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PBA1

DETERMINATION OF URINARY RETINOL AS A NEW POTENTIAL BIOMARKER

KUČEROVÁ, K.,[1,2] KUJOVSKÁ KRČMOVÁ, L.,[1,2] MATYSOVÁ, L.,[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Clinical Biochemistry and Diagnostics, University Hospital in Hradec Králové, Czech Republic

e-mail: kucerok1@faf.cuni.cz

Renal proximal tubule cells are very important for the metabolism and homeostasis of vitamins, especially vitamin A. Some studies show that urinary retinol might be used as an early state biomarker for detection of kidney failure but no clinically useful chromatographic method for simultaneously determination of urinary retinol and creatinine as a urine dilution factor is currently available.[1] Therefore, we developed new UHPLC-UV-MS/MS method using column packed with fluorinated core-shell stationary phase, and acetonitrile with ammonium formate buffer solution as the mobile phase for separation of these two analytes that differ significantly in their physicochemical properties. The method involves very fast and simple sample preparation requiring small amount of sample matrix and solvents. Deuterium labeled internal standard was used for the more precise quantification.[2] The method was tested with real-life samples using urine collected from patients suffering from colorectal or peritoneal cancer, and malignant neoplasm of kidney.

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PBA2

MITOCHONDRIAL ANALYSIS OF HUMAN PLATELETS: COMPARISON OF DIFFERENTIAL CENTRIFUGATION AND CONTINUOUS FLOW APHERESIS

VERNEROVÁ, A.,[1,2] GARCIA-SOUZA, L. F.,[3] GNAIGER, E.,[3] KUJOVSKÁ KRČMOVÁ, L.,[1,2] SOBOTKA, O.,[4,5]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Rep

[2] Department of Clinical Biochemistry and Diagnostics, University Hospital Hradec Králové, Czech Rep

[3] Medical University of Innsbruck, Innsbruck, Austria and Oroboros Instruments, Innsbruck, Austria

[4] 3rd Department of Internal Medicine, University Hospital Hradec Králové, Czech Rep

[5] Department of Physiology, Faculty of Medicine in Hradec Králové, Charles University, Czech Rep

e-mail: verneran@faf.cuni.cz

Using modern high-resolution respirometers (HRR) the analysis of mitochondrial functions and the metabolic profile of platelets (PLT) from a small sample of human blood was made accessible. A standardized isolation procedure for human PLT was developed in the frame of international COST action MitoEAGLE and PLT prepared by this differential centrifugation (DC) secured a high inter-laboratory reproducibility of respirometry results [1]. Continuous flow apheresis (CFA) is a clinical method for PLT isolation aiming for treatment of bleeding diathesis in severe thrombocytopenia with years of good clinical outcomes.

The aim of this project was to introduce a new method of HRR in University Hospital Hradec Kralove and compare mitochondrial respiration of PLT obtained by two different techniques: DC and CFA. HRR was assessed by Oroboros Oxygraph-2k FluoRespirometer (Oroboros Instruments, Austria). According to our preliminary results, the isolation method did not affect maximal respiratory capacity of PLT in this study. PLT isolated by CFA had 30% decrease in succinate oxidation in comparison to DC. Moreover, CFA affected PLT viability. These differences were reversed after washing the PLT by phosphate buffer saline, suggesting possible influence of isolation medium on our results.

We conclude that HRR is a highly sensitive and suitable method to describe the metabolic profile of human PLT. The application of HRR is promising in PLT research in patients with thrombocytopenia, sepsis and metabolic disorders as well as in diagnostics.

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PBA3

THE METHOD DEVELOPMENT FOR THE DETERMINATION OF CISPLATIN IN HUMAN PLASMA

TUROŇOVÁ, D.,[1,2] KUJOVSKÁ KRČMOVÁ, L.,[1,2]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Clinical Biochemistry and Diagnostics, University Hospital in Hradec Králové, Czech Republic

e-mail: turonovd@faf.cuni.cz

Cisplatin is well known for its antitumor activity but it is also associated with side effects, which their relevancy depend on its exposure. Extended exposition can cause nephrotoxicity and other health problems.[1,2] Cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy is a procedure combining maximal surgical removal of the tumor with intra-operative administration of a chemotherapeutic drug that is heated up. The aim of the whole process is to increase the anticancer effect and minimalize the systemic side effects of the chemotherapy in comparison with systemic chemotherapy.[3]

For the purpose of clinical research, a HPLC method with diode-array detection for the determination of cisplatin in human plasma with simple sample pretreatment has been developed and current results will be discussed. The separation was carried out in 8 minutes using C18 core-shell column with mobile phase consisted of methanol, acetonitrile, and water. As internal standard palladium chloride was used.

The study was supported by SVV 260 412, the University Hospital in Hradec Králové MH CZ - DRO (UHHK, 00179906), and by Ministry of Health of Czech Republic via grant NV18-03-00130.

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PBA4

DEVELOPMENT AND VALIDATION OF UHPLC METHOD FOR THE MONITORING OF TARGET ACTIVE SUBSTANCES AND THEIR RELEASING FROM SOLID DISPERSIONS

ARNOLTOVÁ, K.,[1,2] KUJOVSKÁ KRČMOVÁ, L.,[1,2] MATYSOVÁ, L.,[1]

[1] Department of Analytical chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Clinical Biochemistry and Diagnostics, University Hospital in Hradec Králové, Czech Republic

e-mail: arnoltok@faf.cuni.cz

This work is part of a project, that is focused on the development, optimization, and validation of UHPLC methods and their subsequent application on the evaluation of new modern drugs forms based on solid dispersions. The research is based on the development of modern drugs forms, which are using branched polymers as a carrier of active substances with the effort to influence the release of the target drugs and increase their bioavailability. The determination of drug-releasing from the new carrier is for the research crucial. Newly developed chromatographic methods will serve as the main tool for testing various materials used as a carrier of active substances, for the determination of drugs content and detection of possible impurities.

In the presented UHPLC method, miconazole, econazole and its main impurities were chosen as the target analytes. A UHPLC method, using diode-array detection, has been created. A Kinetex^{TM} C18 column, 1.7 μm particle size, 50 x 2.1 mm was used for the separation in combination with the acetate buffer, methanol, and acetonitrile as the mobile phase. Butylparaben was chosen as the internal standard. All substances were detected at a wavelength of 225 nm. The total analysis time was 9 minutes. The new method will be validated and used for testing various branched polymers as a suitable carrier.

The study was supported by SVV 260 412.

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PBA5

IDENTIFICATIONS OF ARTIFICAL MODIFICATIONS INDUCED IN THE COURSE OF LC-MS ANALYSES OF PEPTIDES

LENČO, J.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Re-public

e-mail: lenco@faf.cuni.cz

Column temperature is one of the key parameters that remarkably increases efficiency in bot-tom-up LC-MS proteomic analyses. We noticed, however, that at some point, the benefit of elevated temperature to the peak shape is redeemed by lower number of identified peptides. We hypothesized that an in-column peptide degradation might occur when peptides are separated at elevated temperature using acidic mobile phases. To this end, we scrutinized the effect of temperature on the stability of model proteins trapped in a reversed phase column. We confirmed that temperature as high as 45 °C in combination with 0.1% formic acid may al-ready induce on-column peptide bonds cleavage. We subsequently carried out data-dependent LC-MS analyses of tryptic peptides at various column temperatures. We found out that besides in-column peptide bonds cleavage, peptides trapped in a stationary reversed phase may undergo various artificial chemical modifications in the presence of 0.1% formic acid in mobile phases. Additionally, the risk of artificial peptides formylation due to inappropriate sample handling was discovered within the study.

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PBA6

AUTOMATION OF ON-LINE SAMPLE PREPARATION FOR THE PEPTIDE MAPPING ANALYSIS OF BIOPHARMACEUTICALS

KHALIKOVA, M., LENČO, J., FAISTAUEROVÁ, K., ŠVEC, F.

Charles University, Faculty of pharmacy in Hradec Kralove, Ak. Heyrovskeho 1203, 50005 Hradec Kralove, Czech Republic

e-mail: khalikom@faf.cuni.cz

Peptide mapping is critical methodology in routine biopharmaceutical analysis used to confirm identity, characterize primary structure, monitor degradative events, and meet quality assurance[1]. Peptide mapping is the gold standard method for the in-depth study of post-translational modifications (PTMs) of monoclonal antibodies (mAb). PMTs are critical quality attributes defining therapeutic efficacy and safety[2]. Both PTMs and chemical modifications may arise during production, processing, and storage of proteins[1]. Thus, the identification and quantification of PTM is essential in development and analysis of therapeutic biomolecules.

Sample preparation for peptide mapping requires enzymatic digestion of protein that is typically a time-consuming and labor intensive process. It includes several steps prone to operator error that can induce an increase in artificial modifications such as asparagine deamidation[3].

The multidimensional LC system was used for automation of the digestion procedure of trastuzumab to optimize the sample preparation of this mAb prior to peptide mapping. We will describe ongoing project focused on the setup of automated digestion process as a part of multiattribute analysis of mAb. Our study concerns effects of method conditions on the quality of digests of trastuzumab including type and pH of digestion buffer, incubation time, and temperature.

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PBA7

ELECTROCHEMICAL STUDY OF OLIGONUCLEOTIDE PROBES LABELED BY QUANTUM DOTS FOR THE DETECTION OF BACTERIAL AND VIRAL PATHOGENS

BANÁŠ, D.,[1,2,6] AHMET, D.A.,[1,3,4] NOGUERA, V.M.,[1,3,5] PAY, M.,[1,3,4] HOSNEDLOVÁ, B.,[6] KIZEK, R.,[1,3,6]

[1] Department of Research and Development, Prevention Medicals s.r.o., Czech Republic

[2] Department of Biochemistry, Masaryk University, Czech Republic

[3] Department of Human Pharmacology and Toxicology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

[4] Bezmiâlem Foundation University, Turkey

[5] Faculty of pharmacy in Barcelona, Spain

[6] Veterinary Research Institute, Czech Republic

African swine fever virus (ASFV) is a virus, with high morbidity and mortality, which is infectious for domestic and wild pigs and causes serious socioeconomic impact[1]. Because of the non-existence of suitable vaccine, it is necessary to determine the presence of the virus directly in the focus of infection. Thus, the cornerstone of design of a biosensor for rapid analysis of ASFV was suggested. This work is focused on the electrochemical study of oligonucleotides (ODNs) KING R and KING F which are specifically complementary to ASFV DNA, and quantum dots (CdTe QDs) as a label for these ODNs. The adsorptive transfer technique was used for the accumulation of ODNs to a working electrode. In the first phase, the dependences of the current on concentration and time of accumulation of ODNs were measured. The calibration ranges from 0.4 to 1.7 $\mu g \cdot mL^{-1}$ for KING F and 0.1 to 0.6 $\mu g \cdot mL^{1}$ for KING R were measured and the LODs, LOQs and CVs were calculated. CdTe QDs interacted with ODNs. CA (cytosine and adenine) signals of ODNs were 33.9 ± 8.7 nA and 22.0 ± 3.3 nA for KING F and KING R, respectively. For interaction CA signals were detected as follows: KING F/QDs 15.8 ± 8.6 nA, KING R/QDs 16.1 ± 5.1 nA and cadmium signals were detected as follows: KING F/QDs 61.8 ± 15.7 nA and KING R/QDs 89.3 ± 18.2 nA. This study brought pilot results for the creation of biosensor for detecting bacterial and viral pathogens, which can be useful for the suggestion of suitable pharmaceutical therapy.

The study was supported by the EU programme for education, training, youth and sport ERASMUS+ and project AMOR QK1920113.

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PBA8

LC-MS/MS STUDY OF FIRST PHASE IN-VITRO BIOTRANSFORMATION OF NEW PROMISING TACRINE DERIVATIVES

NOVAK, M.,[1;2] KUCERA, R.,[1] DOLEZAL, R.,[2] PRCHAL, L.,[2]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Biomedical Research Center, University Hospital Hradec Králové, Czech Republic

e-mail: novakm61@faf.cuni.cz

Alzheimer's disease is a neurodegenerative disorder causing decline in cognitive functions, gradual loss of self-control, development of disorientation, and afterwards motoric failure. Current symptomatic pharmacotherapy is primarily focused on acetylcholinesterase inhibitors and NMDA (N-methyl-D-aspartate) receptor blocking.[1] Tacrine molecule, which is one of the acetylcholinesterase inhibitors, was withdrawn from the market due to the hepatotoxicity of its metabolite 7-hydroxytacrine in 2013. The substitution of tacrine molecule may potentially block the formation of toxic metabolites. The introduction of methoxy group to position of 7 the 1,2,3,4-tetrahydroacridine led to 7-methoxytacrine.[2]

The aim of our work was to determine the metabolites of tacrine and 7-methoxytacrine and to evaluate the effects of biotransformation in *in vitro* study. Human liver microsomes (HLM) were used as first phase *in vitro* biotransformation model and HPLC coupled with Q Exactive Plus mass spectrometer was used for the characterization of metabolites.

The new HPLC-MS method for the separation and identification of tacrine and 7-methoxytacrine metabolites was created and structures of metabolites were experimentally designed from Full-MS and MS/MS spectra and confirmed by MassFrontier (MetWorks).

The results of the study show that the main way of biotransformation of both compounds is their monohydroxylation and dihydroxylation. Moreover, several novel *in vitro* metabolites of tacrine and 7-methoxytacrine which have not been reported in the literature so far were found and the relative proportion of individual metabolites was calculated.

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PBA9

ANALYSIS OF ECCRINE SWEAT

PRCHAL, L.,[1]

[1] Biomedical research centre, University Hospital Hradec Králové, Czech Republic

e-mail: lukas.prchal@fnhk.cz

While sweating is usually found unpleasant and annoying it is very important mechanism that provides our bodies with effective temperature control in different situations. Also its composition can be influenced by various substances or state of health. There are two main types of human sweat. Apocrine sweat consists of predominantly lipophilic substances and eccrine sweat, which could be described as a hydrophilic, consists mainly from water and various ions and small amount of other substances[1]. Our research focuses on eccrine sweat and these substances. Sweat is collected by patches worn for one week. Then the patch is collected and extracted and extracts is lyophilised. The samples are then analysed by HPLC coupled with mass spectrometry. As the majority of metabolites are hydrophilic HILIC column was used to at least partially separate them. The analysis was performed in both positive and negative modes. In this initial state we have processed only a small amount of patches, which are considered healthy controls for future research.

The study was supported by MH CZ - DRO (UHHK, 00179906)

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PBA10

ION MOBILITY SPECTROMETRY IN DOPING ANALYSIS

PLACHKÁ, K.,[1] PEZZATTI, J.,[2] NICOLI, R.,[3] VEUTHEY, J.-L.,[2] GUILLARME, D.,[2] NOVÁKOVÁ, L.,[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] School of Pharmaceutical Sciences, University of Geneva, Switzerland

[3] Swiss Laboratory for Doping Analysis, Centre universitaire romand de médecine légale, Switzerland

e-mail: plachkka@faf.cuni.cz

This project aimed to examine the effects of using ion mobility spectrometry – mass spectrometry in doping control analysis using 194 compounds from World Anti-Doping Agency prohibited list, including stimulants and narcotics (Class I) and anabolic steroids, glucocorticoids, and hormones (Class II). MS^e data independent acquisition scan type with high-resolution mass spectrometry was used for the experiments. The analyses were carried out using q-TOF (quadrupole-time of flight) with and without activation of the ion mobility spectrometry dimension. The ultra-high-performance liquid chromatography- mass spectrometry method was developed using standard mixtures to tune the settings of mass spectrometer and to optimize the data processing method. Subsequently, the method was used with urine samples prepared by dilute and shoot approach for Class I and by supported liquid-liquid extraction (SLE) for Class II. Prior to the analysis, SLE procedure was optimized to ensure sufficient sensitivity with final pre-concentration by factor of 10. Finally, the analyses of standard doping agents and urine samples were compared with and without ion mobility function, including the comparison of collision cross section (CCS) values, fragmentation, and quality of spectra. The robustness of the method was proved by intraday, interday, and interweek repeatability of retention times and CCS values, providing RSD values always lower than 2 %. The effect of matrix on CCS values was examined as well as matrix effects and fulfillment of minimum required performance limits. The effect of ion mobility on the quality of spectra, elimination of interferences, and method sensitivity was evaluated with the aim to improve screening capabilities, especially to prevent false positive and false negative results.

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STUDY OF INCREASING SENSITIVITY IN ESI$^-$ BY ANION ATTACHMENT

LHOTSKÁ, I., KOČOVÁ VLČKOVÁ, H., NOVÁKOVÁ, L.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: lhotski@faf.cuni.cz

Increasing detection sensitivity is crucial task in LC-MS method development. Apart from the instrument limits, ionization efficiency is the key to good sensitivity. In case of poorly ionized analytes, alternative LC-MS mobile phase additive can be considered. Besides conventional volatile acids and buffers enhancing deprotonation of analytes in ESI$^-$ and increasing conductivity, the ionization can be achieved by adduct formation or through gas phase proton-transfer reactions.

For this study, ammonium fluoride was selected as anion attachment additive for its strong gas-phase proton affinity. Generally, signal improvement of less polar molecules can be provided by an anion attaching to electropositive region of analyte molecule forming an adduct. Fluoride anion, due to higher gas-phase basicity than most of other anions and deprotonated molecules, is able to abstract the shared proton from the analyte and separate as HF. Finally, ionization efficiency is enhanced by production of $[M-H]^-$ in gas phase.[1]

Mobile phase containing ammonium fluoride (0.1-1mM) was compared to conventional additive 0.1% formic acid and its positive effect on signal intensity was assessed for broad spectrum of compounds varying in molecular weight, polarity, lipophilicity, and structures. Fluoride anion attachment potential as a solution for increasing sensitivity in ESI$^-$ is going to be discussed.

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PBA12

MODIFICATION OF CAPILLARY WALL BY GRAPHENE FOR SEPARATION IN CAPILLARY ELECTROPHORESIS

LOCHMAN, L.,[1] FELIX, O.,[2] DECHER, G.,[2] KUCERA, R.,[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Institute Charles Sadron, CNRS, 23 Rue du Loess, Strasbourg, France

e-mail: lochmanl@faf.cuni.cz

Graphene (G) is two-dimensional sp^2 single-atom-thick carbon sheet with hexagonal structure. High specific area (theoretical value 2630 m^2 g^{-1}) together with the affinity to carbon ring structures via π-π stacking interactions make G and graphene oxide (GO) promising candidates for application in analytical chemistry.[1, 2]

Our work aims on the modification of the inner surface of bare silica capillary by Layer-by-Layer method (LbL).[3] LbL is very simple self-assembly procedure which controls through electrostatic interactions adsorption of negatively charged GO onto wall covered by positively charged polyelectrolyte. Modification of capillary wall by GO is believed to substantially improve the separation properties for analysis of charged/neutral analytes due to the combination of the high CE efficiency and additional interactions with the modified surface. As it is difficult to characterize and optimize a nanoscale coating, the deposition was firstly done on the flat surface (silicon wafer or quartz slide) and different technique (ellipsometry, UV-Vis spectroscopy, AFM) were employed. Different combinations of polyelectrolyte/GO were tested. Based on these results, the coating process was transferred into the capillary and the separation of the model analyte mixture was compared with unmodified capillary.

The study was supported by Dean's fund (PROGRES Q42).

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PBA13

ANALYTICAL STUDY OF THE INFLUENCE OF EXPERIMENTAL CONDITIONS ON THE CHIRAL SEPARATION OF BORON CLUSTER COMPOUNDS

HORÁČEK, O., KUČERA, R.

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: horaceko@faf.cuni.cz

Boron clusters are artificial compounds which create 3D-cage structure and exhibit unique physicochemical properties. Carboranes are prepared by substituting BH units for CH units. They are studied as stereoselective catalysts, monomers for thermostable polymers and in medicine as compounds which enhance the effectivity of boron neutron capture therapy. Steric similarity with rotating phenyl ring enables extensive research of carboranes as new pharmacophores.[1]

Chirality of boron clusters is caused by introducing endo-/exoskeletal substituents, which impair the symmetry of the cage. Due to the chiral character of these compounds, it is vital to evaluate the influence of experimental conditions on chiral separations with respect to their potential use.

Even though zwitterionic carboranes were successfully separated on native beta-cyclodextrin based chromatographic columns, attempts to separate anionic carboranes were unsuccessful.[2] On the other hand, native beta-cyclodextrins were able to resolve some anionic carboranes in capillary zone electrophoresis.[3] To clarify this contradiction, chromatographic behavior of anionic carboranes was tested on a native beta-cyclodextrin column (ChiraDex, Merck).

Our work aims to elucidate the discrepancy between chiral separations of anionic carboranes by high performance liquid chromatography and capillary zone electrophoresis.

The study was supported by SVV 260 401.

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PBA14

LC-MS/MS INVESTIGATION OF CARDIOPROTECTIVE DRUGS

VÁŇOVÁ, N.,[1] BAVLOVIČ-PISKÁČKOVÁ, H.,[1] BRÁZDOVÁ, P.,[2] ŠTĚRBA, M.,[2] ŠŤERBOVÁ-KOVAŘÍKOVÁ, P.,[1]

[1] Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Department of Pharmacology, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic

e-mail: vanovan@faf.cuni.cz

Current anticancer therapy with anthracyclines is, despite its efficiency, limited due to the high risk of cardiotoxic effects. Up-to-date, the only clinically approved cardioprotective agent is dexrazoxane (DEX). Its novel analogue, JAS-2 [4,4'(Butane-2,3-diyl)bis(piperazine-2,6-dione)], exerts greater efficiency *in vitro* (neonatal rat cardiomyocytes model) when compared with DEX. Previously, UHPLC-MS/MS method for determination of JAS-2, its metabolite JAS-2_{met} and its pro-drug with a code name GK-667 was employed in our laboratory using UHPLC system (Nexera, Shimadzu) coupled with a triple quadrupole mass spectrometer (LCMS-8030, Shimadzu). Although this method was successfully applied on *in vitro* stability study on GK-667 and JAS-2 in plasma and DMEM medium, its use in pilot *in vivo* pharmacokinetic study was limited in terms of low sensitivity to GK-667 and JAS-2_{met}. Besides the attempts to enhance the recovery of these analytes by modification of the sample preparation step (i.e. precipitation with methanol), new LC-MS/MS method for determination of JAS-2 and its metabolite in plasma carried on LC system (1290 Infinity II, Agilent) coupled with a triple quadrupole mass spectrometer (Triple Quad LC/MS 6400 series, Agilent) was developed. Chromatographic separation of JAS-2 and JAS-2_{met} was performed on Luna Omega Polar column (100 x 3.0 mm, 2.5 μm, Phenomenex) using gradient elution system consisting of 1mM ammonium formate and methanol. The presented method led to achievement of the LLOQ at the level of 0.01 μM for both analytes, which is 20 times lower than the former procedure. Increased sensitivity enabled determination of JAS-2 and JAS-2_{met} in plasma of rabbits administered with GK-667 (5mg/kg, i.v., n=8).

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PBA15

MONITORING INFLUENCE OF PESTICIDE SPRAY ON ANTIOXIDANTS IN APRICOTS

HOLLÁ, M.,[1] KHOLOVÁ, A.,[1] SKLENÁŘOVÁ, H.,[1] ŠATÍNSKÝ, D.,[1]

[1] Department of Analytical Chmistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: hollama@faf.cuni.cz

Pesticides are substances used in agriculture to control pests and weeds. Their use is controlled, and the highest toxicologically acceptable amount of each pesticide is determined by maximum residue level. Apricots are valuable source of phytonutrients, substances with antioxidant properties, in human diet. Amount of these substances is influenced by cultivar type, harvest time, storage, processing, etc. For the presented study, ´Betynka´ apricot cultivar grown in orchards of Research and Breeding Institute of Pomology in Holovousy, was used.

This study focuses on influence of pesticide spray type applied in permitted level on nutritional properties of apricots regarding phenolics content and potential antioxidant properties. Effect of pesticide spray type was studied in fresh fruits and fruits stored for 10, 20, and 30 days after harvest in ultra-low oxygen warehouse. HPLC separation with diode array detection (DAD) was used to monitor content of 17 selected phenolic substances typically present in apricots. Moreover, HPLC with 8-channel electrochemical coulometric detector (CA) was used for evaluation of overall content of reducing agents, and Trolox equivalent antioxidant capacity assay (TEAC) for evaluation of radical scavenging activity of methanolic apricot extracts.

Results of phenolics determination and antioxidation activity in fresh and stored fruits with individual pesticide spray type obtained by HPLC-DAD, HPLC-CA, and TEAC methods were compared with control sample, without pesticide spray application. For statistical evaluation of the effect of applied pesticide spray on nutritional properties of fresh and stored apricots, ANOVA with repeated measures was applied on results obtained by HPLC-DAD. Based on the comparison, samples of tested spray types and control sample significantly differ from each other, considering the spray type and storage period.

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PBA16

COMPARATIVE STUDY OF VARIOUS SORBENTS FOR DETERMINATION OF OCHRATOXIN A AND OCHRATOXIN B IN ARCHIVE TOKAJ WINES USING ON-LINE SPE-HPLC

KHOLOVÁ, A.,[1] LHOTSKÁ, I.,[1] ŠPÁNIK, I.,[2] MACHYŇÁKOVÁ, A.,[2] ŠATÍNSKÝ, D.,[1]

[1] The Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] The Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Slovakia

e-mail: kholovaa@faf.cuni.cz

Various sorbents for on-line extraction and determination of ochratoxin A (OTA) and ochratoxin B (OTB) in archive Tokaj wine have been compared. Tokaj wine is a special type of sweet wine from grapes infected with mould *Botrytis cinerea* produced in Hungarian Tokaj wine region and in Slovakia. Our method involved on-line extraction using precolumn coupled to liquid chromatography with fluorescence detection and enabled fast and sensitive control of mycotoxin contamination. Four types of fibrous sorbets including polyethylene microfibers, polypropylene microfibers, polycaprolactone microfibers/nanofibers composite, and polycaprolactone microfibers/polyvinylidene difluoride nanofibers composite, typical fused-core C18 sorbent, and commercial molecularly imprinted polymers were compared while varying extraction approaches. The polymer fibers filled in a cartridge were directly connected to HPLC system and the clean-up efficiency and the subsequent chromatography separation optimized. Typically, 50 µL wine was directly loaded and preconcentrated in extraction column. The separation was then carried out using analytical column Kinetex Phenyl-Hexyl (100 × 4.6 mm, particle size 2.6 µm) followed by fluorescence detection (Ex 335 nm, Em 463 nm). Solvents suitable for extraction and separation were methanol or acetonitrile and 0.5% aqueous acetic acid. The separations were carried out in the gradient elution mode at a flow rate of 1.0 $mL.min^{-1}$. These conditions provided reliable validation results with a limit of detection of 0.03 – 0.06 $\mu g.L^{-1}$ and recoveries exceeding 90% were determined for both OTA and OTB in archive Tokaj wines. The maximum tolerable limit for OTA in wines authorized by the European Union is 2 $\mu g.L^{-1}$. Among the tested nanofibers, polyethylene enabled the best results while other nanofibrous materials are unsuitable for the analysis of ochratoxins. Comparable results were obtained using molecularly imprinted polymers, fused-core C18, and polyethylene microfibers. However, the last sorbent excels in the affordability. A more detailed comparison of sorbents will be presented.

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PBA17

PEAR TREES MATERIAL AS A RICH SOURCE OF IMPORTANT PHENOLIC COMPOUNDS

ADAMCOVÁ, A., ŠÍROVÁ, K., ŠATÍNSKÝ, D.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: adamcoa1@faf.cuni.cz

The aim of the study was to determine a spectrum of phenolic compounds and their content in pear trees – leaves, bark, buds, stems, floral ovaries, blossom and also in fruit and seeds. The methanol extracts were obtained from raw material of different pear tree cultivars. Extracted phenolic compounds – arbutin, chlorogenic acid and it´s derivates (1,5 and 3,5-di-caffeoylquinic acid) and rutin were analyzed by high performance liquid chromatography. Finally, ASCENTIS Express RP-Amide 150×4.6 mm, particle size 2.7 μm analytical column was used for analysis due to ability to separate both derivates of chlorogenic acid. Column temperature was 30 °C and injection volume was 1 μl. The separation was performed with gradient elution at flow rate 1 ml/min and analysis time 10.50 min. The mobile phase consisted of acetonitrile and 0.1% phosphoric acid. The detection was carried out at wavelengths 220, 327, 354 nm. The method was validated before the quantification of phenolic compounds in the leave extracts with following parameters: the linearity (R^2 = 0.997-0.999), repeatability (RSD = 0.49-3.09 %), recovery (88.66-114.26 %) and precision (RSD = 0.77-2.87 %). The following concentration range of total content expressed in mg/g of dry mass in defined extracts were obtained: leave extracts (22.62-54.80 mg/g), bark extracts (17.44-55.18 mg/g), bud extracts (14.24-31.93 mg/g), stem extracts (55.00-74.04 mg/g), ovary extracts (50.26-87.60 mg/g) and blossom extracts (38.04-62.83 mg/g). The concentration of evaluated phenolic compounds occurring in fruit and seeds extracts was significantly lower. Arbutin and chlorogenic acid were found as a major component. This research revealed that pear trees material is rich source of phenolic compounds and could be potentially used for further using.

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PBA18

POLYMER INCLUSION MEMBRANE (PIM)-COATED MAGNETIC STIRRER BAR FOR THE PRECONCENTRATION OF SULFAMETHOXAZOLE

ZATROCHOVÁ, S.,[1] ALMEIDA, M.I.G.S.,[2] KOLEV, S. D.,[2] ŠATÍNSKÝ, D.,[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] School of Chemistry, Faculty of Science, The University of Melbourne, Australia

e-mail: zatrochs@faf.cuni.cz

Sulfamethoxazole (SMX) belongs to the group of emerging organic contaminants, which may accumulate in natural waters causing environmental concern.[1] Its monitoring is thus very important. However, the levels of SMX present in natural waters is often very low (ng L^{-1} to μg L^{-1}) and for that reason its analysis consist of a challenge. The aim of this deals with using of magnetic stirrer bars coated with polymer inclusion membranes (PIMs) for the separation and preconcentration of SMX from natural waters. Initially the analyte is selectively retained within the PIM via ion-exchange by stirring the coated magnetic bar in the natural water sample. Following step is dipping the loaded PIM-coated stirrer bar in a smaller volume of a stripping solution to remove the SMX back to an aqueous solution for further analysis. Different types of base polymers, namely cellulose triacetate (CTA), polyvinyl chloride (PVC) or poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF- HFP) were tested for the fabrication of the PIM coating, while Aliquat 336 as used as the extractant. 1-tetradecanol and 2-nitrophenyloctyl ether (NPOE) were used as the modifier or plasticizer, respectively.

This study involves important PIM optimizations, particularly testing of different concentrations of casting solutions and the number of dips into the casting solution required to prepare a sufficient layer of PIM on the stirrer bar surface. Preliminary experiments revealed that the optimum PIM thickness that facilitates the fastest extraction rates is 20 μm, which corresponds to a variable number of dips depending on the PIM composition.

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PBA19

CARBON DIOXIDE EXPANDED LIQUID AS A SOLVENT FOR THE EXTRACTION OF QUERCETIN FROM PLANT MATERIAL

PILAŘOVÁ, V.,[1] KUDA, L.,[1] NOVÁKOVÁ, L.,[1] VAN STADEN, J.,[2] DOLEŽAL, K.,[3]

[1] Department of Analytical, Chemistry Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, South Africa

[3] Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Olomouc, Czech Republic

e-mail: pilarovv@faf.cuni.cz

Supercritical fluid extraction (SFE) using non-polar CO_2 as an extraction solvent is commonly used approach to extract bioactive compounds from natural sources such as dried leaves, herbs, fruits, and flowers. Neat CO_2 is an optimal choice to extract non-polar compounds. Thus, an organic solvent is often added to CO_2 to achieve extraction of more polar compounds. Methanol, ethanol (EtOH), isopropanol, and small water proportion are usually added to change polarity, mass transfer, extraction yield, and analytes solubility. Depending on the amount of added organic solvent, we recognize SFE, SFE with modifier, CO_2 expanded liquid extraction, and pressurized liquid extraction. Other parameters including temperature, pressure, and type of extraction can also improve the extraction yield. In our study, we optimized method for the extraction of quercetin from quince fruit. Placket-Burman model was used to determine the effect of addition of 0- 20 % water in EtOH as a green organic solvent added to CO_2 in ratio 10 – 90 %. We used temperatures in a range of 30 – 80 °C and a pressure of 100 – 300 bar. After its evaluation, the water amount in ethanol was set up on 20% due to low effect of this parameter. The tested ranges were consequently narrowed for other parameters using design of experiment. Then, 4 different flow rates in a range of 1-4 mL min^{-1} were explored to find the final extraction conditions. Optimized method used CO_2/EtOH + 20% H_2O (10/90, v/v) at 66 °C, pressure of 223 bar, and flow rate of 4 mL min^{-1}. These conditions enabled extraction of quercetin in 30 min with recoveries of 120 ng (22% RSD, n=9, 3 different days) from 0.5 g sample.

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3D-PRINTED MAGNETIC STIRRING CAGES FOR THE EXTRACTION OF BISPHENOLS FROM WATER USING MICRO- AND NANOFIBERS

ŠRÁMKOVÁ, I. H.,[1] HORSTKOTTE, B.,[1] ERBEN, J.,[2] CHVOJKA, J.,[2] ŠVEC, F.,[1] SOLICH, P.,[1] ŠATÍNSKÝ, D.,[1]

[1] Dep. of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Technical University of Liberec, Faculty of Textile Engineering, Department of Nonwovens and Nanofibrous Materials, Liberec, Czech Republic

e-mail: sramkovai@faf.cuni.cz

Electrospun nanofibers are currently in the focus of analytical chemists for their great potential as a sorbent in solid phase extraction techniques[1]. Apart from that, 3D printing nowadays holds a stable position in many areas of human activities including analytical chemistry due to the possibility of fast prototyping[2].

Using the 3D printing technology "Fused Deposition Modelling", we designed a cage-like holder with integrated magnetic stirring bar. The cage allowed accommodating up to 60 mg nano-polycaprolactone/micro-polycaprolactone fibers as a lose package for sorbent extraction. The device was immersed into 100 mL sample and was allowed to stir for 50 min for the extraction of eight bisphenols from natural waters. The analytes were then stripped using 5 mL methanol during 35 min. The limits of detection and quantitation were between 0.1 - 2.1 μg L^{-1} and 0.4 - 7.0 μg L^{-1}, respectively. The average analyte recovery at 50 ppb spike level was 99.0% ± 7.0%.

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PBA21

BIOMIMETIC CRYSTALLIZATION OF METAL ORGANIC FRAMEWORK FOR THE PREPARATION OF HYBRID MONOLITH IN CAPILLARY FORMAT FOR THE SELECTIVE EXTRACTION OF DRUGS

FIKAROVÁ, K.,[1,2] MAYA, F.,[2] HORSTKOTTE, B.,[1] SKLENÁŘOVÁ, H.,[1] ŠVEC, F.,[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] Australian Centre for Research on Separation Science, School of Natural Sciences (Chemistry), University of Tasmania, Australia

e-mail: fikaroka@faf.cuni.cz

Organic polymer monoliths are excellent supports for the chromatographic separation of large molecules. Monolithic columns feature high permeability and low back pressure. However, they suffer from a rather small surface area needed for the separation of small molecules. On the other hand, porous metal-organic framework (MOF) crystals are highly porous with widely tunable properties. Unfortunately, their packing in separation columns is challenging due to their small particle size and non-spherical shape. Therefore, we designed a new generation of separation media combining advantages of organic polymer monoliths and MOF while reducing their drawbacks. We prepared polydivinylbenzene monolith containing ZnO nanoparticles as the MOF metallic precursor. ZnO nanoparticles were then converted to zeolitic imidazolate framework ZIF-8 via biomimetic crystallization using 2-methylimidazole as the organic linker with addition of a bioactive molecule - amino acid - that enabled further modulation of MOF size and increased its selectivity. L-histidine, L-valine, phenylalanine, and glutamic acid were selected for our experiment and their effects on crystal morphology and adsorption selectivity were demonstrated. In situ polymerization time, and MOF crystals growth, were optimized to create open tubular capillary columns for enantioselective solid phase microextraction. Chiral selectivity and extraction capacity of the material were studied with batch extraction of propranolol enantiomers.

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PBA22

COMBINING LAB-IN-SYRINGE WITH BEAD-INJECTION FOR PRECONCENTRATION OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS IN SURFACE WATERS COUPLED ONLINE TO HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

GEMUH, C., HORSTKOTTE, B., SOLICH, P.

Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

e-mail: gemuhc@faf.cuni.cz

The modern flow techniques Lab-In-Syringe (LIS) and Lab-On-Valve (LOV) were combined and hyphenated to high-performance liquid chromatography for online micro-solid phase extraction of 5 nonsteroidal anti-inflammatory drugs (NSAID), namely ketoprofen, naproxen, flurbiprofen, diclofenac, and ibuprofen. The combined system ensured adequate mixing of large volume of sample with buffer inside the syringe enabling higher enrichment than typically achieved by simple LOV. SPE on a micro-SPE, packed automatically and in-system following the Bead Injection principle, was carried out in the LOV conduit using 4.4 mg of Oasis HLB® sorbent of particle size 30 μm for each sample analysis. Parameters such as injection volume, volume of sorbent suspension, ionic strength of buffer and the elution and loading flow rates were optimized. After washing the micro-SPE column with water to remove any unretained matrix components, the retained analytes were eluted with acetonitrile: water (50:50%(v/v)) and 350 μL of the eluate loaded into the HPLC injection loop. Separation of 5 NSAID was done on a Symmetry C18 column (4.6 x 150 mm, 5 μm) and C18 OPTI-GUARD® 1 mm guard column using a mobile phase of 30% (v/v) acetonitrile and 30%(v/v) methanol in 25 mmol/L ammonium formate buffer, pH 3.5, in isocratic regime. The method developed was reproducible with RSD values of 1 % to 7 % on 20 μg/L level with linear range of 10 μg/L to 200 μg/L and LOD less than 5 μg/L. Recovery factors between 91 to 109 were obtained for surface water samples at 20 μg/L level.

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PBA23

POLYSULFONE MEMBRANE ENRICHED IN BIOACTIVE COMPOUNDS TO REDUCE OXIDATIVE STRESS AND INFLAMMATION IN DIALYSIS PATIENTS

KOHLOVÁ, M.,[1,3] ROCHA, S.,[2,3] AMORIM G., C.,[3] SANTOS-SILVA, A.,[2] SOLICH, P.,[1] MONTENEGRO, C.,[3]

[1] Dep. of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

[2] UCIBIO\REQUIMTE Department of Biological Sciences, Faculty of Pharmacy, University od Porto, Portugal

[3] LAQV\REQUIMTE Department of Chemical Sciences, Faculty of Pharmacy, University od Porto, Portugal

e-mail: kohlm5aa@faf.cuni.cz

Oxidative stress (OS) and chronic inflammation are commonly present in dialysis patients, due to frequent contact of patients´ blood with artificial membrane. To reduce oxidative stress, vitamin E-coated membranes are used to scavenge the reactive oxygen species formed during the hemodialysis. Although the use of this type of membrane showed diminishment in some inflammatory and OS markers [1], the overall beneficial effect on mortality is still uncertain.

The aim of our work was to evaluate, if the enriched polysulfone (PSf) membranes with other bioactive substances, such as antioxidant lipoic acid or synthetic inhibitors of neutrophil elastase, could have preferable impact on OS and/or inflammation than vitamin E. Lipoic acid was incorporated in PSf membranes alone or together with vitamin E, while synthetic elastase inhibitors were immobilized on the membranes surface through adsorption. The biological activity and biocompatibility of the modified membranes were studied *in vitro*. Both types of bioactive compounds immobilized on PSf membrane showed promising effect on diminishment of OS/inflammation and therefore could be considered for future treatment.

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PBA24

LIQUID-PHASE MICROEXTRACTION OF ORGANOPHOSPHORUS PESTICIDES USING SUPRAMOLECULAR SOLVENT AS A CARRIER FOR FERROFLUID

HASHEMI, B.,[1] ZOHRABI, P.,[2] SHAMSIPUR, M.,[1] HASHEMI, M.,[1,2]

[1] Department of Analytical Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran

[2] Department of Analytical Chemistry, Faculty of Chemistry, Bu-Ali Sina University, Hamedan, Iran

e-mail: hashemib@faf.cuni.cz

Supramolecular solvents (SUPRASs) are water immiscible nano-structured solvents composed of 3D amphiphilic aggregates, which have been used in microextraction procedures. The most important feature of supramolecular solvents is their high solvation potential for a wide range of target analytes (both polar and non-polar ones). As collecting of extracting solvent is of great importance in the liquid-phase microextraction, ferrofluids–suspended magnetic nanoparticles in a carrier liquid-can overcome the drawbacks such as centrifugation and refrigeration. In this study, we used supramolecular solvent as a carrier for ferrofluid and extracted three organophosphorus pesticides (OPPs) in water and fruit juice samples. To this end, oleic acid coated magnetic nanoparticles were prepared to omit the centrifugation step and they were used in combination with SUPRAS in the extraction process. The influence of main variables on the extraction efficiency was investigated using response surface methodology (RSM) based on central composite design (CCD). Under the optimum experimental conditions, the resulting calibration curves were linear in the concentration range of 0.5-400 μg L^{-1}. The intra-day and inter-day precisions were evaluated to be in the range of 2.0-5.3 % and 2.6-5.7 %, respectively. The obtained limits of detection (LODs) also ranged from 0.1 to 0.35 μg L^{-1}.

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