Book of Abstracts

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Chromatography: contribution to a more sustainable world

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Book of Abstracts

INDEX

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List of the plenaries

Number	Title	Presenting author
PL1	Use of ionic liquids and eutectic solvents in biomass extraction and processing: Advantages and challenges	Armando Silvestre
PL2	Bioguided and metabolomic approaches in bioactive natural products research: targeting neuroprotective agents	Gerardo Rivera
PL3	Chromatography in the clinical laboratory	Henrique Reguengo
PL4	Comprehensive two-dimensional liquid chromatography (LCxLC), why to use it in food analysis?	Lidia Montero
PL5	Miniaturizing chromatography techniques using microfluidic structures	João Pedro Conde

PL1. Use of ionic liquids and eutectic solvents in biomass extraction and processing: Advantages and challenges

Silvestre A.[1]

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Ionic liquids (ILs) and Eutectic Solvents (ES) have been attracting a as alternative solvents, due to their greener and sustainable connotation when compared to conventional organic solvents, and their application has been explored in a myriad of domains. Among those, the extraction of bioactive compounds as well biomass fractionation into its macromolecular components has been widely studied.
We have been studying the use of ILs and ES to prepare extract bioactive compounds (e.g., phenolic compounds like hydroxymatairesinol- HMR, and triterpenic acids among other) with improved yields, and in some cases with the possibility of directly using the extracts for biological applications with improved responses. Yet, the specific nature of these solvents raises specific challenges in terms of extraction processing and analysis.
In the domain of biomass deconstruction into its macromolecular components (hemicelluloses, cellulose and lignin) and their subsequent conversion/valorization, ILs and DES also show a high potential, as shown by several studies that we have performed on lignin degradation and in the extraction and conversion of xylans into added value compounds (e.g., xylitol and furfural).
In all cases the potential of these systems as well as the experimental and analytical limitations will be presented and discussed.

PL2. Bioguided and metabolomic approaches in bioactive natural products research: targeting neuroprotective agents.

Gerardo R.[1]

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To uncover the complex relationship between the chemical composition and the observed bioactivity of natural products, bioguided extraction procedures followed by untargeted LC/GC-HRMS/MS data-mining strategies were successfully implemented to characterize bioactive secondary metabolites from agri-food byproducts (e.g., procyanidins, terpenoids, crocins) as well as from valuable plants and algae (e.g., polyphenols, carotenoids and PUFAs). These natural matrices, were shown as promising sources of health promoting compounds with demonstrated antioxidant, anti-inflammatory or anti-cholinergic properties under in-vitrobioactivity testing.

PL3. Chromatography in the clinical laboratory

Reguengo H.[1,2]

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Abstract to be announced

PL4. Comprehensive two-dimensional liquid chromatography (LCxLC), why to use it in food analysis?

Montero L.[1]

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LC-MS and GC-MS have been employed as analytical tools to develop methods for food applications. However, foods are usually very complex matrices, and it is difficult to obtain complete separation and identification of all the analytes present in the sample. For this reason, new analytical techniques able to offer higher resolution power and confidence in the determination of the food profile are needed. Among these techniques, 2DLC can offer significant advantages in comparison to one-dimensional techniques since the sample is analyzed by two different but complementary separation mechanisms, allowing to obtain much higher separation power. Therefore, 2DLC can provide reliable and valuable information about contaminants, potential markers of origin, or potential health promoter ingredients.

Nutritional and organoleptic characteristics of a food product are important properties that the consumers are interested in. However, nowadays, other properties are increasing their importance when a product should be selected. For instance, the fact that a product is safe and free of contaminants, or the importance of products produced in a specific geographical region with characteristic qualities, or even products that enhance or promote the health of the consumers. Therefore, a big effort should be done to ensure all these valuable characteristics of food products.

In this regard, food safety, food authenticity, food traceability, and food bioactivity are four important branches in food analysis that can be responsible for obtaining the required information.

However, 2DLC is complex and has some limitations related to the compatibility of the two dimensions employed for the separation. Consequently, improvements in the 2DLC configuration are essential for increasing the power of this technique.

In this presentation, 2DLC applications for the mentioned food analytical branches as well as a new development for improving the performance of comprehensive 2DLC will be introduced.

PL5. Miniaturizing chromatography techniques using microfluidic structures

Conde J.P.[1,2]

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Microfabricated microfluidic structures with features with characteristic dimensions in the micrometer range are being developed for sensors, reactors, and cell-chips. Microfluidic structures can be explored for both analytical and preparative chromatographic applications. In this presentation, I will present our research in the following topics: (i) screening of chromatographic ligands; (ii) sample preparation for biosensing; and (iii) first steps towards the development of a portable chromatography-on-a-chip device.

List of the keynotes

Number	Title	Presenting author
KN1	LC-MS aplicada à análise de bioativos e contaminantes em alimentos: desmistificar a complexidade, desafios e tendências	Ana Santos Silva
KN2	Targeted and untargeted mass spectrometry for the analysis of biological matrices: the light at the end of the tunnel?	Luisa Barreiros
KN3	Environmental enantioselectivity of chiral drugs	Ana Rita Lado Ribeiro
KN4	Analytical Procedure Lifecycle Management: Implication of ICH Q14 and Q2 (R2) guidelines on liquid chromatography method development	Ricardo Gonçalves
KN5	From Routine Blood Analysis to Cancer Detection: The Transformative Role of Clinical Mass Spectrometry in Diagnostics	Hugo Santos
KN6	Chromatographic approaches in Forensic Toxicological analysis	Mário Barroso
KN7	European Directives in drinking water and surface water – new analytical challenges	Vitor Cardoso
KN8	Artificial Intelligence applications to generate new compounds: a sweet tale	Miguel Rocha

KN1. LC-MS applied to the analysis of bioactives and contaminants in food: demystifying the complexity, challenges and trends

Sanches Silva A.[1,2,3,4]

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Liquid Chromatography coupled to Mass Spectrometry (LC-MS) is a powerful analytical tool allowing the comprehensive analysis of bioactive compounds and contaminants in food. This communication explores the applications of LC-MS in the field of food analysis, focusing on its ability to simultaneously identify and quantify diverse classes of compounds. The versatility of LC-MS is highlighted in its capability to determine bioactive components, such as phytochemicals with antioxidant properties, contributing to a deeper understanding of the nutritional profile of various food matrices. Furthermore, LC-MS plays an essential role in the assessment of food safety by enabling the identification and quantification of contaminants, including pesticides residues and mycotoxins. The sensitivity and selectivity of LC-MS make it an indispensable tool for regulatory compliance and quality control in the food industry.

The communication discusses recent advancements in LC-MS methodologies applied to food analysis, including hyphenated techniques such as LC-MS/MS and high-resolution methodologies such as LC-ToF-MS (Liquid Chromatography coupled with Time-of-Flight Mass Spectrometry). Challenges and future perspectives in the implementation of LC-MS for food analysis are also addressed, emphasizing the need for standardized methods and expanded databases. In this line, the complexity associated with these methodologies is also demystified.

In short, LC-MS stands at the vanguard of analytical techniques for the across-the-board assessment of bioactives and contaminants in food. Its integration into routine food analysis protocols holds great promise to guarantee both food safety and quality, thus ensuring Public Health.

KN2. Targeted and untargeted mass spectrometry for the analysis of biological matrices: the light at the end of the tunnel?

Barreiros L.[1]

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During the last years, tandem mass spectrometry (MS/MS) based techniques have become the method of choice for the targeted analysis of trace levels of bioactive compounds in biological matrices due to their inherent high sensitivity and selectivity. Most strategies rely on the coupling to a separative technique prior to MS/MS detection. The development of such methodologies presents many challenges that will be addressed in this presentation such as an adequate sample treatment, correct assignment of generated ions and efficient separation of target analytes from matrix interferences. Innovative strategies for sample preparation and hyphenation to MS detection will be also discussed.
Whereas a targeted approach allows the accurate quantification of specific molecules of interest, untargeted analysis permits the comprehensive study of all the molecules in a system and may provide valuable insights for the identification of unknown compounds, including health status indicators. The post-experimental data processing in untargeted metabolomics is demanding due to the vast amount of complex information generated. Chemometric tools may help in this challenging task and an example of their application to identify potential biomarkers of the health-to-disease transition will be presented.

KN3. Environmental enantioselectivity of chiral drugs

Ribeiro A.R.L.[1]

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Many pharmaceuticals are chiral, i.e., asymmetric 3D molecules with stereoisomers that can display a differing behaviour in chiral medium, as occurs in the interaction with natural macromolecules (e.g., enzymes, receptors, other binding-molecules). Therefore, enantiomers can present different pharmacokinetic and/or pharmacodynamic properties due to the different attachment to and dissociation from binding sites. Consequently, enantioselectivity can also occur in all biological processes occurring in the environment, different responses in terms of ecotoxicity being expected. Moreover, in the case of antibiotics, another environmental concern is the possible role of enantioselectivity on the development of antibiotic resistance in the environmental settings. This communication aims to highlight that although chirality is rarely considered in environmental studies, the knowledge on stereoselectivity in the environmental fate, distribution, (bio)transformation, (bio)degradation, ecotoxicity, and bioaccumulation is essential to provide a more realistic environmental risk assessment of chiral pharmaceuticals.

KN4. Analytical Procedure Lifecycle Management: Implication of ICH Q14 and Q2 (R2) guidelines on liquid chromatography method development

Gonçalves R.[1]

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The adoption of Quality by Design (QbD) principles and Analytical Procedure Lifecycle Management strategies to ensure the quality of pharmaceutical products has been applied and proposed over the last few years. These concepts focus on the use of systematic knowledge gathering as well as the application of science-based principles during the analytical procedure lifecycle to assure robust procedures that can reliable evolve based on the data collected throughout the application of the analytical procedure are deployed for routine use.
The analytical procedure lifecycle management is a concept that includes three pillars that must be observed during the analytical procedure development, from development up to routine application. The procedure design stage makes use of established and proven QbD concepts to collect the maximum amount of information of each variable and how do they interact with each other. During the procedure performance qualification stage, the performance characteristics of the analytical procedure are evaluated and the maximum variability for the reportable result is determined. By combining the information collected during the two initial stages a risk-based method evaluation is performed to determine the potential for method failures and a control strategy is devised to manage and reduce the risk of failure during routine work. The final step of the procedure lifecycle management is the continued method performance verification in which the procedure performance is continuously monitored to ensure its compliance with the procedure goals. These three stages work synergistically with the goal to increase method robustness, cost reduction, and decrease the risk of failures.
This approach is clearly acknowledged both by regulators and industry as is part of the ICH Q14 and Q2(r2) guidelines for the industry demonstrating the importance of a systematic approach to procedure development with emphasis on understanding the performance of the analytical procedures and the impact of process changes in the reportable result.
In this talk the workflow and implantation path will be discussed with practical examples of applications to the development of analytical procedures for the analysis of pharmaceutical products.

KN5. From Routine Blood Analysis to Cancer Detection: The Transformative Role of Clinical Mass Spectrometry in Diagnostics

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Mass spectrometry (MS) has revolutionised the field of clinical diagnostics, particularly in quantifying blood and solid tumor proteins. This technology has been pivotal in enhancing the accuracy and specificity of various diagnostic assays. In a recent study, we employed the total protein approach (TPA) based on high-resolution MS to delve deeper into renal neoplasms, a group of tumors with shared characteristics that often pose diagnostic challenges. Utilising frozen tissue biopsies from various renal neoplasms and normal adjacent renal tissue as a control, we identified 205 differentially expressed proteins. A panel of 24 proteins were pinpointed as potential biomarkers to differentiate these neoplasms. Notably, proteins such as PLIN2, TUBB3, LAMP1, and HK1 were validated using semi-quantitative immunohistochemistry, underscoring their diagnostic potential[1]. This study exemplifies the power of high-resolution MS combined with TPA in advancing the pathology of renal neoplasms and suggests broader applications in clinical diagnostics. As we progress, the integration of MS in clinical settings promises to bridge the gap between research and its real-world applications, offering novel insights into disease mechanisms and potential therapeutic targets.

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KN6. Chromatographic approaches in Forensic Toxicological analysis

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Chromatography plays a fundamental role in forensic toxicology, serving as a powerful analytical tool for identifying and quantifying toxic substances in biological samples. Indeed, it is the science of separation, and it is used to isolate components from complex mixtures.
Gas and liquid chromatography instruments coupled with mass spectrometry equip forensic laboratories nowadays and enable accurately identifying and quantifying drugs and metabolites in several biological specimens, from the most common blood and urine to the unconventional oral fluid and hair samples.
These techniques allow the efficient separation of the compounds of interest from endogenous interferences (e.g. proteins, lipids) and their detection. For most substances of toxicological interest, this detection is performed by mass spectrometry, as only this highly selective and sensitive technology is capable of unequivocally identifying the substances present in a sample, allowing obtaining forensically valid and sound results.
In this talk, practical routine cases will be presented and discussed.

KN7. European Directives in drinking water and surface water – new analytical challenges

Cardoso V.[1]

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Directive (EU) 2020/2184 of the Parliament and the Council (16 December 2020) on the quality of water intended for human consumption, as well as the revision of the European Directive on environmental quality standards for surface water, will promote new challenges for Water Analysis Laboratories, as well as for Water Management Entities and Regulatory Authorities in each Member State.
The new drinking water quality parameters, as well as the introduction of new priority substances that will be introduced in the list of compounds to be monitored in drinking water and surface waters, will require new investments from water laboratories for the acquisition of more sophisticated analytical equipment in order to meet the requirements established by this legislation in terms of analytical thresholds.
The creation of a European Approval Scheme of Materials in contact with drinking water will require the development of new analytical methodologies for the search of organic compounds that might leach from materials into water, as well as the risk assessment associated with the use of these materials in water supply systems.

KN8. Artificial Intelligence applications to generate new compounds: a sweet tale

Rocha M.[1]

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Generative models from Artificial Intelligence have been increasingly used in many areas to create new text, images or videos, just to name a few examples. In this talk, we will show that AI can also be a fundamental tool in the future to design novel compounds with desired traits, both including chemical properties and biological activities.

List of the sponsor lectures

Number	Title	Presenting author
SL1	Ion Chromatography Applications: Cost-Effective Solutions for Quality Assurance in Drinking Water, Wine Organic Acids and Environmental Analysis	Simão Cardoso
SL2	Solving the PFAS Challenge: Comprehensive Screening of 5,000 Suspects	Rui Rocha
SL3	Work more efficiently with Agilent's new intelligent LC-Triple Quad and LC/Q-TOF solutions	Angel Antelo
SL4	Characterization of Extractables from Common Pharmaceutical Packaging Materials with GCxGC and HR-TOFMS	Julio Lluch
SL5	Analysis of Sugars using Ion Chromatography and Amperometric Detection	Daniel Ettlin
SL6	From the tap to our home: the analysis of per- and polyfluoroalkyl substances (PFAS) in matrices relevant for human exposure	Mario Armelão
SL7	Solutions for Hydrogen Purity Determination	José Manuel Macias
SL8	Advantages of the B.I.P. Technology in Chromatography	Fernando Lourenço
SL9	Measures to replace helium with hydrogen	Pedro Antunes

SL1. Ion Chromatography Applications: Cost-Effective Solutions for Quality Assurance in Drinking Water, Wine Organic Acids and Environmental Analysis

Cardoso S.[1]

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Ion exchange chromatography technology has become increasingly important in the separation and determination of ionic compounds. Its applications are diverse, but the most important are undoubtedly in the food, pharmaceutical and environmental areas. In this presentation we will look in detail at cost-effective solutions for quality assurance in drinking water, wine organic acids and environmental analysis, and how this equipment can be an asset to research laboratories.

SL2. Solving the PFAS Challenge: Comprehensive Screening of 5,000 Suspects

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PFAS, or per- and polyfluoroalkyl substances, represent a group of human-made chemicals characterized by the presence of carbon-fluorine bonds. These compounds have gained considerable attention due to their widespread use in various industrial and consumer products, including non-stick cookware, water-resistant textiles, and firefighting foams. PFAS are persistent in the environment and have been detected in air, water, soil, and even in the blood of humans and wildlife.

The complexity of identifying PFAS in samples arises from several factors. Firstly, the extensive variety of PFAS compounds, estimated to number in the thousands, poses a significant challenge. These substances can have different chain lengths, functional groups, and substitution patterns, making a comprehensive analysis difficult. The diverse nature of PFAS also means that no single analytical technique is universally effective for detecting all of them.

Mass spectrometry is a powerful tool commonly employed for PFAS analysis due to its high sensitivity and ability to provide structural information. However, the analysis of PFAS by mass spectrometry is complicated by their low ionization efficiency and the lack of distinctive fragmentation patterns. Furthermore, isomeric forms of PFAS can exhibit nearly identical mass spectrometric profiles, making it challenging to differentiate between them accurately.

Another challenge lies in the low concentrations at which PFAS are often found in environmental and biological samples. The need for sensitive and selective methods becomes crucial for accurate quantification. Advances in mass spectrometry techniques, such as high-resolution mass spectrometry, have improved the ability to distinguish between closely related PFAS compounds and detect them at trace levels. Additionally, the development of tandem mass spectrometry methods and the use of isotopically labeled internal standards enhance the accuracy of PFAS quantification.

In summary, the identification of PFAS in samples by mass spectrometry is a complex task due to the structural diversity of these substances, their low ionization efficiency, and the challenge of differentiating between isomeric forms.

Bruker, leveraging innovative technology like trapped ion mobility spectrometry coupled with time-of-flight (TIMS TOF), presents a comprehensive and automated solution for the precise identification of PFAS. This advanced technology enhances the capabilities of mass spectrometry by providing an additional dimension of separation based on the mobility of ions in the gas phase. The TIMS TOF approach enables researchers to overcome the challenges associated with isomeric PFAS, offering improved selectivity and accuracy in identifying these substances in complex matrices.

SL3. Work more efficiently with Agilent's new intelligent LC-Triple Quad and LC/Q-TOF solutions.

Antelo A.[1,*], Weidner P.[1], Batoon P.[1], Zekavat B.[1], Fandino A.[1]

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Start building the lab of the future today.
Many laboratories face constant regulatory changes and the need to analyze increasingly complex data, while instrumentation and resources remain constant. Smart, advanced instrumentation that integrates complete workflows and offers greater time control will allow you to focus more on the scientific and analytical aspects. Be inspired by new LC/MS instruments for smarter labs.
We look at the latest advances in Agilent mass spectrometry, such as SWARM autotuning and telemetry logging for maintenance information. We also harmonize method transfer between Q-TOF and TQ mass spectrometers and go a step further in autonomous instrument operation with "Intelligent Reflex".
The new **6495 triple quadrupole LC/MS system** is a high-performance, ultra-sensitive system for research and analytical laboratories. With innovative iFunnel technology, it achieves detection limits in the ppq range for difficult analytes in complex matrices.
It offers high accuracy with the shortest dwell times and features integrated instrument intelligence for uninterrupted routine analysis.
The **Revident LC/Q-TOF** is a state-of-the-art quadrupole time-of-flight mass spectrometer with advanced electronics, implemented instrumental intelligence and ultra-fast detector. It is ideal for routine screening, high resolution mass quantification and identification of unknown substances.
The system is perfect for food safety, environmental analysis, metabolomics, pharmaceutical and forensic applications. Innovative technologies such as "Intelligent Reflex", pre-planned tuning and easy-to-use data logging software boost productivity and increase laboratory efficiency.

SL4. Characterization of Extractables from Common Pharmaceutical Packaging Materials with GCxGC and HR-TOFMS

LECO Applications Team[1]

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Comprehensive two-dimensional gas chromatography (GCxGC) and high-resolution time-of-flight mass spectrometry (HR-TOFMS) were used to characterize extracts from pharmaceutically relevant materials. Butyl rubber stoppers and plastic syringes were extracted with methylene chloride and subsequently analyzed with the Pegasus GCxGC-HRT 4D, equipped with ® a Multi-Mode Ion Source™ (MMS™) (LECO Corporation, St. Joseph, MI, USA). The use of thermal modulation in combination with both a nonpolar and polar column, significantly increased separation selectivity and peak capacity, providing cleaner spectra for interpretation. HR-MS with both electron ionization and chemical ionization (EI and CI) provided spectra for commercial library searching and accurate mass data for formulae determinations and/or to support fragments and molecular ions. Chromatographic elution order in both dimensions—first dimension retention index (RI) and structured GCxGC chromatograms—was also used to support analyte identifications. Several representative materials were evaluated, and several representative analytes are highlighted.

Key Words: Extractable and Leachable, E&L, Pharmaceutical Materials, GCxGC, HR-TOFMS

References

1. USP 1663 “Assessment of Extractables Associated with Pharmaceutical Packing/Delivery Systems”

SL5. Analysis of Sugars using Ion Chromatography and Amperometric Detection

Ettlin D.[1], Alves J.[1], Compianno A.M.[2]

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Numerous biological processes, including cellular communication, gene expression, immunity, organism defence mechanisms, and growth and development, depend on carbohydrates. They are challenging to analyse because they are structurally identical, highly polar compounds, and lack an appropriate chromophore. Silica-based amino-bonded or polymer-based, metal-loaded, cation-exchange columns with refractive index (RI) or low-wavelength ultraviolet (UV) detection have been used in methods for the liquid chromatographic examination of carbohydrates. The uses of RI and low-wavelength UV detection technologies in trace carbohydrate analysis are constrained by their poor sensitivity and selectivity. There is also a common HPLC method known as Hydrophilic Interaction Liquid Chromatography (HILIC), which uses fluorescent tag detection and derivatization. Additionally, HILIC demands mobile phases with a high organic content (50–80% acetonitrile), which could pose issues with sample solubility.

High-performance anion exchange (HPAE), an enhanced chromatographic method, makes use of the weak acidity of carbohydrates to produce extremely selective separations at high pH utilising a strong anion-exchange stationary phase. It enables direct quantification of non-derivatized carbohydrates at high femtomolar concentration levels with minimal sample preparation and cleanup when combined with pulsed amperometric detection (PAD). Analytes that can ionise at high pH levels can be separated using HPAE chromatography. pKas for carbohydrates normally vary from 12 to 13. The analyte is ionised in solution once the pH exceeds its pKa value. Eluents based on hydroxide are used to do this.

Eluents based on hydroxide are used to do this. Additionally, separations at high pH settings are now possible because to the development of strongly cross-linked ethylvinyl benzene-divinyl benzene pellicular resins with a wide pH stability range (0 to 14). Small anion-exchange microbeads with anion-exchange functional groups are permanently electrostatically linked to a bigger cation-exchange resin particle in the nonporous resins of the columns. Because the resin is nonporous, band-broadening is reduced and a wide range of carbohydrates, including branched oligosaccharides, are separated very efficiently. Pulsed amperometric detection can be used to detect underivatized analytes. A waveform is a representation of the possible variations. As a result of the fluctuations, the electrode surface experiences oxidising and reducing conditions, which in turn lead to the oxidation of analytes bound to the working electrode surface. Only substances with functional groups that oxidise at the detecting voltage used are detected by pulsed amperometry. Since many potentially interfering species cannot be oxidised or reduced, they are not identified and detection for electroactive species is sensitive and highly selective. It's also crucial to remember that cationic or neutral sample components in the matrix elute in or very near the column's empty space. As a result, even if the neutral or cationic sample components are oxidised, the important carbohydrate components are not affected.

The technology involved will be demonstrated, and some chromatograms for chlorate/perchlorate and polar pesticides in milk and other matrixes will be shown.

Other special detectors like Orbitrap Analyzer mass spectrometer, have been also tested to support metabolomic profiling using Ion Chromatography.

Acknowledgements: We would like to thank Anne Marie Compiano and the Laboratory Group of Thermo Scientific Excelence Application Center of France for running the samples and helping with their support.

SL6. From the tap to our home: the analysis of per- and polyfluoroalkyl substances (PFAS) in matrices relevant for human exposure

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Poly- and perfluoroalkyl substances (PFAS) are well-known environmental contaminants and are widely detected in humans and wildlife, water, soil, and air. PFAS are primarily used for their stain repellency properties as well as their surfactant characteristics, such as in aqueous film-forming foams (AFFF) to combat petroleum fires. Even though there are an estimated 5,000 unique PFAS manufactured, most monitoring efforts are focused on only 20-30 compounds.
A straightforward targeted detection method was developed with the SCIEX Triple Quad 7500 system using a 2µL injection to reduce matrix interference in complex food matrices. Sub-µg/kg LOQs below the target LOQs for 4 regulated PFAS based on EU 2022/1431 recommendation were achieved.
Non-targeted data acquisition using high resolution accurate mass spectrometry is beneficial for elucidating unknown compound structures, such as PFAS in complex samples. However, candidate structure assignment depends crucially on the collection of high-quality MS/MS spectral data. Traditional fragmentation methods using collision-induced dissociation (CID) can be too aggressive to form diagnostic MS/MS spectra. Alternatively, electron activated dissociation (EAD) has shown potential as a form of fragmentation to produce more robust spectra.
This study evaluated the use of EAD fragmentation qualitative PFAS structure elucidation and compared the results to those produced with MS/MS spectra achieved using traditional CID generated data (ZenoTOF 7600).

Acknowledgements: Holly Lee, SCIEX Concorde, Canada.

SL7. Hydrogen purity: Quality as a differentiator

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In a society immersed in a process of energy decarbonization, green hydrogen plays a very important role and is going to replace hydrogen produced by natural gas reforming.
The presence of impurities will determine its use.
Depending on the impurities present, hydrogen can be used for catalysis, refining, synthesis, fuel and this will determine its value.

SL8. Advantages of the B.I.P. Technology in Chromatography

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Num mercado exigente e cada vez mais competitivo, a qualidade dos gases puros bem como misturas de gases revela-se essencial para assegurar a precisão de resultados analíticos.
Cientes desta necessidade, a Air Products (grupo do qual a Gasin faz parte) tem vindo a investir em tecnologia e na melhoria de processos de modo a poder disponibilizar os produtos que satisfaçam as aplicações mais exigentes. Como exemplo, destaca-se os gases com tecnologia BIP que estabelecem um padrão no que concerne à qualidade dos gases para Cromatografia.
Apresentamos as características mais relevantes da tecnologia BIP (Built-In-purifier) e as suas principais vantagens na Cromatografia Gasosa.
Como complemento apresentamos os equipamentos e serviços relevantes neste segmento de mercado.

SL9. Measures to replace helium with hydrogen

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Helium is running out. The level of global consumption is approaching and threatening to exceed annual production levels, putting pressure on the markets and causing prices to rise significantly. In addition to the economic factor, helium has unique properties that make it irreplaceable in clinical and industrial applications, so in a rationing scenario chromatographic and laboratory applications will be among the most affected as they are catalogued as the lowest priority in terms of access to helium, so replacing or finding alternative methods to using helium has never been a greater priority.
In chromatography, hydrogen is the most viable alternative to helium. Van Deemter curves show that these two gases have similar performance at average linear velocities. Applying slight modifications to existing methods, the use of hydrogen generates results comparable to the use of helium. At higher linear speeds, hydrogen outperforms helium and therefore allows analysis times to be reduced. Combined with the fact that it can be generated internally in the laboratory, the use of helium is in itself a tool for increasing productivity and optimising the operating costs of analysis laboratories. However, hydrogen is not an inert gas. Possible unwanted reactions with analytes must be taken into account and the risk of explosion requires additional safety measures when using it.
The technological challenges faced by manufacturers of hydrogen-generating chromatography equipment in recent times are aimed at mitigating the risks of using hydrogen as a carrier gas in chromatography. The use of hydrogen not only solves the problem of helium consumption but is also economically advantageous.

Acknowledgements: Scion Instruments, VICI AG Internacional

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List of the oral communications

Number	Title	Presenting author
OC1	Comprehensive two-dimensional gas chromatography with TOF MS detector – An effective tool to trace the signature of grape varieties	Daniela Fonseca
OC2	Plasticity of grape varieties related to spatial and temporal biodiversity: the case study of the Portuguese Bairrada Appellation	Cátia Martins
OC3	Volatile terpenic compounds: possible influence on the aroma of broas?	Andreia Bento-Silva
OC4	Construction of a PDO "Pera Rocha do Oeste" barcode supported by GC×GC-ToFMS analysis	Ana M.S. Costa
OC5	Development of a targeted UHPLC-QqQ-MS/MS method for the determination of gut-microbiota metabolites in human plasma	Sara R. Fernandes
OC6	Gas chromatography and mass spectroscopy as reliable tools for essential oil chemotyping	Mónica Zuzarte
OC7	The chromatographic urinary volatilomic biosignature of COVID-19 patients infected by SARS-CoV-2 for disease diagnosis. An exploratory study	José S. Câmara
OC8	New Analytical Approaches for Local Anesthetics Determination in Urine Matrices	Joana R. P. Pereira
OC9	LC-MS/MS method for detection of six PEth homologues in whole blood with minimised interference of phospholipids.	Marisa Henriques Maria
OC10	Chromatography as a tool in the development of Anti-tuberculosis agents	Marta V. Clariano
OC11	Enhancing the methodology for diclofenac detection using tandem mass spectrometry	M. João Nunes
OC12	Evaluating chromatographic methods to analyze cannabinoid and terpenes profiling in cannabis plant	Rita Lousada Falcão
OC13	Optimization and validation of an analytical method for the determination of opiates in urine using microextraction by packed sorbent	Ana Y. Simão
OC14	Determination of amphetamine-type psychostimulants in hair samples using MEPS as sample clean-up and gas-chromatography coupled with mass spectrometry	Bruno Pires
OC15	Development and implementation of a GDME-HPLC-DAD methodology for the evaluation of volatile carbonyl compounds released from particleboards	Fátima Daniela Gonçalves
OC16	Phytochemical characterization of saffron stigmas (*Crocus sativus* L.): a search for compounds with antioxidant effects	Leonor Teixeira da Costa
OC17	Exploring common juniper following the cascade principle: extraction and characterization of essential oil, hydrolat, and phenolic compounds from its biomass	Bianca R. Albuquerque
OC18	LC-MS/MS analysis of proanthocyanidins in red wine fined with microalgae proteins	Elisa Costa

OC1. Comprehensive two-dimensional gas chromatography with TOF MS detector – An effective tool to trace the signature of grape varieties

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Terpenes are the main compounds responsible for varietal aromas and are the compounds that have been most studied in grapes in recent years[1]. These compounds are characteristic of each variety and come from the grapes, mainly from the skinsx[2]. This work focuses on the development of a methodology using HS-SPME-GC×GC-TOFMS, with a flow modulator in the analysis of free varietal volatile compounds in grapes from Trincadeira, Cabernet Sauvignon, Syrah, Castelão and Tinta Barroca from 2021 and 2022[3,4]. To achieve this, it was necessary to optimize the sample preparation methodology and extraction conditions, and after optimizing some parameters it was found that the largest quantity of compounds was obtained using 4 g of grape, 2 g of NaCl and 2 mL of H_2O, with extraction for 40 minutes at 60 °C[5]. The fibre used was a triple carboxen/divinylbenzene/polydimethylsiloxane fibre. The analytical conditions were also optimized so that it was possible to separate the analytes. Thus, it was possible to identify 52 free compounds of which, 17 monoterpenes, 28 sesquiterpenes and 7 C_{13}-norisoprenoids. It was observed that in the year 2021 it was possible to identify more free varietal volatile compounds than in the year 2022. Comparing the varieties for the year 2021, Tinta Barroca was the variety with the highest total relative area. In the year 2022, Trincadeira was the one with the largest relative area. According to the results obtained through linear discriminant analysis, the volatile varietal signature of the grape is significantly different between varieties and between years.

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OC2. Plasticity of grape varieties related to spatial and temporal biodiversity: the case study of the Portuguese Bairrada Appellation

Martins C.[1], Assunção H.[1], Marques J.[1], Rudnitskaya A.[2], Santos A.R.[3], Soares J.P.[3], Rocha S.M.[1]

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Sustainable viticulture and winemaking continue to represent huge challenges, where a better knowledge about the functional role of biodiversity in the vineyard and wine ecosystems is required. Particular attention should be devoted to the spatial and temporal interactions between autochthonous varieties and clime and vineyard conditions (such as soil type, orientation of the lines, age of the vine, density of planting, harvesting practices, among others). Taking advantages of chemometric tools, this research aims to provide advances to examine interactions between climatic conditions, vineyard ecosystem and a set of white and red varieties. Thus, five varieties (Arinto, Cercial, Bical, Maria Gomes, and Baga *Vitis vinifera* L.), from the Portuguese Bairrada Appellation, were selected as case study. For each variety, grapes from at least two different ecosystems were collected during five consecutive harvests (2017 to 2021). For each variety and vineyard, physical-chemical data from grapes (titratable acidity, pH and sugar content, used to estimate the technological maturity state, and free and glycosidically-potential aroma compounds) were combined with edaphoclimatic data. The results unveiled the high biodiversity of the Bairrada Appellation varieties. In addition, to the underlying variability of each vineyard ecosystem, each variety presents a specific pattern, which can be expressed differently in the ecosystems under study. The approach used allowed to hierarchize the weight of the different variables and to estimate the adaptability of the five varieties. This tool has high utility for the management and rational use of endogenous resources.

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OC3. Volatile terpenic compounds: possible influence on the aroma of *broas*?

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The volatile composition of *broa*, a traditional Portuguese maize bread, has recently been explored [1,2]. However, some aroma-impact compounds may remain unexplored due to their presence in trace levels. Such compounds, as monoterpenes and other terpenoids, might also contribute to the aroma of *broas* due to their low aroma thresholds.

In this work, to further explore the volatile composition of *broas*, the compounds of 12 *broas* were extracted by HS-SPME (headspace solid phase microextraction) and analysed by comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–ToFMS). Fifty-nine terpenic compounds (monoterpenes, sesquiterpenes and C13-*nor*-isoprenoids) were identified in *broas*, whereas only two norisoprenoids derived from carotenoids degradation (α-ionone and geranylacetone) and one monoterpene (limonene) have been previously identified by GC-MS [2].

The most relevant terpenes to the aroma of *broas* were suggested by comparison of the ratio of the peak area to odor threshold values described in the literature. Results suggested that β-damascenone and α- and β-ionones may be the most relevant to the aroma of *broas*, contributing to sweet, wooden and flower aromas [3]. A principal component analysis (PCA) was performed to further explore these differences for an easy, rapid and global assessment of the main differences in the volatile composition among the studied *broas*.

To the best of our knowledge, the present study represents the most detailed study on the terpene composition of baking products and may contribute to disclosing possible volatiles of other breads and maize-based foods.

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OC4. Construction of a PDO "Pera Rocha do Oeste" barcode supported by GC×GC-ToFMS analysis

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The "Pera Rocha do Oeste", due to its characteristics, namely its aroma, is an important Portuguese Protected Designation of Origin (PDO) product [1], and its authenticity has an added economic value. The aroma of PDO fruits possess economic significance, contributing to recognition factor. The volatile organic compounds (VOC) responsible for the fruit's aroma originated from metabolic pathways (e.g., lipoxygenase, β-oxidation, and acetate-mevalonate pathways) that exhibited variations depending on the specific cultivar [2]. To identify the unique volatile profile of PDO "Rocha" pears, it is crucial to exclusively select those varietal compounds that remain constant independently from orchard origin, storage conditions, and harvest year [3,4]. The study aimed to establish a distinctive volatile fingerprint for PDO "Pera Rocha do Oeste" pear. The PDO pears from different orchards, subject to varying atmospheres and harvested over two consecutive years, were analyzed. To accomplish this, a methodology based on headspace solid-phase microextraction (HS-SPME) combined with comprehensive two-dimensional gas chromatography-mass spectrometry with time-of-flight analyser (GC×GC-ToFMS) was employed [2]. Among the 130 VOCs identified, 15 were selected as varietal markers. These markers (2 alcohols, 12 esters, and 1 terpene) formed the basis of a volatile signature, converted to a barcode, visually characteristic for PDO pear. This barcode concept was introduced as a supplementary tool for authenticating PDO "Pera Rocha do Oeste", emphasizing their origin and ensuring their distinctiveness. The HS-SPME / GC × GC-ToFMS methodology is an excellent tool to identify the characteristic compounds and to authenticate the origin of the PDO "Pera Rocha do Oeste".

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OC5. Development of a targeted UHPLC-QqQ-MS/MS method for the determination of gut-microbiota metabolites in human plasma

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The gut microbiome is a complex and dynamic ecosystem that plays a critical role in human health and disease [1]. Through the circulating levels of gut metabolites, a snapshot of the host's state can be obtained [1]. However, due to their low concentrations and high molecular diversity, their measurement is still challenging. Thus, the establishment of methodologies that allow the accurate measurement of their levels in biomatrices is demanded. Hence, the main goal of the present work was the development of a method based on ultra-high-performance liquid chromatography coupled to triple quadrupole-tandem mass spectrometry, comprising also a chemical derivatization procedure, for the determination of eleven gut metabolites in human plasma samples, including three short-chain fatty acids, three uremic toxins and five kynurenine pathway compounds. The plasma samples were pretreated by protein precipitation and, afterwards, the analytes present in the supernatant were derivatized using 3-nitrophenylhydrazine. Chromatographic separation was achieved using a BEH C18 column (100 × 2.1 mm; 1.7 µm particle size), maintained at 40 °C. Elution was performed in gradient mode at constant flow rate of 0.2 mL min^{-1} and using 0.1% (v/v) formic acid aqueous solution (solvent A) and ACN containing 0.1% (v/v) formic acid (solvent B) as mobile phase components. The MS was operated in negative ionization mode (ESI-) and data was acquired in selected reaction monitoring (SRM) mode. The method is currently under validation according to EMA guideline on bioanalytical method validation [2], fostering application to human plasma samples collected from patients with chronic diseases.

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OC6. Gas chromatography and mass spectroscopy as reliable tools for essential oil chemotyping

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Portuguese lavenders remain undervalued in global markets due to the lack of high-quality end-products and scarcity of scientific-based studies validating their bioactive potential. Moreover, chemical variability is frequent and can compromise both safety and efficacy. In the present study, we resort to both gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) to identify relevant bioactive chemotypes in both *Lavandula luisieri* and *L. pedunculata*, essential oils, two highly prevalent species in Portugal. Further correlations between chemical composition and anti-inflammatory potential are also disclosed. The main chemical compounds present in the different samples analysed allowed the identification of two chemotypes for *L. luisieri* and three for *L. pedunculata. L. luisieri* chemotypes were distinguished by the amounts of necrodane derivatives, with some samples presenting low concentrations of these compounds (6.9 ± 4.6%) and others showing high amounts (24.9 ± 2.4%). For *L. pedunculata*, differences occurred in the concentrations of three main compounds, with samples rich in fenchone (36.0% ± 12.9), 1,8-cineol (32.9% ± 8.3) or camphor (37.5% ± 8.1). Regarding the anti-inflammatory potential, distinct efficacies and safety profiles, depending on the chemical composition of the essential oils, were found. In fact, *L. luisieri* oil with low amounts of necrodane derivatives was the most potent in mitigating the anti-inflammatory response, via down-modulation of the NF-κB pathway, with significant inhibitions in major inflammatory mediators [1]. Overall, this study highlights the applicability of GC and GC/MS to select bioactive chemotypes and guarantee products efficacy and safety to fulfil the requirements of competitive markets.

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OC7. The chromatographic urinary volatilomic biosignature of COVID-19 patients infected by SARS-CoV-2 for disease diagnosis. An exploratory study

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Coronavirus disease 2019 (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This disease quickly evolved into a pandemic, which had a strong impact on society at the economic, political, social, educational, environmental, and cultural levels. According to the World Health Organization (WHO), 768 million people have been infected and over 6.94 million deaths have been estimated. To assess if SARS-CoV-2 infection induces changes in the urinary volatilomic fingerprint able to be used in the non-invasive COVID-19 diagnosis and management, urine samples of SARS-CoV-2 infected patients (21), recovered COVID-19 patients (14), and non-infected individuals (21) were analyzed using HS-SPME/GC-MS. In total, 101 volatile organic metabolites (VOMs) from 13 chemical families were characterized, including terpenes, phenolic compounds, norisoprenoids, and ketones the most represented groups. A decrease in the levels of terpenes and phenolic compounds was observed in the control group, while norisoprenoids and ketones showed a significant increase. In turn, a remarkable increase was noticed in norisoprenoids and ketones and a milder increase in alcohols, furanic, and sulfur compounds in the recovery group relative to the COVID group. Multivariate statistical analysis identified sets of VOMs with the potential to constitute volatile signatures for COVID-19 the development and progression. These signatures are composed of D-carvone, 3-methoxy-5-(trifluoromethyl)aniline (MTA), 1,1,6-trimethyl-dihydronaphthalene (TDN), 2-heptanone, and 2,5,5,8a-tetramethyl-1,2,3,5,6,7,8,8-octahydro-1-naphthalenyl ester acetate (TONEA) for COVID-19 infection and nonanoic acid, α-terpinene, β-damascenone, α-isophorone, and trans-furan linalool for patients recovering from the disease. To our knowledge, this is the first study to reveal the changes in the urinary volatilomic profile following SARS-CoV-2 infection and recovery from COVID-19 disease. Such changes define volatile signatures with the potential to be used in non-invasive COVID-19 diagnose and management. In this context, the number of samples constitutes a limitation of this study that can be circumvented in future disease outbreaks.

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OC8. New Analytical Approaches for Local Anesthetics Determination in Urine Matrices

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Lidocaine, procaine, benzocaine and tetracaine are local anesthetics commonly used in medical and dental treatments [1]. They are also employed as cocaine and heroin adulterants or as substances of abuse, presenting toxicity to central nervous and cardiovascular systems [2]. In this sense, it is necessary to develop analytical eco-friendly methods that allow, in a rapid and effective way, to monitor these analytes in biological matrices.

In this contribution, two green and innovative analytical techniques, i.e. bar adsorptive microextraction (BAμE) and solid phase microextraction (SPME) LC Tips, were developed, optimized and compared to target four anesthetics in urine samples followed by gas chromatography-mass spectrometry (GC-MS) analysis [3]. The procedure consists of extraction and back-extraction stages and several parameters were optimized using design of experiments approaches. Under optimized conditions, BAμE devices presented better efficiencies than SPME LC Tips with recoveries of 46-112% and 1-151%, respectively. Therefore, the BAμE/GC-MS methodology was validated using 0,5 mL of blank urine samples, showing excellent selectivity, suitable limits of detection (≤80 ng/mL), appropriate linear dynamic ranges (1.0-60.0 μg/mL) with good determination coefficients (r_2≥0.9945), as well as good repeatability at three different levels (RSD≤8 %).

The proposed methodology proved to be an alternative strategy for monitoring these local anesthetics in urine samples, given its great simplicity, use of small amounts of sample and solvent, ease of use, low cost, and excellent analytical performance.

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OC9. LC-MS/MS method for detection of six PEth homologues in whole blood with minimised interference of phospholipids.

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Alcohol consumption is undeniably linked to a multitude of health risks, injuries, and even death, and has significant social and economic consequences worldwide [1]. Phosphatidylethanol (PEth), is a group of promising direct alcohol biomarkers, with a considerably longer half-life in blood compared to ethanol. These biomarkers can be measured to predict various patterns of alcohol consumption [2]. This study's goal was to develop and validate an accurate and precise LC-MS/MS method for detecting six PEth homologues in whole blood, while minimizing interference from unwanted phospholipids. Different organic solvent mixtures for liquid-liquid extraction were investigated aiming to achieve optimal recovery of PEth homologues while eliminating lyso-phospholipids and other interfering phospholipids. For the instrumental analyses, was used an LC-MS/MS. For chromatographic separation was used a BEH C18 column. The mobile phase consisted of 0.025% ammonia in Type I water, pH 10.7, as solvent A, and methanol as solvent B. After method optimization, it was found that the mixture of heptane/2-propanol (80:20, v:v) provided the lowest phospholipid background, satisfactory recovery of all six PEth homologues, and the best signal-to-noise values. The method validation was performed by using blank whole blood as matrix in calibrators and QC samples. Lower limit of quantification was 10 nM for all compounds. The extraction recoveries obtained were within 37-51% and no matrix effects were observed. Quantification of 22 authentic blood samples showed that the developed LC-MS/MS method is sensitive, precise and accurate for the determination of the six PEth homologues in whole blood.

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OC10. Chromatography as a tool in the development of anti-tuberculosis agents

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is one of the world's deadliest infectious diseases. Today, TB still represents a significant public health concern, mainly due to long treatment durations, patient low compliance, and the development and spreading of multidrug and extensively drug resistant TB[1–3]. Furthermore, most available anti-TB drugs do not address latent Mtb forms, prevalent in 90% of infected people. This state can progress into the symptomatic, contagious active disease. Thus, the discovery and development of new drugs with novel molecular structures and potent activity against drug resistant and latent Mtb are essential[3].

In our lab, there has been an effort to develop new compounds with antimycobacterial potential, designed to target both the active and latent Mtb forms. Recently, a library of azaaurones with improved pharmacokinetic properties displayed submicromolar to nanomolar activities against Mtb[4]. Furthermore, a family of novel pyrroloquinolones is now under development.

In a medicinal chemistry point of view, chromatographic methods are essential, as they enable the separation and purification of complex mixtures, through Column and Preparative Thin-Layer Chromatography (TLC), and characterization, through Liquid Chromatography-Mass Spectrometry (LC-MS). Moreover, they allow compound quantification in aqueous solubility, stability, isomerisation, and metabolic studies, through High-Performance Liquid Chromatography (HPLC)[4].

Here, we present the application of different chromatographic strategies in different stages of the development of potential anti-TB agents, from compound purification to the evaluation of physicochemical and metabolic properties.

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OC11. Enhancing the methodology for diclofenac detection using tandem mass spectrometry

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The use of diclofenac (DCF), a nonsteroidal anti-inflammatory drug (NSAID), is widespread for alleviating pain and inflammation in humans and animals. However, its presence in the environment poses significant challenges to sustainability, particularly in water ecosystems. The NATURIST Project addresses this concern by focusing on enhancing fish food safety and implementing vigilant ecosystem monitoring. In this study, a robust LC–MS/MS method was developed to assess the efficacy of materials intended for use in biosensors targeting DCF.

The multiple reaction monitoring (MRM) mode emerged as the most sensitive and selective technique for quantifying DCF concentration, exploring both positive and negative ion modes to optimize detection by tandem mass spectrometry. Solid phase extraction with HLB cartridges was employed for sample preparation, demonstrating excellent selectivity, sensitivity, linearity, accuracy, and precision. The method underwent rigorous validation, encompassing selectivity, limits of detection (LOD), quantification (LOQ), linearity range, recovery, and intra- and inter-day precision.

This study also evaluated the stability of DCF during sample storage and processing procedures. A comprehensive comparative analysis of positive and negative ionization modes was conducted to determine the most suitable option for analytical determination. The proposed method, successfully applied to spiked water samples, sets the stage for future investigations into the efficiency of porous organic materials. This research contributes valuable insights into mitigating the environmental impact of DCF, offering a refined analytical approach for biosensor material selection and detection methods.

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OC12. Evaluating chromatographic methods to analyze cannabinoid and terpenes profiling in cannabis plant

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Cannabis sativa L. has been gaining attention for its therapeutic properties[1]. However, there is variability in analytical methodologies practiced globally and a lack of uniformity. In Europe, the 2018 German Pharmacopoeia (DAB) monograph describes the most widely used method for identifying and quantifying cannabinoids from cannabis flowers [2]. In 2022, the European Pharmacopeia (Ph. Euro.) released a draft of the Cannabis flower monograph that aims to substitute the national pharmacopeias.
The aim of this work was to compare HPLC chromatographic methods in terms of their separation capacity for 17 cannabinoids using Ph.Euro. and DAB. Additionally, cannabis flowers and leaves were characterized (identification and quantification) in terms of cannabinoids by HPLC-DAD and 43 terpenes by GC-MS.
The results revealed that the method described in the Ph. Euro. successfully separated 17 cannabinoids, while the DAB method could only separate 12 cannabinoids. Δ9-tetrahydrocannabinol acid, Δ9-tetrahydrocannabinol and Δ8-tetrahydrocannabinol were the predominant cannabinoids in cannabis flowers and leaves. Regarding terpenes, β-myrcene and D-limonene were the major compounds identified in flowers while squalene, β-myrcene, and β-caryophyllene were the predominant in leaves.
In conclusion, Ph. Euro. method can separate a higher number of cannabinoids from cannabis flowers and leaves than the DAB method. Concerning terpenes, a distinct profile was obtained for flowers and leaves.
This difference suggests that cannabis leaves, a by-product of the cannabis industry, have potential for the extraction of volatile compounds that are not predominant in cannabis flowers.

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OC13. Optimization and validation of an analytical method for the determination of opiates in urine using microextraction by packed sorbent

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According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), approximately 1.3 million individuals have used opiates, both for medical and illicit purposes[1], presenting a significant public health challenge[2,3]. To address this issue, methods for quantifying these substances are needed. Urine is a commonly employed matrix in clinical and forensic toxicological analyses due to its ease of collection and ample availability. Its short detection window is particularly effective for monitoring recent drug exposure[4,5].
This study aimed to optimize a method for determining tramadol, codeine, morphine, 6-acetylmorphine, 6-acetylcodeine, and fentanyl in urine samples (250 µL). The process involved centrifugation, acid hydrolysis, and extraction using microextraction by packed sorbent (MEPS). MEPS offered a rapid, environmentally friendly, and reusable extraction technique[6]. All parameters that influence the extraction were previously optimized. The method was validated following international guidelines, demonstrating excellent linearity [1 to 1000 ng/mL for all compounds, except for fentanyl (10 to 1000 ng/mL), with coefficients of determination of at least 0.99], and presenting coefficients of variation and bias ≤ 15% for precision and accuracy, except for the lowest calibrator (≤ 20%).
Recoveries obtained ranged from 17 to 107%, with lowest percentages for morphine (12 to 17%). Despite the low extraction efficiency obtained for morphine, it was possible to detect concentrations as low as 1 ng/mL for all compounds, except for fentanyl (10 ng/mL). The method was successfully applied to real samples from consumers of these substances.
This is the first method to use MEPS and GC-MS/MS for the simultaneous determination of these six opioids in urine samples.

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OC14. Determination of amphetamine-type psychostimulants in hair samples using MEPS as sample clean-up and gas-chromatography coupled with mass spectrometry

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Numerous protocols for the analysis of amphetamine-type psychostimulants (ATS) in hair have been employed over the years. Microextraction by packed sorbent (MEPS), a downsized version of solid-phase extraction (SPE), has been effectively applied in hair drug analysis, including substances like opiates, cocaine, and ketamine. However, concerning ATS, MEPS has primarily been used to the determination of amphetamine (AMP) and methamphetamine (MAMP) in hair[1-4].

The main objective of this study was to develop and validate a method using MEPS as a sample clean-up, for the determination of AMP, MAMP, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 1-(1,3-benzodioxol-5-yl)propan-2-yl(ethyl)amine (MDE), and N-methyl-1-(1,3-benzodioxol-5-yl)-2-aminobutane (MBDB) in hair.

The extraction procedure involved incubating 50 mg of hair with 1M NaOH at 45 ºC overnight, followed by neutralization with 10M HCl and centrifugation[5]. A Design of Experiments (DoE) approach was employed to optimize MEPS clean-up process, encompassing conditioning, loading, and elution steps. The eluted extract underwent derivatization and was analysed using gas chromatography coupled to mass spectrometry (GC-MS).

The developed MEPS method yielded recoveries ranging from 8 to 52% for the different analytes in hair samples and linearity was obtained between 0.2 (cut-off proposed by SoHT) and 5.0 ng/mg. The precision and accuracy were in accordance to international method validation standards.

This study introduces the first analytical method integrating MEPS with GC-MS for the detection of these specific amphetamines in hair samples. Notably, this method offers a viable alternative to conventional procedures, characterized by its speed, eco-friendliness, and cost-effectiveness.

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OC15. Development and implementation of a GDME-HPLC-DAD methodology for the evaluation of volatile carbonyl compounds released from particleboards

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Wood-based panel (WBP) is a generic term for board products derived from fibres, particles, and/or veneers, which includes particleboards (PBs), medium-density fibreboards, and plywood. During the manufacturing process, an adhesive is incorporated into the wood material, resulting in a blend that will solidify under the influence of heat and pressure [1]. Due to the wood material, chemical additives, and processes involved in their production, WBPs are known to emit volatile organic compounds (VOCs), which can impact indoor air quality and human health [2].

Gas-diffusion microextraction (GDME) [3] serves as a simple sample preparation technique that allows extraction, derivatization, and concentration of volatile compounds from liquid and solid samples. This technique involves capturing, in a closed vessel, the gaseous analytes as they cross a permeable membrane and are subsequently collected in an acceptor solution.

In this study, a GDME-HPLC-DAD methodology, coupled with a derivatization reaction using 2,4-dinitrophenylhydrazine, was developed to extract and analyse volatile carbonyl compounds released from PBs. Several studies were performed, such as the optimization of the GDME extraction parameters, evaluation of the method's precision, assessment of matrix effects, and more. PB samples produced with different particles and resins displayed statistically significant differences in their emission profiles and overall chromatographic peak intensities. Through a Principal Component Analysis (PCA) study, the GDME-HPLC-DAD methodology proved able to successfully differentiate the samples into groups based on the type of resin and the nature of the particle using the volatile carbonyl compounds determined.

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OC16. Phytochemical characterization of saffron stigmas (*Crocus sativus* L.): a search for compounds with antioxidant effects

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Saffron is an expensive spice obtained from the dried stigmas of *C. sativus* [1]. Previous studies demonstrated that stigmas have bioactive compounds - crocins, picrocrocin and crocetin - with health-promoting effects [2]. In our recent review, we concluded that crocins and *trans*-crocetin may have protection against neuro-cognitive disorders [3]. However, factors including sample origin, post-harvest and storage conditions may affect phytochemicals' concentration and compromise saffron bioactivity. In this study, we evaluated the antioxidant activity (ORAC, HOSC, Intracellular Antioxidant Activity and HPLC-DAD-ED) and the phytochemical composition of 38 Algerian samples collected in different regions, by HPLC-DAD-UV (crocins and picrocrocin) and *Folin-Ciocalteu* assay (total phenolic content–TPC). The main bioactives were identified by HPLC-MS/MS. Through principal component analysis, samples were separated according to their bioactivity. Samples with higher concentrations of total crocins (174-240 mg/g_{dw}) and total picrocrocin (39-70 mg/g_{dw}) were positively correlated with higher ORAC (475-614 µmolTEAC/g_{dw}) and HOSC (403-591 µmolTEAC/g_{dw}) values. These samples also registered the highest TPC, which was highly correlated with ORAC (r=0.76). Contrastingly, samples with low concentrations of total crocins (47-80 mg/g_{dw}), crocin 1 (24-47 mg/g_{dw}), crocin 2 (3-11 mg/g_{dw}) and total picrocrocin (8-32 mg/g_{dw}) exhibited lower antioxidant activities (ORAC–316-473 µmolTEAC/g_{dw}; HOSC–337-423 µmolTEAC/g_{dw}). Electrochemical detection (HPLC-DAD-ED) allowed to predict samples' antioxidant effect through the identification of compounds with antioxidant properties (crocin 1 and crocin 2, in particular). Although further analyses are needed to understand the health-promoting effects of minor compounds, this work provides new insights about the bioactivity of stigma components and will contribute to evaluate the impact of harvesting/storage on saffron phytochemical composition.

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OC17. Exploring common juniper following the cascade principle: extraction and characterization of essential oil, hydrolat, and phenolic compounds from its biomass

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Common juniper (*Juniperus communis* L.) is widely cultivated Northern Hemisphere, primarily known for its essential oil production from berries. However, some studies have suggested that its bark and foliage parts can also be sources of essential oils and other interesting compounds [1,2]. Steam distillation generates byproducts, including distilled plant biomass and hydrolat. Distilled plant biomass is employed for industrial heat generation and composting [1]. Within the scope of the BeonNAT Project, this study evaluated the potential of common juniper foliage biomass as a source of three value-added products: essential oils, hydrolat, and phenolic compounds. Essential oils and the hydrolat (containing 0.18% oil) were obtained through steam distillation. The oils were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). The resulting biomass was assessed for its phenolic composition. To obtain extracts, three methods were used: maceration (ME), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE). The extracted compounds were analyzed by HPLC-DAD-ESI/MS. As result, 70 compounds (96.6%) were identified in the essential oil, with α-Pinene, Limonene and Sabinene being the main compounds. In the hydrolat's oil fraction, 39 compounds were identified (94.5%), with terpinen-4-ol being the most abundant. In the extracts, 16 compounds were identified, and the total phenolic compounds (20.86-91.5 mg/g dry weight (dw)) varied depending on the extraction. Flavonoids (7.33-58.05 mg/g dw) and flavan-3-ols (12.14-28.72 mg/g dw) were the main compound classes. The lowest recoveries were achieved by UAE, while MAE produced the best results. In conclusion, common juniper biomass has potential as a source of bioactive molecules.

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OC18. LC-MS/MS analysis of proanthocyanidins in red wine fined with microalgae proteins

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Protein fining agents are crucial to improve wine stability and sensory properties [1-2]. Microalgae offer a high-protein alternative that is non-allergenic-and non-animal origin. Three microalgae protein extracts (Spirulina, *Chlorella vulgaris*, *Tetraselmis chuii*) in comparison with Gelatin were studied to evaluate their suitability as fining agents regard to their capacity to reduce proanthocyanidins of red wine. A laboratory-scale fining experiment was conducted on two red wines at two doses, 25g/hL and 50g/hL [3], for five days. Wine proanthocyanidins were fractionated into two groups: mono- and oligomeric proanthocyanidins and polymeric proanthocyanidins through the use of solid-phase extraction (SPE) [4]. The Proanthocyanidins were analyzed via mass spectrometry (LC-MS/MS) after depolymerizing the samples using thioglycolic acid through chemical depolymerisation [5]. The monomeric and oligomeric fractions of *Chlorella vulgaris* at a concentration of 25g/hL and *Tetraselmis chui* at 50g/hL impact proanthocyanidins, prodelphinidins, and procyanidins in red wine A. In contrast, red wine B is significantly affected only by Spirulina at a concentration of 25g/hL in monomeric and oligomeric prodelphinidins. In red wine A, a concentration of 50 g/L of microalgae proteins had a significant effect on polymeric proanthocyanidins and procyanidins. The protein extracts of Spirulina and Gelatin did not have a significant effect on polymeric prodelphinidins. Meanwhile, significant changes in total polymeric proanthocyanidins were observed in red wine B, with Gelatin at both concentrations affecting prodelphinidins. However, the procyanidins in this wine were not affected. The selection of microalgae protein extracts is dependent on the protein structure and the matrix of the red wine.

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List of the flash communications

Number	Title	Presenting author
FC1	Quick analysis on fatty acids profile, from matrix to chromatogram in 90 minutes, a case study	André F. Jorge
FC2	Caracterização química de amêndoas de intercropping (*Prunus dulcis* Mill.) produzidas sob diferentes sistemas de irrigação no nordeste de Portugal	Eleomar de O. Pires Jr
FC3	The impact of the cooking process in the volatile composition of different legume species: A GCMS-TQ approach	Elsa Mecha
FC4	Analytical techniques for assessing olive oil quality and shelf life	Flávia Freitas
FC5	A GC-MS off-flavour quantification approach helps decipher a new layer of spoilage potential by *Alicyclobacillus* spp.	Inês Carvalho Leonardo
FC6	Empowering bladder cancer patient management though a tailored non-invasive LC-MS/MS approach	Luís B. Carvalho
FC7	Graphene/clay mixtures as sorbent for dispersive solid phase extraction of melatonin and desorption with surfactant aqueous solutions	Lucía Gutiérrez Fernández
FC8	Desenvolvimento e otimização de uma nova metodologia para determinação de antidepressivos em plasma com extração por MEPS e análise por GC-MS/MS	Sofia Soares
FC9	Desenvolvimento de um método por GC-MS para a confirmação de opiáceos em bílis após triagem por Imunoensaios	Pedro Dinis
FC10	Documenting human exposure to cannabinoids using oral fluid	Antunes M
FC11	Unveiling the relationship between stopper and wine by GC×GC: a step forward to pairing wine and stopper	André Viana
FC12	Comprehensive analysis of the aroma profile of Tawny and White Port wines with different indication of age using a multiple headspace solid-phase microextraction method	Juliana Milheiro
FC13	Assessing the Viability of Enhancing Brandy Quality Through the Utilization of a Dual-Wood Blend	Carlos Antunes
FC14	Strategy for reducing the methanol content in strawberry tree-based spirits	Ofélia Anjos
FC15	Protein fraction analysis of six coffee by-products using RP-HPLC-FLD	Marlene Machado
FC16	Identification of Polyethylene terephthalate (PET) photodegradation products by LC-MS	Maria Carolina Melo de Sousa
FC17	A new method for extraction of pesticide residues from soil by direct-immersion Solid Phase MicroExtraction (SPME)	João Brinco

Number	Title	Presenting author
FC18	Analysis of volatile carbonyl compounds released from Medium Density Fibreboards following gas-diffusion microextraction and HPLC-DAD-MS/MS	Rui Miguel Ramos
FC19	Determination of the chemical profile of extracts from the roots of the plant species *Dizygostemon riparius* (Plantaginaceae) using the HPLC-DAD method	Tatiane de Freitas Gomes
FC20	Preliminary chemical profile by HPLC/DAD of organic precursor for green synthesis of metallic nanoparticles	Mirla Cristina Ferreira
FC21	Analysis of biogenic volatile organic compounds in shrub leaves using headspace-bar adsorptive microextraction	Cerqueira, Jéssica
FC22	Recovery optimization and exploring food industrial applications of bioactive compounds in *Thymus* genus through HPLC-DAD-ESI/MS analysis	Eleomar de O. Pires Jr
FC23	Comparative Analysis of the Phytochemical Profiles in the Flowers and Pods of *Acacia mearnsii*	Soraia Inês Pedro
FC24	Chromatographic profiles of selected hydrophilic and lipophilic constituents of edible and non-edible parts of rhubarb leaves	Matilde Rodrigues

FC1. Quick analysis on fatty acids profile, from matrix to chromatogram in 90 minutes, a case study

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For the last 70 years in food science a lot of techniques have been developed to achieve the best sample preparation methodology and the most efficient chromatographic analysis to clarify the fatty acids profile in different types of matrices, from animal and vegetal sources. To accomplish complex samples as fatty acids with less cost, low time and less interference possible, ancient techniques like soxhlet extraction has been abandoned which makes space to sample preparation techniques like ultrasound extraction and accelerated solvent extraction (ASE). To reach a good resolution in fatty acids profile, gas chromatography is a most acceptable technique used (fatty acids are derivatized into fatty acids methyl esters (FAME)), but type of column, detector and temperature program, and other parameters can vary plenty from researcher to researcher.

Based in several tests, we produced a methodology that include sample preparation (ASE[1], derivatization process[2]) to chromatographic analyses (GC-FID) in less than 90 minutes.

This methodology allows us to reduce monetary and time cost in analyses of fatty acids profile per sample, and the most relevant achievement is a chromatographic analysis in less than 20 minutes with identification around 40 fatty acids from Lauric acid (C12:0) to Docosahexaenoic acid (C22:6w3, DHA).

This was the first step to achieve a more robust methodology, with low time and cost consuming and with clear results.

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FC2. Caracterização química de amêndoas de intercropping (*Prunus dulcis* Mill.) produzidas sob diferentes sistemas de irrigação no nordeste de Portugal

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O stress hídrico tem gerado um impacto significativo nas culturas agrícolas, especialmente na região do Mediterrâneo, e espera-se que as mudanças climáticas agravem essa situação nos próximos anos. A prática de intercropping, cultivo de diferentes espécies vegetais no mesmo campo, pode oferecer uma solução viável, reduzindo o escoamento e aumentando a utilização da água disponível no solo, o que resulta num aumento do rendimento das culturas [1,2]. Além disso, esta prática pode também melhorar a qualidade nutricional de alimentos como as amêndoas [3]. Nessa perspectiva, o presente trabalho teve como objetivo analisar as características químicas das amêndoas, visando confirmar os benefícios resultantes da interação entre as culturas sob diferentes condições de cultivo, nomeadamente solo seco e irrigado. A composição química foi determinada através da quantificação dos teores de ácidos gordos utilizando GC-FID, ácidos orgânicos através de UFLC-DAD e açúcares por HPLC-RI. Em relação aos ácidos orgânicos, os resultados mostraram que as amostras de amendoais de sequeiro apresentaram níveis mais elevados de ácido oxálico e málico, registrando 0,38±0,02 e 0,288±0,002 g/100g pf, respectivamente, em comparação com 0,343±0,01 e 0,205±0,05 g/100g pf para amendoais de regadio, o que sugere uma possível associação com as condições de stress enfrentadas pelas plantas. Por fim, em relação aos níveis de açúcares e ácidos gordos, não foram observadas diferenças significativas entre as duas amostras analisadas. Com base nos resultados obtidos até ao momento, a qualidade nutricional das amêndoas manteve-se mesmo sob condições de stress abiótico, podendo ser esta, uma estratégia sustentável à produção alimentar.

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FC3. The impact of the cooking process in the volatile composition of different legume species: A GCMS-TQ approach

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With high nutraceutical value, legumes are highly recommended in the prevention of non-communicable diseases [1]. Nevertheless, in most of the European countries, their intake is below the recommendations [2]. If undesirable volatiles, responsible for the grassy and beany flavors, may contribute to their low consumption, the volatiles associated with pleasant flavors have positive impact in their intake [3]. The abundance of such compounds depends on several factors such as the legume species, the seed storage conditions, and the processing conditions, which may be quite variable for the different species [4]. The present study aims to analyze the volatile composition of six different species (*Phaseolus vulgaris* L., common bean, *Cicer arietinum* L., chickpea, *Pisum sativum* L., pea, *Vicia faba* L., faba bean, *Lens culinaris* L., lentils and *Lathyrus sativus* L., grasspea) using Gas Chromatography Mass Spectrometry – Triple Quadrupole (GCMS-TQ) before and after boiling under the same conditions. Multivariate analyses support a deeper understanding of the compounds associated with the different species. In general, the volatiles were classified into aldehydes (e.g., hexanal), ketones (e.g., 2-heptanone), hydrocarbons (e.g., decane), sulfur compounds (e.g., dimethyl disulfide) and terpenes (e.g., β-Pinene). The formation of volatiles such as furan-2-pentyl and phenylacetaldehyde, responsible by the flowery, and sweet odors [5], with concomitant degradation of compounds in the raw seeds, such as heptanal and nonanal, during the heating process, contributes to increase the appreciation of beans by the consumer. The established difference in the volatile composition allows a better understanding of the reasons underneath legumes acceptability or avoidance.

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FC4. Analytical techniques for assessing olive oil quality and shelf life

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Olive oil is a vegetable oil extracted from olives without the use of solvents or chemicals. It is a cornerstone of the Mediterranean diet due to its health benefits. Its distinctive flavour and aroma result from volatile organic compounds (VOCs), the presence and quantity of which vary due to olive variety, ripeness, processing, and storage[1,2].

VOCs are produced through natural biochemical processes, including the lipoxygenase (LOX) pathway, contributing to the green and fruity flavour of olive oil. However, sensory defects can arise from chemical oxidation and the action of exogenous enzymes, often stemming from microbial activity[3].

Olive oil is the only food product legally required to undergo quality evaluation by a certified sensory panel. This evaluation considers positive attributes such as fruity, bitter, and pungent flavours, as well as negative attributes like rancidity and mustiness. The shelf life of olive oil ranges from 18 to 24 months, thanks to natural antioxidants such as polyphenols[4,5].

Robust analytical methods, such as solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC/MS), are essential to support sensory evaluation. This study aimed to develop an HS-SPME-GC/MS methodology to identify VOCs as markers of both positive and negative attributes, correlating them with concentrations to estimate the risk of disqualification during the olive oil's shelf life.

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FC5. A GC-MS off-flavour quantification approach helps decipher a new layer of spoilage potential by *Alicyclobacillus* spp.

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Industries that produce or use fruit-based products have faced several spoilage events, resulting in economic losses caused by product recalls and loss of consumer confidence. Some of these events correlate to the presence of Alicyclobacillus (ACB) in food products since they can produce off-flavours in the final products. Guaiacol (2-methoxyphenol) and halophenols (2,6-dichlorophenol and 2,6-dibromophenol) have been widely explored as the major culprits of off-flavour spoilage by ACB. These compounds are associated with medicinal, disinfectant, or smoky odours. In this work, relevant metabolites produced by ACB were identified and quantified by GC-MS using a Wax MS capillary column, while simultaneously investigating their potential as spoilage-related compounds. The ability of distinct ACB species (*Alicyclobacillus acidoterrestris*, *Alicyclobacillus acidocaldarius*, and *Alicyclobacillus cycloheptanicus*) to produce off-flavour volatile compounds was evaluated in different conditions (e.g., different time spans, off-flavour precursors added, matrix – growth medium, fruit juice). For the first time, isobutyric acid (2-methylpropanoic acid) and isovaleric acid (3-methylbutanoic acid) were reported, as soon as two days of incubation, as being produced in different conditions at concentrations which could surpass the described odour threshold. The sweaty, sour, and unpleasant profile of these newly reported compounds is often associated with certain metabolites produced during milk fermentation by lactic acid bacteria in cheese production. Most importantly, isobutyric acid and isovaleric acid were found to be produced by all three ACB species, regardless of their ability to produce guaiacol and/or halophenols. This work clearly shows that even ACB species previously identified as non-spoilage bacteria can also pose a threat to the fruit juice and beverage industries. Therefore, the risk assessment currently used in the industry for ACB control may need to be revised to accommodate these new findings.

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FC6. Empowering bladder cancer patient management though a tailored non-invasive LC-MS/MS approach

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Bladder cancer (BC) poses a significant challenge, notably in T1-stage patients, as it carries a high risk of tumor recurrence and progression. Despite significant efforts, there is no reliable method for predicting recurrence, intensifying the need of continuous patient monitoring. In this study, we introduce a non-invasive urine-based approach to follow up BC patients, aiming to transform bladder cancer monitoring and personalized medicine[1]. Liquid chromatography combined with high-resolution mass spectrometry was employed to compare the urinary proteome of T1-stage BC patients with recurring and non-recurring BC. Additionally, six patients underwent personalized monitoring using the same approach. We identified differentially expressed proteins within cancer-related pathways that could predict the patient health status. These pathways variations during the disease course were evaluated using the differential personal pathway index (dPPi) calculation, indicating potential disease recurrence or progression and the necessity for medical intervention. The longitudinal monitoring of the disease course was successfully achieved in BC patients through a combination of urine proteomic analysis and dPPi calculation. Utilizing the information contained in the patient's urinary proteome, the dPPi reflects the individual progression of BC and assists in optimizing the use of more invasive procedures such as cystoscopy. Furthermore, this unique approach tracks patients and, when combined with clinical data, aids in clinical decision-making, ultimately achieving the goal of prescriptomics[2].

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FC7. Graphene/Clay Mixtures as Sorbent for Dispersive Solid Phase Extraction of Melatonin and Desorption with Surfactant Aqueous Solutions

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In this work, a dispersive solid phase extraction method has been developed for an easy and cheap sample preparation method for the analysis of melatonin, a hormone produced by the pineal gland that regulates the sleep-wake cycle. The mixtures of sepiolite (SEP) clay and graphene (G) carbon nanomaterial have been used previously as sorbents for the analysis of riboflavin, polycyclic aromatic hydrocarbons and tryptophan [1-3]. In this work, different G/SEP mixtures have been tested for the analysis of melatonin with the aim to isolate the analyte from the sample matrix and possible matrix interferences. The best conditions of the extraction method were attained for G/SEP (4/96 w/w) mixtures, in which the melatonin retention was quantitative. For the desorption step, a 60 mM aqueous solution of a non-ionic surfactant (Brij L23) was used, which was proven to be a good alternative to conventional organic solvents by comparison of the results with MeOH, which are possible carcinogens to humans. This extraction procedure was tested in herbal tea samples spiked with two different melatonin concentrations, and the extracts were analysed by fluorescence and HPLC, leading to high recoveries of 100 and 102%, which demonstrates the accuracy of the method developed herein.

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FC8. Desenvolvimento e otimização de uma nova metodologia para determinação de antidepressivos em plasma com extração por MEPS e análise por GC-MS/MS

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O consumo de antidepressivos constitui uma problemática mundial e Portugal apresenta uma das taxas de doenças mentais mais elevadas da Europa[1]. A monitorização terapêutica está estabelecida para um pequeno número de medicamentos para os quais se verifica uma relação direta entre concentração e efeito farmacológico. Este trabalho tem como objetivo desenvolver uma metodologia para deteção de antidepressivos (fluoxetina, venlafaxina, citalopram, sertralina, paroxetina e metabolitos) em 250 µL de plasma por microextração em seringa empacotada (MEPS) e cromatografia gasosa acoplada à espectrometria de massa em tandem (GC-MS/MS).

A técnica de MEPS foi otimizada utilizando a ferramenta estatística *Design of Experiments* e o número de *strokes* foi o único fator significativo. O procedimento final de extração foi: adicionar 250 µL de amostra com 500 µL de acetonitrilo, centrifugar, decantar e evaporar; dissolver o resíduo com 1 mL de tampão fosfato 25 mM (pH 5), adicionar o padrão interno e homogeneizar; acondicionamento com 250 µL de metanol e 250 µL de 0,1% de ácido fórmico em H_2O; *loading* de 12x150 µL de amostra; lavagem de 4x50 µL com 1% de ácido fórmico em H_2O; secagem por aspiração de 4x50 µL de ar; eluição de 6x100 µL com 1% de hidróxido de amónia em metanol; evaporar até a secura, derivatizar durante 2 minutos com microondas a 800 W e injetar 2 µL no sistema cromatográfico. Foram obtidas recuperações entre 12-93% e limites de quantificação entre 10-100 ng/mL. Este é o primeiro trabalho a utilizar a MEPS e GC-MS/MS na determinação de antidepressivos e metabolitos em amostras de plasma.

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FC9. Desenvolvimento de um método por GC-MS para a confirmação de opiáceos em bílis após triagem por Imunoensaios

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De acordo com o metabolismo hepático, os xenobióticos e respetivos metabolitos sofrem excreção biliar ou uma possível circulação entero-hepática. Assim, a bílis pode constituir um reservatório de substâncias metabolizadas e excretadas pelo fígado, sendo considerada uma matriz alternativa viável na análise de drogas de abuso. No entanto, são escassos os estudos que se encontram publicados na literatura sobre a sua utilização recorrendo às metodologias analíticas de triagem por imunoensaios seguida de confirmação por cromatografia de gases – espectrometria de massas (GC-MS). O objetivo deste trabalho foi desenvolver um método de confirmação de opiáceos em amostras de bílis por GC-MS após triagem por imunoensaios.
A preparação das amostras foi realizada de acordo com as metodologias analíticas validadas e usadas na rotina do Serviço de Química e Toxicologia Forenses da Delegação do Centro do Instituto Nacional de Medicina Legal e Ciências Forenses, I.P. para a análise de opiáceos em sangue: triagem por imunoensaios, seguida de precipitação proteica, extração dos analitos com colunas Oasis® MCX (3 mL, 60 mg), derivatização por microondas dos extratos secos obtidos (MSTFA/5% TMCS, 90 segundos) e, posterior confirmação dos analitos presentes por GC-MS.
Em todos os casos de triagem positiva para opiáceos, foi possível detetar e confirmar por GC-MS os analitos morfina, codeína e 6-acetilmorfina, com limites de deteção de 10 ng/mL.
O método desenvolvido demonstrou ser útil para a confirmação de opiáceos em bílis por GC-MS, após triagem positiva pela técnica de imunoensaios enzimáticos, sobretudo quando a disponibilidade da matriz *postmortem* é determinada pelo caso sob investigação.

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FC10. Documenting human exposure to cannabinoids using oral fluid

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The importance of studying non-conventional biological matrices such as oral fluid (OF) is increasingly being recognized. This sample presents several advantages, mainly related to its collection procedure: it is non-invasive, easy to perform by non-medical personnel, can be performed under supervision to prevent adulteration, and provides low biohazard risk. OF samples are more likely to contain parent drugs, reflecting recent drug use – a major advantage of this matrix[1].

A fast and robust analytical methodology was developed in OF samples for the determination of tetrahydrocannabinol (THC), 11-hydroxy-tetrahydrocannabinol (THC-OH), 11-carboxy-tetrahydrocannabinol (THC-COOH), cannabinol (CBN) and cannabidiol (CBD) by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), aiming at documenting cannabis consumption. Briefly, 200-µL aliquots of OF were subjected to protein precipitation with a refrigerated methanol/acetonitrile mixture (80:20, v/v). After centrifugation, the extracts were evaporated to dryness, reconstituted in methanol, and 5-µL aliquots were injected into the UPLC-QTRAP-MS 6500+ (SCIEX®) system (in a 14-minute run). The analysis was carried out in MRM mode with two transitions for each compound and one transition for each internal standard.

The method was validated according to the guidelines of ANSI/ASB 036[2]. Parameters such as ion suppression/enhancement, interferents, linearity, precision and accuracy, limits of detection and quantification, dilution integrity and stability were studied and showcased satisfactory results. The 2 ng/mL cut-off for THC[3] was achieved, and the method was successfully applied to real samples (57.95-898.28 ng/mL for THC; 0.17-4.09 ng/mL for THC-COOH; 1.26-44.57 ng/mL for CBN; 0.42-1007.86 ng/mL).

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FC11. Unveiling the relationship between stopper and wine by GC×GC: a step forward to pairing wine and stopper

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The sensory characteristics of wine are a topic studied by several researchers over time, but it continues to be a current and challenging subject. These characteristics are fundamental for the consumer acceptability, which has increasingly aroused their interest to modulate them in line with current market trends and innovation demands. The wine physical-chemical and sensory properties depend on a wide set of factors: they begin to be designed in the vineyard and are later constructed during the various stages of winemaking. Afterwards, the wine is placed in bottles and stored until commercialization. During the storage of bottled wine several physical-chemical changes may occur, modulated by the position of the bottle, type of closure, temperature, and storage time, which impact the oxygen entrance ratio. In fact, the permeability of the stoppers to oxygen is considered one of the most important properties that influences wine sensorial properties during post-bottling (1,2). In the present study, red and white table wines stored in a horizontal position, using natural cork stoppers, different types of microagglomerated cork stoppers and a synthetic one, were characterized. To comprehend the changes that may have occurred during bottling, a set of analysis were implemented: determination of volatile components by comprehensive gas chromatography- mass spectrometry with time-of-flight analyser (GC×GC-ToFMS), sensorial analysis performed by a trained panel, and also determination of colour, acidity (total and volatile), SO^2 (free and total), and pH. The strategy used in this study provides new chemical data that allow evaluating the effect of the stopper among different type of wines. Physical-chemical and sensory analysis unveiled that the type of stopper modulates the characteristics of the wine, and its selection may be used as an oenological tool in the construction of the wine identity.

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FC12. Comprehensive analysis of the aroma profile of Tawny and White Port wines with different indication of age using a multiple headspace solid-phase microextraction method

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Port wine is a fortified wine produced in the Douro Demarcated Region (DDR) in Portugal and commercialised worldwide. The production process involves using specific authorised grape varieties and following traditional winemaking and ageing processes. The aroma profile of wines is a critical quality factor for consumer acceptance. In this work, the aroma profile of Port wine styles obtained through oxidative ageing were investigated, namely sixty-two White and sixty-four Tawny wines, and with increasing indications of age. The aim was to understand the similarities and differences in the oxidative ageing process of these two related but different matrixes. For this purpose, a precise and accurate method using Multiple Headspace Solid Phase Microextraction (MHS-SPME) coupled to GC-MS was developed and validated to quantify the volatile composition of White and Tawny Port wines. The SPME extraction conditions were optimised using a Box-Behnken design with three blocks and two replications, and similar conditions were found for both types of Port wine. The method demonstrated good linearity (0.001-50 mg/L), precision (<5%), and detection limits (<1µg/L), making it suitable for analysing volatile composition of White and Tawny Port wines with reduced costs and manipulation time, eliminating matrix effects. Twenty-three aroma compounds were identified and quantified in Tawny and, for the first time, in White Port wines, including acids, esters, and norisoprenoids [1]. This work aimed to expand the characterisation of the aroma of Port wines obtained through oxidative ageing, both White and Tawny Port wines, and to identify compounds with a significant impact on the characteristic aroma of Port wines.

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FC13. Assessing the Viability of Enhancing Brandy Quality Through the Utilization of a Dual-Wood Blend

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O envelhecimento de aguardentes com madeira é um processo tecnológico valorizador da qualidade final de bebidas. Nesta fase a aguardente adquire novos aromas, melhora as suas características sensoriais e adquire cor característica, que é valorizada pelo consumidor. A aguardente de mel é um subproduto da apicultura obtido pela fermentação do mel e com características aromáticas diferenciadoras[1].

Este trabalho teve como objetivo avaliar a potencialidade de um novo destilado à base de aguardente de mel envelhecida com madeira de Acácia-negra e Carvalho Francês. O ensaio teve uma duração de 3 meses e foi realizado à escala laboratorial usando, no envelhecimento, madeiras com queima média, sendo o grupo amostral composto por seis amostras (3 modalidades de envelhecimento: aguardente com madeira de acácia, carvalho e carvalho+acácia com duas réplicas de cada modalidade).

Para caracterizar este produto foi determinada a massa volúmica, título alcoométrico volúmico, extrato seco, acidez total, volátil e fixa, pH, características cromáticas, análise de voláteis por GC-FID e GC-MS e análise sensorial (com painel treinado).

Os resultados mostraram que para todas as amostras apenas nas análises de compostos voláteis se verificaram diferenças significativas. No entanto, os melhores resultados foram observados na amostra com a mistura das duas madeiras pois apresentou valores acidez total acima de 0,2 g/L Ác. acético e pH com valores perto de 5. Verificou-se a presença de compostos voláteis minoritários característicos do contacto com a madeira (guaiacol, β-metil-γ-octalactona, siringol e acetovanilona). Nos compostos voláteis minoritários verificou-se um teor nulo de metanol nas aguardentes, sendo os álcoois Isoamílicos e o Isobutanol os compostos mais representativos.

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FC14. Strategy for reducing the methanol content in strawberry tree-based spirits

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A aguardente de medronho é um produto muito apreciado e com grande tradição nos países mediterrânicos. Neste contexto foi criada uma estratégia de produção de uma bebida espirituosa a partir do fermentado de medronho ao qual se adicionou mosto de mel, tirando partido das características destes dois ingredientes e limitando assim o teor de metanol no produto final. Para a caracterização desta bebida foram identificados (GC/MS) e posteriormente quantificados (GC/FID) os seguintes compostos: metanol, acetaldeído, acetato de etilo e álcoois superiores. A análise sensorial da bebida foi efetuada por um painel treinado. Comparativamente com a aguardente de medronho o novo destilado apresentou valores inferiores de metanol (359,0 vs. 994,4 g/hL de A.P.), acetaldeído (20,5 vs. 25,6 g/hL A.P.) e acetato de etilo (35,5 vs. 53,9 g/hL P.A.), bem como características intermédias em relação à concentração de álcoois superiores. Relativamente ao perfil sensorial (estabelecido com base em 30 atributos de aroma e sabor), apenas quatro atributos foram significativamente diferentes entre estas bebidas espirituosas: frutos secos, untuoso, verniz e doce. Verificou-se que a estratégia proposta para a redução do teor de metanol em bebidas espirituosa à base de medronho mostrou ser uma opção interessante para o desenvolvimento de uma nova bebida, a qual é também vantajosa do ponto de vista da segurança e qualidade do produto final.

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FC15. Protein fraction analysis of six coffee by-products using RP-HPLC-FLD

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In the face of growing concerns about global food security, there is an increasing need to explore unconventional protein sources. An attractive approach involves the valorization of by-products generated by the food industry. The coffee industry, in particular, produces substantial quantities of by-products, with protein content ranging from 3% (parchment) to 13% (silverskin) in dry weight (dw)[1,2]. This study aims to characterize the protein fraction of six coffee by-products (pulp, husks, parchment, silverskin, defective beans, and sieving residue), including the determination of the amino acid (AA) profile and amino acid score (AAS)[3,4].

Total, non-protein, and protein nitrogen were determined using the Kjeldahl method. For the AA profile, alkaline hydrolysis (KOH 4 M, 110 ˚C, 4 h) and acid hydrolysis (HCl 6 M, 110 ˚C, 24 h) were employed to quantify tryptophan and the remaining AA, respectively. The hydrolysates underwent automatic derivatization with OPA/3-MPA and FMOC and were subsequently analyzed by RP-HPLC-FLD. The AA were identified based on the retention time of the respective standards and quantified using the internal standard method (norvaline)[2].

The results showed that silverskin is the coffee by-product richest in protein (14.34% dw). Except for parchment, all coffee by-products presented a complete profile of essential AA. The AAS ranged from 21.78% (husks) to 32.21% (defective beans), with methionine as the primary limiting AA. In general, the most abundant AA were glutamic acid, aspartic acid and leucine.

In summary, this study identified silverskin, defective beans, and sieving residue as promising protein sources, addressing food security concerns.

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Authors acknowledge FCT/MCTES for the national funds received through the projects UIDB/50006/2020 and UIDP/50006/2020. Macha do M thanks FCT/MCTES and ESF through NORTE 2020 for her PhD grant 2021.04907.BD. Alves R thanks FCT for funding through the Scientific E mployment Stimulus - Individual Call (ref. CEECIND/01120/2017, DOI 10.54499/PTDC/SAU-NUT/2165/2021). The authors also thank to JMV José Maria Vieira S.A. and Quinta do Avô João, for kindly provide the samples for the study.

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FC16. Identification of Polyethylene terephthalate (PET) photodegradation products by LC-MS

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A good chromatographic separation of unknown compounds is always a challenge. The need of mass spectrometry for identification also requires compatibility between the mobile phase and the ion source. Electrospray ionization (ESI) is commonly used with liquid chromatography (LC) because it works well with polar and aqueous mobile phases[1]. Atmospheric pressure chemical ionization (APCI) is also used with LC but for less polar compounds[2].

In this work, we evaluate combinations of two mobile phases, water/methanol, and water/acetonitrile, and two ion sources, ESI and APCI, to study the products released from PET after photoreaction. The study was performed under both positive and negative polarities.

The obtained products were annotated using Compounds Discoverer 3.3 and some were identified after injection of standards. The negative polarity gave more information than the positive polarity, which is related to the presence of carboxylic moiety in the structure of compounds. However, the positive polarity is complimentary as different products were detected. Acetonitrile strongly decreases the signals observed under APCI when compared with ESI. However, the use of methanol instead of acetonitrile improves the APCI signals but changes the retention times. As some compounds are more readily detected under APCI than ESI and *vice-versa*, the identification of unknowns should be made using the two ionization types.

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FC17. A new method for extraction of pesticide residues from soil by direct-immersion Solid Phase MicroExtraction (SPME)

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The analysis of pesticide residues in soil is a changeling task due to the matrix´s chemical and physical variability (texture, pH, mineral nature, organic matter content, etc.), and to the trace concentrations at which the analytes are generally present.
We present a new method for qualitative/quantitative analysis of pesticides in sandy-loam soil by direct-immersion SPME followed by GC-MS/MS analysis. The analytes were: Boscalid, Diflufenican, Epoxiconazole, Indoxacarb, Metalaxyl, Metolachlor, Metribuzin, Penconazole, Tebuconazole and Terbutylazine. The extraction was performed with a new type of semi-disposable SPME configuration using a PDMS/DVB fiber sealed on a micropipette tip. Slurry samples were made by adding an aqueous solution (6% methanol v/v) to 2 grams of soil. The fibers were conditioned and pre-wetted to solvate the coated phase and then inserted, for extraction, into the samples for 75 minutes with constant shaking. Afterwards, the analytes were desorbed onto 100 µL of methanol for 30 minutes. After the addition of analyte protectants [1] (ethylglycerol, gulonolactone, and sorbitol; 500 µg/mL) the extract was analyzed.
Calibration was performed by extracting spiked soil with analyte concentrations of 0.1-50 µg/kg. Isotopically labeled penconazole was used as internal standard. Coefficients of determination were between 0.94-0.97 for all analytes. Limits of quantification range between 0.1-10 µg/kg. This method can be easily automated and generates almost no residual toxic waste. Thus, it has the potential to be introduced as a greener and simpler alternative to the currently used methodologies.

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FC18. Analysis of volatile carbonyl compounds released from Medium Density Fibreboards following gas-diffusion microextraction and HPLC-DAD-MS/MS

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Medium Density Fibreboard (MDF) is one of the most known and used products from the wood-based panels (WBPs) industry. These products are known to emit volatile organic compounds (VOCs) and are a source of indoor air pollution [1]. The emission of VOCs can result from the raw materials, chemical additives, or processes used during panel production. Different studies indicate that aldehydes and terpenes are the most common emitted compounds, with different emission percentages depending on the type of wood [2].
Gas-diffusion microextraction (GDME) is an innovative sample preparation technique for volatile compounds, in which the analytes from the sample pass through a gas-permeable membrane into an acceptor solution, typically a derivatizing agent. With GDME, minimal sample manipulation is required and a clean extract relatively free of interfering compounds can be attained [3,4].
In this work, GDME was combined with a derivatization reaction with 2,4-dinitrophenylhydrazine (DNPH) for the selective extraction of volatile carbonyl compounds from MDF samples, towards their high resolution HPLC-DAD-MS/MS analysis and identification. Thirty volatile carbonyl compounds were able to be identified using this approach, including aliphatic and unsaturated aldehydes, ketones, dicarbonyls and several derivatives of benzaldehyde, resulting from the depolymerization of lignin. Additionally, different emission profiles were observed among samples of coloured MDF, which may indicate the influence of the dye used and other fixing conditions on the formation and potential release of volatile compounds.

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FC19. Determination of the chemical profile of extracts from the roots of the plant species *Dizygostemon riparius* (Plantaginaceae) using the HPLC-DAD method

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Dizygostemon riparius (Platanginaceae) is a recently cataloged species[1,2] found in São Benedito do Rio Preto, Maranhão, Brazil. Chemical studies using the leaves and roots of this plant have shown a predominance of phenolic compounds, mainly flavones[3]. However, a lack of research is available detailing this species' chemical classes and structures. With this in mind, the aim of this paper was to investigate the chemical profile of the extracts from the roots of *D. riparius* through High Performance Liquid Chromatography (HPLC) with Diode Array Detector (DAD). The extracts were prepared ultrasound-assisted extraction with the solvents hexane (HExt), ethyl acetate (EtOAcExt), ethanol (ETOHExt), and water (AQUExt), and analyzed by HPLC with manual injection, volume of 25.0 µL, and C18 column (250 x 4.6 mm - 5 µm). The mobile phase was millikali water (A) and HPLC-grade methanol (B), both acidified to 0.1% formic acid (CH2O2), flow rate 0.9mL/min, at 28 °C, gradient elution, varying B from 5 to 100% in 70 minutes and 100% of B in 10 minutes. The HExt chromatogram (Figure 1), at 254 nm, showed no bands at the start of the chromatographic run, indicating that it is mostly composed of medium and low polarity metabolites. EtOAcEtx had a distribution of peaks throughout the chromatographic run, showing good metabolic diversity when compared to ETOHExt and AQUExt, as well as containing metabolites of different polarities. Therefore, EtOActExt is a good sample for studying this plant species, due to its greater variability in band distributions, representing a larger class of metabolites.

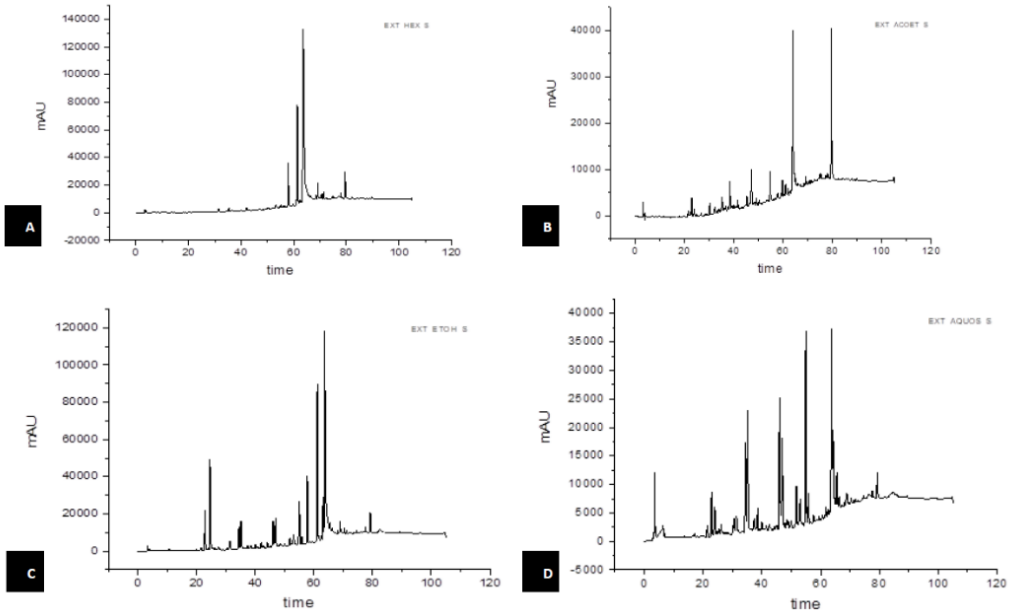

Figure 1 – Chromatograms of hexane (A), ethyl acetate (B), ethanol (C), aqueous (D) extracts of the plant species *D.riparius* (Plantagibnaceae)

Acknowledgements: FAPEMA for granting the master's scholarship; to Professor José Antônio Cantanhede Filho for guiding the work; and to everyone involved in the execution of the project.

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FC20. Preliminary chemical profile by HPLC/DAD of organic precursor for green synthesis of metallic nanoparticles

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Dizygostemon riparius (Plantaginaceae) is a new plant species found in the municipality of São Benedito do Rio Preto, Maranhão, Brazil. The first work of the chemical profile of leaf extracts of the lilac morphotype of the species, revealed the presence of polymethoxyflavones, belonging to the class of polyphenols, phytochemicals with reducing potential. Given the promising potential of the species and the absence of studies on the branches of the plant, the present work used High Performance Liquid Chromatography (HPLC) to investigate the chromatographic profile of the aqueous extract of the branches of the species *D. riparius*. The analyses were performed in a liquid chromatograph of Shimadzu®, coupled to DAD detector model SPD-M20A and column C18 of Luna-Phenomenex (250 mm x 4.6 mm x 5 um). A gradient was used with mobile phase A (0.01% formic acid in water) and mobile phase B (0.01% formic acid in methanol), being operated with flow of 0.9 mL/min at 28 °C, with exploratory gradient with the concentration of B ranging in the range of 5 to 100% in 70 minutes and more 100% of B in 10 minutes. In Figure 1, the chromatogram presents 14 peaks, 3 of which have higher intensity, which can be associated with phenolic compounds, flavones and flavonols, which are detected at wavelengths of 270, 365 or 370 nm. The study is promising, because it is the preliminary chemical profile of the aqueous extracts of the branches by HPLC-DAD, which exhibit bioactives with reducing potential in green synthesis routes of metallic nanoparticles.

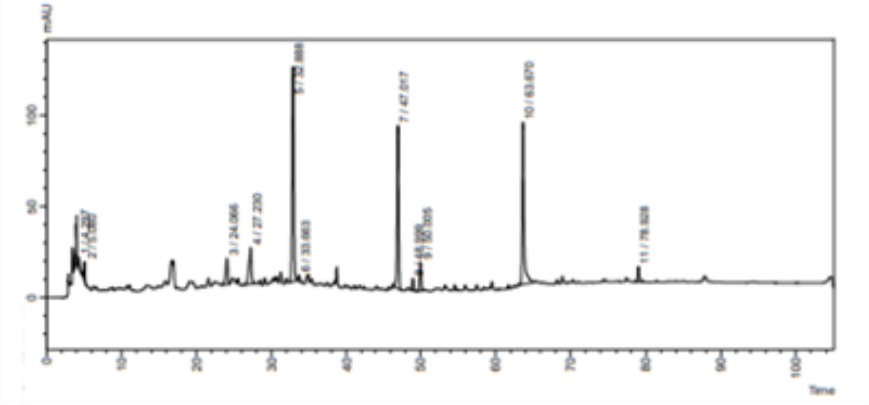

Figure 1. Chromatogram of the aqueous extract of the branches of the lilac morphotype of the plant species *Dizygostemon riparius* (Plantaginaceae). Source: Own Author (2023).

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FC21. Analysis of biogenic volatile organic compounds in shrub leaves using headspace-bar adsorptive microextraction

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Currently, one of the major environmental concerns is the frequent occurrence of forest fires, particularly under extreme atmospheric conditions. Some studies suggest that higher temperatures lead to a greater emission of biogenic volatile organic compounds (BVOCs), produced and accumulated in different plants, becoming extremely flammable gases in the event of forest fires. Consequently, in the presence of an ignition source, BVOCs can contribute to the spread of forest fires, leading to catastrophic events, as was the case with the 'Pedrogão Grande' tragedy (Portugal) in 2017[1,2]. Thus, it becomes relevant to study the composition of BVOCs even more in depth, especially the terpenoid fraction, consisting of compounds such as α- and β-pinene, limonene, 1,8-cineole, and thymol, since they are among the most abundant monoterpenes in trees and shrubs, and even some oxygenated sesquiterpenes, such as caryophyllene oxide[3]. Therefore, it is important to develop and apply effective methodologies that allow the identification of the main BVOCs present in shrubs, highlighting the use of analytical tools such as bar adsorptive microextraction in the headspace mode, a cost-effective, easy-to-use, and eco-friendly technique combined with gas chromatography-mass spectrometry (HS-BAμE/GC-MS)[4].
The present work aimed to apply, optimize, and validate the HS-BAμE/GC-MS methodology to monitor the main BVOCs emitted from several common shrubs in Portugal, namely *Cistus Ladanifer* and *Cistus Monspeliensis*, *Erica Scorparia*, *Lavandula Stoechas* and *Thymus Villosus*. The performance, advantages and limitations of this new analytical approach are also addressed[5] and compared with well-established passive techniques, such as headspace solid phase microextraction (HS-SPME), proving to be a promising alternative methodology.

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FC22. Recovery Optimization and Exploring Food Industrial Applications of Bioactive Compounds in *Thymus* Genus through HPLC-DAD-ESI/MS Analysis

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The natural food preservatives market is continuously expanding, driven by the increasing demand for healthier and safer ingredients, which also have a reduced environmental impact [1,2]. In this study, two species from the *Thymus* genus, native to the Iberian Peninsula (*Thymus mastichina* L. - ETM and *Thymus pulegioides* L. - ETP), underwent a detailed analysis of their phenolic composition and extraction yield, aiming to obtain extracts with high preserving capacity for the food industry. The extraction process was optimized using response surface methodology, employing two extraction methods - heat-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE). The considered variables for optimization included extraction time, solvent concentration, and temperature/power, with extraction yield and the major phenolic compounds identified as the responses to optimize the extraction technique applied. The extracts obtained under optimized conditions were subsequently subjected to the analysis of their phenolic profile by HPLC-DAD-ESI/MS. Results showed that the major compound in both samples was rosmarinic acid. UAE proved to be the most effective technique for ETM, reaching the highest yield at 9.58 minutes, 73.83% (range), and 51.65% (ethanol) for rosmarinic acid. The MAE technique was more suitable for ETP, where rosmarinic acid also emerged as the compound of primary interest under optimal extraction conditions at 78,06 minutes, 81,75°C, and 43,21% (ethanol). It is worth noting that both UAE and MAE extraction techniques exhibited a broader diversity of compounds in the ETM extract compared to the ETP extract. Consequently, ideal conditions for extracting preservative compounds from Iberian herbs (ETM and ETP) were established, allowing the production of extracts with remarkable functional properties, thus validating their potential application in the food industry.

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FC23. Comparative Analysis of the Phytochemical Profiles in the Flowers and Pods of *Acacia mearnsii*

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As espécies do género *Acacia* são plantas invasoras e extremamente agressivas em vários territórios, representando uma ameaça significativa à biodiversidade e ao equilíbrio dos ecossistemas.
Alguns estudos têm sido realizados sobre a composição e atividade biológica em diferentes partes da árvore.
Este trabalho tem como objetivo caracterizar extratos de *Acacia mearnsii* para potencial aplicação industrial.
Nesse sentido, foram analisados extratos de flores e de vagens preparados com material recolhido em 2022 em Alcobaça (Vimeiro), obtidos e tratados de acordo com a metodologia descrita por Pedro *et al.* [1].
Foram analisados os compostos fenólicos e polifenólicos utilizando cromatografia líquida de alta eficiência (HPLC) acoplada a um detetor de díodos (DAD) e cromatografia líquida acoplada a espetrometria de massa de alta resolução de tandem com ionização por *electrospray* (LC-ESI-HRMS/MS).
Foram identificados e quantificados 20 compostos sendo que as flores apresentaram uma matriz mais elaborada no que se refere ao número de compostos. As flores apresentam quantidade significativamente maior de vanilina, enquanto, nas vagens o composto maioritário foi a rutina. Comparando os compostos que aparecem em ambos os extratos, foram encontrados (+)-catequina, ácido *p*-cumárico, naringenina e quercetina.
Esta espécie posiciona-se num nível semelhante relativamente ao número de compostos identificados em vagens comparativamente a outras espécies estudadas por Pedro *et al.* [2]. No que diz respeito às flores, e comparando com a *Acacia retinodes [3],* verifica-se que a *Acacia mearnsii* apresenta menor número de compostos detetados, no entanto, esta apresenta maior concentração do composto vanilina, ácido *p*-cumárico, ácido cinâmico e naringenina.

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FC24. Chromatographic profiles of selected hydrophilic and lipophilic constituents of edible and non-edible parts of rhubarb leaves

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Rhubarb (*Rheum rhabarbarum* L.) is an herbaceous perennial plant with high potential in the food and nutraceutical industry. While the stalks are traditionally used in various culinary preparations, the green leaf is discarded as a by-product due to the high levels of oxalic acid, which can cause kidney lithiasis [1]. Rhubarb is also used in traditional medicine for its anti-inflammatory, neuroprotective, antibacterial, and laxative properties [2-4]. Despite the wide range of potential applications of this species, it remains little explored by both the industry and the scientific community. Therefore, this work was carried out with the aim of characterizing the profiles of hydrophilic and lipophilic compounds in edible stems and inedible leaves of rhubarb. The plant samples were produced in the Bragança region, Portugal. The profiles of free sugars, organic acids and tocopherols were characterized using high-performance liquid chromatography techniques with different types of detection. The fatty acid profiles were analyzed by gas chromatography with flame ionization detection, after derivatization of the lipid fraction obtained by Soxhlet extraction [5]. This study allowed identifying the reducing sugars fructose and glucose and oxalic and ascorbic acids as important hydrophilic constituents of both samples. In tun, α-tocopherol stood out as an abundant fat-soluble vitamer, while the lipid fraction consisted mainly of polyunsaturated fatty acids. Overall, this work contributed to increasing knowledge about the food potential of this plant and the results could be useful to complete food composition tables and to promote the valorization of its by-products.

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List of the posters

Number	Title	Presenting author
P1	Nutrient profile of onions and carrots irrigated with treated wastewater	Joana José
P2	LC-MS/MS as a pivotal tool to steer selenium biofortification strategies in *Pleurotus* species	Matilde Rodrigues
P3	Bioaccumulation of pharmaceutical active compounds in clams (*Ruditapes decussatus*) exposed to a zone influenced by the discharge of a wastewater treatment plant: a case study in the Ria Formosa lagoon	Cristina Almeida
P4	Development and validation of an HPLC method for the quantification of vitamin D3 in wild and farmed salmon	Helena Gonçalves
P5	Development and validation of HPLC method for simultaneous determination of water-soluble vitamins in meat	Cristina Roseiro
P6	Perfil de hidrocarbonetos aromáticos policíclicos em carne de frango grelhada no carvão	Cristina Serrano
P7	Chemical characterization of fermented saffron flowers as a source of high added-value ingredients	Carolina ventura
P8	Development and validation of a high-performance liquid chromatography method for the quantification of gallic acid and rutin in fermented papaya	Patrícia Correia
P9	Impact of different pasteurization methods on the purple passion fruit (*Passiflora edulis* Sims *edulis*) juice potential aroma: on the route of natural characteristics	Silvia Rocha
P10	Importância do sistema de deteção para a avaliação da presença de vitamina E em queijo	Thiago F. Soares
P11	New insights on the isolation of anthocyanins from complex matrices using cation-exchange solid-phase extraction	Hélder Oliveira
P12	Emo-sensory And Volatile Profile of Iberian Wine Vinegar	Catarina Marques
P13	Assessment of Volatile Compounds Associated with the *'Brett Character'* in Wine and Its Sensory Impact	Ofélia Anjos
P14	Does the Use of Different Cork Stoppers Affect the Quality of Sparkling Wines?	Sara Sofia Pinheiro
P15	Exploring Gas Chromatography tools for monitorization of phthalates	Matilde A. Costa
P16	Analysis of the chemical profile by HPLC/DAD of the hydroalcoholic extract of the roots of the lilac morphotype of the plant species *Dizygostemon riparius* (Plantaginaceae).	Jeovana Thaynara Oliveira Martins
P17	Nutritional, chemical profile, and antioxidant activity of wild fruit extracts *Afromomum alboviolaceum* (Ridl.) Hochst. and *Sclerocarya birrea* (A. Rich.) Hochst. pulp and peel from Cuanza Sul, Angola	Claudete Bastos
P18	Insights into the Bioactivities and Chemical Analysis of *Ailanthus altissima* (Mill.) Swingle	Débora Caramelo

Number	Title	Presenting author
P19	Development of an emamectin benzoate insecticide analysis method in *Quercus suber* leaves	Ana I. Mirandela
P20	Validation of a Confirmation Method for Hypoxia-inducible factor (HIF) activating agents in human urine by LC-MS/MS in Doping Analysis	David Lopes Pereira
P21	GC-C-IRMS: An effective tool for identifying the origin of organic compounds - Application to steroids consumption.	Ana Cecília Rocha
P22	Validation of a Quantitative Method for Morphine, Codeine, Ethylmorphine and Norethylmorphine in human urine by LC-MS/MS in Doping Analysis	David Lopes Pereira
P23	Integration of chromatographic and metabolomics data as a useful tool to identify putative urinary prostate cancer biomarkers	José S. Câmara
P24	Preliminary pharmacokinetic assays of CBD administration to cats with Feline Chronic Gingivostomatitis	Andreia Bento-Silva
P25	Quantification of quorum sensing signaling molecule autoinducer-2 in biological samples by gas chromatography - mass spectrometry	Miguel Viegas Rodrigues
P26	Steroid hormone analysis in blood samples of postmenopausal women by solid phase extraction and gas chromatography-mass spectrometry	Ana Teresa Brinca
P27	Determinação da cocaína e metabolitos em águas residuais com recurso à extração em fase sólida e à cromatografia de gases acoplada à espectrometria de massa em tandem	Gonçalo Catarro
P28	Determination of Glyphosate and AMPA in Clean Waters by HPLC-FLD	Georgina Sarmento
P29	Development, validation and application of a high-performance liquid chromatography method for the analysis of 5- Fluorouracil residues in infusion pumps used in chemotherapy treatments	Patrícia Correia
P30	Assessment of mineral and organic compounds from livestock effluents	Eugenia Gallardo
P31	Determination of Cannabinoids in Urine Samples by Bar Adsorptive Microextraction and Gas Chromatography-Mass Spectrometry	Maria Beatriz Pereira
P32	Qualitative determination of Alkyl Amines Stimulants in urine matrices applied to Doping Control Context through Bar Adsorptive µ-Extraction	Carlos Vitor de Paiva e Almeida
P33	A comparative study of SPE and DLLME based methods for the analysis of benzodiazepines and opioids in urine samples by LC-MS/MS	Luana May Rosendo
P34	Monitorização de antidepressivos: comparação de dois métodos de extração em amostras de fluido oral	Sofia Soares
P35	Miniaturized Solid Phase Extraction Techniques Applied to Natural Products: A Review	João Martinho
P36	Microextraction by packed sorbent in forensic drug analysis	Rodrigo Pelixo
P37	Enhancing Pesticide Extraction from Soil with a Novel Solid Phase MicroExtraction (SPME) Fiber Design: Development and Method Optimization	Raquel Carvalho
P38	Bar adsorptive microextraction (BAµE) - An alternative analytical tool to monitor β-blockers in biological and environmental matrices	Mariana F. Mendes

Number	Title	Presenting author
P39	Application of the innovative hollow fiber microextraction (HFµE) to monitor trace levels of pyrethroids in aqueous matrices	Renata Miguéis
P40	Development of a SPE-HPLC-DAD procedure to collect and analyse volatile organic compounds released from wood-based panels	Raquel Torres Fernandes
P41	Determination of trace levels of organophosphate pesticides in water matrices by BAµE/GC-MS(SIM)	Maria Soares
P42	Detecção de pesticidas em azeite: a explorar uma abordagem baseada em sensores	Raquel Garcia
P43	GCxGC using the INSIGHT Flow Modulator: What's going one?	Maria João Cabrita
P44	Special metabolites isolated from the leaf extract of *Amburana acrena* Ducke, a specie native to Brazil	Adrianne Bastos Ferreira
P45	Discovering characteristic molecular markers of fortified wines based on volatilomic pattern obtained by HS-SPME/GC-MS	Teresa Abreu
P46	Unravelling value-added compounds from grape pomace based on chromatographic data analysis	Teresa Abreu
P47	Singularities and applications of bio-oils: a perspective using Two Dimensional Gas Chromatography (GCxGC)	Patricia Borim
P48	Fingerprinting the phenolic composition of spent coffee grounds from different geographical regions using µ-SPEed extraction combined with UHPLC-PDA	Rosa Perestrelo
P49	A chromatographic-based approach as platform to identify putative biomarkers from different apple varieties	Rosa Perestrelo
P50	Prescriptomics	José Luis Capelo
P51	Unraveling Insights through Targeted Tandem Mass Spectrometry with a Triple Quadrupole Mass Spectrometer	Maria João Nunes
P52	Amino acids profile of wild boar (*Sus scrofa* L.) meat using automatic online pre-column derivatization: method optimization and validation	Thiago Freitas Soares
P53	Optimizing Proteomic Analysis: Normalization Methods in Mass Spectrometry for Renal Cell Carcinoma Datasets	Pedro A.D. Teigas-Campos
P54	Decoding the chemical ecology for control of the Western conifer seed bug, *Leptoglossus occidentalis*, with green analytical tools	Sofia Branco
P55	Archaeological biomarkers on cooking ware from the Roman Cistern at Spoletino, Viterbo, Italy	Cristina Barrocas Dias
P56	Application robustness of the 6495 triple quadrupole LC/MS system for non-stop Pesticide analysis in black tea matrix	Angel Antelo
P57	Characterization and antimicrobial activity of a surfactin mixture produced by *Bacillus subtilis* ATCC 6633	Noélia Duarte
P58	A Direct Comparison of Applying Helium & Hydrogen Carrier Gases with HS-SPME-GC-TOF-MS Analysis of Aroma Active Compounds in Whisky	Nick Jones

P1. Nutrient profile of onions and carrots irrigated with treated wastewater

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The agricultural sector is responsible for the consumption of about 75 % of the total water used in Portugal [1]. With the increase in water demand worldwide and the scarcity in some regions, there is a need to find different mechanisms to guarantee that demand, namely in the agricultural sector. One of the solutions for reducing the consumption of freshwater in agriculture is the use of treated wastewaters (TWW) in irrigation. The reuse of TWW contributes to the reduction of the environmental impact by reducing the discharge of water into the environment and to the circular water system. Another advantage of the reuse of TWW is their rich composition in essential nutrients for crop growth, decreasing the need for fertilization. However, the TWW do not only contain beneficial nutrients for plants. Due to industrial wastewater, TWW can be contaminated with toxic metals that can impact the nutrient profile of crops and affect public health.
In this work, the nutrient profile of onions and carrots irrigated with TWW and TWW in the presence of toxic metals (Pb, Cr, Cd and Ni) was studied using ion chromatography. The samples of the vegetables were dried and ground, and the extraction of water-soluble ions was performed using an ultrasonic probe. Before the analysis the extract was filtered. Due to matrix effect, chloride, nitrite, nitrate, phosphate, and sulphate concentrations were determined for both onions and carrots irrigated with TWW and TWW with toxic metals using the standard addition method.
Results show the effect in the concentration of this anions in the vegetables for using these two waters in their irrigation.

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P2. LC-MS/MS as a pivotal tool to steer selenium biofortification strategies in *Pleurotus* species

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Pleurotus are commonly cultivated mushroom species capable of producing a large amount of protein [1]. The nutritional value of these protein-rich foods can be improved through biofortification with essential micronutrients such as selenium. This element is generally added in inorganic form to the mushroom lignocellulosic substrate and is then metabolized into organic compounds such as selenoamino acids [2]. This work aimed to produce *Pleurotus* spp. biofortified with sodium selenite and selenate and to identify which of these inorganic forms produces the best quality protein. For this study, *P. citrinopileatus*, *P. ostreatus*, and *P. djamor* were grown on a lignocellulosic substrate supplemented with different concentrations of sodium selenite and selenate. Control mushroom samples were grown without adding selenium. After harvesting, the levels of selenium and other mineral elements in the mushroom carpophores were determined by AAS, the nitrogen content was quantified by the Kjeldahl method, and total and free amino acid profiles were characterized by LC-MS/MS. The crude and true protein contents were estimated based on the nitrogen and amino acid composition, while protein quality was assessed based on the amino acid profile. After analyzing all the results, it is expected to identify the most promising inorganic selenium form and concentration to biofortify the selected macrofungi and to elucidate how protein quality is affected by selenium supplementation. Therefore, an increase in selenoamino acid levels could result in more nutritious foods since these organic selenium forms are well absorbed by the human body after digestion [3].

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P3. Bioaccumulation of pharmaceutical active compounds in clams (*Ruditapes decussatus*) exposed to a zone influenced by the discharge of a wastewater treatment plant: a case study in the Ria Formosa lagoon

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Many PhACs are not easily removed by conventional treatment processes in wastewater treatment plants (WWTPs) and could adversely affect the aquatic environment and surrounding biota. This study presents the PhACs bioaccumulation in clams *Ruditapes decussatus* exposed to realistic conditions in an area influenced by discharges from a nearby WWTP in the Ria Formosa Lagoon. Clams taken from a pristine site (control) were exposed at four different sites along a spatial gradient of dilution of the discharged effluents (down to 1.5 km) for 1 summer month for three years. Ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) quantified 24 PhACs in clams and marine waters after pretreatment of samples by QuEChERS (quick, easy, cheap, effective, rugged, and safe) method and SPE (solid phase extraction) method, respectively (1). Diclofenac presented the highest concentrations in Ria Formosa water samples from exposure sites, followed by carbamazepine and caffeine (on average ≤ 2 µg/L). However, it is important to emphasize that these PhAC levels in the marine waters represent solely a snapshot of the conditions occurring at the sampling moment. Caffeine was consistently the PhAC with higher levels in clams (0.54-27 ng/g ww), followed by acetaminophen 0.37 -3.7 ng/g ww). Spatially, there was a general decrease of caffeine along the dilution gradient of the effluents, although this was not so evident at the farthest sampling site. This can be associated with external sources of this PhAC due to the closeness of that site to Faro City, the most populated in the region.

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P4. Development and validation of an HPLC method for the quantification of vitamin D3 in wild and farmed salmon

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A vitamina D3 é uma vitamina lipossolúvel que desempenha um papel essencial no desenvolvimento ósseo e na homeostase do cálcio e do fósforo. A sua deficiência tem sido associada ao risco de várias doenças, tais como cardiovasculares, diabetes, certos tipos de cancro, doenças infeciosas ou outras doenças autoimunes e inflamatórias, bem como no risco de doença óssea metabólica[1,2]. A vitamina D3 é sintetizada na pele através da ação da radiação ultravioleta B, no entanto, cerca de 10-20% desta vitamina é obtida através da ingestão alimentar[3]. Alguns alimentos constituem uma fonte importante de vitamina D3, nomeadamente os produtos de origem animal como carne, peixe e ovos. O objetivo deste estudo foi desenvolver e validar um método para a determinação da vitamina D3 em salmão selvagem e de viveiro.
Um total de 20 amostras de salmão (10 amostras selvagem e 10 amostras de viveiro) foram homogeneizadas e saponificadas com uma solução etanólica de hidróxido de potássio. A vitamina D3 foi extraída com n-hexano e quantificada por HPLC-UV em fase normal. Determinaram-se os parâmetros de validação do método como a linearidade, os limites de deteção e quantificação e a taxa de recuperação.
As amostras de salmão de viveiro apresentaram um teor médio de colecalciferol de 8,04µg/100g, variando entre 4,93µg/100g e 12,96µg/100g. Os teores nas amostras de salmão selvagem foram significativamente superiores apresentando média de 20,06µg/100g, variando entre 10,83µg/100g e 23678,04µg/100g. Os limites de deteção e quantificação observados situaram-se em 0,07µg/100g e 0,23µg/100g, respetivamente. A taxa de recuperação do método obtida foi de 80,99%.

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P5. Development and validation of HPLC method for simultaneous determination of water-soluble vitamins in meat

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Water-soluble vitamins perform specific and vital functions in metabolism and are necessary for growth and development, which makes their quantification in food of great importance.
Conventional analytical methods used for vitamin quantification mainly require the determination of each vitamin individually[1,2]. High performance liquid chromatography (HPLC) has assumed wide applications because of its speed, high sensitivity and accuracy, even when the vitamins are present in small amounts and in complex matrices such as foods. The most important advances in the use of HPLC for the determination of water-soluble vitamins include the development of simultaneous determination methods for specific foods, validation of these methods, and improvement in the extraction and clean-up procedures[3]. The aim of this study was to develop and validate a method for the simultaneous determination of six water-soluble vitamins (thiamine, riboflavin, niacin, pyridoxine, folic acid, cobalamine) in meat by HPLC.
For this study, meat samples were obtained from 24 animals produced in Azores. Samples were extracted with sodium acetate 0,4M and submitted to enzymatic hydrolysis. Chromatographic separation was performed in reverse phase with a Waters Atlantis T3 C18 column. Validation parameters of the method such as linearity, limits of detection and quantification and recovery rate (accuracy) were determined[4].
Piridoxine and cobalamine showed the lower DL and QL (0,018mg/100g - 0,054mg/100g and 0,013 µg/100g - 0,039 µg/100g, respectively. Riboflavin presented the highest limits 0,079mg/100g and 0,240mg/100g, respectively. The recovery rates obtained (between 60% and 107%) demonstrate that the extraction method used is suitable for all vitamins under study.

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P6. Perfil de hidrocarbonetos aromáticos policíclicos em carne de frango grelhada no carvão

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Os hidrocarbonetos aromáticos policiclicos (HAPs) são compostos químicos que se formam durante o processamento de alimentos (secagem, fumagem) e cozedura a altas temperaturas (grelhar, assar, fritar)[1,2]. Alguns HAPs são bem conhecidos pelo seu caracter carcinogénico e mutagénico, constituindo, por isso, um risco para a saúde dos consumidores[3]. O objetivo deste estudo foi determinar o nível de contaminação destes compostos por HPLC em amostras de carne de frango grelhada no carvão e recolhidas nas regiões de Lisboa e Alto Alentejo.
Um total de 20 amostras de carne de frango grelhada foram saponificadas com KOH:água:metanol e extraídas com n-hexano. A fração contendo os HAPs foi evaporada até à secura e o resíduo redissolvido em acetonitrilo e finalmente medido por HPLC em fase reversa.
A carne de frango grelhada na região Lisboa apresentou um teor médio de HAPs superior (1687,7 µg.kg^{-1}) à carne de frango grelhada no Alentejo (1099,9 µg.kg^{-1}). O teor de BaP, devido ao seu elevado caracter carcinogénico, é utilizado como marcador da ocorrência de PAHs cancerígenos nos alimentos. Neste estudo, nenhuma das amostras recolhidas apresentaram um teor de BaP superior ao limite de 2,0µg.kg-1 estabelecido pela legislação da UE para este tipo de produtos, apresentando as carnes grelhadas em Lisboa um teor inferior (0,35µg.kg^{-1}) à região do Alentejo (0,40 µg.kg-1). Em relação ao índice PAH4, também as carnes grelhadas na região de Lisboa apresentaram teor médio inferior (8,67µg.kg^{-1}) às carnes grelhadas no Alentejo (11,9µg.kg^{-1}), tendo apenas 2 amostras ultrapassado o limite de 12 µg.kg^{-1} estabelecido pela EU para este índice.

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P7. Chemical characterization of fermented saffron flowers as a source of high added-value ingredients

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Saffron is used in several cultures for multiple purposes, from medicine to the food industry [1]. Usually, only the stigma is consumed, which generates high quantities of by-products. Therefore, it is important to develop strategies to decrease the amount of waste such as the use of these by-products in fermentative processes. Moreover, the bioactive compounds produced by the microorganisms in certain conditions will contribute to the valorization of the by-products.

The aim of this work is to evaluate the effect of fermentation conditions using saffron flowers as substrate in the production of bioactives. The different fermentation conditions were: % of yeast extract, water, glucose and saffron by-products as well as different microorganisms employed, incubation times and fermentative temperatures. The fermented samples were compared to unfermented controls. Chemical analyses were made using analytical methods to identify compounds usually found in saffron flowers [2, 3], namely: HPLC-DAD to quantify seven flavonols and two anthocyanins, Folin-Ciocalteau and ORAC method for the total phenolic content (TPC) and antioxidant activity, respectively, and SPME-GC-MS to determine volatile compounds [4].

Overall, there was no significant variation in antioxidant activity and TPC between fermented and non-fermented samples. The glucoside forms of some anthocyanins and flavonols showed a decrease probably due to the deglycosylation by microorganisms and, by contrast, the quercetin-3-glucoside increased when compared to the controls. Regarding the volatile compounds, samples with higher water content and lower saffron by-products showed more variations between the fermented and non-fermented samples, with the increase and/or formation of compounds in fermented ones.

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P8. Development and validation of a high-performance liquid chromatography method for the quantification of gallic acid and rutin in fermented papaya

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Several nutritional benefits are related with the use of papaya in food as fruit itself or as a fermented product. Phenolic compounds, among the most studied secondary metabolites, have improved the quality of life due to their potential therapeutic effects, being used as anti-inflammatory, antimicrobial and antioxidant agents [1]. The purpose of this study was the development and validation of a high-performance liquid chromatography with a diode array detector (HPLC-DAD) to quantify two phenolic compounds (gallic acid and rutin) in fermented papaya samples. A HPLC-DAD method was implemented using a octadecyl stationary phase and a mixture of acetonitrile and aqueous acetic acid as mobile phase in gradient mode [2]. The linear range obtained was 0.47-6.0 $\mu g \cdot mL^{-1}$ for gallic acid and 0.4-6.0 $\mu g \cdot mL^{-1}$ for rutin. Limits of detection and quantification in the low $\mu g \cdot mL^{-1}$ level (≤ 0.4 $\mu g \cdot mL^{-1}$) were attained. Precision, expressed by the coefficient of variation, ranged from 0.3 to 5.9% in standards of gallic acid and rutin, respectively. Accuracy, expressed as recovery and obtained by back calculation, was 98.8-109.0% for gallic acid and 98.3-113.2% for rutin. Papaya samples, at different fermentation stages, were successfully evaluated using this method and both compounds were quantified: rutin in the range 0.51 ± 0.04–4.47 ± 0.06 $\mu g \cdot mL^{-1}$ and gallic acid in the range 1.43 ± 0.01–3.42 ± 0.01 $\mu g \cdot mL^{-1}$. The proposed HPLC method allowed the efficient separation of the two phenolic compounds and proved to be precise and accurate, enabling their determination in fermented papaya samples.

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P9. Impact of different pasteurization methods on the purple passion fruit (*Passiflora edulis* Sims *edulis*) juice potential aroma: on the route of natural characteristics

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Passion fruit has an average global production of 1.5 million tons, most of which is consumed fresh or processed into juice[1]. Its purple variety (*Passiflora edulis* f. *edulis*) is considered more palatable due to its higher sweetness and lower acidity[2]. For the juice preparation, the fruit is submitted to a juice extraction procedure and then thermally processed to improve its shelf-life[3]. Aroma perception of juices is considered among the most relevant factors that influence the consumer's preferences and it is determined by the emitted volatile compounds. The objective of this work is to evaluate the impact of two different pasteurization methods in the volatile profile of purple passion fruit juice, and consequently to assess the methodology that better preserve the natural aroma of this juice. To fulfil this objective, the volatile compounds released from fresh juice (FJ), thermically pasteurized juice (TP – 88 °C, 15 s), and high-pressure pasteurized juice (HPP – 450 and 550 MPa, 5 min) were analysed by headspace solid-phase microextraction (HS-SPME) combined with comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-ToFMS). A total of 129 compounds were tentatively identified, encompassing several chemical families such as alcohols, carboxylic acids, aldehydes, esters, ketones, and terpenic compounds. It's worth noting that out of these, 76 compounds are being reported for the first time. Through multivariate analysis, it was shown that the volatile profile and passion fruit key aroma compounds composition of high pressure pasteurized juices are more similar to fresh juice than thermal pasteurized juices.

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P10. Importância do sistema de deteção para a avaliação da presença de vitamina E em queijo

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A vitamina E tem normalmente origem vegetal, no entanto, pode estar presente nos alimentos de origem animal, seja por uma alimentação rica em vegetais ou por enriquecimento do produto. A vitamina E é constituída por dois tipos de vitâmeros: tocoferóis e tocotrienóis, tendo cada grupo quatro isómeros (α, β, γ, δ). A análise desses compostos, em alimentos, tem sido realizada por técnicas como a *High-pressure liquid chromatography* (HPLC) acoplada a detetores seletivos e sensíveis, entre eles o detetor de fluorescência (FLD) indicado para a sua quantificação. O uso de 2 detetores acoplados (FLD e UV-visível e/ou arranjo de díodos, DAD) permite, além de quantificar os isômeros presentes, confirmar pelo espectro do pico se efetivamente se trata de um vitâmero de vitamina E. O trabalho que se apresenta pretendeu quantificar os teores de vitamina E em queijos usando HPLC-DAD-FLD. Usaram-se amostras de queijo de vaca, ovelha e cabra que foram trituradas e a seguir extraída a vitamina E, de acordo com Ferreira *et al.* (2023), em triplicata. Procedeu-se à injeção automática de 20 µL do extrato. O detector de fluorescência forneceu cromatogramas com alguns picos de isómeros. Por comparação com os padrões, os picos foram identificados, no entanto, estes isómeros não foram confirmados após utilização do DAD. Conclui-se ser fundamental a presença dos 2 detetores em série para a quantificação correta dos teores de vitamina E.

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P11. New insights on the isolation of anthocyanins from complex matrices using cation-exchange solid-phase extraction

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Anthocyanins are among the most interesting groups of polyphenols due to their several characteristics, ranging from health benefits in different contexts to the use as natural colorants. During the last years, several protocols have been well established regarding the purification of these natural compounds from different sources. However, isolating anthocyanins from specific groups of polyphenols such as flavonol glycosides can be challenging, due to their structural similarity and solvent solubility characteristics. Especially in the case of sources with a high amount of flavonols, this becomes a critical problem. Cation-exchange chromatography as shown to be an efficient way to purify anthocyanins, however, there is a lack on the knowledge about the influence of the anthocyanins structure and source type.
In this study, we tested different solid phases (Reversed Phase Silica C-18®, Amberlite® XAD-7HP, Oasis® HLB, ToyoPearl® HW-40, Sephadex® LH-20, Discovery® DSC-MCAX) to develop a rapid and efficient method to isolate mono and polyglycosylated anthocyanins from different natural sources (purple basil, elderberry, purple sweet potato, cornflower and wild pansy).
The results demonstrated that overall, Oasis® HLB (reversed-phase) and Discovery® DSC-MCAX (cation-exchange) obtained the higher degree of purification for the different anthocyanins regardless of the food source, however, Discovery® DSC-MCAX demonstrated the best performance among the different methods in the case of the sources rich in flavonol glycosides (wild pansy and cornflower). The structural complexity of anthocyanins did not affect the purification efficiency.

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P12. Emo-sensory and Volatile Profile of Iberian Wine Vinegar

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Wine vinegar is primarily manufactured in the Iberian Peninsula and has been widely utilized as a seasoning, a preservative, and, in certain nations, a healthful beverage [1]. This research uses sensory and chemical evaluation methods to analyze four types of wine vinegar from the Douro and Rioja regions.
Quantitative Descriptive Analysis (QDA) was performed for a sensory profile. Fifteen trained panelists assessed a total of twenty-two samples, with fifteen originating from the Douro region and seven from the Rioja region. Initially, the panelists underwent specific training related to vinegar, followed by ranking the samples based on their acidity levels. Subsequently, the panel evaluated three standard samples: white (sample 1), red (sample 2), and Port wine vinegar (sample 3). The assessors then generated sensory descriptors freely, and a QDA test sheet was created employing a 5-point scale.
Moreover, an innovative analysis procedure was developed based on the Rocha et al. [2] protocol using Noldus FaceReader software. To perform this technique, an untrained panel of 40 naïve assessors tasted three samples (white, red, and Port Wine). While in the test, the assessors were filmed, using cameras placed in front of them and adjusted to obtain a correct measurement of their facial microexpressions. Finally, the volatile compounds present in wine vinegar samples were assessed by GC-MS.
These techniques in symbiosis have been revealed to be a relevant methodology to characterize wine vinegar, as it encompasses a dynamic and holistic perspective regarding complex matrices.

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P13. Assessment of Volatile Compounds Associated with the '*Brett Character*' in Wine and Its Sensory Impact

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O caráter conhecido como *Brettanomyces* é gerado por uma levedura contaminante reconhecida há algum tempo, porém explorada em detalhes mais recentemente. O presente estudo tem como base a quantificação de compostos fenólicos voláteis, 4-etilfenol (4-EF) e 4-etilguaiacol (4-EG) e do ácido gordo volátil, ácido isovalérico através da técnica de cromatografia gasosa com detetor de massas. Pretende-se compreender o papel desses compostos na alteração das características aromáticas dos vinhos tintos. Esses compostos são gerados pela atividade enzimática da vinil-redutase da levedura *Brettanomyces*, no caso dos etilfenóis, e pelo seu metabolismo nitrogenado no caso do ácido isovalérico. Os aromas resultantes são frequentemente descritos como suor de cavalo, meias suadas, estábulo ou cavalo. Também foi realizada uma análise sensorial para traçar o perfil sensorial de 10 amostras consideradas relevantes para o estudo, selecionadas de entre mais de 50 amostras analisadas. Foi efetuada a Análise Fatorial em Componentes Principais para compreender a relação entre os resultados da análise sensorial e a concentração dos compostos voláteis.
Concluiu-se que a análise de etilfenóis e ácido isovalérico pode ser realizada nas mesmas condições cromatográficas, e as concentrações de 4-EP e 4-EG estão positivamente correlacionadas entre si, mas negativamente correlacionados com a concentração de ácido isovalérico. Também se constatou que o impacto dos etilfenóis no aroma do vinho é maior em comparação com o ácido isovalérico, uma vez que este último tende a misturar-se com outros aromas do vinho, tornando-se menos perceptível e, em algumas ocasiões, contribuindo com aromas menos desagradáveis em concentrações mais baixas.

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P14. Does the Use of Different Cork Stoppers Affect the Quality of Sparkling Wines?

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Sparkling wine is an alcoholic beverage enjoyed worldwide, produced through the secondary fermentation of still wine, and subsequently stored in bottles sealed with cork stoppers[1]. Cork is a natural and renewable material with unique mechanical properties that when converted into stoppers, contributes significantly to the enhanced preservation of sparkling wine[2,3].

In this work, two Champagne wines, bottled with different types of cork stoppers (one agglomerate body with 2 natural cork discs and one microagglomerate stopper) were analyzed in order to study the differences resulting from different bottling stoppers after 15 months of storage.

The samples were extracted using HS-SPME, and the separation was carried by GC/MS using a non-polar column. The pressure inside the bottle was measured using a laser aphrometer and the wines were subjected to evaluation by a panel of nine expert tasters.

The PCA results revealed a marked differentiation between the two types of stoppers for the first sparkling wine, while the distinctions were less pronounced in the second one. The compounds that differ between bottling cork type are VOCs such as esters and alcohols. On the other hand, wines sealed with microagglomerated cork stoppers exhibited a heightened concentration of oxidation markers.

Regarding the pressure measurements, wines sealed with 2 disk cork stoppers exhibited higher values. In the sensorial evaluation, these wines presented superior aromatic and effervescence (bubble) quality.

These results emphasize the importance of selecting the right cork for preserving wine quality and sensory attributes.

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P15. Exploring Gas Chromatography tools for monitorization of phthalates

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Phthalates are a group of molecules belonging to the class of plasticizers. They are wildly use in large productions to grant several features to polymers[1]. However, they aren't chemically bound to the polymer's, it's easy for them to migrate to their surrounding environment.
Despite being so used doo to their vast applicability, these molecules have very toxic effects in human health. Since phthalates are being more and more linked to greater susceptibility with diseases in the reproductive, endocrine and respiratory system, regulations have been increasing for their use[2,3,4].
In order to enable the identification and quantification of 34 plasticizers in alimentary matrixes a GC/MS/MS method was developed. This method was tested and despite all efforts some coelutions were not able to be solved. To further improve the separation of difficult peak groups, a method bidimensional gas chromatography was used showing promising results.
In this work, 26 phthalates and 8 phthalate substitutes were first separated using a non-polar column and detected using a triple quadrupole in multiple reaction monitoring mode (MRM), and then using a flow modulation GCxGC.

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P16. Analysis of the chemical profile by HPLC/DAD of the hydroalcoholic extract of the roots of the lilac morphotype of the plant species *Dizygostemon riparius* (Plantaginaceae)

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Dizygostemon riparius is a new botanical species found in the Cerrado region of Maranhão, Brazil. A pioneering study of the chemical profile of ethyl acetate and methanol leaf extracts revealed important classes of compounds, such as flavonoids. For a more extensive investigation, this work used HPLC, as a more selective and efficient technique, in evaluating the chromatographic profile of the hydroalcoholic crude extract of the roots of the *D. riparius* species. The analyzes were carried out on a Shimadzu® liquid chromatograph coupled to an SPD-M20A DAD detector and a Luna-Phenomenex C18 column (250 mm x 4.6 mm x 5 um). A gradient was used with mobile phase A (0.01% formic acid in water) and mobile phase B (0.01% formic acid in methanol), operated at a flow rate of 0.9 mL/min at 28 °C and an exploratory gradient with the concentration of B varying in the range of 5 to 100% in 70 minutes and a further 100% of B in 10 minutes. The chromatogram (Figure 1) demonstrates the separation of secondary metabolites with twenty peaks with good resolution, four of which were more intense in the 20 and 30 minute time range, indicating that of the majority compounds are polar, possibly polymethoxyflavones and coumarins, such as isorhamnetin 3-galactoside-7-rhamnoside, 5,7-dihydroxy-3-(3-hydroxy-4,5-dimethoxyphenyl)-6-methoxy-4- benzopyrone and 3',5-dihydroxy-4',6,7-trimethoxyflavone indicated by Martins (2023) as one of the main constituents of the species in the ethyl acetate extract. This preliminary investigation of the crude hydroalcoholic extracts was promising for subsequent studies to isolate the compounds and identify them by HPLC-MS and NMR.

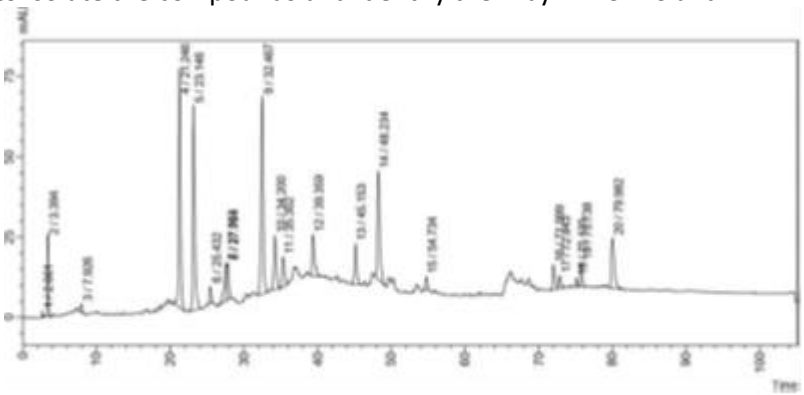

Figure 1. Chromatogram of the hydroalcoholic roots extract of the lilac morphotype of the plant species *Dizygostemon riparius* (Plantaginaceae).

Acknowledgements: We thank FAPEMA for granting the scholarship and IFMA for the assistance provided to participate in the event.

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P17. Nutritional, chemical profile, and antioxidant activity of wild fruit extracts: *Afromomum alboviolaceum* (Ridl.) Hochst. and *Sclerocarya birrea* (A. Rich.) Hochst. pulp and peel from Cuanza Sul, Angola

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Fruits are appreciated worldwide not only for their pleasant taste, but also for being a source of nutrients and bioactive compounds. *Aframomum alboviolaceum* (Ridl.) Hochst. (Jinguenga) and *Sclerocarya birrea* (A. Rich.) Hochst. (Omugongo) are fruit species native to Angola (POWO, 2023; Figueiredo & Smith, 2008; Frazão, 1957) consumed in both natural and processed forms. This study aimed to characterize the nutritional, chemical profiles of both ripe fruits and to assess the antioxidant activity of their pulp and peel. Carbohydrates were the major macronutrients found in both species, followed by proteins and lipids (AOAC, 2016), which were in higher concentrations in both pulps. Fructose, glucose, and sucrose (HPLCRI) were detected in both fruits, with sucrose being the major sugars in the pulp and peel of *S. birrea* (50.0±0.6 and 21.6±0.9 g/100g, respectively). Moreover, phenolic compounds were analyzed through HPLC-DAD-ESI-MSn. Quercetin derivatives were the main compounds in the peel of both fruits: rutin being the major for *A. alboviolaceum* (0.27±0.001 mg/g), quercetin-*O*-pentoside predominating for *S. birrea* (0.17±0.01 mg/g). In the pulp of *S. birrea*, heperetin-*O*-rutinoside was the main compound found (5.95±0.02 mg/g). Although this compound has also been identified in the pulp of *A. alboviolaceum,* where ferulic acid and *p*-coumaric acid were the prevailing (0.53±0.02 and 0.24±0.01 mg/g). The presence of phenolic compounds in the samples may relate to the relevant antioxidant activity (TBARS) observed in the peel extracts. Altogether, this study proved that *A. alboviolaceum* and *S. birrea* are valuable sources of nutrients and bioactive compounds, validating their use by populations and different industries.

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P18. Insights into the Bioactivities and Chemical Analysis of *Ailanthus altissima* (Mill.) Swingle

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In recent years, several analytical methods for characterising the *Ailanthus altissima* (Mill.) Swingle have aroused interest in the scientific community, since this species is not only considered an invasive alien species, but also possesses a wide and complex number of chemical compounds. These compounds are studied with the aim of ascertaining their biological activities, which could help to understand their mechanisms of action, develop new products with potential application in different fields of research. Consequently, it is essential to analyse the optimal extraction method and identify and quantify the main classes of compounds in order to improve knowledge and the potential uses of this species. Based on the review of the different chromatographic techniques for identifying and quantifying the majority of compounds, it was concluded that HPLC-UV and HPLC-DAD are widely used for phenolic compounds and for one of the most important compounds, the ailanthone with concentrations ranged from 6.44 µg/mL to 825 µg/mL. Additionally, the most widely used technique for identifying compounds in the terpene class is GC-MS and GC-FID. Regarding extraction methods, the most commonly used according to the literature is maceration, where the stirring time differs greatly depending on the solvent used. Although there have been a few studies on the bark and leaves of this species which contribute to our knowledge of its bioactivities based on its chemical profile, other parts such as flowers, seeds and stems could be potential study targets for discovering new compounds and optimising the analytical techniques used.

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P19. Development of an emamectin benzoate insecticide analysis method in *Quercus suber* leaves

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Pesticide analysis is crucial for assessing environmental safety and agricultural product quality. Emamectin benzoate is an insecticide used in agriculture to protect a wide range of crops and trees from insect infestations.
This study details the development and validation of a high-performance liquid chromatography (HPLC) method for emamectin benzoate determination in *Quercus suber* leaves, after application focusing on tree protection against the cork borer beetle, *Coraebus undatus*.
Various extraction and cleanup methods were assessed. The better recovery results were achieved by the QuEChERS approach using a d-SPE clean-up with primary-secondary amine (PSA) and MgSO4. After extraction and before analysis by HPLC-FLD the extracts were dried and derivatized. The method was validated, ensuring reliability and accuracy. Recovery, repeatability, limit of detection (LOD), and limit of quantification (LOQ) were assessed. LOD was 1.905 µg/g dried leaf, and LOQ was 6.820 µg/g dried leaf. Emamectin benzoate recovery was 67% at 2 ppm and 63% at 5 ppm, with repeatability having a %RSD (pooled) of 2.86%.
This method was applied to 62 *Quercus suber* leaf samples from trees treated with the insecticide, of which only three showed detectable emamectin levels, but all below LOQ.

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P20. Validation of a Confirmation Method for Hypoxia-inducible factor (HIF) activating agents in human urine by LC-MS/MS in Doping Analysis

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Hypoxia-Inducible Factors (HIF) activating agents have a potential to enhance blood haemoglobin levels after oral administration, leading to an increase of the capacity for oxygen transport. Due to the increased number of HIF stabilizers available, their pharmacological potential and enhancing performance effects in athletes, the use of these substances is prohibited in sport.
The aim of this study was to develop and validate three methods for HIF confirmation in human urine samples by LC-MS/MS.
Sample preparation was performed by *Dilute-and-Shoot* for Daprodustat (GSK1278863), Daprodustat bishydroxylated metabolite (GSK2391220), Desidustat (ZYAN1), FG-2216, IOX2, IOX4, JNJ-42041935, Roxadustat (FG-4592) and Vadadustat (AKB-6548) – method A – and for Enarodustat – method B. For Molidustat (BAY85-3934) and Molidustat Glucuronide (BAY1163348) – method C – a MCX Solid-phase Extraction was used.
Analysis was performed in a AbSciex Qtrap 5500 LC-MS/MS with a 10 (method A and C) or 17 (method B) minutes gradient method and a 0,3 mL/min flow rate (Mobile Phase A - Water with 0,1% Formic acid : acetonitrile (95:5%, v:v) and Mobile Phase B - Water with 0,1% Formic acid : Acetonitrile (5:95%, v:v)) on a XBridge BEH C18 (100x2,1 mm, 2,5 µm) column, with MRM acquisition mode.
The methods were validated for the parameters Selectivity / Specificity, Limit of Identification, Robustness, Carryover, Matrix Effect, Stability and Recovery (only for Molidustat and Molidustat Glucuronide).
The methods shown to be fit-for-purpose, with Limits of Identification below or equal to 1 ng/mL, in compliance with WADA's Technical Documents TD2022MRPL and TD2023IDCR.

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P21. GC-C-IRMS: An effective tool for identifying the origin of organic compounds - Application to steroids consumption.

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The consumption of Endogenous Anabolic Androgenic Steroids (EAAS) is often used to improve performance in sports even though their use constitutes danger to the health and a violation of the World Anti-Doping Code.
The aim of this study is to demonstrate the capability of the GC/C/IRMS (Gas Chromatography/Combustion/Isotope Ratio) methodology to distinguish an exogenous or endogenous origin of EAAS, since these compounds are produced endogenously and their possible exogenous origin cannot be concluded by "traditional analytical methodologies".
The determination of EAAS administration begins with the analysis of the steroid profile, in human urine, by GC-MS/MS, which can be altered by an administration of synthetic EAAS, in particular Testosterone and its precursors or active metabolites.
GC/C/IRMS is a technique that allows the determination, with great precision, of the isotopic composition of a compound, which can differentiate the origin of a steroid. This differentiation is made by comparing the 13C/12C ratio of the Target Compound with the ratio of a Reference Endogenous compound that is not affected by the exogenous consumption of steroids.
The sample preparation begins with a solid-phase extraction clean up, followed by enzymatic hydrolysis, Liquid-Liquid extraction, derivatization, and purification by HPLC, in which the compounds are separated in 6 fractions that are then analyzed by GC/C/IRMS.
In this study, it's shown a real example of two samples with suspicious steroid profiles. From GC/C/IRMS analysis is concluded that one sample has an altered profile due to the exogenous consumption of EAAS, whereas the other is due to an endogenous origin.

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P22. Validation of a Quantitative Method for Morphine, Codeine, Ethylmorphine and Norethylmorphine in human urine by LC-MS/MS in Doping Analysis

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Morphine is a prohibited substance (above 1 µg/mL) in doping analysis by WADA (World Anti-Doping Agency). Codeine and Ethylmorphine aren't prohibited, but Morphine is one of their metabolites. For that reason, is important to understand the origin of Morphine when present in an athlete urine. The aim of this study was to develop and validate a method for confirmation and quantification of Morphine, Codeine, Ethylmorphine and Norethylmorphine (Ethylmorphine metabolite) in human urine samples by LC-MS/MS.
Sample preparation was performed by acid hydrolysis (for the conjugated metabolites of the substances) followed by a MCX Solid-Phase Extraction.
Analysis was performed in a AbSciex Qtrap 5500 LC-MS/MS with a 17 minutes gradient method (Mobile Phase A - Water with 0,2% Formic acid and Mobile Phase B - Acetonitrile with 0,2% Formic acid) on a Zorbax RX C8 (150x2,1 mm, 5 µm) column, with MRM acquisition mode.
The method was validated for the parameters Selectivity / Specificity, Limit of Identification, Robustness, Carryover, Matrix Effect, Stability, Recovery, Linearity, Limit of Quantification, Precision, Accuracy and Uncertainty.
The method showed to be fit-for-purpose, selective, specific, robust, precise, accurate, linear, with Limits of Identification below or equal to 25 ng/mL, without carryover, recoveries above 97% and with 12,7% uncertainty for Morphine in compliance with WADA's Technical Documents TD2022DL, TD2023IDCR and TD2022MRPL.
The method was applied to urine samples and made it possible to distinguish the origin of morphine - one case of Morphine consumption and another case of Codeine consumption.

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P23. Integration of chromatographic and metabolomics data as a useful tool to identify putative urinary prostate cancer biomarkers

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Prostate cancer (PCa) is the second most frequent malignant tumour, the fifth leading cause of cancer death among men worldwide, and the most frequently diagnosed cancer in 105 of 185 of the world countries [1]. Advances in extraction techniques, chromatography, MS instrumentation, and hyphenated systems make increasingly significant contributions to clinical applications, especially in cancer biomarker discovery and verification. The investigation of endogenous volatile organic metabolites (VOMs), which are produced by various metabolic pathways and present in several biofluids, such as plasma/serum, blood, tissue, and urine, is emerging as a novel, effective, and non-invasive source of information to establish the volatilomic biosignature of PCa. In this study, headspace solid-phase microextraction combined with gas chromatography-mass spectrometry (HS-SPME/GC-MS) was used to establish the urine volatilomic profile of PCa in order to identify VOMs able to discriminate the investigated groups. This non-invasive approach was applied to oncological patients (PCa group, n = 26) and cancer-free individuals (control group, n = 30), retrieving a total of 147 VOMs from various chemical families. This included terpenes, norisoprenoid, sesquiterpenes, phenolic, sulphur and furanic compounds, ketones, alcohols, esters, aldehydes, carboxylic acid, benzene and naphthalene derivatives, hydrocarbons and heterocyclic hydrocarbons. The data matrix was subjected to multivariate analysis, namely partial least-squares discriminant analysis (PLS-DA). Accordingly, this analysis showed that the group under study presented different volatilomic profiles and suggested potential PCa biomarkers. Nevertheless, a larger cohort of samples is required to boost the predictability and accuracy of the statistical models developed.

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P24. Preliminary pharmacokinetic assays of CBD administration to cats with Feline Chronic Gingivostomatitis

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Feline chronic gingivostomatitis (FCGS) is a highly painful and debilitating oral inflammatory disease [1,2]. Cannabidiol (CBD) has several recognized therapeutic benefits, namely in the treatment of chronic pain [3-5]. This study aimed to develop a method of quantitation of CBD by UHPLC-MS/MS, to determine the serum concentration of CBD in cats with FCGS after oral administration of a commercially available formulation.
Eleven cats were treated using a fixed dosage of 4 mg per cat. Blood samples were obtained to determine CBD serum concentrations by UHPLC-MS/MS at four time points: 0, 4, 8, and 12 hours after oral administration. The method of extraction of CBD from serum was optimized and general UHPLC-MS/MS validation tests were carried out, namely linearity, precision and accuracy, matrix effect, and recovery assays. The limit of quantitation of CBD was 0.2 ng mL^{-1} in serum. The liquid-liquid extraction allowed the recovery of about 64% of CBD present in the serum. The matrix effect exceeded 50% for higher CBD concentrations. The addition of CBD-d3 as an internal standard allowed the quantitation of CBD in serum samples with good precision and accuracy.
CBD was quantified in all serum samples and ranged from not detectable at 0 hours to 34.81 ng mL^{-1} at 8 hours. Generally, higher concentrations were obtained in serum samples taken 4 hours after taking CBD.

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P25. Quantification of quorum sensing signalling molecule autoinducer-2 in biological samples by gas chromatography - mass spectrometry

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Autoinducer-2 (AI-2) is an important quorum sensing signaling molecule in bacteria regulating microbiota community behavior. Genomic evidence and activity measurements of bacterial cultures show that many gut microbiota members produce AI-2, however, quantification of AI-2 activity in intestinal samples has been difficult. The concentration of this molecule is often estimated through biological assays using specific bacterial reporter strains, which are not quantitative [1].
Previously described quantification methods, namely Gas Chromatography-Mass Spectrometry (GC-MS) [2] and High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) [3] have been solely applicable within bacterial media and saliva samples, but quantification in cecum samples has yet to be achieved.
Herein, we have developed an internal standard method for the quantification of AI-2 in biological samples, such as mice cecum, using GC-MS. For this, the isotopically labelled deuterated AI-2 (AI-d_3) was synthesized for the first time. The addition of this isotopologue enables accurate compensation for losses during sample preparation, assuring the robustness, accuracy, and precision of the analytical procedure. To facilitate GC analysis, a dual derivatization procedure has been adopted to overcome the stability issues of AI-2 and the analyte volatility.
The application of this method provides a valuable tool for investigating AI-2 mediated processes in various biological systems, with potential implications for the development of targeted therapies in microbiome-related disorders.

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P26. Steroid hormone analysis in blood samples of postmenopausal women by solid phase extraction and gas chromatography-mass spectrometry

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The period of a woman's life that elapses since menopause tends to increase further due to the increase in average life expectancy. A series of problems is associated with the deficiency of steroid hormones accompanying menopause. Some of these conditions encompass breast and endometrial cancer, cognition complications, risk of developing type 2 diabetes, and fibrinolysis, and can additionally alter the action of certain analgesics. In this context, women at menopause are deprived of ovarian estrogens and androgens due to the marked decrease in dehydroepiandrosterone. To achieve a more physiological and tissue-specific hormone replacement therapy, a better understanding of the manifestation of these hormones is crucial.
The present study relies on the development and validation of a method for the identification and quantification of 17-beta-estradiol (E2), total testosterone, androstenedione and dehydroepiandrosterone sulphate (DHEA) in blood samples of postmenopausal women by solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS). Linearity was achieved between 0.05 to 100 ng/mL for 17β-estradiol, 0.1 to 100 ng/mL for DHEA and androstenedione, and 0.5 to 100 ng/mL for testosterone. The limits of detection and quantification were 0.05 ng/mL for 17β-estradiol, 0.1 ng/mL for DHEA and androstenedione, and 0.5 ng/mL for testosterone.
The methods were validated with blood samples and applied to 181 authentic samples collected from postmenopausal women. The steroid hormones analysed could be accurately detected and quantified on the samples, confirming the suitability of the developed methods in clinical analysis[1,2].

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P27. Determinação da cocaína e metabolitos em águas residuais com recurso à extração em fase sólida e à cromatografia de gases acoplada à espectrometria de massa em tandem

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A cocaína (COC) é a segunda droga mais consumida na Europa, causando graves problemas de saúde pública, tendo contribuído para um aumento de *overdoses* fatais relacionadas com o consumo de drogas de abuso[1]. A análise de águas residuais (AR's) para determinar o consumo de drogas tem-se tornado cada vez mais comum devido ao seu custo reduzido, elevadas precisão e confiabilidade, e por recorrer a uma matriz ambiental, não invasiva, que apresenta baixa probabilidade de adulteração. Neste estudo, foi desenvolvido e validado um método para determinar COC e os metabolitos ecgonina metil éster (EME), benzoilecgonina (BEG), cocaetileno (COET), e norcocaína (NCOC) em AR's, utilizando extração em fase sólida (SPE) e cromatografia gasosa acoplada à espectrometria de massas em tandem (GC-MS/MS). O método foi otimizado e validado de acordo com as normas internacionais de validação. Foi obtida uma linearidade de 0,00625-5 ng/mL para todos os analitos, tendo sido obtidos coeficientes de determinação superiores 0,9990. O limite inferior de quantificação (LLOQ) para todos os compostos foi de 0,00625 ng/mL. As precisões e exatidão intra-dia, inter-dia e intermédias apresentaram coeficientes de variação abaixo de 15%, e uma exatidão de ±15% para todos os compostos analisados. Além disso, o procedimento permitiu alcançar recuperações entre 72% e 115%. Salienta-se que este procedimento é o primeiro método que utiliza a SPE, com cartuchos Strata TM-X-C, combinada com GC-MS/MS para a determinação de COC e metabolitos em AR's. Este método torna-se então uma alternativa adequada para a monitorização da COC e dos seus metabolitos em amostras de AR's, demonstrando elevada importância ambiental e forense.

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P28. Determination of Glyphosate and AMPA in Clean Waters by HPLC-FLD

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Glyphosate (N-(phosphonomethyl)glycine), commonly known by its original trade name Roundup, is the world's most widely used herbicide. Glyphosate is highly soluble in water and its ability to bind to mineral components makes it persistent in the environment. Due to its low mobility in soil, it is not likely to be found in groundwater, but it can contaminate surface waters by soil erosion and runoffs or even by its direct use on fields near aquatic environments. Glyphosate is chemically stable in water and is not subject to photochemical degradation [1].

In water, glyphosate undergoes rapid conversion to its degradation product aminomethylphosphonic acid (AMPA).

In March 2015, the World Health Organization's International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic in humans" [2]. Since the publication of this report, the use of glyphosate is under debate worldwide.

Glyphosate is currently approved to be used in the EU until 15 December 2023 [3].

The determination of glyphosate and AMPA at low concentrations is considered challenging, because of their unique physicochemical properties such as high polar nature, amphoterism, low volatility, small molecule size, absence of chromophores or fluorophores or others.

A fast, robust and relative easy to use analytical method for the simultaneous determination of the herbicide Glyphosate and it metabolite AMPA in water by HPLC-FLD, has been developed. The 9-fluorenylmethyl chloroformate (FMOC-Cl) was used for derivatization of both compounds. The derivatives were quantified using external calibration with a linear range from 0.05-1.0 µg/L with a quantification limit of 0.10 µg/L.

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P29. Development, validation and application of a high-performance liquid chromatography method for the analysis of 5-Fluorouracil residues in infusion pumps used in chemotherapy treatments

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The treatment of colorectal cancer is based, for most patients, on the administration of the cytotoxic 5-Fluorouracil (5-FU) via infusion pumps on a home basis. Recent reports have revealed concerns about potential spillages of 5-FU from infusion pumps [1]. The main aim of this study was to develop, validate and apply a methodology using high-performance liquid chromatography coupled with a diode array detector to assess the existence of 5-FU residues on the outside of infusion pumps. The extraction method consisted of taking samples using 10 cm^2 gauze pads moistened with ethyl acetate, followed by stirring for five minutes in 15 ml acetonitrile:methanol:water (10:25:65), and filtration (0.2 µm polytetratfluoroethylene filter). Chromatographic conditions consisted of a water mobile phase with 5% acetic acid at a constant flow rate of 0.8 ml/min, and an octadecyl column as stationary phase. The injection volume was 10 µl and the analysis took place during 10 minutes. Ten infusion pumps removed from a central hospital in northern Portugal were analyzed, regarding the presence of 5-FU in three different locations (catheter connection, infusion wire and outer casing). The method was validated for linearity (R^2 = 0.9987), sensitivity (0.040 µg/cm^2 detection limit; 0.120 µg/cm^2 quantification limit), intermediate precision and repeatability (87%-98%), accuracy (85%-100%) and recovery (97%-107%). Residues of 5-FU were found with a retention time of 3 minutes in the catheter connection zones in 80% of the pumps (n=8). The results obtained reinforce the need for care when nurses disconnect pumps, particularly regarding the adequate use of personal protective equipment.

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P30. Assessment of mineral and organic compounds from livestock effluents

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O processo eletrodialítico (ED) permite extrair macronutrientes (nitrogénio (N), fósforo (P), cálcio (Ca), potássio (K) e magnésio (Mg)) de efluentes pecuários, para obtenção de fertilizantes minerais de base biológica. Este processo utiliza corrente elétrica de baixa intensidade e membranas de troca iónica para separar e concentrar iões. Contudo, a avaliação dos compostos orgânicos presentes nos efluentes pecuários não tem sido efetuada. Neste estudo foram avaliados os compostos orgânicos presentes num efluente de suínos (chorume), antes e depois do processo ED. O chorume foi testado através de um reator eletrodialítico para avaliar a eficiência do processo na recuperação de N e P. Em simultâneo, as soluções obtidas no final do processo ED foram caraterizadas quanto ao seu teor de elementos minerais e compostos orgânicos por espetrometria de absorção atómica e cromatografia líquida de alta eficiência acoplada a um detetor de díodos, respetivamente. Os resultados mostraram uma eficiência notável de 100% na recuperação de P e de 57% na recuperação de N. No chorume foi identificada a presença do composto orgânico 2,6-diidopropylnaphthelene (0,02%). Nas soluções do ânodo e do cátodo foram também identificados os compostos 2,6-diidopropylnaphthelene (0,22%) e junipene (0,03%), verificando-se uma maior concentração relativamente à do efluente inicial. O 2,6-diidopropylnaphthelene é um regulador de crescimento vegetal e o junipene é um composto encontrado em oléo essencial de algumas espécies do género *Juniperus* e *Stoebe*. O papel destes compostos como bioactivos das plantas deve também ser avaliado agronomicamente, pois pode acrescentar qualidade ao fertilizante obtido.

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P31. Determination of Cannabinoids in Urine Samples by Bar Adsorptive Microextraction and Gas Chromatography-Mass Spectrometry

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Cannabis sativa L. is an annual herbaceous plant from the botanical family Cannabaceae, used for thousands of years for its therapeutic and recreational purposes. Currently, it is the drug of abuse with the highest prevalence of consumption worldwide and, in recent years, its use has been increasing. Consumption is associated with several adverse psychological and physical effects, which has given rise to growing concern. This plant produces phytocannabinoids, compounds that act on CB1 or CB2 cannabinoid receptors and which are present in the plant in their acid form, namely delta-9-tetrahydrocannabinolic acid, cannabidiolic acid and cannabigerolic acid. These acidic forms, spontaneously in the plant or through heating (e.g., smoked), exposure to light or oxidation, can be decarboxylated into their neutral forms, such as tetrahydrocannabinol (THC) (responsible for psychoactive effects), cannabidiol (CBD) (responsible for therapeutic effects) and cannabichromene [1]. In the present work, the development and optimization of bar adsorptive microextraction followed by gas chromatography coupled to mass spectrometry (BAμE/GC-MS) analysis is proposed for the determination of four cannabinoids metabolites (CBD-OH, CBD-COOH, THC-OH, and THC-COOH) in urine samples [2]. The results shows that the proposed methodology obtained recovery up to 75% for the four analytes, constituting a promising analytical alternative for monitoring these cannabinoids due to its simplicity, easy handling and low cost.

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P32. Qualitative determination of Alkyl Amines Stimulants in urine matrices applied to Doping Control Context through Bar Adsorptive µ-Extraction

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In order to obtain undoubtedly, irrefutable technical and scientific results, as well as in compliance with the World Anti-Doping Agency (WADA) requirements, building analytical methodologies continues to play a great challenge for anti-doping laboratories. Therefore, once sample preparation yet represents a very important step, the introduction of modern techniques brings up added value with boosting analytical enrichment together with strong reduction or elimination of interferents and, thereby, achieving correct identification and quantification of the target analytes. Bar Adsorptive µ-Extraction (BAµE) has emerged as a new perspective in sample preparation following the green chemistry guidelines and proven to be a robust analytical approach, in order to overcome the limitations presented by other technologies.[1-4]
The present contribution proposes a new analytical approach for the qualitative determination of six alkyl amines (1,3-dimethylbutylamine, 1,4-dimethylpentylamine, heptaminol, isometheptene, octodrine and tuaminoheptane) using BAµE followed by gas chromatography coupled to mass spectrometry operating in the selected ion monitoring mode acquisition. After selecting the best sorbent material and achieving the best microextraction conditions using 1 mL of urine sample, a complete validation was performed. The proposed methodology showed excellent selectivity/specificity, suitable limits-of-identification (LOI, 5.0-35.0 ng/mL), appropriate linear dynamic ranges (5.0-200.0 ng/mL) with good determination coefficients (r2>0.9937), as well as good robustness, accuracy and repeatability in intraday and interday conditions at two different levels. To check whether the methodology is fit-for-purpose, four previously analysed proficiency urine samples were successfully tested, in which were unequivocally detected and identified some of the target analytes in compliance with WADA guidelines.

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P33. A comparative study of SPE and DLLME based methods for the analysis of benzodiazepines and opioids in urine samples by LC-MS/MS

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The scientific community has shown increasing interest in detecting illicit drugs due to their widespread consumption, particularly opioids and benzodiazepines[1]. Analytical methods primarily using chromatography have been developed to analyse these drugs in urine, which is a complex matrix that presents low drug concentrations (ng/L or µg/L range). To address these challenges, pre-treatment approaches based on green analytical chemistry principles have emerged[2-5].

This study compares two methods for detecting benzodiazepines and opioids in urine samples. The first method employs solid phase extraction (SPE) with ExtraBond SCX as the sorbent and 7 mL of 5% NH_4OH in methanol as the elution solvent, achieving recoveries between 9 and 107%. The second method relies on dispersive liquid-liquid microextraction (DLLME), using small volumes of chloroform (200 µL) and ethyl acetate (500 µL) as extractant and dispersant solvents, achieving recoveries between 14 and 86%. The second strategy can be considered greener according to the principles of green analytical chemistry since it requires low volumes of solvents, the extraction time is briefer, and the energy consumption is lower compared with the first method developed based on SPE.

These methods were validated with urine samples and applied to 12 authentic urine samples collected from women starting detoxification programs, confirming the suitability of the developed methods in toxicological and forensic analysis. The analysed urine samples showed that most compounds could be detected in the urine samples, but they could not be quantified. Additionally, methadone was the most detected compound, presenting the highest concentrations.

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P34. Monitorização de antidepressivos: comparação de dois métodos de extração em amostras de fluido oral

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O consumo de antidepressivos representa uma problemática mundial, apresentando Portugal uma das taxas de prevalência de doenças mentais mais elevadas da Europa[1]. A monitorização terapêutica é instituída para um pequeno número de fármacos para os quais existe uma relação direta entre a concentração e o efeito farmacológico.
Este trabalho compara duas metodologias desenvolvidas para a determinação de antidepressivos (fluoxetina, venlafaxina, sertralina, citalopram, paroxetina e metabolitos) em amostras de fluido oral com recurso à cromatografia gasosa acoplada à espectrometria de massa em tandem. O primeiro método utiliza amostragem por *dried saliva spots* (DSS) como técnica de extração. O volume de amostra foi de 100 µL e o procedimento foi otimizado utilizando a ferramenta estatística *Design of Experiments* (DoE) para avaliação dos tempos de secagem e extração e do solvente e volume do solvente de extração. As recuperações variaram entre 13 e 46%[2]. A segunda metodologia implementa a microextração em seringa empacotada (MEPS), utilizando 250 µL de amostra. Esta técnica foi otimizada utilizando a ferramenta de DoE para avaliação do número de *strokes* no *load* da amostra, número de lavagens e número de eluições. As recuperações variaram entre 12 e 93%. Ambos os métodos foram validados seguindo diretrizes internacionais. Os limites de quantificação foram estabelecidos entre 10-100 ng/mL e o intervalo de linearidade variou entre 10-500 ng/mL para ambas as metodologias (dentro do intervalo terapêutico). Este trabalho é o primeiro a utilizar DSS e MEPS para determinação destes antidepressivos em fluido oral.

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P35. Miniaturized Solid Phase Extraction Techniques Applied to Natural Products: A Review

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Natural products are increasingly becoming part of our daily lives through their use in industry, food, as therapeutic agents, etc. Different techniques may be used to characterize natural products, including microextraction techniques. This work intends to review the most used solid-based miniaturized sample preparation techniques applied to determining compounds in natural products. Advantages and drawbacks are also presented. A systematic literature search was performed involving three electronic databases: Medline, ISI Web of Knowledge, and Google Scholar. The search considered papers published from 2015 to the present, with exceptions based on the specific method used (the criteria for the search were extended to 2011 due to the low number of publications). The review's findings and discussion revealed that HS-SPME is the most commonly used approach (37.0%) for natural compound analysis due to its "green" characteristics, including solvent-free extraction and sustainability. However, methods using molecularly imprinted polymers for solid-phase extraction (14.8%) and stir bar sorptive extraction (18.5%) are gaining popularity, possibly due to their speed and simplicity. There has been a great deal of attention concerning developing modified sorbents, such as multi-walled carbon nanotubes and MIPs, probably due to their high selectivity.
Chromatographic instruments such as HPLC and GC are preferred (48.1%), and the most common detectors are UV/visible detectors (20.5%) and mass spectrometry (44.9%), respectively.
However, one of the main challenges of using those approaches is obtaining pure and well-characterized materials, as well as the fact that they are not commercially available, which poses a problem to laboratories and industry.

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P36. Microextraction by Packed Sorbent in Forensic Drug Analysis

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Microextraction by packed sorbent (MEPS) efficiently combines extraction, pre-concentration, and clean-up in a single device comprising two parts: the MEPS syringe and the packed sorbent bed[1]. MEPS has been used for several bioanalytical applications, including extraction of endogenous metabolites, biomarkers, and drugs from biological samples. It is particularly useful in metabolomics and pharmacokinetic studies[2,3]. Regarding MEPS applicability to forensic toxicology, urine is the most used specimen, followed by oral fluid (despite of its relatively high viscosity). Protein precipitation followed by centrifugation and with, or without dilution of the supernatant is the most commonly reported approach. The most detected compounds in forensic settings using MEPS are drugs of abuse [opiates and opioids (26%), cocaine (13%) cathinones (11%), dissociative hallucinogens (11%), cannabinoids and amphetamines (9% each) and other drugs (10%)] and medicinal drugs [antidepressants (9%), benzodiazepines/Z drugs (4%)]. MEPS was also applied to a beverage for forensic purposes e.g. to evaluate its composition in drug-facilitated crimes. An important feature in MEPS is the miniaturization of the sorbent. A careful selection of the sorbent will allow working with complex matrices, separating the target analytes from interferences and improve recoveries. The most widely selected sorbent was the silica based C_{18} that is a popular reversed-phase material (41%). Starting in the 2000s, new modifications of sorbents appeared. Overall, what the future holds for MEPS applications in forensic toxicology is promising, and ongoing research and technological advancements are likely to enhance the capabilities of MEPS approaches, making this technique an increasingly valuable tool for toxicological investigations.

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P37. Enhancing Pesticide Extraction from Soil with a Novel Solid Phase MicroExtraction (SPME) Fiber Design: Development and Method Optimization

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Modern agriculture requires the use of pesticides as an economical way to enhance crop yields, thus ensuring food supply for the ever-growing world population. Given that pesticides are a major source of pollution, there is a need to develop new ways of monitoring them qualitatively and quantitatively. This work describes the development of a new methodology, potentially greener and simpler than the ones currently used for extracting pesticides from soil. It employs a new Solid Phase MicroExtraction (SPME) configuration, where the fiber is held on a micropipette tip, followed by Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) analysis.

For method optimization, a Plackett-Burman design was used to analyze 10 variables, each at two levels, performing 12 experiments in triplicate. Ten pesticides (Boscalid, Diflufenican, Epoxiconazole, Indoxacarb, Metalaxyl, Metolachlor, Metribuzin, Penconazole, Tebuconazole and Terbutylazine) and an agricultural soil (sandy-loam texture; 3% of organic matter) were used. The soil was spiked, giving a final concentration of 50 ng g^{-1} of pesticides.

It was concluded that the optimal method consisted of conditioning, followed by the extraction with 2 g of soil with 10 mL of ultrapure water with 6% methanol, kept under shaking to disperse the soil through the entire volume. The extraction time should be 75 minutes for the PDMS/DVB fiber and 120 minutes for the C18 fiber. The retro-extraction should be performed with 100 µL of methanol for 30 minutes. The extract should be combined with 3 analyte protectants [1]: ethylglycerol, gulonolactone, and sorbitol, with a final concentration of 500 mg L^{-1}, before analysis.

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P38. Bar adsorptive microextraction (BAμE) - An alternative analytical tool to monitor β-blockers in biological and environmental matrices

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Cardiovascular diseases are the most common cause of death worldwide, with almost four million deaths per year in Europe [1]. β-blockers play an important role in cardiovascular therapy, but given their great effect in reducing tremors and blood pressure, these drugs are also prescribed in other health situations, such as anxiety control, and were banned in several sports by Word Anti-Doping Agency (WADA) [2]. Furthermore, β-blockers have been detected in wastewater and environmental samples, due to their high global consumption [3]. Develop alternative methodologies to determine trace levels of β-blockers in biological and environmental matrices becomes essential for therapeutic monitoring, doping control and environmental analysis, with chromatographic methods, preceded by microextraction techniques, being an important tool [2].
The present work aimed the development of a novel analytical methodology to monitor trace levels of β-blockers (bisoprolol, carvedilol, nebivolol, pindolol and propranolol) in aqueous matrices, combining bar adsorptive microextraction with high performance liquid chromatography-diode array detection (BAμE/HPLC-DAD) [4]. Under optimized experimental conditions [microextraction - sorbent phase: StrataX-AW polymer, equilibrium time: 4 h (990 rpm), pH 12; back extraction - MeOH/ACN (1:1, v/v), 15 min. under sonication], high recoveries (69.2 - 86.3 %), low analytical thresholds (1.2 ppb < LOD < 10.0 ppb) and good linear dynamic ranges (4.0 - 600.0 ppb; $r^2 \geq 0.994$) were achieved for the five target analytes. The validated methodology was subsequently applied to real matrices (urine, saliva, plasma and wastewater) demonstrating to be an effective, sensitive, user-friendly and lowcost alternative for tracking β-blockers.

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P39. Application of the innovative hollow fiber microextraction (HFµE) to monitor trace levels of pyrethroids in aqueous matrices

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Pyrethroids are chemical compounds belonging to the class of insecticides, widely used today for the control of insect pests, with applications in the areas of agriculture, domestic and veterinary use[1]. Despite their low toxicity characteristic compared to previously employed pesticides, pyrethroids emerge as contaminants that progressively accumulate in agricultural soil, infiltrating into the surrounding waters.
Since this exposure occurs through accumulation, both humans and animals can be affected by consuming these waters or fruits, or by direct exposure, which can result in toxicity to biological organisms[1,2]. According to the European Environment Agency (EEA)[2], it is imperative to establish an agile detection to reduce the consumption of food and water contaminated by pesticides that exceed the limits allowed by current legislation, to reduce their potentially harmful impact on public health and the environment. The present work aims a new analytical approach based on the principles of green chemistry and which is an alternative to conventional. For such purpose, the hollow fibre microextraction technique in combination with gas chromatography coupled to mass spectroscopy (HFµE/GC-MS), will be proposed to monitoring trace level of pyrethroids in aqueous matrices. The HFµE device is composed of a polypropylene-based membrane where convenient organic solvents are incorporated that favour rapid enrichment kinetics, under floating sampling technology, being easy to use and low cost[3]. The main goal is to develop, optimize and validate an innovative methodology that can be applied to real matrices, i.e., biological, food and forensic samples.

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P40. Development of a SPE-HPLC-DAD procedure to collect and analyse volatile organic compounds released from wood-based panels

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The emission of volatile organic compounds (VOCs) by wood-based panels (WBPs), that are widely used in furniture, interior decoration, and construction materials, has been pointed out as the main factor in the degradation of indoor air quality in homes and workplaces [1]. The emission of VOCs can be a result of natural emission from the wood itself or from additives added during industrial production [2]. In addition, the use of different production processes (temperature, surface treatments, among others) can increase and/or vary the type of VOCs emitted [3].
WBPs is a general term used for different board products made with wood fibres or particles. The most known are particleboards (PBs), medium density fibreboards (MDFs) and oriented stand boards (OSBs). For their production, the wood fibres or particles are combined with a resin to create a wood-resin matrix that, through the effect of heat and pressure in a press, forms a solid panel [4].
In this work, extraction procedures based on the sorption of analytes into a stationary phase are being studied for the extraction of volatile analytes from WBPs. A suitable optimization of the most important experimental extraction conditions is presented, together with a proof-of-concept application to the determination of volatile carbonyl compounds released from PBs and MDFs. This approach aims to offer a simple alternative to current reference methods which usually require complex extraction processes and specific and expensive equipment.

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P41. Determination of trace levels of organophosphate pesticides in water matrices by BAμE/GC-MS(SIM)

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To improve agricultural production and ensure a quality harvest and high yield, several approaches have been used to mitigate and control insect pests. Among others, the use of organophosphate pesticides stands out, which replaced organochlorine insecticides, which are now legally forbidden.
However, it's widespread use worldwide and persistent nature, although much less than organochlorine pesticides, continue to raise concerns. After application, part of these compounds persist in the environment at trace levels, contaminating mainly aquatic systems, representing a high risk to public health[1,2]. Therefore, it is imperative to continue to develop innovative and alternative analytical approaches that are even more effective to monitor traces of these contaminants, as is the case of passive microextraction techniques.
The present study proposes the application of bar adsorptive microextraction[3] in combination with gas chromatography coupled to mass spectroscopy (BAμE/GC-MS), to monitor trace levels of organophosphate pesticides in aqueous matrices. The main objective is to develop, optimize and validate an innovative and alternative methodology, compared to the most well-established ones, which can be applied to water samples of different types.

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P42. Detecção de pesticidas em azeite: a explorar uma abordagem baseada em sensores

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Os pesticidas são utilizados no olival no combate de pragas e doenças. A sua desadequada utilização ou o desrespeito pelo intervalo de segurança, pode levar a que estes persistam até à data de colheita das azeitonas. Dado que o azeite é obtido a partir da moenda das azeitonas meramente por processos físicos e/ou mecânicos, poderá ocorrer a presença destes contaminantes químicos no azeite. Dado o efeito nocivo destes contaminantes, é imperativo, no contexto da segurança alimentar, que a concentração destes compostos seja devidamente controlada. Os métodos de referência baseiam-se em técnicas cromatográficas - Cromatografia Líquida de Alta Performance (HPLC) e Cromatografia Gasosa (GC) acoplada a espectrometria de massa (MS)[1]. Apesar de apresentarem uma elevada sensibilidade, estas técnicas são caras, demoradas e manuseadas por técnicos especializados. Recentemente, novas abordagens baseadas em sensores têm vindo a ser exploradas, visando superar os inconvenientes das metodologias convencionais. No entanto, o desenvolvimento de sensores para a deteção de pesticidas em azeite centra-se fundamentalmente em biossensores enzimáticos, os quais apresentam algumas limitações[2]. De forma a ultrapassar alguns destes constrangimentos, este trabalho incidirá sobre o design de um sensor para a deteção de pesticidas em azeite. É expectável que a criação de um dispositivo que alie a seletividade na deteção vestigial destes contaminantes à portabilidade, permitirá a introdução de uma nova e versátil ferramenta analítica.

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P43. GCxGC using the INSIGHT Flow Modulator: What's going one?

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One dimensional gas chromatography has insufficient resolution to separate all the components of complex mixtures. Comprehensive two-dimensional gas chromatography (GCxGC) is regarded as a more powerful technique to achieve the separation goal and has become nowadays a mature technique.
The GCxGC concept uses two different columns with different and "complementary" stationary phases so that all the sample components pass sequentially through both columns (from 1st dimension to 2nd dimension), being separated by both separation mechanisms in a single run. The eluent is transferred between columns through a modulator, a device whose function is to continuously or periodically collect and focus fractions of the effluent from the first column and "re-inject" them to the second column. The most common modulators are the thermal/cryogenic and the flow modulators [1, 2]. Although thermal modulation may provide better resolution and higher S/N and be less constrained in column/flow combinations, the simplicity and low operational costs of flow modulators makes them a potential good choice for routine analysis [1, 3, 4].
In this research, a GC × GC system, set-up with the INSIGHT (SepSolve Analytical) Reverse Flow Modulator and a sample loop with a volume of 50 µL was tested using a Grob Test Mix solution and applied to grape samples. Evaluated parameters are Modulation Period (MP), Duty cycle (transfer rate), Modulation Ratio, Flush Time and Fill Time.
The results show that the system, by providing an efficient separation of the analytes, allows qualitative analyses. Experimental data also shown that for MP>2 seconds the duty cycle decreases, meaning that for trace analysis the system should operate at MPs≤2 seconds.

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P44. Special metabolites isolated from the leaf extract of *Amburana acrena* Ducke, a specie native to Brazil

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A espécie *Amburana acreana*, conhecida popularmente como 'Cumaru-de-cheiro' e 'Cerejeira-da-Amazônia', é nativa do Brasil e pode ser encontrada na região Norte do país. Espécies desse gênero são utilizadas na medicina popular no tratamento de doenças do sistema respiratório, ação anti-inflamatória e analgésica. Embora estudos ressaltem o potencial terapêutico do gênero, ainda são incipientes as informações sobre a composição química, sobretudo dessa espécie. Nesse sentido, o objetivo desse trabalho foi isolar e purificar, utilizando técnicas cromatográficas gravitacional e cromatografia líquida de média pressão (sistema Isolera Prime), os metabólitos especiais extraídos das folhas de *A. acreana* e, identificar estes metabólitos através da análise dos espectros de Ressonância Magnética Nuclear de ^{1}H e ^{13}C, bem como comparar com dados descritos na literatura. Aproximadamente 300 g de folhas de *A. acreana* foram coletadas no município de Ji-Paraná – RO, Brasil. O extrato bruto foi obtido através da remaceração a frio com etanol 94% em 4 ciclos de 24h, obtendo massa final de 88,3 g. O extrato bruto foi ressuspendido com metanol/água (9:1) e particionado com os solventes hexano (HAA), clorofórmio (CAA) e acetate de etila (ACAA), formando suas respectivas frações orgânicas. A fração CAA (14,7 g) foi submetida a coluna cromatográfica (CC), utilizando gel de sílica 60H (Acros, 0,063-0,200 mm) como fase estacionária e, fase móvel hexano/acetona, em gradiente de polaridade. Deste procedimento foi possível isolar 5 substâncias, as quais tiveram suas estruturas identificadas através da análise dos dados de RMN de ^{1}H e de ^{13}C (uni e bidimensionais), obtidos em espectrômetros Bruker, modelos DRX-500, operando a 500 MHz (^{1}H) e 125 MHz (^{13}C), utilizando como padrão de referência interna o TMS (Tetrametilsilano). Através da análise dos dados espectrométricos obtidos, aliados à comparação com dados descritos na literatura, foi possível identificar as substâncias isoladas como sendo a cumarina (**1**), ácido *p*-hidroxibenzoico (**2**), ácido vanílico (**3**), campesterol glicosilado (**4**) e amburosídeo B (**5**). A substância **5**, até o momento, foi identificada apenas em espécies de *Amburana*, podendo ser considerado um provável marcador quimiotaxonômico deste gênero.

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3. Canuto mais atual

P45. Discovering characteristic molecular markers of fortified wines based on volatilomic pattern obtained by HS-SPME/GC-MS

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The aim of the current study was to provide a useful platform to identify characteristic molecular markers related to the authenticity of Italian fortified wines. For this purpose, the volatilomic fingerprint of the most popular Italian fortified wines was established using headspace solid-phase microextraction combined with gas chromatography–mass spectrometry (HS-SPME/GC-MS). Several volatile organic compounds (VOCs), belonging to distinct chemical groups, were identified, ten of which are common to all the analyzed fortified Italian wines. Terpenoids were the most abundant chemical group in Campari bitter wines due to limonene's high contribution to the total volatilomic fingerprint, whereas for Marsala wines, alcohols and esters were the most predominant chemical groups. The fortified Italian wines VOCs network demonstrated that the furanic compounds 2-furfural, ethyl furoate, and 5-methyl-2-furfural, constitute potential molecular markers of Marsala wines, while the terpenoids nerol, α-terpeniol, limonene, and menthone isomers, are characteristic of Vermouth wines. In addition, butanediol was detected only in Barolo wines, and β-phellandrene and β-myrcene only in Campari wines. The obtained data reveal an adequate tool to establish the authenticity and genuineness of Italian fortified wines, and at the same time constitute a valuable contribution to identify potential cases of fraud or adulteration to which they are subject, due to the high commercial value associated with these wines. In addition, they contribute to the deepening of scientific knowledge that supports its valorization and guarantee of quality and safety for consumers.

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P46. Unravelling value-added compounds from grape pomace based on chromatographic data analysis

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Agri-food waste is a worldwide concern that continuously creates problems for society, the environment, human health, and the economy. To help reduce and minimize these concerns, it is necessary to implement transformation strategies that allow the conversion of agricultural waste into a variety of marketable added-value end products including bioactive compounds, biobased chemicals, biofuels, food additives, among others, to functionalize sustainable bio-economy model. In this study, the volatilomic fingerprint of GP obtained from different *Vitis vinifera* L. grapes was established by solid phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS), to explore the properties of the most dominant volatile organic metabolites (VOMs) in a context of its application on marketable products. A total of 52 VOMs belonging to different chemical families were identified. Alcohols, carbonyl compounds, and esters are the most dominant, representing 38.8, 29.3, and 24.2% of the total volatile profile of the investigated GP, respectively. Esters (e.g., isoamyl acetate, hexyl acetate, ethyl hexanoate) and alcohols (e.g., 3-methyl butan-2-ol, hexan-1-ol) can be used as flavoring agents with potential use in the food industry, and in the cosmetic industry, for fragrances production. In addition, the identified terpenoids (e.g., menthol, ylangene, limonene) exhibit antioxidant, antimicrobial, and anticancer, biological properties, among others, boosting their potential application in the pharmaceutical industry. The obtained results revealed the potential of some VOMs from GP to replace synthetic antioxidants, colorants, and antimicrobials used in the food industry, and in the cosmetic and pharmaceutical industry, meeting the increasing consumer demand for natural alternative compounds.

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P47. Singularities and applications of bio-oils: a perspective using Two-Dimensional Gas Chromatography (GCxGC)

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The use of bio-oils is associated with the knowledge of their chemical composition, which in turn contains thousands of different chemicals. One of the most critical problems in bio-oil analysis is the identification of its unknown compounds, which can be achieved using powerful techniques such as two-dimensional gas chromatography (2D-GC)[1-3]. Two-Dimensional Gas Chromatography (GCxGC) is a modern methodology and can be characterized by the sequential use of two chromatographic columns, the combination of them with orthogonal separation mechanisms leads to a significant increase in selectivity, being a useful feature for complex sample analyses. GC has two systems, FID and TOF, the first quantifies and the second identifies the families of chemical compounds. For the modulation of the 2nd dimension the system uses LN2.
The present review reports a comprehensive two-dimensional gas chromatography (GC×GC) for different oils designed to produce sustainable fuels, which showed more than 10,000 peaks and the main classes found were carboxylic acids, alcohols, aldehydes, esters, among others. The chromatographic method uses as first column a Rxi-5MS with 30m and as second column a Rxi-17SilMS with 1.4 m, both with 0.25 mmID and 0.25 µmdf. The injection of the samples uses a split 1:100 and the oven temperature have four ramps from 40ºC to 280 °C totalizing 7220 s, with FID detector temperature of 300 °C and the transfer line temperature of 280 °C to the TOFMS. The next step for this work is to perform a reverse chromatography to verify the possibility of further improving the separation of the compounds.

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P48. Fingerprinting the phenolic composition of spent coffee grounds from different geographical regions using µ-SPEed extraction combined with UHPLC-PDA

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Coffee is one of the most popular beverages in modern society. This popularity is mainly due to its pleasant taste and aroma as well as the stimulating effect of caffeine. Its consumption has continuously increased in recent years, reaching a market value close to 25 billion euros. Although generally treated as waste, spent coffee grounds are a rich source of several bioactive compounds with applications in diverse industrial fields. The present work aimed at the analysis of spent coffee grounds from different geographical origins (Guatemala, Colombia, Brazil, Timor, and Ethiopia) for the identification of bioactive compounds with industrial interest. For this purpose, the identification and quantification of the bioactive compounds responsible for the antioxidant activity attributed to the spent coffee grounds were attempted using miniaturized solid-phase extraction (µ-SPEed), combined with ultrahigh-performance liquid chromatography with photodiode array detection (UHPLC-PDA). After validation of the µ-SPEed/UHPLC-PDA method, this allowed us to conclude that caffeine and 5-caffeoylquinic acid (5-CQA) are the most abundant bioactive compounds in all samples studied. The total phenolic content (TPC) and antioxidant activity are highest in Brazilian samples. The results obtained show that spent coffee grounds are a rich source of bioactive compounds, supporting its bioprospection based on the circular economy concept closing the loop of the coffee value chain, toward the valorization of coffee by-products.

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P49. A chromatographic-based approach as platform to identify putative biomarkers from different apple varieties

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Aroma is a crucial criterion to assess the quality of apple fruits, juices, and ciders. The aim of this study was to explore similarities and differences in volatile fingerprint among apple fruits, juices, and ciders from different apple varieties (Festa, Branco, and Domingos) by headspace solid-phase microextraction gas chromatography–mass spectroscopy (HS–SPME/GC–MS). A total of 142 VOCs belonging to different chemical families were identified, namely, 58 esters, 34 alcohols, 19 aldehydes, 10 ketones, 8 terpenoids, 7 acids, 3 sulphur compounds, 1 dioxolane, 1 lactone, and 1 aromatic hydrocarbon. From these, only 9 VOCs were detected in all analysed matrices (fruit, juice, and cider) and in all apple-tested varieties (Festa, Branco, and Domingos). Moreover, a remarkable difference in terms of the qualitative and semiquantitative profiles was observed, which indicated that apple variety has a significant effect on the volatile profile. Esters and alcohols were the dominant chemical families, contributing 48.81%, 56.75%, and 94.04% on average for the total volatile profile of apple fruits, juices, and ciders, respectively. Moreover, there were unique VOCs for each matrix and for each variety, highlighting the importance of the selection of apple varieties as an important factor in obtaining good sensory and quality ciders, multiple benefits, and legal protection against the misuse of local products.

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P50. Prescriptomics

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Prescriptomics is an emerging field within proteomics and personalised medicine that focuses on deciphering the best drugs to target any individual through the individual proteomic phenotype. Recent advancements in high-resolution mass spectrometry, bioinformatics, and pharmacoproteomics have propelled this field to the forefront of precision medicine.
This work aims to provide an example on prescriptomics, elucidating its objectives, methodologies based on HPLC and mass spectrometry, and implications with two real examples.
High-resolution Mass Spectrometry accomplished to nano-HPLC and bioinformatics sis sued to obtaining the urine proteomic phenotype of bladder cancer patients.
Two bladder cancer patients are presented as case studies, showcasing the application of high-resolution mass spectrometry in prescriptomics. The proteomic variations observed in these patients highlight the potential of this approach for diagnostic and for tailoring drug treatments in cancer therapy.
Prescriptomics, primarily focused on proteomics, represents a promising frontier in personalized medicine. It offers the potential to revolutionize healthcare by tailoring drug therapies to individuals based on their proteomic profiles. As precision medicine continues to evolve, prescriptomics is poised to play a pivotal role in optimizing patient care and treatment outcomes.

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P51. Unraveling Insights through Targeted Tandem Mass Spectrometry with a Triple Quadrupole Mass Spectrometer

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This study was performed using ultra-performance liquid chromatography tandem mass spectrometry (UHPLC–ESI-MS/MS) with electrospray ionization on a TSQ Quantis triple quadrupole mass spectrometer to identify metabolites and establish a metabolic profile of natural products. The approach involved simultaneous determination of chemical compounds using Multiple Reaction Monitoring (MRM) mode and targeted analysis. However, a limitation of the GNPS molecular network was identified, stemming from the requirement for MS/MS spectra in dependent acquisition data (DDA), which is incompatible with the data acquired by the TSQ Quantis. To address this limitation, a database was constructed by consulting various sources, including DrugBank, FoodB, GNPS, HMDB, Metabolomics Workbench, and an extensive literature review. A total of 35 compounds belonging to the benzenoids, dipeptides, indoles, and lipids superclass were used to build the database. Of these compounds, 31 were identified in the samples metabolic profile and chemical characterization was established [1].
This comprehensive approach not only tackled the technical challenges posed by the instrument's data compatibility with GNPS but also provided valuable insights into the metabolic landscape. The constructed database, derived from diverse and reliable data sources, contributed to a robust foundation for compound identification. Overall, the study sheds light on the potential of UHPLC–ESI-MS/MS in tandem with a triple quadrupole mass spectrometer for in-depth metabolomic investigations.

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P52. Amino acids profile of wild boar (*Sus scrofa* L.) meat using automatic online pre-column derivatization: method optimization and validation

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Meat is one of the most important food products due to its nutritional value. Protein composition vary from one tissue to another within the same animal and in corresponding tissues of different species. The nutritional value of meat can vary greatly by the presence or absence of some amino acids, which are the basic units for protein construction [1]. Wild boar (*Sus scrofa* L.) is the most widespread species of wild animal throughout the world due to its great adaptability to the environment. The aim of this work was to optimize both alkaline and acid hydrolyses to obtain the amino acids in the free form and analyse the total amino acid profile of wild boar meat. Different sample/reagent ratios and times of reaction were tested and selected. RP-HPLC-FLD analysis was performed after automatic online pre-column derivatization with o-phthalaldehyde/3-mercaptopropionic acid and 9-fluorenylmethoxycarbonyl chloride and the final method was validated (limit of detection and quantification, linearity, inter-/intra-day precisions, and accuracy). The best conditions to perform alkaline and acid hydrolyses were 4M KOH/110˚C/22h and 6M HCl/110˚C/24h, respectively, both with a sample/reagent ratio of 75-100 mg/3 mL. The results showed that the main amino acids in the meat are glutamic acid (29.2 mg/g), leucine (18.8 mg/g), aspartic acid (18.6 mg/g) and valine (16.2 mg/g), in dry weight. The optimized method proved to be suitable for quantifying total amino acids from this meat with high accuracy and precision, using a microscale derivatization reagent and sample.

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P53. Optimizing Proteomic Analysis: Normalization Methods in Mass Spectrometry for Renal Cell Carcinoma Datasets

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Normalization plays a crucial role in data analysis by adjusting different datasets to reduce variability and ensure fair comparability[1]. While various methods like Z-score normalization, median divide normalization, and quantile normalization have been extensively studied, there is no clear consensus on the best approach, especially when considering factors like the number of experimental groups and sample sizes.
In our study[2], we focus on comparing these normalization methods within the context of renal cell carcinoma datasets. When comparing pairs of datasets, it consistently turns out that Z-score and quantile normalization outperform median divide normalization, leading to better results in protein identification, quantification, and the detection of statistically significant changes. However, when comparing three or more datasets simultaneously, the differences among the methods become less significant. This research provides insight into the effectiveness of these normalization techniques, suggesting that the choice of method should be tailored to the specific analysis scenario.

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P54. Decoding the chemical ecology for control of the Western conifer seed bug, *Leptoglossus occidentalis*, with green analytical tools

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Leptoglossus occidentalis is an invasive insect that causes significant losses to *Pinus pinea* cone production and seed yield, around the Mediterranean. Currently, control relies solely on the use of the insecticide Teppeki (1). If available, semiochemicals may represent an environmentally friendly management alternative. An attractive compound associated to the male emitted aggregation pheromone, leptotriene, has been identified (2). Yet, the synthesis high cost hinders its use as a control tool. Here we present further advances on the decoding of this species chemical ecology.

Pine and insect volatile compounds were collected by headspace solid phase micro extraction. Extracts of male ventral gland were prepared by macerating the glands in methanol. To identify antenally active compounds, gas chromatography simultaneously coupled to a flame ionization detector and to an electroantennographic setup was used. Behavioural biossays were conducted using a dual choice Y tube olfactometer.

Chromatographic analysis allowed for the detection of nine compounds, produced by males, associated to a behaviour of auto-stimulation of the ventral glands. Several of these compounds proved to be antennaly active. Electroantenographic response to 21 host compounds was confirmed. Behavioural biossays showed that secretions of male ventral gland were attractive to both sexes. A compound with kairomonal effect and two potential allomones were identified.

Males of *L. occidentalis* produce specific compounds when stimulating their ventral gland, which are potential components of a sex/aggregation pheromone that differs from the one used for overwintering aggregation. Host emitted compounds with behavioural effect were identified. New control perspectives are discussed.

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P55. Archaeological biomarkers on cooking ware from the Roman Cistern at Spoletino, Viterbo, Italy

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Spoletino cistern is an archaeological site located in the Lazio region in the municipality of Civitella D´Agliano in Italy, dated between the 1st century BC and the 4th century AD(1). Around the mid-1st century AD, it was divided into two sections: one remained as a cistern and the second was turned into a dumped-like deposit from where 21 pottery fragments from cooking ware pertaining to 5 different cooking vessel types, were collected and analyzed by GC-MS for identification of the organic residues preserved on them. The analyses aimed at providing information regarding dietary habits as well as food products used by the human population associated with this archaeological site during the transition from the Republic to the Imperial period.

Total lipid extracts were obtained for all pottery samples, using an organic solvent extraction method(2). All samples exhibited organic residues with analyzable content, and for 15 of them, it was possible to make a correlation between archaeological biomarkers and several food groups. After analyzing the results, it was possible to suggest a mixed diet of vegetable and animal products and a preferential use for *Pentola* (casserole) vessel compared with the *Olla* (pot) and *Tegame* (pan) shapes. For the *Clibano* vessel type, the collected data was not enough to make an association with any food group.

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P56. Application robustness of the 6495 triple quadrupole LC/MS system for non-stop Pesticide analysis in black tea matrix

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System robustness is of utmost importance especially when analyzing samples for routine, in-production, type of analysis. Additionally, when evaluating samples for meaningful scientific results, analysis of a large population of samples is necessary for good population statistics.
The new 6495 triple quadrupole LC/MS system (G6495D) is equipped with VacShield and iFunnel technology that aims to provide high sensitivity and high-performance analysis while being robust and rugged enough to withstand the effects of deposition from a complex and dirty matrix.

- VacShield –ion injector capillary removal mechanism that enables quick routine-maintenance, reduces downtime, and preserves system stability.
- iFunnel Technology – a dual-staged stacked ring ion funnel used to compress and concentrate the ion beam. Innovations within the iFunnel evacuate matrix components while maintaining injection-to-injection MRM precision.
- Instrument Intelligence – built into the overall system architecture to monitor and ensure that the instrument is in good operating condition.

Compared to non-iFunnel systems, the 6495 LC/TQ provides about 10x improvement in signal while providing superior injection-to-injection measurement robustness and precision at sub-millisecond dwell times.

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P57. Characterization and antimicrobial activity of a surfactin mixture produced by *Bacillus subtilis* ATCC 6633

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Surfactin (SF) is a lipopeptide produced by several *B. subtilis* strains. It consists of a ring structure of seven amino acids, with the sequence L-Glu-L-Leu-D-Leu-L-Val-L-Asp-D-Leu-L-Leu, linked to a β-hydroxy fatty acid with different chain lengths, through a lactone bond. As a biosurfactant surfactin not only displays the common beneficial physicochemical properties of a surfactant but also antimicrobial activity towards bacteria, virus and fungi [1].

This work aimed at producing and characterize a mixture of surfactins produced by Bacillus subtilis ATCC 6633 and evaluate its antimicrobial activity. Surfactins were produced in mineral salts medium (MSM), purified through dialysis and further lyophilized. The achieved mixture was then analyzed by reverse phase HPLC-ESI-MS/MS, and its antimicrobial activity evaluated by the minimum inhibitory concentration (MIC) assay.

Surfactin production by *B. subtilis* in MSM was accomplished with yields of 0.847 g L^{-1} and 0.836 g L^{-1} after 72 h and 96 h, respectively. Different surfactin lipopeptide congeners having chains ranging from C12 to C16, were identified by HPLC-MS. Surfactins mixture was able to inhibit the growth of *S. aureus*, *E. faecalis*, and *S. epidermidis* with a MIC ranging from 0.023 mg mL^{-1} to 0.750 mg mL^{-1}. Nevertheless, *P. aeruginosa* or *E. coli* growth was not inhibited under the tested conditions.

The characterization of surfactin mixtures herein produced and the confirmation of its antimicrobial activity against the tested strains shows its suitability for further application on pharmaceutical approaches.

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P58. A Direct Comparison of Applying Helium & Hydrogen Carrier Gases with HS-SPME-GC-TOF-MS Analysis of Aroma Active Compounds in Whisky

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The use of solid-phase microextraction (SPME) hyphenated to gas chromatography and mass spectrometry (GC-MS), for the analysis of aroma active volatiles is widely accepted and applied as an effective technique to provide insights during characterisation, quality screening and process development of food and beverage raw materials and products.
Additionally, SPME is a relatively green analytical approach due to considerable reduction in solvent volumes, the amounts of sample and extraction time required.
With growing emphasis on green analytical approaches, increased analysis throughputs and reducing costs, there is significant interest in using hydrogen (H_2) carrier gas instead of helium (He) for GC-MS workflows. He costs have been increasing year on year in the last decade, whereas H_2 can be safely supplied on-demand, via generators and allows superior gas chromatographic performance, enabling increased analysis speeds whilst maintaining or improving separation efficiency.
However, unlike He, H_2 is a reactive gas, and the possibility exists for the formation of artifacts within the analytical system. Previously, a comparison of He and H_2 carrier gas, being used in conjunction with various SPME fibres, reported hydrogenation of unsaturated species to varying extents, when using H_2, depending on the fibre phase type and desorption conditions used [1].
In this study we analysed the same whisky sample using He and H_2, together with a range of SPME fibre phase chemistries. Due to the high number of conditions being evaluated resulting in multiple data files (Figure 1), supervised statistical review and principle component analysis (PCA), using ChromaTOFSync software was used to compare trends and patterns between fibres and carrier gases.
The use of H_2 carrier gas for HS-SPME studies is attractive due to the significant higher analysis speeds obtainable. However, depending on the SPME fibres used, sample complexity and presence of unsaturated species, care must be taken to avoid erroneous outputs and this interpretation of results. In this study, the use of a triple bed fibre seemed to show much higher levels of hydrogenation, whereas PDMS showed little, if any at all. However, as expected use of PDMS only results in selectivity loss, which must also be evaluated.
Further work will explore use of GCxGC to better separate, identify and quantify artefacts, whilst alternative SPME fibre materials and capacities will also be trialled.