Faster Method Development Using Enhanced Selectivity For Various Fused-Core Bonded Phases

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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.

Most analysts begin their method development by attempting their separation on a C18 bonded phase. However, there are instances when a simple alkyl bonded phase cannot provide the required resolution. This presentation will include comparative selectivity mapping along with 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 rigid aromatic ring, while Phenyl-Hexyl is more retentive for electron-poor compounds. In the case of the RP-Amide phase, polar compounds are better retained compared to the C18 bonded phase. These generalizations, when combined with a defined strategy for column selection, facilitate rapid method development using this array of bonded phase fused-core columns.

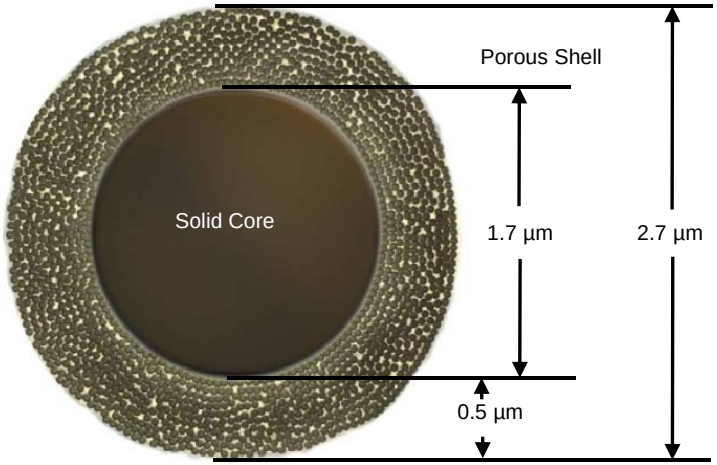
Fused-Core Particles

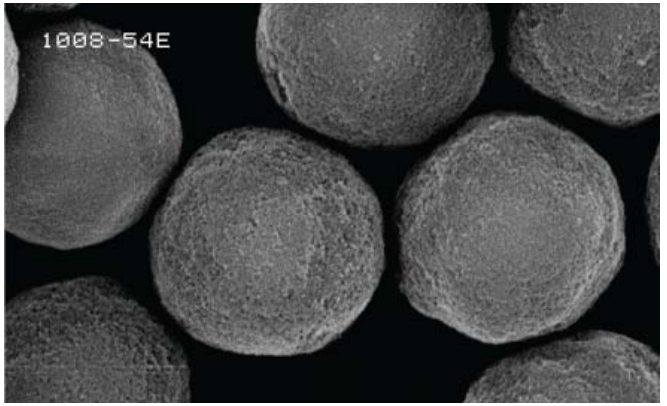
Particle Characteristics

- **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**

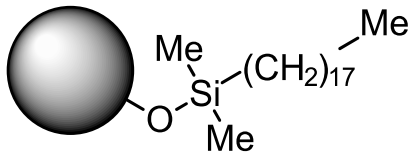
Features and Benefits

- **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**

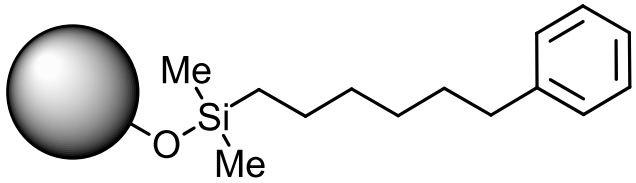




HALO Fused-Core Bonded Phases

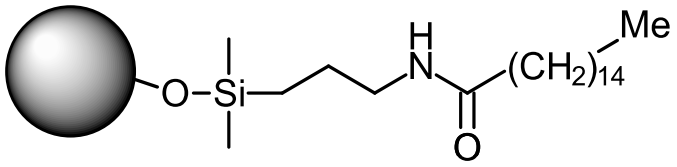


C18 (octadecyl)

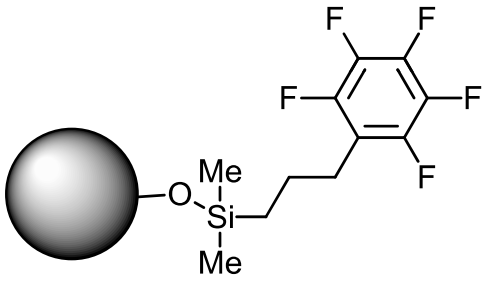


Phenyl-Hexyl

RP-Amide



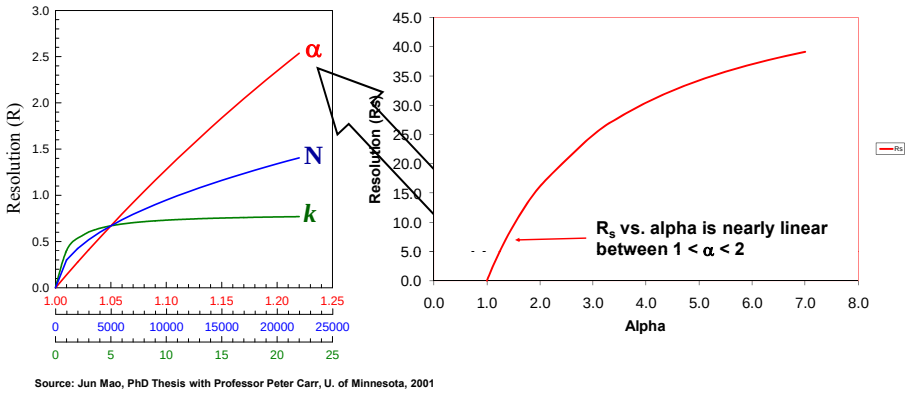
PFP (pentafluorophenylpropyl)



Selectivity and Orthogonal Separations

- Selectivity is the most influential parameter in the resolution equation

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



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
- Shortest length 0.005" ID tubing between modules
- 3.0 µL 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

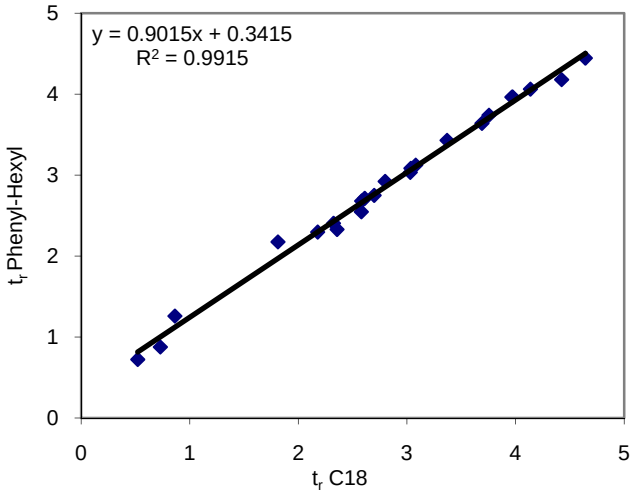
- Temperature: 40 °C
- Flow rate: 0.85 mL/min
- Detection: UV @ 254 nm
- Injection volume: 2 µL

Analytes

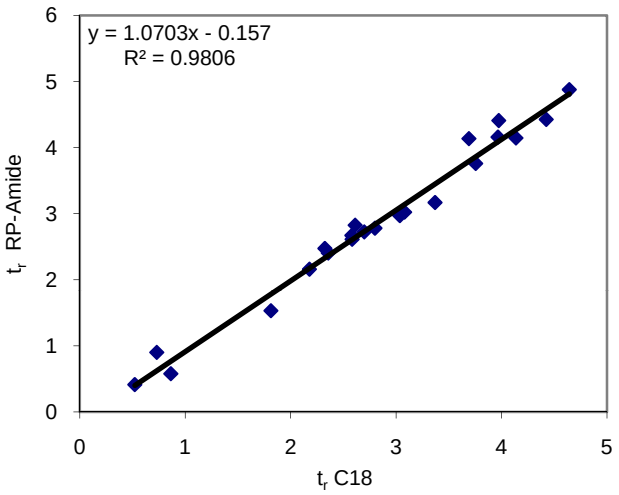
Set represents 23 compounds, specifically pharmaceuticals, including acids, bases, zwitterions, and neutrals.

“Orthogonality” Graphs

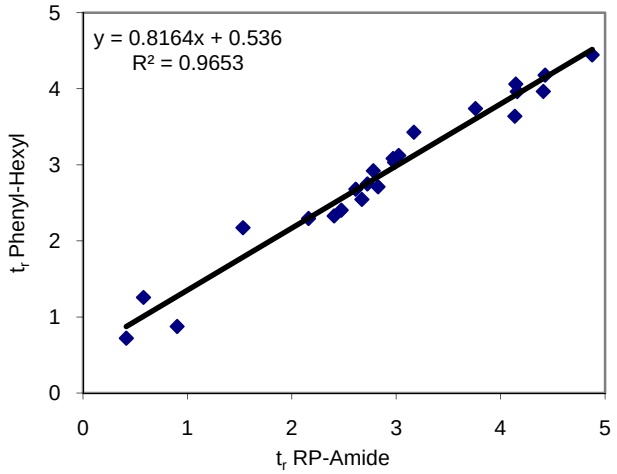
C18 vs. Phenyl-Hexyl
S = 9



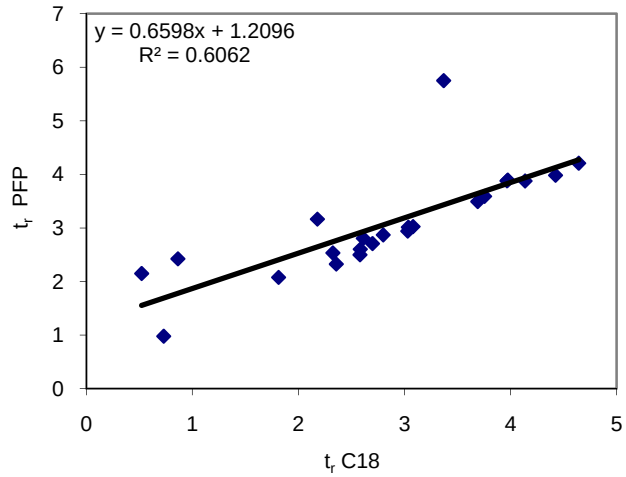
C18 vs. RP-Amide
S = 14



RP-Amide vs. Phenyl-Hexyl
S = 19



C18 vs. PFP
S = 63



"Orthogonality" S Values for HALO Bonded Phases

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

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

Snyder/Dolan Values for HALO Phases

$$\log \alpha \equiv \log\left(\frac{k}{k_{EB}}\right) \equiv \eta' H - \sigma' S^{*} + \beta' A + \alpha' B + \kappa' C$$

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.

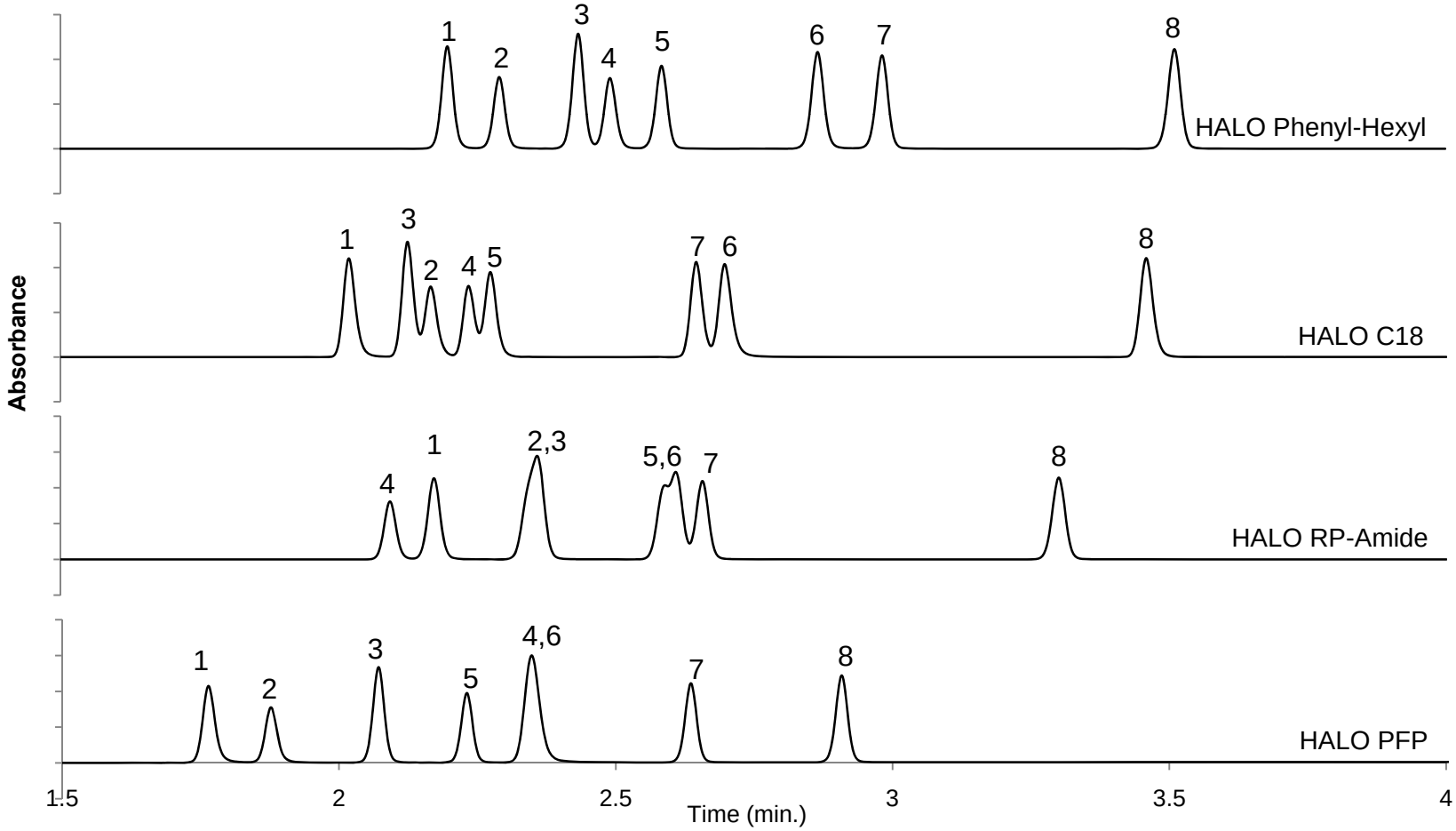
Column	F_s
HALO C18	0.00
HALO Phenyl-Hexyl	17.8
HALO RP-Amide	53.9
HALO PFP	92.7

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 π-π interactions.



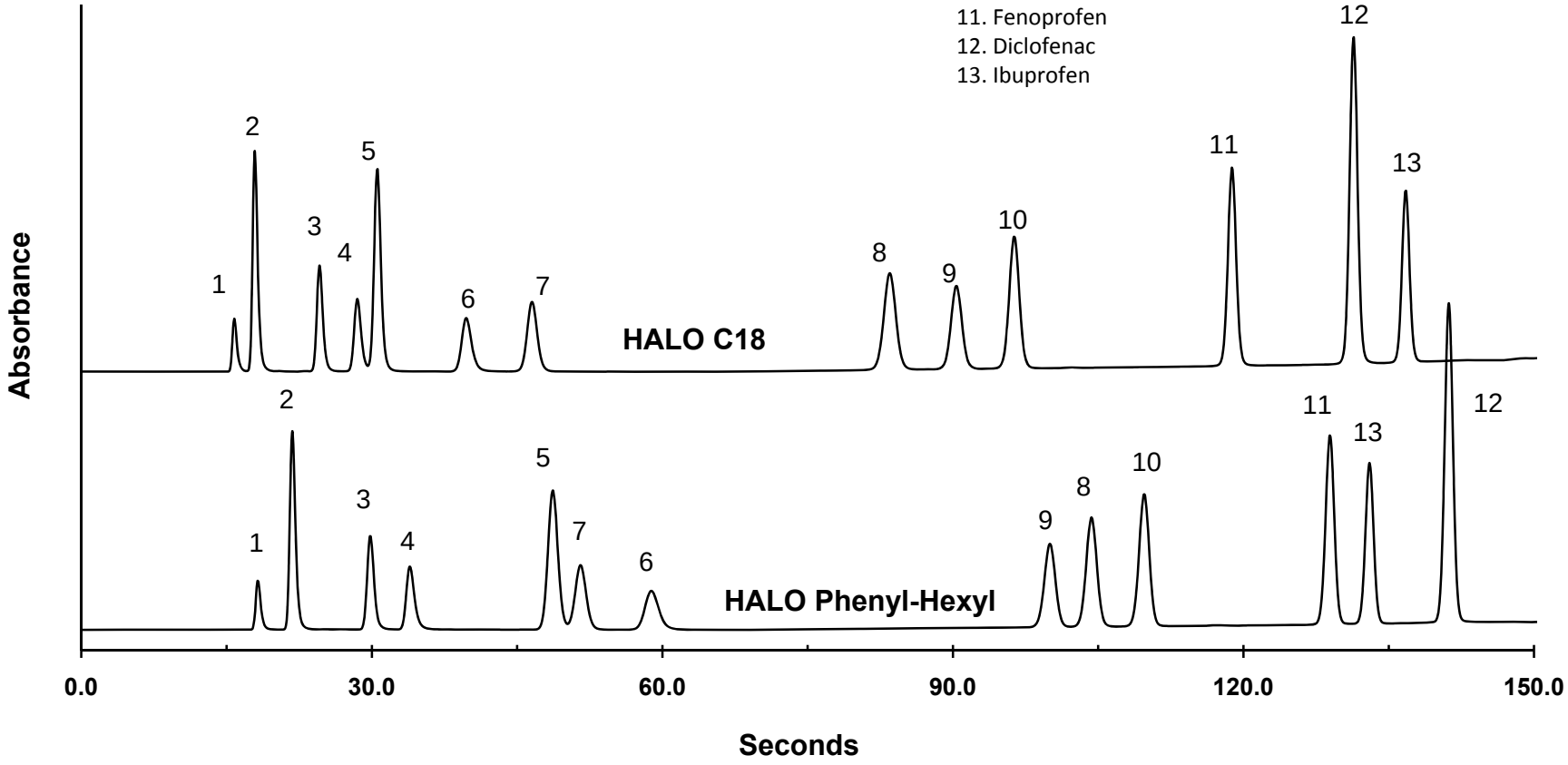
HALO C18 vs. Phenyl-Hexyl: Organic Acids

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

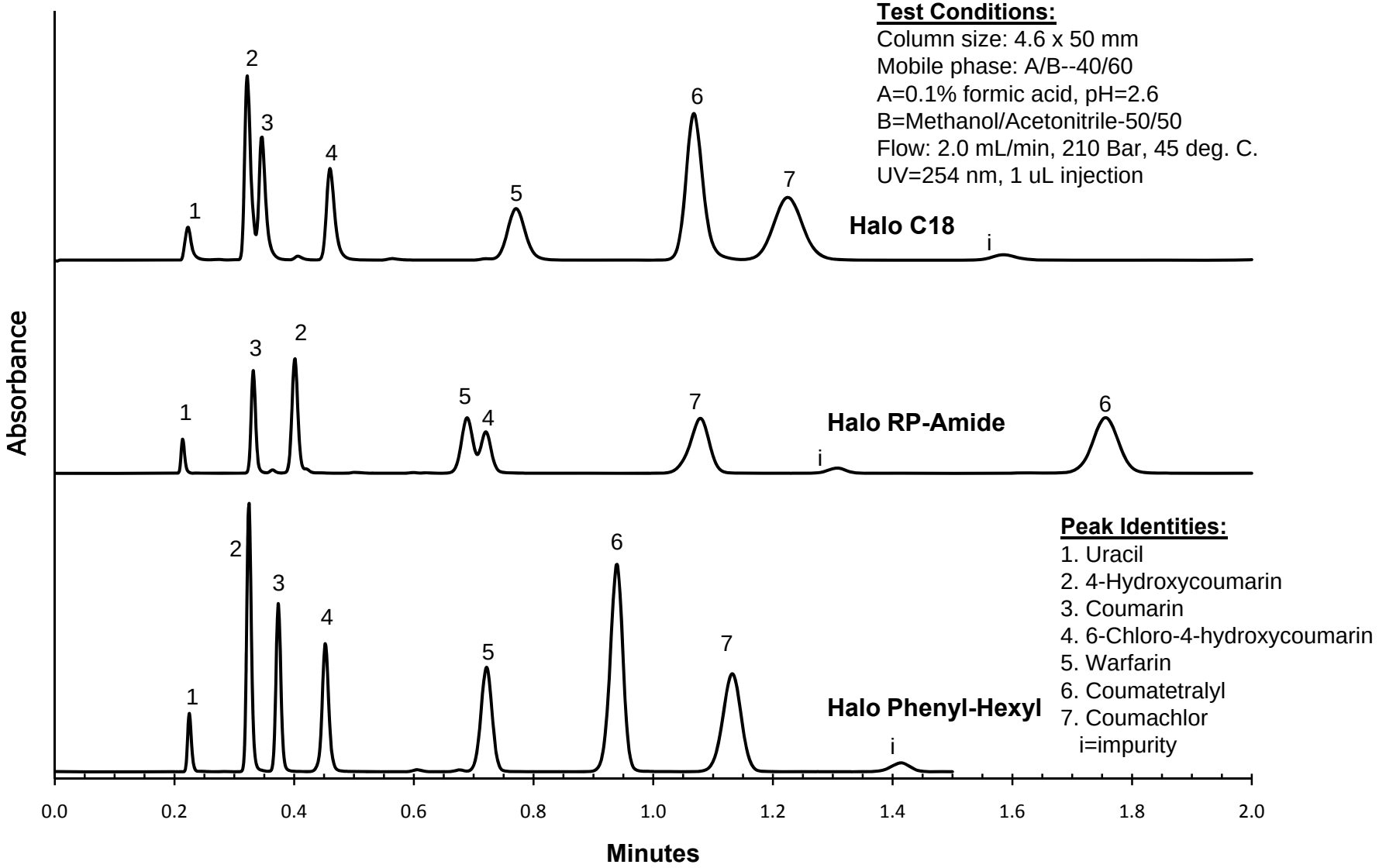
Gradient Program

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



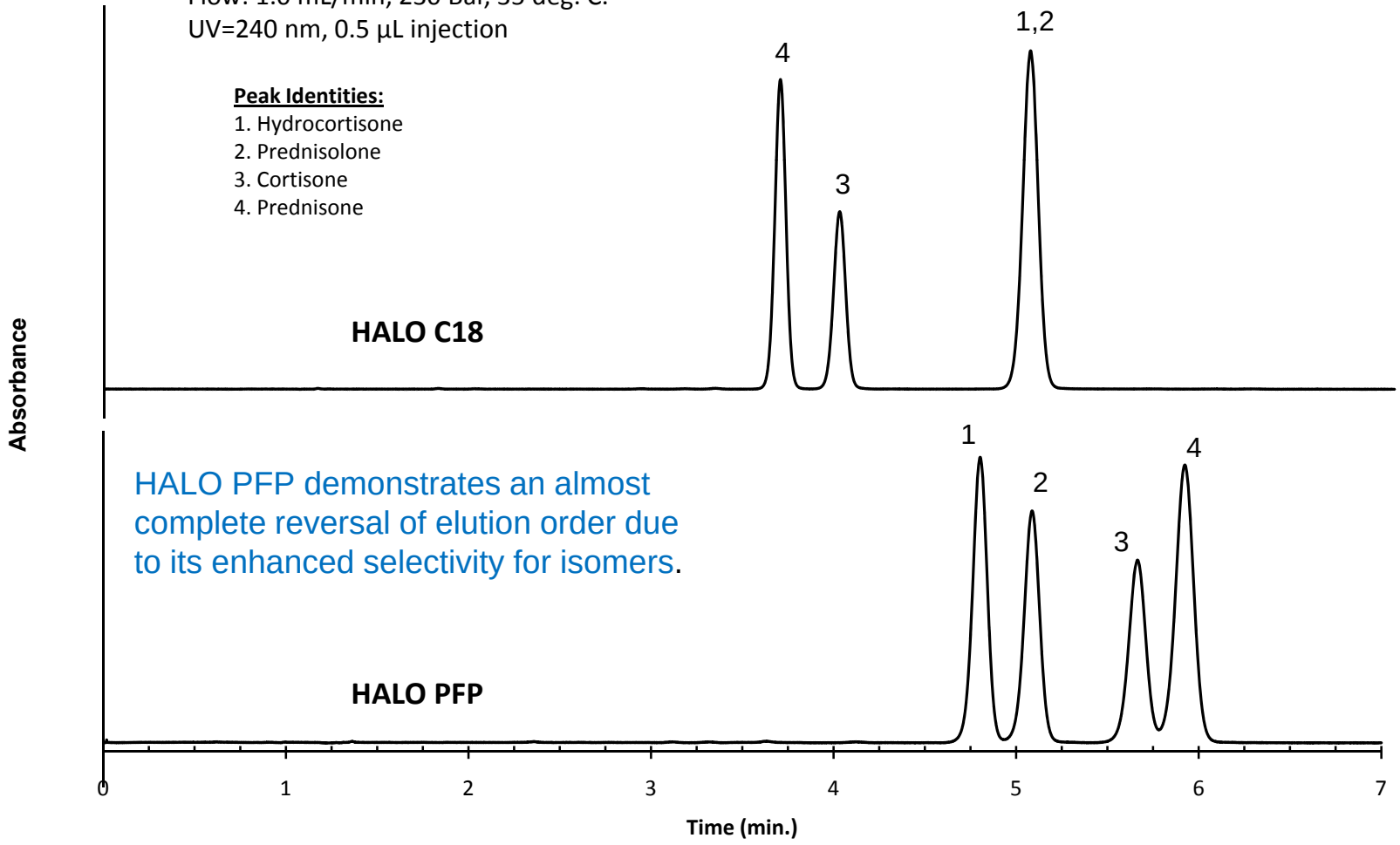
Fast Separation of Anticoagulants on HALO Fused-Core Packings



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 an almost complete reversal of elution order due to its enhanced selectivity for isomers.

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, a 3.0 x 50 mm column is recommended for solvent reduction and faster equilibration.

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–π	Electron-poor compounds, analytes with electron-withdrawing groups, (ketones, nitriles, alkenes, etc)	heterocycles, aromatics, highly aqueous conditions
PFP	Hydrophobic, π–π, hydrogen bonding, dipole-dipole	Electron-rich compounds, analytes with π 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
Silica/HILIC	NPLC: analyte adsorption on silica and displacement by solvent molecules HILIC: partitioning of polar analytes between highly organic mob. phase and water layer near silica surface	NPLC: Polar vs. nonpolar analytes, planar vs. nonplanar HILIC: Polar vs. nonpolar analytes	NPLC: analytes with low or no water solubility, stereoisomers, HILIC Mode: polar acids, bases, and neutrals

Summary

- Demonstrated differences in selectivity among various HALO phases
  - Neue “S” values for orthogonality
  - Snyder-Dolan Hydrophobic Subtraction Model values
- Presented examples of selectivity differences among HALO phases for variety of analytes
- Presented example method development strategies for isocratic and gradient methods.

  More elaborate method development schemes using several different pHs, temperatures, etc. are also advisable for difficult samples, including use of computer simulation such as DryLab® 2010.
- Summarized HALO stationary characteristics and offered suggestions for best application of each.

Acknowledgement

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