Theory & Practice of Developing LC Methods with Solid-Core Particle Columns

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In Partnership with MAC-MOD, our authorized channel partner. MAC-MOD have been a trusted partner for over 20 years with expertise in HPLC & UHPLC

Solid Core Particle Technology

- Solid core[1] particles have gained interest for UHPLC / HPLC due to high efficiencies, rapid separations, method transferability, and low back pressure
- Solid core particles in the 2.X µm range offer the potential of **sub-2 µm efficiencies with HPLC pressures**
- Solid core particles can **accelerate Method Development** on standard HPLC instrumentation
- Extensive theoretical and practical assessments of solid core particles have been reported. A brief summary is provided here

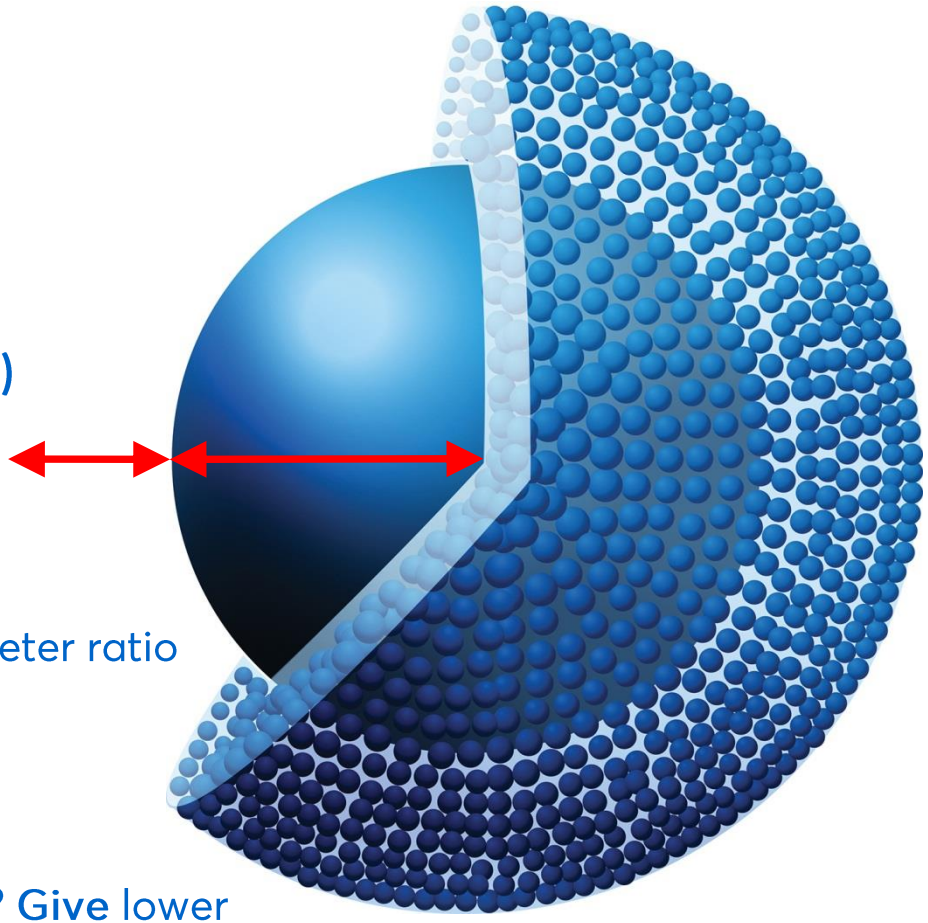
[1] Also known as Fused Core™ , Core Shell, Core Enhanced, Partially Porous or Superficially Porous Particles (SPP).

Solid Core Particle Technology

- Particle architecture

2.4-2.7 µm & 5µm typical

Smaller particles also available (1.3-1.7 µm)
(with their own challenges!)

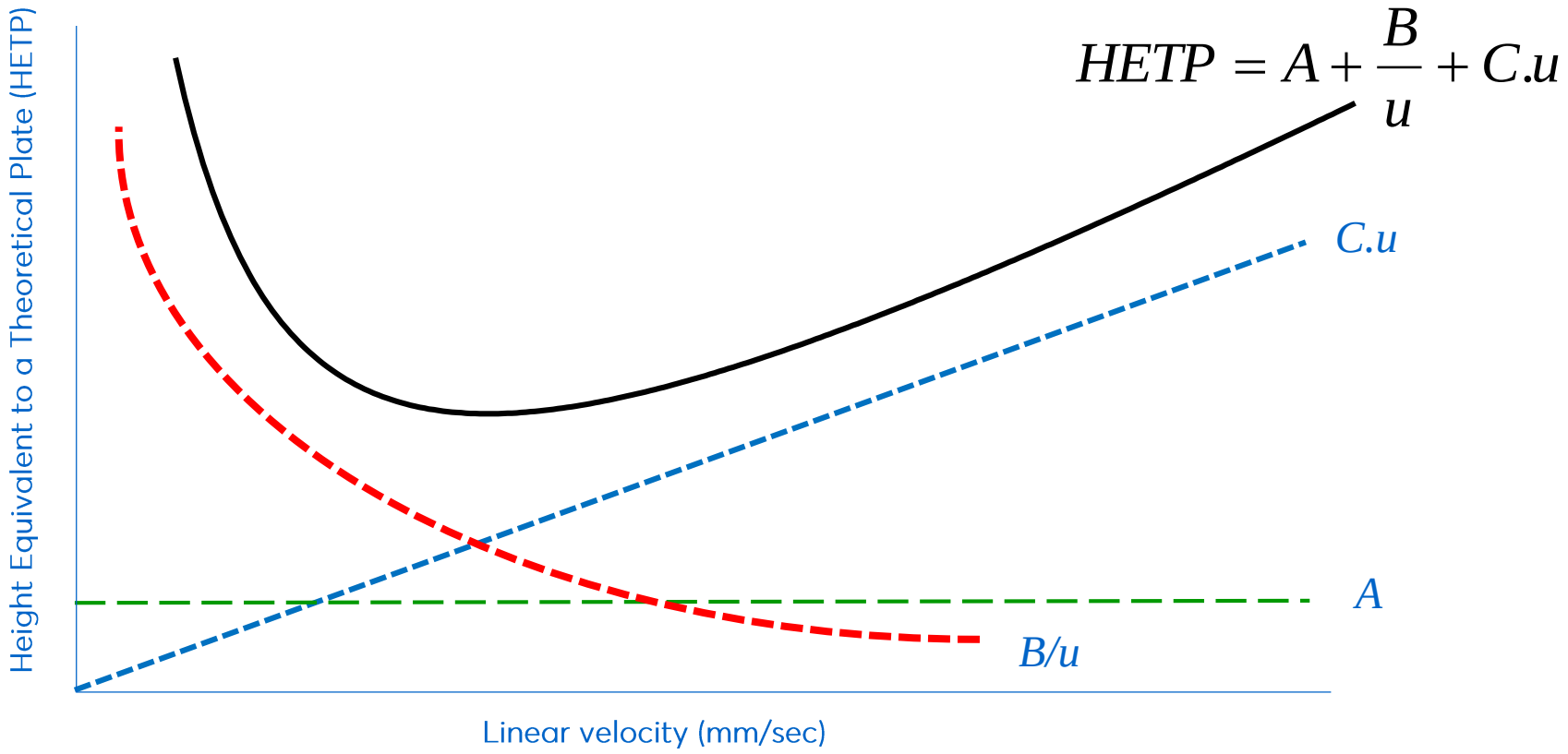


Rho (ρ) = solid core diameter : particle diameter ratio
Typically 0.6 – 0.75 for SPP

- Why do solid core particles
  - **Give** more efficiency? **Give** faster analyses? **Give** lower back pressure?

Efficiency

VAN DEEMTER CURVE

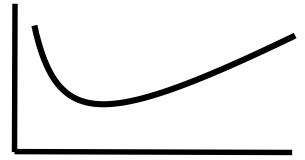


A	Eddy diffusion (analyte paths, packing, wall effects)
B/u	Analyte longitudinal / axial diffusion
C.u	Analyte mass transfer between stationary & mobile phases

Solid Core Efficiency Summary Facts

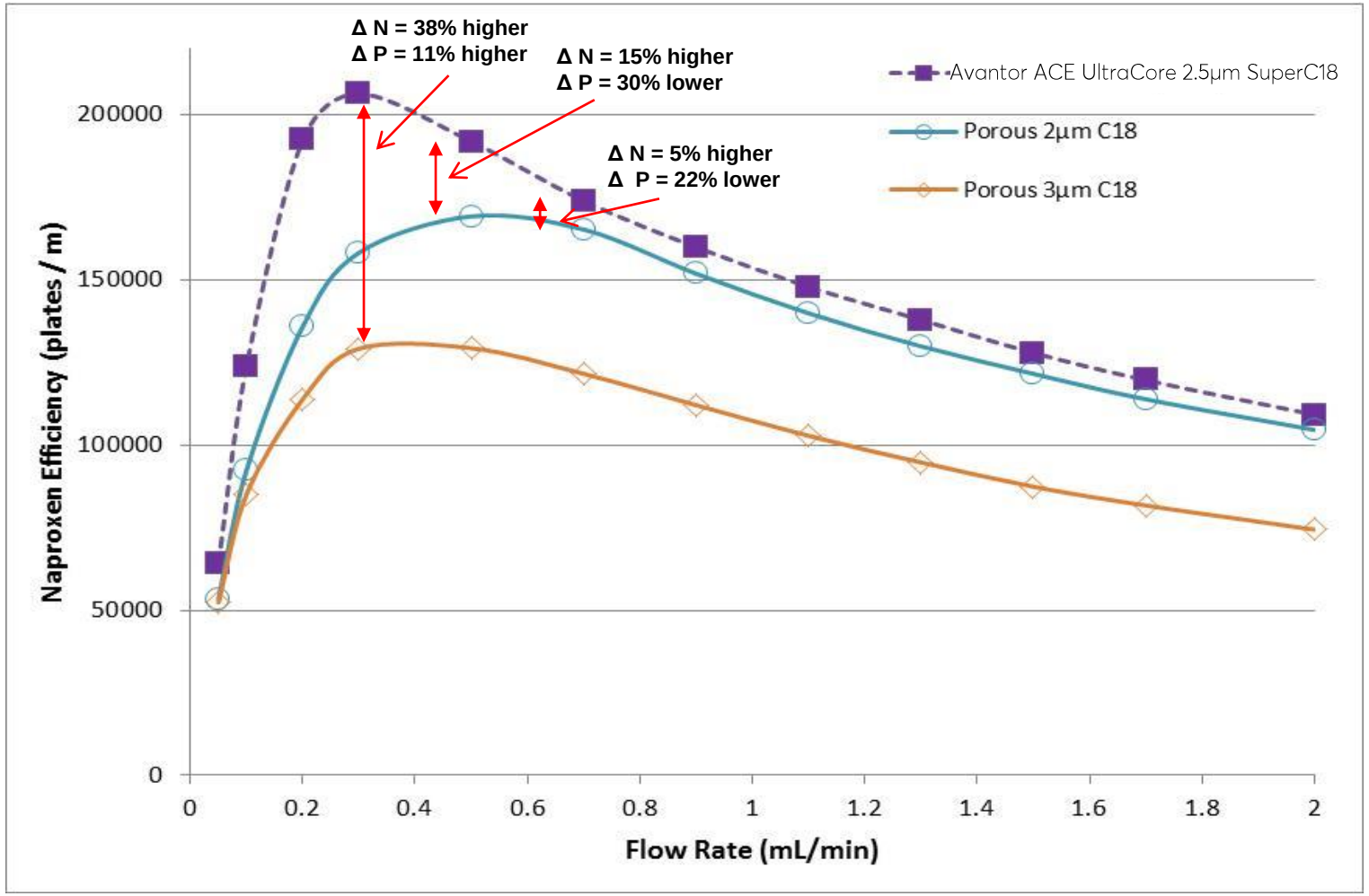
- The A term is not constant at low flows and reductions in trans-column eddy dispersion and wall effects / particle roughness may be significant[2] leading to higher efficiencies
- The B/u term is significant and improves efficiency by reducing analyte molecular diffusion processes
- Shorter molecular diffusion paths (C.u term) are **NOT** the reason for improved efficiency. However they are more dominant for large molecules
- The tight particle size distribution of solid core particles & packing quality have **limited influence** on the improved efficiency[1,2]

$A+B/u+C.u$



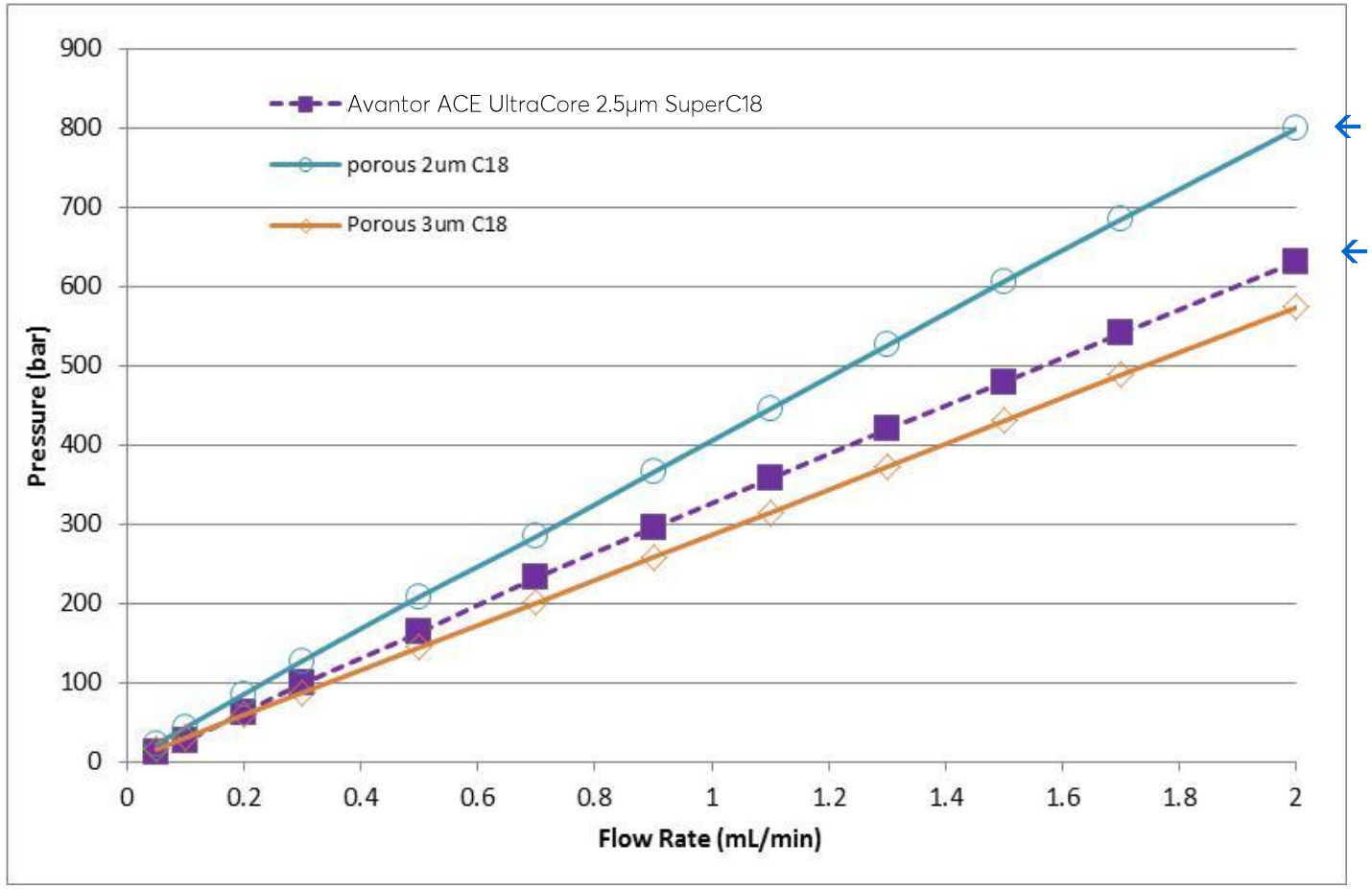
1 A Daneyko et al., Anal. Chem. 83 (2011) 3903-3910.
2. F. Gritti et al., J. Chromatogr. A 1218 (2011) 8209-8221.

Efficiency / Flow Comparison: Porous and Solid Core



Isocratic analysis, 50x2.1mm columns, eluent = MeCN / water + 0.1% TFA, analyte = naproxen, constant k = 10, 40°C, λ=256 nm

Pressure / Flow Comparison: 2µm Porous & 2.5µm Solid Core



Isocratic analysis, 50x2.1mm columns, eluent = MeCN / water + 0.1% TFA, analyte = ketoprofen, constant k = 10, 40°C, λ=256 nm

Solid Core Particles Give Faster Analyses

There are 2 aspects as to why solid core particles offer **faster analyses** than porous equivalents:

Higher linear velocities for a **given efficiency**.

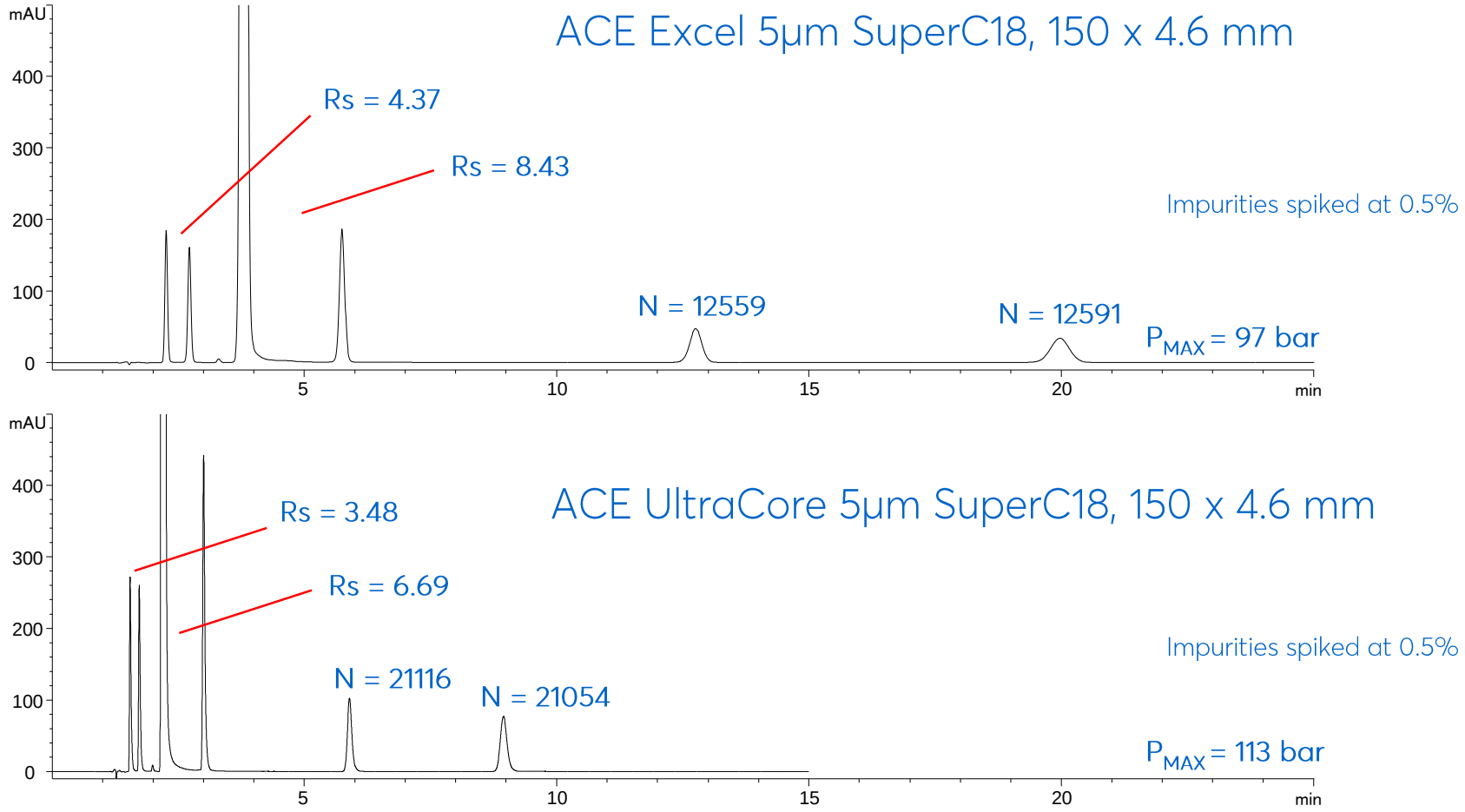
Reduced particle surface area gives **lower hydrophobicity** for a bonded phase.

When moving from a 5µm fully porous particle to a 2.7µm solid core particle, you could trade some efficiency & reduce column length to speed up a method even further

Effect on Peak Capacity

- Peak capacity is a measure of the number of sample analytes that can be separated on an HPLC column per unit time
- Narrow peaks (increase in column efficiency) increase the peak capacity and efficiency of analytical peaks
- Solid core particles have a greater peak capacity & improved S/N then 5 or 3 µm

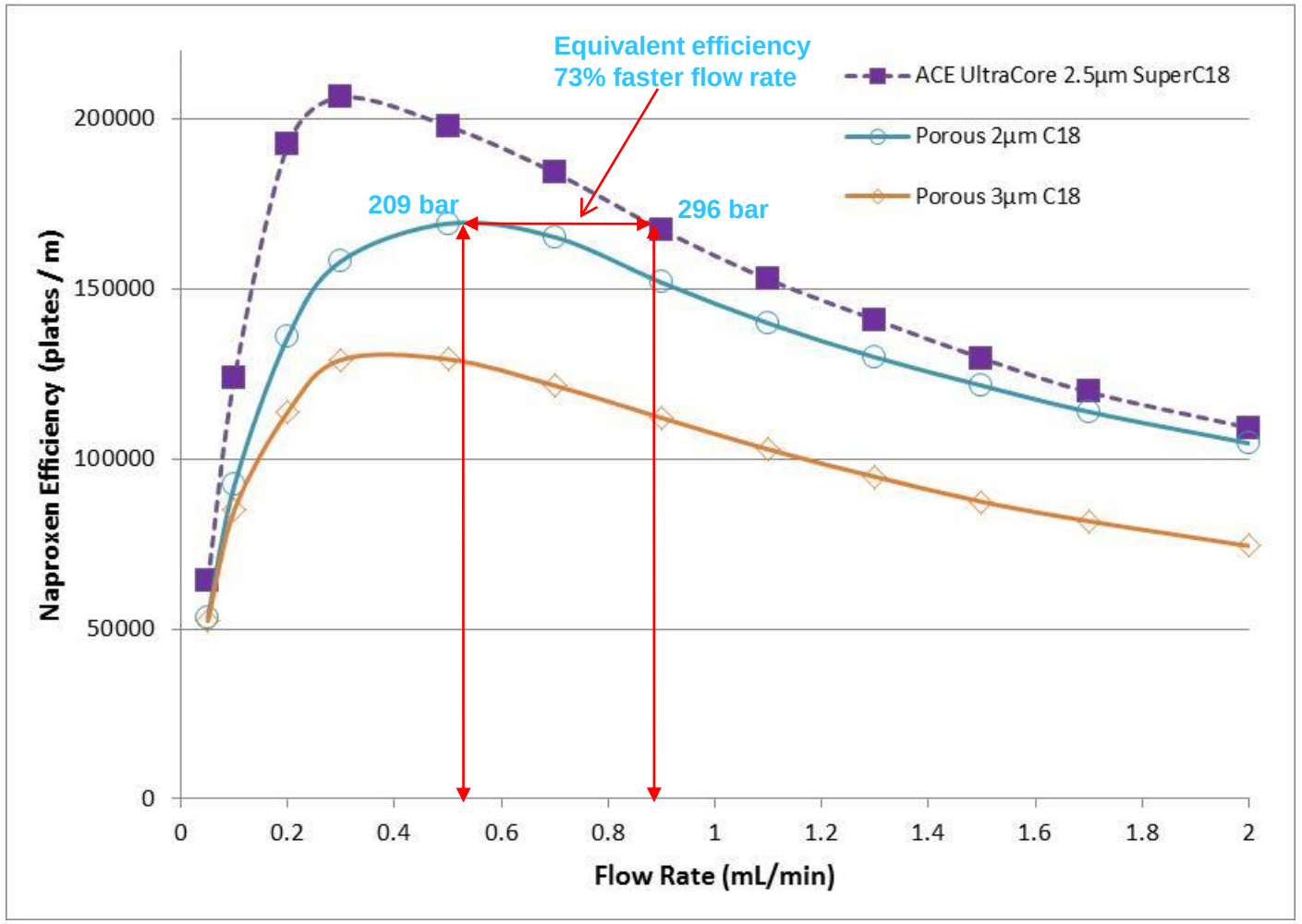
Isocratic Aspirin Analysis: Porous and Solid Core Columns



Reduced hydrophobicity of solid core particles leads to 'faster' analysis

Conditions: (Top): 60:35:5:0.2 v/v/v/v water:acetonitrile:methanol:85% phosphoric acid, 237 nm (2.5 Hz), 25°C, 1 mL/min, 5 µL injection
(Bottom): 60:35:5:0.2 v/v water:acetonitrile:methanol:85% phosphoric acid, 237 nm (20 Hz), 25°C, 1 mL/min, 3.9 µL injection

Efficiency / Flow Comparison: Faster Analyses



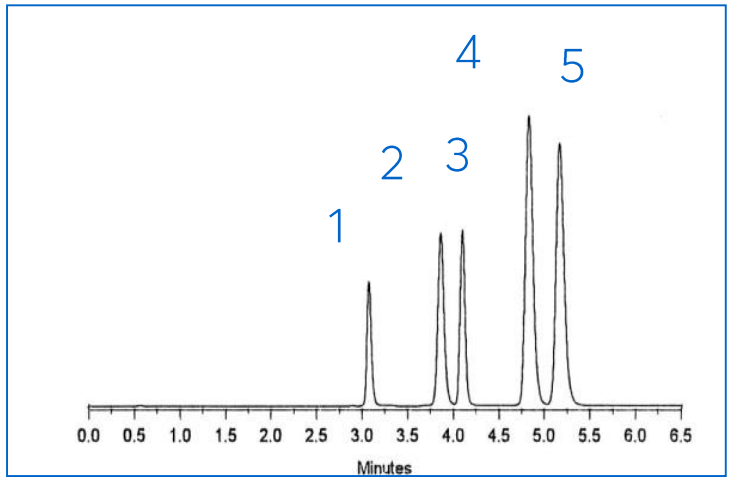
Isocratic analysis, 50x2.1mm columns, eluent = MeCN / water + 0.1% TFA, analyte = ketoprofen, constant k = 10, 40°C, λ=256 nm

Solid Core Particles Are Less Hydrophobic

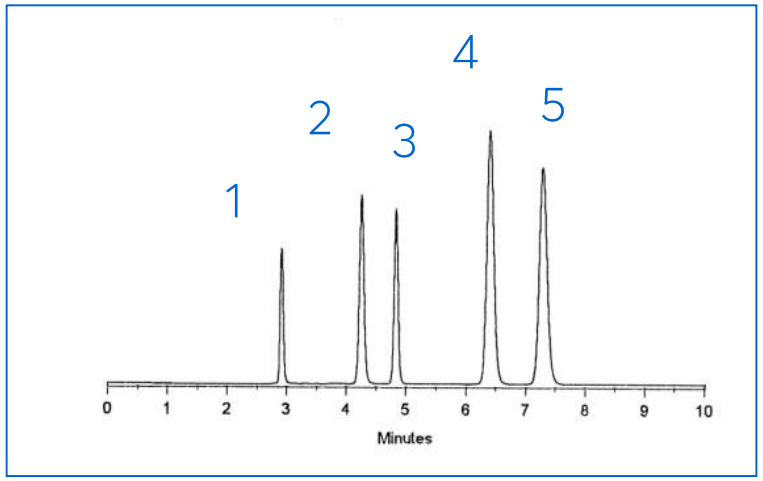
- In RPLC hydrophobicity is a dominant mechanism
- The hydrophobicity of a stationary phase is related to the ligand characteristics (e.g., C4, C8, C18) and amount present on the particle (i.e. %C or μmol/m^2)
- Reducing the hydrophobicity of a bonded phase (e.g., by decreasing the chain length or reducing the coverage on the particle) reduces retention in RPLC
- This can be easily demonstrated under the same conditions

The Influence of Chain Length on Hydrophobic Character

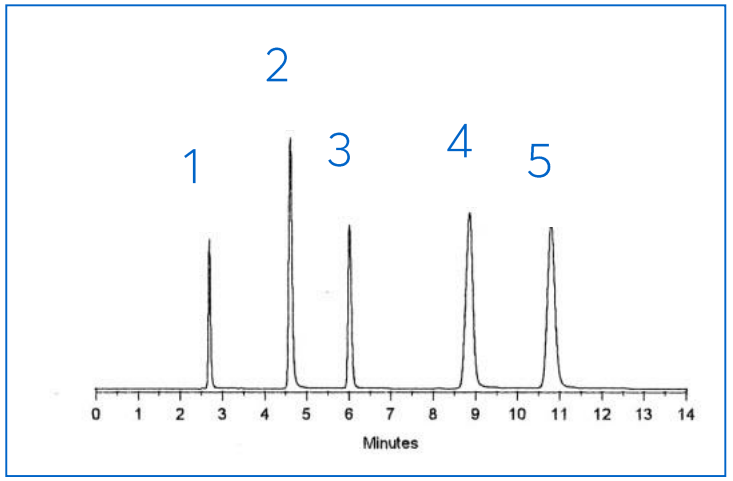
C4



C8



C18



PHASE	USP LISTING	FUNCTIONAL GROUP	ENDCAPPED	PARTICLE SIZE (µm)	PORE SIZE (Å)	SURFACE AREA (m^2/g)	CARBON LOAD (%)	pH RANGE
Avantor® ACE® Traditional Chemistries								
C18	L1	Octadecyl	Yes	1.7, 2, 3, 5, 10	100	300	15.5	2 – 8
C8	L7	Octyl	Yes	2, 3, 5, 10	100	300	9	2 – 8
C4	L26	Butyl	Yes	2, 3, 5, 10	100	300	5.5	2 – 8

Sample: 1. Norephedrine 2. Nortriptyline 3. Toluene 4. Imipramine 5.Amitriptyline
Column: 250 x 4.6mm 5µm Mobile phase: 80:20 v/v MeOH/25mM KH_2PO_4 (pH6.0) Flow: 1.0mL/min, Wavelength: 215nm

Gives hydrophobicity differences

Solid Core Particles Are Less Hydrophobic

- Typical porous C18 phases have %C values >10%
- Solid core particles have lower surface areas so less ligand is bonded leading to lower hydrophobicity / smaller %carbon values & faster elution / analyses

Solid core →
Porous →

	Particle size (um)	Pore size (A)	Surface area (m2/g)	% Carbon load
ACE UltraCore SuperC18 ACE C18	2.5 2,3,5,10	95 100	130 300	7.0 15.5
Agilent Poroshell SB-C18 Zorbax SB-C18	2.7 1.8, 3.5, 5	120 80	130 180	8 10
AMT HALO C18 N/A	2.7 -	90 -	150 -	7.7 -
Phenomenex Kinetex C18 Luna C18(2)	2.6 2.5,3,5,10	100 100	200 400	12 17.5
Thermo Accucore C18 GOLD C18	2.6 1.9,5,8,12	80 175	130 220	9 10
Waters Cortecs C18 ACQUITY BEH C18	2.7 1.7,3.5	90 185	100 130	6.6 17.7

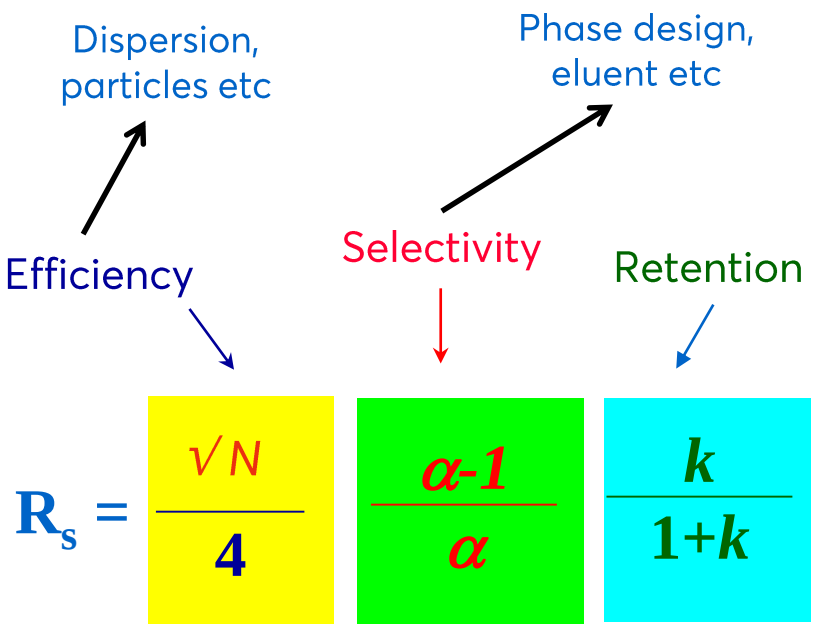
Method Development

SAME CONSIDERATIONS FOR SOLID CORE AS FULLY POROUS

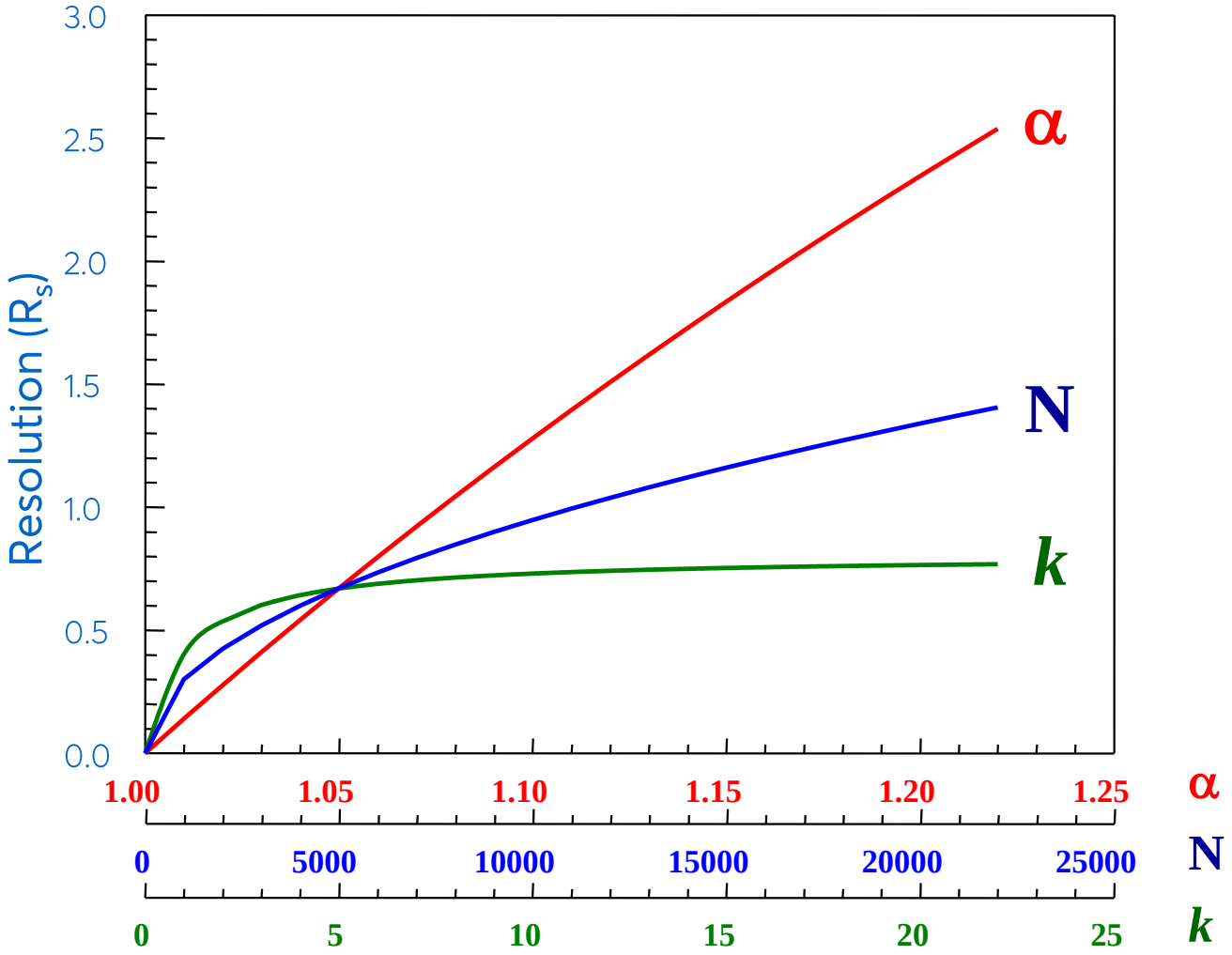
- Pressure
- Column Dimensions / Particle Size / Pore size
- Column Chemistry
- Solvents (type, gradient, modifier etc.)
- Temperature
- pH

Nb. If transferring methods from fully porous to solid core consider injection volume, flow rate, gradient & instrument setup

Resolution, Selectivity, Efficiency & Retention



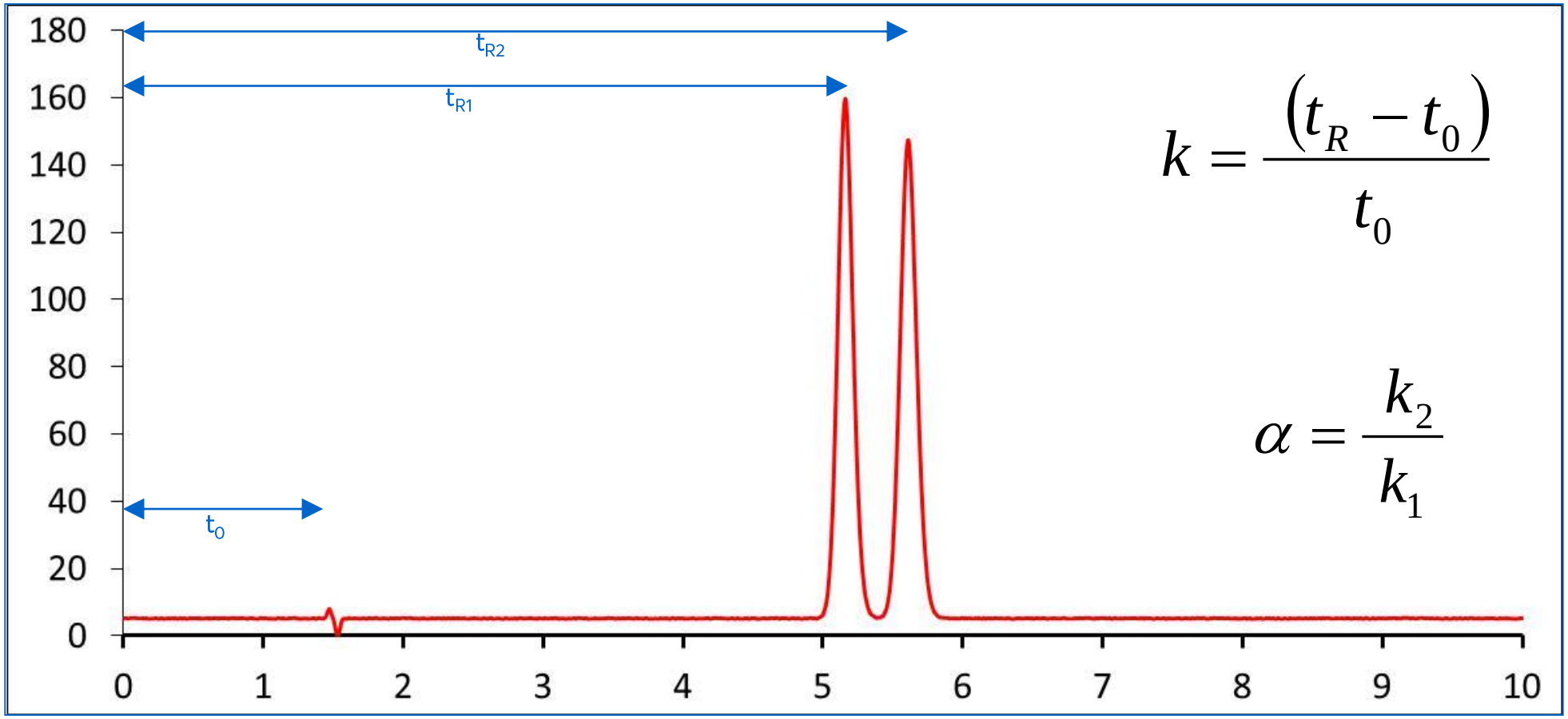
Selectivity is the key to resolution and efficiency boosts performance



Zhao, J.H. and P.W. Carr. Analytical Chemistry, (1999) 71, 2623-2632

What is Selectivity?

- Alpha (α) denotes the separation factor or separation selectivity between 2 adjacent peaks



- Selectivity values > 1.0 indicate the combination of mobile phase and stationary phase are providing some degree of separation for the 2 analytes

Which Factors[1] Affect Selectivity?

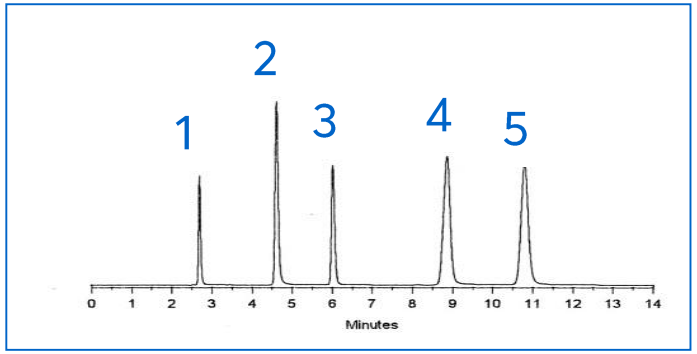
- Strongly influenced by physicochemical properties of the analyte, stationary phase, eluent etc
- From a practical perspective:

Isocratic Separations	MOST Influence ↑ LEAST Influence	Gradient Separations
– Column stationary phase type – pH (ionisable analytes only) – Organic modifier type – % Organic modifier – Buffer selection – Column temperature – Buffer concentration		– All parameters for isocratic PLUS – Gradient steepness, – k* (t_G, F, V_m, $\Delta\Phi$, M), $$k^* = \frac{t_G F}{\Delta\Phi V_m M}$$ – Dwell volume, – Column dimensions.

1 Adapted from 'Introduction to Modern Liquid Chromatography", 3rd Edition, Snyder, Kirkland, Dolan, 2010, p.29, Wiley & sons

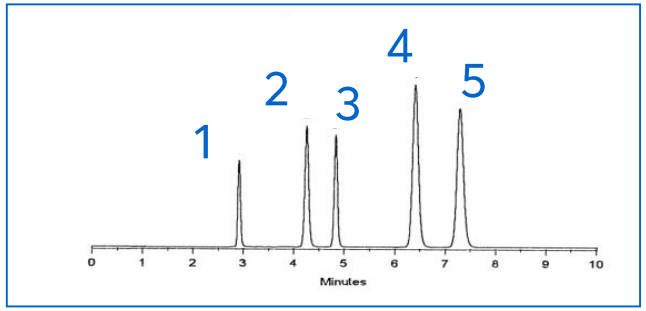
Exploring Selectivity: Porous Silica Bonded Phase Effects

ACE C18 – Increase Retention

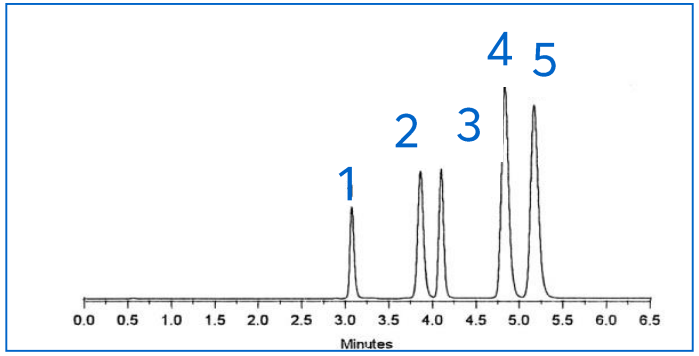


Hydrophobicity Differences

ACE C8 (start point)



ACE C4 – Decrease Retention



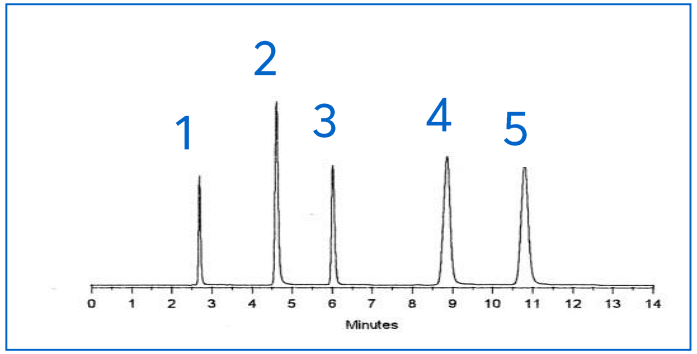
Sample: 1. Norephedrine 2. Nortriptyline 3. Toluene 4. Imipramine 5.Amitriptyline

Column: 250 x 4.6mm 5µm Mobile phase: 80:20 v/v MeOH/25mM KH_2PO_4 (pH6.0) Flow: 1.0mL/min, Wavelength: 215nm

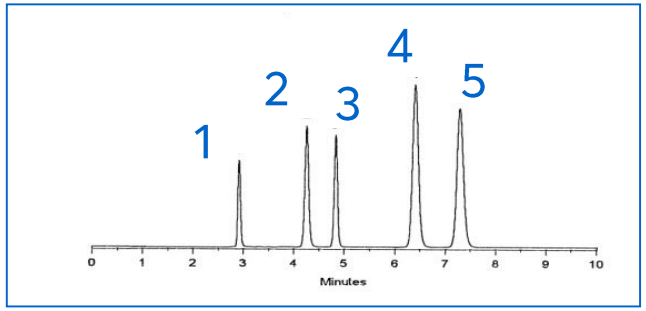
Exploring Selectivity: Porous Silica Bonded Phase Effects

Mechanism Differences

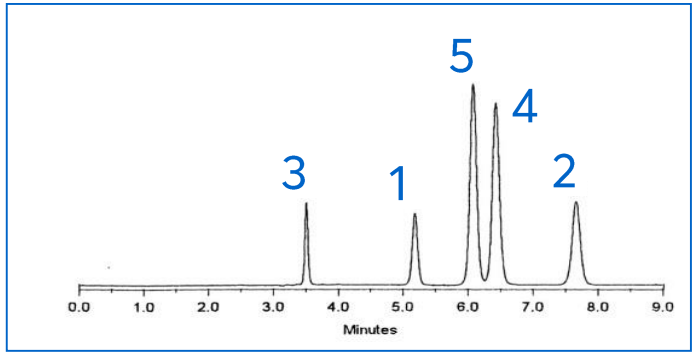
ACE C18 – Increase Retention



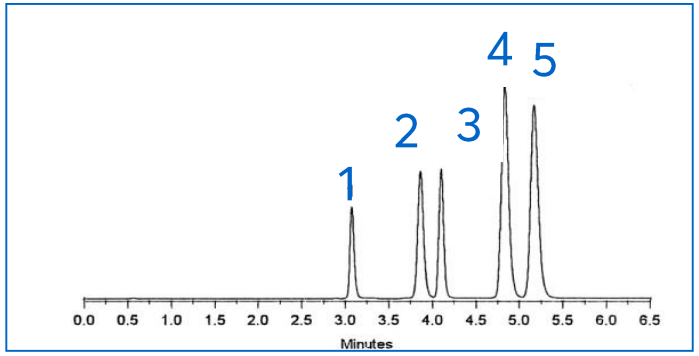
ACE C8 (start point)



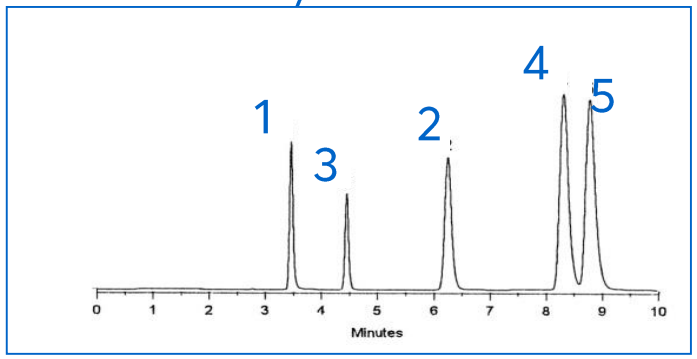
ACE CN – Elution Order



ACE C4 – Decrease Retention



ACE Phenyl – Elution Order



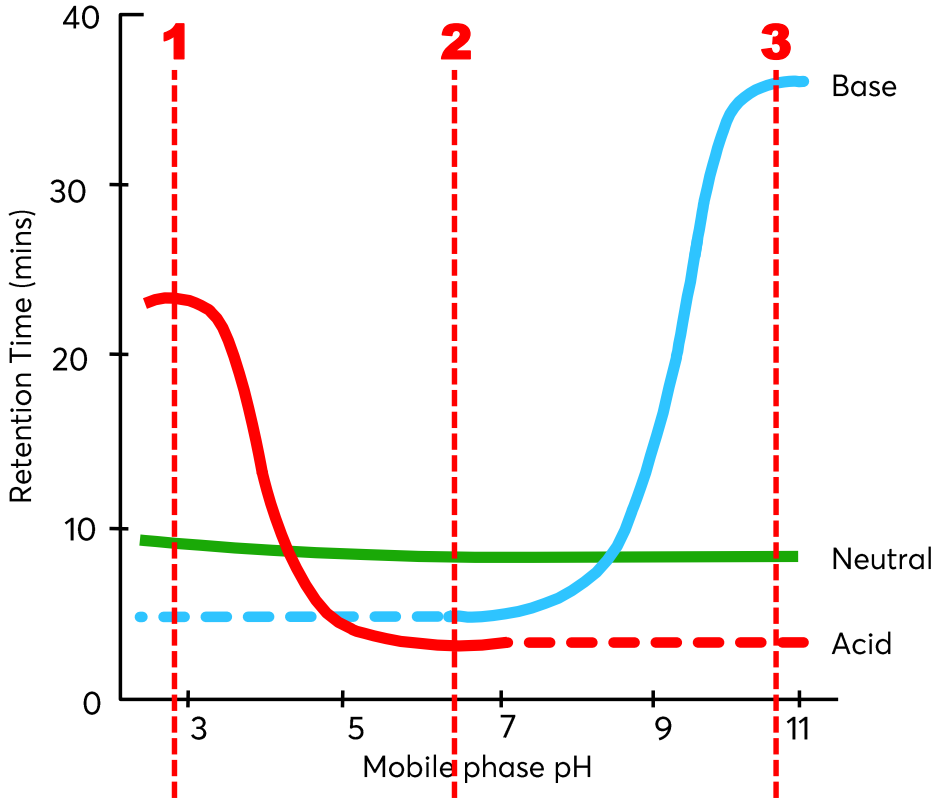
Stationary Phase Is Powerful With Selectivity & Retention

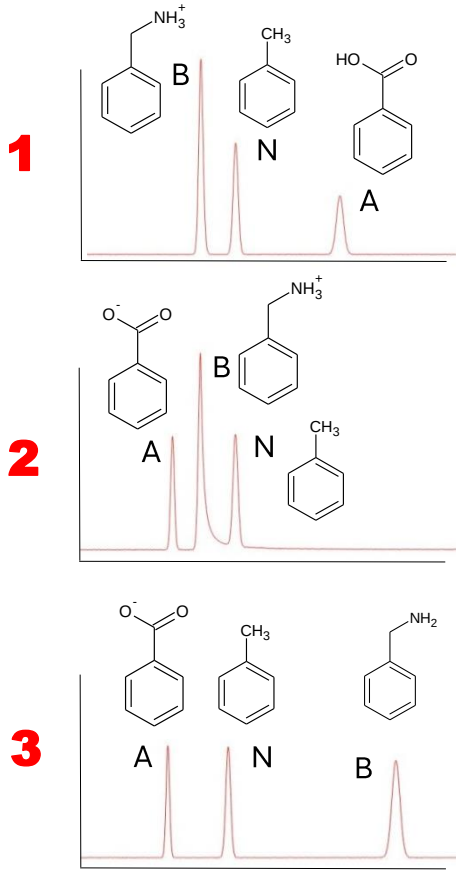
Sample: 1. Norephedrine 2. Nortriptyline 3. Toluene 4. Imipramine 5.Amitriptyline

Column: 250 x 4.6mm 5µm Mobile phase: 80:20 v/v MeOH/25mM KH_2PO_4 (pH6.0) Flow: 1.0mL/min, Wavelength: 215nm

Exploring Selectivity: Eluent pH

ELUENT PH EFFECTS CAN BE LARGE…AND MULTIMODAL.





Eluent pH is Powerful For Selectivity and Retention

Avantor® ACE® UltraCore™ Solid Core Particles: Selectivity

- Silica based solid core particles
- SuperC18 and SuperPhenylHexyl bonded phases for alternative selectivity: hydrophobic / aromatic interactions
- Encapsulated Bonding Technology provides inertness & protects the silica surface from eluent pH 1.5 – 11.0

ACE UltraCore 2.5µm:

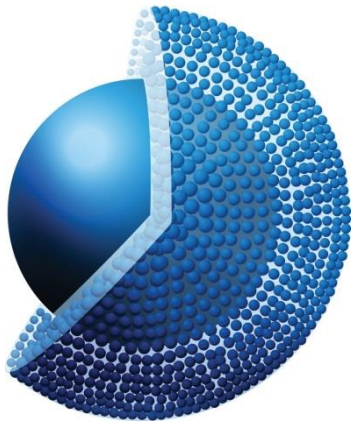
Total particle diameter = 2.5µm

Shell thickness = 0.45µm

ACE UltraCore 5µm:

Total particle diameter = 5µm

Shell thickness = 0.7µm



Method Development / Screening Workflow: Overview

TYPICALLY MULTIVARIATE

- 1 column - 1 temperature - 1 pH - 1 organic modifier - 1 t_G	- 1 column - 2 temperatures - 1 pH - 1 organic modifier - 2 x t_G	- 1 column - 2 temperatures - 1 pH - 2 organic modifier - 2 x t_G	- ≥ 2 columns - 2 temperatures - 1 pH - 2 organic modifier - 2 x t_G	- ≥ 2 column - 2 temperatures - 2 or 3 pH - 2 organic modifier - 2 x t_G
2 x t_G	20C & 60C	MeOH & MeCN	Alkyl chains eg C18, C8 Aromatic eg Phenyl, C18-AR or C18-PFP Polar eg C18-PFP, C18-Amide	pH 2.5 pH 7 pH 10.7

– INCREASING COMPLEXITY … BUT KNOWLEDGE RICH

- Many potential runs to fully explore variables and their effects on retention and selectivity
- Having phases to fully exploit all parameters is helpful
- Would be helpful to reduce parameter options…

Solid Core Method Development / Screening Workflow

- 2 columns - 2 temperatures - 1 pH - 2 organic modifier - 2 x t_G	- 1 column - 2 temperatures - 1 pH - 2 organic modifier - 2 x t_G	- ≥ 2 column - 2 temperatures - 2 pH values - 2 organic modifier - 2 x t_G
SuperC18 SuperPhenylHexyl	MeOH MeCN	pH 3 pH 10.7

– INFORMATION RICH DATA BASED ON SELECTIVITY

2 column method development / screening approach based on selectivity data

General Method Development Initial Conditions

- Perform a broad scouting gradient run on the samples at acidic eluent pH
- How do you calculate your starting conditions?

For a 100 x 3mm column:

t_G = 5 minutes

F = 1.2 mL/min

$\Delta\Phi$ = 0.95

V_m = 0.459 mL

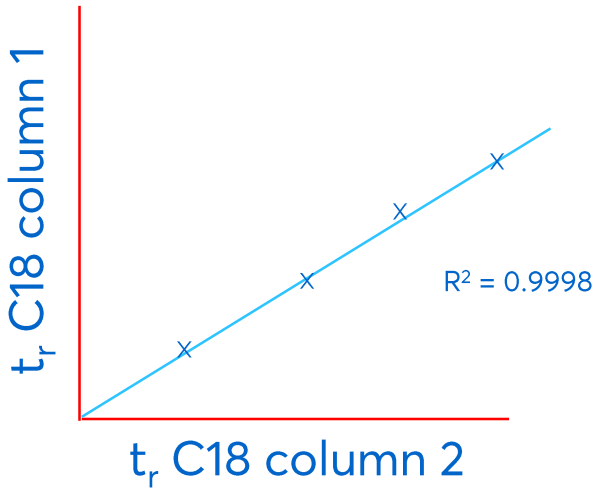
M = 5

$$k^* = \frac{t_G F}{\Delta\Phi V_m M} \quad = \sim 3$$

- Ideally retention (or *k** in gradient elution) should be >2 and <20 for initial method development

Vendor A C18 vs Vendor B C18

$$\text{Selectivity} = 100 \times \sqrt{(1 - R^2)}$$



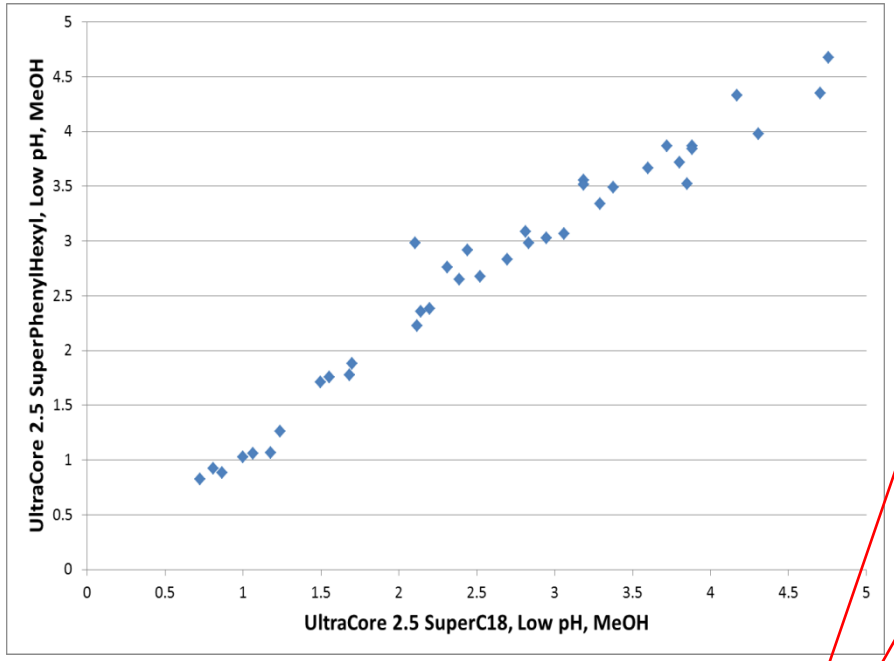
$$\text{Selectivity} = 100 \times \sqrt{(1 - R^2)}$$
$$= 100 \times \sqrt{(1 - 0.9998)}$$
$$= \underline{\sim 1}$$

Low Selectivity Value When Comparing C18 Phases To Each Other

* Neue, O'Gara, Méndez "Selectivity in Reversed-Phase Separations: Influence of the Stationary Phase", J. Chromatogr. A 1127 (2006), 161-174

Selectivity Plot: Exploring The Effect Of Solid Core Phase

SuperC18, low pH, MeOH vs SuperPhenylHexyl, low pH, MeOH



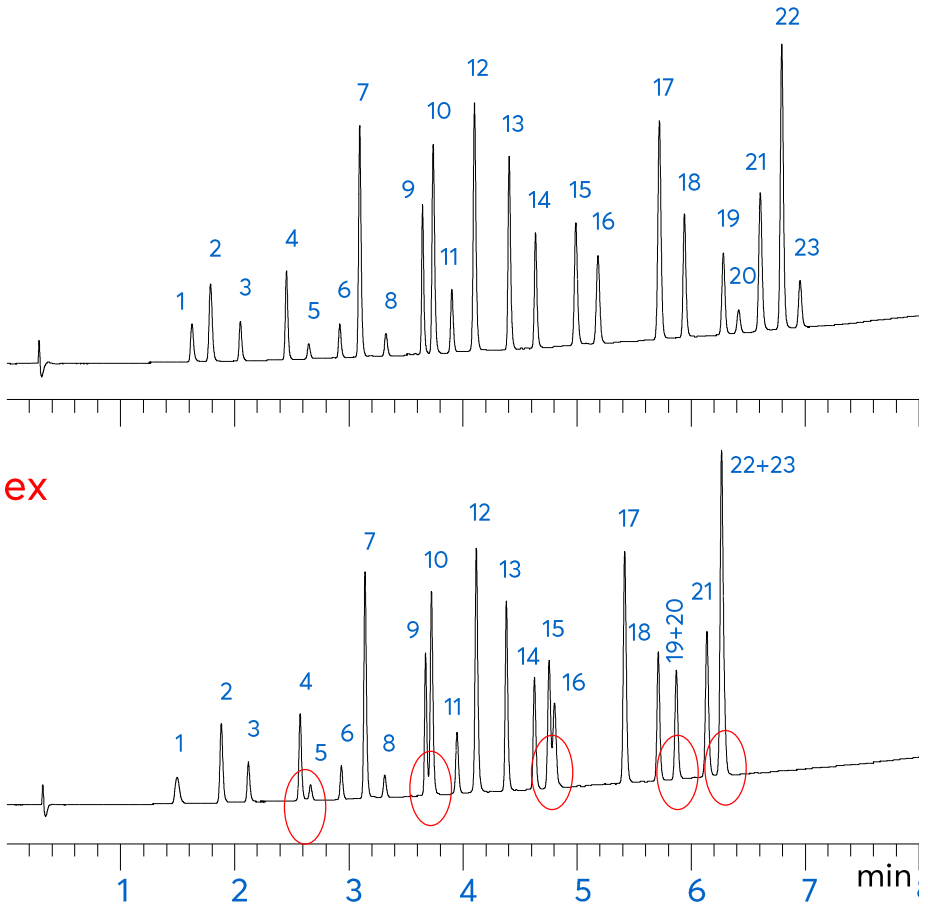
Selectivity = 19

Changes in peak spacing noted

SuperC18

SuperPhHex

Example application:

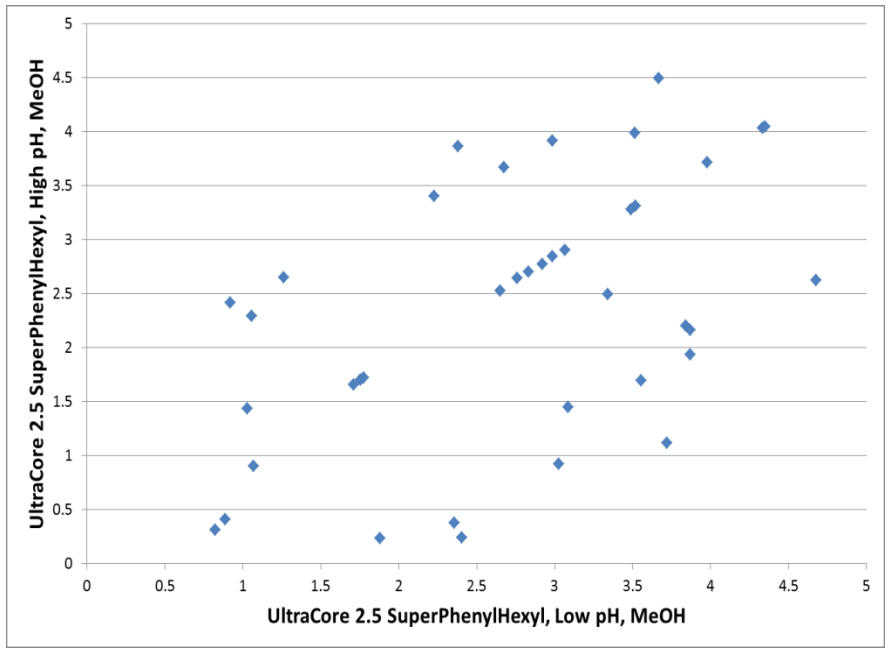


50x2.1mm, 2.5µm, gradient analysis, A= 20mM $HCOONH_4$, pH3 (aq), B= 20mM $HCOONH_4$, pH 3 in MeCN/water 9:1 v/v, 3-100%B in 7.5 mins, hold 100%B for 1.5 mins, 40°C, 0.40 mL/min, 254 nm.

1 amiloride, 2 benzamide, 3 3-hydroxybenzoic acid, 4 vanillin, 5 2-hydroxybenzoic acid, 6 benzoic acid, 7 methyl paraben, 8 p-cresol, 9 cortisone, 10 ethyl paraben, 11 dimethylpthalate, 12 piroxicam, 13 hydro cortisone-21-acetate, 14 ketoprofen, 15 ethylbenzoate, 16 toluene, 17 valerophenone, 18 mefenamic acid 19 hexanophenone, 20 propylbenzene, 21 phenanthrene, 22 heptaphenone, 23 butylbenzene

Selectivity Plot: Exploring Eluent pH With SuperPhenylHexyl

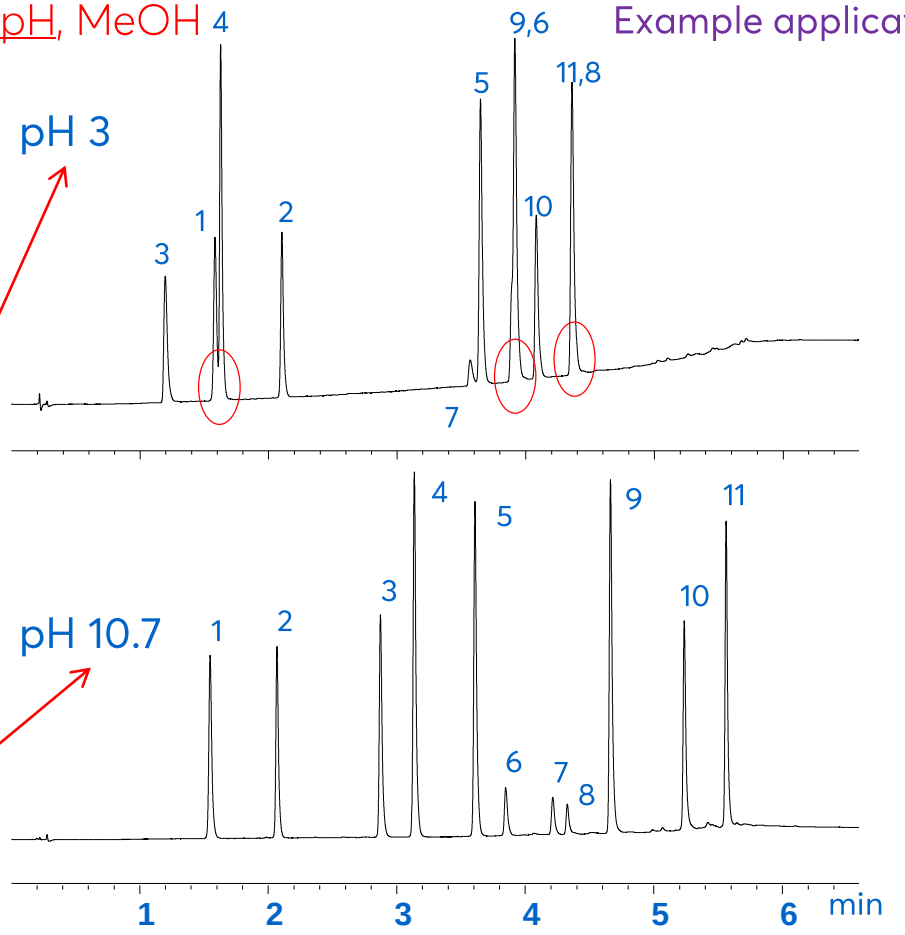
SuperPhenylHexyl, low pH, MeOH vs SuperPhenylHexyl, high pH, MeOH



Selectivity = 83

Significant changes in elution order noted

Example application:

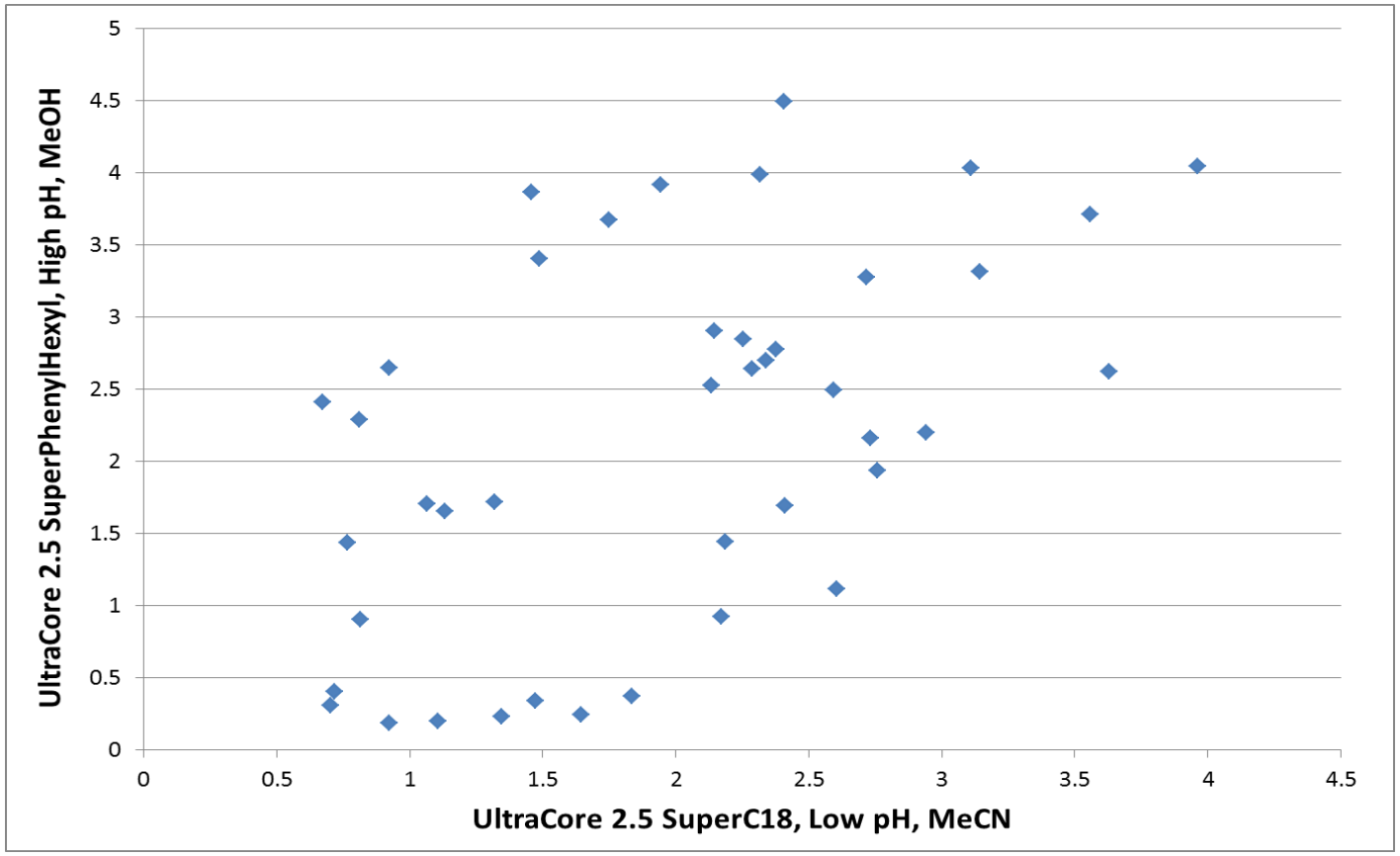


50x2.1mm, 2.5mm, gradient analysis, A1= 10mM $HCOONH_4$, pH3 (aq), B1= 10mM $HCOONH_4$, pH 3 in MeOH/water 9:1 v/v, A2= 0.1% NH_3, pH 10.7 (aq), B2= 0.1% NH_3, pH10.7 in MeOH/water 9:1 v/v, 3-100%B in 5mins, 100%B for 2mins, 40°C, 0.60 mL/min, 254 nm.

1. benzamide, 2 caffeine, 3 procainamide, 4 N-acetylprocainamide, 5 propiophenone, 6 toluene 7 remacemide, 8 ethylbenzene, 9 carvdilol, 10 nortriptyline, 11 clomipramine.

UltraCore: Exploring Phase, Solvent & pH Selectivity

SuperC18, low pH, MeCN vs SuperPhenylHexyl, high pH, MeOH



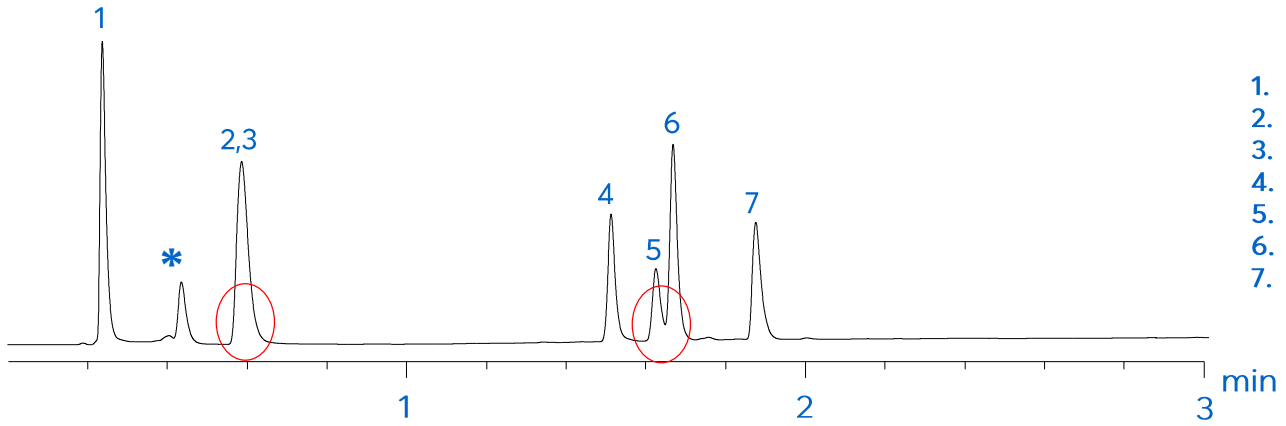
Range of 50 Analytes To Describe Selectivity

Selectivity = 85 → Fully Explore The Selectivity 'Space'

Avantor® ACE® UltraCore™: Exploit Bonded Phase

SELECTIVITY TUNING WITH STATIONARY PHASE TYPE:

UltraCore
2.5 SuperC18



1. Pyridoxine
2. p-Aminobenzoic acid
3. Pantothenic acid
4. Folic acid
5. d-Biotin
6. Cyancobalamin
7. Riboflavin

50x2.1mm, 2.5µm
Gradient analysis

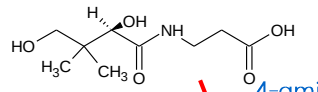
A: 20 mM KH_2PO_4 pH 2.7 (aq)
B: 20 mM KH_2PO_4 pH 2.7 in MeOH:Water 50:50 v/v

T	%B
0	20
1	60
2	70

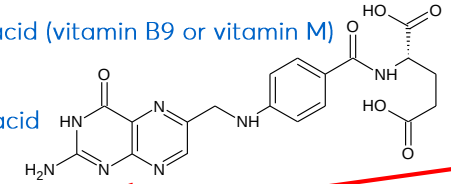
40 °C
0.60 mL/min
205 nm

UltraCore 2.5
SuperPhenylHexyl

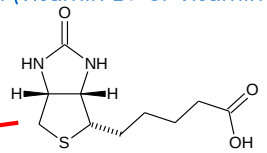
Pantothenic acid (vitamin B5)



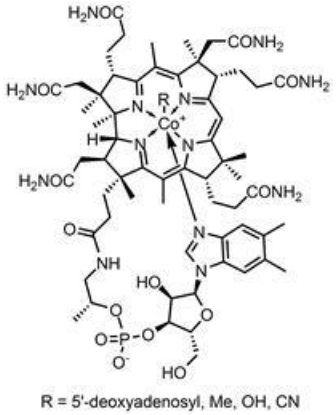
Folic acid (vitamin B9 or vitamin M)



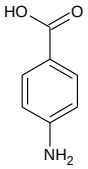
D-Biotin (vitamin B7 or vitamin H)



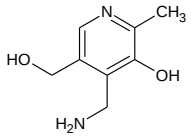
Cyanocobalamin (vitamin B12)



4-aminobenzoic acid



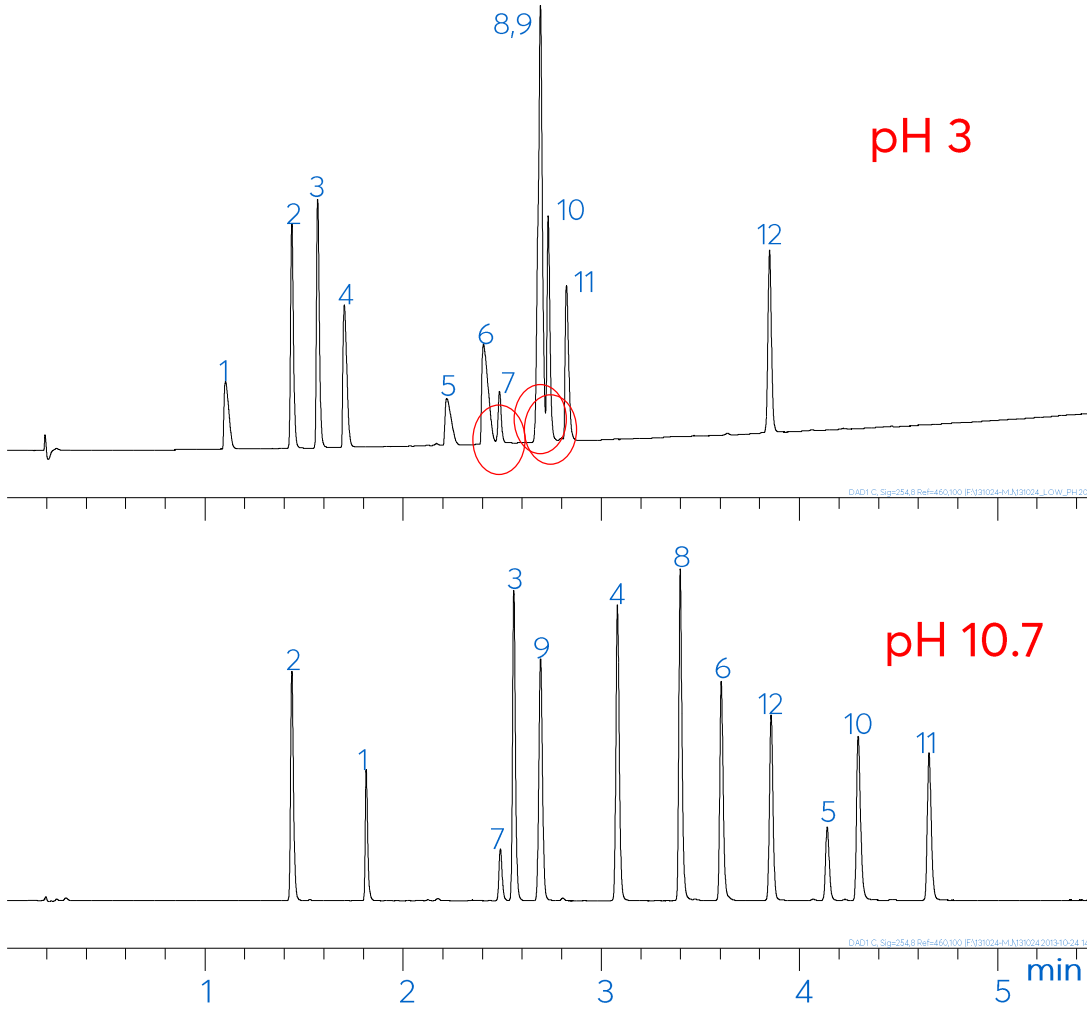
Pyridoxamine or
Vitamin B6



Riboflavin (vitamin B2)

Avantor® ACE® UltraCore™: Exploit Low and High pH Eluents

UltraCore SuperC18: selectivity with pH



50x2.1mm, 2.5µm
Gradient analysis

A1= 10mM $HCOONH_4$, pH3 (aq)
B1= 10mM $HCOONH_4$, pH 3 in MeCN/water 9:1 v/v

A2= 0.1% NH_3, pH 10.7 (aq)
B2= 0.1% NH_3, pH10.7 in in MeCN/water 9:1 v/v

T	%B
0	3
5	100
6	100

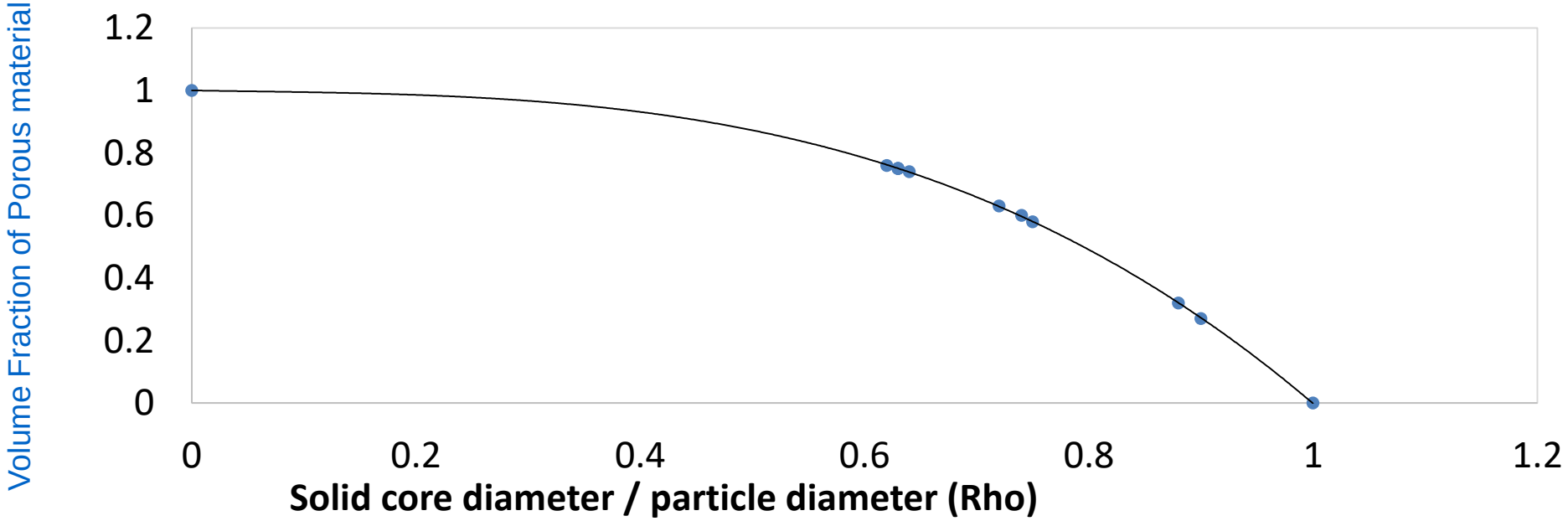
40C
0.60 mL/min
254 nm

1. Atenolol
2. Methylphenylsulfoxide
3. Eserine
4. Prilocaine
5. Bupivacaine
6. Tetracaine
7. 1,2,3,4-Tetrahydro-1-naphthol
8. Carvedilol
9. Nitrobenzene
10. Methdilazine
11. Amitriptyline
12. Valerophenone

Other Considerations

Loading Capacity & Retention

- A thinner shell can be **advantageous for efficiency** (van Deemter), but it **reduces sample loading** and **analyte retention**
- The optimum shell thickness is a compromise between **efficiency, sample loading capacity** and **analyte retention**
- Sample loading capacity and expected retention factor of a given solute are proportional to the stationary phase volume, and is expected to be lower on SPPs than on fully porous particles



SPPs with low volume fractions correspond to large, wide-pore particles / smaller shell thickness.

Scalability

SPPS OF DIFFERENT PARTICLE SIZES ARE SCALABLE FOR SELECTIVITY BUT NOT FOR RETENTION ON ACCOUNT OF DIFFERENT RHO VALUES.

For example :

<u>ACE UltraCore 2.5µm</u>: **ρ = 0.64**

Total particle diameter = 2.5µm

Core diameter = 1.6µm

Shell thickness = 0.45µm

Surface area = 130 m^2/g

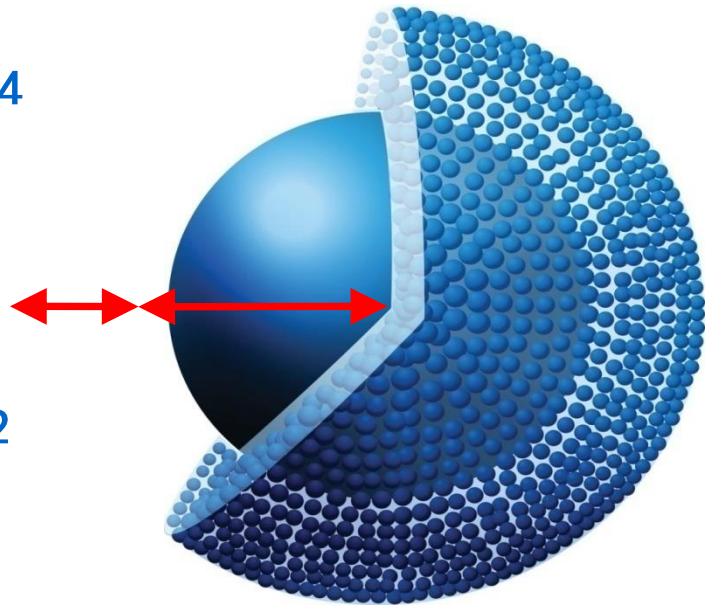
<u>ACE UltraCore 5µm</u>: **ρ = 0.72**

Total particle diameter = 5µm

Core diameter = 3.6µm

Shell thickness = 0.7µm

Surface area = 100 m^2/g



Rho (ρ) = core diameter / particle diameter

Extra Column Band Broadening

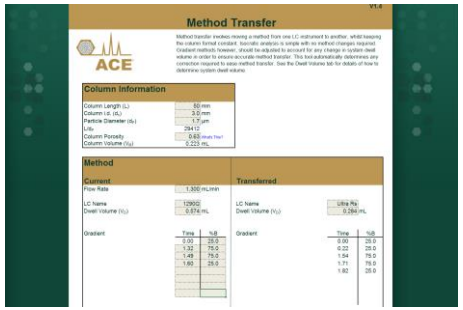
- One of the great advantages of superficially porous particle packed columns is that the back pressures produced often allow the use of standard HPLC instrumentation (not sub 2µm SPP)
- However, the HPLC system needs to be optimised to produce efficient chromatography
- Failure to consider these parameters may result in loss of the increased efficiency offered by the SPP
- Extra column effects are more significant for scaled down separations (as column volume decreases) and for less retained peaks which have a lower peak volume

Summary and Overall Conclusions

- Solid core columns offer efficiency and speed for separations
- Method development is comparable to fully porous particles
- Screening columns with differing retention mechanisms and exploiting eluent pH is useful for method development – explore 'selectivity space'
- Perceived improvements in analysis time are likely to be due to the reduced hydrophobicity of the solid core particles
- The impact of system dispersion is real and can be significant

Useful Resources

- ACE Translation Tool:
  - (+help file)
  - (+ AKN#0023)



- ACE Knowledge Notes (AKNs):
  - AKN0019 – Solid Core Technology
  - AKN0018 – RP Method Development
  - AKN0011 – Practical UHPLC
  - AKN0012 – Understanding the Relationship between Particle Size, Performance and Pressure
  - AKN0017 – How to Determine Extra Column Dispersion and Extra Column Volume
  - AKN0023 – Gradient Method Translation Using the ACE LC Translator

ACE Method Development Kit Brochure and Webinar

Thank you

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