BOOK OF ABSTRACTS

ST'2020

4th International Caparica Christmas Conference on Sample Treatment

Caparica | Portugal

30th November – 3rd December 2020

ST 2020

4th International Caparica Christmas Conference on Sample Treatment

ISBN: 978-989-54822-1-4
Author: José Luís Capelo
Co-author(s): Carlos Lodeiro Espiño, Hugo M. Santos , Gonçalo Martins.
Printed by: Proteomass Scientific Society (Portugal)
Printage: 25 Copies
Electronic support: 200 PDF/ PDF/A
Design: Hugo M. Santos & Gonçalo Martins
Webpage designer: Tomás Miranda

Poster designer: Tomás Miranda

Caparica - Portugal, 2020

©2020 BIOSCOPE Research Group | PROTEOMASS Scientific Society

BOOK OF ABSTRACTS

ST'2020

4th International Caparica Christmas Conference on Sample Treatment

Caparica | Portugal

30th November – 3rd December 2020

INDEX

INDEX 5
INDEX OF PRESENTATIONS 7
WELCOME ST'2020 11
CONFERENCE CHAIRS 17
ADJUNT CONFERENCE CHAIR 17
SCIENTIFIC COMMITTEE 19
LOCAL ORGANIZING COMMITTEE 21
CONFERENCE SECRETARIAT 23
SPONSORS AND PARTNERS 25
PROGRAM BY DAY 29
GENERAL INFORMATION 37
SPECIAL ISSUE - MICROCHEMICAL JOURNAL 39
ZOOM LINKS 41
AWARDS 47
VOTE FOR BEST POSTERS & SHOTGUNS 49
PLENARY LECTURES 51
KPRK TALK 61
KEYNOTE PRESENTATIONS 65
ORAL PRESENTATIONS 73
SHOTGUN PRESENTATIONS 111
POSTER PRESENTATIONS 129
AUTHOR INDEX 139
LIST OF PARTICIPANTS 144

INDEX OF PRESENTATIONS

PLENARY LECTURES 51

PL 1 – Planar sorptive phases, for microextraction and beyond 53

PL 2 – Sample treatment everywhere 54

PL 3 – Towards the influence of metal(-loid)s and metalloproteins in the gut(enteromammary gland)-brain axis 55

PL 4 – New ICP-MS-based strategies for the absolute quantification of proteins and posttranslational modifications 56

PL 5 – 3D printing in microscale extraction and separation science: Reality or utopia? 57

PL 6 – 3D Printing for Plasmonic Biosensing and Microfluidics-Enhanced Single Cell Analysis 58

PL 7 – Global quantitative proteomics using the FASP and TPA methods 59

KPRK TALK 61

KPRK 1 – Structural Biology on the Proteome-Wide Scale: An In-Cell Mass Spectrometry-Based Protein Footprinting Method 63

KEYNOTE PRESENTATIONS 65

KN 1 – Impact of new Raman lineshape in the analysis of paints and biological samples 67

KN 2 – Sample treatment strategies for the extraction and analysis of bioactive cannabinoids from natural products 68

KN 3 – Miniaturisation in bioanalysis: new tools and challenges 69

KN 4 – Omics technologies in the study of neurodegenerative diseases and diabesity comorbidity 70

KN 5 – Silica sequentially coated with polyhexamethyleneguanidinium and sulphonated reagents as universal platform for selective preconcentration and determination of metals 71

ORAL PRESENTATIONS 73

O.01 – Prevalence of equine herpesvirus 2 (EHV-2) in equine ocular disease 75

O.02 – Synthesis of nanoparticle-based hybrid monoliths and their potential in microextraction techniques 76

O.03 – Sampling and Analysis using GC-Ion Mobility Spectrometry 77

O.04 – Analysis of dried blood spots by direct coupling of liquid phase microextraction to capillary electrophoresis 78

O.05 – Volatile composition of Merlot red wine and its contribution to the aroma: optimization and validation of analytical method 79

O.06 – Extraction and analysis of pesticides in polyphenol-rich matrices by SFE, modified QuEChERS and organogel based methods: comparative study 80

O.07 – New PBI-adenine adsorber for API post-reaction stream purification 81

O.08 – Chiral separation of synthetic cathinones in gradient ultra-performance supercritical fluid chromatography coupled to mass spectrometry with a gradient flow of make-up solvent 82

O.09 – Il-based advanced techniques for the extraction of value-added compounds from natural sources and food by-products 83

O.10 – Vibrational Spectroscopic techniques applied to physicochemical characterization of honey 84

O.11 – Identification of terpenes and essential oils by means of static headspace gas chromatography-ion mobility spectrometry 85

O.12 – High-Throughput Measurement of Lipid Turnover Rates Using Partial Metabolic Heavy Water Labeling 86

O.13 – Simplest and label-free determination of intra- and extra-cellular vitamin C dynamics by HPLC-DAD 87

O.13B – Saliva-based Germline T790M Mutation Detection for Familial Inherited Lung Cancer 88

O.14 – Advanced microsampling and LC-MS/MS for the monitoring of patients with neurodegenerative disorders 89

O.15 – Fast and easy-to-use μTAD for creatinine determination in urine 90

O.16 – Determining the most important criteria of sampling and sample preparation applied in prohibited substances analysis from saliva 91

O.17 – EU-OPENSCREEN: Enhancing biochemical research 92

O.18 – Significant molecular and complex association descriptors in QSRR modelling of Green Liquid Chromatography using β-cyclodextrin mobile phases 93

O.19 – Long-term human metabolite of dehydrochloromethyltestosterone 94

O.20 – Determination of trace elements profiles by ED-XRF spectroscopy: a new validation strategy for organic matrices and applications to discriminate their geographical origin and botanical variety 95

O.21 – Multi-analytical profiling of archaeometallurgical samples – A new insight into ancient materials 96

O.22 – Handhelds for food authentication in the fight against food fraud 97

O.23 – A precise and accurate microfluidic droplet dilutor 98

O.24 – Simplified Sample Collection, -Transport and -Storage for Therapeutic Drug Monitoring of Small-Molecule Drugs 99

O.25 – Molecular and cellular FRET-based detection for protein-ligand engagement: the case of PDE5 100

O.26 – Sample preparation for simultaneous determination of phytosterols, cholesterol, and squalene in parenteral lipid emulsions 101

O.27 – New analytical approaches based on the coupling of Gas Chromatography to Mass Spectrometry for the quantitative characterization of energy- and environmental-related samples 102

O.28 – Open-Channel Microfluidics 103

O.29 – Immobilized enzyme technologies in protein phosphorylation and its analysis 104

O.30 – Application of molecularly imprinted polymers for bisphenols extraction from food samples 105

O.31 – SPE: Online, automatically renewable and integrated in -omic workflows 106

O.32 – On-line solid phase extraction using molecularly imprinted polymers and nanofibers coupled to high performance liquid chromatography 107

O.33 – Microfluidic Platform for Label-Free Sorting of Enzymes and Cells 108

O.34 – Simple and highly-available microextraction techniques using menthol 109

SHOTGUN PRESENTATIONS 111

SG.01 – Skin sampling with a flat solid-phase extraction probe followed by on-line mass spectrometric analysis 113

SG.02 – Mass spectrometric analysis of skin excretions by hydrogel-phase sampling followed by automated liquid-phase re-extraction 114

SG.03 – Polyamide coated wooden toothpicks for the determination of drugs of abuse in saliva by DI-MS 115

SG.04 – Potential of air sampling device based on a computer fan for the isolation of volatile organic compounds and nanoparticles 116

SG.05 – Supramolecular solvent-based liquid phase microextraction prior to HPLC-UV determination of sulphonamides in biological fluids 117

SG.06 – Application of hydrogels for bisphenols extraction 118

SG.07 – Impact of sample collection device type on analyte concentration for the HPLC-MS/MS analysis of commonly abused substances from oral fluid 119

SG.08 – Quantitative assessment of cellular uptake and differential toxic effects of HgSe NPs in human cells 120

SG.09 – Determination of mercury in sea waters by magnetic dispersive solid phase extraction prior to quantitation by FI-CV-GFAAS 121

SG.10 – Semiautomatic method for the ultra-trace arsenic speciation in environmental waters via magnetic solid phase extraction prior to HPLC-ICP-MS determination 122

SG.11 – Influence of gut microbiota in the distribution of selenoproteins and selenometabolites in different organs of mice model 123

SG.12 – Human breast milk metabolomic profiling in lactating women with iodine deficiency by UPLC-QTOF-MS 124

SG.13 – Fecal metabolomics approach to evaluate the influence selenium on mice metabolism and gut microbiota 125

SG.14 – Effect of erosive challenge and cigarette smoke on dentin microhardness, surface morphology and bond strength 126

SG.15 – Development of an on-line MSPE-ICP-OES method for the preconcentration and speciation of Cr(III)/Cr(VI) in aqueous samples 127

POSTER PRESENTATIONS 129

P.01 – Comparative study of various sorbents for determination of five bisphenols in milk using on-line solid phase extraction coupled to liquid chromatography 131

P.02 – CO2 efficient hydrogenation into methanol and dimethyl ether 132

P.03 – Alternative methods for upgrading low value sub products and circular economy 133

P.04 – Elucidation of the anticancer mechanism of vitamin C 134

P.05 – Application of primary amine-based supramolecular solvents for the analysis of biological fluids 135

P.06 – Formation of regulated an emerging disinfection by-products in a water treatment plant with ozonation treatment 136

P.07 – Development of a new analytical method for foodomics based on hollow-fiber-liquid phase microextraction (HF-LPME) applied to human breast milk 137

WELCOME ST'2020

4th International Caparica Christmas Conference on Sample Treatment

My career is a constant challenge to do things in the laboratory faster, with high-throughput, cheaper and matching the analytical minimalism rules as explained by halls [1]. I started my PhD with the application of ultrasonics energy to make solid-liquid extraction of metals from biological matrices faster and more efficcient than with microwave energy. Remarkably, we were able to do it for a number of metals. But most interestingly, we were able to move the methodology a step forward towards metal speciation rather than to total metal determination. Thus, the current methods to speciate metals from biological matrices using ultrasound energy were pioneered by my team and co-workers. But our approach did not stop in metal speciation and we were able to deliver one of the fastest methods to cleavage complex proteomes to be interrogated via gel electrophoresis or chromatography, first using MALDI-MS as the technique of protein identification or ESI-MS/MS to develop label free shot gun quantitative proteomics. One of our latest contributions joins ultrasound energy and fast assisted sample preparation proteomics, FASP, in what we consider the universal method for sample treatment in proteomics [2]. In the way to these achievements, we have been able to develop new approaches to extract pesticides, PAHs

and toxins from biological and environmental samples, using ultrasonic energy and gas chromatography and DAD-HPLC as methods of detection.

During my 25 years of career, what incredible moments of science we have passed through!! Yes, during these years I have had the privilege of mentoring human beings. Some are already teachers overseas, in places like the University of Texas or the Scripts in San Diego. As a reader of the Lord of the Rings since I was sixteenth, I like to think myself as one of the components of the company, the one created to deliver the Ring to the Mountain of the Destiny. Carlos Gandalf and Hugo Samwise have been my inseparable companions since the very begging. And in our trip, we have found, and we find, incredible people like Ana Mota, Isabel Moura, Jose Moura, Marco Arruda, Floro Riverola, Manuel Miro, and Jose Luis Ariza (the Master). Also, William Laframboise, Rajiv Dhir or Jacek Wisniewsky. What a privilege to meet and to work with all of them. All united with the same passion, do our job the best we can. And one of the results are the conferences named “Sample treatment”, where we share camaraderie, because this is what we are, comrades, whilst we mentor each other with our best in science. This little yet powerful conference is fueled by the greatest ones in this field of science. And as the time goes by, I witness how new teams and new ideas are created, how new friendships are made. This is the greatness of this conference, to be part of something bigger. It is in this context of hard work and camaraderie that on behalf of the Bioscope Group, I do welcome you to the 2020 edition of the sample treatment conference, where we are going to share the ultimate goal of creating new ideas.

Last but not least, thanks to all of your for participating. Also, my sincere acknowledgement to Gonçalo Martins, Hugo Santos, Carlos Lodeiro and Petro Andriyko for helping me to put this edition in the air, specially to Gonçalo who worked relentlessly during the last year.

Muito Obrigado.

Professor José L. Capelo Martínez.
On behalf of the BIOSCOPE Research Group.

4th ST 2020 Poster

Dear friends, we expect to maintain the high-quality atmosphere of discussion and exchange of excellent science and ideas as in previous editions, as shown in the pictures of the 2016 and 2018 editions shown below. We expect to be able to have the opportunity to join together next year.

3rd ST 2018

2nd ST 2016

The commitment to the internationalization of the 4th ST has been accomplished as 21 countries (70 attendees online, Figure 2). The Sharing of knowledge is thus guaranteed.

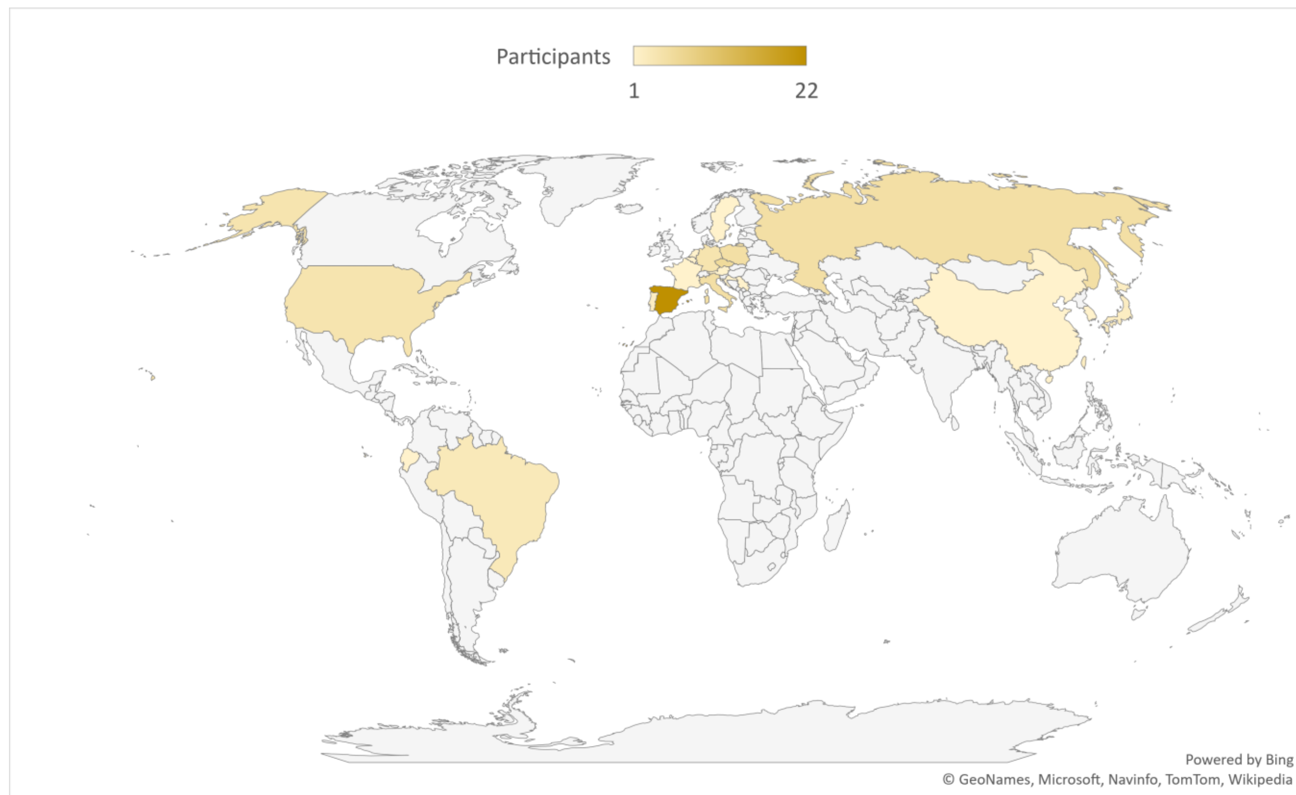


Figure 2. The Sharing of knowledge is thus guaranteed.

4th ST 2020 Attendees: - Spain (22) ; Czech Republic (6); Italy (5); Poland (5); Russia (5); United States of America (4); Germany (4); Brazil (3); Portugal (2); Taiwan (2); Japan (2); Belgium (2); South Korea (1); Sweden (1); Serbia (1); The Netherlands (1); France (1); Ecuador (1); China (1); Croatia (1) and Austria (1).

Finally, on behalf of the organizing committee, the scientific committee, the people of Caparica, the Dean of the NOVA School for Science and Technology of the NOVA University Lisbon, and the President of the Chemistry Department FCT-UNL, we wish you a pleasant stay at home. Keep safe.

José Luis Capelo, Hugo M. Santos, Carlos Lodeiro, Elisabete Oliveira, Javier Fernández Lodeiro, Adrián Fernández Lodeiro, Susana Jorge, Gonçalo Marcelo, Rafael Bento, Luís Ferraz, Frederico Duarte, Gonçalo Martins, Luís Carvalho, Tomás Miranda, Petro Andriyko

BIOSCOPE Research Group | LAQV-REQUIMTE, FCT- NOVA University Lisbon, Portugal

CONFERENCE CHAIRS

José Luis Capelo Martínez| MSc | PhD | FRSC

Head of the Bio-analytics & Proteomics Laboratory

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry

NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Chairman of the PROTEOMASS Scientific Society

e-mail: jlcm@fct.unl.pt

Carlos Lodeiro Espiño | MSc | PhD | FRSC

Head of the Chemistry & Nano-synthesis Laboratory

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry

NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

CEO of the PROTEOMASS Scientific Society

e-mail: cle@fct.unl.pt

Hugo Miguel Santos | MSc | PhD | PhD | MRSC

Head of the Laboratory for Biological Mass Spectrometry & Functional - Associations of Post-Translational Modifications - Head of the Laboratory for Biological Mass Spectrometry – Isabel Moura - BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry - NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal) - Chief Proteomics Technology Officer PROTEOMASS Scientific Society

e-mail: hmsantos@fct.unl.pt

ADJUNT CONFERENCE CHAIR

Gonçalo Martins | MSc

NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

e-mail: g.pinto@bioscopegroup.org

SCIENTIFIC COMMITTEE

José Luis Capelo Martínez| MSc | PhD | FRSC

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Carlos Lodeiro Espiño | MSc | PhD | FRSC

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Hugo Miguel Santos | MSc | PhD | PhD | MRSC

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Elisabete Oliveira | MSc | PhD | PhD

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Javier Fernández-Lodeiro | MSc | PhD

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Adrián Fernández-Lodeiro | MSc | PhD | MRSC

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Gilberto Igrejas | PhD

University of Trás-os-Montes and Alto Douro, ECVA-DGB, LAQV-REQUIMTE (Portugal)

Marco Zezzi Arruda | PhD
Universidade de Campinas (Brazil)

Manuel Miró | PhD
University of the Balearic Islands, Palma de Mallorca (Spain)

Jorge Ruiz Encinar | PhD
University of Oviedo (Spain)

Tamara García-Barrera | PhD
University of Huelva (Spain)

Rafael Lucena | PhD
University of Córdoba (Spain)

Jacek Wisniewski | PhD
Max-Planck-Institute of Biochemistry (Germany)

José Luis Gomez Ariza | PhD
University of Huelva (Spain)

LOCAL ORGANIZING COMMITTEE

José Luis Capelo Martínez| MSc | PhD | FRSC

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Carlos Lodeiro Espiño | MSc | PhD | FRSC

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Hugo Miguel Santos | MSc | PhD | PhD | MRSC

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Elisabete Oliveira | MSc | PhD | PhD

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Javier Fernández-Lodeiro | MSc | PhD

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Adrián Fernández-Lodeiro | PhD | MRSC

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Gilberto Igrejas | PhD

University of Trás-os-Montes and Alto Douro, LAQV-REQUIMTE (Portugal)

Susana Jorge | MSc

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Gonçalo Marcelo | MSc

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Gonçalo Martins | MSc

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Luis Carvalho | MSc

BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Tomás Miranda | MSc

PROTEOMASS Scientific Society (Caparica, Portugal)

Petro Andriyko

PROTEOMASS Scientific Society (Caparica, Portugal)

CONFERENCE SECRETARIAT

José Luis Capelo Martínez| MSc | PhD | FRSC
BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Carlos Lodeiro Espiño | MSc | PhD | FRSC
BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Hugo Miguel Santos | MSc | PhD | PhD | MRSC
BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Gonçalo Martins | MSc
BIOSCOPE Research Group, LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, NOVA University Lisbon (Caparica, Portugal)

Petro Andriyko
PROTEOMASS Scientific Society (Caparica, Portugal)

E-mail: jlcapelom@bioscopegroup.org
E-mail: jlcapelom.sampletreatment2020@bioscopegroup.org
Phone: +351 919 404 933 / +351 916 949 133
Web: https://www.sampletreatment2020.com/

©2020 BIOSCOPE Research Group | PROTEOMASS Scientific Society

SPONSORS AND PARTNERS

SPONSORS

Bruker
www.bruker.com

PROTEOMASS Scientific Society
www.proteomass.org

PROTEOMASS
scientific society | www.proteomass.org

Turismo de Portugal
www.turismodeportugal.pt

Turismo de Lisboa
www.visitlisboa.com

LaborSpirit
www.laborspirit.com

Paralab
www.paralab.pt

NORLEQ
https://norleq.com

Castelbel
www.castelbel.com/en/

Nan@rts
www.nanoarts.org

PARTNERS

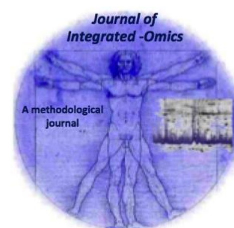
NOVA School for Science and Technology
NOVA Univeristy Lisbon
www.fct.unl.pt

LAQV@REQUIMTE
https://laqv.requimte.pt

Sociedade Portuguesa de Química
www.spq.pt

Câmara Municipal de Almada
www.m-almada.pt

Journal of Integrated OMICS
www.jiomics.com



TAP – Air Portugal
www.flytap.com

BIOSCOPE Research Group
www.bioscopegroup.org

www.bioscopegroup.org
BIOSCOPE
in the forefront of science

Caparica Científica
www.bioscopegroup.org

CAPARICA CIENTÍFICA®
BIOSCOPE Research Group | PROTEOMASS Scientific Society
www.bioscopegroup.org

PROGRAM BY DAY

Monday | November 30th		
ZOOM:	https://us02web.zoom.us/j/88495552274?pwd=SDhBMG95ZVpoc0owS25vTnArUFF4UT09	
Meeting ID:	88 495 552 274	
Password:	448928	
Chairperson:	José L. Capelo	
13:00 - 13:10	Oppening Sesion	
13:10 - 13:30	Plenary Lecture 1. Rafael Lucena (Spain)	
13:30 - 13:50	Plenary Lecture 2. Marco Arruda (Brazil)	
13:50 - 14:00	*Break*	
Chairperson:	José L. Capelo	
	SHOTGUN SESSION I	
14:00 - 14:05	SG.03	*Jaime Millán Santiago (Spain)*
14:05 - 14:10	SG.04	*Francisco Antonio Casado Carmona (Spain)*
14:10 - 14:15	SG.05	*Polina Bogdanova (Russia)*
14:15 - 14:20	SG.06	*Anna Kubiak (Poland)*
14:20 - 14:25	SG.07	*Łukasz Sobczak (Poland)*
14:25 - 14:35	*Break*	
Chairperson:	José L. Capelo	
14:35 - 14:50	Keynote 1. Alberto Tagliaferro (Italy)	
14:50 - 15:00	O.01	*Arnaud Lubin (Belgium)*
15:00 - 15:10	O.02	*Maria Cárdenas-Aranzana (Spain)*
15:10 - 15:20	O.03	*Wolfgang Vautz (Germany)*
15:20 - 15:30	O.04	*Pavel Kuban (Czechia)*
15:30 - 15:40	*Break*	

Chairperson:		José L. Capelo & Hugo M. Santos
15:40 - 15:50	O.05	*Stefany Grutzmann Arcari (Brazil)*
15:50 - 16:00	O.06	*Jean-Christophe Garrigues (France)*
16:00 - 16:10	O.07	*Teresa Esteves (Portugal)*
16:10 - 16:20	O.08	*Michal Kohout (Czechia)*
16:20 - 16:30	O.09	*María Carmen Garrigós (Spain)*
16:30 - 16:40	O.10	*Ofélia Anjos (Portugal)*
16:40 - 16:50	O.11	*Roman Maecker (Ecuador)*

Tuesday | December 1st		
ZOOM:		**https://us02web.zoom.us/j/88904529789?pwd=TWZGOXk5Q2dHL3dDR0xsMmVMeUE3UT09**
Meeting ID:		**88 904 529 789**
Password:		**372382**
Chairperson:		José L. Capelo
09:00 - 09:20		Plenary Lecture 3. Tamara García-Barrera (Spain)
09:20 - 09:35		Keynote 2. Federica Pellati (Italy)
09:35 - 09:50		Keynote 3. Laura Mercolini (Italy)
09:50 - 10:00	O.12	*Tae-Young Kim (South Korea)*
10:00 - 10:10	O.13	*Taiki Miyazawa (Japan)*
10:10 - 10:20	O.13B	*Yanhong Shang (China)*
		PACIFIC SHOTGUN & POSTER SESSION
10:20 - 10:25	P.01	*Aneta Kholová (Czechia)*
10:25 - 10:30	P.02	*Pavel Samokhin (Russia)*
10:30 - 10:35	P.03	*Gema Rodríguez Moro (Spain)*
10:35 - 10:40	P.04	*Ayano Mukaida (Japan)*
10:40 - 10:45	P.05	*Christina Vakh (Russia)*
10:45 - 10:50	P.06	*Antonio Dominguez Tello (Spain)*
10:50 - 10:55	P.07	*Mayka Villegas-Álvarez (Spain)*
10:55 - 11:00	SG.01	*Hsiang-Ting Ni (Taiwan)*
11:00 - 11:05	SG.02	*Decibel Elpa (Taiwan)*
11:05 - 13:15		*Break*

ZOOM:		https://us02web.zoom.us/j/84673828613?pwd=RGZkTTJnQzgrQlRIQW5RL28yT3BGQT09
Meeting ID:		84 673 828 613
Password:		762902
Chairperson:		José L. Capelo
13:15 - 13:35		Plenary Lecture 4. Jorge Ruiz Encinar (Spain)
13:35 - 13:45	O.14	*Michele Protti (Italy)*
13:45 - 13:55	O.15	*Miguel Erenas (Spain)*
13:55 - 14:05		*Break*
Chairperson:		José L. Capelo & Hugo M. Santos
14:05 - 14:15	O.16	*Krzysztof Goryński (Poland)*
14:15 - 14:25	O.17	*Federica Rossela (Germany)*
14:25 - 14:35	O.18	*Ana Protić (Serbia)*
14:35 - 14:45	O.19	*Mats Larsson (Sweden)*
14:45 - 14:55		*Break*
Chairperson:		José L. Capelo
14:55 - 15:10		Keynote 4. José L. Gomez Ariza (Spain)
SHOTGUN SESSION II		
15:10 - 15:15	SG.08	*Laura Cid Barrio (Spain)*
15:15 - 15:20	SG.09	*Juan Carlos García Mesa (Spain)*
15:20 - 15:25	SG.10	*Pablo Montoro Leal (Spain)*
15:25 - 15:30	SG.11	*Sara Ramírez Acosta (Spain)*
15:30 - 15:35	SG.12	*Ana Arias-Borrego (Spain)*
15:35 - 15:40	SG.13	*Belén Callejón-leblic (Spain)*
15:40 - 15:45	SG.14	*Marcela Ferreti (Brazil)*
15:45 - 15:50	SG.15	*Irene Morales-Benítez (Spain)*

15:50 - 16:00		*Break*
Chairperson:		José L. Capelo & Carlos Lodeiro
16:00 - 16:10	O.20	*Michele Ghidotti (Belgium)*
16:10 - 16:20	O.21	*Sanda Rončević (Croatia)*
16:20 - 16:30	O.22	*Sara Erasmus (Netherlands)*
16:30 - 16:40	O.23	*Witold Postek (Poland)*

Wednesday | December 2nd		
ZOOM:	https://us02web.zoom.us/j/82643270359?pwd=azBWcTRPVVZLQVBxY2p4ZUh3cXB5dz09	
Meeting ID:	82 643 270 359	
Password:	936584	
Chairperson:	José L. Capelo	
13:00 - 13:15	Keynote 5. Pavel N. Nesterenko (Russia)	
13:15 - 13:25	O.24	*Georg Hempel (Germany)*
13:25 - 13:35	O.25	*Giulia Di Rocco (Italy)*
13:35 - 13:45	O.26	*Ana Novak (Spain)*
13:45 - 14:00	*Break*	
Chairperson:	Hugo M. Santos	
14:00 - 14:20	Plenary Lecture 5. Manuel Miró (Spain)	
14:20 - 14:40	Plenary Lecture 6. Quan Cheng (USA)	
14:40 - 14:50	O.27	*Mariella Molodovan (Spain)*
14:50 - 15:00	O.28	*Jean Berthier (USA)*
15:00 - 15:10	*Break*	
Chairperson:	José L. Capelo & Hugo M. Santos	
15:10 - 15:20	O.29	*Marcela Slováková (Czechia)*
15:20 - 15:30	O.30	*Magdalena Biesaga (Poland)*
15:30 - 15:40	O.31	*David Jaime Solberg (Austria)*
15:40 - 15:50	O.32	*Ivona Lhotská (Czechia)*
15:50 - 16:00	O.33	*Paul Abbyad (USA)*
16:00 - 16:10	*Break*	
Chairperson:	José L. Capelo & Carlos Lodeiro	
16:10 - 16:20	O.34	*Irina Timofeeva (Russia)*
16:20 - 16:40	Plenary Lecture 7. Jacek R. Wiśniewski (Germany)	

16:40 - 17:00	KPRK Talk. Lisa Jones (USA)
17:00	Closing Session & Awards Ceremony

GENERAL INFORMATION

On-line Conference website

https://conferences.bioscopegroup.org/sample-treatment-2020-online-conference/
Password: **20OnlineST20#**

Conference Language

English is the ST 2020 official language. No simultaneous translation is provided.

Certificate of Attendance

The Certificate of Attendance will be sent to the delegates by e-mail.

Liability and Insurance

Registration fees do not include participants insurance against personal accidents, sickness and cancellations by any party, theft, loss or damage to personal possessions. ST 2020 and the Organization Secretariat accept no liability. Any dispute about payment and participation shall be governed and interpreted according to the laws of that in Portugal. The parties irrevocably submit to the jurisdiction of the courts of Portugal (Lisbon metropolitan area) concerning all disputes or matters arising out of or pertaining to the participation in this conference (ST 2020). Cancellations have to be made in writing form only. In the case of a cancellation 5 months before the conference, 90% will be refunding. Thereafter no refund will be made. If the conference needs to be cancelled or postponed by natural causes or any other cause not linked or due to the organisation committee, a voucher will be given to attend the next edition. No refund is made.

Program Changes

Due to circumstances beyond the control of the Organization and ST 2020, last minute changes to the programme may be unavoidable. All the information in this program is accurate as at the day of printing (November 27th, 2020).

Disclosure of Information

Proceedings Book is available for download at the conference website www.sampletreatment2020.com
Password for the book of abstracts: **20$20ST@Caparica**

Privacy Policy

The right to image is protected by the Constitution of the Republic of Portugal, and Law protects it. Please, do not take pictures or film any presentation, person or activity during ST 2020. No Photography or Videography please without permission of the author.

Follow Us:

@ ProteomassS

Proteomass Scientific Society

#Proteomasssociety

SPECIAL ISSUE - MICROCHEMICAL JOURNAL

A special issue is going to be published in the microchemical journal (Elsevier, impact factor 3.5) in Low Sample Volume Based Methods (analytical Minimalism). Any method requiring volumes ranging from micro liters to some milliliters, or tens of micrograms to tens of milligrams are welcome. Statistics methods ensuring the reliability of such methods are welcome. If you have any doubt, please do not hesitate in contacting me at your earliest convenience. Send us your intention to participate as soon as possible: jlcapelom@bioscopegroup.org.

https://www.journals.elsevier.com/microchemical-journal/

ZOOM LINKS

Monday, November 30th
13:00 – 16:50 Lisbon Time

Join Zoom Meeting

https://us02web.zoom.us/j/88495552274?pwd=SDhBMG95ZVpoc0owS25vTnArUFF4UT09

Meeting ID: 884 9555 2274

Passcode: 448928

One tap mobile

+16465588656,,88495552274#,,,,,,0#,,448928# US (New York)

+16699009128,,88495552274#,,,,,,0#,,448928# US (San Jose)

Dial by your location

+1 646 558 8656 US (New York)

+1 669 900 9128 US (San Jose)

+1 253 215 8782 US (Tacoma)

+1 301 715 8592 US (Washington D.C)

+1 312 626 6799 US (Chicago)

+1 346 248 7799 US (Houston)

Meeting ID: 884 9555 2274

Passcode: 448928

Find your local number: https://us02web.zoom.us/u/kvTKqDP8b

Tuesday, December 1st
09:00 -11:05 Lisbon Time

Join Zoom Meeting

https://us02web.zoom.us/j/88904529789?pwd=TWZGOXk5Q2dHL3dDR0xsMmVMeUE3UT09

Meeting ID: 889 0452 9789

Passcode: 372382

One tap mobile

+13462487799,,88904529789#,,,,,,0#,,372382# US (Houston)

+16465588656,,88904529789#,,,,,,0#,,372382# US (New York)

Dial by your location

+1 346 248 7799 US (Houston)

+1 646 558 8656 US (New York)

+1 669 900 9128 US (San Jose)

+1 253 215 8782 US (Tacoma)

+1 301 715 8592 US (Washington D.C)

+1 312 626 6799 US (Chicago)

Meeting ID: 889 0452 9789

Passcode: 372382

Find your local number: https://us02web.zoom.us/u/kc7A5h85bq

Tuesday, December 1st
13:15 -16:40 Lisbon Time

Join Zoom Meeting

https://us02web.zoom.us/j/84673828613?pwd=RGZkTTJnQzgrQlRIQW5RL28yT3BGQT09

Meeting ID: 846 7382 8613

Passcode: 762902

One tap mobile

+13017158592,,84673828613#,,,,,,0#,,762902# US (Washington D.C)

+13126266799,,84673828613#,,,,,,0#,,762902# US (Chicago)

Dial by your location

+1 301 715 8592 US (Washington D.C)

+1 312 626 6799 US (Chicago)

+1 346 248 7799 US (Houston)

+1 646 558 8656 US (New York)

+1 669 900 9128 US (San Jose)

+1 253 215 8782 US (Tacoma)

Meeting ID: 846 7382 8613

Passcode: 762902

Find your local number: https://us02web.zoom.us/u/kvsWvQR3G

Wednesday, December 2nd

13:00-17:00 Lisbon Time

Join Zoom Meeting

https://us02web.zoom.us/j/82643270359?pwd=azBWcTRPVVZLQVBxY2p4ZUh3cXB5dz09

Meeting ID: 826 4327 0359

Passcode: 936584

One tap mobile

+16699009128,,82643270359#,,,,,,0#,,936584# US (San Jose)

+12532158782,,82643270359#,,,,,,0#,,936584# US (Tacoma)

Dial by your location

- **+1 669 900 9128 US (San Jose)**
- **+1 253 215 8782 US (Tacoma)**
- **+1 301 715 8592 US (Washington D.C)**
- **+1 312 626 6799 US (Chicago)**
- **+1 346 248 7799 US (Houston)**
- **+1 646 558 8656 US (New York)**

Meeting ID: 826 4327 0359

Passcode: 936584

Find your local number: https://us02web.zoom.us/u/kcURgQWwpO

AWARDS

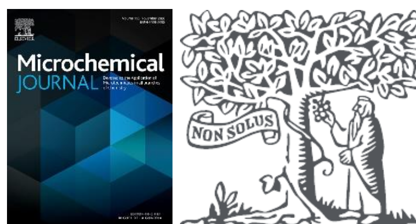
Best Poster Prizes

PROTEOMASS Scientific Society will award the two best poster communications with a certificate and a gift. The Criteria for eligibility will be based on the excellence and originality of the research presented. Selection will be done by an online voting. (Please find all poster in the online url and Book of Abstracts).

Best Shotgun Prizes

PROTEOMASS Scientific Society will award the two best shotgun communications with a certificate and a gift. The Criteria for eligibility will be based on the excellence and originality of the research presented. Selection will be done by an online attendee voting. (Please find all SG in the online url and the Book of Abstracts).

PROTEOMASS
Scientific Society | www.proteomass.org



VOTE FOR BEST POSTERS & SHOTGUNS

Best Poster Prizes – ST P01 – P07

VOTE FOR YOUR FAVOURITE POSTER!

The two best posters presented at the conference will be awarded. Selection criteria will be based on a combination of excellent research, innovation, and presentation. Poster presentation winners will be recognized online in the Closing Session & Awards Ceremony - Wednesday 2nd December at 17:00h. The winners will receive a certificate by PROTEOMASS Scientific Society and a gift.

Participants must vote for the best three posters.

FOR VOTING USE THE LINK

https://forms.gle/gPfE4UzKHiHNQbAv8

Best Shotgun Presentation Prizes –ST SG01-SG15

VOTE FOR YOUR FAVOURITE SHOTGUN PRESENTATION!

The two best shotgun presentations presented at the conference will be awarded. Selection criteria will be based on a combination of excellent research, innovation, and presentation. Shotgun presentation winners will be recognized online in the Closing Session & Awards Ceremony - Wednesday, December 2nd 17:00h. The winners will receive a certificate by PROTEOMASS Scientific Society and a gift.

Participants must vote for the best three shotgun presentations.

FOR VOTING USE THE LINK

https://forms.gle/kpaS8Ws3JTsDFas36

PLENARY LECTURES

ST'2020

4th International Caparica Christmas Conference on Sample Treatment

PL 1 – Planar sorptive phases, for microextraction and beyond

Rafael Lucena[1]*
[1]Departamento de Química Analítica, Instituto Universitario de Nanoquímica (IUNAN), Universidad de Córdoba, Córdoba, España.
*Email: rafael.lucena@uco.es

Abstract
Microextraction techniques evolved from classical extraction approaches following two main driving forces, namely simplification and miniaturization. These trends are applicable to the extraction workflows, but they can be extended to other collateral procedures like the synthesis of the sorptive phases required for analyte isolation. The use of natural materials as sorbents or inert substrates, where the actual active phases are immobilized, is a clear trend in our field [1]. In this communication, the main contributions of our group on the synthesis and design of paper-based sorptive media will be presented, making particular emphasis on the latest development.
Paper-based sorptive phases are very versatile, and they have been prepared following two different routes. Dip-coating has allowed the modification of the paper with commercial polymers, also permitting the coating with nanocomposites and nanoparticles (Figure 1). However, the rich -OH surface of cellulose can be used to bond some phases covalently thus increasing the chemical stability of the coatings. Both approaches will be discussed in detail in the present communication. The versatility of these phases will also be demonstrated with real applications. They can be applied in the classical extraction-chromatography-detection workflow, but other alternatives, like the direct combination to high-resolution mass spectrometry, are attractive to simplify the overall analytical procedure. As a way of example, the application of these materials in sorptive photocatalysis will also be commented on.

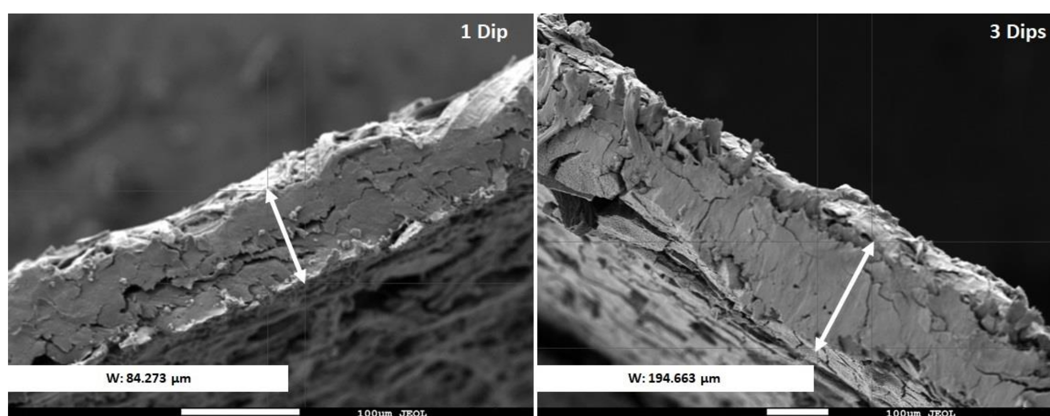


Figure 1. Single wall carbon nanohorns monolithic paper obtained after 1 and 3 dips.

References
[1] G. Mafra, M. T. García-Valverde, J. Millán-Santiago, E. Carasek; R. Lucena, S. Cárdenas, Separations 7 (2020) 2.

Acknowledgements
The financial support of the Spanish Ministry of Economy and Competitiveness through the grant CTQ2017-83175R made possible this research. Many researchers have been involved in this project and their efforts and contributions are acknowledged.

PL 2 – Sample treatment everywhere

M. A. Z. Arruda[1,2]*
[1]Spectrometry, Sample Preparation and Mechanization Group. Institute of Chemistry. University of Campinas – Unicamp, Campinas, Brazil.
[2]National Institute of Science and Technology for Bioanalytics Institute of Chemistry. University of Campinas – Unicamp, Campinas, Brazil.
*Email: zezzi@unicamp.br

Abstract
Sample preparation can be understood as any manipulation that modifies the sample matrix it is as old as any process that reveals one or more sample characteristics [1]. As stated, the best sample treatment is no sample treatment, but direct sample analysis is applicable to few samples and techniques [2]. Although it comprehends ca. 40% of the entire analytical process, new proposals focusing on microwave, lasers, ultrasound energy, among others, may push forward the creativity in terms of sample treatment, reducing drastically the costs and time involved in the process. In this sense, this work presents some examples involving, not only such energies, but also, samples as petroleum, coffee, water, and serum are emphasized.

As conclusion, accurate results are greatly dependent on the quality of the produced data, which are also greatly dependent on sample preparation. As almost the totality of analytical schemes is sample preparation-dependent, either new strategies or modifications on those ones already applied for such task are extremely salutary for an accurate interpretation.

[1] Trends in Sample preparation. Ed. M. A. Z. Arruda, Nova Science, N. York, USA, 304p.
[2] E. L. Lehmann, M. A. Z. Arruda, Anal. Chim. Acta, 1063(2019)9-17.

Key Words: microwave, extraction, ultrasound, petroleum, coffee, water, proteins.
Acknowledgements: FAPESP, CAPES, CNPq and Petrobrás are thankful.
Correspondence: GEPAM group. Institute of Chemistry, Analytical Chemistry Department. University of Campinas – UNICAMP. 13.083-862. Campinas. Brazil.

PL 3 – Towards the influence of metal(-loid)s and metalloproteins in the gut(enteromammary gland)-brain axis

Tamara García-Barrera[1]*, Nieves Abril[2], María Carmen Collado[3], José Luis Gómez-Ariza[1], Ana Arias Borrego[1], Sara Ramírez Acosta[1], Belén Callejón-Leblic[1]
[1]Research Center for Natural Resources, Health and the Environment (RENSMA). Department of Chemistry, Faculty of Experimental Sciences, University of Huelva, Campus El Carmen, Fuerzas Armadas Ave., 21007, Huelva, Spain. [2]Department of Biochemistry and Molecular Biology, University of Córdoba, Campus de Rabanales, Edificio Severo Ochoa, E-14071, Córdoba, Spain, [3]Institute of Agrochemistry and Food Technology (IATA-CSIC), Food Biotechnology, Agustin Escardino 7. 46980 Paterna, Valencia, Spain.
*Email: tamara@uhu.es

Abstract
Some metals are recognized causes of neurological disorders such as methylmercury, which is one of the most known neurotoxin. Moreover, the homeostasis of metals is also interconnected in response to the progression of neurodegenerative disorders. However, other elements and their species are essential and develop important neurological roles such as iodine (thyroid hormones) and selenium (selenoproteins), this last recognized as one of the most known antagonist of metals toxicity, which develop key roles against oxidative stress in neurodegenerative diseases. The role of metals in neurodegenerative disorders via gut microbiota alterations has also been pointed out [1], suggesting that this "hidden organ" acts as a barrier of metals input in the organism and also the colonization of gut microbiota can be shaped by them. Increasing evidence supports the importance of the so called enteromammary gland-brain axis in the mother-offspring microbiota transference to seeding the infant gut. In this context, selenoprotein P has been recently been identified in human breast milk [2], an important protein which transport selenium to the brain among other organs, affects hyperphosphorylated tau aggregation and amyloid-β peptide and possesses signalling functions though neuronal ApoER2 [3]. The hypothalamic-pituitary-thyroid can also be affected by environmental pollution, but in this case the homeostasis of essential iodine species (thyroid hormones) is disrupted by their interaction with other pollutants (persistent organic pollutants) [4].
Our work focus on the influence of heavy metals and essential elements in the host microbiota through the gut microbiota-brain axis. Current analytical methodologies for metaomics, metallomics and metabolomics are also applied, with special focus on heteroatom-tagged proteomics for the absolute quantification of metal or metalloid containing biomolecules using the metal as a "tag" in a sensitive and selective detector such as inductively coupled plasma mass spectrometry (ICP-MS). Integrated microbiome and fecal metabolomic analysis reveals a novel interplay between metals and microbiota. Moreover, we found important and new correlations between selenium and microbiota, demonstrating that Se shapes the composition, diversity and richness in antibiotic treated-mice fed Se supplemented diet. In mice fed Se supplemented diet there is no impact on diversity and richness, but we found some different bacterial groups.
Keywords: Metals; Selenium; neurodegenerative disorders; ICP-MS; gut microbiota

References
[1] T.C. Moulin, L.T.A. van Egmond, Sci. Total Environ. 693 (2019) 133639. [2] A. Arias-Borrego, B. Callejón-Leblic, G. Rodríguez-Moro, J.L. Gómez-Ariza, T. García-Barrera, Food Chemistry 321 (2020) 126692. [3] N. Solovyev, E. Drobyshev, G. Bjørklund, Y. Dubrovskii, R. Lysiuk, M.P Rayman, Free Radic Biol Med 127 (2018) 124. [4] K.J. Oliveira, M.I. Chiamolera, G. Giannocco, C.C. Pazos-Moura, T.M. Ortiga-Carvalho, J. Mol. Endocrinol. (2019) 62

Acknowledgements
This work was supported by the projects PG2018-096608-B-C21 from the Spanish Ministry of Economy and Competitiveness (MINECO) and UHU-1256905 from the FEDER Andalusian Operative Program 2014-2020 (Ministry of Economy, Knowledge, Business and Universities, Regional Government of Andalusia, Spain). Authors are grateful to FEDER (European Community) for financial support, Grant UNHU13-1E-1611. Authors would like to acknowledge the support from The Ramón Areces Foundation (ref. CIVP19A5918).

PL 4 – New ICP-MS-based strategies for the absolute quantification of proteins and posttranslational modifications

Jorge Ruiz Encinar[1]*, Francisco Calderón-Celis[1], Alicia Jiménez Nosti[1] and Juan Jose Calvete[1]
[1]Department of Physical and Analytical Chemistry, University of Oviedo, Av. Julián Clavería 8, 33006, Oviedo, Spain. [2]Instituto de Biomedicina de Valencia, IBV-CSIC, Jaume Roig 11, 46010 Valencia, Spain.
*Email: ruizjorge@uniovi.es

Abstract
Tools that provide absolute quantification of biomolecules, particularly of proteins and their post-translational modifications, without needing suitable specific standards, are urgently demanded nowadays. Inherent analytical features of ICP-MS/MS have placed it as a versatile tool to address such quantification with hyphenated chromatographic (LC) separation. Generic determination of ICP-detectable elements present in biomolecules would enable their absolute quantification without resorting to specific standards.
However, ICP-MS-based quantification of biomolecules in speciation analysis has been limited so far by different reasons. Towering above them all stands the use of carbon containing mobile phases along most reversed-phase and HILIC separations, which significantly affects plasma ionization processes of heteroatoms. As a consequence, elemental response factors in LC-ICP-MS analysis are usually different at each moment of the gradient analysis, which renders the use of a generic element-containing standard for quantification impossible. The most common analitcial solution so far consisted on the use of post-column isotope dilution. Direct measurement of the isotope ratios of the target element in the compound of interest after post-column on line continuous addition of an enriched isotope solution, whose concentration and isotopic abundances are accurately known, can provide perfect correction of sensitivity changes along gradients and therefore, accurate quantification without the need for specific standards. We have extensively used this strategy to carry out the absolute quantification of the multiple protein toxins (up to 40) present in different venom samples using an enriched solution of 34S and ICP-MS/MS.1 Unfortunately, this strategy cannot be considered universal as it is only applicable to multi-isotopic elements, requiring in addition specific enriched isotopes for each one.
We have recently developed a novel and highly sensitive ICP-MS/MS approach for absolute quantification of every target biomolecule containing P, S, Se, As, Br, and/or I (e.g., proteins and phosphoproteins, metabolites, pesticides, drugs), under the same simple instrumental conditions and without requiring any specific and/or isotopically enriched standard.2 Optimized and controlled addition of carbon-containing gases to the plasma leads to the correction of carbon effect in the ionization of heteroatoms. It has proved to be a simpler, more sensitive, and cheaper way of addressing quantitative speciation analysis of biomolecules with LC-ICP-MS. Different gases (CH4 and CO2) were compared in terms of accuracy and detection limits.3 Different quantification approaches making use of both internal and external generic standards will be critically compared as well. Definitive proposed strategy will include in situ column recovery evaluation, which is critical to assure accurate results. Potential for real sample analysis was again demonstrated by the high sensitivity analysis of toxins present in snake venoms. Finally, multielemental speciation capabilities of the approach have been also demonstrated through P and S simultaneous analysis in phosphoproteomics. Simultaneous accurate determination of both absolute protein amount and corresponding phosphorylation degree for intact β-casein, and even impurity traces of κ and α-s1 isoforms present, has been successfully achieved using a simple mixture of inorganic P and S standards.

Keywords: Absolute Protein Quantification, ICP-MS/MS, Generic Quantification, Phosphorylation.

References

[1] F. Calderón-Celis et al., Journal of Proteomics 2017, 164, 33–42.
[2] F. Calderón-Celis et al., Chem. Commun. 2018, 54, 904–907.
[3] F. Calderón-Celis et al., Anal. Chem. 2019, 91, 1105–1112.

Acknowledgements
Financial support of the Spanish Ministry of Economy and Competitiveness through PID2019-109698GB-I00 and BES-2014-068032 (F.C.C.) is acknowledged.

PL 5 – 3D printing in microscale extraction and separation science: Reality or utopia?

Manuel Miró

FI-TRACE group, Department of Chemistry, University of the Balearic Islands, Spain
*Email: manuel.miro@uib.es. Website: www.fitrace.es

Abstract
3D printing is fast evolving as an additive manufacturing technique in (bio)analytical science because of the ample variety of materials and technologies currently available for highly affordable prototyping. This plenary lecture is aimed at surveying via representative examples in the bioanalytical and environmental arenas the current state of the art of 3D printing technologies (namely, fused deposition modelling, stereolithography, digital light processing, laser sintering and photopolymer inkjet printing) in the field of sample preparation and separation science.
Focus will be given to the potential of 3D printing in the design of novel platforms and configurations for miniaturization of solid-phase (micro)extraction procedures including millifluidic devices using:

I. Nanomaterials in tailor-made devices
II. Pristine polymerizable materials or modified physically pre or post-printing
III. Covalent modification of polymerized templates including integration of porous organic monoliths with potential surface modification

References

D.J. Cocovi-Solberg, P.J. Worsfold, M. Miró, Opportunities for 3D printed millifluidic platforms incorporating on-line sample handling and separation, TrAC - Trends Anal. Chem. 108 (2018) 13
D.J. Cocovi-Solberg, M. Rosende, M. Michalec, M. Miró, 3D Printing: The Second Dawn of Lab-On-Valve Fluidic Platforms for Automatic (Bio)Chemical Assays, Anal. Chem. 91 (2019) 1140
E.J. Correa-Carrasco, D. J. Cocovi-Solberg, J. M. Herrero-Martínez, E.F. Simó-Alfonso, M. Miró, 3D printed fluidic platform with in-situ covalently immobilized polymer monolithic column for automatic solid-phase extraction, Anal. Chim. Acta 1111 (2020) 40
H. Wang, D. J. Cocovi-Solberg, B. Hu, M. Miró, 3D printed micro-flow injection analysis platform for on-line magnetic nanoparticle sorptive extraction of antimicrobials in biological specimens as a front end to liquid chromatographic assays, Anal. Chem. 89 (2017) 12541
F. Li, M.R. Ceballos, S.K. Balavandy, J. Fan, M.M. Khataei, Y. Yamini, F. Maya, 3D Printing in analytical sample preparation, J. Sep. Sci. 43 (2020) 1854
I. H. Šrámková, B. Horstkotte, J.Erben, J. Chvojka, F. Švec, P. Solich, D. Šatínský, 3D-Printed Magnetic Stirring Cages for Semidispersive Extraction of Bisphenols from Water Using Polymer Micro- and Nanofibers, Anal.Chem., 92 (2020) 3964

Acknowledgements
Manuel Miró acknowledges financial support from the Spanish Ministry of Science, Innovation and Universities (MCIU) and the Spanish State Research Agency (AEI) through project CTM2017-84763-C3-3-R (MCIU/AEI/FEDER, EU).

PL 6 – 3D Printing for Plasmonic Biosensing and Microfluidics-Enhanced Single Cell Analysis

Alexander S. Lambert, Zhengdong Yang, Bochao Li and Quan Cheng*
Department of Chemistry, University of California, Riverside, CA 92521, USA
*Contact Author and Presenter: Prof. Quan Cheng
Email: quan.cheng@ucr.edu; Tel: (951) 827-2702
Lab Web: chenglab.ucr.edu; Twitter: @ChengLabUCR

Abstract
Three-dimensional (3D) printing has in recent years become one of the most important and exciting fabrication technologies and has experienced a fast growth in both academic research and industrial applications. Well-designed 3D printed architectures hold great promise in bioanalysis and biopreparation, as has been broadly demonstrated in the field of microfluidics. The ability to manufacture components with essentially no constraints on part geometry facilitates faster and more effective analytical method development. In this talk, we will present the design and fabrication of 3D printed optical components with plasmonic microarrays for surface plasmon resonance (SPR) biosensing [1,2]. Specifically, novel custom optical components, particularly those important to spectroscopy and imaging, are printed with high optical features using transparent resin. To overcome the limitation of surface roughness (>100 nm), which is far higher than optical-grade glass (<1 nm), we developed a new approach that combines 3D printing with PDMS molding to produce ultra-smooth optics directly from photopolymer resins. PDMS was used along with a printed component to create a mold of the object, followed by a post-molding smoothing procedure. This process greatly improved surface smoothness, resulting in high quality and ultra-smooth products. The printed prisms were tested in Kretschmann configuration of SPR imaging, as well as in localized SPR (LSPR) sensing. They showed excellent optical performance and sensitivity as compared to conventional glass prisms. In addition, 3D printed Dove prisms were fabricated for special optical settings, which reduce the cost of optical devices and improve their accessibility for broader applications, as dove prisms with unique angles showed lower resonance angle than common equilateral prisms, which yields less image distortion. Benchtop polishing and gold layer deposition was applied to ensure a low surface roughness in SPR biosensing. Plasmonic arrays with micro-dips were fabricated and the 3D printed prisms for SPR detection of a blood biomarker for cerebral concussion have been demonstrated.
In addition, we will discuss the latest work on 3D printed microfluidics for on-chip cell lysis and enrichment. This work aimed to improve single cell analysis by MALDI-based mass spectrometry. Lipid analysis, in particular lipid profiling, has been used to monitor ecotoxicity of herbicide exposure of aquatic microorganisms [3]. For this work, a micropillar structure was printed and incorporated into a microfluidic system where algae cells were lysed and separated. We used the system to detect and identify lipid alterations in *selenastrum capricornuium* exposed to herbicides. Statistical analyses of PCA and volcano plot have been performed, and results show this platform is highly effective for lipidomic research and can be further applied to analysis of other samples including bacterial and cancer cells.

References:
1. Hinman SS, McKeating KS, Quan Cheng, "*Plasmonic Sensing with 3D Printed Optics*", *Anal. Chem.* **2017**, *89* (23), pp 12626–12630.
2. Alexander Lambert, Santino Valiulis, and Quan Cheng, "*Advances in Optical Sensing and Bioanalysis Enabled by 3D Printing*", *ACS Sensors* **2018**, *3* (12), 2475-2591.
3. Peter V. Shanta, Bochao Li, Daniel D. Stuart, and Quan Cheng, "*Plasmonic Gold Templates Enhancing Single Cell Lipidomic Analysis of Microorganisms*", *Anal. Chem.* **2020**, *92* (9), 6213–6217.

PL 7 – Global quantitative proteomics using the FASP and TPA methods

Jacek R. Wiśniewski
Biochemical Proteomics Group, Department of Proteomics and Signal Transduction, Max-Planck-Institute of Biochemistry, Am Klopferspitz 18, D-82152 Martinsried, Germany
*Email: jwisniew@biochem.mpg.de

Abstract
Studying biological system and its perturbations requires information on organization and dynamics of proteome. This is commonly carried out by the LC-MS/MS based proteomics, in which sample preparation and quantitative analysis of the spectrometric data play an essential role.
Filter Aided Sample Preparation (FASP) is a flexible and efficient way of processing protein extracts for bottom-up proteomic analysis. The method repurposes centrifugal ultrafiltration concentrators for depletion of detergents, protein digestion and isolation of pure peptide fractions. FASP can be used for protein cleavage with different proteinases either using single enzymes or in a mode of consecutive multi-enzyme digestion (MED-FASP). The FASP methods are useful for processing of samples ranging in their sizes from sub-microgram to several milligram amounts of total protein. Therefore, FASP is applicable for analysis of minute amounts of laser capture micro-dissected tissue as well as for generation of large amount of peptides required for affinity enrichment of phospho- or glycopeptides. The multi-enzyme-strategy has been found to be a powerful tool for absolute proteomic analysis of clinical samples, such as, tissue biopsies and plasma.
Total Protein Approach (TPA) is a label- and standard-free method for absolute protein quantitation of proteins using large-scale proteomic data. The method relies on an assumption that total MS signal from all identified proteins in a dataset reflects the total protein in a sample and the MS signal from a single protein corresponds its abundance. The method offers a straightforward way to quantify thousands of protein per sample at their specific concentrations. A related method, the 'Proteomic Ruler' enables a conversion of protein abundances into copy per cell values. TPA and the 'Proteomic Ruler' are valuable means for studying dynamics of cell architecture.

KPRK TALK

ST'2020

4th International Caparica Christmas Conference on Sample Treatment

KPRK 1 – Structural Biology on the Proteome-Wide Scale: An In-Cell Mass Spectrometry-Based Protein Footprinting Method

Lisa M. Jones*
Department of Pharmaceutical Sciences, University of Maryland, Baltimore, MD
*Email: ljones@rx.umaryland.edu

Abstract
In recent years, protein footprinting coupled with mass spectrometry has been used to identify protein-protein interaction sites and regions of conformational change through modification of solvent accessible sites in proteins. The footprinting method, fast photochemical oxidation of proteins (FPOP), utilizes hydroxyl radicals to modify these solvent accessible sites. To date, FPOP has been used *in vitro* on relatively pure protein systems. We have further extended the FPOP method for *in vivo* analysis of proteins. This will allow for study of proteins in their native cellular environment and be especially useful for the study of membrane proteins which can be difficult to purify for *in vitro* studies. A major application of the *in vivo* method is for proteome-wide structural biology. In one such application, we used in-cell FPOP (IC-FPOP) to identify on and off targets of the anti-cancer drug Gleevec in triple negative breast cancer cells. By obtaining structural information on proteins across the proteome, we were able to distinguish the differences in the mechanism of action of Gleevec in different racial populations. We have further extended the FPOP method for analysis in *C. elegans*, a member of the nematode family. This allows us to study protein structure directly in animal model for human disease. These methods have the potential to become a powerful tool in the structural biology toolbox.

KEYNOTE PRESENTATIONS

ST'2020

4th International Caparica Christmas Conference on Sample Treatment

KN 1 – Impact of new Raman lineshape in the analysis of paints and biological samples

Alberto Tagliaferro[1,2]*, Massimo Rovere[1], Mattia Bartoli[1], Elisa Padovano[1], Mauro Giorcelli[3]
[1]DISAT, Politecnico Torino, Italy. [2]OntarioTechU, Canada. [3]IIT, Torino, Italy
*Email: alberto.tagliaferro@polito.it

Abstract
Raman spectroscopy is a powerful technique of common use in several fields of application. As it is often the case, however, once a spectrum is recorded further analysis is required to extract the targeted information. In performing this analysis two elements are critical: (i) the subtraction of the background, often due to luminescence contribution, and (ii) the choice of the lineshape used to perform peak decomposition. As per the first item we point out that a proper background subtraction is really critical as otherwise the decomposition might be strongly altered, leading to wrong outcomes. As per the lineshape issue, several lineshapes have been proposed (Gaussian, Lorentzian, Voigt, …) and here again the choice of the lineshape has a strong impact on the outcome of the analysis. Based on the fact that stretched exponential relaxation is a common phenomenon in disordered solids, we have recently developed a new lineshape [1], that has proven itself very useful in the decomposition of Raman spectra of carbon base disordered materials such as biochar. The lineshape is a GauLor one, i.e. an appropriate mixture of Gauss and Lorentz curves (Lorentzian shaped in the peak region, Gaussian shaped in the tails, see figure 1). As both paints (especially black paints powders but not only) and biological materials are partially or totally disordered (i.e. non-crystalline) systems, in the present talk we will discuss the use of the GauLor lineshape to the decomposition of a number of Raman spectra, with an additional focus on how a proper background subtraction can be performed and how it impacts on the info obtained from the analysis.

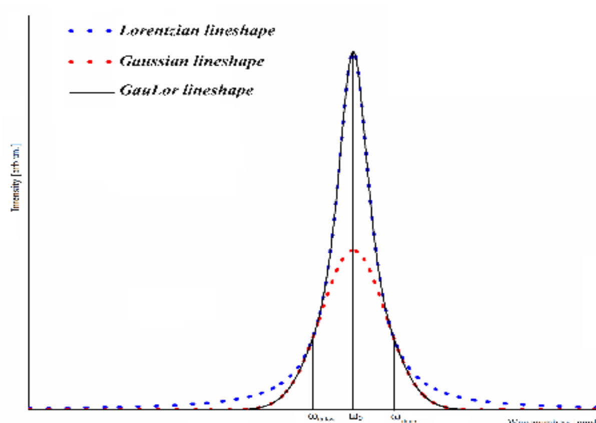


Figure 1. *The GauLor lineshape (Lorentzian shaped in the central region, Gaussian shaped in the tails)*

Keywords
Raman spectroscopy; Lineshapes; Background subtraction; Paints; Biological samples

References [1] Tagliaferro, A., Rovere, M., Padovano, E., Bartoli, M., & Giorcelli, M. (2020). Nanomaterials, 10, 1748.

KN 2 – Sample treatment strategies for the extraction and analysis of bioactive cannabinoids from natural products

Federica Pellati*
Department of Life Sciences, University of Modena and Reggio Emilia, Via G. Campi 103, 41125 Modena, Italy
*Email: federica.pellati@unimore.it

Abstract
In recent years, *Cannabis sativa* L. has been one of the most studied medicinal plant. The taxonomy of this plant has always represented a critical issue. Recently, a monotypic classification has been preferred, in which one species (*C. sativa*) is recognised and it is divided into different chemotypes, according to the specific cannabinoid profile. On the basis of the content in the psychoactive compound Δ^9-tetrahydrocannabinol (Δ^9-THC), it is possible to discriminate between drug-type *Cannabis*, which is rich in Δ^9-THC and it is used for medicinal purposes, from fibre-type *Cannabis* (commonly known as hemp or industrial hemp), which has a content of Δ^9-THC below the legal limit of 0.2% and it is mainly used for textile and food purposes.
The pharmaceutical interest in this plant has been mainly addressed at drug-type *Cannabis*, thanks to its multiple therapeutic applications, while hemp is at the moment under-employed in this ambit, even if it represents a rich source of non-psychoactive compounds, such as cannabidiol (CBD) and related molecules. Due to the complex chemical composition of *Cannabis* plant material and derived products, the increasing scientific interest in its sample preparation and analysis is noteworthy.
Cannabis-based medications are used for the treatment of different clinical conditions. Among galenic preparations, olive oil extracts from medical *Cannabis* are frequently prescribed for their ease of extraction and usage. In this context, this tudy was aimed at the chemical characterization of different medical *Cannabis* oils prepared by following both innovative and existing extraction protocols, with particular attention to their content of cannabinoids and terpenes, in order to set up a suitable method to obtain an extract rich in both chemical classes, due to the so-called "*entourage effect*". Different extraction procedures were followed, of which all but one included a decarboxylation of the plant material. The profile of cannabinoids was studied in detail in both the plant material and the oils by means of HPLC-ESI-MS/MS, while terpenes were characterized by means both GC-MS and GC-FID techniques coupled with solid-phase micro-extraction operated in the head-space mode (HS-SPME).
Due to the increasing number of studies focused on the characterisation of hemp and on the evaluation of the biological potential of its non-psychoactive cannabinoids, this work was also addressed at the optimization of the extraction conditions of these bioactive compounds from hemp inflorescences as well as at the analysis of the extracts by means of innovative methods, using both separation and non-separation techniques. In particular, the profiling of cannabinoids in hemp extracts was performed by HPLC-UV/DAD, ESI-MS and MS^2. As for non-separation analytical strategies, cannabinoids were analysed in the same extracts by means of a new method based on ^{13}C quantitative nuclear magnetic resonance spectroscopy (qNMR). The analytical approach for the determination of cannabinoids involved also an efficient preliminary screening method without the need for any sample preparation by means of attenuated total reflectance (ATR) Fourier transform infrared (FT-IR). In addition to the plant material, essential oils (EOs) belonging to different hemp varieties were fully analysed by means of GC-MS and GC-FID for their terpene and cannabinoid profiling. A GC-MS/MS method was developed and applied for the first time to quantify CBD in these EOs.
Finally, the analysis of cannabinoids in food and food supplements derived from hemp represents another critical issue for both the quality assurance and the dietary intake control of these biologically active compounds. In this ambit, a particular attention is necessary for apiary products, since they are widely consumed and they can be produced by bees starting from different floral sources. In the light of this, the last part of this study was aimed at the development of a new analytical method based on a HPLC-ESI-MS/MS for the determination of CBD and related cannabinoids in honey. A quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction procedure with an un-buffered method was selected and optimised as the more suitable protocol. As regards detection, it was carried out by using a linear ion trap quadrupole (QTRAP) mass analyser, operated in the multiple reaction monitoring (MRM) mode.

KN 3 – Miniaturisation in bioanalysis: new tools and challenges

Laura Mercolini*
Research group of Pharmaco-Toxicological Analysis (PTA Lab), Department of Pharmacy and Biotechnology (FaBiT), Alma Mater Studiorum - University of Bologna, Bologna (Italy)
*Email: laura.mercolini@unibo.it

Abstract

There is a growing interest in the implementation of miniaturised approaches for the determination of therapeutic drugs, drugs of abuse, metabolites and biomarkers in biological samples derived from nonclinical and clinical studies and involved in a wide range of applications and frameworks. This interest is due to the ethical advantages of sampling very minute amounts of biomatrices, particularly for those studies performed in delicate populations, like illicit drug users. Moreover, these technologies facilitate sampling to be performed in locations and occasions usually difficult to be reached. Finally, they enable the collection of samples for additional purposes (extra time points, stability tests, etc..) and allow for feasible, straight-forward and time- and cost-effective analytical protocols [1].

This lecture gives a comprehensive insight about the optimisation and implementation of these advanced strategies in bioanalytical method development and validation, also providing recommendations for best practice in a wide range of applications. In particular, the research group of Pharmaco-Toxicological Analysis (PTA Lab) of the University of Bologna (Italy) recently designed and developed a panel of novel, miniaturised protocols to be applied for the determination of classical drugs of abuse (including cannabinoids, cocaine and methadone together with their main metabolites), new psychoactive substances (NPS), doping agents (ranging from small molecules able to enhance physical performance like clenbuterol, steroids and glucocorticoids, to doping-relevant peptides), as well as prescription drugs.

The microsampling approaches fully designed and developed by PTA Lab include capillary volumetric blood microsampling, volumetric absorptive technologies and microfluidic strategies. These allow not only to collect microvolumes of biological matrices (including hematic samples, urine and oral fluid) in an accurate manner regardless of fluid density, but also able to guarantee sample integrity, subject compliance, a solid chain of custody and feasible, yet effective pre-analytical and analytical protocols [2]. As regards sample pretreatment, miniaturised variants of solid phase extraction have been designed and extensively optimised to be applied to a broad range of microsamples and are represented (among the others) by microextraction by packed sorbent, disposable pipette extraction and stop-and-go extraction.

All the factors and conditions involved in the sample collection, extraction and clean-up steps have been extensively evaluated in order to produce solid and robust protocols. This allowed to obtain a comparative evaluation of procedures and techniques offering peculiarities, advantages and challenges that could guide bioanalytical scientists towards the best miniaturisation choice in relation to the different application scenarios.

References

[1] L. Mercolini, M. Protti. J. Pharm. Biomed. Anal. 130 (2016) 202-219.
[2] M. Protti, R. Mandrioli, L. Mercolini. Anal. Chim. Acta. 1046 (2019) 32-47.

Acknowledgements

This research work was financially supported by the World Anti-Doping Agency (WADA) within the 2017 Research grants [Project 17A20LM] and the 2019 Research grants [Project 19A08LM].

KN 4 – Omics technologies in the study of neurodegenerative diseases and diabesity comorbidity

J.L. Gómez-Ariza[1,2]*, T. García-Barrera[1,2], M. García Alloza[3], A. Arias-Borrego[1,2], B. Callejón-Lebic[1,2], G. Rodríguez-Moro[1,2], S. Ramírez-Acosta[1,2]
[1]Department of Chemistry, Faculty of Experimental Science, Huelva, Spain. [2]Research Centre of Natural Resources, Health and the Environment (RENSMA), University of Huelva, Spain. [3]Division of Physiology. School of Medicine.
University of Cadiz
*Email: ariza@uhu.es

Abstract
The population of people over 65 years of age has grown significantly in the last 20 years and especially in developed countries, so that it is estimated that their number will reach 80 million in 2050. This population is progressively suffering a degradation of its vital functions, associated with the aging process, which leads to a series of pathologies such as arteriosclerosis and cardiovascular diseases, cancer, arthritis, cataracts, osteoporosis, type-2 diabetes, hypertension, and especially Alzheimer's disease (AD) and other neurodegenerative disorders. AD is perhaps the most prevalent suffering among the elderly, generally associated with other comorbidities such as type-2 diabetes and lens degeneration. All these pathological states are associated to processes of cellular senescence and to the control of the brain, which as a directing organ regulating the general state of the organism through allostatic balance.

Neurological diseases, particularly AD, have been normally associated to proteopathies and taupathies of the central nervous system (CNS). Therefore, most of the studies so far have been based on samples of cerebrospinal fluid and brain postmortem tissues from human or model organisms, such as APP/PS1 mice. The use of blood samples in AD studies has been traditionally relegated due to the difficulty to interpret the association between blood-based measures and brain processes. However, recent works have demonstrated close similarities in metabolomic abnormalities observed in serum and brain samples, stablishing growing evidence that AD might be a systemic disorder, which demonstrates the utility of peripheral samples in the study of pathological mechanisms associated with neurological disorders. Numerous metabolites and metal bound to biomolecules are altered during the progress of these disorders that induce changes on multiple essential pathways in the organism. The complexity of these changes requires the application of omics (metallomics and metabolomics) to delve into these pathologies and their diagnosis.

The application of high resolution mass spectrometry (QTOF-MS) introducing the sample by direct infusion (in metabolomic studies) and of inductively coupled plasma plus spectrometry (ICP-MS), generally coupled to liquid chromatography (HPLC-ICP-MS), for metallomic studies, have allowed to decipher the most important metabolic changes observed in the progression from mild cognitive impairment to AD. Degradation of brain phospholipids associated to cellular membranes together with these lipids fatty acid composition, decreasing the presence of unsaturated fatty acids and correlatively increasing the levels of saturated fatty acids. In addition, several plasmalogen species were also down-regulated during the development of AD.

Alterations in total concentrations of metals, as well as metalloproteins or labile species, could be related to the development of Alzheimer. The most significant changes were observed in manganese, aluminium, selenium and zinc, but also important alterations were found in relation to copper and iron homeostasis, as well as the low molecular mass fractions of several elements.

The application of metabolomics to cortex samples from double transgenic mice APP/PS1xdb/db in order to assess comorbidity of type-2 diabetes on AD. The most remarkable results are: Abnormal myo-inositol content whose homeostasis is of vital importance for the central nervous system, since its accumulation in brain tissue is a classic marker of neuronal death. Altered cholesterol levels related to the destabilization processes of neuronal membranes, due to the importance of this compound in the formation of lipid rafts and its participation in the homeostasis of cell membranes. On the other hand, the increase in cholesterol is related to the appearance of hyperlipidemia, one of the vascular risk factors traditionally associated with Alzheimer's disease.

KN 5 – Silica sequentially coated with polyhexamethyleneguanidinium and sulphonated reagents as universal platform for selective preconcentration and determination of metals

Pavel Nesterenko[1]*, Svetlana Didukh-Shadrina[2], Olga Buyko[2], Vladimir Losev[2]
[1]Chemistry Department, M.V. Lomonosov Moscow State University, Moscow, Russian Federation.
[2]Kristall Research and Engineering Centre, Siberian Federal University, Krasnoyarsk, Russian Federation
*Email: p.nesterenko@phys.chem.msu.ru

Abstract
The preconcentration of trace metals retains a strong interest in analysis of complex samples. A numerous types of various chelating resins with functional groups designed for group and individual metal selectivity have been developed. Usually the chelating resins are prepared by covalent bonding of specific organic reagents such iminodiacetic acid, 8-hydroquinoline, amidoxime and others onto the surface of suitable matrix [1]. The preparation of chelating resins is not trivial task and the properties of covalently bound reagents could be different from those observed for free form of these reagents. Also, a highly selective preconcentration of certain metal or few metals can be achieved only with application of specific resin, so normally a set of expensive chelating resins with various is required for different type of analysis.
The application of universal analytical platform for immobilisation of various organic reagents under mild conditions could be solution of above mentioned problem. Recently, a new platform PHMG@SiO_2 comprising porous silica matrix coated with a layer of physically adsorbed cationic polymer (polyhexamethyleneguanidine or PHMG) was designed for this purpose [2]. This simply prepared stable anion-exchange material can easily coated with a third layer of specific to certain metals sulphonated organic reagents including Arsenazo I, Tiron, Alizarine S, Nitroso-R-Salt and others [2,3]. In total, the adsorption of more than 150 organic various reagents have been investigated for the preparation of new selective adsorbents. The strong retention of organic reagents at the of PHMG@SiO_2 is due to electrostatic interactions between protonated primary and secondary amino-groups at the surface of PHMG@SiO^2 and dissociated sulpho-, carboxy- and arsenic acid groups in the molecules. Hydrogen bonding of amino- groups with phenolic and carboxylic groups can also impact in retention of organic reagents.
The regularities of adsorption of transition, heavy and rare-earth metals were studied for the prepared chelating adsorbents. The most selective adsorbents were selected and used for solid phase extraction of metals from complex matrices prior ICP-MS, ICP-OES and spectrophotometric detection. The capability of immobilised photometric organic reagents to form intensively coloured surface complexes with adsorbed metals has been used for determination of metals in the phase of the adsorbent by using diffusive reflectance spectrophotometry, luminescence and visual test-methods.

Keywords

Solid phase extraction; organic reagents; metals; chelation; silica based adsorbents

References

[1] P.N. Nesterenko, P. Jones, B. Paull, High Performance Chelation Ion Chromatography. RSC Chromatography Monographs No.14. 2011. 303p.
[2] V.N. Losev, O.V. Buiko, A.K. Trofimchuk, O.N. Zuy, Microchem. J. 123 (2015) 84-89.
[3] S.L. Didukh-Shadrina, V.N. Losev, A. Samoilo, A.K. Trofimchuk, P.N. Nesterenko, Int. J. Anal. Chem. (2019) 1467631.

Acknowledgements.

The authors would like to acknowledge the financial support of the Ministry of Science and High Education of Russian Federation (grant 4.6343.2017).

ORAL PRESENTATIONS

ST'2020

4th International Caparica Christmas Conference on Sample Treatment

O.01 – Prevalence of equine herpesvirus 2 (EHV-2) in equine ocular disease

Aikaterini M. Zisopoulou[1]*, Šárka Krisová[1]*, Katarína Tóthová[1]*, Dobromila Molinková[2]*, Zita Makra[3]*
[1]University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Medicine, Department of Equine Surgery and Diagnostic Imaging, Brno, Czech Republic. [2]University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Medicine, Department of Infectious Diseases and Microbiology, Brno, Czech Republic. [3]University of Veterinary Science Budapest, Department and Clinic of Equine Medicine, Budapest, Hungary
*Email: zisopouloua@vfu.cz

Abstract
Equine gammaherpesvirus 2 (EHV-2) has been linked to keratitis and keratoconjunctivitis but has also been isolated in horses showing no signs of disease. The aim of the current study was to assess the importance of EHV-2 infection in the aetiopathogenesis of ocular disease, where the applied treatment failed. Seventy-eight horses with nonhealing ocular disease were examined at the Equine Clinic of the University of Veterinary Medicine and Pharmaceutical Sciences, Brno, Czech Republic, between the years 2009 and 2016. In total, 96 conjunctival swabs were taken and, starting from 2014, peripheral blood leukocytes (PBLs) were also examined in 42 patients. Positive EHV-2 results were detected in 53 ocular swab samples (54.64%) and in 22 PBL samples (51.16%). The horses were divided into three groups according to age, up to 3 years, from 3 to 15 years and older than 15 years. Depending on the clinical presentation, horses were also divided into nonulcerative or ulcerative keratitis, keratouveitis, keratoconjunctivitis, and corneal degeneration groups. The group of young horses had a significantly higher ocular swab positivity compared to the middle group (P = 0.01). Increased bilateral ocular occurrence with decreasing age was observed, although it was not significant (P = 0.04). Significant correlation was confirmed between PBL samples and ocular swabs (P = 0.01). This correlation was even higher in cases of bilateral infection. No significant differences were detected when comparing the groups according to the clinical presentation. This study describes the prevalence of EHV-2 in different age group horses with non-healing keratopathies.

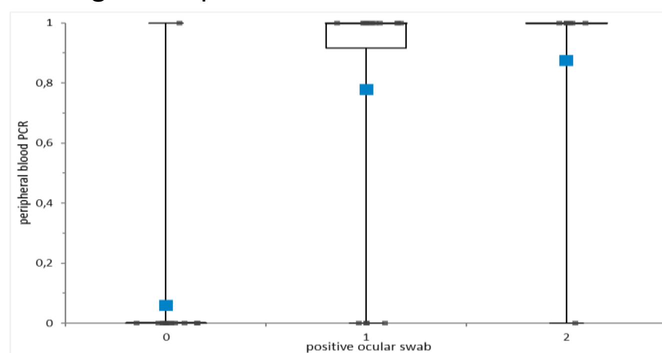


Figure 1. The relationship between PBLs PCR positivity and EHV-2 ocular infection (0 - negative ocular samples, 1- unilateral ocular infection, 2- bilateral ocular infection)

Keywords
Veterinary ophthalmology 1; keratitis 2; conjunctivitis 3; viral infection 4; PCR, horse 5;

References

S.R. Hollingsworth, N. Pusterla, P.H. Kass, K.L. Good, S.A. Brault, D.J. Maggs, Detection of equine herpesvirus in horses with idiopathic keratoconjunctivitis and comparison of three sampling techniques, Vet Ophthalmol 18 (2015) 416-421.
E.S. Hue, G.D. Fortier, C.I. Fortier, A.M. Leon, E.A. Richard, L.J. Legrand, S.L. Pronost, Detection and quantitation of equid gammaherpesviruses (EHV-2, EHV-5) in nasal swabs using an accredited standardised quantitative PCR method, J Virol 198 (2014) 18-25

Acknowledgements

This project was supported by the Internal Grant Agency of the Univerity of Veterinary and Pharmaceutical Sciences (project No. 97/2014 FVL).

O.02 – Synthesis of nanoparticle-based hybrid monoliths and their potential in microextraction techniques

Soledad Cárdenas* and Beatriz Fresco-Cala
[1]Departamento de Química Analítica. Instituto de Química Fina y Nanoquímica (IUNAN). Universidad de Córdoba. Córdoba. Spain
*Email: scardenas@uco.es

Abstract
The continuous and porous structure of monolithic solids makes them an excellent alternative to particulate sorbents as these figures maximize their interaction with the analytes being also compatible with the flow of the liquid samples through them. On the contrary, they are less competitive in terms of superficial area. To overcome this limitation, the combination of the monolithic solids with nanoparticles has been widely described in the literature. The inclusion of the nanostructured solids in the monolithic network (either embedded or anchored on the surface) synergically combines the advantageous properties of both materials in microextraction. The resulting hybrid material exhibits outstanding capabilities for isolating the target compounds from the samples.

This communication reviews the contribution made by the research group on the topic (Figure 1). Different synthesis pathways and microextraction formats will be briefly described. Concerning the synthesis, recent approaches developed using emulsions will be specifically highlighted. Regardless of the synthetic route selected, the hybrid monoliths can be easily adapted for being used in most of the usual formats of micro-solid phase extraction, including capillaries, pipette tips, spin columns, and stirred units.

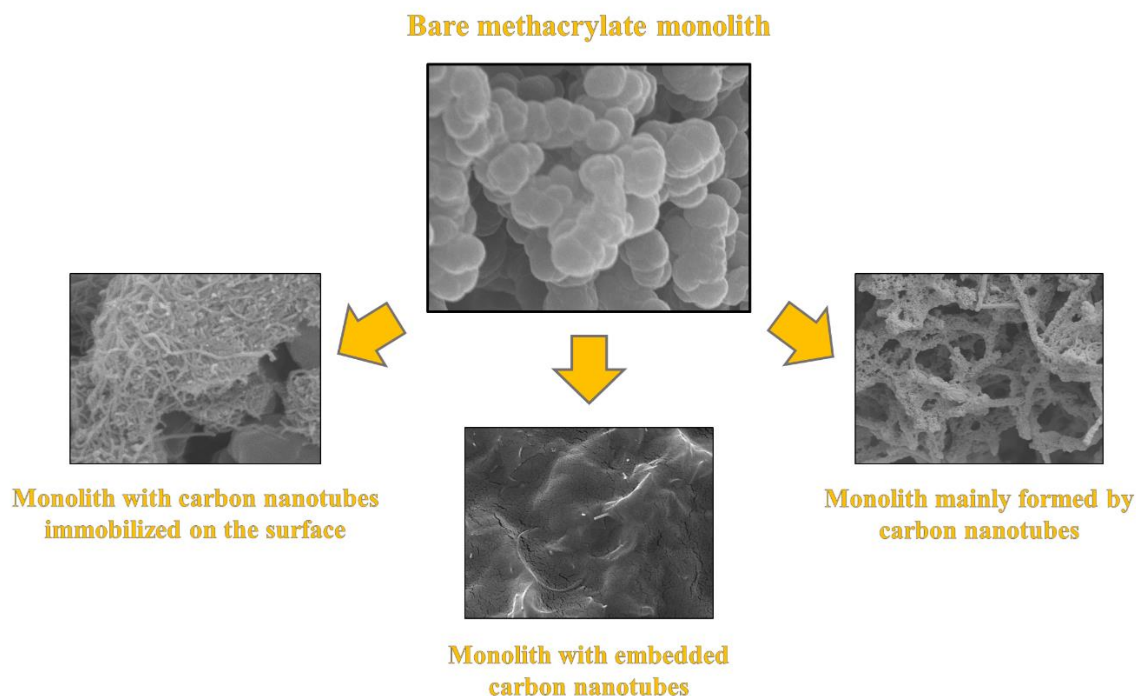


Figure 1. Different hybrid monolithic solids based on the combination of methacrylate polymers and carbon nanotubes.

Acknowledgements
Funding from Spain's Ministry of Economy and Competitiveness (CTQ2017-83175R) is gratefully acknowledged.

O.03 – Sampling and Analysis using GC-Ion Mobility Spectrometry

Wolfgang Vautz[1,2]*
[1]Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Bunsen-Kirchhoff-Straße 11, 44139 Dortmund, Germany
[2]ION-GAS GmbH, Konrad-Adenauer-Allee 11, 44263 Dortmund, Germany
*Email: wolfgang.vautz@isas.de / w.vautz@ion-gas.de

Abstract

Ion mobility spectrometry (IMS) is a fast and sensitive and – in particular combined with rapid GC pre-separation – selective analytical tool. Not requiring extensive experimental setup, GC-IMS can be provided as mobile and relatively low-cost instrumentation. Therefore, a multitude of applications can be covered by GC-IMS analyses, such as (bio-)process and quality control, forensics, biology, medicine, food quality and safety or air quality in general.
It is obvious, that the samples in the different applications mentioned above vary significantly in the complexity and humidity of the sample mixture and in the concentration range. There are extremely low concentrations (ppt or even ppq) e.g. of plant volatiles in environmental air or of (disease related) metabolites in exhaled breath but also high concentrations (ppm or even more) e.g. in process air such as rubber or polymer production.
To cover such a broad variety of applications – each one with specific compounds in complex mixtures and in particular concentration ranges – high flexibility of every single stage of the entire GC-IMS analysis (see Figure 1) is required – and is indeed obtainable. Several meaningful examples of diverse applications with specific challenges, in particular with regard to sampling, pre-concentration and pre-separation will be discussed during the presentation.

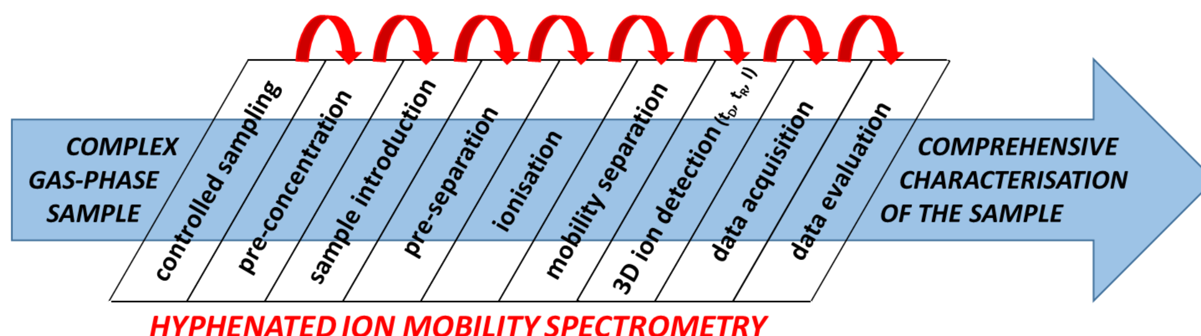


Figure 1. Scheme of the stages of GC-IMS analysis from the sample to the result.

References

[1] W. Vautz, J. Franzke, S. Zampolli, I. Elmi, S. Liedtke, On the potential of ion mobility spectrometry coupled to GC pre-separation e A tutorial. Analytica Chimica Acta 1024 (2018) 52e64

Acknowledgements

Financial support of the *Bundesministerium für Bildung und Forschung* and the *Ministerium für Innovation, Wissenschaft und Forschung des Landes Nordrhein-Westfalen* is greatfully acknowledged. Furthermore, the work was partly funded by the EU in the frame of the project “Detection of olfactory traces by orthogonal gas identification technologies” (DOGGIES, No. 285446, FP7- SEC-20011-1) and of the project “Second Generator Locator for Urban Search and Rescue” (SGL for USaR, No. 217967, FP7-SEC-2007-1).

O.04 – Analysis of dried blood spots by direct coupling of liquid phase microextraction to capillary electrophoresis

Pavel Kubáň*, Miloš Dvořák, Lenka Ryšavá, Blanka Miková
Institute of Analytical Chemistry, Czech Academy of Sciences, Veveří 97, Brno, CZ-60200, Czech Republic
*Email: kuban@iach.cz

Abstract
Sampling of blood is predominantly carried out in wet format and analysis of venous blood serum and/or plasma is considered the golden standard in most clinical applications. An alternative technique for blood collection is sampling of capillary blood in the form of dried blood spots (DBS) [1]. Instead of venepuncture, a drop of capillary blood is collected on a DBS sampling card from a finger/heel prick, is dried for 2 – 3 hours at ambient air, and is used for subsequent analysis. Apart from much less invasive sampling, use of DBS also offers simplified collection, storage at room temperature, transportation by standard mail or parcel delivery companies and thus reduced cost. DBS sampling, therefore, represents an attractive alternative to wet-blood sampling and is widely used for screening of metabolic disorders in newborns, for epidemiological studies and for clinical/therapeutic drug monitoring [2].
DBS analyses are usually hampered by the presence of high concentrations of blood matrix in DBS eluates. Matrix components may interfere with the separation process and even induce damage to the analytical instrumentation. Sample pretreatment is thus applied before the eluate injection, which is traditionally carried out by liquid-liquid extraction (LLE) or solid phase extraction (SPE). However, LLE and SPE are not perfectly compatible with the microliter blood volumes collected during the DBS sampling and liquid phase microextraction (LPME) techniques represent more adequate alternatives for the DBS pretreatment. In addition, capillary electrophoresis (CE) excels in handling minimum sample volumes and thus combination of LPME with CE offers an elegant solution to the DBS analysis. In this contribution, recent achievements in direct coupling of LPME to CE for the analysis of DBS samples are presented and various set-ups are reported, which were developed in our laboratory for the determination of small exogenous compounds in DBS samples. At-line coupling of supported liquid membrane (SLM) extraction and hollow fibre LPME (HF-LPME) to commercial CE instruments is comprehensively described and explained to demonstrate their potential in automated analyses of minute blood volumes colected as DBS samples.

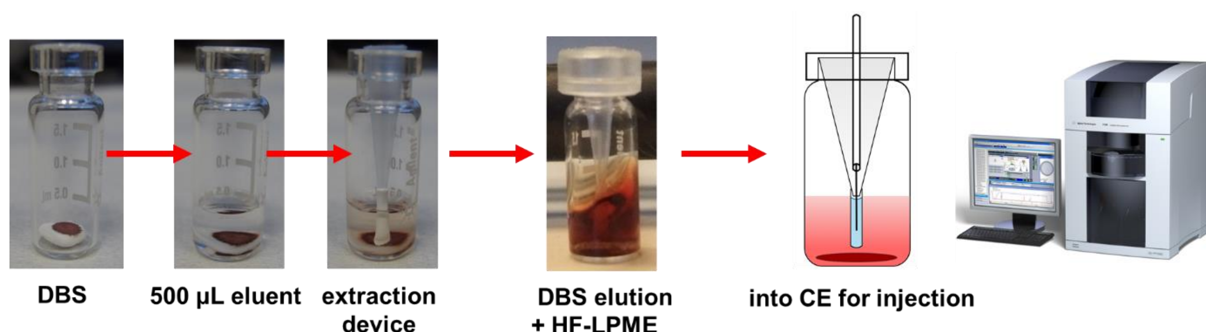


Figure 1. Schematic work-flow for the direct coupling of HF-LPME to CE for the DBS analysis.

References
[1] Guthrie, R., Susi, A. Pediatrics 1963, 32, 338-343.
[2] Tanna, S., Lawson, G. Anal. Methods 2011, 3, 1709-1718.

Acknowledgements
Czech Academy of Sciences (Institute Research Funding RVO:68081715) and Grant Agency of the Czech Republic (Grant No. 18-13135S) are gratefully acknowledged for their financial support.

O.05 – Volatile composition of Merlot red wine and its contribution to the aroma: optimization and validation of analytical method

Stefany Grützmann Arcari[1]*, Vinicius Caliari[2], Marla Sganzerla[3], Helena Teixeira Godoy[3]
[1]Federal Institute of Santa Catarina (IFSC), 89900-000, São Miguel do Oeste, SC, Brazil.
[2]EPAGRI – Experimental Station of Videira, 89560-000, Videira, SC, Brazil.
[3]University of Campinas (UNICAMP), 13083-862, Campinas, SP, Brazil.
*Email: arcari.ste@gmail.com; stefany.arcari@ifsc.edu.br

Abstract
The volatile fraction significantly influences the aroma of wine and is considered one of the most important features about the product quality and consumer acceptance. The volatile fraction of wine is extremely complex, mainly due to the varied chemical nature of the compounds present, such as alcohols, terpenes, esters, acids, aldehydes, lactones, sulfur and nitrogen compounds. A methodology for the determination of volatile compounds in red wine using headspace solid phase microextraction (HS-SPME) combined with gas chromatography-ion trap/ mass spectrometry (GC-IT/MS) and flame ionization detector (GC -FID) was developed, validated and applied to samples of Brazilian red wine. The optimization strategy was conducted using the Plackett-Burman design for variable selection and central composite rotational design (CCRD). The response surface methodology (RSM) showed that the performance of the extraction of the volatile compounds using divinylbenzene/ carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) fiber is improved with no sample dilution, the addition of 30% NaCl, applying an extraction temperature of 56 °C and extraction time of 55 min. The qualitative method allowed the extraction and identification of 60 volatile compounds in the samples studied, notably the classes of esters, alcohols, and fatty acids. Furthermore, the method was successfully validated for the quantification of 55 volatile compounds of importance in wines and applied to a Merlot red wine sample. The calculation of the odor activity value (OAV) showed the most important components of the sample aroma. Ethyl isovalerate, ethyl hexanoate, 1-hexanol, octanoic acid and ethyl cinnamate had the greatest contribution to the aroma of the wines analyzed, which is predominantly fruity with the presence of herbal and fatty odors.

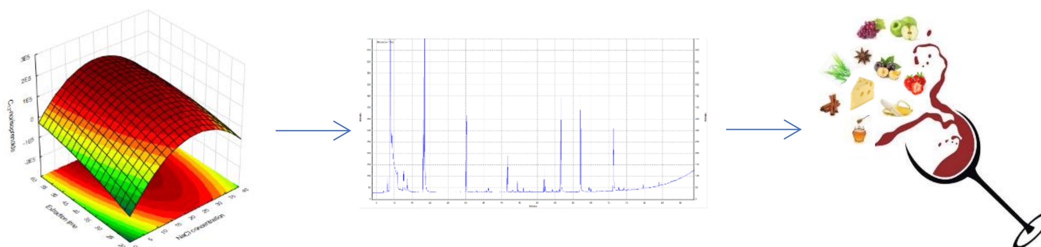


Figure 1. Graphical representation of the analytical work, starting with the optimization of the SPME method by RSM, chromatographic analysis and calculation of the OAV of the wine samples.

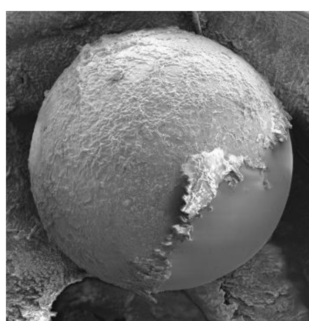
References
[1] M. Revi, A. Badeka, S. Kontakos, M.G. Kontominas. Food Chem. 152 (2014) 331–339.
[2] M. Thompson, S. L. R. Ellison, R. Wood. Pure Appl. Chem. 74 (2002) 835–855.
[3] B. Jiang, X. Zhumei, L. Meijuan, Z. Zhang. Food Res Int. 51 (2013) 482-489.

Acknowledgements
The authors acknowledge the EPAGRI – Experimental Station of Santa Catarina for providing instrumentation for this study and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing Prodoutoral scholarship (S. G. Arcari).

O.06 – Extraction and analysis of pesticides in polyphenol-rich matrices by SFE, modified QuEChERS and organogel based methods: comparative study

Jean-Christophe Garrigues[1]*, Cathy Claparols[2], Emile Perez[1]
[1]IMRCP Laboratory, Toulouse University, 118 route de Narbonne, 31062 Toulouse, France
[2]ICT- UPS CNRS- Service commun de spectrométrie de masse, 118 route de Narbonne, 31062 Toulouse, France
*Email: garrigues@chimie.ups-tlse.fr

Abstract
A rigorous development of analytical method in environmental, bioanalysis or food requires constant innovation in technologies from sample preparation to analytical approaches, to gain early identification, accuracy and sensitivity. Nevertheless, the wide variety of molecular structure of the analytes and their different physicochemical properties (pKa, logP, solubility.) complicate the development of a "universal" analytical method. Moreover, some food samples like tea contains about 20–40% (dry weight) of polyphenol compounds, competing with the target analytes for identification and quantification. During the last years, the tendency to extract and analyze the maximum number of compounds in the minimum of steps has led to the development of generic sample treatments. The weak points of these generic techniques generate contaminated samples (lipids, polyphenols), weak recovery and multiple steps. (1)
Considering the weak points of the various techniques used in sample prep, we decided to evaluate the properties of the porous organogels developed in IMRCP laboratory and Solicore®, (Innochem company) presented in Figure 1.
In a first step, the presentation will reconsider the basic principles, which control an extraction with organogels materials.
In a second step, a comparative study will be discussed between these organogels materials and modified QuEChERS extraction (2). In order to identify the amount of pesticide contained in tea leaves and the quantity extracted during infusion, a supercritical CO_2 extraction (SFE) is carried out. All analyzes are performed in liquid chromatography U-HPLC with HRMS for identification and MS/MS for quantification.
Various analytical parameters will be analyzed: Comparative matrix effect, elemental analysis of identified pesticides and qualitative analysis.
With this new material, it is possible to improve the total sensitivity of an analytical method by decreasing the matrix effect induced by polyphenols in tea samples, after infusion.


A

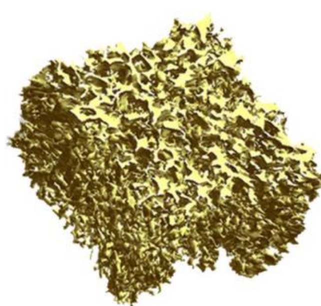

B

Figure 1. SEM of a Solicore® sorbent (A) and microporous organogels 'B)

References
[1] CED. Nazario et al. J Chromatogr B (2017):1043 81–95
[2] J. Guo et al. Food Chemistry 274 (2019) 452–459

O.07 – New PBI-adenine adsorber for API post-reaction stream purification

Teresa Esteves[1]*, Ana I. Vicente[2], Flávio A. Ferreira[1], Carlos A. M. Afonso[2], Frederico C. Ferreira[1]
[1]iBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais, 1049-001 Lisboa, Portugal
[2]Research Institute for Medicine (iMED), Faculty of Pharmacy, Universidade de Lisboa, Avenida Prof. Gama Pinto, 1649-003 Lisboa, Portugal
*Email: teresa.esteves@tecnico.ulisboa.pt

Abstract
Regulatory authorities for pharma industry products have shown increased concern on the presence of impurities in active pharmaceutical ingredients (APIs), especially genotoxic impurities (GTIs), due to their adverse effects on patients' health.[1,2] These electrophilic toxic compounds belong to a wide range of chemical families, including sulfonates, alkyl halides or epoxides, that can act as DNA alkylating agents, and thus are categorized as genotoxins.[3] Furthermore, APIs intensive synthetic pathways, usually carried out in organic solvent matrices, pose additional challenges for the development of efficient adsorbers. To meet this challenge, here, the synthesis and characterization of a new polybenzimidazole (PBI) polymer modified with a DNA base – adenine – is described, able to mimic the process that takes place *in vivo*: the formation of DNA-GTI adducts. This new material is an organic solvent resistant scavenger able to remove more than 80% of several families of GTIs. Its potential is further investigated in the context of an API purification process, presented for several case scenarios, in which the final level of GTI into API reaches the target limits imposed by legislation, meeting the Threshold of Toxicological Concern (TTC) value of 1.5 μg/day [1,2] with minimal API losses.

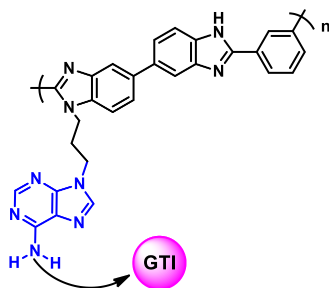


Figure 1. PBI-adenine-GTI adduct formation.

References
[1] EMEA Guidelines on "Limits on Genotoxic Impurities", EMEA/CHMP/QWP/251344/2006, 2006.
[2] Guidance for Industry Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches; U.S. Department of Health and Human Services; Food and Drug Administration; Center for Drug Evaluation and Research (CDER); December 2008.
[3] G. Szekely, M. C. A. Sousa, M. Gil, F. C. Ferreira, W. Heggie, Chem. Rev. 115 (2015) 8182-8229.

Acknowledgements
The authors acknowledge dedicated funding from Fundação para a Ciência e Tecnologia (FCT) through the Project SelectHost (PTDC/QEQPRS/4157/2014) and iBB-Institute for Bioengineering and Biosciences (UIDB/04565/2020), from Programa Operacional Regional de Lisboa 2020 (Lisboa-01-0145-FEDER-007317) and from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Brasil). We also thank Hovione PharmaScience Ltd. for supplying APIs and technical know-how.

O.08 – Chiral separation of synthetic cathinones in gradient ultra-performance supercritical fluid chromatography coupled to mass spectrometry with a gradient flow of make-up solvent

Michal Kohout[1]*, Natalie Kolderová[1,2], Martin Kuchař[2]
[1]Department of Organic Chemistry, University of Chemistry and Technology, Prague, Technická 5, CZ-16628, Prague 6, Czech Republic
[2]Forensic Laboratory of Biologically Active Substances, University of Chemistry and Technology, Prague, Technická 3, CZ-16628, Prague 6, Czech Republic
*Email: michal.kohout@vscht.cz

Abstract
Synthetic cathinones are derivatives of (*S*)-cathinone, the major psychostimulant found in leaves of plant *Catha edulis*. Nowadays, cathinone analogues represent a large group of new psychoactive substances, which are broadly abused as an alternative to classic drugs (such as cocaine, ecstasy or methamphetamine).[1] Cathinones are usually available as racemic mixtures. Their chiral separation can not only further extend information about their production, but also provide the ratio of individual enantiomers in metabolomic studies giving important insight into their preferential biotransformation. In the last decade, supercritical fluid chromatography (SFC) has matured into fully complementary technique to liquid chromatography. Currently, SFC coupled to mass spectrometry (MS) is widely used in drug discovery, food and agricultural industry; increasing popularity of SFC is apparent also in the field of toxicology, particularly when chiral separation is required.[2-4]
The majority of chiral separations is performed using polysaccharide-based chiral stationary phases (CSPs),[4] which offer very high hit rate in chiral resolution of a broad range of organic compounds. Since cathinones are ionisable compounds, which are protonated under weakly acidic conditions, they represent ideal target analytes for chiral cation exchangers that primarily interact with protonated species. On the other hand, ion exchangers require the addition of counter ions (buffer) into the mobile phase, which facilitate the elution of the charged analytes. To enable fast elution of cathinones with a small amount of buffer only, we utilized a zwitterion ion exchange-type CSP. This ion exchanger possesses both the positive and negative charge in its structure under the weakly acidic conditions, thereby having an intrinsic counter ion for acidic and basic analytes.[5]
Using this CSP, we have developed a method for the chiral separation of 25 different cathinones in SFC-MS. The optimum method is based on gradient elution with an increasing amount of buffered methanol in supercritical CO_2. In order to increase the signal intensity in ESI+ MS, we have tuned the amount of a make-up solvent, which is added post-column to enhance ionization efficiency. We show that the best possibility is gradient addition of the make-up solvent, which follows a reversed gradient elution to the gradient of the organic modifier.[6] This is a pioneering approach in the chiral separation using SFC-MS technique, which gives rise to more than 45% signal enhancement without tuning other MS parameters.

References
[1] European Monitoring Centre for Drugs and Drug Addiction. *European Drug Report 2019: Trends and Developments*, Publications Office of the European Union, Luxembourg (2019).
[2] K. De Klerck, Y. Vander Heyden, D. Mangelings, J. Chromatogr. A 1363 (2014) 311-322.
[3] L. Nováková, V. Desfontaine, F. Ponzetto, R. Nicoli, M. Saugy, J.-L. Veuthey, D. Guillarme, Anal. Chim. Acta 915 (2016) 102-110.
[4] R. Geryk, K. Kalíková, M. G. Schmid, E. Tesařová, Anal. Chim. Acta 932 (2016) 98-105.
[5] D. Wolrab, P. Frühauf, C. Gerner, M. Kohout, W. Lindner, J. Chromatogr. A, 1517 (2017) 165-175.
[6] N. Kolderová, M. Kuchař, W. Lindner, M. Kohout, J. Chromatogr. A, 2020, submitted.

Acknowledgements
The authors acknowledge financial support from the Ministry of Interior of the Czech Republic (project no. VI20172020056).

O.09 – Il-based advanced techniques for the extraction of value-added compounds from natural sources and food by-products

Carlos Pelegrín, Marina Ramos, Alfonso Jiménez, María del Carmen Garrigós*
Analytical Chemistry, Nutrition and Food Sciences Department, University of Alicante, E-03690, San Vicente del Raspeig, Alicante, Spain.
*Email: mc.garrigos@ua.es

Abstract
Food by-products are produced during food processing in large amounts around the world (about 38% of the original weight of raw materials). The main current use of agro-industrial wastes is as fertilizers, biofuels or animal feed. However, food by-products as well as their natural sources represent an abundant source of value-added compounds which can be re-used for their potential healthy and rheological properties in different sectors. Growing interest has raised in recent years in food by-products valorisation as well as in the use of natural products with low toxicity instead of synthetic sources. This interest has also led to the need of appropriate extraction methods to obtain bioactive compounds such as polysaccharides, sugars, minerals, dietary fibres, lipids, pigments, organic acids and phytochemicals (polyphenols, carotenoids), showing their high potential for health benefits related to their antibacterial, antioxidant, and anti-inflammatory properties, among others.
The design of sustainable and green extraction methods for natural sources and wastes has become a hot research topic in the last years in order to decrease the use of petrochemical solvents and generation of volatile organic compounds (VOCs). In recent years, the use of ionic liquids (ILs) as green solvents for different extraction techniques has received great attention by their outstanding advantages over conventional organic solvents. ILs are considered environmentally-friendly solvents and they offer some advantageous properties to be used in extraction systems such as low toxicity; non-volatility; non-flammability; high ionic conductivity; and different polarity, hydrophobicity and selectivity. In this sense, their use in combination with advanced extraction techniques, such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) or subcritical water extraction (SWE), is continuously increasing.
This review presents an overview of the latest developments and applications of ILs combined with advanced extraction techniques to obtain bioactive compounds from natural sources and food by-products (Figure 1), which constitute a synergistic approach to contribute to the circular economy by revalorising waste and reducing the use of toxic solvents.

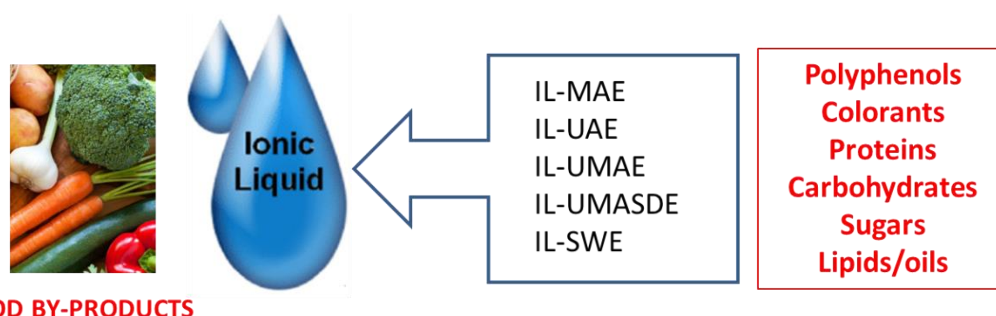


Figure 1. Extraction of bioactive compounds from natural sources and food by-products using ILs.

References
[1] M. Ramos, A. Jiménez, MC. Garrigós, Trends in Analytical Chemistry 119 (2019) 115616.

Acknowledgements
Authors thank the Spanish Ministry of Economy and Competitiveness (MAT2017-84909-C2-1-R) and Consellería de Educación, Investigación, Cultura y Deporte de la Generalitat Valenciana (Spain) (IDIFEDER/2018/007) for their financial support.

O.10 – Vibrational Spectroscopic techniques applied to physicochemical characterization of honey

Ofélia Anjos[1,2,3]*, Letícia M. Estevinho[2]
[1]Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017 Lisboa, Portugal
[2]Instituto Politécnico de Castelo Branco, 6001-909 Castelo Branco, Portugal
[3]Centro de Biotecnologia de Plantas da Beira Interior, Castelo Branco, Portugal
[4]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, 5300-252 Bragança, Portugal
*Email: ofelia@ipcb.pt

Abstract
This study aimed to evaluate the potential of vibrational spectroscopy (particularly FT-RAMAN and FTIR-ATR) to predict the chemical composition of *Lavandula* honey. This type of honey was selected because it is one of the most produced in Portugal, besides being much appreciated by the consumers. For both methodologies, Partial Least Squares (PLS) regression models were applied. The data from FT-RAMAN [1] and FTIR-ATR were similar, even though the spectral information obtained with FT-RAMAN was more informative. Calibration models for electrical conductivity, ash, total acidity, pH, reducing sugars, hydroxymethylfurfural (HMF), proline, diastase index, apparent sucrose, total flavonoids content and total phenol content were obtained. The higher accurate model was obtained with FT-RAMAN with high r^2 (from 90% to 99.9%), with high residual prediction deviation - RPD and low root mean square errors. In Figure 1 it is presented representative spectra obtained with both methodologies.
The results obtained in this study confirm the hypothesis that vibrational spectroscopy is a useful technique for fast current laboratory analysis of honey and their quality control with a more powerful results for FT-RAMAN.

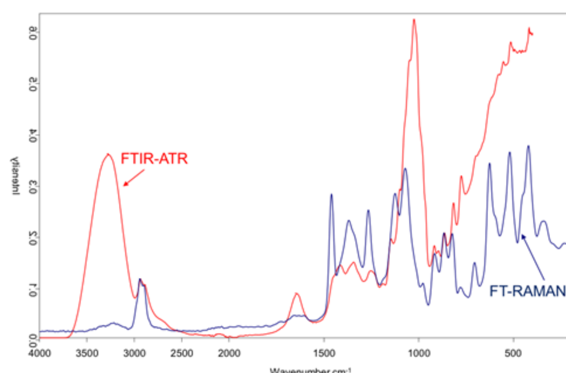


Figure 1. Average FTIR-ATR and FT-Raman spectrum of the *Lavandula* spp. honey for the entire spectral region.

References
O. Anjos, AFA. Santos, V.Paixão, ML.Estevinho, Talanta, 178 (2018) 43–48.

Acknowledgements
Thanks are due to Foundation for Science and Technology FCT, Portugal (FCT/MCTES) for the financial support to CEF (Centro de Estudos Florestais (UIDB/00239/2020) and CIMO (Centro de Investigação de Montanha), Instituto Politécnico de Bragança (UID/AGR/00690/2019).

O.11 – Identification of terpenes and essential oils by means of static headspace gas chromatography-ion mobility spectrometry

Roman Rodriguez-Maecker[1]*, Eduardo Vyhmeister[1], Stefan Meisen[2], Antonio Rosales[3], Andriy Kuklya[4] and Ursula Telgheder[4,5]
[1]Carrera de Ingeniería Petroquímica, Universidad de las Fuerzas Armadas, Ecuador
[2]Solvias AG, Analytical Services, Small Molecule Analysis, Switzerland
[3]Departamento de Ingeniería Química, Universidad de Sevilla, Spain
[4]Faculty of Chemistry, University of Duisburg-Essen, Germany
[5]IWW Water Centre, Germany
*Email: rnrodriguez@espe.edu.ec

Abstract
Gas chromatography-ion mobility spectrometry (GC-IMS) is an analytical technique that has considerable potential for analysis of volatile organic compounds (VOCs). In this study, GC-IMS was used for the identification of terpene components of various essential oils (EOs). Based on the data obtained from 25 terpene standards and 50 EOs, a database for fingerprint identification of characteristic terpenes and EOs was generated, and for authenticity testing of fragrances in foods, cosmetics, and personal care products. The generated database contains specific normalized IMS drift times and GC retention indices for 50 terpenes, and it was used to prepare a fingerprint pattern. The applicability of the method was proven on examples of ten commercially available food, cosmetic, and personal care product samples. The results confirm the suitability of GC-IMS as a powerful analytical technique for direct identification of terpene components in solid and liquid samples without any pretreatment.

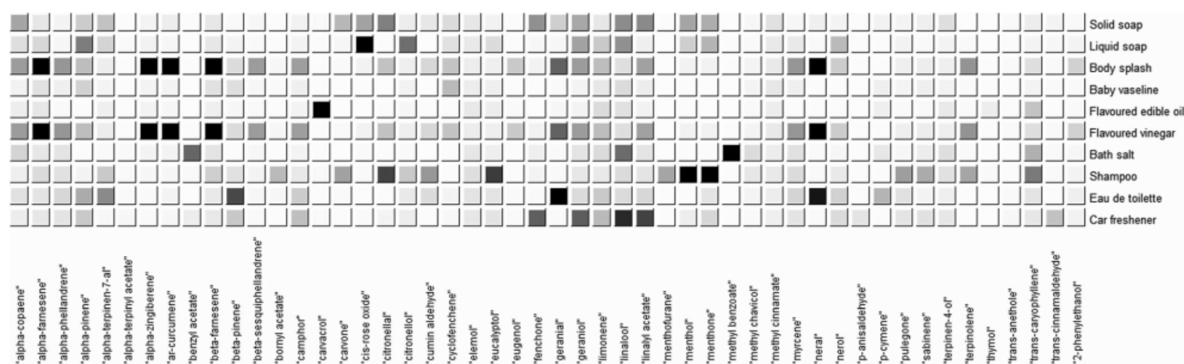


Fig. 1 Terpene fingerprint pattern applied on several commercial products.

References
[1] Do TKT, Hadji-Minaglou F, Antoniotti S, Fernandez X. Authenticity of essential oils. TrAC Trends Anal Chem. 2015;66:146–57.
[2] Talou T, Maurel S, Gaset A. Electronic nose versus multicapillary gas chromatography: application for rapid differentiation of essential oils. Dev Food Sci. 1998;40:79–86.
[3] Gallegos J, Garrido-Delgado R, Arce L, Medina LM. Volatile metabolites of goat cheeses determined by ion mobility spectrometry. Potential applications in quality control. Food Anal Methods. 2015;8(7):1699–709.
[4] Krisilova E, Levina A, Makarenko V. Determination of the volatile compounds of vegetable oils using an ion-mobility spectrometer. J Anal Chem. 2014;69(4):371–6.
[5] Liang F, Kerpen K, Kuklya A, Telgheder U. Fingerprint identification of volatile organic compounds in gasoline contaminated groundwater using gas chromatography differential ion mobility spectrometry. Int J Ion Mobil Spectrom. 2012;15(3):169–77.

O.12 – High-Throughput Measurement of Lipid Turnover Rates Using Partial Metabolic Heavy Water Labeling

Tae-Young Kim[1,2]*, Byoungsook Goh[2], Hoeil Chung[3]

[1]School of Earth Sciences & Environmental Engineering, Gwangju Institute of Science & Technology, Gwangju 61005, South Korea
[2]Department of Chemistry, Gwangju Institute of Science & Technology, Gwangju 61005, South Korea
[3]Department of Chemistry, College of Natural Sciences, Hanyang University 04763, South Korea
*Email: kimtaeyoung@gist.ac.kr

Abstract
Mass spectrometry (MS) has revolutionized biochemical analysis in terms of sensitivity, specificity, and speed. A fundamental drawback of MS as an analytical technique is the poor ability of quantification. To overcome this limitation, stable isotope labeling has been extensively employed for both relative and absolute quantification based on MS. In most quantitative MS experiments, complete labeling of target analytes with a specific type of heavy isotope (e.g., ^{2}H, ^{13}C, or ^{15}N) is designed for simple distinction of isotope-labeled compounds from non-labeled counterparts in a mass spectrum. However, the use of isotope labeling is a cumbersome practice mainly due to high cost and long time to achieve complete labeling. An alternative method to introduce an isotope to biomolecules is indirect deuterium labeling via heavy water ($^{2}H_2O$) administration, which results in strikingly different patterns of mass spectra because of partial isotope enrichment. We have developed novel analytical platforms for turnover rate measurement in a lipidome scale by using partial metabolic $^{2}H_2O$ labeling. In this presentation, the principle and performance of this new lipidomics tool are discussed.

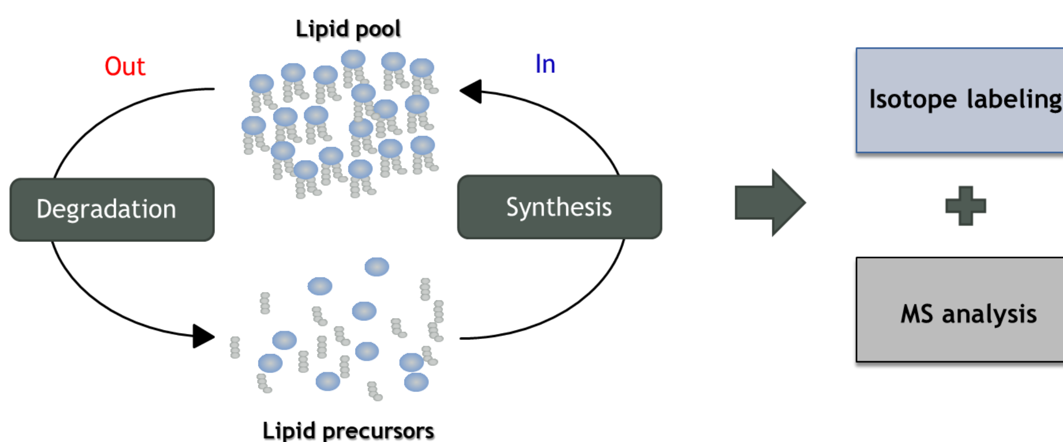


Figure 1. Lipid turnover measurement using isotope labeling combined with mass spectrometry

References
[1] B. Goh, J. Kim, S. Seo, T.-Y. Kim, Analytical Chemistry 90 (2018) 6509–6518.

Acknowledgements
We acknowledge the financial support for this work by the National Research Foundation of Korea (NRF-2014R1A1A1003643).

O.13 – Simplest and label-free determination of intra- and extra-cellular vitamin C dynamics by HPLC-DAD

Taiki Miyazawa[1], Akira Matsumoto[1,2]*, Ayano Mukaida[1], Yuji Miyahara[1]
[1]Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo, Japan
[2]Kanagawa Institute of Industrial Science and Technology (KISTEC-KAST), Kawasaki, Japan
*Email: matsumoto.bsr@tmd.ac.jp

Abstract
Vitamin C is first discovered in the 1920s and is known as one of the most fundamental nutrients for sustaining life. Vitamin C is widely distributed in the body and plays an important role in regulating redox balance, and changes in its concentration are deeply involved in various diseases. Vitamin C involves several vitamers that are inter-convertible depending on the redox state, among which ascorbic acid (ASC) and its oxidized form dehydroascorbic acid (DHA) represent two dominant species. Well known two standard methods for monitoring the dynamics of these vitamers are isotopic labeling and mass spectrometry techniques. However, these methods inevitably involve costly instrumentation and complicated parameter optimization. On the other hand, in this presentation, we report ASC and DHA can be analyzed using a very simple chromatography (HPLC-DAD) protocol.[1] This protocol allows simultaneous and absolute quantification of intracellular and extracellular DHA-ASC pair dynamics. Our protocol commences with a cellular vitamin C extraction process using metaphosphoric acid (MPA), a commonly used stabilizer during vitamin C extraction from food samples. The MPA-compatible and organic solvent-free protocol, hence, provides striking advantages in terms of the robustness of measurement, sample storage, and ease of purification. We demonstrated that our method can be readily applied to in vitro assay both on erythrocytes, as well as pancreatic cancer cell line to trace in detail their glucose transporter 1 (GLUT1)-dependent or DHA-specific cellular uptake and time- and dose- dependent intracellular conversion into ASC. The redox-sensitive inter-conversion of ASC and DHA in intracellular and extracellular environments is of exceptional interest at the forefront of metabolomics and pharmaceutical research, including high-dose vitamin C cancer therapy. [2] Therefore, the presented technique should aid in providing quantitative bases for those therapeutic approaches.

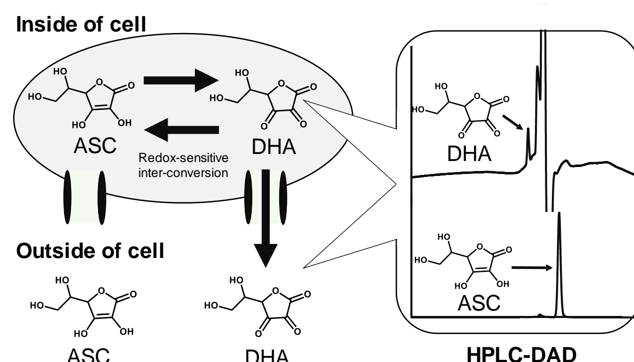


Figure 1. The illustration of the research:
Quantification of intracellular and extracellular DHA-ASC pair dynamics by HPLC-DAD.

References
[1] T. Miyazawa, A. Matsumoto, Y. Miyahara, Analyst 144 (2019) 3483–3487.
[2] T. Miyazawa, A. Matsumoto, Y. Miyahara, G.I.T. Laboratory journal (2019) September 7.

Acknowledgements
This work was supported by Japan Society for the Promotion of Science (JSPS) Postdoctoral Fellowships (KAKENHI (17J09342)) and Grants-in-Aid for Scientific Research (KAKENHI (18K14419)).

O.13B – Saliva-based Germline T790M Mutation Detection for Familial Inherited Lung Cancer

YANHONG SHANG[1]*, RAN HUO[1], JINGHUA LI[1], XIAOFANG LI[1], LIN ZHANG[2] , FENG LI[2] , KUNJIE WANG[1], JIN JIAO[1]
[1]Department of Medical Oncology, Affiliated Hospital of Hebei University, Baoding, People's Republic of China. [2]OrigiMed, Shanghai, People's Republic of China.
*Email: shangyanhong2013@163.com

Abstract
The identification of epidermal growth factor receptor (EGFR) mutations represents a milestone in the treatment of advanced non-small cell lung cancer. Previous studies have shown that saliva-based gene testing can accurately detect EGFR L858R and exon 19 del mutations in patients with lung cancer [1]. However, reports using saliva-based method to detect T790M mutations are lacking. Here we report a 27-year-old patient with lung adenosquamous carcinoma (ASC) harboring a germline T790M mutation, and in his family the T790M mutations were screened with a saliva-based gene detection. This young Chinese patient was diagnosed with ASC in May 2017. The surgically obtained tumour tissues were used for next generation sequencing (NGS) gene panel assay (provided by OrigiMed, the first provider of large panel NGS in China), and EGFR L858R somatic mutations were noted, furthermore, a T790M germline mutation was detected in the paired PBMCs. He was treated with Osimertinib and achieved complete response for more than 30 months, without significant drug-related adverse events. As previous reports indicated germline EGFR T790M mutation is associated with familial inherited lung cancer [2, 3], the family members of the patient were screened for T790M mutations. Saliva samples were collected from all the patient's available relatives for DNA analysis by Sanger sequencing. It was found that 60% (3/5) and 67% (6/9) of his first- and second- degree relatives were germline EGFR T790M carriers, and 67% (2/3) and 33% (2/6) of the carriers had been diagnosed with lung cancer. In carriers detected by saliva-based testing who were subsequently diagnosed with lung cancer, the results of tissue-based testing confirmed the T790M mutation, and all of them benefited from Osimertinib treatment.
Our study demonstrates that the clinical use of NGS could maximize the benefits of precision medicine in patients with cancer, and saliva-based gene detection may provide a feasible approach for germline T790M mutation screening as saliva sample collection is noninvasive, easy and inexpensive.

Keywords
Lung cancer; Epidermal growth factor receptor; T790M; Gene detection; Saliva-based

References

[1] D. Pu, H. Liang, F. Wei, et al. Thorac Cancer 7 (2016) 428-436.
[2] D.W. Bell, I. Gore, R.A. Okimoto, et al. Nat Genet 37 (2005) 1315–1316.
[3] C. Tibaldi, E. Giovannetti, E. Vasile, et al. J Thorac Oncol 6 (2011) 395–396.

Acknowledgements
This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. We owe thanks to the patient and his family. We thank the staff at Affiliated Hospital of Hebei University. We thank OrigiMed for next-generation sequencing technical support and scientific comments.

O.14 – Advanced microsampling and LC-MS/MS for the monitoring of patients with neurodegenerative disorders

Michele Protti[1]*, Camilla Marasca[1,2], Andrea Cavalli[2], Christopher Reist[3], Roberto Mandrioli[4], Laura Mercolini[1]
[1]Research group of Pharmaco-Toxicological Analysis (PTA Lab), Department of Pharmacy and Biotechnology (FaBiT), Alma Mater Studiorum - University of Bologna, Bologna (Italy)
[2]Italian Institute of Technology (IIT), Genoa (Italy)
[3]Department of Psychiatry & Human Behavior, University of California, Irvine (US)
[4]Department for Life Quality Studies, Alma Mater Studiorum - University of Bologna, Rimini (Italy)
*Email: michele.protti2@unibo.it

Abstract
Neurodegenerative diseases refer central nervous system (CNS) disorders caused by neuronal degradations and dysfunctions: Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis are regarded as the major ones. Currently, an effective treatment for major depression and CNS symptoms associated with neurodegenerative diseases involve the use of "new-generation" antidepressant agents (ADA). However, in 20-30% of patients these therapies are inadequate, while no therapeutic drug has until now demonstrated to possess better efficacy and safety for most patients than other agents. In order to help clinicians correctly assess therapeutic options, one of the most useful practices is therapeutic drug monitoring (TDM). TDM provides for the periodic determination of drug and metabolite circulating levels, together with the use of chemical-clinical correlations. TDM can lead to reduced healthcare expenses, due to the possibility of better efficacy, increased patient compliance and enhanced safety [1]. Alternative and patient-friendly microsampling strategies are particularly attractive in order to promote accurate TDM practices, i.e. a more frequent monitoring during pharmacotherapy [2]. To this aim, microsampling approaches have been developed and applied within this study. In comparison to classical in-tube strategies, microsamples are minimally invasive, using a few drops of capillary blood from a fingerprick. Moreover, the dried samples do not require cryopreservation for transport and storage, while usually maintaining high stability. Innovative microsampling strategies using polymeric tips and smart collection tools were designed and tested for sampling and pretreatment, with possible future applications for at-home self-sampling by patients themselves. Within this project, microsampling of whole blood and oral fluid (as an alternative matrix) was studied and several ADA together with active metabolites, as well as neuroprotective agents towards neurodegeneration processes were investigated in both matrices, exploiting LC-MS and LC-HRMS. All the original procedures of miniaturised and automated sampling, pretreatment and analysis were optimised and validated, then compared to traditional, in-tube fluid samples in order to investigate the actual suitability of dried blood and oral fluid as alternative matrices for the TDM of neurodegenerative disorder patients and for the investigation on pathology progression biomarkers and neuroprotective agents. Results were promising, with very good correlations between the two data sets. Such strategies could potentially pave the way towards more frequent patient TDM for precision medicine practices and novel diagnostic tools for at-home testing, when coupled to high-throughput, cost-effective and fully automated analysis workflows.

References
[1] M. Protti, R. Mandrioli, C. Marasca, A. Cavalli, A. Serretti, L. Mercolini. Med. Res. Rev. (2020) 1-39, *in press*.
[2] L. Mercolini, M.A. Saracino, M. Protti. Bioanalysis 7 (2015) 1925-1942.

Acknowledgements
This research work was financially supported by Research Projects of National Relevance (PRIN) 2017 funds and by the Italian Institute of Technology (IIT).

O.15 – Fast and easy-to-use µTAD for creatinine determination in urine

Miguel M. Erenas[1]*, Manuel J. Arroyo[1], Ignacio de Orbe-Payá[1], Kevin Cantrell[2], Jose A. Dobado[3], Alfonso Salinas-Castillo[1], Luis Fermín Capitán-Vallvey[1]
[1]ECsens, Department of Analytical Chemistry, University of Granada, Spain
[2]Department of Chemistry. The University of Portland, Portland, OR, USA
[3]Grupo de Modelización y Diseño Molecular, Dpto. of Organic Chemistry, Univ. of Granada, Spain
*Email: erenas@ugr.es

Abstract
Creatinine is a waste molecule originated from the breakdown of creatine phosphate, a consequence of the muscle and protein metabolism, that goes to the bloodstream and, thanks to the kidney, is removed from blood and it is excreted through the urine. Nowadays, creatinine is used as kidney's biomarker, and its concentration level in blood and urine can be associated to pathologies related to renal function or muscle-level dysfunctions. Therefore, some patients need to monitor its creatinine levels in urine.
In this work, we present a microfluidic Point-of-Care (POC) device that uses cotton thread as support (µTAD), containing all the reagents needed to adjust pH of the sample, and perform the creatine determination by adding a small volume of sample (10µL). The device is an easy-to-use one that permits to non-trained personal to perform the analysis in 30 s, using its smartphone to obtain the analytical parameter and creatinine concentration in urine.
The creatinine determination is based on a ionophore-chromoionophore chemistry[1], where a aryl-substituted monophosphonate-bridged calix[4]pyrrole compound[2] interacts selectively to creatininium cation, by non-covalent interactions, when pH is fixed to 3.8. The color change of the µTAD occurs by ion exchange, due to the input of the creatininium into the membrane where all the reagents are contained, which complexes with the present ionophore and requires the deprotonation of the present lipophilic indicator (ETH7075), in order to maintain the electroneutrality of the system. The µTAD developed permits the determination of creatinine in urine from $1.6 \cdot 10^{-6}$ to $5.0 \cdot 10^{-2}$ M with a precision around 4%.

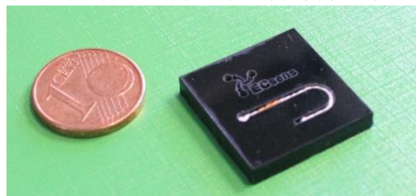


Figure 1. µTAD for the analysis of creatinine in urine.

References
[1] M.M. Erenas, I. Ortiz-Gómez, I. de Orbe-Payá, D. Hernández-Alonso, P. Ballester, P. Blondeau, F.J. Andrade, A. Salinas-Castillo, L.F. Capitán-Vallvey, I. de Orbe-Payá, D. Hernández-Alonso, P. Ballester, P. Blondeau, F.J. Andrade, A. Salinas-Castillo, L.F. Capitan-Vallvey, ACS Sensors. 4 (2019) 421–426.
[2] T. Guinovart, D. Hernández-Alonso, L. Adriaenssens, P. Blondeau, M. Martínez-Belmonte, F.X. Rius, F.J. Andrade, P. Ballester, Angew. Chemie - Int. Ed. 55 (2016) 2435–2440

Acknowledgements
This work was founded by Spanish "Ministerio de Economía y Competitividad" under Project CTQ2016-78754-C2-1-R.

O.16 – Determining the most important criteria of sampling and sample preparation applied in prohibited substances analysis from saliva

Krzysztof Goryński*[1], Łukasz Sobczak[1], Arkadia Ciepłuch[2], Marcin Stachowiak[2], Sławomir Goryński[3], Katarzyna Kuhn[3], Dominika Kołodziej[2]
[1]Bioanalysis Scientific Group, Faculty of Pharmacy,
[2]Scientific Circle of Bioanalysis, Faculty of Pharmacy,
[3] Palliative Care Department, Faculty of Health Science,
Collegium Medicum in Bydgoszcz at Nicolaus Copernicus University in Toruń, Poland *Email: *Email: gorynski@gmail.com

Abstract
One of the directions in the area of monitoring prohibited substances in biological matrices is utilization of alternative specimens i.e. oral fluid, sweat, etc. This forces development of new analytical protocols permitting to meet high requirements of data quality to be considered acceptable by international supervising agencies. Additionally there are a number of compounds that can be possibly used as performance enhancers by sportsmen, the selected method should be capable of screen as many drugs of interest as possible very fast and in one single analysis, without sacrificing sensitivity or time length of analysis. Presently dilute-and-shoot, protein precipitation, liquid-liquid extraction or solid phase-extraction are commonly used in analytical laboratories and two decades ago solid-phase microextraction was introduced into analytical community and have recently made significant advances when applied to variety of exo- and endogenous compounds including drugs and metabolites analysis. Recent preliminary studies have shown pros and cons bench of sample preparation techniques applied on the analysis of doping substances in urine, blood and plasma, as regular matrices, as well as saliva, as alternative fluids.
After presents the applicability of microextraction technique for analysis of prohibited substances in urine, plasma and blood, we will focus on utilization of saliva analysis as an alternative specimen in control of drugs of abuse and potential in doping testing. Recent preliminary studies have shown pros and cons bench of devices to collect oral fluid and sample preparation techniques applied on the analysis of pharmacologically active compounds from saliva. In this talk, based on our experimental results from few studies, we will discuss about strengths and weaknesses different analytical approaches suitable for simultaneous multi-compound bioanalysis of analytes with a wide range of polarities. The complete results from comparison commercially available saliva devices and tailored in laboratory tool will be demonstrated and discussion about the effect of the material of devices are formed on precise analysis will be done. At the end, an alternative sampling and sample preparation solution will be proposed.

References
[1] K. Gorynski, Trends in Analytical Chemistry 112 (2019) 135-146.
[2] E. Boyacı, K. Gorynski, et al., Analytica Chimica Acta 809 (2014) 69-81.

Acknowledgements
This work was supported by The National Centre for Research and Development under Lider IX programme: grant LIDER/44/0164/L-9/17/NCBR/2018 – A new approach to rapid screening of selected drugs and small molecules in oral fluid.

O.17 – EU-OPENSCREEN: Enhancing biochemical research

Federica Rossella[1]*, Edgar Specker[1,2], Martin Neunschwander[1,2] and Wolfgang Fecke[1]
[1]EU-OPENSCREEN-ERIC, Campus Buch, Robert-Rössle str. 10, Berlin
[2]Leibniz-Institut for Molecular Pharmacology (FMP), Campus Buch, Robert-Rössle str. 10, Berlin
*Email: federica.rossella@eu-openscreen.eu

Abstract
EU-OPENSCREEN (EU-OS) is a european research infrastructure for chemical biology founded in 2018. Its mission is to support and enhance the identification and caracterisation of new chemical probes for the development of molecular research tools and possibly therapeutics.[1]
EU-OS hosts the European Chemical Biology Library (ECBD), consisting of 100,000 commercial compounds, selected through chemoinformatics filters to ensure quality and diversity[2], and a growing number of new and often proprietary compounds donated by academic chemists. Scientists can access the entire ECBD through cooperations with the numerous screening partner sites within the EU-OS network (currently 17 in eight member countries across Europe)[1].
The increasing availability of high-thoughput screening (HTS) facilities and of chemical libraries enabled great progress in the identification of possible chemical probes; however, also the number of unreproducible results and false positives increased. Scientists claim the need of higher standards in chemical biology, starting from the quality of library compounds up to the qualityand reproducibility of the HTS data. [3-5]
With this aim, each compound of the ECBD library is analyzed for purity and identity through a Liquid-Chromatographic/Mass Spectrometric (LC-MS) method. Furthermore, a direct pipeline from the samples' registration via the storage in 96-well plates, to the reformatting to 384-well plates was established in order to minimize possible errors. Each compound is also caracterized for its chemical-physical properties, and this results, along with analytical quality control (QC) data are then inserted in a large dedicated database, the European Chemical Biology Database (ECBD). The ECBD, providing open-access to all scientists, will also include each screening result produced with each compounds: the open-access model proved to be beneficial and sustainable for chemical probes caracterization and further on in drug design[6]. If a compound is regarded as a "hit", this compound will be re-analyzed as confirmation of structure and purity, as already reported as a required quality measure. [3]
The data produced and stored in the ECBD will result in an important and freely accessible compendium of today's knowledge of chemical probes that respect rigorous quality standard.

References
[1] P.Brenneke, D. Rasina, O. Aubi, K. Herzog et al, SLAS Discoveries 24 (2019) 398-413.
[2] D. Horvath, M. Lisurek, B. Rupp, R. Kühne et al, ChemMedChem 9 (2014) 2309-2326.
[3] J. Inglese, C.E. Shamu and R.K. Guy, Nat Chem Biol, 4 (2007), 438-441.
[4] N. Thorne, D.S. Auld and J. Inglese, Curr Opin Chem Biol 14 (2010), 315-324.
[5] C.H. Arrowsmith, J.E. Audia, C. Austin, J. Baell et al, Nat Chem Biol 11 (2015) 536-541.
[6] A.Gaulton and J.P. Overinton, Future Med Chem 2 (2010), 903-907.

O.18 – Significant molecular and complex association descriptors in QSRR modelling of Green Liquid Chromatography using β-cyclodextrin mobile phases

Ana Protić*, Nevena Maljurić, Biljana Otašević, Anđelija Malenović, Mira Zečević
[1]University of Belgrade – Faculty of Pharmacy, Department of Drug Analysis
*Email: anna@pharmacy.bg.ac.rs

Abstract
Developing green liquid chromatography is one of the main challenges when dealing with liquid chromatography. One of the strategies includes addition of cyclodextrins (CDs) as mobile phase additives. CDs and diverse hydrophobic organic compounds can form inclusion complexes that are freely dissolved in water compared to free organic compounds. In this way investigated substances are less retained on the hydrophobic stationary phases allowing lower consumption of toxic organic solvents, in the first line acetonitrile and methanol.
CD-modified RP-HPLC systems are dynamic and rather complicated. The solute can be distributed between dissolved CD in the mobile phase, CD adsorbed on the stationary phase, stationary phase and the bulk mobile phase. Therefore, it possesses diverse and complicated equlibria. This makes retention modelling even more complicated in CD-modified chromatographic systems. For developping good predictive Quantitative structure retention relationship model (QSRR) both molecular and complex association descriptors were included in the modeling along with significant chromatographic parameters. In order to assess complex associaton descriptors, docking studies were employed. They revealed the most possible structures of the inclusion complexes formed between β–CD and investigated model substances (risperidone, olanzapine, and their impurities) as well as which part of the solute was most likely incorporated into the β–CD cavity. QSRR model was built using artificial neural network (ANN) technique. The ANN with the best performance was obtained employing multilayer perceptron network with 11-8-1 topology and trained with back propagation algorithm. The network was further used in selection of the most influential molecular and complex association descriptors. Among molecular descriptors, polarizability, dipol-dipol energy, solvent excluded volume and octanol-water partition coefficient were selected as the most important. Considering complex association descriptors, energy of binding, electrostatic energy and unbound system's energy were the most significant for good predictive ability of the formed QSRR model. In this way beneficial solute and complex characteristics could be perceived and further used when deciding whether CD modified RP-HPLC systems are the most suitable solution for the certain set of solutes.
The proposed QSRR model confirmed its applicability in optimization of green chromatographic RP-HPLC methods and in stability constants determination of formed inclusion complexes.

References (Arial, font size 10pt, full justification)

[1] N. Maljurić, J. Golubović, B. Otašević, M. Zečević, A. Protić, Analytical and Bioanalytical Chemistry 410 (2018) 2533-2550.
[2] N.Maljurić, B. Otašević, A. Malenović, M. Zečević, A. Protić, Journal of Chromatography A, article in press

Acknowledgements

The work presented here is financed by the Ministary of Education, Science and Technological Development Republic of Serbia

O.19 – Long-term human metabolite of dehydrochloromethyltestosterone

Mats Larsson*
[1]Department of Physics, Stockholm University, AlbaNova University Center, Stockholm, Sweden
*Email: mats.larsson@fysik.su.se

Abstract
Anabolic androgenic steroids (AAS) are prohibited in sports. One such AAS, dehydrochloromethyltestosterone (DHCMT), also known as oral-turinabol, is an AAS invented in the former DDR and until 2012 believed to have a quite fast elimination rate from the body. Sobolevsky and Rodchenkov [1] claimed in 2012 to have discovered several very long-lived metabolites, and estimated that the detection window for the most long-lived metabolite, denoted M3, is about 40-50 days, substantially longer than the previous window. Several samples were retested based on M3 and found to be adverse analytical findings.
Only a tentative structure for M3 was proposed in [1]. More recently, the metabolite has been synthesized in order to strengthen the detection process [2]. Some details of the fragmentation of M3 were discussed by Larsson in a comment to [2], and some answers were provided [4].
There are remaining puzzling problems with the M3 metabolite. First, several baseball players in the US have tested positive for DHCMT by means of M3, but the source has remained unknown. Most of the players had never heard of oral-turinabol. Secondly, the result of an excretion study (if such even exists) has never been published. The estimated detection window of 40-50 days still derives from the original publication [1]. It was also pointed out in [1] that an excretion study is required in order to obtain a more precise detection window. This fact was also pointed out by Larsson [3], but remained unanswered in [4].

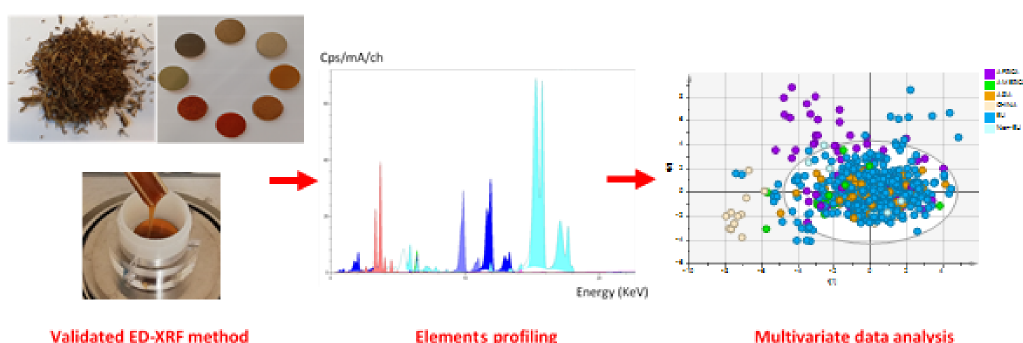
References
[1] T. Sobolevsky and G. Rodchenkov, J. Steroid Biochem. Mol. Biol. 128 (2012) 121-127
[2] G. Forsdahl, T. Geisendorfer, T. Göschl et al., Drug Test Anal. 10 (2018) 1-7.
[3] M. Larsson, Drug Test Anal. 11 (2019) 184.
[4] G. Forsdahl, T. Geisendorfer, T. Göschl et al., Drug Test Anal. 11 (2019) 185.

Acknowledgement
The author is indebted to Arthur Kopylov, Institute of Biomedical Chemistry, Moscow, Russia, for interesting discussions.

O.20 – Determination of trace elements profiles by ED-XRF spectroscopy: a new validation strategy for organic matrices and applications to discriminate their geographical origin and botanical variety

Michele Ghidotti[1]*, Catalina Dumitrascu[1], Yiannis Fiamegos[1], Tereza Zdiniakova[1], Maria Beatriz de la Calle Guntiñas[1]
[1]European Commission, Joint Research Centre (JRC), Health, Consumer & Reference Materials, Fraud Detection and Prevention, Retieseweg 111, B-2440 Geel, Belgium
*Email: Michele.GHIDOTTI@ec.europa.eu

Abstract
Energy Dispersive X-Ray Fluorescence (XRF) spectroscopy has the potential to provide multi-elemental analysis with minimum sample pre-treatment. Solid samples are homogenized, pulverized and subsequently pressed to obtain pellets, which can be stored and analyzed several times [1]. Liquid samples can be analyzed as such under a helium-saturated atmosphere [1]. ED-XRF applications are suitable for both light and heavy elements, they allow trace elements analysis (sub mg kg^{-1}), although the detection limits are considerably higher than in AAS and ICP-based techniques [2]. An ED-XRF method for trace elements analysis in a variety of organic and inorganic samples was developed and validated at the JRC Geel [2]. One single calibration curve was built for each element using a set of 29 reference materials covering different matrices and mass fraction ranges. Method validation was based on the fulfilment of empirically established performance criteria: the use of threshold values for *z*-scores (proxy for the evaluation of trueness) and intermediate precision (calculated on pellet replicates) of 2 and 25% respectively (values below these thresholds were considered satisfactory).
In this study, some of the potential applications of the validated ED-XRF method combined with the multivariate analysis of elemental profiles are reported. Inorganic profiling of the tobacco contained in cigarettes samples is useful to determine its geographical origin, i.e. where the tobacco was possibly produced. This information, combined with the elemental composition of cigarettes legally produced in all around the world, allows the discrimination of counterfeit products and the tracking of their illicit production. The validated ED-XRF method demonstrated a potential in honey classification. The elemental profiles of honey samples analyzed as liquid allow to discriminate geographical origin, botanical varieties and to distinguish products with protected designation of origin. This opens the way for ED-XRF to food authentication.



References
[1] Beckoff, B., KanngieBer, B., Langhoff, N., Wedell, R., & Wolff, H. (2006). Handbook of practical X-ray fluorescence analysis. Heidelberg: Springer-Verlag. ISBN-10 3-540-28603-9.
[2] Fiamegkos Y, de la Calle Guntiñas MB. Validation strategy for an ED-XRF method to determine trace element in a wide range of organic and inorganic matrices based on fulfilment of performance criteria. Spectrochim Acta B. 2018;150:59–66.

O.21 – Multi-analytical profiling of archaeometallurgical samples – A new insight into ancient materials

Sanda Rončević*, Ivan Nemet
Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, Zagreb, CROATIA
*Email: roncevic@chem.pmf.hr

Abstract
Analytical considerations on iron artefacts from ancient bloomery iron production are generally directed to analysis of main and trace elements content in raw materials and wastes. Elemental signature of artefacts usually refers to the carefully selected sample of final iron-making products, as well as to randomly collected samples of slag and pit deposit materials on excavation site. Archaeometric studies reveal the compositional relations between ore, smelting slags and bloom iron products, which is the utmost information for the provenance. Reconstructions of ancient iron making process have confirmed that different types of slag material are formed during smelting and smiting process such as tap slags, bloom slags and ceramic-rich slags.[1,2] However, a significant chemical variability derived from furnace, fuel and fluxes in iron making process cause the provenancing attempts rather ambiguous. Another severe problem arises from representativeness and proper recognition of samples from iron making sites when they were collected from large clusters or dissipated piles of slag. A multi-sample and/or multi-method analytical approach has showed to be capable for collection of enough compositional data, which are adequate for characterization of iron-making sites.[3]
Chemometric methods with univariate and multivariate statistical treatment of large set of spectrometric data are often used in experimental design and analytical method optimisation. In archaeometric studies, a multivariate statistics using principal component analysis (PCA) and hierarchical clustering (HC) are the most often applied modes of exploratory data analysis. It allows that the distinctions among objects with quite similar characteristics at macroscopically scale became visible.
In this research study, the chemical composition of archaeological samples from bloomery iron-making site of NW Croatia was determined using multi-method approach.[4] Determination of elements content by XRF, ICP-AES and ICP-MS methods, as well as morphology study by SEM-EDS method is presented. The discrimination and classification of large group of archaeometallurgical objects was successfully achieved by exploiting of chemical signature as a ground level in PCA and cluster analysis. The main goal of this work was the confirmation of applicability and efficiency of combined multi-analytical and statistical tools in proper recognition of inhomogeneous bloom slags and mutually similar artefacts.

References
[1] A. Eliyahu-Behar, N. Yahalom-MAck, Y. Gadot, and I. Finkelstein, J. Archaeol. Sci. 40 (2013) 4319-4330.
[2] E. Blackelock, M. Martionon-Torres, H.A. Veldhuijzen, and T. Young, J. Archaeol. Sci. 36 (2009) 1745-1757.
[3] H. Portillo, M.C. Zuluaga, L.A. Ortega, A. Alonso-Olazabal, X. Murelaga, and A. Martinez-Salcedo, Microchem. J., 138 (2018) 246-254.
[4] I. Nemet, S. Rončević, A. Bugar, T. Zubin Ferri, L. Pitarević, J. Anal. At. Spectrom. 33 (2018) 2053-2061.

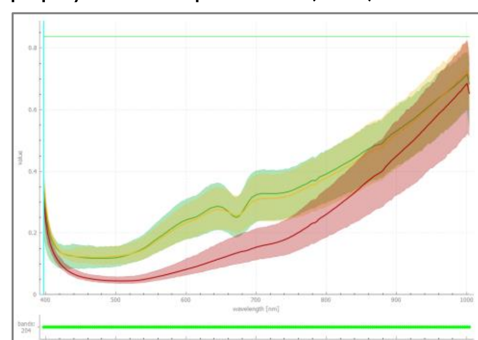
Acknowledgements
We wish to thank the Zagreb City Museum and the curator Mrs. Aleksandra Bugar for allowing excavated early-iron production samples to be analysed. The investigation was performed as a part of project No.705042 financed by Faculty of Science, University of Zagreb, Croatia.

O.22 – Handhelds for food authentication in the fight against food fraud

Sara W. Erasmus[1]*, Saskia M. van Ruth[1,2]
[1]Food Quality and Design Group, Wageningen University and Research, Netherlands
[2]Wageningen Food Safety Research, Wageningen University and Research, Netherlands
*Email: sara.erasmus@wur.nl

Abstract

Fighting food fraud is an ongoing challenge with the globalization of markets, while fraudsters become more innovative and organized in the way they commit fraud. As a way to deter and manage food fraud and ensure product authenticity, quality assurance systems require food manufacturers to perform vulnerability assessments. Within this framework, control measures form part of a mitigation plan to prevent food fraud. Although of limited value for fraud prevention, fraud monitoring prevents escalation when fraud occurs. Food fraud is the intention to cause a mismatch between food product claims and their characteristics [1]. When there is a match between a food product's characteristics and claims, it is deemed authentic. Therefore, with the authentication of food products, the authenticity of the product is verified by means of analytical methods based on chemical, physical and sensory characterization. There are various analytical techniques used to determine the authenticity of foods. The methods can be targeted (detecting specific compounds or markers) or non-targeted (generating characteristic product fingerprints). Currently, there is a great need to have analytical tools for the fast and broad screening and detection of fraudulent samples. The latest handheld spectral devices could help to address this issue as there has also been an increase in the use of spectral devices in the food industry where an increasing number of studies prove the high versatility of the applications of near-infrared (NIR) spectroscopy as well as hyperspectral imaging (HSI) [2,3,4]. For instance, using the latest Specim IQ portable HSI camera, it is possible to generate spectral fingerprints for authentic material and common adulterants. However, the challenge comes in the treatment of spectral data for the screening of adulterated samples. This presentation will cover the use of handhelds to authenticate food products – highlighting the current results, pros and cons, and future prospects.



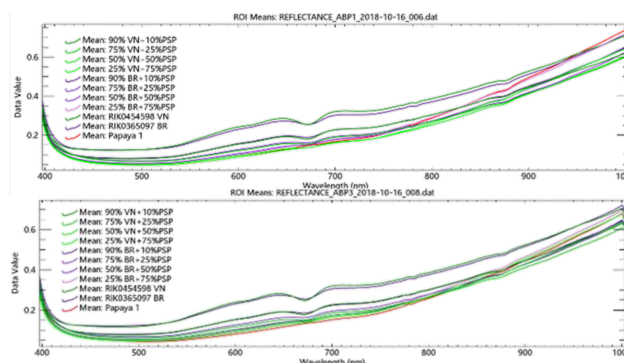


Figure 1. Right: Average spectra of authentic black pepper from Vietnam (green) and Brazil (yellow), and papaya seed powder (PSP) used as adulterant (red); Left: Authentic samples adulterated.

References

[1] CWA 17369:2019, Authenticity & fraud in the feed and food chain - Concepts, terms, and definitions.
[2] N.Liu, H.A.Parra, A.Pustjens, K.Hettinga, P.Mongondry, S.M.van Ruth, Talanta 184 (2018) 128-135.
[3] V.Acierno, G.Fasciani, S.Kiani, A.Caligiani, S.M.van Ruth, Food Chemistry, 289 (2019) 591-602.
[4] S.Kiano, S.M.van Ruth, L.W.D.van Raamsdonk, S.Minaei, LWT 104 (2019) 61-69.

Acknowledgements

The authors would like to express their gratitude towards the students, staff and personnel of the Food Quality and Design Group, and Wageningen Food Safety Research for their help with the different analyses covered in this presentation. Where relevant, their work will be referenced accordingly.

O.23 – A precise and accurate microfluidic droplet dilutor

Witold Postek*, Tomasz S. Kamiński, Piotr Garstecki
Institute of Physical Chemistry of the Polish Academy of Sciences, Kasprzaka 44/52 01-224 Warszawa, Poland
*Email: wpostek@ichf.edu.pl

Abstract
We demonstrate[1] a microfluidic system for precise (coefficient of variance between repetitions below 4 %) and highly accurate (average difference from two-fold dilution below 1 %) serial dilution of solutions inside droplets with a volume of ca. 1 µl. The two-fold dilution series can be prepared with correlation coefficient as high as R^2=0.999. The technique that we here describe uses hydrodynamic traps to precisely meter every droplet used in subsequent dilutions. We use only one metering trap to meter each and every droplet involved in the process of preparation of the dilution series. This eliminates the error of metering that would arise from the finite fidelity of fabrication of multiple metering traps. Metering every droplet at the same trap provides for high reproducibility of the volumes of the droplets, and thus high reproducibility of dilutions. We also present a device and method to precisely and accurately dilute one substance and simultaneously maintain the concentration of another substance throughout the dilution series without mixing their stock solutions. We compare the here described precise and accurate dilution systems to a simple microdroplet dilutor that comprises several traps – each trap for a subsequent dilution. We describe the effect of producing more reproducible dilutions in a simple microdroplet dilutor thanks to the application of an alternating electric field. We briefly show how the dilution series can be emulsified to provide for thousands of replications of a single experimental condition[2,3].

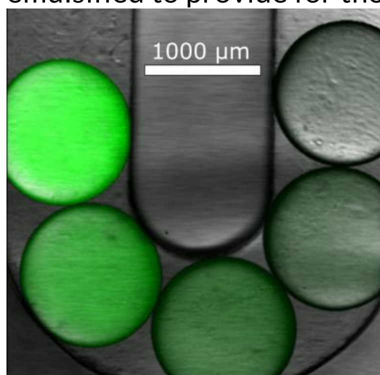


Figure 1. Microliter-sized droplets with precisely diluted fluorescent dye.

References
[1] W. Postek, T. S. Kamiński, P. Garstecki, Analyst, 2017,142, 2901-2911
[2] W. Postek, T. S. Kamiński, P. Garstecki, Lab Chip, 2017, 17, 1323-1331
[3] W. Postek, P. Garguliński, T. S. Kamiński, O. Scheler, P. Garstecki, Lab Chip, 2018, 18, 3668-3677

Acknowledgements
W.P. received funding through a doctoral scholarship from the Polish National Science Centre, scholarship code UMO-2018/28/T/ST4/00318. W.P. and P.G. acknowledge support from the Foundation of Polish Science based on decision POIR.04.04.00-00-16ED/18-00.

O.24 – Simplified Sample Collection, -Transport and -Storage for Therapeutic Drug Monitoring of Small-Molecule Drugs

Hempel, Georg[1]*; Stern, Mike; Weber, Johanna; Liebsch, Simone; Opitz, Patrick; Meistermann, Hannes
[1]Westfälische Wilhelms-Universität Münster, PharmaCampus, Klinische Pharmazie
*Email: georg.hempel@uni-muenster.de

Abstract

Background:
Therapeutic Drug Monitoring (TDM) is an instrument to control drug therapy avoiding over- and under-dosing and thereby improving efficacy and safety. However, classical venous blood sampling requires trained personell and logistics. In order to simplify TDM, we are focussing on developing methods for TDM from capillary blood without laborious sample collection and storage.
Dried blood spot (DBS) analysis is an innovative sampling technique to collect and analyse very small volumes of whole blood. The blood obtained from the finger pulp collected with capillaries coated with anticoagulants, is spotted on a filter paper. After drying, the sample can often be stored at room temperature before extraction and analysis. Thus, DBS facilitates TDM substantially. However, DBS has some drawbacks including an influence of hematokrit on the spot size and chromatography-like effects making the blood spot inhomogeneous. Therefore, we are evaluating alternative methods such as volumetric adsorptive microsampling (VAMS®) device.

Methods:
HPLC-methods for the quantification of antidepressant and antipsychotic drugs, antinfectives and anticoagulant drugs were developed using UV- fluorescence or mass spectroscopy (MS) for detection. Capillary blood samples with a volume of 10 µL were drawn and collected using VAMS devices. Validation was conducted according to the respective EMA and ICH guidelines.

Results:
After successfully establishing a DBS method for mirtazapine, citalopram and risperidone using HPLC-MS [1], the method was successfully extended to nine additional CNS-active drugs using VAMS® devices for sample storage and transport. A similar method is developed for newer anticoagulant drugs rivaroxaban, edoxaban, dabigatran and apixaban using VAMS® and HPLC-MS with column-switching. As internal standards, chemically related substances were used for all methods avoiding expensive deuterated derivatives. A relatively cheap single-quadrupole MS is used for detection. Azole antimycotics can be analyzed in similar way using HPLC-UV detection, whereas the antibiotic drug vancomycin was found to be incompatible with the VAMS device. For vancomycin, capillary blood must be directly prepared for the HPLC analysis.

Conclusion:
Our investigations demonstrate that TDM can be simplified by capillary blood sampling and devices for drying and storage of blood samples such as VAMS®. This enables the establishment of TDM in ambulatory care, for example at nursing homes. In the clinical setting, such methods can be used for TDM in neonates where the sample volume is critical. For most of the analytes, a single-quadrupole MS can be used instead of expensive MS/MS detectors making such methods applicable not only in specialized laboratories and substantially reduces the costs for TDM. Initial experience with patients demonstrate that this method of blood sampling is better accepted by patients and caregivers than classical venous blood sampling.

References

[1] Weber J et al. Validation of a dried blood spot method for therapeutic drug monitoring of citalopram, mirtazapine and risperidone and its active metabolite 9-hydroxyrisperidone using HPLC–MS. J Pharm Biomed Anal 140 (2017) 347–354

Acknowledgements

This work was supported by the Apothekerstiftung Westfalen-Lippe and the Dr. August and Dr. Anni Lesmüller-Stiftung.

O.25 – Molecular and cellular FRET-based detection for protein-ligand engagement: the case of PDE5

Giulia Di Rocco[1]*
[1]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy.
*Email: dirocco.giulia@unimore.it

Abstract
Phosphodiesterases (PDEs) regulate the intracellular levels of cAMP and cGMP. The great clinical success of the PDE5 inhibitors has led to an increasing interest for this class of enzymes. Recent studies have shown a correlation between tumor growth and PDE5 overexpression[1] but even more recent studies on AD confirmed the therapeutic potential of PDE5 inhibitors as cognitive enhancers[2], making PDE5-selective inhibitors attractive molecules in drug discovery. The search for such inhibitors rests today on radioactive assays. We have developed a faster and safer sensor tool for detecting the binding of ligands to the PDE5 conserved catalytic domain able to evaluate their affinities in vitro and suitable for medium-to-high throughput screening of new PDE5 competitive inhibitors and for estimating their potency. The new approach takes advantage of Förster Resonance Energy Transfer (FRET) between, as the donor, a fluorescein-like diarsenical probe able to covalently bind a tetracysteine motif fused to the PDE5 catalytic domain, and a rhodamine probe covalently bound to the pseudosubstrate cGMPS, as the acceptor[3,4]. We have used the FRET signal to quantitatively characterize the binding equilibrium with purified enzyme and in live HEK293 and steroidogenic MLTC-1 cell lines. Competitive displacement experiments were carried out with known inhibitors.

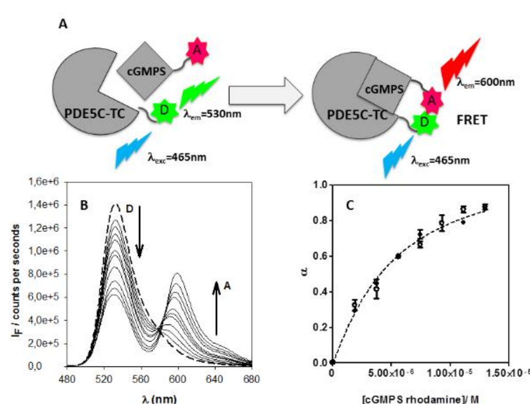


Figure 1. *Scheme of the FRET experiment between PDE5C-TC-FlAsH (the donor) and cGMPS-rhodamine (the acceptor).* ***B)*** *Emission spectra measured during the titration of PDE5C-TC-FlAsH with cGMPS-rhodamine. [FlAsH] = 7 mM, [cGMPS] = 0(dashed line)÷13 μM. The arrows indicate the decrease in FlAsH (D) emission due to cGMPs-rhod (A) addition. λ_{exc}=465 nm.* ***C)*** *Dependence of α, the fraction of occupied PDE5-TC binding sites, on the concentration of added cGMPS-rhodamine. Data represent the mean of three determinations ± SD (when larger than the circle diameter). The dashed line represents the dependence of a on cGMPS-rhodamine concentration computed with the best-fitting value of K_d.*

Phosphodiesterase 5; FRET; competitive displacement analysis; cGMPS-rhodamine;); affinity constant (Kd)

References
[1] Sponziello M. et al. Endocrine (2015) 50, 434–441. doi:10.1007/s12020-015- 0586-x
[2] Zuccarello E., et al., Biochemical Pharmacology, https://doi.org/10.1016/j.bcp.2020.113818
[3] Di Rocco G. et al. J Pharm Biomed Anal. 2018 Feb 5; 149:335-342. doi: 10.1016/j.jpba.2017.11.014
[4] Casarini L. et al. Biochemistry 2019 Feb 12;58(6):799-808. doi: 10.1021/acs.biochem.8b01073.

Acknowledgements
This work was supported by MIUR, Ministero dell'Istruzione, dell'Università e della Ricerca [FIRB grant; RBFR12FI27_005 to Di Rocco]; by Fondo di Ateneo per la Ricerca [FAR2019 to Giulia Di Rocco].

O.26 – Sample preparation for simultaneous determination of phytosterols, cholesterol, and squalene in parenteral lipid emulsions

Ana Novak[1]*, Mercè Gutiérrez-Zamora[2], Lluís Domenech[2], Josep M. Suñé-Negre[1,3], Montserrat Miñarro[1,3], Encarna García-Montoya[1,3], Josep M. Llop[1,3], Josep R. Ticó[1,3], Pilar Pérez-Lozano[1,3]
[1]Department of Pharmacy and Pharmaceutical Technology and Physical Chemistry, Faculty of Pharmacy and Food Sciences, University of Barcelona, Av. Joan XXIII, 27-31, 08028 Barcelona
[2]Service of Development of Medicines (SDM), Faculty of Pharmacy and Food Sciences, University of Barcelona, Av. Joan XXIII, 27-31, 08028 Barcelona
[3]Pharmacotherapy, Pharmacogenetics and Pharmaceutical Technology research group. Bellvitge Biomedical Research Institute (IDIBELL), Av. Granvia de l'Hospitalet, 199-203, 08090 L'Hospitalet de Llobregat
*Email: ana.novakova@gmail.com

Abstract
Analyses of commercially available lipid emulsions for parenteral nutrition are increasing due to the potential clinical effects of phytosterols, which are present in vegetable oils.
It was observed that complex sample matrix of lipid emulsions requires saponification and extraction before the analysis of sterols and squalene by high-performance liquid chromatography (HPLC). The preparation protocol was adapted according to previously described protocols [1,2]. As diluent, MeOH was chosen to avoid incompatibilities with the mobile phase and the final volume of samples was increased to 2 mL in order to ensure proper detection. It was demonstrated that saponification time of 20 min is sufficient to remove medium chain triglyceride as well as to prevent the sterol oxidation and shorten the total preparation time. Ergosterol was established as internal standard due to its good chromophore properties and its absence in lipid emulsions.
The proposed analytical procedure was used on commercially available lipid emulsions with different composition and successfully removed the effect of sample matrix in determination of the sterols and squalene.

References
[1] L. Duelund, Retrieved on July 2016 from: http://memphys.dk/sites/default/files/files/basic_page/ %3Cem%3EEdit Basic page %3C/em%3E Labprocedures/Sterol_UV_121225.pdf.
[2] Z. Xu, K.A. Harvey, T. Pavlina, G. Dutot, M. Hise, G.P. Zaloga, R.A. Siddiqui, Nutrients 4 (2012) 904–921.

Acknowledgements
This research was supported by the Investigation Agency of Spanish Society of Hospital Pharmacy (AISEFH 2014) and Institute of Health Carlos III (AES 2014, PI4/00706).

O.27 – New analytical approaches based on the coupling of Gas Chromatography to Mass Spectrometry for the quantitative characterization of energy- and environmental-related samples

Mariella Moldovan*, Laura Freije-Carrelo, Javier García-Bellido, Jorge Ruiz Encinar
Department of Physical and Analytical Chemistry, University of Oviedo, Oviedo, Spain
*Email: moldovanmariella@uniovi.es

Abstract
Nowadays, the analysis of arsenic in industrial gas and liquefied gas samples is a relevant challenge. For instance, arsenic has to be monitored in the polymer industry since its presence as impurity ethylene or propylene can affect the final polymer characteristics. In this context, the development of a new GC-ICP-MS set up allowed the simultaneous total and speciation analysis in such sample matrices, without the need of a preconcentration step[1]. An arsine in nitrogen standard was used for optimization and evaluation of the system, and good linearity and detection limits, in the very low ppt level for both total and speciation analyses, were found. Liquefied butane pressurized under nitrogen and doped with arsine and a propylene real sample from a cracker plant were analyzed using both external calibration and standard additions methods. The good match between both quantifying approaches demonstrated almost negligible matrix effects, even for the total analysis. Application of the approach to check repartition of volatile elements or species between gas and liquid phases was performed in the real propylene sample.
In parallel, the determination of sulfur compounds in crude oil products is important both for assessing the product quality but also for the characterization of future environmental emissions derived from their use. Considering the wide variety of S-containing compounds (thiols, sulfides, and aromatic sulfur heterocycles) present in fuel samples, generic quantification without specific standards would be desirable. The use of a GC-ICP-MS/MS approach allowed the absolute quantification of sulfur compounds in such products using a simple and certified generic S-containing standard[2][3]. In an initial step, the conditions for the acquisition of the sulfur signal by the GC-ICP-MS/MS were optimized. Then, experimental conditions leading to compound independent calibration were evaluated and optimized with different S-containing standards. Finally, influence of the co-elution with other massive matrix (oil) components was assessed as well. The optimized approach was validated by analysis of the certified reference material ERM-EF213 ("sulfur free gasoline”) and successfully applied to real samples (diesel and gasolines).

Keywords
arsenic compounds; sulfur compounds; speciation; gas chromatography; ICP-MS; ICP-MS/MS; generic quantification

References
[1] L. Freije-Carrelo, M. Moldovan, J.I. García Alonso, T. Thanh VO, J. Ruiz Encinar. Anal. Chem. 89 (2017) 5719-5724.
[2] L. Freije-Carrelo, J. García-Bellido, F. Calderón-Celis, M. Moldovan, J. Ruiz Encinar. Anal. Chem. 91 (2019) 7019-7024.
[3] J. García-Bellido, L. Freije-Carrelo, M. Moldovan, J. Ruiz Encinar. TRAC - Trend Anal. Chem. (2020) in press.

Acknowledgements
This research has been supported by Agilent Technologies, Borealis, Fundación Universidad de Oviedo (project FUO-EM 022-15), Principado de Asturias (project IDI/2018/000166) and Spanish Ministry of Economy and Competitiveness (project CTQ2016-79412-P).

O.28 – Open-Channel Microfluidics

Jean Berthier[1]*, Erwin Berthier[1], Ashleigh B. Theberge[1,2]
[1]Department of Chemistry, University of Washington, Box 351700, Seattle, Washington 98195, United States
[2]Department of Urology, University of Washington School of Medicine, Seattle, Washington 98105, United States
*Email: berthj2@uw.edu

Abstract
Open Microfluidics (OM) is a vast domain encompassing different types of capillary flows, such as droplet open microfluidics, paper-based, thread-based and open-channel microfluidics. The domains where OM is currently used are space science (space plumbing), materials (polymers) microelectronics, and biology and biotechnology. In these two last domains, open structures have the advantage of cheap fabrication and easy access to the fluid.

In this presentation, we focus on open-channel geometry. The geometrical conditions for spontaneous capillary flow will be presented first, using the generalized Cassie angle. Then the dynamics of these flows will be analyzed, based on the concept of average friction length. It will be shown how change of channel cross sections affects the dynamics of the flow. Extending the analysis to the flow in open networks and capillary trees, it is demonstrated that the Lucas-Washburn law must be adapted to take into account complex geometries. The case of capillary trees is especially interesting since it gives insight on capillary pumping. Finally, the flow regimes in two-phase open-capillary flows will be presented.

Numerous open microfluidic capillary devices have been developed for biological applications including cell signaling studies, organotypic models, metabolomics, multi-kingdom studies, biomimetic models, and cell migration studies.

Biography
Jean Berthier is currently with the University of Washington, in Seattle, USA. He received a MS in mathematics from the University of Grenoble, France, an engineering diploma from the Institut National Polytechnique in Grenoble and a PhD from the University Pierre et Marie Curie in Paris. After spending four years at Sandia and Los Alamos National laboratories focused on the interaction between liquid and gases, he joined first the CEA-Leti in Grenoble, then the University of Washington in Seattle. He is presently involved in the development of microfluidic solutions for liquid–liquid extraction and capillary solutions for portable point-of-care devices. In the last years he focused on the theoretical developments of “Open Microfluidics” theory. He is author of the books “Microfluidics for Biotechnology” (Artech House, second edition 2010), “Microdrops and Digital Microfluidics” (Elsevier, second edition 2012), “The physics of Micro-Droplets”, “Open Microfluidics” (Scrivener-Wiley, 2012 and 2016), and recently “Open-channel Microfluidics” (Morgan & Claypool, IOP collection, 2019). He is also the author of many publications in scientific journals, conferences articles and patents.

O.29 – Immobilized enzyme technologies in protein phosphorylation and its analysis

Marcela Slovakova[1]*, Lenka Hromádková[1], Markéta Fojtíková[1], Eliška Strouhalová[1], Rudolf Kupčík[2], Marie Vajrychová[2], Zuzana Bílková[1]
[1]Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Czech Republic
[2]Center of Biomedical Research, University Hospital Hradec Kralove, Czech Republic
*Email: marcela.slovakova@upce.cz

Abstract
Protein phosphorylation, as a major post-translational modification, has a major influence on the physiological functions of proteins, especially those that are in native conformation and are involved in many neurodegenerative diseases. However, structural and functional studies of such proteins require fully defined phosphorylation, including non-physiological ones. In this presentation, application of magnetic immobilized serine, threonine and tyrosine protein kinases will be presented. The principle of magnetic separation of particles with immobilized kinases facilitates and increases the laboratory comfort of the necessary separation of phosphorylated substrates. Immobilized enzyme activity and stability was verified using peptide substrates and evaluated by mass spectrometry analysis, western blot with phosphospecific antibodies and pIMAGO^{TM} dendrimer.
Immobilized kinases have utility in sample preparation and are also part of specialized biosensors. Compared to soluble kinases, immobilized kinases have the advantages of, in particular, reuse, long-term storage and higher enzyme stability. Thus, when phosphorylating proteins and peptides with immobilized protein kinases, the resulting product is highly pure and not contaminated with soluble enzymes. If multiple substrate phosphorylations are desired, carriers with immobilized kinases or immobilized kinases in a mixture can be sequentially used [1]. Methods used to immobilize kinases to solid supports include adsorption [2], covalent bonds [1,3], and glutaraldehyde capture [4].

References
[1] L. Hromadkova, R. Kupcik, M. Vajrychova, P. Prikryl, A. Charvatova, B. Jankovicova, M. Slovakova, The Analyst, 143(2) (2018) 466–474.
[2] L. B. R. Castro, F. F. Silva, A. M. Carmona-Ribeiro, M. Kappl, D. F. S. Petri, The Journal of Physical Chemistry B, 111(29) (2007) 8520–8526.
[3] N. Chauhan, J. Narang, C. Pundir, Indian Journal of Medical Research, 139(4) (2014) 603–609.
[4] I. S. Kucherenko, D. Y. Kucherenko, O. O. Soldatkin, F. Lagarde, S. V. Dzyadevych, A. P. Soldatkin, Talanta, 150 (2016) 469–475.

Acknowledgements
This work was supported by the OP RDE project “IT4Neuro(degeneration)", CZ.02.1.01/0.0/0.0/18_069/0010054.

O.30 – Application of molecularly imprinted polymers for bisphenols extraction from food samples

Magdalena Biesaga[1]*, Anna Kubiak[1]
[1]Faculty of Chemistry, University of Warsaw, 02-093 Pasteura 1 St. Warsaw, Poland
*Email: mbiesaga@chem.uw.edu.pl

Abstract
Bisphenols are the most-known endocrine disrupting compounds (EDCs). Due to the possible migration of these compounds from plastics used as food packaging, the food may become contaminated [1]. During past years, molecularly imprinted polymers (MIPs) were excessively being used as sorbents for bisphenols preconcentration from food samples.
The presentation intended to comprehensively discuss and evaluate the recent applications of molecularly imprinted polymers in determination of bisphenols in food samples. The fundamentals of MIPs devoted to preconcentration of bisphenols are outlined. Recent studies were discussed in terms of food sample preparation, determination methods, MIP synthesis and extraction procedure aspects. The significant aspects of applications of the molecular imprinting polymers in food chemistry are evaluated focusing majorly on literature appeared in the last years.

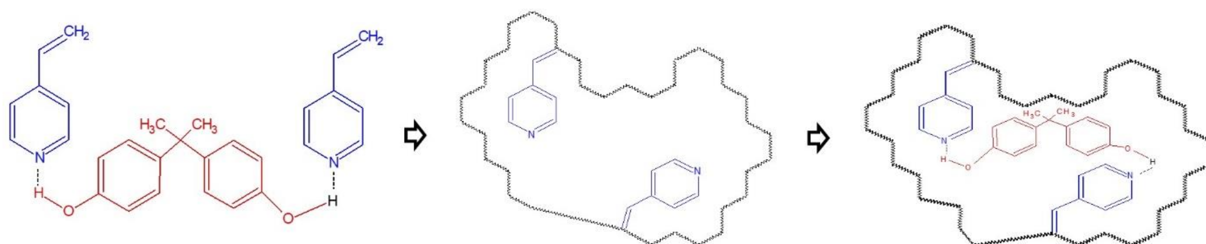


Figure 1. Molecular imprinted polymers scheme synthesized by a thermal polymerization method using bisphenol A as template and 4-vinylpyridine as functional monomer.

References
[1] C. Nerín, M. Aznar, D. Carrizo, Food contamination during food process. Trends Food Sci Technol. 48 (2016) 63–68

O.31 – SPE: Online, automatically renewable and integrated in -omic workflows

David J. Cocovi-Solberg[1]*, Stephan Hann[1]
[1]University of Natural Resources and Life Sciences, Vienna, Muthgasse 18, 1190 Vienna
*Email: david.cocovi-solberg@boku.ac.at

Abstract
The audience has been using SPE over the years for preconcentration, matrix cleanup, desalting and solvent exchange; as a collateral benefit, the analytical instrumentation requires less service and the output data has higher analytical quality. However, 'omics workflows in the frame of exposome evaluation demand extra capabilities as: ultra-clean performance comparable to clean room work, fully automated operation, sample throughput compatible with ultimate spatiotemporal monitoring, simple operation and even portability in the case of in-situ analysis.
In this contribution we present a system based on the manipulation of sorbent beads in closed fluidic manifolds for performing automatic SPE. The idea was implemented in the 90s but was not successful because of poor reproducibility [1]. We have improved the performance using spherical wettable beads, commercial components with narrow tolerances and custom valve prototypes. The automatic control resorts to pressure readings as QC/QA for the automatic manipulation of beads, and tunes all SPE variables, like the surface chemistry of sorbents and their number, loading and eluting volumes and flowrates or number and composition of cleaning steps, to name a few. Advanced scripting also allows the system to be self-optimized, without supervision by the operator.
The described system has been online combined with LC-ICP-MS and LC-ESI-TOF-MS, from which several applications will be presented, including simple enrichment, SPE-based speciation, several tandem SPE opportunities or turbulent flow chromatography, and applied to environmental and clinical samples.
The most striking feature of the presented system is its flexibility, and thus, figures of merit depend on the configuration, but we can highlight the automated unattended operation, preconcentration factors up to 100, manipulation of sample volumes from sub-µL to several mL, possibility of using more than one sorbent per analysis, mass of reusable SPE sorbents ranging from 1 mg to 30 mg per analysis and intermediate precision below 5% relative standard deviation.

References
[1] M. Miró, S. Hartell, J. Jakmunee, Trends Anal. Chem. 27 (2008) 749-761.

Acknowledgements
The authors acknowledge VICI AG International for the kind provision of the fluidic equipment. DJCS acknowledges the Austrian Science fund (FWF) for the postdoc grant through the Meitner-program (Project Number M 2579).

O.32 – On-line solid phase extraction using molecularly imprinted polymers and nanofibers coupled to high performance liquid chromatography

Ivona Lhotská[1]*, Aneta Kholová[1], Martina Háková[1], Dalibor Šatínský[1]
[1]Charles University, Faculty of Pharmacy in Hradec Králové
*Email: lhotski@faf.cuni.cz

Abstract
Sample preparation prior to chromatographic separation plays an important role in the analytical process to reduce matrix interferences and preconcentrate analytes. Current trends that are making progress in analytical methods development are targeted on automation of extraction techniques that can significantly save time and sample handling and increase throughput of routine laboratories. To avoid time-consuming and manual handling sample-prep, automated on-line techniques such as on-line SPE-HPLC by column switching are therefore preferred. Column-switching chromatographic system hyphenizes extraction and separation step by coupling extraction and separation column. Hence, preconcentration and clean-up are performed on extraction column directly in chromatographic system followed by separation on analytical column and detection. The on-line SPE-HPLC methods introduce advantages of significant reduction of analysis time, less handling with toxic sample, preconcentration and clean-up of large volume sample, full automation, and repeatability [1].
Selection of extraction sorbent effects selectivity, clean-up efficiency, robustness, and extraction capacity. In the presented study, advanced polymeric sorbents such as nanofibers with high surface to volume ratio [2] and selective molecularly imprinted polymers were applied in several cases of food and environmental contamination. For example, original molecularly imprinted polymer for mycotoxin citrinin selective extraction was prepared and applied for optimization of on-line SPE-HPLC method for determination of citrinin in food supplements and cereals [3].
Simultaneous optimization of extraction together with separation step is quite problematic, especially in the case of molecularly imprinted polymers and nanofibers. Difficulties and possible solutions concerning column choice, time programming, compatibility of mobile phases and flow inconsistency in column-switching system [4] will be discussed as well as partial steps of optimization, and critical evaluation.

References
[1] C. Fernández-Ramos, D. Šatínský, B. Šmídová, P. Solich, Trends in Analytical Chemistry 62 (2014) 69-85.
[2] M. Háková, L. Chocholoušová Havlíková, P. Solich, F. Švec, D. Šatínský, Trends in Analytical Chemistry 110 (2019) 81-96.
[3] I. Lhotská, A. Kholová, A. Machyňáková, K. Hroboňová, P. Solich, F. Švec, D. Šatínský, Analytical and Bioanalytical Chemistry 411 (2019) 2395-2404.
[4] E. Rogatsky, K. Braaten, G. Cruikshank, H. Jayatillake, B. Zheng, D.T. Stein, Journal of Chromatography A 1216 (2009) 7721-7727.

Acknowledgements
This work was supported by the Czech Science Foundation, project GAČR No 20-19297S.

O.33 – Microfluidic Platform for Label-Free Sorting of Enzymes and Cells

Paul Abbyad[1]*, Claudia Zielke[1], Ching W. Pan[1], Daniel Horvath[1], Samuel Braza[1], Adriana J. Gutierrez Ramirez[1], Cameron Feit[1], Chandler Dobson[1], Trevor Moore[1], Catherine Davidson[1]
[1]Department of Chemistry and Biochemistry, Santa Clara University, USA.
*Email: pabbyad@scu.edu

Abstract
We present a novel method for the label-free sorting of enzymes by activity, and cells by metabolism. In droplet microfluidics, chemical and biological reagents are confined in picoliter droplets transported in inert oil. It was found with the use of a specific surfactant; the interfacial tension of droplets can be very sensitive to droplet pH.[1] This enables the sorting of droplets of different pH when confined droplets encounter a microfabricated trench (see Figure 1). We dubbed this droplet sorting strategy: Sorting by Interfacial Tension (SIFT). The device can be used to sort enzymes, as hundreds of enzyme reactions lead to the production of an acidic or basic product and a concurrent change in solution pH. [1] The technique is relevant for use in high-throughput applications that include enzyme screening and directed evolution of enzymes. The same method can also be used to sort cells as glycolysis leads to the cellular excretion of lactate and acidification of the extracellular environment.[2] Cancer cells, in particular, display high rates of glycolysis even in the presence of oxygen. We show here the specific selection of cells from a population that exhibit elevated glycolysis (Figure 1).[3] This technique holds promise in oncology for isolation of malignant cells as high levels of glycolysis have been associated with metastasis, tumor recurrence and poor outcomes. Moreover, the developed platform can be a general tool in different fields to isolate cells or subpopulations with high rates of metabolism for applications in bioenergy, biotechnology and medicine.

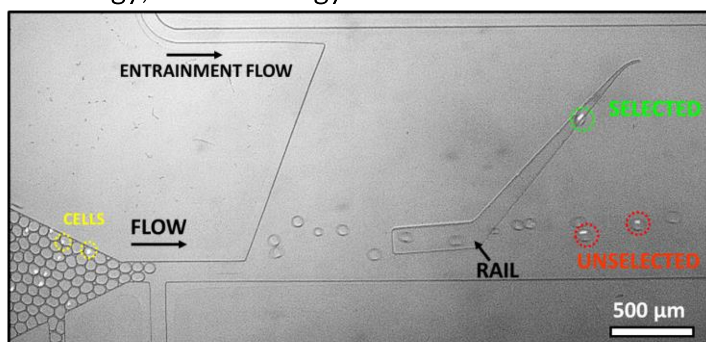


Figure 1. *Selection of droplets of different pH using SIFT. K562 cells (a few circled in yellow) are labelled for better visibility. Droplets containing a highly glycolytic cell (circled in green) ride the rail laterally up, leaving the rail at the top (selected). Droplets containing no cell or less active cells (circled in red) do not ride the rail or are only slightly deflected up the sorting rail (unselected).*

Keywords
microfluidics; droplets; sorting; cells; cancer

References

[1] Horvath, D.G.; Braza, S.; Moore, T.; Pan, C.W.; Zhu, L.; Pak, O.S.; Abbyad, P. Analytica Chimica Acta 2019, 1089, 108–114.
[2] Pan, C.W.; Horvath, D.G.; Braza, S.; Moore, T.; Lynch, A.; Feit, C.; Abbyad, P. Lab on a Chip 2019, 19, 1344–1351.
[3] Zielke, C.; Pan, C.W.; Gutierrez Ramirez, A.J.; Feit, C.; Dobson, C.; Davidson, C.; Sandel, B.; Abbyad, P. Anal Chem 2020, 92, 6949–6957.

Acknowledgements
This work is supported for this project by a National Science Foundation Career Award, Grant Number 1751861, the National Institutes of Health under grant 1R15GM129674-01 and the Henry Dreyfus Teacher-Scholar Awards Program.

O.34 – Simple and highly-available microextraction techniques using menthol

Irina Timofeeva*, Andrey Bulatov, Leonid Moskvin
Department of Analytical Chemistry, Institute of Chemistry, Saint Petersburg State University, RU-198504 Saint Petersburg, Russia
*Email: timofeeva.irina.7@gmail.com

Abstract
Sample pretreatment plays a key role in chemical analysis. As usual, it includes the separation or/and preconcentration of analytes from complex sample matrices with the improvements of selectivity and sensitivity. For effective and green sample pretreatment, the selection of an eco-friendly, selective and cheap extraction solvent is always a challenging task in analytical chemistry. Recently, menthol has been proposed as an alternative to conventional organic solvents for liquid-liquid microextraction due to its low melting point (40 °C), low vapor pressure and its high viscosity, which allows to carry out the green and reproducible extracting process [1]. Moreover, menthol has grown increasingly popular as a cheap and green component used for hydrophobic deep eutectic solvents synthesis [2]. Here we present novel approaches of the dispersive liquid-liquid microextraction using menthol for the simple and highly-available pretreatment of complex sample matrices. The developed techniques combined with HPLC-UV detection were applied for the determination of benzoic and sorbic acids in beverages. These substances are commonly used as preservatives in food industry [3]. However, their excessive use can lead to metabolic acidosis, convulsions, and hyperpnoea in humans [4]. The simplicity of operations, rapidity and low cost can be mentioned as advantages of the developed procedures.

References
[1] M.A. Farajzadeh, L. Goushjuii, Anal. Methods, 5 (2013) 1975-1982.
[2] D. Ge, Y. Zhang, Y. Dai, S. Yang, J. Sep. Sci. 41 (2018) 1635-1643.
[3] C. Guarino, F. Fuselli, A. La Mantia, Lucia Longo, Food Chem. 127 (2011) 1294-1299.
[4] S.A.V. Tfouni, M.C.F. Toledo, Food Control 13 (2002) 117-123.

Acknowledgements
The authors gratefully acknowledge the Russian Scientific Foundation (project no. 16-13-10117) for financial support.

SHOTGUN PRESENTATIONS

ST'2020

4th International Caparica Christmas Conference on Sample Treatment

SG.01 – Skin sampling with a flat solid-phase extraction probe followed by on-line mass spectrometric analysis

Hsiang-Ting Ni[1]#, Pawel L. Urban[1,2]*
[1] Department of Chemistry, National Tsing Hua University, 101, Section 2, Kuang-Fu Rd., Hsinchu, 30013, Taiwan
[2] Frontier Research Center on Fundamental and Applied Sciences of Matters, National Tsing Hua University, 101, Section 2, Kuang-Fu Rd., Hsinchu, 30013, Taiwan
*Email (corresponding author): urban@mx.nthu.edu.tw
#Email (presenting author): a26304865@gmail.com

Abstract

Sweat is a biofluid, which can be utilized in biochemical assays. However, sampling sweat from skin is not straightforward. Previously, our group developed gel-phase microextraction approach for sampling skin excretion residues from various surfaces [1]. Following sample collection, the gel probes were re-extracted into a solvent, and the extract was analyzed by mass spectrometry (MS). Alternatively, hydrogel probes can be analyzed by nanospray desorption electrospray ionization [2]. We also took advantage of liquid microjunction surface sample probe [3] to analyze compounds on surfaces [4], and considered it for analysis of hydrogel probes. However, the above methods have intrinsic limitations. Here, we propose another type of probe and approach for sampling and analysis of skin excretions (Figure 1). It uses disk-shaped probe containing extraction beads. The beads are held between two semi-permeable membranes. The probe is tested by using an "artificial skin" model. An extraction tool is further provided to enable elution of samples analytes from the disk-shaped probe into MS in an on-line system. We believe that this solid-phase extraction variant can provide a viable alternative for skin sampling and analysis.

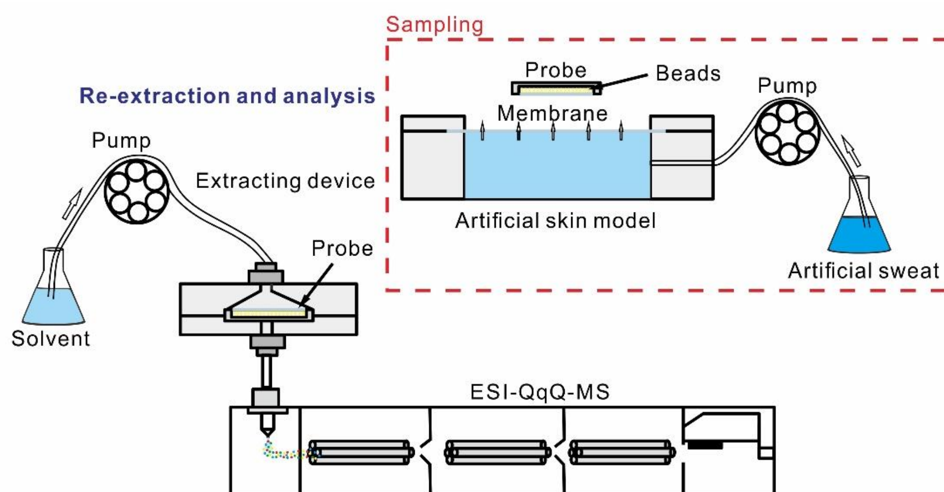


Figure 1. Scheme of experimental procedure.

References

[1] P.-H. Liao, P. L. Urban, *ACS Omega* **2019**, *4*, 19063-19070.
[2] E. P. Dutkiewicz, J. D. Lin, T.-W. Tseng, Y.-S. Wang, P. L. Urban, *Anal. Chem.* **2014**, *86*, 2337-2344.
[3] M. S. ElNaggar, G. J. Van Berkel, *J. Am. Soc. Mass Spectrom.* **2011**, *22*, 1737-1743.
[4] G. R. D. Prabhu, T.-H. Yang, C.-Y. Hsu, C.-P. Shih, C.-M. Chang, P.-H. Liao, H.-T. Ni, P. L. Urban *Nat. Protoc.* **2020**, DOI: 10.1038/s41596-019-0272-1.

Acknowledgements

We would like to thank the National Tsing Hua University, the Ministry of Science and Technology of Taiwan, and the Ministry of Education of Taiwan for their support.

SG.02 – Mass spectrometric analysis of skin excretions by hydrogel-phase sampling followed by automated liquid-phase re-extraction

Decibel P. Elpa[1,2]#, Shu-Pao Wu[1]*, Pawel L. Urban[2,3]*
[1]Department of Applied Chemistry, National Chiao Tung University, 1001 University Rd., Hsinchu, 300, Taiwan
[2]Department of Chemistry, National Tsing Hua University, 101, Section 2, Kuang-Fu Rd., Hsinchu, 30013, Taiwan
[3]Frontier Research Center on Fundamental and Applied Sciences of Matters, National Tsing Hua University, 101, Section 2, Kuang-Fu Rd., Hsinchu, 30013, Taiwan
Email (presenting author): elpa.decibel@gmail.com
* Email (corresponding authors): spwu@mail.nctu.edu.tw, urban@mx.nthu.edu.tw

Abstract
The recently developed ionization and sample introduction techniques for mass spectrometry (MS) workflows have made it possible to directly probe samples with little or no sample preparation [1]. Moreover, the introduction of automation in MS analytical workflows has addressed the constraints of MS applications regarding reproducibility, throughput, and the expertise required to operate MS instruments [2]. Skin excretions can be sampled using a hydrogel probe and analyzed by nanospray desorption electrospray ionization MS [3]. Such a probe is user-friendly and biocompatible. It has proven to be an efficient tool for sampling water-soluble analytes from various surfaces [4]. The so-called liquid microjunction-surface sampling probe (LMJ-SSP) system [5] provides an alternative method to re-extract polar analytes from hydrogel probes exposed to skin. The LMJ-SSP operation, subsequent delivery to MS, data acquisition and processing, and cloud data storage were recently automated [6]. In the present work, the automated LMJ-SSP is used in conjunction with hydrogel probes to provide a tool for skin metabolite analysis. We anticipate that the hydrogel probe skin sampling, combined with the automated LMJ-SSP-MS analysis, will be useful in skin metabolite detection.

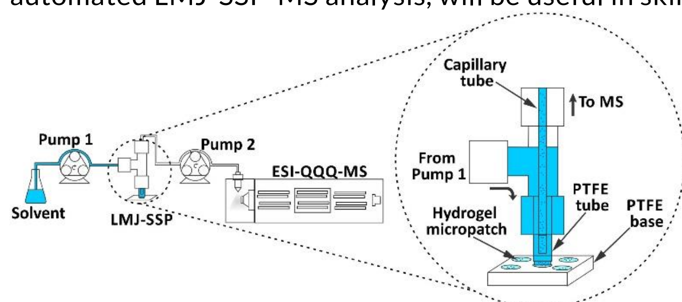


Figure 1. Setup for re-extraction and analysis of skin biomarkers (adapted from [6]).

References
[1] R.M. Alberici, R.C. Simas, G.B. Sanvido, W. Ramão, P.M. Lalli, M. Benassi, I.B.S. Cunha, M.N. Eberlin, Anal. Bioanal. Chem. 398 (2010) 265–294.
[2] D.P. Elpa, G.R.D. Prabhu, S.-P. Wu, K.S. Tay, P.L. Urban, Talanta 208 (2020) 120304.
[3] E.P. Dutkiewicz, J.-D. Lin, T.-W. Tseng, Y.-S. Wang, P.L. Urban, Anal. Chem. 86 (2014) 2337–2344.
[4] P.-H. Liao, P.L. Urban, ACS Omega 4 (2019) 19063–19070.
[5] M.S. ElNaggar, G.J. Van Berkel, J. Am. Soc. Mass Spectrom. 22 (2011) 1737–1743.
[6] G.R.D. Prabhu, T.-H. Yang, C.-Y. Hsu, C.-P. Shih, C.-M. Chang, P.-H. Liao, H.-T. Ni, P.L. Urban, Nat. Protoc. (2020) (DOI: 10.1038/s41596-019-0272-1).

Acknowledgements
We would like to thank the National Chiao Tung University, the National Tsing Hua University, the Ministry of Science and Technology (Taiwan), and the Ministry of Education (Taiwan) for their support.

SG.03 – Polyamide coated wooden toothpicks for the determination of drugs of abuse in saliva by DI-MS

Jaime Millán-Santiago*, María Teresa García-Valverde, Rafael Lucena, Soledad Cárdenas
Departamento de Química Analítica, Instituto Universitario de Investigación en Química Fina y Nanoquímica (IUNAN), Universidad de Córdoba, Campus de Rabanales, Edificio Marie Curie (anexo), E-14071 Córdoba
*Email: q52misaj@uco.es

Abstract
The use of natural products as sorbents such as paper, cotton, wood, cork and pollen, among others, is the latest trend in microextraction [1]. These materials, which are mostly composed by natural polymers, like cellulose, hemicellulose and lignin, are deemed in line with the green analytical chemistry principles, since it implies the use of renewable resources. Among them, wood presents some interesting benefits due to its composition and porosity degree, which allows its interaction with a great variety of compounds. Moreover, its particular structure provides rigidity, being also used as inert physical support. For that reason, wood has been employed in the microextraction context in the form of wooden toothpicks (WT) [2] or wooden sticks [3]. However, in some cases a modification of the wooden surface is required in order to increase the sensitivity and the selectivity of the analytical method [4,5].
In this study, the modification of wooden toothpicks with nylon-6 (WT-N6) to be used as sorbent for the determination of drugs of abuse by direct infusion mass spectrometry (DI-MS) is presented. The proposed coating method simplifies the derivatization process and reduces the use of chemicals, following in this way the environmental consciousness. Different characterization techniques, such as ATR-IR and SEM were used to confirm the correct modification of the wooden tip surface. Additionally, due to the morphology of WT-N6, the extraction process was carried out in a 2 mL vial, attaching the wooden tip to the vial cap. This approach allows the processing of different samples at the same time, providing a high-throughput analysis (20 samples/hour). On the basis of the results obtained, nylon-6 coating increases the extraction efficiency of WT by nearly 40%, proving the enhancement of the sensitivity. The variables involved in the extraction have been studied by means of a design of experiments. Finally, the method has been analytically characterized in respect of its linearity, sensitivity and precision showing suitable results.

References
[1] G. Mafra, M.T. García-Valverde, J. Millán-Santiago, E. Carasek, R. Lucena, S. Cárdenas, Separations 7 (2020) 1–22.
[2] B. Hu, P.K. So, H. Chen, Z.P. Yao, Anal. Chem. 83 (2011) 8201–8207.
[3] H. Karimiyan, M.R. Hadjmohammadi, K.L. Kunjali, M.M. Moein, J. Dutta, M. Abdel-Rehim, Molecules 24 (2019) 1–12.
[4] J. Deng, Y. Yang, L. Fang, L. Lin, H. Zhou, T. Luan, Anal. Chem. 86 (2014) 11159–11166.
[5] J. Deng, T. Yu, Y. Yao, Q. Peng, L. Luo, B. Chen, X. Wang, Y. Yang, T. Luan, Anal. Chim. Acta 954 (2017) 52–59.

Acknowledgements
Financial support from the Spanish Ministry of Economy and Competitiveness (CTQ2017-83175R) is gratefully acknowledged. Also, J. Millán-Santiago expresses his gratitude for the “Beca Semillero de Investigación” grant from the University of Córdoba.

SG.04 – Potential of air sampling device based on a computer fan for the isolation of volatile organic compounds and nanoparticles

Francisco Antonio Casado-Carmona*, Guillermo Lasarte-Aragonés, Rafael Lucena and Soledad Cárdenas
Departamento de Química Analítica, Instituto Universitario Nanoquímica (IUNAN), Universidad de Córdoba, Campus de Rabanales, Edificio Marie Curie, E-14071, Córdoba, Spain
*Email: q92cascf@uco.es

Abstract
The analysis of environmental air is challenging due to the vast volume of this compartment, its heterogenous distribution (both spatial and temporal) and the usual low concentration of the target compounds intended to be monitored. For those reasons, effective analysis should preferably integrate sampling and preconcentration on a single step [1] which can be developed in an active (forced flow though of the sample) and passive sampling approaches [2]. In this context, solid phase microextraction has been extensively applied to the analysis of volatile organic compounds in air [3,4].
In this research work an active sampling device (Patent pending) which is based on micro-solid phase extraction was designed and evaluated. The invention consists of a computer fan where the trap (the extraction media used for the isolation of the targets) is attached to the blades as depicted in Figure 1. Two different traps have been evaluated depending on the targets of the analysis. In this sense, sorptive phases are used for the isolation of volatile/semivolatile organic compounds while sticky phases are used to retain particulate matter.

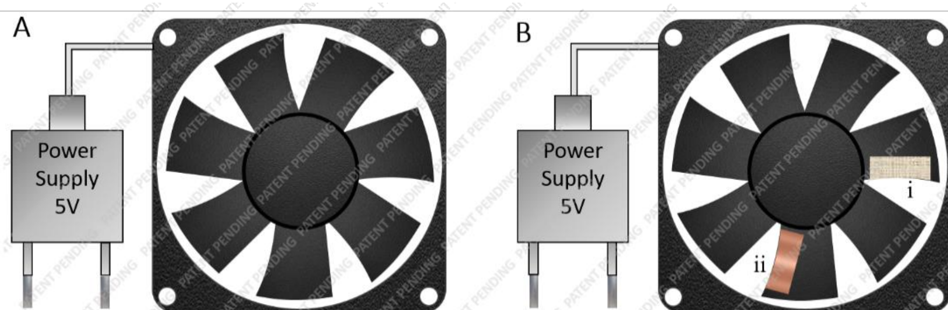


Figure 1. 1a) Sampling device without sorbent phases. 1b) Sampling device with sorbent phases (i- Sorptive phase ii- Conductive double-sided tape).

References
[1] M.C. Alcudia-León, R. Lucena, S. Cárdenas, M. Valcárcel, A. Kabir, K.G. Furton, J. Chromatogr. A 1488 (2017) 17–25.
[2] J.A. Martin, J. Kwak, S.W. Harshman, K. Chan, M. Fan, B.A. Geier, C.C. Grigsby, D.K. Ott, Int. J. Environ. Anal. Chem. 96 (2016) 299–319.
[3] W.-H. Cheng, J.-R. Jiang, C. Lin, J.-J. Liou, Z.-H. Wu, Y.-H. Hsu, Z.-Y. Yang, J. Air Waste Manage. Assoc. 64 (2014) 488–493.
[4] N. Baimatova, B. Kenessov, J.A. Koziel, L. Carlsen, M. Bektassov, O.P. Demyanenko, Talanta 154 (2016) 46–52.

Acknowledgements
Financial support from the Spanish Ministry of Economy and Competitiveness (CTQ2017-83175R) is gratefully acknowledged. F.A. Casado-Carmona expresses his gratitude for the predoctoral contract (ref. FPU16/06069) from the Spanish Ministry of Education, Culture and Sport.

SG.05 – Supramolecular solvent-based liquid phase microextraction prior to HPLC-UV determination of sulphonamides in biological fluids

Polina Bogdanova*, Aleksei Pochivalov, Christina Vakh, Andrey Bulatov
Institute of Chemistry, St. Petersburg State University, 7/9 Universitetskaya nab., St. Petersburg,199034 Russia
*Email: paulinebogdanova@gmail.com

Abstract
Supramolecular solvent-based liquid phase microextraction assumes an application of supramolecular solvent (SUPRAS) as an extractant. The SUPRASs are water immiscible liquids synthesized by self-assembly of two amphiphile solutions in a continuous phase in which self-assembly processes occur on the molecular and nano scale. These supramolecular assemblies of SUPRAS lead to properties useful for extraction of inorganic and organic compounds. A supramolecular solvent-based liquid phase microextraction from aqueous samples usually assumes in situ SUPRAS-rich phase formation. Ionic and nonionic compounds such as alkylcarboxylic acids, alcohol ethoxylates, alkylsulfonates etc. were successfully implemented as SUPRASs in microextraction.
In the present research it was found that SUPRASs can be formed in aqueous solutions containing primary amines with the long hydrocarbon chain and monoterpenoid compounds. When the monoterpenoid was dissolved in primary amine isotropic solution the spontaneous in situ formation of SUPRAS-rich phase was observed (Fig.1). Similar SUPRASs could be potentially applied for sample preparation of biological liquids with the aim to eliminate the negative effect of matrix components and separate target analytes. The developed approach was utilized for the separation of polar sulfonamide antibiotics: sulfamethoxazole, sulfamethazine and sulfapyridine from biological fluids followed by the HPLC-UV determination.
Several parameters affecting the sulfonamides extraction efficiency were investigated: type of primary amine and its volume, amount of coacervation agent and sample volume. The linear dynamic ranges were 0.05-50 mg L^{-1} for sulfamethoxazole, sulfamethazine and sulfapyridine. The LODs were 0.02 mg L^{-1} for sulfamethoxazole, sulfamethazine and sulfapyridine.

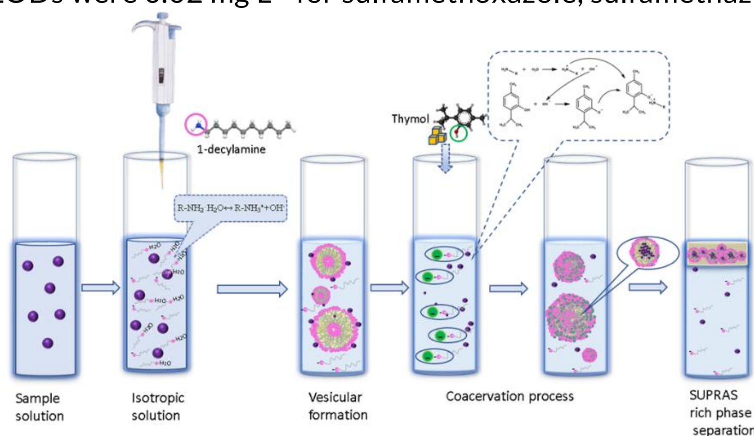


Figure 1. The mechanism of supramolecular solvent microextraction with the use of primary amine and monoterpenoid phenol

References
[1] A. Ballesteros-Gomez, M. D. Sicilia, S. Rubio, Analytica Chimica Acta 677 (2010) 108–130.

Acknowledgements
Authors gratefully acknowledges financial support by Russian Science Foundation (research projects No. 19-73-00067).

SG.06 – Application of hydrogels for bisphenols extraction

Anna Kubiak*, Marcin Maćkiewicz, Marcin Karbarz, Magdalena Biesaga
Faculty of Chemistry, University of Warsaw, 02-093 Pasteura 1 St. Warsaw, Poland
*Email: akubiak@chem.uw.edu.pl

Abstract
Bisphenols are well-known endocrine disruptors and they can easily migrate from plastic and can containers to food [1]. In this study, an extraction-preconcentration procedure based on the use of hydrogel as sorbent has been developed for the determination of five bisphenols (bisphenol A, bisphenol S, bisphenol E, bisphenol B and bisphenol F). Liquid chromatography with UV and fluorescence detection (LC–UV/FLD) was used for the separation and identification of these analytes. Hydrogels were obtained by precipitation polymerization with ethylene glycol dimethacrylate (EDGMA) as cross-linker, oligo(ethylene glycol) methyl ether methacrylate (OEGMA) as monomer and ammonium persulfate (APS) as initiator. The surface morphology of hydrogel was analyzed by using scanning electron microscopy (SEM) and *transmission electron microscopy (TEM).* To evaluate the effectiveness of the synthesized hydrogel for enrichment of bisphenols, the adsorption capacity and equilibrium time was investigated. Figure 1 presents adsorption isotherms of hydrogel for different concentration of bisphenols, where Q is the adsorption capacity. Only 1 mg of hydrogel was sufficient to achieve very good sorption of bisphenols. The hydrogel could be recycled three times without losing adsorption efficiency. The analytical characteristic of this method makes it suitable for monitoring programs, intended for the assessment of human exposure to different bisphenols in environmental samples.

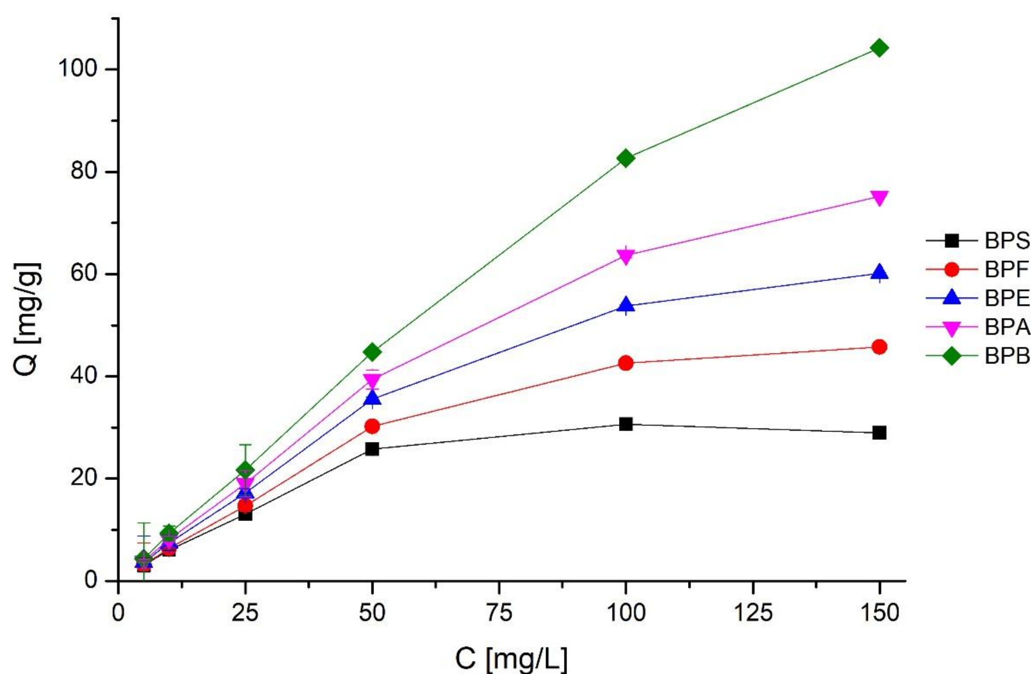


Figure 1. Adsorption isotherms of hydrogel for different concentration of BPS, BPF, BPE, BPA and BPB (1 mL of sample, 1 mg of dried gel).

References
[1] Y. Yang, L. Lu, J. Zhang, Y. Yang, Y. Wu, B. Shao, Simultaneous determination of seven bisphenols in environmental water and solid samples by liquid chromatography–electrospray tandem mass spectrometry. Journal of Chromatography A, 1328 (2014) 26– 34

SG.07 – Impact of sample collection device type on analyte concentration for the HPLC-MS/MS analysis of commonly abused substances from oral fluid

Łukasz Sobczak*, Bartosz Sadowski, Krzysztof Goryński
Bioanalysis Scientific Group, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz at Nicolaus Copernicus University in Toruń, Poland
*Email: lukaszsobczak39@gmail.com

Abstract
Oral fluid is steadily establishing its position as reliable bioanalytical sample, alternative or complementary to plasma or urine – especially in forensics, driving under the influence of drugs and workplace testing. Collection of oral fluid sample is not only convenient and non-invasive, but also preferred by the donors according to survey results. Sample collection is first and one of the most important steps of testing procedure. However its significance seems to be underappreciated, and not often devices are evaluated for analyte recovery in order to select most suitable type for certain application.
This research compared swabs made of absorptive material from 15 various devices produced by 10 different manufacturers. Test was conducted by collecting mixture of 49 popular drugs with swabs and analyzing retrieved samples with HPLC-MS/MS instrument. Commonly abused substances (including drugs of abuse and doping agents) were selected based on the latest European reports on drug abuse and anti-doping testing figures by the World Anti-Doping Agency [1,2]. Analytical method included C_{18} column separation (Agilent InfinityLab Poroshell 120 EC-C18, 3.0x100mm, 2.7µm) in reversed-phase mode with gradient elution (phase A: water with 0.1% formic acid; phase B: acetonitrile with 0.1% formic acid) and detection with triple quadrupole mass spectrometer (Shimadzu LCMS-8060).
As an outcome, 15 device types and 49 analytes resulted in total of 735 results, out of which 324 (44.1%) were within 80-120% drug recovery range. 7 of 15 tested devices provided results within 80-120% range for more than half of tested drugs. Porex Saliva Collection Swabs (4.5x90mm and 8x125mm) and Dräger DCD 5000 performed best and provided results within aforementioned range for 29 analytes. Majority of results were also within aforementioned range with: Salimetrics SalivaBio Oral Swab, StatSure Saliva Sampler, Immunalysis Quantisal and Oasis AccuSAL. Matrix effect (originating mainly from collection swab material) was investigated to reassure that experiment results were unbiased by this phenomenon. As a result, 393 out of 735 (53.5%) calculated matrix effect values were within 80-120% range - suggesting no impact on the results. While the 160 matrix effect values were below 80%, and 182 were over 120% - indicating respectively phenomena of signal suppression or enhancement.
Oral fluid collection method is critical, as it can significantly influence analyte concentration in sample. Thus, many difficulties of improving already existing sample preparation and analysis methods could be easily avoided just by focusing on sample collection step.

References
[1] European Drug Report 2019: Trends and Developments. http://www.emcdda.europa.eu/publications/edr/trends-developments/2019_en, 2019.
[2] 2018 Anti-Doping Testing Figures. https://www.wada-ama.org/en/resources/laboratories/anti-doping-testing-figures-report, 2019.

Acknowledgements
This work was supported by The National Centre for Research and Development under Lider IX programme: grant LIDER/44/0164/L-9/NCBR/2018 – A new approach to rapid screening of selected drugs and small molecules in oral fluid.
Permission to conduct experiments with controlled substances was issued by Kujawsko-Pomorski Wojewódzki Inspektor Farmaceutyczny w Bydgoszczy (permission number WIFBY-KK.857.2.4.2016).

SG.08 – Quantitative assessment of cellular uptake and differential toxic effects of HgSe NPs in human cells

Laura Cid-Barrio[1]*, Diego Bouzas-Ramos[1], Alfonso Salinas-Castillo[2], Yasumitsu Ogra[3], Jorge Ruiz Encinar[1] and José M. Costa Fernández[1]

[1]Department of Physical and Analytical Chemistry, University of Oviedo, Av. Julián Clavería 8, 33006, Oviedo, Spain. [2]Department of Analytical Chemistry, University of Granada, 187071, Granada, Spain. [3]Laboratory of Toxicology and Environmental Health, Graduate School of Pharmaceutical Sciences, Chiba University, Chuo, Chiba 260-8675, Japan.

*Email: cidlaura@uniovi.es

Abstract

Mercury (Hg) is one of the most prevalent toxic elements which is present in the environment in different molecular forms. Additionally, the role of Selenium (Se) in Hg toxicity due to the protective effect of Se against the toxicity of Hg species has been widely studied, although it is not yet unravelled. Recently, the formation of biomineralized mercury selenide HgSe nanoparticles (NPs) has been reported as the ultimate metabolic product of Hg detoxification [1]. In fact, HgSe granules and NPs have been found in different biological tissues of marine mammals and in human brain tissues. However, there is no a deeper knowledge about HgSe NPs metabolisms and toxicity, mainly due to the lack of adequate standards materials that could be used in *in vivo* and *in vitro* studies. Herein, we report the first research on the cellular uptake, location and cytotoxicity of water-stabilized HgSe NPs, using two human cell lines well stablished in cytotoxicity studies, HeLa and HEK 293 cells. Additionally, a critical comparison of Hg toxicity and cellular uptake obtained after cell exposure to different Hg species (inorganic and organic Hg) was also evaluated.

First, a sonochemical synthesis of HgSe NPs was carried out and the resulting suspension was transferred to aqueous media by capping them with dihydrolipoic acid (DHLA) ligands [2]. The resulting HgSe NPs exhibited intense native fluoresce with sharp emission at 575 nm. Such fluorescence emission enabled to assess their cellular distribution and constituted the starting point of a pioneer comparative study of the cytotoxicity of HgSe NPs with those induced by the widely studied inorganic mercury (II) and methylmercury in the two human cell lines. Then, a cytotoxicity study based on the cellular activity by MTT assay was carried out, to evaluate eventual cellular toxicity in the presence of different Hg species. For that purpose, cell viability by HgSe NPs were compared to those induced by inorganic and organic Hg species (Hg^{2+} and $MeHg^{+}$) after their incubation at different Hg concentrations and exposure times. Results revealed that water-stabilized HgSe NPs do not induce any remarkable effect of cell viability, even at higher Hg concentration used. Instead of that, lower exposure levels of Hg^{2+} and $MeHg^{+}$ alone, and even in the presence of other Se species (selenite), caused acute cytotoxicity. Finally intracellular concentration of different Hg species was quantitatively determined by measuring Hg content in digested cells by ICP-MSMS. Results indicated that Hg incorporation when cells were exposed to the same Hg concentration added as NPs (41%) and $MeHg^{+}$ (36%) are quite similar, instead of the decrease on Hg^{2+} uptake (8%). Therefore, the null cell cytotoxicity effect observed for HgSe NPs cannot be ascribed to a lower Hg internalization [3].

Summarizing, water-stable DHLA-HgSe NPs standards synthesized and thoroughly characterized in our laboratory have been successfully applied for cell viability and quantitative cellular uptake studies. Moreover, such standards could be used to obtain a deeper understanding on Hg detoxification pathways. In fact, preliminary findings presented herein could constitute the basis for further environmental studies on HgSe NPs.

Keywords: Synthesis, Nanoparticles, Cytotoxicity, Nanotechnology, Quantification.

References

[1] M. A. Khan and GF. Wang. Chem. Res. Toxicol. 2010, 23, 7, 1202-1206.

[2] D. Bouzas-Ramos, M. Menéndez-Miranda, J. M. Costa-Fernández, J. Ruiz Encinar and A. Sanz-Medel. RSC Adv.,2016,6,19964-19972.

[3] L. Cid-Barrio, D. Bouzas-Ramos, A. Salinas, Y. Ogra, J. Ruiz Encinar and J. M. Costa Fernández. J. Anal. Atomic Spectrom. JA-ART-04-2020-000162.R1.

Acknowledgements

L. C. B. acknowledge the PhD grant FPU15/04989 from the Spanish Ministry of Education and PID2019-109698GB-I00 from Spanish Ministry of Economy and Competitiveness.

SG.09 – Determination of mercury in sea waters by magnetic dispersive solid phase extraction prior to quantitation by FI-CV-GFAAS

J.C. García-Mesa*, P. Montoro-Leal*, S. Maireles-Rivas, M.M. López Guerrero, E. Vereda Alonso
Dpto. Analytical Chemistry, Faculty of Science, University of Malaga, Spain.
*Email: jcgm95@uma.es

Abstract
Mercury is a non-essential trace element that is toxic to humans due to the bioaccumulation effect. In this work, a shell structured Fe_3O_4@graphene oxide nanospheres were used to develop a magnetic dispersive solid phase extraction (MDSPE) method for the extraction and preconcentration of ultra-trace amounts of Hg(II). After first enrichment, a second online preconcentration by cold vapor generation was conducted, followed by the determination of the analyte by graphite furnace atomic absorption spectrometry (CV-GFAAS). The influences of several analytical parameters were optimized for MDSPE and CV-GFAAS. Under the optimized conditions, %RSD, detection limit and determination limit were 2.9%, 0.25 $ng\cdot L^{-1}$ and 4.9 $ng\cdot L^{-1}$, respectively. Thanks to the 500 µL loop, a high preconcentration factor can be achieved even with low sample volume. For example, 5 mL of sample would be preconcentrated 10 times. Moreover, this method is suitable for high sample volume, resulting in a preconcentration factor >250. The accuracy of the proposed method was verified using a certified reference material (mussel tissue NIST 2976) and by determining the analyte content in spiked sea waters and tap water samples collected from Málaga and Cádiz (Spain). The determined values were in good agreement with the certified values and the recoveries for the spiked samples were close to 100% in all cases. The results showed the proposed method is simple, rapid, environmentally friendly and sensitive enough for the accurate determination of mercury.
Keywords
Ultra-trace, Mercury, MDSPE, CV-GFAAS

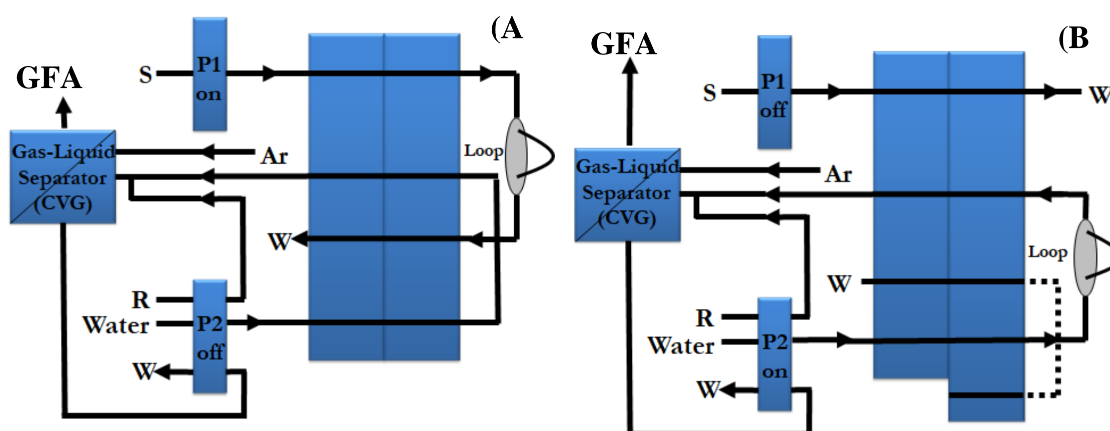


Figure 1. FI system schematic diagram for load step (A) and elution step (B). P1 and P2, peristaltic pumps; W, waste; S, sample; R, reductant.

Acknowledgements
The authors thank "Plan Propio, Universidad de Málaga" for supporting this study and also FEDER funds and Junta de Andalucia (Project UMA18-FEDERJA-060) for financial support of this work.

SG.10 – Semiautomatic method for the ultra-trace arsenic speciation in environmental waters via magnetic solid phase extraction prior to HPLC-ICP-MS determination

P. Montoro-Leal*, J.C. García-Mesa, M.M. López Guerrero, M.T. Siles Cordero, E. Vereda Alonso.
Dpto. Analytical Chemistry, Faculty of Science, University of Malaga, Spain.
*Email: montoroleal.pablo@uma.es

Abstract
A new magnetic functionalized material based on graphene oxide magnetic nanoparticles (MGO) was designed and characterized in order to develop a magnetic solid phase extraction method (MSPE) to enrich both inorganic and organic arsenic species in environmental waters. A FIA system is used to preconcentrate the arsenic species simultaneously, while the ultra-trace separation and determination of arsenobetaine (AsBet), cacodylate, As(III) and As(V) species have been achieved by high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) technique. The sample is introduced in the FIA system where the MSPE takes place, and 1 ml of eluent is collected in a chromatographic vial. After preconcentration, the vial is introduced in the autosampler of HPLC-ICP-MS. Therefore, preconcentration and separation/determination processes are automatic and conducted separately (Figure 1). The strategy of this work was focused on the compatibility between the MSPE eluent and the mobile phase of HPLC column system, resulting in an efficient and reliable semiautomatic preconcentration and detection of inorganic and organic arsenic speciation. To the best of our knowledge, this is the first method combining an online MSPE and HPLC-MS, and one of the first using a magnetic nanomaterial based on MGO for online MSPE. Under the optimized conditions, the preconcentration factors obtained for AsBet, cacodilate, As(III) and As(V) with 12 mL sample solution were 4, 12, 6 and 19, respectively. The LODs for the arsenic species were AsBet 7 ng L^{-1}, cacodylate 0.4 ng L^{-1}, As(III) 1.0 ng L^{-1} and As(V) 0.2 ng L^{-1} and RSDs < 5%. The developed method was validated by analyzing tap water, well water and seawater samples, and Certified Reference Materials; fortified lake water TMDA 64.3 and seawater Cass-6 NRC, promising for routine monitoring of arsenic species in environmental water.
Keywords: Speciation; Arsenic; MSPE; HPLC-ICP-MS

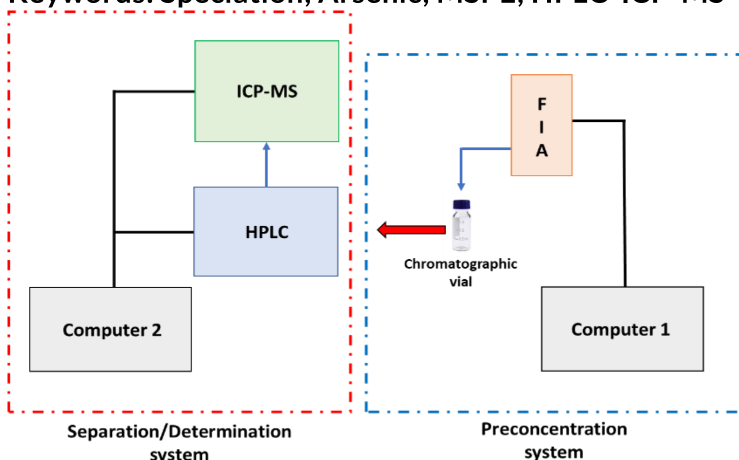


Figure 1. *Online MSPE coupled to HPLC-ICP-MS technique.*

Acknowledgements
The authors thank “Plan Propio, Universidad de Málaga” for supporting this study and also FEDER funds and Junta de Andalucia (Project UMA18-FEDERJA-060) for financial support of this work and the Spanish Ministerio de Ciencia y Tecnología for the fellowship FPU18/05371.

SG.11 – Influence of gut microbiota in the distribution of selenoproteins and selenometabolites in different organs of mice model

Sara Ramírez-Acosta[1,2,3]*, Nieves Abril[4], Marta Selma-Royo[5], María Carmen Collado [5], Francisco Navarro Roldán[6], José Luis Gómez-Ariza[1,2,3], Tamara García-Barrera[1,2,3]
[1]Department of Chemistry, Faculty of Experimental Sciences, University of Huelva, Campus El Carmen, Fuerzas Armadas Ave., 21007, Huelva, Spain. [2]International Agrofood Campus of Excellence ceiA3. University of Huelva, Spain. [3]Research Center for Natural Resources Health and The Environment (RENSMA). University of Huelva, Spain, [4]Department of Biochemistry and Molecular Biology, University of Córdoba, Campus de Rabanales, Edificio Severo Ochoa, E-14071, Córdoba, Spain. [5]Institute of Agrochemistry and Food Technology (IATA-CSIC), Food Biotechnology, Agustin Escardino 7. 46980 Paterna, Valencia, Spain. [6]Department of Environmental Biology and Public Health. University of Huelva. Spain
*Email: sara.ramirez@dqcm.uhu.es

Abstract
Selenium (Se) is an essential trace element in mammals that can be presented in different chemical forms and is used for the synthesis of selenoenzymes (selenoproteins) which are involved in numerous metabolic processes [1]. The human microbiota is defined as a complex and dynamic wide community (bacteria, virus, archaea and fungi) that inhabits human body, and it is involved in immunological, physiological and metabolic functions of the host [2]. It has been demonstrated that dietary Se affects both composition and diversity of the intestinal microbiota which, in turn, influence the host Se status and selenoproteome expression [3].
Forty mice (*Mus musculus)* were randomly divided in four groups (10 mice/group): mice fed rodent diet (group A), mice fed Se supplemented diet (group B), antibiotic treated-mice fed rodent diet (group C) and antibiotic treated-mice fed Se supplemented diet (group D). The extraction of selenometabolites and selenoproteins from organ tissues (liver, kidney, brain and testis) were carried out with CelLytic™ MT extraction reagent following the manufacturer's instructions with some modifications. To this end, 1.5 mL of extraction reagent was added to 0.5 gr of tissue sample. The mixture was homogenized using a glass/teflon homogenizer and centrifuged at 15500 x *g* for 20 min at 4°C. The supernatant was collected and filtered through low protein adsorption filters (PVDF, 25 mm, 0.45 µm). For kidney, testicles and brain extracts, the preconcentration is necessary before the analysis due to the low concentration of selenospecies. The selenoproteins and selenometabolites were determined by two-dimensional column switching, which consisted of two size exclusion columns (5 mL HiTrap® Desalting Columns) and two affinity columns (1 mL Heparin-Sepharose and 1ml Blue-Sepharose columns) coupled to inductively coupled plasma mass spectrometry. The relative abundance of Se species in all organ tissues is: selenoportein P (Selenop)> selenoalbumin (SeAlb)>glutathione peroxidase (eGPx)>Se-metabolites. The highest concentration of selenospecies where found in liver, followed by kidney, testis and finally brain. Significant differences were found among groups in the concentration of eGPx and SeAlb in liver and in eGPx, Selenop and SeAlb in kidney. We found important and new correlations between Se and microbiota, demonstrating that Se shapes the composition, diversity and richness in antibiotic treated mice fed Se supplemented diet (group D). In mice fed Se supplemented diet (group B) there is no impact on diversity and richness, but we found some different bacterial groups.
Keywords: Selenoproteins; gut microbiota; organ tissues; ICP-MS

References
[1] Rayman, M. P. *Lancet (London, England)* 2000, *356* (9225), 233–241.
[2] Collado, M. C.; Rautava, S.; Isolauri, E.; Salminen, S. *Pediatr. Res.* 2015, *77* (1), 182–188.
[3] Kasaikina, M. V.; Kravtsova, M. A.; Lee, B. C.; Seravalli, J.; et al. *FASEB J.* 2011, *25* (7), 2492–2499.

Acknowledgements
This work was supported by the projects PG2018-096608-B-C21 from the Spanish Ministry of Economy and Competitiveness, UHU-1256905 from the FEDER Andalusian Operative Program 2014-2020 (Regional Ministry of Economy, Knowledge, Business and Universities, Spain). Authors are grateful to FEDER (European Community) for financial support, Grant UNHU13-1E-1611.

SG.12 – Human breast milk metabolomic profiling in lactating women with iodine deficiency by UPLC-QTOF-MS

A. Arias-Borrego[1]*, I. Muñoz-Cabrera[1], I. Velasco-López[2], J.L. Gómez-Ariza[1], T. García-Barrera[1]
[1]Department of Chemistry, Campus El Carmen, Faculty of Experimental Sciences, University of Huelva, Fuerzas Ave., 21007, Huelva, Spain. Research Center for Natural Resources, Health and The Environment (RENSMA). [2]Pediatrics, Obstetrics & Gynecology Unit, Hospital de Riotinto, Avda la Esquila 5, 21.660 Minas de Riotinto, Huelva, Spain
*Email: ana.arias@dqcm.uhu.es

Abstract
Adequate human breast milk iodine concentration (BMIC) is essential to provide sufficient iodine supply for the infant. Sufficient iodine intake during infancy is important to ensure optimal thyroid hormone stores and to prevent impaired neurological development [1]. In this sense, breastfed infants are dependent on an adequate supply of iodine in human milk for the production of thyroid hormones, necessary for development of the brain [2]. Despite the importance of iodine for infant health, data on lactating women are scarce.
For this reason, this study characterized for the first time the breast milk metabolic profiles of lactating woman with iodine deficiency and no iodine deficiency using metabolomics based on UPLC-QTOF-MS for polar and lipidic metabolites. The study includes human breast milk with iodine deficiency (BMID, n=30) and adequate human breast milk iodine concentration (BMIC, n=30). The extraction methodology was developed in a single-phase using an optimized methyl-tert-butyl ether solvent system [3]. The application of PLS-DA presented a clear classification of groups of samples, indicating the existence of altered metabolites in BMID. Thirty eight perturbed metabolites in the BMID were annotated in the comparison of BMHC. The pathway analysis indicated that several amino acid metabolic routes were the most affected in BMID. Finally, ROC curves were applied to the dataset and metabolites with an AUC value higher than 0.75 were considered as relevant.

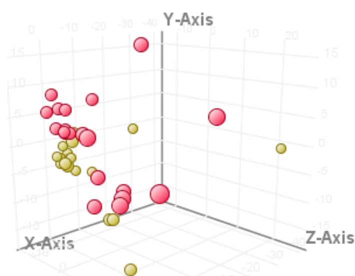


Figure 1. *PLS-DA score plots from human breast milk from lactating woman with iodine deficiency BMID (red dots) and control BMIC (yellow dots).*

Keywords: Iodine; Human breast milk; Metabolomic; Lactating women

References

[1] S. Groufh-Jacobsen, L.M. Mosand, I. Oma, K.S. Bakken, Nutrients 12 (2020) 630, doi:10.3390/nu12030630.
[2] Y. Chen, M. Gao, Y. Bai, Y. Hao, W. Chen, T. Cui, W. Guo, L. Lin, Ch. Wang, J. Shen, W. Zhang, Nutrients 71 (2020)1 10599.
[3] A. Villaseñor, I. García-Perez, A. Garcia, J.M. Posman, M. Fernández-López, A. J. Nicholas, N. Modi, E. Holmes, C.Barbas, Anal.Chem 86 (2014) 8245-8252.

Acknowledgements
This work was supported by the projects PG2018-096608-B-C21 from the Spanish Ministry of Economy and Competitiveness (MINECO) and UHU-1256905 from the FEDER Andalusian Operative Program 2014-2020 (Ministry of Economy, Knowledge, Business and Universities, Regional Government of Andalusia, Spain). Authors are grateful to FEDER (European Community) for financial support, Grant UNHU13-1E-1611.

SG.13 – Fecal metabolomics approach to evaluate the influence selenium on mice metabolism and gut microbiota

Belén Callejon-Leblic[1,2,3]*, Marta Selma-Royo[4], María Carmen Collado[4], Nieves Abril[5], José Luis Gómez-Ariza[1,2,3], Tamara García-Barrera[1,2,3]
[1]Department of Chemistry, Faculty of Experimental Sciences, University of Huelva, Campus El Carmen, Fuerzas Armadas Ave., 21007 Huelva, Spain. [2]International Agrofood Campus of Excellence ceiA3. University of Huelva, Spain. [3]Research Center for Natural Resources Health and The Environment (RENSMA).University of Huelva, Spain. [4]Institute of Agrochemistry and Food Technology (IATA-CSIC), Food Biotechnology, Agustin Escardino 7, Valencia, Spain. [5]Department of Biochemistry and Molecular Biology, University of Córdoba, Campus de Rabanales, Edificio Severo Ochoa, E-14071, Córdoba, Spain.
*Email: belen.callejon@dqcm.uhu.es

Abstract
Microbiota is emerging as an important factor involved in human health and diseases [1]. Gut microbial metabolites analysis is essential to understand the molecular mechanism of the host-microbe interactions. In this sense, gut metabolites would reflect the results of nutrient ingestion, digestion and absorption as well as microbial activity [2]. Selenium (Se) is a trace element that has been shown to modulate gut microbiota composition and ameliorate intestinal mucositis [3]. We aimed to deep insight into the selenium-host microbiota interplay. Thus, we examined the impact of Se on mice gut metabolite profile, gut microbiota composition and metabolism. To this end, forty mice (*Mus musculus)* were randomly divided in four groups (10 mice/group): mice fed rodent diet (group A), mice fed Se supplemented diet (group B), antibiotic treated-mice fed rodent diet (group C) and antibiotic treated-mice fed Se supplemented diet (group D). In our work, fecal metabolomics has been applied to evaluate the influence of gut microbiota on metabolism. On the other hand, the effect of Se on the microbiota was also studied.
The extraction of metabolites in feces was performed by the addition of a mixture of MeOH:$CHCl_3$ (3:1 v/v) to the samples followed by vortexing during 30 minutes. Then, samples were centrifuged at 16000 g, 4° C and the supernatant was dried using a N_2 stream. Finally, the extracts were reconstituted with derivatizing agents (methoxiamine to protect carbonil groups and *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide to convert polar groups-OH to apolar -OSi) before the injection into the gas chromatography-mass spectrometry (GC-MS). The resulting chromatograms were processed to obtain the metabolites present in feces and a multivariante analysis based on partial least square discriminant analysis (PLS-DA) was used to determine the most perturbed metabolites due to the depressed microbiota in mice. Amino acids, organic and fatty acids and derivatives were found altered in feces from microbiota-depressed *mus musculus* mice suggesting that the important role of gut microbiota metabolism balance. Moreover, we found important and new correlations between Se and microbiota, demonstrating that Se shapes the composition, diversity and richness in antibiotic treated-mice fed Se supplemented diet (group D). In mice fed Se supplemented diet (group B) there is no impact on diversity and richness, but we found some different bacterial groups.
Keywords: **Feces, fecal metabolomics, gas chromatography, mass spectrometry, gut microbiota.**

References
[1] J.Xu et al. Trends in Analytical Chemistry 112 (2019) 161-174
[2] A.Heintz-Buschart etal. Trend Microbiol. 26 (2018) 563
[3] M. V. Kasaikina et al. FASEB J. 25 (2011) 2492–2499.

Acknowledgements
This work was supported by the projects PG2018-096608-B-C21 from the Spanish Ministry of Economy and Competitiveness, UHU-1256905 from the FEDER Andalusian Operative Program 2014-2020 (Regional Ministry of Economy, Knowledge, Business and Universities, Spain). Authors are grateful to FEDER (European Community) for financial support, Grant UNHU13-1E-1611.

SG.14 – Effect of erosive challenge and cigarette smoke on dentin microhardness, surface morphology and bond strength

M.A. Ferretti[1]*, J.D. Theobaldo[1], R. Pereira[1], G.M.B. Ambrosano[1], D.A.N.L. Lima[1], F.H.B. Aguiar[1]
[1]Piracicaba Dental School, University of Campinas, Brazil;
*Email: marcela.a.ferretti@gmail.com

Abstract
This study aimed to evaluate the surface microhardness and morphology of dentin, submitted to erosive cycles and cigarette smoke exposure, as well as the microshear bond strength of a self-etching adhesive (Clearfil SE, Kuraray) to eroded dentin, exposed or not to cigarette smoke. Forty dental crowns were divided into 4 groups (n = 10): no treatment (control) (C); erosion (E); erosion + cigarette smoke exposure (ES); cigarette smoke exposure (S). Samples were prepared through third molars polishing until dentin exposure, followed by crown section. Erosive cycles were performed 5 times/day for 30 s at 60 min intervals. Cigarette smoke was produced with twenty cigarettes/day, during 5 days. Microhardness was evaluated initially and after the treatments. Microshear bond strength was tested after the treatments and dentin restoration with flow composite. Failure patterns and dentin morphology was evaluated by Scanning Electron Microscopy. Data were submitted to statistical analyses. Loss percentage of microhardness was observed only in groups submitted to erosion. Bond strength was statistically similar between all groups. The most prevalent failure pattern was of adhesive type. Morphological analysis of dentin showed obliterated tubules in groups submitted to cigarette smoke exposure. Groups submitted to erosion presented greater exposure and diameter of dentinal tubules. Erosion may cause adverse effects at dentin surface, reducing its microhardness. Although cigarette smoke do not affect the dentin microhardness, cigarette combustion products may obliterate the dentinal tubules, which can be long-term prejudicial. Self-etch adhesives are suitable to restore exposed dentin in smokers who present erosion lesions.

Acknologments:
The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Brasil (CAPES- Finance code 001) for the support in this study, and also thank the Piracicaba Dental School (FOP-UNICAMP) for provide the laboratory area to this research development.

SG.15 – Development of an on-line MSPE-ICP-OES method for the preconcentration and speciation of Cr(III)/Cr(VI) in aqueous samples

Irene Morales-Benítez, Juan Carlos García-Mesa, Pablo Montoro-Leal, María del Mar López Guerrero, Elisa Vereda Alonso*
Department of Analytical Chemistry, Faculty of Sciences, University of Málaga, Málaga, Spain
*Email: eivereda@uma.es

Abstract
In this work, a new double-reactor method for the analysis and speciation of trace amounts of Cr(III)/Cr(VI) ions in environmental samples has been developed combining on-line magnetic solid phase extraction (MSPE) with inductively coupled plasma optical emission spectrometry (ICP OES). For the preconcentration and speciation of Cr, a new magnetic graphene oxide (MGO) functionalized with p-sulfanilic acid has been syhthesized. This material presents good capacity of adsorption towards Cr(III) and Cr(VI) species. The FI manifold used for on-line preconcentration and elution is shown in Fig.1. The eight-port valve (V) was changed from position A-B, and vice versa allowing the load of sample of the two reactors (R1 and R2) followed of the elution of Cr(VI) with NH_3 3.2 % in R1 and total Cr with HNO_3 2.3 % in R2 to ICP-OES. Subtracting the signal of Cr (VI) to the signal of Cr(III)+Cr(VI), both ions can be determined. Moreover, several flow and chemical variables were optimized by two multivariate central composite designs (CCD). The optimized method offers good sensitivity and precision. The accuracy of the proposed method was verified using certified reference materials. The obtained results were in good agreement with the certified values and high recoveries were achieved for the spiked samples. Thus, the new adsorbent has demonstrated to be useful for the preconcentration and speciation of Cr(III)/Cr(VI).

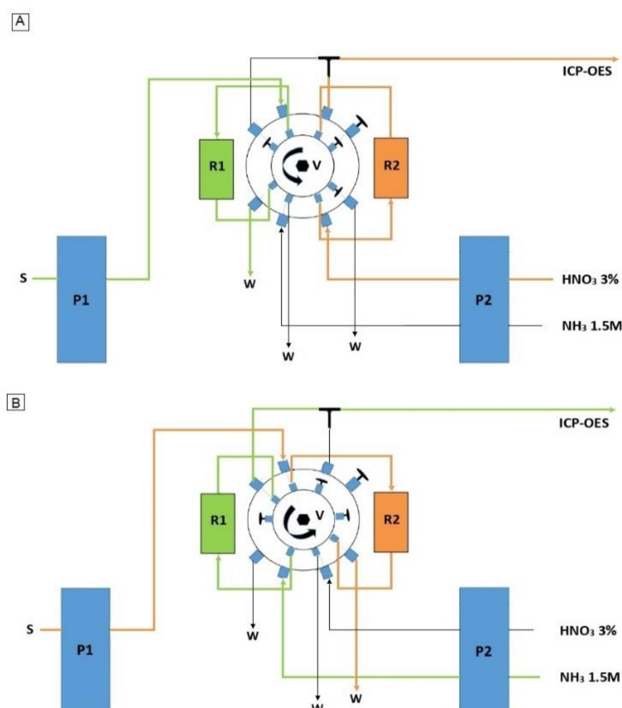


Fig.1. Schematic diagram of the FI system for the preconcentration and separation of Cr(VI) and total Cr. A) Load of R1 and elution of R2 with HNO_3; B) Load of R2 and elution of R1 with NH_3

The authors thank "Plan Propio, Universidad de Málaga" for supporting this study and, and also FEDER funds and Junta de Andalucia (Project UMA18-FEDERJA-060) for financial support of this work.

POSTER PRESENTATIONS

ST'2020

4th International Caparica Christmas Conference on Sample Treatment

P.01 – Comparative study of various sorbents for determination of five bisphenols in milk using on-line solid phase extraction coupled to liquid chromatography

Aneta Kholová*, Hedvika Raabová, Ivona Lhotská, Dalibor Šatínský
The Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
*Email: kholovaa@faf.cuni.cz

Abstract
Various advanced sorbents for the on-line extraction and determination of bisphenol A, bisphenol AF, bisphenol C, bisphenol A diglycidyl ether, and bisphenol F diglycidyl ether in bovine milk have been compared. Milk is a complex matrix containing proteins and lipids. Our approach to sample preparation represents a new on-line method including fast extraction using precolumn coupled to liquid chromatography with fluorescence detection. Five types of fibrous sorbents including polyethylene microfibers, polypropylene microfibers, polycaprolactone microfibers/nanofibers composite, polycaprolactone microfibers/polyvinylidene difluoride nanofibers composite, and polyamide 6 microfibers were compared with commercially available molecularly imprinted polymers for bisphenols and restricted access media sorbent RP-18 ADS in terms of extraction and clean-up efficiency. The cartridge containing polymer fibers and commercial devices were directly connected to HPLC system and the clean-up step and the subsequent chromatographic separation were optimized. The separation was carried out using analytical column YMC-Triart C18 ExRS (150 × 4.6 mm, particle size 3 μm) followed by fluorescence detection (Ex 273 nm, Em 300 nm). Solvents suitable for the separation were acetonitrile and water in gradient elution and 15% methanol for extraction. A flow rate of 1.0 ml/min was used. The sorbents producing best results were used for control of bisphenols contamination in milk packed in plastic bottles. Our results were compared with the migration limit established by the European Union for BPA that is 0.05 mg/kg in food contact plastics.

Acknowledgements
A.K. acknowledge the financial support of the Charles University, project No SVV 260 412 and GA UK No 1134119 and by the Czech Science Foundation, project GA ČR No 20-19297S. This work was supported by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by the ERDF.

P.02 – CO2 efficient hydrogenation into methanol and dimethyl ether

P.V. Samokhin *, G.I. Lin, M.A. Kipnis
Topchiev Institute of Petrochemical Synthesis, Russian Academy of Sciences, Moscow, Russia.
*Email: samokhin@ips.ac.ru

Abstract
Considering the problem of CO2 utilization, the processes of synthesis of methanol and dimethyl ether were investigated in the flow-circulation mode from synthesis gas, vol. % H2 -76.6, CO2 - 19.8, N2 - rest. A bench-scale process was used with flow-type separator, which was cooled by running water. Commercial catalysts were employed: MegaMax 507 (CuO/ZnO/Al2O3) and zeolite HTSVM (SiO2/Al2O3=33) (fraction 1-3.8 mm). Methanol synthesis data: temperature, flow rates of the inlet synthesis gas and the purge gas, circulation rate, CO2 conversion (X), selectivity of CO formation (S), CO2 conversion per pass (K), methanol synthesis productivity (P_{Met}) are shown in table 1.

Table 1. Methanol synthesis at 5 MPa (MegaMax 507. Weight 5 g)

T, °C	Purge gas composition, vol. %				L h^{-1}			X,	Sco,	K,	P_{Met},
	N_2	CO	CO_2	CH_4	Circ.	SG	Purge	%	%	%	kg kg_{kat}^{-1} h^{-1}
220	10.6	4.1	8.7	0.05	200	15	5.0	85.0	8.2	12	0.62
220	9.8	4.5	8.1	0.04	360	14	5.2	84.0	10.4	7	0.49
240	12.9	3.1	6.16	0.16	190	19	5.0	91.6	4.7	22	0.96
240	14.4	3.3	3.7	0.13	380	20	5.1	95.3	4.4	21	0.94
260	15.0	2.2	3.0	0.39	180	22	5.1	96.4	2.6	42	1.24
240	17.4	1.0	0.8	0.44	370	12	2.5	99.2	1.0	44	0.58
260	17.6	0.7	0.4	0.67	380	12	2.5	99.6	0.7	60	0.68

High values of CO2 conversion were reached by variation of temperature, purge gas flow rate, circulation flow rate. There was detected almost complete condensation of methanol in the separator. The largest productivity per catalyst weight at 260°C was 1.24 kg kgkat-1 h-1.
Table 2 reveals the data of experiments, which were carried out at 5 MPa, at the purge gas flow rate of 5 L h-1, at the temperature of separation of 6°C. The bifunctional catalyst was used. Water was the main product at the condensate in the course of DME synthesis unlike the methanol synthesis. The relative yield of DME was in the range of 0.08—0.17 kg kgkat-1 h-1. The conversion of methanol into DME was higher than 42 %.

Table 2. DME synthesis (MegaMax 507/ HTsVM, the weight ratio of 1/1. Weight 10 g)

T, °C	Purge gas composition, vol. %					L h^{-1}		X,	Sco,	P_{Met},	P_{DME},
	CO	CO_2	CH_4	DME	H_2O	Circ.	SG	%	%	g h^{-1}	g h^{-1}
220	3.3	9.4	0.13	3.1	0.5	130	12.2	79	9.5	0.8	1.1
220	4	8.5	0.09	2.9	0.5	370	12.2	82	10.6	1.1	0.8
240	2.7	6.4	0.19	3.5	0.6	110	14.6	88	5.8	1.7	1.0
240	3.3	6.4	0.24	3.2	0.7	120	14	88	7.2	1.1	1.3
240	4.3	6.2	0.15	3	0.6	370	13.9	88	9.3	1.3	1.1
260	1.9	4.1	0.56	3.6	0.6	110	17.8	94	3.1	1.9	1.65
260	2.9	2.8	0.4	3.2	0.7	150	18.7	96	4.5	2.6	1.3

Acknowledgements
The study was supported by a grant from the Russian Science Foundation (project no. 17-73-30046).

P.03 – Alternative methods for upgrading low value sub products and circular economy

Gema Rodríguez Moro[1,2]*, Alejandra Bartolomé[2,3], Juan L. Fuentes[2,3], C Vílchez-Lobato[2,3], Juana María Frontela Delgado[4], Jesús Javier Lázaro Muñoz[4], Mariana da Silva M. M. Lopes[4], José Luis Gómez Ariza[1,2], Tamara García Barrera[1,2]

[1]Department of Chemistry, Campus El Carmen, Faculty of Experimental Sciences, University of Huelva, Fuerzas Armadas Ave., 21007, Huelva, Spain. [2]Research Center for Natural Resources Health and the Environment (RENSMA), University of Huelva. [3]Algal Biotechnology Group, CIDERTA, University of Huelva, Parque Huelva Empresarial, 21007 Huelva, Spain. [4]Cepsa Research Center, Compañía Española de Petróleos S.A., Av. Punto Com 1, 28850 Alcalá de Henares, Madrid, Spain.
*Email: gema.moro@dqcm.uhu.es

Abstract

TAR is a sub-product of the phenol process in Cepsa Química Palos (Cepsa S.A.) generated during its manufacturing process of phenol. The main objective of this study is the analysis of the TAR composition after the application of biodegradation treatments with a microbial consortium (CM) to evaluate the potential for biodegradation of the components of TAR. The second objective is to design an analytical approach to evaluate metabolic products of possible interest produced by biodegradation.

The preparation of the soluble fraction of TAR required for the optimal growth of the microbial community has some drawbacks, since most components of this stream present low solubility in water which decreases with the ionic strength of the medium. This disadvantage has been partially solved by using an emulsion of the sample with an Ultraturrax device. The dilution factor required for the optimal growth of the microbial community makes difficult the direct analysis by gas chromatography mass spectrometry. Due to the dilution of this stream involved in the preparation of these samples, direct analysis using conventional methodology is not possible due to the polarity of the compounds and a new method based on liquid-liquid extraction (LLE) [1] of the soluble fraction with toluene and subsequent analysis by gas chromatography (GC-MS) has been optimized. The main compounds of phenol TAR have been identified and quantified after biodegradation showing the reduction of several compounds. In order to identify metabolic compounds of possible interest after biodegradation, the next step in our study is the analysis by non-targeted mass spectrometry using an ultrafast high performance liquid chromatography coupled to quadrupole time of flight (UHPLC-QTOF) followed by statistical analysis to identify the main discriminant variables (chromatographic peaks) between non treated and treated residues after different times. Thus, the analytical methodology allows determining the capacity to biodegrade TAR components by the microbial community, as shown by the results obtained and the possibility to determine new metabolic compounds after biodegradation is currently under research.

Keywords

TAR, microbial consortium, liquid-liquid extraction and gas chromatography.

References

[1] EPA Method 625.1. United States Environmental Protection Agency

Acknowledgements

This work was supported by the Proyecto: 19/1002; Corporación Tecnológica de Andalucía. Authors are grateful to FEDER (European Community) for financial support, Grant UNHU13-1E-1611.

P.04 – Elucidation of the anticancer mechanism of vitamin C

Ayano Mukaida[1], Taiki Miyazawa[1], Akira Matsumoto[1,2]*, Yuji Miyahara[1]
[1]Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo, Japan
[2]Kanagawa Institute of Industrial Science and Technology (KISTEC-KAST), Kawasaki, Japan
*Email: matsumoto.bsr@tmd.ac.jp

Abstract
Vitamin C is ubiquitous in the body with at least 95% ascorbic acid (ASC) and less than 5% dehydroascorbic acid (DHA). ASC is known as a potential anticancer compound in vitro as well as clinical trials. On the other hand, in 2015, it was reported that DHA was taken into cancer cells via glucose transporter 1 (GLUT1) and lead to apoptosis.1 Since then, the significance of DHA in various physiological actions that have been discovered in ASC research has been controversial and attracting attention. There are two major hypothesis that the anticancer effects caused by DHA: (1) ASC and Hydrogen peroxide (H2O2) generated by the reduction of extracellular DHA causes cell death; (2) DHA is taken up by cells through GLUT1, and cell death occurs when the enzymes consumed in the process of reducing DHA to ASC in the cell. However, there are very few quantitative studies focused on relationships between intra- extra- cellular concentration of vitamer and physiological effects. Therefore, for the elucidation of the anticancer mechanism of vitamin C, we conceived to elucidate the above mechanism by evaluating the relationships between intra- extra- cellular vitamer and cytotoxicity of cancer cells. In this study, two types of colon cancer cells (AGS cells: high expression level, HGC27 cells: low expression level) with different GLUT1 expression levels were used in the different environment (such as different concentration of catalase and glucose). Intra- extra- cellular ASC and DHA were determined by the absolute quantification method of ASC and DHA by HPLC-DAD developed in our laboratory.2 As a result, in the presence of catalase, cell death caused by ASC was greatly suppressed, while that of DHA was not significantly different. Therefore, anticancer effect of DHA was not derived from H2O2 generation. Also, we quantitatively confirmed the following phenomena for the first time in the world; (i) intracellular uptake of DHA were inhibited by the presence of glucose; (ii) High GLUT1 expression of the cells increases DHA uptake; (iii) The time-dependent intracellular reduction of DHA to ASC. These results strongly support the above hypothesis "(2)" (Fig. 1). Currently, we are trying to clarify the effects on intracellular glutathione and active oxygen concentration. In the future, we investigate the new therapy approaches using vitamin C by combining with technologies that increase DHA concentration in cells, such as liposomes.

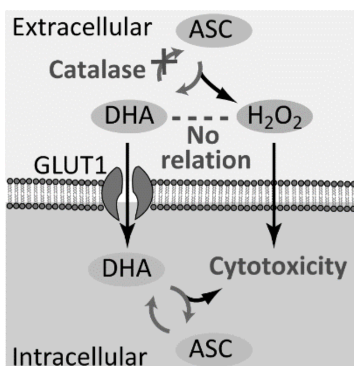


Figure 1. The proposed mechanism of anticancer activity of DHA revealed in this study.

References
[1] J. Yun, E. Mullarky, C. Lu, K.N. Bosch, A. Kavalier, K. Rivera, J. Roper, I.I. Chio, E.G. Giannopoulou, C. Rago, A. Muley, J.M. Asara, J. Paik, O. Elemento, Z. Chen, D.J. Pappin, L.E. Dow, N. Papadopoulos, S.S. Gross, L.C. Cantley, Science 350 (2015) 1391–1396.
[2] T. Miyazawa, A. Matsumoto, Y. Miyahara, Analyst 144 (2019) 3483–3487.

Acknowledgements
This work was supported by Grants-in-Aid for Scientific Research (KAKENHI (18K14419)).

P.05 – Application of primary amine-based supramolecular solvents for the analysis of biological fluids

Christina Vakh*, Andrey Bulatov, Leonid Moskvin
Institute of Chemistry, Saint-Petersburg University, St. Petersburg State University, SPbSU, SPbU, 7/9 Universitetskaya nab., St. Petersburg,199034 Russia
*Email: kristina-fulmes@mail.ru

Abstract
In the recent years the behavior of the supramolecular solvents (SUPRASs) has taken great attention. The SUPRASs are water-immiscible liquids which have been widely used for extraction and preconcentration of various types of substances including organic and inorganic species. SUPRASs have a great potential for the application in sample preparation and multiresidue analysis due to their variety of interactions (i.e. ionic,hydrogen bonding, p-cation and hydrophobic) with the analytes and possibility to tune the polarity by varying the type of the amphiphiles. The SUPRAS based liquid phase microextraction (SUPRAS-LPME) of target analytes from liquid samples is usually carried out using *in situ* procedure of SUPRAS-rich phase formation.
It was found that the primary amines with the long hydrocarbon chain could be used as SUPRASs due to their unique behavior to form isotropic solution in the aqueous phase. SUPRAS based on primary amines could be potentially applied for sample preparation of biological liquids with the aim to eliminate the negative effect of matrix components and separate target analyte.
The present work highlights the most recent application of primary amines SUPRAs for the analysis of biological fluids.

Acknowledgement
Authors gratefully acknowledge The Russian Scientific Foundation (project no. 16-13-10117) for financial support of this work.

P.06 – Formation of regulated an emerging disinfection by-products in a water treatment plant with ozonation treatment

Domínguez-Tello, Antonio[1]*, Arias-Borrego, Ana[2], Gómez-Ariza, José Luis[3], García-Barrera, Tamara[4]

[1]GIAHSA - Water Management Public Company of Huelva. Carretera A-492 Km.4, 21110 Aljaraque, Huelva, Spain

[2,3,4]Research Center for Natural Resources, Health and the Environment (RENSMA). Department of Chemistry, Faculty of Experimental Sciences, University of Huelva, Fuerzas Armadas Av., 21007 Huelva (Spain)

*Email: adtello@giahsa.com

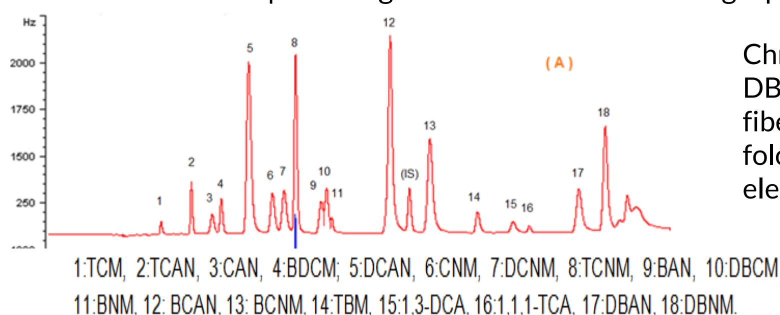
Abstract

During the processes of chemical oxidation and disinfection used in the drinking water treatment plants, secondary pollutants called disinfection by-products (DBPs), may be created by reaction of the oxidizer products, specially chlorine, with the organic precursor compounds contained in raw water. Ozone is the most powerful oxidant which can be used for removal of turbidity, colour, taste, and odour, for disinfection, iron and manganese oxidation, and trihalomethanes (THM) control. Pre-ozonation is capable of breaking down organic precursor compounds into more biodegradable molecules. However, it is necessary to optimize the ozone dose to avoid the formation of oxidation by-products such as bromates, carboxylic acids, aldehydes and ketones derived from these decomposition reactions.

In collaboration with the public water supply company of Huelva (GIAHSA), a project has been proposed for the development and application of a new remote-controlled process management tool. This new process control tool which is applied to full scale at the Lepe's Water Treatment Plant, is based on the run of a predictive model of THMs concentration that calculates the optimal dose of Ozone from the signals of variables measured in plant, especially the Spectral Absorption Coefficient at 254 nm (SAC), pH, temperature, Residual Chlorine and contact time.

The new management tool is controlled by remote control through PLC-SCADA and NEXUS system. The main objectives are the remote control of the purification processes, the improvement of the quality of the supply water avoiding high concentrations of THMs and other DBPs, as well as the reduction of the reagent and energy consumption in the treatments.

In the first phase of the Project, the formation of DBPs in the treated water is evaluated by analyzing different regulated DBPs (Trihalomethanes and Haloacetic Acids) and emerging (Iodo-Trihalomethanes, Halonitromethanes, Haloacetonitriles, Haloketones, Haloacids, Halobenzoquinones) in the treated water. To this end, new methods for the simultaneous analysis of DBPs are developed using HF-LPME and chromatographic techniques (GC-ECD / MS).



Chromatogram showing several DBPs of a water sample by hollow fiber-liquid phase microextraction folowed by gas chromatography-electron capture detector.

Acknowledgements

Authors are grateful to GIAHSA for the private funding and to FEDER (European Community) for financial support, Grant UNHU13-1E-1611.

P.07 – Development of a new analytical method for foodomics based on hollow-fiber-liquid phase microextraction (HF-LPME) applied to human breast milk

M. Villegas-Álvarez[1]*, J.L. Gómez-Ariza[1], A. Arias-Borrego[1], T.García-Barrera1
[1]Department of Chemistry, Campus El Carmen, Faculty of Experimental Sciences, University of Huelva, Fuerzas Ave., 21007, Huelva, Spain. Research Center for Natural Resources, Health and The Environment (RENSMA)1
*Email: tamara.garcia@dqcm.uhu.es

Abstract
In this work, a new analytical method for the determination and quantification of metabolites in breast milk has been developed. The extraction and preconcentration of the analytes was carried out for the first time by 3 phases hollow fiber liquid phase microextraction (HF-LPME) [1]. The chromatographic technique used for the separation of analytes was GC-MS for the unequivocal identification of compounds, due to it allows analysing a large number of metabolites with high sensitivity. Furthermore, by choosing the suitable separation method, a high selectivity can be achieved. To begin with, a time of 60 minutes, a stirring of 600 rpm and a temperature of 25 ° C were selected as extraction parameters, which were optimized after verifying that the extraction is feasible compared to the reference method [2]. The extraction is carried out in 3 phases, where the donor phase corresponds to the milk sample diluted with water, and the organic membrane with n-dodecane. For the acceptor phase, the extractions carried out with two different solvents, acetonitrile and methanol are compared.

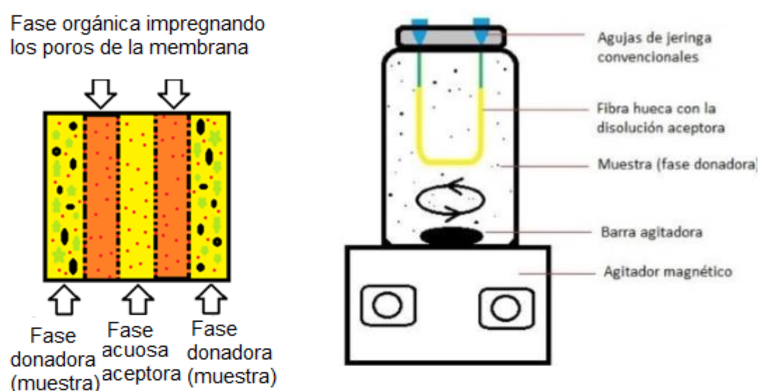


***Figure 1.** Operation of the 3-stage extraction process and final assembly of the device with fiber arrangement in a U.*

Keywords Metabolomics; Breast milk; HF-LPME; GC-MS

References
[1] Tello, A. D. (2017). Desarrollo de métodos de análisis y control de subproductos de desinfección en aguas de abastecimiento público (Doctoral dissertation, Universidad de Huelva)..
[2] Villaseñor, A., Garcia-Perez, I., Garcia, A., Posma, J. M., Fernández-López, M., Nicholas, A. J.,Barbas, C. (2014). Breast Milk Metabolome Characterization in a Single-Phase Extraction, Multiplatform Analytical Approach. Analytical Chemistry, 86(16), 8245–8252.

Acknowledgements
This work was supported by the projects PG2018-096608-B-C21 from the Spanish Ministry of Economy and Competitiveness (MINECO) and UHU-1256905 from the FEDER Andalusian Operative Program 2014-2020 (Ministry of Economy, Knowledge, Business and Universities, Regional Government of Andalusia, Spain). Authors are grateful to FEDER (European Community) for financial support, Grant UNHU13-1E-1611.

AUTHOR INDEX

A

Abbyad, P. 108
Abril, N. 55, 123, 125
Acosta, S. R. 55
Afonso, C. A. M. 81
Aguiar, F. H. B. 126
Ambrosano, G. M. B. 126
Anjos, O. 84
Arias-Borrego, A. 70, 124, 136, 137
Arroyo, M. J. 90
Arruda, M. A. Z. 54

B

Bartoli, M. 67
Bartolomé, A. 133
Berthier, E. 103
Berthier, J. 103
Biesaga, M. 105, 118
Bílková, Z. 104
Bogdanova, P. 117
Borrego, A. A. 55
Bouzas-Ramos, D. 120
Braza, S. 108
Bulatov, A. 109, 117, 135
Buyko, O. 71

C

Calderón-Celis, F. 56
Caliari, V. 79
Callejón-Lebic, B. 70
Callejon-Leblic, B. 125
Callejón-Leblic, B. 55
Calvete, J. J. 56
Cantrell, K. 90
Capelo, J. L. 2
Capitán-Vallvey, L. F. 90
Cárdenas, S. 76, 115, 116
Carmen Collado, M. 123, 125
Casado-Carmona, F. A. 116
Cavalli, A. 89
Cheng, Q. 58
Chung, H. 86
Cid-Barrio, L. 120
Ciepłuch, A. 91
Claparols, C. 80
Cocovi-Solberg, D. J. 106
Collado, M. C. 55
Costa Fernández, J. M. 120

D

da Silva M. M. Lopes, M. 133
Davidson, C. 108
de la Calle Guntiñas, M. B. 95
de Orbe-Payá, I. 90
del Carmen Garrigós, M. 83
del Mar López Guerrero, M. 127
Di Rocco, G. 100
Didukh-Shadrina, S. 71
Dobado, J. A. 90
Dobson, C. 108
Domenech, L. 101
Domínguez-Tello, A. 136
Dumitrascu, C. 95
Dvořák, M. 78

E

Elpa, D. P. 114
Encinar, J. R. 56, 102, 120
Erasmus, S. W. 97
Erenas, M. M. 90
Esteves, T. 81
Estevinho, L. M. 84

F

Fecke, W. 92
Feit, C. 108
Ferreira, F. A. 81
Ferreira, F. C. 81
Ferretti, M. A. 126
Fiamegos, Y. 95
Fojtíková, M. 104
Freije-Carrelo, L. 102
Fresco-Cala, B. 76
Frontela Delgado, J. M. 133
Fuentes, J. L. 133

G

García Alloza, M. 70
García Barrera, T. 133
García-Barrera, T.55, 70, 123, 124, 125, 136, 137
García-Bellido, J. 102
García-Mesa, J. C. 121, 122, 127
García-Montoya, E. 101
García-Valverde, M. T. 115
Garrigues, J.-C. 80
Garstecki, P. 98
Ghidotti, M. 95
Giorcelli, M. 67
Goh, B. 86
Gómez Ariza, J. L. 133
Gómez-Ariza, J. L.55, 70, 123, 124, 125, 136, 137
Goryński, K. 91, 119
Goryński, S. 91
Grützmann Arcari, S. 79
Gutierrez Ramirez, A. J. 108
Gutiérrez-Zamora, M. 101

H

Háková, M. 107
Hann, S. 106
Hempel, G. 99
Horvath, D. 108
Hromádková, L. 104
HUO, R. 88

J

JIAO, J. 88
Jiménez Nosti, A. 56
Jiménez, A. 83
Jones, L. M. 63

K

Kamiński, T. S. 98
Karbarz, M. 118
Kholová, A. 107, 131
Kim, T.-Y. 86
Kipnis, M. A. 132
Kohout, M. 82
Kolderová, N. 82
Kołodziej, D. 91
Krisová, S. 75
Kubáň, P. 78
Kubiak, A. 105, 118
Kuchař, M. 82
Kuhn, K. 91
Kuklya, A. 85

Kupčík, R. 104

L

Lambert, A. S. 58
Larsson, M. 94
Lasarte-Aragonés, G. 116
Lázaro Muñoz, J. J. 133
Lhotská, I. 107
Lhotská. I. 131
Li, B. 58
LI, F. 88
LI, J. 88
LI, X. 88
Liebsch, S. 99
Lima, D. A. N. L. 126
Lin, G. I. 132
Llop, J. M. 101
Lodeiro Espiño, C. 2
López Guerrero, M. M. 121, 122
Losev, V. 71
Lucena, R. 53, 115, 116

M

Maćkiewicz, M. 118
Maireles-Rivas, S. 121
Makra, Z. 75
Malenović, A. 93
Maljurić, N. 93
Mandrioli, R. 89
Marasca, C. 89
Martins, G. 2
Matsumoto, A. 87, 134
Meisen, S. 85
Meistermann, H. 99
Mercolini, L. 69, 89
Miková, B. 78
Millán-Santiago, J. 115
Miñarro, M. 101
Miranda, T. 2
Miró, M. 57
Miyahara, Y 87, 134
Miyazawa, T. 87, 134
Moldovan, M. 102
Molinková, D. 75
Montoro-Leal, P. 121, 122, 127
Moore, T. 108
Morales-Benítez, I. 127
Moskvin, L. 109, 135
Mukaida, A. 87, 134
Muñoz-Cabrera, I. 124

N

Navarro Roldán, F. 123
Nemet, I. 96
Nesterenko, P. 71
Neunschwander, M. 92
Ni, H.-T. 113
Novak, A. 101

O

Ogra, Y. 120
Opitz, P. 99
Otašević, B. 93

P

Padovano, E. 67
Pan, C. W. 108
Pelegrín, C. 83
Pellati, F. 68
Pereira, R. 126
Perez, E. 80
Pérez-Lozano, P. 101

Pochivalov, A. 117
Postek, W. 98
Protić, A. 93
Protti, M. 89

R

Raabová, H. 131
Ramírez-Acosta, S. 70, 123
Ramos, M. 83
Reist, C. 89
Rodríguez Moro, G. 133
Rodriguez-Maecker, R. 85
Rodríguez-Moro, G. 70
Rončević, S. 96
Rosales, A. 85
Rossella, F. 92
Rovere, M. 67
Ryšavá, L. 78

S

Sadowski, B. 119
Salinas-Castillo, A. 90, 120
Samokhin, P. V. 132
Santos, H. M. 2
Šatínský, D. 107, 131
Selma-Royo, M. 123, 125
Sganzerla, M. 79
SHANG, Y. 88
Siles Cordero, M. T. 122
Slovakova, M. 104
Sobczak, L. 91, 119
Specker, E. 92
Stachowiak, M. 91
Stern, M. 99
Strouhalová, E. 104
Suñé-Negre, J. M. 101

T

Tagliaferro, A. 67
Teixeira Godoy, H. 79
Telgheder, U. 85
Theberge, A. B. 103
Theobaldo, J. D. 126
Ticó, J. R. 101
Timofeeva, I. 109
Tóthová, K. 75

U

Urban, P. L. 113, 114

V

Vajrychová, M. 104
Vakh, C. 117, 135
van Ruth, S. M. 97
Vautz, W 77
Velasco-López, I. 124
Vereda Alonso, E. 121, 122, 127
Vicente, A. I. 81
Vílchez-Lobato, C. 133
Villegas-Álvarez, M. 137
Vyhmeister, E. 85

W

WANG, K. 88
Weber, J. 99
Wiśniewski, J. R. 59
Wu, S.-P. 114

Y

Yang, Z. 58

Z

Zdiniakova, T. 95
Zečević, M. 93

ZHANG, L. ... 88
Zielke, C. ... 108
Zisopoulou, A. M. ... 75

LIST OF PARTICIPANTS

A

Abbyad, P................................ pabbyad@scu.edu
Anjos, O...ofelia@ipcb.pt
Arias-Borrego, A. ....ana.arias@dqcm.uhu.es
Arruda, M................................ zezzi@unicamp.br

B

Berthier, J. .... berthj2@uw.edu
Biesaga, M. .... mbiesaga@chem.uw.edu.pl
Bogdanova, P.. paulinebogdanova@gmail.com

C

Callejon-Leblic, B...
...............................belen.callejon@dqcm.uhu.es
Cárdenas, S. ....scardenas@uco.es
Casado-Carmona, F. A. ....q92cascf@uco.es
Cheng, Q. .... quan.cheng@ucr.edu
Cid-Barrio, L. ....cidlaura@uniovi.es
Cocovi-Solberg, D. J.......................david.cocovi-solberg@boku.ac.at

D

del Carmen Garrigós, M. .....mc.garrigos@ua.es
Di Rocco, G...............dirocco.giulia@unimore.it
Domínguez-Tello, A. .... adtello@giahsa.com

E

Elpa, D. P. .... elpa.decibel@gmail.com
Erasmus, S. W..................sara.erasmus@wur.nl
Erenas, M. M................................erenas@ugr.es
Esteves, T.. teresa.esteves@tecnico.ulisboa.pt

F

Forretti, M........ marcela.a.ferretti@gmail.com

G

García-Barrera, T. ....tamara@uhu.es
García-Mesa, J. C...................... jcgm95@uma.es
Garrigues, J.-C....garrigues@chimie.ups-tlse.fr
Ghidotti, M..
...........................Michele.ghidotti@ec.europa.eu
Gómez-Ariza, J. L............... adtello@giahsa.com
Goryński, K. ....gorynski@gmail.com
Grützmann Arcari, S..
...................................stefany.arcari@ifsc.edu.br

H

Hempel, G. ....georg.hempel@uni-muenster.de

J

Jones, L. M. .... ljones@rx.umaryland.edu

K

Kholová, A.........................kholovaa@faf.cuni.cz
Kim, T.-Y. .... kimtaeyoung@gist.ac.kr
Kohout, M. ....michal.kohout@vscht.cz
Kubáň, P.. kuban@iach.cz
Kubiak, A. .... akubiak@chem.uw.edu.pl

L

Larsson, M..................mats.larsson@fysik.su.se
Lhotská, I. ....lhotski@faf.cuni.cz
Lucena, R. ....rafael.lucena@uco.es

M

Mercolini, L. M...........laura.mercolini@unibo.it
Millán-Santiago, J................... q52misaj@uco.es
Miró, M. .... manuel.miro@uib.es
Miyazawa, T. ....matsumoto.bsr@tmd.ac.jp

Moldovan, M. .....moldovanmariella@uniovi.es
Montoro-Leal, P. ..montoroleal.pablo@uma.es
Morales-Benítez, I. .... eivereda@uma.es
Mukaida, A. .... ma190098@tmd.ac.jp

N

Nesterenko, P...
.... p.nesterenko@phys.chem.msu.ru
Ni, H.-T...........................a26304865@gmail.com
Novak, A...................ana.novakova@gmail.com

P

Pellati, F..................federica.pellati@unimore.it
Postek, W. ....wpostek@ichf.edu.pl
Protić, A.......................anna@pharmacy.bg.ac.rs
Protti, M.................... michele.protti2@unibo.it

R

Ramírez-Acosta, S..
.... sara.ramirez@dqcm.uhu.es
Rodríguez Moro, G..
......................................gema.moro@dqcm.uhu.es
Rodriguez-Maecker, R...
.... rnrodriguez@espe.edu.ec
Rončević, S. .... roncevic@chem.pmf.hr
Rossella, F.federica.rossella@eu-openscreen.eu
Ruiz Encinar, J.....................ruizjorge@uniovi.es

S

Samokhin, P. ....samokhin@ips.ac.ru
Shang, Y........... shangyanhong2013@163.com
Slovakova, M. ....marcela.slovakova@upce.cz
Sobczak, Ł.......... lukaszsobczak39@gmail.com

T

Tagliaferro, A.......alberto.tagliaferro@polito.it
Timofeeva, I. ...... timofeeva.irina.7@gmail.com

V

Vakh, C......................... kristina-fulmes@mail.ru
Vautz, W...................... wolfgang.vautz@isas.de
Villegas-Álvarez, M..
.................................tamara.garcia@dqcm.uhu.es

W

Wiśniewski, J. R. ...jwisniew@biochem.mpg.de

Z

Zisopoulou, A. M................ zisopouloua@vfu.cz