# A Comparative Study of the Thermally Tuned Tandem Column ($T^3C$) Concept and Other Two-Variable Optimization Approaches in RPLC

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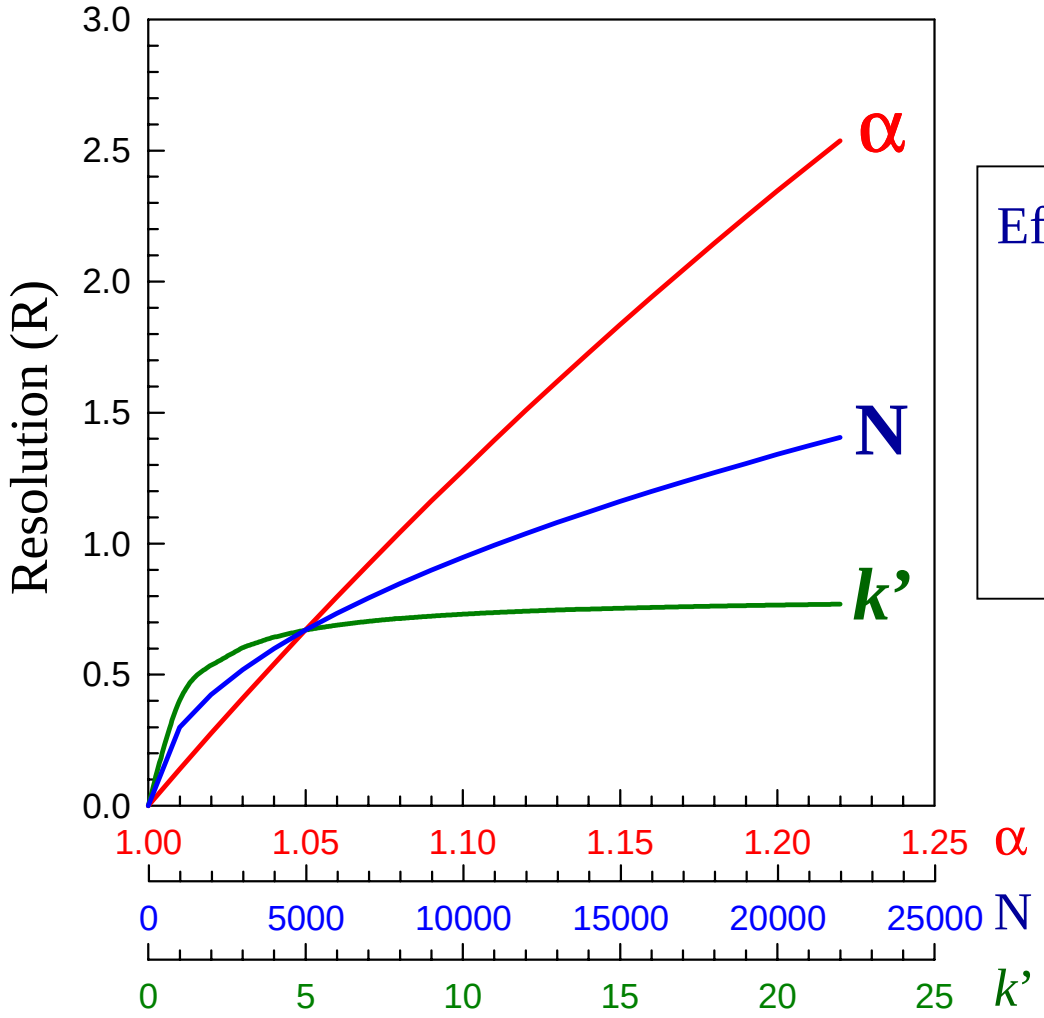
# Outline

- Importance of Selectivity in HPLC Optimization
- Thermally Tuned Tandem Column ($T^3C$) Concept
- An Example – Ten Triazine Herbicides
  - ✓Comparing with Varying Eluent Compositon($\phi$)
  - ✓Comparing with Simultaneous Varying $\phi$ and T.
- Applications
  - ✓Urea and Carbamate Pesticides
  - ✓ Barbiturates
  - ✓ Antihistamine Drugs
- Conclusions
  - ✓ $T^3C$ Works
  - ✓ It Can Save Time or Do Difficult Separations
  - ✓ Only Four or Five Initial Runs Are Needed

# The Ultimate Goal of Separation: Resolution (R)



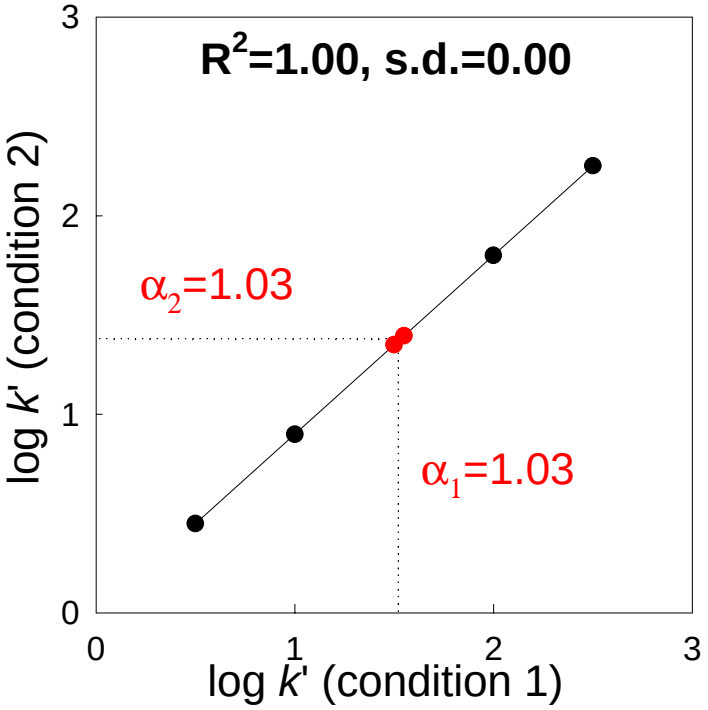

Efficiency Selectivity Retention

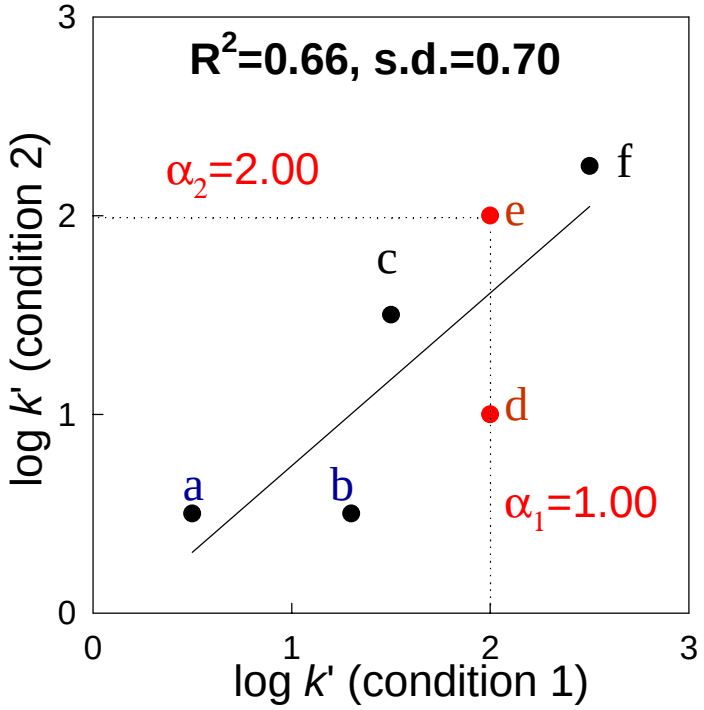
$$R = \frac{\sqrt{N}}{4} \quad \frac{\alpha - 1}{\alpha} \quad \frac{k'}{k'+1}$$

- **Small changes in selectivity (α) have the greatest impact on resolution.**

# Ways to Alter Selectivity

1. Mobile Phase Composition (%B).
2. Mobile Phase Type (MeOH vs. THF).
3. Temperature.
4. Stationary Phase Type.

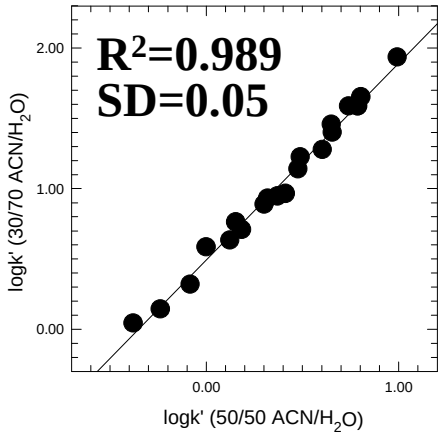


# Comparison of Variables Affecting Selectivity

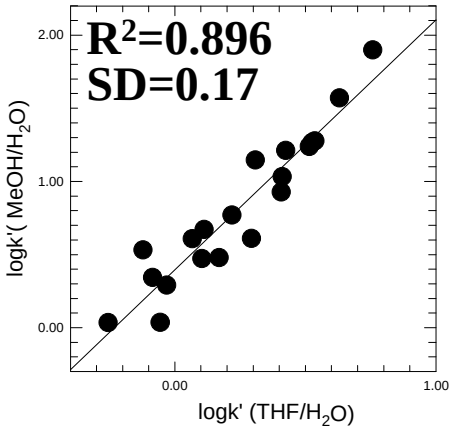
## *1. Mobile Phase Composition*

30% ACN vs. 50% ACN



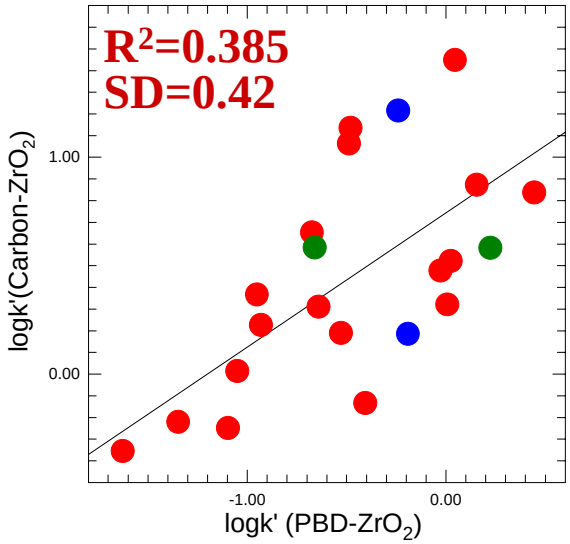

## *2. Mobile Phase Type*

MeOH vs. THF



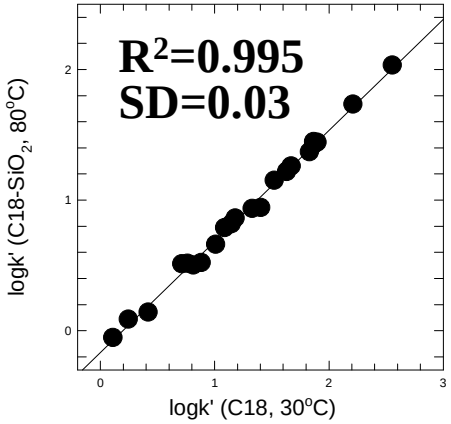

## ***Stationary Phase Type***

Carbon-$ZrO_2$ vs.
PBD-$ZrO_2$



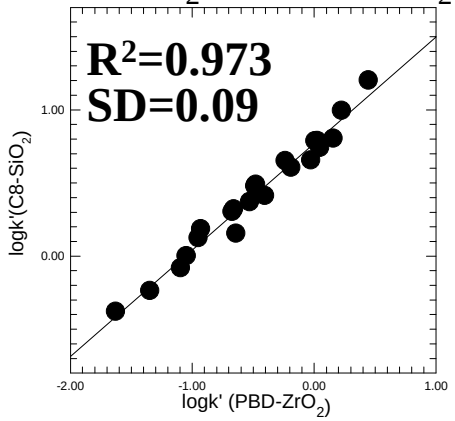

## *3. Temperature*

80°C vs. 30°C




## *4. Stationary Phase Type*

C8-$SiO_2$ vs. PBD-$ZrO_2$

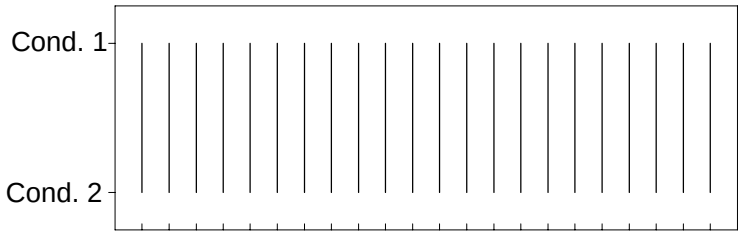



❖ **Stationary phase type can have a very large effect on selectivity.**

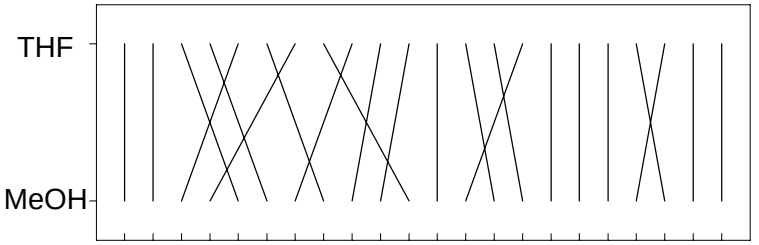
# Comparison of Elution Sequences

***Elution sequence comparison for 22 key solutes under two chromatographic conditions***

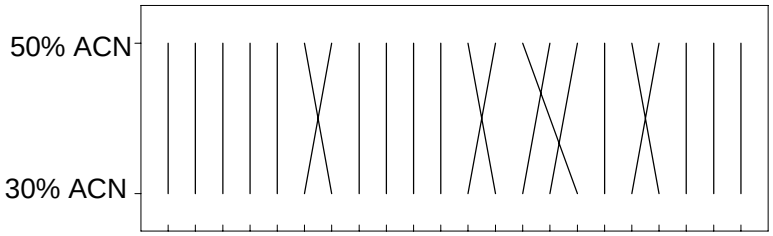
## Same Elution Sequence
## Similar Selectivity



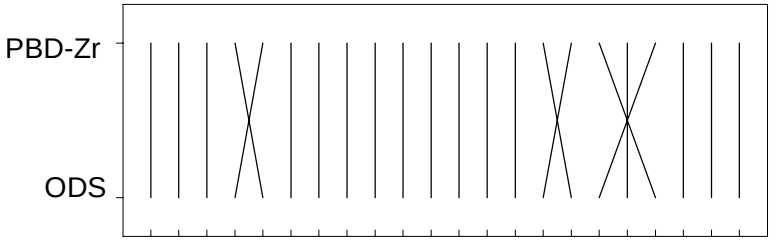

## Mobile Phase Type



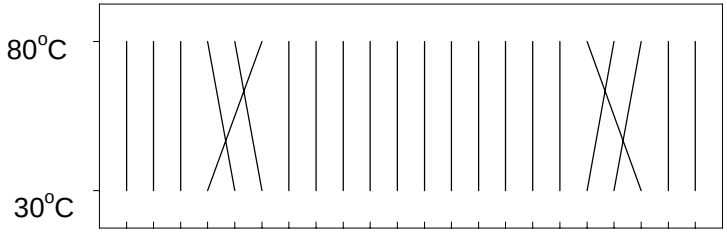

## Mobile Phase Composition



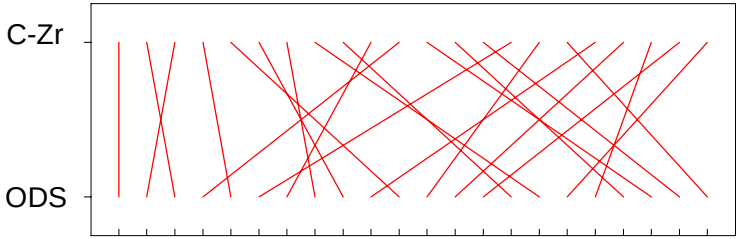

## Stationary Phase Type
## PBD-$ZrO_2$ vs. ODS



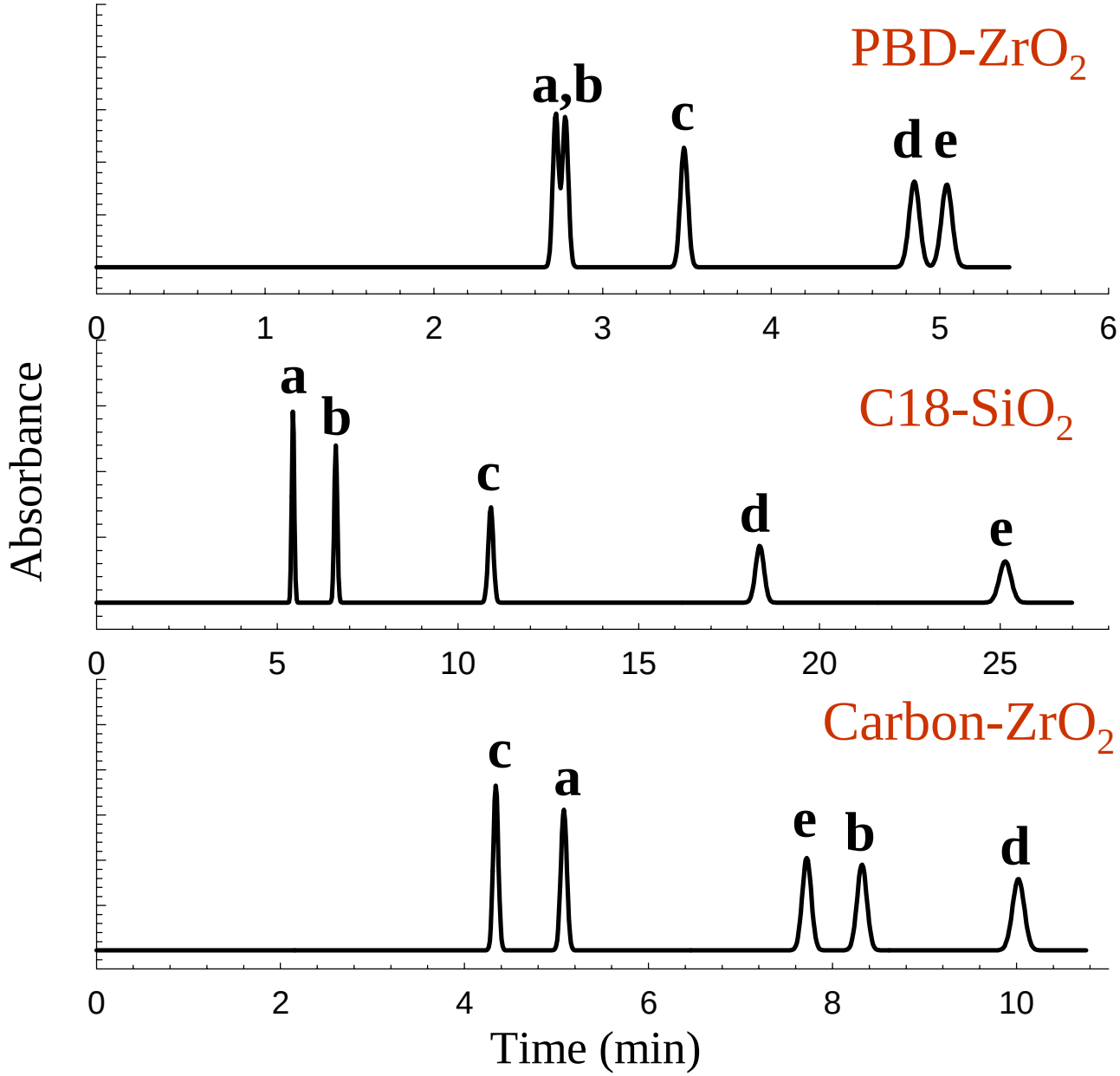

## Temperature




## Stationary Phase Type
## C-$ZrO_2$ vs. ODS



# Effect of Column Type on Simple Analytes




Solutes:

a. 3-phenylpropanol
b. acetophenone
c. benzene
d. bromobenzene
e. ethylbenzene

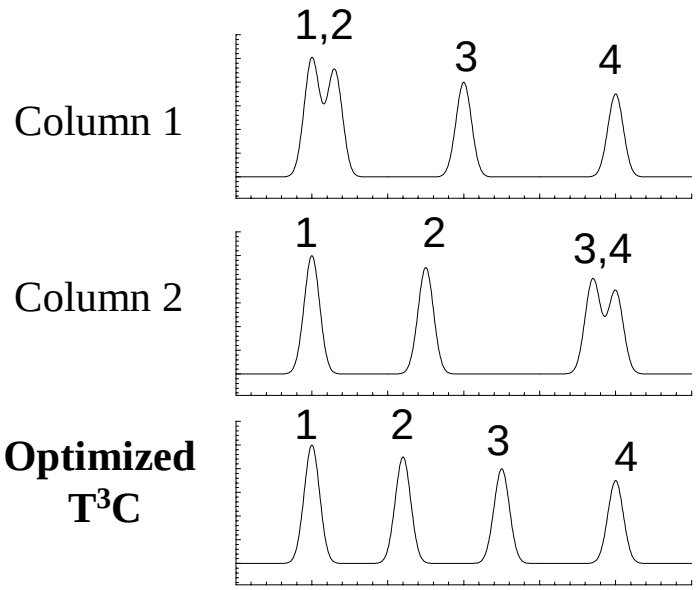
Temperature: 30 °C

Mobile phase:
50/50 acetonitrile/water

Stationary phase:
25cm x 4.6 mm i.d.

# The Concept: Thermally Tuned Tandem Columns ($T^3C$)

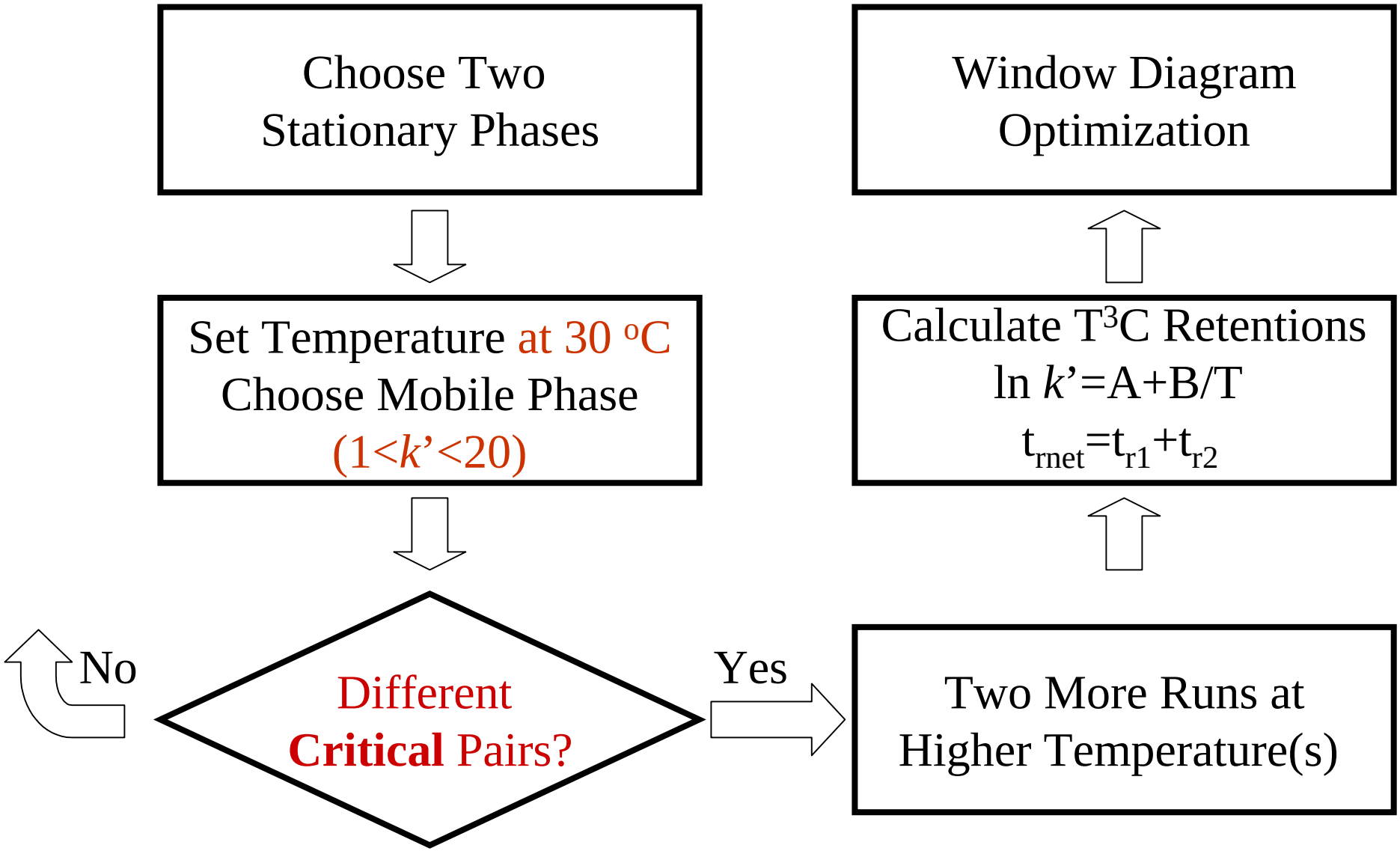
## *A Mechanism to Continuously Adjust the Stationary Phase*

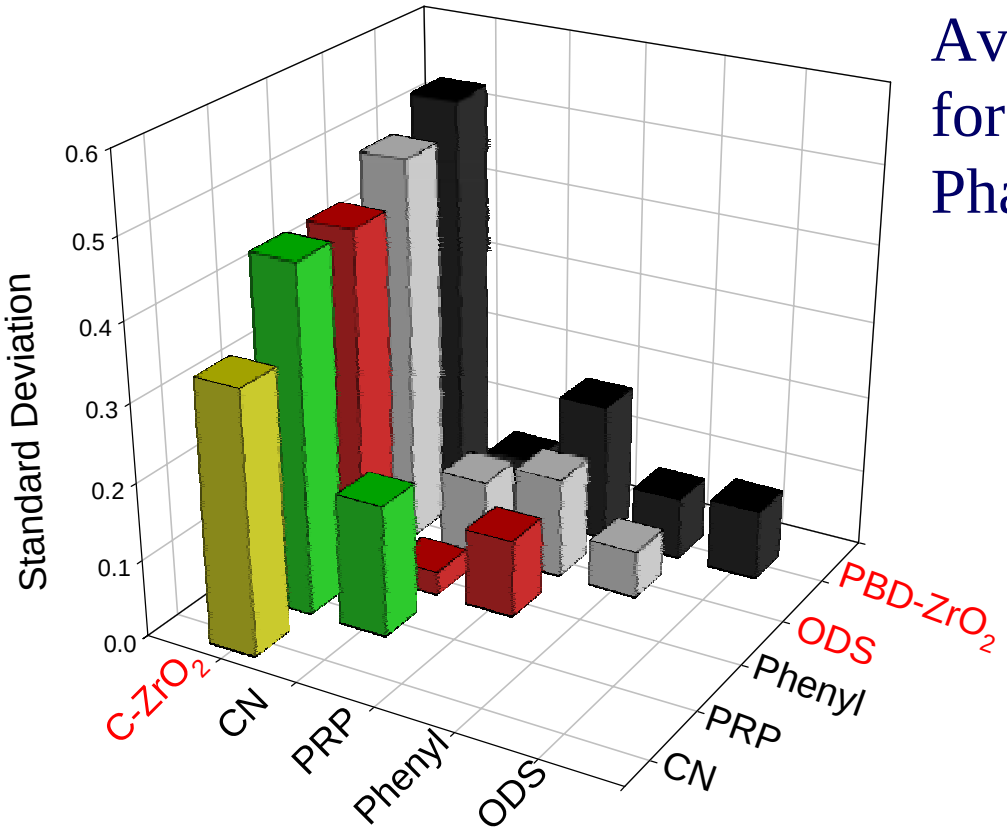

Requirements for $T^3C$:

1. Two columns with different (ideally orthogonal) selectivity.
2. One thermally stable column.
3. **Method development must be easy.**

# Guidelines for Optimizing $T^3C$



# Stationary Phases With Different Selectivity

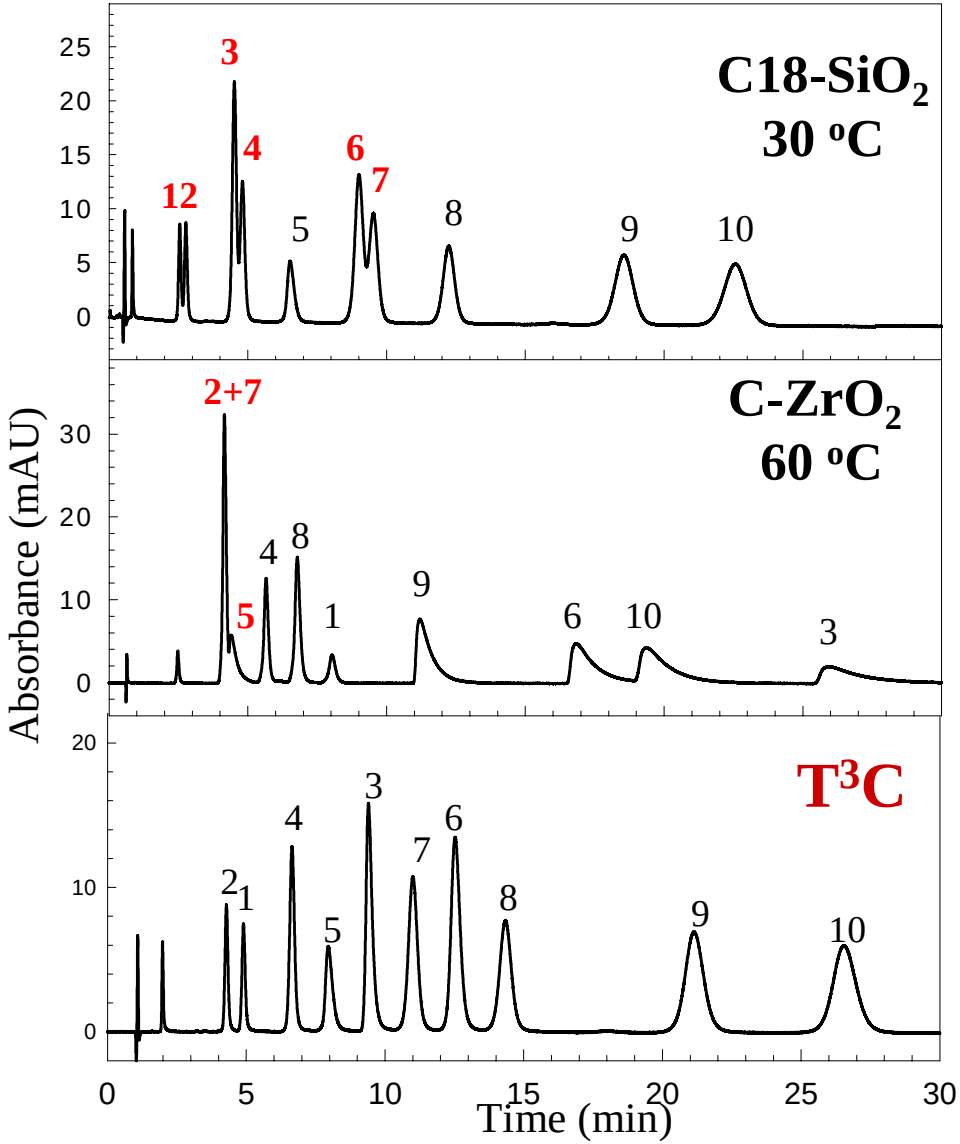


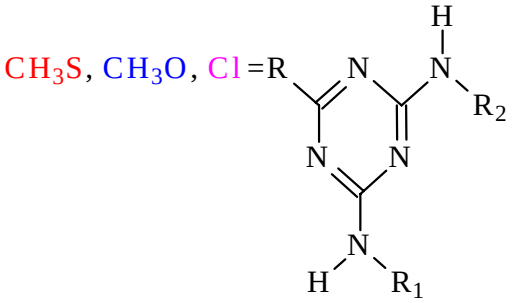

Average Scatter of the κ–κ Plots for Two Types of Stationary Phases Using 22 Solutes

- Carbon-$ZrO_2$
- PBD-$ZrO_2$
- C18-$SiO_2$ (ODS)
- Phenyl-$SiO_2$
- CN-$SiO_2$
- PRP

❖ **For *non-electrolytes*, C-$ZrO_2$ and aliphatic phases have the most different selectivities.**

# Separation of Ten Triazine Herbicides by T$^3$C



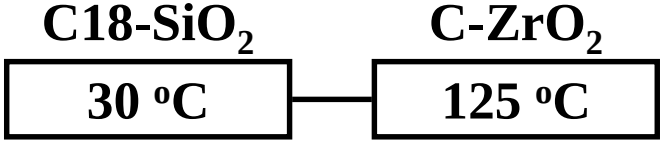

Solutes:

1. Simazine
2. Cyanazine
3. Simetryn
4. Atrazine
5. Prometon
6. Ametryn
7. Propazine
8. Terbutylazine
9. Prometryn
10. Terbutryn

Other conditions:

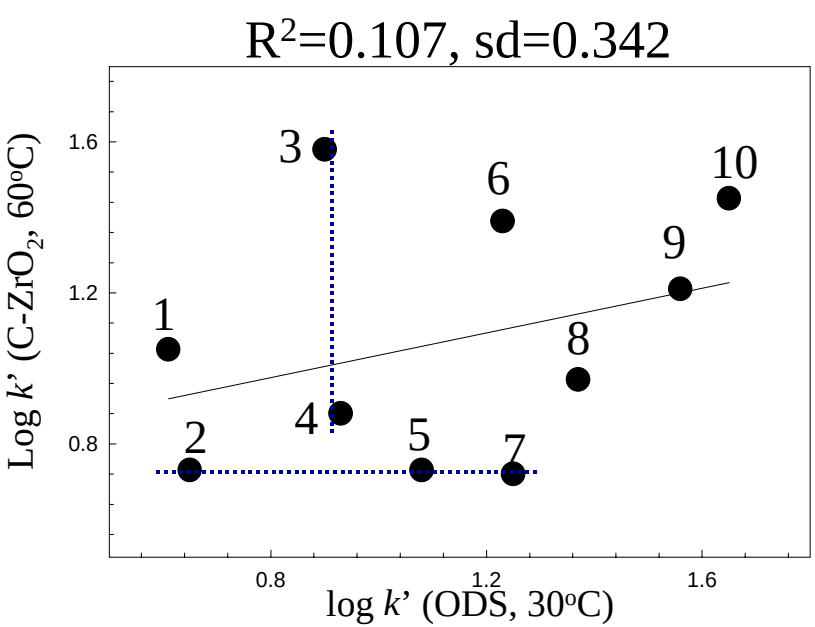
30/70 ACN/water

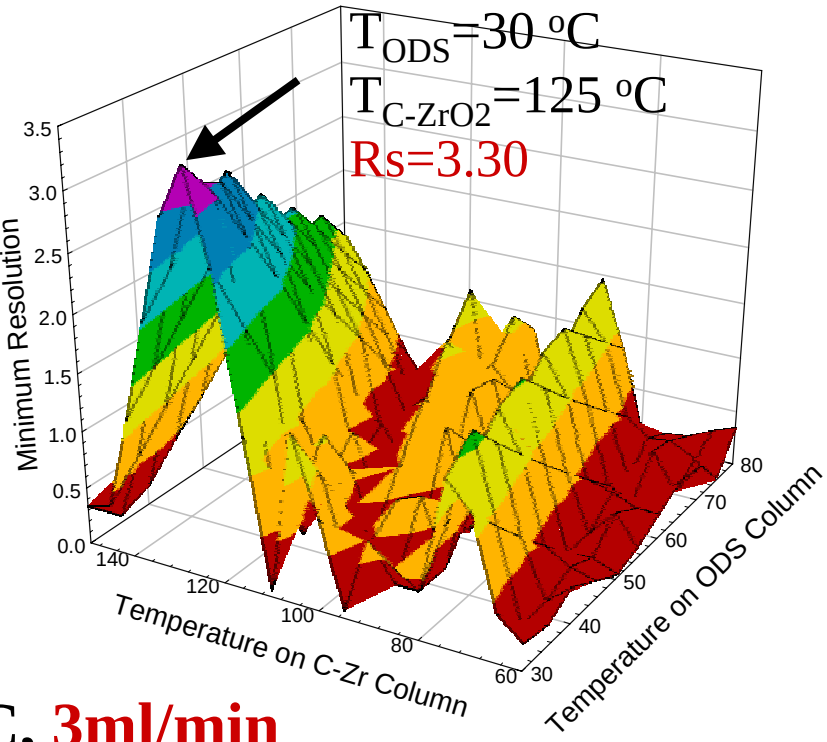
1ml/min; 254 nm detection

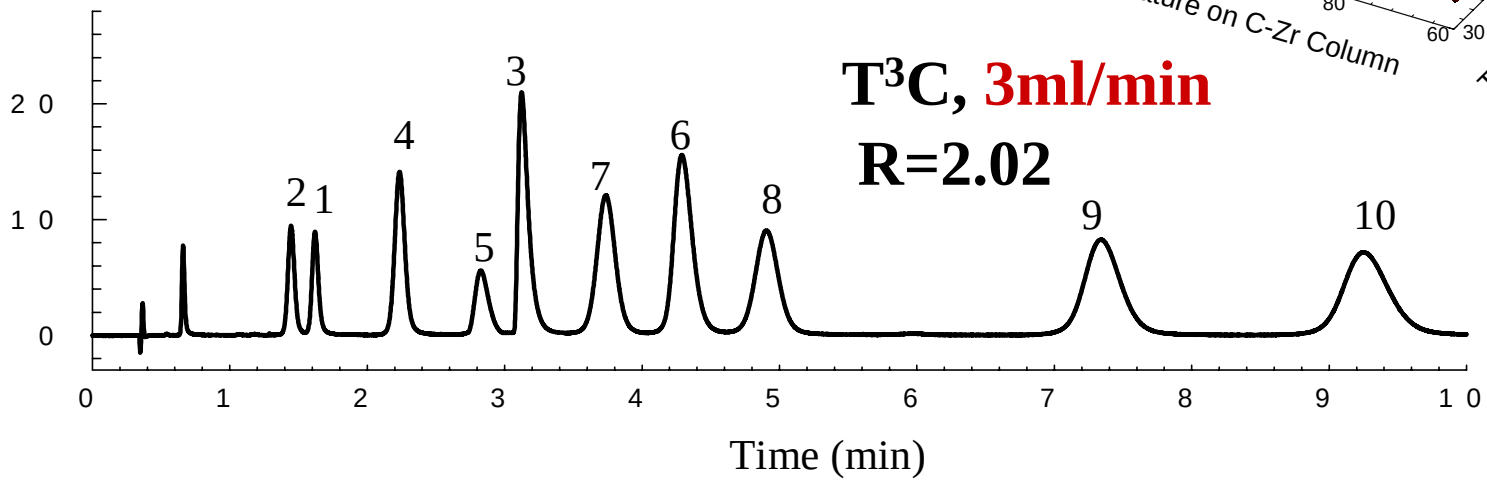



❖ **T$^3$C can improve separation without increasing analysis time.**

# Steps in T³C Optimization of Triazine Herbicides



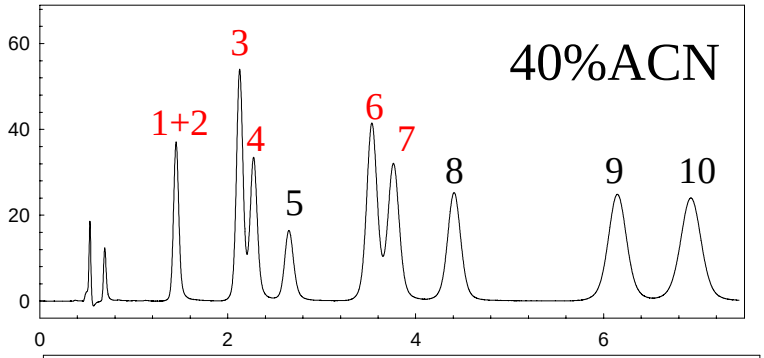



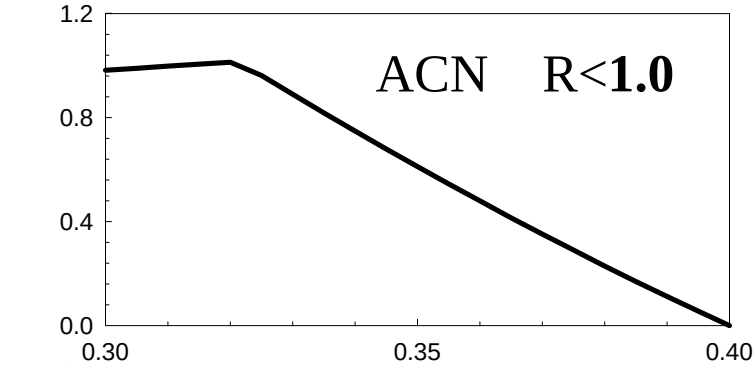




❖ **T³C allows fast analysis due to high resolution.**

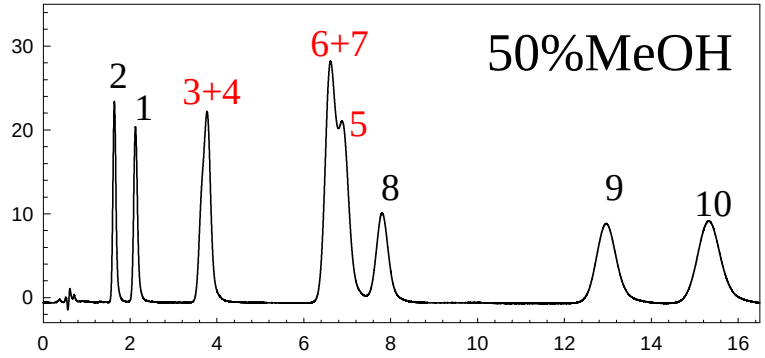
# Compare T³C with Mobile Phase Optimization

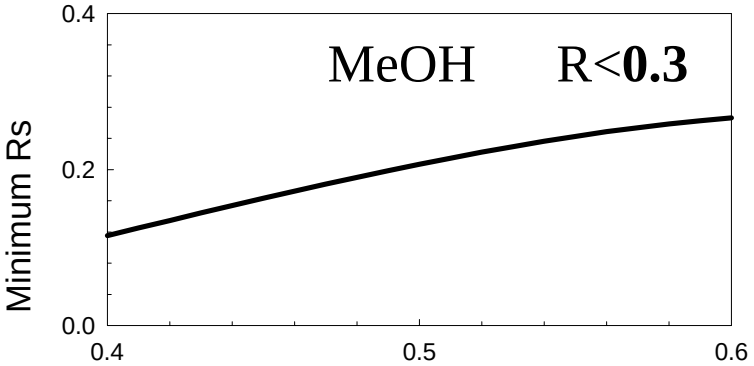
**ACN**



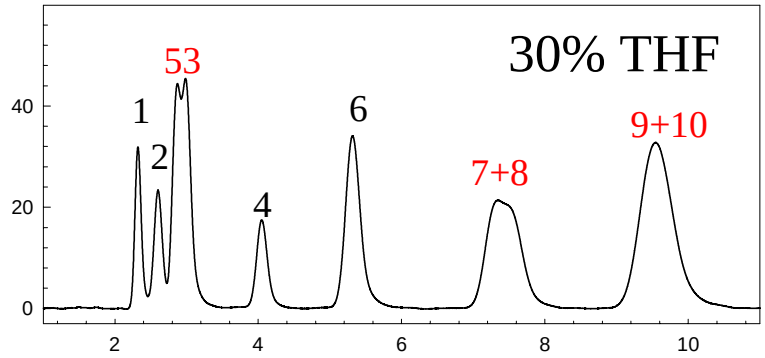



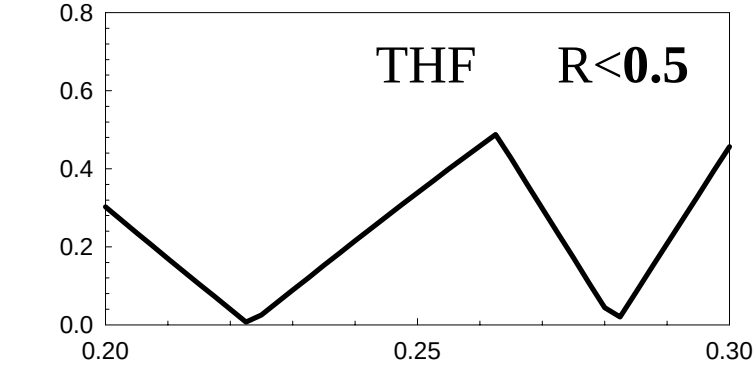

**MeOH**




**THF**




Time (min)

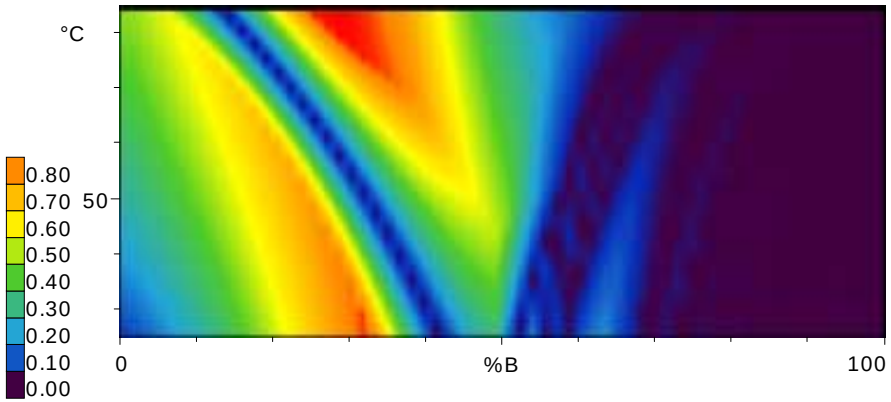
Minimum Rs

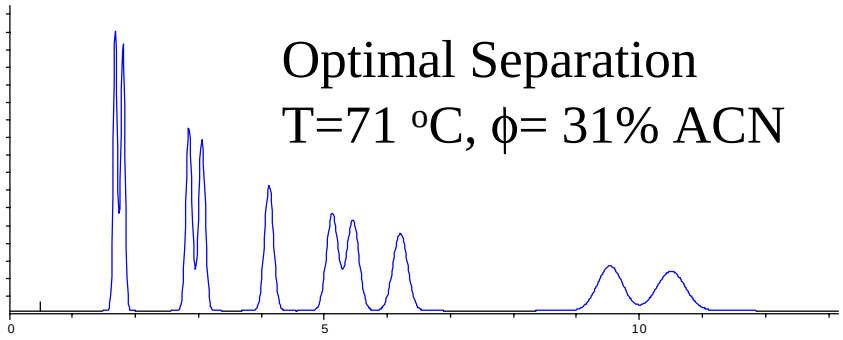
Percentage of organic modifier

❖ **T³C is more powerful than mobile phase optimization on ODS.**

# Compare $T^3C$ with Simultaneous Varying Both Eluent Composition and Temperature

**ODS** (30 °C 30% ACN; 80 °C 40% ACN)

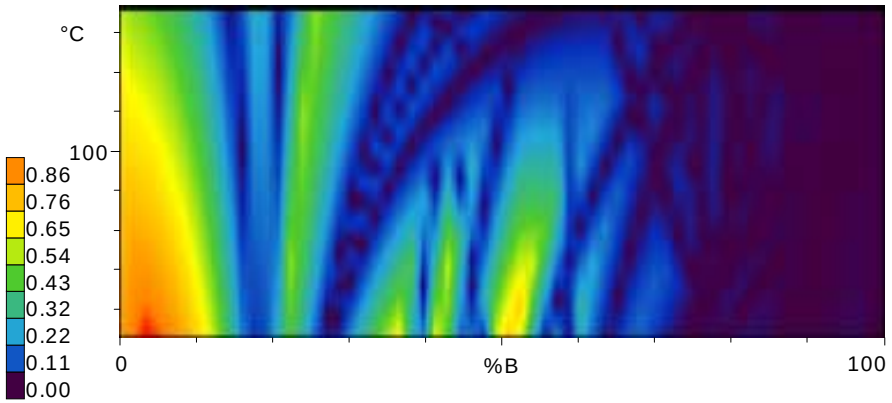


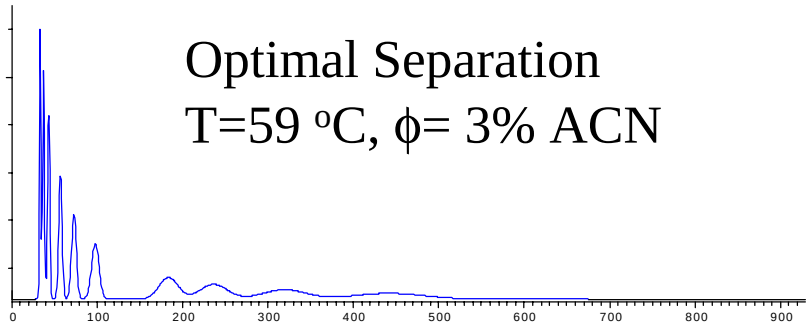




Optimal Separation
T=71 °C, ϕ= 31% ACN

$R_s$=0.86
Analysis time=12 min

**$C\text{-}ZrO_2$** (60 °C 30% ACN; 130 °C 40% ACN)



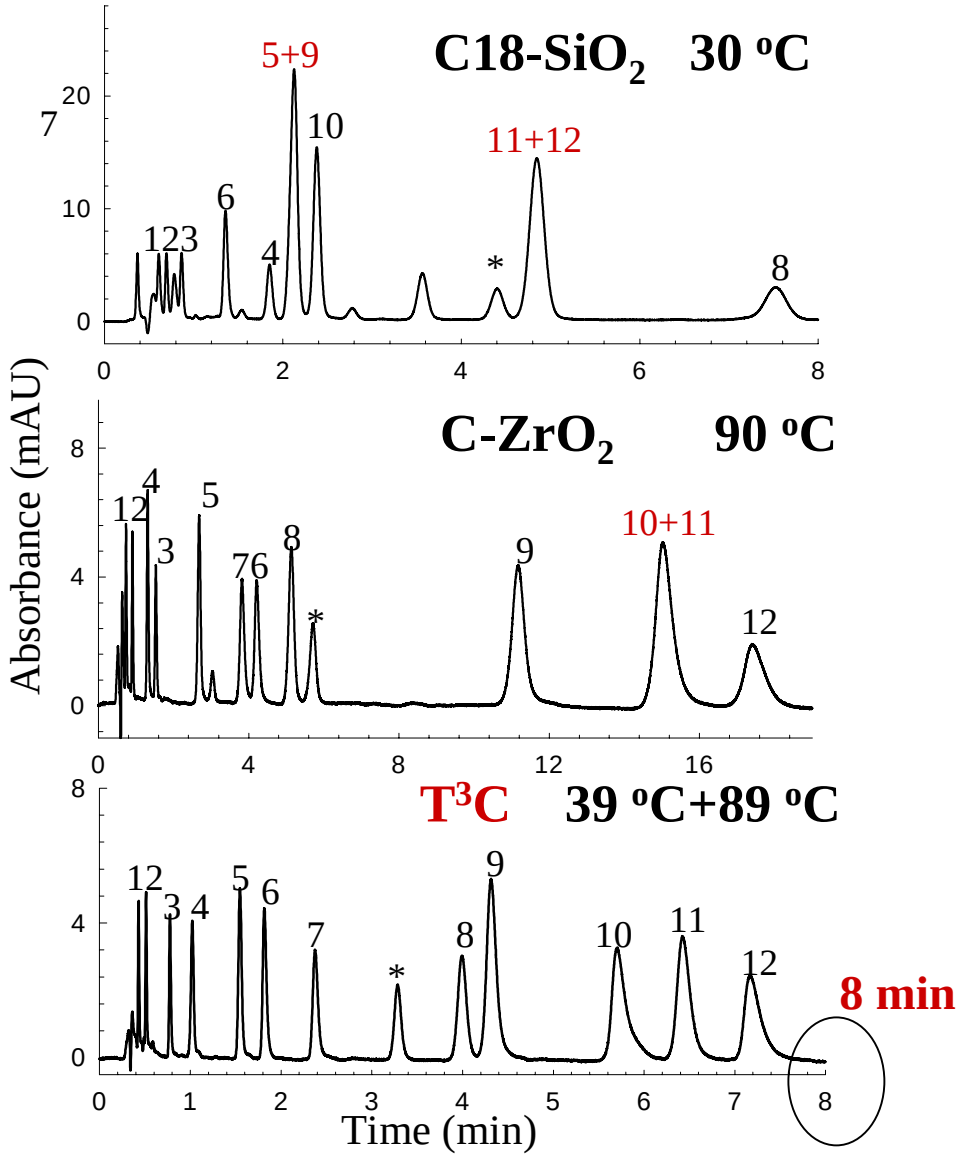

Optimal Separation
T=59 °C, ϕ= 3% ACN

$R_s$=0.93
Analysis time=600 min

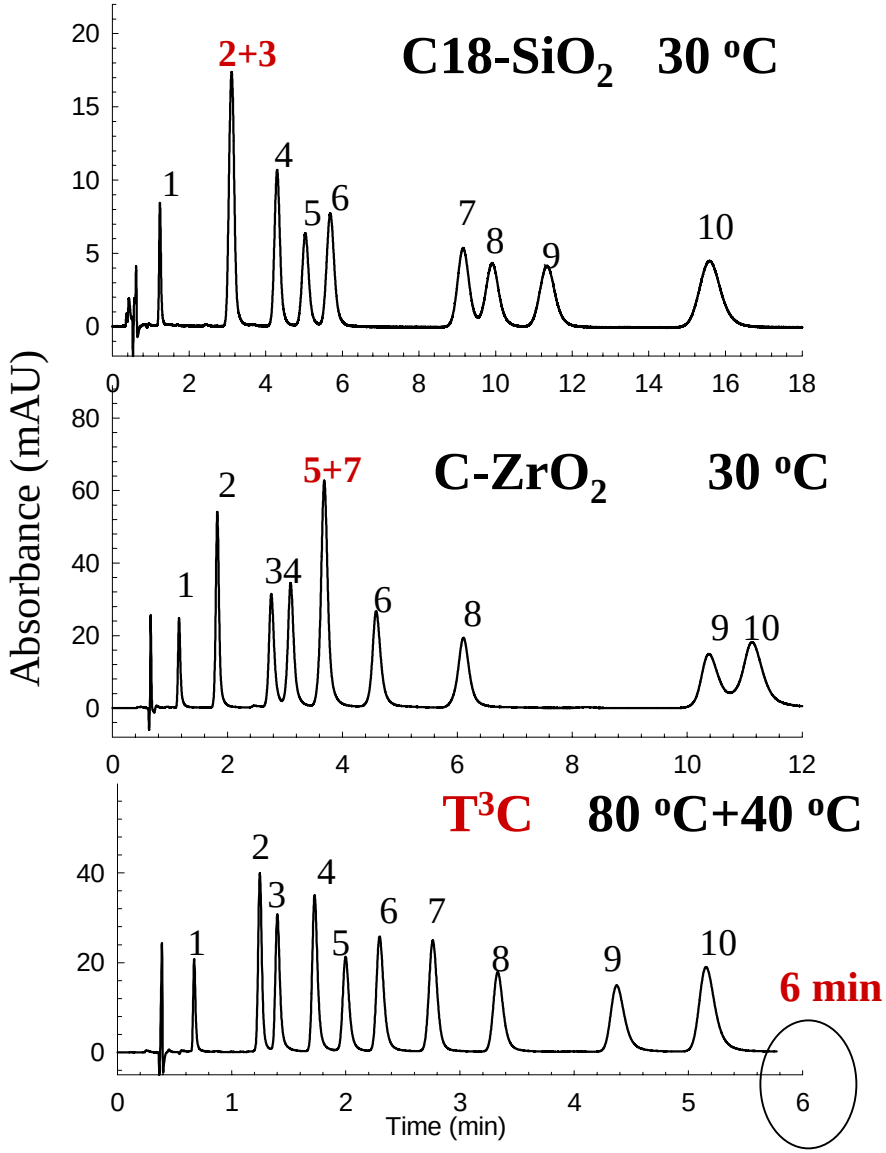
❖ **$T^3C$ is superior to simultaneous eluent and temperature optimization.**

# Applications of $T^3C$ Method

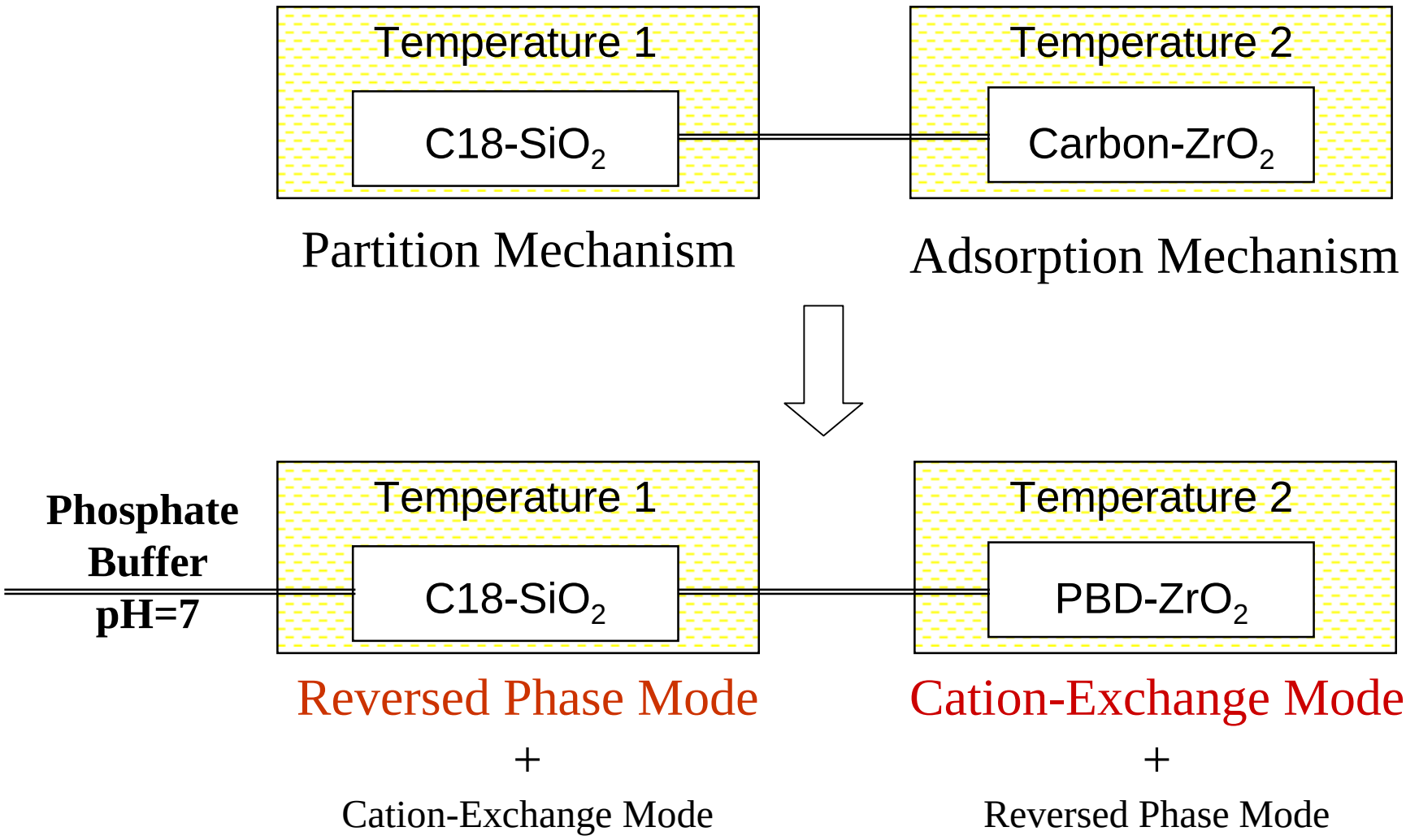
## *Urea and Carbamate Pesticides*



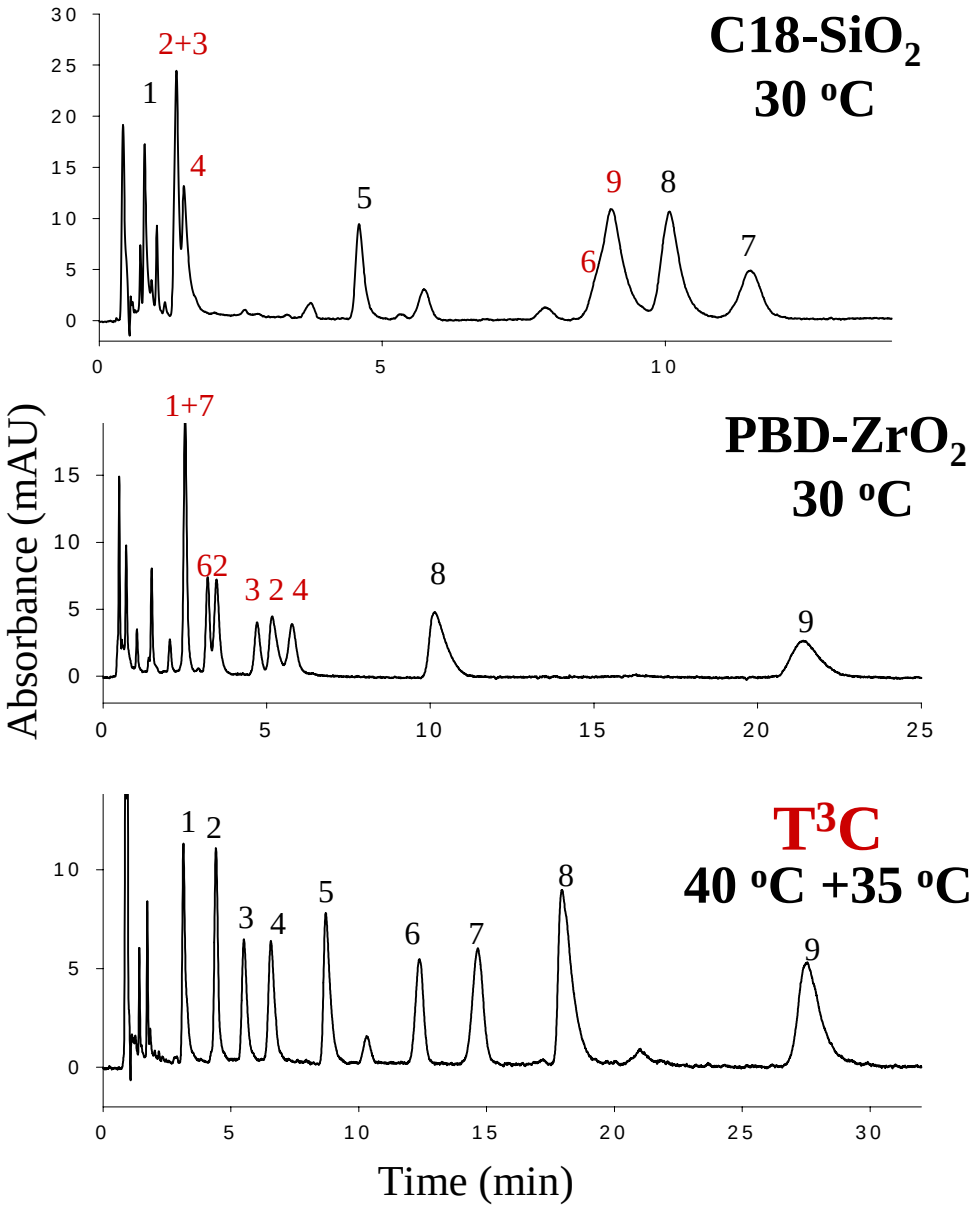

## *Barbiturates*

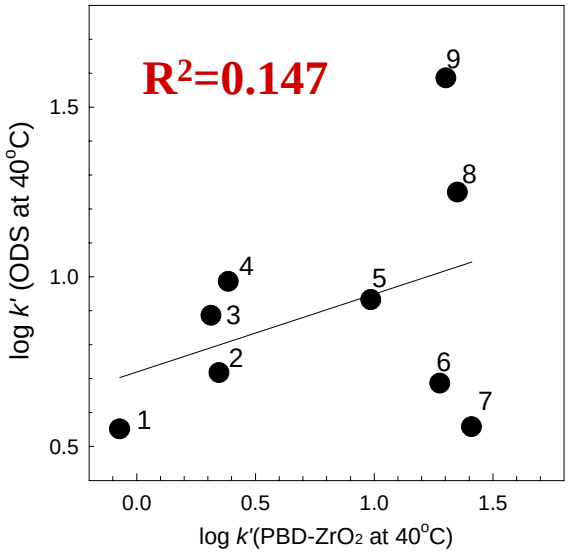


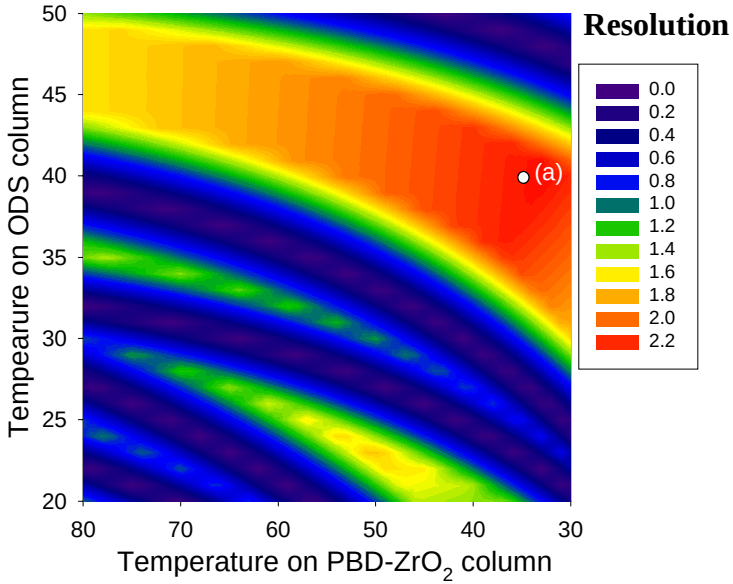
# Combination of ODS and PBD-$ZrO_2$ for the separation of basic drugs



# Separation of Anti-Histamine Drugs by T³C



# Conclusions

- $T^3C$ offers unique selectivity for the separation of complex mixtures.
- $T^3C$ requires that the critical pairs on the two phases are different.
  - Carbon Phase + Aliphatic Phase ⇨ Neutral Compounds
  - Reversed Phase + PBD-$ZrO_2$ Phase Phosphate Buffer ⇨ Basic Compounds
- Optimization needs only 4 or 5 trial runs.
- In many cases, $T^3C$:
  - ✓ is superior to mobile phase optimization.
  - ✓ provides better resolution than a single phase.
  - ✓ improves analysis speed.

# Acknowledgments

**National Institutes of Health**

**University of Minnesota**

**ZirChrom Separations, Inc.**