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L1
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L2

Some Theoretical and Practical Aspects of Microchip Electrophoresis in Bioanalysis

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Some theoretical and practical aspects of microchip electrophoresis (MCE), as a dominant method in lab-on-a-chip analytical systems, are to be discussed. MCE has shown a rapid development over the past few years. This technology, compared to the conventional capillary electrophoresis (CE) systems, has significant advantages in terms of high speed, high throughput, easy integration, low sample consumption and automation. However, a routine analytical use of MCE is still limited because of some serious problems resulted from downscaling.

The analytical possibilities of MCE with on-line conductivity detection in the analysis of complex biological samples have to be shown in this respect. Various types of sample pre-treatment techniques integrated prior to the MCE separations are to be discussed. On-line combinations of electrophoresis methods can be considered as very promising tools in integrating sample pre-treatment with the electrophoretic separations on the microchips. Therefore, we pay a special attention to this topic with a focus on the combinations that integrate (electrophoretic) sample pre-treatment with the electrophoretic separations on the microchip.

In this context, the use of different electrophoresis methods combined on-line on the microchip with coupled channels (CC), e.g. ITP-CZE and CZE-CZE in the analyses of complex biological samples are to be demonstrated. Application possibilities of MCE separation systems with integrated conductivity detection are to be shown on a set of analytical methods suitable for rapid monitoring and analysis of complex biological samples, for example ITP-CZE determination of nitrate and nitrite as biomarkers of various neurological diseases in the cerebrospinal fluid, CZE-CZE determination of metabolic organic acids in body fluids and sequential CZE separations of cations and anions in various body fluids.

Acknowledgements

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L3

System Peak Behavior in Complexing Background Electrolytes

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Capillary electrophoresis offers many useful modifications that employ complexing agents in separation systems and that are widely used for chiral and achiral separations or determination of complexation constants. Such systems are frequently studied theoretically. However, the attention of authors is focused almost exclusively on complexation equilibria of complexing agents with analytes, while the complexation with other constituents of separation systems, *e.g.* buffer constituents, is totally omitted. Only a few papers noticed the interaction of the complex-forming agent with constituents of the background buffer. Gelb *et al.* [1] observed a change in pH of the buffer as a result of addition of a neutral complex-forming agent. Also, Rawjee *et al.* [2] utilized complexation of buffer constituent with complex-forming agents to affect the electrophoretic mobility of the co-ion in order to minimize electromigration dispersion of the analyte. Chen *et al.* [3] and Fang *et al.* [4] reported the origin of additional system peaks after the addition of native cyclodextrins into the electrophoretic system. Unexpected changes of buffer properties (pH, ionic strength, electrophoretic mobility) as well as occurrence of additional peaks in given separation system can significantly impair the quality of separations, may affect the complexation parameters and lead to confusing results in general. Therefore, we utilized our new simulation program Simul 5 Complex, where complexation equilibria are implemented, to investigate changes in the properties of complexing BGE, especially the origin of the additional system peaks as it might have fatal impact on electrophoretic results and their interpretation.

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L4

Why the Enantioseparation is a "Must"?

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L5

Chiral HPLC Separations of Unusual Amino Acids in Reversed Phase and Polar-Organic Modes

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The use of unusual amino acids, i.e. amino acids not encoded by DNA, belongs to the fundamental parts of many scientific fields. The structural and functional diversities of unusual amino acids make them widely used for instance in the research of protein conformations and activities or new drug development.

Unusual amino acids as many others biological substances are chiral. Since the single enantiomeric form can cause different and often serious response of organisms, the separations of unusual amino acids enantiomers are often the first step of their employment.
High performance liquid chromatography using chiral stationary phases dominates over the other separation techniques enabling chiral separations. The aim of our work was to find optimal conditions for chiral separations of unusual amino acids, namely newly synthesized derivatives of phenylalanine and tyrosine. Chiral separations were carried out using two separation modes – reversed phase and polar-organic modes. Overall three chiral stationary phases were investigated. Two of them were based on a macrocyclic antibiotic teicoplanin, Chirobiotic T and Chirobiotic T2 columns. These columns differed in porosity of silica gel carrier used, amount of the chiral selector linked to silica and also in linkage chemistry. Another chiral stationary phase based on amylose tris (3-chlorophenyl carbamate) immobilized to silica gel Chiralpak ID column was also tested.

The retention behavior and enantioselectivity in both modes were compared and discussed with respect to mobile phase compositions and type of chiral stationary phase. It was demonstrated that the derivatization of amino group has significant impact on chromatographic behavior in all systems. Chirobiotic T and Chirobiotic T2 columns performed complementary enantioseparation potential in reversed phase and polar-organic modes, while Chiralpak ID column allowed only limited separation potential in reversed phase mode. All enantiomers of unusual amino acids were successfully separated.

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L6

Application Possibilities of Capillary Electrophoresis on a Chip in Separations of Selected Amino Acids and Their Enantiomers in Body Fluids

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In clinical analysis, amino acids, e.g. aspartic (Asp) and glutamic (Glu) acids, are considered to be the biomarkers of some neurological diseases, e.g. Alzheimer's and Parkinson's diseases and kidney dysfunction. Amino acids are chiral compounds (with the exception of glycine) and therefore, it is a need to monitor their enantiomeric ratio in various body fluids. While L-enantiomers are present in proteosyntesis, e.g. unbound D-Asp is present at the highest concentration levels during prenatal period. Furthermore L-Glu is neurotransmitter of brain functions and together with D-Asp induces acidification of nervous and supportive nervous tissues.

This contribution deals with the application of an electrophoretic chip with coupled channels to the analysis of selected amino acids in body fluids. The conductivity detection integrated on the chip was used due to the fact that studied amino acids have no chromophore groups. The optimization of achiral capillary zone electrophoresis (CZE) conditions for the separation of Asp and Glu and the application of the developed method to the analysis of real biosamples were performed. Chiral separations were optimized with addition of different chiral selectors to achiral separation system to achieve the resolution of enantiomers.

CZE separations were performed in a hydrodynamically closed system with suppressed electroosmotic flow in the carrier electrolyte with pH 6.8 containing 10% methanol as organic modifier. Repeatabilities for migration times of studied amino acids were up to 1% RSD and repeatabilities of their peak areas were less than 5% RSD. Optimized separation conditions enabled us to achieve detection limits at 200 nmol/l concentration levels in a 900 nl injected sample volume. CZE separations of Asp and Glu in CSF and urine samples were performed after electrophoretic removal of ionogenic macroconstituents (chloride, sulphate and phosphate) using the chip in a column-switching mode (CZE-CZE). Baseline resolution of D,L-Glu enantiomers and partial resolution of D,L-Asp enantiomers was achieved with vankomycin as chiral selector.

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L7

Properties of Dual-Cyclodextrin Separation Systems: Experimental Verification of Proposed Model

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In our group, we formerly proposed a model describing effective mobility of an analyte interacting with a mixture of selectors. We have shown that a selector mixture can be regarded as a single selector whose interaction with the analyte is characterized with overall complexation parameters – overall complexation constant and overall mobility of complex (mobility of analyte at infinite concentration of the selector mixture).

The overall complexation parameters can be measured in the same way as parameters of complexation with a single selector. Additionally, when the mixture composition is known, our model enables calculation of the overall complexation parameters based on complexation parameters of the single selectors of which the mixture is composed.

In this work, we choose a dual selector system as the simplest selector mixture to verify the model experimentally. We measured complexation parameters of two analytes (ibuprofen and flurbiprofen) with three cyclodextrins (β-cyclodextrin, dimethyl-β-cyclodextrin, maltosyl-β-cyclodextrin). Although flurbiprofen and ibuprofen are chiral compounds, chiral separation was not in our scope of interest and we performed non-chiral separation of the two individual analytes at the pH used. Then defined mixtures of the cyclodextrins were prepared and overall parameters of complexation of analytes with these mixtures were measured by affinity capillary electrophoresis and also calculated based on our model. Difference between calculated and measured parameters did not exceed 10 % in case of overall complexation constants and 3 % in case of overall mobilities of complexes. Calculated complexation parameters also appeared to be suitable for prediction of dependence of dual system selectivity on total selector mixture concentration. Therefore, the model can be a powerful tool for identifying benefits that might be gained by mixing two particular selectors.

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L8

Identification of genetically modified foods using protein profiling with CE chips

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The important development trend in analytical chemistry in recent decades is miniaturization of instrumentation. Microfabricated devices, which are being developed for analysis of proteins, include different classes of measuring systems. Generally, all of them allow improving performance, increasing speed of determination, reducing costs and reagent consum-ption, hence there is tremendous market potential for such devices. The especially intensively developed group of microdevices for application in proteomics, but also other fields of bioanalysis, are microfluidic devices. These systems are developed mostly for electromigration measurements. A wide demand for miniaturized CE devices is confirmed by increasing number of such instruments available commercially. Various applications have been already published in the literature for the Agilent 2100 Bioanalyzer, but alternative electrophoresis instruments are also available.

Because genetic modifications of plants can cause changes in proteins expression, the protein profiling can be a potentially useful tool for identification of genetically modified organisms (GMO). The most useful methods for protein separation and determination are electro-migration techniques.

The possibility of the application of the chip gel electrophoresis with laser-induced fluorescence detection (Agilent 2100 Bioanalyzer) for protein profiling was examined. The protein profiling was carried out for fractionated and total extracts of maize standards with different content of genetically modified (GMO) plant material. The sensitivity of such determi-nations has been enhanced by lyophilization of extracts or by employing filtering and precon-centration with cut-off filters. For protein extracts, especially effective pretreatment step in the determination of low abundance proteins was application of the ProteoMiner technology, which utilizes a combinatorial library of hexapeptides bound to beads to decrease high-abundance proteins and enrich low-abundance proteins in complex samples. Several reproducible differences were observed for protein profiles between maize standards non-containing the genetically modified organisms (GMO) and those containing GMO, what can be potentially employed for identification of GMO in maize samples and foods of maize origin.

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L9

Comparison of Real-time PCR Assays with Commercial ELISA Kits for the Detection of Potentially Allergenic Mustard

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In persons sensitised to mustard, the ingestion of allergenic food can elicit typical allergic reactions affecting the skin, the gastrointestinal tract or the respiratory system, and also severe life-threatening reactions, like anaphylaxis. Mustard is frequently consumed with various foods or as ingredient in meat-products, sauces, marinades, spices and convenience products. Three mustard species, white mustard (*Sinapis alba*), black mustard (*Brassica nigra*) and brown or oriental mustard (*Brassica juncea*), all belonging to the *Brassicaceae* family, are commonly used in food [1]. Since no therapy for mustard allergy is available, and avoidance of the offending food is essential for allergic patients, mustard has to be labelled on packaged goods according to the EU legislative 2007/68/EC [2]. In order to control correct food labelling, sensitive and selective analytical methods, suitable for high trough put analysis, are necessary. So far, enzyme linked immunosorbent assays (ELISAs) as well as real-time polymerase chain reaction (PCR) methods play the most important role in routine food allergen analysis. However, highly processed food may have an influence on the detectability and may limit the applicability of both methods.

The lecture will present data obtained by the analysis of model sausages with two in house developed singleplex real-time PCR assays, for white mustard [3] and black and/or brown mustard [4], and two commercial mustard sandwich ELISAs, one determining all three mustard species and one specific for white mustard [5]. Two sets of model sausages, containing either white and black mustard or white and brown mustard, were applied.
Validation of the methods was carried out with raw model sausages, and in order to observe any influence on the detectability, also with model sausages brewed at 75°C-78°C for 15 min. In addition, this study includes data obtained by analysing several commercial food products declared with „mustard“ or „may contain mustard“ as well as products that, according to the ingredient list, should not contain mustard.

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L10

Exploring the Interactions of Novel RGD Analogues with Integrin by Docking Studies

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Integrins are family of transmembrane adhesion proteins that mediate cell adhesion and intracellular signaling. Integrin-αvβ3 is expressed on the surface of tumor cells, and can be further induced by chemical stress. The Arg-Gly-Asp (RGD) motif-containing peptides are specifically bound to integrin-αvβ3, and to inhibit neovasculature underlying competition to normal extracellular matrix proteins. Numerous soluble synthetic RGD peptides (linear, circular, and/or chemically modified) have been shown to be capable of mediating cell adhesion in solution and of preventing it in a soluble form and are thus of utility in a wide range of clinical settings, including angiogenesis, inflammation, and metastatic processes of cancer cells.

This study employed docking procedure to explore interactions of novel RGD analogues (RGD-OMe, R(NO_2)GD, CavGD, R(NO_2)GD-OMe, orotic acid-RGD, and orotic acid-CavGD) with integrin- αvβ3. The ability of newly synthesized analogues of RGD to bind integrin-αvβ3 was compared with those of RGD and analogues known from literature. Using computational tools we tried to identify important relationships between structure and biological activity. Correlation between data obtained from *in vitro* assays and docking results could be used in developing a reliable method for designing new analogues with desired biological action.

L11

Methylation Status of Interleukin-1 Receptor type 2 as a Promising Biomarker for Juvenile Idiopathic Arthritis?

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Juvenile idiopathic arthritis (JIA) is the most common form of arthritis in children and adolescents. JIA is a chronic inflammatory autoimmune disease with known genetic predisposition. The aim of the present study was to investigate if DNA methylation, an epigenetic mechanism regulating gene expression, also plays a role in the development of JIA. The study focused on determining the methylation status in the promoter region of genes that are activated during inflammatory processes in the body.

DNA was extracted from dried blood spots of patients diagnosed with JIA and a healthy age-matched control group. By treatment with sodium bisulfite, unmethylated cytosine residues were converted into uracil, whereas methylated cytosine residues remained unchanged. The DNA region of interest was then amplified by the polymerase chain reaction (PCR). After the PCR process high resolution melting (HRM) analysis was carried out to characterize the amplicon according to its dissociation behavior. HRM analysis is performed by continuously increasing the temperature up to the melting temperature of the amplicons, resulting in separation of the double-stranded DNA into the two single strands. In order to ensure that the correct DNA region has been amplified, the length of the PCR product was determined by agarose gel electrophoresis. Information on the methylation status was obtained by sequencing.

Differences between patients diagnosed with JIA and the healthy control group were found in the melting temperature of amplicons obtained from Interleukin-1 Receptor type 2 (IL-1R2), indicating differences in the methylation pattern of its promoter region. These differences were confirmed by sequencing and comparing the methylation status of the individual CpG dinucleotides. The CpG dinucleotides showed partial methylation in all healthy persons, whereas they were completely demethylated in 80% of the patients with JIA. Based on these results, IL-1R2 seems to be a promising biomarker for juvenile idiopathic arthritis.

L12

The Rapid and Orthogonal Assessment of Chiral Recognition in Case of Molecularly Imprinted Polymers Developed for Different B-Blockers by Electromigration Separation and Electrochemical Techniques

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Two different, electrophoretical and electrochemical, approaches in the synthesis and performance evaluation of molecularly imprinted polymers (MIP) for the chiral discrimination and separation of β-blockers were undertaken. The electrochemical approach was expected to offer a much faster method optimization due to the ease of preparation and replacement of the enantioselective film and its high throughput. Due to the high numbers of variables defining the analytical properties of the obtained MIPs, a careful design of experiments was used in the optimization of the polymerization mixtures' composition.

Electrochemical methods have proved to be highly sensitive for the analysis of organic molecules including drugs in pharmaceutical formulations and human body fluids doubled by the simplicity, low cost and relatively short analysis time as compared to the other routinely applied chiral separation techniques. The molecularly imprinted polymeric films were deposited on the surface of a carbon paste electrode by the electropolymerization of methacrylic acid or vinyl-pyridine functional monomers by using cyclic voltammetry (CV) in non-aqueous electrolyte. The efficiency of the chiral recognition of the obtained polymers was assessed by differential pulse voltammetry (DPV). Furthermore, the resulting polymeric films were investigated by electrochemical impedance spectroscopy following the most favorable electrochemical behavior (charge capacity, electron transfer rate, resistance).

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 and in the case of the electrochemical methods on the ratio of the oxidation currents of template and the other enantiomer.

Our results demonstrate that high-throughput synthesis and screening of polymers is a powerful approach for the identification of the best prepolymerization mixture for the chiral analysis of drugs.

L13

Multiple Food Allergen Analysis

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According to EU legislation, 14 potentially allergenic foods, including milk, egg, peanut, tree nuts, sesame, mustard and celery, and products thereof have to be given in the ingredient list of commercial foodstuffs. Since food allergens are commonly present in low concentrations in very complex food matrices, analytical methods must be very sensitive and specific to be applicable to food allergen analysis. Currently, immunoanalytical methods and real-time polymerase chain reaction (PCR) assays are most frequently applied.
Since individuals are frequently allergic to more than one allergenic food and foodstuffs often contain several allergenic ingredients, efforts have been made to develop methods that allow the simultaneous detection of a variety of allergens in one and the same tube or well. These methods offer the big advantage of saving time and costs.
The lecture will give an overview of methods allowing multiple food allergen analysis, including multiplex real-time PCR and mass spectrometry. Results from our research group as well as recent developments found in literature will be presented and critically discussed.

L14

Simple Methods for Determination of Drugs in Binary Systems

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Many pharmaceutical formulations available on the market contain two or more active compounds, in order to strengthen each other's activity, or to obtain double or multiple effects. Some examples of such combinations are: acetyl-salicylic acid + ascorbic acid, acetaminophen + caffeine, ascorbic acid + iron, simvastatin + fenofibrate, acetaminophen + metamizole, prednisolone + azathioprine, metamizole + morphine and many others. Nevertheless, the separation techniques are often too expensive and time consuming methods for routine analysis. Our team's aim was to develop some very simple, efficient, rapid and low cost methods to determine both active compounds, without separation. There are several ways to determine in parallel two analytes from a mixture, the optical methods being the fastest and most efficient.

Our team developed different spectrophotometric methods for simultaneous determination of prednisolone and mesalazine, prednisolone and chloramphenicol, prednisolone and tetracycline, simvastatine and ezetimibe, ascorbic acid and iron, and other mixtures, using the simultaneous equation, izoabsorbtive point, selective complexation or further methods. The methods were tested from analytical point of view, and applied on several drugs available on the market. The developed methods proved to be fast, efficient, low-cost and suitable for routine drug analysis, and also the tested pharmaceutical formulations were suitable to the requirements of the Romanian pharmacopoeia.

Key-words: binary systems, spectrophotometry, drug analysis

L15

ESI MS/MS Investigations of Aroylhydrazones Derived From Nicotinic Acid Hydrazide

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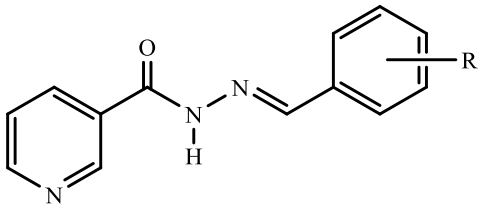
The chemistry of aroylhydrazones has been intensively studied due to their analytical and biological applications. Many compounds of this type possess antimicrobial, antituberculosis and antitumor activities. Recent studies have shown that nicotinic acid hydrazones could be considered as anti-inflammatory and analgesic agents, and as a novel pharmacophore in the design of anticonvulsant drugs.

The aroylhydrazones derived from nicotinic acid hydrazide (Scheme 1) were prepared according to the published procedure [1].



R = H; 2-OH; 2-OH, 3-OH; 2-OH, 4-OH; 2-OH, 5-OH; 2-OH, 3-OCH_3; 2-OH, 4-OCH_3; 2-OH, 5-NO_2

Scheme 1. Aroylhydrazones derived from nicotinic acid hydrazide

In this work the comprehensive mass spectrometric analysis of the title compounds is presented. The ESI MS spectra were acquired both in positive and negative ion mode. The influence of the solvent (methanol, acetonitrile, addition of sodium acetate) on the signal intensities was investigated. MS/MS analyses of protonated molecule $[M+H]^+$ and of fragments ions, as well as of sodiated molecule $[M+Na]^+$ were performed, and fragmentation pathways were proposed. In the MS/MS spectra of all singly protonated aroylhydrazones the signals at *m/z* 148, 123, 121, 106, 105, 80 and 78 were observed and assigned to the fragments arise from the part of molecules originated from nicotinic acid hydrazide. Fragmentation reactions of $[M+H]^+$ ions involved heterolytic or inductive cleavage and a proton rearrangement. In MS/MS spectra of $[M+Na]^+$ ions the signal at *m/z* 23, assigned to the Na^+, was the most intensive one.

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L16

Development and validation of a real-time PCR method for the identification and quantification of *ROE DEER (CAPREOLUS CAPREOLUS)* to detect food adulteration

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According to legal regulations, food manufacturers have to ensure not only the safety but also the authenticity of food products. However, to increase their profit, manufacturers may be tempted to incorrectly label their products and to use lower priced ingredients of inferior quality instead of more expensive ones. In the meat industry, game meat is particularly susceptible for fraudulent labelling since game meat has always been especially appreciated due to its distinctive flavour and its low fat and cholesterol content.

According to the Codex Alimentarius Austriacus (collection of product descriptions determining standards for food identity), in sausages declared as "game sausages" at least 38% of the total meat content has to be game meat. Analytical methods have to be specific and sensitive in order to be applicable for the detection of food adulteration. The real-time polymerase chain reaction (PCR) is a DNA based method that does not only allow the identification but also the quantification of species, e.g. meat species in foods. Quantification of meat species is, however, known to be a difficult task. The quantification strategy is usually based on a reference gene (myostatin gene) that is found in all animal species. This kind of strategy is called relative quantification.

In the present study, a real-time PCR method for the identification and quantification of roe deer *(Capreolus capreolus)* in game meat products was developed and validated. The primer pair and the probe were designed for a sequence of the promoter of the lactoferrin gene. The PCR method was found to be specific for roe deer and does not show any cross-reactivities with other game species (e.g.: red deer, sika deer, fallow deer, wild boar and reindeer), other animal species (e.g.: pork, cattle, chicken and horse) and spices (e.g.: mustard, pepper, soy, onion and garlic) that are often found in processed foods. The analysis of meat mixtures from roe deer and pork showed that the real-time PCR method is applicable to quantify the game meat content in foodstuffs.

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L17

Some Reflections on Lipidomics

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Since lipids may act as endogenous modulators in human diseases involving the disruption of essential metabolic enzymes and pathways, also lipidomics became of considerable interest within the emerging field of –omics sciences. Lipids provide for a number of vital functions in living organisms and must be considered as fundamental molecular constituents therefore. More than 180.000 different molecular species of lipids are known so far and need to be qualitatively and, if any possible, also quantitatively characterized for a better understanding of their metabolic attributes. Lipid droplets e.g. have been recognized as important sub cellular information and energy storage aggregates. They consist of a neutral lipid core of mainly triacylglycerides, covered by a polar lipid monolayer with associated surface molecules. The dominating amount of acylglycerides, however, tends to suppress all the other minor lipid components in a competitive ionization process like ESI and complicates their quantification. Thus, a method yielding unbiased results needs to be concepted.

Chromatographic separation techniques combined with highly mass accurate mass spectrometry are applied to obtain proper information. Differently selective chromatographic separation methods such as ultra high-performance liquid chromatographic technique with reversed-phase and/or HILIC modes are used in order to obtain adequate chromatographic resolution. An online-coupled hybrid linear ion trap-Fourier transform ion cyclotron resonance mass spectrometer with an electrospray ionization source allows high mass accuracy measurement. For positive identification purposes a two-dimensional combined LTQ-FT method is applied. Handling the vast amounts of data generated by high resolution mass spectrometry requires an implementation and development of dedicated software. A true high performance operation can be established by integrating Data Dependent Acquisition MS/MS Analysis and a custom designed Lipid Data Analyzer routine.

L18

Assessment of the Genotoxic Potential of Novel RGD-mimetics Using Comet Assay

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Use of both natural and artificial modifications of bioactive peptides provides opportunities to better understand the basis for bioactivities of the parent structures and to find novel functionality that may be applied for new purposes. Application of unnatural amino acids and peptidomimetics constitutes one of the most powerful methodologies in such chemical approaches to understanding ligand – protein interactions.

Herein we describe the solid phase peptide synthesis and the biological evaluation of a series of short RGD-mimetics containing the sequence **Xaa-GD**, where **Xaa** is Arg-mimetics. Their antiproliferative and cytotoxic potential was examined also. For the determination of cytotoxic effect MTT assay was used.

In an attempt to look deeper in the details of the observed cytotoxicity we investigated the genotoxic effect of synthesized by us RGD-mimetics, and their effect on the cell cycle in HepG2 cells as well. A technique of single cell gel electrophoresis (comet assay) was used for the detection of DNA damage. The method is a fast, sensitive and amenable tool for detection of genotoxicity at a single-cell level.

L19
Lipopolysaccharidomics

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L20

Anitioxidant properties of food samples

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Several studies indicate that the active dietary constituents of fruits, vegetables and beverages prevent free radical-induced diseases and protect against foodstuff oxidative deterioration. Antioxidant capacity is widely used as a parameter to characterize different plant materials (fruits, teas, vegetables, wines, juices, oils).

There are several methods for the evaluation of the efficiency of antioxidants. It should be emphasized that the employed assays are strictly based on chemical reactions *in vitro* and they bear no similarity to biological systems. 2,2-dipheny-1-picrylhydrazyl radical (DPPH) assay is one of the most popular and frequently employed spectrophotometric method to verify the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of food samples. From the analytical point of view it is very difficult to compare the results obtained in different laboratories due to so many methodologies used. Various research groups have used different concentration of DPPH, incubation time, pH value and the way of presenting results.

This work addresses the main analytical problems that arise when determining the antioxidant capacity of food samples by DPPH assay as well as the problems for comparison the results.

L21

Analysis of Illicit Drugs using chromatographic and electrophoretic techniques

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There is still a need to develop new analysis methods for illicit drugs. Besides well known examples, such as marijuana, hashish, amphetamine, methamphetamine, cocaine and heroin, Police and Drug Authorities have to deal with new compound classes in recent years. These new Legal Highs mainly derive from compound classes such as cathinones, amphetamines and tryptamines, but also synthetic cannabinomimetics with various chemical structures entered the black market worldwide.

The goal of this survey is to give examples about common drug analysis for Police and Prosecutors. After checking identity of a sample material, often quantitative analysis has to follow to clarify the extent of penalty. On the other hand, new Legal Highs represent a big challenge in terms of analysis. During the last three years we built up a GC-MS library of these new compounds to be able to identify them. For this purpose, more than 100 samples were purchased from the internet followed by structure elucidation. To date, only few certified substances are commercially available from serious suppliers.

Another aspect is the fact that a huge number of cathinones and new amphetamine derivatives are chiral. It is yet unclear, whether the effect of the two enantiomers differ, since these compounds were synthesized for the drug market and they were never clinically tested. Therefore, methods for enantioseparation of new Legal Highs were developed in our group. As instrumentation, GC, HPLC as well as CE were used. For direct chiral separation, cyclodextrines, crown ethers and polysaccharides served as chiral selectors.

This talk will be dedicated to Gerald Gübitz on the occasion of his 70th birthday.

L22

Complexation with Buffer Constituents: Impact on Determination of Stability Constants

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Stability constants characterize binding interaction between an analyte and complexation agent. These interactions play very important role in separation processes. Affinity capillary electrophoresis (ACE) [1] belongs to very often used methods for determination of stability constants. It was shown that there are many factors which can influence the determination of stability constant – e.g. viscosity of background electrolyte (BGE), Joule heating, ionic strength of BGE [2]. One of the most widely used complexation agents are cyclodextrins (CD).

We show that some buffer constituents can interact with complexation agent and change buffer properties – e.g. ionic strength, pH. We selected several interacting and non-interacting buffers to demonstrate behavior of such systems. We observed that the determined value of stability constant in interacting systems depends not only on type of analyte and complexation agent but also on type and concentration of buffer constituents. Our assumption of complexation of buffer constituents with complexation agent was demonstrated by measurement of neutral complexation agent mobility in interacting buffer (for non-interacting buffers should be zero). These measurements are in perfect agreement with determined stability constants.

Much worse situation is coming up by determination of stability constants of weak analytes, when both comlexation of neutral and charged form of analyte is considered. For such measurements it is necessary to keep constant separation conditions – especially pH of BGE. However, pH of interacting buffer significantly changes with concentration of complexation agent and it is not possible to meaningfully evaluate the experimental data. We show two model systems (interacting and non-interacting) for demonstration of this impact. In the next step, we determined all complexation characteristics describing these particularly systems and used them as input data for simulation by Simul 5 Complex [3, 4].

For these reasons the interaction of buffer constituents with complexation agent should be always considered, as it might have fatal impact on determination of complexation constants and electrophoretical separation itself.

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L23

Calixarene based stationary phases for warfarin enantiomer separation

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Calixarenes represent a new versatile class of compounds which offer good perspectives in host-guest type complex research. They act by forming a cavity inside their macrocycle and form non-covalent interactions with the guest molecules, from which hydrogen bonds are the most important.

Warfarin is the most used anticoagulant worldwide, being used in prevention and treatment of venous thrombosis, stroke, post-myocardial infarction and heart failure. However, it has a low therapeutic index, many drug-drug interactions and many side effects. The enantiomers of warfarin show different metabolic pathways, P450 cytochrome isoenzimes being involved in most of them; furthermore, evidence of their different anticoagulant activity has been reported, the (S)-(-)-warfarin being more active than the (R)-antipode.

Because of the high UV absorbance of calixarene derivatives, they are preferred to be used as bonded stationary phase rather than to be used as mobile phase or background electrolyte additive. For the present study, we prepared different types of methacrylate-calixarene based co-polymers. The mixture containing the functional monomers (methacrylic acid and hexacrotyl-p-tert-butyl-calix[6]arene) was set for polymerization at 50°C (for at least 4 hours), in presence of a radical initiator. Depending on the used dilution, type of used cross-linkers, the concentration of the monomers, different types of open-tubular or monolithic stationary phase were obtained.

All obtained stationary phases obtained were characterized by scanning electron microscopy, in terms of their porosity and particle dimension and by infrared spectroscopy.

The performance of the obtained stationary phases was evaluated for the chiral separation of racemic warfarin.

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L24

Chiral Separation of Some H1-Antihistamines Using Nonaqueous Capillary Electrophoresis

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Nonaqueous capillary electrophoresis (NACE) is a relatively new technique in the domain of chiral separations, offering numerous advantages over "classical" aqueous capillary electrophoresis – unique separation mechanisms, higher efficiency, lower current etc. In this work, the applicability of NACE was tested for the chiral separation of some H_1 antihistamine derivatives.

The selected analytes, namely: pheniramine, brompheniramine, clorpheniramine and promethazine are all first generation, sedative antihistamines, with very similar structure and consequently, similar electrophoretic mobility. In terms of chirality, they all contain one assymetrical carbon, giving rise to a total of eight enantiomers. Our aim was not only the simultaneous chiral separation of the four enantiomeric pairs in nonaquous media, but also to study the effect of different variables affecting the chiral separation (solvent type, background electrolyte composition, chiral selector concentration, temperature, applied electric field).

The chiral selectors derived from two main classes of chemical compounds, cyclodextrins (both charged – sulfobuthylether-ß-cyclodextrin and neutral – ß, γ - cyclodextrin, hydroxipropyl- ß-cyclodextrin) - and macrolide antibiotics (clarithromycin, azithromycin, roxithromycin).

Unfortunately baseline chiral separation was not achieved with the use of macrolides, but almost all cyclodextrins showed chiral recognition towards the analytes. The best separation was achieved using a dual cyclodextrin system in acidic background buffer in formamide, when all antihistamines were baseline separated. Comparing the method to the one developed in aqueous media, we can conclude that due to the diminished intermolecular interactions observed with formamide, much higher concentrations of chiral selectors were needed to achieve comparable results. Also, due to the high viscosity of formamide and the increased concentration of the chiral selector, migration times were also higher. As an advantage NACE offered increased solubility of the chiral selectors, higher selectivity by manipulating the solvent used and lower currents.

L25

Combination of Separation Techniques with Mass Spectrometry Used in the Analysis of Different Biological Samples

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Low- and high-molecular weight compounds occurring in many living organisms, are often analyzed in biological samples of various origin at trace concentration levels because of their bioanalytical importance. The complexity of all biological samples represents the challenges for the analytical chemists, as it is necessary to consider the presence of hundreds of different constituents having various chemical as well as biological properties and moreover, the concentration levels of constituents differ in several orders of magnitude. Because of this, different combinations of powerful separation techniques with sensitive and/or selective detection methods are required for such analyses. Liquid chromatography (LC) and capillary electrophoresis (CE) are two main analytical separation techniques used for solving these problems LC is very often served as "standard" analytical separation technique in such analyses due to its universality and flexibility. However, it is suffering from not sufficient separation efficiency and it also produce toxic waste which are limiting factors for its acceptance as the "universal" analytical separation technique. CE techniques offer some favorable properties, e.g., very high separation efficiencies, very low consumption of the samples as well as the electrolyte solutions, but they suffer from the detection sensitivity. MS detection itself and/or in the combination with LC or CE techniques provides an excellent selectivity while keeping sufficient detection sensitivity. However, because of the complexity of biological samples and different concentration levels of the analytes as well as the matrix components, LC-MS and CE-MS combinations are not always able to solve successfully the above mentioned analytical problems and some sample pretreatment technique is required to be used before final LC-MS and/or CE-MS analysis.

Micropreparative capillary isotachophoresis (pITP), used as the sample pretreatment technique, was performed in a hydrodynamically closed CE separation system. Different biological samples (urine, and saliva samples served as the model matrices) were used alone and/or spiked with several low- and high molecular weight compounds (model analytes) and pITP served for the isolation of analytes and to minimize the influence of the interferents coming from the biological matrices. pITP isolation included the use of discrete spacer constituents as added to the sample solutions. The isolated fractions were lyophilized and then reconstituted for final mass spectrometric analyses in:

1. a direct injection mode,
2. HPLC-MS (UPLC-MS) and
3. CE-MS combinations.

Our work deals with different analytical aspects as regarding the analysis of low- and high molecular weight compounds containing samples. MS and MS/MS spectra were obtained from the reconstructed fractions as included both the pITP clean-up effect and its ITP concentration power as regarding the analyte as present in the complex sample at very low concentration levels. This study is showing high potentialities and compatibility of pITP as the sample pretreatment technique before several modes of MS analysis.

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L26

LC Analysis of Drugs of Abuse in Biological Matrices

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Drugs of abuse represent a major health and legal issue of the 21st century, with high number of problematic consumers, a high level of criminal activity associated with their consumption, their direct negative impact on the health of consumers (lethal overdoses or undesired consequences in case of long-term use) and also the high risk of transmitting infectious diseases, like AIDS or hepatitis (due to risky sexual behavior or sharing of needles). That is why the analysis of this type of illicit compounds represents a major priority for toxicology labs.

When we are discussing about the drugs of abuse testing we think mostly of forensic testing due to the legal implications of these compounds but there is much more than that to be considered. There are multiple goals of the analysis of drugs of abuse, such as: the study of toxicokinetics and toxicodynamics of the drugs, the identification of potential interactions between different drugs of abuse or between drugs of abuse and therapeutic drugs, workplace drug testing, drug testing for traffic safety, identifying cases of drug facilitated aggressions, drugs of abuse testing in the criminal justice system to identify drug offenders and to discourage the abuse of drugs, emergency department drug testing, identification of overdose cases, differentiation between endogenous and drug-induced mental illness, scheduled testing to check on previous drug offenders on parole and probation or periodic drug testing in detoxification centers for compliance monitoring and to ensure continued abstinence.

Urine and blood are the matrices traditionally used for screening and quantification, respectively, but a variety of alternative matrices, such as oral fluid, hair, sweat are available. Depending on the aims of the drug testing and requirements of the drug-testing programs, different matrices and analytical tools can be employed to perform detection, identification and quantification of drugs of abuse present in the biological samples. In case of drug abuse testing an initial screening is performed by multiplex immunoassay (based on lateral flow immunochromatography), followed by confirmatory analysis by GC or LC methods. LC is gaining more and more importance in these types of applications. The general requirements for the LC methods are high sensitivity and selectivity, but high-throughput also. Depending on the goals of the analysis the analyte could be the parent drug and/or the metabolites. The biological matrix and the type of analyte are to be taken into account when selecting the sample treatment previous to the LC analysis. There are multiple sample preparation techniques with different advantages, but several drawbacks also which have to be considered when doing the selection. The sample preparation could become a real challenge when analytes with different properties (polarity, hydro-/lipophilicity) are to be isolated from a matrix. The LC analysis was performed until recently on HPLC columns with 3.5µm or 5µm particle size mainly. A big step in achieving high-throughput with very good separation and high sensitivity consisted in the introduction of UPLC based on 1.7µm particle size of the stationary phase. As detection, photodiode array and fluorescence detectors coupled with the LC instruments can guarantee sometimes the desired sensitivity and selectivity, but it is well known that LC-MS or LC-MS/MS are of choice, guaranteeing a higher degree of confidence in the results.

Nowadays, when we have the possibility to choose from so many analytical techniques the analyst working in a toxicology lab must be aware of the weaknesses of the different analytical tools that he could use for the analysis of drugs of abuse in complex biological matrices.

L27

LC-MS/MS method development for the determination of triazines and triazoles migrating from food packaging materials

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Nutrition is fundamental for our life. Since nowadays most people purchase preprocessed and stored food products instead of farming on their own, a huge amount and a large variety of packaging materials is used for the protection of food quality. However these packaging materials are obviously necessary, we have to take care of the probable migration of toxic compounds from the packaging material into the food.

In 2011 January the European Union published a regulation (10/2011/EC) on plastic materials and articles intended to come into contact with food. Almost a thousand of compounds are listed in this regulation most of which are determined typically with gas chromatography. For some compounds, such as triazines and triazoles, liquid chromatography is preferable. Each compound in the regulation has a specific migration limit (SML). SML refers to the maximum permitted amount of a given substance released from a material or article into food or food stimulants. Those SMLs are expressed in mg of substance per kg of food (mg/kg). According to 10/2011/EC fruit juices have to be modelled with ethanol 10%(v/v) and acetic acid 3%(w/v) and the SMLs for triazines and triazoles range from 0,05 mg/kg to 30 mg/kg.

But when we aim at testing the suitability of a stimulant for modelling fruit juice we have to take into consideration that from retail samples these compounds usually migrate to food products or stimulants at much lower concentration levels than their SML. If we want to measure them at this low concentration level we have to develop a sensitive method. It is essential to choose carefully the proper ion source, column, eluent pH, additives and organic modifier in order to reached low detection limits, good linearity, precision and accuracy both with acetic acid 3%(w/v) and ethanol 50%(v/v).

L28

Determination of Valsartan from Human Plasma by a rapid LC-MS method

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A sensitive liquid chromatography coupled with tandem mass spectrometry method for the quantification of the antihypertensive agent valsartan in human plasma was developed and validated according to the current official guidelines. The internal standard used was irbesartan. The separation was performed on a Kinetex C18 50 x 3 mm, 2.6 µm column under isocratic conditions using a mobile phase of 59:41 (v/v) acetic acid 0.1% (v/v) in water and acetonitrile at 30 ºC with a flow rate of 0.5 mL/min. The detection of valsartan and the internal standard was performed in multiple reaction monitoring mode using an ion trap mass spectrometer with electrospray ionization, operating in negative MS mode. The detected ions were 350; 391 m/z derived from 433 m/z valsartan and 193; 399 m/z derived from 426 m/z irbesartan. The human plasma samples (0.2 mL) were deproteinized with methanol and aliquots of 5 µL from supernatants obtained after centrifugation were directly injected into the chromatographic system. The method shows a good linearity (r^2 > 0.992), precision (CV < 10%) and accuracy (bias < 9.5%) over the range of 0.08- 8 µg/mL valsartan plasma concentration. The recovery was between 98 - 103%. The limit of quantification was 80 ng/mL. The analysis required a 5.8 minutes run. The developed and validated method is simple, rapid and specific for the determination of valsartan in human plasma and was successfully applied in a bioequivalence study, for analysing aprox. 1500 subject's samples.

Keywords: valsartan, LC-MS/MS, human plasma, bioequivalence

L29

Investigations on Biodiesel Fabrication Through Enzyme Catalysis

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Biodiesel has attracted more and more attention in recent years because of its biodegradability, environmentally friendliness, and renewability. Contrary to the conventional chemical catalysis method to produce biodiesel, the biochemical catalysis method developed quickly in the past decade and many immobilized enzymes are commercially available to meet the large-scale industrialization of biodiesel. This review is focusing on the current status of biodiesel production by biochemical catalysis method, especially the commercial enzymes and their immobilization for biodiesel production.

Consequently, we think that biochemical catalysis with immobilized enzymes will be an alternative method instead of chemical catalysis in biodiesel production/

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L30
Green Analytical Chemistry

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L31

Analysis of Lake Sediments

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Recent fluctuation in climate and changes in the environment rise the questions: Were there similar changes in the past? Are the effects of human impacts irreversible or reverible on natural ecosystems? What are the time scales of natural processes? Length of human life and toughness of researchers usually limit the length of time studiing ecological systems. However in the case of lakes we are able to answer these kind of questions. Secrets of lakes are burried deep in the mud deposited in the bottom. All the changes in the catchment area of the lakes have effect on the deposition of sediments. Dead remains of animals and plants settled year by year in the lake bottom together with mineral particles, pollen grains and amorphous coloured precipitates of geochemical reactions.

In this lecture we would like to present results of lake sediment investigations focusing on the inorganic chemistry of sediments. Element analysis of sediments provide useful data for reconstructing environmental changes within the lake and in the catchment area. Lake sediments from high and mindmountain area and from lowlands allowing different look to the past. Application of linear discriminant analysis of chemical composition data will be presented in different time scales, e.g. 15,000, 5000 and 50 years.

L32

Determination of Biliverdin and Protoporhyrin from Avian Eggshells by High Performance Liquid Chromatography

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Ornithologyst have long been fascinated by the coloration and texture of different eggshells laid by birds. Many bird species lay eggs speckled with protoporphyrin-based brown or biliverdin-based blue spots or even have bluish base color. However, the function of eggshell spotting and coloring is still a question. Protoporphyrin shows a pro-oxidant activity while biliverdin possesses anti-oxidant characteristics, so it has been hypothesized that eggshell pigmentation may signal the female's oxidative status [1]. This hypothesis can be confirmed only with the measurement of the pigment content of the eggshell. This necessitates the development of a sensitive and selective analytical method. Protoporphyrin and biliverdin can be determined using high performance liquid chromatography with UV-VIS detection. Prior to analysis, eggshell samples have to be mineralized to free the pigment content of the shell. Formic acid is used for this purpose. The protein content of the eggshell is denaturated by adding ethyl acetate to the extract and then precipitated by centrifugation. Aliquot of the resulting solvent is evaporated to dryness. The pigments are then back-extracted into dimethyl-sulfoxide for injection.

[1]: Avian egg colour and sexual selection: does eggshell pigmentation reflect female condition and genetic quality?, Juan Moreno and José Luis Osorno, Ecology Letters (2003) 6: 803–806

L33

Inductively coupled plasma - mass spectrometry (ICP-MS) and inductively coupled plasma – optical emission spectrometry (IP-OES) analysis of elements in Macedonian wines

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In this study the major, minor and trace elements in 25 Macedonian white, rose and red wines from different wine regions were determined. Analysis was performed with inductively coupled plasma - mass spectrometry (ICP-MS) and inductively coupled plasma – optical emission spectrometry (ICP-OES) for accurate determination of the concentration of 42 elements (Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Ho, La, Lu, Mg, Mn, Mo, Na, Nd, Ni, P, Pb, Pr, S, Sm, Tb, Ti, Tl, Tm, U, V, Yb, Zn, Zr). Statistical treatments, including factor and cluster analysis were performed in order to discriminate wines according to the wine type (white vs. red) and geographical origin. The main observed discriminant elements were Ba, Ca, Cu, P, Na and S. The obtained results show that ICP-MS and ICP-OES elemental analyses are promising techniques regarding the categorization of wine origin.

Keywords: elemental composition, wines, factor analysis, cluster analysis, ICP-MS, ICP-OES.

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L34

Applications of Electrochemistry coupled to Mass Spectrometry

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The off-line and especially the on-line coupling of electrochemistry to different mass spectrometric techniques is a quickly growing research field in analytical chemistry with numerous distinct objectives. Depending on the analytical problem, a separation step can be further integrated according to the instrumental set-up and, most frequently, liquid chromatography is selected for this purpose.

After a short overview of the current state of different applications (drug metabolism studies, the electrochemical study of different bioactive and biomolecules) and some of the opening future perspectives, a specific application of such an experimental setup is presented for the elucidation of colchicine's still unclear redox mechanism.

Apart of the use of different electrochemical techniques (cyclic voltammetry, differential pulse voltammetry, double-potential step chronoamperometry) using various electrodes (glassy carbon electrode, thin film electrodes, graphite-based screen printed electrodes, etc.), the influence of different redox mediators and bioselectors (DNA, β-tubulin, and bovine serum albumin) on the electrochemical behavior of colchicine was also studied.

Moreover, for the monitoring of the intermediates and products resulted in these electrochemical processes, on-line electrochemistry/electrospray ionization mass spectrometry (EC/ESI-MS) was performed using a special microflow electrolytic cell with a graphite felt - glassy carbon working electrode, formerly described by Arakawa [1]. Effects of electrolytic potentials on product ion intensities were examined in aqueous and non-aqueous (acetonitrile, N,N'-dimethylformamide) solutions and the importance of reaction media in the stability of intermediate products was also assessed.

The formation of neutral, radicalic or dimer electrochemical products of colchicine were monitored; these species being distinguished from hydrogen bonded complexes by MS/MS experiments. With the aid of an isotopic pattern generator different overlapping adducts were also separated from the mass spectra.

Finally a full electrochemical reaction mechanism was proposed based both on the obtained voltammetric and MS data and on former knowledge of colchicine's redox behavior.

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L35

Various Types of PAL in the Biotransformation of Unnatural Amino Acids

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L36

Application of HPLC for the monitoring of yield and products of decomposition of organic pollutants in waters using ionizing radiation for environmental protection

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The application of ionizing radiation (gamma from ^{60}Co sources or accelerated electron beam) is especially effective method of Advanced Oxidation Processes (AOP), fast and not requiring the use of additional reagents. This can be also more cost-effective compared to other commonly employed AOP methods. Due to strong oxidative properties of hydroxyl radicals, generated in the large amount in radiolysis of water, this method can be successfully applied in decomposition of numerous classes of organic pollutants, including *e.g.* various types of chlorinated hydrocarbons, pesticides, residues of pharmaceuticals, hormones or polychlorinated biphenyls.

The studies carried out in recent years in our research group concern the radiolytic decomposition of several groups of important environmental pollutants such as chlorophenols, phenolic compounds, different classes of commonly used pesticides, selected mycotoxins and perfluorinated surfactants. Besides examination of efficiency of the use of radiation for their decomposition using chromatographic methods also the monitoring of the toxicity changes of treated solutions containing target compounds was carried out.

The residues of pharmaceuticals in recent years are commonly detected in natural and treated waters. This indicates an insufficient efficiency of their removal from industrial and municipal wastes with commonly employed technologies hence numerous attempts in recent years are focused on development of efficient advanced oxidation processes (AOPs). These studies concerned investigation of radiolytic decomposition of pharmaceuticals analgesic diclofenac, anti-inflammatory drug ibuprofen, and antiepileptic drug carbamazepine using -irradiation. The residues of those drugs are widely detected in waters, municipal and some industrial wastes, and they are only partly decomposed in commonly employed treatment technologies. Based on determined rate constants for radical reactions, it was shown that most efficient decomposition takes place in reaction with $^{\bullet}$OH radicals. The effect of the presence of radical scavengers was examined, and numerous products of decomposition were determined using GC/MS and LC/MS methods. Three different toxicity tests were employed to monitor toxicity change of irradiated solutions. It was concluded that application of radiolytic decomposition is the most efficient, cost effective, and environment friendly water treatment method for the removal of pharmaceutical residues from waters and wastes.

L37

From Aerogels to Artificial Bone Substitution

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Aerogels are the lightest solids characterized with very high specific surface area and porosity, and can be made from a wide range of materials.[1] The most known and used varieties are silica aerogles made from either water glass or organic silane precursors under special conditions. Chemical functionalization with organic moieties or complexing agents led to new chromatographic stationary phases, metal ion selective aerogels and new heterogeneous phase catalysts. We have developed a new technology, which allowed us to produce aerogel foams, as well as composites containing guest particles of any densities and particle sizes from nanometers up to several mm, and the technology can be scaled up and used in continuous mode.[2] The process can also be applied to immobilization of enzymes, and encapsulation of intact cells, like baker's yeast. Lipase enzime preserved its activity, while yeast cells were active only in the aquagel phase and lost their viability upon supercritical drying.

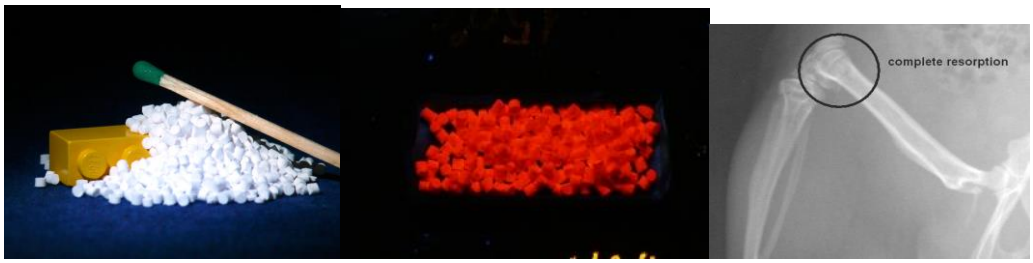

Figure 1 New aerogel-based red fluorescent artificial bone substitute material under visible (left) and UV254 nm light (centre), complete resorption of the cylindrical graft in the femur after 12 weeks (right).

The demand for artificial bone substitution materials is continuously increasing due to limited amount of natural bone tissues available. We have developed a new type of silica aerogel composite containing beta tricalcium phosphate and/or hydroxyapatite particles. The new material proved to be fully biocompatible both *in vitro* in simulated body fluids and in immunology testings. Composites containing red fluorescent labelled phosphates were also developed. Fluorescent and non-fluorescent cylindrical granules have been implanted in the femur of Evans rats and their remodelling was monitored by X-ray for 12 weeks. Cytology studies showed no negative immune answer, no inflammation of the surrounding tissues and verified a complete remodelling of the artificial grafts to new bone tissues.

Acknowledgements

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L38

Designing and preparation of multiple chromatographic packings in microchip

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The dream of the lab-on-a-chip conception is to miniaturize a complete chemical laboratory into a microchip format. Since currently the most widely used separation technique in the analytical laboratories is the liquid chromatography (LC), surprisingly only relatively few chip-based chromatographic systems [1-3]. compared to chip-based capillary electrophoretic devices are known.

Here we report on a simple method to fabricate microfluidic chip incorporating multi-channel systems packed by conventional chromatographic particles without the use of frits [4]. The retaining effectivities of different bottlenecks created in the channels were studied. For the parallel multi-channel chromatographic separations several channel patterns were designed. The obtained multipackings were applied for parallel separations of dyes. The implementation of several chromatographic separation units in microscopic size makes possible faster and high throughput separations.

The simplicity of the replication of the polydimethylsiloxan (PDMS) chips and the minimal consumption of the conventional packing particles (some tens of nanograms for a 10 mm length of packing) makes the chips inexpensive and disposable.

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P1

Elemental analysis of human blood serum of patients with autoimmune diseases by Microwave Plasma Atomic Emission Spectrometer (MP-AES)

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Trace elements play an essential role in many biological processes. The elemental analysis of human blood has been studied for years and is used for building up diagnosis in the clinical practice. The aims of recent study were to investigate the Ca, K, Mg, Cu, Zn and Fe level of blood serum of patients with autoimmune diseases, as well as to study the application of Microwave Plasma Atomic Emission Spectroscopy (MP-AES) for the elemental analysis of human blood.

Microwave plasma is an excitation source that has gained considerable attention recently in the new and powerful MP-AES instruments. For its practical application it is significant to study the matrix effects during the measurements. Matrix effect of Na was investigated since it is an element naturally present in human blood serum in a relatively high concentration. For this purpose a model experiment was carried out. Stock solutions of the selected elements were prepared from analytical reagent grade standards, as well as the stock solution of the matrix element Na was prepared from analytical reagent grade NaCl. The measured solutions were diluted from the stock solutions.

In the second step of recent experiment the blood serum of patients with Systemic lupus erythematosus (SLE) and Sjögren syndrome (SS) was investigated (n=20). Blood was taken from the diseased groups and healthy controls by venipuncture and serum fraction was gained by centrifuging. Samples were then digested prior to analysis in a microwave assisted system along with the mixture of concentrated HNO_3 and H_2O_2. The level of Ca, K, Mg, Cu, Zn and Fe was determined from the diluted solutions by MP-AES and Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Statistical analysis was carried out to evaluate the data.

Considering the results of matrix effect caused by Na, the most appropriate conditions were determined for the elemental analysis of blood serum by MP-AES. There were no significant difference ($p>0.05$) in Mg and Fe concentration between the control, SS and SLE groups. Significantly lower concentration ($p<0.05$) of K and Ca was measured in SS and SLE groups compared to the control. The concentration of Zn was found to be significantly decreased ($p<0.001$) in SS and SLE groups than in the control, while the concentration of Cu significantly increased ($p<0.001$) in both groups.

This research was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support system". The project was subsidized by the European Union and co-financed by the European Social Fund.

P2

Synthesis And Characterization Of Metal Ion Specific Aerogels

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Copper, nickel and cadmium are among the most toxic contaminants released into the environment by industrial processes such as electroplating, mining etc. Due to their non-biodegradability, they tend to accumulate in living organisms and may cause serious health disorders such as lung and kidney dysfunction, cancer, as well as dermatitis, skeletal damage, hypercalciuria and renal dysfunction.

Heavy metal removal from wastewater has gained significant attention in the past decades. One of the most frequently employed methods is adsorption. In recent years, a great number of functionalized ordered mesoporous silicas have been prepared as metal ion adsorbents. [1] The main advantages of these materials are easy chemical surface modification, high specific surface area, large pore volumes and thus good accessibility to active sites.

Another type of mesoporous silicas are aerogels. Silica aerogels are among the lowest density solids in the world, with unique properties such as extremely low bulk density (~0.003 g/cm^3), specific surface area as high as 500-1200 m^2/g and high porosity (80-99.8%).[2]

We report the synthesis and characterization of silica aerogels functionalized with mercaptopropyl groups as well as an equilibrium study of the pH dependence of their Cu(II), Ni(II) and Cd(II) adsorption capacity and selectivity in aqueous solutions.

Silica aerogels were produced by the conventional sol-gel method, followed by supercritical drying with CO_2. The greatest advantage of this method is that this way aerogels can be easily functionalized with a variety of organic moieties including N- and S-containing side chains capable of metal ions binding.[3]

The structures of the synthesized aerogels were characterized by Fourier transform infrared spectra, scanning electron microscopy and nuclear magnetic resonance spectroscopy. Sorption abilities were evaluated by using the batch adsorption technique. Metal ion concentrations were determined by microwave plasma atomic emission spectrometry.

The adsorbents showed high selectivity towards Cu(II) and Cd(II) in the presence of tripotassium citrate providing a unique possibility to take control over the selectivities by changing the chemical environment.

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P3
Ag/TiO_2 based on nanomaterials used in food preservation

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Nanomaterials are unique structures which have dimensions from 1 to 100 nm. Because the size of particles is reduced compared to the macroscopic structures of the same compound, they have different chemical and physical properties. Applications of nanotechnology can be found in different areas: environment protection, pharmaceutical industry, new materials development, agriculture, food processing and packaging.

Nanomaterials can be used in food industry as active scavengers for different compounds (oxygen, ethylene, carbon dioxide, water or undesirable flavours) or sensors (gas indicators, freshness indicators). Scavengers used in active packaging can provide shelf-life extension for food products by eliminating some compounds or by releasing active species on or into food. High oxygen and water vapours concentration from headspace packages accelerates growth of aerobic microorganism and organoleptic changes. Among the oxygen and water scavengers the ascorbic acid, iron powder, glucose oxidase and titanium dioxide are the most used in food industry.

When are exposed to UV light with a wavelength < 390 nm the electrons of TiO_2 are promoted from the valence to the conduction band, migrate to the surface and can enter in a redox reaction with other species present on the surface (oxygen water). By doping TiO_2 mainly with Ag, but also possibly with other noble metals, the response of TiO_2 can be extended even to the VIS region. As the Ag^+ ions can make the permeability of the cellular walls to change, this inducing the lysis of the bacterial cell it becomes obvious that the presence of Ag^+ in the package headspace controls the antibacterial activity of the package.

The paper emphasizes the efficiency of Ag/TiO_2 nanocomposite based on paper and polyethylene packages in preserving the shelf life of wheat bread and fresh juice. A wide array of parameters namely pH, content of fats, protein, sugars and hydroperoxides for bread and acidity, oxidation speed, content of sugars and ascorbic acid for fresh juice are used as markers.

P4

Elaboration of optimal technology and Pharmaceutical AVAILABILITY STUDY OF COMBINED ointments WITH IZOHyDRAFURAL AND METhyLURACIL

*Elena Donici**

Actuality

It was created a new pharmaceutical dosage form - combined ointment with izohydrafural and methyluracil, which combines the antibacterial action of izohydrofural and the regenerating action of methyluracil.

Izohydrofural is a derivative of 5-nitrofuran, invented at the Department of Organic Chemistry, State University of the Republic of Moldova. It has several advantages such as high antibacterial activity, long-time stability, less toxicity. Methyluracil is a derivative of pyrimidine. It stimulates cell regeneration processes and also has anti-inflammatory properties.

This ointment can be used in treating skin diseases, in surgery, obstetrics and gynecology, ophthalmology, proctology due to high efficiency and good way of application.

Keywords: izohydrafural, methyluracil, pharmaceutical bioavailability, dissolution rate constant, half-life time.

Materials and methods: In research it was used the active substances: izohydrafural, methyluracil and excipients: polyethyleneglycol 400, vaseline, stearyl alcohol, cetyl alcohol, propyleneglycol, glycerin, tween 80, sodium laurilsulphate. Also, in research it was used the device Erweka USP for the dialysis method, UV-VIS spectrophotometer Agilent-8453, Milipore membrane 0,22 mm and dimethylformamide as dissolution medium.

Results: First of all it was elaborated the optimal formulation and the manufacturing technology of combined ointments. It was used different excipients, obtaining four models of combined ointments with concentrations of 0,1% for izohydrafural and 5% for methyluracil. Pharmaceutical availability of active principles from ointments was determined by the method of dialysis membrane. It was respected the conditions of method: 50 ml dimethylformamide - as dissolution medium, the temperature - 37 ± 1^0C. From the obtained dialysate, it was dosed the active substances by UV-VIS spectrophotometric method: izohydrafural at wavelength 373 nm and methyluracil at 267 nm. From the results, it was established that the maximum of disposal speed of active substances occurs in composition containing polyethyleneglycol 400, stearin alcohol, glycerin, sodium lauryl sulphate, purified water. Also, it was assayed the dissolution rate constant (K_d) and half-life time ($T_{50\%}$) for all four compositions. It was established that the highest value of the dissolution rate constant has the same ointment containing polyethyleneglycol 400, stearin alcohol, glycerin, sodium lauryl sulphate and purified water. For izohydrafural - K_d = 0,018 min^{-1} and for methyluracil - K_d = 0,064 min^{-1}. At the same composition of ointment was determined the smallest value of half-life time of 38,5 minutes for izohydrafural and half-life time of 42,2 minutes, respectively for methyluracil.

Conclusions: It was concluded that the best pharmaceutical bioavailability of active principles from the four compositions of ointments resulted at ointment containing polyethyleneglycol 400, stearin alcohol, glycerin, sodium lauryl sulphate and purified water.

P5

Chemical stability and pharmacological evaluation of novel endomorphin-2 analogs.

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The application of endomorphins as clinical available analgesic drugs has been impeded by their relatively poor receptor selectivity compared to alkaloid analgesics, rapid degradation *in vivo*, inefficient to penetrate the blood–brain barrier (BBB), and undesirable or toxic effects, such as acute tolerance and physical dependence, respiratory depression, and inhibition of gastrointestinal motility. In order to increase degradation stability, penetration through biological membranes and receptor affinity we modify the structure of endomorphin-2. The analogs we synthesized – Deoxycholic-Tyr-Pro-Phe(pCl)-Phe-OH and Deoxycholic-Tyr-Pro-Phe(pF)-Phe-OH have shown increased hydrolytic stability and unexpected biological activity.

P6

Enantioseparation of Profens on Immobilized Polysaccharide-based Columns in Reverse Phase Separation Systems

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Immobilized polysaccharide-based chiral stationary phases (CSPs) have proved their usefulness for separation of a wide range of chiral compounds in HPLC. The CSPs demonstrate good performance in the areas of enantioselectivity along with high efficiency and solvent-CSP compatibility. However, these columns are mostly used in normal phase HPLC mode.

In this study we worked in reverse phase mode and we used three CSPs that differed in the type of derivatization group or in the nature of the glycosidic linkage of the polysaccharide. The columns CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC are based on tris-(3,5-dimethylphenylcarbamate) of amylose, tris-(3,5-dimethylphenylcarbamate) of cellulose and tris-(3,5-dichlorophenylcarbamate) of cellulose, respectively [[2],[3]].

Profens (non-steroidal anti-inflammatory drugs, NSAIDs) represent an important group of pharmaceutical compounds their chirality is caused by the carbon atom near the carboxyl group. The chromatographic parameters of profens obtained in reverse phase separation systems on three different CSPs are compared. The reversed phase mode was proved to be suitable for separation of the majority of tested profens. The best enantioseparations of the analytes were achieved mostly in the mobile phase containing acetonitrile/acidified water 40/60 (*v*/*v*). The easy-to-prepare aqueous solution was adjusted at pH 2.1 using formic acid. The results show that both the polysaccharide type and substituent affect the separation behavior.

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P7

Determination of Phenylethanoid Glycosides and Iridoid Glycosides from Therapeutically Used *Plantago* Species by CE-MEKC.

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CE methods are valuable tools for medicinal plant screening, analysis, and quality management. Our aim was to optimize and validate a CE-MEKC method for simultaneous quantification of four chief bioactive metabolites from therapeutically significant *Plantago* species. The two most important secondary metabolite groups were aimed to be separated. Different electrolyte and surfactant types were tested. Surfactant concentration, background electrolyte pH, electrolyte concentration and buffering capacity were optimized.

The developed background electrolyte contains 15 mM sodium tetraborate, 20 mM N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), and 250 mM sodium deoxycholate (SDC), pH was set 8.50. Acceptable precision and accuracy, good stability, and high resolution for phenylethanoid glycosides were achieved. Tested analytes were separated within 20 minutes. The proposed method is suitable to quantify the iridoid glycosides aucubin and catalpol; and the phenylethanoid glycosides acteoside (= verbascoside) and plantamajoside from water extracts of different *Plantago* samples. The method was shown to be applicable to leaf extracts of *P. lanceolata*, *P. major* and *P. asiatica*, and a biotechnological product, plant tissue cultures (calli) of *P. lanceolata*. Baseline separation of the main constituents from minor peaks was achieved, regardless of the matrix type. To show applicability in pharmaceutical analysis, decomposition models were run. The proposed method was shown to be suitable to quantify metabolites from these matrices as well.

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P8

Systematic Screening Approach for Chiral Separation of Beta-blockers by Capillary Electrophoresis using Cyclodextrines as Chiral Selectors

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In the field of chiral medicinal substances there is strong bond between the stereochemical characteristics and pharmacologic activity. In the last 20-25 years, a remarkable attention is awarded to enantiomer separation and to the therapeutical use of the enantiomer, which exhibit the maximum efficiency and minimum adverse effects.
In the past few years, in the domain of chiral separations, capillary electrophoresis become a powerful alternative to the more frequently used chromatographic methods due to its remarkable speed and efficiency as well as to its rapid method optimization (especially the high selectivity in choosing and changing the chiral selector).Beta-blockers are a group of chiral substances, which have at least one chiral center in their side chain, frequently used in the treatment of cardiovascular diseases. Although, differences in activity between the two enantiomers are well known, the S-enatiomer being more potent than the R-enantiomer, most of them are usually marketed as racemic mixtures.
In this work the chiral selectivity of six frequently used beta-blockers: bisoprolol, carvedilol, metoprolol, propranolol, pindolol, sotalol was evaluated using six different cyclodextrines. As chiral selectors we used both neutral natural (α, β, γ – CD) and derivatized cyclodextrines (hydroxypropyl-ß-cyclodextrin, randomly methylated ß-cyclodextrin) as well as ionic ones (sulfobutyl ether of β-CD).

Beta-blockers are basic drugs due to the presence of secondary amino group in their structure and have relatively high pKa values (>9), therefore low pH values (pH – 2-4), were selected for the separation in order to obtain ionoselective interactions.

We succeeded in the baseline separation of four of the studied beta-blockers (carvedilol, metoprolol, propranolol, sotalol) and for the other two beta-blockers (bisoprolol, pindolol) partial separation was obtained. In the case of carvedilol and sotalol we achieved baseline chiral separation with several CDs. Were pure enantiomers were available (carvedilol, metoprolol, sotalol) we established also the migration order of the enantiomers.

Our aim was not only the chiral separation of the studied CDs but also the optimization of the analytical conditions (buffer concentration, buffer pH, chiral selector concentration, applied voltage, system temperature, injection pressure and time) in order to achieve an increased chiral resolution and a short analysis time.

We conducted a systematic study of beta-blockers chiral separation comparing the effectiveness of different chiral selectors, the effect of electrophoretic parameters and also tried to establish connections between the chemical structure of the chiral substance and its stereoselectivity towards a specific selector.

P9

Comparison of the DNA Methylation Status of Tumor, Adjacent and Surrounding Normal Tissue of Breast Cancer Patients

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As breast cancer is the most common diagnosed cancer in women there is a great interest in identifying biomarkers for its early detection. Not only genetic changes but also epigenetic modifications, in particular changes in DNA methylation, have the ability to affect the formation of cancer. A high level of methylation of cytosines in CpG dinucleotides in the promoter region of a gene can lead to its silencing.

Previous studies have paid only little attention on differences in the DNA methylation status between tumor, adjacent and surrounding normal tissue in breast cancer patients. The aim of the present study was therefore to identify differences in the methylation status with respect to some breast cancer related genes, e.g. BRCA1 (breast cancer 1, early onset) and genes known to show aberrant DNA methylation in a variety of cancer types, e.g. CDKN2A (cyclin-dependent kinase inhibitor 2A).

The status of DNA methylation was determined by methylation sensitive high resolution melting (MS-HRM) analysis. Sample pretreatment consisted of DNA isolation of tissue samples of breast cancer patients and bisulfite treatment afterwards. Using bisulfite treatment unmethylated cytosines are converted into uracil while methylated cytosines remain unchanged. Next, polymerase chain reaction (PCR) followed by HRM was performed. HRM characterizes the obtained amplicons corresponding to their dissociation behavior as they change from double-stranded in single-stranded DNA with increasing temperature. For determining the methylation status in the samples the melting profile of their amplicons were compared with those of a totally methylated and unmethylated human control DNA.

We could show that in general there is a great difference in the methylation status of the promoter region or the exons of different genes. In addition, we found significant differences between patients related to the same genes. However, the most impressive result was that the ratio of the methylation status in the same gene between tumor, adjacent and surrounding normal tissue differed greatly from patient to patient.

P10

Sediment Analysis Using ICP-AES

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Lake sediments preserve changes in the environment. The generally used multi-proxy studies (i.e. pollen, chladocera, chironomids, macrofossil analysis) are applied in past climate reconstructions. Geochemistry, as a possible indicator of changes is scarcely used, mainly for the lithostratigraphic description.

This study applied bulk lake sediment geochemistry data to reconstruct climate changes in the Southern Carpathians. Sediment core were obtained from a small freshwater glacial lake, Brazi (1740 m a.s.l.) in the Retezat Mts. (Southern Carpathians, Romania). The lake catchment area is small and well-defined. The bedrock composed mainly of granites and granodiorites. The 1 m long core was sliced into 1 cm wide subsamples. Concentration of major elements by means of bulk analysis were determined and calculated in oxide forms (Al_2O_3, SiO_2, TiO_2, CaO, MgO, K_2O, Na_2O, Fe_2O_3, MnO, SO_3). Loss-on-ignition was used to determining the sediment organic matter content (ignition for 4 h at 550°C).

Linear discriminant analysis (LDA) was used to reconstruct past climatic changes on the basis of inorganic composition of sediment..Subsamples were "a priori" classified to “warm” and “cold” groups, according to their age and evidence of cold and warm events in the record, as suggested by proxy correlation with the event stratigraphy of North Greenland Ice Core Project (NGRIP). The discriminant function was calculated using concentrations of Al_2O_3, TiO_2, CaO, MgO, K_2O, Na_2O, Fe_2O_3, and MnO after log ratio transformation.

Under the LDA analysis the 85.1% of the originally grouped cases were correctly classified.

The results of calculated discriminant scores are proportional to the change into colder and warmer periods. Organic matter and sulphur concentrations were not used in LDA computations, they were compared with discriminant scores. The amount of sulphur and organic matter content is high in the samples preserved at the warmer period whereas in the samples deposited during the colder period are significant the major oxides bound into inorganic silicates.

These results are in close agreement with records from the Alps. This method may complete the analyses of sediments and could be a useful tool of environmental reconstructions particularly when the macrofossils are poorly preserved.

P11

The use of capillary electrophoresis methods for the analysis of selected acids in human urine.

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The on-line combination of isotachophoresis (cITP) and capillary zone electrophoresis (CZE) in a column coupling arrangement of the separation unit is a very effective tool for increasing the separation capability and sensitivity of CZE [1].

This work was focused on direct determination of quinolinic acid (QA) and furan-2,5-dicarboxylic acid (FDCA) present in human urine samples with UV detection. CITP was used as a sample clean-up technique for removing of anionic sample macroconstituents and potential interferences. With respect to acid–base properties of QA (pKa1=2.43, pKa2=4.78) and FDCA (pKa1=2.28), the separations at a low pH were favored.

The optimum conditions for cITP separation were achieved by using chloride as leading ion and malate as terminating ion. CZE separations were carried out at pH 5.7 using malate as the carrier ion. The pH and the ratio of single and double charged counter ions in the background electrolyte were the main parameters influencing the effective mobilities of the separated compounds in CZE stage of cITP-CZE.

Very good repeatabilities of total and corrected migration times of analytes were achieved. The RSD values of total migration time, a sum of the time in cITP and CZE steps, evaluated from 80 measurements of different urine and model samples at different dilution were 1.5 and 1.2% for FDCA and QA, respectively. The RSD values of corrected migration times of FDCA and QA in CZE step (cITP time subtracted) were 0.4 and 0.9%, respectively. The proposed method achieved low limits of detection varied from 0.02 to 0.2 μmol L^{-1} when the sample volume injected into the ITP step was 30 μl. The described method allows direct, rapid and sensitive determinations of the furan-2,5-dicarboxylic and quinolinic acids in human urine without additional sample pretreatment step.

Moreover, a single-column CZE separation of urine samples in the background electrolyte selected for CZE stage of cITP-CZE we carried out. Injection of 150 times smaller volume of the samples (200nL), result in 130-180 times higher limit of detection in experiments without cITP concentration step.

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Acknowledgement

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P12

Application of immunotests to identify genetically modified foods

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Legislation concerning the presence of genetically modifies organism (GMOs) in crops, foods and ingredients, requires the development of reliable and sensitive analytical methods for GMO detection. Raw material and processed products (e.g. foods) derived from GM crops might be identified by testing for the presence of introduced DNA or by detecting expressed novel proteins encoded by the genetic material. The western blots, enzyme-linked immunosorbent assay, lateral flow strips and PCR are the most commonly accepted methods for GMO identification.

Immunoassay procedures with antibodies are very attractive methods for qualitative and quantitative detection of proteins in complex matrices when the target analyte is known. Immunoassays with antibodies attached to a solid phase (ELISA) are the most popular immunochemical methods and they can be used in different formats: a competitive assay in which the detector antibody and analyte compete to bind with capture antibodies, or a double antibody sandwich assay in which the analyte is sandwiched between the capture antibody and the detector antibody. The other possibility is application of lateral flow strips. In this case, immobilized double antibodies, specific for the expressed protein, are coupled to a color reactant and incorporated into a nitrocellulose strip, which, when can be placed in extract from plant or food. This colored sandwich flows to the other end of the strip through a porous membrane that contains two captured zones, one specific for the transgenic protein sandwich and another specific for untreated antibodies coupled to the color reagent. The presence of two color lines indicates a positive result.

The aim of presented study is application of lateral flow strips specific for protein Cry1Ab for GMO identification. This protein is expressed after Bt (*Bacillus thuringiensis*) maize modification.

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P13

Immobilization of trypsin for online protein digestion, peptide mapping

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Peptide mapping is a strategy for protein identification, which involves the proteolytic digestion of a sample protein by protease enzyme (usually trypsin) and the analysis of the obtained peptide mixture via mass spectrometry (MALDI/ESI MS) or capillary electrophoresis (CE). The result is a mass spectrum (or an electropherogram) which describes the protein as a fingerprint. The protein can be identified by comparing the result with a peptide map database. The standard procedure for proteolysis is in solution digestion; however, in this case trypsin should be applied in low concentration due to its undesirable self-digestion. The low trypsin concentration brings forth long digestion time (~12 hours). Nowadays, there's plenty of research topic in this area to shorten this procedure.

Self-digestion of trypsin can be debugged by immobilizing it on a surface. Thus molecules of the trypsin are unable to react with each other (the molecules are bound to the surface). In this immobilized form, trypsin can be applied in higher concentration without self-digestion, resulting in short (<10 min) digestion time. Obviously, it is inevitable to find an immobilization method that doesn't reduce the activity of the trypsin, and can be integrated into a system that has high ratio in surface to volume, since the enzyme-substrate reaction occurs only on the surface of the trypsin's carrier. Such systems can be either capillary electrophoresis or microfluidic chips.

In my study the immobilization method is adsorption on polydimethylsiloxane (PDMS) surface. It is well-known that large macromolecules readily adsorb onto PDMS surface. PDMS is also a relatively cheap, porous, biocompatible polymer.

In this work I demonstrate that the adsorption of trypsin is practically irreversible, trypsin doesn't lose its activity and it can be used effectively for protein digestion. Also, the immobilized enzyme reactor is characterized.

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P14

Investigation of active site structure and mechanism of a β(1,6)-N-acetyl-glucosaminidase enzyme

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In our project we focus on an enzyme isolated from an oral pathogen bacterium, which is a ß(1,6)-N-acetylglucosaminidase called DispersinB. This enzyme can hydrolyze the glycoside bonds between two monosaccharide units of bacterial biofilm matrix, which can be destroyed by this mechanism. The *exo*-activity of the enzyme was proved by earlier measurements however some results raise a question to endo-activity. Different length chromofor group containing substrates were applied for my investigation. Cleavage pattern measured by HPLC was received after the hydrolysis of these substrates as well as we used kinetic data determined by HPLC as well. On the basis of homology of similar enzymes the formation of oxazolin derivative was supposed. This intermediate product was attempted to reveal with different analytical techniques: MALDI-TOF-MS, NMR. We investigated whether the wild-type enzyme or inactivated mutant forms of this enzyme could catalyze transglycosylation reaction with oxazolin donor and PNP-GlcNAc acceptor. Neither the supposed intermediate product could be revealed, nor enzymatic product of transglycosylation reactions. Cleavage frequencies, kinetic data and different mathematical models were used in order to create of subsite map. The results represent, that the enzyme can cleave glycosidic bonds inside the chain therefore, it has *endo*-activity. On the basis of the subsite map at least five binding sites was suggested for Dispersin B.

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P15

Expression and Purification of Various Types of PAL and PAM

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Abstract: PAL and PAM are members of ammonia lyase class. These enzymes are interesting not only by their unique mode of catalysis but also for their potential applications. They catalyze the reversible deamination of amino acids.

Based on the considerations regarding the stability differences between eukaryotic and prokaryotic PAL, a modified PAL construct containing the catalytic domain of the eukaryotic PAL and the C-terminal domain of a bacterial PAL were assembled. Several wild type and mutant PAL respectively PAM from various mesophiles and termophiles were cloned into the most proper hosts, followed by their expression and purification. In order to determine the general stability, activity and optimal condition for highest reaction rate, several substrates (**Fig.1.**) were tested. The kinetic constants, *Km* and *Vmax*, were determined and evaluated using unsubstituted derivatives as model compounds.

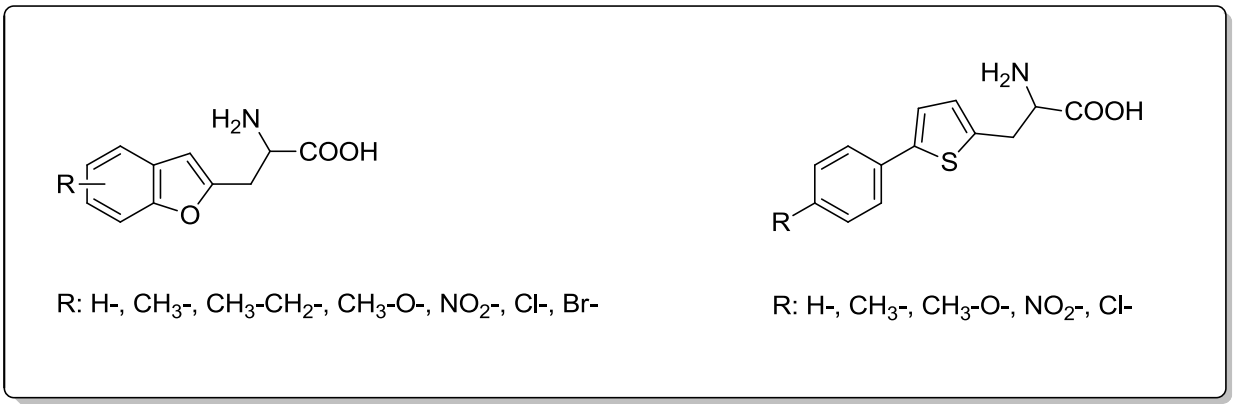

Fig.1. Substituted and unsubstituted derivatives for PAL and PAM

P16

Unnatural Amino Acids Stereoselective Enzymatic Synthesis

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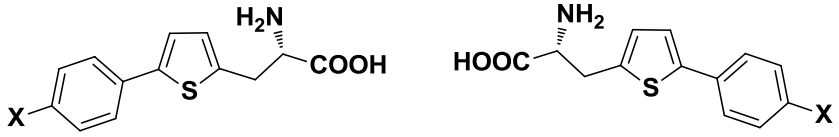
The key role of proteins and amino acids in the structure and function of living matter has stimulated extensive studies. Presently, an important direction in the area of organic and pharmaceutical chemistry consists in the synthesis of unnatural amino acids in homochiral form. Showing similarities in a number of parameters with natural analogues, these compounds possess a high potential for physiological activity. Modified amino acids play an important part in drug design and synthesis of biologically and medically relevant molecules, peptidomimetics and enzyme inhibitors.[4] Several non-proteinogenic amino acids such as ***L***-Dopa, ***L***-homophenylalanine and ***D***-2-naphthylalanine are already known to be used as important pharmaceuticals.[5] Others, like ***D***-phenylglycine, ***D***-*para*-hydroxy-phenylglycine and 2-thienyl-alanine serve as starting materials for the synthesis of various drugs.[6]

In addition to this, aryl-thiophenes were found to be constituents of several important classes of pharmacologically active compounds, being associated with activities like lipoxygenase inhibitors and antiarrhythmics.[7] Thus, the synthesis of enantiopure aryl-thiophene alanines represents an attractive goal. Therefore, the synthesis of four 5-aryl-2-thiophene alanines bearing one or no substituent in the *para* position of the phenyl ring was performed and two different approaches were used to this effect.

For the synthesis of nitro- and cloro-substituted derivatives, the corresponding thiophenecarbaldehydes used as starting materials were prepared by the Meerwein method from the diazonium salts of the substituted anilines and the commercially available 2-thiophenecarbaldehyde.[8] On the other hand, the metoxi-substituted derivative and the one bearing no substituent were obtained by the Suzuki coupling method which consists in the formation of a C-C bond between the corresponding aryl boronic acids and the 5-bromo-thiophene derivatives.[9]

For obtaining both enantiomerically enriched (*R*)- and (*S*)- 5-aryl-2-thiophene alanines, biocatalytic approaches (enzymatic kinetic and dynamic kinetic resolutions) were used.



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P17

CaL-A Mediated Kinetic Resolution of Racemic 2-hydroxy-2-(5-phenylthiophen-3-yl)acetonitrile and its Derivatives

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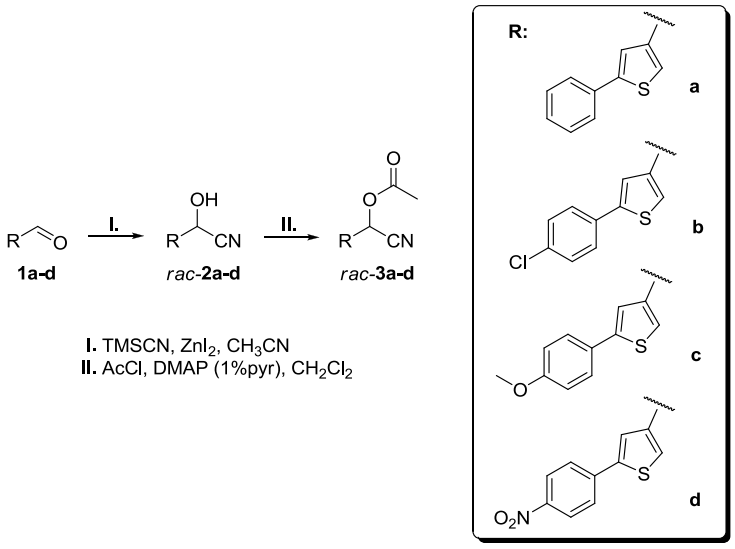
Aryl thiophenes are constituents of a variety of important classes of pharmacologically active compounds, showing activities as lipoxygenase inhibitors[10] and antiarrhythmics[11], while cyanohydrins are industrially important precursors to carboxylic acids and some amino acids.

In order to investigate the stereoselectivity of the reactions involving chiral derivatives of 2-hydroxy-2-(5-phenylthiophen-3-yl)acetonitrile and their esters, the chromatographic separation of the enantiomers was first established using a HPLC column and different mixtures of hexane-2-propanol (v/v) as eluent.



Scheme: Chemical synthesis of cyanohydrins and their corresponding O-acylated esters

Commercially available immobilized lipases were screened in various organic solvents for the enantioselective acylation of the racemic 2-heteroaryl-2-hydroxyacetonitrile *rac*-**2a–d** with vinyl acetate as the irreversible acyl donor. First, the analytical scale enantiomer selective enzyme catalyzed acylation of racemic 2-heteroaryl-2-hydroxyacetonitrile *rac*-**2a–d** was studied using *rac*-**2a** as the model compound and vinyl acetate in the presence of different solvents and various lipases. Most of the enzymes tested, such as immobilized lipase B from *Candida antarctica* (CaL-B, Novozym 435), lipases from *Pseudomonas fluorescens* (AK) on sol-gel and lipase from *Candida rugosa* (CrL) were catalytically inactive after 3 hours. The lipase from *Pseudomonas fluorescens* (AK) immobilized by adsorbtion on Celite showed good enantioselectivity and activity (eep = 98% and ee_S=72% at c = 42% after 3 h, in MTBE). By using CaL-A from *Candida antarctica* immobilized on Celite, the reaction was faster and the selectivity was improved (ee >83%) for both reaction products at 48% conversion), therefore these conditions were further used for the preparative scale enzymatic acylation.

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P18

Alternative Approaches for the Enantioselective Synthesis of (*S*)- and (*R*)- bufuralol

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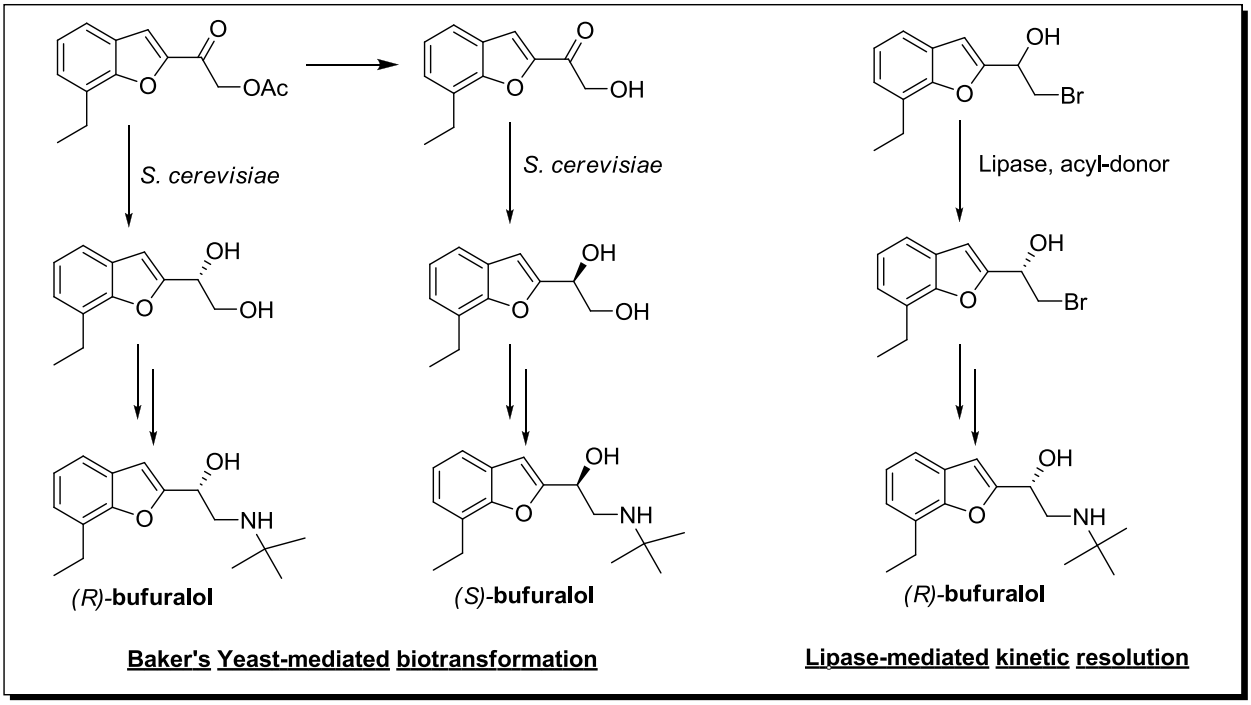
Bufuralol is a potent, nonselective, β-adrenergic receptor antagonist. It is proved to be effective for the treatment of hypertension and it is an inhibitor of testosterone 6β-hydroxylase. Herein we present two alternative approaches for the synthesis of optically pure bufuralol: a Baker's Yeast-mediated reduction of 1-(7-ethylbenzofuran-2-yl)-2-oxoethyl-acetate and 1-(7-ethylbenzofuran-2-yl)-2-hydroxyethanone into the corresponding 1,2-diols and a procedure via lipase-mediated acylation and hydrolysis of 2-bromo-1-(7-ethylbenzofuran-2-yl)ethanol as the key step.



P19

Application of quantum dots as fluorescent sensors in flow analysis

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Quantum Dots (QDs) are fluorescent semiconductor nanocrystals with unique physical and chemical properties that make them widely used in various areas of science.

The aim of this work is to prove that the advantageous features of quantum dots are not only very useful in chemical analysis but could be further improved when combined with the versatile sample handling and reaction zone implementation provided by flow-based systems. Due to the inherent QDs physical properties, such as a high surface-to-volume ratio, most of the reactions they undergo are surface reactions that affect not only their solution stability but also their quantum yield. Effectively, any analyte interacting with the QDs surface could destabilize the organic passivating layer resulting in the QDs precipitation. In discrete batch methods requiring equilibrium conditions for measurements this event could impair detection. In opposition, flow analysis methodologies based on reproducible sample insertion and measurement, under non-equilibrium conditions, are ideal for implementing analytical approaches based on the utilization of QDs as chemosensors allowing the monitoring of QDs-analyte interactions that could affect electron-hole recombination yielding and enhancement or quenching of the QDs fluorescence.

CdSe, CdTe, and CdS QDs were synthesized under different conditions by using distinct capping molecules. The organic capping layer protects QDs core, assuring adequate solution stability, and could also contribute to improve the optical properties. Moreover it could modulate QDs reactivity. GSH, MPA, cysteine, polymers and lipoic acid were used for this propose. The sizes of the water-soluble CdTe nanoparticles were in the range 1.3 - 3.5 nm.

The synthesized QDs were assayed in the determination of organophosphorus pesticides and lipoic acid both in batch and in flow-based analytical systems with distinct configurations: FIA (Flow Injection Analysis) and MPFS (Multi-Pumping Flow System). The developed approaches assured the determination of the referred compounds with good sensitivity and selectivity, and high sampling rate. The mechanisms of the reactions involved in these studies are also discussed.

P20

Application of CE chips for determination of proteins in urine

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The main function of kidney in organism is to filter blood and remove wastes. Urine protein profile and concentration indicates kidney functioning and can be used for diagnosis kidney diseases. For this purpose, very often a ratio of total protein to creatinine amount is utilized. When the ratio is greater than 100 mg protein/1g creatinine (proteinuria) the kidney function is abnormal. When the ratio value greater than 3000, this may indicate the complete destruction of glomerular filters of the kidney. The ratio of albumin to creatinine greater than 30 mg/g, may be due to microalbuminuria. All abnormal proteins concentration may be early symptom of kidney disease, especially in diabetics.

For protein determination in urine many methods have been used *e.g.* precipitation-based, immunochemical and chromatographic ones. The most common and useful are electromigration techniques: capillary electrophoresis, one- and two-dimensional gel electrophoresis and the newest mode - gel electrophoresis on microchips. It is of a great promise for clinical analysis of proteins in physiological fluids.

The aim of this study was to investigate the possibility of application of the Agilent 2100 Bioanalyzer with laser-induced fluorimetric detection for urine protein determination. Bioanalyzer is microfluidic platform for proteins separation and sizing. This microchip system offers short analysis times, limited sample consumption, improved automation and improved data precision and reproducibility. Proteins are separated on polyacrylamide gel as the complexes with sodium dodecyl sulfate (SDS). Sizing and quantitation of protein samples from 14-230 kDa was examined.

P21

Antioxidant Activity and Contents of Anthocyanins from Different Berry Extracts

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Recently, the interest in anthocyanin pigments has increased because of their possible utilization as natural food colorants and especially as antioxidant and anti-inflammatory agents. Different berries from Transylvania, Romania, were successively extracted by different techniques. We obtained several different extracts (methanolic extracts, ethanolic extracts, microwave assisted extracts, enzymatic assisted extracts, ultrasonicated extracts, enzymatic assisted extract, cold pressed extracts and ultrasonicated enzymatic extracts). Antioxidant activity, total phenolic content and content in anthocyanins of extracts were tested and compared.

P22

Analysis of humic acids by off-line combination of capillary isotachophoresis and HPLC

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The aim of this work is an analysis and fractionation of humic acids (HAs) by off-line combination of preparative capillary isotachophoresis (CITP) in discontinuous mode and reverse-phase high-performance liquid chromatography (RP-HPLC). Humic acids are a very complex mixture of organic materials and therefore a combination of two or more analytical methods seems to be a good choice for their characterization.

CITP separations were carried out in electrolyte system at pH 10. Spatial separation of humic constituents was reached by using of three discrete spacers (DSs), injected into the CITP column together with the HAs. Carbonates naturally present in the electrolyte solutions were served also as discrete spacer. The use of micropreparative valve (with a volume of 22 μl), placed behind the conductivity detector in CITP column, allowed fractionation of HAs into five fractions according to the effective mobility intervals defined by pair of DSs.

The correctness of fractionation procedure was verified by means of presence of corresponding DSs zones on the isotachopherograms from the separation of individual fractions in analytical CITP instrument equipped with conductivity detector. Control CITP analyses were performed in the same electrolyte system as used for preparative CITP.

Generally, conductivity detection used in CITP separations is not very convenient for detection of components migrating in "spike-mode" in the interzonal boundaries of regular CITP zones. Detection of HAs positions in the ITP migration configuration was realized by photodiode array detector using detection cell with fiber optics. We found, that HAs substances at higher concentration in the loaded sample migrated not only between two regular ITP zones but also formed mixed zones with spacers.

Isolated and controlled fractions were analyzed by RP-HPLC using 10-step gradient of N,N-dimethylformamide (DMF) in buffered (pH=3.00) aqueous mobile phase and wide-pore octadecylsilica column with fluorimetric detection (ex. 470 nm/em 530). Differences found in the chromatographic profiles of CITP fractions of HAs indicate that ionogenic components with different effective mobilities trapped into the individual fractions have different hydrophobicity and/or interaction with DMF.

Acknowledgement
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P23

Determination of N-acetylcysteine in Mucolytics by Electrophoresis on a Chip

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N-acetylcysteine is an active ingredient in mucolytics. Its sulfhydryl (-SH) group reacts with disulfide bonds in mucoproteins to split them into smaller units, whereby the viscosity of the mucus becomes reduced [1]. This process of cleavage of disulfide bonds also works at a reduction of abnormally thick mucus in patients with cystic fibrosis. Because of its antioxidant properties, N-acetylcysteine is known as an antiviral, anti-tumor and anti-inflammatory agent and also has been used in the treatment of acquired immune deficiency syndrome (AIDS) and hepatitis B, for the prevention of cancer, the treatment of oxidative stress of different origins and paracetamol overdose [2].

The aim of this work was to develop an ITP method for fast and precise determination of the main component, N-acetylcysteine in various pharmaceuticals, such as ACC Long and Solmucol performed on a chip with conductivity detection. The contents of N-acetylcysteine in these pharmaceuticals were evaluated by methods of external calibration and internal standard. External calibration method showed a higher dispersion of the content of N-acetylcysteine in the sample (about 10-fold higher RSD values). Recoveries of N-acetylcysteine in the analyzed samples were evaluated and ranged from 97 to 106%. These values predetermine the proposed ITP method for high-precision determination of N-acetylcysteine in different pharmaceuticals.

Acknowledgement

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P24

The Study of Peptide Digestion by Trypsin under RP HPLC Conditions

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Trypsin is the most widely used protease for protein digestion, especially in proteomic research. Cleavage products of this enzyme are well-characterized due to its high specifity. Trypsin cleaves peptide bonds after lysine and arginine residues, except when they are followed by proline. The speed of peptide digestion may be affected by many factors, such as pH, temperature, presence of organic solvents, autolysis or structure of the protein itself. The tertiary and secondary structure of the protein is usually unfolded by denaturation, but the primary structure, the amino acid sequence, cannot be changed. The acidic amino acid residues are able of making the digestion rate slower if they are near the cleavage site. Their location towards the cleavage site is crucial and will influence the digestion differently.

In this study, synthetic peptides with known sequences are studied in order to determine the change of tryptic cleavage rate in the presence of acidic residues, aspartic and glutamic acids. Peptide digests are analyzed by RP HPLC method with UV detection and the changes in digestion rate due to different positions of acidic residues are compared.

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P25

Analysis of Tryptamines by GC-MS and development of new separation methods using HPLC and CE

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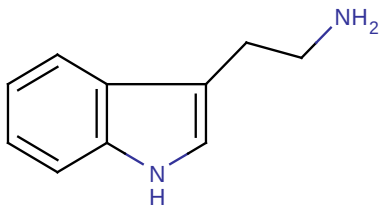
New Legal Highs are sold worldwide on the black market. They represent a huge problem for Police and Drug Authorities. Besides the chemical compound classes of cathinones and cannabinoids also a broad spectrum of tryptamines entered the market recently.

Tryptamines, which are structurally related to the indole alkaloids, and their derivates occur abundantly in animals, plants and mushrooms. They show psychotropic effects, which have been used for ritual and medical purposes since ancient times. Due to the mind-expanding effect these substances have become popular in the drug scene; e.g. Psilocybin, which is found in the well-known "magic-mushrooms". The indole ring, which is a particular structure of the tryptamines, can be recovered from other substances such as LSD. As the tryptamines show a similar but a bit weaker effect than LSD, they are used as a substitute drug in the scene.

Twenty tryptamines were purchased mainly from various Internet-shops. Our first aim was to check identity by GC-MS, verifying the mass fragments to their patterns. Then, separation methods for HPLC and CE were developed and optimized. Besides the use of different mobile phases and buffers, the effect of different cyclodextrines on selectivity was studied.

The goal was to achieve different attempts to separate a broad spectrum of trypamines simultaneously in a short time.



Basic structure of tryptamines

P26

Phytoremediation analysis for removal of Crystal Violet dyes using aquatic plant, Lemna minor

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The free-floating aquatic plants are highly sensitive for many environmental pollutants, and in a few cases have been reported that aquatic plants have a great potential capacity to degrade textile dyes. *Lemna minor* has the characteristics for an ideal test system for water remediation: having a small size, high multiplication rates, and vegetative propagation.

The aim of the study was to show a potential application for phytoremediation of triphenylmethane dye, Crystal Violet, with the free-floating aquatic plant (*Lemna minor*). The plants were left for 3 days in an acclimatization period, and the experimental period was 12 days. The effect of operational parameters such as initial dye concentration, pH, temperature and amount of plant on the efficiency of biological decolorization process was determined. The decolorization capacity by *Lemna minor* decreased whilst increasing the concentration of Crystal Violet (from 40 to 320 mg/L). The phytoremediation process showed that the removal of the dye was effective over a wide pH range. The kinetics mathematical equations: the pseudo-first and pseudo-second order were used to describe the remediation process of the systems and the thermodynamic parameters were also calculated.

P27

Determination of Erythrosine and Parabens in Pharmaceuticals by Microchip and Capillary Electrophoresis

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Erythrosine (ER) is an organoiodine compound, used as a food colorant with potential carcinogenic, hyperactivity and photosensitivity effect. Methylparaben (MP) and propylparaben (PP), used as food preservatives, are slightly toxic causing allergic and hypersensitive reactions with wide spectrum of antibacterial activity. These food additives are used in cosmetic products and pharmaceutics. The aim of this work was to develop electrophoresis methods performed on a capillary and microchip platform for fast determination of ER, MP and PP in various pharmaceutical products. Capillary zone electrophoresis (CZE) separations realized (1) on the microchip with conductivity detection were performed with eliminated hydrodynamic and electroosmotic flow (EOF) and (2) in fused silica capillary with UV detection with suppressed EOF.

CZE separations were carried out in electrolyte system at pH 9.75. Resolution of studied analytes was achieved by addition of β-cyclodextrin to the background electrolyte solution. Microchip with injected volume of a 900 nl has enabled the concentration limit of detection (cLOD) in the range 0.35-10.41 μmol/l for studied analytes. Their cLOD values were in the range 0.02-0.11 μmol/l using conventional capillary electrophoresis. Highly reproducible determinations of the target analytes with RSD <5% were achieved in model and real samples by microchip and capillary electrophoresis methods.

Application possibilities of the proposed analytical methods are presented by analysis of three commercial pharmaceutical products. Only very simple pre-treatment step including centrifugation and appropriate sample dilution prior to the microchip and capillary electrophoresis analysis was needed. Both methods allow fast and reproducible determinations of EP, MP and PP in pharmaceutical products. CZE analyses performed on conventional capillary electrophoresis analyzer with UV detection provided much more sensitive determinations with cLODs up to 2 decade orders of magnitude lower than those achieved on microchip with conductivity detection.

Acknowledgements

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P28

Determination of Temozolomide in Serum and Brain Tumor with Micellar Electrokinetic Capillary Chromatography

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Temozolomide (TMZ) is an anticancer drug and can be considered as the most frequently used alkylating agent for the treatment of malignant primary brain tumors (e.g. glioblastoma). The main problem regarding the chemotherapy against brain tumors is the low drug effectiveness due to the moderate penetration rate through the blood-brain barrier. The TMZ crosses the blood-brain barrier, but its exact local concentration in the human brain tumor tissue has not been determined.

Micellar electrokinetic capillary chromatography (MEKC) was applied to determine TMZ in human serum and brain tumor. In our earlier publication it was demonstrated that the MEKC is a useful technique to determine TMZ in model solution [1]. The TMZ could be detected in in vivo serum samples without sample pretreatment. The brain tumor tissues (0,3 g-0,8 g) were lyophilized and extracted with ethyl acetate to preconcentrate the analyte and obtain an injectable sample. The lyophilized tumor samples were dissolved in minimal volume (300-600 µl) of 0,1 M HCl. The obtained viscous solutions were centrifuged (9000g for 15 min). 50 µl supernatants have been extracted with 3 x 300 µl ethyl acetate (10 min vortex). After removing the organic solvent with rotary vacuum evaporator, the dried material was dissolved into 10 µl 0,1 M HCl, and was injected. The precision of migration times and peak area were 1,07 and 1,48 RSD% respectively. The limit of quantitation (LOQ) was 0,096 µg/ml using on capillary UV detection at 325 nm. The obtained peak concentration (8,2-10,1 µg/ml) and T_{max} (0,5-1,5 h) of TMZ in serum sample [2] were similar to the data reported by others [3], in vivo TMZ concentration found in brain tumor ranged between 0,046-0,117 µg/g.

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P29

High Resolution Melting Analysis of Cell Free DNA from Serum Samples of Breast Cancer Patients

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Breast cancer is the most frequently diagnosed malignancy in women and the leading cause of cancer related deaths. In addition to genetic alterations, epigenetic modifications, such as DNA methylation, were found to be a distinct and crucial mechanism in breast cancer development. Moreover, aberrant methylation patterns seem to be an early event in breast carcinogenesis and are also of potential predictive and prognostic value. DNA methylation therefore represents an interesting target for the development of noninvasive diagnostic, prognostic and follow-up tests for breast cancer.

For the early detection of breast cancer, the development of robust blood-based biomarkers that accurately reflect the host tumor is mandatory. In this study we investigated DNA methylation in circulating cell-free DNA (cfDNA) from serum of breast cancer patients and healthy, age-matched controls to establish a biomarker panel potentially useful for breast cancer diagnosis. The promoter methylation was examined in three different breast cancer relevant genes: RASSF1A (RAS-association domain family 1, isoform A), GSTP1 (glutathione S-transferase P1) and MGMT (O-6-methylguanine-DNA methyltransferase). Serum is readily accessible for molecular diagnosis in all individuals and can therefore be used in order to detect the methylation status of cfDNA in cancer patients. However, the extraction of cfDNA from serum proved to be quite difficult due to poor purity and low yield of the extracted DNA. We therefore compared different methods and conditions to optimize the extraction process. After the extraction, the isolated DNA was treated with bisulfite, converting all unmethylated cytosines into uracil, while all methylated cytosines remain unchanged. The methylation status of the genes was determined using polymerase chain reaction (PCR) and high resolution melting (HRM) analysis. After bisulfite conversion the amplified PCR products showed different base compositions according to whether they originated from methylated or unmethylated variants of the template. Because of the different thermal properties of the DNA strands, melting analysis of the PCR products allows to distinguish between methylated and unmethylated DNA of any unknown sample.

P30

Spectrophotometric Determination of Protonation Constants of Aroylhydrazones Derived from Nicotinic Acid Hydrazide

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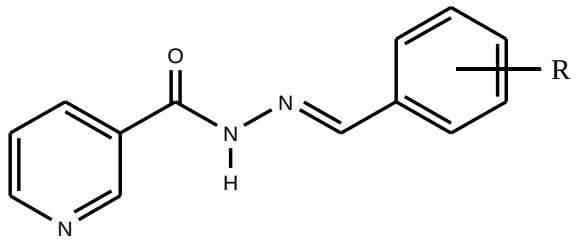
Aroylhydrazones have been extensively studied due to their numerous applications: they can serve as chelating agents, particularly for transition-metal and lanthanide ions, they show biological activity (anticancer, antibacterial and antimicrobial), and have promising properties for various analytical applications [1].

In this work we have determined the protonation constants of aroylhydrazones derived from nicotinic acid hydrazide (Scheme 1). The compounds studied were prepared according to the procedures described elsewhere [1].



R=2-H; 2-OH; 2-OH, 3-OH; 2-OH, 3-OCH_3; 2-OH, 3-Cl; 2-OH, 5-Cl; 2-OH, 3-Cl, 5-Cl; 2-OH, 5-NO_2

Scheme 1. Aroylhydrazones derived from nicotinic acid hydrazide

The protonation constants were determined in methanol/water 1/1 mixture at 25 °C and ionic strength 0.1 mol dm^{-3} (NaCl). Owing to the fact that aroylhydrazones are prone to hydrolysis in solvents containing water, batch spectrophotometric titrations were carried out. The UV-Vis spectra were recorded within first 3 min after the preparation of solutions.

Due to the fact that hydrolysis of arolyhydrazones below pH=2 was rather fast, equilibrium constants corresponding to the protonation of pyridine nitrogen were not determined. The logarithms of constants assigned to the protonation of amide nitrogen were in the range 10.5 to 12.9, whereas those corresponding to the hydroxyl group ranged from 7.1 to 8.9.

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P31

Analyisis of proteins using electrophoretic focusing techniques in combination with mass spectrometric detection

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Keywords: *capillary isoelectric focusing, mass spectrometry, proteins*

In recent years several investigators have emphasized the usefulness of identification and detection of specific proteins in various body fluids such as human plasma, serum, saliva, urine, their structural and regulatory roles in various disorders, because proteins present in human body fluids can often serve as diagnostic indicators of various diseases. The analysis of proteins present in complex biological matrix using mass spectrometry techniques is one of the main interests of biology and (bio)analytical chemistry.

However, due to very low concentration levels of proteins in such samples, their analysis is often challenging and requiring the use of highly sensitive and selective analytical techniques and in many cases also the use of concentrating and sample pretreatment techniques.

The capillary isoelectric focusing (CIEF) is a high-resolution electrophoretic technique for separation and analysis of proteins and peptides based on the differences in their isoelectric points (pI) in a pH gradient. This method has been successfully applied for determination of pI of the amphoteric molecules and analysis of the complex biological samples (human urine, saliva). In recent years coupling of capillary isoelectric focusing with mass spectrometry (MS) tries to substitute the most commonly used methods like two-dimensional polyacrylamide gel electrophoresis and SDS-PAGE electrophoresis to separate proteins.

This work was focused on an on-line coupling of MS with electrophoretic focusing techniques for the analysis of proteins. Capillary isoelectric focusing (CIEF) in on-line coupling with MS was chosen as the electrophoretic focusing technique was selected for the separation proteins, like transferrin, lysozyme and myoglobin, present in complex biological matrices (human saliva and urine) from interfering matrix components.

All CIEF measurement of standards of the above mentioned proteins were performed by using an Agilent HP 3D electrophoretic analyzer with an electrospray (ESI) interface coupled to an Agilent LC/MSD Trap XCT Plus mass spectrometer equipped with ion trap analyzer and controlled with the Agilent LC/MSD trap software. During the measurements mass spectrometer was used in in the positive ionization mode.

The capillary isoelectric focusing method with the sequential injection protocol was successfully combined with an MS instrument equipped with ion trap analyzer.

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P32

Evaluation of the Ingestion of Parabens When Using Dental Care Products by Healthy Human Volunteers

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Parabens are widely used as preservatives in food, cosmetic and pharmaceutics industry. They are known xenoestrogens and there are limits for their daily intake. Many papers focused on their deleterious health effects and percentage of skin absorption. Their ingestion when using dental care products was not monitored.

In our study 25 healthy volunteers were requested to use a mouthwash (in the way recommended by the manufacturer) containing a mixture of methyl and propylparaben. The amounts of ingested and recovered parabens after using the mouthwash were measured using a HPLC-UV technique and were used to estimate the ingestion of the studied substances. Precision, accuracy, linearity, and detectability (LOD and LOQ) were determined for the method we used and good results were obtained.

8 volunteers were requested to use the mouthwash for a shorter period of time (10 seconds) than that recommended by the manufacturer (30 seconds). Another 3 volunteers were requested to use a toothpaste containing parabens to evaluate ingestion from this type of product, too.

Using a mouthwash, ingestion of parabens was found insignificant compared to the ADI. An average person is allowed (EU regulations) to be exposed to 0-700 mg parabens/day. Using the mouthwash as recommended by manufacturer, ingestion never exceeded 6 mg at single use. From toothpaste, ingestion of parabens was even lower: never exceeded 1.5 mg at single use. Even if this products are used 2-3 times/day far lower amounts than those accepted will be ingested. Contrary to expectations, reducing the time of keeping the mouthwash in the oral cavity (from 30 seconds to 10 seconds) increased the amount of ingested parabens.

The results of this study show that the use of dental care products containing parabens is not a health hazard if done as recommended by the manufacturer. However, taking into account Wilson's principles of teratology and the new toxicological findings regarding propylparaben, these types of products should be avoided if possible by pregnant women.

P33

Detection methods for on-chip measurements

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The lab-on-chip methods gained wide popularity during the last decades. They offer today fast and reliable separation methods for a relative low price and equipment needs. One of the main goals of its use is to replace the conventional methods with chip-based ones. As a result of the efforts all the significant chromatographic and electrophoretic methods were already "transferred" to chips.

However, after a successful separation on an in-lab developed chip, the detection still can be difficult and tricky several times, even with optical, electrochemical or mass-spectrometric detection method.

Our poster tries to summarize some ways of the implementation of detection methods into plastic chips, showing its pros and cons from the practical point of view.

P34

New Method for Activity Measurement of Glycogen Phosphorylase B

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Glycosylation is one of the most common post-translational modifications. Most glycoproteins exist as a heterogeneous population of glycoforms or glycosylated variants with a single protein backbone. Glycan analysis means mostly the study of glycan part of glycoproteins. Glycans released from the glycoproteins, can be analyzed by MALDI-TOF mass spectrometry or, after labeling, by either HPLC or MS, or both. On the other hand, glycans are substrates of enzymes that degrade, modify, or create glycosidic bonds.
Our synthetic substrates are oligosaccharide analogues of natural polysaccharides with a reducing end chromophore group. Labeling is important either for UV detection of the substrate and the reducing-end products after HPLC separation and the differentiation of reducing and non-reducing end products on MALDI-TOF spectra.

Glycogen phosphorylase (GP) play an important role in the carbohydrate metabolic pathways and also in therapeutics, inhibition of the enzyme was suggested to the treatment of type 2 diabetes. Although GP is a key regulatory enzyme in glycogen metabolism, studies on GP have been hampered by the lack of a sensitive and convenient assay for GP activity. Most assay methods utilizing the chain-lengthening action of GP measure either the incorporation of ^{14}C-labelled Glc residues from ^{14}C-Glc-1-P into glycogen, or the liberation of P_i from Glc-1-P. Coupled enzyme methods, and radioactive methods have been attempted for measuring Glc-1-P formed in the direction of glycogen degradation

A new chromophor-labelled substrate 2-chloro-4-nitrophenyl-maltoheptaoside (CNP-G7) was used for activity and inhibition measurements. Enzyme reaction of rabbit muscle GPb were studied on CNP-G7 substrate using HPLC technique. Activity measurements were optimized. The enzyme reaction resulted in oligomer products CNP-G6, CNP-G5, CNP-G4 appeared one-by-one. It also turned out, that the pentamer (CNP-G5) was the smallest suitable substrate for the phosphorylase enzyme. Glucopyranosylidene-spiro-thiohydantoin was used for inhibition studies. Our measurement using the oligomer phosphorolysis resulted in inhibition constant K_i= 11 μM with good agreement in Ki = 7 μM obtained earlier on glycogen and Glc-1-P substrate in the direction of glycogen synthesis.

Reductive amination as a simple labeling method was applied for synthesis of similar substrate p-nitroaniline-maltoheptaoside (PNA-G7). The products were identified using MALDI-TOF mass spectrometry and purified by HPLC. The product pattern of GPb catalysed reaction on PNA-G7 was different since endproduct was PNA-G5.

The research was supported by the EU and co-financed by the European Social Fund under the project ENVIKUT (TÁMOP-4.2.2.A-11/1/KONV-2012-0043)

P35

Fabrication of Microchips with Multichannel Systems

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Different microfluidic systems with connected or independent multiple channels were developed. In the channels of these microchips several parallel chromatographic packings can be created.

A new packing procedure was developed, a bottleneck was created in a channel of 100 micron width, and around 1 microliter of suspension of methanolic C18 particles was injected into the chip channel toward the bottleneck and then washed with methanol [1]. The particles retained around the bottleneck, and then the newly arrived particles adhered to the packing; increasing its length. The first particles acted as keystones blocking the other particles [2].

Two different procedures were developed to create the bottleneck in the channels. In the first case the bottleneck was the result of a line in the photolitographic mask. In the other case an 8-10 micrometer wide and 50 micrometer long channel was drawn into the photolitographic mask as a bottleneck. Both procedure was suitable for the making of chromatographic packings in parallel channels. The second procedure was used to integrate the chromatographic packings into the channels because it was more reproducible bottleneck fabrication procedure.

One of the great advantage of these systems is the possibility of making parallel measurements at the same time.

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P36

Hydrolytic stability determination of some peptide analogues by HPLC

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For exhibiting of their effects, biologically active compounds except that they must be delivered to their place of action it must be considered their hydrolytic stability at physiological conditions like: body temperature and pH. However, some peptides are highly susceptible to chemical degradation, resulting in a loss of biological activity. In this, study we determine hydrolytic stability of some peptide analogues in buffers at different pH (basic, neutral and acidic). Reversed-phase HPLC was used as the main tool in determining the percent of degradation of the compounds analyzed. Their quantities were experimentally determined at definite time intervals by HPLC-UV and the dependency concentration / time was plotted.

P37

Contribution of filamentous fungi from *Plantago lanceolata* L. leaves to the pattern and stability of bioactive metabolites: a CE-MEKC and GC-MS study

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The aim of this study was to test contribution of plant-associated microorganism (PAMs) to metabolite pattern and stability/instability in a medicinal plant matrix.

Therefore, PAM strains were isolated and identified from *Plantago lanceolata* leaves. The fungi belong to the genera *Epicoccum*, *Bipolaris*, *Cladosporium*, *Leptosphaerulina*, *Aspergillus*, *Eurotium* and *Penicillium* (pathongens, endophytes, and other species). Sterile water extracts of *P. lanceolata* were incubated with the isolated strains and antioxidants (ascorbic acid (AA), and EDTA) for 15 days, and changes in the concentrations of chief bioactive constituents (aucubin, catalpol, acteoside (= verbascoside)) were quantified by CE-MEKC. Phenolic breakdown-products were identified and quantified by GC-MS.

Some fungi caused significant decomposition of the analytes of interest ($p < 0.001$). Surprisingly, some fungal strains inhibited breakdown of acteoside ($p < 0.001$) with a concurrent increase in the concentration of several phenolic acids ($p<0.001$). Gentisic acid, 4-hydroxyphenyl acetic acid, 4-hydroxybenzoic acid and hydroxytyrosol were only present in fungi-treated extracts. These phenolic compounds are powerful antioxidants and chelators. Concentrations of phenolic acids influenced acteoside stability significantly ($p < 0.01$), as shown by basic data-mining techniques. AA and EDTA also significantly inhibited acteoside breakdown in sterile model solutions ($p < 0.05$).

Our results suggest, that the phenolic acid mixture (produced by the fungi) protected acteoside from breakdown, possibly via its antioxidant activity and metal complexing ability. Thus, it was shown, that PAMs can increase or decrease the stability of chief metabolites in herbal matrices, and can significantly alter the chemical pattern of the plant matrix.

The research was supported by the EU and co-financed by the European Social Fund under the project ENVIKUT (TÁMOP-4.2.2.A-11/1/KONV-2012-0043)

P38

Two Dimensional Liquid Chromatography of Biomacromolecules Isolated from Environmental Sources

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The aims of the presented work is design and development of novel methods of liquid chromatography for analysis and characterization of some from industrial point-of-view distinct biomacromolecules, e.g. humic substances (HS), lignin (L) by utilization of combinations of two or more liquid chromatography methods. HS belong to the most spread envirobiomacromolecules and they have direct influence to various processes playing significant role in an environment. They are created by a complex mixture of amorphous, yellow to black coloured, hydrophilic, polyelectrolyte polydisperse macromolecules. Lignin is a constructional and structure-forming element of higher plants. It creates expressive part of wood mass and it is tightly bound via chemical bonds to polysaccharides (cellulose). HS and L belong to group of poorly defined substances which have not yet exact definition as a chemical individuum and which are so-called uncertainly defined chemical systems.

Multidimensional chromatography has a proven to be useful for the analysis of complex samples such as HS or L samples. From the point-of-view of chemical analysis, characteristic feature of these analytes is diffuse non-distinct analytical signal produced by many detection principles. This signal does not usually result in an exact numerical physical-chemical data, but is described also by their distribution function or range of validity. This dictates the necessity of development of automated complex separation procedures with minimal sample pretreatment, and the use of on-line (off-line) multidimensional chromatographic techniques is a logical solution to these requirements.

With respect to the non-common approach we focused to evaluation of its potential to create orthogonal, i.e. on different separation principles working two dimensional comprehensive separation methods. The coupling of two chromatographic methods, RP-HPLC and SEC was evaluated using the statistical program calculated the Pearson Product Moment Correlation. Comparison of the calculated values of Pearson correlation coefficients for characterization of the examined samples of HA and their fractions by coupling of the RP-HPLC and SEC methods led to the conclusion that the values show a very low level of correlation and the separation system employed behaves as orthogonal.

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P39

Synthesis and Study of New Simulated Body Fluids for In Vitro Testing of Artificial Bone Substitutes

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Bone-bonding ability of a material is often evaluated by examining the ability of apatite to form on its surface in a simulated body fluid (SBF). In 1991 Kokubo et al. (1) proposed that the essential requirement for an artificial material to bond to living bone is the formation of bonelike apatite on its surface when implanted in the living body, and that this in vivo apatite formation can be reproduced in a simulated body fluid with ion concentrations nearly equal to those of human blood plasma. In vivo bioactivity of a material can be predicted from the in vitro apatite formation potential on the surfaces in SBF. However, SBF simulates the inorganic part of human blood plasma and does not contain proteins, glucose, vitamins, hormones, etc. (2)

We have developed new simulated body fluids containing free amino acids, similarly to the real blood plasma. Biocompatibility of the newly synthesized experimental bone substitute materials were tested by soaking them in simulated body fluid of different compositions. It was observed, that a hydroxyapatite layer deposited on the surfaces in all simulated body fluids, meaning that the amino acids have no negative effect on the processes occuring in the fluid. Nevertheless, sterile conditions have to be provided for example by gamma-irradiation, in order to avoid bacterial infections in the incubation periods. Fig. 1 shows SEM analysis of our materials soaked in conventional and new SBFs for 2 weeks.

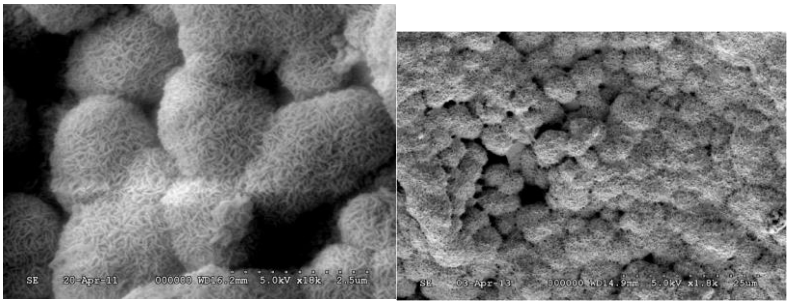

Fig. 1 SEM analysis of aerogel based artificial bone substitutes in conventional SBF (left) and containing amino acids (right). Magnifications are 18k and 1.8k, respectively.

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P40

Cost-effective and rapid digestion method of sediments and rock samples

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Lake sediments preserve the environmental and climatic changes in the catchment area. Study of sediments may be conducted by involving several scientific fields such as chemistry, pollen, chironomid larvae and macrofossil studies. Chemical analysis has a significant importance since it provides information about the chemical composition of the sediments even in the absence of components with biological origin. The chemical analysis covers the determination of the organic and inorgani content (e.g. macro and micro elements) of sediments.

In frame of exploring ancient environmental changes in the Retezat Mountains (Romania) sediment core samples were taken from several lakes (Gales, Taul dintre Brazi, Lia, Bucura) at 1700-2000 m height above the sea level. Increased number of samples constrainded us to develope simple method of sample pretreatment to increase the throughput capacity of our lab.

A rapid and cost effective method of complete digestion was carried out in disposable polypropilene (PP) tubes. Sediments were ashed at 550°C for determination of loss on ignition. The residue of this analysis was forwarded for inorganic analysis. Fine powdered ash (50 mg) were placed into PP test tubes (volume of tubes were 12 cm^3). Digestion was carried out with 1 cm^3 37% (m/m) HCl and 1 cm^3 38% (m/m) HF in a dry oven at 105 ^oC for 2 hour. After cooling, 8 cm^3 boric acid (4% (m/m)) were added to the samples. Sample solutions were diluted up to 20 times with double deionised water for the analysis of major metals.

Standard reference materials were digested to validate the method: BCR 141 (chalky clay), BCR 142 (sandy soil), BCR 143 (sewage-sludge) és BCR 176 (waste destructor ash).

Element analyseis were carried out by Microwave Plasma Atomic Emission Spectrometry (MP-AES 4100 produced by Agilent Technologies). Plasma is run on nitrogen gas supplied by a generator. The plasma was surprisingly resistant to the sample matrix containing 0,2% boric acid. Concentration of Si, Al, Fe, Mg, Ca, K, Na, Mn, Sr, Ba and Ti were measured using different emission lines, respectively.

Comparative measuements were done by ICP-OES method (Thermo Iris Intrepid II XSP Duo produced by Thermo). Both of the applied measuements showed good correlation with the expected values of CRM materials. The developed sample pretreatment method could be a useful tool for rutine analysis of lake sediments.

P41

Ultrafast haplotyping of putative microRNA-binding sites in the WFS1 gene by capillary gel electrophoresis

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The transmembrane protein wolframin (WFS1) plays a crucial role in cell integrity in pancreatic beta cells and maintaining ER homeostasis. Genetic variations in the WFS1 gene have been described to be associated with Wolfram syndrome or type 2 diabetes mellitus. In this work we present on an efficient double-tube allele-specific amplification method in conjunction with ultrafast capillary gel electrophoresis for direct haplotyping analysis of the SNPs in two important miRNA-binding sites in the WFS1 gene. An automated single-channel capillary gel electrophoresis system was utilized in the method that provided dsDNA fragment analysis in less than 240 s. The light-emitting diode induced fluorescence (LEDIF) detection system enabled excellent sensitivity for automated haplotyping of a large number of clinical samples. The detection limit was 0.002 ng/μL using field amplified injection from water diluted samples. The dynamic quantitation range was 0.08–10.00 ng/μL (R2 = 0.9997) in buffer diluted samples.

P42

Application of Split-flow Injection in Microchips

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Keywords: hydrodynamic injection, microchip electrophoresis, poly(dimethylsiloxane), cephalosporin, isotachophoresis

The ability to accurately inject small volumes of sample into microfluidic channels is of great importance in electrophoretic separations. We have developed a new injection method based on the simple patterning of the crossing of channels that does not require sophisticated instrumentation [1]. The sample volume injected into the separation channel is dependent on the ratio of the widths of the crossing channels. This injection method is capable of introducing, into a separation channel, multiple plugs of sample on a large scale. The crossing pattern is applicable in the delivery of pL quantities of solution which is useful for CZE or MEKC on chip. This injection procedure makes it possible to inject a plug of sample of as little as 100–300 μm in length (~200 pL volume). The injected amount can be as little as 1% of the total volume of the separation channel (as in conventional CE). By changing the ratio of the widths of the crossing channels, greater sample volumes can be formed in the separation channel. Reducing the ratio of the widths of the crossing channels will allow for larger sample plugs (in the nL range) to be injected preferred in isotachophoresis (ITP) and preconcentration procedures.

A miniaturized capillary electrophoretic system was developed by combining a microfluidic chip and a conventional fused silica capillary for the separation of different antibiotics (cephalosporin, temozolomide). Here the sample injection was carried out in the PDMS chip based on split-flow injection method [1]. The sample introduction (into the microchip) was accomplished using a microinjector with internal chamber, which was suitable for serial injection of samples. The further advantages of this miniaturized CE system are its portability, flexibility, and the fact that the whole system includes cheap, disposable elements (PDMS platform, CE capillary).

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P43

Analysis of Nucleic Acids using Electrophoretic Techniques

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Keywords: capillary isotachophoresis; preparative capillary isotachophoresis; nucleic acids; complex biological samples,

The aim of the work was the analysis of the nucleic acids (DNA, RNA) present in complex biological samples by using capillary isotachophoresis.
The analysis of high molecular weight compounds present in complex biological samples is a very complicated analytical task as there is a large number of analytes present in complex matrices and, therefore, also the possibility of the occurrence of two or more substances having very close retention or migration characteristics. This fact means that the successful analytical procedure requires the using of powerful separation technique with the sufficiently sensitive and/or selective detection technique.

Capillary isotachophoresis (cITP) is one of the basic modes of capillary electrophoresis (CE) using discontinuous electrolytes system, i.e., leading electrolyte (LE) and terminating electrolyte (TE), which determine the mobility interval for the migration of analytes from the sample. Unlike other CE techniques, isotachophoretic separation provides the self-sharpening effect at the zone boundaries and the inherent concentration capability. After reaching an isotachophoretic steady-state, the zones of separated analytes are migrating according their decreasing effective mobilities with a constant speed. The concentration of the analyte in its own zone is given by Kohlrausch regulation function and does not depend on the concentration of the analyte in the injected sample. Preparative capillary isotachophoresis (pITP) was proved to be a powerful sample pretreatment technique. Main advantages of this technique are rapid simplification and/or reduction of complex ionic matrices, increasing the concentration(s) of the analyte(s) in the collected fraction, well-defined sample pretreatment conditions in ITP separation mode, isolation of analyte into well-defined fraction with known composition when discrete spacer technique is used. These key steps offer, for example, a very suitable analytical tool for some trace analytes present in complex biological samples.

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P44

Using of LC-DAD-MS Techniques for Analysis of Phenolic Compounds in Selected Tea Samples

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Tea is believed to be an important drink for over 1000 years because of its beneficial effect for the human body. It is one of the most consumed soft drinks in the world, next to water. The content of individual phenolic compounds in tea varieties is highly variable which is caused by using of different fermentation procedure, growing season and different geographical region. Phenolic compounds are important components mostly present in different sorts of teas and red wines. They are secondary plant metabolites, which affect organoleptic characteristics of above mentioned drinks. In addition, they show different biological activities such as anti-inflammatory, anti-bacterial, anti-carcinogenic, anti-allergic and further effects.

In this study, a simple, rapid and efficient high performance liquid chromatography (HPLC) method combined with mass spectrometric (MS) detection was used for characterization and identification of several phenolic compounds present in selected tea varieties (black teas and green teas).

HPLC-MS analyses of selected tea samples and their phenolic profiles were performed by means of Shimadzu LCMS-IT-TOF^{TM} (Shimadzu, Kyoto, Japan) analyzer equipped with electrospray ionization (ESI) operating in positive and negative ionization modes. HPLC separations were performed on Kinetex XB-C18 column (100 x 2.1 mm; 2.6 μm) (Phenomenex, Torrance, CA, USA) using gradient elution (water + 0.1% formic acid – acetonitrile + 0.1% formic acid) with 0.2 ml/min flow rate. The column was termostated to 40°C. Data in the MS experiments was acquired automatically within 50-1000 m/z values in both the positive and negative modes and within 190-400 nm wavelengths during DAD detection. Data acquisition and data evaluation were performed by using LCMS Solution ver. 3.51 (Shimadzu). MSXelerator software ver. 2.4 (MSMetrix, Maarssen, Nederland) was used for classification of individual tea samples and their phenolic profiles. Total analysis time was 15 minutes and injected volume was 2 μl.

Selected samples of black and green teas from different producers were obtained in local supermarket. Before the individual samples (2 μl) were injected into the LC-MS ESI-IT-TOF analyzer, the extracts of teas were prepared as follows: individual tea bag (1.5 g) was extracted with 150 ml of boiling water. Extraction time was 5 minutes. The tea infusions were cooled to laboratory temperature before sampling.

Key words*:* green and black teas, phenolic compounds, HPLC-MS analysis, identification

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P45

Electroanalytical Study of Cyclodextrins Interactions with some Pharmaceutical Compounds

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Inclusion complexes between cyclodextrins (CDs) and various drug molecules and their applications in pharmaceutical manufacture and analysis is the issue of many papers published in the last decades[1,2,3]. The CDs effect on the electrochemical behavior of some pharmaceuticals by using cyclic voltammetry and square wave voltammetry at carbon paste electrode (CPE) is presented. The oxidation peak of the analytes is shifted to more positive potential values and the current intensity is decreased, by the inclusion complexes formation, confirmed by FTIR spectra. Carbon paste electrodes modified with β-cyclodextrin (β-CD/CPEs) showed enhanced electrochemical responses for ascorbic and uric acid in comparison with the unmodified carbon paste electrode.

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P46

Zone Electrophoresis On Microchip

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Zone electrophoresis of the labeled amino acids on a commercially available lab-on-a-chip instrument was possible in a background electrolyte (BGE) without stabilizing detergents, which is in contrast to conventional CE; moreover, analysis times were drastically shorter than 10 s range. [1,2]

In our work we label amino acids (glycine, lysine, asparagine acid, glutamine acid) with two fluorescence dyes: fluorescein isothiocyanate (FITC) detected by the blue laser (λ_{ex} 450 nm) and cyanine 5 (Cy5) detected by the red laser (λ_{ex} 630 nm).

In our measurement we used modified DNA script. The running buffer was 100 mM sodium borate (pH 8.3). The amino acid samples were labeled for 24 hours at 4 ^{o}C.

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P47

Biosorption of Cd(II) with chemically modified fir tree sawdust

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Sawdust is one of the cheapest and abundantly available adsorbent that has the capacity to adsorb and accumulate heavy metals from synthetic aqueous solution. This study investigate the potential use of fir tree sawdust (*Abies alba*) treated with concentrate sulphuric acid, for the removel of Cd(II), Cu(II), and Zn(II), from synthetic solutions. After treatement, sawdust is turned into a new material; active carbon. The effects of stirring rate, biomass quantity, initial metal ions concentration, pH were studied. Biosorption kinetics data fitted using first-, pseudo-second-order and intra-particle diffusion. Biosorption process was followed the pseudo-second-order kinetics. Equilibrium adsorption isotherm models were also discussed in detail. Study show that the acid sulphuric treatments provide a good adsorption capacity of Cd(II), Cu(II), and Zn(II) on treated fir tree sawdust.

P48

Study of Drug Metabolism Using EC-LC-MS Technique

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Several analytical methods have been used to metabolic studies of numerous xenobiotics. In vivo experiments with laboratory animals and in vitro experiments with liver microsomes belong to the mostly used approaches. Microsomes show high activity of cytochrome P450 (CYP) enzymes and incubation of drug with liver microsomes can lead to the identification of phase I metabolites. Recently, considerable attention has received the using of a method based on electrochemistry/mass spectrometry (EC/MS) coupling to simulate oxidative phase I metabolism. Mass voltammograms generated by EC/MS technique provide a direct overview of oxidation products that may also be formed in the human body. Electrochemical oxidation is preferable to in vitro oxidation using CYP because it does not use any biomolecules (including enzymes or cofactors). Consequently, obtained oxidation products can be separated easily with minimal contamination or detected without the interferences from biological matrix. Numerous xenobiotics, such as antibiotics, emitted into the environment may directly influent the ecosystem. Most of them undergo chemical and microbial transformations after exposure to terrestrial or aquatic system. It is known that important degradation pathways of xenobiotics in the environment usually involve a redox reaction mechanism.

The aim of this work was to identify possible metabolites of several drugs belonging to the various groups of antibiotics. Simulation of the oxidative phase I metabolism was performed in aqueous medium (acetate buffer) at different pH values and graphite electrode was used as working electrode. After electrochemical oxidation, oxidation media was transferred to the LC-MS analyzer manually. LC-MS analyses were performed using LC-IT-TOF MS® (Shimadzu, Kyoto, Japan). LC experiments were performed on Ascentis C18 column (100 x 2.1 mm; 5 μm) (Sigma-Aldrich, Steinheim, Germany) using a gradient elution (water – acetonitrile) with a 0.2 ml/min flow rate. The $MS\text{-}MS^3$ experiments were set to automatic data acquisition within 50-1000 m/z values in both the positive and negative modes. Data acquisition and data evaluation was performed using LCMS Solution ver. 3.4.151 (Shimadzu). Identification of possible metabolites was performed using MetID Solution ver. 1.1.31 (Shimadzu).

Key words: *electrochemical oxidation, antibiotics, LC-ESI-IT-TOF-MS*

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Synthesis and Adsorption Properties of Collagen-Silica Aerogel Hybrids

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Aerogels are solid materials exhibiting unique properties like extremely high porosity, low density, very high specific surface area and mesoporous structure. Their polarity and surface properties can be modified either by varying of the nature of the starting materials or by the surface treatment in the post-gelation process. We have synthesized special silica-collagen hybrids by the sol-gel procedure and modified their structure by post-gelation silylation to produce hydrophobic materials for biomedical and bioanalytical applications. Both natural and silylated hybrids showed flexible structure and high collagen ratio resulted in exceptional mechanical strength compared to native silica aerogels.

Wetting properties of hydrophobic silica-collagen hybrid (HSC) was found to be quite different from wetting of C18-modified standard chromatographic medium. While C18 particles required a relatively high level of organic component, HSC required approximately 0.1% organic modifier only, which made it more feasible for extraction of moderately polar components from aqueous solutions.

Lutein, α-tocopherol and coffeine were used to test silica aerogels and silica-collagen hybrids as solid phase extraction materials. Different amount of compounds solutions were mixed with 65 mg of aerogels, and were washed with the solvent, then they were extracted with 3 ml of n-hexane, centrifuged and their concentration determined by reversed phase HPLC technique on a 150x4.6 mm ODS column (UV 292 nm, acetonitrile, 0.80 ml/min, and UV 400 nm , methanol, 0.80 ml/min). Results showed that addition of collagen to the silica aerogel structure modify the extraction potential, and surface treatment may increase the efficacy of the sorbents.

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P50

Separation of selected group of PROFENS by RP-HPLC methods

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In this work we dealt with the study of properties and chromatographic conditions of the four selected profens such as fenoprofen, flurbiprofen, ibuprofen and naproxen. Measurements were studied on chiral stationary phase Chiradex and achiral stationary phase Purosphere RP-18e. The drug study was focused on the influence of organic modifier contents, pH and the flow rate of mobile phase in reversed-phase system. We studied profens at DAD and ELS detectors.

Before the HPLC analysis of active substance ibuprofen in a drug MIG 400 two approaches to the sample pretreatment was tried out. One approach was simple dissolving and centrifugation of drug sample. Second one was flow extractions with different types of sorbents on the bottom of small column. We used Silasorb C18, Silasorb Diol, Silasorb Amine, Silasorb Fenyl. From the comparison of the chromatographic relations had emerged that it was not necessary to use flow extraction from solid samples before the determination of ibuprofen in tablet MIG 400. This sample pretreatment approach was used in the determination of active substance flurbiprofen in tablet called Flugalin and a capsule Flugalin Retard as well. The work was given an attention which was dealing with the sample pretreatment of urine samples with an addition of the abovementioned drugs before the HPLC analysis.

The second part is devoted to the study of enantiomeric separation of fenoprofen, flurbiprofen, and ibuprofen on the chiral stationary phase, Chiradex. Naproxen is available as single S-enantiomer. The mobile phase consist of methanol:ammonium formate pH 3.0 flow rate 1.0 ml/min. The chiral resolution of flurbiprofen enantiomers was 0.74 and ibuprofen 0.81. The separation was not achieved for fenoprofen. We also studied chiral resolution of active substance ibuprofen in tablet MIG 400 and active substance flurbiprofen in tablet Flugalin and Flugalin Retard, too.

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