

Rapid Method Screening for High Resolution Aqueous SEC Measurements

Mike O'Leary
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Target Attributes: New Platform for SEC Polymer Characterization

- Column
 - Low energy surface – minimal adsorption
 - High pore volume... and good mechanical strength
 - Range of pore size distributions
 - Good efficiency - small particle size

- Instrument
 - Capable of high pressure operation (1000 bar)
 - Low system dispersion
 - Accurate and precise mobile phase delivery
 - Range of solvent compatibility
 - Good vapor management

The ACQUITY Advanced Polymer Chromatography (APC) System

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Flexible
detection
techniques

Low system
dispersion

Compatibility
with
challenging
solvents

Precise
solvent
management



Stable
refractive
index
detection

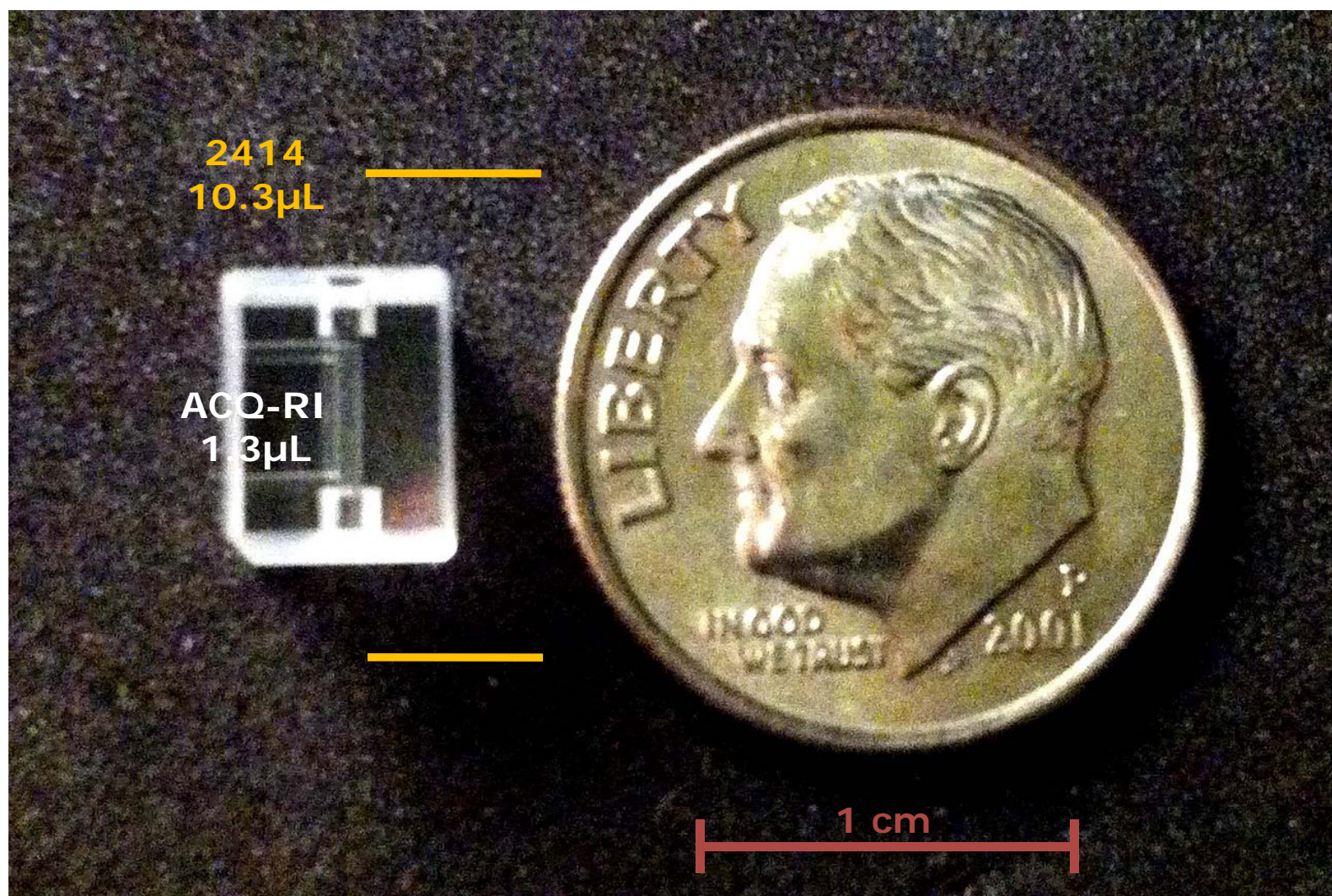
Rigid,
solvent-
resilient
columns

Versatile
column
management

Wide range of
APC
standards

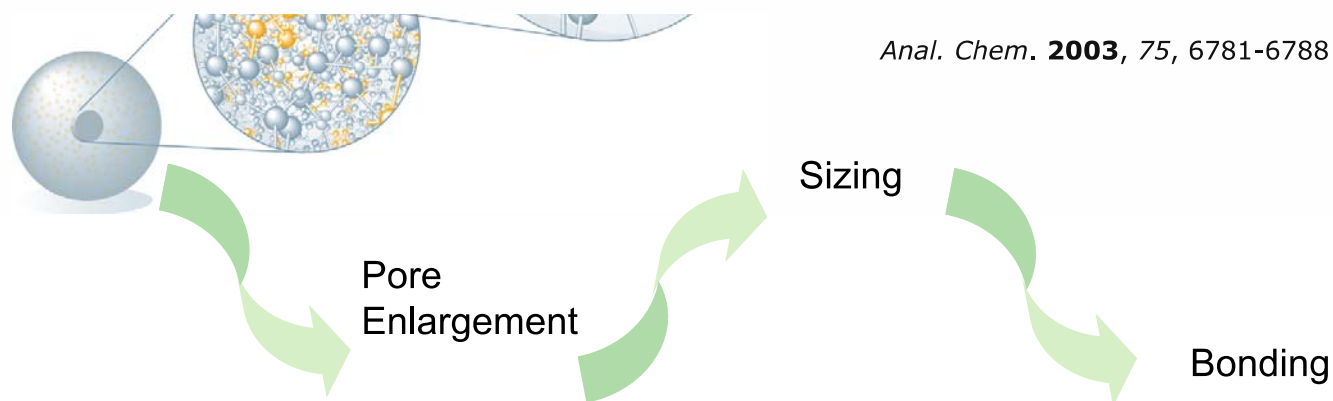
ACQUITY Refractive Index Detector Flow Cell

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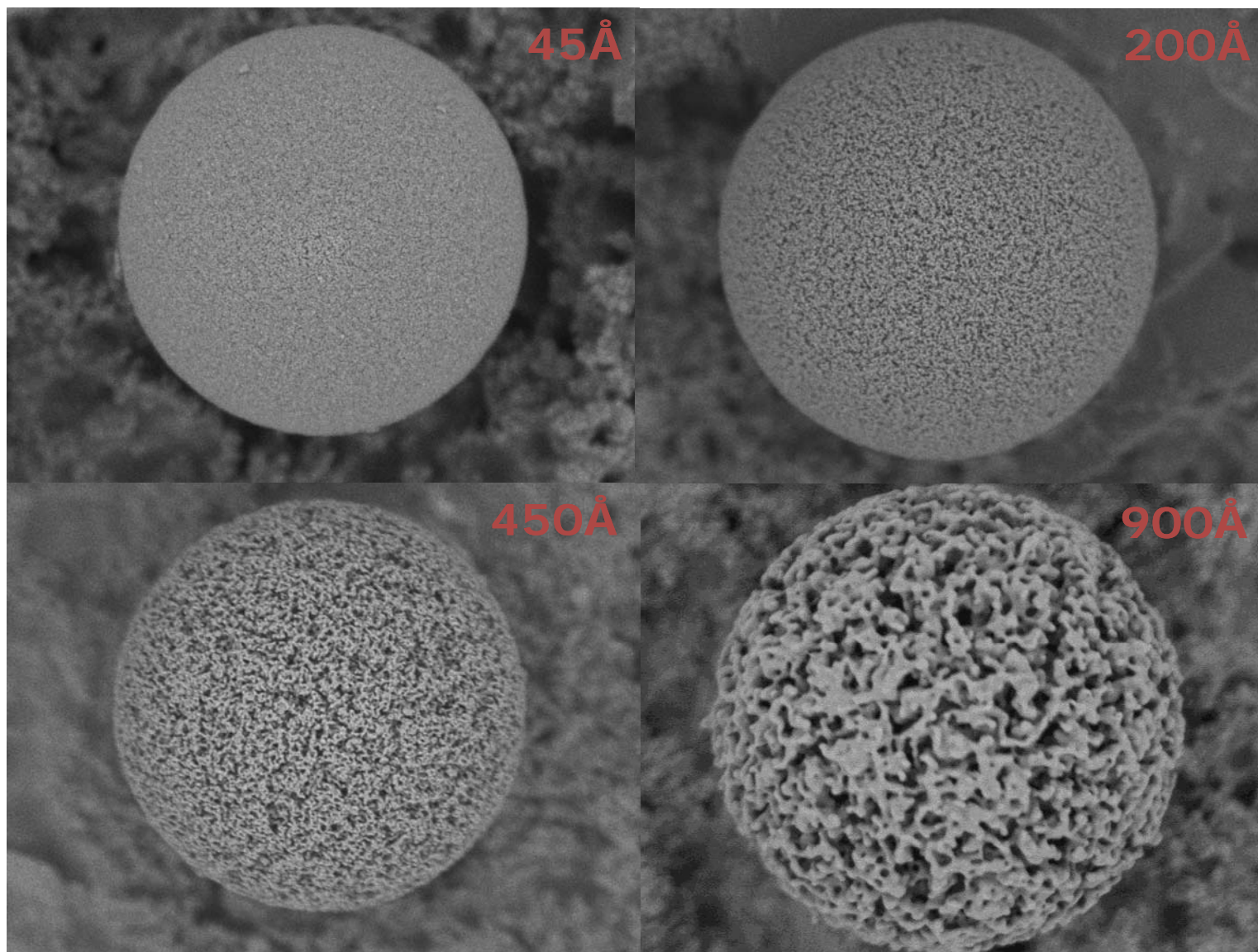
BEH™ Technology Particles Bridged EthylSiloxane/Silica Hybrid

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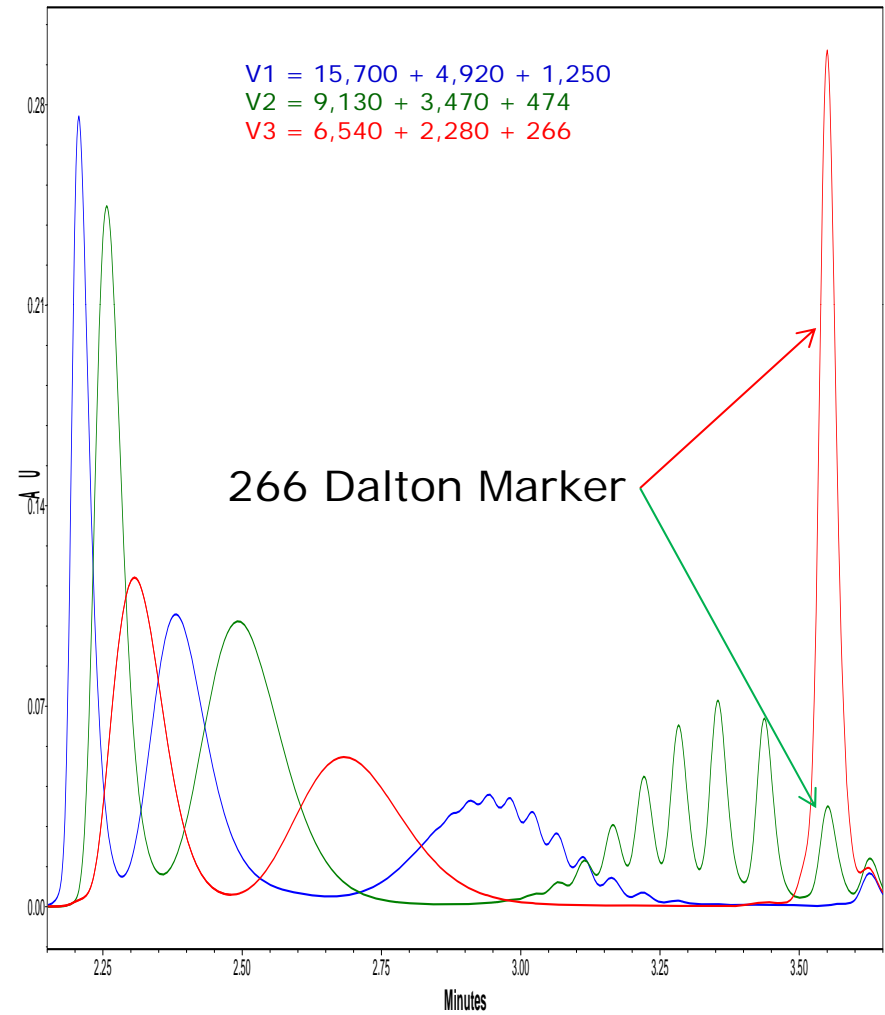
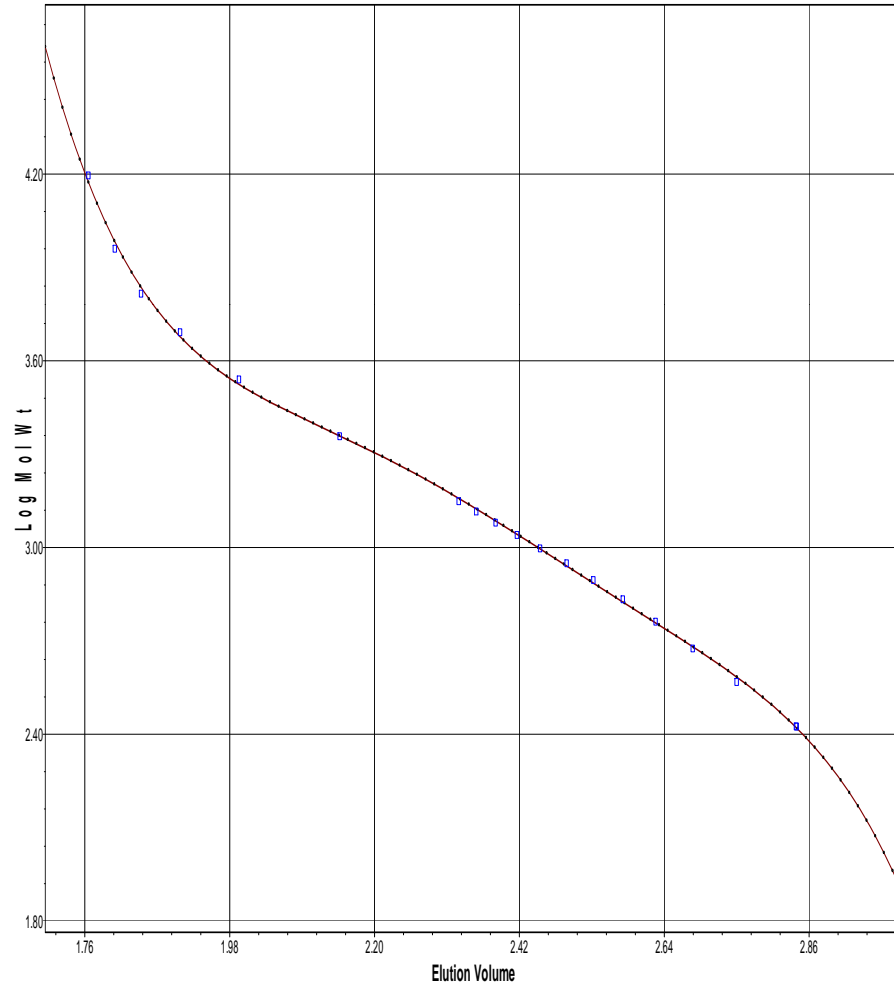
SEM Images: Pore-Enlarged Bridged Ethyl Hybrid

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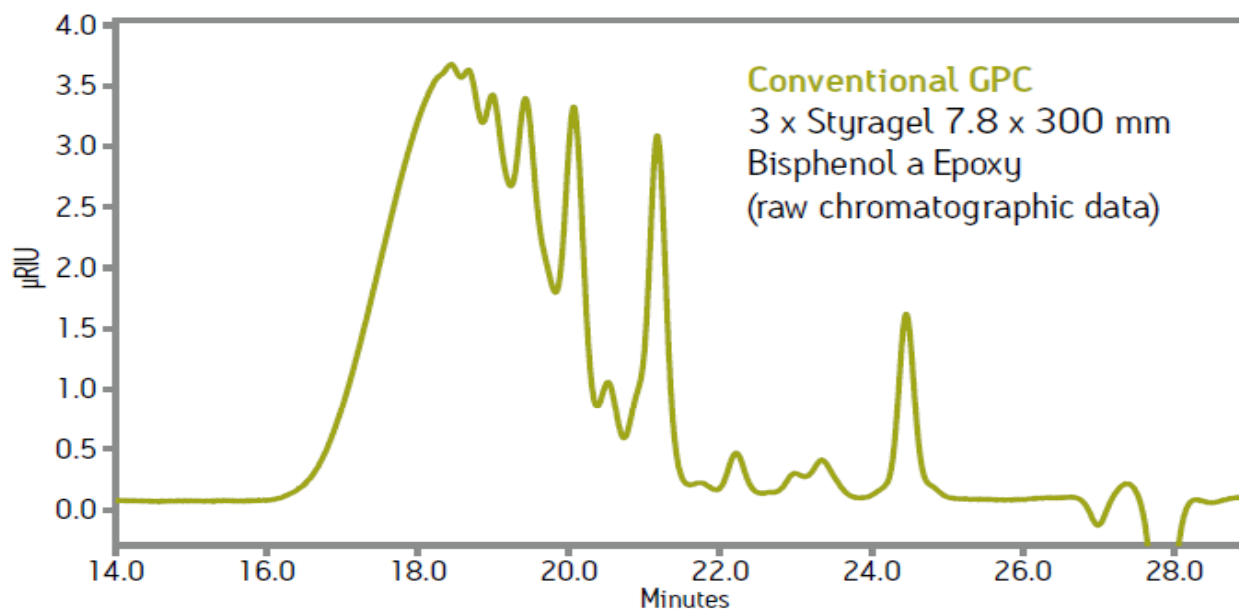
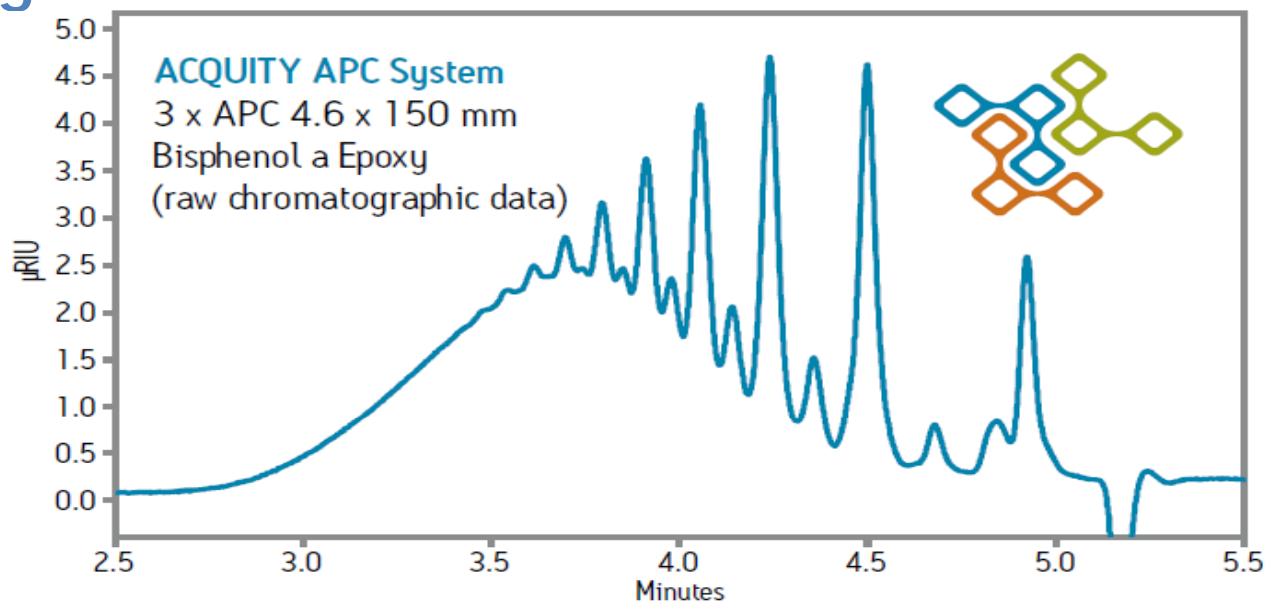
Low MW Standards PS MW: 266 - 15,000

Columns: 2 x 45A APC XT 4.6x150 mm



Higher Resolution Data Faster - Life is good!

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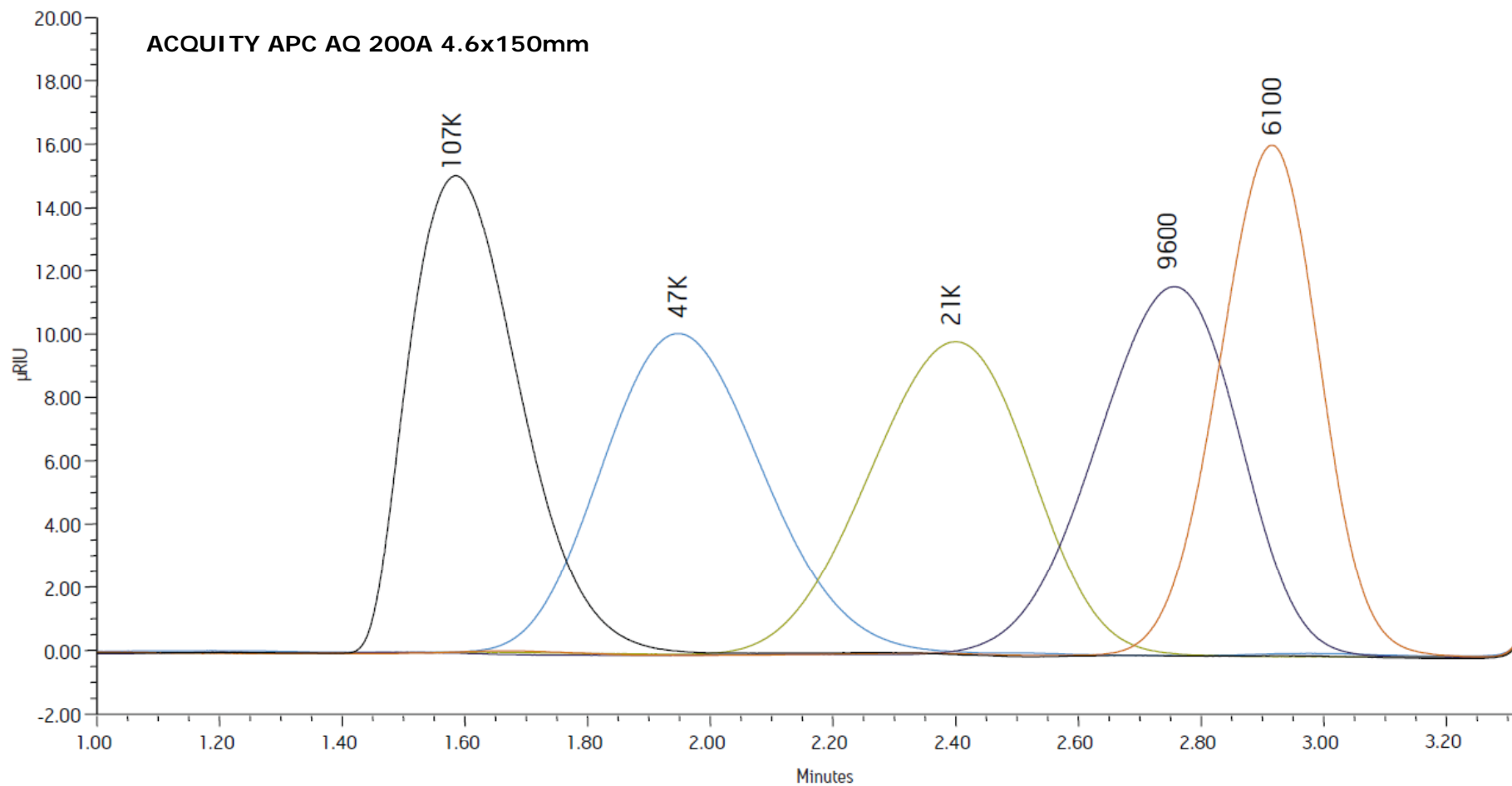
Column Chemistry

- The story so far

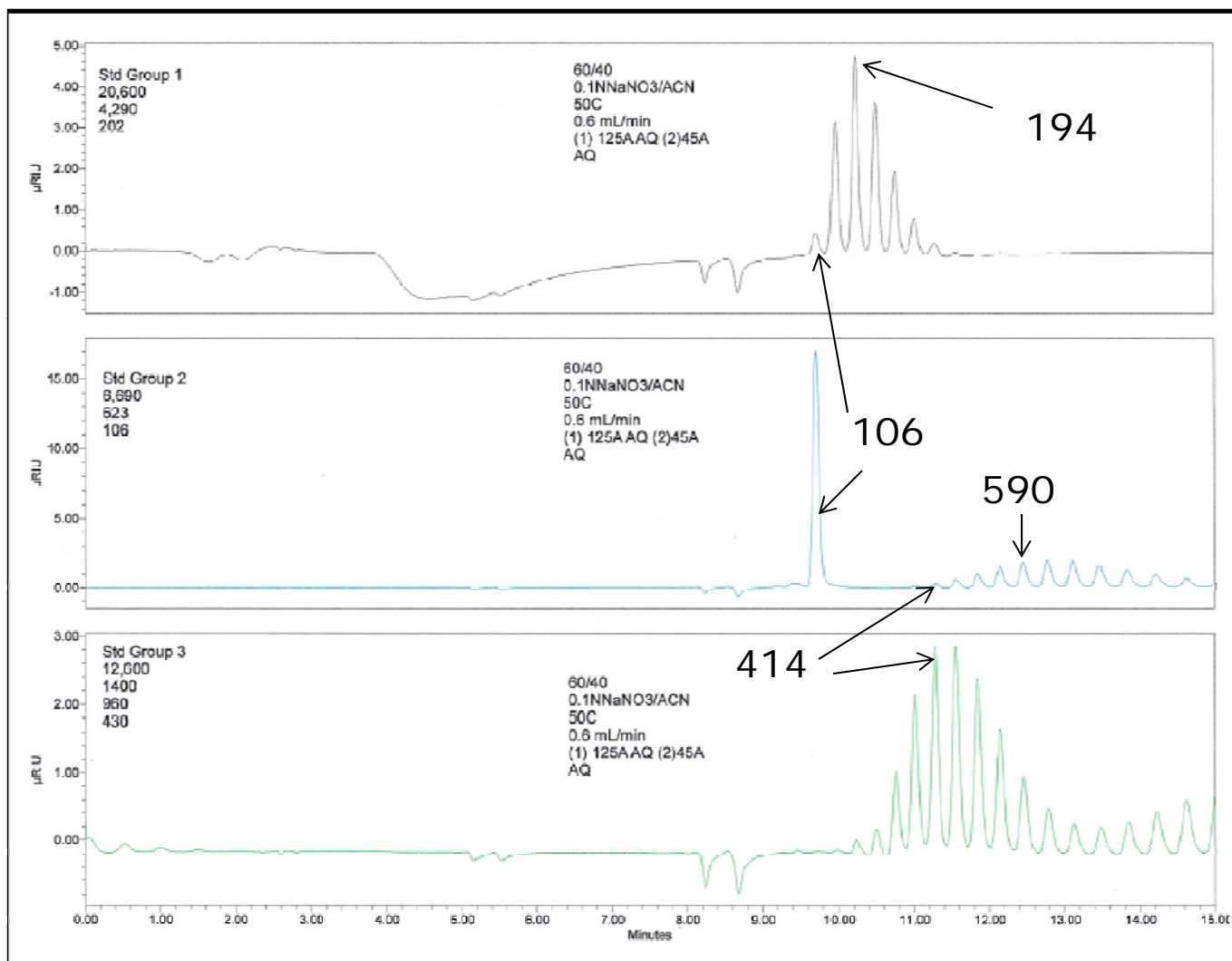
- Maximize accessibility to pore structure, bondings are undesired
- For organic separations, addition of small TMS “end-cap” (APC XT column) acceptable balance of minimizing surface activity and maintaining pore access
- For aq applications, strong salts and modifiers used minimize surface interactions
- For this reason, APC AQ columns have no surface bonding

Pullulan Standards

- 100mM sodium nitrate/CH₃CN : 80/20



PEG Standards: Beautiful Chromatography - Just the wrong kind



Impact of Temperature on Retention

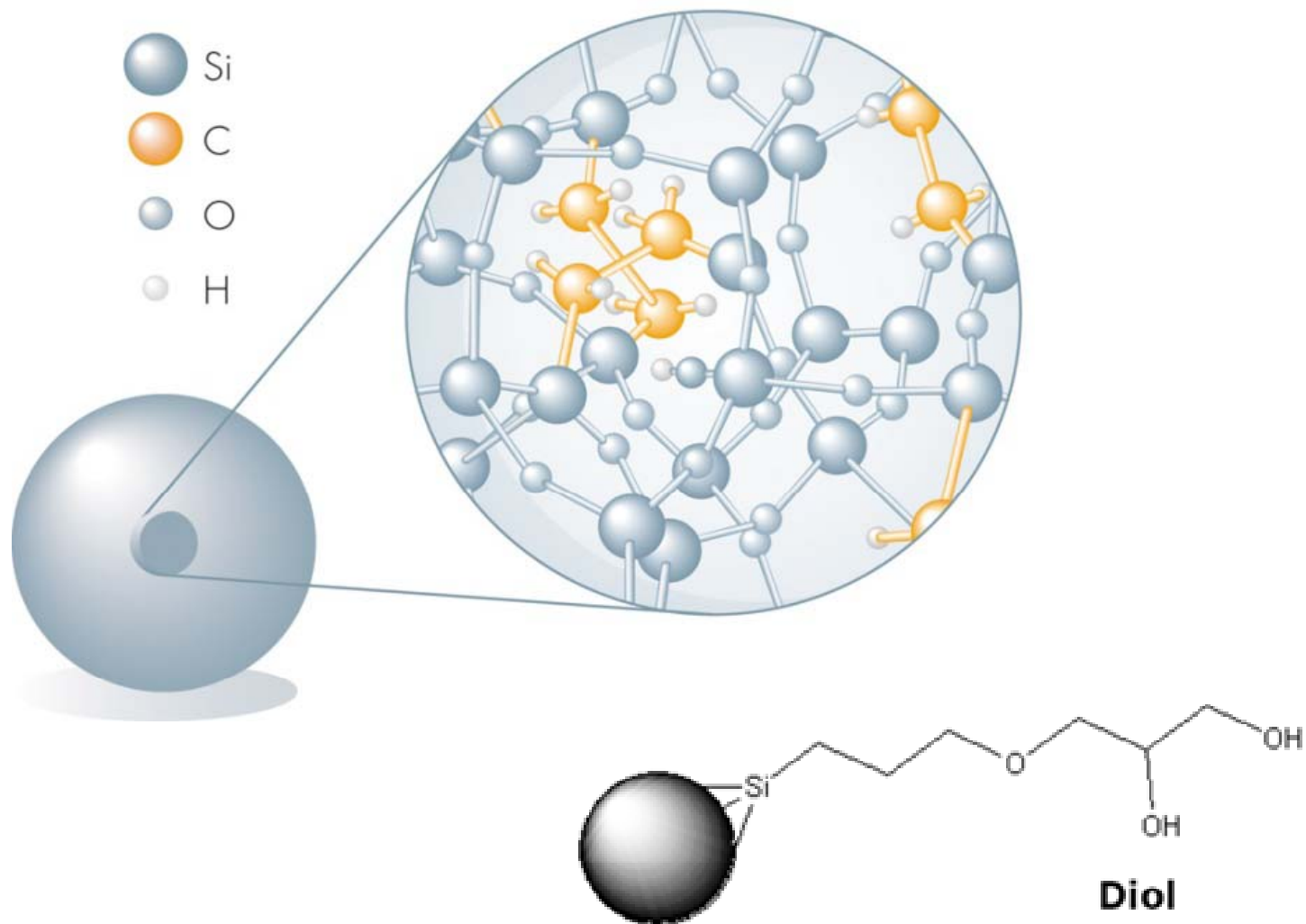
Percent retention time change: 30 °C vs 50 °C

Analyte	Mp	200Å XT	200Å AQ	DVB Brand A	DVB Brand B
Polystyrene	44,200	-1.48	-1.76	-1.84	-1.87
Poly(methyl methacrylate)	31,600	-2.49	-1.96	-1.90	-1.92
Poly (isoprene)	9930	-2.01	-1.82	-1.82	-1.89
Poly (ethylene glycol)	1340	-1.96	-34.99	-2.05	-1.87
Poly (4-vinyl phenol)	11,000	-3.38	Did not elute	-2.01	-1.73
Poly (2-vinylpyridine)	1640	-2.38	Did not elute	-1.62	-1.85
Poly (dimethyl siloxane)	5940	-2.83	-1.65	-2.07	-1.94
Polyurethane	100,000	-1.84	-2.09	-1.48	-3.52
Poly (vinyl acetate)	100,000	-1.89	-1.76	-1.42	-2.69
Cellulose acetate	30,000	-1.42	-2.10	-1.29	-0.44

Mobile phase: THF; Flow rate: 0.6 mL/min; Detection: UV or ELSD

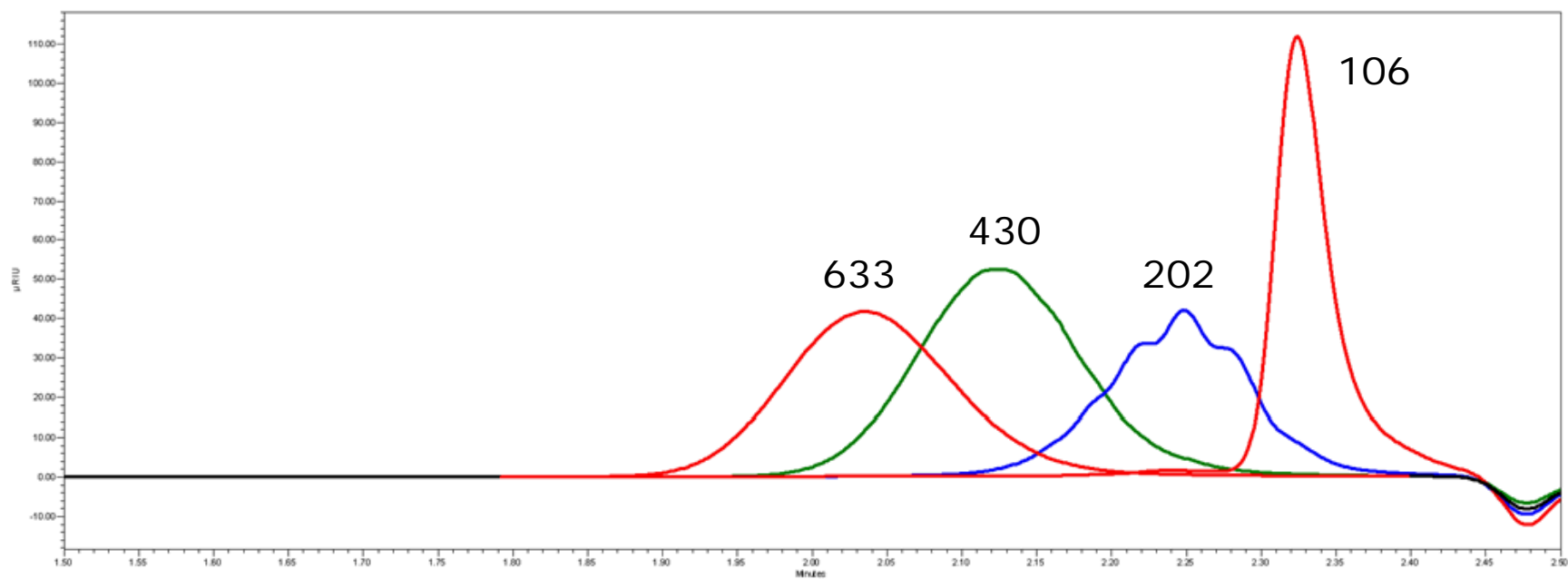
Let's Try Something Else - Protein SEC column

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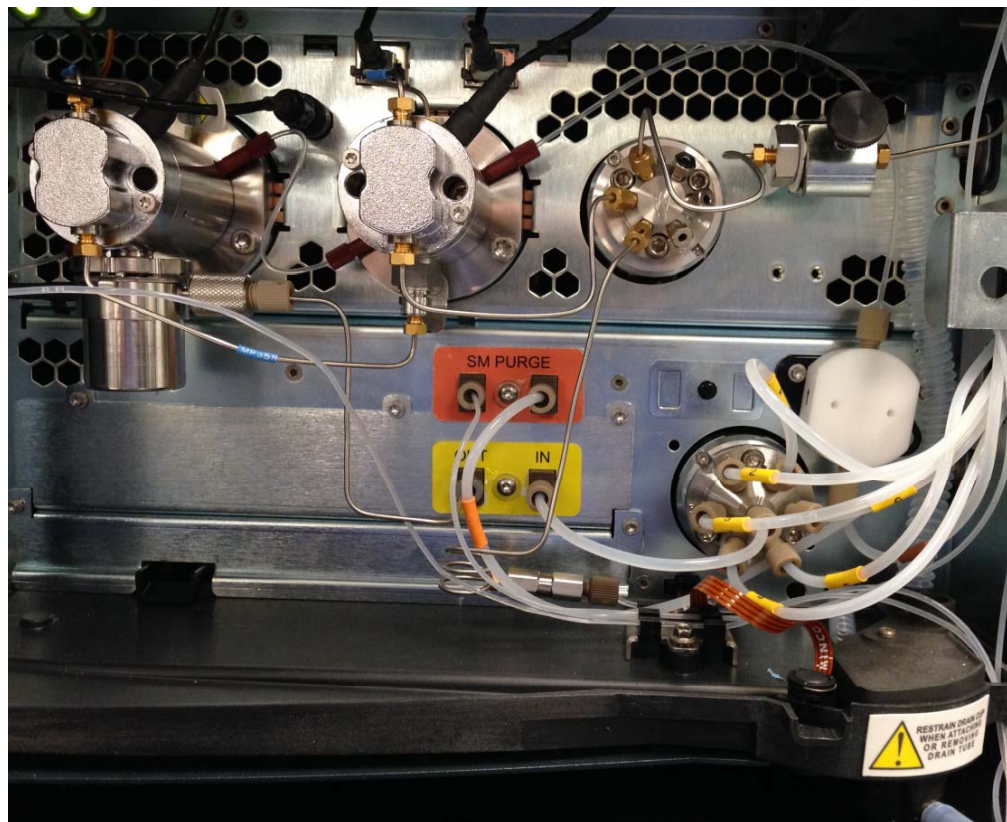
10% ACN in water 0.75 ml/min with one 150 mm 125 A BEH Diol column – PEG Stds

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Optional Solvent Select Valve

- Fully compatible with the ACQUITY APC supported solvents
 - 6 solvent lines
- Enables automated solvent switching on the ACQUITY APC System
 - Solvent resilient ACQUITY APC hybrid particles not susceptible to swelling with solvent switching



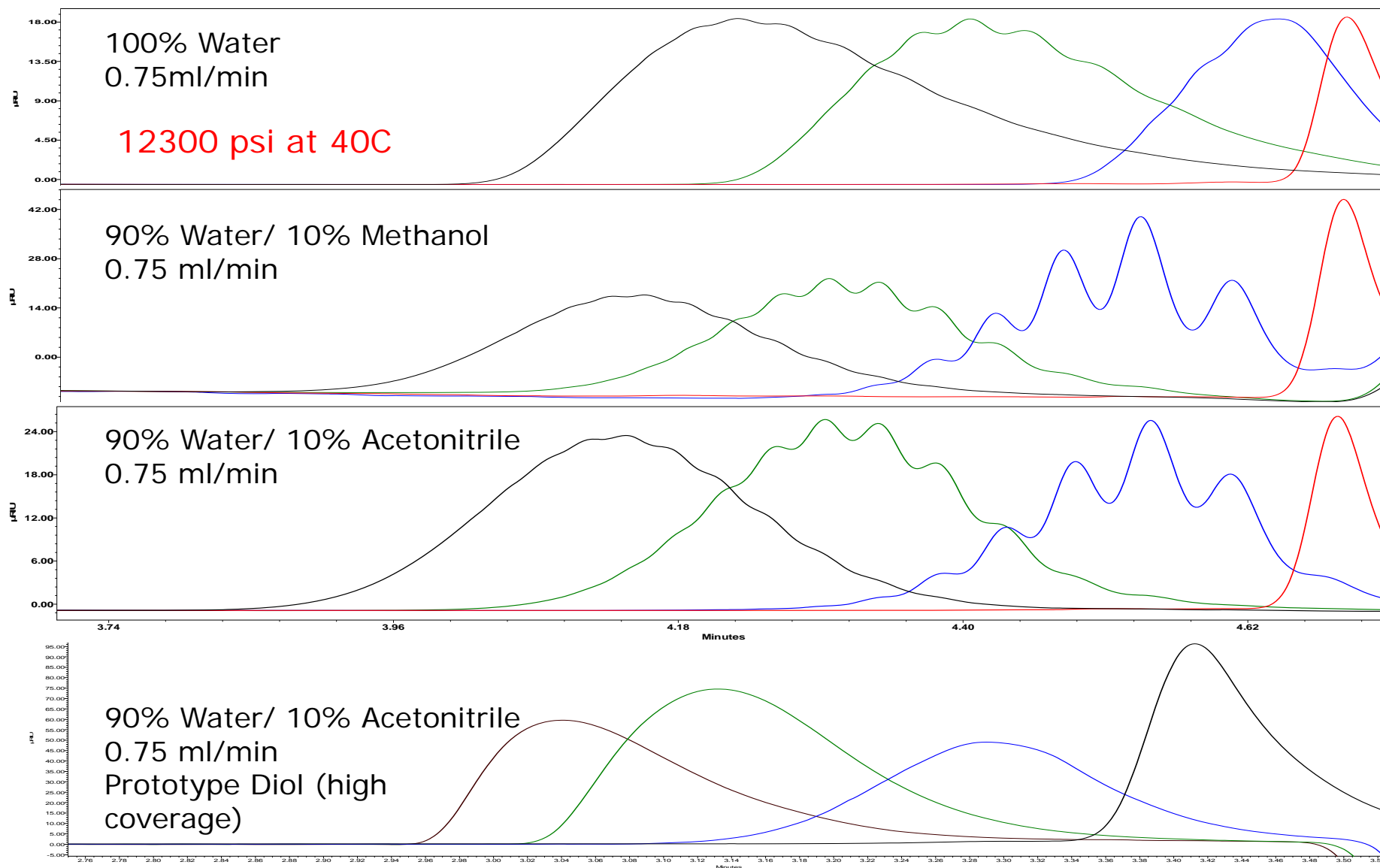
GPC Method Development Screening Protocol

- Completed in 4 hours

	Plate/Well	Inj Vol (uL)	# of Injs	SampleName	Function	Run Time (Minutes)	Method Set / Report Method	Processing
1					Condition Column	45.00	APC AQ 40C S1 ACN 750	
2					Purge Det	5.00	APC AQ 40C S1 ACN 750	
3					Equilibrate	10.00	APC AQ 40C S1 ACN 750	
4	1:A,1	5.0	1	Blank	Inject Samples	5.00	APC AQ 40C S1 ACN 750	Normal
5	1:B,1	5.0	1	PEG 106 10% ACN 40C	Inject Narrow Standards	5.00	APC AQ 40C S1 ACN 750	Normal
6	1:B,2	5.0	1	PEG 202 10% ACN 40C	Inject Narrow Standards	5.00	APC AQ 40C S1 ACN 750	Normal
7	1:B,3	5.0	1	PEG 430 10% ACN 40C	Inject Narrow Standards	5.00	APC AQ 40C S1 ACN 750	Normal
8	1:B,4	5.0	1	PEG 633 10% ACN 40C	Inject Narrow Standards	5.00	APC AQ 40C S1 ACN 750	Normal
9					Equilibrate	45.00	APC AQ 40C S2 MeOH 750	
10					Purge Det	5.00	APC AQ 40C S2 MeOH 750	
11					Equilibrate	10.00	APC AQ 40C S2 MeOH 750	
12	1:C,1	5.0	1	PEG 106 10% MeOH 40C	Inject Narrow Standards	5.00	APC AQ 40C S2 MeOH 750	Normal
13	1:C,2	5.0	1	PEG 202 10% MeOH 40C	Inject Narrow Standards	5.00	APC AQ 40C S2 MeOH 750	Normal
14	1:C,3	5.0	1	PEG 430 10% MeOH 40C	Inject Narrow Standards	5.00	APC AQ 40C S2 MeOH 750	Normal
15	1:C,4	5.0	1	PEG 633 10% MeOH 40C	Inject Broad Samples	5.00	APC AQ 40C S2 MeOH 750	Normal
16					Equilibrate	45.00	APC AQ 40C S3 H2O 750	
17					Purge Det	5.00	APC AQ 40C S3 H2O 750	
18					Equilibrate	10.00	APC AQ 40C S3 H2O 750	
19	1:D,1	5.0	1	PEG 106 100% H2O 40C	Inject Narrow Standards	5.00	APC AQ 40C S3 H2O 750	Normal
20	1:D,2	5.0	1	PEG 202 100% H2O 40C	Inject Narrow Standards	5.00	APC AQ 40C S3 H2O 750	Normal
21	1:D,3	5.0	1	PEG 430 100% H2O 40C	Inject Narrow Standards	5.00	APC AQ 40C S3 H2O 750	Normal
22	1:D,4	5.0	1	PEG 633 100% H2O 40C	Inject Broad Samples	5.00	APC AQ 40C S3 H2O 750	Normal
23	1:A,1	5.0	1	Blank	Inject Samples	5.00	APC AQ 40C S3 H2O sd	Normal

Two 150 mm 125 A BEH Diol columns PEG 106,202,430,633

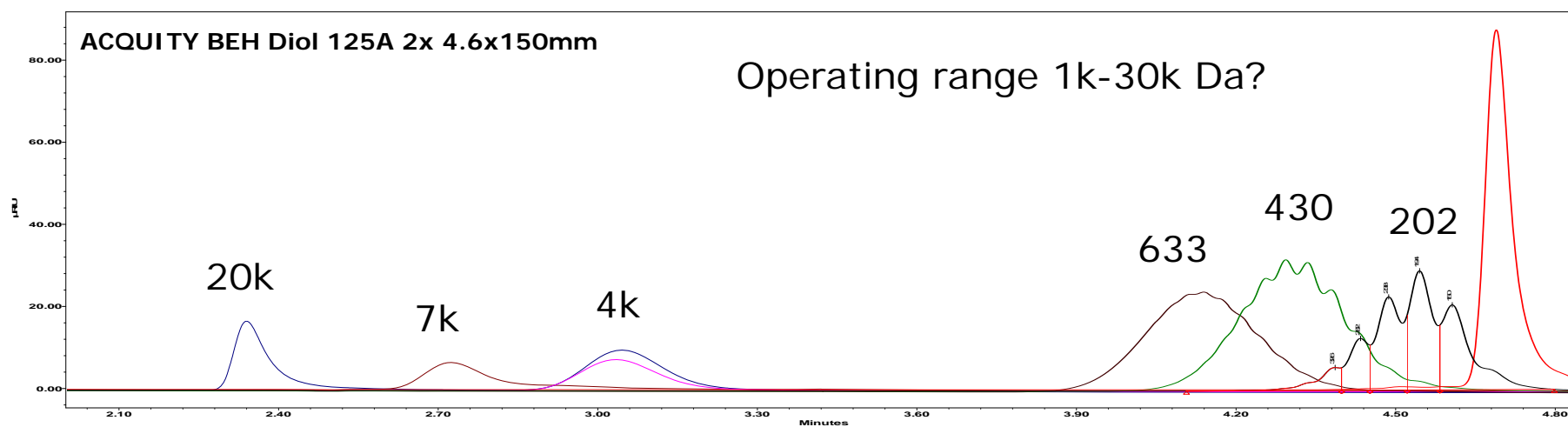
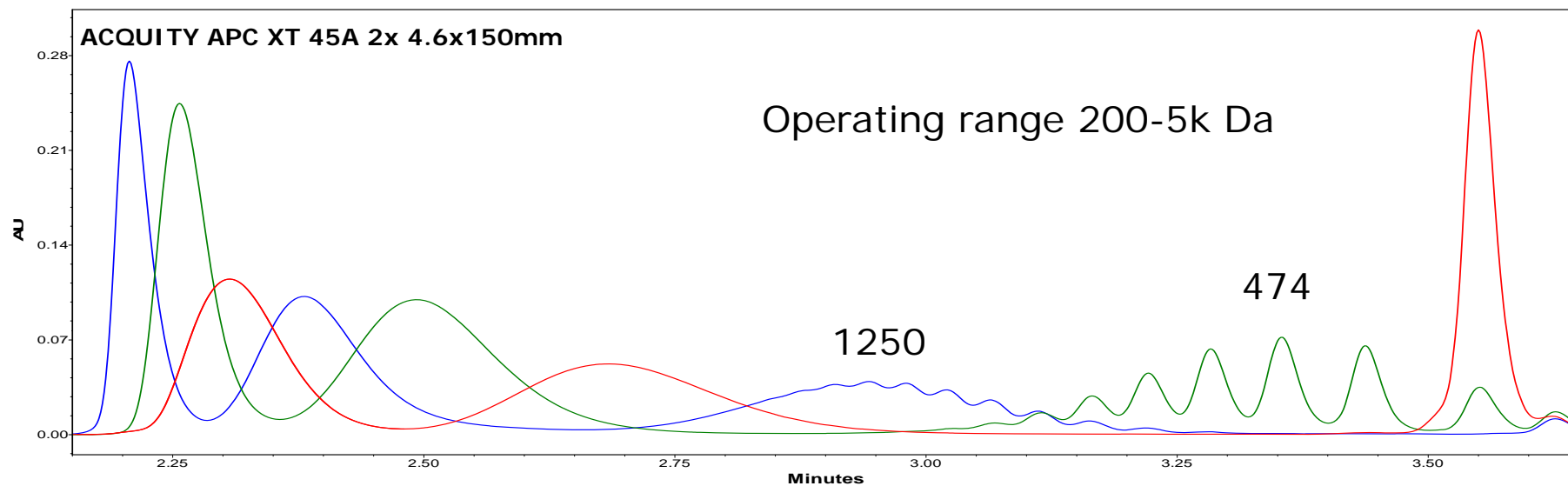
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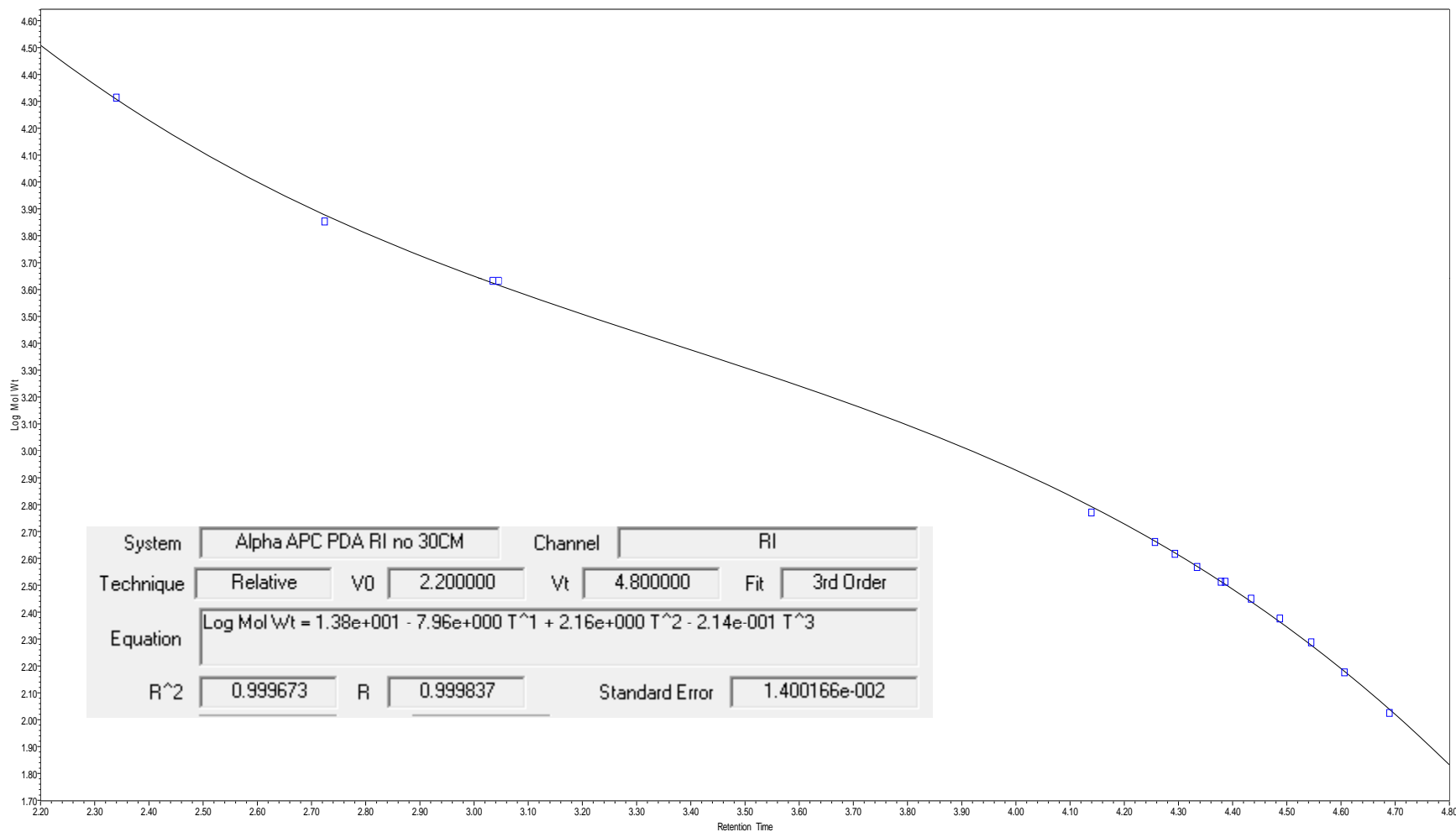
Utility of BEH Diol

- BEH particle structure enabling
 - Mechanical strength and solvent switching capability allows for fast development/screening of conditions
- Diol bonding very effective surface coverage
- Small dp of Diol particles (1.7µm) and high viscosity of water/organic mixtures result in pressures approaching system max, therefore limiting column selection flexibility
- Options for reducing backpressure
 - Increase Temperature
 - Smaller length columns
 - Increase dp
- Is the resolution optimized?

How Does This Compare to the XT 45A Experiment?



Two 150 mm 125 A BEH Diol columns



Summary and Next Steps

- This particular diol coverage has reduced the 125A porosity to effectively ~ 80-90A
- 45A particle bonding not achievable, but there may be “sweet spot” with 90A starting material
- Effect of ligand density/surface coverage levels needs to be better understood
- Alternative OH (and other) ligands to be investigated
- Combination of the low dispersion instrument and robust BEH particle enables fast development experiments

