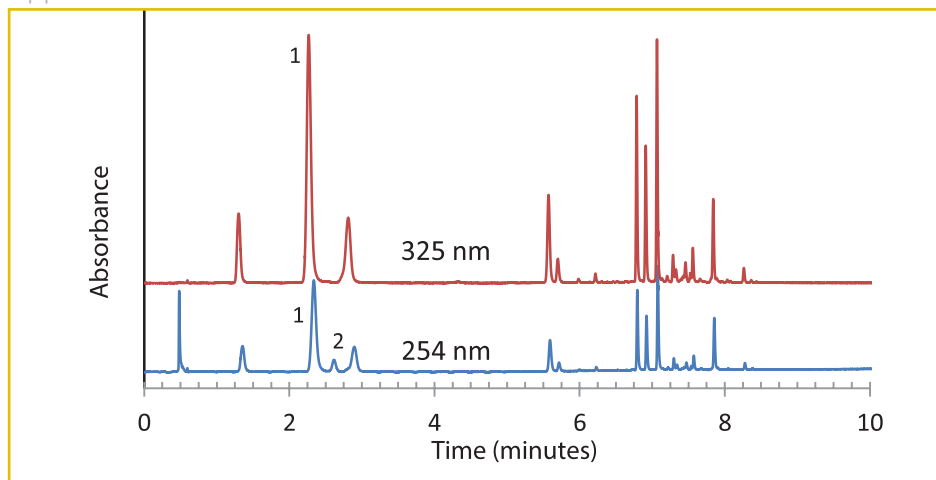
1. Weigh 400 mg of Stevia rebaudiana leaves (Sigma S5381)
2. Crush leaves with mortar and pestle and transfer to vial
3. Add 8.0 mL of 50/50 (v/v) acetonitrile/water
4. Sonicate vial contents for 15 minutes
5. Filter sample using 25 mm syringe filter having 0.2 µm PTFE membrane (VWR 28145-495)
6. Centrifuge @ 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

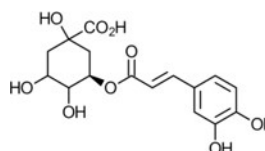
**Data Rate:** 25 Hz

**Flow Cell:** 2.5 µL semi-micro

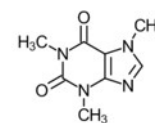
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

### STRUCTURES:



Chlorogenic Acid



Caffeine

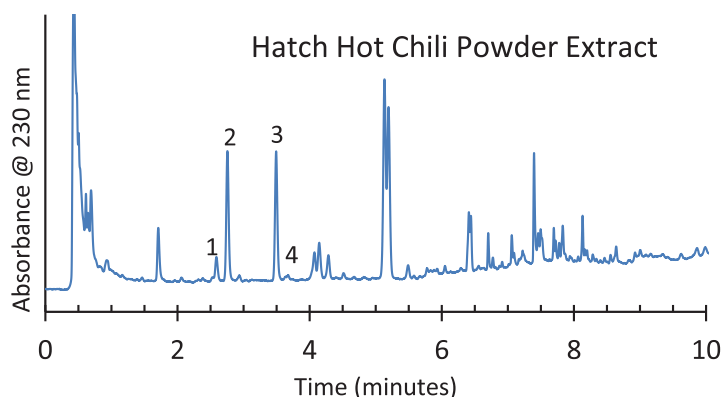
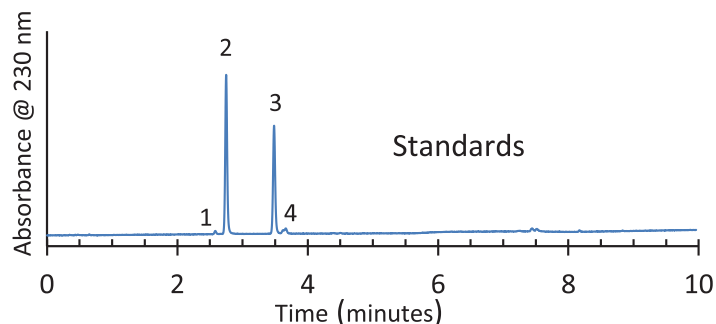


123



## 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

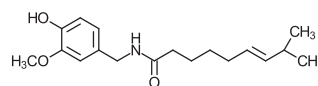
**Sample Solvent:** acetonitrile **Detection:** UV 230 nm, VWD

**Response Time:** 0.02 sec. **Data rate:** 25 Hz

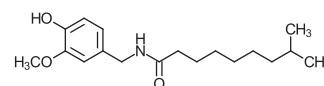
**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Prominence UFLC XR ECV: ~14 µL

### STRUCTURES:



Capsaicin



Dihydrocapsaicin

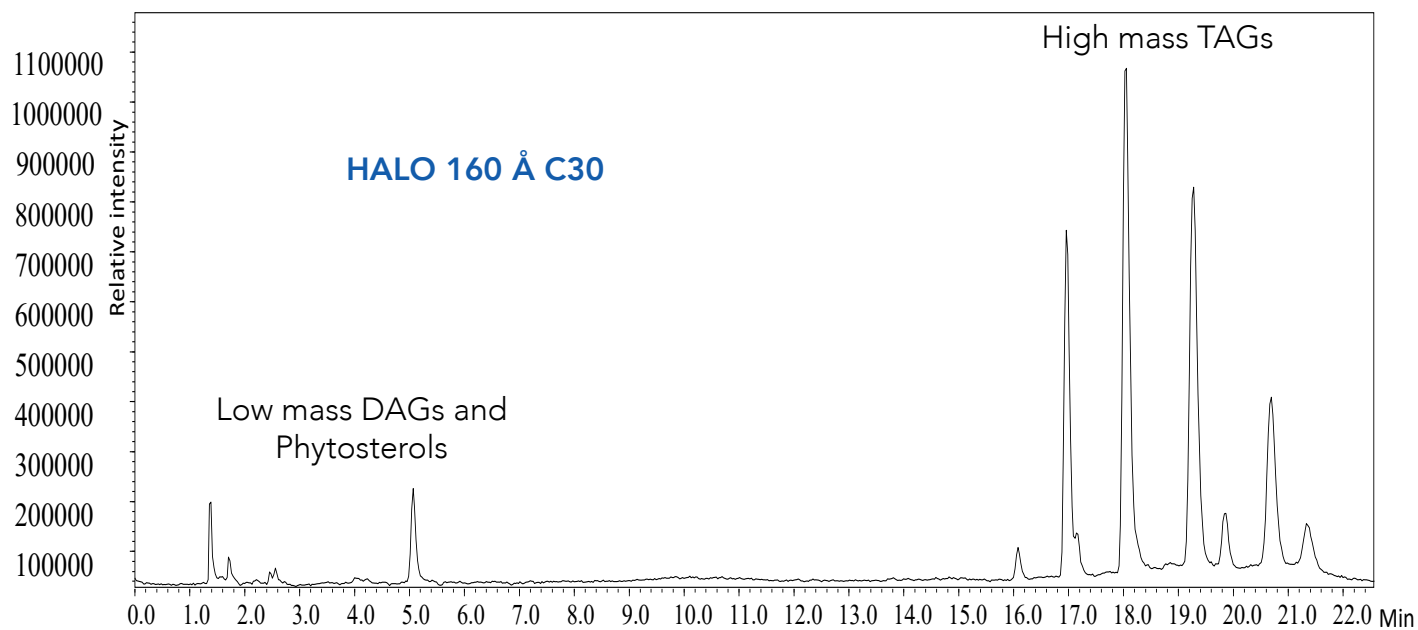
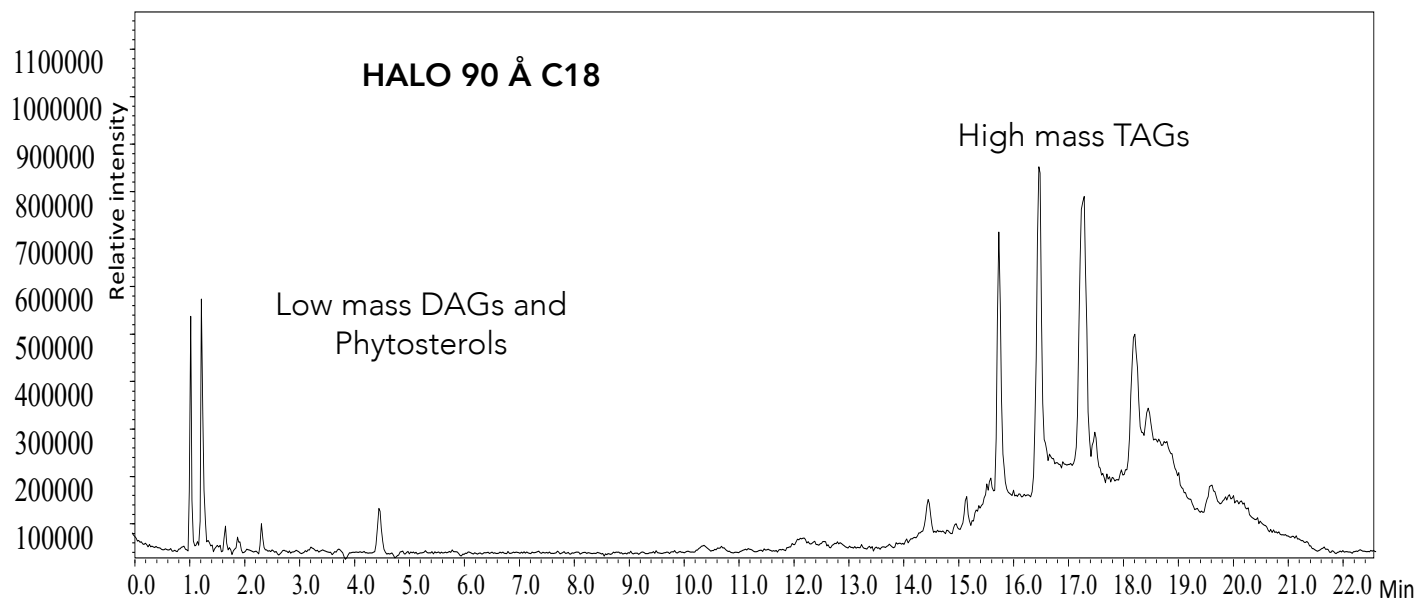






## LC-MS Separation of Corn Oil on HALO® C30 Compared to HALO® C18

Application Note: 208-LI



DAGs = diacylglycerols or diglycerides

TAGs = triacylglycerols or triglycerides



**TEST CONDITIONS:**

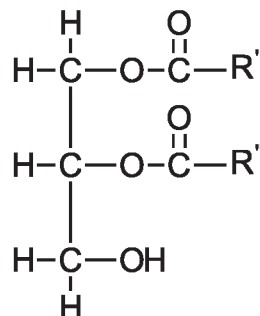
**Columns:** HALO 90 Å C18, 2.7 µm, 2.1 x 150 mm  
**Part Number:** 92812-702  
**Columns:** HALO 160 Å C30, 2.7 µm, 2.1 x 150 mm  
**Part Number:** 92112-730  
**Mobile Phase A:** Methanol  
**Mobile Phase B:** IPA/0.1% Formic acid  
**Gradient:**

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

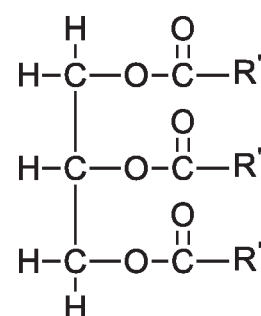
  
**Flow Rate:** 0.3 mL/min  
**Initial Pressure:** 325 bar  
**Temperature:** Ambient  
**Injection Volume:** 2 µL  
**Sample Solvent:** MeOH  
**LC System:** Shimadzu Nexera X2

**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

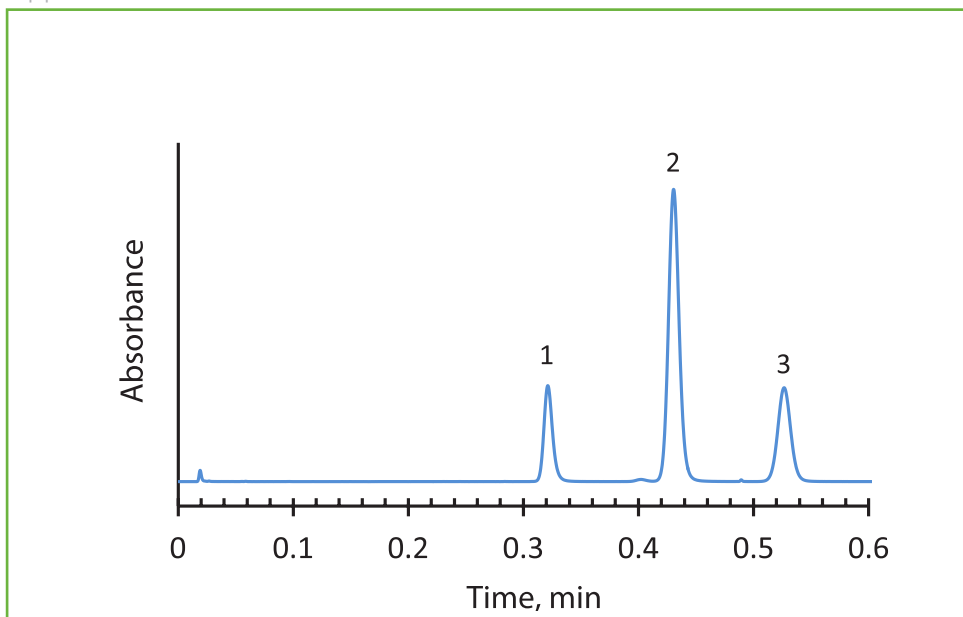
Corn oil, composed mainly of long chain fatty acids and esters, is an edible oil which comprises approximately 5-10% of edible oil consumption. In recent years, corn oil has been used in biodiesel, pharmaceutical, and cosmetic applications as well. The use of a C18 column for the analysis of edible oils 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.





## Separation of Carbamate Pesticides on HALO® ES-CN Phase

Application Note 60-CB



### PEAK IDENTITIES:

1. Carbetamide
2. Protham
3. Chlorprotham

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

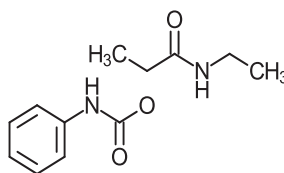
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

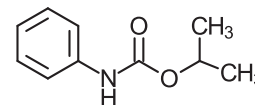
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

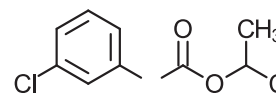
### STRUCTURES:



Carbetamide



Protham



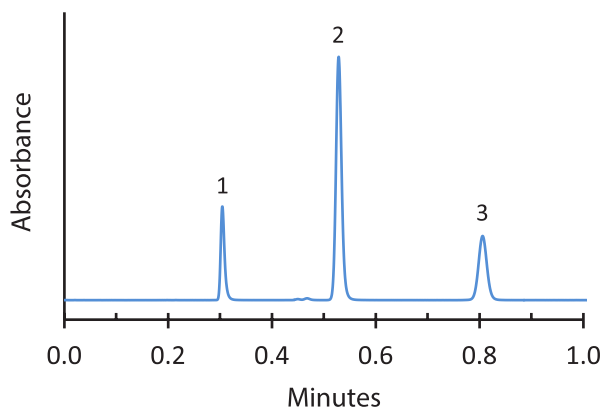
Chlorprotham





## 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

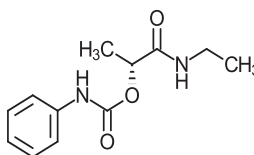
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

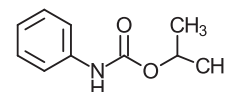
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

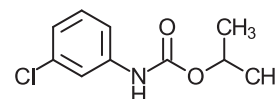
### STRUCTURES:



Carbetamide



Propham



Chlorpropham

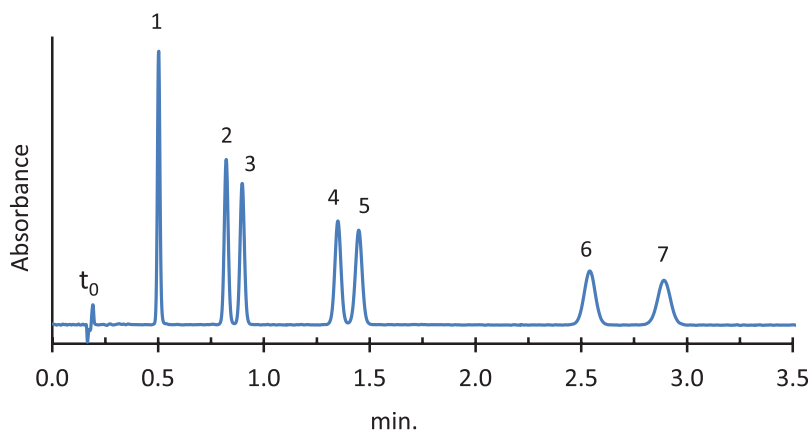


128



## 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

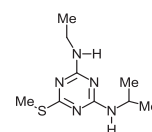
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

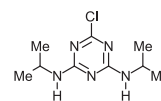
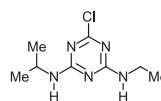
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

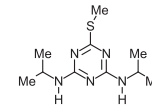
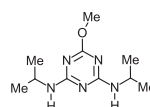
### STRUCTURES:



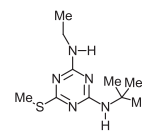
Ametryn



Propazine



Prometryn



Terbutryn



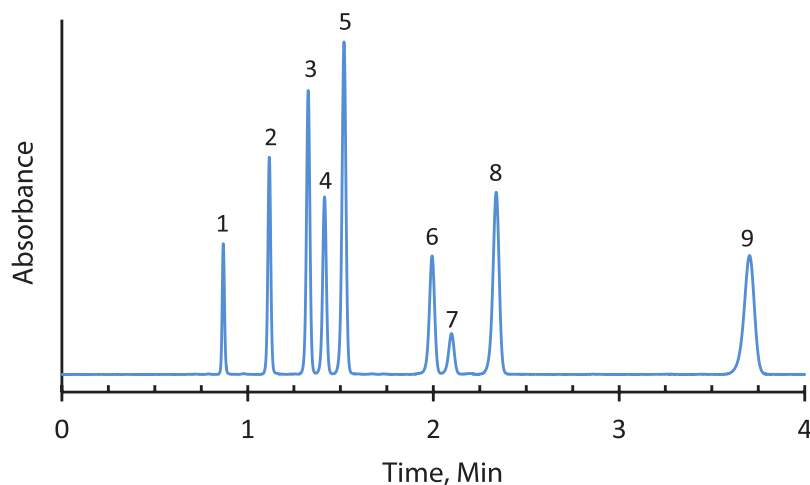
129





## 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

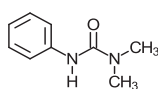
**Sample Solvent:** Acetonitrile

**Response Time:** 0.02 sec

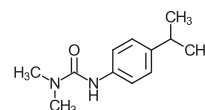
**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Prominence UFLC XR

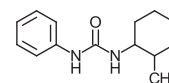
### STRUCTURES:



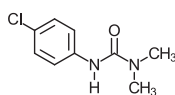
Fenuron



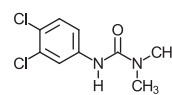
Isoproturon



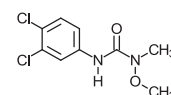
Siduron B



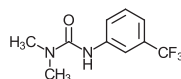
Monuron



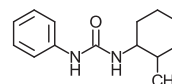
Diuron



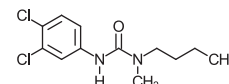
Linuron



Fluomethuron



Siduron A



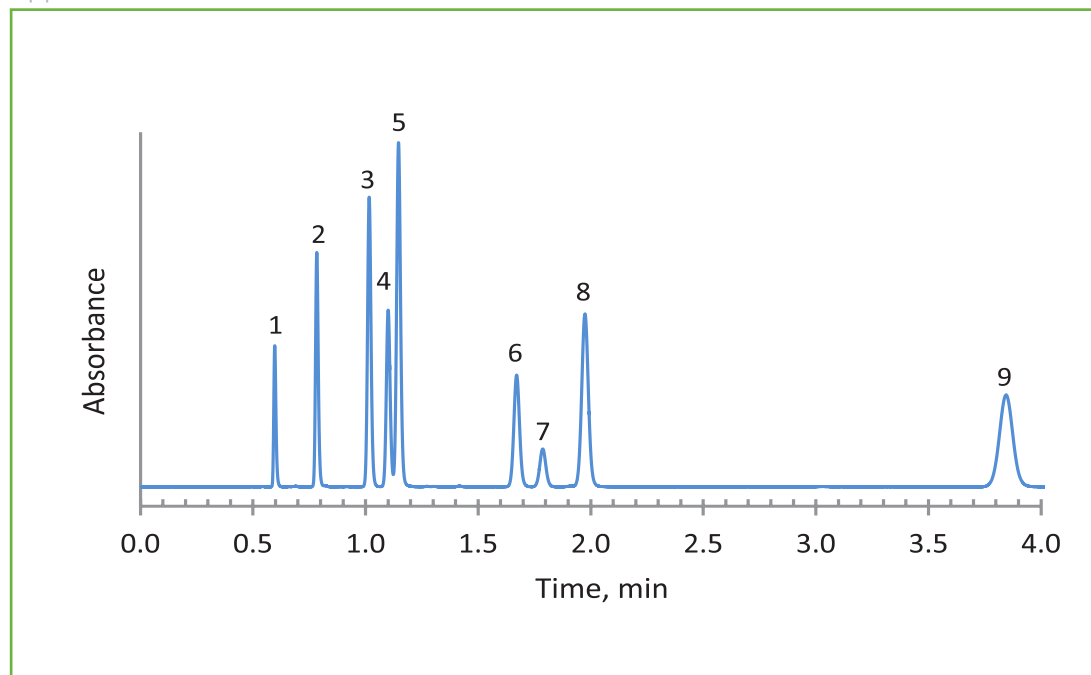
Neburon





## 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

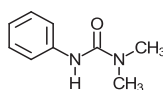
**Sample Solvent:** Acetonitrile

**Response Time:** 0.02 sec

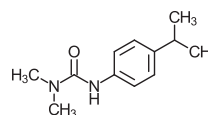
**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Prominence UFLC XR

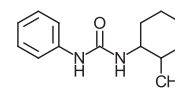
### STRUCTURES:



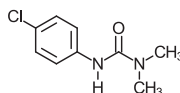
Fenuron



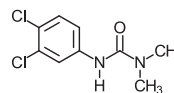
Isoproturon



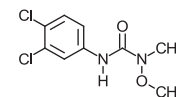
Siduron B



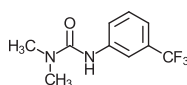
Monuron



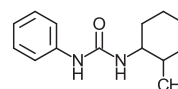
Diuron



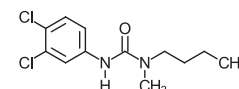
Linuron



Fluomethuron



Siduron A



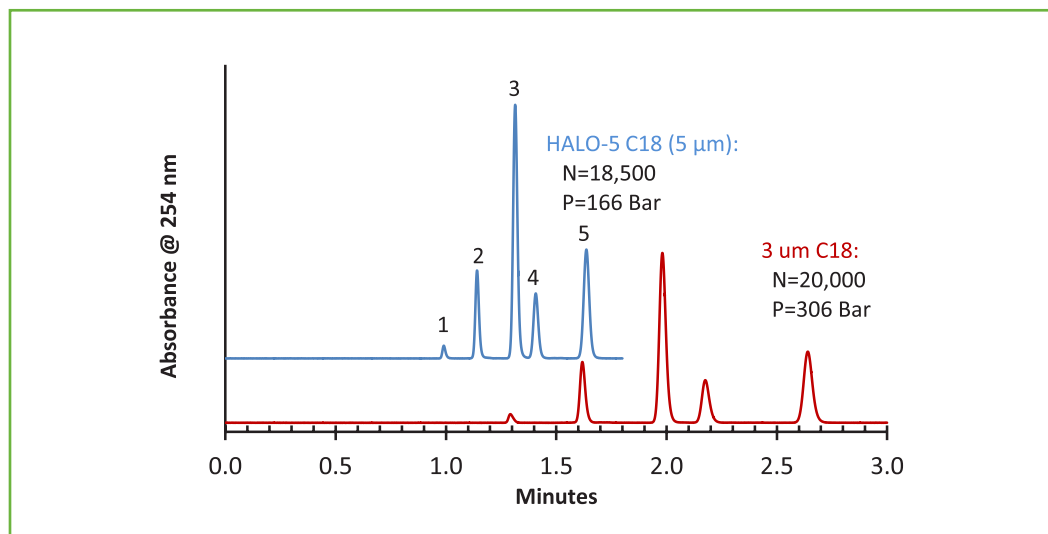
Neburon





## 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 ( $t_R$ )
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

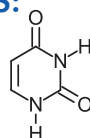
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

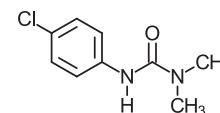
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

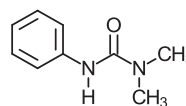
### STRUCTURES:



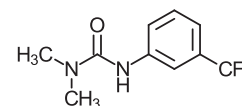
Uracil



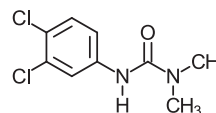
Monuron



Fenuron



Fluometuron



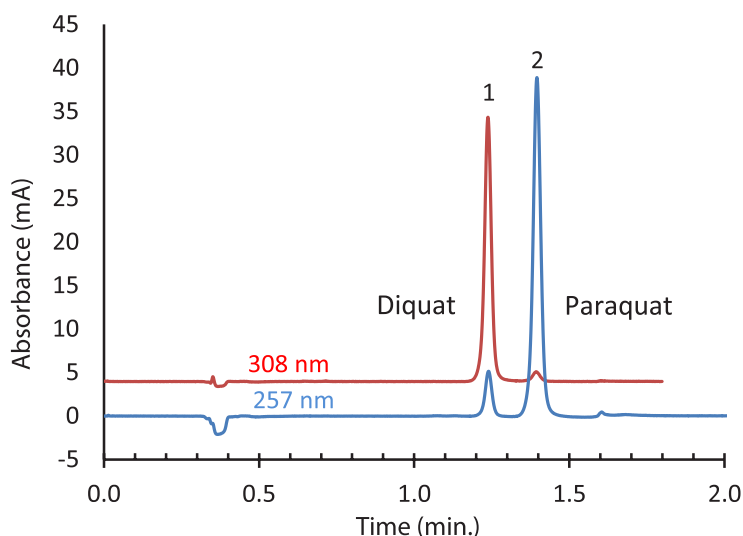
Diuron





## 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

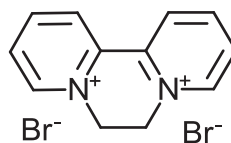
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

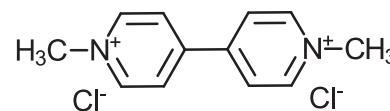
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

### STRUCTURES:



Diquat Dibromide



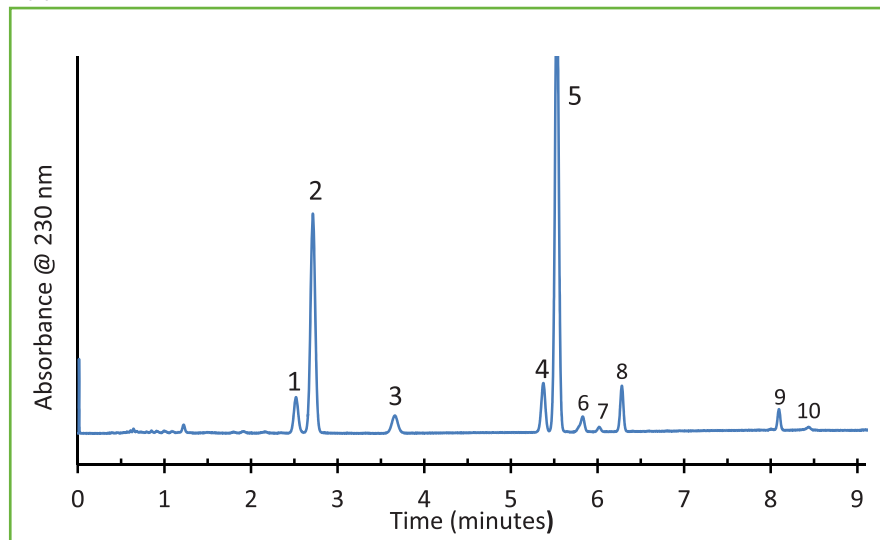
Paraquat Dichloride





## 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

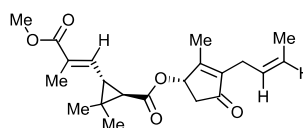
**Data Rate:** 17 Hz

**Flow Cell:** 2.5 µL semi-micro

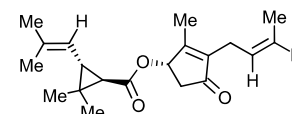
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

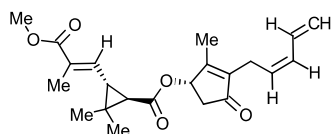
### STRUCTURES:



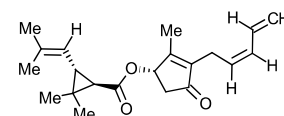
Cinerin II



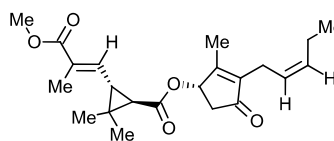
Cinerin I



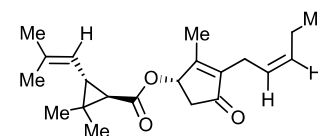
Pyrethrin II



Pyrethrin I



Jasmolin II



Jasmolin I

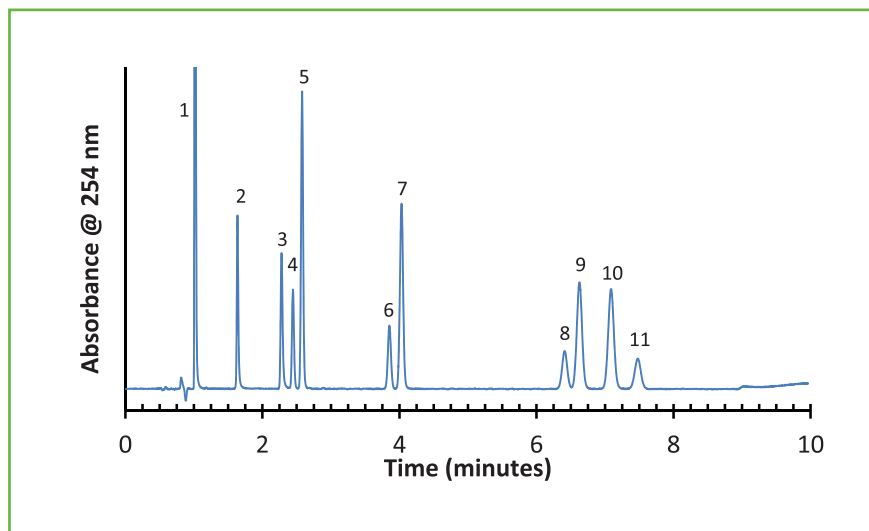






## Separation of Triazine Pesticides on HALO® AQ-C18, 2.7 µm

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. Terbutylazine

Triazines 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

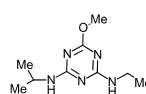
**Response Time:** 0.02 sec

**Data Rate:** 25 Hz

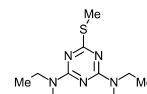
**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Prominence UFLC XR

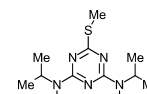
### STRUCTURES:



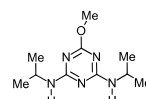
Atraton



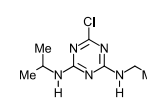
Simetryn



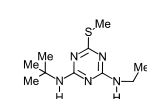
Prometryn



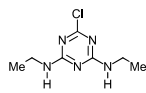
Prometon



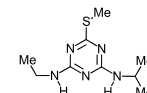
Atrazine



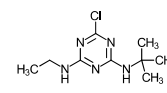
Terbutryn



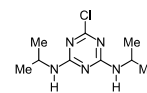
Simazine



Ametryn



Terbutylazine



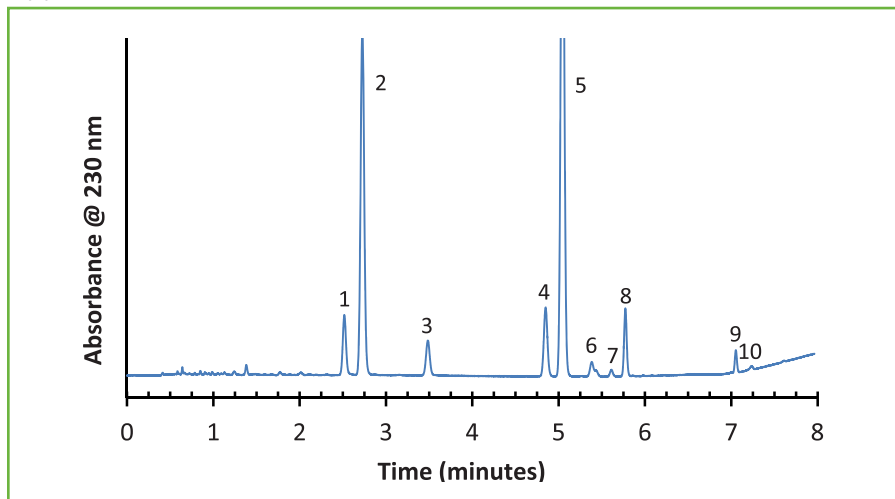
Propazine





## 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

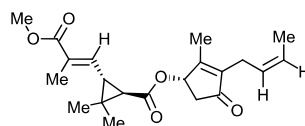
**Flow Cell:** 2.5 µL semi-micro

**Data Rate:** 25 Hz

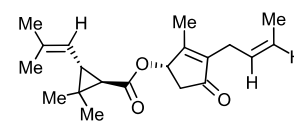
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

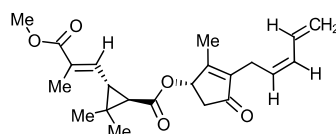
### STRUCTURES:



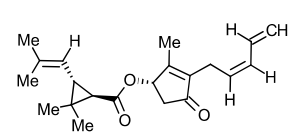
Cinerin II



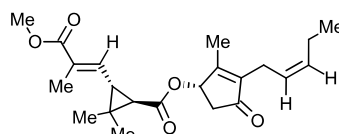
Cinerin I



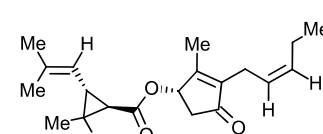
Pyrethrin II



Pyrethrin I



Jasmolin II



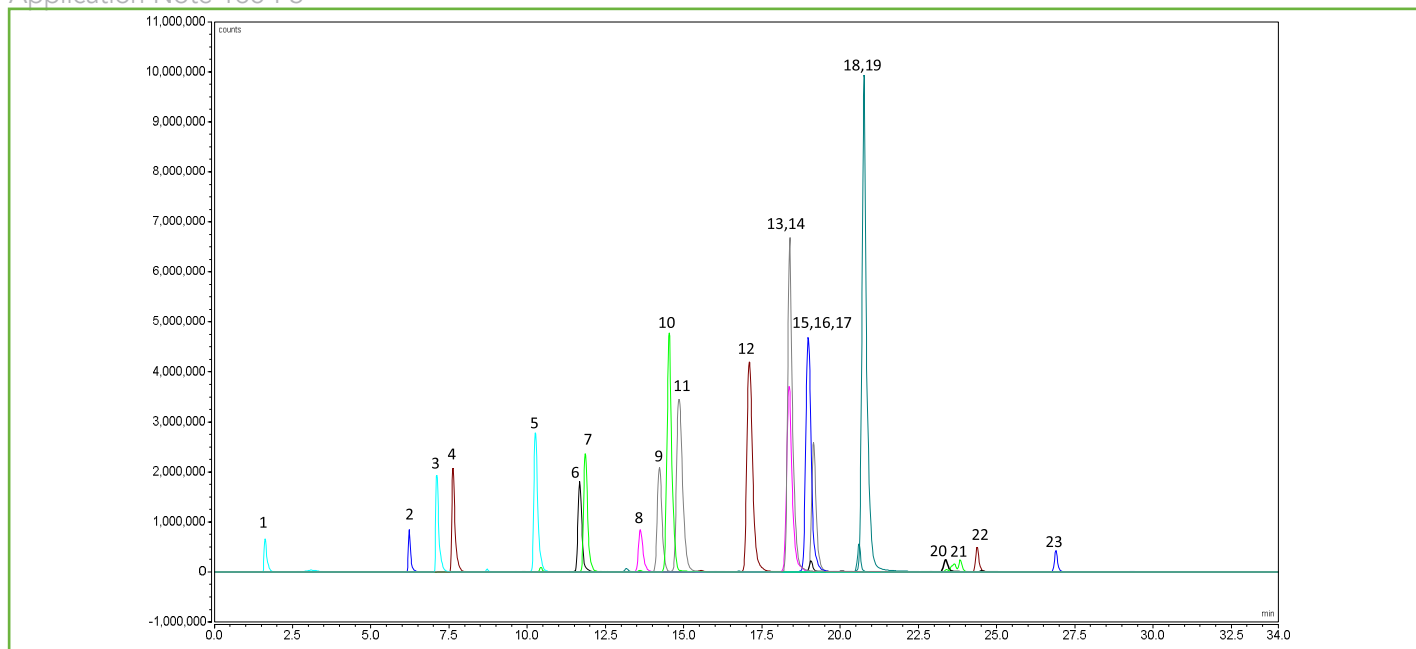
Jasmolin I





## Pesticides Separation on HALO 90 Å Biphenyl

Application Note 168-PS



### TEST CONDITIONS:

**Column:** HALO 90 Å Biphenyl, 2.7  $\mu\text{m}$ ,  
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  $\mu\text{L}$

**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  $\mu\text{s}$ cans/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.

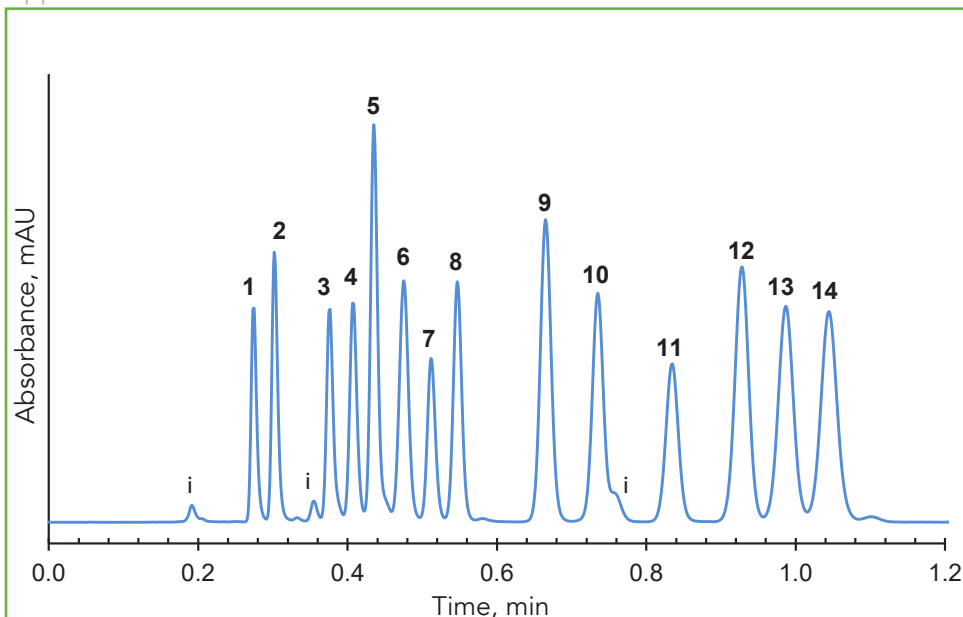






## Rapid HPLC Separation of Aromatic Compounds on HALO® Phenyl-Hexyl

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

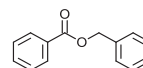
**Flow Cell:** 5.0 µL low-volume

**LC System:** Agilent 1100

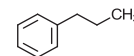
### STRUCTURES:



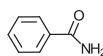
Uracil



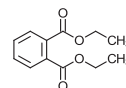
Benzylbenzoate



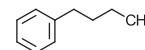
n-Propylbenzene



Benzamide



Diethylphthalate



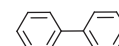
n-Butylbenzene



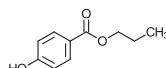
Benzonitrile



Toluene



Biphenyl



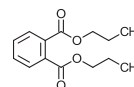
Propylparaben



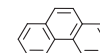
1-Chloro-4-nitrobenzene



Acenaphthene



Di-n-P ropylphthalate



Phenanthrene



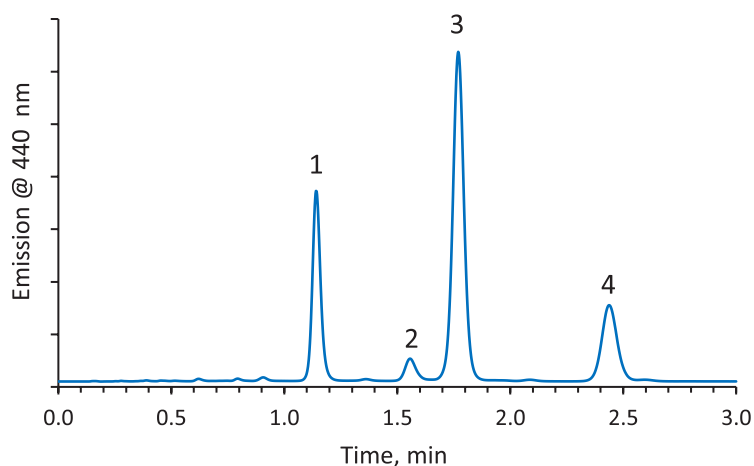
139





## Isocratic Separation of Aflatoxins on HALO<sup>®</sup> 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<sup>®</sup> C18 column.

### TEST CONDITIONS:

**Column:** HALO 90 Å C18, 2.7  $\mu\text{m}$ ,  
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  $\mu\text{L}$

**Sample Solvent:** 70/30 water/methanol

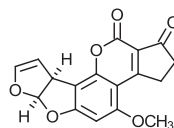
**Response Time:** 0.05 sec

**Data Rate:** 5 Hz

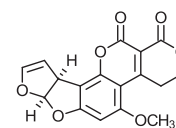
**Flow Cell:** 3.0  $\mu\text{L}$

**LC System:** Shimadzu Nexera X2

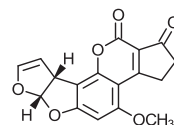
### STRUCTURES:



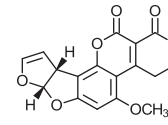
Aflatoxin B1



Aflatoxin G1



Aflatoxin B2



Aflatoxin G2

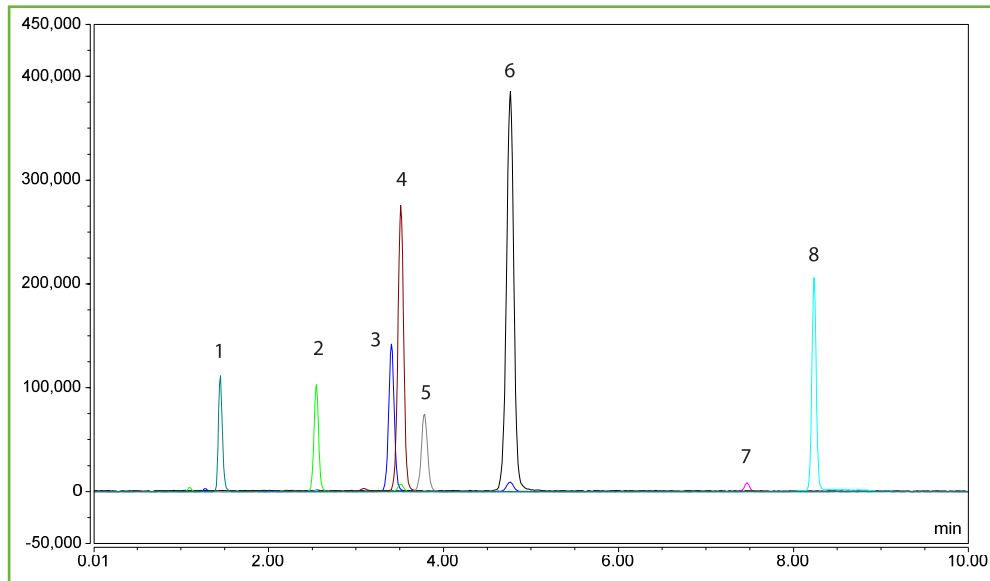


140



## 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. They 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

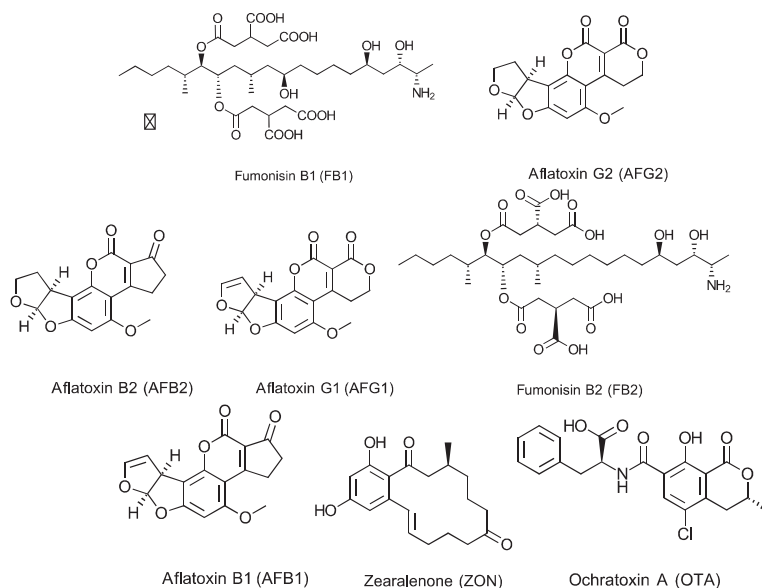
**ESI:** +4

**Heat Block:** 350 °C

**Sheath Gas Flow:** 34.88

**Aux Gas Flow:** 10.00

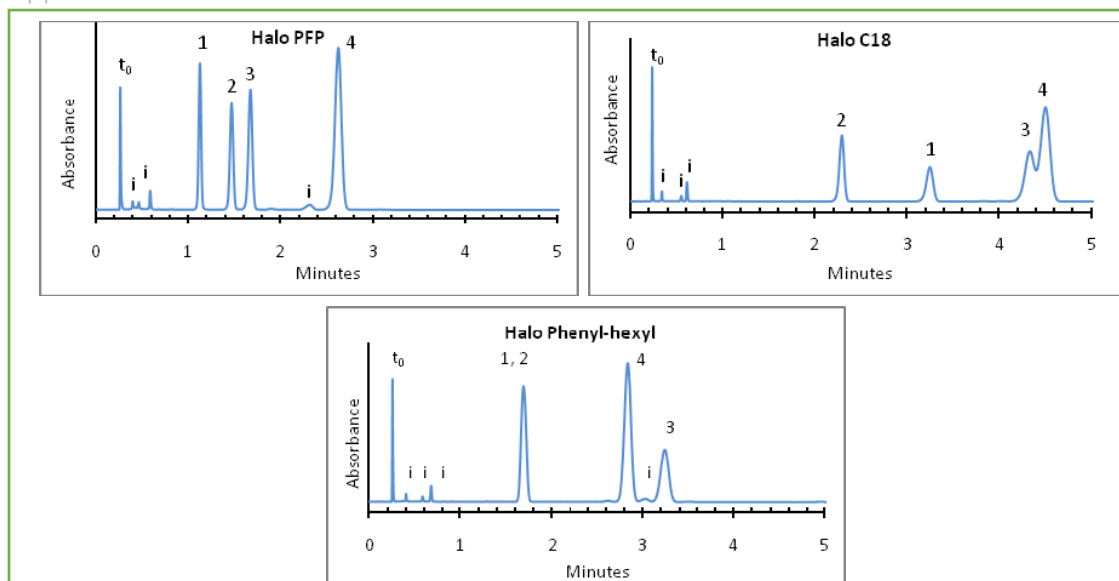
### STRUCTURES:





## Separation of Neutral Aromatics on HALO® PFP, C18 and Phenyl-Hexyl

Application Note 23-N



### 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.

### 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

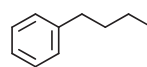
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

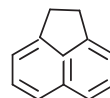
### STRUCTURES:



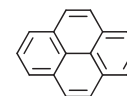
Butylbenzene



1-Phenylnaphthalene



Acenaphthene



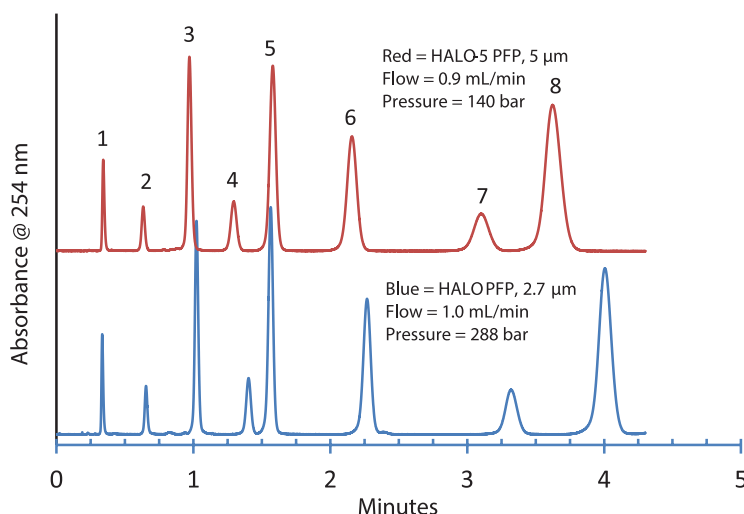
Pyrene





## 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<sub>2</sub>PO<sub>4</sub> 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

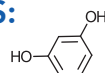
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

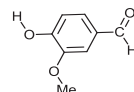
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

### STRUCTURES:



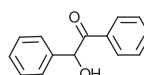
Resorcinol



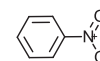
Vanillin



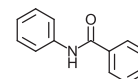
Benzonitrile



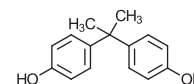
Benzoin



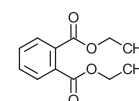
Nitrobenzene



Benzanilide



Bisphenol A



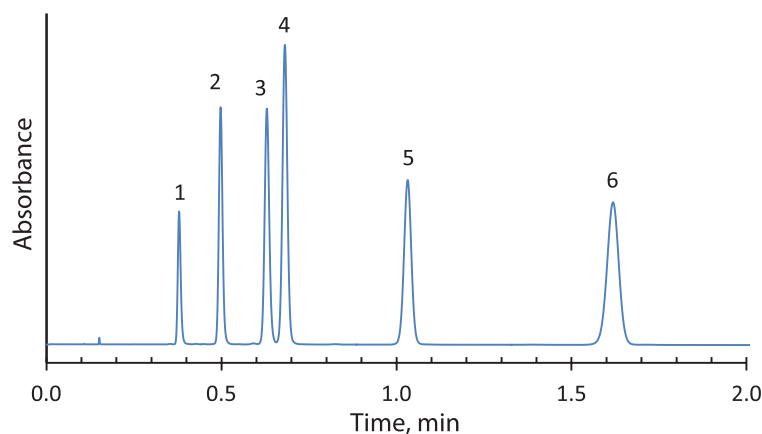
Diethylphthalate





## 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  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** Acetonitrile/water

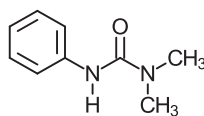
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

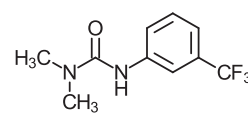
**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu$ L

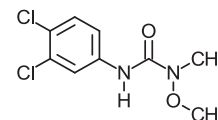
### STRUCTURES:



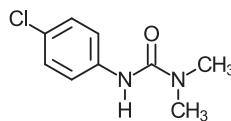
Fenuron



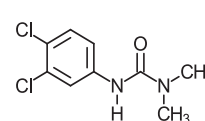
Fluomethuron



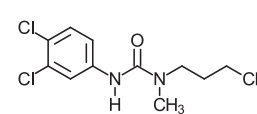
Linuron



Monuron



Diuron



Neburon

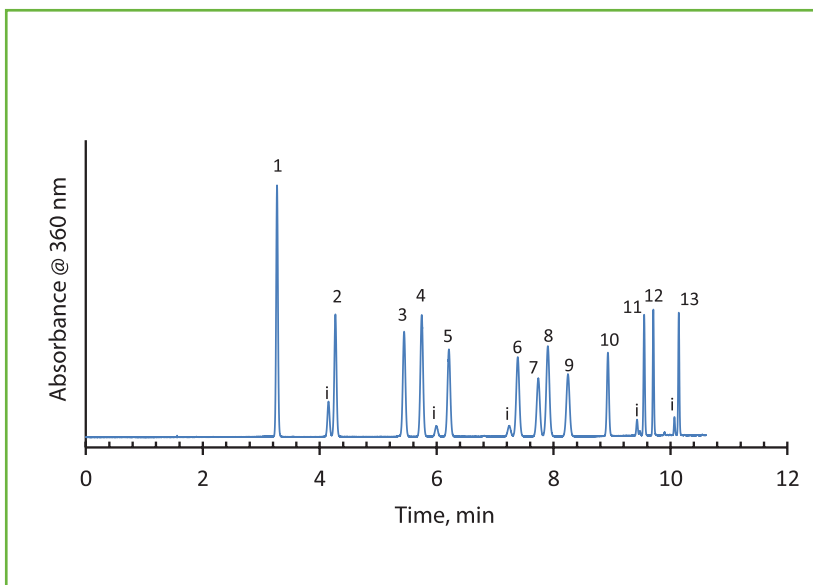






## Separation of Carbonyl Compounds as Dinitrophenylhydrazone Derivatives on HALO® C18, 2.7 µm

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

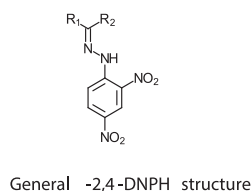
**LC System:** Shimadzu Prominence UFLC XR

**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:

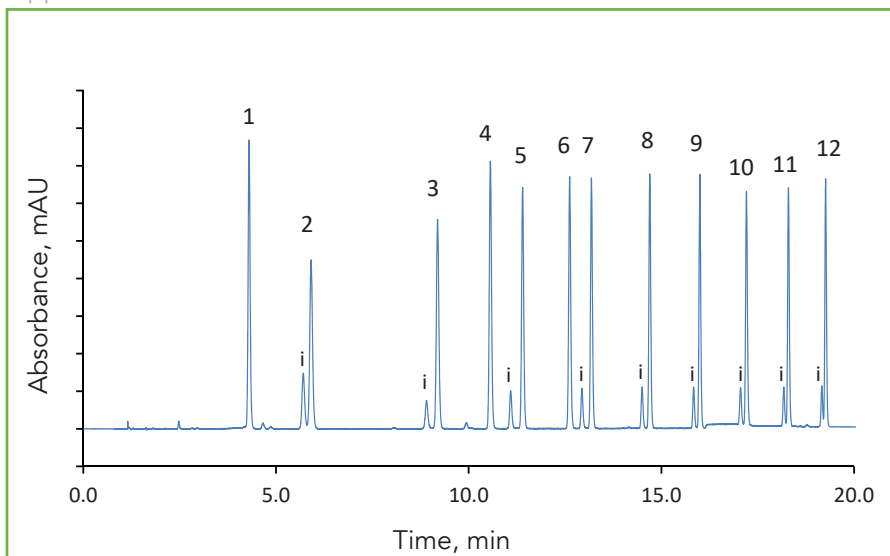
Peak	R1	R2
1	-H	-H
2	-H	-CH <sub>3</sub>
3	-CH <sub>3</sub>	-CH <sub>3</sub>
4	-H	
5	-H	
6	-H	
7	-CH <sub>3</sub>	
8	-H	
9	-H	
10	-H	
11	-H	
12	-H	
13	-H	





## 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

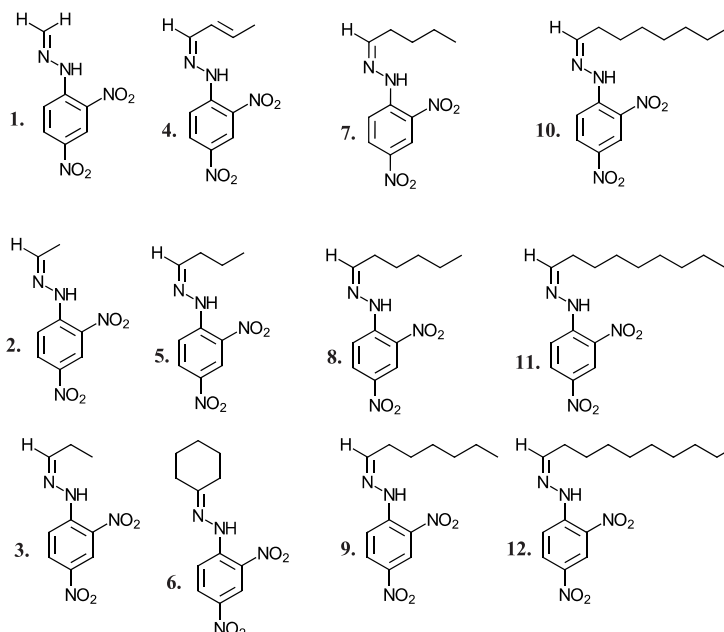
**Sample Solvent:** 50/50 ACN/water

**Response Time:** 0.12 sec

**Flow Cell:** 5.0 µL semi-micro, bypassed

**LC System:** Agilent 1100 Series Quaternary

### STRUCTURES:

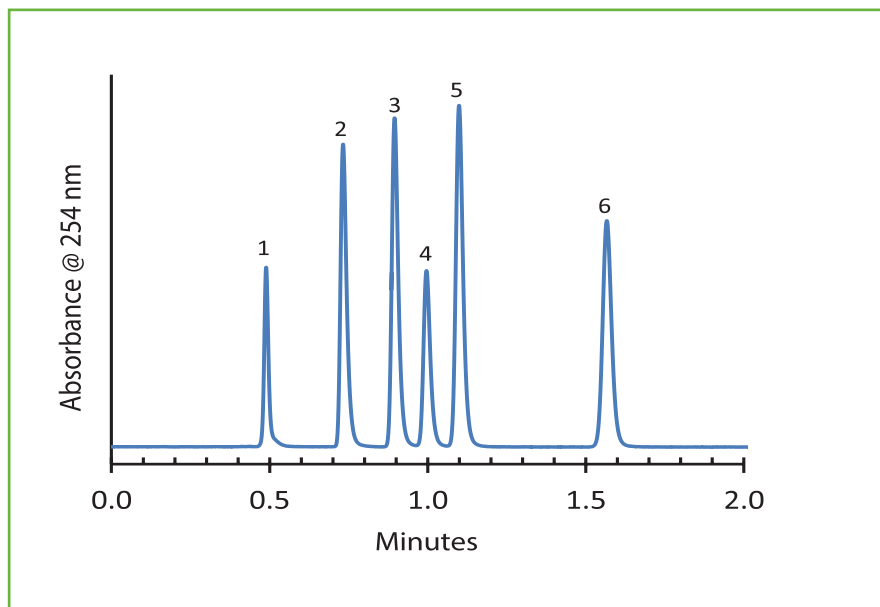


146



## 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

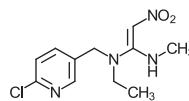
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

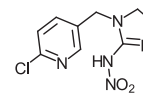
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

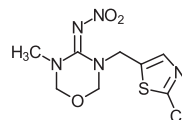
### STRUCTURES:



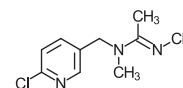
Nitenpyram



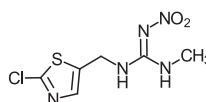
Imidacloprid



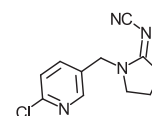
Thiamethoxam



Acetamiprid



Clothianidin



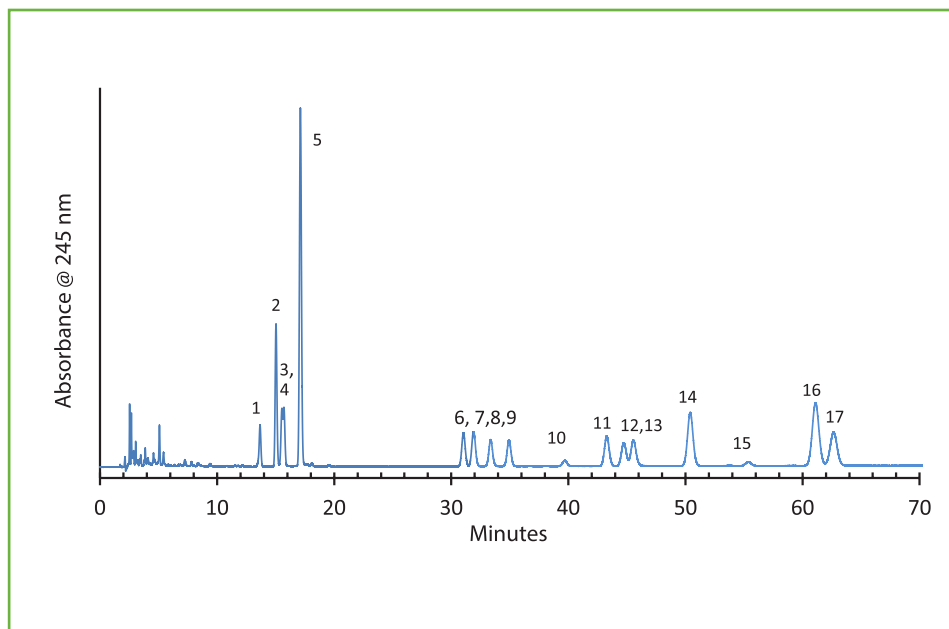
Thiacloprid





## 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

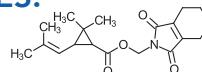
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

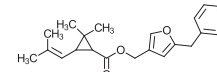
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

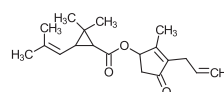
### STRUCTURES:



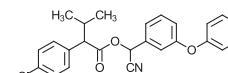
Tetramethrin



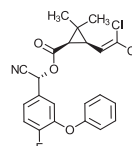
Resmethrin



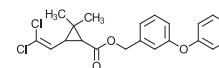
Allethrin



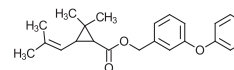
Fenvalerate



Cyfluthrin



Permethrin



Phenothrin

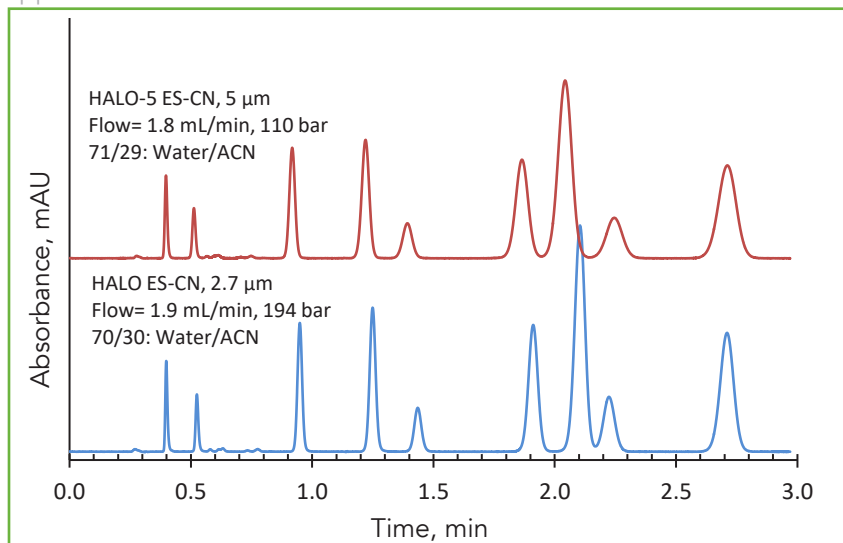






## Comparison of Selectivity of HALO® ES-CN, 5 µm and 2.7 µm Phases

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

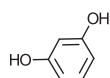
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

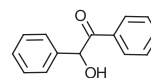
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

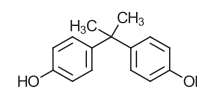
### STRUCTURES:



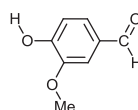
Resorcinol



Benzoin



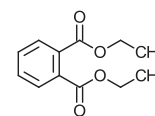
Bisphenol A



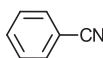
Vanillin



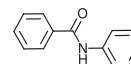
Nitrobenzene



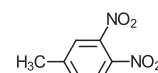
Diethylphthalate



Benzonitrile



Benzanilide



3,4-Dinitrotoluene

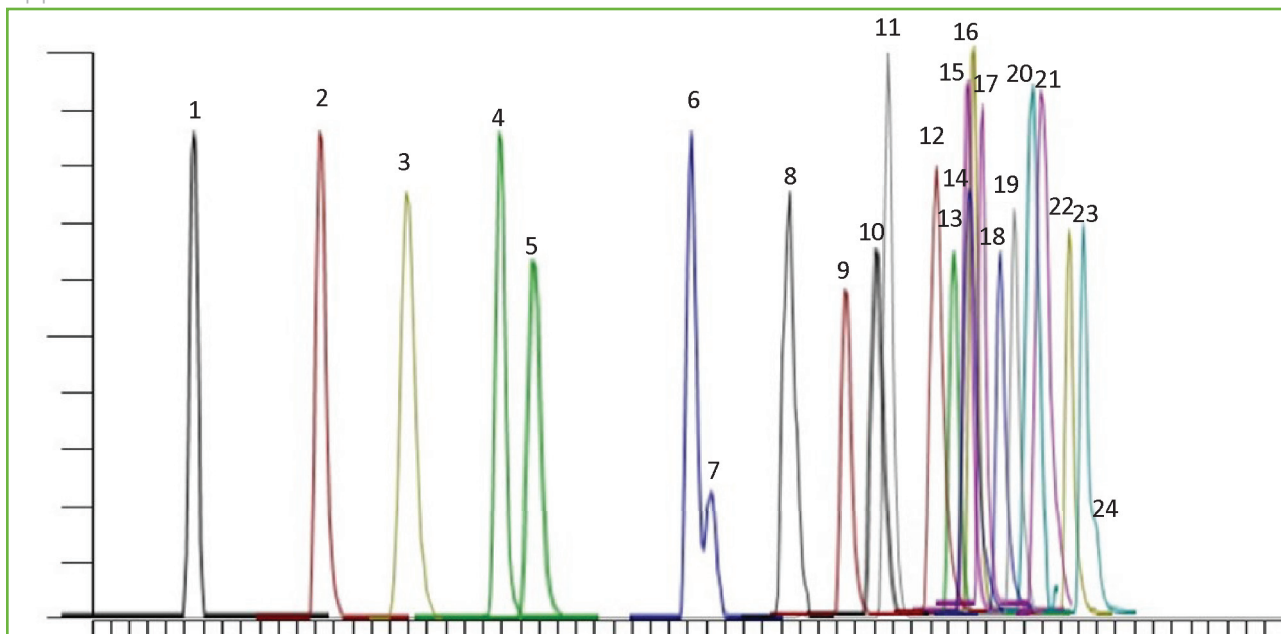






## High Throughput, High speed LC-MS/MS Separation of Mycotoxins on HALO® PFP, 2 µm

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





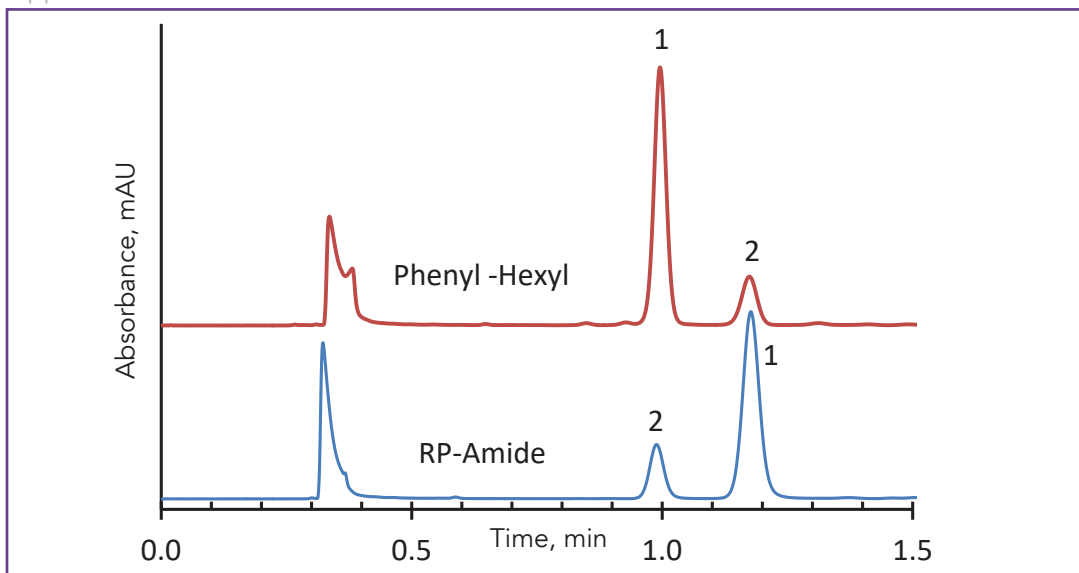
## PEAK IDENTITIES:

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-glucoside	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-Acetyldeoxynivalenol	3.33	339.1378	321.15
7	3-Acetyldeoxynivalenol	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



## Separation of Diosmin and Hesperidin on HALO<sup>®</sup> Phenyl-Hexyl and HALO<sup>®</sup> 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<sup>®</sup> RP-Amide or HALO<sup>®</sup> Phenyl-Hexyl phases. Note the difference in elution order on the two phases.

### TEST CONDITIONS:

#### Columns:

1) HALO 90 Å Phenyl-Hexyl, 2.7  $\mu$ m, 4.6 x 50 mm

**Part Number:** 92814-406

2) HALO 90 Å RP-Amide, 2.7  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** Dimethylformamide (needed for solubility reasons)

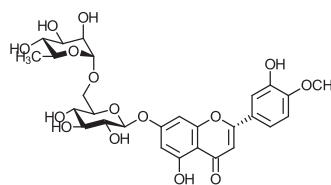
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

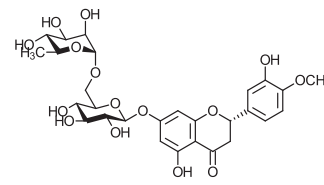
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu$ L

### STRUCTURES:



Diosmin



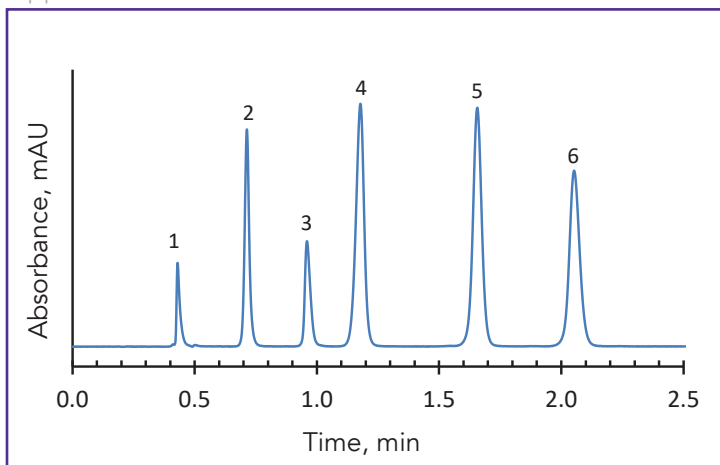
Hesperidin





## Separation of Biogenic Amines on HALO® Phenyl-Hexyl 5 µm by Ion-Pairing

Application Note 140-B



### PEAK IDENTITIES:

1. System peak,  $t_0$
2. L-Tyrosine
3. Octopamine
4.  $\pm$  Synephrine
5. Tyramine
6. Hordenine

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.

### 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

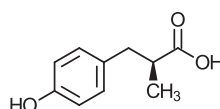
**Data Rate:** 25 Hz

**Flow Cell:** 2.5 µL semi-micro

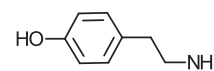
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

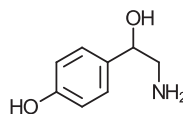
### STRUCTURES:



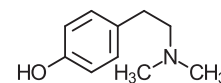
L-Tyrosine



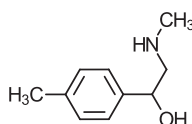
Tyramine



Octopamine



Hordenine



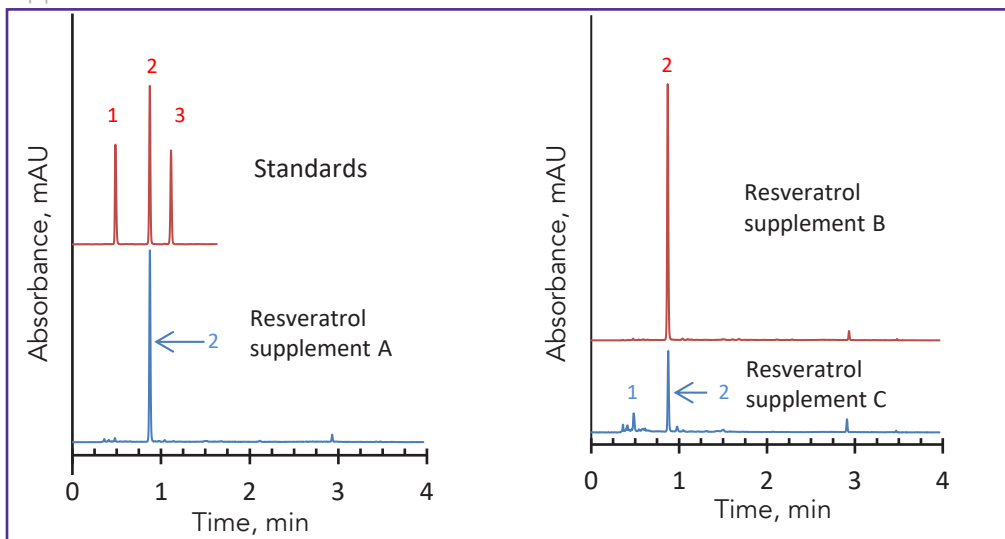
$\pm$  Synephrine





## Separation of Resveratrols on HALO<sup>®</sup> C18, 2.7 $\mu$ m

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<sup>®</sup> Fused-Core<sup>®</sup> C18 columns.

### TEST CONDITIONS:

**Column:** HALO 90 Å C18, 2.7  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** 50/50 acetonitrile/methanol

**Response Time:** 0.02 sec

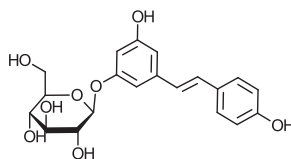
**Data Rate:** 25 Hz

**Flow Cell:** 2.5  $\mu$ L semi-micro

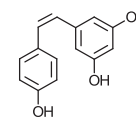
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu$ L

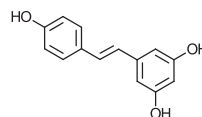
### STRUCTURES:



Polydatin



cis-Resveratrol



trans-Resveratrol

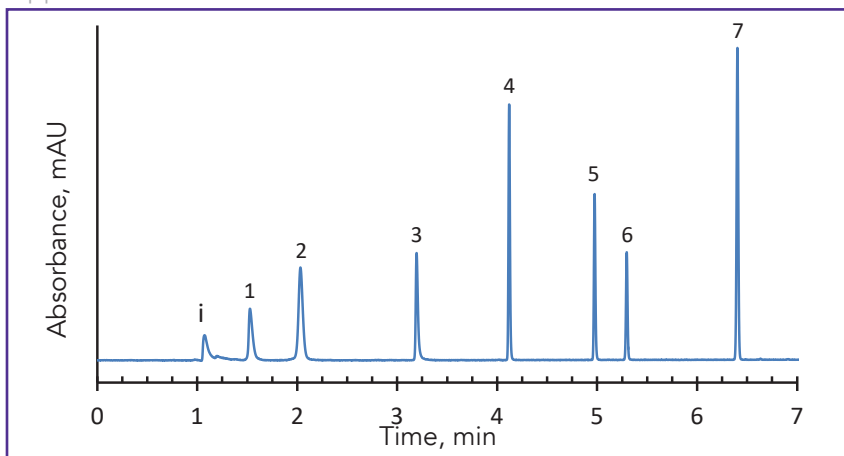






## Separation of Melatonin and Related Compounds on HALO® RP-Amide

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

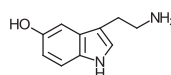
**Data Rate:** 25 Hz

**Flow Cell:** 2.5 µL semi-micro

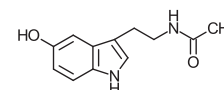
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

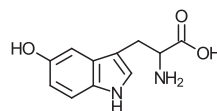
### STRUCTURES:



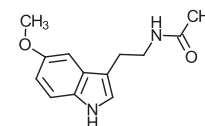
Serotonin



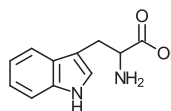
N-Acetyl-5-hydroxytryptamine



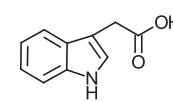
5-Hydroxy-L-tryptophan



Melatonin



L-Tryptophan



3-Indoleacetic acid



Indole

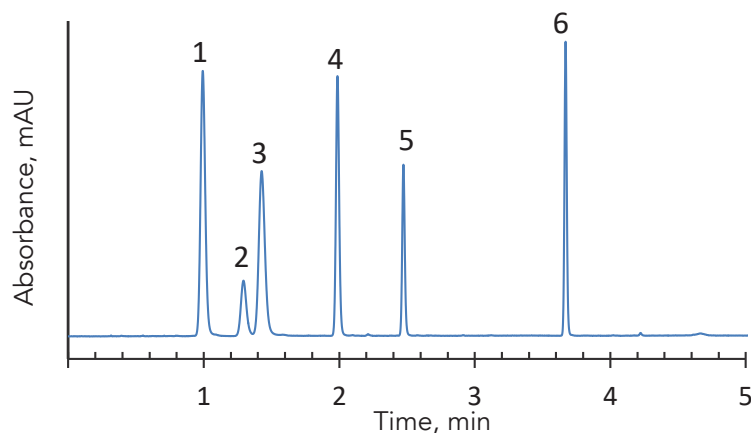


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## Separation of Resveratrols and Related Compounds on HALO® C18, 5 µm

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

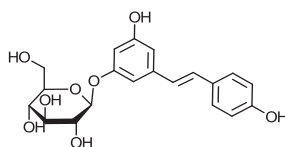
**Data Rate:** 25 Hz

**Flow Cell:** 2.5 µL semi-micro

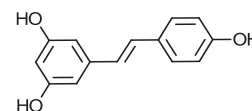
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

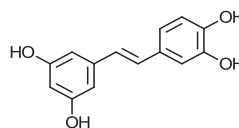
### STRUCTURES:



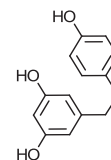
trans-Polydatin



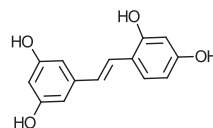
trans-Resveratrol



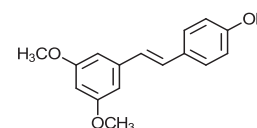
Piceatannol



cis-Resveratrol



trans-Oxyresveratrol



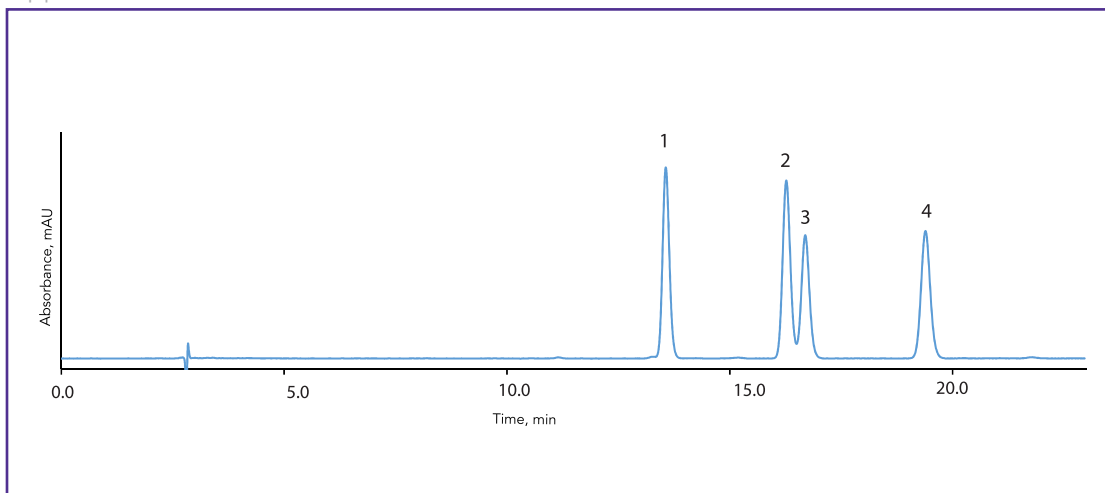
Pterostilbene





## Separation of Tocopherols on HALO® C30 based on GB (Chinese Standards)

Application Note 189-V



### PEAK IDENTITIES:

1.  $\delta$ -tocopherol
2.  $\gamma$ -tocopherol
3.  $\beta$ -tocopherol
4.  $\alpha$ -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  $\mu$ m,  
4.6 x 250 mm

**Part Number:** 92114-930

**Mobile Phase:**

- A: Water  
B: Methanol

**Isocratic:** 95% B

**Flow Rate:** 0.9 mL/min

**Initial Pressure:** 240 bar

**Temperature:** 30 °C

**Detection:** UV 294 nm, PDA

**Injection Volume:** 20  $\mu$ L

**Sample Solvent:** Methanol

**Response Time:** 2.0 sec

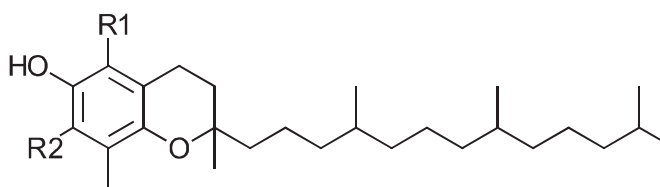
**Data Rate:** 20 Hz

**Flow Cell:** 13  $\mu$ L

**LC System:** Agilent 1100

Data Courtesy of Beijing Institute for Drug Control

### STRUCTURE:



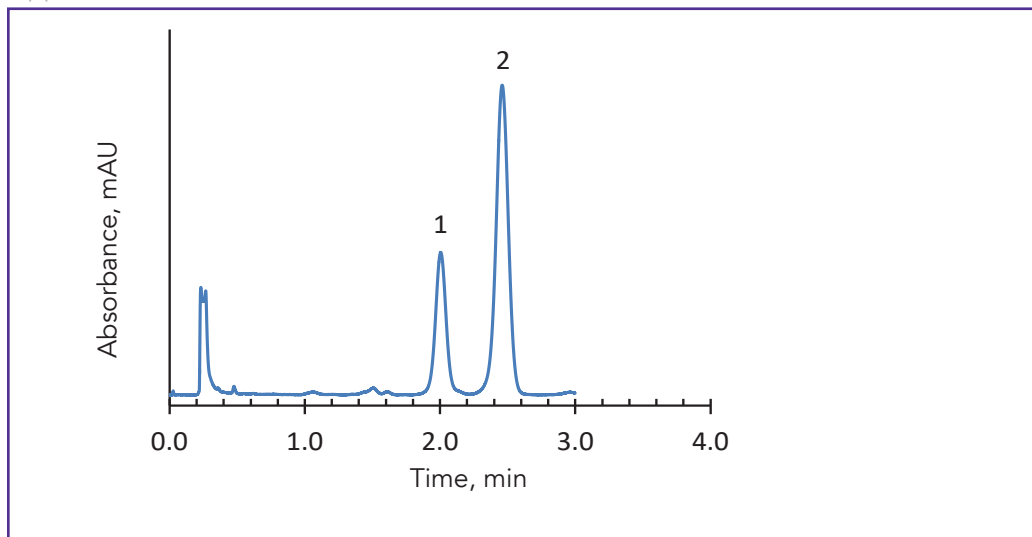
Tocopherol	R1	R2
Alpha ( $\alpha$ )	CH <sub>3</sub>	CH <sub>3</sub>
Beta ( $\beta$ )	CH <sub>3</sub>	H
Gamma ( $\gamma$ )	H	CH <sub>3</sub>
Delta ( $\delta$ )	H	H





## HPLC Separation of Hesperidin and Diosmin on HALO<sup>®</sup> PFP, 5 $\mu$ m

Application Note 84-FL



### PEAK IDENTITIES:

1. Hesperidin
2. Diosmin

These two semisynthetic flavonoids can be rapidly separated using HALO<sup>®</sup> PFP (pentafluorophenyl) 5  $\mu$ m 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  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** Dimethylformamide (needed  
for solubility reasons)

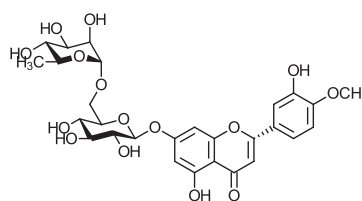
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

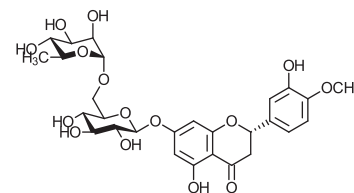
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu$ L

### STRUCTURES:



Diosmin

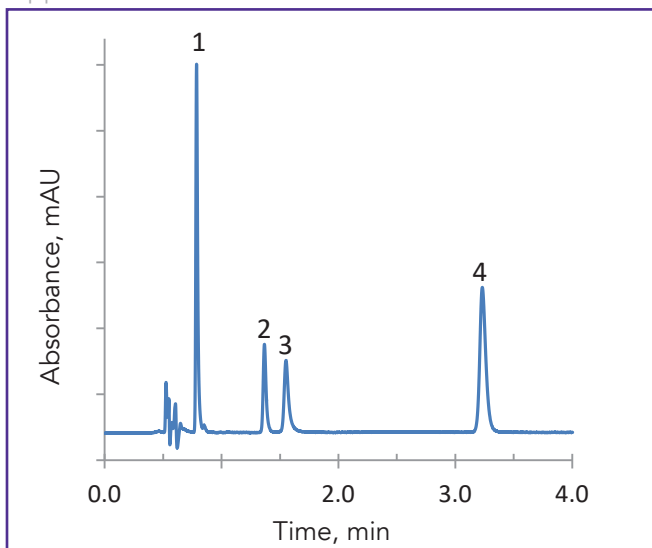


Hesperidin



## 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

A fast separation of four water soluble vitamins is accomplished on a 2.0 µm HALO® HILIC column.

### 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

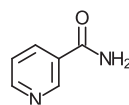
**Response Time:** 0.1 sec

**Data Rate:** 40 Hz

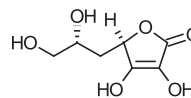
**Flow Cell:** 2.5 µL semi-micro

**LC System:** Agilent 1200 SL

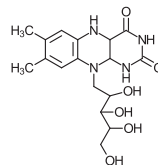
### STRUCTURES:



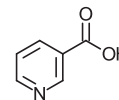
Nicotinamide



Ascorbic Acid



Riboflavin



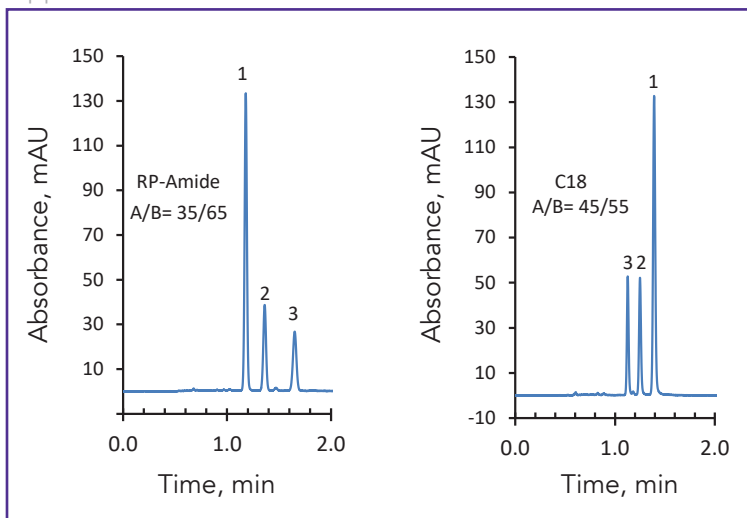
Nicotinic Acid





## Analysis of Curcumins on HALO<sup>®</sup> RP-Amide and HALO<sup>®</sup> C18

Application Note 148-F



### PEAK IDENTITIES:

1. Curcumin
2. Desmethoxycurcumin
3. bis-Desmethoxycurcumin

Turmeric spice contains curcumins that are used as dietary supplements. A methanolic extract of turmeric powder was filtered and analyzed on both HALO<sup>®</sup> C18 and RP-Amide columns, showing the different selectivity for curcumin and two derivatives.

### TEST CONDITIONS:

#### Columns:

1) HALO 90 Å C18, 2.7  $\mu$ m, 4.6 x 100 mm

**Part Number:** 92814-602

2) HALO 90 Å RP-Amide, 2.7  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** Methanol

**Response Time:** 0.02 sec

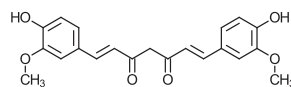
**Data Rate:** 25 Hz

**Flow Cell:** 2.5  $\mu$ L semi-micro

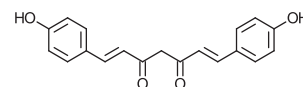
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu$ L

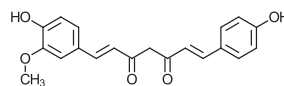
### STRUCTURES:



Curcumin



bis-Desmethoxycurcumin



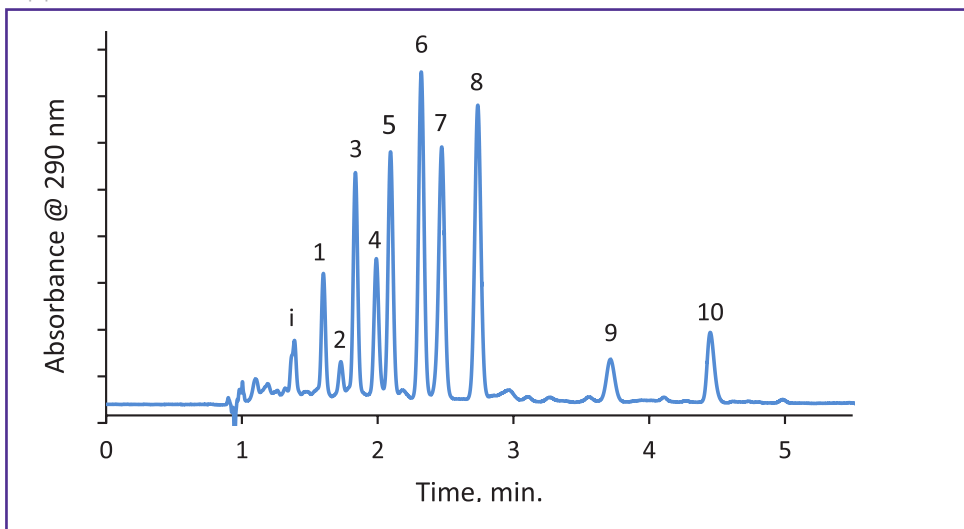
Desmethoxycurcumin





## Rapid Separation of Vitamin E Congeners on HALO® PFP

Application Note 146-V



### PEAK IDENTITIES:

- i = impurity
1.  $\delta$ -Tocotrienol
  2.  $\beta$ -Tocotrienol
  3.  $\gamma$ -Tocotrienol
  4.  $\alpha$ -Tocotrienol
  5.  $\delta$ -Tocopherol
  6.  $\beta$ -Tocopherol
  7.  $\gamma$ -Tocopherol
  8.  $\alpha$ -Tocopherol
  9.  $\alpha$ -Tocopherol acetate
  10.  $\alpha$ -Tocopherol nicotinate

### TEST CONDITIONS:

**Column:** HALO 90 Å PFP, 2.7  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** Ethanol

**Response Time:** 0.05 sec

**Data Rate:** 40 Hz

**Flow Cell:** 1.0  $\mu$ L

**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  $\alpha$ -tocopherol. Only the liquid in the soft gel was used for the analysis. The four tocopherols,  $\alpha$ -tocopherol acetate, and  $\alpha$ -tocopherol nicotinate were standards obtained from SigmaAldrich. The small, unidentified peaks are unknown materials from the soft gel capsule.

### STRUCTURES:

#### Tocopherol/Tocotrienol

Alpha ( $\alpha$ )

Beta ( $\beta$ )

Gamma ( $\gamma$ )

Delta ( $\delta$ )

**R1**

CH<sub>3</sub>

CH<sub>3</sub>

H

H

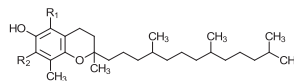
**R2**

CH<sub>3</sub>

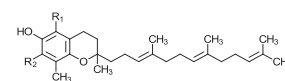
H

CH<sub>3</sub>

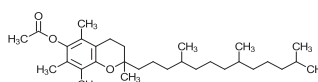
H



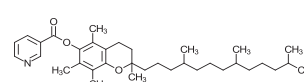
Tocopherol



Tocotrienol



$\alpha$ -Tocopherol acetate



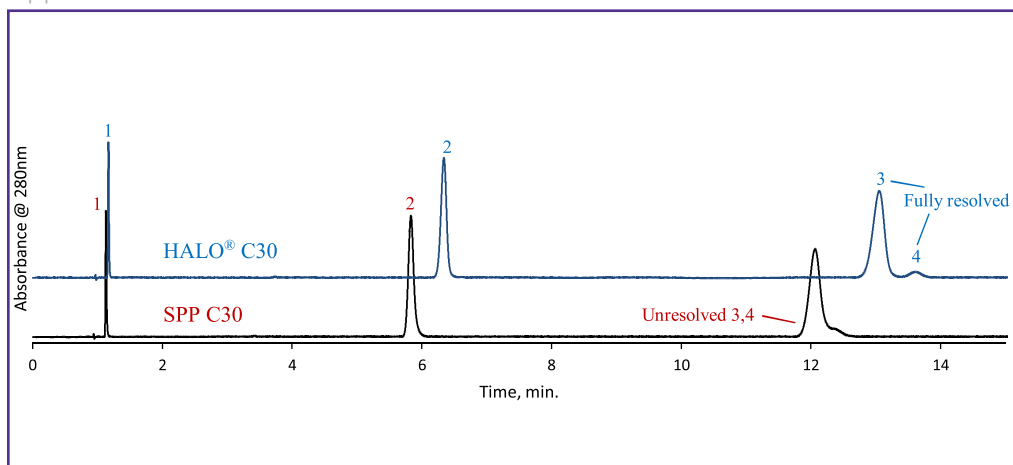
$\alpha$ -Tocopherol nicotinate





## Vitamin K1 Isomer Analysis on HALO® C30

Application Note 180-V



### PEAK IDENTITIES:

1. Menadione (K3)
2. Menaquinone 4 (K2)
3. 2,3-trans-phyloquinone (K1)
4. cis-phyloquinone (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  $\mu$ L

**Sample Solvent:** Methanol

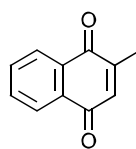
**Response Time:** 0.025 sec

**Data Rate:** 40 Hz

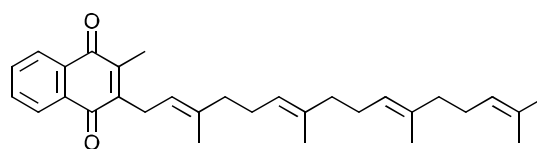
**Flow Cell:** 1.0  $\mu\text{L}$

**LC System:** Shimadzu Nexera X2

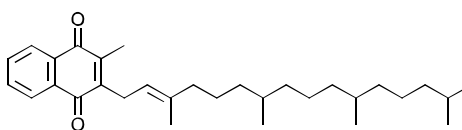
## STRUCTURES:



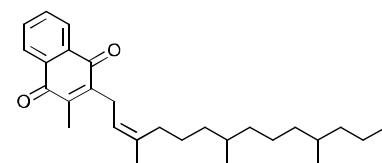
### Vitamin K3: Menadione



Vitamin K2: Menaquinone 4



Vitamin K1: 2,3-*trans*-phyllloquinone



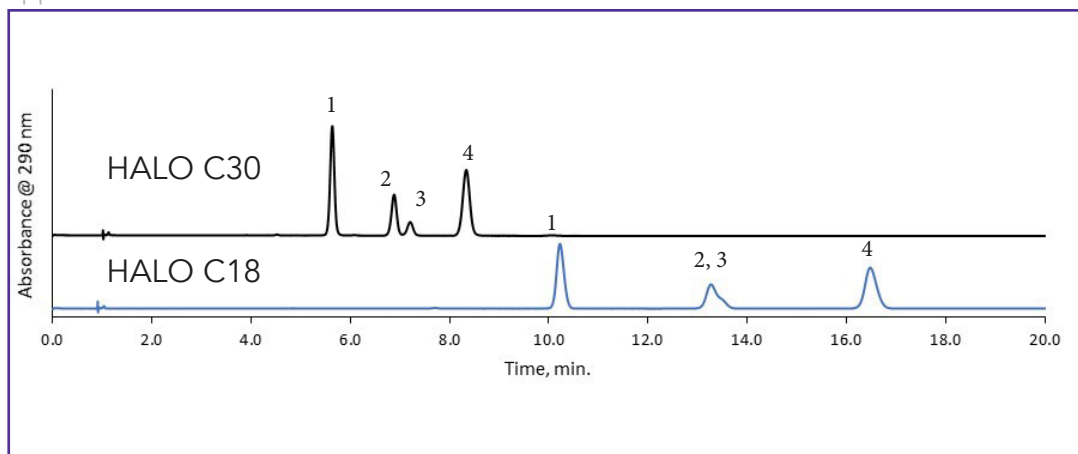
Vitamin K1: *cis*-phyllloquinone





## Separation of Tocopherols on HALO<sup>®</sup> C30

Application Note 185-V



### PEAK IDENTITIES:

1.  $\delta$ -tocopherol
2.  $\gamma$ -tocopherol
3.  $\beta$ -tocopherol
4.  $\alpha$ -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<sup>®</sup> C18 has more surface area (135 m<sup>2</sup>/g vs. 90 m<sup>2</sup>/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  $\mu$ m, 4.6 x 150 mm  
Part Number: 92114-730
- 2) HALO 90 Å C18, 2.7  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** Ethanol/methanol

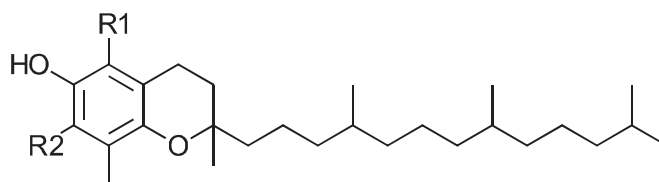
**Response Time:** 0.02 sec

**Data Rate:** 80 Hz

**Flow Cell:** 2.0  $\mu$ L

**LC System:** Agilent 1200 SL

### STRUCTURES:



Tocopherol

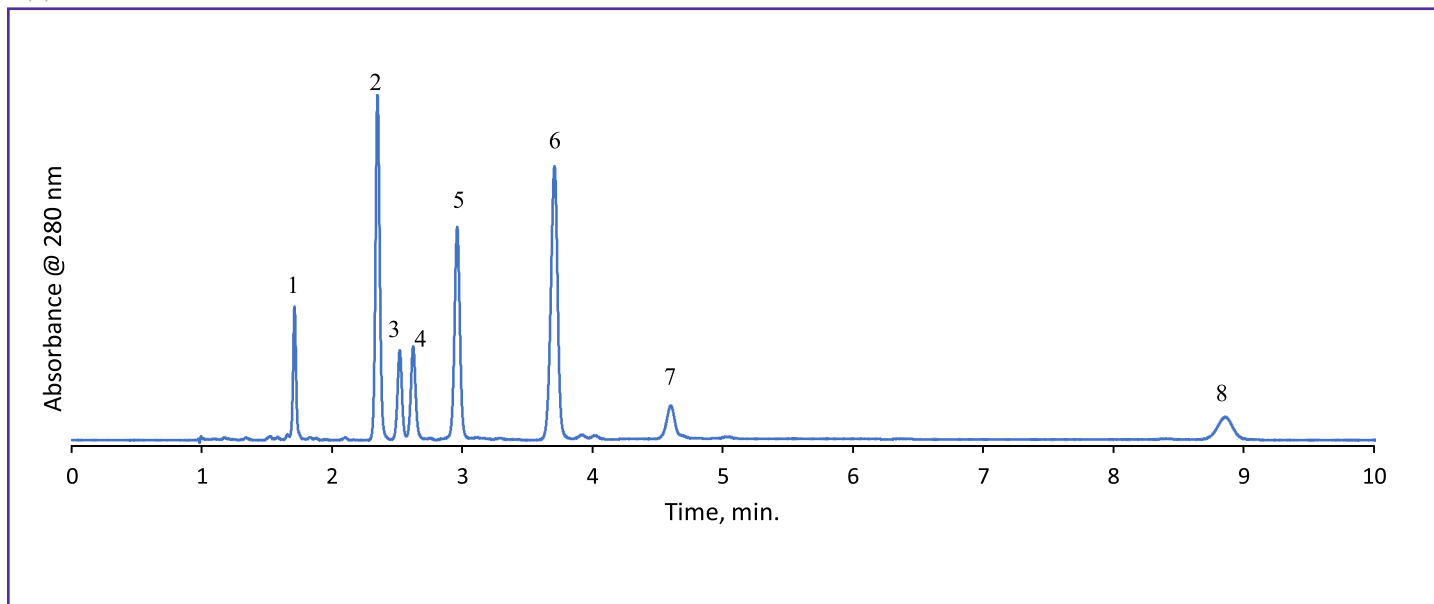
Tocopherol	R1	R2
Alpha ( $\alpha$ )	CH <sub>3</sub>	CH <sub>3</sub>
Beta ( $\beta$ )	CH <sub>3</sub>	H
Gamma ( $\gamma$ )	H	CH <sub>3</sub>
Delta ( $\delta$ )	H	H





## Separation of Fat Soluble Vitamins on HALO<sup>®</sup> 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<sup>®</sup> 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-phyloquinone (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:

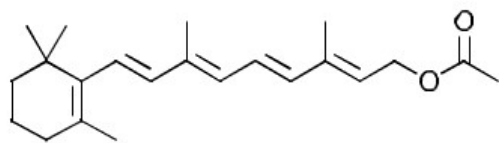
**Column:** HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm  
**Part Number:** 92114-730  
**Isocratic:** 100% methanol  
**Flow Rate:** 1.5 mL/min  
**Pressure:** 262 bar  
**Temperature:** 30 °C  
**Detection:** UV 280 nm, PDA  
**Injection Volume:** 2.0 µL  
**Sample Solvent:** Methanol  
**Response Time:** 0.025 sec  
**Data Rate:** 40 Hz  
**Flow Cell:** 1.0 µL  
**LC System:** Shimadzu Nexera X2



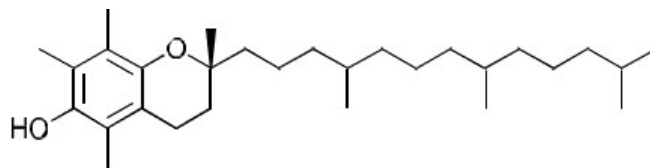




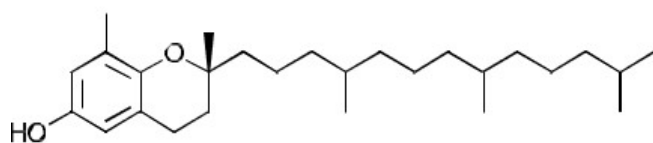
## STRUCTURES:



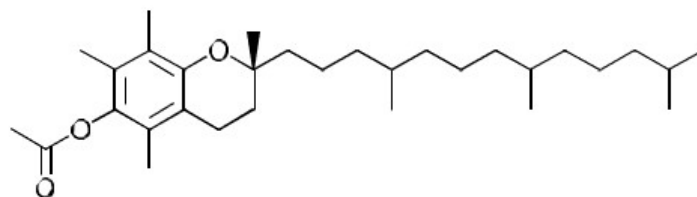
Retinyl acetate (A)



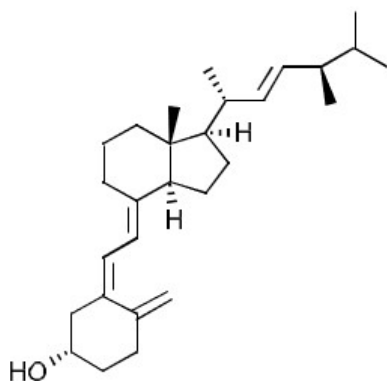
Alpha tocopherol (E)



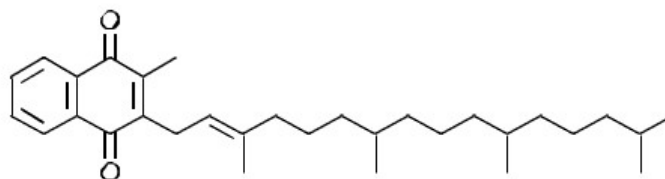
Delta tocopherol (E)



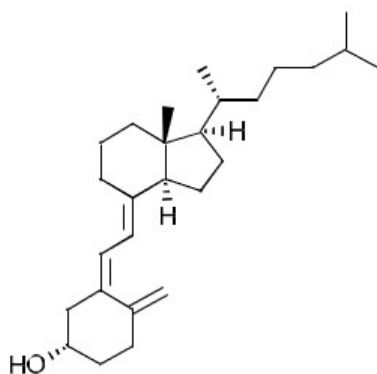
DL-alpha-tocopherol acetate (E)



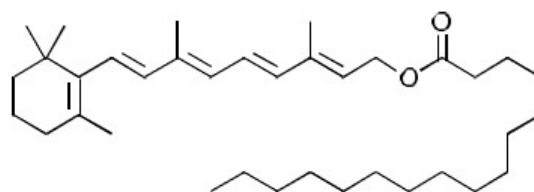
Ergocalciferol (D2)



2,3-trans-phyloquinone (K)



Cholecalciferol (D3)

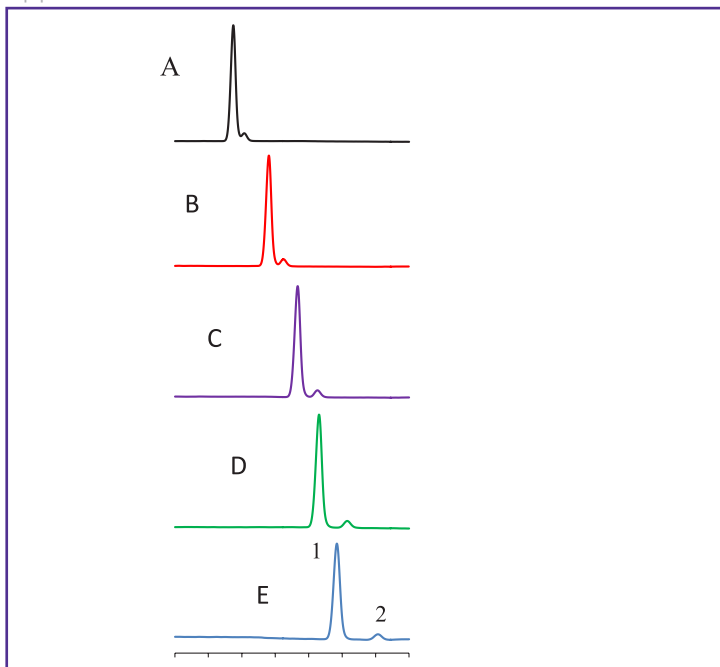


Retinyl palmitate (A)



## Vitamin K1 Analysis: Temperature vs. Resolution

Application Note 197-V



### PEAK IDENTITIES:

1. 2,3-trans-phyloquinone (K1)
2. cis-phyloquinone (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

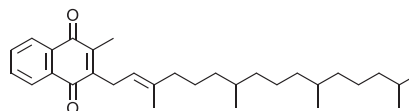
**Sample Solvent:** Methanol

**Response Time:** 0.12 sec.

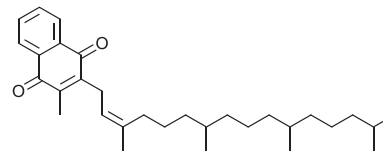
**Flow Cell:** 5 µL Semi-Micro

**LC System:** Agilent 1100 Series

### STRUCTURES:



Vitamin K1: 2,3-trans-phyloquinone



Vitamin K1: cis-phyloquinone

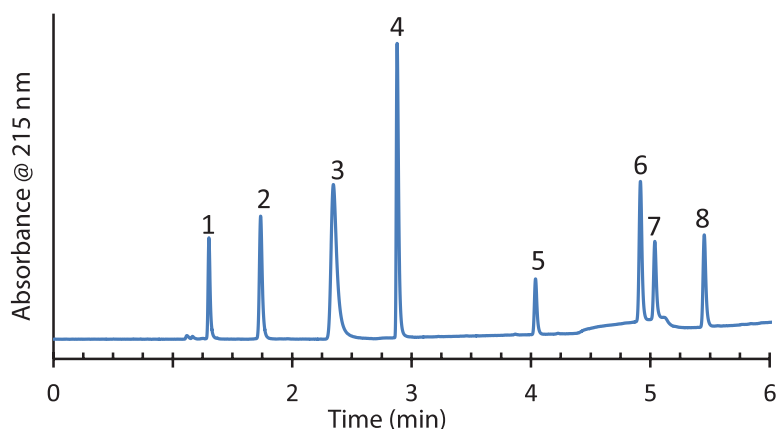
	Resolution	Temperature
A	1.53	35 °C
B	1.58	30 °C
C	1.78	25 °C
D	2.2	20 °C
E	3.03	15 °C





## Separation of Water-Soluble Vitamins on HALO<sup>®</sup> 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<sup>®</sup> 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  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** water

**Detection:** 215 nm, VWD Response Time: 0.02 sec.

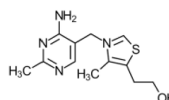
**Data Rate:** 25 Hz

**Flow Cell:** 2.5  $\mu$ L semi-micro

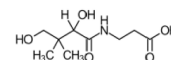
**LC System:** Shimadzu Prominence UFLC XR

**ECV:** ~14  $\mu$ L

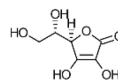
### STRUCTURES:



Thiamine



Pantothenic acid

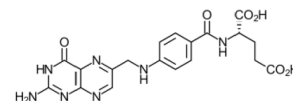


Ascorbic acid

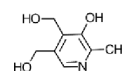
**Cyanocobalamin**  
(structure not included to space constraints)



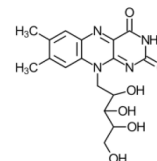
Nicotinamide



Folic Acid



Pyridoxine



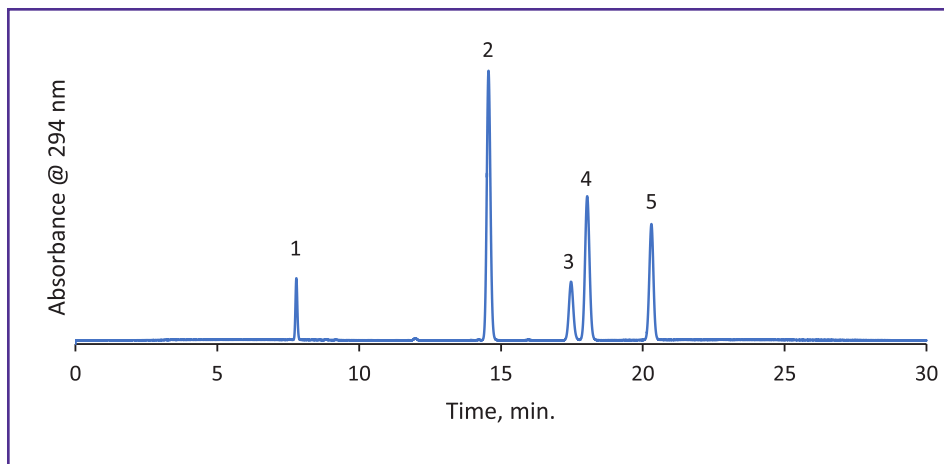
Riboflavin





### Analysis of Vitamin A and Vitamin E Isomers using GB Method

Application Note 210-V



#### PEAK IDENTITIES:

1. Retinyl Acetate
2.  $\delta$ -tocopherol
3.  $\gamma$ -tocopherol
4.  $\beta$ -tocopherol
5.  $\alpha$ -tocopherol

The 2.7  $\mu\text{m}$  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  $\mu\text{m}$   
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  $\mu\text{L}$

**Sample Solvent:** Methanol/ Ethanol

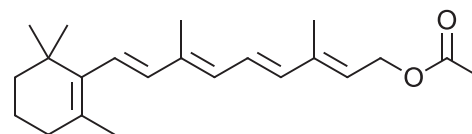
**Data Rate:** 14 Hz

**Response Time:** 0.12 sec.

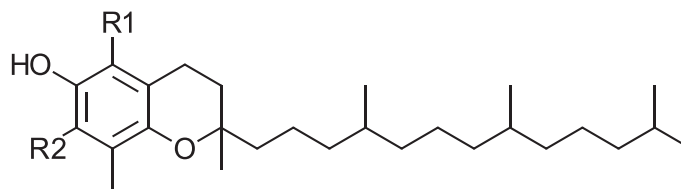
**Flow Cell:** 5  $\mu\text{L}$  semi-micro

**LC System:** Agilent 1100

#### STRUCTURES:



Retinyl acetate



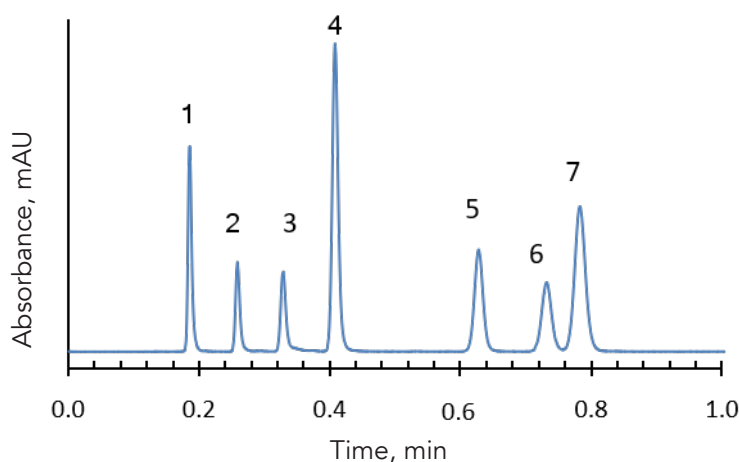
Tocopherol	R1	R2
Alpha ( $\alpha$ )	CH <sub>3</sub>	CH <sub>3</sub>
Beta ( $\beta$ )	CH <sub>3</sub>	H
Gamma ( $\gamma$ )	H	CH <sub>3</sub>
Delta ( $\delta$ )	H	H





## 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

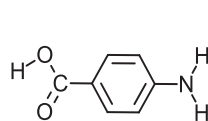
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

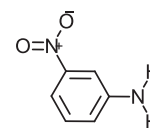
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

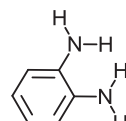
### STRUCTURES:



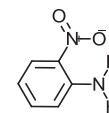
p-Aminobenzoic acid



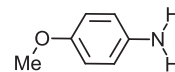
3-Nitroaniline



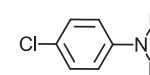
1,2-phenylenediamine



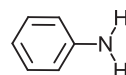
2-Nitroaniline



p-Anisidine



4-Chloroaniline



Aniline

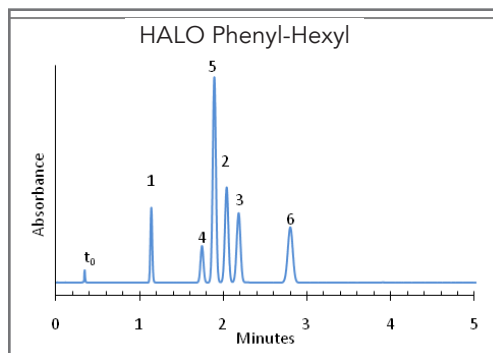
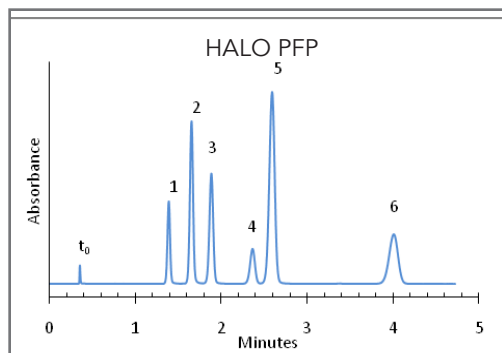






## Separation of Aromatic Nitro Compounds on HALO<sup>®</sup> PFP and Phenyl-Hexyl

Application Note 26-P



### PEAK IDENTITIES:

1. Nitrobenzene
2. 1-Chloro-4-Nitrobenzene
3. 2,6-Dinitrotoluene
4. 4-Nitrotoluene
5. 3-Nitrotoluene
6. 4-Chloro-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  $\mu\text{m}$ , 4.6 x 50 mm

**Part Number:** 92814-409

2) HALO 90 Å Phenyl-Hexyl, 2.7  $\mu\text{m}$ , 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  $\mu\text{L}$

**Sample Solvent:** ~20/80 water/methanol

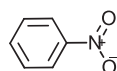
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu\text{L}$  semi-micro

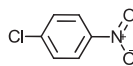
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu\text{L}$

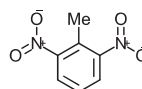
### STRUCTURES:



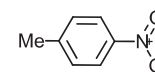
Nitrobenzene



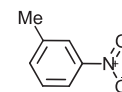
1-Chloro-4-Nitrobenzene



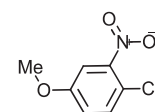
2, 6-Dinitrotoluene



4-Nitrotoluene



3-Nitrotoluene



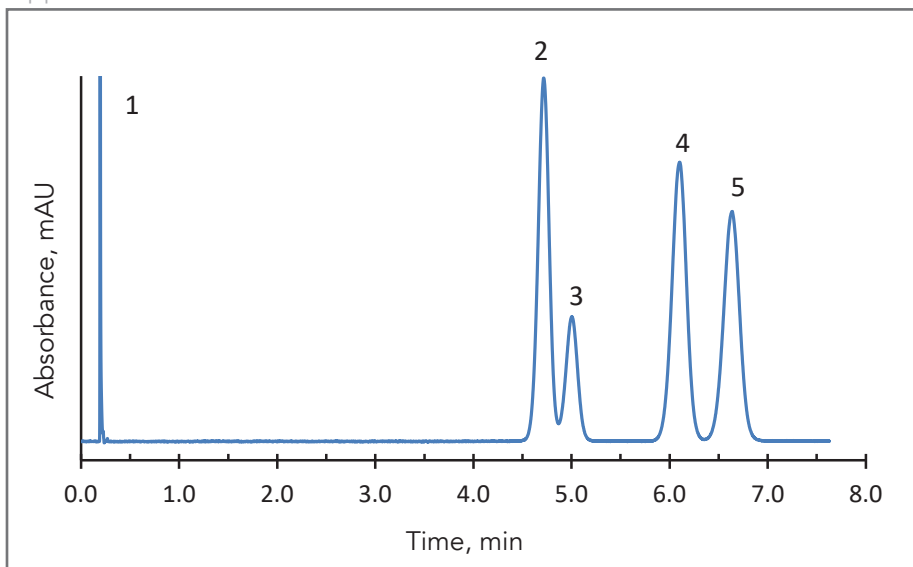
4-Chloro-3-Nitroanisole





## 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

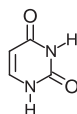
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

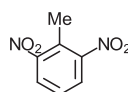
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

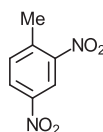
### STRUCTURES:



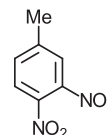
Uracil



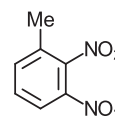
2,6-Dinitrotoluene



2,4-Dinitrotoluene



3,4-Dinitrotoluene



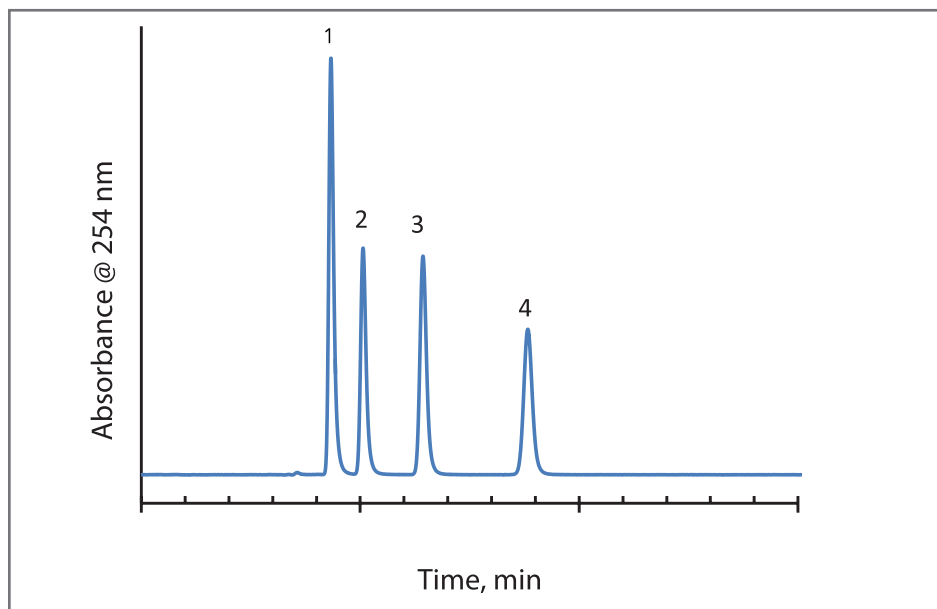
2,3-Dinitrotoluene





## Separation of p-Hydroxybenzoic Acid Esters (Parabens) on HALO<sup>®</sup> C18, 2.7 $\mu$ m

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<sup>®</sup> C18, 2.7  $\mu$ m column at a relatively low pressure.

### TEST CONDITIONS:

**Column:** HALO 90 Å C18, 2.7  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** 50/50 water/methanol

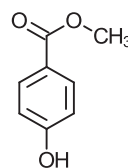
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

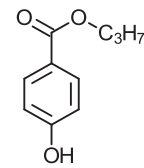
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu$ L

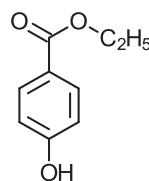
### STRUCTURES:



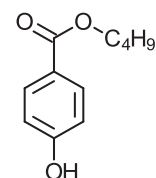
Methyl Paraben



Propyl Paraben



Ethyl Paraben



Butyl Paraben

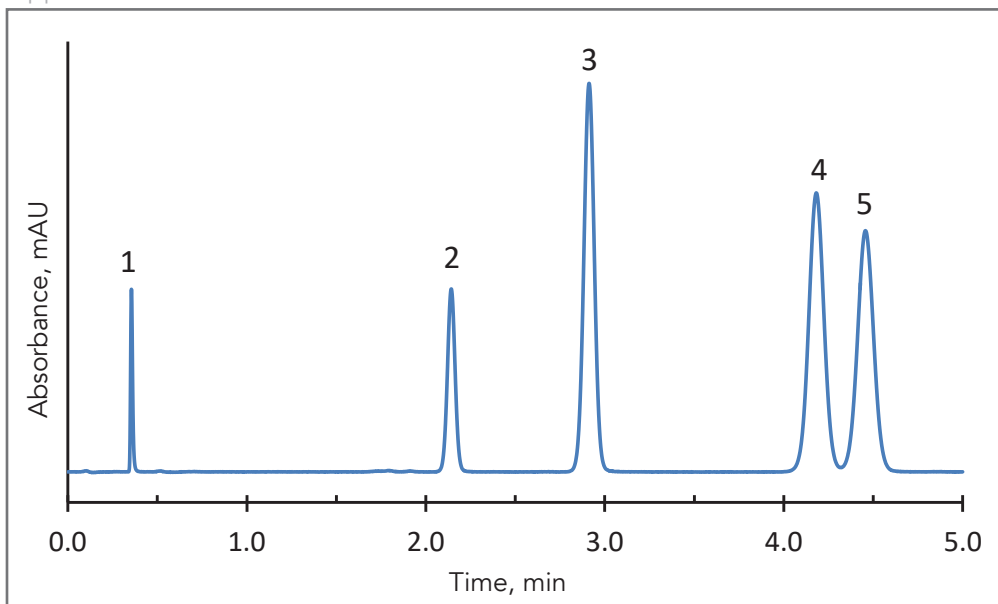


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## Isocratic Separation of Dinitrotoluenes on HALO® PFP Phase

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

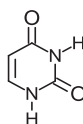
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

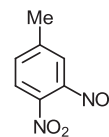
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

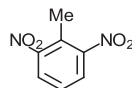
### STRUCTURES:



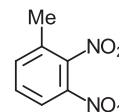
Uracil



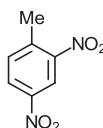
3,4-Dinitrotoluene



2,6-Dinitrotoluene



2,3-Dinitrotoluene



2,4-Dinitrotoluene

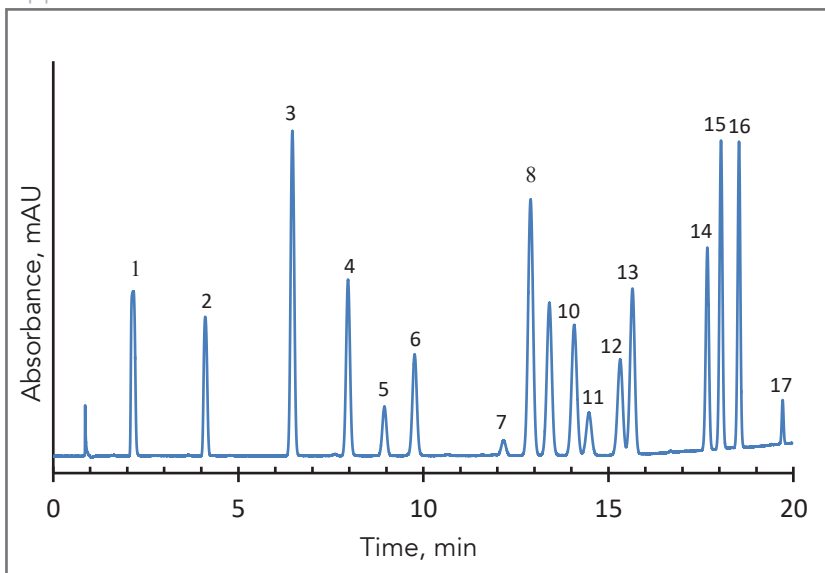


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## Separation of 17 Explosives on HALO® C18, 2.7 µm

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

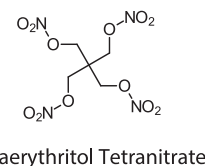
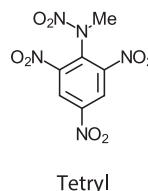
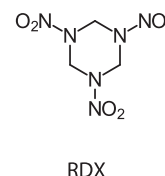
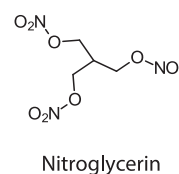
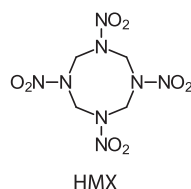
**Data Rate:** 25 Hz

**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

### STRUCTURES:



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.

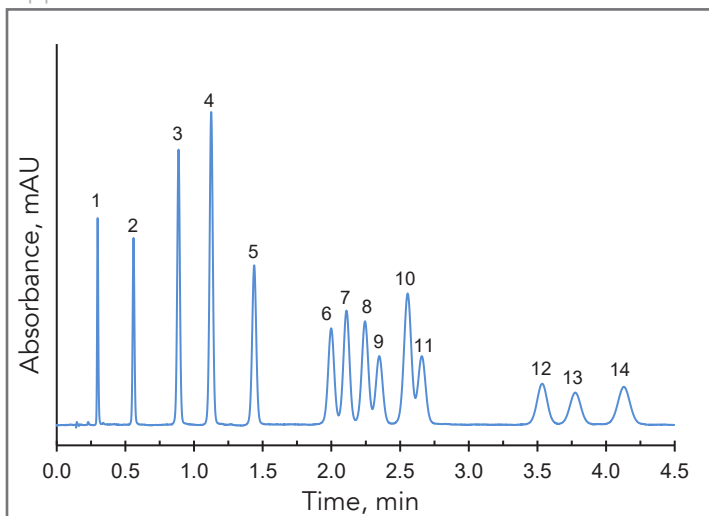






## Separation of Explosives on HALO<sup>®</sup> C18

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<sup>®</sup> 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

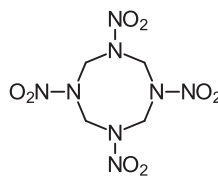
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 μL semi-micro

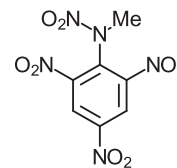
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 μL

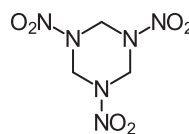
### STRUCTURES:



HMX



Tetryl



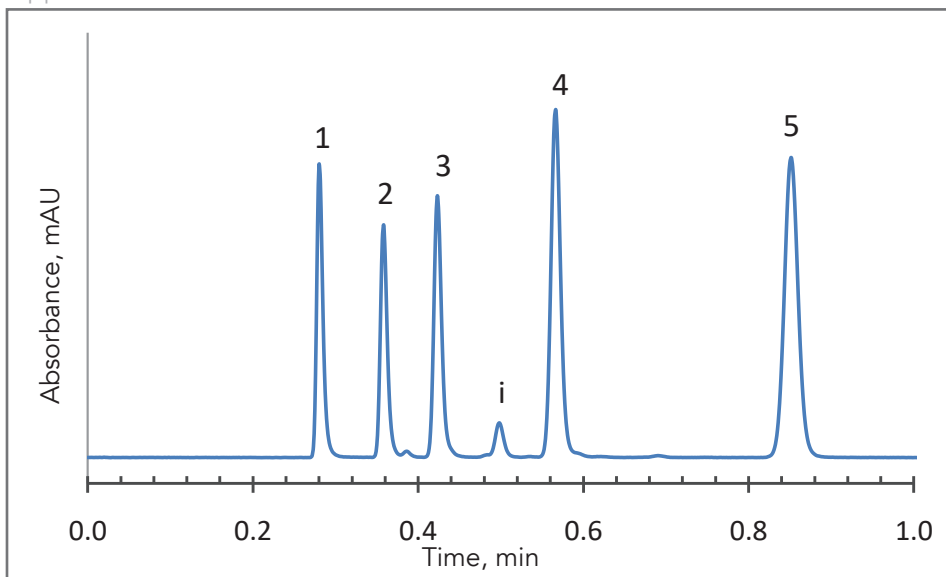
RDX





## Isocratic Separation of Phthalate Esters on HALO<sup>®</sup> C18

Application Note 24-P



### PEAK IDENTITIES:

1. Uracil
2. Dimethylphthalate
3. Diethylphthalate  
i = impurity
4. Di-n-propylphthalate
5. Di-n-butylphthalate

Plasticizers 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<sup>®</sup> C18 column in under one minute.

### TEST CONDITIONS:

**Column:** HALO 90 Å C18, 2.7  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** Acetonitrile

**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

**LC System:** Shimadzu Prominence UFLC XR

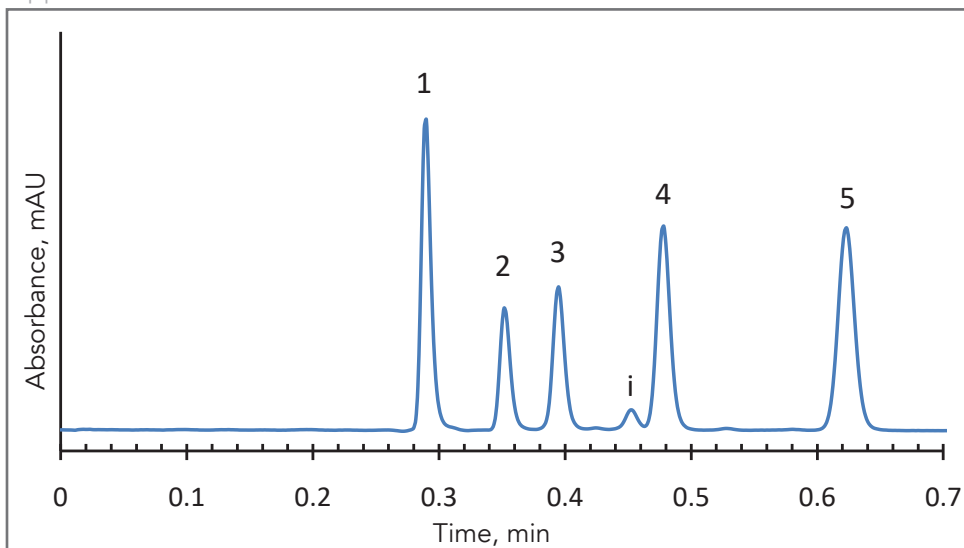
**Extra Column Volume:** ~14  $\mu$ L





## Isocratic Separation of Phthalate Esters on HALO<sup>®</sup> 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<sup>®</sup> 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 issues.

### TEST CONDITIONS:

**Column:** HALO 90 Å RP-Amide, 2.7  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** Acetonitrile

**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

**LC System:** Shimadzu Prominence UFLC XR

**Extra column volume:** ~14  $\mu$ L

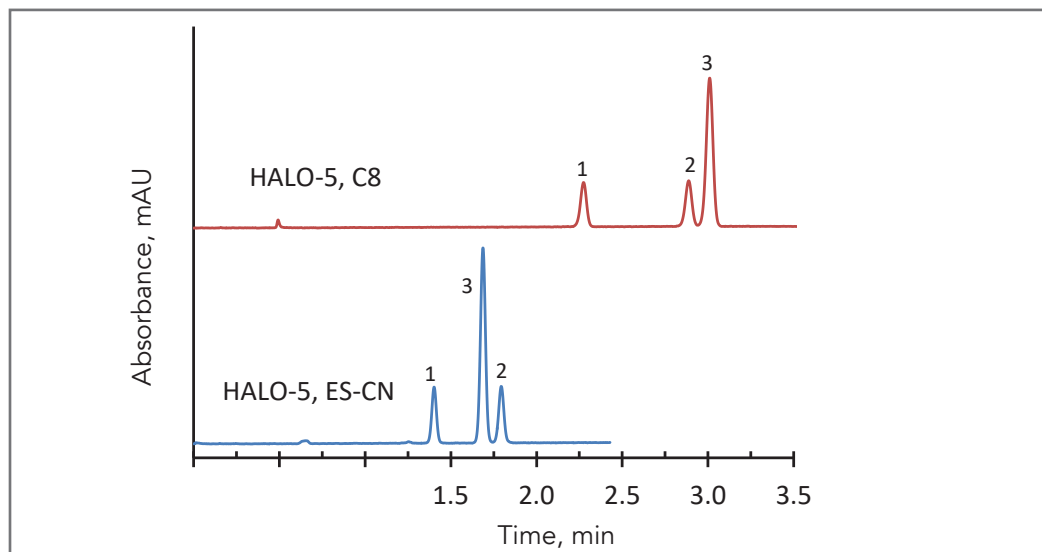


177



## Separation of Stilbenes on HALO<sup>®</sup> C8 and ES-CN, 5 $\mu$ m

Application Note 115



### PEAK IDENTITIES:

1. trans-Stilbene oxide
2. trans-Stilbene
3. cis-Stilbene

These two HALO<sup>®</sup> 5  $\mu$ m 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  $\mu$ m, 4.6 x 50 mm

**Part Number:** 95814-408

2) HALO 90 Å ES-CN, 5.0  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** 50/50 water/acetonitrile

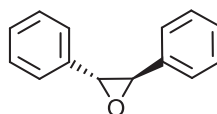
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

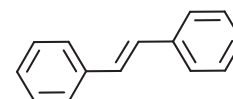
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu$ L

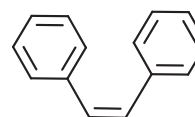
### STRUCTURES:



trans-Stilbene Oxide



trans-Stilbene



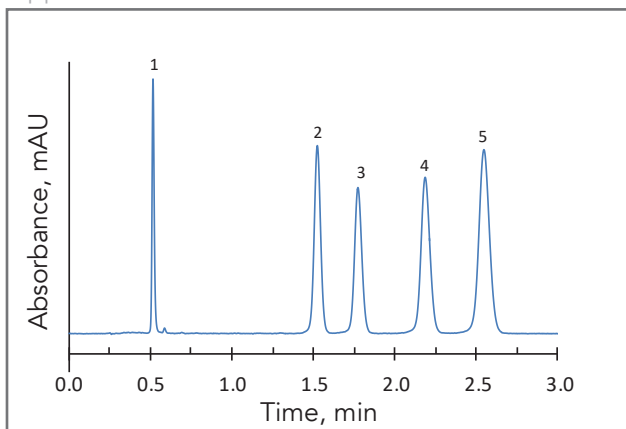
cis-Stilbene





## 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

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.

### 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

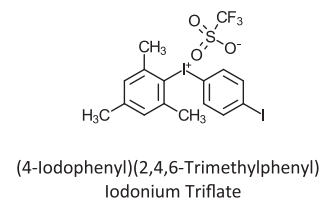
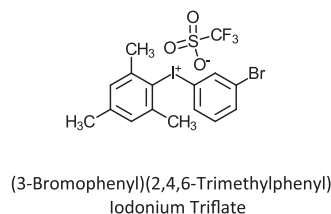
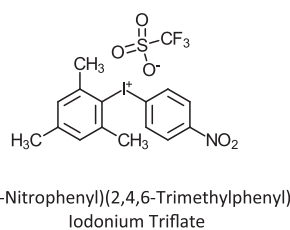
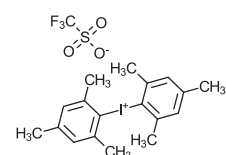
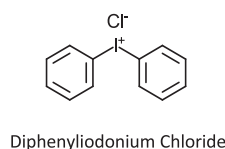
**Data Rate:** 25 Hz

**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

### STRUCTURES:

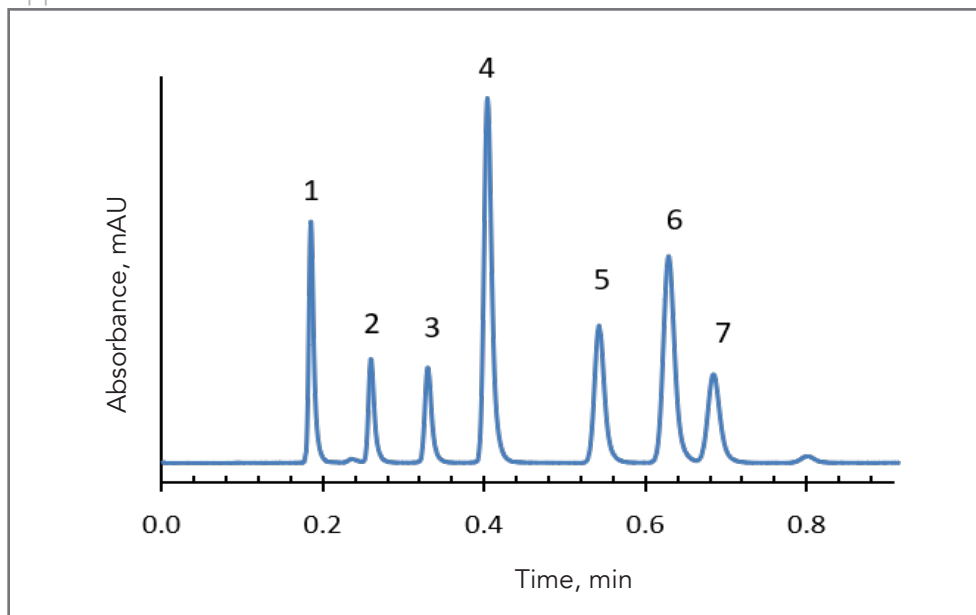






## Isocratic Separation of Anilines on HALO® C18

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

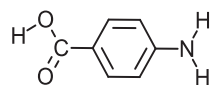
**Response Time:** 0.02 sec

**Flow Cell:** 2.5 µL semi-micro

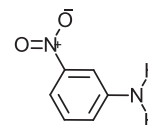
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14 µL

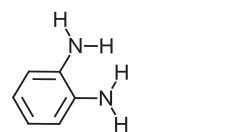
### STRUCTURES:



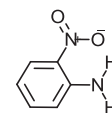
p-Aminobenzoic acid



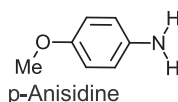
3-Nitroaniline



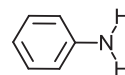
1,2-phenylenediamine



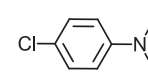
2-Nitroaniline



p-Anisidine



Aniline



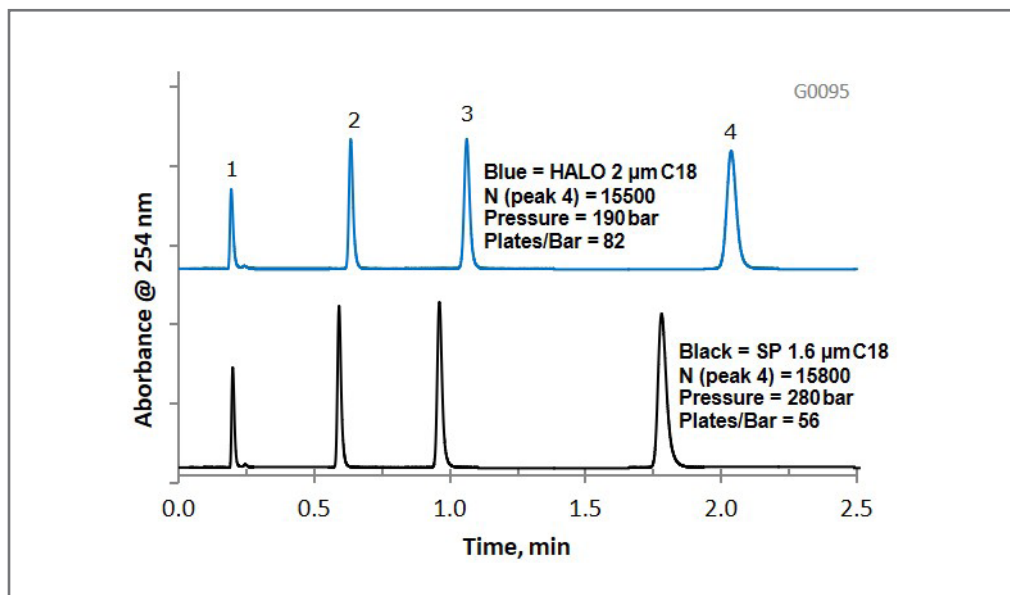
4-Chloroaniline





## Comparable Efficiency of HALO<sup>®</sup> Fused-Core<sup>®</sup> C18, 2.0 $\mu$ m and Superficially Porous (SP) C18, 1.6 $\mu$ m Columns

Application Note 111



### PEAK IDENTITIES:

1. Uracil
2. Pyrene
3. Decanophenone
4. Dodecanophenone

With a HALO<sup>®</sup> 2.0  $\mu$ m C18 column, one can achieve the same performance at only 68% of the back pressure of a competitor's superficially porous 1.6  $\mu$ m C18 column.

### TEST CONDITIONS:

#### Columns:

1) HALO 90 Å C18, 2.0  $\mu$ m, 2.1 x 50 mm

**Part Number:** 91812-402

2) Superficially porous C18, 1.6  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** 20/80 water/acetonitrile

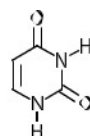
**Response Time:** 0.16 sec

**Flow Cell:** 1.0  $\mu$ L

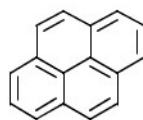
**LC System:** Shimadzu Nexera

**Extra Column Volume:** ~7  $\mu$ L

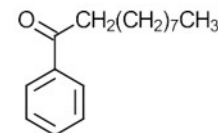
### STRUCTURES:



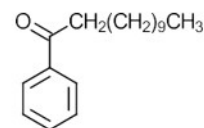
Uracil



Pyrene



Decanophenone



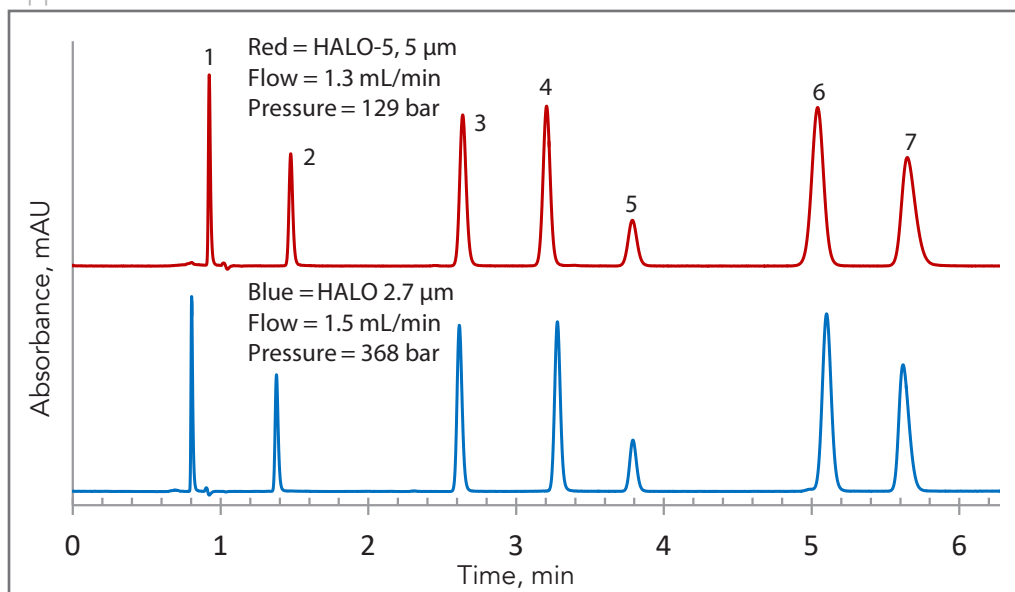
Dodecanophenone





## 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

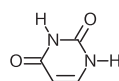
**Sample Solvent:** 50/50 water/acetonitrile

**Response Time:** 0.12 sec

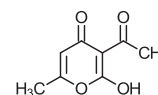
**Flow Cell:** 5.0 µL semi-micro

**LC System:** Agilent 1100

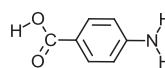
### STRUCTURES:



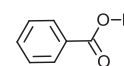
Uracil



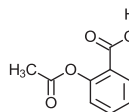
Dehydroacetic Acid



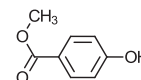
p-Aminobenzoic Acid



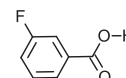
Benzoic Acid



Acetylsalicylic Acid



Methyl Paraben



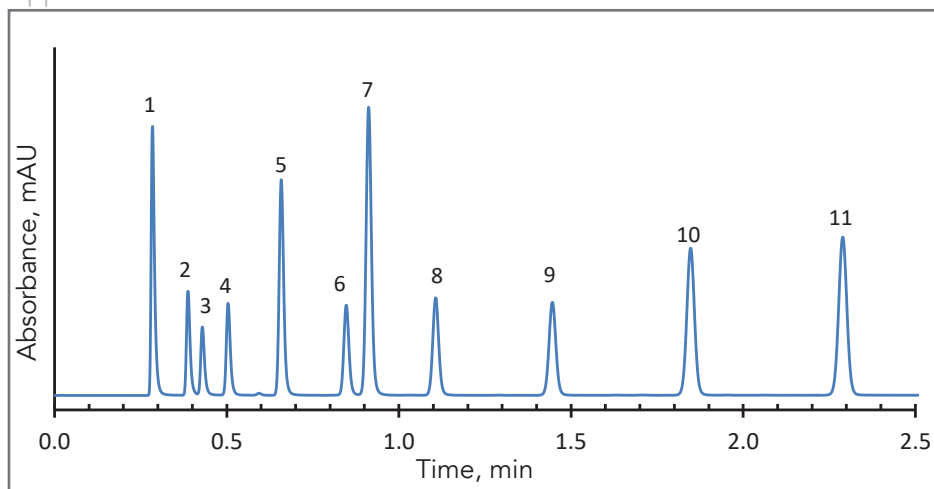
3-Fluorobenzoic Acid





## Rapid HPLC Separation of Phenones on HALO<sup>®</sup> 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<sup>®</sup> C18 column.

### TEST CONDITIONS:

**Column:** HALO 90 Å C18, 2.7  $\mu$ m,  
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  $\mu$ L

**Sample Solvent:** 50/50 methanol/acetonitrile

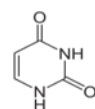
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

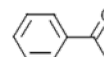
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu$ L

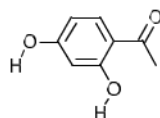
### STRUCTURES:



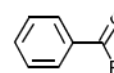
Uracil



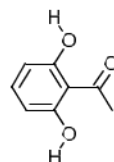
Acetophenone



2',4'-Dihydroxyacetophenone



Substituted Phenones



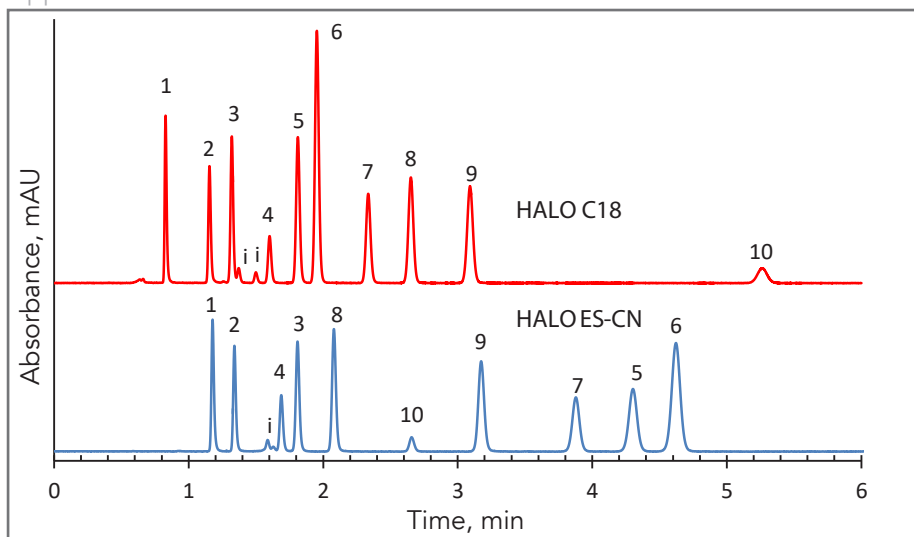
2',6'-Dihydroxyacetophenone





## Separation of Mixed Polarity Compounds on HALO<sup>®</sup> 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<sup>®</sup> C18 and ES-CN stationary phases. Note the increased retention of nitro compounds and reduced retention of non-polar compounds on the HALO<sup>®</sup> ES-CN phase.

### TEST CONDITIONS:

#### Columns:

- 1) HALO 90 Å C18, 2.7  $\mu$ m, 4.6 x 100 mm  
Part Number: 92814-402
- 2) HALO 90 Å ES-CN, 2.7  $\mu$ m, 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  $\mu$ L

**Sample Solvent:** Water/methanol

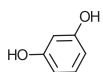
**Response Time:** 0.02 sec

**Flow Cell:** 2.5  $\mu$ L semi-micro

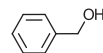
**LC System:** Shimadzu Prominence UFLC XR

**Extra Column Volume:** ~14  $\mu$ L

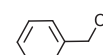
### STRUCTURES:



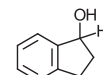
Resorcinol



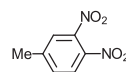
Benzyl alcohol



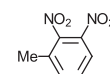
Phenylacetonitrile



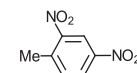
1-Indanol



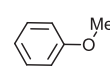
3,4-DNT



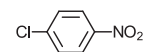
2,3-DNT



2,4-DNT



Anisole



1-Chloro-4-nitrobenzene



Toluene

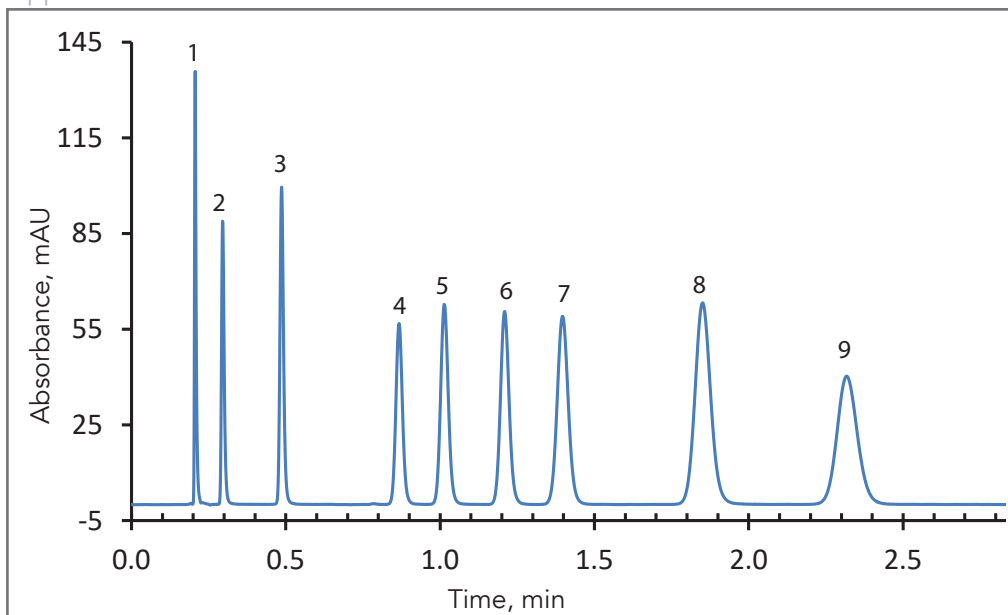






## Polar Compounds Separated by HALO® RP-Amide, 5 µm

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

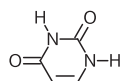
**Sample Solvent:** 50/50 water/acetonitrile

**Response Time:** 0.12 sec

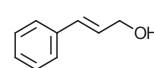
**Flow Cell:** 5.0 µL semi-micro

**LC System:** Agilent 1100

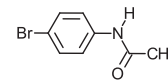
### STRUCTURES:



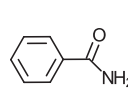
Uracil



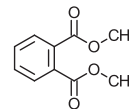
Cinnamyl Alcohol



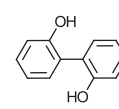
4'-Bromoacetanilide



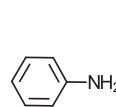
Benzamide



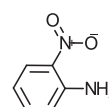
Dimethyl Phthalate



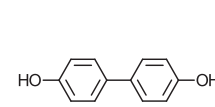
2,2'-Biphenol



Aniline



2-Nitroaniline



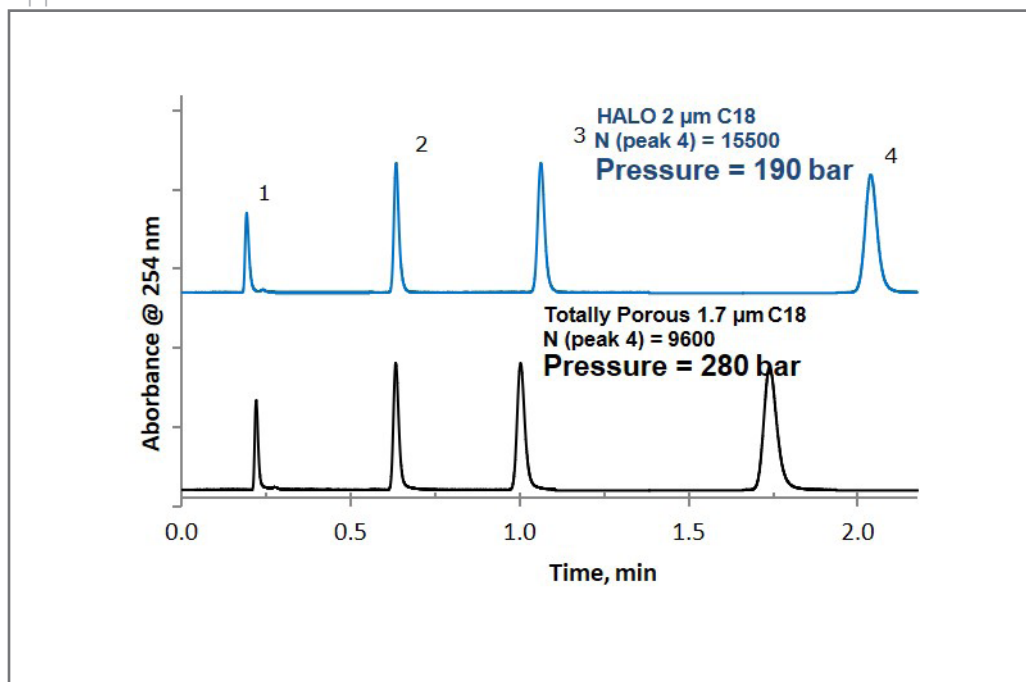
4,4'-Biphenol





## 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

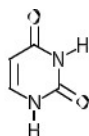
**Response Time:** 0.16 sec

**Flow Cell:** 1.0 µL

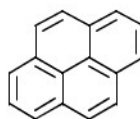
**LC System:** Shimadzu Nexera

**Extra Column Volume:** ~7 µL

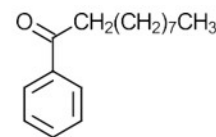
### STRUCTURES:



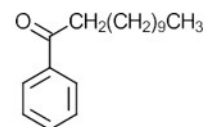
Uracil



Pyrene



Decanophenone



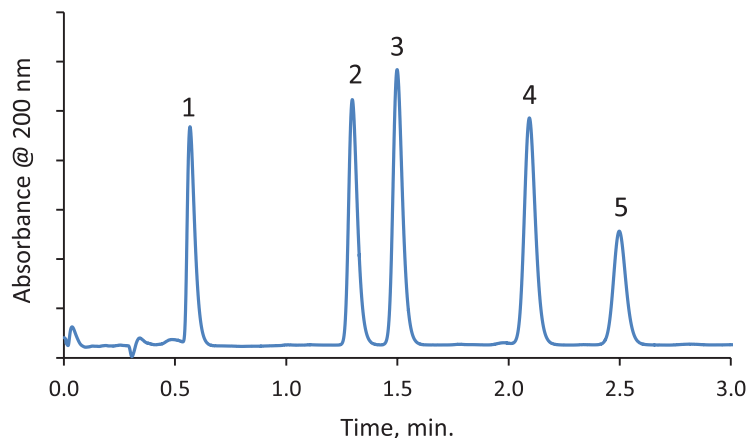
Dodecanophenone





## 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

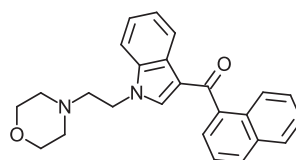
**Sample Solvent:** 50/50 water/acetonitrile

**Data Rate:** 50 Hz

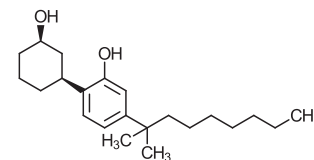
**Flow Cell:** 2.5 µL semi-micro

**LC System:** Shimadzu Prominence UFLC XR

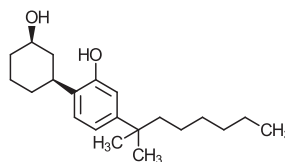
### STRUCTURES:



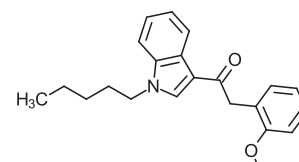
JWH-200



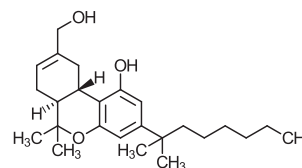
(±)-CP 47, 497 C8 Homologue



(±)-CP 47, 497



JWH-250



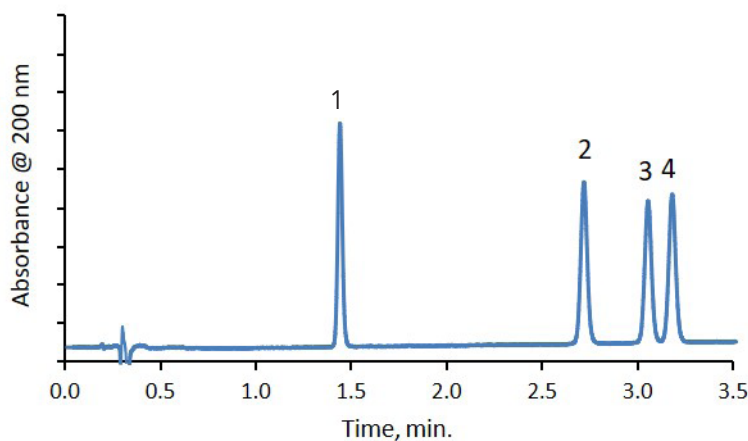
HU-211





## 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

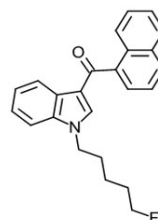
**Sample Solvent:** 50/50 water/acetonitrile

**Data Rate:** 100 Hz

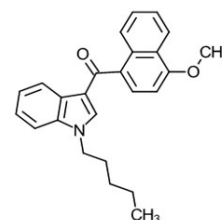
**Flow Cell:** 1.0 µL

**LC System:** Shimadzu Nexera X2

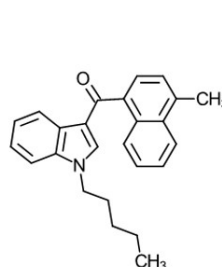
### STRUCTURES:



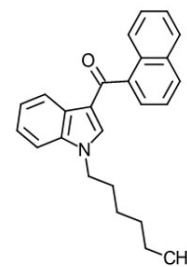
AM2201



JWH-081



JWH-122



JWH-019





### MS TEST CONDITIONS:

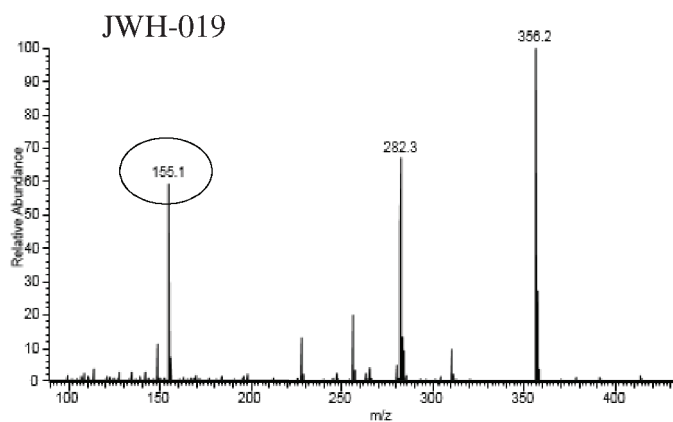
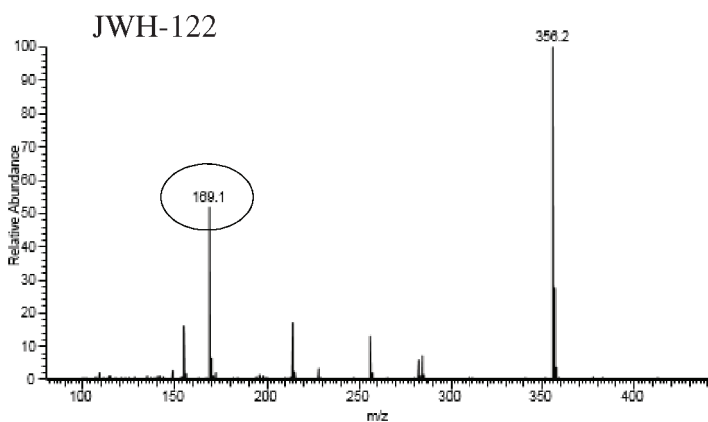
**MS System:** Thermo Fisher Orbitrap VelosPro ETD

**Scan Time:** 6  $\mu$ scans/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.

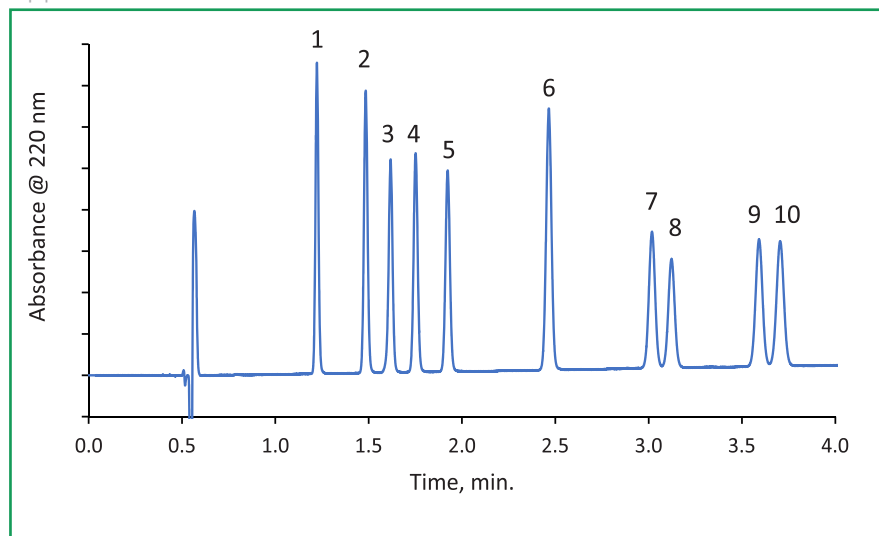






## Fast Separation of Ten Cannabinoids on HALO<sup>®</sup> C18

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 ( $\Delta^9$ -THC)
8. delta-8-Tetrahydrocannabinol ( $\Delta^8$ -THC)
9. Cannabichromene (CBC)
10. delta-9-Tetrahydrocannabinolic acid A (THCA)

A HALO<sup>®</sup> 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  $\mu\text{m}$ ,  
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  $\mu\text{L}$

**Dwell Volume:** 0.471 mL

**Sample Solvent:** 75/25 methanol/water

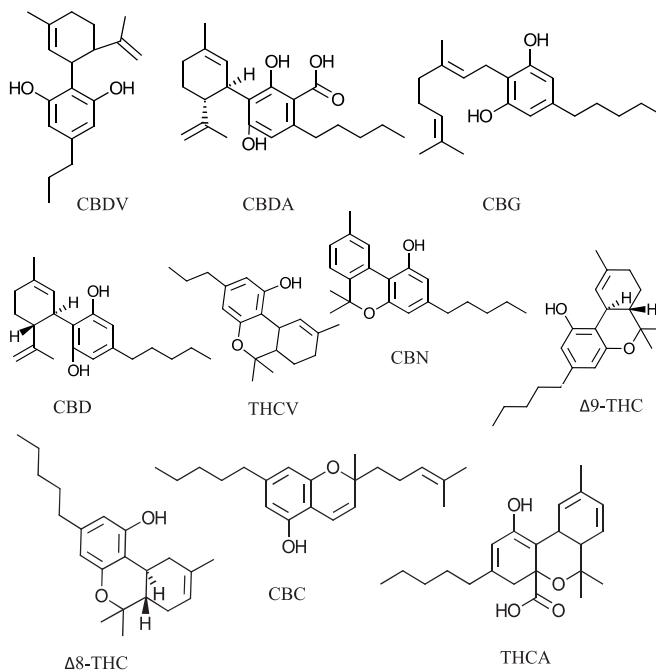
**Response Time:** 0.025 sec

**Data Rate:** 100 Hz

**Flow Cell:** 1.0  $\mu\text{L}$

**LC System:** Shimadzu Nexera X2

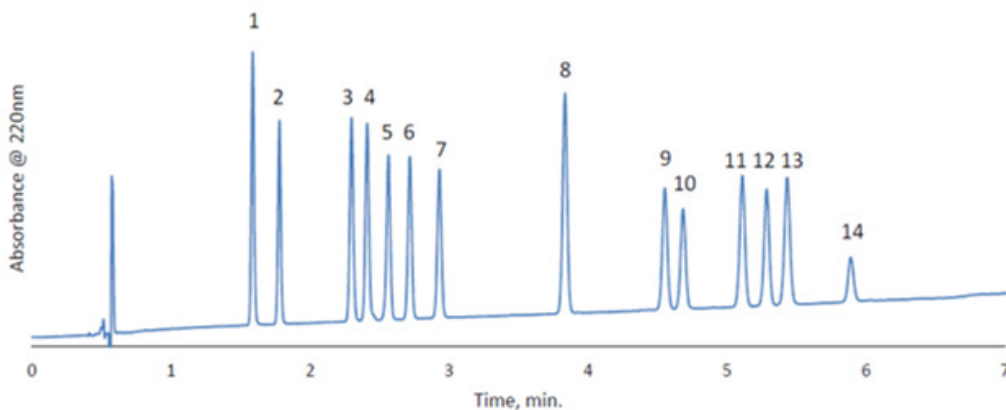
### STRUCTURES:





## Separation of 14 Cannabinoids on HALO<sup>®</sup> 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 ( $\Delta^9$ -THC)
10. delta-8-Tetrahydrocannabinol ( $\Delta^8$ -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  $\mu$ m,  
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  $\mu$ L

**Dwell Volume:** 0.471 mL

**Sample Solvent:** 75/25 methanol/water

**Response Time:** 0.025 sec

**Data Rate:** 100 Hz

**Flow Cell:** 1.0  $\mu$ L

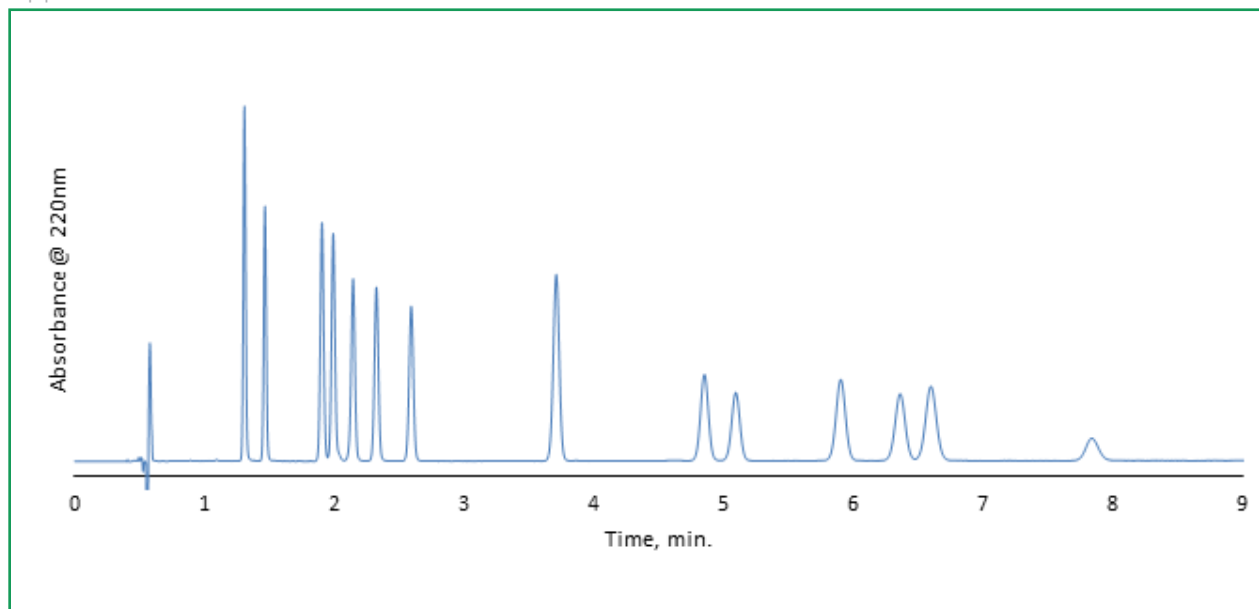
**LC System:** Shimadzu Nexera X2





## Isocratic Separation of 14 Cannabinoids on HALO<sup>®</sup> C18

Application Note 165-CN



### TEST CONDITIONS:

**Column:** HALO 90 Å C18, 2.7  $\mu$ m,  
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  $\mu$ L

**Dwell Volume:** 0.471 mL

**Sample Solvent:** 75/25 methanol/water

**Response Time:** 0.025 sec

**Data Rate:** 100 Hz

**Flow Cell:** 1.0  $\mu$ L

**LC System:** Shimadzu Nexera X2

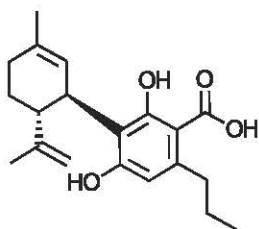
### PEAK IDENTITIES:

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 ( $\Delta$ 9-THC)
10. delta-8-Tetrahydrocannabinol ( $\Delta$ 8-THC)
11. Cannabicyclol (CBL)
12. Cannabichromene (CBC)
13. delta-9-Tetrahydrocannabinolic acid A (THCA)
14. Cannabichromenic acid (CBCA)

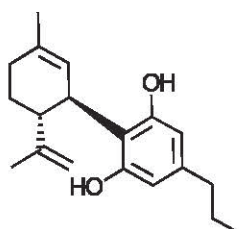




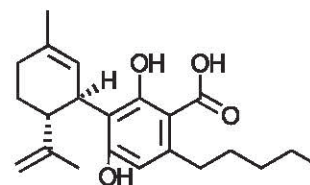
### STRUCTURES:



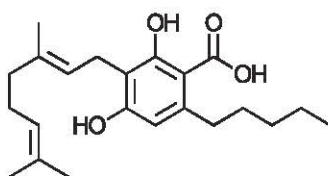
CBDVA



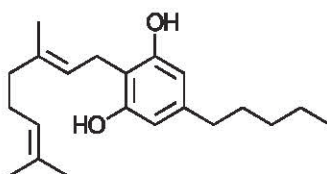
CBDV



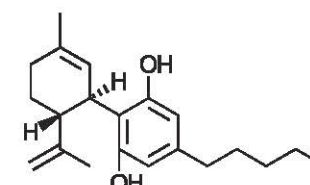
CBDA



CBGA



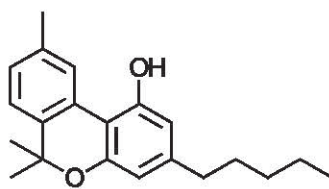
CBG



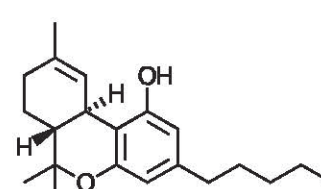
CBD



THCV



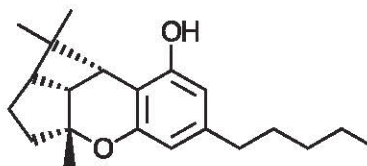
CBN



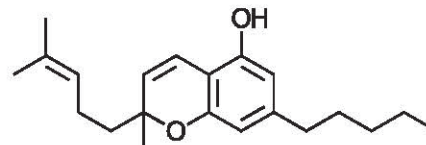
Δ9-THC



Δ8-THC



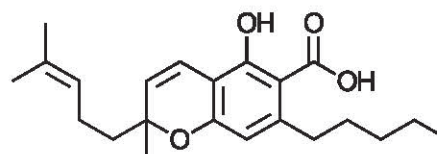
CBL



CBC



THCA



CBCA







# HALO®

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