Stability and Performance of Cyano Bonded Phase HPLC Columns for Reversed-Phase, Normal-Phase and HILIC Applications

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T410171

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Fundamentals of Ascentis® ES Cyano

Column Selectivity Dominates Resolution Equation*

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

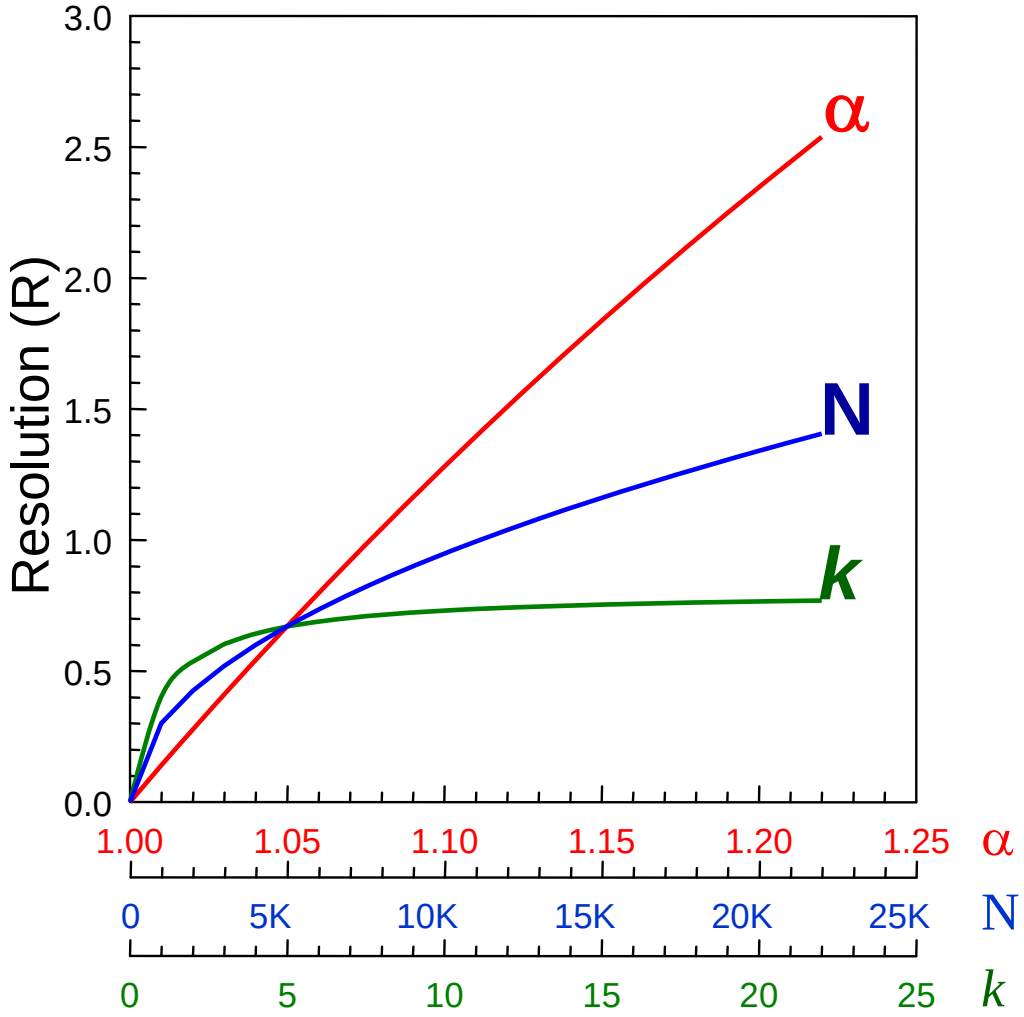
$N = 16\,(t_R/w)^2$ or

$N = 5.5\,(t_R/w)^2$

$k = (t_R - t_0)/t_0$

$\alpha = k_2/k_1$

Selectivity term is powerful being nearly linear at low α values



* Yun Mao, PhD Dissertation, University of Minnesota, 2001.

Important HPLC Stationary Phases on Silica

Choice of column is a critically important variable in determining system selectivity, but chemical interaction at solid-liquid interfaces inside pores is complex and still not well understood.

Reversed Phase

- **C18**
- **C8**
- **Embedded Polar Group (amide)**
- **Phenyl**
- **Cyano**
- **Fluorinated phenyl (PFP or F5)**

Normal Phase

- **Bare silica (no phase)**
- **Cyano**
- **F5 (PFP)**
- **Amino**
- **Diol**

Selectivity Tools in Reversed-Phase HPLC*

Continuous variables (mobile phase):

- Solvent type (methanol, acetonitrile, etc.)
- pH (ionizable solutes most influenced)
- Additives (type and concentration)
- Solvent strength (% ACN or MeOH)
- Temperature (only tool available in GC)

More predictable

(modeling software available)

Discontinuous variable (stationary phase):

- Column type (stationary phase and particle substrate)

Less predictable

(but not hopeless!)

* Used in part with permission by John Dolan

Classification by Chemical Interaction Types[a]

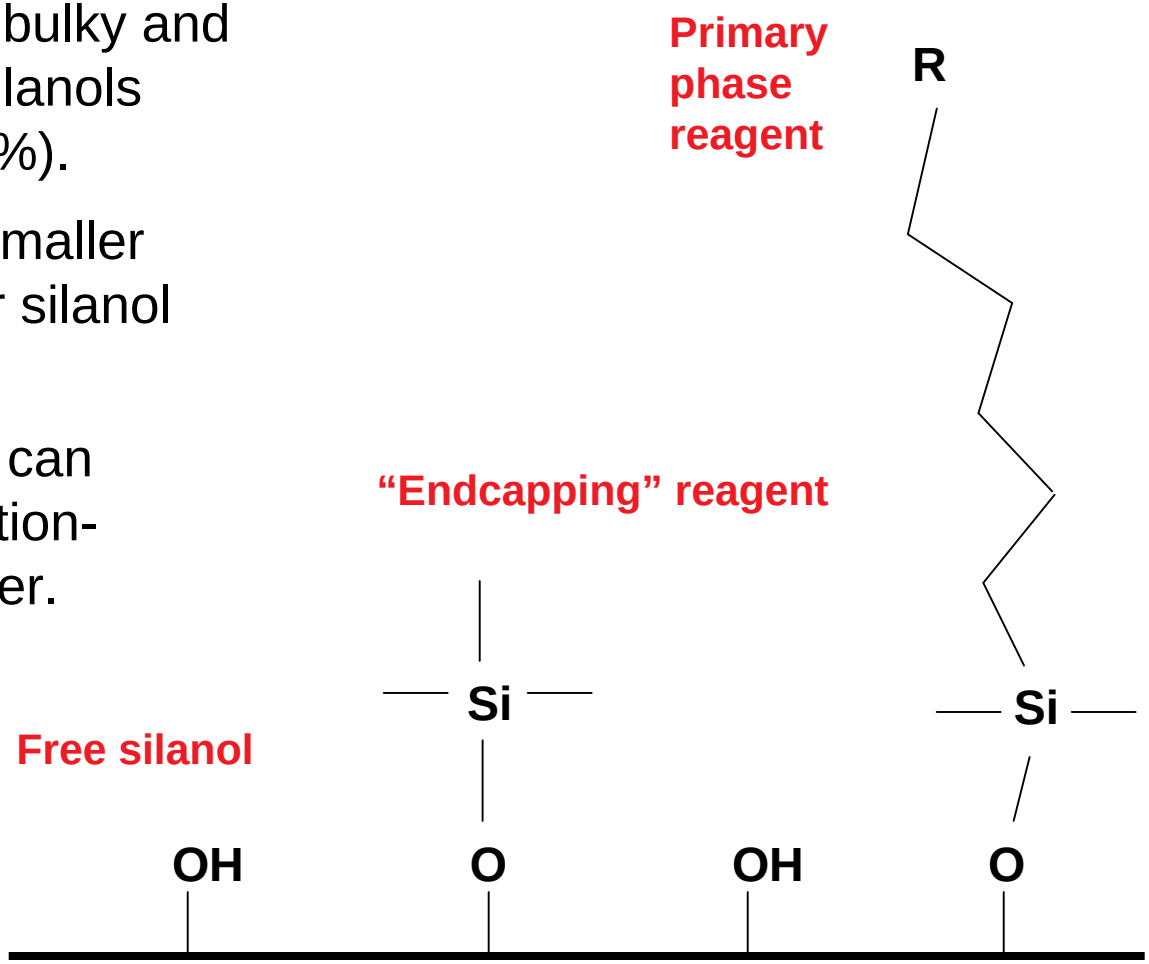
Polarity

Bonded Phase	Dispersive (Hydrophobic)	H-Bonding	Dipolar	π-π	Shape	Ionic
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	Moderate	Weak Acceptor	Weak	Strong Donor	Strong (planar)	Weak
Cyano	Moderate	Weak Acceptor	Strong	Weak	No	Strong
F5/PFP	Moderate	Moderate Acceptor	Strong	Strong Acceptor	Strong (planar)	Strong

a. Using Euerby[2] variation of Snyder-Dolan-Carr Hydrophobic Subtraction Model[3].

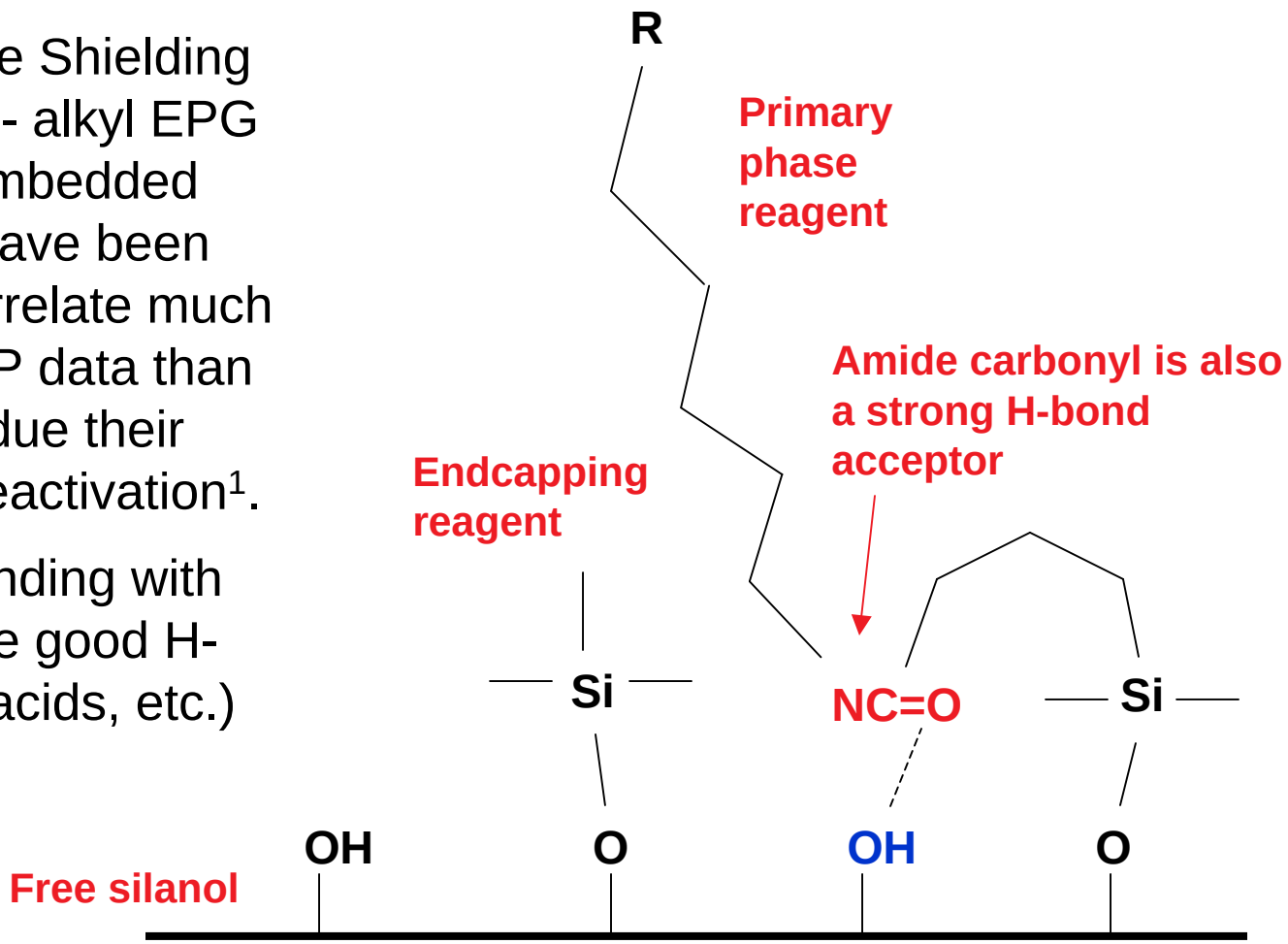
Alkyl Bonded Phase (C18 and C8)

- C18 reagents are bulky 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.



Amide (EPG) Bonded Phase

- 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 their higher base deactivation[1].
- Possible H-bonding with solutes that are good H-bond donors (acids, etc.)



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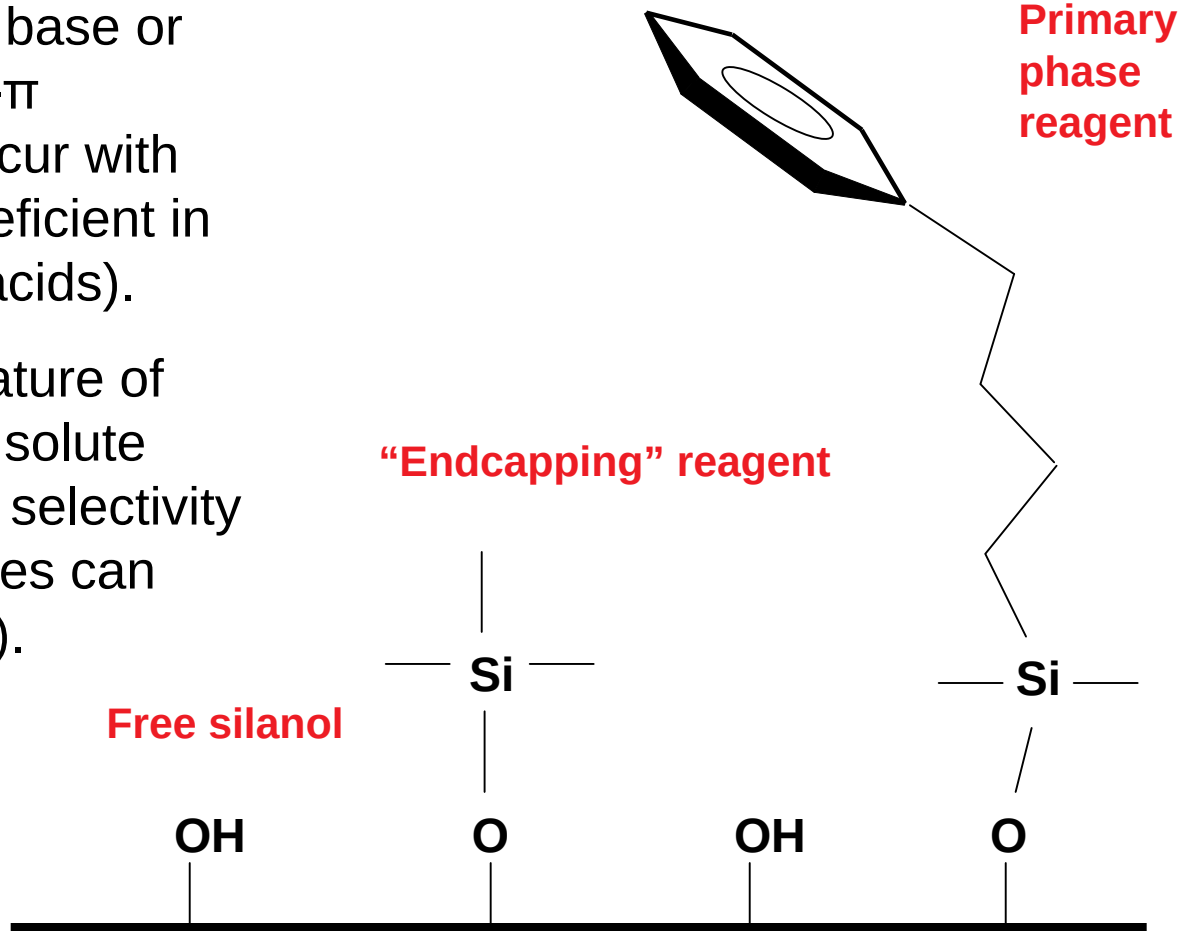
Phenyl Bonded Phase

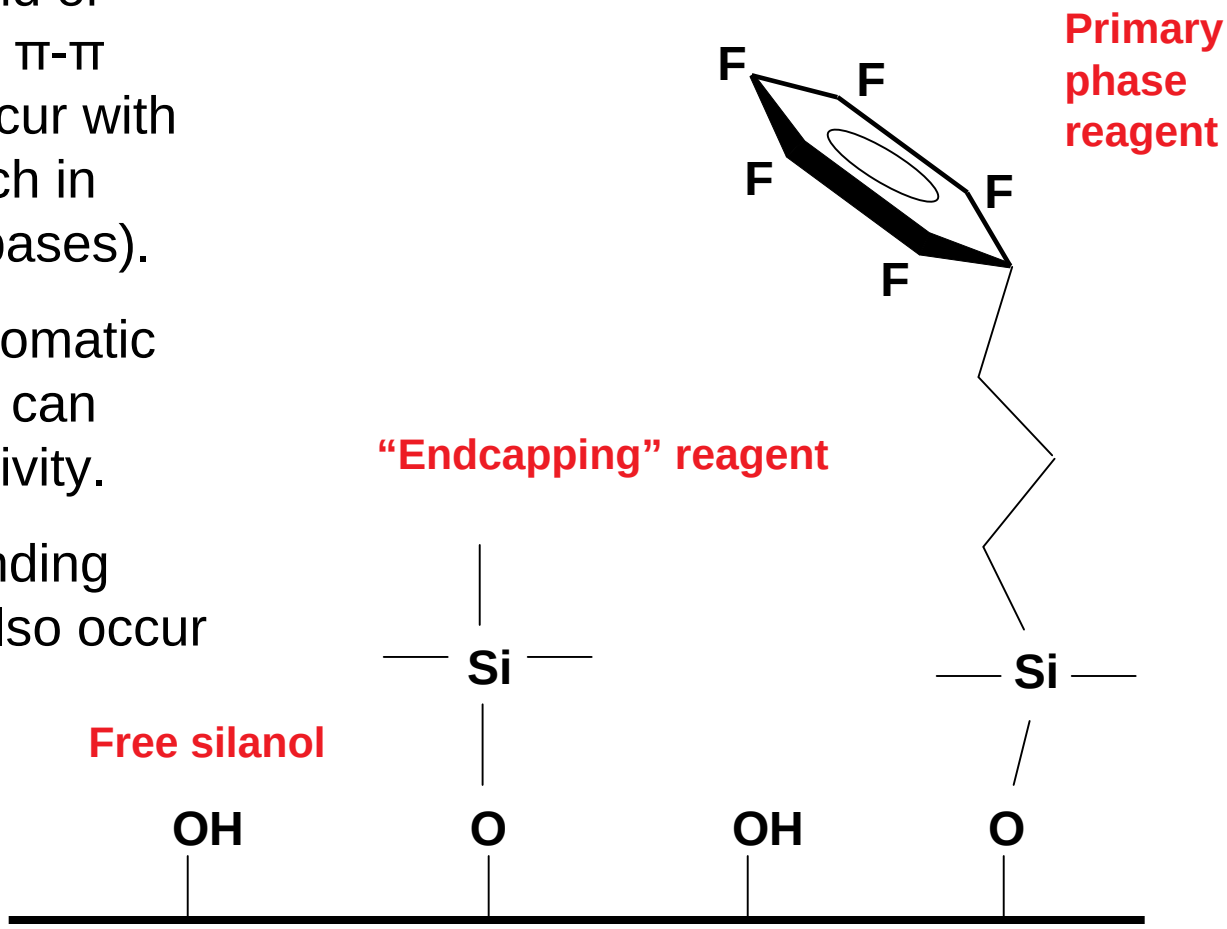
- Phenyl is a Lewis base or electron donor; π-π interaction can occur with solutes that are deficient in electrons (Lewis acids).
- Due to the rigid nature of the aromatic ring, solute shape can dictate selectivity (how closely solutes can approach the ring).



Pentafluoro-Phenyl (F5) Bonded Phase

- 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

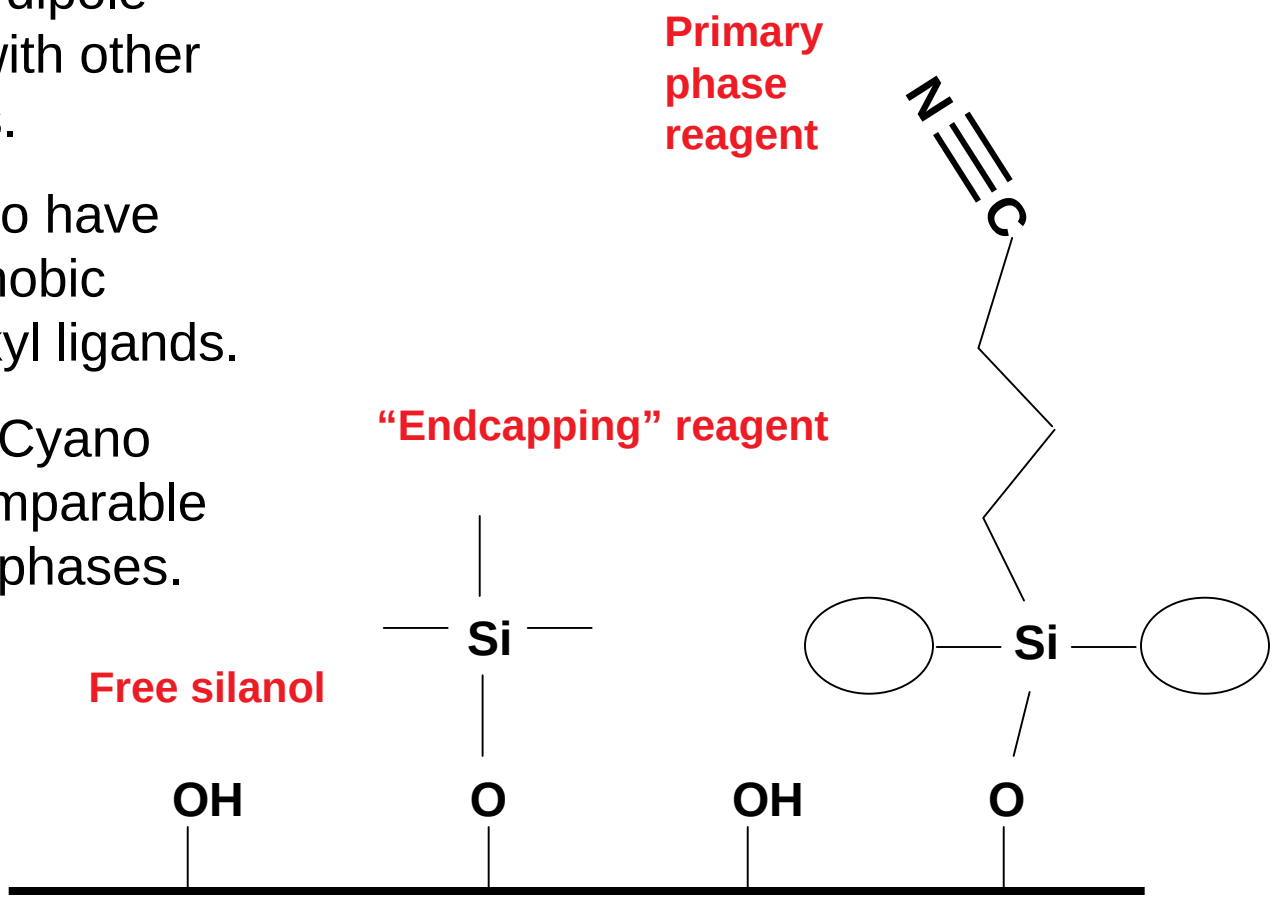
Newest Solid-Core Phase



Cyano Bonded Phase

- Cyano is a strong dipole that can interact with other 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.

Newest Porous Phase



Ascentis ES-Cyano Features and Specififcations

Features: Strong dipole attracts and separates polar molecules; works very well in both reversed-phase and normal-phase modes; extremely useful in HILIC mode; designed for high RP retention and long lifetime; very compatible with LC/MS.

Specifications:

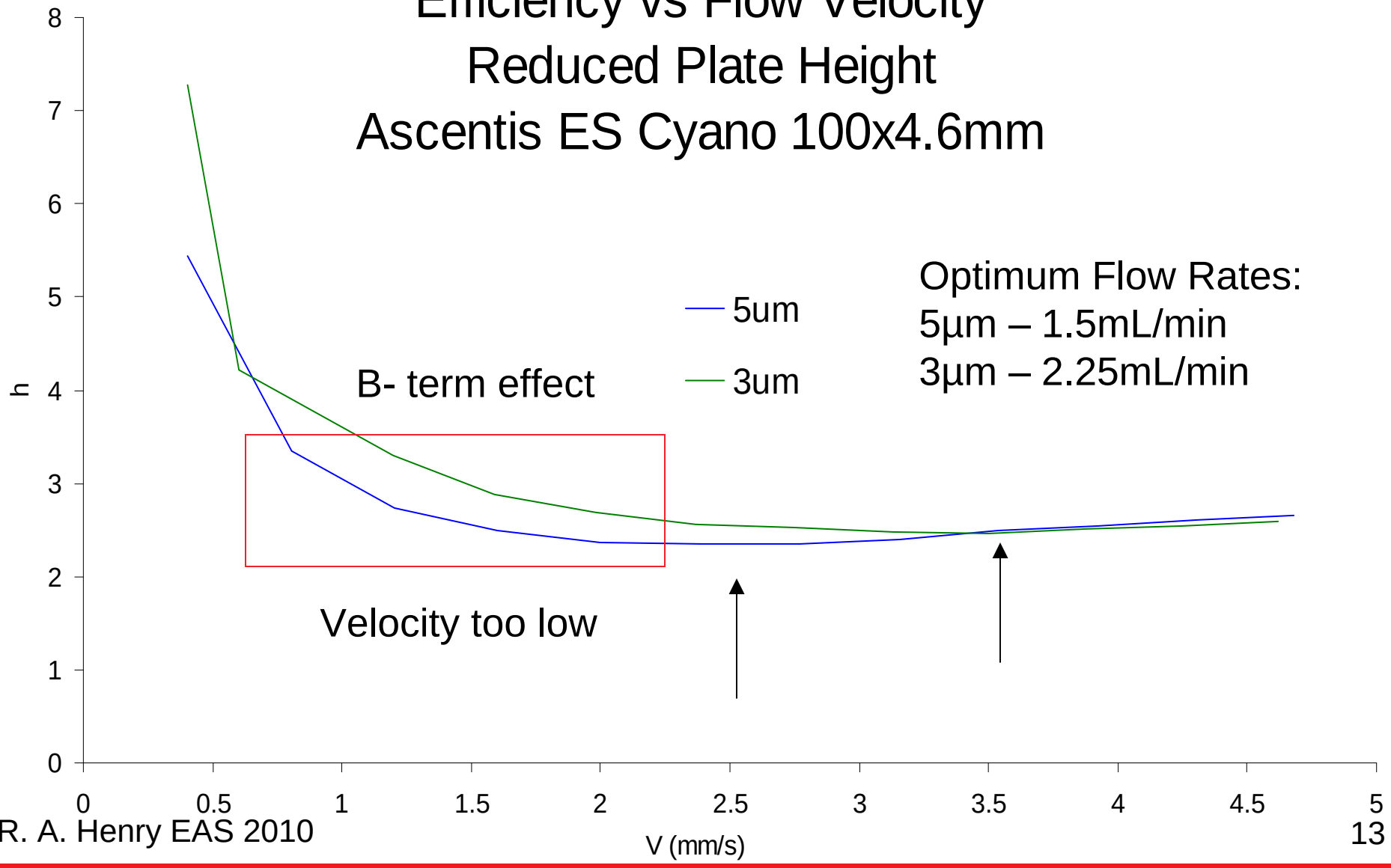
- USP Code- L10 (Cyano)
- Phase- Diisopropylcyanopropyl main phase; silanols endcapped
- Phase load- 10% carbon by wt
- Particle substrate- Spherical silica, type B (<5ppm metals)
- Surface area- 450 m^2/g
- Particle Pore size- 100Å
- Particle size- 3µm and 5µm
- pH range for longest life- 1-8

Toluene Van Deemter Plot

Efficiency vs Flow Velocity

Reduced Plate Height

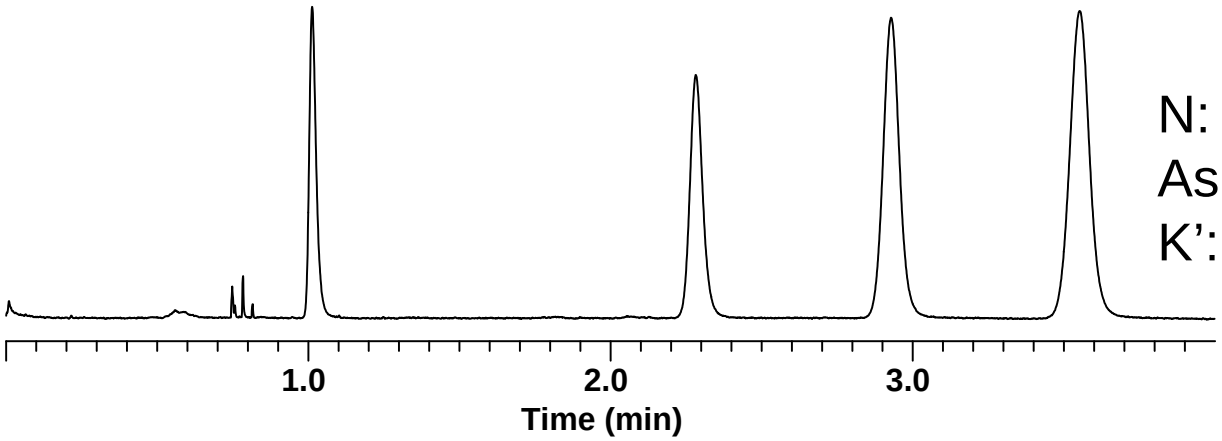
Ascentis ES Cyano 100x4.6mm



Batch Test 1 – Ascentis ES Cyano, 5µm

50% Acetonitrile 50% Water
1.5mL/min, 35ºC, 254nm, 10µL
150x4.6mm 5µm BL: 7122

Elution Order:
1. Uracil
2. Acetophenone
3. Benzene
4. Toluene



N: 14,610
As: 1.05
K': 2.51

Comparison to Express Fused-Core®
Plates: 30-35,000 /15cm

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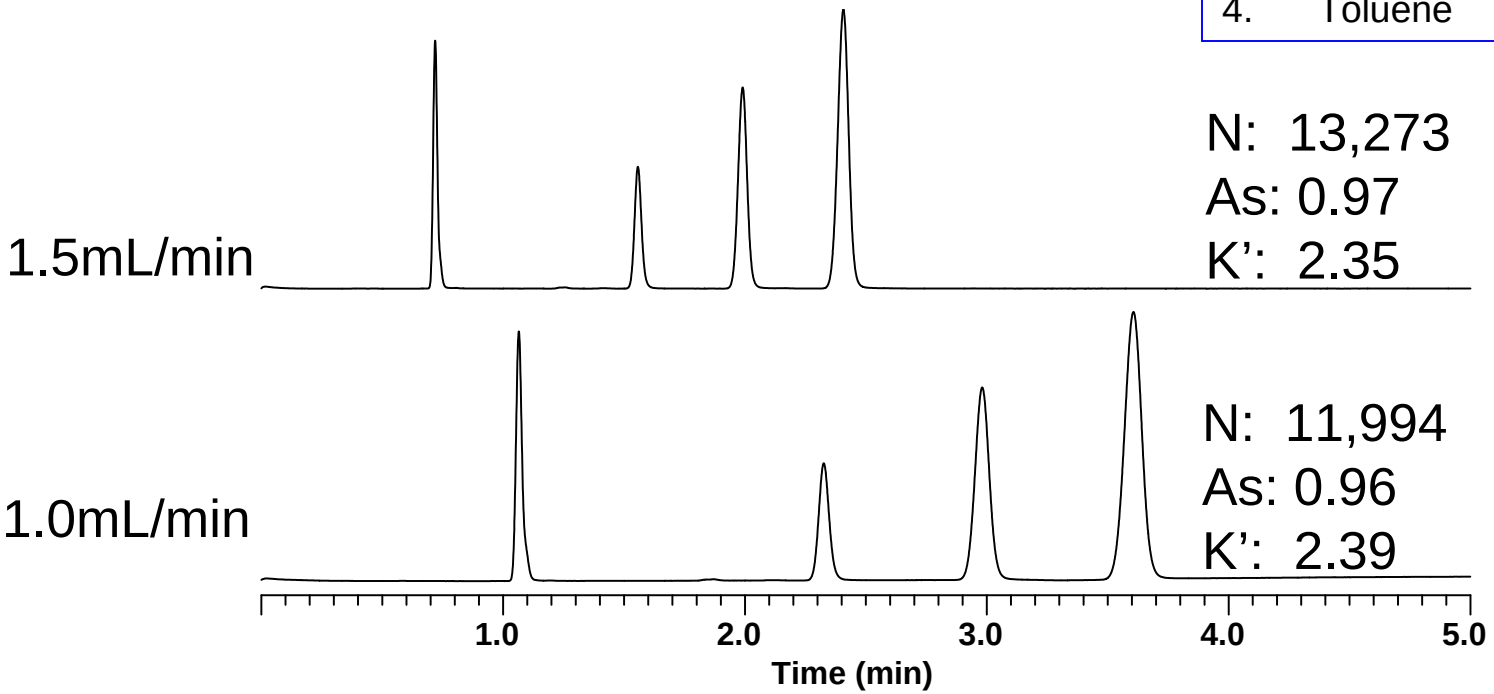
Batch Test 1 – Ascentis ES Cyano, 3µm

50% Acetonitrile 50% Water
35ºC, 254nm, 10µL
100x4.6mm 3um, BL 7141

Elution Order:
1. Uracil
2. Acetophenone
3. Benzene
4. Toluene



Comparison to Express Fused-Core®
Plates: 20-25,000 /10cm

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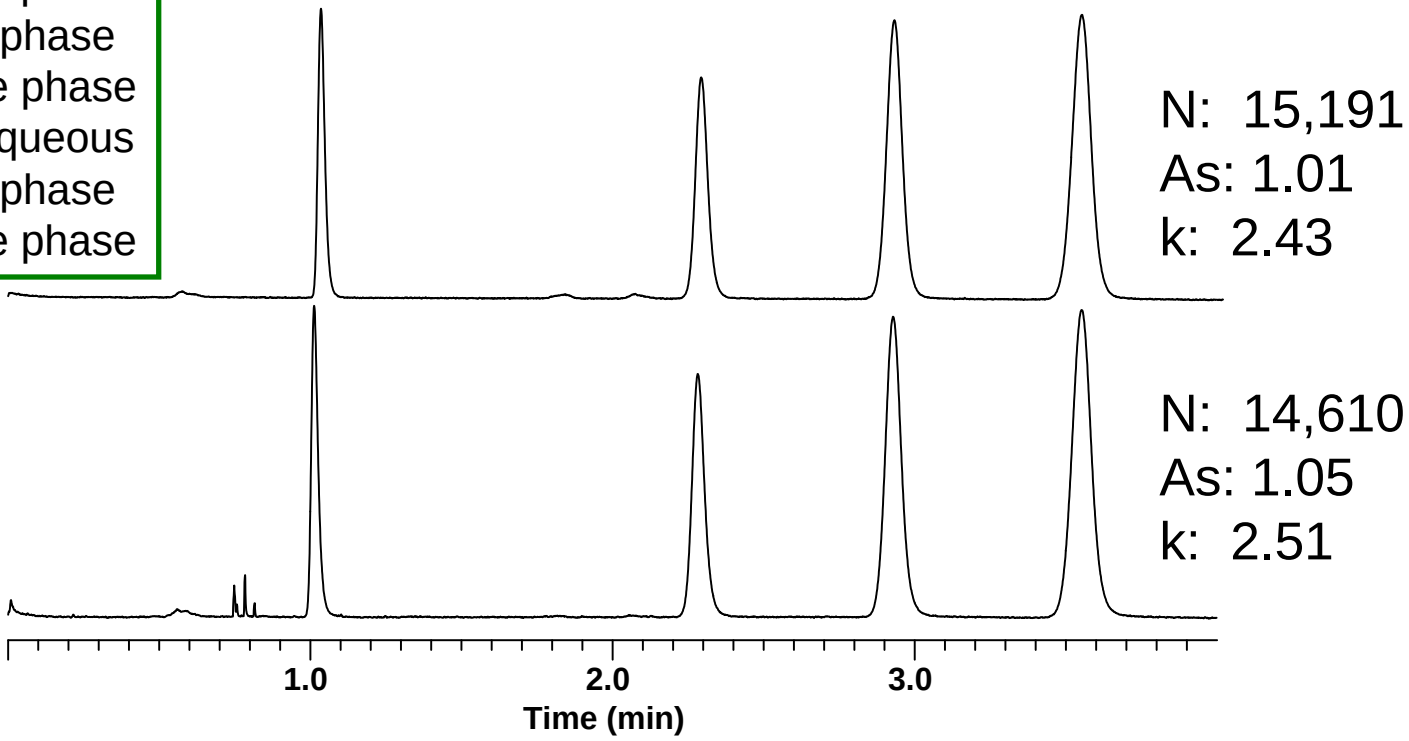
Chromatography Mode Switching*

50% Acetonitrile 50% Water
1.5mL/min, 35ºC, 254nm, 10µL
150x4.6mm 5µm BL: 7122

Elution Order:
1. Uracil
2. Acetophenone
3. Benzene
4. Toluene

Procedure:
1. Reverse phase
2. Normal phase
3. Reverse phase
4. 100% aqueous
5. Normal phase
6. Reverse phase



*Column dedication is highly recommended

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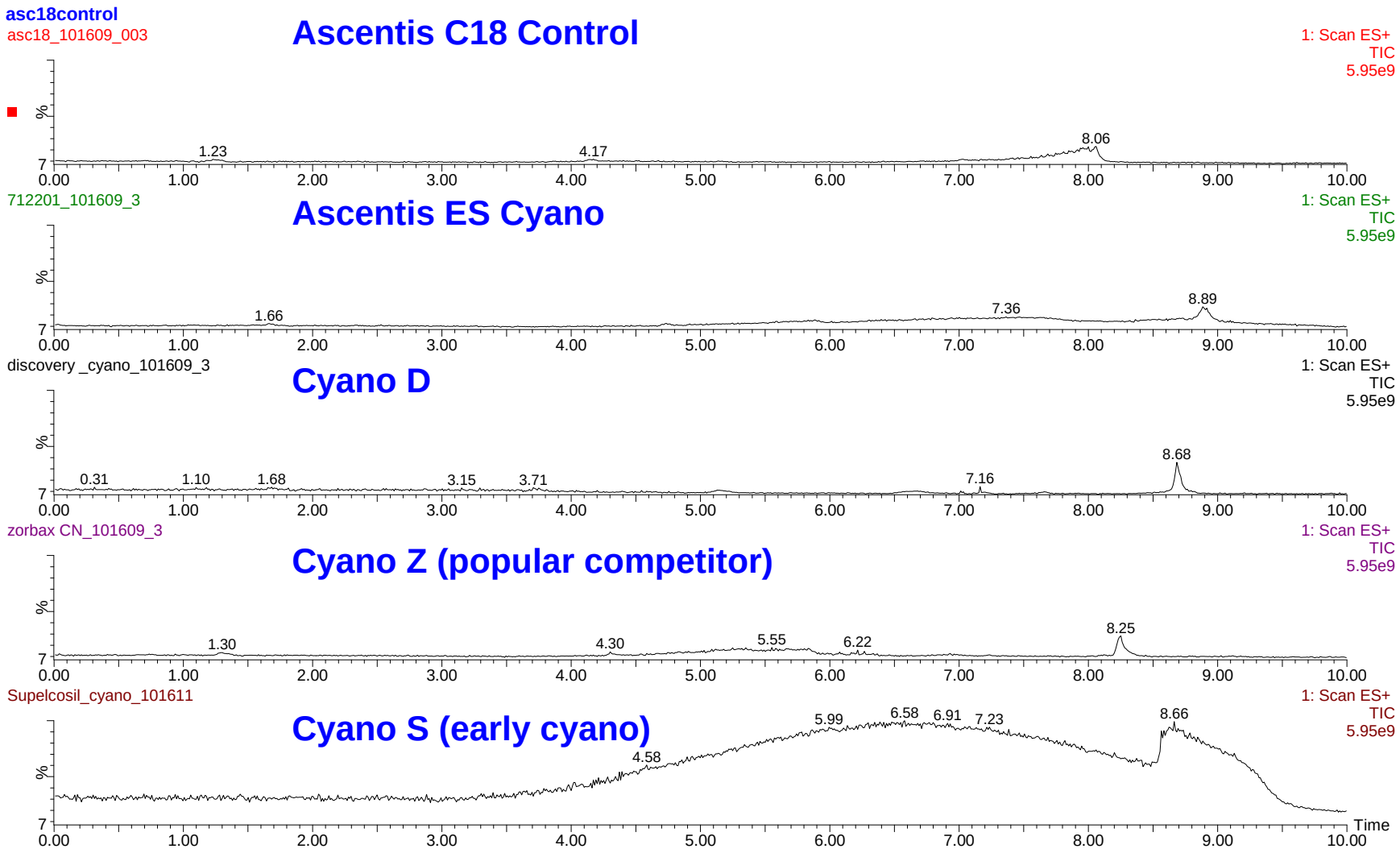
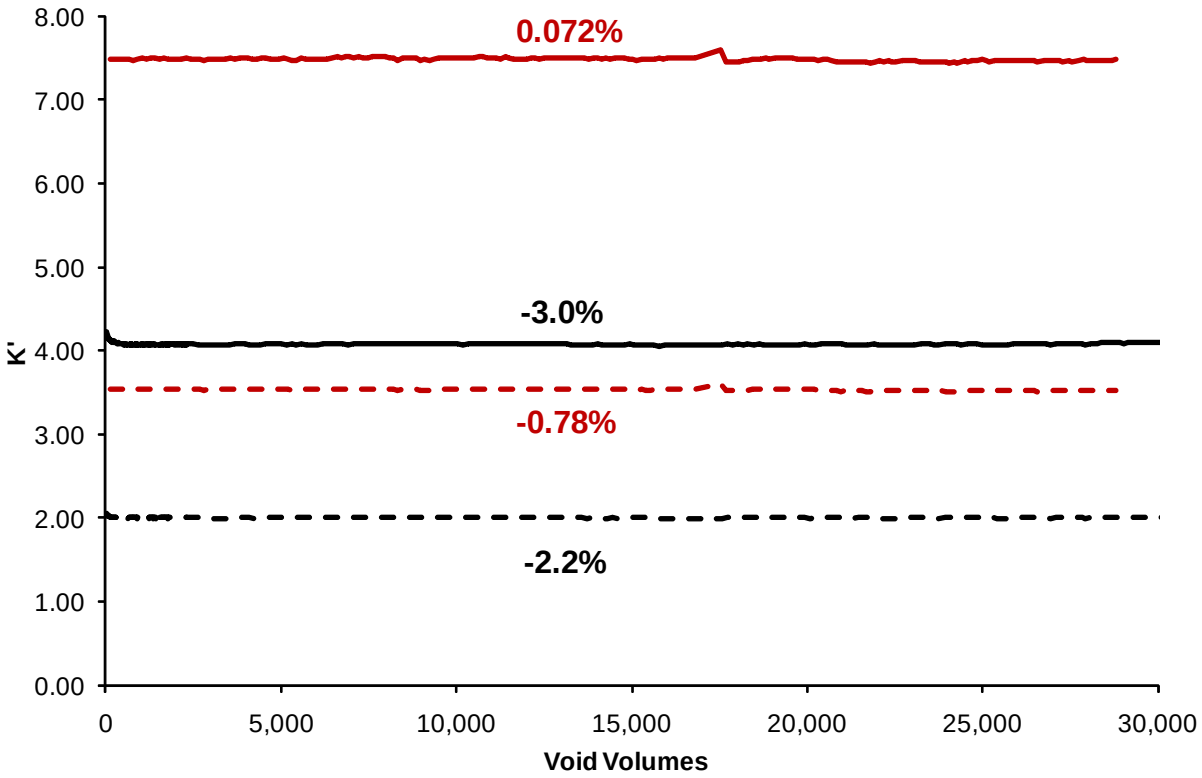
Mass Spectral Bleed TIC – Same Scales



Ascentis ES Cyano Stability: TFA 50° C



Butylparaben
- Ascentis
- Competitor

Nitrobenzene
- Ascentis
- Competitor

Over many column volumes of mobile phase at elevated temperature, Ascentis ES Cyano proved more stable than the leading CN phase.

Mobile Phase: 35% Acetonitrile 65% Water 0.1% TFA
Flow Rate: 0.8mL/min
Temperature: 50°C
Detection: UV-254nm
Injection: 2µL; one injection every 60 minutes
Sample: Uracil (5µg/mL), Nitrobenzene (130µg/mL), Butyl paraben (10µg/mL)
Dimension: 150x2.1mm, 5µm

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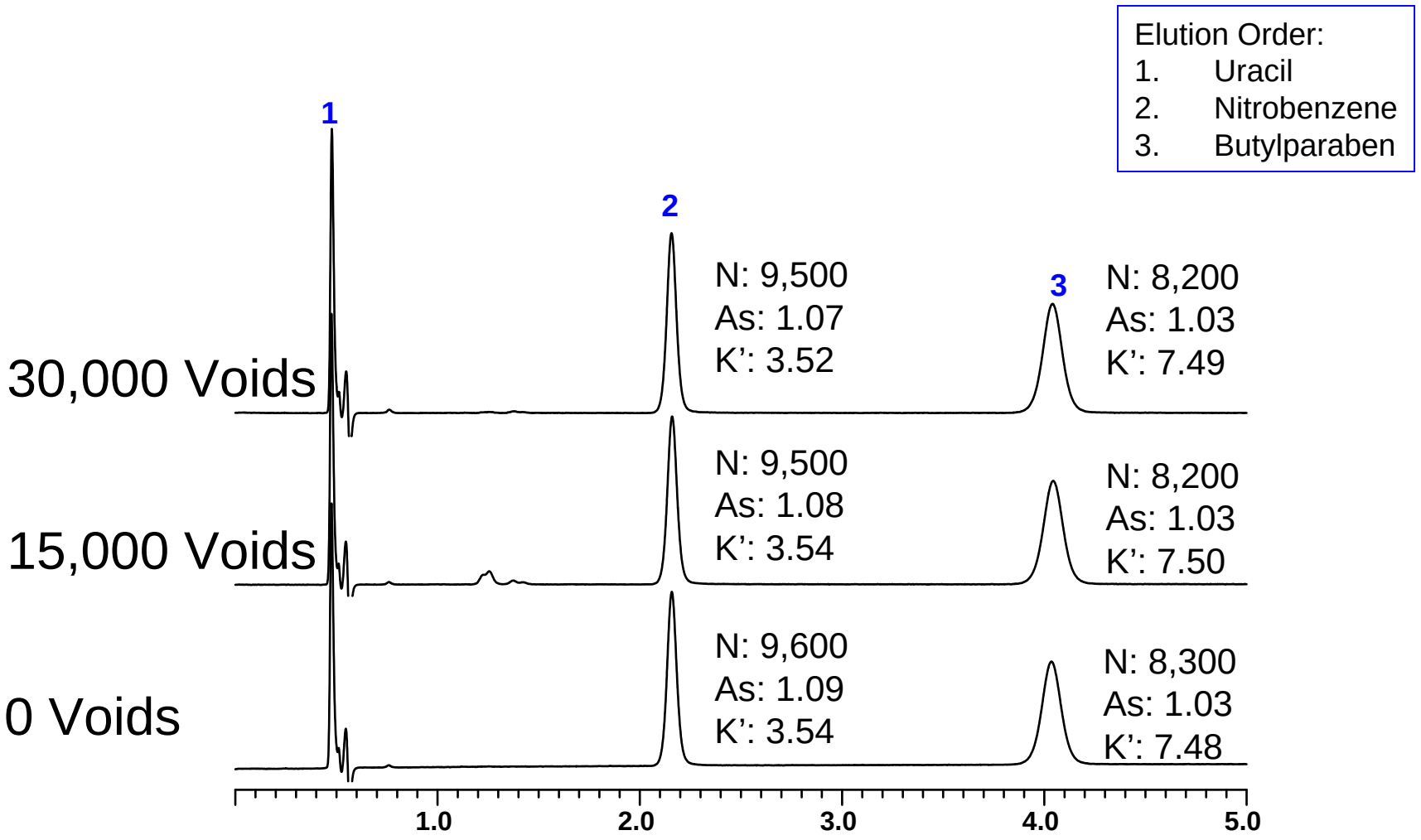
Ascentis ES Cyano Stability- RP Mode 0.1% TFA



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Quantitative Column Classification Data[2]

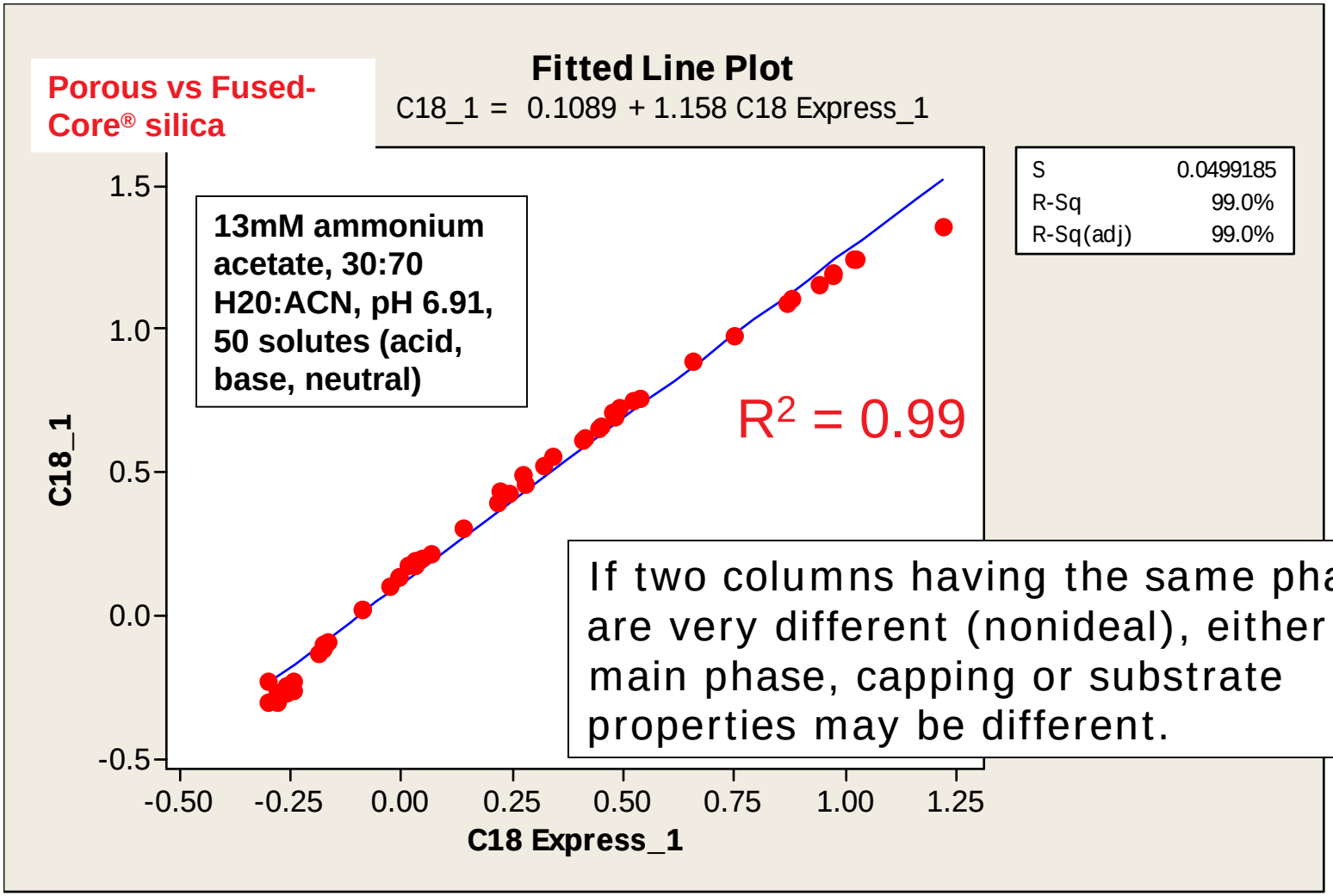
Polarity

Column Type	k_{pb}	α_{CH2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{BA/P}$ (7.6)	$\alpha_{BA/P}$ (2.7)
Ascentis C18	7.35	1.50	1.59	0.37	0.31	0.08
Ascentis RP-Amide	4.91	1.44	1.68	0.22	0.19	0.03
Ascentis C8	3.61	1.41	0.93	0.31	0.25	0.07
Ascentis Phenyl	2.54	1.37	0.97	0.95	0.38	0.11
Ascentis ES-Cyano	0.91	1.21	2.57	0.64	0.88	0.18

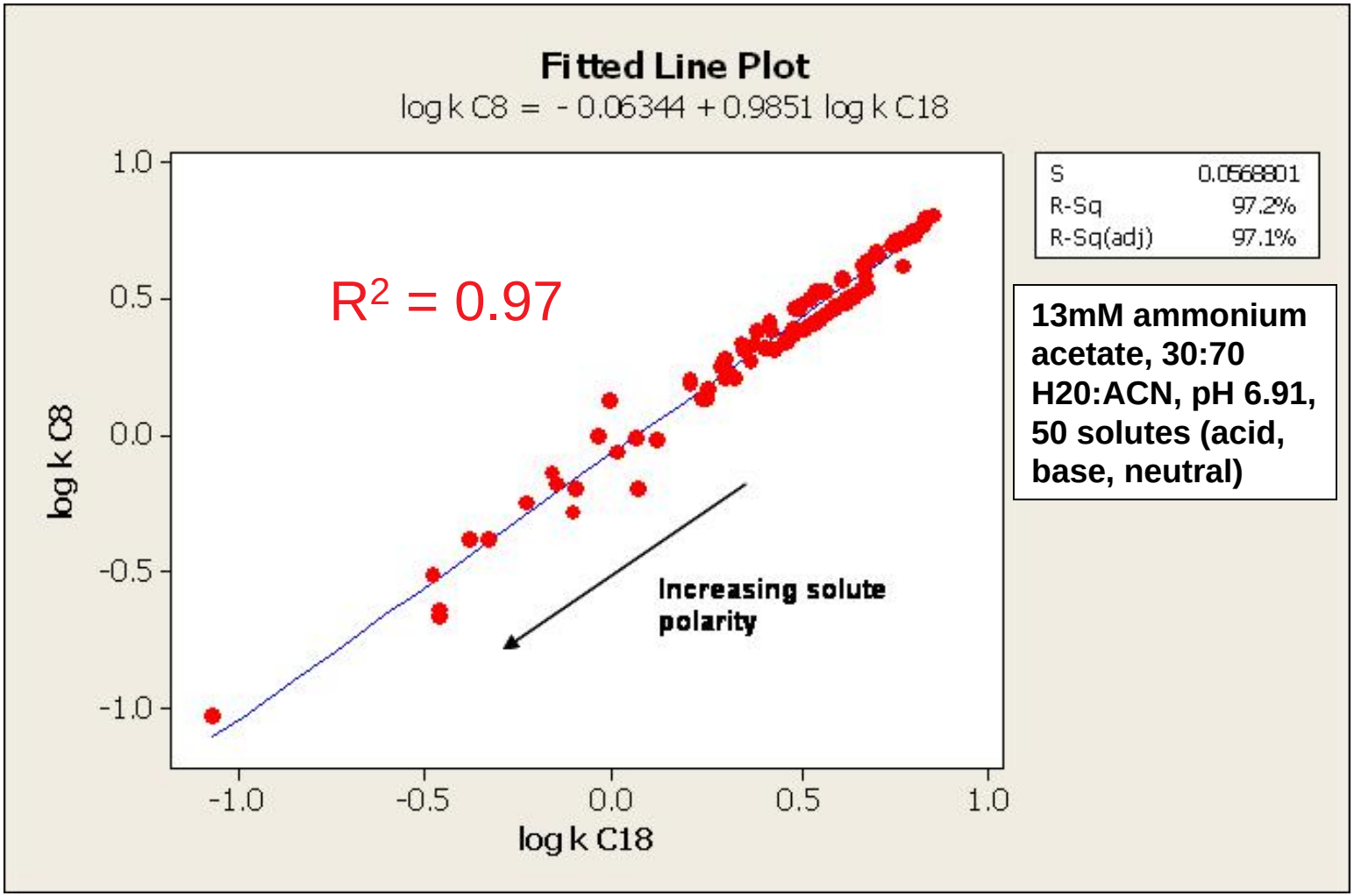
Interaction strength-hydrophobic solute

2. Euerby (Tanaka)

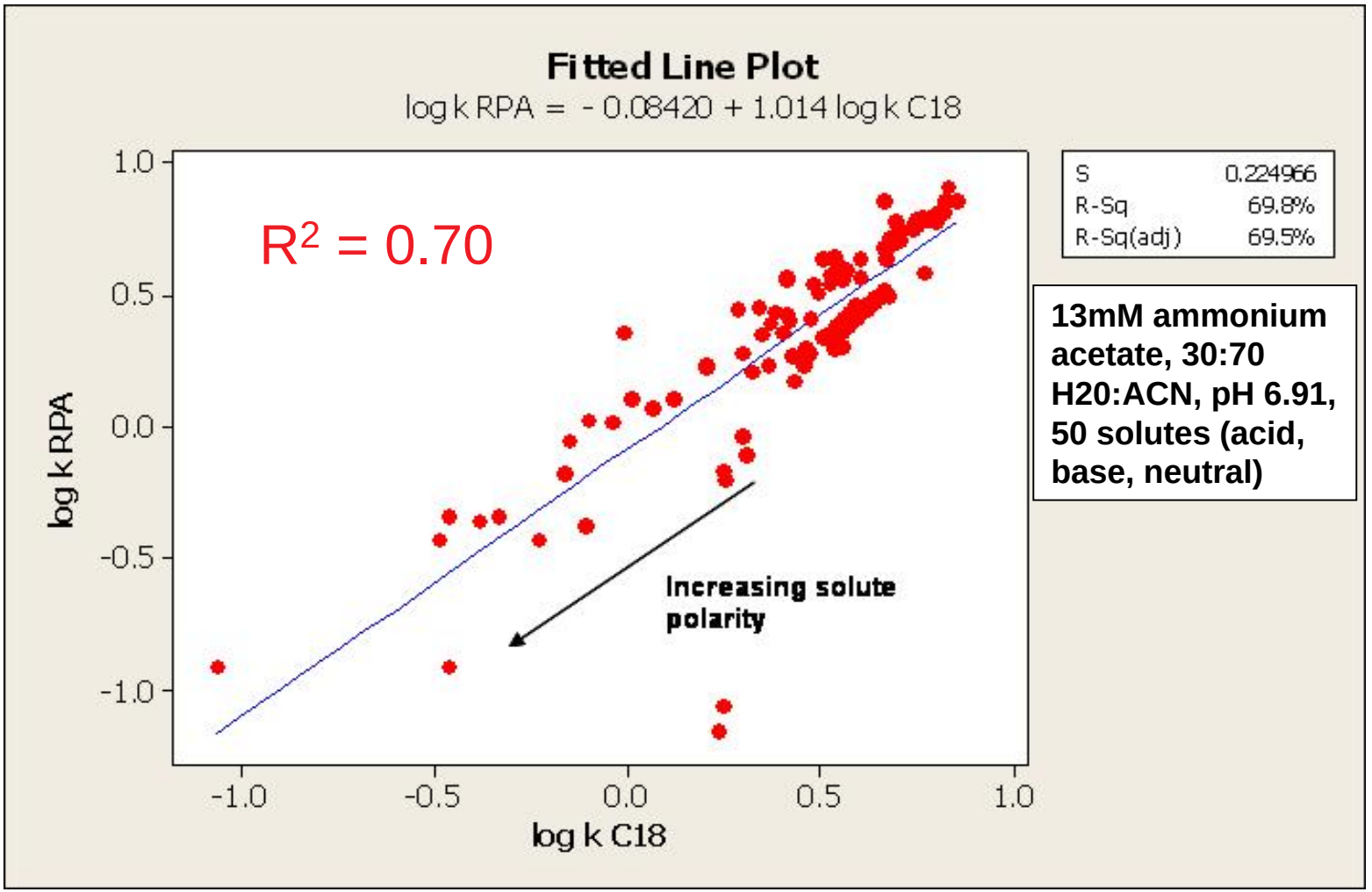
Good Correlation between Same Phases on Different Silica: Ascentis C18 vs Express C18



Columns with Similar Phases Should Also Show High Correlation: Ascentis C18 vs C8

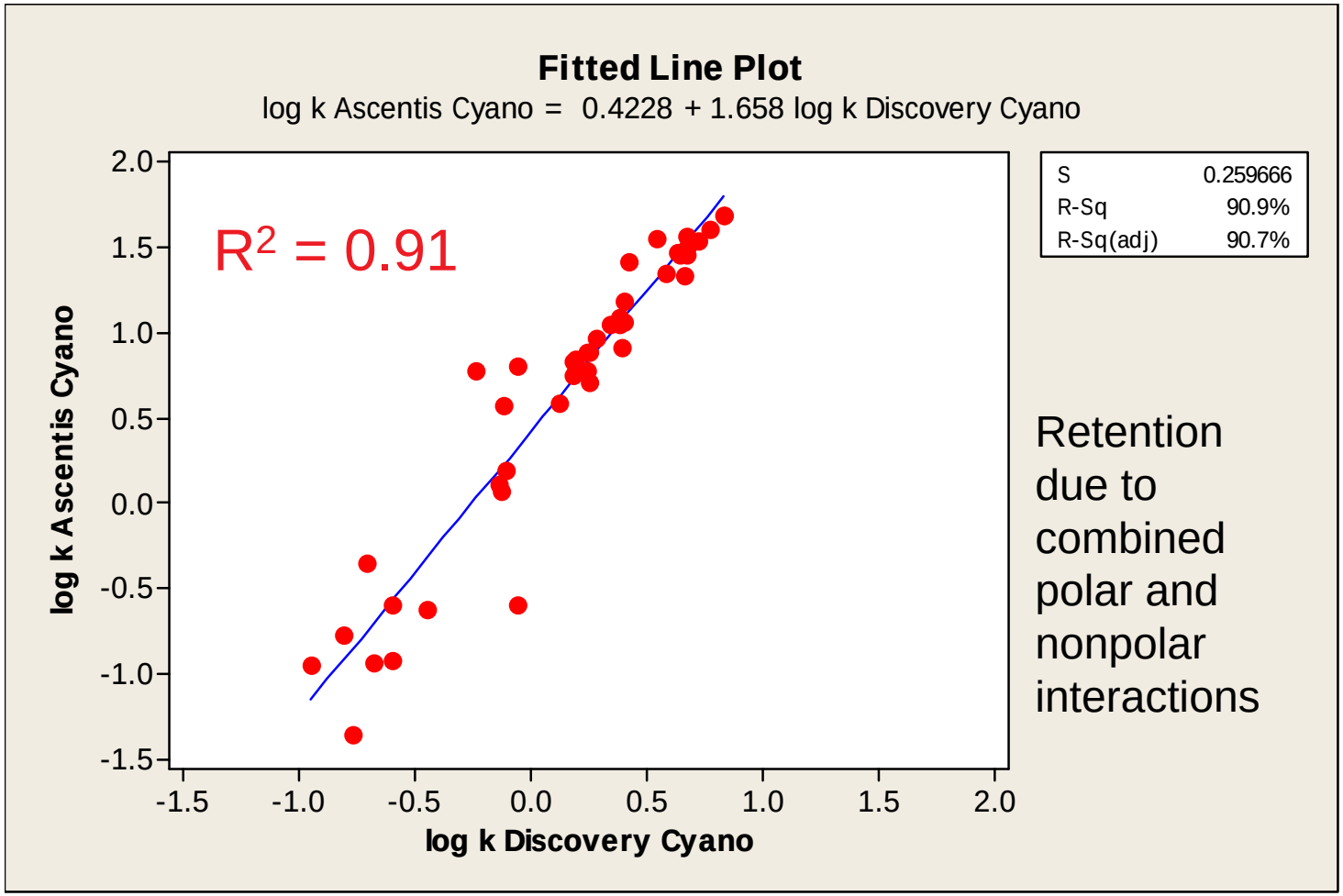


Columns with Different Phases Should Be Orthogonal (Different): Ascentis C18 vs Amide



Ascentis® Cyano vs Discovery® Cyano*, RP Mode

13mM ammonium acetate (70:30 water:acetonitrile) pH 7.0



Retention due to combined polar and nonpolar interactions

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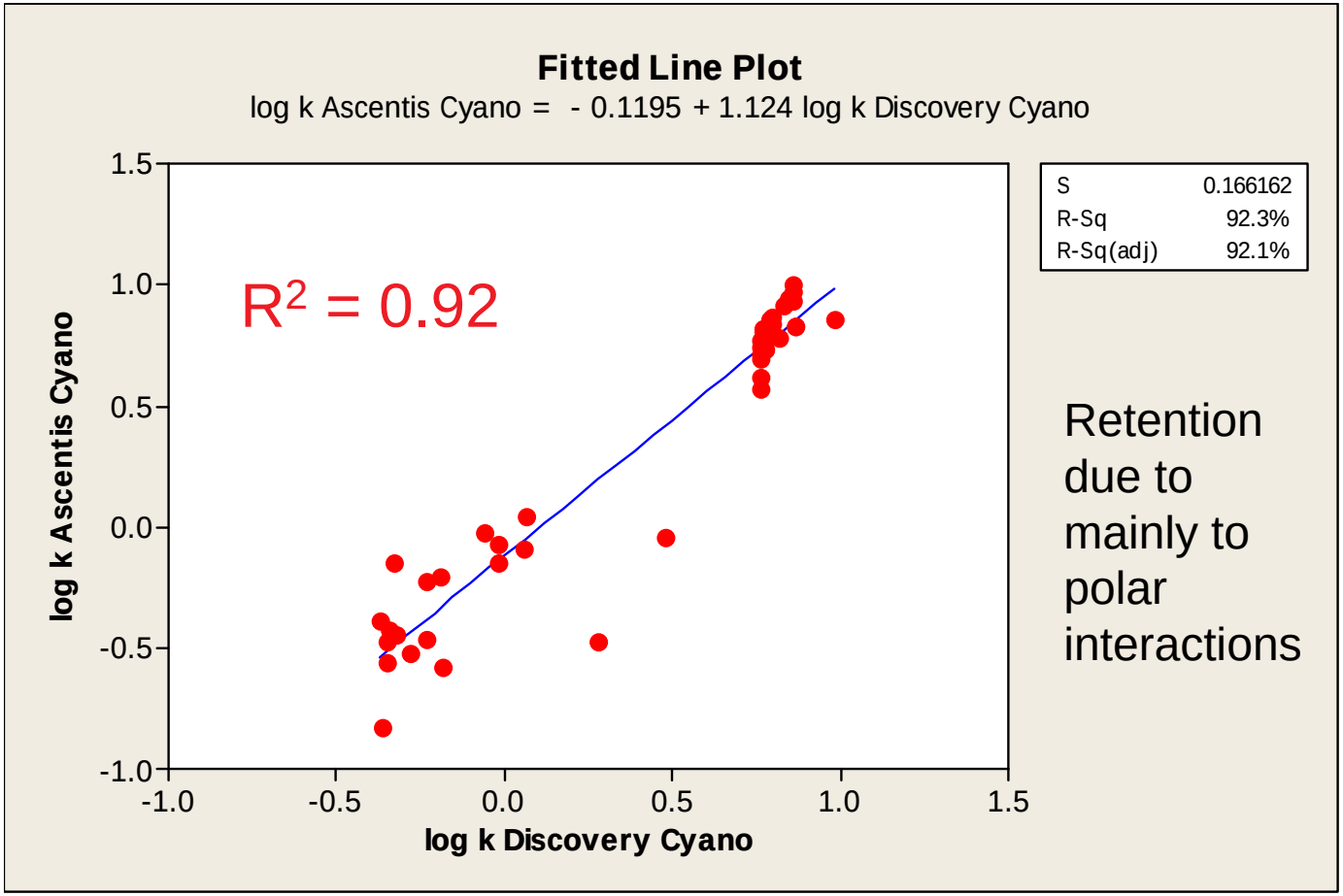
* Discovery silica is 200Å and lower surface area

Ascentis Cyano vs Discovery Cyano, HILIC Mode

2mM ammonium formate (5:95 water:acetonitrile) pH 7.2



Retention due to mainly to polar interactions

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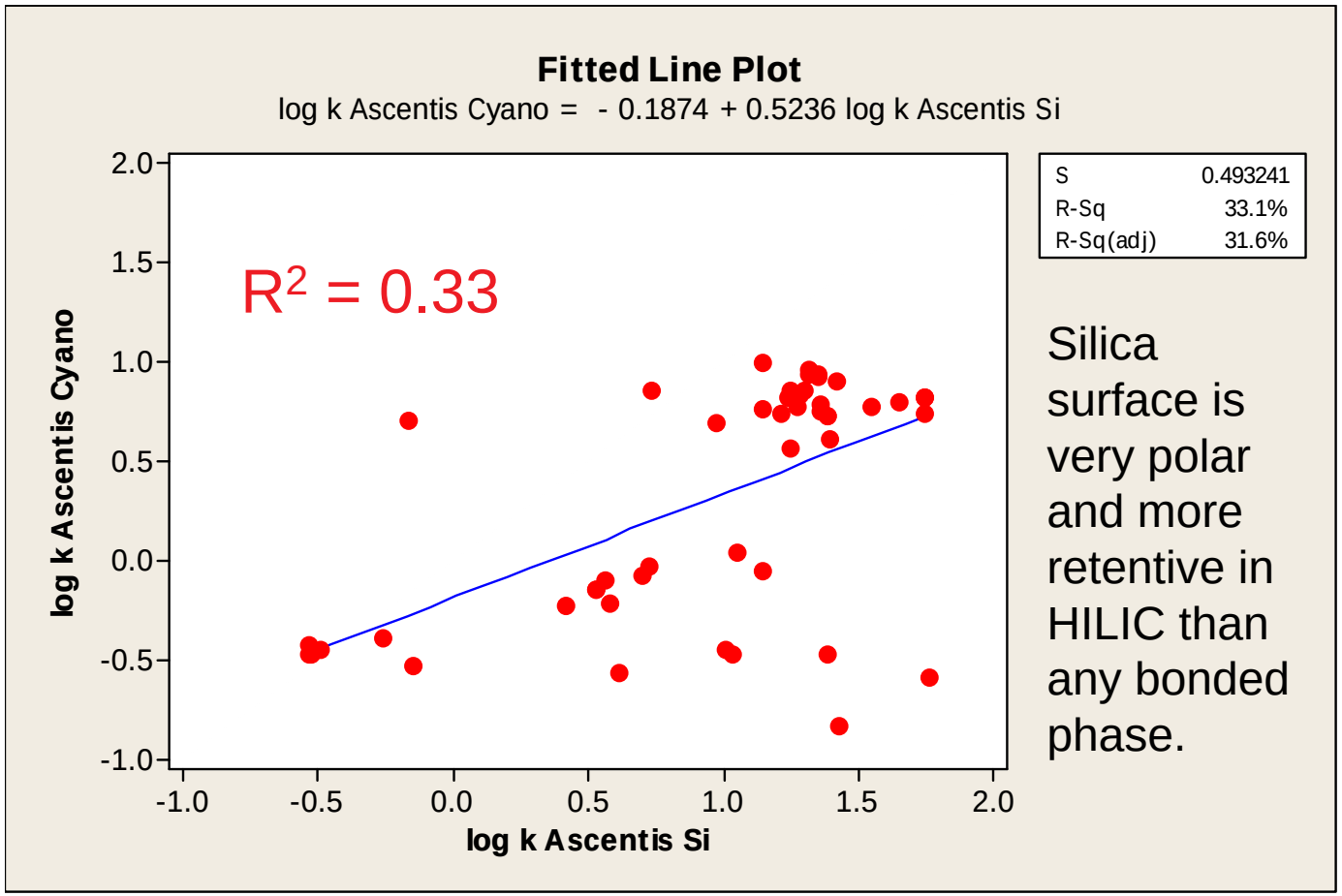
Ascentis Cyano vs Ascentis Si, HILIC Mode

2mM ammonium formate (5:95 water:acetonitrile) pH 7.2



Silica surface is very polar and more retentive in HILIC than any bonded phase.

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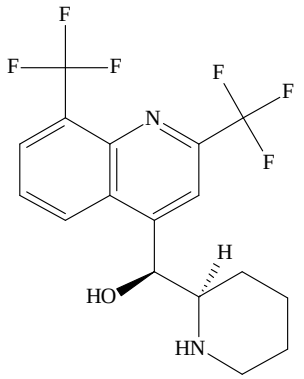
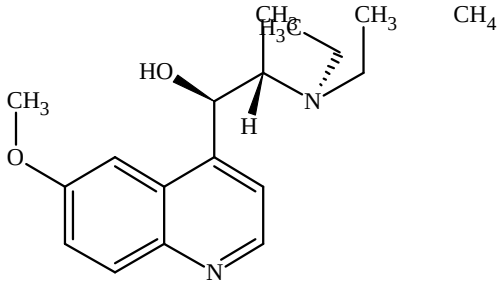
Applications of Ascentis ES Cyano

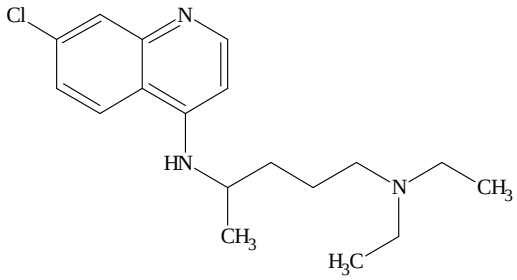
Antimalarial Compounds



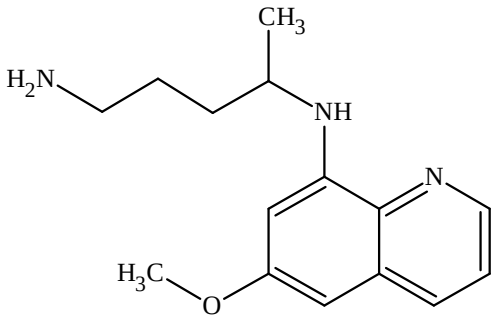
Mefloquine



Quinine



Chloroquine



Primaquine

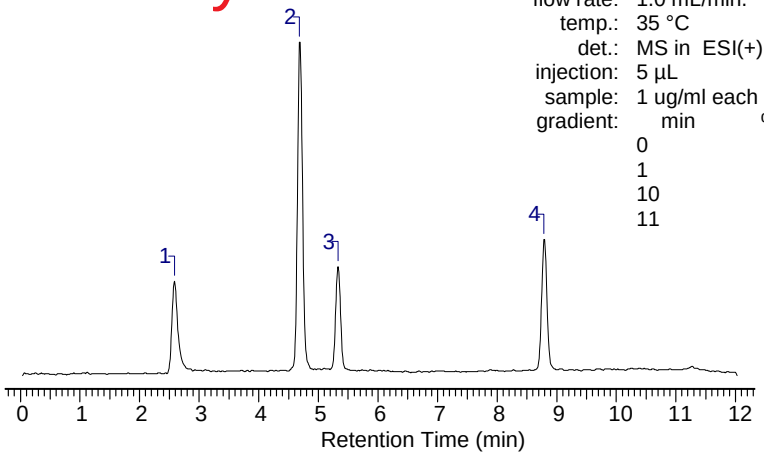
Anti-Malarial Drugs- RP Mode

Ascentis ES Cyano

column: Ascentis Cyano or Competitor CN 5 cm x 4.6 mm I.D. 5 µm particles
mobile phase A: 13 mM ammonium acetate in water (Reidel)
mobile phase B: 13 mM ammonium acetate in acetonitrile (Reidel)

flow rate: 1.0 mL/min.
temp.: 35 °C
det.: MS in ESI(+), SIR mode,
injection: 5 µL
sample: 1 ug/ml each in mobile phase A
gradient:

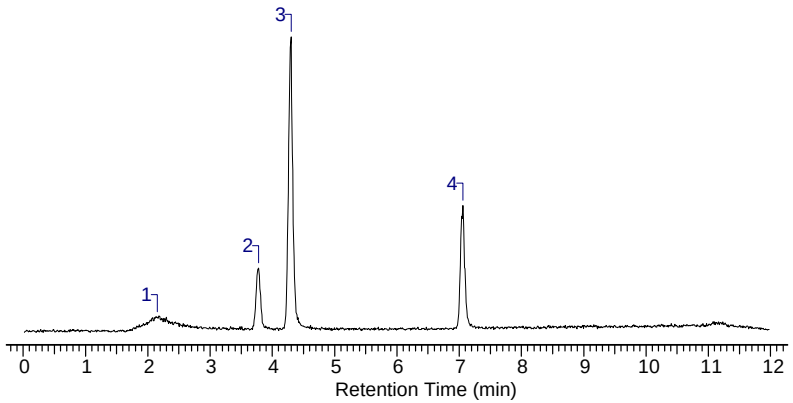
min	% A	% B
0	80	20
1	80	20
10	40	60
11	80	20



Elution Order:

1. Chloroquine
2. Quinine
3. Primaquine
4. Mefloquine

Competitor CN



Elution Order (Note elution order change):

1. Chloroquine
2. Primaquine
3. Quinine
4. Mefloquine

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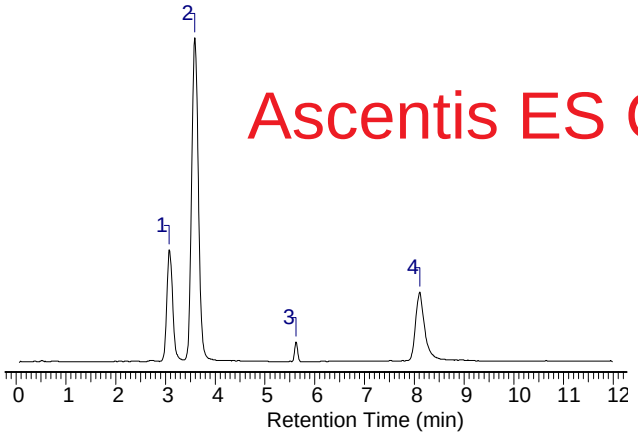
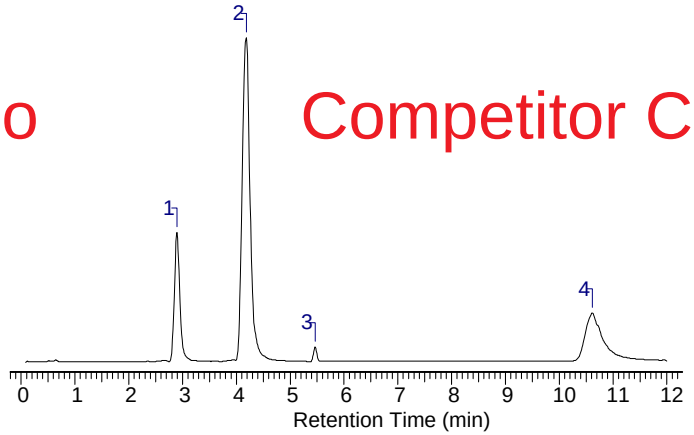
Anti-Malarial Drugs- HILIC Mode





column: Ascentis Cyano or Competitor CN 5 cm x 4.6 mm I.D.
Both 5 µm particles
mobile phase A: Water/Acetonitrile (5:95 v/v); 2 mM ammonium formate
mobile phase B: Water/Acetonitrile (10:90 v/v); 10 mM ammonium formate

flow rate: 1.0 mL/min.
temp.: 35 °C
det.: MS in ESI(+), SIR mode
injection: 5 µL
sample: 1 µg/mL each in mobile phase A
gradient:

min	% A	% B
0	100	0
3	100	0
4	0	100
12	100	0

Elution Order (reversed vs RP):

1. mefloquine
2. quinine
3. primaquine
4. chloroquine

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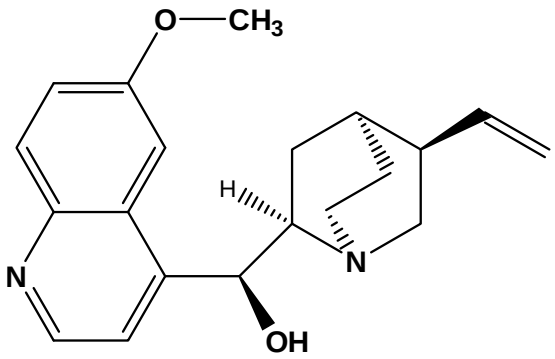
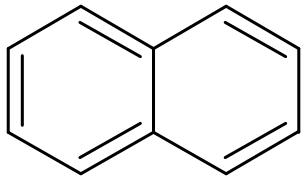
Hydrophobic Bases on ES-Cyano

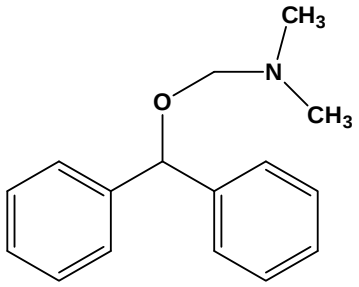
Reversed Phase Mode



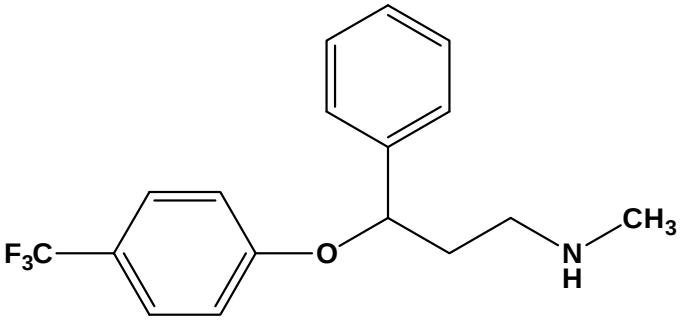
Quinidine



Naphthalene
Neutral Hydrophobic



Diphenhydramine



Fluoxetine
2º Amine and Fluorines (Polar)

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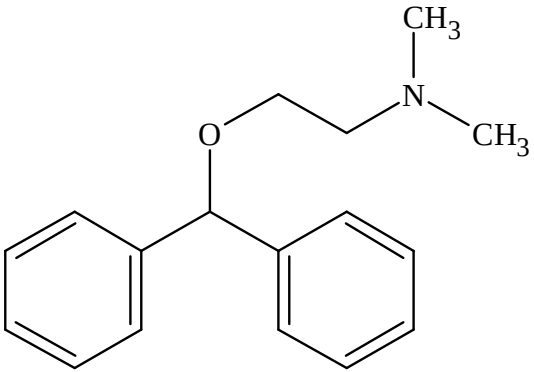
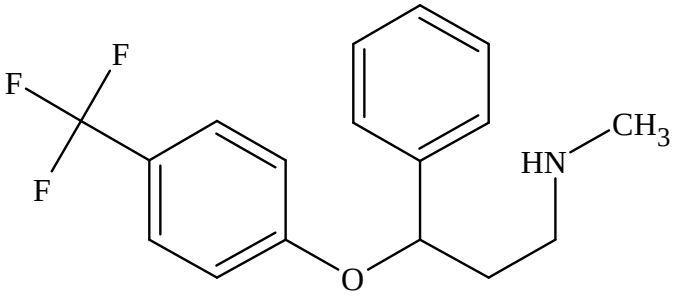
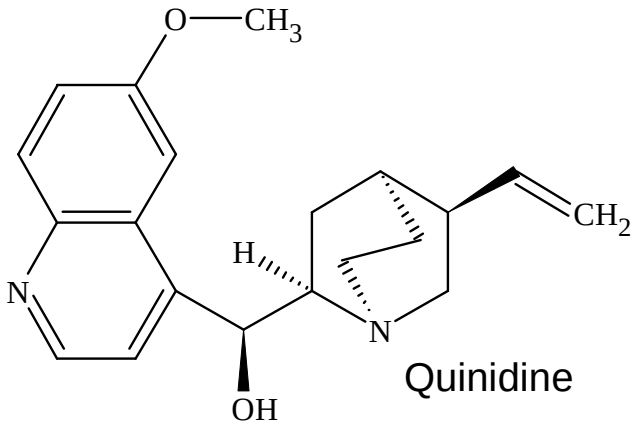


Diphenhydramine

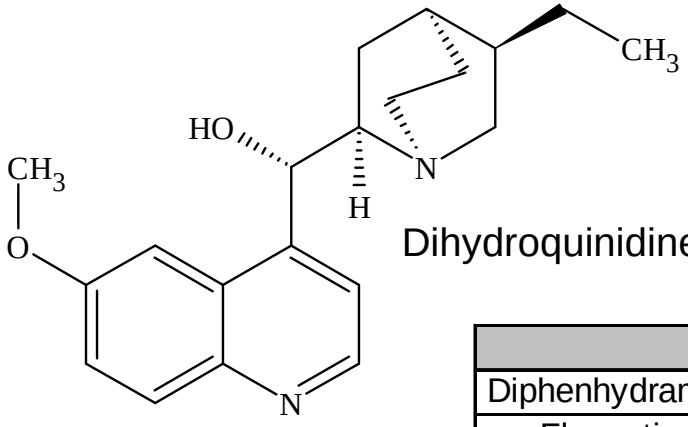


Fluoxetine

ACD Labs Data



Quinidine



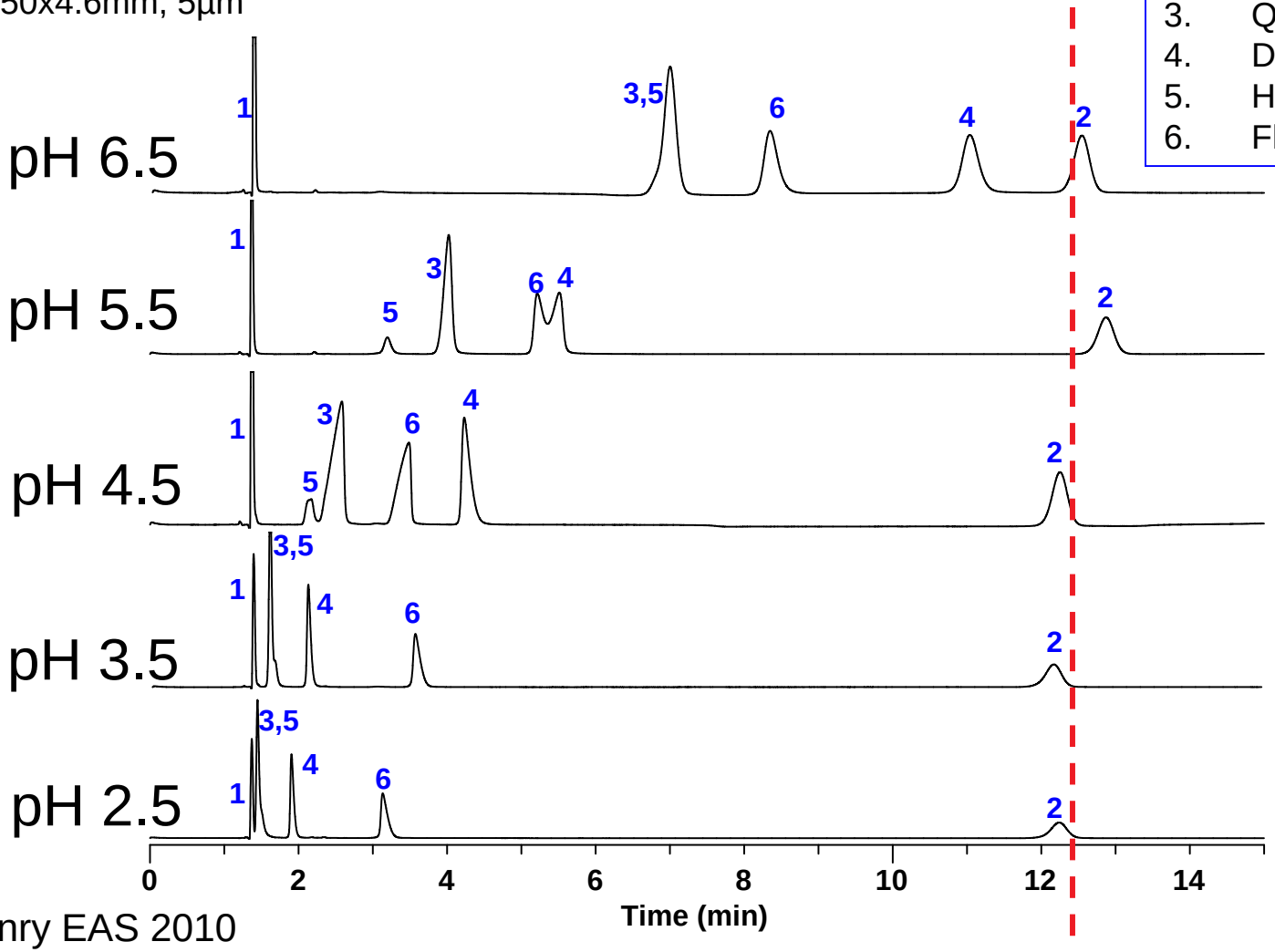
Dihydroquinidine

	pKa	Log P
Diphenhydramine	8.76	3
Fluoxetine	10.05	3.93
Quinidine	12.8	2.82
	9.28	
	4.77	
Hydroquinidine	12.8	3.24
	9.84	
	4.77	

DFQ – pH Change (Ascentis C18)

65% Methanol 35% 10mM Potassium Phosphate
1.0mL/min, 35ºC, 220nm, 10µL(DFQN)
150x4.6mm, 5µm

Elution Order:
1. Uracil
2. Naphthalene
3. Quinidine
4. Diphenhydramine
5. Hydroquinidine
6. Fluoxetine



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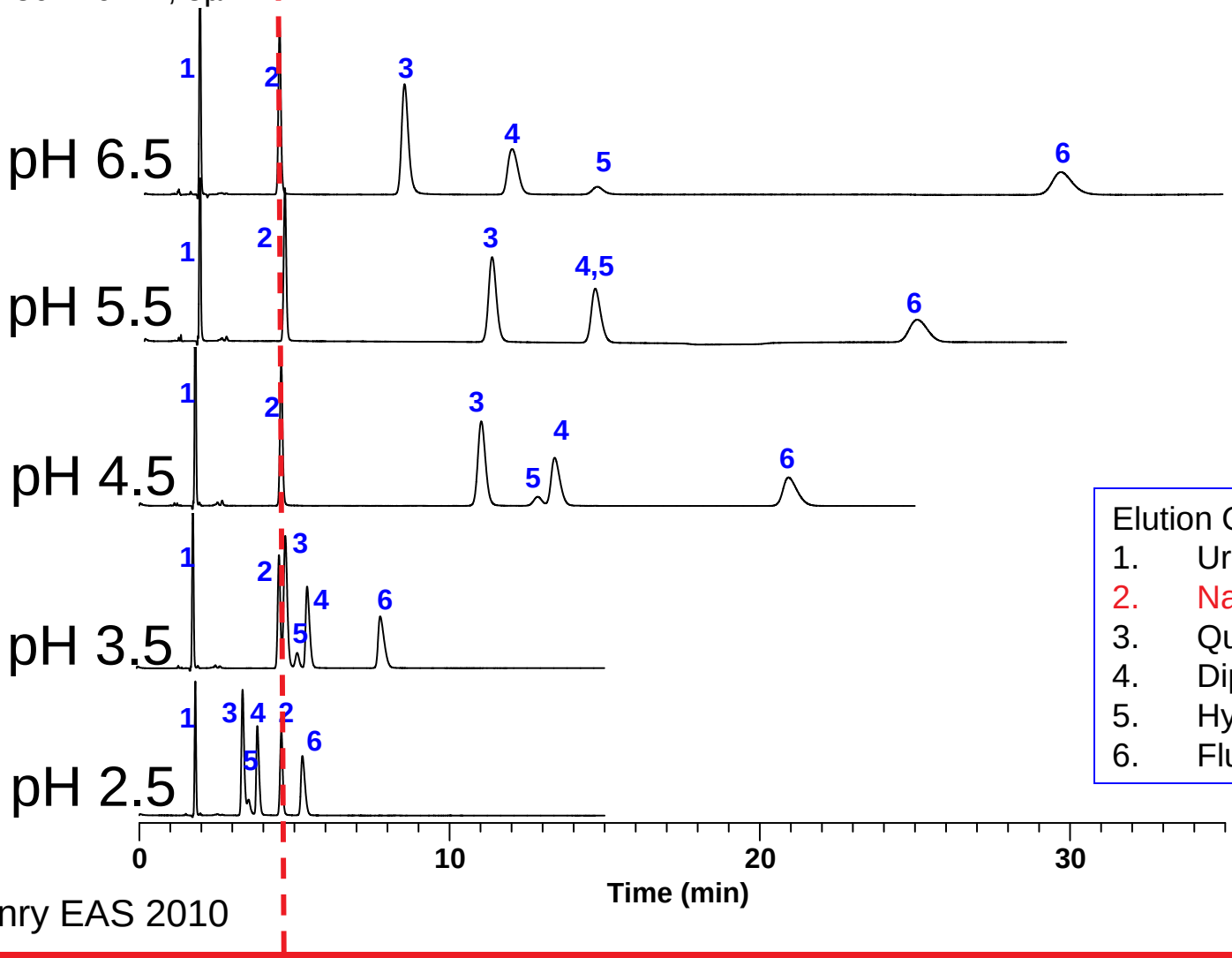
DFQ – pH Change (Ascentis ES Cyano)

65% Methanol 35% 10mM Potassium Phosphate
1.0mL/min, 35ºC, 220nm, 10µL(DFQN)
150x4.6mm, 5µm



Elution Order:
1. Uracil
2. Naphthalene
3. Quinidine
4. Diphenhydramine
5. Hydroquinidine
6. Fluoxetine

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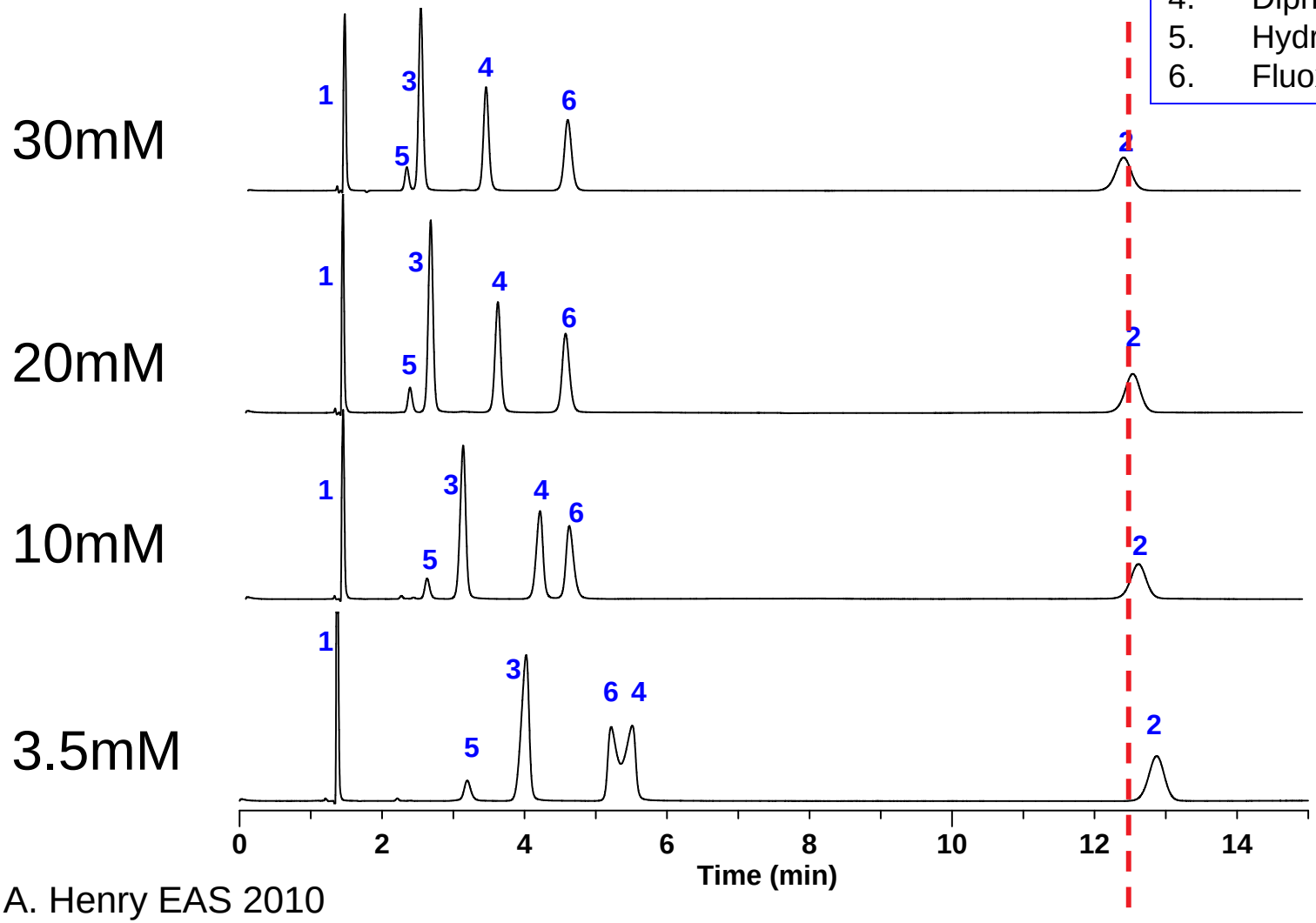
DFQ –Ionic Strength pH 5.5 (C18)

65% Methanol 35% Potassium Phosphate pH 5.5
1.0mL/min, 35ºC, 220nm, 10µL(DFQN)

Elution Order:
1. Uracil
2. Naphthalene
3. Quinidine
4. Diphenhydramine
5. Hydroquinidine
6. Fluoxetine



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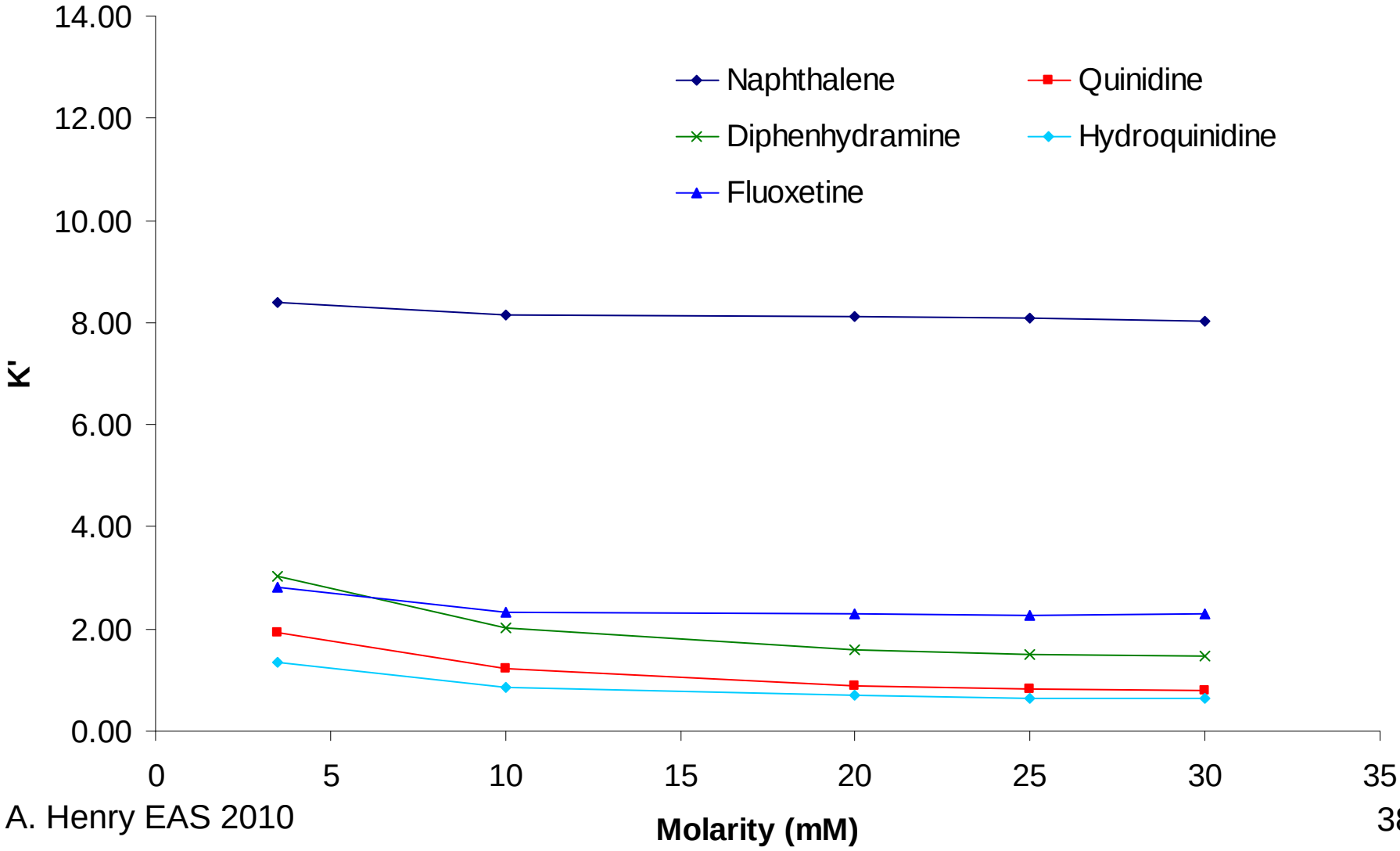
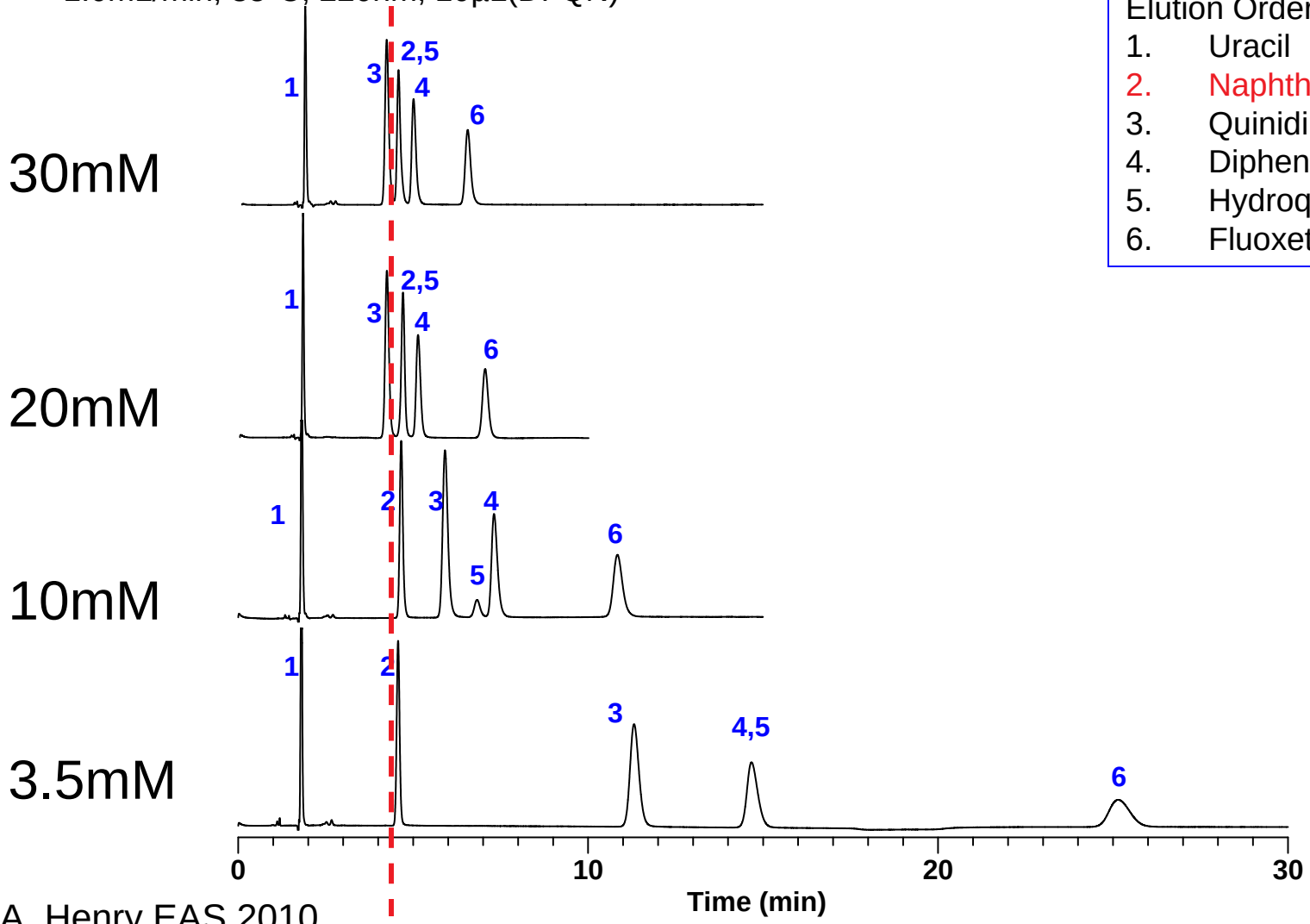
Ascentis C18 (pH 5.5)



DFQ – Ionic Strength pH 5.5 (Cyano)

65% Methanol 35% Potassium Phosphate pH 5.5
1.0mL/min, 35ºC, 220nm, 10µL(DFQN)



Elution Order:
1. Uracil
2. Naphthalene
3. Quinidine
4. Diphenhydramine
5. Hydroquinidine
6. Fluoxetine

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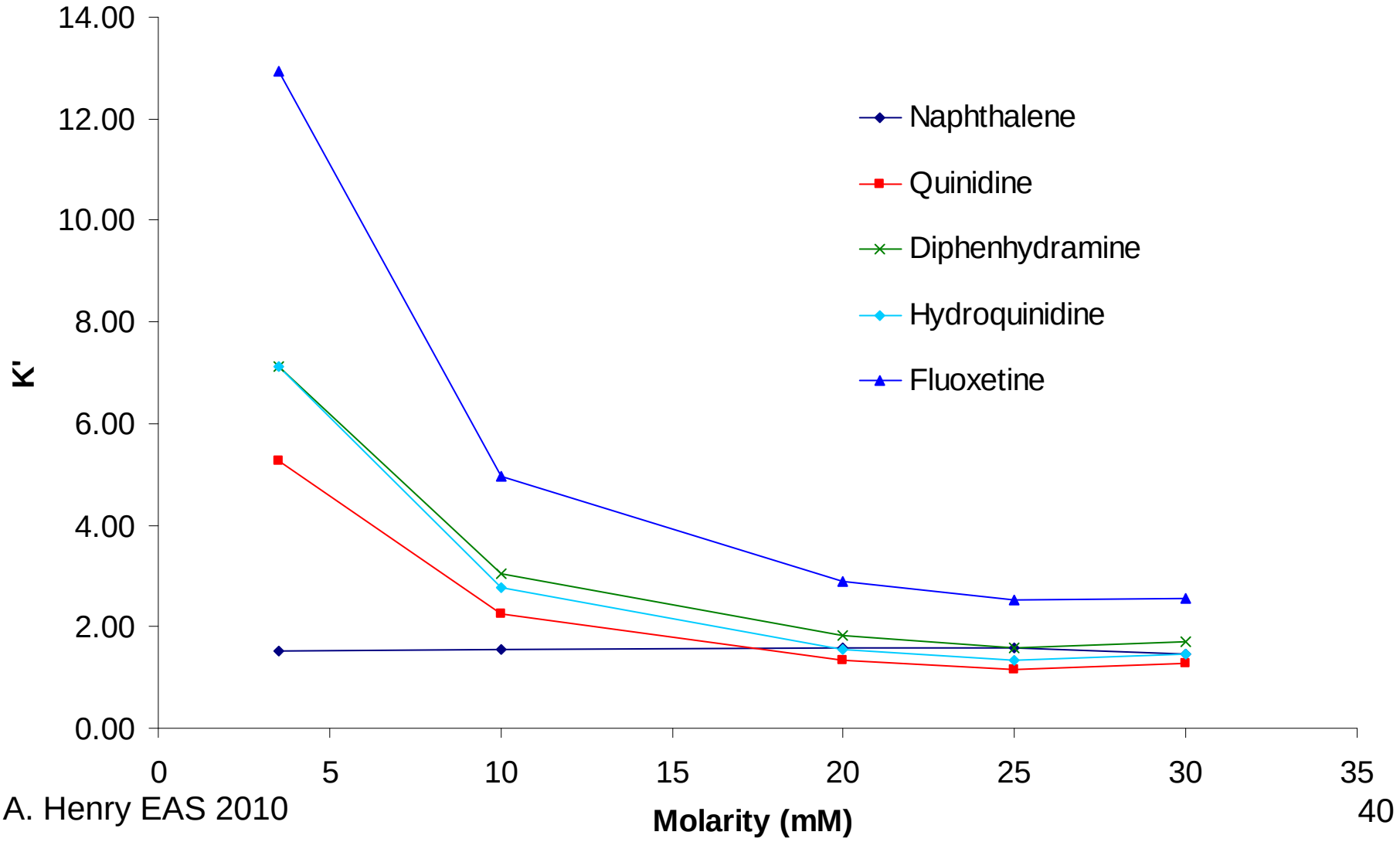
Ascentis ES Cyano (pH 5.5)



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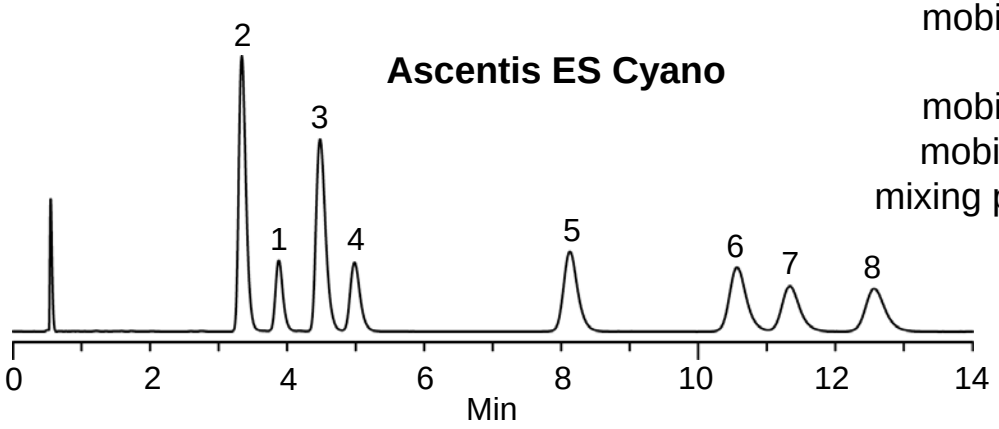
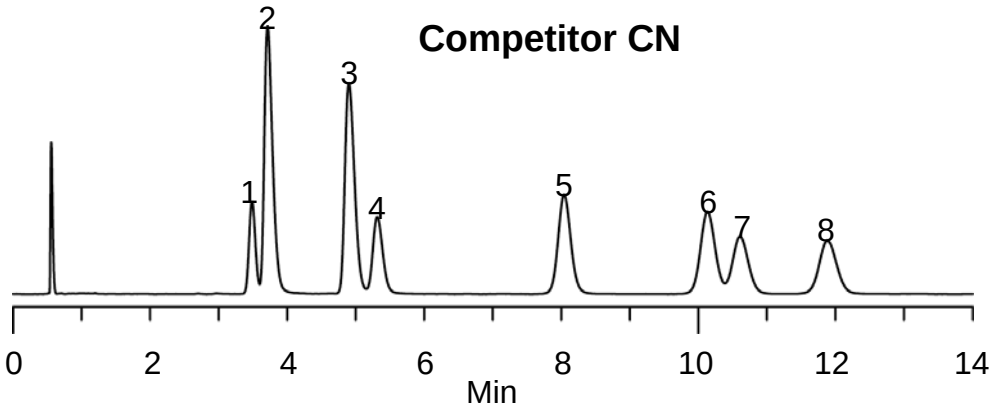
Comparison of Tricyclic Antidepressants- RP Mode

85% RP Correlation





column:	as indicated; 15 cm x 4.6 mm, 5 µm particle
mobile phase A:	20 mM potassium phosphate, dibasic (pH 7.0 with phosphoric acid)
mobile phase B:	acetonitrile
mobile phase C:	methanol
mixing proportions:	A:B:C, 25:60:15
flow rate:	2.0 mL/min
temp.:	25 ° C
det.:	215 nm
injection:	5 µL
sample:	100 µg /mL in 70:30, water: methanol

1. **Trimipramine**
2. **Doxepin**
3. **Amitriptyline**
4. **Imipramine**
5. **Nordoxepin**
6. **Nortriptyline**
7. **Desipramine**
8. **Protriptyline**

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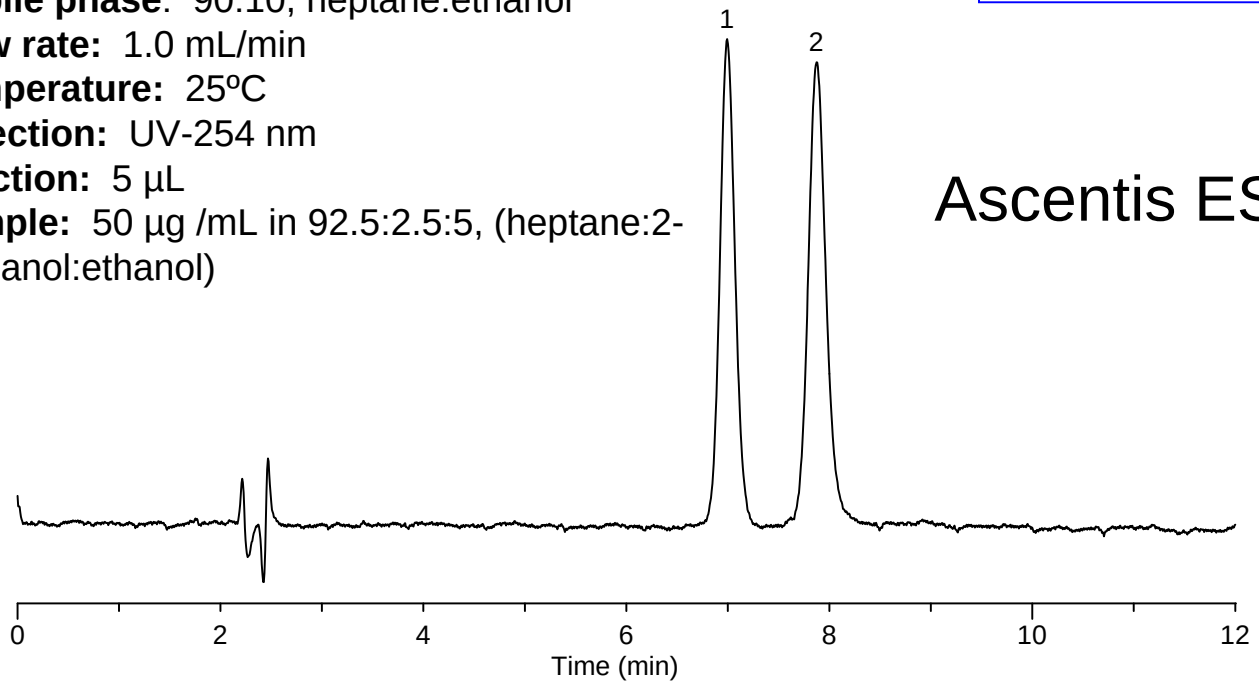
Hydrocortisone and Hydrocortisone Acetate- Normal Phase Mode

Column: Ascentis Cyano 15 cm x 4.6 mm, 5 µm (577306-U)
Mobile phase: 90:10, heptane:ethanol
Flow rate: 1.0 mL/min
Temperature: 25ºC
Detection: UV-254 nm
Injection: 5 µL
Sample: 50 µg /mL in 92.5:2.5:5, (heptane:2-propanol:ethanol)

Elution Order:
1. Hydrocortisone acetate
2. Hydrocortisone



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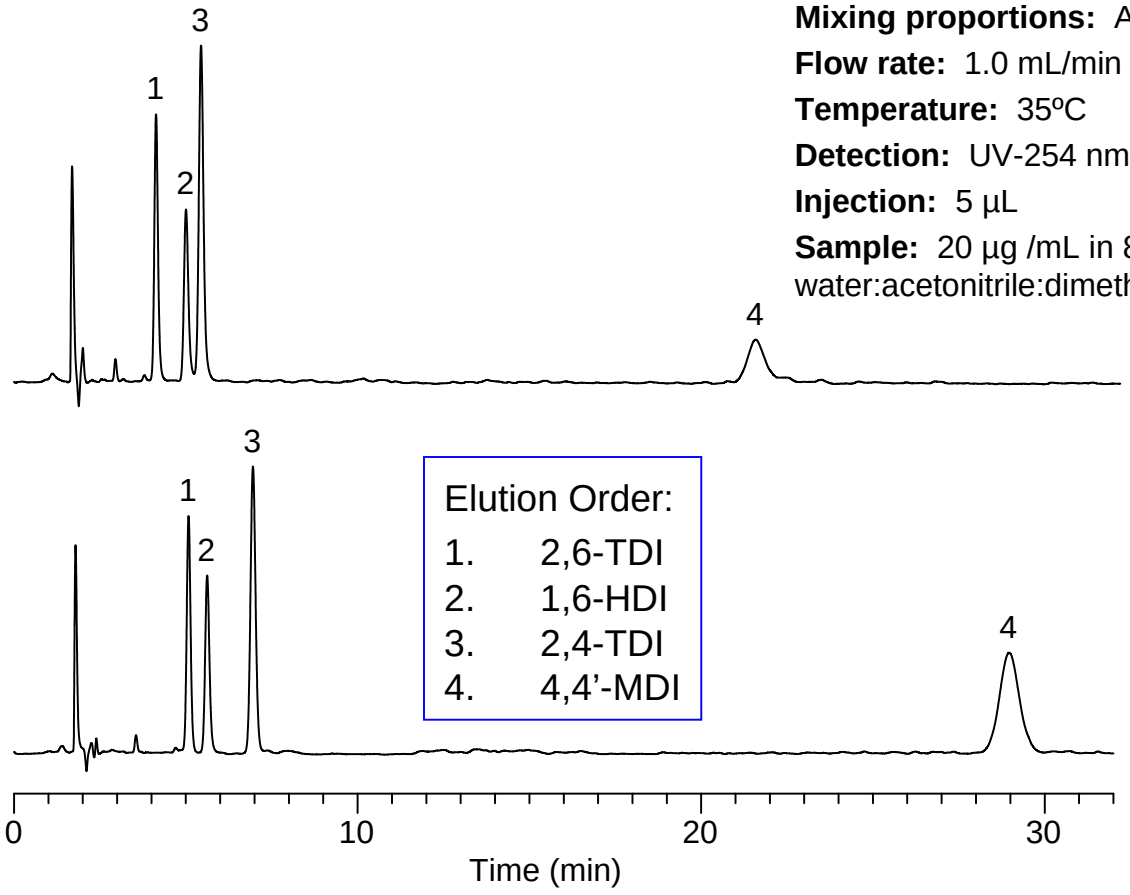
Isocyanate Derivatives- Reversed Phase Mode (30% ACN)

Column: as indicated; 15 cm x 4.6 mm, 5 µm particle
Mobile phase A: water with 0.1% ammonium acetate
Mobile phase B: acetonitrile with 0.1% ammonium acetate
Mixing proportions: A:B, 70:30
Flow rate: 1.0 mL/min
Temperature: 35ºC
Detection: UV-254 nm
Injection: 5 µL
Sample: 20 µg /mL in 80:12:8, water:acetonitrile:dimethylsulfoxide



Competitor CN

Ascentis ES Cyano

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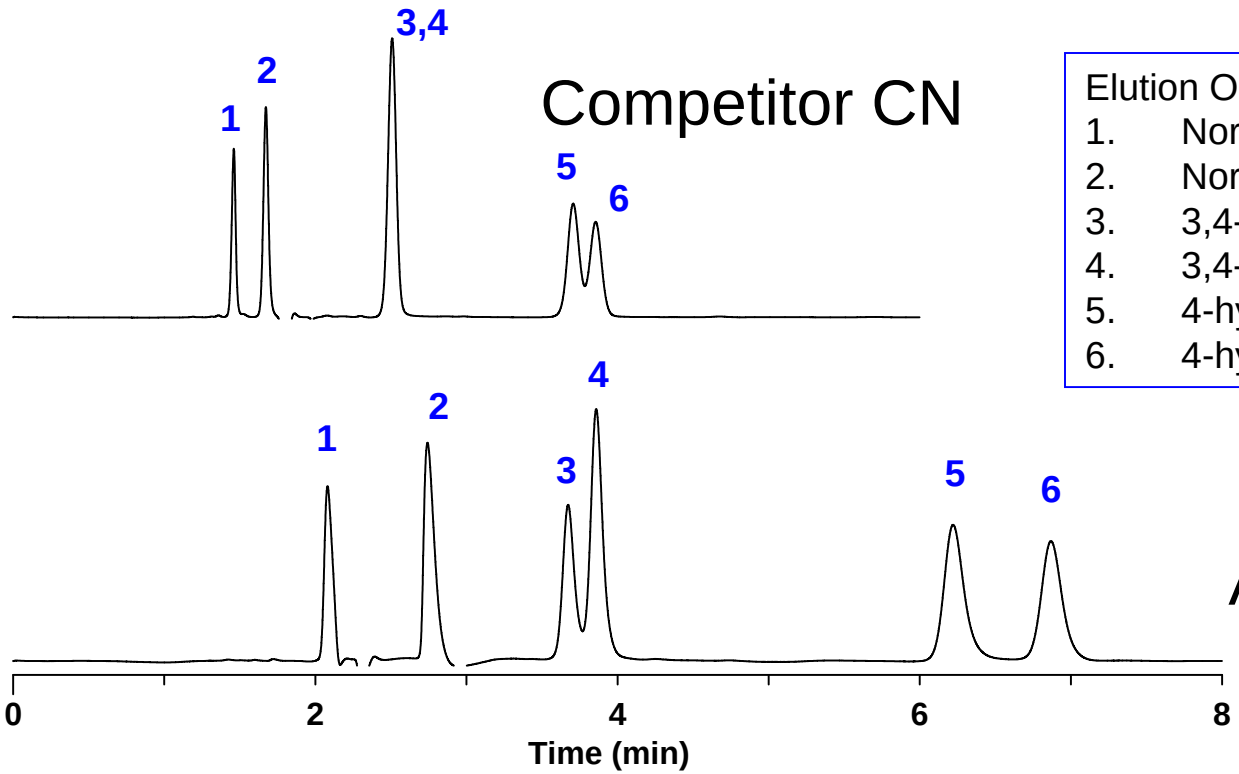
Water Soluble Compounds- Reversed Phase Mode (5% MeOH)



Elution Order:
1. Norepinephrine
2. Normetanephrine
3. 3,4-dihydroxymandelic acid
4. 3,4-dihydroxyphenyl glycol
5. 4-hydroxy-3-methoxymandelic acid
6. 4-hydroxy-3-methoxyphenylglycol

Column: 15 cm x 4.6 mm, 5 µm particle
Mobile phase A: 95%Water 5% Methanol w/ 0.1% Formic acid
Flow rate: 1.0 mL/min
Temperature: 35ºC
Detetion: UV-220 nm
Injection: 10 µL

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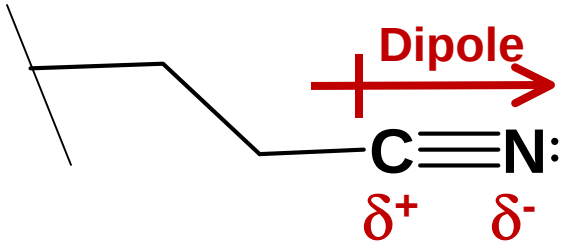
Summary: Ascentis ES Cyano



- The cyano group has a strong dipole that can interact with polar moieties found in most analytes.
- Ascentis porous silica particles are available in range of particle sizes and columns- fast LC to prep LC.
- The stable Ascentis ES Cyano phase may the most versatile of all HPLC phases:
  - Reversed-phase mode (100% aqueous to 100% ACN or MeOH).
    - Ideal for fast LC-MS (balance of retention and selectivity).
  - Aqueous Normal Phase (HILIC) mode (> 70% ACN or MeOH).
  - Normal Phase mode (completely non-aqueous); Cyano may be to normal phase what C18 is to reversed-phase.

Acknowledgements and Trademarks

- The assistance of Craig Aurand, Hillel Brandes, Hugh Cramer and David Bell and others at Supelco Division of Sigma-Aldrich is greatly appreciated.

Selectivity References

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