Using Selectivity Data To Demonstrate A UHPLC / HPLC Method Development Platform Based Upon Solid Core SuperC18 & SuperPhenylHexyl Phases

Alan P McKeown[1], Geoffrey Faden[2]

[1]Advanced Chromatography Technologies Ltd, 1 Berry Street, Aberdeen, Scotland, AB25 1HF UK [2]MACMOD Analytical Inc., 103 Commons Court, PO Box 587, Chadds Ford, PA 19317 USA

THE CHALLENGE

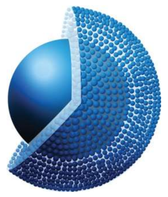
Derive Selectivity data to understand the orthogonality of ACE® UltraCore™ SuperC18 and SuperPhenylHexyl UHPLC / HPLC phases with permutations of phase, pH and organic solvent.

1. ACE® UltraCore™: SOLID CORE PARTICLE TECHNOLOGY

ACE UltraCore 2.5µm: ρ = 0.64
Total particle diameter = 2.5µm
Core diameter = 1.6µm
Shell thickness = 0.45µm
Surface area = 130 m^2/g

Rho (ρ) = solid core diamater : particle diameter ratio

ACE UltraCore 5µm: ρ = 0.72
Total particle diameter = 5µm
Core diameter = 3.6µm
Shell thickness = 0.7µm
Surface area = 100 m^2/g



- Solid core particles have gained interest for UHPLC / HPLC due to rapid separations, method transferability and low back pressure.
- This work explores chromatographic selectivity using permutations of phase, eluent pH and organic solvent with ACE UltraCore.

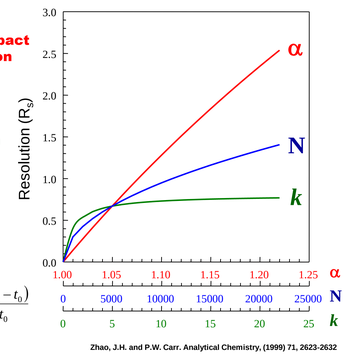
2. RESOLUTION, SELECTIVITY, EFFICIENCY & RETENTION

Selectivity Has The Biggest Impact On Increasing Peak Resolution

Efficiency | Selectivity | Retention

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{1+k}$$

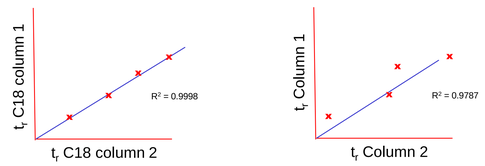
$$N = 5.54\left(\frac{t_r}{w_{0.5}}\right)^2 \qquad \alpha = \frac{k_2}{k_1} \qquad k = \frac{(t_R - t_0)}{t_0}$$



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

3. DETERMINING SELECTIVITY VALUES* FOR PHASES

Selectivity = 100 x √ (1 – R^2)



Selectivity = 100 x √ (1 – R^2)
= 100 x √ (1 – 0.9998)
= ~ 1

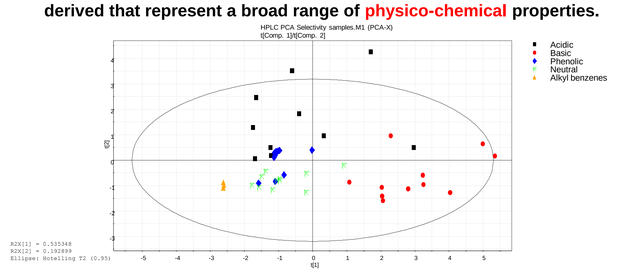
Selectivity = 100 x √ (1 – R^2)
= 100 x √ (1 – 0.9787)
= ~ 15

Higher Selectivity values indicate phase orthogonality for method development / screening

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

4. DIVERSE ANALYTES TO EXPLORE SELECTIVITY

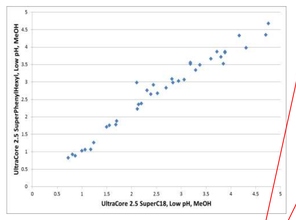
- A mixture of 50 acidic, basic & neutral small molecule analytes were derived that represent a broad range of physico-chemical properties.



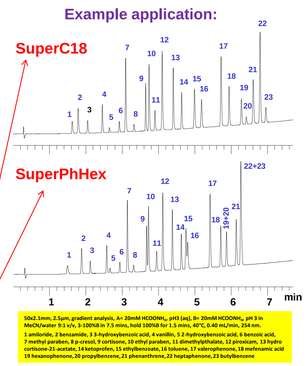
- Challenging stationary phases under different conditions with these probes gives an indication of chromatographic selectivity for each phase and between phases.

5. SELECTIVITY SCATTER PLOT: EXPLORING PHASE TYPE

ACE UltraCore: SuperC18, low pH, MeOH vs SuperPhHex, low pH, MeOH



Example application:



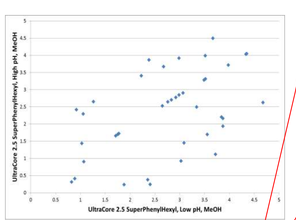
Selectivity = 19

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 dimethylphthalate, 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

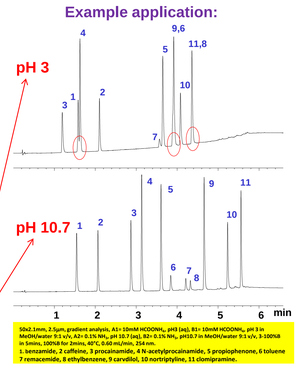
Some changes in peak spacing noted

6. EXPLORING SUPERPHENYLHEXYL PH SELECTIVITY

ACE UltraCore: SuperPhHex, low pH, MeOH vs SuperPhHex, high pH, MeOH



Example application:



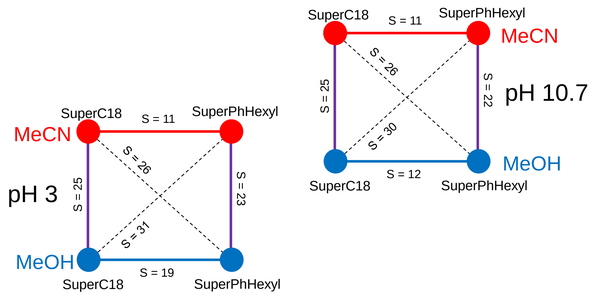
Selectivity = 83

50x2.1mm, 2.5µm, 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 carvidilol, 10 nortriptyline, 11 clomipramine.

Significant changes in elution order noted

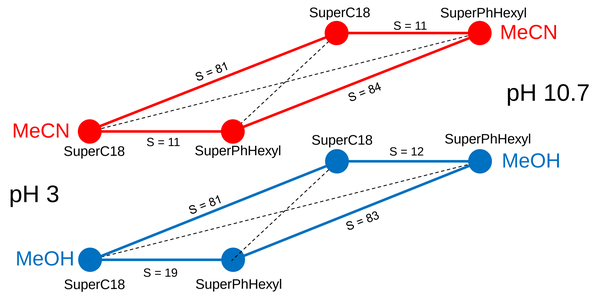
7. SELECTIVITY DIAGRAMS: PHASE, SOLVENT & pH (I)

- SuperC18 & SuperPhHex, MeOH & MeCN, low or high pH



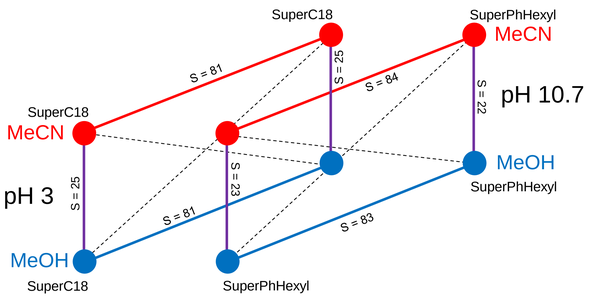
8. SELECTIVITY DIAGRAMS: PHASE, SOLVENT & pH (II)

- SuperC18 & SuperPhHex, low or high pH, MeOH or MeCN

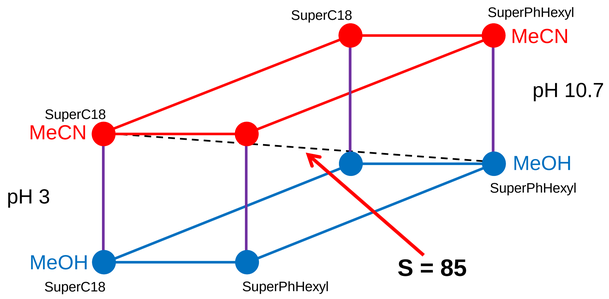


9. SELECTIVITY DIAGRAMS: PHASE, SOLVENT & pH (III)

- Low & high pH, MeOH & MeCN, SuperC18 or SuperPhHex



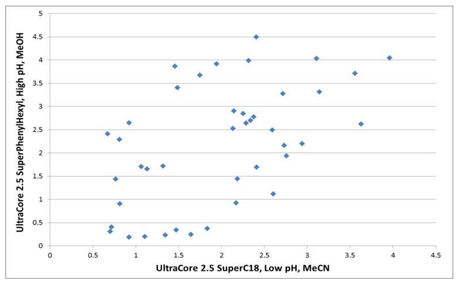
10. SIMPLE, POWERFUL SOLID CORE METHOD DEVELOPMENT



ACE® UltraCore™ Method Development Platform
2 phases, 2 solvents & 2 eluent pH values to explore selectivity

11. ACE® UltraCore™: EXPLORING PHASE & PH SELECTIVITY

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



Range of 50 Analytes To Describe Selectivity

Selectivity = 85 → Orthogonality For Method Development

12. SUMMARY AND CONCLUSIONS

- A solid core method development platform has been explored based upon ACE UltraCore SuperC18 and SuperPhenylHexyl phases using Selectivity Descriptor data from a diverse set of 50 analytes.
- Using permutations of phase type, organic solvent and eluent pH a variety of retention scatter plots were determined.
- Chromatographic separations of analyte mixtures under various conditions showed that larger Selectivity Descriptor values are indicative of increased separation orthogonality.
- The ability to exploit selectivity using stationary phase with a broad eluent pH range (1.5 to 11.0) & solvent type provides analysts with a powerful platform for solid core UHPLC / HPLC method development.

ACE® is a registered trademark of Advanced Chromatography Technologies Limited. ACE UltraCore™ and EBT™ are trademark of Advanced Chromatography Technologies Limited.