About OMICS Group

OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

About OMICS Group Conferences

OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bangalore and Mumbai.

4th International Conference and Exhibition on Analytical & Bioanalytical Techniques

Las Vegas, Nevada, October 15-17, 2013

Why Development of New HPLC Column Technology is Still Alive

Joseph J. Pesek
Department of Chemistry
San Jose State University

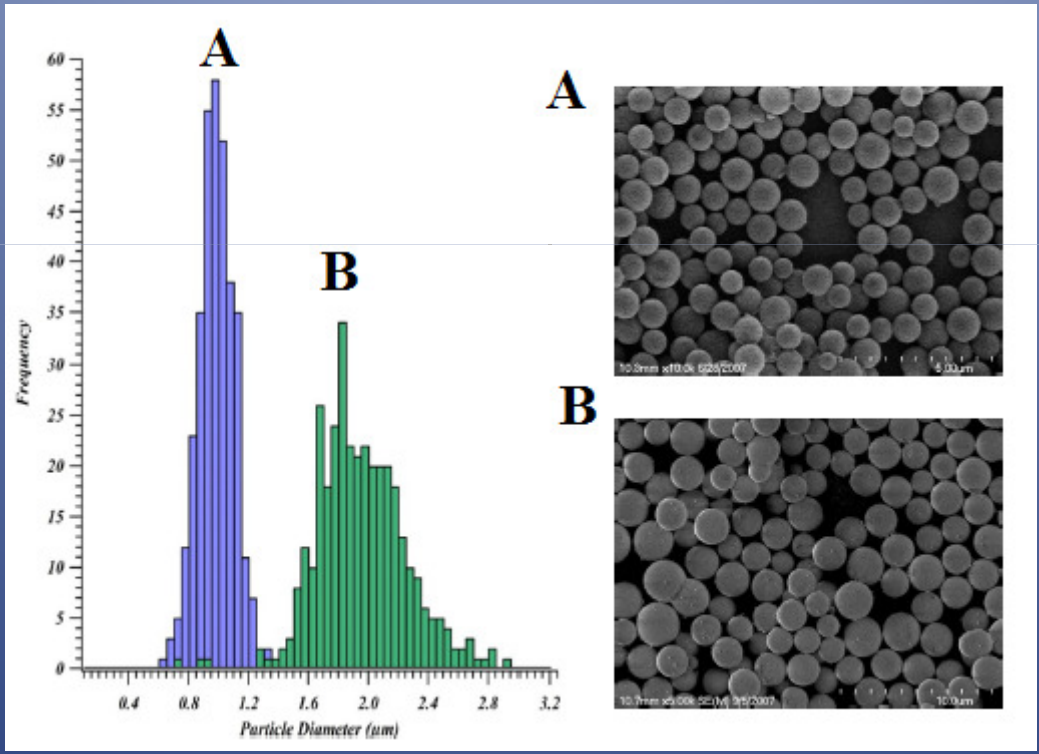
Some reflections on the history of HPLC column development

This presentation will focus on 5 areas of recent HPLC packed column development

1. **Small particles and UPLC**
2. **Fused core particles**
3. **Silica hydride materials**
4. **Mixed-mode stationary phases**
5. **Micro- and nano-separation formats**

Small particles and UPLC

Small particles are generally considered to be 2 microns or less



Small particles result in higher pressure, better instrumentation is needed

BENEFITS OF GOING TO SMALL PARTICLES

HIGHER EFFICIENCIES AT FASTER FLOW RATES

SHORTER ANALYSIS TIMES

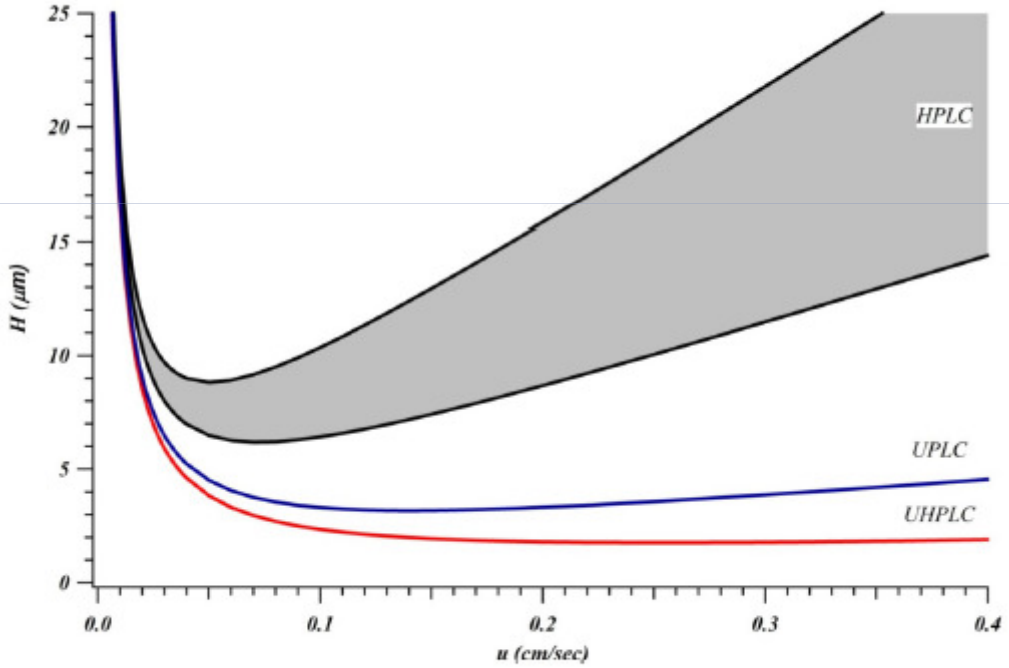
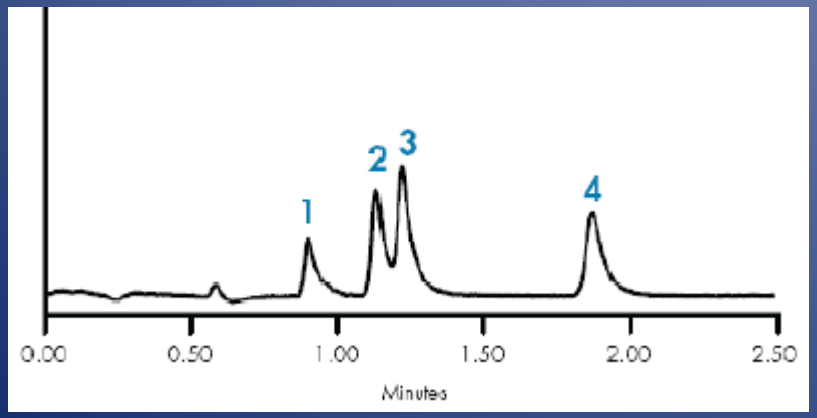


Figure 1-3. Theoretical van Deemter curves for HPLC, UPLC and UHPLC showing the decrease in H_{min}, increase in u_{opt}, and overall decrease in the slope of the high linear velocity region. Particle diameters: HPLC, 3.5 – 5 µm; UPLC, 1.8 µm; UHPLC; 1.0 µm.

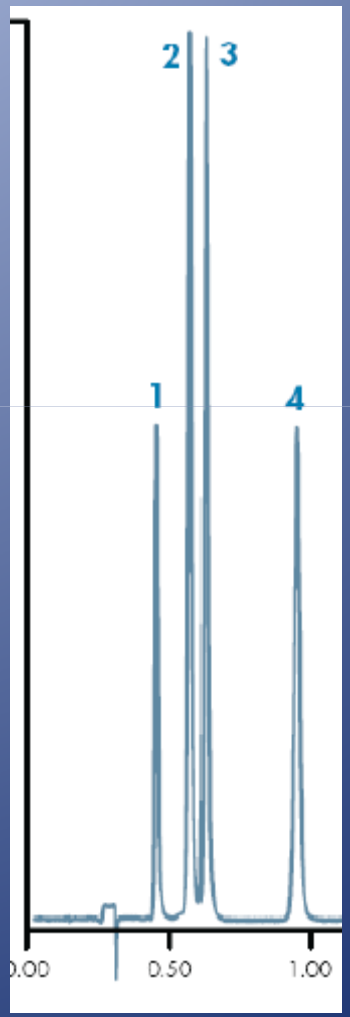
INSTRUMENT DESIGN IS CRUCIAL

Same sample with the same 1.7 µm particle size column run on HPLC and UPLC instruments

UPLC instruments are designed to have minimal extra-column volume that reduces band spreading

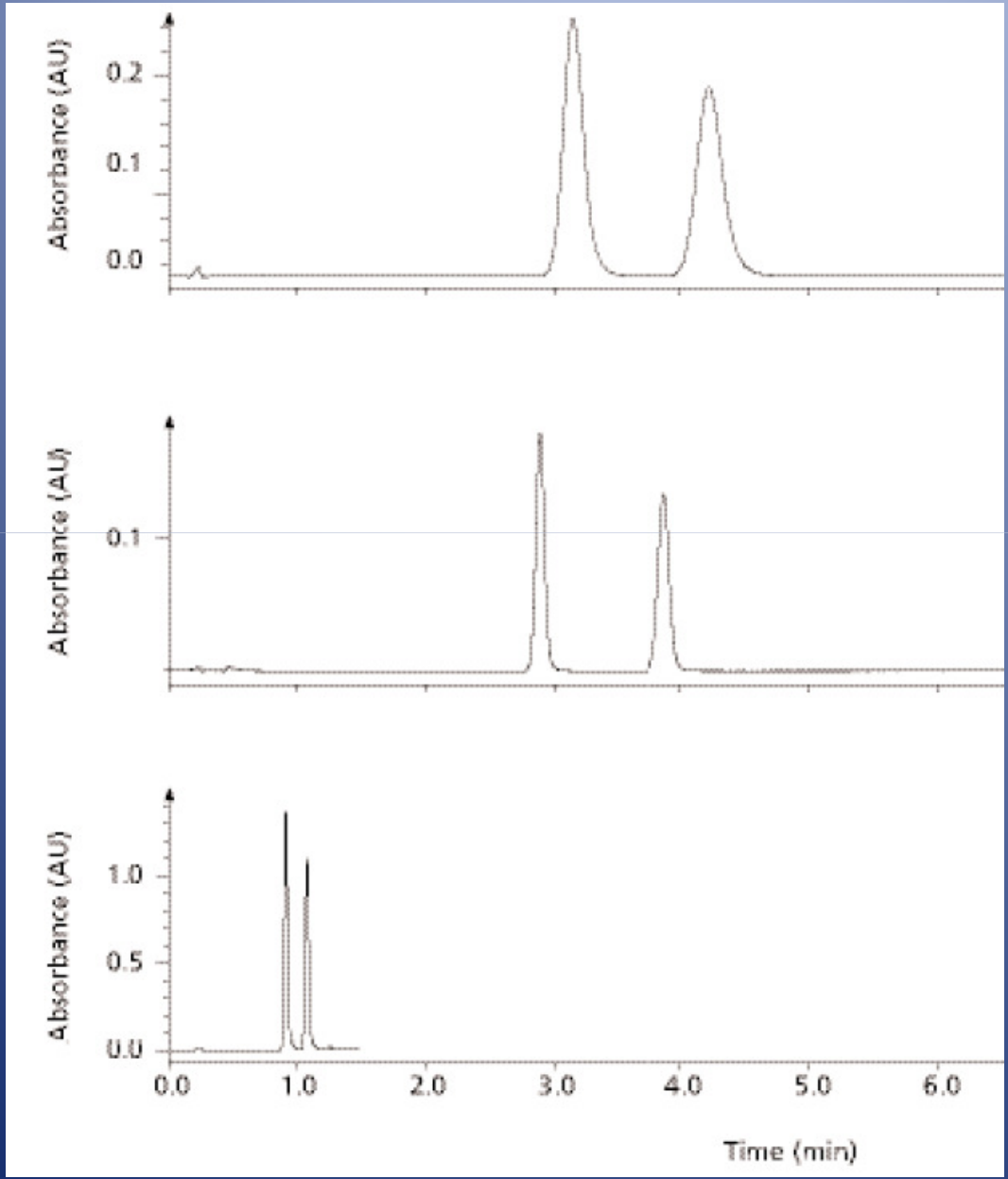


HPLC Instrument



UPLC Instrument

TRANSITION FROM HPLC TO UPLC

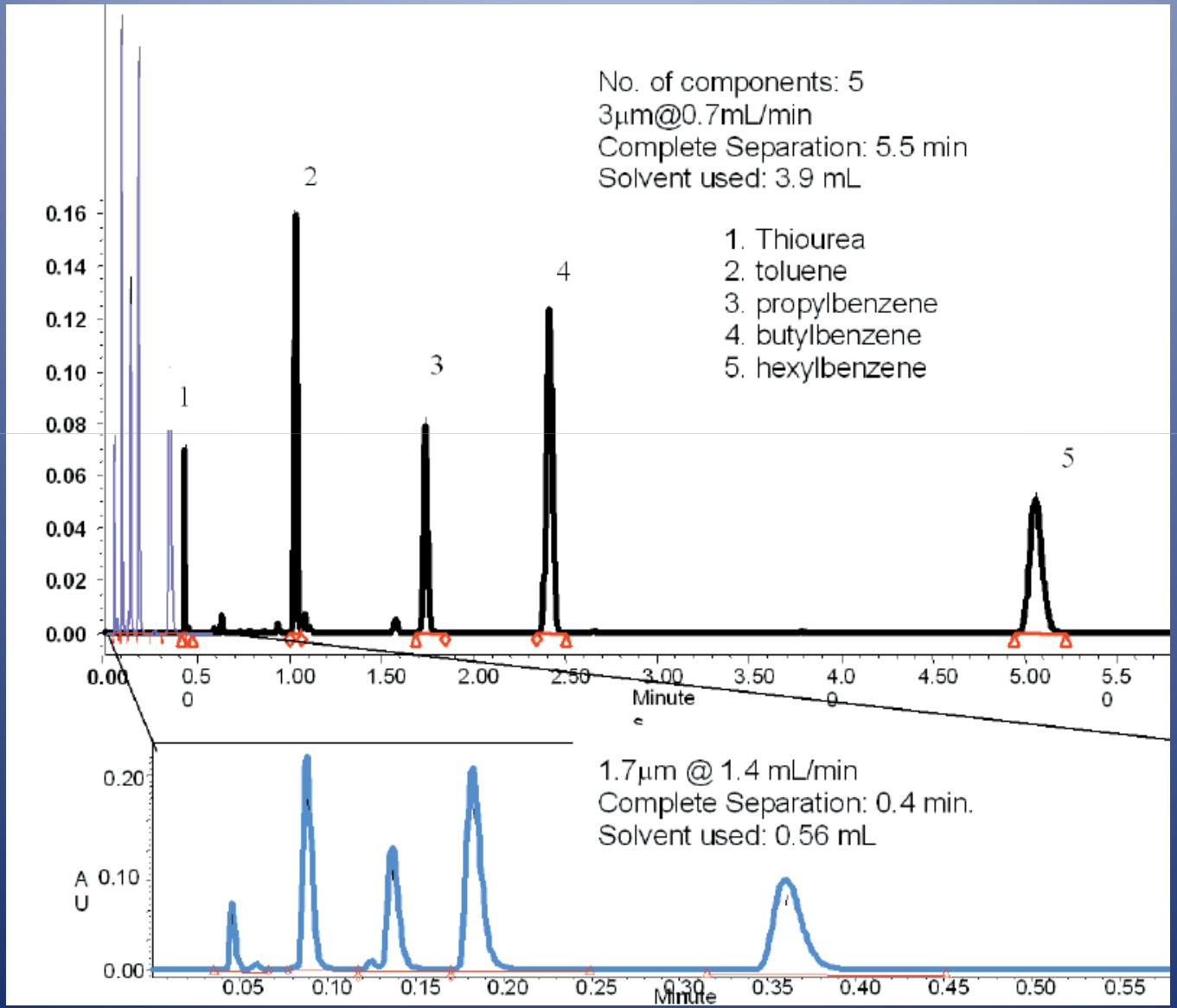


HPLC

Move method to UPLC column and instrument

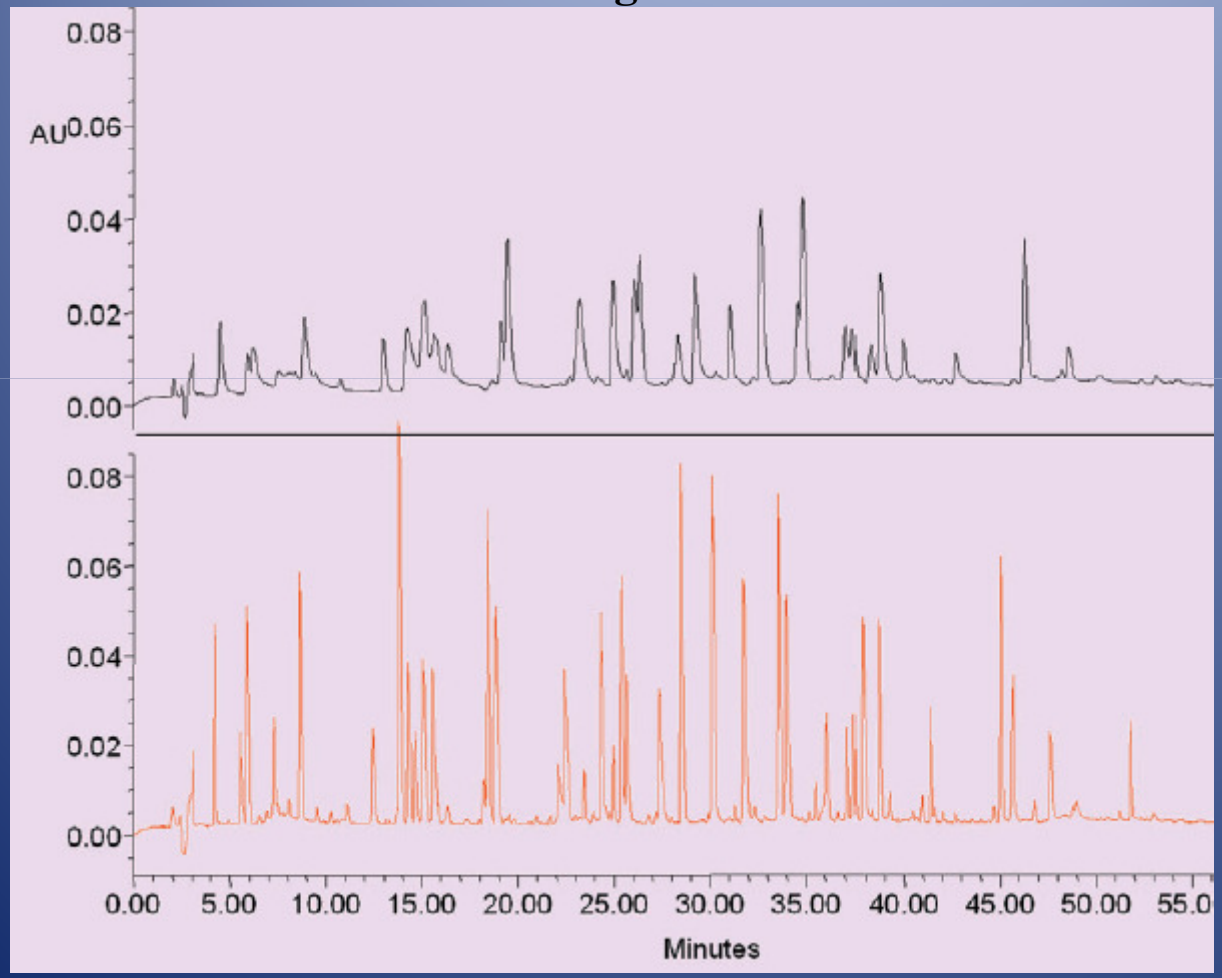
Optimize UPLC method

WHAT HAPPENS AS THE NUMBER OF COMPONENTS IN THE SAMPLE INCREASES



WHAT IS THE BENEFIT FOR VERY COMPLEX SAMPLES

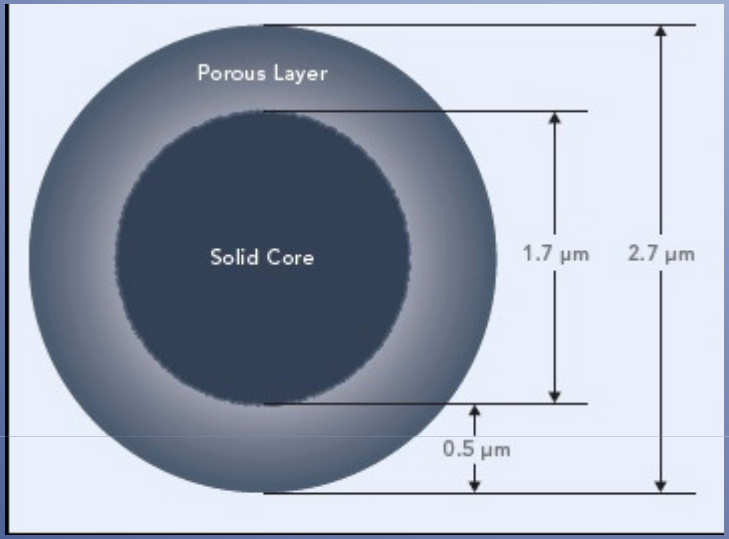
Protein digest



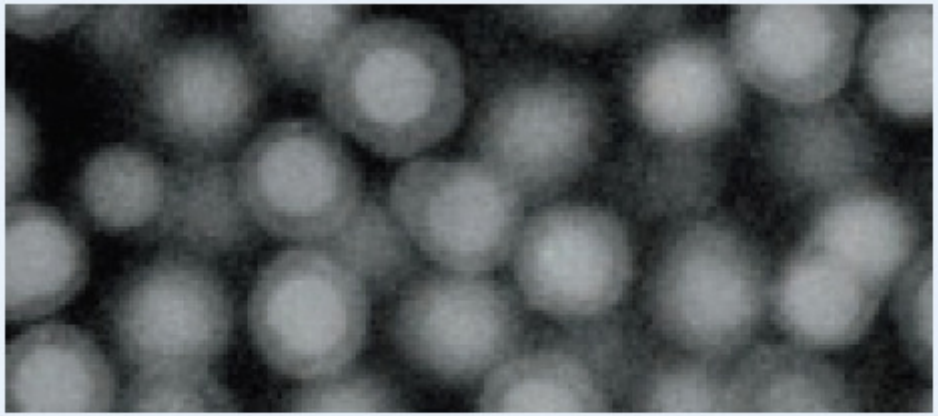
5 µm C18 column
# of peaks: 70
Peak capacity: 143

1.7 µm C18 column
# of peaks: 168
Peak capacity: 360

Fused core particles

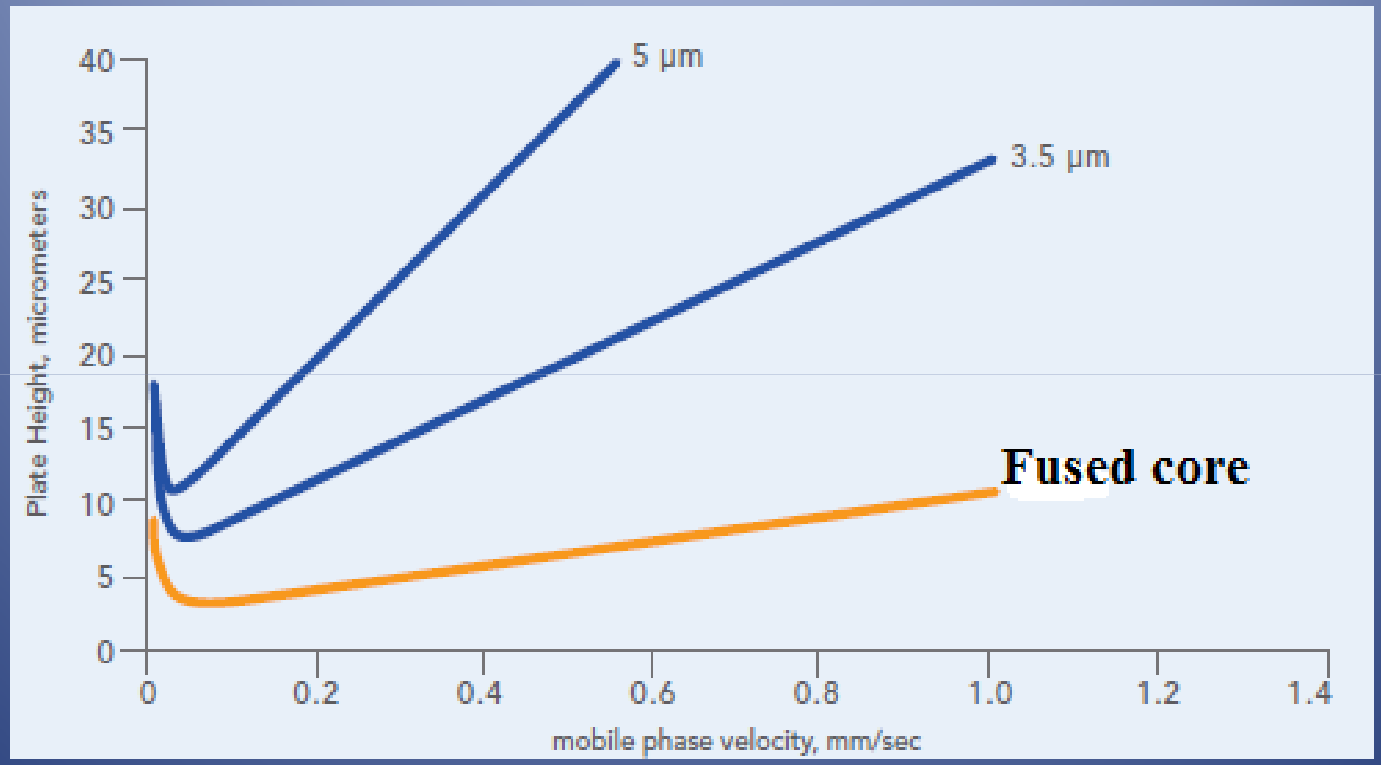


A porous silica layer is "fused on top of a solid silica core



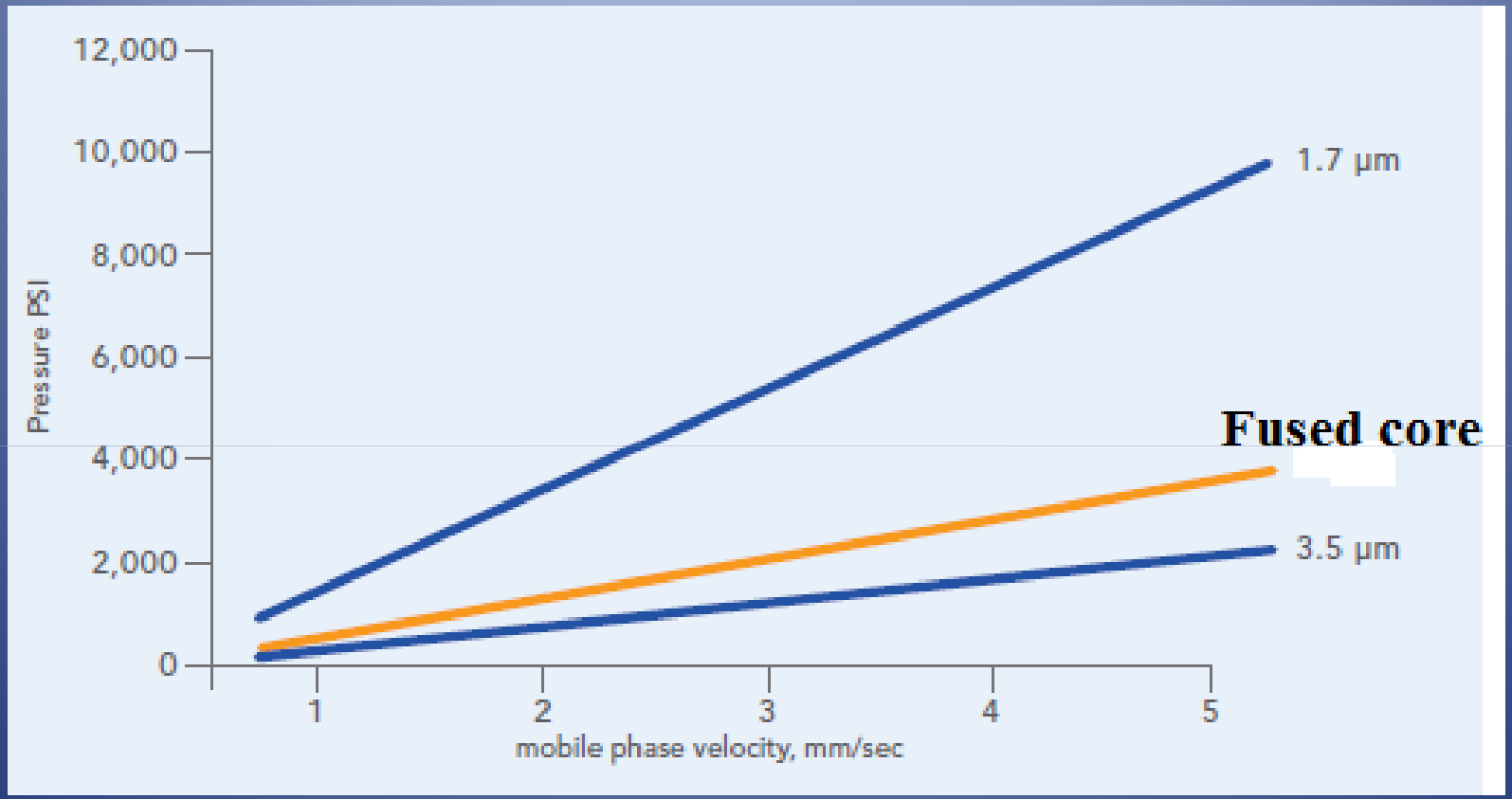
This SEM photograph of "sliced" Fused-Core particles clearly shows the structure; a solid core surrounded by a porous "halo".

COLUMN EFFICIENCY



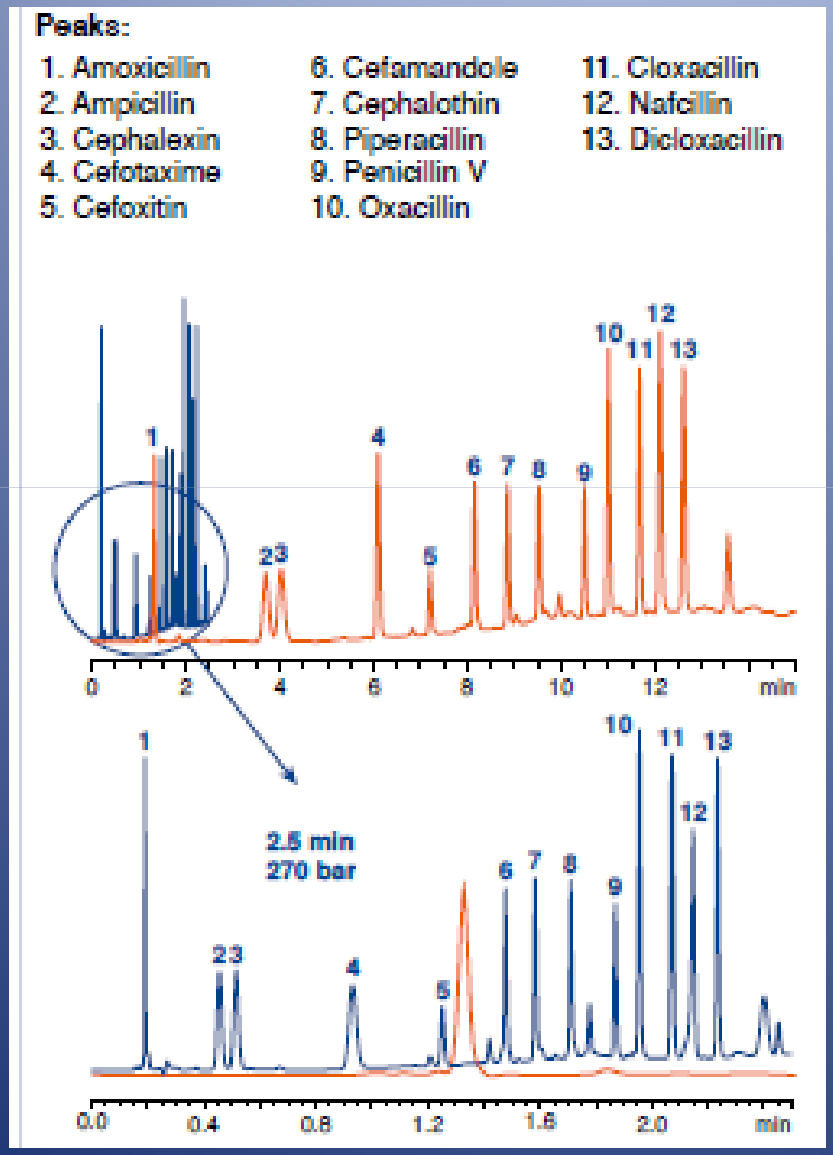
Similar to small particle size columns

ADVANTAGE OF LOWER PRESSURE NEEDED



Most work can be done with conventional HPLC instrument

CORE SHELL VS POROUS PARTICLE

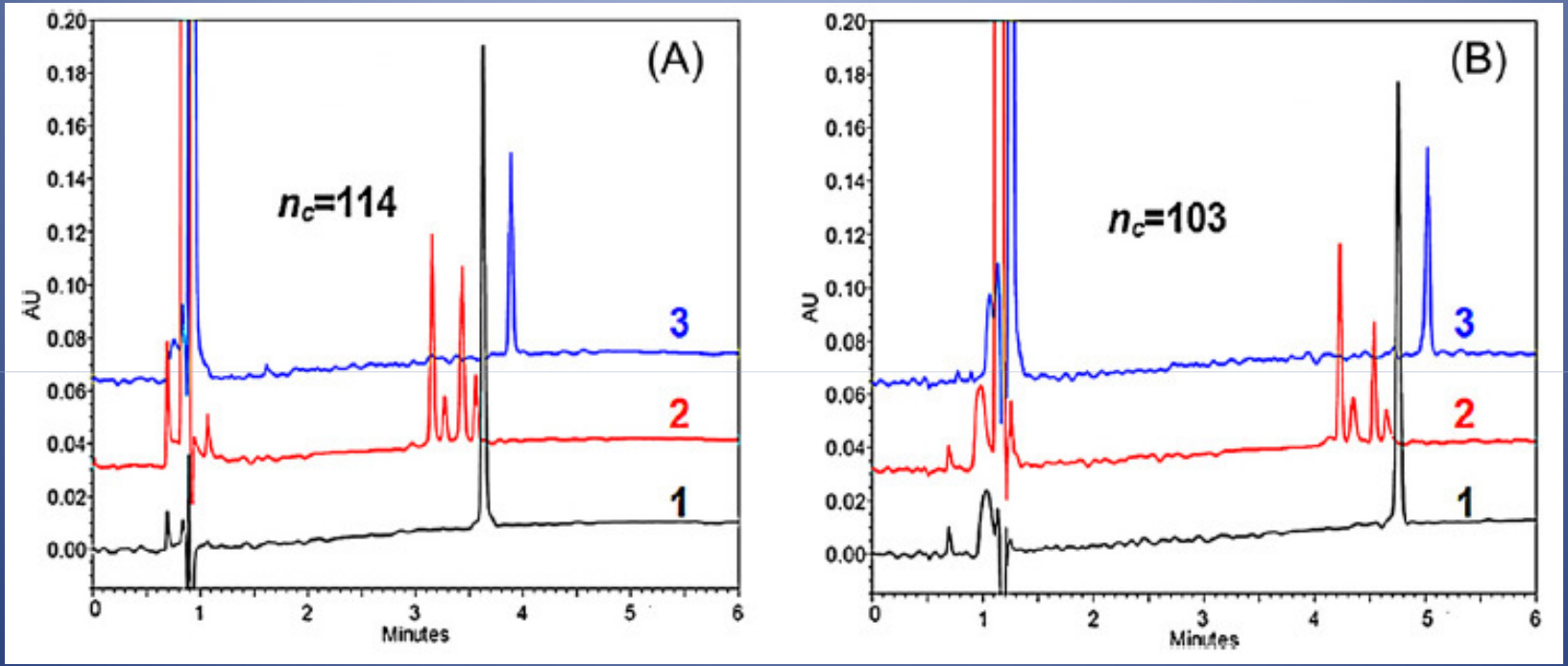


Red peaks are obtained on a standard 5 µm C18 column

Blue peaks are obtained on fused core C18 column

Results are similar to those obtained on smaller particle UPLC columns

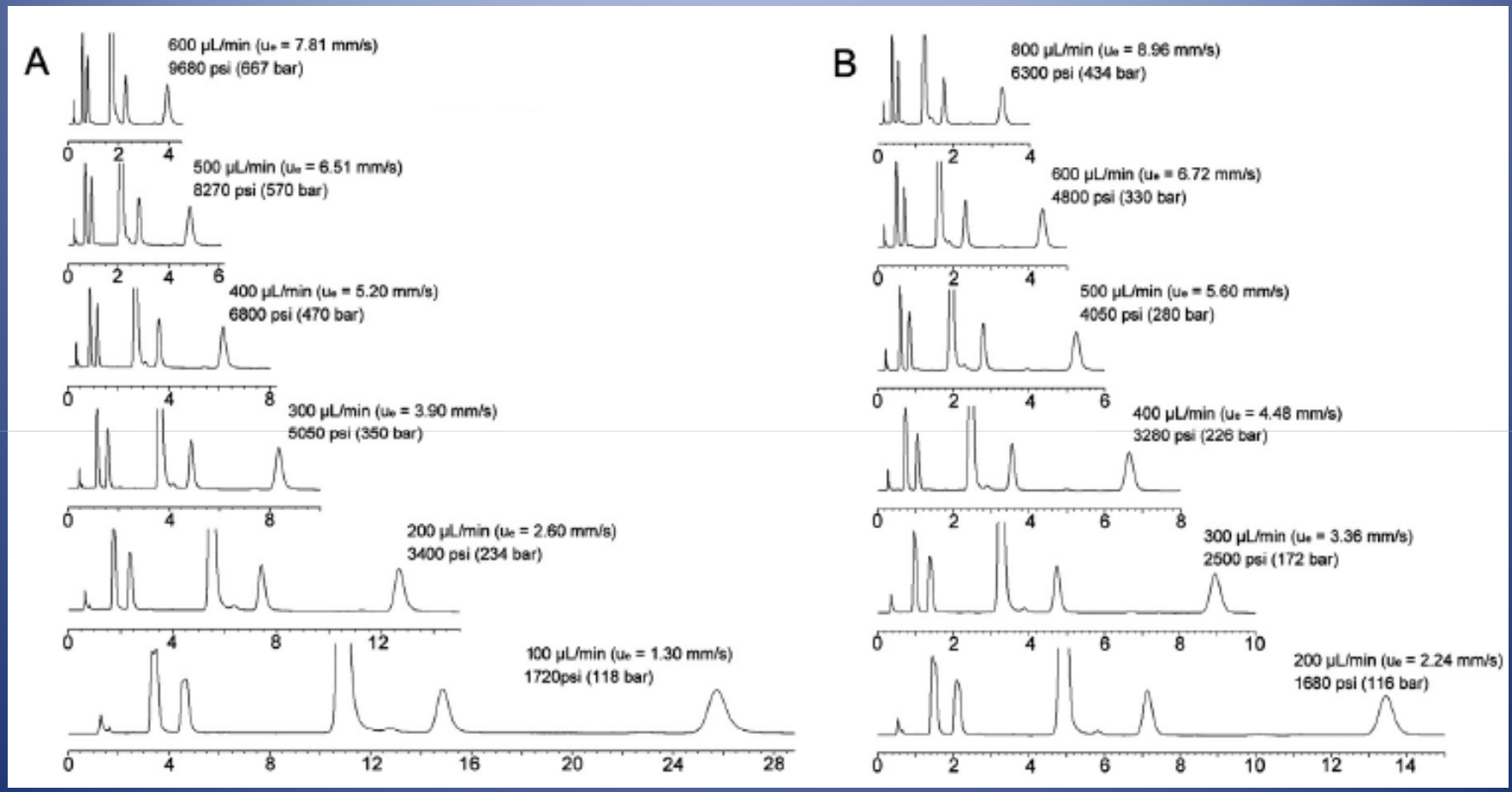
COMPARISON OF PROTEIN SEPARATIONS ON SMALL PARTICLE AND CORE SHELL COLUMNS



(A) Core shell column **(B) Small particle column**

1. Native protein **2. Oxidized forms** **3. Reduced forms**

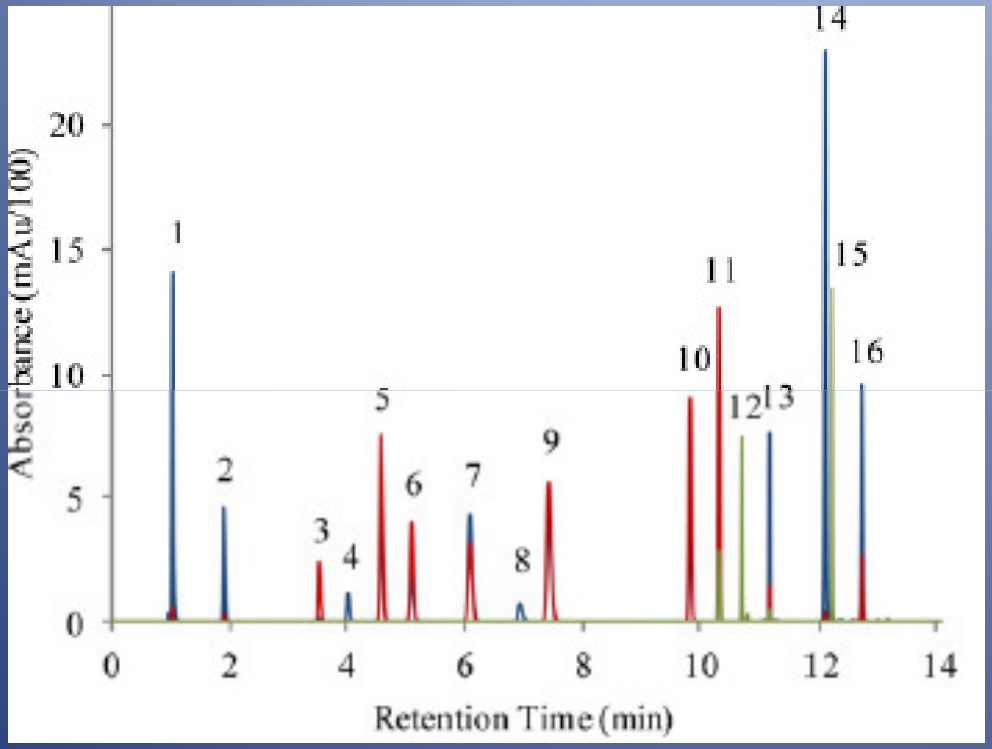
COMPARISON OF ARTICHOKE EXTRACT SEPARATION ON SMALL PARTICLE AND CORE SHELL COLUMNS



A. Small particle column **B. Core shell column**

Comparisons done at various flow rates

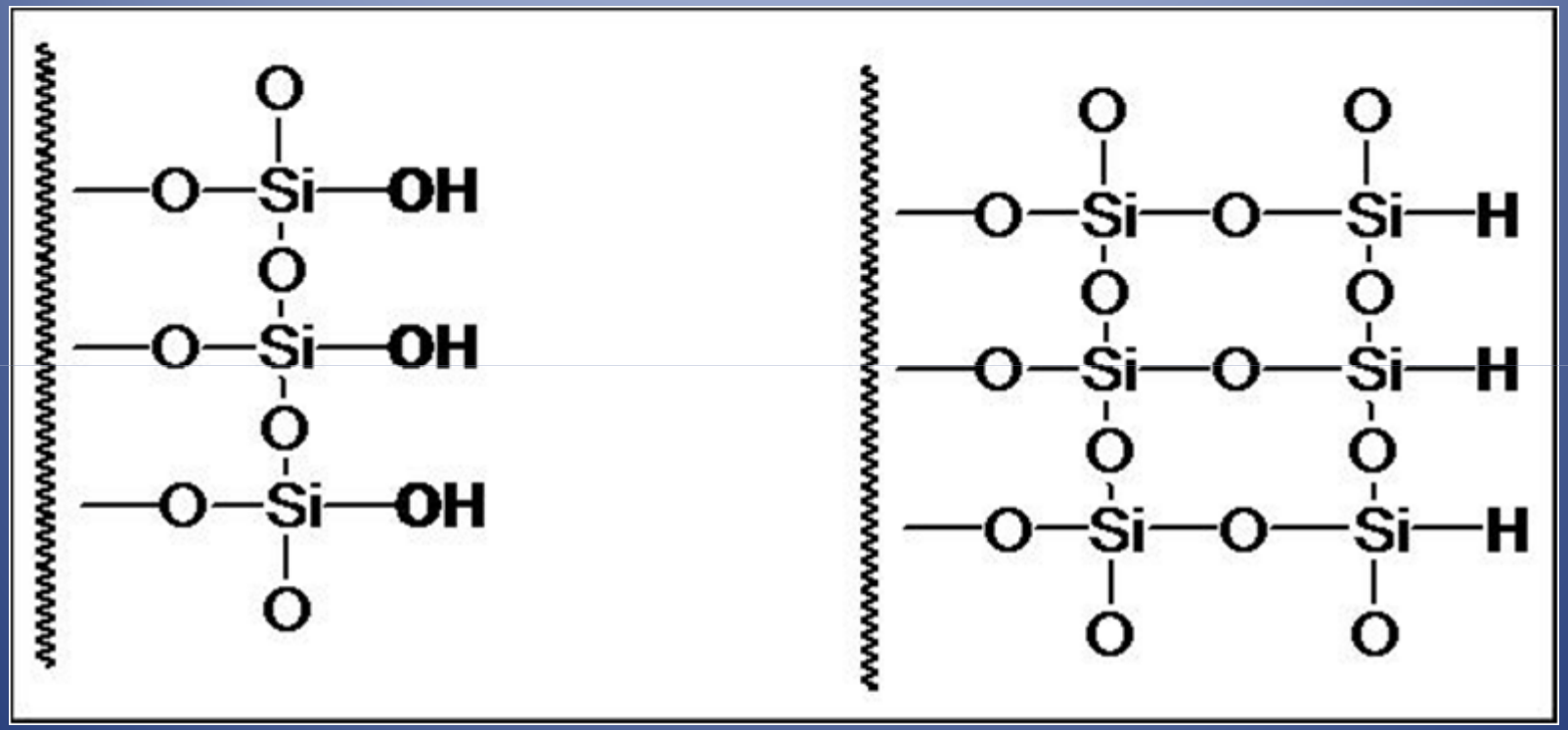
STANDARDS FOR WINE ANALYSIS



Monomeric, non-anthocyanins	
Gallic acid	1
Protocatechuic acid	2
2,5-Dihydroxybenzoic acid	3
Catechin	4
Caffeic acid	5
Chlorogenic acid	6
Vanillin	7
Epicatechin	8
Coumaric acid	9
Ferulic acid	10
Sinapinic acid	11
Rutin	12
Naringin	13
Cinnamic acid	14
Quercetin	15
Naringenin	16

Column: 100 mm × 4.6 mm packed with 2.6 µm particle diameter core shell C18 stationary phase

Silica hydride materials



ORDINARY SILICA SILICA HYDRIDE

WHAT MAKES SILICA HYDRIDE STATIONARY PHASES CHROMATOGRAPHICALLY INTERESTING?

All silica hydride phases can retain compounds in either the reversed-phase or normal phase modes

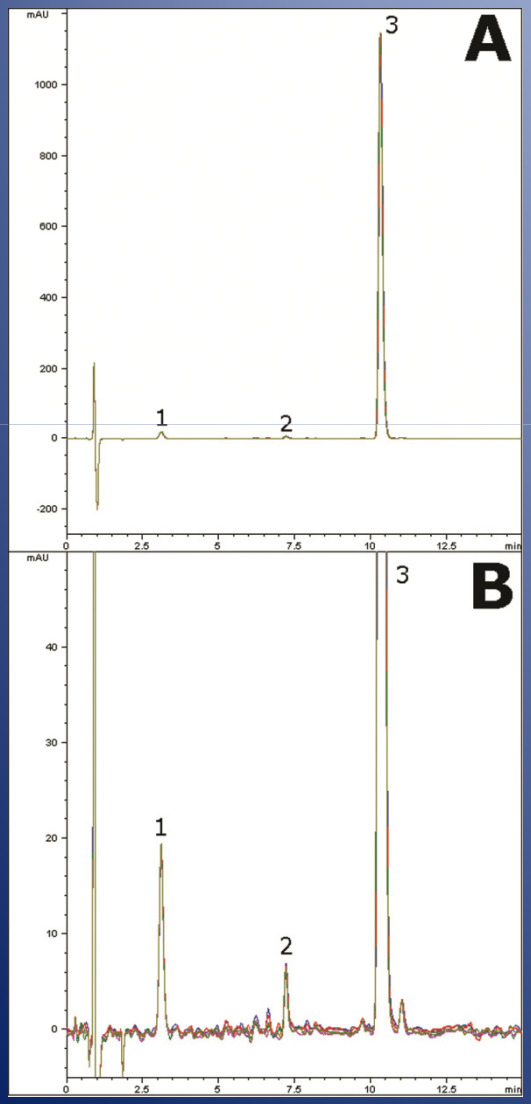
The retention in each mode is determined by the type of organic modifier on the surface and the surface coverage

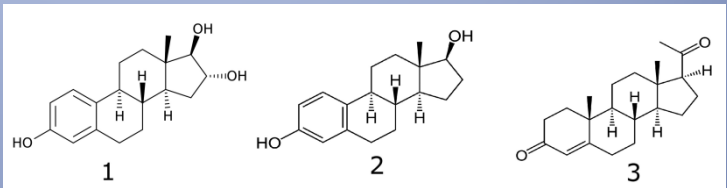
Higher surface coverage and larger bonded groups emphasize reversed-phase retention, but normal phase properties are still present

Lower surface coverage and smaller bonded groups result in greater normal phase retention, but reversed-phase properties are still present

REVERSED-PHASE MODE WITH SHAPE SELECTIVITY

HORMONE REPLACE CAPSULE USING CHOLESTEROL PHASE





Dimensions: 4.6 x 75 mm

Solvents: A: DI H_2O/ 0.1% formic acid (v/v)

B: Acetonitrile/ 0.1% formic acid (v/v)

Gradient:

time (min.)	%B
0	33
2	33
11	65
12	33

Post Time: 3 min

Flow rate: 1.0 mL/min

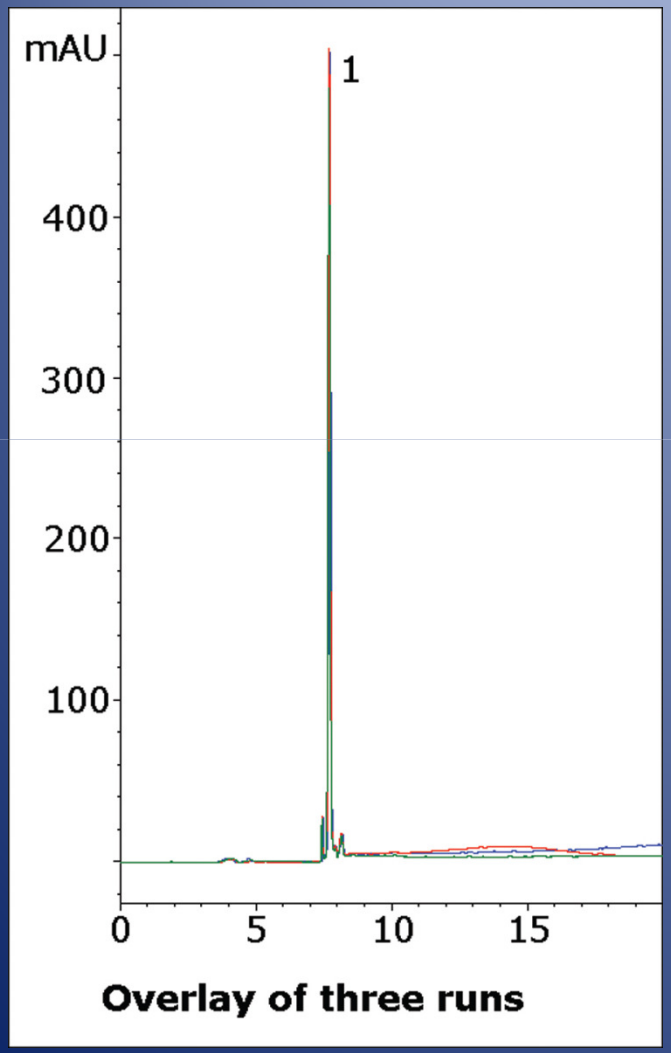
Detection: UV 210 nm

Injection Vol.: 10 µL

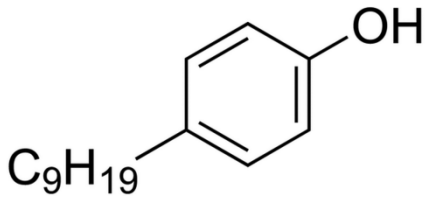
Peaks: 1. 1. Estriol
2. Estradiol
3. Progesterone

Chromatograms are an overlay of five consecutive runs

ORGANIC NORMAL PHASE



Overlay of three runs



Nonylphenol

Dimensions:	4.6 x 100 mm
Mobile phase:	A: Ethyl Acetate
	B: Hexane

Gradient:

time (min.)	%B
0	100
4	100
19	90
20	100

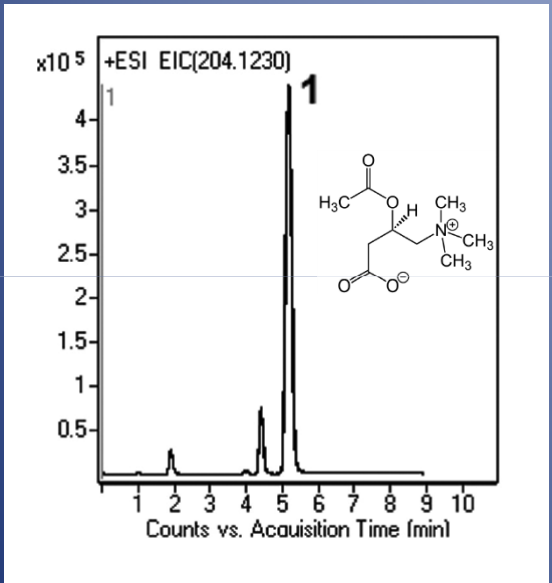
Post Time:	3 min
Flow rate:	1.0 mL/min
Injection Volume:	1 µL

Solvents do not have to be dried.
Silica hydride is not sensitive to small amounts of water in the mobile phase.

AQUEOUS NORMAL PHASE

Typically done with unmodified silica hydride or minimally modified silica hydride (2% carbon)

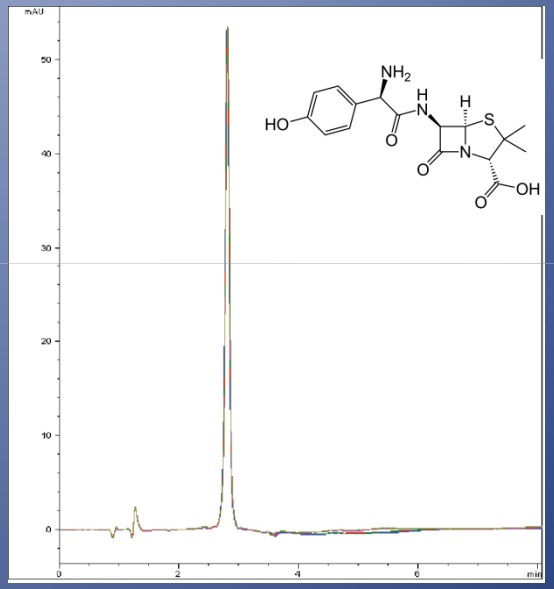
Acetyl-L-carnitine in plasma



Mobile Phase:	A: DI H_2O /0.1% formic acid B: Acetonitrile/ 0.1% formic acid			
Gradient:	time (min.)	%B	time (min.)	%B
	0	80	7	30
	1	80	8	80
	5	30		
Post Time:	3 min			

Peak:	1. Acetyl-L-carnitine: 204.1230 m/z $[M+H]^+$, 3 overlaid injections.

Amoxicillin
Orthogonal method to USP assay



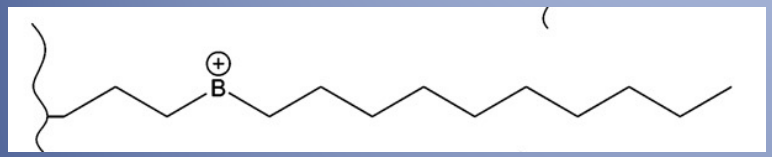
Dimensions:	4.6 x 75 mm
Solvents:	A: DI water + 10 mM ammonium acetate B: 90% Acetonitrile/ 10% DI water/ 10 mM ammonium acetate

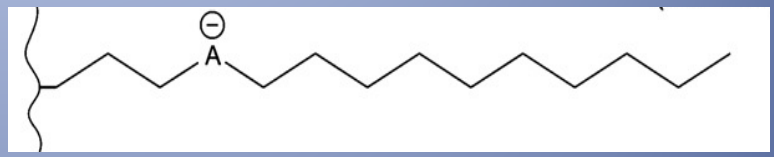
Gradient:	time (min.)	%B
	0	100
	4	90
	5	100

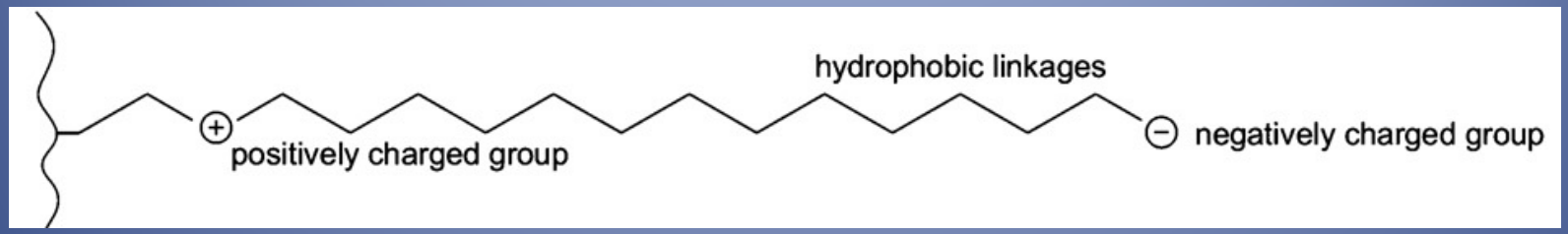
Injection Vol.:	2 microL
Flow Rate:	1.0 mL/min
Detection:	UV 230 nm

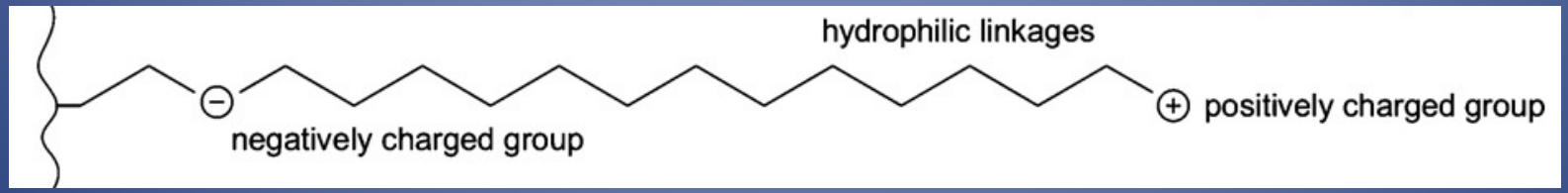
MIXED MODE STATIONARY PHASES

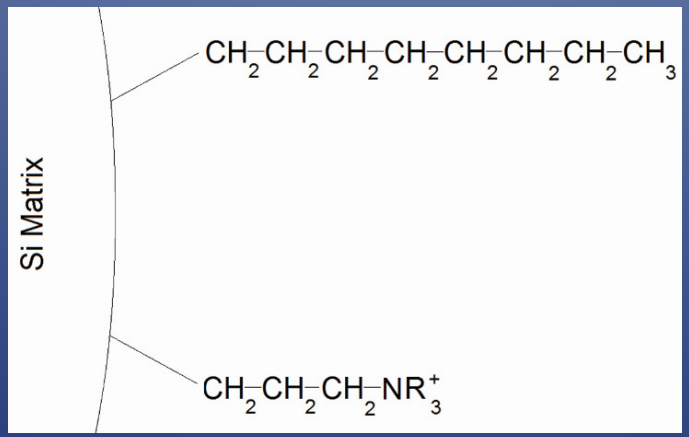
Some examples of mixed mode stationary phases



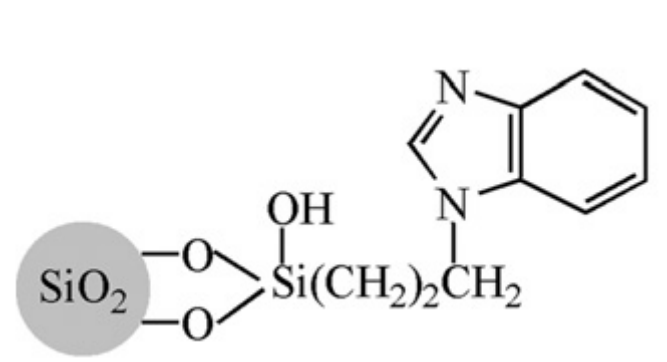


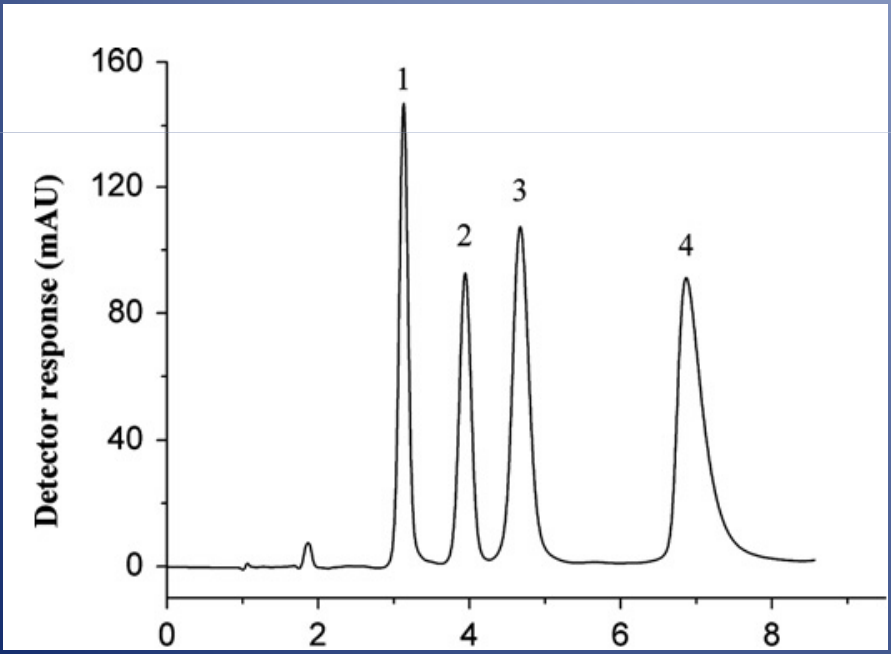




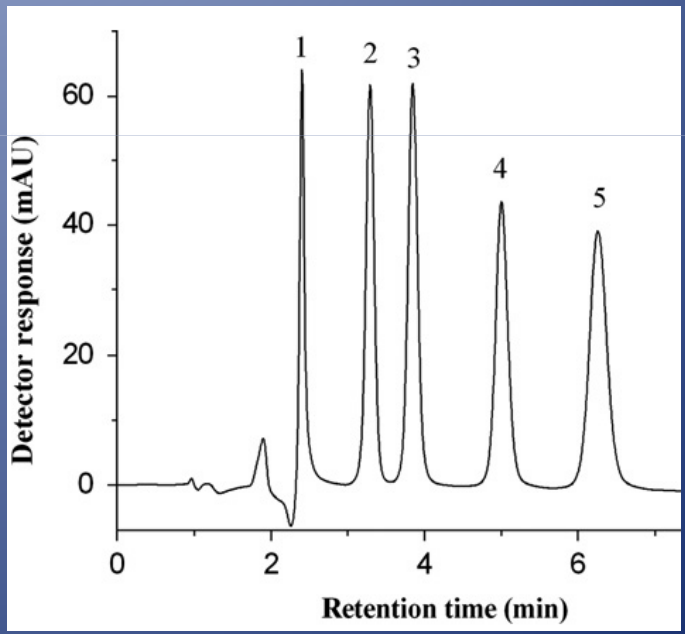


MIXED MODE APPLICATIONS

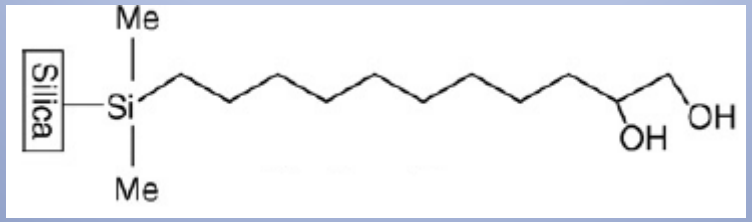


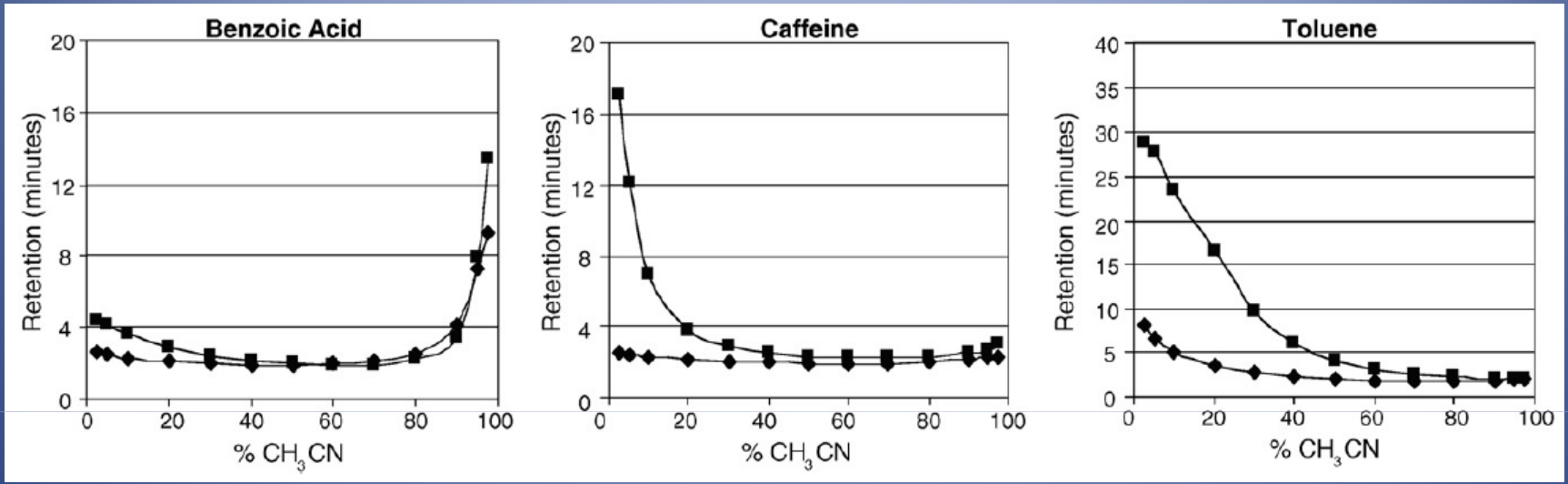


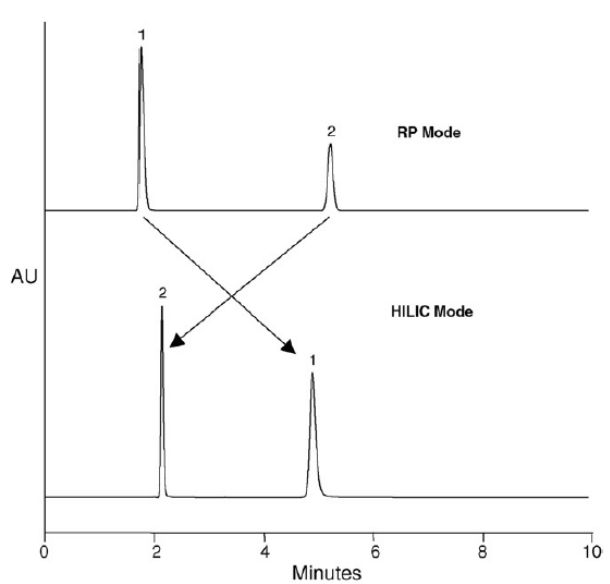
Separation of phthaltes by hydrophobicity
1, dimethyl; 2, diallyl; 3, di-*n*-butyl; 4, dicyclohexyl



Separation of anilines by π-π interaction
1, *p*-methyl; 2, *m*-nitro; 3, *o*-nitro;
4, 1-aminonapthtalene; 5, tribromo

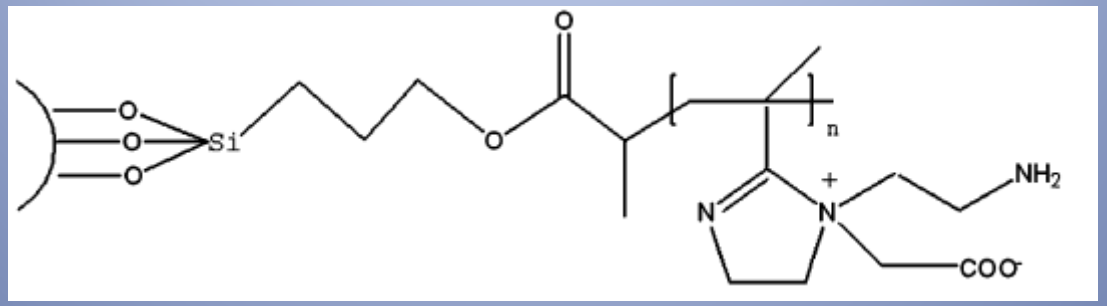




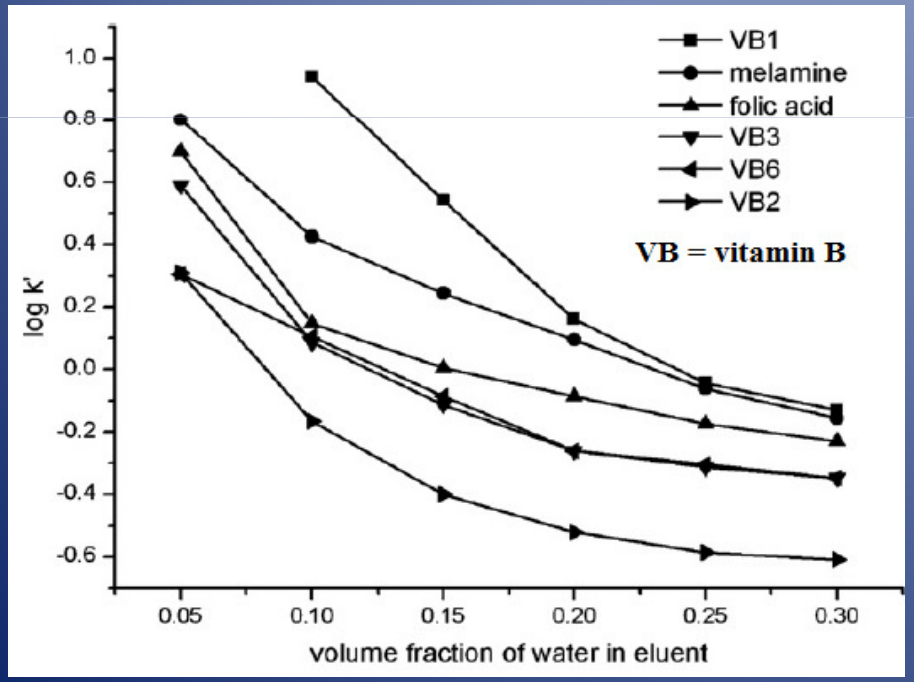


Comparison of retention behavior of two commercial diol phases

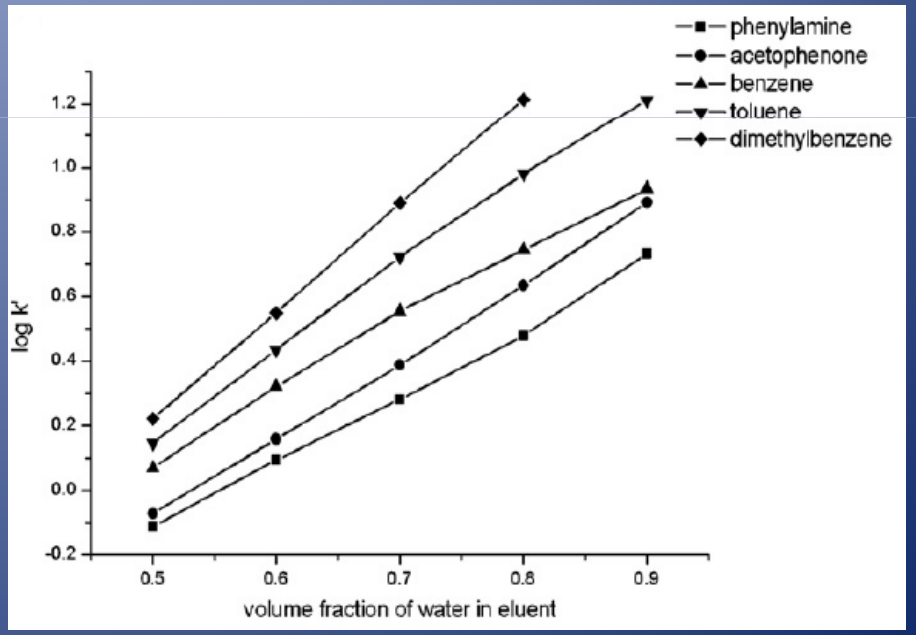
Reversal of retention based on mobile phase content: top 52% ACN; bottom 92% ACN
1, cytosine; 2, naphthalene



HILIC MODE

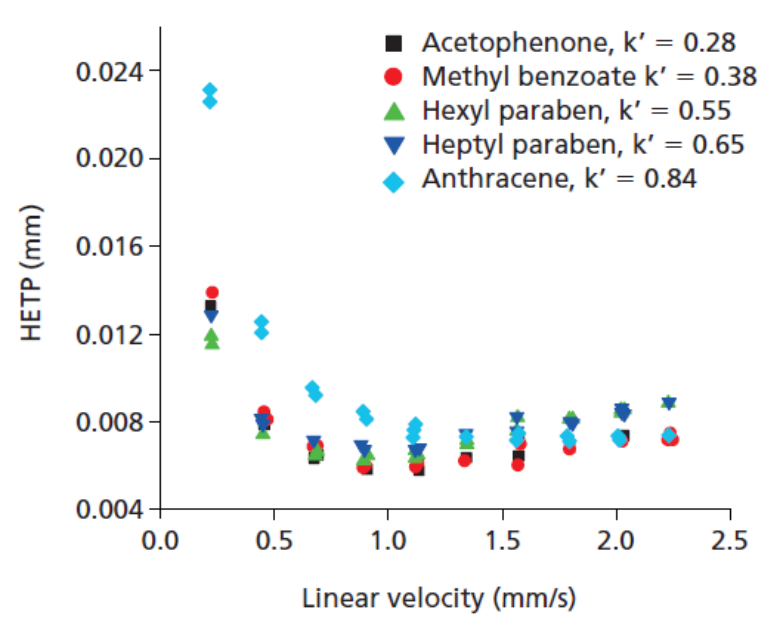


REVERSED-PHASE MODE



MICRO- and NANO-SEPARATION FORMATS

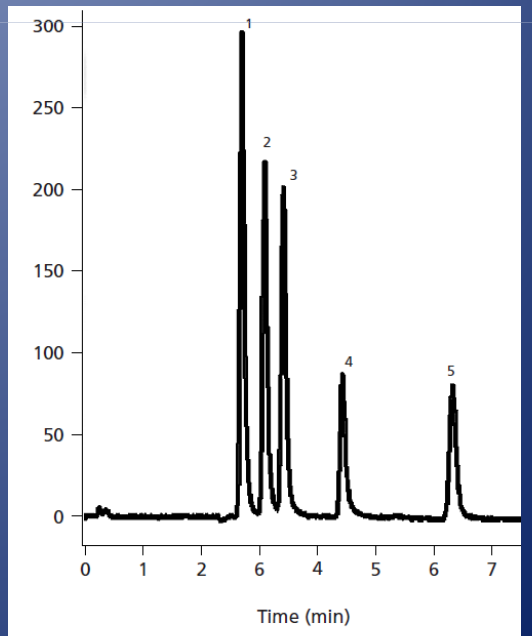
Description	Dimension	Approx. typical flow-rate (velocity 1–10 mm/s)
Open tubular liquid chromatography	< 25 µm i.d.	< 25 nL/min
Nanobore column HPLC	25 µm ⩽ i.d. ⩽ 100 µm	25–4000 nL/min
Capillary column HPLC	100 µm < i.d. < 1 mm	0.4–200 µL/min
Microbore column HPLC	1 mm ⩽ i.d. ⩽ 2.1 mm	50–1000 µL/min
Narrow(small)-bore column HPLC	2.1 mm < i.d. < 4 mm	0.3–3.0 mL/min



CAPILLARY HPLC

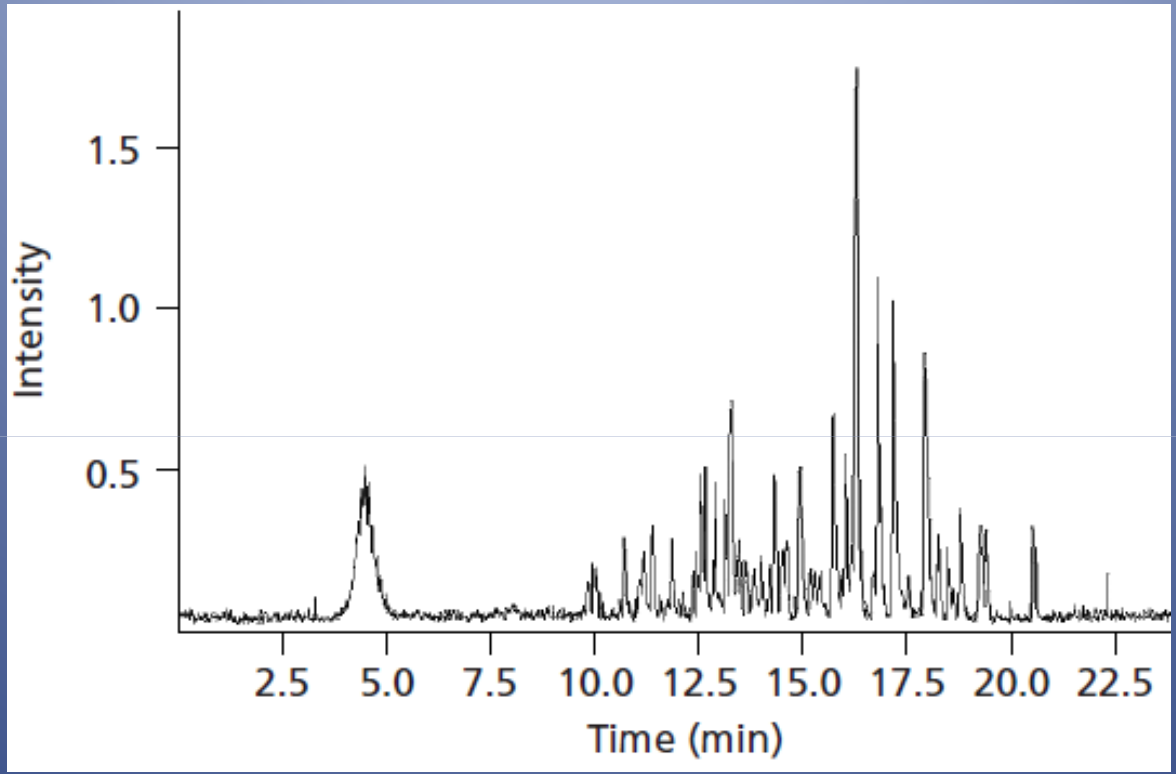
Data obtained on a 150 x 0.3 mm column packed with 3.5 µm C18 stationary phase.

Flow rate: 4 µL/min



Peaks: 1 = thiourea, 2 = dimethylphthalate, 3 = diethylphthalate, 4 = biphenyl, 5 = o-terphenyl.

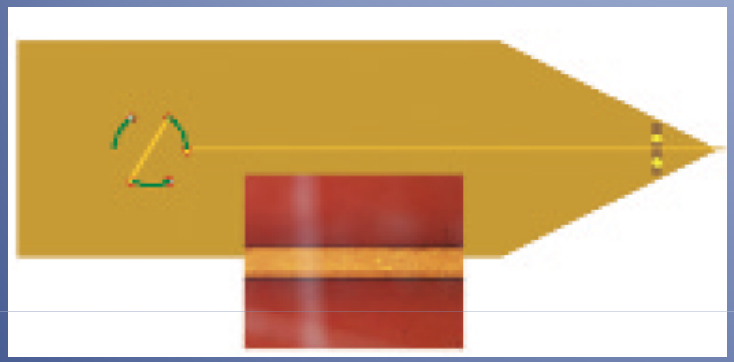
SEPARATION OF A TRYPTIC DIGEST ON A NANOBORE HPLC COLUMN



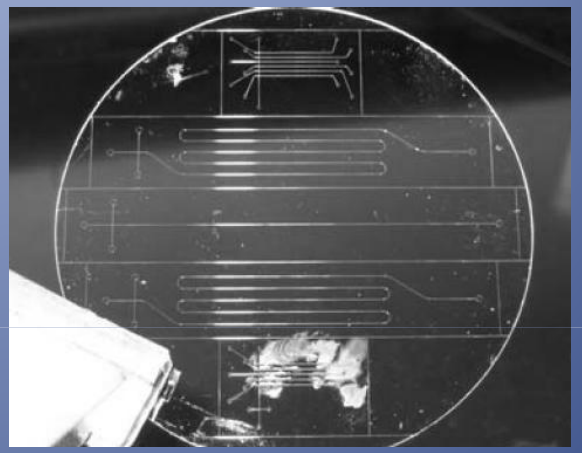
Column: 150 x 0.075 mm packed with 3.5 µm C18 stationary phase.

LIQUID CHROMATOGRAPHY ON A CHIP

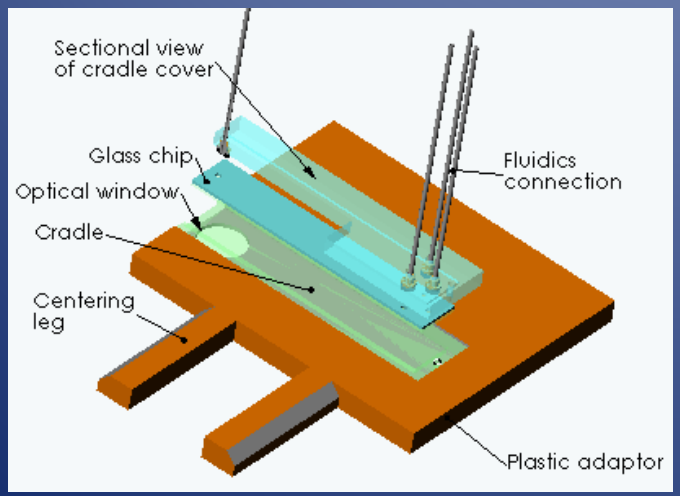
Simple single channel



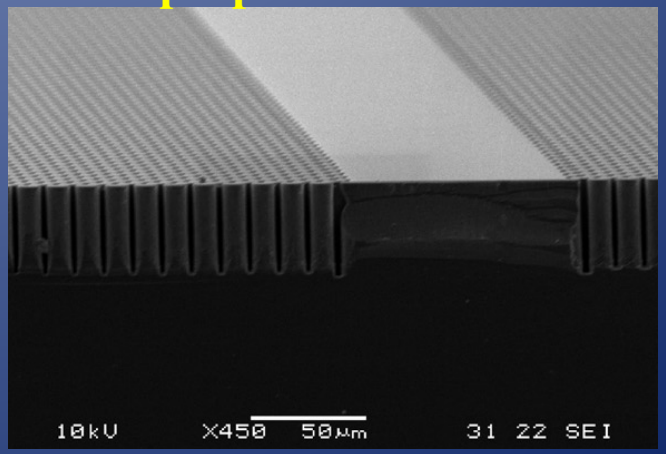
Extended channel design



Chip with connections and holder

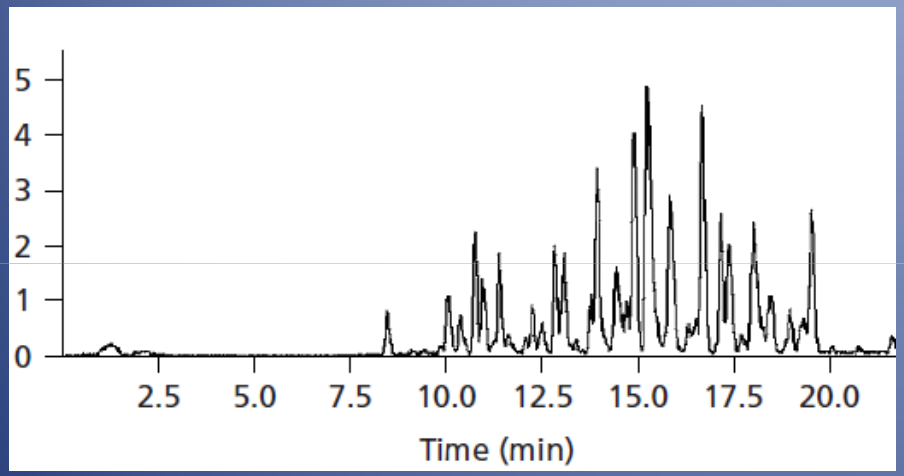


Multiple parallel channels

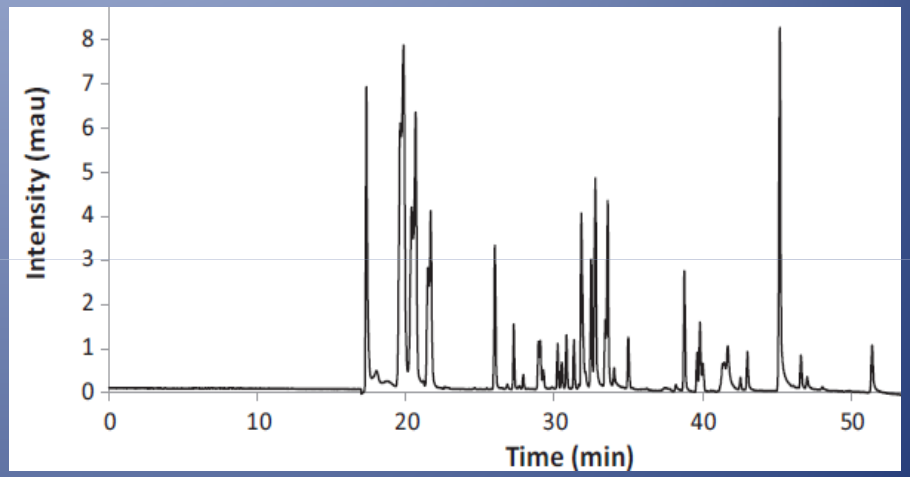


APPLICATIONS OF LC ON A CHIP

Tryptic digest of bovine serum albumin



Separation of steroids



Stationary phases can be either particles packed in the channels of the device or the walls of the channel can be chemically modified with various organic groups similar to bonding on a particle surface.

SUMMARY

EACH OF THESE TECHNOLOGIES HAS A HISTORY,
i.e. they were first postulated at least 20 years ago

TECHNOLOGICAL BREAKTHROUGHS HAVE MADE THEM MORE VIABLE IN THE LAST DECADE

INTENSE DEVELOPMENT EFFORTS ARE CURRENTLY UNDERWAY

THERE IS LIKELY TO BE FURTHER ADVANCES AND GREATER UTILIZATION IN THE FUTURE