1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title: New methods for extraction, separation and determination of therapeutic oligonucleotides in biological samples

1.1. Project goals

The therapy of genetic diseases is very difficult, nevertheless, attempts have been made for years to introduce new drugs to treat these diseases. The active substances of these drugs are synthetic, modified oligonucleotides, with structures analogous to short RNAs. Over the past seven years, there has been an apparent breakthrough in the treatment options, however on the other hand limited information is available concerning the relationship between the genetic therapy effectiveness and the metabolism of oligonucleotides. Such research is of great importance, but the proper analytical tools are needed to obtain all this information. Consequently, the main goals of the project are:

- synthesis and characterization of new materials through modification of silica and polymeric supports with various groups;
- application of synthesized materials as adsorbents for the extraction, as well as stationary phases for the selective separation of therapeutic oligonucleotides and their metabolites;
- utilization of developed methods for extraction, separation, identification, and quantification of various therapeutic oligonucleotides in serum and urine samples.

1.2. Outline

Antisense therapy involves introducing extracellular nucleic acids into the body for therapeutic purposes. Antisense oligonucleotides are an example of this type of compound, as they are synthetic, single-stranded fragments of ribonucleic acid (RNA). These oligonucleotides can bind to a specific fragment of DNA, RNA or protein. This makes it possible to control the synthesis of proteins involved in pathogenesis. In metabolic studies of therapeutic, antisense oligonucleotides and their biotransformation products, it is essential to use selective and sensitive analytical methods.

There are several commonly used techniques, such as isotope labeling, immunoassay methods, capillary electrophoresis and high-performance liquid chromatography (HPLC). However, these methods have low sensitivity and selectivity for metabolites.

There are many methods used in sample preparation for the determination of therapeutic oligonucleotides, namely liquid-liquid extraction (LLE) solid phase extraction (SPE), ultracentrifugation, and gel electrophoresis. Their significant drawbacks are low recovery and significant matrix effects.

Lack of sensitivity and a complex sample preparation process with low throughput remain barriers to successful studies of therapeutic oligonucleotides. Consequently, other analytical solutions should be employed to increase the sensitivity of quantification and extraction efficiency. The solution may be changes made both within the adsorbents used in sample preparation, changes in the stationary phase, and the techniques used to study antisense, therapeutic oligonucleotides.

For these reasons, the research conducted by PhD student will result in the development of new, selective, sensitive and precise methods for sample preparation and qualitative and quantitative determination of antisense oligonucleotides.

1.3. Work plan

- Synthesis of new silica and polymer-based adsorbents.
- Characteristics of novel materials (instrumental and chromatographic).
- Application of synthesized materials for dispersive solid phase extraction, solid phase extraction of therapeutic oligonucleotides.
- Utilization of prepared stationary phases for the separation of therapeutic oligonucleotides and their metabolites by LC-MS.
- Application of developed methods for the complex analysis of therapeutic oligonucleotides in serum and urine samples.
- Statistical evaluation of data.

1.4. Literature *(max. 10 listed, as a suggestion for a PhD candidate)*

Ł. Nuckowski, A. Kaczmarkiewicz, S. Studzińska, Journal of Chromatography B, 1090 (2018) 90–100.
A. Kaczmarkiewicz, Ł. Nuckowski, S. Studzińska, B. Buszewski, Critical Reviews in Analytical Chemistry, 49 (2019) 256-270.
S. Studzińska, Talanta, 176 (2018) 329-343.
A. Kilanowska, S. Studzińska, RSC Advances, 10 (2020) 34501-34516.
S. Studzińska, M. Mazurkiewicz-Bełdzińska, B. Buszewski, International Journal of Molecular Sciences, 23 (2022) 10166.

1.5. Required initial knowledge and skills of the PhD candidate

1. university Master's degree in chemistry;
2. strong motivation for scientific work and an open mind, willingness to conduct scientific research;
3. knowledge of analytical chemistry, knowledge about advanced instrumental techniques, knowledge in the field of liquid chromatography;
4. authorship of publications and/or conference reports is welcome;
5. experience in working with oligonucleotides, DNA or pyridazine derivatives and/or separation techniques is welcome;
6. knowledge of English necessary for independent scientific work (preparation of reports, scientific publications, participation in scientific internships, and conference presentations).

1.6. Expected development of the PhD candidate's knowledge and skills

Acquisition of the ability to synthesize and characterize chemically modified adsorbents for extraction and stationary phases for separation. The ability to independently develop extraction and chromatographic separation methods. The acquisition of the ability to write scientific papers.