BOOK OF ABSTRACT

September 22-25, 2024 – Messina, Italy

Recent developments in enantioselective liquid-phase separations

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Keywords: Capillary electrophoresis, capillary electrochromatography, Chiral recognition mechanisms, enantioseparations, high-performance liquid chromatography.

Objective

The objective of this presentation is to describe developments in the field of enantioselective intermolecular interactions and separation of enantiomers of chiral compounds. The major idea is to show how theory-based approaches can help design and prepare novel powerful materials for solving challenging separation-science problems and also the opposite approach, how application-driven research can advance our understanding of noncovalent interactions playing out in chiral recognition.

Methods

From the separation science portfolio, the methods based on high-performance liquid chromatography, nano- and capillary liquid chromatography, super/sub-critical fluid chromatography, capillary electrophoresis and capillary electrochromatography will be overviewed. Out of the non-separation techniques, primarily nuclear-magnetic-resonance-based methods in combination with molecular modeling will be discussed for specific cases.

Results

The development and commercialization of novel polysaccharide-based chiral selectors for liquid-phase separation of enantiomers, novel chiral adsorbents based on monolithic and superficially porous silica, enantioseparations with exceptionally high selectivity, as well as baseline separation of enantiomers within few seconds in HPLC will be discussed, along with the contemporary theory on enantioselective capillary electrophoresis and new approaches used in capillary electrochromatography [1-3].

Conclusions

The field of enantiosepearations has seen significant advancements over the last three decades. These advancements, specifically the use of capillary electrophoresis, immensely supported the better understanding of enantioselective noncovalent interactions. This latter technique helps in itself the development of new tailor-made chiral selectors for challenging problem-solving in the field of separation science, among others.

ACKNOWLEDGMENTS

B. Chankvetadze thanks Shota Rustaveli National Science Foundation of Georgia for a partial support of his research among other with Grants FR-22–971 and FR-23-24319 for fundamental research.

REFERENCES

[1] Chankvetadze B., (2018). Contemporary theory of enantioseparations in capillary electrophoresis, *J. Chromatogr. A,* 1567, 2-25

[2] Chankvetadze B., (2020). Recent trends in preparation, investigation and application of polysaccharide-based chiral stationary phases for separation of enantiomers in high-performance liquid chromatography, *Tr. Anal. Chem.,* 122, 115709

[3] Chankvetadze B., Scriba G.K.E., (2023). Cyclodextrins as Chiral Selectors in Capillary Electrophoresis: Recent Trends in Mechanistic Studies, *Tr. Anal. Chem.,* 160, 116987.

Exploring the evolution from one- to five-dimensional separation techniques: a personal journey in advancing chromatography and hyphenated detection

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Keywords: hyphenated detection, Multidimensional Chromatography, LC×LC, GC×GC, complex mixtrures

Objective
The advancement in chromatography theory and instrumentation was initially and still is driven by the need for a more comprehensive technique, able to accurately and precisely discriminate targeted and untargeted analytes in challenging sample mixtures, with the highest possible sensitivity and selectivity.

Methods
This presentation will illustrate different analytical solutions implemented in an effort to resolve high- to extremely complex mixtures, relying on heart-cutting (LC-GC, LC-GC-GC-GC-prep., LC-LC-GC, GC-GC) or comprehensive (GC×GC, LC-GC×GC, LC×LC) multidimensional techniques.

Results
Matrix composition and the analytical goal will direct the choice of coupling an additional GC, LC or MS stage, to exponentially extend the capability of the resulting multi-dimensional hyphenated technologies. System-oriented interfaces/devices have been customized in MD instrumentation, based on the optimization of partially concurrent solvent evaporation, the use of valve-based or Deans switch systems with multi-cutting capabilities, and the addition of an automatic collection device as the back-end. The interfacing of two capillary GC columns in a comprehensive way relied on a cryogenic modulator and, more recently, exploited the benefits of split flow and vacuum outlet flow modulation.

Conclusions
The hyphenation to state-of-the art quadrupole and triple quadrupole MS in fast GC and GC×GC approaches, LC×LC approaches and a unified LC-GC×GC-MS/MS platform allowed for the selective determination of trace level contaminants. In parallel, our efforts have been put in developing dedicated data analysis software, to glean useful information from the complex data sets driven by the comprehensive separations, and Linear Retention Indices tool for reliable identification of unknown components in spectral libraries, as also demonstrated in miniaturized LC or SFC-based techniques.

Separation sciences - the key in phytochemistry

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Keywords: phytoanalysis, chromatography, enrichment, purification

Objective

Analytical chemistry and cell biology have significantly advanced natural product research. Developments in chromatography have opened new avenues for the analysis and isolation of natural products, crucial due to the complexity of plant extracts, which contain hundreds to thousands of compounds at varying concentrations.

Methods

Novel enrichment and purification methods particularly advanced solid-phase extraction and dual-flow chromatography [1] combined with high-resolution chromatographic separation and mass spectrometric detection (LC-MS), are essential for accurate phytochemical profiling and the quantification of specific metabolites. Combining separation science with spectroscopy, such as near- and mid-infrared (IR) spectroscopy, facilitates rapid, non-invasive qualitative and quantitative analyses of raw plants and extracts.

Separation methods based on different stationary phases (particle and monolithic based materials) focus on detecting low molecular weight compounds like phenolics. Furthermore, the detection of other biomolecules e.g. DNA and proteins in plant materials is of gaining importance. Plant proteins can serve as active ingredients or allergens, and their identification and quantification are critical for quality control, ensuring the effectiveness, quality, safety, and authenticity. The latest advancements in Next-generation protein sequencing™ (NGPS) allow for the direct sequencing of peptide barcodes with single-molecule resolution.

Results

The integration of advanced chromatographic and spectroscopic techniques enabled the precise enrichtment, isolation, identification, and quantification of key phyto-molecules and proteins within complex plant matrices. Numerous chromatographic applications for phyto-analysis e.g. separation of PAHs, pyrrolizidine-alkaloids or for olive oil compounds have been developed at ADSI and will be presented [2, 3, 4].

Conclusions

The advancements in chromatographic, spectroscopic, and sequencing technologies have revolutionized natural product research by enabling highly accurate and efficient analysis of complex plant extracts. These methodologies not only enhance the identification and quantification of critical phytochemicals and proteins but also significantly improve quality control measures. As a result, they contribute to ensuring the safety, efficacy, and authenticity of phyto-based products, paving the way for more reliable and effective applications in pharmaceuticals, cosmetics, and nutrition.

Many chromatographic developments for phytoanalytics which are presented go back to the ideas and visions of my teacher Prof. Csaba Horvath.

REFERENCES

[1] Bonn, G., Gjerde, D., 2017. Dual Flow Chromatography, *LC-GC Europe*, 30, 404-411.

[2] Stuppner, S., Hussain, S., Märk, B., Gjerde, D., Rainer, M., Jakschitz, T., Bonn, G. K. 2020. An automated preloaded pipet tip SPE method for the accurate quantification of carcinogenic polycyclic aromatic hydrocarbons from tea. *Analytical Methods*, 12(14), 1827-1833.

[3] Handle, K. F., Bakry, R., Bonn, G. K. 2023. Phosphonium based anion exchange resin for enrichment of phenolic acids. *Journal of Chromatography A*, 1700, 464049.

[4] Kreidl, M., Rainer, M., Jakschitz, T., Bonn, G. K. 2020. Determination of phototoxic furanocoumarins in natural cosmetics using SPE with LC-MS. *Analytica chimica acta*, 1101, 211-221.

Electrolyte solution-mediated migration behavior of biomolecules in capillary zone electrophoresis

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Keywords: capillary zone electrophoresis, background electrolyte solution, biomolecules, migration behaviour.

Objective
Capillary zone electrophoresis (CZE) is performed in capillary columns using a background electrolyte solution (BGE) of uniform composition. The separation mechanism is based on differences in the migration velocity of the charged analytes under the action of a constant electric field and, therefore, on their charge-to-hydrodynamic-radius ratio. This communication examines and discusses the effects of the composition of BGE on charge-to-hydrodynamic radius ratio of biomolecules, in order to modulate their migration behavior and regulate the selectivity of CZE for a given separation. The influence of BGE on the electric double layer occurring at the solid-liquid interphase inside the capillary tubes, which generate the electroosmotic flow (EOF), is discussed too.

Methods
The study was performed investigating the influence of a variety of BGE of selected composition on the migration behavior of biomolecules bearing different concomitant functionalities, consisting of ionizable and/or hydrogen-bonding groups, hydrophobic regions, and hydrophilic moieties, capable to interact to different extents with both ionic and nonionic components of BGE. All experiments were performed using either plain aqueous or hydro-organic electrolyte solutions, containing buffering agents and additives [1]. Efficiency, selectivity, and resolution obtained with electrolyte solutions of different composition were compared and the observed variations of the migration behavior of the analytes were correlated to pH, ionic strength and chemical composition of BGE.

Results
Significant variations of the migration behavior of positively charged biomolecules were evidenced by using BGEs containing buffering agents having same cationic and different anionic components, while the ionic strength of solutions was maintained at a commun constant value by adding proper amounts of a neutral salt (NaCl). Under certain conditions, the observed variations included changes in the migration order of the model biomolecules, which were ascribed to selective ion-pairing interactions between the anionic components of the buffer and the cationic analytes. Other experimental data evidenced selective hydrophobic interactions of peptides and proteins with the counterions of ionic liquids employed as dynamic coating agents of bare fused-silica capillaries. Selective interactions also took place between phenolic compounds and 2,2,2-trifluoroethanol, used as an additive of BGE employed for the separation of these compounds as borate complexes. Carbohydrates, lacking easily ionizable functions and chromophore groups, were separated using BGEs at extremely high alkaline conditions (pH 12 and higher), at which they migrate as anions and can be directly UV detected at 270 nm due to the photo-oxidation reaction occurring while passing the detection window.

Conclusions
Appropriate selection of BGE for CZE of biomolecules requires the evaluation of the interactions between analytes and BGE, which should exhibit low conductibility, buffering capacity in a wide pH range, and capability at controlling EOF and untoward interactions of the analytes with the capillary wall. Novel BGEs meeting these requirements comprise aqueous solutions of oligoamines in combination with a polyprotic acid. Carbohydrates can be separated by CZE using strong alkaline BGEs, which also allows their direct UV detection at 270 nm.

ACKNOWLEDGMENTS

Part of this research was funded by the European Union, Project Olive-Net, grant N. 734899, *Horizon 2020.*

REFERENCES

[1] Corradini D., (2023). Buffering agents and additives for the background electrolyte solutions used for peptide and protein capillary zone electrophoresis, *TrAC, Trends in Analytical Chemistry*, e-117080.

Endotoxin quantification by a chemical instrumental analytical (U)HPLC-assay

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Objective

Endotoxins (ETs) are ubiquitous in our environment, leading to the risk of ET contaminations in pharmaceutical and biopharmaceutical products. ETs are non-covalently attached into the outer membrane of all Gram-negative bacteria; they cover about 75 % of their cell surface. ETs are released during bacterial proliferation, growth, and membrane lysis in large quantities. ETs are strong immunostimulants, even quantities as low as pgmL-1, introduced into the human bloodstream e.g. via contaminated medical devices, surgical equipment, or drugs, can trigger severe reactions in the human organism such as fever, sepsis, organ failure and death. Consequently, strict quality control of these contaminants in pharmaceutical products is requested by the health authorities worldwide such as FDA and EMA.

Methods

Today, ET testing is performed usually by biological techniques such as the rabbit pyrogen assay (since 1942 in the USP), the monocyte activation assay (since 2010 in the EP), and the Limulus Amoebocyte Lysate (LAL) (since 1980 in the USP) assays. LAL is considered the gold standard in current compendial but also development phase ET testing. It is based on the blue blood of the endangered horseshoe crab. While highly sensitive, all these biological assays have significant drawbacks. Besides others, they work in a very limited small dynamic concentration range and are all susceptible to strong sample matrix interferences. This may lead to false-negative test results, a phenomenon known as "low endotoxin recovery" which endangers patients' health. Moreover, e.g. the LAL assay is validated with endotoxin recovery (accuracy) values of 50 – 200%, and a precision of 25%, which express the large experimental error of the most common ET quantification assay. A chemical instrumental analytical assay presents a modern solution to reduce experimental errors, extend the dynamic concentration range and solve the specificity issues of the established biological assays. Our group has developed the so called (U)HPLC-Kdo-DMB assay. It is based on the rare, ET specific 3-Deoxy-d-manno-oct-2-ulosonic sugar acid (Kdo), which represents an ET specific marker. Kdo is quantitively released from each ET molecule by mild acidic hydrolysis. Sensitive and selective detection is obtained by specific Kdo derivatization with the fluorophore 1,2-Diamino-4.5-methylenedioxybenzene dihydrochloride (DMB).

Results

The novel assay overcomes problems associated to the huge heterogeneity of different endotoxin preparations, obtained from different bacteria or different purification procedures. Matrix effects are minimized by the separation of Kdo-DMB by RP-(U)HPLC from potential interfering matrix compounds. Consequently, the chemical ET quantification approach strongly reduces the likelihood of the "low endotoxin recovery" phenomenon. The current limit of quantification of the ET assay is 30 EUmL-1, with ongoing research efforts to reduce it to 0.25 EUmL-1, a health authority requirement for pharmaceutical applications. The novel assay has been successfully used to monitor efficiently the time dependent ET release in different crude bioreactor cultivations and for ET removal filter developments. It was further used to assess the efficacy of ET removal by downstream process filtrations and to analyze high protein load samples.

Conclusions

The (U)HPLC-Kdo-DMB assay results and LAL results show high correlation described by a linear function.
The novel, animal free instrumental analytical ET assay brings ET testing to the 21st century.

Csaba Horváth, the father of reversed phase chromatography - The revolution of life science

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Keywords: reversed phase chromatography, solvophobic theory, retention mechanisms, biomolecules.

Objective

In earlier days one worked in Normal-Phase-Chromatography (NPC) in organic eluents such as Hexane or Heptane, with which aqueous samples form a milky suspension, affecting negatively their separation and detection. This became different in Reversed Phase Chromatography (RPC) which is delivering clear solutions and sharp peaks. The kinetics of the interactions between the sample and the C18 ligands is much less distance dependent than in Normal-Phase- or in Ion-Exchange Chromatography (IEC). The force between the sample and the C18 chain is proportional to 1/r6 (r: distance) as long in ion-exchange the force is proportional to 1/r2 . Therefore, the kinetics in RPC is very fast, resulting in sharp bands.

The other main advantage is that in RPC we work in aqueous systems and can deal with aqueous samples, which is a difficulty in NPC. The theory of RPC, called the Solvophobic Theory (ST) is investigating a comparison of the energetic interactions in the Reversed Phase retention process, in summary a recognition of the role of the highly ordered water structure, which is resulting in molecular associations enforces by water, leading to manifold molecular interactions in life science, which made life possible in all of its variable forms. The consequent and systematic application of the theory of RPC to biological systems is possible by modeling the retention process with software tools, leading to the fast discovery of new drugs.

Conclusions

The talk will review some of the work of Csaba Horváth, his devotion to science and to RPC, which changed our world completely, giving us a tool to look deeply in the rules of mother nature and changing the world to a better place for human kind.

Electrophoretic and spectral techniques in the determination of microbiomes

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Keywords: capillary zone electrophoresis, microbiome, pathogens, MALDI-TOF/MS

Objective

The analysis of the microbiome, both in clinical and environmental samples, is of great importance, especially in monitoring and clinical diagnostics. Fast and reliable identification of pathogens in biological samples allows for quick selection of appropriate therapy for the patient. Furthermore, detecting and characterizing environmental microbiomes from different matrices is crucial to identify secondary metabolites that could potentially serve as antimicrobials.

Methods

In this context, electromigration techniques, especially capillary zone electrophoresis (CZE), play a key role in microbiome analysis. These techniques, in addition to the separation and fractionation of pathogen mixtures, enable concentration and preliminary purification of samples. It is also possible to measure the charge on the surface of the microbiome and describe the separation mechanism and interactions.

Results

Laser desorption/ionization (LDI) spectrometry in the form of MALDI-TOF/MS was used to identify individual microbiomes. This technique provides a comprehensive approach in the characterization of diverse microbiomes.

Conclusions

The combination of electromigration methods with spectrometric detection not only facilitates the identification of microorganisms, but also ensures understanding of the structure and composition of the microbiome, which is important in clinical diagnostics and the selection of an appropriate, dedicated medicinal preparation and, consequently, effective therapy.

REFERENCES

[1] Buszewski B., Błońska D., Kłodzińska E., Konop M., Kubesová A., Šalplachta J., (2023). Determination of Pathogens by Electrophoretic and Spectrometric Techniques, *Crit Rev Anal Chem.*, 16, 1-24

[2] Błońska D., Kłodzińska E., Buszewski B., (2023). Fractionation of bacteria by electrophoresis as pre-separation method before MALDI-MS detection, *Electrophoresis*, 44, 1165-76

[3] Jackowski, M., Szeliga, J., Kłodzińska, E., Buszewski, B., (2008). Application of capillary zone electrophoresis (CZE) to the determination of pathogenic bacteria for medical diagnosis, *Anal Bioanal Chem.* 391, 2153-60.

[4] Kłodzińska, E., Szumski, M., Dziubakiewicz, E., Hrynkiewicz, K., Skwarek, E., Janusz, W., Buszewski, B., (2010). Effect of zeta potential value on bacterial behavior during electrophoretic separation, *Electrophoresis,* 31, 1590-6

Challenges in chromatographic analyses of bioactives in plant extracts and food

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Keywords: HPTLC, HPLC, mass spectrometry, effect-directed analysis

Objective

Bioactive compounds with diverse chemical structures are appreciated for their varied health benefits such as: antimicrobial activity, antioxidant activity, enhancement of immune response or cell-to-cell communication, lowering blood pressure and/or cholesterol level, inhibition of enzyme activity, etc. Many bioactives consumed on a daily basis with our diet are still unknown, but at the same time there is also an increased demand for bioactives as ingredients of food supplements and functional foods. Chromatographic techniques play an important role in issues solving challenges in analysis and identification of bioactives and understanding their properties and effects. The lecture will focus on methods based on chromatographic and hyphenated techniques as well as effect-directed analysis (EDA) for targeted and non-targeted analyses of bioactives in plant extracts and food samples.

Methods

Methods based on high-performance thin-layer chromatography (HPTLC–densitometry, HPTLC–image analysis, HPTLC–MS/(MS) and (HP)TLC–EDA) and high-performance liquid chromatography (HPLC–UV/Vis, (U)HPLC–MS/(MS)) for targeted and non-targeted analyses of bioactives in plant extracts (invasive alien plant species: Japanese knotweed, giant knotweed, Bohemian knotweed, tree of heaven; flower pollen, etc.) and food samples (e.g., food supplements, bee pollen, food waste) will be discussed.

Results

The main challenges in the development of chromatographic methods for analyses of bioactives in studied samples were related to the lack of commercial standards and standard reference materials, the lack of chromophores, isomeric structures and stability of the analytes. Challenges in non-targeted (HP)TLC–EDA analyses of antioxidants, antimicrobial compounds and enzyme inhibitors in crude extracts prepared from different parts of invasive alien plant species, flower pollen and bee pollen were mostly related to the influence of the stationary phase, detection reagents and detection modes (UV, Vis, FLD).

Conclusions

Methods based on complementary chromatographic techniques and (HP)TLC–EDA are indispensable in analyses of bioactives in plant and food and samples. Although (HP)TLC–EDA was shown to be a cost-effective approach in discovery of bioactive compounds in plant and food extracts, there is still a lot of room for optimization and further developments.

ACKNOWLEDGMENTS

This study was supported by the Slovenian Research and Innovation Agency (ARIS; research core funding No. P1-0005, project J4-50138, project N1-0235, bilateral project BI-TR/20-23-004 and "Young Researchers" program - Maja Bensa), the Scientific and Technological Research Council of Türkiye (TÜBİTAK; project No: 119N569) and the National Research, Development and Innovation Office of Hungary (NKFIH; project SNN.

The role of HILIC in uni- and two-dimensional separations of pharmaceuticals, supplements, and low-molecular metabolites

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Keywords: hydrophilic interaction liquid chromatography, metabolites, glutathione, two-dimensional separations

Objective
Hydrophilic interaction liquid chromatography (HILIC) is an alternative to reversed-phase liquid chromatography (RP-LC) for separation of highly polar ionic or ionizable analytes, with orthogonal separation mechanims to RP-LC, and straighforward coupling to mass spectrometric detection. A wide range of stationary phases is currently commercially available for HILIC separations ranges from bare silica over various chemical moieties bonded on silica, hybrid silica, or polymeric support [1]. Most HILIC columns provide a dual retention mechanism combining RP and HILIC depending on the composition of the mobile phase, which can be beneficial especially in designing two-dimensional separation systems.

Methods
In this work, a novel approach to the selection of a suitable type of stationary phase for target application was proposed and tested using several mixtures of polar compounds including nucleosides, phenones, oligosaccharides, and low-molecular glutathione metabolites and thiols related to glutathione metabolism.

Results
The glutathione-related metabolites are important indicators of cellular oxidative stress and biomarkers of many diseases, e.g., cataracts, cancer, neurodegenerative disease, pneumonia, and cystic fibrosis [2]. The HILIC separation coupled with mass spectrometry was used for the assessment of glutathione metabolism without the need for derivatization, which was demonstrated on the analyses of cell lysates in the metabolic studies of glutathione metabolism.

Conclusions
In HILIC, water can be partially or entirely replaced by another polar solvent, usually an alcohol such as methanol, ethanol, propan-2-ol. Thus the possibility of modifying the retention and selectivity of separation of polar analytes was tested employing alternative polar modifier to acetonitrile, and the retention model was established and tested for optimization of gradient separations with ternary mobile phases [3].

ACKNOWLEDGMENTS
The work was financially supported by the Czech Science Foundation, project No. 22-09556S.

REFERENCES
[1] Nováková L., Douša M., Česla P., Urban J., (2024). Modern HPLC separations in theory and practice, Czech Chromatographic School, Brno.
[2] Kubát M., Roušarová E., Roušar T., Česla P., (2024). Recent advances in separation methods for characterization of glutathione metabolism and dietary supplementation, *TrAC Trends in Analytical Chemistry*, 176, 117751.
[3] Tošovská B., Mrvková S., Holá K., Česla P., (2024). Gradient HILIC separations with ternary gradients, in preparation.

Wastewater-based epidemiology to assess pharmaceutical consumption in urban populations

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Keywords: wastewater-based epidemiology, NSAIDs, steroid hormones, beta-blockers, SPE-LC

Objective

Wastewater-based epidemiology (WBE), also known as wastewater-based surveillance or sewage chemical-information mining, is a relatively new approach which analyze wastewaters to assess consumption or exposure to chemicals or pathogens in a population [1–3].
The aim of this work was to develop chromatographic methods for the analysis of different pharmaceuticals in wastewater samples collected from the influent of the wastewater treatment plant from Cluj-Napoca, Romania, over a period of time, and to estimate their consumption in the investigated population through WBE.

Methods

The investigated pharmaceuticals were extracted by SPE on Strata X (steroid hormones, NSAIDs) and C18-U (beta-blockers) and analysed by HPLC-PDA (steroid hormones) and LC-MS/MS (NSAIDs, beta-blockers). Procedures were validated according to ICH.

Results

The analysed wastewater samples were collected as follows: NSAIDs and steroid hormones during 18 days, while beta-blockers during 7 days. WBE was applied to LC results from monitoring. The obtained WBE data are expressed as the daily estimation per capita of mass loads of pharmaceuticals (gram/day/1000 inhabitants, g/d/1000inh) and are compared with literature data from other countries.

Conclusions

Our studies support the use of WBE as a tool to monitor trends in pharmaceutical consumption in a community, taking into account additional factors such as their stability (in-sewer and in-sample) and transformation due to microbial activity, the specificity of human metabolism, as well as a good knowledge of the number of inhabitants connected to the sewage system. Parent compounds found in wastewaters can be considered biomarkers that are important in monitoring the presence of various pharmaceuticals in a community, leading to the evaluation of public health indicators.

ACKNOWLEDGMENTS

These WBE studies are performed in the framework of the PhD Programme from Babeș-Bolyai University, Cluj-Napoca, Romania, Environmental Science Doctoral School. The authors thank to ESPCI PSL Paris, France, for the logistical access.

REFERENCES

[1] Zuccato E. et al. (2008). Estimating community drug abuse by wastewater analysis, *Environmental Health Perspectives*, 116(8), 1027–1032.
[2] Boogaerts T. et al. (2024). Current state and future perspectives on de facto population markers for normalization in wastewater-based epidemiology, *Science of the Total Environment*, 935, 173223.
[3] Choi P. M. et al. (2018). Wastewater-based Epidemiology Biomarkers: Past, Present and Future, *Trends in Analytical Chemistry*, 105, 453–469.

Assessment of purity of avocado oil sold in the US marketplace

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Keywords: GC×GC, avocado oil, fatty acids

Objective

Recent publications indicate that avocado oils partially or completely substituted with other edible oils of lower commercial value may be present in the US marketplace. The conclusions reported in these publications were based primarily on the analysis of the fatty acid and sterol composition of oils purchased from retail stores across the US. In June 2022, the US Pharmacopeia (USP) published the first standard of identity for avocado oil, permitting the assessment of avocado oil purity based on the comparison with a reference fatty acid and sterol composition. This study evaluated of purity of avocado oil sold in the US marketplace.

Methods

In this study, the fatty acid composition of avocado oil was studied using mono-dimensional and two-dimensional gas chromatography with online reduction (GC-ORxGC). Selected samples were also fractionated by liquid chromatography before GC analysis. The composition of avocado oil was scrutinized in detail to define which main and minor components including fatty acids may be suitable to detect the undeclared presence of other food oils. This study included the analysis of 47 samples collected from the US marketplace based on the size of their market share, and availability from either online retailers or local grocery stores.

Results

Avocado oil showed a unique fatty acid composition compared to the food oils of lower economic value. The main distinguishing parameters from potential adulterants were the high content in palmitoleic acid (cis 9-16:1) and low content in stearic acid (18:0). The purity of the avocado oil samples collected from the US marketplace was finally assessed by comparison with the USP standard of identity. All samples that did not meet the USP standard of identity failed for both cis 9-16:1 and 18:0 compositional parameters.

Conclusions

The novel multidimensional techniques applied in this study provided a detailed characterization of the avocado oil composition, permitting the selection of markers to detect the presence of other food oils. The targeted analysis using conventional chromatographic techniques permitted to evaluate avocado oil in the US marketplace and assess the chemical composition against other edible oils.

The composition of pyrolysis products of used car tires used as liquid fuel

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***Keywords:** car tires, waste disposal technologies, two-dimensional gas chromatography TOF MS, pyrolysis mixture, alternative fuel

Objective

Waste disposal technologies, which allow obtaining useful products, are one of the most powerful means of environmental protection through the rational use of waste as raw materials for industry. One of such technologies is the pyrolysis of used car tires. As a result of pyrolysis, gaseous, liquid and solid products are formed. These products are used as fuel, in particular, the liquid fraction can be used as diesel fuel.

Methods

The content of the liquid fraction was analyzed by the method of two-dimensional gas chromatography using TOF MS.

Results

More than 6,500 compounds have been identified. The majority of the pyrolysis mixture consists of low-molecular volatile fractions, which largely determine the consumer qualities of the pyrolysis mixture as an alternative fuel. The liquid fraction contains saturated, unsaturated, aromatic hydrocarbons; diene hydrocarbons are fairly widely represented. Derivatives of benzene, diaromatic compounds: naphthalene and indene, trinuclear aromatic compounds - derivatives of anthracene are found among aromatic hydrocarbons. dibenzofuran and fluorene. Polycyclic aromatic compounds containing four or more aromatic rings, which exhibit carcinogenic properties, are practically absent in this mixture.

The pyrolysis liquid contains a significant number of compounds, which include nitrogen, sulfur and halogen atoms. Nitrogen compounds are represented in the form of organic amines, nitroso compounds, heterocyclic compounds - derivatives of pyridine, indole, quinoline, acridine. Sulfur compounds are mainly found as derivatives of thiophene (monoaromatic compounds), dibenzothiophene. The mixture contains a small amount of halogen-containing compounds, mainly in the form of derivatives of monoaromatic hydrocarbons.

Conclusions

When juxtaposing the chromatograms of the liquid fraction of the pyrolysis mixture with the chromatograms of petroleum products, in particular, diesel fuel, it can be seen that they largely contain the same components, which indicates that the properties of the liquid pyrolysis fraction are close to petroleum products of natural origin. Thus, the methods of processing this mixture can be similar to those used in petrochemicals, and the method of recycling rubber car tires by pyrolysis can be successfully implemented for the production of pyrolysis mixture as an alternative fuel.

Characterization of natural biomacromolecules by methods of liquid chromatography

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Keywords: humic substances, lignin, RP-HPLC, SEC, IEC, combination of lignin chromatography methods

Objective
The presented work is focused on methodological aspects of characterization and analysis of selected environmental biomacromolecules - humic substances (HS) and lignin using liquid chromatography methods and their combination. These substances are not yet comprehensively recognized in all details, which is a consequence of their chemical, structural and physical polydispersity, which is reflected in the great uncertainty of the analytical signal in almost all analytical methods that deal with their research from a macromolecular point of view.

Results
A distinct analytical signal of HS and lignin is obtained only by application of simplifying procedures or data, e.g. elemental composition. Structural ambiguity and properties result in a such consequences as are very variable effects of their behavior under various conditions (for instance, strong ability to aggregation and disaggregation, respectively, creation of supramolecular structures- tetramers of macromolecules etc.) These biomacromolecules provide spectral and chromatographic profiles without significant characteristics.

Conclusions
The purpose of their characterization by new approaches is the need to develop analytical methods that are able to elicit a response in them with a characteristic profile of the analytical signal.

ACKNOWLEDGMENTS

This work was supported by the Slovak Grant Agency under the Contract Number VEGA 1/0080/22 and APVV-17-0318.

LC×LC to unravel the complex polyphenolic profile of winery-related products

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Keywords: comprehensive LC, LC × LC, phenolic compounds, by-products

Objective
The main objective of this contribution is to develop new comprehensive two-dimensional liquid chromatography methods applied to the analysis of different winery-related products in an effort to demonstrate the full potential of this analytical tool to address complex separations and problems related to the field of food analysis.

Methods
The combination between a new LC×LC method that combines a HILIC separation in the ^{1}D with a RP-based separation in the 2D coupled to diode array detector (DAD) and tandem mass spectrometry (MS/MS), was used to study the composition and evolution of polyphenols during grape maturation as well as the final composition after winemaking. This method allowed the collection of new data about the evolution of these polyphenols during ripening and winemaking.

Results
Polyphenols are essential in the quality of wine, especially red ones, contributing to sensory properties (colour, flavour, astringency and bitterness). They are characterized by having an enormous structural variability, ranging from simple phenolic molecules to highly polymerized compounds, and are mostly found in conjugated forms. This usually makes the profiling of these compounds a laborious task. However, their determination in grapes, wines and winery by-products is very interesting to diverse aims.
In this contribution we have followed the anthocyanin profile of Malbec grapes with different degrees of ripeness and the phenolic profile of wine produced from them. Moreover, Tannat grape variety pomace is studied in terms of the polyphenols contained that can be considered highly bioactive and could be potentially used for the development of new food supplements.

Conclusions
LC x LC is demonstrated as a useful tool to obtained complex data from food-related products. The new methods provided with a resolving power significantly enhanced compared to conventional one-dimensional approaches. Thanks to the combination of both used detectors, a good number of characteristic anthocyanins and flavan-3-ols have been tentatively identified in the wine, grapes and extremely complex Tannat grape by-products.

ACKNOWLEDGMENTS
The authors thank the funding granted by MCIN/ AEI/ 10.13039.501100011033/ (Spain) for the development of the project PID2020-113050RB-I00 and the National Agency for Research and Innovation (Uruguay) (POS_EXT_2023_1_17530). L.M. thanks the Ramon y Cajal contract (RYC2021-033148-I) funded by MCIN/AEI/10.13039/501100011033 and “EU NextGenerationEU”.

Separations sciences coupled to mass spectrometry how much analyte structural information can we get from a single analysis?

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Keywords: Mass Spectrometry, separations sciences, structural information

Objective

Despite that liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), using electrospray ionisation, has become the method of choice for quantitative or qualitative analysis of metabolites, lipids, pharmaceuticals, or pesticides, the technique still suffers from significant drawbacks. Compound dependent ionisation response, matrix effect, poor MS/MS fragmentation or limited isomers separation are a few of them. For structural identification one relies on public or commercial libraries generated by collision induced dissociation (CID) which limits denovo analytes identification.

Methods

The use of in silico tools for the prediction of chromatographic retention and MS/MS is a strategy which can significantly improve the process without additional analyses. Multimodal mass spectrometry which performs multiple experiments in a single LC-MS analysis, such as differential mobility spectrometry (DMS) or tandem mass spectrometry with fragmentation induced by a gas (CID), electrons (EAD) or photons (UVPD) is another approach to improve information content [1-3].

Results

In this way enhanced structural characterisation or improved selectivity can be obtained without increasing the total analysis time. For example, EAD allows the determine double bonds position in lipids which was not possible with CID and UVPD can generate unique fragments for isomeric differentiation. Better control of the ionisation process is also of importance. Supercritical fluid chromatography coupled to MS offers the possibility to better control the ionisation in ESI but also with atmospheric pressure photoionization (APPI). With dopant assisted APPI the generation of cation radical precursor can be favoured for many classes of analytes in particular for those poorly responding in ESI. The main feature of odd electron ion precursors is that with CID fragmentation they produce electron impact like spectra opening the use of EI libraries.

Conclusions

Various applications of multimodal mass spectrometry with liquid or supercritical fluid chromatography will be presented for the analysis of metabolites and lipids. The first example will discuss the metabolomic analysis of plasma and urine samples from traffic control using LC/MS and SFC/MS, with drivers positive to drug of abuses. The second example will focus on the structural characterization of lipids using robotic off-line multidimensional chromatography (HILIC-C18) followed by CID and EAD tandem MS fragmentation detection.

REFERENCES

[1] Ducati A. O., Ruskic D., Sosnowski P., Baba T., Bonner R., Hopfgartner G., (2021). Improved metabolite characterization by liquid chromatography - Tandem mass spectrometry through electron impact type fragments from adduct ions, *Anal Chim Acta*, 1150, 338207

[2] Mueller P., Bonner R., Hopfgartner G., (2022). Controlled Formation of Protonated and Radical Cation Precursor Ions by Atmospheric Pressure Photoionization with µLC-MS Enables Electron Ionization and MS/MS Library Search. *Anal Chem,* 94, 12103-10

[3] Giraud R., Le Blanc Y.J.C., Guna, M., Hopfgartner G., (2023). Ultraviolet photodissociation and collision-induced dissociation for qualitative/quantitative analysis of low molecular weight compounds by liquid chromatography-mass spectrometry. *Anal Bioanal Chem*, 415, 7117-26.

Application of porous graphitic carbon stationary phases in pharmaceutical analysis

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Keywords: Allopurinol, High temperature liquid chromatography, Porous graphitic carbon, DryLab, Method development, Retention modeling

Objective
Porous graphitic carbon (PGC) stationary phases offer a viable alternative to traditional silica-based phases in high-performance liquid chromatography (HPLC), particularly when solutes have highly variable chemical properties. Although HPLC optimization software based on linear solvent strength (LSS) theory simplifies method development, it may not be compatible with non-conventional phases like PGC. This work aims to study the suitability of the linear solvent strength model for predicting the retention of pharmaceutical ingredients using PGC columns, and to establish a general workflow for HPLC method development with these phases across a broad design space. The effectiveness of this approach is illustrated by developing an analytical method that accurately separates allopurinol and its impurities.

Methods
Retention times of solutes were measured on the PGC phase at different gradient conditions (gradient time: 5-20 min, temperature: 30-90°C, pH: 2-3). The molecular parameters affecting retention behavior were determined by fitting the gradient equation derived from LSS model on the retention times.
The optimization of separation conditions was carried out with DryLab4, a software that was built upon the LSS model principles. A three-dimensional model was set and carried out, aimed to optimize the gradient time, column temperature, and pH of eluent A. Based on the input data of the twelve runs, a three-dimensional design space was created, where the optimal working point was specified, based on the critical resolution

Results
During the fitting process, the solvent strength parameter, S, as well as the slope and intercept of the van't Hoff equation were determined. The relative differences between the calculated and measured retention times were less than 3 %, most typically ± 1 % even in case of the solute with the longest retention. It was found that values of the solvent strength parameter (S) for PGC phases were larger than in reversed phase mode. The values of S decreased as the retention time of the compound increased, a trend not observed in RP separations. The slopes of the van't Hoff equation were similar for each solute, and the results were consistent with the theoretical expectations suggesting that LSS theory can be used with PGC phases. The optimal experimental conditions provided a fast (~5 min), efficient, and robust method for the analysis of allopurinol and its possible impurities.

Conclusions
Our findings indicate that PGC can be effectively modeled using LSS theory, despite its significantly different retention behavior compared to RP-HPLC. The analytical method developed with chromatographic modeling software is both fast and efficient, with calculated and measured chromatograms aligning well. This approach can serve as a general method for testing the LSS behavior of non-conventional phases and developing methods for PGC columns.

ACKNOWLEDGMENTS
This work was supported by the TKP2021-NVA-10 project with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the 2021 Thematic Excellence Programme funding scheme.

Investigation of enantioselective noncovalent molecular interactions by affinity capillary electrophoresis

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Keywords: affinity, binding constant, Capillary Electrophoresis, diquats, cyclodextrins

Objective
The objective of this work was double: i) to study the enantioselectivity and strength of the noncovalent molecular interactions between diquats (DQs), a new class of functional molecules [1], and chiral selectors, randomly highly sulfated cyclodextrins (CDs), using affinity capillary electrophoresis (ACE), and ii) to test applicability of pure enantiomers of DQs as chiral selectors in ACE separation of various chiral compounds.

Methods
ACE was performed in Agilent 7100 CE analyzer (Waldbronn, Germany) equipped with UV-vis diode array detector and bare or hydroxypropylcellulose coated fused silica capillary (ID/OD 50/375 µm, total/effective length 385/300 mm). Analyses were carried out at constant voltage -12 kV (cathode at the injection capillary end), and constant temperature 25°C. The analytes (*ca.* 0.2 mM aqueous solutions of racemic DQs or other chiral compounds) were injected hydrodynamically (700 Pa × 10 s) and detected at 200 or 220 nm.

Results
First, the (*P*)- and (*M*)-enantiomers of a series of newly synthesized DQs were separated by ACE using a background electrolyte (BGE) composed of 22/35 mM sodium/phosphate buffer, pH 2.5, containing 6 mM randomly highly sulfated α-, β- and γ-CDs as chiral selectors. Interactions of dicationic DQs applied as analytes with highly sulfated CDs in the BGE led to formation of highly negatively charged fast migrating complexes that were separated within a short time of 5–8 min. The strength of the DQs-CDs interactions was quantified by the binding constants of their complexes. Second, ACE has been applied for investigation of chiral discrimination capabilities of some DQs. A separation capillary was filled with (*P*)- or (*M*)-enantiomer of a particular DQ chiral selector dissolved in aqueous BGE (50/25 mM Tris/acetate, pH 8.1) and a narrow zone of chiral analytes was introduced at the capillary inlet. Then, in the applied electric field, anionic analyte migrated towards detector whereas the dicationic DQ moved towards the cathode at the capillary inlet. When enantiospecific interaction occurred, two peaks of resolved enantiomers of an analyte (e.g. binol phosphate, vapol phosphate, vanol, warfarin) were observed in electropherogram. This flexible CE setup with both analytes and chiral selector in a free solution was suitable for identification of chiral compounds specifically interacting with pure enantiomers of DQs. Structural diversity of successfully resolved analytes is promising for application of DQs as chiral selectors in CE and LC The availability of both DQ enantiomers is advantageous to control migration/elution order of chiral analytes.

Conclusions
ACE proved to be a suitable method for investigation of the enantioselectivity and strength of the noncovalent interactions between enantiomers of chiral compounds and chiral selectors. The DQs seem to be a promising new type of chiral selectors.

ACKNOWLEDGEMENTS

The work was supported by the Czech Science Foundation, project no. 20-03899S, and by the Czech Academy of Sciences, Research Project RVO 61388963.

REFERENCES

[1] Talele H. R., Koval D., Severa L., Reyes-Gutiérrez P. E., Císařová I., Šaman D., Bednárová L., Kašička V., Teplý F., (2018). Diquats with robust chirality: Facile resolution, synthesis of chiral dyes and application as selectors in chiral analysis, *Chem.–Eur. J.* 24, 7601-04.

Isomer-selective LC in lipidomics and metabolomics

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Keywords: Oxylipins, enantioselective amino acid analysis, phosphoinositide profiling, sugarphosphate isomers

Objective
Lipidomics and metabolomics are analytical approaches widely adopted in biomedical research with the aim to obtain mechanistic insights in biochemical processes, to find diagnostic and prognostic biomarkers, and generate new hypothesis about biological processes or validate concepts from other experimentation. Although common untargeted methodologies allow simultaneous analysis of hundreds of metabolites, they often fall short in full coverage of pathways in which isomeric species play a major role, e.g. the phosphoinositide network, glycolysis and pentosephosphate pathways, enantioselective amino acid analysis, or sugar phosphate isomers. This presentation will focus on challenging lipid and metabolite classes in targeted and untargeted lipidomics and metabolomics, respectively, for which biological interpretations rely on a separation of their structural isomers.

Methods
Isomers cannot be distinguished on MS1 (precursor ion) level. However, many isomers provide characteristic fragmentation and can be distinguished at MS2 level. Ion-mobility mass spectrometry (IMS) allows the gas phase separation of isomers in the ms-time scale ideal for combination with TOF-MS. It enabled separation of double bond-position and -geometry isomers, sn-1/sn-2-positional isomers, and functional group positional isomers in lipidomics. Lipid isomers differing in the double bond position can be distinguished by derivatization. LC and multi-dimensional modes offer a wealth of methods for isomer separations benefitting from unlimited stationary phase-mobile phase combinations.

Results
In the first part, a workflow for phosphoinositide profiling will be presented. It integrates phosphate methylation, positional isomer separations by chiral LC and data independent SWATH acquisition (DIA) as tools to achieve this goal. Compositional (fatty acyl chain length) and positional isomers of (phospho)lipids can often be distinguished by DIA using extracted ion chromatograms at the MS2 level of specific fragments. We have developed a new derivatization concept for double bonds, which allows distinction of double bond positions in unsaturated lipids. It is based on reaction of double bonds with disulfides followed by LC-MS/MS analysis and characterization by specific fragments. A 2D-LC approach with a chiral column in the first dimension and fast RPLC in the second dimension provided a full separation of conjugated polyunsaturated fatty acids (with C18 chain) which were detected as impurities in a pharmaceutical lipid formulation. An important topic in lipidomics is the enantioselective analysis of oxylipins which can be either enzymatically or non-specifically formed by autoxidation or ROS-oxidation. In the second part, the focus will be on polar metabolites focusing on fast and comprehensive enantioselective amino acid profiling as well as dealing with the challenges of an integrated workflow covering the entire glycolysis and pentosephosphate pathways with their numerous phosphorylated carbohydrate structural isomers.

Conclusions
LC and 2D-LC still play a major role in isomer separations in biosciences. New tools like IMS and alternative fragmentation methods in MS/MS complement isomer selective chromatography and provide together enhanced selectivity and identification confidence in untargeted and targeted analysis of real samples.

Chemometrics: a constructive tool for separation sciences

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Keywords: chemometrics, experimental design, multivariate data analysis, signal processing, deconvolution

Objective

Chemometrics is a chemical discipline that makes use of mathematical and statistical methods to design optimal experimental procedures and to extract useful chemical information from chemical data. In the last decades, chemometrics has played a crucial role in the processing of instrumental analytical signals and it has been increasingly employed in the separation sciences, addressing the increasing complexity of data that can be obtained with a single analysis. Nowadays, terms such as artificial intelligence (AI), machine learning (ML) and deep learning (DL) are also permeating different fields, including analytical chemistry. In the present contribution, it will be shown how chemometrics is situated with respect to such disciplines, highlighting its role and effectiveness in the context of separation sciences.

Methods

Univariate processing is the simplest form of analysing data, considering one variable at a time independently of the others, without considering intercorrelation – a feature that can be very informative, if recognised and properly interpreted. On the contrary, multivariate methods play a crucial role for the development of adequate and increasingly automated tools for efficient analytical characterisations of complex systems. Indeed, an appropriate use of chemometric tools involves the overall analytical process: from the definition of the problem, choice of samples, and optimisation of analytical conditions (multivariate design of experiments – MDOE) to the processing of instrumental outcomes (multivariate data analysis – MVDA). Among the multivariate tools most widely used in the field of separation sciences, there is certainly principal component analysis (PCA), an exploratory technique that extracts the highest information contained in the data, looking for their maximum variability. Another fundamental tool is represented by multivariate curve resolution (MCR), a method that addresses the mixture analysis problem using a bilinear model – or a multilinear extension in the case for multidimensional data – to perform signal deconvolution.

Results

In the present contribution, the power of multivariate approaches in the field of separation sciences will be demonstrated, with examples that will range from method optimisation to the processing of complex data.

Conclusions

Chemometrics is confirmed as a valuable tool for addressing several key issues in the separation sciences, at different levels and for different application aims. The possibility of automatising processes, obtaining straightforward analytical responses, makes the chemometric approach even more interesting and, surely, even more implemented in the future.

Navigating the path to sustainable analytical chemistry

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Keywords: green analytical chemistry, circular analytical chemistry, sustainable chemistry

Objective
The present contribution explores and delineates the strategies and methodologies necessary for advancing sustainable practices within the field of analytical chemistry by integrating principles from green analytical chemistry, circular analytical chemistry, and sustainable chemistry. It provides a comprehensive overview of current challenges and opportunities, proposes guidelines for implementing sustainability principles, and highlights case studies.

Methods
The extant research literature from the areas of green analytical chemistry, circular analytical chemistry and sustainable chemistry are analyzed and synthetized to develop the conceptual model of sustainable analytical chemistry.

Results
The extraordinary ability of analytical chemistry to sustain responses to many of the grand environmental challenges comes with a mounting environmental cost. To mitigate the negative environmental impact of analytical chemistry practices, the concepts of green analytical chemistry [1] and green sample preparation [2] were introduced that primarily focused on the environmental impact of laboratory practices and align to linear economy approach. The recent concept of circular analytical chemistry [3] went beyond the greenness of analytical methods and targeted the radical transformation of the entire production, consumption, and disposal system by establishing connections between post-use and production stages. Building on this knowledge and combining it with sustainability factors [4], the concept of sustainable analytical chemistry is discussed. The core of this concept describes, the use of chemical and materials that are readily-available and renewable, operates at optimal efficiency, and employs renewable energy sources; this includes the intentional design, manufacture, use, and end-of-life management of chemicals, materials, and products across their lifecycle that do not adversely impact human health and the environment, while promoting circularity, meeting societal needs, contributing to economic resilience, and aspiring to perpetually use elements, compounds, and materials without depletion of resources or accumulation of waste. Sustainable analytical chemistry is both a path and a goal. As a sociohistorical construction, concept it should be seen as a permeable membrane, with new vital criteria crossing its borders selectively, a subject of continued development, based on frontier scientific foundations.

Conclusions
The analytical chemistry sector is undergoing what seems to be a domain shift, transforming itself in education, research, and practice to reposition as a sustainability science. To arrive at such a turning point for a fully operationalized reoriented analytical chemical practice, it would be necessary to face a reality in which business-as-usual does not work anymore, counting on a critical mass with capacities, intentions, incentives, and technologies that can revolutionize a very complex functioning interconnected production chain to the core.

REFERENCES
[1] Gałuszka, A., Migaszewski, Z., Namieśnik, J., (2013). The 12 Principles of Green Analytical Chemistry and the SIGNIFICANCE Mnemonic of Green Analytical Practices, *TrAC - Trends Anal. Chem*., 50, 78–84
[2] López-Lorente, Á. I., Pena-Pereira, F., Pedersen-Bjergaard, S., Zuin, V. G., Ozkan, S. A., Psillakis, E., (2022). The Ten Principles of Green Sample Preparation, *TrAC Trends Anal. Chem*., 148, 116530
[3] Psillakis, E., Pena-Pereira, F., (2024). The Twelve Goals of Circular Analytical Chemistry, *TrAC Trends Anal. Chem.*, 175, 117686
[4] Kümmerer, K., (2017). Sustainable Chemistry: A Future Guiding Principle, *Angew. Chemie - Int. Ed.*, 56, 16420–1

LC-GC×GC for MOSH and MOAH analysis: unlock new potential and enhance the level of understanding

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Keywords: LC-GC×GC; mineral oil saturated hydrocarbons (MOSH), mineral oil aromatic hydrocarbons (MOAH), foods, contaminants

Objective

Beside the ever standing goal of enhancing separation power, analysts are facing the necessity to increase the efficiency of the sample preparation methods while developing sustainable and greener protocols.
In this context GC×GC can be a powerful alley in the developing of sample preparation methods, particularly in the field of mineral oil hydrocarbon (MOH) contamination in food. The goal of this presentation is to highlight the fudamental role of a recently developed and validated fully integrated LC-GC×GC-TOFMS/FID system [1, 2] in the perspective of improving the reliability of the data generated and mitigate the uncertainties in the analytical workflow.

Methods

A fully integrated LC-GC×GC-TOFMS/FID was used coupled with wisely optimized sample extraction using microwave-assisted saponification/extraction (MASE) and LC purification based on the same analytical column used for the routine separation of MOSH and MOAH but with a different elution gradient.

Results

Constrains and limitations in the analysis of MOSH and MOAH are discussed addressing the support that the completed integrated workflow can bring in untangle the complexity embedded in this application increasing the reliability of the results. Particularly, MASE was properly optimized [3] to overpass the unconsistency problems in the internal standards ratios present in the recently developed official methods and the LC separation was optimized to be used as an alternative to the chemical routine purification (i.e., epoxidation)that cause a substantial loss of MOAH

Conclusions

The fully validate workflow from sample extraction, purification and powerful chromatographic separation allows to obtain better recovery and precision in the determination of the toxicological concerning MOAH fraction, while allowing for a more detailed investiation of the composition of such a toxic fraction.

ACKNOWLEDGMENTS

This work is supported by Fonds de la Recherche Scientifique Belgique (FNRS) (CDR projects-MOHPlatform, J.0170.20 and PDR projects-ToxAnaMOH T.0187.23. The authors thank LECO, Restek, and Milestone for their support. This article is based upon work from the Sample Preparation Study Group and Network, supported by the Division of Analytical Chemistry of the European Chemical Society.

REFERENCES

[1] Bauwens G., Pantó S., Purcaro G., (2021). Mineral oil saturated and aromatic hydrocarbons quantification: Mono- and two-dimensional approaches, *J Chromatogr A,* 1643, 462044
[2] Bauwens G., Barp L., Purcaro G., (2023). Validation of the LC-GC×GC-TOFMS/FID platform for mineral oil analysis exploiting interlaboratory comparison data, *Green Analytical Chemistry,* 4, 100047
[3] Bauwens G., Purcaro G., (2024). Improved microwave-assisted saponification to reduce the variability of MOAH determination in edible oils, *Anal Chim Acta*, 1312, 342788

Fast, but not furious: stepping on the gas pedal in gas chromatography

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Keywords: fast Gas Chromatography, thermal gradient GC, modelling, GC theory

Objective

Gas chromatography can under optimized conditions separate tens to hundreds of volatile and semi-volatile compounds within one run. The price to pay, however, for this high separation power often are extended run times, sometimes in the range of 30-60 minutes per sample. This demonstrates that under standard operation conditions, separation efficiency, separation speed and sample capacity cannot be all optimized at the same time [1]. In this talk we will present how high separation efficiency can be attained for even ultrafast separations.

Methods

An ultrafast gas chromatograph was developed in house. This device consists of a 1.5 m long GC capillary that can be heated segment-wise with a ballistic temperature gradient (up to 4000 K/min). By controlling timing and heating rates of the individually addressable heated segments of the separation column, it is possible to create a thermal gradient that travels along the column. This creates a travelling negative thermal gradient that leads to peak focusing and thus reduced peak width. The entire set-up is modular, allowing to be used with any commercial gas chromatograph and standard detectors, provided they are sufficiently fast which we will demonstrate by using different GC systems and detectors, such as an FID, BID or an MS detector.

Results

The herein developed experimental GC prototype is capable of producing very fast (less than 1-2 min) separations while maintaining very high resolution. Peak widths in the range of 50-100 ms can be obtained with this system, leading to remarkable peak capacities, notably for the short length of separation column.

Hydrocarbon separations in the range up to C20 can be performed in less than one minute, and even the standardized total petroleum hydrocarbon determination according to DIN 9377-2 which normally would require chromatographic runs in the order of 30 min [2] can be performed with cycle times of less than 2 min.

Conclusions

The proposed GC design provides fast, high-resolution gas chromatographic separations with very short cycle times. Its modular design allows its interfacing to any commercial standard GC instrument. Moreover, the fast heating capability make this device interesting for comprehensive two-dimensional chromatography where also the second dimension could be temperature-ramped, thereby further improving coverage as well as peak capacity.

ACKNOWLEDGMENTS

Funding of this project by the Austrian Research Promotion Agency (FFG) under Project no. 879613 ('OPERION') is gratefully acknowledged.

REFERENCES

[1] Regmi B., Agah M., (2018). Micro Gas Chromatography: An Overview of Critical Components and Their Integration, *Anal. Chem.* 90, 13133−13150.
[2] Drozdova S., Ritter W., Lendl B., Rosenberg E., (2013). Challenges in the determination of petroleum hydrocarbons in water by gas chromatography (hydrocarbon index), *Fuel,* 113, 527–536.

Olea europaea: a compelling source of health promoting biomolecules and an inspiration for the semi-synthetic design of bioactive analogues

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Keywords: *olea europaea*, extra virgin olive oil, oleuropein, oleocanthal, oleacein, hydroxytyrosol

Objective

Olea Europaea and its main products olive oil and olive drupes, have attracted scientific attention in recent years because of its biological activities and its attribution in many aspects of human health. Although olive oil primarily consists of oleic acid (up to 80 %) and other fatty acids, some minor phenolic compounds, comprising the 1-2 % of the total content, are generally considered to be responsible for the various health benefits of olive oil. The most characteristic compounds in this group are the simple phenylethyl alcohols such tyrosol, hydroxytyrosol, the seco-iridoids oleacein and oleocanthal, the decarboxylated aglycons of oleuropein and ligstroside respectively [1]. These two compounds, and especially oleocanthal, have been identified as the agents responsible for the pungency of extra virgin oil. Oleocanthal's recent discovery as COX inhibitor, with similar effect to that of ibuprofen, has dramatically increased its interest both for the study of biological properties but also for the development of new non-steroidal anti-inflammatory drugs (NSAIDS) based on its structure [2]. Additionally, according to many data, both compounds demonstrated promising anticancer and neuroprotective activities with no toxic effects.

Methods

Prompted by the outstanding interest of these high-value natural compounds we have initiated a survey of Greek olive oil focusing on polyphenols, representative extra virgin olive oils (EVOOs) from the main producing areas of the country [3].

Results

We developed a concise and scalable procedure for the isolation on pilot scale of these compounds [4] as well as the synthesis and pharmacological evaluation of various analogues [5].

Conclusions

The synthesis is performed by a convenient biomimetic and stereo-controlled approach, starting from oleuropein, isolated from olive leaves, an abundant biorenewable raw material and hydroxytyrosol the major compound of olive mill waste water.

REFERENCES

[1] Skaltsounis, A.L., Argyropoulou, A., Aligiannis, N., Xynos. N., (2015). Recovery of high added value compounds from olive tree products and olive processing, 334-357, in *"Olive and olive oil bioactive constituents*" editor D. Boskou, AOCS PRESS.

[2] Beauchamp R. S. J.; Morel, D.; Lin, J.; Pika, J.; Han, Q.; Lee, C-H.; Smith, A. B.;, G. K. K.; Breslin, P. A. S., (2005). Ibuprofen-like Activity in Extra-Virgin Olive Oil, *Nature*, 437, 45–46.

[3] Nikou T., Liaki V., Stathopoulos P., Sklirou A., Tsakiri E., Jakschitz T., Bonnc G., Trougakos I., Halabalaki M., Skaltsounis A.L., (2019). Comparison survey of EVOO polyphenols and exploration of healthy agingpromoting properties of oleocanthal and oleacein, *Food and Chemical Toxicology*, 125, 403–412

[4] Angelis A., Michailidis D., Antoniadi L., Stathopoulos P., Tsantili V., Renault J.H., Skaltsounis A.L. Pilot extraction of extra virgin olive oil biophenols using Annular Centrifugal Extractor and gram-scale recovery of pure oleocanthal, oleacein, MFOA, MFLA and hydroxytyrosol (2021). *Separation and Purification Technology*, 255, 117692

[5] Sarikaki, G., Christoforidou, N., Gaboriaud-Kolar, N., Smith, A.B., Kostakis, I., Skaltsounis, A.L., 2020. Biomimetic Synthesis of Oleocanthal, Oleacein, and Their Analogues Starting from Oleuropein, A Major Compound of Olive Leaves, *Journal of Natural Products*, 83, 1735-39.

UHPLC-MS/MS investigation of the effect of leaf position on the tree on photosynthesis of polyphenolic compounds

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Keywords: UHPLC-MS/MS, polyphenolic compounds, apple leaves, plant metabolites

Objective
This study examines the polyphenolic compounds in apple leaves, considering that photosynthesis of both primary and secondary plant metabolites predominantly occurs in leaves. The influence of climatic factors (e.g. rain, sunlight, temperature), plant stage (e.g. with buds, with fruits or without fruits), and leaf position on the tree crown, should be considered of great importance to ensure representative sampling. The study focused on the influence of differently positioned leaves on the crown of an apple tree on the synthesis of polyphenolic compounds.

Methods
Advanced analytical techniques, such as ultra-high performance liquid chromatography connected to a triple quadrupole mass spectrometer (UHPLC-MS/MS), was used for comprehensive profiling of phenolic compounds. Principal component analysis (PCA) was used to clarify relationships between variables, while artificial neural network (ANN) was performed in order to develop a predictive model for polyphenols content.

Results
Key phenolic compounds in apple leaf samples included protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, vanillic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, ferulic acid, quercetin, rutin, quercetin 3-*O*-galactoside, quercetin 3-*O*-rhamnoside, isorhamnetin 3-*O*-glucoside, isorhamnetin 3-*O*-rutinoside, kaempferol, aesculin, eriodictyol, phlorizin, and phloretin. The correlation analysis revealed highly significant statistical correlations among polyphenol contents. PCA indicated similar variable interactions, and ANN developed a predictive model for polyphenol content under different conditions.

Conclusions
A significant heterogeneity in the polyhenolic content was revealed among different leaf types due to their positions on the tree. Insights derived from this research could impact horticultural practices, considering that leaf location (e.g. from which stem the leaves were taken), leaf type (e.g. lower, upper leaves), and growing conditions (e.g. with or without the net) affect the photosynthesis of polyphenolic compounds. Furthermore, these findings also could have broader implications for understanding photosynthesis and metabolic processes in plants.

ACKNOWLEDGMENTS

This work was performed within projects supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

On the performance of a new generation micro-pillar array columns

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Keywords: Liquid Chromatography, Micro-pillar Array Columns

Objective
The past decades have witnessed an enormous progress in separation efficiency and peak capacity that can be achieved in 1D liquid chromatography (LC) columns in a practically affordable time. However, there are still areas where the need for more efficiency and speed is imminent. One such area is that of proteomics. Given the typical small size of proteomics samples and the need for sensitivity, micro- and nano-flow LC is the current LC method of choice in proteomics research.
Nano-flow LC is also the area wherein recently the concept of micro-pillar array columns (µPACs) has been introduced. µPACs are produced by silicon micromachining and were developed to benefit from the advantages of perfect order in chromatographic separations. Another advantage of pillar arrays is their low flow resistance, which is owed to the fact that the pillars are freestanding and do not touch each other as is the case in a sphere packing.
In contrast to packed bed columns, which seem to have reached the limit of what is possible in terms of a further reduction of the particle size, µPACs are only at the start of their descent of the feature size ladder. which, Indeed, considering that silicon micromachining offers a spatial resolution well below the 1 µm mark, it is clear that the 1st generation of µPACs (introduced with a pillar diameter of 5 µm and an inter-pillar distance of 2.5 µm) is still amenable to a considerable further reduction.

Methods
In pursuit of higher efficiencies and speeds, a 2nd generation µPAC is now commercially available. In this new format, both pillar size and the inter-pillar distance are halved compared to the 1st generation.

Results
The present contribution compares the performance of Gen1 and Gen 2 µPACs under identical conditions, using the trans-parent cover wall to follow the band broadening process on-chip measurements of the evolution of the band broadening of injected tracer dyes. In this way, the true intrinsic performance is measured, devoid of any extra-column band broadening.
Next to this, the present contribution will also report on the performance of a µPAC wherein the pillars are stretched out radially to obtain rectangular pillars. This concept builds further upon earlier work, where the use of radially-elongated pillars was pro-posed as a means to (1) increase the radial mixing, (2) obtain a more uniform flow field, and (3) quasi completely eliminate the side-wall effect.

Conclusions
Both formats will be compared in terms of plate height curves as well as by using kinetic plots.

Going with the flow: strategies for automating sample treatment

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Keywords: automation, sample treatment, Flow Injection Analysis, bioanalysis

Objective
Flow based sample treatment derives from concepts incepted by flow injection analysis, aiming at the handling of solutions or suspensions in a confined environment with high controlled conditions in time and space. This precise control of operations allows for an increase in reproducibility of events, allowing kinetic control of processes. This communication will provide a glimpse of current developments in automated flow-based sample treatment.

Methods & Results
Online hyphenation to separative techniques will be discussed, namely the issues posed by the compatibility of eluent with conventional HPLC procedures and direct hyphenation to MS detectors [1]. Different flow setups will be compared, namely those based on flow networks with packed columns [2] and those based on the bead injection concept. The bead injection (BI) technique was suggested for automation of solid-phase chemistry with the possibility of renewal through microfluidic handling of beads. The beads can be carriers of different (bio)chemical moieties, providing a platform for operation of different chemistries, aiming at analyte preconcentration, in-situ quantification and separative methods. Lab-on-valve (LOV) devices are used for sample treatment and/or analysis in this context, offering greater potential and compatibility with real-world samples compared to microfluidic devices. The combination BI-LOV enables the implementation of solid-phase extraction schemes, as well as immunoaffinity chromatography. An important feature is that sample treatment can be performed with real-time monitoring of phenomena occurring on the surface of the solid support. Additionally, a high surface-to-volume ratio is attained, utilizing small quantities of samples, sorbents, and reagents, and effectively preventing fouling of the solid phase. Several examples will be critically discussed, including the miniaturization and automation of ELISA protocols, demonstrating a significant reduction in the time required to obtain results (from 2 hours to 5 minutes) for the quantification of carbamazepine and immunoglobulin G [3]. Application to biological samples, namely urine and plasma, will demonstrate the potential for analysis of drugs (tranexamic acid, oral anticoagulants) [1] and endogenous compounds (uremic toxins) [4].

Conclusions
Overall, the automation of sample treatment based on flow techniques is a promising area of research that has the potential to transform the field by improving efficiency and accuracy, with the ability to handle complex samples in a greener way.

ACKNOWLEDGMENTS

FCT is acknowledged for institutional support through national funds (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020).

REFERENCES

[1] Fernandes S. R., Barreiros L., Sá P., Miró M., Segundo M. A., (2022). *Analytical and Bioanalytical Chemistry*, 414, 649-659.
[2] Peixoto P. S., Silva E. M. P., Osório M. V., Barreiros L., Lima J. L. F. C., Segundo M. A., (2018). *Analytical Methods*, 10, 2180-2186.
[3] Ramos I. I., Carl P., Schneider R. J., Segundo M. A., (2019). *Analytica Chimica Acta*, 1076, 91-99.
[4] Fernandes S. R., Barreiros L., Sampaio-Maia B., Miró M., Segundo M. A., (2023). *Analytica Chimica Acta*, 1277, 341668.

Analytical quality by design and design of experiments: a useless complication or an opportunity for separation sciences?

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Keywords: Analytical Quality by Design; Design of Experiments; Pharmaceutical analysis; Separation methods

Objective
The presentation will deal with the application of Analytical Quality by Design (AQbD) and Design of Experiments (DoE) to analytical method development in the field of separation sciences. In order to assure the quality of analytical data, regulatory documents require the definition of the risk of failure map, leading to the identification of the so-called "Method Operable Design Region" (MODR).

Methods
The flowchart of the application of AQbD with DoE will be shown in the field of capillary electrophoresis and chromatography for small drugs quality control and monoclonal antibody infliximab characterization.

Results
The use of DoE is not mandatory for the AQbD approach, but its implementation can be successful for the risk assessment and for the definition of MODR.

Conclusions
DoE allows risk evaluation and risk mitigation and its use should be encouraged to achieve scientific goals, to have greater confidence in the data and to obtain valuable information even from negative results.

ACKNOWLEDGMENTS

The financial support provided by the MUR - Dipartimenti di Eccellenza 2023-2027 (DICUS 2.0) to the Department of Chemistry "Ugo Schiff" of the University of Florence is acknowledged. The research was also supported by project PRIN 2022 (2022AF8KZ3), funded by the Italian Ministry of University and Research (MUR).

Analysis of polycyclic aromatic hydrocarbons in food using cryogenic zone compression gas chromatography-mass spectrometry

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Keywords: Polycyclic aromatic hydrocarbons, extra virgin olive oil, gas chromatography, mass spectrometry, green sample preparation.

Objective
The objective of the study was to develop a straightforward method for analyzing 16 polycyclic aromatic hydrocarbons (PAHs) in Food using the "cryogenic zone compression" (CZC) approach coupled with gas chromatography-mass spectrometry (GC-QMS) to achieve enhanced signal-to-noise ratios, thereby reducing sample preparation steps. The proposed approach perfectly aligns with the principles of green analytical chemistry and enables the attainment of high accuracy and precision values through minimal sample manipulation.

Methods
Only a single extraction (using only 500 µL of acetonitrile) was performed before injection into the GC-QMS. To achieve the CZC approach, the end of the analytical column was looped using the metal support of the cryogenic modulator. A “compression” period of 18 s was employed, with a hot pulse (400 °C) of 350 ms. The cold jet flow was 9 L min^{-1}.

Results
This approach aims to compress the compounds as much as possible before reaching the detector, thereby reducing band broadening and increasing the S/N (lowering the detection limits). The final outcome was achieving peaks with a base width in the 120-420 msec range, with an average value of 217 msec. Considering the S/N values, an average increase of 14 times was observed, with values ranging from 8 to 23 times higher using the CZC approach compared to the conventional GC-QMS approach. A total of ten extra virgin olive oil samples, were subjected to analysis using the CZC-GC-QMS approach. The PAH BcFL was determined in four samples, exceeding 10 µg kg^{-1} in one case, while ranging between 2 and 10 µg kg^{-1} in the other cases. Additionally, a BghiP concentration was determined in two samples. The RSD values consistently remained always below 5 %.

Conclusions
The CZC-GC-QMS method here outlined has proven effective for the PAHs contamination determination in EVOO, offering notable reductions in sample preparation time and solvent usage. Remarkably, the extraction process involved only a single-step liquid-liquid extraction with 500 µL of acetonitrile. A sensitivity comparison between conventional GC-QMS and the CZC-GC-QMS revealed the superior performance of the latter approach

ACKNOWLEDGMENTS

The authors acknowledge the Shimadzu Corporation and Merck Life Science for their support. Funding: This research was supported by the Ministry University and Research whitihn project PRIN 2022 PNRR titled “gReen analytical methods for PAHs detection in EVOO: from lABoratory to smart LabEl (RELIABLE)”, Prot. P2022842MJ, CUP J53D23014520001.

Enhancing non-targeted analysis using comprehensive-two-dimensional gas chromatography coupled to a high-resolution mass spectrometry

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Keywords: GC×GC, HRMS, POPs, NTS, Environmental

Objective
Modern society produces, markets, and uses an increasing number of chemicals, which can be released into the environment through various pathways. The adoption of Non-Target Screening (NTS) workflows to identify these chemicals in the environment and organisms using high-resolution mass spectrometry has rapidly grown within the research community. The combination of comprehensive two-dimensional gas chromatography with high-resolution time-of-flight mass spectrometry (GC×GC-HR-TOFMS) is a powerful analytical technique for NTS of complex samples. This combination offers enhanced chromatographic separation and high-speed acquisition of accurate mass spectra across the full mass range. The generation of such rich data enables insightful retrospective review for vast numbers of unknown compounds. Additionally, target analysis can be performed simultaneously using the same data set obtained for NTS.

Methods
A systematic workflow is presented here for screening both target and non-target contaminants in various high-complexity samples. A combination of non-polar and polar stationary phases was used to enhance separation power of the GC×GC method adopted, resulting in better resolution between matrix and target compounds and, consequently, purer MS spectra for target POPs such as PCB, BDE, and Toxaphenes. Identification and confirmation were achieved through the injection of native standards and the application of dedicated targeted-processing methods. A novel Multi-Mode Source (MMS), capable of EI/CI (both positive and negative), was used to enhance identification confidence by providing library-searchable spectra, accurate mass, and molecular ion information for formula support and/or determinations. For this work, primarily EI and ECNI ionization modes were employed, with ECNI preferred for molecular ion information and formula support in cases where the identity of non-target substances could not be confirmed by EI ionization.

Results
A list of target and non-target molecules is presented, along with information about identification methods (e.g., native standard, retention time, retention index, mass accuracy, etc.). EI high resolution mass spectra will be showed along with ECNI spectra used for confirmation. Formula computation approaches will be highlighted.

Conclusions
The benefits of GC×GC in providing superior separation capabilities and structured chromatograms are highlighted, along with the importance of the novel multi-mode source for HR-TOFMS, which allows for three ionization modes without hardware changes. The combination of ECNI spectra and EI information, such as retention time, retention index, and tentative identification through commercial databases, provided significant information that helped confirm the presence of halogenated POPs in the measured samples.

Developing an analytical method for the near to real-time quantification of microbial volatiles emitted from fungi: a Gas Chromatography-Proton Transfer Reaction Mass Spectrometric investigation of *Trichoderma atroviride*

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Keywords: fast gas chromatography, proton transfer reaction time of flight mass spectrometry, *Trichoderma atroviride*, volatile emissions

Objective
The fast monitoring and precise quantification of microbial volatile organic compounds (MVOCs) at trace levels in biological samples is a major challenge due to the high chemical complexity of the matrices. This complexity results in a large number of volatiles with a wide range of volume mixing ratios from sub ppb_V to ppm_V. This study addresses these challenges by developing a novel method for optimising and applying fast gas chromatographic (GC) separation with proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS), thereby enabling near real-time quantification of the MVOCs with a high level of chemical specificity. Based on previous studies [1,2], the following eleven MVOCs were selected for the GC-PTR-ToF-MS investigation: 6-amyl-α-pyrone, 2-pentylfuran, 1-octen-3-ol, 2-heptanone, 3-octanone, 2-methyl-1-propanol, 2-pentanone, 3-methyl-1-butanol, 3-methylbutanal, acetone and ethanol. The method to quantify the MVOCs was validated analysing the headspace above biological samples of *Trichoderma atroviride*.

Methods
Development and optimisation of the method to quantify MVOCs emitted from fungi were carried out using GC-PTR-ToF-MS by means of gas standards of the selected volatiles to enhance GC separation and production ion formation. *T. atroviride* strain P1 (ATCC 74058) samples were cultivated under standardised conditions in four replicates [2]. Direct headspace measurements by fast GC-PTR-ToF-MS and PTR-ToF-MS were undertaken twice a day for five days.

Results
For many of the MVOCs the product ions are of the form $C_xH_y^+$. This is a problem for PTR-ToF-MS quantification, because a number of unknown fungal and matrix volatiles of lower concentrations were found to make contributions to the product ion signal intensities. With GC separation we were able to correctly quantify the volume mixing ratios for the eleven MVOCs and monitor their unique evolution over time.

Conclusions
A fast-GC-PTR-ToF-MS method has been optimised for the rapid on-line quantification of volatiles in a highly complex biological matrix (fungi and growth medium) to investigate MVOC emissions in near to real-time. Chromatographic separation is essential for the precise identification and quantification of MVOCs emitted from funghi and hence enabling measurements of changes in their concentrations as funtion of time.

REFERENCES
[1] Speckbacher, V. et al. (2020). The Trichoderma atroviride strains P1 and IMI 206040 differ in their light-response and VOC production. *Molecules*, 25(1), 208.
[2] Speckbacher, V. et al. (2021). Monitoring the volatile language of fungi using gas chromatography-ion mobility spectrometry. *Analytical and Bioanalytical Chemistry*, 413, 3055-67.

An ageing study on EVOO by GC and HPLC techniques coupled to mass spectrometry for discriminating olive oil lipid substances in archaeological artifacts

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Keywords: aged lipids, archaeological markers, chromatography-mass spectrometry, pottery

Objective
The identification of archaeological biomarkers is one of the main objectives of analytical chemistry in the archaeological field. However, no information is currently available on biomarkers able to unambiguously indicate the presence of olive oil, a cornerstone of Mediterranean ancient societies lifestyle, in an organic residue. This research attempt to fully characterize the decay products in extra-virgin olive oils (EVOOs) induced by in-lab thermal oxidative treatments, with the aim of revealing specific archaeological biomarkers that could provide insight about the employment of amphorae for olive oil storage. Thirty-three EVOOs belonging to different monocultivar varieties and coming from several Italian regions and Spain were subjected to ageing experiments and following analyses. Multivariate statistical analyses were employed to elucidate decay patterns in olive oils and to identify potential chemical compounds serving as archaeological biomarkers to indicate the presence of olive oil in organic residues.

Methods
The identity of triglycerides (TAGs), diglycerides (DAGs), and their oxidized species was established by means of high-performance liquid chromatography-mass spectrometry (HPLC-MS), while a wide variety of secondary products of oxidation including carboxylic acids, alcohols, hydrocarbons, esters, ketones, lactones, aldehydes, and furans. Head-space solid phase microextraction (HS-SPME) technique coupled with gas chromatography-mass spectrometry (GC–MS) and gas chromatography-flame ionization detection (GC-FID) was employed for the analyses of these volatile substances. Further, all the lipid substances including mono- and dicarboxylic acids, and monoglycerides (MAGs) were derivatized into trimethylsilyl ether (TMS) derivatives by using a silylating reagent and detected by GC–MS and GC-FID.

Results
The complete elucidation of the oxidized lipid species in aged olive oils indicated the mono-carboxylic acids, such as pentanoic, hexanoic, heptanoic, octanoic, nonanoic, and decanoic acids as probable archaeological biomarkers useful for indicating the presence of lipid substances coming from olive oil in archaeological organic residues. Such conclusions were supported by the degradation experiments conducted on standards of triolein, trilinolein and tristearin triglycerides.

Conclusions
Probable archaeological biomarkers have been identified in this research work in order to reveal specific archaeological biomarkers that could provide insight about the employment of amphorae for olive oil storage. However, it must be emphasized that the establishment of specific and selective fatty acids fingerprint indicative of olive oil still remains challenging due to the ubiquitous distribution of oleic and linoleic acids in plant kingdoms, thus complicating interpretations of lipid matter origins.

Joint forces of capillary electrophoresis and ICP-MS/MS in monitoring of liposome-active compound systems formation and changes

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Keywords: electrophoresis, ICP-MS/MS, hyphenated techniques, liposomes, cisplatin, GHK-Cu

Objective

Liposomes are nanometric vesicles built of a double phospholipid layer. Intensive work is being carried out on their wide application in the more effective transport/delivery of various chemical compounds. On the one hand, researchers' great attention is related to the biocompatibility of these chemical objects (imitating cell membranes with their structure), the diversity and simplicity of their synthesis processes, and the encapsulation of crucial chemical compounds inside them. On the other hand, it is essential to examine the mechanisms of their selective transportation into cancer cells (the effect of increased permeation and retention) or the effective incorporation into the membranes of skin cells. Despite the advantages mentioned above, liposomal nanomaterials are problematic analytical objects of interest, making it difficult to understand the pathways of their transportation and release of active compounds in targets. This is due to the lack of unique properties of electromagnetic energy transposition and the poor isotopic composition, which makes it impossible to develop simple tools for their effective determination (especially when compared with metallic nanoparticles). These conditions create a unique research niche and motivation to propose innovative analytical tools to study the formation, stability, and changes of the liposome–active compound systems in variable matrices.

Methods

To solve the abovementioned problems, capillary electrophoresis hyphenated with an inductively coupled plasma tandem mass spectrometer (CE-ICP-MS/MS) was proposed. Its analytical potential in investigating liposome-containing samples will be verified, especially considering the resolution of the optimized methods.

Results

The research aims to develop a method for effectively forming liposomes, which will be able to encapsulate cosmetically and pharmaceutically active compounds inside them, respectively, the GHK tripeptide complex with copper (antiaging agent) and cisplatin (anticancer drug). The studies proved the usefulness of the ethanol injection method for the simple and effective formation of liposome–active compound systems and their stability in the formation medium. Also, it was found that the total lipid content, the lipid composition of the formed liposomes, and the presence of additional steps in the forming procedure influence the encapsulation efficiency of the tested active compounds and the physicochemical properties of probed systems [1].

Conclusions

In the frame of the studies, it was proven that CE-ICP-MS/MS can be considered an effective tool for verifying liposome formation and encapsulating the active compounds inside them. Moreover, due to the elimination of organic solvents from the separation process and imitation of the physiological environment inside the capillary, the changes in the mentioned delivery systems were possible to portray.

ACKNOWLEDGMENTS

Research financed in the frame of WUT Research University Excellence Initiative grant (1820/83/Z01/2022)

REFERENCES

[1] Wróblewska A. M., Łukawska E., Wakuła Z., Zajda J., Keppler B.K., Timerbaev A.R., Matczuk M., (2024). Eur. *J. Pharm. Biopharm*., 198, 114245.

Development of novel fluorescent labels for glycan analysis by CE and LC methods

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Keywords: glycan analysis, labeling, capillary electrophoresis, liquid chromatography, fluorescence

Objective
Sample preparation represents a key step in oligosaccharide and glycan analysis by either chromatographic or electrophoretic methods [1]. Due to the absence of chromophores or fluorophores in the structure of native oligosaccharides and glycans, appropriate modification of their molecules is required for detection by optical methods. Introducing a charged moiety also increases electrophoretic mobility and ionization efficiency for MS detection.

Methods
Standards oligosaccharides and *N*-linked glycans were derivatized by several newly designed and synthesized fluorescent and MS active labels. After purification by SPE methods, the labeled compounds were separated by CE or LC methods followed by fluorescence or MS detection.

Results
Novel fluorescent labels based on pyrene, rhodamine, and fenylpyridine derivatives with different substituents and modifiable linkers were synthesized and applied for oligosaccharide derivatization. The required modification allowed tuning the fluorescence and MS properties (preferred wavelength and quantum yield, charge). Labeling by different synthesized labels was compared with commercially available labels followed by LC and CE analysis. The newly synthesized labels show a significantly higher reaction yield than the traditionally used labels based on reductive amination chemistry, tunable fluorescence properties, and higher ionization efficiency.

Conclusions
This work presents the design, synthesis, and application of novel fluorescent labels that feature fast reaction kinetics, high fluorescence quantum yields, and significantly improved detectability by MS.

ACKNOWLEDGMENTS
This work was supported by the Grant Agency of the Czech Republic [22-00236S] and the institutional research plan [RVO:68081715]

REFERENCES
[1] Smolkova D., Cmelik R., Lavicka J., (2023). Labeling strategies for analysis of oligosaccharides and glycans by capillary electrophoresis, *Trends Anal. Chem.*, 163, 117068.

Semi-untargeted approach for *Lupinus albus* L. traceability

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Keywords: Alkaloids, Lupins, MRM-IDA-EPI, PLS-DA, Traceability

Objective
Lupins, recognized as a potential source of nutrients and phytochemicals with numerous health benefits, have garnered significant global interest as an economical alternative to other legumes. Despite that, they contain quinolizidine alkaloids (QAs), neurotoxic metabolites that act as defense mechanism, whose composition vary significantly due to environmental factors [1]. In our knowledge, few are the methods developed by liquid chromatography-tandem MS (LC-MS/MS) [2] and no one conducted an exhaustive characterization of QA profile or confirmed a geographic classification by multivariate regression models. This study presents the development of a semi-untargeted approach by ultra-high-performance LC coupled with triple quadrupole-linear ionic trap-MS/MS (UHPLC-QqQ-LIT-MS/MS) combined with multivariate analysis. The aim was to deepen the geographical origin of *Lupinus albus* L. samples, through an exhaustive QA profiling, based on mass fragmentation patterns, suggesting the potential use of QAs as markers of traceability, according to samples origin.

Methods
The method was applied on raw *Lupinus albus* L. samples from 4 regions of central-southern Italy, that were extracted and subjected to a suitable clean-up step by Solid Phase Extraction [2]. The analysis involved a UHPLC-QqQ-LIT-MS/MS approach that combines a survey scan in multiple reaction monitoring (MRM) with an information-dependent acquisition (IDA) criteria triggering experiments as enhanced product ion (EPI). MRM transitions were generated via *in silico* experiments and added to the inclusion list. The unknown compounds were then putatively determined by comparison of dependent MS/MS spectra acquired in EPI mode with the few known fragmentations. Subsequently, an unsupervised Hierarchical Cluster Analysis (HCA) was applied, followed by a supervised Partial Least Squares Discriminant Analysis (PLS-DA), to explain phytochemical diversity.

Results
The developed semi-untargeted approach allowed, in an all-in-one analysis, to explore the entire QA profile of all samples, revealing the simultaneous determination of 27 alkaloids, whose fragmentation spectra had not been previously reported. Subsequently, the PLS-DA model allowed a successful and accurate classification of these samples according to their geographical origin, highlighting the huge contribution of the putatively identified QAs, revealing significant differences in alkaloid profiles.

Conclusions
The developed approach allowed to acquire the fragmentation spectra of different QA structures, allowing the exploration of the entire QA lupins profile without the use of analytical reference standards. Coupled with the PLS-DA, it successfully classified lupin samples from all 4 regions, highlighting the synergic contribution of the new alkaloids identified by the semi-untargeted approach. For this reason, the proposed method could represent a helpful tool for broad exploration of the intra-species QAs content, exploiting these compounds as potential markers for geographical origin, in order to verify the authenticity and traceability of a product.

REFERENCES
[1] Bundesinstitut Für Risikobewertung. (2017). *Risk assessment of the occurrence of alkaloids in lupin seeds*.
[2] Eugelio F., Palmieri S., Fanti F., (2022). Development of an HPLC-MS/MS Method for the Determination of Alkaloids in Lupins. *Molecules*, *28*, 1531.

Green techniques and materials for lipid analysis

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Keywords: green analytical chemistry, bio-ethanol, supercritical fluid chromatography, triacylglycerols, vegetable oils

Objective
Preliminary results have already demonstrated the advantages of the use of Supercritical fluid chromatography (SFC) over other chromatographic techniques practiced for lipid analysis, especially triacylglycerols (TAGs). This research aimed at developing a greener alternative to conventional methods used in vegetable oil analysis, with favourable fallout in terms of toxicity, costs, and environmental impact.

Methods
Separation of the main TAG constituents of ten plant oils was achieved on octadecylsilica columns (4x15 cm L, 4.6 mm ID, 2.7 µm fused-core particles), isocratically with 5% of bio-ethanol into carbon dioxide, under subcritical fluid conditions. Ultraviolet detection was used for identification and quantification purposes. Afterwards, gas chromatography with flame ionization detection (GC-FID) was used to obtain the average parameters and fatty acid concentrations of the samples investigated.

Results
In the present work, an SFC method was developed for TAG separation in complex lipid matrices, by using photodiode array detection. The TAGs were separated on four columns serially coupled, based on the partition number and the unsaturation degree, attaining improved chromatographic performance, in terms of peak capacity and resolution. Quantification was attained by the response factor approach, and the results were validated against conventional GC-FID methods.

Conclusions
The method presented in this work is cheaper, faster, and more environmentally friendly than conventional methods used for TAGs analysis, consisting of non-aqueous reversed-phase liquid chromatography (NARP-HPLC), in terms of use of bio-based co-solvent (ethanol from corn), reduced analysis time (30 vs. 110 min), reduced organic solvent consumption and waste (1.5 mL per analysis). The method greenness was quantitatively appraised by different metric tools (Analytical GREEnness calculator, Life Cycle Assessment).

ACKNOWLEDGMENTS

This research was supported by the Ministry of University and Research within project PRIN 2022 PNRR titled “Cephalopod Integrated Analysis to identify the impact Of MARine Environmental pollution (CIAO MARE)”, Prot. P20225SZMX, CUP J53D23013760001. The authors gratefully acknowledge Shimadzu Corporation and Merck Life Science for their continuous support.

Enantioselective liquid chromatography analysis of phytocannabinoids with low- and high-molecular weight chiral selectors

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Keywords: Chiral phytocannabinoids, Enantioselective liquid chromatography, Medicinal cannabis extracts Pirkle's type chiral stationary phases, Polysaccharide-based chiral stationary phases

Objective
While each phytocannabinoid exhibits distinct pharmacological properties, approval for therapeutic indications has thus far been granted only to Δ^9-THC and CBD. Both Δ^9-THC and CBD feature two chiral centers, suggesting the potential existence of four stereoisomers. However, these compounds naturally occur predominantly as a single isomer, with reported levels of other isomers notably low. However, certain cannabinoids such as cannabichromene and cannabicyclol naturally occur as a pair of enantiomers. Importantly, differing biological activities have been demonstrated for the (+)- and (-)-isomers of *trans*-CBD and *trans*-Δ^9-THC as well.

Methods
Over the past decade, there has been an exponential increase in publications addressing the enantioseparation of phytocannabinoids. Enantioseparation methods have been developed utilizing both polysaccharide-type [1] and Pirkle-type [2] chiral stationary phases.

Results
Successful enantioselective liquid chromatography analyses have been conducted using both conventional HPLC and technologically advanced UHPLC systems, operating under the most relevant elution modes [1,2]. This facilitates the analysis of various phytocannabinoids in different matrices. A discussion on selected literature studies will be included in the lecture.

Conclusions
Extensive documentation highlights that from a pharmacological standpoint, the stereoisomers of a chiral compound are non-equivalent and frequently display distinct activities. This holds true also for (phyto)cannabinoids and the production of minor "stereoisomeric impurities" manifests during the synthesis of natural cannabinoids or any cannabis-based product intended for human consumption. The generation of non-naturally occurring enantiomers has been observed in various synthetic routes. Therefore, relying upon efficient enantioselective chromatography methods is of paramount importance.

REFERENCES
[1] Russo F., Tolomeo F., Vandelli M. A., Biagini G., Laganà A., Capriotti A. L., Cerrato A., Carbone L., Perrone E., Cavazzini A., Maiorano V., Gigli G., Cannazza G., Citti C., (2023). Enantioseparation of chiral phytocannabinoids in medicinal cannabis, *Journal of Chromatography B*, 1221, 123682
[2] Mazzoccanti G., Ismail O. H., D'Acquarica I., Villani C., Manzo C., Wilcox M., Cavazzini A., Gasparrini F., (2017). Cannabis through the looking glass: chemo- and enantio-selective separation of phytocannabinoids by enantioselective ultra high performance supercritical fluid chromatography, *Chemical Communications*, 53, 12262-65.

Determination of parabens in cosmetic products by portable capillary liquid chromatography on porous graphitic carbon stationary phases

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Keywords: porous graphitic carbon, parabens, green analytical chemistry, miniaturization, make-up for kids

Objective
The present contriution aims at the development of an eco-sustainable method for the determination of paraben in cosmetic products. Specifically, parabens are antimicrobial preservatives for which interferences with the endocrine system were widely documented, so that limitations were posed to their content by both food and cosmetic legislations. The official method for their quantification is based on a liquid chromatography (LC) separation under reversed phase conditions, employing a large amount of organic solvent. In this regard, current efforts of LC practitioners are addressed to the reduction of toxic solvent through miniaturization and/or decrease of analysis time. In this research, both the miniaturization and the reduction of total analysis time were achieved through the use of a hand-portable capillary LC system (even suitable for in situ analysis) in combination with a porous graphitic carbon (PGC) column, which enabled the separation of parabens at relatively high temperatures (75-80 °C).

Methods
The analyses were carried out on a Axcend Focus CapLC system equipped with a cartridge which contains both column and on-capillary LED based UV detector (255 nm fixed wavelenght). Particularly, a capillary PGC column 50×0.3 mm (L×ID) was installed inside the thermostated compartment of the cartridge and connected to a Z-shaped flow cell for UV detection. Elution was carried out in isocratic mode (40:60, water:acetonitrile, *v:v*). The method was fully validated in terms of linearity, limits of detection and quantification, robustness, accuracy and precision. Intermediate precision was also estimated by replicating the analysis and the entire validation procedure on three different columns, nominally identical, but from different production lots. In order to quantitatively estimate the greenness of the developed method, the AGREE (Analytical GREEnness calculator) tool, based on the assignment of a value between 0.0 and 1.0 for each of 12 principles of Green Analytical Chemistry (GAC), was used.

Results
The developed method was robust enough to enable the transferability between three nominally identical PGC capillary column. Inter-column precision of both retention time and area was calculated on 9 chromatographic runs and CV% was minor than 8% and 21%, respectively. Limit of detection and quantification were below the legislative limits and the accuracy was around 100% on all the tested columns. Methyl- and propylparaben were quantified in the tested samples (make-up for kids were selected for this trial), despite they were not declared on the label.

Conclusions
The use of very low amount of mobile phase on a compact mobile capLC system enabled the development of green and cost-saving analytical methods, which represent a valuable alternative to official methods, often longer and less eco-friendly.

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Isotopologues of amphetamine and methamphetamine: separation, enantioseparation and recognition mechanisms in high-perfomance liquid chromatography

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Keywords: Amphetamine, Enantioseparation, High-performance liquid chromatography, Isotopologues, Molecular recognition

Objective
Kinetic isotope effects were observed for isotopologues resulting from the labelling of molecules with deuterium. The isotope effect may occur in noncovalent interactions and mechanisms underlying intermolecular recognition processes. In chromatography, variations of retention times between isotopologues have been observed with two different elution sequences (isotope effects): the normal isotope effect when heavier isotopologues retain longer than lighter analogues, and the inverse isotope effect featuring the opposite elution order. The molecular bases of these phenomena remain still unclear.

Methods
We studied the separation of isotopologues of amphetamine and methamphetamine on achiral columns by HPLC, as well as simultaneous separation of isotopologues and enantiomers on some polysaccharide-based chiral columns. The effects of the number and location of deuterium atoms introduced in the analyte structure, surface chemistry of adsorbent, mobile phase composition and pH, and temperature on retention and separation of isotopologues and enantiomers were carefully examined integrating experimental and computational analysis.

Results
Normal and inverse isotope effects were observed depending on the content of water in aqueous organic mixtures used as mobile phases.

Conclusions
In this study, it was demonstrated that both number and location of deuterium atoms in the analyte structure impact isotopologue separation. Mobile phase pH also played a critical key role. By integrating experimental and computational analyses, it was possible to deconvolute noncovalent interactions responsible for positive and negative isotope effect, as well as contribution of hydrophobic and dispersion forces.

ACKNOWLEDGMENTS

B.C. thanks Department of Excellence-Biomedical Sciences and Public Health, Università Politecnica delle Marche for providing financial support for his stay in Ancona as Visiting Professor, and Shota Rustaveli National Science Foundation of Georgia for a partial support of this study (Grant N◦ FR-22–971 for fundamental research).

Improving sustainability through modernisation of LC methods

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Keywords: sustainability, environmental impact, method translation, green chromatography, HPLC

Objective
The world is facing increasing environmental challenges, which demands that society re-evaluates daily activities to reduce environmental impact and improve sustainability. This includes the re-evaluation of activities within scientific laboratories. In the analytical laboratory, chromatography is one of the primary tools used for the determination of target analytes, therefore, environmentally friendlier approaches to workflows are required. The 12 Principles of Green Chemistry[1], whilst targeted towards synthetic processes, provides a useful starting point for evaluating greener approaches. Parallels drawn with analytical workflows clearly point towards a number of focus points, including waste reduction, reduced energy use, miniaturisation of analytical methods and minimisation of sample sizes/numbers.
This presentation will examine how the sustainability of existing liquid chromatographic methods can be improved through the utilisation of modern LC techniques and approaches and demonstrate what can be achieved.

Methods
Parameters including column internal diameter, length, particle size and particle morphology are varied to assess the impact of both solvent and electrical energy consumption in the laboratory for a range of methods, across both HPLC and UHPLC formats.
Finally, the use of a 10 mm length cartridge style column is also considered for providing sub-minute separations to further drive method sustainability and sample throughput.

Results
Often, it is perceived that significant gains can only be made by upgrading to high-pressure-limit UHPLC instrumentation; however, it is often feasible to adapt established methods and better utilise existing low-pressure-limit HPLC equipment to realise substantial improvements. This important aspect is considered throughout, and in one specific example a 71.9% reduction in solvent use and 57.3% reduction in electrical consumption and 60.4% reduction in analysis time was easily achieved on a 400 bar HPLC system.

Conclusions
Significant reductions in solvent consumption and waste generation without compromising data quality are possible for both HPLC and UHPLC setups; one of many approaches is to reduce the column internal diameter. This concept can be further extended by employing shorter length columns, packed with smaller particles, to reduce analytical run times. Additionally, the use of solid-core particles can also provide compelling advantages. This also has a demonstrable impact on the electrical consumption per analysis, whilst providing the added benefit of improved laboratory efficiency and reduced running costs. The use of these approaches is supported within updated guidance on allowable changes to pharmacopoeia methods (e.g. USP, EuPh and JP), highlighting great potential for optimisation of both existing and future LC methodologies to drive sustainability improvements.

ACKNOWLEDGMENTS

Many thanks to Tony Edge and Arianne Soliven for their invaluable contributions to both the technical scientific content and a notable portion of the write-up of this presentation, respectively.

REFERENCES

[1] Anastas P. T. and Warner J. C., (1998). Green Chemistry: Theory and Practice, Oxford University Press, New York.

Exploring the capabilities of a cyanopropyl stationary phase for separating cholesterol oxidation products

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Keywords: Oxysterols, Fenton reaction, cyanopropyl stationary phase, RPLC-APCI(+)-FTMS

Objective
The presence of exogenous cholesterol oxidation products (COP) poses a significant challenge to the accurate analysis of endogenous oxysterols (OS) in biological samples. Typically, the concentration of OS is 4 to 6 orders of magnitude lower than that of cholesterol [1], making even slight *ex vivo* COP a potential source of bias [2]. This study aimed to determine how many endogenous OS in BV2 microglial cells can also be generated by exogenous processes triggered by physical and chemical factors, such as heat and metal ions. To achieve this, we compared the OS content in BV2 cells with the OS profile obtained when pure cholesterol was subjected to the Fenton reaction.

Methods
The Fenton reaction was performed by dispersing a dried layer of cholesterol (100 µg) in an acidic aqueous solution containing iron (II) sulfate (0.5 mM) and hydrogen peroxide (0.25 mM) for 16 h at 60°C. The separation of OS was carried out on an Ascentis Express ES-Cyano column (150 x 2.1 mm with 2.7 µm core-shell particle size) operated under reversed-phase liquid chromatography (RPLC) conditions.

Results
The major OS were analysed using RPLC coupled with atmospheric pressure chemical ionization (APCI) high-resolution and tandem mass spectrometry (FTMS/MS). Extensive in-source fragmentation of protonated OS ($[M+H]^+$) generated predominantly $[M+H-H_2O]^+$ ions. The strong similarities among the MS/MS spectra of these ions underscored the critical importance of separation for accurate OS characterization. In this study, we introduced a cyanopropyl column as a powerful alternative to the commonly used C18 stationary phases. This approach allowed us to identify that 6 out of the 7 major endogenous OS detected in BV2 cells, including 7α and 7β-hydroxy cholesterol, cholesterol 5β,6β and 5α,6α epoxides, and 7-ketocholesterol, were also generated by the Fenton reaction.

Conclusions
The significant overlap between endogenous OS in BV2 cells and those generated by the Fenton reaction highlights the need for extreme caution in sample handling. Minimal sample pre-treatment was required for the RPLC-APCI(+)-FTMS characterization in this study, making the method ideal for cross-validating results from analytical techniques that require preliminary derivatization, such as RPLC-ESI-MS and GC-MS. This approach not only enhances accuracy but also streamlines the validation process across different methodologies.

ACKNOWLEDGMENTS
This work was supported by PRIN P2022RW885 funded by the Italian Ministero dell'Università e della Ricerca

REFERENCES
[1] Björkhem I., (2006). Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain, *J Inter Med*, 260, 493-607
[2] Griffith W.J., Abdel-Khalik J., A., Crick P.J., Yutuc E., Wang Y. (2016). New methods for analysis of oxysterols and related compounds by LC-MS, *J Steroid Biochem Mol Biol*, 162, 4-26.

Exploring the enantiorecognition mechanism of 3,5-dinitrobenzoyl-amino acids using two *Cinchona* alkaloid-derived chiral stationary phases: a journey with molecular dynamic simulations

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Keywords: DinitroBenzoyl-amino acids, *Cinchona* Alkaloids-Based Chiral Stationary Phases, Driving Forces

Objective
The molecular dynamic (MD) technique allows the analysis of a range of Selector-Selectand (SO-SA) complexes according to their energy profiles and engaged interactions, offering a more accurate depiction of the enantiorecognition processes happening within the column. Despite this, there is still a lack of standardized procedures and specific guidelines for the computational treatment of enantioselectivity at the molecular level.

Methods
HPLC analyses were performed on an HPLC system ECS05 (ECOM spol. s r.o., Prague, Czech Republic) equipped with a solvent tray, binary pump, column oven, diode array detector, an autosampler and integrated PC. The chromatographic instrument was controlled, and data acquired using Clarity Chromatography Software (DataApex, Prague, Czech Republic). Prototype Chiralpak® QN-AX (CSP 1) and Chiralpak® QD-AX (CSP 2) columns (150 × 3.0 mm I.D.; 3 µm; 120 Å pore size; from Chiral Technologies Europe, Illkirch, France) were used. For the molecular modelling, all simulations with the two CSP systems were performed in the canonical ensemble at 298 K. The temperature in the simulation cell was maintained constant using a Nose–Hoover thermostat. All the other parameters in the simulation study were left to default values in the Desmond Molecular Dynamics System (version 7.3, Schrodinger, LLC, 2023) present in the Schrodinger Suite 2023-1.

Results
Twenty-eight molecular dynamics simulations were performed to study the enantiorecognition mechanisms of seven N-3,5-dinitrobenzoylated α- and β-amino acids (DNB-AAs), occurring with the two quinine- and quinidine-based (QN-AX and QD-AX) CSPs, under polar-ionic conditions. The MD protocol was optimized in terms of box size, simulation run time, and frame recording frequency. Consequently, all the trajectories were analyzed by calculating both the type and amount of the interactions engaged by the selectands (SAs) with the two chiral selectors (SOs), as well as the conformational and interaction energy profiles of the formed SA-SO associates. All the MDs were in strict agreement with the experimental enantiomeric elution order and allowed to establish (i) that salt-bridge and H-bond interactions play a pivotal role in the enantiorecognition mechanisms, and (ii) that the π-cation and π-π interactions are the discriminant chemical features between the two SOs in ruling the chiral recognition mechanism.

Conclusions
The findings of this study clearly highlight the significant role that MD simulations play in understanding the enantiorecognition mechanism involving Cinchona alkaloid-based CSPs. It also became evident from this research that optimizing the MD protocol is essential for ensuring the accuracy and quality of the results obtained.

Method development and environmental assessment for quantitative analysis of oxygen heterocyclic compounds using chromatographic techniques

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Keywords: coumarin, furocoumarin, HPLC, *Citrus*

Objective
Citrus essential oils are popular for their pleasant aroma and are commonly used in food flavoring and hydro alcoholic fragrance formulations. These oils include a non-volatile component that makes up about 10-20% and contains coumarins, furocoumarins, and polymethoxyflavones. Furocoumarins are recognized for their photosensitizing effects and their potential carcinogenic and mutagenic properties. As a result, regulatory organizations like the International Fragrance Association (IFRA) strictly regulate the levels of furocoumarins in cosmetic products. Supercritical fluid chromatography (SFC) has become a cutting-edge technique for analysing these compounds. SFC uses supercritical CO_2 as the mobile phase, combining properties of both liquids and gases, leading to quicker analysis and lower solvent use compared to traditional liquid chromatography. This research aims to develop rapid analytical methods with a minimal environmental footprint using chromatographic technique.

Methods
Two validated methods were created: the first employs Supercritical Fluid Chromatography (SFC) with either a photodiode array detector (PDA) or a triple quadrupole mass spectrometry detector (QqQ-MS), achieving fast separation with minimal solvent use in under 8 minutes. The second method uses Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) with QqQ-MS for rapid screening, completing the analysis in approximately 4 minutes using less than 3 mL of ethanol per analysis. These methods are designed to offer an eco-friendly approach for quantifying coumarins, furocoumarins, and polymethoxyflavones in cold-pressed Citrus essential oils, flavored foods, and cosmetics.

Results
Calibration curves were constructed using distilled lemon essential oils to quantify these compounds in real samples, with satisfactory validation parameters allowing for quantification even at trace levels in finished products. Oxygen heterocyclic compounds were quantified in foods and hydro alcoholic fragrances.

Conclusions
These validated methods offer a green analytical approach for determination of oxygen heterocyclic compounds both in foods and conmetics, with the SFC-PDA method being particularly environmentally friendly compared to previously developed liquid chromatography methods. Thus, SFC is well-suited for quality control of these compounds in citrus essential oils and finished products.

ACKNOWLEDGMENTS

The authors acknowledge the Shimadzu Corporation and Merck Life Science for their support.

Goat milk powder enriched with grape pomace seed extract: Mixture optimisation and UHPLC-Q-ToF-MS characterization

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Keywords: UHPLC-Q-ToF-MS, grape seed extract, goat milk, central composite design, dietary supplement

Objective
Grape pomace seed is a rich source of highly valuable phenolic compounds that can be successfully extracted and used in the formulation of various functional foods. On the other hand, goat milk, especially thermally-treated, possesses unique techno-functional and functional properties, but lacks phenolic compounds [1]. Therefore, the aim of this study was to analyse the bioactive compounds of the optimised goat milk powder enriched with grape pomace seed extract (M/GPE) by UHPLC-Q-ToF-MS technique, as they are important indicators of the functionality of this powder.

Methods
The Central Composite Experimental Design (CCD) was applied to estimate the optimal M/GPE mixture, considering the effects and interactions of two independent variables (pH and GPE content) on total phenolic content, antioxidant properties and ferrous ion chelating ability.

Results
The overall desirability was D=0.9467 for the mixture prepared with 0.703 % GPE, at pH 6.36. The prepared optimal M/GPE freeze-dried powder was extracted with 80% acidified methanol and analysed by UHPL-Q-ToF-MS. A total of forty-three phenolic compounds were identified and quantified, mainly phenolic acids, flavan-3-ols, procyanidins and their derivatives. All phenolic compounds identified in the M/GPE powder were typical for grape seeds, so it can be assumed that they originate from GPE. To facilate the interpretation of the results, all identified compounds were categorised into six different classes. Ellagic acid, dihydroxybenzoic acid and gallic acid were dominant phenolic acids, with contents of 27.77, 21.65 and 59.74 mg/100 g, respectively. The content of other individually detected phenolic acid derivatives was less than 5 mg/100 g. Flavan-3-ols and derivatives were the predominant class of phenolics in the M/GPE powder. Various B type isomers of procyanidin dimer and trimer were also detected, with a predominance of B-type procyanidin dimer isomer II (76.306 mg/100 g) and B type procyanidin dimer gallate (71.89 mg/100 g). All detected flavonol derivatives were present in traces (below the limit of quantification), with the exception of quercetin, quercetin-3-*O*-hexoside and myricetin-3-*O*-hexoside. Among stilbenoids, only complex resveratrol dimer and trimer derivatives were confirmed in small amounts, as well as flavonoid naringenin. Finally, the total content of all phenolic compounds detected in M/GPE powder was 2.42 g/100 g.

Conclusions
M/GPE powder can be used as a dietary supplement or food ingredient containing valuable goat milk proteins and phenolic compounds from grape seed.

ACKNOWLEDGMENTS

This research was supported by the Science fund of Republic of Serbia, FUNPRO Project, #Grant No. 7744714 the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-65/2024-03/200116.

REFERENCES

[1] Pesic, M. B., Barac, M. B., Stanojevic, S. P., & Vrvić, M. M. (2016). Heat-Induced Casein–Whey Protein Interactions in Caprine Milk: Whether Are Similar to Bovine Milk? In R. P. Nedovic V., Tumbas V. and Barbosa-Cánovas G. (Ed.), *Emerging and Traditional Technologies for Safe, Healthy and Quality food*: Springer International Publishing: Cham, Switzerland.

Integrated LC-MS analysis of Maillard-modified lipids in edible insects

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Keywords: Lipidomics, insects, HILIC, C30, High-Resolution Mass Spectrometry

Objective

The rising global population is creating nutritional challenges, and edible insects are emerging as an eco-friendly alternative to traditional meat [1]. However, the consumption of this novel food remains uncommon. More research into the nutritional and health benefits of edible insect species is needed. Pre-treatments such as blanching or pasteurization, often combined with roasting and air or oven drying, are necessary to eliminate pathogenic bacteria and inactivate enzymes [2] in insects intended for human consumption. These processes can cause nutrient modifications. For instance, the Maillard reaction (MR) involves complex chemical reactions between reducing sugars and amino groups of proteins or lipids like phosphatidylethanolamine (PE) or plasmalogen forms (PE-O) when exposed to heat. Initially, a sugar molecule condenses with an amino group to form a glycosylamine, which undergoes further rearrangements, including dehydration, fragmentation, and the creation of a wide range of by-products that could negatively impact food quality and so human health [3]. In this study, we examined several MR by-products on PE and PE-O in edible *Acheta domestica*.

Methods

The study utilized two distinct separation techniques -hydrophilic interaction liquid chromatography (HILIC) and C30 reversed-phase chromatography-both employing core-shell particles. Both columns were coupled with high-resolution mass spectrometry (HR-MS) in positive and negative electrospray ionization (ESI) modes. This advanced setup allowed for the comprehensive identification and characterization of MR products, specifically the derivatives of PEs and PE-Os.

Results

The HILIC column effectively separated the lipid classes based on their modified polar heads. The same sample was separated by the C30 column and from this combination, a detailed analysis of the MR by-products was obtained. At least six classes of lipids generated by PE and PE-O were identified, including formyl-PE, acetyl-PE, Amadori products [4], NAPE, and lyso forms. The most prominent species contained 34 and 36 carbon atoms. A reaction scheme was outlined to account for all these by-products.

Conclusions

While insects are considered the future of human food consumption, extensive research in this field is crucial, particularly concerning molecules that may have adverse health effects. This study represents a pioneering effort in exploring these aspects, specifically addressing MR products.

ACKNOWLEDGMENTS

This work was supported by the project *2023-UNBACLE-0241870 - Lipid7*, financed by Uniba ERC SEEDS project and the project PON Ricerca e Innovazione 2014-2020- 02-G-14853-2, financed by the MIUR

REFERENCES

[1] Aidoo O. F. et al., (2023). Insects as food and medicine: a sustainable solution for global health and environmental challenges, *Front. Nutr.*, 10, 1113219

[2] Liceaga A. (2021). Processing insects for use in the food and feed industry, *Curr. Opin. Insect Sci.*, 48, 32–36

[3] Guo Z. et al., (2012). Identification of novel bioactive aldehyde-modified phosphatidylethanolamines formed by lipid peroxidation, *Free Radic. Biol. Med.*, 53, 1226–38

[4] Nakagawa K. et al., (2005). Ion-trap tandem mass spectrometric analysis of Amadori-glycated phosphatidylethanolamine in human plasma with or without diabetes, *J. Lipid Res.*, 46, 2514–24.

Quantitation of WADA-prohibited and monitored stimulants in dietary supplements using LC-MS/MS and LC-DAD

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Keywords: stimulant, doping, LC-MS/MS, dietary supplement

Objective

The use of stimulants by both professional and amateur athletes is still at the center of interest in the world of sports. Although these substances are banned by the World Anti-Doping Agency (WADA), they are very popular among athletes, which is also supported by WADA's annual doping control statistics. Many top athletes try to find "legal" performance-enhancing products with stimulating effects, especially dietary supplements (DSs) containing high amounts of caffeine (CAF). DS's promising enhanced focus or better performance are widely commercialized in webshops, but they may be contaminated or intentionally adulterated with prohibited stimulants (PSs), thus instead of being safer, their use can provide a high risk of a positive doping test. Consequently, we aimed to analyse these products.

Methods

The analyses of DSs were carried out by means of suitable chromatographic techniques. Our scopes were to quantify the legal active substance, CAF, by means of liquid chromatography coupled with diode array detectors (LC-DAD) and to detect eventual PSs (40 mostly occurring stimulants and narcotics) by means of LC coupled with tandem mass spectrometry (MS/MS).

Results

Our analytical methods were optimized considering the limit of detection, the selectivity, time and solvent consumption to obtain the most suitable ones for the target compound analysis. Furthermore, due to the high variety of the DS's forms and composition sample preparation phases were optimized for normal and swelling DSs. The development of highly sensitive and selective analytical techniques for PS analysis required the optimization of the MRM transitions and gas collision energies (CEs) of 40 target components and the internal standard, allowing their identification and detection at very low concentrations (1-10 ng/g or mL) in commercially available DS. Among 45 analysed samples more than 30% of them contained almost one PS, mainly in the concentration range from ng/g to µg/g, while only in half of them was the CAF content in the range of ±10% concerning the labeled values.

Conclusions

The detection of PSs in DSs used by athletes is a crucial preventive step to avoid unintentional doping. Measurement of active compounds can help also in the evaluation of the cost/benefit of the usage of DSs. The developed methods can support the choice of safe and trustworthy DS used by elite athletes.

Oxysterol profile in Zebrafish by UPLC-APCI-MS/MS: a potential marker for development stage

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Keywords: Zebrafish, UHPLC, tandem mass spectrometry, oxysterols profile

Objective
Oxysterols, which are oxidized derivatives of cholesterol, are reliable indicators of oxidative stress and lipid oxidation. Their investigation in a suitable *in vivo* system could provide useful information on the amount, roles and biological functions of these molecules that are enzymatically generated by cytochrome P450 (CYP450) family, or via autooxidation, or even via both pathways in synergy.
Zebrafish (Danio rerio) is used in toxicology to estimate the effects of xenobiotics and their teratogenic consequences, due to their genetic similarity to humans, presenting several advantageous features as high fecundity, rapid embryonic development (24 h) and external fertilization[1]. The knowledge of the oxysterols profile in zebrafish, during early embryonic stages, provides important information on the role and biological function of these molecules [2] In this work the oxysteriols profile was investigated and correlated with the development of zebrafish from 3 to 96 hpf.

Methods
The study employs liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the quantification and analysis of oxysterols in zebrafish, involving a selective solid-phase extraction (SPE) [3]. Following extraction, separation and detection are conducted using LC-MS/MS, which offers high sensitivity and specificity. The method was validated according to European Medicines Agency (EMA) guidelines, ensuring robustness and reliability. Multivariate analysis was used to understand zebrafish profile modification.

Results
The profile of oxysterols was developed using both unsupervised and supervised multivariate analysis. Both approaches show how the profile changes depending on the developmental stages, particularly in the more advanced stages (48, 72, 96 hpf).

Conclusions
The results demonstrate that specific oxysterols are significantly elevated in embryos exposed to oxidative stress, validating their use as biomarkers. This approach not only enhances our understanding of oxidative stress in aquatic models but also provides a robust tool for environmental and pharmacological studies.

REFERENCES
[1] Teame T., Zhang Z., Ran C., Zhang H., Yang Y., Ding Q., Xie M., Gao C., Ye Y., Duan M., Zhou Z., (2019). The use of zebrafish (Danio rerio) as biomedical models. *Animal Frontiers*., 9, 68–77
[2] Jamadagni P., Patten S. A., (2019). 25-hydroxycholesterol impairs neuronal and muscular development in zebrafish, *NeuroToxicology*, 75, 14-23
[3] Fanti F., Merola C., Vremere A., Oliva E., Perugini M., Amorena M., Compagnone D., Sergi M., (2020). Quantitative analysis of oxysterols in zebrafish embryos by HPLC-MS/MS, *Talanta*, 220, 12139.

Quality assurance of plant-based products through analytical chemistry and biological test systems - applications in phytocosmetics, phytopharmacy and phytonutrition

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Keywords: phytoanalysis, LC/MS, ambient MS, quality control

Objective
The increasing demand for plant-based products in the fields of phytopharmaceuticals, phytocosmetics, as well as food and dietary supplements, necessitates reliable methods for quality assurance. This study aims to enhance the quality assurance processes for these products by integrating advanced analytical chemistry techniques with biological test systems.

Methods
A key component of modern quality control is liquid chromatography coupled with mass spectrometry (LC/MS). This technique was employed to identify and quantify ingredients in plant-based products with high accuracy and sensitivity. LC/MS is particularly suitable for analyzing complex mixtures, such as those found in plant extracts, and can detect both primary and secondary plant metabolites. Additionally, ambient mass spectrometry (Ambient MS) was utilized for rapid and non-destructive analysis directly at the sample site, providing real-time information about the chemical composition without the need for extensive sample preparation. Biological assays were used to investigate the bioactive properties of the ingredients, evaluating their efficacy and safety.

Results
The combination of LC/MS and Ambient MS proved to be effective in providing detailed and accurate analysis of the chemical composition of plant-based products. Biological test systems enabled the identification of substances with specific bioactive effects, such as antioxidant, anti-inflammatory, or antimicrobial properties. These methods facilitated the assessment of the therapeutic efficacy and safety of phytopharmaceuticals, ensured the purity and effectiveness of phytocosmetics, and guaranteed the quality and health benefits of food and dietary supplements.

Conclusions
The integration of LC/MS, Ambient MS, and biological test systems elevates the quality assurance of plant-based products to a new level. These combined methods not only offer precise analysis of the chemical composition but also enable the assessment of biological activity, ultimately leading to safer and more effective products in the fields of phytopharmaceuticals, phytocosmetics, and food and dietary supplements. This approach ensures that plant-based products meet high standards of quality, efficacy, and safety.

Harnessing the potential of 1.0 mm ID columns in UHPLC-HRMS based untargeted metabolomics

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Keywords: Mass Spectrometry, metabolomics, microbore, UHPLC, untargeted

Objective
Untargeted metabolomics offers invaluable insights into the molecular mechanisms underlying diseases and treatments. While LC-HRMS with narrowbore (2.1 mm I.D.) columns is the prevailing method for untargeted profiling, there persists a need for higher sensitivity, especially when analyzing samples in limited quantities. Although microbore (1.0 mm I.D.) columns have demonstrated potential in proteomics, they are not yet widely embraced in metabolomics. In response, we developed an efficient and sensitive microbore UHPLC-HRMS approach using 1.0 mm I.D. columns, achieving superior sensitivity compared to 2.1 mm-based LC-HRMS approaches while ensuring robustness.

Methods
We conducted all analyses using a nano/microflow Vanquish Neo UHPLC coupled with an Orbitrap Exploris 120 mass spectrometer. RPLC and HILIC chromatography were used, withsub-2 micrometer fully porous particles 1.0 mm I.D. columns. HRMS analyses employed data-dependent acquisition (DDA) with a resolution of 60,000 in MS1 and 15,000 in MS2, employing both positive and negative electrospray ionization. Our study utilized human plasma and Patient Derived Organoids (PDO) with colorectal cancer (CRC) treated with two distinct chemotherapies, along with their secretome, as test samples, with comparative analyses on 2.1 mm ID columns. Exploration of flow rate, gradient, injection volume, column temperature, and source parameters were conducted using a mixture of endogenous metabolites with different retention and ionization behaviors.

Results
Optimal conditions were identified with a flow rate of 100 µL/min, yielding excellent reproducibility of chromatographic retention time (≤ 0.5 % coefficient of variation, CV) and peak area (≤ 2.3 % CV), surpassing narrowbore performance. Furthermore, the microbore approach exhibited increased sensitivity in full scan (FS)-DDA, achieving LOD and LOQ values of 0.95 and 3.18 ng/mL, respectively, with a twofold increase in response compared to the narrowbore method, resulting in almost twice MS1 spectral features and metabolite annotations at MSI level 2.
System robustness was validated through 48 hours and over three hundred consecutive injections, with a notable reduction in solvent consumption (300 mL vs. 1.5L), rendering the approach more environmentally sustainable.

Conclusions
As a proof of concept, the microbore LC-HRMS method successfully profiled CRC organoids' metabolome, revealing modulations in several metabolic pathways following chemotherapy. Our findings underscore the robustness, sensitivity, and broad applicability of microbore UHPLC-HRMS in metabolomics, offering a promising alternative to conventional 2.1mm I.D column-based approaches.

A stationary phase with a positively charged surface allows using a mobile phase with reduced formic acid concentration, enhancing electrospray ionization in LC-MS proteomic experiments

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Keywords: proteomics, Reversed-phase Liquid Chromatography, stationary phases, formic acid, Mass Spectrometry

Objective

Despite the availability of very sensitive mass spectrometers, proteomics is constantly striving to achieve better proteome coverage. Unfortunately, chromatographic separation is often disregarded in proteomics as an area with potential for improvement. Most bottom-up proteomic workflows primarily use mobile phase acidified with 0.1% formic acid and traditional C_{18} reversed-phase columns and do not adopt their improved alternatives [1]. This study evaluated the applicability of the mobile phase with reduced formic acid concentration using the C_{18} stationary phase with a positively charged surface (PCS) in bottom-up proteomic workflows [2].

Methods

Three different columns packed with PCS-C_{18} stationary phase were evaluated in terms of ionic interaction, retention, loading capacity, and separation performance using the mobile phase with reduced formic acid concentration. The most suitable column was used to assess the effect of reduced formic acid concentration on the chromatographic behavior and MS response of peptides. The study was carried out using a range of protein samples with varied complexity and on analytical flow, micro-, and nanoflow regimes to expand the applicability in routine practice.

Results

When using the PCS-C_{18} stationary phase, peptides can be efficiently separated even when using the mobile phase acidified with 0.01 % formic acid, which is not possible with the traditional C_{18} stationary phase. Amongst the three selected columns packed with PCS-C_{18} stationary phase, the Acquity Premier CSH column provided superior separation of peptides at 0.01 % formic acid concentration. Using mobile phase acidified with 0.01 % formic acid increased the MS signal response compared to the standard of 0.1 %. The enhanced MS response translated to about 50% improved peptide identifications depending on the complexity and amount of sample injected.

Conclusions

This simple and cost-effective approach can be easily integrated into existing bottom-up proteomic workflows, offering a promising alternative to the expensive and complicated methods used to improve the sensitivity of proteomics analysis.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of the project of the Charles University Grant Agency (GAUK No. 370522) and the SVV Project No. 260 662

REFERENCES

[1] J. Lenčo, S. Jadeja, D. K. Naplekov, (2022). Reversed-Phase Liquid Chromatography of Peptides for Bottom-Up Proteomics: A Tutorial, *J. Proteome Res*, 21, 2846-92

[2] S. Jadeja, R. Kupcik, I. Fabrik, H. Sklenarova, J. Lenčo, (2023). A stationary phase with a positively charged surface allows for minimizing formic acid concentration in the mobile phase, enhancing electrospray ionization in LC-MS proteomic experiments, *Analyst*, 148, 5980-90.

Non-invasive CYP3A4 breath test for predicting individual drug responses

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Keywords: Cytochrome P450, CYP3A4, drug metabolism, non-volatile biomarkers, volatile biomarkers

Objective
Drug metabolism mediated by cytochrome P450 (CYP) enzymes plays a crucial role in drug efficacy and side effects. The CYP3A4 enzymes are particularly important as they are involved in the oxidation of a wide range of therapeutic drugs [1,2]. Conventional methods for assessing CYP activity, such as $^{13}CO_2$ breath tests, have limitations due to the need for isotopically labelled substrates and the use of non-specific volatile biomarkers. Therefore, the aim of this project is to propose a novel approach using non-invasive breath tests with substrates designed to generate unique volatiles upon metabolism by CYP enzymes, with a particular focus on CYP3A4.

Methods
An *in silico* screening was conducted for identifying potential substrates leading to distinct volatile metabolites after biotransformation. To quantify the volatile and non-volatile metabolites, the headspace and liquid sampling setup were developed initially. Analytical methods using proton transfer reaction time-of-flight mass spectrometry and liquid chromatography mass spectrometry were developed to identify the volatile and non-volatile metabolites in biological samples. The biotransformation of the potential substrates were subsequently investigated using CYP3A4-overexpressing human hepatoblastoma (HepG2) cell clones.

Results
Tolterodine is found to be a promising substrate, as CYP3A4 enzymes catalyse the biotransformation to 2-[3-(isopropylamino)-1-phenylpropyl]-4-methylphenol, a non-volatile metabolite, and acetone as a volatile metabolite. Measurements of cell culture headspace and buffer-supernatant confirmed the formation of both metabolites. A time dependent biotransformation indicated an increase in metabolite concentration with incubation time. In addition, the inhibition of the CYP3A4 enzymes with different inhibitors showed a reduced production of both metabolites.

Conclusions
Tolterodine emerged as a promising substrate for assessing CYP3A4 activity in HepG2 cells, producing both non-volatile and volatile metabolites upon biotransformation.

ACKNOWLEDGMENTS
We acknowledge the FWF for funding the Project PREDICT (Project Number: P35312-B)

REFERENCES
[1] Guengerich, F.P., (2008). Cytochrome P450 and chemical toxicology, *Chemical research in toxicology*, 21, 70-83
[2] Pinto, N., Dolan, M.E., (2011). Clinically relevant genetic variations in drug metabolizing enzymes, *Current drug metabolism*, 12, 487-97.

The effectiveness of liquid phase microextraction of beta-blockers from aqueous matrices for their analysis by chromatographic techniques

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Keywords: DLLME, SFOME, beta-blockers, chromatographic techniques, water samples

Objective
Reducing the amount of solvents required in the sample pre-treatment, reducing the steps demanding much labor and energy during the sample treatment, respectively the miniaturization of analytical methods are some of the priorities and strategies for a greener analytical chemistry. The aim of this research is to test the effectiveness of two green liquid phase microextraction procedure for selective extraction of eight beta-blockers (acebutolol, atenolol, betaxolol, bisoprolol, metoprolol, nadolol, pindolol, propranolol) from aqueous matrices for their analysis by gas chromatography or liquid chromatography.

Methods
The efficiency of dispersive liquid-liquid microextraction (DLLME) and solidification of floating organic droplet microextraction (SFOME) was tested. Dichloromethane for DLLME and 1-undecanol for SFOME were used as extraction solvents. Gas chromatography coupled with mass spectrometry (GC-MS) and high-performance liquid chromatography with photodiode array detector (HPLC-PDA) were used for separation and quantification of selected beta-blockers. Prior GC-MS analyses, the selected beta-blockers were subjected to derivatization step using *bis*-trimethylsilyl trifluoroacetamide (BSTFA). The influence of extraction parameters such as the type and volume of extraction and disperser solvents, pH and ionic strength were studied. The efficiency of extraction was expressed by enrichment factor and extraction recovery.

Results
The developed extraction procedures provide a good enrichment factor (80-160), good extraction recovery (72-105%) and good samples cleaning for both extraction procedures. Good limits of detection (0.13-0.69 µg/mL for GC and 0.07 to 0.15 µg/mL for HPLC) and limit of quantification (0.39 to 2.10 µg/mL for GC and 0.20 to 0.45 µg/mL for HPLC) were obtained. The developed procedures were successfully applied to the extraction and analysis of investigated beta-blockers in wastewater samples, proving their applicability to the analysis of real wastewater samples.

Conclusions
The developed DLLME and SFOME are suitable microextraction procedures for the analysis of selected beta-blockers in real wastewater samples. Its offer a sensitivity of the order of micrograms per litter, high enrichment factor comparable to classical extraction methods, high recovery rates and repeatability. The methods are easy to apply in any laboratory, use small volumes of sample and organic solvent and have a short extraction time, being an appropriate and ecological alternative to traditional extraction techniques

ACKNOWLEDGMENTS

This work is part of the doctoral studies, Babeș-Bolyai University, Environmental Science Doctoral School Cluj-Napoca, Romania.

Modifications of polymer nanofibers: advanced extraction materials for sample preparation

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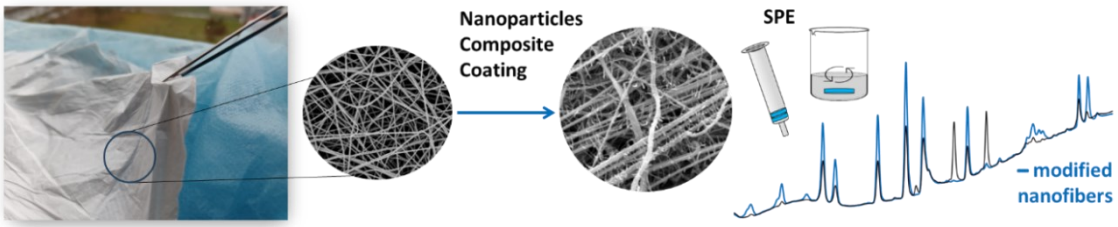
Keywords: sample preparation, Solid Phase Extraction, miniaturization, automation, nanomaterial

Objective

Novel extraction sorbents represent the best strategy to improve selectivity and extraction efficiency. Nanofiber sorbents are a special class of nonparticulate materials that offer the advantages of a great surface area and convenient handling because they can be prepared in large nonwoven sheets or mats that can be shaped into a variety of formats [1].
Generally, polymers provide only nonspecific interactions with a range of pronounced hydrophobicity or the presence of polar functional groups in the polymer. Introducing carbon modifiers, molecular imprinting, coating, or simply combining polymers and fabrication techniques significantly changes their nature. All the modifications enhance various sorbent features such as selectivity, surface area, wettability, and robustness.

Methods

Solid-phase extraction methods, including stirred disk sorptive extraction and online hyphenation to chromatographic analysis via column-switching SPE-HPLC, were developed to compare various polymer nanofiber sorbents with their modified counterparts.



Results

Different polymer/nanofiber modifications led to (i) increased retention of model analytes and shifted selectivity and (ii) changes in physical characteristics, such as higher surface area and wettability with an aqueous sample.

Conclusions

Polymer nanofibers are versatile extraction sorbents with high surface area and porosity. Polymers mainly provide nonspecific hydrophobic interactions, and some feature worse water-wettability. Thus, the presented modifications might be necessary to change the selectivity and surface characteristics.

ACKNOWLEDGMENTS

This work was supported by the Czech Science Foundation, project GA ČR No 23-05586S. I.L. acknowledges financial support by OeAD (Austria's Agency for Education and Internationalisation) for the Aktion scholarship.

REFERENCES

[1] Háková M., Chocholoušová Havlíková L., Solich P., Švec F., Šatínský D. (2019) Electrospun nanofiber polymers as extraction phases in analytical chemistry – The advances of the last decade, *TrAC*, 110, 81-96.

Simplified analytical workflow for contaminants analysis in olive oil using low-pressure gas chromatography

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Keywords: contaminants, pesticides, phthalates, Low-pressure Gas Chromatography

Objective
The aim of this research was to simplify the analytical workflow for the analysis of contaminants (phthalates and pesticides) in fat matrices. Usually, the analysis of contaminants requires several sample preparation procedures that can be costly and time consuming. For this purpose, phthalate contamination was investigated in olive oil by a simple dilution and injection of the sample. While for the investigation of pesticides, an ultrasound-assisted liquid extraction was performed prior to analysis. The methods allowed the identification of 9 phthalates and 149 pesticides.

Methods
Separation was achieved in 10 min by means of low-pressure gas chromatography (LP-GC), followed by multiple reaction monitoring MS detection. The use of a relatively short (5 m) wide bore (0.53 mm I.D.) non-polar column allowed high sample capacity and fast analysis. Sensibility and selectivity were guaranteed using triple quadrupole mass spectrometry.

Results
In both cases, the following figures of merit were measured: the limits of quantification, the accuracy, the reliability of the method also through the evaluation of blinded accuracy analyses as well as intra-day and inter-day accuracy studies. All figures of merit had good values. Different commercial samples, 23 for phthalate and 50 for pesticide contaminations were analysed.

Conclusions
The project here presented simplified the workflow for the analysis of the investigated contaminants in olive oils through the use of reduced sample preparation and a fast gas chromatography system. The LP-GC-QqQMS method is characterised by several advantages such as the use of simplified analytical instrumentation, resulting in reduced energy requirements and much lower He consumption compared to conventional techniques.

ACKNOWLEDGMENTS
The authors gratefully acknowledge the Merck Life Science and Shimadzu Corporation for their continuous support.

Bugs for buffet: optimization of a QuEChERS-GC/MS protocol to assess the organic micropollutant contamination in edible insects

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Keywords: edible insects, PAHs, PCBs, QuEChERS, GC/MS

Objective

Edible insects are consumed by approximately 2.5 billion people, offering a promising solution for sustainable nutrition due to their low environmental impact and high nutritional value. In this regard, the European Commission, through the EU Novel Food Regulation 2015/2283, considers edible insects as a novel food, provided that an authorization that guarantees the safety of consumption for human health is released. The insects authorized so far are frozen, dried, and powder forms of *Acheta domesticus* (the house cricket), *Tenebrio molitor* larva, and *Alphitobius diaperinus*. However, the safety of insect consumption concerning persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) is under-inestigated and requires thorough attention. This chemical contamination mainly depends on the insects' habitat, native plant feed contamination and biomagnification.
Based on previous assumptions, this work aimed to innovatively study the contamination levels of 16 PAHs and 14 PCBs (including six dioxin-like congeners) in two commercially available edible insect species, *Tenebrio molitor* and *Gryllodes sigillatus*, using an analytical protocol optimized for the purpose.

Methods

Insect samples (specifically sold for human consumption) were obtained from an online shop and analysed for PAHs and PCBs using the QuEChERS extraction method coupled with GC-MS. The method was optimized for solvent type and volume and validated for accuracy and repeatability. After pulverization, dehydration of samples, they were extracted by a mixture of dichloromethane and acetone. Analytes were separated and quantified using GC-MS in single ion monitoring mode.

Results

The QuEChERS-based extraction protocol was shown to be effective (50%-110% apparent extraction recoveries for PAHs and 100%-118% for PCBs), as well as reproducible (RSD% <10% for all the target analytes). The overall analytical protocol was validated assessing linearity, method detection and method quantitation limits, repeatability, and matrix effect. The application of the optimized protocol to the analysis of insect samples revealed the presence of ten PAHs in *Tenebrio molitor*, including acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(k)fluoranthene, and benzoperylene. The concentrations observed varied, with benzoperylene showing the highest level (249.4 µg/kg). PCB analysis in *Tenebrio molitor* indicated significant levels (units of ng/kg) of PCB101, PCB118, PCB138, PCB153, and PCB180. *Gryllodes sigillatus* exhibited similar contamination profiles but with lower concentrations overall.

Conclusions

This work suggested that edible insects such as *Tenebrio molitor* and *Gryllode sigillatus* could be prone to contamination by environmental POPs, necessitating comprehensive investigations to ensure consumer safety. In this regard, the optimized and validated protocol provides a robust tool for accurate contaminant analysis in insect matrices. Further research is required to understand the bioaccumulation paths and develop strategies to mitigate this contamination *a-priori*.

ACKNOWLEDGMENTS

This research has received funding from: Project CH4.0 under the MUR program "Dipartimenti di Eccellenza 2023-2027" (CUP: D13C22003520001). Laboratory assistance from Elena Altavilla and Michele Castiglioni is gratefully acknowledged.

Electromembrane extraction of basic drugs from human urine on-line coupled with capillary electrophoresis

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Keywords: Capillary Electrophoresis, clinical samples, electromembrane extraction, microfluids, on-line coupling.

Objective

A new type of efficient extraction technique for the separation of ionized analytes from complex matrices such as human urine or plasma is electromembrane extraction (EME). EME extraction takes place on a Supported Liquid Membrane (SLM), i.e., on a porous polymer membrane whose pores are filled with extraction liquid of suitable properties and analytes migrate across the SLM under the influence of a DC electric field. SLM separates the sample solution (donor solution) from the acceptor solution, in which the extracted substance is analysed. EME experiments are usually performed in off-line mode, i.e., after the extraction is completed, the acceptor solution is manually transferred to the analytical apparatus, which is usually a capillary electrophoresis (CE).

Methods

New designs of a miniature flow-through coaxial EME probes that can be on-line coupled with CE instrument are described and tested. EME probes are on-line coupled with CE instrument by air-assisted or transversal flow gating interfaces (FGI) that are realized by a microfluidic PDMS microchip cast in the laboratory and the entire EME/CE analysis process is automated by the LabView system.

Results

On-line CE/EME approach is applied for the determination of basic amino acids [1], anesthetic ketamine [2] and drug methadone [3] from human urine and serum.

Conclusions

Flow-through probes with a coaxial arrangement of donor inlet and acceptor outlet are more stable in repeated extractions. Another advantage of the described arrangement with a PDMS carrier chip is that the active part of the probe, the SLM, can be carefully withdrawn from the fused silica capillary when damaged and replaced with a new hollow fiber while keeping the overall probe arrangement unchanged.

ACKNOWLEDGMENTS

The financial support from the Czech Science Foundation (22-22398S) is gratefully acknowledged.

REFERENCES

[1] Opekar F., Tůma P., (2023), A new electromembrane extraction probe for on-line connection with capillary electrophoresis for determination of substances in biological matrices, *Talanta*, 254, 124149.
[2] Opekar F., Tůma P., (2023), Electromembrane extraction of anesthetic ketamine on-line coupled to capillary electrophoresis, *Microchemical Journal*, 191, 108886.
[3] Opekar F., Tůma P., (2024), A new coaxial flow-through probe for electromembrane extraction of methadone from clinical samples on-line coupled to capillary electrophoresis, *Analytica Chimica Acta*, 1300, 342461.

How proteomics analysis can be advanced for studying biological, food and cultural heritage samples

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Keywords: protein extraction, sample treatment, protein separation, Mass Spectrometry

Objective
Sample treatment is a critical aspect of proteomics, beginning with the essential step of protein extraction. The choice of buffers and mechanical dissolving methods depends on the sample's type and nature [1]. In our study, we explored various protein extraction and purification techniques applied to a range of matrices, including food, biological, and cultural heritage samples. The selection of extraction buffers was tailored to the specific nature of each sample. Post-extraction, we employed purification processes such as sample filtration using molecular weight cut-off filters and membrane dialysis. For proteomics analysis in positive ion mode, we utilized both top-down and bottom-up approaches. These methods incorporated reversed-phase liquid chromatography (RPLC) coupled with high and low-resolution/accuracy mass spectrometry (MS) to achieve detailed protein analysis.

Methods
Lysis buffer was used for protein extraction from biological sample pellets, whereas milder conditions were necessary for food and cultural heritage samples. During the presentation, various buffer solutions will be illustrated, discussed, and compared in terms of protein recovery. For purification, we utilized dialysis membranes, micro-SPE (both homemade and commercial), and molecular weight cut-off filters. After digestion, samples were analysed by MALDI-MS for peptide mass fingerprinting or by RPLC-MS with properly designed elution gradients.

Results
Protein extraction from serum using a combination of lysis buffer and SDS-PAGE yielded excellent results for protein identification. For novel food sources such as microalgae, we proposed a fast one-pot protocol for proteome characterization. In contrast, conventional protocols based on Tris-HCl buffer showed higher reproducibility for quantifying hidden allergenic proteins. For cultural heritage samples, minimally invasive methods like in situ digestion were effective in identifying proteinaceous binders. However, distinguishing among animal species, such as bovine or rabbit glue, required more extensive preparation, including the use of an alkaline solution, dialysis membranes, and on-filter digestion. All data were collected using MALDI-MS and/or RPLC-ESI-FTMS. Coupled with the optimal preparation protocol, these techniques effectively addressed specific analytical challenges.

Conclusions
The critical aspect of proteomics lies in the extraction and purification of proteins from samples. This work underscores that the composition of buffers and the methods of purification are not one-size-fits-all but must be tailored to the specific nature of each sample. Different combinations of techniques are carefully selected and applied to each type of sample we examined.

ACKNOWLEDGMENTS
This work was supported by the project 2023-UNBACLE-0241869 - NOVAFIND, financed by Uniba ERC SEEDS

REFERENCES
[1] Manoj K., Maharishi T., Jayashree P., et al., (2021). Advances in the plant protein extraction: Mechanism and recommendations. *Food Hydrocolloids,* 115, 106595

Plant labelling with $^{13}CO_2$ to demonstrate root exudates trapping with non-common sampling

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Keywords: root exudates, GC-HRMS, labelling, rhizobox, metabolomics

Objective
The knowledge of the metabolites released by plant roots via root exudation is a major factor in understanding biotic interactions. The lack of knowledge about the composition of root exudates is due to the difficulty of sampling and the complexity of these matrices. At present, most studies carried out are based on soilless experiments, which do not allow access to the processes resulting from interactions with the soil and surrounding species [1]. Our study was focused on the optimization of a trapping device to collect root exudates in soil.

Methods
To consider interactions with the soil matrix and not to disturb exudation, the growth method used in our study was a rhizobox system and an original collection method using sorption filters in contact with root tips, thus providing an environment as close as possible to that found naturally [2]. Also, to make sure that compounds retrieved result from root exudation, a step of plant labelling with $^{13}CO_2$ was performed. After optimizing sample preparation using experimental designs, non-targeted analyses by gas chromatography coupled to Orbitrap-type high-resolution mass spectrometry (GC-HRMS) were carried out to identify labelled compounds released by roots. To do so, sorption filters were placed for one hour on root tips before and after plant labelling (16 h and 22 h after labelling) and were then analyzed. Comparison of isotopic patterns was performed to identify labelled compounds.

Results
A list of targeted compounds, containing small organic acids, amino acids, phytohormones, phenolic compounds and sugars, supposed to be exuded by roots was selected. These compounds were specifically searched with EIC (Extracted Ion Chromatogram) and a comparison of isotopic patterns between specific ions was performed, before and after labelling A discussion on the exactitude of isotopic pattern obtained with Orbitrap analyzer is proposed. For 6 sugars, a significant increase of the ratio between the main ion (containing 100% of ^{12}C) and the ones containing one, two or three ^{13}C was observed. This clearly demonstrates that these sugars came from root exudation.

Conclusions
This study demonstrates that sorption filters can successfully trap root exudates with conditions close to the natural ones. Moreover, the use of GC-HRMS-Orbitrap turned out to be efficient to observe labelled compounds under some precautions. This method should be used to evaluate the influence of microorganisms, fauna and growth conditions on exudate composition, quantity and kinetic of exudation.

ACKNOWLEDGMENTS
The authors would like to thank Charles Lanel, former glass-blower at the Université de Rouen, for designing and manufacturing the rhizoboxes. Also, we would like to thank Antoine Levasseur, former internship at ECODIV laboratory for everything related to the plant labelling. VB is supported by a doctoral grant from the Université de Rouen Normandie and the EDNC.

REFERENCES
[1] E. Oburger et D. L. Jones, (2018). Sampling root exudates – Mission impossible?, *Rhizosphere*, 6, 116-33
[2] G. Neumann, S. Bott, M. A. Ohler, H.-P. Mock et K. Smalla, (2014). Root exudation and root development of lettuce (Lactuca sativa L. cv. Tizian) as affected by different soil, *Front Microbiol*., 5.

Fast screening method for food and natural products analyses by solid-phase-microextraction−transmission mode (SPME-TM) and direct analysis in real time mass spectrometry (DART-MS)

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Keywords: food, natural products, bioactive compounds, SPME-TM, DART-QDa

Objective
The goal of the study was the fast screening and evaluation of bioactive compounds in complex matrices by a reliable and compact analytical instrumentation. Specifically, a solid-phase microextraction-transmission mode (SPME-TM) coupled to a MS system, via direct analysis in real time (DART), was employed as effective tool for the rapid determination of target analytes in natural products and food.

Methods
Edible vegetable oils, medicinal herbs and spices, precious products regularly target of food fraud, were analyzed by an Ambient Mass Spectrometry (AMS) approach, consisting of a DART ionization source connected to a single quadrupole (QDa). Moreover, the system was integrate of sampling devices (SPME-TM) to provide rapid, precise, and accurate results that enable a targeted analyses of bioactive compunds presents in the complex matrices.

Results
DART-QDa method was employed in the present work, as user-friendly and low impact environment instrumentation set-up for untargeted metabolome screening of food and natural products, with the aim to differentiate the samples by means of data statitical analysis. The statistical models developed by applying Principal Component and Linear Discriminant Analyses (PCA/LDA) led to clusterization and classification of the samples according to the geographical origin, cultivar or species, and identification of unknown samples by matching with the database built. In addition, for a qualitative and semi-quantitative evaluation a targeted approach based on the use of SPME-TM for isolation and characterization of bioactive compounds.

Conclusions
The present study employed DART-MS and SPME-TM-DART/MS as fast and reliable methods to be used for preserving the biodiversity and local economies though the rapid screening of the total metabolome of different food and natural products. Both methods led to satisfactory results in terms of rapid identification reliability of unknown samples, and their fast qualitative and semi-quantitative evaluation, confirming the versatile use of AMS for routine control analysis, and safeguard of products against fraudulent activities when employed in combination to multivariate statistical analysis.

ACKNOWLEDGMENTS

The authors acknowledge the Waters Corporation and Merck Life Science for their support. The present research was supported by the Ministry University and Research within project PRIN PNRR –titled: Fast, Reliable and Innovative analytical approaches for the END-to-end quaLity and safetY of spices and aromatic herbs - FRIENDLY, Prot. P2022CAJHW, CUP: J53D23014550001.

Multidimensional preparative gas chromatography as a sustainable analytical approach for the isolation of target analytes prior to biological assays

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Keywords: Multidimensional Gas Chromatography, Preparative Gas Chromatography, volatiles, biological assays

Objective
The interest in terpene components is growing, strictly related to their multiple biological activities, which made them a valid resource for the pharmaceutical, nutraceutical, and food industries. Among this class of compounds, *β*-caryophyllene (BCP), is of primary importance, due to its antioxidant, anti-inflammatory, and ant-hyperglycemic effects, as well as its dietary availability through the consumption of edible plants, including spices. In this research, essential oils having a consistent amount of BCP, obtained from spices, were subjected to preparative gas chromatography to isolate target fractions with and without BCP in order to be assessed in terms of biological activity.

Methods
In this study, a preparative multidimensional gas chromatographic system was exploited, coupled to MS detection, and equipped with wide-bore capillary columns. Biological assays were carried out to evaluate the activity of the fractions collected.

Results
In the first step, the spice's essential oil and the BCP activity were evaluated. In a second step, target terpenes were isolated through preparative gas chromatography, aimed to evaluate possible synergic actions between target terpene components. Preliminary biological tests highlighted that the sesquiterpenes fraction is more active than BCP, able to demonstrate possible synergic effects.

Conclusions
This study demonstrated the efficacy of a combined approach involving gas chromatography and biological studies in elucidating the relationship between biological activity and specific sample components, isolated from preparative gas chromatography.

ACKNOWLEDGMENTS
The project was funded by the Italian Ministry for the University and Research (MUR) with a "PRIN: Progetti di ricerca di rilevante interesse nazionale Bando 2022" Project n. 2022359CZA "Advanced analytical characterization and synergic evaluation of volatile terpenes of selected β-caryophyllene containing spices for diabetes treatment".

Developing fuel property prediction models using comprehensive two-dimensional gas chromatography

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Keywords: sustainable aviation fuel, Two-dimensional Gas Chromatography, chemometrics, machine learning

Objective
Sustainable aviation fuels (SAFs) are crucial to mitigate the carbon emissions associated with the aviation industry. The Fischer-Tropsch (FT) process offers a promising approach for SAF production from non-petroleum sources [1]. However, the complex composition of SAFs poses analytical challenges. Using two-dimensional gas chromatography with mass spectrometric detection (GC×GC/MS) proves highly effective in characterizing SAFs. Composition-property-based prediction models serve as a cost and time-efficient pre-screening strategy in SAF development [2]. This project aims to develop an analytical method for SAF characterization and prediction models using GC×GC/MS and machine learning.

Methods
A total of 36 samples, including surrogates and 35 blend samples, were analyzed using cryogenic modulation comprehensive GC×GC/MS with a reversed column configuration (mid-polar 1D column, nonpolar 2D column). Chromatograms were processed with GC Image software for compound identification and relative quantification. Sample compositions were characterized by compound group class and carbon chain length. For predictive model development, training and test sets were created using multivariate regression, with carbon chain lengths and compound classes as predictors and externally measured fuel properties as predicted variables.

Results
The test and validation datasets were mean-centered before applying multiple linear regression. Venetian blind cross-validation was utilized to ensure accurate representation of the samples in the model validation process. The test set predictions indicated that the models for heating value and density were acceptable. However, further exploration of other chemometric techniques is necessary for predicting additional properties.

Conclusions
The creation of predictive models based on fuel composition would enable effective evaluation of SAF quality and suitability. This approach leverages chemical information to optimize fuel formulations, meet regulatory standards, and enhance fuel performance.

ACKNOWLEDGMENTS

Funding of this project by the FFG under Project no FO 999892264 (‘Max Power to Jet’) and the OMV Downstream GmbH are gratefully acknowledged.

REFERENCES

[1] H. Wei et. al., (2019). Renewable bio-jet fuel production for aviation: A review, *Fuel,* 254, 115599
[2] X. Shi et. al., (2017). Quantitative composition-property relationship of aviation hydrocarbon fuel based on comprehensive two-dimensional gas chromatography with mass spectrometry and flame ionization detector, *Fuel*, 200, 395-406

Selective isolation of chemicals by a customized GC×GC setup

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Keywords: GC×GC, selective isolation, wine

Objective

A novel approach capable of isolating and recollecting nearly any molecule from complex GC×GC chromatograms is presented [1].

Methods

This result was achieved by customizing a GC×GC-Q-TOF system with a Deans switch, a passive splitter, and careful adjustments of flows and capillary dimensions. The setup was evaluated with more than a hundred standard chemicals covering a wide range of volatility (boiling points: 56 – 343 ºC) and polarity (log P: -0.2 – 9.4).

Results

We found that recovery from the initial sample to the isolated sample can become highly efficient if a custom-made adapter is attached directly on the FID port (average recovery rate of 76 ± 7%). Furthermore, we could achieve an isolation down to a minimum distance of 50 ms between baseline separated eluting peaks. In addition, the setup was designed for easy adaptation by repurposing existing instrument control software to define the isolation windows for the compounds of interest (first and second column retention time windows).

Conclusions

We expect this novel development to allow several new applications, e.g., the isolation or selective enrichment of molecules in food and flavour analysis; the investigation of suspect chemicals (incl. unknowns) for effect directed analysis (e.g., bioassays), and the isolation of chemicals for further chemical analysis. An illustrative example relevant to the headspace analysis of a spoiled wine lot is shown.

REFERENCES

[1] F. Di Francesco, M. Ripszam, T. Bruderer, F. M. Vivaldi, EP patent request: 23163823.0, filed on 23/3/2023

Development of HILIC×RP-LC platforms for quali-quantitative screening of bioactive compounds in food and natural products

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Keywords: HILIC×RP-LC, bioactives, gas chromatography, mass spectrometry, green sample preparation.

Objective

Natural products are the chemical ingredients synthesized by living organisms and have been extensively utilized by human beings as a primary source for food, and curative remedies. In the last decades there has been a tremendously high amount of research that has carried out on natural herbs for the search and development of novel therapeutic agents beneficial for human health without or with least side effects. The potential benefits associated to natural products are related to secondary metabolites which exert various pharmacological effects on human health. In general, liquid chromatography (LC) coupled with tandem mass spectrometry is the most employed technique for the investigation of the phytochemical components of complex samples. In some cases, one-dimensional (1D) approaches do not often provide the resolving power and selectivity required for the analysis of this samples. Consequently, this research aims to develop innovative HILIC×LC methods for determination of bioactive compounds in food and natural products.

Methods

HILIC×RP-LC methods were explored to improve separation of bioactive compounds with enhanced practical peak capacity and orthogonality with respect to the current conventional available methodologies. In addition, a quantification approach was carried out through external calibration curves and the method was validated yielding satisfactory LODs, LOQs, intraday and interday precision and recovery values.

Results

The platform, involving the combination of a ZIC-HILIC column in the ^{1}D and a C18 in the ^{2}D, allowed high separation capability with values of practical peak capacity over 1000 and orthogonality as high as 0.80. In all sample investigated more than one hundred different polyphenolic compounds were detected and positively identified by using complementary information from PDA, MS/MS, and literature data information. The employment of a "focusing" modulation procedure with two C18 trapping columns allowed to mitigate the solvent mismatch thus providing an effective "peak focusing" at the head of the ^{2}D column.

Conclusions

The HILIC×RP-LC platforms investigated turned out to be a valuable method for the characterization of various food and natural products samples. Further, their polyphenolic characterization is of valid aid to confirm their potential use for the human health.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Shimadzu Corporation and Merck Life Science for their continuous support.

Application of a Doehlert design in flow-modulated comprehensive two-dimensional gas chromatography

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Keywords: Flow-modulated Comprehensive Two-dimensional Gas Chromatography, Doehlert design, response surface methodology

Objective

The past few years has seen a renewed importance in a flow modulation technology for comprehensive two-dimensional chromatography (FM-GC×GC). This is supported by marketing of improved instrumentation configurations, namely a modulator based on Capilary Flow technology (CFT), INSIGHT^{TM} modulator and FLUX^{TM} modulator. A key issue regarding to FM-GC×GC is more complicated assignment of experimental conditions in comparison to thermal modulation. This study was focused on multivariate optimisation of experimental parameters for efficient analysis of volatile organic compounds with the selected technique.

Methods

Flow modulated GC×GC-MS/FID analysis was performed on an Agilent 7890A GC (Agilent 124 Technologies, USA) equipped with a reverse fill/flush modulator (Agilent Technologies, USA). In the first dimension, a non-polar stationary phase column HP-5 (30 m × 0.25 mm × 0.25 µm, Agilent, USA) was used, in the second dimension, a middle-polarity BPX50 column (2 m × 250 µm × 0.25 µm) and polar columns as Stabilwax (2 m × 250 µm × 0.25 µm) and SLB®-IL60 (2 m × 250 µm × 0.2 µm) were applied. The experimental conditions varied in the range from 0.3 to 1.0 mL/min for 1D flow, from 2 to 5 s for modulation time and from 10 to 30 mL/min for 2D flow. A combination of experimental parameters was set accordingly to a Doehlert design. The bleeding capillaries (2 m × 100 µm, 1.5 m × 100 µm) were attached to the modulator to support the carrier gas direction. A value of the flush time of the modulator was calculated accordingly to a restrictor pneumatic calculator. A 0.5 m ×100 µm restrictor and a 1.2 m × 250 µm restrictor were connected to quadrupole MS and FID, respectively.

Results

Firstly, a length of the 2D column was selected, based on a number of identified compounds and coelutions, a symmetry and width of peaks for both dimensions. Secondary, different combinations of modulation time, flows in the first and second dimensions were tested for better separation of volatile compounds with a 2 m secondary column. The obtained results were calculated with response surface methodology to define optimum conditions. The best results were observed for BPX50 column for the peak tailing, however a number of coelutions and a percentage usage of the separation space were problematic in this case. The sufficient resolution of problematic coelutions was achieved with application of a polar Stabilwax column. A separation of linalool and 2-nonanol were incomplete for all experimental conditions.

Conclusions

The optimal experimental conditions were selected for all columns setups and tested for the analysis of the real wine sample. However, the results for real samples were challenging, due to the presence of compounds at different concentration levels. Thus, the further optimisation of separation conditions should be carried out to achieve better results in terms of peak tailing and symmetry.

ACKNOWLEDGMENTS

This project has received funding from Slovak Research and Development Agency under Contract No. APVV-21-0211 and from Grant Agency of the Ministry of Education of Slovak Republic VEGA 1/0298/23.

Comprehensive two-dimensional gas chromatography–mass spectrometry combined with advanced chemometrics as a powerful tool in the exploration of food authenticity

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Keywords: authenticity, volatilomics, GC×GC, wine aging, food fraud

Objective

Omics is used as an analytical tool to investigate authenticity issues. The untargeted exploration of the volatile fingerprint of foods is currently known as “volatilomics”. GC × GC coupled to mass spectrometry (GC × GC–MS) is considered a powerful technique for the profiling of the volatile constituents. The further processing of the analytical data with chemometric tools improves the breadth of the analysis.

Methods

Solid-phase microextraction (SPME) and SPME-Arrow methods combined with GC × GC–MS and supervised chemometric tools were optimized to adress critical authenticity issues, such as wine aging [1], pomegranate juice adulteration [2], and honey varietal classification [3].

Results

The coupling of SPME-Arrow with GC × GC–MS and advanced chemometrıcs enabled the classification of Protected Designation of Origin (PDO) wine samples belonging to the Xinomavro variety with 100% accuracy according to the vintage age. In pomegranate analysis, a robust prediction model was developed to distinguish characteristic markers that could reveal pomegranate juice adulteration with inferior juices at 1% level. The utilization of SPME Arrow fibers enabled the determination of a signifcantly large number of compounds that define the organoleptic properties of honeydew, flower honeys, and pine honeys.

Conclusions

GC × GC–MS coupled to chemometrics is a powerful tool which opens completely new and hitherto unexplored possibilities for food authenticity testing and confirmation.

REFERENCES

[1] Kalogiouri N.P., Manousi N., Ferracane A., Zachariadis G.A., Koundouras S., Samanidou V.F., Tranchida P.Q., Mondello L, Rosenberg E. (2024). A novel headspace solid-phase microextraction arrow method employing comprehensive two-dimensional gas chromatography–mass spectrometry combined with chemometric tools for the investigation of wine aging, *Analytica Chimica Acta*, 1304, DOI: 10.1016/j.aca.2024.342555

[2] Kalogiouri N.P., Ferracane A., Manousi N., Zachariadis G.A., Tranchida P.Q., Mondello L, Samanidou V.F., Rosenberg E. (2024). A volatilomics analytical protocol employing solid phase microextraction coupled to GC × GC-MS analysis and combined with multivariate chemometrics for the detection of pomegranate juice adulteration, *Talanta*, 266, 2, 12502.

[3] Manousi N., Kalogiouri N.P., Ferracane A., Zachariadis G.A., Samanidou V.F., Tranchida P.Q., Mondello L, Rosenberg E. (2023). Solid-phase microextraction Arrow combined with comprehensive two-dimensional gas chromatography–mass spectrometry for the elucidation of the volatile composition of honey samples, *Analytical and Bioanalytical Chemistry*, 415, 13, 2547-2560. DOI: 10.1007/s00216-023-04513-0

Exploring the flavour profiles of gin using high-capacity sorptive extraction and GC×GC–TOF MS

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Keywords: high-capacity Sorptive Extraction probes, GC×GC–TOF MS

Objective
Gin is a distilled alcoholic beverage with a complex flavour profile, primarily derived from juniper berries and an array of botanicals such as citrus peels, angelica seeds and various spices. The blend of these botanicals creates a multifaceted flavour profile. Unfortunately, traditional analytical techniques often fall short in capturing the full spectrum of volatile and semi-volatile compounds present.
In this study we demonstrate the use of high-capacity sorptive extraction probes in combinatio with Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) as a powerful analytical tool for mre detailed characterization and classification of gin products.

Methods
In this study, high-capacity sorptive extraction probes, offering a larger volume of stationary phase (65 µL) compared to traditional solid-phase microextraction (SPME) (~0.5 µL), were utilized to enhance sample loadings in combinatio with trap-based focusing. Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF MS) was used for enhanced separation.

Results
The use of, high-capacity sorptive extraction probes combined with trap-based focusing, this approach provides improved sensitivity and enhanced chromatographic performance. However, improved extraction capability often results in more complex chromatograms. GC×GC-TOF MS provides superior separation power and allows confident identification of compounds that would co-elute in conventional 1D GC. This approach applied enabled the detection of over 250 distinct peaks in dry gin and up to 400 in flavoured gins. Tile-based chemometrics allowed to automatically identify unique chemical markers across six different gins, including those from the same brand and those with similar flavour descriptions.

Conclusions
In this contribution we show how sorptive extraction and GC×GC-TOF MS can be leveraged for the comparison of gin flavour profiles to help innovate, refine, and tailor products to meet consumer demands.

Greening the downstream of biopharmaceuticals through process intensification and the use of innovative eco-compatible solvents

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Keywords: Liquid Chromatography, green solvents, process intensification, biopharmaceuticals, downstream processing

Objective
Single-column preparative liquid chromatography in reversed-phase (RPLC) mode is the most widely used approach for purifying target biopharmaceuticals from their impurities. During upstream processing, the desired biomolecule is not the only product formed; a series of unwanted product-related impurities are also generated. These impurities have similar chemical and physical properties, as well as chromatographic behavior, to that of the target. As a result, more than one chromatographic step is often required, leading to a significant volume of waste solvent, primarily composed of acetonitrile (ACN), which needs to be disposed of. This solvent is toxic to humans and the environment, prompting the biopharmaceutical industry to seek greener approaches to purify peptides and other biopharmaceuticals.

Methods
In recent years, we have focused on two approaches to enhance the sustainability of biopharmaceutical purification. The first approach involves replacing ACN, which is toxic to humans and the environment, with greener alternatives. The second approach utilizes advanced multi-column countercurrent preparative liquid chromatography platforms, which enable the recycling of overlapping regions of the chromatogram within the system. This mechanism is advantageous not only for automating the purification process but also for significantly reducing solvent consumption.

Results
Among green solvents, we have tested not only alcohols (ethanol and isopropanol) but also dimethyl carbonate (DMC), which is ranked among the greenest candidates by Solvent Selection Guides, but it has been barely applied as organic modifier in liquid chromatography [1,2]. This solvent s characterized by a high elution strength which allows to elute analytes with minimal organic modifier percentages.
As far as regards process intensification, it will be shown that by employing Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) for the purification of Icatibant (a small therapeutic peptide composed of 10 amino acids), it can be reduced by more than 80% with respect to the correspondent single-column process [3].

Conclusions
These findings suggest that these techniques, coupled with the utilization of green solvents and chemicals, have the potential to revolutionize downstream processing entirely.

REFERENCES
[1] Felletti S., Spedicato, M., Catani M., et al. (2023). Dimethyl carbonate as a green alternative to acetonitrile in reversed-phase liquid chromatography. Part I: Separation of small molecules, *J. Chromatogr. A*, 1712, 464477
[2] Bozza D., De Luca C., Catani M. et al. (2023). Dimethyl carbonate as a green alternative to acetonitrile in reversed-phase liquid chromatography. Part II: Purification of a therapeutic peptide, *J. Chromatogr. A*, 1713, 464530.
[3] De Luca C., Felletti S., Catani M., et al. (2021). Process Intensification for the Purification of Peptidomimetics: The Case of Icatibant through Multicolumn Countercurrent Solvent Gradient Purification (MCSGP), *Ind. Eng. Chem. Res.*, 60, 6826-34

Liquid-liquid chromatography as a separation method of natural products

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Keywords: Liquid-liquid Chromatography, Countercurrent Chromatography, Centrifugal Partition Chromatography

Objective

Liquid-liquid chromatography (LLC), i.e. countercurrent chromatography (CCC) and centrifugal partition chromatography (CPC) is a preparative separation method used in the isolation of phytochemicals from complex extracts [1]. In this short overview, the main principles of LLC techniques, scalability and advantages of their application are presented and exemplified by the targeted isolation of bioactive molecules from *Petasites hybridus* (butterbur).

Methods

In the first part the main principles of LLC, such as the concepts of partition coefficients or two-phase solvent systems and advantages over classical liquid-solid chromatography are presented.

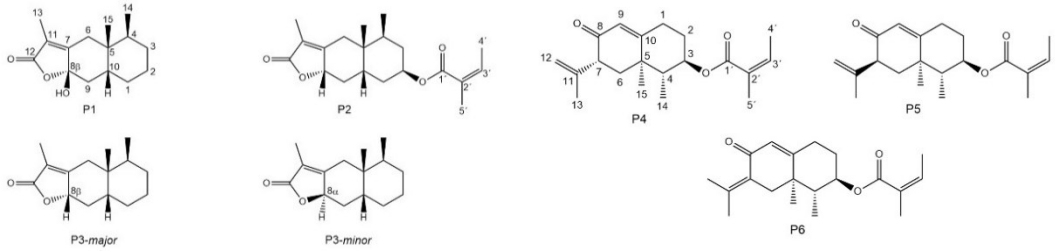

Fig. 1. Structures of isolated petasins from P. hybridus

In the second part, the path from determining optimal separation conditions to the LLC separation procedure is described based on the results from the targeted isolation of petasins from *P. hybridus* for pharmacological evalution (**P1**-**P6**). Strategies towards identifying molecular mechanisms related to anti-migraine, anti-asthmatic, and anti-inflammatory activities of petasins [2] are presented.

Conclusions

The use of LLC for the targeted isolation of phytochemicals provides advantages over conventional liquid-solid chromatography, such as the absence of irreversible adsorption, which allows recovery of the injected sample; reduced solvent consumption; relatively lower time and labor costs, as expensive solid stationary phases or column packing procedures; or the ability to inject raw samples containing compounds with a wide range of polarity [3]. LLC techniques as interesting alternatives for more widespread conventional liquid-solid chromatography in the natural products separation, e.g. plant extracts suitable also for industrial scale isolations.

ACKNOWLEDGMENTS

This study work was financially supported by the National Science Centre, Poland, no 2022/45/N/NZ7/02294.

REFERENCES

[1] K. Skalicka-Woźniak, I. Garrard, (2015) A comprehensive classification of solvent systems used for natural product purifications in countercurrent and centrifugal partition chromatography, *Nat. Prod. Rep*. 32, 1556–1561

[2] Ł. Kulinowski, S.V. Luca, M. Minceva, K. Skalicka- Woźniak, (2022) A review on the ethnobotany, phytochemistry, pharmacology and toxicology of butterbur species (Petasites L.), *J. Ethnopharmacol*., 115263

[3] R. Morley, M. Minceva, (2021) Liquid–liquid chromatography: current design approaches and future pathways, *Annu. Rev. Chem. Biomol. Eng*. 12, 495–518

Preparation of monolith for online extraction and LC–MS analysis of β-estradiol in serum via a simple multicomponent reaction

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Keywords: catalyst supports, on-line extraction, multicomponent reactions, human serum

Objective
This study aims to demonstrate the efficacy of utilizing multicomponent reactions to efficiently and environmentally friendly prepare monoliths suitable for analytical chemistry applications.

Methods
A multicomponent reaction was employed for the one-pot miniaturized synthesis of a poly(propargylamine) polymer within commercially available silica-lined PEEK tubing. The reaction utilized minimal reagents and was characterized by high atom economy. The resulting monolithic column was integrated into an autosampler system for online extraction and cleanup of β-estradiol from human serum. Sample pretreatment involved a simple dilution with methanol followed by centrifugation to remove proteins.

Results
The developed platform enabled LC-MS analysis in multiple reaction monitoring mode for quantitative determination of β-estradiol. Method validation in serum samples showed practical applicability for monitoring fertile women, with recoveries above 94% and LOD and LOQ values of 0.008 and 0.18 ng mL^{-1}, respectively. Comparative analysis with previous methods for solid-phase microextraction of β-estradiol in serum revealed comparable recovery and sensitivity, but with the added advantage of nearly complete automation.

Conclusions
The environmental impact assessment of the process indicated acceptable levels due to the miniaturization of monolith synthesis and extraction automation. The limitation associated with LC-MS technique could be mitigated by including additional analytes in a single investigation. This work underscores the versatility, economy, and potential green nature of multicomponent reactions for producing reversed-phase and mixed-mode sorbents, facilitating miniaturization of the entire analytical procedure from sorbent preparation to analysis.

REFERENCES
[1] A. Cerrato, S. E. Aita, C. Cavaliere, A. Laganà A, C. M. Montone, S. Piovesana, E. Taglioni, A. L. Capriotti, (2024). Preparation of Monolith for Online Extraction and LC–MS Analysis of β-Estradiol in Serum Via a Simple Multicomponent Reaction, *Anal. Chem*, 96, 11, 4639–4646

Microwave-assisted extraction: a sustainable approach to fatty acid production from wheat bran

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Keywords: fatty acid, vegetable oil, extraction, circular economy

Objective

Aiming to protect natural resources and promote a circular economy, the present project focuses on the valorization of by-products from specific agricultural crops through sustainable practices. The primary goal is to develop eco-friendly surfactants by chemically conjugating natural hydrophilic substances extracted from discarded chicory roots with hydrophobic substances derived from by-products of maize starch and flour production. Given the significant role of fatty acids in human health and industrial applications, their sustainable production is crucial. Polyunsaturated fatty acids (PUFAs) are essential for maintaining health and preventing degenerative diseases in adults. Conversely, saturated fatty acids (SFAs) are predominantly used in industrial applications as raw materials for oleochemicals, alternative fuels, lubricants, and coatings. [2] Traditional methods of producing monoglycerides from vegetable oils, rich in triglycerides, involve high-temperature chemical glycerolysis, [3] which is energy-intensive and yields lower-quality products. Thus, exploring alternative methods for extracting and synthesizing fatty acids and monoglycerides from vegetable oils is necessary. Preliminary extractions using microwave irradiation offer a clean, efficient method with higher yields in shorter times compared to conventional heating. [4]

Methods

Fatty acids were extracted and isolated from wheat bran using an alkaline hydrolysis method assisted by microwave heating. The initial method involved heating wheat bran suspended in NaOH solution, followed by filtration and liquid-liquid extraction with n-hexane. The fatty acids were then isolated by acidification and further extraction with n-hexane. To improve the process, an alternative method was developed where wheat bran was treated with ethanol and KOH. The resulting solution underwent liquid-liquid extraction with n-hexane and the crude extract was purified via flash chromatography affording the pure fatty acids mixture. The samples were then esterified and analysed through GC-MS.

Results

The proposed procedure yielded reliable and reproducible results, as the composition of the fatty acid mixtures remained consistent for all the collected samples. The optimized procedure afforded high yields minimizing the use of organic solvents.

Conclusions

The sustainable extraction and isolation of fatty acids from wheat bran represent a promising advancement in green chemistry and circular economy. The study demonstrates that microwave-assisted extraction, combined with alkaline hydrolysis and optimized solvent use, enhances the efficiency and eco-friendliness of fatty acid production. The successful extraction of valuable fatty acids from waste materials not only adds economic value to low-cost by-products but also contributes to reducing environmental impact.

REFERENCES

[1] Dwi Ardiana Setyawardhani, D. A.; Sulistyo, H.; Sediawan, W. B.; Fahrurrozi, M.; Ariyanto, T., (2019). Solid-liquid equilibrium for binary and ternary phases of saturated fatty acid–urea–alcohol in urea complexation, *J. Chem. Eng. Data* , 64, 5066-78

[2] Nitbani, F. O.; Tjitda, P. J. P.; Nurohmah, B. A.; Wogo, H. E., (2020). Preparation of Fatty Acid and Monoglyceride from Vegetable Oil, *J. Oleo Sci.* , 69, 277-95

[3] Moretto, J. A.; Ossanes de Souza, A.; Berneira, L. M.; Brigagão, L. G. G.; Pereira de Pereira, C. M.; Converti, A.; Pinto, E., 2022. Microwave-Assisted Extraction of Fatty Acids from Cultured and Commercial Phytoplankton Species, *Appl. Sci.*, 12, 2407-15.

A miniaturized method for the simultaneous analysis of vitamin D metabolites and total lipidome in human serum

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Keywords: lipidomic, vitamin D, precision medicine, Comprehensive Two-dimensional GC, UHPLC-MS/MS

Objective
The equilibrium of various lipid molecules found in biological fluids serves as a precise indicator of the organism's health status, enabling healthcare professionals to tailor treatments specifically to individual patients, a strategy referred to as precision medicine. Indeed, within the field of clinical research, numerous instances exist where lipidomic assays have been employed to enhance the comprehension of disease pathophysiology, unearth novel biomarkers, pinpoint potential therapeutic avenues, and evaluate the efficacy of treatment in patients. Specifically, the prevalent issue of vitamin D insufficiency is acknowledged as a global health challenge that is linked to various medical conditions. Nowadays, high pressure liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) is considered the gold standard for the analysis of vitamin D metabolites, and for this reason many clinical laboratories all over the world acquired MS instrumentation. In this contest we developed a comprehensive workflow both for the analysis of fatty acids, through flow-modulated comprehensive gas chromatography coupled to mass spectrometry (FM-GC×GC-MS), and for the fast and simultaneous quantification of vitamin D metabolites and assessment of intact lipids in human serum, by using ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS).

Methods
Fatty acids were identified by using FM-GC×GC-MS/FID. Ultra-high performance liquid chromatography coupled to tandem mass spectrometry was used for the simultaneous quantification of vitamin D metabolites and identification and relative quantification of different intact lipid classes.

Results
The evaluation of specific FA ratios, monitored through FM-GC×GC-MS, allowed to get precious information about the health state of a patient. The quantification of five vitamin D metabolites (vitamin D2, vitamin D3, 25-hydroxyvitamin D2, 25-hydroxyvitamin D3, 24R,25-dihydroxyvitamin D3) was achieved using a MRM method, and validated in term of LoD, LoQ, accuracy and precision. A combination of SCAN, precursor ion scan and neutral loss scan, in both positive and negative mode, was used for the identification of 81 intact lipid species, such as phospholipids, cholesteryl esters and triacylglycerols, in less than 25 minutes.

Conclusions
The data obtained were used to build lipidomics maps for the fast screening of different lipid classes, to easily read possible lipid alterations and possibly correlate them with the general health state of the patient.

ACKNOWLEDGMENTS
The authors thank Merck Life Science and Shimadzu Corporations for their continuous support. The research was conducted within the PRIN 2017 “Cutting-edge analytical chemistry methodologies and bio-tools to boost precision medicine in hormone-related diseases” supported by the Ministry University and Scientific Research, Prot. 2017Y2PAB8a.

A click inverse electron demand Diels-Alder reaction for assigning the regiochemistry of carbon-carbon double bonds in untargeted lipidomics

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Keywords: lipidomics, high-resolution mass spectrometry, hyphenated techniques, prostate cancer, click chemistry

Objective
Lipidomics by high-resolution mass spectrometry (HRMS) has become a prominent tool in clinical chemistry due to the proven connections between lipid dysregulations and the insurgence of pathologies. However, it is difficult to achieve structural characterization beyond the fatty acid level by HRMS, especially when it comes to the regiochemistry of carbon-carbon double bonds, which play a major role in determining the properties of cell membranes [1]. Several approaches have been proposed for elucidating the regiochemistry of double bonds [2], such as derivatization before MS analysis by photochemical reactions, which have shown great potential for their versatility, but have the unavoidable drawback of splitting the MS signal as well as promoting unwanted photo-oxidation reaction products. Based on the physicochemical properties of C=C bonds in fatty acyl chains,

Methods
An IEDDA reaction with 3,6-bis(methoxycarbonyl)-1,2,4,5-tetrazine (BMCTz) was employed for the first time for assigning the regiochemistry of C=C bonds in FA and glycerophospholipids (GP) based on untargeted LC-HRMS and MS/MS spectral annotation. The method was tested and optimized using FA and GP analytical standards and then employed to investigate the role of C=C locations in discriminating serum samples from patients with prostate cancer (PCa) and benign prostate hyperplasia (BPH).

Results
Under the optimized conditions, the reaction has shown click characteristics and satisfying yields, and high coverage was obtained for FA and GP with the location of several uncommon minor regioisomers. Thanks to the proposed IEDDA protocol, the role of lipid regiochemistry in distinguishing PCa from benign prostate conditions was investigated in sera for the first time. Indeed, dysregulations in the n-9/n-7 ratio of high abundance PC 16:0_18:1 were associated with PCa and could represent a viable marker for its early diagnosis. Further studies are needed to confirm the results on larger cohorts.

Conclusions
The IEDDA reaction represents a viable alternative to photochemical reactions that suffer from the splitting of the MS signal when chromatography is employed thanks to its click character and compatibility with mild reaction conditions.

ACKNOWLEDGMENTS
Andrea Cerrato was supported by Fondazione Umberto Veronesi through Post-doctoral Fellowships 2024 - 2 Year Grant.

REFERENCES
[1] Cao W., Cheng S., Yang J., Feng J.; Zhang W., Li Z., Chen Q., Xia Y., Ouyang Z., Ma X., (2020). Large-Scale Lipid Analysis with C=C Location and Sn-Position Isomer Resolving Power, *Nat. Commun.*, 11, 375.
[2] Heiles S., (2021). Advanced Tandem Mass Spectrometry in Metabolomics and Lipidomics-Methods and Applications, *Anal. Bioanal. Chem.*, 413, 5927-5948.

Two birds with one stone: FFF-multidetection as isolation and quality control platform for extracellular vesicles

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Keywords: extracellular vesicles, Flow Field-flow Fractionation, Size Exclusion Chromatography

Objective

Extracellular vesicles (EVs) are nanoparticles released by cells which show great potential in diagnostics and drug delivery. However, EVs exploitation requires isolation and characterization, which represent a challenge due to their high heterogeneity and low concentration. Ultracentrifugation, Size-Exclusion Chromatography (SEC) and ultrafiltration, the most used techniques for isolation, have several drawbacks. In particular they can compromise the structural and biological activity of EVs preventing a native characterization. Hollow-Fiber Flow Field-Flow Fractionation (HF5) is a soft and native separative technique which showed a great potential in the isolation of EVs from serum [1]. Moreover, it can be coupled with several detectors, like spectroscopic, light scattering and fluorescence to assess EVs content and size. In this work we present a bivalent approach based on an HF5-Multidetection platform for EVs isolation from plasma and as a quality control technique in vesicles collection, due to its softness and nativeness.

Methods

EVs from healthy donors' plasma sample were both isolated and collected using a common SEC protocol and the optimized HF5 separative system. The fractions were concentrated through an ultrafiltration step and then re-analysed with the HF5-Multidetection platform obtaining a direct comparison of relative size distribution and amount of the collected EVs. The presence of vesicles in the fractions was confirmed with Western Blot and Flow Cytometry.

Results

SEC- and HF5-derived fractions showed differences in population size and composition. Except for the presence of aggregates in SEC-EVs compared to HF5-EVs, no generalized differences between the two isolation methods were measured, while it emerged a high biological variability from donor to donor. Isolated EVs were also characterized via Western blot and were positive for markers including ALIX, confirming the presence of specific EV markers. In addition, Flow Cytometry demonstrated the expression of EV marker (CD9, CD81) and platelet-associated markers (CD41a, CD42b) in EVs from both techniques.

Conclusions

The HF5 platform demonstrated its ability to isolate efficiently EVs with minimal pressure and damage to vesicles enabling an online characterization in native and soft conditions, which still remains a critical point in the study of vesicles, and thereby helping understand the role of circulating EVs in tumour and healthy microenvironment.

ACKNOWLEDGMENTS

Financed by the European Union – NextGeneration EU through the Italian Ministry of University and Research under PRIN 2022 RESOLVE, 202233FTW8 CUP B53D23013360006

REFERENCES

[1] Marassi, V.; Giordani, S.; Placci, A.; Punzo, A.; Caliceti, C.; Zattoni, A.; Reschiglian, P.; Roda, B.; Roda, A., (2023). Emerging Microfluidic Tools for Simultaneous Exosomes and Cargo Biosensing in Liquid Biopsy: New Integrated Miniaturized FFF-Assisted Approach for Colon Cancer Diagnosis, *Sensors*, *23*, 9432

Relative response factors in gas chromatography: a comparison between multiple detectors

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Keywords: GC, response factors, MS, FID, VUV

Objective
When it comes to gas chromatographic analysis, we usually tend to consider MS as the reference detector to identify the analytes, while FID for their quantification. This is because the molecules do not respond in the same way to different detectors, and due to the analogue response of FID, the analytical signal is more linear to the concentration and the dynamic range maintains the linearity over a range of higher concentration.
If the analysis goal is the chemical elucidation of a given sample, the response normalization of the single analytes on the total detected signal (area %), is an effective and common way to express the chemical distribution of within a given sample.
This is reasonable and accurate with a detector as FID, whose response is fairly consistent between different compounds or at least predictable through the calculation of relative response factors (RRFs). Different approaches have been developed for their calculations, among which the effective carbon number (ECN)[1].
Applying total response normalization when using an MS detector is generating an error, especially if different chemical classes are considered: this is due to the different responses of molecules in relation to their different structure. In addition, predicting the RRFs is not yet demonstrated.

Methods
In this contribution the relative response factors of 35 compounds with different chemical structure (n-paraffins, i-paraffins, olefins, naphthenes, aromatics, and N/O-containing compounds) were calculated for GC coupled to different detectors: FID, qMS, TOFMS, and VUV.

Results
Using on-column injection, it was possible to understand the different responses of the various detectors for each class of compounds. In addition, it was studied the inlet splitting influence on RRFs, through the comparison of on-column and split injection analyses.

Conclusions
The understanding of the relationship between different detectors' response, allows a more reliable quantitative characterization of samples. Moreover, this study sets a basis for a pseudo-absolute quantification capability of multiple detectors.

REFERENCES
[1] J.T. Scalon, D.E. Willis, Journal of Chromatographic Science, 1985, 23, 333-340.

Novel strategy based on liquid electron ıonization (LEI) interface for targeted and untargeted analysis in a forensic application

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Keywords: Electron Ionization, Liquid Chromatography, LEI, High-resolution Mass Spectrometry

Objective
Carbofuran is a carbamate broad-spectrum systemic insecticide, nematicide, and acaricide. Due to its toxicity, the European Union banned carbofuran in 2008; however, it is still available in various countries. Recently, cases of animal poisoning occurred in the Czech Republic, but the origin of this pesticide remains unknown. In this study, different carbofuran formulations from countries where it is still available have been characterized to determine their provenance. The analyses were conducted using a Liquid Electron Ionization (LEI) interface coupled with a quadrupole time-of-flight (Q-TOF) mass spectrometer. The LEI interface enables the coupling of liquid chromatography (LC) with electron ionization (EI), providing reliable and highly informative spectra for low volatile and thermolabile molecules

Methods
In this study, different carbofuran granular and liquid formulations were analyzed using LEI-QTOF-MS/MS. For compound extractions, granules were immersed in ultrapure water and sonicated for 10 minutes. The extracts were then filtered using syringe filters, while liquid formulations were diluted in methanol (MeOH) and filtered. For real samples, one liver from an eagle, and the gastric content of a fox and a red kite, were extracted using a modified Accelerated Solvent Extractor (ASE). Reverse phase liquid chromatography (RPLC) separation was performed using a Kinetex column (XB-C18 150x2.1, 1.7 µm) with a gradient elution (5% B hold for 1 min; 5-100% B in 6 min; 100% B hold for 15 min) using ultrapure water + 0.1% formic acid (A) and ACN + 0.1% formic acid (B). The analyses were performed in Scan mode (mass range m/z 85-600) at 70 eV, and low energies (9 and 15 eV), as well as in MS/MS mode.

Results
The carbofuran formulations were analyzed in scan mode, and coeluted compounds were separated by deconvolution. In addition to carbofuran, different compounds were detected in three samples, although carbofuran remained the most abundant component in most cases. The other compounds were investigated and identified using the NIST library and MS/MS high-resolution (HR-MS/MS) fragmentations. The low energy analysis provided prospective molecular ions with an increase in their relative abundance by up to 68%. These ions were used as precursor ions for MS/MS analysis. The structures of the unknown compounds were determined using HR-MS/MS fragmentations with high scores up to 80 and good accuracy below 5 ppm. Real samples were analyzed using the same method and compared with the formulation results.

Conclusions
Combining EI with HRMS using LEI-QTOF-MS/MS allowed for the identification of all compounds detected in the samples, establishing a user-friendly identification method. Because EI is not affected by matrix effects, deconvolution enabled the obtaining of spectra of coeluted compounds necessary for their identification.

ACKNOWLEDGMENTS

The authors thank Agilent Technologies for providing analytical instrumentation and Syngenta UK for financial support.

Analyses of airborne particulate matter in recycling centers using advanced mass spectrometry

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Keywords: particulate matter, Mass Spectrometry, airborne

Objective
Organic dust exposure levels in Material recycling facilities (MRF) were found to be 3.3 orders of magnitude greater than current World Health Organisation limits, raising concern for worker health [1]. To evaluate the dust and chemical exposures for workers, we conducted a four-week sampling campaign and analysed samples using a two-step analytic framework using two-dimensional gas chromatography-time-of-flight-mass spectrometry (GC×GC-TOF-MS). Airborne dust concentrations were 1 order of magnitude greater than ambient concentrations. Known carcinogenic, mutagenic, and endocrine disrupting compounds were detected. Statistical analysis revealed consistent site and temporal chemical variations across the waste management sites. This raises the need for further comprehensive studies to assess ongoing chemical exposures for workers in the sector.

Methods
An active respirable (particulate matter below 4µm aerodynamic diameter; PM4) airborne sampling campaign was conducted at two material recycling facilities, resulting in the collection of one hundred and forty-three PM samples. Quartz filters were pre-conditioned, weighed to determine PM mass, sub-sampled, and analysed using a comprehensive two-step GC×GC-TOF-MS method. Further analysis using high-resolution GC×GC-TOF-MS enabled formula determinations and additional compound annotations. Non-target assignments were performed using the NIST M/Z chemical library, with chemical assignments filtered based on retention index and m/z similarity. Of the thousands of chemical features detected, a list of 30 known carcinogenic, mutagenic, endocrine disrupting compounds were chosen. The samples were statistically compared to assess both site homogeneity and temporal differences using Permutational Multivariate Analysis of Variance and Non-metric Multidimensional Scaling.

Results
The median airborne dust concentration from site one and two were 1168.3 µg/m³ (LQ: 561.92 to HQ: 2386.48 µg/m³) to 178.9 µg/m³ (LQ: 101.3 to HQ: 285.3 µg/m³), respectively. Time-weighted average exposure values for site one and two were 2531.3 and 283.7 µg/m³. Median polymeric compounds were found to range from 62.5 ng (LQ: below limit of detection to HQ: 112.4 ng/m³) to 24.1 ng (LQ: below limit of detection to HQ: 56.7 ng/m³). Statistical analysis revealed consistent site and temporal chemical variations across waste management sites. The type of waste accepted, particularly construction and demolition waste, significantly influenced chemical diversity at the sites. High resolution-GC×GC-TOF-MS analysis allowed a significant number of harmful chemicals to be identified and/or confirmed.

Conclusion
This diversity of chemicals identified raises concern for worker health warranting further comprehensive studies to validate findings across the sector.

ACKNOWLEDGMENTS

AXA Research Fund funded this research project. LECO Instruments for technical support.

REFERENCES

[1] Stagg, et al. *Health and safety Executive* 2013.

Potential of nano-liquid chromatography for the analysis of biomolecules and contaminants

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Keywords: Nano-liquid Chromatography, miniaturized separation technique, sustainable tool, biomolecules, contaminants

Objective
Miniaturized separation techniques have emerged as environmentally friendly alternatives to available separation methods. Among them, nano-liquid chromatography (nano-LC) is a miniaturized method that minimizes reagent consumption and waste generation following the principles of green analytical chemistry. Nano-LC offers high separation efficiency, good resolution, enhanced sensitivity and rapid analysis. The use of capillary columns (i.d. 100 µm), requiring small quantities of stationary phases or expensive additives in the mobile phase (chiral selectors) lead to cost savings. In addition, the reduced flow rate produces a high compatibility with mass spectrometer, providing a high ionization efficiency, improving the detectability of the nano-LC system.[1].
All these positive aspects have made nano-LC a very promising tools in the analysis of molecules of biomedical, agrochemical, food, pharmaceutical and environmental interest [2]

Methods
The aim of the presentation is to briefly discuss the theoretical principles of nano-LC and to show the development of nano-LC methods for the determination of biomolecules and contaminants in agrifood and biological matrices. A rapid nano-LC-UV-MS method was developed for the determination of the main cannabinoids *Cannabis sativa* L. belonging to different varieties in hemp inflorescences. The obtained cannabinoids profile allowed controlling their quality, discriminating between fiber-type and drug-type *Cannabis* samples, and providing valuable information concerning therapeutic and nutraceutical effects [3].

Results
A dispersive liquid-liquid microextraction combined with nano-LC-UV-MS tool was optimized for the analysis of seven neonicotinoids in biological (urine) samples. The good results in terms of precision, sensitivity, extraction yield and rapidity of analysis, make the developed method orthogonal to the conventional LC tool for the analysis of these pesticides in urine samples.

Conclusions
A nano-LC-UV method was optimized to define the polyphenolic profile in plant extracts. The characterization of such secondary metabolites in plant extracts can provide important data in medical and nutrition research. The very satisfactory results so far encourage for further efforts to make nano-LC tool a routine analytical technique in various fields of application.

ACKNOWLEDGMENTS
Dr. Francesca Mariani, Dr. Marco Mazzonna, researchers of Institute for Biological Systems (CNR) Prof. Marialuigia Fantacuzzi, Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara (Italy)

REFERENCES
[1] M. Asensio-Ramos, C. Fanali, C., G. D'Orazio, S. Fanali, (2017). Nano-Liquid Chromatography. In Liquid Chromatography: Fundamentals and Instrumentation; Fanali, S., Haddad, P.R., Poole, C.F., Riekkola, M.-L., Eds.; Elsevier: Amsterdam, The Netherlands, 2017; pp.637–95
[2] L. Shan, B. R. Jones, (2022). Nano-LC: An updated review, *Biomedical Chromatography* 36, e5317
[3] L. Žampachová, Z. Aturki, F. Mariani, Petr Bednár (2021). A rapid nano-liquid chromatographic method for the analysis of cannabinoids in Cannabis sativa L. Extracts, *Molecules*, 26, 1825

SEC-RPLC-MS screening of natural extracts and identification of ligands with high affinity towards PPARα and PPARγ receptors

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Keywords: Size Exclusion Chromatography (SEC); Grating-coupled Interferometry (GCI); Peroxisome Proliferator-activated Receptors (PPARs); drug discovery; plant extracts.

Objective
Peroxisome proliferator-activated receptors (PPARs) are targets for the treatment of type 2 diabetes, dyslipidemia and hyperglycemia associated with metabolic syndrome. Some medicinal plants have been traditionally used to treat this kind of metabolic diseases. However, to date only a few drugs targeting PPARs have been approved. To address this issue, this work describes the development and application of an analytical platform for the screening of natural extracts, the selective fishing of ligands binding to PPARα and PPARγ receptors and the measurement of the affinity of the identified ligands.

Methods
The developed ligand fishing assay included the incubation of the extract with the receptor, the injection of the incubated sample in size exclusion chromatography (SEC) to isolate the extract fraction bound to the receptor and the analysis of the collected fraction by reversed phase liquid chromatography coupled to mass spectrometry (RPLC-MS) to identify PPAR ligands.
The affinity of the identified compounds was then measured by grating-coupled interferometry (GCI), an evolution of surface plasmon resonance (SPR), and *in vitro* tests were performed to confirm the biological activity of the identified ligands.

Results
The analytical platform for PPAR ligand identification and test allowed to screen different extracts from edible plants. From these extracts, three compounds were fished by PPAR receptors and identified as attractive for the treatment of metabolic diseases; the pure compounds were purchased and tested by GCI, allowing to define their affinity constants for PPARs. The affinity was found to be high, with constants in the nanomolar range. In addition, *in vitro* functional assays allowed to define ligand activity (agonist/antagonist).

Conclusions
An off-line bidimensional system based on SEC and RPLC-MS was set-up and successfully applied to the screening of complex samples such as plant extracts, allowing to identify PPAR ligands.
The subsequent GCI and *in vitro* analyses confirmed the validity of the developed system and the identification of compounds of pharmaceutical interest in the treatment of metabolic syndrome.

Sustainable approach in analytical chemistry for the separation of phenolic acids in coffee by nano liquid chromatography

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Keywords: Phenolic acids; nano-LC; coffee; green extraction; deep eutectic solvents; mass spectrometry.

Objective
The present study developed a method based on lab-made nano-liquid chromatography coupled to ultraviolet and MS detection to separate ten phenolic acids (PAs).
The PAs were extracted from coffee powder and its spent grounds by using different green solvents and natural deep eutectic solvents (NADES).
The developed nano-LC-UV method was extended to MS detection by using a liquid–junction ESI interface to confirm the presence of some PA in all coffee samples previously revealed with UV detection by means of the fortification method.
Finally, following the principles of Green Analytical Chemistry, the ecologicality, practicality, and applicability of the method were evaluated by applying the AGREEprep metric.

Methods
By using a 100 µm I.D. capillary column packed in our lab, preliminary experiments were achieved in order to select suitable stationary phases and chromatographic conditions. The baseline separation was obtained for all compounds with C18 core-shell particles (5 µm particle size, 100 Å) in 35 min, working in step gradient mode. The method sensitivity was improved by on-column focusing on studying the type of dilution solvent and injection volume. The usual hot-water extraction procedure for PAs was compared with vortex-assisted liquid phase microextraction (VA-LPME) based on green solvents and NADES.

Results
The whole method was validated, and the resulting RSD% for intra-day and inter-day repeatability, related to retention time and peak area, were below 4.5 and 6.0 %, respectively. LOD and LOQ values were as low as 0.06-6.57 and 3.36-18.8 µg/mL, whereas linearity, assessed over the concentration range of interest for all analytes, was estimated for $R^2 \geq 0.9659$.

Conclusions
A nano-LC system coupled with a UV-mass spectrometry detector, combined with a miniaturised extraction procedure using sustainable solvents, represents an analytical methodology in accordance with the principles of Green Analytical Chemistry. A miniaturised liquid-junction ESI interface is a refined solution for the ionisation of hydrophilic molecules under almost completely aqueous chromatographic conditions. The use of environmentally friendly extraction solvents in miniaturised liquid chromatography is a significant challenge, but the results achieved are promising.

GC-MEMS: development of an ultra-miniaturised gas chromatograph prototype based on lab-on-chip micro electro mechanical systems (MEMS) for space exploration

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Keywords: Nano and Micro Electro Mechanical System (NEMS/MEMS), Gas Chromatograph, miniaturized prototype, organic matter, space exploration

Objective
Gas chromatography GC is an approved technique that have been used since the 1970's in different space missions to characterize the nature and structure of molecules in extraterrestrial environments in order to better understand the physical and chemical properties of solar system bodies and the prebiotic chemistry. While the trend of new space missions is to drastically reduce payloads and resources, actual GC space instruments still bulky, heavy and resource consuming. With these aims, our team developed a miniaturized GC prototype (GC-MEMS) adapted for space missions, using lab-on-chip micro and nano electromechanical system (MEMS and NEMS) technologies.

Methods
Our GC-MEMS prototype is composed of four MEMS/NEMS sub-units: (1) a preconcentrator filled with Tenax® TA, (2) a 5-m chromatographic column coated with PDMS stationary phase (3) a thermal conductivity detector (TCD) and (4) an innovative nano gravimetric detector (NGD, Apix Analytics). The first three lab on chip units are integrated onto a patented fluidic interface [1] adapted for space constraints and ensuring the fluidic connections between sub-units with a negligible dead volume to preserve the system efficiency. The TCD outlet is the coupled in series to the NGD. The prototype was tested using 6-ports valve equipped with a samplig loop (5 – 50 µl) to inject a gaseous mixture of n-pentane, n-hexane, benzene and toluene diluted at 100 ppmv in helium.

Results
The column separation performances are qualified in terms of number of theoretical plates (N) and height equivalent to a theoretical plate (HETP) with 8275 ± 45 plates and 0.6 mm on average, respectively. The retention time is highly reproducible (RSD < 0.2%). The preconcentrator performance for trapping and desorbing are also validated. NGD detector shows a high sensitivity and the limit of detection LOD is evaluated between 3.1 pmol for toluene and 16 pmol for hexane, which is suitable for space exploration since the LOD for the TCD detector, already used on board SAM-GC for Mars Science Laboratory mission, was evaluated at 100 pmol.

Conclusions
The analytical performance of this first prototype of GC based on NEMS/MEMS components are already satisfying and adapted for space studies.

ACKNOWLEDGMENTS

This development is funded by the French space agency (CNES) through a research and technology program.

REFERENCES

[1] Rizk-Bigourd, M., et al., 22 Décembre 2023. Patent FR2315012.

DES-based microextraction of Maillard reaction products in plant-based meat substitutes

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Keywords: deep eutectic solvents, plant-based meat substitutes, Maillard reaction products, LC-MS/MS

Objective

In the presented research a novel method for the quantifcation of acrylamide, 5-hydroxymethylfurfural and furaneol as particular products of the Maillard reaction in pea-based meat substitutes using microextraction based on deep eutectic solvents (DES) followed by liquid chromatography coupled with tandem mass specytrometry (LC-MS/MS) analysis was developed [1]. Plant-based meat substitutes (PBMS) are a novel type of food, designed to be indistinguishable with tipically meat-based products [2], importantly they undergo completely different quality control requirements than meat-based products [3]. However, in the literature there are few analytical reports on PBMS profiling and potential contamination. Maillard reaction products can appear in PBMS in result of plant protein texturizing process [4].

Methods

The 49 compositions of DES were screened by computational prediction to select most suitable option for the extraction of acrylamide, 5-methylfurfural and furaneol. The milled pea was used as a matrix for sample preparation and optimisation. To eliminate the potential undesirable effect of DES for the MS detector, back-extraction into the formic acid solution has been utilized.

Results

The DES based on eugenol and thymol in molar ratio of 2:1 was selected as the one allowing to effective extraction of target analytes. The developed procedure comprised two main steps: extraction — in which the analytes are isolated from the solid sample due to the salting-out effect and pre-concentrated in the DES, and back-extraction — in which the analytes are re-extracted into the formic acid solution for subsequent mass spectrometric detection. The method was fully validated and successfully applied to analysis of eight pea-based meat substitutes.

Conclusions

A novel DES-based microextraction of Maillard reaction products in PBMS followed by LC-MS/MS analysis was developed for the first time and successfully applied to real samples. However, further studies of PBMS and its potential contamination profiling is needed, to monitor this type of food.

REFERENCES

[1] Osiecka D., Vakh C., Makoś-Chełstowska P., Kubica P., (2023). Plant-based meat substitute analysis using microextraction with deep eutectic solvent followed by LC-MS/MS to determine acrylamide, 5-hydroxymethylfurfural and furaneol, *Analytical and Bioanalytical Chemistry*, 417, 1117-1126.

[2] Meat Analogues | Chatham House – International Affairs Think Tank, (2019). https://www.chathamhouse.org/2019/02/meat-analogues (accessed March 18, 2024)

[3] Rubio N.R., Xiang N., Kaplan D.L., (2020). Plant-based and cell-based approaches to meat production, *Nature Communication,* 11, 6276

[4] Singh S., Gamlath S., Wakeling L., (2007) Nutritional aspects of food extrusion: a review, *International Journal of Food Science & Technology,* 42, 916–929

Chemical affinity and binding ability between Prokupac seed phenolic compounds and salivary proteins monitored by UHPLC Q-ToF MS analysis

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Keywords: UHPLC Q-ToF MS, Prokupac, flavan-3-ols, pro(antho)cyanidins, salivary proteins

Objective
Astringency is often an important sensory attribute of red wines, with typical dryness and puckering oral sensation. Previous research has shown that astringency is a consequence of the interaction of flavan-3-ols and their polymerised forms (proanthocyanidins) with salivary proteins, and the formation of insoluble complexes [1]. However, the chemical binding affinity of phenolics and proteins are often strongly depends on the concentration of phenolics/proteins, and the degree of polymerisation/galloylation of the monomeric flavan-3-ols [1]. Prokupac is a traditional red grape variety grown in Serbia. Wine from this variety shows unique sensory properties, with typical astringent and astringent sensations, primarily contributed by tannins derived from seed during fermentation. Previous research has shown that Prokupac seed present a rich source of flavan-3-ols and different proanthocyanidins. The aim of this study was to monitor chemical affinity and binding abilities between Prokupac seed phenolic compounds and salivary protein by UHPLC Q-ToF MS analysis.

Methods
Prokupac water seed extract was prepared according to Pesic et al. [2]. Binding test of Prokupac seed phenolics and salivary proteins was determined according to previously described method by Ma et al. [1]. The filtrate and control seed sample were passed through SPE cartridges, eluated by acidified methanol and analysed by UHPLC Q-ToF MS. UHPLC Q-ToF MS identification of seed phenolics was done on the basis of their monoisotopic mass and MS fragmentation.

Results
The most abundant classes of identified phenolic compounds were flavan-3-ols with relative content of 54.83%, following proanthocyanidins (40.43%) and phenolic acid (4.75%). The best binding ability with salivary proteins were shown by high molecule proanthocyanidins (43.31%), primarily various procyanidin dimer and trimer isomers. Contrary, monomeric flavan-3-ols did not show binding affinity with salivary proteins, except some monomeric gallate derivatives. Binding affinity of phenolic acid was variable (0-80%) and closely dependent on the type of molecules.

Conclusions
In conclusions, polymerised flavan-3-ols showed a high binding affinity to the salivary proteins, suggesting that they contribute the most to the sensory properties of wine and to the precipitation of salivary proteins. However, further research models are needed to confirm the current results.

ACKNOWLEDGMENTS

Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-65/2024-03/200116 and the Science Fund of the Republic of Serbia, #GRANT No. 7744714, FUNPRO.

REFERENCES

[1] Ma W., et al., (2016). Chemical Affinity between Tannin Size and Salivary Protein Binding Abilities: Implications for Wine Astringency, *PLoS One*, 11 (8), e0161095.
[2] Pešić M. B., et al., (2019). *In vitro* digestion of meat- and cereal-based food matrix enriched with grape extracts: How are polyphenol composition, bioaccessibility and antioxidant activity affected? *Food Chem.* 284: 28-44.

Greening liquid chromatography: sustainable solutions for enhanced analytical performance

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Keywords: green chemistry, Liquid Chromatography, eco-friendly solvents

Objective
Liquid chromatography is a standard analytical technique widely employed across pharmaceuticals, biotechnology, food and beverage industries, environmental monitoring, and more. Among its variants, reversed-phase chromatography dominates, accounting for over 75% of applications. Traditionally, this method relies heavily on acetonitrile and large columns (typically 250x4.6 mm), which are not environmentally friendly. However, sustainable alternatives are emerging that do not compromise chromatographic performance. Eco-friendly solvents play a critical role in reducing the environmental impact of liquid chromatography.

Methods
Water, the most used solvent, is non-toxic, inexpensive, and widely available. Hot water has shown potential in reducing organic solvent usage. Ethanol, derived from renewable sources like corn and sugarcane, is biodegradable and non-toxic. Supercritical CO2 and bio-based solvents such as terpenes, lactic acid, and glycerol are also gaining traction for their low toxicity and renewable nature.

Results
Method optimization and greener equipment can further enhance sustainability. Reducing column length, optimizing injection volumes, and employing different gradient conditions can minimize solvent consumption and waste generation. Modern liquid chromatography systems are more energy-efficient and can recycle solvents, thereby reducing environmental footprints. Additionally, recycling waste and choosing sustainable suppliers contribute to greener chromatography practices. Alternative analytical techniques, such as supercritical fluid chromatography, offer more sustainable option with lower environmental impacts.

Conclusions
This presentation will explore various strategies for reducing solvent consumption and improving sustainability in chromatography. Emphasis will be on using smaller internal diameter columns and higher efficiency shorter columns, as well as switching to non-toxic mobile phases like bioethanol. The discussion will include the impact on chromatographic performance and regulatory considerations, supported by multiple examples.

The application of freeze-dried *Lactobacillus plantarum* as an innovative approach for cream enrichment in Vitamin D3 and fatty acid profile modification

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Keywords: food fortification; vitamin D3; probiotics; lyophilisate; fatty acid profile

Objective
The poor vitamin D status is globally recognized problem. The food fortification is considered a promising strategy which allows to increase the bioavailability of vitamin D and to prevent diseases related with its deficiency. The present work aimed to analyze the indirect impact of probiotic bacteria (*L. Plantarum*) on the level of produced vitamin D3 in the cream. Besides, the changes in fatty acids profile, including saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid content were evaluated.

Methods
In this study, two forms of *L. Plantarum* strains were tested: biomass and lyophilizate. The experiment was designed at light/dark and mixing/non-mixing conditions. The level of vitamin D3 in cream was indicated by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS), while the changes in the fatty acid profile was analyzed by gas chromatography-mass spectrometry (GC-MS).

Results
According to results, *L. plantarum* lyophilisate led to more efficient enrichment of cream in vitamin D3 in contrast to biomass. Moreover, the probiotic supplementation induced the decrease of the overall SFA content and increase of MUFA level, while PUFA concentration was mostly dependent on the light exposure and the medium presence.

Conclusions
Based on our studies, probiotics showed a great prospective in the fortification of cream with vitamin D3. The form of added *L. plantarum* strains played a significant role on the cream enrichment with vitamin D. The changes in metabolic activity between both forms of *L. plantarum* might associated with their different survivability. The non-mixing conditions and light exposure were considered the most valid criteria providing to the enhanced production of vitamin D3. Besides, the remodeling of fatty acid content in cream has a beneficial effect by preventing the development heart disease, nevertheless the risk of product's shelf life reduction appears.

ACKNOWLEDGMENTS
This work was financially supported by the Agency for Restructuring and Modernization of Agriculture (ARMA), under the Rural Development Program 2014-2020, grant number 00126.DDD.6509.00244.2022.02. Tetiana Dyrda-Terniuk is a member of Emerging Fields "Cells as Experimental platforms and bioFACTories (CExFact)". Paweł Pomastowski and Justyna Walczak-Skierska are the members of the Toruń Center of Excellence "Towards Personalized Medicine" operating under Excellence Initiative Research University. Dorota Białczak is scholarship holder of the implementation doctorate financed by the Polish Ministry 330 of Education and Science, grant number DWD/6/0121/2022.

Comprehensive characterization of *Piper* essential oils from Brazil by means of gas chromatography analysis and spectroscopic methods

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Keywords: preparative gas chromatography, multidimensional gas chromatography, chiral GC, FTIR, NMR

Objective
This study aimed to characterize the essential oil obtained from the leaves of *Piper gaudichaudianum* Kunth from Brazil by means of gas chromatography based approaches. After GC-MS analysis, a component consituting 27% of the essential oil was not identified, needing a proper analytical strategy to collect and identify the unknown component in reasonable times. In parallel, the main chiral terpenes were investigated by means of enantio-selective multidimensional gas chromatography.

Methods
Leaves of *Piper gaudichaudianum* were collected in Brazil and hydrodistilled in a Clevevenger-type apparatus to obtain the essential oil. While GC-MS analyses were carried out to characterize the main essential oil components, prep-GC, GC-FTIR and NMR were mandatory to collect and characterize the unknown component, respectively. Enantio-selective multidimensional gas chromatography was carried out to define the enantiomeric ratios of the main terpenes investigated.

Results
After the GC-MS analysis, a component constituting 30% of the EO was not identified by exploiting the common filters of mass spectral similarity and linear retention indices. As a result, preparative gas chromatography was needed to collect proper amount of analytes prior to structural elucidation experiments. In a second step, NMR, GC-MS and GC-FTIR, allowed the unambiguous identification of the para-phenol substituted component. Complementarily, to gain more information about the chemical composition of the EO, the enantiomeric ratios of eight terpenes were assessed by exploiting an MDGC approach in heart-cut mode.

Conclusions
The exploitation of multiple and complementary analytical approaches, based on gas chromatography and spectroscopy methods, allowed a proper characterization of the main volatiles in the *Piper gaudichaudinaum* Kunth EO.

ACKNOWLEDGMENTS

The project was funded by the Italian Ministry for the University and Research (MUR) with a "PRIN: Progetti di ricerca di rilevante interesse nazionale Bando 2022" Project n. 2022359CZA "Advanced analytical characterization and synergic evaluation of volatile terpenes of selected β-caryophyllene containing spices for diabetes treatment".

Tracing volatile signature of grapes by GC×GC-ToFMS

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Keywords: HS-SPME-GC×GC-ToFMS, flow modulator, varietal aroma composition, grapes

Objective
This work aimed the development of a methodology for the analysis of free volatile compounds in grapes using HS-SPME-GC×GC-ToFMS and its application to Trincadeira, Cabernet Sauvignon, Syrah, Castelão and Tinta Barroca grapes from the 2021 and 2022 harvests.

Methods
To increase the efficiency of the SPME technique, a detailed optimization of all the steps of the sample preparation technique was carried out. The optimized conditions were 4 g of grapes, 2 g of NaCl, and 2 mL of H_2O. The extraction conditions, using a carboxen/divinylbenzene/polydimethylsiloxane (CAR/DVB/PDMS) fiber were also optimized, and performing the extraction for 40 min at 60ºC allow to identify more varietal compounds. The analyses were performed on a GC×GC-ToFMS (Agilent 8890GC System, Shanghai, China with a BenchTOF-Select detector, Markes International, Bridgend, UK and a CTC Analysis AG autosampler PAL-System, SepSolve Analytical, Zwingen, Switzerland). Data were acquired and analyzed with ChromSpace of Markes International. Chromatographic separation was achieved with the INSIGHT™ flow modulator (SepSolve Analytical), with a 50 µL loop, a BPX5 column (20 m x 0.18 mm i.d. and 0.18 µm film thickness) as the first dimension, and a BPX50 column (5 m×0.25 mm i.d. and 0.1 µm film thickness) as the second dimension. The modulation period was 5s. The oven temperature program began at 40 ºC hold for 3 min; raised at 3 ºC min^{-1} up to 150 ºC, then 4 ºC min^{-1} up to 260 ºC and hold for 10 min. Helium was the carrier gas with a flow of 0.5 mL min^{-1} in 1D and 20 mL min^{-1} in 2D. The MS transfer line and source temperatures were set at 270 ºC [1].

Results
Using the optimized methodology, it was possible to identify fifty-two free volatile compounds, including seventeen monoterpenes, twenty-eight sesquiterpenes, and seven C_{13}-norisoprenoids. According to the results obtained through a linear discriminant analysis (LDA), the differences in volatile varietal signature are observed both among different grape varieties and across different years.

Conclusions
HS-SPME combined with GC×GC-ToFMS provides a suitable and sustainable approach to establish the volatile signature of grapes of different varieties.

ACKNOWLEDGMENTS

This research was funded by "Vine&Wine-Driving Sustainable Growth Through Smart Innovation" project (sub-project-BioGrapeSustain), "Mobilizing Agendas for Business Innovation" under the Recovery and Resilience Program. Authors also aknowledges MED https://doi.org/10.54499/UIDB/05183/2020 and CHANGE https://doi.org/10.54499/LA/P/0121/2020.

REFERENCES

[1] Fonseca, D., Martins, N., Garcia, R., Cabrita, M. J., (2024). Comprehensive Two-Dimensional Gas Chromatography with a TOFMS Detector - An Effective Tool to Trace the Signature of Grape Varieties, *Molecules*, 29, 1989.

Identification of hydrocarbon isomers by means of a novel dual parallel detection based on a GC-FTIR/MS approach

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Keywords: Gas-chromatography, Fourier transform Infrared spectroscopy, Mass spectrometry, hydrocarbons

Objective
Hydrocarbons are the primary components of petroleum. It represents a relevant raw material, as it is utilized in the production of fuels, lubricants, plastics, fibers, rubbers, solvents, industrial chemicals, and other materials. The composition of such complex samples is particularly challenging to elucidate due to the vast number of chemical compounds they contain.
The combination of gas chromatography and mass spectrometry (GC-MS) with an electron ionization (EI) source represents the gold standard for the identification of unknown volatile compounds. However, the high number of geometric and positional isomers of hydrocarbons makes their identification challenging due to their identical molecular weight as well as their similar fragmentation pattern. Conversely, Fourier Transform Infrared Spectroscopy (FTIR) provides a wealth of molecular information of GC-separated analytes, related to the vibration of molecular functional groups, which allows the discrimantion between isomers. This information, in conjunction with LRI and MS, allows for the correct identification.

Methods
Hydrocarbons separation was achieved on a 100 m × 0.25 mm I.D., d_f 0.50 µm polydimethylsiloxane Petrocol DH capillary column (Merck KGaA, Darmstadt, Germany). Analytes were detected by means of a GC-sd-FTIR/MS instrument developed in laboratory, based on post-column GC flow splitting to GCMS-QP2020 NX mass spectrometer (Shimazdu, Kyoto, Japan) and DiscovIR-GC solid phase FTIR detector (Spectra-Analysis Instrument Inc., Marlborough, MA, USA).

Results
The integrated instrumentation developed allowed to obtain three complementary information from a single analysis: the linear retention indices (LRI) of the separated compounds, their *m/z* spectra and IR fingerprint.

Conclusions
Definitive discrimination of hydrocarbon isomers was successfully achieved.

ACKNOWLEDGMENTS

The research was performed within the framework of the Research Project PRIN 2022: Unique analytical workflow involving COMPLEmentary TEchniques for the reliable molecular identification of hydrocarbons (CompleTe), supported by the Italian Ministry of University and Scientific Research, no. Prot. 2022KC2BRL.
The authors acknowledge Shimadzu and Merck for the continuous support.

Insight into bioactivity and phytochemistry of *Cistus laurifolius L.* originated new type of propolis

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Keywords: *Cistus laurifolius*, propolis, HPTLC, LC-MS, anticancer and anti-inflammatory activities

Objective
Botanical origin is an important determinant for the propolis chemical composition which is linked with its pharmacological activity. Lasiocarpin B and Lasiocarpin C rich Eurasian aspen (*Populus tremula*)-type propolis and caffeic acid phenethyl ester, chrysin, and galangin rich black poplar (*P. nigra*)-type propolis are commonly found in Türkiye. The purpose of this study was to evaluate the chemical composition and bioactivities of a new type of propolis originated from *Cistus laurifolius* in which 3-*O*-methyl quercetin and 3,7-*O*-dimethyl quercetin are the marker components.

Methods
Propolis samples were collected from different locations in Türkiye. Resin containing flower and leaf stalks of *C. laurifolius* and propolis samples were extracted with ethanol-water mixture (8:2 *v/v*). HPTLC analysis was comparatively performed by using HPTLC silica gel 60 F_{254} and toluene-ethyl acetate-acetic acid mixture (7:6:2 *v/v/v*) as developing solvent to analyze marker compounds. Unidentified phenolic compounds were detected in HPTLC analysis and to isolate targeted compound from hydroalcoholic extract successive chromatographic methods were used. The chemical structures of the isolated compounds were determined by NMR and MS analysis. LC-MS/MS equipped with a Troyasil C_{18} (150 × 2.1 mm 3.5 µm) column with a gradient solvent system (mobile phase A:1% formic acid-water, B: 10% methanol-10% water-80% acetonitrile mixture) used for screening and quantifying the compounds by using a validated method. Propolis samples at different concentrations (up to 1 mg/mL) were subjected to nitrit assay for evaluating their anti-inflammatory activities. Anticancer activity of these samples was analyzed by two-dimensional (2D) cell culture system and three-dimensional (3D) spheroid formation/growth assay.

Results
HPTLC fingerprint results of the hydroalcoholic propolis extracts were found similar with *C. laurifolius* profile which was indicated the botanical source of propolis samples. The chemical structures of the unknown compounds were elucidated as 3-*O*-methylquercetin, 3,7-*O*-dimethylquercetin. LC-MS/MS method showed that 3-*O*-methylquercetin, 3,7-*O*-dimethylquercetin, and quercetin were the major and marker compounds in propolis samples. Regarding bioactivity studies, propolis samples showed anti-inflammatory and anticancer activities in dose dependent manner.

Conclusions
Botanical origin is a crucial factor effecting the chemical composition and pharmacological activity of the propolis. Therefore, the use of botanically defined and chemically standardized extracts is essential for reproducible pharmacological activity. In this study, the chemical composition of propolis, botanically originated from *C. laurifolius*, was characterized in addition to its anti-inflammatory and anti-cancer activities for the first time.

LC-LEI-HRMS for characterization of PAHs photo-oxidation phenomena in Mars environment

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Keywords: LC-EI-MS, LEI-MS, Mars environment characterization, PAHs analysis, astrobiology

Objective
Polycyclic aromatic hydrocarbons (PAHs) constitute a class of molecules particularly prevalent on Mars. PAHs on Mars often exist in photo-oxidized forms due to the easier penetration of UV light through its atmosphere compared to Earth. In this work, liquid chromatography was coupled with a high-resolution mass spectrometer using the liquid electron ionization (LC-LEI-HRMS) to investigate this phenomenon for molecules of interest in characterizing the Martian environment.

Methods
Standard solutions were prepared in acetonitrile (ACN) or tetrahydrofuran (THF), depending on the solubility of the analytes. Extraction from the spiked mineral MgSO4 was conducted via solid-liquid extraction (SLE). This involved weighing 5 mg of the mineral, adding 400 µL of solvent (ACN for dihydroxynaphthalene (DHN) isomers and THF for coronene), agitating for 5 minutes on an orbital vortex at 3000 rpm, then sonicating the mixture for 10 minutes, and terminating the extraction with another 5 minutes of agitation on an orbital vortex at 3000 rpm. The extract was then filtered with a 0.2 µm PTFE syringe filter to remove the mineral powder. Analyses were performed using an Agilent 7250 GC/Q-TOF adapted to work with an LEI interface. LC separation was performed with an Agilent 1260 Infinity II using a Kinetex 1.7 µm XB-C18 150 x 2.1 mm column at a flow rate of 0.2 mL/min with a gradient from 95%A-5%B at time 0 to 100%B at 19 minutes, then kept constant until 26 minutes (Solvent A: H2O with 0.1% formic acid; Solvent B: ACN with 0.1% formic acid). Injection volume was 4 µL and the flow sent to the MS was set to 500 nL/min using a passive flow splitter. MS acquisitions were performed in full scan mode for molecular structure identification.

Results
The results of the quantitative analysis show an extraction recovery of 100%. Intraday RSD% values were below 15% for both calibration and real sample analysis. Calibration of 1,6-DHN and 2,6-DHN yielded R^2 values of 0.9962 and 0.9963, respectively, within the calibration range of 50 mg/L to 500 mg/L. The analysis of 2,6-DHN powder after 3.7 hours of UV photo-oxidation showed a reduction in the total amount from 100% to 60% m/m, with the formation of a dimeric product and 2-methoxy-6-hydroxynaphthalene.

Conclusions
LC-LEI-MS is a valuable tool for analyzing and identifying unknown substances using the NIST library. This setup enables the chromatographic separation of dihydroxynaphthalene isomers with LC, allowing the acquisition of EI spectra of products potentially incompatible with GC for structural identification thanks to the hard fragmentation and high resolution.

ACKNOWLEDGMENTS

We are grateful to Agilent for providing the instruments used in this project.

Characterization and tracing of *Cannabis sativa L.* essential oils through a comprehensive quali-quantitative, chiral and isotopic investigation

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Keywords: *Cannabis sativa* L., multidimensional Gas chromatography, chiral GC, IRMS, GC-MS

Objective
In this study, twenty-five seized *Cannabis sativa* L. samples were investigated by means of multipe analytical approaches. Alongside the investigation of the cannabinoids content, all the samples underwent to microwave assisted hydro-distillation to produce the essential oils, prior to quali-quantitative, chiral and isotopic analyses.

Methods
Twenty-five seized samples of *Cannabis sativa* L. flowering tops were provided by the Scientific Investigation Department RIS of Messina. Cannabinoids content was evaluated by means of GC-FID analysis, while the terpene profile was assessed by GC-MS and GC-FID analyses. Lastly, a heart-cutting multidimensional gas chromatography (MDGC) approach was implemented for simultaneous chiral and isotopic analysis.

Results
The illicit nature of the seized samples was confirmed by the high content of THC detected (always above >0.2%) through GC-FID analysis. In a second step, all the essential oils, obtained after MAHD extraction, were investigated by means of GC-MS and GC-FID analyses, highlighting the presence of typical monoterpenes and sesquiterpenes, like α-pinene, β-pinene, caryophyllene, and selina-diene derived. After that, target terpene components were investigated by means of a simultaneous chiral and isotopic analysis. Finally, a statistical evaluation was carried out, in order to group the different seized samples, according to their experimental similarities.

Conclusions
This study details the possibility of tracing the origin of seized narcotic samples, by exploiting multiple analytical methods. The combination of the quali-quantitative (volatile fraction and THC content), chiral, and isotopic data was able to suggest a close correlation between specific narcotic samples, able to support police forces.

Towards a database of softwood odors: approaches for the investigation of odor profiles from *Pinus sylvestris* L., *Pinus strobus* L. and *Pinus cembra* L. by (dynamic) headspace extraction thermal desorption-GC-O, GC-FID/MS and human sensory evaluation

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Keywords: aroma chemistry, dynamic headspace, odorant detection, renewable resources, indoor environments

Objective
The composition of softwood materials is increasingly influenced by climate change-related risk factors such as drought and beetle damage [1]. Extractives-rich and resilient species, such as the forest pine *(Pinus sylvestris L.)*, are being promoted in favour of established species, such as the Norway spruce *(Picea abies L.)*. In particular, this change has a decisive impact on olfactory perception in indoor environments, and thus demands a better understanding of the materials' main odorants, as well as odor profiles. In this study we therefore present a comprehensive and robust method with minimal sample preparation for the creation of a database for softwood odors. Of three different softwood types, the odor activity of key odorants is determined, as well as their odor profiles identified.

Methods
Dynamic headspace extraction thermal desorption ((dynamic)-HS-TD) was used for the extraction of volatile species from samples of *Pinus sylvestris L.*, *Pinus strobus L.* and *Pinus cembra L.*. GC with olfactometric detection/flame ionization (GC-O/FID), together with aroma extract dilution analysis (AEDA) and MS detection were performed. A test panel of 20 persons was employed for the evaluation of odor profiles.

Results
Over 40 compounds could be detected at the olfactometric detection port for the three tested wood types. When compared, the sample of *Pinus sylvestris L.* showed the highest number in compounds detected, as well as the highest flavour dilution (fd) factors. 2,6-(Z,E)-nonadienal, 2-nonenal and octanal were found as compounds with fd-factors as high as 768, 1536 and 3072. Evaluation by sensory testing revealed a pronounced musty characteristic for the *Pinus sylvestris* sample, whereas *Pinus strobus* and *Pinus cembra* have similar attributes, such as fruity and sweet.

Conclusions
In this investigation, ((dynamic)-HS-TD) could be successfully employed as sample preparation method for the extraction and detection of odorants from *Pinus sylvestris L.*, *Pinus strobus L.* and *Pinus cembra L.*. The results show the capability of the method to be used as an easy to implement standard method for the conception of a database for different woods and wood-materials.

ACKNOWLEDGMENTS

Financial support of this work in the frame of the FFG (Austrian Research Promotion Agency) COMET program is gratefully acknowledged.

REFERENCES

[1] R. Jandl, (2020), Climate-induced challenges of Norway spruce in Northern Austria, *Trees, Forests and People*, 1, 100008

Qualitative determination of phenolic compounds in *Citrus* fruit molasses by ultra-high-performance liquid chromatography coupled high-resolution mass spectrometry (UHPLC-HRMS)

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Keywords: *Citrus* fruit, untargeted metabolomics, phenolic compounds, UHPLC-HRMS.

Objective
Citrus fruits are rich in phytochemicals and biologically active compounds with numerous health benefits [1]. Their processing generates substantial waste, including peels, seeds, and pomace, often used as animal feed or disposed of, causing environmental issues. Recently, citrus waste has been recognized for its high value in food and non-food industries. *Citrus* fruit production and consumer demand are rising globally, generating significant waste with high disposal costs and environmental risks. Recent guidelines emphasize transforming citrus residues into valuable resources. However, their use remains limited due to the underestimation of their nutritional and economic value. Increasing awareness can support environmental sustainability and harness the benefits of these residues [1,2]. Investigating the phenolic profile of citrus waste is essential for making productive its diverse health benefits and industrial applications. Phenolic compounds serve as antioxidants, anti-inflammatory agents, and materials for food additives, cosmetics, and biofuels [1,2]. Analysing these compounds transforms waste into resources, promoting environmental sustainability and economic opportunities within a circular economy framework. A qualitative determination of the phenolic profile of citrus fruit molasses waste was performed using untargeted analysis. The developed untargeted metabolomics approach by ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) enabled the identification and characterization of phenolic compounds present in two different samples, providing a comprehensive view of their chemical profile.

Methods
Initially, the quantity of phenolic compounds was determined using the Folin-Ciocalteu method, and antioxidant activity was assessed using the 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Subsequently, the phenolic profile was investigated using untargeted UHPLC-HRMS analysis. The analyses were performed in untargeted mode using AcquireX software (ThermoFisher Scientific) for method optimization in Data Dependent Acquisition (DDA). Following this, data were processed using Compound Discoverer 3.3 software, employing a workflow designed to upload LC-HRMS platform data for subsequent analysis.

Results
The developed untargeted metabolomics approach allowed to determine the presence of different phenolic compounds as well as other bioactive molecules in the two samples.

Conclusions
In conclusion, the phenolic profiling of *Citrus* molasses waste reveals its significant potential for various industrial applications and environmental sustainability efforts. Further exploration of these compounds could lead to enhanced utilization of citrus by-products, promoting a more sustainable approach to waste management.

REFERENCES
[1] Shweta Suri, et al., Current applications of citrus fruit processing waste: A scientific outlook, Applied Food Research, Volume 2, Issue 1, 2022, 100050, ISSN 2772-5022, https://doi.org/10.1016/j.afres.2022.100050
[2] Russo, C.; et.al. The Second Life of Citrus Fruit Waste: A Valuable Source of Bioactive Compounds. Molecules 2021, 26, 5991. https://doi.org/10.3390/molecules26195991

Determination of shell-fish emissions by HS-SPME-GC-MS. Mediterranean blue crab odor volatiles for its characterization

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Keywords: blue crab, Mediterranean sea, odor volatile emissions, HS-SPME-GC-MS, sensory analysis, consumer acceptability.

Objective
The blue crab (*Callinectes sapidus* Rathbun, 1896) is a species native to the Atlantic coast of America, from Nova Scotia to Argentina. It lives in brackish areas and coastal marine waters, where it completes its entire life cycle. In these regions, blue crab is considered a valuable seafood and it plays a crucial role in supporting an important fishery and processing industry. In recent years, the blue crab has spread massively throughout the Mediterranean basin, particularly along the coasts of Italy. The significant presence of this invasive alien species in the Mediterranean sea raises concerns about potential impacts on the local environment and economic activities [1]. Unfortunately, its eradication is not feasible. So, while containment strategies are being developed, transforming the blue crab into a food source could be a viable option for gaining economic benefits also for the Mediterranean area. In light of this, the present research aimed to investigate the volatile composition and sensory features of blue crabs from the coasts of Sicily. In addition, their consumer acceptability was assessed and compared with other widely consumed and appreciated crustacean species.

Methods
QDA sensory analysis and Headspace Solid-Phase Microextraction (HS-SPME) followed by Gas Chromatography coupled with Mass Spectrometry (GC-MS) were carried out, and the results were linked to consumer acceptability. Moreover, a comparative acceptability test with Norway lobster and the Caramote prawn, two crustaceans widely consumed and appreciated, was performed and the data were statistically elaborated. Each compound was identified using mass spectra, NIST'18 library (NIST/ EPA/NIH Mass Spectra Library, version 2.0, USA), FFNSC 3.0 database, Linear Retention Indices and data from the literature.

Results
A large number of volatiles were identified in the blue crab meat, with aliphatic alcohols, aldehydes, and ketones as predominant compounds. The samples showed a distinctive odor profile if compared with those of other geographical origins and other crustacean species. Moreover, sensory analysis revealed that blue crab was characterized by fish-like and marine odors and flavor, with notable sweetness, juiciness, and tenderness. Blue crab was well appreciated by consumers, similarly to other widely consumed crustaceans.

Conclusions
The research provides new and advanced insights into the quality and consumer acceptance of blue crabs from the Mediterranean Sea, which could be highly valuable to local fishing and fish processing industries.

REFERENCE
[1] Marchessaux G., Mangano M. C., Bizzarri S., M'Rabet C., Principato E., Lago N., Veyssiere D., Garrido M., Scyphers S. B., Sarà G., (2023). Invasive blue crabs and small-scale fisheries in the Mediterranean sea: Local ecological knowledge, impacts and future management, *Marine Policy*, *148*, 105461.

Use of DES-microwave-assisted extraction with SPE clean-up to characterize oxygen heterocyclic compounds in *Citrus*-scented cosmetics by means of HPLC-PDA

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Keywords: Microwave-assisted extraction (MAE), deep eutectic solvents (DES), Solid-phase extraction (SPE), coumarin, furocoumarins, cosmetics, HPLC-PDA

Objective
This study aimed to investigate the content of oxygen heterocyclic compounds, especially coumarin and furocoumarins, in cosmetics products scented with *Citrus* essential oils. The non-volatile fraction of *Citrus* essences is composed by oxygen heterocyclic compounds (OHCs), which include mostly coumarins (Cs), furocoumarins (FCs) and polymethoxyflavones (PMFs). These compounds show several biological effects on human health but FCs have been investigated mainly for their phototoxicity. For this reason, the European regulation (EC) No 1223/2009 provides the maximum amount of FCs in cosmetics products and International Fragrance Association (IFRA) has issued several opinions.

Methods
All body cream samples were subjected to microwave-assisted extraction (MAE) followed by a solid-phase extraction (SPE). Briefly, 1 g of sample was weighed into a falcon tube and 3 mL of a deep eutectic solvent (choline chloride + urea, molar ratio 1:2) and 100 μL of ethyl acetate were added as extraction solvents. A Synthwave system (Milestone Srl, Bergamo, Italy) was used. The MW conditions were as follows: temperature ramp reaching 70 ◦C in 2 min and hold for 1 min. The procedure was reapeted two times. The supernatant was collected and subjected to solid-phase extraction (SPE) using an Oasis HLB Vac Cartridge (3cc, 60 mg, 30 μm, Waters, Milford, MA, USA). Each sample was analysed in triplicate through a liquid chromatographic system coupled with a photodiode array detector (HPLC-PDA).

Results
The method was validated in terms of extraction recovery and matrix effect. Recoveries obtained for spiked samples ranged from 50 to 91 %. No matrix effect was found. Quantification of OHCs was obtained with calibration curves in ethanol. Coumarin and total furocoumarins content levels in sample analyzed were in agreement with European regulation and IFRA opinions.

Conclusions
The extraction procedure coupled with the HPLC-PDA method here proposed was well applied for the screening of coumarin and furocoumarins in cosmetic samples.

ACKNOWLEDGMENTS
The authors acknowledge the Shimadzu Corporation, Merck Life Science and Milestone Srl for their support.

Characterization and analytical application of lab-made organosilica coatings for sample preparation prior to chromatography determination

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Keywords: sample preparation, chromatography, sorption, aldehydes, sol gel synthesis

Objective
Modern methods of analysis are supposed to be “green” which means using fewer or no solvents and a small volume of reagents and samples. In order to achieve this, different microextraction techniques are applied. Effective sorption materials are required for these techniques. Although there are a number of commercially available sorbents, coatings and devices, new materials for specific tasks are required. In this study, we attempted to synthesize coatings for microextraction using sol-gel technique to obtain uniform, even, durable, less swelling, reproducible in synthesis and sorption coatings.The OH-terminated polydimethylsiloxane was used as organic sol-gel active polymer. For the precursor component, methyltriethoxysilane, methyltrimethoxysilane, phenyltriethoxysilane, aminopropyl-triethoxysilane, cyanopropiltriethoxysilane and others, individually and in mixtures were used. A solution of titanium isopropoxide was used as the inorganic sol–gel precursor. Moreover, the addition of surfactants and other modificators was studied. The reaction to obtain the sol was conducted under acidic conditions. Coatings were applied on glass plates, glass-encased magnetic stir bars, and metal wires. At this stage of the research, the coatings obtained on glass plates served for the characterization of the material, and the stir bars were the most useful for the actual analytical methods of sample preparation. As for the analytical part of the research the determination of Sudan I (dye that is forbidden to be used in food) in spice samples was carried out. Due to the complexity of the matrix and the presence of other colorants in sample, the application of a coating on the stir bar simplifies extraction and reduces matrix contamination of the sample prior to analysis by liquid chromatography.

Methods
In this work, the sol-gel synthesis technique was used to obtain coatings on various substrates. The coatings were characterized by ATR-IR and NMR spectra and by measuring water contact angles. These coatings were applied for the solid-phase extraction and microextraction coupled with spectrophotometry, liquid chromatography, and gas chromatography.

Results
Using the above methods, it was confirmed that the obtained coatings were nonpolar and hydrophobic. The presence of Ti in the coatings synthesized using titanium isopropoxide was confirmed by peak at 925 cm^{-1}. Titanium isopropoxide addition resulted in more stable and less swelling coating. These coatings can be used for the microextraction of non-polar and semi-polar analytes. Ethyl acetate spice extract was used for sorption of Sudan I, and 2,4-dinitrophenylhydrazine derivatives of aldehydes were prepared in a water/methanol mixture. Depending on the coating composition, 0,5 to 10% of the analytes were extracted on synthesized coatings.

Conclusions
Based on sorption characteristics, two methods for sample preparation and determination of the banned dye Sudan I (allowing the detection of 100 µg/g) and derivatives of aldehydes were developed, using different modified in lab-made PDMS coatings. The use of such coatings for the sorption of banned dyes is an effective and simple solution to the problem of the presence of permitted dyes in large excess. The main idea remains that these coatings can be synthesized by a method that is simple enough to be obtained in the laboratory in a short amount of time and without special equipment.

Applicability of MTBE lipid extraction assisted by microwave in food analysis. Case of study: extraction of pistachio oil

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Keywords: Microwave-assisted extraction, pistachio, lipid extraction, MTBE, AGREEprep

Objective
This research aimed to evaluate the effectiveness of a fast and greener lipid extraction method assisted by microwave in fresh pistachios, in which the amount of lipids is up to the 40%. The nutritional and therapeutic significance of Pistachio (*Pistacia vera L.*) oil is largely attributed to its high levels of unsaturated and essential fatty acids. The extraction method used to isolate natural compounds from the raw material significantly impacts product quality, especially in preserving its nutritional value. Consequently, the study focuses on the impact of the proposed extraction method on the quality and yield of the extracted oil, as well as its fatty acid (FA) composition.

Methods
Lipid extraction from fresh pistachios was performed using seven different methods, including microwave-assisted extraction (MAE) with methyl-tert-butyl ether (MTBE), used as one-solvent extraction or in mixtures with methanol and water. In the latter, the same solvents composition and ratio of Matyash method were used (10/3/2.5 v/v/v) [1]. MAE methods were compared in terms of extraction yields and FA composition of pistachio oil to Soxhlet and Matyash, considered gold-standard lipid extraction methodologies in food and biological samples. FAs, derivatized into methyl esters (MEs), were analysed through a gas chromatography-flame ionization detector (GC-FID).

Results
Among the seven methods tested, the MAE-MTBE method showed comparable results to Soxhlet and Matyash methods in terms of extraction yield and FAMEs composition. In all extraction procedures, eight fatty acids were identified in the pistachio samples. The most abundant fatty acid was oleic acid (C18:1n9c), followed by linoleic acid (C18:2n6c) and palmitic acid (C16:0), which together accounted for over 90% of the total FAMEs.

Conclusions
The results obtained by MAE-MTBE were in line, in terms of extraction yields and FAMEs composition, with the other extraction methodologies. MAE-MTBE also proved to be a greener extraction procedure than the reference methods when evaluated based on the AGREEprep metrics [2], highlighting its environmental benefits over conventional methods.

REFERENCES
[1] Matyash V., Liebisch G., Kurzchalia T. V., Shevchenko A. (2008). Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics, *Journal of Lipid Research*, 49, 1137-1146.
[2] Pena-Pereiraa F., Tobiszewski M., Wojnowski W., Psillakis E., (2022). A Tutorial on AGREEprep an Analytical Greenness Metric for Sample Preparation, *Advances in Sample Preparation*, 3¸ 100045.

Miniaturised extraction procedure coupled with a rapid and eco-friendly chromatographic approach for the identification of oxygen heterocyclic compounds in foodstuffs

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Keywords: miniaturised extraction, HPLC-QqQ/MS, coumarin, cinnamon, *Citrus*

Objective
This study aimed to investigate the daily intake of OHCs by food quantifying coumarin and other thirty-five OHCs in thirty food samples flavoured with cinnamon, *Citrus* and carrot products. In order to reduce the amount of solvents utilised and the time required, a miniaturisation of the extraction procedure was implemented, in accordance with the principles of green chemistry.

Methods
All samples underwent a solid-liquid extraction procedure. 1 g of the properly ground sample was weighed into a Falcon tube and 1 mL of ethanol was added as extraction solvent. Each sample thus extracted was then analysed through a liquid chromatographic system coupled with a triple-quadrupole mass spectrometer (HPLC-QqQ/MS).

Results
Coumarin is the only OHC strictly regulated in foods with EFSA proposing a maximum daily intake of 0.1 mg kg^{-1} body weight. All the cinnamon-flavoured samples analysed can be considered safe since the content reported is below the recommended limit. The *Citrus*-flavoured samples presented an higher variability, both in qualitative and quantitative terms, suggesting the addiction of other *Citrus* species.

Conclusions
The miniaturised extraction procedure validated represents a greener approach compared with the previous methods reported in the literature. The use of just 1 mL of ethanol avoids the need for a drying step and the subsequent generation of waste. Furthermore, the sonication step has been decreased resulting in a significant reduction in the overall extraction time from two and a half hours to nearly 30 minutes.

Comparative analysis of innovative chemometric tools for GC×GC-(HR)TOF-MS wine chromatogram processing

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Keywords: wine analysis, comprehensive two-dimensional Gas chromatography, VOC profile, chemometrics

Objective

The interpretation of results from comprehensive two-dimensional gas chromatography-(high resolution) time-of-flight mass spectrometry (GC×GC-(HR)TOF-MS) depends on the analysis's purpose, as complex samples generate extensive data requiring thorough examination. Traditionally, peaks are identified "one by one" to select compounds for statistical interpretation from the 2D chromatogram. Wine analysis is particularly challenging due to its potential to contain several thousand of compounds. This study compares advanced chemometric approaches for analyzing chromatograms to identify volatile organic compounds (VOC) and classify and authenticate wines [1,2].

Methods

A total of 64 wines from Austria, Slovakia, Hungary, and France were analyzed using GC×GC with cryogenic modulation and TOF-MS and HRTOF-MS. They were split into two batches, with 34 and 38 wines each, and 8 samples analyzed by both techniques. VOC were extracted via solid-phase microextraction (SPME) with a 50/30 µm PDMS/CAR/DVB fiber. The GC×GC-HRTOF-MS column setup included ^{1}D DB-FFAP and ^{2}D Rxi-17Sil, while the TOF-MS setup used ^{1}D DB-FFAP and ^{2}D BPX-50.

Results

Chromatograms were divided into 5×5, 10×10, and 15×15 regions of interest (ROI) and analyzed using genetic algorithm (GA) to find the best ROI for identifying wine age-related compounds. The 10×10 sub-ROI was optimal, identifying 163 compounds, including terpenes, alcohols, aldehydes, ketones, phenols, furans, pyrans, esters, and naphthalene derivates. Principal component analysis with a tile-based *Fisher*-ratio approach identified 70 key compounds for classifying samples by geographic origin, with 10 compounds explaining 84.57% of the variance.

Conclusions

Chromatograms were divided into 5×5, 10×10, and 15×15 ROI and analyzed using GA to find the best ROI for identifying wine age-related compounds. The 10×10 ROI was found out to be optimal, with 163 compounds, including terpenes, alcohols, aldehydes, ketones, phenols, furans, pyrans, esters, and naphthalene derivates. Principal component analysis with a tile-based *F*-ratio approach identified 70 key compounds for classifying samples by geographic origin, with 10 compounds explaining 84.57% of the variance.

ACKNOWLEDGMENTS

This project has received funding from Slovak Research and Development Agency under Contract No. APVV-21-0211. N. K. would like to acknowledge the contribution of the STU Grant scheme for Support of Excellent Creative Teams of Young Researchers SOLUTION.

REFERENCES

[1] Koljančić N., Gomes A. A., Špánik I., (2023). A non-target geographical origin screening of botrytizedwines through comprehensive two-dimensional gaschromatography coupled with high-resolution massspectrometry, *Journal of separation science*, 46(19), 2300249.

[2] Koljančić N., Onça L., Khvalbota L., Vyviurska O., Gomes A. A., Špánik I., (2019). Region of interest selection in heterogeneous digital image: Wine age prediction by comprehensive two-dimensional gas chromatography, *Current Research in Food Science*, 8, 100725.

Extractive-liquid sampling electron ionization-mass spectrometry: direct analyses of pesticides in light cannabis and benzodiazepines as pharmaceuticals or rape drugs

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Keywords: real-time Mass Spectrometry, ambient sampling, Electron Ionization, benzodiazepines, pesticides

Objective
Extractive-liquid sampling electron ionization-mass spectrometry (E-LEI-MS) is a real-time technique that combines ambient sampling and electron ionization, allowing a high identification power by comparing the experimental spectra with the electronic libraries for the direct analysis of solid and liquid samples with no or minimal sample preparation. The system's efficiency has already been tested in various applications [1]. The E-LEI-MS capability to provide real-time data was tested for identifying pesticides in light cannabis and benzodiazepines (BDZs) in solution and in a cocktail to mimic their use as rape drugs.

Methods
In E-LEI-MS system, the sampling is enabled by the aspiration of the analytes through the high vacuum of the mass spectrometer. The vaporizing process takes place in the vaporization microchannel (VMC) using the high temperature. BDZs and pesticides were analyzed by E-LEI-MS coupled with an Agilent 7250 GC/Q-ToF-MS and Agilent 7010 GC/QqQ-MS, respectively. VMC and source temperatures were selected according to the molecular weight, boiling point, and degradation temperature of the analytes. Different cultivars of light cannabis were fortified with selected pesticides, then extracted with ACN by sonication and centrifugation. The supernatant was filtered and analyzed by direct aspiration in MRM acquisition mode. Six out of nineteen BDZ solutions, selected for their common use, were used to fortify the cocktails at 20 mg/L and 100 mg/L. 20 µL of the fortified cocktails were spotted on a glass surface and dried. The E-LEI-MS sampling process occurred directly on the glass surface through the coaxial capillaries, allowing the flow of solvent for the extraction of the analytes and their aspiration to the MS [1].

Results
MRM transitions were optimized for all pesticide standards and applied to analyse cannabis extracts. Despite the complexity of the matrix, the MRM mode allowed pesticides detection. Among 19 BDZs analyzed using the E-LEI-Q-ToF-MS configuration, 17 were identified by libraries. The six BZDs used to fortify the gin tonics were properly identified by the experimental library even at the lowest concentration of 20 mg/L, demonstrating the E-LEI-MS potential in forensic applications.

Conclusions
These findings demonstrate the E-LEI-MS capability in real-time targeted and untargeted analysis, extending the application field of this technique in quality control, pharmaceutical, and forensic applications.

ACKNOWLEDGMENTS

This work has been funded by the European Union - NextGenerationEU within the framework of PNRR Mission 4 - Component 2 - Investment 1.1 under the Italian Ministry of University and Research (MUR) program PRIN 2022" - 202224R9NL - CUP: H53D23003760001. The authors thank Agilent Technologies for providing the MS instrumentation and the Toxicology Laboratory in Pesaro, Italy for preparing BDZ solutions.

REFERENCES

[1] Arigò A., Famiglini G., Marittimo M., Agostini M., Renzoni C., Palma P., Cappiello A., (2023). Extractive-liquid sampling electron ionization-mass spectrometry (E-LEI-MS): a new powerful combination for direct analysis, *Sci Rep*, 13, 6429..

Honey bacteria as a source of new potential antibacterial therapeutics

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Keywords: secondary metabolites, honey microbiome, antibacterial properties, antibiotic resistance, MALDI-TOF/MS, Capillary Zone Electrophoresis

Objective
The antibiotic resistance and growth of multidrug resistant bacteria poses a huge treat to human health worldwide, leading to many antibiotics becoming ineffective. It is essential to constantly search for new drugs which will become successful in the treatment of many bacterial infections. Honey and its microbiome may come to the rescue, providing a matrix for producing potential new therapeutic substances. Previous studies have shown that bacteria isolated from a natural substance such as honey can possess antibacterial properties. However, it is related to the production of secondary metabolites that can have bactericidal or bacteriostatic effects even against antibiotic-resistant bacteria. Secondary metabolites can be identified using separation techniques such as capillary zone electrophoresis (CZE), liquid chromatography with mass spectrometry (LC/MS), or the Matrix-assisted laser desorption/ionization (MALDI) spectrometric technique. This may contribute to the identification of new compounds with antibacterial properties. The validity of conducting such research is confirmed by the discovery of new antibiotic - teixobactin, in 2015[1,2]. The study involved 6 strains of *Bacillus pumilus*, isolated from honey samples, which showed the ability to inhibit the growth of pathogens such as *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* or antibiotic-resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

Methods
MALDI-TOF/MS was used to identify microorganisms isolated from honey samples. Determination of antibacterial properties was carried out using Growth Inhibitory Assay. For this step, 8 reference strains were used. The antibiotic resistance of reference strains was detected with the use of MBT STAR Carba Kit. The next stage of the research involved the development of a method for the extraction of bacterial secondary metabolites and their identification using techniques such as capillary zone electrophoresis (CZE) and the MALDI method.

Results
As a result of the Growth Inhibitory Assay approach, 6 strains of *Bacillus pumilus* that showed inhibition against at least 5 different reference strains were selected for further studies. An attempt was undertaken to extract secondary metabolites and remove residual bacterial cells and spores from the obtained sample. The pure extracts were subjected to further studies.

Conclusions
Studies conducted indicate that bacteria from honeys can be a source of potential therapeutic substances. Determination of bacterial metabolites is therefore an incredibly important aspect in the search for new therapeutic agents.

REFERENCES
[1] Pajor M., Worobo R., Milewski S., Szweda P., (2018). "The Antimicrobial Potential of Bacteria Isolated from Honey Samples Produced in the Apiaries Located in Pomeranian Voivodship in Northern Poland", *Inter. J. Environ. Res. and Public Health*, 5(9), 1-14
[2] Brudzynski K., (2021). "Honey as an Ecological Reservoir of Antibacterial Compounds Produced by Antagonistic Microbial Interactions in Plant Nectars, Honey and Honey Bee". *Antibiotics* (Basel). May 9;10(5):551

The role of silver nanoparticles in enhancing detection of low-molecular-weight biomolecules in LDI-MS analysis

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Keywords: Laser Desorption Ionization Mass Spectrometry, silver nanoparticles, chemical vapor deposition, low molecular compounds

Objective
The detection of low molecular weight compounds remains a significant challenge in MALDI mass spectrometry. An approach that is becoming increasingly popular is the replacement of classic matrices with metal nanoparticles, which, thanks to their different optical properties, interact with laser radiation and enable sample ionization. This approach is not only beneficial due to significantly reduced matrix background signals, but also provides greater selectivity, sensitivity and effectiveness of analytical techniques. Proposed technique for silver nanoparticle synthesis via CVD showcased the capability to detect.

Methods
In this investigation, we detail the synthesis of silver nanoparticles (AgNPs) via a precise Chemical Vapor Deposition (CVD) methodology, aimed at augmenting the analytical performance of Laser Desorption Ionization Mass Spectrometry (LDI-MS) for the detection of various low molecular compounds such as: lipids, saccharides, amino acid and carboxylic acids.

Results
XPS and SEM-EDX analyses confirmed the metallic silver composition of the synthesised nanoparticles diameter of 33.5±1.5 nm. Comparative analytical evaluation with traditional MALDI matrices revealed that AgNPs significantly reduce signal suppression, thereby enhancing the sensitivity and specificity of LDI-MS for low molecular compounds i.a.: triglycerides, saccharides, amino acids, carboxylic acids. Notably, the application of AgNPs demonstrated a superior linear response for triglyceride signals, with regression coefficients surpassing 0.99, markedly outperforming conventional matrices. The study further extends into quantitative analysis through NALDI, where AgNPs exhibited enhanced ionization efficiency, characterized by substantially lower limits of detection (LOD) and quantification (LOQ) for tested standards.

Conclusions
These results highlight the significant potential of AgNPs synthesized via CVD to transform the analytical detection and quantifi-cation of low-molecular-weight compounds using nanoparticles-based Laser Desorption/Ionization (NALDI). This approach offers a promising avenue for expanding the scope of analytical applications in mass spectrometry, introducing innovative methodologies for enhanced precision and sensitivity.

ACKNOWLEDGMENTS
This research was funded by National Science Centre Poland grant OPUS 20; 2020/39/B/NZ7/02733).

Capillary electrophoresis analysis of oligosaccharides and glycans using rhodamine B-based labeling

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Keywords: glycan analysis, labeling, Capillary Electrophoresis, Fluorescence

Objective
The analysis and characterization of glycans are becoming increasingly important for the medical and biopharmaceutical industries, as they provide essential information that aids in developing new therapeutic agents. Derivatization of oligosaccharides and glycans released from glycoproteins is a critical step in their analysis by capillary electrophoresis (CE), liquid chromatography (LC), and mass spectrometry (MS). Because saccharides typically lack chromophores or fluorophores in their structure, labeling is necessary for detection using optical methods such as UV/Vis absorption or fluorescence.

Methods
CE/LIF analyses were performed using a P/ACETM MDQ Plus instrument (Sciex) with LIF detection. The instrument was equipped with a 532 nm laser for excitation and a 560/10 nm bandpass filter or 550 nm longpass for detection. The separations were performed in a fused silica capillary (ID 50 µm) L_{tot}/L_{eff} 60/50 cm coated by hexadimethrine bromide (polybrene).

Results
In this study, we present the synthesis of a rhodamine B-based fluorescent tag modified with a hydrazide functionality for saccharide derivatization via hydrazone formation chemistry. Rhodamine B hydrazide was synthesized through the hydrazinolysis of rhodamine B following the method described by Yang et al. [1]. The labeling conditions were optimized to achieve a high reaction yield. The labeled oligosaccharides and glycans were separated in polybrene-coated capillaries, and the separation conditions (BGE compositions, separation voltage, capillary length, etc.) were optimized. These optimized labeling and separation conditions were then applied to CE/LIF analysis of *N*-linked glycans released from model glycoproteins, such as ribonuclease B and immunoglobulin G. The analytical parameters of the developed method, based on rhodamine B labeling and CE/LIF analysis, were characterized in terms of linearity, limit of detection, and repeatability.

Conclusions
We successfully designed and synthesized a novel fluorescent rhodamine B-based label for CE analysis of oligosaccharides and glycans. We optimized labeling conditions to ensure the most efficient derivatization, including reaction temperature and time, solvent composition, and separation parameters. Additionally, the rhodamine B labeling method was employed for *N*-linked glycan profiling of several glycoproteins using CE/LIF analysis.

ACKNOWLEDGMENTS
This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic [9F23002] and the institutional research plan [RVO:68081715].

REFERENCES
[1] Yang X. F., Liu P., Guo X. Q., Zhao Y. B. (2002). Development of a novel rhodamine-type fluorescent probe to determine peroxynitrite. *Talanta*, 57, 883–890

Use of new carbon material as stationary phases in superheated water chromatography for the determination of preservatives in food and cosmetic products

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Keywords: porous graphitic carbon, Superheated Water Chromatography, parabens, green chemistry

Objective
The objective of this study was to develop a green analytical method for the analysis of preservatives in food and cosmetic samples. Specifically, among liquid chromatography (LC) practitioners, current efforts are addressed to the reduction or removal of organic toxic solvent through miniaturization and/or reduction of analysis time. Indeed, the best choice to guarantee the safety of the operator and the environment should consist in the use of pure water as mobile phase. This can be realized by using extreme temperature to drastically reduce the dielectric constant of water and enable the elution of medium polar compounds. In this regard, in the present contribution a high temperature LC (HTLC) system was obtained by coupling an LC instrument with a GC oven, which hosts the LC column, mandatorily characterized by high resistance at elevated temperatures.

Methods
The analyses were carried out on a HPLC system equipped with a photo diode array detector. Water and Ethanol were employed as mobile phase; different columns packed with porous graphitic carbon (PGC) particles and resistant up to 250 °C were tested. The target analytes (Methyl-, Ethyl-, Propyl-, Butyl-paraben) were detected setting the wavelength of PDA at 255 nm and quantified in real sample by using the standard addition method, fully validated.

Results
Parabens were employed as probe analytes to evaluate the performances of different commercially available and prototype PGC columns, in terms of robustness, efficiency and resolution. Promising results were achieved at 200-250 °C, by using mainly or only water in the mobile phase, thus maximizing the greenness of the chromatographic method, according to the last three principles of green analytical chemistry. The HTLC method was validated in terms of linearity, detection and quantification limits, accuracy and precision. All the analytes showed a goodness-of-fit measure for linear regression models ($R2 > 0.98$) in a relatively broad range (three order of magnitude). Chromatographic separations were achieved in less than 10 min, thus enhancing the analytical throughput compared to the official method for the analysis of such additives.

Conclusions
An environmentally sustainable analytical method was developed for the analysis of parabens in both food and cosmetic products. Limit of detection and quantification were below legislative limits. Satisfactory accuracy and precision were achieved at different concentration levels, thus ensuring the suitability of the analytical method for the purpose of the present research.

ACKNOWLEDGMENTS
The authors acknowledge the Perkin Elmer Corporation and Merck Life Science for their support.

UHPLC-QToF MS analysis of phenolic compounds in okara

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Keywords: okara, bioactive components, phenolic components, isoflavones

Objective
Okara is a by-product of soymilk production with a rich nutritional profile, particularly in bioactive compounds. Okara holds promise for making more valuable products, which can bring economic benefits and reduce pollution at the same time. Due to its high content of bioactive compounds, okara products can enhance nutrition and address health concerns [1].

Methods
The aim of this study was to determine phenolic compounds present in okara using UHPLC-QToF MS technique. The QToF-MS system was equipped with Dual Agilent Jet Stream electrospray ionization (ESI) source, operating in negative (ESI-) ionization mode. Fine milled okara was extracted with 10 ml of 80% methanol containing 0.1% HCl, for 1h, centrifuged, filtered through 0.22µm syringe filter and then analysed. Phenolic compounds and their derivatives were identified on the basis of their monoisotopic mass, MS fragmentation and available literature data.

Results
UHPLC QToF analysis revealed the presence of three phenolic acids and thier derviatives, eighteen isoflavones and their derivatives and three other phenolic components. Phenolic acids that were detected are benzoic acid (121.0320 *m/z*), vanillic acid hexoside (329.0943 *m/z*) and syringic acid hexoside (359.1060 *m/z*). Results imply that okara is the most abundant with isoflavones dazdein, genistein gycitein isomers and their acetyl derivates. Other phenolic components that were identified were kaempferol 3-*O*-glucoside (Astragalin) (447.0994 *m/z*), kaempferol 3-*O*-(2"-*O*-rhamnosyl) hexoside (593.1653 *m/z*) and genistein 7-O-(6"-O-hexosyl) hexoside (609.1596 *m/z*).

Conclusions
Food rich with antioxidants especially isoflavones as genistein and daidzein is associated with reduction in the rates of certain cancers, cardiovascular disease, obesity diabetes and other autoimmune diseases [1,2]. This suggests that food enriched with okara possesses functional properties and potential in preventing non-communicable diseases.

ACKNOWLEDGMENTS
This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-65/2024-03/200116 and the Science Fund of the Republic of Serbia, #GRANT No. 7744714, FUNPRO.

REFERENCES
[1] Barnes, Stephen 1998. Evolution of the Health Benefits of Soy Isoflavones. Proceedings of the Society for Experimental Biology and Medicine 217: 386-396. doi: 10.3181/00379727-217-44249
[2] Kamble, Dinkar B and Savita Rani 2020. Bioactive components, in vitro digestibility, microstructure and application of soybean residue (okara): A review. Legume Science 2: e32.

Liquid chromatography as a front-end sample preparation online coupled to gas chromatography-mass spectrometry for polycyclic aromatic hydrocarbons analysis in extra virgin olive oil

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Keywords: polycyclic aromatic hydrocarbons, extra virgin olive oil, Liquid-Gas Chromatography

Objective

The aim of the present research was the development of a rather simple and rapid automated online liquid-gas chromatography method for polycyclic aromatic hydrocarbons determination in extra virgin olive oil. Thanks to the use of a LC silica column, employed to retain the bulk of the matrix (mainly triglycerides), a minimal sample preparation (oil dilution) was performed.

Methods

Triple quadrupole MS was used, with two acquisition modes evaluated and compared, namely, SIM and pseudo multiple-reaction-monitoring (p-MRM). The latter, based on the monitoring of transitions with no mass loss between the precursor and the product ion, enables the reduction or elimination of co-eluting isobaric interferences due to collision-induced-dissociation.

Results

The p-MRM mode provides superior performance in terms of specificity and analytes detectability. The method was linear over the concentration range 1-200 μg kg^{-1}, accuracy was in the 86.9-109.3 % range, intra-day and inter-day precision were in the 1.2-9.7 % and 3.2-10.8 % ranges, respectively. For all the PAHs, a negative matrix effect, from -68 % to -36 %, was observed.

Conclusions

A dilute-and-inject LC-GC-tandem mass spectrometry method is herein proposed fulfilling EU legislation requirements; sample preparation was very simple, inasmuch that it involved only a dilution step, thus avoiding extraction, clean-up, and thus a high consumption of organic solvents. In fact, considering both oil dilution and the LC mobile phase, less than 8 mL of solvents were used.

ACKNOWLEDGMENTS

The authors thank Shimadzu Corporation and Merck Life Science for their continuous support.

The research was performed within the framework of the Research Project PRIN PNRR - MISSIONE 4, COMPONENTE 2, INVESTIMENTO 1.1 - BANDO PRIN 2022 PNRR - DECRETO DIRETTORIALE N. 1409 DEL 14-09-2022: gReen analytical methods for PAHs detection in EVOO: from lABoratory to smart LabEl (RELIABLE), supported by the Italian Ministry of University and Scientific Research, CODICE IDENTIFICATIVO: P2022842MJ, CODICE CUP: J53D23014520001.

Determination of sea-food emissions by HS-SPME-GC-MS. Mussel odor volatiles as indicators of quality and environmental pollution

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Keywords: *Mytilus galloprovincialis* Lamarck, salty waters, odor volatile emissions, HS-SPME-GC-MS, quality, environmental pollution.

Objective
Mediterranean mussels can be potential biomonitors of their living environment, as they are prone to accumulate both organic and inorganic contaminants for their high filtration capacity. Volatile organic compounds (VOCs) are among the most common environmental pollutants, originating from natural and anthropogenic activities. Odorant VOCs of the mussels have been less investigated, although they are fundamental for their real and perceived quality and reflect environmental conditions. This research focuses on the VOCs emitted by native and introduced mussels cultivated in Faro Lake, a small coastal basin in the northeastern corner of Sicily (Italy) [1].

Methods
The experimental plan included: sampling of imported mussels before they were introduced into the Faro Lake (Messina, Italy); periodic collection of water and mussel samples after one week from the introduction into the lake and up to 120 days. Samples of autochthonous mussels were additionally collected to perform an estimation with imported mussels. Water samples were collected at the same depth as the mussel housing. The volatiles of mussels and waters have been studied by Headspace Solid-Phase Microextraction (HS-SPME) followed by Gas Chromatography coupled with Mass Spectrometry (GC-MS). The external standard method has been used for the volatile quantification. Each compound was identified using mass spectra, NIST'18 library (NIST/ EPA/NIH Mass Spectra Library, version 2.0, USA), FFNSC 3.0 database, Linear Retention Indices, and literature data.

Results
Approximately 100 VOCs have been detected and quantified in mussels and the lake water. In particular, alcohols, aliphatic and aromatic aldehydes, ketones, terpenes, sulfur compounds, hydrocarbons, amines, and furans have been identified. Environmental pollutants have been detected as well. The main compound in all samples was dimethylsulfide (DMS), followed by dimethylamine (DMA); both substances exceeded the olfactory threshold. There has been a significant difference in VOC composition of native and imported mussels, even during their growing in the lake water.

Conclusions
In light of our results, mussel VOCs can be potential indicators of their quality and biomonitors of environmental pollution.

REFERENCES

[1] Bordignon F., Aprea E., Betta E., Xiccato G., Trocino A., (2024). Effect of the farming site and harvest time on the nutritional, elemental and volatile profile of mussels: A comprehensive analysis of the PDO 'Cozza di Scardovari', *Food Chemistry,* 140078.

Use of flow-modulation comprehensive two-dimensional enantio-gas chromatography as valid and flexible alternative to heart-cutting multidimensional enantio-gas chromatography

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Keywords: Comprehensive two-dimensional Gas Chromatography, flow modulation, chiral analysis

Objective

This research aims to propose the use of flow-modulation comprehensive two-dimensional enantio-gas chromatography (FM eGC × GC) as a valid, flexible, and potentially superior alternative to heart-cutting multidimensional enantio-GC (eMDGC).

Methods

The analytical instrument consisted of low duty-cycle FM eGC×GC system with time-of-flight mass spectrometry, equipped with an enantioselective ^{1}D column (2,3-di-O-methyl-6-t-butyl silyl β-cyclodextrin derivative) and a ^{2}D polyethylene glycol one. The enantioselective ^{1}D column is the best choice, due to a much higher separation power compared to a short ^{2}D one.

Results

The present investigation was exploited for the simultaneous determination of fifteen target chiral lactones and the investigation of Marsala volatilomes, highlighting their highly complex nature, with over 300 compounds tentatively-identified. Then particular attention was devoted to a side by-side comparison between FM eGC × GC and eMDGC in the chiral only analysis of Citrus essential oils.

Conclusions

The scope of the present research is to propose the use of FM eGC ×GC as a primary analytical technique for the determination of both chiral and untargeted volatile compounds. To demonstrate the capabilities of this analytical platform, complex samples were subjected to analysis. The results obtained confirm the high usefulness of the analytical platform employed, as it can produce two types of information in a single run.

ACKNOWLEDGMENTS

The authors acknowledge LECO Corporation and Merck Life Science.

Application of MBT-STAR-BL and MBT-ASTA tests for antibiotic resistance analysis based on MALDI-TOF MS technique

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Keywords: antibiotic resistant, bacteria, MBT-STAR-BL test, MBT-ASTRA test, mass spectrometry

Objective
For decades, antimicrobial drugs have enabled medical progress. Antibiotics potency and accessibility have led to their overuse, which has resulted in the antimicrobial resistance (AMR). Although outpatient antibiotic consumption has been declining in subsequent years, new evidence from the World Health Organization (WHO) suggests widespread overuse of antibiotics during the Covid-19 pandemic worldwide, which may have exacerbated the spread of AMR [1]. It estimates that if antibiotics are not available by 2050, the total number of deaths from bacterial infections associated with multidrug resistance (MDR) will be about 10 million, exceeding deaths from heart disease and cancer combined [2]. One of the methods for the rapid detection of antibiotic resistance is the matrix- assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) approach. The most frequently used test for β-lactam resistance analysis is the MBT-STAR-BL test, which monitors the hydrolysis of antibiotics by observing specific mass shifts. The disadvantage of this type of test is that only gram-negative bacteria are analyzed and that antibiotic resistance to only one of many types of antibiotics can be detected. The second, newly introduced test is the MBT-ASTRA test based on the assessment of bacterial growth in the presence of the antibiotic. The manufacturers assume that it will allow for the analysis of practically all bacteria and antibiotics. This gives hope for the rapid and accurate detection of antibiotic resistance also in gram-positive bacteria [3].

Methods
The two aforementioned tests were compared by conducting analyses for Gram-negative bacteria and the most common beta-lactam antibiotics (cefotaxime, ceftriaxone, ceftazidime, imipenem, meropenem, ampicillin, piperacillin). The MBT-ASTRA test was also used to analyze Gram-positive bacteria. Additionally, the MBT-ASTRA test was employed to analyze two of the most frequently used antibiotics during the COVID-19 pandemic (azithromycin and teicoplanin).

Results
As a result of the analyses, promising results were obtained in detecting antibiotic resistance

Conclusions
The obtained results can be used to better understand antibiotic resistance in different bacterial populations and to make informed therapeutic decisions

REFERENCES
[1] Akram F., Imtiaz M., ul Haq I., (2023). Emergent crisis of antibiotic resistance: A silent pandemic threat to 21st century, *Microbial Pathogenesis,* 174, 105923
[2] Founou R.C., Blocker A.J., Noubom M., Tsayem C., Choukem S. P., Van Dongen M., Founou L. L., (2021). The COVID_19 pandemic: a treat to antimicrobial resistance containment, *Future Sci OA.,* 7, FSO763
[3] Lange C., Schubert S., Jung J., Kostrzewa M., Sparbier K., (2014). Quantitative Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Rapid Resistance Detection, *Journal of Clinical Microbiology,* 52, 4155-4162.

Microwave-assisted extraction and characterization by GC×GC-MS of solvolysis products of wind turbine blade materials

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Keywords: wind turbine blades, solvolysis, Microwave-assisted extraction, GC×GC-TOFMS, new energy materials

Objective
This project aimed to develop analytical solutions to understand better the recycling of chemicals and valuable materials from new energy material waste, specifically focusing on wind turbine blades. This research is driven by the need to find sustainable methods for managing the end-of-life of renewable energy infrastructure components.

Methods
Wind turbine blades were subjected to 8-hour and 13-hour solvolysis treatments. The solvolyzed samples, referred to as solvolysis soups, were neutralized due to their extremely basic pH levels (around 14). Subsequently, the liquid phases were filtered to separate solid residues, while the solid phases were processed directly after neutralization.
The products of these procedures were then subjected to solid-liquid and liquid-liquid microwave-assisted extraction (MAE) using a solvent mixture of hexane and methanol (10:3 ratio), followed by the addition of water (2.5 ratio) and centrifugation. The upper organic phase was collected, concentrated, and analysed using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS), equipped with a non-polar column in the first dimension and a medium-polar column in the second dimension, connected with a cryogenic modulator.

Results
The MAE-GC×GC-MS methodology enabled the identification of approximately 60 molecules belonging to various chemical classes, such as aromatic, nitrogen-, and oxygen-containing compounds. The identification was based on mass spectral electron ionization (EI) database matching at 70 eV (≥800/1000) and the Linear Retention Index (LRI) window in the ±20 range, according to the non-polar ^{1}D-GC LRI information reported in the NIST database and literature. The location of the investigated molecules on the 2D-GC plane was also considered.

Conclusions
This work contributes to the development of sustainable waste management practices in the wind energy sector, highlighting the potential for improved recycling techniques to efficiently recover valuable materials from wind turbine blades.

Bioactive content identification through liquid chromatography high resolution mass spectrometry and in vitro enzyme inhibitory effect of 19 chili pepper varieties

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Keywords: chili pepper, HPLC-HRMS, chemical composition, biological activity, polyphenols, enzyme inhibition

Objective
Chili peppers have attracted significant interest due to their potential health-promoting properties, mostly related to a a plethora of phytochemicals, particularly polyphenolic compounds, that may confer various biological activities. This study aims to comprehensively characterize the polyphenol profile of 19 distinct Italian chili pepper varieties and evaluate their inhibitory effect on enzymes with important biochemical functions.

Methods
The samples were macerated in deionized H_2O or EtOH at 4°C for 24h and then filtered. Samples were analyzed by UHPLC-HRMS to monitor their phytochemical composition by applying a targeted metabolomic screening. To identify any bioactivity potential, "in-house" developed assays were performed based on the activity of acetylcholinesterase, butyrylcoholinasterase, pancreatic lipase, α-glucosidase and tyrosinase. Lastly, the total antioxidant capacity and the total phenolic content (TPC) were measured using a 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and a Folin assay, respectively.

Results
The results showed significant variation in the types and quantities of polyphenols present across the 19 varieties. Major polyphenols identified included capsaicin, dihydrocapsaicin, luteolin, apigenin, and quercetin derivatives. Moreover, the results showed that the ethanol extracts contained a significantly greater number of polyphenol compounds and exhibited stronger biological activities compared to the aqueous extracts. In addition, the tested extracts were classified to three categories demonstrating i) mild (<40%), ii) medium (40-70%) and iii) strong (>70%) inhibition against each enzyme. Dose-repsonse curves (6.25-100 mg/mL) were prepared for the extracts inducing a strong effect and half maximal concentrations (IC_{50}) were in the range 15-69 mg/mL. Importantly, the Pearson correlation coefficients (r) were calculated and strong correlations were noticed (r>0.5).

Conclusions
This comprehensive investigation of 19 distinct chili pepper varieties has provided insights into the phytochemical profiles and associated biological activities of this economically and nutritionally important crop. The results demonstrate that chili peppers are a rich source of diverse polyphenolic compounds, with significant variability observed across the tested cultivars.

Green separation and isolation of aroma-active components from mixture of natural aliphatic and aromatic esters by reversed phase liquid chromatography

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Keywords: Reversed phase Chromatography, Flash Chromatography, methyl salicylate, ethyl nonanoate

Objective
Aroma-active ethyl nonanoate has a distinct odor, and even less than 2% creates an off-note in natural methyl salicylate. Methyl salicylate, a major component of wintergreen and its essential oil, is colorless with a sweet, minty odor and has analgesic, anti-inflammatory properties used in topical analgesics [1]. Flash chromatography is used in chemistry for isolating natural products, sample enrichment, and purification, offering cost-effectiveness, time efficiency, and high yield [2]. This study presents development of a green, sustainable method for isolating natural methyl salicylate and ethyl nonanoate using reversed-phase liquid chromatography (RP-LC). The transformation from analytical scale method to preparative scale method was performed.

Methods
Preliminary experiments in analytical scale using RP-HPLC-UV enabled to select suitable separation conditions, emphasizing safety and reusability of mobile phase solvent. A gradient mobile phase of ethanol and deionized water (1 mL/min with column at ambient temperature) was tested. For scale-up, a fully automated flash chromatography instrument for large-scale production (up to 1850 g columns) was used. The flash chromatography method in this study used a C18 column, characterized by stability and high load capacity - up to 200 g on a 1500 g column. Chromatographic conditions selected from analytical scale HPLC were applied to a 120 g column with a flow rate of 50 mL/min. Fractions (45 mL) were collected based on chromatographic profiles. Successful separation led to the use a 1500 g column with a 220 mL/min flow rate. The effectiveness of isolating aroma-active esters and determining the purity of each fraction was assessed using GC-FID.

Results
Ethyl nonanoate was successfully isolated, resulting in a final product (methyl salicylate, 99% purity) containing less than 0.1 % of ethyl ester as impurity. Developed chromatographic method achieved a higher recovery yield (> 82%) and reduced organic solvent consumption by 50% compared to silica columns with non-polar organic solvents [3]. The environmental impact of methods was assessed using the Analytical GREEnness calculator (AGREE) and Green Analytical Procedure Index (GAPI).

Conclusions
The developed methodology demonstrates excellent green characteristics, with the main drawbacks being the quantity and toxicity of the ethanol used as the mobile phase.

ACKNOWLEDGMENTS
This work was supported by the Slovak Research and Development Agency under the contract number APVV-20-0317.

REFERENCES
[1] Gondor O. K., Pál M., Janda T., Szalai G., (2022) The role of methyl salicylate in plant growth under stress conditions, *J. Plant Physiol*., 277, 153809
[2] Výbohová V., Hroboňová K., (2023) Isolation of Natural Compounds with Organoleptic Properties Using Selected Advanced Extraction Techniques, *Chem. Listy*, 117, 365-372
[3] Výbohová V., Hroboňová K., in: *Current state and perspectives of analytical chemistry in practice: ACP 2022*. p. 74. Spektrum STU, Bratislava 2022, ISBN 978-80-227-5206-0.

QSRR approach for identification of phenolic compounds and evaluation of their retention behaviour under reverse-phase liquid chromatography

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Keywords: QSRR, flavonoids, phenolic compounds, molecular descriptors, bergamot juice

Objective
Quantitative Structure-Retention Relationship (QSRR) models were developed to identify phenolic compounds using a typical HPLC-PDA-ESI/MS system. A new chromatographic method was developed for the separation of 52 standard phenolic compounds. Over 5000 descriptors for each standard were calculated using AlvaDesc software and then selected through Genetic Algorithm. The selected descriptors were used as variables for QSRR models construction and to obtain a better understanding of the retention behaviour of phenols during reverse-phase separation.

Methods
Three distinct molecule sets, including 52 phenolic compounds (Set 1), 32 flavonoids (Set 2) and 15 mono-substituted flavonoids were divided into training and validation sets to build PLS, MLR and PLS-ANN models, using PLS_Toolbox on Matlab software. To assess the predictivity of the models, these were tested on a bergamot juice sample.

Results
About retention behaviour, the already known variable affecting flavonoids elution were confirmed; moreover, the influence of substituents and their positions was assessed. Amog QSRR models, PLS and PLS-ANN exhibit the lowest prediction error (from 1.9% to 4.8%, depending on the set), and PLS-ANN of Set 1 and 3 showed the best predictive power in real sample recognition.

Conclusions
The building and implementation of such predictive models showed to be a powerful tool to identify phenolic compounds based on retention data and avoiding the use of expensive detectors such as tandem MS

Analytical evaluation of phenols in olive oil by-products

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Keywords: Olive pomace; olive mill wastewater; lipophenols; phenols; HPLC; mass spectrometry

Objective
Olive mill wastewater (OMWW) is characterized by a dark color and high concentrations of organic molecules. Olive pomace (OP) is a solid waste rich in potassium, consisting mainly of water, seed, and pulp with a high organic matter and carbon content. Furthermore, both matrices contain phenolic and lipid compounds. Due to their acidic nature and high concentrations of salts and phenolic compounds, both matrices exhibit a high pollution index, which makes them potentially harmful for the environment.

Methods
Liquid-liquid and solid-liquid extraction techniques were employed for the extraction of phenolic and lipophenolic compounds from OMWW and OP samples. The samples belong to three olive cultivars (*Roggianella*, *Nocellara*, and *Coratina*) sourced from olive oil producers in Cosenza, Messina, and Bari. The sample preparation involved the use of ethyl acetate and methanol. The OP samples were pretreated with n-hexane and then processed. Extracts were subjected to HPLC analysis, which was carried out employing Nexera UHPLC system (Shimadzu, Kyoto, Japan) equipped with an Ascentis Express F5 column (150 × 4.6 mm, 2.7 µm), coupled with a photodiode array detector (PDA) SPD-M20A and an LCMS-2020 mass spectrometer (MS) with an electrospray ionization source (ESI) set in negative mode. Calibration curves were created in Single Ion Monitoring mode (SIM) for a concentration range of 1–100 mg L^{-1}. Several figures of merit were assessed, including linearity, limits of detection (LoD) and quantification (LoQ), precision (intra- and inter-day), accuracy, matrix effect, and recovery.

Results
The OMWW and OP samples demonstrated that the *Coratina* and *Nocellara* cultivars exhibited significantly higher levels of polyphenols, displaying a more diverse and abundant phenolic composition in comparison to the *Roggianella*. Among the cultivars examined, only *Coratina* was found to contain high lipophenolic content. In OP samples, *Coratina* exhibited the highest levels of polyphenols and lipophenols.

Conclusions
This study presents a sensitive and robust analytical method for the quali-quantitative characterization of bioactive compounds (polyphenols and lipophenols) in samples of OMWW and OP. The *Coratina* and *Nocellara* cultivars have been identified as particularly promising for the extraction of bioactive compounds from their by-products.

ACKNOWLEDGMENTS
Research was performed within the framework of the Research Project PRIN PNRR 2022- MISSIONE 4, COMPONENTE 2, INVESTIMENTO 1.1 - BANDO PRIN 2022 PNRR – D.D. N. 1409 DEL 14-09-2022: REcovery and Enhancement of WAste and processing products of the olive oil industry thRough innovative analytical methods for the Development of newly developed NUTRAceutical and COSMetic prOducts (REWARD-NUTRACOSMO), supported by the Italian Ministry of University and Scientific Research, no. Prot. P20224ZHA5, CUP: J53D23014480001.

Forest springs in Czechia. Is the water safe to drink?

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Keywords: springs, forest, chemical pollution, bacteriological contamination

Objective
Springs are classified as natural exits of underground water sources. Springs often serve as a source of drinking water either for random tourists or are specifically targeted by residents as an alternative to tap water. In most cases no information about water quality is available, which can lead to very serious health consequences for consumers. The aim of this study was to determine physical parameters, chemical and microbiological composition of water in selected forest springs in Czechia.

Methods
During the sampling water temperature and spring yield and in laboratory pH, conductivity, chemical oxygen demand, acid neutralizing capacity, chemical and microbiological composition were measured. Chemical analyses included determination of anions by ion chromatography (ICS 2100, Dionex), cations by capillary electrophoresis (Agilent 7100, Agilent Technologies), ammonium by continuous flow analyser with fluorescence detection [1], elements by ICP-MS (Agilent 7700x, Agilent Technologies), total organic carbon and dissolved organic carbon by TOC-VCPH analyser (Shimadzu). Microbiological composition (Intestinal Enterococci, Escherichia coli and Coliform bacteria) of water samples was provided by the Public Health Institute Ostrava.

Results
Forest springs with easy accessibility guided in the database of estudanky.eu as a source of drinking water were selected for monitoring. Quality of water was evaluated according to the requirements for drinking water. Conductivity and pH of most samples complied with required limits. Most of samples also complied with required limits for chemical composition. However, many water samples was microbiologically contaminated (mostly with Coliform bacteria), indicating faecal pollution. A comparison of results in spring and autumn season was also provided.

Conclusions
In most cases water from forest springs did not comply with the requirements for drinking water mostly due to bacterial contamination. Drinking of such water can cause indigestion and worse health troubles. For these reasons tables with detailed results were posted at the springs for future passers-by.

ACKNOWLEDGMENTS
This work was supported by Research Programme Strategy AV21 Water for Life.

REFERENCES
[1] Alexa L., Mikuska P., (2020). Simultaneous determination of gaseous ammonia and particulate ammonium in ambient air using cylindrical wet effluent diffusion denuder and a continuous aerosol sampler, *Anal. Chem.*, 92, 15827-158366.

Nitrogen and hydrogen as alternative carrier gases in GC-FID analysis of bergamot essential oil

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Keywords: Gas Chromatography, Flame Ionization Detector, bergamot, essential oil, carrier gas

Objective

The typical and unique smell of the essential oils generates a particular interest around the global flavor and fragrance market. This research is focused on the development of a gas chromatography-flame ionization detector (GC-FID) method for the analysis of bergamot essential oil by using Nitrogen (N2) and Hydrogen (H2) as carrier gases. Helium (He) is conventionally used as carrier gas in gas chromatography analysis thanks to its great chemical properties but, due to its shortage or slow supply, it is increasingly necessary to find an alternative that maintains similar analytical performances.

Methods

After diluting the cold-pressed essential oil in *n*-hexane (Merck, Darmstadt, Germany), separation and quantification of various components were performed by using Nexis GC-2030 gas chromatograph (Shimadzu Europa, Germany) equipped with a split-splitless injector and a FID detector. Sample injection was performed by using an automatic AOC-20i autosampler. A SLB-5ms fused-silica capillary column (Merck, Darmstadt, Germany) was employed. Pure nitrogen and hydrogen, used as carrier gases in carrying out the analysis, were produced in laboratory by using generators.

Results

More of 60 compounds, including monoterpenes, sesquiterpenes, and oxygenate derivatives (aldehydes, ketones, alcohols, and esters) were satisfactory separated, in accordance with the results obtained in the case of bergamot essential oils analyzed by using helium as carrier gas in GC-FID analysis.

Conclusions

The shortage of helium in recent years, the discontinuity of supplies and the increasing costs of this gas, encourages the study of the use of alternative gases to employ in gas chromatography. Furthermore, the production of these gases directly in the laboratory, by using generators, avoids the problems described above and reduces costs.
The developed method guarantees reliability in the separation of the components of an essential oil, allowing the use of nitrogen and hydrogen as alternative carrier gases in GC-FID analysis.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Merck Life Science and Shimadzu Corporation for their continuous support.

Scouting of separation strategies in comprehensive two-dimensional liquid chromatography for the in-depth metabolites characterization of complex food samples

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Keywords: LC×LC, HILIC×RPLC, RPLC×HILIC, herbal lıqueurs, secondary metabolites

Objective
Achieving a comprehensive characterization of complex food samples often requires the use of advanced chromatographic platforms.
Comprehensive two-dimensional liquid chromatography is one of the most used approaches to obtain high-quality data acquisition for unequivocal identification. In this context, one of the most complex phenolic compounds mixtures is represented by herbal liqueurs, typically produced by macerating several herbs, roots, flowers, bark, and/or citrus peels in alcohol, either neutral spirits or wine, mixing the filtrate with sugar syrup, and allowing the mixture to age in casks or bottles.

Methods
The determination of polar compounds in food analysis is generally performed by means of reversed-phase liquid chromatography (RP) and hydrophilic interaction liquid chromatography (HILIC) separation The aim of this contribute is to compare different platforms (HILIC × RPLC, RPLC × HILIC) for the analysis of complex polyphenols samples. For an in-depht evaluation, more then thirty herbal lıqueurs were analyses. In particular, an SeQuant ZIC-cHILIC column (150 × 1.0 mm 3µm, 100Å, Merck) and Ascentis Express C18 column (50 × 4.6 mm 2.7 µm, Merck) were used in the ^{1}D and ^{2}D, respectively of the HILIC × RPLC set-up. For RPLC × HILIC separation, Ascentis Express ES-CN column (150 × 1.0 mm 2.7 µm, Merck) and SeQuant ZIC-HILIC column (50 × 3.0 mm 2.7 µm, 160Å, Merck) were used in the ^{1}D and ^{2}D, respectively.

Results
In this contribute, the performance of HILIC × RP and RP × HILIC set-up were evaluated. Both configuration were optimised in term of of peak capacity, orthogonality and maximum number of identified compounds. Fixed solvent modulation, involving the addition of a make-up pump of a weak mobile phase after the ^{1}D separation was combined with a large volume of ^{2}D flow. Moreover, hyphenation with PDA and MS/MS detectors and chemometric approaches allowed discriminating between the several isomers presents in the different botanical families.

Conclusions
This study reports in-depth evaluation of the potential pros and cons of HILIC × RP and RP × HILIC for the characterization of secondary metabolites in more than 30 herbal liqueurs. As a result, a significant number of compounds were identified through PDA, MS/MS detectors and literature data

ACKNOWLEDGMENTS
The authors are thankful to Shimadzu and Merck Life Science Corporations for the continuous support.

Effect of water matrix on removal of pesticides by UF/NF/RO membranes

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Keywords: pesticides, environment, water, membrane separation processes, pollution

Objective
Due to their toxicity, persistence, and bioaccumulation, pesticides are considered one of the most toxic groups of chemicals to which humans are exposed [1]. Their harmfulness is also recognized by the European Union, so that the neonicotinoid insecticides (acetamiprid, clothianidin, thiacloprid) were included in the list of substances potentially hazardous to the environment in Diective 2015/495/EU and 2018/840/EU and high-quality data is collected through their mandatory monitoring. Membrane separation processes such as reverse osmosis (RO) and nanofiltration (NF) are used extensively in the production of drinking water and in wastewater treatment due to their high efficiency in removing all undesirable components dissolved in water. In this work, the influence of water matrix on the removal of pesticides by ultrafiltration (UF), NF, and RO membranes was examined.

Methods
The removal of three pesticides (clothianidin, acetamiprid, and thiacloprid) was tested. The solutions were prepared in different water sources (demineralized water and a model solution) with a mass concentration of 10 mg L^{-1}. For the tests, UF (GH), NF (NF, DK, ESNA1-LF), and RO (XLE, ESPA4) membranes were used. The tests were carried out in a UF/NF/RO laboratory device consisting of six cells connected in parallel, each containing a membrane with a membrane surface area of 11 cm^2. The feed and permeate streams were analysed using a high performance liquid chromatograph. The membranes were analysed using Fourier transform infrared spectroscopy (FTIR) to determine the possible interactions between the membrane and the pesticide.

Results
Ultrafiltration membrane with a retention factor < 10% did not prove to be suitable for the removal of pesticides from aqueous matrices. The NF membranes had a retention factor ranging from 40 to 80% for the matrix of demineralized water and model solutions, while the retention factor of RO membranes is > 90%. The results showed that the size exclusion mechanism is the predominant mechanism as the pesticides are hydrophilic and no interactions were confirmed by FTIR.

Conclusions
Membrane separation processes such as NF and RO can significantly increase the efficiency of pesticide removal, including those that are resistant to conventional methods. The integration of membrane separation processes as a complement to conventional water treatment procedures represents an advanced approach that can significantly improve water quality and ensure the safety of the water supply.

ACKNOWLEDGMENT
This research was funded by the NATO Science for Peace and Security Programme under grant id. G6087.

REFERENCES
[1] Biziuk M., Przyjazny A., Czerwinski J., Wiergowski M., (1996). Occurrence and determination of pesticides in natural and treated waters, *J. Chromatogr. A,* 754, 103-123.

A novel LC-ECD/UV method for the simultaneous determination of monoamine, nucleotide and β-endorphin neurotransmitters

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Keywords: LC-ECD/UV, β-endorphin, gradient elution, neurotransmitters, column-switching

Objective
Monitoring of neurochemical dynamics has become an effective tool for psychiatric research in understanding the background of mental health disorders. Neuro-immunological changes, such as an increase in plasma ATP and fluctuations in monoamine levels, have been reported in an animal model of autism [1] and in a study of schizophrenic patients [2]. In some publications, changes in plasma β-endorphin content of patients were reported as a possible predictor of the outcome of schizophrenia. It is known that a significant number of schizophrenic patients show physio somatic symptoms reminiscent of chronic fatigue syndrome. Our aim was to make the examination of our chronic fatigue animal model more informative with neurochemical measurement data. To this end, we have modified our LC-ECD/UV analytical method [3] to enable the simultaneous measurement of β-Endorphin contents in addition to monoamines and adenine nucleotides.

Methods
In the chronic fatigue (CFS) animal model, C57BL/6 and P2X7 receptor deficient mice were injected with Poly I:C systemically and compared to a vehicle control. To test for fatigue-like behaviors, we examined voluntary wheel running. Mouse plasma monoamines (NE, DA, 5-HT), adenine nucleotides (ATP, ADP, AMP), adenosine (Ado), and β-Endorphin (BE) were quantified using an online column-switching liquid chromatographic technique. Solid phase extraction (SPE) was performed on a Phenyl-Hexyl column and coupled to an ACE Super-C18 analytical column for gradient separation. Shimadzu HPLC systems were used. Analytes were detected with Agilent UV and electrochemical detectors. Differences were analyzed with Factorial (ANOVA) and LSD post hoc tests.

Results
β-Endorphin was shown to be particularly sensitive to the concentration of organic modifier within the mobile phase. Thus, the conditions of separation were optimized for compounds with different chemical properties, with special regard to the β-Endorphin component, for which the application of gradient elution is appropriate. In the optimization approach, we took into account the change in the composition of the mobile phase during the analysis, and its effect on the sensitivity of the amperometric detection. The validated method resulted in a 90.5±6.7 % recovery of the applied internal standard, which was not compromised when the volume of 100 µl of the plasma extract was injected in two steps onto the enrichment column. The obtained data indicate that a detection sensitivity of 50 pg/ml for β-Endorphin can be achieved using the HPLC-ECD technique.

Conclusions
Therefore, this analytical method, with the simultaneous measurement of the monoamine, adenine nucleotide and β-endorphin content of the samples, makes an important contribution to the investigation of animal experimental models and psychiatric diseases.

References
[1] Horváth G, Otrokocsi L, Beko K, Baranyi M, Kittel Á, Fritz-Ruenes PA, Sperlágh B., (2019). P2X7 Receptors Drive Poly(I:C) Induced Autism-like Behavior in Mice. *J Neurosci*, 39(13):2542-2561.
[2] Kristóf Z, Baranyi M, Tod P, Mut-Arbona P, Demeter K, Bitter I, Sperlágh B., (2022). Elevated Serum Purine Levels in Schizophrenia: A Reverse Translational Study to Identify Novel Inflammatory Biomarkers. *International Journal of Neuropsychopharmacology*, 25(8):645-659.
[3] Baranyi M, Milusheva E, Vizi ES, Sperlágh B., (2006). Chromatographic analysis of dopamine metabolism in a Parkinsonian mode, *J Chromatogr A*., 1120(1-2):13-20

Analyses of mycotoxins in edible insects: insights from scientific literature

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Keywords: edible insects, mycotoxins analysis, migratory locust, yellow mealworm, novel food

Objective
In some countries edible insects have long been a part of the diet, but only recently the European Union (EU) approved some edible insects - including migratory locust (*Locusta migratoria*, LM) and yellow mealworm larva (*Tenebrio molitor*, TM) as a novel food [1], which can be considered a part of protein diversification. Food safety of novel foods is important, and the novel food regulation covers the aspects such as additional specific labelling requirements and conditions under which the novel food may be used. But it is also important to obtain more information on food safety risks (such as mycotoxins) that could be connected to consumption of novel foods such as edible insects. The aim of this study was to examine mycotoxins analyses in selected edible insects ML and TM through a review of scientific articles.

Methods
A review of scientific literature in the Web of Science and Scopus databases was performed and found a relatively small number of articles studying mycotoxins in edible insects ML and TM.

Results
Limited information is available about accumulation and excretion of mycotoxins by TM and LM. Analyses of various mycotoxins in both TM and LM were performed with HPLC-MS. The list of analysed mycotoxin includes: aflatoxin B1 (AFB1), ochratoxin A (OTA), fumonisin B1 (FB1), zearalenone (ZEN), deoxynivalenol (DON) and trichothecene mycotoxins T-2 and HT-2. Studies investigated the effects of mycotoxins' contaminated feed on TM and found that the larvae did not accumulate detectable concentrations of some of the tested mycotoxins as they were below maximum residue limits for edible matrices, which indicates minor to no contamination. There are also some contradicting findings as larvae were found to accumulate low levels of DON by one study, while another study did not detect DON and its derivatives in larvae, which together with detection of DON in frass indicated mycotoxins degradation by larvae. The ability of larvae to metabolise mycotoxins (ZEN, AFB1) was also indicated because the frass samples and larvae contained metabolites of the analysed mycotoxins. Effects of mycotoxins on survival rate of larvae and their weight gain were also investigated. Mycotoxin residues were eliminated with 24 h fasting.

Conclusions
Mycotoxins do not seem to accumulate in TM, but there is a need for further investigation of mycotoxin enzymatic degradation by TM and the toxic metabolites that could result from this degradation. Further studies of mycotoxins in both TM and LM are necessary to better understand the possible food safety risks of these emerging novel foods.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the Slovenian Research and Innovation Agency ("Young Researchers" program - Maja Bensa, research core funding No. P1-0005) and Special Accounts for Research Grants of the National and Kapodistrian University of Athens (research grants: 14053 and 15363).

REFERENCES

[1] European Commission. 2017. Commission implementing regulation EU 2017/2470. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02017R2470-20220829.

Analysis of coenzyme Q_{10} (CoQ_{10}) and shorter isoprenoid tail lengths (CoQ_n, n = 6-9) by reversed-phase liquid chromatography coupled with APCI-MS

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Keywords: coenzyme Q10, fragmentation pathway, Atmospheric Pressure Chemical Ionization, Mass Spectrometry

Objective
Coenzyme Q10 (CoQ_{10}) and closely related compounds with varying isoprenoid tail lengths (CoQ_n, n = 6-9) play crucial roles in cellular processes. The identification and quantification of CoQ_{10} in foods and biological samples are usually performed using liquid chromatography coupled with mass spectrometry (MS), employing electrospray ionization (ESI) and/or atmospheric pressure chemical ionization (APCI). However, to our knowledge, the complete characterization of the fragmentation pathways of CoQ10 and related compounds using LC-APCI-MS has not been reported in the literature. In this study, we develop a reversed-phase liquid chromatographic method capable of separating CoQ_n species in food samples. Furthermore, we provide a detailed interpretation of various fragmentation patterns starting from the radical anion $[M]^{-\bullet}$ generated as the prevailing species using an APCI source in negative ion mode.

Methods
Coenzyme Q10 standard solution was prepared at a concentration of 100 ppm and then diluted for analysis. CoQ_n species were extracted from lyophilized vegetables using an ethanol/hexane solution. The extracts were analysed by reversed-phase liquid chromatography (RPLC) employing an Ascentis Express C18 column (150 × 2.1 mm ID, 2.7 µm particle size) equipped with an Ascentis Express C18 pre-column (5 × 2.1 mm ID), coupled with high and low resolution/accuracy MS using an APCI ionization source in negative ion mode.

Results
The RPLC-APCI-FTMS conditions enabled the elution of CoQ_{10} in less than 18 minutes and the generation of its radical anion $[M]^{-\bullet}$ at *m/z* 862.684. CID-fragmentation of this radical anion produced two product ions at high *m/z* values, due to the sequential loss of methyl groups from methoxy groups on the ring. Additionally, a series of product ions were highlighted. The major series derived from the ion at *m/z* 832.638 $[M\text{-}2CH_3]^{-\bullet}$ by the loss of oligo isoprene radicals, generating the base peak of the MS/MS spectrum at *m/z* 219.066. From this, three low-abundance series were rationalized, involving the loss of water, of the radical methyl group, and CO, respectively. Specific structures were proposed for product ions, based on our findings and literature. The fragmentation pattern reported here is shared by all CoQ_n species identified in vegetable samples.

Conclusions
The RPLC-APCI(-)-MS method we propose effectively separates CoQ_n species in real samples. Moreover, the detailed analysis of fragmentation patterns allows us to identify diagnostic product ions, making it easier to accurately identify CoQ_{10} and its closely related compounds.

ACKNOWLEDGMENTS
This work was supported by the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of the Italian Ministry of University and Research funded by the European Union – Next Generation EU. Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title “ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods”

Identification of honey microbiome and their antibacterial properties

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Keywords: secondary metabolites, pathogens, honey microbiome, antibacterial properties, natural product analysis, protein extraction

Objective

Bacterial infections and the development of antibiotic resistance among microorganisms worldwide, resulting in a limited choice of drugs for therapy, pose a huge threat to human health and life. In the case of multi-drug resistant pathogens, treating patients becomes even more problematic. The health properties of honey as a natural medicine have been talked about for years, and of particular importance are its antibacterial properties. An important aspect affecting the health effects of honey is also its microbiome, dominated by spore-forming bacteria and yeast. Studies indicate that microorganisms present in honey samples can produce specific metabolites responsible for antimicrobial activity. Due to the potential use of bacterial secondary metabolites as a therapeutic agent, special attention has been paid to the problem of treating hard-to-heal wounds, associated, for example, with diabetic foot syndrome, as a chronic complication of diabetes, or when treating pressure ulcers. The aim of the work is to develop conditions for the isolation and culture of a variety of microorganisms from honey samples from different geographical and botanical regions. A key step is the identification of microorganisms by MALDI-TOF/MS and the preliminary determination of the antibacterial properties of microorganisms contained in honey. This could contribute to the development of new therapeutic options as potential medicinal and cosmetic preparations. In addition, the antibiotic resistance of reference pathogenic strains was determined using two tests: the gradient test (E-test, Biomeriuex, France) and MBT STAR-Carba Assay (Bruker Daltonics GmbH & Co. KG, Bremen, Germany).

Methods

73 honey samples were used for the isolation of microbiome. Different culture temperatures and time, as well as 5 culture media was utilized to variety of microbiome. Isolated microorganisms were then identified with the use of MALDI-TOF/MS technique with the use of extended Direct Transfer (eDT) method of protein extraction. The relationship between the microbiome of the samples and physicochemical properties, such as pH and color, as well as region of origin and botanicals, was also tested. For the next stage of study, 150 strains isolated from honey samples were selected, belonging to families such as the *Bacillacae* and *Paenibacillacae*, in order to determine their ability to inhibit the growth of selected pathogens.

Results

As a result of bacteria isolation on five different microbiological media using various temperature conditions and incubation times, 388 bacterial strains were isolated from 73 honey samples. A study of 150 strains isolated from honey found emerging halo zones against at least one pathogen among 64 strains, accounting for 43% of all strains tested. This indicates that these strains exhibit bacteriostatic or bactericidal activity against the tested reference strains.

Conclusions

The results obtained provide a solid basis for further research, demonstrating the wide possibilities of using honey, a natural product, as a matrix for isolating microorganisms with health-promoting properties. It is necessary to further explore the honey microbiome and their secondary metabolites, which hold potential as new therapeutic strategies, even for infections caused by drug-resistant pathogens.

Enantioseparation of the non-ionic/not-ionizable allantoin with *Cinchona* alkaloid-based zwitterionic chiral stationary phases

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Keywords: CHIRALPAK® ZWIXs phases; h-bonds; molecular dynamic simulations, polar-ionic conditions, retention mechanism

Objective

Allantoin represents a compound widely employed in pharmaceutical and cosmetic fields. Its safety has been acknowledged by regulatory bodies such as the US Food and Drug Administration, the European Commission for Cosmetics and Consumer and Health and European Directorate for the Quality of Medicines & HealthCare. This justifies its wide use in dermatological/cosmetic formulations and allows their safe use [1]. Allantoin exhibits an asymmetric carbon atom, resulting in two enantiomers, with the (*S*)-enantiomer predominating in plants [2], although racemization may potentially occur during manufacturing processes. Notably, literature currently lacks enantioselective LC methods for allantoin analysis. This study aims to fill this gap, developing an atypical enantioseparation for the nature of the investigated analyte.

Methods

In this study, two zwitterionic *Cinchona* alkaloid-based chiral stationary phases (CSPs), commercially known as CHIRALPAK® ZWIX(+) (CSP 1) and CHIRALPAK® ZWIX(-) (CSP 2), were utilized for the enantioseparation of allantoin under polar-ionic conditions. A mobile phase consisting of acetonitrile/methanol/water/acetic acid (96:2:2:0.1, v/v/v/v) was employed, and a molecular dynamic *in silico* protocol was applied to elucidate the retention mechanism in depth.

Results

Following a mobile phase optimization process, a nearly complete baseline separation (with α=1.08) of allantoin enantiomers was achieved in less than 15 min with both CSPs. Due to the "pseudo-enantiomeric" nature of the two chiral selectors (quinine-based in CSP 1 and quinidine-based in CSP 2), an inversion of the enantiomer elution order was observed with the two CSPs under identical experimental conditions [3]. Remarkably, this represents a rare instance where these CSPs demonstrate the ability to enantioseparate a non-ionic, non-ionizable species. Molecular dynamics highlighted the central role of the H-bond formation and the involvement of the anionic moiety of the CSP 1.

Conclusions

The findings of this study strongly indicate that zwitterion-type *Cinchona* alkaloid-based CSPs may possess the capability to enantioseparate non-ionic and not-ionizable compounds. To the best of our knowledge, this is the initial study elucidating the enantioseparation of allantoin, which is known to naturally occur as the (*S*)-enantiomer.

REFERENCES

[1] S. Laneri, R. Di Lorenzo, A. Sacchi, I. Dini, (2019). Dosage of Bioactive Molecules in the Nutricosmeceutical *Helix aspersa* Muller Mucus and Formulation of New Cosmetic Cream with Moisturizing Effect, *Natural Product Communications*, 14 1934578X1986860.
[2] R. Kaur, J. Chandra, B. Varghese, S. Keshavkant, (2023). Allantoin: A Potential Compound for the Mitigation of Adverse Effects of Abiotic Stresses in Plants, *Plants*, 12, 3059.
[3] F. Ianni, Z. Pataj, H. Gross, R. Sardella, B. Natalini, W. Lindner, M. Lämmerhofer, (2014). Direct enantioseparation of underivatized aliphatic 3-hydroxyalkanoic acids with a quinine-based zwitterionic chiral stationary phase, *Journal of Chromatography A*, 1363, 101–108

Red wine volatile organic compounds evolution in winery conditions: inox *versus* oak barrels

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Keywords: HS-SPME-GC-ToFMS, volatile organic compounds, inox, oak barrels

Objective
The main goal of the present work is to describe how the volatile composition of wines is impacted by oak or inox during a short period of time (3 and 6 months) in real winery conditions. To achieve this objective, we used 10 oak barrels, but samples were collected from all the 10 barrels when wines were racked to a stain steel deposit and returned to barrels and compared to the same wine kept in a stain steel deposit. The rationale to this simple experimental design was to overcome the well known barrel-to-barrel variation by considering the wine in the barrels as a unique sample, since the influence of each barrel on the volatile composition of the wine was not the goal.

Methods
HS-SPME-GC-ToF/MS was employed to analyze the volatile profile of the different wines. The GC-ToFMS analysis was performed on an Agilent 8890 GC System (Agilent Technologies, UK) coupled to a Bench ToF-Select detector (MARKES International, China) and the data were acquired and analyzed with ToF-DS 4.1 of Markes International.

Results
With the analytical methodology used we were able to tentatively identify and semi-quantified 150 compounds The majority of the compounds belong to the esters (77) and alcohols (22) families, followed by the terpenes (12) family. For the total content of esters, the only significative difference observed was a decrease that occurs in wines in oak barrels. The total content of alcohols and acids increased significantly in wines aged in oak barrels while no differences were observed for wines in inox.

Conclusions
A polar heatmap with dendrogram of volatile compounds found in wine aged in oak and steel after 3 and 6 months, clearly shows differences among samples. It is possible to use a compose sample from the 10 oak barrels instead of a sample per barrel and still be able to evaluate the influence of oak and the influence of maturation time.

ACKNOWLEDGMENTS
Authors also aknowledges MED https://doi.org/10.54499/UIDB/05183/2020 and CHANGE https://doi.org/10.54499/LA/P/0121/2020.

Determination of micropollutants and transformation products in urban runoff: an application of target and suspect screening with LC-HRMS

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Keywords: suspect screening, HRMS, urban runoff, Liquid Chromatography, micropollutants

Objective
Urban runoff is recognized as a significant non-point pollutant source that impacts the nearby water bodies. Rain washes pollutants from urban surfaces and the atmosphere, creating a complex pollutant mixture. The study aims to determine these pollutants in urban runoff, with samples taken from three Spanish cities.

Methods
Analytes were extracted using solid-phase extraction technique, and then identified using Ultra-Performance Liquid Chromatography High Resolution Mass Spectrometry Orbitrap High Resolution Mass Spectrometry (UPLC-Orbitrap-HRMS). Two acquisition modes were used, All Ion Fragmentation (AIF) and Data Dependent Acquisition (DDA) [1]. AIF data was used for target and suspect screening method using an in-house database. This methodology involved the analysis of over 1 000 emerging pollutants, covering a wide range of compounds. Then, further suspects confirmation was conducted using DDA data with a non-target screening method.

Results
The methods were successfully applied to identify micropollutants in urban runoff water. A diverse set of compounds included in the database were evaluated regarding the extraction efficiencies, achieving satisfactory recoveries for compounds with a wide range of physicochemical characteristics. Moreover, several suspects were unequivocally identified using these methods.

Conclusions
The suspect screening method coupled to the non-target screening method effectively identify and quantify a wide range of compounds in urban runoff samples. This allows the identification and quantification of several pollutants in the same analysis. Moreover, the methods provide a list of suspect compounds to further investigate.

ACKNOWLEDGMENTS

This study was funded from the Spanish Ministry of Science and Innovation, CityPoll project (TED2021-129966B-C31). This work was also partially supported by the Genaralitat de Catalunya through the Consolidated Research Group (SGR ICRA-ENV 2021). The UPLC-HRMS Orbitrap Exploris 120, received support from the CERCA Institute through the CERCAGINYS program, funded by the Spanish Ministry of Science and Innovation.

REFERENCES

[1] D. Gutiérrez-Martín, R. Gil-Solsona, M.W. Saaltink, V. Rodellas, R. López-Serna, A. Folch, J. Carrera, P. Gago-Ferrero, (2023). Chemicals of emerging concern in coastal aquifers: Assessment along the land-ocean interface, *Journal of hazardous materials*, 448, 130876

Diastereomer separation of synthetic phosphorothioate oligonucleotides using one-dimensional and two-dimensional liquid chromatography

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Keywords: oligonucleotides, diastereomers, UHPLC, IP-RPLC

Objective
In the last several years, the number of FDA approved oligonucleotide (ON) pharmaceuticals has been steadily increasing. Unmodified oligonucleotides however have significant drawbacks, as they are rapidly eliminated from the body by renal clearance or nuclease degradation. Therefore, to increase their in-vivo stability, phosphorothioation is a frequent modification of therapeutic ONs which also leads to improved binding affinity facilitating cell internalization and intracellular distribution. A side effect of this modification is the introduction of stereocenters which leads to the formation of Rp- and Sp-diastereomers. This increases the structural diversity. For example, a synthetic oligonucleotide with only 16 phosphorothioate linkages has 65.536 diastereomers. This, in turn, increases the difficulty of chromatographic separation during quality control. Since distinct phosphorothioate diastereomers also have different bioactivities and pharmacological properties, there is increasing interest in implications of stereoisomerism of phosphorothioate oligonucleotides. From a quality and regulatory viewpoint, batch-to-batch reproducibility of the diastereomer profile may be of significant concern. In order to address this issue, this study investigated the stereoselectivity of LC methods for a fully phosphorothioated oligonucleotide (PSO) compound focusing on MS-compatible ion-pairing reversed-phase liquid chromatography (IP-RPLC) and a high resolution 2D-LC approach with a chiral first dimension and an IP-RP second dimension.

Methods
All chromatographic experiments were performed on an Agilent 1290 Infinity II from Agilent Technologies (Waldbronn, Germany). The detection of all oligonucleotides was performed at a wavelength of 254 nm. The LC-System was controlled by the Agilent OpenLab CDS ChemStation. The 2D-LC experiments were carried out on the Agilent 1290 Infinity II 2D-LC Solution from Agilent Technologies.

Results
We present a MS compatible IP-RP method for the separation of 23 of 32 possible diastereomers of a fully phosphorothioated oligonucleotide in 20 minutes by a single 1D LC run. The separation was confirmed by a TOF-MS scan to exclude that other impurities are disturbing the diastereomer separation. Furthermore, a 2D-LC run was performed with a chiral stationary phase in the first dimension and an IP-RP method in the second dimension. Improved diastereomer selectivity was obtained through the 2D-LC experiments and a larger number of peaks could be separated.

Conclusions
Ion-pair reversed phase liquid chromatography is still the first choice for the separation of phosphorothioated ON diastereomers. In this study, a model PSO comprised of six nucleotides has been investigated to maximise the number of resolved diastereomers. It was possible to develop a 1D method that separates 23 of 32 diastereomers while also using minimal buffer content and remaining MS compatible. In order to further increase the number of resolved diastereomers, high resolution sampling 2D-LC experiments were conducted. The 2D-LC approach effectively enabled the separation of even more diastereomers within a total analysis time of 50 minutes.

Development of a new extraction method of pesticides from soil using direct-immersion SPME LC-tips followed by GC-MS/MS

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Keywords: pesticides, soil, contamination, SPME LC-Tips, GC-MS/MS

Objective

Soil contamination is a major global threat to essential ecosystem services. Sustainable environmental strategies, including the European Green Deal and the Circular Economy Action Plan, aim to achieve zero pollution by reducing soil contamination and enhancing restoration. The ISLANDR project supports these efforts through reducing soil contamination and enhancing restoration efforts. Pesticides are widely used in agriculture, but they pose environmental and human health risks. Monitoring their presence in soil is crucial yet challenging due to soil variability and low pesticide concentrations.

Methods

In this study, we introduce a novel approach for extracting ten different pesticides from sandy-loam soil, as a potential greener alternative. Our approach uses direct-immersion solid-phase microextraction (SPME), distinguishing itself from conventional methods through the use of a novel semi-disposable SPME setup, where the fiber is attached to a micropipette tip. A Plackett-Burman experimental design was employed to optimise the method, analysing ten variables at two levels and conducting 12 experiments in triplicate. The final methodology involves creating a soil slurry by adding an aqueous solution (6% methanol v/v) to 2 g of soil. Conditioned and pre-wetted fibers are then immersed into the samples for extraction and kept under shaking to disperse the soil through the entire volume. Afterwards, the analytes are desorbed onto 100 µL of methanol for 30 minutes. The resulting extract is combined with analyte protectants (ethylglycerol, gulonolactone, and sorbitol; 500 µg/mL), and is analyzed by GC-MS/MS in multiple reaction monitoring mode.

Results

To assess the method's accuracy, calibration was performed using spiked soil with analyte concentrations ranging from 0.1 to 50 µg/kg, with isotopically labelled penconazole serving as the internal standard. Coefficients of determination fell within the range of 0.94 to 0.97 for all analytes, with limits of quantification spanning from 0.1 to 10 µg/kg.

Conclusions

This work reports for the first time the use of direct-immersion SPME for pesticide extraction from soil. This new method generates clean extracts with high-concentration factors. It also produces little toxic waste per sample and appears to be very cost-effective.

ACKNOWLEDGMENTS

This work has received funding from the ISLANDR project funded by the European Union´s Horizon Europe Research and Innovation funding programme, agreement number 101112889; and Fundação para a Ciência e a Tecnologia, I.P., Portugal, UIDB/04085/2020 (Research unit CENSE "Center for Environmental and Sustainability Research"); Fundação para a Ciência e a Tecnologia is also acknowledged for J. Brinco (UI/BD/150867/2021) PhD fellowship, and P. Guedes (CEECIND/01969/2020) and N. Couto (CEECIND/04210/2017) contracts established under Individual Call to Scientific Employment Stimulus. This research is anchored at RESOLUTION LAB, an infrastructure at NOVA School of Science and Technology

The synergic interplay between liquid chromatography, UV spectroscopy and high-resolution mass spectrometry for the confident identification of cucurbitacins in bitter unripe melons

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Keywords: cucurbitacins, cyanopropyl stationary phase, Atmospheric Pressure Chemical Ionization, UV spectroscopy, High-resolution Mass Spectrometry.

Objective

Cucurbitacins are a class of highly oxygenated triterpenoids that are responsible for both the bitter taste and the toxicity of some cucurbitaceous fruits [1,2]. The identification of cucurbitacins is crucial to evaluate the health risks related to the consumption of bitter edible fruits. In the present work, a new approach based on reversed-phase liquid chromatography (RPLC) coupled with UV and atmospheric pressure chemical ionization – high-resolution mass spectrometry (APCI-HRMS) detection was exploited to determine the cucurbitacin content in unexpectedly bitter "Scopatizzo" unripe melons (*Cucumis melo L*).

Methods

Cucurbitacins were extracted with an ethanol/water (90:10 v/v) mixture from dried fruits and separated on an Ascentis Express ES-Cyano column (150 x 2.1 mm with 2.7 µm core-shell particle size).

Results

The efficient separation on a cyanopropylic stationary phase paved the way for the characterization of cucurbitacins by UV and APCI-HRMS. On one side, the analysis of UV spectra was crucial to assess the presence of enone groups, *i.e.*, a common and often discriminating structural feature among the known cucurbitacins. On the other side, the interpretation of APCI(±) high-resolution tandem mass spectra supported the unambiguous identification of cucurbitacins B, D, R, and 23,24-dihydro-cucurbitacin B in bitter-tasting "Scopatizzo" unripe melons.

Conclusions

The RPLC-APCI(±)-UV-HRMS/MS analytical workflow proposed here can be used for the untargeted characterization of cucurbitacins in plant samples. Valuable structural information can be obtained from UV-HRMS/MS data, highlighting that even without commercially available standards, the combination of LC with UV and MS detection provides a powerful tool for studying complex classes of plant metabolites.

ACKNOWLEDGMENTS

This work was supported by the BiodiverSO Karpos project funded by Regione Puglia (CUP: B97H22003670009).

REFERENCES

[1] Qing Z., Shi Y., Han, L., *et al*. (2022). Identification of Seven Undescribed Cucurbitacins in *Cucumis Sativus* (Cucumber) and Their Cytotoxic Activity, *Phytochemistry*, vol. 197
[2] Bajcsik N., Pfab R., Pietsch, J. (2017). Simultaneous Determination of Cucurbitacin B, E, I and E-Glucoside in Plant Material and Body Fluids by HPLC–MS, *Journal of Chromatography B*, vol. 1052, pp. 128-34.

Dynamic headspace extraction as an alternative method to collect PFAS from environmental samples

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Keywords: per- and polyfluoroalkyl substances, thermal desorption tubes, emerging contaminants, environmental, GC(×GC)-TOFMS

Objective
Per- and polyfluoroalkyl substances (PFASs) are a family of fluorinated compounds extensively used since 50s by both consumers and industry. Structurally, they consist of a carbon chain bonded to fluorine atoms, with varying functional groups at the end of the chain. Due to their environmental persistence and potential adverse health effects, developing robust analytical methodologies for PFAS is crucial. In this context, thermal desorption tubes, combined with gas chromatography, provide a solvent-free extraction method and high analytical performance alternative to the analysis of PFASs. Therefore, the aim of this research is to evaluate the capabilities of thermal desorption tubes as extraction method, coupled with GC and GC×GC-TOFMS, to characterize (semi)volatile PFAS.

Methods
In order to assess the thermal desorption tubes performance, different sorbents (graphitized carbon black and phenylphenilen oxide polimers) and extraction parameters (conditioning temperature and extraction volume) were evaluated in terms of recovery and selectivity for PFAS extraction. The extraction was optimized using environmental samples (soil and water) spiked with a mix of PFAS standards (MW range 264-571 Da), including fluorotelomer alcohols (FTOH), acrylates (FTAc), and alkyl sulfonamide (N-MeFOSA, N-EtFOSA, N-MeFOSE, and N-EtFOSE) derivatives. For this purpose, both GC and GC×GC, coupled with TOFMS, were used.

Results
The porous polymer poly(2,6-diphenylphenylene oxide) was the most effective sorbent, although graphitized carbon adsorbent also proved useful for extracting certain compounds. The use of GC×GC revealed additional significant molecules in environmental samples, and TOFMS demonstrated its analytical power in distinguishing individual PFAS, even within the same subfamily.

Conclusions
These preliminary tests were essential for understanding the behaviour of these PFAS's families both as standards and in real samples. The thermal desorption tubes have demonstrated to be a valid choice to extract these chemical molecules, minimizing solvents usage. When combined with GC(×GC)-TOFMS, this method represents a new alternative to traditional extraction.

Development of cost-effective system for sample deposition in thin layer chromatography: evaluation and application on plant extracts

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Keywords: TLC; plant extract; polyphenol, injection, spray deposition

Objective
The present study has developed a simple and cost-effective lab-made device for spray-on sample deposition as bands on silica plates for Thin-Layer Chromatography (TLC). The main parameters were carefully optimized, ensuring the method's reliability and effectiveness when applied to selected hydroalcoholic extracts.

Methods
The apparatus design is based on a three-way "T"-shaped junction, with inlets for the sample, gas and an outlet for the nebulized sample. The sample, introduced through an inner diameter 50 µm fused silica capillary with an outlet tip-shape, passed through the "T" and a thin stainless-steel tube, terminating a flat configuration system. Nitrogen gas flowed coaxially to the capillary through the steel tube. The result was a sample homogeneous spray deposited on a TLC plate placed a few millimetres away. The band shape was achieved by oscillating craftsmanship, which ensured sharp and thin deposition.
For the application to the chromatographic separation of medicinal and food plant extracts, tinctures according to the European and US Pharmacopoeias requirements, was used as a reference. Specifically, high-performance reversed-phase TLC (RP-HPTLC) glass plates were used. We modified the main experimental parameters and conducted a thorough visual analysis of the results in fluorescence emission, ensuring the reliability of our method.

Results
One of the critical aspects of chromatographic separation is undoubtedly the sample introduction/injection onto/into the separation system. As in liquid chromatography, the band-broadening deposition effect is dramatic in the separation performance in TLC.
To this end, a new chromatographic band deposition system partially inspired by the commercial one was optimized by changing several technical and chromatographic parameters to achieve the minimum band thickness (i.e., minimum degree of diffusion). In this study, technical and instrumental parameters such as orifice/tip shape, the tip-emitter distance, the nitrogen gas and sample flow, the deposition time, and the solvent dilution were evaluated using a comparative method approach.
The optimized method has also been proposed for use with mixtures of polyphenolic compounds and selected plants hydroalcoholic extracts (artichoke, hypericum, cannabis, propolis).

Conclusions
This innovative and cost-effective system for sample deposition in thin-layer chromatography could provide an attractive alternative to commercial instrumentation. The deposited bands had a width of no more than 2 millimeters, offering high definition and band performance in separating extracts from complex matrixes when compared with those obtained from published experimental studies

Uptake, accumulation, and effects at growth and physiological level of perfluorooctanoic acid (PFOA) in basil (*Ocimum basilicum* L.) plants

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Keywords: perfluoroalkyl substances (PFASs), UPLC-MS, antioxidant activity, chlorophyll fluorescence

Objective
Perfluoroalkyl substances (PFASs) are a group of man-made aliphatic chemicals employed in many industrial and commercial applications. The extensive use and persistence of these compounds has led to their accumulation in the environment, raising concerns about their impact on ecosystems and human health [1]. Owing to the potential transfer of PFASs to humans through the consumption of edible plants, there is a need to develop an efficient approach to assess both the potential toxic effects and the uptake of PFASs in vegetables [2]. In this context, the present study aimed to develop a reliable extraction and clean-up procedure followed by UPLC-MS analysis for the determination of n-perfluorooctanoic acid (PFOA) in basil (*Ocimum basilicum* L.). Furthermore, the effects of PFOA exposure on basil plants were evaluated at morpho-physiological level.

Methods
To assess the accumulation and the potential toxic effects of PFOA in basil, a pot experiment in greenhouse was performed. PFOA bioaccumulation in basil leaves was determined using ultrasonic extraction followed by solid-phase extraction clean-up and UPLC-MS analysis. In addition, the effects of PFOA on the growth and photosynthetic performances of basil plants were assessed.

Results
After three weeks of cultivation, plants grown in PFOA-added substrate accumulated PFOA at different levels but did not display significant differences from the control as for biomass production, chlorophyll content, oxidative status, and antioxidant response in the leaves.

Conclusions
The obtained results showed that, despite a significant accumulation of PFOA in basil leaves, no clear effects of this compound on plant growth and physiological performances were observed.

ACKNOWLEDGMENTS
Authors wish to thank Ms. Laura Lilla for her technical support in UPLC-MS analysis.

REFERENCES
[1] Battisti I., Trentin A. R., Franzolin E., Nicoletto C., Masi A., Renella G., (2024). Uptake and distribution of perfluoroalkyl substances by grafted tomato plants cultivated in a contaminated site in northern Italy, *Science of the Total Environment* 915, 170032.
[2] Pietrini, F., Passatore, L., Fischetti, E., Carloni, S., Ferrario, C., Polesello, S., Zacchini, M. (2019) Evaluation of morpho-physiological traits and contaminant accumulation ability in *Lemna minor* L. treated with increasing perfluorooctanoic acid (PFOA) concentrations under laboratory conditions. *Science of The Total Environment* 695, 133828

High-throughput sample preparation for the evaluation of 104 Pesticides in Hemp Inflorescence According to AOAC SMPR 2018.011

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Keywords: contaminants, pesticides, SPE, hemp inflorescence

Objective
The objective of this study was to develop and validate a comprehensive method for analyzing 104 pesticides in hemp inflorescence, ensuring compliance with AOAC SMPR 2018.011 standards. The method aims to address the complexity of cannabis matrix and provide reliable, high-throughput analysis suitable for commercial laboratories.

Methods
The method involves a unified process for extraction, cleanup, and calibration compatible with GC-MS/MS and LC-MS/MS analyses. Sample preparation includes homogenization of cannabis plant material, extraction with acetonitrile:ethyl acetate (2:1), and clean up using a SPE. Calibration standards were matrix-matched, while validation and method performance were assessed through repeatability, accuracy and recovery tests. Analysis covered 89 compounds quantified by LC-MS/MS and 15 by GC-MS/MS.

Results
The method meets AOAC SMPR 2018.011 specifications. Repeatability and recovery evaluations showed acceptable performance. The method's efficacy was confirmed by recovery and repeatability results, demonstrating its suitability for most target pesticides in cannabis matrix.

Conclusions
The developed method covers a wide range of pesticides required by US state cannabis programs, offering high sensitivity and minimal preparation steps. The study highlights the importance of matrix-matched calibration standards. Designed for high throughput and minimal consumable usage, this method is well-suited for routine pesticide residue analysis in cannabis products, ensuring robust and reliable results.

ACKNOWLEDGMENTS
The authors gratefully acknowledge the Merck Life Science and Shimadzu Corporation for their continuous support.

Lipid profile alteration in diffuse intrinsic pontine glioma cells by drug-candidates ONC201 and THX6

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Keywords: cell cultures, diffuse intrinsic pontine glioma, lipids, drug development

Objective
Alterations in lipid metabolism is a well-documented phenomenon in cancer. Diffuse intrinsic pontine glioma (DIPG) is a aggressive cancer and cause of brain tumor death in children (4-7years). In this study, we analyzed the lipidomic pattern in two DIPG cell lines and the neuroblastoma cells SHSY-5Y, in absence and presence of ONC201 and THX6, two promising drug-candidates.

Methods
DIPG cell lines (SU-DIPG-36, SU-DIPG-50) were cultured in Tumor Stem Media, a 1:1 mixture of DMEM/F12 and neurobasal completed with growth factors. SHSY-5Y were maintained in DMEM/F12. After extraction and separation, the fatty acid methyl esters (FAMEs) identification was performed on a GCMS-system. Intact lipids analysis was performed an LCMS8060 system.

Results
The lipid profile of SU-DIPG-36 and SU-DIPG-50 cell lines and immortalized neuroblastoma cell line SH-SY5Y was determined. It was analyzed in the presence and absence of ONC201 and THX6, two drug-candidates for DIPG treatment. ONC201 and THX6 induced significant alterations in the cell lipidomic. Particularly, in SH-SY5Y and SU-DIPG-50 cells, an increase in C16:0 and C18:0 after treatment with both compounds, and a simultaneous decrease in the content of C16:1n7 and C18:1n9 was observed. A similar pattern is also found in SU-DIPG-36 cells, although in less extent probably due to higher starting levels of C16:0 in untreated cells.

Conclusions
The observed lipidomic pattern suggests an inhibition of the stearoyl-CoA desaturase enzyme, that would appear to be less active in SU-DIPG-36, by ONC201 and THX6. These data could also be related to the efficacy of ONC201 and THX6 in suppressing the tumour growth. Interestingly, this enzyme is known to be involved in cancer pathogenesis.

ACKNOWLEDGMENTS

NEXTGENERATIONEU (NGEU) funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) – A Multiscale integrated approach to the study of the nervous system in health and disease (DD N. 1553 11.10.2022), and project HPC –"National Centre for HPC, Big Data and Quantum Computing – HPC" "Simulazioni, calcolo e analisi dei dati ad alte prestazioni "code CN000000013 (DD N. 3138 16.12.2021); (b) Italian Foundations: Fondazione Progetto GAIA (Marche), Heal Foundation (Lazio), Onlus Mia Neri Foundation (Lazio) and Matibellula Foundation (Piemonte) for their support in the form of studentship

The potential of enzymatic hydrolysis with HS-SPME-GC×GC-ToFMS for the study of volatile signature of grapes

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Keywords: HS-SPME-GC×GC-ToFMS, glycosidic and free varietal compounds, enzymatic hydrolysis, grapes

Objective

This work focuses on the development of a methodology for the analysis of free and bound volatile compounds in grapes using an enzymatic hydrolysis with AR2000 enzyme and HS-SPME-GC×GC-ToFMS. We applied the methodology to grapes of Moreto variety from the 2022 and 2023 harvests.

Methods

HS-SPME extraction was performed according to a previously developed procedure [1], with some modifications for optimization of the amount of enzyme. In a 20.0 mL SPME flask sealed with a Teflon-coated rubber septum/magnetic screw cap, 4 g of grapes previously crushed with the Ultra Turrax T25 basic (IKA Labortechnik, Germany) were weighed, then 2 g of sodium chloride was added, then 50 mg of AR2000 and finally 2 mL of citrate-phosphate buffer solution (pH 5). The flask was then incubated at 35 ºC for 24 hours. After the enzyme acts the vial was equilibrated for 5 minutes at 60 ºC and then extracted for 40 minutes at the same temperature. The thermal desorption of the analytes was carried out by exposing the fiber in the GC injection port at 260 ºC for 3 minutes in splitless mode. GC×GC-ToFMS equipment and chromatographic method are fully described in [1]

Results

After the optimization of HS-SPME-GC×GC-ToFMS, a total of sixty volatile compounds were identified and quantified in the Moreto grapes under analysis, including compounds attributed to the monoterpene family, to sesquiterpenes and to the C_{13}-norisoprenoid family. Differences were observed when comparing grapes from the two harvest years.

Conclusions

A methodology for HS-SPME-GC×GC-ToFMS combined with enzymatic hydrolysis using the AR2000 enzyme was implemented, and the enzymatic hydrolysis conditions were optimized. The developed methodology can be used to study the volatile signature of grapes.

ACKNOWLEDGMENTS

This research was funded by "Vine&Wine-Driving Sustainable Growth Through Smart Innovation" project (sub-project-BioGrapeSustain), "Mobilizing Agendas for Business Innovation" under the Recovery and Resilience Program. Authors also aknowledges MED https://doi.org/10.54499/UIDB/05183/2020 and CHANGE https://doi.org/10.54499/LA/P/0121/2020.

REFERENCES

[1] Fonseca, D.; Martins, N.; Garcia, R.; Cabrita, M.J. Comprehensive Two-Dimensional Gas Chromatography with a TOFMS Detector - An Effective Tool to Trace the Signature of Grape Varieties. *Molecules* **2024**, 29, 1989. https://doi.org/10.3390/molecules29091989

Determination of pharmaceuticals by capillary HPLC-MS/MS

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Keywords: capillary HPLC-MS/MS, pharmaceuticals.

Objective

Demonstrate the use of the compact portable capillary liquid chromatograph Axcend Focus LC coupled to an Agilent Ultivo triple quadrupole mass spectrometer in quantitative analysis of pharmaceutical drugs in model aqueous samples.

Methods

Two test mixtures were analyzed in this study. Mix#1 contained acetaminophen, caffeine, carbamazepine, ciprofloxacin, erythromycin, fluoxetine, sulfamethoxazole, and trimethoprim. Mix#2 was composed of gemfibrozil, ibuprofen, naproxen, and triclosan. All compounds in the test mixtures were identified and calibration curves were constructed in the concentration ranges of 30-1,000 ng/mL for carbamazepine, 50-10,000 ng/mL for trimethoprim, 100-10,000 ng/mL for acetaminophen, 100-5,000 ng/mL for caffeine, 300-10,000 ng/mL for fluoxetine, 300-3,000 ng/mL for sulfamethoxazole, 500-10,000 ng/mL for erythromycin and ciprofloxacin, 30-10,000 ng/mL for gemfibrozil, ibuprofen and naproxen, and 30-1,000 ng/mL for triclosan.

Results

Identification and quantification of pharmaceuticals in mixtures of components in model aqueous samples based on capillary HPLC coupled to tandem MS was successfully performed. Calibration curves with a regression coefficient R^2 above 0.99 were obtained in the concentration ranges typically covering over two orders of magnitude (for example, 30-10,000 ng/mL for acetaminophen).

Conclusions

The developed HPLC-MS/MS methods can serve as a basis for further development of quantitative capillary HPLC-MS/MS determination of pharmaceuticals in biological matrices.

REFERENCES

[1] N.D. Volkow, C. Blanco. The Changing Opioid Crisis: development, challenges and opportunities. - *Mol Psychiatry*. 2021, 26 (1), 218–233.
[2] G. Roda, F. Faggiani, C. Bolchi, M. Pallavicini, M. Dei Cas. Ten Years of Fentanyl-like Drugs: a Technicalanalytical Review. - *Analyt Sci*. 2019, 35 (5), 479-491.
[3] C.E. Rodríguez-Palma, R Herráez-Hernández, P. Campíns-Falcó. A modified micro-solid phase extraction device for in-port elution and injection into portable liquid chromatography: A proof-of-concept study. - *J Chromatogr A*. 2023, 1705, 464216.

Volatile organic compounds in extra virgin olive oil: sensory quality and shelf-life assessment

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Keywords: HS-SPME-GC/MS, extra virgin olive oil, VOCs, shelf-life

Objective
The distinctive aroma and flavor of extra virgin olive oil (EVOO) are closely tied to its volatile composition, which includes various components contributing to both positive sensory attributes and defects resulting from chemical oxidation processes and the action of external enzymes [1]. This study aimed to develop a robust analytical method to identify volatile organic compounds (VOCs) in EVOO as markers of sensory attributes, both desirable and undesirable. We correlated these markers with their relative percentages to estimate the risk of EVOO disqualification during its shelf life.

Methods
Using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC/MS), we monitored VOC levels, particularly those from the lipoxygenase (LOX) pathway, over time. Significant differences ($p < 0.05$) and principal component analysis (PCA) was employed to process the experimental data.

Results
The study identified that the ratio of *E*-2-hexenal to acetic acid could predict the disqualification of monovarietal EVOO by sensory panels.

Conclusions
These findings suggest that the developed HS-SPME-GC/MS method effectively identifies VOCs as markers for sensory attributes in EVOO, providing a valuable tool for predicting the risk of disqualification during the oil's shelf life and aiding in quality control and assurance.

ACKNOWLEDGMENTS

This research was supported by National Funds through the FCT—Foundation for Science and Technology under the Ph.D. Grant of Flávia Freitas (2020.08089.BD DOI 10.54499/2020.08089.BD). This work received support and help from FCT/MCTES through national funds: Associate Laboratory for Green Chemistry—LAQV (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020); MED—Mediterranean Institute for Agriculture, Environment and Development (UIDB/05183/2020 DOI 10.54499/UIDB/05183/2020 and UIDP/05183/2020 DOI 10.54499/UIDP/05183/2020); CHANGE-Global Change and Sustainability Institute (LA/P/0121/2020 DOI 10.54499/LA/P/0121/2020). This research was also anchored by the RESOLUTION LAB, an infrastructure at NOVA School of Science and Technology.

REFERENCES

[1] Freitas F., Cabrita MJ., Gomes da Silva M., (2024). Early Identification of Olive Oil Defects throughout Shelf Life, *Separations*, 11(6), 167

Application of chromatographic techniques for the analysis of urinary bacterial metabolites of the intestinal microflora in Parkinson's disease

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Keywords: bacterial metabolites, Parkinson's disease, chromatographic techniques, GC-MS, LC-MS

Objective
The significance of maintaining a healthy gut microbiota and its influence on host health has gained increasing attention over the past decade. Imbalances in the gut microbiota can disrupt the body's balance, potentially causing new diseases or worsening existing ones. Moreover, these imbalances affect the production of bacterial metabolites, which play a role in gut-brain communication, influencing behavior and brain functions and potentially intensifying disease symptoms. Urinary metabolites are unique bacterial compounds that appear in abnormal amounts. These metabolites, primarily produced through bacterial metabolism, include organic acids such as benzoic, succinic, hippuric, *p*-hydroxybenzoic, *p*-hydroxyphenylacetic acids, and uremic toxins like trimethylamine N-oxide (TMAO) [1-2]. This preliminary study aimed to use chromatographic techniques to determine and compare the levels of organic acids such as succinic, *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, and TMAO in the urine of patients with PD and control subjects. In addition, the possibility of using changes in the amount of the determined compounds as markers in the early stages of disease or to differentiate the severity of the PD was investigated.

Methods
Chromatographic techniques such as gas chromatography (GC-MS) and liquid chromatography coupled to mass spectrometry (LC-MS) were used to quantify bacterial metabolites in the urine of PD patients and healthy volunteers.

Results
Higher levels of succinic, *p*-hydroxybenzoic, and *p*-hydroxyphenylacetic acids and lower levels of TMAO in the urine of PD patients were observed. Statistical analysis showed differences between the levels of succinic acid and TMAO in the PD group with mild disease compared to the moderate and advanced groups.

Conclusions
The application of chromatographic techniques for determining bacterial metabolites in a sample provides a rapid screening test for metabolic disturbances of the gut microflora observed in PD patients. Significant changes in the metabolites of the gut microbiota were observed in patients with mild PD at an early stage of the disease, which were not observed in patients with moderate and advanced PD. This may allow the differentiation of patients regarding disease severity and may be useful in selecting optimal drug treatment.

ACKNOWLEDGMENTS

Work financed under the FU2N program – Fundusz Udoskonalania Umiejętności Młodych Naukowców, supporting scientific excellence of Lodz University of Technology - grant no W-3D/FU2N/7/2024.

REFERENCES

[1] Kałużna-Czaplińska J., Gątarek P., Chartrand M. S., Dadar M., Bjørklund G. (2017). Is there a relationship between intestinal microbiota, dietary compounds, and obesity? *Trends in Food Science & Technology*, 70, 105-113.
[2] Fernández-Veledo S., Vendrell J. (2019). Gut microbiota-derived succinate: Friend or foe in human metabolic diseases? *Reviews in Endocrine and Metabolic Disorders*, 20, 439-447

Towards an AF4-UV-MALS platform online coupled with raman microspectroscopy for the analysis of nanoplastics in commercial bovine milk

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Keywords: nanoplastics, Flow Field Flow Fractionation, Raman Microspectroscopy, milk, online coupling

Objective

Nanoplastics (NPLs) derive from the degradation of plastic waste and from intentional production. Their small size facilitates long-distance transport and cell permeability, with studies showing their ubiquitous presence in water, air, food, and blood. NPLs impact ecosystems by leaching plastic additives and absorbing pollutants due to their high surface area. Detecting and understanding NPLs in biological systems is one of major analytical chemistry challenges. Current methods are limited by sample flexibility, lack of automation, and laborious pre-treatments, often providing only presence data without behavior analysis. Coupling FFF-multidetector platforms with Raman Microspectroscopy (RM) offers a promising solution by combining flexible, automatable, and informative systems with selective plastic detection. However, though FFF platforms were extensively used to study complex colloidal matrixes [1] up to this point this approach has only been tested for the analysis of NPLs in water with added surfactants. We report the first results related to the development of an Asymmetrical Flow Field Flow Fractionation (AF4) platform coupled with UV, Multi Angle Light Scattering (MALS) and online coupled RM detectors for the study of NPLs in bovine milk.

Methods

An AF4 method was developed to separate milk components and NPLs across a broad range of sizes in native-like conditions, providing results representative or the real behaviour of nanoplastics in the milk matrix. Skimmed UHT samples spiked with various amounts of polystyrene (PS) spherical standards of various sizes (300 – 500 nm) were screened. UV and MALS detection allowed the calculation of the masses and radius of gyration of the separated species. Finally, the unique RM online coupling technology allowed to selectively detect the presence of NPLs in the milk samples according to their chemical composition.

Results

The developed platform allowed the separation of milk components from NPLs and the selective detection of the latter within the mixes reaching the low ppm range. The simultaneous size characterization shed also light on the behaviour of 500 nm PS nanoparticles in milk. We observed a stabilizing effect on NPLs, and the absence of aggregation for the 500 nm PS particles when mixed with milk suggesting the real-life stabilizing effect of milk components on NPLs. These results were confirmed by orthogonal techniques such as Scanning Electron Microscopy and Nanoparticles Tracking Analysis.

Conclusions

These state-of-the-art preliminary results illustrate unique potential of AF4-UV-MALS-Raman coupling for the detection of NPLs in complex matrices and the characterization of NPLs-matrix interactions.

REFERENCES

[1] Giordani, S., Kassouf, N... & Marassi, V. (2024). Rapid and green discrimination of bovine milk according to fat content, thermal treatment, brand and manufacturer via colloidal fingerprinting. Food Chemistry, 440, 138206

Concentration sensitivity improving in capillary electrophoresis-frontal analysis for drug-plasma protein affinity studies

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Keywords: Capillary Electrophoresis, affinity interaction, plasma protein, drug,

Objective

A variety of pharmacokinetic processes, including absorption, distribution, metabolism and elimination of drugs, are significantly influenced by their binding with plasma proteins. This is due to the fact that these proteins, particularly human serum albumin (HSA), act as reservoirs and also mediate drug transport. It is therefore imperative that these interactions are characterised at the earliest stages of a new drug development. A comprehensive understanding of the mechanism of interaction necessitates the determination of pivotal parameters, including the binding constant (Kb), the stoichiometry of the interaction, and the identification of the binding sites for drugs on plasma proteins.

Methods

One method for determining these parameters is capillary electrophoresis-frontal analysis (CE-FA). CE-FA is one of the most robust affinity modes of CE, which is a well-established technique for its high resolution, speed of analysis and low reagent and sample consumption. However, it frequently encounters a challenge of low concentration sensitivity due to the narrow capillary inner diameter and ultraviolet-visible (UV-VIS) detection. The objective of these studies was to enhance the concentration sensitivity of CE-FA affinity interaction studies through the implementation of three distinct approaches: the integration of a contactless conductivity detector (C4D) and MS detectors, and the utilisation of an online preconcentration technique, field-amplified sample stacking (FASS).

Results

The three newly optimised methods exhibit enhanced sensitivity for drug detection in comparison to conventional CE-FA UV-VIS methods. The initial two approaches utilise HSA-salicylic acid as a model system. The CE-FA UV-VIS with FASS method demonstrates a seventeen-fold improvement in sensitivity, while the CE-FA C4D method exhibits a six-fold reduction in lower limits of detection. The combination of CE-FA with MS detection allows for the evaluation of binding between HSA and antidiabetics with detection limits that are almost three times lower than those achievable with conventional methods. Furthermore, MS enables the study of drugs with low solubility in aqueous solutions. The estimated binding parameters are comparable with the data measured by conventional methods or with data from the literature.

Conclusions

The newly optimised method contributes to higher sensitivity and universality for more efficient drug-protein affinity studies, extending the use of CE for this purpose.

ACKNOWLEDGMENTS

This work was supported by grant GA19-08358S from the Czech Science Foundation and by project No. NU23-08-00229 from the Czech Health Research Council.

HPTLC analyses of Japanese knotweed and Bohemian knotweed leaves extracts obtained by supercritical fluid extraction

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Keywords: HPTLC, **S**upercritical Fluid Extraction**,** invasive alien plant species, knotweeds

Objective

Japanese knotweed (*Fallopia japonica* Houtt.) and its interspecific hybride Bohemian knotweed (*Fallopia bohemica* J. Chrtek & A. Chrtková) J.P. Bailey) are invasive alien plant species that displace native vegetation in Europe, North America, Australia and New Zealand, and cause environmental and economical problems. Several attempts to eradicate these species in Europe and North America have proven to be unsuccessful. Both knotweeds are rich sources of bioactive compounds (e.g. phenolic compounds, carotenoids) but there are still undiscovered compounds that could due to their activities potentially be applicable for new products. The aim of this study was to perform HPTLC analyses of triterpenoids, phytosterols, lipid classes and proanthocyanidins in extracts from leaves of both knotweeds obtained by supercritical fluid extraction (SFE) using supercritical carbon dioxide or water.

Methods

Different HPTLC methods were applied for the analyses of lipid classes, triterpenoids, phytosterols and proanthocyanidins in SFE extracts of Japanese knotweed and Bohemian knotweed leaves. Lipid classes, triterpenoids and phytosterols in SFE extracts obtained with supercritical carbon dioxide were analysed on HPTLC silica gel (all) or HPTLC C18 (only triterpenoids and phytosterols) stationary phases. Proanthocyanidins in SFE extracts obtained with supercritical water were analysed on HPTLC silica gel. The analytes were detected after post-chromatographic derivatization using the following detection reagents: molybdophosphoric acid (lipid classes), anisaldehyde-sulfuric acid (triterpenoids and phytosterols) and 4-dimethylaminocinnamaldehyde (DMACA; proanthocyanidins).

Results

HPTLC analyses reveled that SFE leaves extracts obtained with supercritical carbon dioxide were rich in lipids, triterpenoids and phytosterols, while SFE leaves extracts obtained with supercritical water were rich in proanthocyanidins.

Conclusions

Supercritical fluid extraction could be in the future used for isolation lipids, triterpenoids, phytosterols, and proanthocyanidins from the leaves of invasive Japanese and Bohemian knotweed. Thus, the huge amount of knotweed biomass that is usually burned could be usefully used for the preparation of food supplements, plant protection products, etc.

ACKNOWLEDGMENTS

This study was supported by the Slovenian Research and Innovation Agency (ARIS; research core funding No. P1-0005 and research project J4-50138).

Performance of a versatile absorbance detector for miniaturized and portable LC systems

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Keywords: absorbance detection, CCD spectrometer, multi-wavelength detection, microcolumn Liquid Chromatography

Objective

Miniaturized and portable LC systems are becoming a popular category of instruments, with ongoing advancements expected to enhance their versatility, particularly in detection capabilities. Due to their lower complexity compared to mass spectrometers, optical detectors, especially UV-Vis absorbance detectors, are preferred for portable LC systems. In this contribution, we present a versatile absorbance detector that incorporates compact deuterium and tungsten lamps as light sources, a cost-effective CCD spectrometer as the light detector, and a specialized 100-nL fused-silica capillary flow cell.

Methods

Conducted experiments included assessments of sensitivity, baseline noise and drift, linearity deviation, dynamic range upper limit, and gradient separation of a test mixture of selected 16 polycyclic aromatic hydrocarbons on a 0.3 × 100 mm microcolumn. The performance of the proposed detector was compared to a benchtop DAD detector equipped with an 80-nL flow cell.

Results

Obtained data from the proposed detector are comparable to data of a benchtop DAD, however, the baseline noise was 4 to 10 times higher, depending on the detection wavelength and this disparity primarily stems from the inherent differences between DAD and CCD array sensors, including their signal acquisition and processing methods. Both detectors exhibited a similar level of baseline drift. Also, the sensitivity data indicated a slightly shorter effective optical path for the proposed detector compared to the DAD detector with the 80-nL cell (5.5 mm vs. 6 mm geometry). The proposed detector showed greater deviation from linearity, resulting in a shorter linear range compared to the DAD detector due to the intrinsic nonlinearity of the particular CCD chip of the proposed detector and a higher level of stray light within the CCD spectrometer body. The results and comparison of chromatography records of 16 polycyclic aromatic hydrocarbons by both detectors showed just tiny difference in peak height given by the different optical paths. Quality of the absorption spectra acquired by both detectors were almost equivalent.

Conclusions

The proposed setup, featuring multi-wavelength detection and absorption spectra acquisition from 210 to 750 nm, is compact and suitable for integration into portable microcolumn LC systems. Experimental evaluation showed that its sensitivity, linearity, and dynamic range are only slightly inferior to the benchtop DAD detector with an 80 nL flow cell. Chromatograms and UV-Vis absorption spectra obtained from a gradient were qualitatively equivalent. The lightweight and compact components connected by the optical fibres, allow for flexible arrangement, making this design ideal for incorporation into portable microcolumn LC systems.

ACKNOWLEDGMENTS

This research work was supported by the Ministry of the Interior of the Czech Republic (grant no. VB02000015). The research was conducted in the laboratories of the Institute of Analytical Chemistry of the Czech Academy of Sciences (Institutional research plan RVO:68081715).

From matrix to FAME: rapid profiling of fatty acids

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Keywords: sample preparation methodologies, Gas Chromatography, fatty acid profiling

Objective
Over the past 70 years, food science has seen the development of numerous techniques aimed at optimizing sample preparation methodologies and enhancing chromatographic analysis to accurately elucidate the fatty acid profiles in diverse matrices, including both animal and plant sources. To address the complexities of fatty acid analysis while minimizing costs, time, and potential interferences, traditional methods such as Soxhlet extraction have been largely replaced by modern techniques like ultrasound extraction and accelerated solvent extraction (ASE). For achieving high-resolution fatty acid profiles, gas chromatography remains the preferred method, however, the choice of column, detector, temperature program, and other parameters can vary significantly among researchers.

Methods
In this study, we developed a comprehensive sample preparation and analytical method that achieves fatty acid profiling from raw samples in under 90 minutes, utilizing ASE [1] (extration procedure), derivatization and a 20-minute chromatographic run, by employing a wax column measuring 30 meters in length, 0.25 mm in diameter, and with a 0.25 µm film thickness.

Results
It was possible to chromatographically resolve the fatty acid profile of complex samples, such as fish tissues. The method effectively separated fatty acids, ranging from capric acid (C8:0, retention time 2.94 minutes) to docosahexaenoic acid (DHA, C22:6ω3, retention time 17.42 minutes).

Conclusions
In conclusion, we believe that this method enables more rapid sample preparation and analysis while achieving superior separation in a shorter time compared to previous methodologies [1].

ACKNOWLEDGMENTS

André Jorge would like to thank to Fundação para a Ciência e Tecnologia (FCT) for a PhD scholarship UI/BD/153381/2022. This work was funding by National Funds through FCT - Foundation for Science and Technology under the Project PTDC/BIA-BMA/030517/2017 and the Portuguese Science Foundation through the strategy plan for MED (Mediterranean Institute for Agriculture, Environment and Development), via project UIDB/05183/2020, for MARE (Marine and Environmental Sciences Centre), via project UIDB/04292/2020, UID/50006/2020, and under the project LA/P/0069/2020 granted to the Associate Laboratory ARNET; and for LAQV-REQUIMTE, via project UIDB/50006/2020 and UIDP/50006/2020. This research was also anchored by the RESOLUTION LAB, an infrastructure at NOVA School of Science and Technology.

REFERENCES

[1] Jorge, A, et al., (2022). Proximate Composition, Nutritional Lipid Quality, and Health Indices of Largemouth Bass (*Micropterus salmoides* Lacépède, 1802) from Several Mediterranean Reservoirs, *Journal of Aquatic Food Product Technology*, 31:1, 19-34.

Breast milk pretreatment for HPLC-MS/MS determination of DINCH plasticizer metabolites

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Keywords: oxo-MINCH, hydroxy-MINCH, breast milk, pretreatment efficiency, HPLC-MS/MS

Objective

Human biomonitoring is the best tool for identifying, controlling and preventing exposure of the population to environmental chemical pollutants [1]. Di(isononyl) cyclohexane-1,2-dicarboxylate (DINCH) gradually replaces phthalates used as plasticizers in many consumer products, especially in food packaging, children's toys and medical devices due to its more favorable toxicological profile [2]. However, as the DINCH production and usage have been rapidly increased, the rate of exposure is increased accordingly, so biomonitoring of exposure is important mainly in sensitive population cohorts such as children in early life [3]. By breastfeeding, the mother can transfer to child potentially toxic chemicals to which has previously been exposed. As biomarkers of exposure to DINCH, mainly two oxidized metabolites - hydroxy- (OH-MINCH) and carboxy- (cx-MINCH) cyclohexane-1,2-dicarboxylic acid monoesters are used. The aim of this work was to study the breast milk sample pretreatment prior to HPLC-MS/MS determination of these two metabolites.

Methods

We examined aqueous fraction of milk obtained after centrifugation, and extracts to ethylacetate and mixture of ethylacetate and hexane. Pretreatment procedures were assessed for overall pretreatment efficiency based on the recovery of cx-MINCH and OH-MINCH from the matrix and on the matrix effects in the ionization source of mass spectrometer. Online SPE-HPLC-MS/MS measurements were performed using C-18 stationary phase, HESI ion source operated in negative mode and monitoring the selective mass transitions for each analyte and corresponding isotopically labeled standards by triple quadrupole MS.

Results

Pretreatment efficiency obtained for aqueous milk phase and ethylacetate extracts were very similar at the level of 50 %. Addition of hexane into the extraction solution resulted in the recovery decrease by half. Extraction with examined organic solvents did not bring any improvement of overall pretreatment efficiency. On the basis of HPLC-MS/MS chromatograms of aqueous fraction of milk samples we estimated limits of quantification, as signal-to-noise ratio 10, for cx-MINCH 0.03 µg/L and for OH-MINCH 0.12 µg/L.

Conclusions

Analysis of the aqueous fraction of breast milk after deconjugation and centrifugation was found out as the most proper for the determination of oxidized metabolites of DINCH, as it is environmentally friendly, cheaper, less laborious and time-consuming, but with comparable limits of quantification as for ethylacetate extraction.

ACKNOWLEDGMENTS

This work was supported by the Slovak Research and Development Agency under the Contract No. APVV-20-0462.

REFERENCES

[1] Angerer J., Ewers U., Wilhelm M., (2007*). Int. J. Hyg. Environ.-Health*, 210, 201
[2] Jurdáková H., Górová R., (2021). *Zdravotnícke listy*, 9(4), 34.
[3] Massart F., Gherarducci G., Marchi B., Saggese S., (2008). *Biomarker Insights*, 3, 159

The quantification of target compounds using GC×GC-high resolution MS. Pitfalls and proposed strategies

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Keywords: quantification, PAH, Comprehensive two-dimensional Gas Chromatography

Objective

The absolute quantification of relevant and toxic chemical compounds in complex environmental matrices is an important part of scientific projects. In addition to valid sampling, sample preparation, the type of quantification and the analytical systems used are of critical importance. When applying complex sample preparation in combination with chromatographic systems and mass spectrometry, the use of isotopically labelled standards for the quantification of known target compounds is a good way to account for possible artifacts caused by the sample preparation and analytical platform. This type of quantification is an accepted approach, e.g. for the determination of PAHs by GC-MS. However, in addition to the target compounds, a non-targeted comprehensive analysis of the matrix is increasingly sought to provide an overall view of the chemical profile of the matrix. Comprehensive non-targeted measurement platforms are suitable for such approaches. For example, two-dimensional gas chromatography with (high-resolution) mass spectrometry can be used and has already been successfully applied. The study shows an approach to combine both methods (targeted & non-targeted) on one platform. However, the transfer of quantification based on isotopically labelled compounds to a GC×GC high-resolution time-of-flight system is associated with unforeseen pitfalls that need to be circumvented.

Methods

Particulate ship emissions are analysed by thermal desorption and two-dimensional gas chromatograph with a high-resolution time-of-flight mass spectrometer. In addition to filter sampling, various quantification methods are being investigated.

Results

The application of GC×GC and ultrahigh-resolution mass spectrometry initially appears to be an ideal platform for the comprehensive investigation of complex matrices. However, the transfer of broadly accepted quantification methods is not easy, which is due to the lack of separation in the first dimension. A high mass resolution can help to overcome this shortcoming and realize alternative quantification approaches, which then allow to combine a non-targeted and targeted approach on one platform.

Conclusions

The use of multidimensional separation methods does not always facilitate the analysis of complex matrices. The necessary effort should be examined for each specific application and whether the implementation of established methods is possible.

ACKNOWLEDGMENTS

We are grateful to our project partner & sponsor association for their support and funding. PlumeBaSe is supported by the German (DFG, 471841824) and Czech Research Foundation (GAČR, 22-03426L=). ULTRHAS is funded under the EU's research and innovation programme Horizon 2020 (955390).

HPLC-HRMS analysis of selected bisphenols and their degradation products *via* target, suspect and non-target approach

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Keywords: bisphenols, degradation products, High Resolution Mass Spectrometry, screening methods, water treatment technique

Objective
One of the main environmental issues is global water pollution caused by micropollutants and plastics, including bisphenols, which are well-known plasticizers. For the primary agent of the bisphenol group, bisphenol A, usage restrictions have been implemented, although other bisphenol analogs also exhibit similar toxic effects. In our study, we focused on the electrochemical removal of selected bisphenols (bisphenol A, S, and AF) using an advanced material, specifically a boron-doped diamond (BDD) film deposited on a porous foam. To evaluate the effectiveness and safety of the advanced water treatment technology a combination of high-performance liquid chromatography and high-resolution mass spectrometry (HPLC-HRMS) was used. The main aims of work were to assess the removal rate and identify the degradation and transformation products.

Methods
The electrochemical oxidation was carried out in 4-liters of distilled water with the addition of salts (NaCl, Na_2SO_4, $NaNO_3$) and bisphenol at a concentration of 1 $mg.L^{-1}$. The samples were analyzed in two ways: i) targeted analysis to evaluate the effectiveness of the degradation process by HPLC-APCI/APPI-HRMS, and ii) suspect and non-target screening to identity the degradation and transformation products with HPLC-ESI-HRMS. For chromatographic separation, an analytical column Hypersil Gold aQ (50x2.1 mm, 5 µm)was used with gradient elution ofmobile phases water and methanol. In both cases, Q-Exactive hybrid quadrupole-orbitrapinstrument was employed.

Results
In this study, the effectiveness of the electrochemical degradation process was evaluated using a targeted approach across three different electrolytes (NaCl, Na_2SO_4, $NaNO_3$), as the type of reactive particles directly influences the elimination of targets. The results showed a high elimination rate for all three persistent bisphenols within 20 minutes, with an efficiency exceeding 98% in NaCl. By adding $NaNO_3$, it was achieved the highest removal efficiency for bisphenol A and bisphenol AF, specifically 65% and 90%, respectively. In general, among the three bisphenols, bisphenol A exhibits the greatest resistance to removal. Additionally, several chlorinated and nitrated degradation products of bisphenols were identified using suspect and non-target HPLC-HRMS analysis. Degradation and transformation products were identified based on mass accuracy below 5ppm, retention characteristics, isotopic pattern and MS/MS fragmentation profile.

Conclusions
This study investigated the electrochemical removal using BDD electrodes of bisphenol A, bisphenol S, and bisphenol AF. The results from targeted analysis suggest that the BDD electrode is a promising treatment technology, achieving over 98% elimination for each bisphenol. Additionally, the suspect and non-target approaches coupled with data software proved as an effective way for identifying newly formed degradation and transformation products to assess the safety of used treatment.

ACKNOWLEDGMENTS
The work thanks for the financial support of the Research and Development Support Agency (APVV-23-0367).

Achiral-chiral HPLC-DAD method coupled with online SPE sample preparation for the analysis of nonsteroid anti-inflammatory drugs in water

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Keywords: NSAIDs, HPLC-DAD, enantioseparation, SPE, sample preparation

Objective
Nonsteroidal anti-inflammatory drugs (NSAIDs) are a group of drugs widely used in human and veterinary medicine. They can enter the aquatic environment (e.g. surface and groundwater) trough effluents from wastewater treatment plants. Due to their adverse effects on ecosystems and human health, it is necessary to monitor water sources using sufficient and sensitive analytical methods, including sample treatment, analytes separation and enantioseparation [1]. The aim of this work was to develop an analytical method for the determining selected NSAIDs in water by achiral-chiral HPLC-DAD coupled with online SPE for analytes preconcentration.

Methods
A 2D-LC-DAD in a heart-cutting mode with a Symmetry C18 (4.6 mm × 75 mm, 3.5 µm) column, a Chirobiotic T (4.6 mm × 250 mm, 5 µm) column and a mobile phase consisting of methanol and 0.1% triethylamine acetate (pH= 4.1) were used for the achiral and chiral separation of ketoprofen, naproxen, fenoprofen, flurbiprofen and ibuprofen. Chromatograms were monitored at a wavelength of 230 nm. The column temperature was 25 and 10 °C for the achiral and chiral column, respectively. Online SPE was performed on an Oasis HLB cartridge (3.9 mm × 20 mm, 5 µm) and sample flow rate of 3 $ml.min^{-1}$.

Results
Several types of achiral stationary phases of C8, C18, Biphenyl, Phenyl-hexyl were tested for the RP HPLC-DAD separation of selected compounds, reaching the best separation on the C18 type with the resolution values of more than 1.2 and run time of 15 min. The chiral separation on teicoplanin, teicoplanin aglycone, vancomysin, rystocetin based stationary phases show the best enantioseparation on the teicoplanin column. The influence of the sample flow rate and the sorption capacity of the SPE adsorbent was investigated to select optimal online SPE conditions. The developed method shows limits of detection of 0.2–0.6 $\mu g.mL^{-1}$, linearity in concentration ranges of 0.6-10 $\mu g.mL^{-1}$ for naproxen, and 1-10 $\mu g.mL^{-1}$ for other NSAIDs under study (R^2>0.99).

Conclusions
The developed achira-chiral 2D-LC-DAD coupled with online SPE was used for the analysis of drinking water and river water in Slovakia. The result showed ketoprofen in river water sample. The proposed sample treatment procedure allows high preconcentration of analytes and was applicable for the monitoring of NSADIs and their enantiomeric form in environment.

ACKNOWLEDGMENTS

This work was supported by the Slovak Research and Development Agency under contract number VEGA 1/0332/24.

REFERENCES

[1] Gómez-Oliván L.M., (2020) Non-steroidal anti-inflammatory drugs in water, emerging contaminants and ecological impact, Springer Cham, ISBN978-3-030-56296-0, Switzerland.

Quantitative assessment of therapeutic peptides via capillary zone electrophoresis with repeated sample injection

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Keywords: Capillary Zone Electrophoresis, hydrodynamically closed separation system, lanreotide, therapeutic peptides, triptorelin

Objective
Triptorelin and lanreotide, both synthetic peptides with significant therapeutic importance, have traditionally been analyzed using liquid chromatography. However, CE is emerging as a promising alternative.
Our goal was to develop green, high-throughput, and practical analythical methods based on hydrodynamically closed system (HCS) capillary electrophoresis. Building on our research group's previous development of a repeated injection (RI) technique to enhance sample throughput in HCSs, we aimed to develop methods applicable for use in quality control laboratories.

Methods
Analyses were conducted using the EA 102 hydrodynamically closed system (HCS). The separation column consisted of an internal diameter of 300 µm and a total length of 90 mm. Analyte detection was achieved using a UV detector set to a wavelength of 214 nm. RI procedure was employed following specific time intervals between successive sample injections. For both therapeutic peptides, three sample injections of the respective sample were performed in each electrophoretic run.

Results
In the first step we tested various background electrolytes (BGEs). Formic acid (HFo) buffer solutions with high concentrations demonstrated superior separation efficiencies, leading us to select a 50 mM HFo solution as the optimal BGE. Next, we optimized the RI procedure, determining that a 100 s interval for triptorelin and an 80 s interval for lanreotide achieved adequate resolution values of the three analyzed peaks.
An extensive validation protocol yielded favorable parameters: low limits of detection (LODs) of 0.25 µg/mL in aqueous matrix and 0.5 µg/mL in synthetic urine, coefficient of determination exceeding the value of 0.99, satisfactory precision (relative standard deviation ranging from 5.2% to 14.9%), and accuracy (relative errors ranging from 91.1% to 107.8%). The method developed for triptorelin was applied for quantification of the peptide in a commercial drug (powder for injection) and in spiked synthetic urine samples. Applicability of the methods was evaluated using the novel Blue Applicability Grade Index (BAGI), highlighting their superior practicality.

Conclusions
This study introduces innovative analytical methods for quantifying therapeutic peptides triptorelin and lanreotide in aqueous and synthetic urine matrices using HCS and RI strategy. The RI approach increased sample throughput threefold compared to the conventional single injection method.
Excellent LODs for triptorelin and lanreotide were achieved due to the enhanced sample loading capacity of the HCS. The developed methods were successfully applied to pharmaceutical and synthetic urine matrices, showcasing their potential for implementation in quality control laboratories and for the quantification of clinical samples.

ACKNOWLEDGMENTS

This research was supported by the projects UK/3023/2024, and VEGA 1/0302/24.

HPLC-MS/MS method development for the determination of free carnitine, creatine and creatinine in tears

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Keywords: tears, HPLC-MS/MS, free carnitine, creatine, creatinine

Objective
Nowadays, tears are increasingly used to determine biomarkers of various diseases, especially those related to the central nervous system (CNS). The advantage of this matrix is its direct connection with the CNS and non-invasive sampling [1]. Autism is a heterogeneous neurodevelopmental disorder known as Autism spectrum disorders (ASD), but the pathophysiological basis of ASD is not yet defined and many factors, including genetic and environmental factors, are involved in the development of this disorder [2]. The onset of ASD can also be related to a lack of brain carnitine, and therefore it is important to know the level of free carnitine in the brain. Creatine deficiency syndromes are a group of inherited metabolic disorders and include disorders of creatine synthesis and transport. A common feature of these syndromes is a lack of creatine in the brain with neurological manifestation of the disease and may also be related to ASD [3]. The aim of this work was to develop a method for the determination of free carnitine and creatine in tears as possible markers of ASD together with creatinine as normalization factor.

Methods
For the determination of analytes, tears were collected using Schirmer strips and after extraction were analysed by HPLC-MS/MS. Extraction recovery, matrix effect and overall process efficiency were evaluated for different pretreatment procedures.

Results
The most optimal procedure was extraction into 1 ml of 80% methanol and subsequent HPLC separation by isocratic elution (95% water, 5% methanol with 1% formic acid), using a Synergi 4 µ Polar-RP column (150x2mm) and HESI-MS/MS detection in the positive ion mode. The LOQ values (S/N=10) for carnitine, creatine and creatinine in the extract were 0.003, 0.02 and 0.1 µmol/l, respectively. Intra- and inter-day precision were up to 4.4 % and accuracy did not exceed 12 % for all analytes.

Conclusions
The developed method is suitable for the determination of selected analytes in tears and meets the criteria for precision and accuracy for the analysis of biological matrices.

ACKNOWLEDGMENTS

This work was supported by the Slovak Grant Agency, Contract Number VEGA1/0080/22 and by the Slovak Research and Development Agency under the Contract no. APVV-20-0462.

REFERENCES

[1] Król-Grzymała A., Sienkiewicz-Szłapka E., Fiedorowicz E., Rozmus D., Cieślińska A., Grzybowski A., (2022). Tear Biomarkers in Alzheimer's and Parkinson's Diseases, and Multiple Sclerosis: Implications for Diagnosis (Systematic Review), *International Journal of Molecular Sciences*, 23, 10123

[2] Demarquoy C., Demarquoy J., (2019). Autism and carnitine: A possible link, *World Journal of Biological Chemistry*, 10(1), 7-16

[3] Jurdáková H., Górová R., (2018). Creatine deficiency syndromes, biomarkers and potential frequency in children with autism spectrum disorders, *Zdravotnícke listy*, 6(3), 43-46

UHPLC 2.0: greener separations, lower footprint - replacing ACN and MeOH with sustainable alternatives

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Keywords: green solvents, Liquid Chromatography, green chemistry

Objective
In recent years, modern society's awareness of global threats such as climate change and environmental issues has been growing steadily. Governments are promoting specific programs to enable sustainable development, such as the European Green Deal, which aims to achieve climate neutrality by 2050 and boost the economy through green technologies. For these reasons, green analytical chemistry (GAC) is increasingly recognized as a new and important area of green chemistry, where replacing harmful solvents with greener ones has become one of the main drivers of sustainability in laboratories and industry. Since the most common chromatographic system includes reversed-phase conditions, most wastewater comprises acetonitrile (ACN) and methanol (MeOH), which are toxic, flammable, and hazardous to the environment and humans. For these reasons, replacing these solvents with greener alternatives is becoming an urgent priority [1,2].
This study investigates the impact of mobile phase composition on the carbon footprint of ultrahigh-performance liquid chromatography (UHPLC) separations. We aim to demonstrate that environmentally friendly organic modifiers like ethanol (EtOH) and dimethyl carbonate (DMC) can achieve comparable chromatographic performance. This investigation contributes to developing green analytical chemistry by identifying effective and sustainable alternatives for UHPLC separations.

Methods
Two mixtures, one containing six polar compounds and another containing seven hydrophobic compounds, were separated using the UHPLC technique with UV/Vis detection. For each mixture, the following mobile phases were tested: water/MeOH, water/ACN, water/EtOH, water/DMC, and water/EtOH/DMC. Separations were conducted on three stationary phases: C18, diphenyl, and pentafluorophenylpropyl (F5).

Results
The results compare key separation metrics across all combinations, including analysis time, solvent consumption, resolution between compound peaks, peak asymmetry, and efficiency. Based on the above-collected data, it was also possible to perform carbon footprint calculations for each solvent studied.

Conclusions
The research compares the effectiveness of green solvents (EtOH and DMC) against conventional solvents (MeOH and ACN) for separating polar and hydrophobic compounds. The study showed that it is possible to achieve equally satisfactory separations using alternative solvents. The carbon footprint of each solvent was calculated to assess the environmental impact of the methodologies.

REFERENCES
[1] Welch C., Wu N., Biba M., et al., (2010) Greening Analytical Chromatography, *TrAC - Trends in Analytical Chemistry*, 29, 667–680.
[2] Felletti S., Spedicato M., Bozza D., et al., (2023). Dimethyl Carbonate as a Green Alternative to Acetonitrile in Reversed-Phase Liquid Chromatography. Part I: Separation of Small Molecules, *Journal of Chromatography A*, 1712, 464477.

Development and validation of a capsule phase microextraction method combined with gas chromatography-tandem mass spectrometry for the determination of polychlorinated biphenyls in water samples

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Keywords: capsule phase microextraction, GC-MS/MS, polychlorinated biphenyls, water samples

Objective

Polychlorinated biphenyls (PCBs) are synthetic organic compounds that are used in a wide range of industrial and commercial applications. Due to the adverse effects of these compounds on human health and ecosystems, their careful monitoring is necessary. In this work, a capsule phase microextraction (CPME) protocol was developed for the extraction of eight PCBs (i.e., 2-chlorodiphenyl, 2,3-dichlorobiphenyl, 2,4,5-trichlorobiphenyl, 2,2',4,4'-tetrachlorobiphenyl, 2,2',3',4,6-pentachlorobiphenyl, 2,2',4,4',5,6'-hexachlorobiphenyl, 2,2',3,3',4,4',6-heptachlorobiphenyl, 2,2',3,3',4,5,6,6'-octachlorobiphenyl) from water samples prior to their determination by gas chromatography-tandem mass spectrometry (GC-MS/MS).

Methods

In CPME, the microextraction device is made of two permeable microporous polypropylene tubes welded together. One polypropylene tube contains a sol–gel hybrid organic–inorganic sorbent, while the other contains a cylindrical magnet. Thus, the extraction device integrates the stirring and filtration mechanisms [1]. The main parameters that affect the adsorption and desorption steps (i.e., type of capsule, sample volume, extraction time, stirring rate, ionic strength, type and volume of eluent, and desorption time) were studied.

Results

The sol-gel Carbowax 20 M encapsulated microextraction capsules were found to be the optimum for the extraction of PCBs. The extraction was achieved within 20 min under constant stirring at 100 rpm using 20 mL of sample. The elution was performed by immersing the capsule in 500 μL of acetone for 5 min. Accordingly, the CPME-GC-MS/MS method was validated in terms of linearity, sensitivity, selectivity, enhancement factor, accuracy, and precision. Good linearity, selectivity, sensitivity, accuracy, and precision. The applicability and the green character of the proposed method were also evaluated using Blue Applicability Grade Index and Complementary Green Analytical Procedure Index.

Conclusions

the combination of CPME and GC-MS/MS can be effectively used for the extraction of the target compounds from real samples with high precision, accuracy, selectivity, and sensitivity.

REFERENCES

[1] Manousi N., Kabir A., Furton K.G., Rosenberg E., Zachariadis G. (2021). Capsule phase microextraction of selected polycyclic aromatic hydrocarbons from water samples prior to their determination by gas chromatography-mass spectrometry, *Microchemical Journal*, 166, 106210.

Untargeted analysis of cigarette leachate extracts using high capacity sorptive extraction followed by TD-GC×GC-MS

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Keywords: cigarette butt leachates, environmental hazard, HiSorb, thermal desorption, GC×GC-MS

Objective
Cigarette butts constitute the main type of litter collected from both urban areas and coasts worldwide [1]. Improperly discarded tobacco products have been proven to leach a variety of toxic and potentially carcinogenic chemicals, such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, as well as nicotine, aromatic amines, nitrosamines, phenols, and BTEX [1, 2]. This study aims to detect and compare the leached compounds from unused and used conventional cigarettes (CCs) and heat-not-burn tobacco sticks (HnBs), using High Capacity Sorptive Extraction (HiSorb) followed by comprehensive two-dimensional Gas Chromatography (GC×GC) coupled to Mass Spectrometry (MS) to assess their potential environmental risk.

Methods
Four different types of leachates were prepared in ultrapure water by soaking unused or used HnBs and CCs at a 10 L kg^{-1} liquid-to-solid ratio for 24 hours at room temperature (25 °C). Subsequently, 9 mL of each leachate was transferred to 10 mL vials and HiSorb units with Polydimethylsiloxane (PDMS) coating were directly immersed (DI) in the samples for 1 hour at 30°C and 300 rpm. After extraction, the probes were thermally desorbed at 250°C for 10 min and compounds separation occurred in a GC×GC using a combination of a low-polar and a mid-polar column, linked by a flow modulator with a 5 sec modulation period. MS scan range was set from 40 to 550 m/z.

Results
In all four leachate types, the two most abundant chemicals were nicotine, from the tobacco plant, and triacetin, from the cellulose acetate filters. Used CCs were the samples that released a significantly higher number of compounds compared to used HnBs, including naphthalene, decanal, large groups of pyridines, quinolines, indoles, phenols, benzonitrile, and some of their alkylated analogues, as well as other nitrogen-containing compounds. These chemical classes were far less abundant or not detectable in the control leachates of unused tobacco products.

Conclusions
The findings suggest that most of the leached compounds occur due to the incomplete combustion of organics during CCs smoking, which takes place at temperatures above 600 °C, in contrast to the simple heating of HnBs at 350°C. Regarding the extraction and analysis methods, the large sorbent phase volume of HiSorb probes enables effective extraction even at mild temperatures (30 °C), which combined with TD-GC×GC-MS allows excellent compound separation and detection of hazardous substances from complex matrices, such as leachates from used tobacco products, highlighting the potential of this technique for environmental risk assessment and monitoring.

ACKNOWLEDGMENTS

Authors wish to thank A.C. Laskaridis Charitable Foundation for the PhD scholarship and Markes Ltd for providing the HiSorb probes, the HiSorb Agitator and the TD100-xr Thermal Desorption Unit.

REFERENCES

[1] Solomou N., Fernández E., Szafnauer R., Psillakis E., (2023). Total and bioavailable polycyclic aromatic hydrocarbons in unused and operated heat-not-burn tobacco products and conventional cigarettes, *Chemosphere*, 335.

[2] Soleimani F., Dobaradaran S., De-la-Torre G.E., Schmidt T.C., Saeedi R., (2022). Content of toxic components of cigarette, cigarette smoke vs cigarette butts: A comprehensive systematic review, *Sci. Total Environ.*, 813, 152667.

Forming of a novel type of HPLC column by bridging of discrete silica particles

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Keywords: Supercritical Water, three-dimensional structure, monolithic column, close-packed bed

Objective

The initial concept aimed to create "hybrid" capillary chromatographic columns, with high porosity, on the border between a packed and a monolithic column that would combine the benefits of both. They prove useful in scenarios where high pressure is necessary because of the high hydraulic resistance of small particles and/or the passing medium with high flow rate.

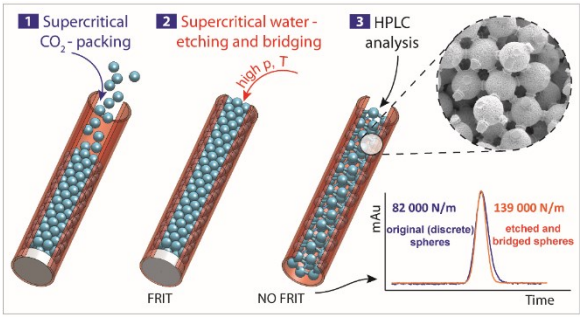
In this first phase of research, we successfully prepared a monolithic column in 100 µm fused silica capillary, packed with 5 µm silica spheres. In the next phase of the research, our aim is to create homogeneous structures from input particles with a standard size of 3µm down to 1.7µm, which could result in structures with an average particle size of 2 or even 1µm, while maintaining a very high porosity and permeability.

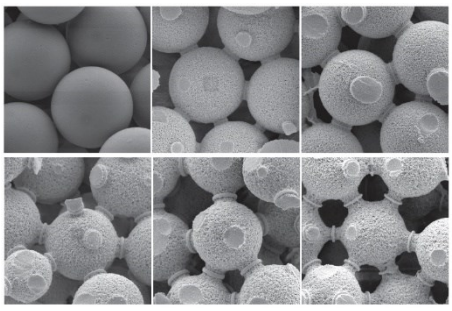
Methods

We used a three-step process to prepare a completely new type of monolithic column. This included capillary filling with supercritical CO_2 (I); etching and bridge-based structure formation with supercritical water (II). Finally, we modified the column surface with a stationary phase (III). Their internal structures were examined using scanning electron microscopy and characterized using microHPLC chromatography.

Results

In order to assess the performance of the new type of monolithic columns, an SCW-treated column and a discrete microsphere-packed column were both chemically modified to introduce C18 stationary phase. A simple comparison using a mixture of alkylbenzenes indicated superior performance of the SCW-treated, bridged-microsphere column over the discrete microsphere-packed column. Columns produced in this way also exhibit high reproducibility of production in both, homogeneity of structure along the capillary and column–to–column fabrication.





Conclusions

This pilot work presents a new and unique method for preparing 3D homogeneous structures using supercritical water's ability to dissolve silica. Chromatographic columns prepared using this method exhibit high separation efficiency while maintaining high permeability. The microspheres are not only connected to each other but also to the capillary wall, resulting in a rigid cartridge that is free from any movement.

ACKNOWLEDGMENTS

This work was supported by the Czech Science Foundation (Grant 23-04703S) and by the Academy of Sciences of the Czech Republic (Institutional Support RVO: 68081715).

The phenolic profile of common European vinegars

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Keywords: vinegar, Liquid Chromatography, Mass Spectrometry, phenolics

Objective
Vinegar is a traditional food product, recognized for its production through a double fermentation process involving yeast and bacteria of the genus Acetobacter. The key component of vinegar is acetic acid, but it also contains a variety of other biologically active substances that contribute to human health benefits. Among these, phenolic compounds are particularly noteworthy due to their antioxidant, antimicrobial, and anti-inflammatory properties [1]. These compounds are naturally present in fruits, vegetables, and other plant-based products. The phenolic content in vinegar varies depending on the raw materials used and the specific production methods. To analyze the qualitative and quantitative presence of these phenolic compounds, liquid chromatography is often employed, coupled with either a photodiode array or mass spectrometry detectors [2][3]. This advanced analytical technique allows a better understanding of their potential health benefits in various vinegar products.

Methods
The HPLC system Agilent 1260 (Agilent, Santa Clara, CA, USA) coupled with tandem mass spectrometer QTrap 4500 (AB Sciex, Framingham, MA, USA) was used for the qualitative and quantitative determination of phenolic compounds. For the separation of phenolic compounds, a Luna Omega Polar C18 column (Phenomenex, Torrance, CA, USA) and a gradient elution of the mobile phase system water - acetonitrile with the addition of 0.1% (v/v) acetic acid were used. Six types of vinegar that are commonly consumed in Europe were selected. Before HPLC analysis, samples were centrifuged and diluted. Quantitative evaluation was carried out by the multiple standard addition method.

Results
A total of 40 phenolic compounds were identified using HPLC-MS/MS method. The phenolic profiles of different types of vinegar (spirit, apple, rice, white wine, red wine and balsamic) were determined. Individual types of vinegar differ from each other in both the qualitative representation of substances and their quantity. The content of selected phenolic compounds in spirit vinegar was negligible. On the contrary, balsamic vinegars seem to be the richest source of phenolic compounds. Phenolic acids and tyrosol can be considered as the main phenolic compounds of fruit vinegars.

Conclusions
Vinegar can be a good source of phenolic compounds, but it depends on the type of vinegar. Significant differences were found in the content of phenolic substances within the given group of vinegar, and therefore the production process and starting material seem to be an important factor.

ACKNOWLEDGMENTS

The work was financially supported by the Czech Science Foundation, project No. 22 09556S.

REFERENCES

[1] Haminiuk C. W. I., Maciel G., Plata-Oviedo M. S. V., (2012). Phenolic compounds in fruits – an overview, *International Journal of Food Science & Technology*, 47, 2023-2044.
[2] Khoddami A., Wilkes M. A., Roberts T. H., (2013). Techniques for Analysis of Plant Phenolic Compounds, *Molecules*, 18, 2328-2375.
[3] Kašpar M., Bajer T., Bajerová P., Česla P., (2013). Comparison of Phenolic Profile of Balsamic Vinegars Determined Using Liquid and Gas Chromatography Coupled with Mass Spectrometry, *Molecules*, 17;27(4):1356

Advanced nanofibrous sorbents for the extraction of pollutants from river waters and protein matrixes

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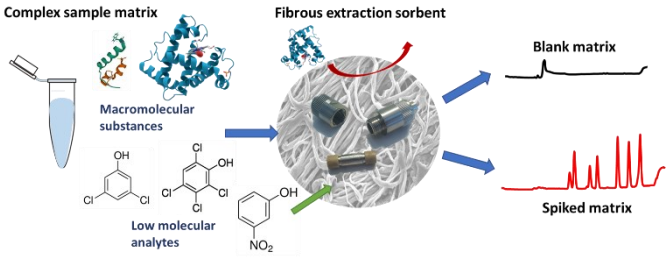
Keywords: nanofiber; polycaprolactone; on-line Solid Phase Extraction; column switching; coating; hybrid fibers; carbon

Objective

The extraction efficiencies of thirty types of fibers produced by meltblown, alternating current electrospinning, and meltblown-co-electrospinning technologies were tested as advanced sorbents for on-line solid-phase extraction in a high-performance liquid chromatography system have been tested and compared with a commercial C18 sorbent.



The properties of each fiber, which were often depended on the production process, and their applicability were demonstrated with the extraction of the model analytes nitrophenols and chlorophenols from various matrices including river water and to purify complex matrix human serum and bovine serum albumin from macromolecular ballast. Polycaprolactone fibers outperformed other polymers and were selected for subsequent modifications including: (i) incorporation of hybrid carbon nanoparticles, i.e., graphene, activated carbon, and carbon black into the polymer prior to fiber fabrication, and (ii) surface modification by dip coating with polyhydroxy modifiers including graphene oxide, tannin, dopamine, hesperidin, and heparin.

Methods

The fiber mats were packed into a cartridge coupled to a column switching chromatography system and the fully automated on-line SPE method was optimized and applied to HPLC-DAD.

Results and conclusions

With the exception of PLA fibers, which collapsed and caused pressurization of the system, all other polymer fibers were sufficiently robust and suitable for analysis at high pressure. PCL fibers outperformed the other fibers and its extraction efficiency via hydrophobic interactions was comparable to that of the conventional C18 sorbent. In addition, PCL fibers were modified to produce advanced hybrid fibers enriched with activated carbon and carbon black, and fibers coated with polyphenolic compounds. These novel modified fibers provided excellent analyte recoveries similar to that of the C18 sorbent and were also tested for extraction from close to real-world aqueous and protein-containing matrices.The on-line SPE-HPLC column-switching method we developed is fast, has a high sample throughput, is fully automated, and minimizes the use of disposable plastic waste and solvents.

ACKNOWLEDGMENTS

This work was supported by the Czech Science Foundation, project GA ČR No 23-05586S.

Effect of vancomycin-modified SPIONS on methicillin-sensitive and resistant *Staphylococcus aureus*

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Keywords: HPLC, nanoparticles, electrochemistry, bacterial infection, nanomedicine

Objective

It has been nearly a century since the discovery of the antibacterial properties of penicillin. Its integration into clinical practice has significantly advanced the treatment of infectious diseases. Subsequently, these substances found utility in agricultural production as well. However, in recent decades, the emergence of bacterial resistance has become an escalating concern, particularly within hospital environments where nosocomial infections caused by resistant strains pose significant clinical challenges, sometimes with fatal consequences. Thus, there is a crucial need for research of novel antibacterial compounds and methodologies. One of the objectives of nanomedicine is to overcome these resistant bacterial strains. The objective of this study is to develop novel nanomaterials to improve the effectiveness of antibacterial therapy.

Methods

For this purpose, SPIONS were prepared using standard procedures, which were then purified on a magnet and lyophilized for 48 hours at -80 °C. The NPs were resuspended by ultrasound for 5 minutes at 100 W. Furthermore, SPIONs were modified with 125 mM chitosan for 24 hours at 300 rpm and 25°C, and then purified on a magnet. Subsequently, SPIONs/Chito were refined with 100 mM vancomycin for 6 h at 300 rpm and 25°C.

Results

The particles were modified with vancomycin (100 µg/mL) resulting in SPIONs/Chito/VANCO NPs. UV-Vis analysis showed maxima at 220, 250 and 400 nm. FTIR analysis demonstrated the presence of both Chito and VANCO on the surface of the nanomaterial through band comparison. TEM and SEM analysis revealed a spherical structure with a particle size of approximately 140-180 nm. The analysis of magnetic properties was performed, and the modification of the nanotransporter resulted in a shift of the zeta potential to positive. The presence of Chito (RT 4.5 min) and VANCO (10.1 min) was demonstrated through their release from the nanoparticles via chromatography. The release of VANCO was studied in various environments, and it was shown that the release decreased with increasing pH (approximately 10% per pH unit). The bacterial culture's growth was monitored using growth curves and evaluated as their integral ($AUC_{0\text{-}24h}$). The average AUC of SA1 was 1900±200, and the total biomass was 21 g/L. For SA2, the average AUC was 2200±250, and the total biomass was 23 g/L. Lastly, for SA3, the average AUC was 1800±150, and the total biomass was 17 g/L.

Conclusions

Specially engineered nanoparticles (NPs) affect bacterial metabolism through various mechanisms, including enzymatic degradation, interaction with the bacterial cell wall and cytoplasmic membrane, the formation of oxygen radicals, and binding to DNA structure, thereby overall altering replication and transcription.

ACKNOWLEDGMENTS

The project is being implemented under the grant project Liga proti rakovině Praha (2399011548).

REFERENCES

[1] Ruttkay-Nedecky, B., Skalickova, S., Kepinska, M., Cihalova, K., Docekalova, M., Stankova, M., Uhlirova, D., Fernandez, C., Sochor, J., Milnerowicz, H., Beklova, M., Kizek, R., (2019). *J. Nanosci. Nanotechnol*. 19, 2762--2769

Transferring the CE-QqQ method to CE-QToF for the untargeted analysis of dietary supplements containing *Boswellia serrata* extract

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Keywords: boswellia serrata, CE, MS, QToF, NSAID

Objective
The recently developed CE-QqQ method [1] was designed for the targeted simultaneous analysis of boswellic acids, the main components of *Boswellia serrata* extract, and 13 nonsteroidal anti-inflammatory drugs (NSAIDs) that belong among potential adulterants of dietary supplements containing *Boswellia serrata* extract. The objective of the presented research is to transfer this CE-QqQ method to CE-QToF for the untargeted analysis of *Boswellia*-containing dietary supplements to detect any additional NSAIDs, as well as other components not listed on the label.

Methods
The separation was carried out in 76 cm fused silica capillary, 50 µm i.d., with the applied voltage of +27 kV. 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 the Agilent 7100 CE system with the Agilent 6530 QToF mass spectrometer was implemented through a coaxial sheath liquid interface using a mixture of water and isopropanol (1:1, v/v) with an addition of 0.5 mmol/L ammonium fluoride as a sheath liquid that was delivered at 6 µL/min. The analysis was carried out in negative ion mode.

Results
The previously developed CE-QqQ method was successfully transferred to CE-QToF. The Auto MS/MS mode was utilized for screening five dietary supplements containing *Boswellia serrata* extract to detect potential adulterants. Before application to actual samples, the method underwent thorough optimization to ensure reliable and repeatable results. QToF-specific parameters were optimized (e.g., fragmentor and skimmer voltage), and some other parameters were reoptimized to achieve adequate sensitivity and repeatability (e.g., gas temperature). Although no NSAID adulterants were found in the five tested samples, the method demonstrated its effectiveness by detecting other compounds such as citric acid or ascorbic acid, which may not pose a threat but were nevertheless not listed on the label.

Conclusions
In conclusion, the successful transfer of the CE-QqQ method to CE-QToF has enabled the effective screening of *Boswellia*-containing dietary supplements, with a primary emphasis on detecting NSAID adulterants. The optimized method demonstrated reliability in detecting potential threats to consumer safety. While the absence of NSAID adulterants in the tested samples reaffirms the integrity of these products, the incidental detection of additional compounds accentuates the method's sensitivity and capability for uncovering undisclosed ingredients. This study highlights the importance of robust analytical methods in safeguarding the quality and transparency of dietary supplements.

ACKNOWLEDGMENTS
The study was supported by the GA UK project No. 214123 and the specific research project SVV 260662.

REFERENCES
[1] Kosolapov D., Bařinka F., Nováková L. (2023). Development of capillary electrophoresis-mass spectrometry method for the simultaneous analysis of boswellic acids and non-steroidal anti-inflammatory drugs, 33rd International Symposium on Pharmaceutical and Biomedical Analysis (PBA 2023), July 2-6, Ankara, Turkey.

Protein profile and ATR-FTIR analysis of optimised mixture of goat milk powder and grape pomace seed extract

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Keywords: protein profile, grape pomace seed extract, goat milk, SDS-PAGE

Objective

Grape pomace seeds are a rich source of bioactive compounds such as falvan-3-ols, procyanidins and phenolic acids with a wide range of biological properties. For this reason, their use in the formulation of functional foods has been reported in the recent decades. Grape pomace seed extracts have been used to improve the antioxidant properites of cheese, yoghurt, ice cream, ceral and meat-based products. Recently, the enrichment of goat milk with GPE has been successfully performed suggesting that goat milk has a good carrier properties for bioactive compounds, especially protective properties during *in vitro* gastrointestinal digestion. However, the optimal ratio of milk and GPE to achieve maximum antioxidant properties of the mixture has not been investigated and it has not been established whether the protein profile of the mixture can be altered by optimization.

Methods

Therefore, the aim of this work was to investigate the protein profile of thermally-treated goat milk (TM) powder enriched with grape pomace seed extract (GPE) using SDS-PAGE and ATR-FTIR technique. The TM/GPE mixture was optimized using the Central Composite Experimental Design (CCD) with two independent variables (pH and GPE content) and five dependent variables: total phenolic content, $ABTS^{\bullet+}$ scavenging activity, $DPPH^{\bullet}$ scavenging activity, feric reducing power and ferrous ion chelating ability. Protein bands from goat's milk corresponding to caseins and whey proteins can be observed on TM and TM/GPE electrophoretic patterns.

Results

New (uncharacteristic) polypeptide bands were not observed on TM/GPE electrophoretic pattern, indicating that grape pomace seeds extract does not affect the protein composition of the thermally-treated goat milk. However, the intensity of all protein bands detected in the TM/GPE sample was decreased, compared to the same bands in the TM control sample, probably due to the decrease in protein content in the formulated ingredient due to the addition of grape pomace seed extract in the milk. The results of the ATR-FTIR analysis confirmed the SDS-PAGE results. No new bands or shifting of bands were observed in the whole M/GPE ATR-FTIR spectra, as a result of the formation of new compounds or significant chemical interactions between constituents.

Conclusions

The newly formulated mixture could be a promising food ingredient or dietary supplement with high antioxidant properties.

ACKNOWLEDGMENTS

This research was supported by the Science fund of Republic of Serbia, FUNPRO Project, #Grant No. 7744714 the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-65/2024-03/200116.

Direct counting approach for quantification of water-dispersed nanoparticles

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Keywords: nanoparticle counting, photon-upconversion nanoparticles

Objective
The number of nanoparticles in a given volume is a principal characteristic of any nanoparticle dispersion. However, estimating this quantity for small nanoparticles (~30 nm) is challenging [1]. We introduce an optical-microscopy method supported by convolutional neural networks (CNNs) for counting-estimating the concentration of nanoparticles after a well-defined immobilization. Photon-upconversion nanoparticles (UCNPs) were selected to demonstrate the method's capabilities because they provide near-infrared excitation, multiple and narrow emission bands, negligible autofluorescence, and high stability [2].

Methods
The method benefits from a well-defined immobilization on glass substrates [2], which preserves the information on the sampled volume of nanoparticle dispersion. Once immobilized, the UCNPs are imaged by an optical epiphoton-upconversion microscope and counted. The brightness of nanoparticles is automatically measured and advanced statistics account for UCNP aggregates. The number of counted nanoparticles is then divided by the sampled volume to estimate the number concentration. For other nanoparticle types, the microscope setup is switched to epifluorescence, bright field, or dark field to fit the optical properties of a particular nanoparticle type.

Results
The limit of detection for UCNPs was 2.0×10^{6} mL^{-1}, and the working range was from 4.4×10^{7} to 2.2×10^{10} mL^{-1}. The accuracy was confirmed using gravimetric analysis and transmission electron microscopy as a reference [3]. The quantification of nanoparticle clusters was achieved by a thorough analysis of the micrographs. Multiplexed detection of two nanoparticle types in a mixed dispersion was feasibly demonstrated. The method supported extremely sensitive imaging, proven by imaging Tm^{3+}-doped UCNPs (17 nm hydrodynamic diameter).

Conclusions
We report an optical counting approach for aqueous dispersions of nanoparticles. The method utilizes the immobilization of nanomaterials in the well-defined gel, which is followed by optical microscopy, and nanoparticle counting. The method supports several imaging modalities such as photon-upconversion, fluorescence, dark-field, and bright-field microscopy and can be applied to other types of nanoparticles.

ACKNOWLEDGMENTS

We acknowledge financial support from the Czech Science Foundation (24-11183S) and Institutional support RVO:68081715.

REFERENCES

[1] Shang, J., Gao, X. (2014) Nanoparticle counting: towards accurate determination of the molar concentration, *Chem. Soc. Rev.* 43, 7267–7278.
[2] Hlaváček, A. et al. (2022) Bioconjugates of photon-upconversion nanoparticles for cancer biomarker detection and imaging, *Nat. Protoc.* 17, 1028–1072.
[3] Hlaváček, A. et al. (2022) Absolute Counting Method with Multiplexing Capability for Estimating the Number Concentration of Nanoparticles Using Anisotropically Collapsed Gels, *Anal. Chem* 94, 14340–14348.

Determination of amino acids in green coffee beans using hydrophilic interaction chromatography coupled to mass spectrometry

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Keywords: Hydrophilic Interaction Chromatography, Mass Spectrometry, separation, amino acids, coffee

Objective

Amino acids are the basic building blocks of proteins, but they also occur in free form in various matrices, such as green coffee beans. During roasting, chemical reactions such as Maillard reactions and Strecker degradation occur in which amino acids react with reducing sugars. These reactions lead to the formation of aromatic compounds and melanoids, which are responsible for the taste, aroma, and color of roasted coffee. It follows that the total amino acid content of coffee beans decreases significantly during roasting. Understanding these processes is crucial for the optimization of the roasting process and for achieving desired coffee properties.

This work aimed to develop a simple method for the determination of free amino acids using the HPLC–MS(/MS) technique. This method is intended for subsequent use in extensive study profiling the substances contained in different varieties of coffee and different stages of roasting.

Methods

Coffee beans were ground and poured with boiling water to extract amino acids. The sample was cooled at laboratory temperature and then centrifuged, filtered, and diluted with acetonitrile before HPLC–MS or HPLC–MS/MS analysis. Amino acids were separated using the ZIC-HILIC column, mobile phase A consisted of 30 mM aqueous ammonium formate with 0.15% formic acid, and mobile phase B was acetonitrile with 0.15% formic acid.

Results

This method enables selective and specific determination of 20 free amino acids using HILIC–MS(/MS). 19 amino acids were found in different varieties of green coffee beans. In the same varieties of roasted coffee beans either no amino acids were found, or their concentrations were under the detection limit of the instruments.

Conclusions

The more affordable single quadrupole offers satisfactory specificity of detection while maintaining comparable sensitivity to triple quadrupole, making it suitable for routine analyses, such as the determination of free amino acids in coffee beans. Although the triple quadrupole offers greater specificity in the case of coeluting compounds, the separation of isomers or substances with very similar molecular mass is crucial for both instruments.

ACKNOWLEDGMENTS

The financial support by the Czech Science Foundation, project No. 22-09556S is gratefully acknowledged.

Development of a magnet-integrated fabric phase sorptive extraction method combined with GC-MS/MS for the determination of 16 polycyclic aromatic hydrocarbons in water samples

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Keywords: PAHs, fabric phase sorptive extraction, sample preparation, GC-MS/MS, water

Objective

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic organic contaminants composed of two or more fused aromatic rings that can pollute aquatic and terrestrial species. Due to their mutagenic or carcinogenic properties, 16 PAHs have been classified as priority environmental pollutants by the Environmental Protection Agency (EPA) of the United States. Thus, the determination of these compounds at trace levels in water samples is of utmost importance.

Methods

In this work, a magnet-integrated fabric phase sorptive extraction (MI-FPSE) protocol was developed and combined with gas chromatography-tandem mass spectrometry (GC-MS/MS). MI-FPSE is a recently introduced sample preparation approach that integrates the stirring and extraction mechanisms into a single sample preparation device. In MI-FPSE, two FPSE membranes are sandwiched together, and a metallic magnetic stirrer is integrated into the device, providing an "all-in-one" extraction device. This technique has been proven to be suitable for the analysis of water samples [1]. The main parameters that affect the performance of the MI-FPSE procedure were studied and the analytical method was validated and used for real sample analysis. The applicability and the green character of the proposed method were also evaluated.

Results

Sol-gel poly(tetrahydrofuran) MI-FPSE media provided the highest extraction efficiency towards the majority of the target analytes. The extraction of PAHs was achieved within 50 min under constant stirring at 1000 rpm. The optimum sample amount was 50 mL and its ionic strength was adjusted using 20% m/v NaCl. Elution was achieved within 2 min using 500 µL of acetone. The developed method showed good linearity, accuracy, and precision. The LOQs for the target analytes were down to 0.01 µg L^{-1}. Moreover, the sol-gel coated MI-FPSE membranes were found to be reusable in accordance with the requirements of green sample preparation.

Conclusions

The MI-FPSE method exhibited good accuracy and precision, wide linear range, as well as low LOD and LOQ values. Among the benefits of this procedure are its operational simplicity, high sample throughput, and rapid extraction kinetics. The overall protocol exhibited good method applicability and green merits. Thus, MI-FPSE serves as a highly versatile tool for the determination of pollutants in environmental samples.

REFERENCES

[1] Manousi N., et al., (2022). Magnet integrated fabric phase sorptive extraction as a stand-alone extraction device for the monitoring of benzoyl urea insecticides in water samples by HPLC-DAD, *Journal of Chromatography A*, 1672, 463026.

Determination of hexavalent chromium in eyeshadow «Toy» cosmetics

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Keywords: cosmetics powder, hexavalent chromium, Inductively Coupled Plasma Mass Spectrometry, extraction, eyeshadows

Objective
The use of children's and recreational makeup kits has become more widespread. Despite being marketed as "toys", these cosmetics are subject to Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of November 30, 2009, which regulates cosmetic products [1]. This Regulation prohibits the use of substances classified as carcinogenic, mutagenic and toxic to reproduction. In particular, Annex II of the regulation prohibits the use of chromic acid and its salts, which have been classified as carcinogenic to humans. In order to minimise the risk of exposure and subsequent repeated absorption or dermal accumulation in the population and in individuals who are already sensitised, it is essential to ensure that the concentration of Cr(VI) in products used on the skin is kept below the set limit [2]. This study proposes an innovative approach for the determination of hexavalent chromium present in cosmetics based on its selective extraction and determination by ion chromatography coupled with inductively coupled plasma source mass spectrometry (IC/ICP-MS) for the separation and identification of the analyte under investigation.

Methods
An extracting solution made of PDCA, $NH_4H_2PO_4$, NH_4AcO, NH_4OH e TBAOH at pH 7,1 was investigated on testing eyeshadow powder to extract all Cr(VI) within. The mixture thus prepared was subjected to mechanical agitation and homogenisation by vortexing, then was treated with an ultrasonic bath and subsequently centrifuged. Two extractions were conducted for each sample, with the resulting supernatant solutions collected together for analytical determination by IC/ICP-MS. The optimised operating conditions ensured the maintenance and stability of the species throughout the extraction and purification stages, preventing the processes of Cr(III) oxidation and Cr(VI) reduction. A mineralisation process, comprising three microwave-assisted acid digestions, was developed for analytical determination of total Cr in the samples.

Results
The method was found to be suitable for the determination of Cr(VI) extracted from the sample in 8 minutes, with a detection limit of 0.3 µg/L, which is sufficient for the determination of safe cosmetic products. The developed method was applied to the determination of Cr(VI) in real toy eyeshadow by means of an external calibration curve drawn in the extracting solution.
A quantitative determination of total chromium was performed by inductively coupled plasma mass spectrometry (ICP-MS) in dynamic reaction cell (DRC) mode, with the calibration curve drawn in the digestion mixture.

Conclusions
The analytical approach developed for the extraction, IC separation, and quantification using ICP-MS as a detector has eliminated the requirement for post-column derivatization reactions in the spectrophotometric determination of Cr, allowing for an accurate identification of Cr(VI) with a very low limit of detection. In conclusion, the quantification of Cr(III) in the samples was determined by the method of difference.

REFERENCES
[1] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products.
[2] Metalli in prodotti cosmetici: procedure raccomandate per la determinazione e valutazione dei rischi per la salute. Alessandro Alimonti, Beatrice Bocca, Franca M. Buratti, Emanuela Fabbri, Emanuela Testai 2019, iv, 57 p. Rapporti ISTISAN 19/18

One step derivatization and switchable hydrophilic solvent microextraction for the determination of adamantine analogues in human urine by HPLC-FLD

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Keywords: adamantine analogues, derivatization, Liquid Chromatography, microextraction, switchable hydrophilicity solvent

Objective
The present study describes an "one-step" derivatization and microextraction using a pH-switchable hydrophilicity solvent (SHS) for the determination of amantadine (AMA) and memantine (MEM) in human urine by HPLC and fluorescence detection. Memantine (MEM) is an extensively prescribed drug for the treatment of Alzheimer's and Parkinson's diseases and amantadine (AMA) has been widely used as an antiviral drug against influenza A virus [1]. Monitoring the concentration levels in biological fluids is of utmost importance in clinical evaluations and in personalized therapeutic strategies.

Methods
The procedure is based on the derivatization of the analytes with o-phthalaldehyde/N-acetyl cysteine at alkaline conditions (borate buffer pH 10.5) in the presence of sodium salicylate as extractant in a homogeneous solution. The liquid-solid transition of salicylic acid was achieved by adding an aliquot of concentrated H_3PO_4 due to the suppression of the carboxylic acid dissociation. The developed scheme enables efficient dispersion, phase separation and derivatization in a single step. The derivatives interact with the benzene ring of salicylic acid via hydrophobic interactions [2]. Due to the moderate melting point of salicylic acid, its solidification is carried out at room temperature without the need for sample cooling.

Results
Good linearity was observed in the range 50-2000 ng mL^{-1}, while the relative recovery values were within 86-113%. The intraday and between the days precision was less than 12% in all cases. The green character of the method was assessed too. The developed method was successfully employed for monitoring amantadine and memantine in human urine samples.

Conclusions
The proposed sample pretreatment protocol offers selectivity, sensitivity, rapidity, handling simplicity and possibility of parallel sample handling. The developed method presented adequate overall performance and could potentially be applied to other biological matrices (e.g. plasma, serum etc).

REFERENCES
[1] N. Manousi., P. D. Tzanavaras, C. K. Zacharis, (2024). Microextraction based on liquid-solid phase transition of benzoic acid: Extraction of statins from human urine followed by chromatographic analysis, *Talanta*, 266, 125088.
[2] Y. Basel, M. Rečlo, Y. Chubirka, (2020). Switchable hydrophylicity solvents in analytical chemistry. Five years of achievements, *Microchemical Journal*, 157, 105115.

Cutting-edge development of an In-Tip MIP-FPSE method for the ultra-selective detection of bisphenol A in urine samples analyzed by HPLC-DAD

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Keywords: In-Tip, fabric phase sorptive extraction, Molecularly Imprinted Polymers, HPLC-DAD, bisphenol A

Objective
Bisphenol A (BPA) is a ubiquitous industrial chemical used in the production of polycarbonate plastics and epoxy resins, found in numerous consumer products such as food and beverage containers, thermal paper receipts, and medical devices [1]. Due to its widespread use and potential adverse health effects, including endocrine disruption and reproductive toxicity, the determination of BPA has become paramount in environmental and public health research. This study introduces a novel miniaturized sample preparation device, namely Molecularly Imprinted Polymers-Fabric Phase Sorptive Extraction (In-Tip MIP-FPSE), for the determination of BPA in urine samples by HPLC-DAD.

Method
The In-Tip-FPSE device was fabricated by integrating FPSE membranes into a 1 mL micropipette tip. The membranes were precisely positioned and secured inside the tip, enabling efficient analyte extraction. Sample extraction involved 20 cycles of aspirating and dispensing the sample through the device, followed by elution with 1 mL of methanol (MeOH). Eluates were filtered through 0.22 nm nylon filters prior to HPLC-DAD analysis. An isocratic chromatographic program was applied consisting of acetonitrile (ACN) and water (H_2O), at a ratio of 70:30, v/v. The total analysis time was 4.2 min.

Results
Accuracy, expressed as recovery rates, ranged between 86.6 and 102%. The proposed method presents good precision, with relative standard deviation (RSD%) for intra and inter-day precision lower than 4.7% and 9.5%, respectively. Linear response was achieved over the concentration range of 0.1 - 10 μg mL^{-1}, with a coefficient of determination (R^2) of 0.999.

Conclusions
The In-Tip MIP-FPSE device represents a significant advancement in miniaturized sample preparation for clinical analyses. Its integration of FPSE membranes into a micropipette tip offers a portable, reusable, and eco-friendly solution for the efficient extraction of BPA from urine samples.

REFERENCES
[1] Kubica P., Kalogiouri N.P., Kabir A., Furton K.G., Samanidou V.F., (2023) Optimization of a Fabric Phase Sorptive Extraction protocol for the isolation of six bisphenols from juice pouches to be analysed by high performance liquid chromatography coupled with diode array detector, *Journal of Chromatography A*, 1708, 464366.

Microwave-assisted extraction and derivatization methods coupled to GC×GC-FID for fatty acid methyl ester profiling: comparison with official methods

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Keywords: Microwave-assisted process, total fat, fatty acid methyl esters, bidimensional Gas Chromatography

Objective
The objective of this work is to compare different microwave-assisted extraction and derivatization methods for the analysis of fatty acids on different food matrices. Their analysis usually involves the step of extraction and then derivatization to have the fatty acid in their methyl esters for the next GC analysis. For routine analysis the method should be robust, green, and matrix-independent. In this context, the use of microwave energy was investigated for the extraction and derivatization, separately and in one-step processes.

Methods
FAMEs were analyzed using seven different methods. The lipid fraction was obtained with two different microwave-assisted extraction methods, one with hydrolysis (MAEH) and one with solvent extraction (MASE). Both different extracts were derivatized either using BF_3, or a microwave-assisted acidic methanol derivatization. These methods were compared also with a one-step microwave-assisted extraction and derivatization [1, 2], and two official AOCS methods, namely AOCS-Ce 2b-11 and Ce 2c-11, used as references.
The FAME were identified and semi-quantified using a comprehensive two-dimensional GC (GC×GC) equipped with a reversed fill/flush (RFF) flow modulator and coupled with an FID.

Results
The seven methods were applied to six different foods. The results obtained from the different methods were compared in terms of the percentage of all the identified FAME and in terms of Saturated, Monounsaturated, and Polyunsaturated fatty acids. The results from all the microwave processes were consistent with the two official methods across all matrices, except for oat. The oat is a special case, for which further optimization is requested to get in agreement with the specific official method.

Conclusions
The microwave-assisted processes were demonstrated to be suitable for FAs analysis in food, showing to be comparable to the official methods and to be robust, rapid, greener when evaluated with the proper tool named AGREEprep, and practical when evaluated with the tool BAGI.

ACKNOWLEDGMENTS

The authors would like to thank Milestone srl, Shimadzu Corporation and SepSolve for their contribution.

REFERENCES

[1] Angelica Fina, Steven Mascrez, Marco Beccaria, Chiara De Luca, Juan Aspromonte, Chiara Cordero, Giorgia Purcaro., (2022) A high throughput method for fatty acid profiling using simultaneous microwave-assisted extraction and derivatization followed by reversed fill/flush flow modulation comprehensive multidimensional gas chromatography, *Adv Sampl Prep*, 4, 100039.
[2] Ferrara Donatella, Cescon Mirco, Giacoppo Giulia, Costa Valentina, Purcaro Giorgia, Spadafora Natasha Damiana, Cordero Chiara, Pasti Luisa, Cavazzini Alberto, Beccaria Marco., (2024). Composition and nutritional values of fatty acids in marine organisms by one-step microwave-assisted extraction/derivatization and comprehensive two-dimensional gas chromatography -flame ionization detector, *J. Chrom. B*, 1236, 124074.

A chromatographic study on ceramic vases from the Milejowice settlement and the Domasław cemetery

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Keywords: chromatographic analysis, organic residues, ceramic vases

Objective

Chromatographic analysis can provide a lot of useful information about the composition of organic residues that can be used to determine their source. The aim of the study was to determine the purpose of ceramic vessels based on the analysis of lipid fractions. The study focused on ceramic vase-like vessels found at the archaeological sites from the Hallstatt period: the Milejowice settlement and the Domasław cemetery. The analyses included 31 vases, 16 funerary and 15 settlement finds.

Methods

The fragments of the vessels, which were ground into powder, were subjected to solvent extraction using a Soxhlet apparatus. The obtained extracts were concentrated, and the analytes were silylated. Samples were analyzed using gas chromatography combined with mass spectrometry (GC-MS). Qualitative analysis of organic compounds was performed using the NIST14 mass spectral library. Quantitative analysis of fatty acids was carried out based on the internal normalization method.

Results

Eighteen acids, mostly saturated, were identified in the samples. The group of unsaturated acids was represented by C16:1 and C18:1. C18:2 acid was determined in only one sample from Domasław. Acids C6:0, C8:0, C9:0, C10:0, C12:0, C16:0, C18:1 and C18:0 were present in all samples. Long-chain acids (C20:0, C22:0) were present in most samples from Milejowice and six from Domasław. C11:0 acid was identified in only two vases from Milejowice. Statistical analyses showed that vessels from settlements and graves differed the most concerning C13:0, C15:0, C22:0 acid content and C22:0/(C16:0+C20:0) ratio. The organic residue study showed that most of the vessels contained mixed residues of plant-animal source, originating from seeds, nuts and berries.

Conclusions

Chromatographic studies provide a base for conclusions about the uses of ceramic vessels and the products they may have contained. These analyses also reveal differences between the contents of vessels in ceremonial and settlement contexts. These discrepancies may be due to specific plants and specific ingredients (such as dairy or oils) that imparted the desired properties.

ACKNOWLEDGMENTS

The research is part of a project: Feeding and ritual practices of the Early Iron Age based on the settlement in Milejowice and the necropolis in Domasław. Between the function and meaning of the ceramic “collections” (2021/41/B/HS3/02531), financed by Narodowe Centrum Nauki (National Science Center)

Spectrometry for everyone: accessibility for individuals with visual impairments/blindness using audio-tactile pictures

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Keywords: audio-tactile images, education, inclusion, spectrometry, visual impairments

Objective

Spectrometric techniques are commonly used for food, pharmaceutical, toxicological, and environmental analysis. Thus, the proper and comprehensive teaching of these techniques to students is of utmost importance. Although significant efforts are being made towards the employment of novel educational approaches in chemistry teaching, the inclusion of people with visual impairment or blindness is still neglected. This results in significant challenges for these students during their studies, especially regarding the hands-on work in a laboratory which is a significant part of their educational journey. In this work, audio-tactile images were developed for teaching the main spectrometric techniques to individuals with visual impairments.

Methods

Audio-tactile pictures have been recently proposed for teaching the main chromatographic techniques to individuals with visual impairments/blindness [1]. For this purpose, a specific touchpad device (IVEO) is used, as well as tactile images, printed on microcapsule paper. These images are placed on the touchpad, and verbal descriptions are provided. In this case, the user with blindness touches the tactile images and at the same time receives by audio a large number of verbal descriptions. This multimodal approach assists in the perception and understanding of images and diagrams by people with blindness or visual impairments.

Results

In this work, three different audio-tactile pictures were developed for teaching the main spectrometric techniques to individuals with visual impairments/blindness. These images depicted the configuration of an ultraviolet spectrometer, an ultraviolet spectrum, and the configuration of a flame atomic absorption spectrometer. These techniques correspond to the configuration of main spectrometric techniques used for organic and inorganic analytes determination.

Conclusions

The development of these audio-tactile images related to spectrometric techniques is expected to assist in the familiarization of people with blindness with these techniques. Thus, they will be able to perform experiments with the equipment that is commonly found in chemistry laboratories, enhance their confidence in working with them, and gain practical experience which is necessary for their further development.

ACKNOWLEDGMENTS

The project was funded by the Royal Society of Chemistry Inclusion and Diversity Fund ('RSC').

REFERENCES

[1] Samanidou,V., Manousi, N., Raptopoulou, F., Isaraj, L., Papadopoulos, K., (2023). Can Chemistry Be Accessible For Students With Visual Impairment? *Column,* 19, 19-22.

Molecularly imprinted membrane extraction combined with HPLC for selective analysis of biologically active substances in cosmetics

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Keywords: Molecularly Imprinted Membrane, sorption capacity, selectivity, swelling, extraction

Objective

Molecularly imprinted membrane (MIM) combines the advantages of molecularly imprinted polymer (MIP) and membrane separation, such as high selectivity, resistance, and stability [1]. Properties as well as separation using MIM are influenced not only by the composition of the polymerization mixture, the preparation conditions, but also by the supporting membrane materials which can be polysulfone, nylon, polyvinylidene fluoride, polyacrylonitrile, or polypropylene [2]. The aim of the this work was to prepare, characterize, and apply MIM in the extraction of D-panthenol from personal care samples.

Methods

MIP was formed by bulk polymerization in N_2 atmosphere at a temperature of 60 °C for 24 h. The polymerization mixture consisted of a template (D-panthenol), a functional monomer (methacrylic acid (MAA) or 4-vinylpyridine (4-VP)), a porogenic solvent (methanol), an initiator (azisobutyronitrile), and a crosslinker (ethylene glycol dimethacrylate) (6:28:1, t:m:i). Nylon (NI), polypropylene (PP) and polyvinylidene fluoride (PVDF) type of membrane were used as supporting materials. For evaluation nonspecific interactions, non-imprinted membranes (NIMs) were prepared.

Results

MIMs were prepared by in-situ multilayer polymerization of MIP. Polymerization on NI and PP membranes was not sufficient, MIP layer was not formed even after repeated polymerization. The most suitable MIM preparation procedure was multilayer polymerization on a PVDF membrane. IR spectra of MIM and NIM confirmed the formation of MIP and the complete remove of the template from the cavities. The specific sorption capacity values were 156 $\mu g.g^{-1}$ and 143 $\mu g.g^{-1}$, for MIM-4-VP and MIM-MAA, respectively. The swelling of MIMs was monitored in water, methanol, and ethanol, detecting maximal swelling in ethanol. The selectivity was tested for a structural analogue, pantothenic acid. The MIM-4-VP was selective for the template (imprinting factor > 2). MIM was used in the analysis of hair-water sample determining the concentration of panthenol of 1.4 $mg.ml^{-1}$.

Conclusions

In this work, a suitable procedure for the preparation of MIM was proposed. MIMs prepared on PVDF membrane showed a higher binding capacity compared to other membrane supports. Among them, MIM-4-VP showed the highest specific binding capacity and selectivity for the target analyte. The membrane proved recoveries over 73% and was successful used in the analysis of real samples.

ACKNOWLEDGMENTS

This work was supported by the call for doctoral students and young researchers of Slovak University of Technology in Bratislava to start a research career (Grant 23-04-01-A).

REFERENCES

[1] Fan J.-P., Cheng Y.-T., Zhang X.-H., Xiao Z.-P., Liao D.-D., Chen H.-P., Huang K., Peng H.-L. (2020) Preparation of a Novel Mixed Non-covalent and Semi-covalent Molecularly Imprinted Membrane with Hierarchical Pores for Separation of Genistein in Radix Puerariae Lobatae. *React. Funct. Polym*. 146, 104439-52

[2] Zhao X., Cheng Y., Xu H., Hao Y., Lv Y., Li X. (2020) Design and Preparation of Molecularly Imprinted Membranes for Selective Separation of Acteoside. *Front. Chem*. 8, 775-86

Introduction of pulsed – post column derivatization coupled to high performance liquid chromatography: development and applications in analytical chemistry

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Keywords: HPLC, pulsed-post-column derivatization, histidine, histamine, thiols

Objective

In the present study, an alternative approach was developed to reduce reagent consumption in High Performance Liquid Chromatography coupled to on-line Post Column Derivatization (HPLC-PCD). In HPLC-PCD, the reagents flow continuously and react on-line with the eluted analytes after the column, forming the derivatives. In the new approach, the reagents are injected into the flowing stream as well-defined volumes of pulses (Pulsed-Post Column Derivatization, Pulsed-PCD) at microliter levels, which overlap the eluting compounds after precise time tuning, forming similar derivatives. The proposed approach can reduce dramatically the consumption of the reagents at microliter levels instead of milliliters.

Methods

The analytical methods that were developed were based on: (1) the determination of histidine in urine samples after derivatization with o-phthalaldehyde in alkaline medium, (2) the determination of the closely eluted thiols cysteine and glutathione in yeast samples after derivatization with ethyl propiolate, (3) the determination of the biogenic amine histamine in food samples after reaction with o-phthalaldehyde in alkaline medium with the general and the specific mechanisms, respectively, and with the expensive reagent naphthalene-2,3-dicarboxaldehyde.

Results

The development of the new HPLC-Pulsed-PCD concept included: (1) the investigation of the tuning of the pulse with the analyte, (2) the study of the profile of the pulse in terms of time and flow rate, and (3) the robustness of the procedure. This approach was applied for the determination of analytes using five different chemical systems, proving the applicability of the approach regardless of the derivatization reaction. New analytical methods were developed and validated for the determination of the above analytes in real samples, with simple sample preparation.

Conclusions

HPLC-Pulsed-PCD was proved to be an alternative approach to post-column derivatization. The consumption of the reagents was dramatically reduced. The determination of closely eluted compounds and the use of expensive reagents in PCD proved the effectiveness of the concept and demonstrated the significant advantage of the technique over the classical technique.

ACKNOWLEDGMENTS

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd call for HFRI PhD Fellowships (Fellowship Number: 5341).

Simultaneous detection and quantification of organic acids and furans in lignocellulosic biomasses hydrolysate

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Keywords: succinic acid, furans, uHPLC, separation, optimization

Objective
Lignocellulosic biomasses can be a valid alternative to the fossil fuels to produce chemicals as succinic acid by fermentation processes. However, due to its complex structure, pretreatment is a pivotal step to break bonds between cellulose-hemicellulose and lignin, and to allow enzymes to convert them into fermentable sugars [1]. Furans (furfural and hydroxymethylfurfural (HMF)) and organic acids are by-products of the pretreatments process. They are detected and quantified by using different and separated HPLC protocols by refractive index detector or UV/DAD (Diode Array) detectors [2].
In this work, a simultaneous identification of furfural, HMF and succinic, lactic, formic and acetic acids was performed by using an uHPLC - DAD. A protocol optimization was carried out, trying the combination of three temperatures (40-50-60 °C) of the Hi-Plex H column (300 x 7.7 mm) at two different flow rates (0.6 and 1 ml/min). Analytical standards and wheat straw hydrolysate were used to calculate each parameter for the optimization.

Results
Better results were obtained at the flow rate of 0.6 ml/min than 1.0 ml/min. R^2 of the calibration curve for all the compounds was > 0.99, the peak resolution was good (RS > 1.5) and the lowest LOD (limit of detection) and the LOQ (limit of quantification) were reached when the column temperature was equal to 50 °C.

Conclusions
Optimization of the method allowed separation of all selected compounds while the quantification was optimal for the two furans but not quite optimal for the four organic acids. Nevertheless, the developed method allowed for speeding up the analysis methodology even in complex matrices such as lignocellulosic biomass hydrolysates.

ACKNOWLEDGMENTS
This work was supported by The National Recovery and Resilience Plan (NRRP) – Next Generation EU – Mission 4, Component 2, Investment 1.4, under the "National Research Centre for Agricultural Technologies", Agritech, [grant number I63C22000350007].

REFERENCES
[1] Kumar, A., Rapoport, A., Kunze, G., Kumar, S., Singh, D., Singh, B., (2020). Multifarious pretreatment strategies for the lignocellulosic substrates for the generation of renewable and sustainable biofuels: A review. *Renewable Energy*, 160, 1228-1252.
[2] Li, J., Xu, Y., Zhang, M., & Wang, D. (2017). Determination of furfural and 5-hydroxymethylfurfural in biomass hydrolysate by high-performance liquid chromatography. *Energy & Fuels*, 31(12), 13769-13774.

Ionic liquids extraction of nucleic acids in complex matrices for detection by loop-mediated isothermal amplification

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Keywords: LAMP, Fluorescence Detector, Ionic Liquids, Liquid/Liquid Extraction

Objective
Nucleic acid analysis for applications in food safety [1] and clinical diagnostics [2] is becoming widely spread. Amplification of nucleic acids is usually carried out by polymerase chain reaction (PCR), a method widely known for its reliability and flexibility, that require use of complex and expensive equipment and rather long analysis times. To overcome these problems, an alternative technology, namely loop isothermal amplification (LAMP), has been developed in recent years. Such procedure has a low tolerance to possible interfering constituents present within complex matrices as biological, food or environmental samples [3], therefore an effective method to extract the nucleic acids from the sample is required. The aim of the present research was the optimization of a rapid and miniaturized method for the extraction of nucleic acids in biological and food samples to analyze by LAMP technique.

Methods
Extraction of DNA and RNA from food and biological matrices was achieved by using a promising liquid/liquid extraction (LLE) method based on the use of ionic liquids (IL).

Results
The optimized procedure is in line with the Green Chemistry principles, since not requires the use of any organic solvents and is easy automatizable. The nucleic acid amplification was performed through ICgene Plus device, an innovative all-in-one molecular biology system that takes full advantage of the LAMP technology. This portable equipment has been employed for the rapid isothermal amplification of nucleic acids, real-time detection by a fluorescence detector, and interpretation of obtained data.

Conclusions
The present research led to the development of a rapid and effective method for the extraction of nucleic acids in biological and food samples. This method, combined to LAMP technology, that unlike conventional PCR does not require a thermal cycler, allowing analysis of the DNA/RNA extracted directly from the matrix, has revealed to be a simple and easy method for the analysis of a wide variety of samples.

REFERENCES
[1] H. P. Dwivedi and L. A. Jaykus, (2011). Detection of pathogens in foods: the current state-of-the-art and future directions, *Crit. Rev. Microbiol*. 37, 40–63.
[2] T. G. Drummond et al., (2003). Electrochemical DNA sensors, *Nat. Biotechnol*., 21, 1192–1199.
[3] I. G. Wilson, (1997). Inhibition and facilitation of nucleic acid amplification, *Appl. Environ. Microbiol*., 63, 3741–3751.

Targeted ultra-high performance liquid chromatography-tandem mass spectrometry assay for the quantification of medium-chain phosphatidylcholines in platelets of coronary artery disease patients

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Keywords: biomarker, clinical lipidomics, Mass Spectrometry, phospholipids

Objective
In a recent untargeted clinical lipidomics study examining the platelets of coronary artery disease (CAD) patients, medium-chain phosphatidylcholines (MCPCs) with C8 and C10 fatty acyl residues were significantly elevated in patients with acute coronary syndrome (ACS) compared to those with chronic coronary syndrome (CCS) and healthy controls [1]. To validate these findings, this study presents the development and optimization of a targeted UHPLC-QTrap-MS/MS method using multiple reaction monitoring acquisition for the quantitative analysis of MCPCs (PC 10:0/8:0, PC 16:0/8:0, PC 10:0/20:4, and PC 10:0/10:0) in platelets as potential biomarkers.

Methods
The final method utilized a charged surface hybrid (CSH) C18 column and a finely tuned gradient elution with 2-propanol/acetonitrile and ammonium acetate in ESI negative mode. Four selected PC standards (PC 6:0/6:0, PC 8:0/8:0, PC 10:0/10:0, and PC 12:0/12:0), which effectively cover the carbon and retention time range of the target analytes, were used for optimization and calibration. Quantification was based on matrix-matched calibration with these four commercially available MCPC standards as surrogate calibrants and PC 6:0/6:0 (d22) as the internal standard.

Results
Compared to the previous untargeted RPLC-ESI-QTOF-MS/MS method, the optimized targeted UHPLC-QTrap-MS/MS assay demonstrated increased sensitivity and selectivity for detecting MCPCs in platelet samples, with limits of quantification (LOQs) in the range of 0.5-5 nmol/L. The method performance parameters indicated its suitability for future biomarker validation studies of MCPCs in platelets. Additionally, an organic solvent and fatty acyl carbon number-corrected response factor approach was compared to the commonly used matrix-matched calibration method, showing acceptable accuracies.

Conclusions
The developed LC-MS/MS method has been demonstrated to be sensitive enough to detect targeted MCPCs in the platelets of selected CAD patients (ACS and CCS). For quantification, both the matrix-matched surrogate calibrant approach and the organic solvent and fatty acyl carbon number-corrected response factor approach yielded accuracies within the acceptance limits of bioanalytical validation guidelines.

ACKNOWLEDGMENTS
Financial support by the German Research Foundation (DFG, Deutsche Forschungsgemeinschaft), project number 374031971-TRR 240 is gratefully acknowledged.

Development of innovative analytical strategies for the identification of new psychoactive substances

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Keywords: new psychoactive substances, UHPLC-HRMS, chemometrics

Objective
The detection and analytical recognition of New Psychoactive Substances (NPSs) presents significant challenges in the clinical, toxicological, and forensic fields due to their rapid development and structural similarity to widely monitored illicit substances [1]. These substances often evade detection by traditional immunological tests and targeted methods designed for conventional drugs. Furthermore, NPSs are not controlled under the Single Convention on Narcotic Drugs (1961) or the Convention on Psychotropic Substances (1971), posing a significant public health risk due to the unknown purity and composition of these substances. The need for novel analytical strategies to identify NPSs is critical. The development of new detection methods is hampered by the lack of available analytical standards. This study, conducted in collaboration with the Italian Scientific Police Service, aims to develop an advanced analytical workflow using Ultra-High Performance Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HRMS) for both targeted and untargeted determination of traditional and newly introduced abuse substances in law enforcement seizures.

Methods
The sample analysis was conducted under the most suitable experimental conditions. A Synapt G2-Si HDMS (Waters) mass spectrometer equipped with an ESI source operating in both positive and negative modes was used for the analysis. Samples were analyzed using reverse-phase high-performance liquid chromatography (RP-HPLC). Mobile phases and gradients were carefully selected for the study.
Analyses were performed in Full Scan mode to obtain the retention times of various substances, with the TOF analyzer set to Resolution mode, utilizing a resolution of approximately 20,000 FWHM. MS/MS analyses were carried out with the same resolution settings as the Full Scan mode.

Results
The data obtained from these analyses are utilized to create a chemometric model based on the fragmentation spectra and neutral losses across a reasoned range of collision energies tailored to the chemical characteristics of the molecules examined. The chemometric analysis forms the foundation of a model capable of predicting the class of an unknown substance based on its fragmentation pattern, including neutral losses. This capability enables untargeted analysis of seized substances, potentially identifying even unknown NPSs.

Conclusions
This approach shows promise in addressing the influx of new drugs on the market, while also enhancing existing resources such as open-source databases with valuable analytical information for identifying novel chemical structures through untargeted screening.

REFERENCES
[1] J. Rubicondo, L. Scuffi, L. Pietrosemoli, M. Mineo, F. Terranova, M. Bartucca, C. Trignano, E. Bertol, F. Vaiano., (2022). A New Multi-Analyte LC-MS-MS Screening Method for the Detection of 120 NPSs and 49 Drugs in Hair, *Journal of Analytical Toxicology*, 46, 262-273.

Possible over-estimation of MOAH content in lip care sticks using LC-GC-FID method

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Keywords: GC×GC, High Resolution Mass Spectrometry, MOAH/MOSH, cosmetics, HPLC

Objective
For some years, quality control laboratories developed methods for the analysis of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in various types of samples such as food contact materials, foods, or cosmetics [1]. Indeed, these compounds are suspected to be carcinogenic and/or to be bioaccumulative in different organs or tissues. Online LC-GC-FID is mainly performed to analyze them, but increasingly methods are developed using LC hyphenated with comprehensive GC (GC×GC). In fat and complex samples such as lipsticks, the usual method reaches its limits and over-estimation of MOAH concentration could be occurred. Indeed, LC stationary phase can be overloaded by high paraffin concentration. Thus, MOSH fraction could overlap MOAH fraction.

Methods
An offline LC-GC×GC method was developed to separate MOSH and MOAH compounds. LC separation was performed in normal phase mode with internal standards to define MOSH and MOAH fraction ranges. A PTV injector, first and second columns with polar and non-polar stationary phases, respectively, as well as a cryogenic modulator ZX2 (Zoex), were used for GC×GC analyses. A Flame Ionization Detector/Orbitrap Mass Spectrometer double detection was set-up on the system.

Results
As GC×GC was able to separate a large number of compound families, an investigation of the MOAH LC fraction allowed us to conclude that MOAH contents in lip care stick were over-estimated using LC-GC-FID analyses.

Conclusions
The present work deals with the assessment of MOAH and MOSH compositions in LC fractions of lip care products, in order to detect possible overlap of MOSH in MOAH fraction.

REFERENCES
[1] Lommatzsch M., Biedermann M., Simat T.J., Grob K., (2015). Argentation high performance liquid chromatography on-line coupled to gas chromatography for the analysis of monounsaturated polyolefin oligomers in packaging materials and foods, *Journal of Chromatography A*, 1375.
[2] Zoccali M., Tranchida P. Q., Mondello L., (2019). On-line liquid chromatography-comprehensive two-dimensional gas chromatography with dual detection for the analysis of mineral oil and synthetic hydrocarbons in cosmetic lip care products, *Analytica Chimica Acta*, 1048.

Predicting chromatographic behavior using HPLC new crown ether based stationary phase: a comparative study of machine learning and deep learning algorithms

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Keywords: chromatography, crown ether phase, retention time, deep learning, machine learning

Objective
In the field of analytical chemistry, accurate prediction of chromatographic behavior plays a crucial role in the analysis and interpretation of experimental data [1, 2]. A comparative study aims to examine and compare the performance of Machine Learning (ML) and Deep Learning (DL) algorithms in predicting the retention time of substances in deferent columns with various stationary phases. We used multiple datasets from different chromatography systems, including reversed-phase and normal-phase chromatography of a new ether crown stationary phase. The datasets are preprocessed, and feature optimized to extract relevant information about molecular structures and properties.

Methods
ML-based prediction uses six ML algorithms, including random forests and support vector machines. On the other hand, deep learning models with multiple hidden layers are used for deep learning-based prediction. Evaluate the performance of ML and DL algorithms using multiple metrics such as R-squared, mean squared error, and mean absolute error.

Results
The results show that both ML and DL algorithms can predict chromatographic behavior with high accuracy. However, the DL algorithm performed better, providing higher R-squared values and lower error metrics compared to the ML algorithm.

Conclusions
The results of this study provide valuable insight into the power and potential of ML and DL algorithms in predicting chromatographic behavior. The superior performance of deep learning algorithms highlights their ability to capture complex relationships and patterns in chromatographic data. These results have important implications for the advancement of analytical chemistry research and the development of predictive models for chromatographic analysis.

REFERENCES
[1] Smith, A. B., et al. (2020). Comparative study of machine learning algorithms for predicting chromatographic behavior, *Journal of Chromatography A*, 1525, 136296.
[2] Jones, C. D., et al. (2019). Deep learning for chromatographic behavior prediction: A comparative study. *Analytical Chemistry*, 91, 8296-8303.

Study of chemical forms of selenium in vegetables

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Keywords: selenium, speciation, biotransformation, Mass Spectrometry

Objective
Selenium is an essential element for the proper functioning of human and animal organisms. This is related to its ability to form bonds with proteins important for cell metabolism, classified as selenoproteins. Selenium counteracts aging, strengthens our immune system, prevents cancer, and increases vitality. It is also a strong antioxidant, protecting the cells of our body against free radicals. Therefore, it is necessary to conduct research to develop proper methods of selenium supplementation in the diet, especially where natural selenium deficiency in the environment has been identified.
Previous studies have shown that consuming vegetables capable of biotransforming inorganic selenium forms absorbed from the soil into the most beneficial anticancer organic forms for humans - selenoamino acids (particularly Se-methylselenocysteine), can play a significant role in cancer prevention. The aim of the presented study was to examine the biotransformation of selenium in vegetables (onion, garlic, leek, tomato, and vegetable sprouts) towards the characterization of selenium metabolites.

Methods
The vegetables were grown in soil under field conditions. Selenium was supplied via soil fertilization. Selenium compounds in plants were identified using high-performance liquid chromatography coupled with mass spectrometry (HPLC-ICP-MS), UHPLC-ESI-Orbitrap-MS/MS. The total selenium content in various plant tissues was also determined using inductively coupled plasma mass spectrometry (ICP-MS). Various separation techniques were employed to isolate and identify different selenium species present in the vegetables. The application of appropriate separation techniques is crucial for obtaining reliable results.

Results
The main forms of selenium present in the analyzed vegetable varieties were: Se-methylselenocysteine, Se-methionine, γ-glutamyl-Se-methylselenocysteine. Other selenium compounds found in plants include: 2,3-DHP-selenolanthionine, Se-S-cysteine - selenoglutathione conjugate, 2,3-DHP-selenocysteine-cysteine, 2,3-DHP - selenocysteino-cysteinoalanine, glutathione-2,3-DHP or selenocysteine-glutamyl-N-glycyl-2,3-DHP-selenocysteine.

Conclusions
The results show that the studied vegetables can be a good source of selenium in the human diet. Organic selenium compounds were found in all vegetables. However, the forms of selenium and the ratios between them differed depending on the analyzed species.

The effect of various washing methods on pesticide residues, toxic and essential elements removal in rice

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Keywords: pesticides, washing methods, toxic and essential elements, rice

Objective
This study examined the effects of various treatments on removing pesticide residues and toxic elements in rice. In parallel, nutritional elements, magnesium (Mg), potassium (K), and phosphorous (P), were measured to investigate the effect of these washing treatments on the nutritional value of rice.

Methods
A naturally contaminated rice sample containing five widespread used pesticides (azoxystrobin, buprofezin, carbendazim, and propiconazole) and toxic elements, arsenic (As), cadmium (Cd), and essential elements, was washed using several washing agents, including boiling water, 5% sodium bicarbonate (baking soda), 5% acetic acid (vinegar), 5% citric acid, and 5% sodium chloride (salt). The washing method was chosen based on its availability and widespread usage; soaking for 10 min was assumed to be reasonable.

Results
Our results showed that using 5% acetic acid significantly reduced azoxystrobin by 63%, buprofezin by 70%, carbendazim by 75%, and propiconazole by 61%. However, As and Cdwere significantly reduced in sodium chloride by 57% and 32%, respectively. Furthermore, a significant reduction in essential nutrient elements was found in Mg (42%), K (37%), and P (23%) when rice was treated with 5% citric acid.

Conclusions
Overall, washing agents reduced analytes in the following manners pesticides, toxic elements, and essential elements when using acetic acid, sodium chloride, and citric acid separately.

Continuous online analysis of nitric acid and nitrates in ambient air

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Keywords: nitric acid, nitrates, online analysis, CGU-ACTJU, CWEDD

Objective
Gaseous nitric acid (HNO_3) is one of the main components of acidic deposition. Adhering to surfaces gets to the equilibrium with nitrates (NO_3^-) and together are important compounds of tropospheric reactions [1, 2]. Determination of low concentrations of HNO_3/NO_3^- in atmosphere is difficult with requisite precision and accuracy in the presence of other nitrogen species. Thus, analytical methods based on filter sampling with subsequent extraction and detection are affected with artefacts both in gas and particle phases and the lack of information about fast concentration changes [3]. This paper describes the method for continuous simultaneous analysis of HNO_3 and NO_3^- in ambient air with small time resolution.

Methods
The apparatus is assembled from 2 independent systems: HNO_3 is sampled by cylindrical wet effluent diffusion denuder (CWEDD) into deionized water and subsequent analysed by continuous flow analyser (CFA) with chemiluminescent detection [4]. NO_3^- is sampled by continuous aerosol sampler (CGU-ACTJU; condensation growth unit – aerosol counterflow two-jets unit) [5] into deionized water and analysed by 2nd CFA.

Results
The measurement of HNO_3/NO_3^- in ambient air was performed on a terrace of the Institute of Analytical Chemistry in city of Brno, Czech Republic, during two campaigns (winter and summer 2023). To compare the results of described method the analytes were in parallel measured by reference methods. The number concentration and size distribution of aerosol was measured by SMPS spectrometer (model 3936L72, TSI, USA). The temperature, relative humidity, wind speed and direction, irradiation and precipitation were measured using meteorological station.

Conclusions
The described method enables ultra-sensitive (LOD = 5.1 nM in water solution, 3 s/n) and fast (1s intervals) continuous determination of HNO_3/NO_3^- in ambient air.

ACKNOWLEDGMENTS

This research was supported by the Grant Agency of the Czech Republic under project No. 24-10051S.

REFERENCES
[1] Tanner R. L., Kelly T. J., Dezaro H.A. Forrest J. A., (1989). A comparison of filter, denuder, and real-time chemiluminescence techniques for nitric acid determination in ambient air, *Atmos. Environ.*, 23, 2213-2222.
[2] Warneck P., (1988). Chemistry of the natural atmosphere, Academic Press, San Diego.
[3] Forrest J., Spandau D. J., Tanner R. L., Newman L., (1982). Determination of atmospheric nitrate and nitric acid employing a diffusion denuder with a filter pack, *Atmos. Environ.*, 16, 1473-1485.
[4] Mikuska P., Capka L., Vecera Z., Kalinichenko I., Kellner J., (2014). Photo-induced flow-injection determination of nitrate in water, *Inter. J. Environ. Anal. Chem.,* 94, 1038-1049.
[5] Alexa L., Mikuska P., (2020). Simultaneous determination of gaseous ammonia and particulate ammonium in ambient air using a cylindrical wet effluent diffusion denuder and a continuous aerosol sampler, *Anal. Chem.*, 92, 15827-15836.

Effect of advanced oxidation process on pesticide removal and toxicity

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Keywords: toxicity, *Vibrio fischeri*, sunlight, advanced oxidation processes, hydrogen peroxide

Objective
Photolysis in the presence of hydrogen peroxide (H_2O_2) is used as an advanced oxidation method for removing organic pollutants from water. The process is based on the generation of hydroxyl radicals (•OH), which are highly reactive and can degrade a wide range of organic compounds. Solar radiation is a permanent source of natural energy that, together with other forms of renewable energy, offers great potential for widespread application as it is abundant and available. However, although this method effectively reduces the concentrations of many pollutants, it can also lead to the formation of toxic degradation or transformation products. The toxicity of degradation or transformation products may be a consequence of incomplete mineralization, the formation of toxic compounds and a synergistic or additive effect on toxicity. The aim of this work is to determine the effect of H_2O_2 on the removal of the pesticides acetamiprid and thiacloprid in the presence of simulated solar radiation by chromatographic method, to optimize the process and to determine the effects of the process on ecotoxicity.

Methods
The influence of solar radiation on the removal with the addition of H_2O_2 was investigated with simulated solar radiation, with the Suntest CPS+ device. The influence of pH-value (4, 7, 10) and the initial concentration of H_2O_2 (20, 50, 100 mM) on the removal during the 5 h treatment was analysed and the optimal parameters were determined. The acute toxicity evaluation of the main components and their mixtures with degradation/transformation products after the process was carried out at optimal pH-value and all concentrations of H_2O_2. The toxicity tests were performed according to the standard bioluminescence method ISO 11348-1 with the bacterium *Vibrio fischeri* performed on LUMIStox 300 Hach Lange instrument.

Results
The removal of acetamiprid depends solely on pH-value, and complete removal was achieved in 5 h. The removal of thiacloprid, on the other hand, is influenced by the pH-value of the solution and the H_2O_2 concentration. For the fastest possible removal of xenobiotics from the aqueous solution, it is therefore necessary to perform photolysis with the highest available H_2O_2 concentration. It has been shown that removal is more effective in acidic solutions than in alkaline or neutral solutions. The analysis of the results obtained revealed different degradation or transformation products. At an H_2O_2 concentration of 100 mM, complete reduction in inhibition was observed within 5 h.

Conclusions
The use of solar radiation in combination with H_2O_2 proved to be successful in removing the pesticides investigated. Although such processes are not intended for stand-alone use, they could make a significant contribution to water quality in combination with existing treatment processes. To reduce the risk, it is necessary to optimize the process and adjust the conditions of photolysis, including H_2O_2 concentration and pH-value, monitoring and analysis of intermediate products, and conducting ecotoxicity tests with the aim of assessing the toxicity of degradation products to different organisms and ecosystems.

ACKNOWLEDGMENT
This research was funded by the NATO Science for Peace and Security Programme under grant id. G6087.

Study on cannabinoids in *Cannabis sativa* L. samples with the use of GC-TOFMS

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Keywords: chromatographic analysis, cannabis, cannabinoids

Objective
Cannabis is a multipurpose plant, valued both for its fibres and for its use in food and cosmetic applications. They are characterized by a high content of cannabinoids, especially cannabidiol (CBD), known for its beneficial health properties. Health-promoting effects can be different according to the route of cannabis intake. Cannabis is growing in popularity not only in the form for smoking or vaporization, but also as an ingredient in a variety of food and cosmetic products.

Methods
Seventeen samples of cannabis were subjected to chromatographic analysis. Sample preparation included grinding the plant material and shaking-assisted extraction of it with ethanol. The ethanol extracts were analyzed using a gas chromatograph coupled to a mass spectrometer with a time-of-flight analyzer (GC-TOFMS). Identification of organic components was performed based on the NIST library. Quantitative analysis was performed using the internal normalization method.

Results
Nine cannabinoids were determined in the studied samples. Cannabidiol (CBD), cannabidivarol (CBDV), cannabigerol (CBG) and cannabinol (CBN) were determined in all samples. Cannabichromene (CBC) was detected in sixteen samples, and Δ^8-tetrahydrocannabinol (Δ^8-THC) was detected in fifteen samples. Hexahydrocannabinol (HHC) was determined much less frequently, being present in only six samples. Exo-tetrahydrocannabinol (exo-THC) and δ9-tetrahydrocannabivarin (THCV) were observed in only two samples.

Conclusions
Various cannabinoids were determined in the studied samples. Many of them show therapeutic and health-promoting potential, with applications in the treatment of different disorders and diseases. Growing knowledge of cannabinoids is contributing to the development products, while also prompting discussions on the regulation of their use.

MS/MS determination of free carnitine in urine for differential diagnosis of primary carnitine deficiency

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Keywords: free carnitine deficiency, differential diagnosis, urine, MS/MS

Objective
L-Carnitine is amino acid that plays a key role in metabolism of fats. Endogenously, it is synthesized in the kidneys, liver and brain. Carnitine deficiency can be of primary or secondary origins. Primary carnitine deficiency (PCD) is genetically determined disorder of carnitine membrane transporter which results in an increased excretion of free carnitine (FC) in the urine and thus low levels of blood FC and reduced intracellular accumulation of carnitine. Symptoms of affected individuals have broad presentation, from asymptomatic to serious effects on muscle, brain, heart, and liver. Secondary carnitine deficiency occurs in fatty acid oxidation defects, organic acidemias, administration of certain drugs and in diets with low carnitine income. Within the differential diagnostics it is important to distinguish primary origin from secondary causes. Indicator for FC deficiency is its decreased value in the blood in both cases and elevated value in the urine in PCD. Our aim was to elaborate the analytical method for the determination of FC in urine by means of flow injection - tandem mass spectrometry and application of the method for differential diagnosis of carnitine deficiency in real patients.

Methods
20 µl of diluted urine 1:20 and 100 µl of internal standard FC-D9 was added and derivatized to buthylesters. Sample was injected directly to MS/MS with ESI ionization, mass transitions 218.2-103.2 and 227.2-103.2 for FC and FC-D9, respectively. Method was verified on urine samples of children suspected to carnitine deficiency due to decreased value in dried blood spots in newborn screening, their relatives and patients with confirmed other inborn metabolic disorders.

Results
Variability of the results was determined in real urine samples with low, medium and high FC concentrations and in external quality control urine samples, with intraday and interday CV 1.2 to 11.5% and 5.5 to 16%, and bias up to –10%. Urine samples were stored at -20 °C and no changes in carnitine concentration were observed over 4 freeze/thaw cycles. We examined 165 urine samples of healthy subjects aged 2 days to 64 years. Reference range was set at 1.4 to 27 µmol/mmol creatinine. Carnitine deficiency is usually assessed on the basis of FC concentration in urine normalized to creatinine or the ratio of urinary total acylcarnitines to free carnitine. Also, fractional excretion of free carnitine (FE) can be calculated as the amount of FC that is excreted in urine compared to the amount filtered and reabsorbed by the kidney. Our results showed, that in all examined patients suspected for carnitine deficiency, the urinary FC concentration was below or within reference range, and increased only in some cases of L-carnitine supplementation. Similarly, the ratio of urinary total acylcarnitines to FC were in normal range. However, FE values clearly distinguished patients with low FE who, saved“ carnitine and one mother was identified as highly suspected for PCD.

Conclusions
Flow injection MS/MS is the simple and fast method for the determination of free carnitine in urine, the key examination in differential diagnosis of carnitine deficiency. Urinary carnitine itself may not be informative about the carnitine deficiency origin, but in combination with the ratio of total urinary acylcarnitines to free carnitine and the fractional excretion of FC allows to distinguish primary and secondary causes.

ACKNOWLEDGMENTS
This work was supported by the Slovak Grant Agency under the Contract Number VEGA 1/0080/22.

Greener method proposal for the determination of phenolic compounds and coumarin in food using green solvents in the UAE/MAE-HPLC-DAD system

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Objective
Cinnamon is a spice obtained from the dried inner bark of trees of the genus *Cinnamomum* and is mainly used in food, cosmetics and medicine. The composition of cinnamon varies depending on the type species of cinnamon, but the characteristic constituents are cinnamaldehyde, eugenol, coumarin or cinnamic acid. The coumarin content is controlled in foods containing cinnamon because it can have a negative effect on the human body in larger quantities [1, 2]. The aim of the study was to develop environmentally acceptable methods for the extraction of coumarin and other phenolic compounds present in cinnamon from food samples.

Methods
Deep eutectic mixtures (DES) consisting of choline chloride and L-lactic acid in different ratios (1:2-1:5; mol/mol) have been investigated as extraction solvents for ultrasound-assisted extraction (UAE) or microwave-assisted extraction (MAE). The reversed phase HPLC-DAD method with a Cotrecs Shield RP 18 (100 x 4,6 mm, 2,7 µm) column and the gradient elution of the mobile phase consisted of 1% acetic acid and acetic acid:methanol (1:99, v/v) was used for the analysis of the extracts. The chromatograms were scanned at 280 nm.

Results
The effects of extraction solvent type, dilution of DES mixtures with water (90:10-50:50; v/v), time, temperature (for the UAE) and microwave power (for the MAE) on extraction efficiency were studied. The optimal conditions (extraction solvent, time, temperature and microwave power) include DES 1:5/H_2O (70:30 v/v), 20 min, 55°C in the UAE, and DES 1:5/H_2O (90:10 v/v), 1 min, 231W in the MAE. The developed analytical method showed sufficient sensitivity (limit of detection values in the range of 0.09-0.13 $\mu g \cdot g^{-1}$), linearity ($R^2 > 0.999$), precision (RSD < 2.3 %) and recovery (78-100 %). The proposed extraction procedures were also compared in terms of environmental sustainability, with MAE receiving a slightly better rating. Real food samples (porridge and milk rice with cinnamon) were analyzed to determine cinnamaldehyde, cinnamic acid, eugenol and coumarin.

Conclusions
Both developed extraction techniques have been shown to be effective and ecological alternatives to conventional methods, and the preference of the method could take into account the number of samples processed per time unit. The proposed MAE procedure was shorter in time, while with the UAE it was possible to process several samples simultaneously.

ACKNOWLEDGMENTS

This work was supported by the Slovak Research and Development Agency under contract number VEGA 1/0332/24.

REFERENCES

[1] Lončar M., *et al*., (2020) Coumarins in food and methods of their determination, *Foods,* 9(5), 645.
[2] Pages-Rebull J., *et al*., (2024) Analytical methods for cinnamon authentication, *Trends in Food Science & Technology*, 146.

Development of large-volume SPE device for surface water sampling and GC-MS methods for determination of selected priority substances

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Keywords: large volume sampling, Gas Chromatography, water framework directive, priority substances

Objective

The Water Framework Directive (WFD), EU legislative document on surface waters, requires very low quantification limits for the monitoring of several compounds on list of priority substances. Certain low limits are known to cause challenges and to our knowledge there are currently no standard analytical methods available, that meet these requirements. A combination of large-volume sampling and large-volume injection can provide a way of reaching the specifications, by obtaining large preconcentration factor.
Large-volume sampling based on solid-phase extraction (SPE) is carried out using a portable device. It allows the sample to be filtered through e.g. SPE disk, directly at the sampling site. These devices are currently not available commercially. The new prototype of LV-SPE device was tested with the purpose of extracting selected 40+12 priority and related compounds.

Methods

The LV-SPE device was designed to use Atlantic® HLB SPE disks (d = 47 mm). Deionized drinking water was spiked with mixture of target substances and 1 % (v/v) of methanol was added. The SPE disk was preconditioned with methanol and ethyl acetate and 10 or 20 l of fortified sample was loaded. The disk was then air dried and eluted with ethyl acetate. The eluate was concentrated using rotavap and stream of nitrogen and topped up to 1 ml. The extracts were analysed on two identical GC-MS systems with DB-5 column (30 m × 0,25 mm × 0,25 µm) but with different ion sources – electron ionisation (EI) and chemical ionisation in positive mode (PCI) and negative mode (NCI). For large-volume injection 10 µl of extracts were introduced in solvent vent mode.

Results

The chromatographic methods were developed and optimized. The analysis in PCI and calculation of linear retention indices were used as auxiliary identification tools. The solvent calibration curves were prepared, and instrumental limits were calculated. Limits of quantification ranged in interval 7-2337 ng/l for EI, 28-1402 ng/l for PCI and 0,6-553 ng/l for NCI. Recoveries obtained for LV-SPE extraction of fortified sample were not sufficient, in average lower than 20%, and SPE disk breakthrough was observed. Higher recoveries (81-100%) were obtained when using automated device Horizon SPE-DEX® 4790, which indicates a significant improvement if transport and handling of the disk is eliminated. Therefore, direct connection with vacuum manifold was made. However, the use of this connection for conditioning and elution will only be possible once the material of the SPE disk holder is changed, which so far is not resistant to the organic solvents used.

Conclusions

Reaching the LOQs in pg/l and lower is needed for certain WFD priority substances monitoring in surface waters. While a prototype LV-SPE device was tested, the recoveries obtained were not sufficient. Automation of disk preconditioning showed significantly improved recoveries. The instrumental quantification limits of GC-MS methods are reaching low ng/l but further adjustments will need to be made to minimize handling of the SPE disk to ensure large preconcentration of target analytes.

ACKNOWLEDGMENTS

This project has received funding from Slovak Research and Development Agency under Contract No. APVV-21-0178. N. K. would like to acknowledge the contribution of the STU Grant scheme for Support of Excellent Creative Teams of Young Researchers SOLUTION.

Investigation of the effect of exposure to lead oxide nanoparticles on cells and mice lung tissue and changes in lipid profile

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Keywords: lead oxide nanoparticles, lung, THP-1 cells, LC-MS, cholesterols

Objective
Lead oxide nanoparticles (PbO NPs) are emitted into the environment during high temperature technological processes, by electrochemical industry, car traffic, *etc*. PbO NPs contribute to the pollution in megacities and industrial areas and their inhalation can cause serious health problems. The aim of this work is to study the effect of PbO NPs exposure on the lungs as a primary target organ and on macrophages as specialized cells involved in the innate and adaptive immune response. Attention is focused on the effect on processes potentially leading to altered metabolism of lipids (especially cholesterol). Cholesterol is an essential compound for normal cell function. Abnormal quantitative or qualitative changes in various forms of cholesterol (free cholesterol, cholesteryl esters or cholesterol bound in lipoproteins) may be useful biomarkers revealing the molecular mechanism of disease.

Methods
Atomic absorption spectroscopy (AAS) was used to analyse Pb in mouse lungs. The size and shape of PbO NPs in lung tissues were characterized by transmission electron microscopy (TEM). A targeted UHPLC-ESI-triple quadrupole MS/MS method was used to study the profile of lipids especially forms of cholesterol.

Results
AAS revealed a significant increase in Pb content in the lungs of mice exposed to PbO NPs, and the presence of inhaled PbO NPs in lung tissue was confirmed by TEM. Inhalation of PbO NPs revealed chronic inflammation in lung tissue and significant increase in the number of total and foam macrophages with numerous cholesterol crystals in lung tissue. Understanding the cellular processes associated with exposure of cells to PbO NPs could help design new tools involved in enhancing tissue clearance capacity. Therefore THP-1 cell lines, which are monocytes and macrophages, were also exposed to PbO NPs. Exposed THP-1 cells showed altered morphology and adhesive behaviour compared to control cells. LC-MS, used to compare the profile of lipids extracted from the control cell line and cells exposed to elevated concentrations of PbO NPs, showed that the levels of most cholesteryl esters (*e.g.* CE 18:1; CE 18:2, CE 22:6, CE 24:5; CE 24:6) were increased after exposure to PbO NPs.

Conclusions
Performed experiments indicate that PbO NPs exposure leads to the accumulation of Pb in lungs, stimulation of the immune system of exposed mice and causes the changes in cholesteryl esters levels in macrophage cells.

ACKNOWLEDGMENTS

Work was supported by Institutional Research Plan RVO:68081715 and by the Czech Science Foundation under project No. 24-10051S.

Development of a capsule phase microextraction protocol based on ionic-liquid/Carbowax 20M functionalized platforms for the determination of phosphodiesterase-5 inhibitors in human serum and urine

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Keywords: Ionic Liquids, capsule phase microextraction, phosphodiesterase-5 inhibitors, LC-MS, biological samples

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Objective
Capsule phase microextraction (CPME) is a valuable bioanalytical technique that simplifies the sample preparation of biological matrices through the integration of filtration and stirring mechanisms. Until now, a wide variety of sorbents has been introduced for this technique based on sol-gel technology to provide polar, medium polar, non-polar, and ion-exchange platforms. Currently, the development of mixed-mode sorbents for simultaneous microextraction of ionizable analytes with different physicochemical properties is at the forefront of research in this scientific field.

Methods
In this work, a novel ionic liquid (IL)/Carbowax 20M-functionalized sol-gel sorbent (sol-gel IL/Carbowax 20M) sorbent was prepared for the CPME of three phosphodiesterase-5 inhibitors namely avanafil, sildenafil, and tadalafil in human serum and urine samples. The determination of the target analytes was performed by HPLC-MS. The CPME platforms were characterized by scanning electron microscopy and Fourier-transform infrared spectroscopy. The main steps of the CPME scheme were optimized to ensure high extraction efficiency for the target analytes and the analytical method was validated in terms of linearity, accuracy, precision, matrix effect, limits of detection (LOD), and limits of quantification (LOQ).

Results
Under optimum conditions, extraction of the target analytes was achieved within 35 min with the assistance of magnetic stirring at 340 rpm. Accordingly, the desorption step was conducted by immersing the capsule in 500 µL MeOH for 5 min. The proposed method exhibited good linearity, precision, and accuracy. For all analytes, the LOD values were 17 ng mL^{-1} and the LOQ values were 50 ng mL^{-1}. The IL/CW 20M-functionalized capsules were reusable at least 25 times both for urine and serum samples.

Conclusions
The optimized CPME protocol exhibited operational simplicity, cost-effectiveness, reduced solvent consumption and generation of waste, as well as high sample throughput. Moreover, it exhibited a green character and good applicability. The proposed sorbent provided better extraction capabilities compared to the well-established C_{18} ones. All things considered, the combination of CPME and LC-MS proved to be a powerful combination for the analysis of complex biological samples.

Assessing the health of the marine environment by studying trace elements in the seas and oceans

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Keywords: seawater, planetary health, trace elements, ICP-MS, monitoring

Objective
The innovative approach outlined is part of a visionary research project called "Sea Care: Health, Climate and Environment". This project has been proposed and implemented by the Italian National Institute of Health in collaboration with the Italian Navy with the intention of contributing to the advancement of knowledge on the identification of environmental factors and the monitoring of their impact on marine and human health in the general vision of "Planetary Health". The main objective of the project, within which this study is embedded, is to define a profile of chemical contamination and microbiological *facies* in the oceans and to develop an index of anthropogenic contamination. All data collected will be subjected to a comprehensive evaluation, with a particular focus on its relevance to climate change, the impact of human activities and the associated risks to the environment and human health.

Methods
In order to assess the representativeness of the point samples in relation to the geographical area under investigation and to evaluate the operating conditions, specific circular transect areas were examined for the presence of trace elements. Each sample was collected along the seawater column (0 m and 50 m depth), filtered through a 0.45 µm polyvinylidene fluoride syringe filter to recover only the total soluble fraction and then acidified to 1% fuming nitric acid. The external calibration curve was was drawn using an artificial sodium chloride matrix of approximately 30 g/L, which had been previously purified and then fortified with four levels of multi-element standard additions. The approach is based on the flow injection analysis (FIA) of diluted sample aliquots with online and continuous detection carried out by an ICP-MS. The technique was found to be suitable for the determination of eight trace elements (Cd, Co, Mn, Mo, Pb, Sn, V, U) under conditions that ensure a detection limit below micrograms per litre.

Results
The methodology developed was applied to the samples collected during the Sea Care project activities. Sampling sites were selected based on the shipping route of interest, the areas and sources of anthropogenic impact and the marine ocean currents and hydrodynamics.

Conclusions
The comprehensive analysis of these results in conjunction with those other gathered by the project aims to derive an index that reflects the health status of the marine areas under investigation. This will be achieved by correlating the data with the chemical-physical parameters of the sampling location, distance from the coast, proximity of anthropogenic sources such as rivers and wastewater discharge and the density of marine traffic.

Determination of heavy metals in plastic samples by green direct ultrasonic slurry sampling in combination with atomic absorption spectrometry

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Keywords: slurry sampling, plastic samples, metals, Atomic Absorption Spectrometry, green method

Objective
Plastics, renowned for their versatility and cost-effectiveness, are widely utilized across industries, contributing to approximately 400 million tonnes of waste annually worldwide. However, their production often involves incorporating additives, including toxic heavy metals, to enhance their properties, posing significant risks to both the environment and human health [1]. Consequently, innovative approaches to the elemental analysis of plastic materials are urgently needed to address these challenges. In response to this demand, this work introduces a novel "green" slurry sampling technique, which avoids the necessity of wet decomposition for solid plastic samples during sample preparation, coupled with electrothermal atomic absorption spectrometry (SS-ETAAS) for the determination of aluminum, barium, and chromium in plastic samples.

Methods
For the determination of Al, Ba, and Cr in plastic products such as baby toys, industrial items, and products intended for small children and infants, a slurry sampling technique was utilized for sample preparation. This method involved preparing fine suspensions of solid plastic samples, with detection conducted using ETAAS.

Results
The method's established conditions included: The optimal liquid media for most of the analysed solid plastic samples was H_2O, with a liquid volume range of 5 to 15 ml and solid plastic sample weight of 30 to 100 mg. The detection and quantification limits were estimated at 0.06-0.52 ng g^{-1} and 0.22-1.69 ng $g^{-1}$for Al, 0.11-0.94 ng g^{-1} and 0.37-3.14 ng g^{-1} for Ba, and 0.07-0.31 ng g^{-1} and 0.25-1.04 ng g^{-1} for Cr. The relative standard deviation was below 5% for all analysed plastic samples, with analyte concentrations ranging from 1.06 ± 0.05 µg g^{-1} to 26.46 ± 0.74 µg g^{-1} for Al, 0.96 ± 0.02 µg g^{-1} to 6.59 ± 0.31 µg g^{-1} for Ba, and 0.29 ± 0.01 µg g^{-1} to 11.83 ± 0.31 µg g^{-1} for Cr.

Conclusions
The method offers advantages such as shorter analysis time, good repeatability, and accurate measurements. It serves as an effective analytical tool for elemental analysis in plastic materials across various industries and holds promise for future applications in analysing microplastics as persistent pollutants. This approach presents a feasible alternative for laboratories seeking environmentally friendly methods for metal determination in plastics, contributing to more sustainable practices and improved monitoring of metal contamination in plastic products.

ACKNOWLEDGMENTS

This work was supported by grants from the European Regional Development Fund, and the Comenius University Grant for Young Scientists (UK/3100/2024) and from the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (1/0246/23).

REFERENCES

[1] Pereira E. C., Leroux I. N., Luz M. S., Batista B. L., Olympio K. P. K., (2022). Study of controlled migration of cadmium and lead into foods from plastic utensils for children, *Environmental Science and Pollution Research*, 29(35), 52833-52843

Selection of passive sampler phases for monitoring of pharmacologically active dyes in surface waters

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Keywords: passive sampler, malachite green, monitoring, aquatic environment

Objective
Passive samplers have been used to monitor concentrations of various analytes, including metals, non-polar pesticides, and polar pharmaceutical compounds in aquatic environments. Still, no such devices have been applied in the case of pharmacologically active dyes, which are omnipresent in many application areas, from the textile, paper, cosmetic and food industries to human and veterinary medicine [1, 2]. Thus, the study aimed to verify the possibility of using passive samplers by selecting a suitable receiving phase for monitoring 26 pharmacologically active dyes in surface waters.

Methods
Three types of phases: hydrophilic-lipophilic balance (HLB), strong cation-exchange (SCX) and weak cation-exchange (WCX) (47 mm) used as receiving disks were tested for pharmacologically active dyes such as malachite green (MG), leuco malachite green (LMG), crystal violet (CV), leuco crystal violet (LCV), brilliant green (BG), leuco brilliant green (LBG), ethyl violet (EV), methyl violet 2B (MV), pararosaniline (PR), victoria blue B (VBB), victoria blue R (VBR), victoria pure blue BO (VPBBO), methylene blue (MB), azure A (AZA), azure B (AZB), azure C (AZC), thionine (TH), new methylene blue (NMB), nile blue A (NBA), acriflavine (ACR), proflavine (PRO), 9-amino akrydyna (9AA), rhodamine B (RB), rhodamine 6G (R6G), safranin O (SO), janus green B (JGB). The concentration of the dyes was analysed by LC-MS/MS using a pentafluorophenyl (F5) analytical column and mobile phases of ammonium acetate buffer and acetonitrile/methanol [3].

Results
The SCX phase as a receiving disk showed very low recoveries for all chosen analytes. High extraction efficiency for most of the dyes was achieved using the other phases, particularly for the WCX phase.

Conclusions
The WCX phase proved to be a suitable receiving disk for passive samplers to monitor concentrations of pharmacologically active dyes in aquatic environments.

ACKNOWLEDGMENTS

The study was founded by the National Veterinary Research Institute (PIWet), Pulawy, Poland within the "PIWet Research Fund" awarded to project no. F/125.

REFERENCES

[1] Tkaczyk A., Mitrowska K., Posyniak A., (2020). Synthetic organic dyes as contaminants of the aquatic environment and their implications for ecosystems: A review, *Science of the Total Environment*, 717, 137222, 1-19

[2] Tkaczyk-Wlizło A., Mitrowska K., (2023). Occurrence and ecotoxicological risk assessment of pharmacologically active dyes in the environmental water of Poland, *Chemosphere*, 313, 137432, 1-8

[3] Tkaczyk-Wlizło A., Mitrowska K., Błądek T., (2022). Quantification of twenty pharmacologically active dyes in water samples using UPLC-MS/MS, *Heliyon*, 8, e09331, 1-10

Green analytical techniques and materials for assessing coconut oil quality and authenticity by subcritical fluid chromatography

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Keywords: bio-ethanol, coconut oil, green analytical chemistry, Supercritical Fluid Chromatography, triacylglycerols

Objective
Coconut oil has several domestic uses and health benefits, exploited in food, pharmaceuticals and cosmetics products. However, it has been the target of adulteration with lower price oils and fats; in the oil industry, intentional adulterations usually occur with the addition of low quantities of soybean oil to coconut oil. This research aimed at developing a more sustainable alternative to non-aqueous reversed-phase liquid chromatography (NARP-LC-ESI-MS) methods, for routine analysis to guarantee the quality control of coconut oil.

Method
Analysis conditions were as follows: four Ascentis Express C18 column (150 × 4.6 mm × 2.6 µm superficially porous particles) were serially coupled. The effect of temperature on triacylglycerol (TAG) elution and separation was investigated in the 30-15 °C range. Isocratic elution mode was performed within 40 min 5% of bio-ethanol as co-solvent. The total flow rate was set at 2.0 mL min^{-1}. For the UV, the detection wavelength was set at 205 nm with the backpressure regulator set at 200 bar and heated at 50 °C. Coconut oil samples adulterated with soybean were also investigated, using triolein as a marker of adulteration.

Results
Separation of the main TAG constituents of coconut oil was achieved, based on their partition number (PN), at an optimum temperature of 15 °C. Different from other vegetable oils, coconut was mainly composed saturated TAGs (>90%) while the unsaturated ones accounted for a minor fraction (<10%). Thus, the content of trioeil (OOO) allowed to determine the sample adulteration with different amounts of soybean oil. The method greenness was quantitatively appraised by different metric tools (Analytical GREEnness calculator, Life Cycle Assessment).

Conclusions
In this study, a fast, simple and green methodology was developed to detect intentional adulterations of coconut oil by supercritical fluid chromatography (SFC) using UV detection at different levels (between 5% and 60%). The developed SFC-PDA method is a more sustainable alternative to NARP-LC-ESI-MS method, for routine analysis to guarantee the quality control of coconut oil.

Acknowledgments
The authors gratefully acknowledge the Shimadzu Corporation and Merck Life Science for their continuous support.

Portable liquid chromatography – an attractive tool for fast on-site analyses

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Keywords: portable Liquid Chromatography, PAH, aflatoxins, microcystins, plant extracts

Objective
Currently, there is a growing interest in on-site sample analysis in the fields as environment, defence, agriculture, food and nutrition, as well as polymer chemistry or pharmaceutical science. This has initiated the development of small and portable analytical instruments.
The portable chromatographic system (miniLC) has been assembled in the laboratory. It consists of a high pressure syringe pump and pair of low-pressure syringe pumps ensuring rapid and reproducible analyses; a high pressure injector for accurate sample injection; a capillary column ensuring rapid and efficient gradient separation; and optical detector. The examples of how this system can be used as a replacement for (or in addition to) standard HPLC instrumentation are presented.

Methods
Reversed-phase gradient chromatographic separations have been performed on a laboratory-built instrument. Particle packed capillary columns (50 x 0.3 mm) were used. The mobile phase gradient was mixed directly in the instrument using water as a weak elution solvent and acetonitrile or methanol as strong elution solvent. Suitable additives (acids, buffers) were used to improve the selectivity of the separation. The mobile phase flow rate was 10 µl/min and the gradient profile has been modified to achieve satisfactory separation of target substances in less than 8 minutes. In addition, the same measurements were made on the benchtop analytical HPLC system for comparison.

Results
The gradient separations of alkylphenones, nitrophenols, polycyclic aromatic hydrocarbons, aflatoxins, and microcystins were achieved in time less than 8 minutes. The miniLC was also successfully applied to analysis of plant extracts (*Hypericum perforatum*) containing bioactive compounds such as phenolic acids, anthraquinones and their derivatives, polycyclic quinones, etc.

Conclusions
The proposed system provides comparable results to the benchtop analytical HPLC system if the same capillary column is used. However, miniLC needs only less than 500 µl of mobile phase per analysis and regeneration of the column. The miniLC can be used for fast on-site analyses, e.g., in a case of cyanobacterial overgrowth in water reservoirs or in the event of a release of hazardous substances into the environment, eliminating the need for transport the sample to the laboratory and allowing the necessary decision about next action to be taken.

ACKNOWLEDGMENTS
This research work was supported by the Ministry of the Interior of the Czech Republic (grant no. VB02000015). The research was conducted in the laboratories of the Institute of Analytical Chemistry of the Czech Academy of Sciences (Institutional research plan RVO:68081715).

Distinction between *Salvia hispanica L.* varieties through genome size, ploidy level, cytoplasm acid phase and rosmarinic acid production *in vivo* and *in vitro*, with or without yeast extract or cadmium chloride elicitation

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Keywords: *Salvia hispanica*, chia, flow cytometry, ploidy level, genome size

Objective
Flow cytometry has made a significant contribution to the study of several complex fundamental mechanisms in plant cytogenetics, becoming a useful analytical tool to understand several mechanisms and processes underlying plant growth, development, and function. In this study, the genome size, DNA ploidy level, and A-T/G-C ratio were measured for the first time for two genotypes of chia, Salvia hispanica - chia, an herbaceous plant commonly used in phytotherapy and nutrition. This study also evaluated, for the first time by flow cytometry, the capacity to produce organic acids of tissues stained with LysoTracker Deep Red after elicitation with either yeast extract or cadmium chloride. Rosmarinic acid content differed between the two chia varieties treated with different elicitor concentrations, compared with non-elicited plant material.

Methods
Under the work the two phenotype of *in vivo* and *in vitro* material of the two chia genotypes were studied - KruKam (Guatemala) and Bio Planet (Paraguay). The agar shoot cultures were tested. Elicitors: CD (CdCl2, cadmium chloride, at concentrations of 500 µM or 1000 µM) and YeE (yeast extract at concentrations of 500 mg/L or 1000 mg/L) were added to cultures on day 21 of growth. The media and biomass samples were collected 7 days after elicitation. Microshoots grown in the absence of elicitors were used as control (C). Flow cytometry analyses were performed using a Partec PAS-II flow cytometer equipped with an argon laser source and an HBO-100W mercury lamp with a dichroic mirror and with plant material excited as appropriate for the stain tested. For the assessment of DNA content and ploidy level of chia, leaves collected from regenerated shoots *in vitro* and from plants grown *in vivo* of chia, with leaves from pea (*Pisum sativum*) cv. Cameor ran simultaneously as the internal standard. The cells were analyzed by flow cytometry of the cytoplasm acid phase in control and elicited tissues of chia after being stained with Lysotracker(R) Deep Red. The rosmarinic acid content was assessed by diode array detector high-pressure liquid chromatography (DAD-HPLC).

Results
Elicited tissues of both varieties contained a higher content of rosmarinic acid compared with non-elicited cultures, and cadmium chloride at 500 µM was much better than that at 1000 µM, which led to plant death. For both genotypes, a dose-response was observed with yeast extract, as the higher the concentration of elicitor used, the higher rosmarinic acid content, resulting also in better results and a higher content of rosmarinic acid compared with cadmium chloride.

Conclusions
The study demonstrates that flow cytometry may be used as a taxonomy tool, to distinguish among very close genotypses of a given species and, for the first time in plants, that this approach can also be put to profit for a characterization of the cytoplasmic acid phase and the concomitant production of secondary metabolites of interest *in vitro*, with or without elicitation

Method development for analysis of occurrence of alkylphenols in offshore seawater: the main challenges

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Keywords: alkylphenols, seawater, Solid Phase Extraction, GC-MS, environmental analysis

Objective
Method development for determination of levels of Nonylphenols (NPs), with isomeric speciation, and 4-tert-octylphenol (4t-OP) in offshore sea water samples using SPE and GC-MS with evaluation of the main challenges of analysis of such kind of pollutants. NPs and 4tOP are alkylphenols (APE) and comes from environmental degradation of alkylphenol ethoxylates [1-2], their use is strictly regulated in UE, USA and in China but despite this, it is still possible to find them in surface waters and so they are monitored under the 2013/39/EU directive [3]. This work aims to fill the gap in knowledge about occurrence of APE in offshore sea water, much less studied than other water bodies, facing the detectability problems, connected to very low concentration (ppt) of these molecules in oceanic environment, and matrix complexity.

Methods
Method development was built for analysis of both surface (30-50 cm) and deep (-50 m) samples collected in offshore seawater around the globe. Main challenges were the salty matrix and the trace concentration levels. 500 mL seawater samples were extracted by SPE using HLB (200 mg) disks, to avoid clogging due to high salt concentration, derivatized to enrich detectability and analyzed by GC-MS using an approximately 30 min run with a 60 m long DB-5ms column.

Results
The obliquitous nature of APE makes method development particularly difficult, as it requires the elimination of any reagents stored in plastic and must contend the difficulties of procuring a blank matrix for method development. Raising the signal level through derivatization is very important and BSTFA showed better performance than TFAA.

Conclusions
Sample preparation is a fundamental step of the process, it is important to know the analytical challenges posed by the salt matrix, and it is very important to consider every possible contamination source.

ACKNOWLEDGMENTS
Authors would like to thank the Sea Care Team members.

REFERENCES
[1] Ying et al., (2002), Environmental fate of alkylphenols and alkylphenolethoxylates—a review, Environment International 28.
[2] Salgueiro-Gonzalez et al., (2017). Trends in analytical methodologies for the determination of alkylphenols and bisphenol A in water samples, Analytica Chimica Acta 962
[3] E.U., (2013). Directive 2013/39/UE

A green liquid phase microextraction using phthalic acid as switchable hydrophilicity solvent for the HPLC determination of sildenafil in human urine

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Keywords: Liquid Phase Microextraction, switchable-hydrophilicity solvent, sildenafil, urine, HPLC-UV

Objective

The present study describes a green liquid phase microextraction protocol using phthalic acid as switchable hydrophilicity solvent (SHS) for the quantification of sildenafil in authentic human urine.

Methods

The analyte was extracted onto phthalic acid solid particles which were produced through acidification of the sample. Its solidification was accomplished at ambient conditions without sample cooling. The determination of the sildenafil was carried out using high performance liquid chromatography-ultraviolet detection (HPLC-UV). The microextraction parameters that affect the extraction efficiency of the drug (i.e. SHS type and its concentration, acid type and concentration, extraction time, filter type) have been studied [1, 2]. The optimized analytical protocol involved the mixing of 300 μL of phthalate solution (0.75 M) with 600 μL of sample, followed by the addition of 50 μL of concentrated H_3PO_4. The produced solid was collected using membrane syringe filter (0.45 μm) and was finally dissolved in 500 μL of CH_3OH.

Results

Method validation data showed determination coefficient ≥ 0.99 for the linear range of 50 – 2000 ng/mL. The limit of detection (LOD) and the lower limit of quantitation (LLOQ) were 30 and 100 ng/mL, respectively. The accuracy (expressed as % recovery) of the method ranged between 88.0 – 108.9% while the precision (expressed as % RSD) was less than 17.8% in all cases. The robustness of the microextraction procedure and the instrumental method were investigated using Plackett-Burman experimental designs. The applicability of the method was demonstrated by analyzing authentic human urine samples after oral administration of drug-containing pharmaceutical formulation.

Conclusions

The developed protocol offers cost-efficiency, handling simplicity, and high throughput. Its green character was evaluated using Green Analytical Procedure Index and Blue Applicability Grade Index.

REFERENCES

[1] N. Manousi., P. D. Tzanavaras, C. K. Zacharis, (2024). Microextraction based on liquid-solid phase transition of benzoic acid: Extraction of statins from human urine followed by chromatographic analysis, *Talanta*, 266, 125088.

[2] Y. Basel, M. Rečlo, Y. Chubirka, (2020). Switchable hydrophilicity solvents in analytical chemistry. Five years of achievements, *Microchemical Journal*, 157, 10511

Unravelling the sources of incorrect lactoferrin quantification by HPLC-DAD in milk

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Keywords: lactoferrin, HPLC, ELISA, co-elution, milk proteins

Objective
Lactoferrin (LTF) is a multifunctional protein, belonging to the transferrin family found in many body fluids including milk, saliva and tears. One of its functions is to capture iron ions, which helps to maintain balanced levels of this micronutrient in the body, preventing its toxic effects. In addition, LTF demonstrates a much more diverse set of biological activities, such as immunomodulation, immunoregulation, anti-inflammatory, antibacterial, antifungal and even anti-cancer. Due to its unique properties, LTF is widely sought and determined in various types of products including milk.
High-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) are most commonly used for LTF detection. Although HPLC is a more cost-effective option due to lower analysis costs, our study found that it may not provide accurate quantitative results for LTF. The aim of this study was to compare the two methods, HPLC and ELISA, in the quantitative analysis of lactoferrin and to understand the reasons for the discrepancies in results between the methods.

Methods
We performed a comparative analysis of the quantitative measurement of LTF by HPLC and ELISA methods. The HPLC separation was performed on the Zorbax 300SB-C8 column with the gradient elution using 0.1% trifluoroacetic acid in water and acetonitrile as phase A and B respectively. Due to the discrepancy in the obtained results, we collected HPLC-separated fractions corresponding to the retention time of LTF. These fractions were then subjected to SDS PAGE analysis in order to examine the protein distribution and identify potential co-eluting proteins that could interfere with lactoferrin detection.

Results
The received results showed significant differences in the amounts of LTF determined by HPLC and ELISA methods. Analysis of the HPLC fractions by SDS-PAGE revealed the presence of other proteins that coelute with lactoferrin, suggesting that these impurities may lead to inaccurate quantitative measurements in the HPLC method.

Conclusions
Our research has shown that the commonly used HPLC method for quantification of lactoferrin does not provide reliable results due to co-elution of other proteins. HPLC methods need to be developed and improved in order to obtain precise and comparable results with ELISA. Further research should focus on optimising HPLC conditions to minimise co-elution and improve the specificity and sensitivity of LTF detection.

ACKNOWLEDGMENTS

The research was funded in the frame of the project titled: "Development of a preparative method for the isolation of biologically active lactoferrin," supported by the National Centre for Research and Development (NCBiR), under the LIDER program, grant number DPWP/LIDER-XIII/6/2023. Oleksandra Pryshchepa, Justyna Walczak-Skierska, and Paweł Pomastowski are members of Torun Center of Excellence 'Towards Personalized Medicine' operating under Excellence Initiative-Research University

Development and validation of a dual molecular imprinted polymer fabric phase extraction method for the selective determination of sulfathiazole in environmental waters analyzed by HPLC-DAD

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Keywords: fabric phase sorptive extraction, Molecularly Imprinted Polymers**,** HPLC-DAD, microextraction, sulfathiazole

Objective
Sulfathiazole is a widely used antibiotic with significant environmental and public health implications. Given the concerns over antibiotic residues in various matrices, the development of accurate and efficient analytical methods to monitor its presence in environmental samples is vital. This study introduces an advanced green analytical method for the determination of sulfathiazole (STZ) using Fabric Phase Sorptive Extraction (FPSE) combined with Molecularly Imprinted Polymers (MIPs), followed by HPLC-DAD analysis.

Method
The MIP-FPSE protocol was optimized using the one-factor-at-a-time method (OFAT). The optimization involved the systematic investigation of the key parameters that affect the performance of the MIP-FPSE procedure, including pH adjustment, absorption time, desorption solvent, volume of desorption, stirring time and ratios and elution time [1]. The optimum involved 20 min of extraction, under 300 rpm of stirring at pH: 3. Methanol was found to be the most appropriate solvent for desorption, and 1 mL was sufficient for the elution of STZ in 30s at 3000 rpm. The total analysis time was 4.6 minutes, employing an isocratic elution program consisting of acetonitrile (ACN) and water (H_2O) at 30:70 ratio, v/v.

Results
The proposed method was validated and was linear over the concentration range of 0.1 - 10 μg mL^{-1}, with a coefficient of determination (R^2) of 0.999. The MIP-FPSE method presented good accuracy, expressed as recovery rates, over the range 90.4 and 98.4%. The relative standard deviation (RSD%) for intra and inter-day measurements was lower than 4.2% and 14.8%, respectively, showing good precision. The limit of quantification (LOQ) was calculated equal to 0.1 μg mL^{-1}, and the limit of detection (LOD) was equal to 0.03 μg mL^{-1}.

Conclusions
The developed MIP-FPSE-HPLC-DAD is proposed as a selective and environmentally sustainable alternative for monitoring sulfathiazole residues environmental waters, thereby contributing to improved environmental stewardship and public health protection.

REFERENCES
[1] Samanidou V.F., Michaelidou K., Kabir A., Furton K.G., (2017). Fabric phase sorptive extraction of selected penicillin antibiotic residues from intact milk followed by high performance liquid chromatography with diode array detection, *Food Chemistry,* 224, 131–138.

Determination of lamotrigine unbound fraction using ultrafiltration and validated LC-MS method

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Keywords: lamotrigine, saliva, LC-MS, quantitation, unbound fraction

Objective
Total plasma concentrations are commonly used for therapeutic drug monitoring of antiepileptic drugs (AED). On the other hand, drug activity and at least some of adverse effects depend on the unbound fraction of a drug in the bloodstream. The determination of free (unbound) concentrations by ultrafiltration is expected to reflect the effective drug concentration. Further, saliva is considered as a plasma ultrafiltrate, thus referring to free drug concentration. Therefore, the aim was to develop a method for the measurement of total and unbound AED concentrations in plasma and correlate them to saliva AED concentration. As a model drug, lamotrigine (LTG) was chosen since it is one of the most widely used AED and is moderately bound to albumin (~55%) [1].

Methods
The developed LC-MS method was performed on the Kinetex C 18 Polar column (3x100 mm, 2.6 μm), which was maintained at 35 °C during the whole acquisition period. The mobile phase consisted of solution A (0.1 % formic acid in aqua) and solution B (acetonitrile containing 0.1 % formic acid). Multistep gradient was set in the range from 5 % to 90 % B within 10 minutes. Following elution from the LC column, ESI-qTOF analysis using the maXis impact Bruker instrument enabled identification and quantification of the drug. Analytical data were processed using Compass DataAnalysis 4.1 and Compass QuantAnalysis 2.1 software (Bruker). The detector response was linear over the concentration range 0.5-15 μg/ml for saliva and plasma samples. Calibration range for plasma ultrafiltrate was from 0.5 to10 μg/ml. The samples of saliva and plasma were obtained from the patients treated at the Neurological clinic of the St. Anne´s Hospital, Brno upon subscription of Informed Consent. The study was approved by the Ethics Committee of St. Anne´s Hospital in Brno (Approval No. 3V/2023).

Results
The method for saliva matrix was fully validated according to European Medicines Agency guidelines. For other matrices, only linearity, intra-day precision, and accuracy were assessed. The extraction efficiency of LTG and its internal standard (LTG-$^{13}C^{15}N_4$) was about 90 % for all matrices. All patient samples were processed within a day of their collection, and thus no analyte stability data were acquired for plasma and ultrafiltrate.

Conclusions
All clinical samples were within the recommended therapeutical range (3-15 μg/ml). Pilot data showed that the concentrations measured in plasma ultrafiltrate and saliva were in close proximity, even though total plasma concentrations are much higher and wildly differ between individual samples. More data must be available to determine the correlation between saliva and plasma concentrations and saliva and ultrafiltrate concentrations.

ACKNOWLEDGMENTS

This work was financially supported by the Czech Health Research Council, project No. NU23-08-00229 and MUNI/A/1580/2023 grant research project.

REFERENCES

[1] Patsalos P.N., Berry D.J. (2013). Therapeutic drug monitoring of antiepileptic drugs by use of saliva, *Ther Drug Monit.*, 35, 4-29.

Monitoring gas phase poly and per fluoroalkyl substances in the environment and from sources

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Keywords: PFAS, TD-GC-MS, sorbent tubes, collection in canisters

Objective
Poly and Per Fluoroalkyl Substances (PFAS) are everywhere. Whilst there has been a lot of focus internationally on PFAS in water researchers globally are turning their attention to volatile gas phase species in air. Gaseous PFAS have many sources including PFAS production facilities, aqueous film forming foams (AFFF), soil gas, materials and remediation of PFAS impacted sites/materials. PFAS in the air can spread easily, transform into more toxic species [1] and impact other matrices such as water and soil through deposition. Understanding and monitoring PFAS in air is an important part of controlling the spread of PFAS and reducing exposure to humans.
In this study we will show how thermal desorption coupled to gas chromatography and mass spectrometry (TD-GC-MS) can offer a robust approach to analyse PFAS in a variety of samples.

Methods
TD-GC-MS was applied to monitoring of hazardous organic compounds in ambient air. The technique is being utilised by environmental agencies and researchers globally to now monitor gaseous PFAS [2].

Results
TD-GC-MS was successfully applied to monitoring of hazardous organic compounds in ambient air for both target and non-target workflows. Volatile fluorinated species can require different sampling techniques depending on their volatility, data are shown for two different approaches: active sampling onto sorbent tubes and whole air collection in canisters.

Conclusions
TD-GC-MS is proved to be an effective approach to target and non-target screening of PFAS in a variety of samples.

REFERENCES
[1] Ashley M. LinJake T. ThompsonJeremy P. KoelmelYalan LiuJohn A. BowdenTimothy G. Townsend, (2024). Landfill Gas: A Major Pathway for Neutral Per- and Polyfluoroalkyl Substance (PFAS), *Environmental Science & Technology Letters*, 11, 730-737.
[2] Other Test Method 50 (OTM-50) Sampling and Analysis of Volatile Fluorinated Compounds from Stationary Sources Using Passivated Stainless-Steel Canisters, available at: https://www.epa.gov/system/files/documents/2024-01/otm-50-release-1_0.pdf.

Use of polyhedral oligomeric silsesquioxane for fabrication of monolithic columns of different polarities and diameters

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Keywords: Liquid Chromatography, monolith, polyhedral oligomeric silsesquioxane, POSS, HILIC

Objective

Polyhedral oligomeric silsesquioxane (POSS) hybrid materials are very popular in various fields of polymer industry, electronics, catalysis and biomedicine. Chemical stability of POSS, its nanoscale-architecture and possibility of surface modification is a challenge to use POSS as a chromatographic materials.
In our work, we studied the possibility to prepare monolithic chromatographic columns of wide range of diameters (50 – 530 µm) so that they could be used in a variety of analytical instruments and applications. Our goal was to find out the composition of polymerization mixtures when the best efficiency for nonpolar and polar stationary phases can be achieved.

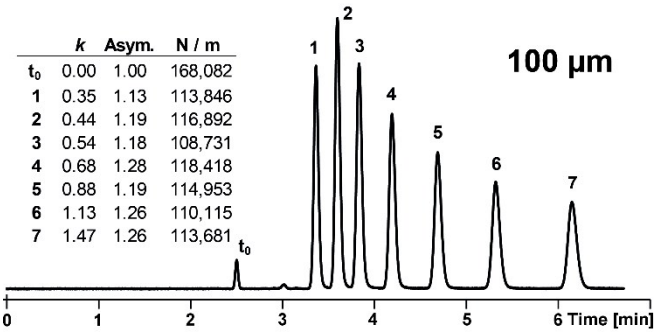
Methods

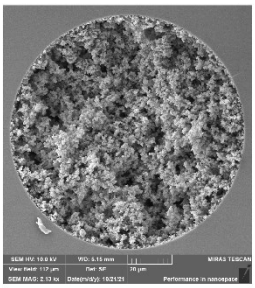
The columns were prepared by radical polymerization of POSS (methacrylate), pentaerythritol tetraacrylate, and n-octadecyl methacrylate (nonpolar C18 stationary phase) or sulfobetaine methacrylate (polar stationary phase). Porogenic system was carefully optimised, the best results were obtained from a mixture of methanol, dodecyl alcohol, and polyoxyethylene (23) lauryl ether.
The columns were evaluated in liquid chromatography employing a water-rich or organic-rich mobile phases. Pore distribution was measured by ISEC in THF mobile phase by elution of polystyrene samples with different molecular mass.

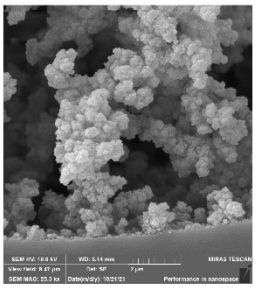
Results

The results confirmed that the columns have high efficiency (exceeding 100,000 theoretical plates/m) and selectivity, regardless of whether hydrophobic (n-octadecyl methacrylate) or hydrophilic (sulfobetaine methacrylate) monomer was used for their preparation. The example below shows separation of uracil and alkylbenzenes (0-6) by C18 monolith in 80% ACN mobile phase.

	k	Asym.	N / m
t_0	0.00	1.00	168,082
1	0.35	1.13	113,846
2	0.44	1.19	116,892
3	0.54	1.18	108,731
4	0.68	1.28	118,418
5	0.88	1.19	114,953
6	1.13	1.26	110,115
7	1.47	1.26	113,681







Conclusions

POSS monomer is a promising material which allows to prepare efficient monolithic columns in a wide range of diameters. We demonstrate the syntheses of non-polar (C18) and polar (sulfobetaine) columns by similar procedures, which differ only in the ratio of solvents in the porogenic system. The whole process of column synthesis is carried out in less than 24 hours.

ACKNOWLEDGMENTS

This work was supported by the Czech Science Foundation (Grant 23-04703S) and by the Academy of Sciences of the Czech Republic (Institutional Support RVO: 68081715).

Synergic profiling of virgin olive oil by coupling vacuum-assisted and multi-cumulative trapping headspace followed by GC×GC-MS

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Keywords: Solid-phase Microextraction (SPME), Comprehensive two-dimensional Gas Chromatography (GC×GC), Vacuum-assisted Headspace (Vac-HS), Multi-cumulative trapping (MCT), olive oil

Objective
In this study, vacuum (Vac) and multiple cumulative trapping (MCT) in headspace solid-phase microextraction (HS-SPME) were compared as potential alternative [1, 2] or combined methods [3] for highly informative virgin olive oil volatile profiling. The ability of these techniques in discriminating among different commercial categories and geographical origins of virgin olive oils was compared.

Methods
Vac-HS-SPME, MCT-HS-SPME, and Vac-MCT-HS-SPME, were coupled with GC×GC-MS. The performance were examined initially using 18 targeted quality markers, previously identified through traditional HS-SPME-GC-MS [4] and later to assess their effectiveness in categorizing virgin olive oil in its commercial categories. Later the data were treated untargetting.

Results
All the three sampling technique demonstrated enhanced extraction performance for semi-volatiles. The synergistic combination of Vac and MCT exhibited up to a 5-fold increase in extraction efficiency for less volatile compounds.
The previously reported markers biased results towards geographical origin over the commercial categories, while using the data matrices in an untargeted way a less biased clusterization was obtained. Notably, no misclassifications were observed, except for one instance where one extra virgin olive oil was erroneously classified as virgin olive oil in the 3×10 min Vac-MCT-HS-SPME method.

Conclusions
The use of these HS sampling approaches is very easily implementable, and it is highly promising, especially for the characterization of less-volatile analytes.

ACKNOWLEDGMENTS

The authors thank Supelco (MilliporeSigma) for providing the SPME fibers, Shimadzu, Markes International, and SepSolve for their support. This article is based upon work from the Sample Preparation Study Group and Network, supported by the Division of Analytical Chemistry of the European Chemical Society.

REFERENCES

[1] Mascrez S., Psillakis E. and G. Purcaro, (2020) A multifaceted investigation of the effect vacuum on the headspace solid-phase microextraction of extrac-virgin olive oil, *Analytica Chimica Acta*, 1103, 106-114.
[2] Mascrez S., and Purcaro G., (2020) Enhancement of volatile profiling using multiple-cumulative trapping solid-phase microextraction. Consideration on sample volume, *Analytica Chimica Acta*, 1122, 89-96.
[3] Mascrez S., Aspromonte J., Spadafora N., and Purcaro G., (2024) Vacuum-assisted and multi-cumulative trapping in headspace solid-phase microextraction combined with comprehensive multidimensional chromatography-mass spectrometry for profiling olive oil aroma, *Food Chemistry,* 442, 138409.
[4] Casadei E., Valli E., Aparicio-Ruiz R., Ortiz-Romero C., García-González D. L., Vichi S., Toschi T. G. (2021). Peer inter-laboratory validation study of a harmonized SPME-GC-FID method for the analysis of selected volatile compounds in virgin olive oils. *Food Control*, 123, 107823.

MAVERIC: development of Miniaturized Autonomous and VERsatIle gas chromatograph for VOC monitoring using nano-gravimetric-detector based on NEMS resonator array.

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Keywords: air quality, miniaturized Gas Chromatograph, Volatile Organic Compounds, Nano Gravimetric Detector NGD

Objective
Environmental monitoring and air quality survey during long-term field campaign tests (especially in low-accessibility or extreme environments) requires robust, standalone and autonomous analyzer with low gas consumption and minimal human intervention. This work presents the development and optimization of MAVERIC, a miniaturized and autonomous Gas Chromatograph system coupled to an innovative Nano Gravimetric Detector (NGD detector) based on NEMS (nano-electromechanical-system) resonator. MAVERIC relies on a technology transfer by adapting SAM-GC technology successfully embedded on MSL science lander.

Methods
MAVERIC instrument operates using three sub-modules: (i) sampling and preconcentration, (ii) separation, and (iii) detection. A homemade thermal-desorption trap (60 mm x 2 mm) filled with 50 mg of Carbopack B 60-80 mesh (Sigma-Aldrich) is coupled to a 26-m capillary column (Rxi-1, 0.25 mm x 1.0 µm PDMS, Restek). The column is coiled on a small stainless-steel cylinder (6 cm), heated-up with an electrical resistor and embedded in a thermally conductive potting ensuring a uniform temperature throughout it. Then NGD detector is used and a homemade software is developed to control the instrument as well as the electronics modules.

Results
Under optimal conditions, the detection limit, the stability, the repeatability and the linearity of the analytical system are assessed. The system operates at a low flow rate (2 $mL.min^{-1}$) of helium used as carrier gas, and allows the measurements of VOCs from C_6 to C_{10} in less than 30 minutes. A detection limit of sub-ppb to few ppt level is determined for C_9 and C_6 compounds respectively. The GC system allows continuous measurements for 1.9 years using only a small helium cylinder of B10 (2 m^3) which is very suitable for long-term field campaign tests.

Conclusions
The system is standalone, portable, robust and is equipped with 4G connection that allows remote control of the instrument and easy data export. It is robust and very adapted for a environmental monitoring and air quality survey outdoors and in low-accessibility or extreme environments.

ACKNOWLEDGMENTS
The development of MAVERIC was partly funded by the Defi – Instrumentation aux limites (AMI2013-INSTRUM) from CNRS and Institut Pierre Simon Laplace in Paris.

Interstitial fluids sampling for contaminants exposure. A preliminary evaluation

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Keywords: interstitial fluids, contaminants, bio-monitoring, SPME, LC-MS/MS

Objective
The interstitial fluid (ISF) represents the aqueous solution that fills the area between the cells. The chemical composition of ISF and blood is similar, and the exchange of substances (including contaminants and residues) between the two compartments is proven. Blood collection is considered the best approach for studying the presence of certain substances inside the body. Nevertheless, it poses some practical issues, as it is often uncomfortable, considered an invasive method and not ideal from an ethical point of view. Other body fluids, such as urine can be considered as an alternative, although many of them alternatives show disadvantages. Firstly, not all contaminants are excreted into urine, and if they are it is through metabolites for which standards are often not available. Besides, in blood samples, hence in ISF, the parent compound as such is our target before any metabolism reaction takes place. This aspect is highly significant for the risk assessment. To overcome these drawbacks, a device should be able to collect the ISF and extract from it the analytes of interest, preferentially during a prolonged period, such as 24 hours. An adsorbent can be used to test for a longer time by slowly storing compounds of interest from the ISF. In this context, the main objective of this project is to carry out a pilot study to develop efficient sampling strategies for classes of compounds (e.g., Steroids) or toxins (e.g., Ochratoxin A), antibiotics (e.g., Chloramphenicol), and pesticides (e.g., Fipronil-sulfone) and others from the ISF.

Methods
The first focus of the study was the selection of contemporary chemical hazards in food that might be detected in the bloodstream (using chemical knowledge and literature search). Afterward, different coated fibers were tested to evaluate the extraction capacities of the selected compounds from the artificial interstitial fluid, plasma and serum as well.

Results
A comparison between the different fluids and the extraction capacity of the fibers will be shown. Moreover, different type of coated fibers (mono- and multi-phase) were evaluated in terms of extraction efficiency and compared to select the best type of fiber for the different classes of analyte. Evaluation of the extraction capabilities was performed by the use of liquid chromatography coupled to tandem mass spectrometry after the desorption of the analytes in the proper solvent.

Conclusions
The proposed approach demonstrated that most of the bio-compatible fibers tested can concentrate a certain amount of analytes from the ISF pioneering the use of this semi-invasive sampling method, compared to blood collection, in the field of risk assessment for humans and animals.

ACKNOWLEDGMENTS

This work was supported by the Dutch Ministry of Agriculture, Nature and Food Quality through the Knowledge development program 'Healthy and safe food systems' (project KB-37-002-012).

Terpenes – non-cannabinoid compounds identified in cannabis vapours

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Keywords: cannabis, chromatographic analysis, terpenes, bioactive compounds

Objective

Products with *Cannabis sativa* L. have several potential health benefits. They show anti-inflammatory effects, can relieve pain and reduce stress [1]. Research also suggests their positive effects on sleep and the immune system [2]. Cannabis seeds are nutritionally valuable and may have neuroprotective properties [3]. One way to intake cannabis is through inhalation of its vapours from vaporization. The dried cannabis is heated in a special device (vaporizer) and the user inhales the resulting vapour. Because there is no combustion, the process is considered safe, as there are no toxic substances typical of smoke. The study focused on analysing 17 samples of died cannabis (commercially available products, without THC) for bioactive compounds in the released vapours, with particular emphasis on non-cannabinoid components – terpenes.

Methods

The research procedure involved grinding hemp before placing it in the vaporizer. Analytes were extracted from vapour by stationary phase microextraction (SPME). Gas chromatography coupled to a mass spectrometer with a time-of-flight analyzer (GC-TOFMS) was used to analyze obtained samples. Identification of organic components was performed based on NIST14 library. Quantitative analysis was performed using the internal normalization method.

Results

Twenty-six terpenes were determined in the studied vapours. (−)-α-Bisabolol, guaiol, linalool, humulene, (−)-β-caryophyllene, and seline-3,7(11)-diene were determined in all samples. Most samples also contained α-pinene, β-myrcene, terpinen-4-ol, α-terpineol, farnesene, β-bisabolene, and caryophyllene oxide. *Trans*-α-bergamotene was detected in three samples, and *cis*-α-bergamotene and p-cymene in only one sample.

Conclusions

Terpenes are biologically active compounds that have health-promoting properties. Their presence not only affects the specific aroma of the vapour but also gives it additional therapeutic properties. Therefore, in further research on the positive effects of cannabis, it is worth taking a closer look at this group of compounds.

REFERENCES

[1] Graczyk M., Lewandowska A.A., Dzierżanowski T., (2021). The therapeutic potential of cannabis in counteracting oxidative stress and inflammation, *Molecules,* 26, 4551

[2] ltman B.R., Mian M.N., Slavin M., Earleywine M., (2019). Cannabis Expectancies for Sleep. *Journal of Psychoactive Drugs*, 51, 405–412

[3] Marsh D.T., Smid S.D., (2020) Cannabis phytochemicals: a review of phytocannabinoid chemistry and bioactivity as neuroprotective agents, *Australian Journal of Chemistry,* 74, 388–404

Creation of a solid deposition GC-FTIR library for hydrocarbon isomers identification

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Keywords: Gas Chromatography, Fourier Transform Infrared Spectroscopy, Mass Spectrometry, hydrocarbons

Objective
Gas chromatography coupled to mass spectrometry detector (GC-MS) with electron ionization (EI) source is a widely used analytical technique for identification of volatile compounds. The highly reproducible fragmentation pattern provided by MS allows reliable identification by comparing experimental spectrum with commercial spectra database. However, in many cases, misidentification can occur due to a high spectral similarity. The Linear Retention Indices (LRI) approach can be used in combination with conventional mass spectral search with the goal of boosting the identification of "challenging" molecules. GC-FTIR, on the other hand provides a high number of information related to the molecular vibrations, but FTIR libraries of such chemical compounds are not widely diffused as the GC-MS ones.

Methods
Alphagaz PIANO Calibration Standards including n-paraffins mix, Isoparaffins mix, Aromatics mix, Napthenes, Olefins Mix and PIANO Mix were used as reference material for the library creation. Separations were achieved on a polydimethylsiloxane Supelco Petrocol DH capillary column, 100 m × 0.25 mm, L × I.D., d_f 0.50 µm. Spectra were acquired using a DiscovIR-GC solid phase FTIR detector (Spectra-Analysis Instrument Inc., Marlborough, MA, USA).

Results
A database containing the spectra obtained by GC-FTIR analyses of hydrocarbon standard mixture was created. It allowed the reliable discrimination of the isomers which presented a very similar mass spectrum, as well as linear retention index. Such isomers, indeed, presented a considerable difference in terms of similarity score in the FTIR library search.

Conclusions
The created library was successfully applied for the identification of unknowns.

ACKNOWLEDGMENTS

The research was performed within the framework of the Research Project PRIN 2022: Unique analytical workflow involving COMPLEmentary TEchniques for the reliable molecular identification of hydrocarbons (CompleTe), supported by the Italian Ministry of University and Scientific Research, no. Prot. 2022KC2BRL.
The authors acknowledge Shimadzu and Merck for the continuous support.

Chiral HPLC method for investigation of chiral impurities in moxifloxacin active pharmaceutical substance

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Keywords: moxifloxacin, chiral impurities, chiral HPLC, isomer, chiral mobile phase

Objective
Moxifloxacin, (1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid) is an anti-infective drug from the group of fourth generation fluoroquinolone antibiotics. It is official according to the European (Ph. Eur.) and American (USP) pharmacopoeia, as active pharmaceutical substance moxifloxacin hydrochloride, while tablets as a dosage pharmaceutical form are official according to the USP. Eight known impurities, related substances A, B, C, D, E, F and H and G are defined within the monograph of moxifloxacin hydrochloride in Ph. Eur. [1]. Potential chiral impurities that can be found in the moxifloxacin active substance (S,S isomer) are: (R,R)-isomer, (R,S)-diastereoisomer and (S,R)-diastereoisomer. The pharmacopoeia requires examination of the chiral impurity G, i.e. R,R isomer. Examining the chiral impurities of a drug involves firstly separating them from the active substance and from each other, using the chiral separation analytical method. The development of such a method is very complex and represents a great challenge for the analyst, given that enantiomers are optical isomers of very similar structures that in an achiral medium have exactly the same physical and chemical properties and are very difficult to be distinguished analytically.

Methods
In this work, a new chiral isocratic HPLC method was proposed for investigation of moxifloxacin hydrochloride and its chiral impurity G using the chiral additive copper(II)-sulfate and isoleucine in the mobile phase with the use of an achiral C18 chromatographic column, Luna C18 150x4.6 mm; 3 µm, as a significant improvement of the official method concerning economy and method eco-friendliness. The mechanism of separation of enantiomers of moxifloxacin using the chiral additive copper(II)-sulfate is also elucidated. In the optimization of the experimental conditions of the chiral HPLC method, the central composite design (CCD) was used, and for multiobjective optimization, the Desirability function with its desired value 1.00 was used.

Results
After the central compositional design procedure was carried out, the best conditions concerning the concentration of chiral agents (0.01 M), organic solvent content in the mobile phase (30%, v/v methanol), pH value of aqueous phase (3.8) and column temperature (33°C) were defined. After optimization, the method was validated according to the ICH regulation. During the method validation and system suitability testing the final conditions of the method: concentration of chiral agents (0.01 M), 30% methanol (v/v) in the mobile phase, pH 3.5 and column temperature 23°C with 10 µl injection volume, were determined.

Conclusions
The method was found to be suitable for quantification of moxifloxacin chiral impurity G ((R,R)-isomer) in moxifloxacin active pharmaceutical substance.

ACKNOWLEDGMENTS
This study was done with the support of the company ZADA Pharmaceuticals d.o.o Lukavac, Bosnia and Herzegovina, in practical contributions and technical support.

REFERENCES
[1] Ph. Eur. Pharmacopeia. 11th edition, (2023). Council of Europe, Strasbourg, pp. 3452-54.

Differentiation of *Cutibacterium acnes* phylotypes by CE utilizing nano-etched fused silica capillary

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Keywords: Capillary Electrophoresis, *Cutibacterium acnes*, phylotype, separation, supercritical water

Objective
Cutibacterium acnes (*formerly Propionibacterium acnes*) is lipophilic, aerotolerant, anaerobic Gram-positive bacterium that is a prominent commensal of the human skin. Although involved in maintaining healthy skin, *C. acnes* is frequently associated with acne vulgaris and many other infections including implant-associated and post-operation infections. Inflammatory and immunogenic properties vary between *C. acnes* phylotypes. Therefore, reliable differentiation between *C. acnes* phylotypes is important for determining the pathogenicity of these bacteria. In this study, capillary electrophoresis in fused silica capillary etched with supercritical water, was used for the discrimination of *C. acnes* phylotypes.

Methods
Tested strains of subtypes IA1, IA2, IB, IC, II and III were isolated from clinical material and identified at the Department of Microbiology, Faculty of Medicine, Masaryk University and St Anne's University Hospital (Brno, Czech Republic). The electrophoretic experiments employed a laboratory-made apparatus. The bacteria were analyzed by a combination of capillary electrophoretic methods, polymer-enhanced transient isotachophoresis and sweeping of the charged bacterial cells in micellar electrokinetic chromatography in the roughened fused silica capillary.

Results
The obtained results showed that the *C. acnes* phylotypes cannot be separated in the smooth fused silica capillary. Only one wide peak of all phylotypes was always detected regardless of optimization of the experimental conditions. Nevertheless, all the phylotypes can be discriminated using CE in the fused silica capillary with roughened part.

Conclusions
The developed CE method for discrimination of *C. acnes* phylotypes provides reliable, fast and low-cost alternative to the traditional microbiological methods. The method can also be used to monitor the presence of individual phylotypes in a sample that will allow more detailed examination of microbiome changes.

ACKNOWLEDGMENTS

This work was supported by Ministry of Health of the Czech Republic (Grant No. NU22-05-00110) and by the Czech Academy of Sciences (Institutional Support RVO:68081715).

Development and validation of a magnet integrated fabric phase sorptive extraction (MI-FPSE) method for the selective determination of seven sulfonamides in human urine analyzed by HPLC-DAD

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Keywords: sulfonamides, MI-FPSE, sol-gel technology, bioanalysis, human urine, HPLC-DAD

Objective

In the present study magnet integrated fabric phase sorptive extraction (MI-FPSE) was employed for the extraction of sulfonamides (i.e., sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, sulfamethoxazole, sulfisoxazole and sulfadimethoxine) from human urine prior to analysis by high pressure liquid chromatography – diode array detection (HPLC-DAD).

Method

The MI-FPSE procedure was optimized after studying the following critical parameters that affect the extraction: type of sol-gel sorbent, extraction time and desorption time, stirring rate, type and volume of elution solvent, the ionic strength and the pH of the sample matrix [1] using the one-factor-at-a-time method.

Results

Sol-gel CW 20M coated on a hydrophilic cellulose fabric substrate was selected. The developed MI-FPSE-HPLC-DAD method was validated in terms of linearity, sensitivity, selectivity, accuracy, and precision and presented satisfactory results. The limits of detection (LODs) and quantification (LOQs) ranged between 0.02 – 0.04 ng/μL and 0.06 – 0.15 ng/μL, respectively. Accuracy was assessed using the percentage of relative recovery and varied from 86.3 – 112.9% (intra-day study) and 85.5 – 106.9% (inter-day study) for all the target analytes. The RSD% values of the intra-day and inter-day assays were found lower than 6.7% and 9.4%, respectively, showing good precision. The proposed method's green character and applicability were investigated using the ComplexGAPI index and Blue Applicability Grade Index (BAGI).

Conclusions

The proposed method combines a low-cost extraction procedure with reduced chemical consumption and waste generation, as well as handling simplicity and rapid extraction kinetics.

REFERENCES

[1] Samanidou V., Kabir A. (2022). Magnet Integrated Fabric Phase Sorptive Extraction (MI-FPSE): A Powerful Green(er) Alternative for Sample Preparation, *Analytica*, 3, 439-447.

A sustainable analytical approach for detecting extra virgin olive oil adulteration using subcritical fluid chromatography with UV detection

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Keywords: adulterations, bio-ethanol, extra virgin olive oil, green analytical chemistry, Photodiode Array Detection, Supercritical Fluid Chromatography, triacylglycerols, vegetable oils

Objective
Current research on the fraud detection of extra virgin olive oil (EVOO) involves a wide array of methods, including infrared spectroscopy, Raman spectroscopy, liquid chromatography (LC), and gas chromatography. In this study, a fast, simple and green methodology was optimized to detect intentionally adulterated EVOO with common seed oils at different levels (between 5% and 50%), by means of subcritical fluid chromatography (SubFC) with UV detection, followed by statistical analysis.

Method
Analysis conditions were as follows: four Ascentis Express C18 column (150 × 4.6 mm × 2.6 µm superficially porous particles) were serially coupled with a column oven temperature maintained at 25 °C. Isocratic elution mode was performed within 40 min with the 95% of SubCO_2 and 5% of co-solvent composed of ethanol. The total flow rate was set at 2.0 mL min^{-1}. Injection volume was set at 0.3 µL for all samples. For the UV, the detection wavelength was set at 205 nm with the backpressure regulator set at 200 bar and heated at 50 °C.

Results
The results demonstrated that using triacylglycerols (TAGs) profile in combination with statistical analysis could differentiate EVOO from seed oils at adulteration level of 5%. In EVOO, the linoleic fatty acid content is much lower than many other oils, thus trilinolein (LLL) is an important parameter that need to be used to distinguish EVOO from other oils types. In fact, LLL levels increase with the percentage of the potential adulterant.

Conclusions
The developed method is a more sustainable alternative to conventional non-aqueous reversed phase methods (NARP-LC), for routine analysis aimed to guarantee olive oil quality. Separation was achieved on four Ascentis Express C18 columns serially coupled, with a CO_2-based green mobile phase, which provided high efficiency without reaching a prohibitive backpressure. Remarkably, small amounts of organic modifier (5%) were sufficient for elution, thus minimizing both solvent consumption and waste (around 1.5 mL per analysis).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Shimadzu Corporation and Merck Life Science for their continuous support.

Enantioseparation of synthetic cathinones by isocratic HPLC-UV using a Lux® i-Amylose-3 column

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Keywords: enantioseparation, amylose tris(3-chloro-5-methylphenylcarbamate), High-performance Liquid Chromatography (HPLC), new psychoactive substances

Objective
In 2024, the European drug market is still undergoing constant changes due to the emergence of New Psychoactive Substances (NPS), as annually around 50 new substances have been registered by the European Union Drugs Agency (EUDA) in recent years [1]. Among them, more than 100 synthetic cathinones, which are often misleadingly traded as "bath salts," play an important role. They possess a chiral centre, leading to the existence of two enantiomers with presumably different pharmacological properties. For them, little is known about the distinct effect of the enantiomers. The aim of this study was to check a commercially available Lux® i-Amylose-3 column by HPLC-UV for enantiomer separation ability of cathinone derivatives.

Methods
Overall, 80 compounds were tested in normal phase mode, where 75 substances were separated under initial conditions. After method optimization, at least partial separation was achieved for the remaining compounds as well. The same set of substances was measured in polar-organic mode, where 63 analytes were resolved into their enantiomers under initial conditions with very short retention times.

Results
All measurements were carried out under isocratic conditions, and intraday and interday repeatability tests were performed. Both modes showed complementary results for the individual compounds [2].

Conclusions
Furthermore, the method in normal phase mode was tested on a real-life sample, the synthetic cathinone N-cyclohexyl methylone, which was identified during a local drug checking program in 2023.

REFERENCES
[1] EMCDDA. European Drug Report 2023: Trends and Developments 2023. DOI: 10.2810/161905
[2] Seibert E., Götz K., Schmid M.G., (2024). Exploring a Lux® i-Amylose-3 column in normal phase and polar-organic mode for chiral separation of cathinone derivatives and pyrovalerones using high-performance liquid chromatography. *Chirality*, 36(5), e23679.

Insight on chemical composition and bioactivity profiles of bee pollen samples botanically sourced from chestnut, willow, and oak

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Keywords: bee pollen, HPTLC, UPLC, antioxidant activity, xanthine oxidase inhibitory activity

Objective
Bee pollen is produced from pollen grains located in anthers of flowering plants. The chemical composition of bee pollen, which is directly related to the botanical source, is responsible for its health benefits [1]. Therefore, the botanical source of bee pollen should be identified as a first step. Although there are various studies on microscopical analysis, there are limited chromatographic studies comparatively analyze the chemical composition of bee pollen and flower pollen grains. This study aims to compare chemical compositions and bioactivity profiles of hydroalcoholic extracts of chestnut (*Castanea sativa*), willow (*Salix alba*), oak (*Quercus pubescens*) flower grains and their bee pollen samples obtained from Türkiye and Slovenia.

Methods
The botanical origins of bee pollen samples were comparatively evaluated by palynological analysis and High-Performance Thin-Layer Chromatography (HPTLC). After, fingerprinting profiles of all samples were evaluated and the dominant and common compound in all samples was purified by successive chromatographic methods and the quantities of the marker components were evaluated by a validated Ultra-Performance Liquid Chromatography (UPLC) method. Then, flower pollen, bee pollen samples and isolated marker component were subjected to comparative bioactivity studies as antioxidant (DPPH, FRAP, CUPRAC, ABTS) and xanthine oxidase (XO) inhibitory activities by *in-vitro* tests and effect-directed analyses (HPTLC-2,2-diphenyl-1-picrylhydrazyl (DPPH) and HPTLC-XO.

Results
It was resulted that bee pollen samples having same botanical origin both from Slovenia and Türkiye showed similar fingerprints. The structure of dominant and common compound in all samples was elucidated as N^1, N^5, N^{10}-tricaffeoyl spermidine by nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS) analyses. Also, rutin, hyperoside, quercetin, quercitrin, isoquercitrin, and myricitrin were determined in flower pollen samples. N^1, N^5, N^{10}-tricaffeoyl spermidine was found to be most bioactive compound that may be responsible from the pharmacological activities of the extracts.

Conclusions
It was concluded that botanical origin identification is a crucial parameter before bioactivity studies and chromatographic analysis comparatively performing with flower pollen grains could be an alternative way to palynological analysis. Standardized *C. sativa* bee pollen extract over N^1, N^5, N^{10} -tricaffeoyl spermidine could have a potential to be used as a food supplement in apitherapy.

ACKNOWLEDGMENTS

This study was supported by Yeditepe University Research Projects and Scientific Activities Support (YAP; Grant No: YAP-US-SAB-22026), The Scientific and Technological Research Council of Türkiye (TÜBİTAK; Project No: 119N569) and the Slovenian Research and Innovation Agency (research core funding No. P1-0005 and the bilateral project BI-TR/20-23-004).

REFERENCES

[1] Sen, N. B., Guzelmeric, E., Vovk, I., Glavnik, V., Kırmızıbekmez, H., Yesilada, E., (2023). Phytochemical and bioactivity studies on *Hedera helix* L. (Ivy) flower pollen and ivy bee pollen, *Antioxidants*, *12*, 1394.

The role of MALDI-MS in detection of changes in urine microbiome during radiotherapy

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Keywords: Laser Desorption Ionization Mass Spectrometry, prostate cancer, microbiome, urinary microbiota, radiation therapy

Objective
The urinary microbiome may have a significant role in the development of complications, yet changes during and after radiotherapy (RT) remain poorly understood. This study utilized matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) to identify the microbiome and examine its alterations in urine samples from 88 patients undergoing prostate cancer irradiation.

Methods
The material consisted of samples of blood for biochemical analysis and urine samples for MALDI at various stages: before gold fiducial implantation (t1), at the start (t2), and conclusion of RT (t3); with subsequent follow-ups at 1 (t4), 4 (t5), and 7 (t6) months post-treatment.

Results
Results revealed 1801 distinct microbial isolates, with 89% (470/528) of samples containing at least one microbial species, 79% (373/470) of which were polymicrobial. Predominant among these were *Staphylococcus* species (51.6% of all isolates), followed by *Micrococcus* (9.1%), *Enterococcus* (7.6%), *Kocuria* (5.6%), *Corynebacterium* (5.4%), and *Streptococcus* (2.2%). A decrease in microbial diversity was observed post-RT, with the total number of species increasing from 50 at t1 to 61 at t2, then returning to 52 at t3. However, biodiversity increased significantly at t4 (68 species), t5 (86 species), and t6 (75 species) post-RT ($p<0.05$). Changes in microbial biodiversity were also reflected in variations in total isolates (261-281 at t1-t3 vs. 292-362 at t4-t6) and detected genera (25-29 at t1-t3 vs. 28-31 at t4-t6).

Conclusions
In conclusion, RT for prostate cancer induced dynamic changes in the urinary microbiome, initially reducing diversity post-RT followed by subsequent increases. These findings underscore the impact of glucose levels in urine and blood on the urinary microbiota, contributing to our understanding of how RT influences the urinary microbiome and patient health. This insight could lead to more personalized approaches in prostate cancer treatment.

ACKNOWLEDGMENTS

This research was funded by National Science Centre Poland grant OPUS 20; 2020/39/B/NZ7/02733).

Analyzing drug-liposome interactions by capillary electrophoresis

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Keywords: Capillary Electrophoresis, interactions, Liposomal Electrokinetic Chromatography, liposomes

Objective
This study aims to develop a capillary electrophoresis method suitable for examining the interactions between selected active pharmaceutical ingredients (APIs) and liposomes. These interactions can influence the kinetics of analytical separations, causing changes in peak shape and/or mobility. Understanding these interactions is crucial for optimizing drug delivery and formulation.

Methods
Liposomes were prepared by the lipid film hydration method from tissue extracts, either from bovine heart or bovine liver, at a total lipid concentration of 5 mg/mL in a 10 mM sodium phosphate buffer at pH 7.10. We utilized sodium phosphate buffer as the background electrolyte during capillary electrophoresis experiments, with the addition of liposomes to investigate the interactions under various physiological conditions, such as different temperatures during separation and different pH levels of the background electrolyte.

Results
We observed that the interactions between APIs and liposomes functioning as a pseudostationary phase in the capillary affected the separation kinetics, in terms of electrophoretic mobility and/or peak shapes of some APIs. Variations in temperature and pH conditions further influenced the separation kinetics, highlighting the sensitivity of API-liposome interactions to physiological changes. Temperature studies revealed that increasing the temperature generally enhanced the effective mobility of most APIs due to lower background electrolyte viscosity and increased liposomal membrane fluidity, although certain APIs like Canagliflozin exhibited unique behavior sensitive to lipid bilayer rigidity. pH studies further complicated the interaction dynamics, showing that both the charge of APIs and the surface charge of liposomes are affected by pH changes, influencing observed interactions.

Conclusions
The developed capillary electrophoresis method provides a platform for analyzing the interactions between liposomes and active pharmaceutical ingredients. Our findings highlight the importance of considering lipid composition, temperature, and pH when evaluating API-liposome interactions, as these factors significantly impact the behavior of APIs. This knowledge can improve the optimization of liposome-based drug delivery systems, enhancing drug encapsulation, release, and targeting under physiological conditions.

ACKNOWLEDGMENTS
The research was supported by Charles University project SVV260690 and by the Grant Agency of Charles University (No 386122).

Wildlife crimes. Poisoning by banned pesticide carbofuran through Czech Republic – investigating the chemical impurity profiles of different carbofuran preparations as a part of forensic analysis

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Keywords: carbofuran, wildlife crimes, High Resolution Mass Spectrometry, chemical attribution compounds

Objective

Carbofuran a highly toxic carbamate pesticide with high toxicological and environmental risk. It was used in agriculture as a pest control and banned in 2008 by EU. Despite this ban, which includes not only the use of carbofuran, but also its possession, acute cases of carbofuran poisoning have recently been reported across the Europe. In the Czech Republic, carbofuran is suspected to be the main substance in more than 90 % of all illegal deaths of raptors and predators. Over the past 10 years, almost 600 cases of wild animal´s deaths in connection with carbofuran have been recorded [1].
Chemical attribution compounds (CAS) encompass the characterization and detection of compounds of interest to find signature impurity, isotopic, and/or elemental profiles. In general, these CAS can be further used to link illegal material to specific manufacturers, stocks, precursors, synthetic routes, or geographical locations.

Methods

Various carbofuran preparations were obtain from Police and from some manufacturers (e.g. Ukraine, where carbofuran is not banned). Animal tissue samples comes from carbofuran-suspicious findings in the wild conducted by the Police of the Czech Republic in cooperation with the Czech Ornithological Society. Carbofuran and its CAS were extracted from animal tissue (liver, contents of the stomach and digestive system) using modified QuEChERS method. Sample extracts were subjected to targeted and non-targeted toxicological analysis (HPLC/QToF MS/MS and HPLC/TQ MS) to confirm the presence of carbofuran and further its CAS.

Results

Multivariate statistical analysis of the results of the iterative measurement of individual formulations of different manufacturers as well as individual batches of a single manufacturer's pesticide showed significant differences in the presence of various chemical attribution compounds. A comparison of analyses originating from samples of poisoned wildlife and materials of poisonous carbofuran-containing material seized by the Police of the Czech Republic yielded that originating from criminal activity made it possible to find suitable characteristic signs in MS/MS analyses.

Conclusions

Fingerprints analysis of chemical attribution compounds of carbofuran commercial products are a promising methodology of toxicologic methods. The use of this methodology in forensic investigations of wildlife crimes will be further investigated and expanded.

ACKNOWLEDGMENTS

This study was conducted as a part of project no. VJ01010026 Effective use of forensic evidence methods to combat wildlife crime financially supported by the Ministry of the Interior of the Czech Republic within the program Strategic Support for the Development of Security Research 2019–2025 (IMPAKT 1).

REFERENCES

[1] https://www.karbofuran.cz

Capillary electrophoresis and isotachophoresis applied for analysis and characterization of peptides regulating food intake

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Keywords: Capillary Electrophoresis, Isotachophoresis, peptides, acidity constant, effective charge

Objective

New lipidized analogs of peptides (ghrelin, prolactin-releasing peptide (PrRP) and cocaine- and amphetamine-regulated transkript peptide (CARTP)) are studied for regulation of food intake. Prior their application, they have to be analyzed and characterized. Hence, the aim of this work was to check their purity and to characterize their acid-base properties by capillary zone electrophoresis (CZE) and capillary isotachophoresis (CITP).

Methods

CZE and CITP analyses were performed in CE 7100 analyzer (Agilent, Waldbronn, Germany) equipped with UV-vis/DAD and/or contactless conductivity detector or P/ACE MDQ DNA System (Beckman–Coulter, Fullerton, CA, USA) with UV-vis/DAD detector. Hydroxypropyl cellulose or polyacrylamide coated capillary ID/OD was 50(100)/375 μm and its total/effective length was 395-500(600)/294-415(515) mm for CZE (CITP). Peptides were introduced hydrodynamically (10-35 mbar × 10-20 s).

Results

Ten newly synthesized (lipo)peptides (3.3-4.7 kDa) containing 7-9 basic groups (His, α-NH_3^+, Lys, Arg) and 3-5 acidic groups (α-COOH, Asp, Glu, Tyr) at variable positions of peptide chain with/without attached fatty acid (octanoic, myristic or palmitic acid) were analyzed by CZE in acidic background electrolytes (BGEs). The purity degree of (lipo)peptides was in the range 85-100%.CZE was employed for the determination of thermodynamic acidity constants (pK_a) and actual ionic mobilities of the above ten (lipo)peptides. Their effective electrophoretic mobilities were measured by CZE in a series of the BGEs within a wide pH range (2.0-10.5), at constant ionic strength (25 mM) and constant temperature (25°C). Thermodynamic pK_a of acidic group such as α-COOH was in the range 1.94-2.07, pK_a of carboxyl group of Asp was in the range 1.96-3.67, pK_a of carboxyl group of Glu was in the range 3.72-5.78, and pK_a of basic group as imidazolium group of histidine residues was in the range 5.83-7.23, pK_a of α-NH_3^+ group was in the range 7.04-10.34, and pK_a of ε-NH_3^+ group of lysine spanned the interval 9.87-10.52, depending on the particular amino acid sequence of the peptides and fatty acid attached to peptide chain.CITP and CZE were applied for the determination of effective charges and ionic mobilities of (lipo)peptides. Effective charges of the (lipo)peptides were determined from the lengths of their ITP zones, ionic mobilities, and molar concentrations, and from the same parameters of the reference compound. CITP analyses were performed in cationic mode using leading electrolyte (LE) composed of 10 mM NH_4OH, 40 mM AcOH, pH 4.0, and terminating electrolyte containing 40 mM AcOH, pH 2.9. Ionic mobilities of peptides and singly charged reference compound (sodium cation) were determined by their CZE analyses in the BGE of the same composition as the LE. The effective charge numbers of (lipo)peptides were found to be in the range 1.37-5.11, i.e. reduced as compared to the theoretical charge numbers by (34-66) %.

Conclusion

CZE and CITP proved to be suitable tools for qualitative and quantitative analysis and determination of the acidity constants, effective charges and ionic mobilities of highly charged peptides and their lipidized analogs.

ACKNOWLEDGMENTS

This work was supported by the Czech Academy of Sciences (Research Project RVO 61388963).

Three formats, one template: enantioselective performance of molecularly imprinted polymers for extraction of D-panthenol

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Keywords: Molecularly Imprinted Polymer, Molecularly Imprinted Polymer on magnetic support, Molecularly Imprinted Membrane, enantioselectivity, panthenol

Objective

Molecularly imprinted polymers (MIPs) are synthetic materials engineered for selective recognition of target molecules, offering significant potential in various analytical and preparative applications [1]. Enantioselective MIPs have an ability of recognize enantiomers, and capturing one preferentially over the other [2]. This study investigates the enantioselectivity of different formats of polymer, MIP prepared by bulk polymerization, MIP on magnetic support (Fe_3O_4@MIP), and MIP on supporting membrane (MIM), all designed for D-panthenol as the template molecule and applicable in sample treatment of complex samples.

Methods

Three formats of sorbent were prepared by bulk polymerization, by precipitation polymerization on Fe_3O_4, and by in-situ multilayer polymerization on supporting polyvinylidene fluoride membrane (identical polymerization mixture, conditions: 60 °C, 24 h). The polymerization mixture consisted of a template (D-panthenol), a functional monomer (4-vinylpyridine (4-VP)), a porogenic solvent (methanol), an initiator (azisobutyronitrile), and a crosslinker (ethylene glycol dimethacrylate). Enantioselectivity of MIP-based sorbents was tested for DL-panthenol. HPLC-DAD with chiral stationary phase Lux i-Amylose-1 (250 x 4.6 mm; 5 µm), and *n*-hexane:ethanol (60:40, v/v) as a mobile phase was used for quantification of enantiomers.

Results

Enantioselectivity of bulk MIP, Fe_3O_4@MIP and MIM was investigated as ability od sorbent preferably captured in the specific cavity enantionmeric form to be imprinted (D-enantiomeric form). Enantiomeric ratio (ratio of elution peaks D- and L-form in extract) reached values of 2.8, 7.4 and 6.0 for bulk MIP, Fe_3O_4@MIP and MIM, respectively. Results demonstrate that all sorbents exhibit high enantioselectivity for the template molecule, preferentially capturing the D-panthenol enantiomer over its L- form. Sorbetns were characterised from the aspect sorption capacity and kinetic. Bulk MIP (in column form), Fe_3O_4@MIP (in batch form), and MIM were used in SPE treatment of personal care products prior the HPLC-DAD analysis.

Conclusions

Three different types of MIP-based sorbents were prepared, tested in terms of enantioselectivity, and successfully used in the analysis of cosmetic samples. Enantiospecificity of all types of sorbents has been demonstrated for D-panthenol. Enantioselective MIP-based sorbents are important for applications in the pharmaceutical and other fields where enantiomeric purity of compounds is essential. Bulk MIP could be used as a chiral stationary phase in HPLC.

ACKNOWLEDGMENTS

This work was supported by the call for doctoral students and young researchers of Slovak University of Technology in Bratislava to start a research career (Grant 23-04-01-A) and Slovak Research and Development Agency under contract number VEGA 1/0332/24.

REFERENCES

[1] BelBruno J. J. (2019) Molecularly Imprinted Polymers. *Chem. Rev*. 119, 94-119

[2] Tiwari M. P., Prasad A. (2015) Molecularly Imprinted Polymer Based Enantioselective Sensing Devices: A Review. *Anal. Chim. Acta* 853, 1-18.

Elevating metabolomic profiling: integrating advanced chromatography and high-resolution mass spectrometry for comprehensive biomarker discovery in clinical studies

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Keywords: High Resolution Mass Spectrometry, untargeted, targeted, metabolomics, Liquid Chromatography

Objective
Metabolomics, a rapidly evolving field, provides profound insights into the quantitative and qualitative assessment of metabolites across various domains, including medicine, environment, nutrition, and agriculture. This scientific discipline facilitates global identification of metabolites, offering crucial insights into metabolic profiles and biomarker discovery. Metabolomics analysis is pivotal in comprehending biological processes and human diseases, demanding advanced analytical methodologies to unravel the complexity of the metabolome. Integration of semi-targeted methodologies, such as the use of internal standards (ISTDs), with untargeted approaches is crucial. This work combines parallel liquid chromatography with high-resolution mass spectrometry (HRMS) as a promising approach to cover metabolites over a wide polarity range in clinical studies.

Methods
The diversity of human metabolites, characterised by a wide range of polarities, also requires the implementation of highly sophisticated liquid chromatography systems. In this regard, the use of parallel chromatography, equipped with two binary pumps, two injectors and two column compartments, is crucial. This approach allows the simultaneous application of two complementary separation techniques: reversed phase chromatography (RP) and hydrophilic interaction chromatography (HILIC). This optimised combination, together with the use of high-resolution mass spectrometry (HRMS), allows maximum metabolite coverage and accurate detection of metabolites with different chemical characteristics.

Results
In this work, with the aid of parallel chromatography and the implementation of high-resolution mass spectrometry (HRMS), it was possible to identify a wide range of metabolites in a broad polarity range. Moreover, metabolites of different classes were identified by means of semi-targeted approaches through the use of internal standards (ISTDs) or non-targeted approaches through the comparison of experimental data with data in the Human Metabolome Database (HMDB).

Conclusions
In conclusion, the integration of semi-targeted methodologies, untargeted approaches and the adoption of advanced liquid chromatography systems represent an effective strategy to address the complexity of the human metabolome, enabling a better understanding of biological processes and the identification of clinically relevant biomarkers.

Electrophoretic analysis of goat milk powder enriched with grape pomace seed and mushroom extracts

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Keywords: protein profile, *A. aegerita*, grape pomace seed, goat milk, SDS-PAGE

Objective

Mushrooms are a valuable source of bioactive compounds with a wide range of biological activites, of which antioxidant, antimicrobial, imunomodulatory and antitumor activities are recent important [1] wheras grape pomace seed are rich source of falvan-3-ols and phenolic acids with high antioxidant properties. For this reason, their use in the formulation of functional foods has increased in the last decades. Mushroom and grape pomace seed extracts have been reported to be used for enrichment of dairy, meat and bakery products. However, the enrichment of goat milk has been neglected, although goat milk contains proteins with high biological value, essential fatty acids, high mineral bioavailability and high vitamin content. Recently, it has been reported that thermally-treated goat milk can be used as a good carrier for bioactive compounds [2], but fortification of goat milk with both extracts has not been performed yet. Therefore, the aim of this work was to investigate the protein profile of thermally-treated goat milk (TM) enriched with an aqueous extract of the wild mushroom *Agrocybe aegerita* (Brig.) Sing (ME) and grape pomace seed extract (GPE) using SDS-PAGE technique.

Methods

Optimization of the TM, GPE and ME mixture was performed using the Central Composite Experimental Design (CCD) with two independent variables (GPE and ME content) and three dependent variables: total phenolic content, $DPPH^{\bullet}$ scavenging activity, and $ABTS^{\bullet+}$ scavenging activity of the formulated mixtures.

Results

Overall desirability was D=0.9377 for the mixture prepared with 0.5% ME and 0.5% GPE at a pH of 6.50. A well-known protein bands of goat's milk corresponding to caseins and whey proteins can be observed on TM pattern. On the other hand, apart from known goat milk protein bands, five new (uncharacteristic) polypeptide bands were observed on TM/ME/GPE pattern, which were not detected on the TM pattern. Moreover, a reduced intensity of the band corresponding to β-casein and a complete absence of the k-casein band were observed on the TM/ME/GPE electrophoretic pattern.

Conclusions

These results indicate the proteolytic activity of specific mushroom enzymes, capable of selectively cleaving goat milk caseins. Further research is needed to evaluate the effects of proteolysis on the nutritional and techno-functional properties of the forumulated food ingredient.

ACKNOWLEDGMENTS

This research was supported by the Science fund of Republic of Serbia, FUNPRO Project, #Grant No. 7744714 the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-65/2024-03/200116.

REFERENCES

[1] Reis, F. S., Martins, A., Vasconcelos, M. H., Morales, P., & Ferreira, I. C. F. R. (2017). Functional foods based on extracts or compounds derived from mushrooms. *Trends in Food Science & Technology, 66*, 48-62.

Affinity capillary electrophoresis applied for the separation of cyclic diadenosine diphosphorothioate and the diastereomers of its difluorinated derivative and investigation of their interactions with 2-hydroxypropyl-β-cyclodextrin

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Keywords: Affinity Capillary Electrophoresis, binding constant, chiral analysis, cyclic dinucleotides, cyclodextrins

Objective

The objective of this work was to propose a novel affinity capillary electrophoresis (ACE) method for the separation of potential anticancer drug, 2′,3′-cyclic diadenosine diphosphorothioate (Rp, Rp) (ADU-S100), and three recently synthesized diastereomers of its difluorinated derivative, 3′,3′-cyclic di(2′-fluoro,2′-deoxyadenosine phosphorothioate [1] employing various cyclodextrins (CDs) as chiral selectors. In addition, ACE should be used for the estimation of binding constants of these dinucleotides (CDNs) with chiral selector that enables their best separation.

Methods

ACE separations were carried out in internally uncoated fused silica capillaries. The analytes were dissolved in deionized water and detected by a UV–Vis absorption spectrophotometric photodiode array detector set at 200 nm. The electrophoretic mobilities were measured at 25°C. The experimental conditions for the separation of CDNs were optimized based on their acid-base and electromigration properties.

Results

Using various CDs, relatively good separations were obtained with background electrolytes (BGEs) containing β-CD, γ-CD or HP-γ-CD, nevertheless, only with HP-β-CD baseline separation was achieved. The anticancer drug, 2′,3′-cyclic diadenosine diphosphorothioate (Rp, Rp) (ADU-S100), and three diastereomers of its difluorinated derivative, 3′,3′-cyclic di(2′-fluoro, 2′-deoxyadenosine phosphorothioate), were baseline separated within 4 min in the BGE composed of 40 mM Tris, 40 mM tricine, pH 8.1 containing 43.5 mM HP-β-CD.
Besides, the average apparent binding constants of the CDNs-HP-β-CD complexes were estimated by ACE. The average apparent binding constants of the analyte-HP-β-CD complexes were obtained from the dependence of effective electrophoretic mobility of the analytes (corrected for viscosity change caused by the addition of CD to the BGE) on the concentration of chiral selector in the BGE using non-linear regression analysis [2]. These complexes were found to be relatively weak with the binding constants in the range of 12.2-94.1 L/mol.

Conclusions

ACE using HP-β-CD as chiral selector allowed fast and highly efficient separation of structurally similar CDNs. The developed method can be applied for the separation, analysis and characterization of the currently investigated CDNs as well as for other, similar CDNs. It is advantageous that it requires only very small amounts of CDNs.

ACKNOWLEDGMENTS

This work was supported by the Czech Academy of Sciences, project no. RVO 61388963.

REFERENCES

[1] Polidarová M. P., Břehová P., Kaiser M. M., Smola M. et al, (2021). Synthesis and Biological Evaluation of Phosphoester and Phosphorothioate Prodrugs of STING Agonist 3′,3′-c-Di(2′F,2′dAMP), *Med Chem*., 64, 7596
[2] Štěpánová S., P. Břehová, V. Kašička, (2024). The separation of cyclic diadenosine diphosphorothioate and the diastereomers of its difluorinated derivative and the estimation of the binding constants and ionic mobilities of their complexes with 2-hydroxypropyl-β-cyclodextrin by affinity capillary electrophoresis, *Electrophoresis*, 45, 1000.

Comparative HPTLC study of fermented and non-fermented smoke tree extract

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Keywords: HTPLC, plant fermentation, bioautography, biologically active compounds

Objective
Our study aimed to apply High-Performance Thin-Layer Chromatography (HPTLC) coupled with various detection methods as a powerful technique for screening of natural products and bioactive compounds in non-fermented (NF) and fermented (F) (*Lactobacillus*) ethyl acetate smoke-tree (ST) extracts. Comparative analysis of the phenolic, flavonoid, and terpenoid fingerprints of ST extracts rationalized the outcomes of bioautographic experiments that determined the antidiabetic, anti-inflammatory, and antioxidant activities of F and NF extracts. To the best of our knowledge, bioautography on ST was conducted for the first time. Through densitometric analysis of chromatograms, the quantitative determination of bioactive compound content was conducted on these two extracts. Additionally, for the first time, ST was fermented and subsequent extract was characterized.

Methods
HPTLC analysis - Chromatographic conditions employed a normal phase mode. The mobile phase used for optimal separation of all compound classes was hexane: ethyl acetate: acetic acid = 15:9:1 (*v*/*v*/*v*). Following chromatographic separation, plates were derivatized using 0.5% NP reagent (*w*/*v*) and 5% PEG (*w*/*v*), as well as $FeCl_3$ and $AlCl_3$ solutions for phenolic profiling, p-anisaldehyde reagent for terpenoid profiling, 0.1% methanolic solution of DPPH radical (*w*/*v*), and solutions of α-amylase and COX-1 for the respective bioautograms. Chromatograms were scanned and saved in TIFF format for image processing using VideoScan software (version 1.02).

Results
Fermentation reduced specific zones in phenolic, flavonoid, and terpenoid profiles, introduced new high-intensity zones, and intensified certain zones compared to the NF sample. This indicates the isolation of new phenolic and terpenoid compounds and increased concentration of existing ones through ST fermentation. Antioxidant capacity nearly doubled (NF-3834 mg GAE/g; F-7109 mg GAE/g), while inhibition of α-amylase (NF-13027 mg acarbose/g; F-6971 mg acarbose/g) and COX-1 (NF-15865 mg SAE/g; F-11539 mg SAE/g) decreased. The increase in phenols, flavonoids, and terpenes suggests that other compound classes may also significantly contribute to inhibiting these enzymes.

Conclusions
Fermentation of smoke tree with *Lactobacillus* increases phenols, flavonoids, and terpenes, suggesting that other compound classes significantly contribute to inhibiting α-amylase and COX-1 enzymes, while greatly enhancing antioxidant capacity. The fermented extract is a green functional extract with potent biological activity.

ACKNOWLEDGMENTS
This research was supported by the Ministry of Science, Tehnological Development and Innovation of Republic of Serbia, contract numbers: 451-03-65/2024-03/200169, 451-03-66/2024-03/200168 and 451-03-66/2024-03/200288.

Application of matrix-solid phase dispersion and molecular imprinted polymer as extraction techniques for environmental samples polluted by pharmaceuticals

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Keywords: sample preparation, Matrix-solid Phase Dispersion, Molecular Imprinted Polymer, pharmaceuticals, Liquid Chromatography

Objective
In accordance with the stated issue, this research is dedicated to the development of the MSPD-HPLC-DAD method for the simultaneous monitoring of 12 pharmaceuticals with different therapeutic effects and physicochemical properties, from sediment collected from the territory of the Republic of Croatia. Various extraction parameters were optimized in order to achieve the best possible recoveries for the extraction of the pharmaceutical mixture, and then the reliability of the method's purpose was examined by determining the validation parameters. The final experiment included the application of the MSPD method on another type of sorbent, specifically a molecularly imprinted polymer (MIP) with an imprinted molecule of sulfamethoxazole, which in previous experiments proved to be a molecule resistant to water removal (lower recoveries by SPE) in comparison with other tested target compounds.

Methods
Sediment samples for MSPD were spiked with pharmaceutical mixture solution and after solvent evaporation catridges were filled with frits and spiked sediment, and ready for elution of analytes with various combinations of solvents. Moleculary imprinted polymer (MIP) of sulfamethoxazole was prepared by polymerization by mixing sulfametoxazole as template molecule dissolved in acetonitrile, methacrylic acid as functional monomer, ethylene glycol dimethacrylate as cross-linker, and azobisisobutyronitrile as initiator (60 °C, 24 h). Before extraction experiments, MIP was washed with methanol and acetic acid, and characterized by FTIR and SEM analysis. All data were obtained by analysis on HPLC-DAD chromatograph, using the gradient method for simultaneous analysis of 12 pharmaceuticals.

Results
By investigating the potential of pure organic solvents with different polarities and solvents in the mixture with some inorganic compounds (acids, bases) and different volumes of elution (in one or two steps), the best MSPD extraction recoveries were with 5 mL of a mixture consisting of 0.1M NaOH and methanol in a ratio 2:8 (*v*/*v*). The suitability of the optimized method was determined by linearity confirmed in the range of 0.0125 – 20 µg/mg with R^2 > 0.99 with LOD and LOQ values below 0.25 and 0.625 µg/mg respectively, and repeatability and reproducibility with RSD values < 16 %. MSPD-HPLC-DAD method was successfully applied on sorbent made of imprinted molecule characterized with better recoveries for pharmaceutical removal.

Conclusions
It is presented developed and validated MSPD-HPLC-DAD method for simultaneous determination of 12 pharmaceuticals in sediment samples which, due to the revealed matrix influence, can be applied in routine analyses. Application of united MIP-MSPD-HPLC-DAD method confirmed the expected increase in the sensitivity of the method.

ACKNOWLEDGMENTS
This study was supported by the Croatian Science Foundation under the project number HRZZ-IP-2022-10-4400 entitled Development of molecularly imprinted polymers for use in analysis of pharmaceuticals and during advanced water treatment processes (MIPdePharma).

Effect of ternary mobile phase composition on the retention of polar substances in HILIC

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Keywords: HILIC, ternary mobile phase, retention, separation

Objective
Recently, the interest in Hydrophilic Interaction Liquid Chromatography (HILIC) applications has been growing significantly. It is caused by the increased need for the separation of highly polar and ionized substances, which are difficult to achieve in other modes of liquid chromatography. The aim of this work was to evaluate the influence of ternary mobile phases on retention and selectivity and to optimize the method for the determination of selected polar substances. The combination of acetonitrile, methanol, and aqueous buffer can influence the character of the diffuse aqueous layer on the surface of the HILIC stationary phases and affect the separation quality.

Methods
A liquid chromatograph with UV detection (Agilent Technologies, USA) was used and five commercial columns were tested: Ascentis® Express HILIC (3 x 150 mm, 2.7 µm, Supelco, USA); InfinityLab Poroshell 120 HILIC-Z (2.1 x 150 mm, 2.7 µm, Agilent Technologies, USA); Luna® Omega SUGAR (3 x 150 mm, 3 µm, Phenomenex, USA); SeQuant® ZIC-HILIC (2.1 x 150 mm, 3.5 µm, Supelco, USA); YMC-Triart Diol HILIC (2.0 x 150 mm, 5 µm, YMC, Japan). The column temperature was set at 25 °C, and a constant linear flow rate of 5.67 cm/min was preserved. In gradient chromatography, water, 98% acetonitrile, and 98% methanol were used as mobile phases A, B, and C with ionic additive 5 mM ammonium acetate. Nucleotide bases, nucleosides, and sulfonamides with a concentration of 10 µl/ml, which belong to the group of polar substances suitable for separation, were chosen as model analytes. Sample volumes of 1 µl were injected in all experiments. The detection wavelength was selected according to the type of substance being measured (254 and 284 nm).

Results
The retention behavior of selected substances was determined using gradient elution with a mobile phase containing acetonitrile in several gradients with different steepness. The columns used were compared in terms of retention and selectivity of substances. The ZIC-HILIC column was selected as the most appropriate, with the best separation of standards (highest resolution) and the highest efficiency achieved among the gradients used. The same series of experiments were also performed for methanol to compare the influence of the organic solvent. For the mobile phase containing methanol, the retention of substances is significantly lower than in the case of a mobile phase containing acetonitrile due to the differences in solvation strength of both solvents. Subsequent analysis of the standards using ternary mobile phases showed that adding methanol to the commonly used acetonitrile/water mobile phase combination affected selectivity and separation. The gradient profiles were chosen to start with 100% acetonitrile and differ in the final acetonitrile/methanol concentration ratio. Using the acetonitrile/methanol concentration ratio during the gradient, the separation of polar substances can be influenced. These findings led to the design of an experiment and the development of a method for detecting selected polar substance representatives.

Conclusions
Analysis of experiments on different columns confirmed that the ZIC-HILIC column is the most suitable for the separation of polar compounds due to its ability to retain these substances and thus provide higher separation efficiency. Subsequent testing of the gradient with ternary mobile phases showed the possibility of using a combination of acetonitrile and methanol in the gradient for the targeted setting of separation selectivity when working with polar compounds.

ACKNOWLEDGMENTS

The work was financially supported by the Czech Science Foundation, project No. 22-09556S.

Astaxanthin extraction from *Paracoccus carotinifaciens* biomass by an ultrasound assisted deep eutectic solvent-based approach

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Keywords: astaxanthin, bacterium, *Paracoccus*, Deep Eutectic Solvent, Ultrasonic Extraction, process intensification

Objective
Astaxanthin (AXT) natural production is gaining more and more attraction due to its superior biological activity compared to its synthetic counterpart. Natural AXT can be obtained from microbial sources, such as microalgae, yeast and bacteria. The recovery of AXT from microalgae and yeast is a challenging process primarily due to the presence of thick cell walls, which make the extraction process difficult and less efficient. As a result, bacterial sources are being explored for their advantages in terms of finer cell wall structure and easier extraction processes that reduces the cost of overall processes to obtain pure AXT extract. However, the current extraction of AXT from bacterial sources heavily depends on traditional solvent extraction methods, giving rise to environmental and safety issues. The aim of this work was to develop an integrated ultrasound-assisted deep eutectic solvent-based process as promising alternatives for the recovery of AXT from *Paracoccus carotinifaciens*, an aerobic Gram-negative bacterium.

Methods
Deep eutectic solvents (DES) composed of choline chloride (HBA) and carboxylic acids/alcohols (HBD) were used as alternative solvents, and the effects of processing intensification parameters on the extraction yield were studied. The investigated parameters included extraction temperature, solid/liquid ratio, amplitude level, ultrasound (US) intensity, DES molar ratio, and extraction time. The extracts have been characterized by HPLC-PDA/MS.

Results
The efficient extraction period for achieving maximum yield was approximately 8 min, extraction temperature 65 ◦C, solid/liquid ratio 0.05 g/mL, amplitude level: 15 %. Sequential five re-extraction cycles were applied, and it was observed that in two cycles, more than 95 % of total extract was recovered. The experimental results showed that the choline chloride:acetic acid (CC-C2) DES combined with US enhanced mass transfer, leading to a remarkable recovery yield increase of up to 900 % compared to the conventional procedure. HPLC analysis showed that around 80% of the extract was represented by AXT isomers, followed by canthaxanthin and adonirubin.

Conclusion
The effect of US on the recovery is largely dependent on the extraction time, temperature, US intensity, HBA:HBD molar ratio and the DES/biomass ratio. Therefore, it is suggested to continue studying the integration of these processes with purification and polishing units to improve the obtaining of pure fractions, considering the need of final application. Moreover, the results reached in this study have been compared with the calculations of the activity coefficient at infinite dilution and the sigma profile delivered by COSMOSAC, concluding that this tool can be used to predict the behavior of DES.

Investigation of sensitizing molecules in hand hygiene gel products by hyphenated chromatography techniques

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Keywords: *Citrus* essential oils, cosmetics, Covid-19, furocoumarins, hand sanitizers, photosensitizers

Objective
The aim of the present research was the optimization of a method for the extraction of thirty-seven oxygen heterocyclic compounds (OHC) compounds in hand gel sanitiziers by using few milliliters of solvent. For the detection of coumarins (Cs), furocoumarins (FCs), and polymethoxyflavones (PMFs), which belong to these molecules class, a rapid liquid chromatography method was developed.

Methods
Separation was achieved by using a UHPLC-MS/MS instrument. Triple quadrupole MS was used in multiple-reaction-monitoring (MRM) acquisition mode to monitor transitions between the precursor and the product ion, eliminating isomer interferences.

Results
An extraction method was optimized for 37 OHC compounds, obtaining absolute mean recovery values in the 73.5–116% range with only few milliliters of solvent consumption. Limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy and precision were determined according to the Eurachem guidelines. Repeatability was evaluated intra-day (n = 5) and inter-day (3 days, n = 15) as the coefficient of variation (CV%) of the peak areas, calculated at 0.005 mg L^{-1}, 0.05 mg L^{-1}, and 1 mg L^{-1}. Apparent extraction recovery (R%) was calculated by adding known amounts (0.005 mg L^{-1}, 0.05 mg L^{-1}, and 1 mg L^{-1}) of a multi-analyte solution to the blank sample prior to extraction, over ten measurements. Matrix effects were evaluated by spiking known amounts (0.05 mg L^{-1} and 0.5 mg L^{-1}) of the 37-analyte standard mixture to the blank sample after extraction.

Conclusions
Although Cs and FCs have been investigated for their anti-inflammatory and antioxidant properties, investigation into the safety of some coumarins used as fragrances or fixatives in cosmetics has identified potential safety concerns of carcinogenicity, hepatotoxicity and skin sensitization. The results of UHPLC-MS/MS analysis performed on commercial hand gel products implies that a regular monitoring of the quality of this product is urgent, considering the current wide use of these products. Indeed, in 2 of the 13 samples investigated of this study, the FC amount exceeded the safe limits recommended, up to a factor of 15. Moreover, 3 hand gel samples did not conform to the labeling requirements, since no “perfume” or “aroma” was declared among the product ingredients, despite the presence of coumarin.

ACKNOWLEDGMENTS
The authors thank Shimadzu Corporation and Merck Life Science for their continuous support.

High performance liquid chromatography coupled to zone fluidics: a new concept to introduce different reagents in post-column derivatization

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Keywords: HPLC, zone fluidics, post-column derivatization, versatility, aminothiols

Objective

In the present study, an alternative approach was developed to introduce different reagents in HPLC coupled to on-line Post Column Derivatization (PCD). In HPLC-PCD, a suitable reagent is selected for the on-line reaction with the eluted analytes, forming the desirable derivatives. The main limitation of HPLC-PCD is that only one chemical system can be used, with compromise of the reaction conditions for all analytes. In the proposed approach, Zone Fluidics (ZF) configuration was used for the injection of different reagents in the eluent, depending on the chemical system and the optimum conditions of each analyte. The reagents are injected into the flowing stream as well-defined volumes at microliter levels, overlapping the eluted analytes.

Methods

The coupling of ZF with the outlet of the chromatographic column was applied as an alternative to the post-column derivatization approach. For this reason, four aminothiols (homocysteine, *N*-acetyl-cysteine, reduced glutathione, and glutathione disulfide) were used and separated using reversed phase chromatography. The development of the new system was based on the derivatization of the aminothiols with *o*-phthalaldehyde (OPA) reagent, with different chemical parameters for each analyte; (i) *N*-acetyl-cysteine reacts with OPA with a primary amine under the classic mechanism, (ii) reduced glutathione reacts with OPA in an alkaline medium under the selective mechanism and (iii) homocysteine and glutathione disulphide react with OPA in a high alkaline medium without the need of the nucleophilic compound. Different reagents can be introduced in the eluent using the ZF configuration through the 10-port valve.

Results

The development of the new HPLC-ZF-PCD system included: (1) the investigation of the configuration for the coupling of ZF with the eluent of the column, (2) the investigation of the tuning of the reagent zone with the analytes, (3) the investigation of repeatability of the developed system and (4) the development and validation of a new method using the proposed concept. The coupling of the outlet of the column with the port of ZF was conducted through a T-connector and a zero dead-volume coupler. The tuning of the reagent zone with each analyte was depended on the retention times and the repeatability was satisfactory (RSD< 5%) using 75 μL of reagent zone. The chemical parameters of each analyte were investigated, and the method was validated for the determination of amintothiols in biological samples and food samples, with simple sample preparation.

Conclusions

HPLC-ZF-PCD was proved to be an alternative "green" approach to post-column derivatization. The development of the new concept HPLC-ZF-PCD allowed the determination of analytes with different reagents, without the need for compromise that used in classic HPLC-PCD. Also, the consumption of the reagents dramatically reduced to 300 μL per run instead of 7500 μL that is required with HPLC-PCD approach.

ACKNOWLEDGMENTS

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd call for HFRI PhD Fellowships (Fellowship Number: 5341).

Microdialysis of microliter volumes of body fluids performed on hollow fiber for monitoring of amino acids by capillary electrophoresis

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Keywords: Capillary Electrophoresis, clinical samples, contactless conductivity detection, microdialysis, microfluids.

Objective

Analysis of body fluids is a continuous challenge for modern analytical chemistry and clinical samples are among the most frequently analysed. Body fluids such as whole blood, serum, plasma, urine, and less frequently saliva, tears, sweat, dry blood spots, and others, represent highly complex matrices containing dozens of macro- and hundreds of micro-components dissolved in aqueous solution with high content of inorganic salts and proteins. In this respect, microdialysis (MD) represents a suitable solution to minimize the consumption of body fluid and the use of harmful chemicals. MD was developed for on-line monitoring of biochemical processes occurring in living tissues and organs as a semi-invasive sampling technique originally designed for neuroscience purposes, but is currently used for sampling metabolites and drugs in most organs and tissues of the human body [1].

Methods

The fabrication of a coaxial MD probe for steady state treatment of blood, blood plasma, dry blood samples and tears is demonstrated. MD probe for discontinuous treatment of as little as 10 µL of clinical sample is proposed, which has close to 100% recovery for amino acids. The MD is made into as little as 2 µL of perfusate solution, which can be reproducibly transferred to a CE vial and subsequently subjected to analysis with contactless conductivity detection for determination of amino acids without the need for further sample preparation. MD is performed through polysulfone hollow fibre directly designed for haemodialysis, which are newly tested for the treatment of blood, plasma, tears, and dry blood samples.

Results

Miniaturization of the MD probe dimensions and performing MD at discontinuous perfusate inflow will achieve 98.3 – 102.5% recovery of MD for amino acids. The electrophoretic separation of amino acids is performed in 8.5 M acetic acid at pH 1.37 as a background electrolyte with analysis time up to 4.5 min and LOD in the range of 0.12 – 0.28 µM.

Conclusions

The methodology is applicable to the determination of the entire profile of amino acids in whole blood, plasma, or tears and has potential for the analysis of dry blood samples.

ACKNOWLEDGMENTS

The financial support from the Czech Science Foundation (22-22398S) is gratefully acknowledged.

REFERENCES

[1] Tůma P., (2023), Progress in on-line, at-line, and in-line coupling of sample treatment with capillary and microchip electrophoresis over the past 10 years: A review, *Analytica Chimica Acta*, 1261, 341249.
[2] Tůma P., (2024), Steady state microdialysis of microliter volumes of body fluids for monitoring of amino acids by capillary electrophoresis with contactless conductivity detection, *Analytica Chimica Acta*, 1287, 342113.

Cross-validation of a solid-phase extraction and a dispersive liquid-liquid microextraction method for therapeutic drug monitoring of breast cancer drugs

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Keywords: bioanalysis, method cross-validation, breast cancer, therapeutic drug monitoring, sample preparation

Objective
We have recently developed two sample preparation methods for the determination of palbociclib, ribociclib, abemaciclib, anastrozole, letrozole, and fulvestrant in plasma samples. The first is an economically and ecologically feasible but labour-intensive dispersive liquid-liquid microextraction (DLLME) [1]. The second is a more automated but costly solid-phase extraction (SPE) [2]. During validation, both showed high extraction recoveries, low matrix effects, remarkable linearity, precision and accuracy. The aim of this work was to assess the agreement between these methods in the analysis of real breast cancer patient samples.

Methods
A total of 38 patient plasma samples were collected, prepared using DLLME and SPE, and analysed with LC-MS, yielding 76 data points per method. These results were statistically compared, with 95% confidence intervals (CI).

Results
Bland-Altman method comparison (% difference between the obtained concentrations vs. their average values) showed agreement within the 15% proposed by the ICH (bias −1.020%, standard deviation 7.265). Deming regression (y (SPE) = 1.022 x (DLLME) − 0.2174) and Spearman's coefficient (R = 0.9963) indicated strong correlation between the results. Wilcoxon test showed that the differences between the results are not statistically significant (median of differences −0.100, CI −1.2 – 2.9, p = 0.0523). The folded empirical cumulative distribution plot revealed that most of the results are centred around the median difference of only −0.02% (CI −15.29 – 13.22).

Conclusions
Both approaches show exceptional agreement and are therefore equally suitable for their designated purpose. The choice of the more favourable protocol depends solely on the available laboratory equipment and workload.

ACKNOWLEDGMENTS

This work has been fully supported by the Croatian Science Foundation (UIP-2019-04-8461, DOK-2021-02-4595), and the European Regional Development Fund (Farminova, KK.01.1.1.02.0021).

REFERENCES

[1] L. Turković, N. Koraj, Z. Mlinarić, T. Silovski, S. Crnković, M. Sertić (2023). Optimisation of dispersive liquid-liquid microextraction for plasma sample preparation in bioanalysis of CDK4/6 inhibitors in therapeutic combinations for breast cancer treatment, *Heliyon*, 9, e18880
[2] L. Turković, D. Mutavdžić Pavlović, Z. Mlinarić, A. Skenderović, T. Silovski, M. Sertić (2023). Optimisation of solid-phase extraction and LC-MS/MS analysis of six breast cancer drugs in patient plasma samples, *Pharmaceuticals*, 16, 1445.

Determination of vitamin D level in biological fluids by using gas chromatography technique coupled to triple quadrupole mass spectrometry

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Keywords: vitamin D, Gas Chromatography, tandem Mass spectrometry, soft ionization, NIST SRM 972a

Objective
The present research aimed to develop and optimize a gas chromatography method coupled to triple quadrupole mass spectrometry with a method under milder electron ionization conditions for the assay of vitamin D metabolites in human serum.

Methods
In order to select the most efficient silylating agent for the conversion of vitamin D species into trimethylsilyl (TMS) derivatives several silylating agents were used. In addition, a fast and efficient GC method has been optimized to achieve rapid separation of target compounds.

Results
As for the optimization of the derivatization procedure, after testing several agents, the most efficient was the MSTFA with 10% TMCS. For the acquisition of vitamin D metabolites, soft ionization (20eV) was used with the aim of increasing the intensity of precursor ions useful for the MRM acquisition mode. Particular attention has also been paid to the optimization of MRM transitions in order to develop a highly sensitive and selective acquisition method. Finally, the accuracy of the developed method was evaluated by the analysis of a standard sample certified by NIST.

Conclusions
The analytical method described can be considered as a valid alternative to standard HPLC-MS/MS procedures, commonly reported in literature for the assay of vitamin D metabolites. The developed strategy included the use of MSTFA with TMCS for the conversion of vitamin D species into TMS derivatives, including 25-(OH) metabolites with specific steric hindrance problems. The ionization of the silylated compounds was performed under milder EI conditions (20-eV energy). The signal acquisition was performed in MRM mode. The developed fast GC-QqQ-MS method under milder EI conditions proved to be highly sensitive and selective for targeted analysis of vitamin D metabolites in human serum.

ACKNOWLEDGMENTS

The Authors thank Merck Life Science and Shimadzu Corporations for their continuous support.

REFERENCES

[1] G. Micalizzi, F. Vento, C. Buzzanca, T.M.G. Salerno, L. Mondello, (2023). *Journal of Chromatography B*, 1227,123813.

Ellagitannin profile in dried walnut shells using liquid chromatography-tandem mass spectrometry

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Keywords: ellagitannins, walnut shells, RPLC-ESI-MS, tandem MS, wastes.

Objective
Walnut shells, often discarded as waste, possess hidden potential as a source of ellagitannins (ETs) [1], compounds with notable antioxidant properties and health benefits [2]. This study aimed to investigate the ET profile in ethanolic extracts of dried, powdered walnut shells, obtained through mill grinding.

Methods
Reversed-phase liquid chromatography (RPLC) coupled with mass spectrometry (MS) using electrospray ionization (ESI) in negative polarity was utilized to analyse the ET profile. Several compounds from ET families were successfully identified as deprotonated molecules ($[M-H]^-$) and characterized, including mono-, di-, tri-, tetra-, and penta-galloyl glucopyranoses. Specific product ions with distinctive relative signal intensity were identified by tandem MS and used to recognize the ET landscape.

Results
The results generally corresponded with theoretical predictions for isomers of mono- and tri-galloyl forms. However, di- and tetra-galloyl species presented a more intricate scenario; di-galloyl-glucopyranoses potentially contained additional structures, while limitations in MS/MS data hindered the conclusive identification of all tetra-galloyl isomers. Characterization of ETs featuring the hexahydroxydiphenoyl (HHDP) group [3] also remained challenging. Despite these constraints, the estimated total content of ETs was more than 400 mg in 10 g of dried powdered walnut shell extracts.

Conclusions
The study indicates potential applications for walnut shells in the food, pharmaceutical, and cosmetic industries by emphasizing the significant ET content. By optimizing the extraction process, effective recovery of ETs from dried walnut waste can be achieved, transforming walnut shells from waste products into a sustainable source of health-promoting compounds and contributing to a greener economy.

ACKNOWLEDGMENTS

This work was supported by the project PON Ricerca e Innovazione 2014-2020- 02-G-14853-2, financed by the MIUR.

REFERENCES

[1] Sánchez-González C. et al., (2017) Health Benefits of Walnut Polyphenols: An Exploration beyond Their Lipid Profile. *Crit. Rev. Food Sci. Nutr,* 57, 3373–3383.
[2] Regueiro J. et al., (2014). Comprehensive Identification of Walnut Polyphenols by Liquid Chromatography Coupled to Linear Ion Trap-Orbitrap Mass Spectrometry, *Food Chem.,*152, 340–348.
[3] Ventura G. et al., (2023) The Effect of Milling on the Ethanolic Extract Composition of Dried Walnut (Juglans Regia L.) Shells, *Int. J. Mol. Sci.* 24, 17.

Separation of chromium species in biological fluids by µLC-ICP-MS

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Keywords: speciation, chromium, method development

Objective
Chromium (Cr) is a transitional element with oxidation states spanning from -2 to +6. The prevalent stable forms of chromium found in the environment are trivalent Cr(III) and hexavalent Cr(VI) compounds. Cr(III) serves as a vital micronutrient for human health, whereas Cr(VI) is notorious for its high toxicity and carcinogenic properties. Separating these two chromium species is crucial due to their disparate toxicities, but separating both species poses a significant challenge as they exhibit instability under varying conditions of temperature and pH. Cr(III) is stable in acidic conditions and Cr(VI) in alkaline conditions. Therefore, interconversions between Cr species may occur in complex matrices like biological fluids and during analysis, hence making their accurate determination very difficult. Moreover, both Cr species are frequently found in low concentrations. Therefore, an improved sensitive and robust method for the simultaneous determination of Cr(III) and Cr(VI) in biological fluids, such as exhaled breath condensate, urine, waste water and nutrition solution, has been developed.

Methods
The method uses a hyphenated micro liquid chromatography (µLC) system coupled to inductively coupled plasma mass spectrometry (ICP-MS). The optimised method incorporates a pH adjusted EDTA complexation step to stabilise Cr(VI) and Cr(III). The µLC system uses an anion exchange micro-sized column to separate the Cr species.

Results
Cr(III) and Cr(VI) were separated with different retention times at 170 and 230 sec, respectively. The method was optimized and validated by spiking Cr(III) and Cr(VI) in various samples of biological fluids. Furthermore, the method was validated using a drinking water proficiency testing material sample.

Conclusions
The developed method can be used for rapid routine determination of chromium species with high precision and reliability.

Determination of selected pharmaceuticals and their metabolites in wastewater and treated water samples

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Keywords: LC-MS/MS, pharmaceuticals, water samples

Objective
Common water treatment procedures in treatment plants are not originally designed to remove pharmaceutical contaminants and often fail to deal with issues such as low efficiency of water purification. Many pharmaceuticals are only partially degraded in the cleaning process and reach concentrations of up to µg/L after being released into surface waters. Therefore, new treatment methods and efficient, sensitive, and reliable analytical methods are needed for determination of pharmaceuticals and their metabolites in wastewater samples. This research focuses on the development of liquid chromatography - tandem mass spectrometry (LC-MS/MS) method for the determination of carbamazepine, sulfamethoxazole and its metabolites in water samples, and monitoring water treatment processes.

Methods
Water treatment was performed in a flow electrochemical device based on the principles of electrocoagulation and electro-flotation provided by the company HOFITECH, s.r.o. Chromatographic analyses were performed on Agilent 1260 Infinity II liquid chromatograph, with an Agilent Technologies 6460 Triple Quad LC/MS tandem mass spectrometer, using Eclipse Plus C18 RRHD column (3.0 x 50 mm, 1.8 µm). The LC-MS/MS method was further used to monitor the efficiency of water treatment by using different types of electrodes (steel, aluminium and carbon), different working currents, electrocoagulation times and electrolyte composition and also by absorption on graphitized carbon (ENVI-Carb).

Results
The first part of the research focused on the development, optimization, and validation of the LC-MS/MS method (selection of precursor and product ions, fragmentor voltage, and collision energy) for the determination of selected pharmaceuticals and metabolites in water samples. The linearity was expressed by R^2 and was in the range of 0.9968 to 1. The limits of detection (LODs) ranged from 1.24 to 1.61 ng/L and the limits of quantification (LOQs) ranged from 4.15 to 5.38 ng/L. In the second part, the effect of the electrode material (C, Fe, Al), the electrolyte composition and the applied working current on the treatment process were monitored. The best results for carbamazepine elimination were obtained by using carbon electrode for treatment. Also, by applying the absorption on carbon, 92.6% of carbamazepine was eliminated from water samples. Sulfamethoxazole was successfully degraded by the electrocoagulation process using all types of electrode materials. The determined concentration of sulfamethoxazole after electrocoagulation on the aluminium electrode was at the level of 0.65 µg/L, on the steel electrode 0.63 µg/L and on the carbon electrode below LOD.

Conclusions
This work dealt with the development and optimization of the LC-MS/MS method for the determination of carbamazepine, sulfamethoxazole and their degradation products in wastewater and treated water samples. The best water purification was achieved after 10 minutes of electrocoagulation with the application of a working current of 10 A for sulfamethoxazole, and 7.5 A for carbamazepine using a carbon electrode and the addition of 5 g/L of salt.

ACKNOWLEDGMENTS

This work was supported by the STU Young Researchers Support Program and the Excellent Teams of Young Researchers Support Program. This work was supported by the Research and Development Support Agency based on Contract no. APVV-19-0149.

LC-MS investigation of the electrochemical degradation of environmentally relevant pharmaceuticals and their degradation products in water

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Keywords: pharmaceuticals, degradation and transformation products, HRMS, water treatment technique

Objective

The presence of organic micropollutants such as pharmaceuticals, in environment is an actual global problem. Their presence in the environment is often associated with the risk of adverse effects on living organisms and therefore new treatment technologies are intensively studied. The aim of the present work was to study the presence of different pharmaceuticals and their metabolites in wastewater based on LC-MS/MS analysis and use different types of advanced oxidation processes (AOPs) for their removal. Additionally, the aim was to identify degradation and transformation products formed during the degradation process by LC-HRMS.

Methods

The water samples before/after treatment were analyzed by LC-MS/MS using the triple quadrupole analyzer TSQ Quantiva with heated electrospray in both ionization mode. Identification of degradation products was performed by LC-MS-IT-TOF and QExactive. Compound Discoverer 3.3 with workflows containing retention time alignment, peak detection, accurate m/z (<5 ppm) elemental composition calculation, halogens isotope pattern detection and statistical analysis were used for data processing and evaluation.

Results

In this study, the effectiveness of the degradation process based on e.g. different type of boron doped diamond electrodes and photocatalysis was evaluated using a targeted LC-MS/MS analysis. The obtained results showed a high elimination rate for all studied compounds in relatively short time. Identification of degradation products of selected pharmaceuticals was performed by suspect and non-target HPLC-HRMS analysis. Both approaches allow us identification of the few degradation products for all tested pharmaceuticals in model and wastewater. The identity of the degradation product was confirmed based on accurate mass, isotope pattern, retention time, and MS/MS data.

Conclusions

This study investigated different type of AOPs for removal of persistent environmentally relevant pharmaceuticals belonging to different therapeutic classes. Non-target LC-HRMS offers valuable insights into the fate of pollutants during advanced treatment processes, facilitating the development of more robust pollution control strategies.

ACKNOWLEDGMENTS

The work thanks for the financial support of the Research and Development Support Agency (APVV-23-0367 and APVV-19-0250). This work was supported by the Czech Science Foundation (GACR 20-04676X).

LC-MS/MS chemoproteomic preliminary study to characterize effect of hydroxynaphthalene-carboxanilides against *Staphylococci*

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Keywords: ABPP, hydroxynaphthalene-carboxanilides, chemoproteomics, HPLC-MS

Objective
The aim of the work is to try to clarify the mechanism of the antistaphylococcal effect of two selected compounds from the group of ring-substituted 1-hydroxynaphthalene-2-carboxanilides against the reference strain *Staphylococcus aureus* and the clinical isolate methicillin-resistant *S. aureus* (MRSA).

Methods
In this study, the method of activity-based protein profiling (ABPP), namely its comparative technique, which allows the identification of protein targets in the proteome of cell lysates, was used. For this purpose, two compounds (probes) with the potential to affect the staphylococcal proteome were used, namely *N*-[2,4-bis(trifluoromethyl)phenyl]-1-hydroxynaphthalene-2-carboxamide (**1**) and *N*-[3,5-bis(trifluoromethyl)phenyl]-1- hydroxynaphthalene-2-carboxamide (**2**). The experiment was performed on the universally sensitive collection strain *S. aureus* ATCC 29213 and the resistant clinical MRSA isolate SA 630. The MS detection itself is always preceded by tryptic cleavage of the proteins. The LC-MS/MS analysis was performed on the Orbitrap instrument (Q Exactive, Thermo Scientific) in a data-dependent mode. Label-free quantification was used to compare the protein expression level between samples. Protein identification and statistical analysis were performed using Proteome Discoverer software with *Staphylococcus aureus* Uniprot database.

Results
Chemoproteomics is a tool for investigating protein targets of potential drugs and has an important place in preclinical drug research. It makes it possible to understand the effect of a bioactive molecule on a living system. The protein profiles of control samples (*S. aureus* and MRSA cells) and those of *S. aureus* and MRSA treated with inactive compound **1** and highly active agent **2** were investigated and compared to each other. More than 1500 proteins were analyzed and found that after exposure to inactive isomer **1** there were huge changes in the proteome, approx. five hundred proteins experienced more significant changes in expression, but ultimately the bacteria survived. On the other hand, there were relatively few changes in the proteome after exposure to active agent **1**, but neither the susceptible *S. aureus* nor the MRSA isolate survived.

Conclusions
LC-MS/MS is often used in many chemoproteomic strategies, such as ABPP, which allows the monitoring of protein expression changes in the proteome of a bacterial cell after exposure to a bioactive compound and helps to estimate the possible mechanism of action. In this work, agents with anti-infective activity were characterized and the issue of chemoproteomics in relation to the mechanism of action of bioactive agents was approached. In addition, this preliminary study found that there were significant quantitative changes in ca. twenty-five proteins that have the character of virulence factors or toxins with cytolytic activity.

ACKNOWLEDGMENTS
This work was financially supported by grant GUK/1143/2024.

Application of SPME arrow technique for analysis of botrytized wines

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Keywords: SPME arrow, Gas Chromatography, botrytized wines, direct immersion

Objective

SPME Arrow has been recently developed as a modified configuration of a Solid-Phase Microextraction (SPME) technique. It is characterised with a larger amount of sorption phase (0.6 µL vs. 3.8 µL for 100 µm PDMS film), as well as an improved mechanical robustness [1]. İn the case of the compounds with log K_{ow}>5 an extraction efficiency of SPME Arrow is comparable with stir-bar sorptive extraction (SBSE) [2]. The purpose of our study was to test this technique for wine analysis in direct immersion extraction mode.

Methods

The samples represented botrytized wines such as Tokaj wines (Hungary and Slovakia), Sauternes (France) and Ruster Ausbruch (Austia). A 250 µm PDMS SPME Arrow fiber was immersed in a 10 mL sample aliquot spiked with a solution of the internal standard. The analysis was performed with a gas chromatograph (Agilent Technologies 7890A) coupled with a quadrupole mass spectrometer (Agilent Technologies 5975 C). The analytes were desorbed at 280 °C and separated with a DB-FFAP column (28 m× 0.25 mm × 0.25 µm). The ion source temperature was set up as 230 ºC and a temperature of the separator as 150 °C. The mass spectra were measured at 70 eV in the m/z range of 28-400 amu.

Results

Primary, the influence of different extraction conditions was tested, e.g. an addition of sodium chloride, a pH value, time of extraction and a stirring rate. A number of the detected compounds and a relative response were selected as the main parameters for a comparison of the results. The optimal conditions for the extraction of the wine profile were found to be 90 min extraction time, 500 rpm rotation speed, a 2 g addition of sodium chloride and a 4.5 pH value. These findings appeared to support application of low values of a stirring rate as well a salt effect for direct immersion extraction with SPME Arrow technique. Overall, 56 compounds were identified in 12 botrytized wine samples from four countries, and the enriched profiles were observed for Tokaj wines originated from Hungary. In comparison with a PDMS covered stir bar (SBSE technique), 14 similar compounds were found with both techniques.

Conclusions

The developed SPME Arrow methods was tested for a classification of the botrytized wines samples accordingly to their geographic origin. A few specific compounds were found for some samples; however, the more reliable results require the larger sample set.

ACKNOWLEDGMENTS

This project has received funding from Slovak Research and Development Agency under Contract No. APVV-21-0211.

REFERENCES

[1] Herrington J. S., Gómez-Ríos G. A., Myers C., Stidsen G., Bell D. S., (2020). Hunting Molecules in Complex Matrices with SPME Arrows: A Review. *Separation*, 7, 12.

[2] Kremser, A., Jochmann, M. A., Schmidt, T. C., (2016). PAL SPME Arrow—Evaluation of a novel solid-phase microextraction device for freely dissolved PAHs in water. *Analytical and bioanalytical chemistry*, 408: 943-952.

Detection of hemoglobin by capillary electrophoresis and FTIR spectroscopy

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Keywords: hemoglobinopathy, Capillary Electrophoresis, UV-Vis, FTIR spectroscopy

Objective
The tetrameric protein hemoglobin (Hb) is responsible for the oxygen transport in red blood cells. More than 1,000 structural mutations causing different biochemical, hematological, and clinical manifestations have been described [1]. Hemoglobinopathies encompass two main categories: structural Hb variants and α- and β- thalassemia syndromes. Hemoglobinopathies, hereditary disorders affecting the structure or production of Hb, were detected by routine HbA_{1c} measurements by capillary electrophoresis (CE).

Methods
Over a four-year period, 59,255 measurements were performed by CE at the University Hospital Motol, Prague, with a detection rate of 0.06 % for Hb variants. CE for HbA_{1c} measurement was performed using the Capillarys3 Octa instrument separating electrically charged molecules based on their electrophoretic mobility in an alkaline buffer with a pH of 9.4. For further analysis, erythrocytes were washed and lyophilized. The potential of ultraviolet-visible (UV-Vis) spectra for hemoglobin variant detection recorded (200 - 800 nm, 1 nm steps) with a single-beam UV-Vis spectrophotometer was elucidated. High-resolution FTIR spectra recorded using the KBr pellet method between the wavenumber range of 4,000 cm^{-1} and 400 cm^{-1} (resolution: 0.5, number of scans: 128) were subjected to mathematical analysis for the identification of changes in secondary protein structures.

Results
CE analysis is used for the routine evaluation of HbA_{1c} and shows mostly normal Hb or increased glycation profiles (HbA_{1c} MT = 70s, HbA_0 MT = 150 s, HbA_2 MT = 240 s). From the 0.06 % detected Hb variants special electropherograms of rare Hb variants obtained including HbS, HbS/HbG-Philadelphia, HbC, HbD, HbE are shown. Selected samples were analyzed by UV-Vis, where unambiguous differences in UV-Vis spectra between patients with normal hemoglobin, a HbC or a HbS/HbG variant could not be demonstrated. However, mathematical processing with band narrowing techniques of FTIR spectra especially of the amide I band (1700-1600 cm^{-1}) indicated slight differences in α-helix (1658-1654 cm^{-1}), β-turns (1670 cm^{-1}), β-sheet (1640-1620; 1699-1689 cm^{-1}) or random coil (1644 cm^{-1}) secondary hemoglobin structures for these mutations.

Conclusions
UV-Vis spectroscopy was unable to differentiate between normal hemoglobin, HbS/HbG and HbC. Nevertheless, samples analyzed with FTIR spectroscopy indicated slight changes in secondary structure of hemoglobin variants. However, further research is required to identify specific changes of hemoglobin variants detected by CE. Analyzing hemoglobin structural modifications can furthermore benefit cancer patients.

ACKNOWLEDGMENTS

The project is being implemented under the grant project Liga proti rakovině Praha (2399011548).

REFERENCES

[1] Thom CS, Dickson CF, Gell DA, Weiss MJ. (2013). Hemoglobin Variants: Biochemical Properties and Clinical Correlates. Cold Spring Harb Perspect Med.

Benzo(a)pyrene concentration in river water in Poland

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Keywords: benzo(a)pyrene, river water quality, national monitoring

Objective
The aim of the study was to assess the pollution of river waters in Poland with benzo(a)pyrene based on the results of national monitoring studies. The state of environmental quality in Poland is controlled as part of the State Environmental Monitoring, which is realized by the Chief Inspectorate for Environmental Protection. In 2022, the chemical status of river waters was classified in most cases (94.6% of surface water bodies) as poor [1]. One of the substances that most often exceeded the permissible standards (almost 80% of surface water bodies) was benzo(a)pyrene [1,2]. It belongs to polycyclic aromatic hydrocarbons. Benzo(a)pyrene is a carcinogenic substance, and its high concentrations are determined, for example, in cigarette smoke, industrial waste and diesel exhaust. In addition to anthropogenic sources, there are also natural sources of benzo(a)pyrene, such as oil deposits and forest fires. The main pathway of human exposure is air, but benzo(a)pyrene can also penetrate soil and water [2,3].

Methods
The research was carried out as part of the State Environmental Monitoring. Water samples were collected at 3685 measurement and control points from a depth of 0.1 to 0.5 m. The concentration of benzo(a)pyrene was determined in river water samples using the HPLC with fluorescence detector (HPLC-FLD) method in accordance with the PB-02/CLB research procedure [4].

Results
The results come from the report of the Chief Inspectorate for Environmental Protection [4]. Some of the results obtained were below the limit of quantification. Maximum concentrations reached 1.15 µg/L. The average concentration was 0.003 µg/L. The highest average concentrations were recorded in the Lower Silesian and Lubusz Voivodeships, while the lowest in the Lublin and Greater Poland voivodeships.

Conclusions
The highest concentrations of benzo(a)pyrene occurred in southern Poland, which is related to industrial activities and natural sources. However, the permissible contents were also exceeded in other voivodeships. This indicates the need to regularly monitor benzo(a)pyrene concentrations throughout the country.

REFERENCES
[1] Reports on the State of the Environment in Poland. Chief Inspectorate for Environmental Protection. Available online: https://www.gov.pl/web/gios/raporty-o-stanie-srodowiska. (accessed on 24 June 2024)
[2] Science flows from the river. Available online: https://blog.p.lodz.pl/komentarze/nauka-plynie-z-rzeki. (accessed on 26 June 2024)
[3] Bukowska, B.; Mokra, K.; Michałowicz, J. Benzo[a]pyrene – Environmental Occurrence, Human Exposure, and Mechanisms of Toxicity, (2022). *Int. J. Mol. Sci,* 23, 6348.
[4] Chief Inspectorate for Environmental Protection. Surface water quality portal. Available online: https://wody.gios.gov.pl/pjwp/publication/367. (accessed on 26 June 2024)

S-scheme BC/$CoFe_2O_4$/Fe_2O_3 photocatalyst for activation of peroxymonosulfate towards degradation of tetracycline

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Keywords: biochar (BC), BC/$CoFe_2O_4$/Fe_2O_3, LC-MS, tetracycline degradation, wastewater

Objective
The aime of this work was to develop a synergistic and highly effective catalyst with strong redox capacity that can activate peroxymonosulfate (PMS) for the degradation of tetracycline present in wastewater.

Methods
The growth of $CoFe_2O_4$/Fe_2O_3 on the surface of biochar was realized by thermal polymerization-hydrothermal two step method. Gaussian simulation calculations were performed on tetracycline molecules to determine the Fukui index of each atom. This analysis identified the sites on each tetracycline molecule that are most susceptible to free radical attack. Following the oxidative degradation, the degradation by-products were analyzed using Liquid Chromatography-Mass Spectrometry (LC-MS). Toxicity Estimation Software Tool (TEST) was used to assess acute and developmental toxicity of tetracycline and its degradation by-products.

Results
BC/$CoFe_2O_4$/Fe_2O_3 can removal 98.84% tetracycline after 60 min with PMS when it is subjected to visible light irradiation. The Total Ion Chromatogram (TIC) in positive ion mode revealed 19 major components within a retention time range of 0-15 minutes. By examining the nucleus-mass ratio in the corresponding mass spectra, comparing the results with relevant literature, and integrating the simulation calculations, we identified and characterized five degradation pathways of tetracycline.

Conclusions
The findings indicated that acute and developmental toxicity of tetracycline can be reduced by oxidative degradation using BC/$CoFe_2O_4$/Fe_2O_3. The acute toxicity of tetracycline is strongly decreased by it degradation under the experimental conditions presentesd above.

ACKNOWLEDGMENTS
This study was performed in the framework of the common PhD program of Babeș-Bolyai University, Cluj-Napoca, Romania, Environmental Science Doctoral School, and Zhejiang University of Science and Technology, Hangzhou, China

Detailed lipid investigation of edible seaweeds by photochemical derivatization and untargeted lipidomics

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Keywords: algae, photochemical cycloaddition, untargeted lipidomics, High-resolution Mass Spectrometry, compound discoverer

Objective

Seaweeds are macrophytic algae that have been gaining interest as alternative healthy food, renewable drug sources, and climate change mitigation agents. In terms of their nutritional value, seaweeds are renowned for their high content of biologically active polyunsaturated fatty acids. However, little is known about the regiochemistry – the geometry and position of carbon-carbon double bonds – of free and conjugated fatty acids in seaweeds. In the present work. In the present work, a detailed characterization of the seaweed lipidome was achieved based on untargeted HRMS-based analysis and lipid derivatization with a photochemical aza-Paternò-Büchi reaction [1].

Methods

A triple data processing strategy was carried out to achieve high structural detail on the seaweed lipidome, i.e., (i) a first data processing workflow with all samples for aligning peak and statistical analysis that led to the definition of lipid sum compositions (e.g., PG 34:1), (ii) a second data processing workflow in which the samples of each seaweed were processed separately to annotate molecular lipids with known fatty acyl isomerism (e.g., PG 16:0_18:1), and (iii) the annotation of lipid regioisomers following MS/MS annotation of the lipid derivatives obtained following the aza-Paternò-Büchi (e.g., PG 16:0_18:1 ω-9).

Results

Once the platform was set up, the lipid extracts from eight seaweed samples from different seaweed families were characterized describing over 900 different lipid species, and information on the regiochemistry of carbon-carbon double bonds uncovered unknown peculiarities of seaweeds belonging to different families.

Conclusions

The overall analytical approach helped to fill a gap in the knowledge of the nutritional composition of seaweeds.

ACKNOWLEDGMENTS

The work was supported by the PRIN2022 PNRR project Prot. P2022PTYWP, entitled “Design of high-pRofit fostEring bioActive coMpounds through integral valorization of seaWEEDs infesting the MEditerranean sea (DreamWEEDme),” provided by the Italian Ministry of Education, Universities, and Research.

REFERENCES

[1] A. Cerrato, A. L. Capriotti, C. Cavaliere, C. M. Montone, S. Piovesana, A. Laganà, (2022). *Anal. Chem*. 94 13117-13125.

Pyrolysis-gas chromatography-mass spectrometry for qualification of microplastics in foods

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Keywords: pyrolysis-Gas Chromatography-Mass Spectrometry, microplastics, qualification; foods

Objective
The occurrence of microplastics in food matrices is still an under-investigated topic. Contamination of food matrices by microplastics has been confirmed in some milk products. Particularly, scientific literature does not provide a preparative method for the determination of microplastics in cheese samples, which could be affected by microplastic's occurrence. The objective of this study is to propose a validated preparative method for the microplastics isolation from cheese. The feasibility of the method is confirmed using pyrolysis-gas chromatography-mass spectrometry (Py–GC–MS).

Methods
To determine the optimal preparative methods, different sample digestion approaches were investigated, using an aqueous solution of KOH in concentrations of 1 and 5 mol L^{-1}, H_2O_2 5.4 mol L^{-1}. Such conditions were tested at room temperature and 50 °C. To confirm whether the preparative method was able to isolate microplastics, polystyrene standard microbeads were spiked to cheese sample (1.0 g) before the digestion tests. Further, before digestion, spiked microbeads were analysed under the optical microscope to count microbeads before and after digestion to assess the recovery rate of microbeads. To check whether the digestion method allow the chemical qualification of microplastics using Py–GC–MS, 50 µL of standard solution of polyethylene terephthalate and polystyrene were spiked to the samples. Spiked samples were digested with the selected digestive protocol and solutions were filtered using Whatmann glass fiber (GF/F, pore size 1.6 µm). GF/F were placed into the liner and analysed using the Py–GC–MS technique.

Results
The most suitable preparative method for the digestion of cheese was the aqueous solution of KOH 5 mol L^{-1} at 50 °C for 48 h. The digestion allowed the removal of the 97.5±0.8 %. Further, the method allowed the recovery rate of the 98.5±0.4 % of spiked microbeads. The Py–GC–MS analysis allowed the identification of polyethylene terephthalate by the identification of its pyrolysis products (i.e., benzophenone), whereas polystyrene was not detected.

Conclusions
This study proposes a method for identifying microplastics in cheese by optimizing the removal of organic matter. This approach showed a minimal impact on PS microplastics and achieved a 97.5±0.8 % digestion efficiency. The method's suitability was confirmed with a 98.5±0.4 % recovery rate and chemical identification of microplastics using Py–GC–MS.

ACKNOWLEDGMENTS

This study has been funded by contributions of the BRIC project.

Dairy analogues in the Algerian market: proportion and substitute fat quality

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Keywords: dairy analogue, vegetable fat, SFA, analogue cheese, analogue cream

Objective
Algeria is the leading consumer of dairy products in the Maghreb, but no data are available on the market of imitation dairy products. This study was conducted to assess the state and the presence of dairy analogs in the Algerian market and their fat quality.

Methods
The study consists of two parts. The objective of the first part is to determine the proportion of dairy analogue, based on the data mentioned on the packaging of 152 products. The second part focuses on the quality of the fat substitute present in a selection of dairy products and analogues (38) by determination of fatty acid composition (GC-FID) and nutritional indices (atherogenicity index (AI) and thrombogenicity index (TI).

Results
The results obtained showed that 74.02% of "dairy products" present on the Algerian market are substitute dairy products. A large proportion contains exclusively vegetable fat (VF) : dairy creams (46.15%), margarines (70.83%) and infant milks (73.91%). In the cheese/cheese preparation category, the proportions are (34.94%) VF exclusively and (37.35%) VF combined with milk fat. The lowest prices in all categories are attributed to vegetable fat based products. The fatty acid composition revealed that the main fats used are palm, palm kernel and copra oils, used alone or in a mixture, having undergone fractionation and/or hydrogenation and/or inter-esterification.

Conclusions
The determination of the nutritional indices revealed that the fats used in the formulation of ice cream and cheese food preparations are the most deleterious to health with a risk of developing cardiovascular diseases.