COLUMN BLEED IN TEMPERATURE PROGRAMMED HIGH TEMPERATURE LIQUID CHROMATOGTRAPHY

INTRODUCTION

Although a number of column heaters have been available for several years that are capable of operation at temperatures up to 100°C, traditional silica based column packings were only stable to about 60°C when used with aprotic solvents. Column bleed due to degradation of the stationary phase or leaching from the packing material at elevated temperatures is a concern. It was not until the creation of zirconia based stationary phases that high temperature liquid chromatography was seriously investigated as a routine laboratory technique. Although these zirconia stationary phases are most often the only ones that come to mind for high temperature use, there are other commercially available columns that can be used at temperatures up to 200°C. This work involved evaluating a number of different stationary phases under temperature programmed conditions to evaluate the amount of column bleed observed with increasing temperature. Six columns were chosen for the temperature programmed high temperature liquid chromatography evaluation. The ZirChrom™ PBD, CARB, and DiamondBond columns from ZirChrom™ Separations, a Selerity Technologies Blaze™ C_8 polydentate silica column, a Hamilton PRP-1® polymeric column, and a Thermo Electron Hypercarb® column consisting of a graphitic carbon stationary phase, were evaluated to assess tested and the level of column bleed was measured.

Table 1. HPLC Columns Evaluated Using Temperature Programmed HTLC

Manufacturer	Column	Packing	Particle Size (micron)	Maximum Temperature
Thermo Electron	Hypercarb®	graphitic carbon pH 0-14	7	200°C
HamiltonCompany	PRP-1®	PSDVB polymer pH 0-14	5	150°C
SelerityTechnologies	Blaze™ C8	polydentate silica pH 2-8	3	100°C
ZirChrom™ Separations	PBD	bonded zirconia pH 0-14	3	150°C
ZirChrom™ Separations	Carb	graphitic carbonp H 0-14	3	200°C
ZirChrom™ Separations	DiamondBond	zirconia-carbon pH 0-14	3	200°C

EXPERIMENTAL

An HPLC system consisting of an Alltech vacuum degasser (Alltech Associates, Deerfield Illinois, USA), Milton Roy CM4000 pump (Thermo Electron, Waltham, Massachusetts, USA), Thermo Separations Spectrasystem UV2000 detector (Thermo Electron, Waltham, Massachusetts, USA), and Alcott 708 AL autosampler (Alcott Chromatography, Norcross, Georgia, USA) combined with a Selerity Series 8000 Oven with temperature programming and independent mobile phase preheating (Selerity Technologies Inc., Salt Lake City, Utah, USA) was used for the evaluation.

Column information is summarized in Table 1. The Blaze™ C_8 column was manufactured by Selerity Technologies, Inc. (Salt Lake City, Utah, USA). The PRP-1® column (part number 79479, serial number 26) was provided by the Hamilton Company (Reno, Nevada, USA). The Hypercarb® column (part number 35007-104646, serial number 1123021A) was provided by Thermo Electron (Bellefonte, Pennsylvania, USA). The ZirChrom™ DiamondBond (part number DB01-1046, serial number OD052302H), CARB (part number ZR01-1046, serial number CARB061802O) , and PBD (ZR03-1046, serial number PBD012502V) columns were provided by ZirChrom™ Separations (Anoka, Minnesota, USA).

A blank temperature programmed run (no sample injected) starting at 40°C and ramping at 15°C/minute using a 50:50 acetonitrile:water mobile phase was performed with each column. The maximum temperature for the temperature program was chosen based on recommendations from the manufacturer for the Blaze™, Hypercarb® and ZirChrom™ columns. The maximum temperature for the Hamilton column was determined from a previous in-house study using isothermal conditions. Chromatograms were generated using a UV detector set at 254 and 220 nm with a range setting of 2 AUFS (absorbance units full scale).

Results and Discussion

Blank temperature programmed analyses for all six columns are shown in Figure 1. The Blaze™ C_8 and PRP-1® columns had an essentially flat baseline. The Hypercarb® column exhibited a slight rise in the baseline (approximately 0.01 AU) starting at about 150°C continuing to 200°C. The zirconia based columns exhibited excessive column bleed under temperature programmed HTLC conditions. The ZirChrom™ PBD column had a steep rise in the baseline with a maximum absorbance of almost 0.20 AU at 220 nm. The ZirChrom™ CARB column also had a steep rise in the baseline with a maximum absorbance of close to 0.45 AU at 220 nm. The ZirChrom™ DiamondBond column had the largest baseline rise with a maximum absorbance of almost 1.5 AU at 220 nm. This large baseline rise was not observed with the Blaze™ C_8, PRP-1® or Hypercarb® columns or when the columns were replaced with a stainless steel union in the instrument. This suggests that the observed baseline rise was caused by some material leaching from the packing of the zirconia columns during a temperature programmed run. This material "bleeding" from the column absorbs in the UV at 254 and 220 nm. To verify this, two or three (depending on availability in our laboratory) of each of the ZirChrom™ columns were tested and the column bleed was observed with each column. A methanol:water mobile phase was also used to conduct temperature programmed runs using the zirconia columns. There was a slight reduction in the baseline rise when compared to the acetonitrile:water chromatograms, but it was still significant. The column bleed would be significant enough to interfere with analyte quantitation.

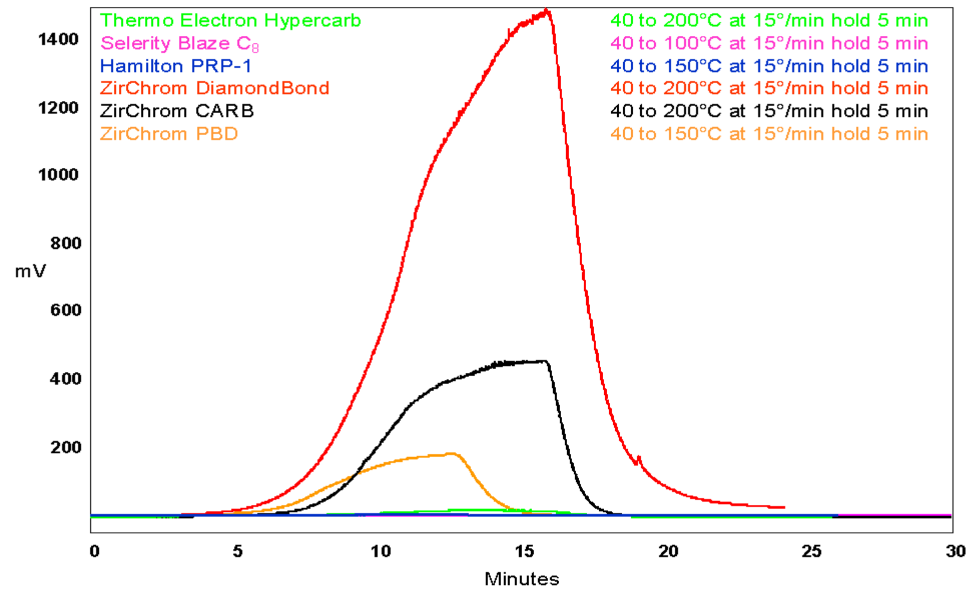

Figure 1. *Blank temperature programs for the six columns studied. The Blaze™ C_8 and the PRP-1® showed essentially no column bleed. The Hypercarb® column showed a slight rise in the baseline. The three zirconia stationary phases had excessive column bleed.*

Conclusions

The Selerity Blaze™ C_8 column can be used for temperature programming up to 100°C with no evidence of column bleed. The Hamilton PRP-1® column can be used for temperature programming up to 150°C with no evidence of column bleed. The Thermo-Electron Hypercarb® column can be used for temperature programming up to 200°C with no evidence of column bleed. The Zirchrom™ PBD, CARB, and Diamondbond columns show significant column bleed and are not good candidates for temperature programming.

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References

J. Chrom. A., 1030, 252-260 (2004)