Abstract

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Title of rigorosum thesis: The development of an HPLC method for monitoring the preparation of oligodeoxynucleotide probes

An artificially prepared oligodeoxynucleotide sequence with the attached label is referred to as a molecular probe. Molecular probes are mainly used to quantify multiplication in RT-PCR and identify mutations in PCR analyses. There are typically two different types of probes – mono-labeled molecular probes and double-labeled molecular probes. The mono-labeled molecular probes are constructed from fluorophore-labeled oligodeoxynucleotide sequence or quencher-labeled oligodeoxynucleotide sequence. Whereas double-labeled molecular probes consist of an oligodeoxynucleotide sequence with a fluorophore attached at one end together with a quencher attached at the other end of the chain. In this work, we first paid attention to the separation of double-labeled oligodeoxynucleotides from mono-labeled oligodeoxynucleotides. Several types of stationary phases were tested and based on that the column Clarity® Oligo-RP™ was chosen to separate a mixture of labeled oligodeoxynucleotides. This column was selective for all tested standards. In the next part, we focused on monitoring the preparation of single-stranded oligodeoxynucleotides labeled with the quencher Q40 from the group of azaphtalocyanines, intercalating agent FK8 and fluorophore BDP16. Labeled molecule played a crucial role during the analysis. We managed to develop three specific HPLC methods. Due to these methods, we were able to evaluate the post-synthetic modification of oligodeoxynucleotide probes prepared by the click reaction and distinguish possible impurities in the sample.

Keywords: HPLC, azaphtalocyanines, acridines, bodipy, oligodeoxynucleotides, molecular probes.