# High Performance Biomolecule Separations Using Zirconia-based Ion Exchange Phases

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**Silica and polymer-based phases used for the separation of biomolecules have traditionally suffered from such limitations as substrate instability in alkali media and shrinking/swelling of the particles upon changes in mobile phase composition. The extraordinary chemical and physical stability of zirconia-based phases allows much greater flexibility in method development for biomolecules. This note shows a separation of a polynucleotide hydrolysate using a ZirChrom®-SAX.**

### Introduction

The long-term reliability of an HPLC method depends greatly on the ruggedness of the stationary phase. In bonded silicas, an Si-O-Si bond is used to attach functional groups to the silica surface. It is well known that this bond is subject to chemical attack, especially at low pH. The silica itself dissolves readily in aqueous mobile phases at high pH. Even sophisticated silica bonding technologies have not solved this problem[1]. The basic instability of bonded silicas causes retention drift, short column life, and frequent replacement of the column and re-qualification of the HPLC system. This is expensive both in terms of actual expenditures and in terms of lost productivity.

### Zirconia-based Phases for Ion Exchange Chromatography

Porous zirconia coated with polyethyleneimine (PEI) has been shown to be very useful for the separation of nucleosides, nucleotides, oligonucleotides, oligodeoxynucleotides and proteins[2]. The PEI coating can be crosslinked with a hydrophobic crosslinker and subsequently quaternarized to produce the stationary phase known as ZirChrom®-SAX which exhibits a mixed-mode characteristic of both reversed-phase and ion-exchange retention mechanisms. Because of the mixed-mode characteristic of the phase, temperature can be used as an effective tool in method development to selectively moderate the contributions of the ion-exchange and reversed-phase retention mechanisms to the overall retention.

### References

(1) J. J. Kirkland et. al., Anal. Chem. 61, 2-11 (1989).

(2) C. McNeff et al., Anal Chem. 67, 2350-2353 (1995).

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### Experimental

A hydrolyzed sample of Poly-G (oligonucleotide) was separated at elevated temperature using a ZirChrom®-SAX column. The separation conditions were as follows:

| | |
|---|---|
| Column: | 4.6 mm x 50 mm ZirChrom®-SAX |
| Mobile Phase: | Gradient elution from 5-95% B<br>A: 0.02 M potassium phosphate dibasic and 0.04 M NaCl, pH 8.5<br>B: 0.20 M potassium phosphate dibasic and 1.0 M NaCl @ pH 8.5 |
| Temperature: | 100 °C |
| Injection Vol.: | 25 µl |
| Flow rate: | 1.0 ml/min. |
| Detection: | UV at 254 nm |

### Results

The chromatogram below illustrates the capability of a zirconia-based ion-exchange phase to resolve nucleotide oligomers in a hydrolysate of poly-G. The separation is performed in a phosphate buffer at 100 °C, further demonstrating the extraordinary chemical stability of the stationary phase.

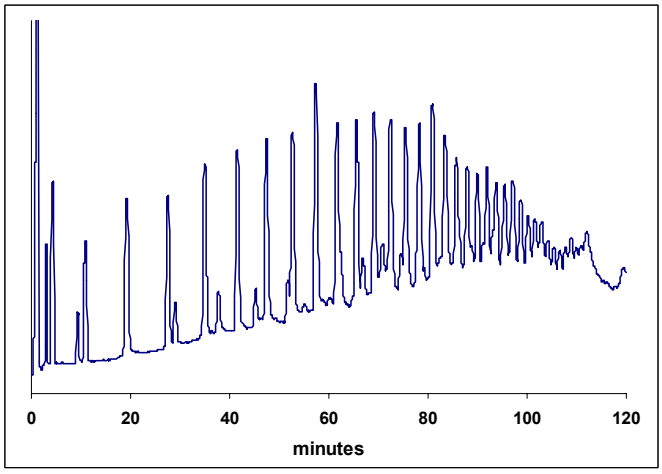


**Figure 1:** Separation Poly-G Hydrolysate

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