Enhanced Selectivity using Various Fused-Core® Bonded Phases to Enable Faster Method Development

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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 been shown by many users to possess unusual efficiency and stability, allowing rapid separations with the needed ruggedness of operation of 5 µm particle columns. These 2.7 µm Fused-Core particles permit separation speeds competitive with sub-2-µm totally porous particles, but with 40–50% of the column back pressure. The general strategy for most RPLC method development has been to use a C18 bonded phase first, then look for alternatives if the C18 cannot provide the required resolution. Correct bonded phase selection at the start of the method development would result in faster method development since time constraints do not always permit the opportunity for quality by design experimentation. This presentation will include the Snyder/Dolan selectivity parameters of the various bonded phases that are available on Fused-Core particles. 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. A new cyano bonded phase shows advantages for the separation of basic compounds. These generalizations, when combined with a defined strategy for column selection, facilitate rapid method development using the diverse bonded-phase selectivities of Fused-Core columns.

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, π-p	Electron-poor compounds, analytes with electron-withdrawing groups, (ketones, nitriles, alkenes, etc)	heterocycles, aromatics, highly aqueous conditions
PFP	Hydrophobic, π-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 ≥ 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 ≥ 80% ACN

Experimental

Instrument

- Agilent 1100 quaternary
- Shortest length 0.005" ID tubing between modules
- 3.0 mL heat exchanger
- Semi-micro flow cell, bypassed ($1 < V_{cell} < 5$ µL)

Analytical Columns

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

Column Screening Conditions

Gradients: 5–95%B in 5 min.

- ACN
- 10 mM ammonium formate, pH 3.0

- Temperature: 40 C
- Flow rate: 0.85 mL/min
- Detection: UV @ 254 nm
- Injection volume: 2 µL

Analytes

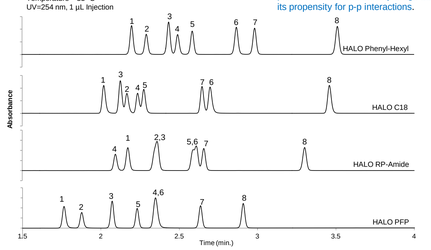
Set represents 23 compounds, specifically pharmaceuticals, including acids, bases, zwitterions, and neutrals.

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

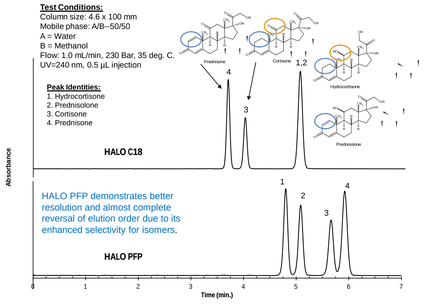
HALO Phenyl-Hexyl is the most retentive phase for these anti-anxiety drugs due to its propensity for p-p interactions.



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



HALO PFP demonstrates better resolution and almost complete reversal of elution order due to its enhanced selectivity for isomers.

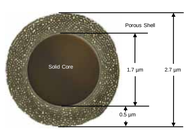
Fused-Core Particles

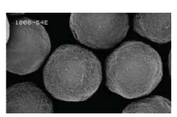
Particle Characteristics

- **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**





Some Suggested Method Development Strategies

Isocratic Method

1. Ensure instrument extracolumn volume is 20 µL or less
   - 5 µL flow cell, 0.005" ID tubing
   - Data Rate > 10 Hz, Response time: £ 0.2 sec
2. Choose 4.6 x 50 mm HALO C18 column.
3. Select pH of aqueous component and buffer.
4. Start with mobile phase having highest ratio of organic modifier to buffer permitted by solubility.
5. Set flow rate at 1.5 to 2.0 mL/min
6. Prepare sample in 50:50 organic modifier/buffer or aqueous.
7. Inject 2 µL.
8. Ensure that all analytes elute at highest % organic.
9. Decrease % organic successively until last component elutes at ~ k = 10–15.
10. Adjust sample solvent composition to keep % organic £ mobile phase % organic.
11. Compare separation at same % organic with HALO RP-Amide, Phenyl-Hexyl and PFP phases.
12. Select phase with best peak shape and band spacing.
13. Optimize % organic, column temp., pH.

Gradient Method

1. Ensure instrument extracolumn volume is 10–20 µL.
   - 2–5 µL flow cell, 0.005" ID tubing
   - Data Rate > 10 Hz, Response time: £ 0.1 sec
2. Choose 4.6 x 50 mm* HALO C18 column.
3. Select pH of aqueous component and buffer.
4. Set flow rate at 1.5 to 2.0 mL/min
5. Prepare sample in minimum ratio of organic modifier/aqueous possible.
6. Inject 2 µL.
7. Run gradient from 5–100% organic in 10 min.
8. Compare 3 x 50 mm HALO RP-Amide, Phenyl-Hexyl, and PFP phases using same gradient profile.
9. Inject 2 µL.
10. Select best phase for further optimization based on band spacing, peak shape, run time.
11. Optimize starting and ending % organic, gradient time, column temp., pH.
12. If more resolution is needed, increase column length as required.

*For those with UHPLC systems and low dispersion volumes, 3.0 x 50 or 2.1 x 50 mm columns are recommended for solvent reduction and faster equilibration.

"Orthogonality" S Values for HALO Bonded Phases

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

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)

	AMT S values	Snyder/Dolan S values
C18 vs. Phenyl-Hexyl	9	18
C18 vs. RP-Amide	14	26
RP-Amide vs. Phenyl-Hexyl	19	35
C18 vs. PFP	63	68

The S values are dependent upon the selected mobile phase and compound set used for screening.

AMT Buffer: 10 mM Ammonium Formate pH 3
AMT Compound Set: 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
Snyder/Dolan Buffer: 30 mM Potassium Phosphate pH 2.8
Snyder/Dolan Compound Set: 5,5-diphenylhydantoin, 5-phenylpentanol, acetophenone, amitriptyline, anisole, benzonitrile, berberine, cis-chalcone, ethylbenzene, mefenamic acid, N,N-diethylacetamide, N,N-dimethylacetamide, n-butylbenzoic acid, nortriptyline, p-nitrophenol, thiourea, toluene, trans-chalcone

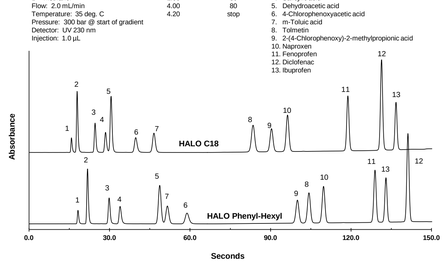
HALO C18 vs. Phenyl-Hexyl: Organic Acids

Test Conditions:
Columns: 4.6 x 50 mm
Mobile Phase:
A = 0.02 M Phosphate buffer. pH =2.5
B = Methanol
Flow: 2.0 mL/min
Temperature: 35 deg. C
Pressure: 300 bar @ start of gradient
Detector: UV 230 nm
Injection: 1.0 µL

Gradient Program

Time	%Methanol
0.00	55
0.75	55
2.00	80
4.00	80
4.20	stop

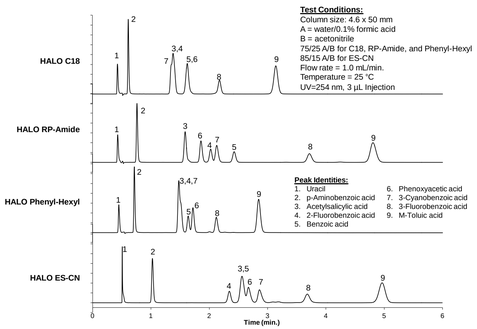
Peak Identities:
1. Acetaminophen
2. Hippuric acid
3. 2-Fluorobenzoic acid
4. Salicylic acid
5. Dehydroacetic acid
6. 4-Chlorophenoxyacetic acid
7. m-Toluic acid
8. Tolmetin
9. 2-(4-Chlorophenoxy)-2-methylpropionic acid
10. Naproxen
11. Fenoprofen
12. Diclofenac
13. Ibuprofen



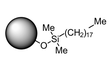
Benzoic Acids on HALO Fused-Core Bonded Phases

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

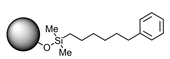
Peak Identities:
1. Uracil
2. p-Aminobenzoic acid
3. Acetylsalicylic acid
4. 2-Fluorobenzoic acid
5. Benzoic acid
6. Phenoxyacetic acid
7. 3-Cyanobenzoic acid
8. 3-Fluorobenzoic acid
9. M-Toluic acid



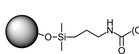
HALO Fused-Core Bonded Phases



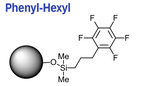
C18 (octadecyl)



Phenyl-Hexyl

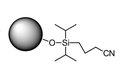


RP-Amide



PFP (pentafluorophenylpropyl)

New at EAS 2011!

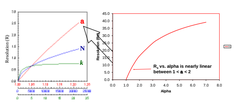


ES-CN (extra stable-cyanopropyl)

Selectivity and Orthogonal Separations

- Selectivity is the most influential parameter in the resolution equation

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



- 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

Snyder/Dolan Values for HALO Phases

$$\log a = \log\left(\frac{k}{k_{EB}}\right) = hH + s\sigma^* + b\beta + a\alpha + kC$$

Column	H	S*	A	B	$C_{2.8}$	$C_{7.0}$
HALO C18	1.107	0.048	0.006	-0.050	0.056	0.040
HALO Phenyl-Hexyl	0.789	-0.094	-0.233	-0.006	0.101	0.456
HALO RP-Amide	0.859	0.080	-0.384	0.190	-0.417	0.312
HALO PFP	0.702	-0.117	-0.073	-0.062	1.168	0.972

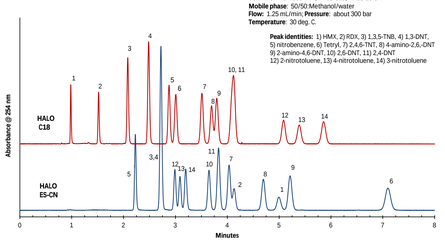
F_S = column selectivity comparison function, based on differences in H, S*, A, B and C for two columns, where the larger the F_s value, the greater the difference in selectivity.

Column	F_S
HALO C18	0.00
HALO Phenyl-Hexyl	17.8
HALO RP-Amide	53.9
HALO PFP	92.7

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

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



Summary

- Presented differences in selectivity among various HALO phases.
  - Neue "S" values for orthogonality
  - Snyder-Dolan Hydrophobic Subtraction Model values
- Demonstrated examples of selectivity differences among HALO phases for variety of analytes.
- Offered example method development strategies for isocratic and gradient methods.
- Recommended more elaborate method development schemes using several different pHs, temperatures, etc. for difficult samples, including use of computer simulation such as DryLab® 2010.
- Summarized HALO stationary characteristics and offered suggestions for best application of each phase.

Acknowledgement

- Chris Heard of BASi Northwest Laboratory for the Snyder/Dolan data