Volume 69 | Supplement 5 | 2023

ACTA MARISIENSIS
SERIA MEDICA

OFFICIAL PUBLICATION OF THE
GEORGE EMIL PALADE UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE, AND TECHNOLOGY OF TARGU MURES

21st INTERNATIONAL SYMPOSIUM AND SUMMER SCHOOL ON BIOANALYSIS (21STISSSB)

10-15 July 2023
Târgu Mureș, Romania

BOOK OF ABSTRACTS

ISSN: 2668-7755 • Online ISSN: 2668-7763 • www.actamedicamarisiensis.ro

UNIVERSITY PRESS
19 99
TÂRGU-MUREȘ

Acta Marisiensis - Seria Medica

Volume 69 | Supplement 5 | 2023

CEEPUS CIII-RO-0010-18-22-23
Teaching and Learning Bioanalysis

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

21st INTERNATIONAL SYMPOSIUM AND SUMMER SCHOOL ON BIOANALYSIS (21STISSSB)

10-15 July 2023
Târgu Mureș, Romania

BOOK OF ABSTRACTS

Acta Marisiensis - Seria Medica

Acta Marisiensis - Seria Medica (ISSN 2668-7755) is the official publication of the George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, being published by University Press, Targu Mures.

The journal publishes high-quality articles on various subjects related to research and medical practice from the all the medical and pharmaceutical fields, ranging from basic to clinical research and corresponding to different article types such as: reviews, original articles, case reports, case series, letter to editor or brief reports. The journal also publishes short information or editorial notes in relation to different aspects of the medical and academic life.

Information for contributors

Manuscripts must be submitted via editorial manager system, available online at
https://ojs.actamedicamarisiensis.ro/

Correspondence

All correspondence should be addressed to the Editorial Office:
Acta Medica Marisiensis
George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures
38, Gh. Marinescu St, 540139 Tîrgu Mureş, Romania
Managing Editor Professor Adrian Man
or sent by e-mail to ammjournal@umfst.ro

Acta Marisiensis - Seria Medica is indexed in the following international databases:

· SCOPUS
· DOAJ
· EBSCO Discovery Service (since 01 July 2010, first indexed number - no.4/2010)
· National Library of Medicine
·Sciendo
·Baidu Scholar
· Celdes
· Google Scholar
· J-Gate
· Publons

DTP and Website Management
Editura Prisma

This journal is printed on environmentally-friendly ECF (elemental chlorine free) woodfree paper.

21st INTERNATIONAL SYMPOSIUM AND SUMMER SCHOOL ON BIOANALYSIS (21STISSSB)

10-15 July 2023 Târgu Mureș, Romania

STUDY OF THE GLYCOSYLATION PROFILE OF A NIVOLUMAB BIOSIMILAR WITH CE-LIF

Csilla Albert[1], Emília Brumă[2], Beáta Albert[3], Csongor Orbán[3]

[1]Department of Food Science, Faculty of Economics, Socio-Human Sciences and Engineering, Sapientia Hungarian University of Transylvania
[2]Doctoral School of Chemistry, University of Pécs
[3]Department of Bioengineering, Faculty of Economics, Socio-Human Sciences and Engineering, Sapientia Hungarian University of Transylvania
e-mail: albertcsilla@uni.sapientia.ro

Monoclonal antibody therapies appeared more than 20 years ago and have since become increasingly popular in medicine, particularly in treating chronic inflammation and tumors. The effectiveness of these therapies lies in their high specificity towards target molecules, but their production is costly, both because they require high technology and because their biological nature means that a lot of time and effort is spent on their development.

In the present work, we investigate the structural similarity of our biosimilar variants of monoclonal antibody Nivolumab, particularly emphasizing the glycosylation profile. Nivolumab is a PD-1 (programmed cell death protein 1) inhibitor, an IgG4-type antibody that is increasingly used in a growing number of therapies. The glycosylation profile is essential because the therapeutic effect of antibodies is mediated not only by the antigen-binding Fab regions but also by the immune effector functions of the Fc regions through antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). The efficacy of both functions depends on the glycan associated with the Fc region Asn-297, which binds to an Fc gamma receptor (Fcgr) on the surface of a nucleated killer cell (NK cell), monocyte, or macrophage.

The glycans are first released from the mAb using a Protein N-Glycosidase F (PNGase) enzyme and subsequently they are labeled using a fluorophore, 8-aminopyrene-1,3,6-trisulfonic acid (APTS). We used Capillary Electrophoresis with Laser-Induced Fluorescence detection (CE-LIF) to analyze the obtained glycans. CE-LIF peak identification was done by a combination of glycan standards

The research was supported by the POC/163/1/3/ Project: Development of a monoclonal antibody production technology at SC CORAX-BIONER CEU SA Project code 121101

Keywords: mAb, glycan, CE-LIF

DEVELOPMENT OF A DNA METABARCODING METHOD FOR THE IDENTIFICATION OF SEAFOOD IN PROCESSED FOODS

Julia Andronache[1,2], Stefanie Dobrovolny[1], Margit Cichna-Markl[2], Rupert Hochegger[1]

[1]Austrian Agency for Health and Food Safety (AGES), Institute for Food Safety Vienna, Department for Molecular Biology and Microbiology, Spargelfeldstraße 191, 1220 Vienna, Austria
[2]Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Währinger Straße 38, 1090 Vienna, Austria
e-mail: julia.saeckler@ages.at

Introduction and objectives: Seafood is highly prone to food adulteration due to significant price ranges between species of similar morphology and complex supply chains. The aim of this project is the development and validation of a DNA metabarcoding method for the identification of seafood in processed foods.

Materials and methods: Reference sequences of the 16S rDNA were downloaded from the National Center for Biotechnology Information (NCBI). Alignment studies were performed with the CLC software (version 10.1.1) for primer design and evaluation of sequence variability. DNA was extracted from single-species reference material by a modified cetyltrimethylammonium bromide (CTAB) –protocol. The primers with the most promising characteristics were tested on the LightCycler 480 II/96 (Roche) with varying primer concentrations (0.2 µM or 0.4 µM) and magnesium chloride was added. First sequencing runs were performed on Illumina iSeq and MiSeq sequencing platforms.

Results and discussion: Four primer systems for the amplification of seven clam families, a subclass of cephalopods (Coleoidea), a class of snails (Gastropoda) and a class of crustaceans (Malacostrata) were created. Additionally, seafood reference material was sequenced.

Conclusions: Identification of seafood samples is possible on genus or species level for all samples with few exceptions such as sea snails. Thus, new primers will be designed and tested for the differentiation of sea snails.

The research was financed by the Austrian Agency for Health and Food Safety (AGES).

Keywords: DNA metabarcoding, 16S rDNA, species identification, seafood, food authenticity

ALCOHOL/ALDEHYDE DEHYDROGENASES IN BIOSYNTHETIC PATHWAYS

Emőke Antal[1], Ildikó Miklóssy[2]

[1]Institute of Chemistry, Faculty of Sciences, University of Pécs
[2]Department of Bioengineering, Faculty of Economics, Socio-Human Sciences and Engineering, Sapientia Hungarian University of Transylvania
e-mail: antalemoke@uni.sapientia.ro

Introduction and objectives: Alcohol and aldehyde dehydrogenases (ADHs and ALDHs) play crucial roles in various biosynthetic pathways. In many organisms, ADHs and ALDHs participate in the primary metabolic pathways, including the catabolism of ethanol and other alcohols as well as the synthesis of biologically and pharmacologically active substrates. These enzymes are extensively studied due to their significant secondary metabolite production which highlights their potential application in areas such as agriculture, pharmaceuticals, and biofuels[1,2].

Materials and methods: In this study we examined the adhE2 (alcohol-dehydrogenase II) enzyme isolated from Clostridium acetobutylicum and the mcr (malonyl-CoA-reductase) enzyme from Chloroflexus aurantiacus. Both enzymes are used in various processes such as the synthesis of active pharmaceutical ingredients (APIs), biopolymers, chiral alcohols, etc. The heterologous expression, purification, in vivo and in vitro examination of these enzymes were carried out in this study.

Results and discussion: The production, purification and examination of the enzymes was carried out successfully. We investigated the 3D structure of these enzymes via in vitro modelling using Alphafold's open access network.

Conclusions: In summary, alcohol and aldehyde dehydrogenases are indispensable enzymes involved in various biosynthetic pathways. Understanding their mechanisms, regulation, and applications can provide valuable insights into metabolic processes, drug discovery, API production, etc.

Keywords: adhE2, mcr, biosynthesis, 3D structure

MOLECULARLY IMPRINTED DRUG RESERVOIR FOR TARGETED GLIOBLASTOMA CELL TREATMENT: IN VITRO AND IN VIVO CHARACTERIZATION

Alexandra-Iulia Bărăian[1], Bogdan-Cezar Iacob[1], Olga Soriță[2], Cosmin Pește[3], Alexandru Florian[4], Sergiu Șușman[5,6], Ede Bodoki[1], Ștefan Florian[4]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [2]Laboratory of Tumor Cell Biology and Radiobiology, Institute of Oncology "Prof Dr. Ion Chiricuță", Cluj-Napoca, Romania; [3]Department of Anesthesiology and Surgical Propaedeutics, University of Agricultural Sciences and veterinary Medicine, Cluj-Napoca, Romania; [4]Department of Neurosurgery, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [5]Department of Morphological Sciences, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [6]Department of Pathology, IMOGEN Research Centre, Cluj-Napoca, Romania
e-mail: alexandra.iuli.baraian@elearn.umfcluj.ro

Introduction and objectives: Within this study, our objective was to address the existing limitations of chemotherapy in treating glioblastoma (GBM) by designing a molecularly imprinted drug reservoir. The aim was to achieve sustained release of the antitumor agent ruxolitinib (RUX) within the tumor post-resection cavity, targeting residual infiltrative cancer cells while minimizing toxic effects. In pursuit of this goal, we successfully developed and characterized four distinct molecularly imprinted polymers (MIPs), one of which progressed to the in vivo assessment stage.

Materials and methods: The synthesis of MIPs involved precipitation polymerization, using acrylamide, trifluoromethacrylic acid, methacrylic acid, and styrene as functional monomers. To assess the cytotoxic efficacy of the polymers, an in vitro evaluation was conducted using the Alamar Blue cell viability assay. Additionally, an in vivo assessment was performed using an orthotropic model in Wistar rats.

Results and discussion: The polymer based on trifluoromethacrylic acid (MIP 2) revealed the most favorable risk-benefit profile over the course of 96 hours. MIP 2 exhibited superior efficacy against GBM cells, while its non-imprinted counterpart showed low toxicity. Within the in vivo evaluation, animals treated with MIP 2 experienced a significant increase in survival time, extending from 20 to 50 days.

Conclusions: The Alamar Blue assay guided the selection of one MIP for further in vivo studies, considering both MIP's efficacy and the potential toxicity of residual monomers. MIP 2 emerged as the most effective, significantly extending the survival time of animals by 30 days.

The research 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 Hațieganu University of Medicine & Pharmacy no. 882/4/12.01.2022.

Keywords: glioblastoma, molecularly imprinted polymers, ruxolitinib

QUANTIFICATION OF PHOSPHATE IN COLA BEVERAGES USING ION CHROMATOGRAPHY

Francisc Boda[1], Lavinia Berța[1], Béla Kovács[2], Ibolya Fülöp[3]

[1]Department of General and Inorganic Chemistry, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania
[2] Department of Biochemistry and Environmental Chemistry, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania
[3] Toxicology and Biopharmacy, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania
e-mail: francisc.boda@umfst.ro

Introduction and objectives: Cola-type non-alcoholic beverages are known to contain a high amount of sugar, sweeteners, and phosphoric acid. Most manufacturers declare on the label the nutritional values of these beverages; however, the amount of phosphoric acid and phosphates remain unexpressed. Studies suggest that cola beverages can lead to hypocalcemia, decrease in bone mineral density, diabetes, high blood pressure, kidney disease and teeth erosion [1]. Several of these symptoms can be correlated with the high phosphoric acid content and consequent low pH of these drinks. The aim of this work was to quantify the phosphate content of cola-type beverages using an ion chromatographic system.

Materials and methods: Phosphate content was determined for 11 commercially available cola beverages, packaged in aluminum cans. Analysis was performed with a Dionex ICS-3000, using an IonPacAS23 Analytical Column (6 µm, 4 x 250 mm), at 30 °C. The mobile phase was an aqueous solution of 4.5 mM Na_2CO_3 / 0.8 mM $NaHCO_3$, at a flow rate of 1.2 mL/min. An in-line conductometric detector was used for signal detection, under suppressed conditions (ASRS300 suppressor at 30 mA).

Results and discussion: Applying the Grubbs' Test on the determined phosphate amounts, one outlier value was identified, namely a cola beverage with 9.2 ppm phosphate content. The label of this product states that it is phosphate-free and uses malic and tartaric acids as acidulants. The phosphate content of the remaining samples ranged from 377.2 ppm to 596 ppm, with a mean value of 500.1 ppm and a median value of 512.8 ppm. Standard deviation was 70.0 ppm, while relative standard deviation was 14%.

Conclusions: As the acceptable daily intake (ADI) of phosphates is 40 mg/kg of body weight, moderate consumption of cola beverages does not lead to a high dietary exposure to phosphates. However, precautions should be taken when underlying diseases are present, e.g. reduced kidney function or hypocalcemia, as a high phosphate intake can exacerbate the symptoms of these conditions.

The research was supported by the University of Medicine, Pharmacy, Science, and Technology „George Emil Palade" of Târgu Mureș Research Grant number 164/8/10.01.2023.

Keywords: ion chromatography, cola, phosphate

BIOSIMILAR MONOCLONAL ANTIBODIES DETECTION WITH ELISA METHOD

Emília Brumă[1], Erika Csató-Kovács[2], Mónika Korodi[1], Csongor Orbán[2]

[1]Department of Analytical Chemistry, Faculty of Sciences, University of Pécs
[2]Department of Bioengineering, Faculty of Economics, Socio-Human Sciences and Engineering, Sapientia Hungarian University of Transylvania
e-mail: bodor.emilia@yahoo.com

Introduction and objectives: Nowadays, the greatest demand among biological drugs is for monoclonal antibodies, as a result, more and more therapeutic monoclonal antibodies (mAbs) are entering different stages of development, and for their production, researchers are rather looking for innovative solutions. Therapeutic mAbs are special antibodies, which are used to treat various diseases such as cancer, Crohn's disease, rheumatoid arthritis, ulcerative colitis, and many others. In the present study, a nivolumab biosimilar antibody produced by us was tested against the original product.

Materials and method: The detection of the biosimilar monoclonal antibody was made by using an enzyme-linked immunosorbent assay (ELISA) method. Standards and samples were incubated in the microtiter plate coated with the reactant for nivolumab. Then we washed the well. Afterwards, horse radish peroxidase conjugated probe was added, and it bound to nivolumab captured by the reactant. Finally, the bound enzymatic activity was detected by addition of tetramethylbenzidine chromogen substrate. The reaction has been terminated with an acidic stop solution.

Results and discussion: Among the clones we produced, we examined the three leader clones, from which two of them we only maintained and one was treated with MTX, and we compared them to the original product. The best was the MTX-treated lead clone, but one of the untreated clones showed almost the same properties in terms of productivity, so it is also a potential stable cell line.

Conclusions: Our results show that the proteins produced by our culture are present in the supernatant in adequate quantity and quality.

Keywords: monoclonal antibody (mAb), biosimilar, ELISA

ELECTROCHEMICAL SENSORS FOR THE ANALYSIS OF PATHOGENS IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

Alexandra Canciu[1], Ana-Maria Tătaru[1], Mihaela Tertiș[1], Andreea Cernat[1], Cecilia Cristea[1]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Hațieganu" Cluj-Napoca
e-mail: alexandra.canciu@elearn.umfcluj.ro

Introduction and objectives: The detection of infectious pathogens (such as Campylobacter jejuni and Staphylococcus aureus) is a priority in the biomedical field to give an early and accurate diagnostic. We aimed to develop two electrochemical sensors based on aptamers (APTs) that can specifically bind to their target: ONS-23 APT for C. jejuni cells and PA#2/8 [S1-58] APT for S. aureus protein A (PrA).

Materials and methods: Carbon-based screen-printed electrodes (SPEs) decorated with Au nanoparticles via chronoamperometry and commercial Au SPEs were employed for the functionalization with the thiolated APTs. 1 µM APT was immobilized onto the surface by multi-pulsed amperometry, followed by the blocking of unbound sites with 100 µM 6-mercaptohexanol using the same technique. The modifications after each step were monitored by differential pulse voltammetry and electrochemical impedance spectroscopy using 5 mM $[Fe(CN)_6]^{3-/4-}$. Scanning electron microscopy was used for the surface characterization. Affinity studies of the PA#2/8 APT to PrA were done by surface plasmon resonance.

Results and discussion: The quantitative determination was performed by measuring the difference in the signal of the redox probe before and after incubation with multiple dilutions of C. jejuni NCTC 11322 cultures and different concentrations of PrA. The resistance to charge transfer in the impedance studies increased proportionally with the tested concentrations, while the intensity of the current in voltammetry decreased. The performance of the aptasensors was also assessed with good recoveries in real samples: wastewater and human serum.

Conclusions: Both developed aptasensors showed promising results for the detection of C. jejuni cells and protein A (as a target of S. aureus) in environmental and biological samples.

The research was supported by the H2020 PathoCERT project, grant no. 883484, by the CNCS-UEFISCDI, project no. TE 89/23.05.2022 and by the UMF Iuliu Hațieganu internal grant no. 773/4/11.01.2023.

Keywords: electrochemical sensor, aptasensors, Campylobacter jejuni, Staphylococcus aureus, protein A

ELECTROCHEMICAL SENSORS FOR THE DETECTION OF BACTERIA IN FISH AND SEAFOOD

Denisa Capatina, Bogdan Feier, Radu Oprean, Cecilia Cristea

Department of Analytical Chemistry, Faculty of Pharmacy, Iuliu Hațieganu" University of Medicine and Pharmacy of Cluj-Napoca
e-mail: DENISA.ELEN.CAPATINA@elearn.umfcluj.ro

Introduction and objectives: Food quality significantly impacts population health, especially regarding fish contamination by Salmonella spp., Pseudomonas aeruginosa, and E. coli, leading to foodborne illness outbreaks in Europe. These bacteria are acquired from polluted aquatic environments, affecting fish and seafood production. Furthermore, the persistent nature of these bacteria on diverse contact surfaces (biofilm) poses a significant challenge across all stages of fish production. Developing innovative methods to detect these bacteria and identify biofilm formation is crucial. This study aimed to develop electrochemical sensors for sensitive and selective detection of biofilm-associated molecules (3-O-C_{12}-HSL, cdGMP, PQS), and an aptasensor for the detection of Salmonella typhimurium.

Materials and methods: Specific aptamers modified with thiol groups were used as recognition elements in the development of the sensors for 3-O-C_{12}-HSL and S. typhimurium. These aptamers were immobilized on screen-printed electrodes (SPEs) modified with gold nanoparticles. To eliminate non-specific interactions, a deposition step with 2-mercaptoethanol was conducted. The sensors were then incubated with the target molecules and the resulting electrochemical signal was monitored. Optimization was performed for all the steps to determine the ideal conditions for target detection, and the modified electrodes were characterized using various electrochemical techniques. For PQS and cdGMP detection, the sensors relied on SPEs modified with nanomaterials, known for their high conductivity and large surface area. Optimization was conducted to determine the optimal experimental conditions, including electrode surface, electrolyte composition, and electrochemical technique.

Results and discussion: All developed sensors exhibited a wide detection range, a very low limit of detection, high specificity, and yielded promising results in detecting the targets in real samples.

Conclusions: These sensors serve as an important foundation for the advancement of "Point-of-care" devices, enabling the detection of bacteria and the monitoring of biofilm formation in real-life scenarios.

The research was supported by the "European integration of new technologies and social-economic solutions for increasing consumer trust and engagement in seafood products (FishEuTrust), Grant Agreement: 101060712/2022

Keywords: electrochemistry, sensors, bacteria, biofilm, seafood.

SIMULTANEOUS CHIRAL SEPARATION OF FIXED DOSE DRUG COMBINATIONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Anca-Gabriela Cârje, Silvia Imre, Alina Balint, Valentin Ion, Camelia-Maria Toma, Adina Bojor, Daniela-Lucia Muntean

Department of Analytical Chemistry and Drug Analysis, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș
e-mail: anca.carje@umfst.ro

Introduction and objectives: Synergy of active substances, high compliance of patients to treatment, decrease in the incidence of side effects, high efficacy compared to the administration of individual components at similar dosages, low manufacturing costs, reduction of associated medication errors are only part of the benefits that the use of fixed drug combinations presents. The aim of the present study was the development of a chiral separation HPLC method that allows the simultaneous determination of active substances in fixed dose drug combinations of amlodipine, indapamide and perindopril from coated tablets using ovomucoid stationary phase as a chiral selector.

Materials and methods: The enantiomeric separations were performed under gradient elution on an Agilent HPLC 1100 series chromatographic system using an Ultron ES OVM chiral column 150 x 4.6 mm with a particle size of 5 µm and UV detection.

Results and discussion: Knowing that small changes in experimental conditions could drastically change the enantioseparation capacity of ovomucoid, the influence of the column temperature, mobile phase nature and pH, was investigated. The optimal chromatographic conditions providing suitable simultaneous enantiomeric resolution of analytes for the amlodipine, perindopril and indapamide combinations studied implied a mobile phase consisting of 10 mM NaH_2PO_4/Na_2HPO_4 at pH 6.5 (A) and methanol (B) as organic modifier, at a 1 mL/min flow rate, with the following gradient elution: 0 min: 15% B, 5 min: 20% B, 12 min: 40% B, 18 min: 40% B and at 25 min: 15% B. The UV detection was performed at 210 nm and the column temperature was set at 25°C. Under these conditions, enantioseparations were achieved with good resolution and selectivity ($R>1.5$, $\alpha>1.2$) in less than 25 minutes.

Conclusions: Ovomucoid proved to be a suitable chiral selector for the simultaneous analysis of the considered substances from a mixture combination and the currently developed HPLC method could be used for quantifications of amlodipine, perindopril and indapamide enantiomers, in a single analysis after validation.

Keywords: ovomucoid, chiral separation, fixed dose drug combinations, HPLC

FOOD AUTHENTICATION BY HIGH RESOLUTION MELTING OF DNA BARCODE REGIONS

Michaela Wildbacher[1], Mina Micic[1], Julia Andronache[1,2], Katharina Pühringer[1], Stefanie Dobrovolny[2], Rupert Hochegger[2], Barbara Siegmund[3], Thomas Rühmer[4], Milena Stojkovska[5], Jasmina Petreska Stanoeva[5], Marina Stefova[5], Margit Cichna-Markl[1]

[1] Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Austria
[2] Department for Molecular Biology and Microbiology, Institute for Food Safety Vienna, Austrian Agency for Health and Food Safety (AGES), Vienna, Austria
[3] Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Austria
[4] Research Centre for Fruit Growing & Viticulture Haidegg, Graz, Austria
[5] Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss Cyril and Methodius University, Skopje, North Macedonia
e-mail: margit.cichna@univie.ac.at

Introduction and objectives: Mislabeling of species and cultivars in food products is a global issue. A number of studies indicate that amplification of an appropriate barcode region by polymerase chain reaction (PCR) and subjecting the PCR products to high resolution melting (HRM) is a cheap and efficient approach to differentiate between species and cultivars. We aimed to investigate the potential of DNA barcoding by HRM analysis to differentiate between berry species, edible insect species, and old Styrian apple cultivars.

Materials and methods: Primer sequences for DNA barcode regions containing microsatellites and/or single nucleotide polymorphisms (SNPs) were taken from databases and publications or designed by using a primer design software. DNA was extracted from reference samples and food products by applying common DNA extraction protocols.

Results and discussion: We developed and optimized PCR-HRM assays to differentiate between the berry species, insect species, and apple cultivars of interest. Various strategies were pursued to increase selectivity, including the combination of more than two primers and the addition of artificial sequences.

Conclusions: DNA barcoding by HRM is applicable for screening food with regard to mislabeling of species and cultivars.

Keywords: Food authentication; species; cultivars; high resolution melting

FLUORESCENT LABELING OF SACCHARIDES FOR CAPILLARY ELECTROPHORESIS

Kateřina Čokrtová[1], Tomáš Křížek[1]

[1]Department of Analytical Chemistry, Faculty of Science, Charles University
e-mail: katerina.cokrtova@seznam.cz

Introduction and objectives: Due to the absence of charged and chromophoric groups, separation and determination of saccharides is a difficult task[1]. Derivazation step is often incorporated before saccharide separation and detection. In this work, saccharide representatives were derivatized with 7-amino-1,3-naphthalenedisulfonic acid (ANDSA) at their reducing ends directly in the capillary using UV detection[2]. Detection using laser-induced fluorescence is more advantageous due to its sensitivity, and therefore suitable conditions were sought for the derivatization of carbohydrates with 8-aminopyrene-1,3,6-trisulfonic-acid acid (APTS), which we are able to detect using an excitation wavelength of 480 nm.

Materials and methods: Experiments were performed on a 7100 CE instrument (Agilent Technologies, Germany) with a diode-array detector operating at wavelength of 254 nm. Measurements were conducted in fused-silica capillary with inner diameter of 50 μm. Background electrolyte was 20 mM phosphoric acid (adjusted to pH = 3.5 with with NaOH). Saccharide zone was injected first and ANDSA as second. Voltage of -5 kV was applied for 90 seconds, substances were allowed to react . After that, a voltage of -30 kV was applied. Other experiments were performed on a 7100 CE instrument with LIF detector. Glucose was mixed with APTS and $NaBH_3CN$. After thermostating at 70° for 60 minutes, the sample was injected hydrodynamically and voltage of -30 kV was applied. Triethylamine in 30 mM concentration with 1M CH_3COOH was used as the background electrolyte.

Results and discussion: Several representatives of different types of carbohydrates, namely xylose, glucose, fucose, N-acetylglucosamine, lactose were successfully derivatized by ANDSA and suitable condition were found for their separation. Offline derivatization using APTS was attempted and the effect of heating on derivatization rate was tested.

Conclusions: In this work in-capillary derivatization of saccharides with ANDSA was performed. For other derivatizing agent suitable conditions were sought.

The research was supported by the Charles University, project SVV260690, and by the Central European Exchange Program for University Studies, network RO-0010-17-2223 – Teaching and Learning Bioanalysis.

Keywords: capillary electrophoresis, saccharide, derivatization

---

NITRITE ANION FORMATION IN PICKLED VEGETABLES UNDER BACTERIAL REDUCTION AND THE TOXICOLOGICAL RISK INVOLVED

Mircea Dumitru Croitoru[1], Ibolya Fülöp[1], Erzsébet Majai[1] , Maria-Corina Barna[1]

[1]Department of Toxicology and Biopharmacy, Faculty of Pharmacy, University of Medicine and Pharmacy of Targu Mures
e-mail: mircea.croitoru@umfst.ro

Introduction and objectives: Recent human studies showed that consumption of pickled vegetables during pregnancy can lead to teratogenic effects (spina bifida). This effect was assumed to be caused by the nitrite anion that can be formed during the pickling process by bacterial reduction of the nitrate ion, present in abundance in many types of vegetables. The observed effect might be attributed to the oxido-reducing ability of nitrite, therefore, a hindrance in the activation of folic acid might occur.

Materials and methods: Several types of vegetables were subjected to the usual preservation process through bacterial fermentation (vegetables, salty water 40 g/L) and samples were obtained at several time intervals during the fermentation process (samples were obtained until the fermentation process was complete). Concentrations of nitrate and nitrite were measured using an HPLC-UV/VIS technique, which employed a combination, in the same run, of a ion-pair method with UV detection for nitrate and a RP method with VIS detection, following a derivatization through diazotation and coupling, for nitrite. Sample preparation was a simple protein removal process with methanol and centrifugation.

Results and discussion: Indeed, in most samples, nitrate was reduced to nitrite and nitrite concentration raised during the first part of the fermentation process and decreased toward the completion. This shows the existence of a health risk brought by the consumption of high nitrate content pickled vegetables through a fermentation process. As risks of nitrite consumption, we can mention the formation of genotoxic and carcinogenic nitrosamines in the stomach and the more recently discovered teratogenic risk that involves hindrance in folic acid metabolism and activation.

Conclusions: Activation of nitrate with the formation of far more toxic nitrite, do take place in the process of vegetables fermentation. A higher risk is present when the products are consumed before the fermentation process is completed. Especially, pregnant women should avoid at least partly fermented pickles, or choose acetic acid (vinegar) preserved pickled products (do not contain nitrite).

Keywords: vegetables, pickles, fermentation, spina bifida, nitrite

STUDY OF DIETARY SUPPLEMENTS CONTAINING VALERIAN ROOT (VALERIANAE RADIX) BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Csongor Csató[1], Elek Ferencz[2], Melinda Urkon[3], Zoltán-István Szabó[4], Eszter Laczkó-Zöld[5]

[1]Pharmacy student, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures; [2]Department of Physical Chemistry; [3]Department of Pharmacology and Clinical Pharmacy; [4]Department of Pharmaceutical Industry and Management; [5] Department of Pharmacognosy and Phytotherapy
e-mail: csatocsongor@gmail.com

Introduction and objectives: Valerenic acids (valerenic acid, hydroxyvalerenic acid and acetoxyvalerenic acid) are specific sesquiterpenes of the valeriana genus, that are mainly found in high amounts in the root of the valerian (Valerianae radix). These substances inhibit the degradation of gamma-aminobutyric acid in the central nervous system, causing a general sedative effect. Therefore, many calming and sleep promoting dietary supplements contain valerian root extract alone or in combination with other herbal extracts, but the product description does not always provide full information on the quantitative and qualitative composition. The aim of this study was to develop a simple, efficient and selective high performance liquid chromatographic method for the identification and quantification of valerenic acids, that can be used for the analysis of dietary supplements with valerian root content, even in the presence of other herbal extracts.

Materials and methods: Starting from the official European and American monographs of valerian root a three-dimensional experimental design framework was used to investigate the effect of method parameters (gradient time, column temperature and ternary composition of the organic mobile phase), in accordance with the quality by design principles. Results were interpreted using the DryLab retention modeling software. The applicability of the virtual separation model was confirmed by experimental runs, and in silico robustness testing was realized to evaluate the effect of method parameters on critical quality attributes, such as analysis time or resolution. The final method was validated regarding selectivity, linearity, accuracy and precision, respectively tested on real samples obtained from commercially available dietary supplements with valerian root content.

Results and discussion: Based on our results, the developed method can be successfully applied for the qualitative and quantitative analysis of valerenic acids content of dietary supplements. Moreover, the described method provides flexibility and robustness based on the established virtual separation model.

Keywords: valerian root, dietary supplements, valeneric acids, quality by design, chromatography

---

WEARABLE ELECTROCHEMICAL MICRONEEDLE SENSOR FOR MDMA SCREENING IN INTERSTITIAL FLUID

Ana-Maria Dragan[1,2], Marc Parrilla[2,3], Cecilia Cristea[1], Radu Oprean[1], Karolien De Wael[2,3]

[1] Department of Analytical Chemistry, 'Iuliu Haţieganu' University of Medicine and Pharmacy Cluj-Napoca, Pasteur 4, 400349 Cluj-Napoca, Romania
[2] A-Sense Lab, University of Antwerp, Groenenborgerlaan 171, 2010 Antwerp, Belgium
[3] NANOlab Center of Excellence, University of Antwerp, Groenenborgerlaan 171, 2010 Antwerp, Belgium
e-mail: Ana.Dragan@umfcluj.ro

Introduction and objectives: The consumption of illicit drugs continues to be a leading concern worldwide, with their increased spread and abuse impacting society on many levels, with severe consequences on the population's health. One of the most frequently consumed illicit drugs is MDMA (3,4-methylenedioxymethamphetamine), a synthetic amphetamine-type substance (ATS) that was consumed by 2.6 million people in 2021, according to the most recent European report. Therefore, an analytical tool for the fast screening of MDMA could be of great importance for the proper management of patients that consume this illicit drug. Thus, we explored the potential of electrochemical screening and monitoring of MDMA in the interstitial fluid using a wearable microneedle sensor.

Materials and methods: Firstly, different nanomaterials were investigated to enhance the performance of the electrochemical MN, according to our previous work. Thereafter, the analytical performance of the sensor was evaluated through the analytical figures of merit, as well as selectivity towards MDMA. Importantly, the performance of the wearable MN was evaluated in artificial interstitial fluid, the stability and reversibility of the response, as well as the influence of temperature being investigated.

Results and discussion: The results obtained showed that the carbon-nanotubes modified MNs could be used for MDMA screening and monitoring in levels encountered in interstitial fluid (0.3 μM and 2.4 μM).

Conclusions: A carbon-nanotubes-based MN array was developed for MDMA screening and monitoring in interstitial fluid, making it a promising tool for individuals on probation and point-of-care tests.

The research was supported by the European Union's Horizon 2020 research and innovation program under grant agreement No 833787, BorderSens.

Keywords: microneedle array, nanomaterials, MDMA, interstitial fluid, forensic analysis

DETERMINATION OF ERBUMINE IN PHARMACEUTICALS BY MICROCHIP ISOTACHOPHORESIS

Marta Ďuriš, Jasna Hradski, Roman Szucs, Marián Masár

Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University Bratislava
e-mail: duris48@uniba.sk

Introduction and objectives: Quality control of pharmaceutical products present in salt form includes, i.a., the determination of the counterion. Erbumine is an organic compound used as a counterion for the active pharmaceutical ingredient perindopril. Perindopril erbumine is used for the treatment of hypertension, heart failure and stable coronary artery disease. Microchip isotachophoresis (ITP) is a miniaturized electrophoretic technique performed in a discontinuous electrolyte system, which is suitable for the accurate determination of the macrocomponents in pharmaceutical products.

Materials and methods: ITP performed on a polymethylmethacrylate microchip with integrated conductivity sensors was used for the determination of erbumine. The ITP quantitative parameter is the length of the zone of the analyte.

Results and discussion: The short-term and long-term repeatability of the erbumine zone length, expressed as the relative standard deviation (RSD), was evaluated. RSD of zone length for short-term repeatability ranged from 0.4% to 2.0% and for long-term repeatability ranged from 0.7% to 1.8%. The concentration of erbumine in the pharmaceutical product was determined using the external calibration and the standard addition method. The recovery of erbumine determination ranged from 98.1% to 102.3%. The determined content of erbumine in the perindopril erbumine pharmaceutical product closely matched the content declared by the manufacturer.

The research was supported by the Slovak Grant Agency for Science (VEGA 1/0116/22) and the Slovak Research and Development Agency (APVV-22-0133 and APVV-17-0318).

Keywords: microchip isotachophoresis, pharmaceutical analysis, erbumine

---

ANALYTICAL METHODS IN SPORT SCIENCE

Tatyana Dzimbova

Department of Anatomy and Physiology, Faculty of Public Health, Health Care and Sport, South-West University “Neofit Rilski”, Blagoevgrad
Department of Biochemical pharmacology and drug design, Institute of Molecular Biology “Roumen Tsanev”, Bulgarian Academy of Sciences, Sofia
e-mail: tania_dzimbova@abv.bg

Introduction and objectives: Proteins, theirs metabolites, electrolytes, and other small molecules may serve as biomarkers for professional athletes and recreationally active individuals. Advances in big data approaches to assessing health and performance of professional athletes suggest that using the newest technology with intrinsic data such as hematological and biochemical data can be powerful in identifying the balance between training and recovery in each unique individual. Numerous biomarkers may be used to assess different aspects of health, sport performance, and recovery, but when tracking profesiional athletes, even useful biomarkers have limitations. The aim of the present study is to present the most frequently used markers used in sports biochemistry and the methods for their determination.

Materials and methods: A literature search was conducted and different research methods were compared, as well as their applicability for routine monitoring of athletes’ condition.

Results and discussion: In training monitoring, evaluation of the body’s metabolic state is usually done by assessment of several metabolites and substrates found in blood, urine, saliva, or sweat. For direct information, biopsy sampling is necessary. In the investigation of muscle metabolism, the value of the information obtained from each method decreases in the following order when metabolites or substrates are determined: muscle biopsy, arteriovenous difference, venous blood, capillary blood, urine and saliva, and sweat. However, the feasibility of each method increases in the same order with muscle biopsy as the least feasible and sweat as the most feasible. Thus, the researchers have to choose the “golden mean,” the method with the most feasibility in the particular circumstances of the activity and that provides sufficient information for the evaluation of the function to be measured.

Conclusions: The low selectivity and sensitivity of the methods used so far can be overcome with the use of modern chromatographic methods for analysis. However, the availability of these methods is limited and in routine practice, although with less accuracy, various field tests are preferred, which give quick results.

Keywords: sport biochemistry, blood analysis, saliva tests

BIOAVAILABILITY AND BIOEQUIVALENCE STUDY OF TWO FORMULATIONS CONTAINING FLUCONAZOLE ON HEALTHY ROMANIAN SUBJECTS

Lenard Farczadi[1,2], Orsolya Fechete[2,3], Laurian Vlase[4]

[1]Center for Advanced Medical and Pharmaceutical Research, George Emil Palade University of Medicine, Pharmacy, Science, and Technology Targu Mures
[2]Clinical and Analytical Research Center Vim Spectrum Targu Mures
[3]Department of Internal Medicine, Emergency County Hospital Miercurea Ciuc
[4]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca
e-mail: lenard.farczadi@umfst.ro

Introduction and objectives: A pharmacokinetic study of fluconazole was carried out to assess the bioequivalence of two oral formulations containing 150 mg fluconazole per capsule. The measured plasmatic concentrations of fluconazole were analyzed to determine the bioequivalence of the test product with regards to the reference product.

Materials and methods: The study was carried out using a single-dose, block randomized, two periods, two sequences, cross-over design. A total of 26 subjects were screened according to the inclusion criteria and 20 subjects were selected for the study. Blood samples were drawn pre-dose and between 0.5 and 168.0 hours after drug administration, a total of 20 samples per subject for each period. The washout period between administrations was 14 days. All blood samples were centrifuged after collection and the plasma separated, ultimately all samples were analyzed using a validated LC-MS/MS method and the results underwent pharmacokinetic analysis.

Results and discussion: The relevant pharmacokinetic parameters (Cmax, AUClast, AUCtot) were determined for both the test and reference product. The mean Cmax value was 5.51 (± 1.60) µg/ml for the test and 5.55 (± 1.68) µg/ml for the reference product. The mean values for the AUClast were 194000 (± 49667) µg/ml x h for the test and 192000 (± 55659) µg/ml x h for the reference product. The Tmax value was 1.95 (± 1.13) h for the test and 1.63 (± 0.82518) h for the reference product, respectively. The 90% confidence intervals for the ratio of means of "Test/Reference" for all relevant pharmacokinetic parameters (Cmax, AUClast, AUCtotal) were within the bioequivalence range of 80-125 %, and there was no statistically significant difference between means of Tmax of the test and reference products.

Conclusions: The results of the study showed that the test product is bioequivalent to the reference product with regards to the rate and extent of the pharmacokinetics of fluconazole.

The research was supported financially by Vim Spectrum SRL Targu Mures, Romania.

Keywords: fluconazole, LC-MS, bioavailability, bioequivalence

---

SMALL RNA AND PROTEIN EXPRESSION PROFILING IN THE DETECTION OF PERSONALIZED PERFORMANCE AND REGENERATIVE CAPACITIES

Gabor Faskerti[1,2], Lilla A. Czuni[2], Peter Urban[2], Jozsef Kun[2], Szabolcs Maar[1,2], Ferenc Gallyas[1,2], Aniko Takatsy[1,2], Ildiko Bock-Marquette[1,2]

[1]Department of Biochemistry and Medical Chemistry, University of Pecs Medical School, Pecs, Hungary
[2]Szentagothai Research Centre, University of Pecs, Pecs, Hungary
e-mail: ildiko.bock-marquette@aok.pte.hu

Introduction and objectives: Responses in our systems to exercise load are undeniably complex and influenced by environmental and genetic factors. Recently, investigators utilize "omics" approaches, such as genomics, proteomics or epigenetics to better understand the complex molecular alterations responsible regarding the beneficial impacts of exercise on our system. A critical element for repeated physical performance is our capability for rapid regeneration, most likely individual specific. Thus, identifying performance specific regenerative molecules or markers to accelerate and state reconstitution processes likely impacts both athletes and clinical settings.

Materials and methods: Plasma samples collected at 4 different time points from three volunteers participating in a 40 km running race were examined. Timing of sample collection: immediately before physical exertion, right after the competition, after a one-hour balneotherapy session, and one day after physical stress. Transcriptomic studies were performed, focusing on the change in the expression profile of miRNAs from the total mRNA isolate, which was determined using NG sequencing. Proteomic studies were also carried out, changes at the protein level were detected by 2D gel electrophoresis. The changes observed between the different samplings were analyzed using statistical methods.

Results and discussion: A number of significant changes at miRNA and miRNP levels were detected in connection with the sampling before and after the physical exercise and after the regeneration period. These differences enable a more precise monitoring of biological processes and a deeper understanding of molecular functions. The heat map display of the miRNA studies clearly shows that the individual differences that appeared in the initial miRNA level, are equalized after the regeneration period (one day) following physical exertion. Studies prove the positive role of balneotherapy in regeneration.

Conclusions: Using miRNA profiling, new, personalized biomarkers describing the physiological state of our body and its regeneration capabilities can be identified and examined.

Keywords: Physical exercise, miRNA profiling, Proteomics, Regeneration, Personalized training

BIOMIMETIC ELECTROCHEMICAL SENSORS FOR ANTIBIOTIC DETECTION

Bogdan Feier, Malek Bibani, Oana Hosu, Cecilia Cristea

Department of Analytical Chemistry, Faculty of Pharmacy, "Iuliu Hatieganu" University of Medicine and Pharmacy of Cluj-Napoca
e-mail: feier.george@umfcluj.ro

Introduction and objectives: Vancomycin (VAN) is a glycopeptide antibiotic, active against Gram-positive bacteria resistant to other antibiotics, indicated parenterally for the treatment of severe bacterial infections. Unfortunately, the treatment with VAN is associated with nephrotoxicity and ototoxicity and the spread of bacterial resistance to VAN. To combat these problems the sensitive and specific detection of VAN is of great importance and the electrochemical aptasensors, using aptamers (short single stranded-DNA or RNA oligonucleotides, artificially selected for their capacity to bind with high specificity the target molecule), are very useful tool.

Materials and methods: Different screen-printed electrodes (SPE) and nanomaterials were tested as a support for the selective aptamer. Various electrochemical methods were used for the aptasensor development and characterization and for the analysis of VAN samples.

Results and discussion: The sensor based on C-SPE modified with gold nanostructures presented the best performance and it was characterized by electrochemical and microscopy techniques. The aptamer immobilization and the electrochemical parameters were optimized. The analytical parameters were determined. The interference study showed the high selectivity of the aptasensor, which was successfully applied for the analysis of VAN real samples.

Conclusions: An innovative electrochemical aptasensor was developed and characterized for the sensitive and selective detection of VAN from serum samples.

The research was supported by the European Union's Horizon Europe programme under grant agreement no. 101060712/2022 – "European integration of new technologies and social-economic solutions for increasing consumer trust and engagement in seafood products" (FishEuTrust)

Keywords: vancomycin detection, aptasensor, electrochemical sensor, real samples

---

OPPORTUNITIES PROVIDED BY COMPUTER-ASSISTED RETENTION MODELLING IN CHIRAL CHROMATOGRAPHY – SEPARATION OF OZANIMOD ENANTIOMERS ON POLYSACCHARIDE-BASED STATIONARY PHASES

Elek Ferencz[1,2], Zoltán-István Szabó[3], Arnold Zöldhegyi[4], Máté Dobó[5], Gábor Németh[6], Imre Molnár[4], Gergő Tóth[5]

[1]Department of Physical Chemistry, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures; [2]Gedeon Richter Romania, Department of Analytical Development; Targu Mures; [3]Department Pharmaceutical Industry and Management, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures; [4]Molnar Institute, Berlin, Germany; [5]Semmelweis University, Faculty of Pharmacy, Department of Pharmaceutical chemistry, Budapest, Hungary;[6]Egis Plc, Budapest, Hungary
e-mail: elek.ferencz@umfst.com

Introduction and objectives: The use of experimental design and retention modelling programs is becoming common in the development of reverse phase chromatography methods, but few examples of optimization of chiral separation systems can be found in the literature. The present study aims to demonstrate the opportunities and limitations of the DryLab retention modelling software for the separation of ozanimod enantiomers on polysaccharide-based stationary phases.

Materials and methods:

The ozanimod in its pure enantiomeric form (S-enantiomer) is authorized as an immunomodulating drug, while the R-enantiomer may be present in the product as chiral impurity and the quantitative determination of this chiral impurity - generally with high performance liquid chromatography - is an official requirement.

Results and discussion: In the first step, nine amylose- and cellulose-based stationary phases were tested in polar organic mode using methanol, 2-propanol and their mixtures in different proportions as mobile phases. The most promising results were optimized in terms of mobile phase composition and flow, as well as temperature, using a two-dimensional experimental design. In silico robustness study was performed to estimate the effect of the method parameters on the separation process and the optimized method was validated according to the current regulatory guidelines.

Conclusions: Our results show that the DryLab retention modeling program can be successfully applied to optimize chiral separation systems and to evaluate the impact of method parameters on the separation process.

This work was supported by the Collegium Talentum Programme of Hungary.

Keywords: ozanimod, DryLab, quality by design, design of experiments, chiral separation

COSMETIC FORMULATIONS BASED ON ESSENTIAL OILS FROM AROMATIC PLANTS, POSSIBLE ANTI-ACNE TREATMENT

Alina Filip, Paula-Alicia Călina, Florin-Dan Irimie, Paula-Veronica Podea

Enzymology and Applied Biocatalysis Research Center, Faculty of Chemistry and Chemical Engineering Babeș-Bolyai University, Cluj-Napoca, Romania
e-mail: alinafilip@ubbcluj.ro

Introduction and objectives: Medicinal plants, since ancient times, have been used to treat various diseases, due to their antibacterial, anti-inflammatory, antioxidant and antiandrogen effects. Their use in the treatment of acne, as a natural alternative that lacks unwanted side effects is of increased interest. At the site of acne lesions, in areas abundant in sebaceous glands, the bacteria Propionibacterium acnes predominates. These, by releasing extracellular lipases, hydrolyze the sebaceous content that contributes to blocking the follicular channel, the emergence of inflammation – comedones. A promising strategy against P. acnes, as well as Staphylococcus epidermidis and other microorganisms that are part of transient microbiota, could be using essential oils from aromatic plants like of fennel, clove and lavender. The main goal of this study is to evaluate the anti-acne effect of these essential oils and to develop efficient anti-acne and antioxidant cosmetic formulations.

Materials and methods: The extraction of the essential oils was carried out by Clevenger hydrodistillation and their chemical composition was determined by the GC-MS. Three types of face lotions formulation (cold cream, stearates cream and gel-cream forms) were obtained and then mixed with different concentrations of volatile oil. The evaluation of the anti-acne activity of the oils and the obtained cosmetic formulas was carried out by the Kirby-Bauer disc diffusion test using three reference species of bacteria: Escherichia coli, Propionibacterium acnes and Staphylococcus epidermis. The results were interpreted by assessing the presence or absence of the previously inoculated culture around the cellulose disks, soaked with the tested lotions and placed on a Petri dish, then measuring the diameter of the inhibition zone.

Results and discussion: The minimum inhibitory concentrations (MIC) were found: 5 mg/disc in the case of clove oil for all three of the studied bacteria and 5 mg/disc for E. coli and P. acnes and 20 mg/disc for S. epidermidis in the case of fennel essential oil. For example, the gel cream (Gc) based on clove essential oil shows very similar results to the essential against the three tested bacteria. Fennel-based creams have also been found to have anti-acne activity, especially stearate cream, which has an inhibitory activity against P. acnes and E. coli. According to the GC-MS analysis the anti-acne properties of the oils are most likely due to content in eugenol and eugenol acetate found in clove oil, and in case of fennel extract due to the presence of limonene and trans-anethole.

Conclusions: Essential oils and the cosmetic formulations obtained have been shown to have anti-acne activity and this can open an interesting research topic in the direction of obtaining valuable cosmetic products on an industrial level.

The research was supported by SEED Grant-FCIC UBB 2020 Development Fund.

Keywords: fennel, clove and lavender essential oils, anti-acne effect, cosmetic formulations;

ENANTIOSELECTIVE POTENTIAL OF MACROCYCLIC GLYCOPEPTIDE-BASED COLUMNS IN SUB/SUPERCRITICAL FLUID CHROMATOGRAPHY

Denisa Folprechtová[1], Martin G. Schmid[2], Daniel W. Armstrong[3], Květa Kalíková[1]

[1]Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University
[2] Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Sciences, University of Graz
[3] Department of Chemistry and Biochemistry, University of Texas at Arlington
e-mail: denisa.folprechtova@natur.cuni.cz

Introduction and objectives: Macrocyclic glycopeptides (MGs) are broadly utilized as chiral selectors in liquid chromatography but not that frequently in sub/supercritical fluid chromatography (SFC). Their complex structure and multiple functional groups allow enantiorecognition of a broad range of compounds. Furthermore, MG-based chiral stationary phases (CSPs) have structural similarities that lead to complementary properties. This work aimed to evaluate the enantioselective potential of MG-based CSPs for biologically important compounds with a strong emphasis on a systematic study of the effect of mobile phase (MP) composition on retention and enantioresolution of tested compounds in SFC.

Materials and methods: A set of structurally different acidic, basic, and neutral chiral compounds was used for the evaluation of the enantioselective potential of MG-based CSPs in SFC. Columns used: TeicoShell (teicoplanin), VancoShell (vancomycin), and NicoShell (modified MG) covalently bonded to 2.7 µm superficially porous particles. Different co-solvents and additives were tested in various MP compositions.

Results and discussion: The TeicoShell column showed excellent enantioselectivity for phytoalexins and derivatives of tryptophan. The VancoShell column was able to enantioseparate almost all tested benzofurans, pyrovalerones, and phenidines. NicoShell column showed some enantioselectivity for basic pharmaceuticals and forensic drugs. In most cases, water as an additive in the MP was beneficial for most enantioseparations of these compounds on polar MG-based CSPs in SFC.

Conclusions: The enantioselective potential of tested MG-based CSPs was assessed in SFC. The influence of the type of co-solvent and a detailed study of the effect of additives were explored. The effect of temperature, back-pressure, and flow rate was also examined. Some complementary behaviour of tested CSPs was observed. The combination of these MG-based CSPs offers a powerful tool for the enantioseparation of a wide spectrum of chiral biologically active compounds.

The work was realized within the cooperation of CEEPUS project No. CIII-RO-0010-17-2223.

Keywords: SFC, enantioseparation, macrocyclic glycopeptides

NABUMETONE/BETA-CYCLODEXTRIN INCLUSION COMPLEXES IN SOLUTION

Nives Galić

Division of Analytical Chemistry, Department of Chemistry, Faculty of Science, University of Zagreb
e-mail: ngalic@chem.pmf.hr

Introduction and objectives: Nabumetone (NAB) is a nonsteroidal anti-inflammatory prodrug used clinically to reduce pain and inflammation in the treatment of patients with osteoarthritis or rheumatoid arthritis. According to the Biopharmaceutical Classification System NAB is categorized as a class II drug - exhibits low aqueous solubility, and consequently low bioavailability. To improve NAB solubility, the complexes with cyclodextrins, a group of cyclic oligosaccharides, can be used.

Materials and methods: Nabumetone (NAB), cyclodextrins: β-cyclodextrin (β-CD), 2-hydroxypropyl-β-cyclodextrin (HPβCD, with an average degree of substitution, DS = 4.5), randomly methylated β-cyclodextrin (RMβCD, DS = 12) and sulfobutylether sodium salt β-cyclodextrin (SBEβCD, DS = 6.5), solid 1:1 NAB: β-CD complex prepared by grinding. UV-Vis, spectrofluorimetry, NMR, microcalorimetry.

Results and discussion: UV-Vis and spectrofluorimetric methods for quantitative analyses of NAB were developed and validated. Phase solubility studies have shown that all β-CDs increase the NAB solubility. Stability constants and complexation efficacy were calculated. Stability constants of NAB-β-CDs complexes were also determined by spectrofluorimetric titrations. Thermodynamic studies were performed by microcalorimetric titrations, and thermodynamic parameters for the complexation reaction were determined (logK, ΔrH^{o}, ΔrS^{o}, ΔrG^{o}). Modes of inclusion were determined by 1D and 2D NMR spectra. In DMSO-d_6, the complexation was about 20%, while in D_2O almost 97%.

Conclusions: NAB forms inclusion complexes with β-CDs in DMSO and water. All tested β-CDs improve NAB solubility in water and simulated biorelevant media (pH = 1; 4.5 and 6.8). RMβCD and SBEβCD had the most significant effect, with an increase of up to ca 160-170 fold.

Keywords: nabumetone, cyclodextrins, spectrofluorimetry, NMR spectroscopy, microcalorimetry

---

STABILITY EVALUATION OF COMBINED EAR DROPS WITH CIPROFLOXACIN, ECONAZOLE AND BASIL VOLATILE OIL UNDER DIFFERENT STRESS CONDITIONS

Vladilena Gandacov[1,2], Livia Uncu[1,2], Ecaterina Mazur[1,2], Vladimir Valica[1,2]

[1]Scientific Center for Drug Research; [2]Department of Pharmaceutical and Toxicological Chemistry
Nicolae Testemitanu State University of Medicine and Pharmacy of Republic of Moldova Chișinău, Republic of Moldova
e-mail: vladilena.gandacov@usmf.md

Introduction: In order to obtain an effective therapy of ear infections, the administration of pharmaceutical forms with local action, complex containing antibiotics, antifungals, anti-inflammatory and antiseptics is justified. Drug stability is an important factor in ensuring its quality and reprezent the ability of the form to maintain its therapeutic properties without changing any of its chemical, physical, microbiological properties during the entire shelf life. In order to establish the degree of influence of various factors on the degradation process, as well as storage conditions, the stability of the studied dosage form was evaluated under conditions of hydrolytic (acid and basic), oxidative, photolytic and thermal stress.

Aim of study: Stability study of combined ear drops with ciprofloxacin, econazole and basil volatile oil under different stress conditions.

Material and methods: Ear drops (laboratory pilot series) containing ciprofloxacin hydrochloride(CH), econazole nitrate(EN) and essential basil oil (Sigma); 3% hydrogen peroxide; solution HCl 0.1 mol/l; solution NaOH 0.1 mol/l; irradiation with a UV lamp; spectrofotometer Shimadzu UV-1800; thermostating at temperatures of 40 °C and 60 °C. After exposure to the action of stress factors, the combined pharmaceutical form was analyzed by the spectrophotometry at time intervals of 0, 3, 24, 48 and 72 hours; the samples were prepared by dilution with methanol to 40 µg/ml (EN) and with methanolic solution of HCl 0.1 mol/l to 10 µg/ml (CH).

Results: The results of the determinations showed that after 24 hours of exposure to hydrolytic stress, CH and EN were resistant in an acid medium, but an alkaline medium CH showed concentration fluctuations, explained by the initiation of degradation processes. Following exposure to oxidative stress with hydrogen peroxide, both substances showed stability within acceptable limits. Under the action of photolytic stress, CH significantly degraded after the first 3 hours and EN degraded at the light after 48 hours, reducing 3.33% of the initial concentration. Under the action of high temperatures, the concentrations of CH and EN were kept within the admissible limits.

Conclusions: Stability studies under stress conditions showed that the studied dosage form is stable in an acidic medium and under the action of oxidizing agents, in an alkaline medium ciprofloxacin is vulnerable to degradation processes. Light has been shown to be a destructive factor for ciprofloxacin and econazole, and both substances are temperature stable.

The research was supported by the project 20.80009.8007.14

Keywords: Stability, stress conditions, ear drops.

INTACT PROTEIN ANALYSIS BY CZE-MS

Attila Gaspar[1], Melinda Andrasi[1], Narmin Hamidli[1], Ruben Szabo[1], Blerta Payaziti[2], Gayatri Vishwakarma[1], Cynthia Nagy[1]

[1]Department of Inorganic and Analytical Chemistry, University of Debrecen, Hungary
[2]Faculty of Pharmacy, Ss. Cyril and Methodius University, Vodnjanska 17, 1000 Skopje, North Macedonia
e-mail: gaspar@science.unideb.hu

Introduction and objectives: Study the applicability of capillary zone electrophoresis using coated and non-coated capillaries with UV and ESI-MS detection for the analysis of intact proteins in model solutions and real samples.

Materials and methods: Measurements were carried out by a 7100 model CE instrument (Agilent) with UV and MS (maXis II UHR ESI-QTOF MS instrument, Bruker) detection. Different capillaries of 85 cm x 50 µm I.D. and 370 µm O.D. were used. UV detection was performed by spectrometric measurement (λ: 200 nm). Background electrolytes were 1 M formic acid (pH=1.8) or 50 mM acetate buffer (pH= 2.6 or 9.5), sheath liquid: 0.1% formic acid in 1:1 isopropyl alcohol.

Results and discussion: Generally, for the separation of intact proteins, the use of coated capillaries is recommended, however, bare fused silica capillary (BFS) is still the most often applied capillary due to its cheapness and simplicity. The performance of BFS capillary for intact protein analysis was compared to that of different coated capillaries (polybrene and permanently coated linear polyacrylamide) using CZE-MS. Good precision, minimal adsorption and separation efficiency similar to or even better than those obtained with the coated capillaries was achieved in cases where low pH (pH = 1.8) was used in BFS capillaries. The polybrene and the linear polyacrylamide capillaries showed their slightly better resolving efficiency in terms of separating the different forms/variants of the same protein. The method was applied for determination of insulin analogs or monoclonal antibodies and the investigation of deamidation of insulin.

Conclusions: The capillary zone electrophoresis coupled with mass spectrometry is an efficient method for separation and determination of intact proteins, however challenges remained.

The research was supported by the CEEPUS, Stipendium Hungaricum and NKFIH, Hungary.

Keywords: intact protein, capillary zone electrophoresis, mass spectrometry, separation

---

HYBRIDIZATION THERMODYNAMICS OF DNA OLIGONUCLEOTIDE COGNATES

Laura-Elena Gliga[1], Bogdan-Cezar Iacob[1], David A. Spivak[2], Ede Bodoki[1], Radu Oprean[1]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy of Cluj-Napoca
[2]Department of Chemistry, Louisiana State University
e-mail: gliga.laura@umfcluj.ro

Introduction and objectives: Nucleic acids (NAs) have demonstrated significant potential as emerging biomarkers for evaluating disease state and predicting and monitoring treatment efficiency. Progress in electrochemical biosensor technology for clinical diagnosis enables the highly sensitive detection of NA sequences using small, affordable devices that are ideal for point-of-care use. Selecting the appropriate recognition component is crucial for ensuring the sensor's high performance, since a strong and selective affinity between the biorecognition element and its target is required for high specificity. NAs represent ideal recognition elements for the construction of biosensors that target other NAs, the specificity of the sensor relying on the unique complementary binding between the two NA fragments. The objective of this study was to document, in terms of thermodynamic and kinetic characteristics, the interaction between oligonucleotide sequences of varying lengths and levels of complementarity.

Materials and methods: A 22-nucleotide model ssDNA sequence, referred to as miR21, served as the target, while eight different ssDNA sequences of varying lengths and complementarities were used as potential probe sequences. Isothermal titration calorimetry (ITC) studies, widely recognized as being reliable for measuring the thermodynamic parameters of interactions in solution, were complemented and cross-validated by capillary gel electrophoresis (CGE) analyses.

Results and discussion: As anticipated, the greater the complementarity of the bases, the stronger the binding affinity between two ssDNA strands. Both ITC and CGE have shown that for two ssDNA sequences to interact, the number of paired bases must exceed a minimum threshold of five, but complete complementarity with the target is not necessary for adequate binding affinity. We also examined several scenarios to record the competitive binding of the target (miR21) by two ssDNA strands that have different degrees of complementarity to it.

Conclusions: Overall, the combined microcalorimetric and electrophoretic data provide a rapid and comprehensive representation of the interaction between ssDNA strands with varying lengths and levels of complementarity. These results will be highly beneficial in the development of high-performance sensors for the accurate detection of NAs.

Keywords: oligonucleotide interactions, oligonucleotide sequence complementarity, microcalorimetry, affinity capillary electrophoresis

DEVELOPMENT OF MOLECULARLY IMPRINTED POLYMER-BASED PLATFORMS FOR THIABENDAZOLE DETECTION

Szabolcs Ianos Győrfi[1], Bogdan Cezar Iacob[1], Bodoki Ede[1]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca
e-mail: szabolcsjanos.gyorfi@gmail.com

Introduction and objectives: Pesticides are widely used in agricultural practices to protect crops from pests and diseases. However, their excessive and indiscriminate use can pose serious threats to human health and to the environment. Thus, the development of sensitive and selective detection methods for pesticide residues is of utmost importance. Employing direct electrochemical techniques can be difficult for the trace analysis. 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. This study presents the design and fabrication of an electrochemical sensor modified with indole-3-acetic acid (3IAA) MIP for the detection of thiabendazole (TBZ), a commonly used fungicide and pesticide.

Materials and methods: The thin 3IAA-based molecularly imprinted polymer film was electrodeposited onto the surface of a gold electrode (GE) using cyclic voltammetry, providing selective recognition sites for the target molecule, thiabendazole. The electrochemical behavior of the modified sensor was investigated using differential pulse voltammetry (DPV) and impedance spectroscopy (EIS). The sensor exhibited enhanced electrocatalytic activity and increased sensitivity towards thiabendazole compared to the bare electrode. Moreover, the fabricated sensor demonstrated excellent selectivity, run-to-run stability, and reproducibility.

Results and discussion: To determine the detection performance, calibration curves were constructed by measuring the electrochemical response of the sensor at different concentrations of thiabendazole in synthetic and real sample matrix. The sensor exhibited a linear response within the concentration range of interest, with a low detection limit, indicating its suitability for thiabendazole detection in real samples.

Conclusions: The proposed electrochemical sensor holds great promise for rapid, on-site detection of thiabendazole residues in different water samples. Its high selectivity, sensitivity, and simplicity make it a valuable tool for monitoring and ensuring food safety, environmental protection, and public health. Further research could focus on optimizing the sensor's performance parameters and investigating its applicability in other real-world scenarios.

Acknowledgements: The research leading to these results has received funding from the NO Grants 2014-2021, under Project contract no. 32/2020.

Keywords: thiabendazole, imprinted polymers, indole-3-acetic acid, electrochemical sensor

---

ANTIBACTERIAL AND CYTOTOXIC EFFECT OF VARIOUS NANOPARTICLES, COMPARATIVE LITERATURE STUDY

Sarolta Halmoczki, Angela Jedlovszky-Hajdu

Laboratory of Nanochemistry, Department of Biophysics and Radiation Biology, Semmelweis University
e-mail: halmoczkisaci@gmail.com

Introduction and objectives: The presence of multidrug-resistant bacteria raises particular difficulties in the healing of chronic wounds. These wounds usually cannot be treated effectively with conventional antibiotics, therefore, alternative methods and agents are investigated to replace them. In this regard, metal nanoparticles (MeNPs) could be promising substances, because they possess significant antimicrobial activity, with the advantage that bacteria are unlikely to develop resistance against them. On the other hand, application of MeNPs may be challenging, since their toxic effect against microorganisms is not selective, and they additionally carry cytotoxic effect. [2] Numerous research articles have been published, which examine the potential of using different MeNPs for wound healing applications. However, it would be invaluable to have a review article that compares the antimicrobial and cytotoxic properties of the most promising MeNPs.

Conclusions: More than 50 scientific articles have been studied, regarding the wound healing potential of silver, gold, copper-, iron- and zinc containing nanoparticles. In the comparison, the main focus was on the antimicrobial activity and cytotoxic effect. Numerous types of Gram-positive and Gram-negative bacteria was treated with metal nanoparticles. In most of the cases, MeNPs were not on their own, rather in a hydrogel, or along with other agents, such as traditional antibiotics. Cytotoxicity was tested on various malignant and normal cell lines. Several cytotoxicity-reducing methods were exhibited, but in general, the effect of the MeNPs is dose-, shape- and size-dependent, but exposition time and the exact cell line used are also not negligible factors.

This research was supported by NKFIH FK 137749

Keywords: metal nanoparticles, wound healing, cytotoxicity, multidrug-resistant bacteria

R OR S: A STORY OF CHIRAL DRUGS

Gabriel Hancu

Department of Pharmaceutical and Therapeutic Chemistry, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureş

Introduction: More than 50% of the drugs currently used in therapy, possessing at least a chiral center in their structure, but only approximately 25% of them are used as pure enantiomers. However, the desired pharmacological effect is usually related to one of the enantiomers called eutomer, while the other enantiomer, called distomer, in most situations is less active, but sometimes can be responsible for the adverse effects of the racemates. The enantiomers of a chiral drug may differ in their pharmacological and/or pharmacokinetic characteristics. The study is an overview of the significance of chirality in drug development, highlighting the challenges of enantiomeric separation, as well as the impact of chiral drugs on therapeutic outcomes and patient safety.

Materials and methods: A comprehensive literature survey was made regarding the tendencies of approving and using pure enantiomers versus racemates in the last 25 years. The clinical significance of chiral drugs is exemplified by numerous examples in medical practice. Selected chiral drugs, such as ibuprofen or omeprazole, were used as case studies to illustrate the pharmacological evaluation of enantiomers.

Results and discussion: The “moment zero” in the field of chirality is considered 1992, when FDA released a guideline on stereochemistry in a report titled “Development of new stereoisomeric drugs”. The pharmacokinetic and pharmacodynamic properties of chiral drugs can differ significantly between enantiomers, leading to variations in drug efficacy, metabolism, and toxicity. The pharmacological differences may arise due to enantioselective interactions with enzymes, receptors, or transporters in the human body.

Conclusions: The utilization of chiral drugs has become increasingly prevalent due to the recognition that enantiomers can exhibit diverse pharmacological profiles. The use of single enantiomer drug use can potentially lead to simpler and more selective pharmacologic profiles, better therapeutic indices, simpler pharmacokinetics due to different rates of metabolism of the different enantiomers or decreased drug interactions. Understanding the stereochemical properties of chiral drugs allows for the design of more effective and targeted therapeutic interventions. The field of chiral drugs encompasses a fascinating interplay between the science of chirality and drug development.

Keywords: stereochemistry, chiral drugs, enantiomers, enantiopure drugs, racemic drugs

---

BIOPHYSICAL CHARACTERIZATION OF CLOT RETRACTION IN PLATELET RICH PLASMA OF PATIENTS WITH PRIMARY ANTI-PHOSPHOLIPID SYNDROME

Jolán Hársfalvi[1], Tímea Feller[1], György Domjan[3], Klára Gadó[3], Katalin Várnai[2], Eszter Barabás[2], Miklós Kellermayer[1]

[1]Department of Biophysical and Radiation Biology, [2]Department of Laboratory Medicine and 3Department of Internal Medicine and Oncology, Semmelweis University, Budapest, Hungary
e-mail: harsfalvi@google.com

Introduction and objectives: Anti-phospholipid syndrome (APS) is an autoimmune process that leads to thrombotic disorders. Based on atomic force microscopy (AFM)-based nano-thrombelastography (nTEG), we questioned, what were the biophysical characteristics of the platelet-rich fibrin network during clot formation and degradation in platelet-rich plasma (PRP) of 38 APS patients versus 18 controls. Patients with APS or venous thromboembolism (VTE) as controls were selected in the study.

Materials and methods: Citrated blood was centrifuged for PRP at room temperature (150 g, 10 min), platelet count was set to 50G/L. An AFM cantilever was submerged in a 0.3mL sample and cyclically moved up and down with 1 μm amplitude and 1 μm/s speed. The sample contained PRP and Ca^{2+} 10mM, clotting was initiated with thrombin, 1 IU/ml final activity. As the sample clotted, the cantilever increasingly deflected during its vertical travel, and viscoelasticity (hysteresis area and force difference), and contractility (baseline force) were measured.

Results and discussion: Compared the APS group to the controls, the medians of the delay until the first force signals were 2-3x [sec]; the slope of the force generation was about 1/2 [nN/sec]; maximal force difference of each cycle -calculated from the maximum deflection signal- was 1/3 [nN]. These results are in line with the Litvinov group’s results which showed a decreased contraction of blood clots in APS patients using a relative macro scale measurement

Conclusions: We could characterize quantitatively the changes in the viscoelastic properties during platelet contractility and fibrin network formation in human PRP. The AFM-based measurement provides nano-scale, numerical parameters, and a standardizable biophysical method.

The research was supported by the TKP2021-EGA-23

Keywords: autoimmune process, thrombotic disorders, atomic force microscopy

COUPLING MICROCHIP ELECTROPHORESIS WITH ION MOBILITY SPECTROMETRY FOR THE ANALYSIS OF CARBOXYLIC ACIDS IN COMPLEX LIQUID SAMPLES

Jasna Hradski, Marta Ďuriš, Roman Szucs, Marián Masár

Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University Bratislava
e-mail: hradski1@uniba.sk

Introduction and objectives: Coupling microchip electrophoresis (MCE) with ion mobility spectrometry (IMS) can be used to improve the identification capability of MCE as well as the separation capability of IMS, which is beneficial for the trace analysis of complex samples. This work describes the development of the MCE-IMS coupling and its application to the analysis of various complex liquid samples.

Materials and methods: The MCE analyzer was connected to the IMS analyzer via a thermal spray based interface. Two MCE techniques, zone electrophoresis (ZE) and isotachophoresis (ITP), were used for coupling with the IMS.

Results and discussion: Various analytical parameters, including sensitivity, linearity and precision, were evaluated for the developed ZE-IMS and ITP-IMS methods. MCE-IMS methods were applied to the analysis of carboxylic acids from the homologous series C_1-C_6 in various environmental, food, biological and pharmaceutical samples.

Conclusions: The developed online two-dimensional MCE-IMS methods proved to be a promising tool for the reliable determination of C_1-C_6 carboxylic acids in complex samples of various origins.

The research was supported by the Slovak Grant Agency for Science (VEGA 1/0116/22), the Slovak Research and Development Agency (APVV-22-0133 and APVV-17-0318) and the Operation Program of Integrated Infrastructure for the project, Advancing University Capacity and Competence in Research, Development and Innovation, ITMS2014+: 313021X329, co-financed by the European Regional Development Fund.

Keywords: microchip electrophoresis, ion mobility spectrometry, hyphenated techniques, complex samples

---

NEXTGENPS – EU PROJECT ON ANALYSIS OF NEW PSYCHOACTIVE SUBSTANCES

Eva Hubner[1], Heike Glänzer[1], Georg Raber[2], Jorge Camarasa[3], Raúl López-Arnau[3], Martin G. Schmid[1]

[1]Institute of Pharmaceutical Sciences, Dept. of Pharmaceutical Chemistry, University of Graz
[2]Institute of Chemistry, Dept. of Analytical Chemistry, University of Graz
[3]Faculty of Pharmacy and Food Sciences, University of Barcelona
e-mail: eva.hubner@uni-graz.at

Introduction and objectives: New Psychoactive Substances (NPS) are slightly modified variants of illicit drugs to circumvent law. They show similar psychoactive effects, compared to classical drugs such as heroin, cocaine, or amphetamine, however, because of their novelty, knowledge about them is scarce. To date, according to the European Monitoring Centre for Drugs and Drug Addiction (EMCCDA), 830 different NPS have been reported. The NextGenPS project was founded with the goal to implement analytical strategies to help speed up possible control measures for newly emerging NPS in the future.

Materials and methods: Eight different NPS – two phenethylamines, three tryptamines and three cathinones – were synthesized and provided by the University of Barcelona. Each sample was investigated by GC-MS to screen the latest drug databases for a positive hit. The second part was to develop a HPLC-UV method for quantification of drug seizures. The next step, which is currently under development, is to establish a solid phase extraction (SPE) method for biological samples. A cation exchanger SPE (Chromabond® PSA) method was implemented using water as a matrix and sample concentration of 100 ng/ml. SPE elutions were measured by means of a Triple Quad LC-MS System.

Results and discussion: The preliminary SPE results show recovery rates between 70 to 100%. As a further step the method will be applied to and optimized for human serum and artificial urine. Final evaluation of results received from the developed methods and validation is not yet completed.

Conclusions: So far, the quantitative methods led to satisfactory first results whereas correct identification via GC-MS libraries was only partially possible. Especially the lack of MS recognition shows the importance of implementing new and quick analytical methods for NPS to keep up with the rapid development of the illicit drug market.

The research was supported by the NextGenPS EU Project

Keywords: SPE, cation exchanger, phenethylamine, tryptamine, cathinone

PROOF-OF-CONCEPT SOLID-STATE MECHANOCHEMICAL SYNTHESIS OF MOLECULARLY IMPRINTED POLYMERS

Bogdan-Cezar Iacob[1], Andreea-Elena Bodoki[2], Ionuț Valentin Creștin[1], Ede Bodoki[1]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2] Department of General and Inorganic Chemistry, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
e-mail: iacob.cezar@umfcluj.ro

Introduction and objectives: Molecular imprinting enables the fast, versatile, robust, and cost-effective synthesis of biomimetic polymeric receptors with tailored selectivity for a wide variety of target molecules. Solvents are a critical component in the synthesis of molecularly imprinted polymers (MIPs), both as a porogen and reaction media, however their use comes with additional challenges, such as environmental concerns, interferences with the binding of the target molecule to the polymer matrix, limited solubility of the template, limiting the range of target molecules that can be imprinted. To address some of the above-mentioned issues, but also to explore potential opportunities or further constraints, we report the first solvent-free mechanochemical synthesis of MIPs via liquid-assisted griding.

Materials and methods: The successful synthesis of the imprinted polymer has been functionally demonstrated measuring its template rebinding capacity, as well as the selectivity of the molecular recognition process in comparison with the ones obtained by the conventional, non-covalent molecular imprinting process in liquid media.

Results and discussion: The proof-of-concept study demonstrated similar binding capacities towards the template molecule and superior chemoselectivity compared to the conventional MIP synthesis method. The adoption of green chemistry principles with all its inherent advantages in the synthesis of MIPs, not only alleviates potential environmental and health concerns associated with their analytical (e.g. selective adsorbents) and drug delivery (e.g. drug carriers or reservoirs) applications, but might also offer a conceptual change in the molecular imprinting technology.

Conclusions: Future studies, besides gaining a deeper understanding on the chemical structure of the resulting imprinted polymer and different mechanochemical variables on the molecular recognition properties of, will also need to address additional aspects, such as the range of template molecules compatible with the mechanochemical synthesis of MIPs, the extent of unwanted degradation of the employed polymerization components, or if mechanochemistry is transferable to other MIP approaches.

The authors acknowledge the COST Action CA18112 'Mechanochemistry for Sustainable Industry.

Keywords: molecular recognition, selective adsorbents, green chemistry, mechanochemistry

---

CUSTOMIZED PLATFORM FOR ELECTROCHEMICAL DETECTION OF INFLAMMATORY BIOAMARKERS FOR WEARABLE APPLICATION

Maria-Bianca Irimeș[1], Mihaela Tertiș[1], Radu Oprean[1], Cecilia Cristea[1]

[1]Analytical Chemistry Department, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, 4 Louis Pasteur St., Cluj-Napoca, Romania
e-mail: maria.bi.irimes@elearn.umfcluj.ro

Introduction and objectives: Biomarkers involved in inflammatory-associated medical conditions such as kynurenic acid (KA) and cortisol (COR) provide information about the existence of inflammatory processes. Thus, their rapid, sensitive detection in biological samples plays an important role in clinical diagnosis and evaluation of medical interventions. In this regard, the development of electrochemical sensors represents a promising approach for the determination of these biomarkers due to their advantages such as capacity for rapid, low-cost detection in unconventional biological samples, with high sensitivity and specificity and suitability for miniaturization with prospects for wearable devices and in situ analysis. The main purpose of this study was to develop customized, flexible platforms for the rapid, sensitive direct electrochemical detection of KA and COR in biological samples for wearable sensors applications.

Materials and methods: Planar electrochemical cells were in-lab printed by using special conductive and insulating inks. Next, the working electrodes were modified using a nanocomposite based on reduced graphene oxide for KA determination and based on Ni for COR detection, respectively.

Results and discussion: The elaborated platforms were characterized, analyzed regarding the analytical performances and will be used for real samples analysis.

Conclusions: The designed platforms allow direct electrochemical detection of inflammatory biomarkers detection and it can be applied for their detection in real samples.

Acknowledgement: This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS-UEFISCDI, project number PN–III–P1-1.1-TE-2021-1543, within PNCDI III. M-B. Irimeș thanks the Iuliu Hațieganu UMF internal grant no. 772/15.11.01.2023.

Keywords: wearable sensors, biomarkers, electrochemical detection, biological samples

CHEMICAL CHARACTERIZATION OF WINE

Violeta Ivanova-Petropulos

Faculty of Agriculture, Goce Delcev University, Stip, Krste Misirkov, 10A, 2000 Stip, Republic of North Macedonia
e-mail: violeta.ivanova@ugd.edu.mk

Introduction and objectives: Wine consists of numerous organic and inorganic compounds which determine its quality. Therefore, chemical characterization of wine is very important to be determined in order to understand how the compounds are changing during the winemaking and influencing the taste and aroma.

Materials and methods: The chemical composition of wine was characterized by chromatographic (HPLC-DAD, HPLC-MS, UPLC-QTOF-MS, GC-MS) and spectroscopic methods (AAS, ETAAS, ICP-OES, ICP-MS) as well as with traditional volumetric analytical techniques.

Results and discussion: Determination of organic acids, biogenic amines and polyphenols was performed by reversed phase high-performance liquid chromatography (RP-HPLC) using C18 column for separation of analytes. Detection of compounds was performed with DAD, MS and Q-TOF-MS detectors. Analysis of aroma compounds was performed by gas chromatography coupled to mass spectrometry (GC-MS), while multielement analysis of wines is performed with electrothermal atomic absorption spectroscopy (ETAAS), inductively coupled plasma - optical emission spectrometry (ICP-OES) and inductively coupled plasma - mass spectrometry (ICP-MS). Wines presented complex chemical composition, confirming the stability and importance to the quality.

Conclusions: Advanced analytical techniques were applied for Macedonian red and white wines analysis, including spectroscopic and chromatographic methods, assessing the chemical composition and quality.

Keywords: wine composition, chromatography, spectroscopy.

MIXED-MODE CHROMATOGRAPHY FOR SEPARATION OF PROTEIN DIGESTS

Květa Kalíková[1], Zuzana Kadlecová[1], Martin Gilar[2]

[1]Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Prague
[2] Waters Corporation, Milford, USA
e-mail: kalikova@natur.cuni.cz

Introduction and objectives: Protein digestion is usually performed with trypsin, a highly specific serine endoprotease. The bottom-up approach is the most commonly used method for protein identification in proteomics. On the other hand, on-line digestion methods, i.e. direct connection of column with cleaving enzyme (e.g. trypsin) and separation column in a series are only rarely used. Therefore, the aim of this work was to evaluate digestion efficiency of trypsin immobilized enzyme reactors (IMERs) differing in trypsin coverage, connected in series with analytical mixed-mode separation column in LC.

Materials and methods: Two trypsin column reactors with the different trypsin coverage on the bridged ethylene hybrid particles were evaluated. Off-line digestion using trypsin spin columns was performed for comparison of digestion efficiency. Mixed-mode Atlantis Premier BEH C18 AX column combining reversed-phase and anion-exchange properties was used for separation of protein digests. The evaluation of trypsin activity was performed by on-line digestion of N_{α}-benzoyl-L-arginine 4-nitroanilide hydrochloride (L-BAPNA). Various proteins as cytochrome C, enolase, myoglobin etc. were on-line and off-line digested by trypsin.

Results and discussion: Based on the results from BAPNA digestion, optimal chromatographic conditions were applied also for digestion of more complex proteins. Column temperature was 37.0°C, pH of aqueous part of mobile phase (MP) was 8.5, to achieve sufficient trypsin activity. During the initial (digestion) state of analysis, flow rate was decreased, and no organic modifier was present in the MP. Trypsin IMERs were utilized for over 300 injections without any noticeable loss of digestion activity.

Conclusions: By directly connecting the trypsin column to the mixed-mode column in a series, a higher sample throughput can be achieved compared to alternative digestion methods. This approach is uncomplicated and can be adopted in most laboratories without need for LC instrument modifications.

The research was supported by the Czech Science Foundation, Grant No. 20-19655S. The work was partly realized within the cooperation of CEEPUS project No. CIII-RO-0010-17-2223.

Keywords: mixed-mode column, immobilized enzymatic reactor, on-line protein digestion, trypsin

SPECTROSCOPIC AND THERMODYNAMIC STUDIES OF NABUMETONE COMPLEXATION BY B-CYCLODEXTRINS

David Klarić[1], Ana Čikoš[2], Ana Budimir[3], Nives Galić[1]

[1]Department of Chemistry, Faculty of Science, University of Zagreb, Croatia
[2]NMR Centre, Ruđer Bošković Institute, Zagreb, Croatia
[3]Department of General and Inorganic Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia
e-mail: dklaric@chem.pmf.hr

Introduction and objectives: Nonsteroidal anti-inflammatory prodrug nabumetone (NAB) is characterized by low solubility and high permeability. Its solubility can be increased by the formation of inclusion complexes with cyclodextrins (CDs). This study aims to interpret the NAB/β-CD inclusion phenomenon together with a detailed characterization of NAB interactions with β-CD, and its derivatives (RMβCD, HPβCD, SBEβCD).

Materials and methods: Complexation of NAB (Cayman Chemical) with selected β-CDs (CyloLab) was studied by fluorescence spectroscopy and isothermal titration calorimetry (ITC) in water. The ^{1}H, ^{13}C, COSY, NOESY, 1D and 2D ROESY, DOSY, HSQCe, and HMBC NMR studies were performed in D_2O and DMSO-d_6.

Results and discussion: Spectrofluorimetric titrations of NAB by β-CDs were performed and stability constants were determined. Other thermodynamic parameters were obtained by ITC (logK, $\Delta_r H$, $\Delta_r S$, $\Delta_r G$). Stability constants were in good agreement with those determined by spectrofluorimetric titrations. The evaluated thermodynamic profile suggested strong and spontaneous interaction. Detailed NMR studies confirmed that the complexes are formed through the inclusion mechanism where NAB enters the β-CD. Two possible modes of inclusion were observed.

Conclusions: All studied β-CDs form stable 1:1 inclusion complexes with NAB by encapsulating the naphthalene moiety of the drug into their central cavity.

Keywords: nabumetone, cyclodextrin, inclusion complexes, characterization

---

ENDORSING BIOPERFORMANCE THROUGH DISSOLUTION PREDICTION USING MULTIVARIATE DATA ANALYSIS (MVDA)

Béla Kovács[1,2], Erzsébet Orsolya-Tőkés[2], Brigitta Nagy[3], Zsombor Kristóf Nagy[3], Éva Katalin Kelemen[2], Katalin Zöldi[2], Francisc Boda[4], Tibor Casian[5]

[1]Department of Biochemistry and Environmental Chemistry, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș
[2]Gedeon Richter Romania, Târgu Mureș, Romania
[3] Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Budapest, Hungary
[4]Department of Inorganic Chemistry, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș
[5] Department of Pharmaceutical Technology and Biopharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
e-mail: bela.kovacs@umfst.ro

Introduction and objectives: Solid oral pharmaceutical formulations containing BCS II active pharmaceutical ingredients (API) pose a challenge in terms of in vitro release kinetics, as the low solubility of these active substances will confine their behaviour *in vivo*. Data mining using powerful computational tools might offer a complementary understanding of product performance alongside traditional experimental approaches, bearing valuable information for scale-up. The present study aimed the application of MVDA in the prediction of the dissolution behaviour of Clopidogrel during technology transfer from laboratory scale (LS) to pilot scale (PS).

Materials and methods: Clopidogrel and chosen excipients were processed by fluidized hot-melt granulation. During LS development the type of plasticizer (Macrogol 6000 / 8000), granulation temperature, and the influence of lubricant were assessed on product performance. In-silico models were developed using the SIMCA 17 software, in order to characterize the similarities and differences in terms of manufacturability of tablets and dissolution profiles recorded at LS and PS.

Results and discussion: Of the tested factors, the type of Macrogol influenced in a significant manner the amount of API released, mainly at the first time-point. Macrogol 6000 increased, whilst Macrogol 8000 hindered the in-vitro release kinetics of the API on both LS and PS. The granulation temperature contributed to a higher resistance to crushing of tablet cores, but did not influence in a compelling way the dissolution of the API.

Conclusions: Computational modelling, through its multivariate and versatile nature can complement the empirical approach based on trial and error during product scale-up, thus ensuring a product performance that can fulfil the quality target product profile requirements.

The research was supported by the University of Medicine, Pharmacy, Science and Technology „George Emil Palade" of Târgu Mureș Research Grant number 510/8 /17.01.2022

Keywords: Clopidogrel, MDVA, scale-up, dissolution prediction

SEPARATION AND PREDICTION OF INTACT N-GLYCOPEPTIDE RETENTION TIME WINDOWS IN HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY

Petr Kozlik[1], Katarina Molnarova[1]

[1]Department of Analytical Chemistry, Faculty of Science, Charles University
e-mail: kozlik@natur.cuni.cz

Introduction and objectives: The examination of protein glycosylation presents difficulties due to the varied and complex nature of the attached glycans. Hydrophilic interaction liquid chromatography (HILIC) is the suitable method for separating glycopeptides, as they cannot be adequately resolved using reversed phase chromatography. In this study, we introduce a user-friendly model for accurately predicting the retention time ranges of glycopeptides in HILIC.

Materials and methods: We conducted LC-MS/MS analysis using a nanoAcquity UPLC system equipped with a binary pump from Waters (Milford, MA, USA) connected to a 6500 Q-TRAP mass spectrometer from AB Sciex (Framingham, MA, USA). For glycopeptide separation, we utilized a HALO® penta-HILIC column with dimensions of 150 mm × 75 µm, packed with superficially porous particles measuring 2.7 µm in diameter, provided by Advanced Materials Technology (Wilmington, DE, USA). The mobile phase composition comprised 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). We employed the following gradient program [(minutes)/% B]: 0/85, 5/85, 50/60, 75/30, 85/30, 87/85, and 100/85.

Results and discussion: We analyzed the retention patterns of multiple glycoforms from six tryptic peptides derived from haptoglobin, hemopexin, and sex hormone-binding globulin using nanoHILIC. The relative spacing between glycoforms attached to the same peptide backbone exhibited minimal variation across different peptides. Consequently, we expressed the retention of individual peptide glycoforms by comparing their retention time to that of the corresponding bi-antennary glycoform. By examining the variances in the relative retention times (RRTs) of individual glycoforms, we developed a user-friendly mathematical model, which we successfully applied to predict the retention time ranges of fetuin glycopeptides in HILIC.

Conclusions: The utilization of relative retention time windows offers additional insights in conjunction with mass spectrometric data, and we deem them valuable for accurate identification of protein glycosylation at specific sites.

The research was supported by CEEPUS, network RO-0010-17-2223 – Teaching and Learning Bioanalysis, Charles University, project SVV260690, and by the Grant Agency of Charles University (No 336421)

Keywords: Glycopeptides, liquid chromatography, HILIC

APPLICATION OF CAPILLARY ELECTROPHORESIS IN CONTROLLED DRUG RELEASE STUDIES

Tomáš Křížek[1], Alice Šimonová[1], Kevin Kotalík[2], Markéta Frejková[2], Tomáš Etrych[2]

[1]Department of Analytical Chemistry, Faculty of Science, Charles University, Prague, Czech Republic
[2]Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic
e-mail: tomas.krizek@natur.cuni.cz

Introduction and objectives: HPLC is the method of choice for monitoring drug release from polymeric nanocarriers and other nanomedicines. Nevertheless, polymeric carriers usually cannot be injected into the HPLC system. On the contrary, CE tolerates problematic sample matrices, and adsorbed polymers can be washed out with a base, acid, or organic solvent. Here we report on the application of CE to the determination of hydrophilic drugs released from polymeric carriers.

Materials and methods: Determination of 5-aminolevulinic acid and its hexyl ester: Fused-silica capillary 20 µm i.d., 50.0/35.0 cm; background electrolyte 1 M HCOOH; injection 5.0 kPa × 20 s; voltage +25 kV + 10 kPa, current 10 µA; contactless conductivity detection; internal standard for quantification Tris·HCl. Determination of acetylsalicylic acid, salicylic acid, and salicyl hydrazide: Fused-silica capillary 50 µm i.d., 50.0/41.5 cm; background electrolyte 20 mM $Na_2B_4O_7$; injection 5.0 kPa × 5 s; voltage +30 kV, current 48 µA; UV detection @ 200 nm; internal standard sodium benzenesulfonate.

Results and discussion: We have developed a method for monitoring the release of 5-aminolevulinic acid and its hexyl ester from N-(2-hydroxypropyl)methacrylamide-based copolymer. The conductivity detection solved the problem of weak UV absorption of the analytes. Using Tris as an internal standard and flushing with 1M NaOH, water, and 1M HCOOH before each run provided very good linearity and repeatability. Applying pressure to the inlet vial stabilized the baseline and shortened the separation to 5 min. The total analysis time was 14 min. Copolymer samples loaded with the drug were incubated at 37 °C and directly injected into CE. Differences in release kinetics at pH 5.0, 6.5, and 7.4 were observed. The second method allowed monitoring of the release of acetylsalicylic acid from the same polymer using UV detection. The separation lasted 4 min. The total analysis time was 9 min. Excellent linearity and repeatability were achieved.

Conclusions: We have demonstrated that CE is a well-suited technique for determining hydrophilic drugs released from polymeric nanocarriers in controlled drug release studies. The monitored solution can be analyzed without any pre-treatment within a few minutes with great precision and accuracy.

The research was supported by CEEPUS, network RO-0010-17-2223 – Teaching and Learning Bioanalysis, and by Charles University, project SVV260690.

Keywords: capillary electrophoresis, controlled drug release, polymeric carriers, HPMA copolymer

FAST AND COST-EFFECTIVE ELECTROCHEMICAL FABRICATION OF SERS SUBSTRATES USING SCREEN-PRINTED ELECTRODES

Petrică-Ionuț Leva, Rebeca-Magda Moldovan, Bogdan-Cezar Iacob, Ede Bodoki, Lucian Barbu-Tudoran

[1]Department of Analytical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy"Iuliu-Haţieganu" of Cluj-Napoca
e-mail: petreleva@gmail.com

Introduction and objectives: Pollutants represent a serious issue for environmental health. Pharmaceutical compounds and Pesticides are two of the most studied pollutant classes. Raman spectroscopy is a fast and high sensitivity analysis method, offering fingerprint-like information about the studied analytes which makes it ideal for detecting multiple compounds from various samples without the need of substrate specificity. Metallic nanostructures generated on the analyzed substrate amplify the Raman signal significantly. Electrochemically roughening the surface of gold screen printed electrodes represent the purpose of this study, in order to obtain substrates suitable for EC assisted SERS determinations

Materials and methods: For this experiment DropSense 220BT gold screen-printed electrodes were used. A Metrohm Autolab PGSTAT204 potentiosta and a portable PalmSens SensitSmart were used for the electrochemical procedures. The SERS experiments were performed using an AvaSpec-ULS2048x64TEC-EVO detector with a 785nm lightsource, conected to a Nikon Eclipse Ni-U microscope. For this purpouse three substances were taken into analysis: Thiabendazole(TBZ), Propranolole(PRNL) and 4-Aminothiophenole(4-ATP). The electrochemical methods used were Chronoamperometry (CA), Fast Chronoamperometry (CAF) and Cyclic voltammetry (CV).

Results and discussion: The maximum surface amplification of the Raman signal was obtained with CA using KCl 0,1M as working electrolyte: 36286 counts at 1081 cm^{-1} for 4-ATP(RSD=12,14%), 1863,77 counts at 1386cm^{-1} for PRNL (RSD=21,4%) and 301,93 for the 784 cm^{-1} peak (RSD 20,32%%) and 441,72 for the 1012 cm^{-1} peak (RSD 21,85%%) for TBZ.

Conclusion: The optimal electrochemical roughening technique for obtaining a SERS substrate from screen-printed gold electrodes is chronoamperommetry. The most suitable electrolyte used for the modification of the electrode surface using chronoamperommetry is KCl 0,1M.

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.(3)

Keywords: pollutants, Raman, electrochemical, nanostructures, spectroscopy

---

IDENTIFICATION OF POTENTIAL STRESS INITIATED PEPTIDES IN SUPPORT OF TISSUE REGENERATION AND REPAIR IN HUMANS

Szabolcs Maar[1,2], Lilla A. Czuni[2], Szilard Rendeki[3], Dominik Cysewski[4], Istvan Farkas[5], Aniko Takatsy[1,2], Tomasz Kowalczyk[4], Michał Ciborowski[4], Adam Kretowski[4], Ferenc Gallyas[1,2], Ildiko Bock-Marquette[1,2]

[1]Department of Biochemistry and Medical Chemistry, [2]Szentagothai Research Centre, University of Pecs Medical School, Pecs, Hungary; [3]Military Medicine, Disaster Medicine and Law Enforcement Medicine, University of Pecs Medical School, Pecs, Hungary; [4]Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland; [5]International Training Centre, Budapest, Hungary
e-mail: ildiko.bock-marquette@aok.pte.hu

Introduction and objectives: As a result of intense psychological and physical stress, numerous biochemical changes are initiated through various signaling pathways in order to maintain and restore cellular homeostasis. Changes at the level of gene expression and at proteomic level are the subjects of intensive studies. The aim of our research was to map biomolecules promoting the regeneration of adult organs and the signaling pathways activated in regeneration.

Materials and methods: Plasma samples were collected from cadets exposed to a strictly controlled intense stress situation. Samples taken before the stress condition served as controls. Proteomic studies were performed with 2D gel electrophoresis, MS-compatible silver staining. Spots showing significant changes were examined with MALDI-TOF analysis after in-gel digestion, and LC-MSMS analysis after trypsin digestion and SP3 protocol purification.

Results and discussion: More than 200 protein targets were identified based on intensity differences. Among others, Apolipoprotein A-I and Alpha-1 antitrypsin, already described as biomarkers by others, were found to be significant with both methods. In addition, a significant decrease in plasma SPARC/SPARCL1 levels could be verified after thirty minutes of psychological stress. An increase in these proteins in mice was associated with right ventricular maladaptive remodeling. A more precise mapping of the relationship between SPARC/SPARCL1 and stress requires further studies in humans.

Conclusions: We believe that the future therapeutic use or inhibition of the potential marker molecules connected to short-term psychological stress response, that were identified during our research can prevent irreversible tissue damage and support the regeneration of organs.

Keywords: Psychological stress, Proteomics, Adaptation, Regeneration

BACTERIAL SECONDARY METABOLITE QUANTIFICATION USING BIOANALYTICAL METHODS

Gyöngyvér Mara[1], Annamária Becze[1,2], Éva Boglárka Vincze[1,2], Klementina Imelda Ilkei[1,2], Csilla Albert[3]

[1]Department of Bioingineering, Faculty of Economics, Socio-Human Sciences and Engineering, Sapientia Hungarian University of Transylvania
[2]Doctoral School of Chemistry, Faculty of Natural Sciences, University of Pécs
[3]Department of Food Engineering, Faculty of Economics, Socio-Human Sciences and Engineering, Sapientia Hungarian University of Transylvania
e-mail: maragyongyver@uni.sapientia.ro

Introduction and objectives: Secondary metabolites are non-essential, small biomolecules that provides to the producer microorganism survival advantage, through improving nutrient availability, protecting against environmental stressors, or enhancing competitive interactions and defense mechanism. The objective of the study was to quantify secondary metabolites of plant associated bacterial strains and also from human microbiome.

Materials and methods: Lipopetides derived from plant associated bacterial strains were analyzed using Agilent Infinity 1260 HPCL, and the detection was performed at 230 nm wavelength. The samples were loaded onto a Pursuit C18 column, a constant flow rate of 1.0 mL/min was applied. SCFAs from human microbiome derived bacterial strains were analyzed using a Coregel87H3 column, with a constant flow rate of 0.6 mL/min. The detection was performed at 210 nm wavelength.

Results and discussion: Both methods have proven to be suitable for the detection of secondary metabolites, although nowadays the aim is to investigate the whole metabolome in order to identify new metabolites.

Conclusions: Microbial secondary metabolites have a great importance, therefore future studies for understanding how to enhance the production of bioactive secondary metabolites are needed.

Keywords: lipopeptides, SCFAs, HPLC

INNOVATIONS IN MICROCHIP ELECTROPHORESIS FOR BIOANALYTICAL APPLICATIONS

Marián Masár, Jasna Hradski, Peter Troška, Marta Ďuriš, Roman Szucs

Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University Bratislava
e-mail: marian.masar@uniba.sk

Introduction and objectives: Considerable progress in life sciences creates growing demand for robust and automated analytical systems capable of immediate response for increasingly complex biosamples. Microchip electrophoresis (MCE) has significant benefits in terms of high-speed, high separation efficiency, high-throughput, reduced consumption of solvents and production of waste, easy automation, and low running costs.

Materials and methods: MCE separations were carried out on an inhouse constructed equipment with two main operational units: electrolyte and electronic unit. Electrolyte unit consists of peristaltic micropumps and membrane driving electrodes. Electronic unit delivers stabilized driving current to the electrodes and controls the peristaltic micropumps. A poly(methyl methacrylate) microchip used in this work consisted of two separation channels equipped with two conductivity detectors. Miniaturized surface enhanced Raman spectrometer and Vis spectrometer were implemented directly on the microchip; ion mobility spectrometer was coupled online using interface based on thermal spray.

Results and discussion: Theoretical and instrumental aspects of MCE as well as its integration with various detection techniques are presented. Practical examples of the separation and the determination of the analytes present in complex samples of biological and pharmaceutical origin are demonstrated. Microchips equipped with coupled channels facilitate combination of different electrophoretic separation techniques. In this context, the benefits of the use of electrophoretic microchips with coupled channels and different miniaturized detectors for determining trace analytes in complex ionogenic samples are shown. For example, the separation and determination of metabolic organic acids in the cerebrospinal fluid, biomarkers of some neurological diseases in urine, or the separation and identification of dyes in pharmaceuticals are presented.

Conclusions: MCE performed on the microchip with coupled channels is a multifunctional analytical tool, which facilitates online integration of sample pretreatment with two-dimensional separation and utilization of various detection techniques for the analyses of complex biological samples.

The research was supported by the Slovak Research and Development Agency (APVV-22-0133 and APVV-17-0318) and the Slovak Grant Agency for Science (VEGA 1/0116/22).

Keywords: microchip electrophoresis, bioanalysis, detection techniques

PREFORMULATION RESEARCHES OF A COMBINED POWDER IN THE TREATMENT OF HYPOKALEMIA

Ecaterina Mazur[1,2], Vladimir Valica[1,2], Vladilena Gandacov[1,2], Livia Uncu[1,2]

[1]Scientific Center for Drug Research
[2]Department of Pharmaceutical and Toxicological Chemistry
Nicolae Testemitanu State University of Medicine and Pharmacy of Republic of Moldova Chișinău, Republic of Moldova
e-mail: ecaterina.mazur@usmf.md

Introduction: Development of effective and stable dosage forms is a long and complex process, requiring well-done data-base and teamwork from various disciplines. Therefore, the first study step is known as the preformulation stage. Preformulation study is focused on the establishment of the physicochemical parameters of the active pharmaceutical ingredient (API) and excipients (Es). Due to recently developed techniques, such as FT-IR spectroscopy, DSC, HPLC, it is more possible to accurately select the Es as a candidate for powder to predict potential interactions (physical or chemical). On the other hand, technological characteristics, such as particle size and particle shape, surface area, bulk density, powder flow properties, density, compressibility, crystallinity, polymorphism and hygroscopicity play important role to select excipients for solid-dosage form to ensure the same dosage of APIs in the powder by filling of the matrix channel and to improve bioavailability of APIs.

Object: The aim of this work was to study the technological characteristic of individual excipients and with API (potassium and magnesium aspartate, potassium orotate, spironolactone) at the preformulation level.

Material and methods: APIs: potassium and magnesium aspartate, potassium orotate (Sigma-Aldrich) and spironolactone, (Acros Organic), Es: microcrystalline cellulose, lactose monohydrate, (Cel.; Lact.; Himedia), fructose, acid stearic (Fr.; Stear.; Chem-Lab) and apparatus: RAD-WAG analytical electronic balance, ERWEKA tapped density tester, VP12A powder flow speed tester were used.

Results: To evaluate the degree of powder flowability scaled by flowability (powder flow rate through the hole), the angle of repose, bulk volume were analyzed and calculated for prepared powder models. According to this technical characteristic: flow speed (<100g/25s), angle of repose (<35^0), Carr's Index (<26%), Hausner's Ratio (<1.35), all prepared powder models were analyzed and were selected: M8 [SA-23.6%, Cel-50%, Lact-26.4%], M13[APIs-23.6%, Cel-74.4%, Stear.-1%], M17 [APIs-23.6%, Cel-37%, Fr-37%, Stear.-1%], corresponding to these established limits.

Conclusions: Following these studies, the technologically optimal powder models for the formulation of stable, safe and uniform powders were selected (M8, M13, M17), which will be used for the next stages of analysis.

The study was carried out with the support of the project 20.80009.8007.14 Cercetări complexe de elaborare a noilor produse farmaceutice antiinfecțioase autohtone pentru optimizarea farmacoterapiei afecțiunilor stomatologice, orofaringiene și auriculare.

Keywords: powder, technological characteristics.

DEVELOPMENT OF A CAPILLARY ELECTROPHORESIS METHOD FOR THE IMPURITY PROFILING OF OMEPRAZOLE USING QUALITY BY DESIGN METHODOLOGY

Adriana Modroiu[1], Gabriel Hancu[1], Luca Marzullo[2], Serena Orlandini[2], Roberto Gotti[3], Sandra Furlanetto[2]

[1]Department of Pharmaceutical and Therapeutic Chemistry, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Targu Mures
[2]Department of Chemistry "U. Schiff", University of Florence, Italy
[3]Department of Pharmacy and Biotechnology, University of Bologna, Italy
e-mail: adriana.modroiu@umfst.ro

Introduction and objectives: Omeprazole (OMP) is a proton pump inhibitor that suppresses secretion of gastric acid by inhibiting the enzyme system of H^+/K^+ ATPase. In recent years the Analytical Quality by Design (AQbD) methodology has been widely used in analytical method development, including in capillary electrophoresis (CE), being based on process understanding and control by quality risk management. In the current study a CE method for the impurity profiling of OMP was developed, applying AQbD methodology to enhance method robustness, efficiency, and reliability.

Materials and methods: The best results from the initial scouting experiments were obtained using a pseudostationary phase composed of micelles of sodium dodecyl sulfate (SDS) and n-butanol in borate buffer. A three-level screening design was used to evaluate the influence of critical method parameters on the critical method attributes, followed by a central composite orthogonal design for the method optimization.

Results and discussion: Probability maps were drawn, making it possible to identify the method operable design region (MODR), that is the multivariate zone where the method requirements were fulfilled with a selected probability. The following optimized conditions were selected: 72 mM borate buffer, pH 10.0, 96 mM sodium dodecyl sulfate, 1.45 %v/v n-butanol, capillary temperature 21°C, and an applied voltage of 25 kV. Using the optimized conditions, the baseline separation of all analytes was achieved.

Conclusions: The newly developed solvent-modified micellar electrokinetic chromatography (MEKC) method enables the simultaneous determination of omeprazole and 7 related substances. By following the AQbD methodology, the CE method for the impurity profiling of omeprazole could be developed in a systematic and controlled manner.

Keywords: Capillary electrophoresis, Analytical Quality by Design, Omeprazole, Impurity control

GLYCOPEPTIDE ENRICHMENT USING SOLID-PHASE EXTRACTION IN HILIC MODE

Katarína Molnárová[1], Michaela Chobotová[1], Petr Kozlík[1]

[1]Department of Analytical Chemistry, Faculty of Science, Charles University
e-mail: katarina.molnarova@natur.cuni.cz

Introduction and objectives: The enrichment of glycopeptides is an essential step prior to their analysis by LC-MS/MS. Solid phase extraction (SPE) on polar stationary phase in hydrophilic interaction liquid chromatography (HILIC) is a promising technique for the enrichment due to its specificity toward glycosylated peptides.

Materials and methods: The glycopeptides of human immunoglobulin G (IgG) were enriched in SPE-HILIC mode on an aminopropyl-modified silicagel column (45 µm, Chromabond Macherey-Nagel). The measurements were carried out on an Agilent 1290 Infinity II LC System with a binary pump (Agilent Technologies, Inc., Waldbronn, Germany) interfaced with maXis™ Q-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The glycopeptide separation was performed in reversed-phase (BEH C18, 2.1 × 100 mm; 1.7 µm; Waters Corporation, Milford, MA, USA). The mobile phase consisted of water (A) and acetonitrile (B) both with 0.1% formic acid. The following gradient was used [(min)/%] 0/5-3/5-15-25-30/80-33-80-35/5-40/5. The glycopeptides were analyzed in data-independent acquisition mode.

Results and discussion: Different elution solvents have been tested on the enrichment of IgG glycopeptides. We observed that the best enrichment efficiency was achieved when acetonitrile was used for the glycopeptide elution. In general, sialylated glycopeptides were eluted from the column later than the neutral ones that was caused by their stronger interaction with the positively charged aminopropyl functional group of the stationary phase. We studied the effect of formic acid and acetic acid as two additives of the elution solvent on the enrichment. At last, three different concentration of acetonitrile (65%, 75% and 85%) were tested during the conditioning and washing step.

Conclusions: The composition of the elution solvent has an important influence on the glycopeptide enrichment, thus the optimization of sample preparation plays a key role in their analysis.

The research was supported by CEEPUS, network RO-0010-17-2223- Teaching and Learning Bioanalysis, Charles University, project SVV260690, and by the Grant Agency of Charles University (No 336421).

Keywords: glycoproteomics, glycopeptide enrichment, hydrophilic interaction liquid chromatography, solid-phase extraction

---

CZE-MS FOR BOTTOM-UP PROTEOMICS: TO DESALT OR NOT TO DESALT?

Cynthia Nagy[1], Melinda Andrasi[1], Robert Bodor[2], Attila Gaspar[1]

[1]Department of Inorganic and Analytical Chemistry, University of Debrecen, Hungary
[2]Department of Analytical Chemistry, Comenius University Bratislava, Slovakia
e-mail: nagy.cynthia@science.unideb.hu

Introduction and objectives: Bottom-up proteomics aims at the extensive characterization of protein samples with analytical platforms such as reversed-phase liquid chromatography (RPLC) or capillary electrophoresis (CE) coupled to tandem mass spectrometry (MS/MS). Sample clean-up prior to analysis is generally considered a necessity, however, the process can lead to peptide loss. The goal of this work was to investigate the significance of desalination, the most often use approach for matrix removal.

Materials and methods: Protein digests of varying complexity (albumin, human tears and yeast cell lysate) were analyzed with CE using UV and MS detection before and after desalination with C18 solid-phase extraction (SPE) pipette tips. The CE (7100 model CE instrument,Agilent) and MS (maXis II UHR ESI-QTOF MS, Bruker) were coupled with a standard coaxial sheath liquid interface (Agilent). Furthermore, ammonium bicarbonate (AB) or acetonitrile (ACN) were added to non-desalinated digests to study sample enrichment phenomena in a capillary zone electrophoresis (CZE) system.

Results and discussion: Desalination, indeed, altered the peptide profile, samples typically lacked small, hydrophilic peptides that were not retained on the reversed-phase SPE packing. In addition to removing the potentially interfering components from the digests, SPE is often used for off-line sample enrichment. Non-desalinated digests actually offer a very attractive alternative to off-line enrichment, since the presence of salts can induce an on-line stacking phenomenon most probably via transient isotachophoresis. Stacking efficiency was further enhanced by the addition of ACN; sequence coverage values and number of protein hits increased.

Conclusions: Although bottom-up workflows often resort to desalination prior to analysis, in our case it did not prove beneficial. It was found that in addition to having a more complete set of cleavage products, non-desalinated digests can also promote the on-line preconcentration of peptides, allowing a more comprehensive characterization of protein samples.

The research was supported by the CEEPUS, NKFIH (ÚNKP-22-3-II), Hungary and the Richter Gedeon Talentum Foundation.

Keywords: bottom-up proteomics, capillary zone electrophoresis, stacking, mass spectrometry

FATTY ACID PROFILES OF OMEGA 3, 6, 9 ENRICHED VEGETABLE OILS

Katalin Nagy[1], Bogdan-Cezar Iacob[1], Ede Bodoki[1], Radu Oprean[1]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy of Cluj-Napoca
e-mail: Nagy.Katalin@umfcluj.ro

Introduction and objectives: The omega-3 and omega-6 fatty acids (FAs) are important components of the human body, due to their multiple biological roles. In the last few years, a wide variety of functional foods enriched with omega-3, 6 and 9 FAs have become commercially available. Hence, this study aims to compare the traditional vegetable oils to their omega enriched counterparts, and to distinguish which are only marketing strategies and which are truly beneficial for the health of consumers.

Materials and methods: The sample preparation involved a transesterification processes, followed by a derivatization with boron trifluoride-methanol solution and an extraction in hexane. Samples were analyzed by GC-MS, using a special column, Zebron™ ZB-FAME.

Results and discussion: Comparing the traditional sunflower oil and the high oleic sunflower oil, there is a huge difference in their fatty acid composition. The traditional one has a larger amount of PUFA, mainly omega-6, which consumed in large quantities supports inflammation. On the other hand, the high oleic sunflower oil is more stable to heat and oxidation, having a higher percentage of MUFA and a lower amount of PUFA. The composition of the high oleic sunflower oil is similar to the olive oil, made up of about 75% oleic acid. However, the olive oil is richer in the omega-3 fatty acid. Vegetable oils marketed with omega 3, 6 label have a high omega-6/omega-3 FA ratio, as is found in today's Western diets, which promote the pathogenesis of many diseases. Not only the components of the vegetable oils can influence the percentage of the omega-3 FA, but also the method of extraction. Results demonstrate that the refining process can reduce the quantity of these type of fatty acids.

Conclusions: The present study shows that unrefined rapeseed oils and high oleic sunflower oils have a better fatty acid composition, although the omega-3 FA content is very low compared to the recommended daily dose.

The research was supported by the European Social Found, Human Capital Operational Programme 2014-2020, project no. POCU/380/6/13/125171.

Keywords: GC-MS, vegetable oils, fatty acids, omega-3 fatty acids, omega-6 fatty acids

---

INSIGHTS INTO THE REGULATION OF LIPOPOLYSACCHARIDE BIOSYNTHESIS BY TWO-COMPONENT SYSTEMS IN SHIGELLA SONNEI

Laura Nagy[1], Ferenc Kilár

[1]Institute of Bioanalysis, Medical School, University of Pécs
e-mail: laura.nagy@aok.pte.hu

Introduction and objectives: Lipopolysaccharides are molecules that cover more than 70% of all Gram-negative bacteria, acting as barriers and providing structural integrity. Lipopolysaccharide biosynthesis - like a variety of other pathways - is under the regulation of bacterial two-component systems. This project demonstrates the connection between the biosynthesis of lipopolysaccharides and bacterial two-component systems, suggesting new possible targets on Gram-negative bacteria.

Materials and methods: Whole genomes of two Shigella sonnei species were sequenced by IonTorrent PGM. De novo assembly of genomes was performed by SPAdes v3.1 and scaffolds of the draft genome were reordered by the Mauve software and the MeDuSa web server. Lipopolysaccharide biosynthesis genes were identified using the KEGG database.

Results and discussion: Analyzing an interesting Shigella sonnei mutant, a hotspot was identified in lipopolysaccharide biosynthesis by the epimerase gmhD. Targeting the gene through the bacterial two-component system by Closantel resulted in a neutral effect.

Conclusions: Bacterial two-component systems can play a role in the regulation of LPS biosynthesis by controlling the expression of genes involved in the biosynthesis. While Closantel did not affect the expression of gmhD, we will continue to test other two-component system inhibitors.

Supported by the ÚNKP-22-3 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund

Keywords: shigellosis, bacterial two-component system, lipopolysaccharide

ELECTROSPINNING NANOFIBROUS MATRICES FOR POTENTIAL WOUND HEALING APPLICATION: THE EFFECT OF SALTS AND HUMIDITY

Mónika Nanys, Ákos György Juhász, Angéla Jedlovszky-Hajdú

Laboratory of Nanochemistry, Department of Biophysics and Radiation Biology, Faculty of Medicine, Semmelweis University, Hungary
e-mail: monika.nanys@gmail.com

Introduction and objectives: Very thin, nano- and microfibers can be created through the technique called electrospinning. The formed fiber meshes are closely resembling the extracellular matrix (ECM) present within the human body. Engrafting cells of a patient onto the fiber structure can assist in healing wounds and replacing missing tissues. If the pores are too small, cells cannot permeate into the material to later help the healing process. The electrospun scaffolds have a very dense form which can be modified through different ways (post-spinning and during the spinning ones) to increase the pore sizes. The aims of my work were to find the right salt and salt concentration to perform this increase in the pore size, create three-dimensional structures, and observe the effects of humidity during the sample preparation.

Materials and methods: During the experiments I used different Polysuccinimide (PSI) (17-25 w/w %) and salt (3-7 w/w % CaCl2, MgCl2 and LiCl) concentrations dissolved in dimethylformamide, and created solid polymer fibers with electrospinning. The mechanical strength of the matrices was measured by a mechanical testing system, the chemical composition by an Infrared Spectrometer and the fibers surface and average fiber diameter was examined by a Scanning Electron Microscope.

Results and discussion: At 20 w/w % PSI concentration we could form thinner fibers than at 25 w/w % (200-360 nm instead of 530-1220 nm). Although beads appeared on the strings these disappeared with adding salt to the solution. On IR spectrum of the meshes new peaks appeared with the added salts. Higher concentration of solution created a higher durability with every sample. I worked on different humidity levels (27.4%-56.8%), after certain % fluffier structures were formed with increased porosity.

Conclusions: My experiments proved our assumption that the presence of salts impact the morphology and diameter of the fibers. I observed that 48% humidity is a threshold, below what all samples became thin, while above with the help of salts a loose, three-dimensional form was created.

The research was supported by the OTKA Foundation (Grant No. NKFIH FK 137749) and the UNKP-21-3-II-SE-56 new national excellence program of the ministry of human capacities.

Keywords: Electrospinning, wound healing, three-dimensional, humidity, salts

---

SELECTION OF APTAMERS FOR CANCER THERAGNOSTIC

Titus Obasi, Bogdan Feier, Magdolna Casian, Oana Hosu, Ioana Manea, Cecilia Cristea

Department of Analytical Chemistry, Faculty of Pharmacy, "Iuliu Hatieganu"University of Medicine and Pharmacy of Cluj-Napoca
e-mail: tcobasi@gmail.com

Introduction and objectives: Aptamers are short sequences of synthetic nucleotides - DNA, RNA or peptides that selectively bind to targets with high specificity, affinity and recognition in 3D-conformation. They are frequently used to target cellular proteins and markers, whose roles are essential for the development and progression of cancer disease. Over the years, the process of aptamer selection has gained prominence via SELEX-technology (Systematic evolution of ligands by exponential enrichment), an approach through which specific sequences are systematically evolved from single-stranded oligonucleotide library, using tools in molecular biology and combinatorial chemistry. Several innovations have been made on SELEX, e.g., Cell SELEX, CE-SELEX, micro-fluidic SELEX etc. Here, we targeted the selection of aptamers specific for Golgi-protein (GP73), for point-of-care early detection, screening, diagnosis and treatment of hepatocellular carcinoma (HCC).

Materials and methods: An in vitro selection technique was employed, using Tosyl-activated magnetic-beads (T-MB) Invitrogen. A PCR machine was also used, while the analysis conducted with Qubit florescence, gel-electrophoresis and UV-Vis spectrophotometer. Our methodology was closely fashioned to mimic the downstream assay, using negative/counter-SELEX procedure to facilitate the removal of non-specific sequences, and to increase the specificity of the aptamer.

Results and discussion: Results show 98% success rate for GP73 target-immobilization on T-MB, while the core-selection process yielded GP73 specific aptamer in shortest duration of cycles. A reduced incidence of "PCR bias" and of generation of "by-products" was recorded.

Conclusions: We developed a new parameter for in vitro selection of aptamer for GP73. Each step in the SELEX procedure was optimized and adapted to the selection of a specific aptamer for GP73, a biomarker of the HCC.

Keywords: Aptamer selection, SELEX-technology, hepatocellular carcinoma, GP73 biomarker

IMPACT OF UNNATURAL AMINO ACIDS IN THE DISCOVERY AND DEVELOPMENT OF BIOLOGICALLY ACTIVE PEPTIDES

Tamara Pajpanova

Institute of Molecular Biology "Acad. Roumen Tsanev", Bulgarian Academy of Sciences
e-mail: tamara@bio21.bas.bg

Peptides show great pharmaceutical tools as drugs and diagnostics in several clinical fields such as neurology, oncology, endocrinology, immunology, urology and recently as new type antimicrobial agents. Peptides are attractive drug candidates because of their high selectivity, low toxicity, and relative ease of synthesis. From a chemical point of view peptides are situated borderline between classical organic drug substances and high-molecular-weight biopharmaceuticals.

Since the properties of peptides are determined by the nature of the constituent amino acids, unnatural amino acids (i.e., those not genetically coded that naturally occur, or also synthetically produced) are important tools for modern drug discovery research. Generally, UAA incorporation is commonly used to enhance affinity and selectivity for a target and to increase the stability of peptides, as it generally induces or stabilizes secondary structures (α-helices, β-sheets, β-turns) increasing at the same time the resistance to proteases degradation.

This presentation summarizes the current use of several UAA, and the emerging new opportunities to development a new type of cationic antimicrobial peptides.

Special attention is paid to the structural characterization and chemical purity determination of the obtained antimicrobial peptides by LC-MS.

The research was supported by the CEEPUS network RO-0010-17-2223 "Teaching and learning Bioanalysis"

Keywords: peptides, cationic antimicrobial peptides, unnatural amino acids, LC-MS

DETERMINATION OF ENANTIOMERIC PURITY OF VILDAGLIPTIN BY CAPILLARY ELECTROPHORESIS

Lajos-Attila Papp[1], Blanka Székely-Szentmiklósi[1], Zoltán-István Szabó[2], Gergő Tóth[3], Márta Kraszni[3], Gabriel Hancu[1]

[1]Department of Pharmaceutical and Therapeutic Chemistry, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș
[2]Department of Drugs Industry and Pharmaceutical Management, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș
[3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Semmelweis University, Budapest
e-mail: lajos.papp@umfst.ro

Introduction and objectives: Vildagliptin (VIL) is a dipeptidyl-peptidase-4 inhibitor, used in the treatment of type 2 diabetes mellitus, a chronic metabolic disorder, with increasing prevalence. VIL possesses one chiral center in its structure and it is commercialized as enantiomerically pure S-VIL. The present study aimed at the development and validation of a cyclodextrin-mediated capillary electrophoretic method for the enantiomeric purity analysis of VIL.

Materials and methods: A systematic screening of cyclodextrins (CDs) as chiral selectors, was performed using native neutral, derivatized neutral, and derivatized ionizable CDs, at three pH levels using phosphate (pH 2.5, pH 7.0) and acetate (pH 4.5) buffers. Method optimization was performed by experimental design approach, by examining the effect of such parameters as the concentration of buffer and CD, buffer pH, capillary temperature, and the applied voltage on the chiral resolution and analysis time. The analytical performances of the optimized method were determined and the method was applied for the determination of enantiomeric purity of VIL from pharmaceutical products. The interaction between VIL enantiomers and alpha-CD responsible for chiral recognition was studied by NMR experiments and molecular docking.

Results and discussion: Based on the initial CD and BGE screening, acetate buffer (pH 4.5) containing alpha-CD proved to be the most suitable separation medium for VIL enantiomers, with favorable migration order (R-VIL followed by S-VIL). The optimized analytical conditions (75 mM acetate buffer pH 4.5, containing 50 mM alpha-CD, 18 kV applied voltage, and 15 °C capillary temperature) provided baseline separation of VIL enantiomers within 10 min. The validated method proved to be reliable and applicable in the determination of enantiomeric purity of VIL including the analysis of drug products.

Conclusions: The developed CE method shows the advantages of short analysis time, simplicity of the analytical conditions, high resolution, and low reagent and analyte consumption. Besides, the complexation mechanism between VIL and alpha-CD has been studied, and the possible interactions responsible for the chiral recognition have been described.

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNSC – UEFISCDI, project number PN-III-P1-1.1-PD-2021-0117, within PNCDI III.

Keywords: vildagliptin, enantiomeric purity, alpha-cyclodextrin, complexation mechanism

DETERMINATION OF TOTAL PHENOLS, ANTHOCYANINS AND COLOR PARAMETERS OF VRANEC WINES

Aleksandar Piperevski[1], Violeta Ivanova-Petropulos[2]

[1]Imako Vino Winery, Mihajlo Apostolski 34/5 2000 Stip, Republic of North Macedonia
[2]Faculty of Agriculture, Goce Delcev University, Stip, Krste Misirkov 10A, Stip, Republic of North Macedonia
e-mail: apiperevski@yahoo.com

Introduction and objectives: In this study, total phenols (TP), total anthocyanins (TA) and colour parameters of Vitis Vinifera red wines Vranec from vintage 2022, produced in the Republic of N. Macedonia, have been evaluated. The wines have been produced with three different fermenters, including classic fermenter, roto fermenter and punch-down fermenter, in order to study and compare the effect of vinification on phenolic composition.

Materials and methods: Total phenols were determined using the Folin-Ciocalteu methot at 765 nm and expressed as gallic acid equivalent (GAE, mg/l). Determination of the total anthocyanins was realized by the method proposed by Di Stefano. For calculation of colour intensity (CI) and hue (H), wine absorbance was measured at 420 nm, 520 nm and 620 nm.

Result and discussion: Vinification technique had an influence of the phenolic content of wines, observing highest content of TP and TA in Vranec wines produced with roto fermenter (TP: 3222 mg/L, TA: 820 mg/L) in comparison to Vranec wines produced with punch-down fermenter (TP: 2987 mg/L, TA: 742 mg/L) and classic fermenter (TP: 2350 mg/L, TA: 572 mg/L). In addition, the values for CI and H were the highest in Vranec wines produced with the roto process of fermentation.

Conclusion: Wines produced with roto fermenters presented highest content of total phenols, anthocyanins, colour intensity and hue, confirming that this fermentation technique is the most suitable for production of stable and complex wines rich in polyphenols.

Keywords: total phenols, anthocianins, Vranec wines, fermenters, spectrophotometry

---

PHOTOCATALYTIC DEGRADATION OF DRUG IN ENVIRONMENTAL SAMPLES

Ewa Poboży, Sylwia Kaczmarek

University of Warsaw, Faculty of Chemistry, Pasteur Str. 1, 02-093 Warsaw, Poland
e-mail: ewapob@chem.uw.edu.pl

Aquatic pollution caused by a great number of pharmaceutical compounds is an environmental problem that became an important public health issue over the last years. Pharmaceuticals such as antibiotics, analgetics, estrogens, anti-inflammatory and antiepileptic drugs are detected in surface waters, groundwater or drinking water. Many of these compounds are resistant to degradation and may remain in the environment over a long time to cause the potential adverse effects.

Conventional biological and chemical treatments are not effective for resistant pharmaceuticals and incompletely treated effluents from sewage plants are released into natural waters. Therefore, it is important to develop effective techniques to treat these pollutants.

In recent years, advanced oxidation processes (AOPs) are the most developed for non-biodegradable, chemically stable, and persistent pharmaceuticals degradation. In these AOPs processes, oxidation reactions are carried out using generated highly reactive oxygen species.

Among AOPs, heterogenous photocatalysis is found to be one of the most efficient methods to degrade pharmaceuticals. The mechanism of photocatalytic oxidation process is based on application semiconducting photocatalyst to initiate the formation of reactive oxygen species.

In this presentation application of advanced oxidation processes, including photocatalytic degradation of pharmaceutical active compounds in environmental samples will be discussed.

Keywords: advanced oxidation processes; photocatalytic degradation; environmental samples

INFLUENCE OF CYCLODEXTRINS ON LORATADINE SOLUBILITY IN WATER AND IN BIORELEVANT MEDIA

Marijana Pocrnić[1], Nives Galić[1]

[1]Division of Analytical Chemistry, Department of Chemistry, Faculty of Science, University of Zagreb
e-mail: mpocrnic@chem.pmf.hr

Introduction and objectives: Loratadine (LOR) is an antihistaminic drug, classified as BCS class II drug due to its low solubility and high permeability. Cyclodextrins (CD) are cyclic oligosaccharides known to improve solubility of poorly soluble drugs by forming inclusion complexes. The influence of natural cyclodextrins (α-CD, β-CD, γ-CD) and its hydroxypropyl derivative on LOR solubility in water and buffered solutions at different pH has already been published. However, solubility in biorelevant media has not been studied yet. The aim of this study was to investigate the influence of β-CD and its hydroxypropyl (HPβCD), randomly methylated (RMβCD) and sulfobutylether (SBEβCD) derivatives on the solubility of LOR in water, simulated intestinal media (SIM, pH 6.8) and simulated duodenal media (SDM, pH 4.5)

Materials and methods: Phase solubility studies were performed according to the method presented by Higuchi and Connors. Excess LOR was weighed and added to the CD solution in the concentration range 0–40 mM (0–12,5 mM for β-CD) in water or biorelevant media. After 72 hours, samples were filtered, diluted and LOR concentration was determined spectrophotometrically or by liquid chromatography. Phase solubility diagrams were constructed, and stability constants, complexation efficiency and solubility enhancement were calculated.

Results and discussion: Presence of β-CD and its derivatives significantly improved solubility of loratadine in water and biorelevant media. High values of obtained stability constants indicate strong interactions of LOR and CD.

Conclusions: Even though CDs greatly improve solubility of LOR, additional analytical techniques are needed for confirmation and characterization of inclusion complexes, such as NMR, HR MS and isothermal titration calorimetry.

Keywords: cyclodextrins, loratadine, phase solubility

DNA METHYLATION ANALYSIS OF MGMT ENHANCERS IN GLIOBLASTOMA

Katharina Pühringer[1], Katja Zappe[1], Simon Pflug[1], Daniel Berger[1], Andreas Böhm[1], Philipp Czarda[1], Sabine Spiegl-Kreinecker[2], Margit Cichna-Markl[1]

[1]Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Austria
[2]Department of Neurosurgery, Johannes Kepler University, Linz, Kepler University Hospital GmbH, Austria
e-mail: katharina.puehringer@univie.ac.at

Introduction and objectives: Promoter methylation of the O^6-methylguanine-DNA methyltransferase (MGMT) gene is commonly found to be negatively correlated with MGMT protein expression. Since high MGMT activity interferes with the anti-cancer effect of the alkylating chemotherapeutic agent temozolomide (TMZ), MGMT promoter methylation has been established as a predictive biomarker for the responsiveness of glioblastoma (GBM) patients to TMZ. However, this negative correlation is not given for all GBM patients. In this study we therefore aimed to determine whether MGMT enhancer methylation is associated with MGMT expression, MGMT promoter methylation and/or overall survival of GBM patients.

Materials and methods: Genomic DNA of primary cell line samples, derived from tumor tissue of GBM patients, was treated with bisulfite for conversion of unmethylated cytosines. Primers targeting CpG sites in enhancers of MGMT were designed. Methylation levels were determined by pyrosequencing, following amplification of the target regions by polymerase chain reaction.

Results and discussion: For one enhancer of MGMT, located in intron 2, we found negative correlation of methylation with MGMT promoter methylation as well as significantly higher methylation levels in MGMT expressing than MGMT non-expressing samples. In addition, methylation levels of some CpG sites in this enhancer showed association with overall survival of GBM patients.

Conclusions: Our results indicate that MGMT enhancer methylation contributes to MGMT regulation and might serve as a potential prognostic biomarker for GBM patients.

Keywords: DNA methylation, MGMT, glioblastoma, enhancer

ELECTROCHEMICAL APTASENSOR FOR THE DETECTION OF HEPG2 CELLS

Alexandra Pusta[1], Mihaela Tertiș[1], Denisa Kezan[1], Diana Bogdan[2], Maria Suciu[2], Ionel Fizeșan[3], Cecilia Cristea[1]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]National Institute for Research and Development of Isotopic and Molecular Technologies, 400293 Cluj-Napoca, Romania
[3]Department of Toxicology, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
e-mail: alexandra.pusta@umfcluj.ro

Introduction and objectives: Hepatocellular carcinoma (HCC) is the most common type of liver cancer and has an increasing incidence and mortality rate. Because HCC is usually diagnosed in advanced stages, it is important to develop sensitive, quick and accessible techniques for HCC screening and diagnosis. The detection of circulating tumor cells, such as HepG2 cells, could represent a promising screening strategy for HCC. The aim of this work was the development of an electrochemical aptasensor for the detection of HepG2 HCC cells.

Materials and methods: Carbon screen printed electrodes were modified with a suspension containing chitosan, graphene oxide (GO) and nanopolymeric particles. The carboxyl groups were activated and aptamer TLS11a was covalently bound to the activated electrode using multipulse amperometry. Bovine serum albumin was used as a blocker to prevent non-specific adsorption. Lastly, the aptasensor was incubated with HepG2 cells. All modification steps were confirmed using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and microscopy techniques. The detection of HepG2 cells was carried out via EIS.

Results and discussion: An increase in the resistance to charge transfer after cell incubation was observed. This was proportional to the concentration of cells, which allowed the construction of a calibration curve. Serum samples were spiked with known concentration of HepG2 cells and good recoveries were obtained.

Conclusions: In conclusion, a label-free electrochemical aptasensor was developed for the detection of HepG2 cells.

The research was supported by the UMF internal grant no 773/9/11.01.2023 and PN-III-P1-1.1-TE-2021-1543 (TE125/23.05.2022).

Keywords: aptasensor, hepatocellular carcinoma, electrochemical impedance spectroscopy

---

INTERNATIONAL RANKINGS OF UNIVERSITIES

Radu Oprean[1], Bogdan-Cezar Iacob[1], Bodoki Ede[1]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy Iuliu Hatieganu Cluj-Napoca
e-mail: roprean@umfcluj.ro

The paper is focused on global international university rankings. The global rankings order institutions in higher education based on factors that vary depending on the ranking. There is much debate about rankings' interpretation, accuracy, and usefulness. The expanding diversity in rating methodologies and accompanying criticisms of each indicate the lack of consensus in the field. Further, it seems possible to game the ranking systems through excessive self-citations or by researchers supporting each other in surveys. For the major global rankings, we are looking in-depth for the indicators and metrics that are used to compute the final score for each university.

THE EFFECT OF SARS-COV-2 VIRUS ON THE ACTIVITY OF THE PANOPTOSIS PATHWAY IN UPPER RESPIRATORY EPITHELIAL CELLS: CORRELATIONS BETWEEN EXPRESSION PATTERNS, AGE, GENDER, AND COMORBIDITIES

Zita Kovács[1], Emese-Éva Bálint[2], Szilvia László[2], Beáta Albert[2,3], Gyöngyi Tar[4,5], Attila András[5], Pál Salamon[2,3,5]

[1]Faculty of Natural Sciences, University of Pécs, Ifjúság útja 6, 7624 Pécs, Hungary
[2]Faculty of Economics, Socio-Human Sciences and Engineering, Sapientia Hungarian University of Transylvania, Department of Bioengineering, Miercurea Ciuc, Libertatii Sq. No 1, Romania
[3]Molecular Diagnostics Laboratory, Emergency County Hospital of Miercurea Ciuc, Dr. Denes Laszlo 2, Miercurea Ciuc, Romania
[4]Faculty of Technical and Human Sciences, Sapientia Hungarian University of Transylvania, Corunca, 1C, Târgu-Mureș, Romania
[5]Public Health Directorate, Harghita county, Mikó Sq. No 1, Miercurea Ciuc, Romania
e-mail: salamonpal@uni.sapientia.ro

Introduction and objectives: The emergence of the SARS-CoV-2 coronavirus had serious consequences, causing a worldwide outbreak of severe acute respiratory syndrome. In the case of SARS-CoV-2 infection, activating a newly described inflammatory programmed cell death pathway called PANoptosis is characteristic, which is controlled by the PANoptosome complex. The PANoptosome can simultaneously operate apoptosis, necroptosis, and pyroptosis, previously considered three distinct pathways.

Materials and methods: In our study, we performed transcriptome profiling of SARS-CoV-2-infected patients to assess the activity of genes comprising the PANoptosome. During transcriptome profiling, we examined the expression levels of MLKL (a necroptotic protein) and Caspase-3 (an apoptotic protein) in the upper respiratory tract epithelial cells using RT-qPCR. Additionally, we analyzed the relationships between age, gender, mortality rate, comorbidities, and major laboratory parameters (such as CRP, creatinine, urea, and lymphocytes) associated with the samples.

Results and discussion: Based on our findings, we can conclude that the SARS-CoV-2-infected patients treated in the intensive care unit with a severe outcome had a higher mortality rate than those not infected with the SARS-CoV-2 virus. The proportion of male patients was higher in the analyzed samples. Among the patients positive for the SARS-CoV-2 virus, significant increases in CRP, creatinine, and urea levels were observed between the sampling periods.

Conclusion: The SARS-CoV-2 infection initiates numerous pathological processes in the body that are associated with severe clinical outcomes and increased mortality.

Keywords: PANoptosis, SARS-CoV-2, transcriptome, RT-qPCR, correlation

---

SEPARATION OF LIPOOLIGOSACCHARIDES BY NON-AQUEOUS CE-MS/MS

Viktor Sándor[1], Dóra Hidegkúti-Németh[1], Anikó Kilár[1], Ágnes Dörnyei[2], Ferenc Kilár[1,2]

[1]Institute of Bioanalysis, Medical School, University of Pécs
[2]Department of Analytical and Environmental Chemistry, Faculty of Science, University of Pécs
e-mail: sandor.viktor@pte.hu

Introduction and objectives: Lipopolysaccharides (LPS) and lipoolygosaccharides (LOS) – also called endotoxins – are constituents of the outer leaflet of the outer membrane of Gram-negative bacteria. Native isolates of endotoxins comprise a high inherent heterogeneity, which contributes to the development of antibiotic resistance. This poster presents the application of a new non-aqueous CE-MS method to separate and online mass analyze the different constituents of underivatized, intact LOS isolates.

Materials and methods: Non-aqueous CE (NACE) measurements were carried out with a 7100 CE system coupled to a 6530 Q-TOF MS (Agilent Technologies) equipped with an Agilent Jet Stream ESI interface. The non-aqueous background electrolyte (BGE) consisted of different mixtures of methanol, chloroform and acetonitrile, in the presence of triethylamine and acetic acid as ionic additives at varying concentrations.

Results and discussion: Based on our previous results in the NACE separation of the hydrolyzed part of endotoxin (*i.e.* the lipid A region) we developed a new electrolyte suitable for the separation and online fragmentation of intact LOS species. The migration of the amphipathic LOS ions was influenced both by the number of fatty acid chains (3 to 7) and by the number of phosphate (1 to 3) and sugar (2 or 3) residues. This resulted in the separation of several LOS species.

Conclusions: The experiments demonstrate for the first time the NACE separation and online fragmentation of small quantities of molecular LOS species in complex bacterial mixtures.

Acknowledgement: The research was supported by the ÚNKP-22-4 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund, NKFIH FK-129038.

Keywords: endotoxin, lipooligosaccharide, non-aqueous capillary electrophoresis – mass spectrometry

DIFFERENT APPROACHES FOR ACHIRAL AND CHIRAL ANALYSIS OF ILLICIT DRUGS

Martin G. Schmid, Eva Hubner, Elisabeth Seibert, Kurt Plöschberger

Department of Pharmaceutical Chemistry, Faculty of Sciences, University of Graz, Austria
e-mail: martin.schmid@uni-graz.at

Introduction and objectives: There is a growing demand for fast and reliable analysis of illicit drugs. On the one hand, efficient prosecution of drug dealers requires a portfolio of methods for identification and quantification of common illicit drugs such as cannabis, cocaine, speed or heroin. Similar applies to harm reduction for drug consumers, if they are informed about the ingredients of their samples purchased from dealers, since there is no quality control. On the other hand, hundreds of so called new psychoactive substances have emerged during the past 15 years to circumvent law and to replace the classic drugs. Since a lot of illicit drugs are chiral, there is a need for the development of chiral separation methods to distinguish between enantiomers, which can exhibit different strength in effect.

Materials and methods: Different chromatographic and electrophoretic approaches for fast and effective qualitative and quantitative analysis of illicit drugs were applied.

Results and discussion: Some special case studies are presented by means of analytical results. Examples will be given by means of chromatographic and electrophoretic techniques such as HPLC, gas chromatography and capillary electrophoresis.

Conclusions: For fast and successful analysis of both classic and new drugs, a solid portfolio of analytical methods is a prerequisite.

The research was partially supported by our CEEPUS program.

Keywords: Analysis, illicit drugs, new psychoactive substances

---

USING SULFATED SS-CYCLODEXTRIN AS MOBILE PHASE ADDITIVE IN HPLC FOR CHIRAL SEPARATION OF NEW PSYCHOACTIVE SUBSTANCES

Elisabeth Seibert[1], Cindy Plöschberger[1], Martin G. Schmid[1]

[1] Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Sciences, University of Graz, Schubertstraße 1, 8010 Graz, Austria
e-mail: elisabeth.seibert@uni-graz.at

Introduction and objectives: According to the EMCDDA drug report 2022, the ongoing development of New Psychoactive Substances (NPS) still represents a huge problem for the European drug market. Around 880 substances have been monitored by the end of 2021, with 52 first appearances in that year. Many NPS possess a chiral centre and are mostly traded as racemic mixtures, making the development of chiral separation methods crucial to gain further knowledge about the pharmacological differences between their enantiomers. So far, many chiral separation methods of NPS by HPLC have been developed employing different chiral selectors. In 2014, Taschwer et al. created a method, using sulfated ß-cyclodextrin as mobile phase additive for enantioseparation of amphetamine derivatives and cathinones. In continuation of this study, this method was tested and adapted for a broader range of NPS.

Materials and methods: All measurements were performed under isocratic conditions. A Nucleoshell RP 18plus column, 150 x 4.6 mm, 2.7 µm, served as stationary phase.

Results and discussion: A set of 8 “benzofuries”, a substance class belonging to the phenethylamines, was tested and for all compounds baseline separation was achieved, for 4 of them within 10 min. Further investigated substance classes showed heterogenous results.

Conclusions: For certain substance classes of NPS this method presents a suitable alternative to the use of chiral stationary phases.

Keywords: Chiral separation, New psychoactive substances, sulfated ß-cyclodextrin

EXPLORING COATING AGENTS FOR CE WITH LIF DETECTION FOR LIPOSOME ANALYSIS

Alice Šimonová[1,2], Tomáš Křížek[1], Vladimír Ďorďovič[2]

[1]Department of Analytical Chemistry, Faculty of Science, Charles University
[2]Zentiva k. s.
e-mail: simonovaa@natur.cuni.cz

Introduction and objectives: Liposomes are small spherical vesicles made up of a closed lipid bilayer. As each lipid has hydrophilic head and hydrophobic tail, various substances can be incorporated either into the membrane or into the core of the liposome. This makes them suitable as a model system for controlled transport of bioactive substances and drugs through organism. The objective of this study is to develop a set of capillary electrophoresis methods using laser-induced fluorescence detection to analyze and characterize liposomes, enabling us to acquire information on drug transport and behavior in the biological environment. These methods can serve as an alternative to commonly used techniques in early drug development. As liposomes are prone to sticking to the capillary wall during separation, this study explored suitable modification of the inner wall with different coating agents.

Materials and methods: The CE experiments were carried out in fused-silica capillaries of 50 µm i.d., 33.0/21.0/8.5 cm; background electrolyte 10 mM phosphate buffer (pH 7.0); injection –50 mbar for 3 s, then pressure –50 mbar for 30 s and voltage of 20 kV for X s; moved with pressure of –50 mbar into the detector; laser induced fluorescence detection at 480 nm and UV detection at 200 nm (for measuring the electroosmotic flow marker – thiourea)

Results and discussion: Liposomes were prepared through the lipid film hydration method in sodium phosphate buffer with a pH of 7.4, they were consisting of four lipids in a total concentration of 5 mg/ml, and they were labeled with 7-nitrobenz-2-oxa-1,3-diazol-4-yl for fluorescence detection. Commercially coated capillary with polyvinylalcohol was initially used for their analysis. Additionally, different approaches of dynamic coating of fused-silica capillaries with polymers, such as Pluronic F-127, polyvinylpyrrolidone K30, polyethylenglycol 6000, and dextran were tested. And lastly permanent coating of the capillary with linear polyacrylamide was tested, which resulted in the fully suppressed EOF.

Conclusions: We have proved that CE with LIF detection is sufficient for liposome analysis and we have optimized capillary coating together with background electrolyte for future CE experiments involving liposomes.

The research was supported by CEEPUS, network RO-0010-17-2223 – Teaching and Learning Bioanalysis; Charles University project SVV260690; and by the Grant Agency of Charles University (No 386122).

Keywords: coating agents, capillary zone electrophoresis, liposomes

---

POST-COLUMN DERIVATIZATION APPROACH IN FLUORESCENCE DETECTION OF OATD-02 -BORONIC ACID ACTIVE PHARMACEUTICAL INGREDIENT

Elżbieta Sobolewska[1,2], Magdalena Tyszkiewicz[2], Magdalena Biesaga[1]

[1]Department of Chemistry, University of Warsaw
[2]Molecure SA, Warsaw, Poland
e-mail: e.sobolewska6@uw.edu.pl

Introduction and objectives: The development process of new drug is under strictly control. Active pharmaceutical ingredient (API) must meet the criteria which are set out in the guidelines such as ICH guidelines. OATD-02, an arginase inhibitor was selected as a clinical candidate for cancer immunotherapy, because of direct antitumor efficacy in animal models. OATD-02 is difficult to analyze because of its weak ultraviolet absorption. The objective of the work was to develop and validate the HPLC method for determination of OATD-02 and its impurities.

Materials and methods: The selective determination of OATD-02 was achieved by HPLC with post-column derivatization and fluorescence detection. Waters column XSelect CSH C_{18} (2.5 µm, 3 mm x 100 mm) was selected and the mobile phase consisted of a mixture of $NaHCO_3$ in H_2O and ACN at 0.50 mL/min flow rate. The solution of derivatization reagent alizarin in MeOH was delivered at a flow rate of 0.50 mL/min. The excitation and emission wavelengths were 470 and 580 nm, respectively.

Results and discussion: The influences of mobile phase composition on retention of OATD-02 were investigated and mixture of ACN and 10 mM $NaHCO_3$ solution was chosen. Various stationary phases in different dimensions and length with various particle sizes were tested. The main chromatographic parameters for OATD-02 and its related substances on different columns were comprised.

Conclusions: HPLC method with post-column derivatization with alizarin solution and fluorescence detection was developed and validated for OATD-02. The developed method showed high precision, linearity and selectivity and it was applied in the analysis of drug active ingredient OATD-02.

The research was conducted in collaboration between University of Warsaw and biotech company Molecure SA in accordance of implementation doctorate program.

Keywords: HPLC, post-column derivatization, chromophore, API

ENGINEERED PALS FOR PHENYLALANINE ANALOGUES

Souad-Diana Tork, Alina Filip, Florin-Dan Irimie

Enzymology and Applied Biocatalysis Research Center, Faculty of Chemistry and Chemical Engineering Babeș-Bolyai University, Cluj-Napoca, Romania
e-mail: souad.tork@ubbcluj.ro

Introduction and objectives: Optically active amino acids play a pivotal role as essential chiral intermediates across diverse industrial sectors, including pharmaceuticals, cosmetics, agrochemicals and food additives. A promising strategy for obtaining optically pure derivatives of L- and D-phenylalanines involves harnessing the potential of phenylalanine ammonia-lyases (PALs, EC 4.3.1.24). PALs are biocatalysts capable of facilitating the reversible deamination of L-phenylalanine. The main goal of this study is the development of an efficient biotechnological procedure to achieve gram-scale synthesis of several unnatural phenylalanine analogues, of high industrial value.

Materials and methods: A focused PAL variant library, obtained through site-directed mutagenesis, was screened for activity towards monosubstituted substrates in both ammonia addition and elimination reactions. In order to determine the efficient reaction conditions, the PAL-based processes were optimized in terms of reaction medium/ammonia source, pH, biocatalyst: substrate ratio, substrate concentration and temperature. Conversions and enantiomeric excesses were monitored using reversed-phase and chiral high performance liquid chromatography (HPLC).

Results and discussion: The successful synthetic applicability of PAL mutants was demonstrated for the efficient biocatalytic synthesis of enantiopure L- and D-Phe derivatives. Optimal conditions for ammonia addition and elimination reactions were achieved through reaction engineering. Our approach surpasses the performance of previously reported scaled-up biotransformations using phenylalanine ammonia-lyase and results in improved space-time yield and reduced environmental impact. The study also demonstrates that mutational approaches can be used to enhance the catalytic efficiency of PAL variants towards non-natural substrates with different substitution patterns. The results suggest that this approach is applicable across PALs of different origins and can be further refined through sequence alignment-based rational modifications.

Conclusions: The successful implementation of the PAL-based procedures described in this study has the potential to make substantial contributions to the advancement of processes for synthesizing active pharmaceutical ingredients (APIs). Furthermore, it promotes a more environmentally sustainable approach to chemical development by employing whole-cell PALs.

The research was supported by the National Research Council-UEFISCDI, project number PN-III-P1-1.1-TE-2019-2118/TE95 and by the Swiss National Science Foundation (SNSF), through PROMYS project, grant nr. IZ11Z0_166543.

Keywords: phenylalanine ammonia-lyase, biocatalysis, preparative-scale biotransformation, whole-cell biocatalysts, site-directed mutagenesis

---

THE ANALYSIS OF MONOCLONAL ANTIBODIES WITH ELECTROSPRAY IONIZATION MASS SPECTROMETRY AND SUBSEQUENT SPECTRAL PROCESSING

Ruben Szabo, Attila Gaspar

Department of Inorganic and Analytical Chemistry, University of Debrecen, Hungary
e-mail: szabodavidruben@gmail.com

Introduction and objectives: Study the electrospray ionisation and mass spectrometric detection of monoclonal antibodies and spectral processing focusing mainly on the deconvolution of ESI-MS spectra.

Materials and methods: Pharmaceutical products containing monoclonal antibodies (Erbitux, Ontruzant) were analysed using a maXis II UHR ESI-QTOF MS instrument (Bruker). Samples were introduced automatically with the pressure system of a 7100 model CE instrument (Agilent) connected to the MS with a CE-ESI Sprayer GI 607B interface (Agilent).

Results and discussion: Mass spectrometry is one of the most important tools in analysis of monoclonal antibodies. Electrospray ionization is prevalent technique that generates numerous charge states in the case of large molecules. The ionization of cetuximab was investigated and found to be promoted by the addition of acids, volatile organic solvents and the removal of the matrix components. The acquired spectra that consist of many peaks corresponding to different charge states. Different algorithms can assign charges to the peaks and create a zero-charge mass spectrum using these charges and m/z values, in a process called deconvolution. This deconvoluted spectrum is similar to each differently charged peak in the recorded spectrum but has higher signal-to-noise ratio and mass accuracy. This facilitates the identification of peaks and determination of masses corresponding to the peak maxima. The deconvolution parameters were studied in order to achieve ideal results, and three different deconvolution software were compared, which produced very similar deconvoluted spectra.

Conclusions: Different matrix components can significantly affect the ionisation of monoclonal antibodies and thus the acquired mass spectrum as well. The deconvolution parameters can have considerable effects on the deconvoluted spectra, but different algorithms provide nearly identical results.

The research was supported by ÚNKP and OTKA NKFIH, Hungary.

Keywords: monoclonal antibody, electrospray ionisation, mass spectrometry, deconvolution

ENANTIOMER MIGRATION ORDER AS A FUNCTION OF EXPERIMENTAL VARIABLES IN CAPILLARY ELECTROPHORESIS

Zoltán-István Szabó[1,2], Szilárd Nagy[1], Tas Molnár[1], Gergő Tóth[3]

[1]Department Pharmaceutical Industry and Management, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures; [2]Sz-imfidum Ltd., Lunga, Romania; [3]Semmelweis University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Budapest, Hungary;
e-mail: zoltan.szabo@umfst.ro

Chirality, or handedness, is a crucial consideration in pharmacology and drug design due to its impact on the interaction between drugs and biological systems. The mirror images of chiral molecules, called enantiomers, can exhibit different pharmacological and/or pharmacokinetic properties and interactions with biological targets, such as enzymes, receptors, and transporters. Thus, the determination of the optical purity of compounds is of utmost importance. As in most cases, the developed methods need to be able to quantify enantiomeric impurities below 0.1%, the elution/migration order of enantiomers is extremely important, as the tailing of the major component can hinder the determination of the enantiomeric purity.

Although, liquid chromatography is still the golden standard in enantioseparations, capillary electrophoresis (CE) has been established over the years as a viable alternative. One of the main advantages of CE enantioseparations is the ease to achieve enantiomer migration order (EMO) reversal. The phenomenon is also important to be studied mechanistically, as EMO reversals indicate interesting changes in the separation process that arise from only minute variations of experimental variables.

The current presentation will give some interesting aspects of EMO reversals that our research group observed when developing enantioseparation methods in CE for a wide variety of analytes using cyclodextrins (CDs) as chiral selectors. Observed EMO reversals based on cavity size, substituent type, and -position of the CDs are presented and critically discussed. The use of dynamic and permanent capillary coatings to achieve EMO reversal is also presented and discussed.

This work was supported by the University of Medicine, Pharmacy, Science and Technology „George Emil Palade“ of Târgu Mureș Research Grant number 10127/3/17.12.2020.

Keywords: enantiomer migration order; cyclodextrin; capillary electrophoresis; capillary coating

---

COMBINED “-OMICAL” ANALYSIS OF BIOFLUIDS IN BIOMARKER RESEARCH

Anikó Takátsy[1,2], Balázs Molnár[1,], Maár Szabolcs[1,2], Lilla Brúnyainé Czuni[2], Jørgen Kosberg Hassve, Gábor Fáskerti[1,2], Dominik Cysewski[3] , Ildiko Bock-Marquette[1,2]

[1]Department of Biochemistry and Medical Chemistry, University of Pecs Medical School, Pecs, Hungary
[2]Szentagothai Research Centre, University of Pecs, Pecs, Hungary
[3]Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland
e-mail: aniko.takatsy@aok.pte.hu

Introduction and objectives: According to FDA, an ideal biomarker should be specific for a particular disease and should be able to differentiate between various physiological states in a safe and easily measurable way. It should provide rapid and accurate results to ensure a fast and correct diagnosis, with similar outcomes in different ethnic groups and genders. Biomarkers are essential in the diagnosis of certain diseases and tracing progression, regression, and treatment. Both identification and detection of biomarkers are challenging partly because of structural and chemical differences of the possible candidates, in addition because of their low concentration in a complex biological matrix.

Chromatographic and electrophoretic techniques can be used to separate substances with different chemical structures, while mass spectrometry provides a fairly accurate and sensitive solution for their detection and structural identification.

Materials and methods: From collected plasma samples total RNA was isolated and miRNA expressions were identified with NG sequencing to cover transcriptomics. In frame of proteomics the possible candidates were separated using 2D PAGE and after in gel digestion they were identified with MALDI/TOF-TOF MS. LC/MSMS measurements were also made.

Results and discussion: Several possible biomarkers, like Apolipoprotein A-I and Alpha-1 antitrypsin were identified.

Conclusions: Combined – “omical” approaches, which have become increasingly popular in recent decades, make it possible to create a more complex picture. With the help of combined transcriptomic, proteomic and metabolomic studies new biomarkers in biofluids can be identified.

Keywords: biomarker, transcriptomics, proteomics

DEVELOPMENT OF AN ELECTROCHEMICAL BIOSENSOR FOR THE DETECTION OF *STAPHYLOCOCCUS AUREUS*

Ana-Maria Tătaru, Andreea Cernat, Alexandra Canciu, Cecilia Cristea

Analytical Chemistry Department, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy, 4 Louis Pasteur St, Cluj-Napoca, Romania
e-mail: ana.mari.tataru@elearn.umfcluj.ro, anamariatataru98@yahoo.com

Introduction and objectives: A major health issue around the globe is represented by the spread of bacterial species highly resistant to the available antibiotics treatment. *Staphylococcus aureus (S. aureus)*, along with its methicillin-resistant variant (MRSA), are the cause of various infections with a high rate of morbidity and mortality. In this context, rapid and sensitive detection of the pathogens would be a useful strategy in the healthcare systems. Using the wide range of virulence factors produced by *S.aureus* (such as the surface component – protein A), the aim of this study was to develop an electrochemical biosensor that can detect and quantify the presence of the bacteria.

Materials and methods: The biosensor was developed on a gold screen-printed electrode (AuSPE), on which the specific SH-modified aptamer for protein A (PA#2/8 [S1-58]) was immobilized via multipulse amperometry (MPA), the remaining unbound sites on the electrode were blocked via MPA, using an agent containing a SH-group and the target, protein A, was incubated in time (30 min). After each step, the electrode surface was analyzed using the ferro/ferricyanide redox probe, by differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS). The solutions used in the analysis were all prepared in TRIS buffer pH 7.1 and DNAase free water.

Results and discussion: The immobilization protocol of the aptamer sequence to the AuSPE was optimized in terms of procedure (incubation in time vs. MPA) and also in terms of parameters of the MPA procedure (pulse step and duration). Various blocking agents were tested (6-mercapto-1-henaxol and 11-mercaptoundecanoic acid) and different methods of blocking the SPE surface. The incubation of protein A was also optimized, choosing the most convenient temperature and time. A wide range of concentrations of the target were tested (10 nM-1000 nM).

Conclusions: An electrochemical biosensor using an aptamer sequence was developed for the fast detection of *S.aureus* and its specific marker (protein A). Also a linear correlation between the concentration of the target and the electrochemical signal was observed in both DPV and EIS analysis.

The research was supported by the Romanian Ministry of Education and Research, CNCS-UEFISCDI, project number TE 89/23.05.2022 and by Iuliu Hațieganu UMF internal grant no. 771/70/11.01.2023.

Keywords: *S.aureus,* aptamers, protein A, electrochemical methods

---

USING HYDROXY BENZALDEHYDES AS AMINO ACCEPTORS FOR KINETIC RESOLUTION OF SUBSTITUTED 1-PHENYLETHYLAMINES

Róbert Tőtős, Laura Barabás, Csaba Paizs, Florin-Dan Irimie

Enzymology and Applied Biocatalysis Research Center, Babeș-Bolyai University, Faculty of Chemistry and Chemical Engineering, Arany János street 11, RO-400028, Cluj-Napoca, Romania
e-mail: robert.totos@ubbcluj.ro

Introduction and objectives: In this study we describe the efficient production of enantiomerically pure (*R*)-1-phenylethan-1-amines, (*S*)-1-phenylethan-1-ols, and hydroxybenzylamines by combining two distinct processes, kinetic resolution of racemic amines catalyzed by ω-transaminases and using carbonic anhydrase II as secondary enzyme to reduce the acetophenones formed in the transamination reaction. The catalytic activity of ω-transaminase from *Pseudomonas psychrotolerans* was studied using hydroxybenzaldehydes as co-substrates, which shifted the reaction equilibrium towards the formation of benzylamines. To increase the efficiency of this combined transamination system the ketone products of the transamination (acetophenones) were stereoselectively reduced to enantiomerically pure (*S*)-1-phenylethan-1-ols.

Results and discussion: Starting from cheap materials and combining two distinct procedures in the same reaction vessel we describe the optimized biocatalytic cascade mediated by ω–TA and human carbonic anhydrase (hCAII). Besides the preparative scale synthesis of two valuable amines (enantiopure (*R*)-1-phenylethan-1-amines and para- or meta-hydroxybenzylamines) we obtained highly enantiomerically enriched (*S*)-1-phenylethanols by the reduction of acetophenones.

Conclusions: The results of this research proved the suitability of hydroxybenzaldehydes as co-substrates in transamination reactions. An efficient and sustainable biocatalytic process was developed to produce amines and alcohols with potential use in pharma industry, in a one-pot two-step enzymatic cascade. Very high conversion rates were achieved for different substrates by whole-cell catalysis.

Keywords: transamination, enzymatic cascade, kinetic resolution, human carbonic anhydrase

PECULIARITIES AND CHALLENGES IN THE DEVELOPMENT OF ANALYTICAL METHODS FOR COMBINED DRUGS

Uncu Livia[1, 2]

[1]Department of Pharmaceutical and toxicological Chemistry, Faculty of Pharmacy
[2] Scientific Center of Medicines
University of Medicine and Pharmacy „Nicolae Testemițanu" of Republic of Moldova
e-mail: livia.uncu@usmf.md

Introduction and objectives: Research and quality control of fixed doses combinations (FDCs) requires the development of robust and accurate analytical procedures that are capable of simultaneously detecting and quantifying several different active substances, which is often a challenge for analysts. By carrying out a review of the most recent publications with reference to the methods and techniques of analysis of FDCs, it was proposed to identify some specific peculiarities in the quality control process, as well as to delimit the challenges that need to be overcome.

Results and discussion: Several key considerations related to the peculiarities and challenges in developing of analytical methods for FDC have been identified. Multiple active ingredients (a): Analytical methods must ensure accurate quantification of each active pharmaceutical ingredient (API), even though each API in the FDC composition may have different solubility, stability and chromatographic behavior. Compatibility and stability (b): Analytical methods should assess the compatibility and stability of the combined drugs, taking into account factors such as pH, temperature and excipients, since the APIs in the FDC may interact with each other. Selectivity and specificity (c): Analytical methods for FDC must demonstrate selectivity and specificity to each API, allowing their differentiation from potential impurities, degradation products or other interfering substances. Chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) coupled with appropriate detectors (ex., UV, mass spectrometry) are commonly used for this purpose. Sample complexity (d): Excipients can complicate analysis, therefore robust sample preparation techniques, such as for example solid-phase extraction or liquid-liquid extraction, are required to isolate and concentrate APIs from the complex matrix. Method Validation(s): The validation process should address the challenges of determining appropriate acceptance criteria for each API, establishing appropriate calibration curves, evaluating matrix effects, and performing forced degradation studies to assess method robustness. Regulatory requirements (f): Regulatory bodies have specific guidelines for the development and validation of analytical methods for FDC. Adherence to these guidelines is crucial to obtaining regulatory approval.

Conclusions: The development of analytical methods for combined drugs requires careful consideration of the unique characteristics of each API, sample complexity, method validation requirements, and regulatory guidelines. Thorough understanding of the physicochemical properties and compatibility of the combined drugs is crucial for the successful analysis of FDCs.

The studies are supported by the research project 20.80009.8007.14.

Keywords: analytical methods, combined drugs.

TOXIC ALCOHOL AND ETHYLENE GLYCOL INTOXICATIONS IN HARGHITA COUNTY

Szende Vancea, Blanka Török, Elek-Attila Ráduly, János Sinkó

Legal Medicine Service, Emergency County Hospital Miercurea Ciuc, Jud. Harghita, Romania
e-mail: vancsa.szende@gmail.com, toxicologie@spitalmciuc.ro

Introduction and objectives: Many cases of accidental or suicidal ingestions were documented with toxic alcohols and glycols in Harghita county. Methanol and ethylene glycol through the generation of toxic metabolites produce severe metabolic acidosis. Therefore, determination of these in an emergency unit requires a rapid, accurate and reliable method.

Materials and method: Cases of fatal intoxications in Harghita county with methanol and ethylene glycol are presented. Serum and/or postmortem blood and urine samples were analyzed by headspace-gas chromatography-FID- for toxic alcohols, while ethylene glycol was confirmed in blood samples by application of an identification reaction after protein precipitation.

Results and discussions: Ethylene glycol poisoning was confirmed by suggestive oxalic acid insoluble salts, which were found in various tissues, especially in kidneys after autopsy, where they cause renal failure. Methanol concentrations in blood and urine were found to be above the toxic/lethal levels. Formic acid is the primary toxic metabolite that accounts for the associated anion gap and end-organ damage.

Conclusions: A rapid, cost-effective headspace GC-FID method for quantification of volatile toxic alcohols in blood and urine reduces turnaround time and provides quantitative results in a hospital setting.

Keywords: methanol, ethylene glycol, intoxication, GC-FID

UHPLC-LC-MS/MS METHOD FOR POLYPHENOLS DETERMINATION

Erzsébet Varga[1], Ibolya Fülöp[2], Lénárd Farczádi[3], Mihály Fazakas[4], Mircea Dumitru Croitoru[2]

[1]Department of Pharmacognosy and Phytotherapy, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, Targu Mureș, Romania
[2] Department of Toxicology and Biopharmacy, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, Targu Mureș, Romania
[3]Center for Advanced Medical and Pharmaceutical Research, Laboratory of Chromatography LC/MS, University of Medicine, Pharmacy, Sciences and Technology of Târgu Mureș, Faculty of Pharmacy, Târgu Mureș, Romania
[4]SC Promedivet SRL, Sovata, Mureș, Romania
e-mail: erzsebet.varga@umfst.ro

Introduction and objectives: The benefits of flavonoids and polyphenols from medicinal plants are very well-known which compounds have an important role in human and in veterinary health too. The aim of this work was to evaluate the polyphenols form indigenous medicinal plants.

Materials and methods: LC-MS/MS separation of analytes was performed using a Nucleodur C18 Gravity, 3 µm, 150x3 mm column using a mobile phase of 0.2 % formic acid in water and methanol with gradient elution and a flow rate of 0.6 ml/min. The studied compounds in the tested medicinal plants were: apigenin, kaempferol, quercetin, luteolin, quercitrin, chlorogenic acid and caffeic acid.

Results and discussion: We determined high concentrations of chlorogenic acid and caffeic acid from the methanolic extracts of medicinal plants. The flavonoid content from the ethanolic extracts of some samples were with higher concentrations.

Conclusions: The obtained results served as a contribution to the polyphenolics and flavonoids determination from the medicinal plants used in modern phytotherapy and veterinary medicine.

The research was supported by the University of Medicine, Pharmacy, Science and Technology of Targu Mures and SC Promedivet SRL, under internal research grant number 17972/07.12.2016

Keywords: medicinal plants, polyphenols, flavonoids, LC-MS/MS

ANALYSIS OF SNAKE VENOMS WITH CZE-MS

Gayatri Vishwakarma[1], Melinda Andrási[1], Péter Hajdú[2], Attila Gáspár[1]

[1]Department of Inorganic and Analytical Chemistry, Faculty of Science and Technology, [2]Division of Dental Biochemistry, Faculty of Dentistry, University of Debrecen, Hungary
e-mail: gayatri.vishwakarma@science.unideb.hu

Introduction and objectives: Venoms comprise of a plethora of biologically active compounds, largely peptides and proteins, that can induce lethal effects. Top-down mass spectrometric technique is efficient enough for the studies for structural and dynamical identification of intact proteins when coupled with capillary zone electrophoresis and can be employed for complex snake venom samples.

Materials and methods: Analyses were conducted using a 7100 model CE instrument (Agilent) with UV and MS (maXis II UHR ESI-QTOF MS instrument, Bruker) detection. Fused silica capillaries of 85 cm x 50 µm I.D. and 370 µm O.D. was used. UV detection was carried out by on-capillary photometric measurement (detection wavelength: 200 nm). Background electrolytes were 1 M formic acid (pH=1.8), sheath liquid: 0.1% formic acid in 1:1 isopropyl alcohol.

Results and discussion: Our study involves the use of fused silica capillaries due to its simplicity and cheapness employing the background electrolytes with very low pH conditions to separate and characterise different components in snake venom samples originated from different geographic locations. We could achieve good precision, minimal adsorption and excellent separation efficiency. We observed similar separation patterns with CE-UV and CE-MS and the molecular mass of the proteins could be determined with MS detection.

Conclusions: CE-ESI/MS analysis provides a rapid, efficient method for the determination of complex natural protein/peptide mixtures such as snake venoms.

The research was supported by the CEEPUS, Stipendium Hungaricum and NKFIH, Hungary

Keywords: capillary electrophoresis, mass spectrometry, snake venom.

OPTIMIZATION OF AN UHPLC METHOD FOR THE ASSAY OF CANNABIDIOL THROUGH QUALITY BY DESIGN (QBD)

Robert-Alexandru Vlad[1], Daniela-Lucia Muntean[2], Paula Antonoaea[1], Adriana Ciurba[1], Emöke-Margit Rédai[1], Lénárd Farczádi[3], Silvia Imre[2,3]

[1]Pharmaceutical Technology and Cosmetology Department, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Gheorghe Marinescu street no. 38, 540142 Targu Mures, Romania
[2]Analytical Chemistry and Drug Analysis Department, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Gheorghe Marinescu street no. 38, 540142 Targu Mures, Romania
[3]Chromatography and Mass Spectrometry Laboratory, Centre for Advanced Medical and Pharmaceutical Research, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Gheorghe Marinescu Street no. 38, 540142 Targu Mures, Romania
e-mail: robert.vlad@umfst.ro

Introduction and objectives: Cannabidiol is an ingredient that can be retrieved from *Cannabis* plants. Numerous analytical methods were used to assay this active pharmaceutical ingredient (alone or in combination with other pharmaceutical ingredients), regularly spectrophotometric or chromatographic *via* HPLC or UHPLC methods. This study aimed to optimize an UHPLC method used for the assay of cannabidiol during the *in vitro* dissolution test.

Material and methods: A stock solution of 1 mg/mL CBD dissolved in acetonitrile was diluted with the dissolution media (0,052 M sodium laurylsulfate, pH=6.8) to obtain a concentration of 10 µg/mL. To establish the optimized conditions, an UHPLC system with PDA-type UV-Vis detection was utilized. In this regard, the InfinityLab Poroshell 120 EC-C18 (Agilent) was used with the following characteristics: 3 X 100 mm, particle size 2.7 µm. The injection volume was varied on three levels 1 µL, 5.5 µL, and 10 µL, whilst the other parameter varied was the flow rate 0.6; 0.8, and 1.0 mL/min. The isocratic mobile phase consisted of a mixture of acetonitrile:water of 70:30. Considering the varied parameters, a 3^2 factorial design was applied with four central points (flow rate of 0.8 µL and injected volume of 5.5 µL) resulting in twelve experiments (N1-N12). The dependent variables considered were the retention time and the signal area. The evaluation and optimization of the UHPLC method were made with the help of Modde 12.1 software.

Results and discussion: The lowest retention time was registered in the case of the N9 run, injection volume of 10 µL and flow rate of 1 mL/min whilst the latest was registered in the case of N1, V = 1 µL and flow rate of 0.6 mL/min. The summary of fit showed values higher than 0.8 for R^2, Q^2, validity, and reproducibility for both retention time and signal area. If the flow rate is decreasing, it might produce a lowered retention time. For the signal area, the factors that produced a lowered area were the flow rate and the interaction between the flow rate and the injection volume. By increasing the injection volume the signal area is increasing substantially. In order to optimize the method, the following parameters were targetted: retention time of 2.5 min and signal area of 200.000 units of absorbance. The software indicated that in order to be close to the targetted values, the injection volume should be 9.97 µL and the flow rate should be 0.74 mL/min.

Conclusion: The UHPLC method was optimized considering the targeted value with very good results regarding R^2, Q^2, validity, and reproducibility. Considering that the validity is higher than 0.8 for both the independent parameters there is a very low risk of a lack of fit.

Keywords: UHPLC, cannabidiol, full factorial design, optimization

EMPLOYING MULTIDIMENSIONAL DESIGN SPACE MODELS AS COMMON PLATFORMS TO CHARACTERIZE AND COMPARE HPLC SEPARATION SYSTEMS – USE CASES

Arnold Zöldhegyi[1], Imre Molnár[1], Elek Ferencz[2], Róbert Kormány[3], Zoltán-István Szabó[4,5]

[1]Molnár-Institute for Applied Chromatography, Schneeglöckchenstrasse 47, 10407 Berlin, Germany; [2]Department of Physical Chemistry, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures; [3]Egis Pharmaceuticals Plc., Keresztúri út 30-38, Budapest, Hungary; [4]Department Pharmaceutical Industry and Management, Faculty of Pharmacy, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures;[5]Sz-imfidum Ltd., Lunga, Romania;
e-mail: arnold.zoeldhegyi@molnar-institute.com

Knowing and controlling chromatographic selectivities in high-performance liquid chromatographic (HPLC) applications are indispensable for subsequent method development success and for robust in-routine use of a method. In this sense, there are three main components in an HPLC-separation system that should equally be considered for holistic understanding and judgement of the separation performance: the stationary phase, the eluent, and the sample. Indeed, the selectivity provided by the column and the corresponding separation depends greatly on the properties of the studied analyte and the applied chromatographic conditions such as the gradient settings, temperature, ternary composition and others. Similarly, additional influences like column batch-to-batch variations and system-to-system differences need to be considered as well.

Benefitting from aligning all system components, multidimensional Design Spaces (DSs) of chromatography-based modeling tools are ideal for delivering comprehensive characterization of separation systems, while minimizing experimental work required. As a result, newest analytical chapters of ICH (Q12 and Q14, Q2(R2) drafts) also point to systematic means of analytical procedure development by urging industry practitioners to follow similar, «enhanced» modeling approaches.

In this seminar we present a streamlined DS-modeling approach (DryLab), with the main focus on building 3-dimensional separation models. Main benefits of applying model-based systematic approaches in comparison of analytical Design Spaces will be demonstrated across several industry-relevant application examples. This includes (1) mapping of Method Operable Design Region (MODR) for individual optimization purpose and for (2) identifying shared MODRs of stationary phases, (3) studying batch-to-batch reproducibility of columns (4) finding suitable replacement column options, (5) describing a general method specification that can be workable across a number of stationary phases and finally, (6) for model-supporting method transfer between instruments equipped with different solvent delivery characteristics.

Keywords: Drylab, design space modeling, HPLC, analytical quality by design

INDEX OF AUTHORS