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 (alpha, 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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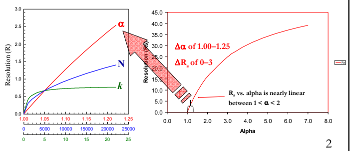
Resolution Equation Shows that Selectivity is Most Effective Parameter to Change

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

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



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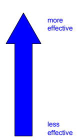
Most Effective Parameters to Change Selectivity

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

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

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

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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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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	differences in hydrophobicity, non-aqueous RPLC
RP-Amide	Hydrophobic, hydrogen bonding	Alcohols, acids, phenols	acidic and basic analytes, heterocycles, proton donors and acceptors, highly aqueous conditions
Phenyl-Hexyl	Hydrophobic, π- π	Electron-poor compounds, aromatic or unsaturated analytes, (ketones, nitriles, alkenes, etc)	heterocycles, aromatics, highly aqueous conditions
PFP (expt'l)	Hydrophobic, π- π, hydrogen bonding dipole-dipole	Electron-rich compounds, aromatics, unsaturated compounds with double and/or triple bonds	stereoisomers, steroids, taxanes, substituted aromatics, highly aqueous conditions

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Analytes and Properties

Analyte Name	pK_a	ACD* Log P
phenylephrine hydrochloride	9.3	-0.03
benzylamine	9.3	1.09
4-aminobenzoic acid	4.6	0.83
acetaminophen	9.7	0.34
benzamide	15-16	0.74
2-benzyloxy-1,3-propanediol	neutral	1.24
benzyl alcohol	15	1.04
3-phenoxy-1,2-propanediol	neutral	0.75
phenol	10.0	1.48
2-phenylethanol	16	1.36
phenoxyacetic acid	3.0	1.34
2-fluorobenzoic acid	3.9	1.86
3-cyanobenzoic acid	3.6	1.48
benzoic acid	4.2	1.86
benzonitrile	neutral	1.66
phenylacetonitrile	neutral	1.45
3-nitrobenzoic acid	3.4	1.82
nitrobenzene	neutral	1.95
4-chlorophenoxyacetic acid	3.0	2.03
4-nitrophenol	7.1	1.57
4,4'-biphenol	9.7	2.42
4-chlorophenol	9.4	2.43
2,2'-biphenol	7.6	1.90
1-chloro-4-nitrobenzene	neutral	2.60

*Advanced Chemistry Development log P calculation

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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%B 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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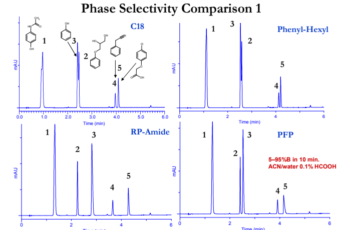
Phase Selectivity Comparison 1



(1) acetaminophen, (2) 3-phenoxy-1,2-propanediol, (3) phenol, (4) phenylacetonitrile, (5) 4-chlorophenoxyacetic acid

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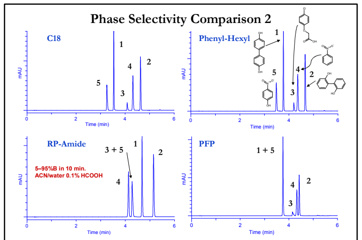
Phase Selectivity Comparison 2



(1) 4,4'-biphenol, (2) 2,2'-biphenol, (3) 4-chlorophenoxyacetic acid, (4) nitrobenzene, (5) 4-nitrophenol

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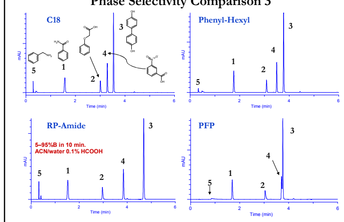
Phase Selectivity Comparison 3



(1) benzamide, (2) phenoxyacetic acid, (3) 4,4'-biphenol, (4) 3-nitrobenzoic acid, (5) benzylamine

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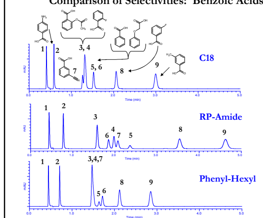
Comparison of Selectivities: Benzoic Acids, Isocratic



1. uracil
2. p-aminobenzoic acid
3. acetylsalicylic acid
4. 2-fluorobenzoic acid
5. benzoic acid
6. phenoxyacetic acid
7. 3-cyanobenzoic acid
8. 3-fluorobenzoic acid
9. m-toluic acid

HALO columns, 4.6 x 50 mm, 2.7 µm,
25:75 ACN/water with 0.1% formic acid, 25°C, 1.0 mL/min

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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 × 2 design (gradient time, temperature; t_g × T)
- Use DryLab® 2010 to identify optimum conditions
- Run predicted optimum conditions
- Compare predicted run with actual run

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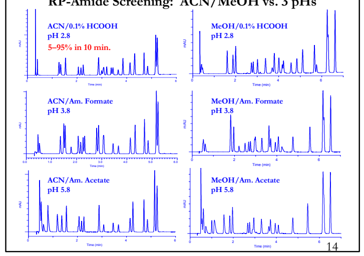
RP-Amide Screening: ACN/MeOH vs. 3 pHs



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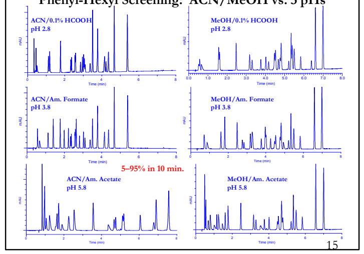
Phenyl-Hexyl Screening: ACN/MeOH vs. 3 pHs



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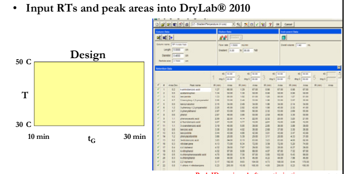
Optimization Approach

- 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



Peak IDs assigned after optimization

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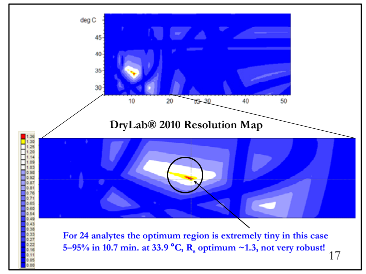

For 24 analytes the optimum region is extremely tiny in this case 5–95% in 10.7 min. at 33.9 °C, R_s optimum ~1.3, not very robust!

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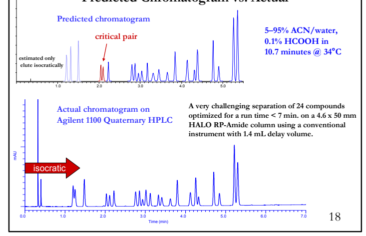
DryLab® 2010 Predicted Chromatogram vs. Actual



A very challenging separation of 24 compounds optimized for a run time < 7 min. on a 4.6 x 50 mm HALO RP-Amide column using a conventional instrument with 1.4 mL delay volume.

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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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