

Alternate Selectivity Using Various Fused-Core[®] Bonded Phases

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Objectives

- The objective of this work is to demonstrate efficient separations while highlighting the alternative selectivity that can be achieved with various HALO bonded phases, especially ES-CN (extra stable-cyanopropyl).
- Orthogonality “S” values are calculated as a measure of how different the phases are using a variety of acids, bases, and neutral molecules, as well as both acetonitrile and methanol organic modifier.

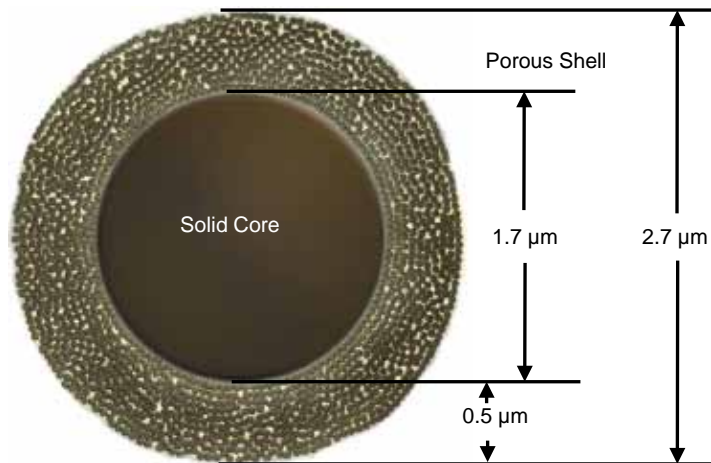
Abstract

HPLC columns of Fused-Core[®] superficially porous particles with an overall diameter of 2.7 μm and a 0.5 μm porous shell have demonstrated unusual efficiency and stability, allowing fast separations with the needed ruggedness of operation of 5 μm particle columns. These 2.7 μm Fused-Core particles permit separation speeds and resolution competitive with sub-2- μm totally porous particles, but with 40–50% of the column back pressure. With the broadest variety of bonded phases available on superficially porous particles, Fused-Core columns allow analysts to efficiently develop methods with superior resolution. Most HPLC method development experiments first utilize columns containing the popular C18 and C8 bonded phases. Added separation capabilities are available with other stationary phases that can widen the separation selectivity possibilities for many applications. The introduction of a new cyano bonded phase augments the alternate selectivities provided by other Fused-Core stationary phases. A systematic evaluation of this bonded phase, including its stability and applications, will be highlighted with emphasis on efficiency and speed. The “orthogonality” S values calculated using a set of acids, bases, neutrals, and zwitterions reveal several generalities that can be made. The PFP (pentafluorophenylpropyl) phase is more retentive for electron-rich compounds, and can show enhanced shape selectivity due to its electronegative aromatic ring, while Phenyl-Hexyl is more retentive for electron-poor compounds due to π - π interactions with a flexible aromatic ring. In the case of the RP-Amide phase, polar compounds are better retained resulting from dipole interactions compared to the C18 bonded phase, especially those with –OH substituents. The ES-CN phase also exhibits increased retention of polar molecules, in particular nitro and nitroamine compounds, in contrast to C18 bonded phase.

Fused-Core Particles

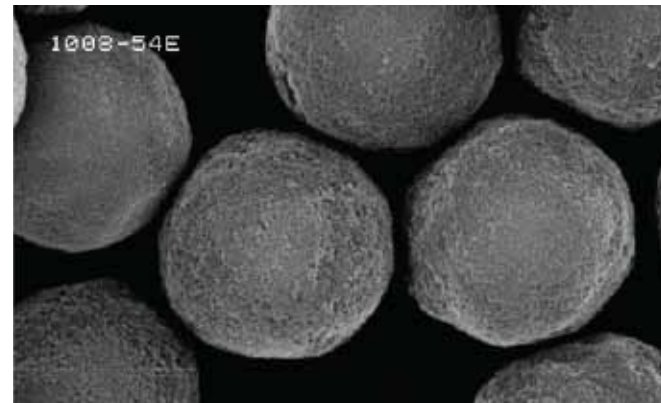
Particle Characteristics

- Silica: High purity, Type B
- Pore Size: 90 Å and 160 Å
- Particle Size Distribution: 5% RSD
- pH range: 2–9
- Efficiency: 230,000 plates/m

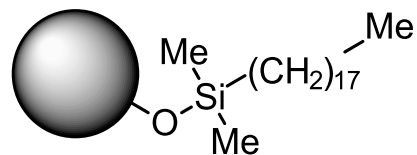


Features and Benefits

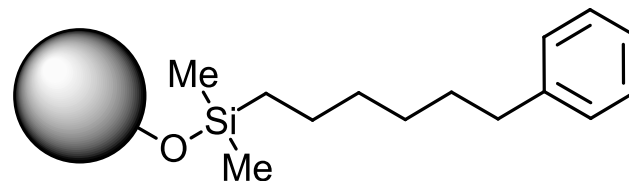
- Ultrafast separations save time and improve productivity
- UHPLC performance without the need for UHPLC equipment
- Low pressures enable the coupling of columns for high efficiency/high resolution



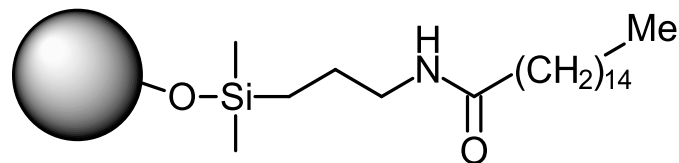
HALO Fused-Core Bonded Phases



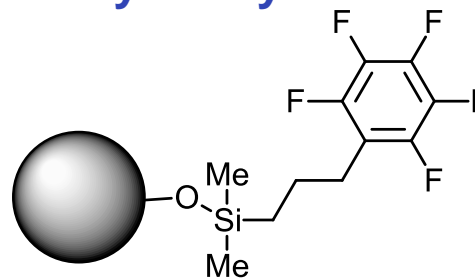
C18 (octadecyl)



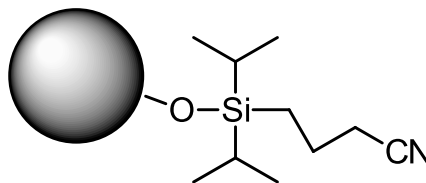
Phenyl-Hexyl



RP-Amide

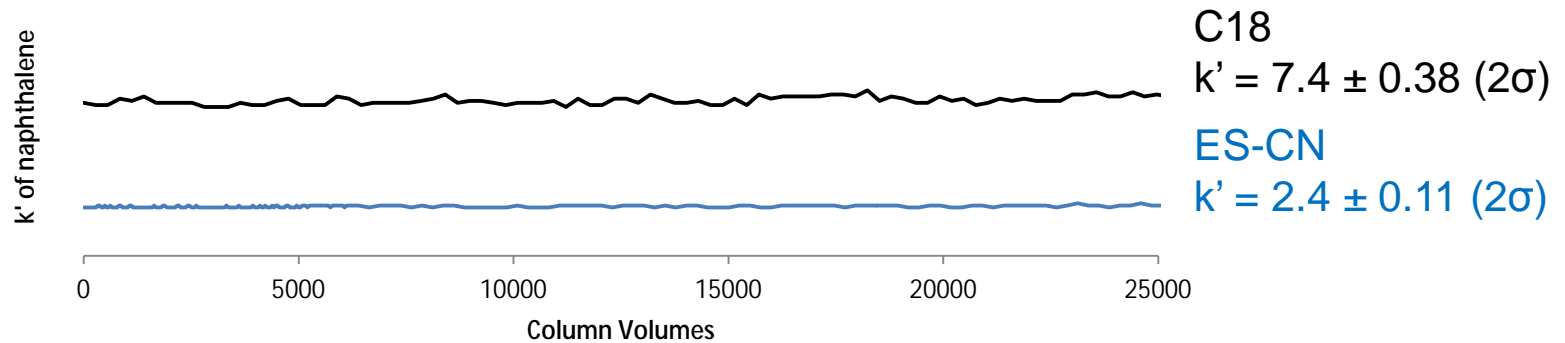


**PFP
(pentafluorophenylpropyl)**



**ES-CN
(extra stable-cyanopropyl)**

Comparison of HALO ES-CN vs. C18: Stability



Columns: 4.6 x 50 mm, 2.7 μm HALO ES-CN or C18

Mobile Phase: 50% Acetonitrile /50% Water/ 0.1% TFA

Flow Rate: 1.8 mL/min

Temperature: 60°C

Detection: UV-254nm

Injection: 1 μL ; one injection every 10 minutes for first 150 injections, then one injection every 60 minutes for ES-CN; one injection every 60 minutes for C18

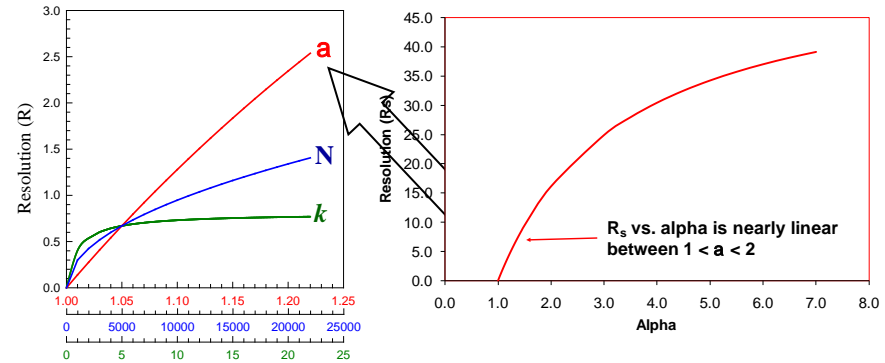
Solute: naphthalene

Instrument: Perkin Elmer FX-15

Selectivity and Orthogonal Separations

- Selectivity is the most influential parameter in the resolution equation

$$R_s = \frac{\alpha - 1}{4} \sqrt{N} \frac{k_2}{\alpha(1 + k_2)}$$



Source: Jun Mao, PhD Thesis with Professor Peter Carr, U. of Minnesota, 2001

- Orthogonal
 - Marked changes in relative retention so that peaks which are unresolved in one (chromatogram) are likely to be separated in the second chromatogram
- Orthogonal separations are conducted on columns with significantly different selectivities

Experimental

Instrument

- Agilent 1100 quaternary or Agilent 1200 binary
- Shortest length 0.005" ID tubing between modules
- 3.0 mL heat exchanger
- 1100: Semi-micro flow cell, bypassed ($1 < V_{\text{cell}} < 5 \text{ mL}$)
- 1200: Micro flow cell (2.5 mL)

Analytical Columns

- 3.0 x 50 mm HALO
 - C18
 - RP-Amide
 - Phenyl-Hexyl
 - PFP
 - ES-CN

Column Screening Conditions

Gradients: 5–95%B in 5 min.

- ACN or MeOH
- 10 mM ammonium formate, pH 3.0
- Temperature: 40 C
- Flow rate: 0.85 mL/min
- Detection: UV @ 254 nm
- Injection volume: 2 mL

Analytes

Set represents 23-29 compounds, specifically pharmaceuticals, including acids, bases, zwitterions, and neutrals.

“Orthogonality” S Values for HALO Bonded Phases

$$S = 100 \cdot \sqrt{1 - R^2}$$

where R^2 is the correlation coefficient of a graph of t_r (phase 1) vs. t_r (phase 2) or $\log k'$ (phase 1) vs. $\log k'$ (phase 2)

	S Values (ACN)	S Values (MeOH)
C18 vs. Phenyl-Hexyl	9	14
C18 vs. RP-Amide	14	21
RP-Amide vs. Phenyl-Hexyl	19	22
C18 vs. ES-CN	20	25
C18 vs. PFP	63	53

The S values are dependent upon the selected mobile phase and compound set used for screening. The larger the S value, the larger the difference in selectivity between the two phases.

Buffer: 10 mM Ammonium Formate pH 3

Compound Set (ACN modifier): 2-fluorobenzoic acid, 3-cyanobenzoic acid, 3-indoleacetic acid, 3-nitrobenzoic acid, 4-aminobenzoic acid, benzoic acid, beta-estradiol, biochanin A, chloramphenicol, cortisone, fenoprofen, ibuprofen, ketoprofen, mefenamic acid, naringin, norfloxacin, nortriptyline hydrochloride, prednisolone, prednisone, procainamide hydrochloride, prunetin, ranitidine, sulfamerazine

Compound Set (MeOH modifier): 2,6-dinitrotoluene, 2-fluorobenzoic acid, 3-cyanobenzoic acid, 3-indoleacetic acid, 3-phenoltoluene, 4-aminobenzoic acid, 4-chlorophenol, 4-nitrophenol, acenaphthene, atrazine, benzoic acid, beta-estradiol, biochanin A, chloramphenicol, cortisone, fenoprofen, ibuprofen, ketoprofen, mefenamic acid, nitrobenzene, norfloxacin, nortriptyline hydrochloride, phenol, prednisolone, prednisone, procainamide hydrochloride, prunetin, ranitidine, resorcinol

Benzodiazepines on HALO Fused-Core Bonded Phases

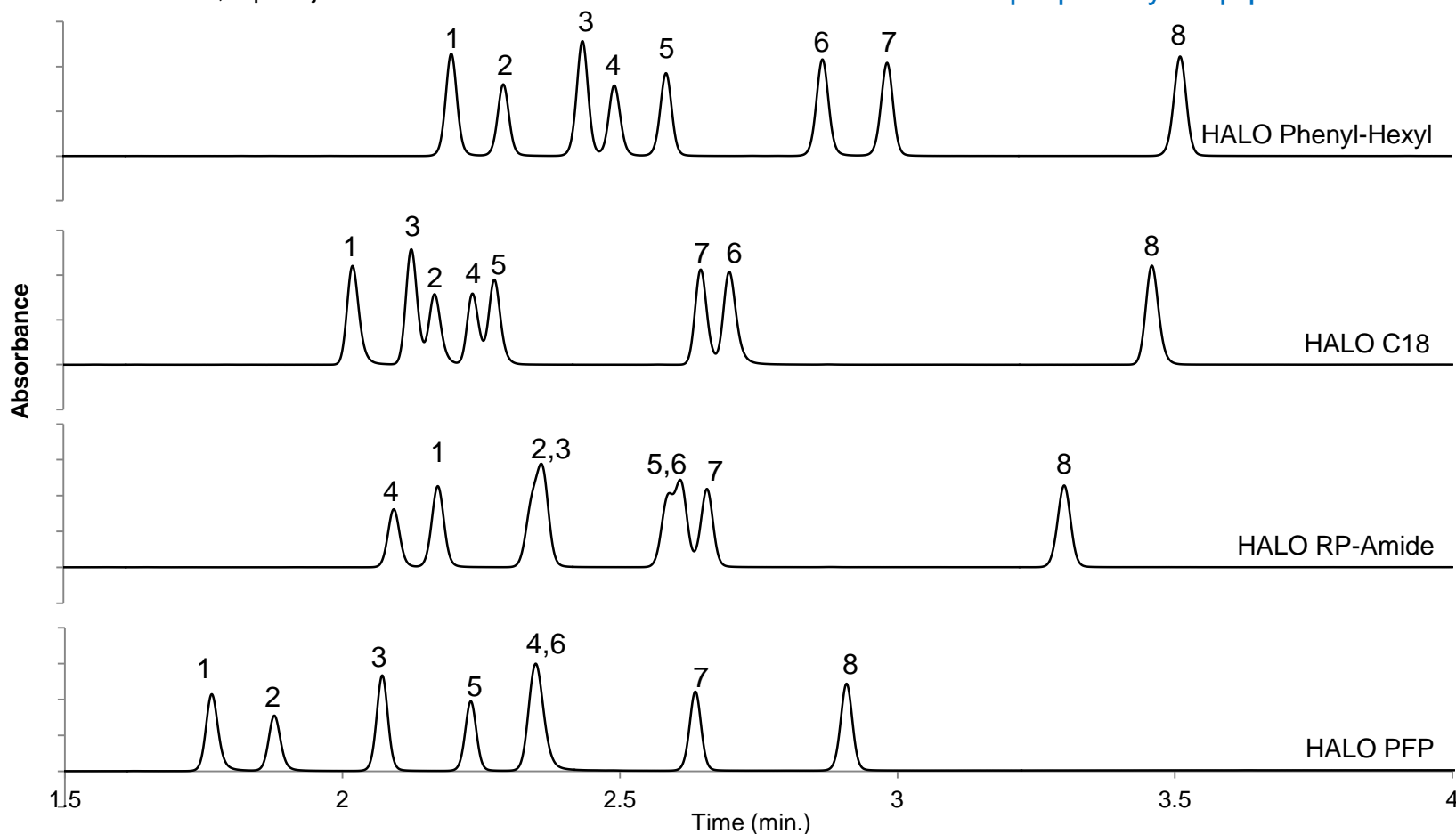
Test Conditions:

Column size: 4.6 x 50 mm
A = 25 mM Ammonium Acetate
B = Acetonitrile
Flow rate = 1.5 mL/min.
Gradient = 34–63 %B in 3.5 min.
Pressure = 200 bar
Temperature = 35 °C
UV=254 nm, 1 µL Injection

Peak Identities:

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

HALO Phenyl-Hexyl is the most retentive phase for these anti-anxiety drugs due to its propensity for p-p interactions.



HALO C8 vs. ES-CN: Antidepressants

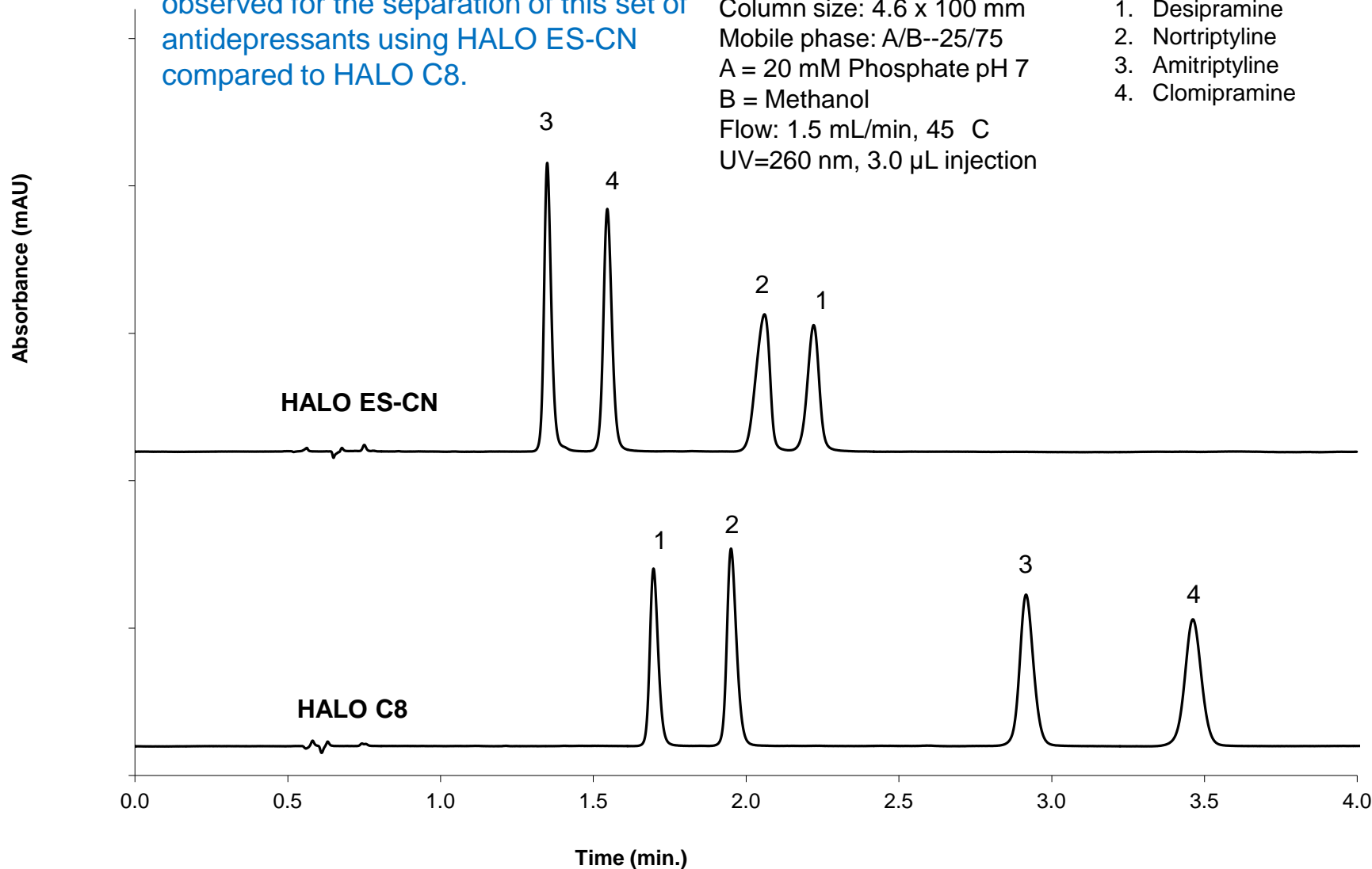
Almost complete elution order reversal is observed for the separation of this set of antidepressants using HALO ES-CN compared to HALO C8.

Test Conditions:

Column size: 4.6 x 100 mm
Mobile phase: A/B--25/75
A = 20 mM Phosphate pH 7
B = Methanol
Flow: 1.5 mL/min, 45 C
UV=260 nm, 3.0 µL injection

Peak Identities:

1. Desipramine
2. Nortriptyline
3. Amitriptyline
4. Clomipramine



HALO C18 vs. ES-CN: Mixed EPA Explosive Standards 8330 A and B

The separation of peaks 10 and 11 on the HALO ES-CN phase is an excellent example of phase orthogonality vs. C18 where the same pair coelutes.

Test Conditions:

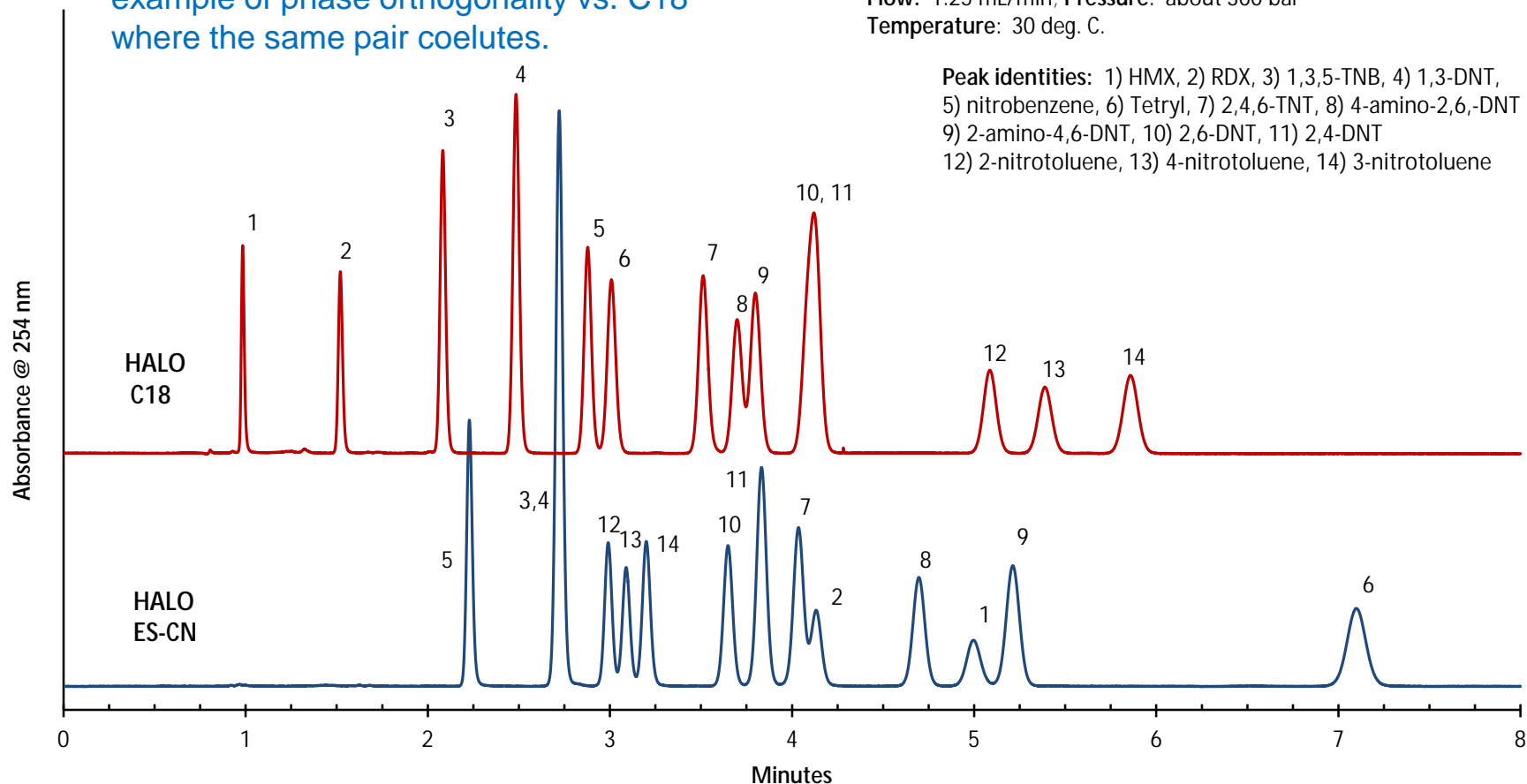
Columns: 4.6 x 100 mm, HALO C18, HALO ES-CN

Mobile phase: 50/50: Methanol/water

Flow: 1.25 mL/min; Pressure: about 300 bar

Temperature: 30 deg. C.

Peak identities: 1) HMX, 2) RDX, 3) 1,3,5-TNB, 4) 1,3-DNT, 5) nitrobenzene, 6) Tetryl, 7) 2,4,6-TNT, 8) 4-amino-2,6,-DNT, 9) 2-amino-4,6-DNT, 10) 2,6-DNT, 11) 2,4-DNT, 12) 2-nitrotoluene, 13) 4-nitrotoluene, 14) 3-nitrotoluene



HALO C18 vs. PFP: Steroids

Test Conditions:

Column size: 4.6 x 100 mm

Mobile phase: A/B--50/50

A = Water

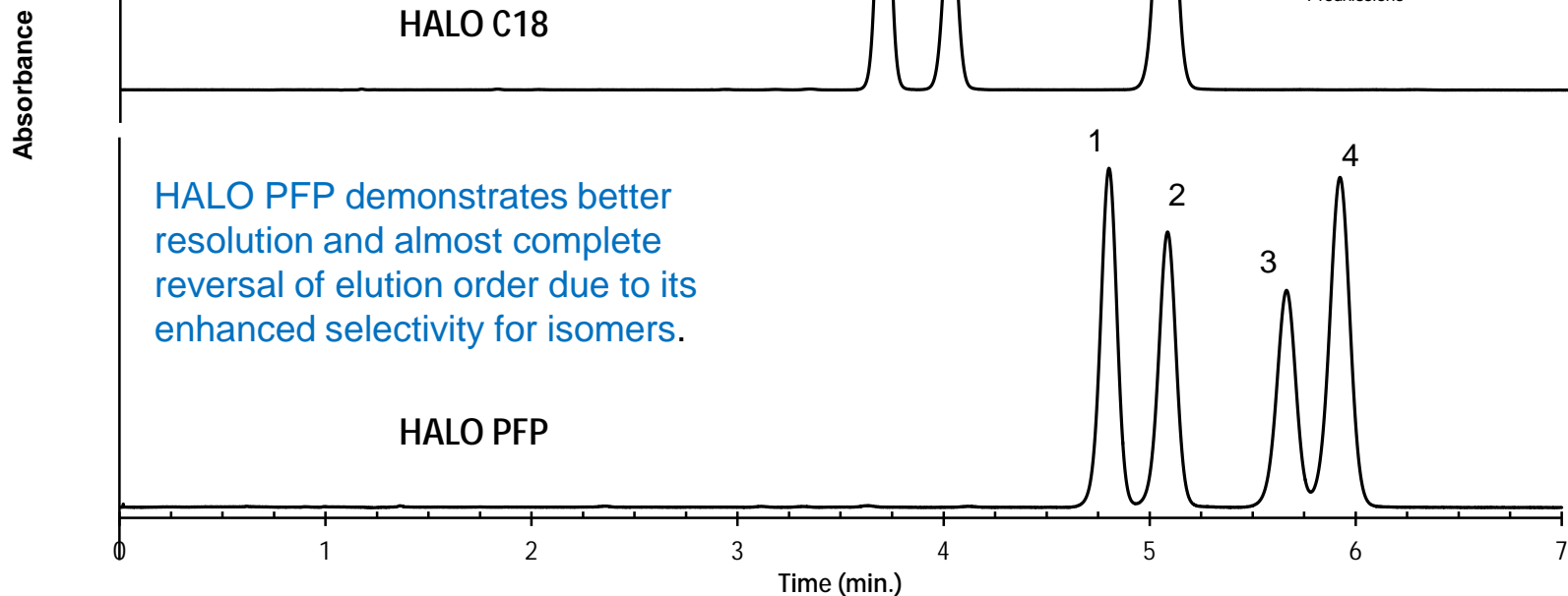
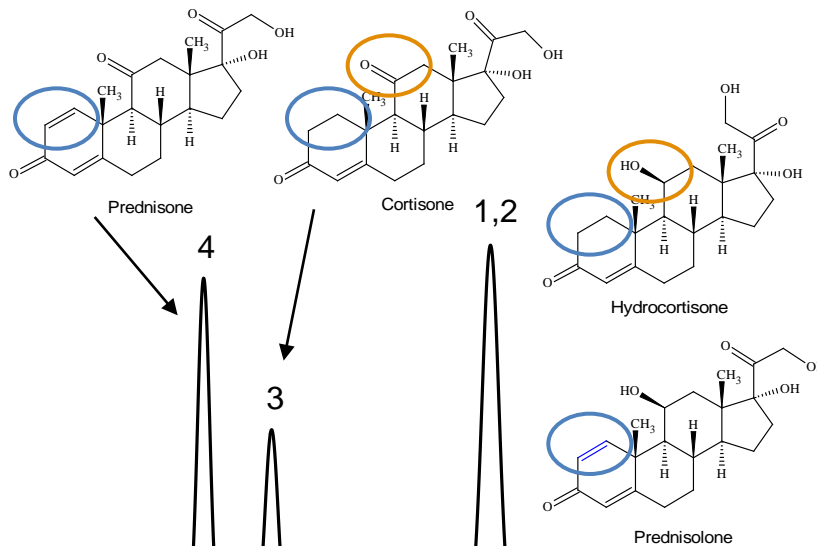
B = Methanol

Flow: 1.0 mL/min, 230 Bar, 35 deg. C.

UV=240 nm, 0.5 µL injection

Peak Identities:

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



Benzoic Acids on HALO Fused-Core Bonded Phases

Test Conditions:

Column size: 4.6 x 50 mm

A = water/0.1% formic acid

B = acetonitrile

75/25 A/B for C18, RP-Amide, and Phenyl-Hexyl

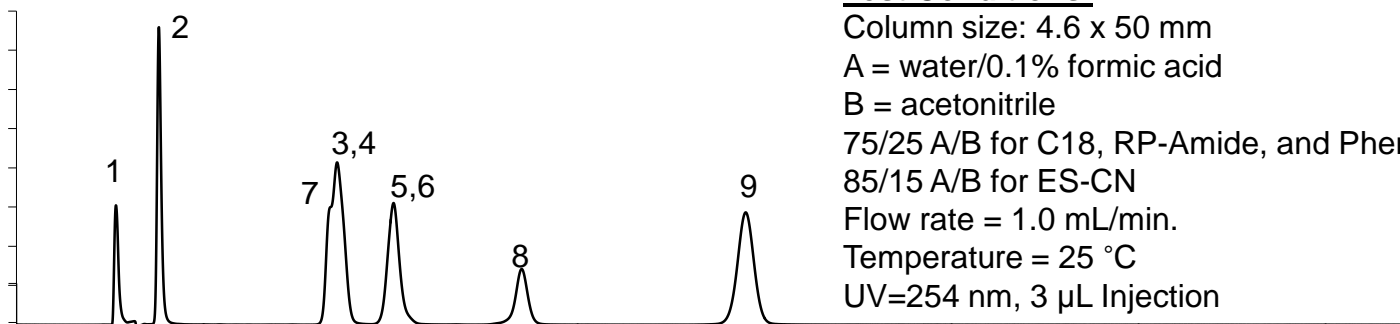
85/15 A/B for ES-CN

Flow rate = 1.0 mL/min.

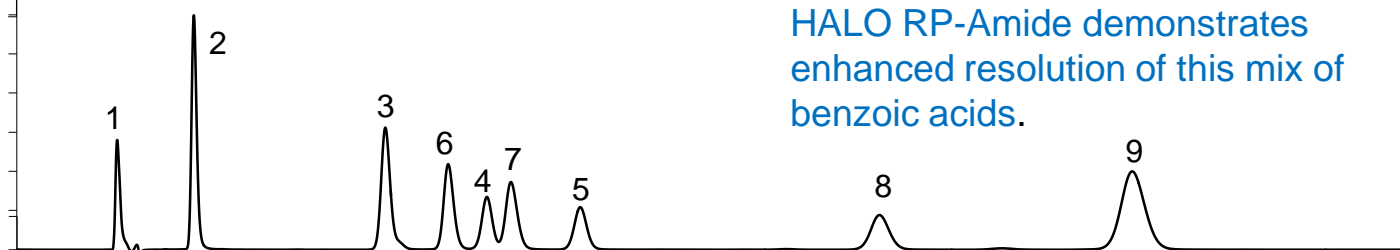
Temperature = 25 °C

UV=254 nm, 3 µL Injection

HALO C18

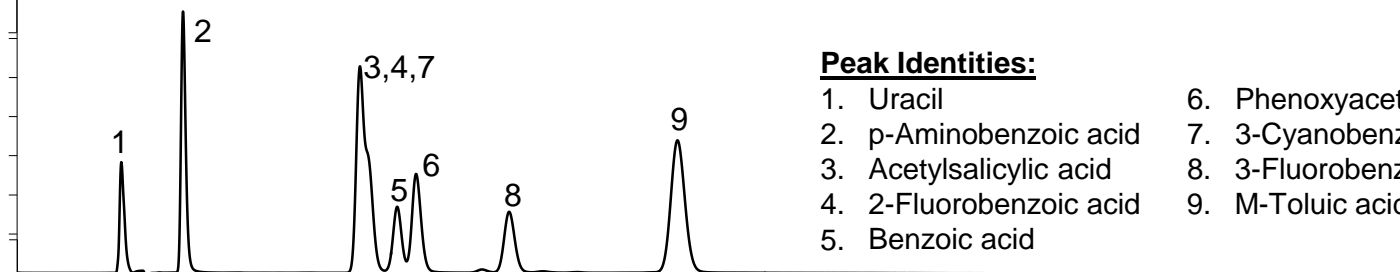


HALO RP-Amide



HALO RP-Amide demonstrates enhanced resolution of this mix of benzoic acids.

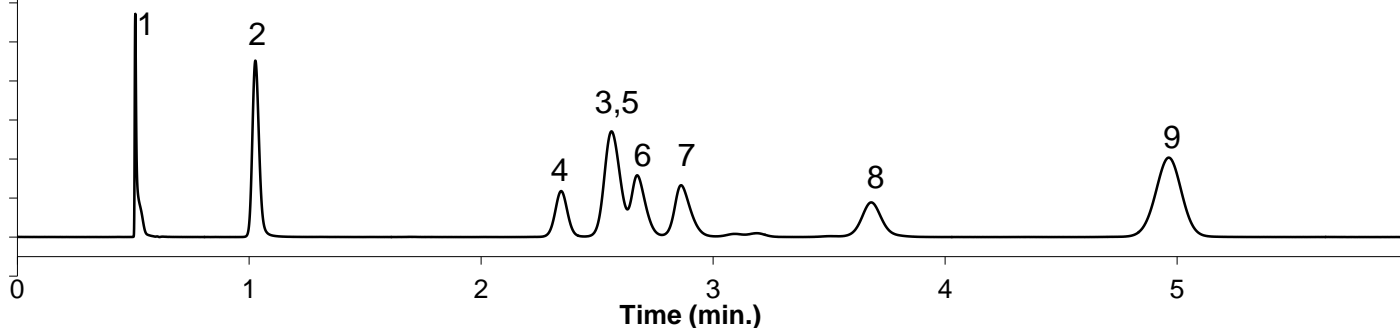
HALO Phenyl-Hexyl



Peak Identities:

- | | |
|-------------------------|-------------------------|
| 1. Uracil | 6. Phenoxyacetic acid |
| 2. p-Aminobenzoic acid | 7. 3-Cyanobenzoic acid |
| 3. Acetylsalicylic acid | 8. 3-Fluorobenzoic acid |
| 4. 2-Fluorobenzoic acid | 9. M-Toluic acid |
| 5. Benzoic acid | |

HALO ES-CN



HALO Bonded Phase Characteristics

HALO Phase	Retention Mechanism	Retention Increased for	Best Application
C18, C8	Hydrophobic interactions	Lipophilic molecules, uncharged acids and bases, strong bases or acids in ion pairing mode	Analytes differing in hydrophobicity, homologues non-aqueous RPLC
RP-Amide	Hydrophobic, hydrogen bonding	Alcohols, acids, phenols	basic analytes, heterocycles, proton donors and acceptors, highly aqueous conditions
Phenyl-Hexyl	Hydrophobic, p-p	Electron-poor compounds , analytes with electron-withdrawing groups, (ketones, nitriles, alkenes, etc)	heterocycles, aromatics, highly aqueous conditions
PFP	Hydrophobic, p-p, hydrogen bonding, dipole-dipole	Electron-rich compounds , analytes with p bonds, electron delocalization and electron-donating groups, proton donors, analytes with different dipole moments	Bases, stereoisomers, steroids, taxanes, substituted aromatics, highly aqueous conditions HILIC separations \geq 80% ACN
ES-CN	Hydrophobic, dipole-dipole	Polar molecules polarity of cyano phase requires 10-20% less organic for retention comparable to other phases, but produces different selectivity	Mixtures of polar and non-polar analytes, explosives, pesticides HILIC separations \geq 80% ACN

Summary

- Stability of the HALO ES-CN bonded phase is comparable to that of HALO C18.
- Calculated Neue “S” values for orthogonality quantitatively show that the selectivity differences among the various HALO bonded phases are significant and can be used to the advantage of method developers.
- Demonstrated examples of selectivity differences among HALO phases for a variety of analytes.
- Summarized HALO stationary characteristics and offered suggestions for best application of each phase.