Purification of a 13-mer DNA Phosphorothioated Crude Deprotected Oligonucleotide by Strong Anion Exchange Chromatography Using TSKgel DNA- NPR

TSKgel
APPLICATION NOTE

Introduction

The TSKgel DNA-NPR column provides excellent chromatography and superior durability against the harsh HPLC method conditions required for oligonucleotide separations.

Due to the unique conditions involved with oligonucleotide separations including elevated pH and aggressive temperatures, adequate separations of the product from the N -1 peak and other impurities can be challenging. Additionally, column degradation as a function of the harsh conditions is common. Therefore, careful consideration of the composition and durability of the stationary phase is required. Recently, a customer focused on the synthesis of oligonucleotides, evaluated the TSKgel DNA-NPR column for use as an analytical tool in the development and quality control tests of a crude deprotected 13-mer oligonucleotide.

Experimental Conditions

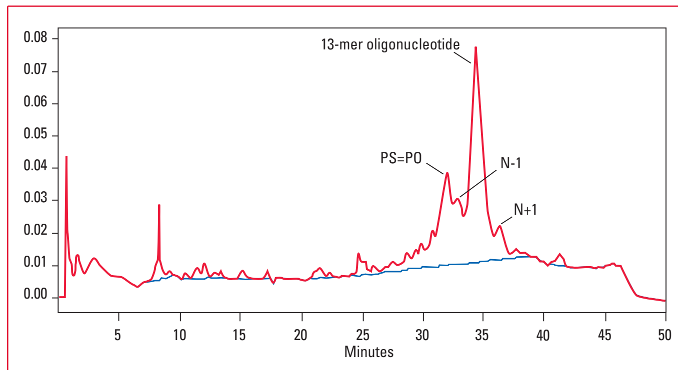
The TSKgel DNA-NPR column is packed with 2.5mm nonporous polymethacrylate beads in a 4.6mm ID x 7.5cm stainless steel housing. The buffer system is delive red at 1.0mL/min and consists of Eluent A (10mM sodium bromide, 20mM NaOH, pH 12, 1% diethylamine) and Eluent B (1 M sodium bromide, 20mM NaOH, pH 12, 1% diethylamine). Step gradients are used to administer Eluent B, starting at 20% from 3.5 min to 12 min and 55% from 12 min to 45 min. The column temperature and sample chamber temperatures are operated at 60 °C and 40 °C respectively.

The method conditions are designed to optimize resolution of all impurity peaks and inhibit any aggregation, secondary structure formation and PS=PO conversion. Specifically, sodium bromide acts as the eluting agent and diethylamine provides the buffering capacity while contributing mild chaotropic effects. The step gradient is designed to remove all the protecting groups from the column before elution the impurity analogs.

Results

Figure 1 contains the chromatographic trace of the crude deprotected 13-mer oligonucleotide. The early eluting peaks from 0–5 min exhibit a lambda max range of 220–230 nm, indicating the presence of protecting groups used in the synthesis. The N-1 peak as confirmed by mass spectrometry elutes just before the main substance peak. The PS=PO peak elutes before the N-1. Structurally, the N-1 analog is completely thioated but is missing one nucleotide. As a result, the N-1 compound is more thioated and hydrophobic than the PS=PO analog. The backside peak is an N+1 impurity verified by mass spectrometry.

Figure 1. Chromatographic trace of a crude deprotected 13-mer olignucleotide.



Although the customers current column, widely considered an industry standard for oligonucleotide separations, provided similar chromatography; the performance declines dramatically after approximately 5 injections. Subsequent work shows the TSKgel DNA NPR column has continued to provide consistent results beyond 50 injections.

Conclusion

The method exhibits good separation of the protecting groups and impurity analogs from the 13-mer oligonucleotide product peak. The TSKgel DNA-NPR column provides superior durability and column lifetime for this application.

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