Silica Based Columns for High Temperature HPLC

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Introduction

New instrumentation has made it possible to perform HPLC separations at temperatures up to 200°C, but most traditional silica columns can only be used to about 60°C. New stationary phases consisting of polydentate silica resist hydrolytic breakdown and can be used at higher temperatures. This paper outlines work done to evaluate a new polydentate silica phase and its durability at high temperatures. This new stationary phase was evaluated using different mobile phase compositions and pH conditions at elevated temperatures. Columns were flushed with 1000 column volumes of different mobile phases, then evaluated for evidence of stationary phase breakdown at 10°C temperature increments.

Benefits of Increasing Temperature

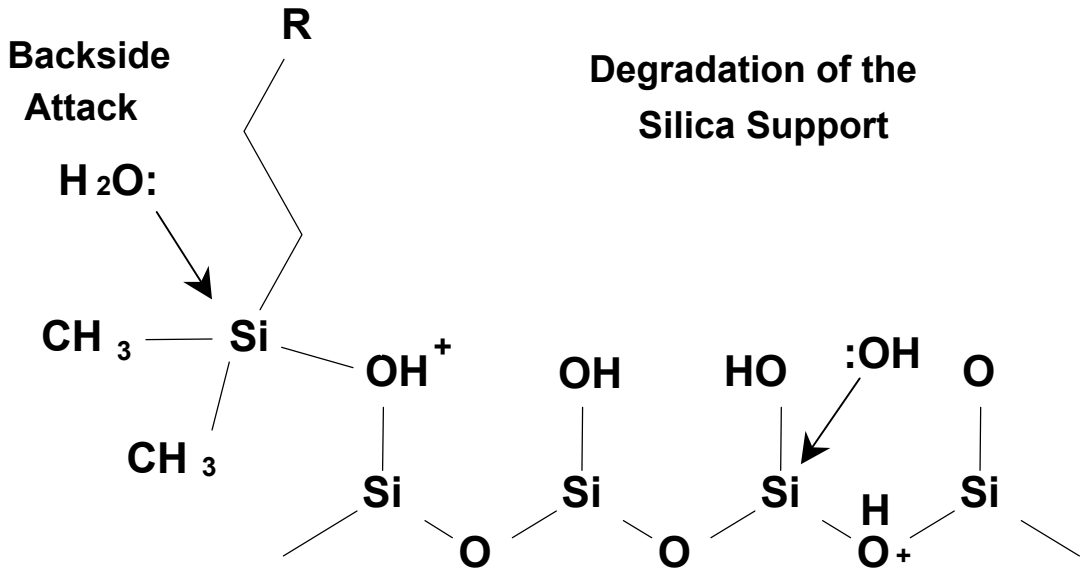
- Speed
- Efficiency
- Lower viscosity and back pressure permits higher flow rates with smaller particle size packings

Benefits of Temperature Programming

- Peak focusing similar to programmed elution in GC
- New selectivity options
- Isocratic elution of a wide range of analytes
- Simultaneous use of compositional gradients or flow programming for additional flexibility

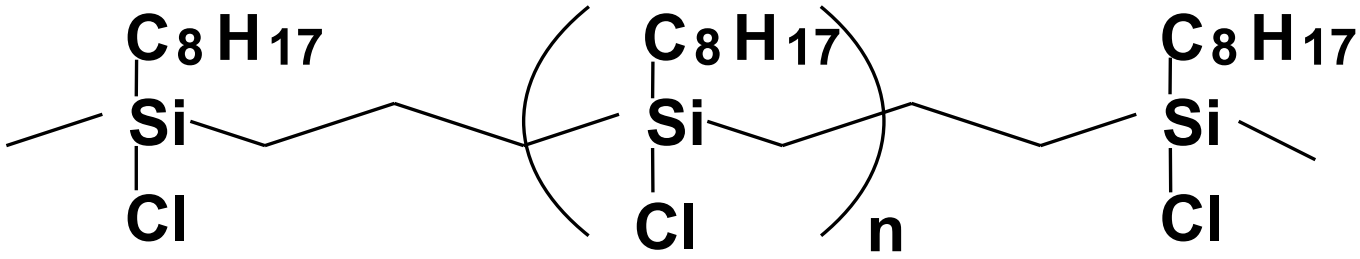
Hydrolytic Breakdown is Accelerated at Higher Temperatures

Water attacks the siloxane bond or behind point of phase attachment



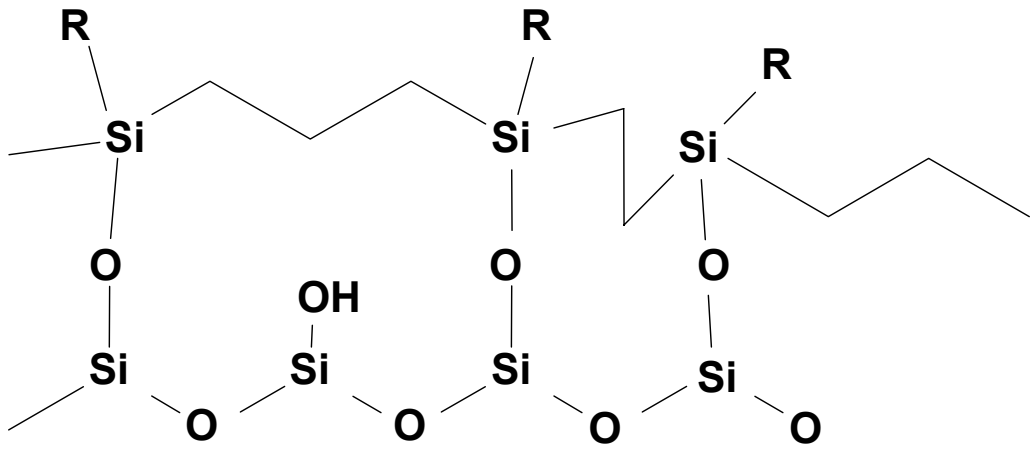
Functionalized Monomers are Prepared Using Grignard Chemistry

Polycarbosilane Bonded Phase Precursor



Polycarbosilane Bonding Structure

- Multiple attachment points to the particle surface
- Multiple crosslinking points to adjacent polymer chains
- Thermal and hydrolytically stable backbone



Patent pending on this new polydentate silica phase

Experimental

Each column was initially tested at 50°C. Thereafter, columns were flushed with 1000 column volumes at 10°C increments. After each high temperature exposure the columns were re-evaluated at 50°C for evidence of stationary phase degradation. All data were collected in triplicate, and the average values reported. Agreement was between less than 1 and 10% (%RSD).

Four parameters were used to monitor the effect of temperature on the columns: theoretical plates (N), resolution (R_s), area-to-height ratio (A/H), and retention time (t_r). Acetophenone and benzene were used for the column evaluation because the peak characteristics of these later-eluting compounds exhibited the effects of the temperature changes more strongly than the dead-time marker and phenol.

Experimental Conditions

Columns: Selerity Blaze C_8, 3 μm, 100 x 2.1 mm
Flow Rate: 0.25 mL/min
Detection: UV 254 nm
Injection: 5 μL (full loop)

Evaluation Mix:

Uracil, Phenol, Acetophenone, Benzene

Four mobile phase compositions:

35:65 acetonitrile:water
35:65 methanol:water
35:65 methanol:10mM ammonium acetate pH 4.0
35:65 methanol:10mM ammonium acetate pH 9.0

Results and Discussion

Figures 1-8 show the effect of temperature on all four parameters for acetophenone and benzene with all four mobile phase compositions. The plate count was normalized to account for total theoretical plate count variation among the different columns and mobile phases. This was done by comparing N after each temperature increment to the initial value of N for each column. A decrease below 80% of the original value of N was considered a good indication that the column material had been seriously affected by the temperature and had irreversibly deteriorated (see Figures 1 and 2). The changes in R_s essentially mirror the changes in N (see Figures 3 and 4).

Results and Discussion

Drastic increases in the area-to-height ratio indicate thermal degradation of the stationary phase (see Figures 5 and 6). Once the columns had been operated beyond their thermal stability limit, significantly reduced retention times were observed (see Figures 7 and 8). Figures 9 through 12 show chromatograms for each of the mobile phase systems studied. Each of these figures contains three traces. The blue trace shows the baseline separation of the column test mixture before the column was subjected to elevated temperature. The red trace shows the column test mixture after the column had been subjected to the highest temperature tolerated by the column without evidence of thermal degradation. The brown trace shows the column test mixture after the column had been subjected to temperatures beyond tolerable limits.

Figure 1
Effect of Temperature on Theoretical Plates for Acetophenone

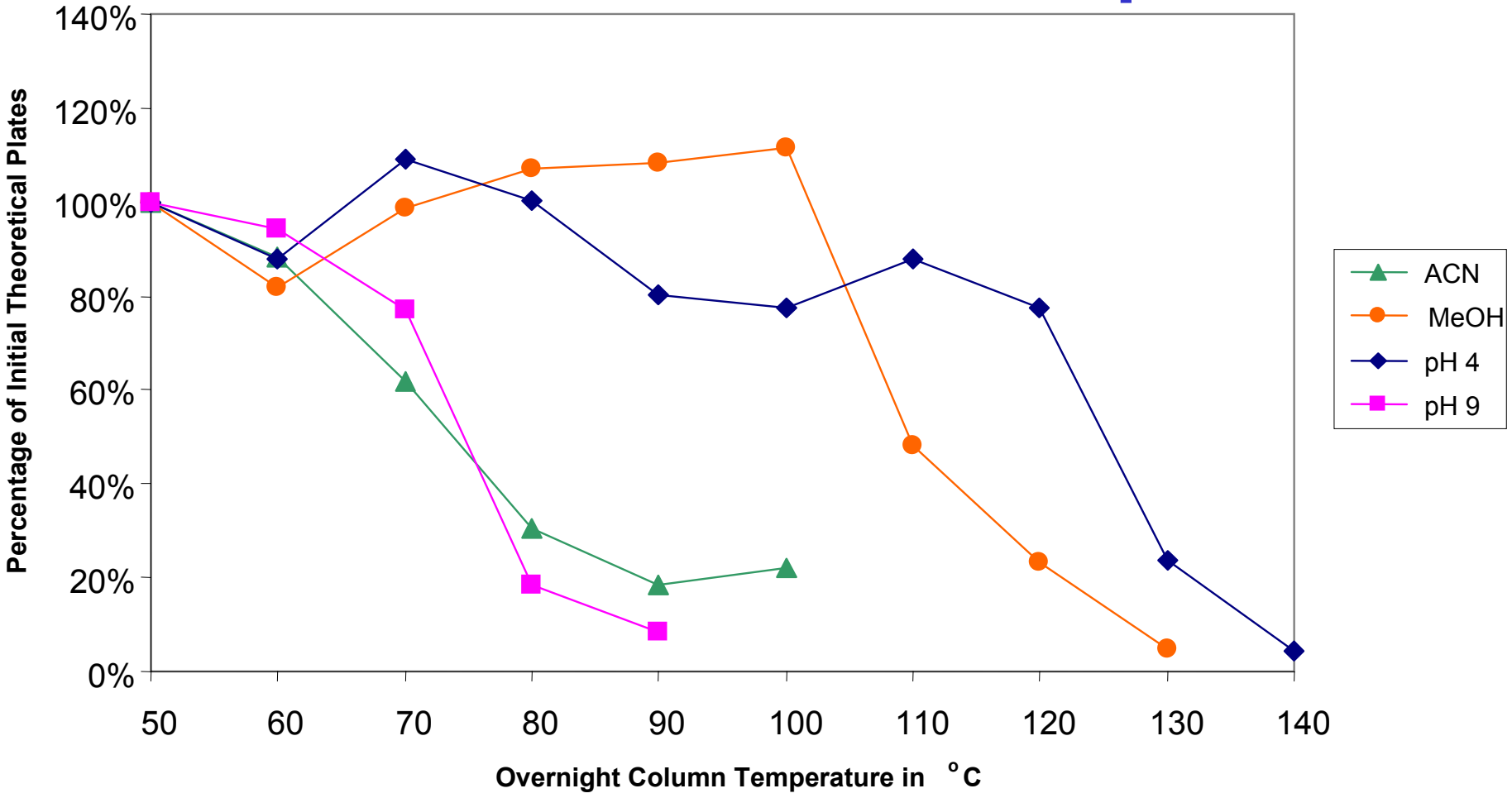


Figure 2
Effect of Temperature on Theoretical Plates for Benzene

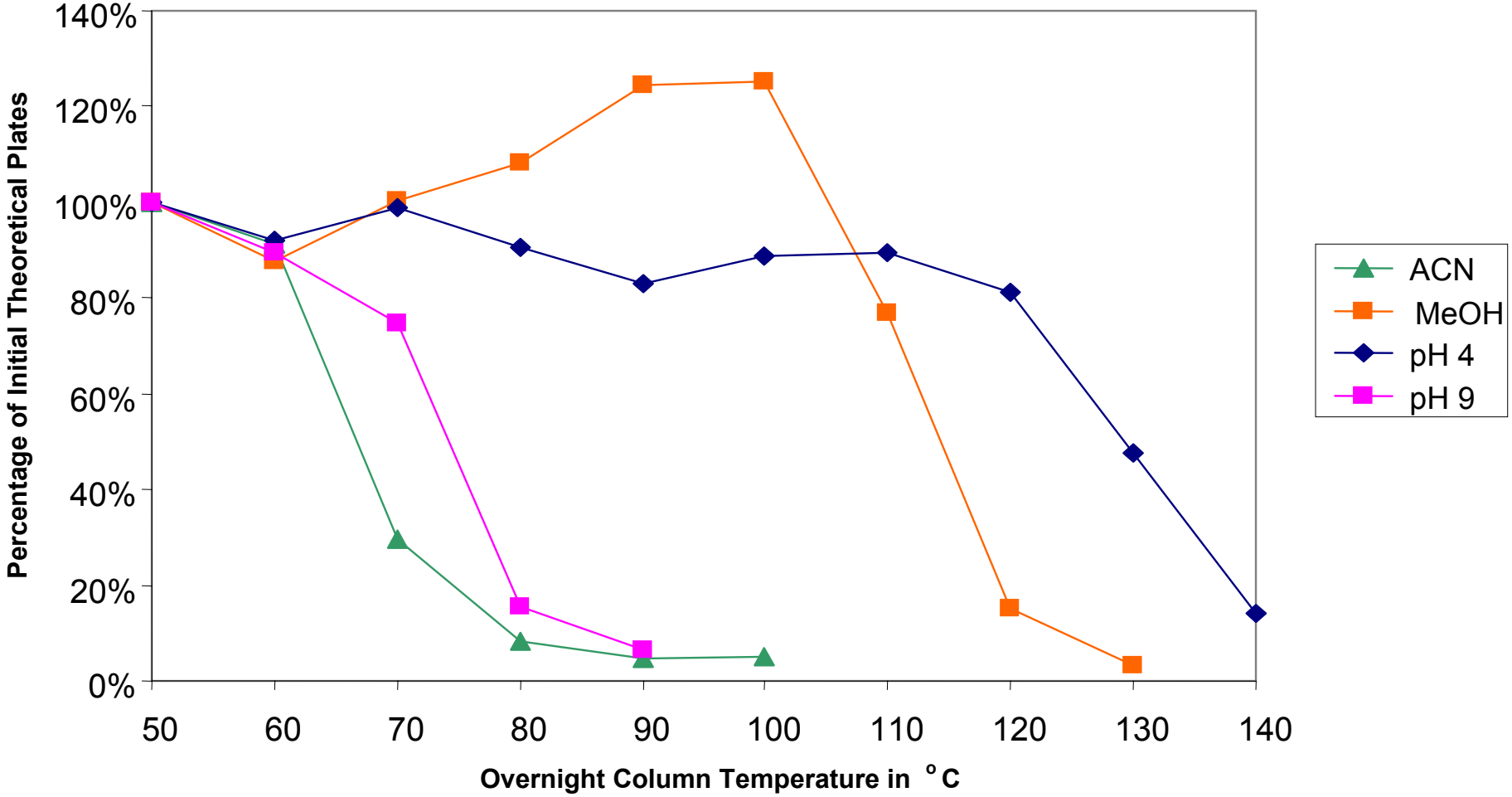


Figure 3
Effect of Temperature on Resolution for Acetophenone

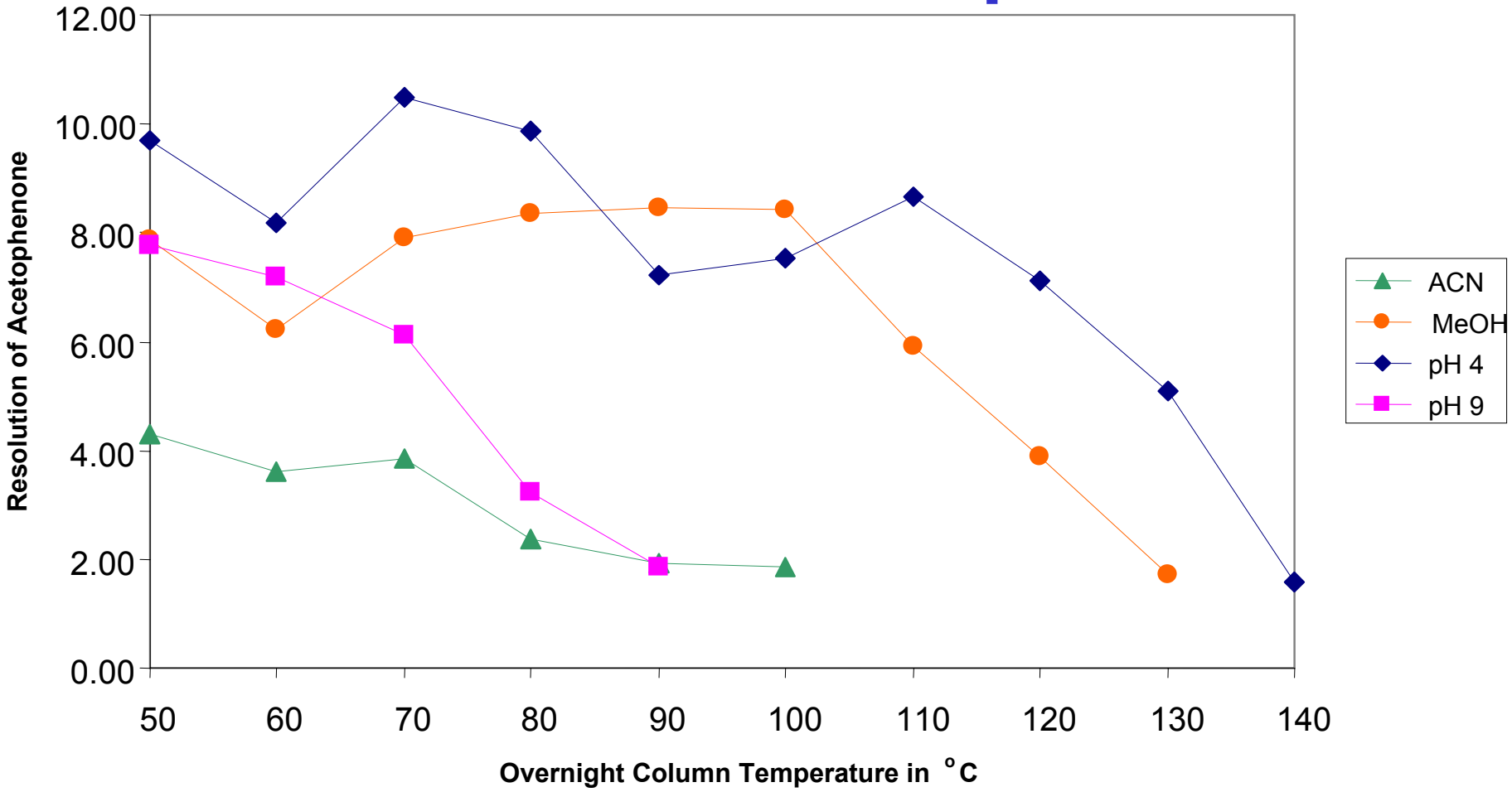


Figure 4
Effect of Temperature on Resolution for Benzene

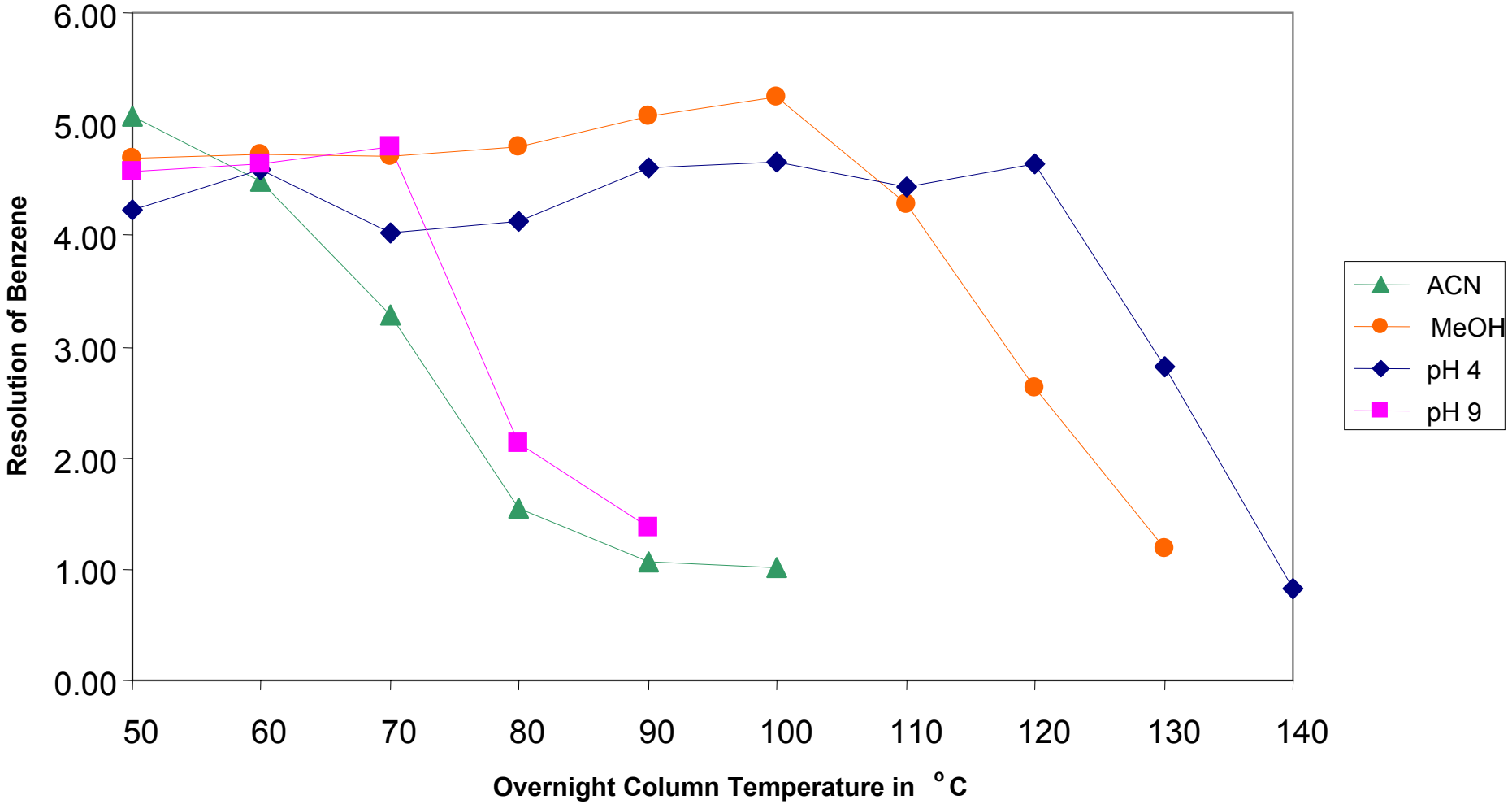


Figure 5
Effect of Temperature on Area/Height Ratio for Acetophenone

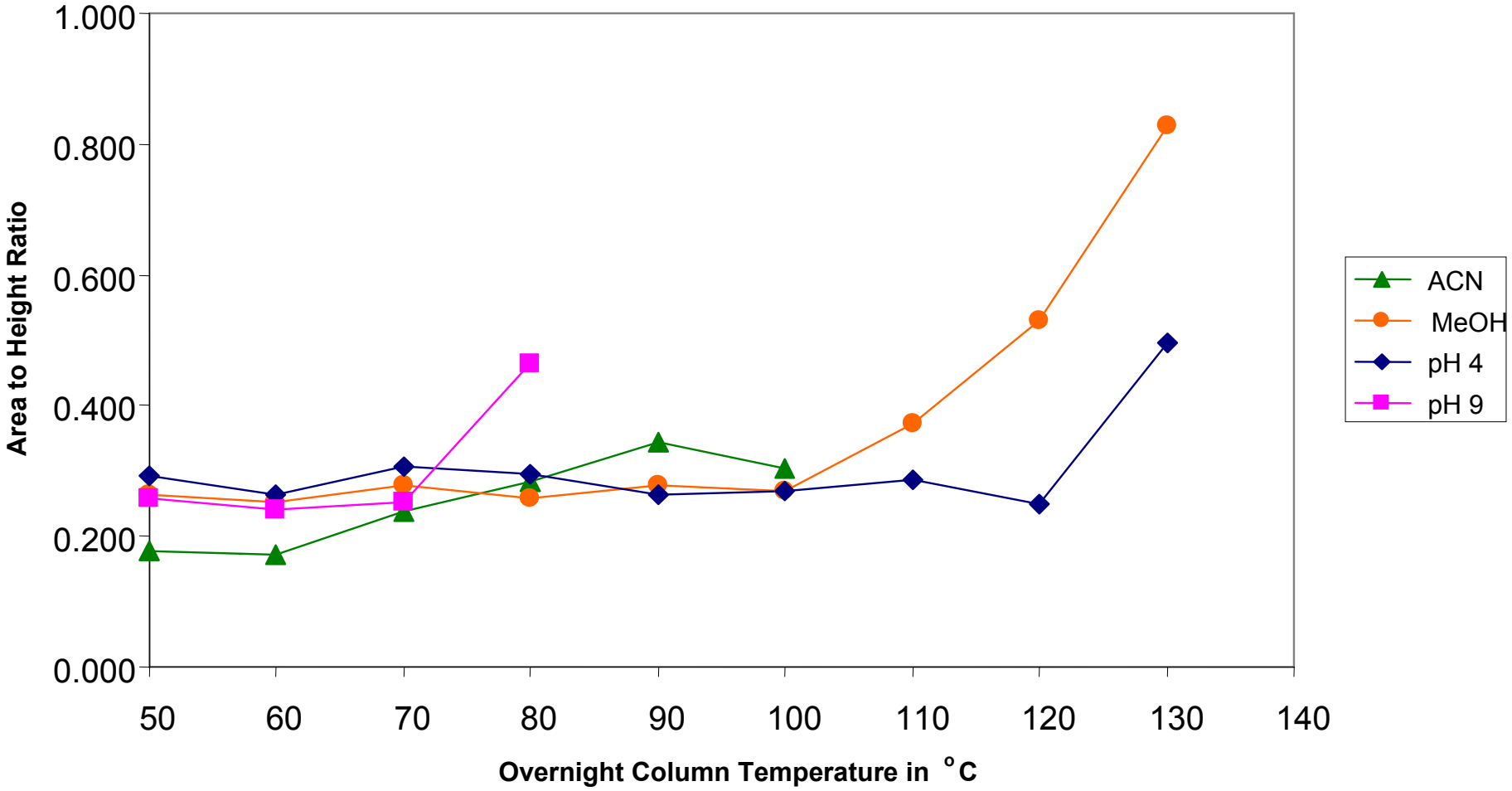


Figure 6
Effect of Temperature on Area/Height Ratio for Benzene

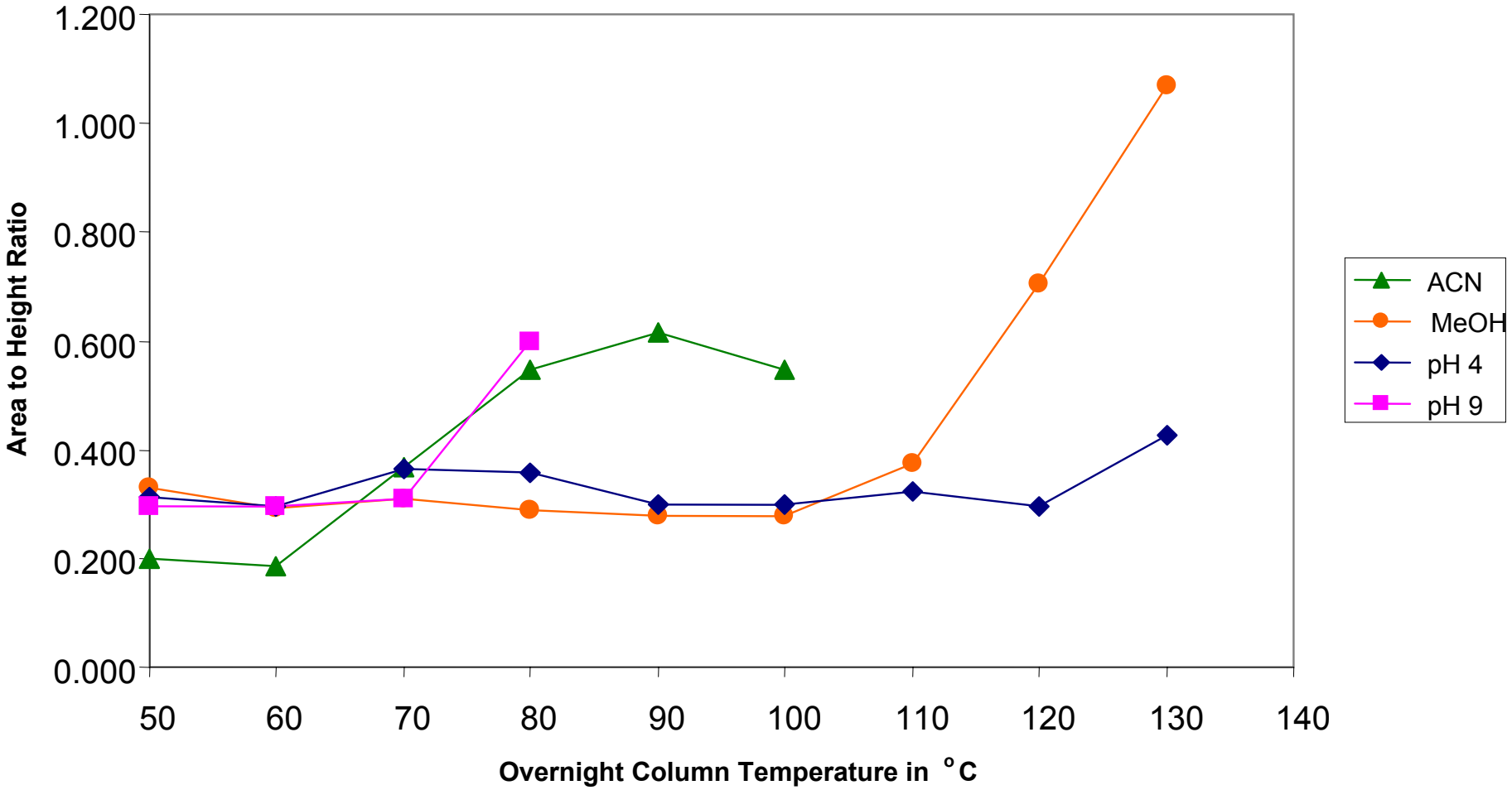


Figure 7
Effect of Temperature on Retention Time for Acetophenone

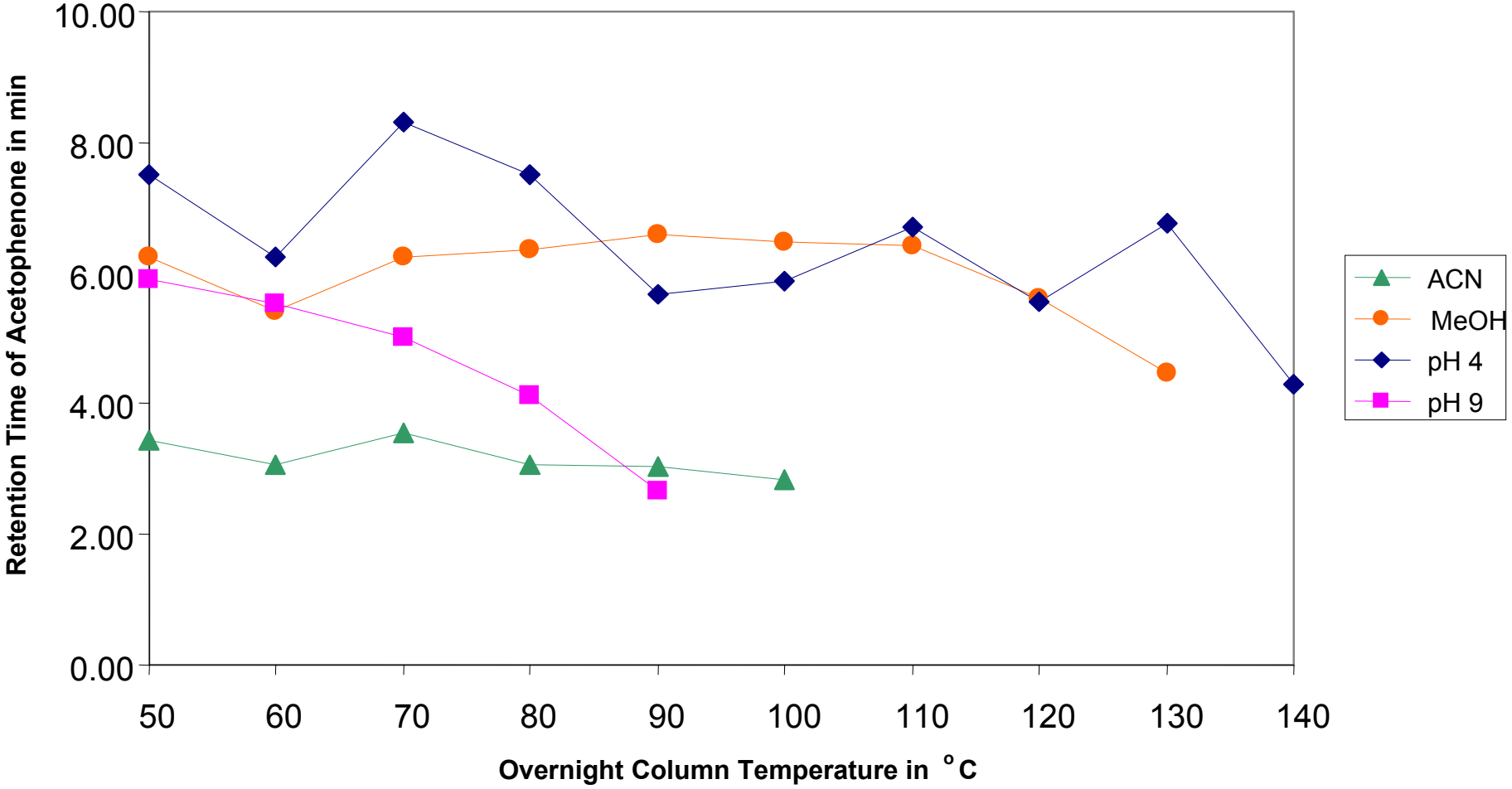


Figure 8
Effect of Temperature on Retention Time for Benzene

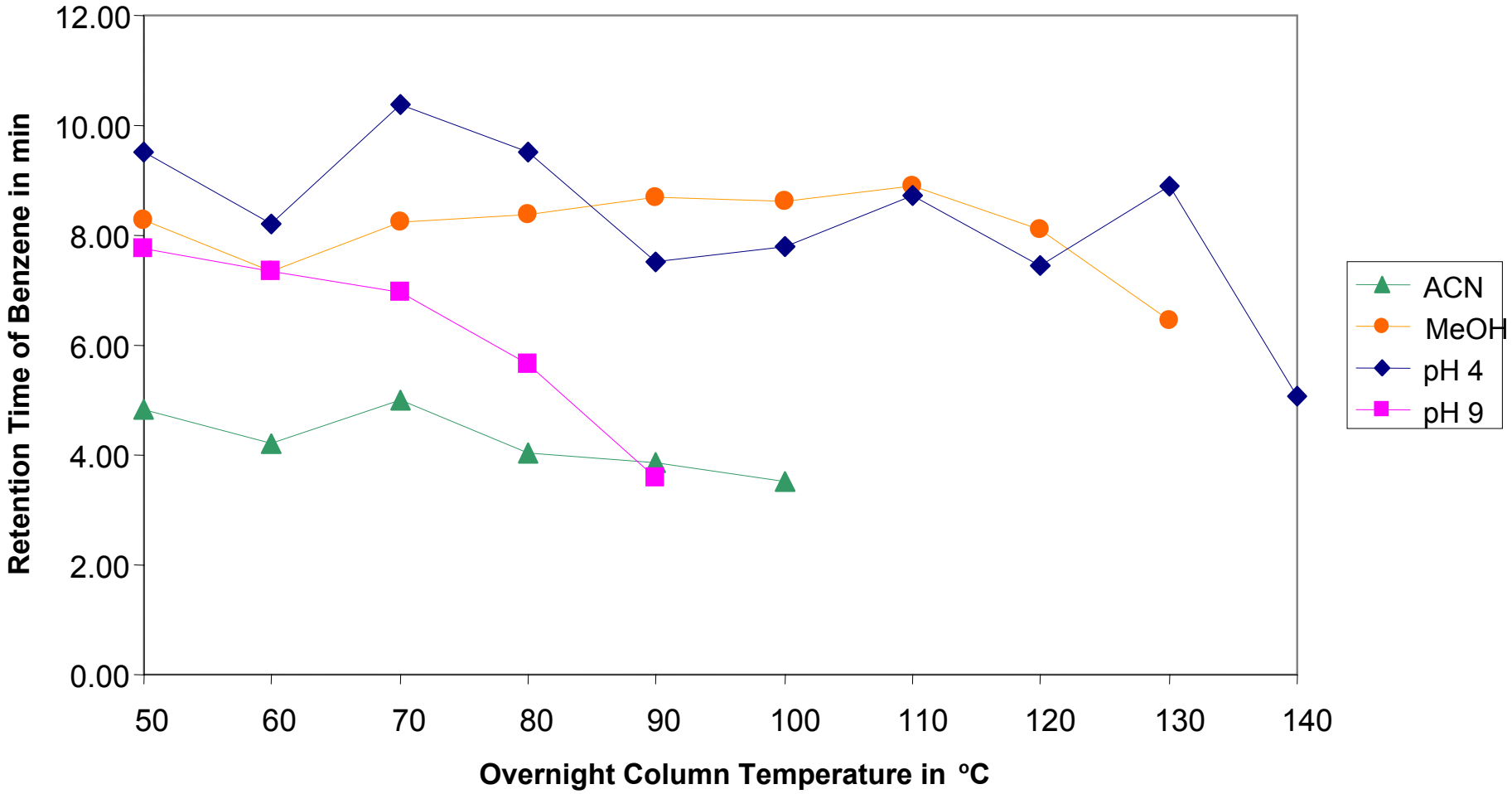


Figure 9
Acetonitrile Profile at 50, 70 and 100 °C

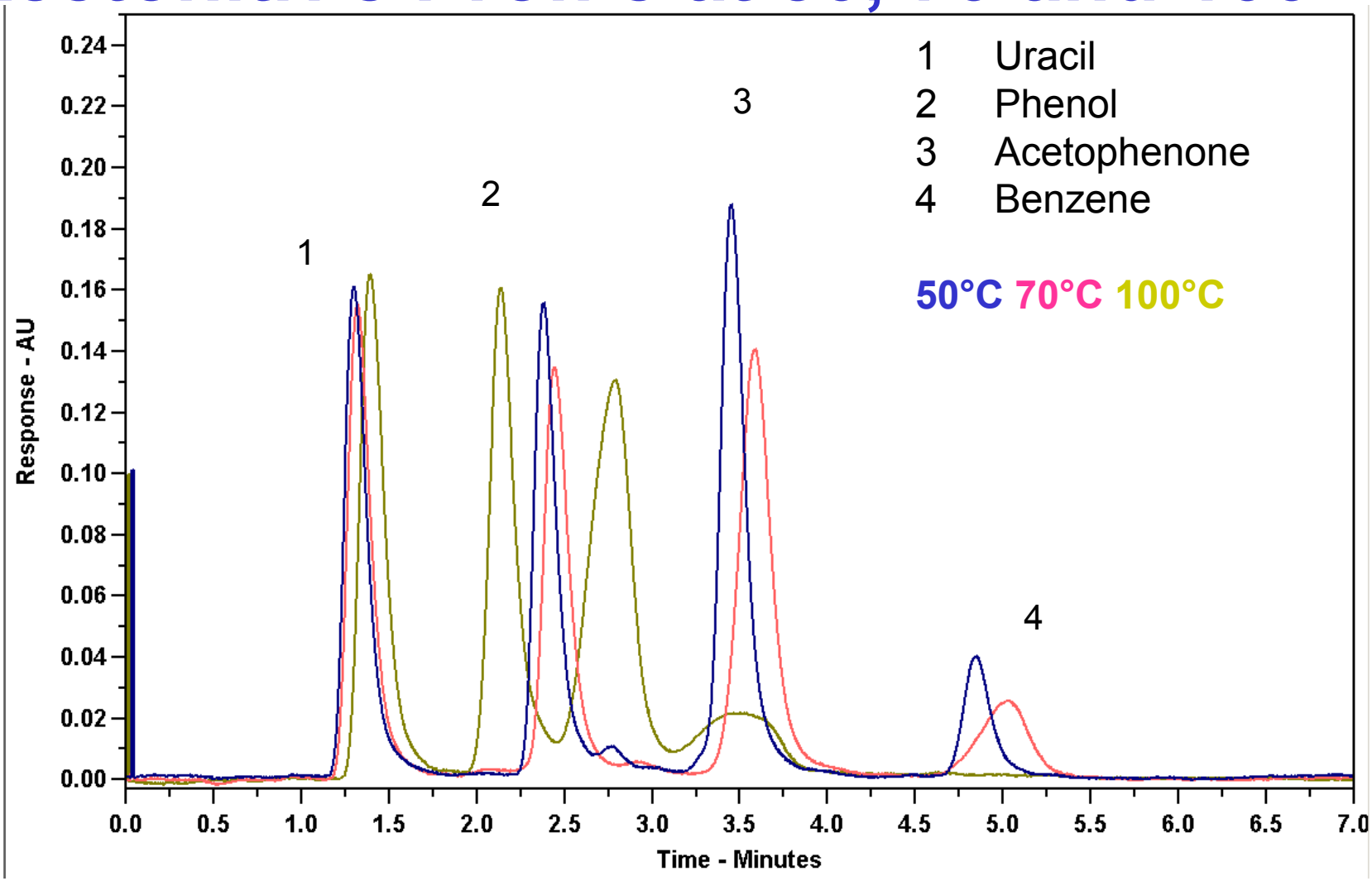


Figure 10
Methanol Profile at 50, 100 and 130 °C

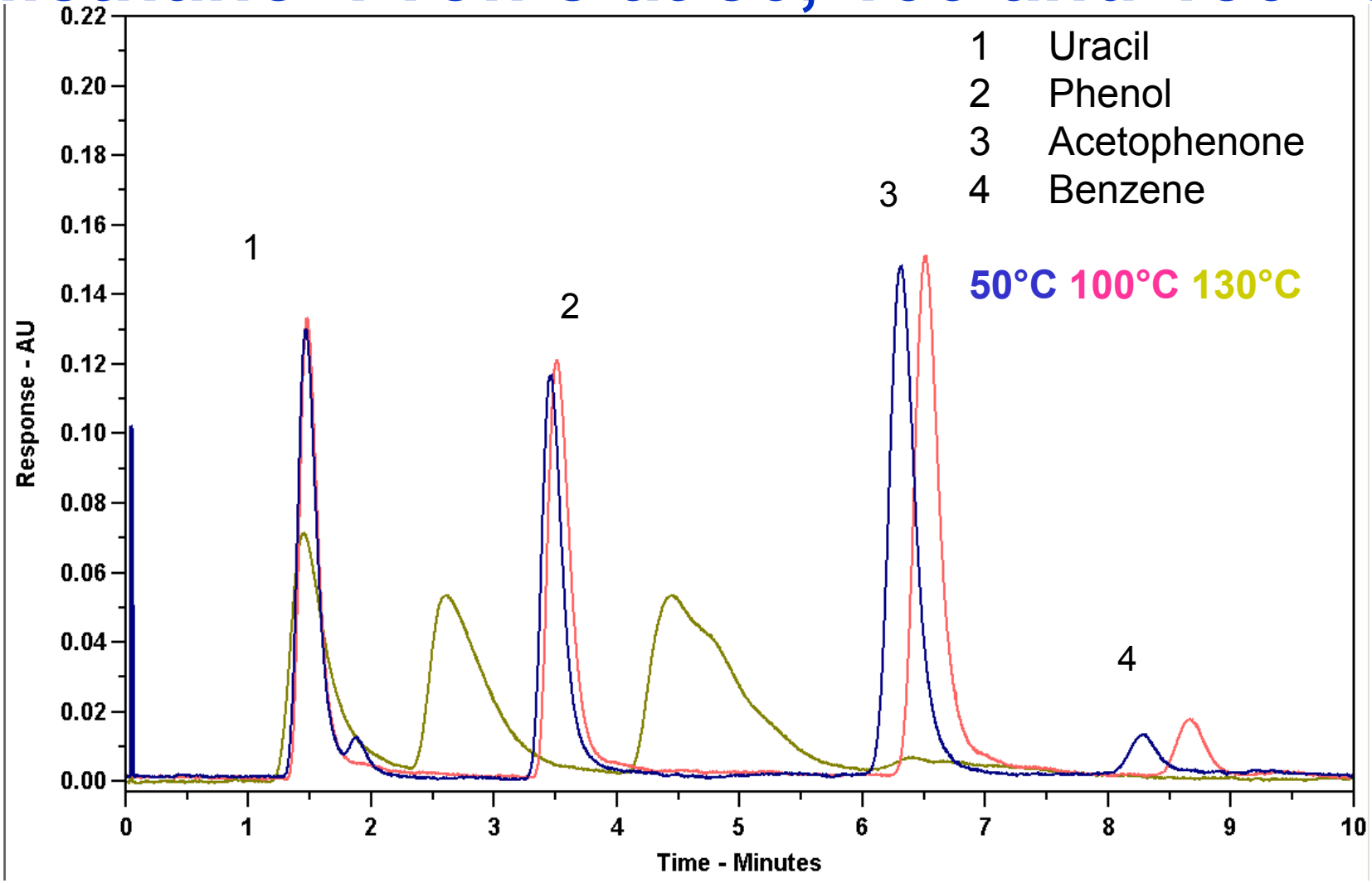


Figure 11
pH 4.0 Profile at 50, 120 and 140 °C

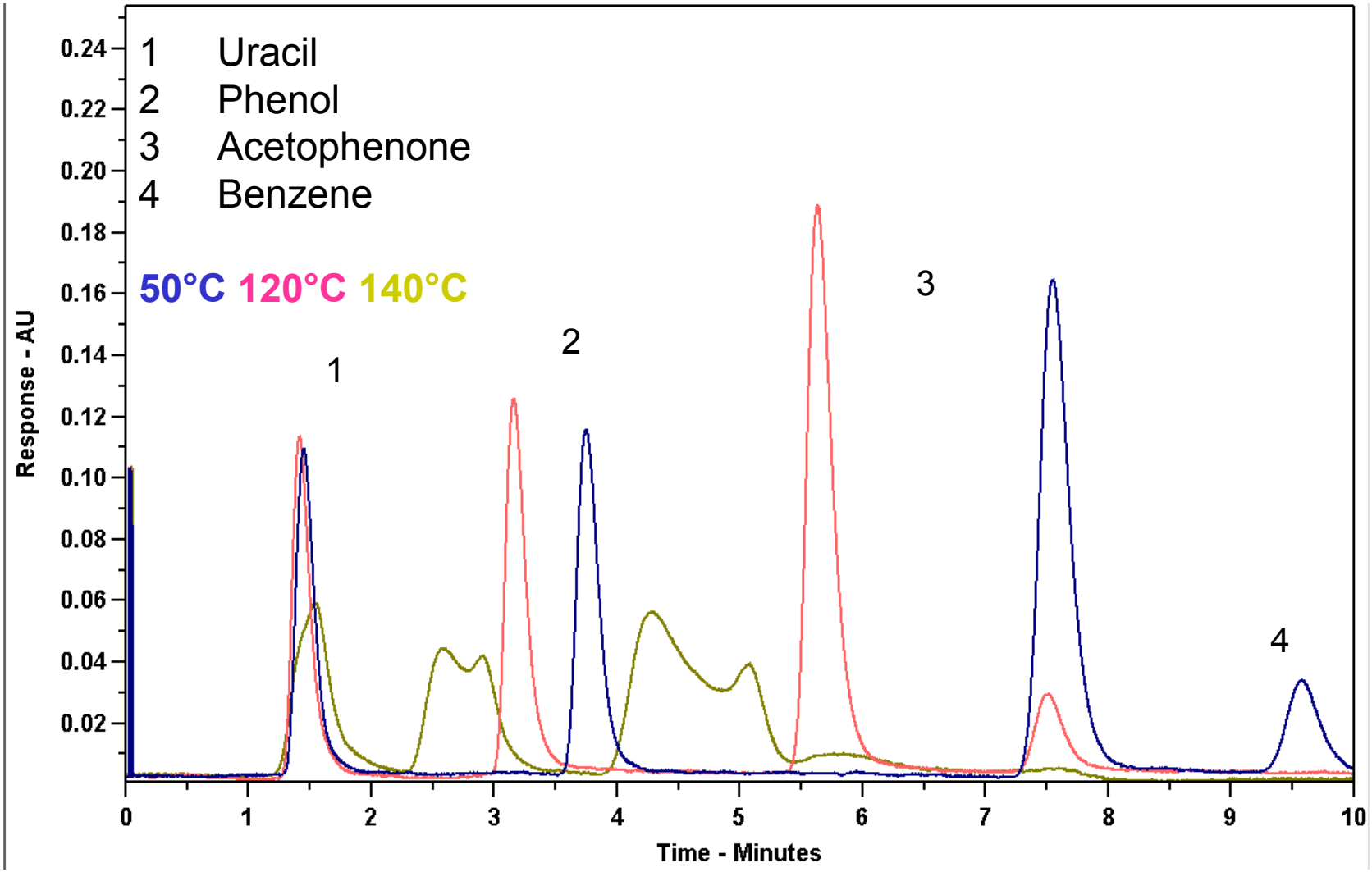
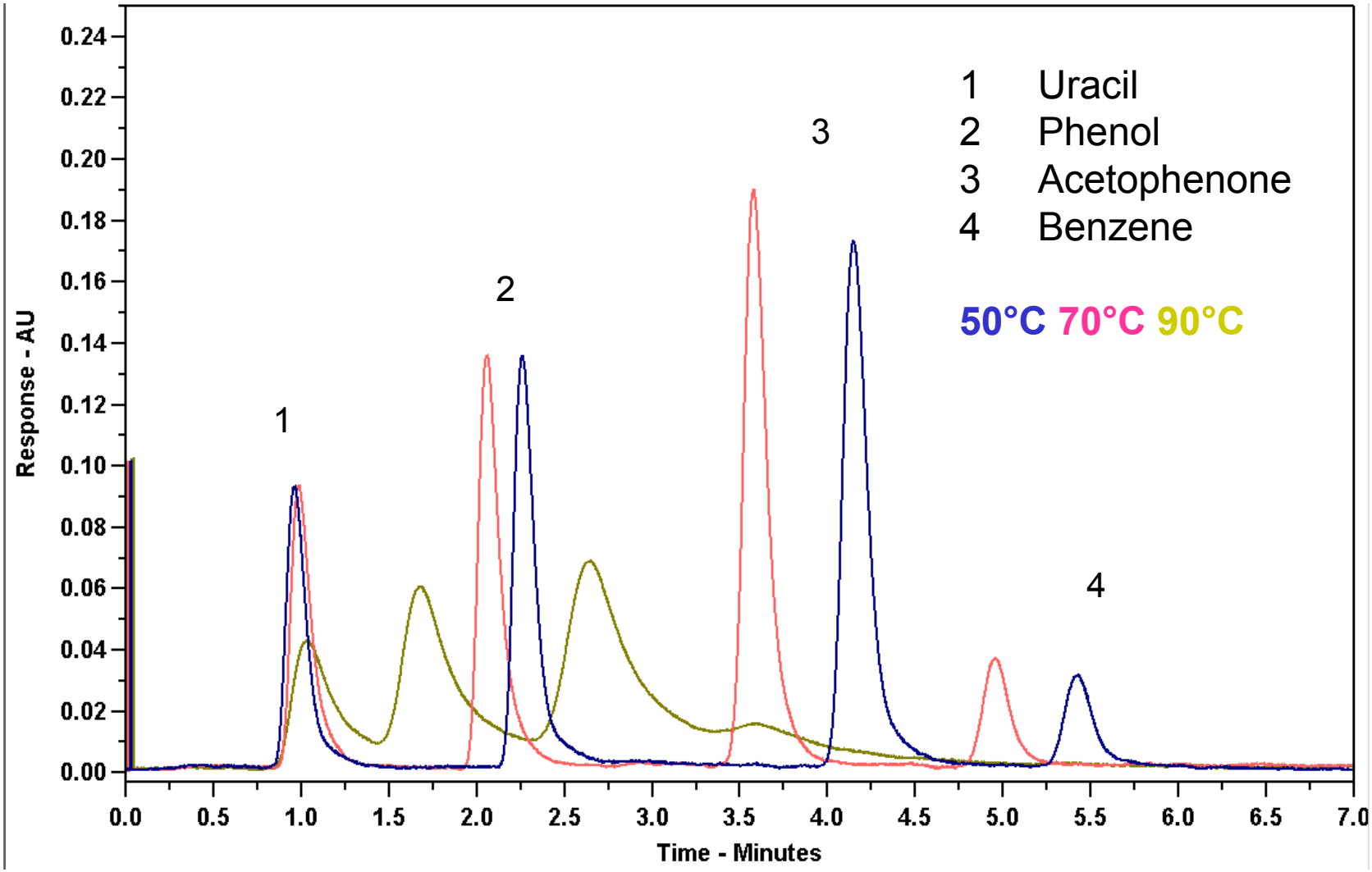


Figure 12
pH 9.0 Profile at 50, 70 and 90 °C



Conclusions

- Column operation at higher temperatures resulted in faster elution times and reduced back pressures for all mobile phases tested.
- Methanol was less aggressive at high temperatures than acetonitrile and extended the usable temperature of the column from 70 to 100^{o}C. An improvement of column characteristics between 70-100^{o}C was observed when a methanol/water mixture was used.
- At pH 4, the column maintained 80% of its efficiency up 120^{o}C. This means that the column tolerated an increase of 20^{o}C over the water/methanol conditions.
- At pH 9, the column quickly deteriorated and behaved very much like acetonitrile/water mixture.
- While maintaining column performance well above base-line resolution, a trend to shorter retention times after high temperature treatment was observed. The causes are not yet understood and are currently under investigation.

Future Work

- Long term stability studies (5,000-10,000 column volumes)
- Lower pH studies (pH 2 and 3)

Acknowledgements

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