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A Simple Method for Predicting HPLC Selectivity in Ternary Reversed-Phase Solvent Systems

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Background/Introduction

- Retention as a function of a single organic modifier has been well characterized in reversed-phase systems. Acetonitrile produces a linear relationship between $\log k$ and amount of solvent. Methanol exhibits a similar, linear trend, but shows different selectivity for many compounds.
- Users will often switch between these two solvents to take advantage of the selectivity differences, which can include elution order changes. For complex mixtures, however, neither binary system may provide optimum resolution of every component.
- An optimum ternary system often can be found using a blend of acetonitrile, methanol and water. This work offers guidance on how to quickly locate the best possible separation using a ternary combination of water with these two organic modifiers.



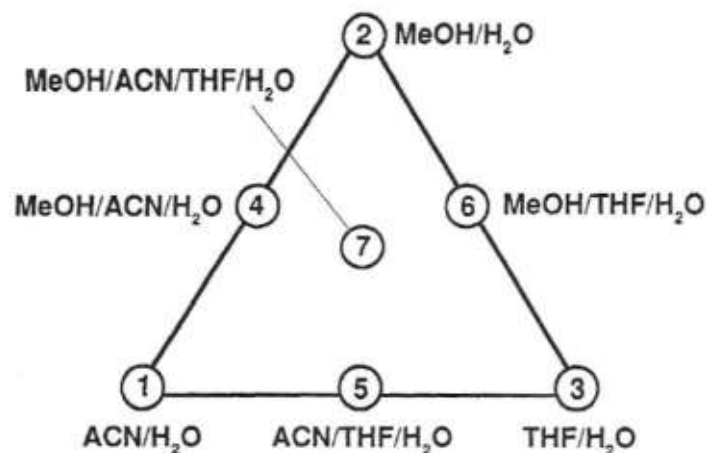
Theory/Purpose

- Retention in reversed-phase systems is usually described using the linear-solvent-strength (LSS) model (1-3):
 - $\log k = \log k_w - S\varphi$
 - Where
 - φ = % Strong Solvent
 - $k_w = k$ for $\varphi = 0$
 - S is a constant
- This relationship is generally valid for both water-acetonitrile and water-methanol systems.
 - Some anomalous behavior is observed when other interactions are present (e.g., H-bonding, ionic interactions).
- These concepts guide method development activities, and changes in selectivity are often observed when changing between different binary systems.
- In some situations, neither binary system produces acceptable separation and other options must be considered.



Theory/Purpose (cont.)

- Ternary solvent systems are well-known (4-5). Snyder et. al. proposed a general screening method for investigating ternary systems.
- The various mixtures combine all possible interactions (hydrophobic, dipolar, H-bonding) in different ways.
 - But little information is available for retention trends within each ternary combination.

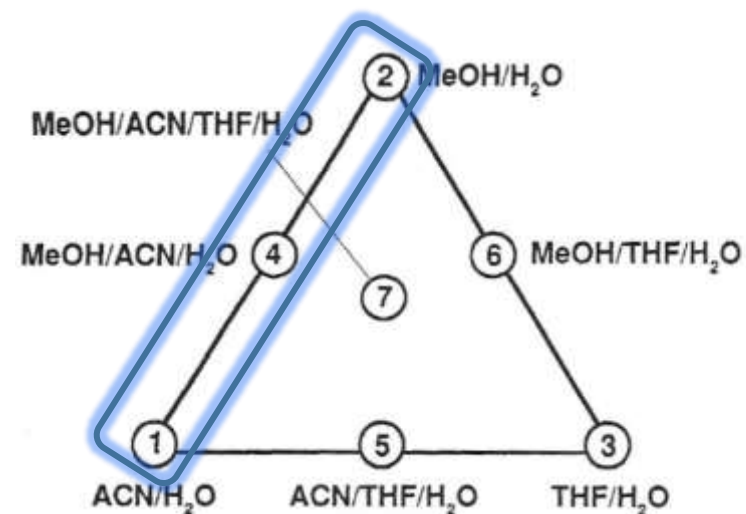


Source: Reference 4.



Objective of this work

- In this poster we explore the water/acetonitrile/methanol ternary system in more detail.
- Our goal is to optimize the separation of a seven-component mixture.
- Retention is studied as the relative amount of each strong solvent is varied while maintaining a constant total volume of strong solvent.
- The retention trends are non-linear but can be easily approximated using a simple second-order polynomial fit.
- These results allow us to propose a simplified procedure for identifying optimum conditions that requires only three injections.





Experimental

- Instrument Conditions
 - Flow: 1.5 mL/min.
 - Injection: 2 μ L
 - Column: 4.6 X 50 mm Halo C18
 - Temperature: 35 $^{\circ}$ C
 - Detection: 250 nm (20 Hz)
 - Mobile Phase
 - A: 0.10 % H_3PO_4 in water
 - B: Acetonitrile
 - C: Methanol



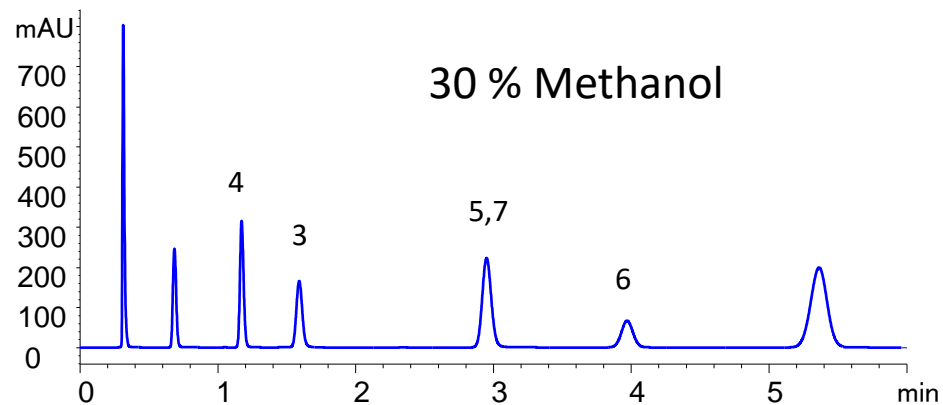
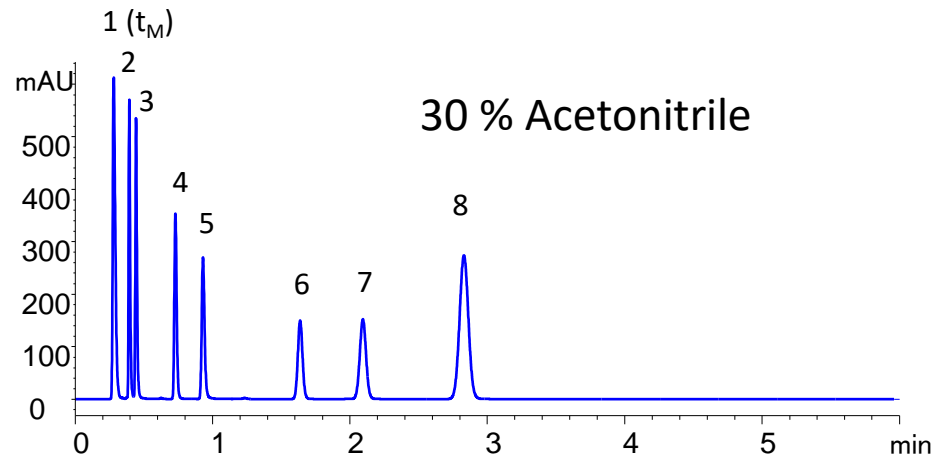
Mobile Phase Combinations

| Aqueous | Acetonitrile | Methanol |
|---------|--------------|----------|
| 70 | 30 | 0 |
| 70 | 25 | 5 |
| 70 | 20 | 10 |
| 70 | 15 | 15 |
| 70 | 10 | 20 |
| 70 | 5 | 25 |
| 70 | 0 | 30 |

| | Code | Name | Log P |
|---|--------|----------------------------------|-------|
| 1 | Ur | Uracil (Void Time Marker) | -1.1 |
| 2 | HBA* | 4-Hydroxybenzoic Acid | 1.6 |
| 3 | HIPhA* | 4-Hydroxyisophthalic Acid | 1.5 |
| 4 | PH* | Phenol | 1.5 |
| 5 | SA* | Salicylic Acid | 2.3 |
| 6 | DMP | Dimethyl phthalate | 1.6 |
| 7 | NB | Nitrobenzene | 2.0 |
| 8 | Ani | Anisole | 2.1 |

* Salicylic Acid and its known impurities.

Results



- Initial Binary Screening

- 30 % Acetonitrile

- Little retention for compounds 2 – 5
 - Minimal separation for compounds 2 and 3

- 30 % Methanol

- Retention order change for compounds 3, 4, and 7.
 - Coelution for compounds 5 and 7.

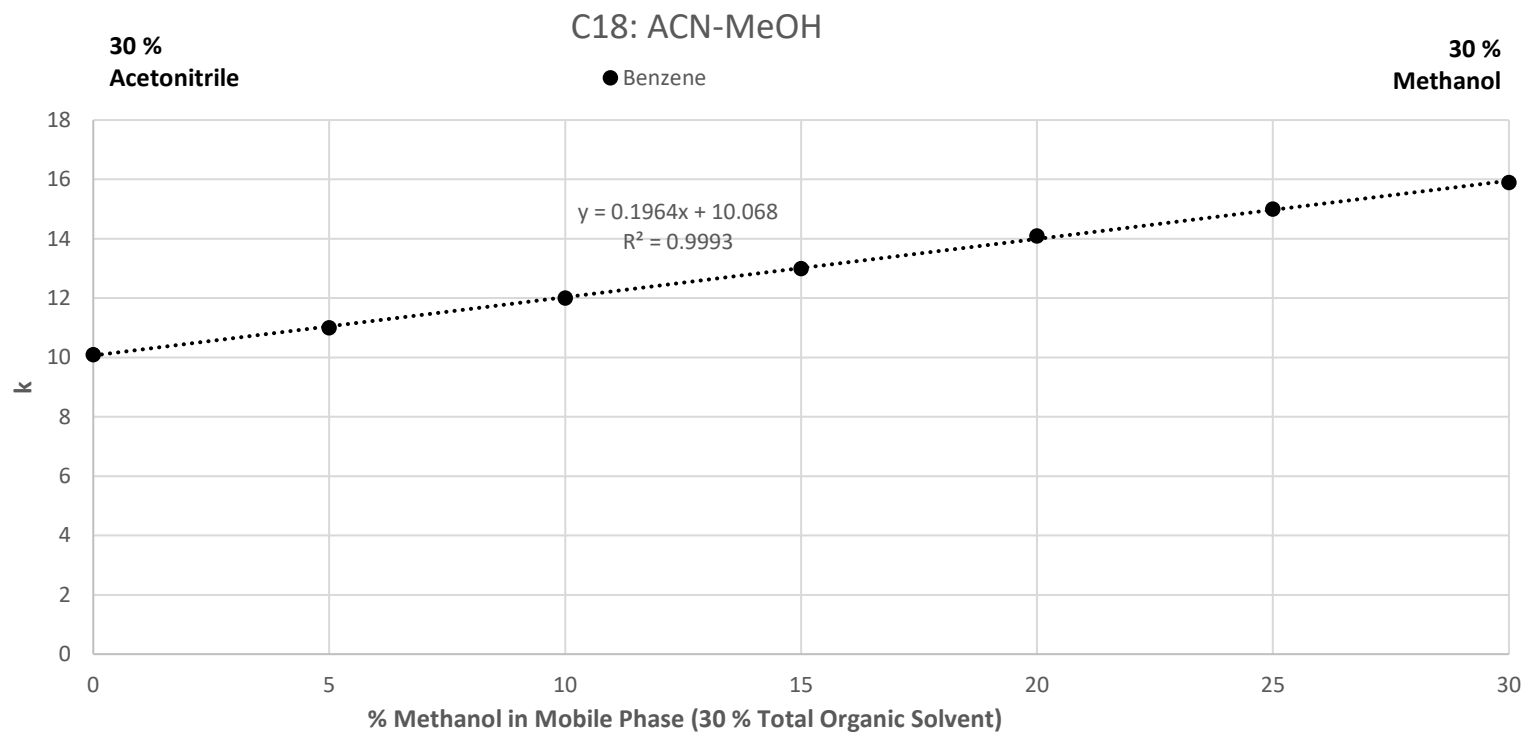
- Questions:

- Would a ternary mixture improve the separation or make it worse?
 - How can we quickly find conditions that produce better retention and resolution?



Initial Ternary Screening with Benzene

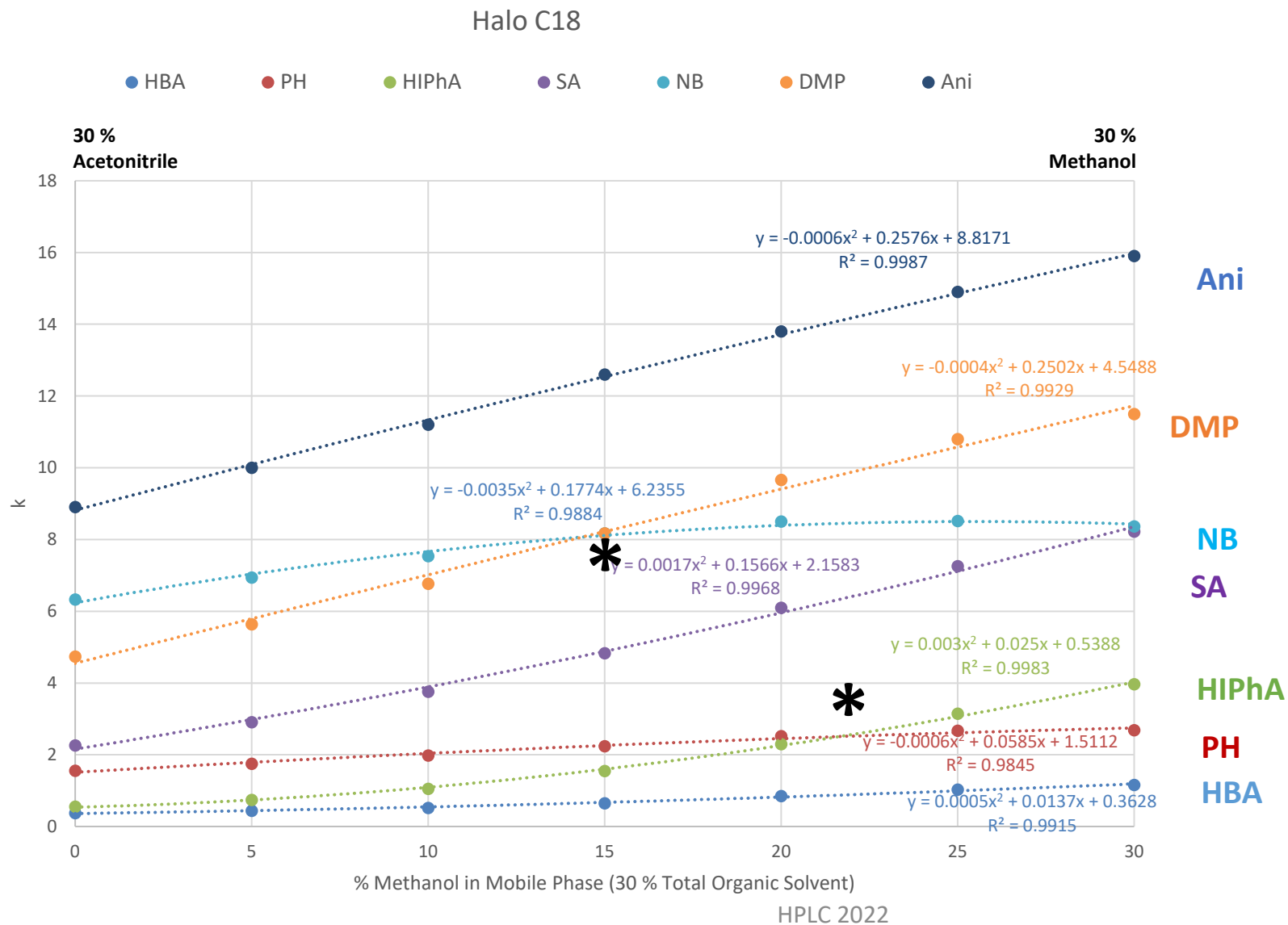
- Regular change in k from 30 % acetonitrile to 30 % methanol for this column
 - Highly linear
 - Suggests simple partition mode for this non-polar analyte



Question:
Will strong solute interactions (Dipoles and Hydrogen-bonding) create departure from linearity?



Retention for All Probes



- Phenol (PH) / HIPhA and Nitrobenzene (NB) / Dimethylphthalate (DMP) show selectivity changes. *



Understanding the Acetonitrile-Methanol System

- The change is regular but not linear.
 - Greatest curvature for SA, HlPhA, and NB
 - SA and HlPhA are carboxylic acids with internal and external H-bonding
 - The curvature is convex
 - NB has an electron-deficient aromatic ring and strong dipoles
 - The curvature is concave
 - Convex curvature is caused by accelerated retention as amount of methanol increases, suggesting stationary phase surface effects.
 - Concave curvature suggests preferential complexes in the mobile phase, probably between the aromatic ring and methanol.
- Across several different functional groups, the change can be predicted by a 2nd order polynomial.
 - Most r^2 values are greater than 0.99.



Predicting Retention in This System

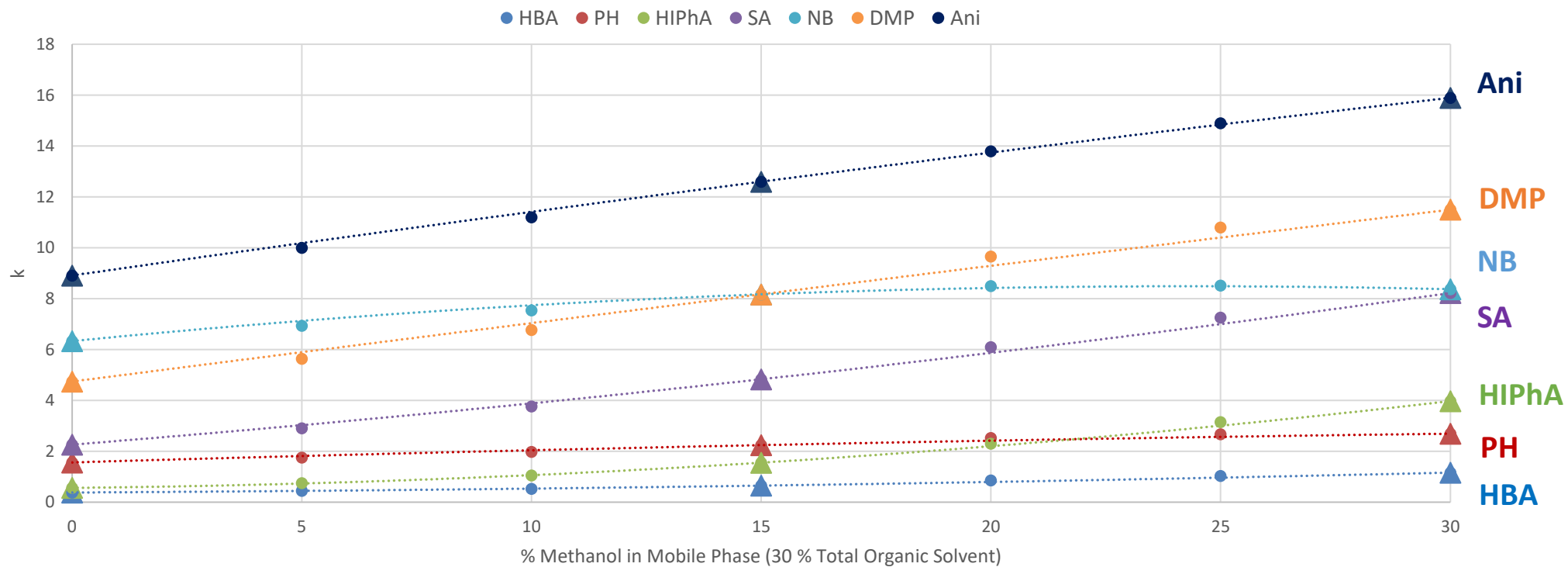
- A second-order polynomial produces an adequate fit to the full data set (seven points).
- Using this information, retention can be modelled using only three injections: the endpoints and one midpoint
 - Aq/ACN/MeOH (70/30/0)
 - Aq/ACN/MeOH (70/15/15)
 - Aq/ACN/MeOH (70/0/30)
- Use fitted equations to predict retention across the series
 - Only relatively simple spreadsheet features required



Overlay Curve Fits With Actual Data

- Triangles are (three) points used for modeling the retention.
 - Trend lines are from three points only.
- Circles are other data points (not used in modeling).
- The maximum deviation from the real value (in k units) is less than 5 %
 - In time units, the time difference is less than 5 sec.

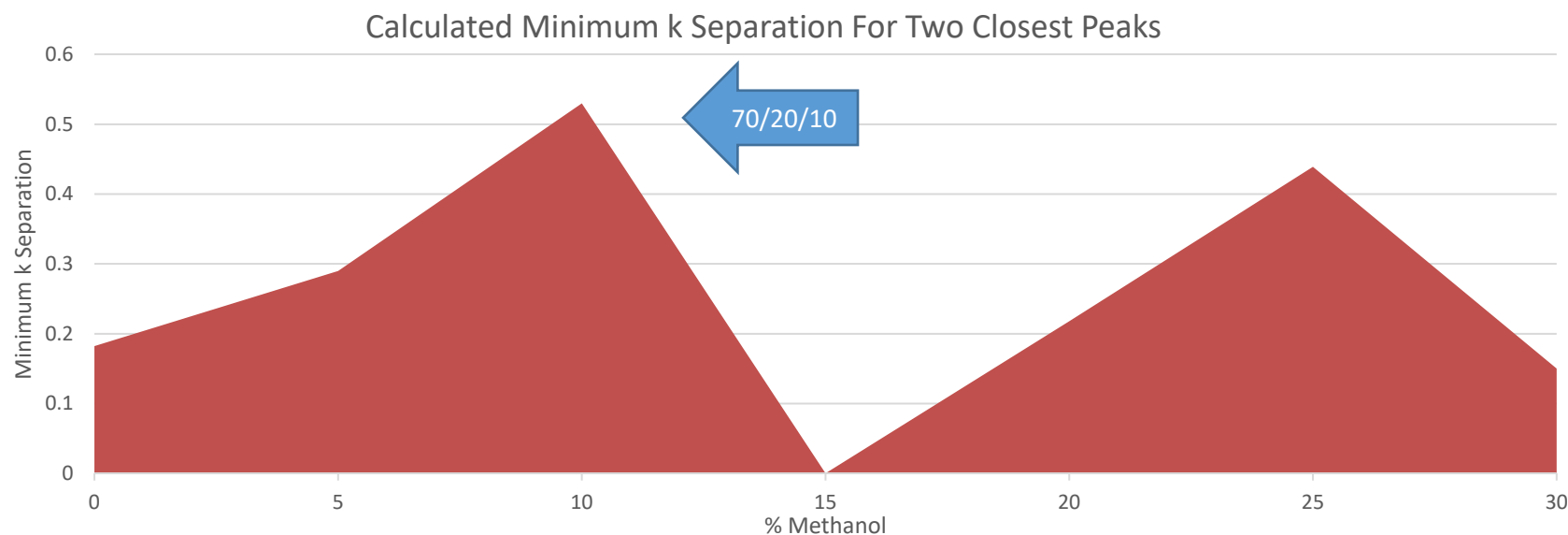
Curve Fit and Actual Data





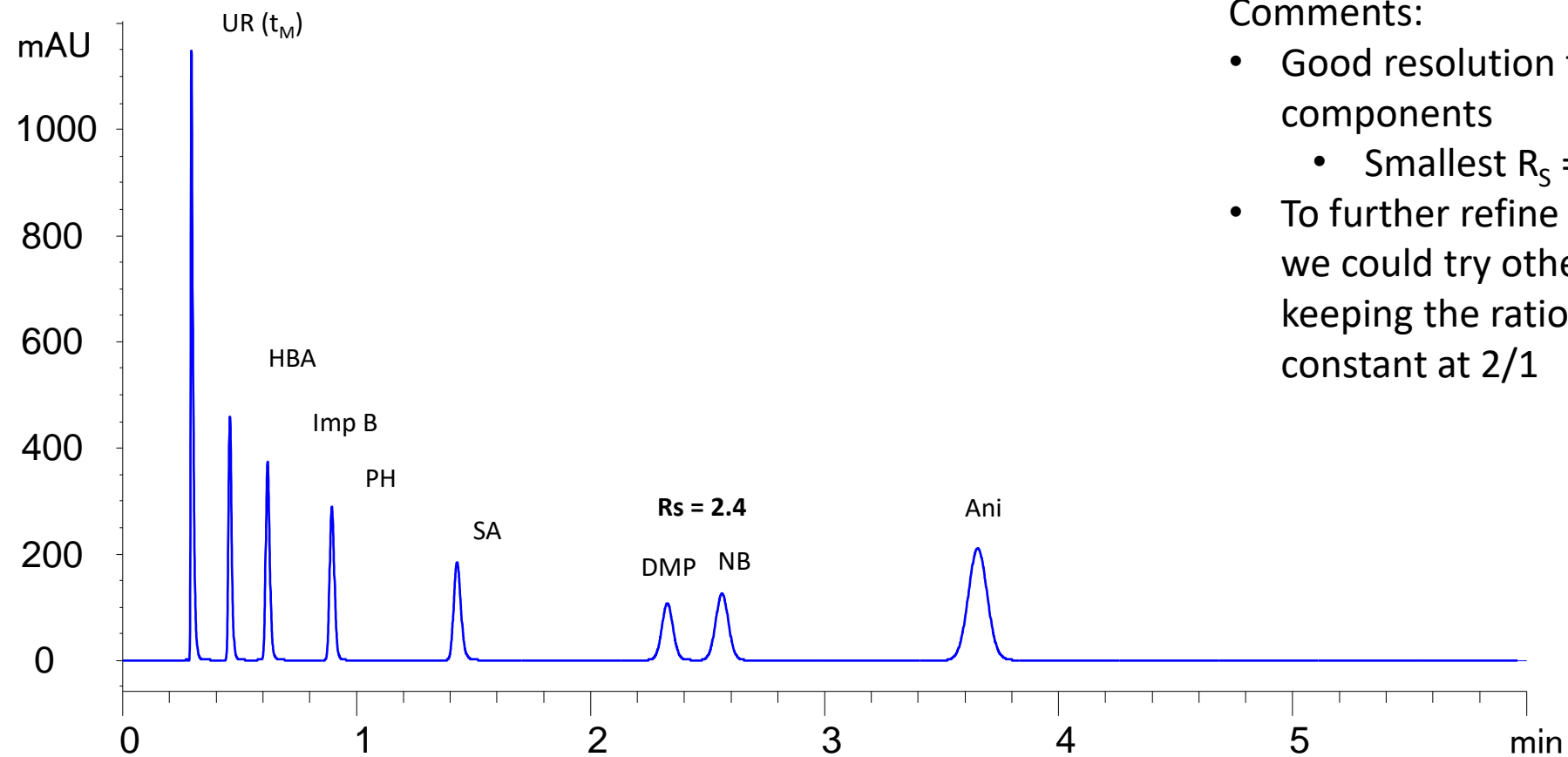
Finding the Best Separation

- Using the calculated k values, we can calculate the predicted separation, in k units, for the two closest peaks across the series.
- Find conditions that maximize that separation.
- The best value is near Aq/ACN/MeOH (70/20/10)



Predicted Optimum Conditions

- Results at Aq/ACN/MeOH (70/20/10)

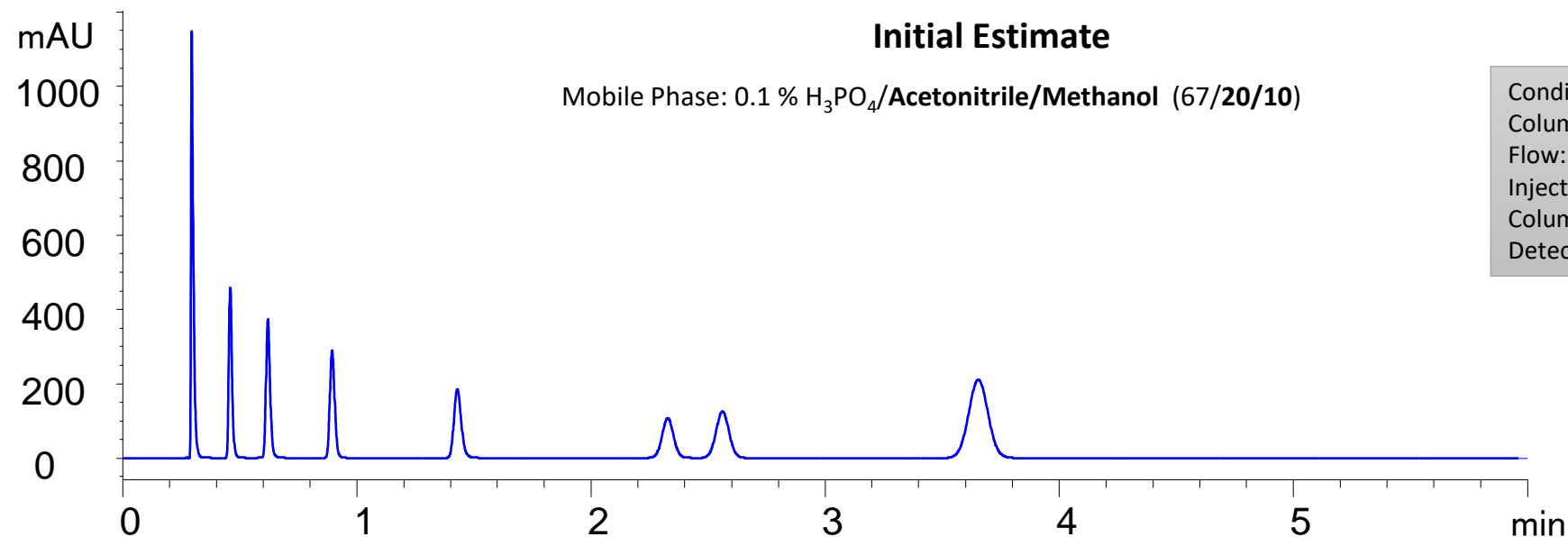


Comments:

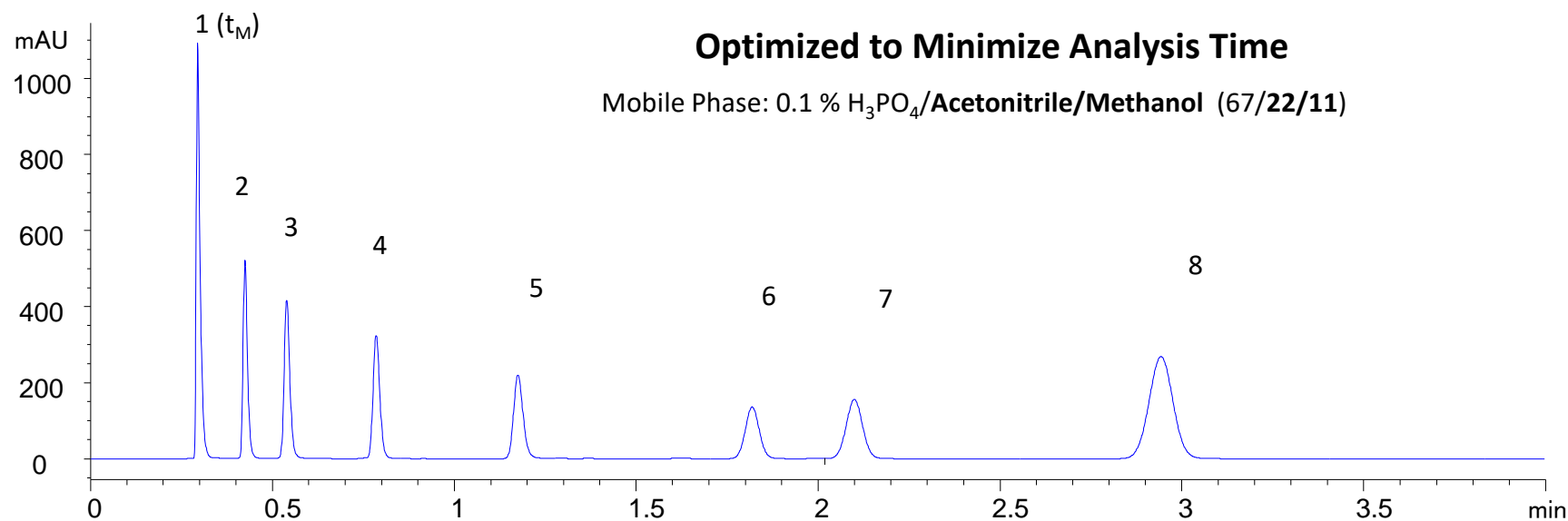
- Good resolution for all components
 - Smallest $R_s = 2.4$ (DMP/NB)
- To further refine this separation, we could try other combinations, keeping the ratio of ACN/MeOH constant at 2/1



Additional Optimization

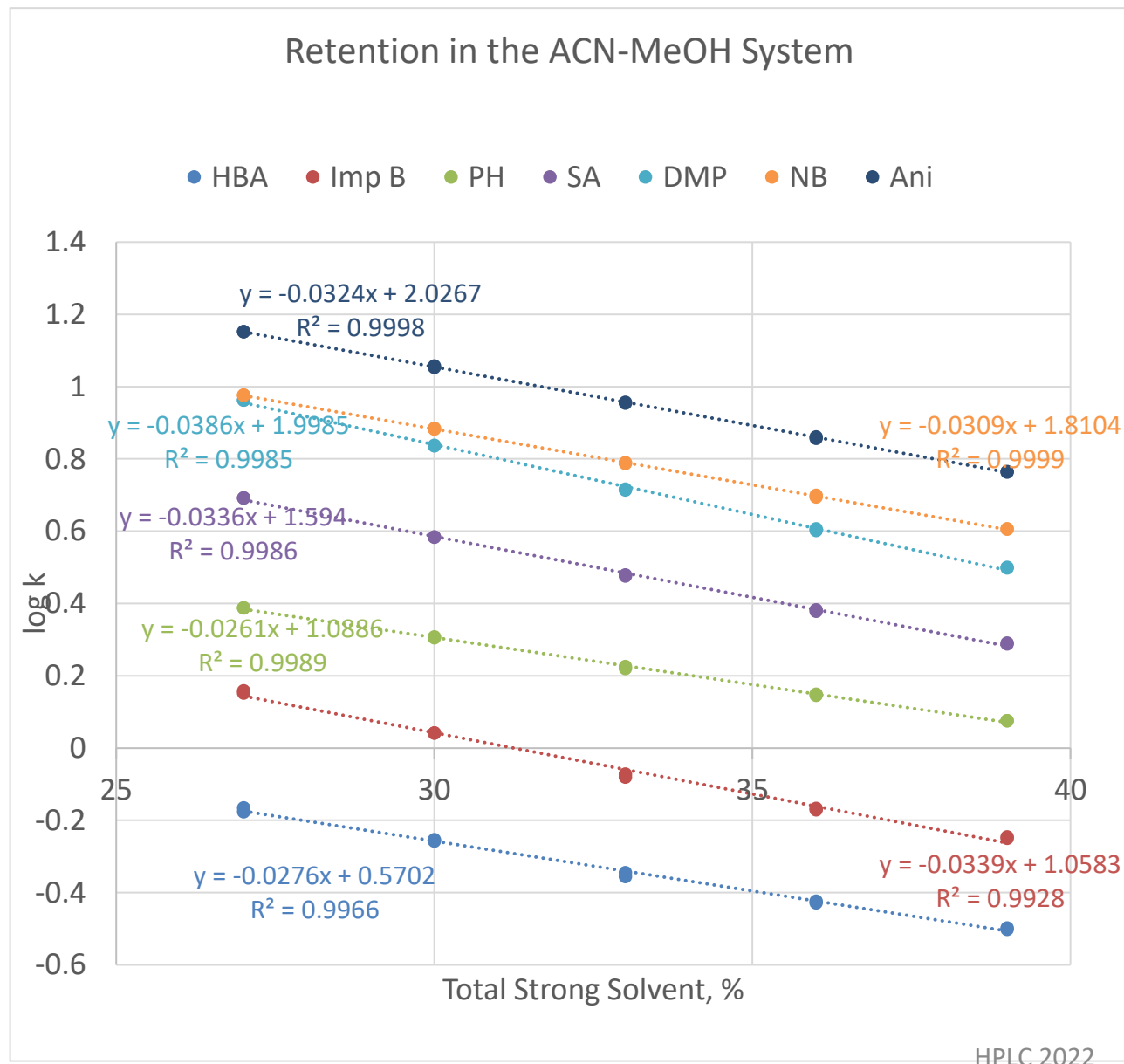


- 1 Uracil
- 2 4-Hydroxybenzoic Acid
- 3 4-Hydroxyisophthalic Acid
- 4 Phenol
- 5 Salicylic Acid
- 6 Dimethyl phthalate
- 7 Nitrobenzene
- 8 Anisole





LSS Retention Trends in Ternary Systems

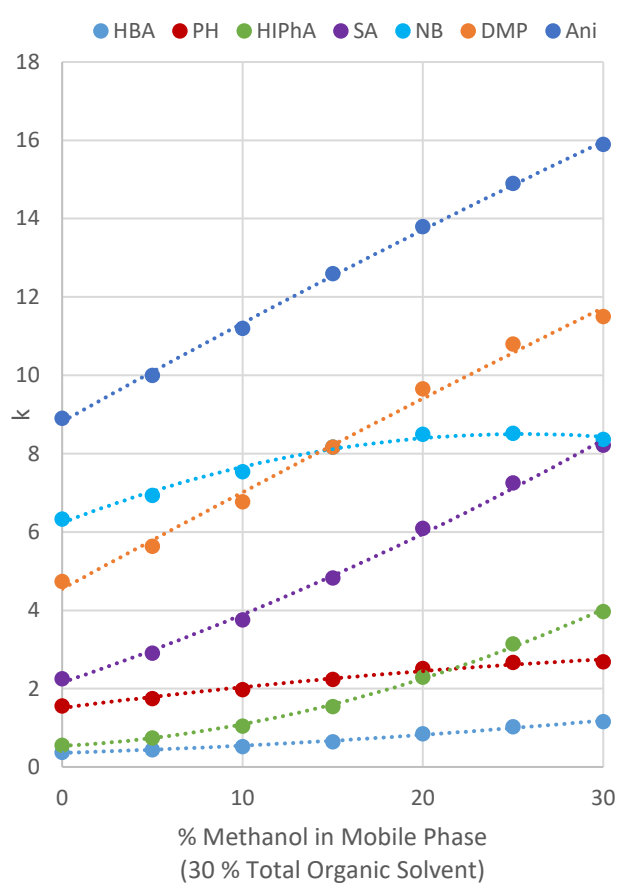


- Log k vs. % Total Solvent are linear for a short range.
 - LLS-type interactions are involved even in ternary systems.
- Note that the curves are not all parallel, suggesting that selectivity changes should be expected even in this ternary system.
 - Nitrobenzene (NB) demonstrates the largest slope deviation from the other analytes.

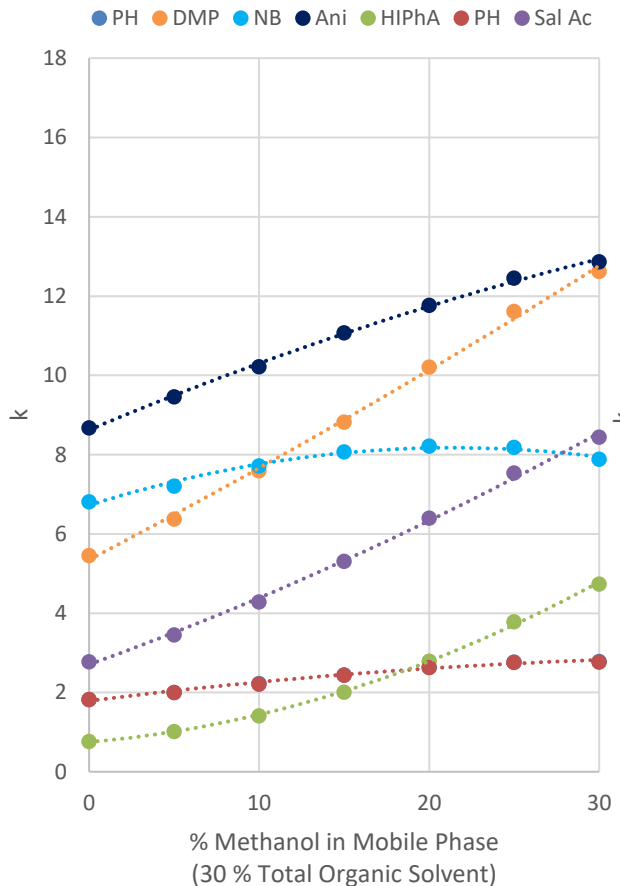


Are Same Trends Observed with Other C18 Columns?

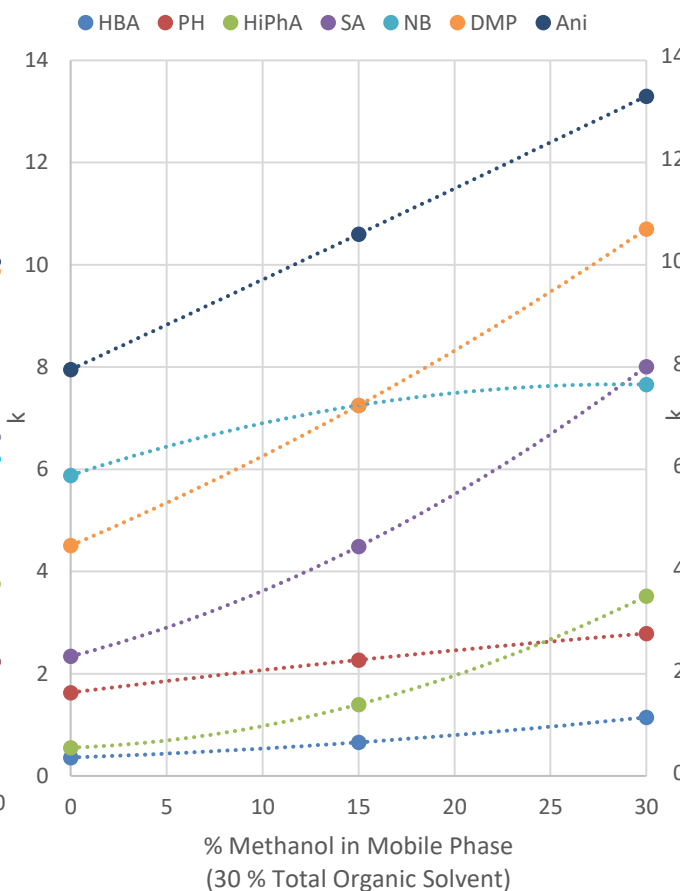
Halo C18 (AMT)



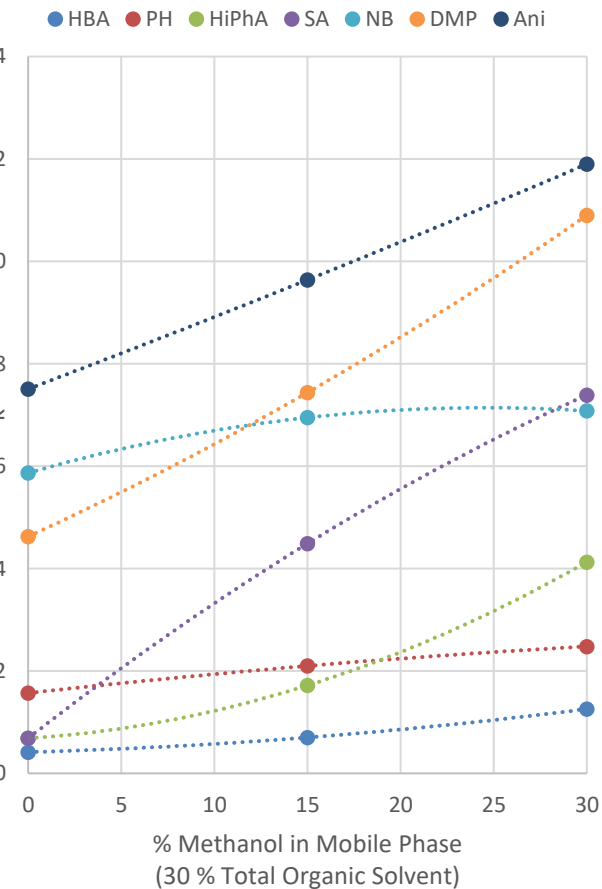
SB-C18 (Agilent)



XBridge C18 (Waters)



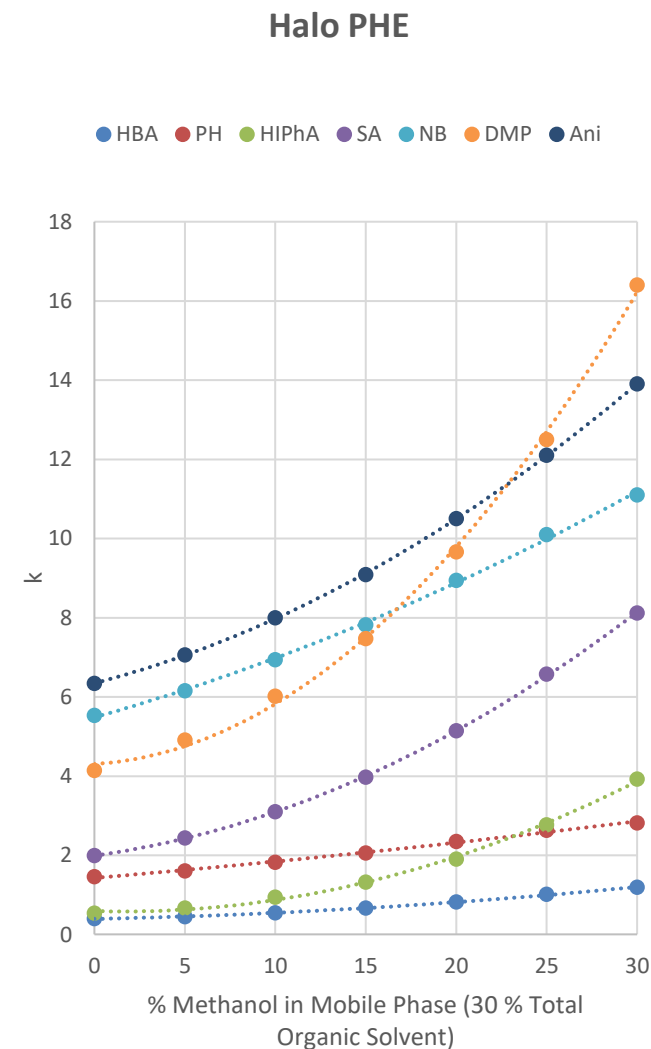
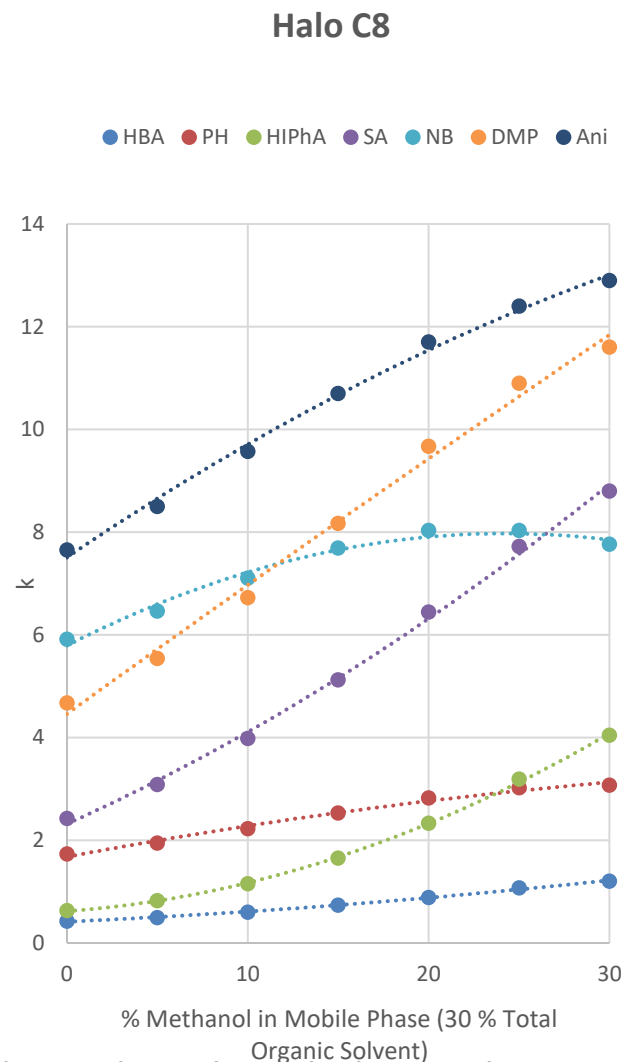
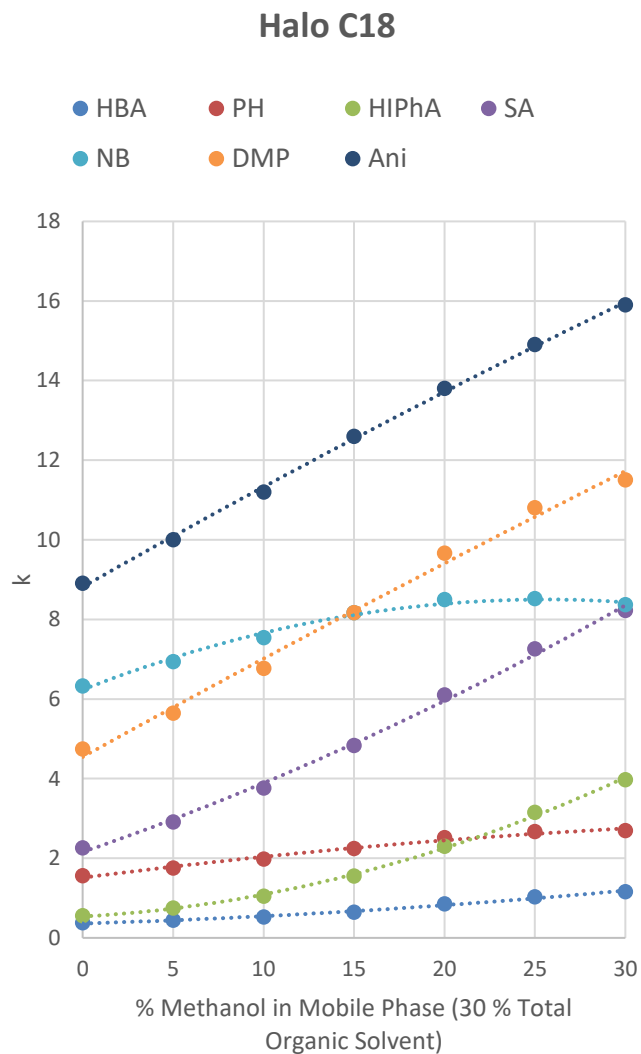
Kinetex XB-C18 (Phenomenex)



Generally, the trends look similar which means that mechanism is same and predictable for same phase chemistry. Subtle differences are noticed by observing the location of the crossover points.



Are Similar Trends Observed with Different Phases?



C18 and phenyl are very similar, as expected. With a phenyl phase, the interactions are more complex and the pattern changes. The same general trends are still present.

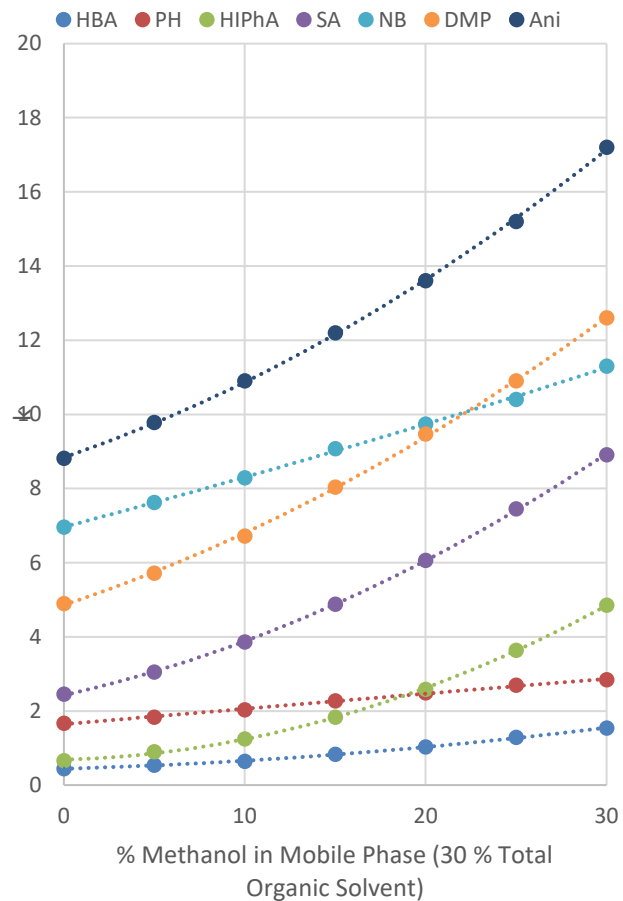


Other Phases

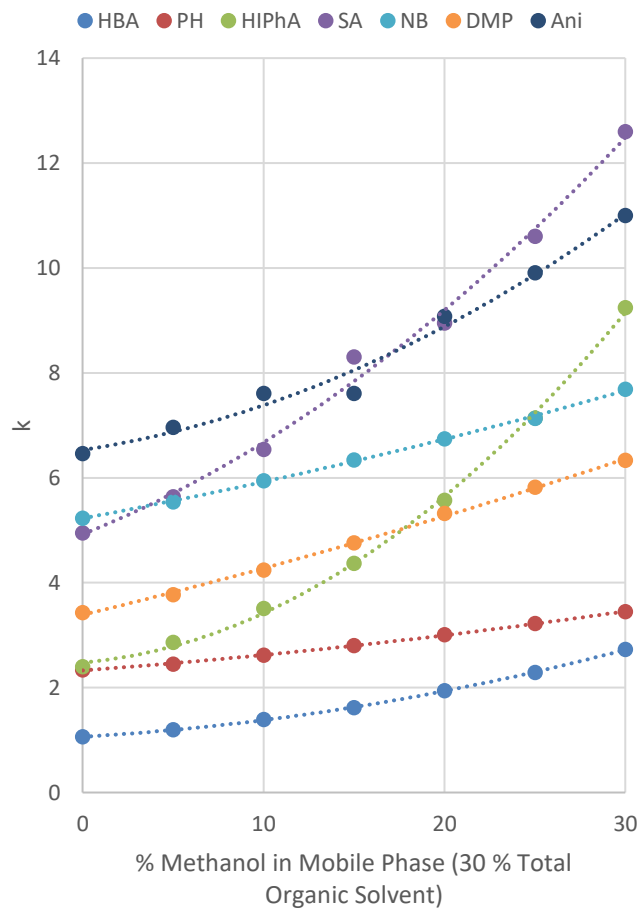
Trends are more complex and may be discontinuous. In general, this simple approach will probably not apply with more polar phases, or where the other non-partitioning mechanisms are present.

A little more scatter.

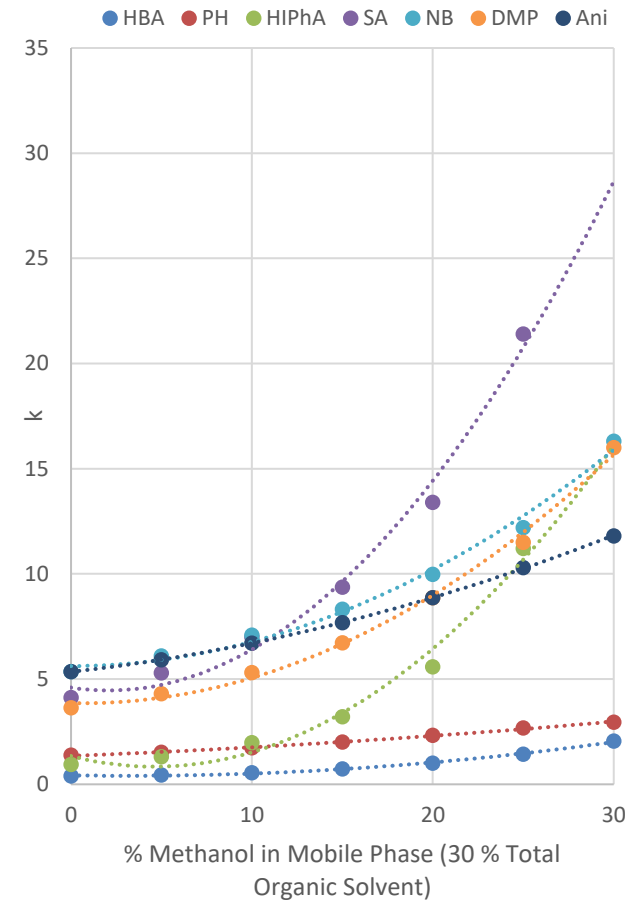
Halo AQ C18



Halo RP Amide



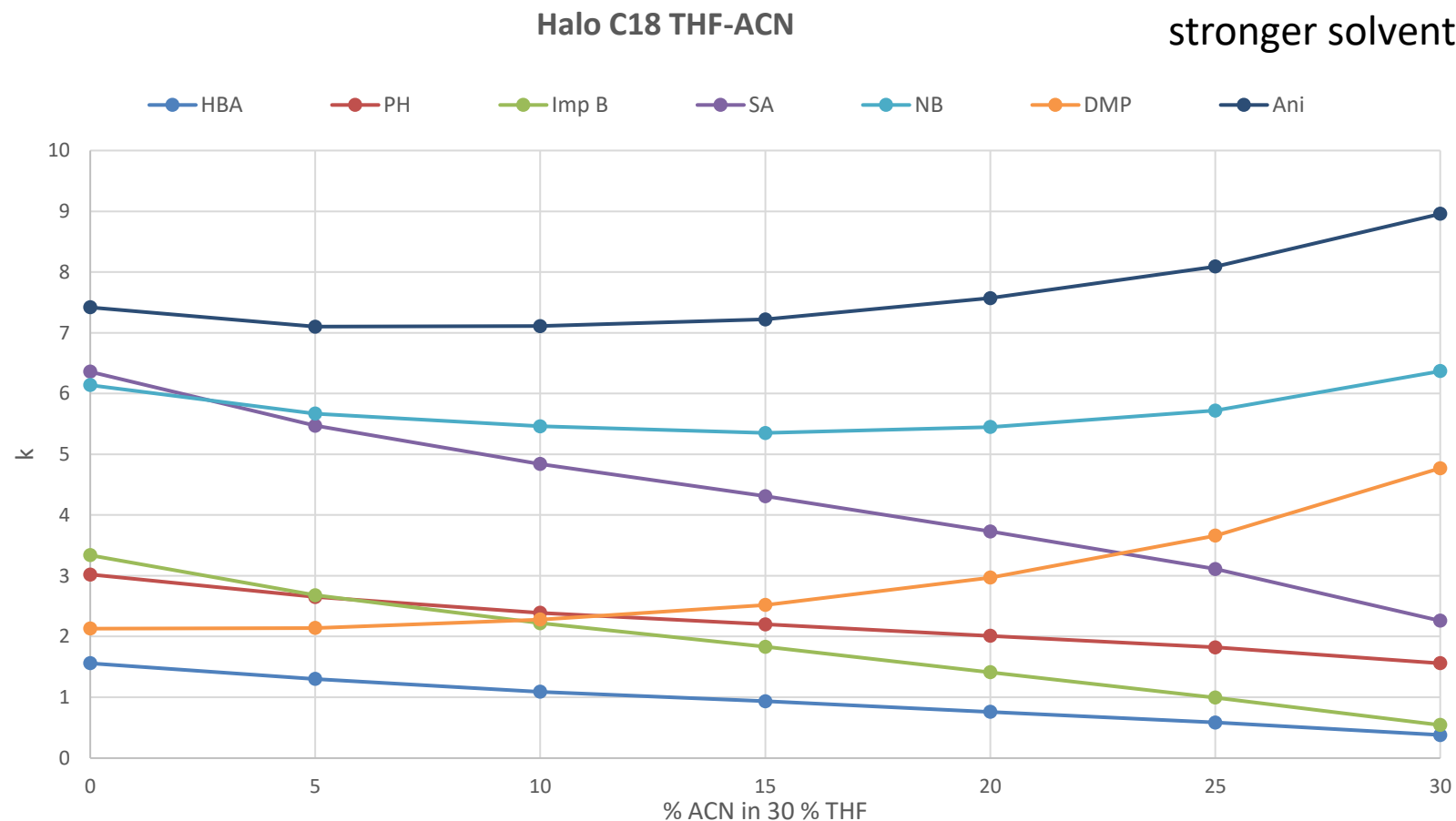
Halo PFP





C18: THF-ACN System

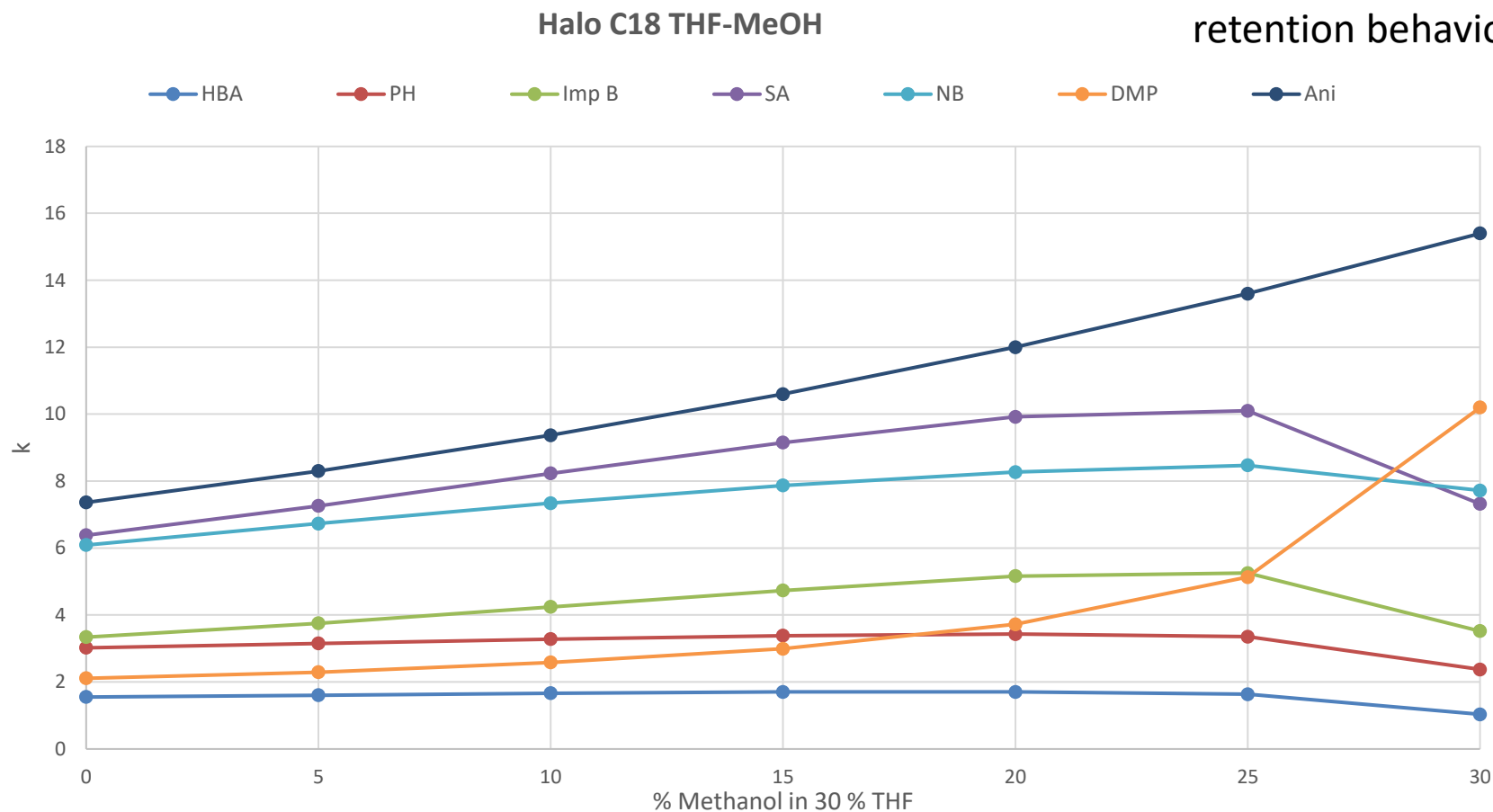
These are preliminary results for this system. The results are much more complex. Among other issues, it is clear that THF is not a universally stronger solvent than ACN.





C18: THF-MeOH System

These are preliminary results for this system. The data appear to be discontinuous; the presence of THF causes a sudden change in retention behavior.





Conclusion

- While binary mobile phases offer useful selectivity changes for simple systems, ternary systems offer important tools for complex systems because binary selectivity changes are often insufficient.
- Aqueous/Acetonitrile/Methanol systems show a regular change in retention when the total organic content is held constant, and the relative amounts of the organic solvents are changed.
- This change can be modelled using a second-order polynomial fit from only three injections.
- Selectivity differences result in elution order changes for some analytes.
 - These changes are predicted by the polynomial fit and can be used to predict the best separation.



Impact of this work/Outlook

- A ternary mobile phase system in reversed-phase methods produces more complex separation behavior for analytes with dipoles and Hydrogen-bonding functional groups.
- The behavior can be approximated for C18 and C8 columns using only three injections and a simple curve fitting technique.
- Additional Work
 - Study/extend this approach to more functional groups, especially basic pharmaceuticals
 - Study ternary gradients



Acknowledgements



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References

- (1) L. R. Snyder, J. J. Kirkland, J. L. Glajch, Practical HPLC Method Development, Wiley, 1997, pg 237.
- (2) L. R. Snyder, J. W. Dolan, High-Performance Gradient Elution, Wiley, 2007, pg 13.
- (3) Linear-Solvent-Strength Model, Advanced Materials Technology (<https://halocolumns.com/wp-content/uploads/2022/05/AMT-UTH-Linear-Solvent-Strength-Model.pdf>)
- (4) L. R. Snyder, J. J. Kirkland, J. L. Glajch, Practical HPLC Method Development, Wiley, 1997, pg 258.
- (5) J. L. Glajch, J. J. Kirkland, K. M. Squire, J. M. Minor, *J. Chromatogr.*, **199** (1980) 57.

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