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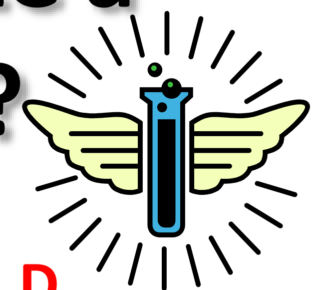
Is A Second Retention Mechanisms in LC a Curse or Cure?



Merlin K. L. Bicking, Ph.D.
ACCTA, Inc.
Richard A. Henry, Ph.D.
Consultant

Minnesota Chromatography Forum 2014
Session: 2D-LC and Novel LC Phases

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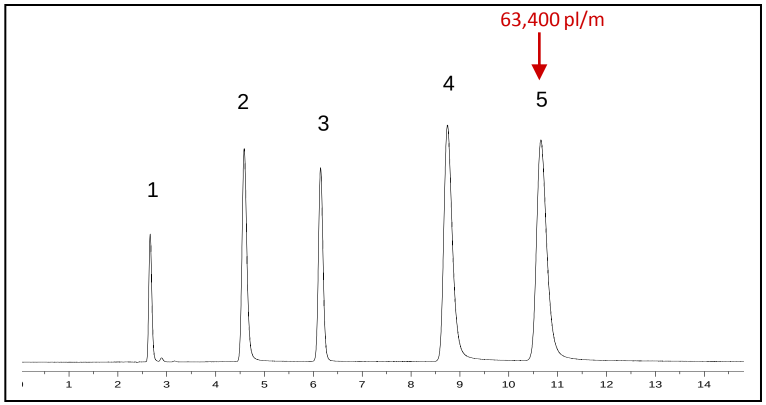
Merlin K. L. Bicking, Ph.D.
ACCTA, Inc.
St. Paul, MN USA 55125
Email: info@accta.com

Richard A. Henry, Ph.D.
Consultant
State College, PA USA 16801
Email: hyperlc@comcast.net

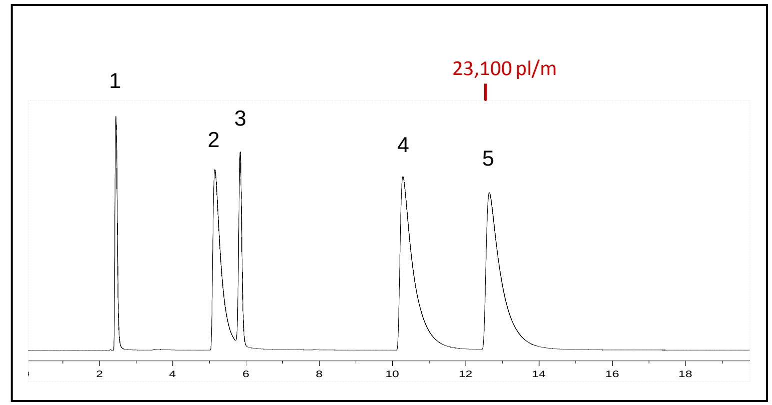
Curse: The Effect of Silica Purity on Selectivity

A second retention mechanism from the silica affected the chromatography

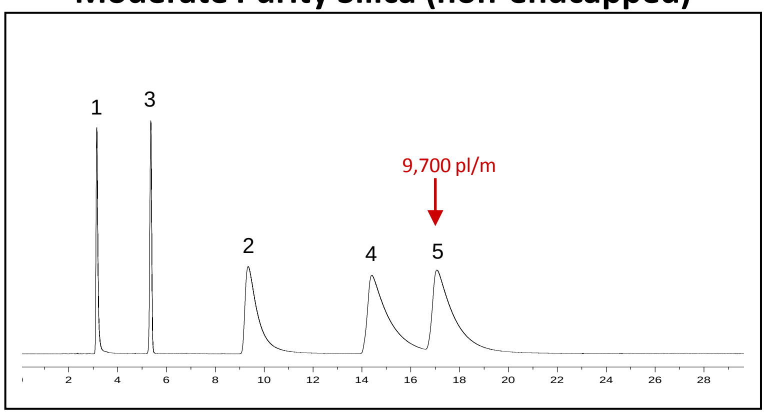
ACE C18
High Purity Silica (fully endcapped)



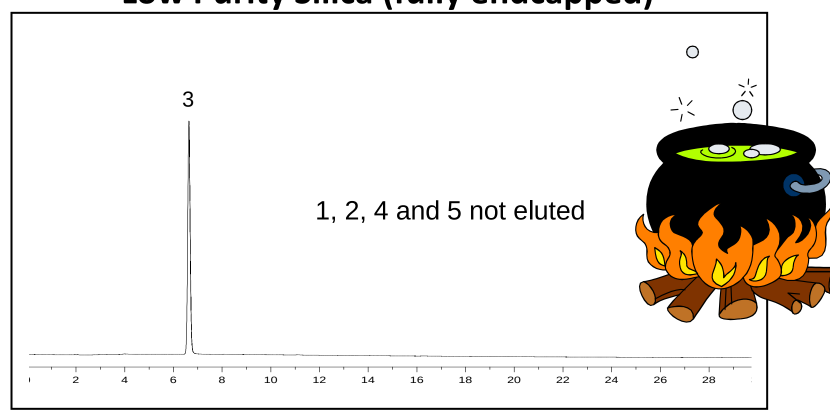
Zorbax XDB C18
Moderate Purity Silica (fully endcapped)



Zorbax SB C18
Moderate Purity Silica (non-endcapped)



Zorbax ODS
Low Purity Silica (fully endcapped)



Decreasing silica purity can change selectivity, but poor chromatography and reproducibility may result from surface interactions.

Column: 250 x 4.6mm, 5µm Mobile phase: 80:20 MeOH/25mM KH_2PO_4 (pH6.0) Flow: 1.00ml/min
Components; 1: Norephedrine, 2: Nortriptyline, 3: Toluene, 4: Imipramine, 5: Amitriptyline

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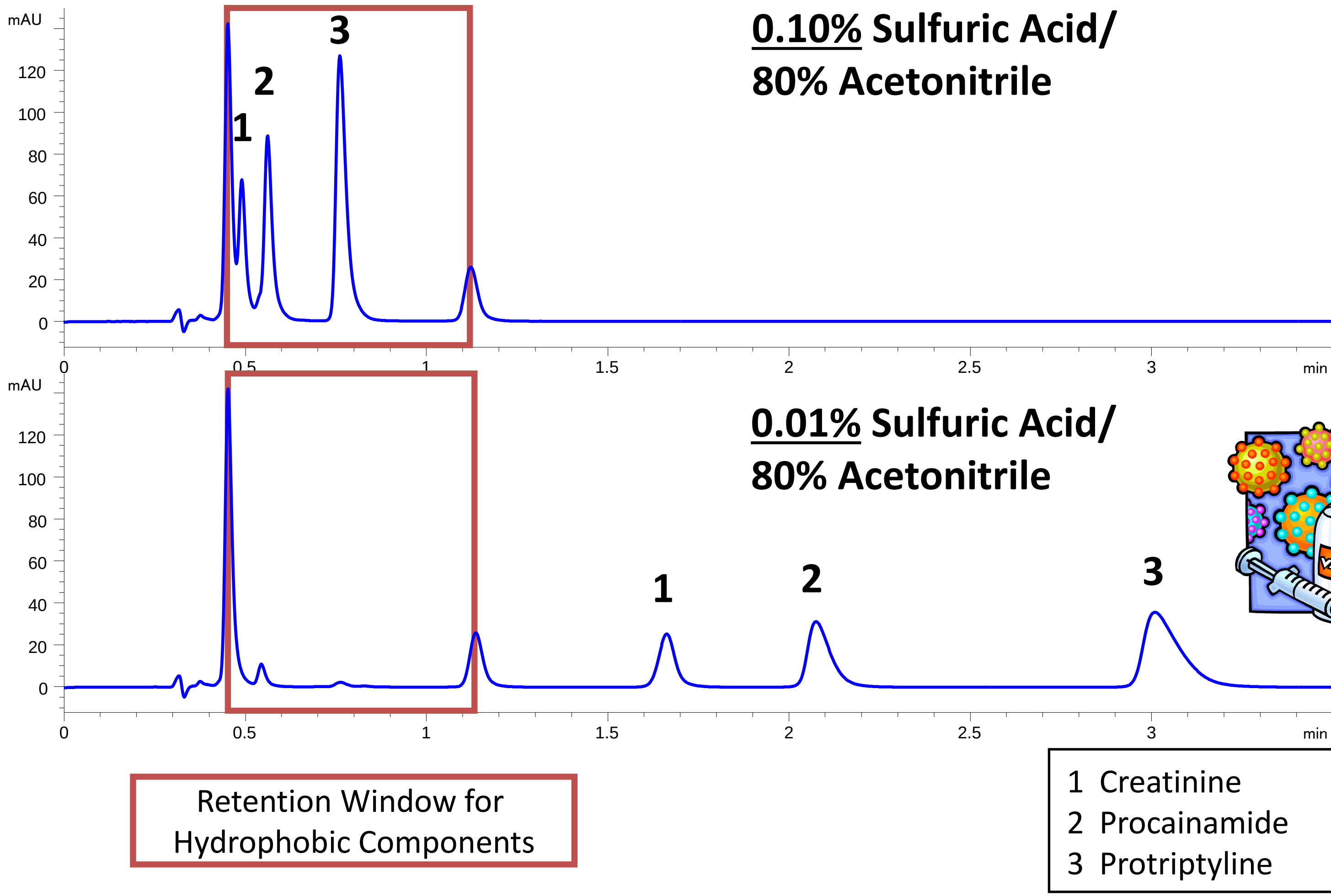
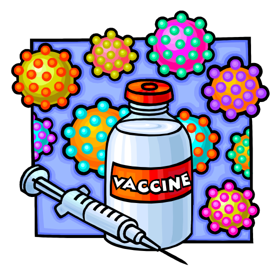
Cure: Change Some Retention Times, Not All

- **Decreasing the acid content causes an increase in the retention of the basic analytes (ionic effects). Since the acetonitrile content has not changed, the hydrophobic retention region does not change.**

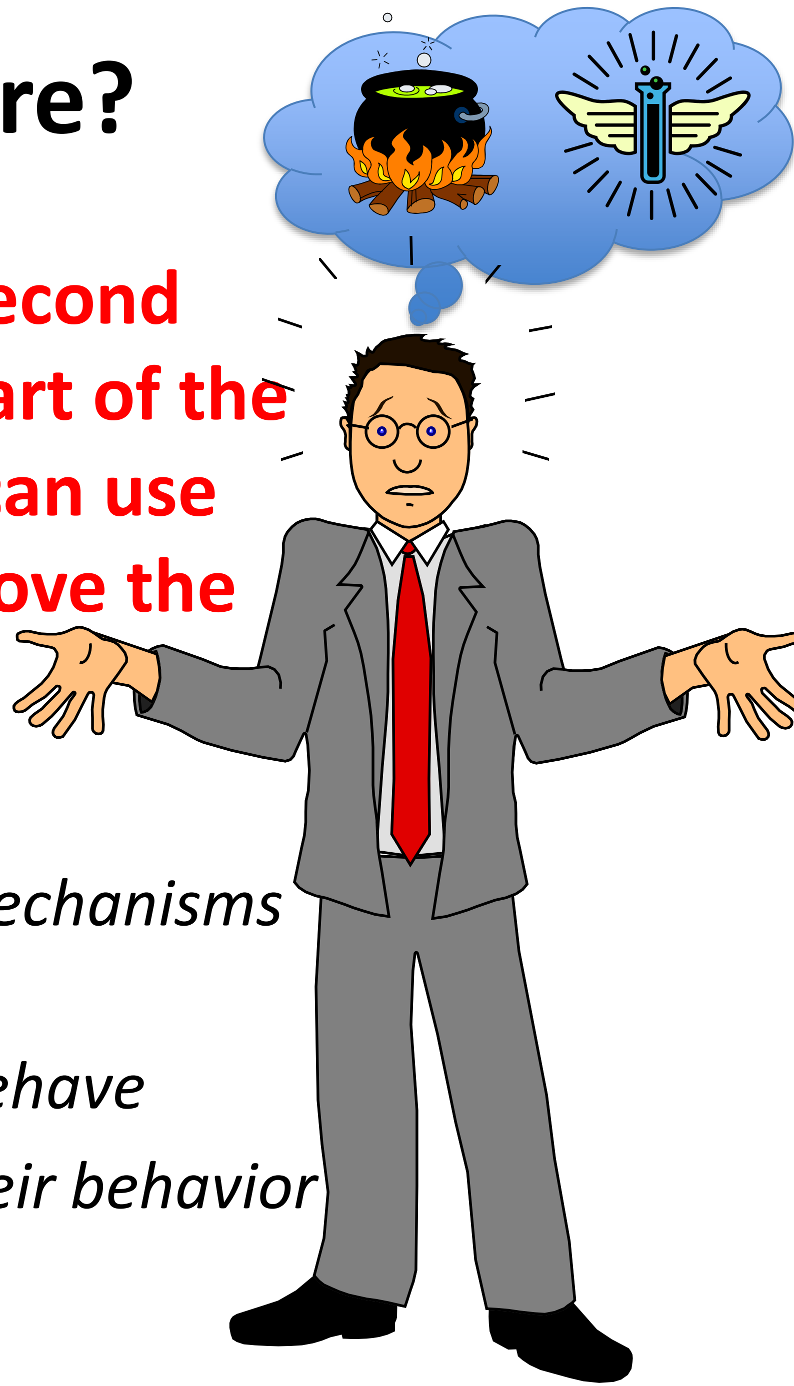
Column: 4.6 X 50 mm PFPP





So, is it a curse or cure?

- **When the presence of a second retention mechanism is part of the phase design, maybe we can use both mechanisms to improve the separation**
- **This is possible only if,**
  - *We understand what the mechanisms are*
  - *We understand how they behave*
  - *We know how to control their behavior*



Experimental Details

- **Equipment**
  - *Agilent 1100 with autosampler, column compartment, and diode array*
  - *OpenLab ChemStation software*
- **Mobile Phase Components**
  - *Acetonitrile (HPLC Grade)*
  - *Ammonium Formate*
    - 10, 20, and 30 mM
- **Operating Conditions**
  - *Flow: 1.75 mL/min*
  - *Injection: 2 uL*
  - *Temperature: 35 C*
  - *Detection: 220 nm*

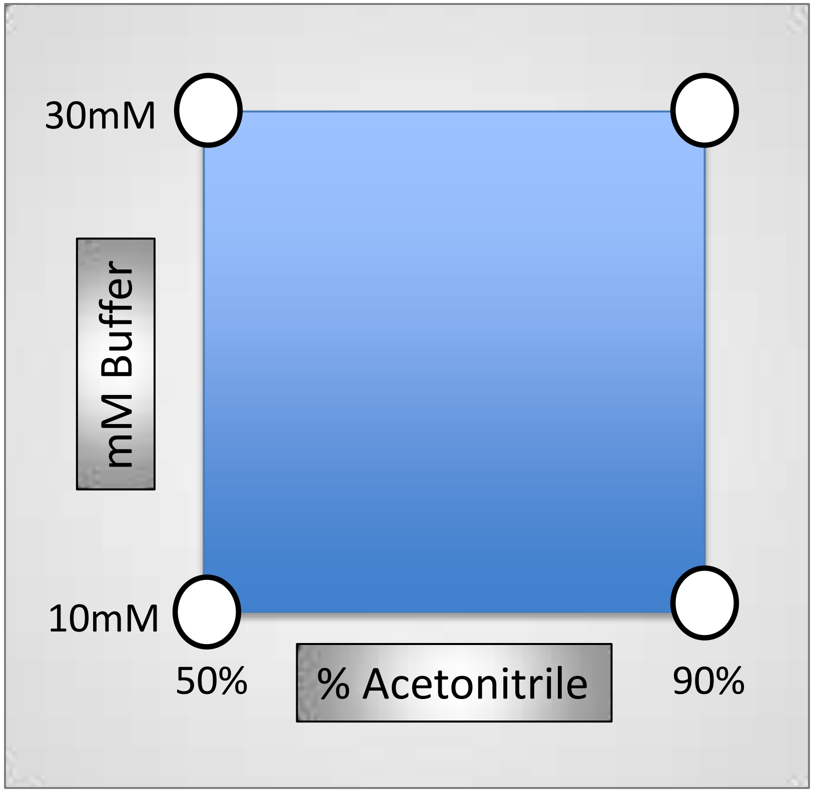
Manufacturer	Phase	Dimensions	Phase
Supelco	Ascentis Express C18	4.6X50 mm, 5 um*	Hydrophobic
Supelco	Ascentis Express F5	4.6X50 mm, 5 um*	Pentafluorophenylpropyl
Supelco	Ascentis Express OH5	4.6X50 mm, 5 um*	OH/Diol
Zirchrom	Zirchrom-PBD	4.6X50 mm, 3 um	Hydrophobic with adsorbed phosphate
Imtakt	Scherzo SM-C18	4.6X50 mm, 3 um	Hydrophobic + Cation Exchange + Anion Exchange
* Fused core design. Equivalent to 3 um particles.			

2^2 Factorial Screening Design

- **Test two mobile phase parameters that may affect retention**
  - *Acetonitrile Content*
  - *Buffer concentration*
- **Test high and low levels of each parameter**
  - *Acetonitrile: 50% and 90%*
  - *Buffer Concentration: 10 mM and 30 mM*

2^2 Factorial Screening Design

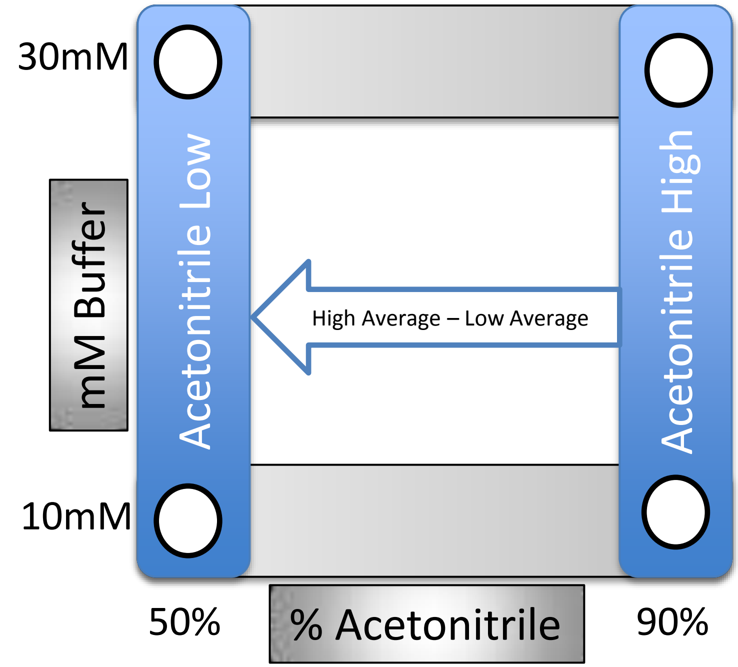
- **Why choose this design?**
  – *Factorials evaluate two or more variables with a minimum number of experiments*
- **Why choose these conditions?**
  – *The choice is arbitrary*
  – *Ideal for general LC and LC-MS applications*

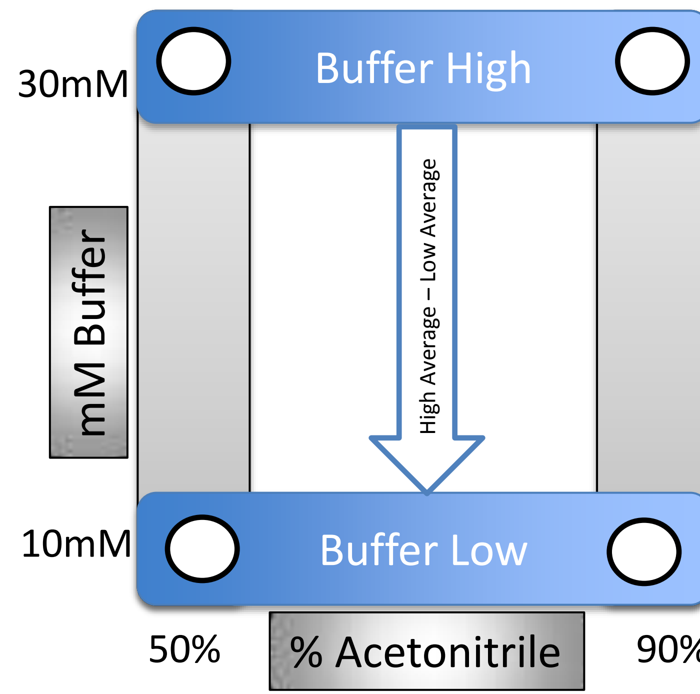


Procedure:
Measure retention
Calculate k
Calculate Main Effects

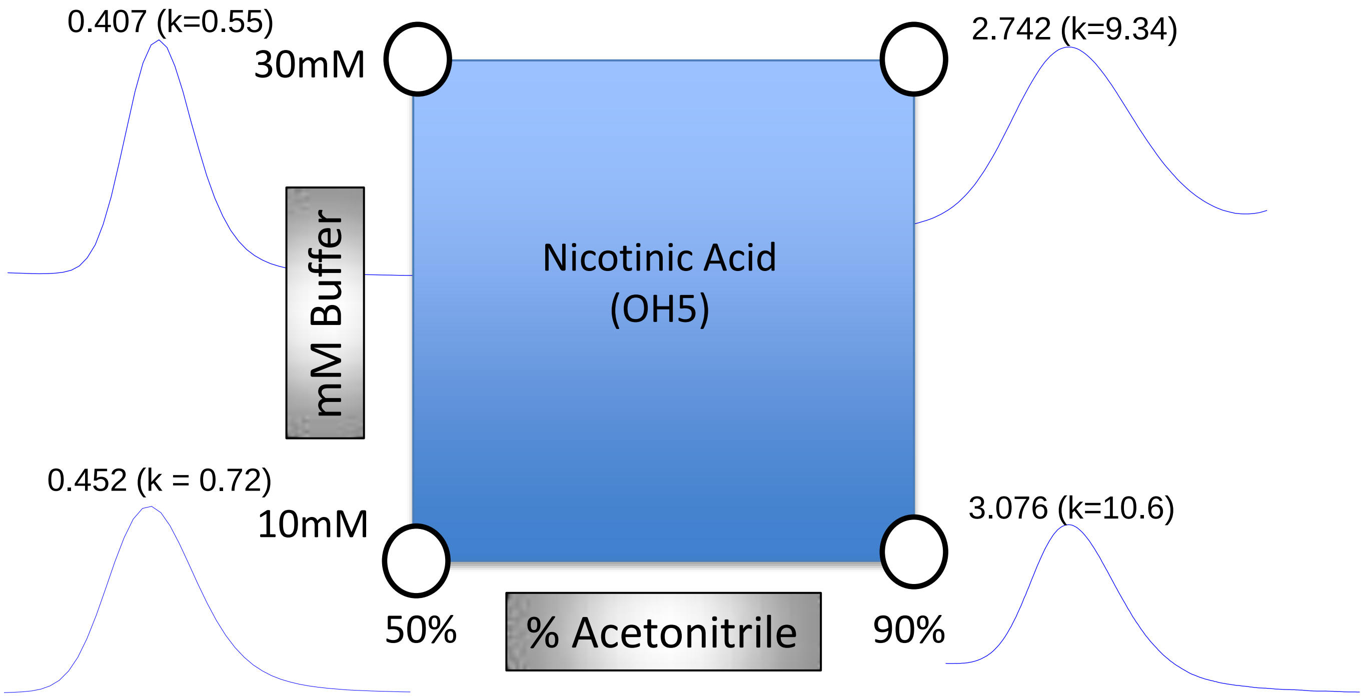
Calculating Main Effects

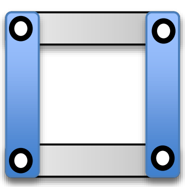
- The Main Effect of a parameter is the difference between the results at high and low settings.
- Larger values mean a larger change in retention over the range studied.
- Small values mean that this variable does not produce a significant change in retention.





Example Calculations

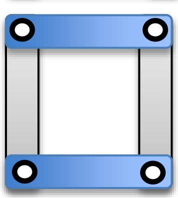




Main Effect for Acetonitrile

$$\frac{(9.34+10.6)}{2} - \frac{(0.55+0.72)}{2} = +9.3$$

- There is a significant increase in retention with an increase in acetonitrile.



Main Effect for Buffer

$$\frac{(0.55+9.34)}{2} - \frac{(0.72+10.6)}{2} = -0.7$$

- There is a small decrease in retention with an increase in buffer conc.

What Do Main Effects Mean?

	Sign of the Main Effect		
	Negative	Near Zero	Positive
Acetonitrile	Reversed Phase Trend	No effect	Normal Phase Trend
Buffer Conc.	Ion Exchange Trend	No effect	Salting Out Trend

Important Note: the existence of a trend suggests, but does not prove, that the stated mechanism is responsible for the effect.

Significant Main Effects for F5 (PFP)

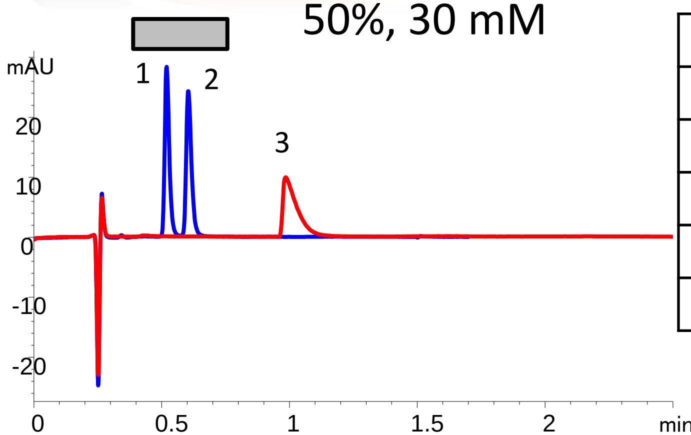
Compound	Class	Main Effects		Trend
		Acetonitrile	Buffer	
BTEX Aromatics*	Non-Polar, neutral	-2.9	-0.1	RP, No ionic
Benzyl Amine	Polar, strong base	+3.6	-2.6	NP, Ion Exchange
Phenethyl Amine	Polar, strong base, more hydrophobic than Benzyl Amine	+4.2	-3.4	NP, Ion Exchange
BTMA**	Polar, cation	+6.0	-5.3	NP, Ion Exchange

- **How do we use this information?**
  - *We want to separate the polar compounds from the less polar 1-ring aromatics.*
  - *Change buffer concentration to move the polar compounds, with little change in retention for the aromatics.*
  - *Change the acetonitrile content to move the polar compounds in the opposite direction from the aromatics.*

*Benzene, Toluene, Ethyl Benzene, Xylenes
**Benzyltrimethylammonium chloride

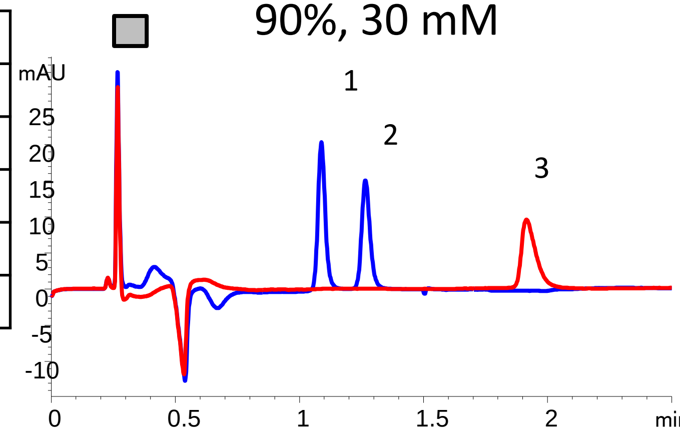
Improving the Separation on F5 (PFP)

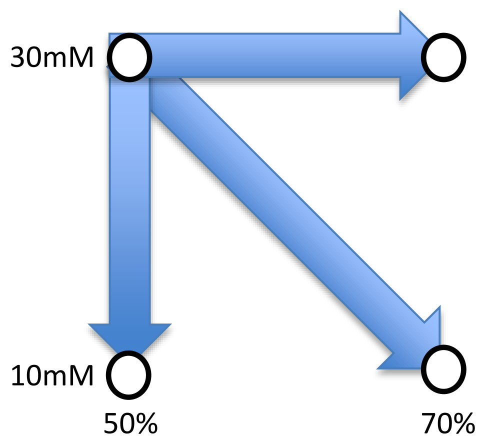
50%, 30 mM



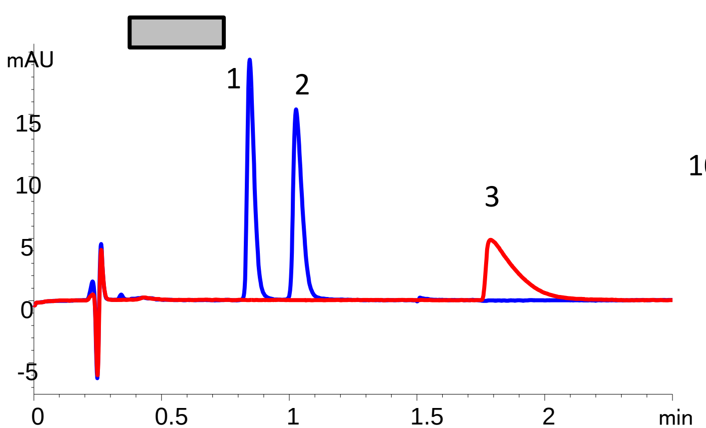
	Main Effects	
	Acn	Buffer
BTEX	-2.9	-0.1
Benzyl Amine (1)	+3.6	-2.6
Phenethyl Amine (2)	+4.2	-3.4
BTMA (3)	+6.0	-5.3

90%, 30 mM

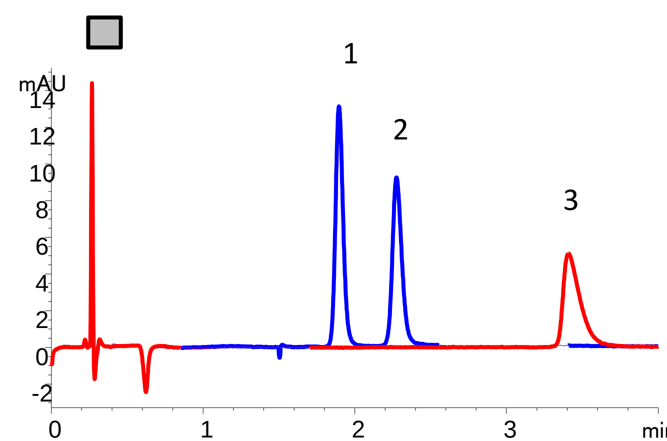




50%, 10 mM

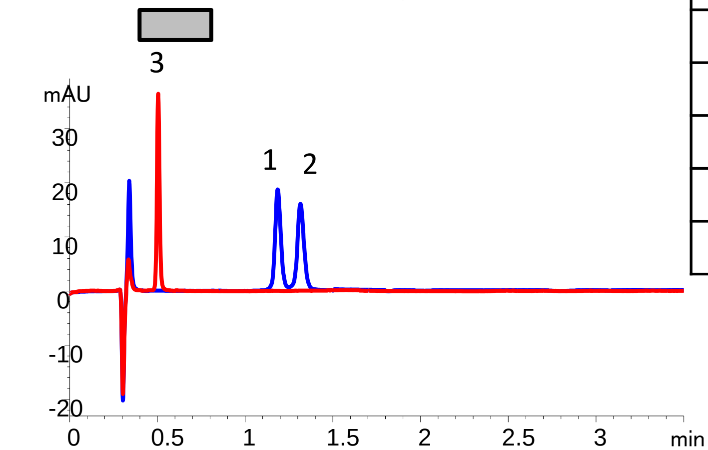


90%, 10 mM



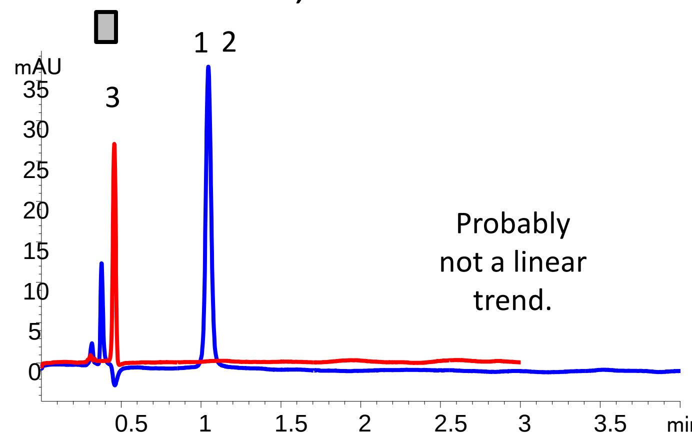
Improving the Separation on PBD

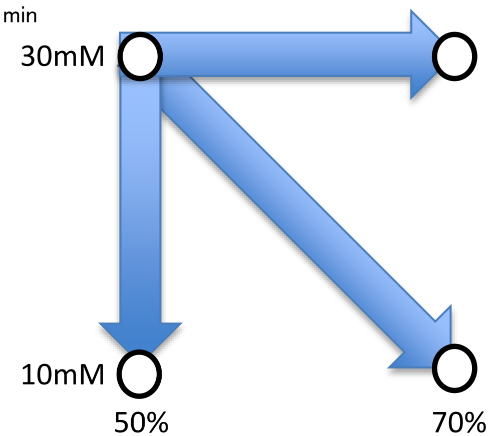
50%, 30 mM



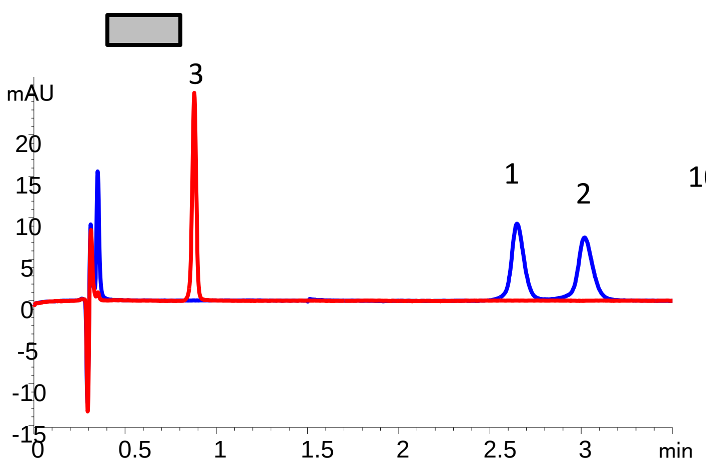
	Main Effects	
	Acn	Buffer
BTEX	-1.6	-0.1
Benzyl Amine (1)	+6.4	-8.0
Phenethyl Amine (2)	+6.3	-8.8
BTMA (3)	-0.1	-1.4

70%, 30 mM

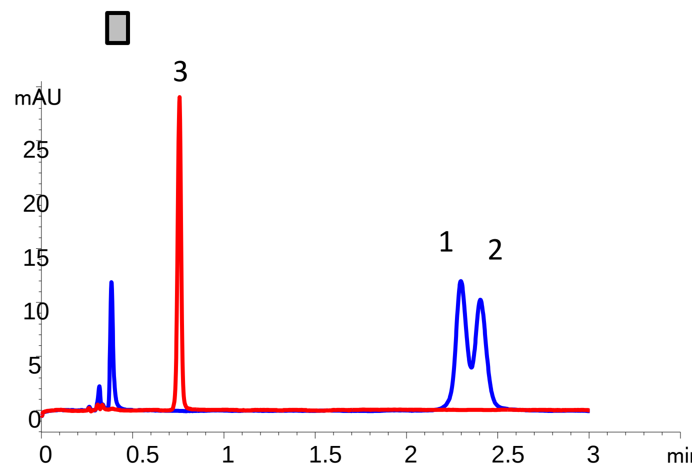




50%, 10 mM

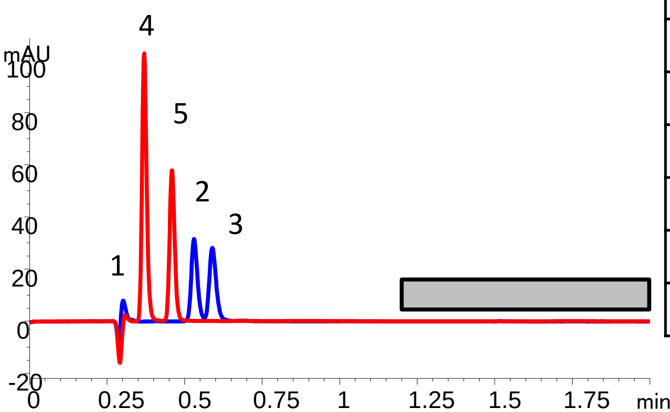


70%, 10 mM



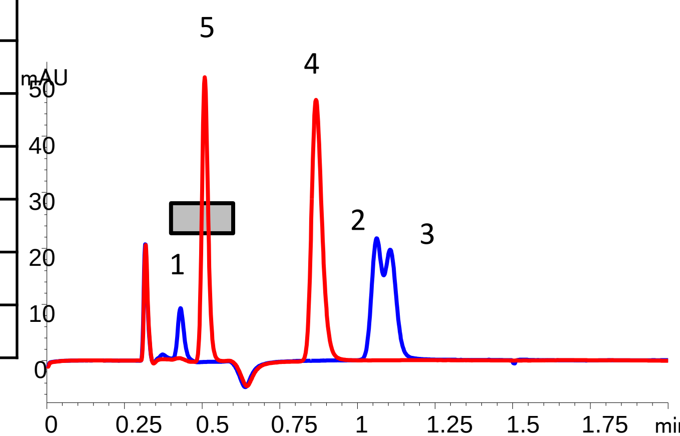
Improving the Separation on SM-C18

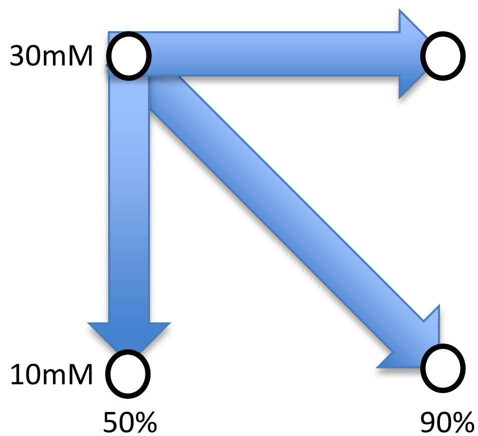
50%, 30 mM



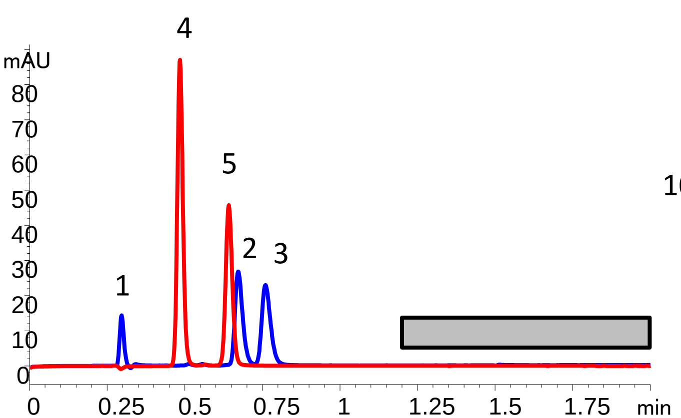
	Main Effects	
	Acn	Buffer
BTEX	-10.0	-0.2
Creatinine (1)	+0.30	0.0
Benzyl Amine (2)	+2.1	-0.9
Phenethyl Amine (3)	+0.5	-0.5
Nicotinic Acid (4)	+2.4	-1.1
Benzene Sulfonate (5)	0.0	-1.1

90%, 30 mM

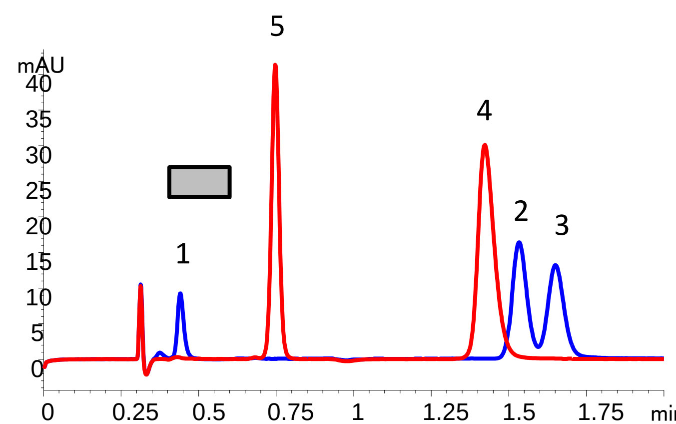




50%, 10 mM

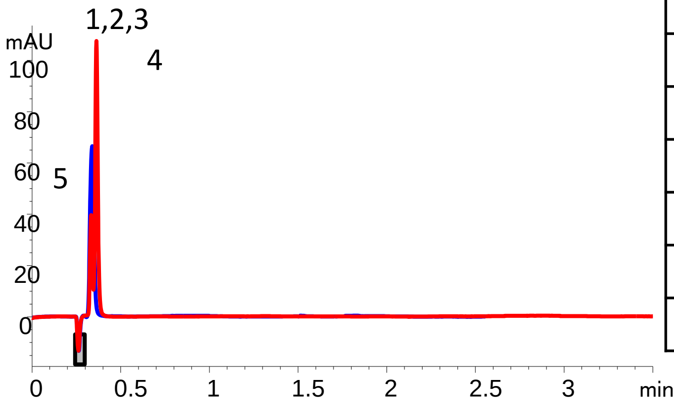


90%, 10 mM



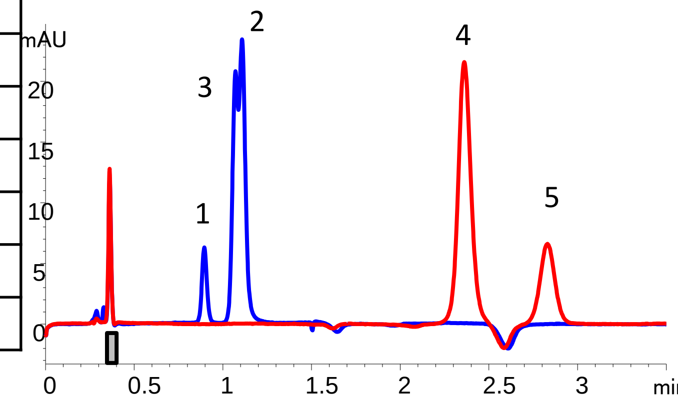
Improving the Separation on OH5

50%, 30 mM

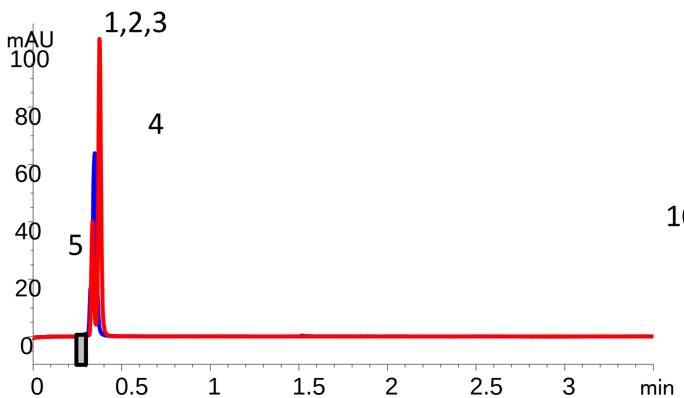


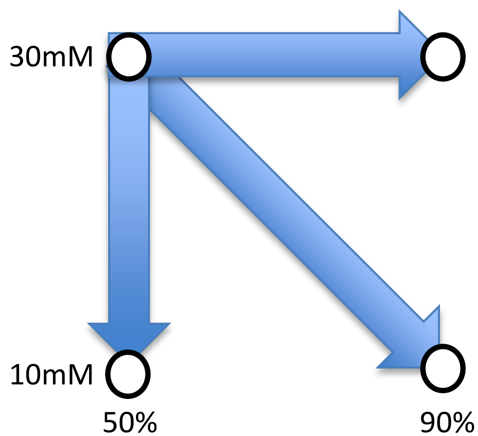
	Main Effects	
	Acn	Buffer
BTEX	0.0	0.0
Creatinine (1)	+2.1	0.0
Benzyl Amine (2)	+2.3	-1.1
Phenethyl Amine (3)	+2.3	-0.1
Nicotinic Acid (4)	+9.3	-0.7
Phenylalanine (5)	+9.6	+0.1

90%, 30 mM

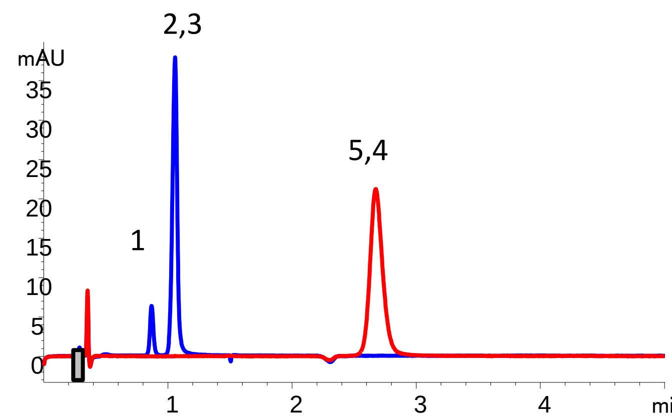


50%, 10 mM





90%, 10 mM



Main Effects Comparisons

BTEX	Main Effect: ACN
SM-C18	-10.0
F5	-2.9
PBD	-1.6
OH5	0.0

Benzyl Amine	Main Effect: ACN
F5	+6.4
PBD	+6.4
OH5	+2.3
SM-C18	+2.1

Benzyl Amine	Main Effect: Buffer
PBD	-8.0
F5	-2.6
OH5	-1.1
SM-C18	-0.9

Summary

- **The 2^2 factorial method can be used to easily identify general retention trends.**
- **Each of the four columns studied demonstrated a unique combination of multiple retention mechanisms.**
- **When two mechanisms are present, it is possible to separately move classes of compounds in different directions by adjusting the mobile phase composition.**
  - *On a single mode column, this would only be possible by changing the chemistry of the mobile phase components (e.g., change from acetonitrile to methanol).*
  - *On a multi-mode column, the retention time selectivity can be changed by adjusting the composition of the mobile phase, <u>without</u> changing the chemistry.*

Acknowledgements

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For more information, contact:
Merlin K. L. Bicking, Ph.D.
ACCTA, Inc.
St. Paul, MN 55125
Email: mbicking2@accta.com
Internet: www.accta.com

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