High Resolution Separations using Fused-Core® Columns with Non-C18 Bonded Phases

J.J. DeStefano, T.J. Langlois, W. L. Johnson and S. A. Schuster
Advanced Materials Technology, Inc.,
3521 Silverside Rd.,
Suite K-1, Quillen Bldg., Wilmington, DE 19810

Objective

The objective of this work is to demonstrate fast separations while highlighting the influence of bonded phase other than C18 on selectivity.

Abstract

HPLC columns of Fused-core® superficially porous particles having an overall diameter of 2.7 µm and a porous shell of 0.5 µm thickness have been shown by many users to possess unusual efficiency and stability, allowing very fast separation speeds with the needed ruggedness of operation of 5 µm particle columns. These 2.7 µm fused-core particles allow separation speeds competitive with sub-2 µm totally porous particles but with up to one-half the column back pressure. Most HPLC separations utilize 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 addition of HILIC, embedded polar phases and other bonded phases now allow alternate separation selectivities to C18 that enhances the use of the highly-efficient fused-core particles for very fast, rugged separations. Illustrative separations with such new fused-core materials and the effects of operating variables such as mobile phases, flow rate, and temperature demonstrate the effectiveness of these new stationary phases for selectivity enhancement.

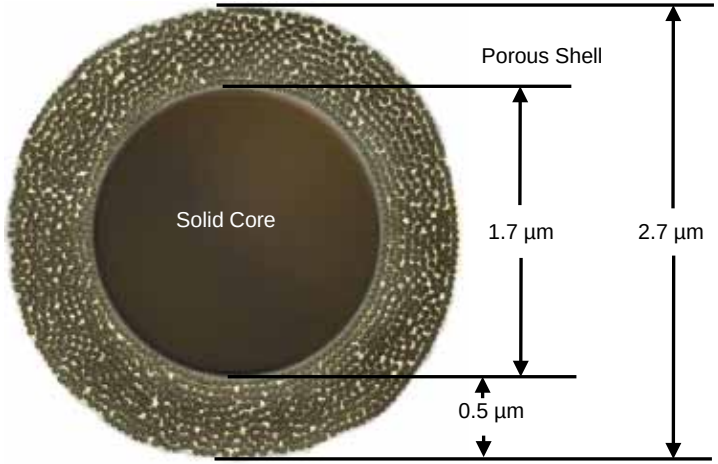
Fused-Core Particles

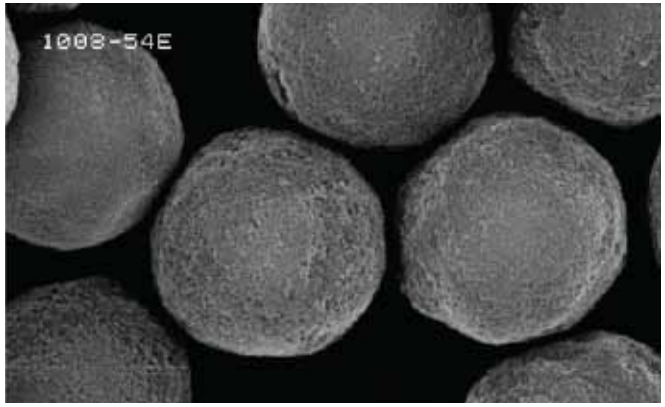
Particle Characteristics

- 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

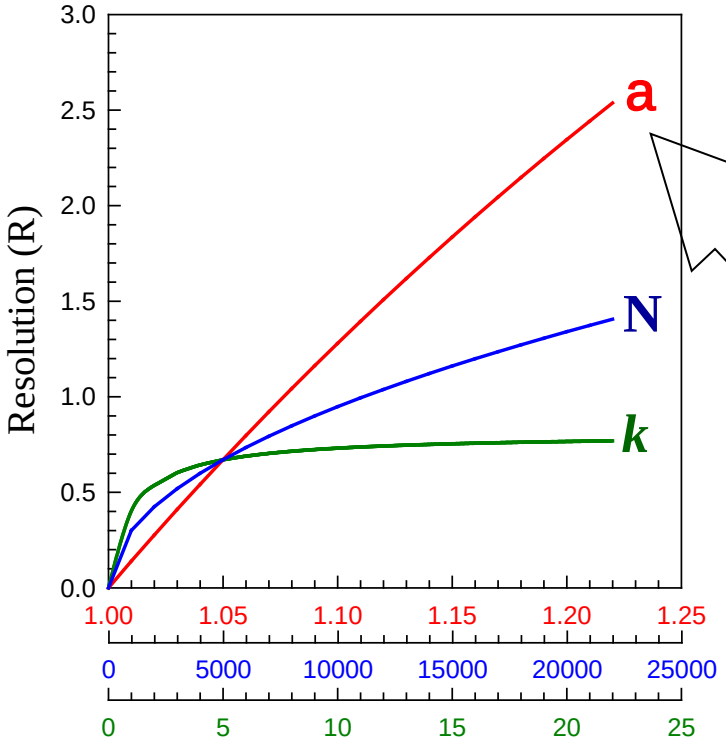


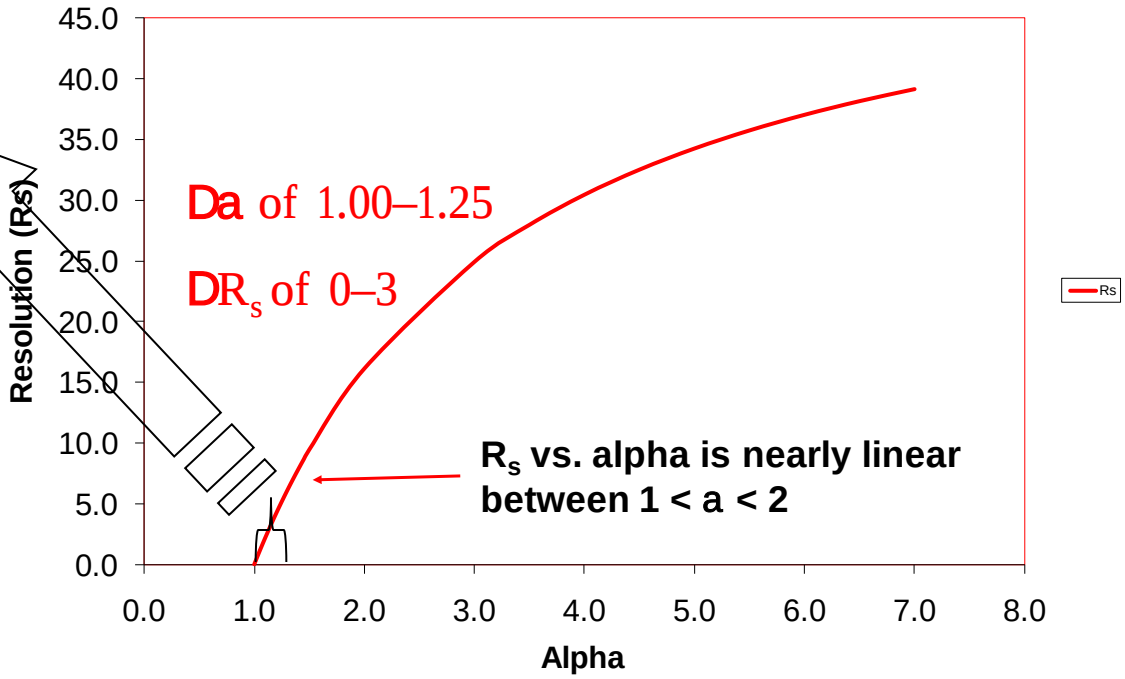


Resolution Equation Shows that Selectivity is Most Effective Parameter to Change

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

Resolution is directly proportional to selectivity (a), but is only proportional to the square root of efficiency, N.



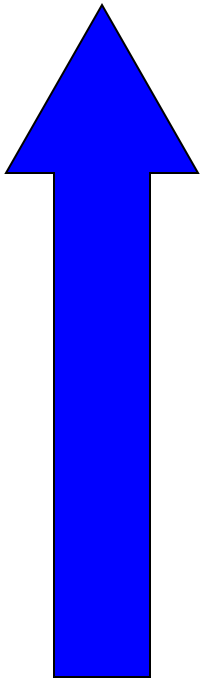


Source: Jun Mao, PhD Thesis with Professor Peter Carr, U. of Minnesota, 2001

Most Effective Parameters to Change Selectivity

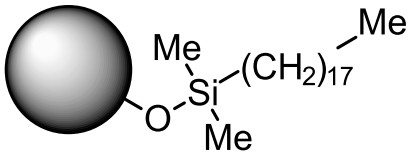
The analysis condition parameters that most affect selectivity, a are[1]:

Column type (C18, phenyl, amide, etc.)	++
B-solvent (acetonitrile, methanol, etc.)	++
Mobile phase pH	++
Ion-pair concentration	++
%B solvent/gradient steepness	+
Column temperature	+
Buffer concentration	+

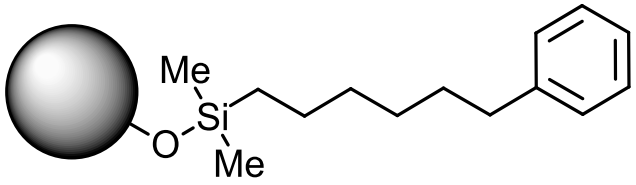


[1]adapted from "Introduction to Modern Liquid Chromatography", 3rd Edition, L. R. Snyder, J. J. Kirkland, J. W. Dolan; p. 29, 2010, John Wiley & Sons, Inc.

HALO Fused-Core Bonded Phases

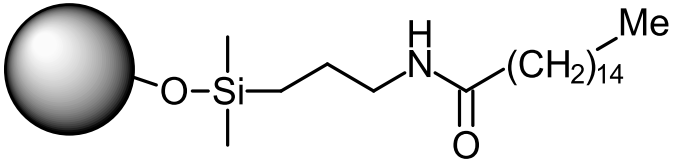


C18 (octadecyl)

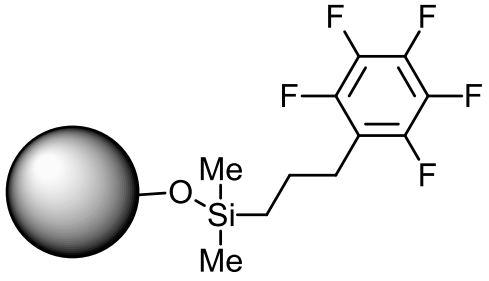


Phenyl-Hexyl

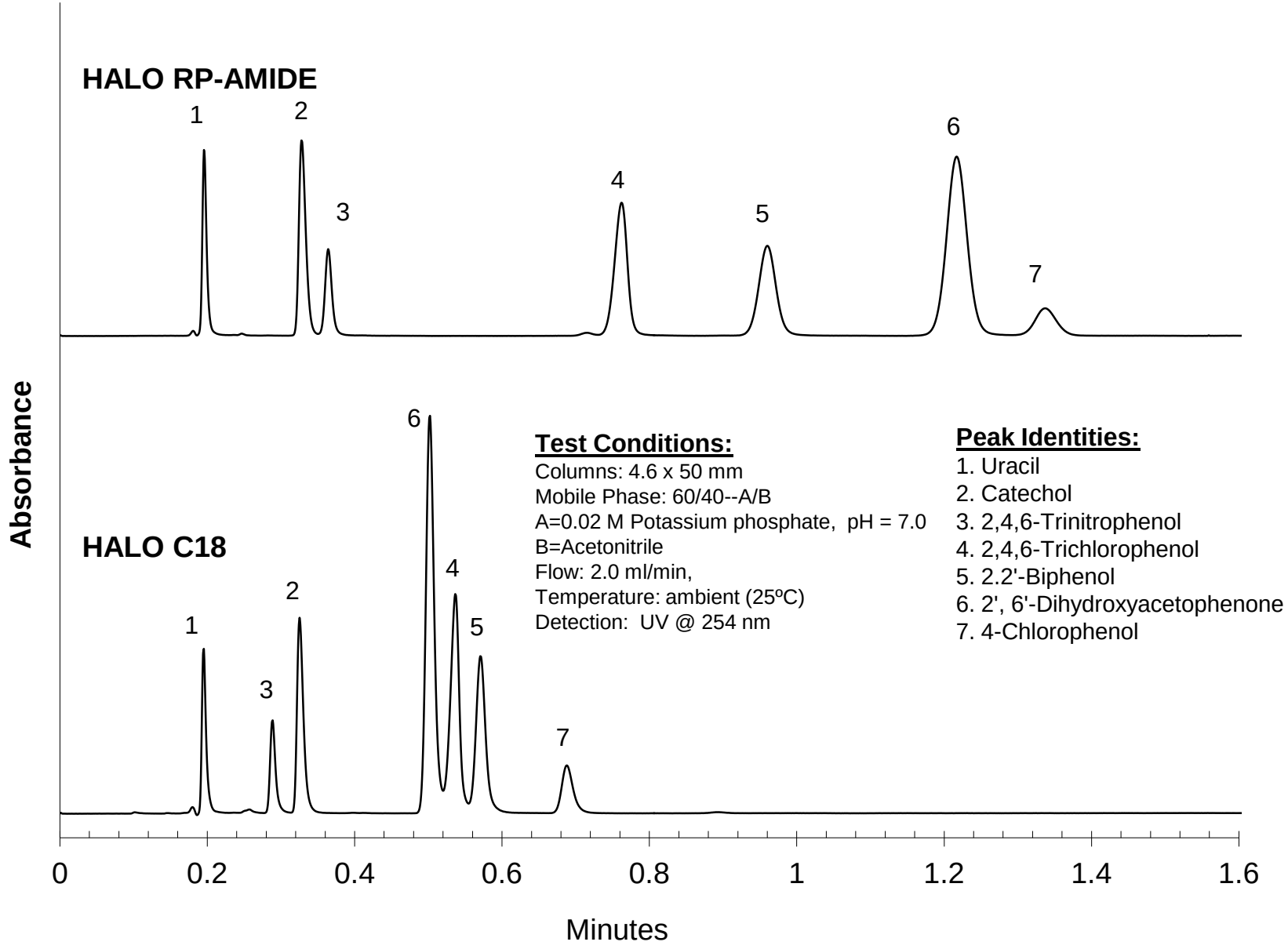
RP-Amide



PFP (pentafluorophenylpropyl)



HALO C18 vs. RP-Amide for Phenolics



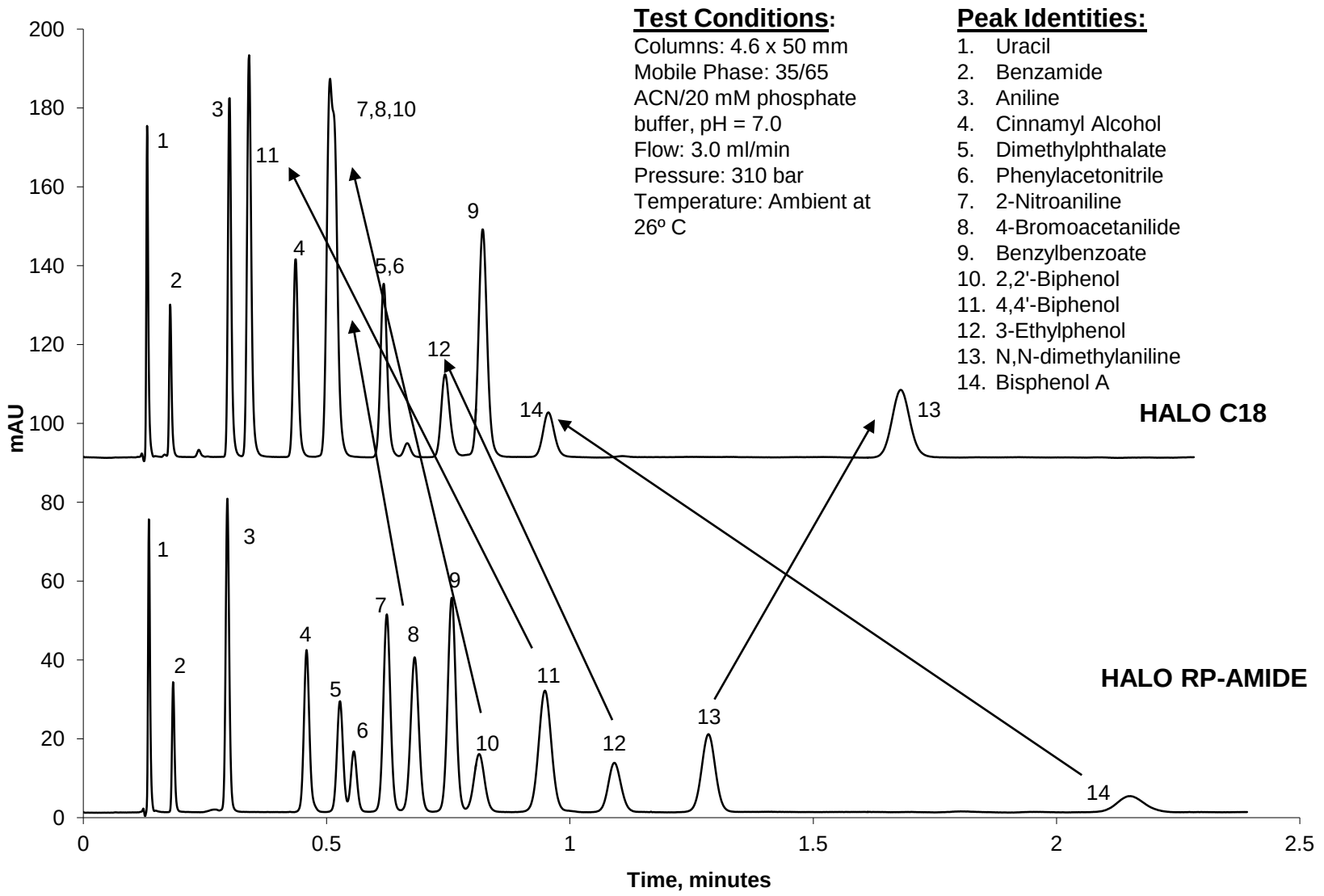
Test Conditions:

Columns: 4.6 x 50 mm
Mobile Phase: 60/40--A/B
A=0.02 M Potassium phosphate, pH = 7.0
B=Acetonitrile
Flow: 2.0 ml/min,
Temperature: ambient (25ºC)
Detection: UV @ 254 nm

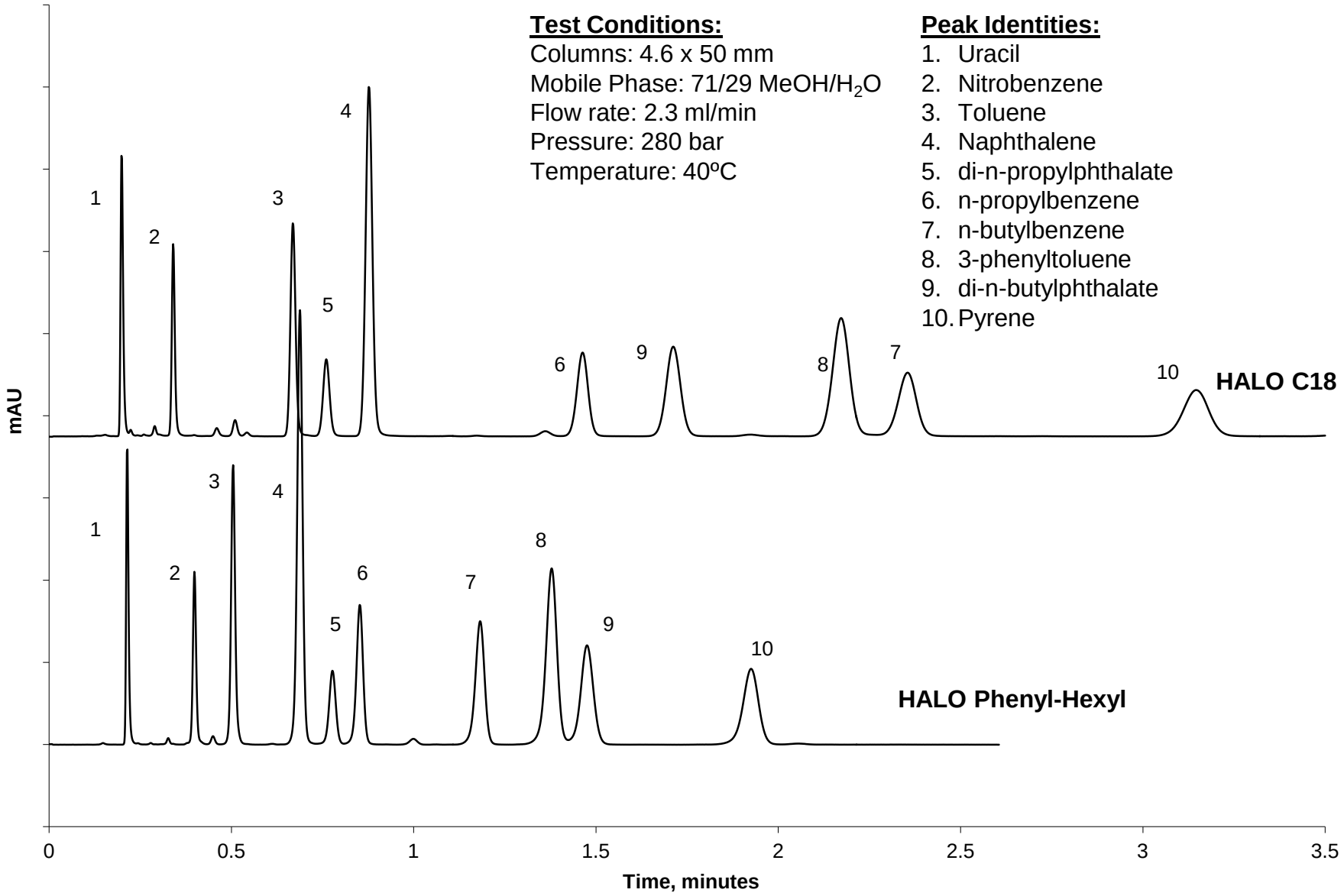
Peak Identities:

1. Uracil
2. Catechol
3. 2,4,6-Trinitrophenol
4. 2,4,6-Trichlorophenol
5. 2.2'-Biphenol
6. 2', 6'-Dihydroxyacetophenone
7. 4-Chlorophenol

HALO C18 vs. RP-Amide: Polar Analytes



HALO C18 vs. Phenyl-Hexyl: Aromatics



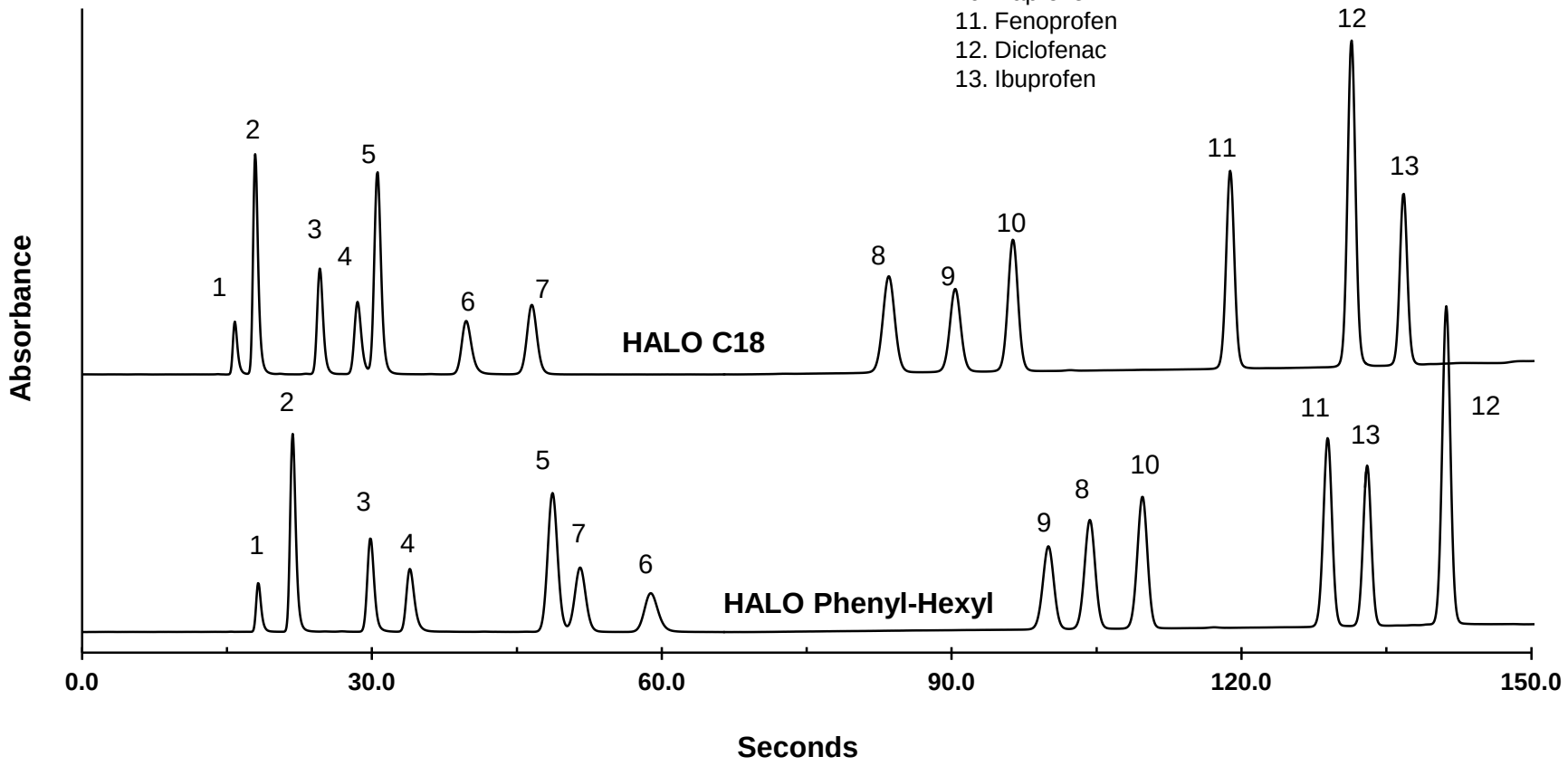
HALO C18 vs. Phenyl-Hexyl: Organic Acids

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 grad.
Detector: UV 230 nm
Injection: 1.0 uL

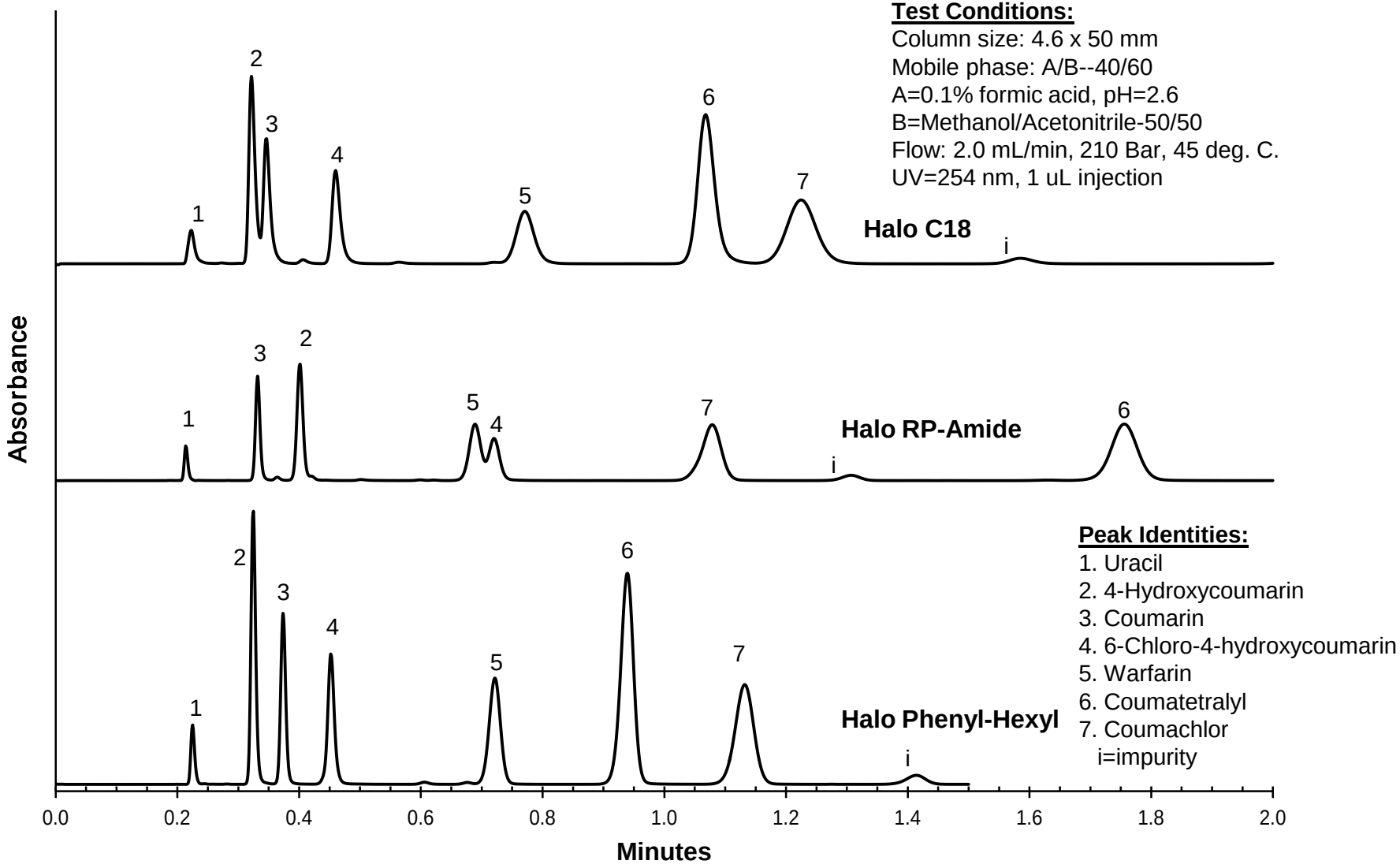
Gradient Program

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



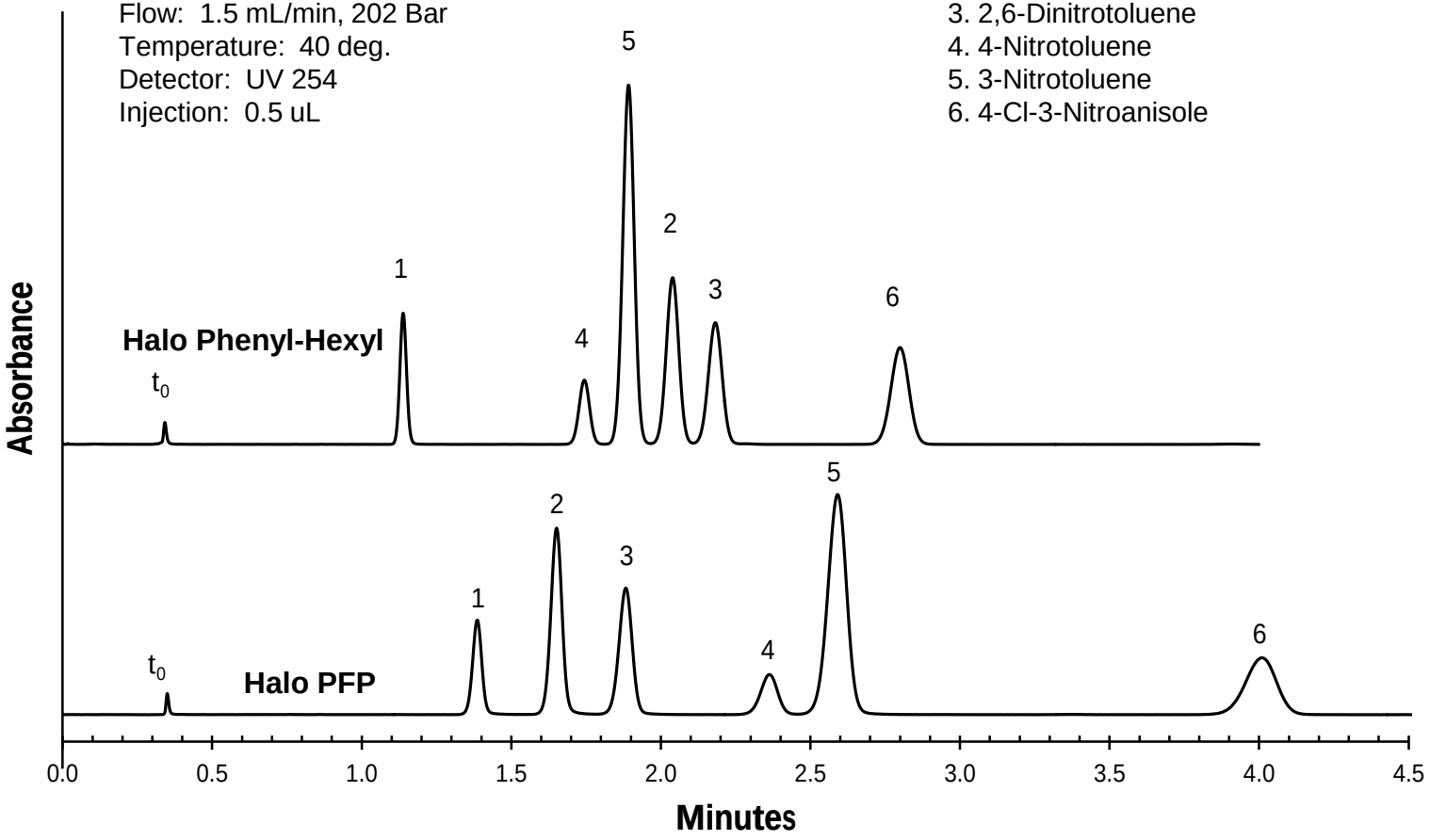
Fast Separation of Anticoagulants on HALO Fused-Core Packings



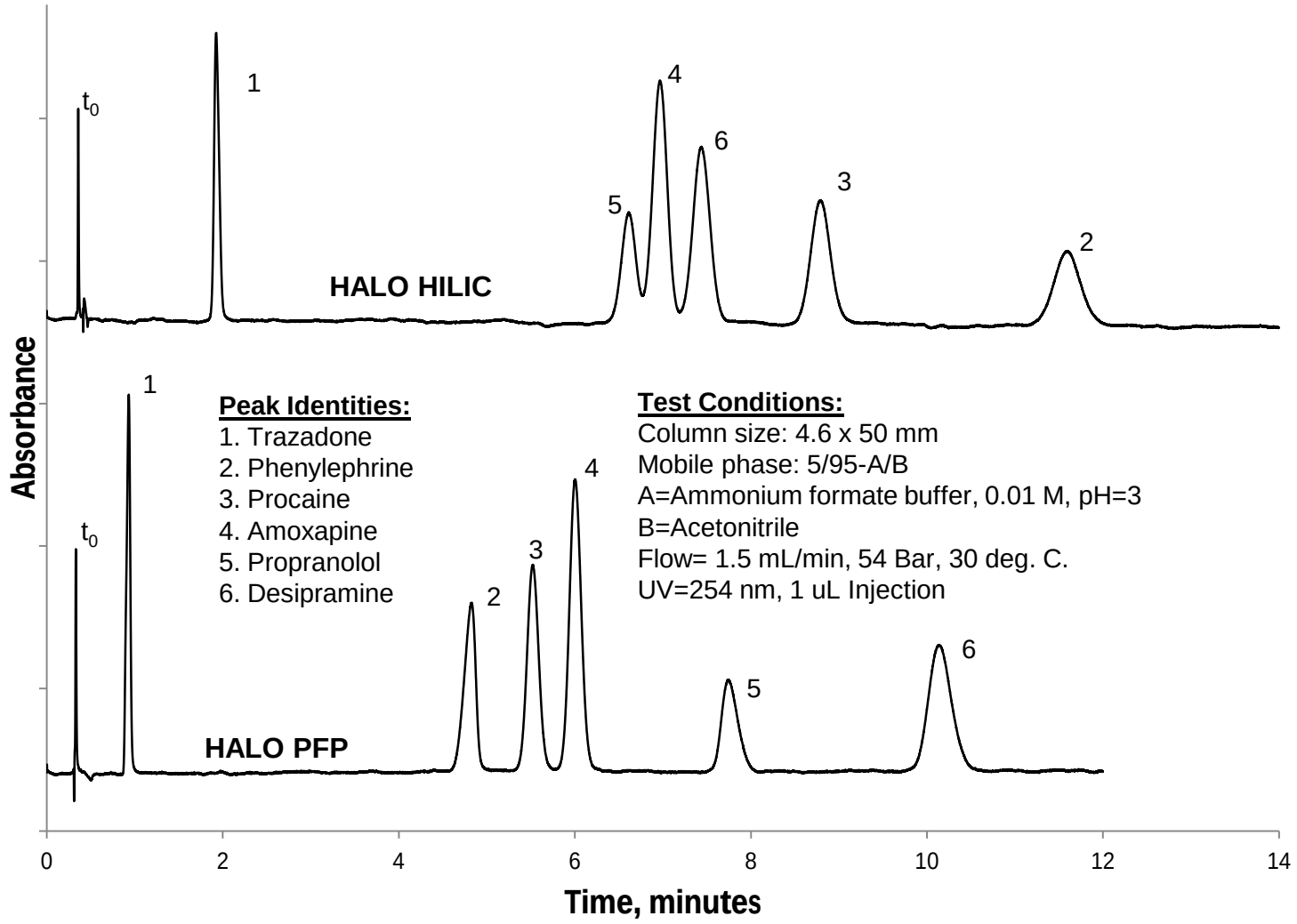
HALO Phenyl-Hexyl vs. PFP: Aromatic Nitro Compounds

Test Conditions:
Columns: 4.6 x 50 mm
Mobile phase: 45/55-water/methanol
Flow: 1.5 mL/min, 202 Bar
Temperature: 40 deg.
Detector: UV 254
Injection: 0.5 uL

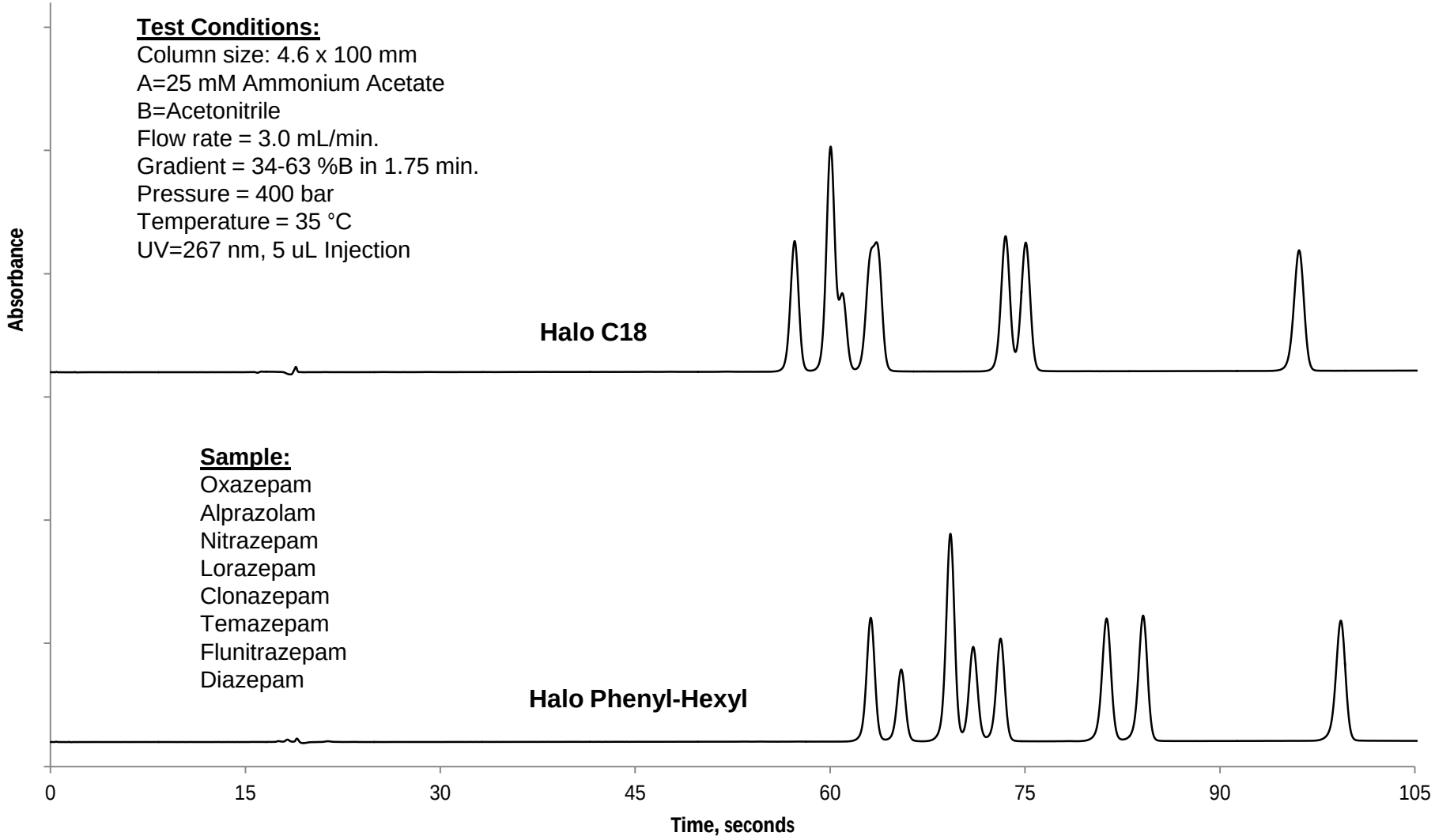
Peak Identities:
1. Nitrobenzene
2. 1-Cl-4-Nitrobenzene
3. 2,6-Dinitrotoluene
4. 4-Nitrotoluene
5. 3-Nitrotoluene
6. 4-Cl-3-Nitroanisole



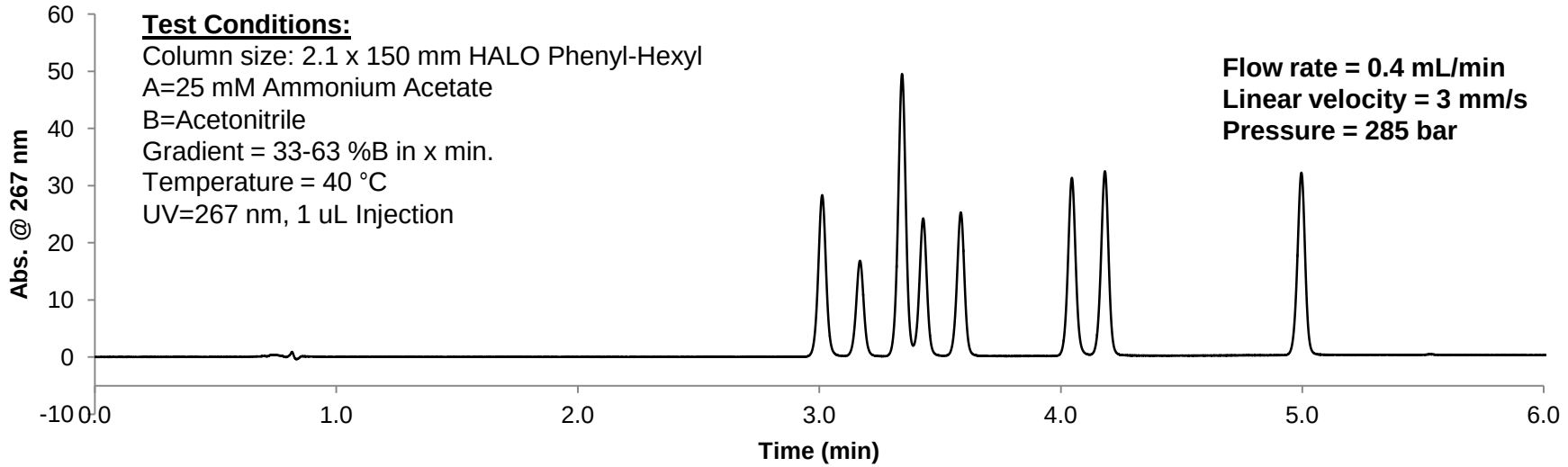
HALO PFP vs. HILIC: Basic Drugs

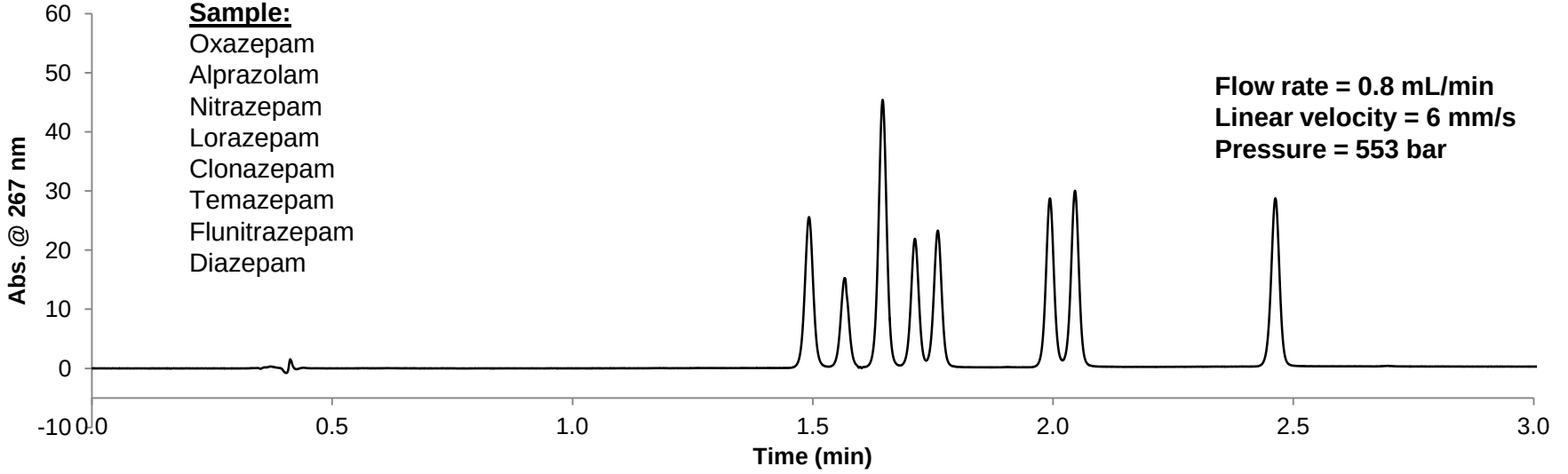


HALO C18 vs. Phenyl-Hexyl: Benzodiazepines



High Resolution at High Linear Velocity





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 ≥ 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

- **Changing selectivity, α, is most effective way to improve resolution and adjust elution order.**
- **Column phases having different retention mechanisms are one of the top 4 parameters for changing selectivity of an HPLC separation.**
- **The HALO Fused-Core column family has a set of orthogonal phases to enable fast, effective method development.**
- **HALO columns enable faster analyses while maintaining high resolution.**