Exploring The Selectivity & Performance Of A New Extra Selectivity / Extended Stability Cyano Phase In UHPLC / HPLC Method Development

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THE CHALLENGE

Engineer a new, unique CN UHPLC / HPLC phase with polar and non-polar retention and alternative selectivity that is reproducibile, robust and gives efficient chromatography

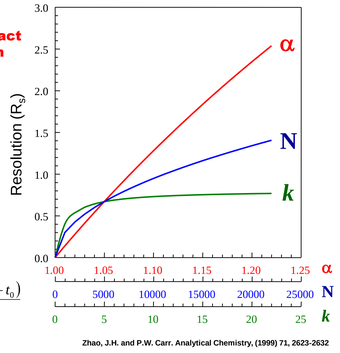
1. RESOLUTION, SELECTIVITY, EFFICIENCY & RETENTION

Selectivity Has The Biggest Impact On Increasing Peak Resolution

Efficiency | Selectivity | Retention

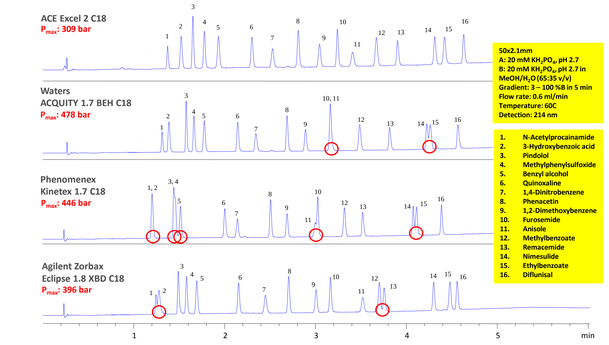
$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{1+k}$$

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



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

2. C18 STATIONARY PHASES SHOW SIMILAR SELECTIVITY



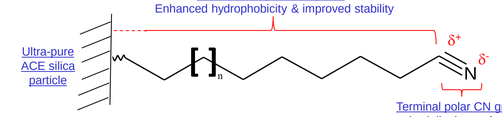
50x2.1mm
A: 20 mM KH_2PO_4, pH 2.7
B: 20 mM KH_2PO_4, pH 2.7 in $MeOH/H_2O$ (65:35 v/v)
Gradient: 3 – 100 %B in 5 min
Flow rate: 0.6 ml/min
Temperature: 60C
Detection: 214 nm

1. N-Acetylprocainamide
2. 3-Hydroxybenzoic acid
3. Pindolol
4. Methylphenylsulfoxide
5. Benzyl alcohol
6. Quinoxaline
7. 1,4-Dinitrobenzene
8. Phenacetin
9. 1,2-Dimethoxybenzene
10. Furosemide
11. Anisole
12. Methylbenzoate
13. Remacemide
14. Nimesulide
15. Ethylbenzoate
16. Diflunisal

C18 phases show 'similar' selectivity...

All trademarks are recognised...comparative separations may not be representative of all applications

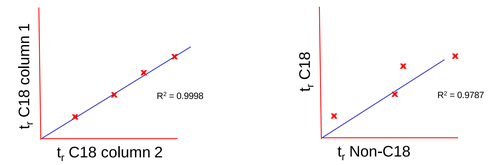
3. ACE® CN-ES: A NEW STATIONARY PHASE OPTION



- **Ligand contains an extended alkyl chain spacer:**
  - Provides enhanced hydrophobicity over short chain CN in reversed-phase.
  - Improves phase stability.
- **Ligand contains terminal polar CN group:**
  - Provides dipole-dipole interactions and different polar selectivity to other polar retention phases such as C18-Amide (ie polar embedded).
- **Usable in 100% aqueous eluents – no pore dewetting.**
- **Usable in Normal Phase eluents – clear polar interactions.**

4. DETERMINING SELECTIVITY VALUES* FOR PHASES

Selectivity = 100 x √ (1 – R^2)



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

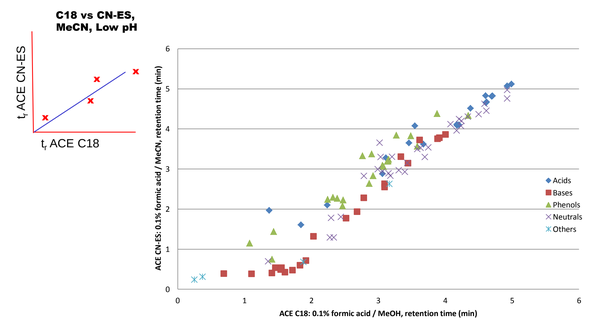
Selectivity = 100 x √ (1 – R^2)
= 100 x √ (1 – 0.9787)
= ~ 15

✓

Higher Selectivity values indicate orthogonality

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

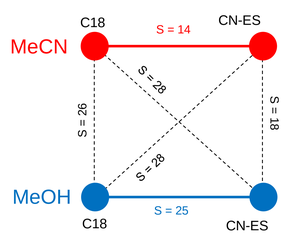
5. ACE® CN-ES EXCELLENT POLAR / NON-POLAR SELECTIVITY



Selectivity = 28 → Powerful For Method Development

6. SELECTIVITY VALUES FOR METHOD DEVELOPMENT

- **45 analytes on 2 columns, 2 solvents**

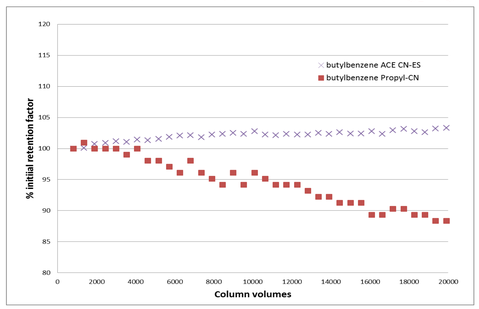


pH 3

Excellent Selectivity Options For Method Development

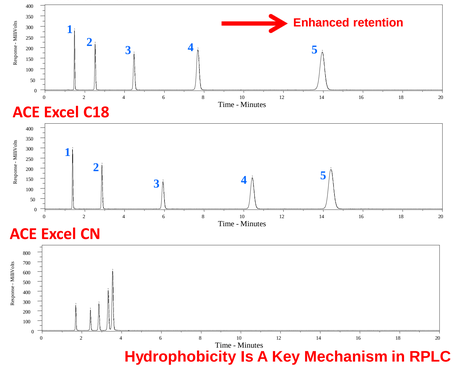
7. ACE® CN-ES SHOWS ENHANCED STABILITY: pH 7, 60C

50x2.1mm, 5µm, isocratic, 0.21mL/min, 1:1 v/v MeOH:20mM $(NH_4)_2PO_4$, pH 7, 60C



Equates to >60 days use (8 hrs / day)

8. ACE® CN-ES SHOWS ENHANCED HYDROPHOBIC RETENTION



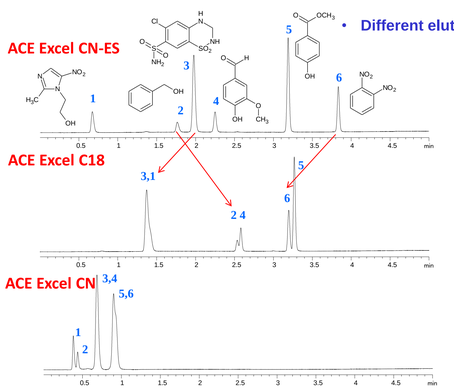
150x4.6mm, 5µm isocratic analysis
6:4 v/v MeCN/water
22C
1.0 mL/min

1. Uracil
2. Dimethyl phthalate
3. Toluene
4. Biphenyl
5. Phenanthrene

Hydrophobicity Is A Key Mechanism in RPLC

9. METHOD DEVELOPMENT: ALTERNATE SELECTIVITY

- Different elution order noted



50x2.1mm, 3µm
Gradient analysis

A = 0.1% formic acid (aq)
B = 0.1% FA in 90% MeOH
40 ºC
0.6 mL/min
254nm
3 – 100% B in 5 minutes

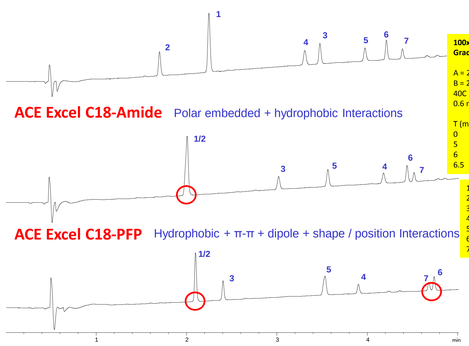
1. Metronidazole
2. Benzyl alcohol
3. Hydrochlorothiazide
4. Vanillin
5. Methyl paraben
6. 1,2 Dinitrobenzene

10. ALTERNATIVE SELECTIVITY TO OTHER ACE® PHASES

ACE Excel CN-ES Dipole + hydrophobic Interactions — **Selectivity differences noted**

ACE Excel C18-Amide Polar embedded + hydrophobic Interactions

ACE Excel C18-PFP Hydrophobic + π-π + dipole + shape / position Interactions



100x3mm, 3µm
Gradient analysis

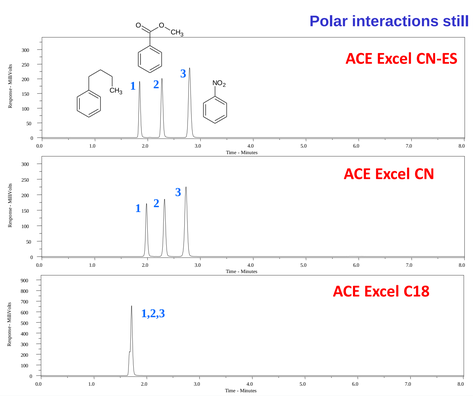
A = 20mM ammonium formate (aq)
B = 20mM ammonium formate in MeOH
40C
0.6 mL/min

T (min)	%B
0	3
5	100
6	100
6.5	3

1. Hydrochlorothiazide
2. Methylphenylsulphoxide
3. 1,3,5 Trinitrobenzene
4. Myricetin
5. P-Cresol
6. Sulindac
7. Toluene

11. ACE® CN-ES: POLAR INTERACTIONS IN NPLC

Polar interactions still present in NPLC



150x4.6mm, 5µm
Isocratic analysis

9:1 v/v Heptane:Ethyl Acetate
22C
1.0 mL/min
254 nm

1.Butyl benzene
2.Methyl benzoate
3.Nitrobenzene

12. SUMMARY AND CONCLUSIONS

- The ACE® CN-ES provides alternative selectivity to C18 based phases which is ideal for method development or sample screening.
- The unique ligand design of the ACE® CN-ES improves the hydrophobic retention mechanism contribution to separations whilst providing enhanced stationary phase stability.
- The ACE® CN-ES is useful for separation of polar and non-polar analytes where traditional CN phases do not provide sufficient retention of column lifetime.
- The versatile ACE® CN-ES provides multiple modes of interaction in RPLC or NPLC to assist in separation of analyte mixtures.