

# **Column Stability and Performance under High Temperature HPLC Conditions**

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# Introduction

HPLC at extreme temperatures offers shorter run times and faster method development to the separation scientist. The real power of High Temperature Liquid Chromatography (HTLC) is the ability to do temperature programming. This allows the use of an isocratic mobile phase and a thermal gradient to alter retention and selectivity. The advantages of performing HPLC analyses at elevated temperatures was documented in the literature over a decade ago (1), but equipment and column limitations made it impractical. The creation of zirconia based stationary phases generated new interest in High Temperature Liquid Chromatography (2). Although zirconia columns are the first to come to mind, several other commercially available columns can be used at temperatures as high as 200°C. Three zirconia columns, a polydentate silica column, a polymeric column, and a graphitic carbon column were evaluated under temperature programmed HTLC conditions.

# Experimental

Columns and their characteristics are summarized in Table 1. Analysis conditions are detailed in Table 2. Seven components consisting of aniline, acetophenone, amitriptylene, Ibuprofen, salicylic acid, styrene glycol and 2-phenyl-2-propanol were analyzed using three different mobile phases under solvent and thermal gradient conditions. The columns were checked with a two component test mix containing uracil and phenol between each mobile phase to monitor for signs of column degradation. Retention times, theoretical plates, and peak areas were documented. UV detection at 254 nm and a flow rate of 1.0 mL/min were used for all chromatographic runs. A blank thermal gradient was initially run on each column. The ZirChrom columns exhibited a significant rise in the baseline under temperature programmed conditions and work with these column was discontinued (see Figure 1).

**Table 1.****HPLC Columns Evaluated Using Temperature Programmed HTLC**

Manufacturer	Column	Packing	Particle Size( $\mu\text{m}$ )	Length (cm)	ID (mm)	Maximum Temperature
Thermo Hypersil-Keystone	HyperCarb <sup>®</sup>	graphitic carbon pH 0-14	7	10	4.6	200°C
Hamilton Company	PRP-1 <sup>®</sup>	PSDVB polymer PH 0-14	5	10	4.1	150°C
Selerity Technologies	Blaze <sup>™</sup> C <sub>8</sub>	polydentate silica pH 2-8	3	10	4.6	100°C

The ZirChrom PBD, CARB, and DiamondBond Columns were also included in this evaluation, but significant column bleed was observed under temperature programmed conditions.

## Table 2.

### Analysis Conditions for Temperature Programmed HTLC Column Evaluation

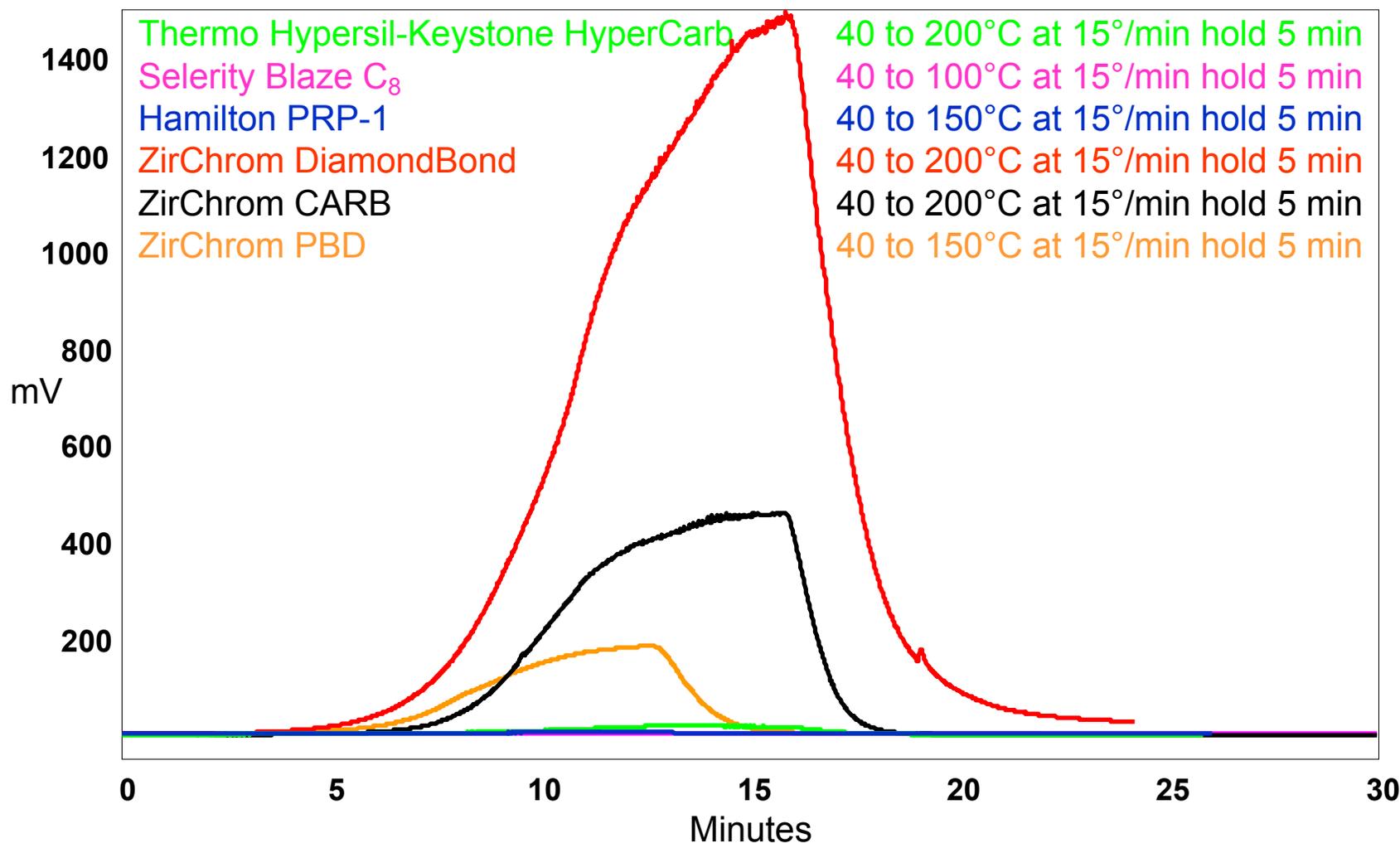
Column	Thermal Gradient	Solvent Gradient	Mobile Phases
HyperCarb <sup>®</sup>	50 to 200°C at 15°/minute, hold five minutes.		50:50 acetonitrile:water
PRP-1 <sup>®</sup>	50 to 150°C at 10°/minute, hold five minutes.	50 to 100% acetonitrile over ten minutes, hold five minutes	50:50 acetonitrile:0.1% TFA pH≈2
Blaze <sup>™</sup>	35 to 100°C at 10°/minute, hold five minutes.		50:50 acetonitrile: 20 mM ammonium hydroxide pH 10

The ammonium hydroxide mobile phase was not used for the Blaze C<sub>8</sub> column evaluation because this silica based column has a pH range of 2-8.

# Results

Figure 1 shows the results of initial blank thermal gradients run on all six columns. Table 3 lists a comparison of peak capacities for the solvent and thermal gradient conditions. Tables 4-6 compare retention time, theoretical plates, and peak areas for the two component test mix used to check the columns between mobile phase changes. Figures 2-8 compare retention times versus degrees Celsius and retention time versus percent acetonitrile for the Blaze C<sub>8</sub>, PRP-1, and HyperCarb column with the different mobile phase compositions under thermal and solvent gradient conditions.

**Figure 1.** Blank thermal gradients for six HPLC columns. The PRP-1 and Blaze C<sub>8</sub> have an essentially flat baseline. The HyperCarb column shows a slight baseline rise (approx 10 mV). The three Zirchrom columns show a significant rise in the baseline.



**Table 3.****Peak Capacity Comparison for Thermal and Solvent Gradients**

Mobile Phase	Blaze™ C <sub>8</sub>		PRP-1®		HyperCarb®	
	Thermal gradient	Solvent gradient	Thermal gradient	Solvent gradient	Thermal gradient	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.

**Table 4.****Comparison of Retention and Efficiency of PRP-1<sup>®</sup> Column During HTLC Evaluation**

Analyte/Stage of Evaluation	Retention Time (min)	Theoretical Plates (USP)	Peak 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 pH 10	0.80	1,357	3,118,760
uracil after pH 10	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 pH 10	1.82	1,230	203,936
phenol after pH 10	1.83	1,358	202,660

## Table 5.

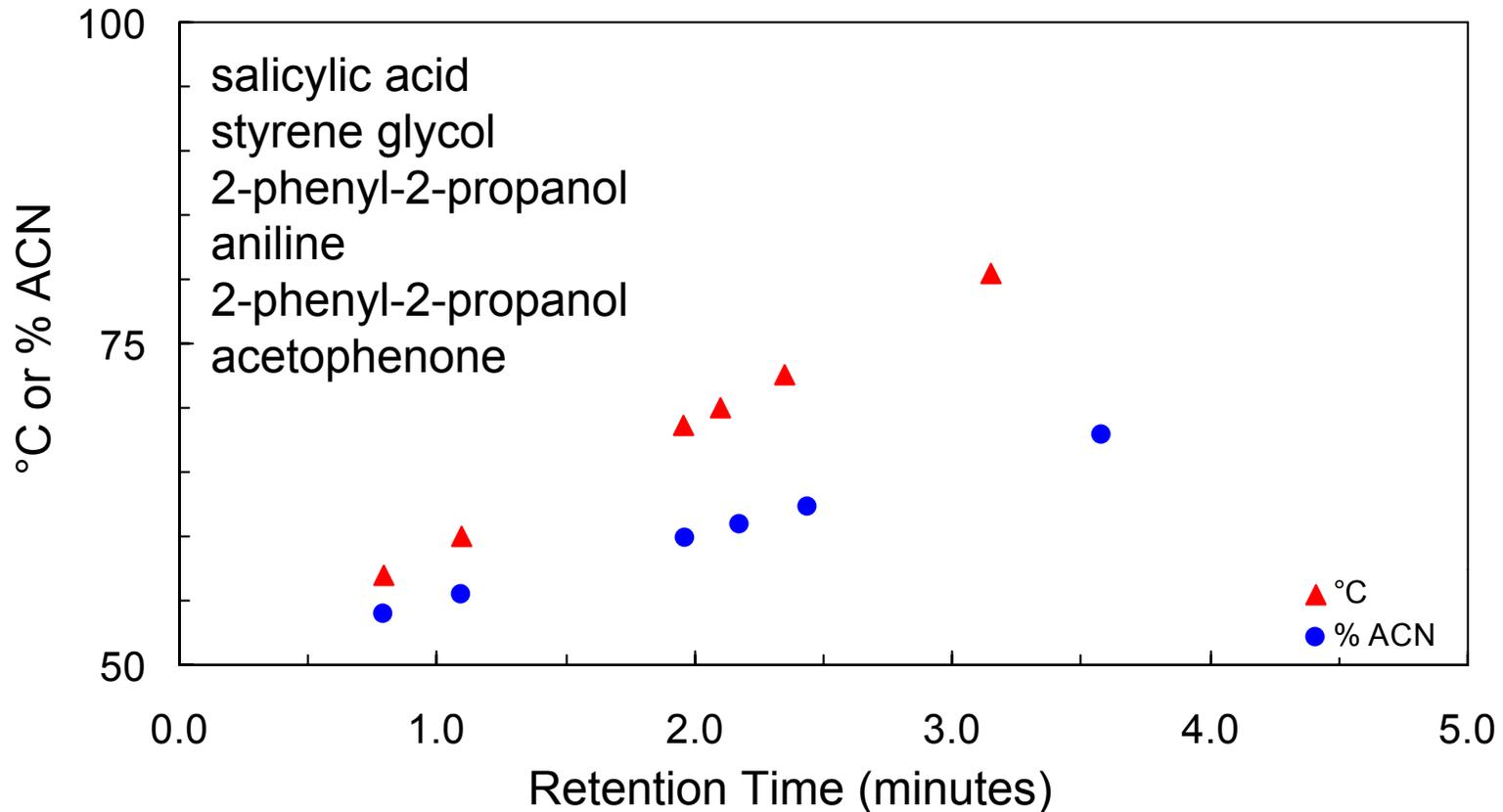
### Comparison of Retention and Efficiency of HyperCarb<sup>®</sup> Column During HTLC Evaluation

Analyte/Stage of Evaluation	Retention Time (min)	Theoretical Plates (USP)	Peak Area
uracil initial	1.43	4,827	3,152,477
uracil after initial runs before pH 10	1.45	5,227	2,698,373
uracil after pH 10 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 pH 10	2.56	5,466	213,160
phenol after pH 10 before TFA	2.36	4,811	209,921
phenol after TFA	2.63	4,944	207,484

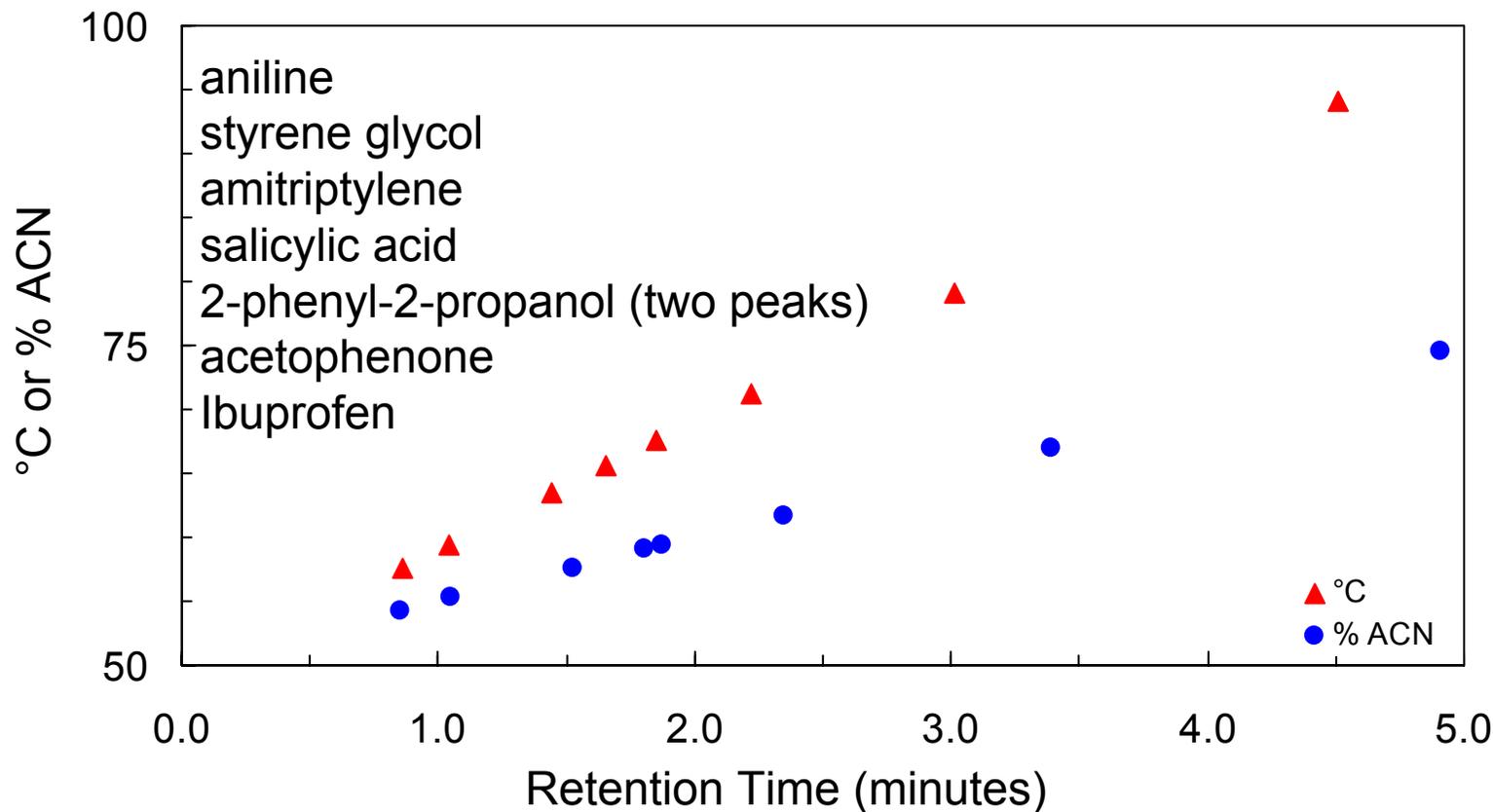
**Table 6.****Comparison of Retention and Efficiency of Blaze™ Column During HTLC Evaluation**

Analyte/Stage of Evaluation	Retention Time (min)	Theoretical Plates (USP)	Peak 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

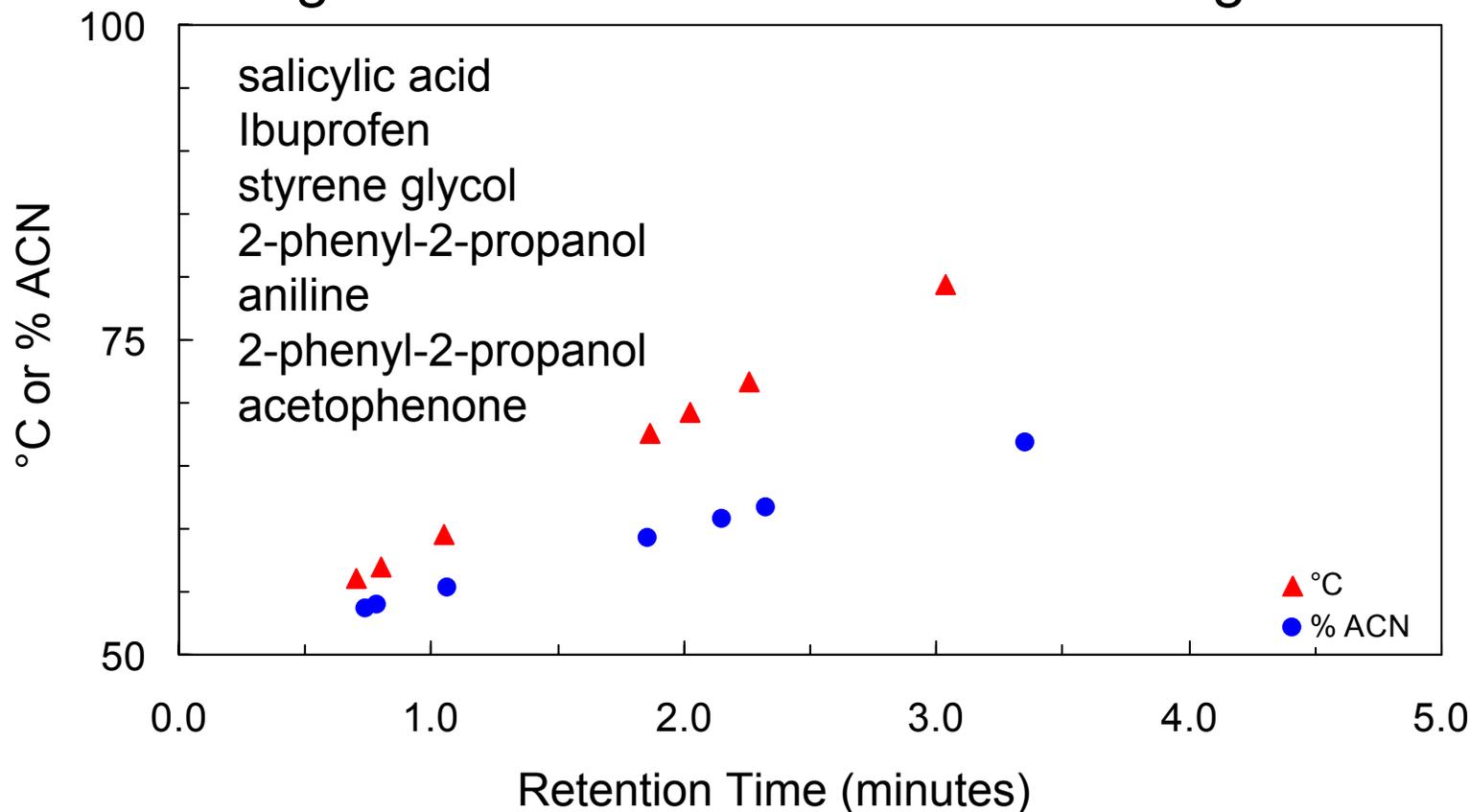
**Figure 2.** Retention time versus °C and retention time versus percent acetonitrile for the PRP-1 column using acetonitrile:water as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the thermal gradient. Ibuprofen and amitriptylene did not elute using either the solvent or thermal gradient within the designated run time.



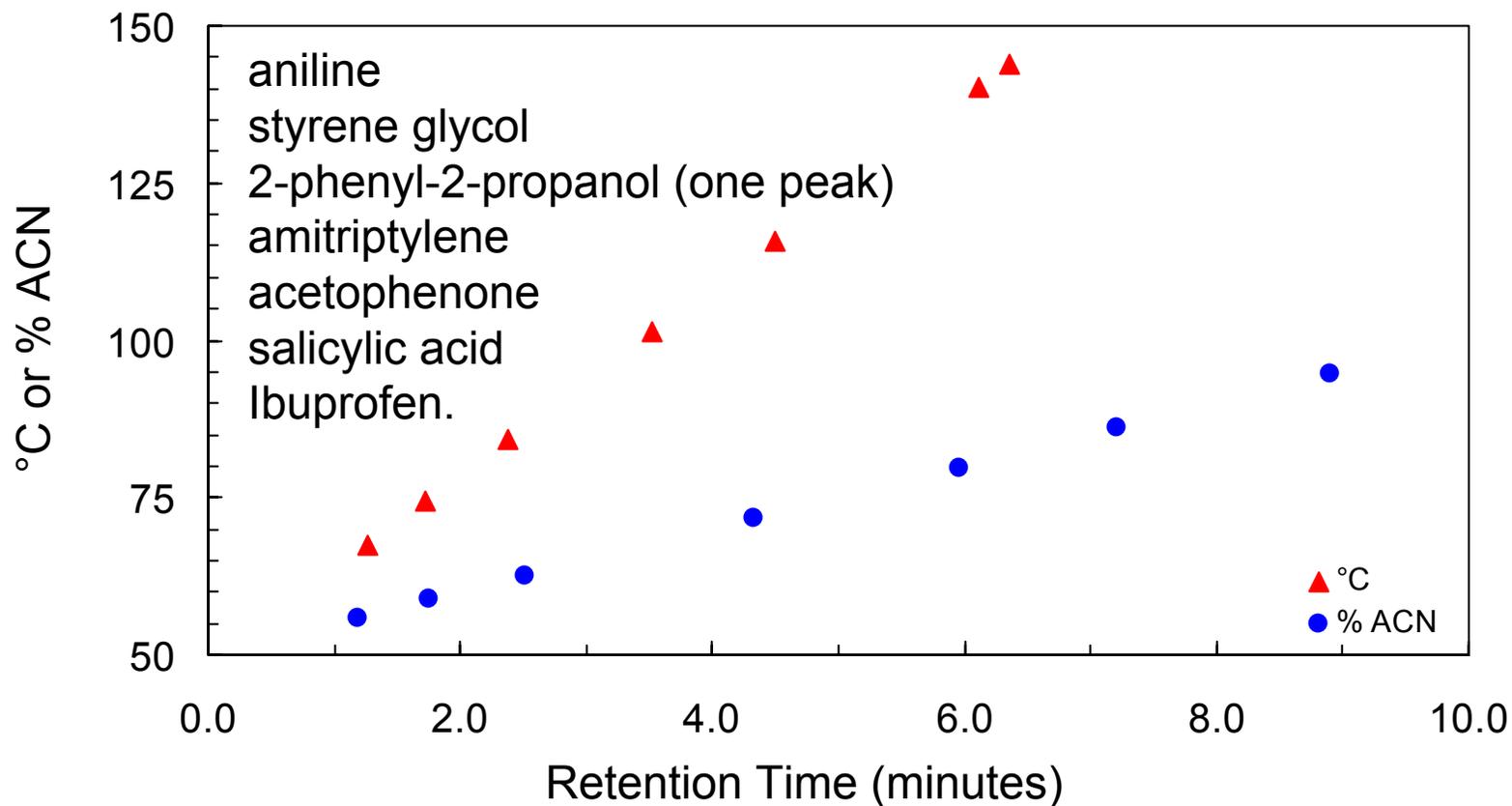
**Figure 3.** Retention time versus °C and retention time versus percent acetonitrile for the PRP-1 column using acetonitrile:water with 0.1% TFA as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the thermal gradient.



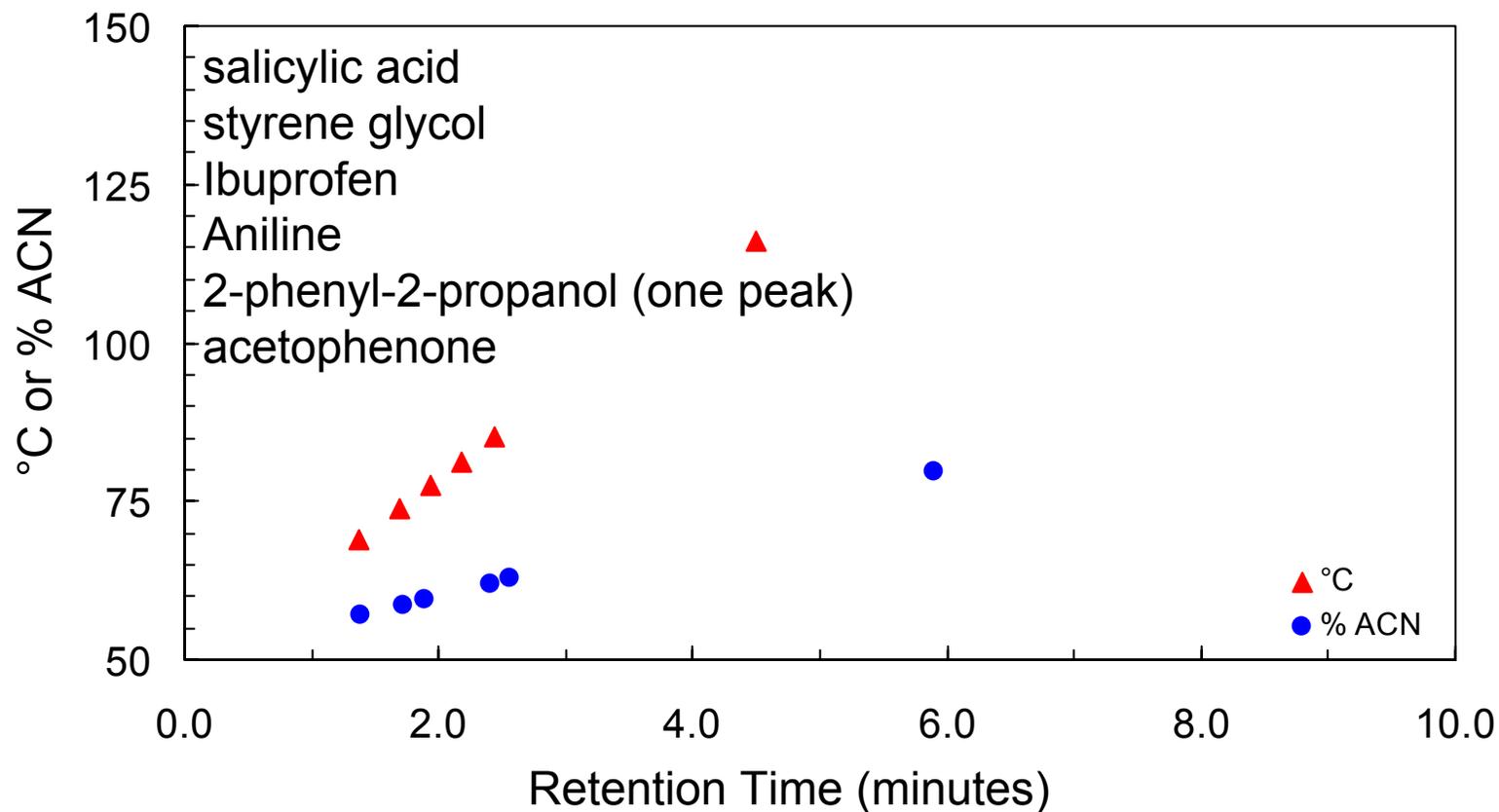
**Figure 4.** Retention time versus °C and retention time versus percent acetonitrile for the PRP-1 column using acetonitrile:20 mM ammonium hydroxide pH 10 as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the thermal gradient. Amitriptylene eluted at 11 minutes with the solvent gradient but did not elute under thermal gradient conditions within the designated run time.



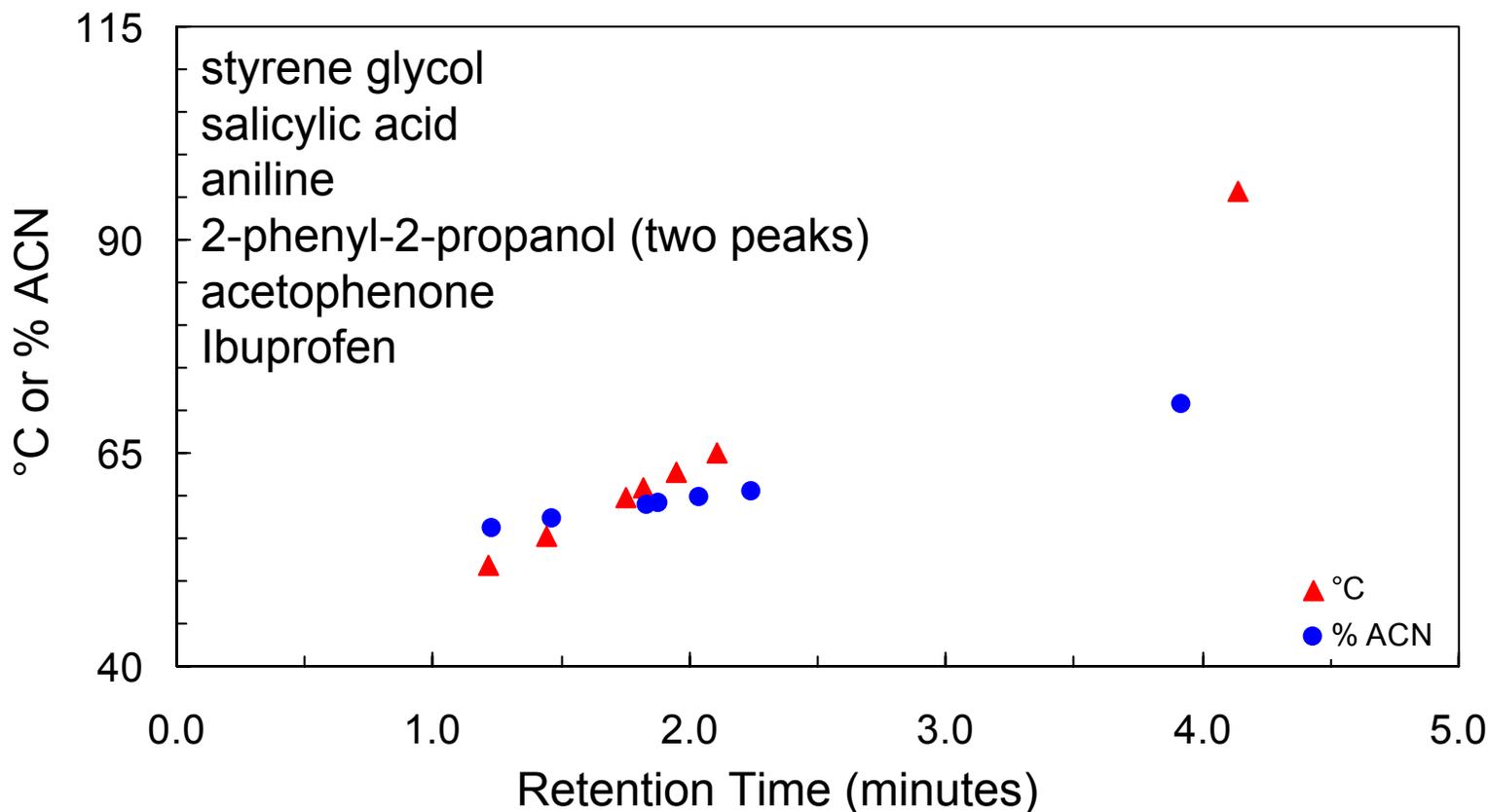
**Figure 5.** Retention time versus °C and retention time versus percent acetonitrile for the HyperCarb column using acetonitrile:water with 0.1 percent TFA as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the thermal gradient.



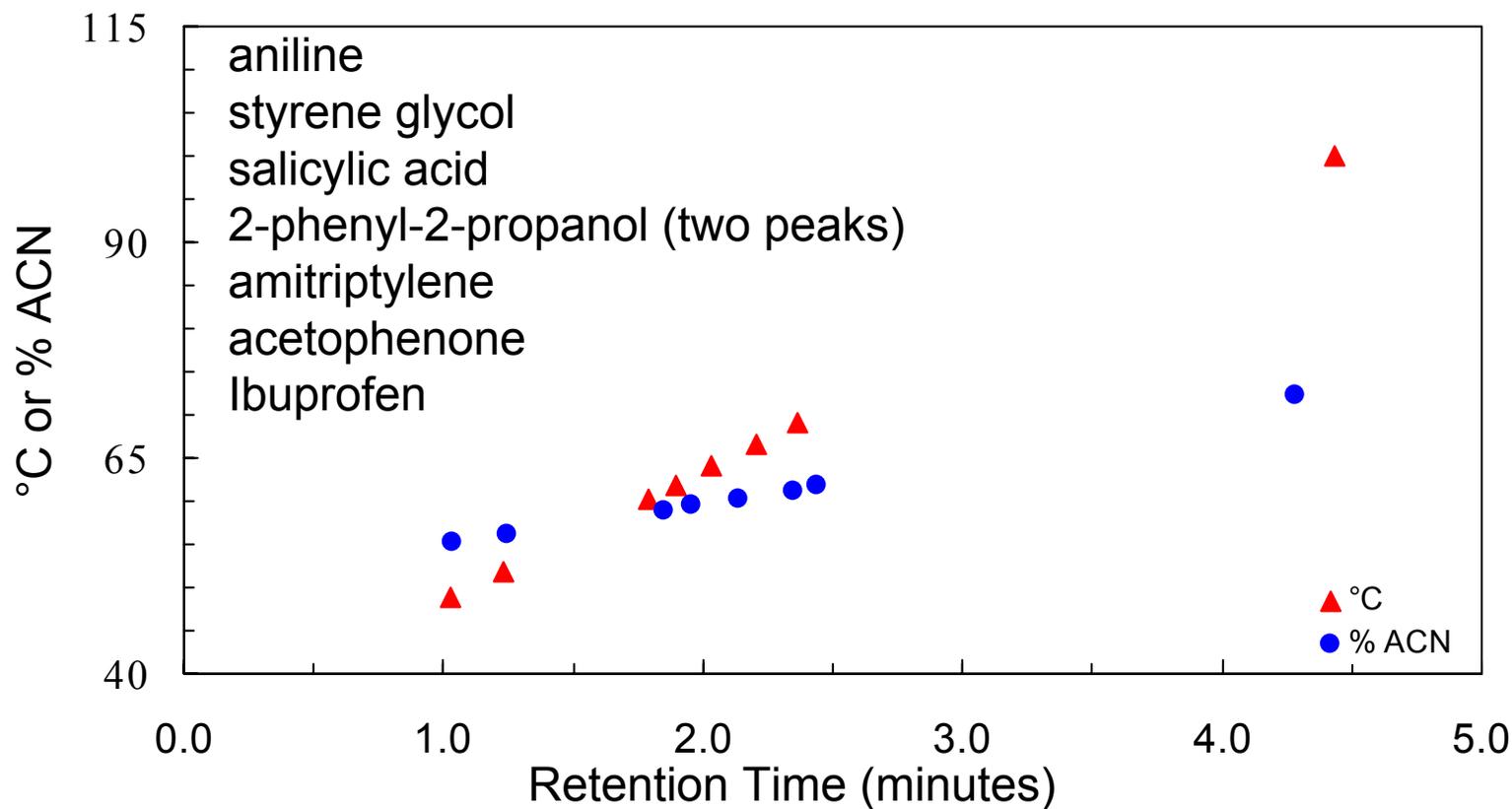
**Figure 6.** Retention time versus °C and retention time versus percent acetonitrile for the HyperCarb column using acetonitrile:20 mM ammonium hydroxide pH 10 as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the thermal gradient. Amitriptylene did not elute under either solvent or thermal gradient conditions within the specified run time.



**Figure 7.** Retention time versus °C and retention time versus percent acetonitrile for the Blaze C<sub>8</sub> column using acetonitrile:water as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the thermal gradient. Amitriptylene did not elute under either solvent or thermal gradient conditions within the designated run time.



**Figure 8.** Retention time versus °C and retention time versus percent acetonitrile for the Blaze C<sub>8</sub> column using acetonitrile:water with 0.1% TFA as the mobile phase. Circles represent retention times for the solvent gradient and triangles represent retention times for the thermal gradient.



# Discussion

Similar retention and comparable peak capacities were observed between the solvent and thermal gradient conditions. There did not appear to be a loss of peak capacity under temperature programmed conditions. The baseline rise was observed only with the Zirchrom columns. It was not present with the Blaze, PRP-1 or HyperCarb columns, or when no column was installed. This suggests that it is caused by some material leaching from the packing of the ZirChrom columns during a thermal gradient. Retention times and efficiency for the Blaze, PRP-1, and HyperCarb column generally agreed within 10% before, during and after the evaluation. It appears that exposure to high temperatures, even at pH extremes, did not damage these stationary phases.

# 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 collapse
- Similar retention characteristics were observed with solvent and temperature gradients
- Comparable or better peak capacities were observed with temperature gradients when compared to solvent gradients for the columns evaluated

## Acknowledgements

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# References

1. F. Antia and C. Horvath, *J. Chrom.*, **435**, 1-15 (1988)
2. T. Greibrokk and A. Anderson, *J. Chrom.*, (in press)



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