Systematic Method Development with Novel, Inert Solid-Core Bonded Phases

Thomas J. Waeghe, Ph.D.,
Geoffrey Faden, Carl L. Zimmerman, Alan P. McKeown*

MAC-MOD Analytical, Inc.
103 Commons Court
Chadds Ford, PA 19317

***Advanced Chromatography Technologies**
1 Berry Street, Aberdeen, AB25 1HF, Scotland

Systematic Method Development with Novel, Inert Solid-Core Bonded Phases

Encapsulated bonding technology (EBT) is a new approach to stationary phase endcapping that provides exceptional inertness and excellent phase stability across a broad pH range from 1.5 to 11.0. This technology has previously been successfully applied to 2, 3 and 5 micron totally porous (non-core) packing materials, but has more recently been implemented for 2.5 and 5 micron solid-core packings.

The ability to use stationary phase chemistry (C18, phenyl-hexyl), organic modifier choice, and pH as variables in a systematic approach is a significant advantage for UHPLC and HPLC method development. The usefulness of such a method development strategy will be described and demonstrated with an appropriate example.

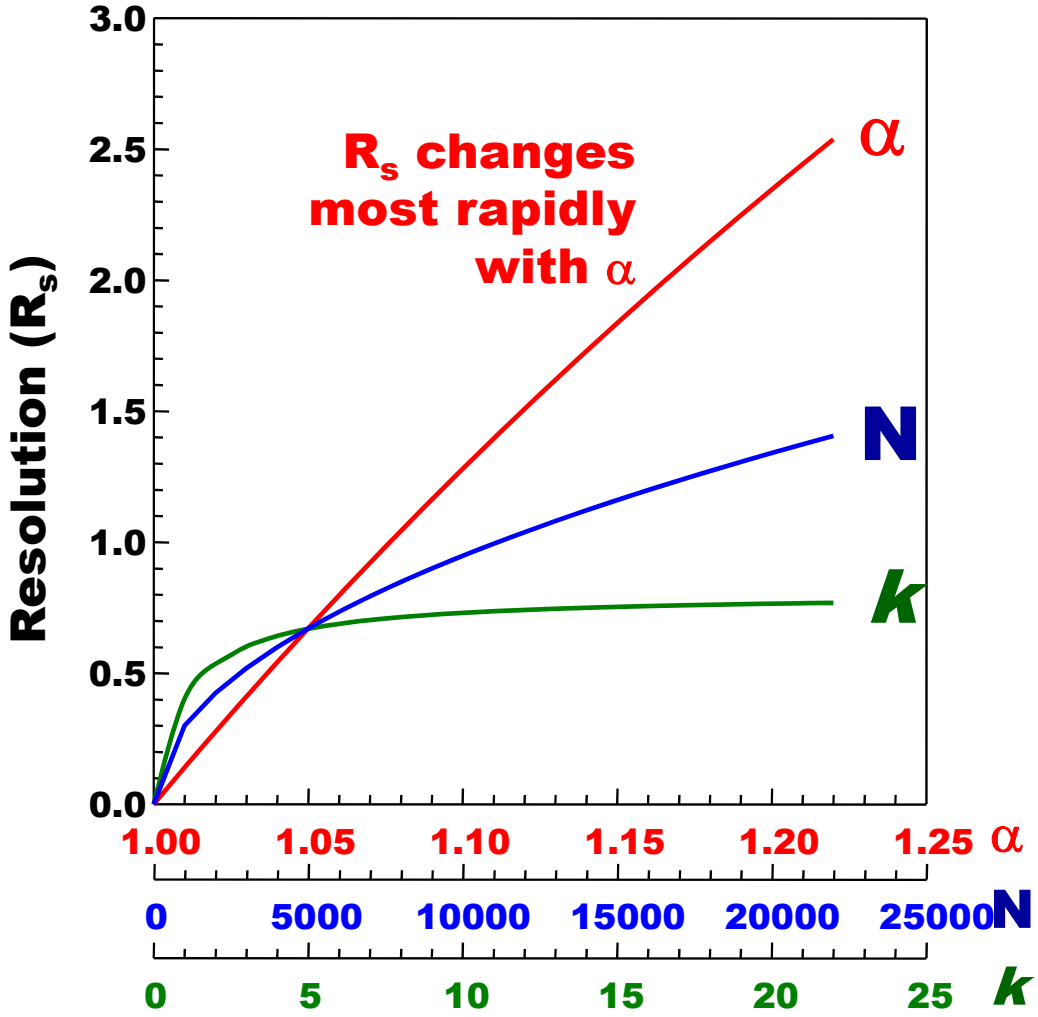
Selectivity: the most powerful variable for increasing resolution

Selectivity has greatest impact on R_s

Efficiency — Selectivity — Retention

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

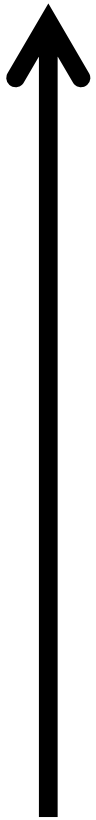
$$N = 16\left(\frac{t_r}{w_{base}}\right)^2 \qquad \alpha = \frac{k_2}{k_1} \qquad k = \frac{(t_R - t_0)}{t_0}$$



Zhao, J.H. and P.W. Carr. Analytical Chemistry, (1999) 71, 2623-2632

Which LC Parameters Affect Selectivity Most? [1,2]

MOST Influence



LEAST Influence

Isocratic Separations

1. **Mobile phase pH (for ionised analytes only)**
2. **Column Stationary Phase**
3. **Organic modifier**
4. **% Organic modifier**
5. **Column temperature**

- **Buffer choice**
- **Buffer concentration**
- **Additive concentration**

$$k^* = \frac{85 \times t_G \times F}{\Delta\Phi \times V_m \times S}$$

Gradient Separations

- **All parameters for isocratic separations and,**
- **Gradient steepness**
- **k* (that is t_G, F, $\Delta\Phi$, V_M, MW)**
- **Delay volume**
- **Column dimensions**

Relative Impact of Different Changes in RPLC Parameters on Selectivity[2]

Parameter	Change in Parameter	Maximum $\lvert\delta \log \alpha\rvert$
pH	5 pH units	0.70
Organic Modifier	$CH_3CN \Leftrightarrow CH_3OH$	0.20
Gradient Time (t_G)	10-fold	0.20
Orthogonal Column	ΔFs ≥ ~65	0.19
% Organic Modifier	10% (v/v)	0.08
Column Temperature	20 °C	0.07
Buffer Concentration	2-fold	0.02

[1] Adapted from 'Introduction to Modern Liquid Chromatography", 3rd Edition, Snyder, Kirkland, and Dolan, 2010, p.29, Wiley & Sons

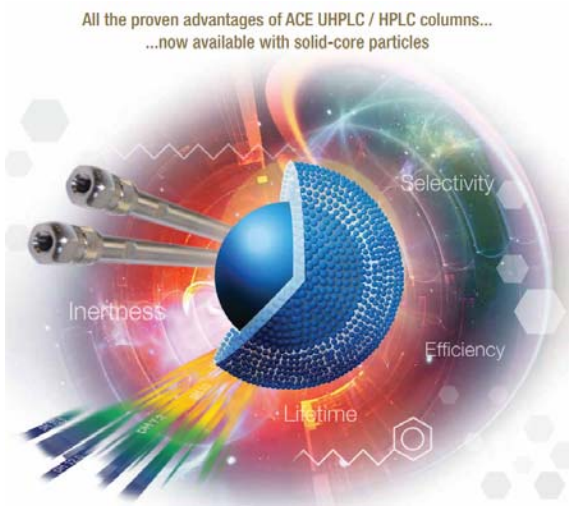
[2] Journal of Chromatography A, 1101 (2006) 122–135, "Orthogonal" separations for reversed-phase liquid chromatography, L.R. Snyder et al.

ACE UltraCore™ Solid-Core Columns

ACE UltraCore 2.5 μm:

Total particle diameter = 2.5 μm

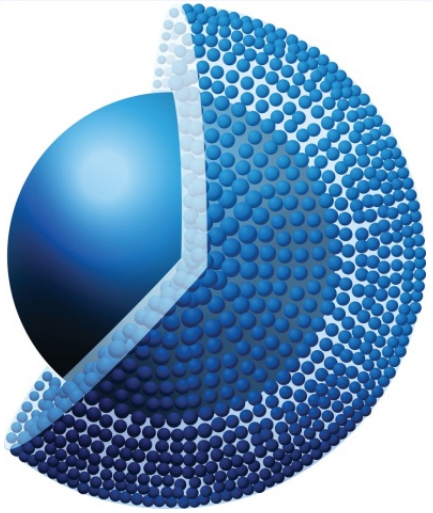
Shell thickness = 0.45 μm



ACE UltraCore 5 μm:

Total particle diameter = 5 μm

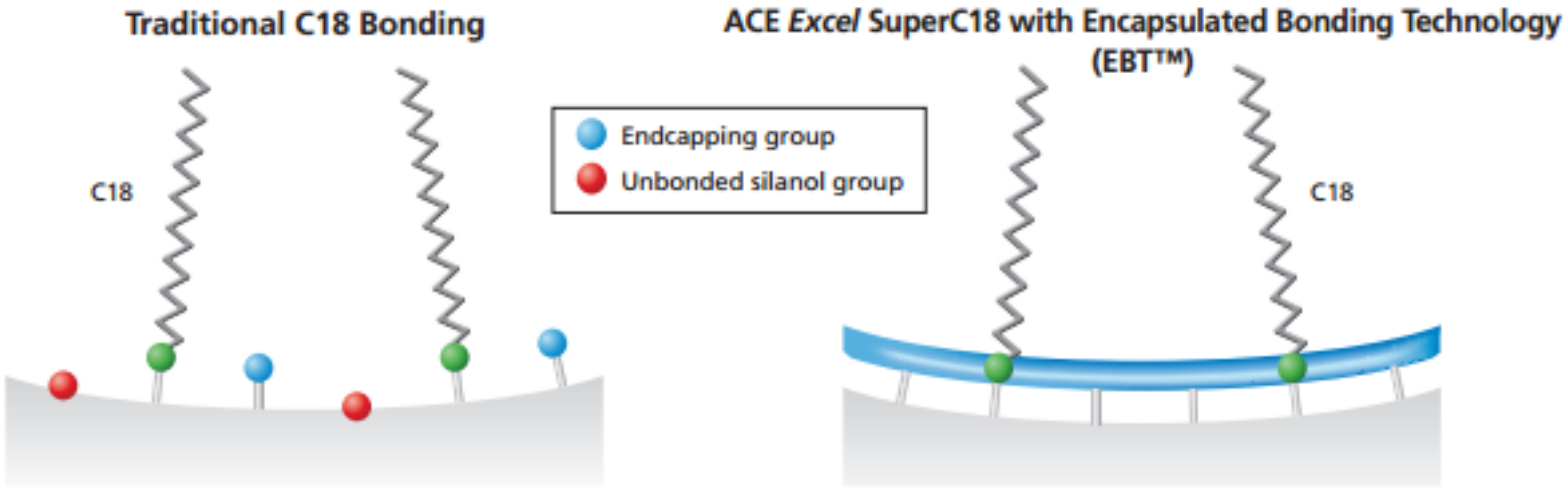
Shell thickness = 0.7 μm



UltraCore SuperC18 and SuperPhenylHexyl Columns

- **Alternate selectivities: hydrophobic and π-π interactions**
- **stable to 1000 bar (14,500 psi)**
- **2 μm frits for improved ruggedness and uptime**
- **20,000 column volume lifetime minimum (≤ 40 °C, pH 8–11.0)**

Advantages of Encapsulated Bonding Technology (EBT™)



Encapsulated Bonding Technology

- **Uniquely developed for ACE UltraCore SuperC18 and UltraCore SuperPhenylHexyl**
- **EBT bonding and endcapping**
  - **dramatically higher ligand coverage**
  - **effectively eliminates the negative effects of unbonded silanol groups**

Benefits of EBT

- **Inertness—superb peak shape**
  - **for bases, acids, and neutrals (pH 1.5–11.0)**
- **Stability**
  - **silica protected from eluent at mid and high pH**
  - **use with volatile buffers for max. stability, ideal for LC and LC-MS (NH_4OH, NH_4OAc, etc.)**
- **Versatility**
  - **no memory effects from switching among eluents at different pHs**

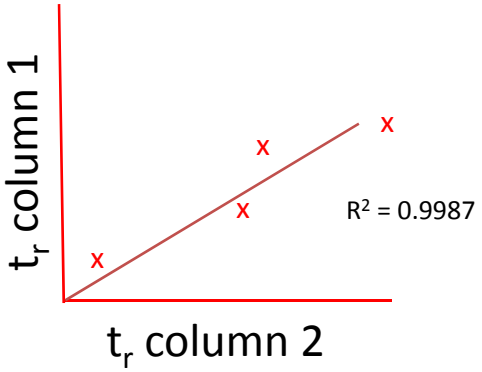
Neue Selectivity Factor

Uwe Neue Selectivity Descriptor, S, as measure of orthogonality

$$S = 100 \times \sqrt{(1 - R^2)}$$

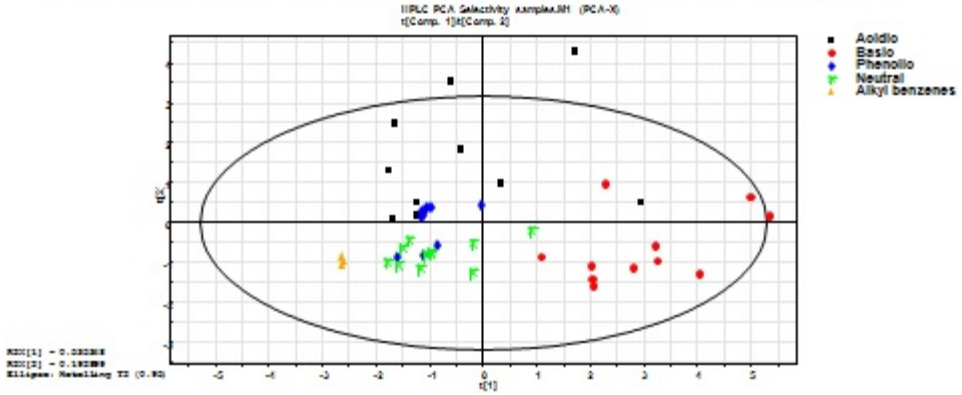
For collection of representative, diverse analytes,

plot analyte gradient retention times (obtained under the same conditions) for different stationary phases, or you can plot gradient RTs for different combinations of analysis conditions and columns) versus



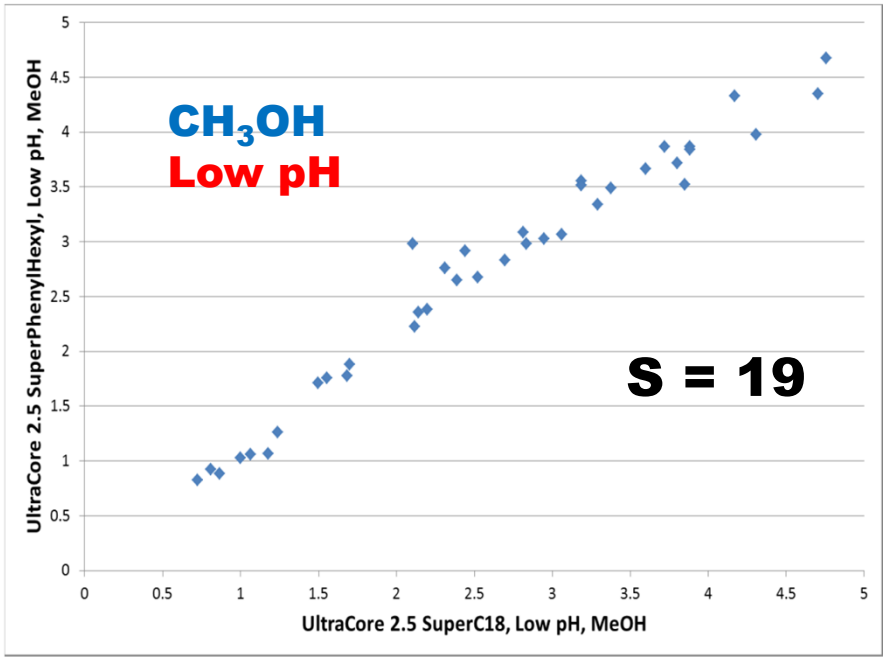
R^2 is coefficient of determination (measure of how close the data are to the fitted regression line.

- A mixture of 50 acidic, basic & neutral small molecule analytes were used that represent a broad range of physico-chemical properties.

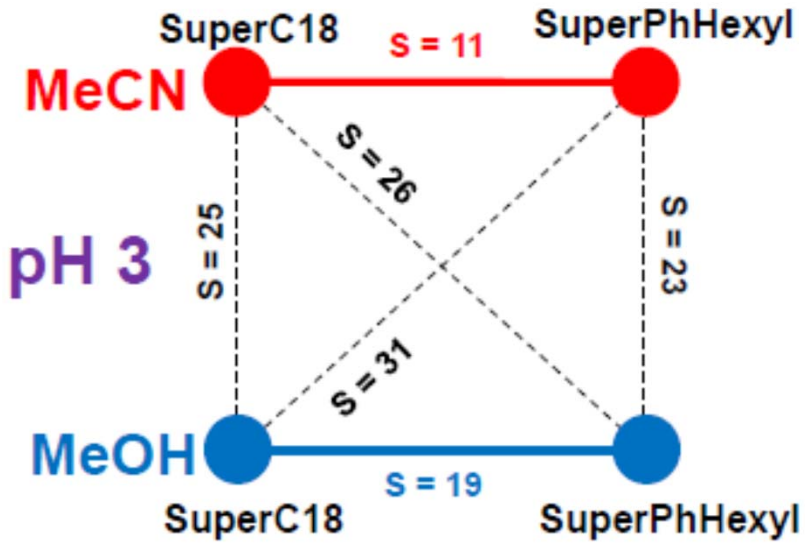


- Challenging stationary phases with these probes gives an indication of chromatographic selectivity for each phase and between phases.

UltraCore Phases: Orthogonality at low pH

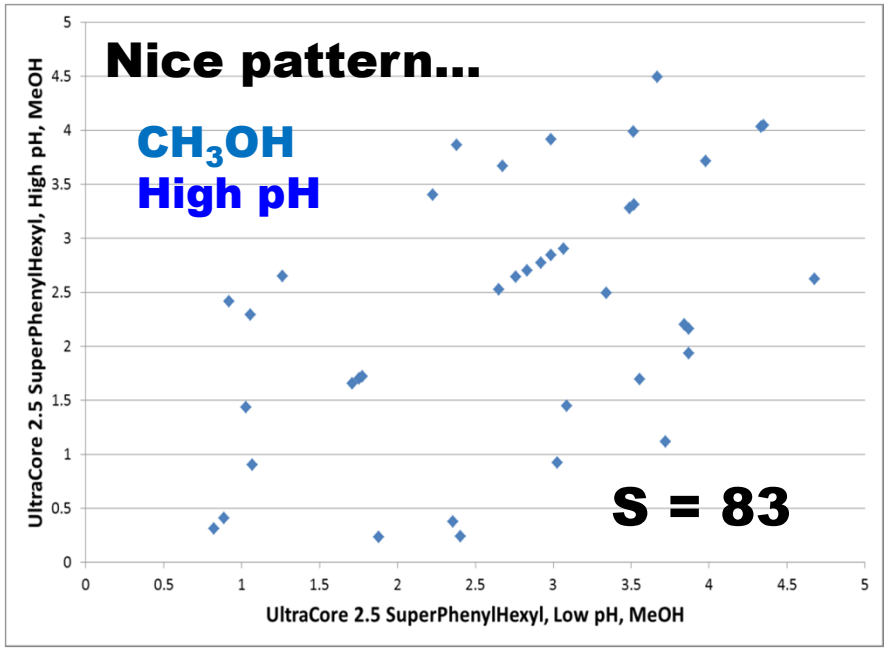


UltraCore SuperPhenylHexyl at low pH vs. SuperC18 at low pH

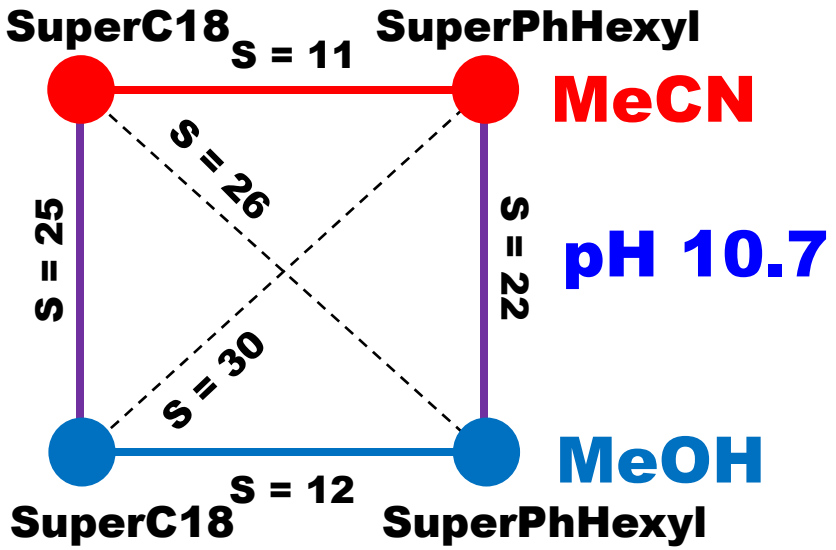


S ~10 or higher is effective for adjusting α

SuperPhenylHexyl Orthogonality: High pH vs. Low pH



UltraCore SuperPhenylHexyl (high pH) vs. SuperPhenylHexyl (low pH)



S values between phases and organic modifiers at high pH

Objective: Explore performance of ACE UltraCore columns for basic analytes

Parameters

- **Stationary phase**
  - ACE UltraCore SuperC18 and SuperPhenylHexyl
- **Organic modifier**
  - CH_3CN, CH_3OH
- **pH**
  - 2.8, 3.8, 8.2, 9.2, 10.2; 9.7 and 10.7

Samples

- 9 Appetite Suppressants
- 16 Drugs of Abuse

Sample 1: Appetite Suppressants

1. methamphetamine
2. amphetamine
3. ephedrine
4. fluoxetine
5. caffeine
6. phentermine
7. fenfluramine
8. chlordiazepoxide
9. phenylpropanolamine

Approach

Columns

- UltraCore SuperC18 and SuperPhenylHexyl, 2.1 x 50 mm, 2 μm

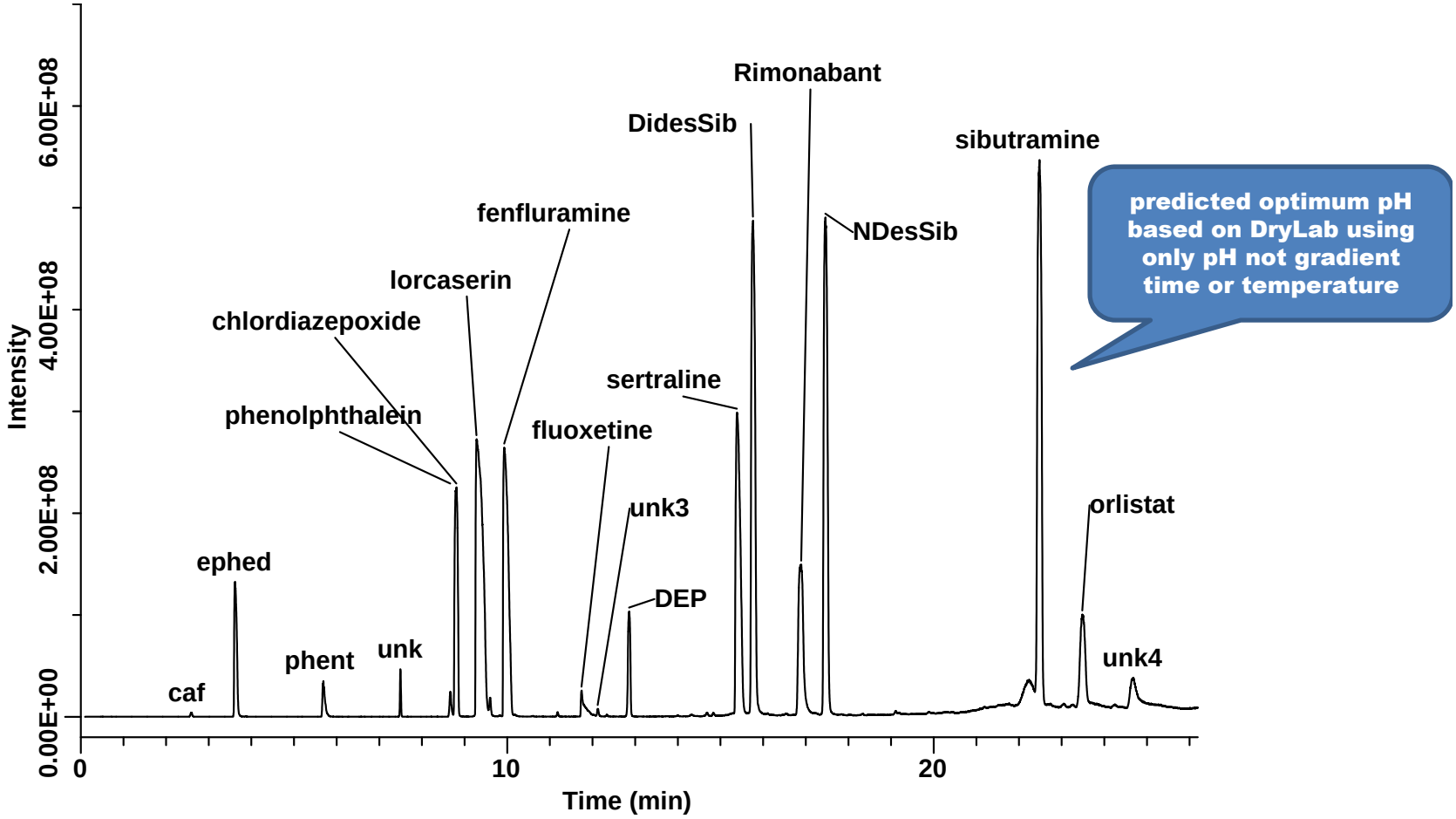
Organic modifiers

- ACN
- MeOH
- ACN/MeOH (1:1)

Aqueous buffers

- ammonium formate, pH 2.7
- ammonium acetate/NH4OH pH 8.7, 9.2, 10.2
- Gradients from 5 to 95% organic in

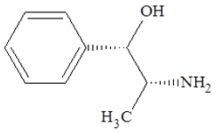
More Complex Appetite Suppressant Mixture: optimum pH from DryLab®

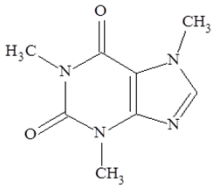


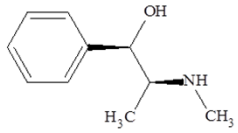
2.1 x 100 mm, 2 μm ACE Excel SuperC18
0.5 mL/min, 25°C, 10–90% CH3CN/20 mM pH 9.3 buffer in 20 min.
MS Detection

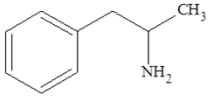
Data courtesy of Phyllis Wilson, U.S. FDA

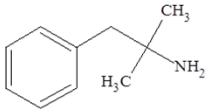
Sample: Appetite Suppressants

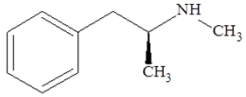

Phenylpropanolamine

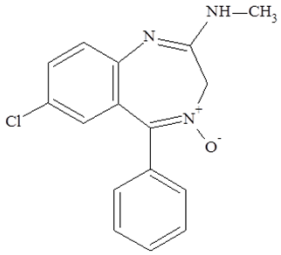

Caffeine

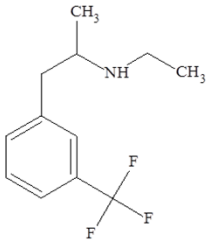

Ephedrine

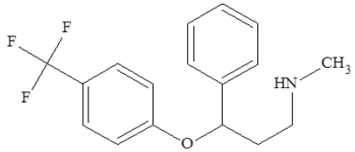

Amphetamine


Phentermine


Methamphetamine

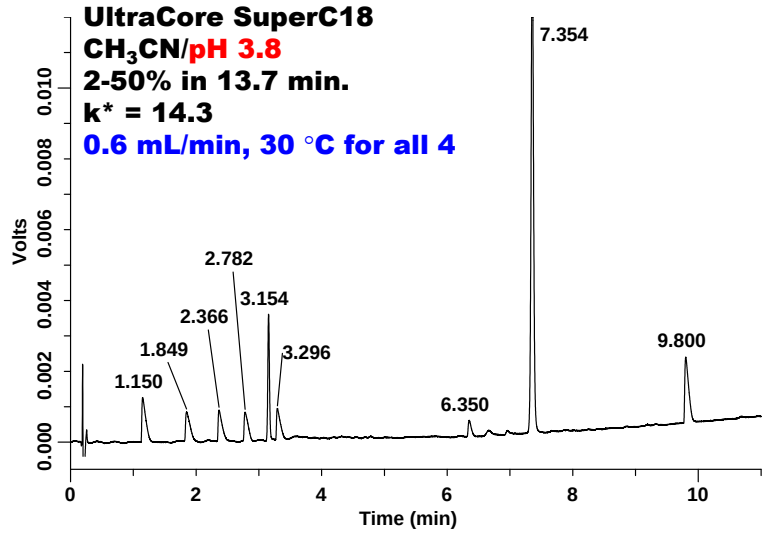

Chlordiazepoxide

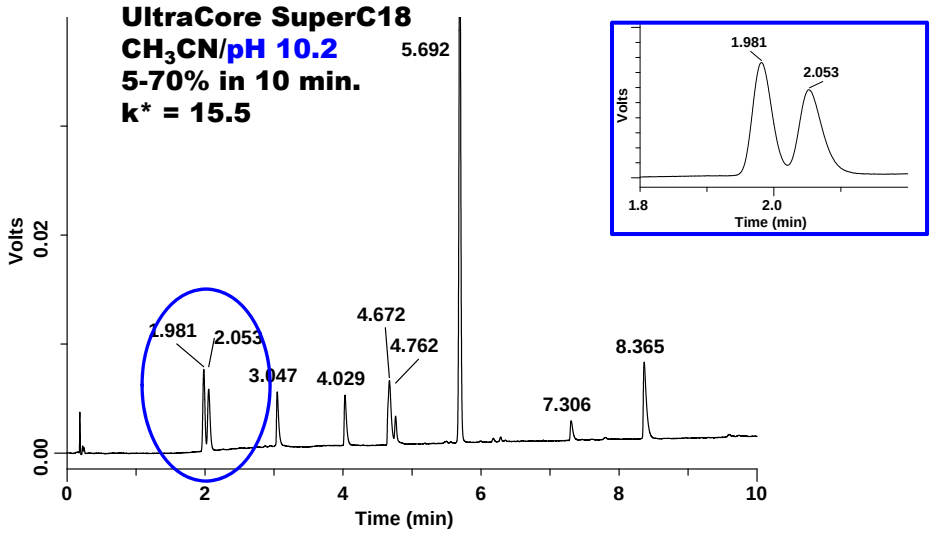

Fenfluramine

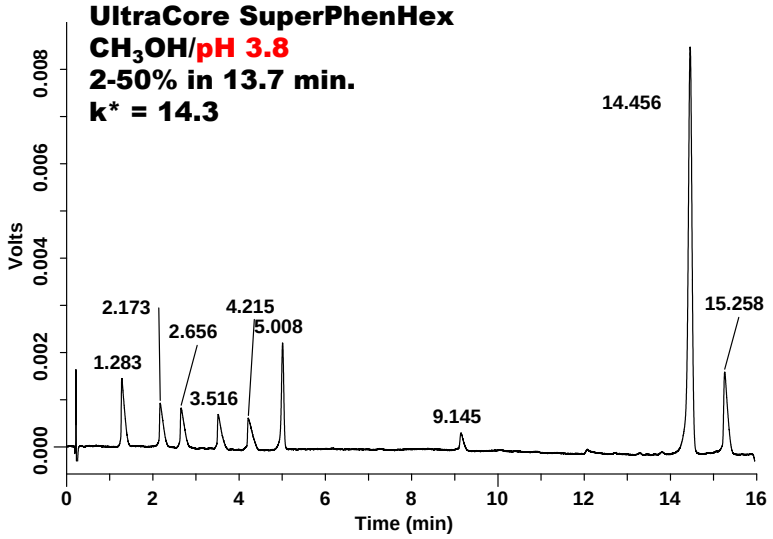

Fluoxetine

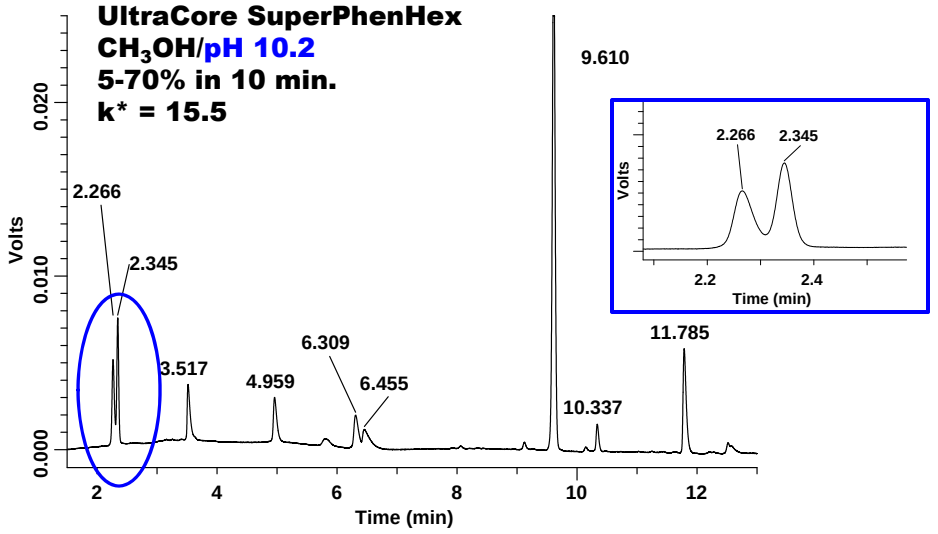
Compound	pKa	log P	pI	RT ACN pH 10.2 2-70% 9.71 min 0.6 mL/min
phenylpropanolamine	9.37	0.89	11.63	2.61
caffeine	14.0	-0.55	none	2.66
ephedrine	9.52	1.32	11.71	3.44
amphetamine	10.10	1.8	none	4.31
phentermine	10.25	2.08	none	4.85
methamphetamine	9.9	2.24	none	4.96
chlordiazepoxide	6.43	3.05	12.47	5.88
fenfluramine	10.22	3.47	none	7.18
fluoxetine	9.8	4.17	none	8.55

UltraCore SuperC18: Examples with CH_3CN and CH_3OH at pH 3.8 and pH 10.2

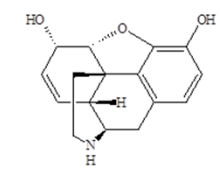


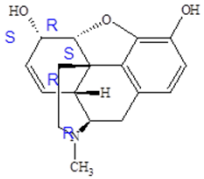


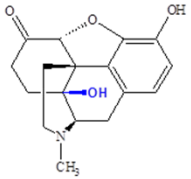


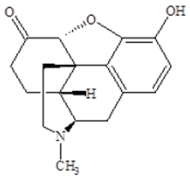


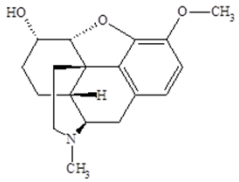
Sample: 16 Drugs of Abuse

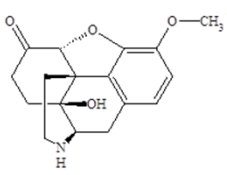

Normorphine

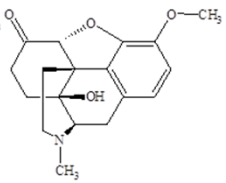

Morphine

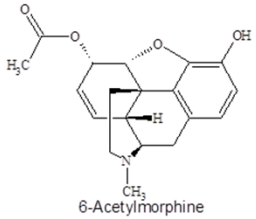

Oxymorphone

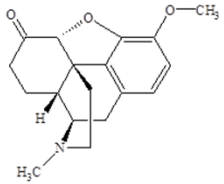

Hydromorphone

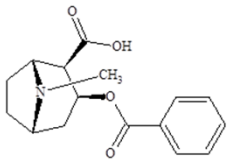

Dihydrocodeine

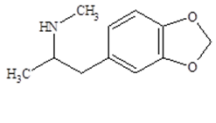

Noroxycodone

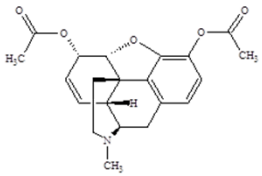

Oxycodone

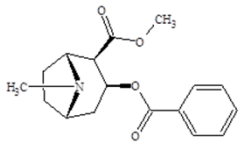

6-Acetylmorphine

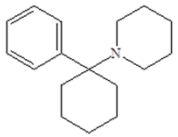

Hydrocodone

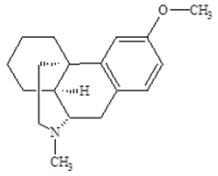

Benzoylecgonine

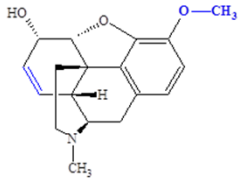

MDMA


Heroin


Cocaine


PCP

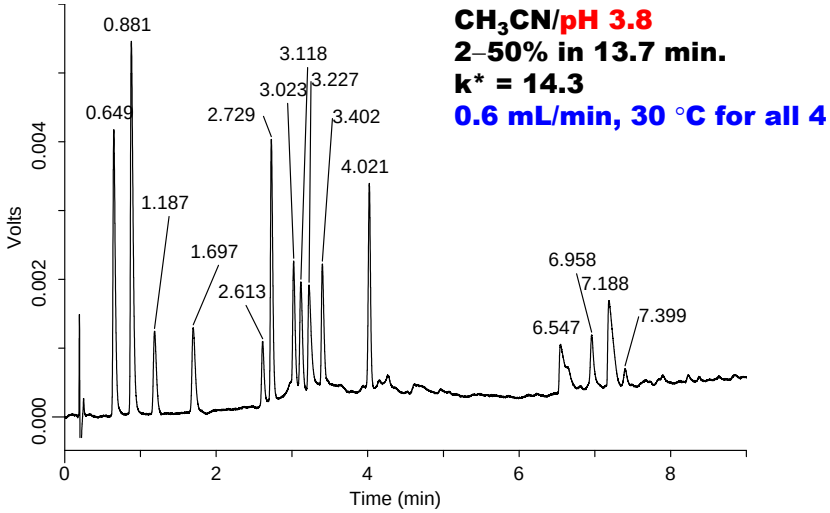

Dextromethorphan


Codeine

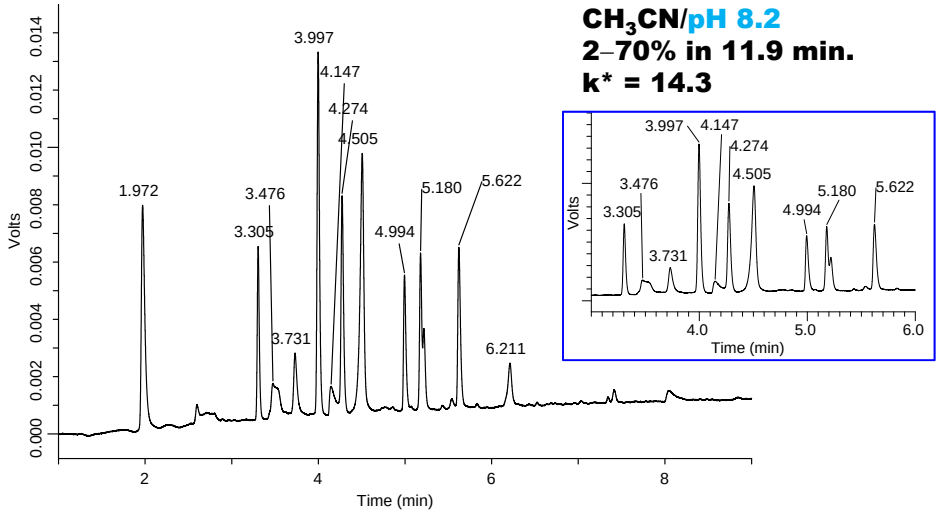
MW	Compound Name	pKa	log P	pI	RT SPH 2-50% pH 2.8
271.31	Normorphine	10.12	0.26	10.12	0.707
285.34	Morphine	9.66	0.90	9.66	1.051
301.34	**Oxymorphone**	**9.14**	**0.78**	**9.14**	**1.405**
285.34	Hydromorphone	9.34	1.62	9.34	1.789
301.38	Dihydrocodeine	9.33	1.55	11.74	2.432
299.36	Codeine	9.19	1.34	11.49	2.519
301.34	**Noroxycodone**	**9.14**	**0.78**	**9.14**	**2.663**
315.36	Oxycodone	8.21	1.03	10.89	2.807
327.37	6-Acetylmorphine	8.42	1.09	9.25	2.874
299.36	Hydrocodone	8.61	1.96	13.30	2.989
289.33	Benzoylecgonine	9.54	-0.59	6.49	3.268
193.24	MDMA	10.14	1.86	NA	3.518
369.41	Heroin	9.10	1.55	NA	3.966
303.35	Cocaine	8.85	2.28	NA	4.051
243.39	Phencyclidine (PCP)	10.64	4.49	NA	4.616
271.40	Dextromethorphan	9.85	3.49	NA	4.793

Isobaric compounds highlighted in same color
RTs for SuperPhenylHexyl 2-50% gradient

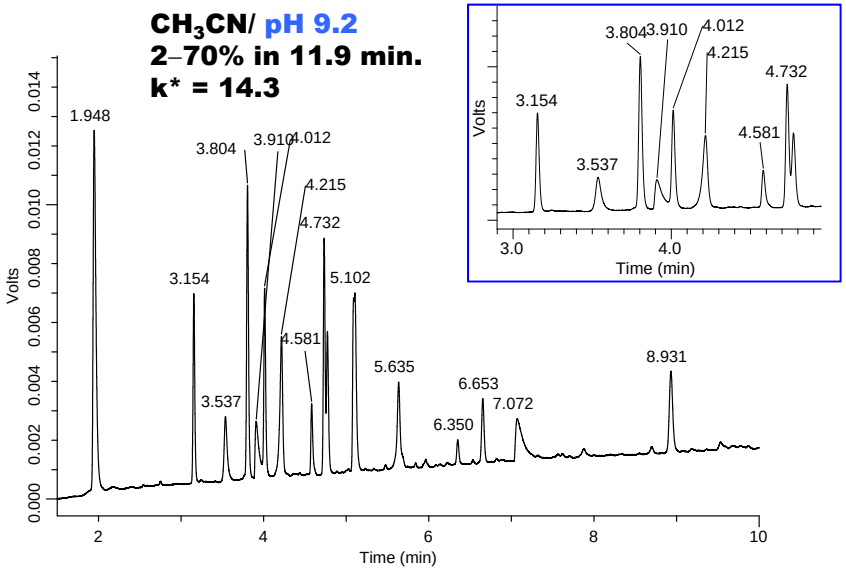
UltraCore SuperC18, 2.1 x 50 mm, 2.5 µm
4 pHs with CH_3CN



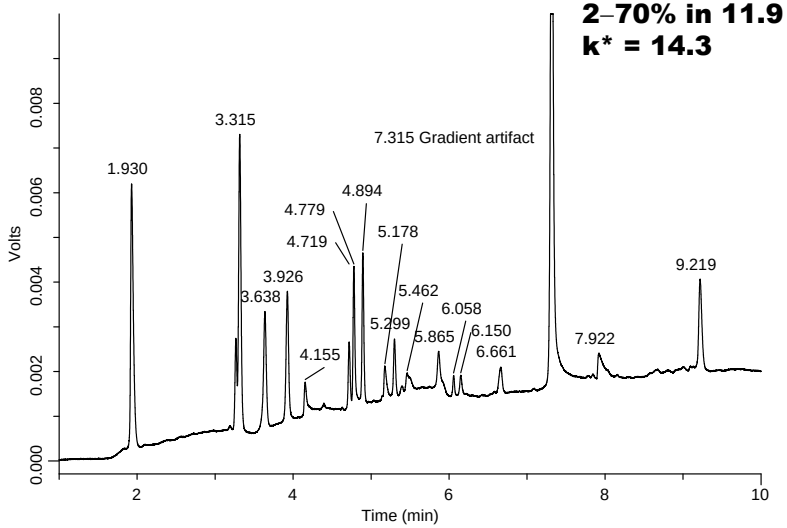
CH_3CN/pH 3.8
2–50% in 13.7 min.
k* = 14.3
0.6 mL/min, 30 °C for all 4



CH_3CN/pH 8.2
2–70% in 11.9 min.
k* = 14.3

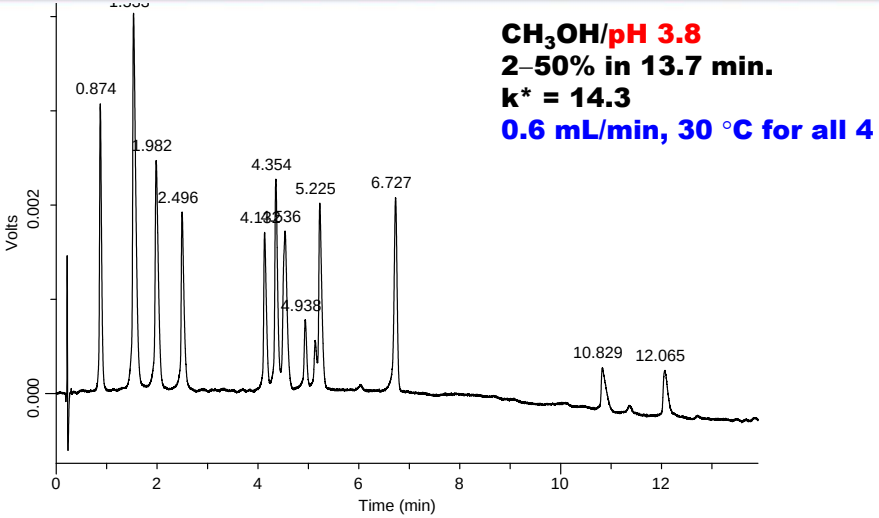


CH_3CN/ pH 9.2
2–70% in 11.9 min.
k* = 14.3

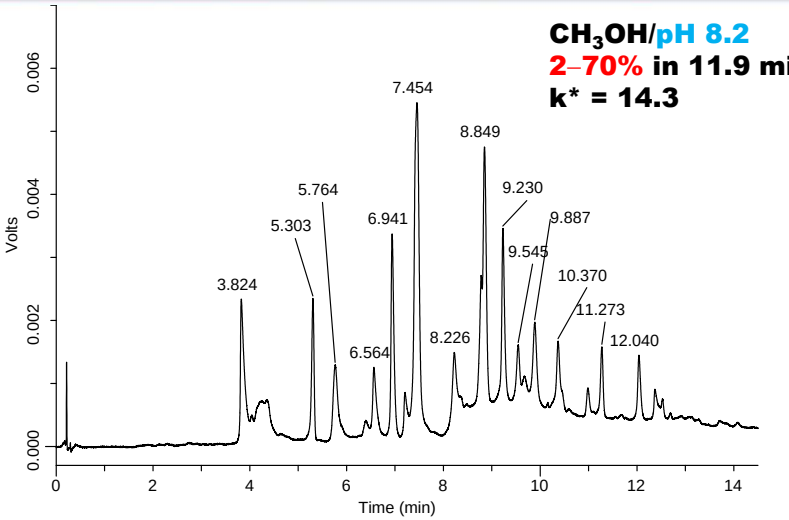


CH_3CN/ pH 10.2
2–70% in 11.9 min.
k* = 14.3

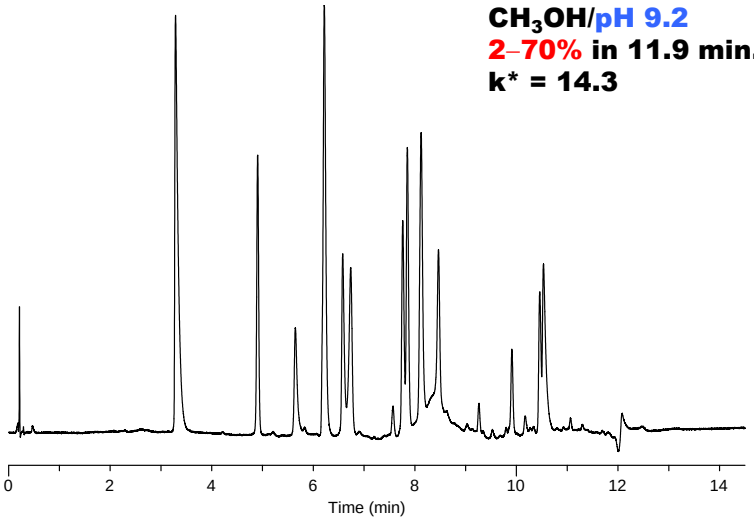
UltraCore SuperPhenylHexyl, 2.1 x 50 mm, 2.5 μm: 4 pHs with CH_3OH



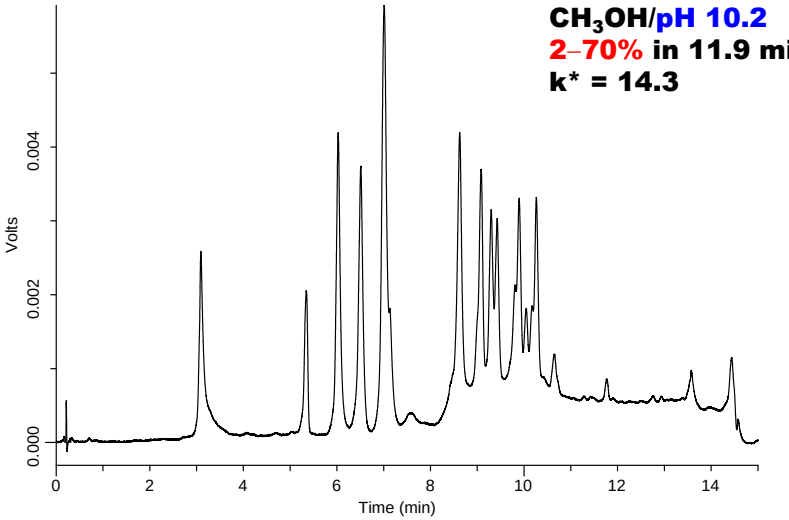
CH_3OH/pH 3.8
2–50% in 13.7 min.
k* = 14.3
0.6 mL/min, 30 °C for all 4



CH_3OH/pH 8.2
2–70% in 11.9 min.
k* = 14.3

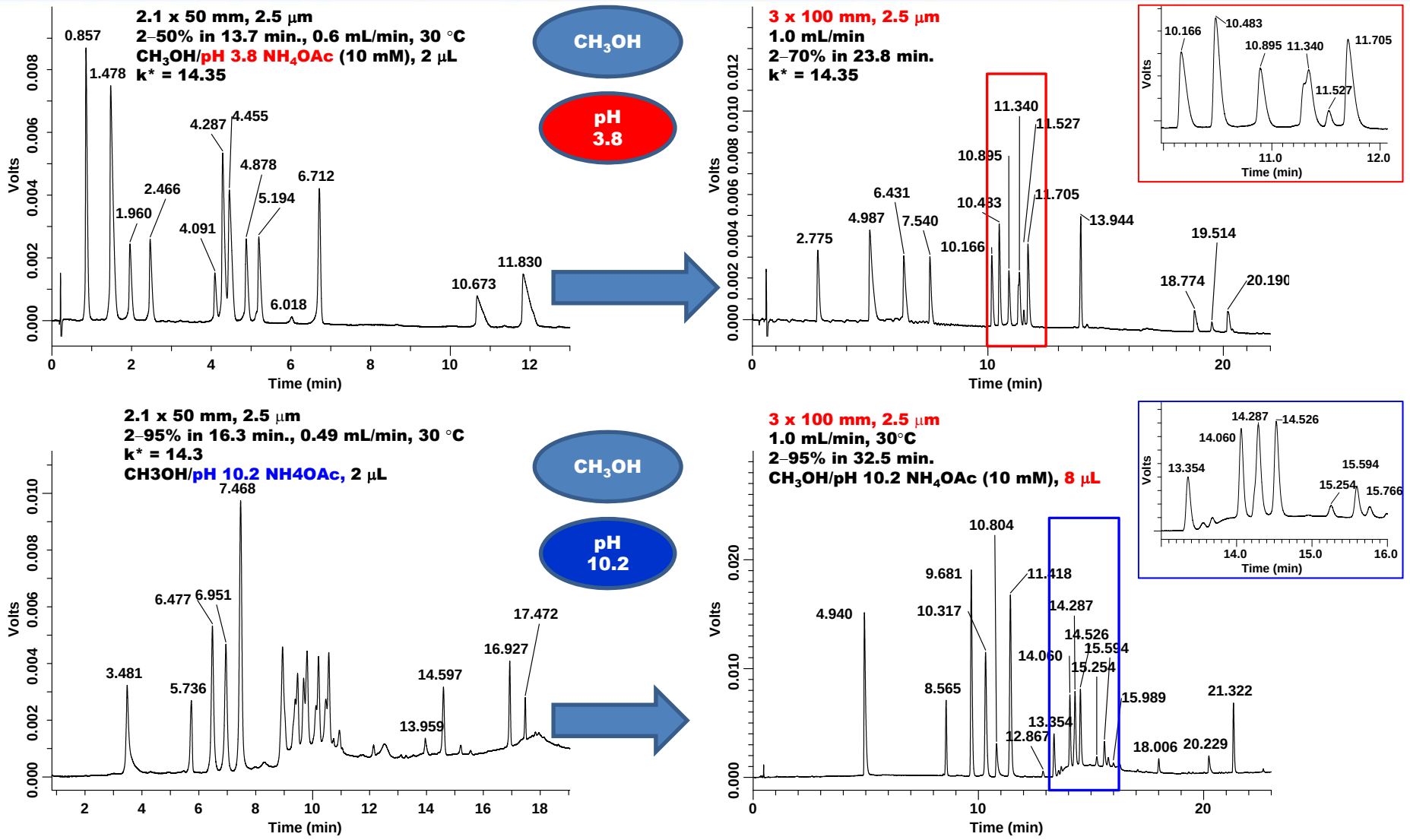


CH_3OH/pH 9.2
2–70% in 11.9 min.
k* = 14.3

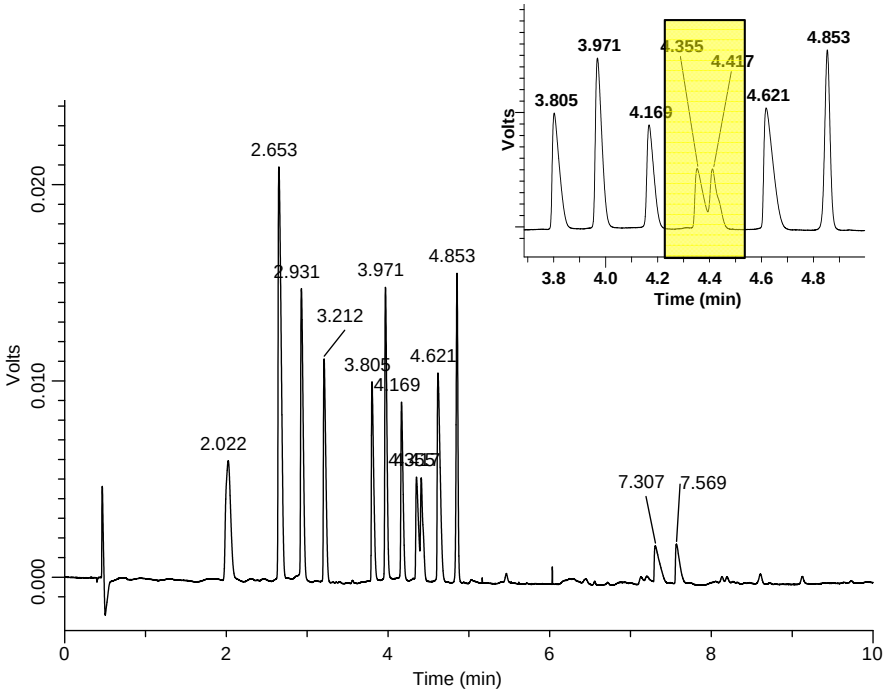


CH_3OH/pH 10.2
2–70% in 11.9 min.
k* = 14.3

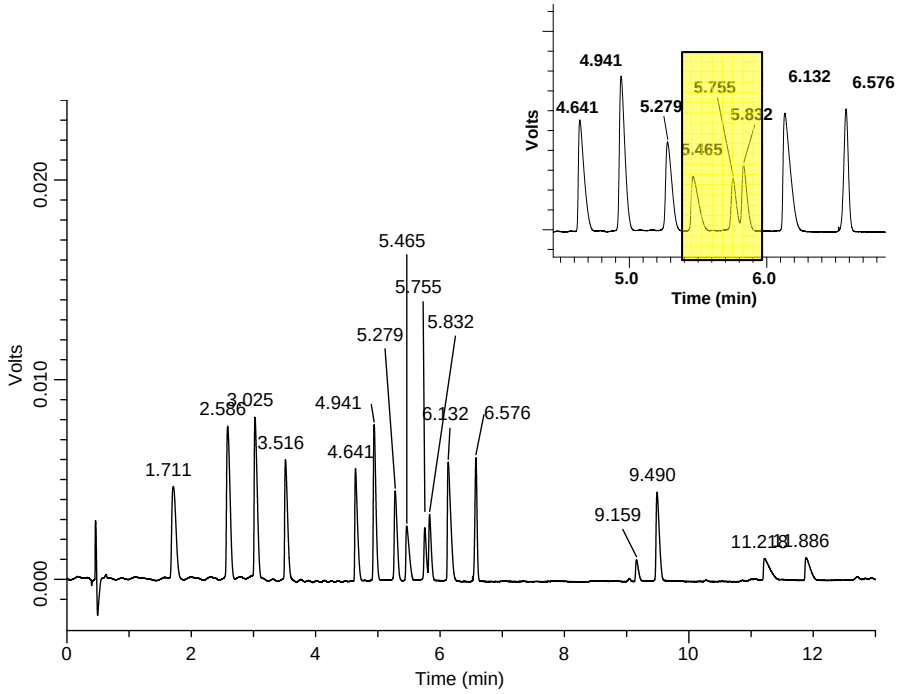
Scaling pH 3.8 and 10.2 Separations to 3 x 100 mm UltraCore SuperPhenylHexyl



Separation on 3 x 100 mm, 2.5 μm SuperPhenylHexyl: All 16 separated



2–70% ACN/pH 3.8 in 15 min. slope



2–70% ACN/pH 3.8 in 30 min. slope

Additional optimization with DryLab

Summary

- **ACE UltraCore SuperC18 and SuperPhenylHexyl columns were evaluated at several pHs for two mixtures of basic analytes.**
- **Peak shape and selectivity were best at pH 3.8 and pH 10.2 with ammonium formate and ammonium acetate/NH_4OH mobile phases, respectively.**
- **Use of 2.1 x 50 mm, 2.5 μm column geometries allows for rapid, yet thorough examination of various combinations of organic modifier, stationary phase and pH.**
- **Method transfer to larger column geometries went well, although inability to use injection delays did not permit completely accurate transfer.**
- **Useable conditions were found for both of the best conditions for each of the columns at low and high pH.**
- **For the drugs of abuse sample, pH 10.7 did not afford as good resolution as pH 10.2.**