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Abstracts

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PBA1

UNRAVELLING THE LIPIDOME OF ATHEROSCLEROTIC PLAQUES USING MATRIX-ASSISTED LASER DESORPTION IONISATION – TRAVELLING WAVE ION MOBILITY MASS SPECTROMETRY

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Atherosclerotic cardiovascular disease is a chronic inflammatory disorder characterised by a gradual build-up of plaques in the arterial wall. Lipids are essential in plaque progression, but their exact mechanistic involvement remains elusive. MALDI-MSI, offering characterisation of the spatial lipid composition of unstable atherosclerotic plaques, has enabled mapping of lipids/lipid classes to defined histologically important regions. To identify lipids elucidated by MS and MS/MS spectra, LIPID MAPS database, ChemDraw, and LipostarMSI was applied, but isobaric lipids were not discriminated. MALDI-TWIMS was explored as an orthogonal separation dimension to increase the confidence level of identification. A method was optimised using 13 odd-/even-chained lipid standards mixed with 10 mg/mL CHCA matrix in a 1:1 ratio and spotted onto a steel plate. The samples were analysed using a Waters Synapt G2Si MS instrument. Favourable ion mobility separation was achieved using a wave: velocity of 700 m/s, and height of 40 V. The method was applied to an New Zealand white rabbit aortic plaque sample, sprayed with 10 mg/mL CHCA matrix using HTX TM-Sprayer™. Drift times of lipids identified in the tissue were correlated to lipid standards using HDI® and DriftScope® software. Key lipid signatures were assessed with and without ion mobility to identify potential isobaric interferents. In conclusion, MALDI-TWIMS can increase the selectivity of detection without adverse effects on sensitivity.

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PBA2

SOLVENT-ASSISTED DISPERSIVE SOLID PHASE EXTRACTION OF BISPHENOLS USING IRON (III) THENOYLTRIFLUOROACETONATE COMPLEX (FE(TTA)$_3$) AS NEW NANOSTRUCTURED SORBENT.

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In this work, we describe the synthesis and application of iron (III) thenoyltrifluoroacetonate complex (Fe(TTA)$_3$) as a novel sorbent for solvent-assisted dispersive solid phase extraction of bisphenols from water samples [1]. The extraction procedure is based on in-situ formation of nanoparticles upon rapid injection of a methanolic solution of Fe(TTA)$_3$ into stirred aqueous sample in a glass vial. Herein, synthesis and characterization of Fe(TTA)$_3$ and study of essential parameters of the preparative procedure were evaluated and reported. The optimized procedure allowed efficient enrichment of bisphenols from various water samples, chosen as model contaminants and matrix, within 2.5 min. The sorbent was collected by centrifugation, re-dissolved in methanol, and injected to HPLC with spectrophotometric detection. Limits of detection (LOD) and quantification (LOQ) obtained ranged from 1.0 to 3.1 and 3.1 to 7.5 μg L-1, respectively. Intra-day and inter-day precisions of < 7 % RSD and < 8 % RSD were obtained with analyte recoveries ranging between 70-117 % (103.8 % on average) for the analysis of river, wastewater treatment plant (WWTP) effluent, and bottled waters.

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

Reference

1. ALADAGHLO, Z, FAKHARI, A. *et al.*; J. Chromatogr. A, 1462, 2016, 27–34.

UHPLC-MS/MS IN ANALYSIS OF ANTHRACYCLINES IN CULTURE MEDIUM AND CARDIAC CELLS

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Daunorubicin (DAU) is a potent anthracycline chemotherapeutic. However, as for other anthracyclines, its administration is associated with cumulative dose-dependent cardiac toxicity.[1] More theories describe this cardiotoxicity, but the exact mechanism is still not fully understood. Therefore, this project is focused on the development of bioanalytical methods supporting the investigation of mechanisms responsible for DAU cardiotoxicity *in vitro*. It was aimed at: 1) optimization of the DAU and the metabolite (daunorubicinol, DAU-ol) extraction from neonatal rat ventricular cardiomyocytes (NVCMs), 2) development and validation of the UHPLC-MS/MS assay for DAU/DAU-ol in culture medium and NVCMs, and 3) application of the assay to investigate the pharmacokinetics of the drugs *in vitro*. The samples were analyzed on UHPLC-MS/MS (1290 Infinity II, 6470 LC/TQ, Agilent) using a Kinetex C18 (100 × 2.1 mm, 1.7 μm, Phenomenex) column and a mixture of 0.0025 % formic acid, acetonitrile, and methanol as a mobile phase. Cell extraction included sonification and liquid-liquid extraction of the analytes to chloroform:methanol mixture (4:1). The method was validated within a concentration range of 0.009–1.896 μM for medium and 0.02 – 2.27 nmol/4.6 million NVCMs. Accuracy and precision were assessed in 4 concentrations and 3 replicates each for medium and 3 concentrations and 3 replicates each for NVCMs. Resulting from the uptake experiments of DAU and DAU-ol, comparable concentrations of exogenous and endogenously produced DAU-ol were measured inside the cells. Efflux experiments showed the involvement of the P-glycoprotein in the transportation of these compounds into the cells.

The study was supported by GAUK 1204120 and SVV 260547.

References

1. SAWYER, DB., PENG, X., CHEN, B., *et al*.: Prog Cardiovasc Dis., 53, 2010, 105–113.

PBA4

COMPLEX SCREENING OF RETENTION BEHAVIOUR OF CATECHOLAMINES

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Nowadays, due to the recently discovered mechanisms influencing the level of serotonin in placental tissue during pregnancy [1], new questions arise, whether concentration changes of other important neurotransmitters do not affect foetal development too. Information about the production of catecholamines (dopamine (DA), norepinephrine (NE) and epinephrine (E)) by the placenta is known. However, there is not fully explored and clarified their role in the foetal 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]. Generally, their retention is low in reversed phase mode. Under HILIC conditions peak broadening was observed despite satisfactory retention. Moreover, catecholamines and their precursors cannot be detected in biological material by native fluorescence because of low concentration levels.

Derivatization with some fluorescent reagents seems to be a solution that promises highly fluorescent products with higher retention and better separation [3]. As part of the development of an HPLC method for the determination of catecholamines in placental tissue, the optimalization of derivatization reaction of tyrosine, L-DOPA, dopamine, noradrenaline, and adrenaline with FMOC-Cl was tested.

References

1. BONNIN, A., LEVITT, P.: Neuropsychopharmacology, 37(1), 2012, p. 299-300.
2. GORBUNOVA, M. V., GUTOROVA, S. V., BERSENEVA, D. A., et al.: Applied Spectroscopy Reviews, 54, 2019, 631-652.
3. BICKER, J.; FORTUNA, A.; ALVES, G.; *et al*.: Anal Chim Acta, 768, 2013, p. 12-34.

PBA5

COMPREHENSIVE CHIRAL GC-MS/MS AND LC-MS/MS METHOD FOR IDENTIFICATION AND DETERMINATION OF N-ACYL HOMOSERINE LACTONES

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N-acyl homoserine lactones are signaling molecules synthesized by gram-negative bacteria to communicate in a process called "quorum sensing." The most common achiral separation methods for the analysis of N-acyl homoserine lactones are biosensors, thin-layer chromatography, liquid chromatography with UV detection, gas chromatography or liquid chromatography coupled with a mass spectrometer.

Recently, the production of both L- and D-N-acyl homoserine lactones have been reported in *Vibrio fischeri* and *Burkholderia cepacia*. The concentrations of the D-N-acyl homoserine lactones were found at limit of quantification. Therefore, more sensitive, reliable, and selective analytical methods are necessary for further studies of D-N-acyl homoserine lactones in the physiology of gram-negative bacteria.

The aim of our work was the development of comprehensive chiral analytical methods for the identification and determination of 18 homoserine lactone derivatives by two analytical techniques, i.e., GC and LC coupled to a triple quadrupole (GC-QqQ and LC-QqQ). The previously used solid phase extraction method has been optimized to pre-concentrate the analytes 100 times and purify the sample from the matrix present in bacterial media.

As a result, the chiral separations of all 18 N-acyl homoserine lactones derivatives were accomplished by the complementary GC-QqQ and LC-QqQ methods. Generally, the limit of detection for the LC-QqQ method was as low as hundreds of parts per trillion. The limit of detection for the GC-QqQ method was found to be three to two orders higher than the LC-QqQ method. The sensitivity of the presented methods will enable studying the role of D-N-acyl homoserine lactones in bacterial "quorum sensing."

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PBA6

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

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When reversed-phase 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%). Results obtained by analyzing tryptic peptides of trastuzumab and a bacterium concluded that 0.01% FA in the mobile phase leads to approx. 50% and 30% enhancement in MS sensitivity and identification. Three different commercially available analytical columns packed with a positively charged stationary phase were compared for their performance when the MS-friendly mobile phase was used. A research study to evaluate alternative weak acidifiers at the reduced concentration for increasing sensitivity of LC-MS analyses for protein analysis are also ongoing.

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References

(1) García, M. C.: J. Chromatogr. B 825 (2), 111-123 (2005)

GRADIENT DESIGN AND OPTIMIZATION FOR SUSPECT SCREENING IN EQUINE DOPING CONTROL APPLICATION

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Equine welfare and maintaining fair practice of horseracing sports are major concerns of horse racing authorities. In general, horse racing authorities advise to assume that any medication that has an effect on horses is prohibited unless a specific announcement that states otherwise[1]. The increased sophistication of doping practices and the virtually unlimited number of prohibited substances have created the never-ending challenges in doping control of equine sports.

The aim of this study was to develop a broad-spectrum screening and confirmatory method for prohibited substances used in equine sports utilizing advanced a LC-MS technique. Minimizing false negative and ensuring the quality of positive results, a reporting criteria of four pillars was designed; the accurate mass, isotope pattern, fragmentation and retention time. Hence, the resourceful HRMS approach that provides the sufficient specificity and selectivity for suspect detection was employed along with unambiguous retention time-assisted identification of analyte(s).

Gradients are typically used to separate a mixture with a wide range of polarity which would be impractical to separate using isocratic conditions. In this study we are challenging the identification of more than 300 compounds reported for illicit use, with a focus on achieving a successful separation of 32 isomeric pairs using a reversed phase ultra-high performance liquid chromatographic system. Maximizing the peak capacity and enhancing the resolution power of a gradient program are done by adjusting “gradient parameters” - gradient time, column length, flow rate and gradient range, before changing column and mobile phase. Therefore, an extensive evaluation of the impact of organic range changes on the resolution and distribution of peaks was done.

References

1. International Federation of Horseracing Authorities, International Agreement: Article 6A. https://www.ifhaonline.org/default.asp?section=IABRW&area=2

PBA8

PHENOLIC COMPOUNDS PROFILING IN ARCHIVE TOKAJ WINE WITH ADVANCED CHROMATOGRAPHIC APPROACHES

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The presented study is focused on the characteristics of archive Tokaj wines, especially in terms of their benefits to the human body in the form of the antioxidant activity of phenolic compounds.[1] Tokaj wine region is one of the few areas with a production of special wine made from grapes affected by noble rot *Botrytis cinerea* under particular environmental conditions, which leads to the production of naturally sweet wines with a unique aroma.[2] More than 60 archive samples were evaluated in terms of phenolic substances profile, including hydroxybenzoic and hydroxycinnamic acids, stilbenes, and flavan-3-ols. The 18 phenolic compounds were identified and quantified by ultra-high performance liquid chromatography method with diode array detection. The correlations among profiles of phenolic compounds and vintage, the amount of nobble rotten berries, and various producers were evaluated. Despite the UHPLC-DAD method being considered acceptable for application in the characterization of these types of complicated matrices, two-dimensional liquid chromatography (2D-LC) has been tested as an effective technique for the separation of chemical compounds in complex sample matrices, such as wine. The 2D-LC achieves high peak capacity, reflected by decreased compound overlap, by a combination of two columns with various separation approaches.[3]

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References

1. STAŠKO, A., POLOVKA, M., BREZOVÁ, V. *et al*: Food Chem., 96, 2006, 185–196.
2. MAGYAR, I.: Adv. Food Nutr. Res., 63, 2011, 147–206.
3. SHUI, J., SHUANG, W., HAISHAN, X. *et al.:* J. Pharm. Biomed., 160, 2018, 301-313.

PBA9

DEVELOPMENT OF CE-MS METHOD FOR THE SIMULTANEOUS ANALYSIS OF BOSWELLIC ACIDS AND NONSTEROIDAL ANTI - INFLAMMATORY DRUGS

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The development of a CE-MS method for the simultaneous separation of boswellic acids and nonsteroidal anti-inflammatory drugs (NSAID) is presented. The purpose of the method is to separate boswellic acids, the main components of *Boswellia serrata* extract, and 13 NSAIDs, specifically carprofen, diclofenac, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meloxicam, niflumic acid, piroxicam, phenylbutazone, salicylic acid, sulindac, and tiaprofenic acid, which belong among known or potential adulterants of anti-inflammatory dietary supplements. The separation was carried out in 76 cm fused silica capillaries, 50 μm i.d., with the applied voltage of +27 kV and at the temperature of 25 °C. The background electrolyte was a mixture of 40 mmol/L ammonium acetate (pH 8.5), MeOH, and ACN (5:1:4, v/v/v). The coupling of Agilent 7100 CE system with Agilent 6495 QqQ mass spectrometer with Agilent Jet Stream ion source was realized through a coaxial sheath liquid interface. A 5 mmol/L ammonium acetate solution in water : methanol (1:1, v/v) was used as sheath liquid in positive ion mode, and a mixture of 25% ammonia solution, water, and methanol (1:49.5:49.5, v/v/v) was used as a sheath liquid in negative ion mode, both at a flow rate of 4 μl/min. The mixture of analytes was analysed in selected reaction monitoring which allowed to achieve selective analysis of all tested NSAIDs and all boswellic acids aside from α- and β-isomers which migrate unresolved under proposed conditions and have identical fragmentation routes. The influence of multiple parameters of ion source and detector on method sensitivity was studied, such as sheath gas temperature and flow rate, nebulizing pressure, nozzle voltage and voltage on sprayer needle, and electron multiplier voltage. Subsequently, the method will be validated and applied in the analysis of dietary supplements.

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PBA10

POSSIBILITIES OF RPLC-UV-MS IN CHARACTERIZATION OF ON-SITE PREPARED DTPA-TRASTUZUMAB CONJUGATE PRIOR TO RADIOLABELING

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Reversed-phase liquid chromatography (RPLC) coupled to mass spectrometry (MS) has been traditionally used for the comprehensive characterization of clinically available therapeutic antibodies (mAbs) and mAb-based drugs currently under development such as radioimmunoconjugates (RICs). RICs are constituted by monoclonal antibody and a radionuclide attached via an appropriate chelating linker bound to certain amino acid residues. Such developments are typically conducted on small, on-site scale, and procedures for localization of the linker within the mAb structure are not well established or even omitted. As the result, it is unknown which parts of therapeutic protein are occupied by a chelating linker, yet it is critical. In the present study, we demonstrated how characterization of intermediate products by RPLC coupled to UV and MS detectors can aid the development of RICs on example of trastuzumab conjugated with diethylenetriamine pentaacetate (DTPA) chelator. Using a reduced IdeS digest of DTPA-trastuzumab, conjugation reaction has been confirmed to occur at all three generated sub-units of the mAb-linker intermediate product. With the bottom-up approach, DTPA was successfully localized in peptides belonging to N-termini of trastuzumab. However, only indirect, but informative assessment of linker attachment to other reactive sites of trastuzumab was feasible as DTPA modification bound through hydroxyl groups of threonine and serine, is unstable under higher-energy collision dissociation.

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PBA11

LC-MS/MS STUDY OF NEW PROMISING TACRINE DERIVATIVES BIOTRANSFORMATION.

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Current symptomatic pharmacotherapy of Alzheimer's disease primarily focuses on acetylcholinesterase inhibitors and NMDA (N-methyl-D-aspartate) receptor blocking. Tacrine, a molecule with both of the above mechanisms of action was withdrawn from the market in 2013 due to the hepatotoxicity of its metabolite 7-OH-tacrine after twenty years of use.[1] The introduction of methoxy- or phenoxy- group to position 7 or chlorine into position 6 on the tacrine moiety led to 7-methoxy-, 7-phenoxy-, 6-chloro-tacrine respectively.[2] These substitutions may potentially hinder the formation of toxic species.

The main aim of our work was to develop a new UHPLC-Orbitrap method to reveal whether the tacrine substitution reduces the formation of 7-OH-tacrine. Moreover, we aimed to select a suitable human-like model for studying of tacrine derivatives biotransformation and their hepatotoxicity.

Our newly developed UHPLC-MS/MS method enabled the separation and identification of sixteen tacrine, fifteen 7-methoxy- and 7-phenoxy-, and fourteen 6-chloro- tacrine metabolites.[3] The main biotransformation pathway of these four compounds is their mono- and di-hydroxylation. Moreover, some new *in vitro* metabolites were found. Based on the relative amount of individual metabolites, it was revealed that 7-phenoxy- and 6-chloro-substitution of the tacrine moiety significantly reduced the formation of the toxic precursor 7-OH-tacrine. In addition, 3D primary human hepatocytes spheroids were chosen as the most suitable model for studying the biotransformation and toxicity of tacrine derivatives.

References

1. ROMERO, A., CACABELOS, R., et al.: Med. Chem. Lett., 23, 2013, 1916-1922.
2. PATOCKA, J., JUN, D., KUCA, K., et al.: Curr. Drug Metab., 9, 2008, 332-335.
3. NOVAK, M., KUCERA, R., et al.: J. Pharm. Biomed. Anal., 2023, in press.

OPTIMIZATION OF UHPLC METHOD FOR THE ANALYSIS OF TRIBULUS TERRESTRIS EXTRACTS

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The aim of the study was to develop, optimize and validate new UHPLC method for simultaneous determination of steroidal saponins in nutraceuticals based on Tribulus terrestris extracts, such as dioscin, diosgenin, protodioscin, pseudoprotodioscin, gitogenin, gracillin, tribulosin, sarsasapogenin and ruscogenin. The optimized UHPLC method is intended to be used for determination of active ingredients in food supplements ADIEL Kotvičník zemní (Ing. Stanislav Kameníček), Clavin (Simply You Pharmaceuticals), Arginmax (Simply You Pharmaceuticals), Tribulus terrestris extract (SWANSON), Erectamin (Nutri Star), 3BULLUS (Dr.Max), Primulus (Primulus Group) and Tribul (ADVANCE).

Optimization of the proposed method is still in process, but according to the so far obtained results, the analysis should be performed under the following conditions: Extraction of the food supplement samples using the mixture of acetonitrile, ethanol and ultrapure water (2:6:3) and their sonication for 10 minutes, and filtration through 0.22 µl PTFE filters. The analysis was performed on the Kinetex® Phenyl-Hexyl (100 × 4.6 mm; 2.6 µm particle size) chromatography column using gradient elution program with mobile phase consisting of acetonitrile and 0.1% formic acid solution at flow rate of 1.0 ml min^{-1} and column temperature 15°C. Due to the absence of chromophores in saponines structure, the detection was carried out using CAD detector.

The UHPLC method was optimized, and the validation parameters will be evaluated. Subsequently, the content of active substances in the preparations will be evaluated and the quality of individual food supplements will be compared.

Keywords: UHPLC; Tribulus terrestris; saponins; tribulus extract; CAD; Phenyl-Hexyl column; nutraceuticals

PBA13

ADVANCED MICROSAMPLING STRATEGIES BASED ON DRIED BLOOD SPOT IN ILLICIT DRUG ANALYSIS

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Innovative attitudes in bioanalysis also reached the field of specimen collection. More patient-friendly and feasible microsampling beats traditional fluids sampling in the term of reduced invasiveness and collected sample volume, as well as in simplification of sample transport and storage or increased analyte stability[1]. Over the decades, in routine practice well-established dried blood spots (DBS) were, unfortunately, dealing with haematocrit and sample volume variability. Regarding the demand for accurate analyte quantification, attention has been paid to advanced volumetric microfluidic strategies.

In this study, we aimed to improve classic DBS methodology with "smart" DBS techniques to analyse cocaine and its metabolites. Progressive miniaturized sample collection followed by simple sample pretreatment was coupled with feasible HPLC-FLD for clinical, forensic, or anti-doping analysis purposes. The results obtained during method optimization will be evaluated with attention to pre-validation data and analytical performance, as well as practical aspects of the attitude will be discussed.

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References

1. PROTTI, M., MANDRIOLI, R., MERCOLINI, L., Anal. Chim. Acta 1046 (2019) 32-47.

PBA14

DISTRIBUTION OF INDIVIDUAL FORMS OF VITAMIN K IN LIPOPROTEIN LAYERS DETERMINED WITH A COMBINATION OF ULTRACENTRIFUGATION AND UHPLC-MS/MS

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Vitamin K, one of the fat-soluble vitamins, consists of several forms, which are separated into two main groups vitamin K1 (phylloquinone) and vitamin K2 (menaquinones or MKs). They differ in lipophilicity when MKs with longer chains are much more hydrophobic. The length and degree of saturation of the isoprene side chain affect their bioavailability and elimination. Thus, MKs have a longer biological half-life. One of our aims was to study transport and distribution of these forms in human body. It is known that vitamin K is incorporated into chylomicrons, excreted into lymphatic capillaries, transported to the liver, and repackaged in lipoproteins. While circulating vitamin K1 is mostly associated with triacylglycerol-rich lipoproteins, vitamin K2 is transported mainly by low-density lipoproteins (LDL). However, there are not many studies focusing on the distribution of vitamin K in individual lipoprotein fractions (VLDL, LDL, HDL). For this reason, we developed a method using a combination of ultracentrifugation with UHPLC-MS/MS for the determination of vitamins K1, MK4, MK7, and MK9. New method was used for monitoring these levels in serum and lipoprotein fractions obtained from 30 human healthy volunteers and 13 laboratory rats. Detailed results will be discussed in the presentation.

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References

1. SIMES D.C., *et al.*: Nutrients, 12(1), 2020, doi: 10.3390/nu12010138.

PBA15

ELECTROMEMBRANE EXTRACTION: A NOVEL AND EFFECTIVE MICROEXTRACTION TECHNIQUE IN BIOANALYSIS

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Electromembrane extraction (EME) is a hybrid microextraction technique laying between liquid-liquid extraction (LLE) and electrophoresis. The technique was firstly introduced in 2006 by Pedersen-Bjergaard and Rasmussen under the name electromembrane isolation [1]. The EME of charged analytes is performed from the aqueous sample through the water-immiscible supported liquid membrane (SLM) to the aqueous acceptor solution. The driving force of the extraction is an electrical potential, which is applied across the SLM. One of the biggest challenges in the development of bioanalytical methods is the sample preparation, since the co-eluting endogenous matrix components may negatively affect the obtained results. Our studies focused on use of EME for isolation of analytes from various biological matrices. EME efficiency was monitored not only in plasma [2] but also in very complex and complicated matrices namely tissues (liver, heart and skeletal muscle) [3] and breast milk [4]. Among the analytes were amphetamines, synthetic cathinones and anthracyclines. The optimized EME procedures were compared with other extraction methods (e.g. LLE, protein precipitation, and salting out assisted liquid-liquid extraction). Both, good recoveries (higher than 70%) and excellent sample clean-up (matrix effects maximally 11%), were achieved from all matrices with EME. The optimized EMEs in all matrices, followed by UHPLC-MS/MS, were successfully validated. In conclusion, EME proved to be simple, reliable, effective, and repeatable microextraction technique, which enables direct enrichment of the sample and provides efficient sample clean-up.

References

1. PEDERSEN-BJERGAARD, S., RASMUSSEN, K.E.: J Chromatogr A 1109(2), 2006, 183-90.
2. BAVLOVIČ PISKÁČKOVÁ, H., OIESTAD, E.L., VÁŇOVÁ, N., *et al.:* Talanta 223, 2021, 121748.
3. BAVLOVIČ PISKÁČKOVÁ, H., KOLLÁROVÁ-BRÁZDOVÁ, P., KUČERA, R., *et al.*: ACA 1177, 2021.
4. BAVLOVIČ PISKÁČKOVÁ, H., NEMEŠKALOVÁ, A., KUČERA, R., *et al.*: JPBA 210, 2022.

PBA16

LAB-IN-SYRINGE FOR HPLC-DIRECTED SAMPLE PREPARATION

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An overview is given of recent research on the implementation of the flow-batch technique Lab-In-Syringe (LIS) [1] in the automation of sample preparation for high-performance liquid chromatography (HPLC). The technique takes advantage of using the void of a computer-controlled syringe as steadily sealed and size-adaptable mixing, reaction, and extraction vessel.

High operational versatility and feasibility for the automation of diverse preparation approaches were proven on the extraction of sulphonamides via homogenous liquid-liquid extraction (HLLE) combined with a secondary cleanup by online SPE [2], dispersive solid phase extraction for selected environmental contaminants [3], and directly immersed single-drop microextraction of fluoroquinolones using deep eutectic solvent as green extractants [4]. Online connection to HPLC was accomplished in all experimental works for the determination of the extracted analytes of interest with achieved efficient matrix clean-up and preconcentration-related gain in sensitivity.

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References

1. HORSTKOTTE, B., SOLICH, P., Molecules 25 (2020) 1612.
2. FIKAROVÁ K., HORSTKOTTE B., MACHIÁN, D., *et al.*.: Talanta 221 (2021) 121427.
3. GEMUH, C.V., MACHÁČEK, M., SOLICH, P., *et al.*.: Anal. Chim. Acta 1210 (2022) 339874.
4. YILDIRIM, S., COCOVI-SOLBERG, D.J., USLU, B., *et al.*.: Talanta 246 (2022) 123476.

DEVELOPMENT OF UHPLC-MS/MS METHOD FOR THE DETERMINATION OF OCHRATOXINS IN URINE

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Ochratoxins are produced by microscopic fungi of the genera *Aspergillus* and *Penicillium* and belong to nephrotoxic mycotoxins, which are associated with a risk of kidney tumor. They often contaminate a wide range of foodstuffs, i.e., cereals, coffee beans, dried fruits, and wine.[1] Biomonitoring is a valuable tool to investigate human exposure to mycotoxins, i.e., the analysis of parent compounds and their metabolites in biological fluids.[2] Urine is the body fluid most often used to measure mycotoxin exposure due to its easy and non-invasive collection. Besides the parent compounds ochratoxin A, B, and C, the metabolite ochratoxin-alpha is often found in urine.[3]

Due to the low levels of ochratoxins in urine, a highly sensitive and selective analytical method is needed. For this purpose, we used ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). Within the development of sample preparation, various techniques have been optimized: supported liquid extraction (SLE), micro-solid phase extraction (μ-SPE) in pipette tips, and salting-out assisted liquid/liquid extraction (SALLE).

Within the optimization of SLE, attention was paid to the acidification of urine and a choice of the most effective eluent. Using 2% formic acid and ethyl acetate, we obtained the best recoveries. However, SLE extracts showed a very high background signal in UHPLC-MS/MS records. Thus, this technique was not suitable for trace analysis. Using μ-SPE, we obtained poor recovery of ochratoxin B and poor repeatability. SALLE represents a cost-effective, time-efficient, and easy-to-use sample preparation method. When ethyl acetate was used as an extraction solvent, its evaporation was identified as the critical point of the process. However, when acetonitrile was used, its compatibility with the LC-MS system enabled the omitting this step. The extraction recoveries of the final method were in a range of 101–130% at the spiking level of 1 ng ml^{-1} urine.

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References

1. MALÍŘ, F., LOUDA, M., TOMAN, J. *et al.*: Food and Chemical Toxicology, 158, 2021, 112669.
2. ESCRIVA, L., MANYSES, L., FONT, G., *et al.*: Toxins, 9, 2017, 330.
3. ALI, N., MUNOZ, K., DEGEN, G. H.: Toxicology letters, 275, 2017, 19–26.

PBA18

PIPETTE-TIP MICROEXTRACTION FOR VITAMIN A ANALYSIS

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The efficient sample preparation based on pipette tip microextraction and used for analysis of retinol in human serum has been developed. The commercial microextraction pipette tips with the variety of sorbents were compared based on their extraction efficiency to select the best device. In addition, other aspects including sample volume, organic solvent uses, difficulty, time consuming, cost, and greenness of the procedure have been considered to achieve the ideal analytical method using in clinical laboratories. The LLE was used as the reference method with 100% recovery.[1] The most satisfying pipette tips were the WAX-S which combine weak anion exchange resin with salting-out assisted liquid-liquid extraction. The tips based on the clean-up workflow were able to routinely and repeatably achieve recoveries of almost 100% for retinol and 80% of retinol acetate (internal standard). Although not all interferences were eliminated, they did not obstruct the peaks of both analytes in chromatographic separations. Moreover, the main advantage of this pipette tip usage was simplicity, eco-friendliness, and cost-effectiveness. Small sample volume, 50 μL, enables to use this technique in clinical research and routine practice. Nowadays, according to the availability of sorbents, the application of this technique can extend to use with wide range of analytes.

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References

1. URBANEK, L., SOLICHOVA, D., MELICHAR, B., *et al*.: Anal. Cnim. Acta, 573-574, 2006, 267-272.

PBA19

TARGETED AND NON-TARGETED ANALYSIS OF FLAVONOID METABOLITES IN PLASMA

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The chromatographic separation of a complex group of flavonoid metabolites and their selective determination remains a complicated analytical challenge. The metabolites include large group of compounds with various physicochemical properties, flavonoids, phenolic acids, phenols, and their sulphate and glucuronide conjugated forms. Moreover, similar fragments and precursor ions are produced during the in-source and collision induced dissociation.

The present study aims to develop and optimize the selective targeted analysis of 6 small phenolic compounds, 4 flavonoids, and their 5 conjugates in plasma using ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) and protein precipitation (PP) as sample preparation method. The optimization was carried out using two SRM transitions for each analyte and included a screening of stationary and mobile phases and gradient program optimization to achieve chromatographic separation of isomeric and structurally similar compounds. The PP procedure could not include the evaporation and reconstitution steps due to low stability of analytes and their various solubility. Therefore, the emphasis was placed on the type and volume of precipitation solvent as it also significantly affected the injection solvent composition and sensitivity of the method. The UHPLC-MS/MS method was validated and applied on rat plasma samples for the description of pharmacokinetic profiles of 4-methylcatechol sulphate and isorhamnetin. The same chromatographic conditions coupled with data independent analysis high-resolution MS were applied to the non-targeted analysis of human plasma. Flavonoid metabolite profiles of healthy persons and patients suffering from metabolic diseases will be evaluated and compared. The metabolites responsible for the significant differences will be searched and identified.

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PBA20

SIMPLE PRECONCENTRATION FROM LARGE-VOLUME SAMPLES USING GRAPHENE-DOPED NANOFIBROUS POLYMER DISKS

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Modifications of polymer nanofibers provide tuning of their characteristics and can enhance their performance as extraction sorbent. For example, hydrophobic interactions are enhanced in carbon-based nanomaterials such as carbon nanotubes, heat-treated polymer fibers, or dispersion of graphene nanoparticles in a composite polymer.[1] In this work, we present for the first time, hybrid nanofibrous material composed of organic polymer highly doped with graphene (30.7%) that was prepared by alternating current electrospinning[2] from a dispersion of graphene in a polycaprolactone (PCL) solution. To our best knowledge, only the use of polymers doped with low content of carbon additive (estimated about 1-2 wt%, 15 wt% max.) has been reported.[3,4] Simple disk-based extraction and preconcentration of trace levels of emerging organic environmental pollutants, bisphenols and pesticides, from surface waters was optimized in terms of conditioning, sample volume, time of extraction, and elution solvent. The SPE disk desorption was carried out in an HPLC vial used for the direct injection into HPLC which significantly reduced sample handling and possible errors. The graphene-doped nanofibers enabled a significant, more than doubled increase in the retention of analytes and a higher specific surface area compared to the native PCL nanofibers.

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References

1. CANLIDİNÇ, R.S.: Pamukkale Univ. J. Eng. Sci., 26, 2020, 1319-1327.
2. ERBEN, J., KLICOVA, M., KLAPSTOVA, A., *et al*.: Microchem. J., 174, 2022, 107084.
3. MEHRANI, Z., EBRAHIMZADEH, H., MORADI, E.: J. Chromatogr. A, 1600, 2019, 87.
4. FANG, X., OLESIK, S.V.: Anal. Chim. Acta, 830, 2014, 1-10.

SUPERCRITICAL FLUID EXTRACTION OF RESINIFERATOXIN FROM PLANT MATERIAL

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Resiniferatoxin (RTX) is a toxic diterpenoid that naturally occurs in the latex of *Euphorbia resinifera*. RTX is an ultrapotent compound enabling the activation of capsaicin receptors (TRPV1). TRPV1 is the potential therapeutic target for controlling hyperalgesia and diabetic neuropathy and treating chronic inflammatory, neuropathic, and cancer pains. RTX isolation procedure is typically carried out by nonpolar solvents such as ethyl acetate and hexane.[1,2] Our study aimed to develop supercritical fluid extraction as a green alternative to traditional liquid-liquid extraction. The key parameters, including CO_2 amount (80 – 100%), type of co-solvent (ethanol, ethyl acetate), extraction temperature (30 – 60 °C), and pressure (100 – 320 bar), were tested using full factorial design of experiment with 19 experiments. CO_2 amount, with 86.5 % contribution, was the most critical parameter affecting the extraction yield, while the change in co-solvent and extraction temperature did not improve the extraction. Among the tested flow rates, 6 mL/min provided the highest RTX extracted yield, 16 μg/g (RSD 16%, n=9), with suitable repeatability and complete extraction only in 45 min. Ethyl acetate was applied to extract residual RTX amount in plant material. It was typically < 0.9 μg/1 g sample. In the next step, the optimized SFE method will be converted to an industrial scale and used for resiniferatoxin isolation in large *Euphorbia resinifera* samples.

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References

1. SHARMA, P., AVULA, B., *et al*.: J. Pharm. Biomed. Anal., 165, 2019, 284–219.
2. FATTORUSSO, E., LANZOTTI, V. *et al.:* Eur. J. Org. Chem. 2002, 2002, 71–78.

PBA22

ORGANOGELS AS AN INNOVATIVE APPROACH FOR EXTRACTION OF LIPOPHILIC COMPOUNDS FROM URINE

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Organogels are based on organized molecular system forming gels in which a small amount of lipophilic solvent is trapped by an organogelator, formulated in chromatography vials (ActiVials). As organogels are solubile in organic solvents, the ActiVials offer an easy-to-handle alternative to liquid-liquid extraction, especially in combination with supercritical fluid chromatography (SFC) analysis. Here, the aqueous sample is put in the ActiVial and mixed so the lipophilic target analytes can be trapped in the organogel. Then, the sample is discarded from the vials. In the next step, the addition of organic solvent dissolves the organogels with trapped analytes and the final extract can be directly injected on SFC instrumentation due to similarity in mobile phase composition.
Eight forms of vitamin E were selected as model analytes for the testing of ActiVial system. The extraction parameters including sample volume and shaking intensity and time were optimized based on the design-of-experiment approach. As several impurities were extracted from the ActiVial system using the final procedure, the separation method had to be also optimized to enable separation of these impurities and target analytes. The final SFC analyses were carried out on Torus 2-picolyl amine column using CO_2-based mobile phase within 6 min. The recoveries for all tocopherols and tocotrienols were determined within 50 – 70% for all analytes and the extraction method was applied on urine samples. Subsequently, the repeatability of the extraction and the variability between ActiVial batches was examined. In the last step, the possibility to down-scale the extraction procedure for cases when only a small amount of sample is available was studied using ActiVials with 5 and 25 µL octanol trapped in organogels instead of the original 50 µL.

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CHROMATOGRAPHIC DETERMINATION OF CARDIOLIPINS AND THEIR OXIDATION PRODUCTS

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Cardiolipins (CLs) represent a special class of glycerophospholipids with four ester-linked polyunsaturated fatty acids located exclusively in the inner mitochondrial membrane. The chemical structure and close proximity to the source of reactive oxygen species (ROS) make CLs the primary target of harmful lipid peroxidation. Currently, the chromatographic determination of oxocardiolipins (oxoCLs) is challenging mainly due to the lack of commercially available standards for developing chromatographic methods as well as sample preparation procedures.[1] In our study, tetralinoleoyl cardiolipin was used as a model substrate to set up the conditions for the generation of oxoCLs via ROS-mediated autooxidation (2M H_2O_2, 200μM Cu^{2+}, 120 min) and to optimize their extraction by the mixture of methyl tert-butyl ether, methanol and H_2O. The lipid extract of HepG2 cells was utilized for CLs profiling and LC-MS/MS method development. The samples were analyzed on Waters Acquity I-class UPLC hyphenated to a Synapt G2-S Q-TOF mass spectrometer with electrospray in negative mode. The MS data were obtained over a mass range of *m/z* 500-1800. The separation was carried on Waters ACQUITY BEH C18 column (100 × 2.1, 1.7 μm) at 50 °C under gradient with a mobile phase consisting of 2mM ammonium bicarbonate, acetonitrile and isopropanol at a flow rate of 0.200 mL/min. In total, 22 CLs were identified, proposing 10 candidates as potential oxidation substrates, which will be the subjects of our further research.

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References

1. VILLASEÑOR, A., GODZIEN, J., BARKER-TEJEDA, T.C., *et al.*: TrAC, 143, 2021.

NEW APPROACHES IN MINIATURIZATION OF SAMPLE PREPARATION - EXTRACTION FROM VAMS TIPS

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Microsampling is a method beneficial for preclinical studies as it allows multiple sampling of a single animal, therefore, the number of animals included in the study can be reduced. Volumetric absorption microsampling (VAMS) is a novel microsampling technique that uses hydrophilic polymer tips to aspirate a precise volume of blood. Compared to dried blood spot, this method is less dependent on haematocrit and sample collection is simpler and more accurate.[1] Electromembrane microextraction (EME) is a relatively new technique based on the extraction using a hollow fibre where the application of a direct current voltage accelerates the isolation of the analyte. Using EME, high recoveries can be achieved in a shorter time, matrix effects are reduced, and a 96-well format is also possible.[2]

The aim of our work was to develop a method for the determination of doxorubicin (DOX) and doxorubicinol (DOXol) from blood absorbed into VAMS tips using EME followed by UHPLC-MS/MS assay. In this work, we tested different EME settings (various supported liquid membranes, voltage, time, acceptor, and donor solution) to achieve the highest recovery. After partial validation, the method was applied for the analysis of blood samples taken after the administration of DOX to mice in a pilot PK study.

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References

1. PROTTI, M., MANDRIOLI, R., MERCOLINI, L.: Anal. Chim. Acta, 1046, 2019, 32–47.
2. HUANG, C., CHEN, Z., GJELSTAD, A., *et al*.: Trends Analyt Chem, 95, 2017, 47-56.