Selecting an "Orthogonal" Column During HPLC Method Development

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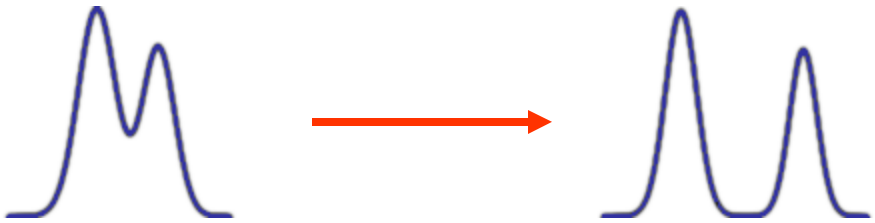
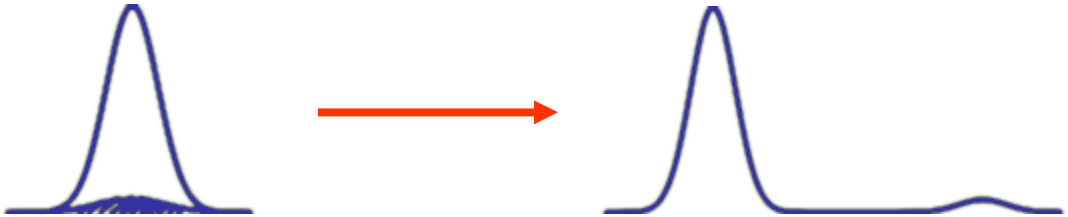
Why Orthogonal Separation?

- **Change of selectivity during method development**

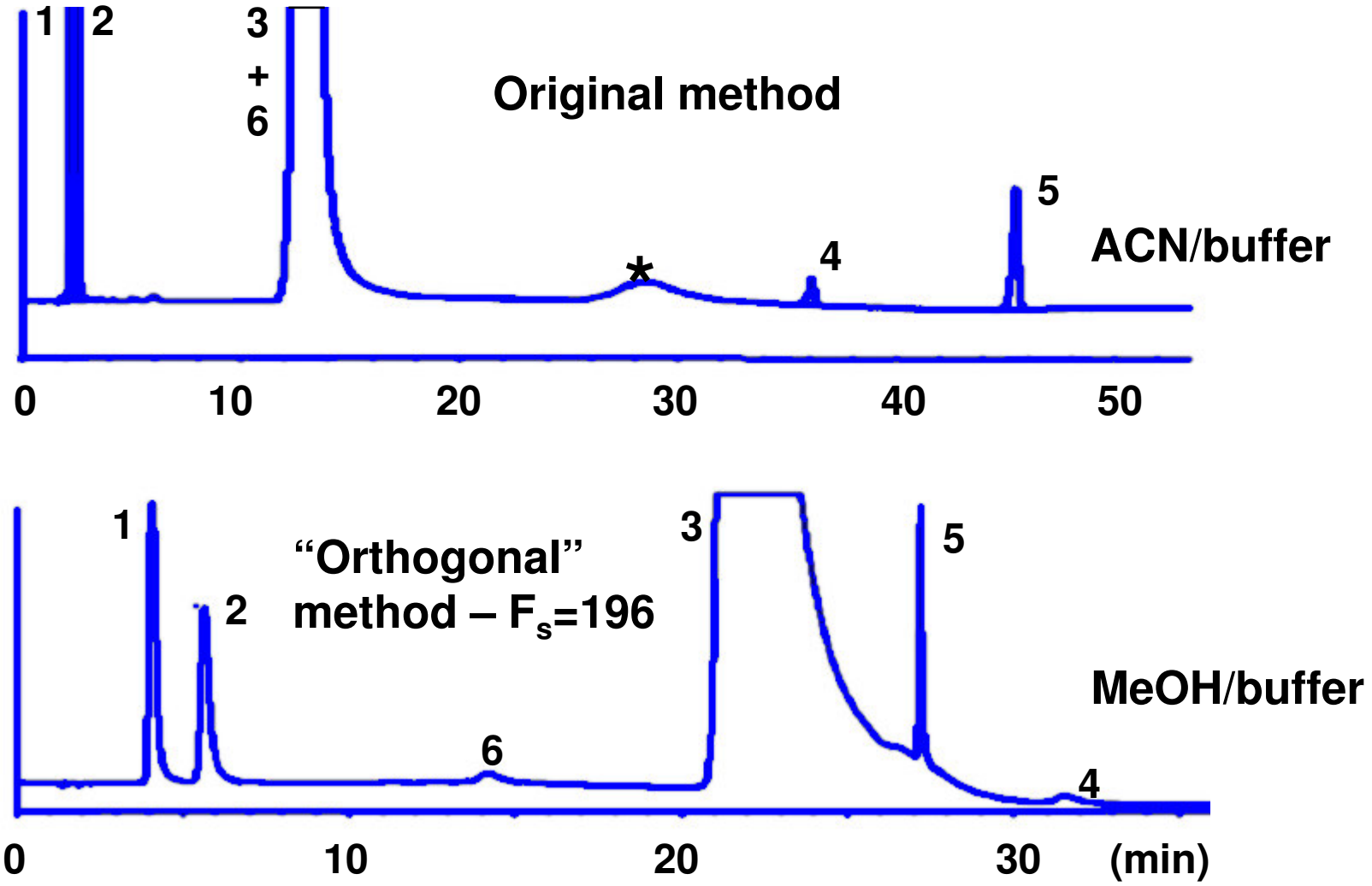


- **Find "hidden" peaks**



- **2-D Separation**

The Need for an Orthogonal Column



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Continuous *vs.* Discontinuous Variables

Continuous variables:

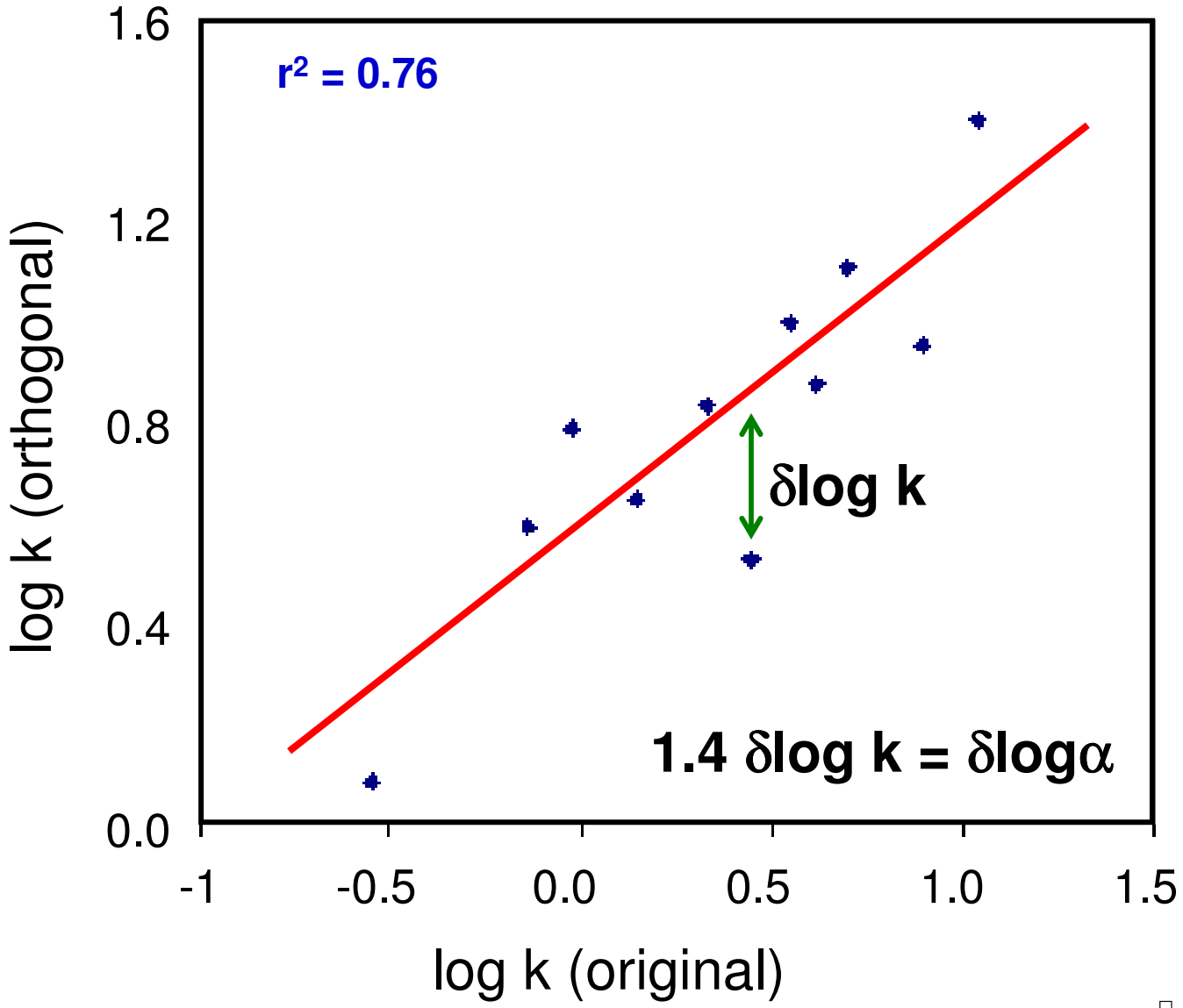
- solvent strength
- temperature
- solvent type
- additives
- (pH)

} ionizable solutes only

Discontinuous variable:

- column type

Comparing Retention



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Comparing "Orthogonal Leverage"

parameter	change	$\lvert\delta \log \alpha\rvert_{avg}$ (need >0.1)
%B	10%	0.08
t_G	3x	0.07
℃	20 ℃	0.07
MeOH (ACN)	ACN (MeOH)	0.20
column	$F_s > 65$	0.19
pH	5 units	>>0.7*
[buffer]	2x	0.02

*ionics only

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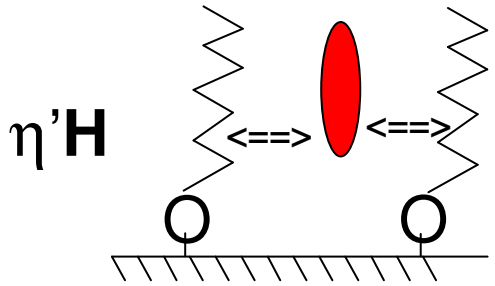
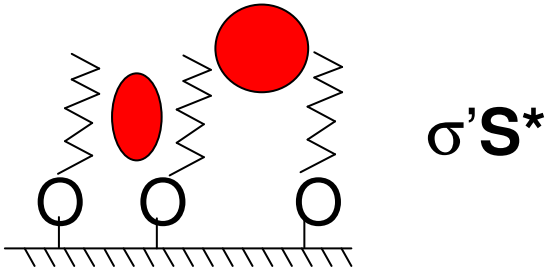
The Hydrophobic-Subtraction Model

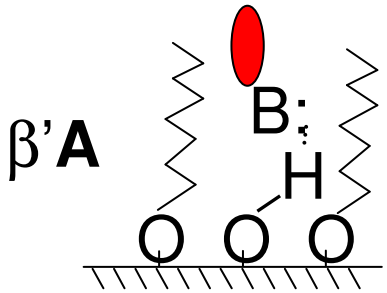
$$\log k/k_{EB} = \eta'H - \sigma'S^* + \beta'A + \alpha'B + \kappa'C$$

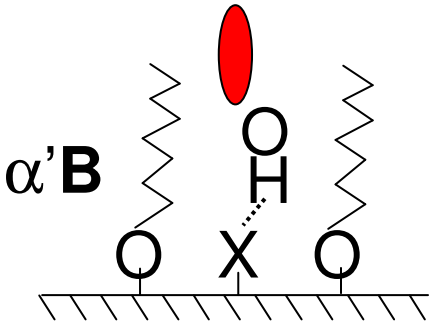


hydrophobic

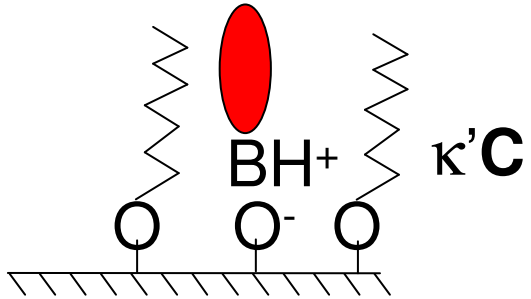


steric





hydrogen bonding



cation exchange

$$F_s = \{(H_2-H_1)^2 + (S^*_2-S^*_1)^2 + (A_2-A_1)^2 + (B_2-B_1)^2 + (C_2-C_1)^2\}^{1/2}$$

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PQRI Column Database
(http://www.usp.org/USPNF/columnsDB.html)

CAPCELL C18 UG120 (Shiseido)

You are viewing different columns.

View Similar ɪnt: ☑ Bases present: ☑ pH of mobile phase: 2.8 Update

Rank	F	Column	H	S	A	B	C(2.8)	C(7.0)	Type	USP Designation	Manufacturer
0	0	CAPCELL C18 UG120	1.007	0.036	0.037	-0.012	0.016	0.001	B	L1	Shiseido
1	133.73	Supelcosil LC-18	1.018	-0.047	0.181	0.162	1.595	1.752	A	L1	Supelco
2	123.41	Adsorbosphere (C18)	0.989	-0.073	0.07	-0.044	1.496	1.683	A	L1	Grace-Alltech
3	123.41	Zorbax C18	1.089	0.055	0.474	0.06	1.489	1.566	A	L1	Agilent
4	121.9	ProntoSIL SpheriBOND 80-5-ODS1	0.7	-0.19	0.367	0.01	1.453	2.4	A	L1	Bischoff
5	118.63	Hypersil PAH	0.949	-0.057	0.234	-0.017	1.439	1.724	A		Thermo Scientific
6	118.6	Hypurity Advance	0.412	-0.056	-0.095	0.249	-1.332	0.785	EP		Thermo Scientific
7	115.76	Apex C8	0.869	-0.071	0.235	0.177	1.364	1.373	A	L7	Grace-Jones
8	102.63	Apex C18	0.985	-0.035	0.013	0.042	1.246	2.311	A	L1	Grace-Jones
9	94.97	Purospher RP-18	0.841	0.235	0.155	0.3	-0.964	0.901	B	L1	Merck KGaA
10	92.88	Supelcosil LC-8	0.834	-0.048	-0.027	0.086	1.117	1.094	A	L7	Supelco

Next | Previous (Total items: 371)

average |(Capcell – other)|

H	S	A	B	C(2.8)	C(7.0)
0.157	0.108	0.168	0.122	1.340	1.588

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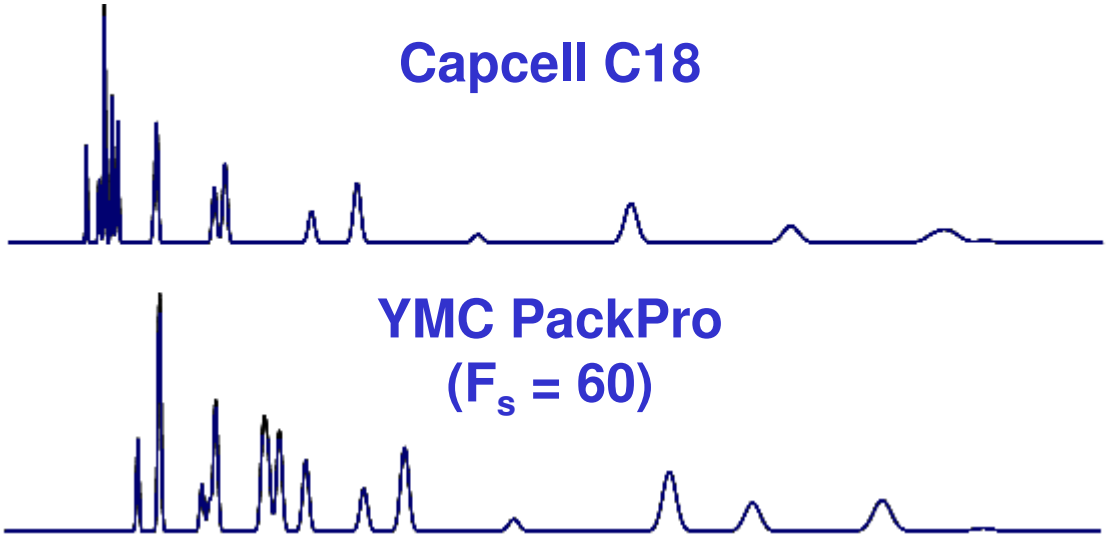
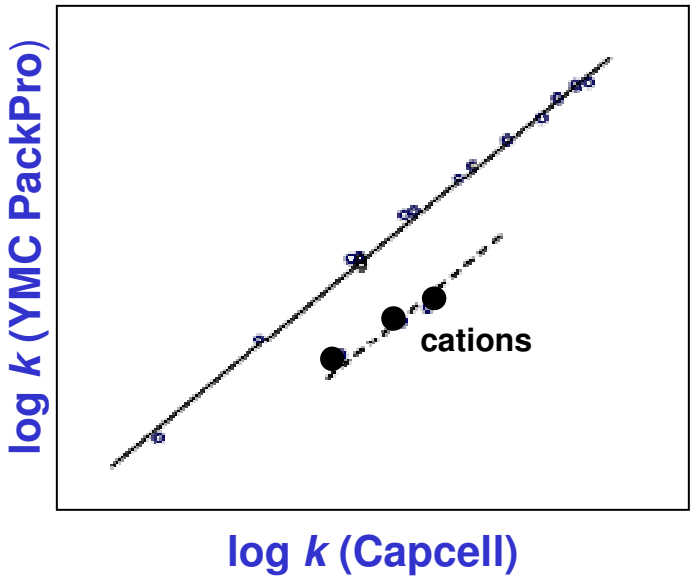
Two Columns with Quite Different Selectivity





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An Alternative Approach for Orthogonal Columns

- Ignore cation-exchange (C)

  (change in mobile-phase pH is equivalent)

$$F_s(H,S^*,A,B) = \{(H_2-H_1)^2 + (S^*_2-S^*_1)^2 + (A_2-A_1)^2+(B_2-B_1)^2 + (C_2-C_1)^2\}^{1/2}$$

- "Equalize" values of S*, A, B

$$(S^*_2-S^*_1)\,(A_2-A_1)\,(B_2-B_1) = \text{maximum}$$

A "Representative" Type-B C_{18} Column

	H	S*	A	B	C-2.8
Avg. for 137 columns	0.99	0.01	-0.03	0.00	0.00
Capcell C18 UG120	1.01	0.04	0.04	-0.01	0.02

$F_s = 2, \quad F_s(H,S^*,A,B) = 0$

Compare Other Columns vs. Capcell

Column	$F_s(H,S^*,A,B)$ (F_s)	$\lvert (S^*_2-S^*_1)(A_2-A_1)(B_2-B_1) \rvert$
Bonus RP	68 (253)	0.029
BetaMax Acid	63 (181)	0.004
Vydac 218MS	57 (78)	0.007
Purospher RP-18	52 (95)	0.009
EC Nucleosil 100-5 Protect 1	51 (268)	0.001
Hypersil Prism C18 RP	51 (236)	0.006
BioBasic Phenyl	50 (50)	0.036

How to Evaluate the Present Approach?

- **Compare column orthogonality for different samples**

  or . . .

- **Use the H-S model for previously studied solutes**

$$\log k/k_{EB} = \eta'H - \sigma'S^* + \beta'A + \alpha'B + \kappa'C$$

90 solutes of diverse structure available
(12 acids, 12 bases, 66 neutrals)

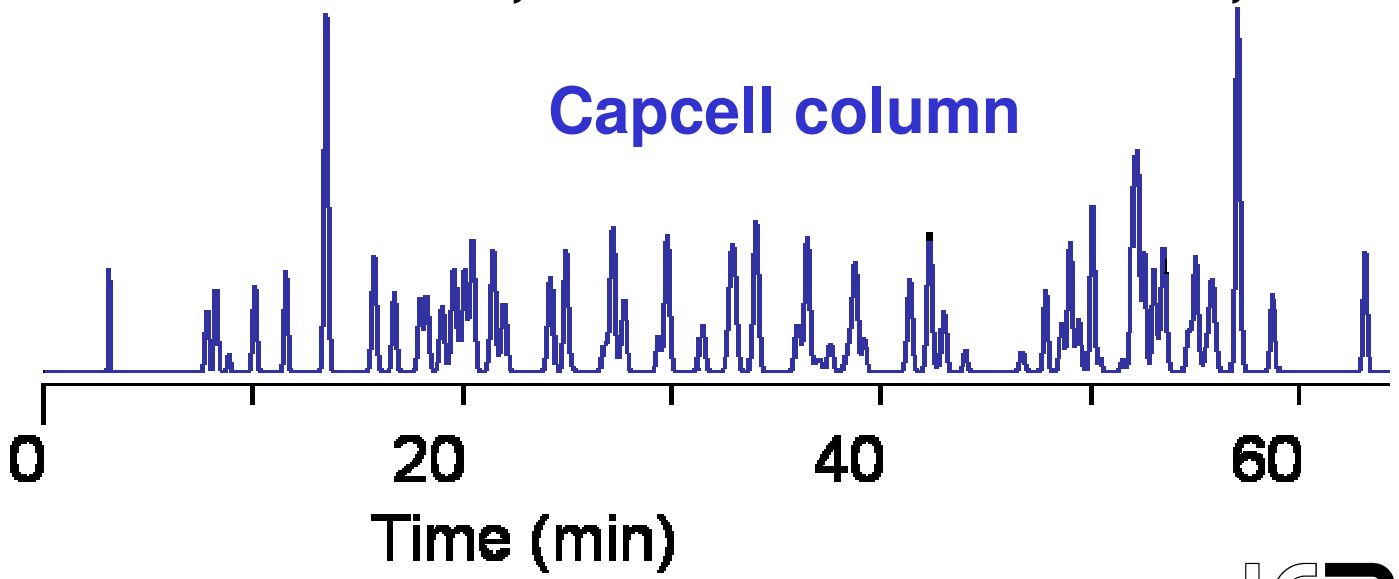
- **Calculate separation for different columns**

Calculating Separation

- use relationship

$$\log k = \log k_w - S\phi$$

- have values of k_w and S for 90 solutes
- conditions 0-100% ACN/buffer (pH-2.8) in 90 min, 35°C, 250 x 4.6-mm columns, 1 mL/min



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Comparing Column Orthogonality

- plots of t_R for column-1 vs. column-2

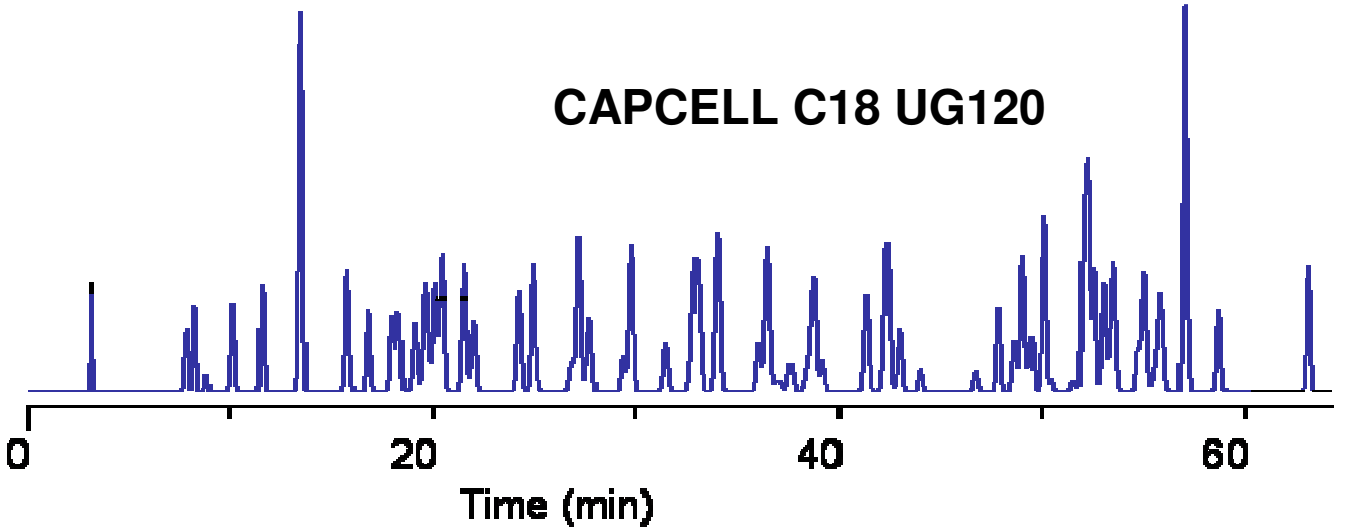
  (r^2, SD ➔ avgΔR_s, visual inspection)

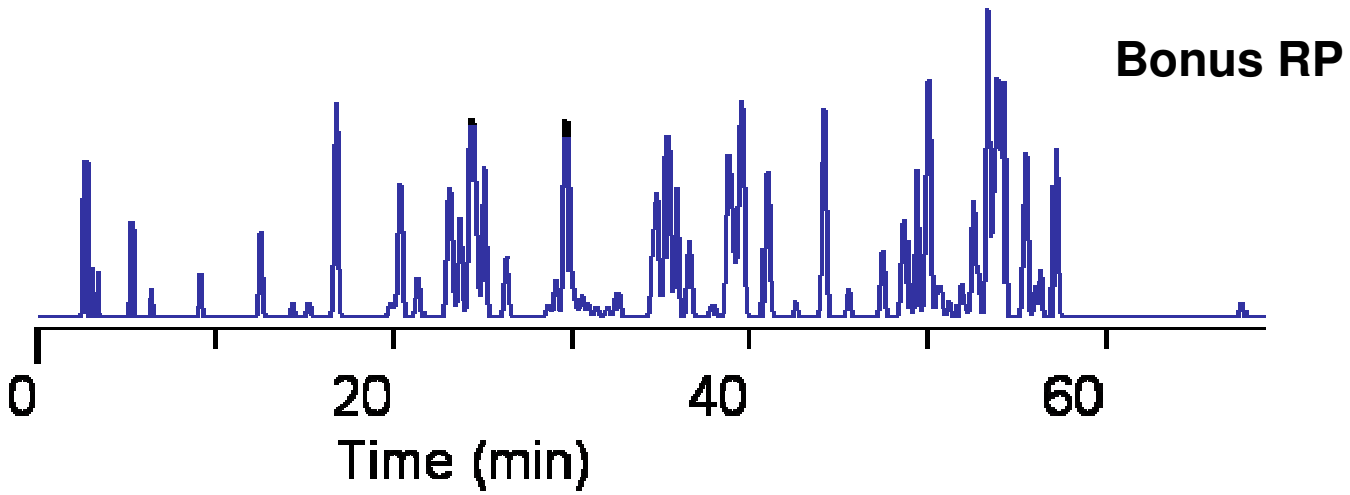
- number of overlaps from column-1

  that are separated on column-2

. . . look for any problems

Calculated Chromatograms





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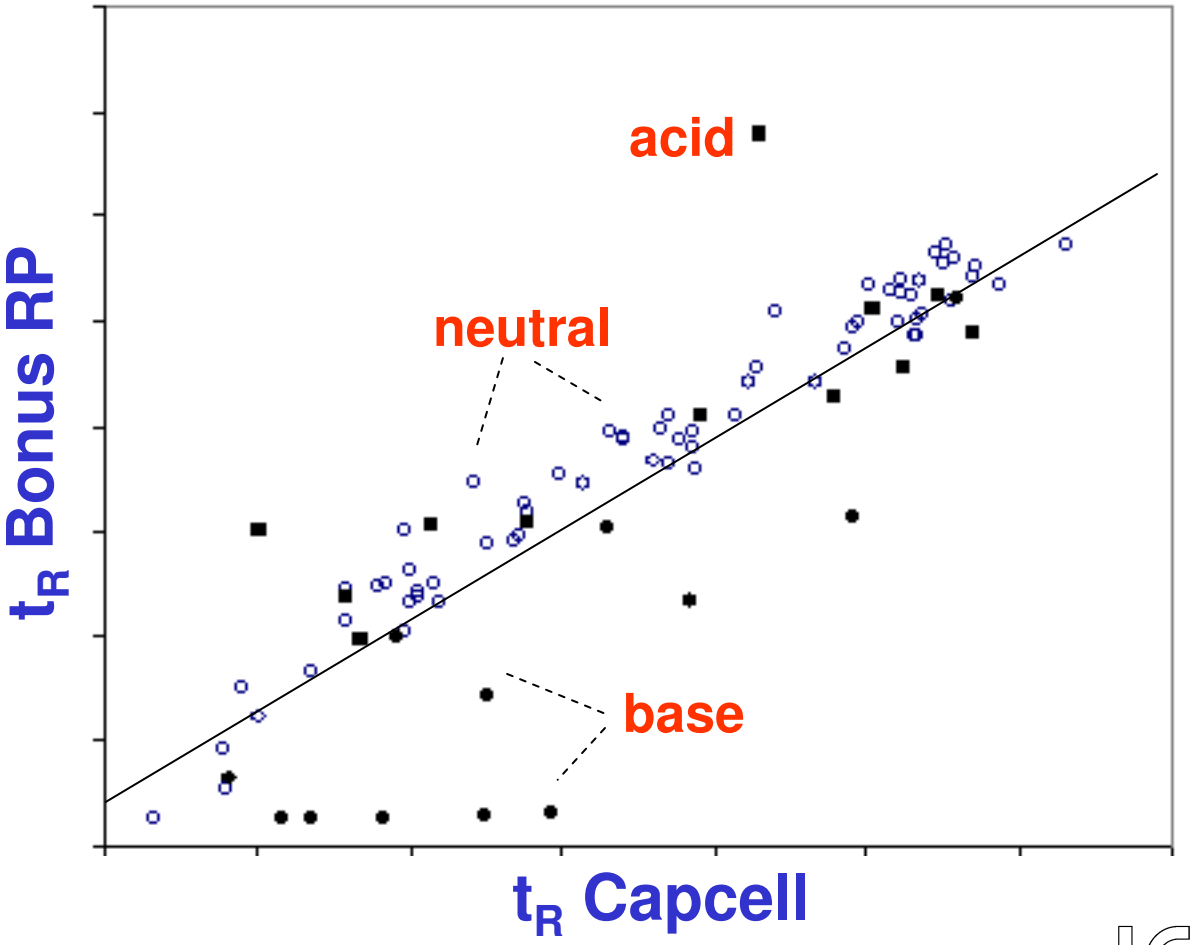
Plot of Capcell vs. Bonus RP

(r^2 = 0.81, SD = 7.1 ➔ avgΔR_s = 25)



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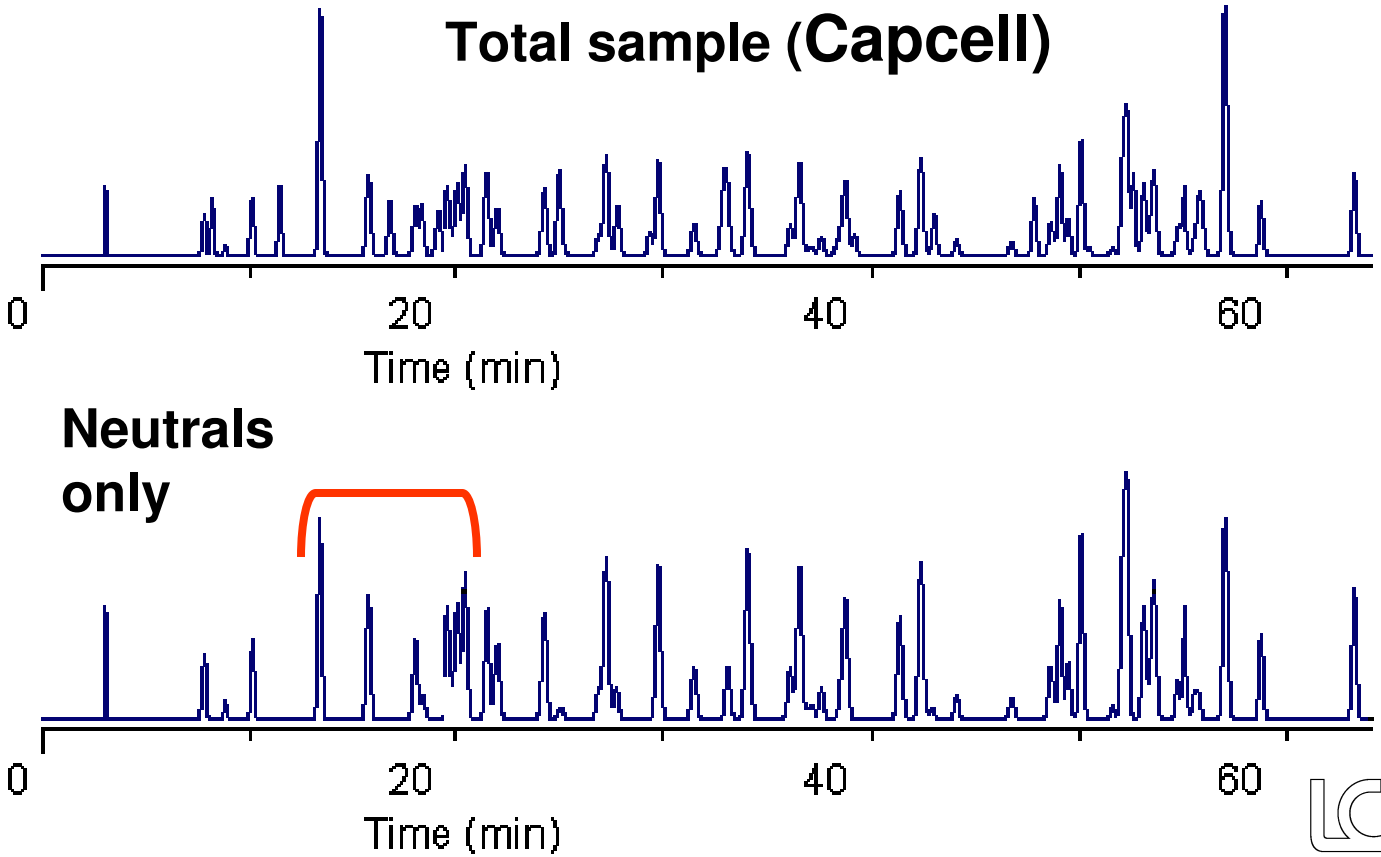
Neutrals Only

- $r^2 = 0.96$, SD = 3.0 ➔ avg$\Delta R_s = 10$
- 26 overlaps, all but one separated with $R_s > 1$ on Bonus RP column



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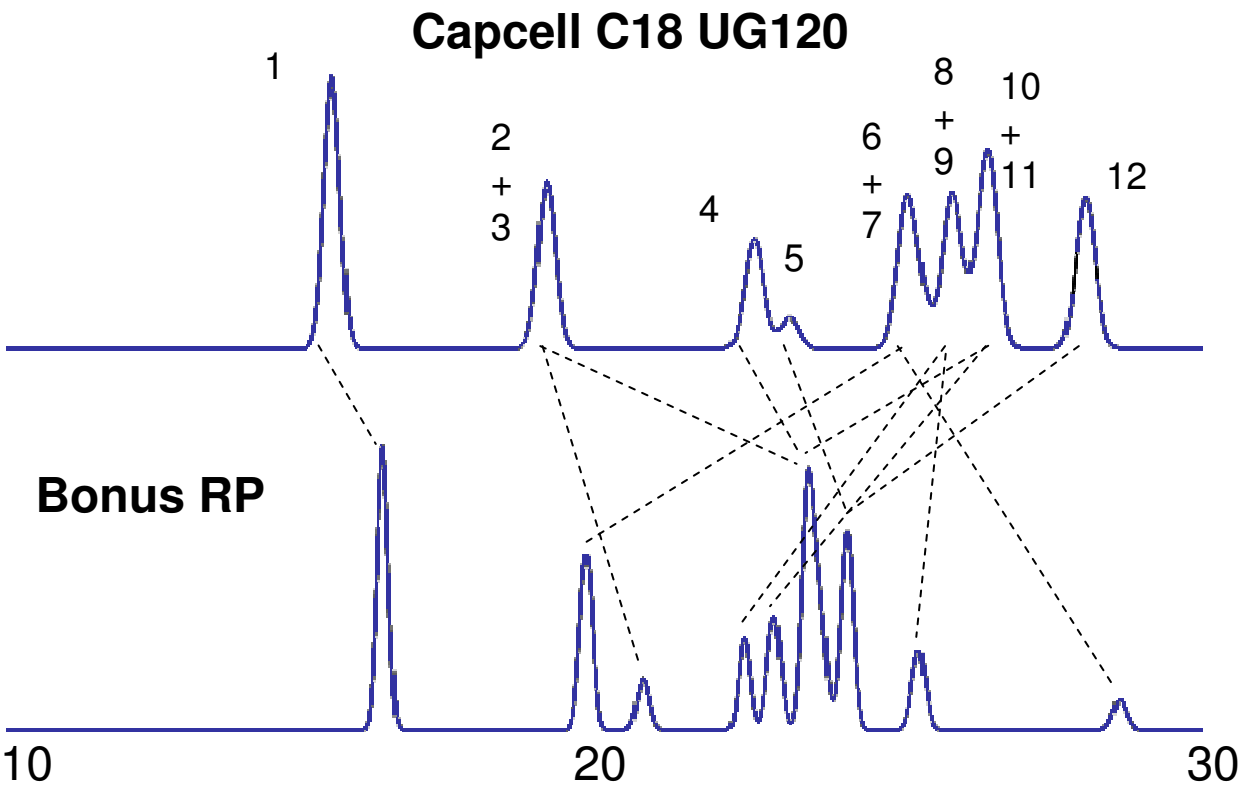
Examples of Changes in Relative Retention



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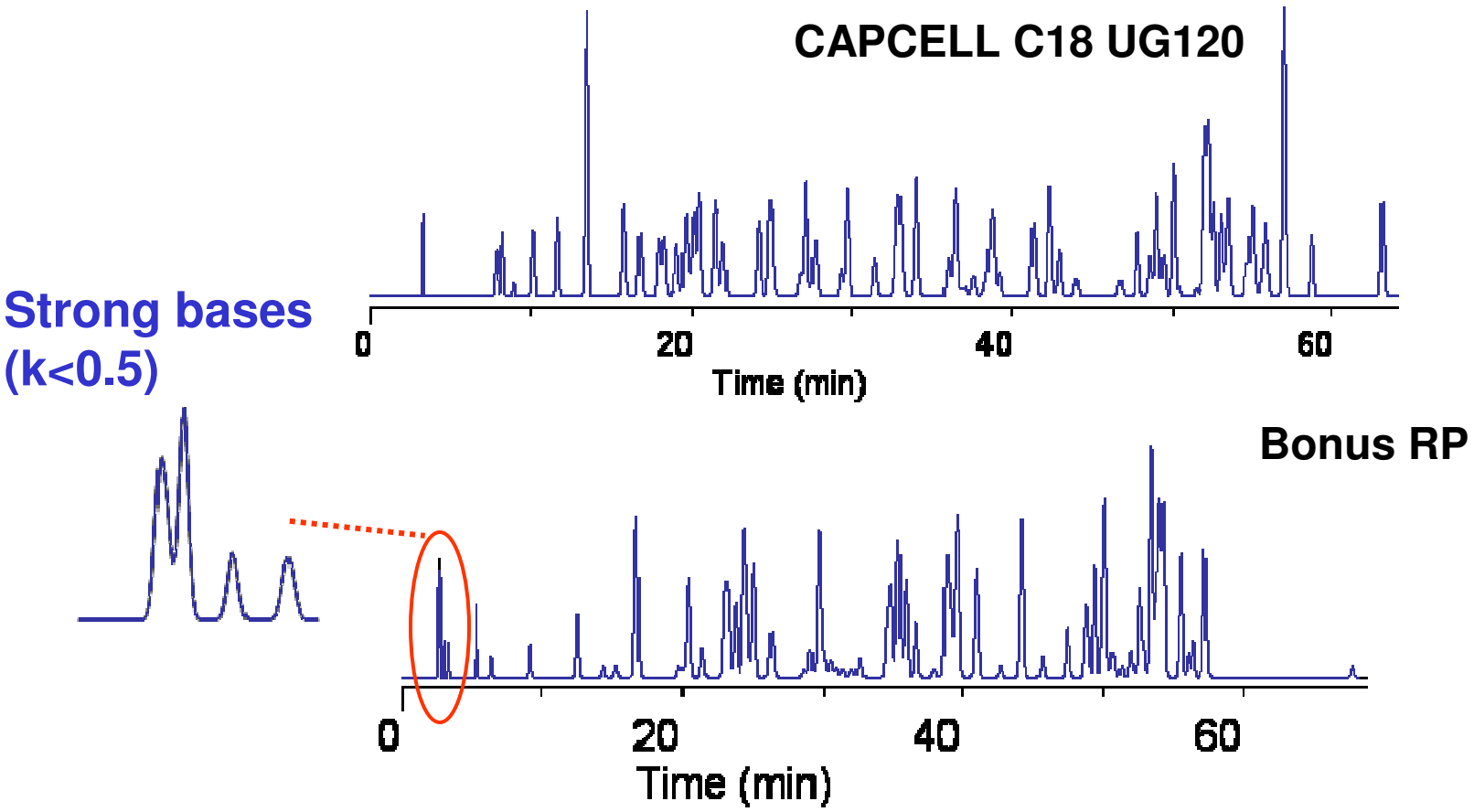
But . . . Early Elution of Bases on Bonus RP



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Compare Other Columns vs. Capcell

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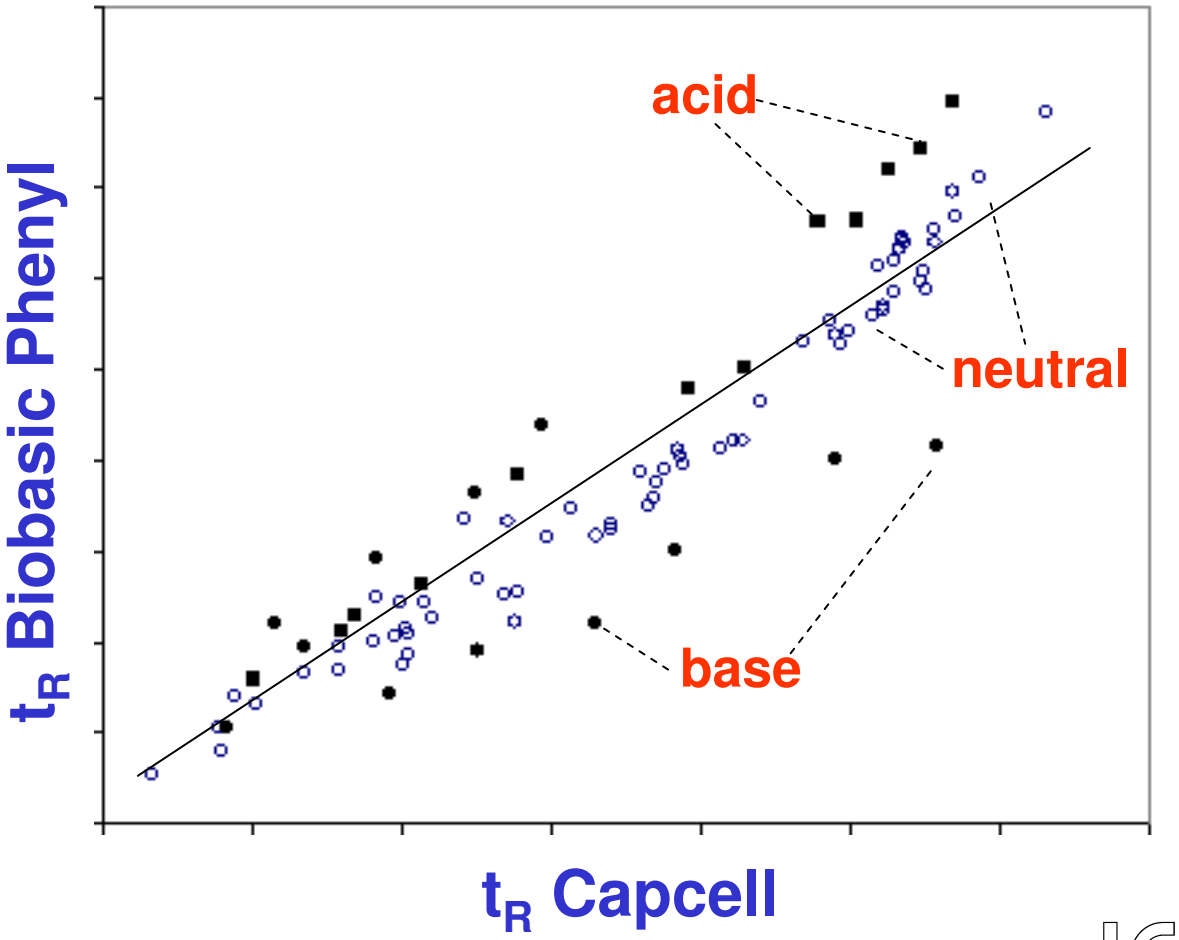
EC Nucleosil 100-5 Protect 1	51 (268)	0.001
Hypersil Prism C18 RP	51 (236)	0.006
→ BioBasic Phenyl	50 (50)	0.036

Plot of t_R for Biobasic Phenyl vs. Capcell

(r^2 = 0.90, SD = 3.1 ➔ avgΔR_s = 11)



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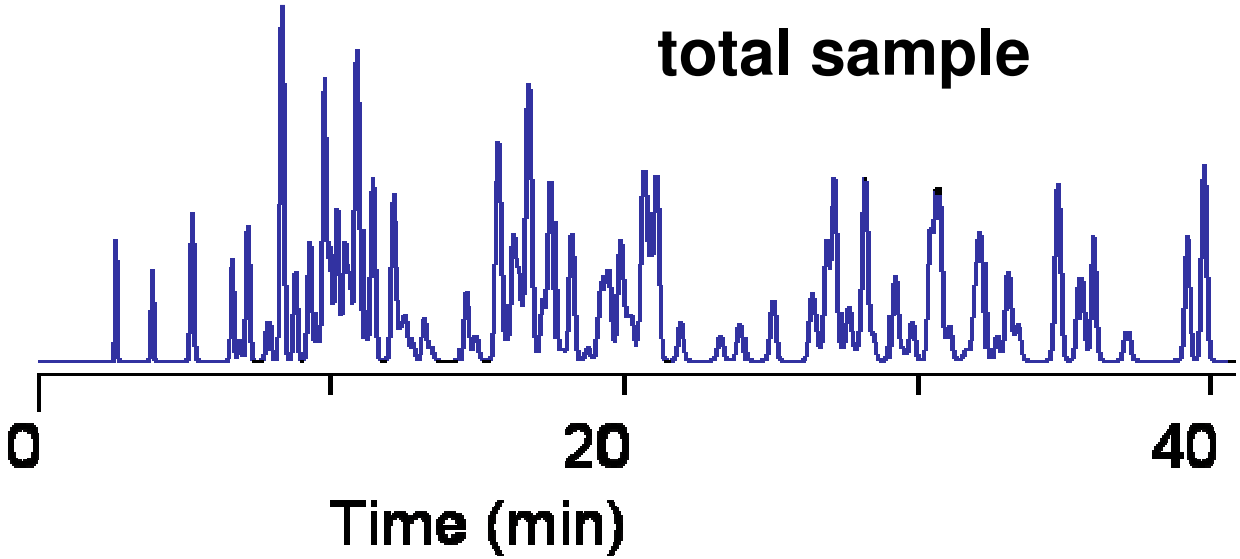
For Neutrals Only

- r^2 = 0.96, SD = 1.8 → avgΔR_s = 6.3
- 25 overlaps, 6 unseparated (R_s<1) on Biobasic Phenyl column

Bonus RP
vs. avgΔR_s = 10
10 overlap,
1 unseparated



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Conclusions

- For *equivalent* columns, minimum-F_s approach with database works well
- For *orthogonal* columns, C-term (cation exchange) dominates
  - may miss selectivity advantages due to S*, A, B for unionized solutes
  - less likely to find best type-B column

so….

So … For Best Results

- **Ignore C:**

$$F_s(H,S^*,A,B) = \{(H_2-H_1)^2 + (S^*_2-S^*_1)^2 + (A_2-A_1)^2 + (B_2-B_1)^2 + (C_2-C_1)^2\}^{1/2}$$

- **Maximize:**

$$(S^*_2-S^*_1)\,(A_2-A_1)\,(B_2-B_1)$$

- **and … Stack the Cards in Your Favor**

  exchange MeOH (ACN) for ACN (MeOH)

it's all about probability

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