



Abstract Book



MS2010
4th Portuguese Mass Spectrometry Meeting
FCUL, 13-15 December 2010



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2. Protection of Nature by pragmatic, technical, scientific and anti-fundamentalist concepts, with particular application in the County of Alcochete, but not limited to this area.
3. Protection of the rights and of the general welfare of animals, with particular interest in the defence of birds and of their natural surroundings, with a special focus on the Tagus Estuary and on the County of Alcochete, but not limited to these areas.

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Welcome

The Organizing Committee cordially welcomes all participants and accompanying persons to Lisbon for the meeting “4th Portuguese Mass Spectrometry Meeting – MS2010” which is to be held at the Faculty of Sciences of the University of Lisbon.

Organizing Committee

António Ferreira	DQB-FCUL
Carlos Cordeiro	DQB-FCUL
Carlos Manuel Borges	DQB-FCUL
Gonçalo Costa	CQB
Paulo Madeira	CQB
Pedro Alves	CQB
Pedro Duarte Vaz	CQB

Scientific Committee

Ana Varela Coelho	ITQB
M. H. Florêncio	DQB-FCUL
Francisco Amado	DQ-UA
Joaquim Marçalo	ITN
M. Conceição Oliveira	CQE-IST UTL
M. Filomena Duarte	DQB-FCUL
M. Tereza Fernandez	DQB-FCUL

General Information

The meeting “4th Portuguese Mass Spectrometry Meeting – MS2010” will take place at Faculty of Sciences – University of Lisbon (FCUL), Campo Grande, Lisbon, Portugal, starting on the 13th December and ending on the 15th December.

Registration and Sessions will take place in building C6. Registration will take place in the atrium of building C6 and Sessions in the Room 6.2.56.

Lunch and Coffee Breaks

Lunch and Coffee Breaks will be served in the atrium of building C6 and are included in the registration fee.

Scientific Program Schedule

Time	Monday, 13th	Tuesday, 14th	Wednesday, 15th	
09:00 – 09:30	Registration & Posters Up	Invited Lecture Jaap Boon	Thermo Lecture Cláudia Martins	
09:30 – 10:00		Coffee & Posters	Coffee & Posters	
10:00 – 10:30		Bruker Lecture Jens Fühser	Advion Lecture Mark Allen	
10:30 – 11:00		OC 7	OC 15	
11:00 – 11:30		OC 8	OC 16	
11:30 – 11:45		Lunch & Posters	OC 17	
11:45 – 12:00			OC 18	
12:00 – 12:15			Awards & Closing	
12:15 – 12:30		Lunch	Lunch	
12:30 – 13:00				
13:00 – 13:30	Opening	Invited Lecture A.Ferrer Correia	Lunch	
13:30 – 14:00	Invited Lecture Albert Heck	OC 9		
14:00 – 14:30	OC 1	OC 10		
14:30 – 15:00	OC 2	OC 11		
15:00 – 15:15	OC 3	OC 12		
15:15 – 15:30	OC 4	OC 13		
15:30 – 15:45	Coffee & Posters	OC 14		
15:45 – 16:00		Coffee & Posters		
16:00 – 16:15	OC 5	RNEM Meeting Posters		
16:15 – 16:30	OC 6	MS Round Table		
16:30 – 17:30	Meeting Dinner			
17:30 – 17:45				
17:45 – 18:00				
18:00 – 18:30				
18:30 – 19:00				
19:00 – 19:30				
19:30 – 20:00				
20:00 –				

Scientific Information

Presentation Preview Room

Speakers are kindly asked to contact the secretariat for their presentation preview 3 h before their lecture/oral communication.

Posters

Posters will be displayed during the whole conference in the hall of C6. Authors are required to display their own posters on the boards provided on Monday, before the opening session.

Conference Awards

One Oral Communication and one poster will be awarded a prize, courtesy of Fundação Jacqueline Dias de Sousa.

Scientific Program

The Scientific Program consists of invited lectures, oral communications and poster presentations.

Monday, December 13, 2010

14:00 Opening Ceremony

Chairperson: M. Helena Florêncio

14:30 IL1 Native Mass Spectrometry in Viral Structural Biology and
Molecular Systems Biology
Albert Heck

Chairperson: A. Varela Coelho

15:30 OC1 Protein glycation in familial amyloidotic polyneuropathy
Gonçalo da Costa

15:45 OC2 Insights in the peptidomics of a functional food prototype
- the buttermilk fermentation product by *Lactobacillus*
delbrueckii subsp. *Lactis*
Kamila Koci

16:00 OC3 Proteomic Analysis of an Interactome for Long-Form
AMPA Receptor Subunits
Bruno Manadas

16:15 OC4 Searching candidate biomarkers for Familial Amyloidotic
Polineuropathy (FAP) in whole saliva
Ana Guerreiro

16:30 Coffee & Posters

Chairperson: M. Conceição Oliveira

17:30 OC5 Phenolic compounds from *Helichrysum obconicum*:
HPLC-DAD-ESI-MSn characterization
Sandra Gouveia

17:45 OC6 Metabolomic Approaches for Food Origin Authentication
Ramon Díaz

20:00 Meeting Dinner

Tuesday, December 14, 2010

Chairperson: M. Helena Florêncio

9:00 IL2 Role of mass spectrometry in the investigation of paintings
Jaap Boon

10:00 Coffee & Posters

Chairperson: Carlos A. Cordeiro

10:30 IL3 Molecular Imaging by Ultra-High Resolution Mass Spectrometry
Bruker Lecture
Jens Fühser

Chairperson: M. Filomena Duarte

11:30 OC7 On the way to predict the antioxidant capability of a phenolic compound?
J. P. Leal

11:45 OC8 Evaluation of glycerophospholipids oxidation profile by LC-MS and MS/MS
Maria do Rosário Domingues

12:00 Lunch & Posters

Chairperson: M. Tereza Fernandez

14:00 IL4 Mass Spectrometry of Oligonucleotide Noncovalent Adducts
A. J. Ferrer-Correia

Chairperson: F. Amado

15:00 OC9 Valproic acid treatment increases ω -oxidation of fatty acids in vivo
Marco Moedas

15:15 OC10 Development and Validation of an Analytical Method for the Detection of Mycotoxins in Drinking Water Matrices
J. P. Ferreira

15:30 OC11 The effect of different HILIC stationary phases on the ionization efficiency of some antiepileptic agents
Hassan Alhazmi

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|-------|------|--|
| 15:45 | OC12 | Quantification of four immunosuppressant drugs by Liquid Chromatography-Tandem Mass Spectrometry using Direct Injection
Maria Joao Barreira |
| 16:00 | OC13 | The influence of different processing methods on the TAG profile of Oils by Maldi-FT-ICR-MS
Pedro Caetano Alves |
| 16:15 | OC14 | Cucurbituril complexes and aggregates in the gas phase
José P. Da Silva |
| 16:30 | | Coffee & Posters |
| 17:30 | | RNEM Meeting & Posters |
| 18:30 | | MS Round Table |

Wednesday, December 15, 2010

Chairperson: J. Marçalo

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|-------|-----|--|
| 9:00 | IL5 | Dodging False Negatives with High Resolution Mass Spectrometry: The Benzophenone Case
Thermo Lecture
Claudia P. B. Martins |
| 10:00 | | Coffee & Posters |

Chairperson: Carlos A. Cordeiro

- | | | |
|-------|-----|--|
| 10:30 | IL6 | From Non Covalent Interactions and Lipid Profiling to Top Down/Bottom Up Beer Protein Analysis and Direct NanoESI MS/MS from Tissue. A Varied Journey on a Single Platform
Advion Lecture
Mark Allen |
|-------|-----|--|

Chairperson: P. Duarte Vaz

- | | | |
|-------|------|--|
| 11:30 | OC15 | The uranium disulfide dication in the gas phase
Joaquim Marçalo |
|-------|------|--|

11:45	OC16	Identification of Early Synthetic Dyes in a Persian Carpet by HPLC-DAD-MS ⁿ Maria da Conceição Oliveira
12:00	OC17	Phytochemical Characterization of Polyphenols by HPLC-PDA-ESI/MS ⁿ : an approach to Structure-Activity Relationship Gustavo Costa
12:15	OC18	Application of Theoretical calculations in Mass Spectrometry: Semi-empirical vs. DFT Paulo J. Amorim Madeira
12:30		Awards & Closing
13:00		Lunch

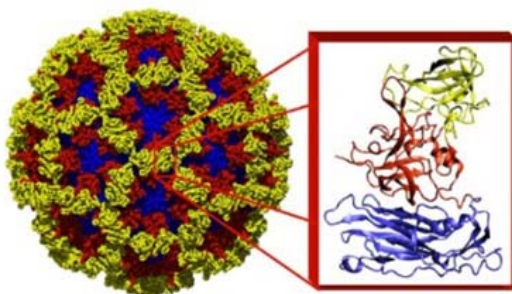
Invited Lectures

Native Mass Spectrometry in Viral Structural Biology and Molecular Systems Biology | IL1

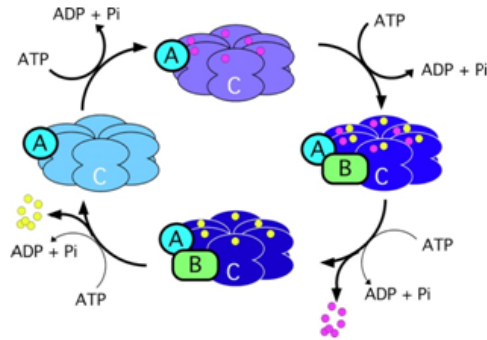
Albert J. R. Heck

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The development of electrospray ionization coupled to mass spectrometry has enabled the analysis of very large intact protein complexes even when they are held together by non-covalent interactions. Together with equally spectacular advances in mass spectrometric instrumentation a new field has emerged, termed native protein mass spectrometry that focuses on the structural and functional analysis of the dynamics and interactions occurring in protein complexes. Native mass spectrometry allows the topological investigation of intact protein complexes with high sensitivity and a theoretically unrestricted mass range. This unique tool provides complementary information to established technologies in structural biology.



Using such mass spectrometry based technologies we have begun to study the biophysical properties of virus structure and assembly, focusing on the important HBV and norovirus human pathogens. The masses of HBV (3-4 million Da) and the noro virus (over 10 million Da) create significant challenges on any structural method to characterize these particles with considerable resolution, are some of the largest particles ever analyzed by high resolution mass spectrometry. For these viruses we have been able to probe several distinct assembly intermediates, which are generally low abundant and therefore difficult to monitor by other techniques. Several biophysical parameters such as self-assembly, stability and shape of the virus particles were monitored as well as their dependence on pH, ionic strength and temperature.



Moreover, we used native mass spectrometry to probe the regulation of a cyanobacterial circadian clock, which is regulated by the interplay of protein assembly and protein phosphorylation, involving three proteins; KaiA, KaiB and KaiC. Native mass spectrometry was used to measure the real-time assembly properties of this self-organizing system and provided the missing link needed to construct a full theoretical molecular model to describe the rhythm, stability and regulation of this clock.

Role of mass spectrometry in the investigation of paintings.

IL2

Jaap J. Boon

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JAAP Enterprise for MOLART Advice, Amsterdam, NL.

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Mass spectrometry plays a crucial role in the correlative analytical studies of painted works of art both for the analysis of pigment and dye composition as well as for the study of binding media, glazes and varnishes. It plays a unique role as tool for the organic chemistry of paintings as a direct analytical method using laser desorption ionization and secondary ion mass spectrometric microscopy of paint cross sections. Microsamples analysed by direct temperature resolved MS using filament evaporation and pyrolysis give information about composition and physical consistency. Nano spray ESIMS using QTOF and FTICRMS proves to be a very strong method for profiling of soluble polar fractions in oil paints and proteinaceous media. Identification of compounds is furthermore achieved by MSMS, GCMS and LCMS.

Many of these approaches have been explored during the MOLART and De Mayerne NWO research programmes. In more recent years, we have been integrated MS techniques with correlative chemical microscopy (Imaging FTIR , SEM-EDX and XTM) in 2D and 3D studies of paint samples.

An overview will be given of the many possible ways that MS can be used to solve complex questions in traditional old master and modern paintings.

Molecular Imaging by Ultra-High Resolution Mass Spectrometry

IL3

Jens Fuchser

FTICR Division BrukerDaltonics Bremen, Germany

The development of electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) were key steps in mass spectrometry to enable this analytical tool to be applied to Biosciences and Biomedicine in proteomics and small molecule analysis. Since more than two decades many different applications evolved based on these ionization techniques. One more recent application is MALDI imaging, where the spatial distribution of molecules in tissues from a wide range of Biospecimens is of interest. This has been spearheaded by MALDI-TOF instruments. However, in the area of small molecules matrix interferences hamper the performance of MALDI TOF.

Fourier Transform Ion-Cyclotron Resonance Mass Spectrometers (FTICR MS) are capable to unequivocally determine the molecular formula of a given compound and to establish its identity, based on accurate mass measurements, isotope pattern and MSMS analysis. Due to the unrivalled resolving power of FTICR no matrix interferences disturb the analysis. This gives rise to applications such as drug molecule measurements using physiological doses where multiple targets can be measured in one run. Therefore, FTICR mass spectrometry can be seen as molecular microscopy giving deeper insight into the spatial distribution of small molecules directly within tissues.

Examples of the latest developments and applications of this cutting edge technology coupled to ultra-high resolution mass spectrometry will be presented.

A J Ferrer-Correia, Graça Santana-Marques, Catarina I V Ramos

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The importance of DNA and other forms of oligonucleotide compounds is well known and needs not be stressed. The basic form of the DNA strand and its double-helix structure, while it is the more common in Nature, is not, by far, the only one found in living organisms. Several aspects of these structures will be briefly discussed, with particular relevance for the study of the formation of noncovalent complexes with oligonucleotides,.

It is now known that Electrospray Ionization Mass Spectrometry (ESI-MS) can be used to detect intact noncovalent complexes, and partly elucidate their structure. In particular, this has been, and continues to be, important in the study of DNA and RNA targeting compounds, which is often relevant for the study of drug mutagenic properties or their use in cancer therapy. This is valid not only for the association of single or double strand oligonucleotide structures, but also of higher order ones¹. The small molecules (usually referred to as “drugs”) that form noncovalent compounds with oligonucleotides are mainly classified as intercalators, when their binding occurs by partially inserting the molecule between consecutive base-pairs, or minor-groove binders, when they form noncovalent bonds with the bases that are exposed in the minor-groove. In this case, some form-specific adaptation is necessary, besides chemical compatibility, for the binding to take place. Other forms of binding will be discussed, such as insertion², or external binding. Mass Spectrometry has also been used in association with other techniques to find details of this association, such as when chemical probes are used in complex structure elucidation³.

The number of ligands attached to the DNA strands and the number of strands that are connected together and remain in the complex can be determined by ESI-MS (and indeed other forms of ionization MS). Their decomposition pathway can be important, as can the strength of the association. For that reason, the value of the binding constants is important, and ESI-MS is one good method of performing its determination. This is possible, since it is established that, under controlled conditions, the gas-phase relative intensities of the different players of the dissociation process are representative of the liquid-phase abundances of the same compounds.

The higher order association of DNA strands gives rise to a variety of interesting structures, some of which form important noncovalent connections to small molecules. Several aspects of this association are discussed and different types of interactions between drugs and oligonucleotides^{4,5} are considered.

One example of this interaction is found when a [d(TGGGGT)₄] quadruplex is stabilised by a group of cationic porphyrins with different number of charges. The

quadruplex is initially stabilized by the addition of three ammonium ions, and the formation of $[d(TGGGGT)_4 + 3NH_4 + \text{porphyrin}]^{n-}$ adduct ions for all the porphyrins shows that these compounds provide an additional stabilization of the quadruplex structure. Under ESI-MS conditions, the number of charges of the porphyrin induces different fragmentation patterns: for lower charged porphyrins the ligand itself breaks away from the complex, while for three and four positive charges the ammonium cations are lost from the complex (in neutral form). The proposed complex structure is that of a ligand binding externally to the four-stranded quadruplex⁶.

1. V. Gabelica, in *Mass Spectrometry of Nucleosides and Nucleic Acids*, ed. J. Banoub and P. Limbach, *CRC Press*, 2010, Ch. 8
3. Zeglis, B. M. *et al*, *Chem. Comm.*, 2007, 4565
2. Mazzitelli, *et al*, *Anal. Chem.* 79(12), 4636, 2007
4. Cuihong Wan *et al*, *Int. J. Mass Spectrom.*, 283 (2009) 48
5. V. Gabelica *et al.*, *J. Am. Chem. Soc.* **129**(2007), 895
6. C.I.V. Ramos *et al.* *J. Mass Spectrom.* **40**(2005), 1439

Dodging false negatives with high resolution mass spectrometry: the benzophenone case

IL5

Claudia P.B. Martins^a, Héctor Gallart-Ayala^b, Oscar Núñez^b, Encarnación Moyano^b,
Maria T. Galceran^b

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In November 2005, the Italian Food Control Authority detected the photoinitiator 2-isopropylthioxanthone (2-ITX) in baby milk at concentrations ranging from 120 to 300 µg/L [1]. It is known that others UV-ink photoinitiators, such as benzophenone (BP), can be used as a component of packaging material. Since this compound can migrate into food, it became crucial to develop an analytical procedure that enables its determination. The latest methodology reported in the literature makes use of Liquid Chromatography coupled to Tandem Mass Spectrometry using Triple Quadrupole (QqQ) analysers due to its specificity and sensitivity when operating in Selected Reaction Monitoring (SRM) mode [2-4].

In this work it is reported the analysis of BP in several food products by UHPLC-QqQ-MS/MS. In compliance to what has been described in the literature, BP was detected at low µg/L levels in most of the samples. However, when using ion ratio calculation ($\pm 20\%$ as in 2002/657/EC [5]) to confirm its presence, most of the samples were concluded as negative. In order to investigate this further, the same samples were analysed using high resolution mass spectrometry (HRMS) making use of an Orbitrap analyser. Another isobaric compound (m/z 183.0917) was found to co-elute with BP (m/z 183.0804) which led to a mass accuracy > 5 ppm for this compound, at a mass resolution lower than 50,000. When working at a mass resolution of 50,000, BP was positively identified in most of the samples with a mass accuracy below 5 ppm. Furthermore, high resolution tandem mass spectrometry (HR-MS/MS) has been applied for an unequivocal confirmation of this compound at low ppb (µg/L).

- [1] 2005 Chronology of Withdrawal of Nestlé and Other Liquid Milks www.ibfan.org/site2005/abm/paginas/articles/arch_art/416-1.doc
- [2] A. Gil-Vergara, C. Blasco, Y. Pico, *Anal. Bioanal. Chem.* 389 (2007) 605.
- [3] G. Sagratini, G. Caprioli, G. Cristalli, D. Giardina, M. Ricciutelli, R. Volpini, Y.T. Zuo, S. Vittori, *J. Chromatogr A* 1194 (2008) 213.
- [4] D-X. Shen, H-Z. Lian, T. Ding, J-Z, C-Y. Shen, *Anal. Bioanal. Chem.* 395 (2009) 2359.
- [5] European Union Decision 2002/657/EC 17 August 2002, Off. J. Eur. Comm. L221 (2002) 8.

From Non Covalent Interactions and Lipid Profiling to Top Down/Bottom Up Beer Protein Analysis and Direct NanoESI MS/MS from Tissue. A Varied Journey on a Single Platform

IL6

Mark Allen, Reinaldo Almeida and Kees Vlask

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A for a variety of workflows and analyses are described using the nanoESI Chip-based TriVersa NanoMate system;

1) Non covalent interactions

Titration of Protein and Ligand followed by MS measurement of the resulting non covalent complex using automated infusion for the determination of dissociation constants.¹

2) Shotgun discovery lipidomics

A workflow enabling quantitative lipid screening by direct infusion following Folch or methyl-tert-butyl ether (MTBE) solvent extraction.²

3) Bottom up and top down analyses for the identification and characterisation of proteins during the brewing process.

The intact mass measurement and primary sequence determination was performed by online UPLC-MS/MS with simultaneous fraction collection and subsequent infusion of intact and digested protein.

4) Direct Liquid Extraction and Surface Analysis (LESA) of drugs and metabolites from blood spots and tissue.

A method is described where a liquid micro junction surface sampling probe is used to extract the analyte directly from a blood spot or tissue and analysed by chip-based nanoESI.³

1. S. Zhang; C.K. Van Pelt, D.B Wilson, *Anal.Chem.* **75** (2003), 3010.

2. V. Matyash, G. Liebisch, T.V. Kurzchalia, A. Shevchenko, D. Schwudke, *J. Lipid Re.* **49** (2008), 1137

3. V. Kertesz, G.J Van Berkel, *J. Mass Spectrom.* **45** (2010), 252.

Oral Communications

Protein glycation in familial amyloidotic polyneuropathy

OC1

Gonalo da Costa¹, Ricardo J. Gomes², Ana Guerreiro¹, Daniel Fonseca¹.
Helia Morais³, Estela Monteiro³, Eduardo Barroso³, Ana V. Coelho.^{2,4}, Ana
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Transthyretin amyloidosis (ATTR) is an autosomic dominant degenerative disease characterized by the formation of amyloid deposits mainly composed of transthyretin (TTR). The disease has been associated with TTR single amino acid point mutations in TTR. TTR point mutations reduce the tetramer stability, leading to its dissociation and subsequent monomer unfolding, aggregation and amyloid formation¹. However, this model fails to explain why non-mutated TTR forms amyloid, causing systemic senile amyloidosis and the wide variation of disease onset time, even in homozygotic twins. Therefore, mutations only accelerate the amyloidotic behavior and other factors beyond genetic ones must be involved. We observed that TTR quaternary structural stability is compromised in TTR mutation carrying individuals. To exclude the hypothesis that the observed minor stability of the dimer could be due to the differential amount of the mutated TTR expressed, we developed a method for the relative quantification of TTR forms using MALDI-FTICR-MS^{2,3}. Here we show that FAP patients present higher levels of argpyrimidine-modified serum proteins. Moreover, fibrinogen, found to be one of the main TTR interacting protein in serum and recently found to be a chaperone, shows increased glycation in FAP patients. We demonstrated that upon glycation its chaperone activity decreases.

1 – Quintas *et al.* [Tetramer dissociation and monomer partial unfolding precedes protofibril formation in amyloidogenic transthyretin variants.](#) J Biol Chem. 2001 276(29):27207-13

2 - da Costa et al. Identification and quantitative analysis of human transthyretin variants in human serum by Fourier transform ion-cyclotron resonance mass spectrometry - Amyloid. 2009 Dec;16(4):201-7

3 - Da Costa et al. *A non-invasive method based on saliva to characterize transthyretin in familial amyloidotic polyneuropathy patients using FT-ICR high-resolution MS; PROTEOMICS - Clinical Applications.* Volume 4 Issue 6-7, Pages 674 – 678

**Insights in the peptidomics of a functional food
prototype - the buttermilk fermentation product by
Lactobacillus delbrueckii subsp. *lactis***

OC2

Kamila Koci^a, Ana Binetti^b, Renata Soares^a, Eliana Haag^b, Gabriel Vinderola^b, Maria do Céu Costa^c, Jorge Reinheimer^b and Ana Varela Coelho^a

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A spray-dried product of buttermilk fermentation naturally enriched of potentially bioactive peptides was analysed using LC-MS/MS and MALDI-MS/MS. In a previous study, mucosal immunomodulation was observed when the fermented product was administrated to BALB/c mice¹. The aim of this work was to identify the peptides responsible to this immune activity. Due to high sample complexity (47% lactose, 29% protein, 12% fat, 7% ash) sample preparation optimization was carried out prior to MS analysis. Four sample treatments were tested in order to obtain peptide enriched extracts. The best results were achieved using 3 successive steps: 1) Extraction of lipids using chloroform; 2) Extraction of peptides using 50% MeOH; 3) Protein precipitation with 10% trichloroacetic acid. LC-MS/MS (Surveyor HPLC/LCQ-Thermo Finnigan) analysis was performed with the supernatant obtained from step 3. For the selective detection of peptides, BCA reaction was performed with 5 sub-fractions collected during the LC-MS run. The LC gradient was modified to improve the separation of the 4 peptide-containing fractions and 1 peptide (IPP) was identified. To overcome sensibility issues and improve fragmentation patterns MALDI-MS/MS (4800 MALDI TOF/TOF-ABI) were performed for 15 fractions using 2 approaches. First, MS/MS spectra for the 10 most intense peaks of each fraction were collected. Subsequently, a more selective MALDI-MS/MS analysis was performed focused on the peaks of interest. Obtained MS/MS patterns were compared with protein sequence databases using ProteinPilotTM search engine. This screening identified 9 proteolytic peptides originating from β -casein and α -lactalbumin bovine proteins. Some of the identified peptides are in good agreement with reported β -casein bioactive peptides released during dairy fermentation using *Lactobacillus*². A guided immune active assay of prepared purified fractions is on its way.

ACKNOWLEDGMENTS: The authors gratefully acknowledge support from CYTED-Project Novel ProBio.

1. P. Burns, F. Molinari, A. Beccarìa, R. Pácz, C. Meinardi, J. Reinheimer and G. Vinderola . *J. Appl. Microbiol.* **Vol 109** (2010), 1370-1378.
2. E.M. Hebert, G. Mamone, G. Picariello, R.R. Raya, G. Savoy, P. Ferranti and F. Addeo *Appl. Environ. Micorbiol.* **Vol 74** (2008), 3682–3689.

Proteomic Analysis of an Interactome for Long-Form AMPA Receptor Subunits

OC3

Bruno Manadas, Sandra D. Santos, Carlos B. Duarte, Ana Luísa Carvalho,

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Glutamate receptors of the AMPA-type mediate fast excitatory synaptic transmission in the central nervous system and play key roles in synaptic plasticity. The binding of these receptors to a variety of proteins is known to regulate their targeting to the synapse and consequently to modulate synaptic strength, as well as to modify receptor characteristics. In this study, a proteomic screening was conducted in order to identify new binding partners for GluR4 AMPA receptor subunit. Immunoprecipitation of GluR4 and associated proteins was performed using rat cerebellum lysates and an heterologous systems overexpressing GluR4 AMPA receptor subunit. Isolated immuno-complexes were resolved by 1-D SDS-PAGE, and analyzed by liquid chromatography tandem mass spectrometry (LCMS/MS). This approach led to the identification of several interactors, most of which are novel AMPA receptor partners, namely, cytoskeleton proteins, motor proteins, RNA processing proteins which are part of neuronal RNA granules, and kinases, among others. This study unravels new constituents of the macromolecular complex of long-form calcium-permeable AMPA receptors.

S. Santos, B. Manadas, *et al*, J Proteome Research, V9 (2010) p1670

Searching candidate biomarkers for Familial Amyloidotic Polineuropathy (FAP) in whole saliva

OC4

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Familial amyloidotic polyneuropathy (FAP) is a neurodegenerative disease, related with amyloid deposition of transthyretin (TTR) in different tissues. In FAP this protein presents point mutations, which can destabilize the tetrameric native structure of this protein and lead to the formation of unfolded monomers with great predisposition to form amyloid deposits¹.

FAP diagnosis is a very important part of an adequate and prompt treatment, and nowadays the search of pre-symptomatic biomarkers is vital. As potential biomarkers we considered advanced glycation end-products (AGEs). These were found recently in FAP patients' sera² and TTR amyloid deposits³.

During this work we were able to observe distinct electrophoretic profiles in some saliva proteins of FAP patients and via tandem mass spectrometry (MS/MS) analysis we also identified new proteins in saliva, such as α -synuclein, which presented an altered electrophoretic profile as well. The majority of the identified proteins were filtered from serum and these were mostly underexpressed. Through the combination of western-blotting and MS/MS we found that some of these proteins were glycosylated, indicating that the filtering process could be affected.

The identification of differentially expressed and glycosylated saliva proteins in FAP individuals, was accomplished, and in the future these FAP biomarkers could be considered as pre-symptomatic biomarkers.

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Phenolic compounds from *Helichrysum obconicum*: HPLC-DAD-ESI-MSⁿ characterization

OC5

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The flora from Madeira Archipelago is well known for its uses in the traditional folk medicine. *Helichrysum obconicum* DC. E (*Asteraceae*) is an endemic species and its aerial parts are used as herbal teas as digestive, stomachic and for intestinal diseases.¹

Hydroxycinnamic acid conjugates, mainly mono- and di-caffeoylquinic acid derivatives, were found to be the major components of the alcoholic extracts; some flavonoid derivatives were also detected in small amounts. Their separation and identification was achieved using a LC-DAD/ESI-MSⁿ method, with special emphasis on MSⁿ fragmentation. Some of these compounds were reported in other *Helichrysum* species.^{2,3} However, the presence of di- and tricaffeoylshikimic acid isomers was reported for the first time, and the spectra of these compounds were mainly characterized by the presence of a [caffeoylshikimic acid-H]⁻ ion at m/z 335 (Figure 1). A lamiridosin-di-O-hexoside, an unusual component in *Asteraceae* species, was also detected (Figure 2).

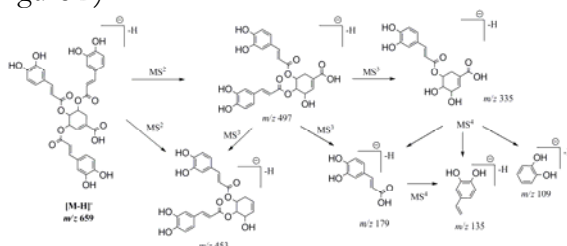


Figure 1. Proposed fragmentation pathway for tri-O-caffeoylshikimic acid.

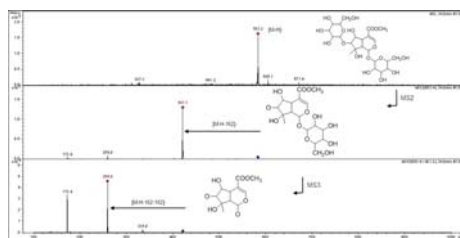


Figure 2. ESI-MSⁿ negative mode of lamiridosins-di-O-hexoside.

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There is an increasing interest by consumers for high quality food products with a clear geographical origin. These products are promoted and suitable analytical techniques are needed for their quality control [1]. Thus, the development of new and increasingly sophisticated techniques for the authentication of food products continues rapidly with increasing consumer awareness of food safety and authenticity issues. Food authentication is also of concern to food processors that do not wish to be subjected to unfair competition [2].

Normally, food authenticity methods are focused on the determination of just few compounds that characterize the sample, as some metals with ICP-MS [3] or on searching for common adulteration substances, as tartaric acids in orange juice [4]. Alternatively, metabolic profiling is presented as a powerful tool for correct origin discrimination in food as it has been widely used in metabolomic approaches [5]. To this aim, HNMR and mass spectrometry are the techniques of choice.

In this work, a hybrid quadrupole time-of-flight mass spectrometer (QTOF MS) coupled to UHPLC has been used for biomarkers identification for correct authentication of valencian region (Spain) oranges. Orange crop is an important component in the Valencia economy and it is well-known that frauds exist. Thus, differentiation from cheaper Argentinean and South African oranges has been carried out using XCMS application and multivariate analysis to UHPLC-(Q)TOF MS data. Thus, Citrusin D, present in sweet oranges has been found as one the possible markers to distinguish the origin.

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On the way to predict the antioxidant capability of a phenolic compound?

OC7

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Phenolic compounds, such as vitamin E, chromanol, trolox and flavonoides, possess therapeutic and pharmacological properties due to their well known antioxidant action.¹⁻⁴ Free radicals, triggering chain-reactions, are determinant in developing diseases such as atherosclerosis, coronary heart diseases and certain types of cancer. Substituted phenols, through their antioxidant action are able to break these chain reactions, reacting with free radicals. The inactivation reaction of free radicals is accomplished by hydrogen atom transfer (HAT mechanism) from the antioxidant to the free radical or by sequential proton loss and electron transfer (SPLET mechanism). In both mechanisms, O-H bond dissociation enthalpy (BDE) is a key property and a measure of the antioxidant capacity of phenolic compounds since the weaker the bond the easier the hydrogen atom transfer. Consequently, it has been extensively investigated in both, gas and condensed phases.

In the gas-phase, it can be determined by the combination of the acidity for a given phenol, the electron affinity of the related phenoxyl radical, and the well known ionization energy of the hydrogen atom.

Till now our work is focused on the determination of phenolic compounds acidity and their dependence on structural features. An overview of the study evolution is proposed.

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Evaluation of glycerophospholipids oxidation profile by LC-MS and MS/MS

OC8

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It is well known that under oxidative conditions, phospholipids undergo structural modifications and the oxidized phospholipids products formed are being implicated as mediators in several pathophysiologic conditions, such as atherosclerosis, neurodegenerative disorders, among others. These modifications result in alterations of the membrane's properties and functions, such as fluidity and permeability, loss of membrane integrity leading to apoptosis, alterations in cell signalling pathways, increase in platelet aggregation and inflammatory response. The oxidation of phospholipids can generate a wide variety of products that result mainly from modification of polyunsaturated fatty acid chains. These modifications can be induced, *in vivo*, by enzymatic or non-enzymatic reactions (induced by radicals generated by radiation or metal catalyzed systems). Detailed identification of the oxidation products generated under oxidative conditions produced by Fenton reaction of phosphatidylcholines (PC), phosphatidylethanolamines (PE), and cardiolipin (CL) have been performed using liquid chromatography tandem mass spectrometry (LC-MS and MS/MS). Oxidation was induced by the hydroxyl radical generated under Fenton reaction conditions (H_2O_2 plus Fe^{2+}). Oxidation products identified included long chain products formed by insertion of oxygen atoms with formation of products bearing keto, hydroxyl and peroxy moieties, and short chain products formed by the cleavage of unsaturated fatty acid chains. LC-MS/MS allows the identification of a huge number of oxidation products of PC, PE, and CL which will be the basis for their sensitive identification in biological samples using a lipidomic approach.

Acknowledgements

Thanks are due to Fundação para a Ciência e a Tecnologia and COMPETE for funding to project PTDC/QUI- BIQ/104968/2008, QOPNA and RNEM.

Valproic acid treatment increases ω -oxidation of fatty acids *in vivo*

OC9

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Introduction: Potential mitochondrial off-targets for valproic acid (VPA), which is used worldwide as an antiepileptic drug, have been thoroughly investigated by our group, namely on fatty acid oxidation (FAO), respiration and oxidative phosphorylation¹. These effects on energy metabolism account for the major underlying mechanisms of microvesicular hepatic steatosis and liver failure, frequently associated with this drug². **Aims:** To study *in vivo* the potential effect of VPA on the plasma concentration of free fatty acids (FFA), using an animal model. Thus, this work aims to gain a new insight on the balance between mitochondrial FAO and microsomal FAO, induced by a subchronic treatment with this drug. **Methods:** Analysis of FFA was performed in plasma samples from Wistar Rats, subjected to subchronic treatment with VPA (100 mg/kg during 15 days, n=9) or saline solution (controls, n=7). The qualitative and quantitative analysis of around 30 individual FFA (up to C18 carbon chain length), was achieved using GC/MS with SIM detection mode according to a published procedure³. **Results:** The present work enabled a detailed monitoring of the changes in plasma fatty acids derived from therapeutic treatment with the drug, including *in vivo* adaptation mechanisms during the subchronic treatment. The experimental GC/MS conditions enabled the complete resolution of different FFA (monocarboxylic, dicarboxylic, saturated, unsaturated and hydroxylated derivatives). No significant changes were observed between the studied groups concerning short-, medium- or long-chain saturated fatty acids. Nevertheless, the quantitative FFA profiling in samples of both animals groups revealed a statistically significant ($p < 0.05$) increase in levels of the dicarboxylic hexanedioic (adipic) acid. **Conclusions:** The accumulation of dicarboxylic acids in VPA-treated rats is strongly suggestive of a fatty acid loading on ω -oxidation pathway as a consequence of the drug interference with mitochondrial β -oxidation. The present results are consistent with our recent findings concerning the inhibitory effect of VPA metabolites (mainly valproyl-CoA and Δ^4 -valproyl-CoA) on the hepatic isoform of carnitine palmitoyl transferase 1 (CPT1A)⁴.

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Development and Validation of an Analytical Method for the Detection of Mycotoxins in Drinking Water Matrices

OC10

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B-type Trichothecenes and Zearalenone are the two most common groups of mycotoxins found in food and feed as result of fungal infection by *Aspergillus*, *Penicilium*, and *Fusarium*. Those toxic metabolites may cause serious acute or chronic adverse health effects on animals and humans. Zearalenone and its metabolites are endocrine disruptors with estrogenic properties; B-type Trichothecenes are responsible for mycotoxicoses in farm animals and induce vomiting, fever, and nausea in humans. Several occurrence studies have been performed using cereal and food matrices but very few studies were conducted to date using water matrices. The low concentration of these compounds in real water samples requires the use of selective and sensitive techniques for their quantification. The analytical technique used in this study was solid phase extraction followed by liquid chromatography coupled with tandem mass spectrometry an electrospray source operating in negative mode and a triple quadrupole as analyser. Chromatographic and mass spectrometry conditions were optimized in order to achieve low limits of detection. For a better sensitivity and selectivity a Multiple Reaction Monitoring mode was used for the quantification, using the best transitions obtained for each compound. Validation parameters were determined and the optimized method efficiently allows the quantification of B-type Trichothecenes and Zearalenone. Further studies are currently being conducted to improve the solid phase extraction conditions and achieve higher concentration capability in order to achieve lower limits of detection.

Acknowledgments: This work was funded by Fundação para a Ciência e Tecnologia (PTDC/AAC-AMB/108303/2008 and REDE/1518/REM/2005 for the LC-MS/MS equipment).

The effect of different HILIC stationary phases on the ionization efficiency of some antiepileptic agents

OC11

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A fast, sensitive and selective method for the detection and quantification of carbamazepine (CBZ) and vigabatrin (VGB) in plasma is described using high-performance liquid chromatographic separation with tandem mass spectrometry. Samples were purified using liquid–liquid extraction and separated on a Phenomenex Luna HILIC 3 μ (2 \times 150 mm) column with a mobile phase consisting acetonitrile/ ammonium formate (5mM, pH 3.5) (75:25, v/v) at a flow-rate of 0.4 ml/min. Detection was performed by a triple quadrupole model G6410A mass spectrometer in the MRM mode using electro spray ionisation (ESI), monitoring the transition of the protonated molecular ion for carbamazepine at m/z 237 and vigabatrin at m/z 130 to the predominant ions of m/z 194 and 71, respectively. The mean recovery was 81% for carbamazepine and 100% for vigabatrin. The calibration curve was linear over the concentration range 250–8000 ng/mL for carbamazepine and 15–960 ng/ml for vigabatrin. The limits of detection for CBZ and VGB in human plasma were 5.43 ng/mL and 3 ng/mL, respectively. The limit of quantification for both analytes in human plasma were 18.2 ng/ml and 10 ng/ml, respectively.

Quantification of four immunosuppressant drugs by Liquid Chromatography-Tandem Mass Spectrometry using Direct Injection

OC12

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Quantification of immunosuppressant drugs in whole blood is essential for therapeutic drug monitoring (TDM) of individuals after organ transplantation. Liquid chromatography tandem-mass spectrometry (LC-MS/MS) is an efficient technology for routine determination of immunosuppressants in whole blood.

The purpose of this study was to implement a rapid and simple LC-MS/MS method for simultaneous determination of immunosuppressants cyclosporine A (CsA), everolimus (EVER), sirolimus (SIR) and tacrolimus (TCR) in whole blood, using cyclosporine D (CsD) and ascomycin (ASC) as internal standards.

This method is based in the iMethod Test¹ for immunosuppressants designed by Applied Biosystems and analytical performance was tested using six levels of whole blood calibrators and three levels of quality controls. Equipment used for method implementation was an API 3200 triple quadrupole, with ESI source.

Linearity of the LC-MS/MS method was evaluated after application of several statistical tests, such as residual analysis, correlation coefficients, coefficient of variation of the method, RIKILT test² and Mandel test (ISO 8466-1). The method was linear for all target compounds within the range of six calibrators used. Provisional limits of quantification (LOQ) are 48,4 ng/mL for CsA, 2,6 ng/mL for EVER, 2,7 ng/mL for SIR and 2,5 ng/mL for TCR. Repeatability was studied using whole blood samples spiked with known amounts of the four immunosuppressants at three concentration levels. Relative standard deviation values for repeatability were all below 15% for EVER and SIR and below 10% for CsA and TCR. Method accuracy was evaluated through participation in the proficiency testing scheme, organized by United Kingdom National External Quality Assessment Service (UK NEQAS). The participant's results are converted into a 'z-score'³ that reflects the actual accuracy achieved. "Z-scores" between -2 and 2 are considered satisfactory. Z-score values from participation in UK NEQAS Scheme ranged from -2,4 to 2,0 for CycA (mean value 0,9; n= 14), from -0,3 to 1,8 for EVER (mean value 1,0; n=8), from -1,8 to 1,9 for SIR (mean value 0,9; n= 19) and from -1,0 to 2,1 for TCR (mean value 1,3; n= 17).

This LC-MS/MS method was used for quantification of everolimus blood levels in 35 clinical samples from patients receiving single therapy. Implemented method assures sensitive and selective simultaneous determination of CsA, EVER, SIR and TCR in 100 µL of whole blood. The pretreatment is extremely simple and rapid allowing handling 20 to 50 samples per day.

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This work forms part of ongoing PhD research: “Molecular aspects of drying processes of oil paints formulated 1890-1940.” The paper will report on preliminary results based on a model using a combined approach: investigating documentary sources, complemented by chemical analysis of freshly pressed oils and oil mixtures appropriate to the period.

The focus will be on finding chemical markers for the oils, and oil Paint from historically accurate paint reconstructions (based on evidence found in the documentary sources). Newly introduced oils of the period include Perilla, Safflower, Cottonseed, Hempseed and Sunflower. They will be prepared and characterized for identification purposes in paint and in mixtures with the traditional oils, linseed, walnut and poppyseed. The ultimate aim is to develop the identification of oils and oil mixtures in oil paintings from the period 1890-1940.

In this first qualitative approach several oils and different processing methods, like Water Washing, heating to 150° C and 300° C, and addition of dryers with and without heat, applied to the raw oils, were selected.

Differences in the spectra are characteristic of processes involved and we aim to relate the chemical changes of initial TAG (TriAcylGlycerides) profile to those processes.

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Cucurbit[n]urils (CB n , $n=5-8$ and 10) are an emerging family of synthetic macrocyclic hosts that have the potential to encapsulate positively charged and neutral organic molecules.¹ These macrocycles function as molecular containers forming 1:1 and 1:2 host-guest inclusion complexes with association constants typically several orders of magnitude higher than that of cyclodextrins in aqueous media. Electro-spray ionization mass spectrometry (ESI-MS) has assumed an important role in investigations of host-guest complexes and other non-covalent complexes.² ESI-MS can transfer CB n complexes intact to the gas phase, allowing this way the access to their intrinsic properties i.e., without solvent effects.^{2,3} We will demonstrate that ESI-MS is a powerful technique to directly observe and identify CB n complexes as well as their aggregates (Figure 1) in the gas phase.

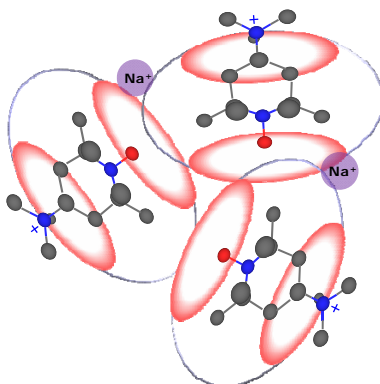


Figure 1. Trimer of 4-(N,N,N-trimethylammonium)-2,2,6,6-tetramethylpiperidyl-N-oxyl@CB8 complexes.

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The uranium disulfide dication in the gas phase | OC15

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The dipositive uranyl ion, UO_2^{2+} , is particularly important in the chemistry of uranium. It is a highly stable hexavalent species ubiquitously found in condensed phases. We have previously carried out the first synthesis of the bare UO_2^{2+} ion in the gas phase using FTICR/MS.¹

We are not aware of any previous work on the sulfur analogue of uranyl. We report here the first synthesis and theoretical description of bare US_2^{2+} in the gas phase. We have used FTICR/MS to study the reaction of U^{2+} ions with COS in which we observed the sequential, efficient formation of US^{2+} and US_2^{2+} . Theoretical studies of US_2^{2+} have shown that, in contrast to linear uranyl, $\text{O}=\text{U}^{2+}=\text{O}$, it has a strongly bent structure at both B3LYP and CCSD(T) levels of theory (Figure 1), with the linear isomer being some 100 kJ/mol higher in energy.

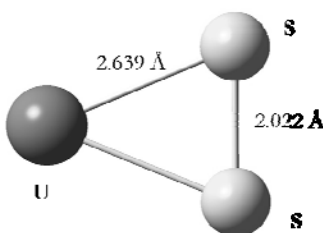


Figure 1. Lowest energy isomer of US_2^{2+} as computed at the CCSD(T) level of theory.

The gas-phase ion chemistry of other singly and doubly charged uranium sulfides is currently under investigation.

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Acknowledgements: This work was supported by Fundação para a Ciência e a Tecnologia («Ciência 2007» Programme), by the CNRS, and by the U.S. Department of Energy at LBNL (Contract DE-AC02-05CH11231). The COS was a generous gift from Dr. João M.A. Frazão from ISEL, Lisbon.

Identification of Early Synthetic Dyes in a Persian Carpet by HPLC-DAD-MSⁿ

OC16

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Standards of 65 early synthetic dyes, both pure dyestuffs and dyed wool, were analysed in order to develop and evaluate an HPLC-DAD-MSⁿ database that can be applied in the field of art, namely to identify and characterize early synthetic dyes in historical textiles. Several chromatographic and mass spectrometry parameters were optimized in order to fully characterize 65 dyes from eleven chemical families.

The analytical methodology was applied on a Persian Carpet (T107) performed by an Armenian weaver owned by the Gulbenkian Museum.

The carpet dated from the 20th century present an Ottoman Turkey style composed by palmettes enriched with golden-metal wrapped silk threads. Nine samples were collected from the back side of the carpet which is protected from light. Samples from five different colours were selected: 2 yellows, 1 green, 4 reds, 1 blue and 1 beige. Extracts of each fiber were analysed by HPLC-DAD-MSⁿ. It was possible to conclude that the carpet was dyed only with synthetic dyes and different colours were obtained with a mixture of at least two synthetic dyes. The yellow and beige colours were obtained with a mixture of Azo Flavine 3R (CI 13090) and Orange IV (CI 13080) and the red colours were obtained with Scarlet N for Silk (CI 15635) and probably with Cotton Scarlet (CI 27290). The blue colours were obtained with synthetic dyes from the triarylmethane family, namely with Patent Blue V (CI 42051). The green colour was obtained with a mixture of the yellow and blue synthetic dyes previously mentioned

Acknowledgements: We thank Museum Conservation Institute-Smithsonian Institute for having granted us the Schwepp's collection and Museu Calouste Gulbenkian for the access to the Persian carpet. Fundação para a Ciência e Tecnologia for financial support under the scope of the Project *Unveiling the secrets of molecules with colour and history*, POCI/QUI-QUI/099388/2008 and Contract REDE/1502/REM/2005.

Phytochemical Characterization of Polyphenols by HPLC-PDA-ESI/MSⁿ: an approach to Structure-Activity Relationship

OC17

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Plant polyphenols are well-known natural antioxidants, and recent studies have reported that they play an important role in prevention and treatment of oxidative-related disorders, such as inflammatory diseases and cancer.

In this work, a phenol-rich fraction (EAF) from *Agrimonia eupatoria* L. dry aerial part was studied. HPLC-PDA-ESI/tandem MS results revealed phenolic acids derivatives (*p*-coumaric and ellagic), flavonol and flavone glycosides, and monomers and oligomers of flavan-3-ols (proanthocyanidins).

Some key features in molecular structure of flavonoids seem to be crucial to a meaningful anti-inflammatory potential: 4-oxo functional group and C2-C3 double bond at C-ring, 5- and 7-OH on A-ring and also OH functions on B-ring¹. Polymerization degree of proanthocyanidins plays a definitive role in their bioactivity, since trimers or higher oligomers are more effective than monomers, in inhibiting NO production, which plays an important feature in inflammatory response. On the other hand, catechol moiety increases antioxidant/anti-radical activity, leading to presume that catechin-type proanthocyanidins are the most active².

The phenolic profile established and the antioxidant and anti-inflammatory activities verified in this work corroborate the traditional use of *A. eupatoria* in inflammatory-related pathologies, since generous amount of the cited compounds were found in the phenol-rich fraction studied.

Acknowledgements: This work was supported by FEDER/COMPETE (FCOMP-01-0124-FEDER-011096) and FCT, by the project PTDC/SAU-FCF/105429/2008 and the PhD fellowship SFRH/BD/46281/2008. A special thank to LEM/UC integrated in RNEM of Portugal for the HPLC/MS analyses.

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From the determination of thermochemical properties of MALDI matrices¹ to the prediction of gas-phase vibrational spectra², theoretical calculations have been used in mass spectrometry studies for quite some time. Recently, density functional theory (DFT) calculations were used to understand and propose mass spectrometric fragmentation pathways.³

Even though DFT calculations provided good results, they are quite time consuming when compared, for example, with semi-empirical calculations. These quite inexpensive computational methodologies have been successfully used in several electrospray ionization mass spectrometry studies⁴⁻⁷ to address fragmentation mechanisms as well as probable protonation sites. Nevertheless, to the best of our knowledge, there are no systematic studies comparing the performance and reliability of DFT with semi-empirical calculations when applied to mass spectrometry specific problems. As such, we present preliminary results of the protonation site for a series of aromatic amines (anilines) using both DFT calculations and semi-empirical calculations.

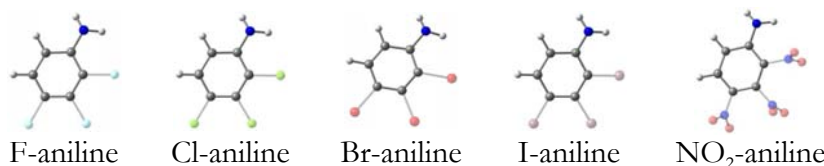


Fig. 1: Structure of anilines tested in this study. Shaded atoms refer to isomers.

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Posters

Analysis of Organometallic Compounds by Using Electrospray Quadrupole Ion Trap Mass Spectrometry | P01

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Electrospray ionisation (ESI) is a ``soft`` ionisation technique that produces gas phase molecular ions of analytes. In ESI, the energy applied in ion source is ``gentle`` enough to sustain molecular ions and prevents fragmentation, thus the molecular weight of an analyte is readily determined. The use ESI process in the mass spectrometry field has greatly facilitated the identification and structural elucidation in molecular material studies, of which one important application is to explore ESI in determination and structural elucidation of organometallic molecules. Herein, a Bruker Esquire 6000 ESI ion trap multistage mass spectrometry (IT/MSⁿ) was applied to explore the determination of series of new synthetic organometallics from our molecular material group in CQM.

Several important factors work together for a successful ESI-MS analysis of organometallic compounds: the compound stability during dissolution in spray solution, sample introduction and ESI ionisation processes, the compatibility of spray solvents with analytes and with ESI, the ionisation conditions and mass spectrometer capacity. In the present ESI-IT/MSⁿ experiments on organometallics, attentions were put on the selection of spray solvents, ESI conditions and IT/MSⁿ capacity for obtaining meaningful ESI-IT/MSⁿ data for organometallics, particularly hydrophobic compounds.

This poster presents the preliminary results obtained from ongoing ESI - MS analyses using different non - aqueous spray solvents (and/or solvent mixtures), including polar (aprotic and protic) and nonpolar solvents: methanol, formic acid, isopropanol, acetonitrile, acetone, dichloromethane, tetrahydrofuran, chloroform (trichloromethane) and hexane. The results showed that mixed aprotic and protic solvent system were more suitable for ESI experiments on organometallics with different polarities, particularly hydrophobic compounds, although still difficult for a successful ionisation of some neutral organic compounds in this study.

The mass spectrometer used in this study is a quadrupole ion trap (QIT) which consists of three electrodes that, when held at appropriate potentials, cause the formation of a trapping pseudo-potential well so that charged particles (ions) can be confined or stored for a long periods of time¹. It is one of the most sensitive mass spectrometers and itself can function as a tandem mass spectrometer (MSⁿ)¹. For these new organometallic compounds, their masses fall in different scan ranges (0 - 3000 and 3000 - 6000) of the QIT/MS, thus the advantages and limits of using QIT in organometallic compound analysis were explored in terms of mass ranges of scanning, mass accuracy and resolution.

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Acknowledgements: We gratefully acknowledge the support of the Portuguese Science Foundation (FCT) through the Pluriannual base funding (CHEM-Madeira-Funchal-674), the research projects PTDC/QUI/64202/2006, PTDC/CTM/098451/2008 and by the NMR and MS Portuguese Networks (REDE/1517/RMN/2005, REDE/1508/REM/2005).

Synthesis and identification of Glutathione Trimethyl Arsonium cation complex by LC-ESI-MSⁿ and HR-ESI-MS

P02

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The protein-Arsenic bond interacting with reactive metabolites such as Sulfur-adenosyl-methionine (SAM) and Methyl-cobalamin (Met-CB12), form intermediate complexes that may have a key role in the Arsenic chemistry of living systems. Particularly, it seems reasonable to assume that the DMAs^{III}GS complex (DMAG) (m/z 412, $[M+H]^+$), formed from Cacodylate (DMA), Glutathione (GSH) and SAM, may have such a role.

In this work the non-enzymatic synthesis of Glutathione Trimethylarsonium cation, (GS-As⁺(CH₃)₃), (m/z 426), from DMAG, obtained *in vitro*, and SAM, apparently through the classical Challenger methylcarbonium attack have been achieved.

Data from full scan and MSⁿ spectra, as well accurate mass measurements obtained by electrospray ionization mass spectrometry (ESI-MS), strongly suggest that such complex (GS-As⁺(CH₃)₃)(m/z 426), was formed in the experiment.

This intermediate, was apparently methylated further by the complex GSH-methylCB₁₂, leading to the synthesis of Tetramethylarsonium ion As⁺(CH₃)₄ (m/z 135) (TETRA) and to the formation of Trimethylarsine oxide (TMAO) (m/z 137, $[M+H]^+$), both detected by LC-ESI-MSⁿ and high performance liquid chromatography (HPLC). Such pathway would explain the accumulation of Tetramethylarsonium ion in the absence of dimethylarsinate, among the arsenic species taken up and produced by polychaetes¹.

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Tandem Mass Spectrometry In The Study Of Nickel(II) Complexation By Nitrobenzyl Azides And As A Probe For Isomers Differentiation

P03

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Azides are versatile compounds extensively applied in synthesis and in industrial applications¹. Their interaction with metals proved to be important in enzyme inhibition² and in the preparation of imido metal complexes³ which due to their reactivity, are relevant in catalysis. The 3'-azido -3'-deoxythymidine (AZT) was the first antiretrovirus used in AIDS treatment due to its ability to inhibit reverse transcriptase.³ Human carbonic anhydrase,² is inhibited by coordination of the protein metal centre to N₃⁻. However, organoazides are better tolerated than inorganic under physiological conditions, and they are relatively inert in the biological milieu⁴. Thus, the use of organoazides as enzyme inhibitors could be beneficial. Our studies are focused in understanding the interaction of organic azides with metals. Our previous studies on aliphatic azides were extended to azides including an aromatic ring. Electrospray Ionization Mass Spectrometry was the technique of choice to investigate both the complex formation and the complexation site. The interaction of three isomers, *ortho*, *meta* and *para* of the nitrobenzylazides with Ni (II) has been studied. Nitrobenzyl azides establish complexes with Ni(II) which present various ligands and different stoichiometries. For *ortho*, singly and doubly charged ions were detected. For *meta*, only doubly charged ions were observed. The *para* showed mixture of the behaviours of the *ortho* and *meta*. The interaction of the three isomers with Ni (II) is different enough to allow their characterization.

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Protein Identification in FTMS: a new scoring system and data analysis platform | P04

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With the development of MS instruments capable of sub ppm mass accuracy, the score of protein identifications based on peptide mass fingerprinting should be reformulated to take full advantage of enhanced MS data quality. Furthermore, with greater accuracy and higher resolution, it is reasonable to expect that better scoring schemes could be developed to distinguish and identify peptides from protein mixtures.

In this work, we present a new scoring system for protein identification by peptide mass fingerprinting that relies on high-accuracy data, and a web platform to support its implementation.

The scoring method takes into account differences between measured and theoretical data, peptide distinctiveness in the database and protein sequence coverage, using a largest relative gap threshold to filter false positives.

The method was tested on several sets of simulated data from protein mixtures. These were designed to cover a range of increasing identification difficulty, with data generated at FTMS accuracy, and at different simulated levels of sequence coverage. The results were compared to the Mascot¹ scoring system as a reference.

Results show that the developed method performs better than Mascot in general, and significantly better for mixtures of up to 20 proteins. As expected, the system's ability to correctly identify proteins is highly correlated with peptide mass accuracy and protein sequence coverage.

The developed web application (alpha stage) runs on a web server with database support, providing a front-end for protein identification. It allows users to group MS datasets into projects and perform analysis with multiple search criteria, saving results for later analysis. The application was designed to store whole organism proteome data and to perform all the computational operations needed for the set up of identification databases, like virtual digestions and inclusion of common residue modifications. The search for post-translational modifications is also being implemented.

1. MASCOT, mass spectrometry search engine to identify proteins from primary sequence databases.F.S. [<http://www.matrixscience.com>]

Characterization of A-type and B-type Proanthocyanidins of *Laurobasidium lauri* by RP-HPLC-ESI(-)- MS/MS

P05

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Laurobasidium lauri (Geyl.) Jülich 1982 (accepted name) syn. *Exobasidium lauri* Geyl. 1874 is a parasitic fungus of old laurel trees. The alcoholic extract of this fungus has been used in traditional medicine as haemostatic, antirheumatic and analeptic. This extract showed a high antioxidant capacity towards the usual *in vitro* assays. To trace the antioxidant origin, total phenol and flavonoid contents as well as the polyphenolic profile were determined. Reversed phase high performance liquid chromatography with electrospray ionization mass spectrometry detection (negative mode) was performed on the defatted alcoholic extracts, revealing flavan-3-ols with a degree of polymerization 1-5, as the main components.

Both A-type and B-type proanthocyanidins (PA) were detected and their structure characterization was performed using MSⁿ, which provides reliable evidence on interflavan linkage.

A-type PAs have attracted increasing attention, despite being present in only a few dietary sources in very small amounts, because of their potential health benefits.^{2,3}

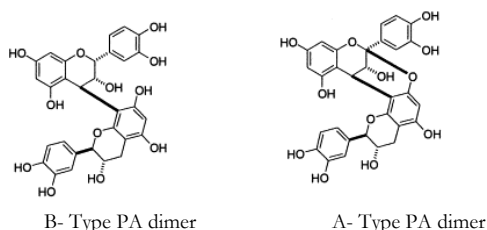


Figure 1. B-type and A-type proanthocyanidin dimers.

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Quality control of the detection and characterization of ignitable liquid residues by GC-MS | P06

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Arson is a crime that is difficult to investigate for two key reasons: physical evidence at the crime scene is, often, scarce, as in the majority of cases it has been destroyed by fire; linking a particular suspect to the physical evidence that has been found is difficult. Fire investigation brings together factual information present at a crime scene and science, through the forensic analysis of collected evidence.

Therefore, upon suspicion that arson may be the cause of fire, forensic laboratories become an extremely valuable tool. Accordingly, samples are collected as part of the fire investigation, and subsequently analysed with the purpose of ascertaining the presence or absence of traces of combustion accelerants. In parallel, they provide, on the one hand, relevant information for the investigation process and, on the other hand, they turn a material proof (fire debris) into scientific evidence that is valid in court. To enable that transformation to take place, results obtained should be reliable.

The Scientific Police Laboratory (LPC) of the Polícia Judiciária (PJ) (Portuguese Police) is responsible for collecting scientific evidence of the use of accelerants in arsons. When analyzing fire debris, LPC uses an ASTM Norm based on visual comparison of total ion chromatograms to reference ignitable liquid (IL) chromatograms followed by the extracted ion profiling, and, finally, the target compound analysis.

This work presents a strategy for the quality control of the performance of the measurement procedure, namely: (1) sample preservation on transport from scene to laboratory, (2) sample extraction efficiency and (3) GC-MS repeatability. Three internal standards with different volatility added in different stages of the measurement procedure allow checking analyte losses by volatility or liquid extraction partition and GC-MS response repeatability. The proposed strategy is robust to the lack of repeatability of sample extraction and dilution using variable binary mixtures of solvents and avoided masking mass spectrometric evaluations with the variability of these mass transference steps.

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Liquors are alcoholic beverages usually prepared by addition of plant materials, such as fruits or leaves, to ethyl alcohol and/or distillates of agricultural origin, in a process known by maceration.¹ Liquors usually possess high content of polyphenolic compounds, a feature associated with antioxidant properties and several health benefits.²

We recently characterized new myrtle berry liquors prepared using fig fruit distillates.³ In this work we identified and quantified the main polyphenolic components of several new pomegranate liquors by LC-MS. The role of the addition of the pomegranate fruit peel on the final content of punicalagin and other tannins will be described in detail (Figure 1).

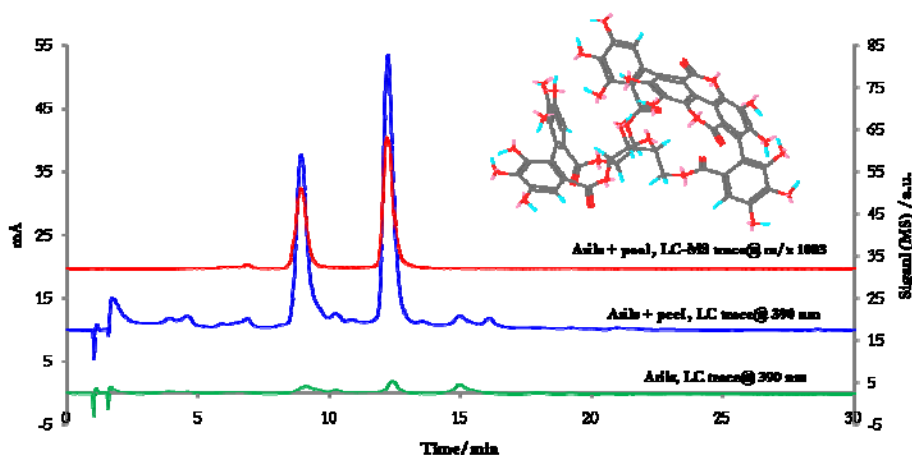


Figure 1. LC and LC-MS traces of liquors prepared with and without pomegranate peel. The inset shows the structure of punicalagin (m/z 1083).

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There is a decrease in radius of the lanthanide ions Ln^{3+} on crossing the series from La to Lu, commonly referred to as the lanthanide contraction. With the increase in the effective nuclear charge as the atomic number increases and as a result of the poor screening of the 4f electrons, the Ln^{3+} ions contract.¹

We used ESI/QITMS to study ethanol solutions of lanthanide (and yttrium) nitrates which readily yielded, in the negative ion mode, species of the type $[\text{Ln}_x(\text{NO}_3)_{3x+1}]^-$. When we examined solutions in which pairs of lanthanides ($\text{Ln} = \text{La}, \text{Ce}, \text{Pr}, \text{Eu}, \text{Tb}, \text{Ho}, \text{Tm}, \text{Lu}$) and also Y were present in equimolar amounts, we observed that the species formed by the smaller ion of the pair systematically showed the higher relative intensity in the spectra (Figure 1). Additionally, MS/MS experiments, in which mixed species $[\text{Ln}^1\text{Ln}^2(\text{NO}_3)_7]^-$ were collisionally fragmented in the QIT, resulted in the preferential formation of the $[\text{Ln}(\text{NO}_3)_4]^-$ ion for the smaller lanthanides (Figure 2).

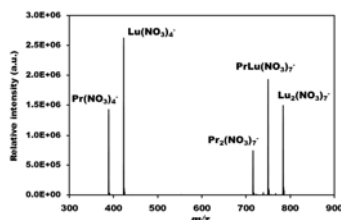


Figure 1. ESI/QITMS(-) spectrum of a $\text{Pr}(\text{NO}_3)_3/\text{Lu}(\text{NO}_3)_3$ 1:1 solution.

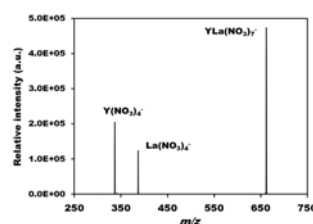


Figure 2. CID spectrum of $[\text{YLa}(\text{NO}_3)_7]^-$.

These observations can be accounted for by the higher charge density of the smaller (although heavier) metal ions, which will confer an increased stability to the nitrate anions formed directly from solution or by fragmentation in the QIT. Y^{3+} confirmed this effect as, although significantly lighter than the Ln^{3+} , it is similar in size to Ho^{3+} (Figure 2).

These and other cluster species derived from simple lanthanide (and actinide) salts are currently under investigation by ESI/QITMS.

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Acknowledgements: The ESI/QITMS was acquired with the support of the Programa Nacional de Reequipamento Científico of Fundação para a Ciência e a Tecnologia (FCT) and is part of RNEM-Rede Nacional de Espectrometria de Massa also supported by FCT. Support from the EC through ACSEPT (FP7-Euratom/CP-2007-211267) and ACTINET-13 (FP7-III-232631/JRP17) is gratefully acknowledged.

Diabetes Mellitus type-1 patient's saliva peptidomics | P09

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Saliva is a biological fluid which plays important physiological functions, constituted by about 40-50 % of small proteins and peptides, usually identified as salivary peptidome that varies in terms of quantity and quality due to several factors including the health status. Hyperglycemia is a key event in type 1 diabetes (T1D) that affects several cells. Signs and symptoms of this pathology can occur in the oral cavity. Alterations in salivary composition may explain the high susceptibility to oral infections, as well as dental and periodontal diseases accompanied by problems in healing.

The present work focused on peptidomic analysis of saliva peptides fraction of controls (n=5) and T1D (n=5) and in the evaluation of the cleavage site frequency of identified peptides. The proteolytic activity of whole saliva samples were accessed by zymography. Our results showed an increment on identified peptides, suggesting an exacerbation of proteolytic activity in T1D patient's oral environment corroborated by a higher proteolytic activity in whole saliva. The protein fragments observed mainly belong to proteins with structural activity and from extracellular matrix. The cleavage site frequency analysis in diabetics showed statistically significant differences in motifs compatibles with proteases like matrix metalloproteinases (MMPs), cathepsins and bacterial proteases. Alterations associated to collagen were observed, namely to type I, as well as augmented proteolytic activity of MMP-9, in accordance with cleavage site analysis. Our data highlight that saliva peptidomics may be useful for early identification of alterations in T1D patient's oral health, related with changes in proteolytic activity.

Bidentate N-donor adducts of trioxidorhenium(VII) and chloridodioxidomolybdenum(VI) – A ESI/QITMS study

P10

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The catalytic activity in olefin epoxidation of $[\text{Re}(\text{CH}_3)_3\text{O}_3\text{L}]$ and $[\text{MoO}_2\text{Cl}_2\text{L}]$ complexes (Figure 1), where L is a bidentate N-donor ligand, is strongly influenced by the electronic and steric nature of L.^{1,2}

The relative stability of a variety of bidentate N-donor adducts of $[\text{ReO}_3]^+$ and $[\text{MoO}_2\text{Cl}]^+$ (as models for the neutral complexes) was investigated by ESI/QITMS, in an attempt to probe the technique as a simple alternative to standard methods (spectrophotometry, NMR) of solution characterization. Experiments with acetonitrile solutions of Re_2O_7 or MoO_2Cl_2 and equimolar amounts of pairs of the ligands showed that their relative stability followed the order 4,4'-dimethyl-2,2'-bipyridine > 2,2'-bipyridine > 3-(2-pyridyl)pyrazole >> 6,6'-dimethyl-2,2'-bipyridine in both cases. The stability of the Mo(VI) adducts appeared to be higher than that of the Re(VII) adducts, as indicated by experiments with solutions containing the two metals in equimolar amounts and one L ligand. These results are in overall agreement with spectrophotometric studies of the stability of N-donor adducts of $[\text{Re}(\text{CH}_3)_3\text{O}_3\text{L}]$ and $[\text{MoO}_2\text{Cl}_2\text{L}]$.³

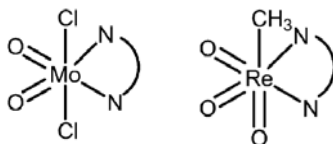


Figure 1. $[\text{Re}(\text{CH}_3)_3\text{O}_3\text{L}]$ and $[\text{MoO}_2\text{Cl}_2\text{L}]$ complexes.

The investigation of the relative stability of monodentate N-donor adducts of $[\text{ReO}_3]^+$ and $[\text{MoO}_2\text{Cl}]^+$ by ESI/QITMS is currently under way.

1. M.-D. Zhou, K.R. Jain, A. Günyar, P.N.W. Baxter, E. Herdtweck, F.E. Kühn, *Eur. J. Inorg. Chem.* (2009) 2907.
2. A. Günyar, F. E. Kuhn, *J. Mol. Cat. A-Chem.* **319** (2010) 108.
3. A.M. Al-Ajlouni, A. Günyar, M.-D. Zhou, P.N.W. Baxter, F.E. Kühn, *Eur. J. Inorg. Chem.* (2009) 1019.

Acknowledgements: The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT), POCI 2010 and FEDER for funding (research project PTDC/QUI/71198/2006). The ESI/QITMS was acquired with the support of the Programa Nacional de Reequipamento Científico of FCT and is part of RNEM-Rede Nacional de Espectrometria de Massa also supported by FCT. B.M. is thankful to FCT for a post-doc grant.

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In nuclear science there is a current interest in the coordination chemistry of lanthanides (Ln) and actinides (An) with N-donor ligands, as related to Ln/An separations within advanced nuclear fuel cycles.¹ MS can be a useful probe of the complexation of ligands and this has already been explored with systems involving the lanthanides.²

The coordination of the new neutral tetradentate ligand L = bis[3-(2-pyridyl)pyrazolyl]methane (Figure 1) towards Ln(III) (Ln = La, Ce, Pr, Eu, Tb, Ho, Tm, Lu) and Y(III) in solution was studied using ESI/QITMS. Experiments with ethanol solutions of the metal nitrates and the ligand in different M/L ratios showed that the 1:1 stoichiometry was preferred, although 1:2 species could also be observed (Figure 2). CID of both species in the QIT indicated that the second ligand is not bonded in a tetradentate fashion, as $[M(NO_3)_2L_2]^+$ easily yielded $[M(NO_3)_2L]^+$, while fragmentation of the nitrate to form $[MO(NO_3)L]^+$ was observed for $[M(NO_3)_2L]^+$.

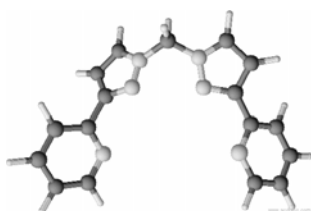


Figure 1. Bis[3-(2-pyridyl)pyrazolyl]methane.

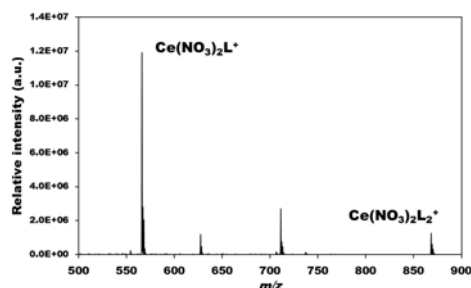


Figure 2. ESI/QITMS(+) spectrum of a $Ce(NO_3)_3/L$ solution.

The investigation of the complexation properties of related ligands towards the lanthanides and actinides by ESI/QITMS is currently under way.

1. Z. Kolarik, *Chem. Rev.* **108** (2008) 4208.
2. S. Colette, B. Amekraz, C. Madic, L. Berthon, G. Cote, C. Moulin, *Inorg. Chem.* **42** (2003) 2215.

Acknowledgements: The ESI/QITMS was acquired with the support of the Programa Nacional de Reequipamento Científico of Fundação para a Ciência e a Tecnologia (FCT) and is part of RNEM-Rede Nacional de Espectrometria de Massa also supported by FCT. Support from the EC through ACSEPT (FP7-Euratom/CP-2007-211267) and ACTINET-13 (FP7-III-232631/JRP17) is gratefully acknowledged. B.M. is thankful to FCT for a post-doc grant.

Behaviour of analytes with different polarity and acid/base chemistry under electrospray ionization mass spectrometry conditions – insights on gas phase ion formation

P12

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In an attempt to understand the behaviour of structurally different compounds, we previously studied [1], aqueous solutions of eight analytes (acetyl-L-arginine, acetyl-L-lysine, L-histidine, creatinine and 3-amino-1,2,4-triazine, L-tyrosine, eugenol and L-phenylalanine), under ESI-MS conditions, were here investigated. Furthermore, the effects of solution concentration and solvent on the ESI ion signal response were studied. The classical ion abundance/concentration plot, where analyte monomer ions abundances change linearly with analyte concentration for concentrations up to 10^{-5} M, and then saturate for concentrations above that value, were only observed for the more basic acetyl-arginine and acetyl-lysine analytes. For creatinine and aminotriazine, a depression in the monomer ion abundance/concentration plots was observed, at concentrations higher than 10^{-5} M. At this concentration range also, ion clusters were observed in the spectra and their charge seems to be maintained as the concentration in solution is raised and high mass clusters are formed. Especial attention was here drawn to gas phase ion formation for creatinine and aminotriazine analytes, in particular creatinine, due to the preservation of solution-phase behaviour in the gas phase during electrospray ionization. This solution-phase behaviour, due to hydrogen bonding between creatinine molecules and water molecules, seems to be influenced by the ionization phenomena and could be regarded as an alternative strategy to study and monitor the evolution of electrosprayed liquids leading to gas phase ion formation during electrospray ionization.

1. M.A. Saraiva; C.M. Borges; M.H. Florêncio. *Eur. J. Mass Spectrom.* **2010**, *16*, 199.

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Several amine/phenolate donor sets proved adequate as supporting ligands of a range of early transition-metal complexes.¹ Our group has been using a diamine bis(phenolate) ligand (Figure 1) for the stabilization of Th(IV) and U(IV) complexes.

Neutral compounds of the type $[\text{An}^{\text{IV}}\{\text{salan-}^t\text{Bu}_2\}\text{Cl}_2\text{L}]$ ($\text{An} = \text{Th}, \text{U}$) are readily formed by reacting ThCl_4 and UCl_4 with the deprotonated $\text{salan-}^t\text{Bu}_2$ ligand in dry tetrahydrofuran or dimethoxyethane, under a N_2 atmosphere. ESI/QITMS was used to investigate the stoichiometry of the $\text{An}(\text{IV})$ - $\text{salan-}^t\text{Bu}_2$ complexes formed in solution. Although these air-sensitive compounds are prone to hydrolysis, and in the case of uranium to oxidation, they are stable enough to allow the observation of $\text{An}(\text{IV})$ ionic species in the MS, provided a dry solvent is used and the solution prepared under N_2 . In Figure 2 we show a spectrum in which a $[\text{An}^{\text{IV}}\{\text{salan-}^t\text{Bu}_2\}\text{Cl}_2\text{K}]^+$ species is present. The presence of anions in the ESI source also allow the generation of species of the type $[\text{An}^{\text{IV}}\{\text{salan-}^t\text{Bu}_2\}\text{Cl}_2\text{X}]^-$. In both modes, we have, therefore, evidence for the neutral nature of the compounds, and this is an advantage of ESI/MS. The MS results are corroborated by the information obtained with other techniques, such as NMR and X-ray diffraction.

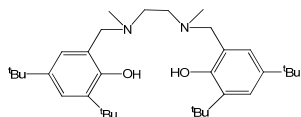


Figure 1. $\text{H}_2(\text{salan-}^t\text{Bu}_2)$.

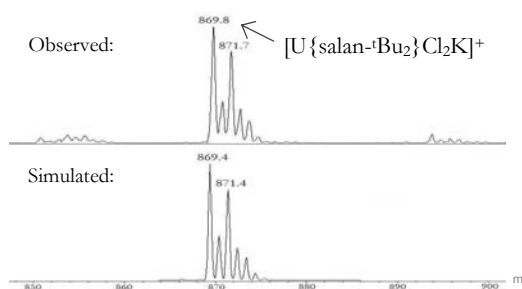


Figure 2. ESI/QITMS(+) spectrum of $[\text{U}\{\text{salan-}^t\text{Bu}_2\}\text{Cl}_2\text{bipy}]$ in acetonitrile/tetrahydrofuran solution.

1. A. Amgoune, C.M. Thomas, J.-F. Carpentier, *Pure Appl. Chem.*, **79** (2007) 2013.

Acknowledgements: The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT), POCI 2010 and FEDER for funding (research project PTDC/QUI/66187/2006). The ESI/QITMS was acquired with the support of the Programa Nacional de Reequipamento Científico of FCT and is part of RNEM-Rede Nacional de Espectrometria de Massa also supported by FCT.

Study of Isomeric Hexose Disaccharides with β -(1-4) linkage by Electrospray Tandem Mass Spectrometry | P14

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Carbohydrates are the most abundant biomolecules in nature, and are responsible for different functions in almost all living. The study of these compounds makes a challenging and important task not only due the functions they perform, but as well the great existing variety. A complete structural description implies exact mass measurement, identification of their monomer composition and anomeric configuration of the glycosidic linkage, and also stereochemical characterization of the different asymmetric centers of the sugar ring. There are various methods of analysis, being nuclear magnetic resonance (NMR) spectrometry the standard tool, with the drawback of requiring large amount of pure sample. Recently mass spectrometry, with precise results, analytical versatility and very high sensitivity, has significantly contributed in glycobiology, and assuming a essential role in many chemical and biological applications. This work describes the analysis of isomeric hexose disaccharides with β (1-4) linkage by electrospray tandem mass spectrometry (MS/MS), in order to detect and distinguish oligosaccharides with same molecular weight but distinct sugar composition. In this context, differences in the relative abundance of ions produced by the dissociation of the $[M+Li]^+$ and $[M+Na]^+$ adducts, in two different spectrometers, a linear ion trap LXQ and Q-TOF, in positive ion mode allowed distinguishing disaccharides four isomeric disaccharides, composed by distinct hexose units but all having β -1,4 type of linkage (Glc β -1,4Glc, Gal β -1,4Gal, Man β -1,4Man and Gal β -1,4Glc).

Acknowledgments

The authors thank the financial support provided to project PTDC/QUI-QUI/100044/2008, QOPNA (Research Unit 62/94), and RNEM by the Foundation for Science and Technology (FCT) and COMPETE.

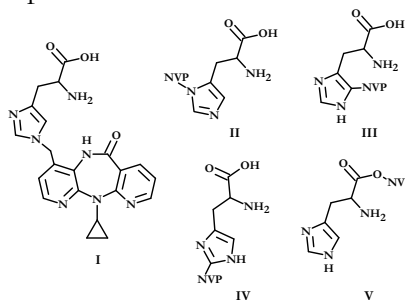
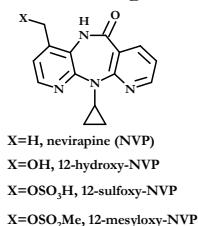
Characterization of regioisomeric histidine adducts from the anti-HIV drug nevirapine

P15

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Nevirapine (NVP), a non-nucleoside reverse transcriptase inhibitor used against HIV-1, is associated with severe toxicities involving liver and skin. Increasing evidence suggests that NVP metabolism to 12-hydroxy-NVP, followed by sulfotransferase-mediated conversion to 12-sulfoxy-NVP, play a role in these processes. We recently demonstrated that the model ester 12-mesyloxy-NVP, used as a synthetic surrogate for 12-sulfoxy-NVP, yields covalent adducts upon reaction with amino acids containing nucleophilic side chains¹. In addition, we obtained evidence for binding of 12-mesyloxy-NVP to blood proteins *in vitro* and *in vivo*^{2,3}. Histidine (His) is one of the amino acid targets for 12-hydroxy-NVP-derived electrophiles. Upon reaction with 12-mesyloxy-NVP we obtained at least four isomeric covalent NVP-His adducts, of which only **I** could be isolated in quantities amenable to full characterization by ¹H and ¹³C NMR. The bulk adduct fraction was separated by semipreparative reversed-phase HPLC with diode array detection and analysed by HPLC-ESI-MS/MS. We discuss herein the characterization of the regioisomeric NVP-His adducts (putative structures **I-V**) based upon their MS/MS fragmentation patterns, particularly the relative propensity for cleavage at the NVP-His linkage. This knowledge should form the basis for identification of specific NVP-His adducts within proteins, to be used as biomarkers for *in vivo* monitoring of NVP toxicity.



- 1.A.M.M. Antunes *et al.*, *Chem. Res. Toxicol.* **23** (2010), 889.
- 2.A.M.M. Antunes *et al.*, *Chem. Res. Toxicol.*, **23** (2010), 1714.
- 3.M.M. Marques *et al.*, 240th ACS National Meeting, Boston, USA, Abstract TOXI 14115 (2010).

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Organic azides are versatile compounds with wide applications in syntheses, in AIDS treatment, in biological labelling and in tumor-growth inhibition¹. The fragmentation of several 2-, 3- and 4-substituted benzylazides species were analyzed through electron ionization mass spectrometry (EIMS) and B/E linked scans². For methyl, methoxy and nitrobenzyl azides elimination of N₂ and N₃[•] were among the most important decomposition channels. Herein, theoretical support for the interpretation of *para* isomers fragmentation was sought.

The optimized structures of the radical cations were calculated at the HF/6-311++G(d,p) and DFT B3LYP/6-311++G(d,p) levels of theory. Selected fragmentation routes were investigated through minimum-energy path following for each reaction, based on intrinsic reaction coordinate (IRC) calculations, obtained with the UHF/6-31G(d) and the UB3LYP/6-31G(d,p) method/basis. From these results, the energy barriers for the N₂ and N₃ elimination from the *para* isomers of methyl, methoxy and nitrobenzylazides (4-MBA, 4-MeOBA and 4-NBA) were estimated to be 33.9 and 19.5 kJ/mol (4-MBA, UHF/6-31G(d)), 202.9 and 91.4 kJ/mol (4-MeOBA, UB3LYP/6-31G(d,p)), and 149.6 and 73.5 N₂ kJ/mol (4-NBA, UB3LYP/6-31G(d,p)), respectively, thus favouring the N₃ elimination over N₂ in all the *para* isomers. This is in agreement with the detection of more abundant ions resulting from the loss of N₃[•] than the ones resulting from loss of N₂, at least for *para* methyl and methoxybenzyl azides.

1. S. Bräse, C. Gil, K. Knepper, V. Zimmerman, *Angew. Chem. Int. Ed.* **44** (2005), 5188.
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Gas-phase behaviour of ruthenium (II) cyclopentadienyl derivatives: an FT-ICR mass spectrometry study

P17

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Ruthenium complexes have been the most widely studied non-Platinum anti-cancer candidates and hold great potential as successful alternatives in cancer treatment.^{1, 2, 3} Results for cytotoxicity against some human tumour cell lines (A2780, A2780cisR, Pc3, MCF7) indicate that the complexes show promising anticancer activity, which varies with changes in phosphines co-ligands and the anionic counter ions.

The present study deals with the gas-phase behaviour of six [Ru(II)(η^5 -Cp)(PP)(1-BuIm)]X (Figure 1), where 1-BuIm = 1-Butylimidazole, PP = Dppe or 2PPh₃ and X = PF₆⁻, CF₃SO₃⁻ or BPh₄⁻.

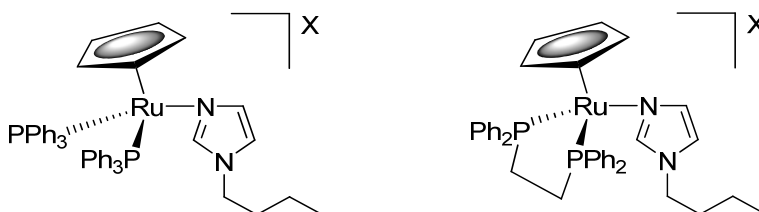


Figure 1. Structure of the Ru(II) cyclopentadienyl derivatives under study.

The preliminary results show that the anionic counterion (X⁻) has a strong influence on the electrospray ionization mass spectra and that the phosphine co-ligands (Dppe or 2PPh₃) greatly influence the fragmentation patterns.

1. P.J. Dyson, G. Sava, *Dalton Trans.* **2006** (2006) 1929.
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3. M.H. Garcia, T.S. Morais, P. Florindo, M.F.M. Piedade, V. Moreno, C. Ciudadm V. Noe, *J. Inorg. Biochem.*, **103** (2009) 354.

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In Madeira Archipelago there are four endemic *Helichrysum* species and three of them are used in the traditional medicine.¹ The aim of this work was to study for the first time *Helichrysum monizii* Lowe, a rare endemism, in terms of its phenolic profile.

Three different methods of extraction were performed and total phenolic content and radical scavenging capacity (ABTS) were assayed. The results revealed a high antioxidant potential mainly related to the phenolic profile of the plant.

Polar components of alcoholic extracts of *H. monizii* were characterized by a high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC-DAD/ESI-MSⁿ) method. 33 compounds were identified and 19 of them were identified as quinic acid derivatives (Figure 1).

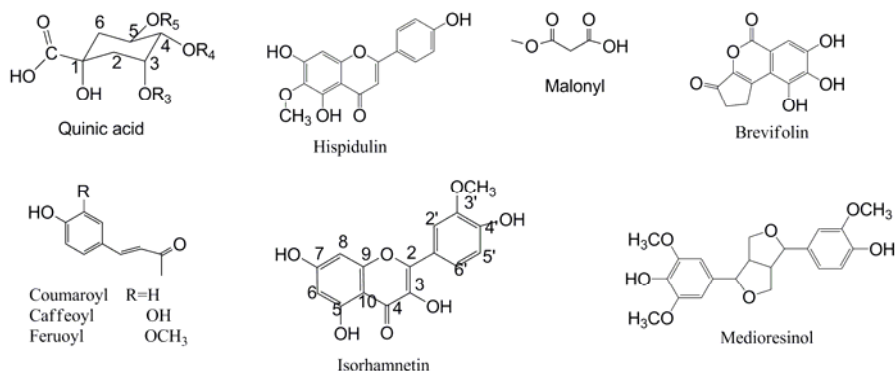


Figure 1. Chemical structures of phenolic compounds detected in *H. monizii*.

1. R. Jardim, *Endemic flora of Madeira*. (2000) First ed. Múchia Publicações, Setúbal.

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The present study aimed to observe the differences and similarities in the PIONA distribution (*n*-paraffins, iso-paraffins, olefins, naphthenes and aromatics) in the various diesel fuels available in market.

For that, we analyzed diesel fuels of different brands and types. These were collected at different points of sale and classified into three types: white label diesels (from pumps of supermarkets), standard diesels (known brands) and Premium diesels (diesel with additives). In total there were 11 different samples under study, including a type of diesel fuel used for heating. For each sample, three trials were performed.

The analysis was performed with the use of two dimensional gas chromatography GC × GC / TOF-MS, a technique increasingly important in the study of the chemical composition for complex mixtures.

The separation was successful and has been possible to observe the separation of more than a thousand peaks/compounds. These were identified/grouped into families PIONA with the help of mass spectrometry taking into account the retention times in the 1st and 2nd dimension, as well as the physical properties of groups and even the automatic identification provided by the software (*Figure 1*).

So, it was possible to estimate the percentage of PIONA compounds in the samples analyzed.

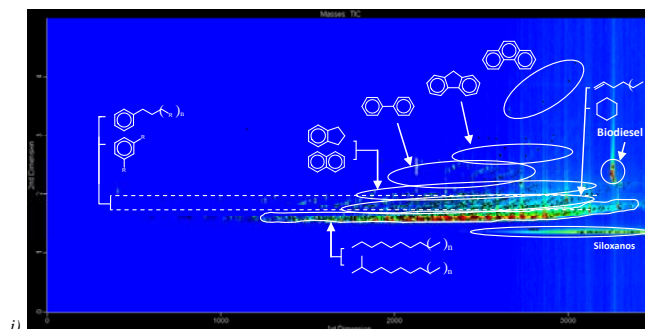


Figure 1. GC×GC TOF-MS contour plot chromatogram of a diesel sample.

Fast methods to measure amidated bile acids in liver transplant patients: Synthesis and SIP-EI⁺-MS fragmentation of the pentachlorophenyl ester of pyrenebutyric acid

P20

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Over a number of years we have developed methods to derivatize bile acids (free and conjugated) with fluorescent or UV-absorbing moieties that can be detected by low-cost HPLC detectors. The pentachlorophenyl (PCP) ester of pyrenebutyric acid (PyPCP, molecular mass 536,67) is a fluorescent activated ester that can tag secondary amines, obtained by bile acid reduction on carbon 24, under mild reaction conditions. The two reactions can be applied in fast methods to obtain complete bile acid profiles, useful in organ evaluation¹ and follow-up of human liver transplants² as has been previously suggested.

PyPCP has been synthesized by carbodiimide coupling using the Kovacs' complex (DCCI:PCP 1:8 molar). The product was purified by column chromatography and crystallized. All attempts to study it by ESI-MS failed so far. MS fragmentation was obtained by solid insertion probe (SIP) electron impact (EI⁺) in positive mode. PyPCP was sublimated at 97°C under reduced pressure and directly introduced into the MS ionisation chamber of an LKB 9000 spectrometer. The mass spectrum was obtained at an ionisation energy generated by a 20 eV potential difference.

Around m/z 536 a group of 6 peaks with a characteristic pattern of intensities due to the 5 chlorine atoms (³⁵Cl or ³⁷Cl) present in the PCP group, each separated from the next by two a.m.u., can be assigned to [M]⁺. The same pattern appears at m/z 266 assigned to a fragment containing the protonated PCP moiety. Fragmentation was observed in the side chain (m/z 271, 253, 243, 227, 201) and in the pyrene nucleus (m/z 189, 164, 107, 69).

However, no evidence was found supporting fragmentation of the PCP group. A gap between m/z 536 and 271 was duly noted, suggesting that the side-chain and pyrene nucleus are easier to fragment than the PCP moiety.

1. H.Vilca-Melendez, M.Rela, K.D.R. Setchell, G.M. Murphy, N.D. Heaton. *Transpl Int*, 17, 6 (2004), 286-92.
2. S.A. Azer; G.W. McCaughan; N.H. Stacey; *Hepatology*, 20, 6 (1994), 1458-1464.

Identification by ESI-MS of the products of the reaction of $\text{BrC}_6\text{H}_4\text{C}\equiv\text{CR}$ with *trans*- $[\text{PdCl}_2(\text{Me}_2\text{NNC}_{10}\text{H}_{14}\text{O})_2]$

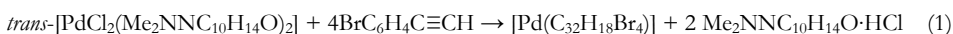
P21

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The activation of the C-C triple bond in alkynes is as a tool for the synthesis of organic species difficult or impossible to obtain in other ways. Within our search for catalysts that are capable of activating alkynes we found that palladium camphor imine complexes are efficient catalysts for the cyclotrimerization of terminal or internal alkynes [1]. We now wish to report the activation of $\text{BrC}_6\text{H}_4\text{C}\equiv\text{CH}$ by *trans*- $[\text{PdCl}_2(\text{Me}_2\text{NNC}_{10}\text{H}_{14}\text{O})_2]$ (reaction 1). The products were characterized by NMR, elemental analysis and ESI-MS.



Structural characterization of $[\text{Pd}(\text{C}_{32}\text{H}_{18}\text{Br}_4)]$ (**1**) (Figure 1) is based mainly on the data obtained by ESI-MS. In methanol, the ESI (+) mass spectrum of **1** displays a group of intense peaks (R.I. = 100%) at m/z 829/831 corresponding to $[\text{Pd}(\text{C}_8\text{H}_4\text{Br})_2(\text{C}_8\text{H}_5\text{Br})_2 + \text{H}]^+$ with an isotopic distribution pattern that agrees with the isotopic distribution pattern calculated for $[\text{Pd}(\text{C}_{32}\text{H}_{19}\text{Br}_4)]^+$ as shown in Figure 1. The less intense peak at m/z 861/862 corresponds to the methanol adduct of the protonated species ($[\text{Pd}(\text{C}_{32}\text{H}_{18}\text{Br}_4)] + \text{H}, \text{MeOH}]^+$) and signals at m/z 723, 755 and 573 were assigned to $[(\text{C}_{32}\text{H}_{18}\text{Br}_4) + \text{H}]^+$, $[(\text{C}_{32}\text{H}_{18}\text{Br}_4) + \text{H}, \text{MeOH}]^+$ and $[(\text{C}_{24}\text{H}_{15}\text{Br}_3) + \text{H}, \text{MeOH}]^+$ respectively. The other products of reaction 1 were also identified by ESI-MS.

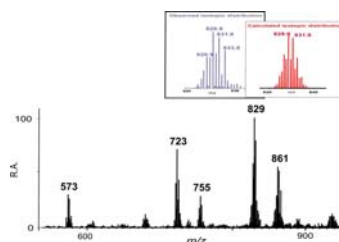


Fig 1-ESI-MS spectrum of $[\text{Pd}(\text{C}_{32}\text{H}_{18}\text{Br}_4)]$

1. M.F.N.N. Carvalho *et al.*, *Inorg. Chim. Acta* **363** (2010)1767-1772.

Acknowledgements

Fundação para a Ciência e Tecnologia for financial support under Transnational Cooperation with ACCR and Contract REDE/1502/REM/2005.

Simultaneous Determination of Carbamazepine and Vigabatrin in Human Plasma using Hydrophilic Interaction Liquid Chromatography-Triple Quadrupole Mass Spectrometry

P22

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A fast, sensitive and selective method for the detection and quantification of carbamazepine (CBZ) and vigabatrin (VGB) in plasma is described using high-performance liquid chromatographic separation with tandem mass spectrometry. Samples were purified using liquid-liquid extraction and separated on a Phenomenex Luna HILIC 3 μ (2 \times 150 mm) column with a mobile phase consisting acetonitril/ ammonium formate (5mM, pH 3.5) (75:25, v/v) at a flow-rate of 0.4 ml/min. Detection was performed by a triple quadrupole model G6410A mass spectrometer in the MRM mode using electro spray ionisation (ESI), monitoring the transition of the protonated molecular ion for carbamazepine at m/z 237 and vigabatrin at m/z 130 to the predominant ions of m/z 194 and 71, respectively. The mean recovery was 81% for carbamazepine and 100% for vigabatin. The calibration curve was linear over the concentration range 250–8000 ng/mL for carbamazepine and 15–960 ng/ml for vigabatrin. The limits of detection for CBZ and VGB in human plasma were 5.43 ng/mL and 3 ng/mL, respectively. The limit of quantification for both analytes in human plasma were 18.2 ng/ml and 10 ng/ml, respectively.

Simultaneous Determination of Phenytoin and Lamotrigine in Human Plasma using Hydrophilic Interaction Liquid Chromatography-Triple Quadrupole Mass Spectrometry

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A fast, sensitive and selective method for the detection and quantification of Phenytoin (PHT) and Lamotrigine (LTG) in plasma is described using high-performance liquid chromatographic separation with tandem mass spectrometry. Samples were purified using liquid-liquid extraction and separated on a Phenomenex Luna HILIC 3 μ (2 \times 150 mm) column with a mobile phase consisting acetonitril/ ammonium formate (5mM, pH 3.5) (97.5:2.5, v/v) at a flow-rate of 0.2 ml/min. Detection was performed by a triple quadrupole model G6410A mass spectrometer in the MRM mode for phenytoin and SIM mode for lamotrigine, monitoring the transition of the deprotonated molecular ion for Phenytoin at m/z 251 to the predominant ions of m/z 102 and protonated molecular ion for lamotrigine at m/z 256. The mean recovery was 90% for phenytoin and 80% for lamotrigine. The limits of detection for PHT and LTG in human plasma were 5.17 ng/ml and 0.13 ng/ml, respectively. The limit of quantification for both analytes in human plasma were 17.24 ng/ml and 0.46 ng/ml, respectively.

Synthesis and identification of Glutathione Trimethyl Arsonium cation complex by LC-ESI-MSⁿ and HR-ESI-MS

P24

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The protein-Arsenic bond interacting with reactive metabolites such as Sulfur-adenosyl-methionine (SAM) and Methyl-cobalamin (Met-CB12), form intermediate complexes that may have a key role in the Arsenic chemistry of living systems. Particularly, it seems reasonable to assume that the DMAs^{III}GS complex (DMAG) (m/z 412, $[M+H]^+$), formed from Cacodylate (DMA), Glutathione (GSH) and SAM, may have such a role.

In this work the non-enzymatic synthesis of Glutathione Trimethylarsonium cation, (GS-As⁺(CH₃)₃), (m/z 426), from DMAG, obtained *in vitro*, and SAM, apparently through the classical Challenger methylcarbonium attack, have been achieved.

Data from full scan and MSⁿ spectra, as well accurate mass measurements obtained by electrospray ionization mass spectrometry (ESI-MS), strongly suggest that such complex (GS-As⁺(CH₃)₃)(m/z 426), was formed in the experiment.

This intermediate, was apparently methylated further by the complex GSH-methylCB₁₂, leading to the synthesis of Tetramethylarsonium ion As⁺(CH₃)₄ (m/z 135)(TETRA) and to the formation of Trimethylarsine oxide (TMAO) (m/z 137, $[M+H]^+$), both detected by LC-ESI-MSⁿ and high performance liquid chromatography (HPLC) Such pathway would explain the accumulation of Tetramethylarsonium ion in the absence of dimethylarsinate, among the arsenic species taken up and produced by polychaetes¹.

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Fast methods to measure amidated bile acids in liver transplant patients: ESI-MS fragmentation and patterns of aggregation of pyrenebutyric acid

P25

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The need for fast methods to obtain complete bile acid profiles, in organ evaluation¹ and follow-up of human liver transplants² has been previously emphasized. Over a number of years we have developed methods to tag bile acids (free and conjugated) with fluorescent or UV-absorbing moieties that can be detected by common HPLC detectors. Pyrenebutyric acid (PyCOOH) has been our starting point to produce fluorescent activated esters that can tag secondary amines obtained by bile acid reduction on carbon 24, under mild reaction conditions.

By using HPLC-ESI-MSⁿ and direct infusion ESI-MSⁿ we have fully characterized solvents, reagents, products and side-products of the above mentioned reactions. PyCOOH (molecular mass 288,35) was dissolved in acetonitrile (10 µg/mL, HPLC grade) and studied by direct infusion into MS (API 2000/3000, Applied Biosystems) and analysed in positive and negative ion modes. The ESI spectra include ions that can be assigned to [M-1]⁻, [M₂-1]⁻ and [M³-1]⁻ at m/z 287, 575 and 864 respectively. This tendency for aggregation in supramolecular structures that precipitate over time is confirmed by Light Scattering Spectroscopy. The ESI⁺ spectra show ions assignable to [M+1]⁺, [M₂+1]⁺, [M+1+18]⁺, [M₂+1+18]⁺, [M+23]⁺, [M₂+23]⁺ at m/z 289, 577, 307, 595, 311, 599 respectively, again demonstrating the tendency for aggregation of PyCOOH with itself and other chemical species (water and sodium).

CID experiments confirmed the identity of the aggregated species and revealed the molecular structure of the monomer.

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Combination of bar adsorptive micro-extraction (BA μ E) with LC/FTICR-MS for the determination of phenolic compounds in water matrices

P26

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Endocrine disrupting chemicals (EDCs) have elicited great concern worldwide because of their adverse effects on wildlife and human health. *In vivo* assays have shown that very small amounts of EDCs may influence wild animals and humans. Phenolic compounds such as 3- and 4-nitrophenols, bisphenol A, 4-octylphenol and 4-nonylphenol are considered potent EDCs and their determination requires highly sensitive methods to evaluate all potential risks.

In this contribution, a novel enrichment technique, bar adsorptive micro-extraction, followed by liquid desorption combined with high performance liquid chromatography coupled to Fourier transform ion cyclotron resonance mass spectrometry (BA μ E-LD/LC/FTICR-MS) was applied to monitor five phenolic compounds in water matrices. Assays performed on 25 mL water samples spiked at the 10.0 μgL^{-1} level under optimized experimental conditions yielded recoveries between in 90 % (3-nitrophenol) and 100 % (bisphenol A).

Portuguese Familial Amyloid Polyneuropathy patients and asymptomatic carries present the same amount of variant TTR (V30M) | P27

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Familial amyloid polyneuropathy (FAP) is a fatal autosomal dominant disease characterized by the formation and deposition of extracellular amyloid fibers, which are mainly composed of transthyretin (TTR). The most widely accepted model of TTR aggregation and amyloid fiber formation relates tetramer stability with TTR mutations. The V30M is the most common one, also called the Portuguese variant¹.

However, this model fails to explain several observations. Among these we highlight the different age of onset disease that varies by decades on different patients bearing the same mutation and the same penetrance levels between homo and heterozygous individuals². Also, it was recently proposed but not verified, that Swedish V30M carriers, which have later age of onset and lower penetrance compared to other populations, display a lower quantity of mutated TTR in circulation³.

We compared symptomatic/asymptomatic carries and healthy individuals using a relative quantification methodology developed by our group⁴. Serum samples from cadaveric and sequential liver transplant individuals were also evaluated.

Our results show that wt TTR is diminished in symptomatic/asymptomatic carriers, but the relation between the native and mutant form in the serum does not change with the progression of this illness.

Liver transplantation is the only viable treatment for ATTR, so it is also relevant to evaluate the two TTR variants in blood circulation for transplanted individuals. Sequential Liver Transplantation using grafts from FAP patients was first performed in 1995 and more than 400 domino transplants were carried out by the end of 2005, according to the Familial Amyloidotic Polyneuropathy World Transplant Registry (<http://www.fapwtr.org>).

In respect to the transplanted samples we observed, as expected, that after cadaveric liver transplant the mutant TTR is no longer present in the serum,

while after sequential liver transplant there is a reduction of the wt TTR proportion, similar to what is observed on non transplanted FAP patients. These data seem to support the hypothesis for the involvement of non-genetic factors in ATTR onset and disease progression.

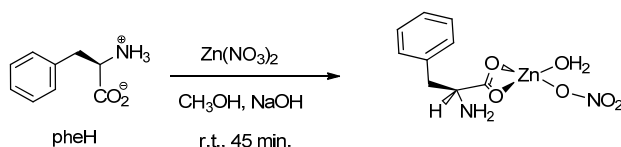
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Oxygen atom transfer (OAT) reactions are known as one of the most important transformations in chemistry with biological systems being mimicked¹. In particular, olefin epoxidation is most relevant since it makes possible the preparation of intermediary products that can subsequently be used in further reactions towards the synthesis of more elaborated compounds. However the pursuit of cheaper still selective systems keeps as a milestone that powers much research. In this context the use of cheaper d-block metals such as Fe, Cu and Zn may be a way of meeting these criteria². Usually olefin epoxidation requires the use of catalysts behaving as Lewis acids Zn^{II} meeting this, although only a few reported works have addressed this reaction using Zn^{II} catalysts³. Biologically zinc is found to play a structural and/or catalytic key role in proteins usually adopting a tetrahedral coordination geometry. Among catalytic roles Zn is found in a variety of enzymes engaged in many aspects of metabolism⁴. When playing this catalytic role, usually Zn is found bound to three ligands with the fourth position being held by a water molecule which is labile thus allowing proteins using it to rapidly shift conformations to perform biological reactions, being taken as the catalytically active position⁵.

In this work a biomimetic Zn complex with phenylalanine was prepared and MS has been used to elucidate on the stoichiometry of the species formed which was found to be formulated as shown in the scheme.



MS data along with results from other spectroscopic techniques concerning structural elucidation will be presented providing further information on this system.

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Genista tenera (Leguminosae) is a plant endemic to the island of Madeira, Portugal, which infusion is used by the local population for the control of diabetes. A previous phytochemical study of the ethanol extract from the plant aerial parts, showed the presence of alkaloids and flavonoids.¹⁻⁴ Pursuing our studies on the research of new bioactive compounds for diabetes prevention and treatment, we present now the flavonoid profile of the aqueous plant extract. Two major compounds were detected at m/z 593.2 and m/z 431.2, corresponding to a trihydroxyflavonoid glycosylglycoside and to a C-glycosyltrihydroxyflavonoid, respectively. Their structure was proposed by means of electrospray tandem mass spectrometry (ESI-MS/MS) in the positive and negative ion modes.

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Acknowledgement: The authors thank Fundação para a Ciência e a Tecnologia for financial support (project PTDC/QUI/67165/2006).

Gas phase affinity scale of $[C_n\text{mim}]^+$ ($n=2, 4, 6$) and $[\text{Phosp}]^+$ cations to $[\text{NTf}_2]^-$ anion

P30

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When ions and neutral molecules encounter each other in the gas phase, they can form a complex that is more stable than the isolated species, because of the strong interaction energies between them. The correlation of the structural factors which mediate the strength of binding interactions can be determined by a gas phase affinity scale of the considered ionic species.

As we previously demonstrated by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) the aprotic ionic liquids 1-methyl-3-alkyl-imidazolium bis{(trifluoromethyl)sulfonyl}amide derivatives, $[C_n\text{mim}][\text{NTf}_2]$, distill under reduced pressure as discrete ion pairs¹, which can further react with the corresponding radical cation or anion formed upon electron ionization.

In this work FT-ICR-MS was used to determine by Cooks method² an affinity scale of the 1-methyl-3-alkyl-imidazolium $[C_n\text{mim}]^+$, with $n = 2, 4$, and 6) and phosphonium $([\text{Phosp}]^+)$ cations to the bis{(trifluoromethyl)sulfonyl}amide $([\text{NTf}_2]^-)$ anion.

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Acknowledgments: This work was supported by FCT, Portugal (Project PTDC/QUI/66199/2006).

Phenolic compounds in *Opuntia spp.* juices: preliminary studies by LC-MS/MS

P31

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Opuntia spp., is a cactus that spontaneously grows in Portugal. Cactus fruits (cactus pear) are known for their high polyphenolic content and several bioactivities as antioxidant and anti-inflammatory have been attributed to these compounds. These fruits contain also carbohydrates, vitamins (for example, vitamin C), amino acids (including taurine) and yellow and/or red betalains (betaxanthins and betacyanins, respectively). In this preliminary study phenolic compounds were analysed in fruit juices collected in different regions of Portugal and were analysed by high-performance liquid chromatography (HPLC) using diode array and mass spectrometry detection. The mass spectrometer used was a triple quadrupole with an ESI ion source operating in negative mode for the identification of phenolic compounds. Chromatographic and mass spectrometry conditions were optimized in order to detect a higher number of peaks in the chromatogram. Some flavonoids were identified based on data from the fragmentation pattern and from absorption spectra.

Acknowledgments: This work was funded by Fundação para a Ciência e Tecnologia (PTDC/AGR-AAM/099645/2008 and REDE/1518/REM/2005 for the LC-MS/MS equipment).

Application of triple-quadrupole mass spectrometry tandem with LC in the determination of residue of Cephalosporins in meat tissues

P32

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Antibiotic resistance has become an increasing important concern in food safety. The incorrect use of antibiotics such as Cephalosporins in veterinary medicine can introduce residues of its in alimentary chain or food of animal origin. The presence of small amounts of antibiotics in food represents a high risk to consumers by the negative effect of antibiotic resistance of pathogens and the increment of allergenic diseases.

To ensure the absence of antibiotics in food of animal origin, EU and other international organizations established maximum residue limits (MRL) for antibiotics in food products and strict requirements of accuracy to the analytical methodologies employed in food safety. MRL defined for Cephalosporins in meat samples are from 50 to 1000 µg/kg. The use of high sensitive techniques like mass spectrometry is necessary for the development of confirmatory methods of determination of antibiotics in animal tissues.

Our work focuses on the development of analytical techniques of confirmatory determination of Cephalosporins in meat samples. Cefalexin, Cefalonium, Cefoperazone, Cefapirin, Cefquinome, Ceftiofur and Cefazolin were determined by using two different clean-up methods for the treatment of the sample: one based on solid phase extraction (SPE) and other based on newly QuEChERS methodologies (Quick, Easy, Cheap, Effective, Robust and Safe). After extraction, samples were analyzed by LC-MS/MS. This technique allows us to reach limits of quantification under the MRL value and to confirm the presence of the Cephalosporins in meat tissue by the identification of their MRM transitions.

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Secoiridoids in Olive Seeds: analysis by MALDI-TOF and LC-MS/MS

P33

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The olive tree belongs to the *Oleaceae* botanical family that have characteristic chemical compositions with emphasis on secoiridoid compounds. These compounds contain elenolic acid in their structure. Some compounds have already been identified in olive seeds such as 11-methyl oleoside and nüzhenide¹. In previous works^{2,3} we reported the analysis and chemical characterization of seed extracts from *Olea europaea* L. using LC-MS with ion trap and triple quadrupole analyzers. MALDI-TOF³ assays were also done for comparison purposes. MALDI-TOF mass spectrometry allowed us to confirm the presence of secoiridoid oligomeric compounds of higher molecular mass than those detected by ion trap or quadrupole analyzers.

In this work we report the optimization of conditions for MALDI-TOF analysis of olive seed extracts using linear and reflectron modes. Results obtained using 2,5-dihydroxybenzoic acid (DHB) and 2,4,6-trihidroxy-acetophenone (THAP) as matrices were compared. Assays were also done without matrix addition. The same samples were analysed by LC coupled with a triple quadrupole mass spectrometer with an electrospray source that was operated in the negative mode.

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

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Rich guanine DNA sequences can form higher-order DNA structures, G-quadruplexes, based on the association of guanine tetrads. Such sequences are found at the extreme 3' end of telomeric DNA, which is located in the end of chromosomes. Telomerase inhibition and telomere maintenance is emerging as an attractive target for anticancer therapies¹.

The use of ESI-MS, allows, through the study of their ionic adducts, the gathering of information on the interactions of potential anticarcinogenic drugs, both with double helices and with higher-order structures of oligonucleotides. It was also found that 5,10,15,20-tetrakis(N-methylpyridinium-4-yl)porphyrin tetra-iodide stabilizes G-quadruplexes², thus being a potential inhibitor of human telomerase¹.

Based in our previous studies³, ESI-MS has been used to study the interactions between the [d(TGGGGT)₄] quadruplex and a group of cationic porphyrins with different number of charges. In the experimental conditions used in this work, the quadruplex is stabilized by the addition of three ammonium ions. Formation of [d(TGGGGT)₄ + 3NH₄ + porphyrin]ⁿ⁻ adduct ions for all the porphyrins shows that these compounds provide an additional stabilization of the quadruplex structure. The number of charges of the porphyrin induces different fragmentation patterns: for porphyrins with one and two charges the ammonium cations remain in the G-quadruplex structure and the porphyrin is displaced, whereas for porphyrins with three and four charges, the porphyrin remains linked to the G-quadruplex but the ammonium ions are displaced through NH₃ losses. Formation of [d(TGGGGT)₄ + 3NH₄ + porphyrin]ⁿ⁻ adduct ions indicates an external binding of the porphyrins to the quadruplex structure⁴. This interaction is strongly dependent on the number of porphyrin charges, increasing with the latter and does not seem to have a marked dependence on the type of substituents.

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ESI/QITMS study of the relative affinity of N-donor bases towards Pr(III) ions in the gas phase

P35

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The coordination chemistry of lanthanides (Ln) and actinides (An) with N-donor ligands is a topic of current research associated to Ln/An separations within advanced nuclear fuel cycles.¹ Some of the ligands used have simple heterocyclic N-donor bases as building blocks.

The relative affinity of a representative number of N-donor bases (Figure 1) towards Pr(III) ions in the gas phase was studied by ESI/QITMS. Ethanol solutions of Pr(NO₃)₃ and equimolar amounts of base pairs yielded the mixed species [Pr(NO₃)₂LL]⁺ which were subjected to competitive CID in the QIT. These experiments indicated that the relative gas-phase affinities of the N-donor molecules followed their gas-phase basicities with the notable exception of pyridazine for which a η² coordination mode seems to prevail, leading to a stronger bonding. These experimental results are in agreement with theoretical studies of the affinities of N-donor bases to Ln(III) ions.²

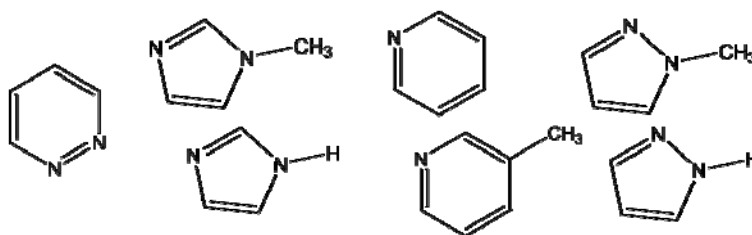


Figure 1. N-donor bases.

Comparative studies of the gas-phase affinities of different bases to lanthanide(III) and actinide(III) ions by ESI/QITMS and FTICRMS are currently under examination.

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Acknowledgements: The ESI/QITMS was acquired with the support of the Programa Nacional de Reequipamento Científico of Fundação para a Ciência e a Tecnologia (FCT) and is part of RNEM-Rede Nacional de Espectrometria de Massa also supported by FCT. Support from the EC through ACSEPT (FP7-Euratom/CP-2007-211267) and ACTINET-13 (FP7-III-232631/JRP17) is gratefully acknowledged.

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Mass spectrometry has always been used for the measurement of microflows; unlike other techniques it allows determining the contribution of each species in the microflow, when it's composed of a mixture of gases. However, its normal use is hindered by the inability to separate the signal due to the microflow from that due to background. The usual technique of measuring the background prior to the test and then subtracting it from the signal is not adequate for long term measurements, during which the background drifts.

A system for dynamic background subtraction was then devised (Figure 1), i.e. a system in which the microflow and background are measured simultaneously. Therefore, any background variation is accounted.

This goal was accomplished using a mechanical chopper for the microflow modulation and a Lock-in amplifier to demodulate the signal acquired by the mass spectrometer. This system was able to measure the contribution of a partial pressure of 10^{-7} mbar of nitrogen in a total pressure of 10^{-5} mbar with a common quadrupole based residual gas analyzer.

An evaluation of the system proved it with good linearity, repeatability and reproducibility. Its lower limit of detection was also evaluated for different gases.

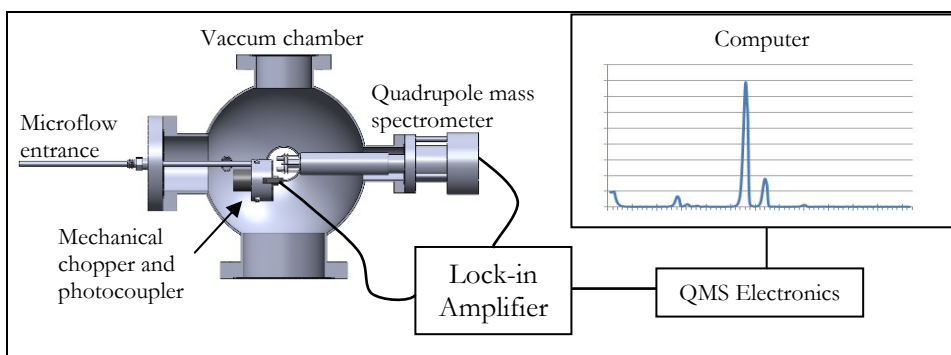


Figure 1. Mass spectrometry system with dynamic background subtraction.

Enhanced MALDI-FTICR MS analysis of glycated Fibrinogen peptides by methylglyoxal

P37

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Transthyretin (TTR) is an extracellular tetrameric protein found in serum and cerebrospinal fluid, described as a transporter of thyroxine and retinol (through binding to Retinol-Binding Protein, RBP)¹. In Familial Amyloid Polyneuropathy, TTR tends to aggregate mainly on the peripheral nervous system, leading to axon degeneration². Despite the fact that mutated TTR is required for FAP to develop, several observations, as the different disease onset age in patients carrying the same mutation (including identical twins), suggest a critical role for non-genetic factors. Therefore, when looking beyond genetic factors, our group described that serum TTR interacts with Fibrinogen, which is also increasingly glycated in FAP individuals³. Being recently characterized as a chaperone⁴, our group also found that Fibrinogen loses chaperone activity upon glycation by methylglyoxal³. We investigated Fibrinogen glycation sites upon *in vitro* glycation by methylglyoxal using MALDI-FTICR. Several MALDI matrices, specific proteases and advanced microchromatography techniques were used to achieve the highest possible sequence coverage. To unveil the role of Fibrinogen glycation in FAP we compared the location of AGEs in fibrinogen with the structural data from its recently established structure (figure 1)⁵.

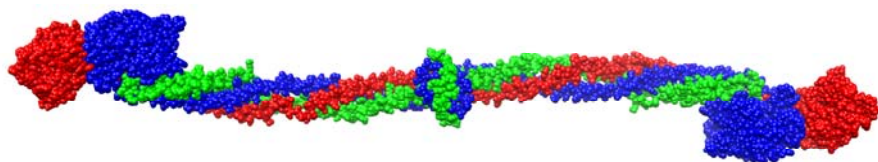


Fig 1. Crystal structure of human Fibrinogen (Protein Data Bank ID 3GHG).

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Development of a multi-residue method for the determination of pharmaceuticals in water by UPLC-(ESI)-MS/MS

P38

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Trace levels of pharmaceutically active substances have been detected in the aquatic environment which has raised great concern regarding the potential chronic adverse effects of these compounds in human health and ecosystems. These substances are commonly derived from municipal, agricultural and industrial wastewater sources and are only partially removed in the wastewater treatment processes, being therefore discharged to receiving surface waters, and subsequently may be found in ground and drinking waters.

In order to evaluate the occurrence of these compounds in Lisbon's drinking water supply, an UPLC-(ESI)-MS/MS method was developed.

The selection of the target pharmaceuticals was based on usage estimates and therefore twenty-eight compounds were chosen from several therapeutic classes, which presents challenges and compromises when applied as a single routine analysis.

Several parameters were optimised in order to get the best formation conditions of the precursor ion for each target compound, using an electrospray source. Two different precursor ion – product ion transitions were selected for each compound, one for quantification and one for qualification, and these ions were monitored under time scheduled multiple reaction monitoring (MRM) conditions, after optimisation of the collision cell energy of the triple quadrupole.

This method showed excellent linearity ranges for all compounds, with determination coefficients (r^2) between 0,9950 and 0,9999 with coefficients of variation lower than 5%.

Instrumental quantification limits (between 0,047 and 60 µg/L) and precision studies were also performed.

Identification and Structural Characterization of Polyphenols in *Myrtus Communis* L. Extracts by HPLC-DAD-ESI/MSⁿ

P39

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Myrtle (*Myrtus communis* L.) is an evergreen shrub belonging to the family of Mirtaceae that grows spontaneously throughout the Mediterranean area. Recent works have shown that myrtle tissues contain essential oils, polyphenols and hydrolizable tannins with important pharmacological and antimicrobial activity¹.

We report herein a method based on HPLC-DAD-ESI/MSⁿ to identify polyphenolic compounds in extracts of leaves of *Myrtus communis* L. collected from the area of Sintra (central west coast of Portugal). The bioactive flavonoids were extracted from leaf tissues by supercritical fluid extraction with carbon dioxide; the optimum extraction conditions were achieved at 48 °C and 10 MPa, the flow rate of CO₂ was maintained at 7x10⁻⁵ kg s⁻¹, and ethanol was used as co-solvent at 5% (w/w) concentration. In the present study, two groups of polyphenols present in the *Myrtus communis* L. extracts were separated and identified, namely galloylquinic acids and flavonoids glycosides. The compounds were identified by comparison of their retention times, UV-Vis spectrum and MS fragmentation patterns with those of standards and published results.

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We acknowledge Fundação para a Ciência e Tecnologia for financial support under the scope of the Contract REDE/1502/REM/2005.

Proteomics of c-kit and Sca-1 expressing populations of mice cardiac stem cells | P40

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Heart failure and other heart diseases are the most important death causes in developed countries. When the heart fails, it heals by scar formation, which compromises its normal ability to pump sufficient blood to meet the metabolic demands of the body¹. Stem cell-based therapy using Cardiac Stem Cells (CSCs) is a very promising approach for myocardial repair. As CSCs are a recent discovery², it is essential to identify the proteins involved in the mechanisms of mobilization, differentiation and proliferation³. In this study, a precision proteomics⁴ based approach using MALDI-FTICR allowed the identification of 122 proteins in CSCs. Mouse heart CSCs expressing the stem cell markers Sca-1 and c-Kit were isolated by Fluorescence Activated Cell Sorting. Nuclei, membrane and whole cell fractions were isolated by differential centrifugation. Proteins were then separated by 1D-SDS-PAGE, sliced in 1 mm lanes and digested in gel with trypsin⁵. Identifications were achieved by peptide mass fingerprint using Mascot as search engine.

Uncharacterized proteins were found in both cell types, together with proteins involved in the proliferation pathways common to both populations (Protein Chibby Homolog 1), specific to c-kit⁺ cells (Alpha Enolase) or Sca-1⁺ cells (47 kDa heat shock protein). Immunity system specific proteins were also identified (Protein 100-A8), suggesting a possible myeloid origin of CSCs. Differentiation involved proteins as Sortilin were identified in the Sca-1⁺ population, which is the most committed CSC population. Also, several protein involved in chemotactic reaction were identified, as is the case of Myristoylated Alanin-Rich C kinase substrate.

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Respiratory chain complexes susceptibility to oxidation and nitration | P41

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Mitochondria are the engines that drive pumping action of the heart. In this organ two populations of mitochondria are described according to their location in the myofiber, with distinct morphological and biochemical properties^{1, 2}. In order to understand the influence of myofiber location in the mitochondria functionality and susceptibility to oxidative and nitrative stress, pure fractions of subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria were isolated from cardiac tissue and further purified in a Percoll gradient, following a methodological approach previously described³. Mitochondrial respiratory chain complexes were separated by BN-PAGE and their susceptibility to nitration and /or carbonylation was assessed by 2D-BN-PAGE followed by western blotting analysis or LC-MS/MS analysis and correlated with OXPHOS activity. Our data evidenced higher OXPHOS activity of IMF compared to SS, paralleled by distinct membrane proteins susceptibility to oxidative damage. Indeed, membrane proteins from mitochondria located beneath the sarcolemma were more prone to carbonylation while the ones from mitochondria trapped in the myofibrils were more susceptible to nitration. Among the preferential targets to posttranslational modifications, ATP synthase subunits alpha and beta were notoriously more carbonylated whereas complex III core 1 and 2 proteins were more nitrated. Our results suggest that unlike nitration, the overall effect of increased carbonylation of OXPHOS complexes subunits may be an underlying mechanism of cardiac mitochondria decreased functionality.

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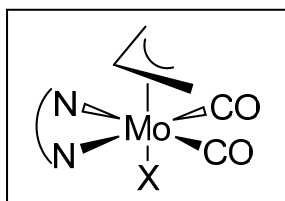
Mo(II) complexes as cytotoxic agents – shedding light on the active species | P42

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Transition metal complexes play a major role in many fields of chemistry, including their applications to medicine^{1,2}. In the last decades, after the success of *cis*-platin, *cis*-[PtCl₂(NH₃)₂], as antitumor agent, the interest in the use of transition metal complexes in medicine has grown rapidly. In particular, the search for new compounds that could overcome cell resistance and toxicity problems associated with platinum complexes led to the study of other metal containing antitumor drugs based on Ti, V, Nb, Mo and Re. A number of Mo containing molecules have since then been described to display cancerostatic activity^{3,4}.

Since Mo is an essential trace metal for organisms, playing a crucial role as cofactor for important enzymes, being transported and excreted as [MoO₄]²⁻, its low toxicity and effects on metabolism should make possible the use of complexes of this metal as therapeutic agents. Cell growth is slowed down by these compounds, so the inhibitory activity might be related to DNA damage probably due to direct action on DNA (e.g. intercalation in the double helix) or by oxidative action of ROS generated by chemical agents.



We describe here the *in vitro* activity of a series of Mo(II) complexes [Mo(η^3 -C₃H₅)X(CO)₂(N-N)] (N-N = bidentate ligand, X = halogen) against human cancer cell lines, cervical carcinoma (HeLa) and breast carcinoma (MCF-7), using a metabolic activity test (MTT). Although results showed significant antitumoral activity (several exhibit IC₅₀ values below 10 μ M) the nature of the actual active species has not yet been proven. MS experiments, which were by Electrospray (ESI-MS) and spectrums of MS and MS2 with CID

(collision induced dissociation), have been conducted to analyze the stability of Mo(II) complexes under *in vitro* conditions and evaluate the formation of new species.

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GC-MS characterization of the components in a mixture of Ibuprofen topic natural permeation enhancers obtained by Soxhlet and ASE from *Prunus lusitanica* L

P43

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Traditionally known in Portugal as "ginjeira brava" or "loureiro de Portugal", *Prunus lusitanica* L. (Figure 1) has never been studied through a "bioguided" strategy nor valued as a source of natural drug leaders, in spite of its potential as a terpene pool¹. There were found only less than 40 papers in literature concerning this plant, one on chemical studies leading to the identification and isolation of ursolic acid and aldehyde, and friedelin.¹ We report here the discovery of skin permeation enhancement properties² for ibuprofen from a major component in a Soxhlet extract (Figure 2) is presented, whose components were screened by NMR and GLC, and identified by GC-MS.³



Figure 1.

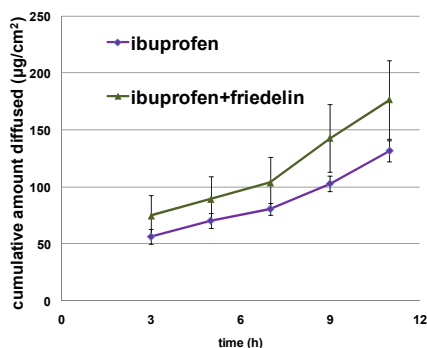


Figure 2.

Besides friedelin (ca. 69%), α - and β -amyrin (ca. 3% each) and minor constituents (<3 %), squalene, hexadecanoic acid, hexacosane, octacosane, nonacosane, 7-tetradecene, β -tocopherol, stigmastan-3,5,22-triene, handianol

and γ -elemene, a noticeable relative amount of 1.4% of 25-epiaplysterylacetate-1 ($C_{31}H_{52}O_2$) was present in the Soxhlet extract, but higher relative content (19%) was detected in a fraction obtained by accelerated solvent extraction (ASE) using the same solvent. Taking into account that ibuprofen for topical use in the skin has been marketed since the late 1990s with a better safety profile (local irritations being the most frequent adverse effects), but cutaneous administration has the disadvantage that a low systemic absorption is achieved after its application (about 5-10% from the concentrations reached when the oral route is used), and most of the drug is therefore wasted, we devise new perspectives in formulations of NSAIDs.

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**Characterization of stains caused by pressure-sensitive
adhesives on 19th and 20th centuries drawings by
THM-GC/MS**

P44

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A set of late 19th and early 20th century drawings stained by pressure-sensitive adhesive tapes were studied within a research project dedicated to the characterization of paper stains, and to the development of treatment methodologies for this kind of degradation.

The drawings belong to the collection of the Museu Bordalo Pinheiro, and were exhibited for a period of six decades, starting in the mid 1930s. At the present time, the drawings present adhesive staining due to the use of tape adhesives placed on the back of the drawings in order to support them.

Thirty four of these drawings were selected for this study. Each stain presented by the drawings was characterized according to its visual appearance and to properties, such as translucence or UV fluorescence, exhibited when illuminated by transmitted visible and UV lights. Based on these observations, the stains were grouped into five batches and then analysed by THM-GC/MS.

THM-GC/MS analyses allowed identifying the type of elastomer, tackifier and plasticizer from the pressure-sensitive adhesives' residues left in the drawings. Moreover, different nature or relative quantities of elastomer, tackifier and plasticizer were found for each one of the five groups of stains.

**CQM/UMa (REDE/1508/REM/2005) Node
Activities
(January 2010 - December 2010)**

P45

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A LC-ESI-IT/MSⁿ system (a system of Dionex UltiMate3000 fast separation liquid chromatography coupling with Bruker Esquire 6000 ion trap mass spectrometer) has been installed in CQM since 2007.

During the period of January 2010 - December 2010, a doctorate of analytical chemistry has been enrolled under the Strategic Program of Activities and Funding of the National Mass Spectrometry, with the responsibility of organization and management of the LC-MS instruments, developing new methodologies based on LC-MS for identification and structure elucidation for (bio)organic, inorganic and organometallic compounds, and providing the technical and scientific LC – MS services to CQM and external users.

As a summary, this poster describes 1) the organisation and management for the LC-MS operation and maintenance; 2) teaching/education activities; 3) research and scientific support provided to CQM members and external analytical tasks; and 4) publications and other scientific outcomes from the multiple disciplinary communications.

As one major focus of the ongoing and future work on LC-MS, the ESI - IT/MSⁿ determination of organometallic compounds and large (bio-) molecules is challenging.

Acknowledgements: We gratefully acknowledge the Portuguese “Fundação para a Ciência e a Tecnologia” (FCT) through the CQM pluriannual base funding (CHEM-Madeira-Funchal-674) and RNEM-REDE/1508/REM/2005 Contract.

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The Metabolomic Core Service purposes to implement advances in lipidomic profiling by providing investigators expert consultation and training for sample preparation and analysis by HPLC/MS/MS.

The equipment is comprised of two main instruments: Waters ACQUITY UPLC™ System with ACQUITY UPLC® Photodiode Array (PDA) Detector and Waters Quattro Premier™ XE benchtop tandem quadrupole mass spectrometer. The UPLC-MS/MS system and the complementary equipment acquired under the REEQ/997/SAU/2005 re-equipment project have allowed the establishment of a Metabolomic facility at IBMC in the year 2007.

The facility major current research topics are qualitative and quantitative LC-MS/MS analysis of key lipids from different biological materials (cells, tissues, serum, blood, urine) determining their basal levels and changes in response to the exogenous agents.

One of the first aims of the Metabolomic Facility is to acquire expertise in sphingolipid metabolites which act as bioactive molecules in signal transduction pathways, affecting cellular processes such as regulation of cell growth, differentiation and proliferation, inflammation, immunity, cancer and angiogenesis, among others.

As an example, we present some data were the levels of Globotriaosylceramide (Gb3), a glycolipid that accumulates systemically at Fabry disease, were measured in the tissues of mice models by tandem mass spectrometry and monitored during enzyme replacement therapy (ERT).

The gas-phase acidity of Trolox | P47

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A large body of phenolic compounds presents a good antioxidant action since they have the capacity to inhibit the oxidative action of free radicals¹. Thus it is crucial to understand the mechanisms involved. The phenolic compounds may transfer the hydrogen atoms, of the OH group, to the free radicals rendering them inactive, this being the most important antioxidant mechanism. As a result the antioxidant activity is related with the OH bond dissociation energy (BDE). This BDE can be determined, in the gas phase by the combination of the acidity of phenol, the electron affinity of the corresponding radical and the ionization energy of the hydrogen atom which is well known². The correlation of these energetic data with the structure and reactivity of these compounds may yield useful information on new antioxidants synthesis.

Trolox is an antioxidant, like vitamin E, and is used in biological or biochemical applications to reduce oxidative stress or damage. In the present study, the gas-phase acidity of trolox was determined by means of mass spectrometry applying the Cooks' kinetic method³. It was found that trolox is much more acidic than phenol. Theoretical calculations support that the deprotonation occurs in the carboxylic group.

These results will integrate a set of gas-phase acidity data on several phenolic compounds, chromanol, vitamin E, caffeic acid, in order to investigate the structure influence on acidity. It is also aimed to measure the electron affinity of phenoxyl radicals, which combined with gas-phase acidities will lead to the BDE of the OH bond, allowing a relationship between structure and antioxidant function.

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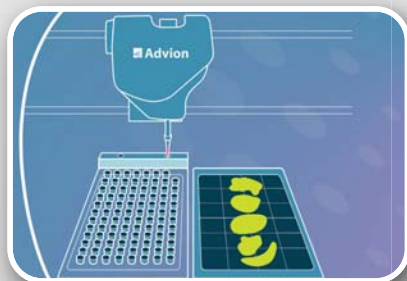
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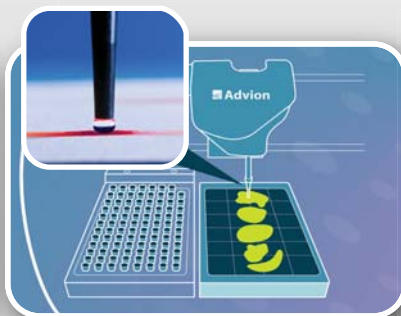
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- TOP-DOWN PROTEOMICS

The TriVersa NanoMate is a chip-based nanoelectrospray ionization source that combines the strengths of liquid chromatography, mass spectrometry, chip-based infusion, fraction collection, and direct surface analysis into one integrated system. It allows analysts to obtain more information from complex samples than with LC/MS alone.

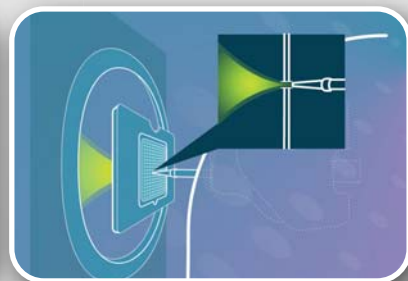
Now with Liquid Extraction Surface Analysis (LESA)



The TriVersa NanoMate picks up a pipette tip from the tip rack, then aspirates extraction solvent from the reservoir.



The robot brings the extraction solvent into contact with the surface of the sample. The analyte is extracted from the surface.



The solvent is retracted into the pipette tip and is analyzed by chip-based infusion.