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

Project title: Synthesis and application of new materials to the analysis of risdiplam used for 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 risdiplam analog and its metabolites from serum samples;
- use of synthesized materials as stationary phases for the selective separation of risdiplam and its metabolites;
- utilization of developed, optimized, and validated methods for extraction, identification and determination of risdiplam and its metabolites 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.
In August 2020, the FDA approved Evrysdi (risdiplam) for treating SMA in adults and children two months of age or older. Risdiplam is a small molecule administered orally in a liquid form. The effectiveness of risdiplam is not clear yet. The effects of clinical trials were evaluated based on the ability to sit without support for five seconds, which was achieved by 29% of patients after 12 months. Risdiplam is a pyridazine derivative metabolized by several enzymes, including flavin-containing monooxygenases and cytochrome P450.
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 link the therapeutic effect, 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 analysis of pyridazine derivatives (which include risdiplam) is liquid chromatography. However, the polar nature of this group of compounds, the presence of nitrogen atoms in the structure often cause problems with the peaks' shape and their resolution (asymmetry, low selectivity), resulting in problems with the sensitivity of analysis. Thus, new strategies focusing on improving the specificity and sensitivity of pyridazine derivatives, as well as 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 pyridazine derivatives.
The reliable metabolism study for each active substance of SMA drugs requires a selective and reproducible extraction method. The research on risdiplam is so early that only one extraction method has been used so far: protein precipitation, which is simple but not always efficient or reproducible. The selectivity also 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, we conclude that 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. All of these attempts will be pioneer for all three classes of compounds used as SMA therapeutics.

1.3. Work plan

- Synthesis of new stationary phases with different types of functional groups bound to different supports for mixed-mode chromatography.
- Instrumental characterization of the obtained stationary phases (elemental analysis, IR, NMR).
- Application of newly synthesized materials for dSPE and SPE extraction of risdiplam from standard solutions and biological samples.
- Application of new mixed-mode stationary phases for separating risidiplam analog and its metabolites.
- Research on the impact of different mobile phase compositions on peak symmetry and resolution of risdiplam and its metabolites.
- Development of a LC-MS method for the quantification of Evrysdi active substance.
- LC-MS analysis of serum samples from SMA patients with the Evrysdi drug (risdiplam).
- 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. 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.