Grouping Modern HPLC Columns Into Chemical Classes for Improving Phase Selection and Method Development

Richard A. Henry, Hillel K. Brandes, William H. Campbell, Carmen T. Santasania, and David S. Bell

Supelco, Div. of Sigma-Aldrich, Bellefonte, PA 16823 USA

www.sigma-aldrich.com

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Importance of Selectivity in HPLC

- In 2001, Mao and Carr[9] published an interesting graphical method to illustrate the critical importance of selectivity in HPLC.

- While selectivity remains as the most powerful resolution tool, there has been recent interest in developing columns with smaller porous and porous-layer particles because column efficiency is easier to use.

- Descriptions of possible solute-phase interaction, such as the Hydrophobic-Subtraction Model[3] for reversed-phase, and guidelines for column phase selection are widely available; however, the process of improving a separation can still be very laborious if the wrong columns are chosen for initial screening.

- Types of phase-solute chemical interaction are reviewed, and columns are classified according to how they are likely to interact with certain solutes.
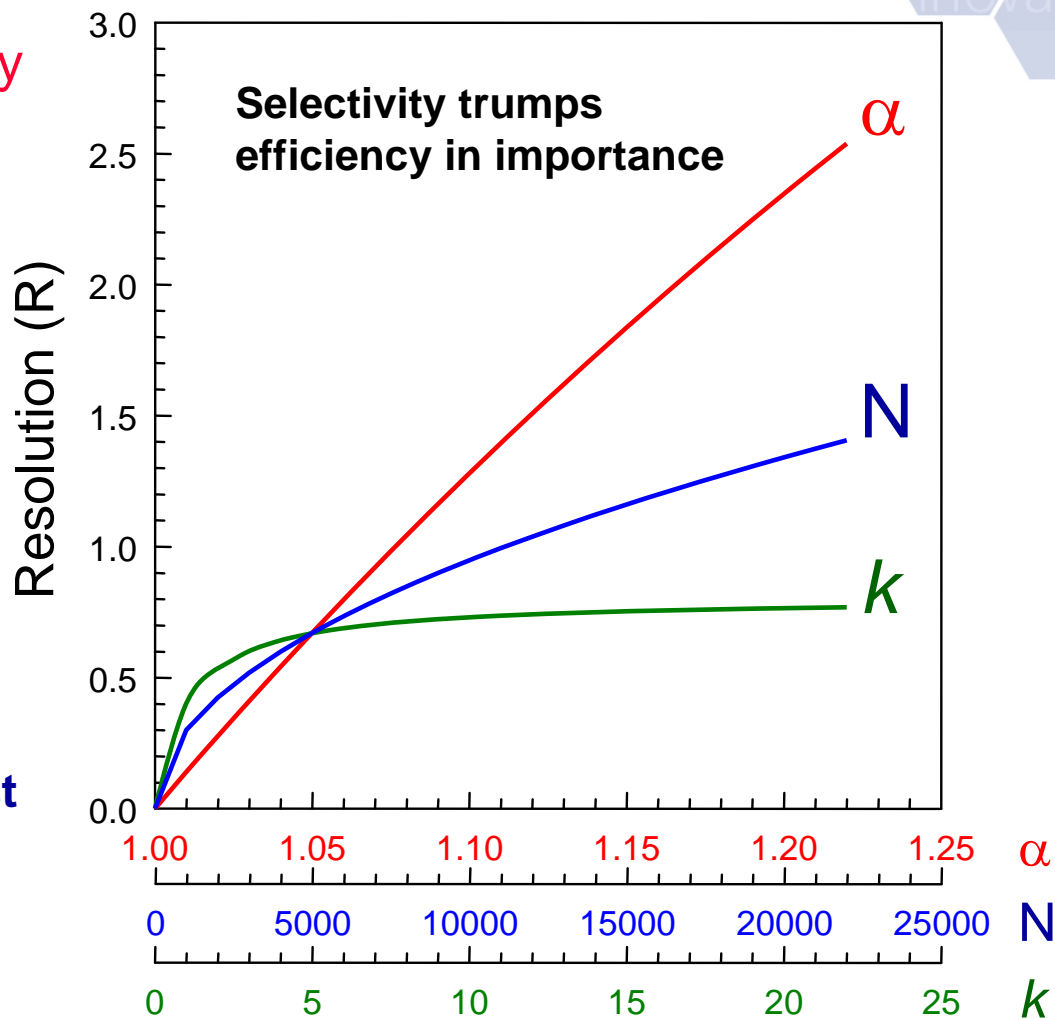
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Resolution: The Importance of Selectivity[9]

Efficiency   Retention   Selectivity

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

$$\alpha = \frac{k_j}{k_i}$$

- ➤ **Retention and Selectivity are closely related.**
- ➤ **Selectivity (α) has the greatest impact on improving resolution.**

Selectivity trumps efficiency in importance

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Important HPLC Stationary Phases

Choice of column is the most important variable in determining system selectivity, but the chemistry is still not well understood.

Reversed Phase (Less polar)

- C18
- C8
- Embedded Polar Groups (amide, carbamate, urea, ether)
- Phenyl
- Cyano
- Fluorinated aromatic (PFP)

Normal Phase (More polar)

- Bare silica (no bonded phase)
- Cyano
- PFP
- Amino
- Diol

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Selectivity Variables in Reversed-Phase[*]

Continuous variables:

➢ solvent type

➢ pH (ionizable solutes only)

➢ additives (type and concentration)

➢ solvent strength

➢ temperature

Predictable
(modeling software available)

Discontinuous variable:

➢ column type (phase and substrate)

Less predictable
(screening required)

[*] Excerpted with permission from J. Dolan, 2009 Minnesota Chromatography Forum Spring Symposium; adapted by R. Henry for EAS 2009 and Pittcon 2010 oral papers.
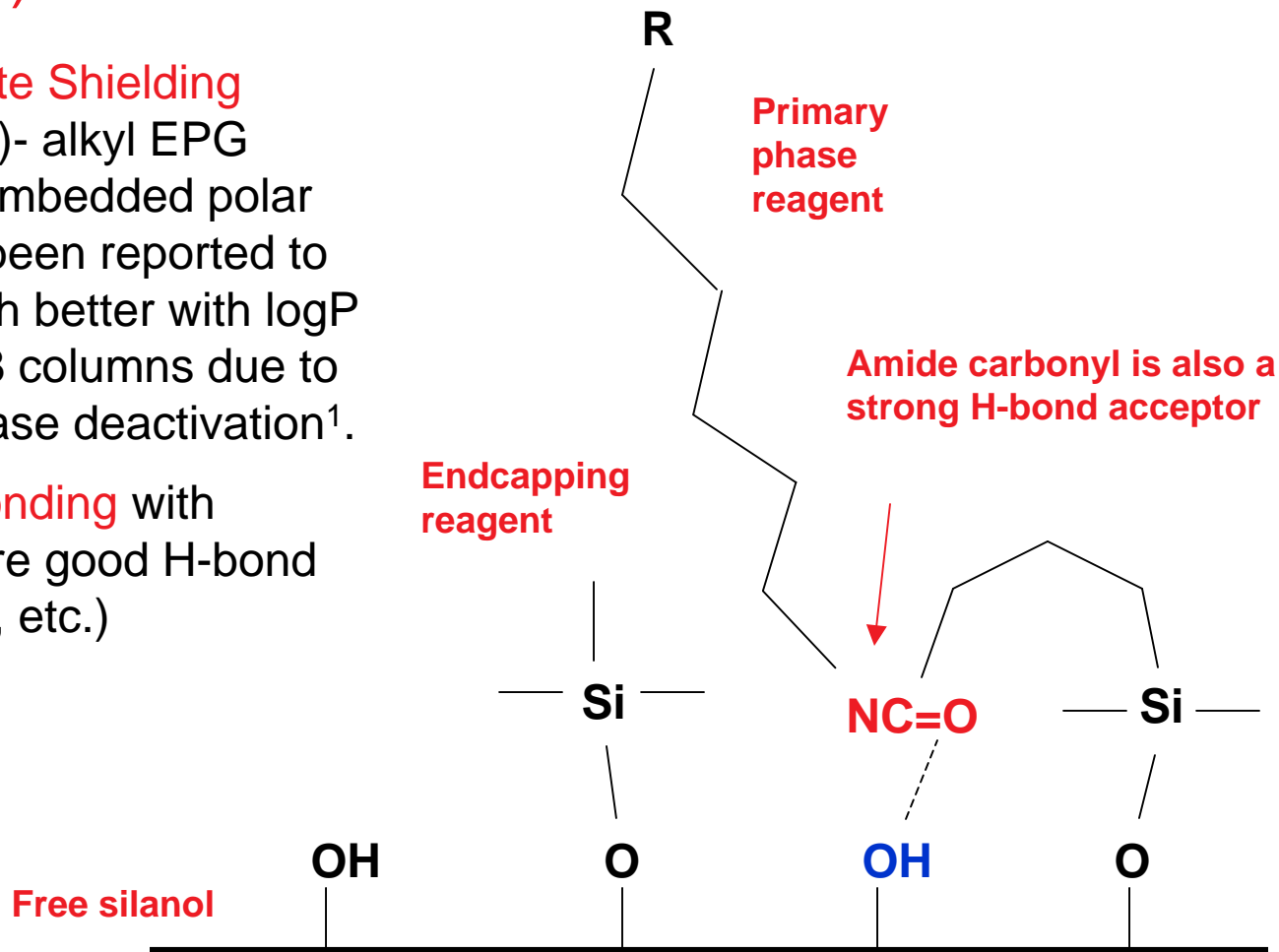
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Alkyl Bonded Phases (C18 and C8)

- C18 reagents are large and can leave some silanols unreacted (ca. 50%).

- C8 reagents are smaller and provide better silanol coverage

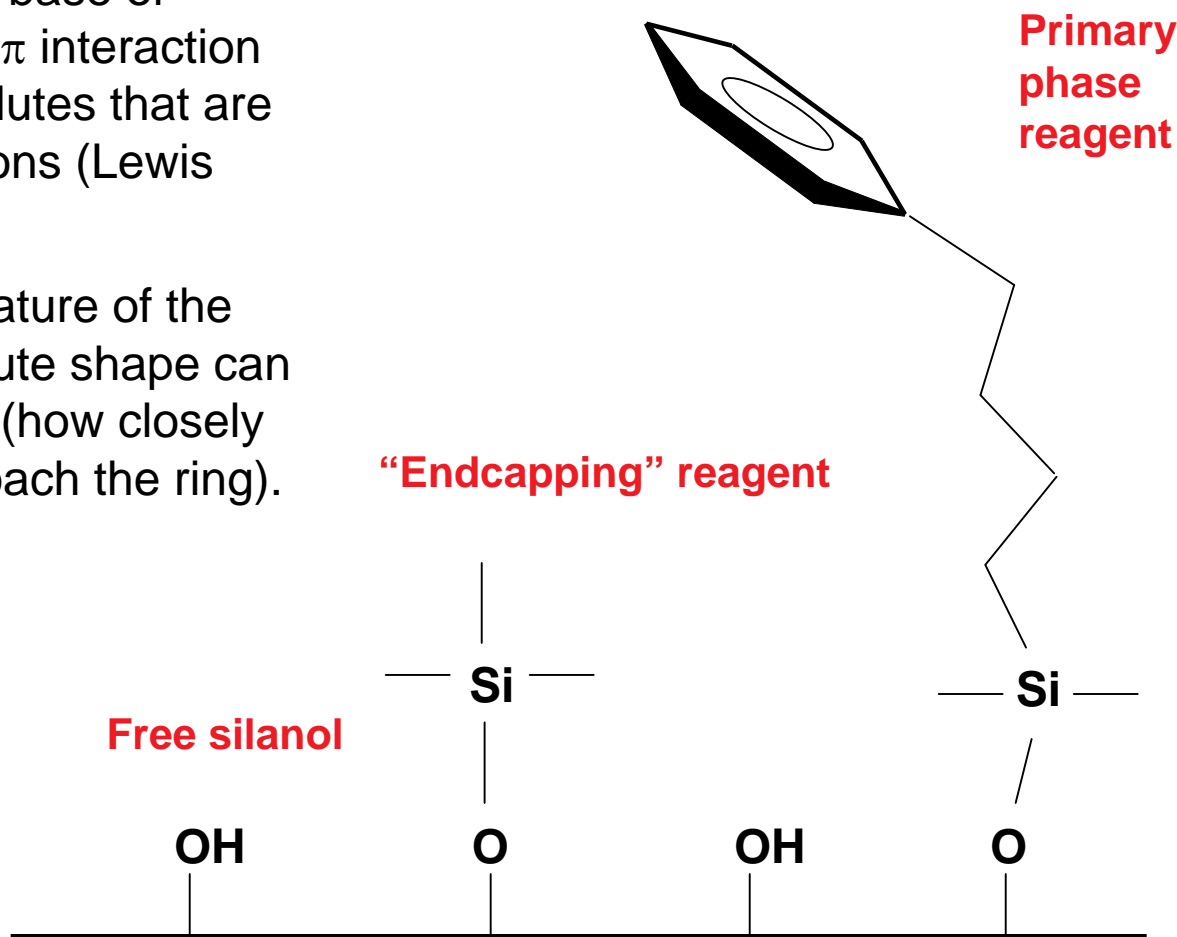- At pH >4, silanols can ionize and add cation-exchange character.

Primary phase reagent

R

"Endcapping" reagent

Free silanol

Si

Si

OH   **O**   **OH**   **O**

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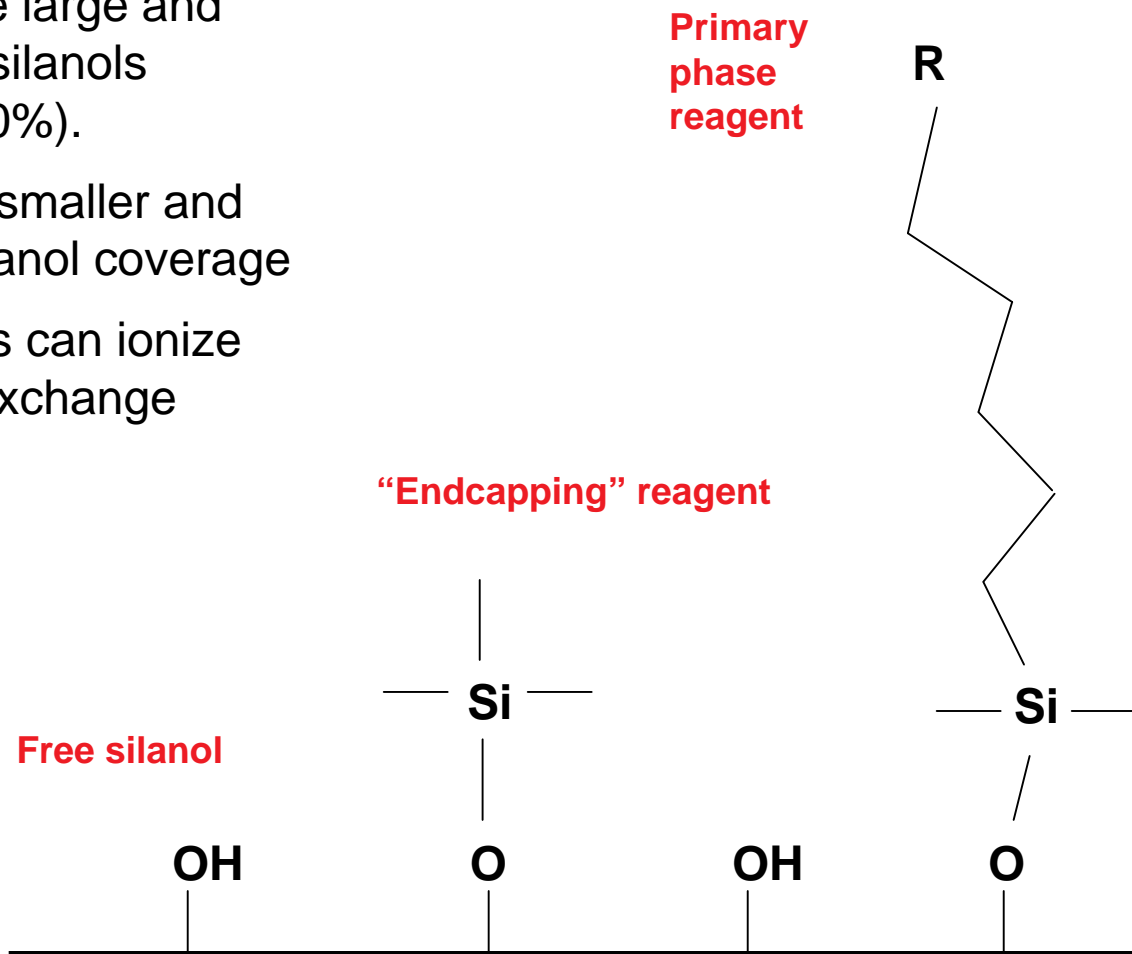
Amide (EPG) Bonded Phases May Shield (Deactivate) the Silica Surface

- Possible Solute Shielding (basic solutes)- alkyl EPG phases with embedded polar groups have been reported to correlate much better with logP data than C18 columns due to their higher base deactivation[1].

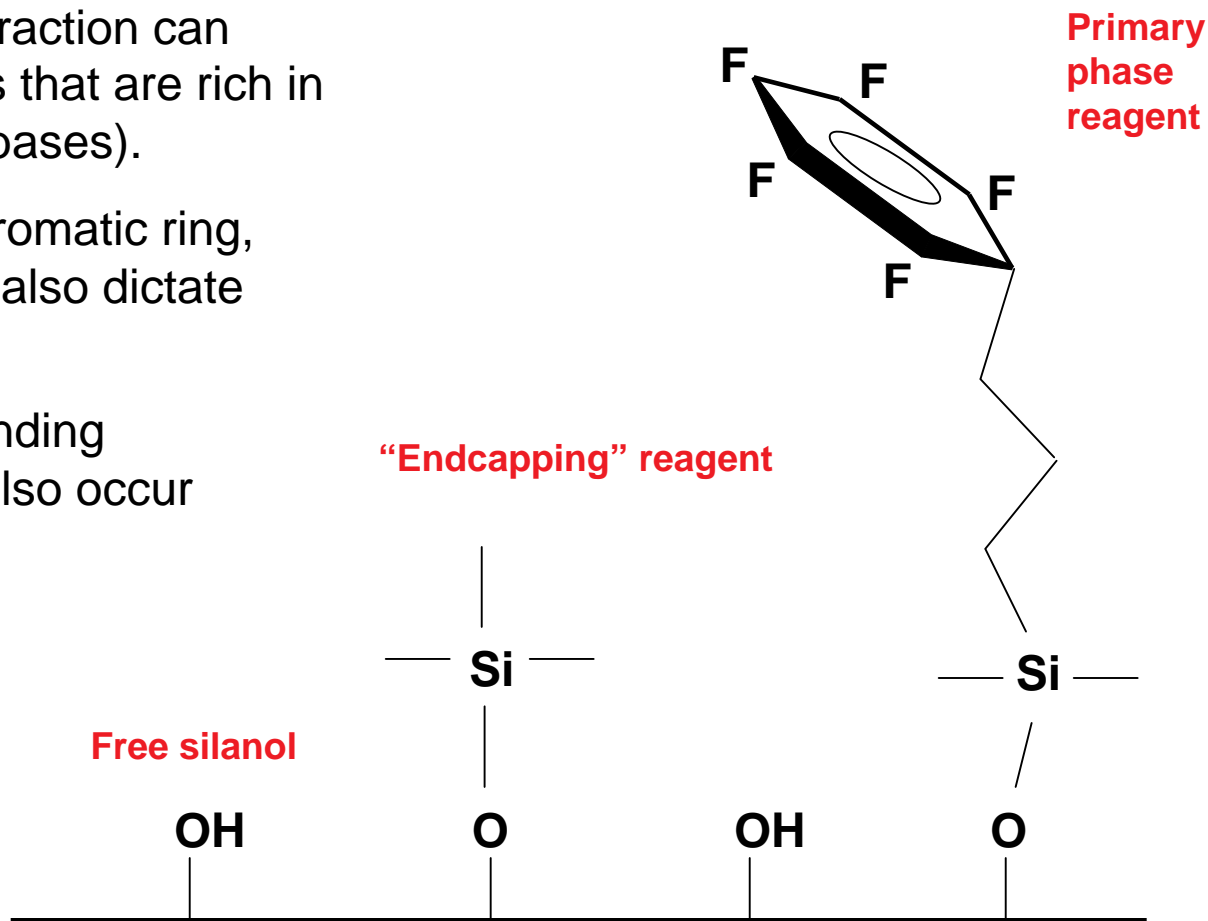- Possible H-bonding with solutes that are good H-bond donors (acids, etc.)

R

Primary phase reagent

Amide carbonyl is also a strong H-bond acceptor

Endcapping reagent

NC=O

Si

Si

OH

O

OH

O

Free silanol

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Phenyl Bonded Phases

- Phenyl is a Lewis base or electron donor; π-π interaction can occur with solutes that are deficient in electrons (Lewis acids).

- Due to the rigid nature of the aromatic ring, solute shape can dictate selectivity (how closely solutes can approach the ring).

Primary phase reagent

"Endcapping" reagent

Free silanol

Si

Si

OH   **O**   **OH**   **O**

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Pentafluoro-Phenyl (F5) Bonded Phases

- PFP is a Lewis acid or electron acceptor; π-π interaction can occur with solutes that are rich in electrons (Lewis bases).

- Due to the rigid aromatic ring, solute shape can also dictate selectivity.

- Dipolar and H-bonding interactions can also occur

Primary phase reagent

F **F**

F **F**

F

"Endcapping" reagent

Si

Si

Free silanol

OH **O** **OH** **O**

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Cyano Bonded Phases

- Cyano is a strong dipole that can interact with other dipoles or induce dipoles on solutes.

- Cyano phases also have moderate hydrophobic character from alkyl ligands.

- Stability of newer Cyano phases is now comparable to all other HPLC phases.

Primary phase reagent

"Endcapping" reagent

Free silanol

$N \equiv C$

Si

Si

OH   **O**   **OH**   **O**
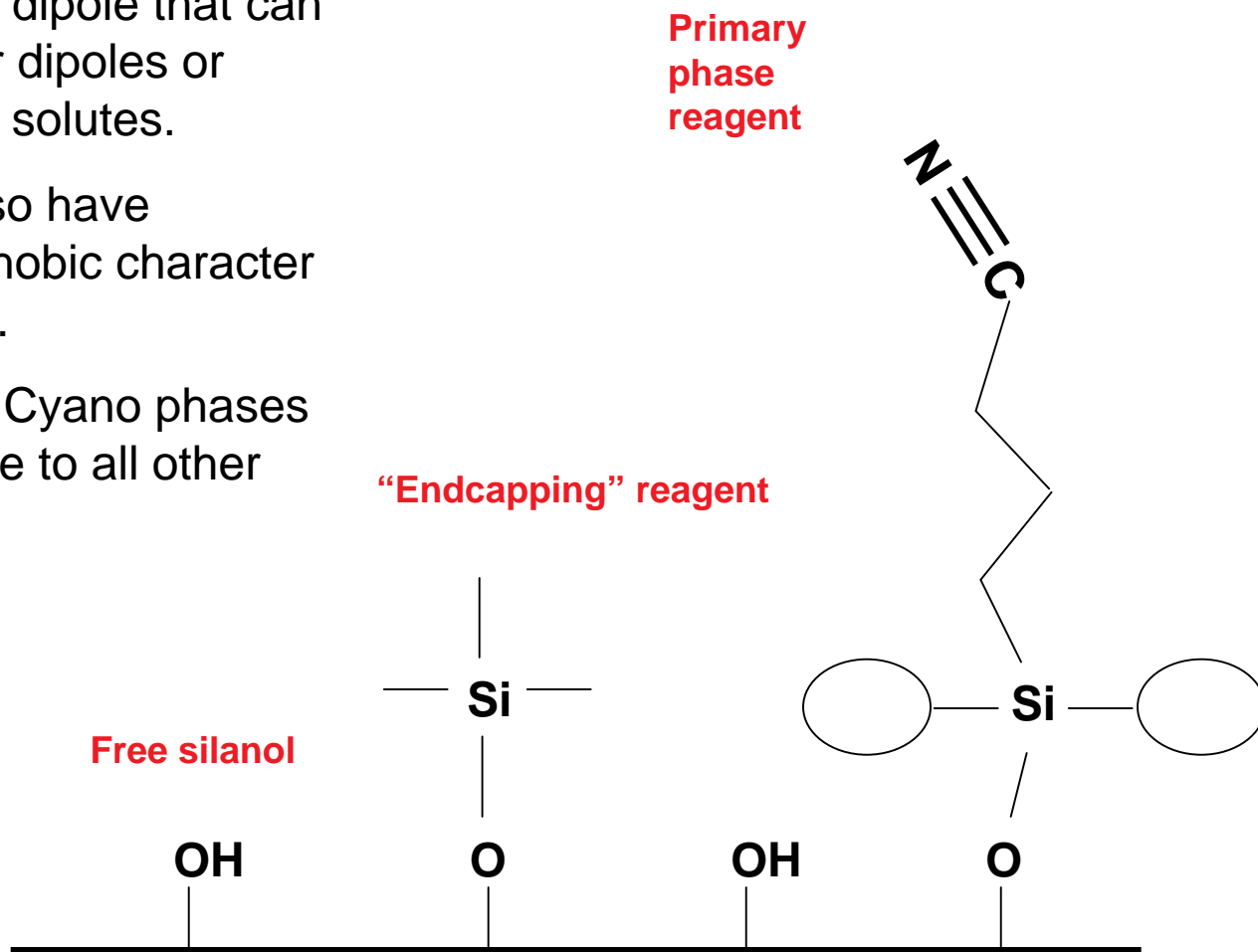
Classification by Chemical Interaction Type[a]

Bonded Phase	Hydrophobic	H-Bonding	Dipolar	π-π	Steric[b]	Ion-Exchange[c]
C18	Very Strong	Weak	No	No	No	Moderate
C8	Strong	Weak	No	No	No	Weak
Amide	Strong	Strong Acceptor	Moderate	No	Weak	Very weak
Phenyl	Strong	Weak Acceptor	Weak	Strong Donor	Strong (Rigid)	Weak
Cyano	Moderate	No	Strong	Weak	No	Very weak
PFP	Moderate	Moderate Acceptor	Strong	Strong Acceptor	Strong (Rigid)	Strong

a. Using Euerby[2] variation of Snyder-Dolan-Carr Hydrophobic Subtraction Model[3].
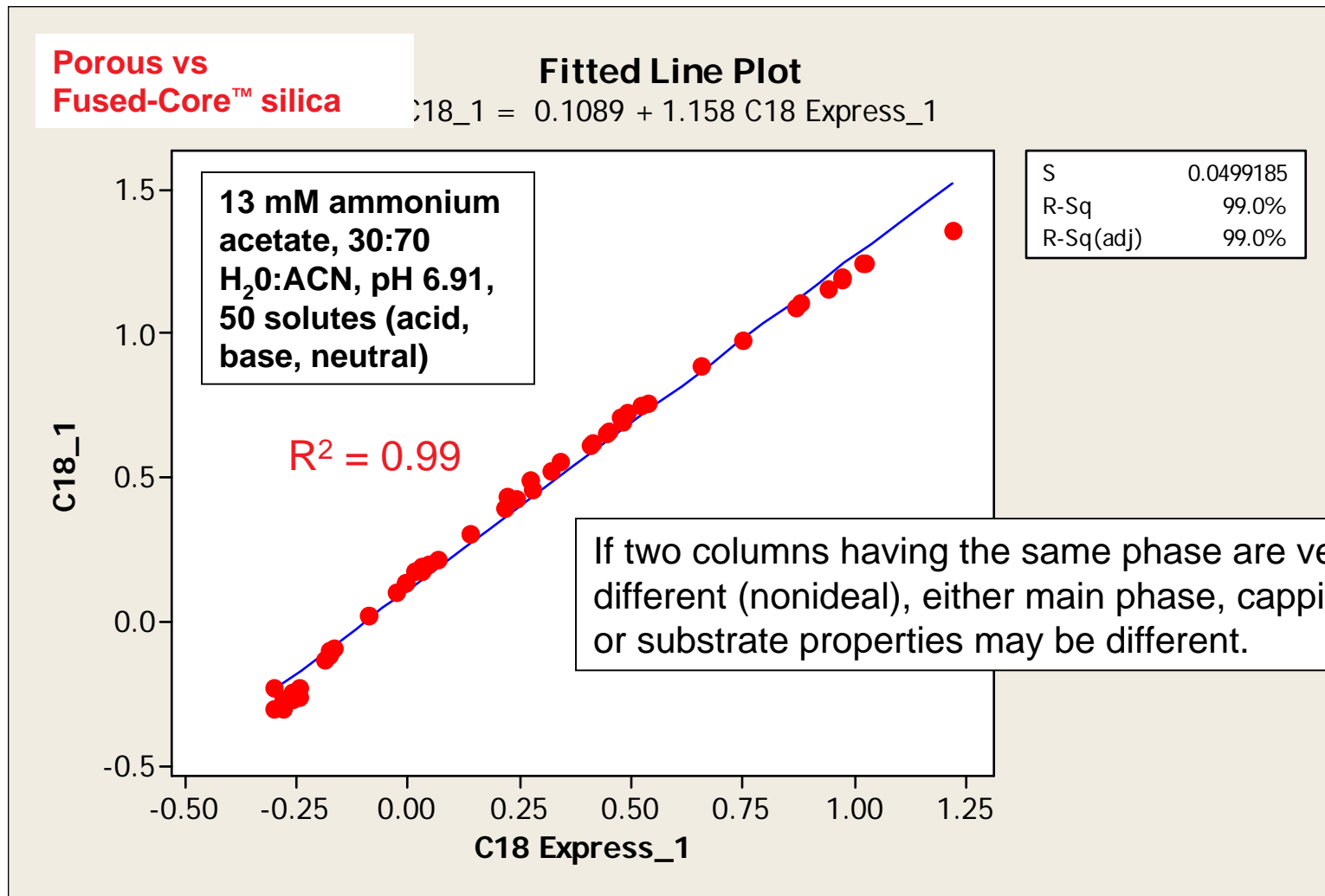
b. Current steric models relate to polymeric phases and are insufficient for polar solutes.

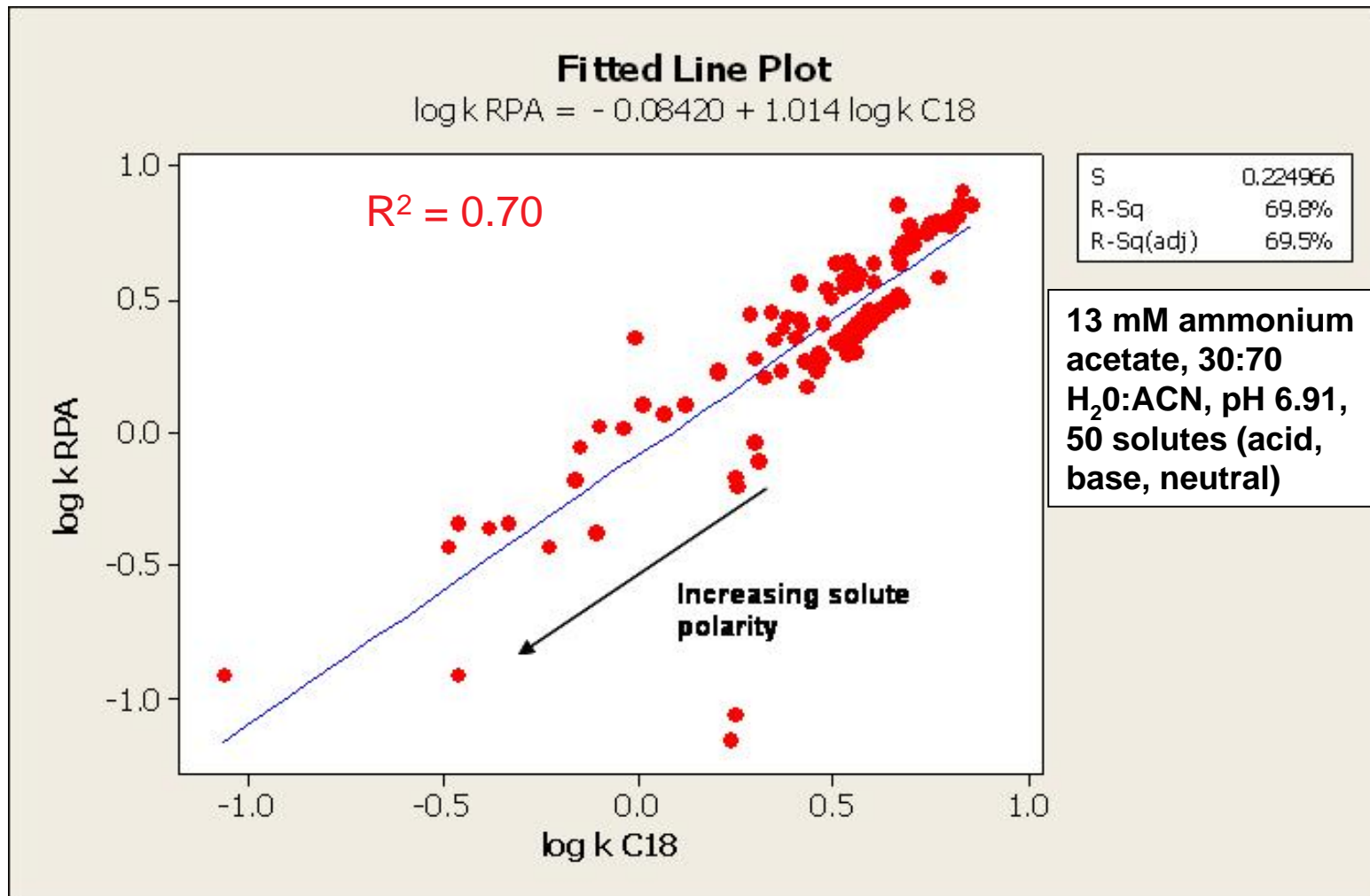c. May include interactions with acidic silica substrate and Lewis acid PFP ring.

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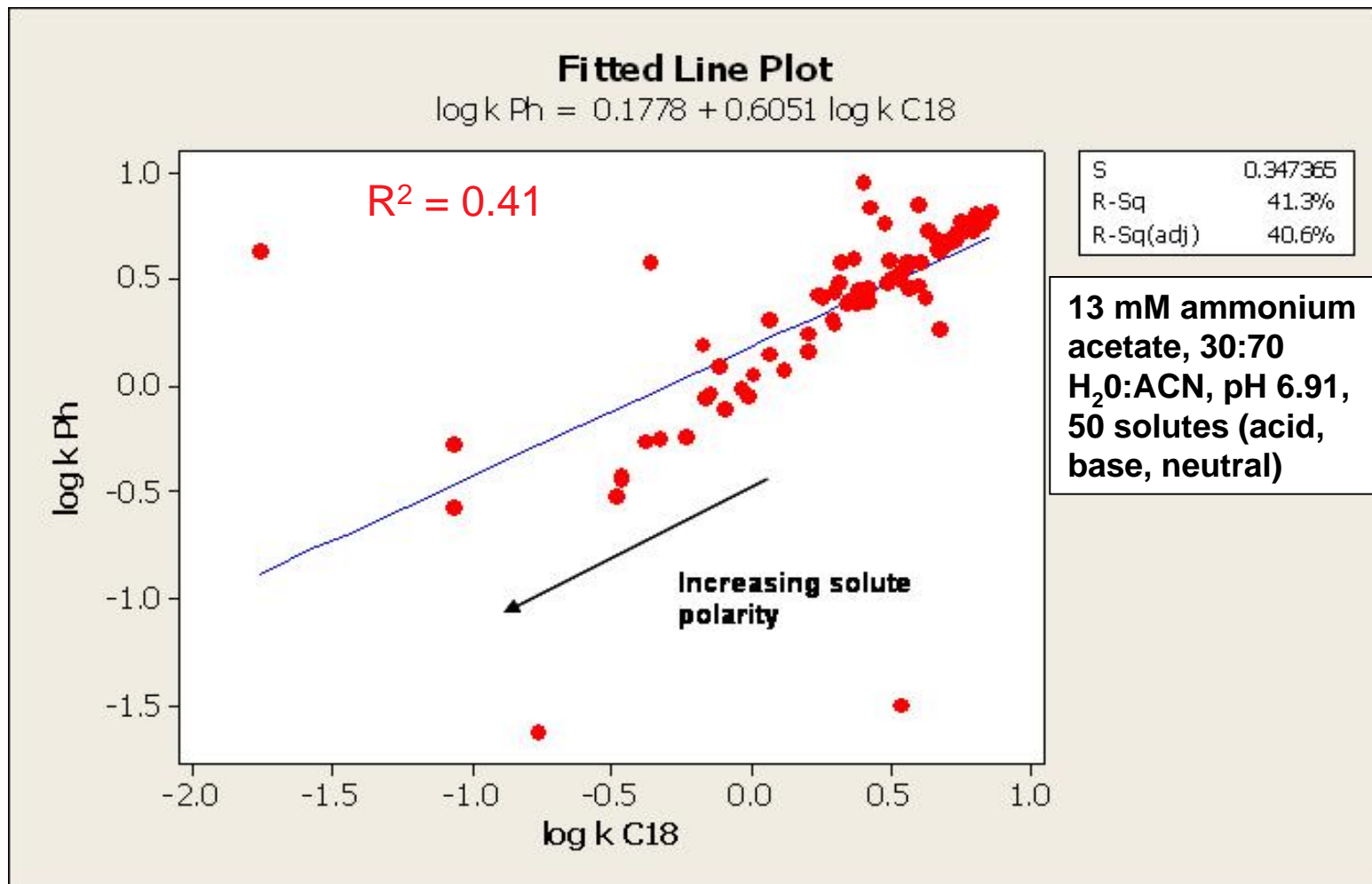SIGMA-ALDRICH®

Columns with Similar Phases Should Also Be Similar: C18 vs C8

Fitted Line Plot

$\log k\ C8 = -0.06344 + 0.9851\ \log k\ C18$

$R^2 = 0.97$

S	0.0568801
R-Sq	97.2%
R-Sq(adj)	97.1%

13 mM ammonium acetate, 30:70 H_20:ACN, pH 6.91, 50 solutes (acid, base, neutral)

Increasing solute polarity

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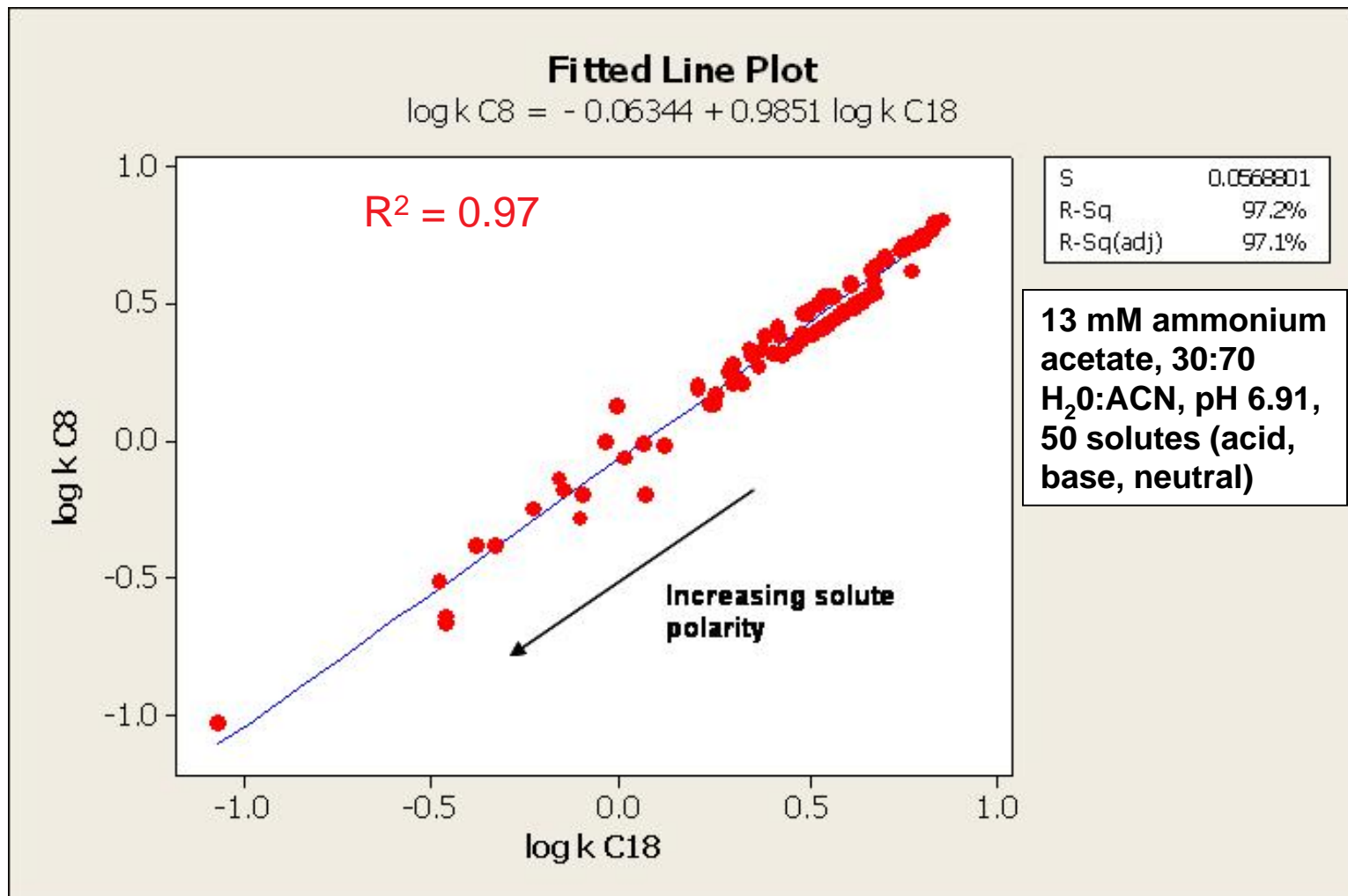
Columns with Same Phase Should Be Similar: Ascentis C18 vs Express C18

Porous vs Fused-Core™ silica

Fitted Line Plot

C18_1 = 0.1089 + 1.158 C18 Express_1

S	0.0499185
R-Sq	99.0%
R-Sq(adj)	99.0%

13 mM ammonium acetate, 30:70 H$_2$0:ACN, pH 6.91, 50 solutes (acid, base, neutral)

$R^2 = 0.99$

If two columns having the same phase are very different (nonideal), either main phase, capping or substrate properties may be different.

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Columns with Different Phases Should Be Orthogonal: C18 vs Amide

Fitted Line Plot
$\log k\ RPA = -0.08420 + 1.014 \log k\ C18$

$R^2 = 0.70$

S	0.224966
R-Sq	69.8%
R-Sq(adj)	69.5%

13 mM ammonium acetate, 30:70 H_2O:ACN, pH 6.91, 50 solutes (acid, base, neutral)

Increasing solute polarity

log k RPA

log k C18

Columns with Different Phases Should Be Orthogonal: C18 vs Phenyl

Fitted Line Plot

log k Ph = 0.1778 + 0.6051 log k C18

$R^2 = 0.41$

S	0.347365
R-Sq	41.3%
R-Sq(adj)	40.6%

13 mM ammonium acetate, 30:70 H_2O:ACN, pH 6.91, 50 solutes (acid, base, neutral)

Increasing solute polarity

log k Ph (y-axis)

log k C18 (x-axis)

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Columns with Different Phases Should Be Orthogonal: C18 vs PFP (F5)

Fitted Line Plot

$C18\_1 = -0.04069 + 0.6013\ HSF5\_1$

$R^2 = 0.63$

S	0.305416
R-Sq	63.6%
R-Sq(adj)	63.0%

13 mM ammonium acetate, 30:70 H_2O:ACN, pH 6.91, 50 solutes (acid, base, neutral)

Increasing solute polarity

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Unique Amide Selectivity - H-Bond Donors

Ascentis Express

100 cm x 4.6 mm, 35% ACN 65% water, 0.2% formic acid
1.8 mL/min, 230 nm, 35 °C, 5 µL

RP-Amide more
retentive than C18
due to hydrogen
bonding

Phenols:
1. π -methoxyphenol
2. phenol
3. π -cyanophenol
4. π -fluorophenol
5. π -methylphenol
6. π -nitrophenol
7. π -chlorophenol

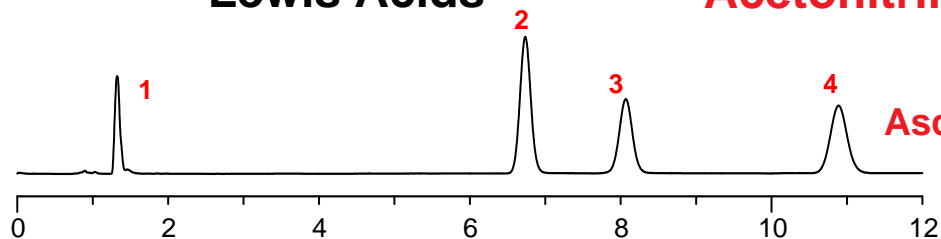**Ascentis Express RP-Amide**
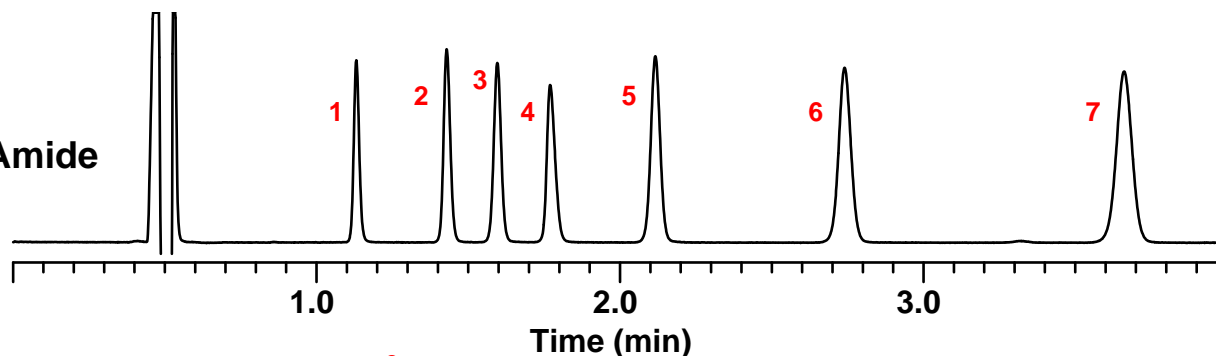
Ascentis Express C18

SIGMA-ALDRICH®
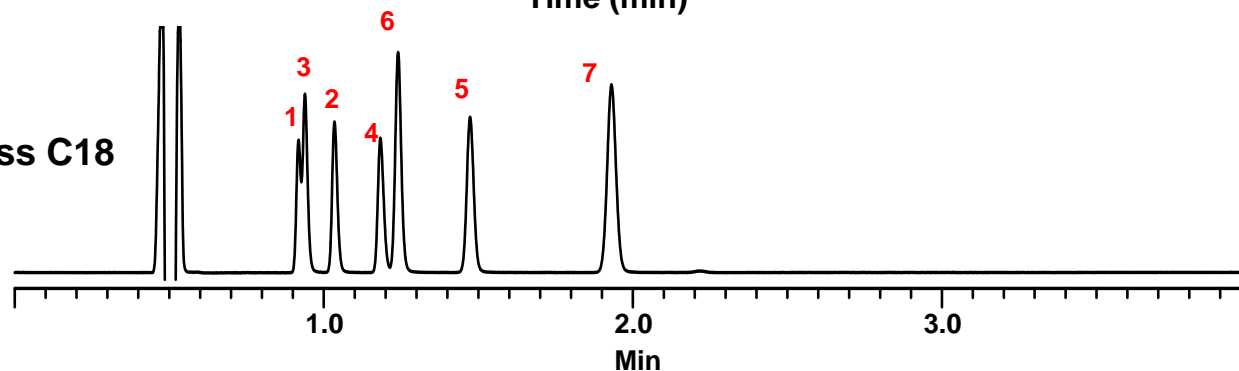
Unique Phenyl $\pi-\pi$ Selectivity

Lewis Acids　　　**Acetonitrile/Water**

Ascentis Phenyl

Ascentis RP-Amide

Ascentis C8
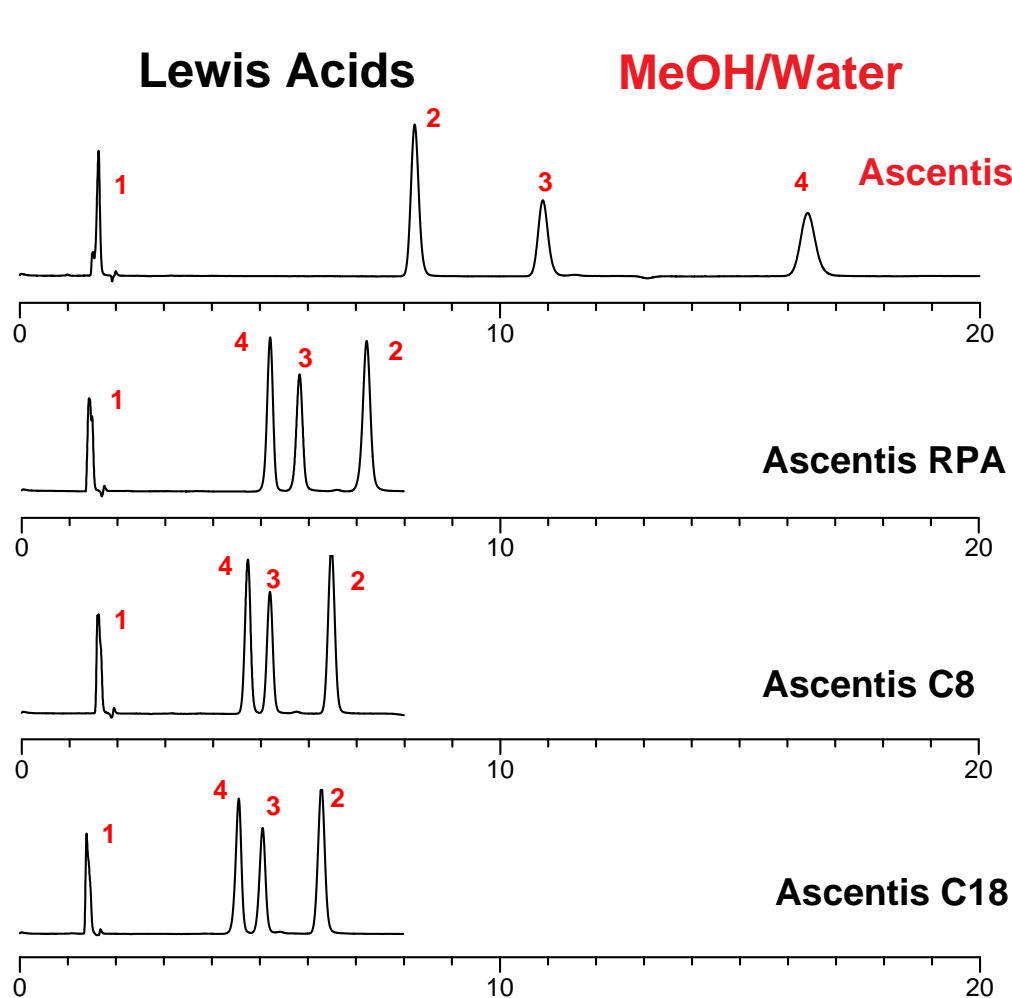
Ascentis C18

Peak ID:
1. Uracil
2. Nitrobenzene
3. 1,4-dinitrobenzene
4. 1,3,5-trinitrobenzene
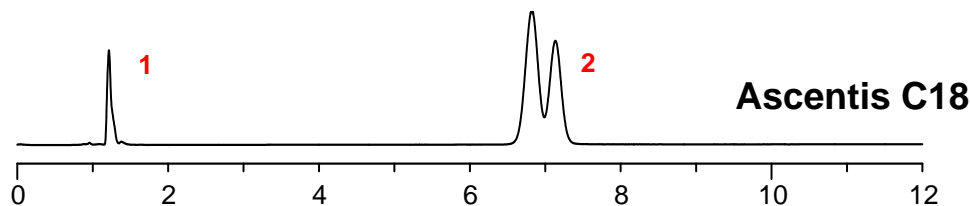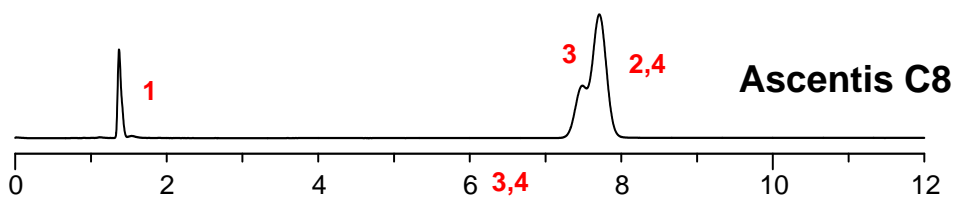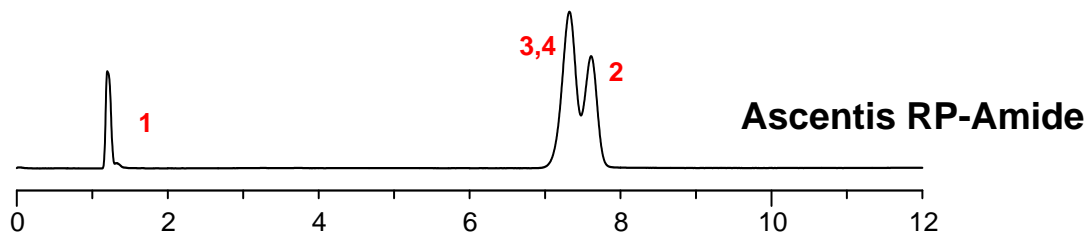
- While the different selectivity of phenyl (due to π-π interaction) is evident from the unusual elution order, selectivity for non-aromatic phases in ACN is poor.

- This may be partially due to enhanced phase solvation that has been reported in ACN (multilayer) over MeOH (monolayer).[8]

column: 150 cm x 4.6 mm, 5 μm
mobile phase: 42% acetonitrile, 58% water
flow rate: 1.0 mL/min
det.: 254 nm
temp.: 40 ºC

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Phenyl π-π Interaction Enhanced in MeOH[8]

Lewis Acids

MeOH/Water

Ascentis Phenyl

Ascentis RPA

Ascentis C8

Ascentis C18

Peak ID:
1. Uracil
2. Nitrobenzene
3. 1,4-dinitrobenzene
4. 1,3,5-trinitrobenzene

- Phenyl phase retention and selectivity is enhanced further in methanol; retention order still increases with # of nitro groups (Lewis acidity).

- Selectivity also improves when methanol is used with other phases, but the opposite (hydrophobic) RP elution order is observed
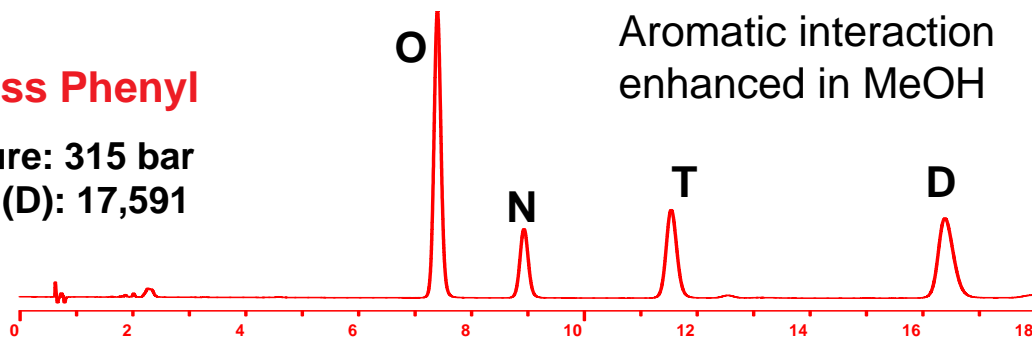
column:	150 cm x 4.6 mm, 5 μm
mobile phase:	50% methanol, 50% water
flow rate:	1.0 mL/min
det.:	254 nm
temp.:	40 ºC

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Application to Complex Heterocyclics

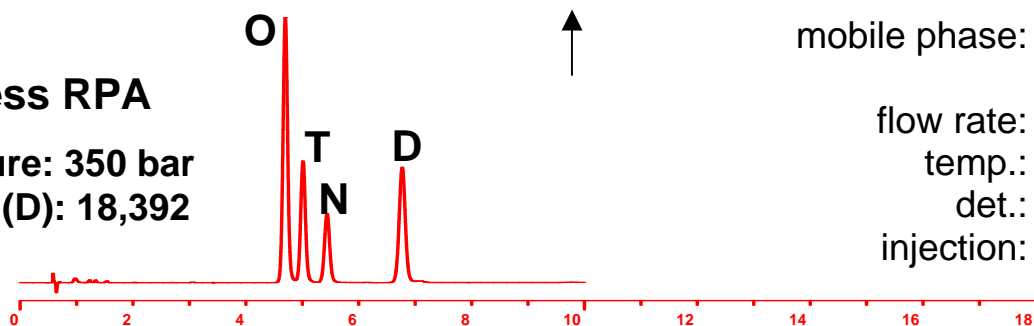**Express Phenyl**

Pressure: 315 bar
Plates (D): 17,591

Aromatic interaction
enhanced in MeOH

Benzodiazepines:
O- Oxazepam
N- Nordiazepam
T- Temazepam
D- Diazepam

Express RPA

Pressure: 350 bar
Plates (D): 18,392

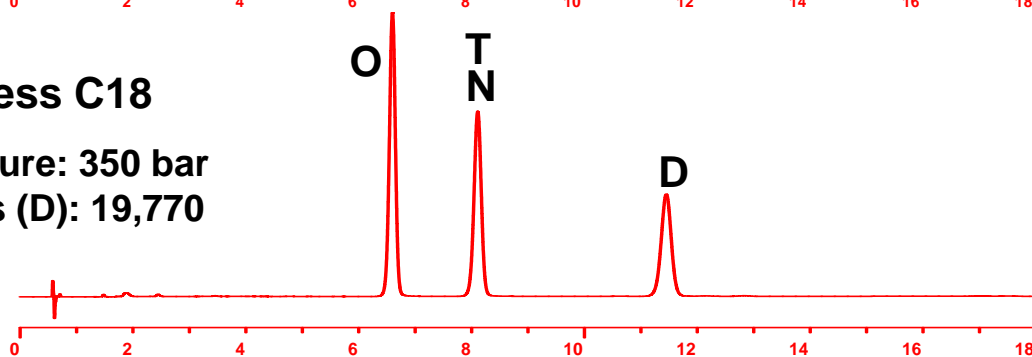mobile phase: 45% MeOH, 0.1%, formic acid,
pH 2.6
flow rate: 1.5 mL/min
temp.: 35 ºC
det.: 230 nm
injection: 10 μL

Express C18

Pressure: 350 bar
Plates (D): 19,770

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Conclusions

- Knowing HPLC column phase structures and visualizing possible phase-solute interactions should be helpful in choosing the most selective columns for initial screening and method development.

- Because solute interactions cannot be entirely predicted from phase structure, other tools such as log k plots are useful to characterize and compare column selectivity for various sets of samples.

- Some common phase structures were shown as simplified drawings and were classified according to types of phase-solute molecular interactions that might occur to cause unique retention and selectivity. The complex effects of mobile phase solvation and molecular shape were not considered.

- Separation evidence was shown to suggest that phase-solute interactions, such as hydrogen bonding and π-π interaction, occur when solvation and molecular shape variables do not interfere.

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References

1. D. Benhaim and E. Grushka, "*Amide Phase for LogP Values*", JCA (2009) in press.

2. M. R. Euerby, et. al., "*Classification of Phenyl Columns*", JCA, 1154 (2007), 138-151.

3. L.R. Snyder, J.W. Dolan and P.W. Carr, "*Hydrophobic Subtraction Model for Classification of Reversed-Phase Columns*", JCA, 1060 (2004), 77.

4. J. W. Dolan and L. R. Snyder, "*Selecting an Orthogonal Column*", JCA, 1216 (2009), 3467-3472.

5. M. R. Schure, et. al., "*Molecular Level Comparison of Alkyl and Polar-Embedded Systems*", Anal. Chem. 2008, 80, 6214-6221.

6. M. Yang, et. al.,"*Impact of Methanol and Acetonitrile on Phenyl Selectivity*", JCA, 1097 (2005), 124-129.

7. D. H. Marchand, et. al., "*Phenyl Column Selectivity*", JCA, 1062 (2005) 65.

8. Y. Kazakevitch, et. al., "*Surface Studies of Phenyl Modified Adsorbents*", JCA, 1082 (2005), 158-165.

9. Mao, Y., *Selectivity Optimization in Liquid Chromatography Using the Thermally Tuned Tandem Column (T3C) Concept*, Ph.D. Thesis (P. Carr), The University of Minnesota, 2001.

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