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DOCTOR OF ENGINEERING SCIENCES

of **Quang-Dong Bui**

The public defense will take place on **Friday 25th September 2026 at 4pm** in room **I.2.01** (Building I, VUB Main Campus)

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# EXPLORATION OF CHROMATOGRAPHIC STRATEGIES FOR IMPURITY PROFILING AND DIASTEREOMER CHARACTERIZATION OF EMERGING OLIGONUCLEOTIDES

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# Abstract of the PhD research

Phosphorothioate oligonucleotides (PS ONs) represent an emerging class of therapeutic agents composed of single- or double-stranded DNA or RNA analogues. During the chemical synthesis of ONs, a variety of side products (impurities) can be generated, and the introduction of PS linkages leads to the formation of diastereomers due to the chirality at the phosphorus center. Reliable detection of these impurities and characterization of diastereomeric patterns therefore require highly selective and high-resolution analytical methods.

To improve impurity profiling workflows using ion-pair reversed-phase liquid chromatography–mass spectrometry (IP-RPLC-MS), critical parameters were systematically optimized, including the tubing configuration, the type of chromatographic column, and the composition of the mobile phase. In addition, the retention behaviour of PS ONs was investigated using anionexchange chromatography (AEX) to gain mechanistic insight into their chromatographic separation and the corresponding separation conditions. Building on these developments, a multidimensional analytical platform combining first-dimension AEX with second-dimension IP-RPLC-MS was established. This approach enabled the characterization of diastereomeric species at the duplex level in the first dimension, followed by sub-minute, post-denaturation strand-specific impurity profiling in the second dimension. A related strategy was also explored by integrating HILIC/RPLC with IPRPLC, in which the native double-stranded structure was preserved during the first-dimension separation and subsequently denatured during the analysis in the second dimension.

Another part of the work focused on improving the separation between the desired PS-ONs and its phosphodiester counterparts. Therefore, a chemical derivatization strategy was developed to selectively modify specific sulfur in the backbone with alkyl chains. This modification improved the chromatographic behavior of the molecules without the use of IP reagents, enabling the separation of the main product from its impurities and improve the separation between diastereomers.

Finally, ONs that are chemically linked to lipid groups were studied using several complementary analytical techniques. Each method provided distinct advantages for for elucidating the structure, purity, and stability of these molecules. Both single-stranded and double-stranded ON constructs were examined, and an efficient separation of closely related variants was achieved even for complex lipid-modified oligonucleotides.