


# ***Investigation of Phenyl Alkyl Bonding Chemistry on Hybrid Organic/Inorganic Particles***



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Poster # A0240  
HPLC 2001  
Maastricht, Netherlands  
June 18-19, 2001

## **Acknowledgments:**

We thank Jim Cook, Dan Walsh, Cheryl Boissel, Bob  
Brennick, and Mike Dion for their assistance.

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## **Summary:**

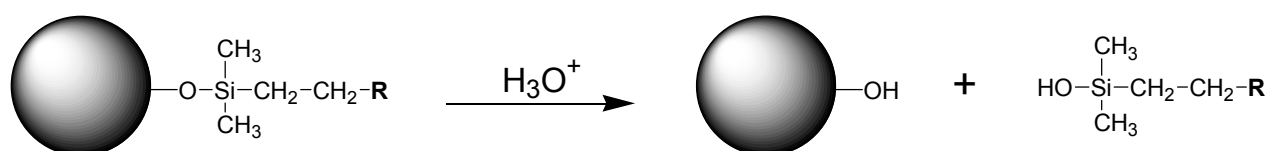
As part of our program to develop phenyl HPLC column packings on XTerra®, we have evaluated the effect of ligand connectivity, chain length, side group steric protection, and endcapping on hydrolytic stability in low and high pH mobile phases. In this report, bonded phase data will be presented that characterizes the differences and similarities between a series of phenyl alkyl substrates on XTerra®. In general, the extension of the alkyl chain length resulted in increased stability in high pH mobile phases. The use of phenyl alkyl substrates based on di- or trichlorosilane or the increase in steric bulk around silicon resulted in increased stability in low pH mobile phases.

## **Acknowledgments:**

We thank Jim Cook, Dan Walsh, Cheryl Boissel, Bob Brennick, and Mike Dion for their assistance.

# ***pH Failure of Bonded Phases***

## **Low pH Failure Mechanism - Ligand Cleavage**



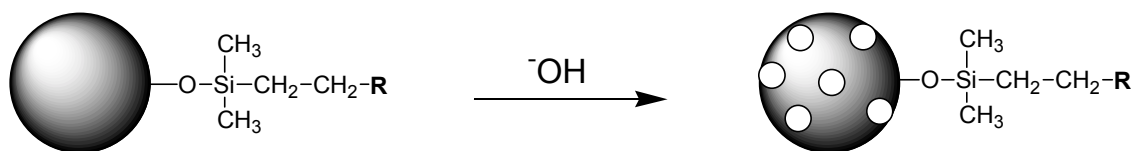
### **What Happens?**

- Decrease in Retention for Neutrals
- Increase in Retention for Bases

### **How to Improve**

- More Siloxane Bonds
- Longer Chain Length
- Steric Protection

## **High pH Failure Mechanism - Silicate Dissolution**



### **What Happens?**

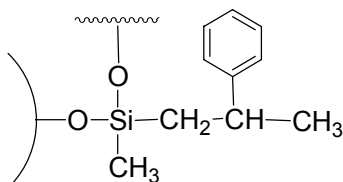
- Loss of Efficiency
- Column Voiding
- High Back Pressures

### **How to Improve?**

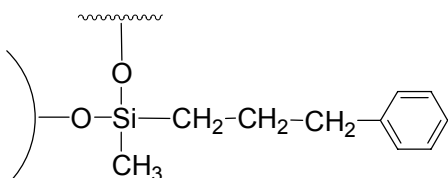
- Higher Ligand Surface Concentration
- Longer Ligand Chain Length
- More Robust Base Particle

# Phenyl Phases Used in this Study

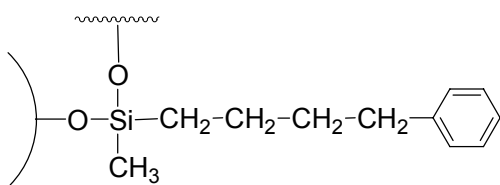
## Difunctional



**D1.** 2-Phenylpropyl

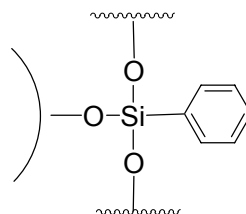


**D2.** 3-Phenylpropyl

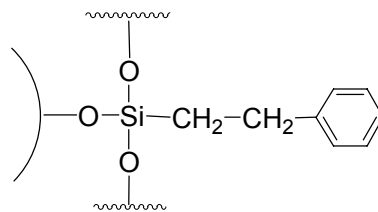


**D3.** Phenylbutyl

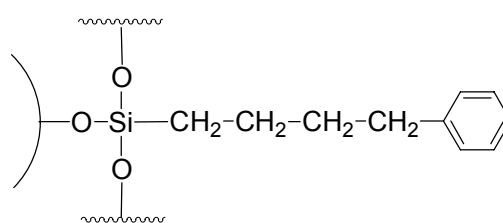
## Trifunctional



**T1.** Phenyl

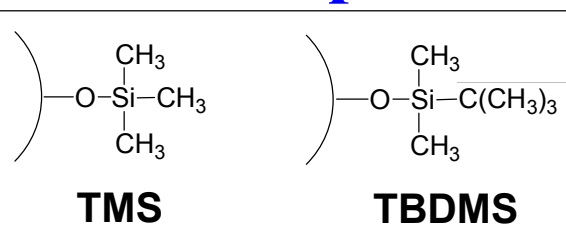


**T2.** Phenylethyl



**T3.** Phenylbutyl

## Endcaps



# ***Experimental Outline and Characterization Data***

- To Evaluate Ligand Dentation,
  - Compare Phases **D3** and **T3**
- To Evaluate Chain Extension,
  - Compare Phases **T1**, **T2** and **T3**
- To Evaluate Steric Protection,
  - Compare Phases **D1** and **D2**

<b>Phenyl Phase</b>	<b>Step 1 %C</b>	<b>Surface Conc. (<math>\mu\text{mol}/\text{m}^2</math>)</b>	<b>Increased %C By TMS-endcap</b>
<b>D1</b>	11.98	2.11	0.45
<b>D2</b>	11.32	2.31	0.93
<b>D3</b>	11.98	2.37	0.78
<b>T1</b>	9.53	2.09	1.21
<b>T2</b>	10.05	2.35	1.55
<b>T3</b>	11.20	2.20	1.37

Properties of XTerra® base material: Carbon content 6.89%; average  $d_p$  5.0  $\mu\text{m}$ ; specific surface area 175  $\text{m}^2/\text{g}$ ; pore volume 0.65  $\text{cm}^3/\text{g}$ ; mean pore diameter 119 Å.

# Test Protocols

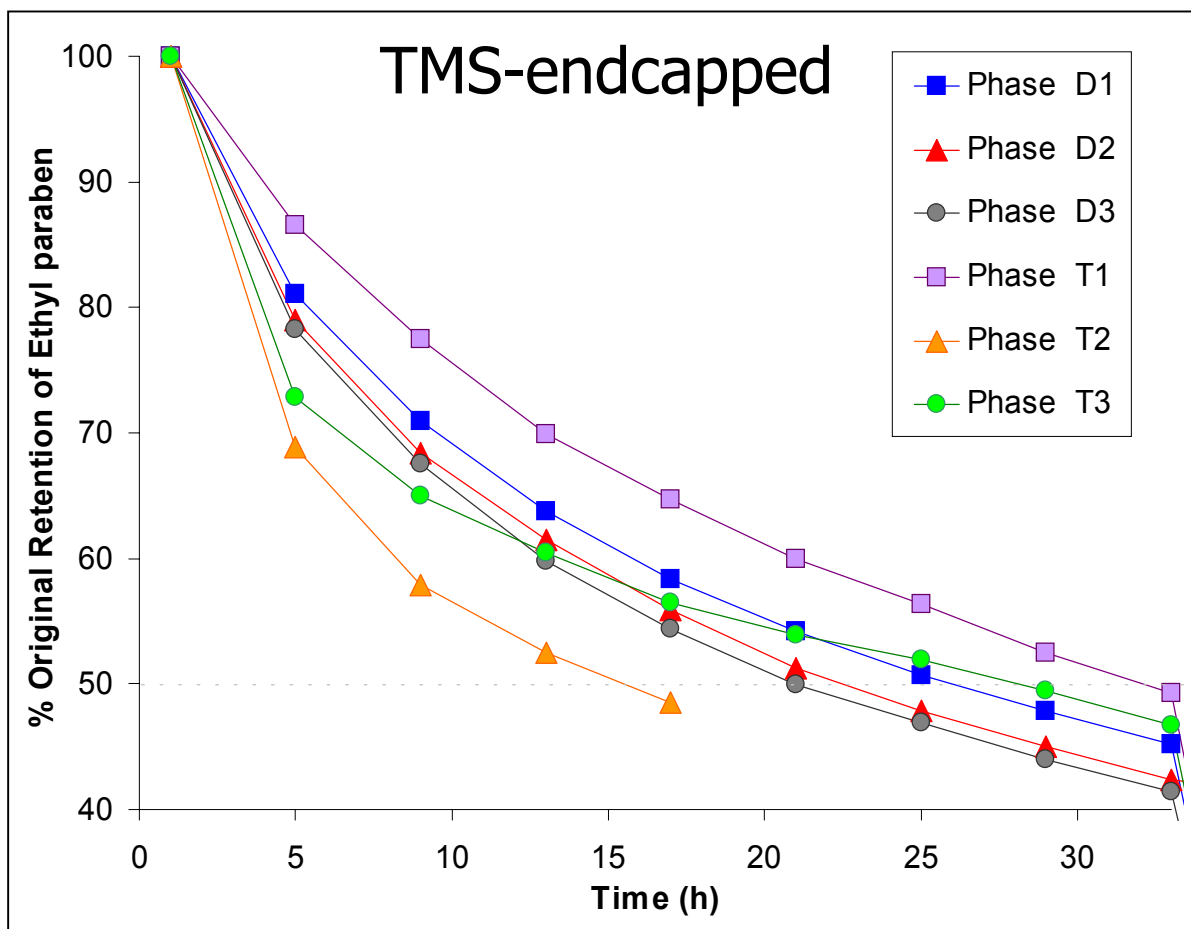
## ● Low pH Column Stability Test (3.0 x 100 mm column)

- ① Measure retention factor ( $k$ ) for ethyl paraben in 1% TFA in water @ 80°C and 0.6 mL/min
- ② Purge column over to 1% TFA in aqueous acetonitrile (40 min at 1.3 mL/min, 80 °C)
- ③ Purge column with 100% acetonitrile (2 h, 80 °C)
- ④ Equilibrate with mobile phase 1
- ⑤ Repeat measurement of ethyl paraben  $k$
- ⑥ Take relative decrease in  $k$  as a measure of ligand loss
- ⑦ Column failure defined as time (h) until 50% loss in  $k$ .

## ● High pH Column Stability Test (4.6 x 150 mm column)

- ① Measure the plate number ( $N$ ) for ethyl naphthalene in 40:60 v/v MeOH/ 20 mM  $\text{KH}_2\text{PO}_4$  / $\text{K}_2\text{HPO}_4$  pH 7.0 @ 50°C and 1.0 mL/min
- ② Purge column over to 50 mM TEA pH 10 at 50°C, run 1.0 h at 2.0 mL/min
- ③ Purge column with 100% water (10 min at 2 mL/min)
- ④ Purge column with 100% MeOH (10 min at 2 mL/min)
- ⑤ Equilibrate with mobile phase 1
- ⑥ Repeat measurement of ethyl naphthalene  $N$
- ⑦ Column failure defined as time (h) until >50% loss in  $N$  or when the HPLC system encounters high column back pressure (> 4000 psi)

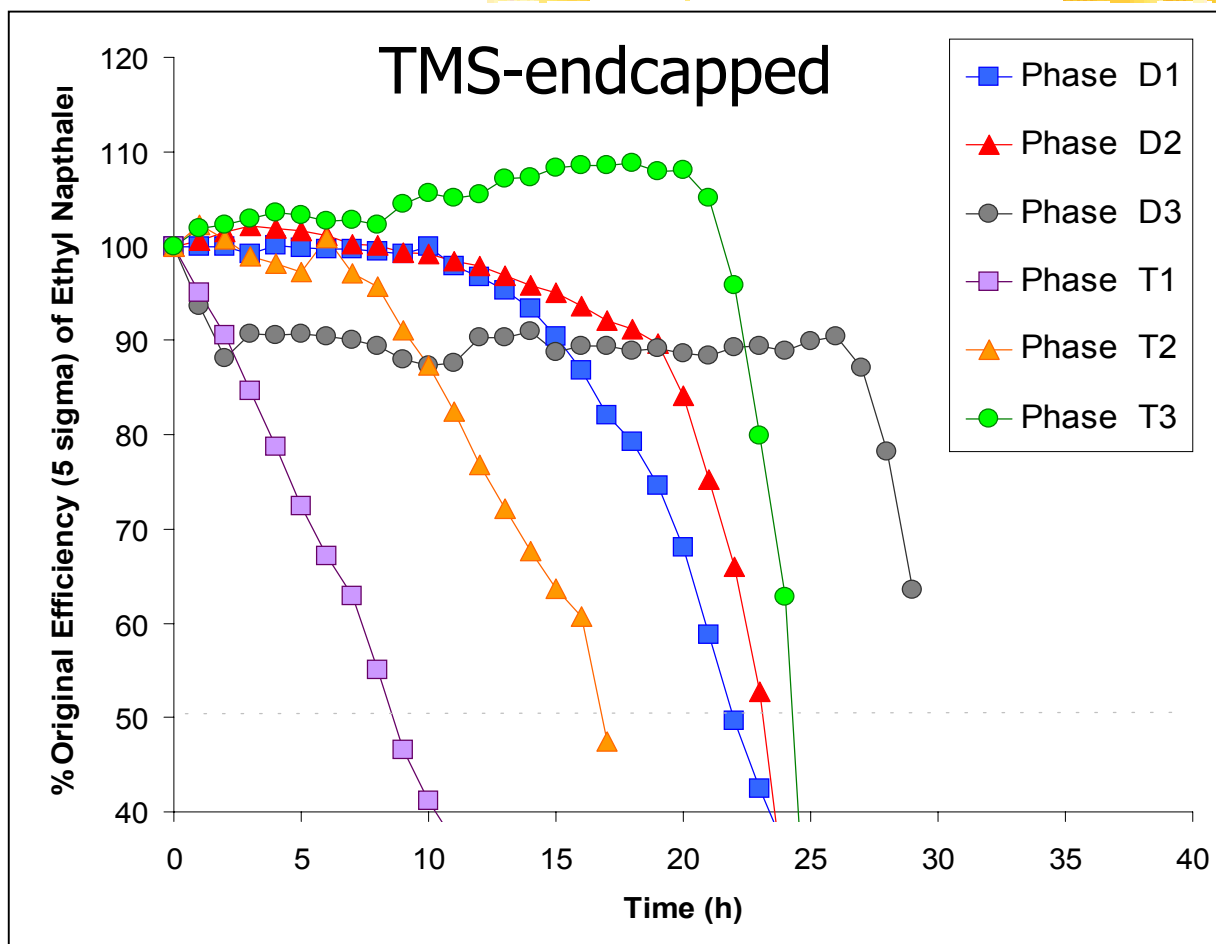
# Low pH Column Stability



## Results

- No correlation in stability of phenyl phases with chain extension. Steric protection is more important.
- **T1** has best acid stability, but shows large peak tailing for Basic Analytes.
- The stability of difunctional phase **D1** is comparable to **T1**, and displays reduced peak tailing for Basic Analytes.

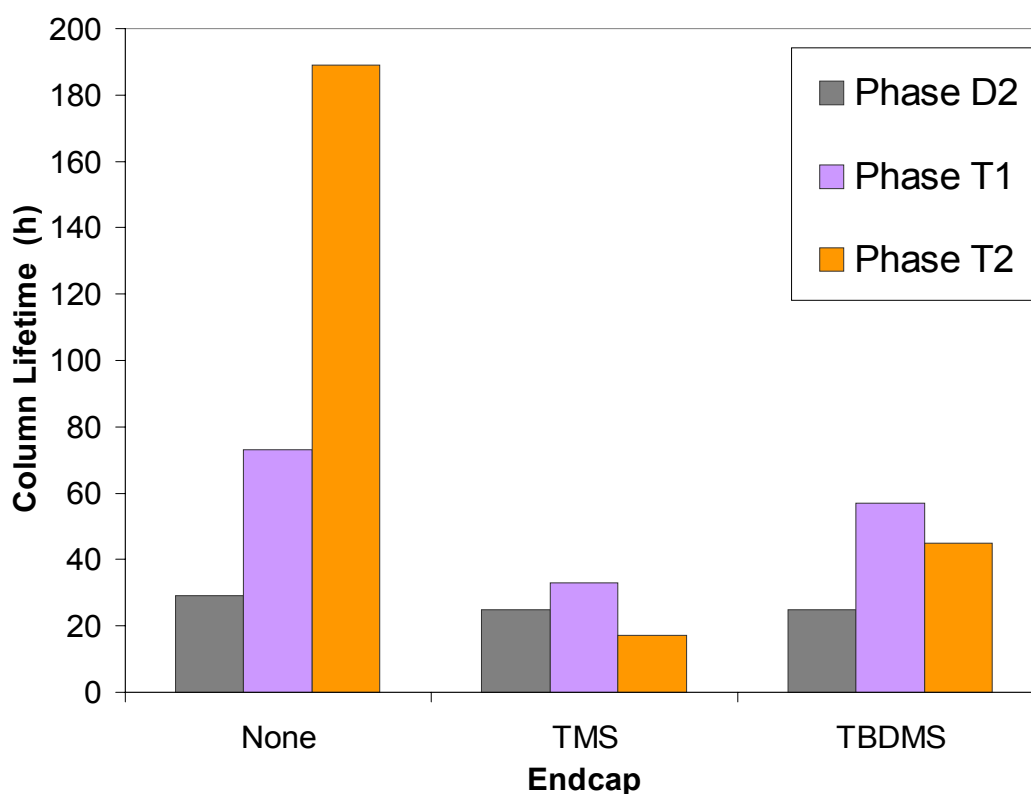
# High pH Column Stability



## Results

- Dramatic increases in stability of trifunctional phases are observed with chain extension.
- Difunctional phases experience increases stability with chain extension, to a lesser extent.
- All difunctional phases are comparable to the best trifunctional phase **T3**.

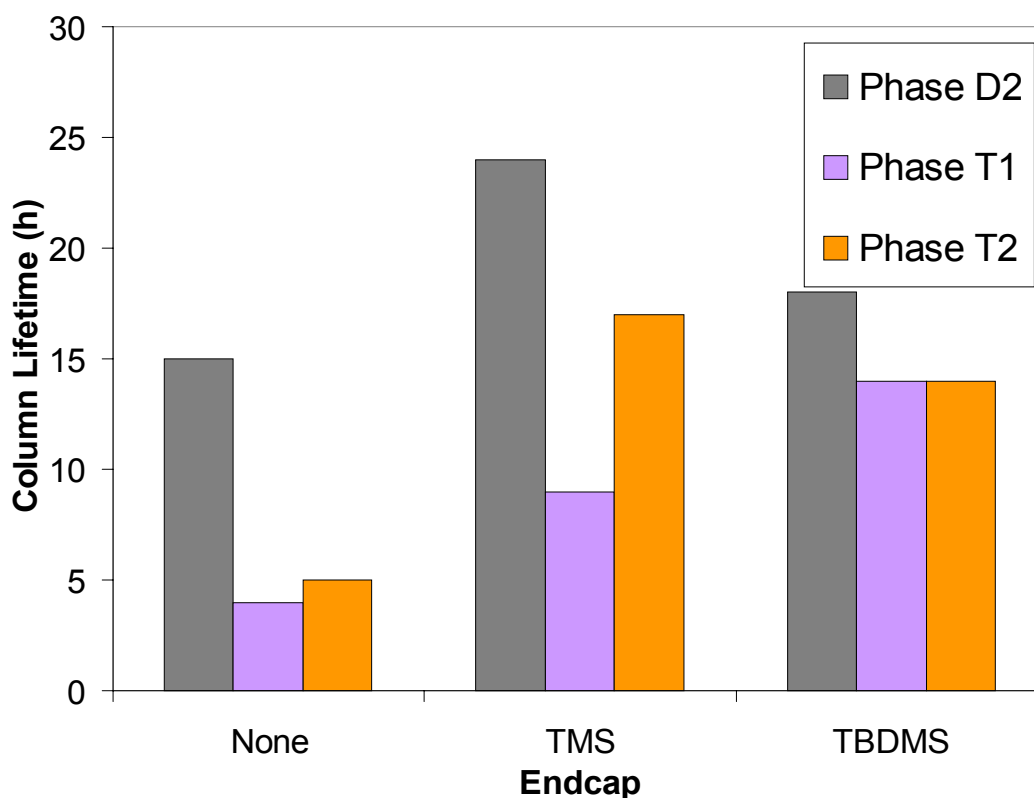
# Effects of Endcapping on Low pH Stability



## Results

- **D2** and **T1** have comparable acid stability with or without endcapping, due to sterics.
- Non-endcapped phase **T2** is more acid stable than endcapped phases, but has large peak tailings for Basic analytes.
- All phases have improved peak shape and increased retention of Non-Polar and Basic analytes by endcapping.

## ***Effects of Endcapping on High pH Stability***



### ***Results***

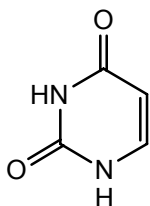
- Non-endcapped phases are less stable than endcapped phases.
- **TMS** and **TBDMS** endcappings afford comparable increases in base stability. Stability of base particle is more important.
- No clear correlation between steric bulk of endcap and base stability.

# Chromatographic Test Conditions

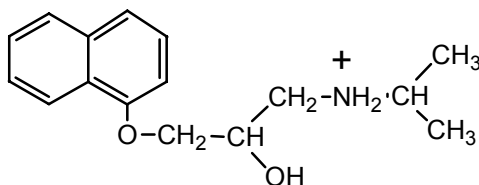
- Column Dimensions: 4.6 x 150 mm
- Solvent: 65:35 MeOH/20 mM  $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ , pH 7.00
- Flow Rate: 1.0 mL/min
- Detector: UV (254 nm)
- Temperature:  $25.0 \pm 0.1$  °C

## Analytes

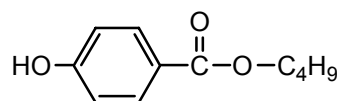
(1) Uracil



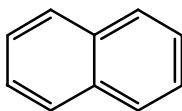
(2) Propranolol



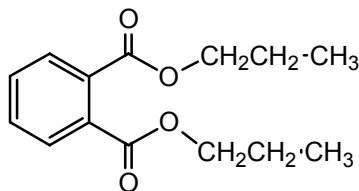
(3) Butylparaben



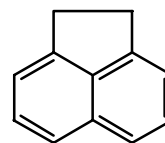
(4) Naphthalene



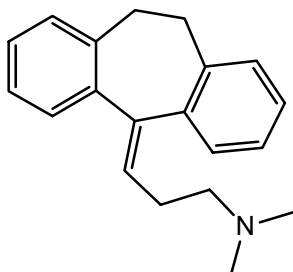
(5) Dipropylphthalate



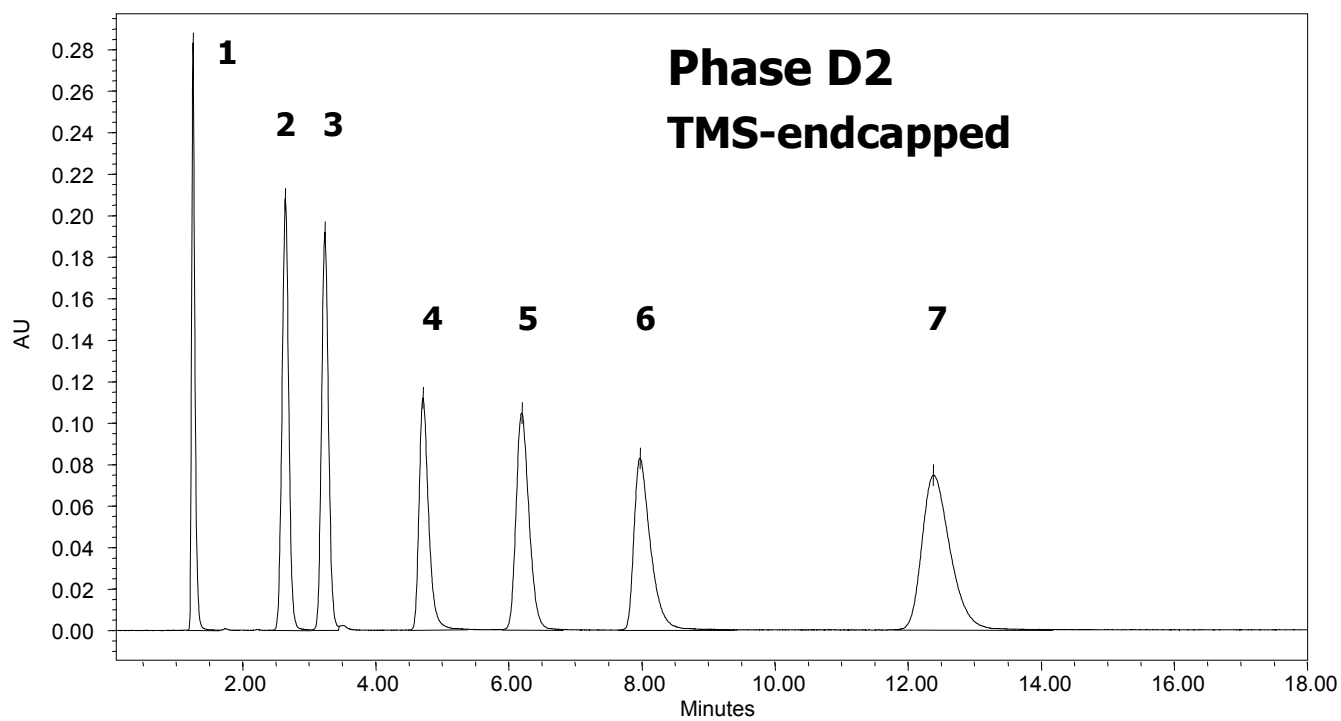
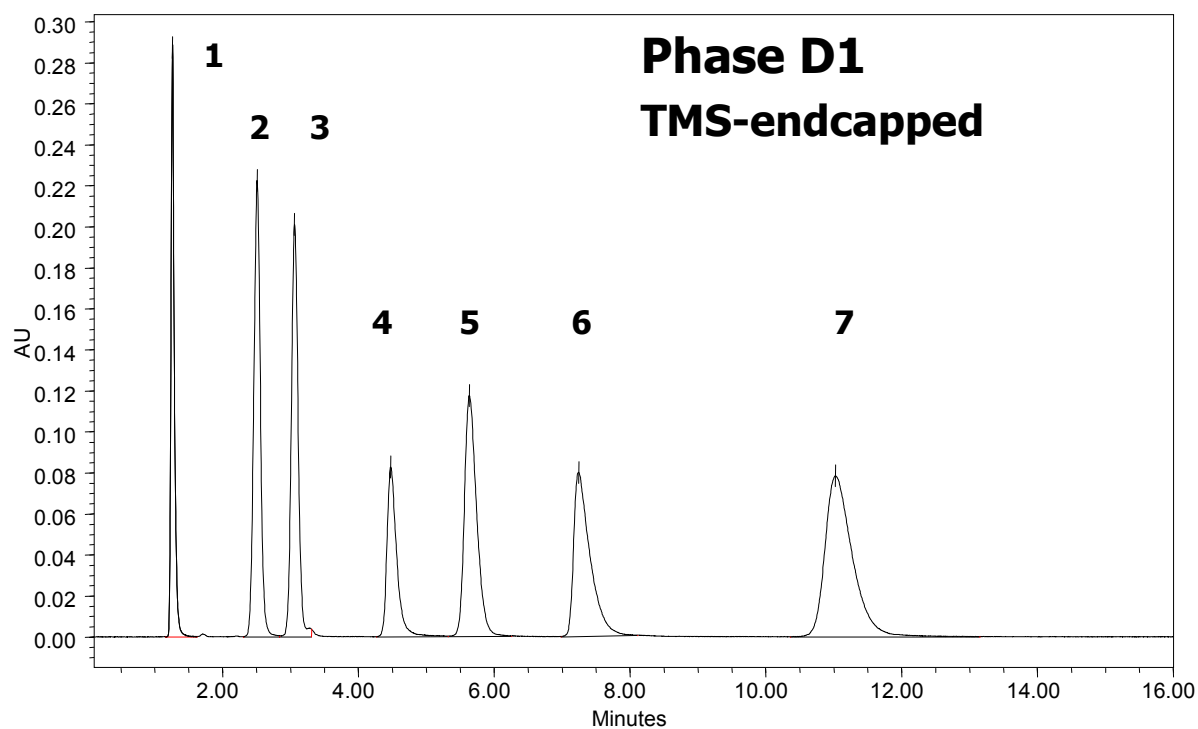
(6) Acenaphthene



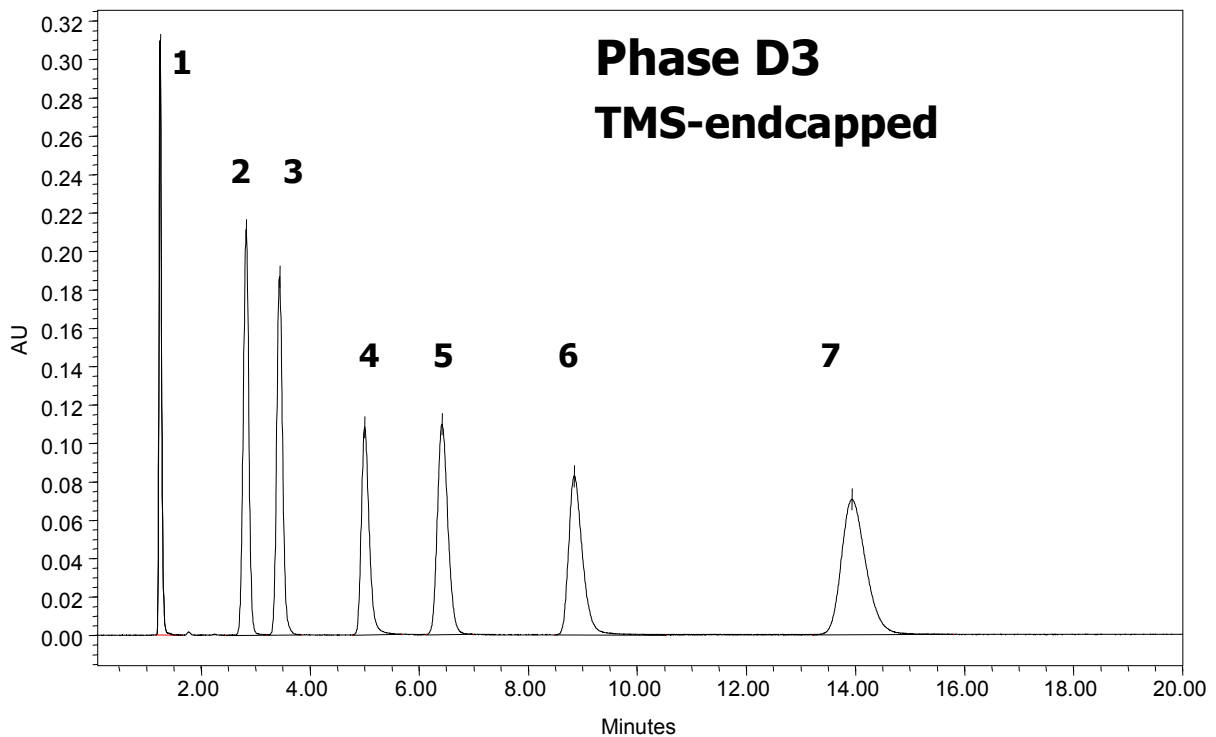
(7) Amitriptyline



# ***Selected Chromatograms***



# Chromatographic Comparison



## Results

- Similar retention factors and peak tailing was observed for difunctional phases
- Increased chain length correlates with increased retention for Non-Polar and Basic Analytes
- Difunctional phase **D1** has best acid stability. All difunctional phases had comparable base stability