Extended Temperature Range Impact on HPLC Column Stability and Performance

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The Selerity Series 9000 Total Temperature Controller

- Forced air oven and chiller
- Isothermal and thermal gradient operation
  - Sub-zero to 200°C
  - Flow rates up to 10.0 mL/min
  - Thermal gradient up to 30°C/min
- Mobile phase pre-heating
- Peltier effluent cooling
- Vapor sensor
- Compatible with any HPLC system

The High Temperature HPLC Advantage

- Temperature Programming
- Speed
- Efficiency

Better Chromatography with Temperature Gradient Programming

- **Change retention through temperature gradient programming**
  - Replace solvent gradients with temperature gradients
  - Water less polar and more like methanol so less organic modifier needed

Faster and More Efficient Separations

- **Speed**
  - Flatter van Deemter curves allow operation at flow rates many times optimal velocity
- **Higher efficiency - better resolution**
  - Increased diffusion rates provide lower plate heights at higher flow rates
  - Lower viscosity and back pressure permits higher flow rates with smaller particle size packings

The Real Power of Temperature in HPLC is Temperature Programming – but what about columns?

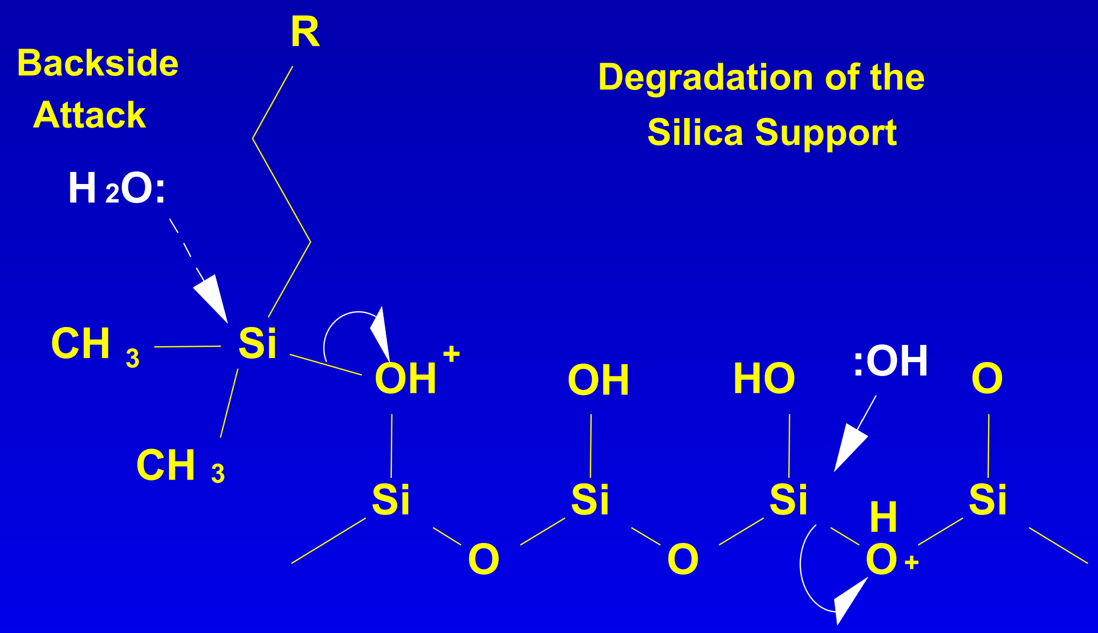
- Evaluated several different types of columns under programmed HTLC conditions
- Compared thermal gradient and solvent gradient results

Column Characteristics

- Selerity **Blaze** C_8
  - Polydentate silica, 3μm, 100 x 4.6 mm
  - Stable to 100°C, pH 2-8
- Hamilton PRP®-1
  - PSDVB, 5μm, 100 x 4.1 mm
  - Stable to 150°C, pH 0-14
- Thermo Hypersil-Keystone Hypercarb®
  - Graphitic carbon, 7μm, 100 x 4.6 mm
  - Stable to 200°C, pH 0-14
- Zirchrom PBD, Diamondbond and CARB

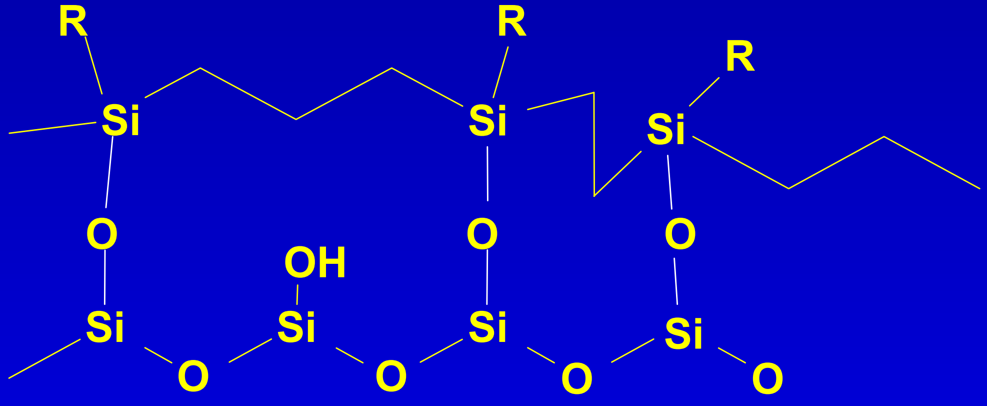
Traditional Silica Columns Can't Take the Heat

- Water attacks siloxane bond or behind point of phase attachment

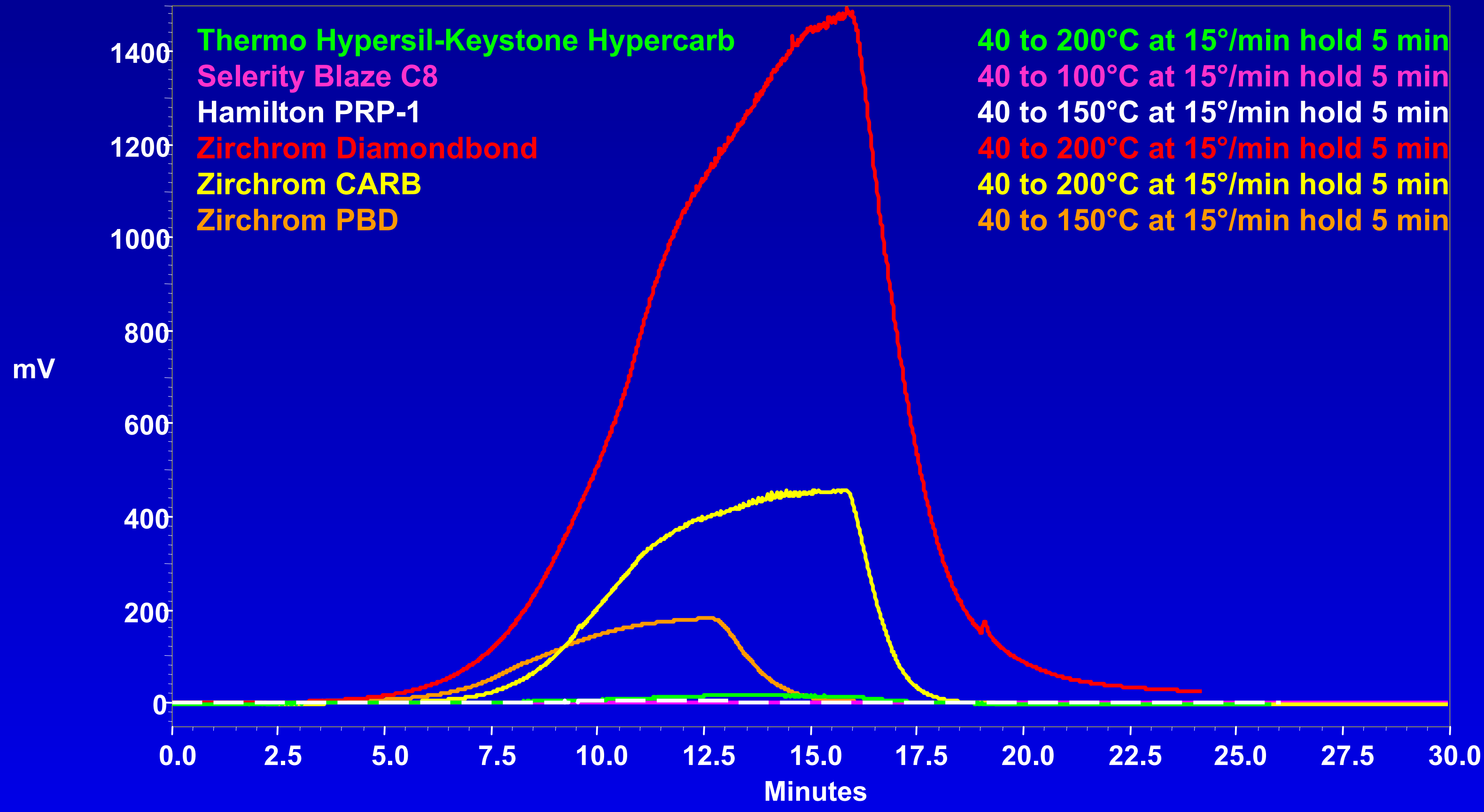


The Selerity Blaze Silica Column Can Take the Heat

- Selerity polydentate phase protects the silanol groups
- Polymer attaches to backbone at several points



Blank Thermal Gradients
50:50 Acetonitrile:Water at 254 nm



Column Evaluation Conditions

- **Seven component mix of acidic, basic and neutral compounds:**

acetophenone
amitriptylene
aniline
Ibuprofen
salicylic acid
styrene glycol
2-phenyl-2-propanol

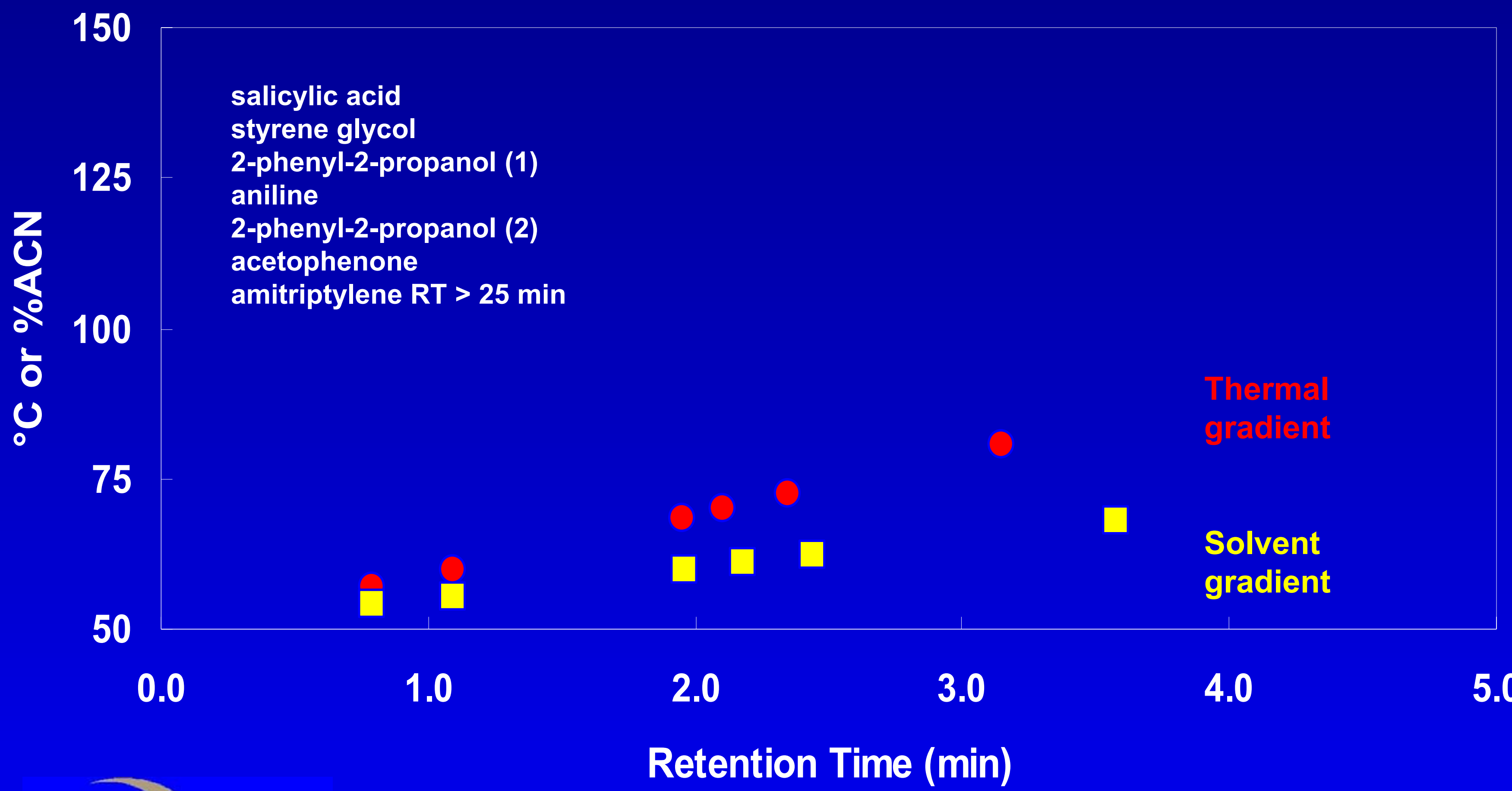
Column Evaluation Conditions

- Three mobile phases:
  - acetonitrile:water
  - acetonitrile:water with 0.1% TFA pH ≈ 2
  - acetonitrile:ammonium hydroxide pH 10
- Analyzed using a solvent gradient and a thermal gradient to give similar retention times.
- 50:50 acetonitrile:aqueous
  - 50% to 100% ACN over 10 minutes, hold five minutes
  - 50°C to 100°C, 150°C or 200°C at 15°/min, hold five minutes

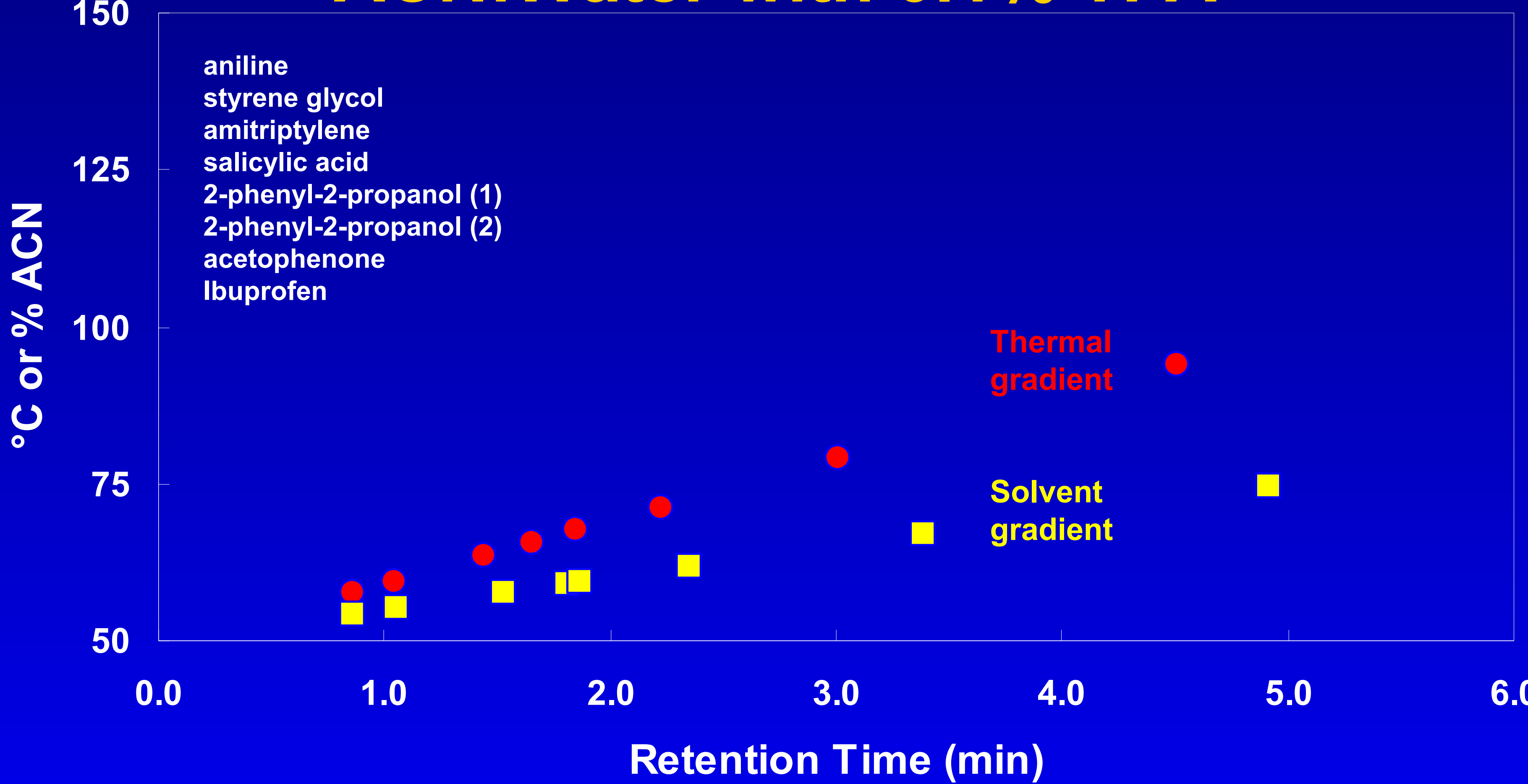
Column Evaluation Conditions

- retention times collected individually
  - Eight solvent gradients and eight temperature gradients per column
- column was evaluated initially and between each mobile phase change with two component test mix
  - uracil and phenol
  - 40°C
  - 50:50 ACN:Water
- retention time, peak area and efficiency were recorded

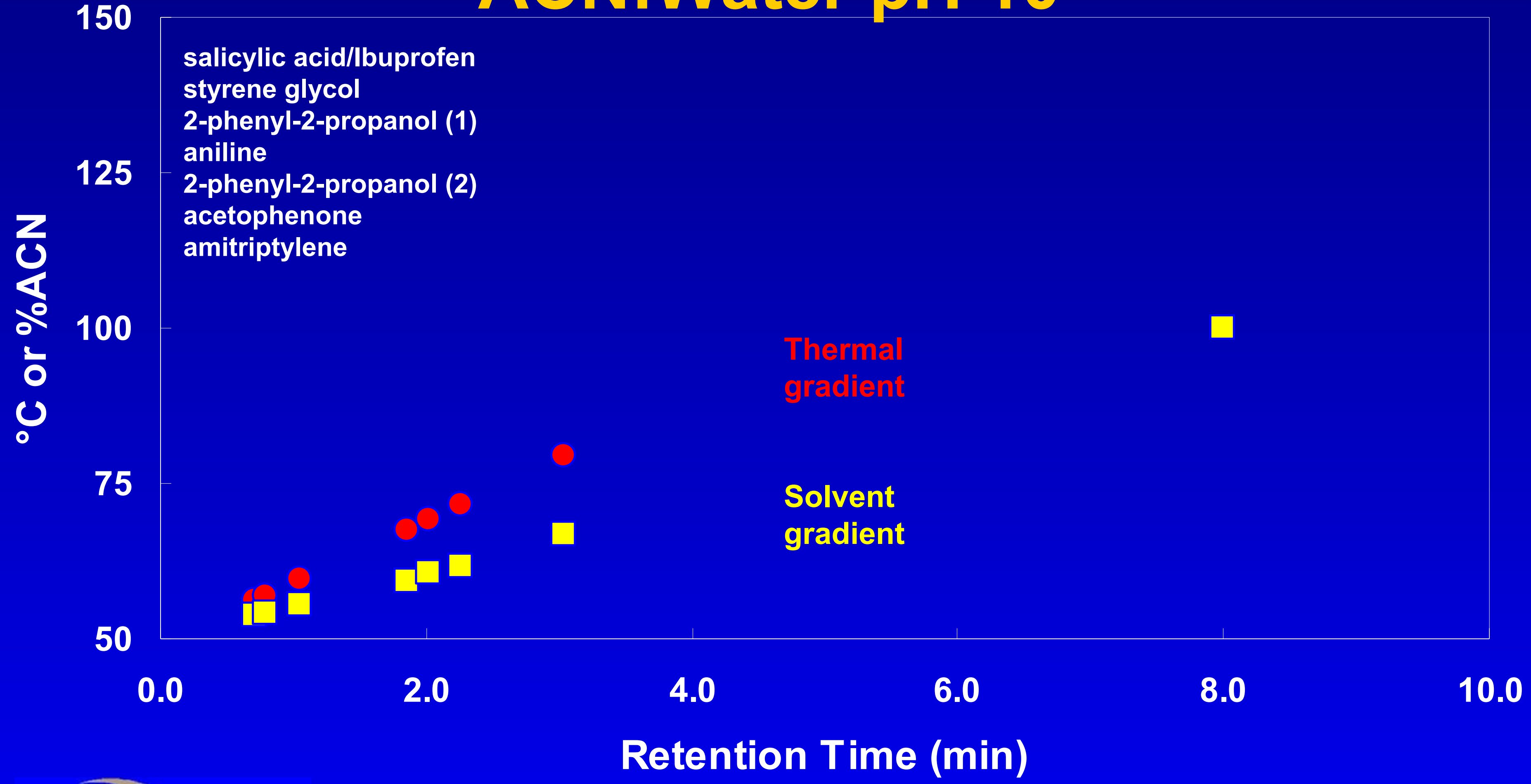
RT vs °C and RT vs %ACN for PRP®-1 ACN:Water



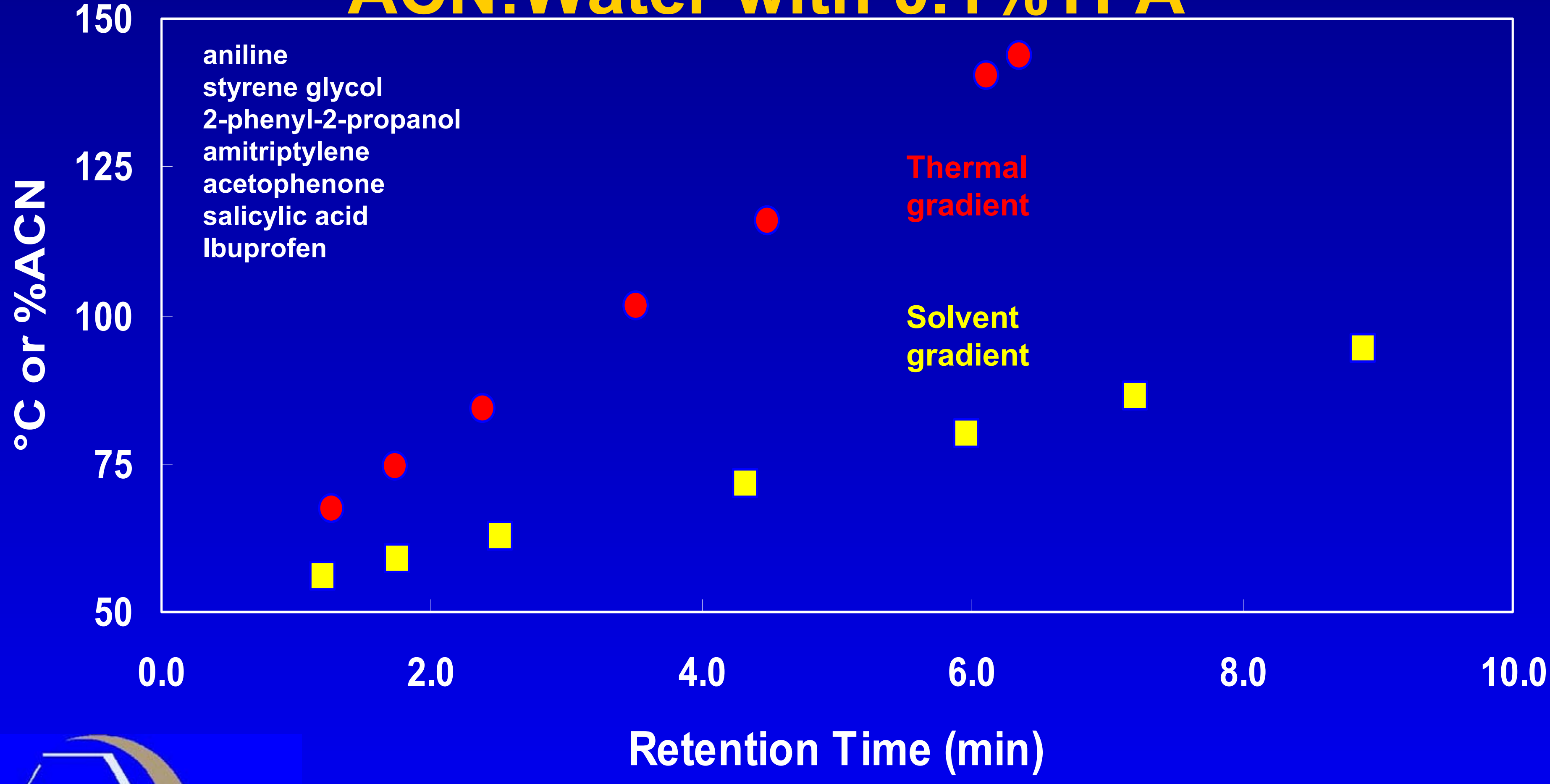
RT vs °C and RT vs %ACN for PRP®-1 ACN:Water with 0.1% TFA



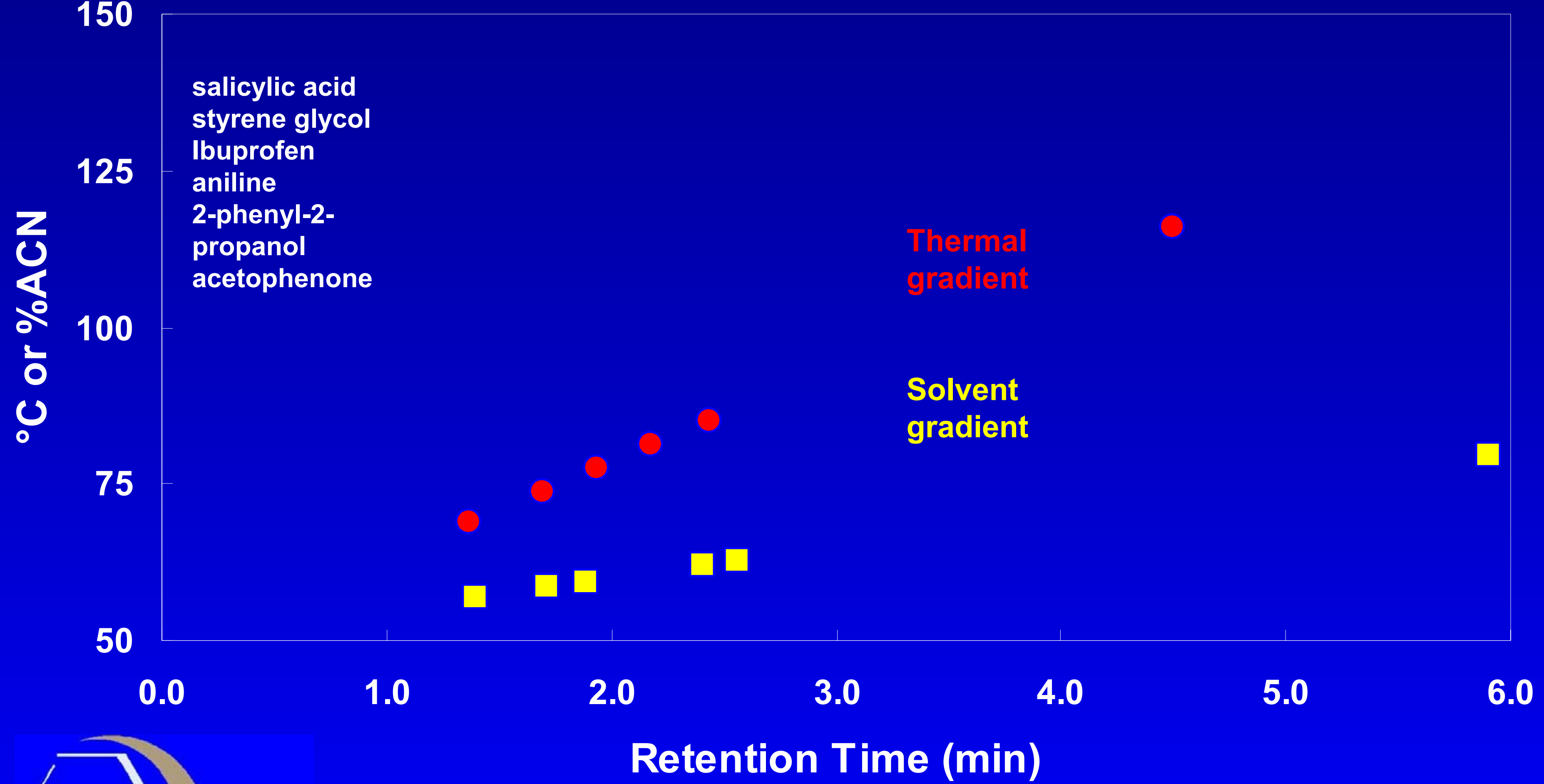
RT vs °C and RT vs %ACN for PRP®-1 ACN:Water pH 10



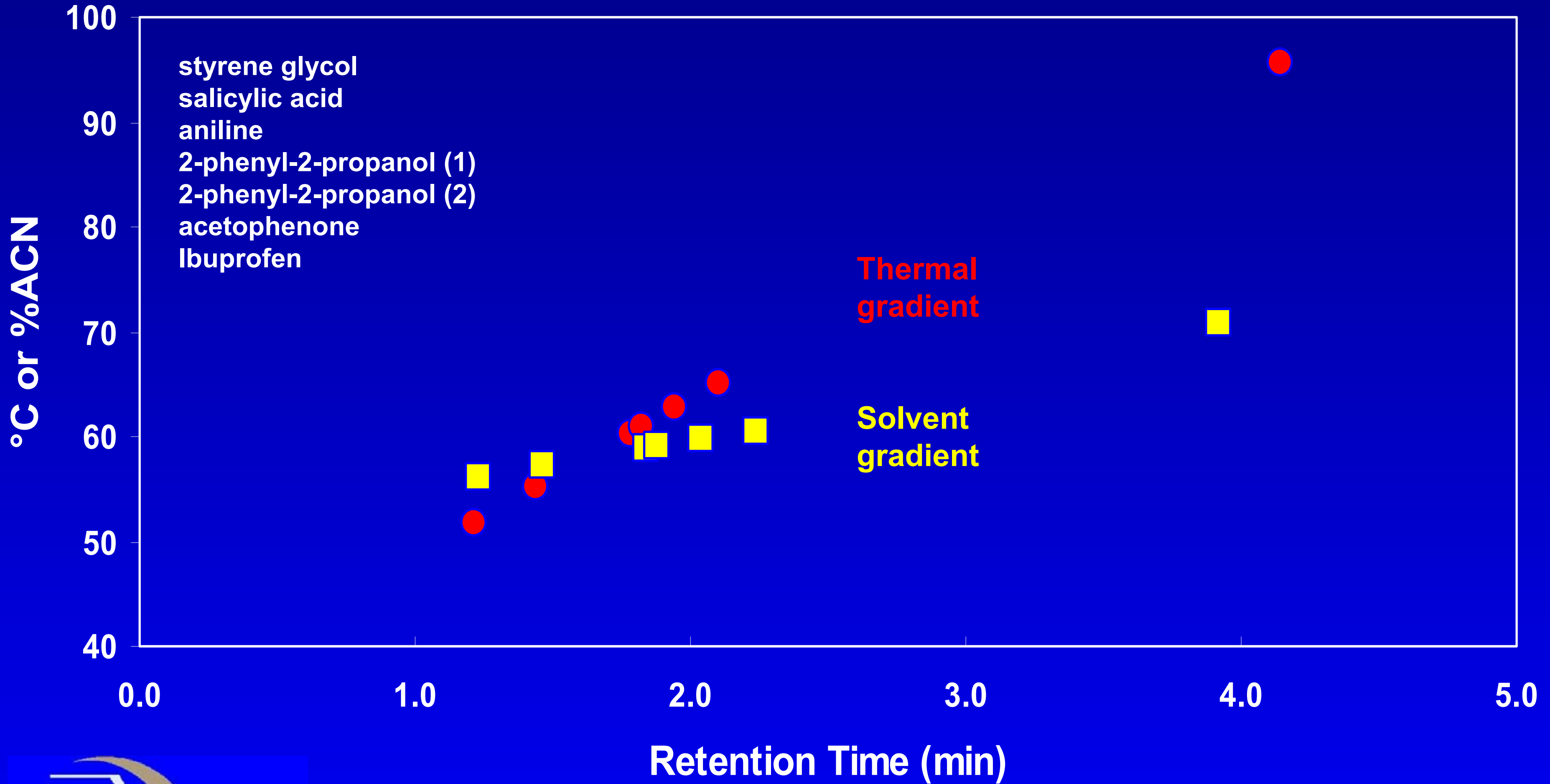
RT vs °C and RT vs %ACN for Hypercarb® Column ACN:Water with 0.1%TFA



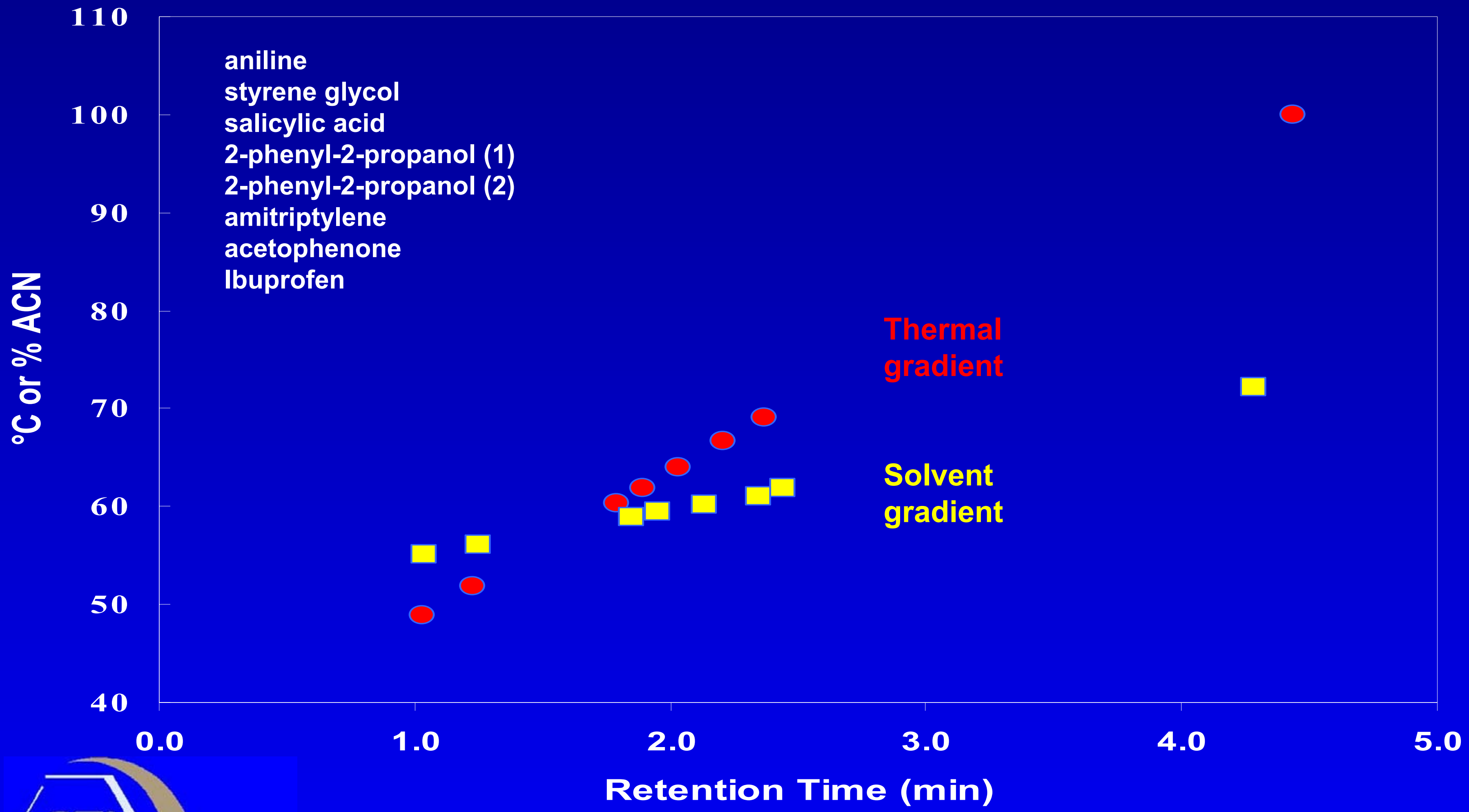
RT vs °C and RT vs %ACN for Hypercarb® Column ACN:water pH 10



RT vs °C and RT vs %ACN for Blaze C_8 ACN:Water



RT vs °C and RT vs %ACN for Blaze C_8 ACN:TFA

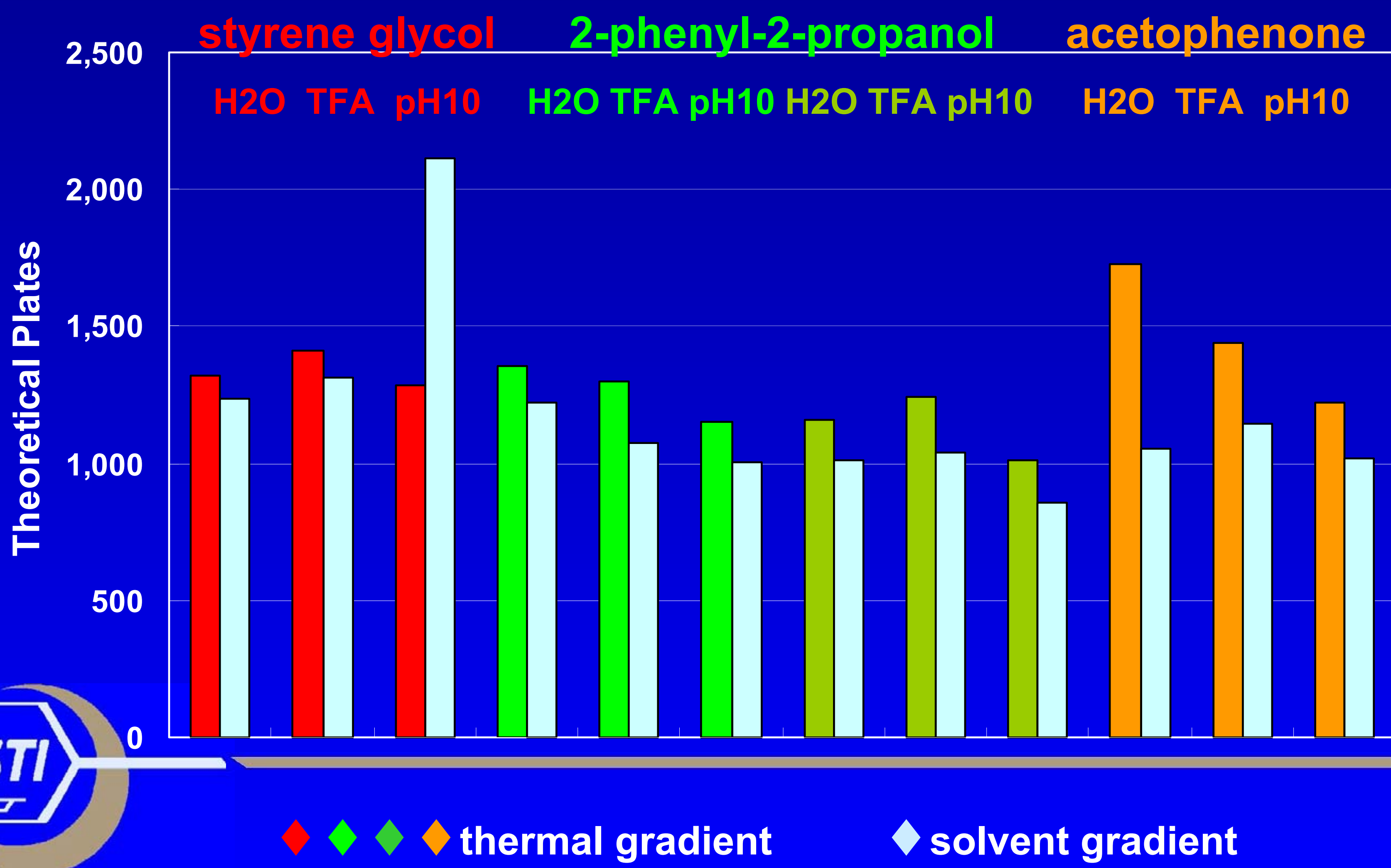


Peak Capacity Comparison for Thermal and Solvent Gradients

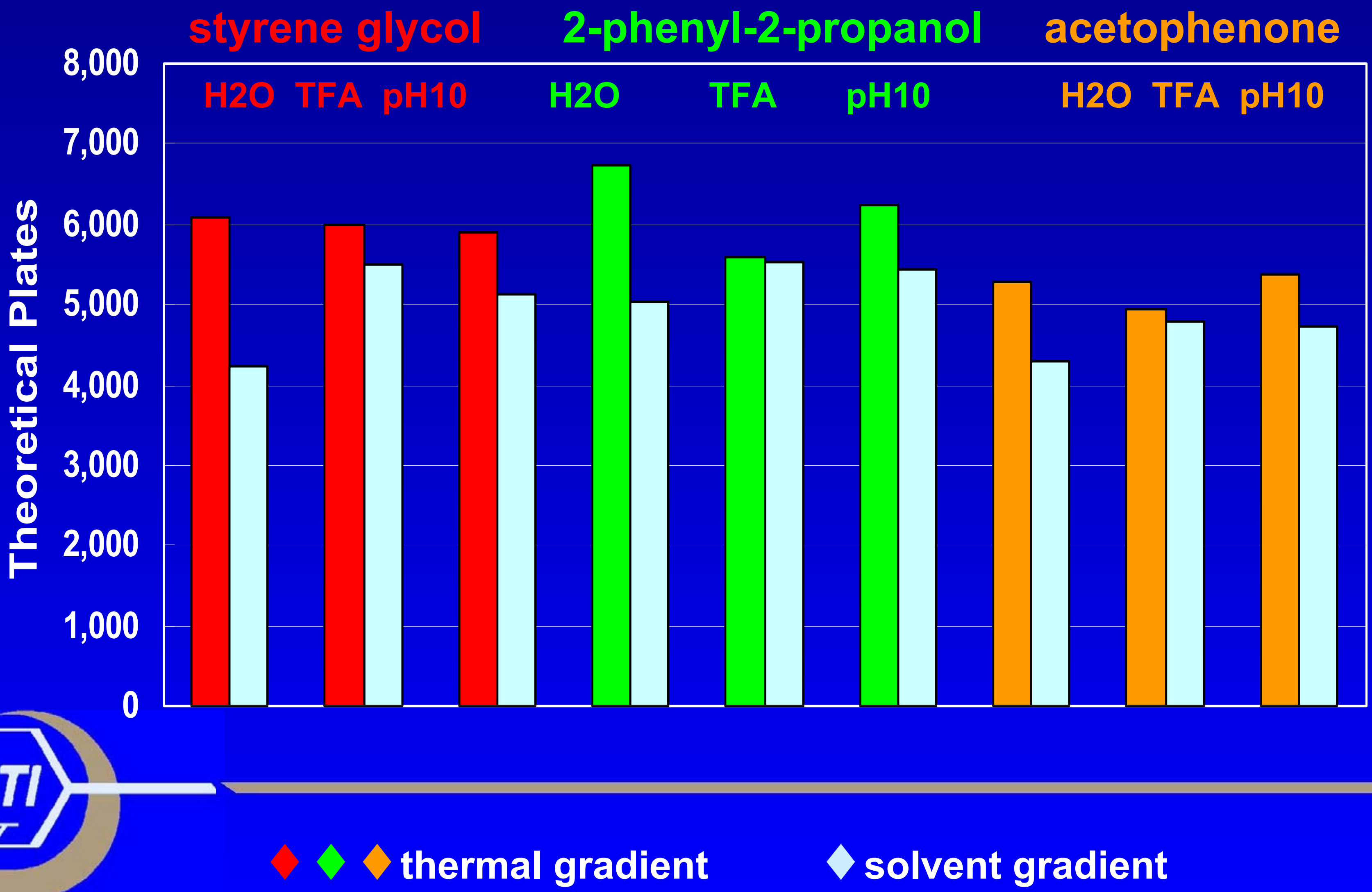
Mobile Phase	Blaze™ C_8 Thermal gradient	Blaze™ C_8 Solvent gradient	PRP-1® Thermal gradient	PRP-1® Solvent gradient	HyperCarb® Thermal gradient	HyperCarb® Solvent gradient
Acetonitrile:Water	44.8	39.7	15.8	15.2	32.7	32.5
Acetonitrile:Water with 0.1% TFA	45.4	43.7	22.1	20.8	31.7	27.9
Acetonitrile: 20 mM Ammonium Hydroxide pH 10	-	-	15.0	14.6	32.4	36.4

Comparable peak capacities were obtained for the solvent and thermal gradient runs.

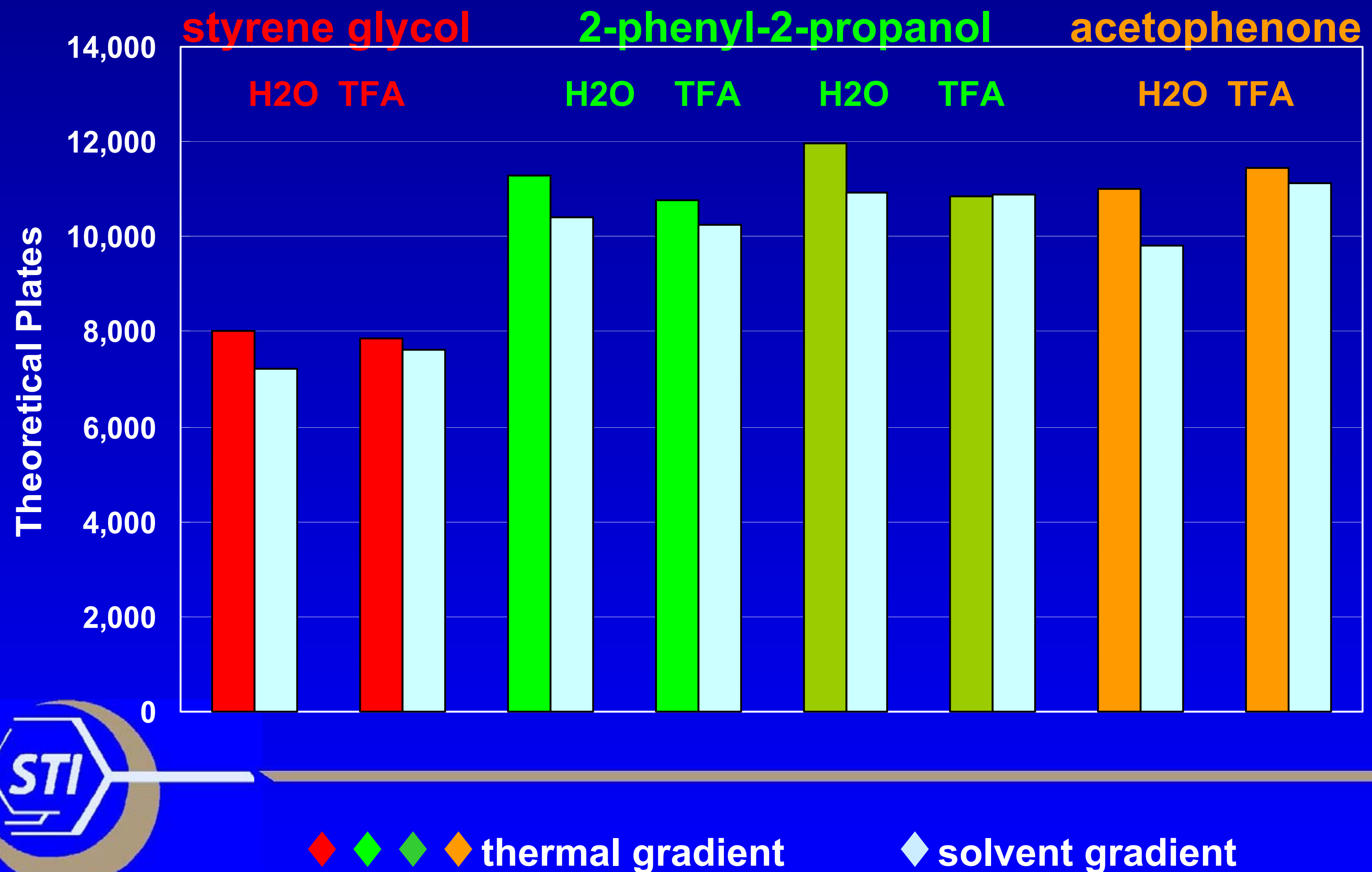
Efficiency Comparison of Solvent and Temperature Gradient for PRP®-1 Column



Efficiency Comparison of Solvent and Temperature Gradient for Hypercarb® Column



Efficiency Comparison of Solvent and Temperature Gradient for Blaze Column



Comparison of Retention and Efficiency of PRP®-1 During High Temperature Evaluation

Analyte	RT	plates	area
uracil initial	0.84	1,234	2,916,552
uracil after initial runs before TFA	0.86	1,300	3,061,639
uracil after TFA before ammonia	0.80	1,357	3,118,760
uracil after ammonia	0.81	1,603	2,931,591
phenol initial	1.84	1,250	193,020
phenol after initial runs before TFA	1.87	1,263	190,543
phenol after TFA before ammonia	1.82	1,230	203,936
phenol after ammonia	1.83	1,358	202,660

Comparison of Retention and Efficiency of Hypercarb® During High Temperature Evaluation

Analyte	RT	plates	area
uracil initial	1.43	3,827	3,152,477
uracil after initial runs before NH3	1.45	5,227	2,698,373
uracil after NH3 before TFA	1.45	4,658	2,991,397
uracil after TFA	1.47	5,298	2,909,248
phenol initial	2.41	5,414	182,970
phenol after initial runs before NH3	2.56	5,466	213,160
phenol after NH3 before TFA	2.36	4,811	209,921
phenol after TFA	2.63	4,944	207,484

Comparison of Retention and Efficiency of Blaze C_8 During High Temperature Evaluation

Analyte	RT	plates	area
uracil initial	1.05	3,898	2,637,763
uracil after initial runs	1.06	4,826	3,112,145
uracil after TFA	1.06	5,006	3,071,317
phenol initial	1.81	7,162	166,090
phenol after initial runs	1.89	7,133	207,308
phenol after TFA	1.89	7,591	201,861

Conclusions

- Column bleed can be an issue with zirconia columns during temperature programming
- Several columns do give good performance under high temperature conditions without evidence of stationary phase degradation
- Similar retention characteristics are observed with solvent gradients and temperature programming
- Comparable or better peak quality is observed with temperature programming when compared to solvent gradients for the columns evaluated

Acknowledgements

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Turn up the Heat!

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