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Presenting a description of the scientific achievements

specified in art. 16 sec. 2 acts

Dr Anna Poliwoda

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1. **First name and last name:** **Anna POLIWODA**

2. **Diplomas and degrees:**

2001	Master of Chemistry, specialty agrobiochemistry, Opole University. Thesis title: *Ekstrakcja peptydów za pomocą membran ciekłych z użyciem Aliquatu 336 jako przenośnika.* [Extraction of peptides with liquid membranes using Aliquat 336 as a carrier.] Supervisor: dr inż. Piotr Wieczorek
2006	PhD of chemical science, Opole University Title of the PhD dissertation: *Wydzielanie i zatężanie peptydów za pomocą membran ciekłych.* [Isolation and preconcentration of peptides using liquid membranes.] Supervisor: dr hab. inż. Piotr Wieczorek

3. **Information on previous employment in scientific institutions:**

1st October, 2002 – 31st January, 2007	Assistant, Institute of Chemistry, Faculty of Mathematics, Physics and Chemistry, Opole University.
1st February, 2007 – 30th September, 2008	Assistant Professor, Institute of Chemistry, Faculty of Mathematics, Physics and Chemistry, Opole University.
1st October, 2008 – now	Assistant Professor, Faculty of Chemistry, Opole University.

4. **Indication of the achievement resulting from art. 16 sec. 2 of the Act of 14 March 2003 on academic degrees and title as well as on degree and title in the field of arts (Journal of Laws of 2016, item 882, as amended in Journal Laws of 2016, item 1311):**

A) Title of scientific achievement:

Separation and spectral methods in the analysis of biologically active compounds.

B) Publications included in the scientific achievement (* - corresponding author):

H-1 **A. Poliwoda***, M. Krzyżak, P. P. Wieczorek, **2010**,
Supported liquid membrane extraction with single hollow fiber for the analysis of fluoroquinolones from environmental surface water samples.
Journal of Chromatography A, 1217(22), 3590-3597.
$IF_{(2010)}$ = 4,194; $MNiSW_{(2010)}$ = 32

My contribution to the creation of this work consisted in creating the concept of work and planning the experimental part, performing experiments from the part of optimization extraction conditions, analysis of real samples and method validation procedure (results included in Fig. 3, 4, 5 and 6, and in Tables 2 and 3). Writing the whole manuscript, preparing tabular summaries, elaborating all received results and their interpreting, co-editing and revising the remarks of reviewers together with co-authors. I estimate my contribution to 75%.

H-2 **A. Poliwoda***, K. Zielińska, M. Halama, P. P. Wieczorek, **2014**,
Determination of muscimol and ibotenic acid in mushrooms of Amanitaceae by capillary electrophoresis.
Electrophoresis, 35(18):2593-2599.
$IF_{(2014)}$ = 3,028; $MNiSW_{(2014)}$ = 30

My contribution to the creation of this work consisted in creating the concept of work and planning the experimental part, performing experiments considering the development of effective electrophoretic separation conditions for compounds tested, as well as method validation procedure (results descried in Fig. 2, 3 and 4). Preparation of all received results and their interpretation, writing the manuscript, co-editing with co-authors the final version of manuscript, along the comments of the reviewers. I estimate my percentage share at 75%

.H-3 A. M. Chrzanowska, **A. Poliwoda***, P. P. Wieczorek, **2015**,
Characterization of particle morphology of biochanin A molecularly imprinted polymers and their properties as a potential sorbent for solid-phase extraction.
Materials Science and Engineering C, 49, 793-798.
$IF_{(2015)}$ = 3,420; $MNiSW_{(2015)}$ = 25

My contribution to the creation of this work consisted in creating the concept of work and planning the experimental part, performing experiments considering synthesis conditions of the the obtained molecularly imprinted polymers, surface characteristic of the obtained sorption materials by means of scanning electron microscopy, as well as optimization of solid phase extraction conditions, preparation the results obtained and their interpretation, writing the manuscript, co-editing with the co-authors the final version of manuscript, following the comments of the reviewers. I estimate my percentage share at 75%.

H-4 A. M. Chrzanowska, **A. Poliwoda***, P. P. Wieczorek, **2015**,
Surface molecularly imprinted silica for selective solid-phase extraction of biochanin A, daidzein and genistein from urine samples.
Journal of Chromatography A, 1392, 1-9.
$IF_{(2015)}$ = 3,926; $MNiSW_{(2015)}$ = 40

My contribution to the creation of this work consisted in creating the concept of work and planning experimental part, performing experiments considering the effective surface modification of the silica with molecular printing, testing the application potential of the obtained sorption materials in the extraction of analysed phytoestrogens from urine samples, analysis of the surface of the obtained polymers using a scanning electron microscope, as well

as elaborating all received results and their interpretation, writing a manuscript, co-editing with the co-authors the final version of manuscript, following the comments of the reviewers. I estimate my percentage share at 75%.

H-5 P. P. Wieczorek*, D. Witkowska, I. Jasicka-Misiak, **A. Poliwoda,** M. Oterman, K. Zielińska, **2015**,

Bioactive alkaloids of hallucinogenic mushrooms.

Studies in Natural Products Chemistry: Elsevier B.V.; 5, 133-168.

$IF_{(2015)}$ = 0,000; $MNiSW_{(2015)}$ = 5

Review work. My contribution to the creation of this work consisted in creating the concept of work and writing a part of the manuscript regarding the introduction, description of fungal alkaloids and analytical methods used in the analysis of these compounds in the fungal material and biological samples, co-editing of the final version of manuscript and revising the remarks of reviewers together with co-authors. I estimate my percentage share at 50%.

H-6 O. Zhuk, I. Jasicka-Misiak*, **A. Poliwoda**, A. Kazakova, V. V. Godovan, M. Halama, P. P. Wieczorek, **2015**,

Research on acute toxicity and the behavioral effects of methanolic extract from psilocybin mushrooms and psilocin in mice.

Toxins, 7(4), 1018-1029.

$IF_{(2015)}$ = 3,571, $MNiSW_{(2015)}$ = 30

My contribution to the creation of this work consisted in co-creation of the concept of work, development of the isolation and UHPLC separation method, and detection conditions using a mass spectrometry in the case of fungal extracts analysis, used then in pharmacological studies in mice, determination of the chemical composition of the analysed extracts, interpretation and discussion of the results from LC-MS analysis and from biological activity experiments. Writing the manuscript and co-editing and revising the remarks of reviewers together with co-authors. I estimate my percentage share at 45%.

H-7 S. Ncube, **A. Poliwoda,** H. Tutu, P. Wieczorek, L. Chimuka*, **2016**,

Multivariate optimization of the hollow fibre liquid phase microextraction of muscimol in human urine sample.

Journal Chromatography B, 1033-1034, 372-381. **$IF_{(2016)}$ = 2,603, $MNiSW_{(2016)}$ = 40**

My contribution to the creation of this work consisted in creating a concept of work, planning an experimental part, and conducting preliminary experiments on the scope of optimization of the extraction conditions of the hallucinogenic substances studied using the HF-LPME technique, interpretation of the obtained results, and writing a fragment of the manuscript regarding the content described in the introduction and in discussion of the results, co-editing with the co-authors the final version of manuscript, following the comments of the reviewers. I estimate my contribution by 50%.

H-8 **A. Poliwoda*,** M. Mościpan, P. P. Wieczorek, **2016**,
Application of molecular imprinted polymers for selective solid phase extraction of Bisphenol A.
Ecological Chemistry and Engineering S, 23(4), 651-664.
$IF_{(2016)}$ = 0,717, $MNiSW_{(2016)}$ = 15

My contribution to the creation of this work consisted in creating the concept of work and planning experiments and co-execution of experiments taking into account the synthesis of molecular-based sorption materials with molecular imprinting, surface analysis using a scanning microscope and examining the application potential of sorption materials for bisphenol A extraction from environmental samples, discussion of obtained results and their interpretation, writing the manuscript, co-editing with the co-authors the final version of manuscript, following the comments of the reviewers. I estimate my percentage share at 75%.

H-9 B. Żyszka-Haberecht, **A. Poliwoda,** J. Lipok*, **2018**,
Biocatalytic hydrogenation of the C=C bond in the enone unit of hydroxylated chalcones—process arising from cyanobacterial adaptations.
Applied Microbiology and Biotechnology, 102(16), 7097-7111.
$IF_{(2018)}$ = 3,340, $MNiSW_{(2018)}$ = 35

My contribution to the creation of this work consisted in creating a concept of work on developing a method for extraction and qualitative and quantitative analysis of hydroxychalcones biotransformation products from cyanobacteria cells, planning appropriate experiments, performing experiments including SPE extraction and LC-MS analysis of compounds tested cyanobacterias, elaborating the obtained results from instrumental analysis and their interpretation, writing a fragment of manuscript regarding the above-mentioned content, co-editing with the co-authors the final version of manuscript, following the comments of the reviewers. I estimate my percentage share at 45%.

H-10 B. Żyszka-Haberecht, **A. Poliwoda,** J. Lipok*, **2019,**
Structural constraints in cyanobacteria-mediated whole-cell biotransformation of methoxylated and methylated derivatives of 2'-hydroxychalcone.
Journal of Biotechnology, 293, 36-46, **$IF_{(2019)}$ – 2,533; $MNISW_{(2018)}$ - 35 pkt**

My contribution to the creation of this work consisted in creating a concept of work on the development a method for extraction and qualitative and quantitative analysis of biotransformation products of 2'hydoxychalcone structural analogues from cyanobacterial cells, planning and performing experiments involving SPE extraction and LC-MS analysis of, discussion of the results obtained from the instrumental analysis and their interpretation, writing a fragment of the manuscript regarding the above-mentioned content, co-editing with the co-authors the final version of manuscript, following the comments of the reviewers. I estimate my percentage share at 45%.

C) Discussion of the scientific purpose of the above-mentioned papers and their possible use.

The complexity of the surrounding environment makes that every day numerous amounts of chemicals have a biological impact on the living organisms functioning therein. It applies to both the substances, which are necessary for correct development and growth (e.g. micro- and macroelements, vitamins, amino acids, carbohydrates, proteins, fats, etc.), the ones having anti-bacterial, anti-oxidant or protective functions (e.g. carotenoids, flavonoids, alkaloids, etc.), as well as so-called foreign substances (xenobiotics), which include mainly synthetic chemical compounds (pharmaceuticals, pesticides, etc.) penetrating the environment as a result of human activity. Therefore, in this case, it is not surprising that for years a significant challenge in the field of analytical chemistry has been searching for techniques and methods enabling effective and efficient determination of various analytes in material samples, often of unknown composition and origin. This applies, for example, to insulation and identification of natural products of vegetable or animal origin with a wide spectrum of biological activity, pro-health compounds formed, for example, in the course of biotransformation processes in microorganisms and numerous pollutants (xenobiotics), usually causing an undesirable biological effect and even death of living organisms exposed to them. In the latter case, monitoring the fate of xenobiotics (their presence, concentration and how they are metabolized and transformed) entered to an ecosystem is aimed at developing and introducing appropriate regulations and legal standards, which define the maximum permissible concentration of harmful compounds in individual elements of the environment.

The chemical structure diversity of biologically active compounds and the complexity of the sample (matrix) material is mostly quite a challenge. Such samples, apart from the compounds we are interested in, frequently present in trace amounts (concentration < 0.01% (100 ppm)), contain a large number of other interfering substances - so-called interferents, which usually prevent conducting a direct chemical analysis. Although there is a group of available, direct chemical analysis techniques, their number is very limited. This is why, it should be emphasized that prior to conducting the specific analysis, it is necessary to previously adequately prepare a sample, which usually includes a stage of separating an analyte(s) from a matrix, its(their) purification and enrichment. Generally, this stage consumes even up to approximately 60% of the total sample analysis time and is still burdened with large uncertainty. An important issue is also searching for efficient methods enabling the detection and determination of the tested compounds. Over the recent years, a particular imperative requiring the development of new analytical study strategies has been the development of biological sciences, which cover explaining the course of metabolism processes, both at a level of a single cell, as well as the entire organism.

The selection of the sample preparation and analyte determination and identification technique shall take into account several important aspects, including the nature of analysed compounds, matrix type and the type of the used detection technique. The currently most frequently applied techniques for separating and enriching analytes include, undoubtedly, extraction techniques. In this case, solid phase extraction (SPE) and solvent extraction dedicated to the analysis of solid sample component analysis are particularly dominant. In the first case, the availability of sorptive materials with very different properties, and in the second case, the possibility for using supporting factors (e.g. microwave radiation or ultrasounds) determine their popularity. The possibility of miniaturization and automation are also factors decisive in regard to their wide application potential. At this point, I should also mention that the so-called membrane extraction techniques, combining the advantages of the membrane and extraction processes, had been widely applied at the sample preparation stage for many years. Simultaneously to the methods dedicated to isolating and enriching analytes, chromatographic and electrophoretic techniques enabling qualitative and quantitative analysis are also important.

Objective of the studies carried out as part of the habilitation process

The major objective of the scientific research described in the papers submitted for the assistant professor qualification procedure **[H 1 – H 10]** was the development of "'tools" enabling efficient and effective analysis of biologically active compounds from the group of xenobiotics, including the residues of fluoroquinolone drugs **[H-1]** and endocrine disrupting substances in environmental and physiological (urine) matrices **[H-3, H-4, H-8],** as well as fungal hallucinogens **[H-2, H-5, H-6, H-7]** naturally occurring in the biological material, and cyanobacteria biotransformation products of selected hydroxychalcones, important from the perspective of biological activity **[H-9 - H-10].** As a result of the conducted research, I developed methods using high-performance liquid chromatography combined with UV detectors (HPLC-UV) and a diode array (HPLC-PDA, HPLC-DAD). I expanded the research through using a mass spectrometer, as the most selective and/or sensitive detection method in combination with ultra high-performance liquid chromatography (UHPLC-MS/MS) and capillary electrophoresis with a diode array detector (CE-DAD). An important task was to develop methods for isolating and enriching analytes in environmental and biological samples using solid and solvent phase membrane extraction. The research projects where I acted as the general contractor played a large role in the execution of the studies.

Given the above issues, my research objectives related to the following problems:

1. The application of the membrane extraction technique using supported liquid membranes in a single fibre configuration for the separation and concentration of antibiotics of the fluoroquinolone group from environmental samples **[H-1]** and isoxazole and indole compounds **[H-7]** from body fluids.
2. The application of advanced separation techniques in research on the quantitative and qualitative determination of fungal hallucinogens and their structural analogues **[H-2, H-5 and H-6].**
3. Developing efficient analytical methods based on the use of molecularly imprinted polymers at the sample preparation stage, in the analysis of endocrine disrupting substances (phytoestrogens and alkylphenols) **[H-3, H-4 and H-8].**
4. The application of the SPE technique and liquid chromatography combined with mass spectrometry for the determination of hydroxychalcone transformation by cyanobacteria **[H-9, H-10]**

Ad. 1. The application of the membrane extraction technique using supported liquid membranes in a single fibre configuration for the separation and concentration of antibiotics of the fluoroquinolone group from environmental samples [H-1] and isoxazole and indole compounds [H-7] from body fluids.

The membrane extraction technique combines elements of the extraction and membrane processes. This applies both to the use of hydrophobic membranes for phase separation within the extraction process, as well as membranes with the organic phase (so-called liquid membranes). Particularly important in terms of analyses are the liquid membranes, and supported liquid membranes (SLM) in particular, which utilize the most optimal three-phase system.

In brief, a liquid membrane is usually a separate liquid phase, which separates two solutions as a selective barrier - donor phase solution, comprised of the analysed sample, from the acceptor phase. In most cases, the donor and acceptor are aqueous solutions, whereas the specific membrane is a hydrophobic organic liquid. An important prerequisite for the formation of such a membrane is the inability of inter-mixing of the liquid phases. Chemical substances found in aqueous solutions adjacent to the membrane can penetrate it, if the dissolve in the hydrophobic layer or reversibly react with the carrier in it. In the case of liquid membranes, where a carrier additive is used, the analytes are separated as a result of reactive-diffusion processes, and in the case of liquid membranes without a carrier, as a result of diffusion processes.

In supported liquid membranes, the organic (membrane) phase is immobilized by capillary forces in a microporous carrier - polymer film, which supports the membrane (Fig. 1). Using such a polymer greatly reduced the volume of the membrane phase, and in consequence, the membrane thickness, resulting in a desirable increase of the active surface to volume factor and the donor phase to

membrane phase volume factor. The values of these factors depend on the geometry of the used membranes, which include flat sheets, hollow fibres and spiral wounds. Both factors increase gradually, in the order of flat sheets < spiral wounds < hollow fibres.

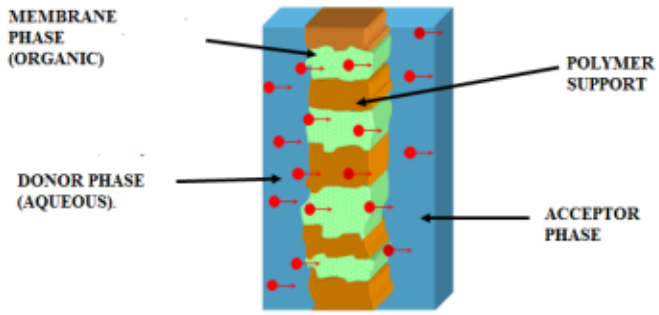

Figure 1. Scheme of supported liquid membrane system.

The aqueous nature of the supported liquid membrane acceptor phase is compatible with liquid chromatography with a reverse phase system and capillary electrophoresis. Starting in the 1990's, SLM technique found wide application at the separation and enrichment phase of a very diverse group of compounds, from samples of complex and complicated matrix composition. Such a broad range of applications results from its numerous advantages. Compared to the commonly used extraction techniques (SPE or LLE), and advantage of SLM is most of all the consumption of minor quantities of organic solvents, which constitute the membrane phase (usually a few µl). This makes this procedure eco-friendly, and the investment and operating costs are low. Furthermore, the ability to change the properties of the membrane phase, its hydrophobicity in particular, as well as the type of the used carrier, enables obtaining high process selectivity. A correct selection of process conditions (donor, membrane and acceptor phase composition) enables transport in contrary to the concentration gradient, which allows to significantly concentrate the acceptor phase with separated analyte. The option of full process automation in the case of SLM is also a great advantage of this technique. The durability of most extraction systems using supported liquid membranes described in the literature is from one week to a month. At the same time, multiple extractions do not cause a decrease in the efficiency and reproducibility of the separation process, which in the case of analytical applications is quite an advantage.

Given my experience gained in the course of my doctoral thesis procedure, which dealt with issues associated with the application of membrane extraction at the separation and concentration stage of short peptides, and my subsequent participation in the implementation of a research project "Nowe standardowe procedury analityczne przeznaczone do badania poziomu zanieczyszczenia ekosystemów

wodnych przez ksenobiotyki należące do grupy farmaceutyków i związków endokrynnych" [New standard analytical procedures developed for studying the level of aqueous ecosystem contamination by xenobiotics from the group of endocrine pharmaceuticals and compounds] (grant N 0536/R/T02/2007/03, 2007-2010), after defending my doctoral thesis, I begun research aimed at defining the possibility of using the SLM technique in analyzing fluoroquinolone antibiotics from environmental samples **[H-1].** Antibiotics are biologically active compounds. which have been commonly used in medicine and veterinary medicine for many years. Their source within the environment are mainly patients treated with antibiotics, who excrete them, both in an unchanged and metabolized form, as well as livestock, which in order to achieve the highest possible efficiency is often given exceeded doses of antibiotics. The consequence of the frequent or improper treatment of people and animals with antibiotics, as well as incorrect disposal of expired drugs is their undesirable appearance in the natural environment. Unfortunately, constant concentrations of antibiotics in the environment, persistent below the inhibition level may cause changes in the biocenosis therein and, in consequence, lead to the formation of immunity reservoirs, shifting the balance between microorganisms towards the ones immune to antibiotics. Spreading of immunity to antibiotics is considered a growing threat both for environmental equilibrium, as well as the possibility of treating numerous diseases in animals and humans. This is why, it is so important to monitor the presence and concentration of this group of compounds, including fluoroquinolones (FQ) (fluoro-substituted derivatives of 7-piperazinile-4-quinolone-3-carboxylic acids), belonging to the 2nd generation quinolone group. These chemotherapeutic agents appeared in the group on antibacterial drugs relatively late. However, they have proven their therapeutic efficacy and there is currently an increased number of indications for their use, both in medicine and veterinary medicine. Unfortunately, the extensive use of FQs did not protect natural ecosystems against contamination. Abuse resulted in fluoroquinolone accumulation in water reservoirs and soils, as well as a growth and multiplication of bacteria resistant to them. It was observed that the bacterial resistance to fluoroquinolones was higher in hospital wastewater than in the case of sewage treatment plants. For many years, numerous reports in the literature indicated the increase of FQ concentration in the aqueous environment, even to a level of several hundred μg/l[1]. This is why, in the light of these concerns and for a better understanding of the presence and fate of fluoroquinolones in a natural aqueous system, there is an undoubted requirement to determine them in various environmental matrices.

[1] Gothwal R, Shashidhar. Occurrence of High Levels of Fluoroquinolones in Aquatic Environment due to Effluent Discharges from Bulk Drug Manufacturers. Journal of Hazardous, Toxic, and Radioactive Waste. 2017;21(3):05016003.

The group of fluoroquinolones analysed in this project was comprised of a mixture of ciprofloxacin (CIPR), norfloxacin (NORF), enrofloxacin (ENRO) and danofloxacin (DANO), and they were extracted using a system of supported liquid membranes (SLM) with a single fiber geometry (HF-SLM, hollow fibre supported liquid membrane or HF-LPME, hollow fibre liquid phase membrane extraction). The obtained SLM extracts were analysed via an author's separation method using the HPLC-DAD technique. Hollow fibers with an acceptor volume of just 57 µl were used as polymer support. Optimization of HF-SLM extraction conditions involved, among others, pH selection of the donor phase, acceptor and membrane phase composition, extraction time and sample salinity. Based on the obtained results I proved that the choice of an organic solvent for membrane preparation determines the stability and efficiency of enriching the used SLM system. Using only organic solvents such as di-n-hexyl (DHE) ether or tri- (2- ethylhexyl) phosphate (TEHP) resulted in obtaining very low values of the enrichment factor for all analytes (below 1). Only adding a D_2EHPA (di-(2-ethylhexyl) phosphoric acid) carrier to the membrane phase, even at low concentrations, significantly impacted the efficiency of analyte transport through a liquid hydrophobic membrane, reaching its maximum for $20\%_{(w/w)}$ of carrier concentration. Given the fact that the driving force behind the transport mechanism was the proton gradient, the studies involved using hydrochloric acid with a 0.1 mol L^{-1} concentration as the acceptor phase. Although higher analyte enrichment efficiency was observed along with increasing HCl concentration in the acceptor, and given the fact that SLM extracts after extraction were analysed with HPLC, using a too high pH of the acceptor phase was not favourable. The developed method of sample preparation using supported liquid membranes enabled achieving high values of extraction efficiency (above 70%), with the enrichment factor value of more than 100. I verified the application potential of the developed method during the analysis of surface water real samples, enriched with known, strictly defined quantities of the determined substances. Whereas the degree of fluoroquinolone extraction was evaluated by comparing the size of signals coming from check (blank) samples and samples enriched with standard substances. The developed method, in the case of environmental samples, was characterized by a wide range of linearity (from 0.1 µg L^{-1} to 4 µg L^{-1}), as well as high precision, below 10%. The detectability limit of analysed fluoroquinolones in real samples ranged from 0.01 to 0.02 µg L^{-1}, and where better compared to the methods described in the literature, using SPE. The obtained results confirmed that the efficiency of an equilibrium process of membrane extraction increases along with decreasing concentration of the tested analytes in donor solution samples, which is extremely important from the analytical perspective, since enriching is necessary only when the analysed substance are present within the studied samples at low concentrations.

I decided to use the advantage of the SLM technique, as a method effectively utilized for the extraction of mildly polar and polar compounds, also in isolating and enriching muscimol, tryptophan

and tryptamine **[H-7]**. This subject matter was associated with my participation, as the main contractor, in the project titled "Wykrywanie substancji halucynogennych [Determination hallucinogenic substances]", financed by EU funds within the Operational Programme Innovative Economy, in the years 2009-2014. At the time of implementing this project, the current state of knowledge in the field of separating, enriching and determining psychoactive fungal substances and/or structural analogs in a biological material was were minor and mainly limited to narcotics listed in the Act on Counteracting Drug Addiction (in this case, this applies to psilocybin and psilocin). The available literature sources focused mainly on the application of classic solvent extraction supported (or not) by ultrasounds (ultrasound-assisted extraction, UAE) or SPE at the sample preparation stage, which was described in detailed and characterized in the review publication I am the co-author of [H-5]. Insofar as solvent reaction is efficient to separate analytes in the case of constant samples of fungal material, the situation with analyzing body fluid samples is different. In this case, the solid phase extraction technique turns out to be much more efficient. Nonetheless, the methods described in the literature applied mainly to determining hallucinogenic indole alkaloids, namely psilocybin and psilocin, sometime also bufotenine. Quite often, when determining these analytes in body fluid samples, in order to obtain satisfactory recovery rates, SPE was insufficient and required the use of additional procedures of the sample preparation stage (enzymatic hydrolysis and/or protein precipitation procedure). It should be emphasized that a definitely small group of available analytical procedures were papers on the possibility of isolating and enriching compounds of the isoxazole alkaloid group, namely, ibotenic acid and muscimol, commonly found in mushroom of the Amanita species. Especially in the case of analyzing urine samples, the low efficiency of used SPE columns hindered obtaining a satisfactory degree of separation, causing co-elution of disturbing components and analytes, particularly the ones with similar physico-chemical properties, which necessitated the use of as many as two extraction techniques (LLE and SPE) at the sample preparation stage.

In the light of the above, in order to simplify the analytic procedure, I initiated a concept of implementing research on the application of the aforementioned HF-SLM technique for the extraction of muscimol, which is a hallucinogenic of the ibotenic acid decarboxylation, and tryptophan and tryptamine **[H-7].** Because muscimol is considered to be the main substance responsible for inducing a hallucinogenic effect after consuming mushroom of the Amanita species, and tryptophan and tryptamine are indole analogs - psilocin precursors. For the extraction I used the same polymer support, as in the case of fluoroquinolone extraction, but with a smaller volume of the acceptor phase in the amount of 22.6 µl. The optimum conditions for separating and enriching an analyte mixture were obtained using also 20% D_2EHPA in DHE as the membrane phase, and a 200 mM hydrochloric acid solution as the acceptor. The total extraction time was 60 min. Urine samples prior to extraction

required only dilution (1:4) and setting a pH equal to 4. The enrichment factor value strongly determined the polarity of the analysed compounds. This factor, in the case of urine samples enriched with a standard additive was 4.1, 19.7 and 24.1 for muscimol, tryptophan and tryptamine, respectively. Here, a particular achievement was achieving a detectability limit of 0.0052 μg mL^{-1} in the case of muscimol, which is compared to detection using a mass spectrometer. Furthermore, the developed method was eco-friendly (minor quantities of used solvents), simple and, most of all, did not require the derivatization stage or advanced and costly instrumental analysis techniques (i.e. CE-ME). Table 1 shows a summary of the obtained results compared to the data found in the literature.

Table 1. Comparison of the effectiveness of extraction methods for muscimol determination from urine samples.

Applied method	Detection limit	Ref.
HF-LPME with HPLC-UV	0.0052 μg mL^{-1}	**[H-7]**
NMR-NOESY spectroscopy	13 μg mL^{-1}	[2]
CE-MS	0.0005 μg mL^{-1}	[3]
Cation exchanger with derivatization (Dowex® 50 W X8) with GC–MS	1 μg mL^{-1}	[4]

Ad. 2. The application of advanced separation techniques in research on the quantitative and qualitative determination of fungal hallucinogens and their structural analogues [H-2, H-5 and H-6].

Given the fact of the growing phenomenon of abusing hallucinogenic mushrooms, it seems justified to develop methods of standard and simple determination of such ingredients, not only in samples of body fluids, but primarily in fungal material. Unfortunately, the legislation in Poland does not stipulate, which fungi species contain hallucinogens. The Act on Counteracting Drug Addiction of 29 July 2005

[2] Deja S, Jawień E, Jasicka-Misiak I, et al. Rapid determination of ibotenic acid and muscimol in human urine. Magnetic Resonance in Chemistry. 2014. doi: 10.1002/mrc.4104.
[3] Ginterová P, Sokolová B, Ondra P, et al. Determination of mushroom toxins ibotenic acid, muscimol and muscarine by capillary electrophoresis coupled with electrospray tandem mass spectrometry. Talanta. 2014 7/1/;125(0):242-247.
[4] Støíbrný J, Sokol M, Merová B, et al. GC/MS determination of ibotenic acid and muscimol in the urine of patients intoxicated with Amanita pantherina. International Journal of Legal Medicine. 2012;126(4):519-524.

and its amendment of 20 July 2018 mentions only psilocybin and psilocin as hallucinogenic fungal substances in the list of psychotropic substances. This is why, in the following years I studied the subject area associated with developing efficient methods of determining fungal extract ingredients.

As I already mentioned, ultrasonic-assisted solvent extraction is most usually used in order to separate hallucinogenic substances from a fungal material. Soxhlet extraction is also used sometimes, but without a doubt, the UAE technique is the more effective procedure in this case. Thus obtained fungal extracts, after filtration and centrifugation are usually analysed with chromatographic techniques, including gas and liquid chromatography. Unfortunately, in order to be able to utilize the GC-MS technique, it is necessary for analyses of such type to complete a stage chemically converting the analytes into their volatile derivatives. This stage is usually labour-intensive and largely extends the sample preparation phase. This is why, the analysis of fungal hallucinogenic substance much more frequently utilizes high-performance liquid chromatography (HPLC) in a system with different detectors, including UV-VIS, diode, fluorescent, and the most popular mass spectrometer. This technique, in contrast to the aforementioned gas chromatography, does not usually require chemical conversion of analytes, which greatly simplifies the entire analytical procedure. However, although a system of combined rings (pyrrole or benzene) in a structure of indole hallucinogens induces absorption and emission of light with a greater intensity, thus determining the high sensitivity and low LOD and LOQ limit of developed analytical procedures, especially the ones using HPLC with UV detection, DAD and fluorescent, in the case of isoxazole alkaloids, obtaining a desired sensitivity is not so simple and requires the application of a much more expensive detection (mass spectrometer) or chemical conversion of the analyte, e.g. into dansyl derivatives.

As a result, my next step involved addressing the issues associated with the application of capillary electrophoresis (CE) with a diode detector for developing an efficient method of separating and determining muscimol and ibotenic acid in fungal extracts of the *Amanita species* **[H-2]**. Capillary electrophoresis is a technique complementary to liquid chromatography and is characterized by many advantages, including high separation efficiency, short analysis duration and most of all, minor consumption of organic solvents. The basis for the separation is the difference in the migration speeds of charged molecules, using a fixed, high voltage. In the course of this project, I simultaneously conducted experiments aimed at optimizing the conditions of electrophoretic separation of the tested analytes, as well as the conditions of ultrasonic-assisted solvent extraction used at the solid sample preparation stage. In the case of analyses with CE, I studied the impact of the type and pH of the separating buffer (BGE) and an organic modifier on separation efficiency. The ionic strength and pH of the carrier electrolyte determined the electroosmotic flowrate and the ionic form of analytes. Due to the chemical nature of muscimol and ibotenic acid, and especially the pKa, it was justified to use BGE

of acidic nature. An organic modifier addition, through changing the BGE dielectric constant and its viscosity, significantly improved the efficiency of the used CE system. The efficiency of the analytical procedure developed using CE-PDA was checked by analysing the real sample of hallucinogenic mushrooms collected in Poland. The conditions of electrophoretic separation induced the modification of previously described methods of ultrasonic-assisted solvent extraction. In this case, using a methanol mixture and 1 mM of a phosphate buffer with a pH of 3 ($1{:}1_{v/v}$) turned out to be more efficient and effective. In total, I analysed samples coming from 41 fungi of the *Amanita* genus (including *Amanita muscaria, Amanita citrina and Amanita pantherina).* Muscimol content range from 46 $\mu g\, g^{-1}$ to 1362 $\mu g\, g^{-1}$ and ibotenic acid from 182 $\mu g\, g^{-1}$ to 2983 $\mu g\, g^{-1}$. The studied fungi included also samples with no identified presence of the ibotenic acid and samples with the presence of both components not detected. Such a diverse content of isoxazole hallucinogenic alkaloids in the tested fungal material resulted from the fact that the presence of fungal metabolites is impacted, among others, by the mushroom growth stadium, weather conditions, ecosystem in which it grows and the mushroom part subject to analysis (cap, stipe), which was also observed and described in the publication by *Deja* et al. [5] where the sued detection technique was the NMR *(Nuclear Magnetic Resonance*). I validated the developed analytical procedure using UAE and CE-PDA, which was aimed at determining the range of its suitability and credibility. For this purpose, I defined the limits of detectability and determination, precision, accuracy, reproducibility, linearity range and the matrix effect. High numerical values of the determination coefficients (R^2) showed the linearity of the calibration curves within the studied concentration ranges, corresponding to the analyte content in real samples. The achieved analyte recovery rate in the case of the developed method of extraction from a fungal matrix ranged from 87% to 95%. The method's precision, determined based on the relative standard deviation (RSD) was below 7%. The obtained detectability limits for muscimol (1.5 $\mu g\, ml^{-1}$) and ibotenic acid (1.8 $\mu g\, ml^{-1}$) were satisfactory and comparable with the chromatographic methods described in the literature, especially the ones using MS detection. What is important, the paper **[H-2]** was the first to describe the possibility of determining hallucinogenic isoxazoles in a fungal material, using capillary electrophoresis. It should be also emphasized that the analytical procedure is characterized by high selectivity and sensitivity, and most of all, does not require chemical conversion of analytes and an additional extraction stage (SPE), despite the fact that a less sensitive selective detector with a diode array was used for detection.

Within the framework of the conducted research in the field of hallucinogens, I also approached the subject area associated with evaluating acute toxicity and pharmacological effects of synthetic psilocin

[5] Deja S, Wieczorek PP, Halama M, et al. Do differences in chemical composition of stem and cap of Amanita muscaria fruiting bodies correlate with topsoil type? PLoS ONE. 2014;9(12).

and dry methanol residues of fungal extracts from the species *Pholiotyna cyanopus* and *Psilocybe semilanceata*. The completion of this project was possible through cooperation with prof. dr hab. Olga Zhuk from the Institute of Biotechnology of the Department of Environmental-Technical Sciences at the University of Opole and a research group from the Chair of General and Clinical Pharmacology from the Medical University in Odessa. As a co-author of a paper published in 2015 in *Toxins* **[H-6]** I participated not only in the part on developing an efficient UHPLC-MS/MS method enabling the identification of the chemical composition of fungal extracts used in pharmacological tests in terms of the presence of psychotropic indole alkaloids, but also in co-creation of the concept of pharmacological tests and discussions, as well as the interpretation of the obtained results. LC-MS analysis of methanolic extracts of *Pholiotin cyanopus* and *Psilocybe semilanceata* confirmed the significant content of psilocybin (about five times higher compared to the content of its main metabolite - psilocin). Generally, the content of psilocin and psilocybin in the analyzed extracts was dominant compared to other identified hallucinogenic indole compounds. In addition to psilocin and psilocybin, the analyzed extracts also contained baeocystin, norbaeocystin and aeruginascin. The data in the literature at that time indicated that indole hallucinogens (including psilocin) belonged to a group of non-specific agonists of serotonin receptors (5-HT), which are bonded with the 5-HT_{2C} receptor with varying affinity degree. One of the ways enabling to evaluate the hallucinogenic effects in laboratory animals is using the head-twitch response (HTR). That literature-based information confirmed the fact that HTR was initiated by the activation of receptor 5-HT_{2C}. The conducted experiments on mice showed a comparable biological effect, taking into account pure, synthetic psilocin and a mixture of indole alkaloids present in the tested extracts. A toxic effect of the studies extracts was significantly stronger in relation to pure psilocin, despite the fact that the overall content of indole alkaloids in them was tenfold smaller, which confirms the existence of a synergistic effect. In this case, hallucinogens as stimulants of the central nervous systems (CNS) may be of interest, owing to the possibility of their application as potential therapeutics. Stimulating the central serotonin receptors is possible through the introduction of serotonin precursor - 5-hydroxytriptophan (5-HTP), which when present in the organisms of nude mice in large doses causes irregular head twitches. The conducted experiments confirmed the assumption regarding the potential antidepressant activity of psilocin and hallucinogenic indole alkaloids. The impact of psilocin and tested fungal extracts on the twitching effect in mice induced by 5-HTP demonstrates the dependence on the recording time and the reliability of the behavioural effect inhibition of 5-HTP by more than 60%. These results prove the presence of a clear antidepressant effect of the studied substances.

Ad. 3. Developing efficient analytical methods based on the use of molecularly imprinted polymers at the sample preparation stage, in the analysis of endocrine disrupting substances (phytoestrogens and alkylphenols) [H-3, H-4 and H-8].

An interesting group of biologically active substances is comprised of the aforementioned xenobiotics, and especially, the ones classified as EDCs (*endocrine disrupting compounds)*. Their presence in the environment around us is associated with, in most cases, human activity and more specifically, with the increasing economic and industrial development, etc. Unfortunately, EDCs, due to their similarity to natural endogenous hormones, already at small concentrations can impact their correct operation, hence disrupting the functioning of the endocrine system. Based on many conducted studies and observations of the animal population inhibiting strongly polluted ecosystems, it was concluded that these compounds can contribute to, among others, infertility and the development of cancers. There is evidence for the occurrence of these adverse changes also among humans. This is why, there is an undeniable need to develop reliable analytical methods for the monitoring of their presence in the environment around us. Due to the minor concentration of these analytes within the analysed samples and the matrix complexity, their determination often requires of time-consuming sample preparation stages. The dominant role in this case belongs to solid phase extraction with the use of commercially available deposits, which are often insufficiently selective, resulting in the co-extraction of other substances with analytes, present in a studied sample material (the ones with complex matrix composition, in particular), which usually hinders both the qualitative, as well as quantitative determination of the tested compounds. In this case, an interesting alternative may be the application of MIPs (*molecularly imprinted polymers*) for solid phase extraction. Molecularly imprinted polymers are characterised by a desirable, high selectivity. The 3D cavities formed during the polymerization process are complementary in shape, size and the type of bonds relative to the compound used as a standard molecule. This allows the obtained molecularly imprinted polymer to be able to selectively bind identical molecules (and structural analogues) with the ones used during synthesis (Fig. 2).

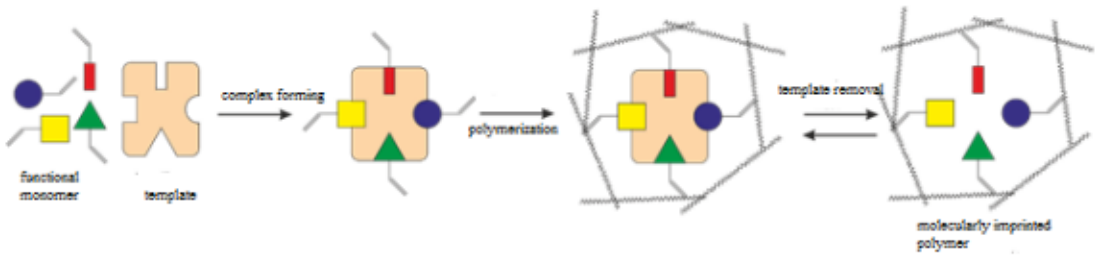

Figure 2. Scheme of molecularly imprinted polymerization process

Selective binding sites, formed during the polymerization process, simulate the binding sites of bimolecular systems - antibodies and enzymes. However, in contrast, the advantage of MIPs is the high chemical, thermal and mechanical stability, and simplicity of acquisition. They can be formed for many compounds and they are significantly more durable and cheaper than their natural antibodies. Moreover, their storage (even for several years) does not impact the "memory capability". In order to confirm the sorptive efficiency of obtained MIPs, at the same time non-imprinted control polymers (NIPs) are used.

A group of the compounds I studied, which exhibited an endocrinal effect was comprised of phenolic compounds, including the naturally occurring flavonoids (phytoestrogens) and synthetic alkylphenols introduced to the environment as a result of human activity (including the popular bisphenol A). Within my research, I was able to optimize the molecularly imprinted polymer synthesis conditions using different polymerization techniques, using the standard molecule in the form of biochanin A - one of the most common natural phytoestrogens **[H-3, H-4]**. and bisphenol A **[H-8]**. All cases involved the determination of the impact and quantity of used reagents and the synthesis conditions on the physico-chemical properties of obtained polymer sorbents. I also prepared the physico-chemical characteristics of the obtained sorptive materials, analysis through infrared spectroscopy and electron scanning spectroscopy, as well as the analysis of the porosity and specific surface using the method of low-temperature nitrogen adsorption. I used the obtained polymers as potential sorptive materials in SPE for separating and enriching the studied xenobiotics. The optimized extraction procedures were then used in the real sample analysis.

As a result, in the paper **[H-8]** I developed an effective method for the isolation and enriching bisphenol A and other selected alkylphenols (2-phenylphenol (2-PP), 4-*tert*-octylphenol (t-OP) and nonylphenol (4NP)), based on using molecularly imprinted polymers. These polymers (MIPs), prepared in the presence of BPA as a reference, were obtained using functional monomers varying in terms of chemical character (methacrylic acid, 4-vinyl-pyridine and acrylamid) and using various blowing solvents (acetonitrile, methylene chloride, toluene, chloroform). I defined the ability of bisphenol A binding as a matrix sample in the case of individual molecularly imprinted polymers and their corresponding non-imprinted polymers differing in terms of the reagents used for the synthesis. The imprinting factor values, defined as a ration between the recovery of bisphenol A obtained with the use of a molecularly imprinted polymer and control polymer in adsorption conditions were close to unit, which resulted from the fact that analyte molecules are bonded not only in the created binding cavities, but also in a non-specific manner, over the entire sorbent surface. I did not observe a significant impact of the type of used functional monomer and blowing solvent on the standard molecule binding efficiency. It was only the experiments, in which I determined the ability of selective

recognition of bisphenol A as a matrix molecule from aqueous mixtures containing the studies analyte and other structural analogues (alkyl-phenyls) that showed the importance of the type of used functional monomer. In this case, the most appropriate monomer in MIP synthesis with an imprinted bisphenol A molecule turned out to be acrylamide, which guaranteed obtaining sorbents able to selectively bind the standard in the presence of other xenobiotics (structural analogues). Unfortunately, in all cases, the 2-phenylphenol, due to the significant similarity to the chemical structure of bisphenol A, was bonded by the sorbent with an almost same efficiency. I was unable to observe the desirable selectivity oriented only on standard molecules (bisphenol A) in any of the cases. Nonetheless, the presence of a long aliphatic chain within the structure of 4-*tert*-octylphenol and nonylophenol hindered effective "matching" of the MIPs used for extraction to the 3D bonding cavity, which in turn, determined the selectivity of the extraction process. The conducted experiments also confirmed that no presence of any of the analysed compounds was detected in the eluates obtained after the sample application stage, in the course of the analyte mixture samples on a MIP deposit. Whereas in the case of similar experiments, but with a NIP deposit, bisphenol A was present. These observations confirmed the assumptions that the impact between BPA and MIP in the case of aqueous solutions was strong but non-specific. This why, in order to eliminate the undesirable non-specific bonding of analytes with an SPE sorbent, the extraction procedure was optimized in terms of selecting the elution solvent capable of selective desorption of 2-phenylphenol and other alkylphenols, which are BPA structural analogues. It turned out that the use of toluene as an elution solvent significantly increases the selectivity of the bisphenol A extraction process from a mixture containing this compound and other alkylphenols. The obtained results showed that toluene used at the elution stage of the MISPE (*molecularly imprinted solid phase extraction*) procedure may, to a certain degree, impact not only the reaction with analytes, but also the structure of the polymer network, which is associated with the appropriate distribution of functional groups in cavities and with the possibility of a more efficient standard bonding, while simultaneously decreasing the availability of recognition sites for molecules of other substances. In this case, using a specified quantity of toluene enabled to even practically eliminate structural analogues (alkylphenols) from MIP surfaces A, hence achieving a high recovery rate for bisphenol A (over 80% with 8:1 BPA selectivity relative to other analytes). The application potential of the developed analytical procedure was evidenced in the course of analysing real samples from the Odra river surface waters. Only selective extraction of bisphenol A was possible in the case of using an elution stage within the MISPE procedure, whereas the other alkylphenols could be detected only, when toluene elution was not used.

For comparison, the research papers **[H-3]** and **[H-4]** describe the procedures for the release of phytoestrogens: biochanin A, genistein and daidzein. For this purpose, the molecularly imprinted

polymers were synthesized with two techniques: bulk polymerization and the synthesis of silica with the surface modified by a layer of MIP. Each of the syntheses involved searching for such a composition and quantities of individual reagents of the polymerization mixture that would allow obtaining a sorptive material with the best possible properties, namely, which would enable selective identification of a specific compound or compound type. In the case of bulk polymerization, the block parameters that had the greatest impact on the quality of the obtained MIPs were the type of the functional monomer and the blowing agent. The best function monomer turned out to be the methacrylic acid and the best blowing agent was acetonitrile. In the case of modified silica, the optimized parameter was the quantity and character of the silica gel used as support. Porosity analysis showed that all molecularly imprinted polymers obtained through both techniques exhibited higher specific areas and higher pore density relative to non-imprinted polymers. Whereas the pore diameter was slightly lower for MIPs, which was probably associated with the presence of a standard in the polymerization mixture, which impacted the formation of selective cavities, which in turn, directly impacted their surface and porosity. Images obtained through the scanning electron microscope confirmed the differences in the morphology of individual sorbents. The polymers obtained via bulk polymerization had a rather compact, irregular structure. Whereas surface-modified silica, both with MIP, as well as NIP layer had a well-developed 3D structure and the main difference between an MIP and NIP was the obtained grain size. In each case, the FTIR analysis confirmed the efficiency of molecular imprinting.

In the case of bulk polymerization, the process selectivity was largely decided by the selection of an appropriate mixture of solvents used at the elution stage. Biding selectivity with respect to biochanin A could be achieved through the application of 1% $NH_3 \cdot H_2O$ in 10% MeOH – then, the BCA, Gen and Da recoveries were: 100.2%, 17.0% and 9.3%, respectively. When using 0.025% $NH_3 \cdot H_2O$ in 10% MeOH, the recoveries where: 94.1%, 93.2% and 91.5%. This stage enabled to eliminate the non-specific impact of analytes (without interfering with the bonds formed in the cavities), and owing to the possibility to change its composition, constituted an important aspect in terms of controlling the degree of selectivity of the obtained polymer (only in relation to the standard molecule or also towards structural analogues). In turn, surface-modified silica enabled achieving high recovery rates for each of the analytes, and the difference in selectivity between individual MIPs and NIPs was very high, which indicated the presence of binding cavities. Compared to bulk polymerization, it was not possible in any case to find an efficient system of solvents enabling effective elution of biochanin A analytes. This was probably associated with the fact that, in the case of the obtained polymer material, the bindings between amine groups of the functional monomer (APTES, (3-aminopropyl)-trietoxysilan) and the

hydroxyl groups of genistein and daidzein were so strong and durable that even an additive of a strongly alkaline aqueous ammonia solution did not break these bonds.

During the next stage, I conducted experiments associated with analysing real samples - human urine. First of all, I analysed undiluted urine samples. Unfortunately, the matrix complexity resulted in a quick contamination of the bed packed in an SPE column, which made it unsuitable for further use. Only after several dilutions of the sample (fivefold in the case of polymers obtained from bulk polymerization - unpublished data), it was possible to obtain satisfactory results. In this case, the obtained results confirmed the efficiency of the studied sorptive material at the stage of enriching the studied analytes. Unfortunately, the effectiveness of eliminating the disturbing substances from the real sample matrix was limited. A similar situation was observed in the case of using silica with its surface modified with molecularly imprinted polymer at the stage of urine sample extraction. Threefold dilution resulted in the desired effect and was sufficient to obtain biochanin A in this case. However, low matrix purification efficiency did not allow for effective genistein and daidzein determination. Only the introduction of an additional sample preparation stage - enzymatic hydrolysis with the use of β-glucuronidase and sulfatase (obtained from *Helix Pomatia)* enabled obtaining high recovery rates for all analysed phytoestrogens, and they were higher compared to the ones for sorbents synthesized with the bulk method. In addition, the analysis of blank samples confirmed the presence of daidzein and genistein therein and a minor amount of biochanin A, which indicated the efficiency of the suggested method.

4. **The application of the SPE technique and liquid chromatography combined with mass spectrometry for the determination of hydroxychalcone transformation by cyanobacteria [H-9, H-10]**

Two of my last publications submitted as my scientific achievements for my assistant professor application concern issues associated with attempting research on developing an efficient analytical method, which enables tracking flavonoid transformations by cyanobacteria, important from the perspective of their biological activity. As photoautotrophic microorganisms with a broad range of tolerance to environmental factors, they turn out to be effective biocatalysts modifying chalcone molecules (biogenetic precursors of all other flavonoid groups), in order to give advantageous properties to biotransformation products. Knowledge in the field of such transformations is still rarely discussed in the literature, and the presented papers are one of the first **[H-9, H-10]**. My task in the course of this project was to find a method allowing to monitor the nature of biotransformation changes of structurally diverse hydroxychalcones and their derivatives, under the impact of different cyanobacteria strains. For this purpose, it was necessary to develop an entire, new analytical method,

select the conditions for their determination and identification, as well as an efficient manner of separating cyanobacteria cells.

Owing to the nature of the conducted research, I used liquid chromatography combined with tandem mass spectrometry for determining the aforementioned compounds, in a mode of monitoring selected MRM (*multiple reaction monitoring*) or SRM (*single reaction monitoring*) reactions. The main advantages resulting from combining these two techniques are high resolution, selectivity and the specificity of determining the chemical structure (mass and fragmentation), which determines the reliability of identified analytes. In the course of selecting LC-MS measurement conditions with ESI (*electrospray ionization*) I took into account the optimization of ion source parameters (e.g. voltage applied to individual ionic optics elements, gas pressure (e.g. shielding and spray-supporting), ion source temperature, etc.)) and the conditions of chromatographic separation (type of the used stationary phase and mobile phase distribution). The MS/MS analyses were conducted in the mode of recording both positive and negative ions, depending on the intensity of fragment ions of the determined compounds. UHPLC separation was conducted with the use of a Gemini-NX C18 column with gradient elution. The mobile phase is a mixture of acetonitrile and water, containing a formic acid additive (0.1% $_{v/v}$). The LC-MS/MS analysis involved cellular extracts of the tested cyanobacteria, obtained from an experimental culture with observed growth inhibition due to the presence of 2'-hydroxychalcone, 2"-hydroxychalcone and 4"-hydroxychalcone **[H-9]** and their methoxy and methyl derivatives **[H-10]**.

The sample preparation stage conditions were optimized simultaneously. Double solvent-based extraction with methanol supported by ultrasounds was used in order to isolate analytes from microorganism cells. The combined extracts were then evaporated to dryness under a nitrogen flux and additionally subjected to SPE, in order to eliminate chlorophyll and carotenoid samples from the matrix, which interfere with chromatographic analysis. The OASIS®MCX ion-exchange bed turned out to be the most effective sorbent of this type among the commercially available. Methanol was also used for SPE elution, and the obtained extract were once again evaporated under a nitrogen flux, with the obtained dry residues solubilized in a methanol:ammonium formate (3:2$_{v/v}$) mixture and analysed with the developed HPLC-MS/MS method. The results confirmed that the outcome of 2'-hydroxychalcone transformation by the tested cyanobacteria were two products: flavanone (more frequently and in a higher concentration) and 2'-hydroxydihydrochalcone. In contrast to 2'-hydroxychalcone, the product of 2"-hydroxychalcone transformation was only 2"-hydroxydihydrochalcone. Whereas the outcome of 4"-hydroxychalcone transformation by cyanobacteria was the formation of three metabolites: 4"-hydroxydihydrochalcone, 4"-hydroxy-1,3-diphenylpropane-1-ol and 4"x-dihydroxy-dihydrochalcone. The tested cyanobacteria strains also

transformed methoxy derivatives of 2'-hydroxychalcone (2",5'-dimethoxy-2'-hydrochalcone and 4",4'-dimethoxy-2'-hydrochalcone) through hydrogenating bioreduction, creating appropriate hydroxydihydro-derivatives. A relationship was observed between the bio-hydrogenation efficiency of tested chalcones and the location of the methoxy substituent and, to a lesser extent, on the biocatalyst strain. 2-hydroxy-4"-methylchalcone was also transformed by cyanobacteria to various products, among which the most interesting were 2'-ethoxy derivatives. Based on the data obtained from MS/MS analyses, fragmentation paths were suggested in all cases. The HPLC-MS/MS experiments confirmed that cyanobacteria were an effective catalyst (highly efficient) for transformations of the chemo-selective reduction of unsaturated carbonyl compounds in mild conditions of a aqueous environment. Such a transformation leads to the formation of dihydrochalcones, which due to their sweet taste and natural origin are sought by manufacturers of multi-ingredient, low-calorie sweeteners. On the other hand, antioxidant and UV-protection properties make dihydrochalcones substances interesting for the pharmaceutical and cosmetic industries.

SUMMARY

The presented cycle of ten publications is a comprehensive elaboration on the procedures of preparing real samples and determining biologically active compounds therein - from the group of phenolic (alkyl phenols, phytoestrogens, hydroxychalcones), fluoroquinolone, and isoxazole and indole alkaloid compounds. The results obtained in the course of the research, which constitute my habilitation application achievement, enable to formulate the following conclusions:

- ✓ I developed selective and effective methods for preparing real samples with varied matrix nature (environmental and biological samples) using SPE, HF-SLM, USA and MISPE techniques, in terms of isolating biologically active substances with different physico-chemical properties.
- ✓ I conducted comprehensive and systematic studies regarding the impact of individual extraction parameters on the efficiency and selectivity of the analyte isolation and enrichment techniques I used. The selection of these conditions enabled achieving satisfactory recoveries of determined compounds and efficient matrix purification, which is why the proposed analytical procedures were a viable alternative to the methods described in the literature.
- ✓ I developed methods for synthesizing sorptive materials of the MIP type for the extraction of a selected group of xenobiotics. I compared the sorptive ability of individual analytes and the selectivity of this process depending on the manner of molecularly imprinted polymers and MISPE extraction conditions (especially the elution stage).
- ✓ I created a set of effective methods for separating, as well as qualitative and quantitative determination of a diverse group of biologically active compounds using advanced instrumental analysis techniques, including liquid chromatography with a spectrometric

detector and a tandem mass spectrometer with electrospray ionization, as well as capillary electrophoresis with a diode array detector. In this case, systematic research on the selection of chromatographic and electrophoretic separation resulted in obtaining new separation systems of high efficiency and resolution, often enabling efficient analysis of particular analytes for the first time.

- ✓ Experiments aimed at verifying the correct functioning of the developed analytical methods (including the range of linearity, LOD, LOQ, precisions, recovery, etc.) confirmed their efficiency and the requirements for quantitative determination.
- ✓ The analytical procedures I developed have been successfully used in the analysis of real samples, enabling the confirmation of the presence and concentration of analysed substances. The obtained detectability limits were higher relative to the methods described in the literature.

5. Discussion of other scientific and research achievements.

Other scientific-research achievements after obtaining a PhD. degree can be divided into the following subject areas:

a) **Continuing the subject of my doctoral thesis from the field of peptide extraction,** which resulted in 3 publications (including 1 review).

b) **Separation of enantiomers of organophosphonates compounds and studying their optical purity.**

The simple execution and low costs of the analysis via zonal capillary electrophoresis was the cause for using it to separate N-benzyl carboxylic enantiomers of phosphonic and aminophosphonic acid analogues. These studies were conducted in cooperation with a group led by prof. Paweł Kafarski from Wroclaw University of Technology, where they were synthesized. I conducted that research in the years 2006-2007 and they resulted in the publication of a research paper in *Electrophoresis* journal.

c) **The application of mass spectroscopy for the identification of newly synthesized organic compounds, their complexes (with metals) and isolated natural products.**

The funding acquired in 2010 from the funds of the European Regional Development Fund within RPO WO (Regional Operational Programme Opolskie Province) 2007-2013 enabled the purchase of a mass spectrometer, which commenced in 2011 my analytical adventure with this advanced spectroscopic technique. Since then, in cooperation with scientist of both my own Department, as well as other domestic and foreign scientific units, I conducted a number

of MS and MS/MS analyses aimed at confirming and describing the chemical structure of a diverse compound group. Within these studies, I was able to, among others:

- ✓ Determine the structure of biosurfactants of the rhamolipid group produced by selected bacteria strains growing on a defined substrate. This project was implemented jointly with prof. dr hab. inż. Grażyna Płaza from the Institute for the Ecology of Industrial Areas in Katowice within the framework of *Zastosowanie odpadów i ścieków z różnych gałęzi przemysłu spożywczego do produkcji biosurfaktantów (The application of waste and sewage from various branches of the food industry for biosurfactant production)*. (Own research project no. N N523 418237)
- ✓ Determine the products of glycerol polymerization in the presence of an alkaline catalyst, in cooperation with prof. Krystyna Czaja from the Chair of Chemical Technology and Polymer Chemistry at my Department.
- ✓ Determine the chemical structure of *Cytisus villosus Pourr* metabolites, which was implemented in cooperation with dr Farida Larit from the Faculty of Chemistry at the University in Constantine, Algeria (Département de Chimie, Faculté des Sciences Exactes, Université des Frères Mentouri, Constantine, Algeria);
- ✓ Identify the structure of oxazoline complexes of titanium and vanadium used as catalysts for ethylene polimerization, in cooperation with dr hab. Wioletta Ochędzan-Siodłak from the Chair of Chemical Technology and Polymer Chemistry at my Department.

The outcome of this cooperation contains five publications in journals in the JCR database, I was the co-author of.

d) **The application of liquid chromatography for the separation of sample analyte mixture of unknown chemical composition.**

Liquid chromatography combined with spectroscopic techniques (mass spectrometry, in particular) provides a wide range of analysis opportunities covering multi-component mixtures, without prior fractioning into individual components. The experience concerning this very technique, which I gained in the course of my career resulted in my participation in numerous projects, in which this type of analysis proved to be an efficient analytic tool. Within these studies, I was able to, among others:

- Develop effective HPLC methods for the separation of phenolic compounds, which are constituents of honey extracts, as well as vegetable Salvia officinalis L, Salvia sclarea L. and UHPLC-MS/MS method fot floral extract analysis from Epipogium aphyllum Sw.

This subject area resulted from my cooperation with dr hab. Izabela Jasicka-Misiak, and a research group from the Institute of Essential Oil and Medical Plants from Simferopol (Ukraine), as well as dr Anna Jakubowska-Busse from the Department of Botanics at the Faculty of Biological Sciences at the University of Wrocław. Up to date, this cooperation resulted in publishing a total of 4 papers from the JCR database, of which I am a co-author.

- Confirm the course TFA-Gly-ZΔPhe to TFA-Gly-EΔPhe isomerization reaction kinetics – project implemented in cooperation with dr Maciej Makowski from my faculty and a research group at the Wrocław University of Technology (paper published in 2017 in Arkivoc)

e) Within the framework of the project on the detection of fungal hallucinogens, apart from two papers listed as achievements in my assistant professor application, 4 other papers in this field were also published, which I co-authored. The outcome in this case was the publishing of papers in the field of mycology, which characterized a new species of fungi gathered in Poland, the *Pholiotina cyanopus* and *Panaeolus antillarium*), as well as an atlas titled *Grzyby neurotropowe* [Neurotropic fungi], which is a sort of a guide book, enabling the inspection services to initially identify the species of fungi producing hallucinogenic substances. The aforementioned atlas, apart from a detailed mycological analysis of the fungal material presents my analysis of the chemical composition of extracts, using CE-PDA and/or UHPLC-MS/MS. as well as the aforementioned atlas of "Neurotrophic fungi". Simultaneously, after completing this project, two doctoral thesis on this subject were defended. I acted as an auxiliary promoter in the case of one of them.

6. Further scientific-research plans:

Within the next stages of my research work, I would like to continue pursuing my interests associated with the techniques of separating and enriching analytes from samples of various origin and composition, as well as develop effective manner for the detection and determination. The issues associated with molecularly imprinted polymers as sorptive materials in SPE, and especially, with the possibility to combine this technique in a system with supported liquid membranes with hollow fibre geometry (HF-SLM). In this case, the project I am currently implementing in cooperation with a research group led by Prof. Antonio Martin-Estebana from Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) from Madrid (Spain) confirms that the application of MIPs but as component of an SLM system acceptor phase determines the high selectivity of obtained extraction systems, but most of all, a high efficiency of matrix purification.

Currently, the first research paper in this field received a „*Major revision*" status in the *Journal of Chromatography A.*

Apart from the aforementioned MIPs, a subject area similarly important to me is capillary electrophoresis and its application possibilities in analyte separation, the identification of which using the HPLC technique is difficult. It especially applies to compounds without chromophore and/or fluorophore matrices in its structure. In such a case, detection using the most frequently used UV-Vis and fluorescent detectors forces the need to introduce a chemical conversions stage, which is a long and labour-intensive process. Then, it is the capillary electrophoresis, which is a real alternative, because thanks to the use of the so-called indirect detection, it is possible to avoid the chemical conversion stage and simplify the analytical procedure as a result. This is why, as part of my research I try to develop an efficient method of analysing organophosphonate compounds, namely polyphosphonates, the analysis of which is quite a challenge.

An additional advantage of capillary electrophoresis is the fact that this technique, apart from analyte determination, enables also their enrichment, directly within a capillary (in *on-line* systems) or before a capillary (e.g. drop concentration in an *in-line system)*, which results in decreasing the detection limit and shortens the sample preparation stage to a minimum. In this case, I am particularly focusing on the use of such techniques for separating, enriching and determining analytes of the hallucinogenic alkaloid group and the aforementioned polyphosphonates in biological and environmental samples. Moreover, my plans include developing my interests in the field of the possibilities regarding coupling capillary electrophoresis with mass spectrometry. The availability of equipment at our Chair and the purchase of an interface last year allowed me to already commence the research on determining phenolic analytes via CE-MS techniques.

7. Summary of scientific and other achievements.

Status by day 22nd February 2019	Before the Ph.D.	After Ph.D.	Including the habilitation cycle	Together
H Index (Web of Science)	10			
Number of publications from the Philadelphia list	4	27	9	31
Number of publications outside the Philadelphia list	3	2	1	5
Summary IF	5,166	60,611	27,332	67,777
Number of citations (We of Science)	62	217	78	279
Role in publications from the habilitation cycle ✓ First author ✓ Corresponding author ✓ Second author ✓ Other	3 5 5 2			
Number of chapters in books	1	3	1	4
Number of oral speeches at national and international conferences: ✓ as presenting author ✓ as co-author	16 27			
Number of poster presentation	78			
Number of projects implemented as: ✓ Manager ✓ Manager of tasks ✓ Main executor (expert)	0 1 5			