Separation of Structurally Similar Steroids on HALO C18 and PFP

**TEST CONDITIONS:**
- Columns: 4.6 x 100 mm, HALO C18
  4.6 x 100 mm, HALO PFP
- Part Numbers: C18, 92814-602
  PFP, 92814-609
- Mobile Phase: 50/50: water/methanol
- Flow Rate: 1.0 mL/min.
- Pressure: About 230 Bar
- Temperature: 35°C
- Detection: UV 240 nm, VWD
- Injection Volume: 0.5 µL
- Sample Solvent: 80% methanol in water
- Response Time: 0.02 sec.
- Flow Cell: 2.5 µL semi-micro
- LC System: Shimadzu Prominence UFLC XR
- ECV: ~14 µL

**STRUCTURES:**
- Prednisone
- Prednisolone
- Cortisone
- 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.
**Isocratic Separation of Amphenicols on HALO Phenyl-Hexyl Phase**

**TEST CONDITIONS:**
- **Column:** 4.6 x 50 mm, HALO Phenyl-Hexyl
- **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 Prominance UFLC XR
- **Extra column volume:** ~14 μL

**STRUCTURES:**

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 @ 240 nm and the second @ 280 nm.
Testing Conditions:
Columns: 4.6 x 50 mm, HALO Phenyl-Hexyl, C18, and PFP Phases
Part Numbers: 92814-406, 92814-402, and 92814-409, respectively
Mobile Phase:
A = 25 mM Ammonium acetate in water (pH=5.8 not adjusted), B = Acetonitrile
Gradient from 34-63% B in 3.5 minutes
Flow Rate: 1.5 mL/min.
Pressure: 200 Bar
Temperature: 35°C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 μL
Standard diluted with Acetonitrile and buffer
Response Time: <0.12 sec.
Flow Cell: 5 μL semi-micro
LC System: Agilent 1100
Gradient dwell volume = 0.88 mL

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

Structures:
Oxazepam
Alprazolam
Flunitrazepam
Lorazepam
Clonazepam
Diazepam
Nitrazepam
Temazepam

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.
Separation of Statin Drugs on HALO C8

PEAK IDENTITIES:
1. Pravastatin
2. Atorvastatin
3. Mevastatin
4. Simvastatin

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Separation of Antiulcer Drugs on HALO Penta-HILIC

TEST CONDITIONS:
- Column: 4.6 x 100 mm, HALO Penta-HILIC
- Part Number: 92814-605
- Mobile Phase: 10/90: A/B
  - A = 0.04 M Ammonium formate, pH=3.0 (adj.)
  - B = Acetonitrile
- Flow Rate: 3.0 mL/min.
- Pressure: 210 Bar
- Temperature: 30°C
- Detection: UV 254 nm, VWD
- Injection Volume: 2 μL
- Sample Solvent: mobile phase
- Response Time: 0.02 sec.
- Flow Cell: 2.5 μL semi-micro
- LC System: Shimadzu Prominence UFLC XR
- ECV: ~14 μL

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.

PEAK IDENTITIES:
1. Cimetidine
2. Nizatidine
3. Famotidine
4. Ranitidine

STRUCTURES:
- Cimetidine
- Famotidine
- Nizatidine
- Ranitidine

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Separation of 5 Beta Blocker Drugs on HALO Penta-HILIC

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

PEAK IDENTITIES:
1. Alprrenolol
2. Propranolol
3. Pindolol
4. Acebutolol
5. Atenolol

STRUCTURES:

The HALO Penta-HILIC stationary phase can rapidly separate highly basic compounds with good peak shapes in a mass spectrometry friendly mobile phase.
Application Note: 60-CB

Separation of Carbamate Pesticides on HALO ES-CN Phase

TEST CONDITIONS:
Column: 4.6 x 50 mm, HALO ES-CN
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: UV240 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

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

PEAK IDENTITIES:
1. Carbetamide
2. Propham
3. Chlorpropham

STRUCTURES:

Carbetamide

Propham

Chlorpropham
Isocratic Separation of Phenyl Ureas on HALO ES-CN

**TEST CONDITIONS:**
- Column: 4.6 x 50 mm, HALO ES-CN
- Part Number: 92814-404
- Mobile Phase: 50/50 A/B
  - A=0.02 M Phosphate buffer, adj. to pH=2.5
  - B=Acetonitrile
- Flow Rate: 2.0 mL/min.
- Pressure: 200 Bar
- Temperature: 20 °C
- Detection: UV 245 nm, VWD
- Injection Volume: 0.5 μL
- Sample Solvent: Acetonitrile/water
- Response Time: 0.02 sec.
- Flow Cell: 2.5 μL semi-micro
- LC System: Shimadzu Prominence UFLC XR
- Extra column volume: ~14 μL

**STRUCTURES:**
- Fenuron
- Fluomethuron
- Linuron
- Monuron
- Diuron
- Neburon

**PEAK IDENTITIES:**
1. Fenuron
2. Monuron
3. Fluomethuron
4. Diuron
5. Linuron
6. Neburon

This separation is an example of a rapid analysis of phenyl urea compounds on a HALO ES-CN column in under 2 minutes.
Rapid Isocratic Separation of Fibrates on HALO C-18 Phase

**TEST CONDITIONS:**
- Column: 4.6 x 50 mm, HALO C-18
- 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

**FIBRATES:**
Fibrates are a class of cholesterol lowering drugs that can be rapidly analyzed using Halo C-18 phase to obtain widely separated peaks in about 30 seconds.

**PEAK IDENTITIES:**
1. Bezafibrate
2. Gemfibrozil
3. Fenofibrate
**TEST CONDITIONS:**

Column: 4.6 x 50 mm, HALO Peptide ES-C18  
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 minute  
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 μL semi-micro  
LC System: Quaternary Agilent 1100  
Gradient dwell volume is 0.88 mL

**DISCUSSION:**

This separation shows the utility of the HALO Fused-Core Peptide 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.