Mixed Mode Mechanisms in LC: Curse or Cure?

Merlin K. L. Bicking, Ph.D.

ACCTA, Inc.

Richard A. Henry, Ph.D.

Consultant

Pittcon 2014
Abstract Number: 570 - 10
Session 570 - Ongoing Enhancements to Chromatographic Methods

Background

- A second retention mechanism (e.g., residual silanols) is usually not desirable in reversed phase LC.
- Several new stationary phases are designed to produce multiple retention mechanisms. Many of these phases are referred to as "HILIC" but the presence of multiple retention modes is the key consideration for this presentation, not the type of separation.
- The chromatographic behavior of these columns is often not clear because of the multiple mechanisms.
- A simple screening approach for identifying the effect of two common mobile phase variables is shown and demonstrated for four different columns.
- We show how the presence of a second separation mechanism can be used advantageously in developing separations of basic and zwitterionic compounds from each other and from neutral solutes, using a basic understanding of the behavior of mobile phase effects on each column.

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

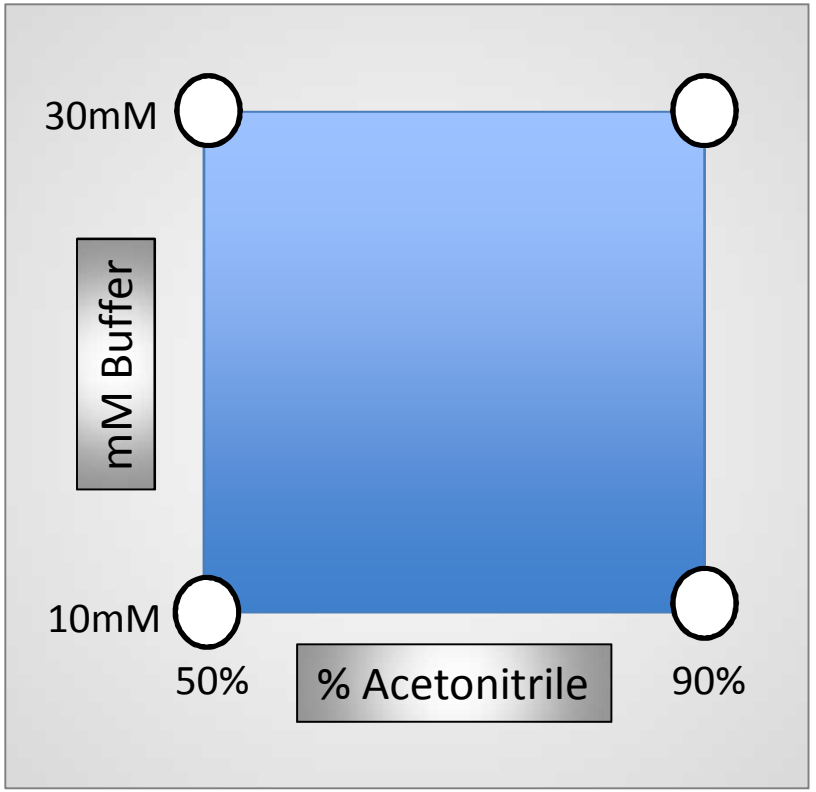
* Fused core design. Equivalent to 3 um particles.

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 of variables and conditions is arbitrary – you can choose any reasonable conditions*
  - *These particular limits were chosen because they are ideal for general LC and LC-MS applications*

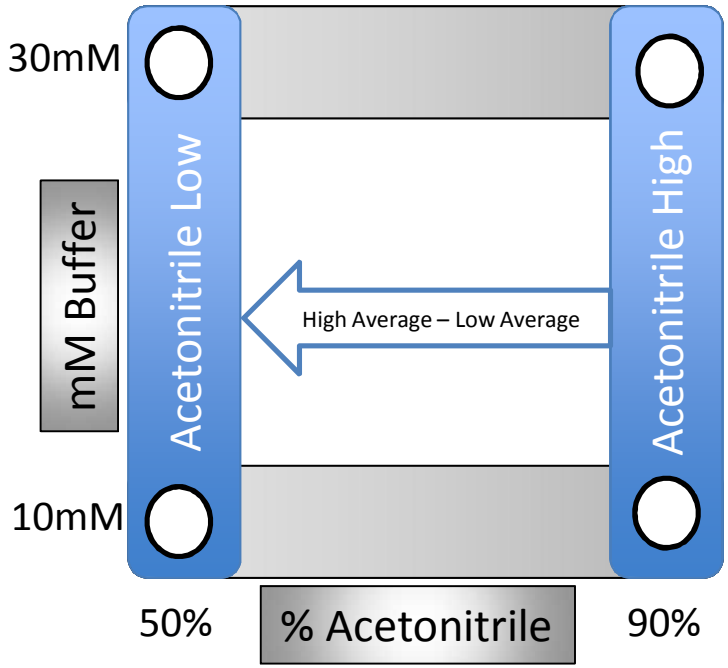


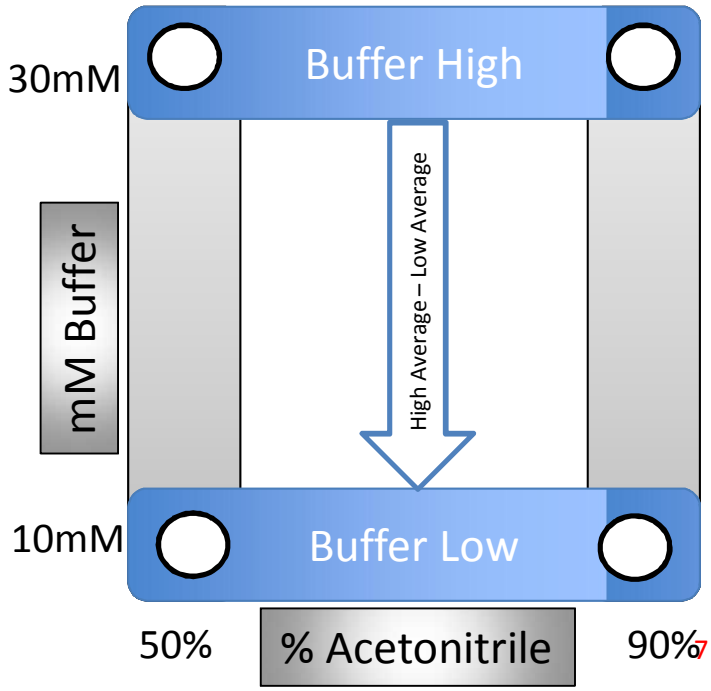
Chromatographic Evaluation

- **Evaluate retention of model compounds under the four mobile phase conditions.**
  - *All compounds studied here had very little retention on a C18 column at 50% acetonitrile.*
- **Calculate retention factor, k, for all injections.**
- **Look for conditions that produce k > 2 for at least one of the four combinations.**
- **Calculate Main Effects for each variable.**

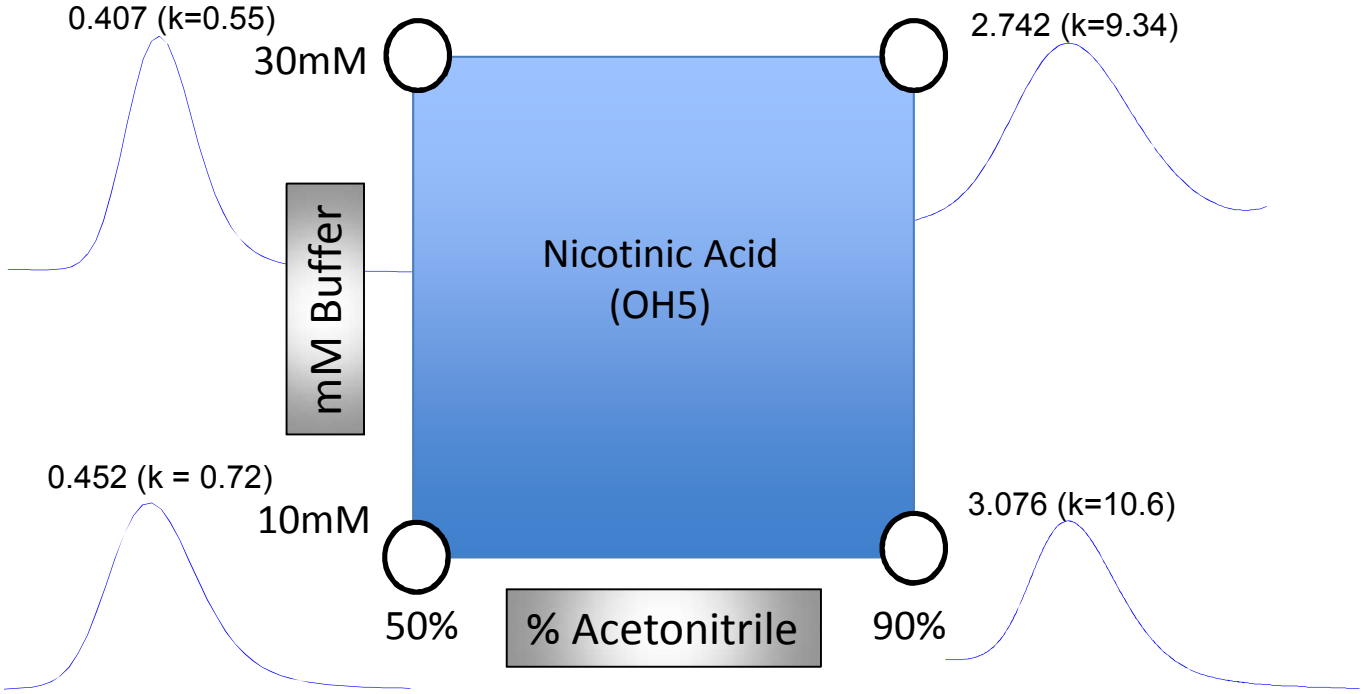
Calculating Main Effects

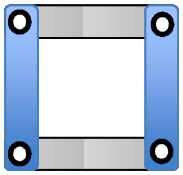
- **The Main Effect of a parameter is the difference between the results at high and low settings.**
- **Larger values mean a larger change in retention over the range studied.**
- **Small values mean that this variable does not produce a significant change in retention.**





Example Calculations

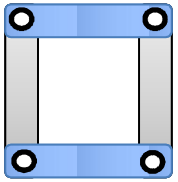




Main Effect for Acetonitrile

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

- 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)

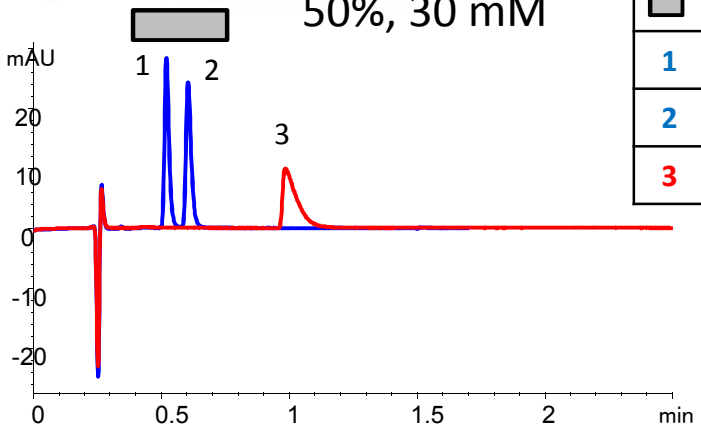
Compound	Class	Main Effects		Trend
		Acetonitrile	Buffer	
BTEX Aromatics*	Non-Polar, neutral	-2.9	-0.1	RP, No ionic
Benzyl Amine	Polar, strong base	3.6	-2.6	NP, Ion Exchange
Phenethyl Amine	Polar, strong base, more hydrophobic than Benzyl Amine	4.2	-3.4	NP, Ion Exchange
BTMA**	Polar, cation	6.0	-5.3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

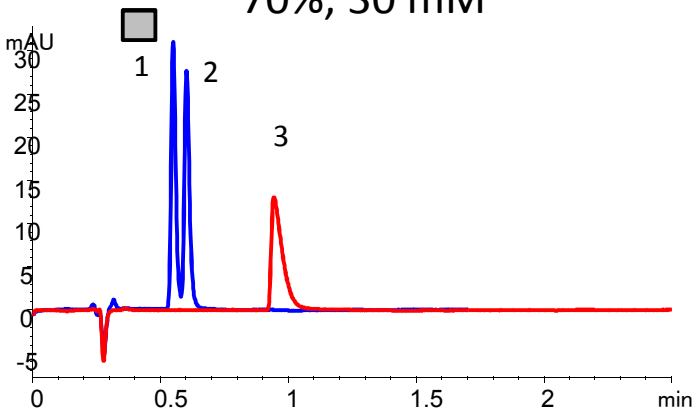
Improving the Separation on F5 (PFP)

50%, 30 mM



	BTEX
1	Benzyl Amine
2	Phenethyl Amine
3	BTMA

70%, 30 mM



Increase Acetonitrile

30mM

Decrease Buffer

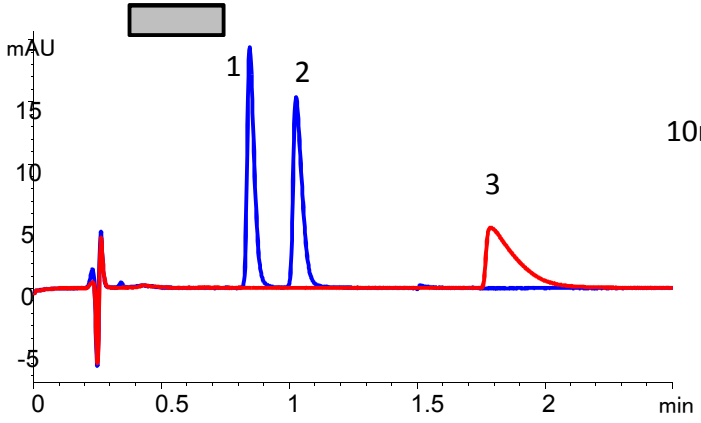
Change Both

10mM

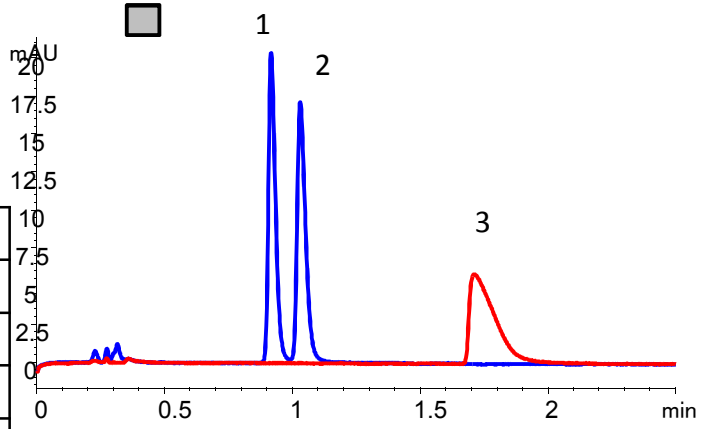
50%

70%

50%, 10 mM



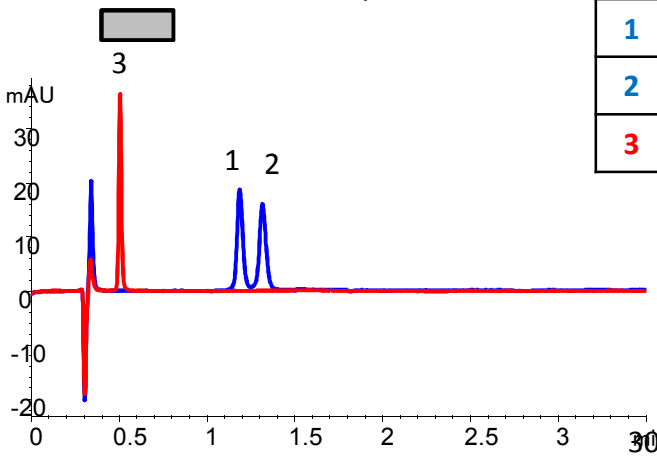
70%, 10 mM



	Main Effects	
	Acn	Buffer
BTEX	-2.9	-0.1
Benzyl Amine	+6.4	-2.6
Phenethyl Amine	+4.2	-3.4
BTMA	+6.0	-5.3

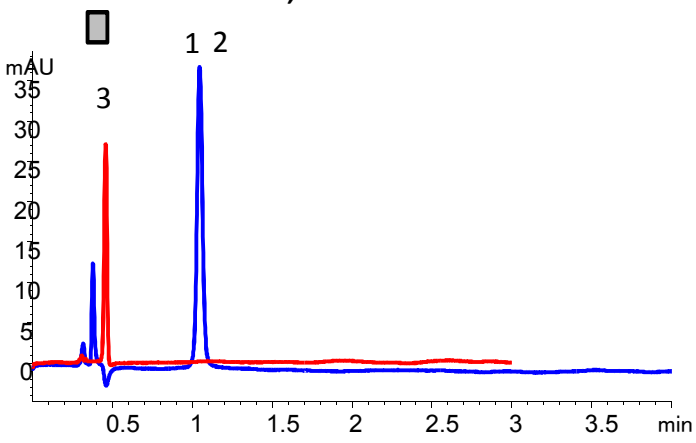
Improving the Separation on PBD

50%, 30 mM



	BTEX
1	Benzyl Amine
2	Phenethyl Amine
3	BTMA

70%, 30 mM

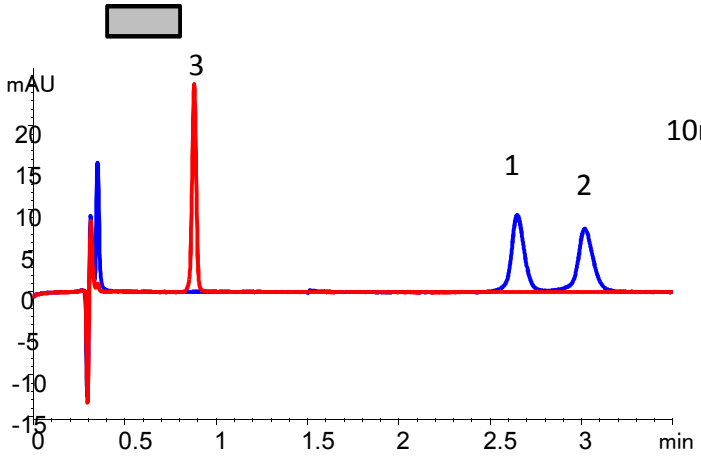


Increase Acetonitrile

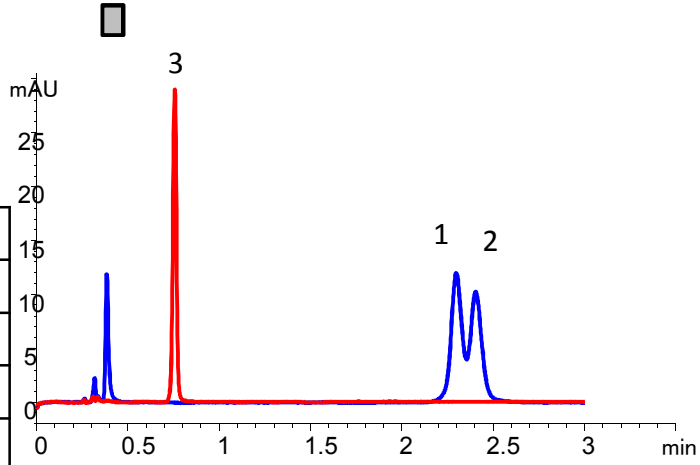
Decrease Buffer

Change Both

50%, 10 mM



70%, 10 mM



10mM

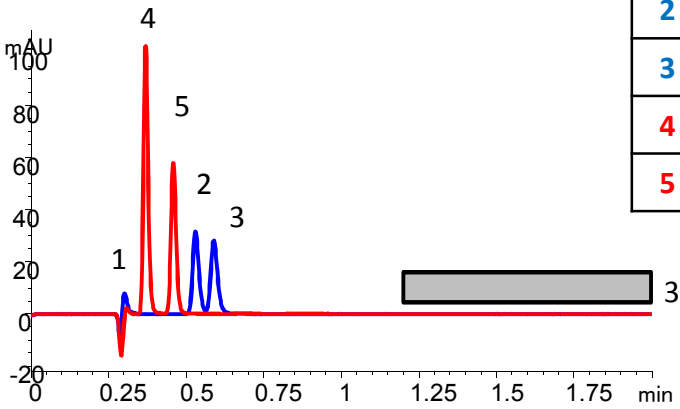
50%

70%

	Main Effects	
	Acn	Buffer
BTEX	-1.6	-0.1
Benzyl Amine	+6.4	-8.0
Phenethyl Amine	+6.3	-8.8
BTMA	-0.1	-1.4

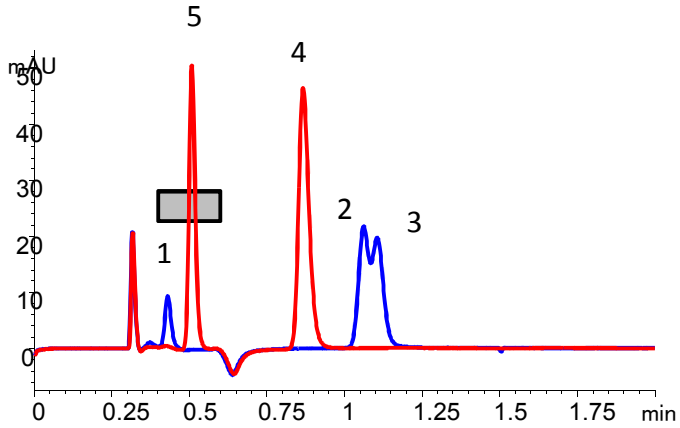
Improving the Separation on SM-C18

50%, 30 mM



	BTEX
1	Creatinine
2	Benzyl Amine
3	Phenethyl Amine
4	Nicotinic Acid
5	Benzene Sulfonate

90%, 30 mM



30mM

Increase Acetonitrile

Change Both

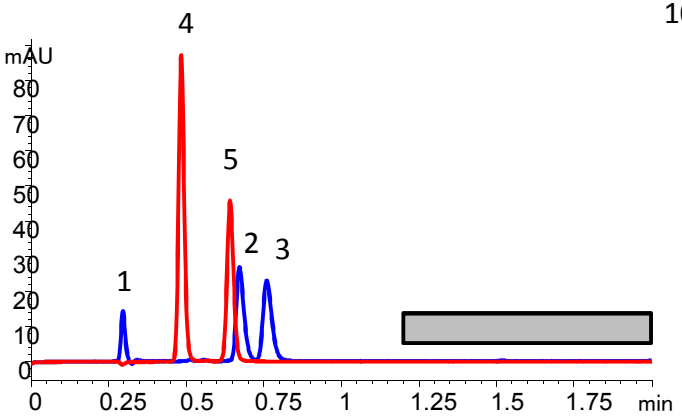
Decrease Buffer

10mM

50%

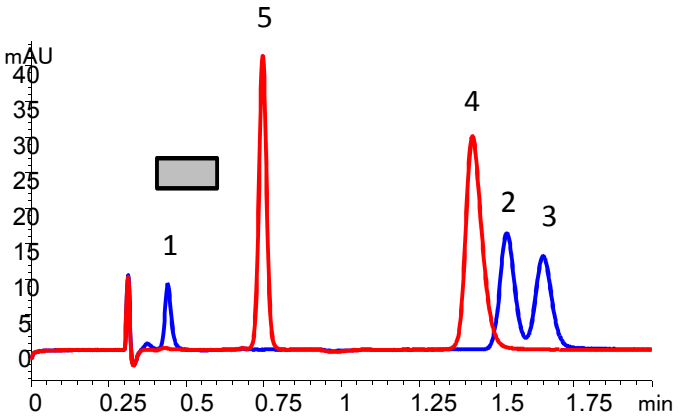
90%

50%, 10 mM



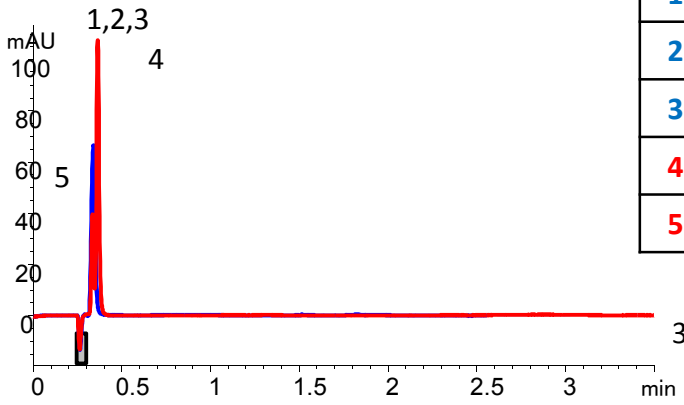
	Main Effects	
	Acn	Buffer
BTEX	-10.0	-0.2
Creatinine	+0.30	0.0
Benzyl Amine	+2.1	-0.9
Phenethyl Amine	+0.5	-0.5
Nicotinic Acid	+2.4	-1.1
Benzene Sulfonate	0.0	-1.1

90%, 10 mM



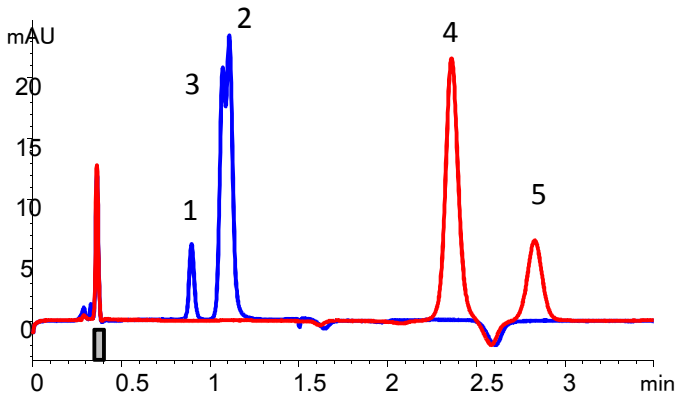
Improving the Separation on OH5

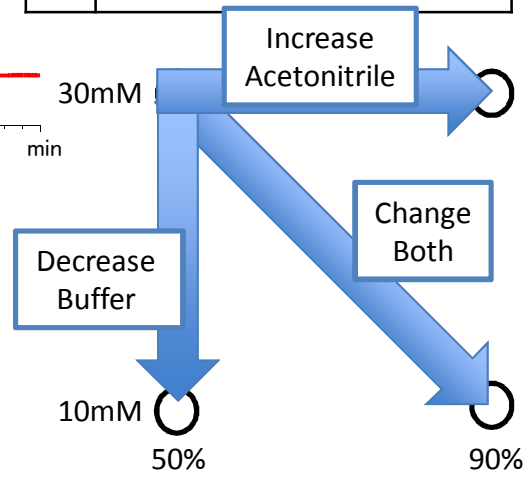
50%, 30 mM



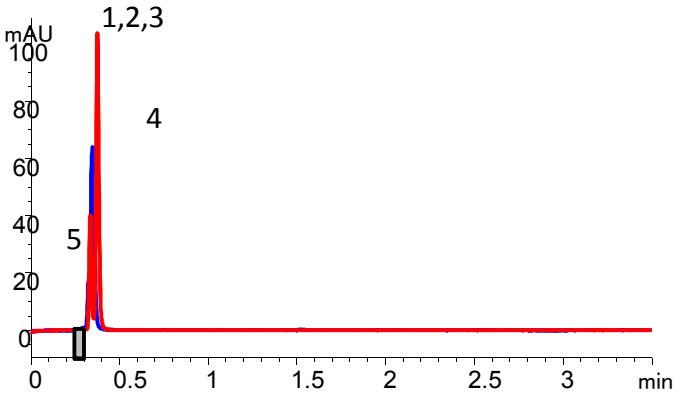
	BTEX
1	Creatinine
2	Benzyl Amine
3	Phenethyl Amine
4	Nicotinic Acid
5	Phenylalanine

90%, 30 mM



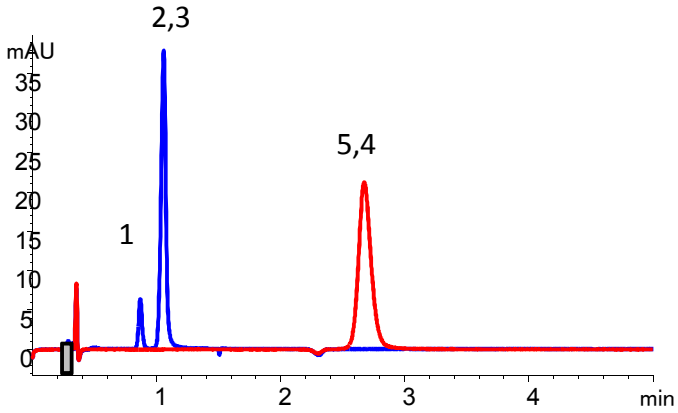


50%, 10 mM



	Main Effects	
	Acn	Buffer
BTEX	0.0	0.0
Creatinine	+2.1	0.0
Benzyl Amine	+2.3	-1.1
Phenethyl Amine	+2.3	-0.1
Nicotinic Acid	+9.3	-0.7
Phenylalanine	+9.6	+0.1

90%, 10 mM



Summary

- **The 2^2 factorial method can be used to easily identify general retention trends.**
- **Each of the four columns studied demonstrated multiple retention mechanisms.**
- **The size and direction of the main effects are unique for each compound on each phase.**
- **The main effects can be used to provide guidance on identifying optimum separation conditions.**
- **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 kind of change in retention order selectivity 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

Columns, reagents, and other resources were kindly provided by:

SUPELCO™ Analytical

ZirChrom®

Imtakt

AVEKA GROUP
Particle Processing & Custom Research

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