Results and Discussion

The results obtained from Agilent 1290 UHPLC system running the ion exchange method are shown in Figure 2 along with the results obtained from customer’s conventional LC. The results show that a significant improvement in resolution is obtained compared with the results obtained from conventional LC. Many impurity peaks from other oligomers are completely resolved. This demonstrates that the high precision of 1290 Infinity pump and the low system delay volume contribute to the huge improvement.

In Figure 4, we show the results of the fraction analysis obtained using a long sub-2 um particle size reverse phase column on the 1290 UHPLC system. The intent of the work is to investigate the possibility of using the same mobile phases and similar methods as those used in semi-preparative purification to run the quality control analysis.

Introduction

Ion exchange and ion pairing reverse phase chromatographic techniques are the main HPLC methods used in both analyses and purifications of wide ranges of oligonucleotides today. Both techniques have shown advantages and disadvantages depending on the required resolution and the sample loading for preparative separations/purifications (1). As the smaller particle size columns become widely available, the improvement in both resolution and mass transfer can be obtained with using new column technologies.

In addition, using UHPLC instrumentation is an important factor in delivering the high resolution separation with higher performance and higher power range (high pressure and flow) when running applications with these new columns.

In this presentation, we demonstrate the oligonucleotide quality analysis using ion exchange chromatography on both conventional HPLC instrument and on UHPLC instrument. The analyses were also done using sub-2 um particle size reverse phase columns with UHPLC instrument.

Experimental

Sample Preparation

A crude customer oligo sample was used in this work. The oligos of interest has a length of 24 base pairs. The sample was purified on Agilent 1200 semi-prep HPLC with an analytical scale fraction collector. The purifications were done using Micro-10 columns (Agilent). 7.5mm packed with 5μm Zorbax SB-Aq. The purity of the fractions was confirmed using a Waters 2695 HPLC system using a 3.9×300mm column with a Poroshell 120 EC-C18 stationary phase with a styrene-based monolithic material (Agilent). The flow rate is 1.25 ml/min.

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Mobile phase A contains 0.1 M NaCl.

Mobile phase B contains 1 M NaCl.

Sample injection at 0.5 μl.

Column temperature 52°C.

DAD acquisition run: 40 Hz

HPLC Instrumentation, Columns and Methods

Agilent 1290 UHPLC system was used to perform quality analysis of the collected fractions using ion exchange chromatography. The 1290 pump is configured to use 50 ml of solvent A and 50 ml of solvent B.

In addition, this further opens the possibility of running both purification and quality analysis using the same UHPLC system to take advantage of the improved resolution demonstrated here.

Conclusions

We demonstrated the benefit of using UHPLC instrumentation in oligonucleotide applications. The Agilent 1290 UHPLC system not only provides the power to run long small particle size (1.8 um) columns with higher flow rate to improve separation efficiency without increase in analysis time, it also delivers high quality performance for conventional ion exchange separation methods with significantly improved resolution.