

Optimizing UHPLC Resolution Using Fused-Core® Column Packings with Different Separation Selectivities

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Abstract

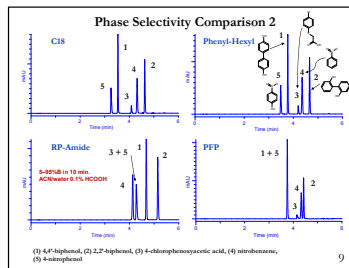
Fused-Core® particles, with an overall diameter of 2.7 µm and a porous shell of 0.5 µm thickness, have demonstrated efficiencies similar to sub-2-µm particles, but with one-half to one-third the column back pressure. Although column efficiency (N) is an important parameter in the general resolution equation, resolution only increases by the square root of an increase in efficiency. However, resolution has a direct linear relationship with separation selectivity (α), and, as a result, selectivity has a much more powerful influence on resolution. Illustrative chromatograms using stationary phases with different selectivities, while maintaining the high plate numbers afforded by the Fused-Core particles, will demonstrate the effectiveness of such an approach for obtaining optimized HPLC separations. The value of using multiple column selectivities as part of a fast method development strategy using DryLab® 2010 software with Peak Match® will also be highlighted.

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HALO Phase Descriptions and Characteristics for Columns Used in this work

HALO Phase	Retention Mechanism	Retention Increased for	Best Application
C18	Hydrophobic interactions	Lipophilic molecules, protonated acids and unprotonated bases, uncharged ion pairs	difference in hydrophobicity, non-aqueous RP/LC
RP-Amide	Hydrophobic, hydrogen bonding	Alcohols, acids, phenols	acidic and basic analytes, heterocycles, protein dyes and acetones, highly aqueous conditions
Phenyl-Hexyl	Hydrophobic, π-π	Electron-poor compounds, aromatic or unsaturated analytes, (ketones, nitriles, aldehydes, etc)	heterocycles, aromatics, highly aqueous conditions
PFP (exp'l)	Hydrophobic, hydrogen bonding, dipole-dipole	Electron-rich compounds, aromatics, unsaturated compounds with double and/or triple bonds	heterocycles, aromatics, esters, ketones, highly aqueous conditions

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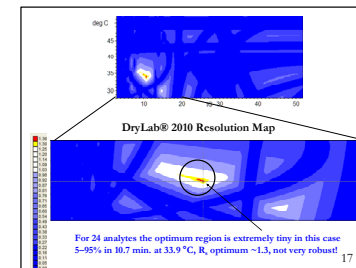
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Column Phase Screening: Conditions

- Organic Modifier
 - ACN
 - MeOH
- Mobile Phase pH
 - 0.1% HCOOH (pH 2.8)
 - 10 mM Ammonium Formate, pH 3.8
 - 10 mM Ammonium Acetate, pH 5.8
- 5-95% organic in 10 min, 30°C
- 1.5 mL/min, µ = 3.0 mm/sec
- Worst case back pressures @ 30°C
 - ACN: 132 bar (1920 psi)
 - MeOH: 210 bar (3051 psi)

Will only show results for RP-Amide and Phenyl-Hexyl for simplicity

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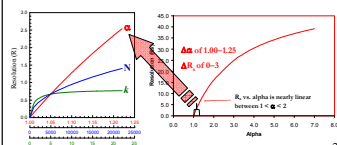


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Resolution Equation Shows that Selectivity is Most Effective Parameter to Change

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

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



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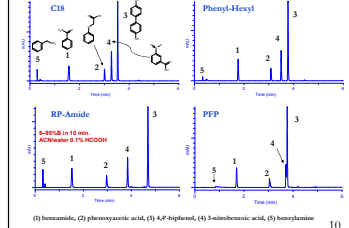
Analytes and Properties

Analyte Name	pKa	ACD	Log P
phenylglyoxal hydrochloride	9.3	4.03	
benzylamine	9.3	1.99	
4-nitrobenzoic acid	4.6	0.85	
acetaminophen	9.7	0.34	
benzamide	15.6	0.74	
2-hydroxy-1,2-propanediol	neutral	1.24	
benzyl alcohol	15	0.75	
3-phenyl-1,2-propanediol	neutral	1.61	
phenol	10.0	1.48	
2-phenylphenol	10	1.56	
phenylacetic acid	3.0	1.14	
2-fluorobenzoic acid	3.0	1.86	
3-cyanobenzoic acid	3.6	1.48	
benzoic acid	4.2	1.86	
benzonitrile	neutral	1.66	
phenylacetic acid	neutral	1.45	
5-nitrobenzoic acid	3.4	1.82	
nitrobenzene	neutral	1.59	
4-chlorophenylacetic acid	3.0	2.83	
4-nitrophenol	7.2	1.57	
4-phenylphenol	9.7	2.42	
4-chlorophenol	9.4	2.43	
2,2'-biphenol	7.6	1.99	
1-chloro-4-nitrobenzene	neutral	2.68	

*Advanced Chemistry Development
log P Calculations

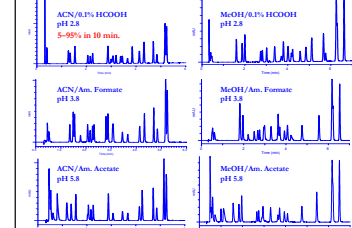
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Phase Selectivity Comparison 3



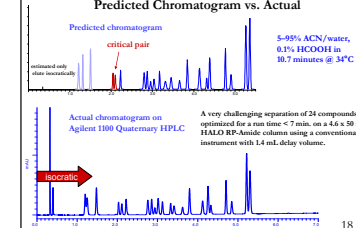
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RP-Amide Screening: ACN/MeOH vs. 3 pHs



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DryLab® 2010 Predicted Chromatogram vs. Actual



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Most Effective Parameters to Change Selectivity

The analysis condition parameters that most affect selectivity, α, are:

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



Note: parameters in blue font were varied in this work

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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

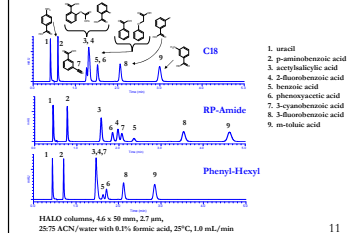
- 4.6 x 50 mm HALO
 - C18
 - RP-Amide
 - Phenyl-Hexyl
 - PFP (experimental)

Column Screening Conditions

- Gradients: 5-95% in 10 min.
 - ACN or MeOH
 - water with 0.1% formic acid
 - 10 mM ammonium formate, pH 3.8
 - 10 mM ammonium acetate, pH 5.8
- Temperature: 30 °C
- Flow rate: 1.5 mL/min
- Detection: UV @ 254 nm
- Injection volume: 5 µL

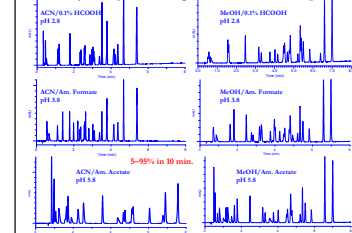
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Comparison of Selectivities: Benzoic Acids, Isocratic



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Phenyl-Hexyl Screening: ACN/MeOH vs. 3 pHs



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Summary and Conclusions

- HALO Fused-Core columns have comparable efficiency yet much lower backpressure than sub-2-micron columns
- Phases such as RP-Amide or Phenyl-Hexyl typically provide very different selectivities compared to C8 or C18 columns
- A variety of different phase selectivities is very advantageous when developing robust and rugged RPLC methods
- Information regarding analyte-stationary phase interactions was provided to guide column choice for different sample types
- Short HALO columns such as 4.6 x 50 mm can be used for fast column and mobile phase screening using all commercially available instrument platforms

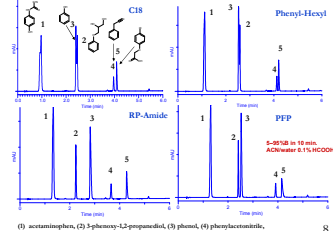
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HALO Fused-Core Silica Particles

- Ultrapure, Type B, low acidity, low metal content
 - 1.7 µm solid silica core
 - 0.5 µm outer porous shell
- Very uniform pore depth
- Extremely narrow particle distribution (RSD 4-6%)
- High N due to small van Deemter "A term"
- Maintain N at higher linear velocities ("C term")
- Back pressures ~ same as for 3 µm particles
- Column efficiencies 220,000 to 240,000 N/m

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Phase Selectivity Comparison 1



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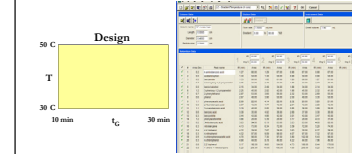
Method Development Strategy Using Multiple Phase Selectivities

- Screen 4 column phases for analyte peak shape and selectivity using combinations of:
 - organic modifier (ACN, MeOH)
 - aqueous component (buffer, pH)
- Choose one of the best combinations to optimize for gradient time and column temperature
- Run 2 x 2 design (gradient time, temperature; t_R x T)
- Use DryLab® 2010 to identify optimum conditions
- Run predicted optimum conditions
- Compare predicted run with actual run

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Optimization Approach

- 5-95% ACN/0.1% formic acid, 1.5 mL/min
- Gradient times of 10 and 30 min.
- Column temperatures of 30 and 50°C
- Input RTs and peak areas into DryLab® 2010



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