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

Project title: ***Synthesis and application of adsorbents for the study of nusinersen and its metabolites during the treatment of spinal muscular atrophy***

1.1. Project goals

- synthesis and characterization of new materials of mixed-mode properties through modification of silica supports with various groups;
- application of synthesized materials as adsorbents for the extraction, purification, and enrichment of nusinersen and onasemnogene abeparvovac from serum and cerebrospinal fluid;
- use of synthesized materials as stationary phases for the selective separation of nusinersen and its metabolites;
- utilization of developed, optimized, and validated methods for extracting, identifying, and determining onasemnogene abeparvovac and nusinersen in the biological samples of healthy individuals and SMA patients**.**

1.2. Outline

Spinal muscular atrophy (SMA) is a rare and genetic neuromuscular disease expanding in the central nervous system, resulting in the loss of motor neurons and progressive muscle wasting. SMA was the most common genetic cause of mortality in infants, mainly due to respiratory failure. Until 2016, SMA was a fatal disease, and treatment included only treating the symptoms. A breakthrough in this field has come in the past seven years, as three drugs have been developed and begun to be administered.
Two drugs are part of an expanding trend of research into antisense and gene therapy, which is particularly promising for genetic diseases. In 2016, the U.S. Food and Drug Administration (FDA) approved Spinraza (nusinersen). The reimbursement for therapy with this drug began in Poland in 2019. Nusinersen is administered directly into cerebrospinal fluid because central nervous system tissue is the primary site of its action. In 2019, Zolgensma (onasemnogene abeparvovac), the first gene therapy drug for neuromuscular disease, was approved. In Poland, reimbursement for treatment started in 2022. Zolgensma is administrated just once by intravenous infusion (over 60 minutes).
Nusinersen is an antisense oligonucleotide, onasemnogene abeparvovec is deoxyribonucleic acid (DNA). Nusinersen is metabolized by hydrolysis mainly with exonucleases at the 3' end of the oligonucleotide. No studies link the effectiveness of therapy to the drug's metabolism. In the case of onasemnogene abeparvovec, the vector levels cross the blood-brain barrier and are distributed to the kidneys, lungs, liver, and heart. However, metabolism or excretion studies were not performed for onasemnogene abeparvovec treatment.
Given that the breakthrough in SMA therapy began only a few years ago and drugs have been in use for a relatively short time, extensive research is needed to accurately determine the fate of drugs in the body and link it to the type of SMA, or the effectiveness of treatment. So far, none of these research links the therapeutic effect, of the patient's condition to the metabolism of the active substances of these drugs. This leaves a huge space for research that needs to be done. However, such studies require appropriate tools and methods. Traditional methods used for the separation and determination of oligonucleotides (as nusinersen) and DNA (as onasemnogene abeparvovec) have some disadvantages: poor sensitivity (nanomolar levels), lengthy hybridization, low dynamic range, and low signal amplification. Thus, new strategies that focus on improving the specificity and sensitivity of oligonucleotides, DNA, and reducing the time are essential. The most promising tool appears to be

liquid chromatography coupled with mass spectrometry (LC-MS). The development should focus on synthesizing and applying stationary phases with greater selectivity to oligonucleotides and DNA.
The reliable metabolism study for each active substance of SMA drugs requires a selective and reproducible extraction method. The most commonly used methods for DNA, RNA, and antisense oligonucleotides is liquid-liquid extraction, enzymatic protein digestion, solid phase extraction and hybridization. These methods have many drawbacks. The selectivity needs to be increased in isolating metabolites from serum and cerebrospinal fluid, as it is a critical step and a limitation of currently used methods. Hence, the synthesis and application of new adsorbents for extracting SMA drugs from patient samples need systematic and extensive experiments to develop new and improved methods.

1.3. Work plan

- Synthesis of new adsorbents with different types of functional groups for the extraction of SMA drugs.
- Instrumental characterization of synthesized adsorbents.
- Application of newly synthesized materials for dSPE and SPE extraction of nusinersen and onasemnogene abeparvovec from standard solutions and biological samples.
- Application of new mixed-mode stationary phases for the separation of analogs of active substances of two SMA drugs (Spinraza and Zolgensma) and their metabolites.
- Research on the impact of different mobile phase compositions on peak symmetry and resolution of nusinersen and onasemnogene abeparvovec.
- Development of an LC-MS method for the quantification of SMA drug active substances.
- Analysis of onasemnogene abeparvovec, nusinersen, and their metabolites in patients' plasma and cerebrospinal fluid extracts using the developed chromatographic method with mass spectrometry detection.
- Statistical analysis 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. authorship of publications and/or conference reports;
4. an additional advantage would be if the candidate could demonstrate honors awarded for scientific research, scholarships and prizes, participation in scientific workshops and training, participation in research projects;
5. knowledge of analytical chemistry, knowledge about advanced instrumental techniques, knowledge in the field of liquid chromatography and extraction techniques;
6. experience in working with oligonucleotides, DNA or pyridazine derivatives and/or separation techniques is welcome;

7. willingness to prepare a valuable dissertation in a short period;
8. 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.