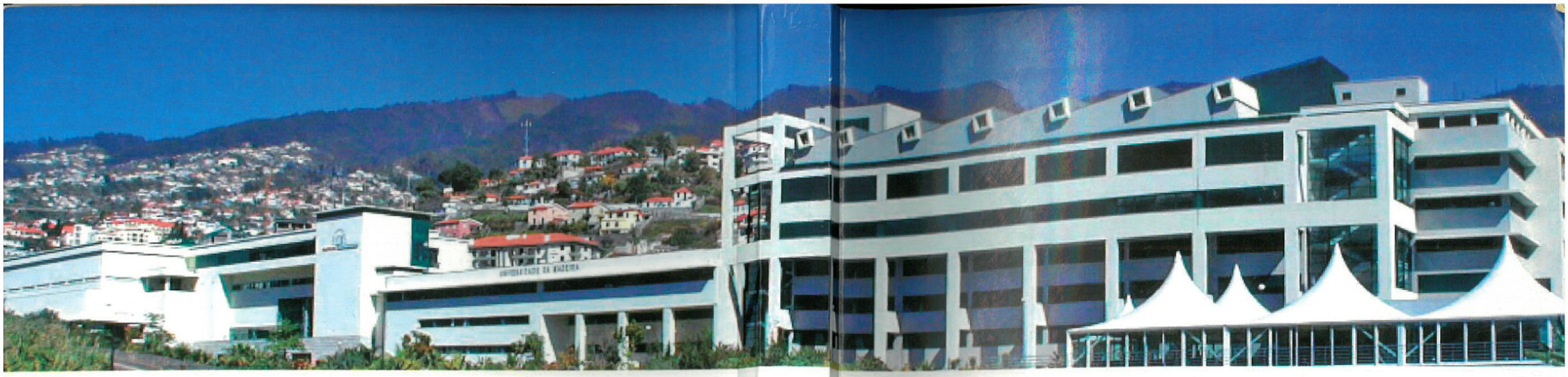

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6º Encontro Nacional de Cromatografia

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Universidade da Madeira

2009

UNIVERSIDADE da MADEIRA
CQM CENTRO DE QUÍMICA DA MADEIRA
SOCIEDADE PORTUGUESA DE QUÍMICA

6º Encontro Nacional de Cromatografia

Sociedade Portuguesa de Química

Universidade da Madeira,
Hotel CS Madeira, Funchal
14 a 16 de Dezembro de 2009

Grupo de Cromatografia

SOCIEDADE PORTUGUESA DE QUÍMICA

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índice

comunicações em poster

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Agradece-se ainda ao Dr. Jorge Pereira, Dra Catarina Luís Silva, Prof. Dra. Sílvia Rocha e Prof. Dr. JM Nogueira pela ajuda, colaboração e participação activa na organização deste Encontro.

informações aos participantes

conferências
As conferências decorrerão nas salas Selvagens I-V e Lobby do **Hotel CS Madeira** (Estrada Monumental, 175-177, 9000-100 Funchal).

expositores de empresas
Os expositores das empresas participantes estarão no Hall de acesso à sala de conferências, onde irá decorrer o 6º Encontro Nacional de Cromatografia, funcionando permanentemente durante os 3 dias do evento.

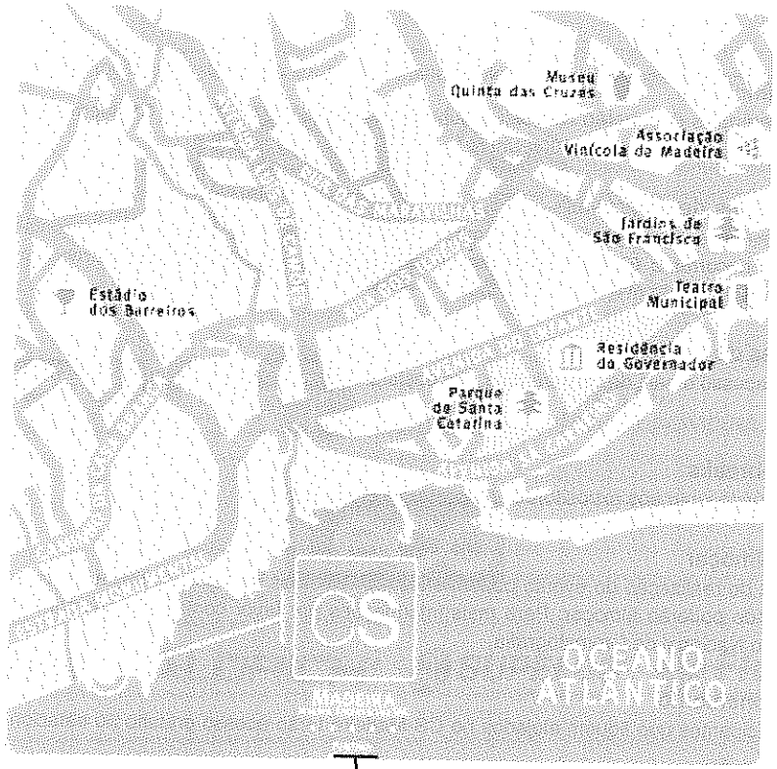
sessões de posters
Hall de acesso à sala de conferências. Os posters deverão estar expostos durante os 3 dias do evento.

coffee-breaks
Será servido junto aos expositores dos posters e das empresas.

onde almoçar
Na área de realização do evento podem encontrar diversos serviços de restaurantes e snack-bars, onde poderão almoçar. Para mais informações por favor contacte o secretariado do 6º Encontro Nacional de Cromatografia.

estacionamento
Os participantes que fiquem alojados no CS-Madeira terão acesso gratuito ao estacionamento do hotel.

outros serviços
Nos arredores do local do evento poderão encontrar agências bancárias, CTT, rent-cars, transportes públicos (www.horariosdofunchal.pt), entre outros tipos de serviços.



encontros anteriores

1º Encontro Nacional de Cromatografia
Fundação Calouste Gulbenkian, Lisboa, 13-15 de Dezembro 1999

2º Encontro Nacional de Cromatografia
Torre do Tombo, Lisboa, 10-12 de Dezembro de 2001

3º Encontro Nacional de Cromatografia
Torre do Tombo, Lisboa, 15-17 de Dezembro de 2003

4º Encontro Nacional de Cromatografia
Universidade de Évora, Évora, 12-14 de Dezembro de 2005

5º Encontro Nacional de Cromatografia
Universidade de Aveiro, Aveiro, 10-12 de Dezembro de 2007

programa

14 de Dezembro > 2ª feira

8:00	Recepção de participantes	
9:15	**Sessão de Abertura**	
		Chairperson: JM Nogueira
9:30	**LP.1**	*IN-VIVO* APPLICATIONS OF SOLID PHASE MICROEXTRACTION **Janusz Pawliszyn** - University of Waterloo, Waterloo, Canada
10:30	**Café e sessão de posters**	
		Chairperson: Celeste Lino e **João Queiroz**
11:00	**LMP.1**	METABOLOMICS, A NEW APPROACH TO BIOANALYSIS WITH SEPARATION TECHNIQUES AND MASS SPECTROMETRY **Coral Barbas** - Universidade de San Pablo, Spain
11:30	**CO.1**	Solid-phase microextraction Ni-Ti fibers coated with particles immobilized in a sol-gel matrix **Manuela Azenha**
11:50	**CO.2**	Development of a simple and solvent free methodology based on HS-SPME for characterization of free and glycosidically-linked components of musts – **Rosa Perestrelo**
12:10	**CO.3**	Analyis of Eucalyptus volatile emissions under different fertilization regimes by HS-SPME, GC-FID and GC/MS **A. Santos**
12:30	**Seminário**	**Tecnocroma**
13:00	**ALMOÇO**	
14:30	**Sessão de posters**	
		Chairman: Marco Gomes Silva
15:30	**LMP.2**	EXHALED BREATH AS A POTENTIAL SOURCE OF DISEASES RELATED METABOLITES: THE ROLE OF THE SPME--GC METHODOLOGIES **Sílvia Rocha** - Universidade de Aveiro
16:00	**Café e sessão de posters**	
		Chairman: Marcela Segundo e **Aquiles Barros**
16:30	**CO.4**	Determination of methadone and its metabolite EDDP in hair by gas chromatography-mass spectrometry **Mário Barroso**
16:50	**CO.5**	Deeper insight into the sesquiterpenoids profile of *Matriarca recutita* L. biosynthesis modulated by the agricultural pratices **Sílvia Petronilho**
17:10	**CO.6**	Characterization of phenolic fraction of cork from *Quercus suber* L. by GC-MS and HPLC-MS **S.O. Santos**
17:30	**CO.7**	Desenvolvimento de uma Metodologia Analítica para Determinação de Antibióticos em Amostras Ambientais **A. Rita Silva**
18:00	***Reunião do Grupo de Cromatografia da Sociedade Portuguesa de Química***	
19:30	***Madeira de Honra no IVBAM***	

15 de Dezembro > 3ª feira

		Chairperson: Ana Maria Freitas
9:00	**LP.2**	RECENT INNOVATIONS IN FLUID-BASED SEPARATION TECHNIQUES AND THEIR IMPACT ON OUR DAILY WORK **Pat Sandra** - Research Institute for Chromatography, Bélgica
10:00	**LMP.3**	ADSORPTIVE MICROEXTRACTION (AμE) TECHNIQUES - NEW TECHNOLOGIES FOR TRACE ANALYSIS OF POLAR COMPOUNDS **José Manuel F. Nogueira** - Universidade de Lisboa
10:30	**Café e sessão de posters**	
		Chairperson: Fernando Nunes e Sílvia Rocha
11:10	**CO.8**	Artificial neural network classification based on high-performance liquid chromatographic data of low molecular weight phenolic compounds – **Maria João Cabrita**
11:20	**CO.9**	Metabolomic analysis of natural products **David Pereira**
11:40	**CO.10**	HPLC-DAD-ESI-MS^n separation and characterization of phenolic compounds in *Helichrysum devium* Johns. flowers and evaluation of its antioxidant activity by online HPLC-DPPH method **Sandra Gouveia**
12:00	**Seminário**	**ILC**
12:20	**ALMOÇO**	
14:00	**Sessão de posters**	
		Chairman: MC Mateus e JC Marques
15:00	**LMP.4**	GC×GC AS A NEW TOOL TO INVESTIGATE THE ELECTROKINETIC REMEDIATION OF ORGANIC POLLUTANTS FROM COMPLEX MATRICES: THE REMOVAL OF CREOSOTE FROM TREATED TIMBER WASTE **Eduardo Mateus** - Universidade Nova de Lisboa
15 :30	**CO.11**	Potentialities of comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOF-MS) in environmental chemistry. An overview of its applications, behaviour and perspectives **Eric Jover**
16:00	**Café**	
16:30	**CO.12**	Automatic flow based sample preparation coupled to liquid chromatography **Marcela Segundo**
16:50	**CO.13**	The use of capillary electrophoresis in drug-drug interactions studies **Hugo Serra**
17:10	**CO.14**	Desenvolvimento e validação de um método qualitativo para a análise forense de tintas esferográficas por HPLC **Daniela Vaz Marques**
17:30	**Seminário**	**Thermo Fisher Scientific**
18:00	**Seminário**	**Dias de Sousa**
20:00	**Jantar do Congresso**	

16 de Dezembro > quarta-feira

		Chairperson: LV Boas
9:00	**LP.3**	PAPEL DE LA CROMATOGRAFIA EN LA ELUCIDACIÓN DEL AROMA DEL VINO **Juan Cacho Palomar** - University of Zaragoza, Espanha
10:00	**LMP.5**	ONLINE SAMPLE PREPARATION AND LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY FOR ENVIRONMENTAL AND FOOD ANALYSIS **Cláudia Martins** – Thermo Fisher Scientific
10:30	**Seminário**	**Sigma-Aldrich**
11:00	**Café e sessão de posters**	
		Chairperson: M Rosário Bronze e JM Oliveira
11:30	**CO.15**	Gas chromatography-olfactometry in aroma characterisation and quality assessment of grapes and wines: an overview **Maria Cristina Clímaco**
11:50	**CO.16**	Membraneless gas-diffusion module for the analysis of volatile and semi-volatile compounds **Luís Gonçalves**
12:10	**CO.17**	DLLME coupled to MD-GC/MS as a reliable method to the assessment of trace contaminant residues in food **J.O. Fernandes**
12:30	**LMP.6**	X-METABOLOMICS: THE X-WINDOWS HIGH-TROUGHPUT MASS SPECTROSCOPY METABOLOMICS PIPELINE **A.C. Silva Ferreira** - Biotechnology Research Centre - Interface A4 Catholic University
13:00	**Divulgação dos Prémios**	
13:10	**Sessão de Encerramento**	

lições plenárias

LP.1 *IN-VIVO* APPLICATIONS OF SOLID PHASE MICROEXTRACTION

Dajana Vuckovic, Shine Zhang, Marcel Musteata, Heather Lord and **Janusz Pawliszyn**
Department of Chemistry, University of Waterloo, Waterloo, Canada, N2L 3G1.

In recent years, there has been a lot of interest in monitoring levels of biologically active compounds in living systems in their natural environments . These efforts are a significant departure from conventional 'sampling' techniques, where a portion of the system under study is removed from its natural environment, and the compounds of interest extracted and analyzed in a laboratory environment. There are two main motivations for exploring these types of investigations. The first one is the desire to study chemical processes in association with the normal biochemical milieu of a living system, and the second one is the lack of availability or impracticality of removing suitable samples from a living system, frequently because of size.
SPME, using an externally coated extraction phase on a microfibre mounted in a syringe-like device seems to be a logical candidate for the development of such tools. As in any microextraction approach, compounds of interest are not exhaustively removed from the investigated system. On the contrary, conditions can be devised where only a small proportion of the total compound is removed, thus avoiding a disturbance of the normal balance of chemical components. Secondly, because SPME is based on a syringe-like device that can be physically removed from the laboratory environment for sampling, it is amenable to monitoring a living system in its natural environment, rather than trying to move the living system to an unnatural laboratory environment. The recently developed fast *in vivo* microextraction technique has the potential to partly replace current methods of analysis based on blood draws. *In vivo* microextraction is faster than conventional methods, interferes minimally with the investigated system, minimizes errors associated with sample preparation, and limits exposure of lab personnel to hazardous biological samples.
The use of solid-phase microextraction (SPME) for *in vivo* sampling of drugs and metabolites in the bloodstream of freely moving animals eliminates the need for blood withdrawal in order to generate pharmacokinetic profiles in support of pharmaceutical drug discovery studies. This is particularly important for small rodents such as mice because it enables the use of a single animal to construct the entire pharmacokinetic profile. This approach was achieved using *in vivo* SPME probes with biocompatible extractive coating. Pre-equilibrium *in vivo* SPME sampling and liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS) were used to simultaneously determine free and total circulating concentrations of carbamazepine and its metabolite carbamazepine-10,11-epoxide in mice after 2 mg/kg intravenous dosing. Standard-in-fibre calibration method was used for quantitative analysis. The method was linear in the range of 1-2000 ng/mL in whole blood with acceptable accuracy (>70%) and precision (<23% RSD). The pharmacokinetic results compare well to the traditional methods relying on blood withdrawal but *in vivo* SPME offers the advantages of speed, decreased animal use, improved accuracy of data due to the elimination of inter-animal variation from the profile, and the ability to obtain both free and total drug concentration from the same experiment.

LP.2 RECENT INNOVATIONS IN FLUID-BASED SEPARATION TECHNIQUES AND THEIR IMPACT ON OUR DAILY WORK

Pat Sandra
Research Institute for Chromatography, Kennedypark 26, B-8500 Kortrijk, Belgium

The current trend of LC analyses is biased toward high throughput, high productivity and high resolution. In response to these increasingly demanding requirements, over recent years, innovative technologies and improvements in instrumentation have emerged which are having a significant impact on our daily work. Ultra High Pressure LC (UHPLC) and Elevated Temperature (ETLC) both have extended speed, productivity and peak capacity for pharmaceutical, environmental, food, and bio analyses. Both developments in combination with the more or less forgotten technique i.e. sub- and supercritical fluid chromatography, offer unforeseen possibilities to produce accurate and precise data.
On the other hand, for the separation of mixtures of very high complexity both the selectivity and peak capacity have to be optimized in multidimensional systems. Recent developments from single to complex configurations will be reviewed with emphasis on hardware design, software approaches and column configurations.
The applicability of the different combinations will be illustrated and guidelines on "what configuration is preferred for a given sample" will be presented. Configurations that will be discussed in detail are: gradient POPLC, selectivity optimization by column coupling (HILIC-RPLC), heart-cutting techniques and the comprehensive techniques NPLC×RPLC, SFC×RPLC, RPLC×RPLC, NPLC×2RPLC and RPLC×2RPLC. A comparison on-line/off-line will be made and the role of mass spectrometry highlighted.

LP.3 PAPEL DE LA CROMATOGRAFIA EN LA ELUCIDACIÓN DEL AROMA DEL VINO

Juan Cacho Palomar
University of Zaragoza, Espanha

lições meias-plenárias

LMP.1 METABOLOMICS, A NEW APPROACH TO BIOANALYSIS WITH SEPARATION TECHNIQUES AND MASS SPECTROMETRY

Coral Barbas[1]; Antonia Garcia[1]; Angel Luis Huidobro[1]; Isabel Garcia-Perez[1]; Santiago Angulo[1]; Jaona Teul[1,2]; Michal Ciborowski[1,2]; Mª Paz Martinez-Alcazar[1]; Javier Ruperez[1]
[1]Facultad de Farmacia. Universidad San Pablo-CEU. Boadilla del Monte. 28668 Madrid. Spain; cbarbas@ceu.es.
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A new approach to bioanalysis looking for biomarker discovery is the so called metabolic fingerprinting, a non targeted analysis where metabolite patterns are acquired and compared with limited a priori knowledge of the metabolites of interest.

The chemical diversity of the metabolites is enormous in addition to a large dynamic concentration range. Therefore, no single analytical platform can capture all metabolites in one sample and a wide variety of techniques and methods need to be used to obtain a non-biased approach to the components of the metabolome. Among them, separation techniques such as CE, GC-MS and LC-MS are adding new information to other classical tools mainly ^{1}H-NMR.

Raw data, coming from different analytical platforms should be pre-treated through baseline correction and normalization to make them ready for multivariate statistical tools. If after that analysis clusters among samples corresponding to a disease, diet or any other situation under study are revealed, the variables accounting for that classification will be identified as the (bio)markers. The identity of the signals of interest from the fingerprint can subsequently be made known by metabolite identification procedures. Finally a biological interpretation is required to transform chemical information into real metabolic knowledge.

Our group is currently working on developing and applying robust analytical methods and chemometric tools for metabolic fingerprinting of different samples and pathologies with separation techniques. Examples will be presented for plasma analysis of patients with cardiovascular disease by GC-MS, Schistosoma infection in mice urine by CE and urine analysis by LC-MS.

LMP.2 EXHALED BREATH AS A POTENTIAL SOURCE OF DISEASES RELATED METABOLITES: THE ROLE OF THE SPME–GC METHODOLOGIES

Sílvia M. Rocha
Departamento de Química. Universidade de Aveiro. 3810-193 Aveiro,
smrocha@ua.pt

Exhaled breath is a potential rich source for countless biomarkers that can provide valuable information about respiratory as well as systemic diseases. Breath analysis, a non-invasive technique, has been used as a promising tool for biomarkers detection. A biomarker has been defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention', that in medicine is used to detect disease states. Several breath biomarkers have been reported: aldehydes and hydrocarbons in cancer, and aldehydes, glutathione, pentane, exhaled nitric oxide and H_2O_2 in asthma, among others. The potential clinical utility of biomarkers has been questioned, as same of them are not specific and because of the lack of information about intra- and inter-individual variability. In spite of the higher potential of these biomarkers, they do not provide information about the metabolic patterns associated to the different diseases. But the exhaled breath should be considered more than a biomarker, it is a complex matrix in which countless compounds may be identified, and until the present, a low turnover has been achieved from that matrix.

The emerging field of metabolomics has been focused on an improved understanding of biological networks by systematic and comprehensive analysis of metabolism, promising immense potential for early diagnosis, therapy monitoring and for understanding the pathogenesis of many diseases. Metabolomic methods are mostly focused on the information-rich analytical technique of NMR spectroscopy. Analysis of the data from this high-resolution method using advanced chemometric approaches provides a powerful platform for clinical research and diagnostic applications. Recent applications in the area of cancer, diabetes, inborn errors of metabolism and cardiovascular diseases have been developed. However, higher detailed information should be achieved if more sensitive methodology will be used. High-sensitivity detection and efficient sample preparation are keys to medical breath analysis. High-performance equipment, such as GC-MS and GCxGC-TOFMS combined with SPME, as well as a high degree of skill are necessary to expand our basic knowledge and to define diseases metabolomic patterns. GC-MS is one of the most frequently used tools for profiling metabolites. Instruments are mature enough to run large sequences of samples; novel advancements increase the number of compounds that can be analyzed, and improved algorithms and databases are employed to capture and utilize biologically relevant information. The separation potential of GCxGC is greatly enhanced when compared to the one-dimensional GC. SPME is a rapid, solvent-free and sensitive approach, highly applied into the field of bioanalysis.

On this talk, particular attention will be done to the role of the SPME-GC based methodologies in the study of the allergic respiratory diseases (ARDs). Nowadays, ARDs represent an important public health issue with a significant growth over the years, including several types of pathologies where rhinitis and asthma allergics play an important role. Particular attention was devoted to the paediatric population as the respiratory diseases represent the major cause of illness in children of developed countries.

LMP.3 ADSORPTIVE MICROEXTRACTION (AμE) TECHNIQUES - NEW TECHNOLOGIES FOR TRACE ANALYSIS OF POLAR COMPOUNDS

J.M.F. Nogueira
University of Lisbon, Faculty of Sciences, Chemistry and Biochemistry Department, Center for Chemistry and Biochemistry, Campo Grande, Ed. C8, 1749-016 Lisbon, Portugal;
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During the last years, sorptive extraction techniques have been extensively developed and applied for trace analysis of many classes of organic compounds in several types of matrices. Solid phase extraction, solid phase micro-extraction and nowadays, stir bar sorptive extraction, are some good examples of the mostly used sample enrichment techniques prior to chromatographic analysis.

Meanwhile, if we focused our attention just on polar compounds, some of these methodologies present great limitations on the recovery yields, even by using convenient polymeric phases. On the other hand, it is well known that polar metabolites are more conveniently adsorbed in grains of specific solids with suitable polar active sites, besides the very difficult way to manipulate these types of fine and divided materials after the enrichment process. Recently, our group has been involved in the development of novel analytical approaches, denominated adsorptive microextraction (AμE) techniques, which represent a great alternative to monitor a wide range of polar analytes in real matrices. These methodologies can be applied through small bars (15 mm in length and 3mm in diameter) or in multi-spheres (diameter ≈ 2mm), where specific adsorbent materials are fixed, such as activated carbons, styrene-divinylbenzene polymers, etc. Since most of the polar compounds presenting non-volatile or termolabile properties, suitable liquid desorption followed by liquid chromatography systems are definitely the methods of choice.

In the present contribution, will be discuss in detail the preparation, development, validation and application of these new analytical technologies (AμE) for the enrichment of trace levels of several classes of polar metabolites (*e.g.* water disinfection by-products, pharmaceutical products, pesticides, drugs of abuse, phenolic acids, etc.) in many types of real matrices.

References

N.R. Neng, J.M.F. Nogueira
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Pedido Provisório de Patente, Instituto Nacional de Propriedade Industrial, Nº 20091000096389 (2009).

LMP.4 GC×GC AS A NEW TOOL TO INVESTIGATE THE ELECTROKINETIC REMEDIATION OF ORGANIC POLLUTANTS FROM COMPLEX MATRICES: THE REMOVAL OF CREOSOTE FROM TREATED TIMBER WASTE

E. Mateus[1], A. Ribeiro[1], J. Zrostlíková[2], M. D. R. Gomes da Silva[3], P. Marriott[4]
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[2] LECO Instrumente Pilsen – Aplication Laboratory Prague, Sokolovská 219, 190 00 Prague 9, Czech Republic
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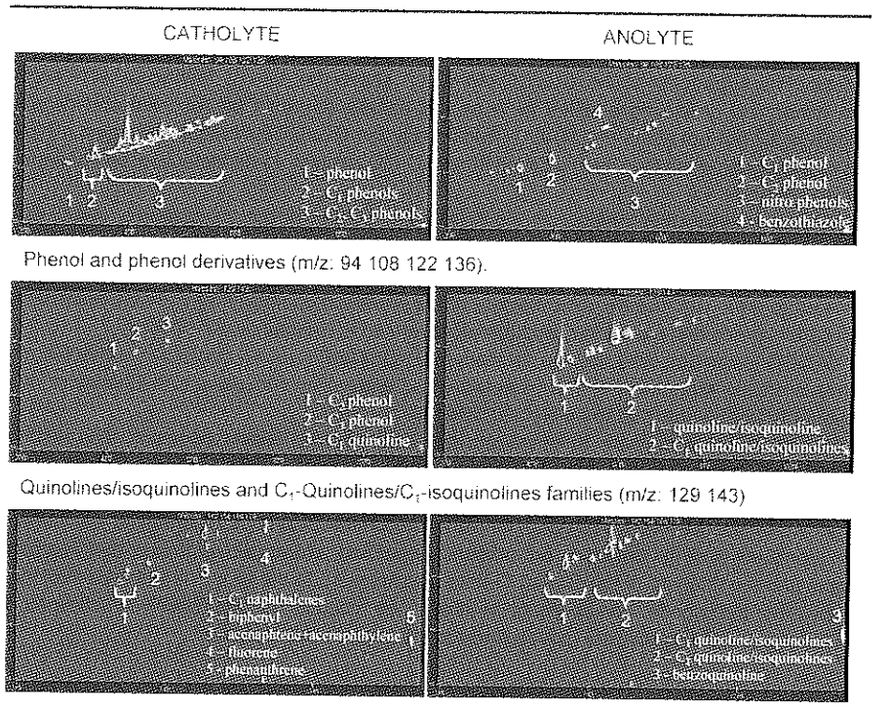
[4] ACROSS, Department of Applied Chemistry, RMIT, University, GPO Box 2476V Melbourne Victoria 3001, Australia

A study has been conducted to illustrate the usefulness of the GC×GC-technique over the classic 1D-GC technique to monitorize the remediation of organic pollutants from complex matrices. The superiority of the GC×GC-technique above the 1D-GC technique were proven when minor and co-eluting analites such as the azaarenes could be resolved and detected using GC×GC but not using the 1D-GC. Additionally the GC×GC-technique displayed a higher level of accuracy in analyte identification than 1D-GC system when detecting compounds in complex mixtures.

Creosote is a distillation product of coal tar that is one of the most widely used wood preservatives. Chemically creosote is a variable and complex mixture composed of various compounds, where around 300 have been identified, among the 10000 chemicals that are estimated to be in the mixture. The majority of the identified chemicals have been classified as toxic, carcinogenic and mutagenic, and therefore related to harmful health effects

The GC×GC technique was applied to study the applicability of electrokinetic remediation to remove creosote contaminants from treated wood wastes and to assess the behavior of its components when submitted to an electric field.

The electrolyte solutions were collected and extracted by solid phase extraction. The resulting extracts were analysed by one dimensional gas chromatography hyphenated with mass spectrometry (1D-GC/MS) and comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC/TOFMS). The chemical groups of creosote components were identified and its behavior on process described. Polycyclic aromatic hydrocarbons, phenols and the majority of the S- and O-heterocycles were found to move in the electrokinetic cell towards the anode compartment whereas the majority of the positively charged N-heterocycles (aza-heterocycles) moved towards the cathode compartment.



F1 Structured GCxGC/ToF-MS extracted ion chromatograms for the anolyte and catholyte solutions for some group family compounds.

LMP.5 ONLINE SAMPLE PREPARATION AND LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY FOR ENVIRONMENTAL AND FOOD ANALYSIS

Cláudia P. B. Martins[1], Dipankar Ghosh[2]
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Achieving low limits of detection for the analysis of pesticides, emerging pollutants, veterinary drugs and many other low level residues is essential to meet European regulatory guidelines. LC-MS/MS is the main tool used to perform quantitative and analysis of these residues in both environmental and food samples. However, most of the methodology proposed for these analytes requires extensive offline sample preparation, which can be time consuming and expensive. As a response to this, new methods are being developed for the determination of these compounds in a cost effective way, which combines on-line sample preparation and LC-QqQ-MS/MS. In addition, minimizing sample handling improves the performance characteristics of the method like recovery, repeatability and reproducibility.

This presentation will discuss the applicability of on-line sample preparation tools on the analysis of both environmental and food samples. Data will be shown regarding the analysis of pesticides and emerging contaminants in environmental samples using Equan-LC-QqQ-MS/MS. Furthermore, information will be discussed regarding the analysis of multi class antibiotics in honey and milk samples using TurboFlow™ technology combined with LC-QqQ-MS/MS.

In addition, new software (TraceFinder™) which has been developed specifically to help improve throughput in residue laboratorie, by incorporating built-in LC-MS methods for a large number of contaminants, will be discussed. The use of on-line sample preparation tools combined with Trace Finder software significantly increases productivity by drastically reducing analysis time.

LMP.6 X-METABOLOMICS: THE X-WINDOWS HIGH-TROUGHPUT MASS SPECTROSCOPY METABOLOMICS PIPELINE

Martins, R.C.[1*], Castro, C.C.[2] Teixeira, J.A.[2], Silva Ferreira, A.C.[3]
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X-metabolomics is our mass spectroscopy signal (MS) processing pipeline implemented with the X-window interface, based on R statistical programming environment for comprehensive statistical computing and access to bioconductor bioinformatics platform under Unix, Linux and Mac OS X. X-metabolomics is primarely intented for fast MS spectral validation by multivariate process analytical technology and metabolomic scientific research by spectral fingerprinting, high-troughput compound identification and quantification. For increased flexibility, we included direct importation of processsed chromatograms from MetaAlign and MzMine. Xmetabolomics imports MS chromagrams in the NetCDF format and can use also standard XCMS peak alingment and feature extraction algorithms (e.g. bin-lin and centWave). All configurations for filtering and feature extraction are located on a text file (X-metabolomics.conf) which can be changed and reloaded for new configurations.

X-metabolomics processing pipeline works as follows: i) importing chromatograms (directory or selected samples); ii) peak extration and aligment; iii) supervised filtering; iv) fragment classification, identification and quantification; v) fingerprinting methodologies using multivariate statistics; vi) building the metabolites identification and quantification tables.

The software is currently a tool designed for aiding our metabolomics research, providing a usefull laboratory tool for fast GC-MS and LC-MS fingerprinting diagnosis. assessment of composition and results quality control by multivariate chart control.

comunicações orais

CO.1 SOLID-PHASE MICROEXTRACTION NI-TI FIBERS COATED WITH PARTICLES IMMOBILIZED IN A SOL-GEL MATRIX

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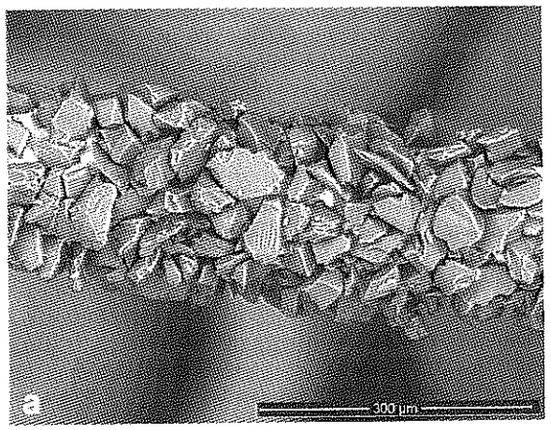
One of the possible approaches for the development of novel solid-phase microextraction fibers is the physical deposition of porous materials onto a support using high temperature epoxy glue. However, a major drawback arises from decomposition of epoxy glue at temperatures below 300ºC and instability in some organic solvents. This limitation motivated us to explore the possibility of replacing the epoxy glue with a sol-gel film, thermally more stable and resistant to organic solvents.
We found that functionalised silica particles could be successfully attached to a robust Ni-Ti wire by usage of a UV-curable sol-gel film (figure 1).



F1 SEM image of a NiTi wire covered with sol-gel immobilised silica particles

The particles were found to be more important than the sol-gel layer during the microextraction process, as shown by competitive extraction trials and by the different extraction profiles observed with differently functionalised silica particles. If a quality control microscopic-check aiming at the rejection of fibers exhibiting unacceptably low particle load was conducted, acceptable (6-14%) reproducibility of preparation of C18-silica fibers was observed, and a strong indication of the durability of the fibers was also obtained.
A cyclohexyldiol-silica fiber was used, as a simple example of applicability, for the successful determination of benzaldehyde, acetophenone and dimethylphenol at trace level in spiked tap water. Recoveries: 95-109%; limits of detection: 2-7µg/L; no competition effects within the studied range (≤ 125µg/L)[1].
The developed method is intended to be generalised to other specialized materials such as restricted access materials or molecularly imprinted materials existing in the particulate format only (or not amenable to obtain as a thin film for SPME), thus allowing that unique sorption properties can be put to use of SPME technique. The present communication will include results from ongoing work related with the usage of molecularly imprinted polymer particles.

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CO.2 DEVELOPMENT OF A SIMPLE AND SOLVENT FREE METHODOLOGY BASED ON HS-SPME FOR CHARACTERIZATION OF FREE AND GLYCOSIDICALLY-LINKED COMPONENTS OF MUSTS

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The aroma is one of the most important factors to determine the wine character and quality. Several studies recognized a relationship between the wine character, and the grape and musts volatile compounds, namely terpenoids, norisoprenoids and C_6 alcohols[1]. Volatile compounds appear in the free and/or glycosidically-linked forms. The formers present the volatile compounds as their aglycons, which may be released by acid and/or enzymatic treatments. The volatiles are generally present in trace amounts and require a previous step of isolation and concentration, followed by gas chromatographic analysis. Several works have been developed for characterization of free and glycosidically-linked volatile compounds in grapes, musts and wines using solid-phase extraction (SPE) and liquid-liquid extraction (LLE) followed by gas chromatography - mass spectrometry (GC-MS)[3]. Nevertheless, SPE and LLE are based on the use of organic solvents, and present some drawbacks, such as the possibility of introduction of solvent artefacts, the loss of analytes during the concentration step, and are time-consuming. Thus, the establishment of a suitable extraction procedure combined with an analytical methodology is always an important challenge to the chemists and biochemists that work on wine chemistry. Solid-phase microextraction (SPME) appears as a potential tool, as integrates sampling, extraction, concentration and sample introduction into a single step. SPME is rapid, easy to use, solvent free, sensitive and not requires any concentration step prior analysis, preventing production of artefacts[2].

In these work, it was developed a headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) methodology for the characterization of free and glycosidically-linked volatile compounds of musts. This work was done using musts obtained from Malvazia Cândida *Vitis vinifera* L., harvest 2008, from Madeira Island. Characterization by SPE and LLE were also applied as a comparative approach.

The free fraction contained a total of 55 volatiles identified by HS-SPME/GC-MS whilst using the SPE/GC-MS methodology only 27 compounds were identified. The glycosidically-linked volatiles were extracted, after enzymatic hydrolysis, by LLE and HS-SPME methodologies. A total of 43 volatiles were detected by HS-SPME/GC-MS, a value higher than that obtained by LLE/GC-MS (15 compounds). The SPME allowed the detection of 2 to 3 times more volatiles than the SPE and LLE. In order to increase the SPME extraction efficiency, several experimental parameters were tested: type of coating fibre, extraction time and temperature, ionic strength, sample volume (expressed as 1/β), and desorption time and temperature. According to the data obtained, it was selected the following parameters: divinylbenzene-carboxen-polydimethylsiloxane SPME coating fibre (50/30μm), extraction temperature of 60 °C and 45min of extraction time, 1/β of 0.5, desorption temperature of 250°C and 7min of desorption time. This simple and solvent free methodology allowed to identify up to 55 volatile compounds, in the free and glycosidically-linked fractions, distributed over the chemical groups of mono and sequiterpenoids, norisoprenoids, C_6 alcohols and aldehydes. This data allowed to establish the aroma potential of each variety, which represents a powerful approach to help the winemaker decision.

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CO.3 ANALYSIS OF EUCALYPTUS VOLATILE EMISSIONS UNDER DIFFERENT FERTILIZATION REGIMES BY HS-SPME, GC-FID and GC/MS.

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In order to assess the influence of nutrient availability upon the main carbon-based secondary metabolites emitted by eucalyptus leaves, the emission patterns from leaves of *Eucalyptus globulus* under different fertilization regimes were analized by gas chromatography (GC-FID) and mass spectrometry (GC/MS) after headspace extraction by solid phase microextraction (HS-SPME).Only monotorpenes and sesquiterpenes were taken into account once these terpenes are considered as the most important carbon based secondary metabolites in plant–insect olfactory communication[1,2].
The leaves of juvenile plants of *E. globulus*, submitted to different fertilization treatments and kept under green house conditions were compared. The *E. globulus* samples were took from experiments conducted at Quinta do Furadouro, Óbidos – Portugal, by SilviCaima.
The volatiles emitted by the eucalyptus leaves were extracted using a Supelco® 100μm PDMS (polydimethylsiloxane) fiber. Each extraction was performed for 45 minutes. Samples were analysed by gas chromatography (GC-FID). All samples were injected by splitless and the volatiles separated in an apolar column (DB-5) and in a polar column (DB-Wax), allowing the elimination of possible co-elution events.
Compounds were identified by mass spectrometry (GC/MS) in combination with authentic reference compounds and their estimated retention indices (GC-FID and GC/MS). The obtained spectra and the experimental retention indices were compared with the Adams[3] and the NIST 98 mass spectra database libraries.
Significant differences among fertilization treatments were encountered, regarding emissions by *E. globulus* leaves of α-pinene ($P<0.01$), α-phelandrene ($P<0.001$), limonene ($P<0.05$), 1,8-cineol ($P<0.05$), γ-terpinene ($P<0.001$), α-terpineol ($P<0.01$), β-charyophillene ($P<0.01$), alloaromadendrene ($P<0.05$) and bicyclogermacrene plus γ-amorphene ($P<0.05$). Furthermore, a factor analysis showed that fertilization treatments could be discriminated based upon the previous monoterpenes and sesquiterpenes.
Results shows indicate a clear influence of fertilization upon the volatile emissions of *E. globulus*, and suggest that different nutrients may influence the emission of different terpenes.

Key Words – HS-SPME, GC-MS, terpenes, *Eucalyptus globulus*, leaves, fertilization

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CO.4 DETERMINATION OF METHADONE AND ITS METABOLITE EDDP IN HAIR BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Alternative specimens are gaining increasing relevance in toxicological analysis. Amongst these samples, hair plays a special role for several reasons, e.g. the fact that its collection is not invasive, its adulteration is difficult, and the windows of detection for certain drugs can be strongly enhanced. Hair is often called a timetable of drug exposure, and this characteristic allows segmental analysis, which assumes particular importance in workplace drug testing and methadone maintenance programmes.

Ten milligrams of finely divided hair were incubated with 1M sodium hydroxide for drug extraction (50ºC for 45min). After this incubation, the samples were neutralized with an equimolar amount of hydrochloric acid, and centrifuged at 3500rpm for 3min. The samples were decanted to polypropylene test tubes and 6mL of 0.1M potassium dihydrogenphosphate were added. Fifty microlitres of an internal standard mixture (deuterated analogues of both compounds at 2μg/mL) were added, and after being homogenized by rotation/inversion movements, the samples were subjected to mixed-mode solid-phase extraction. The extracts were evaporated to dryness at 50ºC under a gentle nitrogen stream, dissolved in 50μL of ethyl acetate, transferred to autosampler vials and an aliquot of 3μL was injected onto the chromatographic system with a split ratio of 3:1. The ions were monitored at m/z 277, 276 and 262 for EDDP; and at m/z 72, 223 and 294 for methadone. Only one ion was monitored for the internal standards, at m/z 280 for EDDP-d_3 and at m/z 297 for methadone-d_3.

No interferences by endogenous compounds or several other drugs that might be present in an authentic sample have been observed by analysis of blank specimens of 10 different origins. The method was linear ($1/x^2$) from 0.1-30ng/mg for both analytes, presenting correlation coefficients higher than 0.9914. Repeatability, intermediate precision and accuracy were in conformity with internationally accepted guidelines for bioanalytical method validation[1,2]. Additionally, imprecision has been calculated by analysis of an authentic sample (n=15), and was less than 16% for both analytes. Clean-up efficiency, calculated at four concentration levels, was higher than 90% for both analytes. The method was successfully applied to authentic postmortem hair samples.

The presented method has shown to be adequate for the determination of methadone and EDDP in hair samples, and can be used in forensic and clinical situations were these compounds are involved.

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CO.5 DEEPER INSIGHT INTO THE SESQUITERPENOIDS PROFILE OF *MATRICARIA RECUTITA* L.: BIOSYNTHESIS MODULATED BY THE AGRICULTURAL PRACTICES

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Matricaria recutita L., known as chamomile, is included into all over the world pharmacopoeias due, among others, to its relevant health benefits. In modern phytotherapy, chamomile has been used for its anti-microbial, anti-malarial, anti-inflammatory, anti-oxidant, and other activities. These health benefits are related to several groups of biologically active compounds, among which the sesquiterpenoids of inflorescences are of greatest importance[1]. This group comprises the α-bisabolol, nerolidol, and chamazulene, which are known as therapeutically active compounds. The sesquiterpenoids of chamomile has been studied directly in the inflorescences, or in the essential oil usually obtained by steam-extraction distillation (which represents a time-consuming methodology). The headspace solid-phase microextraction (HS-SPME) followed by one-dimensional gas chromatography coupled with mass spectrometry detection (GC-MS) has been frequently applied to characterize the sesquiterpenoids of several natural products. Although such methods often provide rewarding analytical results, the complexity of many natural matrices exceeds the capacity of any single separation system. As a consequence, comprehensive two-dimensional gas chromatography (GCxGC) represents an interesting approach. This work aims to obtain a detailed characterization of sesquiterpenoids released to the headspace by the inflorescences of *M. recutita* L. To fulfil this objective, a HS-SPME/GC-qMS methodology was implemented.

The proposed methodology was applied to fourteen populations obtained from different geographic origins, agricultural practices, and harvest times. For the populations under study, the samples from biological production systems showed a tendency to have lower amount of sesquiterpenoids when compared to those obtained from the conventional production systems. Moreover, the biosynthesis of sesquiterpenoids seems to be modulated by the agricultural practices. In order to obtain a deeper insight into the sesquiterpenoids from *M. recutita* L., GC×GC–ToF-MS was also applied as a comparative approach. A set comprising a non-polar (HP5) and a polar (DB-FFAP) capillary column was used. The plot of the first dimension retention times *versus* the second dimension was obtained using the *m/z* 93, 161 and 204 ions. This study proposes a methodology and provides data that can be used as a prediction tool to evaluate the potential of *M. recutita* L. populations as sources of secondary metabolites of interest to human health just by direct analysis of their inflorescences. Twenty seven sesquiterpenoids were reported for the first time in inflorescences of chamomile.

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CO.6 CHARACTERIZATION OF PHENOLIC FRACTION OF CORK FROM *QUERCUS SUBER* L. BY GC-MS AND HPLC-MS

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The valorization of the by-products of the forestry industry constitutes an ever more important challenge on the development of a sustainable economy and of environmentaliy friendly industrial processes. These by-products are seen, in recent years, as promising sources of renewable chemicals, materials and fuels and as a response to the inevitable depletion of fossil resources within the emerging "bio-refinery" concept[1].

Cork is the outer bark of *Quercus suber*, a common species in the Mediterranean region and because of its peculiar properties[2], cork has a large variety of applications. Portugal produces about 157000ton of cork per year, which represents about 53% of the world production. This industry generates substantial amounts of a residue, called "cork powder", which represents in Portugal, about 40000ton per year, 20% of the total cork production. This by-product, that is not suitable for current industrial uses, is currently burned to produce energy[3].

In the new "bio-refinery" concept, the full exploitation of this resource and specially the detailed study of its chemical composition is a key step towards the recovery of this sub-product and will play an important role in the national context.

The detailed chemical composition of the lipophilic extractives of cork and cork by-products has been recently investigated by Sousa *et al.*[4], demonstrating that this fraction could be an interesting source of bioactive triterpenic compounds. However, the information available on the phenolic fraction of cork is scarce, despite the fact that this group of components be known by its vast range of applications and also by their easy extraction from those residues.

In the present work we report the quantification and detailed characterization of the phenolic fraction of cork extractives by gas chromatography-mass spectrometry and by high pressure liquid chromatography-mass spectrometry. After removal of the lipophilic fraction from milled cork samples by Soxhlet extraction with dichloromethane, the phenolic fraction was extracted by two different multiple extraction approaches: sequential extraction with methanol and water; and a single extraction with methanol:water (80:20) followed by fractionation with diethyl ether[5]. A large number of phenolic compounds were identified in these extracts, including compounds identified for the first time as cork constituents.

Acknowledgments
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CO.7 DESENVOLVIMENTO DE UMA METODOLOGIA ANALÍTICA PARA DETERMINAÇÃO DE ANTIBIÓTICOS EM AMOSTRAS AMBIENTAIS

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A sociedade tem vindo a acentuar a preocupação com os poluentes emergentes ainda não regulamentados. Um grupo diversificado de substâncias químicas bioactivas que tem recebido especial atenção como poluentes prioritários ambientais são os produtos farmacêuticos, de higiene e cuidado pessoal. Entre os produtos farmacêuticos, a presença de antibióticos no ambiente aquático tem tido enorme relevância, uma vez a exposição prolongada mesmo para concentrações vestigiais poderem promover resistência a microrganismos. O elevado consumo destes compostos, a excelente solubilidade em água e a incompleta remoção nas estações de tratamento de águas residuais tem originado a sua presença no ambiente. A ocorrência vestigial destes compostos, a complexidade de algumas matrizes ambientais assim como as propriedades físico-químicas, nomeadamente a elevada polaridade, constituiem um desafio para o desenvolvimento de métodos alternativos para enriquecimento prévio para combinação com técnicas cromatográficas adequadas.

No presente trabalho, propõe-se uma nova metodologia para determinação de diversos antibióticos (trimetoprim, enrofloxacina, sulfatiazol, sulfadimetoxina e sulfametaxazol) em amostras ambientais utilizando uma nova técnica de extracção com fase polimérica à base de poliestireno-divinilbenzeno, seguida de análise por cromatografia líquida de alta eficiência com detecção por rede de díodos e hifenada a espectrometria de massa *Tandem* por *electrospary* (SBSE(PU)-LD/HPLC-DAD-(ESI)MS/MS). O método foi optimizado tendo demonstrado boa gama dinâmica de linearidade, limites de detecção ao nível das partes-por-trilião (ppts) e boa resposta por aplicação a amostras ambientais. O método desenvolvido demonstrou ser simples, sensível, com utilização reduzida de solventes torna-o ambientalmente preferível em relação a outros métodos de microextracção para a determinação de antibióticos em amostras ambientais.

Agradecimentos
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CO.8 ARTIFICIAL NEURAL NETWORK CLASSIFICATION BASED ON HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DATA OF LOW MOLECULAR WEIGHT PHENOLIC COMPOUNDS

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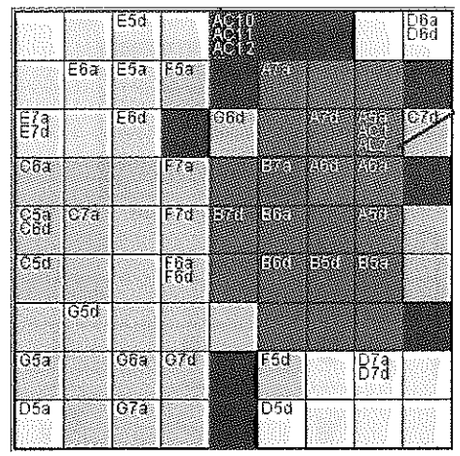
The importance of phenolic compounds in wine is very well established due to their influence in colour, flavour and astringency. Non-flavonoid compounds include benzoic, cinnamic acids and aldehydes, usually named low molecular weight phenols. Although being present in small amounts in wines they play an important role in sensory quality and may contribute, through an additive effect, to bitterness and harshness, specially cinnamic acids.
In this work, a reversed phase liquid chromatography-DAD method is proposed for analysis of major non-flavonoid phenolic compounds, during three years, in monovarietal wines of seven different varieties: Trincadeira, Cabernet Sauvignon, Aragonez, Touriga Nacional, Castelão, Alforcheiro and Alicante Bouschet. The method was used to evaluate the impact of spontaneously malolactic fermentation (MLF) in low molecular phenolic compounds.
For Trincadeira we also analyzed wines that underwent different treatments, like the addition of a pectolytic enzyme or lysozyme, and the way malolactic fermentation was carried out, spontaneously or with the inoculation of two different commercial lactic bacteria.
The use of an artificial neural network allow to separate the seven varieties in seven different groups and among each group according to year.
The impact of the MLF was also verified for Trincadeira wines.



F1 A – Trincadeira; B – Cabernet Sauvignon; C – Aragonez; D – Touriga Nacional; E – Castelão; F – Alforcheiro; G – Alicante Bouschet; 5 – 2005; 6 – 2006; 7 – 2007; a – before MLF; d – after MLF

CO.9 METABOLOMIC ANALYSIS OF NATURAL PRODUCTS

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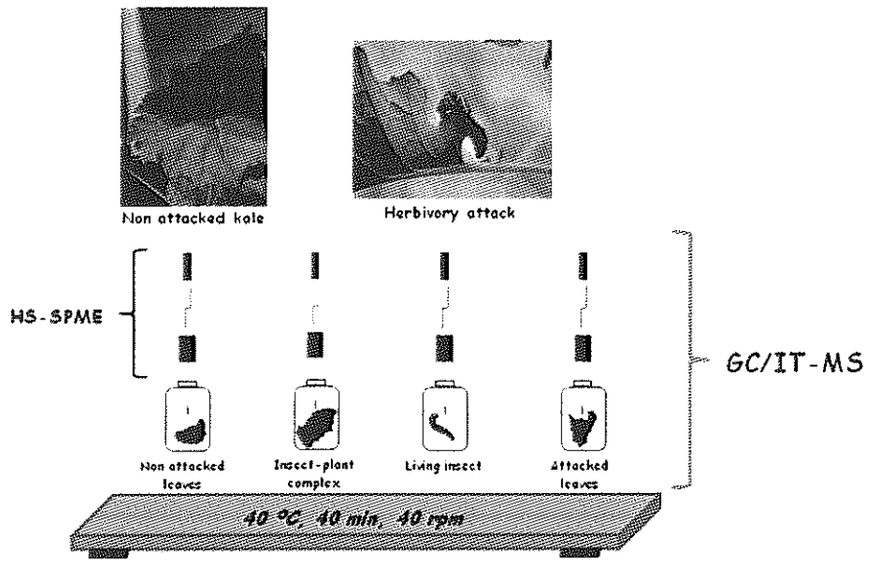
The metabolome comprises all metabolites in a biological organism, which constitute the end products of its gene expression. Metabolomics consists on the systematic study of the chemical fingerprints resulting from specific cellular processes or, more particularly, the study of an organism's profile of low molecular weight metabolites. Thus, metabolomics is perhaps the ultimate level of post-genomic analysis, as it can reveal changes in metabolite fluxes that are controlled by only minor changes within gene expression.
Classical phytochemical approaches often comprised a rather tedious and time consuming process of isolation, dereplication of known substances, followed by structure elucidation and quantification. However, it is important to highlight that, in many situations, the effects of natural products are not due to a single compound, but to a mixture of related and unrelated ones. Thus, metabolomics provides an efficient tool for the quality control and authentication of medicines of natural origin, contributing as well to the characterization of different species. Several combined techniques have been applied in the measurements of intracellular metabolites, whether qualitative or quantitative, which reveal the biochemical status of the organism. This work offers an insight on the methods used in the metabolomics analysis (LC-MS, GC-MS, HPLC-DAD, NMR) of several natural matrices (Figure 1) with protective health potential, with special emphasis on the determination of phenolics profiles, once these represent the most abundant and widely spread class of plant natural compounds, additionally exhibiting interesting biological activities, also discussed.



F1 HS-GC-MS analysis of a living insect.

CO.10 HPLC-DAD-ESI-MS^n SEPARATION AND CHARACTERIZATION OF PHENOLIC COMPOUNDS IN *HELICHRYSUM DEVIUM* Johns. FLOWERS AND EVALUATION OF ITS ANTIOXIDANT ACTIVITY BY ONLINE HPLC-DPPH METHOD.

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Helichrysum devium Johns. is an endemic sub-specie of *Helichrysum* (*Asteraceae*) growing up in Madeira Archipelago (Portugal). This plant is used in the local traditional folk medicine for treating bronchitis and pharyngitis.

In this study, the methanolic extract of the flowers of *H. devium* was analysed by an HPLC-DDPH on-line method for screening the presence of radical scavenging compounds: when radical scavengers are present, the DPPH radical is reduced to a colourless product[1]. This reduction is observed after an HPLC separation by a visible wavelength detector as a decrease in absorption at 515 nm. The main radical scavenging compounds were identified as quinic acid derivatives by their UV and mass spectra.

In order to fully characterize this sample in terms of its phenolic composition, a different method was applied using high-performance liquid chromatography with on-line UV and electrospray ionization mass spectrometry. A total of 23 compounds were identified or tentatively characterized based on their UV and mass spectra and retention times. Three of these compounds were positively identified by comparison with reference standards. The phenolic compounds included derivatives of quinic acid, flavonols *O*-glycosides (quercetin, isorhamnetin and kaempferol), flavones *O*-glucosides (apigenin and luteolin), caffeic acid derivative and protocatechuic acid derivative. The characteristic loss of 206Da from malonylcaffeoyl quinic acid was used to confirm the malonyl linkage to the caffeoyl group.

This contribution presents one of the first reports on the analysis of phenolic compounds from *Helichrysum devium* flowers using LC-DAD-ESI-MS^n.

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CO.11 POTENTIALITIES OF COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY TIME-OF-FLIGHT MASS SPECTROMETRY (GC×GC-TOF-MS) IN ENVIRONMENTAL CHEMISTRY. AN OVERVIEW OF ITS APPLICATIONS, BEHAVIOUR AND PERSPECTIVES.

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Comprehensive two-dimensional gas chromatography (GC×GC) technique has been developed in the late nineties but it is still in expansion. Its main benefits include: (i) an increased chromatographic resolution, (ii) improved analyte detectability due to the focusing in the modulator and (iii) chemical class ordering in the contour plots. When coupled to a time-of-flight mass spectrometer (TOF-MS) its potentialities are still increased as the fast mass detection system is specially fitted to the GC×GC requirements.

A general trend in environmental monitoring is the periodical updates of the target analyte lists by the regulatory agencies. Both EU and USEPA have issued dangerous and hazardous contaminants lists, and several emerging pollutants are under scrutiny. Therefore, new analytical methodologies are needed in order to monitor those contaminants. Both dedicated and multi-rersidue approaches have to be developed and these methodologies need to be able to determine trace level target analytes (in the ppt range) in complex samples such as aqueous matrixes. In this communication, we will overview the existing environmental chemistry methodologies based on GC×GC-TOF-MS highlighting its suitability for these kind of applications and we will present some new results on emerging contaminants detection and multi-residue approaches based on a Leco Pegasus 4D system.

We have seen that one of the main characteristics of GC×GC is that we can obtain chemical class ordering in the contour plots. We will present the behaviour of this chemical ordering for a broad range of chemicals and using different chromatographic systems. Moreover, the potential application of this feature in target analyte identification confirmation, emerging pollutants detection and new metabolites identification will be discussed.

CO.12 AUTOMATIC FLOW BASED SAMPLE PREPARATION COUPLED TO LIQUID CHROMATOGRAPHY

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Analyte enrichment and sample cleanup by using solid-phase extraction (SPE) is a common sample preparation step prior to liquid chromatographic (LC) determination of organic compounds. SPE is a successful technique due to the large variety of commercially available sorbents, the reduced amounts of sample and organic solvents used and also the possibility of automation, resulting in high enrichment factors with a wide range of applications.[1] Hence, the present communication addresses the automation of SPE protocols using flow analysis manifolds with direct coupling between sample pretreatment and chromatographic determination.

To achieve this goal, several strategies can be implemented, from which two will be highlighted. Generally, the SPE sorbent is packed in a flow through column, where sample is fed and the target analytes are retained. Subsequently, eluent is propelled through the column, analytes are desorbed and directed to the injection loop of a chromatograph, where a portion of the eluent is injected by a heart-cut approach. After regeneration of the column, the next sample can be processed.

The other, more recent strategy presented here is based on the bead injection concept, associated to Lab-on-valve (LOV)[2] equipment. This technique allows the handling of micro-amounts (in the order of μL) of bead suspensions with high precision in an automated fashion. Thus, it is possible to implement SPE protocols based on the renovation of the sorbent between consecutive samples. This can be achieved by sequential aspiration of beads, followed by packing them in one of the channels of LOV piece in order to assemble a SPE micro-column. Then sample is propelled through the micro-column, followed by washing solution and eluent, which is directed to the chromatograph injection valve.

Advantages and limitation of both strategies (and their hyphenation to liquid chromatography equipment) will be discussed along with presentation of selected examples, namely the determination of phenolic compounds in environmental samples and the determination of riboflavin in foodstuff.

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CO.13 THE USE OF CAPILLARY ELECTROPHORESIS IN DRUG-DRUG INTERACTIONS STUDIES

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In recent years the issue of drug-drug interactions has generated significant concern within the pharmaceutical industry and among US and European regulatory authorities. The co-administration of different drugs or dietary supplements can affect the pharmacokinetics and consequently the therapeutic outcome of a drug due to alterations in its absorption, distribution, metabolism and excretion (ADME). For that reason the development of *in vitro* methods to access information about these kinds of interactions has become very important.
Capillary electrophotesis (CE) has been well proven as an effective method, capable of performing fast analysis using only small amounts of sample, which is ideal for following the kinetics of reactions where low quantities of reactants are available and multiple injections are made in order to obtain kinetic profiles. CE has been successfully applied on the determination of kinetic parameters like Michaelis-Menten constants (K_m)[1] or inhibition constants (K_i)[2].
Clopidogrel is a potent antiplatelet and antithrombotic agent which acts as a pro-drug, requiring oxidation by the hepatic cytochrome P450 and subsequent hydrolysis to generate the active metabolite, a thiol compound. However, after oral administration to humans, the main metabolite circulating in plasma (85 %) is the inactive carboxylic acid derivative which results from ester hydrolysis by carboxylesterases[3,4].
Flavonoids have been reported to affect positively ester drug metabolism and absorption due to esterase inhibition[2,5]. Being present in food, dietary supplements and over the counter medicines, the relevance of such interactions should not be ignored.
A fast MEKC method was developed for the analysis of clopidogrel bisulphate and its carboxylic acid metabolite and applied to the evaluation of the enzymatic inhibition of clopidogrel hydrolysis by some flavonoids (aglycones diosmetin and hesperitin) present in our diet and in some pharmaceutical formulations.
The optimized method demonstrated to be selective, precise, exact and linear in the concentration range tested for clopidogrel and its metabolite. The inhibition studies showed that the flavonoid aglycones diosmetin and hesperitin have a strong inhibitory effect on porcine liver carboxylesterase and also in primary rat hepatocytes. Kinetic profiles were obtained and the parameters K_m and K_i were determined, in order to understand the mechanism of the inhibition.

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CO.14 DESENVOLVIMENTO E VALIDAÇÃO DE UM METODO QUALITATIVO PARA A ANALISE FORENSE DE TINTAS ESFEROGRAFICAS POR HPLC

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Os laboratórios de análise forense são diariamente confrontados com casos que envolvem a falsificação de documentos. Questões relacionadas com alterações subtis a documentos como sejam declarações de impostos, testamentos, contratos, recibos, declarações de seguros, etc. podem ter implicações financeiras significativas. Estas alterações passam pela adição/acrescento de partes, rasura/eliminação de partes e até pela reinscrição/sobre inscrição de algumas partes importantes.

A detecção destas alterações, o eventual acesso à idade relativa das mesmas e possível relacionamento entre materiais suspeitos, envolve regularmente análise de tintas. A comparação de tintas utiliza métodos físicos e químicos tais como a microscopia óptica, a reflectância e a luminescência no IV, a fluorescência no UV e a microespectrofotometria. Os métodos destrutivos, como a cromatografia, fornecem mais informação sobre a composição química dos vários materiais analisados, o que é importante quando se pretende testar a existência de uma origem comum.

No caso das tintas esferográficas, estamos perante uma mistura complexa e pouco homogénea de produtos químicos. Este tipo de tintas tem na sua composição 45% de corantes e 55% de solventes e aditivos (resinas e ácidos gordos). A Cromatografia em Camada Fina de Alta Resolução, HPTLC, tem sido a técnica usada com maior sucesso na separação e comparação destes componentes, sendo rápida, relativamente simples e económica. No entanto, falta-lhe sensibilidade e algum poder de resolução. Na realidade, muitas tintas podem ter a mesma composição em termos de corantes, mas a proporção na qual os mesmos se encontram pode variar em larga escala em diferentes formulações. A técnica de HPLC tem o potencial de permitir uma melhor separação dos componentes da tinta, possui uma maior sensibilidade de detecção, para além de possibilitar a identificação do tipo de corantes utilizados e a sua proporção na mistura.

Neste estudo usamos o HPLC para separar e detectar vários componentes em tintas esferográficas azuis extraídas de documentos. O método de análise qualitativa desenvolvido foi optimizado e validado atendendo às exigências analiticas e às particularidades da aplicação forense a que se destina.

Estamos perante um método robusto, com boa precisão e elevada repetibilidade e reprodutibilidade. Foram igualmente determinados os limites de detecção do equipamento e do método de modo a assegurar a sua fiabilidade.

Uma aplicação prática é demonstrada.

CO.15 GAS CHROMATOGRAPHY-OLFACTOMETRY IN AROMA CHARACTERISATION AND QUALITY ASSESSMENT OF GRAPES AND WINES: AN OVERVIEW

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Over the years, many attempts have been made to identify food or alcoholic beverage odourant profiles.

Gas chromatography-olfactometry (GC-O) is a unique analytical technique which associates the resolution power of the capillary column in GC with the selectivity and sensitivity of the human sense of smell and its introduction was a breakthrough in analytical aroma research.

The human nose is commonly more sensitive than any instrumental detector, and GC-O is a powerful tool for measuring odour activities of volatile compounds. However, in GC-O only single compounds are assessed. This technique doesn't provide information on their behaviour in mixtures. Wine aroma is the expression of complex mixtures of volatiles, some of these compounds derive from grapes and others are formed during fermentation processes or wine aging.

There is a great interest about the application of GC-O in the study of wine aroma compounds since the 70's. GC-O analysis strongly contributed to the characterisation of odour-active compounds in white and red musts and/or wines. In fact, GC-O has been used to identify odour-active compounds in wines from Chardonnay[1], Gewürztraminer[2,3], Scheurebe[2], Merlot and Cabernet[4], Tempranillo[5] and Touriga Nacional[6]. Furthermore, quantitative GC-O analyses made possible to find out key differences in odour profiles of monovarietal young red wines[4], Madeira wines from Malvazia, Boal, Verdelho and Sercial cultivars[7] or among clonal red wines from Trincadeira[8].

The odourant compounds that are responsible by quality or defects in grapes and/or wines have been studied by different authors[9-11]. For example, 2-methylisoborneol, (-)-geosmin, 1-octen-3-one, 1-octen-3-ol, 2-octen-1-ol, and 2-heptanol were identified or tentatively identified as odourant compounds responsible for mushroom, mossy or earthy odours, by gas chromatography-olfactometry, in extracts of rotten grapes and musts[10].

This presentation will show numerous examples of studies aimed at determining the importance of odourant compounds and its contribution for the final aroma of grapes and wines. The application of GC-O for the quality control of the grapes and in the characterisation of aroma quality of wines will be also presented and discussed.

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CO.16 MEMBRANELESS GAS-DIFFUSION MODULE FOR THE ANALYSIS OF VOLATILE AND SEMI-VOLATILE COMPOUNDS

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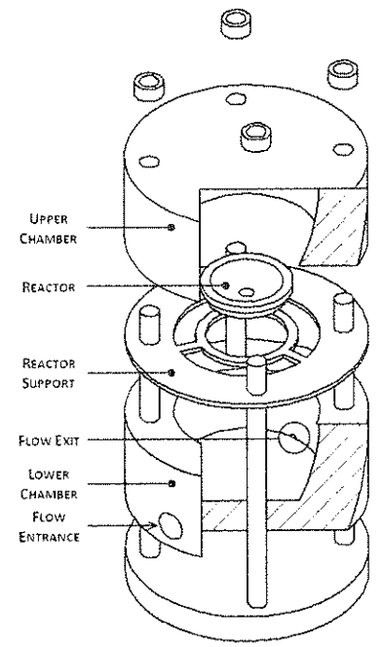
A membraneless extraction module (MLEM) for the sample preparation of volatile compounds is reported. The extraction process is based on the same principles of gas-diffusion (GD) and pervaporation; however it does not use a membrane. This module has a lower chamber where the sample continuously flows, while volatile compounds evaporate to the headspace. Inside the module there is a suspended small reactor, where a small volume of a suitable acceptor solution is placed.
This extraction module has been tested, so far, in the chromatographic analysis of vicinal diketones (VDKs) in beer (CV = 5%; L.O.D. = 4µg/L)[1] and in the voltammetric analysis of sulphites in wine (CV = 5%; L.O.D. = 6 mg/L)[2] Several parameters of the extraction process, such as temperature, sample flow and extraction time, were studied and optimized. This module proved to be a good tool for the sampling of volatile compounds, since the extraction is made without using a membrane avoiding all the robustness problems related with its use.



F1 Membraneless gas-diffusion module.

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CO.17 DLLME COUPLED TO MD-GC/MS AS A RELIABLE METHOD TO THE ASSESSMENT OF TRACE CONTAMINANT RESIDUES IN FOOD

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During production, processing, and storage of foods, organic contaminants like agricultural, industrial and environmental residues, heat-induced toxins and food packaging migrants, may enter in the food chain[1]. For many of these contaminants, legal limits in food have been established or are under evaluation in order to protect human health from their toxic properties. Taking into account the low levels authorized, analytical methods used for monitoring purposes should be highly selective and sensitive, rapid, robust, and if possible covering the desired scope of matrices and analytes. The aspects of green chemistry and environmental protection should also be taken into consideration into the evaluation of analytical methods[2]. For these purposes in the field of sample preparation a new microextraction procedure called dispersion liquid-liquid micro-extraction (DLLME) have been recently developed[3]. The DLLME consists in the formation of a cloudy solution promoted by the fast addition to the aqueous sample of a mixture of extractor and dispersive solvents. The tiny droplets formed and dispersed among the aqueous sample solution are further joined and sedimented in the bottom of a conical test tube by centrifugation, allowing great enrichment factors and good yields. The resultant sedimented phase is directly analyzed by gas chromatography (GC) or liquid-chromatography (LC)[4]. In the field of instrumental analysis the development of alternative methods, such as Heart-cutting multidimensional GC coupled to mass spectrometry (MDGC/MS), allowed simultaneously a higher sample capacity, increased separation of trace compounds in complex matrixes, and enhanced system reliability and robustness. The Heart-cutting MD-GC is achieved with a Deans Switch device which permits the selection of a region of a primary column separation, based upon retention time, to pass to the start of a second column for MS analysis or to go to a restrictor column for waste[4].

The aim of this work is to present the results obtained in development and validation of DLLME-MDGC/MS methods for the analysis of multipesticide residues in juices and for the analysis of bisphenol A and bisphenol B in soft drinks.

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comunicações em poster

P.1 DETECTION AND QUANTIFICATION OF SELECTED ENDOCRINE DISRUPTORS IN DRINKING WATER SUPPLIES IN THE NORTH OF PORTUGAL

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Endocrine disrupting compounds (EDC) have become a major subject of environmental worldwide concern and include a wide variety of pollutants, namely substances of different sources of contamination: domestic (estrogens), industrial (plasticizers and heavy metals) and from agricultural practices (some pesticides)[1,2]. Pesticides, even those which are not EDC, are also known to be toxic and have a recognized impact in human health[3].

The aquatic environment is particularly susceptible to pollution due to intentional or accidental release of chemicals[4] and groundwater contamination might be anticipated when the mitigation capacity of the soil system is exceeded, especially when enhanced productivity agriculture is carried out on hydrogeologically vulnerable soils[5,6]. Pesticide contamination of groundwater is a national issue as it is often used as drinking water. This concern is especially acute in rural agricultural areas where population uses frequently small private groundwater supplies, regularly without any laboratory surveillance.

The Ministério da Agricultura, do Desenvolvimento Rural e das Pescas published a list of pesticides to be researched by authorities in drinking water in each Portuguese region[7,8]. However, water analysis from wells revealed other pesticides that are not included in the government list and are even outside the advised pesticides list[9]. Some of them, as organochlorine, are very persistent and lipophilic, which favors their widespread accumulation in adipose tissue and liver[10] and their detection may be due to an application years before, or they may be transported from other places, or even due to some forbidden application. Exposure to less persistent but widespread EDC is also important and has only recently been addressed[11].

EDC and pesticides analytical technologies for environmental samples use chromatographic procedures as standard methods. Optimization and validation of solid phase extraction (SPE) methodology coupled with GC-MS for water samples analysis were developed for 15 compounds as, for example, dimethoate, atrazine, alachlor linuron, dieldrin, methoxychlor, folpet, estrone and β-estradiol.

The aim of this study was to assess the evidence for EDC and other pesticides in aquatic environment of some chosen problematic regions of the North of Portugal, to evaluate their possible presence and consequent exposition of population, in order to develop a reliable health risk assessment, to define a new more accurately organic pollutants governmental list and to define possible strategies for environmental and health remediation.

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P.2 HPLC-ESI-MS/MS DETECTION AND CHARACTERIZATION OF COVALENT DEOXYTHYMIDINE ADDUCTS IN DNA MODIFIED WITH THE NEVIRAPINE METABOLITE, 12-HYDROXY-NEVIRAPINE

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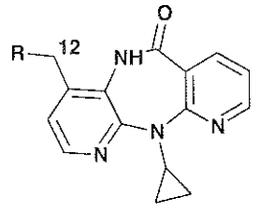
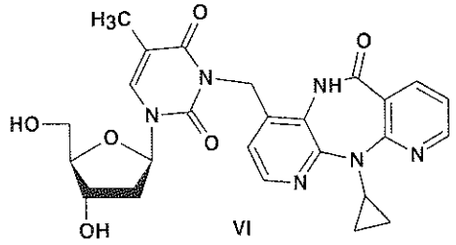
Nevirapine (**I**, NVP) is a non-nucleoside reverse transcriptase inhibitor used against the human immunodeficiency virus (HIV-1), mostly to prevent the vertical transmission of HIV-1 from mother to child. However, one of the shortcomings of NVP use is severe hepatotoxicity, which raises concerns about chronic administration of the drug, particularly to children[1]. NVP metabolism involves oxidation of the 4-methyl substituent to 12-hydroxy-NVP (**II**), and the formation of phenolic derivatives. Further metabolism, either through oxidation of the phenols to quinoid derivatives or Phase II esterification, may produce electrophilic species capable of reacting with DNA to yield covalent adducts. These adducts could potentially be involved in the initiation of mutagenic and carcinogenic events.

We have previously reported the synthesis and characterization of a series of covalent adducts from reaction of 2'-deoxynucleosides (deoxyguanosine, deoxyadenosine, and deoxycytidine) or DNA with 12-mesyloxy-NVP (**III**), used as a surrogate for the putative electrophilic metabolite from **II**, 12-sulfoxy-NVP (**IV**)[2].

We report herein, the synthesis and characterization of novel covalent deoxythymidine (dT)-NVP adducts. The synthetic strategy involved reacting 12-bromo-NVP (**V**) with 3',5'-*O*-bis(*tert*-butyldimethylsilyl)-dT under palladium(0) catalysis and using cesium carbonate for catalyst regeneration. Following desilylation of the deoxyribosyl substituent with tetrabutylammonium fluoride, two products were isolated by reversed-phase HPLC. Based upon ^{1}H and ^{13}C NMR and HPLC-ESI-MS/MS, the major product was characterized as a dT-NVP adduct, with a connectivity through N3 of deoxythymidine and C12 of NVP (**VI**). Although the amount of the minor product was insufficient for full characterization, the MS/MS profile was also consistent with covalent binding between dT and NVP. On the basis of retention times and HPLC-ESI-MS/MS patterns, adduct **IV** was detected in an enzymatic hydrolysate from DNA reacted with **III**. Our data suggest that, similarly to other deoxynucleosides, dT can plausibly be modified *in vivo*, as a result of NVP metabolism to **II**. Furthermore, HPLC-ESI-MS/MS should be useful for monitoring DNA-NVP adduct formation *in vivo*.



I. R=H
II. R=OH
III. R=OSO_3Me
IV. R=OSO_3H
V. R=Br



VI

Ackowledgement
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P.3 HPLC-ESI-MS/MS CHARACTERIZATION OF COVALENT PROTEIN ADDUCTS FROM THE NEVIRAPINE METABOLITE, 12-HYDROXYNEVIRAPINE

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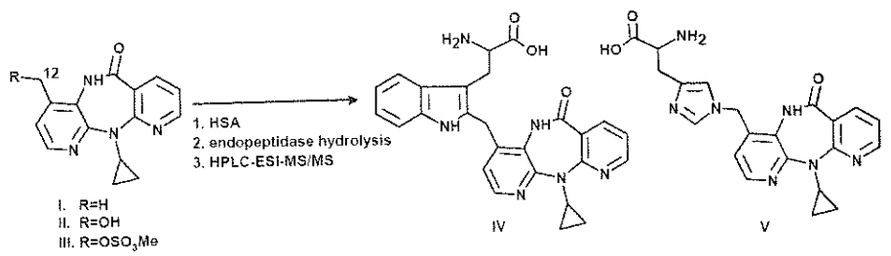
The non-nucleoside HIV-1 reverse transcriptase inhibitor nevirapine (I, NVP) is one of the most prescribed antiretroviral drugs in developing countries, particularly to prevent mother-to-child transmission of the virus. However, NVP administration is associated with a variety of toxic responses, ranging from serious adverse cutaneous effects to severe hepatotoxicity[1]. As such, the recent suggestion[2] that extended-dose daily NVP regimens may be adequate to decrease the risk of HIV transmission to breastfed infants in low-resource settings has met criticism, on account of toxicity considerations[3]. One of the Phase I NVP metabolites is 12-hydroxy-NVP (II); further Phase II metabolism of II may produce electrophilic derivatives capable of reacting with bionucleophiles to yield covalent adducts[4,5]. Recent data have implicated II in NVP-induced skin rash[6], which could be due to protein adduct formation.
We report herein the development of HPLC-ESI-MS/MS methodology for the detection and characterization of NVP residues covalently bound to proteins. With this purpose, we have synthesized the model electrophile III, as a surrogate for plausible Phase II metabolites of II, and investigated its reactivity in vitro towards amino acids (AA) bearing nucleophilic side chains (cysteine, tryptophan, histidine and lysine). Covalent adduct standards involving binding of the AA through C12 of the NVP moiety were isolated and characterized in all instances. Based upon comparison with the retention times and fragmentation patterns of the synthetic NVP-AA standards, NVP adducts with tryptophan (IV) and histidine (V) were detected in an endopeptidase hydrolysate of human serum albumin (HSA) incubated with III. Our data suggest that NVP administration may lead to in vivo protein modification, via Phase II activation of II, in a manner similar to that observed in vitro. Moroever, the HPLC-ESI-MS/MS methodology reported herein should be suitable for the detection of NVP-protein adducts, as potential biomarkers of NVP toxicity.



Ackowledgement
We thank the Portuguese NMR and MS networks (IST-UTL Center) for providing access to the facilities. Fundação para a Ciência e a Tecnologia (FCT) and FEDER are gratefully acknowledged for a research grant (POCI/QUI/56582/2004; PPCDT/QUI/56582/2004). SAF thanks FCT for a BII fellowship. Support by the U.S. National Toxicology Program (NTP) is also acknowledged.

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P.4 FLUOROQUINOLONES DETERMINATION IN TURKEY MUSCLE BY LIQUID CHROMATOGRAPHY AND FLUORIMETRIC DETECTION

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Fluoroquinolones (FQs) use in veterinary medicine and the emergence of bacterial resistance had an adverse impact on human medicine, because of the possible failure of human antibiotic therapy[1].

Effective control in food matrices is necessary to ensure that they are not present at levels that may pose health risks to the public. Maximum residue limit (MRL) set by the European Union (EU) in muscle is 100μg.kg^{-1} for all species. For enrofloxacin (Enro) the marker residue is the sum of Enro and its main metabolite, ciprofloxacin (Cipro)[2].

The overall goal of this study was to develop an analytical methodology for the rapid, accurate, and sensitive analysis of norfloxacin (Nor), Cipro, Enro and sarafloxacin (Sara), in turkey muscle tissue. The method involves extraction of the residues from the samples with 0.15M HCl and cleanup by Oasis HLB cartridges. High performance liquid chromatographic separation was carried out on a C18 TSK gel column, in the isocratic mode, with 0.025M H_3PO_4 solution adjusted to pH 3.0 with TBA and methanol (78:22), as mobile phase. The spectrofluorimetric detection, which is more sensitive and selective, was carried out at 278nm and 450nm for λ excitation and λ emission, respectively. The mobile phase pH contributes for a highest fluorescence, since at low pH (from 2.5 to 4.5) the neutral and cationic species prevail for the piperazinylquinolones.

Nor, Cipro and Enro had good linear response within the range of 0.625 to 12.5ng and Sara within the range of 1.25 and 15.0ng of injected amount. The limits of quantification were 15μg.Kg^{-1} for Nor, Cipro and Enro, and 30μg.Kg^{-1} for Sara.

Overall percent recoveries yield for spiking samples ranged from 73% to 91%.

The method has been successfully applied to determine these four FQs in turkey samples, collected from markets of Portugal. This study confirmed widespread misuses of FQs in turkey production.

Acknowledgments:
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P.5 OCHRATOXIN A IN BREAD FROM BRAGANÇA AND ALGARVE IN SUMMER 2008 – CONTRIBUTION TO THE DIETARY INTAKE

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Ochratoxin A (OTA), mainly produced during storage by *Aspergillus ochraceus* and *Penicillium verrucosum*, is a natural contaminant of a variety of foodstuffs, mainly cereals. This mycotoxin possesses carcinogenic, nephrotoxic, teratogenic and neurotoxic effects[1]. Its levels are unpredictable and can vary both between seasons and different growing areas or under different storage conditions. Furthermore, various studies concerning human OTA exposure indicate a continuous and widespread distribution of this mycotoxin as assessed by the analysis of various foodstuffs and the monitoring of biological fluids[2]. From all the various foodstuffs analyzed, cereals and their derivatives, namely bread, rise as the major contributors to human exposure[3,4].

The purpose that steered the present work was thus the determination of the OTA levels in the bread marked in the Bragança and Algarve regions during the summer of 2008, and the estimation of its contribution to the daily intake. In order to do so, wheat bread samples (30 from Algarve and 20 from Bragança) were collected from various bakeries and supermarkets during the period of August and September of 2008. OTA was extracted with methanol:PBS (50:50, v/v). The extract was filtered and passed through immunoaffinity Ochratest® columns, which were afterwards washed with 10mL of distilled water. The toxin was eluted with 3ml of methanol then evaporated under a gentle nitrogen flow at 50ºC. Before detection and quantification with an LC-FD equipment (λ exc=333nm; λ em=460nm) – a setup which resulted in a LOQ of 0.1ng/g – the dried extract was dissolved in 250μl of mobile phase (acetonitrile/water/acetic acid (49.5:49.5:1.0, v/v). The aforementioned analysis revealed a much higher contamination by OTA in the samples from Algarve than in those from Bragança (averages of 0.158 and 0.054ng/g, respectively). This is in contradiction with the expected tendency, seeing as, both in general and in the summer of 2008 in particular, Bragança's climate is consistently colder and more rainy (i.e., wetter) than Algarve's, conditions which favour fungal OTA production. A look at the results from the previous season further corroborates the apparent incongruity of the results. In that season, Algarve featured samples with slightly less contamination than Bragança (0.12 vs. 0.16ng/g, respectively)[5]. Therefore, and with the onset of summer, Bragança's samples' contamination was subjected to a substantial decrease to ~30% of its previous level, in accordance to the new environmental conditions. Algarve's samples', however, have suffered an increase of 33% with the changing of the season. Meteorology reports discard the possibility of environmental abnormalities skewing the results, perhaps suggesting a laxness in storage conditions during the warmer months, which is further worsened by the growing touristic influx observed during this season in Algarve. These differences in the average levels resulted in estimated daily intakes (EDI) of 0.275 and 0.188ng/kg b.w./day for Algarve and Bragança, respectively. The same regions, in the previous season featured EDI of 0.26 and 0.38ng/kg b.w./day5. Thus, the EDI for the considered regions in both seasons were well below the one introduced by EFSA (~17ng/kg b.w./day). The present study, therefore, both reveals a need for continuous monitoring of OTA levels (so as to detect unexpected developments) and warns of the dangers of improper food storage.

Acknowledgements:
To FCT and FEDER/POCTI (Project PTDC/AGR-ALI/65528/2006 and grant SFRH/BD/37409/2007).

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P.6 DETERMINAÇÃO DE PESTICIDAS PRIORITÁRIOS NO SISTEMA LAGUNAR DA RIA FORMOSA POR SPE-GC/MS

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A metodologia de análise implementada com aplicação a alguns pesticidas pertencentes à lista das substâncias prioritárias da Directiva/60/CE (Atrazina, Simazina, Clorfenvinfos, Clorpirifos e Endossulfão), bem como aos insecticidas metidatião e clorotalonil, envolveu a realização de estudos de eficiência de extracção em fase sólida (SPE) utilizando cartuchos C8, Easy (ambos Chromabond) e Oasys (Waters). O volume de solução padrão de trabalho extraída ($1{,}0x10^{-5}$M para cada um dos pesticidas) foi de 250mL para atingir um factor de concentração de 1000. Os cartuchos tipo C8 apresentaram o melhor desempenho para o conjunto de pesticidas estudado (69-93%) pelo que foram seleccionados para acompanhar a eficiência de extracção das amostras, aplicando o método a níveis de concentração próximos dos limites de quantificação dos pesticidas em estudo (c.a.$1{,}5x10^{-7}$M).

O estudo quantitativo, utilizando o método do padrão interno (P.I – fenantreno D10), foi realizado através das rectas de calibração dos diversos pesticidas, obtidas por GC-MS em modo SIM, e análise da razão sinal/ruído, permitindo assim calcular os limites de detecção e quantificação para cada um dos pesticidas estudados.

Foram colhidas amostras de água em durante o período de um ano (de quatro em quatro meses) no sistema lagunar da Ria Formosa junto à cidade de Faro. Foram escolhidos dois pontos de colheita potencialmente afectados pela presença de campos de golfe e um ponto de colheita potencialmente limpo desta influência. As amostras, colhidas em duplicado, após filtração, foram sujeitas a processo de extracção simples e com amostra enriquecida. Os pesticidas atrazina, simazina e clorpirifos apresentaram sinal positivo em diversas amostras analisadas, tendo sido quantificados em modo SIM através das respectivas rectas de calibração, após confirmação da sua presença em modo SCAN usando o método de extracção de iões.

P.7 SCREENING OF THE VOLATILE PROFILE OF ANNONA CHERIMOLA MILL. CULTIVARS BY SOLID-PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY-QUADRUPOLE MASS SPECTROMETRY DETECTION

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The analysis of volatile compounds in Funchal, Madeira, Mateus and Perry Vidal cultivars of *Annona cherimola* Mill. (cherimoya) were carried out by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography–mass spectrometry (GC–qMSD). HS-SPME technique was optimized in terms of fibre selection, extraction time, extraction temperature and sample amount to reach the best extraction efficiency. The best result was obtained with 2g of sample, using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre for 30min at 30ºC under constant magnetic stirring (800 rpm).
After optimization of the extraction methodology, all the cherimoya samples were analysed with the best conditions that allowed identify about sixty volatile compounds. The major compounds identified in the four cherimoya cultivars were methyl butanoate, butyl butanoate, 3-methylbutyl butanoate, 3-methylbutyl 3-methylbutanoate and 5-hydroxymethyl-2-furfural. These compounds represent 69.08 ± 5.22 %, 56.56 ± 15.36 %, 56.69 ± 9.28 % and 71.82 ± 1.29 % of the total volatiles for Funchal, Madeira, Mateus and Perry Vidal cultivars, respectively. This study showed that each cherimoya cultivars have 40 common compounds, corresponding to different chemical families, namely terpenes, esters, alcohols, fatty acids and carbonyl compounds and using PCA, the volatile composition in terms of average peak areas, provided a suitable tool to differentiate among the cherimoya cultivars.

P.8 CHARACTERIZATION OF VOLATILES CONSTITUENTS OF APPLES FROM *ROSACEAE* FAMILY BY HS-SPME/GC-QMS

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The volatile profile of apple subspecies of *Malus domestica Borkh* apple subspecies from different locations at Madeira Islands, namely Ponta do Pargo (JPP), Porto Santo (JPS) and Santo da Serra (JSS) was established using a dynamic headspace solid-phase microextraction (HS-SPME) procedure followed by gas chromatography-mass spectrometry (GC-qMSD) analysis. The experimental factors that might influence the extraction efficiency of the HS-SPME technique such fiber coating, extraction temperature, extraction time, sample amount, dilution factor, ionic strength and desorption time, were evaluated and optimized. The SPME fiber coated with 50/30μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) afforded highest extraction efficiency of volatile compounds, providing the best sensitivity for the target volatiles, particularly when the samples were extracted at 50°C for 30min. A qualitative and semi-quantitative analysis between the investigated apple subspecies has been established. It was possible to identify about one hundred of volatile compounds among pulp (44, 43 and 37), peel (60, 56 and 63) and pulp+peel (63, 42 and 48) samples from JPP, JPS and JSS apples, respectively. Ethyl esters and higher alcohols were found to be the most representative volatiles in the studied apple subspecies. Some of the identified volatile compounds with relatively high relative amount percent in apples were, in average α-farnesene (24.71%), hexan-1-ol (14.06%) and hexyl 2-methylbutanoate (10.80%). The principal component analysis (PCA) was carried out to visualize data trends and to detect possible clusters within the samples. Then, linear discriminant analysis (LDA) was performed in order to detect the volatile compounds able to differentiate the three kinds of apples investigated.

P.9 SOLID-PHASE MICROEXTRACTION FOLLOWED BY GAS CHROMATOGRAPHY-QUADRUPOLE MASS SPECTROMETRY FOR SYSTEMATIC STUDY OF VOCS AND SVOCS IN DIFFERENT BANANA CULTIVARS

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Five different banana cultivars with certified botanical origin (Dwarf Cavendish, Prata, Maçã, Ouro and Platano) belonging to the *Musaceae* family, the most common genomic groups cultivated in Madeira Island (Portugal) were analysed by dynamic headspace solid-phase microextraction (HS-SPME) followed by gas chromatography-quadrupole mass spectrometry detection (GC-qMS) system, in order to define their volatile (VOCs) and semi-volatile (SVOCs) composition, as pertinent criteria of differentiation.
In order to achieve highest recovery, the isolation procedure was optimised by selection of the appropriate fibre and optimization of extraction temperature and extraction time. The optimum set of conditions, in terms of the maximum signal obtainable for the total volatile and semi-volatile composition, were afforded using a 75μm PDMS/DVB coating particularly when the samples were extracted at 50°C for 60min under constant stirring at 750 rpm, after saturating the samples with salt (NaCl). Using this method about seventy compounds, distributed by chemical groups of esters, acetates, alcohols, carbonyls and ketones, were extracted, tentatively identified and used to profile the free volatile and semi-volatile compounds in different banana cultivars, thus emphasizing the sensitivity and applicability of solid-phase microextraction for volatile profile of plant secondary metabolites. Ethyl esters were found to comprise the largest chemical class in the studied banana cultivars, accounting 80.9%, 86.5%, 51.2%, 90.1% and 6.1% for the volatile fraction in Dwarf Cavendish, Prata, Ouro, Maçã and Platano cultivars, respectively. As found for *Prata banana* cultivar (31.7%), isopentyl 3-methylbutyl butyrate was the most abundant component in Maçã banana samples (33.8%), while isopentyl butyrate (27.1%) occur as the major compound in Dwarf Cavendish and 3-methylbutyl butyrate (44.0%) in Ouro banana samples. In Platano variety, isoamyl acetate (27.4%) was the major identified compound. Elemicin, which give the product its typical mellow aromas, was also identified. The semi-quantitative results were then submitted to statistical evaluation, namely principal component analysis (PCA) in order to differentiate between banana cultivars.

P.10 MONITORIZATION OF VOLATILE COMPOUNDS AS POTENTIAL BIOMARKERS IN INDIVIDUALS WITH ONCOLOGIC PATHOLOGIES BY HS-SPME-GC-QMSD

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There is an increasing interest on the determination of volatile biomarkers for clinical diagnosis and therapeutic monitorization. Since the early 90´s, several groups of investigators described some volatile compounds as markers of metabolic processes and clinical diagnosis from various pathologies, including cancer. This pathologic state leads to the production of several volatile organic compounds (VOC´s) namely, aldehydes (pentanal, hexanal, octanal, nonanal), alcanes (decane, n-undecane) and aromatic hydrocarbons (benzene, xylene, toluene), in biological fluids like blood and urine[1].

Several methods have been employed for the discovery of biomarker patterns of major human diseases, especially for various types of cancer. The development of solid-phase microextraction (SPME) has experienced significant growth since its introduction as a new approach to sample preparation in the 90´s[2]. The biological samples used in this work were obtained from 81 patients with oncologic pathologies and 21 controls (healthy volunteers) in Serviço de Hemato-Oncologia and Banco de Sangue of Centro Hospitalar in Funchal.

The purpose of this work was to identify the volatile organic compounds in biological fluids by HS-SPME-GC-qMSD (headspace solid phase-microextraction) described in the literature as possible biomarkers of cancer from individuals with oncologic pathologies (oncologic group) and without pathology (control group). More than 80 volatile compounds were identified in both groups, belonging to several chemical families, namely aldehydes, ketones, benzene derivates, among others. The major chemical families identified in control group were ketones, sulfur compounds, and for oncologic group were ketones, sulfur compounds and volatile phenols. The most representative compounds of these families were 4-heptanone, methanethiol and 4-methyl-phenol. Acetone, 4-heptanone and 2-pentanone were common in both groups, with higher values for the oncologic group.

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P.11 DESENVOLVIMENTO DE UMA METODOLOGIA ANALITICA POR HPLC-ESI-MS/MS PARA A DETERMINAÇÃO DE AMINAS BIOGENICAS EM TUNIDEOS

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As aminas biogénicas são compostos azotados, de baixo peso molecular, formadas principalmente pela descarboxilação de aminoácidos livres, que se encontram naturalmente presentes nos alimentos em baixas concentrações. No pescado fresco, as aminas biogénicas resultam sobretudo da acção bacteriana, podendo a sua formação ser potenciada pela refrigeração inadequada, más condições de higiene e pelo mau acondicionamento do pescado durante o seu manuseamento.

O consumo de pescado com elevados teores destas aminas está associado ao aparecimento de diversos sintomas de intoxicação, nos quais se incluem náuseas, vómitos, diarreia, hipotensão e urticária. Estes sintomas são fundamentalmente atribuídos à acção de uma amina biogénica, a histamina, que se encontra legislada com um teor médio por lote de 100 ppm (Decreto Lei n.º 375/98 de 24 de Novembro de 1998, Regulamento CE n.º 2073/2005 DA COMISSÃO. de 15 de Novembro de 2005). Este limite aplica-se, somente a pescado pertencente às familias *Scombridae* (tunídeos), *Clupeidae*, *Engraulidae* e *Coryphaenidae*. A identificação e quantificação de aminas biogénicas é importante, não só devido à sua toxicidade, mas também pelo facto de servirem como indicadoras do grau de frescura e degradação do pescado, das condições de pesca e, no caso das conservas de pescado, da qualidade da matéria prima.

Neste âmbito, o presente trabalho teve como objectivo desenvolver e validar uma metodologia analítica de rotina para determinar o teor de aminas biogénicas (histamina, tiramina, triptamina, cadaverina e espermina) no pescado, por HPLC-ESI-MS/MS. O método de preparação da amostra é relativamente simples, envolvendo uma extracção dos analitos presentes no músculo do pescado com ácido clorídrico e uma purificação da amostra através da extracção em fase-sólida (SPE). Algumas condições e parâmetros experimentais foram optimizados, nomeadamente as condições de ionização, os parâmetros de fragmentação, o volume de injecção, fluxo de corrida, fase móvel e os cartuchos de SPE. A validação do método teve em conta os seguintes parâmetros: coeficientes de correlação, coeficientes de variação, determinação dos limites de quantificação e detecção e percentagem de recuperação.

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P.12 DIFFERENTIATION OF WINES FROM DIFFERENT GEOGRAPHIC REGIONS BASED ON VOLATILE PROFILE

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Identification of wine aroma compounds and the relationships between their relative content may be a useful tool on the differentiation of wines from different varieties and for the establishment of genuineness criteria, in order to improve the quality of the wines, preventing fraud and guarantee their origin[1].

The aim of this study was the characterization of volatile profile of wines from Madeira, Azores and Canary Islands obtained from the same varieties using solid phase extraction (SPE) methodology combined with GC-qMSD. More than eighty compounds were detected and tentatively identified in wine extracts and their percent amounts determined. Quantitatively, higher alcohols (2-phenylethanol, benzyl alcohol and hexan-1-ol) are the most abundant compounds in Madeira, Azores and Canary Islands analyzed wines. The other main volatile compounds pertaining to ethyl esters and fatty acids. Diethyl succinate, ethyl 3-hydroxyhexanoate, hexanoic and octanoic acids are the major ones. These volatile compounds make a positive contribution to the general quality of wine being responsible for their "fruity" and "floral" notes to the wine sensory properties. Significant quantitative differences on volatile composition of Madeira, Azores and Canary wines were verified. Principal components analysis (PCA) of chromatographic data, in terms of peak areas, was used as suitable tool to differentiate these samples.

Acknowledgment
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P.13 ESTABLISHMENT OF THE VOLATILE PROFILE OF *PASSIFLORA* FRUITS SPECIES

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The volatile composition of different varieties of Passiflora species (*Passifloraceae* family) was established by dynamic headspace solid-phase microextraction (HS-SPME) followed by thermal desorption gas chromatography-quadrupole mass spectrometry analysis (GC-qMS). The performance of five commercially available SPME fibres: 65μm polydimethylsiloxane/divinylbenzene, PDMS/DVB; 100μm polydimethylsiloxane, PDMS; 85μm polyacrylate, PA; 50/30μm divinylbenzene/carboxen on polydimethylsiloxane, DVB/CAR/PDMS (StableFlex); and 75μm Carboxen/polydimethylsiloxane, CAR/PDMS; was evaluated and compared. Several extraction times and temperature conditions were also tested to achieve optimum recovery. The SPME fibre coated with 65μm PDMS/DVB afforded the highest extraction efficiency, when the samples were extracted at 50°C for 40min with a constant stirring velocity of 750 rpm, after saturating the sample with NaCl (16.7%, w/v – 0.2g). A comparison among different Passion fruit species has been established in terms of qualitative and semi-quantitative differences in volatile composition. By using the optimal extraction conditions and GC-qMS it was possible to tentatively identify seventy one different compounds in Passiflora species: 51 volatiles in Passiflora edulis Sims (purple passion fruit), 24 in *Passiflora edulis Sims* f. *flavicarpa* (yellow passion fruit) and 22 compounds in *Passiflora mollissima* (banana passion fruit). It was found that the ethyl esters comprise the largest class of the passion fruit volatiles, including 82.8% in *Passiflora edulis* variety, 77.4% in *Passiflora edulis Sims* f. *flavicarpa* variety and 39.9% in *Passiflora mollissima*.

P.14 TRIHALOMETANOS COMO SUBPRODUTOS DO PROCESSO DE CLORAGEM DA ÁGUA EM PISCINAS COBERTAS

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A inclusão de processos de desinfecção por cloro no tratamento de águas, para salvaguardar o utente de doenças do foro microbiológico, conduz à formação de subprodutos (Desinfection ByProducts - DBPs), por reacção deste com a matéria orgânica presente na maior parte das águas. Neste grupo de produtos incluem-se o clorofórmio, o diclorobromometano, o dibromoclorometano e o bromofórmio, conhecidos como Trihalometanos - (THM's) bem como outros compostos voláteis e não-voláteis. O clorofórmio é o composto que habitualmente aparece em maior concentração.

A formação destes produtos é condicionada pelas características e origem da água, nomeadamente o pH e a temperatura a que o tratamento é realizado bem como concentração e tempo de contacto do desinfectante.

Os estudos epidemiológicos realizados na última década, têm vindo a sugerir uma possível correlação entre a existência deste tipo de subprodutos e o aparecimento de doenças, devido à inalação de THM's pelo tracto respiratório.

As águas de piscinas sofrem recirculação e só esporadicamente é que são renovadas estando continuamente a ser poluídas pelos utilizadores que são fontes de microorganismos e de diversos compostos orgânicos. Por este motivo, é necessário que seja efectuada uma desinfecção contínua destas águas, por exemplo com cloro, devendo este desinfectante manter-se numa concentração suficiente de forma a proteger os utilizadores de microorganismos remanescentes e de outros que estão a ser introduzidos continuamente. Deverá assim minimizar-se o risco de ocorrência de poluição microbiológica e consequentemente o aparecimento de doenças nos utilizadores das piscinas.

Uma vez que as águas de piscina não são substituídas frequentemente, os subprodutos formados atingem concentrações mais elevadas do que as normalmente encontradas, por exemplo, nas águas de consumo. Devido à sua volatilidade, estes compostos são libertados no ar ambiente.

Várias organizações estabeleceram para as águas de consumo humano um valor máximo admissível para os THM's. Para o caso de águas de piscinas e ar interior, não existe legislação aplicável nem valor alvo para este tipo de compostos.

A quantificação dos THM's nas águas de piscinas, já está bem estudada em termos internacionais, não acontecendo o mesmo no ar ambiente e consequentemente os problemas de saúde originados pela exposição continuada dos utilizadores e dos trabalhadores a estes químicos.

Para além das vias respiratórias existem outras vias de entrada destes compostos nos organismos dos utilizadores através da pele e por ingestão de água da piscina.

Face ao exposto, verifica-se que não tem havido uma atenção muito grande relativamente às questões ligadas à higiene, segurança e saúde no trabalho, pelo facto de não existir ainda uma consciencialização alargada para este tipo de assunto e por não ter sido ainda efectuada uma adequada avaliação dos riscos da exposição por parte dos trabalhadores e utilizadores aos compostos citados.

Em Portugal, os laboratórios só analisam os Trihalometanos nas águas de abastecimento porque estão referenciados na legislação em vigor. Consideramos no entanto, de todo o interesse, o desenvolvimento de uma metodologia analítica robusta, rápida e fácil de utilizar

que permita analisar estes mesmos compostos na matriz ar interior, nomeadamente em piscinas.
A determinação destes parâmetros é realizada pela técnica de desadsorção térmica acoplada a um cromatógrafo gasoso com detector de massa, Perkin-Elmer TurboMatrix Clarus 500 (ATD-GC-MS).

P.15 RP-HPLC/UV METHODOLOGY FOR ASSESSMENT QUALITY OF *HYPERICUM* COMMERCIAL SPECIES IN PORTUGAL

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Hypericum (Guttiferae) is a large genus (*ca* 450 species) of herbs or shrubs used in folk medicine since the antiquity. Their health benefits demonstrated through the number of pharmacological and clinical trials have been attracting attention to the scientific community for the study of the *Hypericum* genus[1]. HPLC coupled with UV or MS detection has been widely applied for the analysis of the pharmacologically relevant compounds of *Hypericum* species. Usually, HPLC analyses are carried out with C18 columns. However, most of the methods described in literature are not economical in terms of time (60-90min or more) and consequently in solvent consumption or show poor resolution of some important compounds[2,3].
In this study, a reversed-phase C_{12} column (Phenomenex Synergi MAX-RP 80Å, 150×4.6mm i.d., 4μm particle size) was used and the analytical conditions were optimized using a ternary gradient mobile phase consisting of: phase A – ammonium acetate buffer equilibrated to pH 5.0 with glacial acetic acid, phase B – ACN:THF:H_2O (3:1:1), and phase C – MeOH. Under these analytical conditions all compounds were eluted within 60min and the use of acetonitrile was highly reduced as compared to other published methods[2,3]. The internal standard method was used for the calibration and further quantification of twelve known phenolic compounds in *Hypericum* species. Detection was performed at 270nm, for phenolic acids, flavonoids and phloroglucinols, and 590nm for naphtodianthrones. The results presented good linearity between the peak areas and concentrations, with correlation coefficients ranging from 0.9929 for 3-caffeioylquinic acid to 0.9986 for quercetin. The precision of the chromatographic method was evaluated by performing the repeatability of the HPLC analysis of the major *Hypericum* components in intraday and interday measurements of the retention time (RT) and peak area (PA) by repeated injections (n=5) and then checking the percent relative standard deviation (%RSD). The results revealed that the RSD intraday repeatability of the RT ranged between 0.97% and 6.72%, whereas the interday precision (data acquired over a period of five days) was better than 6.15%. The RSD intraday repeatability of the PA ranged from 2.94% to 4.20%, indicating a good repeatability in the peak areas. The accuracy of the used methodology for the *Hypericum* components determination was also evaluated by determining the recovery of rutin in the *H. perforatum* sample of a known level. Three different amounts of rutin standard were added to the sample which was subjected to the HPLC chromatographic analysis. The RSD was better than 3.69% and the mean recovery ranged from 99.74% to 104.39%.

The HPLC method described above were applied to the separation and quantification of the major phenolic compounds presented in the infusions of three widely consumed *Hypericum* species in Portugal: *H. androsaemum*, *H. undulatum* and *H. perfuratum*. Results revealed, as expected for the aqueous systems, that the yield of phenolic constituents decreased with increasing of its lipophilicity. These infusions are rich in highly antioxidant caffeioylquinic acids and flavonoid glycosides, while naphthodianthrones and hyperforin (the characteristic active compounds from *H. perforatum*) were detected in trace amounts or were not detected, due to its nonpolar characteristics. In conclusion, this RP-HPLC/UV methodology is suitable for the assessment quality of *Hypericum* infusions.

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P.16 SEPARATION OF OPEN CIRCULAR AND SUPERCOILED PLASMID DNA ISOFORMS USING A NEW CHROMATOGRAPHIC STRATEGY WITH LYSINE-AGAROSE

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The interest in producing large quantities of supercoiled plasmid DNA (sc pDNA) has increased with the rapid development of gene therapy with non-viral vectors and DNA vaccination technologies. Owing to the commercial interest in these approaches, the development of innovative procedures to easily and efficiently purify the sc plasmid isoform is enforced. Several chromatographic and non-chromatographic methods have been reported to accomplish this purpose, but not all strategies allow the efficient separation of pDNA isoforms. The recent application of amino acids (histidine and arginine) as immobilized ligands in affinity chromatography (AC) has lead to interesting results[1].

The present study describes a new strategy that uses a lysine ligand in affinity chromatography to efficiently separate supercoiled and open circular (oc) plasmid DNA isoforms. The retention behaviour of sc isoform was characterized and adjusted to achieve higher specificity in this chromatographic operation. After the separation of sc from oc isoform, using a linear sodium chloride gradient the temperature effect on plasmid retention was also studied, and it was verified that the retention of sc isoform increased with increasing temperature. To better understand the mechanism for specific recognition of sc pDNA with lysine–agarose, some experiments of competitive elution with buffers containing different amino acids were also performed.

Overall, it was verified that lysine-agarose support can promote a specific interaction with the sc conformation favouring the total plasmid isoforms separation. The results suggest that the underlying mechanism involves biorecognition between the lysine matrix and pDNA isoforms, including hydrogen, electrostatic and hydrophobic interactions[2].

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P.17 MORTE ACIDENTAL VS HOMICÍDIO: UM CASO DE ESTUDO*-1

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"Um incêndio de grandes proporções destruiu ontem uma habitação em Carcavelos, provocando a morte de um jovem adulto, encontrado no seu interior já sem vida. As autoridades suspeitam de curto-circuito e de que a vítima terá morrido por inalação de fumo. A Polícia Judiciária está a investigar o caso."

No decorrer da perícia ao local de incêndio é excluída a hipótese de curto-circuito, concluindo tratar-se de um fogo iniciado por acção humana, acidental ou dolosa. O que à primeira vista parecia uma morte acidental acaba por se tornar uma investigação de homicídio. É efectuada a recolha de material para pesquisa de produtos inflamáveis no ponto de início do incêndio, tendo sido encontrado um bilhete de ameaça na roupa da vítima. Na posse do suspeito é encontrado material que se pensa poder estar relacionado com o bilhete de ameaça – canetas esferográficas e uma agenda de onde se pensa ter sido retirado o papel do documento. O material é apreendido e enviado para análise ao laboratório de documentos do LPC.

Pretende-se comparar a tinta do instrumento de escrita utilizado na inscrição dos dizeres do bilhete, com as dos instrumentos de escrita apreendidos ao suspeito, bem como determinar se o papel do bilhete tem origem na agenda.

Na comparação das tintas utilizam-se métodos físicos e químicos tais como a microscopia óptica, a microespectrofotometria, a espectroscopia de Raman, HPTLC e HPLC. Para análise e comparação do papel foram realizados testes de espessura, luminescência no IV, reacção à luz UV e determinação do tipo de pasta do papel. Para a detecção e visualização de dizeres gravados ou vincados na agenda suspeita, eventualmente relacionados com os dizeres manuscritos no bilhete, recorreu-se à utilização de um equipamento de detecção electrostática, o ESDA.

* O caso apresentado, bem como o material descrito é, por motivos de sigilo profissional, totalmente fictício, embora pretenda simular algumas situações reais. No entanto, os resultados analíticos apresentados, quer da análise das tintas, quer do papel, correspondem a dados de um caso real.

P.18 MORTE ACIDENTAL VS HOMICÍDIO: UM CASO DE ESTUDO* - 2

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"Um incêndio de grandes proporções destruiu ontem uma habitação em Carcavelos, provocando a morte de um jovem adulto, encontrado no seu interior já sem vida. As autoridades suspeitam de curto-circuito e de que a vítima terá morrido por inalação de fumo. A Polícia Judiciária está a investigar o caso."
No decorrer da perícia ao local de incêndio é excluída a hipótese de curto-circuito, concluindo tratar-se de um fogo iniciado por acção humana, acidental ou dolosa. Assim, o que à primeira vista parecia uma morte acidental acaba por se tornar uma investigação de homicídio. É efectuada a recolha de material para pesquisa de produtos inflamáveis no ponto de início do incêndio, tendo sido encontrado um bilhete de ameaça na roupa da vítima.
O material carbonizado recolhido foi sujeito a pesquisa de produtos inflamáveis pelas técnicas de GC/FID e GC/MS. A presença destes produtos, passíveis de serem utilizados como acelerantes de combustão, pode indicar tratar-se de um incêndio doloso, que configura o crime de incêndio. Porém, muitos compostos que fazem parte da composição de produtos inflamáveis existem nos próprios materiais que sofreram carbonização, que, juntamente com os seus produtos de combustão/pirólise podem originar um efeito matriz importante. Assim, estes factores, juntamente com o efeito da combustão na composição dos produtos inflamáveis produzem alterações no perfil cromatográfico dos produtos originais, factores importantes a ter em consideração na interpretação dos resultados.
Os laboratórios forenses fazem parte do sistema judicial de administração da justiça, auxiliam a investigação criminal e possibilitam aos tribunais a aplicação da justiça com base na apreciação da prova material, valorada desde finais do século XVIII.

* O caso aqui apresentado é uma compilação de várias situações reais que, por motivos do sigilo profissional, não podem ser divulgados.

P.19 ANÁLISE MULTI-RESÍDUOS DE PESTICIDAS EM FRUTAS E VEGETAIS – APLICAÇÃO DO MÉTODO QUECHERS, GC-ECD-NPD E GC-MS

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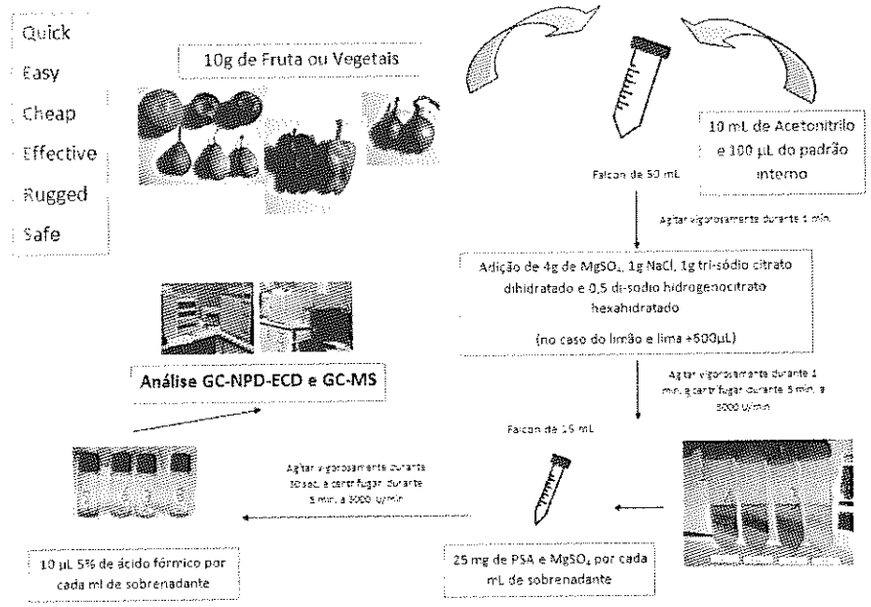
A utilização e massificação de pesticidas, em virtudes dos seus efeitos benéficos exercidos a nível da produtividade agrícola, marcaram a produção de alimentos das últimas décadas. As preocupações com o ambiente, bem como a incerteza dos efeitos nefastos por parte de alguns dos pesticidas, têm levado ao desenvolvimento de técnicas para a detecção destes produtos. Neste sentido a Escola Superior Agrária de Coimbra (ESAC), nomeadamente o laboratório de Química e Bioquímica com o intuito de responder a várias solicitações externas, procedeu à implementação das técnicas de rotina para a análise simultânea de múltiplos pesticidas em produtos alimentares.
O método de extracção utilizado foi o método de QuEChERS (Figura 1). O método QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) é um novo método para a extracção de resíduos de pesticidas em frutos, legumes e outros alimentos.



F1 Método de extracção: QuEChERS

A detecção de pesticidas nos extractos efectua-se num cromatógrafo HP 6890 Series com injector Split/Splitless com injecção em simultâneo em duas colunas (Coluna 1: Teknokroma, TRB-5ms, 30m x 0,25mm. Coluna 2: JW Scientific, DB-5ms, 30x0,25µm), acopladas respectivamente a detectores ECD e NPD. Nos casos em que se detectam bandas cromatograficas nestas condições, submetem-se os extractos a confirmação/identificação dos

resultados num cromatógrafo GC Varian 3900 acoplado a um detector de massa MS Varian Saturn 2100T.
A quantificação nas amostras positivas é efectuada recorrendo a padrões autênticos dos pesticidas (Mix A e Mix B da Supelco para os pesticidas organoclorados e Mix A e Mix B da Restek para os pesticidas organofosforados) após obtenção de rectas de calibração.
Foram analisadas 50 amostras de frutas e vegetais, cuja amostragem foi realizada em diferentes supermercados de Coimbra.
Este trabalho permitiu-nos concluir que o método de extracção QuEChERS é um método rápido, simples, de baixo custo, baixo consumo de solventes, não necessitando de equipamento especiais.
Nas amostras analisadas apenas foram encontrados valores vestigiais de pesticidas, abaixo dos limites máximos de resíduos (LMR) permitidos. Este facto provavelmente deve-se as crescentes medidas de fiscalização e prevenção implementadas.
Actualmente, o laboratório encontra-se a efectuar estudos que alarguem o âmbito de utilização do método a cerca de 140 substâncias activas.

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P.20 VALIDATION OF A CHROMATOGRAPHIC METHOD FOR VITAMIN B2 QUANTIFICATION IN FOODS

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The use of reliable, accurate and precise analytical methods for nutrients is essential for the calculation of dietary intake of nutrients from food. Method validation has become increasingly important as a mean of ensuring its reproducibility and results accuracy.
The aim of this study was first to optimise and validate a high performance liquid chromatography method with fluorometric detection for the determination of vitamin B_2 based on EN 14152 .This work was done as part of the process of achieving the quality insurance requirements needed to obtain method accreditation according to ISO 17025

Methodology: The analytical method involved an enzymatic treatment with isocratic chromatographic separation performed on a HPLC system (Waters Milford, MA, USA) equipped with a fluorescence detector.
Samples (2 to 10g) were submitted to an acid hydrolysis with HCl 0,1M for 30min in an autoclave at 121°C followed by an overnight enzymatic treatment (mixture of α-amylase and β-amylase), at 40°C with agitation. After filtration, 12.5 to 100μl of the solutions, were injected and separated at 37°C on a reversed phase column (Phenomenex Luna C18 (250x4, 6mm, 5µm). The mobile phase consisted of an aqueous solution of sodium acetate buffer (0.05mol/l): Methanol (70:30, v/v) with a flow rate of 1mL/min. Riboflavin detection used excitation and emission wavelengths at 422nm and 522nm, respectively. Quantification was made with an analytical calibration curve obtained with six concentrations of the riboflavin standard solution (0.04-0.14μg/mL).
The validation process consisted on the evaluation of the calibration curve parameters (working range, linearity, correlation coefficient (r), and analytical limits) followed by evaluation of precision, accuracy and detection and quantification limits, of the method, according to the criteria established by ISO 8466 and ISO 5725. Working range was established by testing homocedasticity of signals obtained with the two extreme calibrators of the analytical curve. Linearity was verified using Mandel's test and residual analysis. Detection and quantification limits were calculated based on residual standard deviation and slope of the regression line. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Accuracy evaluation was done by testing certified reference materials and through participation on proficiency testing schemes (FAPAS and BIPEA).

Results: Homocedasticity of the analytical calibration curve was verified through an F-test at confidence level of 99%, for the working range of 0.04-0.14ug/mL. The linearity was checked at a significance level of 1% and residuals were <10%. The values obtained for detection (LOD) and quantification (LOQ) of the method were, respectively, 0.0035 and 0.0106mg/100g. The r values of the 10 calibration curves obtained and used to the previous referred tests and calculations were ≥0.995.
For repeatability and intermediate precision RSD %, was ≤8.
The results for the analysed certified reference materials and the participation on proficiency testing schemes (FAPAS and BIPEA) showed absolute value of Z-scores <2.

Discussion: The method was checked against routine conditions in laboratory and has demonstrated that the results are appropriate for the purpose. The evaluation of the calibration

curve fulfilled all the established criteria and the method proved to be precise and accurate. The present methodology fulfils the requirements of ISO 17025.

P.21 OPTIMIZATION AND VALIDATION OF A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD TO MEASURE MALONDIALDEHYDE IN HUMAN SERUM: APPLICATION TO PORTUGUESE TYPE 2 DIABETIC PATIENTS

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Background: Over the last few decades, there has been a great interest in the role of oxidative stress in several pathological disorders, such as atherosclerosis, diabetes and cancer. Malondialdehyde (MDA), one of the major secondary oxidation products of peroxidized polyunsaturated fatty acids (PUFA), is known as a universal biomarker of lipid peroxidation[1]. Evaluation of MDA serum levels are commonly used to measure the degree of lipid peroxidation. There are several methods for measuring MDA, but the most widely employed is a high performance liquid chromatographic (HPLC) method based on the reaction of MDA with 2 thiobarbituric acid (TBA) in acidic medium with fluorescence detection. Unfortunately, the TBA assay is non-specific for MDA, because TBA is also reactive to other compounds that may be present in biological samples. This lack of sensitivity and the occurrence of side reactions were the main reasons for the development of new HPLC methods[2,3].

Aim: The present work describes an optimized and validated HPLC method with UV detection for analysis of MAD in human serum. This method was applied in order to assess MDA serum levels of a Portuguese population of 150 type 2 diabetic patients and its possible influence on angiopathy.

Methods: The assay was performed on an AcquityTM ULPC equipment with a PDA detector from Waters. A Phenomenex, SynergiTM Hydro-RP (150x4.6mm, 4.0µm) column with a SecurityGuard Cartridge AQ C18 (4.0x2.0mm) was used for separation. The mobile phase was potassium dihydrogenphosphate (0.03M; pH 4.0):methanol (95:5, v/v). The flow rate was 1.0mL/min and UV detection at 254nm. Validation procedure was performed according to Food and Drug Administration (FDA) or International Conference on Harmonization (ICH) guidelines[4,5]. The population in this study was divided in two groups: group I (36 males and 39 females) - 75 type 2 diabetics patients with angiopathy; group II (31 males and 44 females) - 75 type 2 diabetics patients without angiopathy.

Results: Detection and quantification limits were 0.016µM and 0.05µM, respectively. The standard curve for MDA was linear over the range of 0.1-10.3µM. The within-day precision varied from 0.53% to 3.07% and between-day precision was 8.52%. The overall recovery was calculated in fortified serum with 0.8, 1.8 and 2.8µM MDA and the values obtained for recovery were 102.75%, 92.51% and 94.14%, respectively.
The prevalence of retinopathy, macroangiopathy and two types of angiopathy (macroangiopathy and retinopathy) in group I was 57.3%, 17.3% and 25.3%, respectively. For both group, the overall mean of MDA levels in patients was 2.76 ± 0.78 µM. For group I, the mean values of MDA levels in males were 3.12 ± 0.66 µM and in females 2.68 ± 0.78 µM. For group II, the mean values for males were 3.82 ± 0.68 µM and for females 2.45 ± 0.70 µM.

Conclusion: A simple, sensible, rapid, precise and accurate HPLC method with UV detection has been validated for routine use in epidemiological studies. Although MDA serum levels were higher in Group I (2.91 ± 0.78 µM) than in Group II (2.60 ± 0.75 µM), no statistical differences were detected between MDA levels in patients with and without angiopathy. Our results show

that elevation of MDA serum levels in type 2 diabetic patients is not associated with the presence of angiopathy.

Keywords: Malondialdehyde, HPLC, Validation, Diabetes

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P.22 EFFICIENCY AND VOLATILE COMPOSITION OF COMMERCIAL PHEROMONE LURES OF CYDIA SPLENDANA IDENTIFIED BY GAS CHROMATOGRAPHY-QUADRUPOLE MASS SPECTROMETRY

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Cydia is a major threat to chestnut trees in Europe. In Madeira Island, this moth causes also heavy infestations to chestnut fruits. The endocarphophagous life cycle of this species together with the abandonment of infested fruits on the ground, partly due to the steep relief of chestnut fields, limits the application of prevention and control measures on this Island[1]. As result of this, populations of *Cydia splendana* are large representing a major threat to the culture and associated local traditions[1]. In order to outline a pest control program, the efficiency of six different commercial pheromone lures was tested in field trials in the three largest chestnut production areas of Madeira from 2004 to 2006[2]. Of all pheromones tested, those commercialized by Pheronet were the most efficient to collect *C. splendana*. In order to relate the results obtained with the volatile composition of these pheromones, a headspace solid phase microextraction followed by gas chromatography-mass spectrometry analysis was performed. The extraction methodology was optimized and all pheromones samples were analysed with the best conditions. As a whole 133 volatile compounds were identified in the six pheromones: 29 in *Pheronet* of which 17 are exclusive to this pheromone; 22 in *Oecos* with 5 exclusive; 47 in *Isca Technologies* with 25 exclusive; 41 in *Isagro* with 23 exclusive, and 26 in *Tomagro* with 5 exclusive. The chemical composition of each pheromone was compared to the pheromone efficiency in moth capture in the field.

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P.23 THE ROLE OF LC-MS(MS) IN WORKPLACE DRUG TESTING SCENARIOS

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Workplace drug testing is a well established branch of forensic toxicology, which aims at reducing workplace accidents caused by impaired workers. Several classes of abused substances may be involved, such as alcohol, amphetamines, cannabis, cocaine, opiates and also prescription drugs, like the benzodiazepines[1].

Drug testing can be performed in several situations, including pre-employment, reasonable cause/suspicion, accident or incident-related, after drug treatment or as follow-up procedures, and on a scheduled routine basis.

Urine has been traditionally the main biological specimen used for testing for drugs in the workplace, and this was partially due to the often higher concentrations at which the drugs (and metabolites) appear in this specimen when compared to blood, the ease of sampling and the low invasiveness of the collection procedure. However, urinalyses do present a number of disadvantages, from which the possibility of sample adulteration or substitution, and the consequent examinee's loss of privacy (because of the need of controlling the sample collection process) are the most important.

To overcome these limitations, several unconventional biological specimens, such as hair, oral fluid or sweat, are being evaluated for their possible inclusion in workplace drug testing programmes. These samples do present a number of advantages over urine, from which the fact that sample collection is totally non-invasive to the donor is the most important. Moreover, the time window for drug detection can be increased, for instance in the case of hair, allowing the documentation of long-term drug exposure. However, these specimens also present some drawbacks, essentially related to the low sample availability for analysis. This is relevant particularly for oral fluid, since drug concentrations of most drugs are very small in this specimen, and the volume available for analysis is often of less than 1mL.

Therefore, the use of high sensitive techniques is mandatory for most applications involving these specimens, and GC-MS based confirmation methods seem unsatisfactory to enable the detection of these low levels of drugs.

Consequently, the use of LC-MS(MS) appears to be a prerequisite to successfully meet the desired sensitivity, bringing a new light into bioanalytical and forensic sciences.

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P.24 SUPERCOILED PLASMID QUALITY ASSESSMENT BY ARGININE-AFFINITY CHROMATOGRAPHY - AN ANALYTICAL APPROACH

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Supercoiled plasmids are an important component of gene-based delivery vehicles, applied in new therapeutic strategies like gene therapy or DNA vaccination. However, aiming the general distribution of the plasmid DNA (pDNA) therapeutics it is required a procedure to easily and efficiently purify this sc plasmid isoform.

Taking advantage of affinity interactions between amino acids and nucleic acids it was established a new chromatographic strategy for the purification of pDNA[1]. In fact, arginine-agarose matrix was first used to fully separate supercoiled (sc) and open circular (oc) pDNA isoforms[2], at preparative scale.

Based on the technique previously described using amino acids as ligands to exploit a specific interaction with pDNA, an arginine affinity methodology with UV detection was developed to quantify and to control the quality of sc plasmid biopharmaceuticals. The application of an increased sodium chloride gradient resulted in the separation of oc and sc plasmid isoforms. The fact that this new technique is able to distinguish both plasmid isoforms represents a great advantage, since it allows the selective quantification of the biologically active pDNA topology, and a more accurate analysis of the quality of the plasmid isolated.

The analytical experiments were performed in 10 minutes and the method was found to be accurate, precise, reproducible and linear for a sc plasmid concentration range between 5 and 150μg/mL. In comparison with other established methods used in the quantification of native pDNA (oc+sc), the main advance introduced by this new method is the possibility to quantify the sc plasmid in a sample containing other plasmid topologies, assuring the purity of plasmid products to be therapeutically applied.

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P.25 ASSESSMENT OF THE PRESENCE OF 4-(5-)METHYLIMIDAZOLE IN SOFT DRINKS AND DARK BEERS

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4-(5-)Methylimidazole [4(5)MI] is a nitrogen heterocyclic that has been shows to be a potent convulsing agent that elicits neurological signs in mice, chicks, rabbits and cattle[1]. Studies *in vivo* have demonstrated its ability to inhibit the Cytochrome P450 isoenzyme, which catalyses the oxidation of many known or suspected carcinogens of low molecular mass in human liver[2].
For humans, the main sources of 4-(5-)MI are the ammonia caramel colours, a group of additives widely used in the food industry to colour a large number of commonly consumed foods and drinks, notably beers, carbonated beverages, cakes and biscuits, meat products, pickles, sauces and confectionery.
The aim of this work was to quantify by gas chromatography-mass spectrometry (GC-MS) the 4-(5-)MI in 19 darks beers and 40 soft drinks (colas, energy drinks, juices) commercialized in Portugal. The sample preparation was based in a method previous validated[3] which entails the following steps: i) extraction with an ion-pair (di-2-ethylhexylphosphoric acid) ii) back-extraction with 0.1 M HCl iii) derivatization with isobutulychloroformate iv) extraction of derivatives with isooctanol. GC-MS analysis was performed in SIM mode using for quantification 2 ethylimidazole as internal standard.
In general, the levels of 4-(5-)MI found in the dark beers were lower than those found in the soft drinks. The compound was found in 15 of 19 dark beers samples at levels that ranged from 6 to 425μg/l. In soft drinks 4-(5-)MI was found in 28 of 40 samples at levels that ranged from 34 to 613μg/l.

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P.26 INFLUENCE OF MALT IN THE PRODUCTION OF A XANTHOHUMOL ENRICHED BEER

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The hop plant (*Humulus lupulus* L.) is used in the brewing industry to add bitterness and aroma to beer[1]. Xanthohumol (XN) is the main prenylflavonoid of hops (0.2-1.1%) and is largely converted into isoxanthohumol (IXN) during the wort boiling[1]. This is the main reason why commercial beers have a low content of XN (max. of 0.15mg/L in pale beers) and a high content of IXN (ranging from 0.04 to 3.44mg/L)[2]. XN has been found to have a range of interesting biological properties in vitro that may have therapeutic utility including hormonal[2], antioxidant[2], inhibition of HIV-1[2], as well as its multimechanism classification as a potential "broad-spectrum" anticancer and cancer prevention agent[2].

XN and IXN can be determined by high-performance liquid chromatography with diode array detection (HPLC-DAD), according to a specific method, recently developed by our group[1]. It was verified that XN was largely converted into IXN during wort boiling. However, the use of dark malts revealed a positive effect on the thermal isomerization of XN. These results are indicative of the isomerization-inhibiting effect of the stout production process, which resulted in remarkable high levels of XN in the beer. Presumably, XN is bound to the roasted substances, present in roasted malt, during wort boiling preventing isomerization. The roasted substances may act as a carrier, transporting XN throughout the brewing process[2]. In order to investigate which substances are responsible for this inhibition, fractionation of roasted malt was carried out using size exclusion chromatography (SEC) in the form of gel permeation chromatography (GPC). It was performed by a Superdex 200 column from molecule sizes from 100 to 600kDa. Approximately 30 fractions were collected in the fractionation of roasted malt.

After molecular exclusion chromatography experiments it was verified that the adsorption of XN to PVPP (strong adsorbent of polyphenols) is lower for the high molecular weight fractions which lead to the conclusion that in these fractions (probably melanoidins) the XN preferentially adsorbs to other compounds of roasted malt, with which it forms more thermically stable complexes. In addition, it was verified that the ratio XN/IXN is significantly higher for the high molecular weight fractions in comparison with the low molecular weight ones, suggesting that the isomerization of XN into IXN was inhibited. On the other hand it was observed that the content of melanoidins was much higher in the higher molecular weight fractions, suggesting that probably these compounds are the main responsible for the isomerization inhibition of XN.

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P.27 DETERMINATION OF OCPS RESIDUES IN SOIL – COMPARATIVE STUDY OF SPE, SFE, LLE AND HYDROMATRIX

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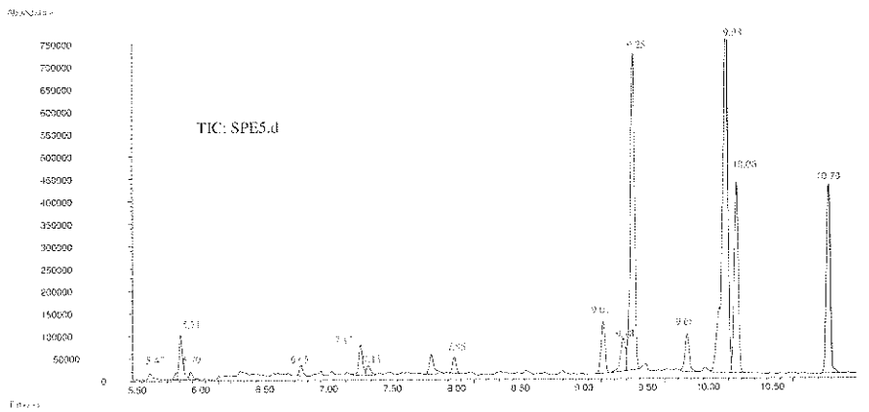
A solid phase extraction -gas chromatographic–mass spectrometric (SPE-GC–MS) method was developed to quantify organochloride pesticides (OCPs) residue in soils.
Optimal parameters of extraction are defined as well as GC/MS conditions for detection and quantification of analytes. Supercritical Fluid Extraction, Solid Phase Extraction and Extraction on Hydromatrix of OCPs have been compared. By comparision of the obtained experimental results, taking into account the other factors which influence the choice of sample preparation method, the optimal procedure for extraction of investigated OCPs from soil is chosen. Efficiency and recovery of Supercritical Fluid Extraction with CO2 of analyzed organochlorine pesticides from soil matrix is established. Recovery values are between 11.54% and 129.55%. Standard deviation values are in the range from 0.03 to 1.13, RSD value laid between 4.00 and 37.84%. After supercritical fluid extraction, further clean up is necessary. New solid phase extraction is developed for extraction of OCPs from soil matrix using C18 as a sorbent. Result for recovery, SD and RSD are between 69.87 and 105.04%, 0.01 and 0.48, 2.40 and 12.57%, respectively. Extracts obtained by SPE are clean, and there is no need for further clean up procedure. Results obtained with extraction on hydromatrix Extrelut are not comparable with results obtained by other extraction methods. Value for SD and RSD are extremely high for analytical methods (from 0.28 to 2.42 and 31.97% to 68.00% respectively), and method is not reproducible. Recovery values are between 11.00 and 180.67%. This extraction procedure has to be introduced for further clean up of samples. The results emphasized that the SPE procedure is significantly efficient and fulfilled the original aim - obtaining the fast, simple, reproductive, efficient and cheap method for extraction of the most frequently used OCPs residue from the soil matrix.



F1 Chromatogram of OSP's mixture extracted using C18-SPE column
(Retention times of OCP's: α HCH- 5.47; β HCH- 5.71; γ HCH- 5.79; heptachlor- 6.64; hlorpyrifos (ISTD) - 7.11; aldrin- 7.17; heptachlorepoxid- 7.85; p,p'-DDE- 9.01; dieldrin- 9.17; o,p'-DDD- 9.22; endrine- 9.66; p,p'-DDD- 9.94; o,p'-DDT- 10.03; p,p'-DDT-10.76)

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P.28 CHROMATOGRAPHIC AND ELECTROPHORETIC ANALYSIS OF SOLUBILIZED FULLERENE C_{60} AND ITS DERIVATIVES

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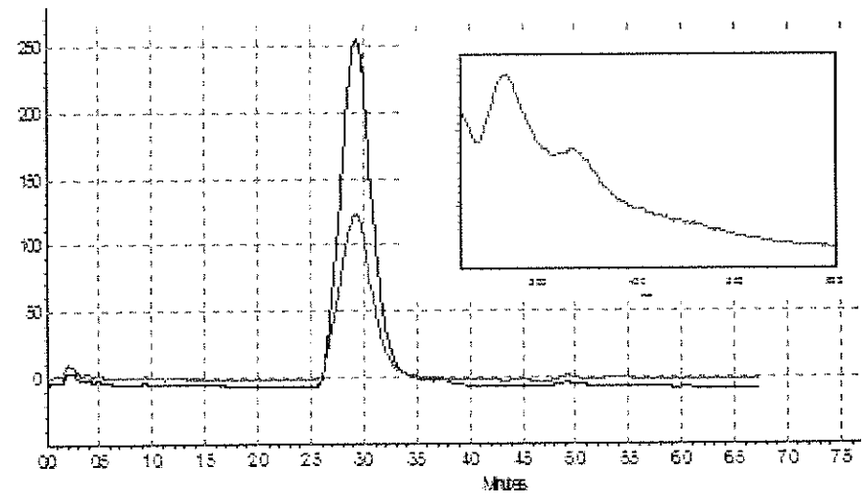
Since the discovery of fullerene C_{60}, various biologically activities of their derivatives have been claimed: antiviral and antibacterial properties, antioxidative and neuroprotective activities, cell signaling and apoptosis. Fullerenes are extremely hydrophobic molecules best dissolved in benzenes, naphthalenes and alkanes[1], so potential biomedical applications of fullerenes have been hampered by their extremely poor solubility in polar solvents. There are several possible approaches to transfer fullerene into water: solubilization with surfactants, chemical modification of the carbon cage of fullerene, solvent exchange and long term stirring of C_{60} in water.
In our laboratory, we have been focusing on the techniques that do not involve chemical modification, to help solubilize hydrophobic fullerene derivatives. The development and evaluation of solubilized forms must be supported by suitable analytical methodologies. Previous results published by different authors report HPLC analysis of fullerene suspended in aqueous media, using re-extraction with toluene before analysis. On the other hand, analysis by micellar electrokinetic chromatography (MEKC) of C_{60} has been performed using SDS (sodium dodecyl sulfate) as a solubilizer of fullerene. Due to its ionic properties, SDS is not suitable for application in biological systems[2,3] and therefore we have solubilized C_{60} and its derivatives (conjugates with BHT) with a more biocompatible surfactant, Tween-80, by incorporating fullerene into this water-soluble supramolecular structure. We have also developed HPLC and MEKC techniques for analyzing fullerenes directly from the aqueous solutions, without any pretreatment.



F1 Chromatogram of C_{60}–Tween80 and corresponding UV/VIS spectra recorded at 263 and 335nm (legend: 263nm; 330nm). In square is presented characteristic UV/VIS spectra of C_{60} from 200 – 600nm.

For HPLC analysis, a Waters Spherisorb ODS2 column, 250mm x 4.6mm x 5μm, was used with a mobile phase composed of acetonitrile and water (1:1) at a flow rate of 0.5mL/min. Solubilized fullerenes were detected between 2 and 3min as confirmed by UV/VIS spectra (Figure 1).
For MECK we used 40cm fused silica capillary, 0.5μm i.d., at 16kV separation voltage in 10mM, pH=9.2, phosphate-borate buffer with 150mM SDS.
With both techniques we succeed to analyze fullerenes and fullerene derivatives which give us the possibility of choosing the technique according to sample availability or sensitivity needed.
HPLC has some advantages in comparison with MEKC, being faster, more reproducible and allowing obtaining better UV/VIS spectra. MEKC offers the possibility of less sample consumption which may be important when analyzing compounds prepared in very small amounts.
These methods will be used for identification and detection of these forms of fullerene from biological samples. Results obtained in our lab are, to our knowledge, the first that allow preparing and analyzing soluble forms of unsoluble fullerenes and open new perspectives in the evaluation of the biological activity of these compounds.

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P.29 CAPILLARY ELECTROPHORESIS - ELECTROOSMOTIC FLOW MODULATION BY IONIC LIQUID CATIONS

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Due to interesting physical and chemical properties such as thermal stability, high ionic conductivity, negligible vapor pressure and large electrochemical window versatility, ionic liquids (ILs), have become a matter of research in many fields. The study of their thermodynamic properties has been the main subject for many scientists, but it is in the large potential for future applications that remains the true asset of these compounds.
The main application so far, has been in synthetic chemistry where ILs have been used as alternative recyclable environmentally benign reaction media for chemical processes. ILs can solubilize a large range of compounds and their physical properties (such as melting point, viscosity, density, hydrophobicity and chirality) can be tuned by modification of their cations and anions in order to achieve the adequate properties for the intended reaction.
Other applications have been found in separation science such as chromatography and capillary electrophoresis (CE). In the field of capillary electrophoresis, ionic liquids have been applied in aqueous and nonaqueous separations to enhance resolution, peak efficiency and peak symmetry but the application of ILs for chiral separations remains a relatively unexplored topic. This is a highly interesting application since enantiomeric compounds frequently display different biological activity and toxicity and therefore the development of reliable, inexpensive, environmentally friendly and fast analytical techniques is of upmost importance for the development of new drugs.
The ability of cations for establishing ionic bonding with the capillary silanol groups, allows modulating the EOF and reducing adsorption of the analytes to the capillary walls and therefore, the IL's cations can be used also with this purpose.
As could be expected, several ionic liquids based on imidazolium and ammonium cations, have demonstrated EOF reversal ability.
In this paper, we have studied this property in these and other types of cations that have been used in the preparation of ionic liquids including guanidinium, phosphonium, cysteinium and sulfonium.
Analyses were carried out in a Beckman P/ACE MDQ capillary electrophoresis system coupled with a diode array detector (DAD) (Palo Alto, CA, USA). A 50µm, 40cm (30cm to detector) fused silica capillary was used and maintained at 25°C.
The test substance was DL-propranolol hydrochloride at 100ppm in water with 1% DMSO as EOF marker. The running buffers were composed of sodium phosphate at 100 mM and pH=3 or pH=10 plus the chloride or bromide salt of each cation, at 1.25 or 2.5mg/mL.
For evaluation of the ability to improve chiral resolution, 7.5mM of hydroxypropyl-β-cyclodextrin (HP-β-CD) was added to each of the previous buffers.
The results confirm the ability of the above stated cations for the modulation of EOF. Most guanidinium and phosphonium cations reversed the EOF at lower concentrations than immidazolium cations.
Some cations also had a positive effect in the resolution of the enantiomers of propranolol separated by HP-β-CD. In this case, the most effective cations were the immidazolium derivatives, which demonstrate that the resolution improvement is not related to the EOF modulation effect.

P.30 PHENOLICS METABOLISM IN INSECTS: *PIERIS BRASSICAE* - *BRASSICA OLERACEA* VAR. *COSTATA* ECOLOGICAL DUO

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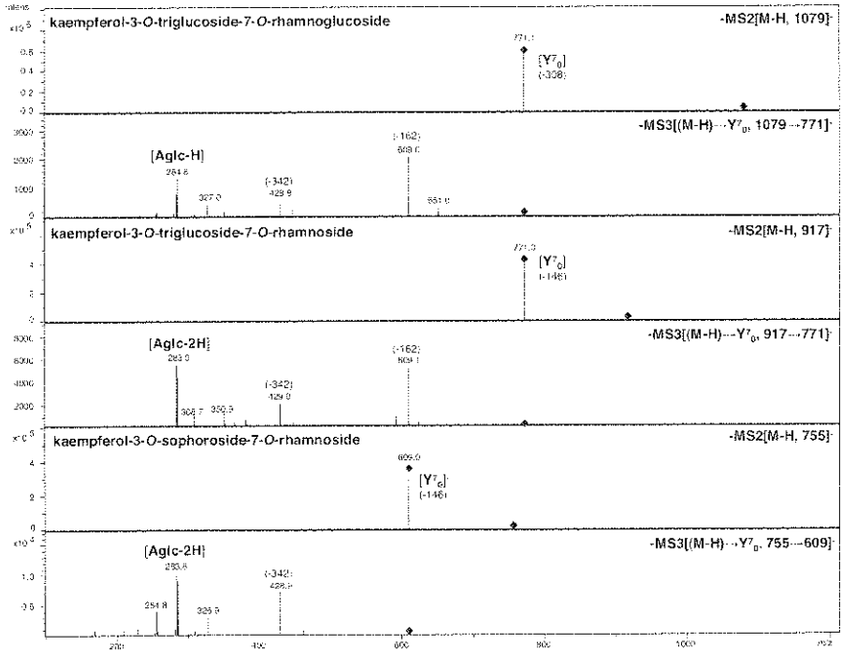
Changes in phenolics composition of *Pieris brassicae* larvae fasted for distinct periods (1, 2, 4, 6, and 8h), their excrements and also *Brassica oleracea* L. var. *costata* DC leaves were determined by HPLC/UV-PAD/MSn-ESI. This is the first report following phenolics' metabolism in *P. brassicae*. The results evidence that *P. brassicae* sequesters and metabolizes the phenolic compounds from the host plant. In a general way, deacylation was the main metabolic reaction, but deglycosylation and sulphate conjugation reactions also occurred. Additionally, several kaempferol derivatives containing rhamnose (Figure 1) that is not common in *Brassica*, were found in the host plant. Attending to the bioactivities recognized for the type of identified compounds, the different materials may constitute an interesting source of bioactive compounds, namely of highly glycosylated and acylated kaempferol and quercetin derivatives, representing an economic advantage for producers who have great losses caused by this pest. In addition, a deeper understanding of phenolics metabolism in insect was pursued.



F1 MS2 and MS3 spectra of some flavonoids derivatives with rhamnose, not common in *Brassica*.

Acknowledgments: The authors are grateful to Fundação para a Ciência e a Tecnologia (PTDC/AGR-AAM/64150/2006) for financial support. D. M. Pereira (BI) and F. Fernandes (SFRH/BD/37963/2007) are grateful to FCT for the grants.

P.31 SIMPLE AND REPRODUCIBLE HPLC-DAD-ESI-MS/MS ANALYSIS OF ALKALOIDS IN *CATHARANTHUS ROSEUS* ROOTS

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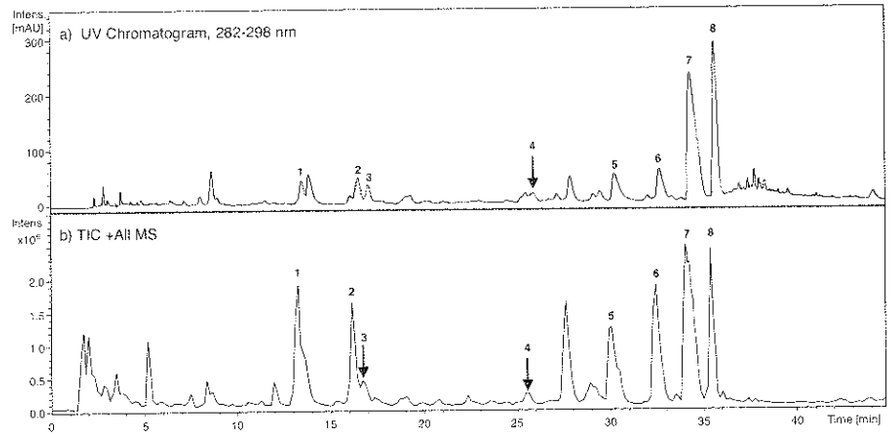
Catharanthus roseus remains one of the most important medicinal species worldwide. Its leaves constitute the only source of the indolomonoterpenic alkaloids vincristin and vinblastin, powerful therapeutics for many cancers, such as Hodgkin lymphoma. Usually, the remaining plant parts go to waste. This work describes a phytochemical study on this species roots. Alkaloids in aqueous extract, the usual form of consumption of this matrix, were studied using HPLC-DAD-ESI-MS/MS, which allowed the identification of 19-*S*-vindolinine, vindolinine, ajmalicine and an ajmalicine isomer, tabersonine, catharanthine, serpentine and a serpentine isomer (Figure 1). Quantification of these compounds revealed that serpentine and its isomer were predominant (64.7%) over the other alkaloids, namely vindolinine and its isomer (23.9%), catharanthine (7.7%) and ajmalicine (3.8%). The used procedure revealed to be simple, sensitive and reproducible.



F1. HPLC-DAD-ESI-MS/MS of the alkaloids of *Catharanthus roseus* roots. a) UV chromatogram at 282-298nm; b) Total Ion Current (TIC) chromatogram. (1) 19-*S*-vindolinine; (2) vindolinine; (3) ajmalicine (isomer); (4) tabersonine; (5) ajmalicine; (6) catharanthine; (7) serpentine; (8) serpentine (isomer).

Acknowledgments

The authors are grateful to Fundação para a Ciência e a Tecnologia (PTDC/AGR-AAM/64150/2006) for financial support. D. M. Pereira (BI) and J. Faria (BII) are grateful to FCT for the grants.

P.32 CHROMATOGRAPHIC DETERMINATION OF TOCOPHEROLS, SUGARS AND FATTY ACIDS IN WILD FRUITS

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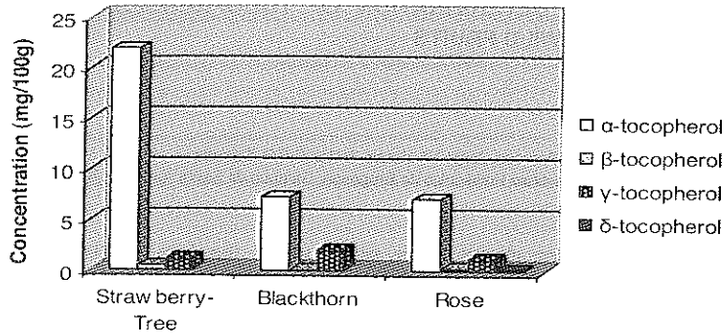
Food and medicinal plants have always been significant in the folk traditions of the Mediterranean area. Similar diets and ailments treated with plants, as well as, related practices in preparation and administration of the folk remedies reflect an important heritage, which constitutes a base for phytochemical and pharmacological studies that can lead to new therapeutic and nutraceutical products[1].

Three wild fruits (strawberry-treeand blackthorn berries and dog rose hips) were analyzed for tocopherols by high performance liquid chromatography (HPLC) coupled to a fluorescence detector, sugars by HPLC coupled to a refraction index detector (RID), and fatty acids by gas-chromatography (GC) coupled to a flame ionization detector (FID).

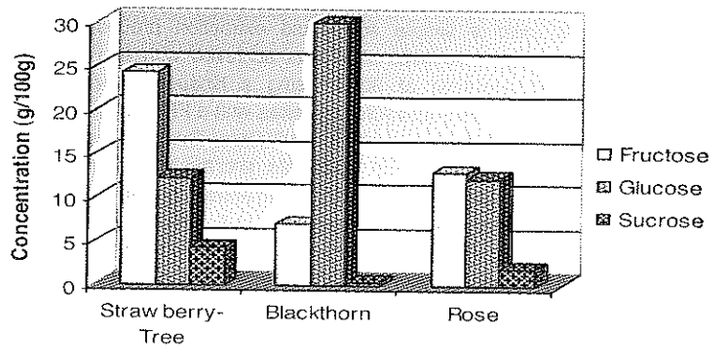
For tocopherols analysis it was used a simple solid-liquid extraction procedure without saponification step and the chromatographic separation was achieved with a a YMC-Pack Polyamine II column (250x4.6mm) operating at 30ºC, using a Knauer Smartline HPLC equipment with a 2500 UV detector at 295 nm connected in series with a FP-2020 fluorescence detector programmed for excitation at 290nm and emission at 330nm. The mobile phase used was hexane/ethyl acetate (70:30, v/v) at a flow rate of 1.0mL/min, and the injection volume was 20μl. For sugars analysis it was used a solid-liquid extraction procedure and the chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6mm x 250mm, 5mm) operating at 35ºC, using a Knauer Smartline HPLC equipment with RID. The mobile phase used was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1mL/min, and the injection volume was 20μl. The fatty acid profile was analyzed, after a trans-esterification procedure, with a DANI model GC 1000 instrument equipped with a split/splitless injector, a FID and a Macherey-Nagel column (30m x 0.32mm ID x 0.25μm df)[5]. The oven temperature program was as follows: the initial temperature of the column was 50ºC, held for 2min, then a 10ºC/min ramp to 240ºC and held for 11min. The carrier gas (hydrogen) flow-rate was 4.0mL/min (0.61 bar), measured at 50ºC. Split injection (1:40) was carried out at 250ºC.

The values obtained in the analysis of the samples point to the existence of differences in what concerns tocopherols composition (Figure 1). α-Tocopherol was the major compound in all the fruits, and δ-tocopherol was only detected in blackthorn fruits. Strawberry-tree fruits presented the highest content of tocopherols (23.46mg/100 g of dry weight) while rose fruits revealed the lowest content (8.33mg/100g). α-Tocopherol was the principal form of vitamin E, it is a lipid-soluble antioxidant and it functions as a chain-breaking antioxidant for lipid peroxidation (LP) in cell membranes and also as a scavenger of reactive oxygen species such as singlet oxygen. It is considered to serve as the first line of defence against LP, and it protects PUFAs (polyunsaturated fatty acids) in cell membranes from free radical attack through its scavenging activity in biomembranes at early stages of LP.[2]



F1 Tocopherols composition (mg/100 g of dry weight) of the wild fruits.

In what concerns sugar composition (Figure 2) the wild fruits presented fructose, glucose and sucrose as main sugars.



F2 Sugars composition (g/100 g of dry weight) of the wild fruits.

For strawberry-tree (24.21g/100g) and rose fruits (12.89g/100g) fructose was the most abundant sugar, while glucose predominates in blackthorn samples (29.84g/100g). Strawberry-tree fruits revealed the highest total sugars content, and highest levels of fructose and sucrose (Figure 2), which is in agreement with its sweet taste. Otherwise, rose fruits showed the lowest levels in total sugars (26.90g/100g).

The major fatty acids found in strawberry-tree and rose fruits were α-linolenic acid (C18:3) and linoleic acid (C18:2), contributing to the prevalence of PUFA in these samples. In blackthorn fruits, MUFA (monounsaturated fatty acids) predominated over MUFA due to the abundance of oleic acid (C18:1). These fruits also present high levels of linoleic acid but significant lower amounts of α-linolenic acid than the other two wild fruits.

Overall, strawberry-trees revealed the highest contents in sugars and tocopherols while rose fruits showed the highest content in PUFA. This study contributes not only to a better knowledge of these wild fruits but also to their valorisation.

Acknowledgements
FCT grant SFRH/BPD/4609/2008.

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P.33 OPTIMIZATION OF THE DETERMINATION OF TOCOPHEROLS IN LAMIACEA SP. BY A NORMAL PHASE LIQUID CHROMATOGRAPHIC METHOD

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Many wild plants gathered from the scrubland were preserved and used for medicinal and food purposes in the north-eastern region of Portugal. Since they are important ingredients of the folk pharmacopoeia and traditional cuisine some of these wild botanicals have been semi-domesticated and are still cultivated in homegardens and present in every homesteads. Several ethnobotanical surveys conducted in this Portuguese region have highlighted the use of three particular *Lamiaceae*[1-3]. Ground ivy (*Glechoma hederaceae*), oregano (*Origanum vulgare* subsp. *virens*) and mastic thyme (*Thymus mastichina*) are widespread Mediterranean perennial herbs widely considered as medicinal plants, although it has also been reported some other common uses.

Epidemiological and experimental studies have consistently shown an inverse association between consumption of vegetables and fruits and the risk for chronic diseases related to oxidative stress, such as cardiovascular diseases, arthritis, chronic inflammation and cancers[4]. Therefore, there is a growing interest in natural antioxidants for their potential role in the prevention of those oxidative stress-related diseases; furthermore, synthetic antioxidants are being questioned due to their potential carcinogenic activity.

In the present study, the tocopherols (powerfull antioxidants) contents of three *Lamiaceae* often used in Portuguese folk medicine were determined. The individual profiles were obtained by a normal-phase high-performance liquid chromatography (NP-HPLC). The linearity and sensitivity of the HPLC analysis were determined and the method was validated by the repeatability and accuracy, using Thymus mastichina.

It was used a simple solid-liquid extraction procedure without saponification, in which a BHT solution (100μL) and IS solution (250μL) were added to the sample prior to the extraction procedure. The samples (~500mg) were homogenized with methanol (4mL) by vortex mixing (1min). Subsequently, hexane (4mL) was added and again vortex mixed for 1min. After that, saturated NaCl aqueous solution (2mL) was added, the mixture was homogenized (1min), centrifuged (5min, 4000g) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 1mL of n-hexane, dehydrated with anhydrous sodium sulphate, filtered through a 0.22μm disposable LC filter disk, transferred into a dark injection vial and analysed by HPLC.

The chromatographic separation was achieved using a HPLC equipment integrated with a Smartline pump 1000, a degasser system Smartline manager 5000, an AS-2057 auto-sampler and a 2500 UV detector at 295nm connected in series with a FP-2020 fluorescence detector programmed for excitation at 290nm and emission at 330nm. Data were analysed using Clarity 2.4 Software. The chromatographic separation was achieved with a Polyamide II (250x4.6mm) normal-phase column from YMC Waters operating at 35°C. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1mL/min, and the injection volume was 20μl. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method (0.075 to 16.0μg/mL; α-tocopherol y=2.12899x; β-tocopherol y=0.51248x; δ-tocopherol y=0.7359x; γ-tocopherol y=0.65148x). Tocopherol contents in the samples are expressed in μg per g of dry matter.

For each compound, 7-level calibration curve was constructed using the peak-area ration between the tocopherol and tocol versus concentration of the standard (ng/mL). The average of triplicate determinations for each level was used. The correlation coefficients were always higher than 0.999 for all the compounds. The limits of detection (LOD), calculated as the concentration corresponding to three times the calibration error divided by the slope, ranged from 14.79 and 25.82ng/mL. The limits of quantification (LOQ) were calculated using the concentration corresponding to ten times the calibration error divided by the slope, and ranged from 49.32 to 86.07ng/mL.

In order to evaluate the instrumental precision, the sample extract was injected six times. The chromatographic method proved to be precise (CV% between 0.22 and 2.36%). Repeatability was evaluated by applying the whole extraction procedure 6 times to the same sample. All the obtained values were low (CV% ranging from 2.18 to 4.45%). The accuracy of the method was evaluated by the standard addition procedure (% of recovery) with three addition levels (0.5, 1.0 and 2.5μg/mL of each one in duplicate). The standard mixture was added to the sample, and all the extraction procedure was carried out. The results demonstrate good recovery for the compounds under study (ranging from 89 to 95%).

Glechoma hederacea revealed higher content of tocopherols (369mg/100g), while the other *Lamiaceae* presented the opposite. The four tocopherols (α, β, γ, δ-tocopherols) were quantified in all the samples. α-Tocopherol was the major tocopherol vitamer for *Glechoma hederacea* (272mg/100g) and *Origanum vulgare* (10.1mg/100g). For *Thymus mastichina*, γ-tocopherol was the most abundant vitamer (3.8mg/100g), revealing this species the lowest total tocopherols content (4.1mg/100g).

Acknowledgements
FCT grant SFRH/BPD/4609/2008.

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P.34 CHROMATOGRAPHIC ANALYSIS OF IMPORTANT PHYTOCHEMICALS IN AROMATIC PLANTS

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The number of individual phytochemicals already identified in fruits and vegetables is estimated in >5,000, but a large percentage still remain unknown and need to be identified before we can fully understand the health benefits of phytochemicals in whole foods[1]. However, more and more convincing evidence suggests that the benefits of phytochemicals may be even greater than is currently understood, because the oxidative stress induced by free radicals is involved in the etiology of a wide range of chronic diseases[2]. Phytochemicals can include secondary metabolites such as phenolic compounds but also vitamins, sugars and fatty acids, and these compounds can be used as nutraceuticals.

Cistus ladanifer and other species of *Cistaceae* are used as general remedies in folk medicine for treatment of various skin diseases, as antidiarrheics, and as anti-inflammatory agents[3]. According to literature the foliage of *Cupressus lusitanica* is used in indigenous practices to treat catarrh, headache and dermatitis. The essential oil of the leaves is used against rheumatism, whooping cough, and styptic problems[4]. For the production of phytopharmaceuticals, essential oils rich in 1,8-cineole (called also "eucalyptol"), are of special importance. These products are applied for relief of head colds, rheumatism, muscular pain, and as expectorant in cases of bronchitis (added to cough syrups).

In the present work, the phytochemical composition of three aromatic plants (*Cistus ladanifer L.*, *Cupressus lusitanica Mill.* and *Eucalyptus gunnii Hook.* f) were evaluated, in order to valorize them as sources of nutraceuticals. Samples were analyzed for ascorbic acid by spectrophotometric assay, tocopherols by high performance liquid chromatography (HPLC) coupled to a fluorescence detector, sugars by HPLC coupled to a refraction index detector (RID), and fatty acids by gas-chromatography (GC) coupled to a flame ionization detector (FID).

For tocopherols analysis it was used a simple solid-liquid extraction procedure without saponification step and the chromatographic separation was achieved with a a YMC-Pack Polyamine II column (250x4.6mm) operating at 30°C (7971R Grace oven), using a Knauer Smartline HPLC equipment with a 2500 UV detector at 295nm (Knauer, Germany) connected in series with a FP-2020 fluorescence detector (Jasco, Japan) programmed for excitation at 290nm and emission at 330nm.5 The mobile phase used was hexane/ethyl acetate (70:30, v/v) at a flow rate of 1.0mL/min, and the injection volume was 20μl. For sugars analysis it was used a solid-liquid extraction procedure and the chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6mm x 250mm, 5mm, Knauer) operating at 35°C, using a Knauer Smartline HPLC equipment with RID[5]. The mobile phase used was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1mL/min, and the injection volume was 20μl. The fatty acid profile was analyzed, after a trans-esterification procedure, with a DANI model GC 1000 instrument equipped with a split/splitless injector, a FID and a Macherey-Nagel column (30m x 0.32mm ID x 0.25μm df)[5]. The oven temperature program was as follows: the initial temperature of the column was 50°C, held for 2min, then a 10°C/min ramp to 240°C and held for 11min. The carrier gas (hydrogen) flow-rate was 4.0mL/min (0.61 bar), measured at 50°C. Split injection (1:40) was carried out at 250°C.

Eucalyptus sample presented the highest content of tocopherols (1558.27μg/g of dry weight). Otherwise, ascorbic acid was the most abundant vitamin in *Cupressus lusitanica* and *Cistus ladanifer* leaves, and particularly for the latter sample it presented a very high level (647.64mg/g). The aromatic plants presented fructose, glucose, sucrose and raffinose as main

sugars. *Cupressus lusitanica* revealed the highest total sugars content (82.96mg/g), and highest levels of fructose and raffinose. The present study describes for the first time the sugars composition in these aromatic plants. The major fatty acids found in *Cupressus lusitanica* and *Eucalyptus gunnii* samples were α-linolenic acid (C18:3), followed by linoleic acid (C18:2) and palmitic acid (C16:0). For *Cistus ladanifer* leaves, eicosadienoic acid (C20:2) was the most abundant fatty acid, and was followed by arachidic acid (C20:0) and α-linolenic acid. Twenty four fatty acids were identified and quantified. As far as we know, nothing has been reported on fatty acid composition of the leaves of the three aromatic plants, despite the extensive reports on their essential oils composition[4,6].

Overall, the combination of the useful phytochemicals found in the analysed aromatic plants with the precious contribution of vitamins (tocopherols and ascorbic acid) and reducing sugars (glucose and fructose) make them a possible source of compounds to be used as remedies for diseases related to oxidative stress, or dermatological applications, as also for cosmetics. The polyunsaturated fatty acids (linoleic, α-linoleic and eicosadienoic acids) including omega-3 and omega-6 families detected in the plants constitute another important class of phytochemicals due to their generalised beneficial health effects.

Acknowledgements
Foundation for Science and Technology (Portugal) for financial support to L. Barros (SFRH/BPD/4609/2008).

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P.35 SELECTED NUTRIENTS IN PORTUGUESE WILD MUSHROOMS ANALYSED BY GAS CHROMATOGRAPHY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Wild edible mushrooms have been used as food and food flavouring material in soups and sauces for centuries, due to their unique and delicate flavour and they have also been traditionally eaten seasonally by specific groups of people (local people, enthusiasts and gourmets) providing a source of minerals and vitamins when fresh vegetables were not available. Wild edible mushrooms are rich in trace minerals, and have high water, protein, fibre, and carbohydrate contents, and low fat/energy levels making them an excellent food for use in low caloric diets[1]. Our research group has been interested in the nutritional characterization of wild mushrooms, and in the last years we studied sixteen different mushroom species from the Northeast of Portugal, one of the European regions with higher wild mushrooms diversity[2,3]. Nevertheless, we intend to go on in the study of this biological matrix, documenting the nutritional composition of all these unique species, and making the information available for a better management and conservation of this natural resource and related habitats.

In this work, we report the chemical composition of ten different Portuguese wild mushrooms, with reference to the contents of moisture, proteins, fat, carbohydrate and ash. On the basis of the samples composition, an estimation of the mushrooms nutritional role was also performed. Among the individual components, fatty acid and sugar profiles were obtained by gas chromatography coupled to a flame ionization detector (GC/FID) and high performance liquid chromatography coupled to a refraction index detector (HPLC/RID), respectively, the latter methodology being then completely validated. The fatty acid profile was analyzed, after a trans-esterification procedure, with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID) and a Macherey-Nagel column (30m x 0.32mm ID x 0.25μm df)[4]. The oven temperature program was as follows: the initial temperature of the column was 50°C, held for 2min, then a 10°C/min ramp to 240°C and held for 11min. The carrier gas (hydrogen) flow-rate was 4.0mL/min (0.61 bar), measured at 50°C. Split injection (1:40) was carried out at 250°C. For sugars analysis, it was used a solid-liquid extraction procedure and the chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6mm x 250mm, 5mm, Knauer) operating at 35°C (7971R Grace oven), using a Knauer Smartline HPLC equipment with RI detector[4]. The mobile phase used was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1mL/min, and the injection volume was 20μl.

The macronutrient profile in general revealed that the wild mushrooms were rich sources of protein (24.32 to 76.63g/100g) and carbohydrates (10.35 to 55.48g/100g), and had low amounts of fat (0.36 to 2.63g/100g). The highest energetic contribution was guaranteed by *Hygrophoropsis aurantiaca*, while *Hypholoma capnoides* gave the lowest energy contribution.

The analysis of fatty acid composition allowed the quantification of twenty five fatty acids. Unsaturated fatty acids and, in particular, oleic (C18:1) and linoleic (C18:2) acids, were predominant (17-61% and 20-54%, respectively). Both linoleic and oleic acids have been related to decreased risk of cardiovascular disease, contributing to the recommendation of mushrooms in the diets of people with high blood cholesterol[5]. Furthermore, linoleic acid is the precursor of 1-octen-3-ol, known as the alcohol of fungi, which is the principal aromatic compound in most fungi and might contribute to mushrooms flavour[6].

In the analysis of free sugars, all the compounds were separated in a period of time of 10min; the method used proved to be precise (CV% ranged between 0.82 and 1.47), reproducible (CV% ranged from 1.02 to 2.09) and accurate (recovery %) between 91.04% and 92.11%). Arabinose (1.53 to 7.66g/100g), mannitol (0.38 to 18.41g/100g) and trehalose (0.21 to 18.66g/100g) were the most abundant sugars.

Overall, the rich nutritional composition (high contents in protein and carbohydrates, low contents in fat with the precious contribution of unsaturated fatty acids, and absence of trans fatty acids) makes wild mushrooms very special. This study contributes to the documentation of the nutritional composition of wild mushrooms, which are highly consumed and appreciated, but most of the times without a scientific base of support.

Acknowledgements
Foundation for Science and Technology (Portugal) for financial support to L. Barros (SFRH/BPD/4609/2008).

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P.36 LIMITATIONS TO THE APPLICATION OF EMMA METHODS TO KINETIC STUDIES: ACETYLCOLINESTERASE INHIBITION BY PLANT EXTRACTS

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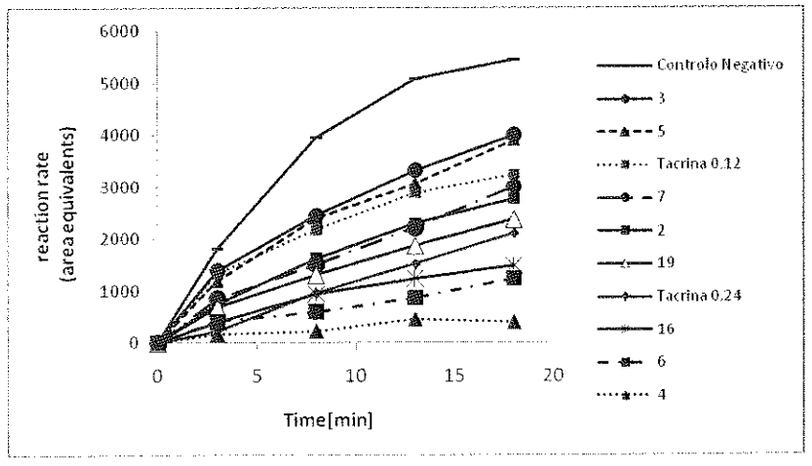
Electrophoretically mediated microanalysis (EMMA) presents several advantages in the study of enzyme activity. The correct evaluation of the kinetic parameters and/or inhibition constants by this technique is however dependent on the appropriate establishment of the analytical parameters which may, in some cases, be limited by the sensitivity of the detector. Moreover, as reported by Tang *et al.*[1], the Michaelis-Menten and inhibition constants of acetylcholinesterase by tacrine, are not always in agreement with the parameters obtained by traditional techniques.

All EMMA methods for enzyme kinetics are based on the assumption that the enzyme and substrate concentrations inside the capillary are the same as the concentrations in the original solutions, however this may not be the case namely due to stacking effect or diffusion. By careful tuning of the assay conditions such as concentrations, injection pressure, voltage and contact time, it is possible to obtain the same kinetic parameters that are determined with traditional methods.

In the present work, we optimized a previously published EMMA method[1] in order to obtain the some kinetic constants as determined with the spectrophotometric method, however the use of EMMA for the study of enzyme kinetics of unknown samples may be limited if the mobilities of the reagents is not known.

Among several plants, the ethanolic extract of Hypericum undulatum (St. John's Wort), was found to exhibit acetylcholinesterase inhibition potential, but interference at the detection wavelength prevented the test at higher concentrations by the traditional Elman's method[2].

Although EMMA has been used several times for screening inhibitors in plant extracts, we found that there is a risk of false negatives due to differences in the electrophoretic mobility of the sample components. In this work we report the low performance of an EMMA method[1] when searching for acetylcholinesterase inhibitors in different extracts of in vitro produced cells of St. John's Wort.



F1 Decrease in the reaction rate (equivalent to the decrease in area of the product) in the presence of extracts.

As an alternative, we propose an off-line capillary electrophoresis method which provides evidence of inhibitory potential of the extracts, but still avoids interference found when applying the Elman's method.

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P.37 ALIMENTARY SUPPLEMENTS: AN OBJECTIVE COMPARISON OF DIFFERENT BRANDS OF THE SAME PRODUCT

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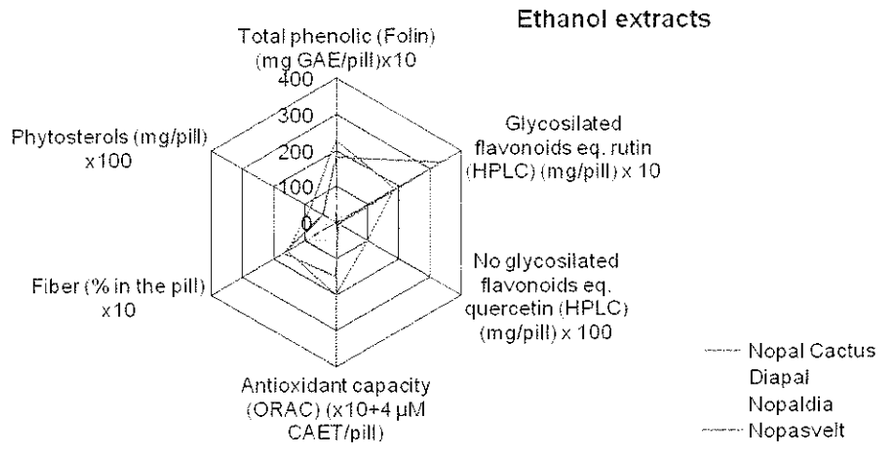
With the discovery of new active substances in medicinal plants and with the increase in the demand for natural products, synthetic drugs are sometimes substituted by the ones of botanical origin. However this may be in the origin of a problem of public health, due to reduced legislation and harmonization of products and procedures.

Therefore, several products reach the market without proper description of their characteristics and claims as well as without adequate quality, safety and efficacy that are necessary for the preservation of consumer health.

This work consisted in the comparison of different food supplements derived from the same plant (*Opuntia ficus indica*), that are available in the world-wide market, with different brands and from different producers. Comparisons were based on the chemical composition as well as on their potential pharmacological properties and claims alleged in the package. Phenolic constituents were quantified in different extracts of the chosen samples, namely flavonoids which present significant antioxidant capacity. The fiber and phytosterol content were also determined. These constituents are reported because they have alleged beneficial properties to the human health, namely: cholesterol-lowering effect, antidiabetic, analgesic, antiulcerogenic and anti-atherogenic.

It was concluded that, in fact, there are products in the market that despite having similar descriptions in the label, present different compositions and concentrations. The doses recommended for each product are not related with their relative compositions and there are probably some differences in the production processes that lead to deglycosilation of phenolic compounds in some cases but not in others.



F1. Comparison of ethanolic extracts obtained from different market products from *Opuntia ficus indica*.

So, it is extremely necessary to invest in the standardization and in the definition of quality criteria, composition and efficacy evaluation of dietary supplements, as well as in training of qualified persons for the rigorous evaluation and application of these regulations and criteria.

P.38 EVALUATION OF ESTERASE ACTIVITY IN SERUM AND IN MAMMAL CELL EXTRACTS BY ELECTROMEDIATED MICROANALYSIS

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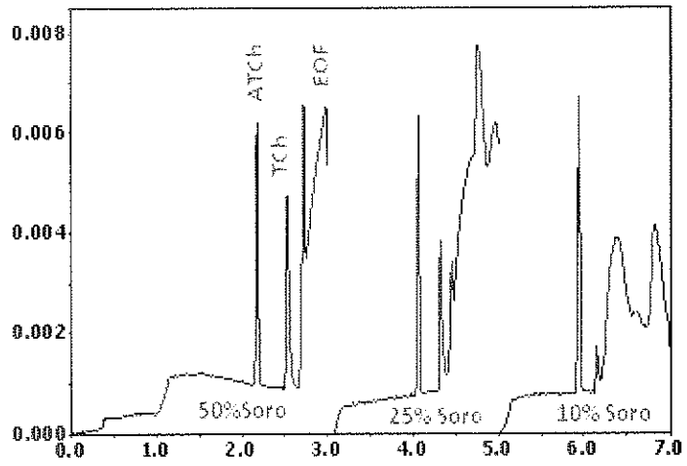
Electrophoretically mediated microanalysis (EMMA) is a branch of capillary electrophoresis that presents several advantages in the study of enzyme activity. The methodology conjugates the potential of a separation technique with the convenience of on-line reaction, thus allowing simultaneous, unattended reaction and detection. EMMA methods also have the advantages of the reduced amount of sample needed and avoiding interference by sample components that might absorb at the same wavelength as the substrate or product.
The method has been frequently used for inhibition and activity studies of pure enzymes but not for the study of the activity of biological samples and extracts. This work demonstrates that EMMA can be used and is discriminative for the evaluation of cholinesterase and carboxylesterase activity in mammal blood and for the evaluation of expression of carboxylesterase by recombinant technology.
Dog, cat, cow, horse, sheep, rat and human serum were used after dilution in the appropriate buffer and compared in terms of the ability to hydrolyse common acetylcholinesterase, butirylcholinesterase and carboxylesterase substrates. The relative metabolic responses for the different esterases and species were generally consistent with the observations presented in the literature and obtained with traditional methods.
The method was dose dependent as illustrated in the Figure 1 for conversion of acetylthiocholine to thiocholine by acetylcholinestrase in horse serum.



F1 Hydrolysis of acetylcholine by horse serum at different concentrations.

This approach will allow the establishment on *in vivo - in vitro* correlations, for interspecies metabolic evaluation, that can subsequently be applied pre-clinically to the study of metabolism and bioavailability of ester containing drugs and pollutants.

An EMMA method was also developed and applied for the determination of carboxylesterase activity in human cell extracts resulting from recombinant production of human carboxylesterase-2 and can be used for in-process control.

P.39 DETERMINATION OF VOLATILE COMPOUNDS IN PORTUGUESE *FICUS CARICA* L. VARIETIES BY HS-SPME AND GC/MS

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Fig (*Ficus carica* L.) is a tree belonging to the *Moraceae* family, one of the first plants cultivated by humans as a seasonal food. It is an important constituent of the Mediterranean diet.[1] Volatile compounds present in fresh and processed fruits affect significantly their flavour and aroma quality, which is formed by a complex group of chemical substances.[2] Volatiles can have multiple functions in the plant, being involved in species-specific ecological interactions, and often exert defensive and attractive roles. Additionally, the interest in volatiles from plants is focused on their biological potential, which includes antioxidant, antibacterial and antifungal activities.[3]

In this work, the volatile compounds profile of two characteristic Portuguese white varieties ("Pingo de Mel" and "Branca Tradicional") and three dark ones ("Preta Tradicional", "Borrasota Tradicional" and "Vebera Preta") was determined by headspace solid phase microextraction (HS-SPME), combined with gas chromatography/mass spectrometry (GC/MS).

This work permitted the identification of several types of compounds, namely aldehydes, alcohols, ketones, fatty acids and their esters and terpenoids, among others. All varieties exhibited a similar volatile profile. Volatile terpenes exist in higher amounts in leaves when compared with pulps and peels.

Many of these compounds have an important biological role in human organism and could be useful in health promotion.

Acknowledgments
A. P. Oliveira (SFRH/BD/47620/2008), D. M. Pereira (BI) and M. Taveira (BIC) are grateful to FCT for the grants.

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P.40 FATTY ACID PROFILE OF 18 POTATO CRISPS COMMERCIALIZED IN PORTUGAL AND IDENTIFICATION OF THE FAT/OIL USED IN THE FRYING PROCESS

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Gas chromatography (GC) has been a reference technique to analyse fatty acid (FA) profile in foods. Nowadays, GC methods with high-quality capillary columns allow sensitive and reproducible results in FA analyses, as well as the characterization of complex mixtures of geometric isomers when combined with other chromatographic separations[1]. The current concern for fat intake has raised the question of the individual fatty acids impact on health. This important issue has strengthened the awareness of nutritionists and food manufacturers for the control of the FA profile of food products[2].

Snack food such as potato crisps are an important example of remarkable industry and consumer demand for more stable food products with increased shelf life[3]. The variety of this product in the market has greatly increased in the last few years. Nowadays, it is possible to find in the market, potato crisps in different types of fat, with or without added salt or new flavours. Potato crisps present a high fat content and most of the fat comes from the fat used in the frying process. The aim of this study was to determine the FA profile in potato crisps commercialized in Portugal, and to identify the type of fat/oil used in the frying process and to compare it with those of different fats and oils.

Methods:

Eighteen samples of potato crisps, acquired in local supermarkets at two different seasons (December 2008 and March 2009), were analysed in order to study their profile regarding thirty six fatty acids. Preparation of fatty acid methyl esters (FAMEs) was achieved by transesterification using a methanolic solution of potassium hydroxide (KOH). Analyses were performed in a gas chromatograph (HP 6890 series) equipped with a flame ionisation detector. A Supelco™ 2380 capillary column (60m x 0.25mm, 0.2µm film thickness) was used with the following oven ramp: begin at 60ºC, hold for 1min, increase to 168ºC at 17ºC/min, hold for 28min, increase to 235ºC at 4ºC/min and hold for 15min. The injector and detector temperatures were 260ºC and 290ºC, respectively. Identification of chromatographic peaks was carried out by comparison of their retention times with appropriate standards of FAMEs (Supelco® 37 Component FAME Mix C4:0 – C24:0).

Results:

Our results show that the brands with more saturated FA were: 7, 12 and 14 (>15 g saturated FA /100g of sample); with more monounsaturated FA were 10,15 and 18 (>20g de monounsaturated FA / 100g of sample); with more polyunsaturated FA were 4-6 and 13 (>15 g polyunsaturated FA /100g of sample); and with more TFA were 5-8, 11,12, 14 and 16 (≥0.1g of TFA/ 100g of sample).

In order to identify the fat/oil used in the frying process, the FA profile in potato crisps was analyzed, and compared with the legislation for these fats/oils[4,5]. The eighteen samples were divided into three groups according to the major FA. Group 1 (brands 2, 3, 10, 15, 17 and 18) has C18:1 as the major FA. Brands 2 and 15 have C16:1 lower than 0.1% and in the other brands, C16:1 content is higher than 0.5%. This is in line with the FA profile of olive oil and high oleic acid sunflower oil. In Group 2 (brands 1, 4, 5, 6 and 13), the major FA is C18:2. Therefore,

the fat/oil used for frying was either sunflower or soybean oil. Comparison between the FA profile in potato crisps and in the oils lead to the conclusion that brands 1, 4 and 5 used soybean oil and brands 6 and 13 used sunflower oil to fry the potato crisps. The main FA used to distinguish them was C16:0. In the case of sunflower oil, C16:0 values are in the range of 5.0-7.6% of total FA, whereas in the case of soybean oil, they are in a higher range (8.0 - 13.5%). Group 3 has similar profiles of C16:0 and C18:1 (brands 7-9, 11, 12, 14 and 16). The FA profile is very similar among all these brands and it corresponds to the profile of palm fat.

Conclusion:

Our results show that in some brands of potato crisps commercialized in Portugal, there is still a high amount of saturated FA. The level of TFA found in the selected potato crisps was always lower than 1%, which is the TFA intake goal of World Health Organization.

Food and Drug Administration (FDA) requires that the amount of TFA should be listed on a separate line under saturated fat in the nutrition label, when the total fat is higher than 0.5g per serving. In Portugal, there is still no legislation that regulates TFA content in the label. Because the worldwide use of hydrogenated oils is considerable, consumers should be able to find the levels of TFA on labels, in order to better select the products containing partially hydrogenated oils and avoid their possible adverse effects to health.

From the results, it was possible to identify two brands of potato crisps that were fried in sunflower oil, two in high oleic acid sunflower oil, three in soybean oil, four in olive oil and seven in palm fat. Most of the potato crisps labels do not indicate the type of fat/oil, with the exception of those fried in high oleic acid sunflower oil and olive oil, which also corresponds to the most expensive brands.

Acknowledgements

This work was financially funded by Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P. Lisbon, Portugal. The authors are grateful for the Postdoctoral contract awarded to Ana Sanches Silva financed by the "Foundation for Science and Technology" under the frame of the Program "Science 2007".

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Available:http://www.fda.gov/Food/LabelingNutrition/ConsumerInformation/ucm109832.htm

P.41 EVALUATION OF α-TOCOPHEROL STABILITY DURING THE PROCESSING OF ACTIVE PACKAGING FILMS

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Low density polyethylene (LDPE) is the most widely used food packaging material. In order to minimize the polymer degradation during processing and storage, antioxidants (AO) are frequently included into packaging at levels of several hundred parts per million (ppm).

The most common AO are synthetic such as Irganox 1010, Irganox 1076 and BHT. However, some studies have mentioned the possibility of toxicity of some synthetic antioxidants[1]. Therefore, nowadays there is an increased interest on replacing synthetic by natural antioxidants. α-tocopherol was chosen as AO because of its capability to protect polyolefins during processing oxidation, already reported in the literature and also because, being one of the most effective natural antioxidants toward e.g. polyunsaturated fatty acids, it can be used to protect packaged foods from oxidation during storage[2].

In the present work, active films with different α-tocopherol contents were prepared through a two-step process. 50g of polymer and α-tocopherol (0.1; 0.5 and 1% w/w) were fed into a mixer (HAAKE Rheocord 9000). Rotors' speed of rotation was set equal to 20rpm and mixing time was 6min, the melting temperature was 140°C. The mixed matter was afterwards collected, pressed and then, pellettized. A twin co-rotating screw extruder was used to transform pellets into a film. The temperature profile from the feeding zone to the die was 100 to 150°C. The extruded film was pressed with a calender into a thin film (150μm).

In order to measure the concentration of α-tocopherol in pellets and of active films, the following extraction procedure[3] was used: 1g of film was extracted with 40mL of tetrahydrofuran (THF) for 24h. Extracts were evaporated to dryness in a rotavapor at 30°C and finally dissolved in n-heptane to a volume of 20mL.

α-tocopherol was determined with a High Performance Liquid Chromatograph (HPLC) with fluorescence detection. A Lichrosorb® Si 60 (250x4mm, 5μm) column was used at 37°C. The mobile phase used was isocratic 98:2 n-heptane/isopropanol. The flow rate was 2mL/min and the injection volume was 10μl. The obtained chromatograms showed a well defined peak of α-tocopherol at 1.8min. Excellent linearity was obtained (r^2=0.99985), indicating suitability for quantification in the range (2.9-10.6μg/mL).

Results showed that about 50% of α-tocopherol was lost during the whole process (first + second step) in the case of 0.5 and 1% loaded film. The 0.1% loaded film lost 50% during the first step and about 30% during the extrusion. The obtained data are in accordance with the one found in the literature and they show, as expected, that a great amount of AO is lost during the processing due to the oxidative degradation of the material.

Acknowledgments

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P.42 COMPARISON OF DIFFERENT SPME FIBBER COATING FOR EXTRACTION OF PESTICIDES IN LETTUCE BY HPLC/DAD

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Pesticide is a generic term that identifies a wide spectrum of synthetic compounds with biocide activity, worldwide used to contrast weeds, fungi and insects with the scope of increasing productivity in agriculture.
Multiresidue method development for pesticides analyses is difficult, due to the fact that compounds of different polarities, solubility's, volatilities and pKa values have to be simultaneously extracted and analysed[1].
Solid-phase microextraction (SPME) is a very successful sample concentration technique that has been largely employed for the extraction of many types of volatile and semivolatile organic compounds mainly from environmental, biological and food sample matrices. The main SPME features include simplicity, high sensitivity, reliability, portability and easy of automation. It represents an environmentally friendly alternative to traditional sample preparation techniques since it eliminates the use of undesirable organic solvents. In SPME, a small piece of fused silica is coated with a thin film of polymeric phase (e.g. poly(dimethylsiloxane) (PDMS), polyacrylate) or dispersions of solid adsorbents in polymers (e.g. PDMS/divinylbenzene (PDMS/DVB), PDMS/Carboxen). This film has the ability to both sorb and concentrate the organic analyte[2]. Solid-phase microextraction coupled with HPLC techniques is gaining increased applicability[3].
In order to study the application of SPME/HPLC/DAD to the analysis of pesticides in lettuces, a group of 11 allowed pesticides (e.i. Acetamiprid, Azoxystrobin, Cyprodinil, Fenhexamid, Fludioxonil, Folpet, Iprodione, Metalaxyl, Pirimicarb, Phosmet and Tolyfluanid) has been selected. The aim of this work was to study four different fibber coating for extraction of selected pesticides.
The fibber coatings assayed in this work were: PDMS (100mm), PDMS/divinylbenzene (PDMS/DVB, 60mm), carbowax/templated resin (CW/TPR, 50mm), and polyacrylate (PA, 85mm).
CW/TPR fibber presented the best extraction performance for most of the pesticides. Acetamiprid, Metalaxyl and Pirimicarb showed the lowest peak areas and in some cases (at least under the selected preliminary conditions) some of them could not even be extracted.

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P.43 CHARACTERIZATION OF SUGARS IN ALMOND (*PRUNUS DULCIS*) BY HPLC-RI

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Almond tree (*Prunus dulcis*), is the most important nut tree produced in the world, especially in the Mediterranean region, from which about 28% of the world production is obtained. In Portugal, almond is a traditional crop, mainly spread through Algarve, Baixo Alentejo and "Terra Quente Transmontana", with 24522 crops spread trough 36530ha[1].

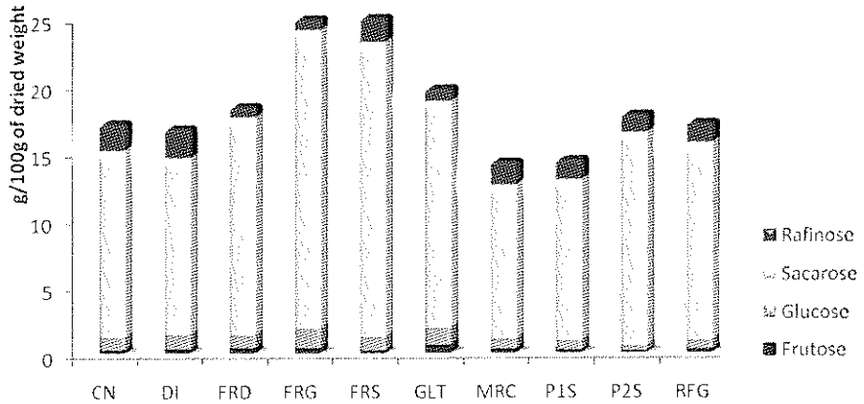
In this work, we selected commercial [*Ferraduel* (FRD), *Ferragnes* (FRG), *Ferrastar* (FRS), *Gloriette* (GLT) and *Marcona* (MRC)] and regional [*Casanova* (CN), *Duro Italiano* (DI), *Pegarinhos one seeded* (P1S), *Pegarinhos two seeded* (P2S) and *Refego* (RFG)] cultivars, produced in Trás-os-Montes region, to be screened in what concerns to their sugars composition.

Carbohydrates are relevant components in almond, especially starch, which is followed by sucrose. They are known as the most important parameter in the assessment of fruit quality, once sugar content and composition can be lowered or modified by several conditions, like storage temperature, relative humidity, harvest time, oxygen level or packaging[2]. Together with sucrose, glucose, fructose and raffinose are present in significant amounts and may contribute for cultivar characterization.

Free sugars profiles were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI). The HPLC equipment consisted of an integrated system with a Smartline pump 1000, a degasser system Smartline manager 5000, a Smartline 2300 RI detector (Knauer, Germany), and an AS-2057 auto-sampler (Jasco, Japan). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with an Eurospher 100-5 NH_2 column (4.6x250mm, 5mm, Knauer) operating at 35ºC (7971R Grace oven). The mobile phase used was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1mL/min, and the injection volume was 20µl. The compounds were identified by chromatographic comparisons with authentic standards. The results are expressed in g/100g of dried weight, calculated by internal normalization of the chromatographic peak area. Sugar identification was made by comparing the relative retention times of sample peaks with standards.

The linearity and sensitivity of the HPLC analysis was determined and the method was validated by the instrumental precision, repeatability and accuracy, using the fruits of *Ferraduel* cultivar.

Sucrose (Figure 1) was always the main sugar (11.46±0.14 in *Marcona* to 22.23±0.59 in *Ferragnes* g/100g of dried weight). Raffinose was the second major sugar (0.71±0.05 in *Ferraduel* to 2.11±0.29 in *Duro Italiano*), followed by glucose (0.42±0.12 in *Pegarinhos two seeded* to 1.47±0.19 in *Ferragnes*) and fructose (0.11±0.02 in *Pegarinhos two seeded* to 0.59±0.05 in *Gloriette*).



F1 Proportions of each individual sugar in assayed almond cultivars.

The obtained results highlight sugar composition as a useful discrimination factor among different cultivars, making them possible to be used in authenticity studies, which are an important feature in fruits with such elevated economical importance due to their numerous applications. Sugars profile, and especially sucrose, may also be used as an indicator of almond quality, due to the already brought up importance of this parameter in the assessment of fruit quality.

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P.44 COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY TIME-OF-FLIGHT MASS SPECTROMETRY ANALYSIS OF VOLATILE COMPOUNDS FROM MARINE SALT

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Marine salt is a natural product that is obtained in saltpans. Saltpans are man-made systems where the salt is produced by the evaporation of seawater due to the combined effect of wind and sunlight. Nowadays, there is a growing concern for protection and re-examining the value of saltpans that should be intrinsically associated to the quality of the marine salt. This can be evaluated by the physico-chemical properties of marine salt. In a previous study, a headspace solid-phase microextraction method combined with gas chromatography–quadrupole mass spectrometry (HS-SPME/GC–qMS) methodology was developed to study the volatile composition of marine salt[1]. In spite of the useful data extracted from the one-dimensional chromatographic separation processes, the complexity of the volatile composition of marine salt exceeds the capacity of one single separation system, and the observed chromatographic co-elution limited reliable MS identification. This suggests that if a more powerful separation technique is used, a better characterization of the marine salt volatile compounds can be obtained. In addition, the presence in marine salt of compounds in trace amounts also supports the use of a more sensitive technique. Thus, the aim of this work was to perform the analysis of volatile composition of marine salt by headspace solid-phase microextraction (HS-SPME) using comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC/TOFMS) methodology.

Samples from two saltpans of Aveiro, in Portugal, with distinct locations, produced in three different years (2004, 2005, and 2007) were analysed. A 50/30μm divinylbenzene/carboxen/polydimethylsiloxane SPME coating fibre was used. The volatiles present in the headspace of the solid salt samples (crystals) were extracted overnight at 60ºC prior to injection in the GC×GC/TOFMS. One hundred and fifty volatile and semi-volatile organic compounds were identified, distributed over the chemical groups of alcohols, aldehydes, aromatic compounds, esters, furans, haloalkanes, hydrocarbons, ketones, monoterpenoids, C_{13} norisoprenoids, and sesquiterpenoids. Furans and haloalkanes were identified for the first time in marine salt. The use of GC×GC/TOFMS enabled the separation and identification of a higher number of volatile compounds than when the volatile compounds were analysed by gas chromatography-quadrupole mass spectrometry (GC–qMS)[1]. This work also indicates that the aging of the salt accompanies the loss of volatile compounds and confirms the importance of the saltpan environment in the volatile composition of marine salt.

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P.45 APPLICATION OF AMINO ACIDS LIGANDS AS A NEW CHROMATOGRAPHIC STRATEGY FOR hSCOMT ISOLATION

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Catechol-*O*-methyltransferase (COMT; EC2.1.1.6) play an important role in the metabolism and inactivation of biologically active and toxic catechols, since catalyzes the transfer of a methyl group from the donor s-adenosyl-L-methionine to a catechol substrate in the presence of magnesium cation. Nowadays, this enzyme has been a relevant focus of research, due to the normal brain function, especially in the regulation of both the dopaminergic and noradrenergic neurotransmitter systems, which has a close relationship with a variety of mental disorders, such a Parkinson's diseases. As a result, while the development of pharmaceutical human Soluble COMT (hSCOMT) trials for a rational drug design depends on the availability of high purified samples, more suitable purification strategies[1] must be develop and emerge in order to satisfy the requirements of pharmaceutical industry.

The diversity of biomolecules presents in hSCOMT recombinant extracts from *Escherichia coli*, with structural and chemical similarities, are the foremost challenge in order to establish novel and suitable laboratory purification protocols[2]. So, the aim of this study was to develop a selective purification of hSCOMT, using distinct pseudoaffinity supports with amino acid as immobilized ligands. This strategy could be particularly promising while combines a natural biological interaction, improving the selectivity's onto the support and purity achieved in the target protein fractions maintaining the desirable kinetic properties.

In particular, using L-histidine, L-arginine, L-glutamine, L-aspartate and L-leucine as amino acids immobilized ligands, a variety of hSCOMT adsorption and elution methods has been tested and developed, incorporating more than one mode of adsorption, commonly based on hydrophobic and ionic interactions. Particularly, the majority of the group tested, excluding arginine, allowed a total retention of hSCOMT at higher salt concentrations above 1M of ammonium sulphate. Only the arginine ligand allows the binding of the target protein without salt in the mobile phase. Also, additional studies by non-specific desorption profiles based on pH manipulation will be presented since previous stability trials showed that hSCOMT maintains its activity over pH ranges from 3 to 8.

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P.46 AGEING OF MADDER DYE IN WOOL – AN LC-MS STUDY

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Natural dyes and dyeing are as old as textiles themselves. Man has always been fascinated by colour; the art of dyeing has a long past and many of the natural dyes still in use nowadays go back into pre-history[1,2]. Red plant dyes can be obtained from the roots of different species of the Rubiaceae family which include madder (*Rubia tinctoria L.*)[3]. Alizarin and purpurin are the main chromophores of madder and both present an anthraquinone ring system[3]. Different dye-mordant combinations can produce a wide array of colours. However, when exposed to light these colours easily fade or change. Understanding how these processes occur is of extreme importance in the field of textile conservation.

Madder was used in this work to dye wool mordanted with different amounts of copper, aluminium and iron salts. Two different dyeing methods were also used. Differences in colour hues were measured by colorimetry. To simulate natural ageing, fibres were subjected to artificial light ageing in a solar box. Samples were collected at different time intervals and colour parameters were measured by colorimetry. Mordant ion quantification was done after fibre acid digestion by ICP-AES and AEE. After dye extraction from the wool samples, LC-ESI-MS was used to evaluate the chromophore variation throughout the ageing process.

It was found that the chemical nature of the mordant ion, its concentration and the dyeing process has a strong impact on the wool final hue. Stronger and brighter colours were obtained using alum as mordant.

The amount of metal ions in the wool samples is very small when compared to the metal ion available in the dyeing bath, and increasing mordent concentration in the dyeing bath leads to a small increase in the fibre metal ions contents.

Chromophore degradation was found to be faster in the beginning of the light exposure. Degradation rates for the alizarin and purpurin are not the same and, in general, alizarin degrades faster than purpurin, which is probably the reason why the wool colour not only fade but change hue when exposed to light.

The chemical nature of the mordant also influences the photodegradation of the madder chromophores. Aluminium was found to promote degradation, a finding particularly important for textile conservation due the historical significance of alum as mordant.

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P.47 RADIATION EXPOSURE: DETERMINATION OF 8-HYDROXY-2'-DEOXYGUANOSINE (8OHDG) IN AIRLINE PILOTS BY GC/MS-SIM AFTER SOLID-PHASE EXTRACTION

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Ionizing radiation, is a type of radiation that contains enough energy to displace electrons and break chemical bonds, being able to remove, at least, one electron from an atom or molecule, creating radical species such as Reactive Oxygen Species (ROS). ROS can be divided in radical (e.g. superoxide, hydroxyl or peroxyl) and non-radical (e.g. hydrogen peroxide, ozone or singlet oxygen). At higher concentrations these species will promote damage to cellular structures by oxidizing lipids, reducing sugars and amino acids or causing a large number of purine and pyrimidine modifications[1-3]. In humans, the major concern focuses the occupational exposure to this radiation. This is particular relevant when one consider that airline pilots or radiologists are considerably exposed to this type of radiation. Extreme exposure conditions can lead to severe damage in cellular structures (DNA, proteins and others) and, therefore, to diseases such as cancer.

The urinary level of 8-hydroxy-2'-deoxyguanosine (8OHdG) is regarded as an important biomarker of endogenous oxidative damage to DNA and its precursors. In this preliminary study a simple method was applied[4] in order to measure the urinary level of 8OHdG in airline pilot's population. The results obtained for the volunteers were normalized considering the creatinine concentration. The pilot's population were compared with a control population living near the sea level. After SPE the samples were analysed as BSTFA derivatives by GC/MS-SIM. LOD and LOQ were 82 and 272ppb respectively. This preliminary results show that the pilot's population present slightly higher 8OHdG levels then the control population.

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P.48 THE IMPLEMENTATION OF A CLASSIFICATION METHODOLOGY FOR AGED MADEIRA WINE BASED ON VOLATILE PROFILE

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The present work aims to deepen our understanding of relevant phenomena occurring during "Malmsey" Madeira wine ageing in casks, in order to characterize how its aroma composition evolves during ageing, and to develop a classification framework for predicting its age.
Among all sets of variables that could be considered to address this subject, aroma composition was chosen for our study, as it provides a rich source of information which can be easily characterized and is widely recognized as a major contributor to the overall perception of wine features[1]. Consequently, the screening of volatile compounds was carried out using the solid phase extraction method proposed by López *et al* [2]. The great enrichment of the aromatic compounds in the extract, achieved while using a small quantity of organic solvent, the possibility of selecting the most adequate solid phase according to the analytes under study and the ability to extract several samples simultaneously[3,4], do consubstantiate our choice to evaluate the screening of wine aroma prior to chromatography analysis in this study.
A large amount of data was achieved through GC-MS analysis on wine samples covering an extended ageing period of twenty years. A total of 128 volatile compounds for the set of 26 samples analyzed were identified and quantified relative to an internal standard. Under this regard, it is a far from trivial to simultaneously address the following goals: to identify wine ageing trends, to find the natural 'tracers' of ageing and to establish classification methodologies for aged wine task. In this context, Chemometrics arises as a natural framework, an indispensable tool to process and to analyze the large volume of data. Therefore, statistical feature extraction methodologies and classification techniques were applied in order to taking advantage of the maximum amount of information available.
The major outcomes achieved with this study reveal interesting ageing trends and information concerning which chemical compounds are contributing to them. Furthermore, according to volatile information, a quite remarkable classification methodology can indeed be achieved for predicting wine age.

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P.49 SPME APPLIED TO THE ASSESSMENT OF PAH LEVELS IN PINE TREES

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Since its introduction by Dr. Janusz Pawliszyn and his co-workers in the early 1990s[1], SPME has been firmly established as a valid alternative to traditional extraction methods. Its application is reported in many studies of sampling and analysis of environmental samples, food and pharmaceuticals and a considerable number of review articles have also been published on the subject[2] and references therein.

It has been used to extract a wide range of compounds, with particular incidence given to priority pollutants. For instance, polycyclic aromatic hydrocarbons (PAHs) were extracted using this technique from waters, soils, sediments, air, vegetable oils or human samples like blood serum or urine[3]. Nevertheless, to our knowledge, SPME was never applied in the biomonitoring of PAHs by vegetation matrices.

Even so, plant species have been used as natural monitor of contaminants since the 1960s[4]. But the same matrix properties which allow the capture of such contaminants may also bring difficulties when it comes to the preparation of samples for multiresidue extraction and clean-up.

Our purpose is to study a new alternative, applied to two materials of different characteristics sampled from pine trees: needles and bark.

Microwave-assisted SPME followed by GC-MS quantification was tested and validated for the determination of the 16 EPA polycyclic aromatic hydrocarbons (PAHs) on samples from the two main pine species in Portugal: Pinus pinea L. and Pinus pinaster Ait. A subsequent sampling of naturally contaminated samples in locations of different environmental stresses in Portugal was performed to answer the following questions: Do bark and needles have the same entrapment ability towards PAHs? Is it possible to establish differences between both pine species? Are there important differences on the contamination levels according to the sampling location?

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P.50 CHARACTERIZATION OF VOLATILE COMPOSITION OF PORT WINE – DEVELOPING OF AN ANALYTICAL METHODOLOGY

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In recent years there is a growing trend in the study of the volatile fraction, because these compounds contribute to the consumer perception of quality, determining the acceptance or rejection of many products. Often called "off-flavours" imply microbial contamination which makes it the target of food security[1]. Analysis of volatile compounds typically includes two steps: i) extraction, where the compounds are removed from the sample matrix and isolation of non-volatile, and ii) separation and identification by chromatographic methods[2] (usually by gas chromatography coupled to mass spectrometry –GC/MS). The main objective of this work is to characterize the composition of the volatile profile of Port Wine.

The volatile compounds represent various chemical groups, some of which are reactive and present in low concentrations[2], and are present in very complex matrices, such as wine[1]. They will also have different characteristics: polarity, volatility, contributing to the challenges associated with analytical measurements of these compounds, with the limited specificity of detection systems[1,3,4]. The impact of each component in the aroma of the wine depends on the specific properties of the wine[5] because in another wine may be different, resulting in an "off-flavour". The difficulties encountered in qualitative and quantitative analysis are based on these characteristics. González[6] argues that the complexity of the aroma of a wine is in line with its chemical complexity being the result of interactions between many chemical components with odor.

The extraction of volatile compounds can be made using different methodologies: processes of distillation, liquid-liquid extraction, solid-phase extraction (SPE), solid phase microextraction (SPME), among others. The methodology of this study has as innovation the application in the matrix of Port Wine of the analytical technique of vacuum distillation in conjunction with others. Extracts enriched in the volatile fraction are analyzed by chromatography in order to obtain their structural characterization. This work was carried out as follows: after the vacuum distillation, a passage by a column of SPE and subsequent concentration in a Danish Kuderna. For analysis of the extract obtained and its volatile component GC/MS was applied. We compare the chromatographic profiles of samples as well as identifying some of the compounds of this type of wine (using the digital library – NIST98). After the vacuum distillation was applied SPME followed by GC/MS in order to perform the same type of analysis. Moreover, the sample was subjected to a steam distillation, passed through a SPE column and after elution was subjected to analysis by high performance liquid chromatography with diode-array detector (HPLC-DAD) in reverse phase.

The sample obtained during the vacuum distillation and the sample obtained after the concentration process in the Danish Kuderna were analyzed by HPLC-DAD either by normal phase or reverse phase and compared with a sample of wine after liquid-liquid extraction with dichloromethane. For reasons of polarity and hydrophobicity is in the reverse phase where we get results, but less prominent, but noted that this technique is not the most desirable itself for the determination of aromatic compounds (often used in the fractionation of samples). In an attempt to try to identify these compounds by liquid chromatography (LC) used electrospray ionization (ESI) with mass spectrometry (LC/ESI-MS).

Regarding the techniques used it can be said that applying SPME after a vacuum distillation is a more appropriate method (under these conditions although it is necessary to optimize certain parameters) than doing a vacuum distillation and then SPE and further concentration. The first

method raises a few questions: the study of the influence of ethanol during the vacuum distillation and the condition of SPE tube pack (with or without the use of water). The chemical compounds found belong to different chemical families: ranging from esters to alcohols, acids and terpenes (norisoprenoids). Few phenolic compounds are detected and there are no sulfur compounds to be found.

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P.51 DEVELOPMENT OF A COMPLETE FACTORIAL DESIGN TO ASSESS ALLERGIC RESPIRATORY DISEASES METABOLOMIC PATTERNS BY A SOLID-PHASE MICROEXTRACTION GAS CHROMATOGRAPHY-MASS SPECTROMETRY BASED METHODOLOGY

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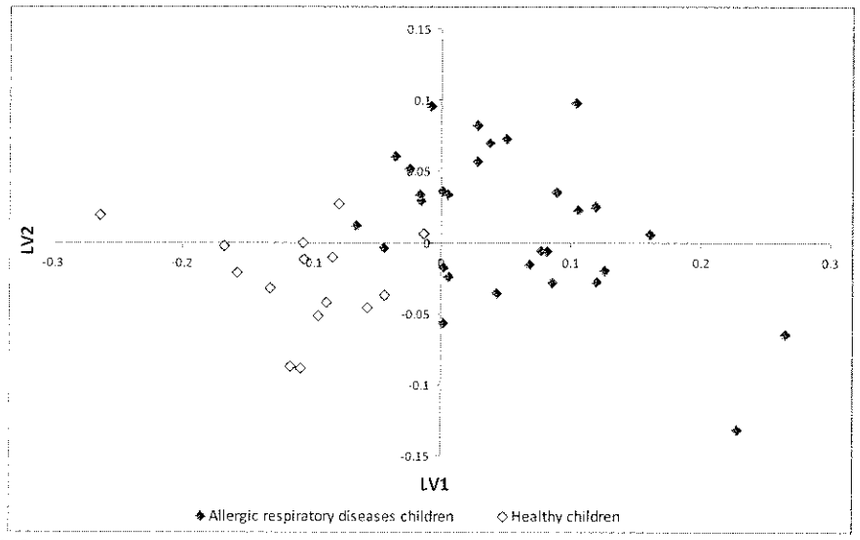
Solid-phase microextraction (SPME) has been introduced in the 1990s as a new sample preparation approach, which many offers advantages including simplicity, speed, and is a solvent free extraction, that presents good linearity and high sensitivity. Over the years, SPME has been widely applied in the characterization of biological matrices (blood, urine, plasma, saliva and hair) for the discovery of biomarkers or biomarker patterns of human diseases, which can allow a further understanding and characterization of such diseases. More recently, there has been an increased interest in the analysis of disease-related volatile organic compounds in exhaled breath with over 200 compounds being identified. The analysis and characterization of exhaled breath is a recent approach with potential to provide valuable information about respiratory and systemic diseases leading to a deeper knowledge of the human health status. Allergic respiratory diseases (ARDs) represent an important public health issue with a significant growth over the years. ARDs affect individuals of all ages from infancy to seniors. Particular attention was devoted to the paediatric population as respiratory diseases represent the major cause of illness in children of developed countries. ARDs are also associated with high direct and indirect health costs, especially related with diagnosis and treatment.

The lack of a prominent study by HS-SPME led us to develop an experimental design to assess ARDs metabolomic patterns by SPME combined with gas chromatography quadrupole mass spectrometry (SPME/GC-qMS) methodology. To fulfil these objectives three important SPME experimental parameters that influence the extraction efficiency, namely extraction temperature and time, and coating fibre were considered in this study. Different biologically relevant standards pertaining to different chemical families (hydrocarbons, aldehydes, ketones, aromatic and aliphatic alcohols) were selected to yield the experimental design. It was selected the conditions that promoted the higher extraction efficiency, corresponding to the higher GC peak areas and number of analytes: DVB/CAR/PDMS coating fibre, and 22ºC and 60 minutes as the temperature and time of extraction, respectively. The developed methodology was then applied to the analysis of children exhaled breath with ARDs (30) and healthy children (15), allowing to identified up to 44 compounds distributed over the chemical families of aliphatic alkanes, aromatic compounds, carbonyl compounds, and alcohols among others. PLS-DA was applied to the GC chromatographic raw areas in order to establish a preliminary classification model and assess the relationships between the compounds and the children understudy. Figure 1 shows the PLS-DA scores scatter plot (LV1xLV2) of the volatiles chromatographic areas identified in exhaled breath of children with ARDs and healthy children. The classification model was assessed by interval cross-validation (7 blocks) given a model with 4 LVs with a classification rate of 91%. This work will show the compounds that promote the observed discrimination by the analysis of the corresponding loadings weights. One will show that a small number of compounds are responsible for the difference between ARDs and healthy subjects, which may facilitate the analysis of such an important subject.



F1. Scores scatter plot of the chromatographic areas of the volatiles identified in exhaled breath of children with allergic respiratory diseases and healthy children.

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P.52 ISOFLAVONAS EM CAFÉ: VALIDAÇÃO E APLICAÇÃO DE UMA METODOLOGIA ANALÍTICA

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Diversos estudos têm associado a ingestão de isoflavonas a alguns efeitos benéficos na saúde, nomeadamente, acção hipocolesterolémica, anticarcinogénica, antiosteoporótica e alívio da sintomatologia menopáusica. Estes compostos possuem uma estrutura química muito semelhante à do 17β-estradiol e actividade estrogénica fraca[1]. A soja e os seus derivados são conhecidos como as principais fontes de ingestão de isoflavonas[2]. No entanto, é importante avaliar também os teores destes compostos em outros alimentos, essencialmente naqueles que são parte integrante de dietas Ocidentais, nas quais a soja é ainda pouco utilizada.
Este trabalho teve como objectivo desenvolver e validar uma metodologia analítica simples para a quantificação de daidzeína, genisteína e formononetina em café (em grão e em bebida), por HPLC/DAD. Para obtenção dos compostos na sua forma livre, foram testados vários processos de hidrólise (ácida e enzimática) e o método mais eficiente (em termos de recuperação, resolução cromatográfica e áreas de pico relativas) foi optimizado para as diferentes amostras. A metodologia final proposta baseia-se numa hidrólise ácida, na presença de metanol, BHT (antioxidante) e 2'-metoxiflavona como padrão interno. Obtiveram-se elevados coeficientes de correlação (r>0,999), boas precisões intra- e interday (<7%), recuperações na ordem dos 95±1% e limites de quantificação entre 14 e 25ng/mL.
O método foi aplicado a 2 amostras de café arábica, 2 de café robusta (ambas com torra média) e 2 misturas comerciais (arábica e robusta). Foi avaliada também a extractabilidade dos compostos para a bebida (cafés expresso - 6.5g/30mL), preparados a partir de cada amostra.
Os cafés robusta apresentaram teores de isoflavonas totais (89μg/g) significativamente superiores (p<0,05) aos de café arábica (23μg/g), diferença que se deve essencialmente à formononetina. As misturas comerciais (arábica + robusta) apresentaram teores intermédios (50μg/g). Nos cafés expresso (30mL) os teores de isoflavonas totais variaram entre 46μg (100% arábica) e 238μg (100% robusta). O grau de extracção dos compostos para a bebida "expresso" rondou os 30-50%.

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P.53 ANALYSIS OF SELECTED PESTICIDES IN BOVINE MILK BY DISPERSIVE SOLID-PHASE EXTRACTION FOLLOWED BY GAS CHROMATOGRAPHY-ELECTRON CAPTURE DETECTION

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The presence of contaminants in foodstuffs is a matter of growing scientific and public concern worldwide, due to the adverse effects of these toxicants in human health and environment[1].
Pesticides have been widely used in agriculture to control pests, weeds or plant diseases. Their use results in economic benefits related to production and postharvest storage. However many pesticides can make their way into food after application and subsequently be available for human consumption. Although most of the chlorinated pesticides (OCPs) have been banned decades ago, they can still be found in the environment due to their lipophilicity and their resistance to metabolism processes. Hence, environmental exposure of living organisms to these compounds results in their accumulation and persistence in fat tissues[2].To protect consumers and to promote trade, legal limits (Maximum Residue Levels or MRLs) have been established for residues of pesticides on products of plant and animal origin[3].
Milk-producing animals, such as cows, may accumulate residues of these pesticides through carry over processes from contaminated feed, grass/hay, water, top layer soil and inhaled air. As a result, several residues of OCPs as well as compounds from other pesticide classes can be detected in bovine milk samples[1, 2].
To achieve effective extractions of pesticides from milk samples, several sample preparation methods have been developed aiming at the quantification of residues in milk. One such method is dispersive solid-phase extraction (DSPE), which is a relatively new method belonging to the quick, easy, effective, rugged, and safe (QuEChERs) methods. QuEChERs are based on liquid-liquid partitioning with acetonitrile followed by a clean-up step with DSPE[1, 4, 5].
The main purpose of this study was to assess the use of DSPE-GC/ECD for the analysis of selected pesticides in commercial bovine milk samples. The pesticides investigated were: azinphos-methyl, chlorfenvinphos, chlorpyrifos, chlorpyrifos-methyl, deltamethrin, diazinon, dimethoate, endosulfan I, endosulfan II, endosulfan sulphate, ethion, folpet, malathion, methidathion and thiobencarb. These were selected due to their relevance in milk pesticide residue analysis.
Sample preparation and extraction of pesticide residues was based on the QuEChERs protocol applied by Lehotay[5] on a more diverse type of samples and for a different set of target pesticides. The temperature program for the GC/ECD was based on that by Cunha[4]. Pesticide standard working solutions of 20mg/L and 100mg/L were prepared in methanol and used to create composite mixture solutions of concentrations in the range of 0.1-6 mg/L from which calibration curves were obtained. Commercial milk samples were spiked at levels of approximately 50 and 500ng/g. Acceptable recoveries were obtained for most of the compounds.

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P.54 USING RADIELLO™ SAMPLERS TO MEASURE BTEX IN INDOOR AIR

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There is a growing public awareness regarding the health risks associated with poor indoor air quality in the home and in the workplace[1]. Personal inhalation of volatile organic compounds (VOCs) has been shown to be dominated by indoor source contributions since individuals spend most time indoor where concentrations are usually typically higher than outdoors[2]. Among VOCs, BTEX (benzene, toluene, ethylbenzene and xylenes) are particularly abundant in the indoor environments, and their sources include tobacco smoke, construction materials emissions and fuel burning. Benzene is one of the most dangerous pollutants and its concentration in indoor air is regulated by European legislation[4].

In this work, an analytical methodology involving the use of Radiello™ passive samplers[3] was established for quantifying BTEX in indoor air. Several locations were chosen for sampling and included three residential homes (one with an attached garage and another with a fireplace) and two commercial locations (a coffee house and a tire garage).

RAD130 samplers are made of activated charcoal and were left in contact with the indoor air for a period of seven days as suggested by the manufacturer. After the sampling period, the cartridges were extracted with carbon disulfide and the solution analyzed by GC/MS in the SIM mode, using two quantifying ions for each analyte. The limits of quantification (LOQs) of the analytical method ranged from 0.11mg.L^{-1} for the p-xylene to 4.88mg.L^{-1} for toluene.

Results obtained showed that benzene was measured in most of the locations at concentrations higher than 5μg.m^{-3}, the maximum value recommend by the European legislation[4]. Fireplaces and garages were found to be important sources of BTEX pollutants inside the residential areas. Pollution was higher in the immediate vicinity of the pollutants sources and tend to diminish as the air sampling place was moved away from them. The highest amount of pollutants was measured inside the garage of one of the residential homes: 133.33 μgm^{-3} of benzene; 536.79μg.m^{-3} of toluene; 134.19μg.m^{-3} of ethylbenzene; 146.63μg.m^{-3} of p-xylene; 271.23μg.m^{-3} of m-xylene and 152.78μg.m^{-3} of o-xylene.

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P.55 VOLATILE PHENOLS IN RED WINES: A METHODOLOGY

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The accumulation of volatile phenols in wine has been a cause of great concern in modern enology, being considered, nowadays, a key point in the control of wine quality. Vinylphenols (4-vinylphenol (4VP) and 4 vinylguaiacol (4VG)) and ethylphenols (4-ethylphenol (4EP), 4-ehtylguaiacol (4EG) and 4-ethylcathecol (4EC)) may be produced in wine, in a sequence pathway, due to microbial activity, imparting undesirable odours and flavours

A number of microbial species have been reported as being able to decarboxylate hydroxycinnamic acids, such as ferulic, *p*-coumaric and caffeic acids. The products of these decarboxylations are ethylphenols that give rise to strong smoky and aromatic odours and flavours and are regarded as the source of phenolic "off-flavours" in several wines.

This work describes a sample preparation method based on liquid-liquid extraction (LLE) with diethyl ether:pentane (1:1 v/v) and a GC chromatographic method to measure these compounds in red wine samples. The chromatographic conditions were: a RTX-Wax column from Resteck (60m × 0,25mm × 0,25µm), injection 1µL in splitless mode at 230°C, split vent of 2min, helium at 1,4mL/min and a flame ionization detector at 250°C. Oven was at 100°C, raised to 220°C at 10°C/min, to 240°C at 3°C/min (7min).

The recovery of the extraction procedure was assessed: 84% for 4-vinylguaiacol, 106% for 4-ethylphenol, 102% for 4-ethylguaiacol and 111% for 4-ethylcathecol.

Calibration parameters and LOD and LOQ of each compound were also determined:

Compound	Equation	R^2	LOD (mg/L)	LOQ (mg/L)
4-VG	y = 0,489x + 0,0503	0,9998	0,29	0,83
4-EP	y = 1,1036x + 0,0959	1	0,27	0,66
4-EG	y = 0,7163x + 0,0937	0,9998	0,40	1,13
4-EC	y = 0,2536x - 0,03	0,9997	0,09	0,36

We tested this methodology in synthetic wine samples and in red wine samples.

Several red wines were analized and we found mainly 4-ethylphenolin them. In one red wine it was possible to quantified 4-ethylphenol, 4-ethylguaiacol and 4-ethylcatechol. We found no vinylphenols in the wines analyzed.

P.56 FAST, SIMPLE AND RELIABLE HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF ASCORBIC AND ISOASCORBIC ACIDS

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A reliable method for the determination of total vitamin C must be able to resolve ascorbic acid (AA) and the epimeric isoascorbic acid (IAA), and determine simultaneously AA and dehydroascorbic acid (DHA). AA and IAA are polar molecules with a low retention time in conventional reversed phase systems, and hence of difficult resolution. Hydrophilic interaction chromatography using a TSK gel Amide 80 with isocratic elution with a 0.1% TFA aqueous mobile phase was successful in resolving the two epimers. The column was compatible with injections of high concentrations of metaphosphoric acid, Tris(2-carboxyethyl)-phosphine and EDTA without drift of baseline and retention time. Total AA and IAA were extracted, stabilized and reduced in one step at 40ºC, using 5% *m*-phosphoric acid, 2mM of EDTA and 2mM of Tris(2-carboxyethyl)-phosphine as reducing agent. This simple, fast and robust HILIC-DAD method was applied for the analysis of food products namely fruit juices, chestnut and ham and pharmaceutical and multivitamin tablets. Method validation was performed on the food products including parameters of precision, accuracy, linearity, limit of detection and limit of quantification. The absence of matrix interferences was assessed by the standard addition method and Youden calibration. The method was fast, accurate and precise with a LOQ_{AA} of 1.5mg/L and LOQ_{IAA} of 3.7mg/L. The simple experimental procedure, completed in 1h, the possibility of using IAA as an internal standard, and low probability of artifacts are the major advantages of the proposed method for the routine determination of these compounds in a large number of samples.

P.57 DETERMINATION OF ALIPHATIC AMINES AND AMMONIA IN WINES BY HPLC-UV USING A GAS-DIFFUSION EXTRACTION MODULE FOR SAMPLE PREPARATION

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In this work, an innovative gas-diffusion extraction module was applied for the HPLC-UV analysis of aliphatic amines and ammonia in wines.

The extraction module consists of a Teflon module of our own design with a microporous hydrophobic membrane (PTFE) at its bottom that avoids the diffusion of the aqueous solvent, but allows the mass transfer of volatile compounds.

The determination of ammonia and aliphatic amines by HPLC-UV is achieved through a reaction of derivatization with phenyl isothiocyanate (PITC), producing phenylthioureia derivatives[1]. Typically, the module was immersed in 25mL of the sample at a selected temperature; a solution of PITC (1%) in acetonitrile with 0.1M carbonate buffer (1:5) was placed inside the module. At the end of the extraction time, the acceptor solution, containing the derivatized amines, is collected and injected in the HPLC system.

Several parameters with influence in the extraction process were studied, such as: temperature, time of extraction, type of acceptor solution, type of membrane, and others.

The results obtained demonstrated that the proposed method has a good precision (RSD<4%) and a broad linear range for the concentrations tested of the three aliphatic amines (methylamine, dimethylamine and ethylamine) and ammonia.

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P.58 COMPARAÇÃO DAS TÉCNICAS DE TOFMS E DE SIMPLES QUADRUPOLO ACOPLADAS À CROMATOGRAFIA GASOSA NA IDENTIFICAÇÃO DE COMPOSTOS ORGÂNICOS EM ÁGUAS

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As técnicas de espectrometria de massa (MS) têm sofrido uma evolução assinalável nos últimos anos como ferramenta de detecção de compostos orgânicos em diversas matrizes ambientais e alimentares. O seu acoplamento à cromatografia gasosa (GC-MS) e à cromatografia líquida (LC-MS) permitem a análise de diversos tipos de compostos orgânicos em águas de um modo rápido e fiável. A análise de compostos alvo tem sido efectuada com recurso a espectrómetros de massa de simples quadrupolo (modo SIM), de triplo quadrupolo (modo MRM) ou de ion-trap, que garantem uma selectividade elevada, ao mesmo tempo que permitem atingir limiares analíticos extremamente baixos, muitas vezes na ordem dos ng/L.

Os laboratórios de águas deparam muitas vezes com a necessidade de analisar compostos poluentes desconhecidos em amostras de água superficial ou subterrânea, ou de compostos lixiviados a partir de materiais em contacto com a água destinada ao consumo humano. A identificação destes compostos é normalmente efectuada por GC-MS, com recurso ao espectrómetro de massa de simples quadrupolo (modo full scan) ou ao TOFMS. A técnica de TOFMS de elevada resolução permite não apenas a identificação desses compostos por comparação com bibliotecas de espectros de massa, obtidas em modo full scan, mas ainda a medição da massa exacta de modo a calcular a composição elementar desses compostos, sendo uma ferramenta adicional para a identificação de compostos desconhecidos.

Neste estudo foi efectuada a comparação do desempenho do TOFMS com o espectrómetro de massa de simples quadrupolo, em termos de exactidão, na identificação de compostos desconhecidos através de diversas distribuições de ensaios interlaboratoriais Aquacheck organizados pela entidade LGC standards, de modo a demonstrar a fiabilidade de ambas as técnicas e a avaliar o desempenho do Laboratório Central da EPAL, com vista à sua validação e posterior acreditação pelo IPAC.

Paralelamente foi efectuada a comparação de resultados obtidos na identificação de compostos orgânicos existentes em lixiviados obtidos após ensaios de migração de materiais (tubagens de PE e PVC, provetes revestidos com tintas epoxídicas) usados em contacto com a água destinada ao consumo humano.

Os resultados obtidos foram equivalentes no que respeita à identificação por comparação com as bibliotecas de espectros de massa existentes. No entanto, o uso do TOFMS com a ferramenta de análise da composição elementar recorrendo à medição da massa exacta permite ter uma confirmação inequívoca dos compostos identificados, eliminando prováveis candidatos obtidos no processo de identificação. Por outro lado, a rapidez de processamento de dados é substancialmente superior no caso do TOFMS, permitindo obter uma resposta de análise mais eficiente, em caso de ocorrência de um problema na qualidade da água ou na resposta a um ensaio analítico a materiais.

P.59 ANÁLISE DE MISTURAS FENOL/NONILFENOLETOXILADOS/ÁGUA POR HPLC

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A presença de poluentes em efluentes líquidos industriais tem causado grande problemas ambientais, principalmente no que diz respeito aos cursos de água. Dentre esses poluentes se destacam os compostos fenólicos que podem ser formados apenas pela molécula simples do fenol ou de compostos que apresentem o fenol em sua cadeia. O método mais utilizado para determinação da concentração de derivados de fenóis na água é a análise por espectrofotometria de absorção molecular, no comprimento de onda 260nm. O problema deste tipo de análise engloba a contribuição da absorbância de todas as moléculas contendo grupamentos fenólicos, impossibilitando assim a quantificação de cada uma das moléculas. Com o objetivo de solucionar este problema foi utilizada a cromotografia líquida de alta performace, HPLC, (High Performance Liquid Cromatography), onde as amostras são introduzidas no sistema com o auxílio de uma micro-seringa, em uma coluna cromatográfica (C8), utilizando como eluente a mistura (fase móvel) contendo água (42,5% vol), acetonitrila (50% vol) e metanol (7,5%), com uma vazão de 1,2mL/min, previamente filtrado e desgaseificado[1]. O sensor é o de absorção molecular e é regulado no comprimento de onda 260nm. Foram utilizados os reagentes fenol, e os tensoativos nonilfenolpolietoxilados com etoxilação variando de 8 a 15, e as concentrações variaram de 0,5mg/L a 1000mg/L. A concentração permitida pela Legislação Brasileira (Resolução Conama Nº 35, 17/03/2005) limita em 0,5mg/L.[2] A escolha dos tensoativos nonilfenolpolietoxilados para o estudo neste trabalho baseou-se na necessidade de determinar a concentração residual, tanto do fenol quando do tensoativo na fase aquosa após a extração do fenol pelo processo de separação por ponto de turbidez de tensoativos não iônicos. Os resultados mostraram que o tempo de retenção do fenol foi de 2,5min e o dos tensoativos variaram de 15 a 33min, indicado que o aumento da etoxilação do tensoativo, promove um aumento do tempo de retenção da molécula na coluna, pois uma molécula com maior etoxilação interage mais fortemente com a coluna dificultando seu escoamento através da coluna.

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P.60 LIQUID CHROMATOGRAPHY ASSAY FOR GLIADINS QUANTIFICATION: APPLICATION TO GLUTEN-CONTAINING AND "GLUTEN-FREE" FOOD PRODUCTS

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In the present work a high performance liquid-chromatography UV/Vis method was developed to quantify gliadins in foods. Gliadins are prolamins and are one of the constituents of wheat gluten (approximately 50%). These compounds have particular importance in food analysis since they are responsible for the celiac disease, which is an intolerance or hypersensitivity to ingested prolamins. The only known treatment for celiac disease is a gluten free diet. Therefore, the development of analytical methods to assess the gliadins contents in food products is crucial, namely in those labeled as "gluten-free". A "gluten-free" alimentary product cannot contain more than 100ppm of gluten, which corresponds to 50ppm of gliadins.

Fifteen wheat based foods (7 "gluten-free" and 8 gluten-containing food samples, according to the label) were purchased in commercial supermarkets and analyzed using the HPLC method developed. The samples studied included flours, biscuits, breakfast cereals and bread.

The HPLC equipment consisted of a Varian chromatographic system, equipped with a Varian Prostar 220 pump and a 7725i Rheodyne manual injector with a 10μL loop. A Varian ProStar 330 Photodiode Array detector was used. The equipment was controlled using the Star Chromatography Workstation software (version 4.5), which also controlled the solvent gradient, the data acquisition and data processing. The chromatographic separation was performed with a chromatographic column PLRP-S (polystyrene divinylbenzene stationary phase, particle size 8 microns, pore 300Å and 150×4.6mm id). The column was placed inside an oven (Jones, Model 7981) and kept at 40±0.1°C. The best chromatographic resolution for the gliadins was achieved using a gradient elution with acetonitrile/TFA/water. The elution was performed at a constant flow of 0.6mL/min and at a temperature of 40±0.1°C. The detection was made at a wave-lenght of 210nm. Each sample analysis took 30min.

The gliadins concentrations of the calibration standard solutions (different standard gliadin masses dissolved in ethanol/water mixed in the proportion of volume 7/3) were confirmed using a Pierce Coomassie Plus kit test.

The gliadins were extracted from the food samples using an ethanol/water solution (70:30, v/v) solution and, after vortex homogenization and centrifugation, the solutions were filtered through a Whatman 0.2μm nylon filter, before HPLC analysis.

Calibration results showed a linear relationship between peak areas related to gliadins and concentrations with a correlation coefficient of 0.9993. The calibration curve was established by the external standard calibration method. The dynamic range of gliadins concentrations studied was between 50 and 1309ppm. The detection and quantification limits obtained were 46.5 and 140.91ppm of gliadins, respectively. Data refer to the overall area of all peaks. The values obtained for repeatability and intermediate precision were acceptable since the relative standard deviation percentage values were lower than 4%.

The gliadins peaks in the food samples were identified based on the retention times obtained for the standard solutions of gliadins and by analysis of the UV spectrum associated to each peak. Results confirmed the presence of gliadins in all food samples indicating gluten-containing label. Moreover, gliadins were also detected in two "gluten-free" food samples. Furthermore, in one of

them it was also possible to quantify the gliadin level, which was greater than 50ppm, indicating that the "gluten-free" label was incorrect.
The results obtained showed that the HPLC method developed in this work can be a useful tool for assessing gliadin contents in "gluten-free" products, although some additional effort must be made to decrease the detection and quantification limits of the proposed method.

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P.61 MEMBRANE EXTRACTION MODULE BASED IN GAS-DIFFUSION FOR THE ANALYSIS OF VOLATILE AND SEMI-VOLATILES COMPOUNDS

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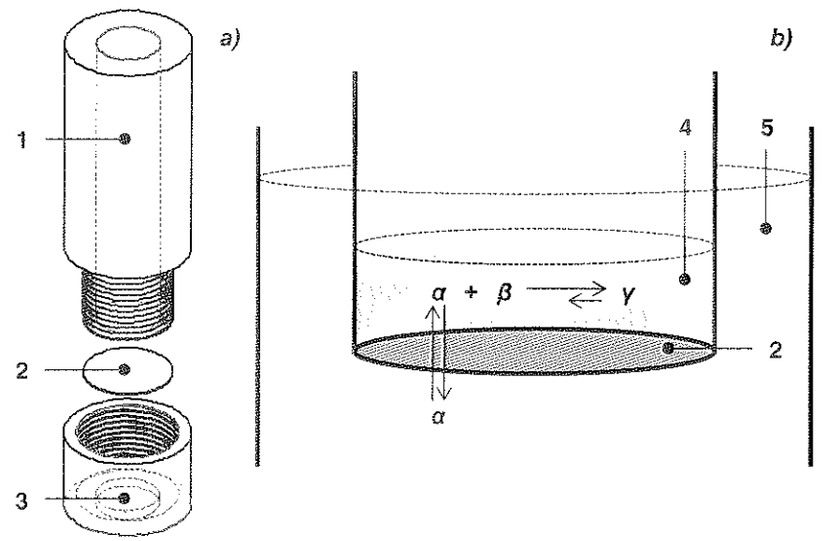
In this work a novel extraction approach[1] for volatile and semi-volatile compounds is presented. The extraction module consists in a Teflon module of our own design with a microporous hydrophobic membrane (PTFE) at its bottom that, due to its hydrophibicity avoids the diffusion of the aqueous solvent but allows the passage of volatile compounds. Inside the module d a small volume of an acceptor solution, which is collected and analysed at the end of the extraction. It is a very precise system as well as robust and reproducible; furthermore it has very low reagent consumption. By means of a derivatizing reaction, simultaneous extraction and concentration of the analyte is achieved allowing a simpler instrumental detection. Sampling times are in the minute time span and little operator intervention is required during the process. Moreover, since it is very small and easy to operate it is perfect for in-situ sampling. It is also very versatile: it can be used with many analytes, matrices and many kinds of analytical instruments like HPLC, GC or voltammetry. Several parameters of the extraction process, such as temperature and extraction time, were studied and optimized.
This extraction module has been tested, so far, in the chromatographic analysis of vicinal diketones in beer, aliphatic amines in water and wines, sulphur dioxide and acetaldehyde in wine and beer.



F1 a) Membrane gas-diffusion extractor and b) detail of the extraction with a derivatizing reaction.
1 – extractor's superior piece, 2 – hydrophobic membrane, 3 – extractor's lower piece, 4 – acceptor solution, 5 – donor solution, α – analyte, β – derivatizing agent, γ – derivatized species

[1] Pedido de Patente PT104789 UPIN Pat.56 Módulo extractor e processo de extracção de espécies voláteis e semi-voláteis baseado em difusão gasosa, 16/10/2009

P.62 DEVELOPMENT OF A NEW METHOD FOR EXTRACTION AND ISOLATION OF ANTIOXIDANTS FROM HOPS (*HUMULUS LUPULUS L.*): CHARACTERIZATION BY HPLC-ESI-MS/MS

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An important problem that researchers are often faced with, when investigating complex mixtures, is the need to isolate the analytes from natural sources and thoroughly purify the extracts prior to the analysis. Until nowadays, several techniques for the extraction, isolation and purification of phenolic compounds from complex mixtures are often used such as supercritical fluid extraction (SFE), semi-preparative high performance liquid chromatography (SP-HPLC), high-speed counter-current liquid chromatography, precipitation-adsorption and solid-phase extraction[1-3]. These methods, however, have the disadvantages that they are time-consuming and only allow small volumes of sample. In addition, SFE methods require a high investment cost for the equipment acquisition and, on the other hand, the yield of polyphenols is very low.

Since adsorption is a low cost separation technique, it is preferred for the selective recovery of phenolic compounds from complex matrices. The use of adsorbents might also be a useful tool not only to concentrate plant phenolics but also to fractionate the crude extract or at least to enrich certain compounds. Numerous of sorbent materials such as cyclodextrin, Sephadex, cellulose triacetate, Amberlite XAD and other kind of resins have been applied for adsorbing valuable polyphenols from plant extracts[1-3]. These adsorbents, however, have the disadvantage that either have a high adsorption performance or a high elution performance, but that do not simultaneously have both a high adsorption performance and a high elution performance. The main objective of the present work is to provide a method for the extraction and isolation (by adsorption to PVPP) of phenolic compounds from hop extracts, in order to facilitate their isolation for subsequent characterization and quantification, removing the prior art drawbacks. The characterization and quantification of these compounds were reached with high-performance liquid chromatography with diode array detection (HPLC-DAD) and tandem mass spectrometry using electrospray ionization (HPLC-ESI-MS/MS).

It was possible to obtain high adsorption (≥80%) and recovery yield (≥70%) values for several polyphenols from hops such as xanthohumol, catechin, epicatechin, quercetin and kaempferol glycosides. Moreover, it allows the identification of about 30 polyphenols by HPLC-DAD-ESI-MS/MS. It has been also observed that the adsorption of the studied phenolic compounds by PVPP displayed classical Langmuir characteristics over a wide range of PVPP concentrations (0-5.0mg/mL), and the isotherms obtained were compared to determine the extent of selectivity. PVPP preferentially adsorbed the more highly hydroxylated phenolic compounds in model solutions. The hydroxyl derivatization (methylation and glycosylation) of the phenolic molecule resulted in greatly diminished binding to PVPP. We suggest that the methodology could be used in the future to other complex matrices in order to obtain polyphenolic-enriched extracts for application in food, cosmetic and pharmaceutical industries.

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P.63 HPLC ANALYTICAL METHOD IN THE EVALUATION OF FRAGMENTATION, PURIFICATION AND REDUCTION OPERATIONS APPLIED IN ANTIBODY FRAGMENTS ISOLATION

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The protection conferred by antibodies is due to its ability of antigen linkage and to several functional properties, such as the propensity to activate phagocytic cells. Also, monoclonal antibodies have interesting properties for several biological domains, including the interesting specificity for in vivo diseases. Indeed, suitable development of new diagnostic tests has been recently enhanced, specifically related with radio-immunoassay detection and immunotherapy. These methods can allow an earlier detection and a more real prognostic, for instance, in a variety of neoplasic diseases. Despite these advances, the success of this methodology obliges the discovery of new chromatographic strategies that lead to highly purified antibody fragments, with satisfactory recoveries and lower process costs. Also, the downstream design should attend on the target antibody, source (recombinant or natural) and final application.

This work is aimed to employ an analytical method as an assessment and quality control tool during the fragmentation, purification and reduction process of the targeted antibody fragment. Specifically, the qualitative and quantitative analysis by HPLC with UV detection system of the antibody fragment allowed the: (1) adjustment of the fragmentation conditions of the whole antibody, (2) control of the purity levels achieved with the chromatographic operation (3) establishment of the optimal reducing agent concentration and dialysis conditions to guarantee the antibody stability during the reduction step and (4) investigation of appropriate storage conditions of antibody fragment samples in order to improve the stability of the antibody fragment for further biochemistry applications.

P.64 CHARACTERIZATION OF *PINUS SPP* NEEDLES EXTRACTS BY MULTIDIMENSIONAL ANALYTICAL TECHNIQUES

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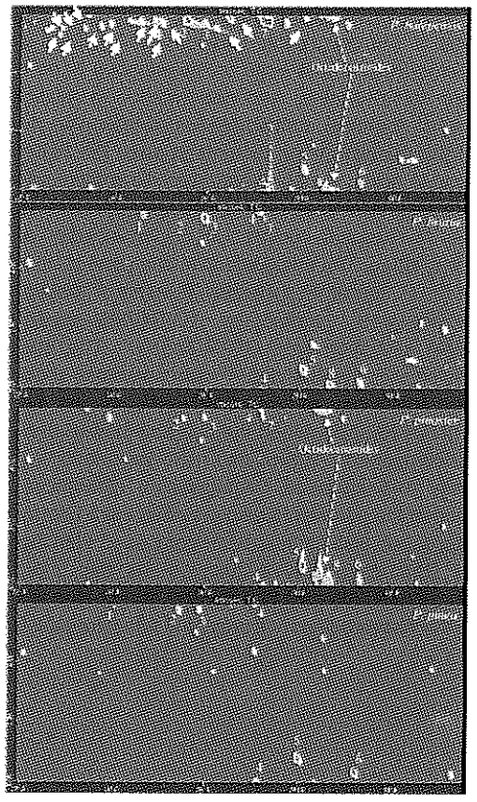
The susceptibility to *T. pityocampa*, within the genus *Pinus*, varies both intraspecifically and interspecifically, according to the geographic location and previous defoliation history of the trees. However, contradictory results and large variations have been reported in the literature, concerning *T. pityocampa* larval survival and performance in relation to different host pine species and the physical, or chemical, characteristics of their pine needles. In relation to the chemical composition of the needles, their contents in silica (an hardness indicator), nitrogen, soluble carbohydrates, amino acids, terpenes and phenolic compounds have been studied and tentatively related with larval survival and performance. Nevertheless, no studies concerning the contents and composition in diterpene resin acids of pine needles and *T. pityocampa* larval performance have been published. The composition in diterpene resin acids of the needles of *P. pinaster*, *P. pinea*, *P. brutia*, *P. kesiya*, *P. sylvestris*, *P. nigra*, *P. patula*, *P. radiata*, *P. elliottii*, *P. taeda* and *P. halepensis* were studied by 1D-GC/MS (quadrupole and ion trap), GC×GC/TOFMS and Field Ionization (1D-GC/FI-ToFMS). The use of GC×GC resulted in enhanced separation efficiency of the analytes, maximizing mass spectra quality and improving compound detection and identification. The GC/TOFMS, using field ionization as a soft ionization method (GC/FI-TOFMS), permitted the characterization of the needles based on the molecular weight of their components. It provided rapid information regarding the mass range, molecular weight diversity and evidence for significant differences due to different mass patterns in the composition of the needles. It also provided extra information, which can be used to identify compound classes, as well as to support compound identification performed by other ionization methods and chromatographic techniques. As this information reports to exact molecular weights, it can be used to assign, or confirm, the presence of particular compounds, or compound classes, in the needles and thus providing a tool for their future characterization.



F1 Expanded view of the resin acids retention time domain from the GC×GC/TOFMS contour plots of methylated extracts from pine needles. Pine species reported in the plots.
Key: 1 – pimaric acid; 2 – sandaracopimaric acid; 3 – isopimaric acid, 4 – palustric/levopimaric acids; 5 – dehydroabietic acid; 6 – abietic acid; 7 – copalic acid; 8 – communic acid. White arrows – compounds with similar mass spectra.

P.65 HPLC-UV ANALYSIS OF GALACTURONIC ACID IN FRUIT JUICES AFTER DERIVATIZATION WITH P-AMINOBENZOIC ACID

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Pectins are additives used in food for their gelling and stabilizing properties[1]. They are a group of polysaccharides consisting almost of α-D-galacturonic acid (GA) residues linked by α-(1,4) glycosidic bonds in which the carboxyl groups of the GA can be present in a free form or esterified. Pectin is not only an effective or even necessary additive to form the structure of food products but also has medicinal benefits which include lowering low density lipoprotein cholesterol (LDL), lowering glucose levels in diabetic patients as well as lowering the risk of several types of cancer[2]. Many food processors and pectin ingredient suppliers need to determine pectin content to control the quality of their products.

The principal aim of this work was to apply a selective and sensitive method for the determination of GA in fruit juices by high performance liquid chromatography (HPLC). The separation was carried out on a C18 column using precolumn derivatization with p-aminobenzoic acid (p-AMBA) and UV detection at 304nm. The derivatization procedure consisted in the reaction of GA with p-AMBA solution in the presence of sodium cianoborohydride, as described previously in other work with proper modification[3]. The GA derivative was separated within 28min using a gradient of water and methanol eluent, both containing 1% of HCOOH and 20 mmol.L^{-1} of ammonium formate. This methodology was optimized in terms of time, temperature and p-AMBA concentration of the derivatization reaction. Further studies on the identification of GA by tandem mass spectrometry (HPLC-MS/MS) were carried out. Mass spectra were recorded using a LQC quadrupole ion trap mass spectrometer with electrospray ionization in positive ion mode. The described method was applied to the quantitative analysis of GA in different fruit juices.

The concentration of GA in the samples analyzed ranged from 18.0 to 316$mg_{GA}.L^{-1}$. Within the analyzed samples, soybean-based juices demonstrated to be the richest in GA content, showing to be a good source of pectins. The detection and quantification limits of the described methodology were 1.1 and 3.5$mg.L^{-1}$, respectively. Quantitative GA recoveries in the beverages were in the range of 90 to 98%. The results showed that the proposed HPLC method was precise and suitable for the identification and quantification of GA in beverages. The developed methodology is able to isolate GA from complex matrices, enabling its structural analysis by HPLC-ESI-MS/MS.

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P.66 OPTIMISATION OF DIRECT INJECTION- LC/MS METHOD FOR PARAQUAT DETERMINATION IN WATERS

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Paraquat is a bipyridylium herbicide widely used for broadleaf weed control. It is also used as a crop desiccant and defoliant, and as an aquatic herbicide. This compound is legislated by the European Union with maximum contaminant level of 0.1μg/L, according to the Drinking Water Directive 98/83/EC. This extremely low value is due to the high toxicity of this kind of compounds. Bipyridylium herbicide's analysis is not easy. Literature reports some problems such as: interaction of the solvent used in standards preparation with the chromatographic analysis, paraquat sorption to glass and other reservoirs and paraquat stability (temperature and light effects)[1-2,6-7].

Literature survey indicates that liquid chromatography (LC) with ultra-violet (UV) or mass spectrometry (MS) detections are the advisable techniques for determination of paraquat in water matrices[1-7].

In this work a simple approach using direct injection in LC/MS was optimised and validated in order to allow a rapid screening of the presence of this contaminant in waters.

Concerning MS optimisation direct infusion allowed the definition of the parameters: capillary voltage, RF loading, needle voltage, drying gas temperature, drying gas pressure and nebulising gas pressure. Afterwards the mobile phase composition was optimised. The calibration curve was carried out by direct injection of paraquat analytical standards and the validation parameters were obtained: linearity range, detection and quantification limits, repeatability and accuracy (by recovery percentage of standard additions). It was found that the calibration curve was linear in the range of 0.1-10mg/L. The detection limit and quantification limits were calculated based on a Signal to Noise Ratio of 3 and 10, respectively and they were 0.04 and 0.12mg/L, respectively. The average precision, at three concentration levels, for standards and spiked tap water were 17% and 10%, respectively. The recovery of paraquat for samples spiked at three concentration levels (0.1; 0.5 and 10mg/L) was 104%.

The present approach is not yet capable of attaining lower concentrations (as expected to meet the criteria for the legislated maximum level of 0.1μg/L), meaning that a further pre-concentration step is required for that purpose. Nevertheless the direct injection developed in this work represents a simple, rapid and reliable methodology that may be used in sorption or degradation studies.

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P.67 RAPID TOOL FOR ASSESSMENT OF C13 NORISOPRENOIDS IN WINES

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C13 norisoprenoids are wine varietal components which have been associated with high quality wine characteristics due to their low odour threshold ($\mu g.L^{-1}$) and pleasant odour descriptors related to tea, violet, exotic flowers, stewed apple, eucalyptus, and camphor[1]. Compounds such as β-damascenone, β-ionone, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and vitispirane isomers[2] have been frequently found in wines. In this work we propose two novel methodologies for quantification of C13 norisoprenoids in wines. The first methodology, method A (reference method) was based on the headspace solid-phase microextraction combined with gas chromatography–quadrupole mass spectrometry operating in selected ion monitoring mode (HS-SPME–GC–qMS-SIM). This methodology allowed selecting the GC conditions for an adequate chromatographic resolution of wine components. The second methodology, method B (rapid method) was based on the HS-SPME–GC–qMS-SIM, using GC conditions that allowed obtaining a C13 norisoprenoid volatile signature. In the later, the GC capillary column of 30m at 220°C was used acting as a transfer line of the components sorbed by the SPME coating fibre to the mass spectrometer, which acts as a sensor for m/z fragments 142 and 192. It does not require any pre-treatment of the sample, and the C13 norisoprenoid composition of the wine was evaluated based on the chromatographic profile and specific m/z fragments, without complete chromatographic separation of its components. For quantification purposes, external calibrations curves were constructed with β-ionone chemical standard. Calibration curves with regression coefficient (r^2) of 0.9940 and 0.9968, R.S.D. of 1.08 and 12.51%, and detection limits of 1.10 and 1.57 $\mu g.L^{-1}$ were obtained for Methods A and B, respectively. These methodologies were applied to seventeen white and red table wines. Two vitispirane isomers (158 to 1529$\mu g.L^{-1}$) and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) (6.42 to 39.45 $\mu g.L^{-1}$) were quantified. The data obtained for vitispirane isomers and TDN using the two methods was highly correlated (r^2 of 0.9756 and 0.9630, respectively). Associated to the fast and robust character of the proposed rapid method B and considering the extraction time, it is important to focus its selectivity and potential applicability if specific m/z fragments would be established for new analytes.

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P.68 AMOXICILLIN ABATEMENT BY FENTON OXIDATION

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The exponential and continued growth in populations has created an increase in the demand for the Earth's limited supply of freshwater. This fact has resulted in one of the most essential environmental issues of the 21st century: the claim for the quality protection of water resources[1]. Although the occurrence of antibiotics in the ecosystem has been known for almost 30 years, it was only in mid-1990s, when the use of these compounds was widespread, that their presence became an emerging concern[2,3]. Human and veterinary antibiotics are continually released to the environment, as a result of metabolic excretion, waste effluents of manufacturing processes and discharges from wastewater treatment plants (WWTPs), which are not often designed to remove these chemicals[2,4].

The bioaccumulation and persistence of antibiotics in the environment can induce toxic effects in the ecosystems in low concentrations levels (ng/L to µg/L). In addition to these facts, they are suspected to be responsible for the appearance of resistance in natural microbiological populations, causing several problems of public health. Amoxicillin is one of the most prescribed antibiotics in EU and USA. When ingested, 80 to 90% of this antibiotic is excreted unmodified5 and therefore, it has been detected in several matrices. To prevent environmental matrices contamination it is necessary to develop degradation or removal processes.

The main purpose of this work was the development of a methodology to degrade amoxicillin (AMOX), from water matrices by Fenton oxidation. Chromatographic detection and quantification of the studied compound were performed by high performance liquid chromatography (HPLC) with diode array detection (DAD). The analytical methodology was validated (linearity range: 10-500µg/L, detection limit: 10µg/L; average intermediate precision of 4.9% and average recovery of 91.1%) and the global uncertainty evaluated using a bottom-up approach (uncertainty varied from 23% to 5%).

A laboratory set-up was designed to evaluate the main parameters that influence the oxidation process: temperature (30-60 °C), H_2O_2 concentration (400-4280µg/L) and Fe^{2+} concentration (95-350µg/L). The oxidative process was optimized through an experimental design methodology (DoE). A percentage removal between 72 and 100% was achieved after a maximum reaction time of 120min. The results of this study proved the efficiency of Fenton oxidation process for amoxicillin abatement.

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P.69 AMINO ACIDS PROFILE OF SWEET AND DRY TINTA NEGRA MOLE MADEIRA WINES SUBMITTED TO ESTUFAGEM

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Amino acids have been the goal of many studies as they are the most important nitrogen fraction of must and wines, and it has been demonstrated that they influence the winemaking. Their content may be influenced by vinification methods, grape variety, geographical region and vintage. Together with sugars and organic acids, nitrogen compounds are essential to the growth and metabolism of yeasts in fermentation. Furthermore, amino acids also have an important effect on the aromatic complexity of wines, namely as metabolic precursors of higher alcohols, the major group of wine aroma compounds. Additionally, the amino acids remaining in wine after fermentation can influence the composition of aromas during the maturing process. On the other hand, some amino acids can generate adverse compounds, such as ethyl carbamate, biogenic amines, ochratoxin A and β–carbolines.

The main purpose of this work was the determination of amino acids profile and behaviour after the vinification process used in Madeira wines: reduced or extended fermentations followed by a baking step surrounding 45°C for 3 months, known as estufagem, common in the Tinta Negra Mole (TNM) wine production. For this purpose, 2 types of Madeira wines, dry TNM and sweet TNM were heated at 45°C during 3 months. The wines were sampled monthly during this period. The amino acids were derivatized through a in loop orthophthalaldeyde derivatization procedure followed by RP-HPLC-FLD determination, as described by Pereira[1], adding an intermediate step to include cysteine derivatization. Before the heating step, the amount of amino acids in sweet TNM wine, as the sum of the individual contributions of the 20 amino acids determined, was 3.6 times higher (644.4mg/L) than in the dry one (117.6mg/L). This was expected given that sweet wines are less fermented than dry wines and the amount of amino acids which was metabolized by yeast is lower. After the baking step the total amino acid fraction declines about 12% in both wine types, revealing that amino acids undergo other reactions during this period. The two main amino acids found were arginine and alanine, for both sweet and dry wines. Arginine reaches 355.9mg/L in the sweet wine and 11.1 times less for the dry one, while alanine was 2.9 times higher in the sweet wine than the 28.7mg/L found in the dry wine. The majority of the amino acids decreases during the heating period, namely cysteine in dry wines (about 79%) showing the probable participation in flavours production as precursor.

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P.70 INFLUENCE OF THE HEATING PROCESS IN THE ORGANIC ACIDS PRESENT IN MADEIRA WINES PRODUCED FROM TINTA NEGRA MOLE GRAPES

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Wine tasting is the sensory examination and evaluation of a wine and comprises, besides the visual and olfactive analysis, the sensations that are generated in the mouth, namely acidity, essential for the quality and taste of a wine. High acidity originates wines with a sour and sharp taste while low acidity makes them taste flat and insipid. Regarding to wine quality, the acidity have to be well balanced with sweetness and bitter components, and controlled, given the direct influence on colour and bacterial stability of the final product. The compounds responsible for wine acidity are organic acids and are already present in grapes, namely tartaric, malic and citric acids. During the fermentation process they play a central role in winemaking, inducing the growth and vitality of yeasts and hence increase the sensory complexity of wine, contributing to the sharpness of flavours. During this step, other acids like succinic, lactic and acetic acids are produced. Eventually, others acids can occur in small amounts which may be derived from ethanol oxidation.

The main goal of this work was the determination of the profile of these compounds in Madeira wines. Tinta Negra Mole (TNM) is a red *Vitis Vinifera* variety which represents between 80 to 85% of Madeira wine production. Dry to sweet fortified wines can be produced from this variety depending on the alcoholic fermentation extension. The vinification process usually includes a banking step during 3 months up to 45°C. Due to this unique vinification it can be assumed that Madeira wine should have a profile of organic acids different from other fortified wines. To satisfy these reservations, organic acids determinations of TNM wines, one dry and other sweet, were monthly followed during the heating step at 45°C during a 3 month period. The quantification was achieved using a RP-HPLC-DAD methodology recently developed by our research group, under publication.

The most abundant organic acid found in the studied Madeira wines was malic acid, in concentrations of about 3.8g/L, followed by acetic acid (about 1.3g/L) and tartaric acid (about 0.6g/L). In the vinification of Madeira wines malolactic fermentation (MLF) is not encouraged, so high levels of malic acid are expected, adding acidic and astringent flavours to these wines, since this acid is more acidic than lactic acid, the resulting product from MLF. The amount of some acids decreases after the heating process, specially succinic acid in the dry wine which is reduced in about 75% of the initial amount. This can be explained by the transformation of this acid into ethyl esters derivatives.

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P.71 ESPECIAÇÃO DE COMPOSTOS ORGANOESTÂNICOS EM MATRIZES AMBIENTAIS POR EXTRACÇÃO SORTIVA EM BARRA DE AGITAÇÃO E ANÁLISE POR CROMATOGRAFIA GASOSA-ESPECTROMETRIA DE MASSA

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De entre as actividades humanas que podem introduzir substâncias químicas nocivas no ambiente marinho, destaca-se o uso das tintas anti-vegetativas aplicadas para revestimentos dos cascos das embarcações. De um modo geral e apesar da proibição das Organizações Ambientais internacionais, o princípio activo destas tintas continua muitas vezes a ser à base de compostos organoestânicos, como é o caso do tributilestanho (TBT), tendo vindo a originar a ocorrência de efeitos de toxicidade nefastos ao nível da fauna marinha.

O presente trabalho consistiu no desenvolvimento, optimização e aplicação da técnica de extracção sortiva em barra de agitação com derivatização in-situ, usando tetra-hidreto borato de sódio ($NaBH_4$), seguida de dessorção líquida e análise por cromatografia gasosa acoplada a espectrometria de massa com aquisição no modo de monitorização de iões seleccionados (SBSE($NaBH_4$)in-situ-LD/GC-MS(SIM)), para determinação de TBT em amostras ambientais. Do trabalho desenvolvido, verificou-se que o $NaBH_4$ proporcionou boa especificidade como agente derivatizante para o TBT em meio aquoso, permitindo a formação de hidreto de tributilestanho (TBT-H), compatível com a análise por GC-MS. O método optimizado permitiu boas recuperações (43,9 ± 8,3%), tendo evidenciado excelente linearidade (r^2>0,9985) na gama de trabalho compreendida entre 14,0 e 1450,0μg/L, permitindo alcançar limites de detecção de 23,0ng/L e precisão adequada (RSD<9 %). Com recurso ao método da adição de padrão, a aplicação da presente metodologia a matrizes estuarinas do rio Tejo, demonstrou elevada sensibilidade ao nível vestigial. A metodologia desenvolvida provou ainda ser uma boa alternativa comparativamente com os métodos convencionalmente usados em rotina, sendo rápida, fácil de manipular, sensível e requerendo pequena quantidade de amostra para monitorizar o TBT em matrizes ambientais.

P.72 APLICAÇÃO DE NOVAS METODOLOGIAS ANALÍTICAS PARA DETERMINAÇÃO DE COMPOSTOS FENÓLICOS EM MATRIZES ALIMENTARES

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O crescente interesse no estudo dos compostos fenólicos, em particular os ácidos fenólicos, deve-se às propriedades antioxidantes que apresentam. A acção antioxidante destes compostos no sistema biológico manifesta-se através da neutralização dos radicais livres gerados no organismo, que estão associados a diversas doenças, como é o caso do cancro ou das doenças cardiovasculares. Os ácidos fenólicos presentes nos alimentos podem desempenhar um importante papel na prevenção destas doenças. Neste sentido é de interesse inquestionável o desenvolvimento de métodos analíticos capazes de determinar e quantificar os compostos fenólicos numa vasta gama de alimentos.

O presente trabalho consistiu no desenvolvimento, optimização, validação e aplicação de um novo método para a determinação de ácidos fenólicos (clorogénico, ferúlico, p-cumárico e cafeico) em produtos alimentares, recorrendo à micro-extracção adsortiva em barra com dessorção liquida, seguida de análise por electroforese capilar com detecção por rede de díodos (BAμE-LD/CE-DAD). Numa primeira abordagem, seleccionou-se o material adsorvente mais adequado para a extracção dos ácidos fenólicos, nomeadamente carvão activado, polidimetilsiloxano, co-polímero de divenilbenzeno e alguns dos seus derivados, tendo o derivado de co-polímero de divenilbenzeno com propriedades de troca aniónica e fase reversa (Oásis® MAX Sorbent) demonstrando ser o mais eficaz. Para optimização da metodologia desenvolvida (BAμE(MAX)-LD/CE-DAD), foram efectuados estudos sistemáticos a diversos parâmetros, tendo 3h de extracção (1000rpm; pH 6) e o uso de solução metanol/ácido fórmico (2%) durante 15min para retro-extracção, permitido obter recuperações compreendidas entre 35 e 40%. O método revelou ainda, excelente linearidade (r^2>0,99) na gama de trabalho compreendida entre 0,8 e 8,0mg/L, tendo-se alcançado limites de detecção e de quantificação entre 18 - 82 μg/L e 61 - 273μg/L, respectivamente.

Com recurso ao método da adição de padrão, a aplicação da presente metodologia a matrizes alimentares, nomeadamente chá verde, mel e sumo de frutos vermelhos, demonstrou bom desempenho analítico tendo-se verificado a ocorrência de níveis de alguns dos ácidos fenólicos estudados. A metodologia proposta (BAμE(MAX)-LD/CE-DAD), apresentou como principais vantagens a utilização de pequenas quantidades de amostra e solventes, tempo analítico reduzido e fácil manipulação, associada à rapidez, simplicidade e bom desempenho analítico.

P.73 DETERMINAÇÃO DE ALDEÍDOS E CETONAS COM ORIGEM NA DESINFECÇÃO DA ÁGUA PARA CONSUMO HUMANO POR MICRO-EXTRACÇÃO ADSORTIVA EM BARRA (BAμE)

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O interesse em analisar compostos carbonilos no meio ambiente, tem-se revelado uma preocupação crescente por parte da comunidade científica, devido à presença ubíqua e ao seu potencial toxicológico para a saúde pública. Recentemente, os compostos carbonilos também têm sido descritos como metabolitos que ocorrem após a desinfecção da água para consumo Humano através do processo de ozonização. Diversos estudos demonstraram que alguns desses compostos, nomeadamente, o formaldeído e acetaldeído, se relacionam com diversos efeitos adversos na saúde humana, particularmente carcinogénicos, tendo já sido classificados pela Agência de Protecção Ambiental norte-americana como sub-produtos prioritários da desinfecção.

O presente trabalho consistiu no desenvolvimento, optimização e aplicação da micro-extracção adsortiva em barra com derivatização in-situ usando pentafluorofenilhidrazina (PFPH), seguida de dessorção líquida e análise por cromatografia líquida de alta eficiência com detecção por rede de díodos (BAμE(PFPH)in-situ-LD/HPLC-DAD), para determinação de compostos de carbonilos de cadeia curta (formaldeído, acetaldeído, propanal, acetona, butanona e 2-hexenal) em amostras de água para consumo Humano.

O método optimizado permitiu a obtenção de recuperações compreendidas entre 47 e 85%, tendo evidenciado excelente linearidade na gama de trabalho (1.0-80,0μg/L), permitindo alcançar limites de detecção convenientes (42 - 137ng/L), e elevada precisão (RSD<13%). Para avaliar da robustez e versatilidade da metodologia proposta, efectuaram-se ensaios de aplicação em diversos tipos de amostras de águas para consumo Humano sujeitas a diferentes tipos de tratamento de desinfecção. Por implementação da metodologia proposta verificou-se a ocorrência deste tipo de contaminantes ao nível vestigial.

P.74 APPLICATION OF POLYURETHANE FOAMS TO CHARACTERIZE AROMA COMPOUNDS FROM COFFEE BLENDS

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Commercially available roasted coffees derive from two species. *Coffea arabica* and *Coffea canephora* var. *robusta* that are cultivated in every continent except Europe. Different blends, composed by mixtures of arabica and robusta types of coffee, have distinct aroma and flavour. Nowadays, headspace solid-phase microextraction (HS-SPME) is one of the most used analytical approaches for sampling aroma compounds from coffee matrices. SPME using carboxen-polydimethylsiloxane (CAR/PDMS) polymeric phase is considered the most efficient fiber coating for analysis, due to its capacity to extract aroma compounds in a wide range of polarity.

In this work, we compare HS-SPME(CAR/PDMS) performance with headspace sorptive extraction using polydimethylsiloxane (HSSE(PDMS)) and polyurethane foams (HSSE(PU)) polymeric phases, followed by gas chromatography coupled to mass spectrometry (GC-MS). For the HSSE assays in particular, liquid desorption and large volume injection was adopted for sensitivity enhancement. From the data obtained by HSSE(PU), much higher extraction yields for particular classes of compounds, in particular ketones, pyrroles, pyrazines, furans and acids, which are the most relevant in coffee aroma. In short, the efficiency yields obtained by HSSE(PU) was considerably much higher when compared with other sorptive microextraction techniques.

P.75 ADVANTAGES ON THE APPLICATION OF POLYURETHANE FOAMS FOR STIR BAR SORPTIVE EXTRACTION OF TRIAZINIC METABOLITES IN WATER MATRICES

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Atrazine is probably the most widely used herbicide all over the world and one of the most common pollutants in ground and surface waters, whose use is restricted. Nevertheless, atrazine and its degradation products are still detectable, due to its previous extensive use, high persistence, high water solubility and weak adsorptivity, contaminating the aquatic environment through agricultural run-off and leaching. In water and soil, parent triazine herbicides are subject to various biotic and abiotic degradation processes, such as photolysis, oxidation, hydrolysis and biodegradation, leading to the dealkylation of amine groups, dechlorination, hydroxylation at position 2, deamination and, to a minor extent, ring cleavage. The main degradation products in water are the dealkylated chloro metabolites, mainly desethylatrazine (DEA), desisopropylatrazine (DIA) and didealkylatrazine (DDA), while in soils we can found the hydroxylated degradation compounds, such as 2-hydroxyatrazine (OH-ATR). The triazinic herbicides and their major degradation products are considered as a group to be endocrine-disrupting chemicals by the U.S. Environmental Protection Agency. The inclusion of the degradation compounds in analytical procedures is highly important, since some of them are as toxic as their parent compounds (or even more).

In this work, polyurethane foams (PU) were applied for SBSE of five triazinic metabolites, desethyl-2-hydroxyatrazine (OH-DEA), desisopropylatrazine (DIA), desethylatrazine (DEA), 2-hydroxyatrazine (OH-ATR) and desethylterbuthylazine (DTBZ) in water matrices. The best experimental conditions using stir bars coated with PU were 5h of extraction (1000 rpm), 5% (v/v) of methanol as organic modifier for the analysis of OH-DEA and OH-ATR and 15% (w/v) of NaCl for the remaining compounds, followed by liquid desorption with 5mL of acetonitrile under ultrasonic treatment (60min) and high performance liquid chromatography with diode array detection (SBSE(PU)-LD-HPLC-DAD). This methodology provided recoveries between 2.6% (OH-ATR) and 26.3% (DTBZ) and remarkable precision (RSD<2.4%). Excellent linearity (r^2>0.9993) ranging from 5.0 to 122.1μg/L and reasonable detection limits (0.4 - 1.3μg/L) at trace level were achieved. This analytical approach was applied for the analysis of the five triazinic metabolites in tap, river and ground waters, with remarkable performance and negligible matrix effects using the standard addition methodology. The comparison between SBSE (PU; 86μL) and SBSE (PDMS; 126μL) was also performed. The latter does not extract OH-DEA and OH-ATR, while for the others compounds the recoveries with PU were up to ten times higher (DIA), proving that the PU is more suitable to extract the polar triazinic herbicides.

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P.76 DESENVOLVIMENTO DE UMA METODOLOGIA ANALÍTICA PARA DETERMINAÇÃO DE ANTIBIÓTICOS EM AMOSTRAS AMBIENTAIS

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A sociedade tem vindo a acentuar a preocupação com os poluentes emergentes ainda não regulamentados. Um grupo diversificado de substâncias químicas bioactivas que tem recebido especial atenção como poluentes prioritários ambientais são os produtos farmacêuticos, de higiene e cuidado pessoal. Entre os produtos farmacêuticos, a presença de antibióticos no ambiente aquático tem tido enorme relevância, uma vez a exposição prolongada mesmo para concentrações vestigiais poderem promover resistência a microrganismos. O elevado consumo destes compostos, a excelente solubilidade em água e a incompleta remoção nas estações de tratamento de águas residuais tem originado a sua presença no ambiente. A ocorrência vestigial destes compostos, a complexidade de algumas matrizes ambientais assim como as propriedades físico-químicas, nomeadamente a elevada polaridade, constituiem um desafio para o desenvolvimento de métodos alternativos para enriquecimento prévio para combinação com técnicas cromatográficas adequadas.

No presente trabalho, propõe-se uma nova metodologia para determinação de diversos antibióticos (trimetoprim, enrofloxacina, sulfatiazol, sulfadimetoxina e sulfametaxazol) em amostras ambientais utilizando uma nova técnica de extracção com fase polimérica à base de poliestireno-divinilbenzeno, seguida de análise por cromatografia líquida de alta eficiência com detecção por rede de díodos e hifenada a espectrometria de massa Tandem por electrospary (SBSE(PU)-LD/HPLC-DAD-(ESI)MS/MS). O método foi optimizado tendo demonstrado boa gama dinâmica de linearidade, limites de detecção ao nível das partes-por-trilião (ppts) e boa resposta por aplicação a amostras ambientais. O método desenvolvido demonstrou ser simples, sensível, com utilização reduzida de solventes torna-o ambientalmente preferível em relação a outros métodos de microextracção para a determinação de antibióticos em amostras ambientais.

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P.77 ANALYSIS OF AROMA COMPOUNDS IN GOURMET COFFEES BY HSSE(PU)

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A coffee beverage has particular aromatic characteristics depending on the composition of the blend from which it is prepared. It must be taken into consideration that the different blends available are produced according to secret recipes of the producer companies. Some coffee blends are considered gourmet, exquisite products whose quality control is tighter, since they have to maintain their aroma characteristics over time. The coffee aroma is quit complex, involving a large number of compounds with different volatilities and a wide range of functional groups. Their analysis is performed on coffee aroma extracts, using several methods, such as vacuum steam-stripping, simultaneous distillation-extraction, press oil aroma extraction, supercritical fluid extraction and solid phase microextraction, usually in the headspace mode.
In a previous work, we propose, discuss and optimize a novel analytical approach using headspace sorptive extraction with polyurethane foams followed by liquid desorption and large volume injection-gas chromatography coupled to mass spectrometry (HSSE(PU)-LD/LVI-GC-MS) for the characterization of roasted coffee aroma compounds.
In this contribution, we intended to apply this optimized methodology in the aroma characterization of six gourmet coffees from two different brands.

P.78 NOVAS ESTRATÉGIAS PARA DETERMINAÇÃO DE HORMONAS ESTERÓIDES SEXUAIS EM MATRIZES AQUOSAS

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As consequências da presença de desreguladores endócrinos (EDCs) no meio ambiente tem recebido cada vez maior atenção por parte da comunidade científica, das agências reguladoras e do público em geral, uma vez que estes compostos têm vindo a ser introduzidos continuamente no meio ambiente, estando comprovado que interferem com as funções normais do sistema endócrino, afectando o desenvolvimento e a reprodução da vida animal e humana. Do grande grupo de substâncias de que se suspeita ou está comprovado que actuam como EDCs, as hormonas sexuais esteróides são consideradas como os compostos com as propriedades estrogénicas mais acentuadas. No presente trabalho, propõe-se a optimização simultânea de duas metodologias para microextracção analítica de oito hormonas esteróides sexuais (estriol, 17β-estradiol, 19-noretisterona, estrona, dietilstilbestrol, D-(-)-norgestrel, progesterona e mestranol) em amostras aquosas. As metodologias desenvolvidas são análogas à extracção sortiva em barra de agitação (SBSE), no entanto utilizam diferentes materiais de extracção, nomeadamente fases poliméricas à base de poliuretanos e poliestireno-divinilbenzeno. A técnica instrumental utilizada foi a cromatografia líquida de alta eficiência com detecção por rede de díodos (HPLC-DAD). Durante o desenvolvimento de ambas as metodologias, estudos sistemáticos evidenciaram condições experimentais óptimas, no qual o tempo de extracção, a força iónica assim como os solventes e o tempo para retro-extracção demonstraram ser parâmetros críticos na eficiência de recuperação. Dos resultados obtidos pode concluir-se que os novos polímeros permitem obter maiores recuperações ao nível vestigial quando comparado com as barras de SBSE comerciais com polímeros à base de polidimetilsiloxano.

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P.79 COMPARISON OF POLYURETHANE FOAMS AND POLYDIMETHYLSILOXANE POLYMERIC PHASES FOR THE CHARACTERIZATION OF AROMA COMPOUNDS IN VINEGARS BY STIR BAR SORPTIVE EXTRACTION

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The volatile composition of wine vinegars is quite complex, with dozens of compounds accounting for low concentration levels. Nowadays, to characterize the aroma of vinegars, solventless extraction techniques, such as solid-phase microextraction and more recently, stir bar sorptive extraction (SBSE) are widely used in the headspace (HS) mode prior to thermal desorption (TD) and analysis by gas chromatography-mass spectrometry (GC-MS). Nevertheless, SBSE had shown much higher sensitivity since the amount in polydimethylsiloxane (PDMS) involved is greater than in SPME.

Lately, our group optimised several parameters through HS-SBSE/TD-GC-MS technique in order to characterize the aroma compounds in Sherry wine vinegars. Although good performance was attained for the identification and quantification of more than 50 volatile compounds, SBSE using non-polar polymeric phases such as PDMS, the only one commercially available, present some limitations particularly to identify important aroma compounds with higher polar characteristics, in particular those ones absent in the HS of the vinegar matrices. To overcome this limitation, the use of polymeric phases presenting different polarity characteristics, such as polyurethane (PU) foams, have been successfully developed and proposed in many applications.

The present contribution aims the optimisation and comparison in between SBSE(PDMS) and SBSE(PU) techniques with liquid desorption (LD), followed by large volume injection (LVI) and GC-MS analysis, to characterize seven varieties of vinegars (Jerez, Jerez Reserva, Jerez Gran Reserva, Balsamic, Apple and two red wines) elaborated by the traditional methodology in two kinds of wood (cherry and oak). The data obtained shown that SBSE extraction capacity when using PU polymeric phases is 5 to 80 times higher than with PDMS and several new compounds with aroma impact could also be identified (e.g. furaneol, solerone, etc).

P.80 MICRO-EXTRACÇÃO ADSORTIVA EM BARRA (BAμE) - UMA METODOLOGIA INOVADORA PARA ANÁLISE VESTIGIAL

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Nas últimas duas décadas, diversos tipos de abordagens para preparação de amostras têm sido propostos para o enriquecimento de várias classes de compostos orgânicos em matrizes aquosas para posterior análise cromatográfica. São bons exemplos, a extracção em fase sólida, a micro-extracção em fase sólida e, mais recentemente, a extracção sortiva em barra de agitação (SBSE), como algumas das técnicas de extracção sortiva mais comuns em análise vestigial.

SBSE, em particular, é uma técnica de preparação de amostras emergente, proposta na última década, que apresenta um desempenho notável, fácil manipulação, elevada precisão e grande sensibilidade. Neste sentido, esta técnica analítica tem sido largamente aplicada com recurso à fase polimérica à base de polidimetilsiloxano (PDMS), que evidencia grande afinidade para compostos apolares. No entanto, quando em presença de compostos com características mais polares evidencia grandes limitações para microextracção.

Na presente contribuição, propomos a descrição detalhada de uma nova metodologia para enriquecimento vestigial de compostos polares, microextracção adsortiva em barra (BaμE). A grande vantagem desta nova abordagem, para além da grande capacidade extractiva é a possibilidade de se poder seleccionar o adsorvente mais conveniente para cada caso em estudo.

O desempenho da BAμE é ilustrado com a aplicação analítica a diversas classes de compostos polares em matrizes aquosas, nomeadamente a subprodutos de desinfecção da água, produtos farmacêuticos, pesticidas, drogas ilícitas e ácidos fenólicos. Para a microextracção dos compostos seleccionados, os limites de detecção alcançados por esta metodologia associada a cromatografia líquida de alta eficiência com detecção por rede de díodos situam-se nas partes-por-trilião (ppts).

P.81 OPTIMIZAÇÃO DO MÉTODO DE ENSAIO PARA ANÁLISE DE HORMONAS ESTERÓIDES SEXUAIS EM MATRIZES AMBIENTAIS POR LC-(ESI)MS/MS

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As hormonas esteróides são responsáveis pelo desenvolvimento e diferenciação dos orgãos sexuais femininos e masculinos, assim como pelas características sexuais e comportamentais. Uma vez presentes no meio ambiente, podem interferir com o funcionamento normal do sistema endócrino, perturbando a reprodução e desenvolvimento de diversos organismos vivos incluindo o ser humano, sendo considerados compostos desreguladores endócrinos.
Seleccionaram-se como modelo para o presente estudo oito hormonas esteróides sexuais (estriol, 17β-estradiol, 19-noretisterona, estrona, dietilstilbestrol, D-(-)-norgestrel, progesterona e mestranol). Do ponto de vista instrumental, recorreu-se à cromatografia líquida de alta eficiência acoplada à espectrometria de massa Tandem por electrospray (HPLC-(ESI)MS/MS), tendo-se optimizado as melhores condições para a formação do ião percursor para cada hormona e o padrão de fragmentação MS/MS, usando-se o modo de ionização positivo e como analisador de massas a "armadilha de iões". Estabelecidas as condições do sistema HPLC-(ESI)MS/MS, definiu-se a gama de trabalho, limiares analíticos e repetibilidade instrumental. Posteriormente, estudou-se a validação da metodologia recorrendo a duas abordagens analiticas para enriquecimento com fases poliméricas à base de poliuretanos e poliestireno-divinilbenzeno. As metodologias propostas demonstraram serem simples, sensíveis ao nível vestigial (ppb) para alguns dos compostos estudados.

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P.82 POTENTIALITIES OF TWO SOLVENTLESS EXTRACTION APPROACHES - STIR BAR SORPTIVE EXTRACTION AND HEADSPACE SOLID-PHASE MICROEXTRACTION FOR DETERMINATION OF HIGHER ALCOHOL ACETATES, ISOAMYL ESTERS AND ETHYL ESTERS IN WINES

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A stir bar sorptive extraction with liquid desorption followed by large volume injection coupled to gas chromatography–quadrupole mass spectrometry (SBSE-LD/LVI-GC–qMS) was evaluated for the simultaneous determination of higher alcohol acetates(HAA), isoamyl esters (IsoAE) and ethyl esters (EE) of fatty acids. The method performance was assessed and compared with other solventless technique, the solid-phase microextraction (SPME) in headspace mode (HS). For both techniques, influential experimental parameters were optimized to provide sensitive and robust methods. The SBSE-LD/LVI methodology was previously optimised in terms of extraction time, influence of ethanol in the matrix, liquid desorption (LD) conditions and instrumental settings. Higher extraction efficiency was obtained using 60min of extraction time, 10% ethanol content, n-pentane as desorption solvent, 15min for the back-extraction period, 10mL.min^{-1} for the solvent vent flow rate and 10°C for the inlet temperature. For HS-SPME, the fiber coated with 50/30μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) afforded highest extraction efficiency, providing the best sensitivity for the target volatiles, particularly when the samples were extracted at 25°C for 60min under continuous stirring in the presence of sodium chloride (10% (w/v)). Both methodologies showed good linearity over the concentration range tested, with correlation coefficients higher than 0.984 for HS-SPME and 0.982 for SBES-LD approach, for all analytes. A good reproducibility was attained and low detection limits were achieved using both SBSE-LD (0.03 to 28.96μg.L^{-1}) and HS-SPME (0.02 to 20.29μg.L^{-1}) methodologies. The quantification limits for SBSE-LD approach ranging from 0.11 to 96.56μg.L^{-1} and from 0.06 to 67.63μg.L^{-1} for HS-SPME. Using the HS-SPME approach an average recovery of about 70% was obtained whilst by using SBSE-LD obtained average recovery were close to 80%. The analytical and procedural advantages and disadvantages of these two methods have been compared.

Both analytical methods were used to determine the higher alcohol acetates, isoamyl esters and ethyl esters of fatty acids content in "Terras Madeirenses" table wines. A total of 16 esters were identified and quantified from the wine extracts by HS-SPME whereas by SBSE-LD technique were found 25 esters which include 3 higher alcohol acetates, 3 isoamyl esters and 19 ethyl esters of fatty acids. Generally SBSE-LD provided higher sensitivity with decreased analysis time.

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P.83 AN LC-MS/MS HIGH THROUGHPUT SCREENING METHOD FOR THE DETERMINATION OF ANTIBIOTICS IN MILK

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Veterinary drugs are being used on a large scale and administered as feed additives or via drinking water to prevent the outbreak of diseases. As a response to this, large numbers of food samples are analyzed and new, cost-effective methods have to be developed for these compounds. The combination of high sample throughput with high sensitivity and reproducibility is the focus of many recently developed methods. We propose the combination of TurboFlow™ technology and Liquid Chromatography-Tandem Mass Spectrometry for an efficient screening of multi-class antibiotics in milk.

The advantage of this method is the drastic reduction of sample preparation time when comparing with the normal offline sample preparation methods. The automated on-line extraction was performed using TurboFlow technology which exploits the difference between large and small molecules and column chemistry to retain compounds of interest while matrix molecules flow to waste.

Albendazole, difloxacin, oxytetracycline, phenylbutazone, salinomycin, spiramycin, sulfamethazine and tetracycline were detected and quantified by LC-ESI-MS/MS in positive selective reaction monitoring mode (SRM). Peak areas of spiked milk samples were compared to those of neat standards to assess matrix interferences and carry over. Even without internal standards, quantitative results proved to be linear in the concentration range of 5 to 500μg/L as well as reproducible and precise (RSD 0.4-14%). The limits of detection were between 0.1 and 5.2μg/L. Also, the results obtained show good reproducibility even when testing different milk brands. Higher fat content seem to influence the precision of the method only at the highest level of the range studied (500μg/L). At the 100μg/L level, the matrix effect is minimal for salinomycine Na, spiramycin, tetracycline, oxytetracycline and sulphametazine. However, values between 70 and 85% were found for phenylbutazone, albendazole and difloxacin.

The method was tested by simulating a routine screen procedure with real milk samples. The result was the detection of all the antibiotics present in the sample.

The developed automated TurboFlow-LC-MS/MS method permits simple sample preparation while minimizing matrix interferences for the detection of various antibiotics in milk products down to μg/L levels. Accurate quantitation of those compounds subject to residual matrix interferences could be accomplished by using a suitable internal standard.

P.84 THE DETECTION OF PHARMACEUTICAL CONTAMINANTS IN WATER SAMPLES BY ON-LINE LIQUID CHROMATOGRAPHY- MASS SPECTROMETRY

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Pharmaceuticals residues constitute a group of emerging contaminants which have been receiving considerable attention in recent years. Although these compounds are not yet controlled, many studies have been published where the detection of some the studied contaminants reach concerning levels. The detection of these compounds at low ng per litre levels is still a challenge for the analyst. Techniques that can meet this challenge are mainly chromatographic techniques coupled with mass spectrometry.

A rapid, sensitive and precise method for the determination and quantification of pharmaceutical residues in water samples is presented. It employs the use of on-line solid phase extraction and liquid chromatography - tandem mass spectrometry. The columns employed in this study were: Hypersil Gold 12μm, 50x2.1mm as a pre-concentration column and Phenomenex Luna C18 (2) 5μm, 150x2.0mm as an analytical column. This turnkey method allows water sample injections up to 20mL. However, this study was developed by injecting volumes of 1mL and 5mL. The sample preparation method was reduced to a simple filtration when handling ground water or waste water samples or direct injection when handling drinking water samples.

The advantage of the method is the drastic reduction on sample preparation time, when comparing with the normal offline sample preparation methods, and the lower limits of detection achieved.

The emergent contaminants studied included compounds belonging to different families, such as anti-inflammatories, antibiotics, antidepressants, lipid regulators and β-blockers. This method was employed in the analysis of drinking water, ground water and waste water samples. Linearity, reproducibility and precision were studied. Two transitions (Selected Reaction Monitoring mode) were selected for each compound and quantification was performed by isotopic dilution.

The limits of detection were found to be at low ng/L levels.

P.85 OPTIMIZATION OF AN UNIVARIATE HEADSPACE SOLID-PHASE MICROEXTRACTION EXPERIMENTAL DESIGN FOR THE QUANTIFICATION OF HALOANISOL COMPOUNDS IN MADEIRA WINES

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Haloanisoles constitute a family of organic compounds responsible for the taste and odours associate to fungal and earthy-musty descriptors in wine This undesirable change in the organoleptic properties affects 2–5% of bottled wine, prevents its consumption and causes great economic losses in the viticulture and related industries and for that the determination their origin and causes is one of the priority objectives of wine companies.

From a biological point of view the origin of haloanisoles can be found in methylation of the corresponding halophenols, by certain fungi such as the aspergilius family[1].

Due to the extremely low concentration of these off-flavour compounds in wine (in range of ng/L) it becomes necessary to select an analytical technique with suficient sensitivity and reprodutibility. Therefore, in these work, it was developed a headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) methodology was optimized in order to quantify 2,4,6-trichloroanisole (TCA), 2,4-dichloroanisole (DCA), 2,4,6-tribromoanisole (TBA), 2,6-dichloroanisole (DCA) and pentachloroanisole (PCA) in wines.

The quantification involved the selection and optimization of the parameters which might affect the extraction efficiency such as type of coating fibre, extraction time and temperature, ionic strength, sample volume (expressed as 1/β), stirring, desorption temperature and time.

The SPME method optimization was been conducted by considering one parameter at a time and keeping all other variable constant (univariate optimization design). According to the data obtained, it was selected the following parameters: polydimethylsiloxane-divinylbenzene (PDMS/DVB) SPME coating fibre, extraction temperature of 35°C and 45min of extraction time, 1/β of 0.5.

The optimized and validated method will be then applied to the analysis of real wine samples produced at Madeira Island.

Acknowledgements
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P.86 ANALYSIS OF VIRGIN OLIVE OIL PHENOLIC METABOLITES IN HUMAN PLASMA AND URINE BY LC-MS/MS: A PRELIMINARY STUDY

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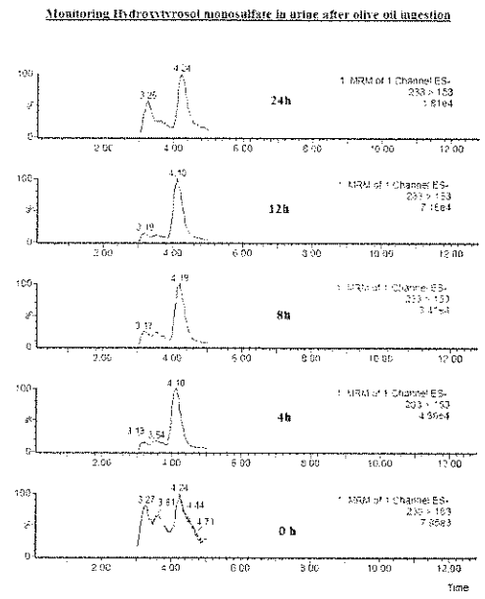
Olive oil is a typical component of the Mediterranean diet and has been associated with a reduced incidence of coronary heart disease. This is likely due in part to the high amount of monounsaturated fatty acids but also to its phenolic content. Olive oil has in its phenolic composition oleuropein and ligstroside derivatives, which are secoiridoid compounds, and also hydroxytyrosol and tyrosol. Several studies have shown that these phenolic compounds are absorbed. Glucuronide, sulphate and methyl conjugates of hydroxytyrosol and tyrosol have been found in plasma and urine in both human and animal experiments[1].

The aim of this study was to optimize an SPE and LC-MS/MS procedure for the isolation and analysis of metabolites, in biological samples, of typical phenolic compounds in olive oil such as hydroxytyrosol monoglucuronide, hydroxytyrosol monosulfate, tyrosol glucuronide, tyrosol sulfate. Other metabolites related to secoiridoid compounds were also screened by means of LC-MS/MS.

Two volunteers were provided with 50mL of extra virgin olive that was ingested in a single dose with some bread. Urine was collected before and after the ingestion period (at 0, 4, 8, 12 and 24h) and stored at -20°C until analysis. Blood was also collected at time 0, 0.5, 1, 2, 4 and 6h. After centrifugation plasma was pooled and stored at -20°C until analysis.

Figure 1 shows the chromatograms corresponding to hydroxytyrosol monosulfate, by means of MRM, detected in urine after olive oil ingestion.



F1 Hydroxytyrosol monosulfate detected in urine: 0, 4, 8, 12 and 24h.

Acknowledgements
Fundação para a Ciência e Tecnologia (FCT), REDE/1518/REM/2005

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P.87 CONTRIBUTION TO THE CHARACTERIZATION OF THE PORTUGUESE *OPUNTIA SPP.*

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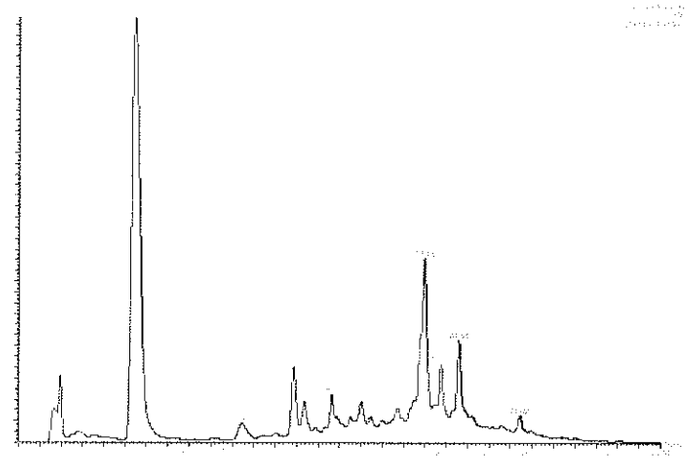
Opuntia spp., a cactus which spontaneously grows in Portugal, is a xerophytic plant that mainly grows in arid and semi-arid regions. Cactus cladodes, fruits and flowers have been traditionally used as medicines in several countries, particularly in Latin America but also in northern Africa. Examples of this use are the treatment of gastritis, fatigue, dyspnoe, rheumatic disorders and erythemas, the improvement of digestion and the enhancement of the general "detoxification process". Cactus uses as cosmetic have also been described, mainly using seed oil.
The fruits (named prickly pears) are a good source of carbohydrates, vitamins (for example, vitamin C), amino acids (including taurine) and yellow and/or red betalains (betaxanthins and betacyanins, respectively). Cactus flowers contain many interesting chemical compounds: carbohydrates, mucilage, minerals and flavonoids (such as luteolin, kaempferol and quercetin). The cladodes are rich in fiber and used in dietary supplements often referring ability for regulating blood sugar or cholesterol levels.
In this preliminary comparative study cladodes (collected in Portugal) were extracted with different solvents and analysed by high-performance liquid chromatography (HPLC) using diode array, electrochemical and mass spectrometry detection. In figure is presented an illustrative chromatogram of a cladode extract detected at 280nm.



F1 Chromatogram of the ethanol:water (50:50) extract from cladodes (280nm).

Fruit juices of different *Opuntia* varieties were also analysed. By using LC-MS/MS it was possible to obtain some results concerning molecular weight of peaks detected and possible fragmentation paterns.
Results obtained are discussed in terms of the chromatographic profiles and the chemical composition is correlated with the antioxidant activity of the different samples analysed.

Acknowledgements: Fundação para a Ciência e Tecnologia (FCT), REDE/1518/REM/2005.

P.88 AVALIAÇÃO DA APLICABILIDADE DA METODOLOGIA DE SPME/GCXGC-TOFMS NA OBTENÇÃO DO PERFIL METABÓLICO VOLÁTIL DE AZEITES EXTRA-VIRGEM

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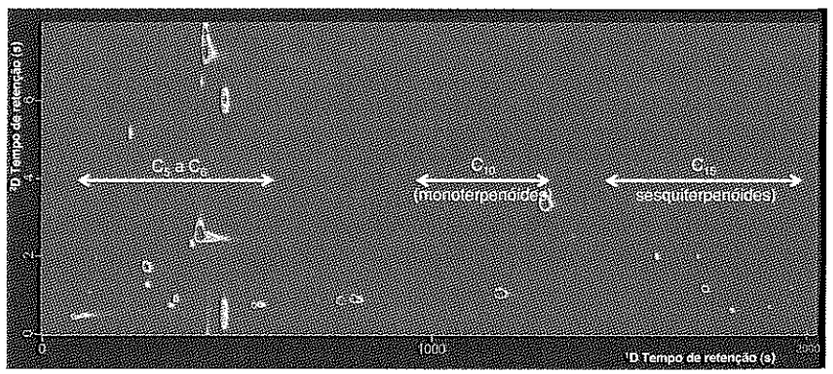
O objectivo do trabalho é avaliar a aplicabilidade da metodologia de SPME/GCxGC-TOFMS na obtenção do perfil metabólico volátil de azeites extra-virgem, com vista a estudar a sua influência no aroma do produto e no estabelecimento de potenciais marcadores varietais.

No controlo de qualidade do azeite e a sua subsequente classificação nas diversas categorias é imprescindível, por um lado, a quantificação de um conjunto de parâmetros químicos e por outro a avaliação das suas características organolépticas[1]. Para a avaliação organoléptica recorre-se a um painel de provadores, que faz a apreciação das amostras definindo atributos e/ou defeitos. Sob, o ponto de vista do aroma, os atributos comummente descritos num azeite são o frutado, o herbáceo, a maçã ou a amêndoa e são conferidos pela variedade e estado de maturação da azeitona assim como pelo tipo de processamento utilizado e boas práticas de fabrico. Analisando a fracção volátil de azeites classificados como extra-virgem e com características organolépticas de excelência, detectou-se uma grande percentagem de compostos em C_5 e C_6, nomeadamente aldeídos. Estes compostos são considerados descritores de *aromas herbáceos*[2].

Existem diversos estudos sobre a composição volátil do azeite envolvendo cromatografia unidimensional por GC-MS, no entanto é necessário recorrer à compilação de diversos resultados para se obter um perfil o mais completo possível[2, 3]. Atendendo à complexidade da composição volátil do azeite, e à elevada sensibilidade e resolução cromatográfica da cromatografia compreensiva bidimensional, a aplicação da metodologia de SPME/GCxGC-TOFMS ao estudo da fracção volátil de amostras de azeite apresenta-se bastante promissora[4]. Assim, após uma fase de optimização da metodologia, foram estudados diversos azeites extra-virgem da mesma marca comercial. Os resultados obtidos permitiram obter cromatogramas estruturados que por combinação com *m/z* específicos levaram à definição de espaços cromatográficos associados aos analitos de interesse, nomeadamente aos compostos em C_5 e C_6, e, ainda, a diversos compostos terpénicos (Fig.1).



F1 Cromatograma estruturado com selecção dos iões *m/z* 67, 93, 161 e 204 para algumas famílias químicas: álcoois e aldeídos em C_5 e C_6, e mono e sesquiterpenóides.

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P.89 CHARACTERIZATION OF VOLATILES IN INDIVIDUALS WITH BREAST CANCER BY HS-SPME-GC-qMSD

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Breast cancer is the most common major malignancy in women and the second leading cause of cancer death. Several groups of investigators have described some volatile compounds as markers of metabolic processes and clinical diagnosis from various pathologies, including cancer[1]. The progress in the development of early detection of breast cancer makes it a case study to identify the tools that will be effective for detection and diagnosis of other types of tumors.

Various methods have been employed for the discovery of biomarker patterns of major human diseases, especially for various types of cancer. The appropriate biomarkers may be able to define risks and identify the early stages of tumor development, assist in tumor detection and diagnosis, predict outcomes of the disease, and help in surveillance for disease recurrence.

The methodologies used in the study of cancer pathologies includes solid-phase microextraction (SPME) introduced in the 90´s as a new approach to sample preparation[2].

The biological fluids (urine) used in this work were obtained from 27 patients with breast cancer and 21 controls (healthy volunteers) in Serviço de Hemato-Oncologia and Banco de Sangue of Centro Hospitalar in Funchal.

The purpose of this work was to identify the volatile compounds in biological fluids by means of HS-SPME-GC-qMSD (headspace solid phase-microextraction) described in the literature as possible biomarkers of cancer. More than was 80 volatile compounds were identified in both groups, belonging to several chemical families, namely aldehydes, ketones, benzene derivates, among others. The major chemical families identified in control group were ketones, sulfur compounds and volatile phenols, for the group with breast cancer were ketones, sulfur compounds and volatile phenols. The most representative compounds of these families were 4-heptanone, methanethiol, dimethyl disulfide, *p*-tert-butyl-phenol, 2,4-bis(1,1-dimethylethyl)phenol for the control group, and 4-heptanone, 2-pentanone, methanethiol, dimethyl disulfide, phenol, 4-methyl-phenol, *p*-tert-butyl-phenol for the breast cancer group.

Acknowlegments
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P.90 ANALYSIS OF EARLY SYNTHETIC DYES WITH HPLC-DAD-MS

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Early synthetic dyes from the Smithsonian's Helmut Schweppe Collection, Reference Collection DYE 16850-16915, "Important Early Synthetic Dyes" and dyed fibers "Colourings of Early Synthetic Dyes", were analyzed with High Performance Liquid Chromatography – Diode Array Detection – Mass Spectrometry (HPLC-DAD-MS^n). This Schweppe collection has circa 11 chemical families, as azo, nitro, quinoline, just to name a few, being a challenge in terms of chromatographic separation. Several elution gradients for the HPLC-DAD-MS analysis of early synthetic dyes were tested and the results obtained will be presented. The best results were obtained using an improved elution gradient of MeOH and TEA 3mM/H_2O, pH=6.4 which allowed the separation and characterization of the almost 65 dyes from the collection. In this work particular attention will be given to the characterization of purple pos-Perkin dyestuffs, which can reveal important contributions to establish the production date of textiles from this period. This will be exemplified with textiles from the XIX century.

Ackowledgement
We thank the Portuguese MS Network (IST-UTL Center) for providing access to the LC-MS facilities.

P.91 DETERMINATION OF SULFUR COMPOUNDS IN FERMENTED RASPBERRY BEVERAGES BY GC-PFPD

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Volatile sulfur compounds are known to have very powerful and characteristic odours, and can contribute to pleasant or unpleasant aromas of a wine, according to their nature and concentration. Their concentration has a great influence on sensory properties, often being strongly dependent on threshold values, normally low. The determination of sulfur compounds, eluted in a chromatographic column, has been performed using mainly flame photometric detection (FPD), sulfur chemiluminescence detection (SCD), atomic emission detection (AED) and more recently, pulsed flame photometric detection (PFPD).

The objective of this study was to determine the sulfur compounds in a fermented raspberry beverage using GC-PFPD. The analyses were made on a Varian CP-3800 gas chromatography equipped with a PFPD detector operating in sulfur mode. Aromatic extracts, obtained by liquid-liquid extraction with dichloromethane, were injected into a 1079 split/splitless injector (splitless for 30s). The separation was performed with a CP-Wax 52 CB column (50m x 0.25mm i.d., 0.2μm film thickness; Chrompack). The oven temperature was programmed from 60 °C (5 min) to 200°C, at 20°C/min (final hold for 5min). The carrier gas was helium with a constant flow rate of 1.2mL/min. The temperature of the injector and detector was set to 250°C. The detector voltage was 570V, the gate delay for sulfur compounds was 6ms and the gate width was 20ms. All sulfur compounds were identified by comparing their retention times with those of the pure standards. Raspberry pulp was diluted with water (1:1), sucrose was added to adjust the initial sugar concentration to 160g/L (sucrose+glucose+fructose) and the pH was corrected to 4 with $CaCO_3$. Batch fermentations (3L of raspberry must) were carried out at 22°C in a bioreactor, after inoculation with 1g/L of *Saccharomyces cerevisiae* CAT^{-1} previously grown in YPD medium.

In fermented raspberry beverage were identified and quantified 7 sulfur volatile compounds: furfuryl mercaptan (16.9μg/L), 2-mercaptoethanol (4.7μg/L), 2-(methylthio)ethanol (158.1μg/L), 3-mercapto-3-methylbut-1-ol (4.0μg/L), 3-methylthio-1-propanol (128.9μg/L), 4-mercapto-1-hexanol (1.5μg/L) and benzothialoze (5.7μg/L). The highest concentrations were found for 2-(methylthio)ethanol and methionol (3-methylthio-1-propanol). Methionol, with cauliflower and cabbage descriptors, can be related to the metabolism of yeasts, when methionine is metabolized.

It was concluded that liquid-liquid microextraction with dichloromethane followed by GC-PFPD analysis is effective to quantify volatile sulfur compounds produced in fermented raspberry beverages. As expected, the PFPD detector exhibited a quadratic behavior for these compounds.

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P.92 A CARQUEJA COMO FONTE DE AÇÚCARES FERMENTÁVEIS EM ETANOL

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A produção de bioetanol a partir de biomassa vegetal é uma forma importante de obtenção de um biocombustivel que permite reduzir o consumo dos combustíveis fósseis, bem como evitar o seu esgotamento, preservando o ambiente, principalmente reduzindo a quantidade de dióxido de carbono libertado para a atmosfera. Portugal possui uma extensa área florestal com uma grande quantidade de espécies arbustivas, as quais podem ser utilizadas como matéria-prima para produção de bioetanol. Esta biomassa lenhocelulósica pode ser considerada um material bastante atractivo para a produção de etanol no futuro.

Um dos passos cruciais para a produção de etanol a partir de espécies vegetais é a hidrólise da hemicelulose e celulose a açúcares monoméricos. O método mais promissor é o uso de enzimas, contudo é necessário tornar este material acessível a estas através de um pré-tratamento, sendo esta etapa de grande importância na bioconversão de materiais lenhocelulósicos em bioetanol. No presente trabalho foi estudada a optimização das condições de um pré-tratamento usando ácido sulfúrico diluído, para uma espécie arbustiva da biomassa florestal, Pterospartum tridentatum (carqueja). A biomassa vegetal foi caracterizada quanto à composição química, e apresentou valores que colocam esta espécie arbustiva como matéria-prima de elevado potencial para a produção de bioetanol.

Os ensaios foram realizados sob diferentes condições operacionais utilizando uma ferramenta estatística de Response Surface Methodology para modelar o pré-tratamento de modo a obter um elevado rendimento de açúcares redutores totais.

As condições operacionais estudadas foram: temperatura (80-180°C), tempo de residência (30-120min), concentração de ácido sulfúrico (0,5-5%) e razão biomassa/líquido (1,5g/10mL-3g/10mL). A concentração de açúcares redutores totais nos filtrados resultantes dos pré-tratamentos foi determinada pelo método do ácido dinitrosalicílico (DNS). Os açúcares individuais foram determinados por HPLC, usando uma coluna aminex HPX-87H (Bio - Rad, Hércules, CA) usando como eluente 0,05M H_2SO_4, com um caudal de 0,4mL/min a uma temperatura constante de 65°C. Esta determinação apresenta particular relevância porque quando passamos para a etapa de fermentação, é necessário conhecer quais os açúcares e em que quantidades se encontram no nosso filtrado, dado que pentoses e hexoses são fermentados por microorganismos diferentes.

A concentração máxima de açúcares totais obtida foi de 306,1mg/g de biomassa, quando a reacção foi realizada a 130°C durante 75min, 5% de ácido sulfúrico e 2,25g/10mL de razão biomassa/líquido. A análise por HPLC dos filtrados resultantes dos tratamentos efectuados nas condições anteriores mostra que a xilose foi o principal açúcar obtido, sem que produzam grandes quantidades de produtos de degradação dos açúcares.

As variáveis operacionais que mais influenciam o pré-tratamento são o tempo de residência, a temperatura e a percentagem de ácido.

P.93 FAST DETERMINATION OF PLASMA AMINOTHIOLS BY ISOCRATIC HPLC ELUTION AFTER PRECOLUMN DERIVATIZATION

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Low molecular weight plama thiols such as homocysteine (Hcy), cysteine (Cys), cysteinylglycine (Cys-Gly) and glutathione (GSH) are fundamental cellular components that play several important roles in metabolism and homeostasis. In recent years there has been a growing interest in identifying the effects of aminothiols in the pathogenesis of human disorders, namely cardiovascular diseases[1]. Plasma aminothiols content is frequently measured by HPLC with fluorescence detection. The aim of our study was to develop a new methodology for a simultaneous determination of the four aminothiols, taking into account the reduction of their retention times, which will have a significant effect on the number of the daily sample analysis performed.

In this method, thiols were reduced from plasma proteins with tri-n-butylphosphine (TBP), followed by protein precipitation with trichloroacetic acid (TCA) and derivatized with a thiol-specific fluorogenic reagent ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate (SBD-F). The thiols adducts were separated by an isocratic elution on a Platinum EPS C18 analytical column (53x7mm I.D., 3μm particle size) kept at 35°C that was protected by a C18 guard-column (30x2mm I.D.), using 0.1mol/L KH_2PO_4 buffer pH 2 containing 40mL/L of acetonitrile as a mobile phase. The detection was performed by a fluorescence detector (excitation at 385nm and emission at 515nm). Peaks of the four referred aminothiols were quantified by comparison to a standard curve prepared by plotting peak areas versus the different levels of known standard solutions after normalization with internal standard and the results are expressed in μM per 20μL of sample injection. Results indicated an excellent linearity for all the analytes over their respective concentration ranges with correlation coefficients r≥0.99. The precision of the chromatographic methodology was evaluated by performing the repeatability of the HPLC analysis of the four plasma thiols in intra- and inter-day measurements of the retention time (RT) by repeated sample injections. The RSD for the intraday (n=10) repeatability of the RT ranged from 0.54% to 1.86%, and for the interday (n=4) precision varied from 0.32% to 1.71%. The accuracy of this method was evaluated by determining the recovery of Hcy, Cys, Cys-Gly and GSH in samples with known concentrations of aminothiols. Three different standard amounts of each of the thiols were added to the plasma sample which was subjected to the HPLC chromatographic analysis. The recovery was calculated based on the difference between the total concentration determined in the spiked samples and the concentration in the non-spiked samples. Results show that recovery ranged from 92% to 100% indicating a high degree of the method's accuracy for the determination of aminothiols in the analyzed plasma samples.

This method allows a complete analysis in 6min, whereas other published studies show analysis times ranging from 15 to 20min[2,3] using HPLC gradient elutions. By reducing the analysis time, a larger number of analysis can be performed daily with the advantage of determining the levels of the four aminothiols at the same time. This assay is well suited for high-throughput quantitative determination of aminothiols in clinical studies, and can be used to support investigations of oxidative stress in patients with cardiovascular and chronic kidney diseases.

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P.94 SEPARATION AND CHARACTERIZATION OF THE PHENOLIC COMPOUNDS IN *HELICHRYSUM MALALECEUM* BY HPLC-DAD-ESI-MS^n

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Madeira archipelago has four endemic sub-species of *Helichrysum* (*Asteraceae*). Two of them, *Helichrysum malaleucum* and *Helichrysum devium*, are used in folk medicine for the treatment of respiratory diseases such as bronchitis and pharyngitis.
In the present work, a simple and rapid method has been used for the screening and identification of the main phenolic compounds present in methanolic extracts of *H. malaleucum* using high-performance liquid chromatography with on-line UV and electrospray ionization mass spectrometry detection (LC-DAD-ESI-MS^n).
Plant material was harvested in the wild in two locations of Madeira Island: Fajã da Nogueira, a mountainous location, and São Vicente, in the north coast.
A total of 38 compounds were identified or tentatively characterized based on their UV and mass spectra and retention times. Two of these compounds were positively identified by comparison with reference standards.
The phenolic compounds detected included several derivatives of quinic acid, flavonols *O*-glycosides (quercetin, isorhamnetin and kaempferol), flavones *O*-glucosides (apigenin and luteolin), caffeic acid derivatives.
This contribution presents one of the first report on the analysis of phenolic compounds from *Helichrysum malaleucum* flowers using LC-DAD-ESI-MS^n.

Acknowledgements:
S. Gouveia thanks FCT for a PhD grant SFRH/BD/24227/2005.
This work made use of equipments from MS National Network REDE/1508/REM/2005 and NMR National Network REDE/1517/RMN/2005.

P.95 CHROMATOGRAPHY: THE ANALYTICAL TOOL TO THE DETERMINATION OF PESTICIDE RESIDUES – A MADEIREAN OVERVIEW

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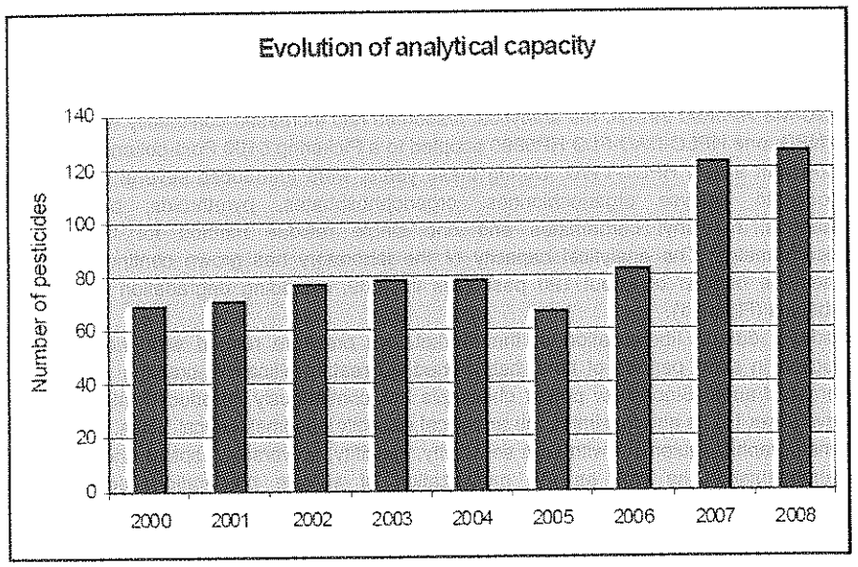
The monitoring programmes of pesticide residues in Autonomous Region of Madeira started in 1992 with the installation of Agricultural Madeira Laboratory in Camacha. During the initial exploratory years the main objective was to increase the analytical capacity of the laboratory in order to achieve the necessary scope. Initially equipped with one GC-ECD/NPD HP5890 and one GC-MS HP5890 5971A, the analytical resources had increased continually with a HP6890 NPD/NPD, one HPLC-DAD-FLD HP5890 acopled to a Pickering 5100 Post-column system and, in 2006 with a new HPLC-VWD. During these years, were introduced several multi-residues methods to analyse organochlorines, organophosphates, pyrethroids, benzamidazole carbamates, N-methyl carbamates and one single residue method for the family of dithiocarbamates. The analytical capacity of the laboratory has grown continually and has reached the number of about 120 pesticides in 2009, excluding degradation products and metabolites (Figure 1).

Nowadays, due to liberalization of the market, the pressure related with the increase of production in short periods of time, has gained a great significance, creating conditions for alimentary frauds. Since new potent chemicals are continuously introduced into the market, achieve lower LODs and maximise the analytical capacity becomes critical. Thus, investment in chromatographic equipment with high sensitivity, specifity, universality, speed and high degree of automatization as well as the minimization of sample preparation techniques, the complementarity of the installed techniques and the investment in new substracts and analytes are crucial for a residues laboratory.

In this way, with the new Regional Laboratory of Veterinary and Food Security in Santa Rita, in 2009, our Division was reinforced with a new GC-TOFMS from LECO, one LC-MS-MS Micromass Quattro-micro from Waters, one HPLC-FLD Agilent 1200, one GC-ECD/FPD Agilent 7860 and a new automated GPC from Gilson. With these new chromatographic equipments, our laboratory has become one of the best equipped laboratories in Portugal in analysis of residues. Our challenge for the near future is to introduce a new line for the control of pesticides in water, for micotoxines in food, start to analyse pesticides in animal origin products, implement an analytical line for single-residue methods and increase our analytical capacity, in routine methods, for 200 pesticides. This is our Mission: challenging but attractive.



F1. Evolution of the residues analytical capacity of RAM, since 2000.

Keywords: pesticide analysis; chromatography, GC, GC-TOFMS, HLPC, LC-MS/MS

P.96 PESTICIDE RESIDUES MONITORING IN MADEIRA ISLAND AGRICULTURAL PRODUCTS, 2006-2008

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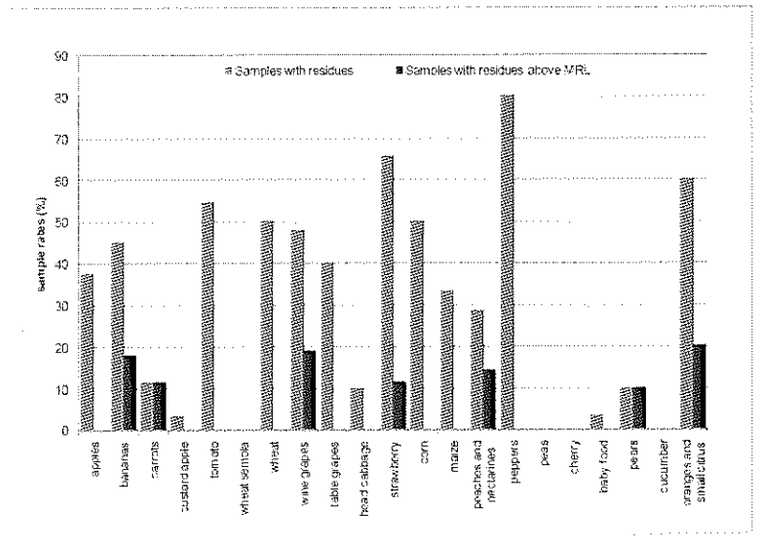
Between 2006 and 2008, a total of 575 samples, including regional and imported commodities, were collected on wholesale and retail market, in Madeira Island, to determine the degree of exposition of madeirean population to the pesticide residues. Pesticides residues were determined by multi-residue methods (MRMs) and single residue methods (SRMs) followed by gas chromatography analysis with Nitrogen Phosphorous Detector (NPD), Electron-Capture Detector (ECD) and Mass Spectrometry Detector (MSD), as well as by liquid chromatography with Variable Wavelength Detector (VWD) and Fluorescence Detector (FLD).

From the total analysed samples, 76% had no detectable residues and 11% showed pesticides above the respective Maximum Residue Level (MRL). During this period, the violation rates decreased significantly (from 12% to 8%) in 2008 when compared with 2006 and 2007 whereas the detection rates in 2008 increased slightly (from 27- 32 to 49%) in comparison with the previous years.

Of the 120 pesticides that were monitored (excluding metabolites and degradation products), 43 pesticides were actually found. The most frequently detected pesticides were dithiocarbamates (17%), dimethoate and omethoate (13%), chlorpyriphos (11%), thiabendazol (10%) and acrinathrin (8%). Agricultural products with pesticide residues were pepper, strawberry, oranges and tomato in decreasing order (Figure 1).

The pesticides involved in violative samples where mainly dimethoate and acrinathrin. Despite the oranges, wine grapes, bananas and peaches showed the highest violation rates (14-20%) (Figure 1), since 2006, the number of violative samples has decreased significantly namely in banana (from 28 to 5%), wine grapes (from 22 to 11%) and strawberries (from 23% to 0%).



F1 Percentage of each sample with residues and violative (above MRL) pesticides monitored in 2006-08.

P.97 PESTICIDE RESIDUES: DATA VALIDATION BY GAS AND LIQUID CHROMATOGRAPHY

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Pesticides-residues testing of agricultural products and foods is carried out worldwide and is probably the most extensive studied contaminants in the food chain. Pesticides monitoring is mainly undertaken to check the compliance with maximum residue limits (MRLs) as part of regulatory testing programmes. Since regulatory limits for pesticides have progressively decreased and residue data is used in exposure assessment by safety and health authorities, accurate measurement of any level of residues above the reporting limits have been imposed. The challenge for pesticide residues analysis laboratories is therefore to be capable to test a large number of widely different pesticides, to have methodologies validated for a diversity of food types/pesticides combinations and to be capable to demonstrate a competence in achieve the detection of low levels of pesticides. Laboratories therefore need to validate methods and provide mechanisms to estimate the uncertainty of the obtained data.

In the present work, we describe the results obtained from the validation of about 120 pesticides (excluding metabolites and degradation products) using multi-residue (MRMs) and single residue (SRMs) methods. Chromatographic analysis was performed using Gas chromatography with Nitrogen Phosphorous (NPD), Electron-Capture (ECD) and Mass Spectrometry (MSD) detectors, as well as by liquid chromatography with Variable Wavelength (VWD) and Fluorescence (FLD) detectors tecnhiques. Linearity, Pearson correlation coefficient of calibration curves, instrumental limit of detection/quantitation (LOD/LOQ) and matrix recoveries at several levels of concentration were evaluated[1].

Good linearity of the calibration curves was obtained over the range from 0.01 to 0.32mg/kg, with Pearson correlation coefficients ≥0.99 to the majority of the pesticides. Instrumental LOD values generally varied from 0.001 to 0.007 mg/kg. The instrumental LOQ values were around 0.01mg/kg for almost all pesticides and more than 90% of pesticides analysed by each mode met the acceptability criteria of recovery (70-120%) and RSD (≤ 20%)[2], at the studied levels.

Keywords: Pesticides, Method Validation, Linearity, Limit of Detection, Limit of Quantification

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P.98 IDENTIFICAÇÃO POR HPLC-PDA DE METABOLITOS SECUNDÁRIOS DE EXTRACTOS ETANÓLICOS DE PLANTAS AROMÁTICAS COM APLICAÇÃO EM INDÚSTRIA ALIMENTAR

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Actualmente, existe um conhecimento crescente do impacto dos antioxidantes naturais na promoção de saúde humana, combinado com estudos que demonstram os efeitos adversos que alguns antioxidantes sintéticos, como o butil-hidroxianizol (BHA) ou o butil-hidroxitolueno (BHT), podem trazer para a saúde, levou ao aumento das pesquisas sobre os antioxidantes naturais, principalmente os encontrados nas plantas. Os extractos de plantas aromáticas têm-se revelado excelentes fontes de compostos fenólicos com actividade antimicrobiana e antioxidante podendo ser usadas como conservantes naturais em alimentos.

O presente trabalho pretende ser um contributo para este tema estudando os metabolitos secundários de um conjunto de plantas aromáticas com aplicação na indústria alimentar, usadas em Portugal, as quais foram já objecto de análise preliminar das suas potencialidades antioxidantes, antifúngicas e antibacterianas (Matos et al, 2009). Com o objectivo de identificar os compostos responsáveis pela bioactividade referida utilizou-se a técnica de extracção sequencial em fase sólida (SPE) para separação dos compostos iónicos (tais como alcalóides) e não iónicos (tais como terpenóides e compostos fenólicos), seguida da técnica de cromatografia líquida de alta resolução, com detecção por fotodiodos (HPLC-PDA) para identificação qualitativa dos compostos estudados.

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P.99 EVOLUÇÃO DE COMPOSTOS VOLÁTEIS EM FERMENTADOS DE MEDRONHO

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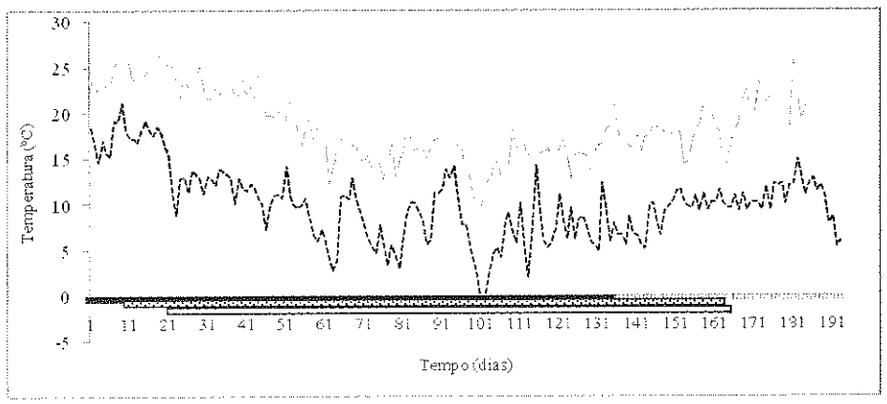
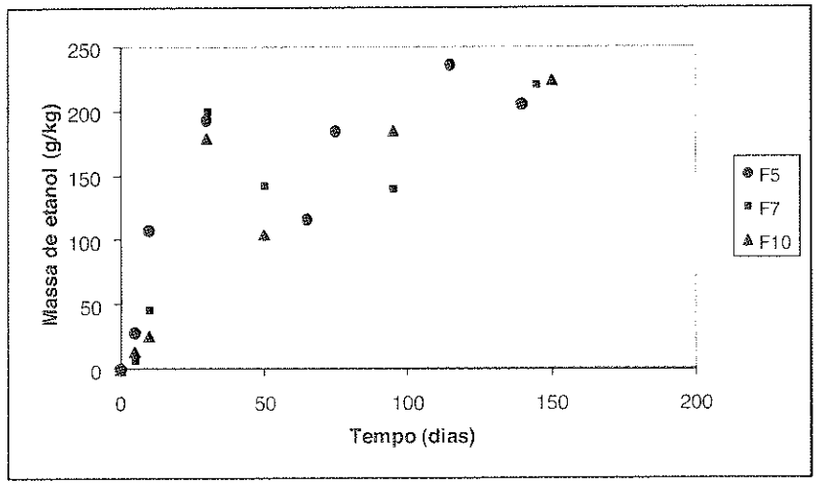
O destilado do fruto *Arbutus unedo* L., apresenta grande importância económica para os habitantes da zona serrana do Algarve. Vários trabalhos têm sido desenvolvidos no sentido de melhorar o processo produtivo. Primeiramente, dando particulare importância ao acompanhamento das fermentações e à análise do produto final e nesta fase dando maior relevância à formação de etanol e a sua ligação com a microbiota durante a fermentação. Neste sentido, foram seguidas 3 fermentações de um produtor tradicional de São Bartolomeu de Messines no concelho de Silves. As fermentações decorreram de forma natural, desde finais de Outubro a início de Março, durante 130 a 150 dias. Neste período, as temperaturas na serra algarvia são muito baixas chegando a atingir valores negativos durante a noite, figura 1.
Foram recolhidas 7 amostras de cada fermentador em diferentes fases da fermentação e de diferentes pontos do fermentador. As amostras de fermentado foram sujeitas a diversas análises entre elas a quantificação de etanol, de acetato de etilo e de isopentanóis, por HS-SPME-GC, usando o método da adição de padrão e uma fibra de poliacrilato.
Em todas as fermentações verificou-se que a contagem de microrganismos totais e de leveduras era da mesma ordem de grandeza e que se atingiu um máximo ao quarto dia de fermentação, valor que se manteve muito semelhante até ao final.



F1 Variação das temperaturas máximas (.....) e mínimas (-----) da sala de fermentação. Período de fermentação 5 ( ), 7 ( ) e 10 ( ) dias.

A quantidade de etanol inicial não era detectável pela metodologia usada mas após 30 dias de fermentação este composto atingiu mais de 80% do valor que apresentava no final figura 2.



F2 Evolução da massa de etanol por kg de massa de fermentado para os 3 fermentadores em estudo.

É notório que a partir dos 30 dias de fermentação existe um abaixamento no teor de etanol em todas as fermentações estudadas, o que certamente está relacionado com o abaixamento da temperatura ambiente. O acetato de etilo e os isopentanois têm uma evolução semelhante embora com teores de 1,5% e 0,5% respectivamente, quando comparados com os teores de etanol.

P.100 QUANTIFICATION APPROACH FOR ASSESSMENT OF SPARKLING WINE VOLATILES FROM DIFFERENT SOILS, RIPENING STAGES, AND VARIETIES BY STIR BAR SORPTIVE EXTRACTION WITH LIQUID DESORPTION

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Stir bar sorptive extraction with liquid desorption followed by large volume injection coupled to gas chromatography-quadrupole mass spectrometry (SBSE-LD/LVI-GC–qMS) was applied for the quantification of varietal and fermentative volatiles in sparkling wines[1]. Two grape varieties, *i.e.* 'Fernão-Pires' (FP) and 'Baga' (BG), obtained from different ripening stages and soils were used. The analytical data were performed by using suitable standards of monoterpene hydrocarbons (α-pinene), monoterpenols (linalool), sesquiterpenoids (*E*,*E*-farnesol, *Z*-nerolidol, and guaiazulene), C_{13} norisoprenoids (β-ionone), aliphatic and aromatic alcohols (hexanol and 2-phenylethanol), and esters (hexyl acetate and ethyl decanoate) as model compounds. The wine volatiles were quantified using the structurally related standards. The SBSE-LD/LVI-GC–qMS methodology allowed, in a single run, the quantification of 71 wine volatiles that can be quantified accurately at levels lower than their respective olfactory thresholds. The variety and soil influenced significantly the volatile composition of sparkling wines; lower effect was observed for the ripening stage of grapes picked up one week before or after the maturity state. From all sparkling wines studied, those of wines of FP variety, from a clay-calcareous soil with ripe grapes seem to provide the wine with the highest aroma potential[2].

Acknowledgements: This work was financially supported by AGRO Nº 38 and Fundação para a Ciência e Tecnologia (SFRH/BD/25336/2005).

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P.101 DIRECT HPLC ENANTIOSEPARATION OF CHIRAL XANTHONES ON POLYSACCHARIDE AND MACROCYCLIC ANTIBIOTIC-BASED CHIRAL STATIONARY PHASES

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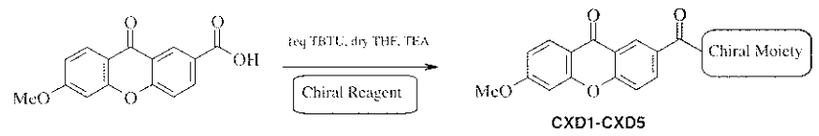
Chiral xanthone derivatives (CXD) can be associated with important pharmacological activities such as anticonvulsant, antidepressant, anti-inflammatory and antitumor[1,2], with mechanisms of action many times associated with enantioselectivity[1]. The use of Chiral Stationary Phases (CSP) in HPLC has proven to be the most helpful among the currently methods to achieve chiral separations[3]. Thousands of different chiral compounds have been efficiently enantioresolved by polysaccharide-based columns[3] and, in our group, analytical HPLC methods using this type of CSPs were already used for resolution of racemic xanthonolignoids[4]. In recent years, the CSPs with covalently bonded macrocyclic antibiotics have proven to be versatile and selective chiral selectors for separation of different classes of chiral compounds[3].

This work describes the investigation on the resolution of a small library of CXDs on three CSPs (one polysaccharide and two macrocyclic antibiotics), under different elution modes (normal, reversed-phase, and polar organic).

The CXD were synthesized by coupling 2-carboxy-6-methoxyxanthone with both enantiomers of valinol (CXD-1), leucinol (CXD-2), 2-amino-1-propanol (CXD-3), 1-amino-2-propanol (CXD-4) and α,4-dimethylbenzylamine (CXD-5) as chiral reagents (Figure 1).



F1 Synthesis of different CXDs (1-5).

The polyssacharide-based CSP consisted of cellulose *tris*-3,5-dimethylphenyl carbamate coated onto APS-Nucleosil (CSP-1). The macrocyclic antibiotic-based were the Chirobiotic T (CSP-2) and Chirobiotic R (CSP-3).

The optimized chromatographic conditions that allowed the best enantioseparations are presented in Tables 1 and 2.

T1 Best enantioresolution achieved on polyssacharide-based CSP*.

CXD	CSP	Mobile Phase	K_1	α	R_s
1	1	MeOH:H_2O (80:20) with 0.1% TFA	1.5	1.2	1.0
2	1	MeOH:H_2O (80:20) with 0.1% TFA	1.9	1.2	1.3
3	1	Hex:EtOH (80:20)	2.1	1.2	1.0
5	1	MeOH	0.6	1.5	2.3

T2 Best enantioresolution achieved on macrocyclic antibiotic-based CSPs*.

CXD	CSP	Mobile Phase	K1	α	Rs
1	2	Hex:EtOH (80:20)	5.3	1.5	2.1
2	3	Hex:EtOH (50:50)	1.0	1.4	1.5
3	2	Hex:EtOH (80:20)	9.0	1.3	1.5

Hex = hexane, EtOH = ethanol, MeOH = methanol, TFA = trifluoroacetic acid
*flow rate of 0.5mL min^{-1}, UV detection at 254nm

HPLC method leaded to the enantioresolution of the CXDs 1-3 and 5 with good selectivity and resolution. Normal elution mode using ethanol as modifier was found to be the most efficient for chiral separation of xanthones on macrocyclic antibiotic-based CSPs. The polyssacharide-based CSP have proved to be effective to separate this class of compounds under normal, reversed-phase, and polar organic conditions.

Acknowledgments
FCT (I&D, nº4040/2007), FEDER, POCI, FCT-GRICES/CAPES 00770 29/05/08, CESPU U. Porto and Santander for financial support.

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P.102 ANALYSIS OF *PINUS* SPP ESSENTIAL OILS BY ENANTIOSELECTIVE COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY/TIME-OF-FLIGHT MASS SPECTROMETRY (GCXGC/TOFMS).

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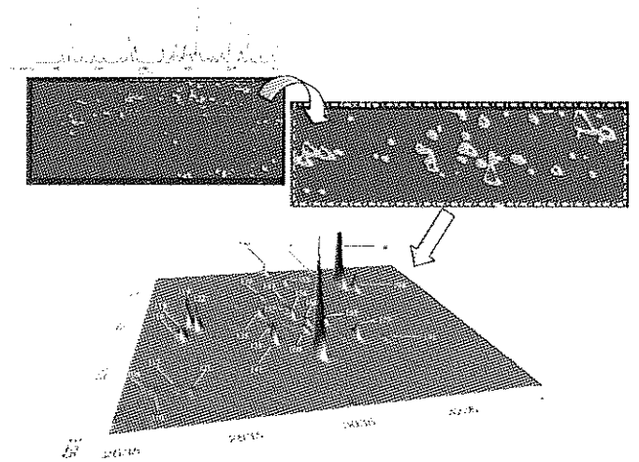
The enantiomers of chiral terpenes present in the volatile blends emitted by *Pinus spp.* should analytically be considered as individual components, due to their inherent potential role in insect-host plant relationships, and in the chemical discrimination of pine species. The separation of enantiomeric monoterpenes by GC×GC analysis was performed with a taylor made fused silica capillary column with 30m × 0.25mm I.D., coated with 0.25µm film of 15% heptakis (2,3-di-O-methyl-6-O-tert-butyldimethylsilyl)-β-cyclodextrin in SE52 (2,3-DiMe) on the first dimension. The column was coupled to a polar column (Supelco-Wax) composing an original set of columns for GC×GC. In order to collect information and evaluate the set performance in GC×GC, a solution with a mixture of 43 standards was analysed before the Pinus samples.
The chemical composition of the needles of *P. pinea*, *P. pinaster*, *P. halepensis*, *P. nigra*, *P. brutia*, *P. patula*, *P. radiata*, *P. taeda*, *P. elliotti*, *P. kesiya* and *P. sylvestris* was investigated. Steam distillation extraction (SDE) was used to collect the volatile fractions. The use of GC×GC resulted in enhanced separation efficiency and increased signal to noise ratio (sensitivity) of the analytes, maximizing mass spectra quality and improving compound detection and identification. The mass spectra obtained by GC×GC showed a better quality than those obtained by 1D-GC/TOFMS, providing higher match library factors with the NIST/Wiley searchable libraries, and thus a stronger identification of the compounds present in the samples than by one-dimensional GC analysis. The enantio-GC×GC analysis performed for all the *Pinus spp.* under study achieved the detection of 422 different compounds.



F1 Enantio-GC×GC/TOFMS overview of the separation: first dimension reconstructed chromatogram with contour and 3D plots (*P. pinaster* needles).

P.103 CHARACTERIZATION OF VOLATILE COMPOUNDS IN SELECTED FRUITS FROM R.A.M. BY SOLID PHASE MICROEXTRACTION FOLLOWED BY GC-qMS

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Flavor is one of the most important attributes of foods in determining consumer acceptance. Many methods have been used for flavor volatile analysis. The most typically utilized methods for extraction and preconcentration are headspace techniques, purge-and-trap, liquid-liquid extraction, and simultaneous distillation and extraction. The majority of these methods are time-consuming, require exhaustive concentration steps, have memory effects, and/or require dedicated headspace sampling devices.

The low concentration of the most volatile compounds makes enrichment as a basis for identification and quantification, in which liquid-liquid extraction (LLE) followed by gas chromatography coupled to mass spectrometry (GC-MS), have been the analytical method of choice. Nevertheless, LLE is a time consuming and labour intensive technique, involving multi-step procedures and use of solvents Recently, for the purposes of determining fruit aroma volatiles, the solid-phase microextraction (SPME) technique has been applied as an alternative sample preparation strategy, to overcome the problems associated with conventional sampling methodologies, such as elevation costs, time-consumption, and the use of large volumes of organic solvents.

In this study the volatile compounds in two selected fruits from R.A.M. (Madeira Island), São Jorge lemon (*Citrus limon*), and kiwi (*Actinodia deliciosa*), were characterized. The volatile compounds from the headspace of the citrus and kiwi juices were isolated by solid-phase microextraction (SPME) prior to separation with gas chromatograph (GC) and identification by mass spectrometry (MS). For each extraction, fibres were exposed to the headspace of a 4mL septum-sealed glass vial containing 2 ± 0.001g of sample, 0.5mL of water, 1μL of internal standard and 0.10g of NaCl during 30min at 25 ± 1ºC under constant magnetic stirring.

The HS-SPME procedure allowed for the identification of 25 compounds in São Jorge lemon juice. Most of these compounds were tentatively identified as terpenes and esters, found in large numbers and exhibiting intense peak areas. Limonene, γ-terpinene, o-cymene and α-terpinolene were the major compounds, all of which have been reported as potent odors in other aromatic fruits. In the kiwi aroma, 47 substances were tentatively identified being the most intense aldehydes and ethyl esters families. The two major compounds in the fruit pulp were (E)-2-hexenal (59.0%) and ethyl butyrate (36.4%). Furfural, hexanal, 5-hydroxymethylfurfural and 2,5-furandicarboxaldehyde were present in levels higher than 5%.

P.104 COMPARISON OF THE FIBER EFFICIENCIES IN THE EXTRACTION OF VOLATILE AND SEMIVOLATILE COMPOUNDS FROM *CARICA PAPAYA* L. FRUIT

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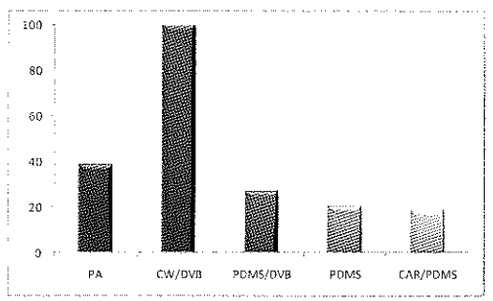
Among the many attractive and desirable attributes that create demand for fruits from the tropics and subtropics, their characteristic flavor is the most noticeable to consumers. In addition, these fruits are often inexpensive, extremely rich in vitamins, and can be used in a wide range of food products. Despite the characteristic desirable flavor of this fruit, the chemical composition of the volatile compounds of Madeira Island *Carica papaya L.* has not previously been the object of study.

For the analysis of the volatile compounds, some publications are available using gas chromatography-mass spectrometry (GC-qMSD) followed by liquid-liquid extraction and steam distillation. These techniques, however, have some disadvantages such as higher costs, extent time-consumption and larger volumes of organic solvents used. Recently, the headspace solid-phase microextraction (HS-SPME) technique emerges as an attractive alternative for volatile analysis because offers many advantages like high sensitivity and reproducibility, is a rapid, versatile, sensitive, solventless and economical sample preparation technique and has been applied in a variety of analytical applications since first being described by Belardi and Pawliszyn. Since the first HS-SPME fibres became commercially available, they have been used in several applications, including a wide range of food analysis, like volatile composition in wines, beers, whiskeys, honeys, medicinal plants and several kind of fruits.

In this work as an attempt to fully characterize papaya puree flavor, a lot of fibers from low polarity to high polarity (for flavors) were used to reduce discrimination toward very polar and very non-polar volatiles. The performances of each commercially available SPME fiber in this study were determined based on the intensity of the response observed. The sum of the identified peak areas in the total ion chromatogram (TIC) obtained for each fiber was normalized in relation to the sum of the identified peak areas in the TIC obtained for the fiber with highest peak areas. In this study, each extraction was done in triplicate and the repeatability (RSD%) was lower than 10%. Among the commercial SPME fibers, it was observed that the CW/DVB fiber exhibits better extraction efficiency for highly volatile compounds. This fiber is covered with a porous solid coating, which suggests that the analyte extraction occurs via adsorption, which is particularly efficient for volatile compounds. The major compound in the fruit pulp was benzyl isothiocyanate (42.3%). Linalool oxide, furfural, hydroxypropanone, linalool and acetic acid were present in levels higher than 7%.



F1 Normalized extraction efficiencies measured for PA, CW/PDMS, PDMS/DVB, PDMS and CAR/PDMS

P.105 HEADSPACE SOLID PHASE MICROEXTRACTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY BASED METABOLOMIC APPROACHES IN URINARY BIOMARKER STUDY OF CANCERS

Catarina Luís, J.S. Câmara

Cancer is a disease characterized by abnormal growth and development of normal cells beyond their natural boundaries. Despite of global efforts to limit the incident of this disease, cancer has become the leading cause of death in the last 50 years. The management of high risk cancers, such as breast and prostate cancer, requires diagnosis at an early stage, which specifies the need for specific and sensitive biomarkers. A biomarker is a quantifiable laboratory measure of a disease specific biologically relevant molecule that can act as an indicator of a current or future disease state. Sometimes, certain molecules are differentially expressed in cancer cells relative to their normal counterparts and their altered levels could be measured to establish a correlation with the diseased state. Diagnostic biomarkers of cancers could be valuable diagnosis, to evaluate the risk and stratification. The cancer biomarker study includes several steps such as risk assessment, noninvasive screening for early-stage disease, detection and localization, disease stratification and prognosis, response to therapy, and screening for recurrence using a variety of patients samples such as urine, plasma, hair, and tissues with the conventional statistics. Metabolomics helps to understand metabolic state of biological systems with comprehensive insight by detecting the metabolomes. It involves establishing the relationships of phenotype to metabolism, which is a key aspect to describe the distribution of metabolites with their biological functions. The HS-SPME/GC-MS based metabolomics have therefore become a promising technique in the clinical diagnosis. In many biomedical applications, MS can play an important role to identify mixtures and create libraries along with metabolite patterns, which are useful for to prepare metabolic fingerprints.

The metabolomic approaches for mining biomarkers of cancers based on headspace solid phase microextraction (HS-SPME) followed by gas chromatography-mass spectrometry combined with multivariate analysis are described. To identify urinary potential biomarkers, the qualitative and quantitative analyses were introduced with 27 breast, 14 leukemia, 12 colon and 7 lymphoma cancer patients as well as 21 normal controls. Urine samples obtained from clinically diagnosed patients of breast, leukemia, colon, and lymphoma cancers were analyzed and compared to healthy normal controls. Four groups into the normal controls and the patients with breast, leukemia, colon, and lymphoma cancers were comprised. Normal controls (n=21, age=44.1±10.2) were volunteered and selected with subjects have no pathological evidences of all of breast, leukemia, colon, and lymphoma cancers, who attend at Hospital Central do Funchal (HCF, Madeira, Portugal). Breast cancer patients (n=27, age=54.8±9.41) were from females with a diagnosis of invasive breast cancer who attend at HCF. Breast cancer patients underwent the diagnostic procedures, i.e., breast physical examination, mammography, and ultrasonography. Leukemia (n=14, age=50.1±12.9), colon (n=12, age=62.0±10.9) and lymphoma cancer patients (n=7, age=42.0±19.0 y) were identified and categorized through biopsy. The study protocol was approved by the ethical committee of HCF attended, and all subjects had an agreement to use their urine samples on the scientific purpose. Urine samples for normal and oncologic patients were collected and were stored at −20 °C until being used.

Two different approaches as qualitative and quantitative profiling analyses of volatile and semivolatile were performed by gas chromatography coupled to mass spectrometry (GC-MS) followed by multivariate data analysis. The one-way ANOVA was applied in order to evaluate if there were differences between the analyzed groups (normal and oncologic). The principal component analysis (PCA) allowed the differentiation of the referred groups with 81.02% of the

total variance. The compounds 1-octanol and 3-heptanone influence breast and leukemia cancers, colon cancer is influenced by 4-methyl-phenol, anisole and 1,2-dihydro-1,1,6-trimethylnaphtalene. Hodgkin by heptanal and 2-methyl-3-phenyl-2-propenal, while the control group is influenced by *p*-cymene, 1,4,5-trimethylnaphtalene and dimethyl disulfide.

Acknowlegments
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P.106 APPLICATIONS OF SOLID-PHASE MICROEXTRACTION: FROM ENVIRONMENT TO MEDICAL CHEMISTRY

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Solid phase microextraction (SPME), one of the six "*great ideas of the decade*", was invented by Pawliszyn in late 1989 as a new pre-concentration technology, in which a fused-silica fiber coated with a stationary phase was used. Is based on the sorption of analytes present in the sample or in its headspace by a thin film of an extracting phase immobilized over the surface of a fused-silica fiber. Fibers are available coated with pure liquid polymeric phases or with porous solid phases (more accurately, porous solids dispersed in liquid polymer matrixes). For liquid polymeric phases such as polydimethylsiloxane (PDMS), the physico-chemical mechanism responsible for the extraction is partition, and, when porous solid coatings like Carboxen/PDMS are used, the process also involves adsorption of the analytes by the fiber coating. Under practical conditions, the extracted mass, **n**, of an analyte is proportional to its concentration, $\mathbf{C_0}$, in the sample- which is the basis of the quantitative use of SPME. Extracted masses also depend on several other controllable and/or measurable parameters: the coating/sample distribution constant, K_{fs}; the headspace/sample distribution constant K_{hs} (when a headspace is present); sample and coating volumes; temperature; etc. $\boldsymbol{K_{fs}}$ is a function of the affinity between analyte and coating, and the proper choice of coating phase can lead to selective extractions.
The development of solid-phase microextraction (SPME) has experienced significant growth since its introduction as a new approach to sample preparation in the early 1990s. In comparison to existing technologies, such as liquid-liquid or solid-phase extraction, SPME offers many advantages, including simplicity, speed, solventless extraction, and a convenient format for the analyst, is simpler and faster, and provides markedly cleaner extracts than methods based on liquid-liquid extraction or solid-phase extraction. In the relatively few years of its use, SPME has become a mature technique and a useful alternative to contemporary techniques in various scientific and research fields.
When SPME was first introduced, much attention was devoted to the extraction of pesticides from environmental, biological and food matrices. Since then, work in this field has continued, and HS-SPME is often used as a routine technique for the extraction of pesticides from liquid and solid samples. Clearly, organophosphorous pesticides (OPs) and organochlorines (OCs) are the most extensively investigated pesticide groups by the HS-SPME approach due to their thermal stability and volatility. Since 2000, numerous articles have described the determination of OPs by HS-SPME in different matrices, including water, juices, fruits, vegetables, oil, passiflora infusions, and human biological specimens, such as whole blood, blood plasma, urine, cerebrospinal fluid, liver and kidney.
Although SPME was initially applied only for the analysis of organic compounds from rather clean samples (air, water), it is now increasingly being used in bioanalysis (*in vitro* and *in vivo*) for the determination of proteins, polar alkaloids, pharmaceuticals and surfactants, because of its successful coupling with liquid SPME is an excellent alternative to classical methods for separating drugs and biomolecules from biological samples. Applications of SPME in bioanalysis can be divided into eight main groups, according to the type of analyte: toxicological analysis; forensic analysis; drugs of abuse; clinical chemistry; analysis of pharmaceuticals in biological samples; biochemical analysis; chemical analysis; and, analysis of natural products. In the past 10 years, the number of reports on the applications of SPME in bioanalysis increased from 10 to more than 300.

In vitro applications of SPME developed to date include the analysis of drugs from serum, plasma, whole blood, milk, urine, saliva and hair, by headspace, direct immersion SPME and in-tube SPME. Generally, the analysis of biological fluids is encumbered by the presence of dissolved biopolymers.
SPME methods can be optimized by selecting extraction temperatures that result in satisfactory sensitivity in an acceptable period of time. Applications of SPME in bioanalysis can be divided into eight main groups, according to the type of analyte: toxicological analysis; forensic analysis; drugs of abuse; clinical chemistry; analysis of pharmaceuticals in biological samples; biochemical analysis; semiochemical analysis; and, analysis of natural products.
Early *in vivo* investigations with SPME focused on fragrances emitted by insects, fungi and bacteria. These investigations were extended to biogenic volatile organic compounds emitted by animals and plants. In a more recent application, SPME technology was used for the in vivo analysis of intravenous drug concentrations in a living animal. It should be pointed out that some clinical chemistry SPME applications could also be considered *in vivo* bioanalysis.
Bioanalytical chemistry is playing an increasingly central role in the fields of academic and industrial science. It overlaps with a diverse range of disciplines, including biotechnology, biopharmaceuticals, and diagnostics. In this field SPME has also been applied to analysis of VOCs in human breath. In comparison to the sorption on solid sorbents followed by thermal desorption, SPME is faster, less complicated and easier to perform. As a sample preparation method of human breath SPME offers some advantages: it is highly sensitive (limited to compounds at a concentration of more than ~0.1 ppb); it uses short extraction time in the order of minutes; it does not require any other device connected to the gas chromatograph (e.g., cryotrap or additional desorber); it can easily be automated and is comparatively easy to use. Hundreds of papers have been published, in which the SPME method was applied to pre-concentrate VOCs in many gases matrices, such as indoor air, atmospheric air, in vivo emission air, and human biological samples including urine, blood, and breath. Recently SPME was developed to investigate aldehydes in lung cancer blood.

P.107 SECOIRIDOIDS IN OLIVE SEEDS OF *OLEA EUROPAEA* L. VAR. *SYLVESTRIS*: CHARACTERIZATION AT DIFFERENT MATURATION STAGES

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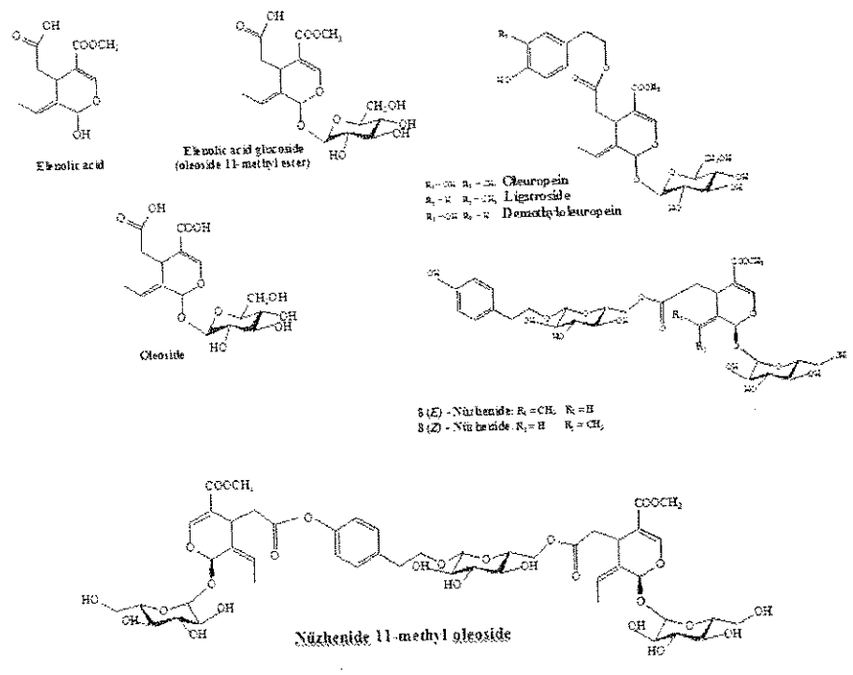
The olive tree belongs to the genus *Olea* of the Oleaceae family. Members of the Oleaceae family are characterized by the presence of secoiridoids (figure). These compounds have in their structures elenolic acid or its derivatives. Derivatives of elenolic acid include oleosides. Oleosides are not necessarily phenolic compounds but may include a phenolic moiety as a result of esterification. While phenolic acids, phenolic alcohols and flavonoids occur in many fruits and vegetables belonging to various botanical families, secoiridoids are present exclusively in plants belonging to the Oleaceae family which includes *Olea europaea* L.[1]. Oleuropein, ligstroside and nüzhenide are the most abundant secoiridoids in olives[1] and their structures are presented in figure 1 as well as other secoiridoids.
Some phenolic compounds have been identified in olive seeds like salidroside, nüzhenide, hydroxytyrosol, nüzhenide 11-methyl oleoside, oleuropein, tyrosol, and demethyloleuropein[1]. In this paper we report the analysis and chemical characterization of seed extracts from *Olea europaea* L. var. sylvestris using reversed phase HPLC, with electrochemical and diode array detection, hyphenated with mass spectrometry (electrospray ionization). Seeds were collected at different maturation stages and extracts were prepared using several solvents: acetone, methanol and water:methanol mixture.
As previously reported nüzhenide and nüzhenide 11-methyl oleoside among other 11-methyl oleosides are the most important compounds detected in olive seeds[1]. Methanolic and acetone extracts seem to present higher concentration of compounds and were chosen for further comparisons. As expected the chromatograms obtained at 240 nm (maximum wavelength absorption for these compounds) are more complex for the fruits in a less mature stage and some peaks detected present electrochemical activity and can be responsible for the antioxidant activity of the extracts.



F1 Chemical structures of secoiridoid compounds found in Oleaceae

Acknowledgements: Fundação para a Ciência e Tecnologia (FCT), REDE/1518/REM/2005.

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P.108 DEVELOPMENT OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ASSAY FOR 17α-METHYLTESTOSTERONE IN WATER SAMPLES AND SEDIMENTS OF TILAPIA AQUACULTURE

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The hormone 17α-Methyltestosterone (MT), a synthetically steroid hormone with potential anabolic and androgenic actions, is frequently used in many parts of the world to induce sex reversal in farmed tilapias. It is a simple and reliable way to produce all-male tilapia stocks, which consistently grow to a larger/more uniform size than mixed sex or all-female stocks. Adding MT to fish feed is the easiest and most convenient mode of hormone-induced sex reversal. The *Nile* tilapia and the other *Oreochromis* species dominate commercial tilapia farming. One of the main concerns with the use of MT is the fact that larger volumes of water used in the *Nile* tilapia aquaculture sites are discarded without previous treatment. Thus, uneaten and nonmetabolized food may release significant amounts of MT into the tank, exposing hatchery workers as well as aquatic and terrestrial organisms to the hormone.

Usually, high performance liquid chromatography (HPLC) and liquid chromatography-mass spectroscopy (LC-MS) methods have been used for the quantification of MT in biological matrices and pharmaceutical preparations[1-5] and not in water tanks and effluents.

In the present work, we report a HPLC method for determination of MT in water and sediments using testosterone as internal standard (IS). Hormone determinations were carried out using UV/Vis detection (245nm) and an RP-C18 column at room temperature with an isocratic mobile phase of acetonitrile:water (45:55, v/v) at a flow rate of a 1.0mL.min^{-1}. The analytical curve was linear (r^2=0,998) over a wide concentration range (100-2000μg.L^{-1}). Two procedures were used to extract MT from water and sediments samples. The recovery assay was performed in three levels of fortification 100, 600 and 2000μg.L^{-1}. The HPLC method developed in this study showed specificity, linearity in the working range, good precision and accuracy, making it very suitable for quantification methyltestosterone in water and sediments.

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P.109 DESENVOLVIMENTO DE UM MÉTODO EXPEDITO PARA A ANÁLISE DE 2,4,6-TRICLOROANISOLE EM CORTIÇA (*QUERCUS SUBER* L.) POR HS-SPME-GC-MS-MS

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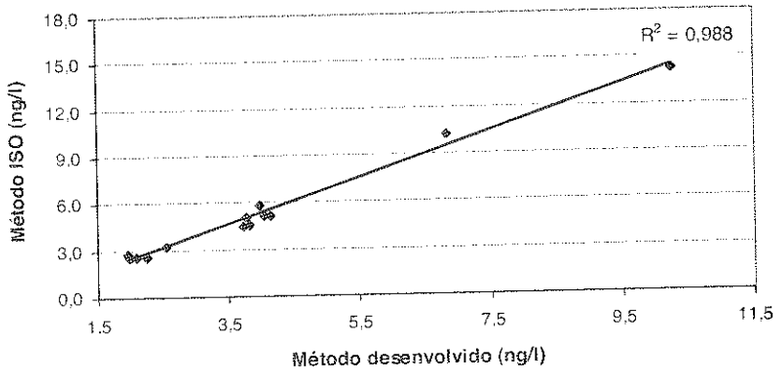
O 2,4,6-tricloroanisole (TCA) é um metabolito que tem sido detectado em vários materiais lenhosos, resultante do desenvolvimento de determinados microrganismos[1]. Este composto tem sido associado ao aparecimento de defeitos sensoriais em vários produtos, nomeadamente cortiça, vinho e água de consumo humano, o que tem motivado o desenvolvimento de inúmeras metodologias para a sua detecção e quantificação. O limite de percepção sensorial (LPS) do TCA é muito baixo (*ca.* 2ng/L), pelo que as metodologias analíticas devem apresentar uma sensibilidade adequada à sua quantificação em concentrações menores ou iguais ao respectivo LPS. Enquanto em matrizes líquidas a sua análise pode ser efectuada directamente, em matrizes sólidas, como a cortiça, com o objectivo que incrementar a eficiência extractiva procede-se a uma pré-extracção com uma fase liquida, a partir da qual se procede à análise[2,3].

O método de controlo da qualidade das rolhas de cortiça encontra-se normalizado internacionalmente[4], e inclui uma fase de maceração de um grupo de 50 rolhas em vinho modelo durante 24 horas, seguindo-se a extracção por HS-SPME e quantificação por GC-MS ou GC-ECD. Este método ISO apresenta algumas limitações associadas ao tempo necessário para a obtenção do resultado e o facto de apresentar um resultado médio de um grupo de 50 rolhas. Assim, pretende-se desenvolver um método expedito que permita a análise de TCA em produtos de cortiça, diminuindo significativamente o tempo de extracção e análise, de modo a dar resposta em tempo útil às necessidades de controlo de qualidade da produção nas indústrias, permitindo, ainda, obter dados de produtos individuais. Para o desenvolvimento desta metodologia, foram optimizados parâmetros que podem influenciar a eficiência extractiva por SPME, tais como, quantidade de cortiça, tempo de maceração da cortiça numa fase liquida a partir da qual decorrerá a SPME, e o modo de análise em 2 e 3 fases. Como termo de comparação, as amostras foram também analisadas pelo método ISO, utilizando o método da adição padrão.

De acordo com os dados obtidos, foram seleccionadas as seguintes condições de trabalho: 2 horas de maceração, para uma razão de massa de cortiça/volume de vinho modelo de 0,5g/20mL, num sistema de 2 fases (ausência de espaço de cabeça). Uma aliquota de 10mL do extracto é posteriormente transferida para o *frasco* de 20mL, onde será efectuada a extracção por HS-SPME. Para a gama de concentração de TCA testada (1 a 50ng/L), o método desenvolvido apresenta uma boa linearidade (r^2=0,9855), uma repetibilidade de 10%, avaliada como o coeficiente de variação do menor padrão (1ng/L) e reprodutibilidade de 14%, avaliada como o maior coeficiente de correlação de todos os níveis de concentração testados.

O método foi aplicado à análise de amostras obtidas numa indústria produtora de rolhas de cortiça, tendo-se obtido concentrações de TCA entre 2 e 14ng/L, determinadas pelo método desenvolvido e pelo método ISO. Os resultados obtidos pelos dois métodos estão altamente correlacionados (r^2=0,988, Figura 1). O método desenvolvido apresentou uma melhor reprodutibilidade comparativamente com o método ISO, apresentado CVs entre 3-13% e 7-30%, respectivamente. Além disso, verifica-se uma redução no tempo de análise de 25 para 3 horas (extracção + análise por GC).



F1 Correlação entre a quantificação obtida pelo método desenvolvido e o método ISO.

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tecnocroma
Inovação em cromatografia

CROMATOGRAFIA LÍQUIDA DE ULTRA-ALTA PERFORMANCE (UHPLC) EM QUALQUER SISTEMA DE HPLC

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Os desafios de produtividade, sensibilidade e qualidade de resultados apresentados pelos utilizadores de HPLC aos fabricantes têm produzido várias soluções instrumentais, que obrigam necessariamente a avultados investimentos na aquisição de novos equipamentos.
O objectivo deste seminário é apresentar, de forma simples e acessível, uma forma de incrementar dramaticamente a performance e a produtividade através de uma nova e altamente eficiente tecnologia de partícula, a qual determina melhorias significativas no tempo de análise, sensibilidade e qualidade de resultados em qualquer sistema de HPLC ou UHPLC.

TECNOLOGIAS MAIS PRODUTIVAS EM CROMATOGRAFIA

Guilherme A. Santos
ILC – Instrumentos de Laboratório e Científicos, Lda.

Durante 32 anos a ILC tem acompanhado a evolução tecnológica e disponibilizado os mais inovadores instrumentos e produtos para a cromatografia.
Para além da eficiência na separação, na sensibilidade e na automação, as tecnologias recentes em cromatografia têm também por objectivo um aumento da produtividade, com menos recursos e menor intervenção do utilizador, desde a preparação da amostra até à obtenção dos resultados.
Em cromatografia gasosa convencional, o sistema analítico Clarus 600 GC da PerkinElmer permite um aumento de produtividade de 30%, ao reduzir em 4 minutos o tempo de espera entre injecções, sem alteração de métodos previamente estabelecidos e com colunas convencionais. Na base deste desempenho está o forno inovador de parede dupla, com capacidade para arrefecer de 450 °C a 50 °C em menos de 2 minutos, e a pré-programação das lavagens do amostrador automático.
Em cromatografia líquida os sistemas analíticos de UHPLC Flexar da PerkinElmer, com operações até 15.000 psi, permitem uma produtividade até 10 vezes superior com um consumo 15 vezes inferior de fase móvel, reduzindo os custos com os solventes e o seu impacte ambiental.
Os sistemas de amostragem automática de headspace TurboMatrix HS da PerkinElmer, acopláveis a qualquer sistema de cromatografia gasosa, permitem a análise de compostos voláteis em matrizes líquidas e sólidas, isenta de preparação das amostras e extracções com solventes. A tecnologia de balanço de pressões garante uma precisão e sensibilidade superiores.
Ao nível da preparação de amostras para cromatografia líquida e gasosa, o sistema MEPS da SGE constitui um processo de micro-extracção que permite reduzir o tempo, da extracção à injecção, a minutos e numa única seringa, com volumes de solventes e amostra na ordem dos microlitros.

APPLICATION OF MULTI-RESIDUE SCREENING AND QUANTIFICATION IN FOOD MATRICES, USING LIQUID CHROMATOGRAPHY AND HIGH RESOLUTION ORBITRAP MASS SPECTROMETRY

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As a consequence of potential presence of pesticide residues and other contaminants in food and animal feeds, strong legislations have been established globally, to protect the consumer. The increasing list of compounds and matrices requires sophisticated techniques to be able to identify and quantify these residues and contaminants at a low level. The target compounds are mainly small molecules with molecular weights in the range of 100-1000 Dalton (majority m/z 200-400). Typical examples are residues of pesticides and veterinary drugs, natural contaminants, environmental and processing contaminants. Traditionally pesticide residues are detected with Liquid Chromatography and Tandem Mass Spectrometry (LC-MS/MS). This technique can quantify large numbers of analyte, however has limitations as it is considered to be targeted screening.

In the field of analytical chemistry there is a clear tendency to combine and expand existing analytical methods within the different contaminant classes and beyond. This is more efficient and more information can be obtained during the analysis[1].

Mass spectrometers based on the unique performance of established Orbitrap™ of mass analyzer are routinely achieving mass resolution up to 100.000 and mass accuracy below 2 ppm. Those parameters significantly improve the efficiency and accuracy of the residue screening methods and allow successful screening of various residues at even very low concentration levels. In this presentation, data will be shown the identification and quantification at high resolution at low levels (ppb) of pesticides in food matrices.

References
[1] H.G.J. Mol, P. Plaza-Bolaños, P. Zomer, T.C. de Rijk, A.A.M.Stolker and P.P.J.Mulder, Anal. Chem. 80 (2008) 9450-9459.

Dias de Sousa
INSTRUMENTAÇÃO ANALÍTICA E CIENTÍFICA, S.A.

ULTRA-HIGH-PERFORMANCE NANOLC-MS/MS ANALYSIS OF COMPLEX PROTEOMIC SAMPLES

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Introduction

Determination of the proteome and identification of biomarkers is required to monitor dynamic changes in living organisms and predict the onset of an illness. One popular method to tackle contemporary proteomic samples is called shotgun proteomics, in which proteins are digested, the resulting peptides are separated by high-performance liquid chromatography (HPLC), and identification is performed with tandem mass-spectrometry. Digestion of proteins typically leads to a very large number of peptides. For example digestion of a cell lysate easily generates 500,000 peptides. The separation of these highly complex peptide samples is one of the major challenges in analytical chemistry.

Methods

The main strategy to improve the efficiency of packed columns is either to increase column length or by decreasing the size of the stationary phase particles. However, to operate these columns effectively the LC conditions need to be adjusted accordingly. Naturally, the on-line coupling to MS systems has to be taken into account in the optimization process.

Results

Here, we report on the performance of nanoLC columns operating at ultra-high pressure. The effects of column parameters (particle size and column length) and LC conditions (gradient time, flow rate, column temperature) were investigated with reversed-phase (RP) gradient nanoLC. High-resolution LC-MS separations of complex proteomic peptide samples are demonstrated by combining long columns with 2 μm particles and long gradients. The effects of LC parameters on performance and the influence on peptide identification are discussed.

Innovative aspects

- Ultra-high performance nanoLC
- Combined optimization of stationary phase, column length and LC conditions.

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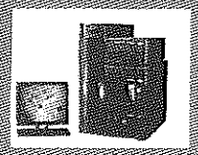
In recent years HPLC has been challenged with more complex and faster separation demands. The aim was, and is, to achieve a greater number of separations in a shorter time to increase sample throughput.

A trend towards smaller, sub-2μm particles provides greater efficiency although at the price of high backpressures, resulting in the development of UHPLC instrumentation. Fused Core™ particles in the Ascentis Express range of columns have been used successfully as an alternative to sub-2μm particles. They comprise a 2,7μm diameter particle with a 1,7μm solid (fused) core. Improved diffusion properties result in these particles exhibiting the same efficiency as a sub-2μm particle, but at a backpressure only slightly higher than regular 3μm particles, maintaining robustness.

This versatile and proven technology combines high efficiency and high speed with robustness for HPLC, LC/MS and UHPLC applications and as such, can be used on all types of instrumentation. With the advent of recent new bonding chemistries, such as the RP Amide, HILIC and Phenyl, alternative selectivities can be used to extend and optimise the types of separations now possible.

This seminar will describe the technology, properties and areas of applications for this technology.



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