FACULTY OF PHARMACY
IN HRADEC KRÁLOVÉ
Charles University

12th Postgraduate and Postdoc Conference

1. – 2. February 2022

Abstracts

Section 4 - Pharmaceutical Analysis and Bioanalytical Chemistry

EFSA-CDN

This conference is supported by the EFSA-CDN project (Reg. No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by the European Union

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APPLICATION OF UHPLC-MS/MS METHOD FOR DETERMINATION OF INFLAMMATORY BIOMARKERS IN GINGIVAL CREVICULAR FLUID

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Gingival crevicular fluid (GCF) is a transudate or an exudate of plasma, which is secreted via gingival crevice / periodontal pocket.[1] Since GCF is secreted at the site of inflammation, analysis of GCF provides an information about current inflammation level of periodontal tissue.[2] Determination of GCF inflammatory biomarkers such as neopterin, kynurenine, and tryptophan can contribute to diagnosis, evaluation of treatment and progression of periodontal diseases (gingivitis and periodontitis). We developed a new UHPLC-FLD-MS/MS method using core-shell stationary phase for determination of neopterin, kynurenine, tryptophan, and creatinine in GCF. The separation of these four substances was achieved using very simple sample preparation technique requiring small amount of sample and was completed within 4 min. Deuterium labelled internal standard was used for the precise quantification. The method was tested with real-life samples using GCF collected from patients suffering from periodontitis and healthy controls.

The study was supported by Project SVV 260 548, by institutional grant PROGRES Q40/13, and by University Hospital in Hradec Králové, 00179906.

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PBA2

OPTIMIZATION OF UHPLC MS/MS METHOD FOR DETERMINATION OF VITAMIN K IN SERUM

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Vitamin K is an essential factor in blood coagulation, and also its protective effect on bone metabolism and vascular calcification is well known. It consists of many similar compounds, vitamin K1 (phylloquinone) and K2 (menaquinones, MK4-MK13)[1].

Determination of vitamin K is a challenge in many aspects of bioanalysis. In addition to the low concentration, there are also obstacles such as its high photosensitivity, the matrix interference occurrence, and its adhesion to plastic and glass surfaces[1]. These limitations can be solved by the development of a new UHPLC-MS / MS method with a modern sample pre-treatment procedures.

During the optimization of the method, the attention was paid mainly to the right choice of stationary and mobile phase, appropriate mobile phases additives, and optimization of sample preparation. Columns based on C18 or biphenyl phases with various lengths and particle sizes were tested. Solvents like methanol, acetonitrile, water, and isopropanol in combination with additives such as acetic acid, formic acid, ammonium acetate, and ammonium formate were experimented with to select a suitable mobile phase. Several microextraction procedures were tested as part of the vitamin K extraction process from serum. The best choice seems to be the Supported Liquid Extraction in combination with protein precipitation and ultrasonication. The detailed results will be presented and discussed.

The study was supported by the Czech Health Research Council (NU21J 02 00021), the Charles University (SVV 260 548, GA UK no 3602/2021), and MH CZ DRO (UHHK, 00179906)

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

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Current symptomatic pharmacotherapy for Alzheimer's disease is primarily focused on acetylcholinesterase inhibitors and blocking of the NMDA receptor (N-methyl-D-aspartate). Tacrine, a molecule with both of the mechanisms of action, was withdrawn from the market in 2013 after 20 years of use due to the hepatotoxicity probably caused by its 7-hydroxytacrine metabolite.[1] A rationale substitution of the tacrine molecule can potentially hinder the formation of a toxic species. The introduction of the methoxy or phenoxy group to position 7 or chlorine to position 6 on the 1, 2, 3, 4-tetrahydroacridine moiety led to 7-methoxytacrine (7-Meota), 7-phenoxytacrine (7-Phota), and 6-chlorotacrine (6-Cl-ta), respectively.[2]

The aim of our work was to determine and compare emerging metabolites of tacrine and its three derivatives. Human liver microsomes (HLM) were used as the first phase *in vitro* biotransformation model, and HPLC coupled with Q Exactive Plus (MS/MS) was used for the characterization of emerging metabolites.

The new HPLC-MS method was developed for the separation and identification of tacrine, 7-Meota, 7-Phota and 6-Cl-ta metabolites. The structures of metabolites were experimentally designed from full-MS and MS/MS spectra and some of them were confirmed by *de novo* synthesis. The results of our study enabled the comparison of the biotransformation of these four compounds. Generally, monohydroxy and dihydroxy metabolites were observed. Moreover, several novel *in vitro* metabolites, which had not been reported in the literature so far, were found. The relative proportions of individual metabolites were calculated on the basis of chromatographic data and their importance assessed.

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COMPLEX SCREENING OF RETENTION BEHAVIOUR OF CATECHOLAMINES

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Nowadays, in the light of relatively new information about the effect of serotonin and its metabolites level on fetal brain development [1], the question arises as to whether catecholamines also affect fetal development. Despite many published methods, the determination of catecholamines is still a challenge due to their chemical nature and low concentrations in complex biological materials [2].

As part of the development of an HPLC method for the determination of catecholamines in placental tissue, the screening of retention behaviour of tyrosine, *L*-DOPA, dopamine, noradrenaline, and adrenaline is presented. The most used chromatographic system in the determination of catecholamines is a reversed phase chromatographic system [3]. But low retention with poor resolution was observed by our screening in reversed phase system.

The alternative for analysis of polar compounds is HILIC [4]. This part of screening includes the behaviour of catecholamines using different concentration of organic solvent from 5 to 90 % on commercially available PFP stationary phase in two value of pH and describes behaviour of catecholamines under HILIC conditions on neutral, negatively charged and zwiterionic commercially available HILIC stationary phases. Detection of catecholamines is based on their absorption in the UV range and their fluorescence.

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PBA5

CHROMATOGRAPHIC DETERMINATION OF ALDEHYDIC LIPID PEROXIDATION PRODUCTS IN VITRO

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Overproduction of reactive oxygen species, if not adequately counteracted by the endogenous antioxidant system, may lead to severe damage to intracellular biomolecules. Malondialdehyde (MDA) and 4-hydroxynonenal are the most abundant individual aldehydes formed through lipid peroxidation and they are frequently used as oxidative stress biomarkers. For the assessment of total MDA levels in cells, intracellular content released by ultrasonication is subjected to alkaline hydrolysis of protein bounded MDA by NaOH followed by protein precipitation by trichloracetic acid. MDA, present in the supernatant, is derivatized by 2,4-dinitrophenylhydrazine (DPNH) and the hydrazone formed by this reaction is extracted by solid-phase extraction. Reversed-phase liquid chromatography coupled to an atmospheric pressure chemical ionization-tandem mass spectrometry (RP-LC-APCI-MS/MS) in a selected reaction monitoring (SRM) mode was used to monitor the transitions of *m/z* $[M+H]^+$ 235 → *m/z* 159,189 for MDA-DNPH and *m/z* $[M+H]^+$ 237 → *m/z* 161,191 for d_2-MDA-DNPH (internal standard).

Since drugs represent a diverse group of oxidative stress triggers, evaluation of their potency to induce oxidative damage *in vitro* is an essential part of toxicity studies in drug development and discovery. However, the exact mechanism of drug-related oxidative stress and, furthermore, the relationship between chemical structure (or particular structural features) and the resulting oxidative damage is still largely unknown.[1]

The study was supported by Charles University [Progress UK/Q42].

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PBA6

DEVELOPMENT OF UHPLC-UV STABILITY INDICATING METHOD OF IXAZOMIB AND HRMS IDENTIFICATION OF ITS DEGRADATION PRODUCTS

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Ixazomib (IXA) is highly selective, reversible, and the only orally active proteasome inhibitor approved for clinical use in the EU in 2016 for the treatment of multiple myeloma. The aim of this project was to 1) develop and validate a first stability indicating analytical method for IXA, 2) apply it to investigate the degradation kinetics of the drug under stress test conditions, and 3) identify the main degradation products. The stability study of ixazomib was evaluated using an UHPLC-DAD system (Nexera, Shimadzu), and the identification of degradation products was performed on an orbitrap mass spectrometer with an ESI ion source (Q-Exactive™, Thermo Scientific). Different chromatographic columns were tested during the method development: Zorbax Bonus-RP (100 × 3.0 mm, 1.8 μm, Agilent), Kinetex C18 and Kinetex F5 (both 100 × 2.1 mm, 1.7 μm, Phenomenex) and Acquity UPLC BEH C18 (100 × 2.1 mm, 1.7 μm, Waters). The best peak shape was achieved on the last column, and its length was finally shortened to 50 mm to reduce the analysis time. A mixture of ammonium formate, acetonitrile, and methanol was used in gradient mode as the mobile phase. The method was validated according to the ICH guidelines in a concentration range of 2.5 – 100 μg mL^{-1}, accuracy and precision were assessed in 3 concentrations and 4 replicates each. Ixazomib was exposed to acidic, alkaline, oxidative, neutral, and photolytic stress conditions. Ixazomib was prone to decomposition under oxidative conditions, on the contrary, it slowly degraded under neutral conditions. The main degradation products, which resulted from the splitting of an amide bond, were identified. In this study, the UHPLC-UV stability indicating method and identification of IXA degradation products were described for the first time.

The study was supported by SVV 260 547.

PBA7

APPLICATION OF CAPILLARY ELECTROPHORESIS FOR THE SIMULTANEOUS SEPARATION OF BOSWELLIC ACIDS AND NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

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This presentation describes the development of a capillary electrophoresis (CE) method for the simultaneous separation of boswellic acids and nonsteroidal anti-inflammatory drugs (NSAID). The method was optimized for the mixture of *Boswellia serrata* extract and 15 NSAID, specifically 4-dimethylaminoantipyrine, carprofen, celecoxib, diclofenac, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meloxicam, niflumic acid, piroxicam, phenylbutazone, salicylic acid, sulindac, and tiaprofenic acid. The effect of the background electrolyte (BGE) composition including its concentration, pH, and the type and amount of organic modifier on the separation selectivity for the model analytes was examined. Only volatile buffers were tested to assure BGE compatibility with mass spectrometric detection. The separation was carried out in 40/48.5 cm and 88/96.5 cm fused silica capillaries, 50 μm i.d. with the applied voltage of +30 kV and at the temperature of 25 °C. The analytes were detected at 200 and 250 nm.

The best resolution for the individual NSAID (15 compounds resolved into 12 peaks) was achieved in acidic BGE (50 mmol/l acetic acid, pH 4.5 : MeOH : ACN; 10:3:12). The boswellic acids, however, migrated unresolved with EOF under these conditions. The employment of alkaline buffers decreased the separation selectivity for the NSAIDs, but it allowed to improve the resolution of boswellic acids. The components of *Boswellia serrata* extract no longer migrated at the rate of EOF and were separated into 4 peaks when using 40 mmol/l ammonium acetate (pH 8.5), MeOH, ACN (5:1:4) buffer, and into 2 peaks when using 40 mmol/l ammonium bicarbonate (pH 8.5), MeOH, ACN (5:1:4) buffer. The mixture of 15 NSAID was separated into 9 peaks in both alkaline BGE.

The study was supported by SVV 260 548 and by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

PBA8

UHPSFC-MS/MS ANALYSIS OF VITAMIN D IN LIVER TISSUE

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Fat-soluble vitamin D plays an essential role in biological processes and its deficiency can be associated with various diseases. Its metabolism and the storage forms are not fully elucidated yet, although many forms, including hydroxylated metabolites and esters, were described. Analysis of vitamin D is challenging due to the similarity in compounds structures, different physico-chemical properties of metabolites groups, chemical instability, and complexity of the analyzed matrix.

Our study aimed to develop a fast, selective, and sensitive method for analysis of vitamin D, its hydroxylated metabolites, and esters using ultra-high performance supercritical fluid chromatography with tandem mass spectrometry detection (UHPSFC-MS/MS). 1-Aminoanthracene column providing good selectivity for hydroxylated metabolites and mobile phase containing CO_2 and methanol (MeOH) was used as the starting point [1] for following optimization where the effect of various modifiers, pressure, and the temperature was tested. The separation was finally achieved with gradient elution using MeOH/isopropanol mixture with 8% water at 140 bar and 60 °C. MS with atmospheric pressure chemical ionization and photoionization were compared in terms of matrix effects (ME) and limits of quantification (LOQ). Two solid phase extraction strategies, "bind and elute" and "removal", using a hydrophilic-lipophilic balance (HLB) copolymer sorbent with preceding acetone solvent extraction, were optimized for the liver tissue extraction. Both methods were compared in terms of recovery, ME, and LOQ, and finally applied to the clinical liver samples, where some esters were successfully detected for the first time.

The study was supported by the Carl Trygger Foundation (project: CTS 18:395) and STARSS reg. No.: CZ.02.1.01/0.0/0.0/15_003/0000465 funded by ERDF.

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PBA9

RETENTION TIME STABILITY IN UHPSFC BASED ON VARIABLE COLUMN HISTORY

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Organic modifiers and additives are necessary to achieve analyte elution and symmetrical peak shapes in the state-of-the-art ultra-high performance supercritical fluid chromatography (UHPSFC) methods. Besides their undisputable advantages, the presence of organic modifier and/or additive can cause several problems. Column selectivity varies during column use depending on the column history and the amount of adsorbed additive, especially when switching additive and additive-free conditions. Moreover, organic alcohol modifiers also interact with the stationary phase. The free silanol groups on the silica surface react with alcohol in the mobile phase resulting in the formation of silyl-ether structures (SEF) on the stationary phase's surface. The silyl-ether formation decreases the number of free silanols and thus decreased retention can be observed over time. The SEF is a condensation reaction reversible by water hydrolysis and catalyzed in basic/acidic environment. The aim of this study was to describe retention time shifts over 1-year-use of different stationary phases with various organic modifiers of CO_2-based mobile phase.

The set of 112 compounds with a wide range of physicochemical properties was analyzed using 4 stationary phases and 3 organic modifiers such as pure methanol, 10 mmol/L ammonia in methanol, and 2% water in methanol. The analyses were carried out using a generic SFC method to enable the elution of at least 60 compounds using each chromatographic conditions (stationary phase/organic modifier). Several data points were used for the retention time shifts evaluation, i.e., the 1^{st} injection and measurements after 1, 2, 3, 6, and 9 months. Finaly, the percentual differences between the 1^{st} and subsequent injections were calculated and correlated to used stationary phase, organic modifier, and physicochemical properties of analytes.

The study was supported by the Czech Science Foundation project GAČR n. 21-27270S and by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

PBA10

NON-TARGETED UHPLC-DIA-HRMS APPROACH FOR EXAMINATION OF IMPURITIES IN LEVOTHYROXINE FORMULATIONS

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Levothyroxine is a synthetic hormone that is used in standard therapy for patients with hypothyroidism. Since it has a narrow therapeutic index, even a little change in its concentration can have a significant influence on therapeutic activity. To comply with a regulation, the Merck company changed the formulation of Levothyrox: the previous formulation containing lactose as an excipient was replaced by mannitol and citric acid, with the aim to prevent a reaction between lactose and levothyroxine.[2] However, this substitution led to many adverse effects, which were reported by approximately 1.43% of patients treated with the new formulation of Levothyrox.[3]

The aim of this study was to develop an analytical method for the comparison of various levothyroxine formulas. For this purpose, a non-target approach employing ultra-high performance liquid chromatography coupled with a data-independent acquisition high-resolution mass spectrometry (UHPLC-DIA-HRMS) was used. The data acquisition method, so-called MS^E, provides simultaneous acquisition of HR mass spectra of all the ionizable compounds together with their respective fragmentation spectra. This combination is very helpful for the identification of impurities and reactive products. All the acquired ions were processed by principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), with a focus on the selection of compounds important for the classification of the samples and evaluation of differences between individual batches. Important markers discriminating the samples among the groups were mostly identified as reaction products between mannitol and other excipients. In addition to that, undeclared phospholipids were also discovered in the tablets. These compounds can affect the bioavailability of the active compound.

This work was supported by the project EFSA-CDN (No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF.

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PBA11

COMPARISON OF TWO MATRIX EFFECT EVALUATION APPROACHES

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The matrix effects (MEs) are one of the important drawbacks of LC-MS methods. Although these effects of matrix compounds on MS response can be minimized or compensated, it is usually not possible to totally mitigate them from LC-MS analysis. Therefore, MEs need to be considered during method validation to enable reliable quantification of target analytes. Two main strategies, the post-extraction addition approach and the comparison of calibration curve slopes, were proposed for the ME quantification. The goal of this study was to systematically compare two evaluation approaches using samples with complex matrix, namely plasma, and only a non-selective sample preparation method such as protein precipitation. Nowadays, the post-extraction addition method is the method of choice as it is defined by several guidelines, for example in EMA guideline. The main advantage is that analyte concentration is taken into account. Therefore, this approach enables to obtain more accurate results of ME. The standard and spiked matrix calibration curves were measured for 27 compounds in positive and negative ionization modes. The suitability of four linear calibration models, i.e., a least square, logarithmic, and weighted 1/X and $1/X^2$ for individual compounds were evaluated with regards to coefficient of determination and %-errors. MEs calculated from calibration curve slopes were then related to the MEs calculated by the post-extraction addition approach at all concentration levels. MEs in negative ionization mode with maximal value of 151% were significantly lower compared to positive mode with maximal value of 8970%. The same trend was observed for both evaluation approaches. However, the calibration curve slope approach provided significantly lower MEs. For all compounds with ME < 20%, the ME results of both evaluation approaches corresponded. The rest of compounds provided different ME results for both approaches and significantly depended on the type of calibration model used for the evaluation.

The study was supported by the project EFSA-CDN (No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF.

PBA12

ANALYSIS OF BIOGENIC STEROIDS IN MOUSE PLASMA SAMPLES: FROM CHROMATOGRAPHIC METHODS TO SAMPLE PREPARATION

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The analysis of biogenic steroids remains a troublesome analytical challenge despite many published studies using traditional GC-MS and LC-MS methods. Structural similarity resulting from a typical steroid skeleton and minor structural modifications is often multiplied by the common loss of water molecules in MS and MS/MS spectra, creating additional isobars. This fact, another time beneficial for the development of sample preparation methods, imposes requirements on the achievement of full chromatographic separation in terms of elimination of observed interferences and correct quantification of target analytes in biological samples.

The present study aims to develop and optimize fast and sensitive UHPLC-MS/MS and UHPSFC-MS/MS methods for the quantification of 35 biogenic steroids, from the groups of C19 (androstanes), C21 (pregnanes), and 2 synthetic steroids in mouse plasma. A total of 37 steroidal compounds generated 11 critical pairs/groups of analytes and resulted in a challenging separation of 28 of them. The development of both UHPSFC and UHPLC chromatographic methods consisted of extensive screening of analytical columns with various stationary phase chemistries, different types of particles, and particle sizes. The columns with the best separation score of isomers were selected for further optimization of the gradient elution, using various gradient programs and gradient curves, analysis time, temperature, and also organic modifiers in SFC. The protein precipitation, supported liquid extraction, and combination of these methods are tested for the pretreatment of mouse plasma samples, to ensure the release of binding to transport proteins, sample purification, and minimization of matrix effects.

The study was supported by the GA UK project No. 348221, STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF, the project of specific research SVV 260548.

PBA13

A UHPLC-DIA-HRMS METHOD FOR A SELECTIVE AND BROAD-SPECTRUM DOPING CONTROL ANALYSIS IN EQUINE BIOFLUIDS: A SCREENING AND CONFIRMATORY APPROACH

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The main scope of anti-doping laboratories is to detect the administration or use of prohibited substances and methods by the analysis of biological samples of an athlete or racing animal. Therefore, there is an extensive need for continuous development of bioanalytical methods capable of providing qualitative and quantitative measures of the dope and-or its metabolite(s) to serve for better sports practice and ensure athletes and animals welfare[1].

The aim of this study was to develop a broad-spectrum screening method for prohibited substances used in equine sports utilizing the UHPLC-MS technique, with the advanced HRMS approach that provides the sufficient specificity and selectivity for multi-target analysis.

A major concern was given to develop the analytical method using the C18 columns that offer wide compatibility for separation of compounds with different physico-chemical properties, challenging the chromatographic separation of some isomeric drugs commonly reported by anti-doping laboratories without the need for using a specific chiral separation system. Also, to enable the simultaneous isolation of all the compounds of interest at fast and cost-effective strategies, a generic extraction procedure based on Liquid-Liquid Extraction technique using a mixture of tert-Butyl methyl ether - Dichloromethane – Ethyl acetate was utilized for both horse urine and plasma samples.

Combination of the above has established an efficient analytical method for doping control of more than 300 compounds of different acid-base properties in a single injection of 20.5min. Moreover, the full scan accurate-mass data obtained adds the possibility for the retrospective investigation without the need for reprocessing of test samples.

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PBA14

ALTERNATING CURRENT ELECTROSPINNING METHOD FOR PREPARATION AND MODIFICATIONS OF NANOFIBROUS SPE SORBENTS

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The electrospinning method using alternating current (AC) was firstly used for the production of nanofibrous material as a sorbent for extraction. The nanofibrous samples were produced from polyamide 6 (PA 6) using AC electrospinning and reference material via conventional direct current (DC) electrospinning. AC electrospinning yields higher productivity of fibers that are more porous and mechanically stable in high-pressure LC system. Moreover, the AC electrospun PA 6 nanofibers showed enhanced hydrophilicity caused by higher overall porosity and slight changes in surface chemical composition[1]. The applicability of the new material was demonstrated in a case study for extraction of bisphenol A, bisphenol S, butylparaben, and fenoxycarb followed by HPLC-UV.
In advance, AC electrospinning brings new possibilities of sorbent modifications. For example, it is possible to fabricate nanofibers with a higher load of graphene doping compared to the conventional method. New polycaprolactone nanofibers with 1-8% graphene were tested for simple solid phase extraction. Graphene content in nanofibers increased analytes retention and enabled trace-level contaminants preconcentration from high-volume environmental samples.

The study was supported by GAČR project no. 20-19297S and the Charles University project No. SVV 260 548.

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PBA15

STIR BAR SORPTIVE EXTRACTION USING HYBRID MONOLITHS INCORPORATING METAL-ORGANIC FRAMEWORKS COUPLED WITH HPLC-FD FOR DETERMINATION OF ESTROGENS

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A novel coating based on hybrid monolith with metal-organic framework (MOF) onto conventional Teflon coated magnetic stir bars was prepared. The surface of the Teflon stir bar was firstly vinylized in order to immobilize a glycidyl methacrylate (GMA)-based polymer onto the magnet. After this step an amino-modified MOF of type MIL-101 (NH_2-MIL-101(Al)) was covalently attached to the GMA-based monolith. The resulting hybrid monolith was evaluated as SBSE sorbent for extraction of three estrogens (estrone, 17β-estradiol, estriol) and synthetic 17β-ethinylestradiol from water and human urine samples followed by HPLC with fluorescence detection. Several parameters affecting extraction of target estrogens by stir bar sorptive extraction (SBSE) including pH, ionic strength, extraction time, stirring rate, desorption solvent and desorption time were investigated. Under the optimal experimental conditions, the analytical figures of the method were established, achieving satisfactory limits of detection in the range of 0.015-0.58 μg L^{-1}, recovery results ranging from 70-95% with RSD less than 6%, and precision values (intra- and inter-extraction units) below 6%.

The study was supported by the Charles University (Project SVV 260 548), Charles University Grant Agency (Project GAUK No. 1070120), and EFSA-CDN project (no. CZ.02.1.01/0.0/0.0/16_019/0000841) cofunded by ERDF.

USED NANOFIBER RESPIRATORS AGAINST COVID 19: JUST A WASTE OR EFFICIENT EXTRACTION MATERIAL

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A coronavirus pandemic all around us produces tons and tons of medical waste every day. Respirators, which are made of various nanofibrous materials, also belong to this "unnecessary" waste. In this study we tested commercially available respirators made of several types of nanofibers as an extraction tool for the preconcentration and extraction of various analytes – nitrophenols and chlorophenols from different matrices – river water and protein-containing biological samples. These model analytes were selected to cover a wide range of lipophilicity and thus possible further use of nanofibers. A small amount of nanofibers from respirators have been manually packed into an extraction cartridge and connected to the column-switching chromatographic system using on-line solid-phase extraction. While the ballast protein macromolecules were quantitatively eluted from the nanofibrous sorbent, the analytes were retained. After the mobile phase was switched to stronger acetonitrile, these analytes were then eluted from the extraction sorbent, directed in the analytical column, and finally separated. The separation was carried out using analytical column YMC C18 ExRS (100 × 4.6 mm, particle size 5 μm) followed by photodiode-array detection (210 nm). Solvents suitable for separation were acetonitrile with water under gradient elution. 5% methanol was used for the first clean-up step for the matrix purification. The result of our study is to confirm the possible use of used respirators for the extraction of various samples and thus find a way to use this waste further. The results of the tested respirators will be presented.

A.K. acknowledges the financial support of the Charles University, project No SVV 260 548 and GA UK No 1134119, and by the Czech Science Foundation, project GA ČR No 20-19297S. The STARSS project supported this work (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by the ERDF.

PBA17

HOT WATER AS AN EFFICIENT SOLVENT FOR EXTRACTION OF PHENOLIC COMPOUNDS FROM PLANT MATERIALS

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The aim of study was to demonstrate hot water as an efficient solvent to extract major phenolic compounds from dry pear leaves. Based on eco-friendly approaches in analytical chemistry, water is defined as the mostly green extraction solvent, which is non-toxic, abundant, cheap, safe, eco-friendly. Firstly, the stability of standards solution in temperature range of 40 – 90 °C for 10 minutes was tested. Then, the comparison between water and methanol extraction was performed with real sample of leaves. Finally, the water as a solvent was applied to extract 10 different pear leave cultivars. Extracted phenolic compounds – arbutin, chlorogenic acid, rutin and 3,5-di-caffeoylquinic acid were analyzed by high performance liquid chromatography with using Ascentis Express RP Amide 150 × 4.6 mm, 2.7 μm particle size analytical column. Column temperature was 30 °C and injection volume 1 μl. The separation was performed with gradient elution at flow rate 1 ml/min and analysis time 10.50 minutes. The mobile phase consisted of acetonitrile and 0.1% phosphoric acid. The detection was carried out with using Diode Array detector at wavelength 220, 327 and 354 nm. The results revealed the optimum extraction temperature of 60 °C and comparable results in using water and methanol as a solvent. Ten different cultivars of pear leave were extracted by water in 60 °C for 10 minutes and the concentration range of total phenolic compounds was 39.96 mg g^{-1} – 66.91 mg g^{-1} of dry weight (DW). This study reveals that pear leave is rich source of phenolic compounds, mainly chlorogenic acid, that can be easily extracted by water. In addition, prepared extracts showed antimicrobial activity against *Francisella tularensis, Listeria monocytogenes,* and *Pseudomonas aeruginosa*. This finding can lead to apply pear leave for further using, such as tea food supplements with beneficial effect to human health.

The study was supported by GAUK project no. 1152120.

PBA18

DEVELOPMENT AND OPTIMIZATION OF UHPLC METHOD FOR DETERMINATION OF GINGEROL AND CURCUMIN DERIVATIVES IN NUTRACEUTICALS BASED ON GINGER AND TURMERIC EXTRACTS

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The aim of the study is to develop, optimize and validate new UHPLC method for simultaneous determination of gingerol, curcumin, and their derivatives in nutraceuticals containing the extract of Zingiber officinale, Curcuma longa, and Piper nigrum. The optimized UHPLC method was used for determination of active ingredients in food supplement BIO Turmeric + Ginger – VANAVITA (GymBeam, s.r.o.), and it is planned to use the method also for Kurkumin (LIFTEC CZ, a.s.), Kurkumin Advance (ADVANCE nutraceuticals, s.r.o.), Kurkumin-Piperin Plus (Vieste group, s.r.o.), Kurkumin 550mg (Jamieson laboratories), and KURKUMIN-PIPERIN complex (Natural Medicaments, Vivantis, a.s.). For the analysis, extracts were obtained from the samples of the food supplement using the mixture of acetonitrile, methanol and acetic acid (38:60:2). Extracts were sonificated for 10 minutes and filtrated through 0,22 µl PTFE filters. The analysis was performed on the YMC Triart C18 ExRS (150mm × 3.0mm; 1.9µm) chromatography column using gradient elution program with mobile phase consisting of acetonitrile and ultrapure water. The separation was performed at flow rate of 0.4 ml min^{-1} and the detection was carried out at wavelength of 230 nm using PDA detector. The column temperature was 35°C. The UHPLC method is optimized, the evaluation of validation parameters is still in progress. Subsequently, the content of active substances in the preparations will be evaluated and the quality of individual food supplements will be compared.

Keywords: UHPLC; gingerol; curcumin; curcuma extract; ginger extract; C18 column; nutraceuticals

QUALITY CONTROL OF FOOD SUPPLEMENTS FROM *CURCUMA LONGA* AND *BERBERIS ARISTATA* EXTRACTS

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Food supplements are considered as concentrated source of nutrients or other substances with nutritional or physiological effect and they are taken because of their presumed health benefits. In the European Union, food supplements are regulated the same as food, with that come lot of risks, especially because of lack of safety guarantees and documentation about purity.[1] Also, the producers proclaim their supplements contain a certation amount of plant extract, but they usually don't inform about the content of active substances in the extract. In relation to that, the presented experiment is focused on the determination of bioactive compounds of food supplements from *Curcuma longa* and *Berberis aristata* extracts. *Curcuma longa* extracts contain phytochemicals including curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin, and other bioactive compounds;[2] isoquinoline alkaloids were detected in the *Berberis aristata* extracts, namely berberine, palmatine, jatrorrhizine, magnoflorine, and other alkaloids.[3] Bioactive substances in both extracts were separated and quantified by ultra-high performance liquid chromatography method with diode array detection. The majority of plant extracts are in the solid state, so ultrasound-assisted solid-liquid extraction followed by filtration was applied before analysis. The declared content of berberine in berberine extracts was compared with the measured amount of berberine; almost all of the supplements have a lower amount of berberine in berberine extracts than declared values on the label of each product. The *Curcuma longa* extracts label only informs about the curcumin extract's content, so only the quality of the curcumin extracts, in terms of percentage of curcuminoids in the extract, was evaluated.

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DEVELOPMENT OF CE-UV METHOD FOR DETERMINATION OF PICOLINIC HERBICIDES

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Herbicides picloram, clopyralid, aminopyralid, and fluroxypyr belong to the picolinic acid family of herbicides. These substances are used in greater amounts to effectively control broadleaf weeds and woody plants in pasture, rangeland and forestry. The main drawback of these compounds is the ability to persist in the environment from weeks to more than one year (soil, dead plants, compost, water), which can be followingly harmful to other grown crops. Moreover, via contaminated manure can picolinic herbicides enter the food chain.

This ongoing study aims to develop a fast capillary electrophoresis method with UV detection (CE-UV) for selective and sensitive determination of four herbicides. During method development, the main limitations were poorly absorbing analytes at the nonspecific wavelength of 220 nm and the need to apply selective and effective preconcentration step allowing quantitation of trace levels of contaminants. Thus, solvents were selected concerning their cut off value to prevent interferences at 220 nm. Solid-phase extraction using cartridges with molecularly imprinted polymers (SPE-MIP) designed for picolinic herbicides was applied for the preconcentration step. The optimization of the CE-UV method was divided into two main parts. First, the CE method was optimized (capillary, buffer, separation voltage, injection, temperature, conditioning) to enable separation with an appropriate resolution of analytes in a reasonable time. The second part was focused on the SPE-MIP preconcentration step. The method enables separation in less than 7 min and the preliminary parameters are very promising. The further step is to optimize the method with matrices of food samples.

The study was supported by specific research project No. SVV/260548, and GAUK No. 1466119.

PBA21

DEVELOPMENT OF THE LAB-IN-SYRINGE METHOD FOR ANTIBODY COATING OF MAGNETIC BEADS WITH ANTI-SPIKE PROTEIN IGG

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The extraction of bioanalytes from a larger volume is the first step in many diagnostic and analytical assays. Therefore, immunomagnetic separation is a method of choice when isolation of a specific analyte is required. In this method, the affinity reagents such as antibodies, affimers, or other molecules are non/covalently bound to the surface of preactivated magnetic beads. In a research lab, the batch arrangement inside an Eppendorf tube using a magnetic separator and rotator is the most widely used procedure. Here, we present the Lab-In-Syringe (LIS) system that was used for the automation of magnetic bead coating with a monoclonal antibody against the SARS-CoV2 surface Spike protein. The system is composed of a 12-port multiposition valve head containing one open-port interface, 1 ml glass syringe surrounded by a 3D printed ring containing two magnets. Optimization of many aspects of the device was tested. We developed the method on anti-ApoE Abs and when the procedure was ready, we switched to anti-Spike monoclonal antibodies. Evaluation of magnetic immunosorbent was done at the University of Pardubice on samples from patients with SARS-CoV2 in the BSL3 lab. The automated system provides many advantages over the "batch" procedure such as shorter time, higher precision, and low batch-to-batch variability.

The study was supported by Dean's fund (PROGRES Q42) and the OP RDE project "Strengthening interdisciplinary cooperation in research of nanomaterials and their effects on living organisms" (Reg. No. CZ.02.1.01/0.0/0.0/17_048/0007421).

PBA22

APPLICATION OF BENZYL-IMIDAZOLIUM BASED NANOGUMBOS AS A NOVEL SORBENT FOR SOLVENT-ASSISTED DISPERSIVE SOLID PHASE EXTRACTION OF BISPHENOLS FROM SURFACE WATER.

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A novel benzyl imidazolium-based solid phase material called GUMBOS (Group of Uniform Materials Based on Organic Salts) was synthesized from organic salts by simple one-step ion exchange reaction [1] and applied for the development of a solvent-assisted dispersive solid phase extraction method to enrich 6 bisphenols. The synthesized GUMBOS was dissolved in organic solvent (methanol) and an aliquot was rapidly injected into an aqueous sample in a vial under stirring and a precipitate formed. This process, referred as reprecipitation, led to the in-situ synthesis of nanomaterials which were dispersed in the sample for extraction of the analytes [2]. The mixture was left to stir for a defined period to ensure analyte extraction and the nanomaterial recovered by centrifugation. Afterwards, the recovered adsorbent material was dissolved in methanol and 20 μL was injected into HPLC for analysis. Essential analytical parameters influencing extraction efficiency, such as sorbent volume, extraction time, effect of salt, and sample volume, were optimized. Optimum extraction conditions were found to be 150 μL of GUMBOS solution volume, 5 min of dispersion time, 15 %w/v NaCl salt and 15 mL of sample volume. Under these conditions, the method would be validated and applied for the preconcentration and determination of bisphenols from surface waters.

The study was supported by Charles University SVV 260 548 and GUAK project no. 1070120.

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PBA23

THE USE OF MOBILE PHASE WITH LOW FORMIC ACID CONCENTRATION IN LC-MS PROTEOMIC ANALYSIS LEADS TO ENHANCED ELECTROSPRAY IONIZATION AND IMPROVED SENSITIVITY.

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When liquid chromatography is hyphenated to mass spectrometry for analysis of protein samples, the use of a mobile phase with high ionic strength is not recommended as it causes inefficient electrospray ionization.(1) Formic acid at a concentration of 0.1% has been a popular choice as an acidifier for its decent ion-pairing ability and relatively low MS signal suppression. In this study, analytical columns packed with a charged surface hybrid (CSH) stationary phase, designed to function efficiently with mobile phases of low ionic strength, were used to evaluate MS sensitivity gain and extent of peptide identification at a varied concentration of formic acid in the mobile phase (0.1% to 0.01%). Well-characterized peptides, a digested monoclonal antibody, and a complex bacterial sample were included in this study to evaluate the effect on varying sample complexity. About 40% to 50% gain in the MS sensitivity was observed using mobile phases with reduced formic acid. Furthermore, the number of identified peptides was increased when using the mobile phase with lower formic acid concentration. CSH stationary phases have opened doors for proteomic LC-MS analysis to use mobile phases with a minimal concentration of formic acid as an acidifier and exploit the advantages of the enhanced MS detection with no peak distortion.

The study was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) cofounded by ERDF. and by specific research project No. SVV 260548.

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REVERSED PHASE AND HILIC LIQUID CHROMATOGRAPHY FOR ANALYSES OF RAMUCIRUMAB AND ITS CONJUGATE

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Reversed-phase liquid chromatography (RPLC) has been traditionally used for the comprehensive characterization of therapeutic proteins (mAbs) due to its easy compatibility with mass spectrometry. Eventually, HILIC separation mode has become an alternative to RPLC. Along with a novel class of therapeutic proteins, antibody-drug conjugates, conjugates of antibodies and chelating linkers, such as diethylenetriamine pentaacetate (DTPA), are produced in-site by research groups for investigation purposes. The chelating linker is bound to the reactive side chains of amino acids, which makes analysis of such molecules challenging as they are the mixtures of unevenly conjugated species. Their separation is imperative for monitoring the conjugation sites and their quantification. In the case of antibody conjugates produced in-site, fast and simple but reliable methods are needed. RPLC and HILIC demonstrated the potency to suffice a need of this kind at intact, subunit, and peptide levels. In turn, RPLC and HILIC analyses can be complicated for free and in-site conjugated mAbs, demanding more efforts to optimize the analytical conditions. We found optimal conditions for analyzing ramucirumab at intact, reduced, and subunit levels. Neither RPLC nor HILIC were able to separate the mixture of conjugated ramucirumab molecules. Using both modes, conjugation reaction has been confirmed by the resulting peak shape of ramucirumab-DTPA against the previously obtained one from the free antibody. Our pilot experiments suggest that peptide mapping is the only level at which qualitative and quantitative analysis of the conjugation sites is possible.

The study was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) cofounded by ERDF. and by specific research project No. SVV 260548.

PBA25

LIPOSOMES AS AN ARTIFICIAL BIOMIMETIC CELL MEMBRANE FOR INVESTIGATION OF MEMBRANOTROPIC EFFECTS OF XENOBIOTICS

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Liposomes are a versatile tool in a wide range of applications. Although the primary use of these lipid nanoparticles is operating as protective nanocarriers for bioactive compounds, they can be used as a simplistic model of the cell membrane. In fact, liposomes allow us to study the interaction of xenobiotics across the lipid bilayer. The use of fluorescent membrane probes located at different depths of the membrane give information about membranotropic effects[1] caused by the compound (e.g., order, hydration, viscosity.). Membrane damage can be estimated by the leakage of hydrophilic probes encapsulated in the aqueous core of liposomes. Alternatively, NMR spectroscopy, can elucidate specific interactions between the molecule and the phospholipid and detects conformational changes and diffusion coefficients[2]. Furthermore, theoretical calculations provide additional information such as permeation kinetics and distribution into the membrane as obtained by molecular dynamics[2], or interaction energies and the geometries of the complexes as obtained by density functional theory calculation (DFT)[1]. A holistic approach combining experimental and theoretical techniques will help gaining a broad insight into the behavior of xenobiotics within the lipid bilayer. Even though liposomes are basically composed of the lipidic part of a eucaryotic cell membrane they can be useful as a first approach. In fact, they have shown a similar trend to that observed in cytotoxic assays performed on intestinal cells[3]. The addition of cholesterol, a mixture of phospholipids or even some membrane protein increases the complexity of the composition of liposome and serves as a better mimic of a specific organ or tissue.

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APPLICATION OF DERIVATIZATION IN ANALYSIS OF PLATINUM COMPOUNDS

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Determination of platinum drugs can be challenging. Their retention on reverse-phases is demanding, as platinum drugs are very hydrophilic complexes[1]. Tricky is also their poor absorption in UV spectra[2]. Derivatization of the analyte changes and optimizes a chemical structure and its properties, thus allows to change the behaviour of the analytes during analysis. The derivatization reaction should be quantitative, the process should be simple, and the reaction should be achieved easily. Also, it is desired that the final derivated complex should be stable.
Methods applying derivatization with diethyldithiocarbamate (DDTC) are universal for determination of various platinum compounds, as the derivatization agent provides with them the same complexes that can be analysed[1]. Results discussing the problematics of pre-column derivatization using DDTC during sample preparation applied for the HPLC-DAD determination of oxaliplatin and cisplatin in human plasma, peritoneal fluid, and urine will be presented.

The study was supported by Charles University by project SVV 260 548, the University Hospital in Hradec Králové MH CZ - DRO (UHHK, 00179906), and by Ministry of Health of the Czech Republic via grant NV18-03-00130.

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