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Seznam prezentovaných plakátových sdělení

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Pokyny pro prezentaci plakátových sdělení

- *Prosíme všechny prezentující, aby svá plakátová sdělení vyvěsili v průběhu nedělního večera nebo pondělního dopoledne.*
- *Diskuse u plakátových sdělení budou probíhat volně v průběhu přestávek na kávu, případně po ukončení odborného programu.*
- *Hodnocení plakátových sdělení provedou členové nezávislé hodnotící komise.*
- *Vyhlášení tří nejlepších plakátových sdělení proběhne po ukončení poslední odborné sekce ve středu.*

Optimisation of extraction method for determination of PAHs in malt using HPLC-FLD method

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Keywords: *PAH, QuEChERS, malt*

The aim of this study was to develop a practical method for the determination of 15 polycyclic aromatic hydrocompounds (PAHs) in the samples of barley malt. Two extraction methods were compared; QuEChERS method and the pressurized extraction by acidified acetonitrile performed by EDGE extractor. Subsequentely the clean-up by dSPE method was performed (PSA and Supel™ QuE Z-Sep in the case of QuEChERS method and SPE PSA tubes in the case of the EDGE extractor). The chromatographic analysis was carried out by HPLC with fluorescence detection. The two methods were compared using spiked malt specimens. The recovery of individual PAHs was used for the comparison of both methods. Considering generally better recovery of the analysed compounds with the use of QuEChERS method (Recovery ranged from 75 to 92 %), its simplicity, price, and material availability the QuECHers method was selected as more convenient for routine determination of PAHs in malt matrix. The values of PAH content in samples of common malt were under or close to the LOQ values. The PAH residues over the LOQ values were found in samples of smoked malt.

UHPLC-HRMS STUDY OF IMPURITY PROFILES IN LEVOTHYROXINE TABLETS

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The purpose of this study was to identify impurities in different formulations of tablets containing levothyroxine using an UHPLC-HRMS method and to compare the profiles of known and unknown impurities. These impurities may be related to the reported adverse effects of levothyroxine tablets. In particular, the intent of the method was to (1) identify known and unknown impurities (molecular or supramolecular) from the active principle ingredient (API) and, from the excipients, such as mannitol, lactose, and stearic acid; (2) study the molecular interactions between API and the excipients and (3) optimize the metabolomic workflow including experimental design, detection, data analysis, statistical evaluation, and compound identification. Three different experimental designs were compared: *1-individual measurement, 2-all in one measurement* and *3-one batch processing* in terms of sample preparation and UHPLC-HRMS analysis. Data processing involved testing of different settings (mass accuracy, thresholds) as well as testing different software platforms.

The data were acquired in MS full scan mode and MS^e, both ESI negative and positive mode, and will be used in both targeted and non-targeted data processing. In targeted analysis we confirmed the presence of mannitol stearate in levothyroxine drug formulations containing mannitol as an auxiliary ingredient. Levothyroxine-lactose adducts were identified in some drug formulations with lactose. On the other hand, the presence of supramolecular levothyroxine-mannitol complex and levothyroxine-mannitol adduct was not confirmed in formulations.

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

UHPLC coupled with charged aerosol detector for rapid separation of steviol glycosides in commercial sweeteners and extract of *Stevia rebaudiana*

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Keywords: *artificial sweetener; charged aerosol detector; Stevia rebaudiana Bertoni; steviol glycosides; ultra-high performance liquid chromatography*

A fast gradient UHPLC coupled with charged aerosol detection enabling quantitation of stevioside, rebaudioside A-D, and steviolbioside in commercial sweeteners and *Stevia rebaudiana* plant extracts has been developed. All steviol glycosides were baseline-separated in less than 4 min with a total run time of 7 min (Figure 1). Buffer-free eluents were used in the separations and only 2.45 mL solvent were needed per analysis. Critical pair of glycosides, stevioside and rebaudioside A, were baseline separated. The most abundant glycoside detected in sweeteners was stevioside followed by rebaudioside A. A leaf-to-stem ratio describing the dominant accumulation of steviol glycosides in leaves affected the differences in the amount of steviol glycosides among plant samples.

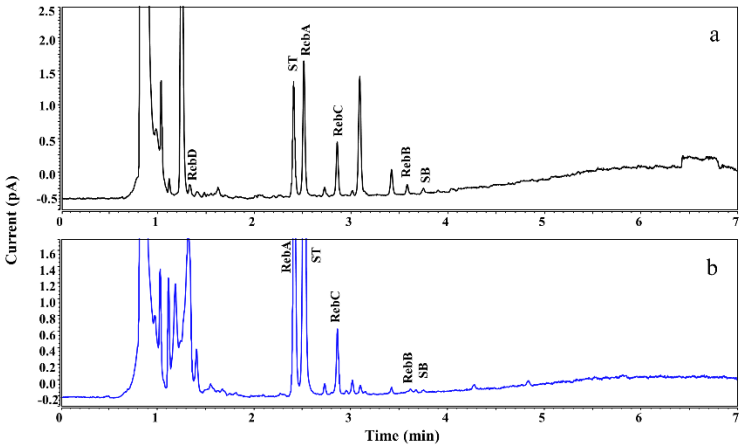

Figure 1. Chromatographic separation of extracts from tablet 6 (a) and stevia leaves 8 (b) using optimized UHPLC-CAD method.

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Improving HPLC separation of oligonucleotides by a novel recycle HPLC approach

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Keywords: *recycle HPLC, repetto, oligonucleotides, separation, resolution, efficiency*

Separation of biologically important substances such as proteins, peptides and nucleic acids, has become an important area of application for various modes of high-performance liquid chromatography in recent years. One of those applications is preparative chromatography for isolation of pure oligonucleotide synthesis products as they are of a fundamental importance in a variety of molecular methods including polymerase chain reaction (PCR), DNA sequencing, detection of pathogens by specific hybridization etc. The preparation of well-defined and pure oligonucleotides is often crucial for the success of these methods, so the efficiency of isolating the target sequence from incomplete synthesis products or other unwanted impurities is very important.

The concept of recycling chromatography, where the separation is improved by reinjecting the analytes back to the column for repeated passage, has been known in preparative chromatography since the 1960s. Recently, however, a completely new concept referred to as "repetto", where segments of the separated mixture are sequentially returned to the column head without the need to pass through the pump shows new possibilities for analytical HPLC. The repetto approach exhibits minimal overall dead-volume and, unlike in twin column chromatography (an alternate recycle approach) neither the column nor detector are exposed to a back pressure.

The main goal of this work was to optimize conditions for an isocratic separation of a mixture of oligonucleotides differing in sequence length or content of high-melting G and C bases and subsequent demonstration of significant improvement in resolution of their separation using the above-mentioned recycling chromatography approach. The benefits of recycling in the separation of mixtures of different types of oligonucleotides, including types with different lengths

or relative proportion of guanine and cytosine in the sequence defining the melting temperature, was demonstrated. The results of the thesis proved the applicability of the chosen recycling principle.

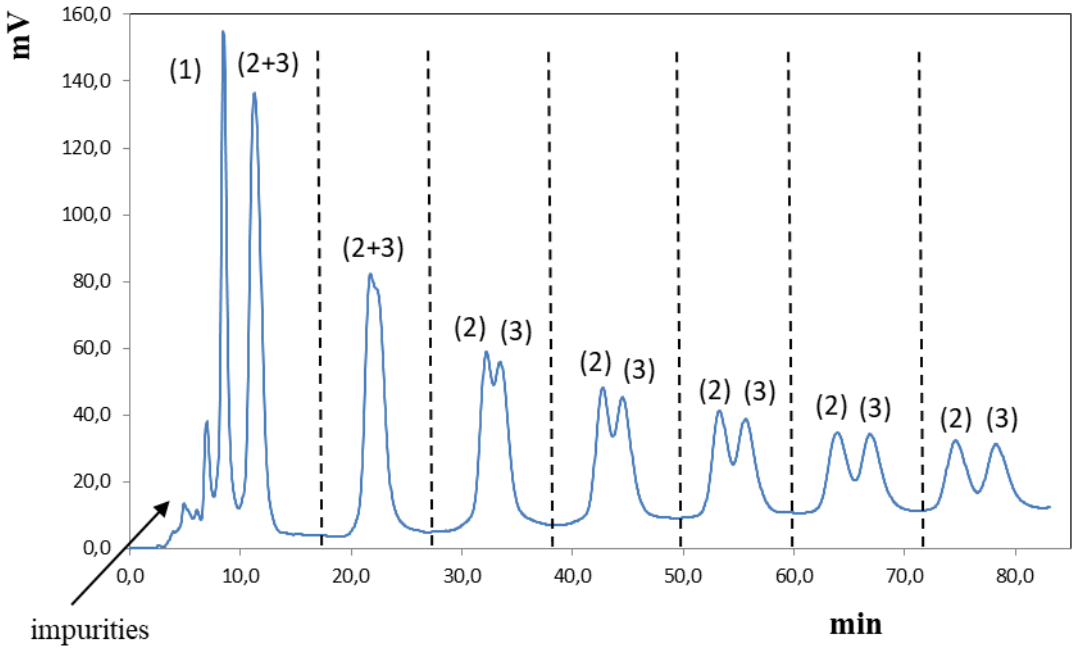

Figure 1. Improvement of separation of long oligonucleotides (54, 55 and 56nt, (~40 % GC), using repetto approach.

HPLC conditions: Column: XTerra C18, 50x4,6 mm, 100 Å, 2,5 µm, mobile phase composition: A (5% ACN v 0,1 mol/dm3 TEAA, pH 7), B (15% ACN v 0,1 mol/dm3 TEAA, pH7), C (100% ACN), A:B (50:50, v/v, 10 % ACN), flowrate: 0,4 ml/min, separation temperature: 60°C, injection: 20 µl, UV detection at 260 nm.

This work was supported by TACR project no. TO01000232

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The effect of tandem coupling of NicoShell and TeicoShell columns in sub/supercritical fluid chromatography on enantioresolution

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Keywords: *enantioseparation, NicoShell, SFC, tandem column coupling, TeicoShell*

A coupling of columns in sub/supercritical fluid chromatography (SFC) represents a possible way to influence the separation efficiency and to extend the selectivity of the separation system [1]. A suitable combination of different types of chiral stationary phases may lead to an improvement in the enantioresolution of chiral compounds if the columns coupled in series are complementary to each other. Opposing properties of tandem columns can result in the loss of the enantioresolution [2]. In this work, two superficially porous particle (2.7 μm) macrocyclic glycopeptide-based columns, i.e., TeicoShell and NicoShell, were serially coupled and tested in SFC for the first time. The influence of tandem column arrangement on the enantioseparation of structurally different biologically active compounds, i.e., benzodiazepines, β-blockers, phenylalanine, and its related compounds, was studied. The obtained data showed how the column order crucially affected the enantioresolution of the compounds tested while the retention was negligibly affected in most cases. In the second part of this work, single TeicoShell and NicoShell columns were shown to be suitable for the development of highly efficient and fast/ultrafast SFC enantioselective methods for structurally diverse chiral compounds. The optimized methods for sub-minute enantioselective separation of certain biologically important compounds were proposed.

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Chromatographic methods of phytochemical analysis of *Cannabis*

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Keywords: *Cannabis sativa; hemp; cannabinoids; terpenes; phenolic compounds; liquid chromatography; gas chromatography*

Cannabis sativa is a multi-purpose crop used in medicine, agronomy and different industries. It is an annual herbaceous plant with a unique composition of secondary metabolites. Technical cultivars (also known as hemp) are limited by the content of psychoactive cannabinoid tetrahydrocannabinol (THC) less than 0.2 % in the states of the European Union (EU) [1]. The presence of cannabinoids in food is controlled and the products containing cannabinoids are referred to as novel foods in the EU [2]. Moreover, hemp seed oil and cannabinoids are incorporated into cosmetic products. Presently, products with non-psychoactive, yet biologically active cannabidiol (CBD) are widely sold as hemp-derived dietary supplements, for example in the form of CBD oil or capsules. The legal status of CBD is different depending on its origin. Synthetic CBD is not subject to international control and can be marketed freely. Conversely, CBD extracted and/or isolated from the plant is a scheduled controlled substance and is under regulation [3]. Its origin can only be distinguished by analytical methods and chemical analysis of the impurity profile.

Medicinal cannabis is used for the treatment of many indications (chronic pain, multiple sclerosis, Parkinson's or Alzheimer's disease, epilepsy, anxiety). The biologically active constituents of cannabis are involved in its various therapeutic effects [4]. More than 500

compounds have been reported in this species and cannabinoids, terpenes, and phenolic compounds are the most abundant classes of phytochemicals in cannabis plants [5]. The presence of the particular secondary metabolites in the medicinal formulations is essential for the pharmacological activity due to the synergistic interactions between the constituents termed the entourage effect [6].

In this work, we present methods for the analysis of phytochemicals in cannabis samples using liquid or gas chromatography with mass spectrometry or ultraviolet detection. The presented methods could be applied for qualitative and quantitative control of *Cannabis sativa* and its derived preparations and products. The analysis is crucial for the understanding of the biological effect and the appropriate application of cannabis preparations/therapeutics and the product and food safety. In addition, the composition of secondary metabolites varies among cannabis genotypes and the complex phytochemical analysis could be used for the chemotaxonomic classification of cannabis.

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The effect of ion-pairing systems on phosphorothioate oligonucleotides diastereomer separation in ion-pairing reversed-phase chromatography

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Keywords: *phosphorothioate oligonucleotides, ion-pairing reversed-phase chromatography, diastereomer separation, ion-pairing agents*

Therapeutic oligonucleotides are commonly modified to increase their *in-vivo* stability against exo/endonuclease activities. One of those modifications, phosphorothioation, introduces a chiral centre into a phosphate backbone, which leads to the formation of 2^n diastereomers (where n is the number of modifications) [1]. The huge number of diastereomers and thus potentially separable peaks makes chromatographic analysis and quantification quite challenging. For these reasons, the suppression of diastereomer separation is necessary. Utilization of ion-pairing reversed-phase chromatography allows to suppress the hydrophobic interactions responsible for diastereomer separation and give priority to electrostatic interactions [2].

In our study, ion-pairing reversed-phase ultra-performance liquid chromatography was utilized for the analysis of native and phosphorothioate therapeutic oligonucleotides (21 mers) with the identical nucleobases sequence. The effects of ion-pairing agents´ character and concentration on retention, diastereomer separation and resolution of mers of different length were investigated. Nine different ion-pairing agents, i.e. alkylamines at two concentrations in an arrangement ion-pairing agent + acetic acid were tested. Results show that increasing hydrophobicity and concentration of ion-pairing agent causes higher suppression of diastereomer separation, but contrary to expectations, not in a whole hydrophobicity range. Results also confirmed that increasing hydrophobicity and concentration of ion-pairing agents provide higher resolution of n and n-1 mers. Using hexafluoroisopropanol as a counter-ion instead of acetic acid significantly improves the ability of ion-pairing systems to suppress diastereomer separation, even though their concentration was lower.

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Determination of steroids with neuroactive effects in human serum by UHPLC–MS/MS

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Keywords: *Neuroactive steroids, Neurosteroids, Ultra-high-performance liquid chromatography–tandem mass spectrometry*

Steroid hormones are primarily known for their involvement in adolescence, reproduction, or for their metabolic effects [1]. Less well known is the ability of some of them to affect the nervous system [2]. All substances of steroid origin that are able to influence the function of the nervous system are referred to as neuroactive steroids (NASs) [3]. Steroids are biosynthesized in the "typical" steroidogenic organs, which are the gonads (testes and ovaries), placenta, and adrenal glands, but also in the skin, adipose tissue, intestinal tissue, gut microbiota or directly in the nervous system [3–5]. This specific subgroup of NASs produced in the central and peripheral nervous system by neurons or glial cells has been named neurosteroids. NASs also include synthetic steroid molecules. Based on the interactions of NASs with different types of receptors, their mechanisms of action can be divided into genomic and non-genomic [6]. A less known non-genomic mechanism of action is mediated by the interaction of NASs with ion channels and membrane receptors. The resulting effect is almost immediate, unlike the genomic mechanism where gene transcription is regulated.

The levels of some NASs can be altered by pathological processes in the nervous system [3]. Changes in their levels have been observed, e.g., in multiple sclerosis, Parkinson's disease, Alzheimer's disease, psychiatric disorders, etc. This work aims to develop and validate a comprehensive method for profiling nine steroids with neuroactive effects in human blood serum. The method combines a relatively simple and rapid purification and extraction protocol based on the precipitation of serum proteins and their subsequent removal, followed by the detection and quantification of steroid analytes by ultra-high-performance liquid chromatography combined with electrospray tandem mass spectrometry (UHPLC–MS/MS). The selected analytes include steroids

classified as androgens, specifically testosterone, 5α-dihydrotestosterone, androstenedione, dehydroepiandrosterone and epiandrosterone, and as progestins, namely pregnenolone, progesterone and 5α-dihydroprogesterone. The developed method can be an effective tool for finding new biomarkers useful in the prevention, diagnosis, or monitoring of diseases associated with changes in NAS levels. The acquired knowledge may contribute to the development of new drugs and therapeutic approaches.

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Development of capillary electrophoresis method for the simultaneous separation of nonsteroidal anti-inflammatory drugs and boswellic acids

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

The acidic BGE (50 mmol/l acetic acid, pH 4.5 : MeOH : ACN; 10:3:12) allowed to achieve the best resolution for the individual NSAID (15 compounds resolved into 12 peaks). However, poor selectivity for the boswellic acids was observed under these conditions; they migrated unresolved with EOF. On contrary, the application of high-pH buffers allowed to improve the resolution of boswellic acids at the cost of worsening the separation selectivity for the NSAID. Using the 40 mmol/l ammonium acetate (pH 8.5), MeOH, ACN (5:1:4) BGE, it was possible to separate the Boswellia serrata extract into 4 peaks, while the mixture of 15 NSAIDs was separated only into 9 peaks. A 40 mmol/l ammonium bicarbonate (pH 8.5), MeOH, ACN (5:1:4) BGE has also shown promising separation resulting in 9 peaks of NSAID and two peaks of Boswellia serrata extract.

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ITP Analysis of Amine Derivatives of Adamantane

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Keywords: *ITP analysis; Amantadine; Rimantadine; Memantine*

The amine derivatives of adamantane (Amantadine, Rimantadine, Memantin) are widely used as antiviral drugs against various strains of flu [1] and/or for treatment of Parkinson disease. SQ109 based on etylenediamine-skeleton of adamantane is active against both drug susceptible and multi-drug-resistant tuberculosis bacteria while it is assumed that adamantane skeleton is responsible for biological activity as consequence of its high lipophilicity and hydrophobicity.

The development of determination of above-mentioned compounds is paid high attention [1]. The analytical methods of molecular spectroscopy (absorption, luminescence, NIR), flow-injection analysis and potentiometry with ISE were tested [1-2] while hyphenated chromatographic methods, mostly HPLC-MS, were employed for analysis of biological samples [1]. In addition, capillary electromigration techniques (electrophoresis, isotachophoresis - ITP) were utilized for analysis of these compounds in mixture [2, 3, 4] and their sufficient resolution in CZE was achieved by addition of cyclodextrines when inclusion complexes of high stability are formed [4, 5]. The application of cyclodextrins as complexing agents in capillary ITP is less often than in CZE, therefore the new analytical method for determination of Amantadine and Rimantadine in mixture was developed.

Acknowledgment

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Optimization of UHPLC method for analysis of vitamin K in plasma and serum

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Keywords: vitamin K, UHPLC, human serum, human plasma

Vitamin K is a fat soluble vitamin, well known as an essential factor in blood coagulation. It consists of many similar compounds, vitamin K1 known as phylloquinone and K2, menaquinones (MKn), that exists in 10 different analogs from MK4 to MK13. Separation of more than one form hence could be quite challenging, because of structural similarities and low concentrations in human fluids [1]. So optimization of suitable UHPLC-MS/MS conditions and modern sample preparation could be the solution for achieving a sensitive and selective method.

Different stationary phases, C18, PFP, and biphenyl, in combination with various mobile phases, were tested for separating vitamin K1 and vitamin K2, specifically MK4, MK7, MK9 in human serum and plasma. Also, a sample pre-treatment procedure suitable for clinical research and practice was developed with the tendency on simple, fast, and ecological techniques. The results will be presented and discussed.

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MONITORING OF ALBENDAZOLE TRANSFER FROM OVINE FAECES TO FODDER PLANTS AND CONNECTED SOIL BY UHPLC-MS

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Keywords: *veterinary drugs, environmental analysis, UHPLC-MS*

The anthelmintic drug albendazole (ABZ) with a broad-spectrum anthelmintic effect is used for the treatment of helminthiasis caused mainly by gastrointestinal worms in veterinary and human medicine. Frequent dose, overdose or underdose make a risk of developing resistance, which can be a serious global problem in the treatment of helminthiasis. The anthelmintics can enter the environment unchanged as a parent compound or as a metabolite through the faeces or urine. These chemicals can be found in soil and groundwater where there is the potential for uptake into plants.

Laboratory experiments have shown that the plants can uptake and even biotransform the ABZ in laboratory-controlled conditions, however, it was not known whether these phenomena occur in real field conditions. The present study monitors the transfer of ABZ and its transformation products (TPs) albendazole sulfoxide (ABZ-SO) and albendazole sulfone (ABZ-SO_2) from the faeces of treated sheep to common fodder plants *Medicago sativa* and *Trifolium pratense* and adjacent soil. This was observed from different distances (plants, soil) and depths (soil) to which the compounds could spread from piles of the faeces.

Our study successfully revealed the occurrence of ABZ TPs (ABZ-SO and ABZ-SO_2) in the soil-plant system. Even two months after the first contact of fodder plants with faeces, ABZ-SO was still present in plants and the adjacent soil.

The UHPLC-MS method for the analysis of ABZ, ABZ-SO, and ABZ-SO_2 was developed and validated. To ensure that the method is suitable for its intended use, the method validation has been performed according to the SANTE/11813/2017 guideline. Pharmaceutical compounds were extracted from soil and plant samples using validated methods chosen for their high percentage recoveries and cost convenience. The QuEChERS (soil) and LLE (plants) sample preparation methods were applied.

UHPLC-MS/MS method for determination of glucosinolates in minute sample

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Keywords: Brassicaceae metabolism, glucosinolates, weak anion exchange solid phase extraction, sample miniaturization, standard addition method, spiked combined matrix quantification, UHPLC-MS/MS analysis

Glucosinolates are specialized metabolites present in Brassicaceae plants that are involved in different aspects of plant life cycle. Except their biological activities *in planta*, their nutritional value through the consumption of cabbage vegetables (Brassica crops) is recognized too. Modern analytical techniques present valuable tools to study their impressive chemical diversity, which counts more than 120 so far known compounds [1]. In addition, great challenge in accurate quantification of glucosinolates is their natural abundance in plants that varies from picomolar to micromolar concentrations. The general approach still employed by numerous research groups is the determination of desulfated glucosinolates using a high-performance liquid chromatography coupled with diode array detector (HPLC-DAD) according to ISO standard (ISO 9167-1:1992). This technique has several limitations in terms of duration, accuracy, sensitivity and amount of plant material (>30 mg of dry weight) required for analysis. Significant technical improvements of mass spectrometry-based instruments enable more accurate and precise detection of analytes in minute amount [2].

Herein, we have developed a method for profiling nineteen intact glucosinolates in less than one milligram dry weight of plant tissues. Using our method, we were able to analyze the following (i) aliphatic glucosinolates (glucoiberin, glucocheirolin, sinigrin, progoitrin, epiprogoitrin, gluconapin, glucobrassicanapin, glucoraphanin, glucoraphenin, glucoberteroin and glucoerucin), (ii) aromatic glucosinolates (glucotropaeolin, gluconasturtiin, glucosibarin, glucobarbarin and sinalbin), and (iii) indole glucosinolates (glucobrassicin, 4-metoxyglucobrassicin and neoglucobrassicin). The optimized protocol includes (i) a sample purification step by minimized *in-tips* micro-solid phase extraction using a weak anion exchanger sorbent and (ii) targeted analysis

by an ultra high-performance liquid chromatography coupled with a triple quadrupole mass spectrometer (UHPLC-MS/MS) in less than 6 min. The lack of stable isotopically labeled internal standards required for accurate quantification was overcome by spiking the plant matrix with known amounts of standards. The plant matrix used to construct the calibration curves presented a homogenous mixture of all samples from the same type of tissue. Different dilution factors were employed due to the high dynamics in natural abundance of glucosinolates and the short linear dynamic range of UHPLC-MS/MS method covering only three orders of magnitude (0.01-1 pmol injected). The adopted quantification approach resulted in 95-100% accuracy compared to quantification using the classic standard addition method and halved the number of samples required for validated analysis.

Our developed highly accurate and precise UHPLC-MS/MS method can be efficiently applied for fundamental and applied biological studies of glucosinolate metabolism in various Brassicaceae species.

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UHPSFC-MS/MS analysis of vitamin D and its metabolites in liver tissue

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Keywords: *vitamin D, supercritical fluid chromatography, mass spectrometry, liver tissue*

Vitamin D plays an important role in many biological processes. Despite the fact, that metabolites such as various hydroxylated forms and esters were described, the exact metabolism and storage in human body is still unknown. Analysis of vitamin D is challenging with bottlenecks originating from its lipophilic nature, chemical instability, and matrix complexity.

The aim of our study was to develop fast, selective, and sensitive method enabling analysis of vitamin D and its metabolites in biological matrix using ultra-high performance supercritical fluid chromatography with mass spectrometry detection (UHPSFC-MS/MS). Based on the previous study [1], 1-aminoantracene column with good selectivity for hydroxylated metabolites and mobile phase containing carbon dioxide with methanol (MeOH) as a modifier were used as the starting point for the further optimization. Isopropanol, acetonitrile and their mixtures with MeOH were tested to enable separation of esters, vitamin D, and hydroxylated forms. Separation was finally achieved using methanol/isopropanol mixture (50/50) with 8 % water. The effect of temperature, pressure, and gradient affecting mostly hydroxylated forms was studied. MS with atmospheric pressure photoionization using organic solvent as a dopant was optimized for the detection. Two solid phase extraction strategies, “bind and elute” and “removal”, using HLB sorbent with preceding acetone extraction were optimized for the liver tissue extraction. Both methods were compared in the terms of recovery and matrix effects.

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Capillary electrophoretic peptide profiling of in-bone digested proteins – a promising tool for evaluation of pathological states in oral surgery

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Keywords: *alveolar bones / capillary electrophoresis / in-bone protein digestion / linear discriminant analysis / principal component analysis*

Since the commonly used histological assessment of pathological states of alveolar bone tissues in oral surgery needs a laborious and time-consuming processing by the experienced histologist, easier and faster methodology is required in this field. Following this demand, we report here a straightforward approach using specific proteolytic (tryptic) cleavage of proteins directly in the bone without its demineralization, followed by CE-UV analysis (profiling) of the yielded protein digest. Cleavage-derived peptides were separated as cations by CE in acidic background electrolytes, pH 2.01-2.54. The best resolution of peptide fragments with the highest peak capacity was achieved in the background electrolyte composed of 55 mM H_3PO_4, 14 mM Tris, pH 2.01. The differences in the obtained CE-UV profiles with characteristic patterns for the particular bone samples were subsequently discriminated by linear discriminant analysis over principal components. This approach was first verified on porcine bone tissues as model samples and subsequently applied on human alveolar bones. Samples of porcine jawbone cleaved by trypsin could be discriminated with accuracy of 100% from those cleaved by chymotrypsin. The same degree of accuracy was achieved for discrimination between samples of porcine jawbone and porcine calf bone, both of them cleaved by trypsin. Finally, the method proved the capability to differentiate unequivocally human healthy and inflammatory alveolar bone tissue samples obtained from an oral surgery. The reported procedure [1] seems to be promising to become a complement or even an alternative to the traditional histological distinguishing of healthy and pathological bone tissues in the oral surgery.

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Application of CO_2 enriched fluids for extraction of polar phenolic compounds

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Keywords: *supercritical fluid extraction, phenolic compounds, apples*

Advanced technique of supercritical fluid extraction (SFE) was applied for the extraction of bioactive substances from dried apples. SFE allows to minimize the use of toxic solvents and is suitable for the extraction of valuable bioactive compounds which can easily degrade in the presence of air or light. Thanks to low viscosity and high diffusivity, liquids enriched with CO_2 possess great transporting properties resulting in shorter extraction time and greater extraction yields. Solvating power of such a fluid can be easily modified by pressure, temperature or even by the addition of a small portion of polar co-solvent. We studied the SFE conditions for 14 phenolic compounds. Analyses of extracts were carried out by the UHPLC-DAD method.

To study the influence of individual variables the Plackett-Burman design was created to monitor the importance and influence of studied variables (CO_2/ co-solvent ratio 10-90%, the water content in co-solvent 0-20%, pressure 100-300 bar, and temperature 30-80°C). The optimal extraction conditions were adjusted for the 6 most abundant substances (chlorogenic acid, epicatechin, phloridzin, quercitrin, catechin, guajaverin) in extracts. The influence of CO_2 percentage was identified as the most important factor followed by the water content and pressure. The effect of temperature was rather minuscule, but a decrease of some compounds was observed while employing higher temperatures. In the next steps, the extraction kinetics and influence of glass beads size were optimized. The optimal conditions were following: 10 % CO_2, ethanolic co-solvent with 20% of water, extraction temperature 59°C, pressure 300 bar, extraction medium flow rate 3 mL/min, extraction time 30 min, and glass beads size 3 mm.

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Partial filling affinity capillary electrophoresis applied for study of interactions between concanavalin A and (glyco)peptides

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Keywords: *PF-ACE, concanavalin A, glycopeptides, binding constant*

A new method, partial filling affinity capillary electrophoresis (PF-ACE), has been developed to study interactions of concanavalin A with octapeptide and its two analogs modified by mannose. Concanavalin A (ConA) is a lectin (tetrameric protein with four identical 28 kDa subunits) specifically binding sugars, glycopeptides and glycoproteins, mainly internal and non-reducing terminal α-D-mannosyl group.

PF-ACE experiments were carried out in Agilent CE7100 analyzer (Agilent, Waldbronn, Germany), equipped with photodiode array UV-vis detector set at 200 and 210 nm and with the fused silica capillary internally coated with polyanionic covalent coating (4% PAMAMPS, copolymers of acrylamide and 2-acrylamido-2-methyl-1-propanesulfonate), total/effective length 325/240 mm, id/od 50/375 μm, with outer polyimide coating (Polymicro Technologies, Phoenix, AZ, USA). The capillary temperature was set to 25°C and sample carousel was cooled to 20°C.

The apparent binding constants, K_b, of the ConA-(glyco)peptide complexes were determined from the dependence of the effective migration time changes of the above (glyco)peptides on the variable zone lengths of ConA dissolved in the background electrolyte (BGE) and hydrodynamically introduced into the capillary close to the UV detector [1]. The migration time of (glyco)peptides in alkaline BGE, 20/9.5 mM HEPES/NaOH, pH 7.4, 20 mM NaCl, 3 mM $CaCl_2$, 3 mM $MnCl_2$, in bare fused silica capillary was too short; therefore, coated capillary with tunable EOF was selected to obtain shorter time of analysis and to prevent adsorption of analytes to the inner capillary wall. The ConA interaction with the non-glycosylated peptide was very weak (K_b = 142 L/mol) whereas the ConA interactions with the glycopeptides were much stronger, with K_b values 24.0×10^3 L/mol, and 35.9×10^3 L/mol, respectively.

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Determination of pK_a values of cyclic dinucleotides diphosphates by CE

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Keywords: *acidity constant, capillary electrophoresis, cyclic dinucleotides diphosphates*

Capillary electrophoresis (CE) was employed for the determination of the acid-base properties of cyclic dinucleotides diphosphates: cyclic diadenosine diphosphate (c-diAdiP), cyclic diguanosine diphosphate (c-diGdiP) and cyclic guanosine-adenosine diphosphate (c-GAdiP), and their difluorinated derivatives. The mixed acidity constants, p$K_{a,mix}$, and the actual ionic mobilities of these compounds were determined by non-linear regression analysis of the pH dependence of their effective electrophoretic mobilities using the commercial software Origin Pro 8.5. The effective mobilities were measured by CE in a series of the background electrolytes in a broad pH range (0.92-11.48), at constant temperature of 25ºC, and mostly at constant ionic strength of 25 mM. Thereafter, the p$K_{a,mix}$, values were recalculated to thermodynamic pK_a values using the Debye-Hückel theory. In addition, a new freely available program AnglerFish [1] was employed, which enabled direct calculation of both the thermodynamic pK_a values of acid-base groups and the limiting ionic mobilities of cyclic dinucleotides from CE data. From the limiting ionic mobilities, the Stokes hydrodynamic radii of the particular ionic forms of the studied nucleotides were calculated. Additionally, the acid-base properties of mononucleotides, adenosine monophosphate and guanosine monophosphate, were investigated and compared with those published in the literature.

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Challenges and pitfalls of in-situ derivatization in bioanalysis

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Keywords: *Derivatization, diethyldithiocarbamate, biological matrix, platinum drugs*

Application of derivatization allows to change and optimize a chemical structure, thus changing the properties of the analyte for analysis. In bioanalysis, optimization of sample preparation is key to obtaining correct results, especially when the biological matrix differs from patient to patient.

Determination of platinum drugs can be tricky. Their retention on reverse-phases is demanding, while platinum drugs are very hydrophilic complexes [1]. Problematic is also their poor absorption in UV spectra [2]. Protocols applying derivatization with diethyldithiocarbamate (DDTC) can be used to determine various platinum drugs, as the derivatization agent provides with them the same complexes that can be detected [1]. Challenges and pitfalls of in-situ derivatization with DDTC applied for the HPLC-DAD determination of oxaliplatin and cisplatin in various biological matrices will be discussed.

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Monitoring the levels of B vitamins in different human groups using HPLC method

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Keywords: *Vitamin B, HPLC, methanol*

The B vitamin family is made up of eight B vitamins. Although they are commonly recognized as a group and often work together in the body, each of the B vitamins performs unique and important functions. At present, increase the requirements on measurement vitamins B1 and B6 in different population groups and also in search of the possible range specifying certain diseases. The main objective of this work was to monitor the levels of vitamin B in specific human groups and its determination as a marker of early diagnosis.

The level of vitamin B1 was studied in the group of 100 patients after intoxication with methanol and vitamin B6 in a group of 20 patients with autosomal dominant disease. Vitamin B1 was extracted from whole blood, vitamin B6 from plasma using two types of appropriate separation kits (RECIPE, CHROMSYSTEMS). Subsequent analysis was performed by high-performance liquid chromatography (HPLC, Agilent Technologies 1260).

We found out, that people after methanol intoxication had values of vitamin B1 in an appropriate reference range. Vitamin B6 was significantly increased in patients with autosomal dominant disease.

The results have interesting predictive value among specific human groups. The correctness of the analysis was confirmed by two different separation kits.

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ClinRep® Complete Kit for Vitamin B6 in Plasma / Whole Blood for 100 assays, RECIPE (CHEMICALS + INSTRUMENTS GmbH), München, Germany

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Determination of inflammatory biomarkers in gingival crevicular fluid as a tool for diagnosis of periodontal disease

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Keywords: *gingival crevicular fluid, chromatography, inflammation, mass spectrometry, periodontal tissue*

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 more precise quantification. The method was tested with real-life samples using GCF collected from patients suffering from periodontitis and healthy controls.

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Cibulové odpady jako zdroj biologicky aktivních látek

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Cibule kuchyňská (*Allium cepa* L.) je druhá nejpěstovanější zelenina na světě. Během posklizňového a potravinářského zpracování vzniká velké množství odpadů, zejména slupek, které jsou velmi bohaté na vlákninu a různé biologicky aktivní látky, zejména polyfenoly, konkrétně kvercetin a jeho glykosidy. Tyto látky mají mnoho pozitivních vlivů na zdraví, jsou to zejména antioxidanty [1]. Antioxidanty jsou látky, které dokáží organismus chránit před tzv. oxidačním stresem, což je faktor, který přispívá ke vzniku mnoha civilizačních onemocnění [2].

Cibulové odpady by mohly být zakomponovány do receptur některých potravin, např. pekařských výrobků. Takové produkty by mohly být označovány jako tzv. funkční potraviny.

Potenciální zdravotní efekt polyfenolů ale závisí na jejich biodostupnosti a jejich tepelné stabilitě nebo transformaci během přípravy potravin. Některé polyfenoly totiž nejsou tepelně stabilní a vůbec nemusí být přítomné v hotové tepelně upravené (např. upečené) potravině [3].

Cílem tohoto pokusu bylo zjistit vliv přídavku cibulových slupek červené odrůdy Lisa do bezlepkového pohankového chleba, se zaměřením na:

i. tepelnou stabilitu polyfenolických látek během pečení a antioxidační aktivitu těst a chlebů a
ii. vliv konzumace obohaceného chleba na *in vivo* antioxidační aktivitu krve dobrovolníků.

Výsledky pokusu ukazují, že 5% nahrazení bezlepkové mouky mletými cibulovými slupkami přineslo významné zvýšení antioxidační aktivity (metodou DPPH) chlebů v porovnání s chlebem kontrolním (neobohaceným). Tento významný nárůst je způsoben přítomností polyfenolických látek z cibulových slupek, které byly identifikovány a kvantifikovány pomocí HPLC-MS/MS. Jednalo se zejména o kvercetin (aglykon), kvercetin-4‘-monoglukosid, kvercetin-3,4‘-diglukosid a také o dimery kvercetinu a jeden trimer. Pečení chleba (180 °C/30 minut) způsobilo významné rozdíly v kvalitě i kvantitě derivátů kvercetinu (před x po pečení). Ukázalo se, že kvercetin je teplotně poměrně stabilní, zatímco jeho deriváty, zejména dimery a trimer byly během tepelného opracování téměř úplně rozloženy, což mělo za následek, že obsah kvercetinu (aglykonu) byl vyšší po pečení než v těstě.

Dalším velmi zajímavým zjištěním bylo, že pečený chléb obohacený o cibulové slupky měl oproti těstu před pečením vyšší antioxidační aktivitu. Z toho lze vyvodit, že pokud je kvercetin vázán ve složitějších komplexech, např. ve formě glykosidů či dimerů nebo trimeru, tak je jeho antioxidační aktivita nižší. Naopak po rozvolnění těchto vazeb je kvercetin antioxidant silnější.
V cross-overovém experimentu, kterého se zúčastnilo 14 dobrovolníků, bylo každému z nich podáno nejprve 200 g kontrolního neobohaceného chleba a po wash-out periodě 200 g chleba obohaceného cibulovými slupkami. Dobrovolníkům byla odebrána krev vždy před a 90 minut po konzumaci chleba. Následně byla stanovována *in vivo* antioxidační aktivita krve pomocí kitu FORD (Callegari SpA, Parma, Itálie). Z výsledků vyplývá, že konzumace obohaceného chleba o cibulové slupky dokáže zvýšit antioxidační aktivitu krve, čímž tak může preventivně oddalovat či tlumit oxidační stres.

Výsledky práce naznačují, že cibulové slupky, které jsou většinou jako odpad kompostovány nebo využívány jako palivo pro bioplynové stanice, jsou bohatým zdrojem polyfenolů a mohou být využity i pro potravinářské účely.

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