11th NATIONAL MEETING ON CHROMATOGRAPHY

9-11 Dezembro 2019
Caparica | Portugal

Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa
Title
11th National Chromatography Meeting

Título
11º Encontro Nacional de Cromatografia

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SCIENTIFIC AND SOCIAL PROGRAM

SATURDAY, DECEMBER 7

09:00 Short courses registration and FCT NOVA

1. Sample preparation methods for chromatographic analysis. **9:30 to 12:30**
   Eduardo Mateus, Resolution Lab, CENSE-FCT-NOVA, Portugal

2. MS hyphenation with LC and GC. **14:30 to 17:30**
   Marco Gomes da Silva, Resolution Lab, LAQV-FCT NOVA, Portugal

3. Validation of Chromatographic Methods. **14:30 to 17:30**
   Alice Mosca – AIM, Portugal and Ricardo Bettencourt Silva – FCUL, Portugal

SUNDAY, DECEMBER 8

4. Comprehensive gas chromatography – GC x GC. **9:30 to 12:30**
   Philip Marriott, School of Chemistry, Faculty of Science at Monash University – Australia

5. HPLC. **14:30 to 17:30**
   Marco Gomes da Silva, Resolution Lab, LAQV-FCT NOVA, - Portugal

WEDNESDAY, DECEMBER 11

6. Large-scale efficient extraction of chemical information from untargeted chemical profiling (GC/MS) data. **14:30 to 17:30**
   Rasmus Bro, Copenhagen University, Faculty of Sciences – Denmark
Short Courses

1. **Sample preparation methods for chromatographic analysis.**

   *Eduardo Mateus, Resolution Lab, CENSE-FCT NOVA, - Portugal*

**BRIEF SUMMARY:** The course will include an overview of extraction techniques, from classical to advanced and green methods for LC and GC analysis. Extractions involving solid, liquid, and vapor phases will be discussed with key examples including static and dynamic headspace extraction, sorptive micro-extractions (SPME, SBSE, SPE), and liquid-phase micro-extractions (LLE, MLLE). Participants will be asked to bring a sample preparation problem from their own work experience for discussion by the group.

**WHO SHOULD ATTEND:** Analytical scientists seeking a quick overview of sample preparation techniques for chromatography as well as 11ENC attendees seeking complementary information to the symposium. This course is also recommended for chromatographers seeking advice and ideas for difficult sample preparation problems.

2. **MS hyphenation with LC and GC.**

   *Marco Gomes da Silva, Resolution Lab, LAQV-FCT NOVA, - Portugal*

**BRIEF SUMMARY:**
This course covers the practical theory you need to know about LC and GC and hyphenation with MS and detector analysers. It will be also included, general data analysis and quantitative analysis. Tandem MS systems will be also covered.

**WHO SHOULD ATTEND:**
Scientists & technicians familiar with LC and GC but who need to analyze and quantify from complex samples.
3. Comprehensive gas chromatography – GC x GC.
Philip Marriott, School of Chemistry, Faculty of Science at Monash University - Australia

BRIEF SUMMARY:
The course will begin with a basic outline of the principles and developments underlying procedures of GCxGC. It will describe modulation strategies and detection strategies, choices of column sets and dimensions, as well as data processing and visualisation. Applications will target a variety of areas and be selected from the following: petrochemical, environmental, foods/flavours/fragrances, metabolomics, amongst others.

WHO SHOULD ATTEND:
Scientists & technicians familiar with GC but who have an interest in extending their understanding of multidimensional gas chromatography and GCxGC, and who need to analyse samples of increasing complexity.

4. HPLC.
Marco Gomes da Silva, Resolution Lab, LAQV-FCT NOVA, - Portugal

BRIEF SUMMARY:
The course will provide an expedited overview of basic HPLC techniques and applications. Chromatographic modes and detection systems will be outlined. Special emphasis on novel stationary phases will be given.

WHO SHOULD ATTEND:
Analytical scientists & technicians.
5. Large-scale efficient extraction of chemical information from untargeted chemical profiling (GC/MS) data
Rasmus Bro, Copenhagen University, Faculty of Sciences - Denmark

BRIEF SUMMARY:
GC/MS data may be challenging due to the high complexity of data including overlapped, embedded, retention time shifted and low S/N ratio peaks. This course will provide tools for processing raw data, namely GC/MS data as well as solutions for analysts dealing with complex chromatographic data. It allows extraction of chemical/metabolite information directly from the raw data and provides automatically methods to perform peak identification based on deconvoluted mass spectra using integrated NIST search engine, generating an identification report.

WHO SHOULD ATTEND:
All chromatography and mass spectrometry users.
6. Validation of Chromatographic Methods
Alice Mosca – AIM, Portugal and Ricardo Bettencourt Silva – FCUL, Portugal

BRIEF SUMMARY:
This course overviews the main concepts of chromatographic methods validation, both quantitative and qualitative, and covers the most important method performance parameters including ways of estimating them. Concepts like Accuracy (trueness and precision), selectivity, sensitivity, linearity, LoD, LoQ and robustness will be addressed. Statistically sound criteria for low-resolution GC-MS and LC-MS identifications will be presented.

WHO SHOULD ATTEND:
Analytical scientists and technicians seeking a quick overview of method validation techniques as well as 11ENC attendees seeking complementary information to the symposium.
CONGRESS INFO

Congress Office

The Congress Office is located at the entrance hall of Hotel Aldeia dos Capuchos Congress Center.

Opening hours:

Short Courses, Registration at FCT NOVA: Saturday, Sunday and Wednesday, Dec. 7 and 8 from 9:30 to 17:30 and Dec. 11 at 14:30. Courses on hyphenated and multidimensional techniques, sample preparation and data treatment.

11ENC
Monday, Dec. 9, 8:00 -18:45
Tuesday, Dec. 10, 8:45 - 18:25
Wednesday, Dec. 11, 8:45 - 13:15

Presentations:

The plenary (PL), oral communications (O) and flash oral communications (FO), will take place at the NERUDA ROOM. Poster presentations (P), will be presented at the DIAMONDS ROOM, LOBBY and NERUDA ROOM. For discussion, please meet the authors at their numbered poster board at the time indicated in the scientific program. Special stickers to hang up the posters are available at the Registration Desk.

Social Program

Monday, Dec 9, 19:00 “Welcome Cocktail”
Local: Hotel Aldeia dos Capuchos

Tuesday, Dec 10, 20:15 “Congress Dinner”
Local: Quinta do Miratejo
The 11ENC organization will provide transportation from the venue to table and back.

Wednesday, Dec 11, 13:15 “Farewell Cocktail”
Local: Hotel Aldeia dos Capuchos
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Programme

MONDAY, DECEMBER 9

8:00 Congress registration open
8:45 Opening Session – Opening Ceremony, NERUDA ROOM

Marco Gomes da Silva - Chairman of 11ENC, Universidade NOVA de Lisboa
Adelino Galvão, General Secretary, SPQ
Ana Costa Freitas, Rector Universidade de Évora
João Sáágua, Rector Universidade NOVA de Lisboa

MORNING SESSION
Session 1  Chair: Marco Gomes da Silva – Universidade NOVA de Lisboa

9:20  PL01  From amino acid analysis in 1969 to characterization of protein biopharmaceuticals in 2019
Pat Sandra¹
¹ Research Institute for Chromatography, President Kennedypark 26, B-8500 Kortrijk, Belgium

10:00  O1  Impurity Profiling: know the unknown by HRMS
Liliana Silva¹, Marco Galesio¹
¹ Hovione Farmaciência S.A., Analytical Development, Estrada do Paço do Lumiar, Campus do Lumiar, Edificio S, 1649-038 Lisboa, Portugal

10:20  O2  High sensitivity applications with High Resolution MS-QTOF: Analysis of PCB’s and PCDD’s in fish tissue by GC-APCI-QTOF
Miguel Ángel Pérez¹
¹ Bruker Applications Development Laboratory, Madrid, Spain

10:45 Coffee Break & Posters Session

MORNING SESSION
Session 2 Chair: Maria João Cabrita- Universidade de Évora

11:10  O3  Effect of gamma radiation on bioactive compounds of olive wastes
Madureira J¹, Dias MI², Barros L³, Santos-Buelga C³, Margaca FMA¹, Ferreira ICFR³, Cabo Verde S¹
¹ Centro de Ciências e Tecnologias Nucleares (CZTN-IST), Universidade de Lisboa, E.N. 10 ao km 139.7, 2695-066 Bobadela LRS, Portugal
² Centro de Investigación de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
³ Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno s/n, 37007 Salamanca, Spain
Seasonal effect on the Polycyclic Aromatic Hydrocarbons contents of *F. spiralis*, *Porphyra spp.* and *Ulva spp.* seaweed species harvested in the Portuguese coast

Vieira EF\(^1\), Soares C\(^1\), Ramalhosa MJ\(^1\), Sousa S\(^1\), Oliva-Teles MT\(^1\), Correia M\(^1\), Carvalho AP\(^1\), Domingues VF\(^1\), Morais S\(^1\), Delerue-Matos C\(^1\)

\(^1\) LAQV, REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, R. Dr. António Bernardino de Almeida 431, 4200-072 Porto, Portugal

How far can you get in the analysis of complex mixtures through 2D-LC?

António Chana\(^1\)

\(^1\) Agilent Technologies, C/ José Echegaray, 8, 28232 Las Rozas, Spain

An improved method for determination of sotolon in Port wines

Milheiro J\(^1\), Vilamarim R\(^1\), Filipe-Ribeiro L\(^1\), Cosme F\(^2\), Nunes FM\(^3\)

\(^1\) CQ-VR, Food and Wine Chemistry Lab, Chemistry Research Centre, University of Trás-os-Montes and Alto Douro, School of Life Sciences and Environment, Chemistry Department, 5000-801, Vila Real, Portugal

\(^2\) CQ-VR, Food and Wine Chemistry Lab, Chemistry Research Centre, University of Trás-os-Montes and Alto Douro, School of Life Sciences and Environment, Biology and Environmental Department, 5000-801, Vila Real, Portugal

\(^3\) Department of Pharmaceutical Botany, “Iuliu Hațieganu” University of Medicine and Pharmacy, Romania

Cytinus hypocistis (L.) L. extract as a source of anti-aging cosmeceutical ingredients

Ana Rita Silva\(^1,2\), Taofiq Oludemi\(^1\), José Pinela\(^1\), Maria Inês Dias\(^2\), Ricardo C. Calhelha\(^1\), Maria José Alves\(^1\), Andrei Mocan\(^3\), Pablo A. García\(^2\), Lillian Barros\(^1\), Isabel C.F.R. Ferreira

\(^1\) Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Portugal

\(^2\) Facultad de Farmacia, CIETUS-IBSAL, Universidad de Salamanca, Salamanca, España

\(^3\) Department of Pharmaceutical Botany, “Iuliu Hațieganu” University of Medicine and Pharmacy, Romania

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\(^1\) Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Portugal

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\(^3\) Department of Pharmaceutical Botany, “Iuliu Hațieganu” University of Medicine and Pharmacy, Romania

Extraction of chemical information from untargeted chemical profiling (GC-MS) data

Rasmus Bro\(^1\)

\(^1\) Department of Food Science, University of Copenhagen, 1958 Frederiksberg, Denmark

Combining analytical pyrolysis and chemometrics: A powerful approach to study complex organic matrices

Jiménez-Morillo NT\(^1,2\), Miller AZ\(^2\), Palma V\(^1\), Dias Barrocas C\(^2\), Cabrita MJ\(^1\)

\(^1\) ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7006-554 Évora, Portugal

\(^2\) Laboratório HERCULES, Universidade de Évora, Palácio do Vimioso, 7000-809 Évora, Portugal

Integration of data from GC-MS and UPLC-QTOF-MS to better understand wine ageing: a new graphical interface

A.R. Monforte\(^1\), A. C. Silva Ferreira\(^2\)

\(^1\) Centro de Biotecnologia e Química Fina (CBQF), Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

\(^2\) Institute for Wine Biotechnology (IWBT), Department of Viticulture and Oenology (DVO), University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa

\(^3\) Cork Supply Portugal, S.A., Rua Nova do Fial 102, 4535 São Paio de Oleiros, Portugal
15:40 O10  The Use of Ion Mobility-MS to Resolve and Discover Sample Complexity In Small Molecule Analysis
Alberto Méndez1
1 Waters Corporation

16:05 O11  Analysis of skin volatiles using a membrane-SPME/GC-MS approach to unveil putative biomarkers for neurodegenerative diseases
Beatriz Andrade1, Jorge Pereira1, José Câmara1,2
1 CQM – Centro de Química da Madeira, Universidade da Madeira, Campus Universitário da Penteada, 9020-105 Funchal, Portugal
2 Faculdade de Ciências Exatas e da Engenharia, Universidade da Madeira, Campus Universitário da Penteada, 9020-105, Funchal, Portugal

16.25 Coffee Break & Posters Session

AFTERNOON SESSION
Session 4 Chair: Cristina Dias - Universidade de Évora

17:00 O12  Determination of the phenolic composition of vine-canies subcritical water extracts and its utilization for production of a topical formulation
Manuela M. Moreira1, Francisca Rodrigues1, Olena Dorosh1,2, Diana Pinto,1 Andreia F. Peixoto,3 Paulo Costa4, Simone Morais5, Cristina Freire5, Cristina Delerue-Matos1
1 REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072, Porto, Portugal
2 Instituto de Tecnologia Química e Biológica António Xavier, Av. da República 2780-157 Oeiras, Portugal
3 REQUIMTE/LAQV, Dep. de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
4 UCIBIO/REQUIMTE, MedTech-Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto

17:20 O13  HPLC and UHPLC Selectivity – Finding a Selectivity Starting Point
Zeshan Aqeel1, Felipe Silva2, PhD. Jason Anspach1, and Ryan Splitstone1
1 Phenomenix, Inc., 411 Madrid Ave., Torrance, CA 90501 USA
2 Phenomenex C/ Valgrande 8, planta 2.1.B., 28108 Alcobendas, Madrid, Spain

17:45 O14  Separation of Nadolol Racemates by High pH Reversed-Phase Fixed-Bed and Simulated Moving Bed Chromatography
R. Arafah1,2, A. Ribeiro1,2, A. Rodrigues2, L. Pais1,2
1 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Apartado 1134, 5301-857 Bragança, Portugal
2 Laboratory of Separation and Reaction Engineering, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal

18:05 O15  Pharmaceutical drugs as emerging pollutants in aqueous media of Northeast Portugal
A. Oliveira1, A. Ribeiro1,2, P. Brito1, A. Queiroz1
1 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
2 Laboratory of Separation and Reaction Engineering, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal
18:25 FO1 (P04)  Chemical characterization of the hydrodistillation residual water and essential oil of Crithmum maritimum
Jorge M. Alves-Silva¹,²,*, Inês Guerra¹,*, Maria José Gonçalves¹, Carlos Cavaleiro¹, Maria Teresa Cruz³, Artur Figueirinha⁴, Lígia Salgueiro¹
¹ CIEPQPF and Faculty of Pharmacy, University of Coimbra, Azinhaga de Santa Comba, Celas, 3000-548 Coimbra, Portugal
² iCBR, Faculty of Medicine, University of Coimbra, Azinhaga de Santa Comba, Celas, 3000-548 Coimbra, Portugal
³ CNC, Faculty of Pharmacy, University of Coimbra, Azinhaga de Santa Comba, Celas, 3000-548 Coimbra, Portugal
⁴ LAQV, REQUIMTE, Faculty of Pharmacy, University of Coimbra, Azinhaga de Santa Comba, Celas, 3000-548 Coimbra, Portugal
* Authors contributed equally to the work

18:29 FO2 (P18)  Occurrence of polybrominated diphenyl ethers and their metabolites in Douro river biota
D. Menezes-Sousa¹,², S.C. Cunha¹, F.H.S. Fogaça³, M.B. Alonso², J.P.M. Torres², A. Marques⁴,⁵, L. Guilhermino⁶,⁷, J.O. Fernandes¹
¹ LAQV, REQUIMTE, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal
² Laboratório de Radioisótopos Eduardo Penna Franca, Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil
³ Empresa Brasileira de Pesquisa Agropecuária, Brasil
⁴ 2IPMA, Divisão de Aquacultura e Valorização, Instituto Português do Mar e da Atmosfera, I.P., Avenida de Brasília, 1449-006, Lisboa, Portugal
⁵ CIIMAR, Universidade do Porto, Rua dos Bragas 289, 4050-123, Porto, Portugal
⁶ ICQAS-LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal
⁷ ICQAS-LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal

18:33 FO3 (P26)  The use of chromatographic methods to study the contribution of oral cells in polyphenols-salivary proteins interaction
Soares S.¹, Brandão E.¹, Guerreiro C.¹, Mateus N.¹, de Freitas V.¹, Soares S.¹
¹ LAQV, REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal

18:37 FO4 (P30)  Analysis of phenolic and lipophilic compounds of elderberry stalks from northern Portugal using high performance chromatographic techniques coupled with mass spectrometry
Samuel Patinha¹, Juliana V. Murteira¹, Ângelo C. Salvador¹,², Sónia A. O. Santos², Armando J. D. Silvestre², Sílvia M. Rocha³
¹ QOPNA/LAQV-REQUINTE, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal
² CICECO-Aveiro Institute of Materials Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

18:41 FO5 (P34)  Evaluation of antiglycation potential of Sambucus nigra L.
Sandrine S. Ferreira¹,², Amélia M. Silva², Fernando M. Nunes¹
¹ CQ-VR, Chemistry Research Centre-Vila Real, Food and Wine Chemistry Lab, University of Trás-os-Montes and Alto Douro, Quinta dos Prados, 5000-801, Vila Real, Portugal
² CITAB-UTAD, Center for Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro Quinta dos Prados, 5000-801, Vila Real, Portugal

19:00 Welcome Cocktail LOBBY

Molecular Gastronomy Moments
MORNING SESSION
Session 5 Chair: José Manuel Nogueira – Universidade de Lisboa

8:45 PL03  “Smart” Gradients for Enhancing Peak Capacity in Comprehensive Two-dimensional Liquid Chromatography under Reversed-phase Conditions: Application to Polyphenols in Food and Natural Real-world Samples
Paola Dugo1,2, Francesco Cacciola3, Katia Arena1 and Luigi Mondello1,2,4,5
1 Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy
2 Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy
3 Department of Biomedical, Dental, Morphological and Functional Imaging Sciences, University of Messina, Messina, Italy
4 Unit of Food Science and Nutrition, Department of Medicine, University Campus Bio-Medico of Rome, Rome, Italy
5 BeSep s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

9:25 O16  New coloring strategy for dairy products using anthocyanin extracts from edible flowers
Tânia C.S.P. Pires1,2, Rúbia C.G. Corrêa1,3, Maria Inês Dias4, Lillian Barros1, João C.M. Barreira1, Celestino Santos-Buelga2, Isabel C.F.R. Ferreira1
1 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
2 Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno s/n, 37007 Salamanca, España
3 Program of Master in Science, Technology and Food Safety, Cesumar Institute of Science, Technology and Innovation (ICETI), University Center of Maringá (UNICESUMAR), Maringá, Paraná, Brazil

9:45 O17  Natural colorants in cookies: evaluation of the incorporation effects on the physico-chemical composition
Custódio L. Roriz1,2, Eliana Pereira1, Sandrina Heleno1, Márcio Carocho1, Lillian Barros1, Isabel C.F.R. Ferreira1
1 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
2 Dpto. Nutrición y Ciencia de los Alimentos. Facultad de Farmacia. Universidad Complutense de Madrid (UCM), Madrid, Spain

10:05 O18  Setting New Benchmarks of Intelligence, Efficiency, and Design in Chromatography
Raymond Wong1, Anja Grüning2, Gesa J. Schad2, Jan Stenzler3, Manuel Lucini4
1 Shimadzu UK, Milton Keynes, United Kingdom
2 Shimadzu Europa, Duisburg, Germany
3 Shimadzu Deutschland, Duisburg, Germany
4 Izasa Scientific, Spain

10:30 O19  High Throughput Bar Adsorptive Microextraction (HT-BAµE): A simple and effective tool for the simultaneous enrichment of ketamine and norketamine from large number of urine matrices
S. M. Ahmad1, J. M. F. Nogueira2
1 Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

10:50 Coffee Break & Posters Session
MORNING SESSION
Session 6 Chair: Ana Maria Seca – Universidade dos Açores

11:10 O20 Validation of a method to quantify acrylamide in biscuits
João Siopa¹, Fernanda Cosme¹,², Fernando M. Nunes¹,³
¹ Centro de Química de Vila Real (CQ-VR) – Food and Wine Chemistry Lab, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5001-801, Vila Real, Portugal
² Departamento de Biologia e do Ambiente, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5001-801, Vila Real, Portugal
³ Departamento de Química, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5001-801, Vila Real, Portugal

11:30 O21 Very Fast analysis of TCA in cork Disks by HS-SPME GC/MS/MS – A Proof-of-concept
Cátia Santos¹, Renato Cres², Marco Gomes da Silva¹, Eduardo Mateus³
¹ LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
² SOQUÍMICA – Sociedade de Representações de Química, Lda., Rua Coronel Santos Pedroso, 15 - 1500-207 Lisboa, Portugal
³ CENSE – Center for Environmental and Sustainability Research, DCEA, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal

11:50 O22 Polar Pesticides Anions in water and food using a new and unique Ion Chromatography and Mass Spectrometry High Resolution MS or MSMS method
Ettlin Daniel¹, Jorge Alves², Anne Marie Compianno³
¹ Unicam Sistemas Analíticos Lda. - Alameda Antonio Sergio 22 -7B -1495-132 Mirafl ores, Portugal
² Unicam Sistemas Analíticos Lda - Alameda Antonio Sergio 22 -7B -1495-132 Mirafl ores, Portugal
³ Thermo Scientific – Laboratoire de CSC – Villebom sur Ivette – Les Ullys – France

12:15 O23 Chemical characterization of Cistus ladanifer L. lipophilic fraction: an underexploited raw material of biologically active terpenes
Valentina F. Pinheiro¹, Olinda Guerreiro¹,², David Soldado¹, Patricia A. B. Ramos³,⁴, Sônia A. O. Santos³, Carmen S. R. Freire³, Armando J. D. Silvestre³, Eliana Jerónimo¹,²
¹ Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal
² Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAM), Universidade de Évora, 7000 Évora, Portugal
³ CICECO and 4QOPNA & LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

12:35 O24 Chemical characterization of new psychoactive substances belonging to the class of synthetic cathinones in seized materials in Portugal
Gonçalves JL¹, Alves VL¹, Caldeira MJ², Teixeira HM¹,², Câmara JS¹,⁵
¹ CQM - Centro de Química da Madeira, Universidade da Madeira, Campus Universitário da Penteada, 9020-105 Funchal, Portugal
² Laboratório de Polícia Científica da Polícia Judiciária, Novo edifício-sede da Polícia Judiciária, Rua Gomes Freire 1169-007 Lisboa
³ Instituto Nacional de Medicina Legal e Ciências Forenses, I.P., Delegação Centro, Largo da Sé Nova, 3000-213 Coimbra, Portugal
⁴ Faculdade de Medicina da Universidade de Coimbra, Azinhaga de Santa Comba, Celas, 3000-548 Coimbra, Portugal
⁵ Faculdade de Ciências Exatas e da Engenharia, Universidade da Madeira. Campus da Penteada, 9020-105 Funchal, Portugal

12:55 Lunch & Posters Session
AFTERNOON SESSION
Session 7 Chair: José Câmara – Universidade da Madeira

14:30 O25 Selective pre-enrichment of pesticide residues in olive oil samples: from MISPE technology into smart MIPs
Raquel Garcia1, Elisabete Carreiro2, Nuno Martins3, Marco Gomes da Silva3, Anthony J. Burke2, Ana Maria Costa Freitas1, Maria João Cabrita1
1 ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7006-554 Évora, Portugal
2 Centro de Química de Évora, Universidade de Évora, Colégio L.A. Verney, 7000 Évora, Portugal
3 LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

14:50 O26 Application of Tape Adsorptive Microextraction to Determine Benzophenone & Related Compounds in Water Matrices
N.R. Neng1, J.M.F. Nogueira1
1 Centro de Química e Bioquímica e Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Campo Grande Ed. C8, 1749-016 Lisboa, Portugal

15:10 O27 Application of Deep Eutectic Solvent in extraction of PAHs in soft drinks
Lucas Caldeirão1,2, Helena Teixeira Godoy1, José O. Fernandes2, Sara C. Cunha2
1 Departamento de Ciência de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Rua Monteiro Lobato, 80, 13083-862, Campinas, São Paulo, Brasil
2 LAQV/REQUIMTE, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo, Ferreira 228, 4050-313, Porto, Portugal

15:30 O28 An Innovative and Robust Triple-Quadrupole Tandem Mass Spectrometer Aid to Meet Standards and Regulatory
Ignazio Garaguso1
1 PerkinElmer LAS Germany GmbH, Ferdinad-Porsche-Ring 17, 63110 Rodgau, Germany

15:55 O29 Evaluation of the QuEChERS and magnetic micro dispersive solid-phase extraction of brominated flame retardants in red fruits with determination by GC/MS
Virgínia Cruz Fernandes1, Maria Freitas1, João G. Pacheco1, Valentina Fernandes Domingues1, Cristina Delerue-Matos1
1 LAQV, REQUIMTE, Instituto Superior de Engenharia do Porto, Politécnico do Porto, Rua Drº António Bernardino de Almeida, 431, 4200-072 Porto, Portugal

16:15 O30 AQbD and High-throughput analytical toolkit combination to support RP-LC Method Development for Itraconazole Quantification
Rute Nunes1, Patrícia Nunes1,2, Lúcia Volta e Sousa1
1 Hovione Farmacêutica S.A., Analytical Development, Estrada do Paço do Lumiá, Campus do Lumiá, Edifício S, 1649-038 Lisboa, Portugal
2 Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal

16.35 Coffee Break & Posters Session
AFTERNOON SESSION
Session 8 Chair: Cristina Delerue Matos – Instituto Superior de Engenharia do Porto

17:00 O31 Phytochemical composition and in vitro antioxidant and antimicrobial properties of Aloe vera leaf tissue extracts
Mikel Añibarro-Ortega¹, José Pinela¹, Ana Ćirić², Cristina Caleja¹, Olga Ferreira¹,³, Marina Soković², Lillian Barros¹, Isabel C.F.R. Ferreira¹
¹ Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
² Department of Plant Physiology, Institute for Biological Research “Siniša Stanković”, University of Belgrade, Bulevar despot Stefan 142, Belgrade, Serbia
³ Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

17:20 O32 A Systematic UHPLC/HPLC Method Development Strategy with Complementary Stationary Phases to Maximise Selectivity and Resolution
Gemma Lo¹
¹ Hichrom /VWR, part of Avantor, Rua da Indústria n°6, 2610-088 Amadora, Portugal

17:45 O33 LC-MSMS for Human Health Diagnosis: Identification of Stress Biomarkers in Sweat
M. João Nunes¹, Cristina M. Cordas¹, José J.G. Moura¹, Luís Branco¹, João Paulo Noronha¹
¹ LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

18:05 FO6 (P58) Determination of Perfluorooctanesulfonic Acid (PFOS) in river water and biota matrices by UPLC-MS-MS
Ana Fernandes Cruz¹, Joana Silva¹, Georgina Sarmento¹, Paula Viana¹, Margarida Santos Romão¹,²
¹ LAIST, Laboratório de Análises do Instituto Superior Técnico, 1049-001 Lisboa, Portugal
² Agencia Portuguesa do Ambiente, 2610-124 Amadora, Portugal

18:09 FO7 (P03) New capillary zone electrophoresis method for the determination of cinchocaine hydrochloride and hydrocortisone
Ahmed S. Saad¹,², Mohamed R. El-Ghobashy¹,², Nada S. Ayish¹ and Badr A. El-Zeany¹
¹ Cairo University, Faculty of Pharmacy, Analytical Chemistry Department, Kasr Elaini St., P.O. box 11562, Cairo, Egypt.
² October 6 University, Faculty of Pharmacy, Chemistry Department, October 6 city, Giza, Egypt

18:13 FO8 (P01) Determination of N-nitrosodiethanolamine in shampoo using on-line solid phase extraction-ultra-high performance liquid chromatography coupled to tandem mass spectrometry
Alyne Tada¹,², Susanne Rath²
¹ IFB, Campus Gama, Institute Federal of Brasilia, 72429-005 Gama, DF, Brazil
² Institute of Chemistry, Department of Analytical Chemistry, University of Campinas, P.O, Box 6154, 13084 - 971 Campinas, SP, Brazil

18:17 FO9 (P65) The use of Statistic Tools for QuEChERS Content Optimization in the Extraction of Ibuprofen and its Metabolites in Different Types of Soil Samples
Paíga P¹, Delerue-Matos C¹
¹ REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal
18:21 FO10 (P09)  

*Qualitative doping analysis of β-blockers in urine by GC-MS/MS*

Rocha Gomes T.¹, Gomes S.¹, Salema B.¹, Ruivo J.¹

¹ Laboratório de Análises de Dopagem, Av. Professor Egas Moniz (Estádio Universitário), 1600-190 Lisboa, Portugal

18:30 Chromatography Group Meeting

20:15 Congress Dinner
MORNINGS SESSION

Session 9 Chair: Silvia Rocha – Universidade de Aveiro

8:45 PL04  Comprehensive Two-Dimensional Gas Chromatography – Expectations beyond Design?
Marriott P

1 Australian Centre for Research on Separation Science, School of Chemistry, Monash University, Clayton 3800, Victoria, Australia

9:25 O34  Use of GC×GC-ToFMS to evaluate the impact of plant-based coatings in the preservation of ‘Rocha’ pears during long-term storage
Alexandre M. A. Fonseca1, Cindy Dias2, Ana L. Amaro3, Né lion Isidoro3, Manuela Pintado2, Armando J. D. Silvestre1, Sílvia M. Rocha4

1 CICECO, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro
2 Universidade Católica Portuguesa, Centro de Biotecnologia e Química Fina – Lab. Associado, Escola Superior de Biotecnologia, 4169-005 Porto
3 Cooperativa Agrícola dos Fruticultores do Cadaval, CRL (COOPVAL), Estrada Nacional 115, Km 26 2550-108 Cadaval
4 QOPNA/LAQV-REQUIMTE, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro

9:45 O35  Influence of the growth cycle on the chemical composition and biological properties of Cynara cardunculus L. var. altillis blades and petioles
Fi lipa Mandim1,2, Ângela Fernandes1, Maria Inês Dias1, José Pinela1, Spyridon A. Petropoulos3, Marina Kosti ć4, Marina Soković4, Lillian Barros1, Celestino Santos-Buelga2, Isabel C.F.R. Ferreira1

1 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal
2 GIP-USAL, Faculdad de Farmacia, Universidad de Salamanca, Salamanca, Spain
3 University of Thessaly, Department of Agriculture, Crop Production and Rural Environmental, Magnissia, Greece
4 Institute for Biological Research “Siniša Stanković”, University of Belgrade, Belgrade, Serbia

10:05 O36  Quantitation and Non-Target Detection of Pesticides in Spinach Extract with Pegasus BT 4D. Improvement to Targeted & Untargeted Pesticide Residue Analysis: Fast and Flexible Analyte Finding For GC-MS and GCxGC-MS
Julio Lluch1

1 LECO Sep Science Sales Engineer, Madrid, Spain

10:30 O37  Selection of the best urine sampling period based on the volatomic profile
Priscilla Porto-Figueira1, José Figueira1, Margarida Baptista1, Sofia, Cristina Berenguer1, Jorge Pereira1, Valdemar Máximo2,4, José S. Câmara1,5

1 CQM - Centro de Química da Madeira, Universidade da Madeira, Campus Universitário da Penteada, 9020-105, Funchal, Portugal
2 i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto, R. Alfredo Allen, 4200-135, Porto, Portugal
3 IPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, R. Júlio Amaral de Carvalho 45, 4200-135, Porto, Portugal
4 Departamento de Patologia, Faculdade de Medicina da Universidade do Porto, Universidade do Porto, Alameda Prof. Hernâni Monteiro, 4200-319, Porto, Portugal
5 Faculdade de Ciências Exatas e da Engenharia, Universidade da Madeira, Campus Universitário da Penteada, 9020-105, Funchal, Portugal

10:50 Coffee Break & Posters Session
MORNING SESSION
Session 10 Chair: José Oliveira Fernandes – Universidade do Porto

11:10 O38  The Influence of Soil Fertilization on the Amino Acid and Volatile content of Aragonez Wine
Catarina Pereira¹, Nuno Martins², Davide Mendes³, Pedro Alpendre⁴, Marco D. R. Gomes da Silva³, Maria João Cabrita¹
¹ IIFA, ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Núcleo de Mitra, Ap. 94, 7006-554 Évora, Portugal
² ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Núcleo de Mitra, Ap. 94, 7006-554 Évora, Portugal
³ Departamento de Química, Faculdade de Ciências e Tecnologia, LAQV, REQUIMTE, Universidade Nova de Lisboa, Campus da Caparica 2829-516 Caparica, Portugal
⁴ Departamento de Fitotecnia, Escola de Ciências e Tecnologia, ICAAM, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

11:30 O39  Comprehensive aroma profiling of food and beverages by GC×GC-TOF MS/FID/SCD
Laura McGregor¹, Anthony Buchanan¹, Aaron Parker¹, Hannah Calder² and Jody Dunstan²
¹ SepSolve Analytical, 22 Commerce Road, Lynch Wood, Peterborough, PE2 6LR, United Kingdom
² Markes International Ltd, Gwaun Elai Medi-Science Campus, Llantrisant, Wales, CF72 8XL, United Kingdom

11:55 O40  Using chromatography to unveil the secrets of the past
Ana Manhita¹, Mafalda Costa², Catarina Miguel¹, Silvânia Afonso³, Cristina Barrocas Dias¹,²
¹ Laboratório HERCULES, Universidade de Évora, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal
² Departamento de Química, Escola de Ciências e Tecnologia, Universidade de Évora, Rua Romão Ramalho 59, 7000-671 Évora, Portugal

12:15 FO11 (P68)  Chemical characterization of the sacred wood: final resting place of Benedictine Abbots of St. Margaret, Bijela
A. Fundurulic¹,², A. Janeš², A. Manhita³, A. Celant¹, C. Barrocas Dias³, D. Magri¹
¹ Department of Environmental Biology, Faculty of Mathematical, Physical and Natural Sciences, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
² Department for Archaeology, Division for Archaeological Heritage, Croatian Conservation Institute, Kožarska 5, 10 000 Zagreb, Croatia
³ HERCULES Laboratory, University of Évora, Palacio do Vimioso, Largo Marques de Marialva 8, 7000-809 Evora, Portugal

12:19 FO12 (P73)  Discrimination of Lavandula essential oil growing in Castelo Branco region by GC-MS and FTIR-ATR
Joana Domingues¹,², Daniela Coutinho¹, Fernanda Delgado¹,³,⁴, José Carlos Gonçalves⁵,⁶,⁷, Ofélia Anjos¹,³,⁷
¹ CBPBI- Plant Biotechnology Center of Beira Interior, Quinta da Senhora de Mêrcules, Apartado 119, 6001-909, Castelo Branco, Portugal
² CICS-UBI- Health Sciences Research Centre, Universidade da Beira Interior, Av. Infante D. Henrique 6200-506, Covilhã, Portugal
³ IPCB-ESA- Instituto Politécnico de Castelo Branco, Escola Superior Agrária, Quinta da Senhora de Mêrcules, Apartado 119, 6001-909. Castelo Branco, Portugal
⁴ CERNAS-IPCB- Research Centre for Natural Resources, Environment and Society, Instituto Politécnico de Castelo Branco, Portugal
⁵ CEF - Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

12:23 FO13 (P17)  Using SPME/GCxGC-ToFMS approach for a rapid and early evaluation of food contamination based on A. niger biomarkers pattern
Carina Costa¹, João Raul Belinato de Souza², Adelaíde Almeida³, Fabio Augusto², Silvia M. Rocha¹
¹ Department of Chemistry & QOPNA/LAQV- REQUIMTE, University of Aveiro, 3810-193 Aveiro, Portugal
² Institute of Chemistry, University of Campinas and National Institute of Science and Technology in Bioanalysis (INCTBio)
³ Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal
12:27 FO14 (P74)  *Development of methodologies to evaluate odour retention capacity in textiles*
Inês Pinheiro¹, Ana Magalhães¹, Catarina Costa¹, Lorena Coelho¹
¹ CeNTI, Centro de Nanotecnologia, Materiais Técnicos, Funcionais e Inteligentes, 4760-034 Vila Nova de Famalicão, Portugal

12:31 FO15 (P75)  *Rapid determination of some of the most used pesticides in Northeast Portugal as emerging contaminants in rivers by SPME/GC-MS.*
A. Oliveira¹, R. Ben Hmida¹, A. Ribeiro¹,², P. Brito¹, A. Queiroz¹
¹ Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
² Laboratory of Separation and Reaction Engineering, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal

12:35 Closing Ceremony & “Best Presentation” Awards

13:15 Farewell Cocktail
Pat Sandra is known for his expansive knowledge across a wide spectrum of analytical techniques, blending the world of academia and private enterprise, and bringing separation science into the mainstream consciousness. He has combined analytical excellence, innovation, and a unique gift for problem solving into a career which has lasted almost 50 years. Pat has written hundreds of publications in peer-reviewed journals and is a well-known speaker at prestigious events around the world. He started his own chromatography group, the RIC group in Kortrijk, Belgium, that focuses on separation science and mass spectrometry. As a long-time professor at the Ghent University, from which he recently retired, he has supported many scholarships and international cooperation with numerous universities and companies. Pat was also the driving force behind the popular symposium on capillary separation techniques that is organized biannually in Riva del Garda, Italy.

The Research Institute for Chromatography (R.I.C.) was founded in 1986 by Pat Sandra and has been involved in the development and promotion of chromatographic and mass spectrometric knowledge and know-how from the very start. R.I.C.’s mission is to transfer chromatographic and mass spectrometric knowledge and know-how. Ever since its foundation, the research activities and the method development projects have been situated in a broad range of application areas, such as such as (bio)chemistry, biotechnology, petro-chemistry, clinical chemistry, food, flavors and natural products, polymer sciences, pharmaceutical sciences, toxicology, environmental chemistry and life sciences. Parallel with these services, R.I.C. is offering total analytical solutions and is involved in instrumental innovations as such and in collaboration with manufacturers.

PL - Plenary Lectures – Invited Speakers

PL01 From amino acid analysis in 1969 to characterization of protein biopharmaceuticals in 2019
Pat Sandra
Research Institute for Chromatography, President Kennedypark 26, B-8500 Kortrijk, Belgium
Rasmus Bro is performing research on most aspect of chemometrics and in particular on multi-way analysis both from a theoretical and a practical point of view. He is heading an industrial research consortium, ODIN, focusing on Process Analytical Technology (PAT) and has also started a new master of science in the same area. He has been an editor on Journal of Chemometrics for many years and is the author of a number of matlab toolboxes that are made available at www.models.life.ku.dk.

The Primary fields of research are Chemometrics, Data Mining, Multivariate Calibration, Classification. Multi-way Analysis, Exploratory Analysis, Experimental Design, Fluorescence spectroscopy, Numerical Analysis, Spectroscopy, Metabonomics, Process Analytical Technology and gas chromatography.
Luigi Mondello is full Professor of Analytical Chemistry at the University of Messina in Italy. He is co-president and co-founder of The Mediterranean Separation Science Foundation Training and Research Center, founder and Chief Operating Officer of Chromaleont S.r.l., a Society committed to the development of analytical instrumentation and software, as well as the training of PhD students and post-doctoral fellows from around the world.

His research is mainly focused on multidimensional instrumentation and dedicated software, LC, GC, SFC and hybrid combinations, mainly hyphenated to state-of-the-art MS. Advanced technologies are applied for the analytical characterization of complex and unknown samples, as well as for assessment of the impact of bioactive molecules on the biochemistry in living organisms. He has co-authored more than 400 scientific papers (h-index of 53 in Scopus database), and has shared research projects and collaborations with universities and corporations all around the world.

In recognition of the global visibility and innovation of his research, he has received several awards: HTC Award, COLACRO Medal, Silver Jubilee Medal, Liberti Medal, TASIAs, IFEAT Medal, GC×GC Lifetime Achievement, Golay Award, Robert Kellner Lecture Award, Dutton Award. The Analytical Scientist Journal has named Luigi Mondello to the “Power List” of the top 100 most influential people in the analytical sciences worldwide, and to the “Magnificent Tens” in the field of Separation Science.

Luigi Mondello is editor of Journal of Essential Oil Research, Analytical and Bioanalytical Chemistry, Food Analytical Methods.

He splits his work time between research and seminars/meetings, as witnessed by over 1000 conference presentations, among which are 200 invited talks. He is permanent member of the scientific committee of international symposia (ISCC, ISEO, HTC, SIMCRO, COLACRO) and since 2012 he is Chairman of the International Symposium on Capillary Chromatography (ISCC), featuring advances from leading academia and industrial experts in the field of miniaturized separation techniques, comprehensive chromatography and mass spectrometry, with an attendance of >700 participants from 43 countries. Next Symposium (44th ISCC) will be held in 2020 in Riva del Garda, Italy, jointly with the 17th GC×GC Symposium.
Philip Marriott is Professor in the School of Chemistry, Faculty of Science at Monash University. His previous academic appointments were at the National University of Singapore, Department of Chemistry, and RMIT University. This was preceded by a postdoctoral position at the University of Bristol and a PhD in Chemistry (LaTrobe Univ., Melbourne). His main research activities are in Analytical Chemistry, with primary research interests in GC and MS, specifically in very high resolution comprehensive 2D GC (GCxGC) and multidimensional GC, with MS and other detectors. Research includes fundamental method development and a broad applications base – petrochemicals, essential oils, natural products, pollutants and pesticides, fatty acids, and chiral analysis. Professor Marriott is recipient of an ARC Discovery Outstanding Researcher Award. He has recently been awarded a Special Visiting Researcher on a Brazil CNPq grant, administered by the Federal University of Rio de Janeiro, in conjunction with the Agricultural Institute EMBRAPA on a project on coffee and natural oils.

Prior to this, he was awarded a World Class University (WCU) Distinguished Professorship, under the Korean Research Foundation, with Chung-Ang University, Seoul, and Australian Academy of Science professorial visits in collaboration with the Chinese Academy of Science to China, and to Portugal. He has published 368 journal papers, and 27 book chapters.

His Monash [profile](#) has a more complete research profile, activities, and publications, and a link to his Research Group webpage where a "GCxGC and MDGC tutorial" can be found.

**PL04 Comprehensive Two-Dimensional Gas Chromatography – Expectations beyond Design?**

*Marriott P*

Australian Centre for Research on Separation Science, School of Chemistry, Monash University, Clayton 3800, Victoria, Australia
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* Denote Flash Oral communication
Plenary lectures
From amino acid analysis in 1969 to characterization of protein biopharmaceuticals in 2019

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In 1969 I finished my Master studies at the Ghent University, Belgium with a Master thesis entitled “Developing gas chromatographic methods for the analysis of amino acids and sugars”. The final goal of the work was the analysis of amino acids and mono- and disaccharides in beer, our national drink. This was nearly ten years later than the invention of the amino acid analyzer by Moore-Stein-Spackman \(^1\) and its commercialization by Beckman. This was still far from routine analysis as we know now, reason to evaluate at that time GC instead of ion exchange chromatography (IEX).

Over the years, amino acids and sugars were key words in my research and in the development of analytical methods for their elucidation and quantification in different matrices. In the last decade my interest moved to life sciences, needless to say why, and especially to biopharmaceuticals and biosimilars. Key molecules are again amino acids and sugars but now in the form of large identities namely proteins and glycans. Developing analytical methods for detailed characterization of these molecules is extremely challenging as molecular weights can differ with a factor of 1000 as illustrated in **Figure 1** for the amino acid phenylalanine (left) and the biopharmaceutical herceptin for HER2 positive breast cancers (right).

![Figure 1: Phenylalanine (left) versus herceptin (right)](image)

Protein biopharmaceuticals have emerged as important therapeutics for the treatment of various diseases including cancer, cardiovascular diseases, diabetes, infection, inflammatory and autoimmune disorders. Protein biopharmaceuticals have substantially reshaped the pharmaceutical market and today over 350 products have been approved for human use in the United States and the European Union. In 2017, these biopharmaceutical products accounted for a total sales value of $ 188 billion which represents around a quarter of the total pharmaceutical market.

Together with a huge therapeutic potential, these molecules come with an enormous structural complexity highly demanding towards analytics. Opposed to small-molecule drugs, biopharmaceuticals are large and heterogeneous (due to the biosynthetic process and subsequent manufacturing and storage). Despite the fact that only a single molecule is cloned, hundreds of possible variants differing in post-translational modifications (N-glycosylation, asparagine deamidation, aspartate isomerization, methionine oxidation, etc.), amino acid sequence, higher order structures, etc. may coexist, all contributing to the safety and efficacy of the product. Complexity further increases when antibody-drug conjugates are considered since the heterogeneity associated with the mAb is superimposed with the variability associated with the drug conjugation.

An emerging tool to tackle this complexity is two-dimensional liquid chromatography (2D-LC). The current lecture will highlight the power of 2D-LC in hyphenation to high resolution mass spectrometry (MS) for the detailed characterization of protein biopharmaceuticals. Platforms based on both heart-cutting LC-LC and comprehensive LC×LC will be presented and their performances highlighted.
PL2 Extraction of chemical information from untargeted chemical profiling (GC-MS) data

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Modern GC-MS systems combined with efficient sampling techniques produce chromatograms with a large number of peaks of which many are not well-resolved. Well-designed experiments and screening investigations include many samples and replicates. The result is unavoidably a heavy workload on the investigator to process such data and extract the chemical information. Many approaches have been used from simple analysis of total ion chromatograms over single-ion techniques to different kinds of deconvolution techniques, but they all have significant drawbacks: most are very time-consuming, results can be user-dependent to different degrees, and for almost all techniques, chromatograms are treated independently of each other. Furthermore, many approaches can only handle moderately overlapping peaks and also often experience problems with low signal-to-noise peaks. Furthermore, non-detects remain an issue as well.

Here a completely different approach using the so-called PARAFAC2 tensor modelling (PARAllel FACtor analysis 2) is demonstrated. Until now, PARAFAC2 modelling has only been available for mathematical users and has required extensive coding for efficient use. An integrated approach called PARAFAC2 based Deconvolution and Identification System (PARADISe) has, however, become available. The solution is user-friendly, time-saving, and produces reliable results that are less user-dependent. It is freely available from University of Copenhagen.

PARADISe benefits from the ability of PARAFAC2 to resolve co-eluted chromatographic peaks for all investigated chromatograms simultaneously as well as automatically handling baseline. It overcomes the limitation of PARAFAC2 which only works on time intervals, and it can perform all the necessary steps from visualization of data to generation of a final table of identified compounds for an entire set of chromatograms.

Examples will be shown demonstrating how the PARADISe approach allows a very efficient extraction of chemical information from complex data (Figure 1). It is concluded that treatment of large datasets with PARADISe results in extraction of more information, the extracted information is more reliable, and the time-consumption when treating datasets with numerous complex samples/chromatograms is dramatically reduced.

Figure 1: Example of a part of a set of elution profiles (left) and how that data is resolved into baseline and co-eluting peaks.

References:
"Smart" Gradients for Enhancing Peak Capacity in Comprehensive Two-dimensional Liquid Chromatography under Reversed-phase Conditions: Application to Polyphenols in Food and Natural Real-world Samples

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Polyphenols in food and natural products do comprise a complex group of molecules associated to a plethora of pharmacological effects on human health. Besides, they do have important functions e.g. protection against UV radiation, inhibition of pathogen development, as well as industrial applications, e.g. natural colorants and preservatives. Several studies have focused on the chemistry and bioactivity of these molecules in various food and natural products, thus promoting an ever increasing interest in the discovery/identification of "target" novel compounds. As a consequence, powerful and sensitive analytical methods are deemed as mandatory for their determination.

In the last three decades comprehensive two-dimensional liquid chromatography (LC×LC) has emerged as a valuable analytical tool for the analysis of complex samples. Such a technique implies the coupling of at least two stationary phases remarkably increasing the overall separation power compared to the one-dimensional liquid chromatography counterpart.

In this context, our research group have successfully developed different LC×LC methodologies with complementary column sets (e.g. NP×RP, RP×RP and HILIC×RP) including dedicated software for data processing, which were successfully applied for the characterization of bioactive molecules in food and natural products.

In this contribution selected LC×LC applications for determination of the polyphenolic content in complex samples will be presented and discussed with particular emphasis on the use of "smart" gradients in RP×RP separations.

Acknowledgements: The authors are thankful to Shimadzu and Merck Life Science Corporations for the continuous support. The researches were performed within the framework of the Research Project PRIN 2015: Securing and ensuring sustainable use of agriculture waste, co- and by-products: an integrated analytical approach combining mass spectrometry with health effect-based biosensing, supported by the Italian Ministry of University and Scientific Research, no. 2015FFY97L.
PL4 Comprehensive Two-Dimensional Gas Chromatography – Expectations beyond Design?

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When a new technique is proposed, and initially demonstrated, it is useful either at that time, or in retrospect, to speculate the (future) value of the technique, whether it has delivered on promised expectations, or to ask if analysts had expectations that exceeded the design principles of the technique. In fact, we can interpret most research based on this question!

In respect of comprehensive two-dimensional gas chromatography (GC×GC), we might ask if the original proposers, Liu & Phillips1, anticipated the potentialities of the technique. Did they envisage how it could and would be applied to all manner of samples, and what was required of the data processing challenges thrown up by GC×GC? Was the proposed ‘design’ capable of delivering what analysts might want? For analysts, who were initially intrigued and fascinated by GC×GC, did they expect it to do everything they could possibly want in sample analysis, that had either been difficult or nigh on impossible by 1D GC? Were the expectations of analysts unrealistic in demanding GC×GC provide a universal solution to their problems?

Can we look back in the past to understand the future? Was the design and original fundamentals of GC×GC a portent of the power that we now understand? Were any of the expectations of analysts unable to be realised by GC×GC? We introduced cryogenic modulation to GC×GC.2 Our on-line tutorial outlines and updates various novel studies we investigate.3 The general technology of MDGC has been reviewed,4 and systems integrating MDGC and GC×GC approaches described.5 Capabilities for organophosphate environmental pollutants in difficult matrices is a logical application,6 and novel GC processes and information can be revealed by GC×GC.7 I will try to give a progressive evaluation of the status of GC×GC, and at the same time discuss the nexus between design and expectations. But for me – and for many others – who continue to be mesmerised by seeing what GC×GC can accomplish, maybe the most relevant question is: Why aren’t more GC users flocking to GC×GC?

Acknowledgements: I thank the many research students in my group, and visitors to my group, who have collectively contributed in many ways to our experience in comprehensive two-dimensional GC. They have joined our group with great expectations, have designed numerous application studies and new separations systems, and whose research studies have inspired the above reflections on GC×GC technology.

References: [Calibri 8]
Oral Communications
The characterization of impurities present in pharmaceutical products is a matter of great concern at all stages of the drug development and manufacturing process. Impurity identification and profiling is essential to the assurance of patient safety and drug efficacy. Regulatory authorities have established strict guidelines to limit the presence of impurities according to their toxicological effect.\(^1\)

Impurities are unwanted chemicals that remain with the Active Pharmaceutical Ingredient (API) throughout the process chemistry development and formulation or may result from the chemical degradation of the pharmaceutical product.\(^2\) Traditionally, their control is performed using chromatographic methodologies, such as, high performance liquid chromatography (HPLC) coupled to UV/Vis detectors or gas chromatography (GC) coupled with FID detectors. Despite the general use of these techniques, the data provided has limited information to completely understand the impurity profile obtained during the development process.

The use mass spectrometry (MS) in combination with liquid chromatography has proven to be a key technique for impurity identification and structural elucidation.\(^3\) Mass spectrometry allows higher specificity and sensitivity when compared with other techniques. Additionally, advances in MS instruments, such as, user friendly single quadrupole detectors that have the capacity to provide real-time process information, deeply improved the way process chemistry development is performed. Furthermore, the introduction of state-of-the-art high-resolution MS instruments in R&D laboratories, such as, renewed Time-of-flight mass spectrometers (TOFMS), allowed the application of high-resolution accurate mass measurements that amplified the capacity to obtain comprehensive process understanding. The aim of this talk is to demonstrate the effective use of this technique for impurity profiling and process understanding at Hovione. The characterization of the impurities present in 4-fluorophenylacetic acid (4-FPAA), commonly used in several chemical processes worldwide, was achieved using a LC UV/Q-TOF HRMS system. With a data dependent acquisition strategy that allows hypothesis-driven analysis of selected ions of interest, all visible impurities were identified.

In this work, the rapid identification and structural elucidation of the main impurities present in 4-FPAA allowed a clear understanding of the possible reaction pathways when this material is used.

References:
In recent years, the use of GC-APCI in combination with HRMS has been dramatically increased for the analysis of non-polar compounds in both food and environmental samples, mainly. The "soft" ionization produced by the APCI source enhances the formation of the molecular ion and/or protonated molecule in comparison to the traditional "hard" EI ionization technique where ionization typically produces highly fragmented spectra and very low intensity of the molecular ion for many compounds. Conversely, APCI produces a very intense molecular ion, which can be selected as an excellent precursor ion for MS/MS analysis, increasing the sensitivity and selectivity of the method and reducing interferences drastically.

A high sensitivity method based on GC-APCI-QTOF has been developed for the analysis of PCBs and PCDDs (Dioxins) in fish tissue. GC-APCI-QTOF is a good and cost effective option to meet the requirements demanded by EU and US-EPA regulations for Dioxins and PCBs like-dioxins in all food/feed/environmental samples.

Very good results regarding sensitivity, selectivity and precision has been obtained addressing these requirements with confidence.
Effect of gamma radiation on bioactive compounds of olive wastes

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The olive pomace is an environmentally detrimental residue from olive oil industry. This residue contains large amounts of bioactive compounds, such as hydroxytyrosol and tyrosol, secoiridoid derivatives, phenolic acids and flavonoids that might be used by the food industry as preservatives. The aim of this work was to study the gamma radiation potential to improve the extractability of the bioactive compounds present in olive wastes. Gamma radiation is an eco-friendly technology that can be used to enhance the benificial properties of different agro-industrial products. Olive pomace samples (crude olive pomace - COP - and extracted olive pomace - EOP) were collected from UCASUL - União de Cooperativas Agrícolas do Sul, located in Alentejo region, in Portugal. The irradiation experiments were carried out at room temperature in a Co-60 semi-industrial facility (absorbed doses: 5-22 kGy; dose rate: 16 kGy/h). The characterization of the phenolic profile in the extracts of olive pomace and the identification of the radiolytic products were carried out by HPLC-DAD-ESI/MS.

The major phenolic compounds present in olive pomace extracts were hydroxytyrosol, hydroxytyrosol-β-glucoside, tyrosol, syringic acid and luteolin-7-O-rutinoside. Caffeic acid, vanillin, verbascoside and its derivatives and oleuropein aglycons were also found in the extracts although in lower concentrations. The obtained results demonstrated that gamma radiation significantly improved the extraction of phenolic compounds from both olive pomace extracts, obtaining the highest yield at 10 kGy for EOP and at 22 kGy for COP. At these doses, the total concentration of phenolic compounds in the extracts was 159±7 mg/g in the EOP and 161±2 mg/g in the COP ones. Comparing with non-irradiated samples, these values represent an increase in extractable phenolic compounds of 2.5 and 2.4 fold, respectively. Nevertheless, for EOP it was found that an absorbed dose of 5 kGy was capable to increase the phenolic content with no significant difference from the higher applied doses.

These results demonstrated that gamma radiation could be a suitable technology for the valorization of olive oil by-products, contributing to enhance extraction of phenolic compounds. This outcome can help the olive oil industry to adopt clean processes and promote the sustainable development.

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References:
Dietary intake has been reported as the most important route for human exposure to Polycyclic aromatic hydrocarbons (PAHs). Considering the increasing interest of using seaweeds for human consumption it is crucial to assess its safety regarding the presence of these persistent pollutants that have been raising global concern due to their carcinogenic and mutagenic properties. To the best of our knowledge, limited information exists concerning the assessment of PAHs in seaweeds from the Portuguese coast. Moreover, the effect of the seasonal variation on the PAHs composition has never been studied. In this study, a total of 18 PAHs (16 USEPA priority compounds, benzo[j]fluoranthene and dibenzo[a,l]pyrene (Figure 1) were assessed in 18 seaweed samples [brown species *F. spiralis*, n=6; red species *Porphyra* spp., n=6; and green species *Ulva* spp., n=6] harvested in Aguda (Vila Nova de Gaia; GPS coordinates: 41°03’05.4”N 8°39’20.3”W) between April and July 2016 and between October and November 2016. All samples were collected and prepared following the procedures described by Vieira et al. (2018) and analysis was based on microwave-assisted extraction (MAE) and liquid chromatography with photodiode array and fluorescence detection (LC-FLD), according to Ramalhosa et al. (2012) procedures. For comparison purposes, three commercial European dried seaweeds (*F. vesiculosus* from wild origin; *Porphyra* spp. from aquaculture; and *Ulva* spp. from aquaculture) were purchased in local health products stores and analyzed for PAHs contents. The concentrations of ∑PAHs in the seaweed samples harvested on the Portuguese coast ranged between 0.027 and 0.646 μg/kg fresh weigh (fw). The species *F. spiralis* and *Ulva* spp. collected during October and November presented significantly higher (0.328 and 0.167 μg/kg fw, *p*<0.05) contents of ∑PAHs compared to those collected between April and July (0.076 and 0.031 μg/kg fw respectively). The PAHs with 2-3 rings were the predominant compounds (86-92% of ∑PAHs), mostly represented by Ace, Phe, Fln and Pyr. The commercial seaweeds presented ∑PAHs concentrations in the same range as the wild samples. None of the samples contained detectable amounts of benzo[a]pyrene, the marker used for evaluating the occurrence and carcinogenic effects of PAHs in food. The potential health risks due to regular consumption of 20 g fw of seaweed will be assessed by the determination of total toxic benzo(a)pyrene equivalent and through non-carcinogenic and carcinogenic risks (USEPA, 2017).
How far can you get in the analysis of complex mixtures through 2D-LC?

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Bidimensional liquid chromatography, as a natural extension of traditional liquid chromatography, truly expands the chromatographic choices to the next level. The possibility of using commercially available 2D-LC solutions gives also a level of robustness and reproducibility that has a great deal of influence in the popularization of the technique.

2D-LC applications nowadays are so spread that it is routinely used in industries such as Pharma/Biopharma, Food industry or Petrol analysis just to name a few. During this presentation we are going to see how 2D-LC has become a first-choice technique in traditionally complex tasks such as impurity profiling of drug products, food product authentication or fingerprint analysis. We are going to review some real-life examples of these approaches for critical evaluation of the technique a future applications.
An improved method for determination of sotolon in Port wines

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Port wine is a fortified wine produced by a specific winemaking practice in the Douro Demarcated Region (DDR) of Portugal, widely known throughout the world. During the aging process, Port wine undergoes many changes in colour and aroma, with the levels of some compounds decreasing over time while other increase or appear [1]. Some of the compounds formed or accumulated over the aging time can be considered age markers, like sotolon (3-Hydroxy-4,5-dimethyl-2(5H)-furanone) (Figure 1). Sotolon can be related to the descriptors “nutty” or “spicy” and it plays an important role in the “perceived age” of Port wines. The odour threshold value is 19 µg L⁻¹ in Port wines [2]. This research aimed to develop a simple analytical method to assess sotolon by using high-pressure liquid chromatography (HPLC) coupled with UV detection. The availability of a fast, sensitive, and reliable analytical method could allow a better monitoring of the aging process of Port wines.

Figure 1: HPLC-UV chromatogram of sotolon (1) and internal standard (2).

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O07 *Cytinus hypocistis* (L.) L. extract as a source of anti-aging cosmeceutical ingredients

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Plant-derived compounds have been extensively used for cosmeceutical applications, especially because humans have once again turned to Nature to mitigate the relative void of combinatorial chemistry, to find new molecules and the toxicological effects associated with the synthetic ones [1]. *Cytinus hypocistis* (L.) L. is a wild edible parasitic plant on various members of the Cistaceae family. Although its biological properties were potentially attributed to its hydrolysable tannins content, to the author’s best knowledge, its chemical composition is largely unknown, and active biomolecules are not yet identified [2]. According to a semi-quantitative study, where 100 extracts obtained from plants collected in India, Africa, and the Mediterranean area, *C. hypocistis* figures on the top 10 group of plants that potently inhibited both elastase and tyrosinase, two main enzymes involved in skin aging [3]. Thus, studying the bioactive properties and chemical composition of *C. hypocistis* plant will give comprehensive clues on its potential cosmeceutical applications.

Plant specimens of *C. hypocistis* were collected in June 2018 in Castro Daire, Portugal. After lyophilisation, the phenolic compounds were analysed in the hydroethanolic extracts of four different parts of *C. hypocistis* (whole plant, nectar chamber of the flower, petals, and stalks) using a HPLC-DAD-ESI/MSn system. The antioxidant activity of the four extracts were evaluated using OxHLIA and TBARS methodology. Anti-tyrosinase enzyme inhibitory assay was performed using L-DOPA as substrate and kojic acid as standard. *C. hypocistis* extracts were also tested for their antibacterial activity based on minimum inhibitory and bactericidal concentrations and the anti-inflammatory activity was evaluated through NO inhibition, in LPS-activated murine macrophage (RAW 264.7).

A total of 17 phenolic compounds were identified, being galloyl-bis-HHDP-glucose, digalloyl-bis-HHDP-glucopyranose, and pedunculagin the most abundant. UV radiation generates oxidative stress, being mainly responsible for cell membrane oxidation and, although through different mechanisms, OxHLIA and TBARS are equally a consequence of lipid peroxidation. All the tested extracts showed high antioxidant capacity, with the petals exhibiting the most promising results for both OxHLIA (IC<sub>50</sub> = 279 ± 5 ng/mL) and TBARS (IC<sub>50</sub> = 342 ± 2 ng/mL) assays.

Considering the anti-tyrosinase inhibitory assay, the main enzyme involved in skin pigmentation, the stalks presented the lowest IC<sub>50</sub> values, 0.09 ± 0.02 mg/mL. All tested extracts displayed a broad-spectrum microbial inhibition against both Gram-positive and Gram-negative bacteria. Moreover, being chronic inflammation one of the molecular mechanisms behind skin aging, the petals result for NO inhibition (IC<sub>50</sub>: 127 ± 8 µg/mL) is an important evidence on the versatile profile of this plant.

Although for the four studied samples the 17 identified phenolic compounds were the same, its concentration was higher in the petals extract, followed by the stalks, being these two plant parts of *C. hypocistis* unveiling the strongest bioactive potential. These results point a potential correlation between the phenolic profile of *C. hypocistis* and its properties. For its bioactivity validation and mechanism investigation, further studies on fractionation, isolation and characterization of compounds of the extracts of *C. hypocistis* are currently ongoing.

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References:
Combining analytical pyrolysis and chemometrics: A powerful approach to study complex organic matrices

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Assessing the molecular composition of organic matter present in a wide variety of complex matrices has been of great interest, owing to the enormous information that can be obtained, such as: 1) the authenticity and provenance of foodstuff (fraud detection and product safety); 2) the fire impact on the molecular composition of soil organic matter; 3) organic signatures preserved in speleothems from volcanic caves. Therefore, the use of state-of-the-art analytical techniques is of utmost importance for the accurate characterization of organic molecules preserved in complex matrices.

The diagnostic techniques based on pyrolysis-gas chromatography, such as traditional mass spectrometry (Py-GC/MS) and compound-specific isotope analysis (Py-CSIA), are cutting-edge methods used to obtain valuable information on molecular and isotopic (e.g., δ13C, δ15N and δ2H) fingerprinting of solid materials not amenable by conventional GC/MS-isotope ratio mass spectrometry (GC/MS-IRMS) approach. The analysis can be done using small sample amounts with minimum handling and pre-treatment, minimizing the chance of contamination and artefacts.

As the data obtained by analytical pyrolysis techniques can be complex and not always with a straightforward interpretation, the use of chemometrics (multivariate statistical analysis), like principal component analysis (PCA), multidimensional scaling (MDS) or partial least squares (PLS) regression, is required to achieve a correct interpretation of chemical data. In addition, the chromatographic data (relative abundance of specific compounds) can be analysed by graphic-statistic tools, such as the classic van Krevelen diagram. This graphical tool has the advantage of showing the density (chromatographic area) of different regions of the atomic H/C vs. O/C ratios, facilitating the comparison among samples (Figure 1).

In this communication, we will introduce the combination of analytical pyrolysis techniques and chemometrics as a novel approach for the detection of organic compounds in complex matrices. Case studies on speleothems from lava tubes, fire-affected soil organic matter and extra virgin olive oils will be presented as well as the potential application in the field of geomicrobiology, environmental disaster and food safety.

**Figure 1:** 3D van Krevelen diagrams (H/C vs O/C vs Pearson’s R² coefficient) of A) whole, B) burnt, and C) unburnt soil samples (mixture of burnt and unburnt samples. Convex surface corresponds to positive R² values, whereas, concave surface corresponds to negative R² values.

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**References:**

Integration of data from GC-MS and UPLC-QTOF-MS to better understand wine ageing: a new graphical interface

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Given the global trends in the food and flavor industry, i.e. naturality, organic food, authenticity, transparency as well as ‘clean label’, it has become essential to revisit the way flavor is imparted in foods and beverages, as well as the way it is measured with fast reproducible methods, close to the overall consumer experience. In the last decade, there has been an increase in analytical methods allowing the acquisition of volatile and non volatile fractions of different matrices like GC and LC-MS. The application purpose of this technique includes a variety of projects from origin and history assessment of food and raw products, to food quality monitoring, to flavor release and/or generation profiles as function of product composition, to sensory related studies. However, the limitation of these high-throughput techniques remains with the considerable amount of data generated in a single analysis, in a complex data structure, and the required prior number of steps to a robust analysis of the acquired information. Hence, the need to develop alternative data treatment concepts, capable to extract relevant information, supporting the usage of fast real time measurements, close to the overall consumer experience. The present talk will elaborate on a few of the these current issues and on how the food industry is transforming challenges into opportunities, with the development of a pipeline, consisting on: data importation, peak apex’s extraction to reduce the number of variables and integrating several statistic tools for data comparison and visualization. In particular unravelling the chemical changes, occurring during ageing, that are responsible for the wine flavor, constitutes a critical task when one attempts to address issues related to authenticity and sensory quality. In order to have a holistic view of the chemical system a pipeline was developed based on UPLC-MS-QTOF and GC-MS data acquisition followed by data fusion. The process is hyphenated with an in-house peak picking interface, coupled with multi- and univariate statistics to get the most relevant compounds related in this case with Ports stored from 1 to 150 years old.

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References:
Small molecule profiling and qualitative multiresidue screening analyses are a challenging task to undertake, considering the number of compounds and the dynamic range over which a large number of compounds may need to be detected. The ever increasing requirement to increase sample analysis throughput has resulted in the evolution of multi-method/generic chromatographic strategies alongside generic sample extraction.

Full scan TOF MS and mass accuracy offers high specificity with theoretically no limitation in the number of compounds detected, but it is still a challenge to rapidly and efficiently identify targeted compounds present in a sample with a large number of co-extracted matrix components.

Development of screening strategies incorporating a CCS metric has provided an added analysis dimension to help overcome the challenges of sample complexity. The opportunity exists to advance conventional working strategies, generate more information, enhance specificity and screening efficiency, with increased flexibility in the tolerance parameters applied.

Advances and new observations in pesticide, natural products, veterinary drugs and steviol glycosides analysis will be used to illustrate, reduction in false detections, deconvoluted isomeric quantitation, profiling of knowns and known unknowns, CCS finger prints, charged isomers (multi-protomeric charged species) and IMS multivariate analysis.
Analysis of skin volatiles using a membrane-SPME/GC-MS approach to unveil putative biomarkers for neurodegenerative diseases

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Neurodegenerative diseases (NDDs) are a heterogeneous group of disorders characterized by the progressive degeneration of the structure and function of the central or peripheral nervous system. Alzheimer’s and Parkinson’s diseases are the most prevalent NDDs and their incidence is increasing alongside with the average life expectancy. While there aren’t yet strong biomarkers for most NDDs, their diagnosis relies basically on the clinical symptoms, however, neurodegeneration begins long before the patient experiences any symptoms. Consequently, there is an obvious interest in the early diagnosis of NDDs. This would allow the anticipation of the treatment and attenuation of the negative effects of neurodegeneration.

The characterization of the volatile organic metabolites (VOMs) composition of different human biofluids is being explored as a promising and non-invasive tool to unveil new biomarkers for the diagnosis of human diseases and infections. The VOMs profiles can provide important metabolic information, particularly about the metabolic changes caused by different clinical conditions, including NDDs.

In this project we are developing new sampling procedures using polydimethylsiloxane (PDMS) membranes followed by solid phase micro extraction (SPME) and gas chromatography coupled with mass spectrometry (GC-MS) to explore the potential of skin VOMs as putative biomarkers for the non-invasive diagnostic of NDDs. This methodology is highly amenable for studies with patients affected by Alzheimer’s and Parkinson’s diseases and ultimately will constitute a seamless tool to unveil putative volatile biomarkers for these NDDs. Furthermore, such approach has a great potential for the integration in POCT devices for the clinical environment.

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Determination of the phenolic composition of vine-canefour subcritical water extracts and its utilization for production of a topical formulation

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Grapes are one of the major fruit crops produced throughout the world, and for many countries, such as Portugal, these fruits represent a crucial part of the economy. Nevertheless, grape production is not a fully optimized process, and it is very far from being considered an agro-industrial sector based on a circular economy. Despite the richness of grapes wastes in polyphenols, usually they end up discarded; in the case of vine-canefour they are typically incorporated in the soil or incinerated. Depending on the vine varieties, it is estimated that for each hectare of vineyard 1,75 tons of vine-canefour wastes are produced. Considering that polyphenols possess a powerful antioxidant capacity with a wide range of health benefits, vine-canefour could be used to obtain bioactive extracts which could be further applied in the nutraceutical, cosmetic, pharmacological, enological, and food additive industries.

The present work aims to characterize the phenolic composition of Portuguese vine-canefour extracts obtained by subcritical water extraction (SWE). For that, two grape varieties, namely Touriga Nacional (TN) and Tinta Roriz (TR) from Dão region, were used and the influence of temperature on the extraction yield was evaluated. The extracts phenolic content and antioxidant activity were evaluated through spectrophotometric and chromatographic techniques.

The increase of extraction temperature from 125 to 250 °C resulted in vine-canefour extracts with highest phenolic content and antioxidant activity. Concerning the differences in the vine-canefour varieties, TR presented the highest amount of total phenolic (181 ± 12 mg GAE/g dry extract) and total flavonoids compounds (51 ± 6 mg EE/g dry extract), as well as the highest antioxidant activity. The capacity of vine-canefour extracts to capture reactive oxygen species superoxide (O₂⁻) was also studied and a IC₅₀ value of 83.67 ± 5.84 µg/mL was obtained. Furthermore, no adverse effects were observed in the HFF-1 (fibroblasts) dermal cell line viability after exposure to extract concentrations below 100 µg/mL. The HPLC analysis enabled the identification of phenolic compounds belonging to different families, with gallic acid, and the flavonoids catechin and quercetin being the major contributors to the demonstrated antioxidant properties of the produced vine-canefour extracts. Finally, the most promising vine-canefour extract, namely TR at 250 °C, was selected and incorporated into a topical formulation. The stability evaluation of the topical formulation over 30 days suggested that it should be kept at room temperature.

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References:
O13 HPLC and UHPLC Selectivity – Finding a Selectivity Starting Point

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A systematic approach to determining a rational reversed-phase selectivity starting point based on the physicochemical properties of analyte(s) of interest and system constraints. The approach leverages HPLC/UHPLC column particle morphology and the related impact of efficiency (N) to improve chromatographic parameters.

Key Objectives
- Impact of efficiency (N) on the relative chromatographic resolution, sensitivity, and productivity and understand the impact of particle morphology
- Overview of HPLC/UHPLC selectivity and how to leverage the most appropriate stationary phase for the application
- Method development tips with stationary phase categories and how to employ multi-modal selectivities to recognize immediate method gains

Speaker: MSc Felipe Silva, Technical Consultant, Phenomenex
O14 Separation of Nadolol Racemates by High pH Reversed-Phase Fixed-Bed and Simulated Moving Bed Chromatography

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Nadolol is a pharmaceutical chiral drug worldwide prescribed to the relief of some diseases mainly related with the cardiovascular system. Although some studies refer that the therapeutic effect of this drug is related with only one enantiomer, nadolol is still being marketed as a mixture of four stereoisomers, in a form of a racemic mixture of two racemates. The separation of all the four stereoisomers, despite being a very challenging task, will be very helpful to provide the pharmaceutic industry of any amounts of pure compounds to perform individual pharmaceutic and pharmacologic studies.

Recently, our research group reported the pseudo-binary separation of RSR-nadolol stereoisomer by simulated moving bed (SMB) technology using both coated Chiralpak AD and immobilized Chiralpak IA chiral stationary phases, with an eluent normal-phase mode. In this work, we present an alternative strategy, implementing a first achiral separation step, by using C18 columns to perform the separation of the two nadolol racemates under reversed-phase mode. This introduces much more deep and new challenges involving selection of the packing to be used, optimization of the solvent composition, and the strategy design for defining the different separation steps and its sequences. Different separation strategies can be designed and optimized, enlarging the packing materials possibilities, from fully chiral (Chiralpak) to achiral (C18) – chiral (Chiralpak) separation combinations and, so, the use of both normal and reversed-phase chromatography. For each step, the optimization of the solvent composition will be carried out, using pure alcohol, alcohol-hydrocarbon and alcohol-water mixtures, all with a basic modifier, considering the strong basic nature of the nadolol stereoisomers. The separation technology to be used will also be tested, including fixed-bed and SMB liquid chromatography. The different alternatives will be evaluated in terms of the real capacity to achieve complete separation of all the four nadolol stereoisomers and in terms of system productivity and solvent consumption.

Considering the previous tasks, both modelling-simulation and experimental tools will be fully used, namely in what concerns the knowledge of the equilibrium adsorption isotherms, kinetic data (axial dispersion and resistance to mass transfer), and the prediction of preparative fixed-bed and SMB performances. This chemical engineering approach will allow the deep knowledge of all the separation processes and its optimization at preparative scale. Extensive experimental and simulation results will be presented, including solvent screening, measurement of equilibrium adsorption isotherms, breakthrough measurements, preparative HPLC (Azura pilot unit) and SMB (FlexSMB-LSRE unit) experimental separations of nadolol racemates using C18 columns. At the end is expected the clear definition of the best separation strategy for the complete separation of nadolol stereoisomers and the experimental availability of all the four pure stereoisomers [1-4].

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References:
Emerging pollutants are potentially toxic substances that although found in very small concentrations can produce hazard effects to the environment. Due to their very small concentrations they are not yet included in the water quality monitoring programs neither in national or international environmental control regulations. Pharmaceutical and Personal Care Products (PPCPs) represent an important group of emerging pollutants owing to increased worldwide consumption and to their inherent capacity to induce physiological harmful effects in very low doses, which raises several concerns related with the potential adverse effects on humans, animals and environmental systems.¹

In this work, it will be presented the development and validation of a complete experimental methodology proposed for the monitoring of pharmaceutical drugs. The method is based on solid phase extraction (SPE) followed by analysis with high performance liquid chromatography with a diode array detector (HPLC-DAD)². Experimental results obtained with two different columns will be presented. An analytical Nucleosil 100-5 C18 column, 150 mm x 4.6 mm, obtained from Macherey-Nagel for compounds with lower pKa values and a SiliaChrom XT C18 column, 4.6 mm x 250 mm, obtained from SiliCycle for compounds with higher pKa values. The method is validated by the analysis of real aqueous matrices samples obtained from different water media sources, such as, swimming pools, rivers and wastewater treatment plants. To extend the scope of the analytical method and thus obtain a broader study, several drugs were selected, belonging to five different pharmacological classes: non-steroidal anti-inflammatory (ibuprofen, acetylsalicylic acid, ketoprofen, naproxen and diclofenac), analgesic (paracetamol), antibiotic (sulfamethoxazole), an anticonvulsant (carbamazepine) and a central nervous system stimulator (caffeine). These compounds were selected due to their high level of use and medical prescription and, consequently, leading to a high probability of environmental contamination. Figure 1 shows the overlay chromatograms of individual drugs standards with Nucleosil 100-5 C18 column and a concentration of 100 ppm in the optimum wavelength. Figure 2 represents the chromatogram of a mixture of four selected drugs standards (sulfamethoxazole, paracetamol, caffeine and carbamazepine) with a SiliaChrom XT C18 column and 100 ppm concentration using the optimum wavelength for each compound.

References:
New coloring strategy for dairy products using anthocyanin extracts from edible flowers

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Among dairy fermented products, yogurt is certainly one of the most popular, being consumed worldwide due to its organoleptic and nutritional properties. Nevertheless, some of the yogurts available in the market are prepared with artificial colorants, and the recent concerns with the safety of these compounds push forward the development and application of natural alternatives. Edible flowers, rich in natural pigments, arise as promising sources for the development of new coloring strategies for food products.

The central aim of the present work focuses on the use of anthocyanin-rich extracts from edible petals of *Rosa damascena* “Alexandria” and *R. gallica* “French” draft in *R. canina*, as an alternative to the E163 (anthocyanin commercial extract) as a new way of coloring dairy products (specifically yogurts). The anthocyanin aqueous extract from rose petals was characterized for its anthocyanin profile by HPLC-DAD-ESI/MS. Furthermore, it was incorporated in natural yogurts and the stability of the developed color was assessed on two distinct periods (preparation day and after 7 days of storage at 4 ºC). Likewise, the nutritional composition (AOAC methods), free sugars (HPLC-Ri) and fatty acids (GC-FID) profiles were also evaluated in the developed yogurts. For better comparison a negative control (yogurt without any coloring agent) and a positive control (yogurt with the commercial colorant - E163) were prepared and analyzed considering the same parameters.

Two anthocyanins were detected in the aqueous extract of rose, but cyanidin-3,5-O-diglucoside was by far the major compound, as it corresponds to 98% (13.19 ± 0.01 µg/g extract) of the identified anthocyanins (the second was cyanidin-3-O-glucoside). In what concerns its incorporation in yogurts, it should be bear in mind that the use of additives on dairy products has to consider the consumers’ acceptability, being therefore highly dependent on its organoleptic and rheological properties. Likewise, it is crucial that new developed products maintain the same nutritional properties as those available in the market. In this study, the yogurts prepared with the anthocyanin extract of rose petals and the negative and positive controls showed similar nutritional composition, free sugars and fatty acids profile throughout the assayed storage period.

In conclusion, rose petals represent a potential coloring alternative (in the yellow-orange range) for dairy products without withdrawing the consumers’ acceptance requisites. With the incorporation of these natural extracts for the development of new food products with high added value, a second objective related with the functionalization of the products might also be achieved, potentiating the health benefits to the consumers.

Acknowledgements:
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References:
O17 Natural colorants in cookies: evaluation of the incorporation effects on the physico-chemical composition

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In order to respond to the industry’s need for additives of natural origin, the exploration of new natural sources for different compounds has increased. Compounds with dyeing capacity are of great interest because, throughout the industrial processing of foods, they eventually see their initial color compromised.\textsuperscript{1} Thus, it is necessary to add coloring compounds, preferably of natural origin, to meet the demands of consumers, who are aware of the harmful effects that are attributed to some food additives of artificial origin. The addition of a natural colorant to a particular food has several advantages, besides imparting color, it also enriches the food, due to its intrinsic biological benefits. Betacyanins, for instance, are pigmented compounds that have a powerful pink color, being quite abundant in \textit{Gomphrena globosa} L. flowers, which have strong coloring ability, in addition to their high antioxidant and chemopreventive effects\textsuperscript{2}.

In the present work, betacyanins were obtained from purple-colored flowers of \textit{G. globosa} by ultrasound assisted extraction (UAE) and were further submitted to stabilization processes. The obtained coloring formulations were incorporated in cookies and the influence of this incorporation in the chemical profile regarding fatty acids (GC-FID), sugars (HPLC-RI) and tocopherols (HPLC-fluorescence) of the cookies were evaluated along a shelf-life of 30 days. Furthermore, the cookies were also analyzed for their physical characteristics through the evaluation of the color using a colorimeter (D65 illuminant), analyzing the L\textsuperscript{*}, a\textsuperscript{*} and b\textsuperscript{*} coordinates, where L\textsuperscript{*} represents lightness, a\textsuperscript{*} represents the redness and b\textsuperscript{*} the yellowness. Texture was analyzed with a TA.XT texturometer, evaluating the effects on the hardness, adhesiveness, springness, cohesiveness, chewiness and resilience over the 30 days. Cookies with a commercial colorant and ones with no colorant were used as control samples. As expected, the natural colorants incorporation caused no significant differences among the chemical analyses of the cookies, as the definition of colorants implies that beyond the color imparting no other parameter of the food should be altered. In terms of the cookie profiles, the most abundant individual fatty acid was palmitic acid (C16:0), followed by oleic (C18:1) and linoleic acid (C18:2) contributing to the major prevalence of the saturated fatty acids (SFA), followed by the monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Sucrose was the only detected sugar and corresponded to \(\approx 48.5 \pm 2.0\) g/100 g dw. Regarding the tocopherols, only three of the four isoforms were detected, namely \(\alpha\)-, \(\beta\)-, and \(\delta\)-tocopherol, being \(\alpha\)-tocopherol the most abundant (\(\approx 29.2 \pm 0.8\) mg/100 g dw), followed by \(\delta\)-tocopherol (\(\approx 8.0 \pm 0.8\) mg/100 g dw), and \(\beta\)-tocopherol (\(\approx 2.0 \pm 0.2\) mg/100 g dw). Regarding the physical parameters, among the prepared formulations, the one stabilized by lyophilization presented a loss of the original color. Comparing the developed formulations with the pink commercial colorant, the natural ones showed a better pink color, since this commercial colorant displays a more reddish color, highlight the potential of the developed formulations as real pink colorants. Concerning the texture profile, no significant differences were verified between the samples over the shelf-life of 30 days, although hardness decreased due to retrogradation. The results demonstrated the strong potential of \textit{G. globosa} as a promising source of betacyanins with a stable pink colour with high applicability in the food industry.

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References:
O18 Setting New Benchmarks of Intelligence, Efficiency, and Design in Chromatography

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The Nexera UHPLC series maximises reliability and uptime with fully unattended workflows that span from startup to shutdown. Operators can set the Nexera to start up at a specified time, so that it can complete auto-purge, equilibration, baseline checks and system suitability in advance and be ready for analysis before they arrive at the lab. In addition, FlowPilot ramps up the flow rate gradually, reducing the possibility of damage to columns. The Nexera also has auto-diagnostics and auto-recovery capabilities that allow it to monitor pressure fluctuations to check for anomalies. With remote mobile phase monitoring and integrated consumables management, the system maximises uptime and reliability. Real-time monitoring of mobile phase levels allows lab personnel to efficiently run batches and respond accordingly if there isn’t enough mobile phase before starting a run. In addition, Nexera tracks consumable usage and sends alerts when parts need replacing, allowing users to keep the system running at peak performance. The Nexera UHPLC series allows analysts to confirm parameters and monitor chromatograms in real time directly from a web browser on their smart device. The Nexera UHPLC increases efficiency by automating workflows and maximising throughput analysis. The SIL-40 autosampler can process the entire injection cycle time in as little as seven seconds and continuous analysis can be carried out on up to 44 MTPs (using 3 plate changers). The SIL-40’s plate changers enable non-stop temperature-controlled analysis of thousands of samples. The Nexera automatically blends mobile phases at any set ratio, which speeds up the preparation of buffer solutions and the dilution of solvents. Because it prepares the exact amounts required for analysis, the Nexera reduces waste and labour. The Nexera’s compact design saves bench space and because it uses more than 80% less electricity when on standby, it significantly reduces running costs and supports an environmentally-friendly lab.
High Throughput Bar Adsorptive Microextraction (HT-BAμE): A simple and effective tool for the simultaneous enrichment of ketamine and norketamine from large number of urine matrices

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Modern sample preparation techniques such as sorbent-based passive or static sampling modes have gained more acceptance in almost all scientific areas, including solid-phase microextraction and stir bar sorptive extraction. Additionally, novel static-based microextraction techniques, such as bar adsorptive microextraction (BAμE), have also demonstrated to be a very effective and alternative analytical tool. However, these techniques are not dedicated to routine analysis and have trouble coping with the simultaneous enrichment of large number of samples.

Ketamine is a widely used veterinary medicine. Its medical application in humans is limited to children because in adults it induces severe psychedelic episodes. In recent years, teenagers have abused ketamine as a recreational and "club drug" because of its hallucinogenic and stimulant effects. Ketamine is also misused as a "date-rape" drug (to induce amnesia in unsuspecting victims). For this reason, simple, fast, reliable and cost-effective methods are needed for the determination of these compounds in urine matrices, especially in a large number of samples.

In these work, we present the development, optimization, validation and application of a simple, fast, reliable and cost-effective sample preparation approach using High Throughput Bar Adsorptive Microextraction (HT-BAμE) in combination with gas chromatography–mass spectrometry operating in the selected-ion monitoring acquisition mode, for the simultaneous enrichment of ketamine and its major metabolite (norketamine) from a large number of urine samples.

The target compounds were extracted in a HT-BAμE apparatus, which allows for simultaneous microextraction and subsequent back-extraction of up to 100 samples, resulting in a sample preparation time of only 0.45 min/sample. Under optimized experimental conditions, the developed methodology allowed for linear dynamic ranges between 5.0 and 1000.0 μg L⁻¹ with determination coefficients of 0.997 and 0.999, as well as average recovery yields of 84.9-89.8 % and 96.5-97.8 % for norketamine and ketamine, respectively. The developed methodology was applied for the analysis of 24 samples (in triplicate), where no target compound was detected (≤ LOD).

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References:
Validation of a method to quantify acrylamide in biscuits

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Acrylamide (2-propenamide) is a product of Maillard reaction.1 It’s mainly generated through the reaction of the carbonyl group of reducing sugars and the amine group of asparagine. Acrylamide formation has been the object of many studies since 2002 when it was discovered in large quantities in heat-processed foodstuffs. It’s considered to be potentially carcinogenic and neurotoxic.2 It is present in a variety of widely consumed products, such as fries and potato chips, breakfast cereal, biscuits, bread, coffee, etc.3 Due to its toxicity and wide distribution in the daily diet, there is an interest to study strategies to mitigate the formation of acrylamide in those foods, without negatively affecting the organoleptic characteristics. In 2017 the EU has set maximum levels of acrylamide in a range of food products, including biscuits.4 Therefore the determination of acrylamide nowadays is still important for the food industries. In this work a method was developed and optimized for its application in biscuits and cookies by GC-SIM-MS by combination of various methods previously described in the literature. The aim of this new method was to be simpler without losing precision and accuracy. Therefore the use of an isotopically labeled internal standard, the use of bromination for obtaining 2,3-dibromopropionamide (2,3-DBP) followed by in-situ debromination by co-injection of trimethylamine was used. The method developed presented good precision and accuracy. This method has been applied with success to a range of biscuits and cookies.

Figure 1: Chromatogram of 2-DBP(1) and 13C-2-DBP(2), obtained by GC-MS.

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O21 Very Fast analysis of TCA In cork Disks by HS-SPME GC/MS/MS – A Proof-of concept

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There has been always an interest for faster GC methods. In recent years, this interest has grown due to the fact that the number of samples to be submitted for GC analysis has greatly increased. This reality is often making sample throughput the most important aspect to consider in the routine analytical applications. Portugal is the world leader in the production and supply of cork stoppers. Cork is the material of choice used to produce wine stoppers and they represent around 70% of all cork industrial production. However the wine industry is always looking for products that threat cork’s dominance such as screw tops and plastic stoppers. This happens because cork may occasionally suffer from a problem of contamination with 2,4,6-trichloroanisole (TCA) that transfers to wines a “cork taint” off-flavor. TCA is therefore responsible for significant economic losses in wine and cork industries, since it has a low sensory threshold that varies widely among people. Some individuals can detect it in wine at concentrations of only 1 ng/L (1 ppt). For a cork company considering the routine methods, only few of thousands of cork discs produced per day can be analysed.

The aim of this work was to develop and test as proof-of-concept a new Very Fast GC methodology targeting the reduction of the analysis cycle to less than 2 min that allows the cork disks analysis capacity to be multiplied tenfold with only one equipment, performing the detection and quantification of TCA at a concentration as low as 0.5 ppt. The developed analytical method employs an Agilent GC/MS/MS system coupled with a robotic PAL 3 Dual Head RSI autosampler equipped with two sampling SPME Arrow Tool towers and two Single Magnet Mixers. The chromatographic and headspace extraction parameters such as SPME Arrow fibre coating, extraction temperature, agitation, speed, salting out (Ionic strength), sample volume, matrix effect (aqueous vs hydroalcoholic solutions), extraction time, desorption temperature, oven gradient temperatures, MRM transitions and retention time precision and reproducibility have been evaluated. The Intuvo GC system, leaded to high chromatographic throughput, enabling fast ballistic temperature ramps and coolings, with accurate and reproducible retention times through its direct heating furnace. The robotic system enabled SPME sample preparation cycles to overlap as a result of simultaneous operation of both towers, allowing two consecutive samples to be staggered and processed in parallel, crossing off the dead time between consecutive chromatographic cycles HS-SPME-GC/MS/MS. The resulting total time obtained was 186 sec plus 30 sec cooling time, allowing short analytical cycles of around 98 seconds in average. The obtained data (good linearity of R2 > 0.98 and LOD below 0.5 ppt) supports that the fully automated method is selectiv with high sensitivity compared to conventional detection methods (GC-EAD; GC/(SIM)MS) with an Increased productivity due to a runtime reduction of 10 times.

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O22 Polar Pesticides Anions in water and food using a new and unique Ion Chromatography and Mass Spectrometry High Resolution MSM or MSMS method

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The use of polar pesticides has been widely spread over the last years. The control in food and on all water supply is mandatory in many countries, and of extreme importance for environmental and health purposes.

But the routine analysis of Polar pesticides has been a big challenge for the chemists in the last years. The difficulty of having a normal or reversed phase method that performs well is in the base of the problems. Additionally, 3 or four different methods and columns must be used in order to detect a large list of species and metabolites. (1)

We have developed and tested a new method, that uses a new approach for this analysis: Ion Chromatography, coupled with High Resolution or Tandem Mass spectrometry. Most of the species are negatively charged at high pH. We can use that property in order to make possible a good separation using its capability to be charged. The Ion Chromatography, is better suited for doing this type of job. It improves selectivity and offers a good separation. As separation occurs with an strong basic eluent, it is mandatory to suppress the highly ionic signal whilst neutralizing the eluent. The result shall be a stream of water. This unique feature opens the door for other hyphenated technologies like mass spectrometry. We can use either High Resolution mass spectrometry (featuring the Orbitrap Mass analyzer) or Tandem mass spectrometry (using Triple quads technology) to be sure that the highest sensitivity is achieved. High resolution Mass spectrometry provides another dimension of being unequivocal in its quantitation and identification, representing the best approach for analysis of pesticides and contaminants (2)

The goal of this unique methodology as well, is avoiding any additional sample preparation, or any concentration, for water or food samples. In general, the samples are highly concentrated in several ionic species, and it is quite common to observe some ion suppression in the Electrospray source. Being capable to achieve a good separation strategy is crucial in order to achieve good limits of detection, and minimize the impact of the sample matrix.

We will show some of the chromatograms produced for different samples, with some of the polar pesticides species, on different type of samples. Some of the analytical challenges that we have faced will be shown as well.

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O23 Chemical characterization of *Cistus ladanifer* L. lipophilic fraction: an underexploited raw material of biologically active terpenes

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*Cistus ladanifer* L. (Cistaceae) is a Mediterranean shrub, highly adaptable to arid soils and hot summers; besides it grows abundantly in burnt and uncultivated fields. This aromatic plant is much appreciated in the cosmetic industry, due to the essential oil and labdanum exudate. However, its fast colonization rises severe concerns, mainly related with the large amounts of accumulated biomass and/or by-products and, consequently, the high fire risk. Nevertheless, *C. ladanifer* L. extracts have exhibited antioxidant and antimicrobial activities, as well as modulating effect on *in vitro* rumen biogeneration, which are mainly associated with the phenolic composition. Furthermore, lipophilic compounds identified in *C. ladanifer* L., like lab dane-type diterpenes, also demonstrate interesting biological activity, namely anti-inflammatory and antimicrobial actions. However, a detailed lipophilic chemical characterization of the distinct morphological parts of *C. ladanifer* L. is missing. Hence, this work aims to valorize *C. ladanifer* L. biomass, through the identification and quantification of lipophilic compounds present in flower buds, flowers, seed heads, stems and leaves, by gas chromatography–mass spectrometry (GC–MS) analysis.

Five lipophilic families were found in the studied morphological parts of *C. ladanifer* L., namely aromatic compounds, long chain aliphatic alcohols, fatty acids, terpenes and sterols (Figure 1). Leaves and flower buds presented the highest concentration of detected lipophilic compounds, mainly composed of terpenes. Thus, two sesquiterpenes (e.g. viridiflorol) and six labdane-type diterpenes, like labdanolic acid, were detected. Other valuable lipophilic compounds include three unsaturated fatty acids (9Z,12Z-octadeca-9,12-dienoic acid, 9Z,12Z,15Z-octadeca-9,12,15-trienoic acid and 9E-octadeca-9-enoic acid) and two sterols.

Overall, these promising insights can boost the integrative and sustainable use of *C. ladanifer* L. biomass, towards biomedical, food, nutraceutical and feed applications, along with the current cosmetic use.

**Figure 1:** GC-MS chromatogram of trimethylsilylated lipophilic extract of *C. ladanifer* L. leaf. IS—Internal Standard.

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**References:**

O24 Chemical characterization of new psychoactive substances belonging to the class of synthetic cathinones in seized materials in Portugal

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Over the last decade, a tremendous change in the illicit drug market has become evident with the appearance of a “new generation” of psychoactive substances. Typically known as “legal highs”, the new psychoactive substances (NPS) include a vast number of drugs that are intentionally synthesized for recreational purposes, providing similar effects to the conventional drugs of abuse, but with slightly altered chemical structures, to avoid drug legislation. In Portugal, the phenomenon of NPS appeared in earlier 2007 with the opening of the first sales point in Aveiro. This new reality quickly reached the Autonomous Region of Madeira, where dozens of hospitalizations and even deaths associated with NPS consumption were recorded. Given the seriousness of the situation, the government of Madeira was forced to take legal measures, through the implementation of the Legislative Decree nº. 28/2012M of 25th October, which prohibited the sale and distribution of such substances. In 2013, new legislation was introduced in Portugal (Decree-Law nº. 54/2013 of 17th April), which prohibits the production, export, advertisement, distribution and sale of 159 NPS. Despite the legislative efforts against the NPS problem, new substances continue to emerge on the clandestine drugs market. Currently, synthetic cathinones (SCAT) are one of the NPS groups with the highest number of seizures recorded in our country and given the constantly evolving nature of these substances in the illegal drug market, extra efforts must be made in order to develop analytical strategies for their identification. In this sense, the present study aims the chemical characterization of “legal high” products, suspected to contain SCAT, by attenuated total reflectance Fourier transform infrared spectrometry (ATR-FTIR), gas chromatography coupled to mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR). For this purpose, eleven seized products, “Charlie”, “Kick”, “Bliss”, “Blow”, “Kick”, “Bliss” (two packages), “Bloom” (five packages) and “Flakka”, apparently containing SCAT, were provided by the Laboratory of Scientific Police of the Judiciary Police of Portugal. All samples were primarily analysed by ATR-FTIR, followed by GC-MS operated in electron-impact (EI) ionization mode. All mass spectra were compared with NIST 14 MS library and SWGDRUG MS library version 3.4. The characterization and structural elucidation of SCAT were also realized by one- and two-dimensional NMR (1H NMR, 13C NMR, 19F NMR, COSY and HSQC).

ATR-FTIR spectra of products were consistent with the molecular structure of SCAT intense peak at 1677–1699 cm⁻¹ (stretch C=O), intense peak at 1589–1603 cm⁻¹ (stretch C=C) and bands with relative low intensity at frequencies near to 3200 cm⁻¹ corresponding to an amine). By GC-MS it was possible to identify seven SCAT, namely methylvone, methedrone, 3-ethylcathinone, buphedrone, pentedrone, 3-fluoromethcathinone and α-pyrrolidinohexanophenone (α-PHP). Caffeine, a common active adulterant in illicit drugs, was found in several seized materials, including “Bloom”, “Bliss”, “Blast” and “Kick”, while ethylphenidate, a psychostimulant compound analogue of the prescription drug methylphenidate (Ritalin®), was detected in “Bloom” and “Charlie” products. Isopentadone a by-product of the synthesis of pentedrone was also identified in one seized product (“Kick”). NMR analyses were also of great importance during the identification of SCAT. Methylvone, one of the first ring-substituted SCAT to be reported in the EU, was identified as the main component in “Bliss” products, while α-PHP was the main component detected in “Flakka” product. These results highlight the prevalence of SCAT in seized materials from the Portuguese market. Analytical standards are generally required for confirmation, but when standards are not available, mass spectrometry in combination with spectroscopic techniques are fundamental for the structural characterization of unknown substances.

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References:
Selective pre-enrichment of pesticide residues in olive oil samples:
from MISPE technology into smart MIPs

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Owing to the huge complexity of olive oil samples, the trace analysis of pesticide residues in this food matrix constitute a demanding task. The preliminary step of sample pre-treatment is mandatory encompassing the selective pre-enrichment of these target contaminants from the olive oil matrix. It enables to achieve more clean extracts minimizing the presence of interferents and providing a further accurate trace analysis of these harmful compounds. Molecularly imprinted polymers (MIPs), which action mimics the natural receptors, proves to be promising as selective sorbents for solid phase (SPE) applications. Their molecular recognition abilities bring high levels of selectivity and specificity into SPE approach.

In this communication will be highlighted the use of MIPs-based sorbents in the pre-treatment step of olive oil samples by means of SPE, named as MISPE. The design and preparation of those imprinting systems will be discussed, as well as their integration on the analytical workflow for the trace analysis of pesticide residues in olive oil samples (Figure 1).

Figure 1. Analytical workflow for the trace analysis of pesticide residues in olive oil using MISPE technology in the pre-treatment step.

The development of advanced functional materials “smart”-MIPs, which combines a controllable mode of action by an external stimuli, with the selectivity/specificity imprinted by MIP technology shows a huge potential to be integrated in the sample preparation step. Thus, this communication will be also focused on the usefulness of these new functional materials on pre-concentration of pesticide residues but remaining higher selectivity to target analytes. Improvements on the sorption-based extraction techniques by using these “smart”-MIPs will be discussed and emphasised.

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O26 Application of Tape Adsorptive Microextraction to Determine Benzophenone & Related Compounds in Water Matrices

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The use of personal care products containing ultraviolet (UV) filters, such as, sunscreens, skin lotions, hair sprays, lipsticks, among others, has increase over the years due to the awareness of the risks of sunlight exposure. The most common organic compounds used as UV-filters are benzophenone and its derivatives. Due to intensive usage, these compounds can easily reach environment and, several toxicity studies have shown that they are hazardous to plants, mutagenic in some bacteria cell systems and are linked to cancer and endocrine disruption in humans.1

In last decade, bar adsorptive microextraction (BAμE) was introduced as a novel sample enrichment approach and has already proved to be remarkable alternative for trace analysis of medium-polar to polar compounds in aqueous media, being already successfully applied for trace analysis of several classes of emerging or priority compounds with high effectiveness. BAμE that operates under the floating sampling technology present several advantages such as the possibility to choose the most suitable sorbent phase (e.g. activated carbons, polymers, etc.) for each particular type of application.2,3 In this contribution, a novel sorption-based microextraction technique, tape adsorptive microextraction (TAμE, Figure 1) followed by microliquid desorption combined with high performance liquid chromatography-diode array detection (TAμE-μLD/HPLC-DAD), is proposed to monitor benzophenone and related compounds in aqueous media. By using this new approach, some improvements were introduced, including the downsizing of the analytical device, as well as the reduction of the solvent volume of the back-extraction stage. These new advances allow the elimination of the solvent switch step, making possible the back-extraction in one single step and turning the manipulation much simpler. Assays performed on 25 mL of ultrapure water samples spiked at the 8.0 μg L−1 level yielded average recoveries ranging from 80.9 to 102.54 % and demonstrated suitable detection limits (0.1-0.3 μg L−1) for the compounds studied using optimized experimental conditions.

Figure 1: Tape adsorptive microextraction (TAμE).

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Deep eutectic solvents (DES) are composed by two components, one capable to be a hydrogen bound acceptor (HBA) and one hydrogen bound donor (HBD), some advantages of DES are ease preparation, low cost components, low toxicity, biodegradability, non-flammability and modeling of physical properties by changing HBA and HBD components and molar ratios. One challenge towards DES is the production of hydrophobic solvents for extraction of non-polar compounds, since several components applied as HBA and HBD are hydrophilic and the interaction between them are destroyed by water. Polycyclic aromatic hydrocarbons (PAHs) are group of harmful organic compounds containing only carbons and hydrogens that are composed of multiple aromatic rings which make them nonpolar molecules. Many of these PAHs exhibit mutagenic and carcinogenic activity. An important source of PAHs is food especially those submitted to smoking and heating treatments such as tea, coffee, oils and chocolate. The aim of this work was to compare the extraction efficiency of different DES in determination of PAHs in black tea and toasted yerba mate based soft drinks. Different DES based on fatty acids [hexanoic acid (C6), heptanoic acid (C7), nonanoic acid (C9), decanoic acid (C10), dodecanoic acid (C12)], thymol (Th) and camphor (CAM) were tested as extractive solvent in dispersive liquid-liquid microextraction (DLLME) for PAHs extraction: C6-C7 (1:1, molar ratio), C9-C10-C12 (3:1:1), Th-CAM (1:1), Th-CAM-C9 (1:1:1), CAM-C6 (1:1), CAM-C7 (1:1), CAM-C8 (1:1), CAM-C9 (1:1) and CAM-C6-C7 (1:1:1). The analysis of the compounds was achieved by gas chromatography coupled with a triple quadrupole mass spectrometer (Agilent Technologies Inc., Palo Alto, CA, USA). The criteria used for DES selection were height, width and shape of the peaks and number of extracted PAHs.

Fatty acid based DES (C6-C7 and C9-C10-C12) extracted lower amount of compounds (10 out of 18 selected PAHs) and showed several peaks with tailing, fronting and less resulted separation when they were compared to the other DES. Th-CAM was the solvent with lower intensity for all peaks however when C9 was added, creating a ternary solvent (Th-CAM-C9), peaks intensities were considerably bigger, however only 12 compounds were extracted. Camphor-fatty acid based DES (CAM-C7 and CAM-C6) were the solvents that extracted higher number of PAHs (15) and showed peaks with higher intensity, nevertheless, CAM-C7 revealed peaks with fronting and tailing when compared with CAM-C6 DES. Therefore, CAM-C6 (Figure 1) were the best tested DES in this study and it was elected for development and validation of a DES-DLLME-GC-MS/MS method for determination of 15 PAHs in black tea and toasted yerba mate based soft drinks.

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References
O28 An Innovative and Robust Triple-Quadrupole Tandem Mass Spectrometer Aid to Meet Standards and Regulatory

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Triple quadrupole (TQ) mass spectrometry (MS) is widely for targeted quantitation. In particular, the capability of TQ-MS systems to perform multiple reaction monitoring (MRM) data acquisition has fostered their application in quantitative confirmatory methods. The stringency of current regulations in food, environmental and clinical analysis has increased the demand for selective and sensitive methods, which has driven the development of MS systems with increased sensitivity and robustness.

Here we introduce a high sensitivity and robust liquid chromatography (LC) TQ-MS system, the QSight®, that enables high levels of efficiency and productivity to meet both standard and regulatory requirements. In the ion source an efficient desolvation and ion entrainment is reached employing hot-surface induced desolvation (HSIDTM) and a unique laminar Flow ion guide, which minimize ion scattering and maximize sensitivity. This MS system do not employ axial electrical fields allowing minimal tuning and minimal maintenance of the ion path. Moreover, a near-simultaneous detection of positive and negative ions without the need for high-voltage switching is obtained with the use the UniField Detector™.

Here we present advantages and limitations of this innovative LC-MS/MS system. To assess robustness, selectivity and sensitivity of QSight LC-MS system we evaluated and show analyses of a number compound classes. QSight® LC-MS/MS system can be used where low sensitivity limits and very wide analytical scope are necessary. (Figure 1).

Figure 1: Long term stability data for injections of milk spiked sulfamethazine.

Acknowledgements: Feng Quin, jingcun wu, Jamie Foss, Avinash Dalmia, Miguel portela

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References: ]
A sample preparation method, QuEChERS extraction combined with a magnetic micro dispersive solid-phase extraction (MµdSPE) (Fig 1), was optimized and evaluated for the trace analysis of 9 brominated flame retardants in red fruit samples (strawberries, blueberries, and raspberries) using gas chromatography-mass spectrometry [1]. Magnetic nanomaterials were used as sorbents providing an extraction of the target compounds. The good performance of the new methodology was demonstrated by the cleaner chromatograms with peaks very well defined. Linearity was established for all the analytes (from 10-200 µg kg\(^{-1}\)). Seven concentration levels were analyzed with three measurements at each concentration. Linear responses (\(R^2 > 0.99\)) were obtained, recoveries of all target analytes were within the range of 65-141%, relative standard deviations were <20% at all three spiking levels, while intraday and interday precisions were below 20%. This study demonstrated that the new sample preparation with magnetic nanoparticles could potentially be expanded to extract and pre-concentrate the BFRs in different red fruit samples [2]. The method has been successfully applied to study BFRs in 12 samples from conventional and organic farming.

**Figure 1**: Sample preparation scheme

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**References**:

O30 AQbD and High-throughput analytical toolkit combination to support RP-LC Method Development for Itraconazole Quantification

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Reversed Phase Liquid Chromatography (RP-LC) is a key analytical technique in pharmaceutical industry to support both drug discovery and drug development. Frequently, RP-LC method development is a complex process and not automated, leading to a significant time consuming procedure and quite often less robust methods.¹ In order to increase the efficiency of the chromatographic method development tasks, the combination of a systematic and scientific based approach, based on Analytical Quality by Design (AQbD) principles, with the right instrumentation, automation and software enables a fast, modernize and efficient method development process.

The main goal of this work was to develop a fit-to-purpose method to determine the antifungal Itraconazole (ITRA) and its degradation impurities in a single run, using AQbD combined with a high-throughput toolkit, based on automated instrumentation, software and in silico tools, to systematically screen the most critical chromatographic method parameters during its development and optimization.

ITRA is a triazole antifungal agent that has a broad spectrum of activity. This agent is highly efficacious, particularly because hydroxy-itraconazole (its main metabolite), also has considerable antifungal activity. ITRA is available in capsules for oral administration and is used to treat fungal infections not only in the lungs and in the central nervous system, but also in other body parts, such as serious fungal infections of the skin and nails. The ITRA oral solution is used to treat yeast infections of the mouth, throat, and esophagus and also available for intravenous dosage form. This drug is highly lipophilic in nature and practically insoluble in water. It is an extremely weak base (pKa = 3.7) that is ionized only at very low pH.²,³

Prior to any development activities, prior knowledge was gathered. The analyte physicochemical properties prediction that are likely to affect RP-LC method development were assessed using in silico tools. The most relevant parameters are the pKa values, hydrophobicity, electrical charge and sample solubility (Figure 1). This knowledge supported the selection of the optimum pH working range (≥ 6) and the selection of the stationary phase chemistry.

With this information and through a risk assessment exercise, experimental strategy was defined using DoE based software. The use of a high-throughput toolkit combined with a structured and scientific based approach (AQbD) is key to achieve robust methods and deeply improve method understanding. With this approach, a new method was developed to determine ITRA by RP-LC coupled to an ultraviolet detector.

Currently, forced degradation studies and method optimization are ongoing to assure the method is capable to selectively detect main degradation products of ITRA.

As a conclusion, the use of predictive tools and screening approaches combined with an AQbD approach lead to a fast method development with increased robustness and reliability along the method lifecycle.

![Figure 1: Itraconazole physicochemical properties prediction (a) %microspecies distribution vs pH; (b) Log D vs pH.](image-url)

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References:
O31 Phytochemical composition and *in vitro* antioxidant and antimicrobial properties of *Aloe vera* leaf tissue extracts

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Over the last decades, *Aloe vera* has been subject of several scientific studies that aimed to characterize compositional and biological properties. Despite this, a lack of information on the exact part of the plant analysed or even the species involved is common in many of these works. There are confusing descriptions, mostly about the inner part of the leaf, due to the different terms that have been used interchangeably, such as fillet, pulp, mucilage, gel, and parenchyma, among others. However, these terms do not refer to the same part of the leaf. Therefore, this study was performed to evaluate and compare chemical and bioactive features of *Aloe vera* leaf fillet, mucilage and rind. Freshly cut *Aloe vera* samples of certified organic production were provided by an agricultural company located in Elvas, Portugal. The green rind was separated from the inner fillet and the transparent slippery exudate consisting mainly of gel was collected from the mucilage layer of the outer leaf pulp adjacent to the rind. After lyophilisation, the powdered samples underwent a solid-liquid extraction with an hydroethanolic mixture to obtain the extracts. These were analysed by HPLC-DAD-ESI/MS to characterize their phenolic profile, and *in vitro* screened for antioxidant (by the capacity to inhibit the oxidative haemolysis (OxHLIA), the formation of thiobarbituric acid reactive substances (TBARS), and the β-carotene bleaching) and antimicrobial (against skin-associated pathogenic bacteria and fungi) activities.

Up to seventeen phenolic compounds were identified in the *Aloe vera* leaf extracts and classified into four groups: phenolic acids, flavonoids, chromones, and anthrones. The chromones aloesin and 2′-p-methoxycoumaroylaloresin and the anthrones aloin A and B, 10-hydroxyaloin A and B, and malonyl aloin A and B were detected in the three leaf extracts. The mucilage contained the highest level (131±3 mg/g extract) of phenolic compounds, mostly anthrones (62.1%) and chromones (34.6%), followed by two luteolin glucosides (3.3%). The rind was ranked second, with 105±3 mg/g extract of these secondary metabolites was found in fillet. In addition, the chromosome levels found in the rind did not differ statistically from those of the mucilage. Although the phenolic profiles of fillet and mucilage were similar, a significantly lower concentration (11.2 ± 0.2 mg/g extract) of these secondary metabolites was found in fillet. In addition, this leaf part had an equal ratio of anthrones and chromones. The mucilage and rind extracts revealed interesting antioxidant properties. On the other hand, fillet and rind extracts showed a powerful antifungal activity against *Aspergillus flavus*, *A. niger*, *Penicillium funiculosum*, and *Candida albicans*, higher than that of the positive control ketoconazole. This study showed that the three studied extracts of *Aloe vera* leaf have a different content of phenolic compounds and a high antifungal activity. Since the rind of this plant is often discarded as a biowaste, in future studies we intend to evaluate the potential of green solvents with a different number of hydroxyl groups to extract phenolic compounds from this matrix.

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In this seminar, we review the importance of chromatographic selectivity in RPLC from a theoretical and practical perspective and how this relates to analyte resolution for method development. With an understanding of selectivity, and using a variety of chromatographic data, we discuss phase design principles and how it is possible to introduce functionality to enhance selectivity through mechanisms such as hydrophobicity, π-π, dipole-dipole and shape selectivity interactions. An overview of the method development workflow is discussed. Based upon the key parameters to maximise selectivity, a systematic and optimised method development screening platform is described and an example related substances method development activity is illustrated using the complementary stationary phases with MeOH and MeCN solvents.
O33 LC-MSMS for Human Health Diagnosis: Identification of Stress Biomarkers in Sweat

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The ability to use sweat as a biofluid provides the opportunity for non-invasive sampling for early and continuous diagnostics. To date, the contents of sweat and the mechanisms of biomarkers transport from blood and interstitial fluid are relatively unexplored compared with other diagnostic biofluids. As excessive sweating is a common symptom in patients with infectious disease, the identification of stress sweat biomarkers can lead to rapid detection and monitoring of disease states. The aim of this work is to develop a simple, rapid and versatile method able of identifying Stress Biomarkers in sweat by ultra high performance liquid chromatography (UHPLC) coupled with multiple-reaction monitoring (MRM) tandem mass spectrometry (LC-MSMS).

Analysis of biological samples poses complex challenges in chromatography. The pre-requisites that we must consider are sampling procedure and sample preparation for forthcoming LC-MSMS analysis using electrospray ionization (ESI) source. Considering that some of the major components of sweat samples are protein and salts although the pre-treatment of samples is necessary, this should hinder the loss of biomarkers molecules that are intended to be identified. Also, sampling protocols need to be developed considering the detection technique and possible degradation of the sample. In this context it is necessary to develop a methodology without extensive sample treatment in order to remove molecules that can cause clogging in particle packed columns and ESI signal suppression, which demands protein precipitation and centrifugation of samples and salts removal by filtration prior detection analysis.

This study has been performed for a global identification analysis on pooled sweat samples collected from ten healthy individuals. All twenty seven selected analytes of stress biomarkers were identified using negative and positive ionization modes and multiple reaction monitoring.

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Use of GC×GC-ToFMS to evaluate the impact of plant-based coatings in the preservation of ‘Rocha’ pears during long-term storage

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‘Rocha’ pear (Pyrus communis L. cv. Rocha), is a Denomination of Protected Origin cultivar from west region of Portugal. It is a fruit highly appreciated by the consumers, in both internal and external markets due to its unique sensory properties, namely its distinctive aroma. With an average annual production of 173 000 tons, ‘Rocha’ pear represents the main pear cultivar in Portugal and occupies the fourth position in Europe. Currently, pears from this cultivar are harvested in August and may be stored up to 9 months under controlled atmosphere (CA) conditions (at 0 to 1°C and 0.3% O2). During this long-term cold storage period, pears are susceptible to physiological disorders that are highly dependent on pre- and postharvest factors. One of such disorders, the so-called “superficial scald”, is characterized by the appearance of brown or black patches on the fruit’s surface that greatly depreciates its appearance, taste, texture and flavour, and inevitably their economic value. The superficial scald has been associated with α-farnesene oxidation into conjugated trienes (CTs), although a full understanding of its mechanism remains unclear. CTs disrupt cell membranes, leading to polyphenoloxidase-mediated browning of the fruit peel and subsequent necrosis of the hypodermal cell layers. Some strategies to prevent or minimize superficial scald have been developed, however they still show limited success, which open opportunities for the development of sustainable and more efficient methodologies to prevent superficial scald and preserve the peculiar characteristics of ‘Rocha’ pears. Thus, the objective of this study is to evaluate the impact of plant-based coatings in the preservation of ‘Rocha’ pears during long-term storage. To fulfil these objectives, a pilot scale assay was performed across a 4-month storage period in both modified (2°C; 0.3% O2) and normal atmospheric conditions (2°C). For each storage condition, 3 coatings were tested: pectin, and pectin combined with two different plant extracts. The volatile compounds released from uncoated pears, used as control, and coated pears were followed by headspace solid-phase microextraction (HS-SPME) combined with comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-ToFMS). This methodology allowed the detection of hundreds of instrumental features, among which a set of 64 compounds potentially related with oxidative processes and peculiar aroma of ‘Rocha’ pears were selected. Brix and CTs were also quantified along the time of storage. Hierarchical clustering analysis and heatmaps were performed combining all the domains of information (volatile components, Brix and CTs) for the conditions under study (uncoated pears and three types of coated pears, storage under two conditions) across a 4-month storage period. Coating with pectin and plant extracts seems to delay ripening and oxidation processes, contributing to the preservation of pears longer compared to control conditions. These effects are more evident if combined with storage under modified conditions (2°C; 0.3% O2).

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O35 Influence of the growth cycle on the chemical composition and biological properties of
*Cynara cardunculus* L. var. *altilis* blades and petioles

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*Cynara cardunculus* L. (cardoon) is a typical Mediterranean species comprising the ancestor wild cardoon (var. *sylvestris*) and the cultivated leafy cardoon (var. *altilis*) and globe artichoke (var. *scolymus*). It has a worldwide distribution as a result of the high adaptability to climate change, such as resistance to temperature extremities, water stress, and soils with variable pH.1,2 It is also considered a multipurpose crop due to its nutritional, pharmacological and industrial potential. It is consumed as an antidiabetic and anticholesterolmic agent and to increase liver function. In recent years, it has been recognized as a promising energy crop as a result of its possible use in new and environmentally friendly industrial applications for energy production.2,3 Due to the increase of its commercial and economic value, this study aimed to evaluate the chemical composition and bioactive potential of cardoon blades and petioles in relation to plant growth cycle.

Samples of *Cynara cardunculus* L. var. *altilis* were collected in central Greece at different maturation stages. The individual profiles in tocopherols, free sugars, organic acids, and fatty acids were determined by chromatographic methodologies. The polyphenolic profiles of their hydromethanolic extracts were analyzed by HPLC-DAD-ESI/MS. The antioxidant potential was assessed through the cell-based TBARS and OxHLLA assays. The cytotoxic activity against four human tumor cell lines (HeLa, HepG2, MCF-7, NCI-H460) and a non-tumor cell line (PLP2) was screened by the sulforhodamine B assay. The anti-inflammatory activity was evaluated by the inhibition of NO production. The antibacterial and antifungal activities were evaluated by the broth microdilution method.

Thirteen phenolic compounds were tentatively identified in blade extracts and quantified in higher amounts in samples at an intermediate maturation stage. On the other hand, eleven phenolic compounds were identified in petiole extracts and the immature samples revealed the higher contents as also the best antioxidant capacity. Alpha and gamma tocopherols were found in both cardoon parts (petioles and blades), while beta-tocopherol was present only in petioles; the higher amounts were quantified in more mature samples. Regarding free sugars, fructose, glucose, sucrose, trehalose, and raffinose were detected in both plant tissues, mostly in immature samples. Oxalic, quinic malic, citric, and fumaric acids were detected and quantified in higher quantities in mature blades and immature petioles. Finally, twenty-six fatty acids were found in blades and twenty-seven in petioles. Among them, palmitic, linolenic and alpha linolenic acids stood out for their relative abundance. In addition, blades had more saturated fatty acids, whereas petioles had a higher polyunsaturated content. In terms of bioactivity, both blade and petiole extracts revealed anti-inflammatory and cytotoxic potential, especially the samples at an intermediate maturation stage, as well as antibacterial and antifungal activities. Extracts of mature blades and immature petioles were those with higher antibacterial activity. Regarding the antifungal potential, the results varied according to the tested fungi, since some fungi were inhibited by immature sample extracts (e.g. *Aspergillus versicolor*, *Penicillium ochroloron* and *P. aurantiogriseum*) and others by mature sample extracts (e.g. *Aspergillus fumigatus*, *A. niger* and *P. fusicolus*).

In conclusion, this study showed how chemical features and biological activities of cardoon blades and petioles are affected by the relation to plant growth cycle. However, further studies are needed to better understand which compounds are responsible for the observed bioactivities.

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**References:**
O36 Quantitation and Non-Target Detection of Pesticides in Spinach Extract with Pegasus BT 4D. Improvement to Targeted & Untargeted Pesticide Residue Analysis: Fast and Flexible Analyte Finding For GC-MS and GCxGC-MS

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Accurate detection, identification and quantitation of compounds in high matrix food extracts often proves challenging even to experienced analysts. This work becomes more challenging as limits of detection (LODs) are constantly driven lower by regulatory agencies while they simultaneously increase the number and types of compounds that must be targeted. Selected ion monitoring and MS/MS techniques can help mitigate matrix interferences but may not be selective enough for all compounds in the most challenging matrices. Furthermore, these types of targeted analysis techniques remove the possibility for retrospective nontarget analysis of the data, preventing analysts from detecting new or emerging contaminants. In contrast, comprehensive two-dimensional gas chromatography (GC×GC) dramatically improves chromatographic resolution of analytes within a sample often completely separating target compounds from would-be matrix interferences. Additionally, new time of flight mass spectrometers (TOF-MS) allow for full scan collection at SIM level sensitivities obviating the need for quadrupole based systems. In this article, we demonstrate the use of GC×GC TOF-MS as a methodology to combat matrix interferences, quickly target and quantify suspected contaminants while still allowing non-target analyte detection in a single sample injection.
**O37 Selection of the best urine sampling period based on the volatomic profile**

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The worldwide high incidence and cancer mortality justify the development and implementation of effective and non-invasive strategies leading to early diagnosis. Biological fluids such us urine are rich in several metabolites including volatile organic metabolites (VOMs) that can reflect imbalances of all biochemical pathways. Theoretically, urinary VOMs profiles could be useful to characterize the disease itself, as well as disease progression and response to therapy. However, the presence and concentrations of VOMs may depend not only on biochemical or pathologic processes but also on physiological parameters. As urine sampling can be done in different periods (e.g. fasting, after or before food ingestion), there is a lack of comprehensive studies on the impact of sample collection period on volatile composition of human urine. Therefore, in order to overcome this weakness, we aimed to investigate the influence of the sampling period through the urine collection at different periods - first urine morning, before/after lunch and afternoon, from healthy volunteers, over a period of 25 days. For that, the variability of VOMs was analysed by means of HS-SPME/GC-MS combined with multivariate statistical tools in order to find if the volatile urinary fingerprint is affected by the period of urine collection. The preliminary results are very promising, showing significant differences between some sampling periods which might allow an in-deep and comprehensive understanding regarding the best collection period as a tool to select the more adjusted sampling period and reduce the presence of external confounding factors.

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The Influence of Soil Fertilization on the Amino Acid and Volatile content of Aragonez Wine

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Vineyard fertilization is an important practice as it provides soil nutrients to the levels required for optimum grapevine growth and yield. Most soils contain adequate amounts of micronutrients, however Nitrogen (N), Phosphorus (P) and Potassium (K) (principal macro-nutrients) as well as Magnesium (Mg), Calcium (Ca) and Sulphur (S) (secondary macro-nutrients) are the ones that usually can limit grape production. Mg is required as a component of chlorophyll molecules and for metabolic processes and influences fruit formation and berry ripening. In Alentejo region soils usually tend to have low pH, which translates in deficiency of Magnesium.

The aim of this work was to understand the influence of several nutrients applications to soil vineyards on the amino acid and volatile content of the wines from Aragonez grapes. The experiment was conducted in a randomized block design, with three replications, in a split-plot arrangement. Two different doses of Mg were applied (D1 and D2). For each one there was six different treatments: 1) with N, P, Ca, S, K; 2) with P, Ca, S, K; 3 with N, Ca, S, K; 4) with N, P, S, K; 5) with N, P, Ca, K; 6) with N, P, Ca, S. A control plot with N, P, Ca, S and K without Mg addition was also considered, in a total of 13 plots. The amino acid content of the wines was quantified using a HPLC-DAD system from Waters, USA. The column used was an ACE HPLC column (5 C18-HL) particle size 5 μm (250 mm x 4.6 mm). Prior to injection, samples were derivatized. The volatile content of the wines was also analyzed using a GC/MS system from Bruker, USA. Chromatographic separation was achieved on a ZB-WAX PLUS capillary column (60 m x 0.32 mm i.d., 1.0 μm df). Prior to injection, an HS-SPME extraction was performed on the samples. Results shown that some differences can be observed among the amino acid and volatile content of the wine samples.

Figure 1: Statistical representation of the amino acids content from the Aragonez wine samples with different doses of Mg.

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Aroma profiles of food and beverages are composed of a broad range of chemical classes, including terpenes, phenolics, fatty acids, esters, lactones, aldehydes, as well as nitrogen- and sulfur-containing compounds. It is important to be able to confidently identify these volatiles, for quality control and authentication purposes, as well as in the engineering of new aromas.

In this study, we will demonstrate the use of trap-based secondary focusing to extend the performance of conventional sampling techniques (e.g. headspace and SPME) while retaining fully automated methods. Using real world examples, we will demonstrate unique, high-performance workflows including SPME-trap with enrichment and high-capacity sorptive extraction which allow significant improvements in profiling applications.

Nevertheless, the aroma profiles are often highly complex, with important compounds, such as trace-level off-odours, frequently masked by higher-loading components. The enhanced separation capacity of comprehensive two-dimensional gas chromatography (GC×GC) is now frequently used to tackle this challenge.

Here, we apply multi-hypenated analytical system to obtain comprehensive aroma profiles. The use of parallel detection by three different techniques ensures that three complementary datasets are obtained from a single run:

- Robust quantitation of high-loading species by flame ionisation detection (FID)
- Highly-sensitive, confident identification of aroma-active species by time-of-flight mass spectrometry (TOF MS)
- Highly specific detection of sulfur odour taints by sulfur chemiluminescence detection (SCD)

We will show that the result of using this multi-functional setup is confident aroma profiling and off-odour detection, with fully automated workflows and simple data processing.
O40 Using chromatography to unveil the secrets of the past

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Historical objects usually yield very complex samples for analysis. The diversity of materials, along with the manufacturing process of the artefacts turns sample analysis into a challenging task. This mission can be further complicated by the chemical changes induced by the ageing of materials. The questions that need to be addressed depend on the object in study, and are usually related with the history and the manufacture of the object (where and how it was produced, how was it used throughout times), or with the decay processes of the constituent materials (crucial in terms of object conservation). Chromatographic techniques can be a powerful solution to uncover the secrets behind historical artefacts, especially when coupled to mass spectrometry.

In this presentation, three case studies will be presented to show the wide range of questions and analytical methodologies needed to address them. The identification of the previous contents of archaeological pottery is usually done using GC/MS, and this will be exemplified in the identification of the illuminant used in Roman oil lamps from two archaeological sites from the south of Portugal¹. Py-GC/MS technique requires very little sample preparation when compared to common GC/MS and is usually the method of choice for the identification of oils, varnishes, resins, waxes or gums in historical objects. Red glass beads from the Kongo Kingdom (Democratic Republic of the Congo) were analysed by Py-GC/MS for identification of the waxy coating layer². Finally, LC/DAD/MS technique was applied to the study of red lake micro-samples collected from the Manizola 116c codex, a 16th century antiphonary³.

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Flash Oral Communications

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Posters
FO1 (P04) Chemical characterization of the hydrodistillation residual water and essential oil of Crithmum maritimum

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Crithmum maritimum L. (Apiaceae, sea fennel) is a spontaneous aromatic plant that grows in high salt environments along the coast of Portugal with several medicinal and cosmetic uses ascribed. Several reports highlighted the phenolic composition of sea fennel showing that this plant is characterized by chlorogenic acid and other phenolic acids, however other classes of compounds such as flavonoids can also be found. In addition, C. maritimum can also be distilled to obtain essential oil with a high chemical variability, as reviewed elsewhere. The hydrodistillation process yields essential oil however also originates high amounts of residual water, which is usually discarded. However, due to the characteristics of the extractive process, is conceivable that this water may be rich in non-volatile compounds which might have interesting biological properties. Nevertheless, no study on the residual water was carried out. Having this in mind, the aim of the present study was to highlight the bioactive potential of C. maritimum L. essential oil and hydrodistillation residual water (HRW), the by-product of the distillation process. The chemical composition of the essential oil and HRW was carried by GC-MS and HPLC-DAD-MS/MS, respectively. The antioxidant potential of the HRW was unveiled using the DPPH radical scavenging assay. Using TLC, a composition-activity relationship was unveiled. Furthermore, the bioactive potential of the essential oil was disclosed by assessing its anti-inflammatory potential using the lipopolysaccharide-stimulated macrophages. Chemical analysis of the essential oil showed that γ-terpinene (33.6%), sabinen (32.0%) and thymol methyl ether (15.7%) are the major compounds whereas HRW is characterized by hydroxycinnamic acids and small amounts of flavone and flavonol glycosides. The HRW shows a promising antioxidant activity (IC\(_{50}\) = 0.65 ± 0.16 mg/mL) which seem to be attributable to the presence of chlorogenic acid and quercetin glycosides. Regarding the anti-inflammatory potential, our results show that at 3.125 µg/mL the essential oil of C. maritimum decreases the production of nitric oxide in LPS-stimulated macrophages by 35% without affecting cell viability.

Summing up, the present work highlights the bioactive potential of Crithmum maritimum essential oil and HRW thus endorsing the industrial exploitation of this plant.

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References:
FO2 (P18) Occurrence of polybrominated diphenyl ethers and their metabolites in Douro river biota

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Especially added on too many industrial and domestic products as flame retardants (FRs), polybrominated diphenyl ethers (PBDEs) are among the chemicals of high environmental importance because of their high association with endocrine disruption in animals and their potential harmfulness for human health. In this study, PBDEs and their metabolic products (methoxylated PBDEs – MeO-PBDEs) were quantified in several biotic compartments from Douro river (Porto, Portugal) for assessing the presence of those compounds in that environment. The analyses were performed thoroughly using an environmental friendly method (QuEChERS)-based extraction\textsuperscript{1} in several matrices (algae (n=03 pool), mussels (n=03 pool), crabs (n=06 pool), and three fish species muscle (n=57)). Instrumental analyses were performed using a gas chromatography coupled to triple quadrupole mass spectrometry with electron ionization source (GC-MS/MS, Agilent). Seven PBDEs (28, 47, 99, 153, 154, 183) and eight MeO-PBDEs (6-MeO-BDE-47, 2-MeO-BDE-68, 5-MeO-BDE-47, 4-MeO-BDE-49, 5-MeO-BDE-100, 4-MeO-BDE-103, 5′-MeO-BDE-99, 4′-MeO-BDE-101) were studied in the present study. According to the results, PBDE-47 followed by PBDE-28 were the compounds with the major relative contribution for PBDEs studied in those different matrices. The major relative contribution for MeO-PBDEs comprised the 6-MeO-BDE-47 and 2-MeO-BDE-68, respectively; in edible fish from Brazil (Rio de Janeiro), the most presence of 2-MeO-BDE-68 was reported\textsuperscript{2}. Sum of PBDEs and MeO-PBDEs revealed MeO-PBDEs as compounds with the high contribution in those matrices. Although the low presence of PBDEs and MeO-PBDEs in Douro River, it is important to highlight the similarity of relative occurrence (sum of PBDEs and MeO-PBDEs) in contrast to several other organisms such as yellowfin tuna from South Atlantic Ocean\textsuperscript{3}. The presence of brominated FRs in the Douro river environmental compartments can be used for assessing the health status of the Douro river living organisms, as well as, the potential harmfulness for human health.

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References:
FO3 (P26) The use of chromatographic methods to study the contribution of oral cells in polyphenols-salivary proteins interaction

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Plant-based foods have been attained a high importance between consumers and scientific community due their relation with health benefits and wellbeing. These benefits have been attributed to different classes of compounds, mainly to polyphenols. Nevertheless, the importance of these compounds in the consumer’s preference is related not only with its well documented beneficial properties but also with organoleptic properties, since polyphenols have an important effect on some of these properties, specially color and taste. Regarding taste, polyphenols are related with astringency and bitterness, that could be unpleasant when they are perceived with high intensity, and thus interfering with food quality and consumers perception1,2. Astringency sensation is a tactile sensation described as puckering, dryness and shrinking of the oral surface upon the ingestion of astringent substances3. Aiming its understanding, several studies have been performed to explain its onset, based on the polyphenols-salivary proteins interactions, oral cells or taste receptors. But the most accepted one relies on the interaction and precipitation of salivary proteins by one important group of food polyphenols, the tannins group. However, astringency proved to be a complex phenomenon and further studies have demonstrated that it’s probably related with more than one physical-chemical mechanism. Thus, this work aims to develop a novel cell-based assay, containing human saliva, mucosa pellicle (formed through mucin addition) and an oral cell line (HSC-3), to study the contribution of the most relevant oral constituents on astringency perception by using chromatographic methods. Mucin from porcine stomach was used due its high similarly with human mucin. The interactions were studied using a procyanidin fraction (PF) to investigate the polyphenols-salivary proteins-oral cells interactions. The supernatants of all conditions were collected and analysed by HPLC. In general, results revealed higher interaction (synergism) for the model with all oral constituents when compared to the interaction with individual constituents, the PF+cells or PF+saliva. Although, mucin does not seem to contribute for this interaction, it seems important to the formation of oral pellicle. Analysing the procyanidins individually with the several oral constituents, a significant interaction was observed for B7, B2G, ECG and trimer C1. In conclusion, this work presents a more realistic in vitro oral model, able to study polyphenols-salivary proteins-cells interactions through the use of chromatographic methods, thus providing a breakthrough not only for the astringency study, but also with outcomes in other physiological effects, such as nutrition and bioavailability.

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References:
FO4 (P30) Analysis of phenolic and lipophilic compounds of elderberry stalks from northern Portugal using high performance chromatographic techniques coupled with mass spectrometry

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Elderberries (Sambucus nigra L.) have been widely used since ancient times in folk medicine. However, in recent years an increasing interest on these berries has been witnessed, notably throughout Europe and North America, due specially to better knowledge about their effects in the promotion of human health and well-being. This growing interest in elderberries has resulted in an increased plantation reaching an estimated production of around 1500-2000 tons per year of fresh fruit in Varosa Valley (northern part of the country)¹. Elderberry stalks are the main by-product from the industrial processing of elderberries, accounting for about 10% of fresh weight, currently being mostly used for composting or simply disposed. Currently, there is a great concern to apply circular economy guidelines in the management of residues and by-products of the agro-food industries. In this sense, the stalk of elderberry is of potential interest, whose valorisation implies a previous phase of exploration of bioactive compounds with potential for industrial applications. In this vein, a detailed prospection of the polar (enriched phenolic fraction) and lipophilic fractions of elderberry stalks was performed by ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-DAD-MS²) and by gas chromatography – quadrupole mass spectrometry (GC-qMS), respectively, after a soxhlet extraction with dichloromethane for lipophilic fraction and a sequential methanol: water extraction for polar fraction.

The S. nigra stalks polar fraction accounts for ca. 35% of dry weight and was constituted mainly of phenolic acids, flavonols glycosides (predominantly quercetin-3-rutinoside and quercetin-3-glucoside); and anthocyanins (namely cyanidin-3-O-sambubioside and cyanidin-3-sambubiosyl-5-glucoside). On the other hand, lipophilic fraction accounted for ca. 2% of dry weight and is mainly composed by triterpenes (such as ursolic acid), phytosterols (mainly β-sitosterol) and fatty acids.

Overall, this study uses high resolution chromatographic tools in order to achieve a detailed phytochemical characterization of a complex matrix, in which, both polar and lipophilic fractions represent ca. 37% of dry weight of elderberry stalks. These results allow to infer the elderberry stalks potential as a valuable source of phenolic and lipophilic compounds.

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References:
FO5 (P34) Evaluation of antiglycation potential of *Sambucus nigra* L.

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In the last years it has been an increase in the awareness about the relation of diet and health, especially about the health benefits of the ingestion of fruits, plants and vegetables. This has prompt the need to develop a deeper knowledge of many species, including their composition, new potential uses and products. *Sambucus nigra* L. commonly named European elderberry, are known as a good source of bioactive compounds, mainly phenolic compounds. Indeed, elderberries are very attractive for the industries, due to their high concentration of anthocyanins where the extracts are used as colorants, and different studies proved that elderberries presented several bioactivities such as antioxidant, anti-inflammatory activities, immune-stimulating, chemopreventive and atheroprotective effects, presented also beneficial effects against degenerative diseases (cardiovascular and inflammatory diseases), cancer and diabetes.1-4

The main purpose of this study was the evaluation of the potential antiglycation effect of elderberries from Varosa Valley, Portugal. In order to accomplish this goal, elderberries extracts were incubated with methylglyoxal and the adducts formed were analysed by LC-MS. The results indicate that among the phenolic compounds present in elderberries, the anthocyanins cyanidin-3-glucoside and cyanidin-3-sambubioside formed adducts with methylglyoxal (Figure 1), proving in this way the potential antiglycative effect of elderberries.

![Figure 1: Anthocyanins profile of elderberries extracts obtained without (A) and with (B) methylglyoxal incubation.](image)

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References:
Determination of Perfluorooctanesulfonic Acid (PFOS) in river water and biota matrices by UPLC-MS-MS

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The LAIST was interested in developing and validating an analytical method for the determination of perfluorooctanesulfonic acid (PFOS) in drinking water and biota matrices. PFOS's are persistent, bioaccumulative, toxic substances belonging to the family of the perfluorinated compounds (PFC). Such characteristics placed these compounds in the priority action substances list, with subsequent definition of the environmental quality standards, under the Portuguese law.

The detection of PFOS's was performed in an ultra-performance liquid chromatography coupled with tandem mass spectrometer (UPLC-MS-MS) with the extraction method differing according to the type of matrix. Water matrices were extracted using the solid phase extraction (SPE) with SDB-XC discs whereas the biota was extracted using an ultrasonic bath, with an optimized protocol. Biota samples from different locations in Portugal were analyzed with concentrations varying between 0.26 to 3.78 µg/kg wet-weight. A comparison was performed between the concentrations of PFOS's obtained in the biota and those in respective river waters, which were further compared with the Portuguese legislation (Decreto-Lei 218/2015).

The use of optimized extraction protocols coupled with the use of state-of-the-art equipment and analytical procedures ensured an appropriate detection of PFOS's in environmental samples allowing to determine whether the environmental quality standards were being respected and to better understand the risks to the human health.

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FO7 (P03) New capillary zone electrophoresis method for the determination of cinchocaine hydrochloride and hydrocortisone

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In the recent years, applications of green chemistry have been vastly growing worldwide which involve elimination or at least reduction of hazardous and toxic chemicals, minimizing sample size, shortening of analysis time and reduction of produced wastes\textsuperscript{1}. Capillary electrophoresis is a powerful analytical technique that gained the attention of the international scientific community as an alternative powerful technique for the separation and analysis of compounds of industrial and pharmaceutical interest\textsuperscript{2}. The present work represents a novel green capillary zone electrophoresis method for the simultaneous determination of Cinchocaine hydrochloride (CIN) and Hydrocortisone (HYD) in the presence of the two co-formulated excipient Methyl parapen (MP) and Propyl parapen (PP). Uncoated fused silica capillary ((50 \textmu{}m i.d. × 33 cm and 24.5 cm effective length) was used. Conditions were optimized to achieve better sensitivity in short run time. The finally optimized conditions involve the use of 30 Mm monobasic sodium phosphate of pH 8 as a running buffer and 20 Kv as a positive mode of applied voltage. The method was validated according to the ICH guidelines and was found to be suitable for the determination of the selected drugs in their pure form and in pharmaceutical formulation \textbf{Figure 1}. Bieng simple, sensitive and eco-friendly technique it can be used for routine analysis in quality control laboratories.

\textbf{Figure 1}: Electropherogram of a resolved mixture of 50 \textmu{}g/mL CIN ($t\textsubscript{R}=1.621$), 50 \textmu{}g/mL HYC ($t\textsubscript{R}=1.926$), 50 \textmu{}g/mL MP ($t\textsubscript{R}=2.410$) and PP ($t\textsubscript{R}=2.505$).

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\textbf{References}:
Determination of N-nitrosodiethanolamine in shampoo using on-line solid phase extraction-ultra-high performance liquid chromatography coupled to tandem mass spectrometry

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Contaminants can be present in cosmetic and personal care products due to the contaminated raw material, degradation of ingredients or by reactions between ingredients during the formulation process and need to be controlled1,2. Carcinogenic N-nitrosamines, including N-nitrosodiethanolamine (NDELA), has been detected in personal hygiene products and cosmetics. The objective of this work was to develop an analytical method for the determination of NDELA in baby shampoo using solid-phase extraction on-line ultra-high-performance liquid chromatography associated with sequential mass spectrometry (SPE-UHPLC-MS/MS). Different sample preparation procedures were evaluated for extraction and clean-up of the sample matrix. Due to complex matrix of shampoo, an off-line solid phase extraction, using a C18 (1.5 g) sorbent was additionally used to the on-line SPE. The NDELA concentration was performed in the SPE on-line, using an Oasis HLB column, whose optimization was performed by a central composite experimental design. The response surface is shown in Figure 1. The optimum conditions were: sample loading flow rate and time of 0.87 mL min<sup>-1</sup> and 0.27 minutes, respectively. Sample loading solvent, water and an injection volume of 200 µL. The electrospray source (ESI) was operated in the positive mode and the quantification was performed in the selected reaction monitoring mode. The analytical column used was a UPLC<sup>®</sup> CSH C18 (2.1 x 100 mm, 1.7 µm), with a mobile phase composed of 0.1% formic acid in water: methanol, and gradient elution. NDELA quantification was done by internal standardization using NDELA-d<sub>8</sub> as surrogate. The mass spectrometry conditions comprised: capillary voltage (1.0 kV); source temperature (150 °C); desolvation temperature (350 °C), desolvation gas flow rate (N<sub>2</sub>, 600 L h<sup>-1</sup>) and cone gas flow rate (50 L h<sup>-1</sup>). For quantification of NDELA the transitions (m/z) 135→104 (quantitation, cone voltage 15 V and collision energy 4 eV) and 135→74 (identity confirmation, cone voltage 15 V and collision energy 12 eV) were monitored. The method showed linearity in the solvent between 1.0 to 20.0 ng mL<sup>-1</sup> and in the shampoo sample matrix between 10 to 100 ng g<sup>-1</sup>. The LOQ was 10 ng g<sup>-1</sup>. Intra-day precision ranged from 9.1 to 19.2% (n = 3, within 3 days) and the inter-day value was 15.3% (n = 9). Of the four analyzed samples, one presented NDELA at the concentration of 54 ng g<sup>-1</sup>; two samples presented NDELA less than the limit of detection of the method and one sample a concentration lower than the limit of quantitation.

Figure 1. Response surfaces for NDELA, showing the area as a function of significant parameters. A: x<sub>2</sub> (loading time, min) and x<sub>3</sub> (loading volume, mL min<sup>-1</sup>) B: x<sub>1</sub> (% of H<sub>2</sub>O) and x<sub>3</sub> (loading time, min). C: Characteristic chromatogram of NDELA.

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References:
The use of Statistic Tools for QuEChERS Content Optimization in the Extraction of Ibuprofen and its Metabolites in Different Types of Soil Samples

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Pollutants in water or in air generally are more easily extracted than those associated with soil, due to its interaction. Strong chemical and physical forces may act to bind the contaminants to the soil particles. Thus, if the monitoring technique requires that the chemicals be extracted or removed from the soil prior to analysis, the efficiency of the extraction process becomes crucial to the overall success of the analysis. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) sample preparation poses an alternative method that is able to provide consistent results, the use of low amount of solvent, and requires little labour and materials commonly used in laboratories. The sample preparation has been widely used since its development and publication by Anastassiades et al. 2003 and different matrices and different pollutants have been studied. The objective of the proposed work is the use of Response Surface Methodology to optimize the proportion of salts present in the QuEChERS content for soils with different organic matter using a $2^4$ factorial design. Liquid chromatography with fluorescence detector was used for the analysis.

Figure 1 - QuEChERS procedure used for the extraction of ibuprofen and its metabolites from soil sample.

Different proportions of the amounts of salts were tested and results were compared with pre-packet QuEChERS kits. Good recoveries were obtained for the studied compounds. The proposed QuEChERS method was applied to sediments and agriculture soils were collected and twelve soil samples were extracted. Ibuprofen was found in all analysed samples (between 0.44 to 4.96 ng/g), hydroxyibuprofen was found in two sediment samples (3.53 and 5.86 ng/g), and carboxyibuprofen was detected in one agricultural sample (0.851 ng/g).

The benefits and costs of the optimized QuEChERS content were compared to the commercial kits. The optimized QuEChERS content conducts to the reduction of the expenses when compared to commercial QuEChERS kits.

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References:
FO10 (P09) Qualitative doping analysis of β-blockers in urine by GC-MS/MS

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The World Anti-Doping Agency (WADA) was established in 1999 as an international independent agency composed and funded equally by the sport movement governments of the world. In 2004, the World Anti-Doping Code (Code) was created to harmonize, coordinated and regulate anti-doping programs at the international and national level.

WADA stands by the slogan "Play True", and embraces it every year, becoming stricter and increasing the number of compounds included in the List of Prohibited Substances (annually reviewed). This extensive list describes the substances for which the intake is forbidden to athletes that want to compete on events organized by Code Compliance Signatories.

Only a restricted number of forensic laboratories are authorized by WADA to perform the analysis of doping controls for sports under the Code. It is mandatory that these laboratories are accredited both by an ILAC (International Laboratory Accreditation Cooperation) member under ISO/IEC 17025 and by WADA itself, under the International Standard for Laboratories (ISL).

WADA-accredited laboratories must have the ability to detect the over 400 enhancing drugs in different biological matrices (urine and blood). The strategy is to screen for these substances in faster comprehensive methods, to clearly distinguish negative samples form suspected ones and then to use more specific/selective methods to confirm the presence/absence of any prohibited drug.

In order to give a “positive” case (Adverse Analytical Finding) for a certain compound, WADA strongly recommends that laboratories have that substance in the scope of the accreditation. It was, then, necessary to developed and validated methods for all classes of substances mentioned in the list, approving that all substances could be accredited under the global flexible scope, allowing the addition/removal of substances and/or its metabolites.

One of the classes that are prohibited in-competition and out-of-competition in some sports (e.g., automobile, golf, archery, shooting, etc.), are β-blockers. β-blockers - beta-adrenergic receptor blockers - are a class of drugs widely used in clinical pharmacology to treat cardiovascular diseases and related conditions (e.g. controlling acute panic symptoms in anxiety-provoking situations). These drugs reduce blood pressure, manage cardiac arrhythmias and are cardioprotective after myocardial infarction (heart attack). β-blockers are competitive antagonists that bind to beta-adrenoceptors and block the receptor sites for the epinephrine (adrenaline) and norepinephrine (noradrenaline) of the sympathetic nervous system.

In the illicit pharmacological support to sport competition, β-blockers are used to reduce the cardiac frequency and to minimize tremors, in order to improve the performance in skill-based sport disciplines.

The present work develops an analytical method for the qualitative determination of nine (9) β-blockers present in WADA’s list (metoprolol, propranolol and its metabolite 4-hydroxy propanolol, sotalol, timolol, celiprolol, bisoprolol, atenolol and nebivolol) in urine, a noninvasive and easy-to-collect biological matrix with a high potential in the detection of prohibited substances, by GC-MS/MS.

The method proved to be selective, robust and presents good identification capacity where the LOD (Limit of detection) determined for all substances was 50 ng/mL (according to TD2019MRPL, the minimum criteria performance levels (MRPL) for chromatographic-mass spectrometric confirmation of the identity of analytes for this class of substances is 100 ng/mL). No carryover has been verified and the method presents adequate recoveries of the analytes.

Once completed the validation, the method was subsequently applied to two interlaboratory positive samples for β-blockers and the results were in accordance with WADA’s reporting rules, so the developed method can be an important new tool to confirm the presence of the β-blockers analyzed and being under flexible scope of accreditation it can be easily incremented with other β-blockers.

References:
FO11 (P68) Chemical characterization of the sacred wood: final resting place of Benedictine Abbots of St. Margaret, Bijela

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Archaeological wood, as organic material, is prone to biodegradation during deposition and physical and chemical decay, especially after the change of environment conditions post excavation. Loss of cellulose and hemicellulose and alteration of lignin causes formation of water filled cavities that after drying of the material, deform the original wood. Since the wood structure is anisotropic, shrinkage is uneven and may lead to warping and cracking. The porous and fragile structure is low in polysaccharides and mainly composed of residual lignin, which is often highly oxidized and fragmented. This makes the correct taxonomic identification and consolidation treatments more difficult and sometimes impossible. Furthermore, it is important to note that different plant species and areas of wood succumb to decay at different rates, also depended on the presence and amount of other compounds naturally present in wood, like tannins and resins. Consequently, chemical analysis has proved to be a valuable approach in the diagnosis and conservation of archaeological woods$^{1,2}$.

Samples of archeological wood were recovered from the ruins of the Benedictine monastery of St. Margaret, located in Bijela in northeastern Croatia. The monastery became one of the most important Benedictine centers in Medieval Slavonia, however, by the turn of the 15th century, it was used as a fortification and defense against the Ottoman threat. Still, life in Bijela persisted as evidenced by burials in the church, even in the 16th and the 17th century$^3$. Burials revealed a predominant male community as well as the presence of women and children who endured stress, hard physical labor, low health standards and intentional violence$^4$. Remains of wooden coffins were preserved in connection with some burials, despite the non-preferable conditions in aerated soil environment, demonstrating a preferred burial ritual.

To confirm taxonomic identification of decayed and deformed archaeological wood and to distinguish between the type of wood used for different burials as well as gain further understanding of wood components and degradation processes samples were studied by FTI-IR/ATR spectroscopy and Py-GC/MS analysis. We examined chemotaxonomy of each sample and difference between gymnosperms (softwood) and angiosperms (hardwood) species. By observing degradation and chemical alterations in the samples, we present the application and the value of chromatographic and spectroscopic techniques in the study of archaeological wood.

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References:
FO12 (P73) Discrimination of Lavandula essential oil growing in Castelo Branco region by GC-MS and FTIR-ATR

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The genus Lavandula L. belongs to the Lamiaceae family that are endemic to the Mediterranean region. In Portugal, namely in Beira Interior region, this species is a valuable resource for beekeeper activities and for essential oils production in a sustainable way. The literature described 39 species and numerous hybrids. This genus had undergone several taxonomic modifications due to its morphological variability and hybridization ability. Therefore, for its description and classification, it is important to consider the polymorphism and chemotypic phenomena. Lavandula species are considered important medicinal and aromatic plants due to the production of bioactive compounds, found mainly in their essential oils with interesting biological properties. L. stoechas subsp. luisieri deserves particular attention because its essential oil is composed by necrodane derivatives, and at the moment have no reports that this valuable compound is present in another plant species.

The aim of this study was to discriminate and identify the different species of Lavandula present in this region through the essential oil profile. Three different locals in the Castelo Branco region (Penamacor, Vila Velha de Ródão and Proença-a-Nova) were studied. In each local 10 aerial flowering plants of Lavandula were collected, according the good agricultural and collection practices to ensuring the natural regeneration and propagation of the species. A copy of the each collected plant was deposited in the herbarium of the biology laboratory of IPCB-ESA (Polytechnic Institute of Castelo Branco- Agrarian School).

Lavandula luisieri is a valuable resource for beekeeper activities and for essential oils production in a sustainable way, for this reason, for ensuring the natural regeneration and propagation of the species, the application of FTIR-ATR appears to be a powerful technique for discriminating the quality of Lavandula essential oil from different geographical species, further research will be needed to confirm these preliminary results.

Keywords: Lavandula stoechas subsp. luisieri, Essential oil, GC-MS, FTIR-ATR.

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References:
FO13 (P17) Using SPME/GCxGC-ToFMS approach for a rapid and early evaluation of food contamination based on A. niger biomarkers pattern

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Recently, the Aspergillus niger volatilome was explored using advanced gas chromatography tools tandem with multivariate analysis, which allowed to propose a molecular biomarkers pattern for this fungi. This pattern comprises a set of 44 volatile metabolites, related with amino acid metabolism, biosynthesis and metabolism of fatty acids, degradation of aromatic compounds, mono and sesquiterpenoid synthesis and carotenoid cleavage [1]. The main objective of this research was to test the applicability of this pattern in real food samples. Actually, A. niger is a ubiquitous fungus responsible for food contamination [2,3], being reported as one of the main agents of the black mould disease, a serious post-harvest pathology of table grapes [4]. Thus, red and white table grapes (Red Globe and Dominga, respectively) were inoculated with A. niger and storage at 25°C and 90% of humidity. In order to check the presence of A. niger biomarker pattern, a metabolomics targeted analysis was performed by direct analysis of metabolites released from inoculated grapes based on the HS-SPME/GC×GC-ToFMS methodology (Headspace Solid Phase Microextraction combined with Two-Dimensional Comprehensive Gas Chromatography-Time of Flight Mass Spectrometry), after 1, 4 and 7 days of inoculation) [1]. Unsupervised multivariate analysis were performed (PCA and clustering analysis), which revealed that after 1 day of inoculation it was possible to determine the previously established A. niger biomarkers pattern [1], which allows to notice its presence. Furthermore, the follow-up of this set of metabolites showed that white variety exhibited at 4 days a level of these metabolites similar to those obtained at 7 days for red variety, which suggest that white variety is more susceptible to fungi development than red ones. The results obtained confirm the potential applicability of the pattern of A. niger biomarkers to early detect the fungi (after 1 day of contamination) and also may be further explored for access food susceptibility to fungi contamination, based on a direct analysis of food item and taking advantage of the high sensitivity of the GC×GC-ToFMS.

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References:
Development of methodologies to evaluate odour retention capacity in textiles

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In general, a person releases different compounds (such as sweat) and is surrounded by unpleasant odours (such as cigarette smoke). This odours tends to get trapped by textiles and, the capacity to retain and neutralize odour is a property of increasing interest in substrates for various applications and it is, therefore, important to find ways to assess it. ISO 17299:2014 defines the main chemical components of odours and specifies test methods using various types of instruments, namely gas chromatography with flame ionization detector (GC-FID), where the odour reduction rate of gas in contact with textiles is objectively determined. Most odour-related studies include testing with a sensory evaluation panel, since the broadest and most sensitive odour detector is undoubtedly the mammalian olfactory system. This methodology combined with an instrumental method, becomes an ideal method for both internal and external odour analysis.

In this regard, two different substrates were analysed, textile A of 67% PES + 29% CV + 4% EL and textile B of 63% CO + 37% PES. The textile A was used as a positive control, as it already has intrinsically great deodorant capacity and textile B for having greater potential for improvement. In this sense, textile B was analysed as control and functionalized with milk protein by padding techniques. To determine the reduction capacity of these textiles, the samples were analysed by GC-FID with isovaleric acid (IVA), which corresponds to body sweat odour, following ISO 17299-3:2014. In addition, a sensory analysis was performed with 19 volunteers for the same odoriferous marker, to qualify the odour felt and correlate with the results obtained by GC.

According to the standard, and for IVA marker, a reduction capacity of ≥ 85% is required for a textile to be considered deodorant and only the textile A can be considered as such (95% of reduction). Although, the functional textile B (78% of reduction) demonstrated a high ability to reduce IVA odour and improvement compared to the substrate control (45% of reduction). Since a greater ability to reduce an odour by a textile decreases the odour present in the atmosphere, it was possible corroborate the results of sensory analysis with the results obtained by GC. This because the volunteers were able to distinguish and considered that the less intense atmosphere is that of textile A, followed by the functional textile B and control textile B. Statistically, by Friedman’s test using the Newell and MacFarlane table for a 95% probability, it is further found that there are no significant differences between textile A and functional textile B.

In conclusion, it is possible corroborate the obtained results and to verify the added value of the correlation of the two techniques in the results analysis in order to prove the interactions between the odorant marker and the functional fabric.

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References:
FO15 (P75) Rapid determination of some of the most used pesticides in Northeast Portugal as emerging contaminants in rivers by SPME/GC-MS

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In this communication the development of an analytical methodology is presented for the monitoring of five emerging pollutants, namely, alachlor, metolachlor, heptachlor, dimethoate, and terbuthylazine, represented in Figure 1. These compounds are among the most used pesticides in the northeast region of Portugal and Tunisia. The complete experimental methodology is optimized based on the simultaneous extraction and concentration of all five pesticides from aqueous matrices, by means of solid-phase micro-extraction (SPME) followed by detection and quantification using gas chromatography with mass spectrometry (GC-MS).

Fig. 1 Chemical structure of the five pesticides.

The optimization of the extraction step is performed using a polydimethylsiloxane-divinylbenzene (PDMS-DVB) coated SPME fiber by direct immersion (DI-SPME) in the aqueous sample. Experimental conditions, such as extraction temperature and time, pH value, salt addition, and desorption time and temperature in the GC injector port were studied. The optimum value for each one of these parameters was selected based on the maximum total area value obtained in MS detector, using Full Scan Mode, for the mixture of five pesticides. The extraction optimized conditions were achieved by immersion of a PDMS-DVB fiber in the sample mixture with 10% NaCl, a pH value of 2, at 60 °C for 80 min. Desorption of the compounds from the fiber is done in the GC port at 250 °C during 4 min. The Shimadzu GC-MS equipment, model QP2020, operating conditions were also studied, and the main separation and detection parameters were selected. Samples were analyzed using a Rxi-5MS Low Bleed capillary column from Restek and the following oven temperature program: initial temperature of 120 °C (held for 2 min), increased by 15 °C.min⁻¹ to 190 °C (held for 4 min) and, finally, increased by 10 °C.min⁻¹ to 227 °C and held for 1 min. The MS instrument operating in the Electron Ionization mode (EI) was used for a full scan. The acquisition was performed in the range of 35–450 (m/z). The ion source temperature was 200 °C and the interface temperature was 270 °C.

The identification and quantification were carried out using calibration curves obtained from the extraction of a standard mixture of the five selected pesticides for at least six concentration levels, in the same experimental conditions used for the real samples. Detection limits ranged from 4.2 to 6.6 ng/mL. For pesticides with low values of Kow, like dimethoate, the use of a fiber of relatively non-polar nature would be more favourable. The developed experimental methodology was implemented by the analysis of different samples collected from the surface water of three rivers from Bragança, namely, Fervença, Sabor and Onor. All three rivers showed different types and levels of contamination.

References:
**P02 Essential oil from Chenopodium ambrosioides**: chemical profile in CG/MS and phenotypical analysis of subpopulation T lymphocytes in rats infected with *T. cruzi*

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Chenopodium ambrosioides is a perennial herb with a typical strong and unpleasant odor, widely distributed in Brazil and Latin America. In folk medicine, this plant is frequently used for anthelmintic agent, although other biological activities attributed to the secondary metabolites of this plant have been identified, like as antitumor, analgesic, anti-inflammatory and antimicrobial activity. *C. ambrosioides* essential oil is rich in ascaridol, which is a component associated with the trypanocidal and leishmanicidal action of this species. The aim of this work was to evaluate the chemical profile of the essential oil from *C. ambrosioides* leaves, as well as to evaluate its immunomodulatory potential by analyzing the T lymphocyte cell population in rats infected with *Trypanosoma cruzi*. The essential oil was extracted from the 40g the leaves by hydrodistillation for 90 minutes using a modified Clevenger apparatus. After the extraction, the essential oil was collected and its chemical analysis was performed by gas chromatograph coupled to mass spectrometer (GC/MS – Shimadzu CG-17A), with EN5MS column (30m x 0,25mm x 0,25 µm), helium as a carrier gas at a flow rate of 1,5 mL/min and selective mass detector (QP 2010). For analysis the oil’s immunomodulatory effect, we used as a parameter the evaluation of the T lymphocyte cell population (T CD3+CD8+ and TCD3+CD4+) from rats infected with *T. cruzi* (Chagas’ Disease), in the acute and chronic stages of disease and treated with daily doses of 0,5 mg of the essential oil. The cell quantification was performed by flow cytometry. The identification of chemical components of the essential oil was based on the chromatogram, mass spectra generated for the detected substances, comparison with standards (NIST08, WILEY7 and FFNL1.3 library) and Kovats Retention Index (IK) calculation. Thus, it is possible to determinate seven substances: α-terpinene, p-cymene, Limonene, γ-terpinene, Ascaridole, Piperitone and Isoascaridole. T lymphocyte populations were determined and evaluated for acute (9th day) and chronic (60th day) infection groups. According to the results, that animals treated with *C. ambrosioides* essential oil showed an increase in CD8+ T cell percentages and it may contribute to prevent the parasitic spread during the acute phase of the disease. It was also verified that these same cells, in the chronic phase, remained stable. Data also revealed that the treatment of the rats with essential oil contributed to the immunomodulatory effect, when the CD4+ T cell population is evaluated. It is an efficient way to stop the advance of *T. cruzi* in organism. In the chronic phase, the results showed CD4+ T cell population decrease, useful to contribute to prevent an exacerbation of an inflammatory response, which may cause further damage to the organism. Therefore, the study showed the potential of *Chenopodium ambrosioides* essential oil which its secondary metabolites may serve as the basis for the design of new drugs with immunomodulatory properties, especially for Chagas’ disease.

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References:
Bar Adsorptive Microextraction (BAμE) applied to the Determination of Six Tricyclic Antidepressants

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Depression is a common mental disease and one of the biggest causes for incapacity around the globe. It is estimated to affect around 300 million patients worldwide, the number of antidepressants consumed has been increasing. Antidepressants allow people diagnosed with depression to live almost ordinary lives, with no restrictions. Tricyclic antidepressants (TCAs) were the first antidepressants to be commercialized (1955) and are still to this day in large use. However, antidepressants, especially classic ones, can cause serious intoxications if used in higher quantities than prescribed. It is also worth noting that TCAs have a narrow therapeutic range. The determination of TCAs levels is necessary for emergency toxicological screenings, abuse drugs testing and forensic exams for fatalities likely to have been caused by TCA overdose. For this reason, it is crucial the development of user-friendly, with high sensibility and precision methods for the determination of this compounds in biological samples (e.g., urine). In general, the methodologies proposed for the determination of TCAs in biological matrices demand for sample preparation techniques prior to separation systems, such as gas or liquid chromatography or capillary electrophoresis using convenient detectors or coupled to mass spectrometry (MS).

This work entailed the development, optimization, validation and application of a novel analytical approach, based on bar adsorptive microextraction followed by liquid microextraction in combination with large volume injection gas chromatography coupled to mass spectrometry operating in the selected ion monitoring (BAμE-LVI-GC-MS(SIM)) mode, for the determination of six tricyclic antidepressants (amitriptyline, imipramine, trimipramine, imipramine, mirtazapine and clomazolin) in urine matrices.

Detection and quantification limits of 0.2 - 1.6 μg/L and 0.1 μg/L were achieved, respectively. A weighted linear regression plot was performed for each analyte, where a weighting factor of 1/y was applied. Linear dynamic ranged in between 10.0 and 1000.0 μg/L were selected, showing remarkable linearity (r² > 0.9960). Matrix effects ranged from 90.2 to 112.9 % (RSD < 13.9 %), high recovery yields (92.3 to 111.5 %), RSD < 12.3 %) and remarkable efficiencies (84.9 to 124.3 %; RSD < 15.9 %) of the analytical process were obtained. The optimized methodology was successfully applied to 52 anonymous real samples obtained from a medical clinic, in which levels of TCAs were detected.

The proposed methodology proved to be an alternative strategy for the analysis of TCAs in urine matrices, having as main advantages the use of small sample volume, negligible amounts of organic solvents, easy manipulation, simplicity and excellent analytical performance in accordance with the recent trends in analytical chemistry.

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P06 Ergosterol rich-extracts from *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm: A comparative study between mushroom and its bio-residues


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Edible, medicinal, and wild mushrooms are the three major components of the global mushroom industry, recently accounted for US$ 38.13 billion, and expanding at a compound annual growth rate (CAGR) of 7.9% from 2018 to 2026 [1]. Depending on the mushroom industry size, a large amount of bio-residues is generated and often discarded (20 to 35% in weight of fresh mushrooms), even though their content in biomolecules is not necessarily compromised [2].

*Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm is one of the most produced edible mushrooms worldwide due to its ability to colonize and degrade a large variety of lignocellulosic substrates [3]. In the present work, *P. ostreatus* bio-residues (POR) and intact mushrooms (POG) were compared for their ergosterol content. Response Surface Methodology (RSM) was applied using heat-assisted extraction methodology. The combined effect of time (10-150 min) and temperature (30-90°C) was performed using a circumscribed central composite design (CCCD), and the response criteria determined using the HPLC-UV were ergosterol content in mg/g (ergosterol purity) and mg/100g dw (ergosterol extraction yield). Response surface models were fitted by using the following second order polynomial equation:

\[ Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} \sum_{j=i+1}^{n} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{i} X_i^2 \]

The global optimum conditions predicted by the model were 65.6 min, 30°C, and 10 min, 30°C for POR and POG, respectively. Under these conditions, 43.72 and 57.61 mg of ergosterol per 100 g of dry weight sample were recovered from POR and POG, correspondingly. Regarding the ergosterol content in dry weight basis (mg/100g dw), 290.90 and 246.31 were obtained for POG and POR, respectively. The values predicted by the model are in close agreement with the experimental observations with very low residual distribution, proving the validity of the applied model. The results also showed the usefulness of the predictions for future scale up based on the desired responses. Ergosterol and its well-known anti-inflammatory, anti-proliferative, and anti-tyrosinase activities, together with its use in new drug formulations associated with antibiotics, confirm the enormous potential of the under-exploited *P. ostreatus* bio-residues as a source of ergosterol-rich extracts.

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**References**:

Phytochemical profile and biological activities of 'Ora-pro-nobis' leaves (Pereskia aculeata Miller), an underexploited superfood from the Brazilian Atlantic Forest

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Pereskia aculeata Miller, known worldwide as ora-pro-nobis, is a highly nutritious species of the Cactaceae family from the Brazilian Atlantic Forest. From South to Northeast of Brazil, ora-pro-nobis leaves are used in traditional cuisine, featuring as an ingredient of various sweet and savoury dishes. In some low-income communities, it is known as the ‘meat of the poor’, being the main protein source available. The aim of the present work was to perform an in-depth study on the phytochemical profile and some biological activities of *P. aculeata* leaves, with the view of expanding the current knowledge on the potentialities of this superfood. For this purpose, its hydroethanolic extract was characterized in terms of phenolic composition, antioxidant, and antibacterial performances and finally the hepatotoxicity of the extract was evaluated.

A total of ten phenolic compounds were identified via high-performance liquid chromatography coupled to diode array detector and mass spectrometer (HPLC–DAD–ESI/MSn): two phenolic acids (caffeic acid derivatives) and eight flavonoids (quercetin, kaempferol and isorhamnetin glycoside derivatives). Caftaric acid was the extract’s major phenolic constituent, accounting for more than 49% of the phenolic content, followed by quercetin-3-O-rutinoside (14.99%) and isorhamnetin-5-O-pentoside-3′-rutinoside (9.56%). The total phenolic content found in the hydromethanolic extract was relevant (23.75 mg/g).

A broad study of the extracts’ antioxidant activity was carried out, using a set of five distinct methods: two cell-based methods (1) oxidative haemolysis inhibition assay (OxiHLIA); (2) the inhibition of the production of thiobarbituric acid reactive substances (TBARS); besides three chemical in vitro assays (3) reduction of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH); (4) reduction of the 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonate) cation (ABTS) and (5) hydroxyl radical scavenging assay. Overall, the *ora-pro-nobis* leaf extract showed relevant values of antioxidant activity, even higher than Trolox in the DPPH and ABTS trials. The antioxidant activity verified for the hydroethanolic leaf extract of *P. aculeata* is possibly related to its phenolic composition; the predominant constituents of the extract, i.e. caftaric acid and rutin, have widely proven antioxidant activities. Furthermore, the evaluated extract showed no toxicity against a non-tumour liver primary culture PLP2, at the highest tested concentration (400 μg/mL). The antimicrobial activity exhibited by the extract against both Gram-positive (*Enterococcus faecalis, Listeria monocytogenes, MRSA – Methicillin-resistant Staphylococcus aureus*) and Gram-negative (*Escherichia coli, Klebsiella pneumoniae, Morganella morganii, Proteus mirabilis, and Pseudomonas aeruginosa*) bacteria (MIC values between 5 to 20 mg/mL) suggests the presence of a broad spectrum of phytochemicals with antimicrobial activity. The extract was more active against *K. pneumonia* than the antibiotic ampicillin, whereas against *M. morganii* it presented equivalent inhibitory effect.

Therefore, the information reported here not only corroborates the importance of the production and consumption of *P. aculeata* leaves by the low income population to improve nutrition, but also reinforces their potential as a sustainable source of promising food ingredients to be used both for food enrichment and preservation.

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2. 308.
3. 503.
P08 Targeted metabolites’ analysis of *Hibiscus sabdariffa* L. calyces from Guinea-Bissau (West Africa)

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Numerous plants have been used all over the world and in addition to the food properties, their consumption seems to be associated with different beneficial properties for consumers’ health. In this way, several scientific studies have been carried out with the objective of confirming these properties besides the assessment of their nutritional quality¹. *Hibiscus sabdariffa* L., is an annual or perennial plant with red stems and calyces, belonging to the Malvaceae family. It is cultivated mainly in the tropical and subtropical areas of both hemispheres².

The present study aimed to perform a targeted metabolites’ analysis (free sugars, organic acids, tocopherols and fatty acids) of *H. sabdariffa* dried calyces (Guinea-Bissauan origin), as well as the individual phenolic composition of its infusion and hydroethanolic extracts. Free sugars were identified through an HPLC-RI system, the organic acids by UFLC-PDA, tocopherols by HPLC-fluorescence, fatty acids by GC-FID and the individual phenolic compounds were analysed through HPLC-DAD-ESI/MS.

Glucose and quinic acid showed the major concentration for sugars and organic acids, respectively. Palmitic acid and α-tocopherol were the most abundant lipophilic compounds. In the individual phenolic profile, thirteen compounds were identified, five phenolic acids and flavonols, and three anthocyanins. The hydroethanolic extract presented all the identified compounds, while the infusion revealed the presence of twelve molecules, with the absence of caffeic acid. In general, the hydroethanolic extraction seems to have favoured the extraction of non-anthocyanin phenolic compounds, while the infusion was the ideal methodology for the anthocyanins’ extraction. Nevertheless, 3-O-caffeoylquinic acid was the major non-anthocyanin compound, while, delphinidin-3-O-sambubioside was the most abundant anthocyanin, in both extracts.

This study demonstrates the high potential of this species highlighting its usage as a functional food or beverage, and as a source of a possible nutraceuticals and natural pigments, giving an added value for future applications in several industrial sectors.

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Mushrooms are low-calorie foods with good quality proteins, vitamins and minerals, besides holding potential for some medicinal applications. In fact, mushrooms could be a source of many different nutraceuticals such as steroids, phenolic compounds, and others. Thus, they might be used directly in diet and promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present. The edible mushroom *Pleurotus ostreatus* var. Florida known as "Hiratake" is one of the most consumed mushrooms in the world, mainly due its easy cultivation, economic potential, nutritional quality, as well as therapeutic and biological properties. Silicon (Si) is known to play an important role in the mineral nutrition supplementation of mushrooms and plants, including increased productivity, through the availability of nutrients, increased biomass and resistance to biotic and abiotic stresses. In this study, cultivated *Pleurotus ostreatus* var. Florida was supplemented with calcium silicate (0.5, 1, 2 and 4 %) and the effects of this supplementation on chemical and bioactive composition were evaluated. Ergosterol and vitamin D2 were determined by high performance liquid chromatography coupled to a UV detector; organic acids and phenolic compounds were determined by ultrafast liquid chromatography coupled to a photodiode array detector. The supplementation with calcium silicate exerted remarkably positive effects in the evaluated parameters. Specifically, higher vitamin D2 contents were obtained in samples treated with 1% and 2% (866 and 862 µg/100 g dw). A similar increase was obtained in organic acids (5.15 g/100 g dw), considering the three identified compounds (oxalic, malic and fumaric acids) in samples treated with 0.5% of calcium silicate. The calcium silicate supplementation also increased the total phenolic compounds (protocatechuic acid, p-coumaric acid and cinnamic acid) relatively to the control sample. Calcium silicate supplementation was effective in improving the chemical profiles of *P. ostreatus*. Therefore, this practice may represent an effective way to increase the compounds of interest in different mushrooms.

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UV-C radiation increases Vitamin D2 content in *Pleurotus ostreatus*

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Mushrooms have been traditionally recognized for their nutritional and medicinal value, and countless investigations have reported highly nutritious content as well as medicinal properties such as anticancer, antimicrobial, hypocholesterolemic, antioxidant, among others. These properties are attributed to bioactive compounds such as ergosterol (precursor of vitamin D2) and other steroids, phenolic compounds, and vitamins. Vitamin D2, in particular, plays an important role in many human metabolic processes. Mushrooms are the only non-animal food source of vitamin D2, which is also formed during UV exposure. Since dietary sources are scarce, vitamin D deficiency can cause serious health problems; studies of vitamin D have received considerable attention in recent years, supported by the growing number of deficiency reports, e.g. rickets, osteoporosis, multiple sclerosis and cardiovascular disease, among others.

The present work reports the effectiveness of UV-C radiation on increasing the content of vitamin D2 in sliced *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. samples. The irradiation was performed in a UV chamber at the intensity 0 (non-irradiated), 200, 800, 3200 mJ/cm² and different exposure times: 0, 2, 6 and 10 min. Vitamin D2 was determined using high performance liquid chromatography coupled to a UV detector.

It was verified a significant (*p*<0.050) interaction (exposure time x UVC) among factors, indicating that the effect of each UVC dose was modulated by exposure time and vice versa. Nonetheless, it is obvious that the application of UV-C radiation induced a clear increase in the quantity of vitamin D2, most likely due to the conversion of some of the ergosterol content naturally present in these mushrooms. In what concerns, exposure time, the adequate choice would be 6 min (125 µg/g DW of vitamin D2), as no significant increases were attained with the maximum assayed time (10 min). On the other hand, and despite the statistically significant differences, the advised UV-C dose would be 200 mJ/cm², particularly considering that this option would be less expensive, without relevantly compromising the increase in vitamin D2.

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P12 HPLC-PDA-MS/MS analysis of the leaves of two *Jasminum* species from Egypt

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*Jasminum officinale* L., commonly known as true Jasmine and *Jasminum multiflorum* Burm.f. (furry Jasmine) are two climbing shrubs with white slightly fragrant flowers belonging to Family *Oleaceae*.¹ Both plants are widely cultivated in Egypt for the production of the concrete of the flowers.² HPLC-PDA-MS/MS analysis allowed the identification of a total of 50 and 51 metabolites in the methanolic extract of the defatted leaves of *J. officinale* (JO) and *J. multiflorum* (JM), respectively. The main components of both species were secoiridoids (monomeric, dimeric or trimeric) as oleuropein and its derivatives³, represented by 26 and 29 components in JO and JM, respectively. Flavonoids, 11 and 7 components in JO and JM, respectively were mainly quercetin and kampferol mono-, di- and tri-glycosides.⁴ On the other hand, JO was typified by the abundance of phenolic acids (11 components) with rosmarinic acid as major constituent. Phenylethanoids as tyrosol and hydroxytyrosol or their glycosides were identified in both species.⁵ Also, lignans as hydroxyariciresinol and pinoresinol were identified in JO and JM.⁶ The presence of secoiridoids and lignans are characteristic for the genus *Jasminum*. From our results it could be concluded that the extracts obtained from the defatted leaves of the plants could be a rich source of many bioactive constituents.

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References:
P13 Insights into the phenolic composition and bioactive properties of Aloe vera flower

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Aloe vera leaf has been subject of several scientific studies that aimed to characterize compositional and biological properties.1,2 However, the flower (Figure 1) remains an underexploited plant part. Aiming the prospection of bioactive phytochemicals in this plant matrix, this study was focused on the analysis of phenolic compounds and in vitro antioxidant, antimicrobial, and tyrosinase inhibition activities. A dried powder of Aloe vera flower underwent a solid-liquid extraction with an hydroethanolic mixture for 1 h, and the phenolic profile of the obtained dried extract was characterized by HPLC-DAD-ESI/MS.5 Regarding biological activities, the antioxidant activity was evaluated by the cellular assays of OxHUA and TBARS, using sheep erythrocytes and porcine brain cells as oxidizable substrates, respectively, and by the β-carotene bleaching inhibition assay; the antimicrobial activity was screened against skin-associated pathogenic bacteria and fungi; and the capacity to inhibit the activity of the tyrosinase enzyme was tested using L-DOPA as a substrate. It was found a phenolic profile constituted mainly by the flavonoids apigenin,5,6,8-C-diglucoside, apigenin-2''′-O-pentoxide-C-hexoside, apigenin-6-C-glucoside, and traces of luteolin glucoside derivatives (accounting for 93.4% of the extract), and by the phenolic acid 5-O-caffeoylquinic acid. As far as we know, it is the first time that some of these compounds are described in Aloe vera flower. No anthraquinone glycosides were detected in this part of the plant. The extract revealed an interesting antioxidant activity, being able to protect the erythrocyte membranes and the β-carotene from the free radical generated in the in vitro reaction system. It was also able to inhibit and kill multidrug-resistant bacteria such as Pseudomonas aeruginosa and Escherichia coli and some fungi, including Candida albicans. It also inhibited the tyrosinase enzyme activity, which translates its potential as a skin whitening agent. Based on these results, it was concluded that the Aloe vera flower could be exploited by industrial sectors interested in bio-based ingredients due to its composition in flavonoids and antioxidant, antimicrobial, and tyrosinase inhibition properties.

Figure 1. Aloe vera flower.

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**P14 Determination of MOSH and MOAH by CGXCG-TOFMS**

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Nowadays across the European Union food contamination by mineral oils from production processes and packaging is becoming a serious problem for public health institutions and governments. In this study comprehensive two-dimensional gas chromatography (GCXGC) in combination with time-of-flight mass spectrometry was evaluated, in order to find a robust one run analytical method. LECO GCxGC system takes advantage of a dual-stage, quad-jet thermal modulator positioned between the two columns and a secondary oven allows independent temperature control of the second dimension column, combined with high acquisition rate, full range TOF mass spectra. The combination of two different polarity columns led to effective separations between compound families, identifications within families were easily reached by high acquisition rates TOFMS systems and ChromaTOF software classification capabilities defined chromatogram regions to locate clearly each compound family.
P15 Chemical Profile of Nutraceutical Formulations with Natural Preservatives

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Nutraceutical is a recent term, not having a formal regulatory definition, it was first defined by Stephen DeFelice as “food or part of a food that provides medical or health benefits, including the prevention and/or treatment of a disease”. From early ages, the medicinal properties of plants were well known, and there are now many scientific studies that support such claims. Aloe Arborescens Mill., one of the most abundant species of the Aloe genus, native to South Africa having been imported into many countries as an ornamental and medicinal plant due to its biological effects (antimicrobial, anti-inflammatory and healing properties), has had its benefits proven by scientific studies. Growing concerns about the quality and safety of food and pharmaceutical products leads to consumers preferring products with minimal added additives. Thus, in line with consumer demand, the food industry has been working on uncovering natural preservatives.

The objective of this work was to study the influence of natural preservative ingredients (citric acid and chestnut flowers) on the chemical profile (composition in free sugars, organic acids, fatty acids and phenolic compounds) of a nutraceutical based on Aloe arborescens and honey.

Free sugars were identified by an HPLC-RI system, organic acids by UFLC-PDA, fatty acids by GC-FID and phenolic compounds by HPLC-DAD-ESI/MS. The results were compared with those obtained in the formulations with an artificial preservative (sodium benzoate) and with no preservative.

Regarding the results, in general, there were no significant differences between formulations. In the evaluation of hydrophilic compounds, three sugar molecules (fructose – 13 g/100 g fw; glucose – 12 g/100 g fw; trehalose – 0.3 g/100 g fw) and two organic acids (malic acid – 0.40 g/100 g fw; citric acid - 0.04 g/100 g fw) were found. Regarding the fatty acid profile, seventeen compounds were identified, especially palmitic (C16:0 37%), followed by stearic acid (C18:0 14%); all the other fatty acids were below 10%. Saturated fatty acids (SFA) were the most abundant group with 89%, followed by 7% of monounsaturated fatty acids (MUFA) and 4% polyunsaturated fatty acids (PUFA).

Regarding phenolic compounds, ten molecules were identified and quantified (two phenolic acids, three flavonoids, two aloins and three other anthraquinones), with a highlight for aloenin (flavonoid) as the major compound (0.14 µg/100 g fw).

The results obtained in this study allow us to coclude that the natural preservatives used have no influence on the chemical composition of the nutraceutical studied. However, further research is needed to conclude that these natural preservatives are a good alternative to sodium benzoate or any other synthetic preservative.

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References:
P16 Analytical strategies based on tandem mass spectrometry detection for quantification of bioactive compounds in biological matrices

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Fast and accurate analysis, providing reliable results at trace concentration levels, is a current demand of the modern world. This pressure is justifiable in limit situations but also in our daily life, for instance when waiting for a diagnosis based on lab results in a hospital or when wondering about the quality of water running from our taps. During the last years, tandem mass spectrometry (MS/MS) based techniques have become the method of choice for determination of chemical compounds in complex matrices due to their inherent high sensitivity and selectivity. MS/MS techniques allow the achievement of low limits of detection and therefore prompt for the quantification of trace analyte levels generally present in environmental and biological samples. The majority of applications rely on the coupling to a separative technique prior to MS/MS detection. In this work, relevant applications of the association HPLC-MS/MS for quantification of bioactive compounds in biological matrices will be critically discussed. The steps of sample preparation and analytical determination will be addressed. Moreover, the main analytical features of each developed method, including selectivity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), stability and matrix effects will be highlighted.

First, despite the recognition of tranexamic acid (TXA) as an important antifibrinolytic drug, there is a lack of pharmacokinetic and pharmacodynamic data concerning variable age groups undergoing surgeries with high blood loss. Clinical trials performed so far suggest a wide variability in response to TXA and, therefore, the implementation of a methodology based on UHPLC-MS/MS for monitoring TXA in human plasma samples at sub-microgram per milliliter levels was pursued. In a different context, millions of people worldwide live with human immunodeficiency virus (HIV) infection raising the continuous search for new prevention and treatment strategies, including topical microbicide products combining antiretroviral drugs such as tenofovir (TFV) and efavirenz (EFV). An HPLC-MS/MS method was developed targeting the quantification of antiretrovirals in mice tissue and fluid samples recovered from a pharmacokinetics study with nanoparticles and it was fully validated for the different biological matrices. Finally, BIBP 3226 is a potent and selective neuropeptide Y Y1 receptor antagonist that has been successfully used in in vitro studies showing a positive impact in bone turnover and thus providing good perspectives towards its application as a pharmacological tool for bone regeneration. Having in mind the therapeutic potential of BIBP 3226 and also the need to elucidate receptor-antagonist internalization mechanisms, the challenge was to develop a methodology based on HPLC-MS/MS that permitted to quantify the low quantities of antagonist expected to be internalized by cells.

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References:
P19 Determination of Micro Pollutants in Sediment of the Portuguese Coast

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This study is part of the project AQUIMAR (01-08-218 to 31-07-2021) (project MAR2020 nº MAR-02.01.01-FEAMP-0107) that will evaluate the quality of aquatic systems for aquaculture activity along the Portuguese coast.

Surface sediment samples were collected along five different sites of oceanic areas in 2018 and 2019 (Figure 1), using a Smith McIntyre dredge, aiming at studying the contamination with organic micro pollutants of those areas. The compounds analysed were hexachlorobenzene and the seven polychlorinated biphenyl (ICES7) indicators, congeners (PCB28, PCB52, PCB101, PCB118, PCB138, PCB53, and PCB 180).

Figure 1: Areas of Portuguese coast.

Extraction were performed using pressurized liquid extraction (System ASE) followed by adsorption chromatography clean-up through, alumina and silica gel glass column. These assessments were supported on detailed quality control. Identifications and quantifications were performed by capillary gas chromatography with an electron-capture detector (GC-ECD). Compounds identify was confirmed by elution and quantification in two capillary columns with different polarity.

The main objectives of this study are: (1) to provide a perspective of the profiles from PCBs and HCB in the sites studied recognizing the most abundant pollutants, (2) and to perform an assessment of sediments quality. Two approaches were used to assess sediments quality, namely the comparison of micro pollutants concentrations with two sediments quality guidelines (SQGs) in order to evaluate the potential risks of the contaminants to aquatic life. Two sets of SQGs were considered: threshold effect level (TEL) and probable effect level (PEL)1, low-range effects (ERL) and median-range effects (ERM)2. In addition, compounds concentrations may be compared with Environmental Assessment Criteria (EACs) which set values below which marine species are protected from chronic effects (LOWER EAC) and maximum values for which they are not expected to promote toxic effects (UPPER)3,4.

The uncertainty of the performed environmental monitoring, including the sampling uncertainty, was quantified5. The comparison of the observed contamination with defined limits was based on a decision rule that guarantees a consumer’s risk smaller than 5%6.

References:
P20 Wine aroma and SO₂ influence on white must fermentation

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The last decade Portugal established in the European Union (EU) a key role among the main wine producers. The growth of this industry has contributed to a solid economic position regarding wine-exporting and the visibility of its products. To preserve global competitiveness is necessary to meet the challenges on agriculture, new consumer preferences and EU normative alterations related to processes and addition of substances on winemaking process.1

One substance well accepted as a preservative agent in winemaking process, due to its anti-oxidative and anti-microbial properties is sulfur dioxide (SO₂).2 However, excessive exposure to this compound in some cases can cause cumulative toxicity in humans been reported symptoms of allergic response.3 To respond public health concerned EU has required a warning in the product indicating that sulfites are present if concentrations are higher than 10 mg/L.4,5

Wine aroma depends on many factors, being grape variety, that contributes to the varietal aroma, and winemaking process that contributes to most volatile compounds found in wines, the so-called fermentative aroma, the two most important ones. The volatile compounds formed during pre-fermentative and fermentative steps of winemaking can be modulated through several additives, namely the use of SO₂.6,7

The aim of this work was to evaluate the impact of SO₂ on the volatile organic composition (VOCs) of three varietal white wines, Arinto, Siria and Gouveio. Arinto must was fermented with 0, 15, 30, 45, 50 and 100 mg SO₂/L, Siria must was fermented with 0, 15, 30 and 45 mg SO₂/L and Gouveio must was fermented with 0, 50 and 100 mg SO₂/L. All fermentations were done in duplicates. After fermentation, the volatile organic composition was analysed by HS-SPME-GC/MS. VOCs were tentatively identified by matching mass spectra with spectra of reference compounds in NIST library, also by determination of the linear retention indices (LRI) using a commercial hydrocarbon mixture (C8-C20) and comparing LRs with the literature.

Regarding VOCs composition on each varietal wine were tentatively identified 56 compounds for Arinto wines, 55 compounds for Siria wines and 43 compounds for Gouveio wines. The chemical functional groups observed were esters, ethers, alcohols, aldehydes, ketones and carboxylic acids being esters the most representative in all wines. Observe within the same wine when different doses of SO₂ were applied there is a distinction expression of same compound, for example ethyl 9-decanoate increases with increasing dose applied on Arinto an Siria wines. However, on Gouveio wines ethyl 9-decanoate reduces when increasing dose applied. The PCA analysis indicates that resulting wines showed different VOCs composition, depending on the SO₂ doses applied. Results obtained for the three white wines shows that the recommendation to reduce the addition of SO₂ will impact the aroma of wines. Therefore, these changes should be considered when alternative substances are applied to maintain or improve the quality of the final product produced.

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References:
P21 Valorization of sugars from aqueous fraction resulting from liquefaction of eucalyptus wood

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The depolymerization process of lignocellulosic biomass can be a very useful tool for the utilization of various types of chemicals, such as sugars obtained from wood waste biomass (Isikgor e Becer, 2015). The biomass liquefaction process can be carried out by the method of direct non-catalyzed liquefaction and increasing the temperature (250 to 500 °C) under high pressure, or by acid catalyzed liquefaction with a selected solvent system under atmospheric pressure and moderate temperatures (120 to 180 °C). Although there are some studies on the eucalyptus biomass liquefaction process, the applied methodologies have not yet been sufficient to elucidate the characteristics of the mixtures resulting from the liquefaction, as well as to identify, quantify and characterize the sugars originated during this process.

This work has as main objective to study the valorization of the sugars present in the aqueous fraction, coming from the processes of liquefaction of the eucalyptus biomass, allowing its use in industrial or agro-industrial processes. The methodology used in this work is the use of chromatography techniques, namely high performance liquid chromatography (HPLC). After identification of the major components (sugars), the preparative high performance liquid chromatography technique (HPLC-Prep) will be used as a resource for the isolation of sugars. Therefore, this work is of great relevance since the study of eucalyptus biomass liquefaction will allow to isolate and identify the major components in previously separated fractions. In addition, the valorization of sugars from eucalyptus biomass may serve as a low cost alternative for the pharmaceutical and agro-industry, as well as for the production of compounds with antioxidant potential, which are essential in the production of various products / drugs.

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Phytotherapy is a growing science that deals with the treatment of diseases or as health-promoting agents through plant-based products. The increasing consumption of these products has generated public health concerns since herbs and plants can be contaminated with toxigenic fungi, which can produce mycotoxins, causing many severe health effects in humans, from allergic responses to cancer. Tribullus terrestris is a vine plant frequently used in supplements or medicines worldwide growing in moderate and tropical climates in the United States, Mexico, Eastern Europe, India and China. Both the root and fruit of the plant have been used medicinally in Traditional Chinese Medicine and Indian Ayurveda medicine for a variety of potential effects, including to enhance libido, keep the urinary tract healthy and reduce swelling. This work aims to develop and validate a method, according to Commission Regulation (EC) nº 401 February 23, 2006 to determine the levels of aflatoxins (AFB1, AFB2, AFG1, AFG2), ochratoxin A (OTA) and zearalenone (ZEA) in Tribullus terrestris, using immunoaffinity columns (IAC) for extraction and HPLC-FLD for quantification. The method was adapted from the IAC supplier, VICAM, and the detection limits (LOD) and quantification limits (LOQ) for aflatoxins ranged from 0.191 μg kg⁻¹ (AFG1) to 0.588 μg kg⁻¹ (AFB1), and 0.635 μg kg⁻¹ to 1.902 μg kg⁻¹, respectively. The LOD and LOQ for OTA were 1.404 μg kg⁻¹ and 2.701 μg kg⁻¹, respectively; and for ZEA were 1.288 μg kg⁻¹ and 3.590 μg kg⁻¹, respectively. The average recoveries determined at different spiking levels (10 μg kg⁻¹ for AF and OTA and 50 μg kg⁻¹ for ZEA) were 57% (AFG2), 105% (AFG1), 134% AFB2 and 165% (AB1) for Aflatoxins and 77% (OTA) and 103% (ZEA). Results indicate that the method comply with the provisions of Commission Regulation (EC) nº 401/2006, only for AFG1, OTA and ZEA. The performance of the mycotoxins extraction and quantification from T. terrestris matrixes and other plant drugs will be further discussed.

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References:
Monitoring tranexamic acid in human urine by automatic solid-phase extraction combined with liquid chromatography-mass spectrometry

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Tranexamic acid (TXA) is an important antifibrinolytic agent in the treatment of different haemorrhagic conditions.1,2 However, the information about pharmacokinetics and pharmacodynamics is scarce. Therefore, the development of analytical methods for the quantification of TXA in different types of biological samples is required, because this information will be relevant in the establishment of adequate doses. TXA has been determined in different biological matrices but the quantification in urine samples assumes particular importance because urinary excretion is the main route of elimination.1,2 Sample preparation is a critical step in analytical procedures for the elimination of interfering compounds and also for analyte pre-concentration.2,5 Among the different sample preparation techniques, solid-phase extraction (SPE) is one the most versatile sample-processing methods and the automation of this strategy increases precision by reducing human intervention, sources of error and also analysis time and cost.5,6 Hence, the main goal of the present work was the development of an automated micro-solid-phase extraction (μSPE) methodology using bead injection (BI) in a mesofluidic lab-on-valve (LOV) flow system combined to liquid chromatography and mass spectrometry for the determination of TXA in urine samples. For the μSPE-BI-LOV methodology, three sorbents were tested, namely OASIS-HLB, -MCX and -MAX, and different parameters were evaluated, including eluent and carrier composition, composition of matrix removal solution and sample loading volume. All steps of SPE were defined and implemented by computer programming. The processed samples were analysed using a method based on ultra-high-performance liquid chromatography coupled to triple quadrupole-tandem mass spectrometry (UHPLC-MS/MS).5 Chromatographic separation was achieved using a BEH Amide column (50 × 2.1 mm; 1.7 µm particle size), maintained at 40 °C. The mobile phase consisted of a mixture of acetonitrile-aqueous ammonium bicarbonate (pH 7.4; 10 mM), at a flow rate of 0.1 mL min⁻¹. The MS was operated in positive ionization mode (ESI+) and data was acquired in selected reaction monitoring (SRM) mode (m/z 158.25 > 95.15 for quantification, and m/z 158.25 > 123.20 for identification).

Firstly, studies were performed using TXA standards, in order to establish the optimal conditions for SPE. The results revealed that OASIS-HLB sorbent permitted to achieve higher recovery percentages (ca. 80%) and higher repeatability compared to the other tested sorbents, particularly OASIS-MCX. Consequently, OASIS-HLB was selected for the further experiments. The eluent composition and the sample loading volume were also studied, and the best results were obtained using a mixture of acetonitrile-aqueous ammonium bicarbonate (pH 7.4; 10 mM) (75:25, v/v) and 1000 µL of sample, respectively. Furthermore, the use of 0.1% (v/v) of formic acid as washing solvent for sample preparation permitted to increase analyte recovery from 55% to 80%. The use of aqueous ammonium bicarbonate (pH 7.4; 10 mM) or water as carrier was also tested, and the obtained analyte recoveries were similar. The method is currently under development targeting the application to urine samples recovered during scoliosis surgery and the implementation of a strategy for hyphenation of the automated SPE system with mass spectrometry.

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**P24 Analysis of Environmental Contaminants in Smoked and Cured Codfish Samples**

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Codfish stands out for its content in high biological value proteins, in minerals (iodine, phosphorus, sodium, potassium and iron) and complex B vitamins. For centuries codfish has been the main ingredient in the Traditional Portuguese cuisine (Figure 1). Codfish is worldwide mainly consumed as salt-cured due to the greatly appreciated sensory characteristics promoted by salt.¹

![Figure 1. Fresh and salted codfish.](image)

It is widely recognized that the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) sample preparation is relevant in pesticides extraction from vegetables and fruit samples. Many studies around world use routinely this extraction due to the simplicity and efficiency. It has been proved that QuEChERS is effective for the extraction of other pollutants (pharmaceuticals, mycotoxins, and polycyclic aromatic hydrocarbons, etc.)²⁻⁴ in different matrices (fish, bread, soils, etc.)²⁻⁴. Thus, QuEChERS was the selected extraction methodology. Homogenised fish sample (5 g), acetonitrile (10 mL), and QuEChERS powder were placed into a Falcon tube and shaken during 5 min in a vortex mixer. After centrifugation (5 min at 3500 rpm), 5 mL of the supernatant was evaporated to dryness with a gentle stream of nitrogen². The residue was redissolved and injected in liquid chromatography system²⁻⁵. Results of the different analysed samples were compared and discussed.

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**References:**

Assessment of Contaminants in Salmon Using QuEChERS Methodology and Liquid Chromatography

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Salmon (Figure 1) is an excellent source of high-quality protein, vitamins and minerals (such as selenium, potassium and vitamin B12), but its content in omega-3 fatty acids is the most highlighted quality. This fat is responsible for oily fish’s reputation as a valuable “brain food”.

Salmon represents a valuable part of a healthy diet and can be found as steaks or fillets, fresh, frozen, smoked or canned.

Due to the presence of several contaminants in the environment food safety is, nowadays, one of the most pressing issues for industry, governments and consumers. The analysis of contaminants (metals, pesticides, pharmaceuticals, polycyclic aromatic hydrocarbons, dioxins, polychlorinated biphenyls, etc.) in different environmental compartments are target of studies by the scientific community.

Despite the benefits of salmon consumption, the assessment of some chemical compounds in this fish is utmost important. Therefore, the objective of the present work was the assessment of environmental contaminants in salmon fish using QuEChERS methodology for extraction and liquid chromatography for the analysis.

The extraction procedure optimized by the authors for horse mackerel (Trachurus trachurus), chub mackerel (Scomber japonicus) and sardine (Sardina pilchardus) using the original QuEChERS extraction was applied to salmon and the extracts were analysed using high-performance liquid chromatography. The obtained results of all studied samples were analysed, discussed and compared.

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References:

P27 Optimization of extraction procedure for Brominated Flame Retardants evaluation on Shrimp samples

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Brominated Flame Retardants (BFRs) are mixtures of man-made chemicals applied in high quantity to reduce the flammability of polymers. They are usually used in plastics, textiles and electronic equipment. This group of chemicals consists of tetrabromobisphenol A (TBBPA), polybrominated diphenyl ethers (PBDEs) that comprises 209 congeners, polybrominated biphenyls (PBBs), and hexabromocyclododecane (HBCDD). These compounds are lipophilic and can persist in the environment. The harmful health effects of these chemicals can be related to their persistency, bioaccumulation and biomagnification potential through the food chain. Since they are lipophilic, they have the capacity to accumulate in animal fats including aquatic species. BFRs have already been found in the atmosphere, sewage sludge, sediments, soils, biota, blood and breast milk. BFRs can act as endocrine disruptors and the continuous human exposure to them are associated with several disorders, including diabetes, cancers, neurological effects, thyroid disorders and reproductive disorders. Shrimp are one of the most popular consumed seafood in the world and can be a healthy addition to our diet, they are rich in proteins and omega-3 fatty acids. This shellfish is also a good source of phosphorus, choline, copper, selenium, zinc, iodine, carotenoid astaxanthin, as well as B-complex vitamins (B3, B6 and B12), vitamin A and E. However, they can also accumulate in pollutants such as BFRs. The shrimp specie Palaemon serratus is widely distributed in Portuguese coastal areas and estuaries, is the subject of small-scale fisheries.

In this work, the extraction procedure for BFRs in shrimp samples was optimized. For that, 5 g of the edible portion of the shrimp samples were used, and BFRs residues were extracted using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) approach. Three different QuEChERS were tested: i) AOAC (6 g MgSO4, 1.5 g NaAcetate); ii) EN (4 g MgSO4, 1 g NaCl, 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate; and iii) Original (4 g MgSO4, 1 g NaCl). Two volumes were tested of prepared aliquot: i) 1 mL; and ii) 1.5 mL, sampled from the upper layer into a 2-mL centrifuge vial containing a clean-up sorbent. The clean-up composition was 150 mg MgSO4, 50 mg PSA, 50 mg C18, and to the pigments removal it was tested: i) 2 mg of graphitized carbon black and ii) 1 mg of multiwalled carbon nanotubes. An aliquot of the supernatant was transferred to a vial, and the extract was concentrated just to dryness. The sample residue was reconstituted with hexane and placed into an auto sampler vial for analysis. The extent of the environmental contamination was reached through the quantification of 12 BFRs: 1,2-Dibromo-4-(1,2-dibromoethyl) cyclohexane (TBECH), pentabromotoluene (PBT), pentabromobenzylbenzene (PBBE), 2-ethylhexyl-2,3,4,5 tetrabromoobenzocate (TBB), 1,2-bis(2,4,6-tribromophenoxo) ethane (BTBTE) and polybrominated diphenyl ethers 28, 47, 99, 100, 153, 154 and 183 using gas chromatography coupled with electron-capture detector.

The best results were obtained using QuEChERS AOAC, clean-up containing 2 mg of graphitized carbon black with 1 mL of prepared aliquot. The recoveries obtained were between 72% and 122%.

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References:
P28 Optimization of Synthetic Musks in Human Adipose Tissue by Gas Chromatography Mass Spectrometry

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Synthetic Musks (SM) are present in personal care products such as perfumes, body lotions, shampoos, soaps, deodorants and antiperspirants. SM were described as bioaccumulative and persistent xenobiotics, that could lead to distinct types of dermatitis, carcinogenesis and endocrine disorder. In Europe, the Regulation (EC) no.1223/2009 prohibited the use of musk-amberette, musk-tibetene and musk-moskene and limited the concentrations in cosmetics for musk-ketone (MK), musk-xylene (MX), phantolide and tonalide (AHTN). Dermal absorption is thought to be the major route of human exposure to SM and since these are lipophilic contaminants, adipose tissue (AT) is the preferred matrix providing integrated measure of lipophilic chemicals accumulated over time. However, very few studies have reported SM levels in human AT.1-3 SM are not included in routine monitoring and health impact data is lacking. Nonetheless, the body of literature supports the conclusion that SM bioaccumulate in human body and can even be passed on through breast milk or perinatal exposures.1,4

In this work, the efficacy of hexane extraction with different volumes accomplished with an ultrasonic homogeniser, frozen time and cleanup methods and solvents were evaluated for the detection and quantification of four SM [Galaxolide (HHCB), AHTN, MK and MX] in human AT. The relative sample cleanup was evaluated by measurement of total lipid content and SM quantification was performed by gas chromatography mass spectrometry (GC-MS) with deuterated d2-AHTN as internal standard (IS). For the SM identification, the retention time, the ratio of specific ions and the NIST and Wiley libraries were used. Human AT samples were collected in 2009 from patients undergoing bariatric surgery at the Surgery Department of Hospital de São João, Porto, Portugal (protocol approved by the Ethics Committee of Hospital São João).5 The chosen analytical method was optimized and validated. Recoveries were performed with three replicates with the SM mixture at two concentration levels 0.125 and 0.250 µg/g of AT. Non-spiked samples (blanks) were also analysed and the levels found subtracted from those obtained for spiked samples.

Extraction with 6 mL of hexane in an ultrasonic homogeniser followed by 2 hours at -18°C obtain better SM extraction from human AT. The dispersive SPE with 50 mg PSA, 150 mg MgSO4, 100 mg C18EC and 50 mg Z-Sep clean provide the most effective cleanup, removing the greatest amount of interfering substance including lipids and simultaneously ensuring good analyte separating and recoveries higher than 70%. Recoveries ranged from 89% (AHTN) to 94% (MX) in human AT samples and method detection limits were in the 0.002 µg/g (MK) to 0.009 µg/g (AHTN) range.

A fast, reliable and efficient extraction in a single step method was achieved with hexane extraction and dispersive SPE cleanup for SM assessment in human AT. This methodology can easily will be applied to biomonitoring of HHCB, AHTN, MK and MX in human AT samples. More studies are needed to fill the lacking information regarding SM bioaccumulation properties and the consequently impact on human health.

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References:
P29 Evaluation of QuEChERS for the determination of organic pollutants in Portuguese cherries from conventional and organic farming

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Persistent organic pollutants (POPs) are chemicals of global concern due to their potential for long-range transport, persistence in the environment, they have an ability to bio-accumulate in ecosystems. With time, they accumulate in fatty tissues of a living organism and are found at the highest concentration in every step of the food chain[1]. At the present time, some organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are prohibited by European Union. Despite the prohibition of OCPs, PCBs, and PBDEs, we still find them in the environment and in the food[2].

In this study, a sample preparation method, QuEChERS extraction combined with dispersive solid-phase extraction, was optimized and evaluated for the trace analysis of 23 POPs in Portuguese cherry samples. The analysis was performed using gas chromatography with electron capture detector. After the evaluation of different QuEChERS, the AOAC version was selected because of the best performance in terms of extraction efficiency. The developed method was validated in terms of linearity, recoveries, matrix effects and precision. Globally, the method proved to be effective and versatile for routine analysis of fruit samples.

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Acrylamide is a major food contaminant in products submitted at high temperatures, such as biscuits, bread, or French fries. Acrylamide (or propenamide, C₃H₅NO) is a water-soluble compound, mostly formed by Maillard reactions between reducing sugars and free asparagine. This substance has been classified since 2002 as a possible carcinogen (class 2a)¹ by the International Agency for Research on Cancer. Because Maillard reactions are also responsible for the browning during thermal processing of foods, contributing to important sensorial properties as texture and flavour, monitoring acrylamide balanced with the final properties of the foods is of high importance.

Up to now, determination and quantitation of acrylamide in food products requires sample pre-treatment and several steps of solid-liquid and liquid-liquid extractions, interleaved with cleaning and purification steps, making it a complex and time-consuming process. Consequently, a methodology that allowed the simultaneous extraction and quantification of acrylamide in foods is a major need. Thus, the aim of this study was to develop a fast and simple methodology to quantify acrylamide by directly determine its presence in the headspace of biscuits dispersed in an aqueous based solution (single-step extraction) using headspace-solid phase microextraction (HS-SPME) followed by the analysis gas chromatography coupled with quadrupole mass spectrometry detection (GC-qMS). In order to improve extraction efficiency, the influence of 3 extraction conditions were evaluated: extraction temperature (40, 50 and 60 °C), extraction time (15, 30 and 45 min), and the amount of organic solvent (propanol) in the aqueous solution (10, 20 and 30 mL for a total of 40 mL of solution). The statistical Box-Behnken experimental design, using a response surface methodology (RSM) for the analysis of the p-values, was used to select the best experimental parameters. The best results were obtained when higher solvent amount (30 mL) and extraction time (45 min) were used, while the extraction temperature showed non-statistically (p>0.05) significant effect. For quantification purposes, the standard addition method was performed using the ion extraction chromatography (IEC) mode (m/z 71). Good linearity was obtained with a regression coefficient (R²) higher than 0.96. Furthermore, the developed methodology showed good precision (RSD < 21.5%), with detection (LOD) and quantification limits (LOQ) of 200 µg/kg and 675 µg/kg, respectively, being the EFSA reference value for biscuits, 350 µg/kg, within these limits.

This methodology was further tested in biscuit samples, which are food products with high acrylamide incidence as well as high consumption levels, especially among youngsters. The results obtained by the developed methodology were in accordance with the ones given by an external certified laboratory determined by the conventional method (HPLC). Moreover, as pH modulates acrylamide formation, a mitigation strategy using pectin addition⁴ was also tested. The results showed a mitigation effect with reduction on the acrylamide values detected in the new formulated biscuits below the benchmark level recommended by EFSA for children (150 µg/kg).⁵

In summary, this new HS-SPME/GC-qMS approach allowed the simultaneous detection and quantification of acrylamide directly on biscuits without the need of multi-step analysis usually applied in acrylamide quantification. Furthermore, the optimized methodology was successfully applied to commercial samples, including those were a mitigation strategy was applied, showing its applicability to routine analyses towards acrylamide monitoring in biscuits, as well as other food products.

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P32 Evaluation of the composition in organic acids, vitamin E and phenolic compounds of lovage (Levisticum officinale W.D.J. Koch) roots

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Since ancient times, several aromatic plants and spices have been used worldwide in traditional medicine, in addition to its common usage for food purposes. Lovage (Levisticum officinale W.D.J. Koch) is an aromatic plant from the Apiaceae (Umbelliferae) family used as a condiment in several regions of Europe, being also described to have medicinal properties. In particular, lovage roots are described as possessing carminative and spasmyloptic activity. According to the assessment report of the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency on Levisticum officinale Koch, radix, this root is known as a medicine since ancient times in Greece, nowadays being authorized as a traditional herbal medicine in several countries of the European Union. This report also includes information regarding lovage root chemical composition, referring the presence of different phthalides, coumarines, phenylpropanoids (chlorogenic, caffeic and ferulic acids) and polyacetylenes (falcarindol and falcarniol). Apart from this information, the scientific literature reports mainly the chemical composition of the essential oil of lovage root, with no information being available regarding other bioactive compounds. Therefore, to address this gap, in this work, the organic acids, vitamin E and phenolic compounds of lovage roots were determined. Dried lovage roots were acquired from an herbal shop in Spain. Organic acids were determined by ultra-fast liquid chromatography coupled with a diode-array detector (Shimadzu Corporation, Japan) in the lyophilized sample, which was extracted using a methodology previously described and optimized. Tocopherols were determined in the lyophilized sample using a HPLC system coupled to a fluorescence detector as previously described. Phenolic compounds were analysed in two different extracts, namely hydroethanolic and decoction, after those being re-dissolved in ethanol/water (80:20, v/v) and water, respectively, to a concentration of 5 mg/mL. The compounds were evaluated using a Dionex Ultimate 3000 UPLC equipped with a quaternary pump and a diode array coupled in-series to an electrospray ionization mass spectrometry detector (LC-DAD-ESI/MSn) operating as previously described. The obtained results showed the presence of 3 organic acids in lovage root, namely oxalic (2.23±0.02 g/100 g d.w.), malic (1.48±0.04 g/100 g d.w.) and fumaric (0.007±0.011 g/100 g d.w.) and two tocopherols, α-tocopherol (0.83±0.03 mg/100 g d.w.) and γ -tocopherol (0.48±0.03 g/100 g d.w.). Regarding phenolic compounds’ composition, a total of 9 compounds, including phenolic acids and flavonoids, were identified and quantified, with vanillic acid being the predominant one in both types of extracts. Comparatively to the hydroethanolic extract, the decoction allowed the extraction of a significantly higher amount of total phenolic compounds (24.3±0.5 mg/g extract versus 3.07±0.04 mg/g extract). To our knowledge, this study represents the first report on the organic acids, vitamin E isoforms and phenolic compounds composition in lovage roots.

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References:
P33 Comparison of the volatile profile of the essential oils extracted from the aerial parts and roots of lovage (Levisticum officinale W.D.J. Koch)

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Lovage (Levisticum officinale W.D.J. Koch.) is a perennial aromatic plant from the Apiaceae (Umbelliferae) family cultivated in several European countries. The aerial parts of this plant are used in culinary due to their strong taste like celery combined with parsley with a scent of aniseed and curry. The aroma and flavour of the aerial parts of the plant somehow remember some commercial condiments, therefore being commonly designated in Portugal as “planta do knorr” and in several countries as “Maggi plant”. Although, currently, other aromatic herbs from the same family are much more used than lovage, this species was once much recognized, being considerably used either by the condiment’s industry as well as by households in soups, stews, meat dishes, etc. The root of L. officinale has been known for centuries as a traditional medicine possessing carminative and spasmyloytic activity and is also known to contain essential oils in its composition. It has been described to present also a warm-spicy note, although not as intense as the leaves and seeds.

In this work, the volatile profile of the essential oils extracted from edible aerial parts and roots of lovage were determined. Fresh aerial parts (leaves and stems) were commercially acquired in 2018 at Porto, Portugal, while the roots were obtained dried, being acquired from an herbal shop in Spain. The essential oil was extracted by hydrodistillation in a Clevenger apparatus in accordance with the European Pharmacopoeia. Because of the low yield obtained for the root’s oil, 2 mL of hexane were added to the distilled mixture of water/essential oil. The oil from the aerial parts was recovered directly without adding any solvent. Samples were analysed in a GC-2010 Plus (Shimadzu) gas chromatography system with a AOC-20iPlus automatic injector and a mass spectrometry detector. Separation was achieved on a SH-RX1-5ms column (30 m x 0.25 mm x 0.25 μm; Shimadzu, USA). Compounds identification was based on the NIST17 mass spectral library and in the linear retention index calculated based on the retention times obtained for a reference mixture of n-alkanes. Comparisons were also performed with published data and, when possible, with commercial standards. Quantification was performed as relative percentage of total volatiles using relative peak area values obtained directly from the total ion current (TIC) values.

GC–MS analysis enabled the identification 99.1% of compounds, corresponding to a total of 44 identified compounds in the aerial parts, those belonging mainly to monoterpenes (74.0%) and phthalides (24.3%). α-Terpinyl acetate was found to be the major compound (33.6%), followed by p-cymene (20.5%), (Z)-ligustilide (22.2%), β-phellandrene (4.7%) and myrcene (4.2%). For the root’s a total of 60 compounds were identified, corresponding to a total of 88.4%, with the phthalides group being the major group (61.7%). Different phthalides were present, being (Z)-butylenephthalide (29.0%) the major compound, followed by neocnidilide (8.9%) and (Z)-ligustilide (8.5%). Among the remaining compounds, the sesquiterpene alcohol spathulenol (6.3%) was the one in higher amounts. The obtained results evidence the presence of high amounts of phthalides in the essential oils of both botanical parts of lovage. Phthalides are believed to play a major role in the aroma of lovage. Moreover, different biological properties, including antioxidant activity, antihyperglycemic activity, analgesic and neurological effects, have been ascribed to these compounds, in particular to (Z)-ligustilide, which can support the use of lovage, particularly the roots, use in traditional medicine.

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References:
Identification of possible authenticity markers of PDO “Pera Rocha do Oeste” using HS-SPME/GC×GC-ToFMS

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The food products with Protected Designation of Origin (PDO), legally framed by the European Union, is a guaranty of authenticity, traceability and sustainability. The “Rocha do Oeste” pear due to its sui generis characteristics is a Portuguese PDO product [1], namely its aroma. The aroma compounds with pear and fruity odour description such as esters, alcohols, aldehydes and terpenes [2], are influenced by environmental conditions of orchards and storage of fruits [3]. To identify authenticity markers based on volatile compounds, it is important to select only the contribution of compounds that do not vary according to the storage conditions of the fruits.

In this study, a method based on headspace solid-phase microextraction (HS-SPME) coupled with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-ToFMS) was used to analyse volatile compounds of pears from different orchards of the PDO appellation. “Pera Rocha do Oeste” pears were stored in normal atmosphere and in controlled atmosphere along 1 and 5 months, respectively. Each pear was placed in a 1L closed glass vial overnight at 20 °C. Then the SPME fiber (DVB / CAR / PDMS) was exposed for 1h in a thermostated bath at 25 °C. Sorbed volatiles on the SPME fiber coating were determined using a LECO Pegasus 4D (LECO, St. Joseph, MI, USA) GC×GC-ToFMS [4].

From a total of 130 volatile compounds, 42 were identified with pear or fruity odour descriptor, mainly esters (28), followed by alcohols (9), aldehydes (3), and terpenes (2). From these 42 compounds were 14 selected compounds (1-butanol, 1-hexanol, ethyl acetate, butyl acetate, pentyl acetate, butyl butanoate, ethyl hexanoate, hexyl acetate, heptyl acetate, ethyl octanoate, hexyl hexanoate, ethyl 2,4-decadienoate and α-farnesene). The profile and relative abundance of the selected compounds showed small variation between pears harvested from different orchards in PDO region (8) stored in different atmosphere conditions (normal and controlled). These 14 compounds could be proposed as possible markers to trace authenticity of PDO “Rocha do Oeste” pear. The HS-SPME/GC×GC-ToFMS methodology is a powerful tool to identify and quantify the compounds characteristic of the fruits that remain stable under different storage conditions.

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References:
Aliphatic Hydrocarbons of seaweeds from the North Portuguese Coast

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Seaweeds are recognized for their richness in minerals and vitamins, as well as for the presence of bioactive substances such as polysaccharides, proteins, lipids and polyphenols, which are claimed to have medicine-like effects in treating or preventing certain diseases. In Europe, although the inclusion of seaweeds in the diet has been gaining high importance, its consumption continues low. Seaweeds are able to accumulate heavy metals, aliphatic hydrocarbons (AHs) and pesticides, being sometimes used as pollution indicators in coastal waters due to their limited mobility, abundance in aquatic systems and ability to uptake lipophilic organic compounds from seawater. Hence, their safety should be carefully evaluated before its use as “new food” and introduction into the European market. AHs are considered high priority pollutants, being introduced into the environment through spills or leaks, from industrial releases or as by-products from commercial or domestic uses. Dietary exposure to AHs ranged between approximately 0.03 and 0.3 mg/kg body weight per day in the general population across Europe. A limit of 50 μg/g was imposed to mineral paraffin (saturated hydrocarbons in the range C10-C56 from external sources minus the alkanes C27, C29 and C31) by the European Commission (EC) in sunflower oils, but no limit was established to the concentration of AHs in seaweeds.

In this work, ten species of seaweeds were collected from 4 beaches across the Portuguese north coast on different seasons during 2016: Ascophyllum nodosum, Chondrus crispus, Fucus spiralis, Gracilaria spp., Laminaria ochroleuca, Osmundea pinnatifida, Porphyra spp., Saccorhiza polyschides, Ulva spp., and Undaria pinnatifida. In addition, six species of European seaweeds were purchased in local shops: Chondrus crispus, Fucus vesiculosus, Laminaria spp., Porphyra spp., Ulva spp. and Undaria pinnatifida. The analysis of eight AHs (C18, C19, C20, C22, C24, C28, C32 and C36) was performed by gas chromatography with flame ionization detector (GC-FID), after Soxhlet extraction and purification using silica microcolumns.

The total AHs content ranged between 0.16-6.5 μg/g dw in A. nodosum, 0.05-1.5 μg/g dw in C. crispus, 0.11-2.8 μg/g dw in F. spiralis, 0.13-0.58 μg/g dw in Gracilaria spp., 0.07-0.37 μg/g dw in L. ochroleuca, 0.18-4.0 μg/g dw in O. pinnatifida, 0.30-16 μg/g dw in Porphyra spp., 0.17-3.4 μg/g dw in S. polyschides, 0.12-3.6 μg/g dw in Ulva spp. and 0.15-5.8 μg/g dw in U. pinnatifida. Among each species, some differences were observed according to sampling season. Considering the EC limit for AHs in oil as reference, the seaweeds collected along the Portuguese north coast are all below the threshold, being the highest value (6.5 μg/g dw) obtained for the C18 AHs in A. nodosum. Except for Laminaria spp., Ulva spp. and U. pinnatifida species, the concentrations of total AHs found in seaweeds purchased in local shops were higher than the total concentration of AHs found in wild seaweeds collected in the Portuguese coast. Therefore, seaweeds harvested in the Portuguese north coast are safe for human consumption in terms of the AHs levels.

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References:
The cultivation of *Vitis* (Vitaceae) grape varieties is one of the most important economic activities in Portugal. According to the International Organization of Vine and Wine, in 2017 the Portuguese vitiviniculture area cultivated was 193,672 hectares. Therefore, one of the biggest challenges for wine-producing is to create alternatives for processing the vast amount of grape wastes generated during the harvest season. Vine-canes constitute one of the most abundant vineyard wastes, being estimated that for each hectare of vineyard, 1,75 tons of vine-canes wastes are produced. Traditionally, these vineyard pruning are used as a heating source or left on the ground, however using this raw material as a source of phenolic compounds could increase its economic value.

In this work, an ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) method using BEH C18 analytical column was developed for the separation and quantitation of 32 phenolic compounds. The separation was accomplished using gradient elution with a mobile phase consisting of methanol and 0.1% formic acid. Electrospray ionization (ESI) in both positive and negative ion mode was optimized to reach high sensitivity and selectivity for quantitation using multiple reaction monitoring (MRM) with the selection of proper product ions for each transition. ESI in negative ion mode was found to be more sensitive for quantitative analysis of most of the analyzed phenolic compounds (26), while catechin, epicatechin, tiliroside, rutin and quercetin-3-O-glucopyranoside were analyzed in the positive ionization mode, as [M+H]+ ions. The proposed UHPLC-MS/MS method was fully validated for the separation and quantitation of the 32 phenolic compounds regarding linearity (0.996 < R² < 0.999), limit of detection (0.01 – 7.02 mg/L), limit of quantification (0.033 – 23.4 mg/L) and inter/intra-day precision (RSD < 10%). Work is in progress in order to apply the optimized methodology and quantify the phenolic compounds present in Portuguese vineyard pruning from different grape varieties.

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References:
P38 Molecular fingerprinting of the Brazilian sugarcane spirits to go further on the understanding of their aroma properties

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Cachaça is the typical sugar cane spirit that is exclusively produced in Brazil, and has 38 to 48% alcohol content in volume. Aroma is one of the main factors that contribute for the consumers’ acceptance; nevertheless its volatile composition is still far to be completely known. The volatile composition represents the main contributor for the global and peculiar aroma characteristics of cachaça, which can be modulated by a network of several chemical and biochemical processes that include, for instance the raw materials composition (e.g. sugarcane varieties); the production process, including the fermentation and distillation; and also aging. Therefore, this work intends to perform an in-depth characterization of the cachaça’ volatile composition, using the combination of headspace solid-phase microextraction (HS-SPME) with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-ToFMS). This high throughput and highly sensitive methodology was chosen once the GC×GC-ToFMS orthogonal mechanism (combination GC columns with different stationary phases, connected in series through a cryomodulator), and also the ToF analyzer increases the chromatographic and spectral resolution and sensitivity. These features are important for the simultaneous analysis of major and trace analytes, improvement of the detection limits, separation of chemical components from background, and/or deconvolution of co-eluted peaks. For this work, 12 commercial cachaças from four Brazilian states were selected as case study, which included different: producers, production processes (e.g. direct fire, indirect fire, internal serpentine), and aging (e.g. stainless still or wood aging). Before the samples characterization and in order to increase chromatographic resolution and peak capacity, several chromatographic conditions were tested, namely different set of columns, with and without the same diameters in primary and secondary columns. The SPME conditions were adapted from Zhao et al.: 6 mL of cachaça (adjusted to 10% of ethanol) were placed into a 25 mL glass vial, with 2g of NaCl and an internal standard (3-octanol), in a thermostated bath adjusted to 50 °C for 10 min; then, SPME fiber (50/30 µm DVB/CAR/PDMS) was inserted into the headspace for 30 min.

The results revealed that higher resolution and peak capacity were observed with non-polar/moderately polar set of columns, using the same column diameter in both dimensions. The number of instrumental features per analysis varied between ca. 400 and 1000, depending on the sample under study. A wide set of volatiles were putatively identified distributed over different chemical families, namely alcohols, esters, furans, furanones, ketones, lactones, norisoprenoids, sulfur and terpenic compounds. Chromatographic data was processed through different statistical methods, namely hierarchical analysis that allowed achieving a molecular fingerprinting of the cachaças under study. Unveiling cachaças’ volatile composition allows to relate the components with their aroma notes, and to understand which of them can have an impact on the aroma perception. In fact, despite the alcoholic notes that may be highly perceived in the cachaça, the perception of sweet, fruit, vegetable, and wood notes are considered good aroma characteristics, which can be due to, for example, the presence of esters (fruit notes) and lactones (sweet and wood notes). Moreover, these data may be further useful to relate cachaças’ volatile composition with its intrinsic factors, such as raw materials composition, production steps (namely fermentation and distillation), off-flavors, cachaça aroma, cachaça fingerprinting, among others.

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References:
P39 Phytochemical characterization of *Moringa oleifera* Lam. Extracts

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*Moringa oleifera* Lam. a native tree from Asia, nowadays widely distributed by tropics and subtropics regions, has recently attracted great interest due to its high nutritional, therapeutic and industrial potential. *M. oleifera* is an excellent source of micronutrients, vitamins, minerals and health-promoting phytochemicals. The phenolic compounds present in this plant, such as flavonoids and glucosinolates, are known to have antioxidant, antimicrobial and anticarcinogenic properties,¹ and so has an important effect in prevention of some diseases and to be consumed as food supplement or as infusion tea.

The aim of this study was to compare the phytochemical profile of three *M. oleifera* extracts obtained from the powder of two commercial food supplements (Nosófis and Nutribody brands), and from dried leaves collected in a farm from Angola. The separation and structural characterization of the major compounds present in *M. oleifera* was evaluated by high efficiency liquid chromatography coupled with diode array detector, and sequential mass spectrometry electrospray ionization (HPLC-DAD-ESI(-)/MS³), as shown in Figure1. A semi-quantitative analysis of the main compounds identified on the extracts, indicates that quercetin-3-O-β-glucoside is the most abundant phenolic in the three extracts, whereas higher amounts of glucosinolates were found in the commercial food supplement extracts, when compared to those found in the dried leaves extract. The data obtained in the extracts of the food supplement powders are a mixtures of leaves, roots and seeds, and the *M. oleifera* tissue seeds presented a higher glucosinolates contend.²

It was also investigated the influence of two drying methods, spray drying and freeze drying, on the physical-chemical properties of dried leaves powder, with maltodextrin as carrier. The stability of the phytochemical compounds was evaluated by mass spectrometry-based techniques.

![Figure 1: LC-DAD-MS analysis of an extract of a fine powder of dried leaves of *M. oleifera*.](image)

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**References:**
P40 Analysis of *Cistus ladanifer* extracts by gas chromatography coupled to mass spectrometry and its interest in the cosmetics and pharmaceuticals fields.

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The analysis of plant extracts in terms of their biological activity is a subject that has been exposed to investigations in order to verify their applicability in the cosmetic and pharmaceutical fields. The biological activities of the different extracts depend mainly on the chemical compounds that characterize them, so it is important that phytochemical studies be carried out with the aid of high specificity equipment. Gas chromatography coupled with mass spectrometry (GC-MS) is an important tool for chemical characterization of plant extracts. This study aimed to evaluate the applicability of two extracts of a Portuguese plant, the essential oil of *Cistus ladanifer* (CLEO) and the hydrolate of *Cistus ladanifer* (CLH), in pharmaceutical and cosmetic formulations, through a multidisciplinary approach.

CLEO and CLH were commercially purchased from Aromas do Valado, Castelo Branco, and were analysed by GC-MS. The CLH with a concentration of 1 mg/mL was injected (0.5 µL) using a 1:10 split and the CLEO sample was injected (1 µL) using a 1:350 split. The antimicrobial activity of CLEO and CLH was determined upon a relevant group of skin pathogens (*Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 178970, Enterococcus hirae ATCC 179954, *Streptococcus agalactiae* ATCC 181324, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404), by a microdilution method published by CLSI [1,2,3]. The minimum inhibitory concentration (MIC) corresponded to the minimum concentration of the extract that completely inhibited the microorganism. The minimum lethal concentration (MLC) was determined by plating in agar medium 10 µL of all the wells with absence of turbidity, corresponding to the minimum concentration of the extract that prevented the formation of colony forming units. The antioxidant effect was evaluated by determining the CLEO and CLH capacity to reduce the compound 2,2-diphenyl-1-picrylhydrazyl (DPPH) [4]. The extracts were serially diluted (100-1 µL/mL) in methanol and incubated at room temperature in the dark with 50 mM DPPH. Ascorbic acid was used as the validation control. EC50 was calculated as the amount of extract able to reduce at least 50% of DPPH initial concentration.

The major compounds of CLEO were alpha-pinene (35,7%), 2,2,6-Trimethylcyclohexanone (6,7%), camphene (6,6%) and bornyl acetate (4,9%), and for CLH were 4-Hydroxy-3-methoxybenzaldehyde (21,5%), (-)-Mirtenol (11,1%), p-Cimen-8-ol (10,6%) and D-Verbenona (9,8%), relative to the percentage of total compounds. Regarding the antimicrobial activity of CLH, at 500 µL/mL (CMC = 500 µL/mL) it was only able to completely inhibit the growth of *S. agalactiae* and *C. albicans*, without being lethal (CMC > 500 µL/mL). Regarding the antimicrobial activity of CLEO, it was able to completely inhibit the growth of all microorganisms in test showing MIC values for a concentration range of 2-32 µL/mL. Regarding the antioxidant activity, 50% of DPPH reduction was achieved in the presence of a CLEO concentration of 12.5 µL/mL (EC50). The CLH was not able to reduce DPPH at study concentrations and was not shown to have relevant antioxidant activity under the conditions in which the study was performed. Biological activity of CLEO showed that this extract presents higher antimicrobial and antioxidant activities comparred to CLH. Thus depending on the purpose of the cosmetic and pharmaceutical product, the essential oil emerges as an ingredient with a more active preservative and antioxidant power than hydrolate. The major compounds appear as the main difference in bioactivity shown by the extracts.

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**References:**

P41 Fate of emerging organic contaminants in soil after irrigation with reclaimed wastewater: decay and by-products identification

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As world demands for water grow, effluent reuse – reclaimed wastewater (RWW) - becomes increasingly important as an indispensable component of the integral water resource management being regarded as a sustainable approach in agricultural irrigation [1]. Effluent reuse in agriculture also contributes to nutrients recycling, as phosphorus, alleviating pressure on over-exploited resources (e.g. phosphate rock, included in the EU list of 27 Critical Raw Materials). However, it has been reported that emerging organic contaminants (EOCs) are not completely removed from effluent in wastewater treatment plants (WWTPs). Nowadays the dissemination of these contaminants in agricultural soils irrigated with RWW has been made apparent raising concerns for environmental quality and human health [2].

In this work a clay soil was irrigated with either deionised water or RWW, both of which were spiked or not with a mix of nine EOCs. At each condition under study, the soil was extracted using a procedure based on the quick, easy, cheap, effective, rugged and safe (QuEChERS) principle in which a salting-out extraction with a solvent (acetonitrile) followed by a dispersive solid phase extraction (d-SPE) are performed. The soil extracts were then analysed by high-performance liquid chromatography (HPLC) with diode array (DAD) and fluorescence (FLD) detectors for total decay. Selected extracts were then dried in a N₂ flow, resuspended in Acetone and analysed by gas chromatography with time-of-flight mass detector (GC-TOFMS) for the analysis and identification of potential degradation products.

These experiments disclosed the contribution of the indigenous soil microorganisms to the remediation process. Decays ranged between 30 to 100% in 14 days yet their degradation kinetics were, in general, altered when RWW was used for the irrigation, possibly due to the load of nutrients and/or EOCs (under further analysis). So far, 15 potential/known degradation products were detected and identified. Our final aim is to unveil potential links between the decay of each EOC and their degradation products with the soil microbiota composition and functioning for the removal of toxicants from the soil.

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References:
P42 MONITORING ANTIBIOTICS IN SURFACE AND WASTEWATER SAMPLES IN A RIVER BASIN

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Antibiotics are bioactive compounds, with both natural and semi-synthetic nature, that belong to the antimicrobial group [1]. In the European Union their use is restricted to treatment of diseases, in humans and animals, however in other parts of the globe these compounds are allow to be used as growth promoters and to increase feed efficiency in livestock [1].

The occurrence of pharmaceuticals in the environment is a growing concern. Pharmaceutical ingredients, including their metabolites and conjugates, are mainly excreted in urine or feces [2]. Wastewaters generated by hospital, industrial and domestic sources are regarded as one of the most important source of pharmaceuticals to the aquatic environment [3].

The overarching goal of this study was the evaluation of the presence of 29 antibiotics in WWTP influent and effluent samples, and surface water samples in a River Basin. The samples were extracted by SPE and analyzed by UHPLC-MS/MS[3], with ESI mode.

In the influent samples the maximum concentration value was obtained for azithromycin (3600 ng/L) and ofloxacin (2219.5 ng/L). The most frequently detected antibiotics belonged to the sulfonamides family (sulfamethoxazole and sulfapyridine), followed by azithromycin and ciprofloxacin.

In the effluents samples azithromycin and ofloxacin were the most frequently detected. Azithromycin was the antibiotic present at highest concentrations (7905.9 ng/L) followed by ofloxacin (1784.8 ng/L) and ciprofloxacin (1267.8 ng/L).

For the river water samples clarithromycin was the antibiotic most frequently detected and azithromycin and sulfamethoxazole the ones determined at highest concentrations (maximum detected values of 55.81 and 22.05 ng/L, respectively). The Risk Quotient values for the detected antibiotics in river water samples were below 1, which indicates that these concentrations do not pose a risk for aquatic organisms.

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P43 Optimization of a SPE-GC-MS method for the determination of 25 pesticides in surface water

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Humans have always used pesticides to protect their crops. However, these compounds volatilize into the air, runoff and leach into surface water and groundwater, ending up in all main ecosystems. The main problems of pesticides contamination are their high toxicity, bioaccumulation and slow degradation [1-2]. In this work, 25 pesticides were evaluated in surface water samples, specifically: aldrin, o,p-DDT, p,p-DDD, o,p-DDE, HCB, dieldrin, HCH isomers, endosulfan, endrin, chlorothalonil, methoxychlor, α-endosulfan, β-endosulfan, cypermethrin, λ-cyhalothrin, deltamethrin, fenvalerate, fenpropatrin, vinclozolin, chlorpyrifos, chlorpyrifos-mehtyl, pendimethalin and cyprodinil. Solid-phase extraction (SPE) method coupled to gas chromatography–mass spectrometry (GC–MS) was optimized namely; sample volume; different solvents; time of drying and pH. The optimized parameters provide the best performance showed recoveries between 83% and 125%. at three fortification levels spiked in the water sample. Calibration curves in matrix for the studied pesticides were linear in the range 0.03-1.00 µg/L (coefficient of determination higher than 0.98). The majority of the pesticides were identified by the retention time, and the ratio of three ions, the NIST and Wiley pesticide libraries with MS/MS optimized parameters [3]. An efficient SPE-GC-MS method was obtained from the detection of 25 pesticides in surface water samples.

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Funding: This work was financially supported by REWATER project (Reference FCT: WaterJPI/007/2016).

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(1) EPA, 2019, http://www.epa.gov/pesticides/about/index.html#what_pesticide
Chromatographic separation of phenolic compounds from monovarietal extra virgin olive oils

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Phenolic compounds present in extra virgin olive oils (EVOO) are well known for their health benefits, being the focus of some attention within past few years. After the approval by the European Food Safety Authority of a health claim, which associates virgin olive oil polyphenols consumption to the protection of blood lipids from oxidative stress, a growing interest on the EVOO phenolic profile has been observed, and consequently an increased demand has emerged for better and more precise analytical techniques for the identification and quantification of specific EVOO phenolic compounds. Our research team has been studying new methods for a more rigorous and precise identification, and quantification, of EVOO phenolic compounds. A new method was developed using a traditional HPLC-UV equipment, but with a new biphenyl core-shell stationary phase column, instead of the typical C18 columns. For this, a comparative study where hydrophilic phenolic extracts were produced form 'Galga vulgar' EVOO was performed. These extracts were then characterized using three different HPLC stationary phase columns, namely a Kinetex biphenyl core-shell (A), a UChrompher C18 column (B), and a Spherisorb ODS2 C18 (C). From the tested columns, the biphenyl column (A) proved to be the most suitable for the HPLC separation, identification and quantification of specific hydrophilic phenolic compounds, such as dihydroxyphenyl glycol, tyrosol, hydroxytyrosol and oleuropein aglycone isomers (Figure 1). Compared to others, biphenyl column presented better selectivity for target analytes, shorter retention, higher peak capacity and better peak symmetry, leading to an overall higher resolution capacity, therefore more appropriate to be applied in the quantification of hydrophilic phenolic compounds. The method development also proved to be within the demanded requirements for new methods, showing to be simple, rapid, accurate and robust, which can be easily applied by any laboratory for the quality control of EVOO, with particular interest upon phenolic profiles. Biphenyl column also showed to provide a lower limit of detection (LOD), which is very important for the detection and quantification of minor compounds present in these extracts. For further method validation within different EVOO cultivars, hydrophilic phenolic extracts from seven monovarietal EVOO were produced and analysed, namely 'Galga vulgar', 'Cobrança', 'Verdeal Alentejana', 'Cordel de Serpa', 'Azeteira', 'Biancane' and 'Carrequinha da Elvas'. Results also show a good reproducibility of the method for different EVOO cultivars when applying the biphenyl column.

Figure 1. High performance liquid chromatography (HPLC)-UV chromatograms of the EVOO hydrophilic phenolic obtained at 280 nm with (A) Kinetex biphenyl column, (B) UChrompher RP18 column, and (C) Spherisorb ODS2 column.

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In the current days there is a huge concern related to frequent oil spills resulting from intentional discharges (e.g. tank wash, ballast release, etc.) or vessels accidents (e.g. collision, grounding, etc.). Among the various problems associated with these incidents, stand out the socioeconomic impacts as a result of the cleaning operations and conditioning access to the affected areas, and the environmental impacts since oils are harmful substances and, being transported in large quantities, can lead to huge spills. For these reasons, science and law work together making possible to identify the spill source, especially where the origin is unknown and/or are multiple suspects as a source of the pollution incident. However, court proceedings are opened for attribution of responsibility but require reliable evidences.

Oil spill identification involves the characterization and comparison of samples collected in spills and suspected sources. These procedures assume that crude oils have singular compositional characteristics obtained during their formation, commonly referred as oil fingerprint, enabling differentiation among crudes and refined products. Identification quality benefits significantly from oil spill samples analysis by gas chromatography hyphenated with mass spectrometry (GC-MS). This analytical method obtain a wide range of chemical data, with great information about hydrocarbon mixtures, subsequently processed using statistical treatment tools, which can lead to a high level of reasoning and reliability when chemical composition of spill and source samples are compared. For spill and source samples are determined diagnostic ratios using chromatographic signals of chemical components which are later submitted to correlation analysis employing the Student’s $t$ test. Nevertheless, this statistical tool assumes that results have a normal distribution and sometimes deviations to normality are observed.

This work describes a computational tool for setting statistically sound criteria for abundance ratios of characteristic oil spill signals by the Monte Carlo Method simulation of these correlated signals. The modelling is based on previous records of signals precision and correlation from sample analysis. This work allows quantifying the uncertainty associated with the chromatographic signal equivalence between an oil spill and suspected oil source as a likelihood ratio. A likelihood ratio larger than $10^6$ indicates and extremely strong chromatographic equivalent between signals.

References:
Soil acidity is one of the most serious edaphic problems affecting agricultural systems worldwide, since it leads to increased bioavailability of H⁺, aluminum, iron and manganese, causing toxicity in plants and a decrease in crop yields. In Alentejo region, in the south-east of Portugal, soils occupied by the main agro-forestry-pastoral system - the Montado, are generally acidic and under weather conditions that promote increased Mn bioavailability.

Arbuscular mycorrhiza fungi (AMF) can protect plants against Mn toxicity. This protective capacity is greatly enhanced if colonization is initiated by an intact extra-radical mycelium (ERM), previously developed in the soil from native AMF in association with a mycotrophic tolerant plant (developer). AMF can differentially affect Mn detoxifying mechanisms resulting in changes in the absorption of other ions, the production of antioxidant enzymes, plant hormone balance or gene expression under metal stress. The present work focused on the optimization of the extraction and analysis of plant hormones through liquid (LC) and gas (GC) chromatography coupled to mass spectrometry (MS) on wheat shoots, grown for 3 weeks, in disturbed or undisturbed soil, with previously grown ERM associated to the developer *Ornithopus compressus* (ORN).

Manganese toxicity was ascertained by elemental quantification through shoot acid digestion followed by quantification by inductively coupled plasma mass spectrometry (ICP-MS). Five adapted extraction methodologies were tested to determine the most effective extraction procedure of phytohormones from plant samples. The most successful extraction technique was used to optimize the analytical conditions to quantify abscisic (ABA), jasmonic (JA) and salicylic (SA) through LC-MS and GC-MS, in wheat shoot samples, grown in disturbed or undisturbed soil, with previously grown ERM associated to the developer *Ornithopus compressus* (ORN). Wheat shoots showed about 30% greater biomass, measured in shoot dry weight, when grown in undisturbed soil. In shoots from this treatment, manganese concentration was almost 60% less than in shoots of wheat grown in disturbed soil.

Extraction with 2-propanol/H₂O/HCl (2:1:0.002, v/v/v) resulted in higher recovery percentages. Overall, LC-MS technique allowed obtaining lower limits of detection and quantification (LOD and LOQ) values, so it was chosen to identify ABA, JA and SA on shoot samples of wheat. Wheat grown in undisturbed soil revealed the presence of JA while wheat grown in disturbed soil showed the presence of SA. The presence of an intact AMF induced higher growth, decreased Mn uptake, decreased SA (a stress-related phytohormone) and increased JA (known to trigger the production of defensive phenolics and phytoalexins). The use of native AMF can contribute to the improvement of an environmentally friendlier agricultural productivity.

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**References:**
P47 Method optimization for profiling fatty acids content in *Micropterus salmoides* using gas chromatography – mass spectrometry

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The importance of inland recreational fisheries increased significantly in the past 20 years in Portugal, contributing for the development of many rural areas located near the main fishing sites1. The largemouth-bass is one of the most interesting species for sports fishing in the world. In Portugal, especially in the innermost regions of the country, is considered a delicacy, and in some places, it is described as a regional cultural landmark. However, nutritional characterization of this species is scarce, so it is important to characterize its nutritional profile to assess if largemouth-bass meat could be considered a healthy practice in the diet of consumers2.

GC/MS is one of the most used techniques to quantify or qualify fatty acids (FA) profiles. Nevertheless, the separation between FA geometrical isomers is in most cases very difficult because they have similar polarity and boiling point, occurring in several cases coelution between isomers. We studied a method using different conditions (initial temperature, increase rating of temperature, flow, run time, injection mode – split vs. splitless) to achieve separation between geometrical isomers throughout the FA chromatographic profile (Figure 1).

The final method was applied to the separation, identification and semi-quantitative determination of individual fatty acid contents3.

Figure 1: TIC’s of optimization process.

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**References:**
P48 Sugar profile of honey by ion chromatography

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Honey is a sweet food product mainly composed of carbohydrates (60-80 %), lower amounts of water and a great number of minor components. Fructose and glucose are the main sugars in honey. Furthermore, it is possible to find more than 20 different oligosaccharides.

The aim of this work is to evaluate the possibility of distinguishing three types of honey by their sugar profiles. The sugar content and profile (trehalose, arabinose, glucose, fructose, sucrose, maltulose, melezitose, maltose, turanose and erlose) were evaluated of three groups of honey samples commercially classified as Castanea sativa honey (10 samples), honeydew honey (6 samples) and Rubus honey (3 samples) from Spain.

Pollen analysis was carried out using the method recommended by the International Commission of Bee Botany (ICBB). Qualitative analysis was conducted by examining each of the preparations under the optical microscope (Nikon Eclipse 80 i) at 400 and 1000 magnification. An average of 650 pollen grains in each honey sample were identified using various keys and literature and the pollen data base of Department of Biodiversity and Environmental Management of the University of León.

The honey’s sugar content was analysed in Dionex™ IC53000™ ion chromatograph. Separation was performed in a column “CarboPacTM PA20 3×150mm” with a precolumn “CarboPacTM PA20 3×30mm”. Electrochemical detector in Integrated Pulsed Amperometric Detection (IPAD) mode was used. The elution was performed with a gradient with two NaOH solutions (15 and 200 mM). Standard solutions were used to identify and quantify the individual sugar components. All analyses were carried out in duplicate.

Concerning the principal component analysis made with different sugar content and the percentage of the pollen (higher than 10%) the Rubus honey samples are very well separated from the other samples and are characterized by higher content in maltose and the absence of melezitose. Comparing the Castanea sativa monofloral honey and honeydew honey, the more relevant differences are observed in the contents of melezitose, maltulose and trehalose. Overall the melezitose is almost null for Castanea sativa monofloral honey, whereas for honeydew honey maltulose is 32% lower and trehalose is 21% higher than in the floral honey.

Keywords: Castanea, honey, pollen analysis, Rubus, sugar.

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Matrix pH Effects on Bar Adsorptive Microextraction Efficiency

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Over the last few years, the high complexity of real matrices has been encouraging the optimization of analytical microextraction techniques to enhance analysis at the trace level. Bar adsorptive microextraction (BAμE, figure 1), a novel alternative static-based microextraction technique, has been proposed for trace analysis of polar to nonpolar analytes in aqueous media. This analytical approach allows the selection of the most convenient sorbent coating (e.g. activated carbons (ACs), polymers (Pds), etc.) according to the target compounds involved, using the floating sampling technology process, which has already shown high effectiveness in many applications. In order to maximise the microextraction efficiency, several parameters are usually optimised, including sorbent selectivity, equilibrium time, agitation speed and matrix properties, such as pH, polarity and ionic strength. Nevertheless, the matrix pH can have a significant impact on the recovery yields, once it influences the overall charge of the target analytes of the matrix, as well as of the surface charge of the sorbent phases involved. In this sense, the present work aims the study of the effect of matrix pH and its relation with pK values and log D values on the recovery efficiency of particular organic compounds, using different ACs, to better understand the mechanisms behind those interactions. Thus, model organic compounds belonging to different chemical classes and having different pK values were selected, namely ibuprofen, clofibric acid, 3-hydroxybenzophenone, 2,2′,4,4′-tetrahydroxybenzophenone, estrone and prazepam, using high-performance liquid chromatography-diode array detection as the monitoring system. The results obtained suggest that the interactions between the analytes and the sorbent phases, such as ACs, is a very complex process that affects the BAμE efficiency, govern through the type of the molecules involved, the sorbents characteristics, as well as the matrix pH.

Figure 1: BAμE enrichment process through the floating sampling technology.

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P50 Screening of ‘Spice’ herbal mixtures using GC-MS and NMR as complementary techniques

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In Portugal, the phenomenon of new psychoactive substances (NPS) appeared in 2007 with the opening of the first sales point in Aveiro and since then, it has been observed a growing utilization by users and an increase in the number of substances introduced into drug market. Among NPS, synthetic cannabinoids (SCs) represent the largest, most diversified, and fastest growing group with a total of 179 substances notified from 2008 to 2017 and more than 250 have been reported worldwide to the United Nations Office on Drugs and Crime. Sprayed on natural herb mixtures with the aim to mimic the euphoria effect of cannabis and sold as “herbal smoking blends” or “herbal incense” under brand names like ‘Spice’ or ‘K2’, SCs are available from websites for the combination with herbal materials or more recently, for the use in e-cigarettes. Little is known about how these substances work and their toxic effects in humans and the same product could vary not only in the amount and in the type of substance added. More potent than natural cannabis, SCs have been associated with deaths and acute intoxications in Europe and given the worldwide spread of these herbal mixtures, it poses a steep challenge for forensics and clinical toxicology laboratories with appropriate analytical methods for their detection and identification. In this sense, the present work aims the identification and characterization of herbal incenses suspected to contain SCs by gas chromatography coupled to mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR). Nine seized products namely, “Caramba”, “Maá”, “Mandala”, “Maya 2012”, “Esfinge”, “Atomic Bomb (Strawberry)”, “Atomic Bomb (Blueberry)”, “Radioactive (Strawberry)” and “Radioactive (Blueberry)” were provided by the Laboratory of Scientific Police of the Judiciary Police of Portugal. Each herbal incense was, primarily, extracted in methanol and filtered before analysis by GC-MS operated in electron-impact (EI) ionization mode. All mass spectra were compared with NIST 14 MS library and SWGDRUG MS library version 3.4. The characterisation and structural elucidation were realized by one- and two-dimensional NMR (1H NMR, 13C NMR, 19F NMR, COSY and HSQC). The analysis of all samples of herbal incenses has allowed the initial identification and characterization of 7 SCs (JWH-018, JWH-073, JWH-122, JWH-210, AKB48, XLR-11, MAM2201) by GC-MS and NMR. Also, it was possible to identified tocopherol (vitamin E) and oleamide, two adulterants frequently added to herbal products in order to mask the active ingredients or added as preservatives. The methodology applied proved to be useful, allowing the preliminary identification of the different SCs in the mixture. Furthermore, the examination of mass spectral product ions, as well as the study of both one- and two-dimensional NMR experiments enabled the characterization of the molecular structure of SCs and may also assist the structure elucidation of these NPS. This work was done within the scope of the protocol established between UMa and LPC/PJ with financial support by Fundação para a Ciência e Tecnologia (FCT) through two doctoral projects (SFRH/BD/116895/2016 and SFRH/BD/117426/2016).

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P51 Evaluation of bioactive potential and volatologic pattern of foods from vegetable origin

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In recent years, there has been growing interest in studying the benefits of food on human health. The presence of certain bioactive compounds (BACs) in certain foods seems to contribute to their functional properties allowing an increased prevention in the development of certain diseases. Fruits and vegetables are natural sources of fibers, vitamins, minerals and a large number of phytochemicals responsible for their bioactive and organoleptic properties (1). Recently, numerous scientific studies have shown that regular consumption of these foods is directly related to decreased risks associated with chronic diseases such as coronary heart disease (2,3), some cancers (4) and neurological diseases (5). Most of these beneficial effects are associated with the antioxidant capacity of some phytochemicals that interacts with free radicals to predict cellular oxidative damage (6). Among the wide range of chemical compounds in fruits and vegetables, polyphenols, carotenoids, organosulfur compounds and terpenes are the predominant classes that exhibit antioxidant activity (7). The antioxidant power of these compounds in plant foods depends essentially on their chemical structure and concentration. In this work, the bioactive composition and antioxidant activity of 11 vegetables - beetroot, red and yellow onion, orange and white carrot, watercress, broccoli, spinach, garlic, tomato and tamarillo, were analysed. Through the methanolic extracts obtained from these samples, the antioxidant capacity was determined by DPPH, ABTS and FRAP methods, and the higher capacity was observed in red onion, tamarillo and beetroot. Using the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction methodology combined with ultra-high performance liquid chromatographic with photodiode array detector (UHPLC-PDA), it was possible to identify and quantify some polyphenols. Catechin, gentisic and ferulic acids were found the most abundant polyphenols. Additionally, the volatile composition of these samples was studied using headspace solid phase microextraction (HS-SPME) followed by gas chromatography coupled to mass spectrometry (GC-MS). 320 volatile compounds were identified and grouped into different chemical families. Based on the results, terpenic compounds were found the most predominant group in beetroot (61%), orange carrot (58%) and white carrot (61%), while organosulfur compounds are dominant in onion, garlic and watercress. Regarding to broccoli and spinach, these vegetables are, essentially, constituted by alcohols and aldehydes, while fruits of Solanaceae family are characterized by esters, in tamarillo, and aldehydes in tomato. The results highlight the importance of fruits and vegetables as a promising source of functional ingredients and increase their potential use in the food and nutraceutical industries.

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P52 The chemical profile of *Lobophora variegata* chloroform extract by GC-MS

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Although macroalgae have promising applications within the food, cosmetic and health industries,¹ there are no commercial applications for *Lobophora variegata*, a small brown seaweed of large geographic distribution that occurs along the Macaronesia coast, and in great quantities on Gran Canária island. The only way to evaluate and propose some application to this biomass imply its chemical and biological characterization. The first results showed the cytotoxic and antiaging activities of some extracts of beach-cast *Lobophora variegata* biomass.² The present study analyses the chloroform extract of the *Lobophora variegata* by GC-MS, to establish its chemical profile and identify new or known bioactive compounds, in order to evaluate the potential of this seaweed as source of value added phycometabolites.

The gas chromatography coupled to mass spectrometry allowed the identification of several compounds present in this *Lobophora variegata* chloroform extract which chromatogram is present in the Figure 1. Several fatty acids such as lauric, myristic, hydroxymyristic, palmitic, myristoleic, linoleic and oleic acids, gamma- and delta-tocopherol and a 1,2-benzenedicarboxylic acid ester derivative were identified easily, based on the comparison with the mass spectra of data base Wiley and NIST. However, various extract constituents like cyclic ketones, fatty lactones, sterols and phytol derivatives were identified based on the deep study of fragmentations pattern. More detailed information about extract GC-MS analysis and compounds identification will be presented and discussed.

![Figure 1: GC-MS chromatogram from chloroform extract of *Lobophora variegata*.](image)

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References:
P53 Development of a SPME/GC-MS method based on Quality by Design approach, for evaluation of fish deterioration progress through TMA and DMA quantification.

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Volatile amines - trimethylamine (TMA) and dimethylamine (DMA), could be used as an important freshness index for fish and other seafood-related food products. The presence of DMA and TMA in fish and seafood products is related to decomposition of trimethylamine oxide (TMAO). TMA is associated with the classic ‘fishy’ odor of spoiled seafood. Its formation occurs through bacterial reduction of TMAO, which is naturally present in the living tissue of many marine fish species. DMA is formed during frozen storage from the enzymatic breakdown of TMAO (trimethylamine oxide aldolase). Its production increased by improper storage and/or handling conditions [1-2]. The present study describes the development and validation, under quality by design approach [3], of a solid-phase micro-extraction technique in headspace mode (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) methodology, for quantification of partial volatile basic nitrogen (PVB-N), expressed in TMA, and DMA levels, in fish products. Two fish species, Spaurus aurata and Seriola dumerili, from aquaculture production, in Madeira Island, Portugal, were studied to quantify TMA, and DMA levels during degradation progress, under ice storage at 0ºC. This optimization approach, enabled the selection of the best parameters for extraction, separation, and quantification, of targeted amines. The best extraction conditions were achieved with DVB/CAR/PDMS fiber, exposed into the vial headspace for 30 min, containing 0.5 mL of sample, 1 mL of a 15 M NaOH solution, and 1 mL of saturated NaCl solution (35%) in a 35ºC water bath. The optimal point into the GC-MS equipped with Agilent BP-20 column, was initial temperature 80ºC (2 min hold), ramped to 220ºC (50ºC min⁻¹), and kept for 5 min. Carrier gas flow (helium) was set at 1.0 mL min⁻¹ under splitless mode. The concentration-response relationship for TMA and DMA, were described by polynomial function models, being confirmed by Fisher variance (F-test). The% recoveries were in a range of 90-99%. Good method precision was observed, yielding relative standard deviations (RSDs) less than 9.6% for repeatability and 9.7% for intermediate precision. The limits of quantitation for the analytes ranged from 1.9 μg.mL⁻¹ to 2.8 μg.mL⁻¹. The developed method revealed an effective and potential analytical tool for TMA and DMA quantification in order to monitor the deterioration progress in fish species.

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Fermentation can occur in any sugar pulp from fruit, berries or honey, where specific yeast convert the sugar into alcohol. The spirit can be produced from the distillation of the obtained musts. The different raw material used in the spirit production give them specific characteristics and flavours.

The aim of this study was to evaluate the quality of the volatile composition of different samples of fruit spirits purchased from the market. It was analysed 13 samples labelled as produced from different fruits: orange (1); Ficus carica (1); Opuntia ficus indica (2); strawberry tree fruit (2); carob (1); cherry (2); plums (1); pear (2); raspberry (2).

Methanol, acetaldehyde, ethyl acetate and fusel alcohols concentration was measured by gas chromatography with flame ionization detection GC-FID using internal standard (1 ml of 4-methyl-2-pentanol with 9 ml of sample) according to the method described and validated by Luis et al.1, and the results are expressed as g/hL of P.A.

The volatile compounds identification was done in a GC–MS (Magnum, Finnigan Mat, San Jose, CA, USA), equipped with a fused silica capillary column of polyethylene glycol (INNOWAX of J & W Scientific, Folsom, CA, USA), 30 m, 0.25 mm i.d., 0.25 μm film thickness. Identification of volatile compounds was achieved by comparison of the GC retention times and mass spectra with those of the pure standard compounds. All mass spectra were also compared with those of the data system library (NIST).

The values of methanol content are according to the limits of the regulation (EC) no. 110/2008. The higher values are observed for the spirit produced with strawberry tree fruit (994 and 857 g/hL P.A.) and plums (980 g/hL P.A.), and the low concentration of methanol content observed for orange and carob spirit. Acetaldehyde and ethyl acetate, when in high concentration are usually associated with off-flavour’s. The spirit produced with carob has the higher concentration of acetaldehyde and ethyl acetate with values of 83.09 g/hL P.A. and 504.10 g/hL P.A., respectively.

Higher alcohols pay an important role in the sensory qualities of the spirit and the values of the different fruit spirits ranging from 1.38 g/hL P.A and 631 g/hL P.A. This variation seems indicate a very different quality and characteristics of the fruits as well as different technological steps namely fermentation and distillation process. Concerning the Principal component analyses performed with the results of major volatile compounds profile, it was possible discriminate some beverages for their kind of fruit used in the must preparation as well as to identify some spirits with low quality.

Keywords: fruits, distillate, volatile composition.

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P55 Os desafios da quantificação motivada pelos efeitos de matriz observados na análise de Pesticidas em amostras de água por SPE-GC/MS, SPE-LC-MS/MS e Injeção direta em UPLC-MS/MS

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A análise de pesticidas em amostras de água é um problema de enorme preocupação para os Laboratórios de Análises devido ao nível de toxicidade desses compostos e ao seu risco para a saúde pública. A Diretiva 98/83/CE do Conselho, de 3 de novembro de 1998, relativa à qualidade da água destinada ao consumo humano, estabelece um valor paramétrico de 0,10 µg/L para cada pesticida individual e 0,50 µg/L para a soma de todos os pesticidas monitorizados.

Para avaliar o impacto destes compostos no sistema de abastecimento a Direção de Laboratórios e Controlo da Qualidade da Água da EPAL tem implementados diversos métodos de ensaio para a monitorização de 52 pesticidas e metabolitos. Os métodos de ensaio implementados incluem preparação da amostra e análise cromatográfica associada à espectrometria de massa, nomeadamente SPE-GC-MS, SPE-LC-MS/MS e injeção direta em UPLC-MS/MS.

Durante o processo de validação dos diferentes métodos, uma das maiores dificuldades sentidas foi a definição de metodologias para ultrapassar os problemas relacionados com o impacto dos efeitos de matriz no processo de quantificação.

Para alcançar os limites de quantificação descritos na legislação para estes compostos torna-se muitas vezes necessário recorrer a métodos de preparação da amostra, tais como o SPE, que permitem obter um extrato da amostra mais concentrado. Esta necessidade de concentrar o analito na amostra traduz-se num extrato de matriz igualmente mais concentrado, o qual poderá ter um forte impacto nos fenómenos de efeito de matriz.

Neste trabalho foram analisados os efeitos de matriz associados à análise destes pesticidas por três métodos de ensaio diferentes. No método de ensaio por SPE-LC-(Esi)-MS/MS, dependendo dos analitos em questão, verificaram-se efeitos de supressão iónica com decréscimos na intensidade de sinal que atingiram cerca de 80% (bentazona), assim como efeitos de enriquecimento iónico em que os acréscimos da intensidade de sinal atingiram valor até cerca de 300% (carbendazima). No método de ensaio por SPE-GC-(Ei)-MS verificaram-se sistematicamente efeitos de enriquecimento iónico com acréscimo de sinal instrumental que pode atingir cerca de 400% dependendo da matriz e do tipo de pesticidas. No método de ensaio por injeção direta em UPLC-(Esi)-MS/MS apenas foram observados efeitos de supressão iónica, com decréscimos na intensidade de sinal na ordem dos 40%.

Neste sentido, foram testadas diversas metodologias de quantificação que permitissem anular o efeito de matriz, nomeadamente o método do padrão interno com padrões marcados isotopicamente, método da adição padrão em matriz ajustada, diluição da amostra e fortificação da amostra (método adição de padrão). Neste trabalho pretende-se igualmente descrever as dificuldades associadas aos diversos métodos de quantificação assim como as vantagens e desvantagens na aplicação destas metodologias.
Optimization and validation of an SPE-HPLC-DAD method for glyphosate quantification in surface waters

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Glyphosate is an organo-phosphorated post-emergence broad spectrum herbicide used for plant growth control. It is considered practically not toxic and not carcinogenic¹, although the scientific literature disagrees². Thanks to the call from the scientific community, the European commission asked for a revaluation of glyphosate, whose results will be disclosed by the end of the year 2022. From this evaluation, a new directive can introduce a stricter control of glyphosate in water³.

The main goal of this work was the development, optimization and validation of a methodology able to quantify glyphosate in surface waters.

The methodology developed and optimized involved the use of solid phase extraction as a concentration and cleaning step, as well as high pressure liquid chromatography in reverse phase and a diode-array detector, relying on FMOC-Cl for derivatization, increasing this molecule’s absorption in the ultra-violet region.

The validated method allows the quantification of glyphosate from 90 ng/L in river sample, with recoveries between 71% and 118% for the working range in study (20-100 μg/L).

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References:
P57 UPLC-HRMS applied to surfactants

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Agrochemical formulations are homogeneous and stable mixtures containing one or more active ingredients, which are available in different forms and are designed to be safe and efficacious when applied to a specific target. The active ingredient, and the inert or solvent, represent generally around 90% of the mixture, being surfactants the remaining components of the agrochemical formulation. [1]

Surfactants, or surface active agents, are amphipathic molecules which mean that they have hydrophilic and hydrophobic parts. They are categorized based on their structure as anionic, cationic, amphoteric or non-ionic depending if the hydrophilic part of the structure is negatively charged, positively charged, both or uncharged. These components can act as emulsifiers, dispersing agents, wetting agents or others. [1]

Separation and identification of this type of compounds is a challenge since they are available in a wide chemical diversity. Taking this into account, Mass Spectrometry (MS) reveals to be a powerful tool to solve analytical questions related to surfactants since it provides high selectivity and also structural information. This technique is a sensitive and specific method of analysis that is applicable to complex mixtures when coupled to separation techniques, e.g. liquid and gas chromatography. [2] [3]

In the present communication, we will discuss the applicability of liquid chromatography coupled to mass spectrometry to study surfactants in complex mixtures of formulated plant protection products.

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References:


P59 Development of target methods for triclosan monitorization in effluents at trace levels using gas chromatography-triple quadrupole mass spectrometry and electronic tongues

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Triclosan (TCS) is a bacteriostatic used in household items that has been raising health concerns, due to the promotion of antimicrobial resistance and endocrine disruption effects both to humans and biota. TCS is being released to the environment by wastewater treatment plants processing at sub-ppb effluent concentrations, demanding therefore new tools for its continuous supervising and monitoring in complex matrices. Gas chromatography-triple quadrupole mass spectrometry (GC/MS/MS) is one of the most commonly employed method for emerging organic contaminants (EOCs) detection, due to its selectivity and sensitivity. In the other hand, also sensors devices are under the spotlight when fast and non-complex monitoring tools are needed. Electronic tongues (e-tongues) emerged as promising devices for EOCs detection in complex liquid matrices. Thus, two methods for detection and quantification of TCS in effluent were developed: (1) a GC/MS/MS method without the analyte derivatization, after operational optimization (GC parameters: splitless time, liner type, injector; MS parameters: source and interface temperature, Q1 and Q3 resolution (peak shape and area offset), collision gas pressure, collision energy and dwell time); (2) an e-tongue based on layer-by-layer films, [poly(ethyleneimine)/ poly(sodium 4-styrene sulfonate)]p, prepared onto gold interdigitated electrodes, using electrical impedance spectroscopy as a means of transduction. GC/MS/MS selectivity and sensitivity allowed the detection and quantification of TCS in effluent, with a limit of detection of 132 ppt and a limit of quantification of 395 ppt. The data reported demonstrated that standard methods should be adjusted “case-by-case”. Using the e-tongue, was accomplished an “analytical” sensor for the detection and pseudo-quantification of TCS in effluent. A Principal Component Analysis supported and demonstrated the sensor’s ability and potential to discriminate TCS concentrations using the second principal component, achieving a sensitivity value of 0.19±0.02 and a resolution of 0.13 pM (limit of quantification and detection of 0.2 and 0.1 pg/L, respectively). Finally, e-tongues are not intended to replace traditional methods, but they can be useful and complementary tools when speed and a lower-cost routine response are required.

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P60 Expanding capabilities in multi-residue pesticide analysis using SFC-MS/MS

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As the global population is expected to be 9.7 million people by the year 2050, the Food and Agriculture Organization of the United Nations (FAO) projects the increase in food projection will be derived from increasing yields and the number of times per year crops can be grown on the same land. The current panel of over 1000 pesticides is likely to play a significant role in agriculture, however, the effects on humans and the environment of exposure to pesticides are a continuing concern.

The World Health Organisation (WHO) in collaboration with FAO is responsible for assessing the risks to humans of pesticides. Acceptable daily intakes are used to establish maximum residue limits (MRLs) or tolerance information (EPA) for pesticides in food. A default value of 0.01 mg/kg is applied for MRL enforcement and as this level is equivalent to the lower limit of quantification it requires highly sensitive and specific analytical technologies to monitor an increasing number of pesticides.

This work describes the capability of SFC-MS/MS in measuring a panel of 164 pesticides in three different matrices (tomato, orange and leek). Compared to conventional LC-MS/MS methods, SFC-MS/MS resulted in higher response for pesticides most notably polar pesticides and a faster sample cycle time.
Unraveling the spatial and temporal biodiversity of grape varieties: the case study of the Portuguese Bairrada Appellation

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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, 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 comprehensive two-dimensional gas chromatography (GC×GC) tandem with chemometric tools, this research aims to provide advances to examine the impact of climatic and vineyard ecosystem conditions on 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 two consecutive harvests (2017 and 2018). For each variety and vineyard, physical-chemical data from grapes (titratable acidity, pH and sugar content, used to estimate the technological maturity state) were determined. Also, comprehensive two-dimensional gas chromatography–time of flight mass spectrometry (GC×GC-ToFMS) was used for in-depth characterization of the free and glycosidically-potential aroma compounds. Relationship between the data sets were studied using Component and Specific Weight Analysis (ComDim).

Except for Cercial, the varieties are clustered by year of growth rather than vineyard environmental conditions. Secondary metabolism showed sensitivity towards different climatic conditions, as confirmed by metabolomic terpenic data, which supported the observed differences on aroma notes among harvests. In summary, the results unveiled the high biodiversity of the Bairrada Appellation varieties, as each variety presents a specific pattern, which can be expressed differently in the ecosystems under study, and through harvests. 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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C18 vs Biphenyl Selectivity Comparison of Aromatic Compounds in Sunscreen

using Superficially Porous or Core-Shell Kinetex HPLC/UHPLC Columns

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The reversed-phase selectivity implications of 5 aromatic compounds of a commercially available sunscreen were evaluated on a Kinetex 2.6 μm C18 and a Kinetex 2.6 μm Biphenyl column. Both columns are manufactured based on the same superficially porous (core-shell) particle morphology but with unique selectivity characteristics4. In this example, we observed improvements and changes in relative peak shape, height, and overall compound elution order when both columns were compared. These changes were seen using the identical system and mobile phase conditions and are therefore attributed to the orthogonality of the two column’s stationary phases2. For these 5 aromatic compounds of interest, the Kinetex Biphenyl phase demonstrated overall chromatographic improvements and validated the benefits in leveraging the most applicable column selectivity for a given HPLC method.

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The changes in chemical composition during storage are considered, nowadays, the main quality problem of beer. These chemical changes, in contrast with wines, are usually considered negative for the beer flavour quality. Carbonyl compounds, particularly aldehydes, are considered to play an important role in the deterioration of beer flavour and aroma during the storage. In levels higher than their sensory threshold values, aldehydes are responsible for the appearance of oxidized flavours. E-2-Nonenal has received particular attention as the major source of the papery/cardboard character developed in aged beers and presents a very low threshold value (0.05-0.1 µg/L). β-damascenone, a terpenic ketone, was considered to be a significant flavour in some beverages such as whiskey, brandy, run and beer because of their very low threshold value in water (0.02-0.09 ng/g). β-damascenone has been identified as the responsible for sweeted apple, fruity and honey-like flavours.

This work aims at improving an analytical method developed by our research group for the simultaneous determination of E-2-nonenal and β-damascenone in beer. The modifications involved the addition of 2,4-dinitrophenylhydrazine (DNPH) as derivatizing agent, in order to improve the method’s sensitivity, as well as the inclusion of E-2-decenal as internal standard, to minimize experimental deviations caused by the steam distillation and solid phase extraction steps. The objective of this optimization was the enhancement of the extraction and quantification conditions of these two compounds by high-performance liquid chromatography (HPLC). The optimized method has been applied to monitor the profile of E-2-nonenal and β-damascenone for fresh and aged beers (naturally aged beers and forced aged beers).

The obtained results confirm that the sensitivity of the method was substantially improved by the addition of DNPH. In addition, the loss of the volatile compounds throughout the steam distillation has been compensated by means of the internal standard. Analysing the impact of different storage conditions on the concentration values of the analysed compounds, an approximately two-fold increase on the E-2-nonenal and β-damascenone concentrations, was observed for aged beers.

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References:
The intensive production of organic pesticides is an increasingly public health and environmental problem. Due to the high level of consumption, some organic compounds became ubiquitous in air, soil water and biota. There has been an increasing public pressure on environmental authorities to draw up regulatory measures to irradicate, or if not possible, to limit the presence of some chemical organic substances in the environment, namely some pesticides, pharmaceutical drugs and micro-plastics. The European Union has been very aware of this problem, and so in each revision of Environmental Directives, new organic substances are systematically incorporated—therefore its important and necessary to establish monitoring programs that allow us to know their levels in the different environmental compartments.

In order to accomplish the limits required in the latest European Environmental Directives the analytical laboratories need to develop, rapid and expeditious methodologies, able to identify and quantify trace and ultra-trace amounts of organic compounds in a diversity of environmental matrices. Technics such as Solid Phase Extraction (SPE), Solid Phase Micro Extraction (SPME) or more recent procedures such as ultra pressure liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) proceeded by online-SPE allow to achieve levels of quantification at the order of ng/L or even less as pg/L.

The present methodology allows the determination of 27 pesticides in a single run of 8 minutes through direct injection of the water sample in UPLC-MS/MS system. Chromatographic separation of the pesticides is achieved by using a 5 cm column (Phenomenex, Synergi Fusion – RP 80 Å) and the analysis is perform in QTRAP 4500 ABSCIEX in multiple reaction monitoring (MRM) mode with two selected transitions for each compound to ensure method selectivity. Electrospray ionization (ESI) is required at the source of the first mass spectrometer and both positive/negative mode were used depending on the pesticide.

<table>
<thead>
<tr>
<th>PESTICIDE</th>
<th>Z-SCORE</th>
<th>RECOVERY (%)</th>
<th>CONCENTRATION LEVEL (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bentazole</td>
<td>0.07</td>
<td>101</td>
<td>54</td>
</tr>
<tr>
<td>Chlortoluron</td>
<td>-0.65</td>
<td>93</td>
<td>107</td>
</tr>
<tr>
<td>Linuron</td>
<td>0.67</td>
<td>107</td>
<td>81</td>
</tr>
<tr>
<td>Mecopropre</td>
<td>1.06</td>
<td>110</td>
<td>101</td>
</tr>
<tr>
<td>Simazine</td>
<td>-0.43</td>
<td>96</td>
<td>116</td>
</tr>
<tr>
<td>Desethylatrazine</td>
<td>0.28</td>
<td>103</td>
<td>91</td>
</tr>
</tbody>
</table>

**Figure 1**: Example of pesticides calibration curves at ng/L level.

**Table 1**: Z-Score of interlaboratorial exercice and recoveries obtained for 6 pesticides.

Acknowledgements: We want to express our gratitude to the other members of the APA, IP especially the LRA partners.

References:
1. DIRECTIVE 2013/39/EU of the European Parliament and of the Council – amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy (12 August 2013);
3. L. Maldaner, J. Jardim, Determination of some organic contaminants in water samples by solid-phase extraction and liquid chromatography–tandem mass spectrometry, Talanta 100, 2012.
P66 Determination of antibiotics and psychiatric drugs in Douro and Leça Rivers (waters and sediments samples)

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The presence of pharmaceuticals and its metabolites and degradation products in the environment requires continuous research and monitoring studies to assess their potential risks to the human and ecosystem’s health. To our knowledge, this is the first study in Portugal for the analysis of both antibiotics and psychiatric drugs in river waters from Douro and Leça rivers and its sediments (Figure 1). Samples were extracted using solid phase extraction and QuEChERS procedures and the analysis was performed using liquid chromatography with tandem mass spectrometry.

Carbamazepine and fluoxetine were detected in Douro river and thirteen pharmaceuticals were detected in Leça river. The higher concentration was found in samples (river water and sediments) collected in Leça when compared with samples collected in Douro. The highest concentration was found for azithromycin in both types of samples with µg/L level in river water. Carbamazepine and venlafaxine showed a potential risk to algae in Leça river.

In sediments, the antibiotic sulfamethoxypyridazine was found in Douro sediment and the antibiotic azithromycin was detected in Leça sediment. Psychiatric drugs were also found in the studied sediments: four in Douro sediment and six in Leça sediments. The concentration found in the top and at the bottom in each sample were compared.

Acknowledgements

Funding: This work was also supported by UID/QUI/50006/2019 with funding from FCT/MCTES through national funds. The authors would like to thank also the EU and FCT / UEFISCDI /FORMAS for funding, in the frame of the collaborative international consortium REWATER financed under the ERA-NET Cofund WaterWorks2015 Call. This ERA-NET is an integral part of the 2016 Joint Activities developed by the Water Challenges for a Changing World Joint Programme Initiative (Water JPI).

References:
P67 Transporting olive oil in Roman times – chemical analysis of Dressel 20 amphorae from Pax Iulia civitas

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Although the origin of the edible olive is uncertain, it has coexisted with humans for about 6000 years, being associated with the development of the civilizations around the eastern Mediterranean basin and Asia Minor. During the Roman period, while cereal cultivation and viticulture gradually extended to the northern regions, olive growing remained restricted to the Mediterranean area due to its vulnerability to cold. The Guadalquivir valley in Andalucia, Spain, was one of the largest areas of production of the ancient olive oil, as it is today. Kilns to produce Dressel 20 amphorae, used to transport the Baetican olive oil across the empire, have been found sited along the banks of the river Guadalquivir and its tributaries between Seville and Córdoba1. This amphora could transport between 7 and 8 modii (i.e. 61-70 L) of olive oil, and is the most widely distributed amphora, being mainly found in military sites along the German and British limes and in Rome’s Monte Testaccio1,2.

Pax Julia civitas, known as Beja nowadays, was founded in the 1st century BCE by Octavian or August, but the site, located in a hill 277 m high, presents a strategic vantage point over vast plains, and is known to have been occupied at least since Iron Age. The studied Dressel 20 amphorae were unearthed in 1995/1996 during the excavation of the Main Square of Beja’s Castle and were part of dump levels dating from the end of Flavian’s government and the beginning of the second century, roughly situated between 80 A.D. and 100 A.D3. The organic extracts recovered from 14 potsherds were analysed by GC/MS. Biomarkers of plant oils (saturated and unsaturated fatty acids, alcohols and alkanes arising from plant waxes) were identified in 10 samples, and Pinaceae spp. pitch biomarkers were also identified in 8 samples. The absence of additional biomarkers in the studied Dressel 20 suggests that, even considering reuse, it should have been for the same or a similar commodity, i.e., storage or transport of a plant oil. The presence of the pitch in the amphorae can only be explained if it was used to reduce the porosity of the ceramic paste and minimize the oil diffusion through it.

Funding: This work was financially supported by FCT under project “MedOomics – Mediterranean Extra Virgin Olive Oil Omics: profiling and fingerprinting-Arimnet2/0001/2015” and project UID/Multi/04449/2013 (POCI-01-0145-FEDER-007649).

References:
Separation of positional isomers of fluorophenyl acetic acid

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4-Fluorophenyl acetic acid and its positional isomers are common building blocks in the synthesis of a large array of chemical compounds. Therefore, these compounds are commonly used in the pharmaceutical industry as raw materials and its characterization is of great importance. However, only few analytical methods are described in literature to deliver acceptable selectivity, since the similarity of these compounds, regarding physical and chemical properties, presents a certain degree of challenge when trying to achieve total chromatographic separation 1.

In this study, an analytical methodology to selectively determine these compounds was developed. Several techniques are available in the market that have demonstrated to be capable of aid in this type of separation, such as capillary electrophoresis (CE), gas chromatography (GC), supercritical fluid chromatography (SFC) and high performance liquid chromatography (HPLC) or ultra-high performance liquid chromatography (UHPLC). For this development, UHPLC was selected due to its increased chromatographic resolution. Moreover, this type of instrumentation is commonly present in most quality control analytical laboratories.

Method parameters and conditions, known to affect separation, were studied and taken into account, in the early stages of the method development. These parameters included, primarily, stationary and mobile phase selection and, in an optimization and final stage, column temperate and organic solvent contribution in the mobile phase. Also, of extreme importance was the evaluation of the molecules’ physicochemical properties, being the most relevant the pKa value, hydrophobicity, electrical charge and sample solubility. Using the appropriate in-silico predictive tools, it was possible to predict the pKa values, log D and log P for 4-fluorophenylacetic acid and its positional isomers. The visualization of the distribution plot of all the micro species under different pH conditions played a major role in the selection of the mobile phase pH. Moreover, the prediction of the log D/ log P for all isomers allowed the selection of the most suitable organic modifier to be used.

The initial chromatographic conditions to separate 4-fluorophenylacetic acid and its isomers - 2, 3-fluorophenyl]acetic acid, phenyl acetic acid and 4-hydroxyphenyl acetic acid - were selected. The first experiments were performed by running a scouting gradient, using an Acquity UPLC BEH C18 (2.1 mm x 100 mm; 1.7 µm) and acetonitrile as the organic modifier. Even though the pH range was within the one initially predicted and the column was adequate for these type of compounds, the compounds 4-fluorophenylacetic acid and 3-fluorophenylacetic acid co-eluted.

After performing several experiments with different organic modifiers, it was decided to use a not so commonly organic modifier in the mobile phase – tetrahydrofuran (THF). THF has been described in literature to play a key role in the separation of fluorophenyl acetic acid isomers by HPLC 3. The use of THF deeply increased the chromatographic resolution between 4-fluorophenylacetic acid and 3-fluorophenylacetic acid. The initial column selection was also changed to an YMC Triart ExRs (2.1 mm x 100 mm; 1.9 µm) due to column availability, showing similar results than the BEH column from Waters. After optimization of the analytical method, the final method was defined as an isocratic elution, at 0.2mL/min using water (0.1 vol% H3PO4) with THF (19 vol%) as organic modifier.

After method development, all critical method performance parameters were successfully evaluated. The LOQ was defined as being 0.001 mg/mL. Linearity was proven between 0.001mg/mL and 2.6 mg/mL. Precision, repeatability and accuracy were also proven for the method at the method target concentration (2 mg/mL).

As a conclusion, the separation of 4-fluorophenylacetic acid and its positional isomers was effectively achieved with a 20 minutes isocratic method. The use of predictive tools and the choice of the organic modifier played an important role in attaining this objective.

References:
P70 DETERMINATION AND QUANTIFICATION OF PHOTOINITIATORS FROM PRINTING INKS IN BIBS

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Plastic baby Bibs are used extensively today. According European framework legislation, baby bibs are considered a food contact material (FCM) as they can reasonably be expected to be brought into contact with food or to transfer their constituents to food under normal or foreseeable conditions of use. In a previous study focusing in screening analysis of baby bibs, several photoinitiators (PI) were detected. PI are used for UV-curable printing and varnishes present in bibs and potential transfer into food should be assessed.

A gas chromatography mass spectrometry (GC-MS) method was developed to detect and quantify thirteen PI. Calibration curves, using deuterated benzophenone as internal standard (IS), were prepared according to the regulatory status of the specific PI and ranged between 0.10 – 1.20 mg L⁻¹ for benzophenone (BP), 0.4 - 4.8 mg L⁻¹ for 2-Ethylhexyl 4-(dimethylamino)benzoate (2-EDAB) and 0.002 – 0.02 mg L⁻¹ for 4-Methylbenzophenone (4-MBP), 2-Isopropylthioxanthone (ITX), Ethyl 4-(dimethylamino)benzoate (EDB), 4,4’-Bis(diethylamino)-benzophenone (DEAB), 2-Ethylanthraquinone(EAQ), Methyl 2-Benzoylbenzoate (MBB), 2,2-Dimethoxy-2-phenylacetophenone (2,2-DPAP), 1-Hydroxycyclohexyl phenyl ketone (1-HPCK), Triphenyl phosphate (TPP), 4-(4-Methylphenylthio)benzophenone (4-MPBP) and 4-Morpholino benzaldehyde (4-(4-Morpholimyl)benzaldehyde) (4-MLB). Linearity (R²) was between 0.9966 for 4-MLB and 0.9999 for BP. Limit of detection (LOD) ranged from 0.0007 mg L⁻¹ for 2,2-DPAP and 0.2018 mg L⁻¹ for 2-EDAB while limit of quantification ranged between 0.0024 mg L⁻¹ and 0.6725 mg L⁻¹ for the same compounds. Repeatability, expressed as coefficient of variation (CV), was lower than 10% for all compounds except for TPP (23%). For recovery determination a printed bib purchased in a commercial store was extracted as follow: triplicate analysis of 1 g of bib printed areas was cut into small pieces and extracted with 10 mL of DCM, with IS and known amounts of PI at 2 different levels, for 24 hours at 40 °C then submitted to ultrasound for 30 min, followed by centrifugation 5 min at 2500 rpm and filtration with a 0.025 mm mesh prior to injection into the GC-MS system. Recovery where between 116% and 129% for all PI but except TPP where recovery was 300% probably due high variability of the sample as printed areas where very heterogenic. In the bib used for recovery calculation only TPP was detected at an average concentration of 0.0023 mg g⁻¹ which, assuming total migration, correspond to 0.0197 mg kg⁻¹ of foodstuff. Although TPP is not included in the positive list of EU Regulation 10/2011, this PI is referred in Swiss regulation on printing inks and should not migrate into a detectable amount into foodstuff by a method of analysis with a limit of detection of 0.01 mg kg⁻¹. As this method of analysis is based on extraction of PI from bibs instead of migration further testing is needed to assess compliance as a FCM.

This method of analysis is able to quickly detect and quantify PI in a material that often stakeholders consider as clothing or toy but is a FCM intended to be use by a particularly vulnerable population group, infants and young children.

References:

1. European Union Regulation nº 1935/2004
Optimization and validation of two methods to determine the levels of AFM1 in milk and cheese samples using immunoaffinity columns for extraction and HPLC-FLD for quantification

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Consumption of dairy products has expanded rapidly over the past decade and constitutes an important source of dietary protein.1 Afatoxin M1 (AFM1) is a potent carcinogen metabolite that can be present in milk from dairy cows that consume feed contaminated with Afatoxin B1. Even though it is less toxic than its parent compound, AFM1 is hepatotoxic and carcinogenic, and is stable during milk pasteurization, storage and preparation of various dairy products.2,3 Due to the toxicity of this molecule, its detection and quantification is extremely important.

The objective of this work was to optimize and validate two methods, according to Commission Regulation (EC) nº 401/2006 of 23 February, to determine the levels of AFM1 in milk and in cheese, using immunoaffinity columns (IAC) for extraction and HPLC with fluorescence detection for quantification.4

The method for milk samples was adapted from VICAM – the supplier of the IAC, and for cheese samples was from r-biopharm and VICAM.5,6 For both methodologies, three levels of spiking in triplicate on two different days were performed. The calibration curve was linear from 0.047 to 4.7 µg L⁻¹ and the detection and quantification limits for milk and cheese were 0.001 µg L⁻¹ and 0.003 µg L⁻¹, and 0.006 and 0.02 µg kg⁻¹, respectively.

For milk samples, average recoveries determined at spiking levels of 0.020, 0.050 and 0.10 µg L⁻¹ were in the range of 62 % – 87 %, with intra-day precision (RSD) in the range of 3.4 % – 9.5 %, and inter-day precision (RSD) in the range of 5.4 % – 6.2 %. For cheese samples, average recoveries determined at spiking levels of 0.050, 0.10 and 0.25 µg L⁻¹ were in the range of 54 % – 78 %, with intra-day precision (RSD) in the range of 2.8 % – 8.7 %, and inter-day precision (RSD) in the range of 3.7 % – 6.2 %.

Results of the validation process indicate that, except for the recovery in cheese samples with spiking level of 0.25 µg L⁻¹, both methods are in agreement with the provisions of Commission Regulation (EC) nº 401/2006.

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References:
P72 The isotopic composition (δ^{13}C, δ^{2}H and δ^{18}O) of Mediterranean extra virgin olive oils

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This study aimed to assess if EVOOs produced in three different Mediterranean regions could be discriminated on the basis of multivariate statistical analysis of geoclimatic and isotopic data. To that end, a total of 138 EVOO samples from 3 Mediterranean regions, Portugal, France and Turkey (67, 50 and 21, respectively) and in 2 different cultivation years (2016 and 2017) were sampled. Each sample was geo-referenced, obtaining data of altitude (m.a.s.l), longitude (UTM), latitude (UTM), mean annual precipitation (mm), mean annual temperature (°C) and oceanic distance (km). The isotopic composition (δ^{13}C, δ^{2}H and δ^{18}O) of EVOOs was obtained using an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS).

One-way analysis of variance (one-way ANOVA) using δ^{13}C as independent variable (IV) indicated that of Portuguese and Turkish EVOOs are significantly (P <0.05) different among them but not among its cultivation years. Nevertheless, French samples were significantly different (P <0.05) between cultivation years. In the case of δ^{2}H, it was observed that only the samples from Turkey were significantly (P <0.05) different according to the cultivation year. In contrast, the samples from Portugal and France are significantly (P <0.05) different from each other, which may be directly related to a very different climate. On the other hand, δ^{18}O values showed that there were significant (P <0.05) differences between cultivation years for Portuguese and French samples. Further, the δ^{18}O composition of EVOO samples cultivated in 2017 was considerably different between the three analyzed countries.

Principal component analysis (PCA), using both the geoclimatic information and the isotopic composition of the 138 EVOO samples analyzed, clearly sorted the samples in three clusters, which correspond to each of three Mediterranean regions studied. In light of the results, the multivariate isotopic analysis of EVOO samples may discriminate not only between geographical regions but also among cultivation years.

Funding: This work was financially supported by FCT under project "MedOOmics – Mediterranean Extra Virgin Olive Oil Omics: profiling and fingerprinting: Arimnet2/0001/2015".
The use of pesticides is a common practice in modern agriculture. These compounds are commercially available in liquid solutions, purchased in plastic containers. In 2018 in Portugal, over 700 tons of pesticide packing was sold to farmers. After their use, the plastic containers must be recycled or destroyed, depending on the amount of toxic pesticides still present in the residue. With the aim of classifying these residues as hazardous or not, according to EU law, different chromatographic approaches were tested to quantify 21 of the most widely used pesticides in Portugal, in post-consumer packing. The solid matrix was liquid extracted after grounding to 0.5 mm. Extraction was performed using tetrahydrofuran (THF) and a solution of 1,1,1,3,3,3 isofluoro-2-propanol in THF (5% v/v). The performance of GC/TOFMS and GC/MS/MS was compared in terms of identification, amount of information obtained, and limit of detection. Gas chromatographic separation was achieved in an apolar stationary phase column, for both techniques.

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References:
Preventing Future Health Risks: Exploring GC/MS tools for monitoring phthalates in food matrices

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Phthalate esters (PE’s), better known as phthalates, are a group of chemical compounds widely used since 1960 as plasticizing agents in order to impart flexibility, durability and longevity to plastics.¹ Given their unique physicochemical properties, some phthalates and their metabolites have a severe toxic effect on human health, primarily in the reproductive, endocrine and respiratory systems.²,³ Several studies have led the EU and the USA, among other countries, to intervene and regulate these compounds⁴. The control must be rigorous with very low levels of detection (ppb or lower), so it is important to define methodologies that respond to this need. Traditionally, the analysis of PE’s is performed using 1D gas chromatography techniques, e.g. GC/MS and GC/MS/MS. However, these have shown several problems both in identification and quantification, mainly due to co-elutions between different PE’s, and also with compounds in the matrix. In this work, GC/TOFMS and GC/MS/MS were both used to try and solve some of the problems inherent to the analysis.

Up to this moment, eight phthalates have been quantified in Portuguese olive oil and different materials used in its production process, such as hoses. Liquid extraction with hexane/methanol was performed, and chromatographic analysis was carried on a GC/TOFMS and a GC/MS/MS, with an apolar capillary column.

In the future, this project will apply classical and alternative 2D analytical methodologies (GCxGC and/or MD-GC and/or LC-GC) in order to obtain better separation, detection and sensitivity for PE’s in complex food matrices, such as wine and olive oil.

References


Acknowledgements This work was supported by the Associate Laboratory for Green Chemistry- LAQV which is financed by national funds from FCT/MCTES (UID/QUI/50006/2019), ICAAM funding by FCT - Foundation for Science and Technology under the Projects UID/AGR/00115/2019, and anchored by the RESOLUTION LAB an infrastructure at NOVA School of Science and Technology.
The eucalyptus weevil, Gonipterus platensis (Coleoptera, Curculionidae), is a major defoliator of eucalyptus worldwide. The use of pheromones for monitoring and control strategies against this species was, until now, hindered by the difficulty of identifying compounds emitted by these weevils. Here we report the collection, detection and identification of pheromones of G. platensis. The weevil’s volatile compounds were collected by solid phase micro extraction (SPME) and monolithic material sorption extraction (MMSE - MonoTrap™disks). In order to detect and identify insect emitted compounds Gas Chromatography and Mass Spectrometry (GC/MS) analysis was used, complemented by GCxGC/FID analysis to clarify some coelution zones. Eleven insect specific compounds were identified and three of these compounds, verbenene, cis-verbenol and trans-verbenol, were shown to be male-specific. Gas chromatography - mass spectrometry electroantennographic detection (GC-MS/EAD) was used to determine which compounds were perceived by the antennal olfactory system of the insect. Olfactometer biossays were conducted to access the behavioural effects of the collected MMSE extracts and to standards of the identified compounds. Extracts from virgin males, proved to be attractive to virgin females in olfactometer biossays. Further behavioural biossays showed that both virgin females and virgin males were attracted to the male specific compound cis-verbenol and that virgin females were attracted to trans-verbenol. Regarding 2-α-hydroxicineole and 2-oxo-cineole, which are produced by both sexes, the alcohol was attractive to virgin males and both the alcohol and the ketone were repellant to mated females.

This is the first identification of pheromones in Gonipterus spp. and also the first evidence of cineole metabolites acting as semiochemicals.

Acknowledgements:
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Control of the dry wood termite Cryptotermes brevis – semiochemicals potential role

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The West Indian dry wood termite Cryptotermes brevis (Walker) is an urban pest that causes extensive economic damage to structural and decoration timber. Due to its high reproductive potential, infestation ability and capacity to survive inside wood with very low moisture content, C. brevis has spread round the world, reaching Terceira Island, Azores, in 2004. It has since colonized most of the Archipelago islands (Borges et al. 2014)1, where costs to treat all affected buildings were estimated at > € 50 million (Guerreiro et al. 2014)2. Existing control methods are not effective because re-infestations by seasonal swarmers often occur, rendering eradication unfeasible. A study of the chemical ecology of C. brevis is ongoing, aiming at the identification of pheromones and other semiochemicals, given these compounds potential as management and control tools. Bioassays were performed in the laboratory with virgin alate males and females, collected during the swarming period of July 2016, from infested dwellings in the capital, Angra do Heroísmo. In the laboratory, bioassays were performed in an Y-tube olfactometer, to determine if an attraction response of males to females and/or of females to males, would take place. Results showed that males spent significantly more time in the Y-arm of the olfactometer where one female had been placed, inside a small chamber enclosed by a net mesh, than in the inlet chamber and in the Y-control arm. On the contrary, females spent significantly more time inside the inlet chamber and in the Y-control arm of the olfactometer, than in the Y-arm where one male had been previously placed. It can thus be hypothesized that the behavioural responses of the males were triggered by semiochemicals emitted by the females, acting as sex pheromones; by contrast, females did not respond significantly to an eventual emission of semiochemicals by the males. Alate C. brevis termites from the same population used for the bioassays, were captured, placed in hexane vials separated by sexes and analysed by GC-MS at RESOLUTION LAB, FCT, UNL. A comparison between the chromatograms obtained for males and for females, showed that both sexes had very similar chromatographic profiles, mainly composed by cuticular hydrocarbons and wood metabolite compounds. To date, sexual attraction in the genus Cryptotermes has not been studied. This study presents a first contribution to the identification of compounds that would be relevant to tackle the serious and widespread problem caused by C. brevis.

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References:
Finding a needle in a haystack: Dereplication of complex extracts from marine-derived actinomycetes using LC-MS/MS-based Molecular Networking

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Ocean environments constitute a major source of biodiversity, harboring life forms capable of producing a variety of new molecules with unmatched biochemical diversity and structural complexity, which should be explored to address complex human health challenges such as antibiotic resistance. Our screening rationale aims to unveil new and effective strategies to fight multidrug resistant bacteria by reducing their capacity to form biofilms. The inhibition of such recalcitrant bacterial communities can be an advantageous alternative to antimicrobials, as this approach does not lead to the development of resistance mechanisms. Boosting the identification of new biological activities, MS/MS-based Molecular Networking, facilitated by the Global Natural Products Social (GNPS) platform, is rapidly changing the way in which we dereplicate known natural products in complex mixtures, find new analogues in bioactive structure classes, and identify new chemical entities. Here we report the dereplication of complex extracts from marine-derived actinomycetes using MS/MS-based Molecular Networking assisted by the Global Natural Products Social (GNPS) platform. Six MAR4 Streptomyces strains isolated from the Madeira Archipelago,3 were used to study the chemical diversity of produced hybrid isoprenoids. These marine actinomycetes were investigated by analysing their crude extracts using LC-MS/MS and their metabolomic profiles were compared using multivariate statistical analysis (principal component analysis), showing a separation trend closely related to their phylogeny. Molecular networking unveiled the presence of a class of metabolites not previously described from MAR4 strains and new chemical derivatives belonging to the napyradiomycin and marinone classes. Furthermore, these MAR4 strains produce metabolites that inhibit biofilm formation of Staphylococcus aureus and Marinobacter hydrocarbonoclasticus.4

Acknowledgements: We would like to thank W. Fenical, P. R. Jensen and C. A. Kauffman from SIO-UCSD for the sustenance given to perform the samples collection. We acknowledge P. Castilho from UMA and M. Freitas from EBM for logistic support during the collection expedition.

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References:
P81 Electro-reactor as a polishing step for the removal of emerging organic contaminants from wastewater: microcosm scale

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The use of electro-based technologies to remediate contaminated matrices may be of great interest to public and the environmental health as it presents itself as a versatile and promising technology with potential for reducing the environmental and human risks associated with the spread of contamination. Emerging organic contaminants (EOC) are a large group of unregulated compounds, which decrease in effluents is needed aiming a safer discharge to the receiving water bodies and/or to promote a safer water re-use in agriculture. Electrokinetic process may be a cost-effective key solution for this situation.

The ex situ effluent remediation was tested in one electro-chemical cell and their controls (without electric current). The target EOC were: caffeine, sulfamethoxazole, carbamazepine, diclofenac, oxybenzone, bisphenol A, estradiol, ethinylestradiol, ibuprofen, as they present different physical and chemical characteristics, belonging to different categories and were already detected in various environmental compartments. The effect of electrode material (mixed metal oxide electrodes) and different treatment times (2, 4 and 6 hours of treatment) with a fixed current density (50 mA) was assessed in terms of EOCs removal. The organic component was extracted by solid phase extraction (SPE) and analysed by high-performance liquid chromatography (HPLC) with a diode array and fluorescence detectors (HPLC–DAD-FLD). A Poroshell column was used for analytes separation. A mixture of ACN/Mili-Q water/formic acid was used as eluent. Calibration curve was performed in the range between 0.5 and 8.0 mg L⁻¹. The limits of detection in this work were between 0.55 and 3.0 µg L⁻¹, and the quantification limits were between 1.7 and 9.0 µg L⁻¹. The recovery percentages were between 80 and 120% in all cases.

For all the cases, the electric current enhanced contaminants removal (up to 55% in 2 hours and 70% in 6 hours). This treatment does not require the addition of reagents and represents low energetic costs, making it more environmentally friendly.

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P82 Analysis of Histamine and Spermidine in commercial fish species by GC-MS and ELISA: an assessment of seafood quality

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There is a strong need for food quality and safety [1], with the chemical and biological nature of hazards being the major concern. Therefore, there is a major concern regarding food spoilage making it unsuitable for human ingestion. Fish deterioration is a complex phenomenon where a series of events occur simultaneously, influencing each other and beginning immediately at the time of organism death [1,2]. Thus, there is a strong need for developing reliable seafood quality analysis methods. The main aim of the present study was to develop an analytical approach based on sample treatment and GC-MS methodologies to detect histamine and spermidine as products of fish deterioration. In the present study we surveyed histamine and spermidine in two fish species (Trachurus trachurus and Sarda sarda), acquired in a Portuguese traditional market. As a complementary approach, the fish samples were also analysed for the same biogenic amines by Enzyme Linked Immunosorbent Assay (ELISA).

The selected species (T. trachurus and S. sarda) were carefully fileted and exposed to room temperature for nine days (T. trachurus) and 13 days (S. sarda). Samples were collected every day with the aid of a clean scalpel and frozen (-80°C) until later analysis. Following the exposure time, the samples were homogenized in 75:25 Methanol: 0.4N HCl. Then, samples were treated and then analysed by GC-MS essentially as described by Richard et al. [2]. Standards of mixed Biogenic Amines (Sigma-Aldrich), were used to detect and quantify the target biogenic amines. For ELISA assay, samples were processed by homogenizing samples in a phosphate buffer saline solution, centrifuged (10,000xg at 4°C) for 15 min and then stored at -80°C until analysis. The ELISA assay was performed following the same methodology described by Madeira et al. [3]. Following GC-MS analysis, spermidine emerged in two days followed by a decrease below LD (limit of detection) after nine days of exposure showing a different profile over exposure time. GC-MS analysis detected Histamine in S. sarda after seven days of exposure followed by a decrease through time. Spermidine and Histamine were also detected and quantified by ELISA. The biogenic amines profiles (histamine and spermidine) are compatible with the bacterial activity in sea food, namely biogenic amines which are usually produced by decarboxylation of free amino acids and transamination of aldehydes and ketones by the action of several microorganisms. Moreover, although GC-MS seems to be reliable technique to analyse spermidine in seafood samples more analysis should be performed focused on other sample treatment methodologies for histamine determination and cross-checking with other analytical techniques (e.g. LC-MS/MS and HPLC) until full validation.

Figure 1: representative chromatogram of standard Biogenic Amines (A). Legend: PUT (Putrescine), FEN (Phenylethylamine), CAD (Cadaverine), TIR (Tryptamine), IS (Histamine), TRIP (Tryptamine), ESPD Spermidine), ESP (Spermine).

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This work was supported by the Applied Molecular Biosciences Unit- UCIBIO which is financed by national funds from FCT/MCTES (UID/Multi/04378/2019) and by the Associate Laboratory for Green Chemistry- LAQV which is financed by national funds from FCT/MCTES (UID/QUI/50006/2019) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728) and the project Q3s for quality - Development of new devices and techniques for seafood quality assessment (PTDC/MAR-BIO/6044/2014).

References:
P83 Simultaneous isolation of polar and non-polar functional extracts from Opuntia robusta peels

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Extraction of essential oils or vegetable oils generally requires the vegetable matrix to be pre-dried in order to allow transfer of those metabolites to non-polar solvents such as hexane or petroleum ether. The drying operation may include air drying for extensive periods or oven drying at temperatures close to 100 °C, methods that may cause oxidation and decomposition of antioxidant species present in the vegetable matrix, therefore precluding their subsequent recovery and valorisation. An alternative method for extraction of lipids from fresh vegetable or animal tissues is the Bligh and Dyer method, that may be applied to wet matrices but includes a halogenated solvent (chloroform) and is not easily adapted to an industrial scale. Fruit peels are biological tissues that may simultaneously contain vegetable or essential oils and phenolic components, but the recovery of those non-polar and polar metabolites is generally performed separately because the solvent systems used in extraction are generally different. In this work it was developed a one-step approach to recover vegetable oil and antioxidant compounds from the peels of Opuntia robusta fruits. The peels were separated from the pulp and mixed with the extraction solvents (50% petroleum ether + 50% acetone or 50% petroleum ether + 50% ethanol). The mixture was mechanically homogenized to promote the extraction and the solid residue was eliminated by filtration. The liquid extract was diphasic comprising an upper organic phase mainly composed of petroleum ether and a lower aqueous phase composed of the vegetable water and most of the hydrophilic solvent (acetone or ethanol). Non-polar metabolites such as vegetable oils were dissolved in the organic phase while the aqueous phase contained polar metabolites such as phenolic compounds. The extraction was repeated with 100 % hydrophilic solvent (acetone or ethanol) to maximize the recovery of polar components and the liquid extracts were combined. The organic and aqueous phases were separated by addition of 200 mL of water and decantation and an aliquot was evaporated to dryness to determine the extract yields. The organic phase was also characterized by other analytical procedures: Methodology, available at https://www.fda.gov/downloads/drugs/guidances/ucm073384.pdf (Accessed January 24, 2018).

The peel oil is characterized by the presence of significant amounts of peel waxes and phytosteroles characteristics that may support the application of these oils in cosmetics applications. The aqueous phase was characterized by HPLC-DAD and the extract composition was also characterized by colorimetric tests for evaluation of total phenols, total flavonoids, DPPH antioxidant activity and ferric reduction antioxidant power (FRAP) (Table 1). The extracts obtained presented total antioxidant activity and total phenols comparable to those determined for Passiflora peels extract. The acetonite extract presented higher values of total flavonoids, DPPH antioxidant activity and FRAP activity. On the other hand, the ethanol extract presented higher extraction yield and higher phenolic compounds probably due to the co-extraction of some sugars from the residual pulp attached to the peels. Reducing sugars are more soluble in ethanol than in acetone and they are also positive interferents of the Folin-Ciocalteu reaction, what explains the higher value of total phenols in the ethanol extract. The use of this one-pot method enables the recovery of added value products with possible applications in the cosmetic and nutraceutical industries from a residual biomass that is not presently valorized. The residue after extraction may still be included in animal feed or converted to activated carbon.

### Table 1. Characterization of extract composition for total phenols, total flavonoids, DPPH antioxidant activity and ferric reduction antioxidant power (FRAP).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extraction yield (% w/w)</th>
<th>Total phenols (mg EAE/g extrato)</th>
<th>Total flavonoids (mg catechine/g extrato)</th>
<th>DPPH antioxidant activity (mg Trolox/g extrato)</th>
<th>Ferric reduction antioxidant power (mmoles FeSO₄/g of ext)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP/Ace</td>
<td>0.71</td>
<td>34.3</td>
<td>9.9</td>
<td>17.3</td>
<td>0.7</td>
</tr>
<tr>
<td>EP/AE</td>
<td>0.83</td>
<td>56.7</td>
<td>8.4</td>
<td>17.2</td>
<td>0.6</td>
</tr>
</tbody>
</table>

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References:
The present study aims to evaluate the impact of HPP on seaweeds volatile organic compounds (VOCs). Samples of Codium tomentosum seaweed were collected in Buarco beach (Portugal), and processed at 400 and 600 MPa for 5 min. (23 °C), being stored at 3 °C for 90 days. At times 0 (control), 1, 7, 15, 30 and 90 days the samples were frozen in liquid nitrogen and preserved at -24 °C for further analysis. VOCs were extracted by headspace solid-phase microextraction (HS-SPME) using a triple phase fiber (DVB/CAR/PDMS) and analyzed by gas chromatography coupled to mass spectrometry (GC-MS) according to the methodology proposed by López-Pérez, et al., with minor changes. A total of 33 volatiles were identified in samples processed at 400 and 600 MPa: 12 aldehydes, 10 hydrocarbons, 7 ketones, 5 alcohols, 1 sulfur- and 1 bromo-containing. The most abundant compounds in all chromatograms were the hydrocarbons Heptadecane and 8-Heptadecene which should not significantly contribute to overall aroma due to their high threshold values. However, 8-Heptadecene, described as having earthy and mossy notes, can impart an off-flavor in seafood. Considering the odor detection threshold (ODT) values and relative concentration of the compounds in the sample, Dimethyl sulfide (DMS), (E,E)-2,4-Decadienal, trans-2-Nonenal, Hexadecanal, and Epoxy-3-Decadienal were the most prominent compounds in the Codium tomentosum aroma profile. Dimethyl sulfide is described as having a sulfuric, cooked vegetables, seashore aroma; (E,E)-2,4-Decadienal a fatty, oxidized, green, characteristic seaweed aroma; and trans-2-Nonenal a grassy and cucumber-like aroma. These findings are in accordance with previous work by the author and small differences are probably due to the methodology applied, ODT references and environmental conditions of the collected samples. Results from 11 chromatograms show that DMS relative participation was reduced during storage (Figures 1 and 2). This compound results from many different factors such as enzymatic, alkaline, and thermal decomposition of its precursor Dimethyl-propiothetin (DMPT). There was a shift in DMS at the 90th day of the samples treated with HPP at 600 MPa a relevant increase in a- and ß-Ionone after processing and over time was noticed (Figure 2). Usually the presence of these ketones in food matrices is related to enzymatic or autoxidative cleavage of ß-carotene. It is known that HPP has a limited effect on covalent bonds within the food product, although, the findings evidence the breaking of chemical bonds specially in carotenoids and polysaturated fatty acids (PUFAs). Due to the fact that epoxides are uncommon in nature, the presence of Dill ether (3,9-epoxy-1-p-menthene) and 7,8-Epoxy-a-ionone in the samples treated with 600 MPa suggests that the higher pressures could also promote the oxygenation of double bonds in some compounds. The behavior of the aldehydes in the sample was inconclusive. There is a possible trend in the reduction of their relative concentration, probably because of oxidation reactions, primarily by bacterial spoilage. However, the liberation of intracellular enzymes, such as lipoxigenase, can act on polysaturated fatty acids producing new aldehyde molecules. The main consequence of these dynamics in terms of flavor is the development of a rancid or unpleasant off-flavors. Despite of the fact that some other aldehydes showed erratic behavior (data not shown), it can be seen in the Figures 1 and 2 that Hexanal decreases constantly with time. D-Limonene and Bromofuran compounds do not seem to be affected by storage conditions. 1-Hexanol and Benzyl alcohol appear in the samples treated at 400 Mpa, particularly after 90 days of storage. The production of this compound can be connected with the activity of lactic acid bacteria dehydrogenases that could act on Hexanal and the reduction of Benzaldehyde by enzymes from yeast. In a similar work with Laminaria ochroleuca, HPP achieved better odor characteristics and overall sensory acceptance than other preservation procedures. Thus, it can be concluded that HPP is a good technique to preserve fresh seaweeds maintaining sensory qualities. Most of the VOCs were stable during storage period what implies that in a greater extent the microbial activity, enzymatic and autoxidative reactions were avoided.

Figure 1: Compounds evolution after HPP (400 MPa) and storage.

Figure 2: Compounds evolution after HPP (600 MPa) and storage.

Acknowledgements: We would like to thank LAQV (Requimte) and Nova.ID.FCT for the research scholarship; University of Aveiro that provided the HPP equipment for the essay; Prof. Marco Silva and the organization of the 11th ENC for the invitation to present this work.

Funding: This work was developed under the Project MAO-01.03.01-FEAMP-0016 – AlgaFood that has the financial support of the European Maritime and Fisheries Fund and is co-financed by Operational Program Mariculture financed by the Operational Program for the competitiveness and productive investment of the European Union through the European Regional Development Fund (POCI-01-0145-FEDER-000019). The present study aims to evaluate the impact of HPP on seaweeds volatile organic compounds (VOCs). Samples of Codium tomentosum seaweed were collected in Buarco beach (Portugal), and processed at 400 and 600 MPa for 5 min. (23 °C), being stored at 3 °C for 90 days. At times 0 (control), 1, 7, 15, 30 and 90 days the samples were frozen in liquid nitrogen and preserved at -24 °C for further analysis. VOCs were extracted by headspace solid-phase microextraction (HS-SPME) using a triple phase fiber (DVB/CAR/PDMS) and analyzed by gas chromatography coupled to mass spectrometry (GC-MS) according to the methodology proposed by López-Pérez, et al., with minor changes. A total of 33 volatiles were identified in samples processed at 400 and 600 MPa: 12 aldehydes, 10 hydrocarbons, 7 ketones, 5 alcohols, 1 sulfur- and 1 bromo-containing. The most abundant compounds in all chromatograms were the hydrocarbons Heptadecane and 8-Heptadecene which should not significantly contribute to overall aroma due to their high threshold values. However, 8-Heptadecene, described as having earthy and mossy notes, can impart an off-flavor in seafood. Considering the odor detection threshold (ODT) values and relative concentration of the compounds in the sample, Dimethyl sulfide (DMS), (E,E)-2,4-Decadienal, trans-2-Nonenal, Hexadecanal, and Epoxy-3-Decadienal were the most prominent compounds in the Codium tomentosum aroma profile. Dimethyl sulfide is described as having a sulfuric, cooked vegetables, seashore aroma; (E,E)-2,4-Decadienal a fatty, oxidized, green, characteristic seaweed aroma; and trans-2-Nonenal a grassy and cucumber-like aroma. These findings are in accordance with previous work by the author and small differences are probably due to the methodology applied, ODT references and environmental conditions of the collected samples. Results from 11 chromatograms show that DMS relative participation was reduced during storage (Figures 1 and 2). This compound results from many different factors such as enzymatic, alkaline, and thermal decomposition of its precursor Dimethyl-propiothetin (DMPT). There was a shift in DMS at the 90th day of the samples treated with HPP at 600 MPa a relevant increase in a- and ß-Ionone after processing and over time was noticed (Figure 2). Usually the presence of these ketones in food matrices is related to enzymatic or autoxidative cleavage of ß-carotene. It is known that HPP has a limited effect on covalent bonds within the food product, although, the findings evidence the breaking of chemical bonds specially in carotenoids and polysaturated fatty acids (PUFAs). Due to the fact that epoxides are uncommon in nature, the presence of Dill ether (3,9-epoxy-1-p-menthene) and 7,8-Epoxy-a-ionone in the samples treated with 600 MPa suggests that the higher pressures could also promote the oxygenation of double bonds in some compounds. The behavior of the aldehydes in the sample was inconclusive. There is a possible trend in the reduction of their relative concentration, probably because of oxidation reactions, primarily by bacterial spoilage. However, the liberation of intracellular enzymes, such as lipoxigenase, can act on polysaturated fatty acids producing new aldehyde molecules. The main consequence of these dynamics in terms of flavor is the development of a rancid or unpleasant off-flavors. Despite of the fact that some other aldehydes showed erratic behavior (data not shown), it can be seen in the Figures 1 and 2 that Hexanal decreases constantly with time. D-Limonene and Bromofuran compounds do not seem to be affected by storage conditions. 1-Hexanol and Benzyl alcohol appear in the samples treated at 400 Mpa, particularly after 90 days of storage. The production of this compound can be connected with the activity of lactic acid bacteria dehydrogenases that could act on Hexanal and the reduction of Benzaldehyde by enzymes from yeast. In a similar work with Laminaria ochroleuca, HPP achieved better odor characteristics and overall sensory acceptance than other preservation procedures. Thus, it can be concluded that HPP is a good technique to preserve fresh seaweeds maintaining sensory qualities. Most of the VOCs were stable during storage period what implies that in a greater extent the microbial activity, enzymatic and autoxidative reactions were avoided.

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