20th International Symposium and Summer School on Bioanalysis

Book of Abstracts

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Dear Colleagues,

On behalf of the Organizing Committee, we are glad to present You the Book of Abstracts of the 20th International Symposium and Summer School on Bioanalysis.

As the meeting is held annually it provides a unique opportunity to collaborate with colleagues from around Europe, share ideas and expertise, while enabling the development of science and skills within this research field.

Bioanalysis is a developing area which has grown significantly in the last decades, especially due to the advances in modern biochemistry and new analytical tools. It provides a theoretical framework for analyzing, interpreting and understanding biological data acquired from biological experiments. Bioanalysis provides tools for solving real world problems.

This book offers a front-row seat to the exciting developments in this field and puts forward the state-of-the-art in this scientific area.

We expect this conference to be beneficial in terms of knowledge, creativity and future collaborations.

Organizing Committee

Oral presentations

L1

QA BENCHMARKING OF BIOANALYTICAL BACHELOR PROGRAMS. WE HAVE 2 AND/OR WE NEED IT?

Ede Bodoki, Bogdan Iacob, Radu Oprean

Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Romania

Study programs should be subjected to an internal and external evaluation process. ENQA sets out the main guidelines for the internal evaluation process. The purpose of this presentation is to compare the main ways of internal evaluation in the countries participating in the CEEPUS network. The main question that arises is: do we have to do an internal evaluation or do we need it? Discussions on this topic will shape the trend of partner universities in this network, especially for joint programs and microcredentials.

L2

BIODIESEL, A GREENER AND A GREENER FUEL

Florin-Dan Irimie, Mihai Lacatus, Csaba Paizs, Monica Tosa

Enzymology and Applied Biocatalysis Research Center, Babeș-Bolyai University, Faculty of Chemistry and Chemical Engineering, Arany János street 11, RO-400028, Cluj-Napoca, Romania

Biodiesel is a green alternative to petrodiesel because it's made from vegetal/algal oils or animal fats. It could be produced through enzymatic routes by alcoholysis, esterification or interesterification. Additionally, biodiesel additivation with natural substrates gets biodiesel closer to nature. A state of the art on these topics, supported by experimental results, will be presented.

L3

PERIODIC PHENOMENA AND PATTERN FORMATION IN CHEMICAL AND BIOLOGICAL SYSTEMS

Gabriella Donáth-Nagy

George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Department of Physical Chemistry

Periodic phenomena can be often observed at all levels of matter organization, from molecular level to level of organisms, or a whole population. They also have very large time interval of period time: from milliseconds to years. These systems are still the focus of interest because of the wealth of spatio-temporal behavior these systems exhibit. This includes bistability, multistability, excitability, complex oscillations, chaos, and high sensitivity towards both physical and chemical perturbations, the formation of target and spiral patterns, or mosaic structures of transient appearance in a thin, unstirred layer. The pattern formation in biological systems can also be described using mathematical models.

A special group of chemical oscillators shows periodic pH variation. The pH oscillators with suitable period time and amplitude can be used for designing periodic drug delivery devices. Another use of these systems is to obtain oscillations in concentrations of elements possessing only one stable oxidation state (ex. Ca^{2+}, Al^{3+}, F^-). Besides the composition and temperature, there are other factors – the presence of perturbants, the stirring speed, the flow rate, etc. – that influence the oscillation parameters (period time, the length of induction period, amplitude, number and shape of oscillations).

L4

QUALITY EVALUATION OF BIOLOGICALS

Dashnor Nebija[1*], Attila Gáspár[2], Blerta Pajaziti[3], Melinda Andrási[2], Blin A. Pajaziti[1], Rumenka Petkovska[3]

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In contrast to chemically synthesized drugs, biologics are produced using biological processes involving recombinant DNA technology and other cutting-edge technologies. Biologics are made using highly complex manufacturing processes that involve living cells and different process conditions can result in different product characteristics therefore for biologics production process defines the product [1,2]. Biological medicines precisely target molecular processes and have fundamentally revolutionized the treatment of many medical conditions for which no other treatment options are available [3]. Biologics demonstrate high degree of inherent heterogeneity and complexity and the combination of different techniques should be used for the extensive characterization of their quality attributes, including identity, structural integrity, purity and stability [4]. In this presentation the application of orthogonal analytical methodologies for the evaluation of quality of different classes of biopharmaceuticals is presented. 2-D gel electrophoresis complemented with MALDI-TOF MS analysis, after tryptic digest, was used for the qualitative characterization and identification of therapeutic rmAbs trastuzumab and rituximab, and fusion protein, abatacept. In addition, the impact of post-translational modifications of these glycoproteins on the electrophoresis behavior has been evaluated. Moreover, CZE and CGE were applied for the separation of human insulin, insulin analogues and their degradation products. In summary, peptide mass fingerprinting (PMF) analysis allowed rapid identity confirmation of rmAbs and fusion proteins, whereas CE-MS has been proven to be a suitable technique for the evaluation of the quality of insulins. These analytical strategies may represent an important strategy for the assessment of the quality of biologics in research and regulatory environment.

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L5

A COMPARATIVE STUDY OF MALDI-TOF MS AND 16S RDNA IDENTIFICATION OF PGPR BACTERIA

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Plant growth promoting rhizobacteria (PGPR) are free living soil bacteria that are able to promote plant growth and development. Due to the fact that they can be used in conservative and sustainable agricultural practice as biopreparates, many bacterial strains are isolated and characterized from the soil environment. Therefore, a rapid identification of these microorganisms that are accurate and cost effective is indispensable in applied microbiology. There have been several tools developed in the past few years for rapid microbial identification, using spectrophotometry (Biolog system), DNA sequencing, gas chromatography (GC-FAME) or matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS).

In the present study, bacterial strains isolated from different natural soils were identified with Bruker MALDI Biotyper using intact cells. At the same time 16S rDNA sequencing was realized for the isolated 25 salt tolerant and 13 heavy metal tolerant rhizobacteria. The isolates were identified with MALDI-TOF MS as belonging to *Bacillus sp, Pseudomonas sp., Lysinibacillus sp, Micrococcus sp., Variovorax sp., Serratia sp., Artrobacter sp., Variovoax sp* and *Microbacterium sp.*. The same genera were identified through sequence analysis, differences were observed mainly at species level. Comparative analysis for the isolated strains indicated that the MALDI-TOF MS identification results correlates with 16S rDNA identification results. The misleading results or identification failure of MALDI-based identification was attributed to the lack of the organism data from databases rather than to the methodological error.

L6

GENERAL ASPECTS ON SARS-COV-2 DETECTION. A TWO YEAR SUMMARY.

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The rapid spread of the SARS-CoV-2, which causes severe acute respiratory syndrome, broke out a pandemic in 2019 that continues to this day. It has become important to efficiently identify those infected by molecular diagnostic methods, for which quantitative RT-PCR has proven to be the most reliable method. However, at the beginning of the outbreak, there was a limited worldwide availability of sample processing materials and equipment required for RNA extraction prior to qRT-PCR as well as for PCR itself. In addition, this process is time consuming and unfavorable for the processing of emergency samples.

The aim of our research is to optimize the proposed RNA extraction and the RT-PCR method to diagnose hospital patients infected with the SARS – CoV-2 virus, during which the virus can be detected without RNA extraction. This will significantly reduce the time required for testing, lower costs, simplify the methodology, and reduce the environmental impact of the process.

In the course of our work, we determined the appropriate parameters of the heat treatment in order to have a sufficient correlation between the two methods (with and without extraction). After evaluating the results, it can be concluded that the whole extraction procedure can be eliminated in the diagnosis of patients infected with SARS-CoV-2 virus by RT-PCR. By diluting the sample in the appropriate proportions and treating the samples at the optimum temperature, the RNA to be detected was obtained with the same efficiency as by extraction. Thus, it has been demonstrated that the presence of SARS-CoV 2 virus in a sample can be detected without isolation, only by heat treatment.

L7

ANALYSIS OF INTACT PROTEINS WITH CAPILLARY ZONE ELECTROPHORESIS COUPLED TO MASS SPECTROMETRY USING UNCOATED AND COATED CAPILLARIES

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Proteins are the actuators of many vital biological processes. Understanding the structural characterization, identification of macromolecules in their intact states entails the studies of cell biology, disease prevention and treatment [1]. Top-down mass spectrometric (MS) technique is sensitive to enable the studies for structural and dynamical identification of intact proteins when coupled with capillary zone electrophoresis (CZE) [2]. However, a serious concern is the analyte adsorption on the bare fused silica (BFS) capillary surface, which necessitates the application of extreme pH or the use of coatings to minimize the analyte-wall interactions [3]. Our study involves the use of BFS capillaries employing the background electrolytes with very low pH and compares the analytical performance with those coated with polybrene as a dynamic and linear polyacrylamide (LPA) as a static coating material. The work demonstrates the differences in the ideal operating conditions (optimal pH, proper capillary conditioning etc.) of each capillary.

The results suggested that the analysis in BFS capillaries with BGE of very low pH (pH=1.8) resulted in good precision (0.56-0.78 RSD% and 1.7-6.5 RSD% for migration times and peak areas respectively) and efficiency values with minimum adsorption into the capillary surface. Coated capillaries showed higher resolving power for the separation of different forms (subunits of hemoglobin) of the protein. However, the separation performance in LPA coated capillary distinguished from others based on their stability, reproducibility over 25 runs and shorter analysis time in less than 10 min. The applicability of the proposed methods was also supported by the analysis of protein rich samples (e.g., snake venom). Hereby, the application of BFS capillaries for the analysis of intact protein mixtures would be considered also an efficient choice compared to coated capillaries when ideal conditions are applied.

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L8

DEVELOPMENT AND APPLICATION OF MICROFLUIDIC IMMOBILIZED ENZYMATIC REACTORS

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The field of microfluidics enables the manipulation of minute volumes of fluids in channel systems of micrometer range dimensions. Microfluidic devices have their inherent advantages of high surface area-to-volume ratio, low reagent and sample consumption, reusability, which are exceptionally beneficial in cases when high-efficiency catalysis needs to be carried out. A typical area of application is for bottom-up proteomic studies as microfluidic immobilized enzymatic reactors (μ-IMER).

The conventional approach for proteolysis utilizes in-solution digestion, however, enzymes can only be applied in a low concentration to minimize autolysis. Such low concentrations necessitate the use of prolonged incubation times (2-16 h), which is not compatible with the increasing demand for high throughput proteome analysis. The issue of enzyme autolysis can also be overcome by binding enzymes to solid supports, thereby restricting their access to one another. This results in the possibility to apply these enzymes is high (surface) concentration, which can decrease reaction times considerably. Recently, it was shown that the channel surface of a polydimethylsiloxane (PDMS) microchip readily adsorbs trypsin [1]. Our further goals were centered around enhancing the specific surface area of such PDMS microchips, for which an array of pillars was accommodated into the microchannels [2]. The successful formation of the pillar array structure required the thorough optimization of the soft lithographic method [3]. In addition to channels with increased specific surface area, channel patterns that induce passive mixing also proved efficient [4].

Apart from the off-line μ-IMER arrangements, it is possible to fully integrate the reactor into the analytical system, establishing an in-line platform [5]. Here, the reactor was formed only on a short, initial section of a capillary. The peptides were then separated in the same capillary using capillary zone electrophoresis (CZE) and detected by mass spectrometry (MS). The μ-IMER-CE-MS system allowed full automation.

Real samples of high complexity (tear, saliva, venom) were used to test the applicability of the reactors. The CZE-MS/MS analysis of the peptides obtained confirmed the efficieny of all three μ-IMERs.

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L9

MOLECULARLY IMPRINTED POLYMER-BASED DRUG DELIVERY SYSTEM FOR THE SUSTAINED RELEASE OF RUXOLITINIB IN CANCER TREATMENT

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In the past years, molecularly imprinted polymers (MIPs) have extended their biomedical applications to drug delivery, due to their ability to provide an extended release of a desired drug. MIPs have the ability to protect de active pharmaceutical ingredient from degradation, thus increasing its bioavailability [1,2]. Ruxolitinib (RUX), an inhibitor of the JAK/STAT-3 signaling pathway, has recently shown cytostatic activity in various types of cancer, including ovarian, pancreatic, colorectal, breast cancer, and glioblastoma [3,4].

We planned to overcome the current limitations of chemotherapy by developing a MIP-based drug delivery system for the sustained release of RUX, providing high biocompatibility with minimum toxic effects. Three different acrylic-based MIPs were developed and characterized, using acrylamide (AM) for MIP1, trifluoromethacrylic acid (TFMAA) for MIP2, and methacrylic acid (MAA) for MIP3 as functional monomers, trimethylolpropane trimethacrylate (TRIM) as the cross-linker and 2,2'-azobis(isobutyronitrile) as the initiator of the photopolymerization process. Isothermal titration calorimetry assay showed that TFMAA has the highest affinity towards RUX, followed by MAA and finally AM. However, MTT cell viability assay showed that TFMAA and TRIM have the highest cellular toxicity, followed by AM and MAA.

MIPs were first characterized in terms of their particle size and zeta potential by dynamic light scattering. Smallest particle diameter was observed for MIP2 with 484 (±19.92) nm, followed by MIP1 with 1780 (±44.29) nm, and MIP3 with 2677 (±37.07) nm. Moreover, particle morphology was observed by scanning electron microscopy. MIPs' loading capacity, measured in μg RUX/mg polymer, varied from 38.01 (± 8.03) for MIP2, to 31.48 (±0.56) for MIP1 and 31.45 (±5.44) for MIP3. In vitro release studies were subsequently performed, showing that after 96 hours, MIP1 released 28.23 (±7.07) % of the loaded drug, whereas MIP2 41.99 (±9.70) % and MIP3 31.50 (±11.61) %, respectively. Drug release kinetics was fitted with the Korsmeyer-Peppas model in all cases.

To sum up, three different acrylic MIPs were developed and characterized within this study, with the purpose of RUX delivery in cancer treatment. Although MIP2 turned out to be superior in terms of loading capacity and drug release, its residual toxicity may imply safety concerns. Therefore, MIP1 and MIP3 showing similar drug release profiles and without notable toxicity issues, became more promising for further in vivo studies.

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Acknowledgements: This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI-UEFISCDI, project number PN-III-P2-2.1-PED-2019-1387 within PNCDI III, as well as by an internal grant of Iuliu Haţieganu University of Medicine & Pharmacy no. 882/4/12.01.2022.

L10

BIOCHAR AS THE "GREEN" TOOL IN SOIL REMEDIATION

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Soils play an important role in the biogeochemical cycle of elements. They are responsible for the retention or mobilization of nutrients and hazardous substances. Platinum group elements (PGEs) is deposited along the roads and pollutes the environment [1]. Many soils require remediation, one of the methods seems to be enriching them with biochar [2]. Biochar (BioC) is a fine-grained, porous substance characterized by a high content of organic carbon and low susceptibility to degradation. Obtained from biomass in the pyrolysis process [3]. Preliminary data showed that biochar limits the mobility of the ionic forms of potentially toxic elements in the soil. Elements determined by ICP MS technique.

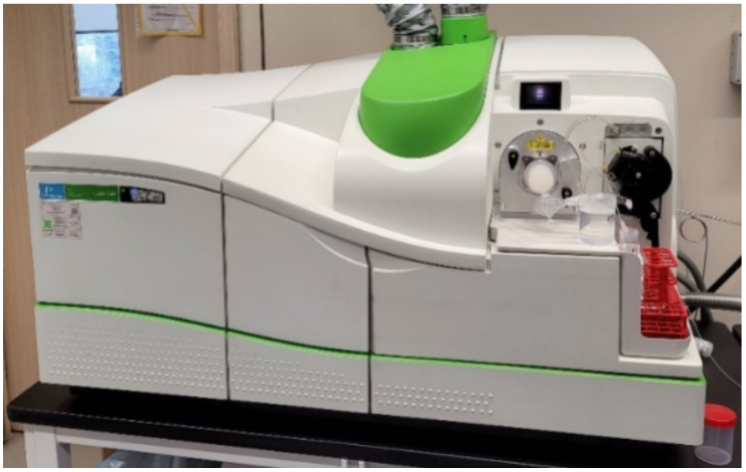

Figure 1. Mass spectrometer used for the determination of metals using the ICP MS technique.

There are plans to collect and test samples in high-traffic areas. Future research may prove the high application potential of BioC in controlling the transformation of nanoparticles of platinum group elements in soil.

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L11

COMPARISON OF MEASURED AND SIMULATED CALIBRATION CURVES OF A NOVEL CHRONOPOTENTIOMETRIC ANTIOXIDANT MEASUREMENT METHOD

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We recently proposed a novel antioxidant activity measuring method. The method employs a thin redox mediator film immobilized on glassy carbon electrode (GCE). In performing the measurement, first a short controlled potential step is employed where the film is brought to its oxidized state. Upon exposing the electrode to reducing samples, the redox potential changes. The initial slope of the electrode potential – time function is used for assessing the antioxidant activity[1]. Meldola Blue (MB)+(N,N dimetyl-7-amino-1,2-benzophenoxazinium ion) mediator layer was used in the measurement as mediator.

Different antioxidant species could be measured in different sample solution. Recently a model for understanding the response character and shape of calibration curves obtained with the method has been introduced. The details of the model and using it calculating the response traces and calibration curves will be discussed.Our recent experimental results obtained with the new method and calculated by the model equations will be presented and compared at the conference.

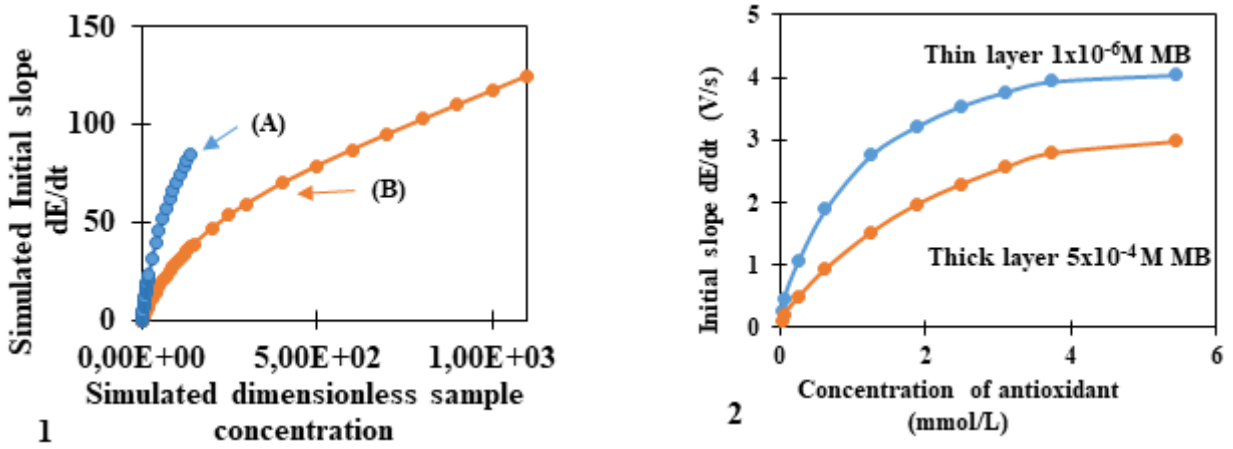

Figure 1: 1 (A). The simulated calibration curves of chronopotentiometric measurement under conditions of simulated thin mediator layer (A) and simulated thick mediator layers (B). 2. The initial slope of antioxidant concentrations in PBS pH =7 measured using reagentless chronopotentiometric technique in thin layer compared to thick layer of Meldola blue mediator.

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L13

ACHIRAL AND CHIRAL ANALYSIS OF ILLICIT DRUGS AND DESIGNER DRUGS

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Besides consummation of well known illicit drugs such as cocaine, heroine, MDMA and speed, recreational abuse of novel synthetic psychoactive drugs has become a challenging problem worldwide. Every year, dozens of new compounds enter the drug market and due to their similar substitution patterns, full characterization is difficult. At the end of 2020, the European Monitoring Centre for Drugs and Drug Addiction was monitoring around 830 new psychoactive substances, 46 of which were first reported in Europe in 2020.

Many of these compounds, particularly stmulants, contain a stereogenic centre and therefore, pharmacological potency of the enantiomers might differ as known from various pharmaceutical drugs. Therefore, the development of analytical methods for chiral separation of new psychoactive substances is of big forensic interest.

This work gives an overview of different methods for achiral and chiral separation of different drug compound classes including opioides, cathinones (“Bath salts”), amphetamines, benzofuries, thiophenes, phenidine and phenidate derivatives by high-performance separation techniques such as HPLC, GC, CE and CEC. Most of these analytes were either purchased at various Internet shops or seized by Austrian police, since they are hardly available at serious sources. Before serving as analytes, they underwent characterization by MS or NMR.

For successful enantioseparation, either commercially available chiral columns for HPLC and GC or chiral selectors as chiral phase additives for capillary electrophoresis were used. More than 100 chiral new psychoactive compounds were resolved successfully. Obviously, all tested new psychoactive substances were traded as racemic mixtures.

L14

INCREASING THE POWER OF DISCRIMINATING SNP GENOTYPE COMBINATIONS BY COUPLING HIGH RESOLUTION MELTING ANALYSIS TO PYROSEQUENCING

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Single nucleotide polymorphisms (SNPs) - the most abundant type of genetic variations in the human genome - have been associated with a variety of diseases, including cancer. Since SNPs are considered as potential diagnostic, prognostic, and/or predictive biomarkers, many methodologies have been developed for genotyping candidate SNPs.

High resolution melting (HRM) analysis is a very cost-efficient approach. It is based on amplifying a DNA template containing the SNP of interest in the presence of a saturating intercalating dye, followed by subjecting the PCR products to an increasing temperature gradient. HRM analysis is very applicable for discriminating between homozygous and heterozygous variants. Several studies demonstrated that it even allows discriminating combinations of SNPs in one and the same amplicon, the discriminatory power is, however, limited.

Thus, we aimed to investigate whether the discriminatory power can be increased by subjecting PCR products after HRM analysis directly to pyrosequencing (PSQ). PSQ is a sequencing-by-synthesis method in which the deoxynucleotides are added one after the other. In case the deoxynucleotide is incorporated, pyrophosphate is released and, via an enzyme cascade, a chemiluminescence signal is generated.

We targeted three C>T SNPs in the promoter of the *MGMT* gene, located within a distance of 45 bp. The *MGMT* gene encodes a DNA repair protein preferentially repairing O6-methylguanine lesions.

Direct coupling of HRM analysis with PSQ made it necessary to optimize the composition of the reaction mixes, because commercial PCR-HRM mixes are designed to obtain optimal HRM results, PCR-PSQ mixes to achieve high signals in PSQ.

For investigating the discriminatory power of HRM analysis, PSQ, and HRM coupled to PSQ, we analyzed oligodeoxynucleotide controls representing the 36 possible variant combinations for diploid human cells. 22 of the 36 variant combinations could be unambiguously genotyped by HRM analysis, 16 by PSQ. By coupling the two complementary methodologies, the discriminatory power could be drastically increased: 34 out of the 36 variant combinations could be unambiguously genotyped.

Our approach was successfully applied to commercial cancer cell lines of different origin, primary human tumor cell lines from glioma patients, and breast tissue samples.

Zappe K; Pirker C; Miedl H; Schreiber M; Heffeter P; Pfeiler G; Hacker S; Haslik W; Spiegl-Kreinecker S; Cichna-Markl M. Int. J. Mol. Sci. **2021**, 22, 12527

L15

INTACT PROTEIN ANALYSIS BY CAPILLARY ZONE ELECTROPHORESIS – MASS SPECTROMETRY

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Top-down proteomics is often applied for a pure protein component without any separation method, but when several proteins are measured, the fractionation of these proteins is inevitable. Capillary electrophoresis (CE) is well-known for its high resolving power, simple instrumentation, minimal sample consumption and short analysis time. Capillary zone electrophoresis (CZE) is the simplest CE separation mode based on the differences in the electrophoretic mobilities of the analytes (defined by the size, charge and the shape of the molecule). CZE is relatively easy to on-line hyphenate with ESI-MS, thereby providing molecular mass and fragmentation pattern information about the separated components [1].
One of the most significant issues in CZE analysis is the adsorption of proteins onto the capillary wall through electrostatic or other intermolecular (e.g., dipole-dipole or H-bond) interactions due to the forces acting between charged or neutral surfaces and the biomolecules of interest. In order to overcome this problem, several strategies have been applied, including the appropriate choice of pH, the use of different BGE additives or capillary coatings. With an uncoated capillary, the simplest way to reduce interactions is if very low or very high pH values are chosen for the BGE. In our work, human insulin and its several analogues were separated and determined using CZE-MS. Three different capillaries (bare fused silica (BFS), dynamic successive multiple ionic-polymer layer (SMIL) and static linear polyacrylamide (LPA) coated) were compared based on their separation performances in their optimal operating conditions [2].
The applicability of CZE for the separation of the deamidated forms of insulin has been also studied. 50 mM NH_4Ac (pH = 9) with 20 % v/v isopropylalcohol was found optimal for efficient separation of insulin from its even 10 deamidated forms. The developed method was efficiently applied for monitoring the degradation rate of insulin and the formation of different deamidation isoforms. Two months after the acidification more than thirty peaks can be observed in the electropherogram, because degradation products other than deamidated components were formed as well. The recorded mass spectra enabled us to assign the exact mass of the components, and thus the identification of insulin isoforms could be accomplished [3].

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L16

ANALYSIS OF HONEY SAMPLES BY CAPILLARY ELECTROPHORESIS USING FLUORESCENT DETECTION

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The number of different compounds (sugars, amino acids, proteins, vitamins, enzymes, flavonoids, minerals) in honey exceeds several hundred. A significant part (80-90%) is a mixture of fructose and glucose monosaccharides, but other mono-, di- and oligosaccharides are also present in smaller concentrations. The sugar composition influences the physical properties of honey and the relative proportion of monosaccharides is characteristic of honey. The determination of sugar composition can be used to characterize the quality of honey and also provide information about its authenticity.

Sugars do not posses UV / Vis absorption and charge therefore their analysis by capillary electrophoresis (CE) is a challenging task. Usually their anionic complexes formed with the borate content of the background electrolyte are used for the separation using indirect UV detection. The determination of saccharides after derivatization is also possible by fluorescence detection [1]. Analysis of the sugar content of honey was performed by capillary zone electrophoresis (CZE) using LED-induced fluorescence detection. Derivatization was performed by reductive amination using a 8-aminopyrene-1,3,6-trisulfonate (APTS). The APTS-sugar conjugate has absorption maximum at 480 nm and an emission maximum at 520 nm. The LEDIF detection provided high sensitivity determination of sugars (LEDIF S / N = 81000, UV S / N = 30). The labeling reaction allowed the CZE separation of triple negative charge carbohydrates. Saccharides of honey samples were identified with the help of a standard maltooligosaccharide ladder.

High separation efficiency was achieved using a buffer containing 0.3% polyethylene oxide (PEO) (25 mM ammonium acetate, pH 4.5) (theoretical plate number: 280,000 to 630,000 N / m). The standard deviation of the migration time of the labeled mono- and disaccharides was 0.49-2.53 RSD%, and that of the area was 2.48-4.41 RSD%. The sugar content of honeys of different botanical origins and the relative proportions of each sugar component were different.

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L17

FROM SCRATCH TO BATCH. MONITORING ENZYMATIC SYNTHETIC PATHWAYS

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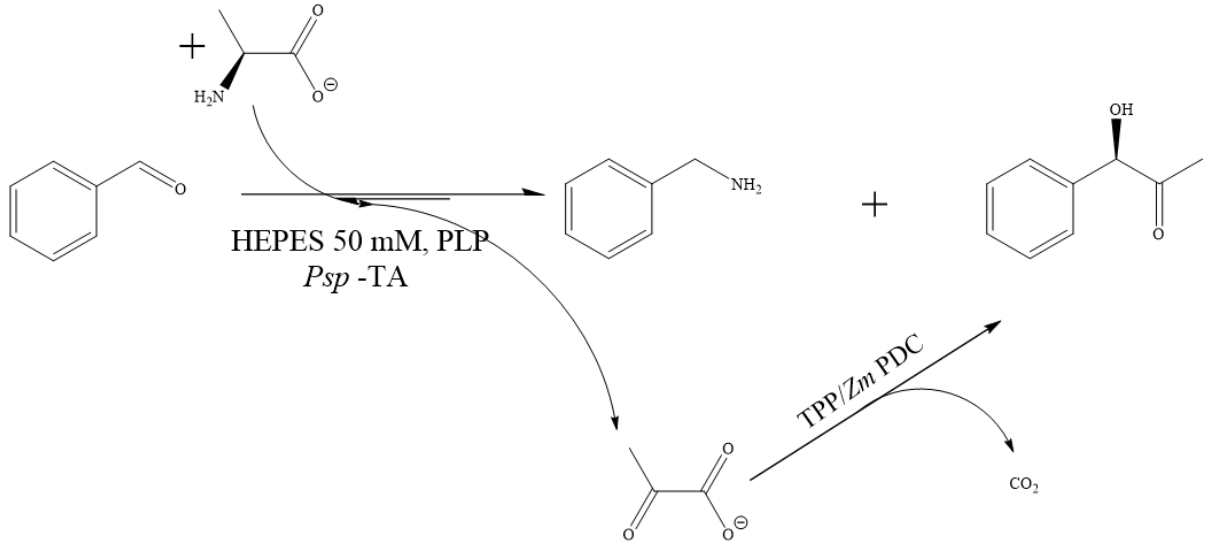
Nowadays several industry fields use biocatalysts to produce optically active API's, pharmaceutical intermediates, fragrances, pesticides or building blocks for organic synthesis.
We will describe the development of a multienzymatic biocatalytic system based on the combined action of a ω-transaminase and a pyruvate decarboxylase providing valuable intermediates for organic synthesis.



The monitoring of the progress of the reaction would not be possible without a proper analytical method that can determine simultaneously and with suitable precision the reaction products: benzylamine and the (R)-acyloin formed in the carboligation side reaction catalyzed by ZmPDC used the shift the reaction equilibrium toward benzylamine formation.
The determinations were made using a triple quadrupole LC-MS system, equipped with an ESI source set to positive polarity and scan type set to MRM mode. The calibration ranges were ~5÷640 ng/ml for both analytes. The developed method showed very good interday precision, the regression coefficients were >0.99 for all performed batches.

L18

BIOANALYSIS IN THE RATIONAL DESIGN OF FERULIC ACID DECARBOXYLASE VARIANTS

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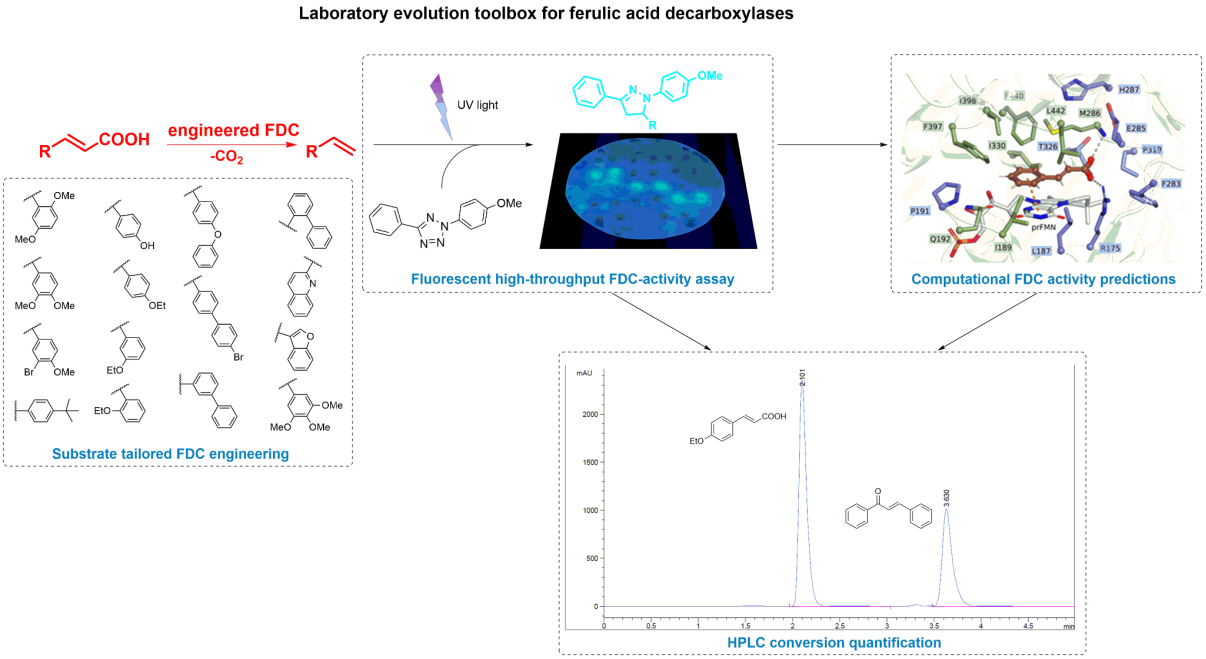
The recent studies elucidating the mechanism of ferulic acid decarboxylase (FDC1) and mapping the active site of the enzyme, also established it to be an ideal candidate for substrate tailoring through rational design protein engineering. Herein we report the development of a collection comprising 24 single and double mutant variants which was tested against a substrate panel of various cinnamic acid derivatives. With one exception, we have identified, for each substrate, at least 3 variants with improved activity compared to the *wild-type* enzyme, including several cases where the native FDC1 provided no detectable conversion, yet certain mutants transformed up to 83% of the substrate. Furthermore, we describe a high-throughput solid-plate assay suitable for the qualitative assessment of a larger collection of decarboxylases. Our results are supported by docking calculations revealing more fitting substrate poses in the engineered active sites.



L19

PRIMERS FOR AUTHENTICATION OF A WIDE RANGE OF FOOD PLANT SPECIES USING DNA METABARCODING

Alexander Petritsch[1], Stefanie Dobrovolny[1], Margit Cichna-Markl[2], Verena Peterseil[1], Elisabeth Muck[3] and Rupert Hochegger[1]

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Food adulteration can impair both the quality and safety of food and is a punishable offense [1, 2]. It can involve the replacement of food plant species with material of cheaper plant species [3]. Toxic or allergenic adulterants can pose health risks [1]. Consequently, there is a need to detect certain plant species and to differentiate between plant species in processed foods, when morphological features are no longer recognizable. DNA barcoding is an amplicon sequencing method that is increasingly used for food authentication [1]. DNA barcodes are short distinctive standardized DNA-sequences that are utilized as genetic markers [4]. DNA metabarcoding is the analysis of DNA barcodes in samples containing DNA mixtures using next generation sequencing (NGS) [2, 4, 5]. This allows for multiplexing and sequencing of large numbers of DNA-fragments in a massively parallel manner. The application of universal polymerase chain reaction-(PCR-) primers in DNA metabarcoding enables simultaneous detection of a wide range of species in a single sequencing experiment. This universality is an advantage over other DNA-based methods like real-time PCR [2, 5]. The aim of this study is to develop and validate barcode-marker systems for identifying a broad range of food plant species using DNA metabarcoding. The method should be applicable to processed foods and should be able to distinguish most plants important for food production, as well as plants that are often subject of adulteration. The barcode internal transcribed spacer 2 (ITS2), is frequently used for plants [6] and was chosen as first candidate barcode for this study.
A prerequisite for the DNA metabarcoding procedure is the extraction of sample DNA. Prior to sequencing, a DNA-library is prepared: This involves an initial PCR during which the universal barcode-specific primers are applied. After that, an index-PCR and further library preparation steps are performed before loading the NGS-sequencing instrument. The obtained sequences are identified by comparison with a customized reference database [2]. Method validation involves species differentiation in DNA-mixtures containing DNA from multiple selected plant species and tests on commercial foods. Alignment studies on ITS2-sequences from over 400 plants were conducted to characterize primer-binding sites and to analyze the interspecific variation. Three ITS2-specific primer pairs, based on a previous study [6], were developed at the AGES and tested on a broad range of plants. During ITS2-amplicon sequencing of 163 single-species plant samples, 75-78 % of samples were assigned to the correct genus while 43-69 % were assigned to the correct species. A minor part of samples showed suboptimal PCR-efficiency. These preliminary results indicate that the DNA metabarcoding method is suitable for authentication of most tested plants. Suboptimal PCR amplification was likely either due to mispriming or low DNA-extract quality. Further tests are necessary to verify these findings and to test the applicability on different processed foods before validation experiments can be conducted. It is planned to complement our ITS2-specific method with the application of a second barcoding marker, possibly the chloroplast barcode trnL [7].

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L20

COMPARISON OF ACTIVE AND PASSIVE AIR SAMPLING OF ANALYSIS OF SEMIVOLATILE ORGANIC COMPOUNDS NEAR A HCH DUMPSITE

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Hexachlorocyclohexane (HCH) isomers are classified as persistent organic pollutants (POPs) and there are more than thirty HCH dumps in the world that cause contamination of all media and bring serious concern due to their harmful effects to ecosystems and human health. The usage and production of these chemicals is prohibited in most countries around the world, but because of their persistence and ability for long-range transport via the atmosphere they can be still found in the environment. Due to their low concentrations in ambient air (ranging from few $pg \cdot m^{-3}$ to $ng \cdot m^{-3}$) and the complex air composition, sampling is a crucial step in the accurate measurements of HCH and other semivolatile compounds in ambient air.

In this study a low-volume active and passive air sampling on polyurethane foam adsorbent were used and compared in order to estimate the concentrations of HCH isomers in the air around the landfills at the former chemical plant OHIS (Organic Chemical Industry Skopje), which is the most critical environmental "hotspot" in Macedonia. Active sampling was performed using a low-volume air pump with flow rate 4 L/min equipped with polyurethane foam (PUF) plug that was operated for a certain time periods. The passive sampler consisted of a PUF disc adsorbent placed between two stainless steel domes that was exposed to ambient air for a whole month. Concentrations of four HCHs (α-HCH, β-HCH, γ-HCH, δ-HCH) and hexachlorobenzene (HCB) in air determined by the two different sampling approaches were compared at a time scale of one month. Good agreement of active and passive air sampling trends was observed for all HCH isomers (detected organochlorine pesticides). Besides these, other compounds from the classes of polychlorinated biphenyls, polycyclic aromatic hydrocarbons and volatile organic compounds were retained and detected on the passive samplers.

These results confirm passive air sampling as a convenient and appropriate alternative for determination of long-term concentrations of HCH and other volatile and semivolatile compounds in air, and highlights the importance of continuous monitoring with suitable analytical methods.

L21

DEVELOPMENT OF CAPILLARY ELECTROPHORESIS METHOD WITH LIF DETECTION FOR CHARACTERIZATION OF LIPOSOMES

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Liposomes are small spherical vesicles formed by a closed lipid bilayer. Due tó their amphiphilic character, various substances with different properties can be incorporated into them, making them especially suitable as a model system for drug delivery throughout the organism. The composition makes them biocompatible, biodegradable, and non-toxic, and based on the lipids used when preparing them, they can mimic compositions of biological membranes can be prepared [1,2].
The aim of this work is to develop a set of capillary electrophoresis (CE) methods with UV/VIS and LIF detection for fast physico-chemical characterization of liposomes. These methods can be used as an alternative to commonly used ones and they can be later applied in early drug development. For example, the leakage during body circulation of liposome-based drugs can be studied in capillaries. The leakage, as well as the liposome behaviour in the body, depends on liposome size, composition, hydrating buffer, and the method of preparation. The amount of released drug during stability studies or after immediate degradation and removal of the membrane and thus the encapsulation efficiency can also be determined by CE [2-4]. The information regarding the transport of drugs and their behaviour in the biological environment can be obtained by the developed methods.
Liposomes used as a model system were prepared by the lipid film hydration method [5] and were composed of distearoylphosphatidylcholine (DSPC), distearoylphosphatidyl-glycerol (DSPG), polyethylenglycolated (PGE) dimyristoyl glycerophosphoethanolamine (DMPE) and phosphatidylcholine labeled with 7-nitrobenz-2-oxa-l,3-diazol-4-yl (NBD-PC). The liposome sample DSPC:DSPG:DMPE-2000PEG:NBDPC (molar ratio 75:22:3:0.5) was prepared in 10 mM sodium phosphate buffer at pH 7. The PEGylated lipid was added to support the stability of liposome and NBD-PC for LIF detection.

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L22

THE USE OF MICROCHIP ELECTROPHORESIS IN BIOANALYSIS

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Microchip electrophoresis (MCE) is a miniaturized analytical separation technique that offers high separation efficiency, high throughput, easy automation, low reagent consumption, reduced waste production and low running costs. Despite these attractive features, its applicability in bioanalysis is limited. The complexity of bioanalytical samples and the increasing requirements for high detection sensitivity often require sample pretreatment prior to the MCE and/or its hyphenation with sophisticated detection techniques.

Methodological and instrumental aspects of the MCE, as well as its coupling with various detection techniques, are presented. Different electrophoresis techniques, e.g., zone electrophoresis, isotachophoresis and their online combinations are performed on a microchip with coupled channels and separations are monitored directly on the microchip using integrated conductivity sensors [1]. Spectrophotometry [2], surface enhanced Raman spectroscopy [3] and ion mobility spectrometry [4,5] are additional detection techniques used in coupling with MCE.

The practical applicability of MCE in bioanalysis using different detection techniques are shown in this work, e.g., for trace determination of nitrite and nitrate, biomarkers of neurological diseases, in body fluids [1]; determination of carminic acid, a natural red food dye, in various food and pharmaceutical products [2]; identification and determination of synthetic food dyes in pharmaceuticals [3]; identification and determination of carboxylic acids in food, pharmaceutical and biological samples [5].

MCE performed on the microchip with coupled channels is a multifunctional tool that facilitates the online integration of sample pretreatment with two-dimensional separation and the use of various detection techniques. Fast, reproducible and sensitive analyses are achieved using single or combined electrophoretic techniques on the microchip. The coupling of MCE with special detection techniques is beneficial for increasing the sensitivity as well as the selectivity of bioanalytical methods. Moreover, the presented results show the great analytical potential of MCE to fulfill the requirements for a lab-on-a-chip concept.

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L23

ANTHOCYANIN FINGERPRINTING AS AN AUTHENTICATION TOOL FOR BLUEBERRY, ARONIA AND POMEGRANATE JUICES

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In the last twenty years berry fruits have been intensively studied for the presence of different classes of polyphenols (phenolic acids, anthocyanins and flavonoids), which occur in different concentrations depending on the berry type. These studies were motivated by their potential health benefits since many epidemiological studies suggested that regular consumption of fruits reduces the risk of chronic and degenerative human diseases. But, high prices of polyphenol rich fruits often lead to adulteration of their juices with synthetic substitutes and/or cheaper fruits.

Therefore, the main focus of the study was to establish anthocyanin markers and use them to detect potential frauds in commercial fruit juices. The strategy was to establish the typical anthocyanin profile of various berry fruits, analyze the commercial samples and then compare the obtained profile with the declared composition. Differences in the presence and ratio of individual polyphenols can be an indicator that the fruit product has been adulterated.

Methods based on high-performance liquid chromatography coupled to UV-Vis spectroscopy and mass spectrometry MS are essential for separation, identification and quantification of anthocyanins. This technique was used for qualitative and quantitative analysis of anthocyanins as a tool for authentication of commercial juices containing blueberries, aronia and pomegranates.

The results showed that delphinidin 3,5-diglucoside, cyanidin 3,5-diglucoside and pelargonidin 3,5-diglucoside can serve as markers for pomegranates, cyanidin 3-galactoside for aronia and the content of malvidin and delphinidin derivatives can distinguish blueberries from bilberries. Based on the established markers, the study has revealed that fifteen juice samples were authentic, since they contained the declared fruits, but six of them had lower total anthocyanin content than expected from the declared fruit content. For juices containing several different fruits it is difficult to distinguish the origin of anthocyanins and correlate to all fruits present, but still the total anthocyanin content can be used to estimate the fruit content and thus for quality control.

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L24

PHTHALATES – HEALTH AND ENVIRONMENTAL CONCERNS

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Phthalates (diesters of phthalic acid) are widely used as plasticizers and additives in many consumer products including medical devices, food wrap, building materials, packaging, automotive parts, children's toys, and childcare articles made of polyvinyl chloride. Consequently, phthalates are found in human residential and occupational environments in high concentrations, both in air and in dust. Phthalates are also food and environmental contaminants. An increasing number of studies sampling human urine reveal the ubiquitous phthalate exposure of consumers in industrialized countries. Excessive use of phthalate esters has raised numerous questions about their possible health effects or damage to the environment.

Recent toxicological studies have demonstrated the potential of the most important phthalates to disturb the human hormonal system and human sexual development and reproduction.

This presentation overviews of the interest in the control of phthalate esters concentration and the availability of analytical methodologies for areas such as environmental and food analyses. It discusses usefulness of chromatographic techniques and pre-treatment steps.

L25

CHEMICAL AND PHENOLIC PROFILE OF STANUŠINA RED WINES DETERMINED BY HPLC-DAD

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Stanušina is a red grape variety indigenous of the Republic of N. Macedonia and herein only cultivated, i.e. it is found nowhere else in the world. In this study Stanušina wine was studied for the first time, and the extraction of phenolic compounds during maceration was monitored with the time (3, 6 and 9 days). Spectrophotometric analyses were performed at the following wavelength: 280 nm (total phenols), 420 nm (browning degree), 520 nm and 620 nm (anthocyanins) and 515 nm (antioxidant activity) with a UV-VIS spectrophotometer. In order to determine the general chemical composition of wines, official methods of analysis of wines (OIV 2016) were used and the following parameters were determined: alcohol (OIVMA-AS312-01 A), dry extract (OIV-MA-AS2-03B), specific density (OIV-MA-AS2-01 A), total acidity (OIV-MAAS313–01), volatile acidity (OIV-MA-AS313–02), total SO_2 and free SO_2 [1]. In addition, a total of 19 individual phenolic compounds belonging to the groups of anthocyanins, phenolic acids and flavan-3-ols were identified and quantified by HPLC-DAD [2]. On average, the content of total phenols of Stanušina wines was 795 mg/L and the effect of skin maceration time was highest at day 9. The observed antioxidant activity of wines was 102, 100 and 105 mg/L TE for wines macerated for 3, 6 and 9 days, respectively, and as expected, the total phenols content was well correlated with the antioxidant activity. Among antocyanins, malvidin-3-glucoside and its derivatives were the major compounds, while caftaric acid was the predominant cinnamic acid derivative, followed by catechin, the main flavan-3-ol. The concentration of hydroxycinnamic acids, anthocyanins and (+)-catechin ranged from 454 to 511 mg/L, 113 to 153 mg/L and 139 to 262 mg/L, respectively and peaked at 3rd, 6th and 9th day of maceration, respectively. In general, Stanušina wines presented relatively low levels of anthocyanins, due to the variety characteristics and high levels of hydroxycinnamic acids, such as caftaric and caffeic acids, and high antioxidant activity, confirming that this variety has a high potential for producing typical autochthonous Macedonian red wines.

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L26

ON-CAPILLARY DERIVATIZATION OF SACCHARIDES FOR CE-LIF

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The intensity of research activities in the field of glycoproteomics is constantly growing. Saccharide chains offer a vast number of combinations regarding the identity and order of monomer units, but also their linkage and possible branching. For these reasons, saccharide components of biomolecules play a crucial role in molecular signaling. Alteration of glycosylation pattern is related to several diseases and thus analysis of glycosylation can serve as a tool for early diagnosis of cancer and other diseases [1, 2]. Glycoproteomics is of imminent importance also in biopharmaceutical research and industry. The occurrence of proper glycosylation is vital for the effectiveness and immunogenicity of therapeutic monoclonal antibodies. Glycoproteomics is thus an indispensable tool in the field [3].

One of the glycoproteomic approaches is based on the release of glycans from glycoproteins using the peptide-*N*-glycosidase F enzyme [4]. The released glycans can then be analyzed using different analytical methods, among them capillary electrophoresis. Due to the absence of changed and chromophoric groups, their separation and determination present a challenging task. One of the options how to deal with saccharides is their derivatization. By derivatization at the reducing end, chromo- and/or fluorophoric readily ionized moieties can be introduced into the analyte molecules [5], facilitating their electrophoretic migration as well as sensitive detection. The derivatization can be highly automatized by utilizing the on-capillary derivatization performed directly in the inlet part of the separation capillary [6].

In this work, saccharide representatives were derivatized by 7-amino-1,3-naphthalenedisulfonic acid (ANDSA). Zones of saccharides and ANDSA were injected separately and then mixed using the electrophoretic migration of ANDSA. Several experimental parameters were optimized to achieve the highest product yield possible, namely, duration of the mixing, composition of the reaction solution, and reaction time. The results obtained from the optimization step were compared with the results of simulations performed by the Simul software [7]. Representatives of various saccharide classes, namely, xylose (pentose), glucose (hexose), fucose (hexose), *N*-acetylglucosamine (aminosaccharide), and lactose (disaccharide) were successfully derivatized, separated and detected using direct UV detection.

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L27

ANALYSIS OF PHOSPHOROTHIOATE OLIGONUCLEOTIDES BY ION-PAIRING REVERSED-PHASE LIQUID CHROMATOGRAPHY

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Phosphorothioation of internucleotide linkages is one of the widely used modification of therapeutic oligonucleotides to increase their *in-vivo* stability against endo- and exo-nucleases degradation [1]. While the phosphorothioate (PS) modification increases *in-vivo* stability of oligonucleotides, it also makes their chromatographic analysis more challenging due to the formation of diastereomers. This is caused by the introduction of a chiral center into the phosphate group leading to the formation of 2^x diastereomers, where x is the number of PS modifications. The presence of multiple potentially separable isomers may cause broadening of chromatographic peaks and complicate the oligonucleotides analysis [2]. For this reason, the suppression of diastereomeric separation can be beneficial for the resolution of the target peak from the shorter impurities. For this purpose, ion-pairing reversed-phase chromatography (IP RPLC) using IP alkylamines seems to be suitable method.
In this work the effects of ion-pairing alkylamine nature (alkyl chain length, hydrophobicity) and concentration, and separation temperature on retention, resolution of the target peak (*n* mer) and its impurities (*n*-1, *n*-2 and *n*-3 mers), and suppression of diastereomeric separation of 21 mer phosphorothioate oligonucleotides were assessed using octadecyl stationary phase.
The obtained results showed that to some extent increasing separation temperature and IP agents´ hydrophobicity and concentration lead to higher suppression of diastereomeric separation together with higher resolution of *n* mer and its shorter impurities.

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L28

MINIATURIZED ELECTROPHORETIC TECHNIQUES COUPLED WITH ION MOBILITY SPECTROMETRY FOR THE ANALYSIS OF CARBOXYLIC ACIDS IN LIQUID SAMPLES

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Microchip electrophoresis (MCE) is a widely applied miniaturized separation technique characterized by fast analysis, low sample and reagent consumption, and waste production. However, due to the reduced dimensions of the separation path, it is necessary to implement a selective and/or sensitive detection technique in the MCE for the analysis of complex samples. Ion mobility spectrometry (IMS) is a separation and strong identification technique that is characterized by a fast response but limited separation capacity that can be improved by its coupling with separation techniques [1]. This work shows the development of the MCE-IMS coupling and its application to the analysis of complex liquid samples. Carboxylic acids from the homologous series C_1-C_6 (formic acid, acetic acid, propionic acid, butyric acid, valeric acid and caproic acid) were analyzed in various liquid samples of environmental (wastewater), food (apple vinegar, wine, fish sauce), pharmaceutical (ear drops) and biological (saliva) origin.

Both MCE and IMS techniques are based on the separation of ions with different mobility in an electric field. However, the analyzes are performed in different phases, i.e., MCE in the liquid phase, while IMS in the gaseous phase. Therefore, it is necessary to develop an interface between the MCE analyzer and the IMS analyzer suitable for transfer of the separated sample components with minimal impact on the achieved resolution in the MCE.

Zone electrophoresis (ZE) and isotachophoresis (ITP) were used as the MCE techniques for coupling with the IMS in this work. MCE separations were performed on a poly(methyl methacrylate) microchip in a hydrodynamically closed separation system with suppressed electroosmotic flow. A thermal spray-based interface was used for online coupling of the MCE and IMS analyzers. An auxiliary liquid consisting of dilute electrolytes, which were used during the separations on the microchip, was used to transfer analytes from the MCE analyzer to the IMS analyzer. Reduced ion mobility of the analytes was used as an identification parameter in the IMS. Various analytical parameters, including sensitivity, linearity and precision, were evaluated for the developed ZE-IMS and ITP-IMS methods.

The developed online two-dimensional MCE-IMS methods can be used for the separation and reliable determination of C_1-C_6 carboxylic acids present in complex samples of various origins.

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L29

DIAGNOSIS OF PATHOGEN BACTERIA WITH ELECTROCHEMICAL SENSORS

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Detection of pathogen bacteria represents a highly researched topic in the biomedical field given their critical implication in food borne and waterborne infections. Bacterial contamination is often the source of outbreaks posing major concern to human health and leads to large number of deaths every year. The alarming spread of nosocomial infections as well as antimicrobial resistance being among the top ten global health threats (according to the World Health Organization) [1] call for urgent interventions, which also include fast and reliable detection tools for the pathogen agent in order to prevent the severe risks and the economic burden on the healthcare systems.

Electrochemical sensors have emerged as complementary methods to conventional analytical techniques, such as microbiological, molecular or immunological methods, that fall short on analysis speed, the need for pre-enrichment steps, expensive equipment and specialized analysts. In recent years, advancements have been made in the field of electrochemical sensors with smart sensing technologies being developed, overcoming the drawbacks of conventional sensing techniques and allowing for increased analytical performances (high sensitivity and low limits of detection), real time response, rapid and selective, culture-free, *in situ* detection, with simplicity and minimum sample pretreatment [2]. Electrochemical sensors can be wearable, custom-designed, inexpensive/cost-effective, portable with proven utility in remote settings, overcoming the drawbacks of conventional sensing techniques.

The electrochemical individual detection of *Pseudomonas aeruginosa* via its markers, pyocyanin and/or pyoverdine, was achieved through different approaches: with the help of graphite based screen-printed electrodes modified with reduced graphene and gold nanoparticles, with thermosensitive hydrogel and Au/Ag nanoalloy and with wearable sensors printed on gloves [3, 4, 5]. These platforms were also employed for the individual detection of *Escherichia coli* via its siderophore, enterobactin (with a limit of detection of 1.66 μM and a sensitivity of 0.154 μA/μM). The simultaneous detection of previous mentioned pathogen bacteria was performed using the procedures optimized for their individual detection (with good recoveries for the two analytes).

The results obtained show promise for further development of custom-designed wearable and portable sensors suitable for point-of-care testing in both environmental applications and clinical settings.

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L30

ELECTROCHEMICAL SENSORS BASED ON APTAMERS AND MOLECULARLY IMPRINTED POLYMERS FOR THE DETECTION OF QUORUM SENSING MOLECULES IN *PSEUDOMONAS AERUGINOSA*

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Bacterial infections caused by *Pseudomonas aeruginosa* are associated with a high mortality rate due to the ability of the bacteriumto develop multidrug resistance and form an adhesive biofilm that leads to treatment failure and systemic dissemination. With antibiotic resistance on the rise, it is crucial to develop new methods to identify *P. aeruginosa* infections. There are new detection methods that target representative structures, such as quorum sensing (QS) molecules. QS is a form of cell-to-cell communication between bacteria with the aid of substances called autoinducers, such as N-acyl derivatives of homoserine lactone (AHLs). QS plays a key role in determining virulence and biofilm formation, and the detection of these molecules in clinical samples is an important tool for diagnosing early-stage *P. aeruginosa* infections [1,2].
In recent years, biomimetic systems such as aptamers and molecularly imprinted polymers (MIPs) have attracted great interest due to their affinity for target molecules. Aptamers are short single-stranded oligonucleotides artificially synthesized to bind targets with high affinity. Due to their advantages (high specificity and stability, low cost, easy functionalization), aptamers have gained great interest as recognition elements in the development of electrochemical sensors [3]. MIPs represent another promising direction in the development of electrochemical sensors for the detection of QS molecules. These recognition elements also offer several advantages such as high selectivity, wide versatility, good resistance and low cost, and have proven to be very useful in the dosage of a wide variety of molecules. *In situ* electropolymerization of MIP using electropolymerizable monomers provides a simple one-step method to obtain thin layers directly on the electrode surface, allowing selective detection of the analyte of interest from the sample [4].
In this study, we developed two sensitive and specific electrochemical sensors based on an aptamer and MIP for the detection of QS molecules in *P. aeruginosa*. For the development of the aptasensor, the specific aptamer was selected based on information from the literature (3) and was functionalized with thiol groups. The electrode surface was modified with gold nanoparticles to facilitate the immobilization of the aptamers. Then, a deposition step was performed with 2-mercaptoethanol to eliminate non-specific interactions on the Au surface. For the development of the MIP-based sensor, the monomer exhibiting the strongest binding interactions with the molecule of interest was selected based on the results from the preliminary tests and methylene green was chosen as the monomer for our study. In both cases, all the steps in the development of the sensors were optimized to determine the optimum conditions for the detection of AHLs, and the modified electrodes were characterized using various electrochemical techniques. The developed sensors showed good results in detecting QS molecules in *P. aeruginosa* real samples.

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L31

EXTRACTION AND CLEAN-UP STRATEGIES FOR THE ANALYSIS OF PYRROLIZIDINE ALKALOIDS IN HONEY SAMPLES

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Pyrrolizidine alkaloids (PAs) and related *N*-oxides (PANOs) are secondary plant metabolites produced by many common plant species and exhibiting hepatotoxic properties to humans. As a consequence, beehive products are prone to be contaminated with those compounds by bees foraging PA-producing plants. PAs/PANOs are transferred to honey by their presence in nectar, honeydew, and pollen, which are collected from the flora by bees.

The structural diversity of more than 400 known PAs and the broad range of polarity, typically caused by the co-existence of free base PAs and their *N*-oxides (PANOs), represent a particular analytical challenge. Sample preparation is also crucial in analyzing PAs/PANOs in complex food matrices, and it can often be complicated. Therefore, it is of great importance to establish the simplest, yet successful extraction and clean-up strategy. The aim of this study was to make a comparison between the efficiency of several extraction mixtures as well as different sample purification methods.

Systematic examination of the extraction efficiency of PAs and PANOs was carried out using 12 different solvent mixtures containing methanol or water combined with different acids (sulfuric, formic or hydrochloric acid). Considering the complexity of honey matrices, clean-up steps are required to make the extract adequate for LC-ESI-MS analysis. Three purification methods including solid phase extraction (SPE) using a strong cation exchange cation, reversed phase C18 SPE, and QuEChERS were evaluated. Due to its specificity and sensitivity, a fast and simple method was developed to determine pyrrolizidine alkaloids in honey using liquid chromatography tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI)

Extraction efficiency was studied by analysis of samples spiked with working standards containing 10 different PAs and 5 related *N*-oxides (PANOs) at concentration level of 100 μg/L. Evaluation of the results for contents of all compounds indicate that the type of solvent is an important parameter that influences the extraction. Using this data, combinations of the most compatible solvent with clean-up procedures can be made. Presence of acid in the solvent mixture seems to negatively affect the clean-up step by SPE using strong cation exchange. Comparing the clean-up techniques, SPE using C18 column shows biggest loss of analytes.

Further research should include constant tracking of analytical data related to PA, in order to ensure the quality of food or prevent PA intoxications in humans, especially in products from the beehive.

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L32

ANALYSIS OF LEACHING OF CHROMIUM FROM LEATHER PRODUCTS – CHROMIUM SPECIATION

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The subject of my research will be natural hides used for leather products. Natural leather is a popular material for clothing or other items because of its viscoelasticity, pore size distribution, and breathability. However, the protein structure of leather makes it sensitive to attack by microorganisms that cause stains and degradation of leather, which ultimately shortens the life of leather products. To obtain leather with antibacterial properties, leather surfaces are treated with metal nanoparticles [1].
During the research, I will pay particular attention to the optimization of a simple, cheap and repeatable method for the separation and determination of trace Cr content in extract samples using SPE columns and the HPLC technique combined with a UV-Vis detector. The total content of Cr in the tested samples will give little information because it is necessary to pay attention to the speciation of this chemical element, mainly Cr (VI), which is toxic.

Chromium on the sixth oxidation state is attributed with carcinogenic properties - it penetrates biological membranes relatively easily, inside the cell it is reduced to chromium(III). Its adverse effect leads to lipid peroxidation, DNA damage, and as a result, often to cell death.

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L33

ELECTROCHEMICAL MIP SENSOR FOR THIABENDAZOLE DETECTION AT INDOLE-3-ACETIC ACID MODIFIED GOLD ELECTRODES

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Thiabendazole (TBZ) is a systemic fungicide and the lead molecule of the benzimidazole group. TBZ is increasingly used on seeds, leaves or for post-harvest treatment of fruits and vegetables [1]. The benzimidazole structured molecules are of high chemical stability and consequently, they remain persistent contaminants of food and the environment. Even though TBZ has a low acute toxicity, the US Environmental Protection Agency (EPA) classified it as likely carcinogenic if it reaches a high enough concentration to perturb the thyroid hormone balance [2]. The conventional techniques used for the detection and quantification of TBZ are usually high-performance liquid chromatography and gas chromatography, coupled with fluorescence, UV or mass spectrometric detection. However, these techniques require expensive equipment, extensive sample pre-treatment and long analysis times. Therefore, the development of rapid, sensitive and cost-effective detection techniques of TBZ is of great importance. Electrochemical sensors are explored in this regard, presenting also the advantage of miniaturization and potential enclosure in lab-on-a-chip systems that could be used on field [1].

Direct electrochemical techniques can be rarely employed for the trace analysis of pesticides. However, a thin electrochemically-synthesized molecularly imprinted polymer (MIP) layer able to selectively preconcentrate the target molecule in the vicinity of the electrochemical transducer may represent a viable option [3].

A MIP-based sensor on gold electrodes using indole-3-acetic acid as a functional monomer, and TBZ as a template molecule has been obtained by cyclic voltammetry. Both differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) in the presence of 1 mM ferro/ferricyanide as redox probe has been employed for the successful detection of TBZ in aqueous medium. The surface topology of the electrodeposited films has been investigated by SEM, whereas the analytical performances of the optimized electrochemical sensor were evaluated both by DPV and EIS. By EIS, upon a 10 minutes preconcentration, a linear response in the range of 0.01 – 1 μM TBZ has been achieved, with an estimated limit of detection of 5 nM. Binding efficiency and selectivity have been comparatively assessed also on non-imprinted polymeric layers in the presence of various environmentally relevant interferents.

Furthermore, the potential of an dual spectroelectrochemical (EC-SERS) detection of TBZ has also been studied.

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L34

SPECTROELECTROCHEMICAL DETECTION OF THIABENDAZOLE RESIDUES IN FRUIT JUICE

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The discovery of surface enhanced Raman scattering (SERS) from an electrochemical (EC)-SERS experiment is known as a historic breakthrough [1]. Since then, great progress has been made in using this technique for various analytical purposes, Raman spectroelectrochemistry representing now a promising alternative to conventional analytical methods.
By manipulating the surface charge through polarization, the physico-chemical interaction between the substrate and the target molecule can be controlled and facilitated. As a result, a higher enhancement of the Raman signal is observed. The EC modulation of substrate potentials during SERS analysis brings a series of other benefits, such as higher selectivity, possibility of preconcentration, reusability and improved reproducibility [2]. Furthermore, the electrochemical methods for "roughening" (activation) of the low-cost screen-printed electrodes offer a convenient, accessible, and ready-to-use alternative to commercially available SERS substrates that are expensive and sometimes irreproducible.
In this work we report the first EC-SERS detection of thiabendazole, a systemic fungicide used in agriculture that can be found as a contaminant in various foods, including fruit juices. The EC-SERS measurements were performed using a portable Raman spectrometer and a potentiostat. Commercially available gold screen-printed electrodes were used after an optimized electrochemical activation procedure. An applied potential to the substrate (-0.8V vs. Ag/AgCl) further increases the SERS signal of thiabendazole allowing its detection down to 0.06 ppm (0.3 μM), with a relatively wide linear range (0.5 - 10 μM) and good intermediate precision (RSD% < 10). As acidity of juice has a high variability, the pH-dependence of the SERS response was also investigated. The recovery of thiabendazole from unprocessed, spiked juice samples was found to be more than 80%. Furthermore, the possible integration of the developed EC-SERS sensor in a microfluidic chip was successfully demonstrated, suggesting a feasible implementation of miniaturized systems for real-time detection.

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L35

HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY IN THE SEPARATION OF GLYCOPEPTIDES

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Hydrophilic interaction liquid chromatography (HILIC) is an alternative separation technique for polar analytes which are insufficiently retained in reversed phase chromatography. It combines the three major chromatography methods as it uses polar stationary phase (similarity with normal phase chromatography), but the mobile phase is similar to those used in reversed phase chromatography. In addition, HILIC can be used for the separation of charged analytes (similarity with ion chromatography) [1]. The retention mechanism of polar analytes in HILIC is based on the combination of adsorption and partitioning processes. Moreover, in case of charged substances electrostatic interactions can be observed [2].
Glycosylation is a process by which glycans are attached to proteins or lipids via series of enzymatic reactions. Protein glycosylation is the most common and important post–translation modification, over 50% of the plasma proteins are glycosylated. They play an important role in many biological processes, are used as disease biomarkers and biopharmaceuticals [3]. Due to the diverse character of glycoproteins, their separation prior to their identification by mass spectrometry is highly required.
In this work, we show the potential of HILIC hyphenated to high resolution mass spectrometry in the separation of glycopeptides. As a model glycopeptide human immunoglobulin G was chosen. The separation was carried out on HILIC columns recently developed by Advanced Chromatography Technologies: HILIC–A (containing unfunctionalized silica), HILIC–B (containing aminopropyl functionalized silica) and HILIC–N (containing polyhydroxy functionalized silica) and was compared with the separation on C18 BEH column. The fragmentation was achieved via collision−induced dissociation (CID) while collision energy was set at 70 eV and the mass spectra were acquired simultaneously at lower and higher collision energies. In case of HILIC separation the glycopeptides with different glycan composition were successfully separated. Moreover, in case of HILIC–A and HILIC–B columns we observed mixed–mode separation character of the charged substances. On the other hand, the C18 BEH column was not able to adequately resolve these glycoforms. We also show the ability some of the columns to partly separate the isobaric glycoforms, which only differ in the position of the attached glycan.

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L36

STUDY OF THE PHENOLIC PROFILE OF VRANEC AND MERLOT WINES PRODUCED UNDER DIFFERENT VINIFICATION CONDITIONS

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Polyphenols are large family of naturally occurring, structurally diverse, organic compounds abundant in plants. Phenolic compounds such as anthocyanins, flavonols and tannins are important constituents of red wine contributing to the taste, color, mouthfeel and quality. They are also associated with the health-promoting properties of red wine. The proportion of the different polyphenols in wine vary according to the type of grape,maturity and the type of vinification. In this study, phenolic profile of *Vitis Vinifera* red wines Vranec and Merlot (vintage 2021), produced in Republic of N. Macedonia, has been evaluated. Wines have been produced with three winemaking techniques, including classical fermentation, roto process and punchdown method in order to study and compare the effect of vinification on the individual phenolic compounds. The phenolic profile was determined using an UPLC technique coupled with DAD and MS detectors. ESI-IT-MS method with alternating ionization polarity was used for identification of the phenolic compounds [1]. In total, 50 phenolic components were identified, divided into the following groups: phenolic acids and derivates, stilbens, flavonols, dihydroflavonols, flavan-3-ols and antocyanins. Individual standard solutions of 9 phenolic compounds (gallic acid, caffeic acid, 4-coumaric acid, ferulic acid, 2,4-dihidroxibenzoic acid, syringic acid, rutin, quercetin, and cis-resveratrol) were prepared and used for construction of calibration curves as well as for quantification of the individual phenolics. Considering the influence of winemaking method, it was observed that wines from both varieties produced with roto process had highest content of phenolic compounds. Compared to the classical fermentation, the content of phenolic compounds was about 30% higher in the wines obtained by the roto method.

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L37

INVESTIGATION OF PRAZIQUANTEL/CYCLODEXTRIN INCLUSION COMPLEXATION

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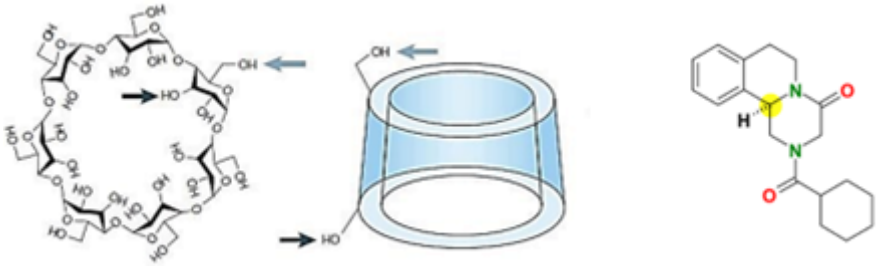
Praziquantel (PZQ) is according to a biopharmaceutical classification system (BCS) class II anthelmintic drug characterized by poor solubility and a bitter taste, both of which can be efficiently solved by inclusion complexation with cyclodextrins (CDs). Both natural and chemically modified CDs can improve the *in vitro* dissolution properties, provide efficient taste-masking and/or alternative ways of drug delivery through inclusion complexation.



b-cyclodextrin praziquantel

In this work [1], a comprehensive investigation of praziquantel/cyclodextrin (PZQ/CD) complexes was conducted by means of UV-Vis spectroscopy, spectrofluorimetry, NMR spectroscopy, LC-HRMS/MS and molecular modelling. Phase solubility studies revealed that among four CDs tested, the randomly methylated β-CD; RMβCD and the sulfobutylether sodium salt β-CD; SBEβCD resulted in the highest increase in PZQ solubility (approximately 16-fold). The formation of 1:1 inclusion complexes was confirmed by HRMS, NMR and molecular modelling. Both cyclohexane and the central pyrazino ring, as well as an aromatic part of PZQ are included into the CD central cavity through several different binding modes, which exist simultaneously. Furthermore, the influence of CDs on PZQ stability was investigated in solution (HCl, NaOH, H_2O_2) and in the solid state (accelerated degradation, photostability) by UPLC-DAD/MS. Complexes in the solid state were prepared by grinding and analyzed as described previously [2].

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L38

THE PHYTOCHEMICAL PROFILE OF SOLIDAGO SPECIES FROM THE REPUBLIC OF MOLDOVA FLORA

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Background. The genus *Solidago* L. (family Asteraceae) is represented in the Republic of Moldova flora by two species: *S. virgaurea* L. – spontaneous and *S. canadensis* L. – cultivated, known as adventive one. They have been used since ancient times as medicinal plants with diuretic, anti-inflammatory and antioxidant properties. The multi-pharmacological effects of *Solidago* species are due to the presence of a broad spectrum of bioactive compounds: flavonoids, phenolic compounds, triterpene saponins, carotenoids and essential oils.
Aim of the study. Comparative phytochemical study of flavonoids, saponosides, carotenoids, chlorophylls and hydroxycinnamic acids (THC) of *Solidago* species from the flora of the Republic of Moldova.
Material and methods. European goldenrod *S. virgaurea* and Canadian goldenrod *S. canadensis* plants harvested in the flowering phase from the central area of the Republic of Moldova served as biological material. The dry extracts have been obtained through fractional maceration and concentrated using rotative evaporator Laborata 4011. Phytochemical analyzes were carried out by UV-VIS spectrophotometric methods. The measurement of the optical densities has been performed on the *Metertech UV/VIS SP 8001* spectrophotometer, at the corresponding wavelengths (412 nm – flavonoids, 540 – saponosides, 448 nm – carotenoids, 644 nm – chlorophyll *b*, 663 nm– chlorophyll *a,* 518 nm – THC). The results of the data have been statistically processed using the GraphPad Prism 7.0 program.
Results. The experimental obtained data of flavonoids analysis denote that Canadian goldenrod flowers were characterized by a maximum content (%) of flavonoids (9.06), while the aerial parts of European goldenrod – by a minimum one (2.13). The results of the qualitative study of saponosides demonstrated the presence of triterpene saponosides and the lack of steroids ones in all analyzed vegetal products of both *Solidago* species. The comparative quantitative study of saponosides indicates that the dry extracts obtained from the vegetal products of *S. canadensis* plants have a higher saponoside content, as opposed to the same dry extracts of *S. virgaurea* plants. The same consecutive order between the saponoside content for the same type of dry extract is also maintained for both species. Thus, the highest content (mg/l) of saponosides is characteristic for leaves extracts (*S. canadensis* – 291.12, *S. virgaurea* – 244.87); in descending order are placed the extracts from the aerial parts (*S. canadensis* – 252.37, *S. virgaurea* – 228.62); followed by the flowers ones (*S. canadensis* – 188.62, *S. virgaurea* – 143.62). The assay of total content of THC indicates the following data: the highest values (%) of THC were determined for the Canadian goldenrod vegetal products and on the forehead are situated the flowers (1.25), followed by the aerial parts (1.20) and leaves (0.94). The leaves of both species are highlighted by a maximum content (%) of carotenoids (*S. canadensis* – 54, *S. virgaurea* – 44) and chlorophyll (*S. virgaurea* – 27.63, *S. canadensis* – 26.90).
Conclusions. This phytochemical study reveals that *Solidago* species from the Republic of Moldova flora could serve as source of raw materials rich in natural chemical compounds in order to use for phytotherapeutic purposes. Also, it has been shown that vegetal products of *S. canadensis* are characterized by a higher content of chemical compounds such as flavonoids, saponosides, carotenoids and THC than *S. virgaurea.*

L39

PEPTIDES AND PEPTIDE MIMETICS: POTENTIAL TOOLS FOR THERAPY

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Biomedical research is constantly oriented towards the development of new therapeutics based on peptides. Peptides have been widely investigated across the therapeutic spectrum and successfully developed as drugs, vaccines, cosmeceuticals, providing treatment for diabetes, endocrine disorders, cancer, Alzheimer's disease, cardiovascular disease, and many more.

Over the last decades the increase of generation of peptidomimetics, as well as the maturity of peptide synthesis technology managed to overcome the limitations of peptide drugs and highlight their attractive pharmacokinetics profile, including their remarkable potency, selectivity, and low toxicity.

This lecture will present recent results from the design, synthesis and biological evaluation of some peptides, such as neuro, anticancer and antimicrobial peptides.

In addition, our joint research on the analysis and characterization of these peptides, within our Net work will be presented.

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L40

ASSESSMENT OF DISTRIBUTION AND DIVERSITY OF PYRROLIZIDINE ALKALOIDS IN THE MOST PREVALENT BORAGINACEAE SPECIES IN MACEDONIA

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Pyrrolizidine alkaloids (PAs) are secondary plant metabolites and their 1,2-unsaturated derivatives that contain retronecine, heliotridine or otonecine type of necine base have raised concern due to their ability to form hepatotoxic intermediates and exhibit serious toxic effects. Over 660 PAs and their corresponding *N*-oxide derivatives have been identified in more than 6000 plant species in more than 12 plant families, in particular *Boraginaceae*, *Asteraceae* and *Fabaceae*

In this work, liquid chromatography (LC) tandem mass spectrometry (MS) method utilizing electrospray ionization (ESI) for qualitative and quantitative analysis of 17 most common PA and PANOs in plant material was developed and validated. With the optimized LC-ESI-MS/MS method using ion trap, the distribution and diversity of PAs and PANOs in plant material (leaves, flowers and stems) from wild-grown *Echium vulgare*, *Echium italicum*, *Symphytum officinale* L., *Cynoglossum creticum* Mill. and *Onosma heterophylla Griseb* species from Macedonia was assessed.

Characteristic fragment ions and their abundance in the mass spectra of different PAs were used to reveal typical fragmentation patterns for various classes of PAs that can be further employed to distinguish monoesters (retronecine, heliotridine type), open chain diesters and macrocyclic diesters and corresponding *N*-oxides.

These widespread *Boraginaceae* species contain various PAs and PANOs and 25 of them were identified. Based on these qualitative and quantitative analyses, the profiles of 1,2-unsaturated PAs for each sample were obtained and their toxic potential was estimated. The toxic potential of *O. heterophylla* and *C. creticum* were assumed to be highest (containing up to 4753 mg/kg and 3507 mg/kg), followed by *E. vulgare* (up to 1340 mg/kg), *S. officinale* L. (up to 479 mg/kg) and *E. italicum* (up to 16 mg/kg). This method can be used for monitoring the inclusion of these secondary metabolites in the food chain in order to contribute in their risk management.

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L41

MOLECULAR APPROACHES IN MICROBIOME RESEARCH: BEST PRACTICES

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Studies of microbial communities - the microbiome - have become quite popular in recent years. These studies are powered by the high throughput sequencing and bioinformatics tools, which provide sequenced profiles of microbial communities from different sources and compared the data for elucidating the associated pattern of microbiota.
The lecture will present the major challenges in microbiome research and the approaches to address them. Best practices in laboratory work and experimental design, important for microbiome analysis, and touch on computational and statistical methods will be discussed. Most examples will be from 16S rRNA marker gene sequencing and from ITS marker gene sequencing from fungi. Lastly, our research on the ecological diversity of soil microbiome in heavy metals contaminated soils will be present.

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L42

MOLECULAR IMPRINTING IN ELECTROANALYSIS. NEWS AND TRENDS

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Combining the nature inspired molecular recognition concept with the processability of manmade materials, molecularly imprinted polymers (MIPs) became well-established and robust receptor-like synthetic binding sites with widespread applications in analytical sciences. Moreover, the number of recent publications focused on the use of these functional polymers in electroanalysis demonstrates their increasing popularity and the rising interest in further development and exploitation to full potential. The tailored selectivity, long-term stability, ease of fabrication and cost effectiveness are just a few of the inherent advantages of these engineered biomimetic recognition elements which may be put to good use in affinity sensor development. The well controlled, in-situ electropolymerisation of imprinted thin films on the transducer's surface further promotes the facile integration of these recognition elements into electrochemical sensors. The good mechanical adherence and easily tunable properties (charge transfer, capacitance) of the resulting (semi)conducting polymeric sensing layer offer various signal transducing strategies and enable the analysis of a plethora of both electroactive and inactive analytes, from low molecular weight molecules (pharmaceuticals, natural products, food contaminants, pollutants, metabolites, biomarkers, chiral molecules, etc.) to very complex biological structures (biomacromolecules – proteins, DNA & RNA sequences; microorganisms – bacteria, viruses). The considerable progress in their computational chemistry-assisted rational design and synthesis protocols, as well as the significant achievements of the last few years in the imprinting of macromolecules further consolidates the reliability and the amazing versatility of the molecular imprinting technology. Nevertheless, as their commercial development and exploitation is still lagging behind, there is still plenty of room for further research and innovation to better understand the intimate electrochemical processes occurring at the MIP-film coated electrodes (e.g. "gate effect" phenomenon), to find more clever ways in imprinting large molecules, to pursue the implementation of MIP-based multiplex assays and to further gain in sensitivity or selectivity by the integration of synergistic molecular recognition strategies (e.g. dual aptamer-MIP systems) and signal triggering (e.g. photoelectrochemistry) or instrumental hyphenation (e.g. spectroelectrochemistry).
A brief overview addressing the most recent advances, current challenges and trends with respect to the application of molecularly imprinted polymers in electroanalysis will be presented.

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Posters

P1

RENEWAL OF AN OLD COMPENDIAL METHOD FOR THE DETERMINATION OF THE RELATED SUBSTANCES OF DALFAMPRIDINE

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Introduction: 4-aminopyridine or fampridine (syn. dalfampridine) is a broad-spectrum potassium channel blocker, approved in 2010 for the treatment of multiple sclerosis-associated motor symptoms. Due to the hydrophilic nature of the molecule and its related substances, the chromatographic separation of these compounds is challenging and the use of special conditions, such as ion-pairing reagent in the mobile phase are needed. The aim of the study was to improve the selectivity of the underperforming compendial chromatographic method for the determination of related substances of dalfampridine, using software-assisted retention modeling and experimental design-based principles.

Materials and methods: The systematic experiments were realized in different multi-dimensional experimental frameworks, successively changing the method parameters, such as column temperature, gradient, pH of the aqueous part of the mobile phase and the ternary composition of the organic mobile phase, at different levels. The effect of the stationary phase was also studied by testing columns with different chemistries. The virtual retention model of the different separation systems, *in silico* robustness testing and method optimization were performed using DryLab modeling software.

Results: The pentafluorphenyl-type stationary phase offers multiple mechanisms for orthogonal selectivity to traditional C18 phases. Indeed, this column showed higher selectivity and better peak performance characteristics, regarding theoretical plates and peak shape. Based on the obtained retention models the most critical method parameters were the pH of the mobile phase and the structure of the gradient. It was also observed that greater critical resolutions are achievable at higher column temperatures. The *in silico* robustness testing proposed safety confidence intervals for each studied chromatographic parameter to ensure the suitability of the method during its lifecycle. Based on the robustness study, the control strategy of the method was also established.

Conclusion: The experimental design-based framework using mechanistic retention modeling is an efficient way of chromatographic method development and optimization. Furthermore, a better understanding of the separation process is achievable by exploring the individual and combined effect of method parameters on the critical method attributes. Using this approach, the fast identification of optimal and robust chromatographic conditions is possible, based only on a limited number of experiments.

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P2

REGULATION OF LIPOPOLYSACCHARIDE BIOSYNTHESIS BY BACTERIAL TWO-COMPONENT SYSTEMS

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Lipopolysaccharides cover about 70-75% of all Gram-negative bacterial surfaces. Their role goes beyond structural function, as our project demonstrates, thermosensitivity, biofilm-forming ability, and antibiotic susceptibility are all related to the proper biosynthesis of these molecules. Bacteria with lipopolysaccharide deficiencies often survive in standard laboratory conditions, making it difficult to analyze the impact of different mutations.

Two-component regulatory systems play a key role in the regulation of many aspects of bacterial growth and virulence, reacting to the environment of cells. Bacteria use two-component regulatory systems to control and modulate the expression of many genes, such as lipopolysaccharide biosynthetic enzymes.

With the help of two-component systems, bacteria synthesize different lipopolysaccharides according to the availability of different ions, different temperatures, and we see that intracellular pathogens create very special lipopolysaccharides to survive intracellularly. Bacteria need this flexibility to thrive in different niches.

Novel approaches are sought in the development of new lipopolysaccharide targeting antimicrobial agents to combat Gram-negative bacterial infections. The studies of the connections between lipopolysaccharide biogenesis and known bacteria two-component system inhibitors have the potential to find new antibacterial targets.

P3

A PROTEIN-BASED APPROACH OR THE ENANTIOSEPARATION PF AMPHETAMINE DERIVATIVES VIA HPLC-UV

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Among other substance classes of New Psychoactive Substances (NPS), new amphetamine derivatives have led to fundamental changes in the global drug market during the last years. Also known as “legal highs”, they are sold under various names like "bath salts", "air fresheners" and so forth. To circumvent law, they were created by slight structure modification of classic illicit drugs such as amphetamine, methamphetamine and MDMA and samples are traded worldwide via various internet shops. Many of these new derivatives possess a chiral centre and the pharmacological differences of the two enantiomers are not yet investigated for many of them. This makes the continuous development of analytical methods for their chiral separation crucial.

This study aimed to develop a simple HPLC-UV method for enantioseparation and to test this method on a set of 26 amphetamine derivatives, including latest products on the drug market. With the protein Cellobiohydrolase I as chiral selector, a Daicel Chiralpak® CBH 150 x 3.0 mm column (5 µm particle size) was used for all experiments. For method optimization the influence of various parameters, such as type of organic modifier and type, molarity and pH of the buffer on separation results was investigated. UV detection was performed at 210 and 254 nm and all measurements were conducted under isocratic conditions. With a mobile phase consisting of 5 mM sodium dihydrogen phosphate buffer (pH = 6.9) / methanol (85 : 15 v/v) all 26 tested amphetamines were separated successfully into their enantiomers. The results showed that all analytes were traded as racemic mixtures.

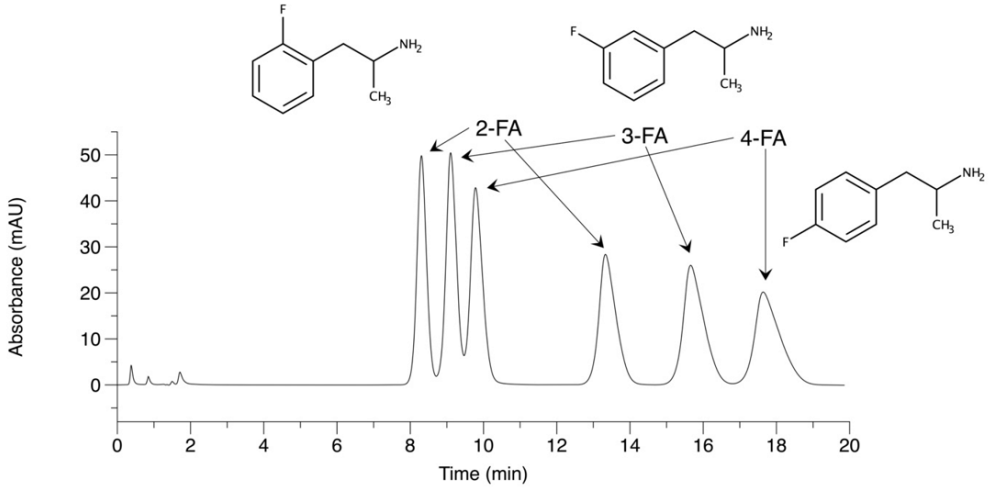

Figure 1: Simultaneous enantioseparation of 2-FA, 3-FA and 4-FA

Conditions: Column: Chiralpak® CBH 150 x 3.0 mm (5 µm), mobile phase: 5 mM sodium dihydrogen phosphate buffer (pH=6.9) / methanol = 85 : 15, 25±1°C, flow: 0.5 mL/min, UV: 210 nm, injection volume: 1 µL

P4

ANALYSIS OF INSULIN FORMULATION BY CAPILLARY ELECTROPHORESIS

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Capillary electrophoresis (CE) has been proven to be an effective technique for determination of the quality of biological medicines. The main advantages of this technique are simplicity, high speed, excellent resolving power, sensitivity, low sample size requirements, low solvent consumption, and ease of automation. Capillary zone electrophoresis (CZE) and capillary gel electrophoresis (CGE) were developed for the separation of charge and mass variants of human insulin and its analogs. CZE permitted the separation of molecules on the basis of their *m/z* ratios, making it a useful approach for the isolation of insulin deamidation products, whereas for the isolation of higher molecular weight transformation products, the CGE method was applied. The Design of Experiment (DoE) technique was employed to increase method performance, optimization, and development of CE methods for the determination of insulin and its analogs. This methodology provided information on the impact of key factors on method development as well as the interaction of significant components in terms of reducing analysis time. The effect of the main influential factors, such as background electrolyte pH and concentration, applied voltage, and temperature, was studied applying response surface methodology (RSM) design using Central Composite Face Centered (CCF) Design. In summary, capillary electrophoresis due to its sensitivity, selectivity is a very suitable method for the determination of quality of insulin formulations.

P5

CHARACTERIZATION OF ISOMERIC LIPID-A SPECIES FROM PSEUDOMONAS AERUGINOSA BY NON-AQUEOUS CE-MS/MS WITH COLLISION-INDUCED DISSOCIATION

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Pseudomonas aeruginosa is a ubiquitous nosocomial pathogen, one of the deadliest bacteria in hospitals. Moreover, this gram-negative bacterium has numerous intrinsic resistance mechanisms against the majority of antibiotics, leading to most antibiotics becoming useless. For these reasons, *P. aeruginosa* is ranked by the World Health Organization (WHO) as a priority number one pathogen for the research and development of new antibiotic strategies. One of the new directions in antibiotic strategy development involves membrane active agents that induces bacterial cell membrane destabilization leading to the loss of its integrity. For this purpose, it is necessary to know the detailed composition and structure of the bacterial cell wall constituents.

The outer leaflet of the gram-negative outer membrane is mainly a highly ordered lipopolysaccharide (LPS) monolayer. LPS molecules (or often called endotoxins) are built up of three distinct structural regions: a polysaccharide called the O-antigen, the core oligosaccharide and the hydrophobic and endotoxic lipid-A portion. Lipid-As – as the membrane-integrated part of LPSs – generally consist of a β-(1′→6)-linked 2-amino-2-deoxy-D-glucose disaccharide backbone carrying phosphate groups at the C4′ and C1 positions, as well as amide and ester linked hydroxy fatty acids or O-acylated hydroxy fatty acids at the C2’/C2 and C3’/C3 positions, respectively. Naturally, lipid-A isolates are heterogeneous mixtures of various lipid-A molecules that differ in the number, the position and the type of acyl chains and phosphate groups.

Herein, a non-aqueous capillary electrophoresis (NACE) method coupled to tandem mass spectrometry (MS/MS) in the positive and negative ionization modes with collision-induced dissociation (CID) activation technique was applied for the deep characterization of the lipid-A isolate of *Pseudomonas aeruginosa* as one of the critical priority targets for antibiotic research. This new and unique strategy has revealed hitherto unreported isomeric monophosphorylated lipid-A constituents in the naturally heterogeneous sample. The electrophoretic separation – orthogonal to the former chromatographic techniques – of *P. aeruginosa* lipid-A ions allowed for the observation and structural evaluation of both, acyl chain and phosphate positional isomers. CID of the separated monophosphorylated species using only the obvious negative ionization mode is insufficient to fully characterize lipid-A, as the phosphate positions remain unresolved. Our new strategy involved the parallel fragmentation of the ions in the complementary positive and negative ionization modes as well, facilitating the full structural assignment of this class of molecules.

The research was supported by the grants ÚNKP-21-2 and ÚNKP-21-4 New National Excellence Program of the Ministry for Innovation and Technology from the National Research, Development and Innovation Fund, NKFIH FK-129038 and K-125275. The research was performed in collaboration with the Mass Spectrometry Core Facility at the Szentágothai Research Centre of the University of Pécs.

P6

HRMS CHARACTERIZATION OF DOXAZOSIN DEGRADATION PRODUCTS GENERATED BY GAMMA IRRADIATION

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In the last decade, pharmaceuticals have attracted increasing attention as emerging pollutants due to their presence in natural waters and their potential negative impact on the environment. It is well known that conventional wastewater treatment processes are not capable of completely removing them from wastewater. Therefore, new and more efficient methods need to be investigated, such as oxidation by highly reactive radicals generated photochemically or by ionizing radiation.[1,2] Several studies have shown that gamma irradiation is a successful and cost-effective method for the degradation of variety of pharmaceuticals.[1,3]

Doxazosin (DOX) is a selective alpha blocker, used in the treatment of hypertension. Due to its widespread use, there is a possibility of its occurrence in aquatic environment. Therefore, development of a suitable degradation method is of great importance. The photolytic and photocatalytic degradation od doxazosin has already been studied, and the main degradation products have been characterized.[4]

In this work, the degradation of DOX by gamma irradiation in the presence of air and N_2O was studied. High-resolution quadrupole time-of-flight (QTOF) mass spectrometry was used for detection and characterization of degradation products. The structures of degradation products were determined based on MS and MSMS spectra, and degradation pathways were proposed under different experimental conditions.

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P7

ANALYTICAL PERSPECTIVES IN THE STUDY OF OLIGONUCLEOTIDES INTERACTIONS

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Affinity capillary electrophoresis occupies a key role among different non-separation and separation-based analytical methods for interaction studies. The interaction of two or several oligonucleotide strands modulate vital physiological phenomena. Moreover, the high affinity and/or selectivity of oligonucleotide-mediated binding offers a myriad of therapeutical and analytical applications, whose rational design implies an accurate knowledge of the involved molecular mechanisms, concurring equilibrium processes and key affinity parameters. Considering the general lack of studies involving ternary ssDNA interactions, a complementary analytical workflow involving capillary gel electrophoretic mobility shift assay and microcalorimetry has been deployed for the characterization of a series of free and surface-bound oligonucleotide interactions. As a proof of concept, the DNA analogue of miR21, a well-known oncogenic short miRNA sequence has been chosen as target molecule, simulating limiting-case scenarios involved in dual molecular recognition models exploited in affinity (bio)sensing. Oligomer-functionalized gold nanostructures are often tested as attractive analytical platforms for the development of fast, label-free optical or electrochemical biosensors. In our study the analytical perspectives of short ssDNA-modified gold nanorods (A2-GNRs) and their affinity towards DNA miR-21 mimic have been assessed.

The CGE separations, with reversed polarity, were performed on a G1600 CE system (Agilent Technologies, Germany) using fused-silica capillaries filled with a buffered dextran-based gel (pH = 8.3) containing 190 mM TRIS, 190 mM boric acid and 0.1 mM EDTA (further referred TBE buffer). High pressure hydrodynamic sample injection (48s at 5 bars) and the default DAD-detection (260 nm) was employed.

The salt conditions (100 mM NaCl, 10 mM $MgCl_2$) potentially involved in conformational changes both during the hybridization and CGE separation process were studied. Even though the presence of inorganic cations are not significant variables in the interaction of DNA miR-21 sequence with the free complementary aptamers, it turns out to be detrimental in the specific binding with the oligomer-modified GNRs. Given the unique features of the separation-based methods, the hybridization stoichiometry (miR21:A1/A2-GNR = 3:1) of the binary and ternary DNA complexes were also conveniently established in the given experimental conditions.

Considering the obtained results, the developed GCE method proved to be an efficient, straightforward and cost-effective investigational tool for the further development and optimization of affinity-based sensors exploiting various signal transduction strategies (i.e. electrochemical or surface-enhanced Raman spectroscopy) for the selective and sensitive detection of miR21 from various biological samples.

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P8

DETERMINATION OF PHENOLIC COMPOUNDS USING CAPILLARY ZONE ELECTROPHORESIS COUPLED TO MASS SPECTROMETRY

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Phenolic compounds are molecules having at least one hydroxylated aromatic rings. These compounds can be divided into several groups, such as simple phenols, phenolic acids, flavonoids, tannins, coumarins, etc. Phenolic compounds as phytochemicals can be found in most plant tissues.

The antioxidant activity and the human health beneficial effects of phenolic compounds in plant foods, red wine, honey or tea is well-known and intensively studied [1].

The phenolic compounds are generally analyzed using GC or HPLC, however CE can be an alternative or complement method to chromatographic separations. The coupling of capillary electrophoresis with mass spectrometry via electrospray ionization combines the fast and efficient separation of CE with the selectivity and sensitivity of MS. Thus it counts an important tool in the characterization of phenolic compounds in samples including complex matrix materials.

In our work 15 phenolic compounds were separated by capillary zone electrophoresis. As a simpliest choice and MS compatible electrolyte a 0.5 M NH_4OH solution was applied as background electrolyte. At this high pH of the electrolyte (pH=11) all phenolic compounds were ionized and completely separated. The electrolyte of high pH also generated a strong electroosmotic flow, which resulted in fast separation. A further advantage of the highly basic running electrolyte was that it provided proper basic conditions (after its merging with isopropanol:water (1:1) sheath liquid) for the negative mode MS detection. The separation and the MS detection parameters were optimized and the analytical performance data were determined. The linear detection ranges for the analyses of the components were generally between 1-200 mg/mL.

An optimized CZE-MS method was applied for the determination of several phenolic compounds in honey. The MS detection revealed several constitutional isomers of the studied phenolic compound, which could be separated from each others.

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P9

FORCE DEPENDENT MULTIMER GLYCOPROTEIN ELONGATION

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The von Willebrand factor (VWF) is a multimeric glycoprotein essential for anchoring platelets to the subendothelium or to other platelets. VWF is a sensor of forces induced by blood-flow variation which are the major stimuli for clotting. The lack of sufficiently long multimers or the excess of too long ones pose risks of bleeding or thrombotic events, respectively. The smallest unit or protomer of VWF is the dimer. A protomer consists of two large, terminal nodules and one small, central nodule which are connected by elastic rods. The overall length of the protomer is 80-120 nm. Considering that the protomer is a head-to-head dimer, the large and small nodules correspond to the N- and the C-terminal domains of the VWF monomer, respectively. Here we investigated the hierarchy of topological rearrangements of the protomer within multimeric VWF exposed to mechanical forces. Samples of plasma-derived VWF were dropped on mica and either incubated for 1 min or immediately extended by molecular combing. Tapping-mode AFM images were collected in air. Unstretched multimers displayed a relaxed random-coil conformation and appeared as flexible, beads-on-a-string filaments. The protomer nodules were nearly touching each other, and the interconnecting elastic rods were rarely visible. VWF multimers exposed to molecular combing displayed axial orientation, chain straightening and extension. The contour length of the investigated stretched multimers ranged between 416-3090 nm, which correlated with protomer lengths of 19-329 nm. Notably, the number of small nodules between the large, terminal ones increased from zero to three with increasing stretch, pointing at the presence of polymorphic structural intermediates. The topographical height of the nodules became progressively reduced with increasing stretch, indicating that the VWF protomer extends at the expense of gradual domain unfolding and uncoiling.

P10

CELLULASE FROM *TRICHDERMA REESEI* AS PSEUDO STATIONARY PHASE IN CE FOR CHIRAL SEPARATION OF AMPHETAMINE DERIVATIVES

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About 830 different New Psychoactive Substances (NPS) are currently observed on the European drug market according to the latest European Drug Report. Though there was a slight decrease in newly created substances during the last years, regulating synthesis and trade of designer drugs remains a difficult task for authorities, which leads to a particular interest in developing specialised analytical methods.

A certain number of these NPS possess a stereogenic centre, especially substances derived from amphetamine. The two enantiomers of each NPS are supposed to have different effects or side effects, which is already known for the parent compound amphetamine, b b ut until today there is little information available about the huge number of derivatives. Therefore, further investigation and development in the field of chiral analysis is of great importance.

For chiral separations, proteins can be used as chiral selectors. Among them, cellulase from the fungus *Trichoderma reesei* turned out to be a potent selector and is already commercially available as a chiral stationary phase for HPLC. However, these chiral columns are cost intensive, considering this, capillary electrophoresis (CE) for enantioseparation can be considered as a cheaper alternative because of low consumption of chiral selector, electrolytes and analytes.

According to literature, pH 3.9 represents the isoelectric point of cellulase. To keep the chiral protein zone pseudostationary in the capillary, this pH was chosen along with 10 mM disodium hydrogen phosphate buffer as background electrolyte (BGE) as well as for sample preparation (1 mg/ml) and solvent of the purchased solid cellulase. Preliminary experiments reveiled cellulase concentration of 500 mg/ml in electrolyte to be the optimal. Prior to measurements, this enantioselective zone was created in the capillary. To prevent undesired interactions between cellulase and silanol groups of the capillary inner wall as well as to suppress potential occurrence of electroosmotic flow, capillaries with a special coating was used. Among different variants which were pre-tested (unpublished results), a methacrylate-coated capillary was found to be advantageous for the enantioseparation experiment.

To date, success of this novel approach was shown by means of amphetamines being abused as NPS, since several were separated or even baseline separated using this method.

P11

PARTICIPATORY STUDY OF CHEMICAL AND BIOLOGICAL CHARACTERISTICS AND PUBLIC HEALTH ASPECTS OF FOG AND PRECIPITATIONS IN CLOSED BASINS OF HARGHITA COUNTY (ROMANIA)

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A growing body of evidence suggests correlation between air pollution, especially particulate matter (PM) concentrations, and morbidity/mortality associated with cardio-respiratory diseases. However, available statistics reflect merely data from studies conducted in North America and Western Europe, while data from Eastern-Central Europe area are scarcely available even on a local scale. Several specific factors – demographic, genetic, meteorological and other local factors – can determine health effects of airborne particles. Air quality monitoring data suggest that people of Eastern-Central Europe inhabit an area with higher air pollution indexes compared to other European territories (http://airindex.eea.europa.eu/,http://berkeleyearth.org/air-quality-real-time-map/).

Based on relevant literature data, PM particles activate pro-inflammatory cytokine response in the respiratory tract involving oxidative stress which causes trachea hyperactivity, increased mucus production and damage of alveolar cells (e.g. alveolar macrophages), which in turn, are responsible for decreased lung and local immunity functions. Moreover, PM exposure can affect the vascular endothelium, the sympathetic nervous system and by inducing general inflammatory responses, can increase especially ischemic cardio-vascular morbidity and mortality rate. Meta-analysis based data (23 mortality and 10 hospital admission databases) suggest correlation between short-term high PM10-2.5 concentration and morbidity as well as mortality rates, especially concerning respiratory, and at a lower rate, cardio-vascular pathologies.

The closed basins of Harghita County represent areas with unique and specific microclimate, where temperature inversions can occur during the winter, with foggy episodes and high PM concentrations, while to our knowledge, there has been no complex investigation concerning physico-chemical parameters, microbiological load and dispersion modelling of air pollutants in fog and precipitation from a public health perspective in the region.

Considering the above, the tackled research topic consists of analysis of fog and precipitation chemistry, PM long-range transport (HYSPLIT modelling approach) microbiome-scale diversity, determination of the occurring chemical processes and possible prediction of health-related effects of particulate pollutants of the specific closed basins of Harghita County.

The aim of our project is to contribute to the development of open science strategies in Harghita County, by tackling a participatory approach to point out, refine and implement a pilot research study concerning possible public health aspects of air pollution.

P12

STATIONARY PHASE INDEPENDENT CHROMATOGRAPHIC METHOD FOR THE SEPARATION OF EZETIMIBE AND ITS RELATED SUBSTANCES

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Introduction: A new scientific approach in chromatographic method development is the experimental design-based retention modeling, supported by special modeling tools or pure statistical-based softwares. This concept is highly welcomed by the regulatory authorities, as outlined in the recently introduced ICH Q14 guideline, which encourages the pharmaceutical industry to implement experimental design-based approaches and to facilitate the understanding of the separation process. The aim of this study was to investigate the impact of stationary phase chemistry on chromatographic method performance using the analytical quality by design approach for the separation of ezetimibe and its related substances.

Materials and methods: A tridimensional experimental design framework with 12 corner runs was realized by systematically changing critical method parameters such as temperature, gradient time, and the ternary composition of the mobile phase. The retention behavior of the molecules was investigated using the DryLab modeling software by establishing the virtual separation models for different stationary phases. The obtained design spaces were compared to reveal overlapped areas, where baseline separation of all peaks is achievable. Ultimately, *in silico* robustness testing was realized to identify critical method parameters with a high impact on the separation process.

Results: In the preliminary phase nine different chromatographic columns were tested with different chemistries and all separation systems were optimized individually to obtain the highest critical resolutions. Afterwards, the design spaces were compared and from the common area a column independent working point was selected and tested experimentally for all nine stationary phases. This common working point was able to present higher than 2.0 critical resolution, achieving the baseline separation of the peaks on each of the columns tested. Based on the robustness testing the most critical method parameter is the structure of the gradient, independently of the stationary phase type.

Conclusion: Using this experimental design-based approach and retention modeling it was proven that the separation of the model analytes can be achieved on multiplex stationary phases and column-independent working points can be identified, which will allow baseline separation of all analytes, regardless of the column employed. Our results underline that a deep understanding of the separation process is of utmost importance and that adequate selectivity is achievable in some cases independently of the chemistry of the stationary phase.

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P13

LINK BETWEEN BIOANALYSIS AND BIOPHYSICS

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Bioanalysis is a sub-discipline of analytical chemistry, quantitative measurement of xenobiotics (drugs and their metabolites, and biological molecules in unnatural locations or concentrations) and biotics (macromolecules, proteins, DNA, large molecule drugs, metabolites) in biological systems.
Biophysics is the study of physical phenomena and physical processes in living things, on scales spanning molecules, cells, tissues, and organisms. Biophysicists use the principles and methods of physics to understand biological systems. It is an interdisciplinary science, closely related to quantitative and systems biology.
See below some presentations of CEEPUS Summer School 2022 completed with citations of recent publications which explore links between bioanalysis and biophysics.

Anna Harsányi, Attila Kardos, Imre Varga; Preparation of advanced intelligent materials by chemical modification of carboxylic acid groups of pNIPAm copolymer microgels [1].

Gyongyver Mara: A comparative study of MALDI-TOF MS and 16S rDNA identification of PGPR bacteria [2].

Alexandra-Iulia Bărăian: Molecularly imprinted polymer-based drug delivery system for the sustained release of ruxolitinib in cancer therapy [3].

Kamil Bortka: Biochar as the "green" tool in soil remediation [4].

Nives Galić: Investigation of praziquantel/cyclodextrin inclusion complexation [5].

I believe that common interests and possibilities in our Department (Semmelweis University, Budapest, Hungary; http://biofiz.semmelweis.hu/index.php?mid=1) elicit fruitful collaboration with the CEEPUS friends in the near future.

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P14

DEVELOPMENT OF HIGH-RESOLUTION MICROFLUIDICS METHODS FOR FAST DETERMINATION OF L-HISTIDINE AND β-ALANINE IN DIETARY SUPPLEMENTS FORMULATIONS

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Expanding usage of dietary supplements in today's modern society arouses the question regarding their safety. International regulations corresponding to their content and safety controls have not been strictly established. Several risks can be related considering the weak control of these products; for instance, contamination of dietary supplements, amino acids overdose, or in the adverse case, the consumer can use an ineffective dose [1]. Enacting legislation for regular control of amino acids in dietary supplements would raise a new issue - demand for eluding the common problems associated with the conventionally utilized analytical methods. This report describes the development of new microchip electrophoresis (MCE) method to determine L-histidine and β-alanine in complex dietary supplement formulations. L-histidine and β-alanine containing dietary supplements are used by athletes since they improve endurance during intense exercise. Furthermore, L-histidine is used in dietary supplement formulations to enhance the immune system. For quantification of L-histidine and β-alanine, this study employed a contactless conductivity detector (C^4D). The effect of various parameters was observed, aiming to achieve an appropriate detector response and optimal electrophoretic conditions. Established parameters were applied during the analysis of three different dietary supplement samples. All three samples contained one amino acid and different secondary active ingredients. Sample ingredients were baseline separated, and the calculated resolution was high without exception. The measured concentrations of amino acids were agreed with the labeled content. The separation was rapid; migration time for L-histidine was 38 (±0.2) s, and for β-alanine was 26 (±1) s. Detector response to both analytes showed high linearity in the tested range of 10 - 100 µmol / L. Detection limits were below 1 mg / L. The analytical characteristics of the proposed microfluidic method showed great potential for further analysis of different amino acids in these types of samples. Furthermore, the method is simple, fast, and cost-effective. It belongs to green analytical chemistry since negligible amounts of samples and reagents are required.

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P15

Influence of β-cyclodextrin and its various derivatives on cinnarizine solubility in water

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Cinnarizine (CIN) is a piperazine derivative with antihistaminic and calcium channel blocking activity which is widely used for the treatment of vertigo and prevention of motion sickness. Due to its extremely poor aqueous solubility, CIN is classified as a Class 2 drug substance according to The Biopharmaceutical Classification System [1]. β-Cyclodextrins (β-CDs) are cyclic oligosaccharides consisting of seven D-glucopyranose units which are α-(1,4)-linked in a ring formation. It is already well known that β-CDs can interact with poorly soluble drugs by taking up lipophilic drug moieties into their central cavity hence forming inclusion complexes and thus enhancing drug aqueous solubility and bioavailability [2].

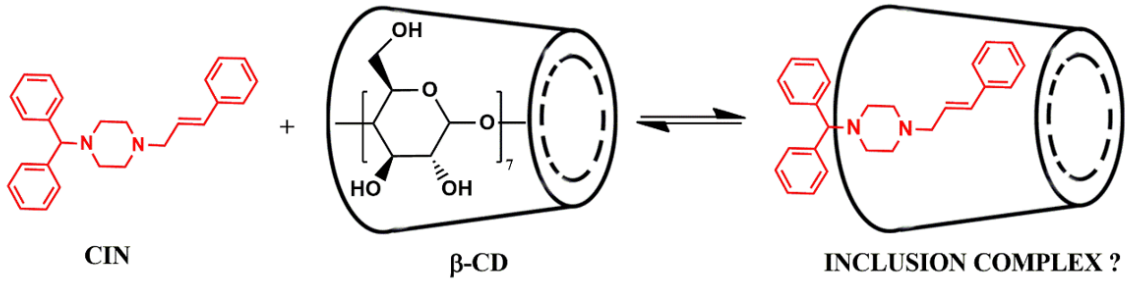

Figure 1. Cinnarizine and β-cyclodextrin

Our study aimed to evaluate the influence of β-CD and its randomly methylated (RMβCD), sulfobutylether (SBEβCD), and hydroxypropyl (HPβCD) derivatives on intrinsic solubility of CIN in water through phase-solubility studies according to existing Higuchi-Connors method [2]. To evaluate total drug solubility changes in the presence of increasing CD concentration phase-solubility diagrams were constructed. Quantitative determination of CIN was performed by developed and validated UV/Vis spectrophotometric and spectrofluorimetric methods.

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P16

MOLECULARLY IMPRINTED POLYMER-BASED EC-SERS SPECTROELECTROCHEMICAL DETECTION OF MOXIFLOXACIN

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Bacterial resistance is a subject of high importance these days, more and more common infections being treated with late generation antibiotics, that were used as the last resort for complicated infections in the past. Moxifloxacin is an antibiotic belonging to the fluoroquinolone pharmacological class, used to treat complicated urinary, respiratory and skin infections. Fluoroquinolone antibiotic class was registered on the pollutants watch list of the European Commission, and since late generation antibiotics are starting to be used in treating common infections, monitoring its levels in different environmental samples would be of great importance.

Molecularly imprinted polymers (MIPs) constitute a class of biomimetic receptors designed for the specific recognition of a certain molecule or it's structural class, by assembling around it during the polymerization step, and following template removal, tridimensional sites complementary to the template are left inside the polymer. The imprinted cavities are thus capable of selectively trapping the same molecule in the polymeric matrix. The advantages they offer over the biological counterparts, like antibodies and aptamers, is their reduced time and cost of synthesis, resistance to all kinds of harsh contidions like temperature, oxidation and number of uses.

Electrochemical (EC) methods are exploited with the aid various of analytical devices and sensors, providing fast and accurate results, but with a limited level of selectivity in the absence of a proper recognition element. Raman spectrometry is a technique that provides structural and fingerprint-type molecular information, and even though on a limited range of concentrations, but filling the need also for quantification purposes. On the other hand, surface-enhanced Raman scattering (SERS) in the presence of metallic nanostructures (i.e. nanoparticles, nanorods, nanostars, etc.), like gold and silver as the detection substrate is able to offer a Raman signal amplification up to 10 orders of magnitude. Despite the prospects of combined advantages, such as the sensitivity brought by electrochemistry and the selectivity provided by molecular spectroscopy, analytical applications describing their simultaneous use is rather scarce in the current literature.

As such, various means by which electrochemistry may assist the SERS detection of traces of moxifloxacin using MIPs as biomimetic molecular recognition elements has been investigated in the present study. The MIPs designed for moxifloxacin detection were synthesized using electro- and photopolymerization. The thin layer of MIP resulting from the electrochemical polymerization of p-aminothiophenol: p-aminobenzoic = 1:3 (molar ratio) demonstrates selectivity towards moxifloxacin, but its functionality needs careful adjustments along with the nanostructured SERS substrate for their fully functional integration for Raman spectroelectrochemical analysis.

This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2019-5473, within PNCDI III.", also the internal grant of Iuliu Haţieganu University by PCD grant no. 881/27/12

P17

THE COMPATIBILITY STUDIES BETWEEN EXCIPIENTS AND ACTIVE SUBSTANCES IN POWDER DOSAGE FORM USED IN HYPOPOTASSEMIA BY FT-IR ANALYSIS

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Introduction: The Fourier Transform Infrared (FT-IR) spectroscopy is one of the methods used in the drug-excipient compatibility studies to detect the possible interactions between active pharmaceutical ingredient (API) and excipients. Definitely, not all drug-excipient interactions are so bad, but the majority of them are life-threatening, therefore it represents an essential phase in the development stage (pre-formulation) of dosage forms. Potassium aspartate, magnesium aspartate, potassium orotate and spironolactone were used as APIs applied in treatment of hypopotassemia (the serum potassium level less than 3.6 mEq/L), because this fixed-dose combination can ensure both the potassium supplementation and etiological treatment. Hypopotassemia is a common electrolyte disorder with symptoms: weakness and discomfort of muscles to the arrhythmias, or abnormal heart rhythms, muscle cramps (paralysis), respiratory failure, etc. Despite the FT-IR spectroscopy the Differential Scanning Calorimetry (DSC), High-Performance Liquid Chromatography (HPLC) are carried out to show drug-excipient compatibility.

Object: The aim of this work was to study the compatibility between excipients and API (spironolactone) during the elaboration of the powder dosage form used in hypopotassemia.

Material and methods: FT-IR measurements were carried out on spironolactone and excipients (dextrin, saccharin, D-mannitol and magnesium stearate) as well as on the physical mixture of both components (50:50) in the transmission mode. Spectra were recorded on a FT-IR Spectrometer Spectrum 100, Perkin Elmer in the range of 4000-650cm^{-1} at a resolution of 4cm^{-1}. Then transmittances in terms of the peak frequency, peak intensity, peak width were compared and analyzed by absences or appearance of new peaks.

Results: The FT-IR spectrum of spironolactone showed the following characteristic bands: C-H stretching vibration bond at 2949*cm*-1, a band for stretching vibration of carbonyl group of γ-lactone at 1764*cm*$^{-1}$, a band for a stretching vibration of thioacetyl carbonyl group at 1689*cm*$^{-1}$, stretching vibration of the carbonyl C=O bond of the ring at 1672*cm*$^{-1}$, and stretching vibration of the C=C bond at 1617*cm*$^{-1}$. These bands were considered as specific markers to recognize spironolactone. The FT-IR spectrum of each excipient has its own unique vibrational characteristics and therefore results in unique IR spectral signatures. Following the dates through FT-IR of mixture of both components (50:50)**,** there is no change in functional group region of spironolactone when mixed with excipients.

Conclusions: According to analysis of FT-IR spectra on spironolactone and excipients, as well as on the mixture of both components, it was shown the compatibility of these components in the future fixed-dose drug combination**.**

P18

DESIGN OF INNOVATIVE NANOSTRUCTURED PLATFORMS FOR THE ELECTROCHEMICAL DETECTION OF GLUTEN

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Gluten is the generic name for the proteins found in many types of cereals, including wheat, rye, barley and oats. This compound helps maintain the structural integrity of these foods, acting as a binding agent. Despite of its importance in this context, gluten is also an allergen, its presence in these foods being dangerous for people who are allergic or intolerant to this compound. Moreover, gluten can be found even in products where it is not expected, raising food safety concerns for allergic persons or celiac disease patients. Therefore, the detection of the presence of gluten in food is of particular and practical importance [1].

The main aim of the study was to design a nanostructured platform for the rapid, sensitive and selective detection of gluten in foods with prospects for applications in food industry.

The first approach was related to the direct electrochemical detection of gluten on in-house printed carbon electrodes. Two different platforms were used: bare printed electrodes and printed electrodes functionalized with gold nanoparticles (AuNPs). An attempt was thus made to correlate the electrochemical signal obtained by the electrochemical oxidation of gluten with its concentration in the analyzed sample. The results obtained on real samples of wheat flour were promising, but the recovery rates for spiked samples were quite low, justifying the need for a more complex experimental approach, involving components that aim to increase the selectivity for the target analyte.

The second approach refers to the development, optimization and testing of an aptasensor for the selective and sensitive detection of gluten. To do this, we started from the same printed platform functionalized with AuNPs on which a specific aptamer for Gliadin 4 (an important component of gluten) was immobilized through stable thiol-gold bonds.

This aptasensor has been shown to be sensitive and selective for the target analyte, even in real samples of wheat flour, which recommends it for applications for food quality control, to prevent some serious health complications such as food allergies and celiac disease.

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P19

PROCESS OPTIMIZATION FOR ULTRASOUND-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM *ALLIUM URSINUM* LEAVES

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Allium ursinum L. is widely used as a spice as well as a traditional medicine in Romania. The aim of this study was to optimize the extraction process of *A. ursinum* leaves. The experiments were carried out according to tree level, three variables D-optimal design, combined with response surface methodology (RSM). Solid to liquid ratio (SLR, from 1:5 to 1:15 (*w*/*v*)), ethanol concentration (from 30% to 70%) and extraction time (from 5 to 30 min) were investigated as independent variables in order to obtain the optimal conditions for extraction and to maximize the total phenolic content of obtained extracts. Experimental results were fitted to a model where multiple regression and analysis of variance were used to determine the fitness of the model and optimal condition. The predicted values for the TPC (167.552 μg GAE/mL extract) were determined at the optimal conditions for ultrasound assisted extraction: 66% ethanol, for 5 min, with a SLR of 6 (*w*/*v*). The predicted results matched well with the experimental results obtained using optimal extraction conditions which validated the RSM model with a good correlation. These results suggest that the method can be used in order to produce extracts enriched in bioactive compounds, obtaining high extraction yields by applying optimal parameters.

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P20

WORKFLOW FOR STRUCTURAL ELUCIDATION OF PROTONATED AND DISODIATED LIPID A MOLECULES USING LOW ENERGY CID TANDEM MASS SPECTROMETRY

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Lipid A, the inflammatory part of lipopolysaccharides (LPS, endotoxins), is the major component of the external membrane of Gram-negative bacteria. Its bioactivity in people and animals depends on its chemical structure. In the present study, the application of positive-ion electrospray ionization multistage mass spectrometry (ESI-MS^n) in conjunction with tandem mass spectrometry (MS/MS), using low-energy collision-induced dissociation (CID) for the analyses of the protonated form and disodium adduct of monophosphorylated lipid A derivatives is presented. Several synthetic and native lipid A samples were included in the study. We have seen a great similarity between the fragmentation pattern of lipid A as a disodium adduct $[M - H + 2Na]^+$ and a deprotonated molecule $[M - H]^-$ (usually detected with negative ion mode), both revealing the distribution of fatty acids, whereas the fragmentation pattern of lipid A as a protonated molecule $[M + H]^+$ is unique for the determination of the phosphorylation site. In short, the complementary information obtained from the fragmentation of these two types of precursor ions enables complete structural characterization of a 4'-monophosphoryl lipid A species in a single ionization mode, even from complex mixtures. In addition, this study allows a better understanding of the overlap of MS signals often observed during the fragmentation of native lipid species A containing both, C-1 and C-4' phosphorylation isomers.

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P21

DIRECT ELECTROCHEMICAL DETECTION OF KYNURENIC ACID IN BIOLOGICAL SAMPLES - A STEP TOWARDS POINT-OF-CARE DIAGNOSIS

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Kynurenic acid (KA) is one of the active metabolites of tryptophan obtained through the kynurenine pathway [1] that plays a crucial role in several physiological and pathological processes due to its effects such as antioxidant, neuroprotective and anti-inflammatory function [2]. It has been observed that KA is present in various biological fluids and its levels change in some diseases. For example, its level decreases in schizophrenia and prostate cancer, but increases in type 2 diabetes and chronic kidney disease [2]. Sensitive and selective detection of KA in biological samples is therefore of major importance in clinical diagnosis, monitoring the evolution of chronic diseases and the response to drug treatment. Electrochemical methods represent a better approach for the analysis of biological samples due to their many advantages, such as high sensitivity and specificity, low cost, suitability for miniaturization and *in situ* analysis [1]. Among electrochemical sensors, wearable sensors present a real interest particularly for the detection and monitoring of biomarkers in less conventional biological samples like sweat and saliva. This interest arises from the possibility of monitoring a person's health status in real-time, in an inexpensive and noninvasive way [3].
The main purpose of the study was to design a platform for the rapid, sensitive and selective electrochemical direct detection of KA in biological samples with prospects for biomedical applications in the diagnosis and monitoring of patients.
To obtain integrated portable and wearable devices, respectively, to ensure reproducibility, the planar carbon electrodes were in-lab printed by using conductive and insulating inks. Firstly, the electrochemical behavior of KA was analyzed regarding the influence of electrode material, pH, electrolyte solution and scan rate. After this, the proper electrode material, electrolyte solution and scan rate were chosen and a differential pulse voltammetry (DPV) procedure was optimized for the detection. Finally, the analytical parameters such as dynamic range, detection limit, limit of quantification, and sensitivity for KA were also determined. The selectivity of the optimized sensor was tested in the presence of compounds that are usually found in biological fluids. Future applications of the elaborated sensor in sweat and saliva will be presented.

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P22

APTAMERS AS BIORECOGNITION ELEMENTS FOR THE ELECTROCHEMICAL DETECTION OF QUORUM SENSING MOLECULES

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An important medical concern is represented by the failure of antibiotics to treat bacterial infections, which leads to high mortality rates. *P. aeruginosa* is among the most highly virulent and antibiotic-resistant bacteria, its virulence is owned to the ability to form an adherent biofilm on the affected surfaces that increases the resistance to disinfectants, UV light and antibiotics. The bacteria communicate through a system called quorum sensing (QS) by producing molecules like N-3-oxo-dodecanoyl L-homoserinelactone (3O-C_{12}-HSL) and cyclic dimeric guanosine monophosphate (c-di-GMP). When a threshold of population density is achieved, it triggers the switch from planktonic form to biofilm and the production of virulence factors [1]. To increase the chances of survival of a *P. aeruginosa* infection, early diagnosis is required.

In this study, new electrochemical biosensors were developed as a diagnosis tool for the selective and sensitive detection of molecules involved in QS (3O-C_{12}-HSL) and biofilm formation (c-di-GMP). The support electrode used was a portable and easy to use carbon screen printed electrode (C-SPE) that was then modified with gold nanoparticles (AuNPs). Biomimetic elements represented by thiol functionalized aptamers, specific for each QS molecule, were integrated into the AuNPs-based sensing platform to increase the specificity of the method. A suitable redox probe was chosen and every modification step was optimized. The electrochemical characterization methods were represented by differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) using $[Fe(CN)_6]^{3-/4-}$ as a redox probe. The developed aptasensors were used to identify the analytes from biological and real culture samples with good recoveries. This method showed good sensibility and specificity and can act as a promising tool in detecting healthcare associated infections.

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P23

DEVELOPMENT OF DNA METABARCODING ASSAY FOR SPECIES IDENTIFICATION OF EDIBLE *MOLLUSCA*

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Seafood is the second group of foodstuff that is considered to be mostlikely mislabelled especially in case that the original ingredients are no longer recognizeable [1].Additionally, seafood might cause severe allergic reactions, thus a reliable detection isalso a matter of food safety [2]. The term seafood includes crustaceans, bivalves (suchas oysters, mussels, scallops, clams, razor shells and sheath shells) and cephalopods aswell as frogs, turtles and sea cucumbers. Bivalves and cephalopods are taxonomicallyclassified as molluscs. Other molluscs that are included in the human diet and present analternative protein source to ’traditional’ meat are snails.

For the identification of food fraud with regard to marine and non-marine molluscs aDNA metabarcoding method is developed. The method consists of different steps:first,an extraction of DNA from the sample is performed. Then a specific target region of theDNA is amplified by real-time polymerase chain reaction (PCR). A library is preparedthat contains all specifically labelled PCR products. These are sequenced with next generation sequencing (NGS) technology. The received sequences are then analyzed withthe application of a bioinformatic pipeline and compared to reference sequences whichallows the identification of the species in the sample [3]. In contrast to real-time PCR, the goal is not to design specific but universal primerswhich bind to the DNA of a variety of species from different classes of molluscs. Formetabarcoding analysis, mitochondrial DNA(mtDNA)is usually appliedas a marker sincemore copies of themtDNA are available than of genomic DNA. Due to its ring struture, the mtDNA is morerobust, too [4]. Both of these aspects are important since degradation of DNA often occursin processed foodstuffs due to harsh influences such as temperature [5]. For the identification of different seafoods, mitochondrial cytochrome oxidase subunit 1 (COI)and cytochrome b (cytb)genes were frequently used astarget loci because they proved to have high intraspecies diversity [1]. Previous workshave shown the suitability of the 16S rDNA gene as the target locus because of its highconservation between classes. This makes it possible to design primers of higher universality for this gene [6]. DNA metabarcoding methods are already applied for the identification and determination of different foodstuffs like fish feed, milk and dairy products, rice, noodles, meatproducts and candies [5]. Meanwhile, not many metabarcoding methods are in placefor the analysis of molluscs from different classes or families [3]. While some of thesemethods used primers designed for the COI and cytb gene, other methods were developed with regard to the 16S rDNA gene (sometimes combined with the COI gene) forthe identification of species from the same family or class that are of interest to this work[3]. An in-house method was alreadydeveloped for the identification of mussels (Mytilidae), oysters (Ostreidae) and scallops (Pectinidae). For each class, specific primerswere designed that bind to the 16S rDNA gene,resulting in an amplicon of 150 bp. DNA extraction was achieved by extraction with hexadecyltrimethylammonium bromide (CTAB) buffer and Proteinase K. Afterwards, DNAisolation was performed with the Maxwell®16 FFS Nucleic Acid Extraction SystemCustom-Kit. The following conditions were applied during PCR: DNA input amount of12.5 ng,HotStarTaq Master Mix Kit, annealing temperature

of 62 °C, 25cycles. Instruments fromIllumina were applied. The method was succesfully used for theidentification of pure samples and sample mixtures; even minor components were identified aswell as ingredients in highly processed foods [4]. The current aim of this workis to create a new primer system, so that processed food samples can be screened for thepresence of additional molluscs like snails and cephalopods in the longterm.

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P24

ENZYME ASSISTED EXTRACTION OF AVOCADO (*PERSEA AMERICANA MILL*) SEEDS

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Enzyme-assisted extraction is a feasible, efficient alternative extraction method which is gaining consideration in food industry. A considerable part of bioactive compounds from plants are often bound in cell wall from carbohydrates, peptides and lipids, being difficult to be extracted by conventional extraction methods. Enzymes are used as a plant material pretreatment to facilitate extraction step, to increase the yield and to enrich the extract in bioactive compounds.

Avocado (*Persea americana* Mill) is originated from Mexico, and its benefits have been lately recognized. The consumed part from avocado is the pulp while the skin and seeds are considered wastes. These residues contain a high amount of bioactive compounds, but are unexploited.

The objective of this work is to assess the bioactive compounds extracts obtained by applying an enzymatic treatment on avocado seeds and to evaluate the potential incorporation of these extracts in cosmetic sun protection emulsions.

Seven hydrolytic enzymatic preparates part from pectinase, lipase, amylase and protease classes were used for an enzymatic pretreatment of seeds material. Spectrophotometric methods were used to analyze the achieved extracts in terms of total polyphenolic content (TPC), flavonoid content, to determine the solar protection factor (SPF) and antioxidant activity. The amino acids content and fatty acids profile analysis were performed on GC-MS. There emulsions were prepared as water/oil formulations containing different concentration of the obtained extract.

The effect of enzymatic treatment on avocado seeds was observed on all bioactive compounds analyzed. An increasing with 25% in content of polyphenols was found for the extract obtained after using pectolytic preparate pretreatment (commercial named Pectinex). A similar trend was observed for the flavonoid content and antioxidant activity. The highest SPF 26.52±0.08 and 24.39±1.43 resulted for the extracts achieved after using Pepsine and Lypozyme pretreatment, two enzymatic preparates with proteolytic and respectively lipolytic activity. In the case of free amino acids quantification, Pepsine showed an important effect. Regarding the fatty acids extraction, lypolitic preparates Lypozyme and CalB demonstrate a significant influence. Analyzing the solar protection factor of the obtained emulsions, by incorporating 2% of 100 mg/ml enzymatic avocado seed extract in the cosmetic emulsion, a 6.91±0.30 SPF was achieved.

The high content of antioxidants, free amino acids and fatty acids found in avocado seeds extract, offers a new potential application in cosmetic formulation and food industry. The really high obtained SPF and antioxidant activity makes avocado seeds extract a natural sunscreen with beneficial effects for the skin health and a feasible substitute to traditional harmful chemicals. Enzyme-assisted extraction shows a real alternative in obtaining plants extracts with higher content in bioactive compounds.

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P25

NACE-ESI-MS/MS METHOD FOR THE SEPARATION AND CHARACTERIZATION OF PHOSPHATE AND ACYL CHAIN POSITIONAL ISOMERS OF BACTERIAL LIPID A

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Bacterial lipid A is a glycophospholipid moiety present in endotoxins (lipopolysaccharides) that build up the outer cell membrane of Gram-negative bacteria. Once lipid A enters into the blood circulatory system of the human body, it is capable of stimulating an immune response that (during a severe bacterial infection) may lead to endotoxic shock and sepsis. Not only the quantity, but also the fine structure of lipid A from different bacterial species determine the strength of the induced immune response.

With the help of separation science, we directed our research to accurately understand the structure of lipid A and to explore the type of phosphorylation sites and fatty acyl chains attached to the basic sugar backbone. We succeeded in developing a pressure-assisted non-aqueous capillary electrophoresis (NACE) – tandem mass spectrometry (MS/MS) method for the baseline separation of both, phosphate and acyl chain positional isomers. We have studied both, the effect of the solvent composition and the electrolyte composition to separate the different lipid A species present in a single bacterial strain (*Shigella sonnei*). As a result, we could achieve for the first time separation and full structural identification of C1‑ and C4'‑monophosphorylated isomers, which are known to differently activate the innate immune system. Structural information specific to phosphorylation was provided by MS/MS mass spectra recorded in the positive ion mode (it should be noted here that most studies use the negative ionization mode MS/MS for the structural analysis of lipid A). Based on our results, diagnostic B_2-type ions (used in sugar chemistry) identify the phosphorylation position, while B_1-type ions allow the characterization of acylation isomers. The application of constant external pressure (3 mbar) from the inlet was needed to overcome the cathodic mobility of the EOF as to ensure detection at the anodic end.

The present method can complement the LC-MS/MS methods previously developed to probe bacterial endotoxin samples, moreover, it can be a good screening procedure of phosphate positional isomers in different lipid A‑based therapeutics.

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P26

PREPARATION OF ADVANCED INTELLIGENT MATERIALS BY CHEMICAL MODIFICATION OF CARBOXYLIC ACID GROUPS OF PNIPAM COPOLYMER MICROGELS

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Intelligent materials attracted significant interests due to their favorable and tunable physicochemical properties, which make them a promising candidate in many fields of applications (e.g., biosensors, advanced microreactors, responsive microcapsules etc.).

One of the most popular intelligent materials is the temperature sensitive pNIPAm (poly(N-isopropylacrylamide) microgels. pNIPAm microgels are synthesized with precipitation polymerization. The advantages of this technique are the monodisperse particle size distribution and the easy copolymerization of several kinds of comonomers. However, precipitation polymerization also imposes some limitations on the quantity and quality of the applied comonomers and imprinted materials, e.g., biomacromolecules.

To overcome these difficulties our group has applied a simple, yet robust synthetic route for the preparation of advanced pNIPAm based materials. First, acrylic acid containing pNIPAm microgel particles with different radial comonomer distributions (homogenous and core-shell like structure) were synthesized. Then, the carboxylic acid groups of the microgels were functionalized, e.g., with one or two steps EDC/NHS coupling reaction.

In one example, the shell of hierarchical pNIPAm-shell-pAAc core-shell microgel particles were modified in a single step EDC coupling with an amine-containing cationic polyelectrolyte (polyethylene imine - PEI) to obtain pNIPAm-shell-PAAc-PEI mixed shell nanocapsules. A series of experiments was conducted to examine the pH and synthesis time dependence of the reaction conditions on the composition of the nanocapsule.

In another example, a simple approach was developed for the preparation of Bovine Serum Albumine (BSA) functionalized pNIPAM microgels and it was demonstrated that the microgel bound proteins can form red fluorescent complexes with Au(III) ions similarly to the free proteins. For the preparation of pNIPAm-co-AAc/BSA/Au complex microgel a two-step EDC coupling was applied. This approach allowed not only the preparation of bright red fluorescent nanogel beads but by the functionalization of the external shell of the nanogel particles their targeting could also be achieved.

P27

PHARMACEUTICAL AVAILABILITY STUDY OF THE COMBINED CAPSULES USING DISSOLUTION TEST

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Introduction. During the development of new drug formulations one of the main objectives is to develop an effective and stable drug, starting from an active pharmaceutical ingredient (API) with optimal characteristics and ending with the production of a robust formulation. Ensuring of the bioavailability constant is a challenge for the drug combinations, due to the changes that may occur in the release (dissolution from dosage form) of APIs and their pharmacokinetic parameters. In the process of design selection, formulation technology requires control over the release and absorption to ensure the effective therapy. The use of the dissolution test *in vitro*, which would reflect gastrointestinal conditions *in vivo*, is a common practice for predicting the pharmacokinetic behavior of the future drug formulations. The dissolution test is the only test used to measure the *in vitro* drug release from pharmaceutical forms as a function of time. That is based on the evaluation of the dissolution profiles, or release, of APIs. Due to this, it allows to understand the processes occurring at the level of the gastrointestinal tract, in particular, the influence of critical composition or manufacturing process parameters on the dissolution and subsequent absorption. In the present study, combined capsule formulations containing nicergoline, piracetam and Hawthorn dry extract with application in neurosensory hearing loss medication were investigated.

Aim of study. Application of the dissolution test in assessing of the pharmaceutical availability of combined capsules containing nicergoline, piracetam and Hawthorn dry extract.

Material and methods. Agilent 8453 UV-VIS Spectrophotometer; Electrolab TDT8 Dissolution Tester; 4 formulations of combined gelatin capsules (experimental laboratory series) with piracetam, nicergoline and Hawthorn dry extract; dissolution media: 0.1 M hydrochloric acid HCl (pH=1-2) and phosphate buffer (pH=6.8); sampling at 5, 10, 15, 20, 30, 45 and 60 min; sampling volume - 10 ml, dilution with methanolic solution of 0.1 M HCl to concentrations of 24 μg/ml for piracetam and 9 μg/ml for nicergoline.

Results. Following the dissolution test in acid medium, the absorbance of samples taken at different time intervals was within 0.0006 – 0.8 for nicergoline and 0.1-1.3 for piracetam. After calculating the concentration of each substance, the optimal release of nicergoline from formulation F2 (78.71% for 45 min) and piracetam from formulations F1 and F2 (90.89% and 75.52% for 45 min., respectively) was found. Analysis of the dissolution profiles of nicergoline and piracetam from combined capsules in the phosphate buffer medium with pH=6.8 showed insufficient release for all four formulations with concentrations of samples taken at 45 min. for nicergoline and piracetam ranging from 25-40% and 10-16%, respectively.

Conclusions. Following the analysis of the dissolution profiles for nicergoline and piracetam from the tested formulations, it was found that the release in the acid medium was better than in the phosphate buffer. Both substances were found in the dissolution medium at concentrations above 75% in 45 min., that suggests close pharmacokinetic profiles.

P28

ssDNA APTAMER SELECTION FOR GLYCOPEPTIDE ANTIBIOTICS

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Since the discovery of penicillin many decades ago, multiple species of bacteria have responded to the use of antimicrobial agents with their ability to develop and transmit antimicrobial resistance, becoming a threat to human and animal health. Glycopeptides such as vancomycin or teicoplanin are among the most frequently used antibiotics for the treatment of severe infections like endocarditis, pneumonia and meningitis caused by antibiotic-resistant Gram-positive pathogens, particularly methicillin-resistant Staphylococcus aureus (MRSA) or enterococci. As bacterial resistance is a more and more potent global healthcare issue, antibiotic monitoring is highly required [1].

Aptamers are short nucleic acid strands capable of selectively interacting with the target molecule similarly to the antigen-antibody interaction but come with the advantages of having superior stability to antibodies and can be artificially selected in the laboratory through a process called systematic evolution of ligands by exponential enrichment (SELEX). In the recent years, with the continuous development of the SELEX technology, magnetic beads have become more and more used in the selection process. Having good dispersion properties, uniform particle size distribution and the ability to respond quickly to the applied magnetic field they make the separation process between target and sequences fast and effective. After the selection and characterization steps, the selected aptamer can be incorporated into different electrochemical sensors for fast and specific detection and monitoring of different analytes [2].

Our current progress in the selection of a vancomycin specific new aptamer *via* magnetic beads-based SELEX technology is presented in this poster. The selected aptamer will be further incorporated into an electrochemical sensor for the detection of vancomycin from different biological and environmental samples.

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P29

DETERMINATION OF TRACE PERCHLORATE IN RADISH SAMPLES BY ON-LINE COMBINATION OF CAPILLARY ISOTACHOPHORESIS AND ZONE ELECTROPHORESIS

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It is important to determinate perchlorate in food because as a contaminant, perchlorate can have significant impact on human health [1], because it hinders the production of hormones by thyroid simply by blocking the iodine uptake route [2]. Method US EPA (314.0) [3] have been set as reference method for determination of perchlorate, however, among routinely applied analytical techniques used for perchlorate determination are spectrophotometry, ion chromatography, mass spectrometry and capillary electrophoresis (CE) [4]. CE is an analytical separation technique with attributes such as low reagent consumption, high separation efficiency, easy automation and low running costs. Thanks to column coupling technology, combination of isotachophoresis (ITP) and zone electrophoresis (CZE) can be utilized for perchlorate determination. ITP's large sample loading capacity, ability to eliminate interferents and its high concentrating power in combination with high efficiency and short analysis time of CZE make cITP-CZE suitable for analysis of complex samples in general [5].

The aim of this work was to develop fast and sensitive cITP-CZE method for the determination perchlorate in radish samples. Separations were performed in a fully automated separation system working in column coupling configuration which allows on-line combination of these two techniques. Perchlorate in radish samples was identified and quantified using standard addition method. Addition of polyvinylpyrrolidone into leading electrolyte in ITP step assured separation of perchlorate from other anions, especially anionic macroconstituents, while α-cyclodextrine was used to resolve perchlorate from anionic impurities originating from electrolytes and from thiocyanates naturally present in radishes.

The linear range of the method was from 5 to 800 nmol/L (R^2 value based on peak area equal to 0.9995). Optimized method showed very good repeatability of both migration times and peak areas, RSD calculated from results of eight times repeated analysis of radish sample at 3 concentration levels were less than 2%. The estimated limit of detection (LOD) for the presented method was less than 5 nmol/L with good repeatability of results. This method shows good selectivity and precision while maintaining high sensitivity and sample throughput around 100 samples per day.

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P30

CHARACTERIZATION OF APTAMER-FUNCTIONALIZED MAGNETIC NANO-CARRIERS FOR THE TARGETED TREATMENT OF HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) is the most common type of liver cancer and is ranked among the first five causes of cancer-related mortality worldwide. Sorafenib, the drug of choice for advanced-stage HCC has certain limitations that can be overcome by its encapsulation into nano-carriers for targeted drug delivery. To increase the selectivity of nano-carriers towards tumors, they can be functionalized with cancer cell-specific aptamers.

In this work, two types of magnetic nano-carriers were used, namely: azelaic acid-functionalized magnetic nanoparticles (MNP) and poly tartaric acid-functionalized magnetic nanoclusters (MNC). The carboxyl groups on the surface of the magnetic nano-carriers were activated for one hour using NHS/EDC coupling and the activated nano-carriers were incubated with the amino-terminated HepG2 cell-specific aptamer, TLS11a. Aptamer functionalization was confirmed by spectroscopic and electrochemical techniques (UV-Vis spectrophotometry, Raman spectroscopy and electrochemical impedance spectroscopy).

The aptamer-modified magnetic carriers were then loaded with sorafenib and this step was confirmed by UV-Vis spectrophotometry and Raman spectroscopy. The amount of sorafenib released over time from the modified carries was also quantified by UV-Vis spectrophotometry.

In order to develop a faster and simpler strategy for sorafenib quantification for loading and release studies, an electrochemical method for the detection of sorafenib was developed. The electrochemical behavior of sorafenib was analyzed using cyclic voltammetry on different types of electrodes and the best surface was chosen. Scan rate and pH influence were also studied to identify the optimal detection conditions.

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P31

SIMULTANEOUS DETERMINATION OF SEVERAL CYTOSTATIC COMPOUNDS IN SEWAGE SLUDGE AND SEDIMENT BY COMBINATION OF MICROWAVE-ASSISTED EXTRACTION AND UHPLC-MS/MS

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Cytostatic compounds are pharmaceutics, which are designed to stop cell division or to kill cells. Due to their mechanism, they are often classified as mutagenic, carcinogenic and or toxic to reproductive systems. However, they effect all cells, not only cancer cells [1]. This may cause side effects at even low concentrations during prolonged exposure [2]. Therefore, several papers described determination of cytostatic compounds in environmental samples, mostly in aqueous samples. Some studies also suggest that cytostatic compounds may be adsorbed within sludge or sediment [3].

For this, the aim of our work was to develop an analytical methodology based on microwave assisted extraction coupled with ultra-high-performance chromatography with tandem spectrometry detection (MAE-UHPLC-MS/MS). Microwave-assisted extraction was used for its capability of rapid extraction of micropollutants from solid matrix using small amounts of samples and solvent volume [4].

The optimization of extraction was performed through an experimental design using Minitab 17.1.0. All the assays were established by a randomized experimental design 2^5 (five parameters at two levels) and then 3^2 (two parameters at three levels), which allowed study of contribution of each variable individually and relation among variables. The extraction efficiency was analyzed, and condition favoring the extraction of the greatest number of analytes were chosen [4].

Optimized method was used for analysis of real samples of sewage sludge acquired from main wastewater treatment plant and sediment obtained close to the marine outfalls from different wastewater treatment plants. These samples were acquired every three months for two years [4].

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P32

NOVEL HYPHENATION PF MICROCHIP ISOTACHOPHORESIS WITH ION MOBILITY SPECTROMETRY FOR THE ANALYSIS OF COMPLEX SAMPLES

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Microchip isotachophoresis (μITP) is an electrophoretic technique characterized by high separation capacity and concentration power. μITP is implemented by two electrolytes, the leading electrolyte and the terminating electrolyte, and the sample is introduced between them. After reaching an ITP steady-state, analytes with different effective mobilities will form discrete zones with sharp boundaries between them [1]. However, due to miniaturization, the separation capacity and detection sensitivity of microchip electrophoretic techniques are limited. Therefore, it is necessary to use a selective detection technique when analyzing complex samples. Ion mobility spectrometry (IMS) is based on a similar separation principle as electrophoretic techniques, but the biggest difference is type of the phase in which the separation is performed [2]. To perform the μITP-IMS analysis, it is necessary to transfer the separated components from the first dimension (μITP, liquid phase) to the second dimension (IMS, gaseous phase), which is done by implementing an auxiliary liquid to the microchip using an external syringe pump. To ensure minimal dispersion during the transfer of the separated components between the two dimensions, the auxiliary liquid was introduced in the opposite direction to the electrophoretic movement of the separated components on the microchip. An evaporation unit was used as an interface between the μITP analyzer and the IMS analyzer.

The potential of the developed μITP-IMS method was shown in the analysis of carboxylic acids from the homologous series C_1-C_6 (formic, acetic, propionic, butyric, valeric and caproic acid). For each of the carboxylic acids studied, several analytical parameters of the μITP-IMS method were evaluated, including sensitivity, linearity, accuracy, and precision. The practical applicability of the μITP-IMS method was demonstrated in the analysis of food (apple vinegar, fish sauce, wine), biological (saliva) and pharmaceutical (ear drops) samples. Based on the values of reduced ion mobility (K_0), used as a qualitative parameter, the presence of acetic acid was confirmed in all analyzed samples, while propionic acid was also identified in the saliva sample. Due to the potential matrix effects, the presence of carboxylic acids in the analyzed samples was also verified using a standard addition method. The K_0 values in the real samples did not significantly differ from those measured in the model sample. Hyphenation of μITP with IMS into a two-dimensional system contributes to improving the identification potential of μITP and the resolving power of IMS.

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P33

APPLE VINEGAR PRODUCTION USING WILD APPLE VINEGAR BACTERIAL CONSORTIA

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Vinegar production has ancient roots, including several substrates as raw materials, numerous manufacturing methods, and an end product with innumerable beneficial properties. Two well-known processes are used for biotechnological production. Using the traditional method, the acetic acid bacteria (AAB) grow and develop on the surface of the liquid. Still, the result is a complex and high-quality product. In the case of the submerged vinegar production, the AAB are cultured in the medium with constant oxygen addition, which gives relatively fast fermentation (24-48h).

We used wild apple vinegar as inoculum in submerged fermentation in our experiment. The fermentation was conducted using apple wine as substrate in a bioreactor set-up with continuous oxygen addition. The temperature was set to 30°C, and continuous stirring was applied with 0,3-0,5 L/min flow to reach a 1-3 ppm dissolved oxygen rate.

The acetic acid bacteria of the traditional inoculants used were isolated and phenotyped. The most abundant strains were identified using Maldi-TOF and metabolic changes during fermentation were monitored using HPLC techniques.

P34

DETERMINATION OF PAH CONTAMINATION LEVELS INBREAST MILK SAMPLES FROM HUNGARIAN VOLUNTEERING MOTHERS USING HPLC/FLD

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Polycyclic aromatic hydrocarbons (PAHs) –a large group of different organic compounds-can be extremely hazardous to human health due to their carcinogenicity and toxicity. PAHs are present in the environment as products of incomplete combustion processes of organic compounds. The general population is exposed to PAHs through diet, air inhalation and/or dermal contacts (1). Following mothers' exposure, a part of PAHs might be transferred into breast milk and PAH exposure may results in adverse effects in breastfeeding infants (2).The aim of this study was to determine PAH content in 28 breast milk samples obtained from Hungarian volunteering mothers using high-performance liquid chromatography with fluorescence detection (HPLC/FLD).

The sample preparation includes liquid-liquid extraction of 5 ml mother milk with of 65:35 acetonitrile/water ratio followed by clean up and concentration step with solid phase extraction (SPE). 14 US EPA PAHs (United States, Environmental Protection Agency) were determined by HPLC/FLD. The method was validated using parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and reproducibility.The concentration of total PAHs was calculated on each breast milk sample, and toxicity was assessed using toxic equivalent quantity (TEQ). All 28 breast milk samples were positive for at least 6 PAHs. Benzo (a) Anthracene were the most frequently detected PAHs in the samples, followed by Phenanthreneand Fluorene. Benzo[a]pyrene and Chrysene were the least detected. Dibenzo (a,h) anthracene and Benzo(g,h,i) perylene were not detected in the analyzed samples.

The developed method provides a fast and sensitive tool to detect PAHs in human breast milk.

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P35

MODERN MICROFLUIDIC ANALYSIS FOR DETERMINATION OF THE PROTEIN CONTENT IN DIFFERENT TYPE OF PLANT-BASED DRINKS

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Nowadays, consumers are increasingly looking for alternatives to cow's milk. The

development of functional and specialty beverage products is gaining ground around the world, driven by cow's milk allergies, lactose intolerance, calorie concerns, high cholesterol levels and the need to follow a vegan diet.

Thus, the application and further development of low-sampling, rapid, efficient and automated analytical methods is essential for the qualitative and quantitative analysis of proteincomponents in plant-based milk replacer beverages.

In our study, we used one of the state-of-the-art separation techniques, the microchip gel electrophoresis with High Sensitivity Protein 250 kit, to analyze the protein profile and total protein content of a big range of plant-based milk beverages (22 rice, 20 oat, 15 soy, 15 almond, 13 coconut, 4 cashew, 4 spelt, 1 quinoa, 1 buckwheat, 1 Brasil nut, 1 chickpea, 1 millet, 1 brown rice, 1 peanut and 1 hemp). We compared not only the same plant-based drinks from different manufacturers, but different expiry dates from the same manufacturer, too. We compared the obtained total protein concentration with the nominal concentration values. The analyses and statistical evaluation showed that the protein content of the same vegetable beverages from different manufacturers did not differ significantly. In contrast, the protein profiles of different plant-based drinks of different origins differ significantly.

Greater emphasis should be placed on the composition of plant-based beverages consumed as a substitute for cow's milk, not only in terms of protein but also in other macro- and micronutrients, to meet the requirements of a quality, health-conscious diet.

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