

1 Introduction

One major problem of the routine use of high temperature liquid chromatography is the choice of suitable stationary phases which can withstand high temperatures over a long period. Generally, silica based stationary phases are considered less suitable for high temperature liquid chromatography since the silica may rapidly dissolve at high eluent temperatures. Nevertheless, column manufacturers have currently improved and modified the stability of these silica based stationary phases. Therefore, the aim of this investigation was to study the temperature stability of various commercially available reversed phase stationary phases.

2 Experimental

2.1 System set-up

A Shimadzu LC-2010C HPLC system (Duisburg, Germany) has been used to collect the chromatographic data. To maintain the temperature of the mobile and stationary phase a Metalox Model 200-C high temperature column oven (System, Inc., New Brighton, USA) was used. All investigated columns were 150 x 4.6 mm, 5 µm except the ZirChrom PBD column which was 150 x 4.6 mm, 3 µm.

2.2 Test procedure

For the first five heating phases a mobile phase consisting of 90/10 (v/v) deionized water and methanol was used. For the next five heating cycles a mobile phase of 90/10 (v/v) phosphate buffer adjusted to pH 2.2 and methanol was used. For the last five heating cycles a mobile phase of 90/10 (v/v) phosphate buffer adjusted to pH 12.0 and methanol was used. The columns were heated up to 150 °C at a flow rate of 1.0 mL/min for a period of five hours. Subsequently, the columns were cooled down to 25 °C. After re-equilibration with a mobile phase consisting of water and methanol, the test mixture as proposed by Neue was analyzed to calculate the retention factors, asymmetry (tailing factor in 5 % peak height), resolution and theoretical plate numbers. All chromatograms depicted in this presentation were collected using identical chromatographic conditions: mobile phase: 35/65 (v/v) phosphate buffer pH 7/methanol; detection: UV@254 nm.

3 Results

Figures 1 to 10 show the results of the column stress study.

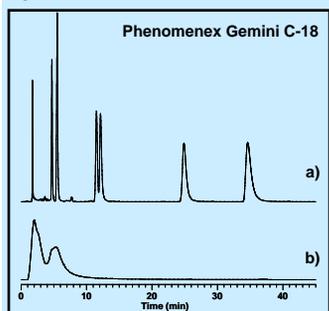


Figure 1: (a) test chromatogram obtained before the column was heated up to 150 °C; (b) test chromatogram obtained after the fifth heating cycle.

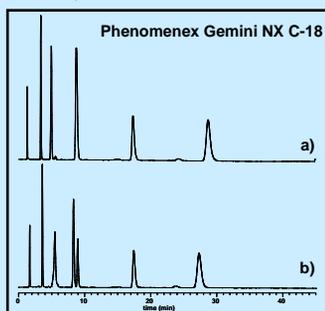


Figure 2: (a) test chromatogram obtained before the column was heated up to 150 °C; (b) test chromatogram obtained after the fifth heating cycle.

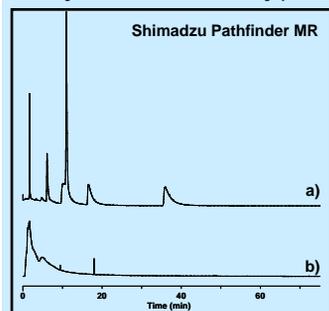


Figure 3: (a) test chromatogram obtained before the column was heated up to 150 °C; (b) test chromatogram obtained after the fifth heating cycle.

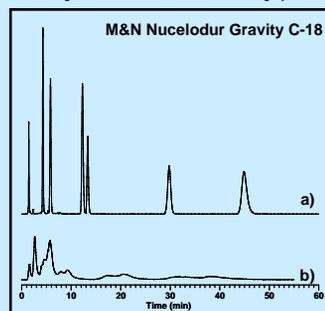


Figure 4: (a) test chromatogram obtained before the column was heated up to 150 °C; (b) test chromatogram obtained after the fifth heating cycle.

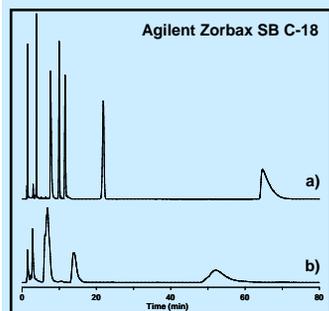


Figure 5: (a) test chromatogram obtained before the column was heated up to 150 °C; (b) test chromatogram obtained after the fifth heating cycle.

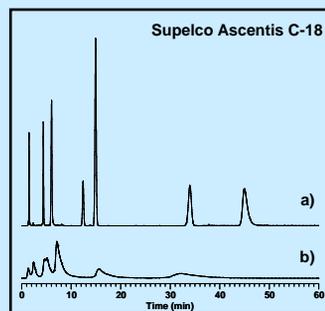


Figure 6: (a) test chromatogram obtained before the column was heated up to 150 °C; (b) test chromatogram obtained after the fifth heating cycle.

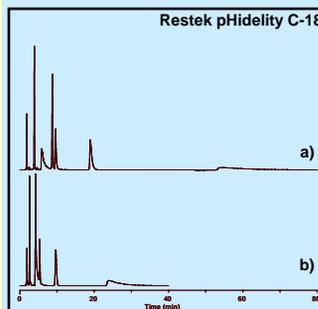


Figure 7: (a) test chromatogram obtained before the column was heated up to 150 °C; (b) test chromatogram obtained after the fifth heating cycle.

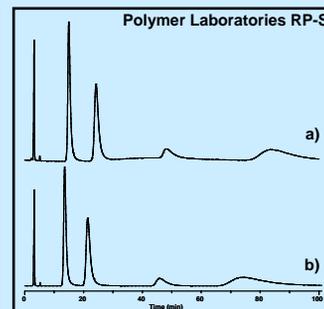


Figure 8: (a) test chromatogram obtained before the column was heated up to 150 °C; (b) test chromatogram obtained after the fifth heating cycle.

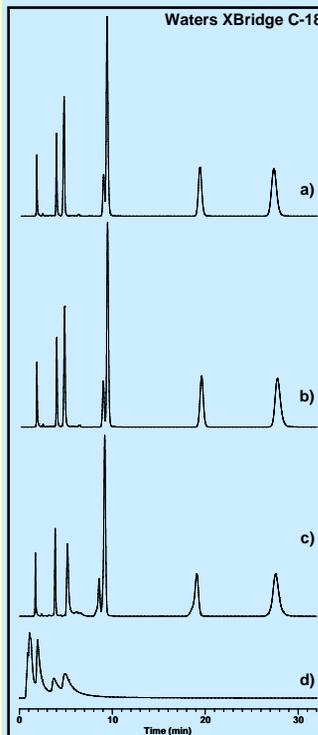


Figure 9: (a) test chromatogram obtained before the column was heated up to 150 °C; (b) test chromatogram obtained after the fifth heating cycle, (c) test chromatogram obtained after the tenth heating cycle, (d) test chromatogram obtained after the fifteenth heating cycle.

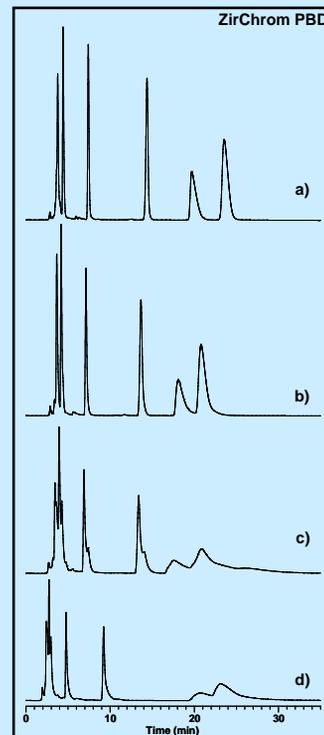


Figure 10: (a) test chromatogram obtained before the column was heated up to 150 °C; (b) test chromatogram obtained after the fifth heating cycle, (c) test chromatogram obtained after the tenth heating cycle, (d) test chromatogram obtained after the fifteenth heating cycle.

4 Discussion

The test procedure revealed that new silica based stationary phases have a much higher stability at high eluent temperatures than was anticipated. It could be shown that the XBridge column from Waters was virtually not affected by a mobile phase consisting of 90/10 water/methanol (v/v) at 150 °C for 25 hours (Figures 9 a and b). Even after adjusting the pH to 2.2 using phosphate buffer and purging the column for another 25 hours, the column performance was virtually unchanged except the fact that for some peaks a fronting became visible (Figures 9 a and c). Also, the pHidility column from Restek proved very stable under neutral elution conditions. However, the acidic elution phase caused a severe degradation of the overall performance of this column (data not shown in this presentation). In contrast to this, the polybutadiene coated zirconium stationary phase has an unparalleled stability at high temperatures and extreme pH. Unfortunately, the performance of this column was also affected since the efficiency and peak asymmetry worsened, although there was only a slight loss in retention compared to the silica based columns even after the column was flushed with phosphate buffer at a pH of 12 for 25 hours. Also, the Gemini NX column proved to be much more stable than the Gemini phase (Figures 1 and 2).

The results very clearly point out that manufacturers are continuously improving the stability and ruggedness of silica-based reversed phase columns, which is a prerequisite for the routine application of high-temperature liquid chromatography in industry.

5 Acknowledgements

Financial support by the Federal Ministry of Economics and Technology (BMWi) initialized by the German Federation of Industrial Cooperative Associations „Otto von Guericke“ e. V. (AiF-FV 14514 N) and member organization VEU is gratefully acknowledged.