BOOK OF ABSTRACTS

PL-1

Novel Results with Capillary Electrophoresis Coupled to Mass Spectrometry

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Keywords: lipopolysaccharide, endotoxin, structure-function relationship

One of the strongest challenge in separation science is the coupling the microfluidic techniques with mass spectrometry. We are working on two different fields, i.e. in LC-MS and CE-MS, where the mass spectrometry detection provides new and very important results, whenever the problems are solved.

Lipopolysaccharides (also called as endotoxins) are physiologically active components of the outer membrane of gram-negative bacteria and are released during growth, division and lysis. They have been recognized as the most potent stimulants of mammalian immune systems, causing a wide spectrum of pyrogenic and toxic reactions. LPSs consist of a lipid region, termed lipid-A covalently attached to a polysaccharide region. Both regions have extremely high variability in their structures, which directly affects their physiological impact. A comprehensive study has been continued to explore the complex structure of various lipid-A regions to clarify the phosphorylation and acylation patterns using UHPLC-Q-TOF MS for analyses.

The other very interesting question is the coupling capillary isoelectric focusing (CIEF) to mass spectrometry, which provides a high resolution separation and a high accuracy mass measurement of proteins. Applying the sequential injection of ampholytes, and the sample, when the pH gradient does not cover the pI of the amphoteric compounds, the disturbing effect of ampholytes can be avoided. Sample compounds are forced to migrate through the pH gradient, and then migrating outside the ampholyte zone they will reach the MS detection in the absence or in the presence of low ampholyte concentration.

The research was supported by CEEPUS RO-0010 and the grant OTKA K-100667.

PL-2

Non-routine Approaches of Chemical and Biochemical Investigation Towards Obtaining Complementary Information About Biological Systems

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Increased attention is focus on the investigation of the mechanisms of bio-transformation and translocation of various chemical species in biological objects, which become to be of fundamental interest of e.g. toxicologists, physiologists, medical doctors, plant physiologists and those interested in environment. The main issue is to understand the respective mechanisms of those processes occurring in tissues of plant's and animal's origin. It should be highlighted that the biological activity of several substances depends strongly on their chemical form, means that investigation towards evaluation of their presence in the object of interest become remarkable important.

Due to the incredible fast development of modern instrumentation the investigation allowing in-deep view into the biological process occurring in living organisms become possible. In this presentation those amazing facilities will be exemplified with selected cases of the examination of the structural composition of unknown compounds, e.g. metabolites of parent compounds; examination of the isotopic compositions of selected metabolites of cholesterol; in-vivo examination of the oxidation stage of biologically active selenocompounds in plant tissues; examination of the positional isomer of amphetamine; examination of the distribution of the chemical species of interest over the plant or animal tissues. Also, various scenarios tailored towards obtaining complementary information of the biological and chemical characterization of the cells, organelle and tissues, will be discussed.

All those investigation should be performed in a wide context, means to linked several discipline, allowing to start with a properly established question, following by the designing the properly tailored analytical procedure, especially when multi-techniques approaches is used toward obtaining the complementary information about the given object, finally to collect reliable data allowing to verify the preliminary hypothesis.

The study was carried out at the Biological and Chemical Research Centre, University of Warsaw, established within the project co-financed by European Union from the European Regional Development Fund under the Operational Programme Innovative Economy, 2007 - 2013.

PL-3

Physicochemical Characteristics of Pharmaceutical Substances - Case Studies

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Keywords: capillary electrophoresis, pseudopolimorphism, particle size and shape distribution, pharmacutical analysis, pharmaceutical active substance

A finding of the answer to the question which set of complementary methods should be used to fully define the physicochemical properties of the pharmaceutical active substance (API) is a challenge of the modern pharmaceutical analysis. A complete evaluation of the API's properties, requires the consideration of aspects related to the quality, safety and utility in the drug formulation technology. The aim of the pharmaceutical analysis is not only to collect the data of the quality of pharmaceutical substances and products, raw materials, reagents, excipients and packaging materials, but also to propose solutions and to develop methods enabling the complete qualitative and quantitative characterization of the API and drug product. The methods used in the pharmaceutical analysis have to provide the data of structural and physicochemical properties of the tested substance, including the assay and impurity profile determination, identification of the polymorphic forms, determination of the morphological characteristic and the particles size distribution, particularly important in the development of pharmaceutical technology and the study of stability and bioavailability of the drug.

The presented case studies of the electrophoretic, simultaneous determination of the counter ion and possible impurity from the synthetic route in the API [1], the diagnostics of the API's polymorphism [2] and the determination of particles size and shape distribution of the API [3] demonstrate the necessity to use a broad spectrum of analytical methods to perform a comprehensive characterization of the physical and chemical properties of the API, beyond the pharmacopoeial monographs and routinely used techniques, such as IR, HPLC or GC.

References:

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PL-4

Trends in Lab-on-a-Chip Technology

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Keywords: lab-on-a-chip, microfluidic chip, chromatography, electrophoresis

During the past decade microfluidic “lab-on-a-chip” technology has revolutionized laboratory experimentation, thereby demonstrating the potential of miniaturization, integration, and automatization to research-based industries. To create “lab-on-a-chip” devices, manufacturing methods from the microchip industry were coupled to techniques involving fluid dynamics, analytical chemistry, and biochemistry resulting in miniature integrated biochemical processing systems. Miniaturization is not only about shrinking current technology but involves opening new windows of opportunity for novel analytical systems. The key to success of “lab-on-a-chip” systems does not only lie in the development of novel microfabricated components but also in demonstrating that these systems offer novel capabilities and improved performance over current technologies.

This lecture aims at surveying the main recent trends of lab-on-a-chip technology, focusing on the developments and the applications of analytical separation (chromatography and electrophoresis). The goals of the research in our laboratory to design and fabricate new types of microfluidic chips for analytical determinations based on different separation methods are also discussed. We made efforts to develop multiplex analysis, where several electrophoretic or chromatographic separations can be carried out in the same time in a single chip in order to largely increase the rate of the analysis (and largely decrease the cost of the measurements).

The research was supported by the the National Research, Development and Innovation Office, Hungary (NKFI K111932).

PL-5

Microchip Electrophoresis. Bioanalytical Applications

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Keywords: Microchip electrophoresis; Coupled channels; Sample cleanup; Biological and pharmaceutical samples

This presentation is focused on the methodological aspects of microchip electrophoresis (MCE) with coupled separation channels (CC) and using various detection techniques. MCE has significant benefits in terms of high-speed, high separation efficiency, high-throughput, easy automation, and low sample consumption. The use of MCE in bioanalysis is, however, limited due to a high complexity of biological matrices. Simplification of the complex samples and/or analyte preconcentration by suitable pretreatment technique is therefore required. The online combination of various electrophoresis techniques, e.g., isotachophoresis with zone electrophoresis on the CC microchip can be considered as a highly effective tool for integration of the sample preparation as well as preconcentration to the MCE.

A coupling of the MCE with various detection systems has paid a wide attention. The present CC microchip employs universal conductivity detection. MCE separations performed on the CC microchip can be monitored by more selective and sensitive detectors, e.g., Vis spectrometry or surface enhanced Raman spectroscopy to solve difficult bioanalytical tasks.

From the application point of view the benefits of the use of CC technology on the microchip combined with various detection techniques will be presented on a set of developed analytical methods suitable for fast analysis of complex biological samples, e.g., determination of various biomarkers of neurological diseases (metabolic organic acids) in cerebrospinal fluid using conductivity detection, determination of oxidative stress marker (3-nitrotyrosine) in urine with Vis spectrometry detection, and identification of drug additives (synthetic dyes) in antipyretics, etc.

The research was supported by the Slovak Research and Development Agency (APVV-0259-12) and the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA 1/0340/15).

PL-6

Looking for a Suitable Method to Determine the *MGMT* Promoter Methylation Status, a Prognostic and Predictive Biomarker in Glioblastoma

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Keywords: DNA methylation, MGMT, glioblastoma, biomarker, bisulfite pyrosequencing

Glioblastoma multiforme (GBM) is the most common and aggressive type of brain tumor in adults. With a median survival rate of ~15 months, prognosis of GBM is extremely poor. Standard treatment consists of surgical resection, followed by radiotherapy and concomitant chemotherapy with temozolomide (TMZ).

TMZ is an alkylating compound, causing the formation of alkyl adducts at the O6 position of guanine. Subsequent interstrand cross-links lead to replication arrest and ultimately to the death of the tumor cell. Responsiveness of GBM patients to TMZ varies considerably and critically depends on the expression level of *O6-methylguanine-DNA methyltransferase (MGMT)*. *MGMT* encodes *O*6-alkylguanine DNA alkyltransferase, a DNA repair enzyme removing alkyl groups from the O6 position of guanine. In general, the lower the expression level of *MGMT*, the better is the response to TMZ treatment. Since *MGMT* expression is regulated epigenetically via DNA methylation, the promoter methylation status of *MGMT* is considered a prognostic and predictive biomarker in glioblastoma.

Various methods have already been published for *MGMT* promoter methylation analysis. Studies show that bisulfite pyrosequencing is the method of choice because it allows determining the methylation status of individual CpG dinucleotides (CpGs) and is applicable in routine analysis. Since the *MGMT* promoter contains 98 CpGs, it is, however, challenging to design primers that do not contain CpGs and do not yield unspecific PCR products due to mispriming.

The lecture will discuss the challenges in MGMT promoter methylation analysis in more detail and present our approaches to overcome these problems.

PL-7

Determination of Bacterial Cell Viability Under The Influence of Propiltiodiazolochinazolin-one

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Keywords: cell viability, cytometry, Propiltiodiazolochinazolin-one.

In process of evaluation of antibacterial mechanism of action is particularly important to know the impact of the active substance on the efflux pumps of the bacterial cell. We aimed to evaluate the viability of the bacterial cell under the influence of a new antibacterial agent – Propiltiodiazolochinazolin-one.

Determination of the influence of Propiltiodiazolochinazolin-one on the activity of bacterial efflux pumps was performed by flow cytometry techniques: determining cell viability by marking with propidium iodide and assessing the activity of bacterial efflux pumps in the marking with ethidium bromide. The method is based on the measurement of scattered light in relation to incident laser radiation, coupled with the cellular complexity, cytoplasmic granularity and parameters of fluorescence when a linear laminar flow of cell passes the laser beam at a right angle.

Alternative marking of samples (microbial strains grown in the presence of Propiltiodiazolochinazolin-one at a concentration MIC/2) with two fluorochromes, allowed to assess the mechanism by which it influences on microbial strains, namely the permeabilising of cell coatings or the inhibition of efflux pumps microbial. This last mechanism is of particular interest since it can cause the conversion of resistant microbial phenotypes to sensitive phenotypes by targeting the activity of an efflux pump with the help of inhibitor, which should restore the sensitivity of microorganism to antimicrobial substances.

Correlating quantitative data with qualitative aspects of histograms, we can conclude that Propiltiodiazolochinazolin-one has an correlated activity with affinity for Gram coloration of the studied strains: works by permeabilising of cell coatings on Gram-negative bacteria and by inhibition of efflux pumps of Gram-positive bacteria.

The research was conducted in the bilateral project between Faculty of Pharmacy Medical and pharmaceutical University "Nicolae Testemitanu" and Faculty of Pharmacy of Medical and pharmaceutical University "Carol Davila", Bucharest, Romania ".

PL-8

Heteroelement-incorporated Full-length Protein Standards for Protein Quantification by Mass Spectrometry

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Among numerous technologies to study the proteome, mass spectrometry (MS)-based techniques are capable to provide the most accurate and reproducible quantitative data. Proteomic studies can provide two types of data: relative and absolute. For absolute quantification suitable standards are required. Use of stable isotope-labelled proteins as internal standards is recognized as an optimal analytical approach for absolute protein quantification at present.

Here, a novel approach for production intact protein standards, which are quantified very accurately, is presented. Standard protein production is achieved by cell-free synthesis with incorporation of heteroelement- (e.g. selenium) and stable isotope-labelled amino acids (e.g. $^{13}C/^{15}N$-labelled arginine and/or lysine). Standard protein absolute quantification is carried out by ICP-MS via heteroelement detection. Standard proteins containing selenium as heteroelement have been named RISQ standards (Recombinant Isotope-labelled and Selenium Quantified) [1]. While RISQ proteins can be quantified highly accurately by ICP-MS due to their exclusive selenium content, their stability might be influenced by the objectionable reactivity of selenium. Therefore, to further develop the RISQ protein concept, we generated Se-quantified but Se-free standard proteins. For this purpose we introduced the two types of label into two separately expressed proteins (RSQ and RIQ standards) [2]. The heteroelement-containing protein (RSQ, Recombinant Selenium Quantified) is quantified by ICP-MS and is then used as a standard for quantification of the purely stable isotope-labelled standard (RIQ, Recombinant Isotope-labelled and Quantified) by LC-ESI-MS/MS. The latter then represents the final heteroelement-free protein to be used as internal standard for endogenous protein quantification. The extra quantification step by LC-ESI/MS can be performed with a relative standard deviation around 1% [3]. Standard preparation, purification, and characterization are described in detail.

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PL-9

Covalent Anchoring of Human Transferrin to Carbon-encapsulated Iron Nanoparticles in Presence of Magnetic Field as a Way of Preservation of its Conformational Integrity and Electroactivity

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Keywords: human transferrin, protein layers, core-shell ferromagnetic nanoparticles, quartz crystal microbalance with dissipation, magnetic field

Any realistic biomedical application of magnetic nanoparticles requires that they maintain a specific ability to recognize and bind the selected molecular targets (e.g. tumor receptors, pathogens, antigens and proteins) without losing their biological activities. It is known that the contact of transferrin with SPIONs leads to irreversible changes in the protein structure and finally to metal ions release. The application of carbon-encapsulated iron nanoparticles eliminates this problem.

The covalent anchoring of human transferrin (Tf) to carbon-encapsulated iron magnetic nanoparticles functionalized with carboxylic groups (Fe@C-COOH Nps) in the presence of magnetic field, results in its conformational integrity and electroactivity. We showed that it is possible to attach, without changing pH, more than one single layer of transferrin to the Fe@C-COOH Nps. This is a very rare phenomenon in the case of proteins. We proved, using various experimental techniques, that the proposed methodology does not lead to release of iron from Tf molecules, what was the major problem so far. We believe that this finding opens new possibilities in drug delivery systems and medical diagnostics.

The research was supported by the Polish NCN grant: no. 2014/15/D/ST4/02989.

PL-10

Interactions of Biologically Active Species with Model Biological Membranes.

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Keywords: Langmuir technique, anticancer drugs, drug carriers.

Langmuir technique can be successfully used to prepare phospholipid monolayers at the air-water interface, which can be treated as simple models of one leaflet of cell membranes. Such model systems are commonly used in the studies on the interactions of drugs, toxins and other biologically important species. Although very simplified, this model has already proved to be useful in explaining the interactions of anticancer drugs. The main advantage of such approach is the fact that the composition of a model layer may be adjusted to mimic different types of cellular membranes. In order to obtain a better model of a real biological membrane, the phospholipid monolayers may be transferred onto solid support to form a bilayer. Additionally, the transfer allows one to employ other techniques such as spectroscopy, microscopy and electrochemistry to investigate the model membranes.

The formation and characteristics of biomimetic membranes of different composition corresponding to healthy and cancer cell membranes will be reported. In addition to Langmuir monolayer studies at the air-water interface along with Brewster angle microscopy, the layers were transferred onto solid support by means of Langmuir-Blodgett and Langmuir-Schaeffer method and aforementioned techniques were employed in order to characterize these systems in more detail. Model membranes were also employed to study the interactions of typical anticancer drugs: daunorubicin and doxorubicin. Both monolayer studies at the air-water interface and electrochemical experiments performed with supported model membranes revealed that the composition of the membranes and its order of organization determines the type of the prevailing driving forces responsible for the interactions of drugs with phospholipids: electrostatic or hydrophobic. The effect of free drugs on model membranes will be also compared with the interactions of drugs attached to carbon nanotubes, which are potential drug carriers.

The research was supported by the Sinergia project no. CRSII2_154451 financed by the Swiss National Science Foundation.

PL-11

Development of Analytical Methods for Psychoactive Drugs by High-Performance Separation Techniques

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Besides consumation of well known illicit drugs, such as cocaine, heroine, MDMA and speed, recreational abuse of novel synthetic psychoactive drugs has become a challenging problem worldwide. Every year, dozens of new compounds enter the drug market and due to their similar substitution patterns, full characterization is difficult.

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

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

For successful enantioseparation, either commercially available chiral columns or chiral selectors as chiral phase additives on RP-columns were used. More than 40 new psychoactive compounds were resolved successfully. Obviously, all tested novel psychoactive drugs were traded as racemic mixtures.

PL-12

Design, Synthesis, Analysis and Evaluation of Neurotensin (8-13) Analogues Containing Non-protein Arginine and Lysine Residues

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Keywords: opioid peptides, neurotensin, analgesics, CE, HPLC

Opioid receptors are important targets for the treatment of pain and potentially for other disease states (e.g. mood disorders and drug abuse) as well. The endogenous ligands for opioid receptors are peptides. Since their initial discovery in the 1970s, there have been extensive studies of the structure-activity relationships (SAR) of opioid peptides for each of the opioid receptors. They have the potential to be pharmaceutical agents for the treatment of pain, devoid of side-effects accompanying morphine. In addition small neuropeptides are currently the state-of-the-art tool in the development of new site-directed radiopharmaceuticals. Lower molecular weight, faster washout from the bloodstream and better tumor-to-background ratios at early times than high-molecular-weight compounds.

Neurotensin (NT) is a tridecapeptide first isolated from bovine hypothalamus. It is known to play a crucial role as a neurotransmitter in the central nervous system (CNS) and as a neuromodulator in the periphery. These actions are mediated by interactions with specific receptors found at the cell membrane of target cells. Besides their numerous central and peripheral functions it was reported that NTRs are overexpressed in various human tumors. Thus, in the last decade many efforts dealt with radiolabeled NT analogues to target NTR1-bearing tumours.

This report focuses on the development, analysis and pharmacological evaluation of novel NT(8-13) analogues demonstrating activity after systemic administration.

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PL-13

Scientometric Indicators for Bioanalysis

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Keywords: scientometrics, bioanalysis, key indicators, journals

Scientometrics is covering in quantitative fashion the development of science. The science itself is depending on fashion. Nowadays, words beginning with bio-, eco-, nano- or ending in -omics are part of our scientific life. Nevertheless, words as colorimetry or potentiometry look to be obsolete. The main purpose of this paper is to draw the pathway of a successful paper in the field of bioanalysis. Several journals in the field were analyzed according to their scientometric indicators compared with the "fashioned" topics. The contribution of Central and Eastern Europe research groups was sized according to the outcome indicators. Moreover, the partnerships established within our CEEPUS network were clustered.

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PL-14
Chemistry and Genetic Information

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Keywords: (evolution, mutagenesis, directed-evolution)

Evolution, regarded as a continuous change, is the result of adaptations to the environment. These adaptations are possible due to a genotypic diversity of populations, created by random mutagenesis which is actually a chemical transformation.

Biocatalysts can be improved by man, through rational mutagenesis, or by directed evolution protocols.

The chemical basics of mutagenesis and a practical example of site directed mutagenesis on an enzyme will be presented.

The research was supported by the National Research Grant PNII-ID-PCE-2011-3-0775.

PL-15

Air Pollution Study with Heavy Metals in Mine Environments. Case Study Bregalnica River Basin, Republic of Macedonia

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Keywords: Heavy metals, air pollution, mine environment, Bregalnica River, Macedonia

Application of moss species and attic dust for monitoring of anthropogenic impact on heavy metals air pollution in Bregalnica River Basin, Republic of Macedonia, was studied. Moss samples were reviewed for their potential to reflect heavy metals air pollution. The attention was focused on their quantification ability, underlying the metal accumulation within moss plant tissue and attic dust “historical archiving”. Potential “hot spots” were selected in areas of copper mine (Bučim mine) and lead and zinc mines (Zletovo mine and Sasa mine) as main metal pollution sources in the Eastern part of the Republic of Macedonia. Continuously, dust distribution from ore and flotation tailings occurs. Several moss species (*Hypnum cupressiforme*, *Homalothecium lutescens* and *Scleropodium purum*) were used as plant sampling media. Determination of chemical elements was conducted by using both instrumental techniques: atomic emission spectrometry with inductively coupled plasma (ICP-AES) and mass spectrometry with inductively coupled plasma (ICP-MS). Combination of multivariate techniques (PCA, FA and CA) was applied for data processing and identification of elements association with lithogenic or anthropogenic origin. Spatial distribution maps were constructed for determination and localizing of narrower areas with higher contents of certain anthropogenic elements. In this way the influences of selected anthropogenic activities in small scale air pollution can be determined. Summarized data reveal real quantification of the elements distribution not only in order to determine the hazardously elements distribution, but also present complete characterization for elements deposition in mines environs.

PL-16

Experience with Saccharide-based Chiral Stationary Phases for Separation of Enantiomers

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Saccharide-based chiral stationary phases (CSPs) belong to the most popular CSPs that can be used for separation of a broad spectrum of structurally different enantiomers. Their broad applicability results from the possibility to work in various separation modes, i.e. they are compatible with different mobile phases. These stationary phases are prepared by coating or by immobilization of derivatized polysaccharides, amylose and cellulose, or oligosaccharides - cyclodextrins and cyclofructants and their derivatives. In general, native saccharides have mostly limited capabilities to serve as chiral selectors while their derivatives exhibit interesting separation possibilities for a variety of analytes.

In this presentation we want to share our experience with saccharide-based CSPs applied for enantioselective separation in high performance liquid chromatography (HPLC) and also in supercritical liquid chromatography (SFC). Possibility of use of cyclofructans, the newest group of chiral selectors in capillary electrophoresis will also be briefly mentioned. Based on our experiments/results we try to correlate structures of the analytes tested with their retention and resolution values in order to get some insight on the separation mechanism.

PL-17

Structural Investigations of Aromatic Hydrazones in Solid State and in Solution

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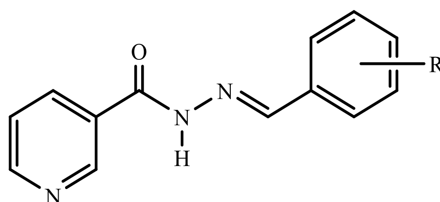
Keywords: aroylhydrazones, MS, NMR, IR spectroscopy, UV-Vis spectrometry

Aroylhydrazones derived from nicotinic acid hydrazide and differently substituted benzaldehydes were prepared according to the published procedure [1].



R = H (**1**); 2-OH (**2**); 2-OH, 3-OH (**3**); 2-OH, 4-OH (**4**); 2-OH, 5-OH (**5**); 2-OH, 3-OCH_3 (**6**); 2-OH, 4-OCH_3 (**7**); 2-OH, 3-Cl (**8**); 2-OH, 5-Cl (**9**); 2-OH, 3-Cl, 5-Cl (**10**); 2-OH, 5-NO_2 (**11**)

Such compounds can be involved in keto-enol tautomeric interconversion. Different tautomeric forms have different spectral properties and can be distinguished according to their IR, UV-Vis and NMR spectra. Structural investigations of title compounds in solid state and in solution were performed using following spectroscopic techniques: NMR (^{1}H, ^{13}C NMR, solid state NMR), IR spectroscopy, mass spectrometry (MS), UV-Vis spectrometry and spectrofluorimetry. Structural isomers of aroylhydrazones were distinguished by tandem mass spectrometry. Compounds **3** and **4** (derived from 3- and 4-hydroxy salicylaldehyde) as well as **8** and **9** (derived from 3- and 5-chloro salicylaldehyde) were distinguished by MS/MS as a result of "*ortho* effect" [2]. The changes in UV-Vis spectra in DMSO during time were observed for **11**, as well as for starting aldehyde 2-hydroxy-5-nitrobenzaldehyde.

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PL-18

Applications of In Situ UV-Vis-NIR Spectroelectrochemistry

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Keywords: spectroelectrochemistry, UV-Vis-NIR spectroscopy, pharmaceutical analysis, multivariate data analysis

Spectroelectrochemistry represents a valuable analytical tool for studying the redox chemistry and characterizing chemical structures in charge transfer reactions, as well as monitoring interfacial processes, able to offer complementary data, for both qualitative and quantitative purposes. Oxidation states are changed electrochemically by addition and/or removal of electrons while spectral measurements on the solution adjacent to the electrode are made simultaneously. Spectroscopic analysis may be performed by various techniques on various spectral ranges, but by far the most commonly used hyphenation of electrochemistry is done with UV-Vis/NIR spectroscopy.

Some applications of in situ UV-Vis-NIR spectroelectrochemistry concerning the analysis of various active pharmaceutical ingredients (drugs) will be discussed. Valuable qualitative and quantitative information is usually extracted from the acquired data matrix by various multivariate data analysis techniques which will be briefly outlined as well. Moreover, some future perspectives in the development of hybrid spectroelectrochemical sensors will also be presented.

PL-19

Gabor Dibo

PL-20

Flow Analysis vs. Flow Chemistry

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The effectiveness of the carrying out chemical reactions, especially in terms of conducted in laboratory or on technological scale syntheses, is a target of the optimization of chemical conditions and physico-chemical parameters of a given process. Since the publication of pioneering works in early of 1970-ties [1], it is increasingly accepted opinion that carrying out chemical reaction in a continuously flowing streams rather than in batch configuration has numerous advantages, the flow chemistry can be at present considered as a separate and rapidly increasing area of modern chemistry. Four decades of the development of those methods resulted in thousands of original research works, numerous reported attractive technologies, and also numerous specialized instruments, which are available on the market.

That vast literature and numerous reported successful applications indicate numerous advantages of flow chemistry in chemical synthesis compared to batch processes [2-4]. It is first of all a convenient way to precise controlling of the reaction time, fast carrying out mixing reagents, easy handling solutions containing gases and a simplification of carrying out multi phase liquid reactions. Numerous achievements of laboratory scale chemistry, which were developed for synthesis, can be successfully employed also for design of analytical instrumentations, and developing of new analytical methodologies.

The performing of chemical syntheses and analytical determinations under continuous flow conditions can be then considered as two areas of modern chemistry with numerous common features. In both cases, be it for the preparation of a required product, a chemical transformation of an analyte or its isolation from complex matrices, there are carried out similar physico-chemical operations and chemical reactions in similar instrumental modules exploiting different kinetic effects. Similar advantages can be gained in both fields with the miniaturization of instrumentation [5].

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OC-1

Determination of Organic Acids in Wines Using Capillary Zone Electrophoresis-Electrospray Ionization /Quadrupole-Time-of-Flight-Mass Spectrometry (CZE-ESI/QTOF-MS)

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Keywords: organic acids; CZE-ESI/QTOF-MS, validation, red wine.

Organic acids, inlcuding tartaric, malic, lactic, citric, succinic and shikimic, were determined in red wines using capillary zone electrophoresis hyphenated to electrospray ionization/quadrupole-time-of-flight-mass spectrometry (CZE-ESI/QTOF-MS). Separation of the analytes was perfomred using 50 mM ammonium acetate buffer, with pH 6, as a background electrolyte. The capillary was coated with 1 % (*m/v*) solution of hexadimetrine bromide. The applied voltage for the capillary electrophoretic separation was – 20 kV with anodic detection. The method was validated presenting best recoveries ranged from 98.4 % to 112 % for all organic acids. The calibration curves were linear with correlation coefficients r^2 > 0.99, ranging from 0.9902 for shikimic acid to 0.9990 for tartaric acid. Developed method was applied for analysis of Vranec wines from different wine regions. Tartaric acid was the main ogranic acid in wines (range: 2.09 – 4.96 g/L), followd by malic acid (range: 0.29 to 4.03 g/L). The total content of oraganic acids ranged between 3.53 to 8.5 g/L, concluding that climate conditions in the wine areas inlfuenced the acids amount in grapes and wine.

The research was supported by the grants from the: CEEPUS, CII-HU-0010-03-0809 Network and Research Fund of the University "Goce Delčev" – Štip, R. Macedonia for the project titled "Polyphenolic and aroma profile of Vranec wines fermented with isolated yeasts from Tikveš wine area", covering the study stay of Violeta Ivanova-Petropulos at the Institute of Bioanalysis, Faculty of Medicine, University of Pecs, Pecs, Hungary, where the capillary electrophoresis analyses of wines were performed.

OC-2

Determination of Pharmaceutical Additives by Microchip and Capillary Electrophoresis

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Keywords: Microchip electrophoresis, Capillary electrophoresis, Pharmaceutical additives

The electrophoretic separation of the pharmaceutical additives, such as erythrosine (ER), methylparaben (MP) and propylparaben (PP) by capillary zone electrophoresis (CZE) on a microchip with conductivity detection and on a conventional electrophoretic analyzer with UV detection is described. Synthetic dye ER is considered potentially harmful because of its carcinogenic impact, and may cause hyperactivity and increased photosensitivity. Preservatives MP and PP are slightly toxic causing allergic and hypersensitive reactions with wide spectrum of antibacterial activity.

The analytes were separated in the developed CZE electrolyte systems at high pH (9.8). In order to improve the resolution of the separated analytes β-cyclodextrin at a 1 mmol/L concentration was added to the background electrolyte. High repeatabilities of the qualitative parameters of ER, MP and PP in the model samples were guaranteed by preferred working conditions which eliminated a negative influence of electroosmotic flow. RSDs of migration time were in the range of 0.8–1.9% and 0.4–1.7% on the microchip and capillary, respectively. The concentration limits of detection (cLOD) for studied pharmaceutical additives obtained in CZE separations on microchip with conductivity detection were in the range of 0.4-6.8 μmol/L. The cLOD values on capillary with UV detection were in the range of 0.1-0.4 μmol/L.

The proposed CZE method on microchip and capillary was successfully applied to the determination of ER, MP and PP in three real samples of pharmaceutical preparations.

The research was supported by grants APVV-0259-12, VEGA 1/0340/15, UK/312/2016 and CEEPUS CIII-RO-0010-10-1516.

OC-3

Study of the Oxidative Metabolic Pathway of beta-blockers by Capillary Electrophoresis Coupled with Mass Spectrometry

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Keywords: beta-blockers, CE/MS, metabolism pathway, oxidation mechanism

The evaluation of the oxidative transformation of drugs within the human body is of high importance in the process of understanding the phase I reactions in drug metabolism. A full comprehension of the involved oxidative mechanisms is essential for the evaluation of the possible toxicity of new drugs as well as for various correlations with the data collected by pharmacovigilance of the already approved ones. Because of their high reactivity, in some cases, an accurate identification and quantification of the metabolism products is known to be very difficult.

The present study aims to evaluate the possibility that a purely instrumental electrochemical method can be used as a prediction tool for the bioconversion of beta-blockers. For this purpose, a comparative study was performed, between the microsomal oxidation (human liver microsomes) of chosen beta-blocker representatives (propranolol, atenolol, alprenolol, oxprenolol) and their non-enzymatic oxidation induced by an electrochemical flow cell. For the detection of the emerging oxidative products by both approaches, capillary electrophoresis coupled with mass spectrometry was used.

The conducted studies not only reconfirmed the viability of electrochemical simulation of the oxidative metabolism as a complementary screening tool for the prediction of metabolic conversions in early stages of drug development, but also helped in elucidating unknown electrochemical reaction mechanisms of known drugs, namely beta-blocker representatives.

The research was supported by PhD Research Project no. 7690/21/15.04.2016 Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania and by the European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/136893.

OC-4

Determination of Acetate in Buserelin Acetate by Microchip Isotachophoresis

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Keywords: Microchip isotachophoresis; Quantitative analysis; Conductivity detection; Counterion; Buserelin acetate

This study is focused on the evaluation of the basic methodological aspects of the quantitative analysis by microchip isotachophoresis (ITP) using conductivity detection. From the application point of view determination of acetate as an anionic macroconstituent in a cancer treatment drug buserelin acetate was studied.

ITP separations were carried out in a separation system with suppressed hydrodynamic and electroosmotic flow. Acetate quantitation was evaluated using external calibration and internal standard methods, with succinate as an internal standard. RSD of the corrected acetate zone lengths ranged from 0.1 to 0.7% that indicates high precision of the developed method. The long-term validity of the ITP quantitative parameters for determination of acetate on two microchips and two electrophoretic devices was verified. This favors ITP over other microchip electrophoretic techniques, when chip-to-chip and/or device-to-device transfer of the analytical method is required.

The robustness of the ITP quantitative analysis was studied in terms of impact of fluctuations in various working and separation conditions, e.g., fluctuations in the driving current, concentration of the leading ions, pH of the leading electrolyte and impurities in the electrolytes, on the precision of determination.

The recovery values in the range of 98-100% indicate very accurate determination of acetate in buserelin acetate. In summary, ITP method performed on the microchip with conductivity detection is suitable for reliable determination of main components in relatively simplified pharmaceutical preparations.

The research was supported by the Slovak Research and Development Agency (APVV-0259-12), the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA 1/0340/15), and the Grant of Comenius University in Bratislava (UK/205/2016).

OC-5

The Application of Non-covalently Immobilized Trypsin in a Poly(dimethylsiloxane) Microfluidic Device for Rapid Protein Digestion

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Keywords: immobilization; trypsin; digestion; peptide mapping

We described an immobilized enzymatic microfluidic device (MD) capable of rapid and efficient proteolysis. The MD was made of poly(dimethylsiloxane) (PDMS), a supreme adsorbent of proteins, which enables non-specific trypsin adsorption on the channel walls of the MD. Trypsin activity on the PDMS surface was investigated with sequential peptide mapping of bovine serum albumin. Rapid and efficient proteolysis occurred within 2 hours after immobilization of trypsin CZE peptide map of BSA. The BSA samples were digested with good reproducibility (RSD% values for migration times were less than 1%). The immobilized trypsin MD was capable of rapid digestion of different proteins (hemoglobin, myoglobin, lysozyme and BSA) in a wide size range (15-70 kDa) with a contact time less than 1 min. The number of the separated peaks correlated well with the expected number of peptides formed in the complete tryptic digestion of the proteins. The simplicity of the channel pattern, the immobilization procedure and the easily regeneratable or disposable feature make this MD to one of the simplest but efficient enzymatic microreactors.

This work was supported by grants from the TAMOP-4.2.2.A-11/1/KONV-2012-0036 and NTP-NFTÖ-16-0038 project. The authors also acknowledge the financial support provided to this project by the National Research, Development and Innovation Office, Hungary (K111932). We would like to thank to József Bakó and Prof. Dr. Csaba Hegedűs for measurement possibilities in the Biomaterials Research Lab on the Faculty of Dentistry and Dr. István Csarnovics for AFM measurement possibilities in the Department of Experimental Physics.

OC-6

Complexation of Charged Complexation Agents and Buffer constituents: Influence on Properties of Buffer

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Keywords: electrophoresis, complexation, ionic strength, buffer constituent

The most commonly used complexation additives to background electrolyte (BGE) in capillary electrophoresis are cyclodextrins (CDs) and their derivatives. Presence of CDs in a BGE offers additional interactions to analytes and thus selectivity of separation systems can be enhanced, chiral separation can be achieved and even neutral analytes can be mobilized.

However, these additives can also interact with BGE constituents. This type of interaction can significantly change BGE properties even in case of addition of neutral CDs. Moreover, when charged CDs are used, high number of charges on CD molecule unpredictably affects ionic strength of the BGE. This work is focused on revealing complexation between charged CDs and BGE constituents and proposing noninteracting buffers for electrophoretic measurements with charged CDs. Further, the influence of presence of multiply charged CDs in BGE on its ionic strength of BGE is investigated.

The equations for calculations of the pH changes of BGE caused by complexation of complexation agents with buffer constituents have been derived. These equations have showed that the pH of the buffer should increase or decrease depending on which form of weak electrolyte interacts more strongly with the complexation agent. We used pH measurement for revealing complexation among buffers tested. To confirm the results of the pH measurements, measurements of the changes of the effective electrophoretic mobilities of buffering constituents with the addition of selected charged CDs were carried out. Further, for the determination of influence of charged CDs on the ionic strength of BGE, the concept of ionic strength marker was utilized. The results from the ionic strength marker measurements suggest that influence of multiply charged CDs on the ionic strength is significantly lower than would be expected for these multiply charged species.

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OC-7

Characterization of Oligonucleotides-Ligand Interactions with Implications in Myotonic Dystrophy

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Keywords: Nucleic acids, Affinity capillary electrophoresis, Myotonic dystrophy

Myotonic dystrophy type I, or the Steinert disease, is an autosomal dominant inherited genetic disorder characterized by wasting of the muscles, cataract, cardiac conduction abnormalities and endocrine changes (insulin resistance). At genetic level, the mechanism of the disease is related to a mutation at the DMPK gene characterized by abnormal repeats of the CTG triplet. The transcription of this mutated segment leads to abnormal CUG repeats in the tRNA which in turn can sequestrate an important splicing factor (MBNL-1) thus leading to the symptoms of the disease.

Several in vivo studies showed that different small molecules could disrupt this complex by binding competitively to the CUG region and release the MBNL-1 splicing factor restoring its function. An analytical approach could be complementary to in vivo techniques and could facilitate the fast screening of different ligands.

The present work illustrates a capillary electrophoresis (CE) method developed for the study of nucleic acids-ligand using pentamidine as the lead compound. Different compounds, mainly antibiotics, were tested and their interaction with the nucleic acids (both DNA and RNA) were estimated in terms of binding constant and stoichiometry.

The results suggest that upon additional optimization this CE method can be employed for the rapid screening of potential ligands. In a next step, the most promising ligands may be further tested by in vitro or cells cultures, thus saving time.

The research was supported by the PCD, FRMH and CEEPUS CIII-RO-0010-10-1516 scholarships.

OC-8

Development and Validation of a Cyclodextrin-Modified Capillary Electrophoresis Method for the Enantiomeric Quality Control of *R*-praziquantel

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Keywords: antihelmintic; chiral separation; enantioseparation

The enantiomers of praziquantel, the drug of choice in schistosomiasis were separated by cyclodextrin-modified capillary zone electrophoresis. 10 anionic cyclodextrin derivatives were screened for their ability to discriminate between the uncharged enantiomers. Among the tested selectors, 7 presented chiral interaction with praziquantel enantiomers, the best results being obtained when using sulfated-β-cyclodextrin. Interestingly, the enantiomer migration order was the same in all cases, *R*-praziquantel, followed by its antipode. In order to achieve reversed migration order, a simple polarity switch was employed, which resulted in extreme resolution values (Rs>35).

Using the optimized method (50 mM phosphate buffer pH 2.0, supplied with 15 mM sulfated-β-CD, reversed polarity, applied voltage -15 kV, capillary temperature 25 °C, short-end injection -50 mbar x 2 seconds), analysis times under 10 minutes were obtained, while still maintaining high resolution values, thus enabling the detection of *S*-praziquantel as an enantiomeric impurity at 0.1 %. Validation of the method was carried out according to the ICH guideline for sensitivity, linearity, accuracy, precision and robustness. Application of the method was tested on commercial, combination tablets containing racemic praziquantel and the method was found to be suitable for routine analysis.

The research was supported by the Domus Scholarship of the Hungarian Academy of Sciences.

OC-9

A New CE-MS Approach Using In-Capillary Derivatization for the Chiral Analysis of Amino Acids

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Keywords: in-capillary derivatization, FLEC, amino acids, chiral separation

In the context of bioanalytical procedures, the automatization of the methodology is nowadays a necessity in order to save time, improve method reliability and reduce costs. For the first time, a fully automatized in-capillary derivatization MEKC-MS method was developed for the chiral analysis of D- and L- amino acids using (−)-1-(9-Fluorenyl) ethyl chloroformate (FLEC) labelling reagent. The derivatization procedure was optimized using an experimental design approach leading to the following conditions: sample and FLEC plugs in a 2:1 ratio (15s, 30mbar: 7.5s, 30mbar) followed by 15 minutes of mixing using a voltage of 0.1 kV. The formed diastereoisomers were then separated using a background electrolyte formed of 150 mM ammonium perfluorooctanoate (pH=9.5) and detected by mass spectrometry. Complete chiral resolution was obtained for 8 amino acids, while partial separation for 6 of them. The method showed good reproducibility and linearity in the low micromolar concentration.

OC-10

Promoter Methylation Patterns of *ABCB1*, *ABCC1* and *ABCG2* in Drug-Sensitive and Multidrug-Resistant Human Cancer Cell Lines

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Keywords: ABC transporter, multidrug resistance, cancer cell, DNA methylation, bisulfite pyrosequencing

Drug-resistant tumors are able to grow and spread even in the presence of chemotherapeutic drugs. Resistance may exist not only against one drug, cancer cells also can acquire resistance to multiple chemotherapeutic agents. This phenomenon is called multidrug resistance (MDR). In tumors, overexpression of the ATP-binding cassette (ABC) proteins P-gp (encoded by the *ABCB1* gene), MRP1 (encoded by *ABCC1*) and BCRP (encoded by *ABCG2*) is a major cause of limited efficacy of anticancer drugs, because ABC transporters are functioning as efflux pumps. Current research aims at overcoming MDR by administering drugs that have the potential to inhibit these efflux pumps.

Studies indicate that epigenetic mechanisms including DNA methylation are involved in regulation of P-gp and BCRP, data on MRP1 is, however, scarce. Our aim was to determine the promoter methylation patterns of *ABCB1*, *ABCC1* and *ABCG2* in 19 human cancer cell lines derived from different cancer types. This data will help selecting an appropriate cancer cell line for testing the mode of action and/or for testing the efficacy of potential chemotherapeutic drugs. In addition, we determined the promoter methylation patterns in several MDR cell models in order to investigate the role of DNA methylation in developing a MDR phenotype.

The lecture will present methylation levels obtained by methylation-specific bisulfite pyrosequencing and gene expression data on both the mRNA and protein level. Our results indicate that the promoter methylation status of *ABCC1* is not associated with expression of MRP1. In contrast, in several cancer cell lines and MDR cell models the promoter methylation status of *ABCB1* was found to correlate with the expression of P-gp.

OC-11

Voltammetric Detection of Paramagnetic Components in Blood

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Keywords: magnetic nanoparticles, hemoglobin, ceruloplasmin, transferrin, voltammetry

The efficient, reliable, rapid and low cost monitoring of disease biomarkers is an analytical challenge in several technological fields. The application of electrochemical detectors seems to be very competitive compared to the present clinical methods due to their low cost, no need to use the toxic substances and short analysis time.

In our work we clearly demonstrated that voltammetric detectors were excellent for the fast determination and quantification of paramagnetic blood components: ceruloplasmin (Cp), hemoglobin (Hb), transferrin (Tf) within one step measurement without special treatment of the blood sample. Our method is based on electrode ferromagnetic modifier (carbon-encapsulated iron nanoparticles) and the presence of a weak external magnetic field (~ 40 mT). The nanomagnets at the electrode surface play the role of the specific and selective filter, which during the first few minutes enhance the transport to the electrode surface of the paramagnetic species only. Additionally, all paramagnetic molecules reaching the voltammetric detector maintain their electroactivity. Moreover, the direct contact of the enzyme with ferromagnetic modifier did not lead to its deactivation. The determination of Cp, Hb and Tf appeared to be highly sensitivity, well reproducible and of very low detection limit (pM level).

The research was supported by the Polish NCN grant 2015/17/N/ST4/03933.

OC-12

A MCFA Strategies for Determination of Proteins in Real Human Samples

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Keywords: Flow Analysis, Clinical Analysis, Proteins

In this contribution, the versatility of MCFA (MultiCommutated Flow Analysis) systems will be shown in the field of clinical diagnosis. The presented measurement strategies will be focused on determination of proteins, which are significant in routine clinical analysis such as total protein and albumin. Both of this parameters are the components of general urine test carried out in clinical laboratories throughout the world. The pathological level of total protein authorizes to diagnose proteinuria, in turn albumin indicates the states of microalbuminuria. Both states are important as prognostic markers for kidney diseases. The parameter of total protein is also determined in cerebrospinal fluid (CSF), which is requested in the case of infection, neoplasm or degeneration of the central nervous system.

The main strategies of determination of total protein in urine and CSF samples and albumin in urine samples will be presented. Detection systems will be based on the concept of paired emitter detector diode (PEDD). For determination of total protein, Exton method with sulphosalicylic acid were used. In the case of albumin determination, the kinetic measurements of turbidity caused by the reaction between albumin and antibodies were carried out. The measurements were fully mechanized by microsolenoid devices providing relatively small volume of injected sample and satisfactory flow-throughput. After the optimization, to prove the usefulness of mentioned analytical systems the physiological and pathological urine samples were analyzed in validation process.

The research was supported by the Polish National Science Centre (project OPUS NCN no. 2014/13/B/ST4/04528)

OC-13
Analysis of Latex Protein Content with High Performance Liquid Chromatography Coupled to the Tandem Mass Spectrometry

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Keywords: amino acids, mass spectrometry, HILIC, latex gloves

In this work, protein content of latex gloves production by-products was analyzed with high-performance liquid chromatography coupled to the electrospray ionization tandem mass spectrometry (HPLC-ESI/MS/MS). Up-to date, latex protein content was studied with different spectrophotometric methods; unfortunately these methods are very susceptible to sample matrix interferences, which often leads to unreliable results.

Latex proteins were hydrolyzed to the corresponding building blocks and resulting free amino acids mixture was subsequently quantified by HPLC-ESI/MS/MS. Fifteen underivatized amino acids were separated with hydrophilic interaction stationary phase (ZIC-HILIC column); by using this approach is was possible to establish the total protein content of the latex sample. The ZIC-HILIC also column allows for underivatized amino acids separation while eliminating the need to use problematic ion-pairing agents, such as trifluoroacetic acid (TFA). After chromatographic separation, the analytes were introduced into the electrospray ion source (ESI) of the triple quadrupole mass spectrometer operating in the selected reaction monitoring mode (SRM). By utilizing this very selective and sensitive detection method it was possible to minimize interferences from the sample matrix.

The optimized method was used to study protein content of the *Hevea brasiliensis* (rubber tree) ammonia stabilized natural rubber latex concentrate and vulcanized latex gloves. The obtained results were used to evaluate the performance of a different protein removal methods used during the latex gloves manufacturing.

The research was supported by the Human Capital Operational Programme 2007-2013: support the cooperation of scientific environment and enterprises as well as by the European Regional Development Found in the Sectoral Operational Programme "Improvement of the Competitiveness of Enterprises 2004 –2005. This study was carried out at the Biological and Chemical Research Centre, University of Warsaw, established within the project co-financed by European Union from the European Regional Development Fund under the Operational Programme Innovative Economy, 2007 – 2013.

OC-14

Control and Determination of Organic Compounds During Recombinant Protein Expression in E. coli BL21

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Keywords: fermentation, glucose, organic acids

Different factors can affect the course of a fermentation influencing the adaptation and metabolism of a microorganism. The determination and precise control of these fermentation conditions plays a key role in achieving a high yield recombinant protein expression. During a fermentation especially in a bioreactor, usually the main physical (temperature, pressure, gas flow rate, agitator speed) and chemical parameters (pH, oxygen concentration) are monitored on-line and controlled automatically. Substrates and metabolites are monitored off-line and controlled manually, which makes it difficult to achieve a high yield protein expression due to the late feed-back (from 1 to 24 hours). Because in the inorganic, well defined media (used in the fermentation process), the sole carbon source is glucose, it is highly important to control and determine its change in concentration during fermentation. However there are on-line bioprocess analyzers for measuring glucose and lactose compounds based on single-use enzymatic sensors which are impractical for those who are on a low budget. We controlled the glucose concentration during fermentation by determining a glucose dosage rate in a fed-batch fermentation system. We used off-line glucose concentration measurements as data points to determine the glucose uptake rate of the E. coli BL21 expressing the IAP1 and XIAP inhibitor of apoptosis proteins respectively. The uptake rate was specific for the fermentation conditions used: pH 7, aerobe environment, biomass formation on 37°C and protein expression on 18°C for 12 hour. The glucose uptake rate combined with the specific growth rate calculated from the biomass formation was used to determine the necessary glucose concentration in function of time during the fermentation process. Nevertheless we determined the change in concentration of different organic acids during the fermentation for the better understanding of the metabolism and carbon utilization of the microorganism.

This work was supported by the Romanian Ministry of Education, Executive Agency for Higher Education, Research, Development and Innovation Funding, PNCDI II, project no. 119/2014.

OC-15

A Novel Reference Real-Time PCR System for the Relative Quantification of Roe Deer and Deer Species in Food

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Keywords: meat species, real-time PCR, relative quantification, thermally treated food, game

According to the Codex Alimentarius Austriacus, a “game” sausage must contain at least 38% of game meat. In order to verify the authenticity of game products, DNA based methods are preferentially used over protein based ones, due to the higher stability of the analyte and the fact that DNA is present in the majority of cells. Nonetheless, quantification by PCR is still a challenge that has to be overcome, especially the correlation between the concentration or copy number of the target sequence determined and the meat content given in weight percent. So far, the relative quantification method published by Laube et al. in 2007 seems to be the most promising one. It uses two PCR systems targeting single copy genes: one PCR system to analyze the content of the species of interest and a reference PCR system, targeting the myostatin gene, to determine the total amount of mammals and poultry. With the help of calibration curves, the percentage of the animal of interest in the food is calculated.

However, when we applied the reference system published by Laube et al. to determine the game meat content in thermally treated food, recoveries were substantially higher than 100%. Our idea was to shorten the target region (from 97 bp to 70 bp) in order to make it less prone to degradation by food processing.

To investigate the applicability of the novel reference system, aliquots of a model game sausage were subjected to various thermal treatment steps (e.g. boiling, brewing, microwave treatment). Results obtained by analyzing DNA extracts from these aliquots and DNA extracts from heat treated commercial game products indicate that by using the novel reference system, more accurate results are obtained than with the reference system published by Laube et al.

The research was supported by the Austrian Agency for Health and Food Safety.

OC-16

Development of a DNA Metabarcoding Method for Meat Species Identification

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Keywords: Food authenticity, Meat species, DNA barcoding, Next-generation sequencing

Since commercial foodstuffs must be both safe and authentic various analytical methods have already been developed to detect meat adulteration. These methods must be selective, sensitive, and applicable to raw and processed meat products and should allow high sample throughput to be used in routine by food control authorities.

Most of them are based on the analysis of proteins or DNA sequences. In general, DNA-based methods are better applicable because DNA is more stable than proteins. Most DNA assays for meat species identification in foods rely on the use of the polymerase chain reaction (PCR). Currently, real-time PCR plays an important role in species authentication in foods, but next-generation sequencing (NGS) technologies, especially the massively parallel sequencing of amplicons is assumed to be a promising alternative.

In this study we focus on developing a DNA metabarcoding method to simultaneously identify 21 animal species in food by using a NGS instrument. DNA barcoding is based on the analysis of species-specific differences in short DNA sequences. We started by testing the applicability of two universal primers for mammals previously published. In order to allow the identification of both mammals and poultry species in foods, we had to modify the primer system. With the new primer system we were able to obtain PCR products for the 21 animal species of interest, including 16 mammals and five poultry species. Preliminary results obtained by analyzing binary mixtures of DNA extracts indicate that in general, the metabarcoding method allows the identification of meat species down to 0.1%. Further experiments are necessary to investigate the applicability to DNA extracts from foodstuffs.

The lecture will give an overview of food authentication, explains the principle of DNA metabarcoding coupled with NGS and presents our work.

The study is supported by the Austrian Agency for Health and Food Safety.

OC-17

Bioactive Compounds and Antioxidant Properties of Green Coffee Brews

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Keywords: Green coffee, HPLC/MS, caffeine, antioxidant activity

Coffee is one of the most popular drink nowadays all over the world. It contains more than 700 compounds which are responsible for its aromatic and unique flavor. Genus *Coffea arabica* and *Coffea canephora* var. *robusta* are most important species of Coffea and they constitute 60% to 40% of world production. Coffee as a functional food with antioxidant properties reduces the incidence of cancer, diabetes and liver disease, protects against Parkinson's disease and reduces mortality risk. Green coffee bean extract shows a hypotensive effect in rats and reduces visceral fat and body weight. These properties are connected with bioactive compounds, not only chlorogenic acids and their derivatives, but also caffeine, theophylline and theobromine, tocopherols, cafestol, kahweol and trigonelline. There is only a few studies about polyphenolic composition of green coffee brews. Twelve green coffee beans of different origin: Coffea arabica (Brazil, Rwanda, China, Laos) and Coffea robusta (Vietnam, Vietnam decaffeinated and Vietnam steamed, India, Indonesia, Laos, Uganda Sc and Uganda Bugishu) obtained from Strauss Café, Poland were involved in this study. Different assays - determination of reducing power (Folin-Ciocalteau and CUPRAC methods), DPPH radical scavenging activity and metal chelating activity – were used for this purpose. The contents of chlorogenic, caffeic and ferullic acids were determined by HPLC-ESI-MS.

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OC-18

Equilibrium and Structural Characterization of Rufinamide-Cyclodextrin Complexation

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Keywords: rufinamide, water solubility, cyclodextrin, complex formation

Introduction: Rufinamide (RUF), the novel antiepileptic drug, with triazole structure was characterized in terms of cyclodextrin complexation, using a set of complementary analytical techniques. Materials and methods: In an attempt to increase the aqueous solubility of the drug, three different cyclodextrins were tested, namely native β-cyclodextrin (β-CD) and two of its substituted derivatives: randomly-methylated-β-cyclodextrin (RAMEB), and sulphobutylether-β-cyclodextrin (SBECD). Complex stability constants were determined by using phase solubility studies and capillary electrophoresis, while stoichiometry of the complexes was elucidated using ESI-TOF-MS and 1H NMR Job plot method. Elucidation of inclusion complex geometries was made by 1H and 2D ROESY NMR measurements and further verified by molecular modeling. Results: Phase solubility studies revealed A_L-type of diagrams in all cases, characteristic of 1:1 stoichiometry, with a maximum of over 15-fold increase in solubility of RUF, when complexed with RAMEB. Observations regarding stoichiometry were further confirmed by MS experiments and Job's plot analysis, while ^{1}H and 2D ROESY investigations revealed the inclusion of the triazole moiety of the compound, which was also revealed by molecular modeling. Apparent stability constant of the SBE-CD-RUF complex was also determined by affinity capillary electrophoresis and results were in good agreement with the value obtained by phase solubility studies. Conclusion: Cyclodextrin complexation is suitable for achieving higher aqueous solubility of rufinamide, which in terms might pave the way for a drug formulation with improved bioavailability.

The research was supported by the joint project of Studium Prospero Foundation and Hungarian Academy of Sciences.

OC-19

Is there any Recreational Value in the Codeine Containing OTC Analgesics?

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Keywords: codeine, OTC, analgesics, recreational use

Codeine containing OTC analgesics are used for the treatment of low intensity pain with the advantage of combining low doses of an opioid analgesic (codeine or other weak opioid in some countries) with a non steroidal anti-inflammatory drug (NSAID: acetaminophen, ibuprofen, etc). These types of combinations have two main advantages:

- lower side effects due to the completely different toxicity profile of the two ingredients,
- impossibility to recreationally use the combination due the need of a huge number of doses that will bring NSAID toxicity (gastric bleeding, liver toxicity, etc).

Due to this reasons, codeine is made available as OTC product only in combinations. However, the so called "harm reduction" internet forums instruct the users how to separate codeine from the NSAID and obtain a recreational usable codeine with harmless levels of NSAID. The codeine can be used as it is or it can be transformed in more dangerous drugs as "krokodil" (desomorphine) with horrifying toxic effects. The extraction of codeine employs a very simple method: tablets are suspended in water, the water is cooled in a freezer until first crystals of water are formed and then the suspension is filtered through a regular coffee filter. This way, the low solubility NSAID will be precipitated leaving in the solution the highly soluble codeine.

The purpose of this work was to measure the recovery of codeine and NSAID (using a validated HPLC-UV method) from commonly used codeine containing OTC products in Romania: Fasconal, Aspaco, Codamin P, Solpadeine, Nurofen Plus, Tusocalm, and Ultracod which is not an OTC product. The results show that codeine can be very easily separated from NSAID as aspirin, acetaminophen, ibuprofen using the cold water extraction method. However, very large differences (20 to 90%) were recorded for the recovery of codeine depending on the OTC product that was used. That large difference increases the risk of potentially lethal overdoses when the user switches between "similar" products.

OC-20

Study Regarding the Stereochemistry of Modern Antidepressants

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Keywords: antidepressants, stereochemistry, enantiomers, chiral separation

Depression is a common and serious mood disorder, characterized by severe symptoms that affect the quality of daily life and normal functioning of the individuals.

In modern therapy selective serotonin reuptake inhibitors (SSRI) and selective noradrenaline and serotion reuptake inhibitors (SNRI) are mostly used in the treatment of depressive disorders. Almost all SSRIs and SNRIs contain in their structure at least a chiral center (fluoxetine, citalopram, venlafaxine), and some have two chiral centers (sertraline, paroxetine). Some derivatives are used in therapy as racemic mixture (fluoxetine, venlafaxine), other are used both as racemic mixture and pure enantiomers (citalopram – escitalopram) and others are used as pure enantiomers (sertraline, paroxetine, duloxetine).

The differences between the pharmacokinetic, pharmacologic, pharmacotoxicologic properties of the analytes in question have been studied extensively in the last 25 years; there are opinions and arguments for and against using racemic mixtures or pure enantiomers at most of the studied substances.

Taking in consideration the aspects presented above development of new analytical methods for the chiral determination of modern antidepressants is a necessity and also a permanent challenge for any analyst.

A fundamental part of the quality control of pharmaceutical formulations is the determination of enantiomeric excess and enantiomeric purity; for this purpose, efficient and reliable analytical methods are needed and electrophoretic techniques (capillary electrophoresis) are very efficient and inexpensive candidates for the role. In this study, the enantioselective capillary electrophoresis methods available for the analysis of second-generation antidepressant are presented and discussed.

OC-21

Determination of Midazolam and its Main Metabolites in Biological Samples - Comparison of LC-UV and LC-MS

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Keywords: Midazolam, HPLC-UV, HPLC-MS, Metabolites.

Midazolam is a sedative compound routinely used in intensive care patients. Due to its pharmacological properties, i.e. a half-life of about 3 hours and only minimal influence on hemodynamics, its use in sedation is widely accepted, even for long periods. In patients with renal and liver failure, metabolites of midazolam may, however, accumulate, leading to unwarranted prolongation of sedation.

Methods published so far allow the determination of midazolam and its hydroxy metabolites 1-hydroxymidazolam (1-OHM) and 4-hydroxymidazolam (4-OHM), but not 1-hydroxymidazolam-β-D-glucuronide (1-OHMG) and 4-hydroxymidazolam-β-D-glucuronide (4-OHMG) in one and the same run. 1-OHMG and 4-OHMG are usually determined after enzymatic cleavage to 1-OHM and 4-OHM. We developed two independent methods, a LC-UV and a LC-MS method, allowing the direct determination of midazolam and its four metabolites. Both methods include sample clean-up by solid phase extraction. The lecture will compare the two methods with regard to the most important analytical parameters, including selectivity, applicability to serum and urine samples, matrix effects, limit of detection (LOD), limit of quantification (LOQ) and recovery. In addition, the lecture will present data on the metabolism of midazolam.

OC-22

Degradation of Zein Nanoparticles of Interest to Drug Delivery

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Keywords: zein, nanoparticles, degradation, capillary gel electrophoresis

Zein is the main protein found in corn endosperm, and has the ability to form NPs, due to its hydrophobicity, and incorporate hydrophobic molecules, such as drugs, pesticides or dietary supplements [1,2]. Although zein is a natural polymer, generally regarded as safe for human consumption, in the form of NPs its reactivity and chemical properties change and further evaluation of its toxicity and stability is required.

The present study aims to characterize the degradation process of zein NPs in aqueous media, as well as in reconstituted biological fluids. Accelerated degradation studies were carried out in aqueous media within a wide pH range (4-9) and the changes in parent compound (two monomers of ~24-28 kDa) was monitored by capillary gel electrophoresis (CGE) with UV detection. Changes in parent compound and apparition of degradation products were also monitored after degradation in reconstituted gastric and intestinal fluids, simulating digestion. Most studies focus on evaluating the behavior of active substances throughout digestion, and not that of the nanostructured carrier system. The data provided by the current study are critical for the assessment of nanoparticles' degradation and offers a better understanding of the processes zein NPs undergo after ingestion.

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OC-23

Recent Advances in the Chiral Discrimination of β-Blockers Using Molecularly Imprinted Polymers

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Keywords: molecularly imprinted polymers, β-blockers, chiral analysis, capillary electrochromatography

In order to further investigate the variables defining the analytical properties of the molecularly imprinted polymers (MIPs) and the mechanism of recognition in the polymer matrix, *R*(+)-atenolol (ATNL) was employed in the study as a template. Based on our previous results, 2-(trifluoromethyl)methacrylic acid was proved to offer the strongest interactions with the template and therefore was selected as the functional monomer. Six cross-linkers (trimethylolpropane trimethacrylate, ethylene glycol dimethacrylate, pentaerythritol triacrylate, pentaerythritol tetraacrylate, 3-(trimethoxysilyl)propyl methacrylate and divinylbenzene) and four solvents (acetonitrile, methanol, n-butanol and dimethylformamide) were employed in the MIP synthesis.

Due to the high numbers of variables affecting the imprinting process, a careful design of experiments was used in the optimization of the polymerization mixtures' composition. The polymers were prepared by precipitation polymerization in different vials and their enantioselectivity was tested using batch rebinding studies by adding the racemic mixture of ATNL over the obtained MIPs.

The results obtained by enantioselective MIP were compared with the ones obtained by capillary electrochromatography using monolithic capillaries with the same MIP. The polymer selection and the optimal working conditions were based on the resolutions obtained in the electrophoretic separations.

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OC-24

Synthesis of Cu-Porphyrin Complex for the Potential Application in Radiopharmaceutical Preparation

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Positron emission tomography (PET) is one of the modern medical imaging technique. It utilizes radionuclides that undergo β^+ decay. One of the isotopes of interest is ^{64}Cu ($t_{1/2}$ = 12.7 h), that can serve as a positron emitter. The other advantage of ^{64}Cu is properties that facilitate the use of ^{64}Cu based radiopharmaceuticals as a therapeutic agent. Potential ligands for PET ^{64}Cu radiopharmaceuticals are porphyrins. They are useful agents for photodynamic therapy and fluorescence imaging of cancer. Additionally, porphyrins are excellent metal chelators, and are known for forming stable complexes with copper. Thus, ^{64}Cu chelation with porphyrin photosensitizer may become a simple and versatile labelling strategy for clinical positron emission tomography.

The present study reports a convenient method for the synthesis of Cu complex with *tetrakis*(4-carboxyphenyl)porphyrin (TCPP). The experimental conditions for labelling, such as the metal-to-ligand molar ratio, pH and time of reaction were optimized to achieve a high complexation efficiency in a short period of time as possible. In order to accelerate the metallation, the use of substitution reactions of cadmium or lead porphyrin and the presence of reducing agent, such as ascorbic acid, hydroxylamine and flavonoid - morin, were evaluated. The optimum conditions for the synthesis of the copper complex was borate buffer at pH 9 with the addition of 10-fold molar excess, with respect to Cu^{2+} ions and TCPP and ascorbic acid which resulted in reduction of the reaction time from 120 min to below 1 min.

OC-25

Identification of Radionuclidic Impurities and Determination of Metal Content Along the Synthesis Path of ^{18}F-fluorodeoxyglucose

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^{18}F-fluorodeoxyglucose (^{18}F-FDG) is the main radiopharmaceutical used in routine clinical positron emission tomography (PET). The applications of ^{18}F-FDG cover oncology, neurology and cardiology. Important step in radiopharmaceutical preparation is quality control. The product has to meet several criteria before it can be injected. Radionuclidic and chemical purity are one of the parameters checked.

In this study the identification, determination and distribution of metallic and radionuclidic contaminants in the synthesis of ^{18}F-fluorodeoxyglucose are presented. Samples of irradiated ^{18}O-enriched water, purification columns (anionic, C18, Al_2O_3), final product and wastes were examined. Metallic contaminants were determined by ICP-MS and the radionuclide impurities by high resolution germanium gamma-spectrometry. Fifteen radionuclides were identified in the samples. The enriched water contained cationic contaminants (^{55}Co, ^{56}Co, ^{57}Co, ^{58}Co, ^{57}Ni, ^{52}Mn, ^{7}Be) while the anionic (^{95}Tc, ^{95m}Tc, ^{96}Tc, ^{183}Re) were mostly retained in the QMA column. The sources of contamination by metals and radionuclides were determined. Results obtained by ICP-MS generally confirmed the radionuclide distribution obtained by gamma spectrometry.

OC-26

Sulfobutylether-β-CD as a Coating Agent and Mobile Phase Additive in Chromatography

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Keywords: HPLC, sulfobutylether-β-cyclodextrin, dynamic coating, mobile phase additive

Various amino acids, dipeptides and their isomers were (enantio)separated using sulfobutylether-β-cyclodextrin as chiral selector. Two different approaches were employed: First, dynamic coating of sulfobutylether-β-cyclodextrin onto the strong anion-exchange stationary phase and second the use of the sulfobutylether-β-cyclodextrin as a mobile phase additive in separation system with C18 column. Measurements were carried out in RP-HPLC and hydrophilic interaction liquid chromatography. Mobile phases composed of organic modifier (methanol) and four different aqueous parts: (i) deionized water, (ii) aqueous solution of formic acid (pH 2.1), (iii) ammonium acetate buffer (pH 4.7), and (iv) ammonium acetate buffer (pH 8.8) in various volume ratios. Under these separation conditions the majority of analytes were separated. Sulfobutylether-β-cyclodextrin proved to be suitable for separation of chiral and also achiral analytes. The use of sulfobutylether-β-cyclodextrin as dynamic coating agent or as a mobile phase additive depends on the particular chromatographic system and analytes of interest.

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OC-27

Comparison of Three Immobilized Trypsin Reactors used in HPLC

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Keywords: digestion efficiency, trypsin column, HPLC

Trypsin is the most widely used enzyme in proteomic research for its high specificity. Although the in-solution digestion is still frequently used, it is burdened with several drawbacks, such as long digestion time, autolysis, intolerance to high temperatures and organic solvents [1,2]. To overcome these shortcomings, immobilization of trypsin on solid support is convenient, since it ceases autolysis, allows use of high enzyme concentration and is compatible with modern LC-MS instruments [3]. In this work, three trypsin columns were tested for their catalytic activity and compared using Nα-benzoyl-L-arginine 4-nitroanilide hydrochloride as a substrate. Two commercial trypsin columns, Perfinity and Poroszyme, and one column prototype from the University of North Carolina were used. For this purpose, various buffer pH, flow rates and temperatures were used. Relative standard deviation values were determined to describe repeatability of digestions and the whole separation process. Activities of columns differed the most using buffer pH 9.0, when the highest digestion activity was possessed by the column prototype and the lowest by Poroszyme column at all conditions tested.

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OC-28

Profiling of Phenolic Metabolites in Human Urine Samples using LC-MS/MS

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Keywords: polyphenols, funcional food, metabolism, LC-MS/MS

Polyphenols are plants secondary metabolites characterized by a high complexity and diversity of structures. These natural compounds are widely present in plants and food of plants origin. They are considered to play a protective role against oxidative stress related pathological states of organisms. Epidemiological studies show the strong correlation between the dietary polyphenols intake and the reduction of risk of some chronic diseases such as cardiovascular diseases, cancer, diabetes and also aging. Due to this facts, the protective effect of these natural compounds has been a topic of increasing research in the past decade. However, the biological properties of polyphenols strongly depends on their metabolism and bioavailability in human body.

The aim of this study was to investigate the transformation of different polyphenols in human digestive track. For that purpose healthy human volunteers were set on polyphenol-free diet during 48 hours. The study was conducted in two days cycles. First 24 hours were used in order to “wash-out” the organism. Then, polyphenols rich food was consumed and urine samples were collected after different times from the consumption for a total period of next 24 hours. All samples were analyzed using high performance liquid chromatography tandem mass spectrometry (LC-MS/MS). Studies allowed to determine the transformation pathways the polyphenols undergo in human digestive track and create the pharmacokinetic profiles of polyphenols’ metabolites.

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The research was carried out with the approval of the ethics committee, KEIB – 5/2016

OC-29

Analysis of Blue Natural Dyes with Purple Hue and Products of their Degradation by LC/MS/MS

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Keywords: HPLC, natural dyes, historical textiles

In this work, high performance liquid chromatography coupled to the electrospray tandem mass spectrometry (LC/MS/MS) was used for the identification of natural dyes from historical textiles samples. In the ancient times, several compounds of both plant and animal origin were used to textiles dyeing. From the chemical point of view, natural dyes contained a number of different compounds, such as flavonoids (yellow dyes), anthraquinones (red dyes), tannins (brown dyes) and indigotins (blue dyes), etc. [1]. The obtained data shown a presence of orchil, a dyes prepared from lichens, e.g. *Roccella/Ochrolechia/Varialaria* [2], in addition to indigo and brazilwood.

Additionally, degradation of the major orchil components: orcein derivatives was studied in the aging chamber. The aging chamber constructed in the laboratory allows for exposing the studied textile fiber to UV-Vis irradiation at different wavelength and intensities for various periods of time. Obtained results allowed for proposing the currently unknown orchil decomposition mechanism. For example compound with molecular mass of 376 Da, a likely orchil decomposition product, was detected in the aged sample.

The research was supported by the DEC-013/09/B/HS2/01197.

As the part of the detailed analysis of 192 medieval liturgical vestments currently stored in the National Museum in Gdansk, the identification of selected natural dyes present in these textiles was carried out.

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OC-30

Molecular Trapping of Saponins in Self-assembled Micellar-films / Aggregates:

An alternative Method for Separation and Purification

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Keywords: Purification of saponins, amphiphilic-monomers, self-assembled micelles, collapsed micellar-films, trapped molecules.

A pure sample of saponins was obtained from methanolic root extract of *Securidaca longipedunculata* by liquid-liquid extraction, in a process whereby the molecules are trapped within the structural building-blocks of self-organized micelles and segregated (via phase-separation) to forms a cluster of easily-extractable solids. On TLC, it yielded two prominent fractions of triterpenic saponin **4a2** and **4a4** (RP-C18). Having the properties of surfactants, it emulsifies fat and forms micelles (self-assembled aggregates) in aqueous media, at level above critical micelle concentration (CMC).

For purpose of validation, 50mg sample was introduced into a mixture of organic solvent and water (2:1) to produce a partially thermodynamic-stable emulsion. Substituting the organic solvent with ethyl-ether, a severe instability occurred, resulting to phase-separation - (creaming, flocculation and coalescence). Observed movements of particles were seen in compliance with gravitational force / Stoke's law. Finally at the interface, was formed a deposit of thick whitish-solid mass considered to be the constituents monomers ("trapped molecules") released from the debris of collapsed micellar-films. This was carefully collected, dried and analyzed using gravimetric and semi-quantitative (HPTLC) methods. After 5 continuous cycles, total percentage recovery of solute was estimated at 94%, having a ratio of 5:1 when compared with popular liquid-liquid extraction (n-Butanol and Water). Selectivity was quite significant ($P<0.05$)*. Product of degradation was limited to the upper layer (ethyl-ether), with value 2.15% on average, (RSD±61.32%).

The research was supported by the Iuliu Hatieganu University of Medicine and Pharmacy (UMF), Cluj-Napoca, Romania. Grant for PhD research project Nr.7690/81/15.04.2016.; CEEPUS CIII-RO-0010-10-1516 Network.

OC-31

Stress Protein Determination from Metal Stressed Bean Plants

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When plants are subjected to high metal exposure, different responses to the metal-induced stress were observed for taxonomically different plants. Based on their response to metal exposure four groups of plants can be distinguished: metal-sensitive species, metal-resistant excluder species, metal-tolerant non-hyperaccumulator species, and metal-hypertolerant hyperaccumulator species. In each plant - either metal sensitive or tolerant - different molecular mechanisms were developed to deal with the metal stress (resistance/tolerance to metal toxicity). Plant responses to heavy metals are regulated biochemically by homeostatic processes, that includes regulation of the metal-induced reactive oxygen species (ROS) signaling pathway. ROS generation and signaling plays an important role in heavy metal detoxification and tolerance. Plant-associated bacteria can enhance trace element availability in the rhizosphere and uptake bz the plant and also can modify the plant response to heavy metal.

The aim of our research was to detect the effect of plant growth-promoting bacteria (PGPR) on heavy metal uptake, plant growth and stress response.

In case of Cd^{2+} and Zn^{2+}treatment of bean plants the decrease of root lenght was observed, but no dofferences in root biomass was recorded. The Cd^{2+} accumulation in bean plants was higher in root than in shoot, due to the limited translocation, whereas the accumulated quantity of Zn^{2+} was higher in shoot than in root. The PGPR bacterial strain used had no effect on shoot and root growth of the metal stressed plants, but in case of Cd^{2+} treatment the PGPR bacteria decreased the accumulation of the metal in bean plants. Differences in poliphenol oxidase and peroxidase activity was observed between metal stressed and control plants.

OC-32

Synthesis of Phytochelatins Induced by As, Tl and PGEs in White Mustard (*Sinapis alba* L.)

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Keywords: phytochelatins, arsenic, thallium, platinum group elements, white mustard (*Sinapis alba* L.)

Phytochelatins (PC), which are polymers of glutathione, belong to a family of proteins made up of glutamic acid, cysteine and glycine ((γ-Glu-Cys)$_n$-Gly). They play an important role in detoxification and homeostasis of various plant species. Xenobiotics complexed by thiol groups of phytochelatins show lower toxicity than free metal ions. The synthesis of phytochelatins is discussed on the example of selected plant species under the influence of various metal and metalloid ions. Special attention is paid to research related to the synthesis of PCs by white mustard (*Sinapis alba* L.) grown in the presence of As, Tl, Pt, Rh and Pd ions, applied in various concentrations, chemical forms and combinations of xenobiotics. Phytochelatins PC2, PC3 and PC4 were determined in plant extracts prepared from roots, shoots and leaves. Derivatization of thiol compounds was done using monobromobimane (mBBr) in the presence of DTPA. In the course of analysis, two methods were used for PCs determination: liquid chromatography with fluorimetric detection (HPLC FLD) and electrospray ionization mass spectrometry (HPLC ESI MS).

The research was supported by the National Science Centre (NCN), Poland, grant number UMO-2014/15/N/NZ8/00326 and the Faculty of Chemistry, University of Warsaw, grant number 501/86-DSM-110200/2015.

OC-33

Non – Wood Forest Products (NWFPs) As a Source of Antioxidants

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Keywords: biophenols, antioxidant activity, HPLC-MS, spectrophotometric methods

Non-wood forests products are the group of goods of biological origin other than wood derived from forests. One of the most common forest shrub growing in Poland is heather (Calluna vulgaris). Traditionally, flower and herbal material are used to treat urinary tract disorders and as an antiseptic, antirheumatic and choleretic remedy.

The objective of this study was to determine the content of some biophenols as well as antioxidant activity of selected heather collected from forest and to compare them with cultivated plant.

Ethyl acetate, water, ethanol and its mixture were used for the extraction. The content of some biophenols was determined by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS). Antioxidant capacity of the prepared extracts was screened by several spectrophotometric methods. The total reducing capacity was evaluated by Folin-Ciocalteu (FC) method (so-called total phenolic content) and cupric ion reducing antioxidant capacity (CUPRAC). The content of flavonoids was determined by two mostly applied spectrophotometric methods based on the formation of Al(III)-flavonoid complexes. The total content of anthocyanins was estimated according to pH differential method.

The research was supported by the Forest Research Institute, project 'The possibility of utilisation non-wood forest products (NWFPs) as a source of antioxidants in beneficial for health supplements', topic no. 260801.

OC-34

Reagent-free Photodegradation - Simplifying of the Procedure for Sample Preparation before Voltammetric Measurements

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Keywords: photocatalysis, photodegradation, ASV, Fe_2O_3, WO_3

Voltammetry cannot be applied for determination of metal ions in samples containing significant amounts of surfactants. The presence of organic compounds contributes to the changes of the redox potentials of the analyte, deformation of the signals and/or decrease of the sensitivity of the method and selectivity or repeatability of the measurements. Therefore, an important element of the analytical procedure is mineralization of the sample, which is usually done using conventional methods of wet mineralization. An alternative is reagent-free photodegradation, which takes place at room temperature and atmospheric pressure. Degradation caused by sole radiation is inefficient so semiconductors are applied as catalysts for the process. The most widely used is titanium dioxide, but it requires application of UV lamps and quartz vessels. An interesting solution is application of stable and durable photocatalysts that are activated with radiation from the visible range (solar light). This lowers the cost of equipment and vessels. Such catalysts are e.g. iron and tungsten trioxides. Their effectiveness was checked for different compositions of the active layer, which consisted of: WO_3, a mixture of Fe_2O_3 and WO_3, a three-layer structure made of $Fe_2O_3/WO_3/Fe_2O_3$ and a three-layer structure made of $WO_3/Fe_2O_3/WO_3$. The samples containing SDS were irradiated for 2 and 4 h and the samples containing Triton X-114 for 2, 4 and 6 h. The quality of Pb signal (DPASV) was an indicator of the degree of decomposition of these surfactants.

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OC-35

Covalent Immobilization of Lipases on Functionalized Single-Walled Carbon Nanotubes for Biodiesel Production in Batch and in Continuous-Flow Modes

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Keywords: Lipases, Single-walled carbon nanotubes, Covalent immobilization, Biodiesel production

Carboxylated single-walled carbon nanotubes ($SwCNT_{COOH}$) were used as support for the covalent immobilization of lipase B from *Candida antarctica* (CaL-B) and Amano lipase from *Pseudomonas fluorescens* (L-AK). The nanostructured biocatalysts with low diffusional limitation were used in the ethanolysis of sunflower oil in batch and in continuous- flow modes. The immobilized enzyme preparations proved to be highly efficient and stable biocatalysts.

Using the CaL-B enzyme preparation ($SwCNT_{COOH}$–CaL-B), through several optimization rounds, the conversion reached 83.4% after 4 h at 35°C in acetonitrile as cosolvent. Reusability studies revealed that the immobilized lipase preserves more than 90% of its original activity after 10 reaction cycles.

The immobilized L-AK on carboxy functionalized single-walled carbon nanotubes proved to be also an efficient biocatalyst in the production of biodiesel from sunflower oil in batch mode, the conversion reaching 90% after the optimization procedures. The long term stability of the immobilized lipase was further tested in a continuous-flow reactor.

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P-01

Screening of Silage Bacteria for Antagonistic Potential

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Keywords: silage bacteria, antagonism, secunder metabolites, HPLC

Fungal and bacterial phytopathogens affect worldwide the crops and are responsible for significant losses, and also decrease the quality of agricultural products. The biocontrol plant growth promoting bacteria (PGPB) can suppress or prevent the phytopathogen damage. The PGPB can affect plant growth through direct or indirect mechanisms. The direct effects of PGPB include nutrient mobilization and phytohormone production. Mechanisms responsible for antagonistic activity include inhibition of the pathogen by different antibiotics, lipopeptides, cell wall degrading enzyme production, competition for minerals, nutrients, and colonization sites.

The aim of our research was the selection biocontrol bacterial strains in order to use them in bacterial biopreparates. 29 bacterial strains isolated from corn, alfalfa, and grass silage were used in our work. All bacterial strains were able to degrade one type of structural carbohydrate (cellulose, xylan, carboxymethyl cellulose). The bacterial strains used were the followings: *Bacillus aryabhattai, B. subtilis subsp. subtilis, B. simplex*, *B. subtilis subsp. inaquosorum, B. licheniformis, Paenibacillus amylolyticus, Paenibacillus pabuli, Weissella paramesenteroides.* The antifungal effect of the selected bacterial strains was tested against 6 phytopathogenic fungi. We also studied the siderophore production ability, organic and anorganic phosphorous mobilization, chitin degrading capacity, indole acetic acid and iturin production of the studied bacterial strains.

Our results showed that from the studied bacterial strains those belonging to *Bacillus sp.*, *Stenotrophomonas sp.* and *Weissella sp.* genera were the most efficient PGPR strains, and have the higest potential to be used in bacterial biopreparates.

The research was supported by the Romanian Ministry of Education, Executive Agency for Higher Education, Research, Development and Innovation Funding, PNCDI II, project no. 159/2014, acronym CERES.

P-02

Kinetics of Hydrolysis of Aromatic Hydrazones

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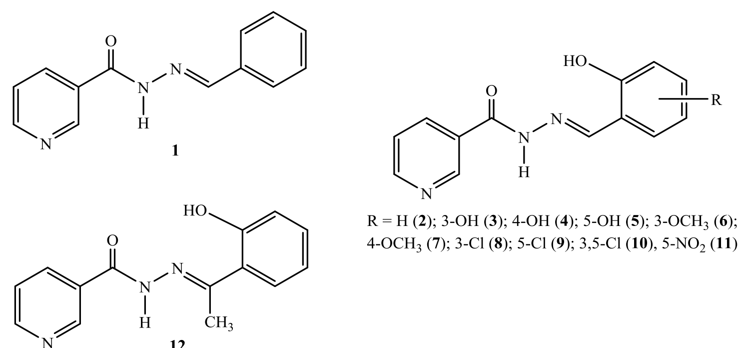
Keywords: aroylhydrazones, hydrolysis, spectrophotometry, chromatography

The kinetics of hydrolysis of compounds **1**–**12** (Scheme 1) were investigated in acidic and basic methanol/water 1/1 solutions by means of UV-Vis spectrophotometry. Hydrolytic stabilities of **3**, **4**, **10** and **11** were also studied by HPLC. Hydrolytic products of **3**, **4**, **5**, **7** and **10** in basic media were determined by ESI-MS.



Scheme 1. The structure of aroylhydrazones derived from nicotinic acid hydrazide.

The electron-withdrawing substituents decreased the hydrolysis in acidic media, whereas the electron-donating groups (–OH, –OCH_3) had the opposite effect. The hydroxyl group at position 4 at salycilidene part of the molecule, and methyl group at azomethine carbon atom, prevented the hydrolysis of hydrazones in basic media. In addition, the hydroxyl groups at position 3 and 5 caused the autooxidation of the hydrolytic products, and conversion of aldehydes to respective quinones.

The results obtained by different techniques were in excellent agreement.

The research was supported by the Croatian Science Foundation (project IP-2014-09-4841)

P-03

Size-Exclusion Chromatography for the Characterization of Klason Lignin Samples Using Microbore and Narrow-Bore Columns

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Keywords: lignin, size-exclusion chromatography, microbore, narrow-bore

Wood is composed of many chemical components, primary extractives, carbohydrates, and lignin. Lignin is the second most abundant biological macromolecule, and comprises of 15 – 25 % of the dry weight of woody plants. For the characterization and determination of the relative molar mass distribution of lignin, size-exclusion chromatography (SEC) is often used.

The fact that macromolecules (including lignin) have small diffusion coefficients is of vital to consequence to chromatographic practice [1]. Hence, chromatographic separation of lignin must use lower flow rates of mobile phase, which may result in peak band broadening. In order to improve separation efficiency and peak resolution, narrow-bore (3 – 6 mm i.d.) and microbore (< 3 mm i.d.) columns may be used.

The aim of this work was characterization of Klason lignin samples isolated from four woods (spruce, beech, eucalyptus, aspen) by size-exclusion chromatography using microbore and narrow-bore columns filled with 2-hydroxyethyl methacrylate gel (HEMA), and N,N-Dimethylformamide as mobile phase.

The research was supported by projects VEGA 1/0897/15 and VEGA 1/0899/16.

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P-04

Separation of As(III) and As(V) Based on Differences in Precipitation with DDTC – Speciation Analysis

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Keywords: arsenic, dithiocarbamates, diethyldithiocarbamate (DDTC)

Arsenic belongs to a group of very toxic elements. They are listed as priority hazardous substance and are considered within the top 20 contaminants by USPEA. As toxicity depends on the chemical forms and redox state of the elements. The most toxic forms of arsenic are non-organic compounds – arsenous hydride, arsenites and arsenates. Less toxic are methyl derivative like monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Arsenic organic compounds – arsenocholine (AsC) and arsenobetaine (AsB) are non-toxic.

Dithiocarbamates were applied to preconcentrate and isolate the arsenic from water samples. Dithiocarbamates are organic compounds which have a high chelating capacity. In literature there are described various examples of dithiocarbamates e.g. diethyldithiocarbamate (DDTC) or pyrrolidine dithiocarbamate (APDC). They form complexes with metal of general formula $(R_1R_2NCS_2)_3M$, where M is a trivalent metal (e.g. As(III)). The complexes are insoluble in water and soluble in nonpolar organic solvents, e.g. chloroform.

DDTC was added to the As(III) and As(V) standard solutions. Then the standard solution was filtrated through 0.45 µm membrane filter. For separation and preconcentration of As(III) the following sequence of solutions was applied to the membrane filter: 2 mL of sample - phase 1 (P1), 3 mL HNO_3 pH 2 (to elute As which can not be precipitated with DDTC) - phase 2 (P2) and mineralization of the filter – phase 3 (P3). As(III) is retained on the filter in phase 1 (recovery 20.1 – 31.3 %) and was eluted from the filter in phase 3 (recovery 72.4 – 55.4 %), while a large amount of As(V) is not retained on the filter and was eluted in phase 1 (recovery 82.4 – 89.3 %). Then equimolar mixture of As(III) and As(V) was applied on the membrane filterAs(V) is found in P1 (43.4 – 41.0 %) and As(III) in P3 (43.4 – 36.7 %). Cr(III) and Cr(VI) ions have negative influence to precipitation of arsenic. As(III) should be eluted in step 3 (phase 3) because it can be retained on the filter like a As(III)DDTC complex however in the presence of Cr(III) and Cr(VI) more amount of As(III) is eluted in phase 1 (73-80%).

P-05

Elution Behavior of Aliphatic Acids on Zwitterionic Stationary Phases in Hydrophilic Interaction Chromatography

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Keywords: hydrophilic interaction chromatography, retention behavior, aliphatic acids

This study deals with elution behavior of seven aliphatic acids (pyruvic, gluconic, 2-oxoglutaric, tartaric, malic, oxalic and citric acid) in hydrophilic interaction chromatography. Two silica based stationary phases with zwitterionic ligands has been used in this study. The first column was ZIC-HILIC (150 × 4.6 mm I.D, 5 µm) with sulfobetaine functional groups and the second column was ZIC-cHILIC (150 × 2.1 mm I.D, 3 µm) with phosphorilcholine functional groups. The mobile phase composition (ionic strength and volume of aqueous ammonium buffer solution) and the temperature effect on retention and separation selectivity of aliphatic acids were investigated. At increasing temperature, the retention factors of aliphatic acids decrease except for oxalic and tartaric acids. Also some deviation from the linearity of the van't Hoff plots *ln k* versus *1/T* was observed, indicating an involvement of a secondary mechanism. The standard molar entropy and standard molar enthalpy were calculated from the van't Hoff plots and the enthalpic and entropic contributions to retention were compared.

The research was supported by the grant of VEGA 1/0897/15, CEEPUS mobility grant CIII-PL-0706-02-1314 and grant UK/87/2016.

P-06

Antiangiogenic Peptidomimetics – Investigation of Their Degradation Pathway in Human Serum with HPLC-MS

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Keywords: angiogenesis, peptides, mimetics, HPLC-MS, proteolytic degradation

Angiogenesis is the process of new microvessels formation from preexisting vascular network, which physiologically occurs during embryogenesis, wound healing and reproduction. Pathological angiogenesis is crucial to the progression of cancer, particularly in metastasis and was first proposed by Folkman [1] as a therapeutic target in treatment of cancer. One of the most important growth factors involved in angiogenesis process is vascular endothelial growth factor-165 (VEGF165). Its chemical signal is transduced via VEGF receptors and significantly enhanced by association with co-receptor neuropilin-1 (NRP-1).

It has been previously shown, that heptapeptide ATWLPPR (A7R) binds to NRP-1 and selectively inhibits VEGF-165 binding to NRP-1. *In vivo* treatment with A7R resulted in decreasing breast cancer angiogenesis and growth in xenografted nude mice with MDA-MB-231 cell line [2].

In the frame of structure activity relationship research (SAR) we have proposed a family of very potent analogues of A7R [3]. Each of compounds possess higher inhibitory activity in comparison with A7R, however stability of peptides in proteolytic environment should be considered. For this purpose a human plasma sample was added to solution of investigated compound. After incubation at 37 °C a small volume was taken, enzymatic reactions were quenched with enzyme denaturating agent and after centrifugation sample was injected to HPLC-MS. In this communication we present results of this experiment obtained for our best peptidomimetic and triazolopeptidic analogues.

The research was supported by the NCN grant no N 204 350940.

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P-07

Electrochemical and Fluorescent Properties of Tetrazines in Aqueous Solution

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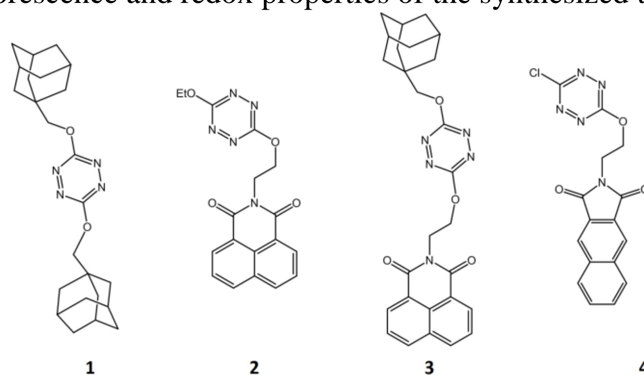
Keywords: tetrazines, β-cyclodextrin, gold nanoparticles, electrochemistry, fluorescence

Tetrazines are colored and electroactive heterocycles with a very high electron affinity. Tetrazines substituted with heteroatoms display interesting fluorescence properties that can be electrochemically monitored, aspect which makes them especially attractive in view of sensing applications [1, 2].

Four tetrazines substituted by linear 2,3-naphtalimide antennas and/or adamantane groups (scheme 1) were analyzed both in organic and aqueous media. For the first time, these hydrophobic compounds were successfully solubilized in aqueous solutions by using β-cyclodextrin (β-CD) and gold nanoparticles modified with β-CD, due to the formation of the inclusion complexes. The tetrazine itself and the organic anchoring groups fit the requirements for inclusion within β-CD cavity. The obtained inclusion complexes allowed the study of both fluorescence and redox properties of the synthesized tetrazines in water [3].



Scheme 1. Schematic representation of the tetrazine derivatives [3]

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P-08

Presence of Xenoestrogens in the Urine and Placenta Blood Samples of Pregnant Women

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Keywords: xenoetrogens, LC-MS, pregnant women, urine, blood

Xenoestrogens are widely used as preservatives in foods or cosmetic products. They may have weak estrogenic effects, therefore may be involved in different hormone-related diseases (effects on male reproductive system, breast cancer, etc.).

In this study xenoestrogens (phenoxyacetic acid, AFA; methyl paraben, MP; propyl paraben, PP; monobuthyl phthalate, MButF; bisphenol A, BFA; mono-benzyl phthalate, MBenzF, benzophenone, BenzF; mono-ethyl phthalate, MEP; triclosan, TCS) were measured in urine and blood of pregnant women using an HPLC-MS method. 44 volunteers were involved in the study; urine samples were collected during different month of pregnancy (between 5-9 month); blood samples were collected from placenta at birth. An LC-MS method was developed for the quantification of the studied xenosterogens. The LC-MS conditions were as follows: Luna C18 column (150 x 4.6 mm, 3 µm); mobile phase: 0.2% acetic acid:ACN 50:50; flow rate: 0.7 ml/min; triple quadrupole mass spectrometer with electrospray ionisation using MRM mode for AFA, MP, BenzF and SIM mode in the other cases.

The concentration of the xenoestrogens in the urine samples ranged between: 12.42 – 29395 ng/ml, 0.69 – 396.98 ng/ml, BLQ-103.12 ng/ml, 3.00 – 1537.60 ng/ml, BLQ – 40.67 ng/ml, 27.00 – 115.12 ng/ml in the case of AFA, MP, BenzF, MButF, MEP, TCS respectively. In blood samples the concentration of the studied xenoestrogens were 5.11 – 67.70 ng/ml, 3.99-30.21 ng/ml, 33.78 – 444.76 ng/ml, 1.15 – 20.31 ng/ml, 9.42 – 18.05 ng/ml, 12.34 ng/ml, 6.35 – 14.82 ng/ml for AFA, MP, BFA, PP, MButF, MBenzF, MEP, respectively.

P-09

Assessment of Perfumes with Synthetic Human Pheromones by Gas Chromatography-Mass Spectrometry

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Keywords: gas-chromatography, perfume, pheromones, qualitative analysis, quantitative analysis

In the present study the composition of twelve commercially perfumes claiming to contain human pheromones from different brands has been evaluated by gas chromatography-mass spectrometry. The identification of the perfume ingredients was performed by comparing the obtained electron ionization mass spectra of relevant chromatographic peaks with corresponding spectra from the Wiley MS library. Only in seven perfumes synthetic human pheromones have been identified. Androstenone is the most used pheromone by perfumes manufacturers being found in five fragrances for both women and men but of all analyzed pheromones the highest concentration has androstenol in a perfume for women. Also the results indicate that in some cases the difference between perfume for women and men is due to the other substances added in fragrance and not because of pheromones which are the same and have the same concentration in both versions.

P-10

Influence of Maceration Time on Bioactive Phenolic Compounds and Antioxidant Activity of Stanušina Wines

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Keywords: phenolic compounds; HPLC; maceration; Stanušina wine.

Bioactive phenolic compounds of red wines from Stanušina, a grape variety indigenous of the Republic of Macedonia, were determined using high-performance liquid chromatography coupled to diode array detector (HPLC-DAD). Wines were produced by different maceration time (3, 6 and 9 days) in order to study its influence on the phenolics extraction. Anthocyanins and phenolic acids were observed to be present in the highest content after 6 days of maceration (153 and 674 mg/L, respectively), while (+)-catechin content was highest 9 days after the skin maceration (262 mg/L). Malvidin-3-glucoside was the main anthocyanin in wines, ranging from 87.8 to 115 mg/L, while caftaric acid was the predominant cinnamic acid derivative, ranged from 373 to 428 mg/L. In general, Stanušina wines showed low level of anthocyanins, but relatively high content of hydroxycinnamic acids, such as caftaric and caffeic acids, and antioxidant activity as well (on average: 102 mg/L, Trolox equivavlents).

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P-11

Determination of Trace Elements in Macedonian Grape Brandies Using GFAAS

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Keywords: Rakija (grape brandy); trace elements; GFAAS.

A graphite furnace atomic absorption spectrometry (GFAAS) was used for trace elements (Cu, Fe, Mn, Zn, Cd, Cr, Ni, Pb) determination in "Rakija" samples, a grape brandy traditionally produced in Republic of Macedonia by distillation of grape pomace or wine for characterization. Principal component analysis was used to establish pattern recognition of the Macedonian brandies and classification depending of aging mode (oak barrels/oak chips) and distillation system (home-made/industrial). Two home-made brandies showed Cu, Fe and Zn concentrations higher than in the industrial distillates. These brandies were found to be not safe for consumption because of Cu and Zn over the maximum allowed values. For industrially produced brandies, Mn was identified to be a suitable marker related to aging with oak chips regardless variety, while Cu a marker for the influence of oak chips type.

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P-12

Application of Isotachophoresis in Commercial Capillary Electrophoresis Instrument using C^{4}D and UV Detection

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Keywords: capillary isotachophoresis, capillary electrophoresis, capacitively coupled contactless conductivity, UV detection

Although the different electrophoretic techniques are commonly used in commercial CE instruments, only a very few CITP applications are known [1, 2]. In this work we tested the applicability of a commercial (Agilent) CE instrument for CITP. The fused silica capillaries (50 µm I.D.) were flushed with polyvinylpyrrolidone (PVP) solution before each sample injection to suppress the EOF. As a dual detection mode commercial C^{4}D and UV detectors were applied. The experiments showed that the detection gap of the C^{4}D limits the achievable LOD and the separation resolution when the analyte CITP zones are very narrow, therefore long (120 cm) CE capillary was used and it was largely filled with the sample solution. The C^{4}D in commercial CE instruments was applicable for wider zones of analytes (>2 mm) which was mainly determined by the concentration of the analyte, the sample volume injected into the capillary and the concentration of the LE.

CITP analyses of several real samples (leather extract, red wine, juice, fizzy drink) have been demonstrated. In peak mode of CITP when the zone of a chromophore analyte positioned between non-chromophore zones, excellent sensitivity could be achieved by UV detection. The trace amount of hazardous chromate (1.3 µM) was determined in the aqueous extract of tanned leather.

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P-13

Stereoselective Acyloin Condensations of Aromatic Aldehydes with Lyophilized Yeast Cells

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Keywords: acyloin, yeast, condensation, lyophilized, aldehyde

The biocatalytic synthesis of asymmetric chiral acyloins as intermediates in the production of active pharmaceutical ingredients is an attractive goal. For example, *R*-(-)-phenylacetylcarbinol (*R*-PAC), is the key chiral precursor for pseudoephedrine production. The use of pyruvate decarboxilase (PDC, E.C.4.1.1.1), a Mg(II) and thiamine diphosphate-dependent enzyme from yeasts as a biocatalyst, has been extensively studied and is now a well-recognized method for condensing aldehydes and pyruvic acid to form acyloins(α-hydroxyketones).

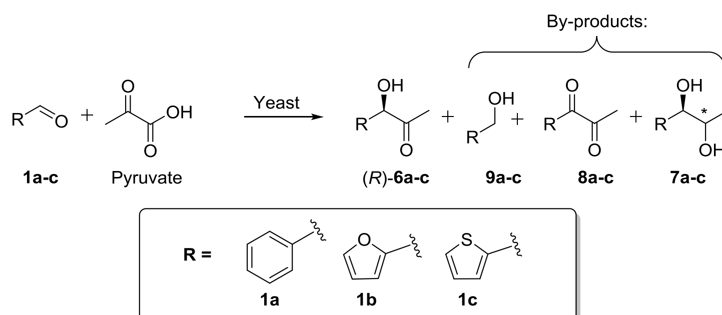

Figure 1. *Yeast promoted biotransformation of aldehydes **1a-c** for the efficient stereoselective synthesis of acyloins (R)-**6a-c**.*

In biotransformations mediated by whole cell microorganisms (**Figure 1**), by-product formation cannot be avoided. Consequently, screening for new microorganisms with high pyruvate decarboxilase and low oxido-reductase activity is still a challenging task. Moreover, by-product formation can be minimized by optimizing reaction conditions. In this work, the screening of several newly isolated yeasts in lyophilized whole cells form for acyloin production in aqueous media was investigated using aromatic and heteroaromatic aldehydes **1a-c**.

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P-14

Interaction of Bacterial Endotoxins with Human Serum Proteins by Microchip Electrophoresis

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Keywords: bacterial endotoxins, sepsis, serum proteins, microchip electrophoresis

The presence of microbial pathogens in the bloodstream triggers systemic inflammation and can lead to sepsis, which often defeats the most powerful antibiotic therapies and causes multiorgan systems failure, septic shock and death. Endotoxins, also known as somatic O-antigens in bacterial serology, are lipopolysaccharides (LPS) in biochemical aspects, derived from the outer membrane of Gram-negative bacteria. The presence of endotoxins released from the bacterial cell wall in the blood stream can cause violent pathophysiological reactions.

It is known that several proteins (HDL, LDL, LBP, CRP, apolipoprotein, transferrin, holotransferrin, hemoglobin and albumin) can interact with endotoxins and form complexes [1].

In our previous work we developed a new microchip electrophoresis method which was suitable for the detection of endotoxins labeled with covalent binding of a fluorescent dye [2].

The microchip gel electrophoresis method was applied to trace the presence of endotoxin in blood. We could show the characteristic endotoxin peaks from blood and it has been found that the endotoxin bound to serum proteins was shifted in migration time.

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P-15

The Motions of Insulin Through a Core-Shell Adsorbent

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Keywords: General rate model, Adsorption rate constant, Insulin

In this study we investigated the migration and adsorption process of a protein sample both in isocratic and gradient elution mode. To determine the adsorption kinetics, and the factors affecting the retention and band broadening of insulin on core-shell reversed phase adsorption bed we applied the general rate model of chromatography. The general rate model provides the most detailed and most accurate point of view to describe molecular movements in a chromatographic column. The focus of our work was to determine how the pressure and the adsorption rate constant affects the retention factor and efficiency of the separation of this particular macromolecule.

To model effective separations it is crucial to have a detailed understanding of the physico-chemical processes taken place between the adsorbent and adsorbate. The determination of the adsorption rate constant is a difficult task in porous adsorbent materials, and it is often neglected during the estimation of the column efficiency. Our study will reveal if the rate of the adsorption of a macromolecule can be neglected or should be included in numerical calculations deals with column efficiency in the case of biopolimers.

The determination of such kinetic parameters embedded in the general rate model allows us not just to determine thermodynamic coefficients, but helps to design separations of complex samples of proteins, both using isocratic or gradient elution mode.

P-16

Optimising Peptide Mapping by Capillary Zone Electrophoresis

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Keywords: peptide mapping, in-solution digestion, trypsin, CZE

Peptide mapping is a strategy developed in the early 1990s for the identification of proteins. An analysis is started with the denaturation of the protein (usually by adding urea or guanidine hydrochloride). Then, reduction of the disulphide bonds are acquired with dithiothreitol or mercaptoethanol and finally iodoacetamide or iodoacetic acid is added as alkylating agent. Standard tryptic digestions are performed using 1:50 trypsin:protein ratio for 16 hours and the reaction can be stopped by adding formic acid to the mixture. The obtained reaction mixtures were analyzed by means of CZE. The aim of this study was to optimize the peptide map of bovine serum albumin (BSA) and to develop the capillary zone electrophoresis (CZE) method.

Examining the parameters of in-solution digestion, we optimized the peptide map of BSA. The protein samples were denaturated, reduced and alkylated using special reagents and digested by trypsin, which cleaves the peptide bonds at the C-terminal side of arginine (Arg) and lysine (Lys). We used CZE for separating peptide fragments and forming peptide maps. For the complete digestion, all the reagents and trypsin should be used. Developing the CZE method, we achieved an appropriate resolution for 12 proteins, such as cytochrome c, lysozyme and hemoglobin. The effects of the pH- and concentration of buffer, temperature and bandwidth on the separation have been studied. For peptide mapping, two types of buffer composition are suitable: 25 mM phosphate buffer (pH=7) and 25 mM phosphate buffer (pH=9). The analytical parameters of the developed method were determined with good reproducibility. Migration times and peak areas were less than 1 RSD% and 7,43 RSD% respectively.

This work was supported by grants from the TAMOP-4.2.2.A-11/1/KONV-2012-0036 project. The authors also acknowledge the financial support provided to this project by the National Research, Development and Innovation Office, Hungary (K111932).

P-17

Expanding the Substrate Range of the MIO-enyzme Toolbox Towards bi-(hetero)aryl alanines

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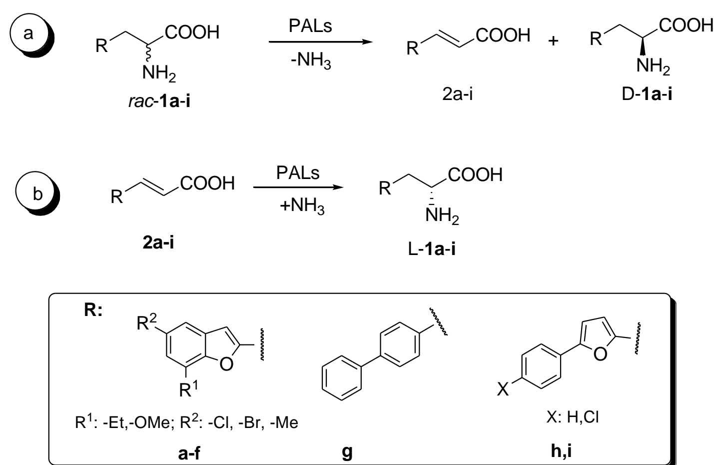
Keywords: biocatalysis, unnatural amino acid synthesis, phenyalanine ammonia lyases, site-directed mutagenesis, rational design

In frame of creating a MIO-enyzme toolbox, consisting of phenylalanine ammonia lyases (PALs) with larger substrate promiscuity, we aimed the broadening of the substrate scope of PAL from *Petroselinum crispum* (*Pc*PAL) towards bulkier heteroaryl or biaryl alanines.

A series of heteroaryl and biaryl racemic alanines *rac*-**1a-i** as well as their corresponding acrylates **2a-i** were tested previously by us in the ammonia elimination and addition reactions catalyzed by *wt-Pc*PAL (Scheme 1) and shown to be accepted as poor substrates or competitive inhibitors for the enzyme. Redesign of the hydrophobic pocket of the enzyme‘s active site, resulted in active mutants, showing highly improved catalytic properties towards the studied non-natural substrates.



Scheme 1. The studied dehydroamination (a) and dehydroamination (b) reactions catalyzed by *Pc*PAL

The research was supported by the PN-II-RU-TE-2014-4-1668 project, granted by the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI.

P-18

The Study of Separation and Determination of Aliphatic Organic Acids in Wine and Partialy Fermented Grape Must by Ion-Exclusion Chromatography

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Keywords: ion-exclusion chromatography, organic acids

Ion-exclusion chromatography (IEC) has been used for the separation of relatively small acids, weak bases and hydrophilic molecular species. Chromatographic separation system consists of three phases: mobile phase, occlude liquid and Donnan membrane. Completely dissociated strong acids are repelled by the positively charged cation-exchange functional groups that make up the so-called Donnan membrane. Undissociated or partially dissociated weak acid pass through the membrane and are thus retained on the column longer [1].

The content of aliphatic organic acids is one of the main factors affecting the quality of wine. They have a major role in the microbiological and physiochemical stability and sensory properties of wines. The most common acids in wine are acetic acid, tartaric acid, oxalic acid, malic acid, citric acid, lactic acid, and malonic acid [2]. The aim of proposed work was to develop a simple method for the determination of organic acids in partially fermented grape must and wine by IEC with spectrophotometric detection at 210 nm. Twelve acids (including succinic, maleic, malic, malonic, oxalic, tartaric, citric, fumaric, acetic, propionic, lactic and itocanic) were separated by IEC using an analytical column Alltech Prevail^{TM} organic acid 5 µm (150 mm x 4.6 mm I.D). The effect of various mobile phases and its pH as well as the column temperatures on the retention of the test acids has been investigated.

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P-19

Determination of Ciprofloxacine in Multicomponent Mixture by HPLC Method

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Keywords: ciprofloxacine, HPLC, multicomponent mixture.

Ciprofloxacin is a fluoroquinolone with potent bactericidal activity and broad spectrum of activity. The bactericidal action is due to inhibition of DNA gyrase susceptible organisms, thereby blocking bacterial protein synthesis. The absolute bioavailability of ciprofloxacin is 70-80%. Several analytical methods for the quantitative determination of pharmaceutical formulations of ciprofloxacin were reported in the scientific literature as capillary electrophoresis, UV spectrophotometry, titration, and high performance liquid chromatography (HPLC). We aimed to determine ciprofloxacin in a complex mixture by HPLC method.

A simple technique for determining the ciprofloxacin in a multicomponent mechanical mixture (ciprofloxacine + loratadine + dexamethasone) was developed. Optimal separation was obtained using Nucleosil 100 C18, 5 μm, 15 x 0.4 cm chromatography column. The mobile phase was a mixture composed of 0,1% phosphoric acid and acetonitrile (65:35) mixed with methanol in 80:20 ratio. The pH was adjusted to 3.0 with triethylamine. Mobile phase flow rate - 1 ml/min, column temperature 40^0C. Spectrophotometric detection was performed at 275 nm. For evaluation of the chromatographic peaks were recorded chromatograms of standard solutions of ciprofloxacin, dexamethasone and loratadine dissolved in the mobile phase, on the same chromatographic conditions.

The chromatographic parameters were optimized, to give very short retention time - 6 minutes: 1,72 min for ciprofloxacin, 3,49 for dexamethasone, and 5,80 for loratadine. The recovery rate of ciprofloxacine was between 98,6 to 104,1 %, and the relative standard deviations was lower than 5 % for repeatability, and lower than 5,21 % for intermediate precision. The limits of detection was 0,041 μg/ml. The RSD value was satisfactory: 0,5 for n=5. The developed method is simple, fast and accurate, and can be used for ciprofloxacin determination in multi-component pharmaceutical formulation.

The research was supported by the institutional project of Scientific Center of drugs, State Medical and Pharmaceutical University "Nicolae Testemitanu".

P-20

Metabolism of Flavanones

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Keywords: flavanones, metabolism, oranges juice

Flavonoids are a wide groups of biological molecules which play a protective role in plants. Plant flavonoids are secondary metabolites that exhibit a lot of physiological effects in living organism, including humans. Flavonoids are a large group of compounds, divided in several subgroups, containing a characteristic skeleton C6-C3-C6, Flavanones (such as naringin and hesperidin) are the most abundant flavonoids in citrus plants.

The aim of this study was to determine the metabolism of two flavonoids glycosides: naringin (naringenin-7-O-rhamnosidoglucoside) and hesperidin (hesperetin-7-O-rutinoside) in human urine samples. The study was carried out in two days cycles during which healthy volunteers were set on polyphenol-free diet. First 24 hours were used in order to wash out the organism. After consumption a cup of orange juice, urine samples were collected for 24 hours and analyzed for flavonoid metabolites content by LC-MS. The major metabolites in urine were identified as glucuronide and diglucuronide The highest metabolite levels appear at approximately 2-9 hours after oranges juice consumption. In addition, small amounts of aglycones and glycoside were found in sample of urine. Area under the metabolite in urine concentration time curve (AUC) for glucuronide metabolites were significantly higher than that for free glycosides and aglycones. This indicates higher bioavailability of conjugated forms that unconjugated. These results show that orange juice flavonoids, hesperidin and naringin are intensively metabolized in human digestive track.

P-21

HPLC Separation of Amino Acids and Dipeptides by Sulfobutylether-β-CD

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Keywords: HPLC, chiral selector, sulfobutylether-β-cyclodextrin, (enantio)separation

Cyclodextrins (CDs) still represents important tool for analysis of structurally different compounds. They are used either as chiral stationary phase (SP) or mobile phase (MP) additives [1-3]. Thus, our main objectives of this work were to prepare SP based on SBE-β-CD by coating procedure on the suitable strong anion exchange surface and further to discover and optimize MP compositions for separation of chiral underivatized and derivatized amino acids, dipeptides and mixtures of dipeptide isomers. However, as CSs could serve also as MP additives, we tested SBE-β-CD also in this manner. C18 column was used for measurements with SBE-β-CD present in MP.

Analytes of interest were chosen with the respect to their nature. Our focus lies particularly in amino acids Tyr, Trp, Phe and their derivatives as well as dipeptides derived from the mentioned amino acids. Dipeptide isomers form unique group of analytes examined in this study. Dipeptides used for mixtures have the same molecular formula but the sequence of amino acids constituting the dipeptide is reversed.

Sulfobutylether-β-cyclodextrin proved to be suitable for separation of chiral and also achiral analytes. The use of sulfobutylether-β-cyclodextrin as dynamic coating agent or as a mobile phase additive depends on the particular chromatographic system and analytes of interest.

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P-22

Speciation Analysis of Chromium in Water Samples by Solid Phase Extraction Using NANO adsorbent ZrO_2 and Electrothermal Atomic Absorption Spectrometry

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Keywords: species, chromium, ZrO_2, SPE, ETAAS

Toxic heavy metal ions are major sources of pollution in environmental and water resources. Speciation study of heavy metal ions is very important since toxicity of some of them depends on their chemical properties, oxidation states, and bioavailabilities. Chromium is one of the most abundant elements having the potential to contaminate groundwater, and so can be a major source of drinking water contamination. In aqueous solution, chromium can usually be found in two different oxidation states including hydrated Cr(III) and Cr(VI) species with different physiological effects. Cr(III) is a trace element essential for maintaining glucose, cholesterol, and fatty acid metabolisms in biological cells. On the other hand, Cr(VI) with a high oxidation potential and relatively small size can easily permeate through biological cell membranes, and is known as a carcinogenic and mutagenic substance for humans. Development of simple, accurate, and sensitive analytical methodologies for separation and determination of trace chromium species in water samples takes an important role in analytical chemistry. In the present work a nanometer-sized zirconium oxide (ZrO_2) was used as a solid-phase extractant for the preconcentration and speciation of trace amounts of Cr(VI) and Cr(III) prior to its determination by electrothermal atomic absorption spectrometry (ETAAS). The main factors having influence on the preconcentration of analytes such as pH of the sample, amount of ZrO_2, extraction time, sample volume, eluent concentration and volume were studied, and the optimal experimental conditions were established. The proposed procedure was successfully applied to the determination and speciation of Cr(VI) and Cr(III) in real water samples and certified reference materials.

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P-23

Microchip Electrophoresis Determination of 3-Nitrotyrosine in Human Fluids

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Keywords: Oxidative stress biomarker, Microchip electrophoresis, Spectrophotometric detection, Body fluids

Reactive nitrogen and oxygen species are implicated in many disease conditions. A product of tyrosine nitration, 3-nitrotyrosine (3-NT), is considered to be a marker of cell damage, excessive inflammation and many other diseases. The increased levels of 3-NT in body fluids are formed when peroxynitrite (ONOO-) reacts with accessible tyrosine residues in proteins. Monitoring of concentration levels of 3-NT in human fluids can be a significant tool for proper diagnosis and noticing early symptoms of many diseases. High performance separation methods combined with sensitive detection techniques are needed when the levels of 3-NT are expected to be low (at nM to μM concentrations in human plasma and urine). Microchip electrophoresis (MCE) has many advantages which include high separation efficiency, low sample consumption, low running cost and a wide range of application. A significant drawback of MCE in trace analysis is a requirement for sensitive detection technique to reach an adequate limit of detection for target analyte.

This work was focused on developing a simple and fast microanalytical method for the determination of trace concentrations of 3-NT in complex samples of human urine and blood plasma. Online combination of isotachophoresis with zone electrophoresis performed on the microchip with VIS detection at 400 nm wavelength was used to improve sensitivity of the method. Under the developed separation conditions a 0.16 μM limit of detection (S/N=3) for 3-NT was achieved. The values of relative standard deviation of qualitative and quantitative parameters for 3-NT in real samples did not exceed 4.1%. The total analysis time was less than 10 min. Concentration levels of 3-NT in the analyzed urine and blood plasma samples were in range 0.7–0.9 and 0.4–1.1 μM, respectively.

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P-24

DNA Methylation of Monoamine Oxidase B: A Diagnostic Biomarker for Late-Onset Alzheimer's Disease?

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Keywords: Bisulfite Pyrosequencing • Diagnostic Biomarker Discovery • DNA Methylation • Late-Onset Alzheimer's Disease (LOAD) • Monoamine Oxidase B (MAOB)

Alzheimer's Disease (AD) is an irreversible neurodegenerative disorder clinically characterised by progressive loss of memory and other cognitive functions. It is the most common form of dementia in Western societies. The prevalence of sporadic AD is dramatically elevated among people older than 65 years (Late-Onset Alzheimer's Disease, LOAD). As dementia is very care-intensive and the world population as a whole is getting older, an enormous burden to health care systems around the globe is imminent: In 2015, Alzheimer's Disease International projected an almost threefold increase of dementia patients by 2050 (then 131.5 million people).

Despite decade-long scientific efforts, definite diagnosis of AD is possible only by post-mortem neuropathological examination to date. For LOAD, a genetic risk factor (*APOE ε4* allele) was identified, but current approaches also suggest a substantial contribution of epigenetic processes to its complex pathogenesis. DNA methylation in the context of CpG dinucleotides is a fundamental component of epigenetic gene regulation and emerged as a potential tool in the discovery of diagnostic biomarkers for LOAD.

Monoamine oxidase B (MAOB) catalyses the breakdown of various amines, among them dopamine. Expression of MAOB was found to be upregulated in platelets of AD patients, and platelets proved to be a good model system for neuronal pathways, but nucleated cells are required to assess the DNA methylation status of a gene. Therefore, whole blood and T lymphocytes were used to investigate whether there is an indicative, DNA methylation-dependent regulation of the *MAOB* gene. Using bisulfite pyrosequencing, DNA methylation analysis of eleven CpG loci in the promoter region of the *MAOB* gene was performed. Comparing a group of ten neuropathologically confirmed AD patients with ten age-matched, healthy control individuals, we report that no significant difference was observed for any CpG.

P-25

Predicting Chromatographic Behavior of Several Chiral Beta-Blockers from Molecular Structure by QSPR Analysis

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Keywords: Molecular descriptors, MOE, β-blockers, chiral HPLC, multivariate data analysis

The chiral HPLC separation of a series of fourteen beta-blockers previously performed on four commercially available chiral columns (Chiralpak IA, Chiralpak IB, Chiralpak IC and Chiralpak ID) was evaluated through computational techniques, using a set of 340 molecular descriptors, calculated with the Molecular Operating Environment (MOE) software. The polymeric selectors in the above mentioned HPLC columns are polysaccharide derivatives (amylose and cellulose) chemically immobilized to the surface of the support material.

Several model-building approaches were employed to establish a quantitative structure-propriety relationship (QSPR) between the set of calculated molecular descriptors and the chromatographic parameters obtained experimentally. Various multivariate data analysis techniques were used - PLS, OPLS, O2PLS, PLS-tree clustering, followed by external validation to generate gradually more refined and robust semi-empirical models which revealed that there were certain classes of descriptors that significantly correlated with the dependent variables (HPLC separation data), several classes that had a particular behavior towards the employed chiral stationary phase (CSP).

From the results obtained we could speculate how some features and proprieties of ligands may affect the different binding with the chiral selector.

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P-26

Separation of Very Long Chain Fatty Acids by GC-MS in Human Serum

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Keywords: very long chain fatty acid; gas-chromatography; mass spectrometry

Fatty acids are most commonly separated by gas-chromatography, after derivatization to their methyl esters (FAME). Long-chain fatty acids have been identified to present higher profiles in patients with peroxisomal disorders (e.g. adrenoleukodystrophy) compared to healthy volunteers. The aim of this study was to develop and optimize an efficient method for the separation of fatty acids in the serum of a patient diagnosed with adrenoleukodystrophy, with a focus on very long chain fatty acids.

For this purpose, we have developed two methods in two stages: derivatization and separation, which were also optimized. One method also contained an extraction step before injection of FAME. The fatty acids were derivatized with methanol in acidic, non-aqueous media (70°C, 3 hours)/ The samples were analyzed using a GC-MS system, through an HP-5ms column (30 m, 0.25 mm, 0.25 μm), in a gradient temperature program. The mass detector was set in SIM mode.

Results showed a good separation in terms of resolution of the fatty acids methyl esters. The temperature gradient was focused on the resolution of very long FAME, which would be quantified. For this purpose, the internal standard method was used, using heptadecanoic acid as an internal standard.

In conclusion, we have developed and optimized the gas-chromatographic separation of fatty acids obtained from the serum of a patient with adrenoleukodystrophy, after their derivatization to their methyl esters.

P-27

Determination of Bacterial Cell Viability Under The Influence of Propiltiodiazolochinazolin-one

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Keywords: cell viability, cytometry, Propiltiodiazolochinazolin-one

In process of evaluation of antibacterial mechanism of action is particularly important to know the impact of the active substance on the efflux pumps of the bacterial cell. We aimed to evaluate the viability of the bacterial cell under the influence of a new antibacterial agent – Propiltiodiazolochinazolin-one.

Determination of the influence of Propiltiodiazolochinazolin-one on the activity of bacterial efflux pumps was performed by flow cytometry techniques: determining cell viability by marking with propidium iodide and assessing the activity of bacterial efflux pumps in the marking with ethidium bromide. The method is based on the measurement of scattered light in relation to incident laser radiation, coupled with the cellular complexity, cytoplasmic granularity and parameters of fluorescence when a linear laminar flow of cell passes the laser beam at a right angle.

Alternative marking of samples (microbial strains grown in the presence of Propiltiodiazolochinazolin-one at a concentration MIC/2) with two fluorochromes, allowed to assess the mechanism by which it influences on microbial strains, namely the permeabilising of cell coatings or the inhibition of efflux pumps microbial. This last mechanism is of particular interest since it can cause the conversion of resistant microbial phenotypes to sensitive phenotypes by targeting the activity of an efflux pump with the help of inhibitor, which should restore the sensitivity of microorganism to antimicrobial substances.

Correlating quantitative data with qualitative aspects of histograms, we can conclude that Propiltiodiazolochinazolin-one has an correlated activity with affinity for Gram coloration of the studied strains: works by permeabilising of cell coatings on Gram-negative bacteria and by inhibition of efflux pumps of Gram-positive bacteria.

The research was conducted in the bilateral project between Faculty of Pharmacy Medical and pharmaceutical University "Nicolae Testemitanu" and Faculty of Pharmacy of Medical and pharmaceutical University "Carol Davila", Bucharest, Romania ".

P-28

Speciation of Chromium in the Air by Ion Chromatography

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Keywords: chromium species, ion chromatography, analysis of air in workplace

The toxicity and bioavailability of chromium depends essentially on the oxidation state of this element. Chromium (Cr) occurs in the environment mainly on two different oxidation states, trivalent chromium Cr(III) and hexavalent chromium Cr(VI). Cr (III) is considered as an essential trace element for the proper functioning of living organisms. It is involved in carbohydrate, lipid and protein metabolism. In contrast, Cr(VI) is dangerous for humans due to its toxicity and carcinogenic properties. Therefore determination of total chromium is insufficient for a complete toxicological evaluation. Separation and determination of Cr(III) and Cr(VI) in environmental samples still represents a challenge to the analytical chemistry.

The Ion Chromatography method has been proposed for determination of hexavalent and trivalent chromium in the air at the workplace. Chromium compound are detected by UV detection. The procedure was based on reaction of Cr(III) with pyridine-2,6-dicarboxylic acid (PDCA) forming a violet, stable complexes. The influences of various parameters on the complexation were evaluated: mole PDCA/Cr ratio (4,17), complexation reaction time (30 min). Also tested dependence the pH of the form of chromium (optimal pH is 5,9). The next step was the separation of the resultant complex Cr(III)-PDCA from Cr (VI) ion on exchange column (CS5A, 250 mm). After separation, in the post column reaction the Cr (VI) reacts with 1,5-diphenylcarbohydrazide (DPC) to form a colored complex. Our results showed that the optimal concentration and flow rate post column reagent (DPC) is respectively: 2 mM and 0,5 mL/min. The air samples collected onto glass filter (Ø 25 mm) of air flow rate 2 mL/min. Extraction of chromium from the filter was used 50 mM nitric acid and subjected to agitation in ultrasonic bath for 30 min at ambient temperature. The recovery of chromium from samples collected at the weld was also investigated (Cr(III) 73% ; Cr(VI) 89%). The determination method of the IC / UV-VIS is: Cr(III) 0,07 $mg/m^3$and Cr(VI) 0,007 mg/m^3.

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Chemical Characterization of Stationary Phases for Fast Liquid Chromatography

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Keywords: Tanaka-test; base-deactivated; end-capping; advanced stationary phase

Introduction

Many kinds of C_{18} stationary phases are available on the market. For chromatographers, the understanding of the stationary phase properties is required, so they can choose the appropriate column for certain separations, to reproduce separations or find another column with dissimilar selectivity.

The Tanaka test is a simple characterization measurement for C_{18} phases. By means of a few simple chromatographic test injections we will get information about surface area, surface coverage, amount and functionality of silanols.

Materials and methods

Chromatographic analyses were performed on a Waters Acquity I Class UPLC equipment. Six 2,1 × 50 mm C_{18} columns were used for the experiments. 0,02 M phosphate buffer (pH 7) were used as eluent A and methanol was used as eluent B for measurements. The flow rate was set to 0.3 mL/min, and the column was thermostated at 40 °C.

Results

	k'_{AB}	$\alpha(CH_2)$	$\alpha_{t/o}$	$\alpha_{C/P}$
InertSustain Swift C_{18}	1,545	1,406	1,209	0,463
InertSustain C_{18}	3,31	1,455	1,167	0,427
InertCore C_{18}	1,56	1,496	1,133	0,491
Monotower C_{18}	2,036	1,482	1,858	0,444
Kinetex C_{18}	1,543	1,626	1,393	0,431
Kinetex EVO C_{18}	1,305	1,44	1,118	0,42

$$k'_{AB} = k'(\text{amylbenzene})$$

$$\alpha(CH_2) = \frac{k'(\text{amylbenzene})}{k'(\text{butylbenzene})}$$

$$\alpha_{t/o} = \frac{k'(\text{triphenylene})}{k'(\text{o} - \text{terphenyl})}$$

$$\alpha_{C/P} = \frac{k'(\text{caffeine})}{k'(\text{phenol})}$$

Based on the k'_{AB} values, the InertSustain column has the largest surface area, and then follows Monotower. Since similar values of $\alpha(CH_2)$ were obtained, similar surface coverage can be assumed for the various columns. The Monotower offered the best steric selectivity. The columns show similar hydrogen bonding capacity according to $\alpha_{C/P}$ values.

Conclusion

According to the above-defined results, these six columns have similar properties, even though these stationary phases are fundamentally different - such as monolithic, core-shell or fully porous packings. The higher $\alpha_{C/P}$ value suggest more residual silanols, in our case $\alpha_{C/P}$ values are quite similar, but these columns are base-deactivated, and end-capped, that is why we have to confirm with other tests more accurately the amount of silanols.

Reference

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P-30

Characterization of Heavy Metals Contents in Various Plant Foods from Polluted Sites

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Keywords: heavy metals, pollution, food, contamination risk, Macedonia

Food safety and quality are a major public concern worldwide, regarding the risk associated with consumption of food stuffs contaminated with heavy metals as toxins. Bioavailability of metals occurring in soil is the basic source of its accumulation in plant food. The impact of soil pollution (due to urban and mining areas) on the food chain presents a challenge for many investigations. Bioavailability of metals in a potentially polluted soil and their possible transfer and bioaccumulation in several vegetable species and herbs was examined. Microwave digestion was applied for total digestion of the plant tissues, while on the soil samples open wet digestion with a mixture of acids was applied. Three extraction methods were implemented for determination of bioavailable metals in the soil. Atomic emission spectrometry with inductively coupled plasma was used for determination of the total contents of 21 elements. Significant enrichments in agricultural soil for As, Pb and Zn (in urban area), Cd, Cu and Ni (in a copper mine area), compared with the respective values from European standards were detected. On the basis of three different extraction methods, higher availability was assumed for both lithogenic and anthropogenic elements. Translocation factors higher than 1 were obtained for As, Cd, Cu, Ni, Pb and Zn. Higher root to shoot translocation of these metals indicated that plants species have vital characteristics to be used for phytoextraction of these metals. The obtained data also suggested that *S. oleracea* and *R. acetosa* were singled out to have a phytostabilization potential for Cd, Cu, Ni and Pb, while U. dioica only for Cu. *Rumex acetosa* has a potential for phytoextraction of Cd in urban and copper polluted areas.

P-31

Novel AVPI-RGD bi-functional Hybrids and Study of their Potential Cytotoxic Effect

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Keywords: AVPI, RGD, apoptosis

Targeting critical apoptosis regulators is a promising strategy for the development of new classes of anticancer drugs. Another approach in pharmacy is design of hybrid molecules comprised of functionally different subunits so affecting different pathways simultaneously.

We focused our work on synthesis and study the effect of bi-functional AVPI-RGD peptides as well as their combined effect with TRAIL (trigger of the death-receptor apoptotic pathway) and cisplatin (trigger of intrinsic apoptotic pathway) drugs over different cell lines. AVPI tetrapeptide is the essential functional region of pro-apoptotic Smac molecule (second mitochondria-derived activator of caspases) that inhibits several IAPs proteins (inhibitors of apoptosis). IAPs are over-expressed in cancer cells so their inhibition may lead to activation of both apoptotic pathways via reactivation of caspases. RGD is a peptide sequence known to interact with specific over-expressed proteins on the membrane of cancer cells thus it's interesting for its targeting potential.

All AVPI-RGD hybrids were synthesized by Fmoc solid-phase method. Modifications were made in proline residue of AVPI and arginine residue of RGD in order cytotoxicity and selectivity of our compounds to be increased. We studied the potential cytotoxic effect of the compounds by calorimetric MTT analysis. We were also interested if these compounds sensitize pretreated cancer cells to TRAIL and cisplatin, as it is supposed by literature data. We used several cell lines (MCF-7, MDA-MB-231, HepG2) in order to check for cell line-selectivity.