Science with Passion

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Column screening for oligonucleotide analysis and quality control

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Sepapure® oliGO

SUMMARY

What properties does a column need to be suitable for oligonucleotide analysis? It needs to withstand certain conditions like elevated temperature and pH values. Therefore, stability is a crucial point for maintaining robustness and repeatability. The following data shows the results of a column screening for oligonucleotide analysis.

INTRODUCTION

The interest in oligonucleotides in various areas of research, but especially in medical research, has been enormous since the corona pandemic. A reliable and high-resolution analytical method is the basis for any application of the variant-rich oligonucleotide molecules. One of the most chosen methods for oligonucleotide analysis is ion pairing reversed phase (IP-RP) chromatography. IP-RP uses an ion-pairing reagent to mask the charge on the molecule, that would prevent retention on a classical RP column. The quantification of oligonucleotides can easily be done by UV detection because oligonucleotides show strong absorption at 260 nm. For comparison of the screened columns, the purity of an oligonucleotide was determined. The crude product, the final product and a purified strand from a DNA full thiolate 43mer was used.

In this work once more we collaborated with the BianoGMP GmbH. The company specializes in the production of high purity and quality oligonucleotides and has many years of experience in the development of therapeutic oligonucleotides with a focus on GMP services and oligonucleotide analytical methods.

Column screening for oligonucleotide analysis and quality control

SAMPLE PREPARATION

The data shown was provided from BianoGMP GmbH.

RESULTS

The oligonucleotide samples were measured under IP-RP conditions. The same method was applied to measure the samples on different columns. **Fig. 1** und **Fig. 2** show the comparison for the measurement of two different crude products after synthesis, cleavage and deprotection, before any purification steps have been performed.

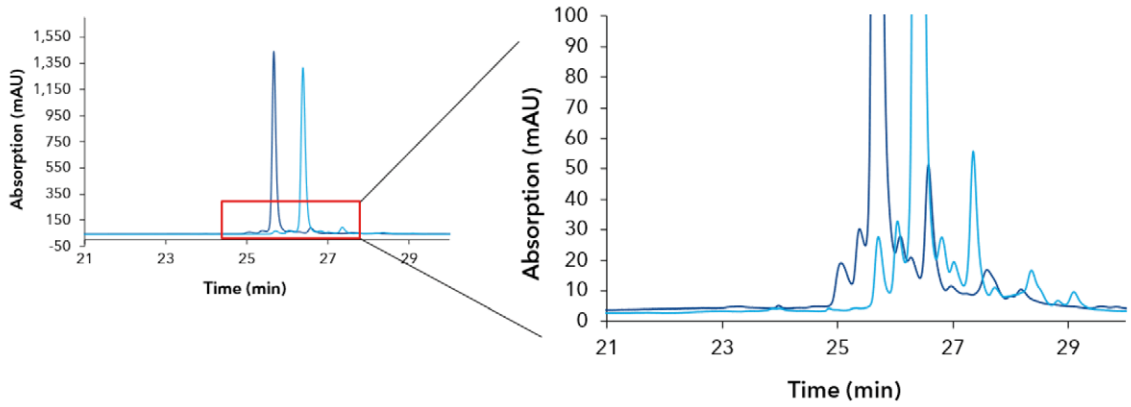

Fig. 1 Overlayed chromatogram of crude product of 43mer; dark blue – KNAUER Sepapure® oliGO column, light blue - Waters XBridge™ BEH oligonucleotide column.

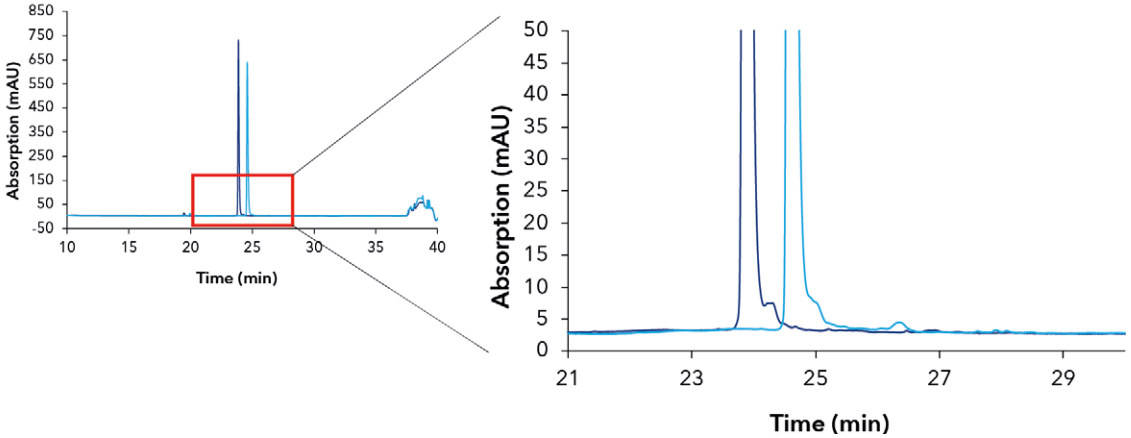

Fig. 2 Overlayed chromatogram of crude product of dT18 linker; dark blue – KNAUER Sepapure oliGO column, light blue - Waters XBridge™ BEH oligonucleotide column.

The columns show a similar behavior. Referring to the shoulder on the right of the peak, the KNAUER column showed a slightly better resolution. The shift in retention time can be explained with the different chemical properties of the stationary phases as well as slight differences in pore- and particle size **(Fig. 3)**.

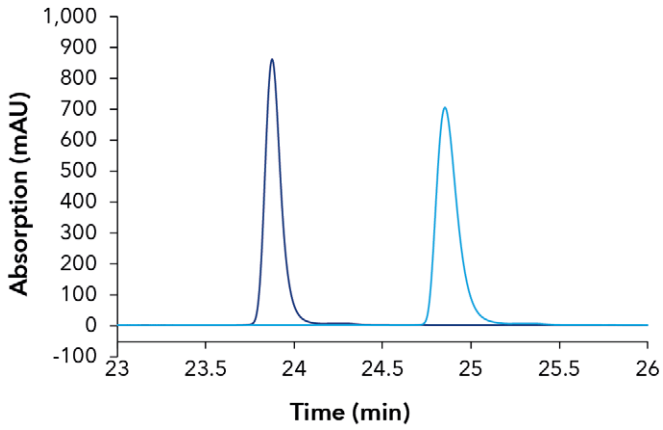

Fig. 3 Overlayed chromatogram of purified dT18 linker; dark blue – KNAUER Sepapure oliGO column, light blue – Waters XBridge™ column.

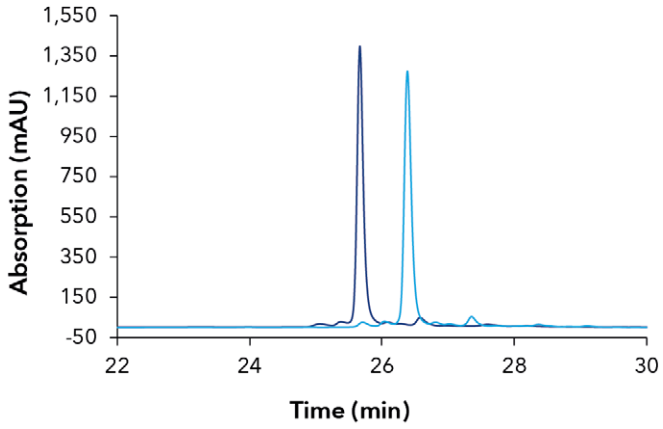

Fig. 4 Overlayed chromatogram of final product of 43mer (DNA full thiolate); dark blue – KNAUER Sepapure oliGO column, light blue – Waters XBridge™ BEH oligonucleotide column.

During all measurements the screened columns showed very similar behavior regarding resolution, peak symmetry and peak areas **(Fig. 4)**. The achieved purities were comparable to former results determined from BianoGMP.

CONCLUSION

The measurements show nearly no differences between the columns. The KNAUER Sepapure oliGO column is a good alternative to the commonly used competitor column for oligonucleotide analysis.

MATERIAL AND METHODS

Tab. 1 Instruments

Instrument	Description	Article No.
Column	KNAUER Sepapure oliGO, 50 x 4.6 mm ID	05EF18NSPG
Column	Waters XBridge™ BEH oligonucleotide column	