Selectivity and Particle Size Considerations for High Resolution and High Speed LC

Agilent Technologies

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Abstract

The new focus in LC columns is columns with sub 2-micron particles. These small particles provide very high resolution in short and traditional column lengths. But efficiency, N, is just one parameter in overall resolution in a separation. Selectivity has always been viewed as the most important parameter in a separation. Different bonded phases and different mobile phases provide changes in selectivity in a separation to optimize resolution. Most chromatographers like to have the option of at least 3 bonded phase choices available to obtain the needed resolution for their sample. These typically include C18, Phenyl and an alternate phase with a different polarity. We can look at the need for these different selectivity choices to manipulate resolution when columns with different particle sizes are used by comparing results with different samples with different polarities and with varying numbers of analytes and calculating potential resolution.

Efficiency in Hi Res, Hi Speed LC

Sub 2-micron particles deliver efficiency and productivity

This is the basic premise from which we operate.

Plates — Selectivity — Retention

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

$$N \propto \frac{L}{d_p}$$

To Maintain Rs:

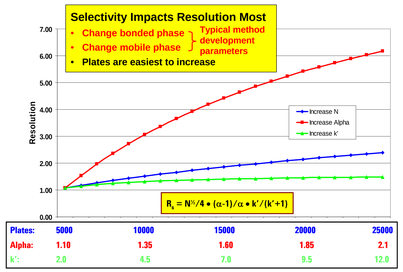
e.g.: L/2 → $d_p/2$

Column Length = ↓ N

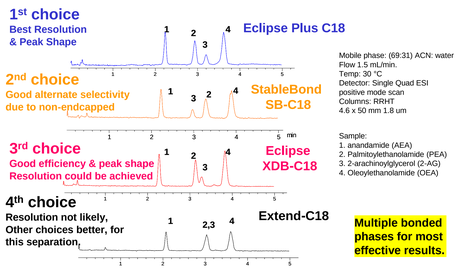
Particle Size = ↑ N

Selectivity Impacts Resolution Most

Typical Method Development Parameters: Effects of Selectivity, Efficiency and Retention



Each ZORBAX RRHT C18 Bonded Phases Provides Different Selectivity to Optimize a Separation



Sub 2-Micron Columns Provide the Efficiency of Longer Columns for More Productivity

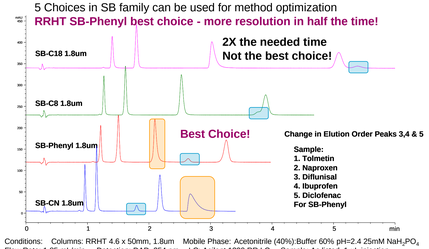
Column Length (mm)	Resolving Power N(5 µm)	Resolving Power N(3.5 µm)	Resolving Power N(1.8 µm)	Typical Pressure Bar (1.8 µm)	Analysis Time*
150	12,500	21,000	32,500	550	
100	8,500	14,000	24,000	420	-33%
75	6000	10,500	17,000	320	-50%
50	4,200	7,000	12,000	210	-67%
30	N.A.	4,200	6,500	126	-80%
15	N.A.	2,100	2,500	55	-90%

Analysis Time / Peak Volume / Solvent Usage

* Reduction in analysis time compared to 150 mm column
• pressure determined with 60:40 MeOH/water, 1ml/min, 4.6mm ID

More RRHT Bonded Phase Choices Allow for Optimized Method Development

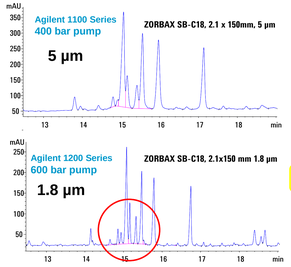
5 Choices in SB family can be used for method optimization
RRHT SB-Phenyl best choice - more resolution in half the time!



Conditions: Columns: RRHT 4.6 x 50mm, 1.8um Mobile Phase: Acetonitrile (40%):Buffer 60% pH=2.4 25mM NaH_2PO_4
Flow Rate: 1.85 mL/min Detection: DAD, 254 nm LC: Agilent 1200 RR LC Sample: As listed, 1 uL injection

Separate More Peaks with 1.8 µm

Complex Natural Product Extract from Ginseng

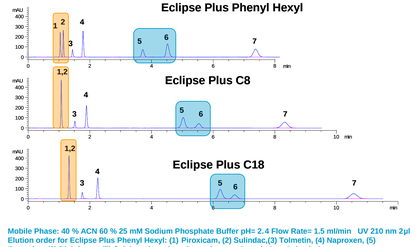


Mobile phase: A = H2O + 0.1% TFA
B = ACN + 0.1% TFA
Gradient: 10% to 95% ACN in 40min, hold for 1min
Flow: 0.4 mL/min
Inj. volume: 3 µ L, partial loop filling
DAD: 220 nm (20 Hz)
2µl flowcell

Improved Resolution

Agilent Application Note:
Analysis of a complex natural product extract from ginseng – Part I
Publication Number 5989-4506EN

Comparison of Eclipse Plus Family – C18, C8 Phenyl-Hexyl NSAIDS



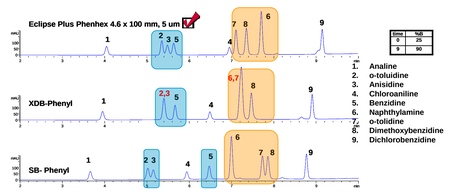
Mobile Phase: 40 % ACN 60 % 25 mM Sodium Phosphate Buffer pH= 2.4 Flow Rate= 1.5 ml/min UV 210 nm 2µl
Elution order for Eclipse Plus Phenyl Hexyl: (1) Piroxicam, (2) Sulindac,(3) Tolmetin, (4) Naproxen, (5) Ibuprofen, (6) Diclofenac, (7) Celebrex (equal portions of approximately 1 mg/ml solutions

Phenyl Selectivity

Multiple Types of Phenyl Columns Provide Selectivity Differences

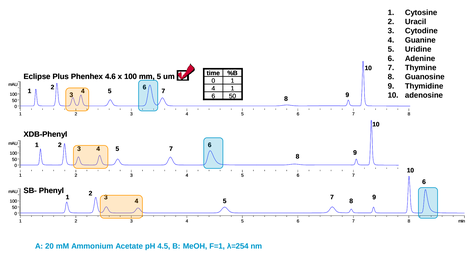
Phenyl Bonded Phase Type	Endcap	Silica Type	Agilent Column	What distinguishes it?
Phenylhexyl	Yes	B	Eclipse Plus Phenyl	Longer hexyl spacer adds hydrophobicity to the bonded phase.
Phenylethyl	Yes	B	Eclipse XDB-Phenyl	Original Type B Silica Phenyl Offering
Phenylpropyl	No	B	SB-Phenyl	Non-endcapped increases hydrophilic and silanol interactions
Phenylethyl	Yes	A	ZORBAX Phenyl	Original phenyl offering Type A silica has different selectivity.

Different Selectivity of ZORBAX Phenyl Columns Amines from Azo Dyes



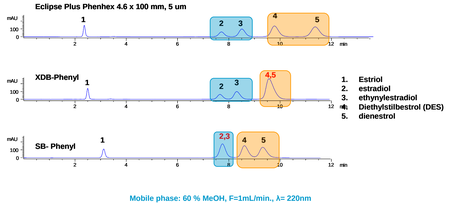
Gradient: A: 10 mM NH4Acetate, pH 4.7, B: MeOH

Different Selectivity of ZORBAX Phenyl Columns Nucleobases and their nucleosides



A: 20 mM Ammonium Acetate pH 4.5, B: MeOH, F=1, λ=254 nm

Different Selectivity of ZORBAX Phenyl Columns Estrogens



Mobile phase: 60 % MeOH, F=1mL/min., λ= 220nm

Phase Orthogonality

Retention Comparison of Aliphatic and Aromatic Compounds – C18 vs. Phenyl Phases

Experimental Process

- A series of over 40 aliphatic, nitro substituted benzenes and substituted benzenes were injected onto 4.6 x100 mm 5 micron columns using an Agilent 1200 system. The solvent consisted of either 40 % Acetonitrile 60 % Water or 60 % Methanol 40 % Water, at 1 ml/min, 205 nm. log k' of each compound was determined and plotted.
- The scatter plot indicates that nitro substituted aromatics had the greatest difference in retention on the C18 vs. Phenyl, but the aliphatics and substituted benzenes also varied. This is shown by the correlation coefficient of the plot.
- Therefore Phenyl columns are a good alternate selectivity to choose during method development.
- A variety of phenyl columns were also compared and differences in all the columns are there, but the C18 vs. Phenyl shows the most difference. A methanol organic modifier changes selectivity more than acetonitrile and can be used to also change selectivity.

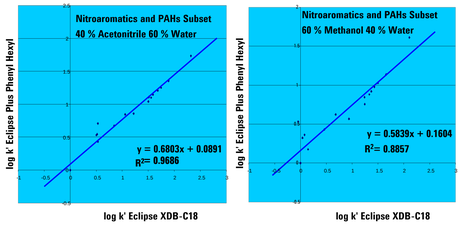
Scatter Plot Explanation

Hydrophobic interactions generally dominate reverse phase HPLC; deviations from linearity can be attributed to other solute interactions: π-π, dipole-dipole etc.

The scatter plot data shows Phenyl columns can take advantage of π-π interactions when using Methanol rather than Acetonitrile. The compounds used consist of nitrogen substituted aromatics and polyaromatic hydrocarbons to clarify this point. Other compound groups were also examined.

The suppressed retention of some aromatic compounds with Acetonitrile can be attributed to competitive π-π interactions.

Phenyl Phases can take advantage of π-π interactions and thus show different selectivity than C18s, which primarily utilize hydrophobic interactions

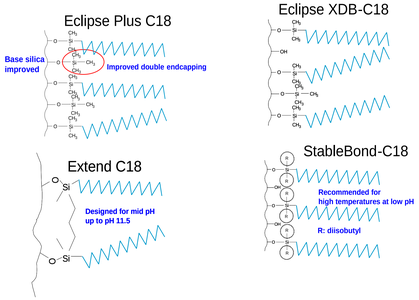


Results of C18 vs. Phenyl with ACN and Methanol

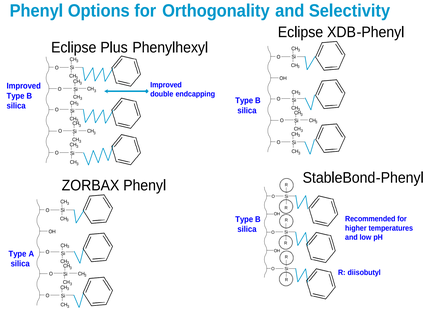
Column A	Column B	Solvent	Slope	R^2
C18	Phenyl Ethyl	Methanol	0.6168	0.8908
C18	Phenyl Ethyl	Acetonitrile	0.7011	0.9695
C18	Phenyl Hexyl	Methanol	0.7718	0.9236
C18	Phenyl Hexyl	Acetonitrile	0.7712	0.9422
C18	Phenyl Propyl	Methanol	0.5750	0.9176
C18	Phenyl Propyl	Acetonitrile	0.6768	0.9705
Phenyl Ethyl	Phenyl Propyl	Methanol	0.9062	0.9782
Phenyl Ethyl	Phenyl Propyl	Acetonitrile	0.9565	0.9912
Phenyl Ethyl	Phenyl Hexyl	Methanol	1.248	0.9837
Phenyl Ethyl	Phenyl Hexyl	Acetonitrile	1.125	0.9860

Phenyl Ethyl = Eclipse Phenyl; Phenyl Propyl= StableBond Phenyl
Phenyl Hexyl = Eclipse Plus Phenyl Hexyl

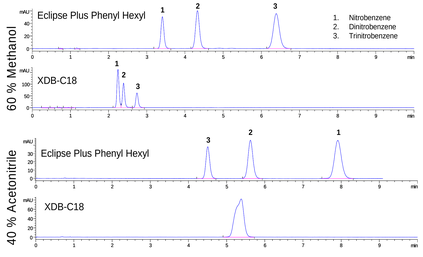
C18 Options for Orthogonality and Selectivity



Phenyl Options for Orthogonality and Selectivity



Selectivity Differences used in Scatter Plots Nitro Substituted Aromatics



Conclusion

- RRHT columns make separations fast and provide superior efficiency
- Selectivity choices are critical for best results and is independent of particle size.
- Phenyl vs. other C18 phases in terms of orthogonality – Yes, it is different and worth trying for method development.
- Phenyls vs. each other – range of choices means there is always one to optimize your separation
- Non endcapped phases such as StableBond C18 or Phenyl are good alternate third phases for selectivity differences