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International Conference

MASSA 2016

Istituto Superiore di Sanità
Rome, September 6-8, 2016

ABSTRACT BOOK

Edited by
M. Fiori, G. Giorgi, C. Civitareale, V. Patriarca and E. Gregori

ISTITUTO SUPERIORE DI SANITÀ

International Conference

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Edited by

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Valeria Patriarca (a) and Emanuela Gregori (a)

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The series of conferences "MASSA", instituted by the Division of Mass Spectrometry (DSM) of the Italian Chemical Society (SCI), is an opportunity for the scientists of academia world, of inspection bodies and of those working in the productive sector, to compare their knowledge and experiences in this field. For the first time, in the 2016 edition, the Istituto Superiore di Sanità is the active subject in the organization of the Congress together the SCI. In the edition of 2016, Prof. Richard M. Caprioli, of Vanderbilt University (Nashville, Tennessee) will be awarded with the Italian Chemical Society Honorary Membership for his valuable contribution in the development of MALDI Imaging Mass Spectrometry and its application for molecular mapping of tissues and biological materials. The DSM, as every year, offers 10 fellowships and two prizes for the best posters presented.

Key words: Mass spectrometry, Imaging mass spectrometry, Food safety, Environment, Omics sciences, Doping and dependence, Nanomaterials, Ion spectroscopy, IRMS, Stable isotopes.

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La serie di convegni "MASSA", istituita dalla Divisione di Spettrometria di Massa (DSM) della Società Chimica Italiana (SCI), rappresenta un momento di confronto tra il mondo accademico, gli enti di ricerca, gli enti di controllo e il settore produttivo, con l'obiettivo di presentare gli elementi di innovazione tecnologica e applicativi della spettrometria di massa. L'edizione del 2016 vede per la prima volta l'Istituto Superiore di Sanità come soggetto organizzatore insieme alla SCI. Nell'edizione 2016 viene conferita la carica di socio onorario della DSM della SCI al Prof. Richard M. Caprioli, della Vanderbilt University (Nashville, Tennessee), per il suo prezioso contributo nello sviluppo della spettrometria di massa MALDI *Imaging* e della sua applicazione per la mappatura molecolare dei tessuti e dei materiali biologici. La DSM, come ogni anno, mette a disposizione 10 borse di studio e due premi per i migliori poster presentati.

Parole chiave: Spettrometria di massa, Spettrometria di massa per immagini, Sicurezza alimentare, Ambiente, Scienze omiche, Doping e droge d'abuso, Nanomateriali, Spettroscopia ionica, IRMS, Isotopi stabili.

Si ringrazia la Divisione di Spettrometria di Massa della Società Chimica Italiana (DSM SCI) per il lavoro di collaborazione paritaria e per aver finanziato l'evento.

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NOTE FOR THE READER

This volume gathers the abstracts of the contributions presented at the Conference. Abstracts are divided into Plenary Lecture (PL), Oral Presentations (OR), and Poster (P).

For easy consultation, Plenary Lecture and Oral Presentations are listed in the order of the programme and Posters are arranged by topics.

At the end of the volume, the Authors' Index is provided for the reader's convenience.

PREFACE

Mass spectrometry is a methodology which is increasing more and more importance, development and applications in many branches of science, from research to controls and screening in chemistry, biomedicine, from the operating room to the clinical biochemistry laboratory, food science and technology, environment, metabolomics, proteomics, toxicology, forenics, cultural heritage and many others.

Innovation in instrumentation and in methods and applications in constant development allow one to perform analyses in a short time, with great sensitivity, specificity and accuracy with robust, effective and reliable methods of molecules present at trace level in complex mixtures.

In this background, the MASSA 2016 conference inserts. Organized by the Istituto Superiore di Sanità with the Division of Mass Spectrometry of the Italian Chemical Society (SCI DSM), MASSA 2016 is a conference with an international impact, dedicated to all aspects of mass spectrometry and its applications, innovations and developments. It is an useful occasion to present the state-of-the-art and look at the future of this constantly expanding and evolving discipline.

Organized in plenary lectures, oral and poster communications, the scientific program is articulated into different sessions regarding mass spectrometry imaging, biomedical applications, ion spectroscopy, food and food safety, nanomaterials, stable isotopes, doping and dependency.

The conference is a good opportunity for meeting and discussion among scientists coming from both public and private institutions, universities, hospitals, industries, regulatory and research institutions, for comparing industrial needs and academic knowledge, promoting opportunities for strengthening collaborations and projects already established or for creating new ones.

Noteworthy is the wide participation of young people who, with their skills, energy, enthusiasm and passion, are the lifeblood for the development of science. Ten of them are recipients of fellowships awarded by the Division of Mass Spectrometry.

In the opening ceremony the Honorary Membership of the Italian Chemical Society will be awarded to Prof. Richard M. Caprioli, Vanderbilt University (USA) for his pioneering studies of imaging mass spectrometry. The "DSM-SCI 2016 award for young operating in the field of mass spectrometry" will be also assigned.

Maurizio Fiori, Gianluca Giorgi
Conference Chairpersons

PREMESSA

La spettrometria di massa è una metodologia che sta assumendo sempre più importanza, diffusione e applicazioni in molti ambiti scientifici, dalla ricerca ai controlli e allo screening, in ambito chimico, biomedico, dalla sala operatoria al laboratorio di biochimica clinica, nelle scienze e tecnologie alimentari, in ambito ambientale, metabolomico, proteomico, tossicologico, forense, dei beni culturali e in moltissimi altri.

Innovazioni strumentali, metodologiche e applicative in continuo sviluppo consentono di poter effettuare analisi in tempi estremamente ridotti, con grande sensibilità, specificità e accuratezza con metodi robusti, efficaci, affidabili di molecole presenti in tracce in miscele complesse.

In questo contesto si inserisce il convegno MASSA 2016. Organizzato dall'Istituto Superiore di Sanità con la Divisione di Spettrometria di Massa della Società Chimica Italiana (DSM SCI), MASSA 2016 è un convegno di rilevanza internazionale, dedicato a tutti gli aspetti della spettrometria di massa e alle sue molteplici applicazioni, innovazioni e sviluppi per fare il punto sullo stato dell'arte e guardare al futuro di questa disciplina in continua espansione e evoluzione.

Organizzato in conferenze plenarie, comunicazioni orali e poster, il programma scientifico si articola su varie sessioni riguardanti la spettrometria di massa imaging, le applicazioni biomediche, la spettroscopia ionica, cibo e sicurezza alimentare, i nanomateriali, gli isotopi stabili, il doping e le dipendenze.

Il convegno costituisce una buona occasione di dialogo e confronto tra operatori provenienti da istituzioni pubbliche e private, università, ospedali, industrie, enti di controllo e di ricerca, per confrontare esigenze industriali e conoscenze accademiche, rafforzare collaborazioni e progetti già in atto e crearne di nuovi.

Il convegno ha un'ampia partecipazione di giovani che con la loro competenze, energia, entusiasmo e passione sono linfa vitale per lo sviluppo della scienza. Dieci di loro sono stati assegnatari di borse di studio da parte della Divisione di Spettrometria di Massa.

Nella cerimonia di apertura verrà conferita la carica di Socio Onorario della Società Chimica Italiana al Prof. Richard M. Caprioli, Vanderbilt University (USA) per i suoi studi pionieristici sulla spettrometria di massa imaging. Verrà anche assegnato il "Premio DSM-SCI 2016 per giovani operanti nel campo della spettrometria di massa".

Maurizio Fiori, Gianluca Giorgi
Responsabili scientifici

AWARDS

Italian Chemical Society Honorary Membership

The Italian Chemical Society awards **Prof. Richard M. Caprioli**, Vanderbilt University School of Medicine, for the development of MALDI Imaging Mass Spectrometry and its application to molecular mapping of tissues and biological materials, plenary speaker at many conferences organized by the Italian Chemical Society, for his invaluable contribution to the progress of science.

DSM-SCI Awards for Young Researchers 2016

The Division of Mass Spectrometry of the Italian Chemical Society has established since 2000 the "DSM-SCI Award for young working in the field of mass spectrometry" that awards a joung scientist uder 35 years for the best work PhD thesis or publication having mass spectrometry as a reference methodology. The 2016 winner is **Dr.ssa Sara Granafei** from Università degli Studi di Bari, for lipidomics analysis of extracts from different animal and bacterial matrices carried out by liquid chromatography coupled with electrospray ionization (RPLC-ESI-MS^n).

Previous winners: **Dr. David Bongiorno** (2000); **Dr. Antonina Gucciardi** (2001); **Dr. Maria Pia Vitale** (2002); **Dr. Sara Rinalducci** (2003); **Dr. Ester Marotta** (2004); **Dr. Ingrid Zagnoni** (2005); **Dr. Vera Muccilli** (2006); *ex-equo* **Dr. Francesca Attanasio, Maria Chiara Monti** (2007); **Dr. Valeria Cavatorta** (2008); **Dr. Caterina Fraschetti** (2009); **Dr. Alessandra Tata** (2010); **Dr. Giovanni Caprioli** (2014); **Dr. Cristiana Labella** (2015).

Session 1

Imaging mass spectrometry, proteomics and metabolomics

Chairpersons

Donatella Caruso, Gianluca Giorgi

PL1. IMAGING MASS SPECTROMETRY: MOLECULAR MICROSCOPY FOR BIOLOGY AND MEDICINE

Richard M. Caprioli
Departments of Biochemistry, Chemistry, Pharmacology and Medicine, and the Mass Spectrometry Research Center, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

MALDI Imaging Mass Spectrometry (IMS) produces molecular maps of peptides, proteins, lipids and metabolites present in intact tissue sections. It employs desorption of molecules by direct laser irradiation to map the location of specific molecules from fresh frozen and formalin fixed tissue sections without the need of target specific reagents such as antibodies. Molecular images of this nature are produced in specific m/z (mass-to-charge) values, or ranges of values. Thus, each specimen gives rise to many hundreds of specific molecular images from a single raster of the tissue. In a complementary approach where only discrete areas within the tissue are of interest, we have developed a histology-directed approach that integrates mass spectrometry and microscopy. Mass spectra are collected from selected areas of cells within the tissue for laser ablation and analysis.

We have employed IMS in studies of a variety of biologically and medically relevant research projects. One area of interest is the molecular mapping of molecular changes occurring in diabetes in both a mouse model and in the human disease. Major molecular alterations have been recorded in advanced diabetic nephropathy involving both proteins and lipids. Other applications include developmental studies of embryo implantation in mouse, assessment of margins in renal cancers as well as that in other organs, and neurodegenerative disease. Molecular signatures have been identified that are differentially expressed in diseased tissue compared to normal tissue and also in differentiating different stages of disease. These signatures typically consist of 8-10 or more different proteins and peptides, each identified using classical proteomics methods. One such application described is that concerning the differentiation of benign skin lesions from melanomas using our PIMS (Pathology Interface for Mass Spectrometry). In addition, IMS has been applied to drug targeting and metabolic studies both in specific organs and also in intact whole animal sections following drug administration.

This presentation describes recent technological advances both in sample preparation and instrumental performance to achieve images at high spatial resolution (1-10 microns) and at high speeds so that a typical sample tissue once prepared can be imaged in just a few minutes. Applications will include the use of MS/MS, ultra-high mass resolution, and ion accumulation devices for IMS. Finally, new biocomputational approaches will be discussed that deals with the high data dimensionality of IMS and our implementation of “image fusion” in terms of predictive integration of MS images with microscopy and other imaging modalities.

PL2. THE APPLICATION OF MASS SPECTROMETRY TO THE STUDY OF THE INTACT HUMAN SALIVARY PROTEOME

Massimo Castagnola (a,b), Tiziana Cabras (c), Federica Iavarone (a), Claudia Desiderio (b), Irene Messana (b)

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(c) Department of Life and Environmental Sciences, Università degli Studi, Cagliari, Italy

Saliva is a very attractive bodily fluid for the diagnosis and prognosis of diseases for several reasons: i) its collection is usually low cost, "easy", "safe" and it can be performed without the help of health care workers (it allows for home-based sampling); ii) it is considered an acceptable and non-invasive process by the patients because it does not provoke pain and can be easily collected also from newborns.

Our group started more than fifteen years ago to study this bodily fluid with an integrated proteomic top-down/bottom-up platform. The lecture will describe the proteoforms of the main families of human salivary proteins, i.e. proline-rich proteins, histatins, statherins, cystatins, α-defensins and β-thymosins, characterized by these methods. Several post-translational modifications occurring before, during and after their secretion will be described, underlying the proteolytic fragmentations from the pre-pro-proteins, which have been characterized thanks to the top-down strategy applied. Saliva contains specific families of proteins of secretory origin. However, they are submitted to post-translational modifications (phosphorylation, sulfation, glycosylation, cyclization, fragmentation) which are due to enzymes commonly distributed in other exocrine and endocrine glands and tissues. Salivary protein profiling in relation to age, with a particular concern to the physiological variations observed in pre-term newborns and in the pediatric age range, will be also discussed. The putative role of some salivary proteoforms identified in the oral cavity and the demanding issues arising from the proteomic results until now obtained will be finally pointed out.

OR1. MASS SPECTROMETRY: FROM RESEARCH TO CLINICAL APPLICATIONS

Giulio Mengozzi, Manuela Lucchiari, Fabio Settanni
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In recent years the re-organization of the clinical laboratory network in our region lead to the consolidation and centralization of specialistic or second-level investigations in very few centres, thus serving a large population. At the same time, there was an increase in the clinical needs for appropriateness not only in terms of requests but also of quality performances.

As an hub laboratory we have to guarantee both high throughput automated results and specialty tests, in the great majority of cases within a daily working period, with short turn-around-time (e.g. for therapeutic drug monitoring).

We are now facing the challenge to introduce mass spectrometry technology in the clinical routine of the laboratory through the development of a working cell with dedicated highly specialized operators. The pre-analytical step will be included in that of the corelab, whereas preparation of samples should be carried out by automated tools and the whole analytical and post-analytical process should be highly standardized and as far as possible automated.

Clinical applications include therapeutic drug monitoring (immunosuppressant, anti-convulsivant, neuroleptics, anti-fungal, benzodiazepines), steroid profiles, aminoacids and other metabolites, biological amines.

The introduction of mass spectrometry in the clinical laboratory setting allows us to meet clinical needs for high-quality accurate results associated with prompt responses to help clinical decision making.

OR2. UHPLC COUPLED WITH TANDEM MASS SPECTROMETRY APPLIED TO THERAPEUTIC DRUG MONITORING (TDM) IN SOLID ORGAN TRANSPLANT

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The recent advances in mass spectrometry and the development of new technologies for desorption from surface tissues, started the revolution of the diagnostic laboratory. Different approaches are involved to reach the goal of "Precision Medicine": from tissue imaging to biopsy and "omics" big data for a Personalized Medicine that transforms the approach of the patient care from "reactive" to "pro-active".

Automation procedures in pre-analytic sample matrix preparation (e.g. whole blood analysis of therapeutic immunosuppressant drugs), certified and standardized methods for the TDM with high specificity, high sensitivity and fast throughput analysis are driving forces for a clinical laboratory to move from immunomediated techniques to LC-MS/MS methods. TDM for immunosuppressants is recommended by International Guide Line to improved patients transplant outcome.

Because of the narrow therapeutic window, recent advances in the drug/dose optimization, taking into account specific clinical situations and the analytical method currently available, drew some recommendations and guidelines to help clinicians with the practical use of the drug. Pharmacokinetic, pharmacodynamic and more recently pharmacogenetic approaches help physicians to set up individualized long term therapies and to diminish risk of graft versus host disease. Immunosuppressants like Tacrolimus, Cyclosporine A, Everolimus, Syrolimus take undoubtedly benefit from TDM, but interpretation of the blood concentration is confounded by the relative differences between the assays (immunomediated techniques versus LC-MS/MS).

Single time points, limited sampling strategies and the area under concentration curve have all been considered to determine the most appropriate sampling procedure that correlates with therapeutic efficacy.

The choice of an appropriate method and potential pitfalls should all be considered when determining immunosuppressants concentration. Performing careful validation when implementing a new analytical assay, participating in external proficiency testing programs, promoting the use of certified material as calibrators in HPLC/UPLC with mass spectrometric detection methods are recommended procedures to drive clinicians in an appropriate immunosuppressants drug therapy for maintaining a viable organ allograft**.**

OR3. PROTEIN ANALYSIS BY MASS SPECTROMETRY: FROM IDENTIFICATION TO CHARACTERIZATION

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Mass spectrometry is a powerful and versatile instrumental technique applicable to a variety of inorganic, organic, and biological molecules. When applied to proteins, it can uncover a large number of features, ranging from identity to physical interactions and from Post-Translational Modifications (PTMs) and adduct formation to tridimensional structure.

Protein mass spectrometry can be performed with different instruments, which all share basic elements, which can be variously exploited to study the protein(s) of interest and their relevant characteristics.

To illustrate the power and flexibility of protein mass spectrometry, we have chosen a recent investigation of the 14-3-3 protein of the human parasite Giardia duodenalis. The Giardia signal transduction protein 14-3-3 (g14-3-3) is post-translationally modified in a stage-specific manner and is directly involved in the differentiation of the parasite into cyst. To elucidate the functions of g14-3-3, we have investigated its protein interactome, using an *in vivo* approach, to maintain physiological PTMs in both the free-living and encysting parasites. A FLAG-tagged g14-3-3 was expressed in G. duodenalis under its own promoter and purified by affinity chromatography; co-purified proteins were analyzed by LC-MS/MS and candidate g14-3-3 interactors were identified. To distinguish real interactors from precipitation contaminants, we adopted a discriminating statistical approach. Proteins were quantitated using a label-free method (EmPAI) to identify candidate interactors, some of which were then orthogonally validated. The large number of putative interactors identified, 314 including direct, indirect, stage-specific, and ubiquitary ones, is witness to the role played by 14-3-3 as a hub of many and diverse functional pathways. A hypergeometric distribution analysis of binding site frequencies established an enrichment in g14-3-3 binding motifs among the presumptive interactors, suggesting that at least a subset of them do directly bind g14-3-3.

We focused our attention on TTL3 and gDIP, because of their identity and characteristics, and hypothesized that they are involved in the control of polyglycylation, a peculiar PTM of g14-3-3. Mass spectrometry was employed to demonstrate that indeed TTL3 and gDIP are responsible for elongating and shortening, respectively, the polyglycine chain on g14-3-3.

In a search for new drugs against Giardia, we used mass spectrometry to identify protein targets of NBDHEX, a molecule studied for its antineoplastic properties but endowed with activity against Giardia. Several proteins were found to form adducts with NBDHEX on their cysteine residues, suggesting possible mechanisms of action.

OR4. TOP-DOWN ANALYSIS OF PROLACTIN-INDUCIBLE PROTEIN AND ITS GLYCOFORMS IN HUMAN SALIVA

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Prolactin-Inducible Protein (PIP) is a small glycoprotein present in seminal plasma, human milk and saliva. Several functions have been attributed to this protein, and, among them, it has been demonstrated that PIP acts as an aspartyl proteinases with specific fibronectin-degrading ability, suggesting its potential role in processes such as tumor progression and fertilization. Moreover, salivary PIP may play an important role in mucosal immunity. The pre-protein, consisting of 146 amino acid residues, after cleavage of the signal peptide produces a protein of 118 residues. The four cystein residues of PIP form two disulfide bonds (Cys37-Cys63 and Cys61-Cys95) and the N-terminal Gln is cyclized to pyroglutamic acid. Chemical studies showed that one glycan chain is attached to Asn77, however due to carbohydrate microheterogeneity multiple PIP glycoforms are present in human secretions.

During a top-down proteomic characterization of the acidic soluble fraction of whole human saliva performed by high-resolution mass spectrometry, we observed a cluster of proteins with monoisotopic monoprotonated ions spanning from 13493.9 to 16357.0 ± 0.2 *m/z*. Based on *m/z* differences, they corresponded to different glycoforms of the same protein for the presence/absence of deoxyhexose moieties, N-acetylneuraminic acid, and N-acetylhexosamine-hexose units. MS/MS analysis performed on the major species with monoisotopic monoprotonated ions at 13493.9, 13696.9, 13843.1, and 13860.2±0.2 $[M+H]^+$ *m/z* allowed identifying four proteoforms of PIP with 2 disulfide bridges and, except the fourth species, the N-terminal pyroglutamic acid. The protein with $[M+H]^+$ at 13493.9 m/z corresponded to non-glycosylated PIP, and the proteins with $[M+H]^+$ at 13696.9, and at 13843.1 m/z, corresponded to proteoforms carrying an additional N-acetylhexosamine (theor. Δ = 203.08 *m/z*), and a fucosylated N-acetylhexosamine moiety (theor. Δ = 349.14 *m/z*), respectively. The two proteoforms with $[M+H]^+$ at 13860.2 m/z and 13843.1 m/z differed only for the cyclization of the N-terminal glutamine residue. Although it has been reported that the major N-glycan species linked to salivary PIP are the complex fucosylated diantennary structures, the present data show that the PIP glycoforms present in the acidic soluble fraction of human saliva are mainly linked to small sugar moieties.

OR5. STRUCTURAL CHARACTERIZATION OF A NEW PROTEOFORM OF THE HUMAN SALIVARY CYSTATIN D BY TOP-DOWN MASS SPECTROMETRY

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The cystatins constitute a superfamily of related proteins which are natural inhibitors of C1 (papain-like) and C13 (legumain) cysteine proteinases. The mammalian superfamily includes: type 1 cystatins (cystatins A and B), cytoplasmic proteins without signal peptide and disulfide bridges; type 2 cystatins (C, D, E, F, S, SN, SA), secreted inhibitors with two disulfide bridges; type 3 cystatins, or kininogens, multidomain proteins.

Human cystatin D, encoded by *CST5*, was originally purified from human saliva and tears; the product of *CST5* is a preprotein of 142 amino acid residues, with the first 20 residues constituting a typical signal peptide, and contains, as other type 2 cystatins, four Cys residues forming two characteristic disulfide bonds. Even if the main biological role of cystatin D is related to extracellular regulation of cathepsins, it has been recently demonstrated its ability to inhibit proliferation, migration and invasion of colon carcinoma cells indicating tumor suppressor activity unrelated to protease inhibition.

Due to gene polymorphism, cystatin D is present in two proteoforms with either Cys or Arg at position 26 (in the mature form). Moreover, mass spectrometry analysis evidenced the presence of two N-terminal truncated forms, des 1-4 and des 1-8, carrying the Arg^{26} polymorphism. Here, we report the structural characterization, by a top-down high-resolution mass spectrometry approach, of a new truncated proteoform of cystatin D detected in human saliva together with des 1-4 and des 1-8 cystatin D.

The MS/MS spectrum of the deca-charged ion at *m/z* 1352.67±0.02 was consistent with the sequence of the N-terminal truncated des1-5 cystatin D, carrying the Arg^{26} polymorphism, 2 disulfide bridges and the N-terminal Gln converted to pyroglutamic acid residue (theor. monoisot. *m/z* 13509.65, $[M+H]^+$). Since the N-terminal portion of cystatin D determines the different inhibition profiles shown against cathepsins S, H and L, and it is involved in the antimigratory and antiproliferative effect on colon carcinoma cells, the characterization of this new truncated proteoform can open new perspectives on the biological functions of this protein.

OR6. MASS SPECTROMETRY-BASED LIPIDOMICS INVESTIGATIONS

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The basis for lipidomics development is mainly due to recent improvements in Mass Spectrometry (MS). In this communication I will be described a lipidomics investigation involving three organisms (projects), the bacterium *Rhodobacter sphaeroides* and the fish *Sparus aurata* and *Dicentrarchus labrax*.

Different were the proposed goals in each project. As concerns the lipidomics characterization of samples extracted from *R. sphaeroides* cells and chromatophores, all polar lipids were extracted according to Bligh-Dyer standard protocol and several focused chromatographic separations, including GC and LC coupled to MS detection were devised in order to examine the profile of free Fatty Acids (FA), Phospholipids (PL), Ornithine Lipids (OL) and Sulfoquinovosyldiglycerides (SQDG). The phospholipidome of lipids extracted from *Sparus aurata* fillets was performed by LC-ESI-MS. At first, lipids of farmed and wild *S. aurata* were analyzed by hydrophilic interaction liquid chromatography with electrospray ionization and Fourier-transform mass spectrometry (HILIC-ESI-FTMS) and identified untargeted. A multivariate analysis was applied to compare experimental data.

The next step was a focused characterization of the lipid extracted; the identification along with regiochemical assignments (i.e., location of alkyl or acyl side chains on the sn1 and sn2 positions of glycerol) was performed through CID MS/MS and MS^3 fragmentation. Finally, a new mixed-mode chromatography method using two HILIC-RPC columns in series was developed and used for a fine characterization of *S. aurata* phospholipidome.

The last part of the Ph.D. project was focused on the characterization and quantification of the main PL, free FA and sphingomyelins occurring in the brain of European sea bass. Two previously validated methods, direct flow-injection ESI-MS/MS and GC-MS, were chosen to establish the brain's lipid content.

Two bio-specimen storage technologies, i.e., the gold standard frozen tissue and the lyophilization process were compared as well as two lipid-extraction procedures, the Bligh-Dyer and the chloroform-free methyl-*tert*-butyl ether.

The results of these early hints will be presented and discussed.

Session 2

Food safety and environment

Chairpersons

Maurizio Fiori, Roberta Galarini

PL3. ANALYTICAL OPTIONS FOR FORBIDDEN SUBSTANCES DETECTION IN LIVESTOCK. WHERE ARE WE NOW?

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To make progresses in food safety and thus improving consumer's confidence, technological companies and control laboratories have to develop and implement hyphenated analytical tools to be able to monitor efficiently residues and contaminants in food items at any concentration level whatever the complexity of the matrix submitted to the analysis.

These methods are furthermore expected to deliver tailored information such as the emergence of unfamiliar or unknown hazards. Corresponding signals may be detected and the identity of the compound identified. A review of up-and-coming analytical technologies will be provided with a particular emphasis given to the resolution of difficult issues encountered in the food safety area.

Several examples related to residues of banned substances (growth promoters) will be selected to explain 1-how Ambient Mass Spectrometry (DART, ASAP) can be considered as an alternative strategy for rapid identification of chemical hazards, 2-how Supercritical Fluid Chromatography or GC coupled to Atmospheric Pressure Chemical Ionization may change the way of thinking when dealing with large family of compounds characterized by a wide range of physic-chemical properties, 3-how Ion Mobility Mass Spectrometry may give a valuable extra dimension to characterize chemical residue at trace level in complex matrices, 4-how ultra-high resolution mass spectrometry offers today an essential piece of information both for classical targeted measurement or pioneering untargeted fingerprinting.

All benefits in terms of time analysis, specificity/selectivity or sensitivity of signals will be widely discussed together with limitations/pitfalls associated with these cutting edge technologies.

OR7. FOOD SAFETY: OPTIMIZATION OF PERFORMANCE IN MYCOTOXIN ANALYSIS IN FOOD

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The analytical scenario of food analysis is constantly shacked by the progress and improvement of knowledge and expertise of researchers and by the technical innovation of the instrumentation available. During the last 10 years the mass spec has gained the first place of attention especially for some topics, such as mycotoxins in food and feed sector, upgrading both the high throughput and the reliability of the analytical measurements. However, still a lot of challenges exacerbate the laboratory that deals with an extraordinary, i.e. not routinely, daily work.

Following the need that was highlighted to carry out a study on the assessment of the exposure to mycotoxins by a Total Diet Study (TDS), the list of bottlenecks that hamper the analyses and the shortcuts that smoothed the study will be presented as an example on how to deal with multifaceted situation.

The TDS represents a complex situation where composite mixtures of food commodities are analyzed for a number of mycotoxins.

In this study, 10 mycotoxins were analyzed by LC-MS/MS method reaching very good low limits of detection with a validated matrix matched method.

OR8. "FOOD PROFILING": NEW HORIZONS OF HIGH-RESOLUTION MASS SPECTROMETRY APPLICATION

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Among the European countries, Italy owns one of the highest numbers of food products labelled with brands of protected geographical indications and traditional specialities. A traceability system, or rather the ability to track the production chain, from processing to distribution, and locate the food origin, becomes essential to protect people from food frauds. High resolution mass spectrometry (QOrbitrap) can help us to achieve the necessary results. Practical applications are the combined use of sugars and simple phenols analyses for the botanical traceability of commercial tannins, and of vitamins and amino acids for the characterisation of grapes, musts and fermentation adjutants. High resolution mass permitted also to characterize simple phenols and glycosylated phenols, using targeted and untargeted approaches, in red wines (Primitivo di Manduria and Negroamaro). With this approach we could also characterize vanilla, cocoa, green coffee and tannins.

In this work we will also present in more detail a targeted and untargeted method for alkaloid analyses. On-line concentration/purification was performed with a SolEx HRP spe cartridge, while the chromatographic separation with a Raptor Biphenyl analytical column. The mass spectrometer was operated in positive ion mode using the following parameters: sheath gas flow rate set at 30 arbitrary units; aux gas flow rate at 10 arbitrary units; spray voltage at 3.5 kV; capillary temperature at 330°C; aux gas heater temperature at 300°C; Mass spectra were acquired in full MS-data dependent MS/MS analysis (full MS-dd MS/MS) at mass resolving power of 140.000.

The method was applied to a broad characterization of alpine pasture herbs (N=67), herbal mixes (48) representative of the natural daily intake of cows (8) grazing on alpine pastures (2) in north-eastern Italy, and milks (48) produced by the same cows. The aim was to evaluate the possible transfer of alkaloids from herb to milk in order to differentiate different mountain origins, or mountain products from lowland ones. 35 alks were identified and quantified in reference to the pure analytical standards, 48 were confirmed for chromatographic retention time and fragmentation profile analyzing the extracts of herbs already well documented in literature, and other 200 alks were identified using literature information regarding exact mass and isotopic pattern.

OR9. ANALYSIS OF BISPHENOLS AND ALKYLPHENOLS IN FOOD BY LC-MS/MS

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Relationships between environmental pollution and food contamination have been widely described in several outbreaks all over the world. Endocrine Disruptor Chemicals (EDCs) are a panel of chemical substances belonging to many groups, that can interfere by different mechanisms with several endocrine pathways, mainly mimicking and affecting the activities of sexual hormones and thyroid. Among EDCs, Bisphenol A (BPA) and alkylphenols, in particular Nonylphenol (NP) and Octylphenol (OP), have been widely studied because of their estrogen-like activity. BPA is employed in industry as a plasticizer and to produce epoxy-resins for inner coating of cans for food; alkylphenols are present in PET and PVC plastics. Food and beverages represent for humans the main route of exposition to BPA and alkylphenols, which have been correlated to obesity, cognitive disorders and malformation of reproductive apparatus. These substances can contaminate food as a consequence of environmental pollution due to industrial production of plastics, but can also migrate directly from some packaging materials.

In this work, we describe the development of test methods based on LC-ESI-MS/MS to determine nonylphenol, octylphenol, BPA and other bisphenols (BPF, BPB, BPS, BADGE, BFDGE) in food, beverages and feed. Molecularly imprinted polymers specific for BPA were successfully employed for purification of BPA, 4-NP, *tert*-NP, 4-OP e *tert*-OP from different matrices. These methods were employed to determine bisphenols, nonylphenol and octylphenol in fish, canned seafood, beer, energy drinks, feed.

The effects of temperature and mechanical damaging of cans on the migration from inner coating to food have been also studied.

A monitoring of BPA in urines from kids was carried out, to evaluate possible relationship with obesity.

OR10. MASS SPECTROMETRY IN THE CHEMICAL CONTROL OF DIETARY SUPPLEMENTS, NOVEL FOODS AND ENERGY DRINKS

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In recent years the consumption of certain foods, such as energy drinks, novel food and dietary supplements is having an great increasing, in particular those derived from natural sources such as extracts of plants or marine organisms.

Although it is common idea that natural products do not pose a risk to consumer health, the illegal addition of synthetic substances to products marketed as dietary supplements poses a serious threat to consumers health. These compounds are typically added to supplements to produce a biological effect or enhance the action of the natural products.

Considering that these products, administered without medical supervision, may contain toxic components or substances whose safety has never been examined, and whose interaction with medications can be unpredictable.

The aim of our study was to develop a method in mass spectrometry to detect the presence of illegal drugs, in these particular foods.

To this end, we have developed two analytical procedures, a GC-MS and LC-HRMS in order to identify and quantify the presence of illegal drugs in various forms of food supplements, energy drinks and novel food. After a suitable extraction and purification of the active principles with SPE cartridges, the extracts are analyzed by mass spectrometry.

The results showed that about 6% of the samples analyzed were found to not conform for the presence of hormones and unauthorized drugs

OR11. FAST LCMS & GCMS APPROACHES FOR EXPANDING PRODUCTIVITY IN MULTI-RESIDUE PESTICIDES ANALYSIS

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National programs for pesticide monitoring in the US, Europe and Japan have set Maximum Residue Levels (MRLs) or tolerance information for pesticides in food products. A default value of 0.01 mg/kg is applied for MRL enforcement, thus requiring highly sensitive and specific analytical technologies to monitor an increasing number of pesticides. Therefore, LC-MS and GC-MS represent the analytical gold standard.

We have developed a LC-MS/MS method able to detect 646 pesticides in 15 minutes, with 1919 transitions in total. The fast polarity switching (5 msec) and the high speed of the analysis (30,000 amu/sec) enables the precise quantitation of compounds even with high data density. Indeed, the average variation in peak area response was observed to be less than 3% by monitoring 25 compounds eluting in 9 seconds. Linearity of the method was assessed over a six point calibration curve from 0.001 to 0.2 mg/kg (R^2>0.99). Robustness was evaluated through 24 hours and peak area variance was reported to be <5.7%. One of the major challenges in the quantitative LC-MS/MS analysis for pesticides in food is that compound and matrix-dependent response suppression or enhancement may occur. The method was evaluated on three different matrices (apple, mint and tomato). The response for 3 selected pesticides analyzed in a single batch sequence (about 72 hour) showed that within a matrix, variance was less than 5.9% RSD for all compounds.

In order to be able to satisfy the whole pesticides panel requested from the EURL-SRM we have integrated the LC-MS/MS approach with a GC-MS/MS method, able to detect and quantify 360 pesticides in less than 10 minutes on different food matrices.

Matrix calibration curves were measured over the EURL-SRM requested concentration range and linearity was assessed with a correlation factor >0.99. Peak widths at half maximum were decreased below 1 sec by using fast GC separation. In order to obtain a robust quantitative analysis and to enable the acquisition of at least 10 data points/peak, a loop time of 0.18 was selected. As in some parts of the chromatogram up to 30 compounds elute in the same processing window and 2 transitions/compound are required, 60 data points are necessary, thus leading to a 3 msec dwell time. RSD values were reported to be <10% for most of the compounds.

LC and GC methods proposed represent a fast, selective and highly sensitive approach to satisfy the EURL-SRM requests for pesticides analysis on food matrices.

OR12. ILLEGAL ADMINISTRATION OF DEXAMETHASONE IN BOVINES: TARGETED PROTEOMICS AS A NEW TOOL FOR AN INDIRECT SCREENING?

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Despite the ban of use of growth promoters in Europe dates more than twenty years, illegal practices for enhancing food-producing animals weight gain, are still a matter of concern for the European authorities and consumers.

Laboratories in charge of official controls are constantly engaged in a battle for the detection of the lowest possible residual concentrations of forbidden compounds in biological fluids or tissues. Analytical methods are mainly based on HPLC-MSMS for the determination of the residues of the administered drugs or related metabolites. Notwithstanding the recent technological advances reached by highly sensitive mass spectrometers, surveillance of such practice is still challenging, due to low dosage of administration and/or to fast depletion/elimination processes, so that analytical methods may not detect residues of past illegal treatments.

In the last 10 years, several alternative analytical approaches focusing on the indirect biological effects of growth promoters have been developed. Among them, targeted protein markers profiling has been suggested as a screening approach for the detection of illegal treatments in meat production. In this study, an analytical method based on selected reaction monitoring for the absolute quantification of protein markers in skeletal muscle for the indirect detection of illicit dexamethasone treatments has been developed and evaluated. A set of protein markers of treatments with dexamethasone, previously identified by 2D-DIGE proteomics approach, was analysed by standard HPLC-MSMS, typically adopted for residue analysis. The gathered data enabled to define a promising predictive model based on logistic regression to detect dexamethasone treated animals.

OR13. A RAPID EXTRACTION AND SCREENING METHOD FOR MYCOTOXINS FROM CEREAL PRODUCTS USING QUECHERS AND LC-MS/MS

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Although aflatoxins and ochratoxins are of major significance, the FDA is also actively concerned with other mycotoxins, including fumonisins, trichothecenes, patulin, and zearalenone. Depending on the intended use of the product, the FDA has established mycotoxin action levels in the parts per billion range; 20 ppb for aflatoxins in all products intended for human consumption, except milk. As such, a specific and sensitive method is necessary to proactively monitor mycotoxins in food.

In this study, we developed a screen to detect different classes of mycotoxins. We demonstrate a rapid sample preparation and LC-MS/MS method for screening all major mycotoxins from cornmeal. Sample cleanup using QuEChERS extraction and dSPE kits containing PSA/C18E successfully removed interferences from the ground corn-meal matrix. Recoveries were all acceptable, ranging 66 to 118% for most mycotoxins. By increasing the concentration of formic acid from 0.1%, 0.5% and 1.0% we improved analyte partitioning into the organic layer. Although an increase in concentration of formic acid helped with recovery of Ochratoxin A (2% recovery with 0.1% formic acid to 86% recovery with 1% formic acid), results on the other mycotoxins varied.

Compared to previously published data, the extraction using 50% acetonitrile under acidic conditions helped increase extraction efficiency for most analytes except fumonisins (FB1 and FB2). Future experiments should be considered to improve fumonisin recovery.

Extracts were analyzed using a Kinetex® 2.6 µm XB-C18 Core-shell Technology HPLC column. Compared to LC separation with fully porous particles, this technology allows for faster separations and better sensitivity.

In addition, using a novel Kinetex® 2.6 µm Biphenyl CoreShell stationary phase in place of the frequently used C18 columns, results in a significant retention improvement thus lowering the matrix effect.

Compared to C18 phases, the polar retention mechanisms put in place by the biphenyl ring are able to better retain polar mycotoxins, such as M1 and move them away from the ion suppression area typically located at the beginning of the chromatogram.

OR14. IMPROVEMENT OF A MULTICLASS SCREENING METHOD TO DETECT BANNED SUBSTANCES IN BOVINE URINE BY LC-HRMS

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European Union banned anabolic substances in food-producing animals because of their possible toxic effects on public health. In order to control the abuse of these compounds included in the group A (Annex I of Directive 96/23/EEC), National Residue Plans provide to analyze urine, that is the matrix of election.

In Italy, the official laboratories apply several analytical methods to fulfil the monitoring plans. Generally, a preliminary screening test, followed by the relevant confirmatory method, in case of suspect sample. Therefore, the routine controls involve the use of several single-class methods to cover all the required molecules/classes with high times and costs. It must be also underlined that each method applied needs a preliminary intensive work to be developed, validated and accredited.

The aim of this research was the improvement of a previously developed multiclass screening method to detect different classes of banned substances in bovine urine: lactones of Resorcylic Acid (RALs), nitroimidazoles, steroids, stilbenes and two banned veterinary drugs, dapsone and chloramphenicol. Two classes, belonging to group B, corticosteroids (B2f) and sedatives (B2d), were included too, since the first ones can be associated to illegal growth promoting treatments and sedatives can be used in cattle to prevent stress and death after steroid treatments. Liquid Chromatography coupled to hybrid high-resolution mass spectrometry was applied (LC-Q-Exactive Plus). Because of the several interfering substances, which vary from urine-to-urine, a Parallel Reaction Monitoring (PRM) acquisition was necessary to achieve the suitable selectivity. Two chromatographic runs were carried-out: negative ESI for chloramphenicol, RALs, stilbenes, and positive ESI for all the other analytes. Improving the method involved the sample preparation. The previous protocol provided, after the deconjugation step, a tandem SPE purification (Oasis HLB followed by NH_2 cartridges), whereas the new one involves a parallel clean-up of two aliquots with Strata X-C and Oasis HLB/NH_2 cartridges, respectively, joining the purified extracts. In this way the detection capabilities (CCβ) of the most critical analytes, such as β-nortestosterone, stanozolol, terbutaline and salbutamol improved significantly. Thanks to this revised strategy, the modified screening test is now suitable to detect all the investigated compounds at 1 µg/L (except diethylstilbestrol).

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OR15. MULTI-COMPOUND AND MULTI-CLASS IDENTIFICATION AND QUANTIFICATION USING HIGH RESOLUTION LC-MS/MS

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LC-MS/MS is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues and contaminants. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants.

However, the use of triple quadrupole based mass analyzers limits the number of compound to quantify and identify. In addition there is an increasing demand for retrospective and possibly non-target data analysis. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC-MS/MS run.

Here, a generic QuEChERS procedure was used to extract residues and contaminants from fruit and vegetable samples. Extracts were diluted to minimize potential matrix effects and subsequently analyzed by LC-MS/MS using a SCIEX QTOF system operated in high resolution accurate mass MS and MS/MS mode. Full scan MS and MS/MS data was explored to identify targeted compounds using extensive XIC lists of target compounds. Analytes were identified with high confidence based on retention time matching, mass accuracy, isotopic pattern, and MS/MS library searching. Quantitation was achieved in the same data processing step.

The developed method was successfully applied to the analysis of store-bought samples. It was found that the use of MS/MS information is crucial to minimize false positive results.

OR16. MEASURING DIOXINS AT THE ATTOGRAM LEVEL: SCIENCE OR FICTION?

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Dioxins, furans and Polychlorinated Biphenyls (PCBs) are organohalogeated compounds of great interest because of the high toxicity, bio-persistence and bio-accumulation of some congeners. For this reason, very low detection limits are required for such compounds and they are reached with the combination of sensitive mass spectral detection (GC-HRMS Magnetic Sector or GC-MS/MS Triple Quadrupole instruments) and careful chromatography, as well as combining increased sample size with increasingly selective sample preparation (extraction and clean-up) to reduce matrix effect and avoid possible interferences in congener quantification.

However in some case sample is v small, as for dried blood spots routinely sampled from new born in many countries and containing 50-150 µL of blood. In such cases, residue levels, especially for lipophilic compounds, are very small, and a unique analytical challenge is presented. Cryogenic peak modulation is a well established technique used for comprehensive GC×GC applications since 1991, and due to the narrow modulated peak widths, it is best used in combination with fast acquisition Time Of Flight (TOF) analyzer, which unfortunately does not have adequate sensitivity and reliability required for trace analysis of dioxin and dioxin-like compounds. GC×GC modulator was used on a single and short GC column coupled to a High Resolution Mass Spectrometer (HRMS) to exploit the signal enhancement of the cryo-focusing and the high sensitivity of the Magnetic Sector mass spectrometer, a technique they termed Cryogenic Zone Compression Gas Chromatography (CZC GC-HRMS). The main difference between CZC GC-HRMS and GC×GC-HRMS is that entire 1^{st} dimension peaks are trapped, followed by reinjection of the complete refocused peak onto the 2^{nd} dimension column, producing only one single 2^{nd} dimension chromatogram (whereas in GC×GC each 1^{st} dimension peak is modulated into several 2^{nd} dimension chromatograms), maximizing the peak enhancement effect.

In our laboratory, we are testing a CZC module on a new DFS Magnetic Sector, as, although this technique has been conceived few years ago, there are still questions and issues open, related to the main aspects of dioxin analysis, such as the purity of the internal standard used for Isotope Dilution, blank levels, LOQs, as well as sample extraction and clean-up, to be reproducible and robust with such small amounts of samples. Not to mention the instrumental challenge related to the Magnetic Sector instrument, which is required to be faster but still accurate and very sensitive.

OR17. CYCLIC IMINES (CIS) IN MUSSELS FROM MIDDLE ADRIATIC SEA (ITALY): LC-MS/MS IDENTIFICATION AND MONITORING

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Cyclic Imines (CIs) are lipophilic marine toxins produced by dinoflagellate microalgae that bioaccumulate in filter-feeding bivalves and can be conveyed to humans. CIs include Spirolides (SPXs), Gymnodimines (GYMs), Pinnatoxins (PnTXs), Pteriatoxins (PtTXs), spiro-prorocentrimine and prorocentrolides. Their structure is characterized by a cyclic-imino moiety, responsible for a rapid onset of systemic neurotoxicity in mice when administered intraperitoneally (Fast Acting Toxins). Until now, human intoxication by CIs has been not yet reported, nevertheless, because of their growing geographical distribution, CIs are considered "emerging" toxins worldwide. Therefore, the Community Reference Laboratory for Marine Biotoxins (CRLMB) suggested the member state official laboratories to include CIs in the routine bivalve molluscs monitoring (XVII Workshop for marine biotoxins, Lisbon, 23-24 October 2014).

Here we report the results of the CIs monitoring in mussels breed along the Marche coast (Middle Adriatic Sea), during 2014. The analysis were performed by the official LC-MS/MS protocol used for the marine lipophilic toxins (AESAN vers.5, 2015) opportunely modified to include eight CI analogues.

Mass spectral experiments were performed using a hybrid triple-quadrupole/linear ion trap 3200 Q TRAP mass spectrometer (AB Sciex, Darmstadt, Germany). To investigate the presence of CIs in mussel samples Multiple Reaction Monitoring (MRM) experiments were conducted using two transitions for each analogue. To identify unequivocally the CIs, the suspected chromatographic peaks were further investigated doing Collision Induced Dissociation (CID) experiments in the Linear Ion Trap (LIT) and the obtained results were compared to those of certified CIs standards.

Two SPX analogues (13-desMeSPX C and 13,19-didesMeSPX C) and GYM A (the latter never reported before in Italy) were often detected with maximum levels in January-March for SPXs (25 µg/kg as sum of the 2 analogues) and in June-August for GYM A (12 µg/kg). The geographical distribution of the toxins was the same for SPXs and GYM A with higher levels in mussels collected in the south of the Region. The low levels detected, compared to the guidance level of 400 µg ΣSPXs/kg proposed by the CRLMB, do not represent a risk for the public health. However their presence in the Adriatic Sea confirms the need to monitor and identify their biogenic origin, because the occurring environmental and climatic changes might promote the toxic microalgae proliferation. The LC-MS/MS approach implemented has proved to be sensible and specific for the determination of CIs in shellfish even at low ppb levels.

OR18. DETERMINATION OF POLYCHLOROBIPHENYL (PCB) IN WATER BY HS-SPME/GC-MS-MS

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The aim of this work is to test the performances of a method suitable for the monitoring control of PCB in water at low legislation level: 0,05 µg/L. The method is based on AUTO HS-SPME-GC-MS/MS technique and it has been found to be simple, fast, accurate, selective and sensitive. Analytes in water samples were directly concentrated on a solid-phase micro-extraction fiber SPME 30µm PDMS (Polydimethylsiloxane) using the headspace mode. For headspace SPME analysis 10 mL of water sample were transferred in a 20 mL screw-cap vial with silicone/PTFE septa, added with internal standard solution at a final concentration of 10 ng/L and placed in a HS-SPME autosampler. The fiber was heated at 90°C for 15 minutes for the extraction step, then the desorption took place in the GC injector at 280°C. Analyses were performed using a GCMS-TQ8040 Shimadzu (Shimadzu Corporation, Kyoto) equipped with autosampler GERSTEL MPS2 and "RXI-17 Sil MS" column (Restek) 10 mt × 0.15 mm ID × 0.15 µm df. The carrier gas was helium at a total flow 16 mL/min and the temperature program used was 40°C for 1 min, 50°C/min to 170°C, 10°C/min to 260°C, 50°C/min to 310°C. The splitless injection port temperature was 280°C, with a pressure of 150 kPa. The mass spectrometer was used in the positive electron impact ionization mode at 70 eV; the MS parameters were: interface temperature 300°C, ion source temperature 260°C, acquisition mode MRM (Multiple Reaction Monitoring), acquisition start time 5.0 minutes and end time 13.54 minutes. The overall analysis time was 26 min, including 15 min for the extraction step. The calibration curve was obtained by dilution of "ISS PCB Calibration Mixture" (Ultrascientific) from 0.1 ng/L to 50.0 ng/L and the internal standards ^{13}C labeled PCB Mix solution, were purchased from Cambridge Isotope Lab (Andover, MA).

The present study shows that HS-SPME combined with fast GC-MS/MS can be efficiently used for the quantification of semi-volatile compounds, like PCB. Analytical parameters, such as linearity, limit of quantification, precision and recovery were evaluated and satisfactory performances were reached. The method is completely automatized, the extraction process avoids the use of solvent, sample preparation and analysis times are minimized, compared to traditional methods, based on liquid-liquid extraction, SPE purification and conventional chromatography, that require about 2 hours.

Session 3

Doping and dependence

Chairpersons

Donatella Caruso, Paolo Stacchini

PL4. DOPING ANALYSIS: MASS SPECTROMETRY AND BEYOND

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Anti-doping analysis represents a peculiar field of analytical chemistry applied to forensic toxicology, with the aim to detect the recourse to substances and methods illicity used for the non-physiological enhancement of sport performance from the analysis of biological samples (urine and blood).

This communication presents a general overview on the activity of the antidoping laboratories accredited by the World Anti-Doping Agency (WADA) and belonging to the World Association of Anti-Doping Scientists (WAADS), outlining the evolution, over the last five decades, of the analytical procedures for the detection of prohibited substances and methods, with special emphasis on mass spectrometry-based techniques. For indeed, a great portion of the analytical procedures set up and applied in the WADA laboratories relay on mass spectrometric techniques, and specifically on the combination between chromatography (both gas and liquid chromatography) and mass spectrometry and/or tandem mass spectrometry, using different ionization sources and acquisition modes. All low molecular weight xenobiotics, most of the peptides and the "pseudo-endogenous" steroids (i.e. testosterone and its precursors and metabolites) are indeed successfully screened for and confirmed by either GC-MS(/MS), GC-IRMS and LC-MS(/MS). At the same time, an overview is also given of those areas in which the application of mass spectrometry-based techniques is not yet complete (i.e. detection of blood transfusions, and, more in general, of "blood doping", of recombinant growth hormone and of peptide and glycoproteic hormones).

Despite the drastic improvement in the efficacy of the anti-doping analytical procedures recorded in the last years, some "grey zones" still exist, and are primarily related to the use of (i) still unknown doping agents and/or "masking agents"; (ii) substances that are virtually identical to those naturally produced by the human body; (iii) substances whose pharmacokinetic profile has not been fully clarified yet; and (iv) substances whose concentration in blood/urine fall below the limit of the detection of the available analytical techniques. Future perspectives on the advancement of anti-doping knowledge in these areas are finally outlined.

OR19. NEW TRENDS IN CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY APPLIED TO PSYCHOTROPIC DRUGS AND DOPING AGENTS

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The use of psychotropic drugs and doping agents represents two distinct social phenomena, sometimes overlapping since the substances consumed are the same or of both classes. The dependence that can create some performance-enhancing substances can be similar to that of drug users.

One of the main problems related to the diffusion of psychoactive substances and doping agents is the difficulty of their analytical identification, both in non-biological products and in biological samples collected in cases of intoxication or misuse potentially related to their consumption.

Although Gas Chromatography coupled with Mass Spectrometry (GC-MS), represents the most commonly used technique for identification molecules of clinical/forensic interest, Liquid Chromatography coupled with Mass Spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS) is the most versatile technique for non-biological samples and biological ones where the parent drug is often present with hydrophilic metabolites.

The systematic toxicological analysis that extracts the samples at three different pH (acidic, neutral with a non-polar solvent and carries out a qualitative analysis by GC-MS followed by a quantitative LC-MS is the analytical strategy to identify and quantify the largest number of samples with unknown analytes. Finally, the ultra performance liquid chromatography coupled to tandem mass spectrometry allows to analyze a large number of analytes, characterized by the previous methods in a very short time, allowing, he screening of many samples in a short time with very high sensitivity.

Methods for identification and quantification of psychotropic substances, drugs and doping agents have been developed in cases of unexplained intoxication in neonatology, pediatrics and adults and to identify unknown substances seized at the Internet web sites, sexy shops or shops that sell herbal products.

OR20. SYSTEMATIC TOXICOLOGICAL SCREENING USING LC-MS

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Forensic toxicology laboratories require reliable screening techniques that can detect a wide variety of toxicants in highly complex biological matrices, such as ante and postmortem specimens.

This approach uses an UPLC coupled with triple quadrupole. Data is acquired in scanning mode using in-source fragmentation and library searching. Libraries are built by Waters using the NIST format and are dedicated to Toxicology.

This method is suited for screening of more than 950 drug substances and metabolites. Runtime from sample to sample is only 15 minutes, including column conditioning. Sample deconvolution and library searching are managed by a dedicated software specific for this kind of application.

This solution has been successfully and routinely used in toxicology laboratories worldwide providing a simple and sensitive method for forensic toxicology screening of compounds in various biological matrices (Hair, blood, plasma, urine, oral fluid).

OR21. FORENSIC INVESTIGATION OF KERATIN MATRIX: DEVELOPMENT OF A METHOD BY TURBOFLOW™ HPLC-MS/MS FOR CANNABINOIDS QUANTITATIVE ANALYSIS

Valentina Castelli (a), Sergio Indelicato (b), Serena Fanara (a), Manuela Fontana (a), Rossella Pisciotta (a), Fabio Venturella (c), Francesca Di Gaudio (d)

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The products of cannabis are the most frequently used illicit drugs of abuse detected in workplace drug-testing programs or in cases of driving under the influence of drugs. For this reason, there is a great need for sensitive and specific methods which can confirm the chronic exposure and which take account of the cut-off values that have been proposed in Italy by the GTFI Guidelines (Group of Italian Forensic Toxicologists). The method currently more appropriate for quantification of Δ9-Tetrahydrocannabinol (THC) and its major metabolite 11-nor-9-Carboxy-Δ9-THC (THCA) appears to be one that involves HPLC-MS/MS and keratin matrix. The purpose of this work, performed at the Mass Spectrometry Laboratory for Clinical Risk and Quality Control (CQRC) in A.O.U.P. "Paolo Giaccone" of Palermo, is the developing of an accurate method equally or more sensitive than those used routinely in forensic toxicology laboratories. A pre-analytical phase has seen the preparation of hair samples, comprising basic hydrolysis, and a subsequent Liquid-Liquid Extraction (LLE). In the analytical phase, the step of detection was performed using the Thermo Scientific™ Transcend™ II system which combines the online sample preparation technology TurboFlow™ with the chromatographic separation technique of High Performance Liquid Chromatography (HPLC) and the detection technique of tandem Mass Spectrometry (MS/MS) using a high sensitive Thermo Scientific™ TSQ Quantiva™ Triple Quadrupole Mass Spectrometer. During the phase of the developing method a series of experimental tests regarding the analytical conditions have been performed, on six different chromatographic columns. This has highlighted the main advantages of TurboFlow™, the adequate selectivity in the sample cleaning and the possibility of injecting more volumes of sample than the normal LC-MS techniques, leading to an appreciable increase in the sensitivity. According to the aim of the work, the latter steps of sample preparation, including LLE, were replaced with a simple hydrolyzate filtration and direct injection into TurboFlow™ system. In the light of the results we obtained it can be said that the method developed by TurboFlow™ -HPLC-MS/MS that involves the short phase of sample preparation procedure allows accurate quantification of cannabinoids in the keratin matrix. This technique, showing an adequate sensitivity, can be

compared and preferred to used routine techniques. The short phase of preparation of the sample, also, results in a considerable saving of time and materials and in the reduction of possible errors caused by the operator.

OR22. RAPID MINIATURIZED DISPERSIVE LIQUID/LIQUID MICROEXTRACTION METHODS (DLLME) FOR THE DETERMINATION OF DRUGS OF ABUSE IN BIOLOGICAL FLUIDS BY CHROMATOGRAPHIC TECHNIQUES COUPLED WITH MASS SPECTROMETRY DETECTION

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Sample pre-treatment is a fundamental step when analyses are performed on highly complex matrices, such as blood, plasma, urine, oral fluid, and hair extract. Recently great attention has been paid to miniaturised extraction techniques, due to the capability to use them as high-throughput, low-solvent "environment-friendly" methods for the extraction of various classes of analytes from different matrices, spanning from air, waters, soils, food, to biological samples. Dispersive Liquid Liquid Micro Extraction (DLLME) is a miniaturized extraction technique firstly developed for the analysis of organic and inorganic species in water and food, that is gaining popularity also in the field of clinical and forensic chemistry for urine and blood pretreatment. DLLME allows an efficient extraction of analytes with a limited consumption of organic solvents by using very small amounts of a binary mixture of solvents, one water-miscible disperser solvent, and one immiscible extractant solvent.

This technique has been applied in our laboratory for the development of methods for the determination of drugs of abuse/therapeutic drugs and their metabolites in biological fluids (urine and blood) by Gas Chromatography-Mass Spectrometry (GC-MS) or by Liquid Chromatography/tandem MS (LC-MS/MS).

Urine or deproteinized blood, after addition of deuterated internal standards and hydrolysis of conjugates, are added with water containing the appropriated buffer. DLLME is performed through the rapid injection of a mixture of chloroform (extractant) and methanol (disperser), with the formation of a cloudy solution with a high contact surface among the phases, that allows a very quick extraction of the analytes. The sample is then centrifuged. The sediment phase (about 70-90 μL) is transferred into a vial, evaporated, reconstituted in mobile phase for LC-MS/MS or derivatised in case of GC-MS analysis, and then injected. The total extraction time is about 5 minutes, including centrifugation.

Limit of detection and of quantification obtained are suitable for the purposes of the forensic analysis. The developed methods have been fully validated prior to their application to authentic samples. The minimal use of solvents, cheapness, and quickness render the method attractive for its routine application in forensic analyses.

Session 4

Ion spectroscopy and inorganic elements

Chairpersons

Simonetta Fornarini, Giorgio G. Mellerio

PL5. ION SPECTROSCOPY. STRUCTURES OF SILICON IONS AND CLUSTERS: SILANE IONS AND DOPING ON THE NANOSCALE

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Silanes and their derivatives and ions are fundamental species in a variety of chemical disciplines, ranging from organic chemistry and materials science to astrochemistry and theory of chemical bonding. In contrast to hydrocarbon ions, almost no information is available for the corresponding $Si_xH_y^+$ cations. Here, IR spectra of $Si_xH_y^+$ produced in a supersonic plasma expansion of SiH_4 are inferred from photodissociation of cold Ne and Ar complexes obtained in a tandem quadrupole mass spectrometer coupled to an electron impact ionization source, an octopole ion trap, and an IR-OPO laser. The clusters are characterized in their ground electronic states by quantum chemical calculations to investigate the effects of ionization/protonation and Ar/Ne complexation on their geometric, vibrational, and electronic structure. We present initial results for $Si_2H_6^+$, $Si_2H_7^+$ and $Si_3H_8^+$, which have complex potential energy surfaces, with low-energy isomers featuring unusual three-center two-electron (3c-2e) bonding. Results on elusive protonated silanols will also be presented.

Doping of bare silicon clusters at the nanoscale is relevant for applications in materials science and astrochemistry. We present IR spectra and calculations of neutral and charged $Si_nX_m^{(+)}$ clusters with first row elements X=Be-O generated in a laser desorption cluster source coupled to a molecular beam, a time-of-flight mass spectrometer, and an IR free electron laser. IR spectra of neutral Si_nX_m clusters are obtained by IR-VUV resonant photoionization, while those of cationic $Si_nX_m^+$ clusters are measured from photodissociation of cold mass-selected $Si_nX_m^+$-Xe clusters. The structures are assigned by comparison to sophisticated DFT calculations combined with effective basin hopping and/or genetic algorithm search routines. It is shown, that the cluster structures sensitively depend on the type and number of dopant atoms.

PL6. MOBILITY SEPARATION AND IR CHARACTERIZATION OF ION ISOMERS USING A SINGLE INTEGRATED MS/MS SET-UP

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The focus of this lecture will be put on the separation and structural characterization of isomers and tautomers of small molecules with tandem mass spectrometry coupled with an ion mobility device and a tunable infrared laser for specific ion action. This coupling provides two additional dimensions to the analytical technique, and can be interesting from both analytical and fundamental points of view. From an analytical point of view, an enhancement of the resolving power could be achieved using a selected structure-specific activation wavelength for inducing the fragmentation of DIMS selected ions. From a more fundamental point of view, the proposed coupling allows for the IR spectroscopic characterization of mass- and mobility-selected ions.

Separation and identification of oligosaccharides, for example, remains a challenge and an important advance could be achieved with the integration of ion mobility spectrometry (IMS) techniques to MS/MS. We will show that the additional coupling with an IR laser allows for the structural characterization of mobility separated epimers and anomers of saccharides.

Separation and structural characterization of small molecules is also of interest for metabolomic where our aim would be to contribute to the targeted analyses of unknown metabolites. In this context we will focus our attention on the auto-oxidation reactions of tetrahydrobiopterin which is involved in hydroxylation reactions of living organisms. We will show that the combination of mobility separation and infrared spectroscopy allows for the identification of tautomers involved in the oxidation process of tetrahydrobiopterin.

Differential Ion Mobility Spectrometry (DIMS) is used. This approach is interesting because DIMS-selected ions can be continuously accumulated in the ion-trap, before being subjected to MS/MS experiments. MS/MS experiments presented here are performed with a commercial Bruker Esquire 3000+ which is also coupled to infrared laser sources, which can be the free electron laser at CLIO (Orsay) or a bench-top optical parametric oscillator/amplifier (OPO/OPA) laser.

OR23. EXPLORING LIGAND SUBSTITUTION MECHANISMS AT THE MOLECULAR LEVEL WITH IRMPD/MS: THE CASE OF CISPLATIN

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The applications of Mass Spectrometry (MS) are innumerable and diverse. Mostly applied to address analytical issues, MS is also recognized as one of the most useful tools to gain insight into reactive processes at the molecular level. In a relatively recent expansion of MS-based methods, IR Multiple Photon Dissociation (IRMPD) spectroscopy has provided valuable contributions, allowing to obtain information on vibrational features and consequently structural identification about MS sampled species.

In this contribution the reactivity of cisplatin, one of the most widely used antineoplastic drugs, with pyridine, trimethylphosphate and 4(5)-methylimidazole, models of biological targets, was assayed employing MS and IRMPD spectroscopy supported by DFT calculations. Ionic products obtained by addition and/or substitution by the selected ligands have been isolated in the gas phase and characterized by IRMPD spectroscopy. The IR spectroscopic analysis has covered both the fingerprint region (1000-2000 cm^{-1}) and the N-H/O-H stretch region (2800-3800 cm^{-1}). The assignment of the vibrational features in the experimental IRMPD spectra has been assisted by computations employing the classical B3LYP functional, and the ωB97XD one, which has been shown to better take into account long range and dispersion effects.

Intriguingly mass spectrometry permitted to reveal the addition complex generated by the interaction of the first hydrolytic product of cisplatin, namely *cis*$[PtCl(NH_3)_2(H_2O)]^+$ with the model ligands. This species was interrogated by IRMPD spectroscopy and direct evidence was obtained for the substitution reaction occurring when the ions are activated by resonant IR photons. Differences into the reactivity of the ligands with cisplatin are also described and compared with the gas phase reactivity obtained in the cell of an FT-ICR mass spectrometer. Finally calculations of the potential energy surface have shed light on the behavior of the different complexes.

OR24. THALLIUM REMOVAL WITH GAS-WATER MIXTURES FROM NATURALLY-CONTAMINATED URBAN DRINKING WATER NETWORKS: EFFICACY ASSESSMENT BY ICP-MS

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In September 2014, drinking-water samples collected from two connected waterworks in the municipality of Pietrasanta (Tuscany, Italy) resulted contaminated by thallium in significant concentrations (≤14 µg/L), well above the US-EPA limit of 2.0 µg/L. The contamination was originally caused by the water supplied from a spring located near to some abandoned mine sites. Surprisingly, spring disconnection and water flushing did not restore a safe water supply but determined a fluctuating increase in the concentration of thallium (up to 60 µg/L) due to its migration from the distribution system, where it had accumulated as sediments, sludge and adsorption products.

A new inexpensive procedure to clean contaminated pipes was developed by the Italian Institute of Health in co-operation with the water supplier GAIA SpA The procedure, developed on the basis of preliminary tests, consisted of the following steps. The pipeline under treatment was isolated from the remaining part of the distribution network by closing valves and water meters. Then, a mixture of water and air at high pressure (5-6 atm) was introduced into the pipe as pulses for a time sufficiently long to induce mechanical ablation of contaminated coatings from the internal surface of the pipe.

The progress of the treatment was continuously monitored by recording water turbidity at the end of the pipe. The residual contamination absorbed and/or occluded in iron oxides and hydroxides was removed from the pipe surface by continuously introducing a mixture of water and CO_2 at high pressure for one hour, at least. Finally, the pipeline was reconnected to the distribution network and the water was made to flow to proximal drainpipes for 1-5 days to remove possible pockets of contamination accumulated near valves or temporary-closed connections.

The optimization of the experimental conditions adopted in the final procedure as well as the overall efficacy of the treatment were carried out by elaborating kinetic data on the release of total and soluble thallium into the water flow during preliminary tests and the following application of the cleaning procedure.

To that end, samples of the sewage produced in the course of every test were collected at prefixed times and analysed by ICP-MS.

OR25. CRITICALITIES OF DETERMINATIONS OF INORGANIC ELEMENTS IN FOOD BY MEANS OF ICP-MS: EXPERIENCES OF THE ITALIAN NATIONAL REFERENCE LABORATORY

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In compliance with Regulation (EC) No 882/2004 Member States shall arrange for the designation of one or more National Reference Laboratories (NRL) for each Community Reference Laboratory (EURL) referred to in Article 32. Since 2010 the Italian NRL for Heavy Metals in Food (NRL-HM) is hosted by Istituto Superiore di Sanità, Department of Food Safety and Veterinary Public Health. The NRL-HM shall: collaborate with the EURLs within its area of competence; coordinate, for its area of competence, the activities of official laboratories responsible for the analysis of samples in accordance with Article 11; where appropriate, organize comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing; ensure the dissemination to the competent authority and official national laboratories of information that the EURLs supply; provide scientific and technical assistance to the competent authority for the implementation of coordinated control plans adopted in accordance with Article 53; be responsible for carrying out other specific duties provided for in accordance with the procedure referred to in Article 62(3), without prejudice to existing additional national duties.

In particular, the LNR coordinates the activities of the official laboratories responsible for the analysis of food samples, through the regular collection of information about the types and performance of the analytical methods they use and the verification of their compliance with the legal requirements; the development, validation and transfer of methods of analysis. This cooperation enables a shared and harmonized approach on several critical issues that may arise in the activities described.

The current analytical methodologies available for the control activities can be affected by certain critical aspects from sampling up to the expression of the result involving the entire analytical process (preparation, instrumental interpretation, evaluation of the effect due to the presence of certain analytical interferences). As part of the determination of inorganic elements by ICP-MS, spectral interferences (isobaric, and those due to polyatomic ions with double charge) and non-spectral interferences (physicochemical and the memory effect) are critical. It is necessary, therefore, to recognize them and monitor them in relation to the analytical method used. In certain circumstances the resolution of these critical issues may not be simple or immediate. Therefore, the collaboration and the application of uniform and shared holistic approaches, represent the strong point of the activities conducted within the network of the official control laboratories for heavy metals in food.

OR26. ADVANCED ICP-MS-BASED METHODS FOR THE CHARACTERIZATION OF INORGANIC NANOMATERIALS AND THEIR ANALYTICAL DETERMINATION IN COMPLEX MATRIXES

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Inorganic nanomaterials (metals, their compounds, and oxides) - e.g. Ag, SiO2, TiO2, ZnO, TiN, Fe oxides/hydroxides, nanoclays - are a prominent class of nano-sized materials with a number of current or projected applications. For instance, in the food sector the latter range from food additives, to antibacterial agents, to food packaging. It is thus essential to have analytical methods available to characterize them as such (pristine materials), and in complex matrices (e.g. in tissues from *in vivo* toxicological studies needed to assess their safety).

State-of-the-art mass spectrometric techniques for the analytical determination of inorganic nanoparticles in dispersion and (after proper sample extraction) in complex matrices have recently become available. Being based on atomic mass spectrometric, they are element-specific (i.e. provide information on the chemical identity) and have the potential to measure size, size distribution, number and mass concentration of particles.

Single particle inductively coupled plasma mass spectrometry (sp-ICP-MS) is based on time resolved analysis of diluted nanoparticle dispersions using short dwell times (≤10 ms). Each particle gives rise to a signal clearly distinguishable from random background noise and, by means of appropriate algorithms, signal frequency distributions are converted into size frequency distributions. In principle, the signal arising from ionic (i.e. soluble) forms of the element constituting the particles, if any, can be distinguished from that due to the presence of the particles themselves. Therefore, it is a particle-specific technique with sizing capability, presently having limitations mainly in the size detection limits (from ~10 to several tens nm, depending on the element).

Another powerful technique is asymmetric flow field flow fractionation (AF4), which provides separation of particles according to their size, combined on-line with optical detectors for size determination (MALS, DLS, UV) and elemental detection/quantification by ICP-MS. With AF4-ICP-MS particles having diameters down to 1 nm can be determined, with the additional advantage of multi-detector capability, but with some limitations in the mass concentration detection limits.

Our laboratory was one of the few - on a worldwide level - that participated to the three interlaboratory studies (ISs) promoted by the European Commission on the application of these techniques to food analysis, namely two ISs on sp-ICP-MS and one IS on AF4-ICP-MS, all focusing on the determination of Ag nanoparticles. However, far more challenging is the application of these techniques to the characterization of oxides such as SiO_2 and TiO_2 owing to a number of issues, including spectral interferences in the detection of Si and Ti. Selected applications, mainly from nanotoxicological studies, are presented.

OR27. SIMULTANEOUS DETECTION BY ISOTOPE RATIO MASS SPECTROMETRY AND QUADRUPOLE MASS SPECTROMETRY COUPLED TO MULTIDIMENSIONAL GAS CHROMATOGRAPHY

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Isotope Ratio Mass Spectrometry (IRMS) is commonly recognized to be able to provideinformation about the geographical, chemical, and biological origins of substances. The ability to determine the source of substances stems from the relative isotopic abundances of the elements which comprise the material.

By performing a separation prior to isotope ratio analysis, hyphenated techniques such as GC-C-IRM, can provide isotopic analysis of a complex mixture, thereby providing additional information and higher discriminatory power. Since its introduction, the use of this analytical approach was not widespread due to a series of drawbacks related to chromatographic and isotopic issues. In fact, dead volumes due to the typical instrumental setup, requiring the combustion of the components followed by a drying step, often limit the separation efficiency, driving to an increased band broadening and peak asymmetry producing peak coelutions, thus falsify the measurements.

Moreover, the reduced chromatographic performance increases the gas chromatographic isotope effect (or inverse isotopic effect) that generates GC peak not isotopically consistent because composed of lighter isotopes (12C, 1H and 16O) that elute after the isotopomers containing heavier organic compounds because of their higher volatility. The present research deals with the development of an MDGC-MS/IRMS prototype characterized by the improved resolution capability of the heart-cut mode, exploiting two different GC stationary phases, and the simultaneous qMS and IRMS detection of the 2D chromatographic bands.

The IRMS system was optimized in terms of dead volumes enabling to overcome the extra-column band broadening effect that usually affects the commercial systems. Different applications on food and flavour and fragrance samples are reported showing the enhanced performances of the prototype described.

Poster Communications

P1. ANALYSIS OF MELAMINE IN FOOD SUPPLEMENTS

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Melamine, also known as tripolycyanamide, is a trimer of cyanamide with a 1,3,5-triazine skeleton and contains 66% nitrogen by mass; this is the reason because this molecule is often used for food adulteration. It can be produced from urea, dicyandiamide or hydrogen cyanide but commercially melamine is usually manufactured from urea with purity approx 99%. It is use in the production of glues and plastic, in plants and as a fertilizer and when mixed with resins it has fire retardant properties. Low levels of melamine may be present in water effluents as a result of industrial-scale uses, production and disposal. Melamine is a metabolite of Cyromazine, which can be used as a pesticide or veterinary drug, residues of these products have been detected on vegetable crops after spray application. The Melamine residue in food can be originate from unavoidable background (food contact material, pesticides use, etc.) and unacceptable adulteration. European Food Safety Authority has proposed a legal Maximum Residue Level (MRL) of 1.0 mg/kg for infant food and 2.5 mg/kg for other foods.

The aim of this study was to develop and to validate a reliable analytical method for the detection and quantification of melamine in food supplements and raw material and milk based products.

Different strategies were applied in order to find the best analytical performances: High Performance Liquid chromatography with Diode Array Detection (HPLC-DAD), Ultra High Performance Liquid Chromatography coupled with tandem Mass Spectrometry (UHPLC-MS/MS) and Gas Chromatography coupled with Mass Spectrometry (GC-MS) both in Single Ion Monitoring (SIM) and Scan monitoring.

The clean-up process was very important because samples were very complex so the removal of the interfering components was required. Different Solid Phase Extraction (SPE) products were tested both in terms of sorbent characteristics (melamine specific, Reversed, Phase as High Capacity C18, C18 Endcapped and Ion Exchange Sorbent (SCX)) and of sorbent mass. Several Mobile Phases and analytical columns were tested and the optimal chromatographic separation was achieved by using SCX column with both water (H_2O) and Acetonitrile (ACN) modified with 2.5mM ammonium acetate.

The presented method was validated by testing linearity, recovery, limit of detection (LOD) and Limit Of Quantification (LOQ), selectivity, precision and accuracy. Signal response was linear from 0.1 µg/g to 8 µg/g with an r^2 0.9992 and the recovery range for three concentrations is between 67 and 104%.

P2. DETERMINATION OF LIPOPHILIC MARINE BIOTOXINS IN OFFICIAL CONTROL OF LAZIO AND TUSCANY REGIONS BY LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

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Consumption of shellfish contaminated with marine biotoxins can cause severe intoxications in humans such as Diarrhetic Shellfish Poisoning (DSP). In order to protect public health, EU regulatory limits for marine biotoxins in bivalves molluscs are established by regulation (EC) No 853/2004 and regulation (EU) 786/2013.

Historically, the mouse bioassay (MBA) and the rat bioassay (RBA) were the official methods for the control of shellfish on the presence of lipophilic marine biotoxins. As these methods were considered not appropriate for this purpose due to lack of detection capability and specificity, besides being perceived as unethical, in 2011 the European Commission decided to replace MBA and RBA methods with LC-MS/MS as EU reference method: it is mandatory from January 2015.

According to the European Union Reference Laboratoy for Marine Biotoxins (EURLMB) guidelines, a LC-MS/MS method for the separation and detection of marine lipophilic toxins, including Okadaic Acid (OA) and its esters, dinophysistoxins (DTX-1, DTX-2) and their esters, azaspiracids (AZA-1, AZA-2, AZA-3), pectenotoxins (PTX-1, PTX-2) and yessotoxins (YTX, hYTX, 45-OH-YTX, 45-OH-h-YTX), has been validated on different molluscal shellfish matrices, both fresh and cooked.

The method consists of an extraction procedure with methanol, followed by LC separation carried out with acidic mobile phase for AZA and PTX groups and alkaline mobile phase for OA, DTX and YTX groups and tandem mass spectrometry detection in positive and negative ion mode respectively.

Different purification procedures (LLE, SPE) on sample extracts are in progress in order to reduce ion suppression effects due to matrix complexity.

The method is currently used for official control program and it has been included in the scope of the accreditation according to ISO/IEC 17025 criteria.

Since 2014, Chemistry Departement of Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri has analysed over 350 samples of molluscal shellfish coming from primary production, end production, markets, restaurants and self-checks all over Lazio and Tuscany. Only in a few samples from Latina and Anzio, levels of Okadaic Acid above 40 µg kg^{-1} and levels of YTX group above 120 mg kg^{-1} were found.

P3. MULTI-ANALYTE HPLC COUPLED TO HRMS METHOD FOR DETECTION OF TROPANE AND PYRROLIZIDINE ALKALOIDS IN HONEY

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In June 2015 the European Commission issued the Recommendation (EU) 2015/976 on the monitoring of the presence of Tropane Alkaloids (TAs) in food and in February 2016 the Commission Regulation (EU) 2016/239 amending Regulation (EC) 1881/2006 as regards maximum levels of TAs in certain cereal-based foods for infants and young children. Analytical data on occurrence of Pyrrolizidine Alkaloids (PAs) in honey provided to EFSA in recent years led to an external scientific report in 2015. TAs and PAs are natural toxins produced by plants. PAs are known to be present in more than 6,000 plant species and over 350 PAs have been identified. The acute disease is associated with high mortality, and a subacute/chronic onset may lead to liver cirrhosis, affecting livestock, wildlife and humans. TAs are present in more than 3,000 plants, comprise 200 compounds but the only data available concerning the occurrence in food regard scopolamine and atropine. TAs cause symptoms like tachycardia, decreased gastrointestinal motility but they are not bioaccumulative and do not exhibit chronic toxicity. There is a growing interest in natural toxins in recent years, due to their potential hazard for human and animal health related to their presence in food and feed. A modified QuEChERS sample treatment and Hybrid Quadrupole-Orbitrap Mass Spectrometer were used for the determination of the alkaloids in honey samples. The following alkaloids were investigated: echimidine, heliotrine, intermedine, lasiocarpine, lycopsamine, retrorsine, senecionine, seneciphylline and senkirkine, for PAs, and atropine and scopolamine for TAs. We used senecionine-D_3 and atropine-D_3 as internal standard for PAs and TAs, respectively. The developed method was validated according with Commission Regulation (EC) n. 333/2007. The accuracy and precision of the assay were measured at 2, 5 and 10 μg kg^{-1}, the percentage recoveries were in the range of 92.3-114.8% with repeatability (RSD%) in the range of 0.9-15.1 and the reproducibility (RSD%) in the range of 1.1-15.6. LOD and LOQ were determined using a matrix matched calibration curve and ranged from 0.5 to 1.4 μg kg^{-1} and from 0.9 to 2.5 μg kg^{-1} respectively. This work provides a fast, simple, selective and sensitive method for the simultaneous detection and quantification of nine PAs and two TAs in a complex food matrix as honey.

P4. QUANTITATIVE DETERMINATION OF NATURAL HORMONES IN BOVINE BLOOD SERUM BY LC-MS/MS: METHOD DEVELOPMENT AND VALIDATION

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Directive 96/23 EC has banned in EU hormones and other anabolic substances in livestock. Therefore, Member States are requested to include the analyses for these growth promoters in their Official Monitoring Plans. In Italy, D.M. 14/11/1996 has fixed Maximum Physiological Limits (LM) for natural hormones in bovine blood serum, taking into account sex and age. Hence, natural hormones blood concentrations above those LM suggest an illicit treatment ongoing, and causes destruction of not compliant animals.

Because of the strong legal impact involved in such a kind of investigation for the official controls in Food Safety, we developed a rapid and very effective procedure for quantitative determination of testosterone, progesterone and 17β-estradiol in bovine blood serum, according to Commission Decision 2002/657/EC criteria. During method development a range of extraction solvents, clean up procedures and LC-MS/MS conditions were tested. Matrix effect was also evaluated and its correlation with ionization conditions was tested.

The final procedure involved sample extraction with 2 x 5 ml of tert-butyl methyl ether, centrifugation and supernatant evaporation to dryness; for testosterone and progesterone, residue was dissolved with methanol/water (50/50 v/v) and directly analyzed; for 17β-estradiol, a clean-up and concentration step on SPE NH_2 cartridge before LC-MS analysis was required.

The analytes were detected using a LC-MS/MS (QQQ) equipped with a H-ESI, operating in positive and negative multiple reaction monitoring (MRM) mode.

The separation of analytes was performed on a Phenomenex Synergy Max RP80 column (150x2 mm, 4 μm), with gradient elution. As internal standards 17β-estradiol-d4, 17β-testosterone-d3 and progesterone-d9 were used.

The method was validated as a quantitative confirmatory method, according to Commission Decision 2002/657/EC, taking into account the following parameters: specificity, linearity, CCα, CCβ, stability, within-laboratory reproducibility, trueness, ruggedness.

For validation experiments, different blank blood serum samples were spiked with the analytes (females < 6 months age for testosterone, males < 6 months age for progesterone and estradiol) at different concentration levels, to include all the LM values. Results fell within the requisites expressed by the legislation.

The method is able to detect residues in serum between 0.020-0.080 μg/L for 17β-estradiol, between 5-45 μg/L for testosterone and between 0.5-20 μg/L for progesterone.

P5. DEVELOPMENT OF LC-HRMS METHOD AND STABILITY STUDY OF BETA-AGONIST RESIDUES IN BOVINE HAIR

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The aim of the present work was the development of a fast and reliable analytical multiresidue method for confirmation 20 β-agonist compounds in animal hair. It was based on alkaline digestion, LLE with organic solvents, SPE clean up and Liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis.

Validation was performed according to Commission Decision 2002/657/EC requirements. Independent samples spiked with the investigated compounds in the range 0.2-10.0 µg kg-1 are showing intra-day and inter-day precision (RSD) lower than 16.2% and 19.8%, respectively. Drugs linearity in the range of 0.1-10.0 µg kg-1, resulted with r >0.996. The decision limits (CCα) for the all investigated beta agonists resulted in the range of 0.1-1.5 µg kg-1.

The proposed method was tested on real hair samples obtained from cattle, resulted positive to clenbuterol, to check the stability of β-agonists and the effectiveness of the method. This method is suitable for the public official control of β-agonist residues in hair sample.

P6. CONTAMINANTS IN PAPER AND BOARD FOR FOOD CONTACT. GC-MS SCREENING ANALYSIS AND IDENTIFICATION OF RESIDUES FROM RECYCLED FIBERS

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The use of Recycled Paper and Boards (RPB)is a valuable source of environmental sustainability. However the use of RPB for food contact packagings paper requires other considerations, concerning food safety and, of course consumer safety. In fact, potential residues from previous use of the paper/board could survive the recycling process and potentially migrate into the food, when the P&B is used in food packaging.

Therefore, a screening analysis on the P&B packaging is suitable to identify potential organic migrants and the eventual illegal use of RPB in fields where it is not allowed (eg. pizza boxes, etc). The paper presents a GC-MS screening method developed to identify the residual contaminants in P&B food packaging, to map the most frequently found substances and to suggest a set of markers for RPB that could be used in a survey.

The method is based on solvent extraction of the paper and GC-MS analysis. Different solvents (acetone, isooctane, dichloromethane, acetonitrile) were used, alone or in combination depending on the analytes. The extracts are analysed by GC-MS, run in Scan mode. From the Total Ion Chromatograms the peaks of the extracted substances are identified and qualified by distinction between components of the cellulose based packaging or extraneous substances. Single Ion Monitoring (SIM) analysis on selected fragments may be performed to quantify the substances. The method was tested in real samples randomly collected and is suitable to identify residues from glues (eg phthalates) and inks (benzophenones, aliphatic and aromatic hydrocarbons) derived from previous uses of the P&B.

P7. IDENTIFICATION AND QUANTIFICATION OF MIGRANTS FROM PLASTIC PACKAGINGS FOR FOOD CONTACT: GC-MS APPLICATIONS

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Plastic food contact materials and articles are manufactured using polymers and additives, according to Good Manufacturing Practice and specific EU rules (Reg. EU 10/2011).

These Intentionally Added Substances (IAS) are regulated and very often there are Specific Migration Limits to be respected into the foods. A number of possibilities exist that other substances are present in the final food contact material and article, obtained from side reactions or as by products, or impurities, that is, Non Intentionally Added Substances (NIAS). These substances, whether migrateable, might endanger human health, depending on their toxicological profile, the migration amount, the food into contact etc.

Therefore, the characterization of IAS and NIAS is one of the fundamental step in risk assessment for food safety either done by the business operators or by the enforcement authorities. In view of this, the paper presents applications of GC-MS based methods developed to identify and quantify migrated substances (IAS and/or NIAS) from plastic food packagings. In migration tests, plastic samples are put into contact with food simulating solutions (liquid or solid) for predefined time and temperatures, then the migrants are extracted from the migration solutions (by liquid/liquid, SPE extraction or ASE extraction) and the extracts are analysed by GC-MS, run in scan mode. From the TIC the peaks with at least S/N>3 are considered and identified as most probable substances. From the characteristic fragments a quantitative determination may be performed in SIM mode by using the selected fragments with external multilevel calibration with respect to a target substances.

Applications are presented concerning a) migration of a phenolic derivative from plastic film for food packaging, b) migration of an antioxidant from monolayer rigid plastic packaging, c) migration of IAS and NIAS into solid simulant into contact with plastic monolayer.

P8. GC-MS ANALYSIS OF 1,2-AND 1,4 ISOMERS OF BENZEN DICARBOXYLIC ACID, DI-2 ETHYLHEXYL ESTERS USED AS PLASTICIZERS FOR PVC INTO CONTACT WITH FOODS

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Esters of 1,2-benzendicarboxylic acid, with C2-C10 linear and/or branched alkyls are used from a long time to soften plastics, mainly PVC and rubber articles, into contact with foods.

They are commonly known as the family of phthalate esters. Among them 1,2-benzendicarboxylic acid, di-2 ethylhexyl ester (CAS 117-81-7), di-2-(ethylhexyl)phthalate, (DEHP) was the most used for decades due to its advantageous technological performance. In the mixtures of phthalic plasticizers DEHP is often present together with minor amount of its linear isomer Di-N Octyl Phthalate, (DNOP). Toxicological studies revealed adverse effects on human health and along with the increasing knowledge on toxicity of DEHP and in general of phthalate family, its use was progressively restricted and/or limited, to prevent consumer exposure. (e.g. not for articles in contact with baby foods, not for oily contact, etc. Reg EU/10/11).

To make available alternative plasticizers, chemical industry introduced a new member in the phthalate family: 1,4 benzen dicarboxylic acid, di-2 ethylhexyl ester.

The substance is structurally related to DEHP, but is indeed its 1,4- isomer, namely di-2-(ethylhexyl)terephthalate (CAS 6422-86-2 DEHTP). The EFSA risk assessment of the substance allowed DEHTP to be introduced in the EU list of the additives authorized for food contact plastics, without specific limitations. Therefore the analytical differentiation DEHP/ DEHTP is crucial to confirm compliance or to highlight illegality and potential risk for consumer health. The paper presents a GC-MS method developed to identify and quantify DEHP and DEHTP extracted from plastic matrices. The extraction is carried out by repeated solvent dissolution/ precipitation steps. The extracts are GC-MS analysed in scan mode. From the TIC (m/z 45/450) the characteristic fragments are identified and the quantitative determination is performed in SIM mode with external multilevel calibration. 3 selected ions (DEHP: 149-167-279, DEHTP: 149-70-261) have been assigned to identify fragmentation of DEHP and DEHTP.

The method was developed on a HP6890 GC coupled with a HP5973 MSD. Separation of phthalate isomers was performed with Rtx 5MS 30m × 0.25mm df 0.5 0 µm. It is worth to note that there is the analytical risk to mislead DEHTP for DNOP because the technical mixtures containing DEHP very often contain other phthalates.

P9. EVALUATION OF PESTICIDE RESIDUES AND MYCOTOXINS IN DRIED HERBS, TEA AND SPICES BY LC-MS/MS, GC-MS/MS AND HPLC-FLUORESCENCE

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The role of medicinal plants in diet and in lifestyle has become more and more important due to the occurrence of different conditions. Several opportunities, in recent years, have promoted the development of new agricultural enterprises operating in this sector. The increase of the amount of productions of herbs are in line with consumer preferences. Consumers prefer to get herbalist's shop preparations, but also industrial, based on natural active ingredients, extracted from herbs obtained without resorting to the use of synthetic chemical products. Although the field of medicinal plants (aromatic, medicinal, seasonings, perfume) is considered a minor crop, considering that the cultivated species have increased by about ten times, should not be underestimated the problem linked to the recruitment of active substances in diet.

50 samples of different herbs were collected from different sites as markets or herbalist's shops. The samples had different provenances (European and NO-EU) and different productions (natural harvest, industrial production or biological cultivation). The samples were analyzed for the determination of mycotoxins (Aflatoxins and Ocratoxin A) and pesticides residues.

SPE immunoaffinity columns and HPLC equipped with FLD detector were used for the determination of the amount of mycotoxins. The Limits Of Quantitation (LOQ) were set at 0,375 mg/kg for aflatoxins and 1,0 mg/kg for OTA.

Determination of pesticides residues was performed by QuEChERS extraction and clean up method following a GC-MS/MS (QqQ) and LC-MS/MS analysis.

GC-MS/MS analysis was performed in MRM mode using MassHunter software; LC-MS/MS analysis was performed in MRM positive mode. The whole method includes 283 molecules (216 by GC and 67 by LC). Limit Of Quantification (LOQ) was set at 0,01 mg/kg. The methods were validated and accredited according to ISO 17025 and SANTE Document.

P10. DEVELOPMENT AND VALIDATION OF LC-MS/MS METHODS TO ASSESS CONCENTRATIONS AND RATIOS OF SPHINGOID BASES AND THEIR PHOSPHATES AS FUMONISIN BIOMARKERS OF EFFECT IN PIGS: THE ROLE OF SAMPLE COLLECTION TECHNIQUES

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Fumonisins are potent inhibitors of ceramide synthase, an enzyme involved in the sphingolipid metabolism. As a consequence of the ceramide synthase inhibition, the concentrations of the free sphingoid bases Sphinganine (Sa) and - to a lesser extent - also Sphingosine (So) increases. The Sa/So ratio can be used as a blood biomarker of effect to assess fumonisin exposure in pigs. Recent studies in pigs revealed that also the concentration of Sphinganine-1-Phosphate (Sa-1-P) in blood increases upon exposure to fumonisins. Red blood cells and platelets lack some of the enzymes necessary for dephosphorylation and metabolism of sphingoid base-1-phosphates, allowing them to effectively store these bioactive lipids.

The collection of the biological fluids is one of the key points during the design and development of animal trials. Invasive needle procedures are unavoidable to obtain volumes of blood or serum that are greater than a few hundred microliters. However, blood can also be obtained by minimally invasive sampling using e.g. Whatman® protein saver cards or Mitra™ microsampling devices (Neoteryx). The aim of this study is the development and validation of LC-MS/MS based methods for the quantification of Sa, So, Sa-1-P and So-1-P in whole blood of pigs collected by the two above mentioned devices. The method performance was evaluated by assessing the apparent recovery (R_A), the Signal Suppression/Enhancement (SSE), the recovery of the extraction step (R_E), limits of detection and quantification, and repeatability.

Our results showed negligible SSE for So and Sa. The R_E's were higher for Mitra™ than for protein cards, resulting in higher R_A's for Mitra™ than for protein cards. For Sa-1-P and So-1-P severe matrix enhancement was observed. The R_E was approximately 40% and the R_A's resulted to be between 118% and 180%. In addition to the unsatisfactory R_A the analysis of the phosphates was accompanied by further analytical difficulties: Sa-1-P is not readily soluble in aqueous or organic solvents, and the preparation of the respective standard solutions is tricky.

Furthermore, during the LC-MS/MS analysis, So-1-P and Sa-1-P were susceptible to carry-over. From a biological point of view, the Sa-1-P/So-1-P ratio is a better biomarker of effect than Sa/So ratio for assessing fumonisin exposure in whole blood. The complexity of the analytes makes the use of this biomarker not fitting for purpose, though. Nevertheless protein cards and Mitra™ microsampling devices are suitable to collect, store and analyze animal blood in order to assess the Sa/So ratio as a biomarker of effect for fumonisin exposure.

P11. CONTROL ACTIVITIES FOR PESTICIDES RESIDUES ON FOOD FROM NON-EU COUNTRIES

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The food control from non-EU areas are part of our laboratory control activities. In the past two years about 400 samples, from the third zone, were analyzed for the presence of pesticide residues. About 6% has been rejected as not in compliance with standards set for at least one pesticide residue.

Sixty-two percent of the positive samples are from the South East Asia and even if is not possible to establish a prevalence in the types of residue is observed that the classes are more easily found was organophosphorus and triazoles.

The analyzed matrix ranges from infants products to formulas derived from the cereals milling, berries and exotic fruit, from honey to the preserves. The most frequently analyzed matrices are the stone fruits, fresh herbs and exotic fruit.

Matrices that most frequently showed non-compliance were gotu kola and chili peppers with residues of profenophos.

The question that raises concern in these samples is the presence of pesticides with concentration above the LOQ and the co-presence, also in positive samples, of pesticides below their MRLs.

The current legislation has led certainly to a greater examination of active substances used in formulations and to more stringent controls on the correct use of pesticides in agriculture. However European and national control plans of phytosanitary residues in food don't devote proper attention to the multi-residue phenomenon and to its possible impact on consumer health. This excluding the evaluation of synergistic effects that may result from the concomitant presence of multiple chemical residues, even at low concentrations and within the legal limits.

P12. SEMI-UNTARGETED *VS* SEMI-TARGETED APPROACH FOR THE DETERMINATION OF MORE THAN 400 COMPOUNDS WITH HIGH RESOLUTION MASS SPECTROMETRY IN FOOD MATRICES

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The enormous evolution of High-Resolution Mass Spectrometry (HRMS) has permitted to overcome the traditional targeted analysis applying new analytical strategies such as untargeted and semi-untargeted ones. The untargeted approach is highly supported to promote the HRMS technique; however, it has been scarcely applied in targeted compound analysis because the association of an accurate *m/z* value to a unique compound is a very challenging goal. For these reasons, in the last years, the semi-untargeted approach is spreading more than untargeted one. The semi-untargeted analysis uses a database only for searching the substances present within it and, possibly, confirm them by MS/MS experiments. As well known, one of the main problems related to semi-untargeted analysis is its lack of selectivity. In fact, because of different chromatographic conditions and LC equipments applied in the different laboratories, the retention times are not generally set, producing difficult and time-consuming interpretation of results.

The approach here proposed is based on a custom database, in which three different groups of substances have been included: veterinary drugs (banned and authorized), mycotoxins and pesticides by more than 400 compounds. To enhance selectivity, for each compound, the database considers the *m/z* ion and, in addition, its retention time. In case of a suspect peak in a real sample, the MS/MS spectrum is compared to confirm the identity of the found substance applying targeted data depending experiment (semi-targeted approach). The database has been built on a UHPLC-Q-Exactive (Thermo Fisher Scientific) platform. A standard LC gradient (0.1% formic acid and methanol) has been developed allowing a fast transferability of database parameters to another LC column/system, just injecting few probe compounds. Another critical point is the sample preparation: a generic extraction procedure has been developed able to recover the considered analytes in several food of vegetable and animal origin.

The complete procedure, extraction and result interpretation, is performed on a series of "blind" spiked and proficiency test materials comparing semi-untargeted to semi-targeted approach. The results obtained showed how the use of semi-targeted approach reduces drastically the numbers of false suspect positive results (-90%) and, above all, reduces the number of false negative results (-60%). The ambitious final aim is the possible decreasing and rationalization of the dozens of different screening protocols applied

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P13. SCREENING OF SYNTHETIC PDE-5 INHIBITORS IN FOOD SUPPLEMENTS BY LC-MS/MS

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Sildenafil citrate (Viagra, Pfizer), Vardenafil hydrochloride (Levitra, Bayer) and Tadalafil (Cialis, Elli Lilly), are prescription drugs used to treat erectile dysfunction because they are strong inhibitors of the cGMP-specific phosphodiesterase type 5 enzyme (PDE5). The adverse effects of these drugs are well documented. Among others they are particularly dangerous for people with diabetes, hypertension or ischemic heart disease, conditions often associated with erectile dysfunction; such patients may look to herbal products as an alternative treatment. As a consequence of the great commercial success of these over-the-counter alternative treatments some manufacturers added synthetic PDE-5 inhibitors to their herbal products without listing them on the label, causing a serious health threat. Several cases of adulteration of herbal products with Sildenafil, Vardenafil and Tadalafil have been reported over the years. In order to avoid the detection of the adulterants some manufacturers started to counterfeit their products with analogues of the well-known prescription drugs; such compounds are not detected by targeted analytical methodologies. With the use of NMR spectrometry one analogue of Sildenafil, also known as homosildenafil was founded in a beverage. In the following years several other sildenafil analogues were isolated from oral capsules advertised as herbal alternatives for sildenafil, they were found and identified by several techniques.

The aim of this work was the development of a screening method for the detection of inhibitors of the PDE-5 in herbal samples marketed for erectile dysfunction. The method was performed using Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS). Mass spectrometry was performed in the precursor ion scan acquisition mode, operating in the positive mode using ESI source. This was possible because all compounds, inhibitors of PDE-5, are structurally modified from Sildenafil, Vardenafil and Tadalafil; so they have similar structural fragments. This method was used on real samples of dietary supplements and is valid for analysis of counterfeit natural products.

P14. DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF AMINOGLYCOSIDE AND POLYPEPTIDE ANTIBIOTICS IN FOOD: PRELIMINARY RESULTS

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Antibiotics are the most used veterinary drugs and they are authorized to treat or prevent animal diseases but they can also be illegally administered as growth promoters. For authorized substances the EU has set Maximum Residue Limits (MRLs) for residues in food and in target tissues. Aminoglycosides belong to a group of antibiotics used to treat Gram-negative bacterial infections and they are basic and highly polar compounds containing several amino groups with different pK_a values. Polypeptide antibiotics are a chemically diverse class of antibiotics containing non-protein polypeptide chains. The compounds included in the study were amikacin, apramycin, dihydrostreptomycin, gentamycin, kanamycin, neomycin, paromomycin, spectinomycin and streptomycin (aminoglycosides), and bacitracin and colistin (polypeptides).

The chromatographic separation of these veterinary drugs can be performed with reversed-phase columns, using ion-pairing agents to achieve suitable retention. However, when mass spectrometry is used as the detection technique, ion-pairing reagents can cause ion suppression and contaminate the LC-MS equipment. In this scenario, HILIC (Hydrophilic interaction liquid chromatography) has been receiving increased attention and several methods based on this quite new chromatography approach have been proposed so far.

For the present study, during the development of chromatographic conditions, three LC columns for HILIC applications have been tested: 1) Thermo Hypercarb (100x2.1 mm, 5 µm); 2) Thermo Synchronis Hilic (100x2.1 mm, 5 µm); 3) Agilent Poroshell 120 HILIC (100x2.1 mm, 2.7 µm). Thermo Hypercarb is a stationary phase composed by 100% of porous graphitic carbon and (even though it is not a "pure" HILIC phase) it is highly suggested for the separation of very polar compounds. Unfortunately it did not retain more polar analyte, spectinomycin, and, on the other hand, more apolar, neomycin, did not elute within usable run time. Testing Thermo Synchronis (zwitterionic phase), neomycin was highly retained with unacceptable peak shape. Finally, Agilent Poroshell (non bonded silica) gave satisfactory results using acetonitrile and formic acid (0.5%) as mobile phases. Experiments are in progress to optimize mass spectrometric detection.

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P15. RAPID DETERMINATION OF TETRACYCLINES AND THEIR METABOLITES IN SHEEP MILK BY LIQUID CHROMATOGRAPHY COUPLED TO ORBITRAP HIGH RESOLUTION MASS SPECTROMETRY (HRMS)

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In Sardinia (Italy), the sheep industry is based on a local breed of dairy ewes. The island is one of the most important EU regions for sheep dairy production, with more than 3.5 million animals bred (3.7% of the EU total in 2009) that totally produce more than 300,000 MT of milk. This quantity corresponds to about 4% of total world production and the sheep milk is processed into different types of cheese.

Antibiotics are widely used on dairy small ruminant livestock to treat mastitis and other infectious diseases; tetracyclines (TCs) account for 39 % of total prescriptions of veterinary drugs in Sardinia, and in 98% of cases oxytetracycline (OTC) is used.

This paper describes the development and validation of ultrahigh performance liquid chromatography Orbitrap mass spectrometry (UHP-Q-Orbitrap) method for the rapid and simultaneous determination of 8 antibiotic residues in sheep's milk: 4-epioxytetracycline (4EOTC), oxytetracycline (OTC), 4-epitetracycline (4ETC), tetracycline (TC), 4-epichlortetracycline (4ECTC), chlortetracycline (CTC), 4-epidoxycycline (4EDC), doxycycline (DC). Milk samples were extracted using MCIlvaine buffer solution (pH = 4) and purified with solid- phase extraction (SPE) Oasis HLB cartridge. The method was optimized and validated for the complete analysis of TCs and their metabolites within 8 min, each compound was validated at least at the concentrations 0.5, 1.0 and 1.5 MRL (Maximum Residue Limit). The calculated relevant validation parameters, e.g. the decision limit CCα, the detection capability CCβ, the repeatability, the within-laboratory reproducibility and the recovery, are in an acceptable range and in compliance with the requirements of Commission Decision 2002/657/EC. The method is rapid, sensitive, convenient and robust, and can be used to simultaneously confirm residues of tetracyclines and their metabolites in milk.

P16. MONITORING OF ENVIRONMENTAL CONTAMINANTS IN BREAST MILK OF THE LAZIO REGION

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Exposure to persistent organic contaminants of anthropogenic origin, direct and indirect, diffused into the environment, leading to chronic toxicity due to extremely low dose taken daily from the diet. The main effects of the substances of the study are carcinogenicity, genotoxicity, mutagenicity, teratogenicity. Finally yet importantly and perhaps, the most insidious are the effects of contaminants such as dioxins and PCBs dioxin like that mimic the activity of endogenous substances of organisms. Doses taken by infants and young children through breast milk have a negative impact certainly different from that of an adult, for that purpose and contrast the benefits of lactation and breast milk.

The study linked the concentration levels of pollutants in human milk in the provinces of Rome, Rieti, Viterbo, Latina and Frosinone, with the eating habits and the residence of the donor mothers. The results obtained are compared with other Italian and European studies. Breast milk samples (n=81) issued from primi and multiparae mothers were collected between 2013 and 2015 in different birth centers of the Lazio region and assisted in the Bambino Gesù hospital in Rome. The samples were individually subjected to the analysis of PCDD, PCDF and PCB-dl by HRGC-HRMS.

Statistical evaluation of the data shows no correlation (p value <0.05) between the levels of contamination of milk and the variables considered, as also confirmed by literature. These results show that the levels of dioxins found in the Lazio's region are lower than of 2008 for the region Campania (8.47 WHO-TEQ_{1998} pg/g on fat basis, and lower than recorded values for the city of Rome between 1998 and 2001 (WHO-TEQ_{1998} PCDD/F of 9.40 pg/g on fat basis).

In conclusion, the present study shows the homogeneity of the concentration levels of PCDD/F and PCBs-DL in human milk for the provinces of Lazio, with the exception of the province of Rome, which shows higher values. The standard profile of dioxin congeners is in agreement with all the provinces of Lazio and found a correlation of 99% with the human milk profile analyzed in Ireland in 2010. The PCDD/Fs average value of 2.76 pg WHO-TEQ_{1998}/g on fat basis in milk of women living in the Lazio region is significantly inferior to the data reported in the literature for other European countries, while remaining high compared to the European standard limits for baby foods.

P17. DEVELOPMENT OF A LC-MS/MS CONFIRMATORY METHOD FOR THE DETERMINATION OF 9 THYREOSTATS IN MUSCLE AND THYROID TISSUE USING A QUECHERS APPROACH

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Administration of thyreostats to livestock causes an increase in the live weight of animals prior to slaughter due to higher retention of water in the edible tissue and the gastro-intestinal tract. Meat derived from treated animals is therefore of lower quality, but may also contain harmful residues. According to the International Agency for Research of Cancer (IARC), some thyreostats possess carcinogenic and teratogenic properties. For this reason, their use for animal fattening is banned in the European Union since 1981. However, the control of their illegal use in breeding animals is particularly difficult because of their low molecular weight, high polarity and existence of tautomeric forms.

With the present study, we optimised and validated a robust method for the routine determination and confirmation of nine thyreostatic drugs in muscle and thyroid gland samples using liquid chromatography tandem mass spectrometry. The optimized procedure involves the QuEChERS approach: residues were first extracted with acetonitrile and partitioning salts (sodium chloride, magnesium sulphate, sodium citrate dihydrate and sodium hydrogencitrate sesquihydrate), the extracts were then purified by dispersive SPE. A new clean-up sorbent, Supel^{TM} QuE Z-Sep^{+}/$MgSO_4$, was used and proved to be effective for interference removal from muscle and thyroid extracts. After purification, the compounds were derivatized with 3-iodobenzylbromide in basic medium (pH 8) in order to improve retention and separation on the reserved-phase support and to achieve higher sensitivity of the measurements and lower detection limits. Thyreostatics were determined as 3-IBBr derivatives using conventional HPLC columns and instrumentation and for quantification purposes, three deuterated internal standards, tapazole-d3, propylthiouracil-d5 and mercaptobenzimidazole-d4 were tested.

The method was found to be effective for the determination of tapazole, thiouracil, methylthiouracil, dimethylthiouracil, ethylthiouracil, propylthiouracyl, phenylthiouracil, mercaptobenzimidazole, benzylthiouracyl, dimethylthiouracil. Validation of the confirmative quantitative method was carried out according to Commission Decision 2002/657/EC. For all compounds, decision limit (CC_α) and detection capability (CC_β) were found to be below the recommended value set at 10 µg kg^{-1} by the Community of Reference Laboratories (CRLs). The results obtained from the validation were highly satisfactory and fulfilled all requirements for confirmatory quantitative purposes.

P18. ANALYSIS OF PHYTOESTROGENS BY GC-MS/MS: STUDY OF THE INSTRUMENTAL CONDITIONS AND DERIVATIZATION PROCEDURE

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Phytoestrogens are a class of natural nonsteroidal compounds, widespread in the plant kingdom. The chemical structure of some phytoestrogens resembles that of natural estrogens and it has been demonstrated that they can interact with the endocrine system of animals and humans, through a range of mechanisms, thus acting as Endocrine Disrupting Chemicals (EDCs). The major sources of phytoestrogens are red clover, legumes, licorice, hop and, above all, soy. Since the attribution of beneficial or detrimental effects on humans is still controversial, it is important to determine phytoestrogens content in food and environmental matrices, in order to evaluate the possible intake of these compounds.

Analysis of phytoestrogens is usually performed by chromatographic techniques coupled with different detectors. Liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) is the most employed system when low detection limits are required. Only a few applications of Gas Chromatography (GC) are found, due to the required derivatization step. Nevertheless, GC-MS technique remains widespread and less expensive in comparison to HPLC-MS/MS.

In our laboratory, a systematic study has been carried out to find the best instrumental conditions for a GC-MS/MS method for the determination of five phytoestrogens, after derivatization. The compounds under consideration were the isoflavones Biochanin A, Formononetin, Genistein and Daidzein and the coumestan Coumestrol. The instrument used was a GC coupled with an Ion Trap mass spectrometer, that allows to perform tandem mass spectrometry experiments.

The derivatization procedure has been studied, testing different derivatization reagents and conditions. The following sylilating reagents were tested: *N,O-bis*-(trimethylsilyl) trifluoroacetamide (BSTFA), *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MtBSTFA) and *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA). BSTFA was chosen as the best derivatization substance and an experimental design was carried out to evaluate possible interactions between variables involved in the process. Various instrumental conditions were tested to optimize the sensitivity of the GC-ion trap system. First of all, mass spectrometric detection and tandem mass spectrometric detection were compared, and, as expected, the highest sensitivity was reached with the MS/MS method. Then, two instrumental parameters were evaluated: the effect of ion source temperature and the influence of different values of collision energy on the fragmentation and signal intensity of the five compounds. A source temperature of 250°C and optimal values of collision energies for each analyte were selected for the GC-MS/MS method.

P19. DETERMINATION OF TWENTY-ONE CYANOTOXINS IN ITALIAN DRINKING WATER CHAIN BY LC-MS/MS ANALYSIS

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The cyanotoxins are a group of natural toxins different from both chemical and toxicological point of view, responsible for acute and chronic poisoning in animals and humans. These compounds might be produced by certain genera of cyanobacteria, a group of prokaryotic organisms that occur worldwide in natural and artificial water bodies under different climatic conditions.

This study describes a developed LC-MS/MS method used as analytical approach to the management of a proliferation of *Planktothrix rubescens*, a cyanobacterium responsible of cyanotoxins production, in Vico Lake (Lazio Region, Italy). The method was applied within a monitoring plan conducted in the period 2010-2013 in Vico Lake and the whole drinking water chain of the municipality of Caprarola (Viterbo Province). To analyze cyanotoxins in water sample, an analytical protocol based on solid phase extraction followed by detection with liquid chromatography-tandem mass spectrometry has been employed. Several new cyanotoxins classes are recently available as certified standards, so a previous method (1) was upgraded, optimizing it in terms of instrumental response, field of application and extraction efficiency for these analytes. In particular, the number of target cyanotoxins was enlarged, including Anabaenopeptin A, Anabaenopeptine B, CYP 1007, CYP 1041, Microginin 527, Microginin 690, Microginin 704, Microginin 527 methyl ester, Microginin 690 methyl ester, MC-HtyR, MC-HilR and MC-WR. A good linearity was achieved, with correlation coefficients in the range $0.9925 \leq R^2 \leq 0.9998$. The method has been proven to be robust, precise and accurate with recovery percentages above 85% and with relative standard deviations ≤16%, fit for the intended purposes at the concentrations of interest. The LOD obtained applying the procedure SPE-LC-MS/MS were within the range 0.002 to 0.025 μg/L, at least 50-fold lower than the guideline value proposed by the WHO for drinking water (1.0 μg/L for microcystin-LR). The analytical method was then applied for a monitoring plan during a drinking water emergency occurred in Vico Lake. The systematic study of the contamination phenomenon in the drinking water chain has shown in raw water the presence of [D-Asp3]-MC-RR, MC-RR and [D-Asp3]-MC-LR respectively, at concentrations of up to 1.520, 0.100 and 0.159 μg/L. Other toxins (LW, LY, LF, HilR, WR, HtyR CYP 1007, CYP 1042, Anabaenopeptins, Ana-a and Microginins) have never been detected during the sampling period. CYN has been sporadically found. The data obtained on the treated water showed that the treatments conventionally carried out on the raw water generally prove effective in reducing almost all of the contents of the analytes in the influent water to WTP.

P20. SCREENING OF PRESERVATIVES BY HPLC-PDA-ESI/MS: A FOCUS ON BOTH ALLOWED AND RECENTLY FORBIDDEN COMPOUNDS IN THE NEW EU COSMETICS REGULATION

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Commission regulation (EU) No 358/2014 amending the new regulation (EC) No 1223/2009 on cosmetics has prohibited the use of isopropyl-, isobutyl-, phenyl-, benzyl- and pentylparaben. Furthermore, Commission regulation (EU) No 1004/2014 has lowered the maximum permitted concentration of butyl- and propylparaben in cosmetics and it has also banned them in leave-on products designed for application on the nappy area of children under three years of age.

A HPLC-PDA-ESI/MS method has been developed herein for the detection of seventeen preservatives, both the most utilised and the recently forbidden by the new EU regulations. The separation of these compounds, including benzoic acid and its derivatives in a 1.10-3.04 log P_{ow} range, has been performed with a gradient elution on a Symmetry® C18 column (250×4.6 mm i.d., particle size 5μm) with water and acetonitrile (0.1% formic acid) as mobile phase. Quantification has been carried out by HPLC-PDA.

The method has been validated and successfully applied to the analysis of a large number of cosmetics with different functions like rinse-off and leave-on, or composition like skin, hair, face and oral products and with a wide range of lipophilicity like shampoo, deodorant, hair conditioning, oral care product, lotion, sunscreens, conditioning, moisturizing and cleansing creams. It is consequently suitable for the analysis of CPs which must be accomplished by control laboratories involved in control of safety of CPs in compliance with the new regulation on cosmetic products.

P21. PHYTOCHEMICALS IN FERMENTED CEREALS TYPICAL OF SUB-SAHARAN AFRICA AND TUSCAN TRADITION: IDENTIFICATION BY HPLC-ESI-MSN AND TIME-OF-FLIGHT MASS SPECTROMETRY

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Cereal grains constitute a major source of dietary nutrients all over the world. Although cereals are deficient in some basic components (e.g. essential aminoacids), fermentation may be a simple and economical way of improving their nutritional value, sensory properties and functional qualities. In this study we determine the qualitative profile of phenolic compounds (free and bound fraction) and their content after fermentation in selected matrices: ancient grains grown in Tuscany (wheat and spelt) and varieties of millet consumed by the populations of Burkina Faso. For this purpose HPLC-DAD-MS TOF and HPLC-ESI-IT MS^n analysis were performed on the extracts of these cereals.

Fermented foods constitute a very important component of the diet of African populations, and local cereals are fermented by spontaneous processes using household methods. Hence, information about polyphenols in these cereals after fermentation could be useful for understanding the benefits associated to this process and for augmenting its consumption as sources of nutraceuticals.

Tentative identification of phenolic compounds in both free and bound fraction extracts, before and after fermentation, was performed using an optimized HPLC-DAD procedure, accurate mass measurements and comparison with literature data.

HPLC-ESI-MS^n technique has been increasingly used in the structural characterization of complex matrices and in this study was successfully applied to identify a wide of phenolic compounds, including phenolic acids, flavones and flavonols.

The results of this research indicated that fermentation can significantly increase the phenolic acid content. Also, it was observed that insoluble bound form of phenolics attached to the cell wall material of cereals is a major contributor to the total phenolic content.

P22. ANTIOXIDANT CAPACITY OF PHENOLIC COMPONENT OF DIFFERENT CULTIVAR OF TUNISIAN OLIVE LEAF EXTRACTS

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Polyphenols are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants. Plant polyphenols have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases such as cancer. In the last few years, the identification and development of phenolic compounds or extracts from different plants has become a major area of health and medical related research. In olive tree, which is one of the most important fruit tree in Mediterranean countries, studies concerning biological activity including antioxidant, antimicrobial and therapeutic properties of phenolic compounds of olive oil and olive leaves has recently been increasing.

The present study was undertaken to investigate the antioxidant capacity of phenolic component of different cultivar of olive leaf extracts (Chemlali, Sayali, Meski and Neb jmel). For the quantitation of phenols, all samples were analysed by Electro Spray Ionization tandem Mass Spectrometry (ESI-MS/MS) using a MSD Sciex Applied Biosystem API 4000 Q-Trap mass spectrometer. The LC-MS was operated in the negative ion mode using Multiple Reactions Monitoring (MRM).

The phenolic contents of different OLE cultivar was calculated based on the corresponding standard concentration-response curves. Four main groups were identified in all studied samples: oleuropeosides (oleuropein and verbascoside), flavonols (rutin), flavones (luteolin-7-O-glucoside, apigenin-7-O-glucoside and luteolin) and substituted phenols (hydroxytyrosol, vanillin, vanillic acid and coumaric acid).

Oleuropein aglycon was found in all three cultivars with low level, the concentration of this compound ranging from 4.7 to 8.9 mg/kg of dray weight. High level of oleuropein aglycon in olive extract is generally a sign of oleuropein hydrolysis. Flavonoids, another important group of compounds in olive leaves, have also been characterized in all the olive leaves extracts (OLEs). In all of the cultivars studied, apigenin-7-O-glucoside was the most abundant flavonoid.

P23. IS COFFEE SILVERSKIN ACTUALLY A SUITABLE SOURCE OF EITHER FOOD SUPPLEMENTS OR ADDITIVES? UPLC-PDA-ESI-TOF/MS METABOLIC PROFILING OF AQUEOUS EXTRACTS: NATURAL ANTIOXIDANTS *VS* PHYTOTOXINS

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In the last decades coffee has been widely recognized as an important source of antioxidants and radical scavengers, with increasing evidence supporting its health-promoting potential. Many of these benefits are ascribed to chlorogenic acids (CGAs) remarkably contained in coffee beans. A growing interest in the valorization of coffee wastes like spent coffee grounds and by-products like Silverskin (SS) is justified by their richness in polyphenols. As a consequence of the increasing consumption of Green Coffee (GC) as antioxidant-rich dietary supplement, concern has been expressed about its use as food additive or supplement. In fact, atractyligenin and its derivatives, compounds structurally related to the phytotoxin atractyloside, have been recently extracted from raw coffee and their phytotoxic activity has been evaluated. Three compounds inhibit the activity of adenine nucleotide translocase in mitochondria and among them 2-O-β-Glucopyranosylcarboxyatracyligenin (GPCA) shows the highest toxicity. A deeper knowledge about the presence of such compounds also in SS, is supported by the recent interest in the use of SS as a source of food additives or dietary supplements, beverages for body weight control and also as a new cosmetic ingredient. In this contribution, the UPLC-PDA-ESI-TOF/MS metabolic profiles of SS aqueous extracts of different coffee varieties have been determined and compared to that of GC. Atractyligenin, carboxyatractyligenin and five glucosyde derivatives were identified. In contrast to GC, where only atractyligenin glucopyranosyl derivatives were detected, in all SS also the aglycones were identified and their content was higher in Arabica than in Robusta. Compared to GC, GPCA reaches higher concentration in Arabica and comparable concentration in Robusta SS. Interestingly, the content of CGA in aqueous extracts of SS is reversed compared to atractyligenin derivatives. Moreover, an atractyligenin derivative not previously detected in GC, has been detected in GC. Concluding, the use of SS as food or dietary supplements needs careful evaluation and further and adequate controls on SS raw material are necessary in order to assess its suitability as functional ingredient for food or nutraceutical products.

P24. CHARACTERIZATION OF DIFFERENT TUSCAN HONEYS BY HPLC-DAD-TOF-MS AND HS-SPME-GC-MS TO IDENTIFY MOLECULES RESPONSIBLE FOR ANTI-DIABETIC EFFECT OF HONEY

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Despite the availability of various antidiabetic agents, recent epidemiological studies predict that at 2035 the number of people affected by diabetes will exceed 580 million and the oral hypoglycemic drugs are not enough to maintaining glycemia under strict control avoiding the onset of pathological complications. In the last years, the interest in the use of honey to ameliorating glycemic control in diabetic patients or to reduce inflammation status has grown, but the action mechanism of the compounds present in the different honeys remains to be clarified. Recent reports demonstrated that polyphenols present also in honey are able to inhibit the enzyme PTP1B, one of the main negative regulator of insulin receptor. Its pharmacological inhibition leads to increasing the insulin sensitivity. To date, it is considered one of the most interesting target to evaluate new antidiabetic drugs.

In our recent experiments several honeys from different floral and geographic origin were screened to evaluate their ability in inhibition of PTP1B. The final goal of the present study is the identification of the molecule or group of molecules responsible for the inhibition of PTP1B by the active honeys. The chemical composition of all samples was investigated by HPLC-DAD-TOF-MS and HS-SPME-GC-MS. Moreover, the more active sample was fractionated and the biological activity of the fractions was evaluated. A further step of the study will regard the characterization of honey active fractions by 1H-NRM.

For HPLC-DAD-TOF-MS analysis 5 g of samples were dissolved in H_20 (pH=3.2 for HCOOH) and extracted twice with ethyl acetate. The analysis of both organic and aqueous extracts obtained was performed by an HP Liquid Chromatograph, equipped with a Poroshell 120, EC-C18 column (i.d. 3 mm, l. 150 mm, p.s. 2.7 μm), and a DAD coupled to an ESI-TOF Mass Spectrometer (Agilent Technologies, Palo Alto, CA, USA). Different samples showed different profiles and some never detected molecules were pointed out.

For the HS-SPME-GC-MS analysis, 1 g of samples was dissolved in a aqueous supersaturated solution of NaCl. The headspace of the obtained solution was adsorbed on a DVB/CAR/PDMS Stableflex (2 cm) fiber. The separation of the molecules adsorbed by the fiber was conducted by GC; the detection of the molecules was carried out by the comparison of the MS spectra with a reference NIST 2011 library. To date, up to 80 volatile compounds were identified.

P25. REGIONAL FEATURES OF NORTHERN ITALIAN SPARKLING WINES, IDENTIFIED USING SOLID-PHASE MICRO EXTRACTION AND COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED WITH TIME-OF-FLIGHT MASS SPECTROMETRY

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We carried out comprehensive mapping of volatile compounds in 70 wines, from 48 wineries and 6 vintages, representative of the two main production areas for Italian sparkling wines, by HS-SPME-GC×GC-TOF-MS and multivariate analysis. The final scope was to describe the metabolomics space of these wines, and to verify whether the grape cultivar signature, the pedoclimatic influence of the production area, and the complex technology were measurable in the final product. The wine chromatograms provided a wealth of information, with 1695 compounds being found.

A large number of putative markers influenced by the cultivation area was observed. A subset of 196 biomarkers fully discriminated between the two types of sparkling wines investigated.

Among the new compounds, safranal and alpha-isophorone were observed. We showed how correlation-based network analysis could be used as a tool to detect the differences in compound behaviour based on external/environmental influences.

P26. MULTI-OMIC APPROACH TO UNDERSTAND RESISTANCE IN GRAPEVINE AGAINST *PLASMOPARA VITICOLA*

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Grapevine (Vitis vinifera L.), a major fruit crop worldwide, is susceptible to many microbial infections. Downy mildew is one of the most severe disease caused by the obligate biotrophic oomycete Plasmopara viticola (Berk. et Curt.) Berl. et de Toni. The current strategy for grapevine disease control is based on the extensive and expensive use of pesticides with negative impact on the environment and human health. To reduce sprayings, V. vinifera cultivars were crossed in the past with resistant Vitis spp to select resistant hybrids. Given that plant resistance and plant-pathogen interactions are complex biological processes and are up to now poorly understood, a multi 'omic' approach is most suited for this kind of studies.

The aim of the work was to correlate differentially expressed genes with the metabolites which significantly differ between the infected and non infected leaf disks at different time points. Among metabolites at present some polyphenols and lipids seems to be involved in plant-pathogen interaction and alterations in their biosynthetic pathways may be responsible for resistance phenotype against Plasmopara viticola.

In this study we focused our work on the perturbation of different classes of metabolites in leaf disks of resistant variety 'Jasmine' induced by the infection with Plasmopara viticola pathogen after 0, 12, 24, 48 and 96 hours. In targeted metabolomics approaches phenolics and lipids were determined using a LC-MS/MS methods. In order to better understand the changes in lipid profile untargeted lipidomic approach was also applied.

For these analysis LC-MS was coupled to high resolution Exactive Orbitrap mass spectrometer (Thermo Fisher). HCD fragmentation was performed with three different energies 30, 60 and 100 eV. On the same set of samples the transcript profiling (RNAsequencing) was done using the Illumina Next Generation Sequencing (NGS) technology.

The results of this study will allow us to identify signaling metabolic pathways and provide new knowledge in plant-pathogen interaction.

P27. MASS SPECTROMETRY-BASED METABOLIC SCREENING OF KALE SEEDS AND SPROUTS

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Vegetables belonging to the *Brassicaceae* family (cabbage, cauliflower, broccoli, Brussels sprouts) give health benefits for the presence of active components such as polyphenols, flavonoids, vitamins E and C and glucosinolates, all with antioxidant and anti-inflammatory properties. These molecules can be identified and quantified by mass spectrometry and recently by "Ambient Mass Spectrometry" which consists of sample analyses in its "natural state", eliminating or limiting extraction and purification steps.

The present study is aimed at identification, structural characterization and semi-quantitative analysis of polar metabolites in *Brassica oleracea* var. *acephala* (black cabbage) in seeds, sprouts and young leaves by using mass spectrometry, tandem mass spectrometry and high-resolution mass spectrometry.

The experiments have been carried out by using electrospray ionization in "ambient" conditions, without a separation system, by direct infusion of the analytes into the ion source, coupled to an ion trap and an Orbitrap analyzer for high-resolution measurements.

A pool of polar metabolites belonging to glucosinolates, hydroxycinnamic acids and galactolipids have been identified together with their variations in seeds and in different developmental stages of plantlets. Our determinations demonstrated that kale have a consistent amount of compounds with well known positive properties on health and high antioxidant activity. Sprouts in particular have higher nutritional value than mature leaves.

For this reason, regular consumption of kale sprouts should be encouraged to obtain sufficient assumption of antioxidants.

The reported experimental approach, rapid, sensitive, specific and efficient can be extended to the study of metabolome of other biological systems and it is particularly effective for evaluating variations of metabolites based on age, development, environmental stimuli and pathological states of an organism.

P28. METABOLIC FINGERPRINTING OF MYRTLE BERRIES BY LC-ESI-ORBITRAP-MS: NOVEL MARKERS FOR DISCRIMINATION OF GEOGRAPHIC ORIGIN

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An untargeted metabolomics approach followed by multivariate data analysis and phytochemical characterization, using liquid chromatography coupled to high resolution mass spectrometry (LC-ESI-Orbitrap-MS), was developed in this work for the discrimination of different cultivars of myrtle berry, whose seeds where collected from two geographic area of Sardinia (Cagliari and Sassari) and then grown in the experimental station of the University of Sassari located in Oristano (Sardinia Italy).

Aqueous methanol extracts of whole berries, peel and pulp and seeds of myrtle berries were analysed by LC-ESI-Orbitrap-MS with the aim to identify novel markers in different parts of myrtle berries and to understand which is the fruit part most influencing on the metabolomics classification of berries, based on the geographic origin of the plant.

35 compounds, mainly flavonols, flavanols, antocyanins and hydrolysable tannins, were identified or tentatively identified on the bases of their retention time, UV/Vis spectra, MS spectra and MS fragmentation patterns. 19 compounds were detected for the first time in the species of *Myrtus communis* and in particular among these identified compounds, a new class of hydrolysable tannins was detected.

Moreover, by using Multivariate Statistical Analysis, and in particular by using score scatter plot and loading plot obtained by Principal Component Analysis (PCA), predictive classification models for authenticity and geographical origin assessment were carried out, and phenolic compounds were identified as putative markers to assess the geographic origin of these berries.

P29. LC-ESI-LIT-ORBITRAP-MS/MS BASED METABOLOMICS IN ANALYSIS OF *MYRTUS COMMUNIS* L. LEAVES

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Myrtus communis L. tissues are generally used in the preparation of extracts with important pharmacological activity that is usually ascribed to essential oils, polyphenols and hydrolizable tannins. In addition leaves were used in Sardinia for the preparation of the white Myrtle liqueur, typical production of this area. Thus identification and quantitation of polyphenolic compounds in leaves of *M. communis* L. appears interesting from both a biological and ecophysiological point of view.

In the present study the discrimination of phytochemical content of *M. communis* L. leaves from different geographic origin and cultivars, was explored by Liquid Chromatography-Elettrospray-Linear Ion Trap-Orbitrap Mass Spectrometry (LC-ESI-LIT-Orbitrap-MS) metabolic profiling followed by multivariate data analysis and phytochemical characterization. Experiments were carried by collecting leaves from myrtle plants grown in an experimental area of Sardinia region, obtained by the germination of seeds taken from berries collected in each part of the region. An untargeted approach on leaves' extracts was performed by collecting LC-ESI-LIT-Orbitrap-MS data obtained by operating in negative ion mode and sending data to Principal Component Analysis (PCA). LC-ESI-LIT-Orbitrap-MS/MS experiments was performed to identification of metabolites. 39 compounds were tentatively identified on the bases of their retention time, UV/Vis spectra, MS spectra and MS fragmentation patterns. In a second step a pseudo-targeted analysis with a reduced number of variables, was realised. A data matrix was obtained by LC-MS results, by using as variables the peak area of each known compound. PCA results were compared and used to discriminate the different geographical areas of the seeds used for the cultivation. Finally an LC-DAD (Liquid Chromatography-Diode Array Detector) method was developed, validated and applied for the quantitative analysis of extracts based on 9 commercial standard compounds.

P30. BASIL PHENOLIC ACIDS IDENTIFICATION: MSPD EXTRACTION AND UHPLC-MS/MS CHARACTERIZATION

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Common basil (*Ocimum basilicum L.*), is a member of the Lamiaceae family, worldwide cultivated under a variety of ecological conditions and of widespread use in preparing, cooking and serving food. This plant is also well-known for its high content of Polyphenolic Compounds (PCs), secondary metabolites existing in a variety of structures and functions and displaying different biological properties in human health. Thanks to PCs content, basil is a digestive stimulant, moreover some antimicrobial, antibacterial, anticonvulsant and anticarcinogenic properties have been demonstrated.

Among the main classes of PCs (phenolic acids, flavonoids, stilbenes and lignans), the basil contains high levels of phenolic acids (derivatives of benzoic acid and cinnamic acid) that contribute to its strong antioxidant capacity and medicinal properties, in particular related to the considerable presence of rosmarinic acid and caffeic acid derivates.

Commonly, the recovery of phenolic acids is carried out with sequential liquid-liquid extractions followed by long purification procedures requiring specific equipments, materials and large amounts of organic solvents. More recently, a valid extraction alternative method is represented by Matrix Solid-Phase Dispersion (MSPD), that requires small quantities of matrix samples and solvents, and can be performed more rapidly.

In this work, a MSPD extraction procedure followed by UHPLC-MS/MS analysis of phenolic acids in basil samples has been optimized.

The results of this study has allowed the optimization of a MSPD method using a C18T sorbent for the isolation of phenolic acids present in basil. The model can be translated to similar matrices and could provide also a basis for further investigation of other classes of PCs. Furthermore, the use of limited sample quantities, combined with lower solvent consumption, remarks the convenience of this technique for the extraction/isolation of compounds of interest.

P31. PLANT BIOACTIVE COMPOUNDS: ANTIOXIDANT ACTIVITY AND CHEMICAL CHARACTERIZATION

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Naturally occurring Bioactive Compounds (BCs) are an extremely large family of compounds capable to interact with one or more component(s) of living tissues with the possibility of generating a wide range of effects. Often, the plant BCs consist of complex multi-component mixtures, so their extraction and their chemical and biological activity determinations need different methodological approaches (extraction techniques, purification methods, chromatographic identification of the components and antioxidant potential assays).

Among the large variety of BCs we focused our attention on those contained in Essential Oils (EOs) and water and ethanolic extracts obtained from different vegetal matrices. EOs were collected by steam distillation, while water and ethanolic extracts were produced by the Rapid Solid-Liquid Dynamic Extraction (RSLDE) technique performed with the Naviglio Extractor apparatus.

For all the EOs and RSLDE extracts were carried out: (i) the evaluation of their antioxidant activity and the estimation of the total phenolic content, and (ii) the investigation of their chemical.

The antioxidant properties were evaluated through different assays: (a) FRAP - evaluating the ferric reducing antioxidant power; (b) DPPH - measuring the scavenging abilities to 2,2′-diphenyl-1-picrylhydrazyl stable radicals; (c) ABTS - assessing the [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation inhibition; (d) Folin-Ciocalteu - estimating the total phenolic content.

EOs were chemical characterized by GC-MS, while, the RSLDE extracts were subjected to concentration and clean-up procedures, in order to be able to investigate their chemical composition by the mean of different chromatographic techniques.

P32. COMPARISON OF ANALYTICAL TECHNIQUES TO PRODUCE DATA FOR MULTIVARIATE ANALYSIS: THE CASE STUDY OF *ARBUTUS UNEDO* ANTIOXIDANT EXTRACTS WITH HPTLC AND HR LC-MS

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A combined metabolomics approach was proposed to discriminate *Arbutus unedo* (*A. unedo*) plants by comparing the merging of High-Performance Thin-Layer Chromatography (HPTLC) assay and High Resolution Liquid Chromatography Mass Spectrometry (HR-LC-MS) with Principal Component Analysis (PCA). Rapid fingerprints of *A. unedo* extracts (leaves, yellow fruit and red fruit collected in Sassari and in archipelago of La Maddalena) were obtained by using HPTLC technique. By HPTLC fingerprints, a rapid visual comparison of secondary metabolites in *A. unedo* samples from different places of collection was obtained and PCA was performed providing an overview of the capacity of the HPTLC variables to discriminate samples. HPTLC fingerprint assisted by PCA results a reliable untargeted approach for the discrimination of different samples on the basis of the tissues and of their geographical area. Similarly, same extracts were submitted to non-targeted liquid chromatography coupled to high resolution mass spectrometry (LC-ESI-FT-MS) metabolite profiling. With the aim of analyse the acquired dataset by multivariate methods, LC-MS chromatograms were pre-processed using MZmine to compensate variations in retention time and *m/z* values between the chromatographic runs. Obtained data were used through an approach of untargeted analysis and treated with an unsupervised Multi Variated Data Analysis (PCA).

All the differences between leaves, yellow fruit and red fruit of *A. unedo* from different geographical origin were highlighted. In addition, compound identification by LC-ESI-Orbitrap-MS and LC-ESI-Orbitrap-MS/MS was performed to identify which secondary metabolites contribute most to the differentiation of the samples. In conclusion our results showed that data obtained by coupling both HPTLC-PCA that HR-LC-MS-PCA are in agreement.

Thus, HPTLC could be applied as a simple and reliable technique to rapidly distinguish extracts from different tissues and/or geographical origins while HR-LC-MS could be used to identify which metabolites are responsible for the discrimination of the samples.

P33. ISOTOPIC PATTERN ANALYSIS APPLIED TO MS-BASED LABELLING EXPERIMENTS IN METABOLOMICS

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MS-based metabolomics has been gaining more and more popularity, also because of recent advances in terms of instrumentation performances. As a consequence, many are the metabolomics applications to medical and biological problems. However, this analytical field has to face some limitations and challenging issues, including the elucidation of metabolic pathways, which would require the direct measurement of metabolic fluxes. To date, most of the commonly used approaches only measure pool sizes of metabolites, from which they infer changes in flux.

Some metabolomics limitations can be overcome by using stable isotopes to label metabolites in living cells, and stable isotope tracer-based metabolomics approaches have already been established for metabolite identification, quantification, and pathway analyses. In the past, radioactive tracers were commonly used, and they were easily detected by scintillation counters. The ever-improving sensitivities in analytical techniques such as Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS), however, have made it possible to replace the dangerous radioactive tracers with stable isotopes, mainly ^{2}H and ^{13}C.

MS is particularly suitable for labelling experiments in metabolomics, because if analytes incorporate the label, their natural isotopic pattern will be affected, and therefore it will change its "shape", to a degree that depends on the label incorporation. Although many software packages have been devised to predict natural isotopic patterns, there is limited software as yet available for dealing with "labelled" experimental isotopic patterns.

We will discuss how it is possible to estimate the relative isotopic abundance of the labelling isotope inside target analytes by analyzing their experimental isotopic patterns. We will also present the informatics tool we have recently developed for tackling this important issue in biological labelling experiments, where some of the metabolites could have partly incorporated the "label", thus resulting in its unknown relative abundance within them. Our software tool will soon be made available to the scientific community as an R package.

P34. IN SILICO PREDICTION OF 4,4'-DMAR METABOLISM AND *IN VIVO* CONFIRMATION IN RATS BY HIGH RESOLUTION MASS SPECTROMETRY

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4-methyl-5-(4-methylphenyl)-4,5-dihydrooxazol-2-amine (4,4′-DMAR) is an analog of the known psychostimulants 4-methylaminorex and aminorex. In the light of reports of deaths associated with its abuse, and the easy access from Internet vendors, it has been recently added to the schedule II of 1971 Convention on Psychotropic Substances. No information is currently available about the drug's pharmacokinetic profile and its metabolic pathway.

In the present study, metabolism profilers available within OECD QSAR ToolBox Software were used at first to identify potential metabolites. The predicted ones were then checked in plasma and brain of rats treated intraperitoneally with 10 mg/kg 4,4'-DMAR, and sacrificed at different time points. Analysis were performed by high performance liquid chromatography - high resolution mass spectrometry (HPLC-HRMS) with electrospray ionization (ESI) in positive ion mode. The instruments used were an Agilent 1200 HPLC system and an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher), equipped with a Prosolia desorption ESI source. Full scan at 70000 resolving power and MS/MS spectra (resolving power 17500, CID 35eV) were acquired simultaneously using a targeted method.

The predicted hydroxylated, oxidated, dealkylated and deaminated metabolites were found in plasma and brain samples. Structures of metabolites were confirmed with MS/MS spectra, identifying specific fragments for each molecule. Moreover, the chromatographic separation allowed the detection of different isomeric forms of hydroxylated metabolites, suggesting different sites of hydroxylation.

Determination of 4,4'-DMAR concentrations were also obtained by a validated HPLC-MS/MS method, using a triple quadrupole with ESI positive ionization in SRM mode. Pharmacokinetic profile in plasma showed a rapid absorption (15 min) and a rapid clearance, whereas analysis of brain tissues showed that drug levels reached Cmax at 30 minutes after the treatment and then declined as plasma levels. The ratio "brain AUC0-inf/plasma AUC0-inf " was 24.5 and showed a high brain uptake of 4,4'-DMAR.

In parallel, semi-quantitative analysis were performed for the hydroxylated and oxidated metabolites. Notably, we observed that the levels of the latter were higher than those of 4,4'-DMAR in plasma but not in the brain, suggesting a low brain uptake of this main metabolite.

In conclusion, the HRMS analysis, associated with the in silico prediction, allowed the identification for the first time of the *in vivo* metabolites of 4,4'-DMAR. Moreover, investigation of the pharmacokinetic profiles in plasma and brain showed that 4,4'-DMAR (but not its main metabolites) readily reached the brain with a high brain-to-plasma ratio.

P35. A NEW HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF PACLITAXEL AND 6A-HYDROXY-PACLITAXEL IN HUMAN PLASMA: DEVELOPMENT, VALIDATION AND APPLICATION IN A CLINICAL PHARMACOKINETIC STUDY

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Standard dosing of chemotherapy does not take into account the many inter-patient differences that make drug exposure highly variable, thus leading to the insurgence of severe toxicity. This is particularly true for paclitaxel (PTX) considering that a relationship between haematological toxicity and plasma exposure was found making Therapeutic Drug Monitoring (TDM) necessary in order to treat patients with the correct dose.

Several methods have been published for the determination of PTX in human plasma anyway, to our knowledge, just seven offer the possibility to quantify even the main metabolite, 6α- hydroxy-paclitaxel (6α-OH-PTX). Among these methods, five require a high volume of plasma being the sample size range between 200 and 500 µL.

Moreover, the extraction method used in all these methods, was LLE (liquid-liquid extraction or SPE (solid-phase extraction), which are both time-consuming procedures and therefore not suitable for TDM assays.

In order to quantify paclitaxel PTX and its main metabolite, 6α-OH-PTX, in patients' plasma, we developed a new, sensitive and specific HPLC-MS/MS method applicable to all PTX dosages and therefore suitable for TDM application and for being applied to a dose-escalation study.

Chromatographic separation was done on a SunFire^{TM} C18 column (3.5 µM, 92 Å, 2,1 x 150 mm) using 0.1% formic acid/bidistilled water and 0.1% formic acid/acetonitrile as mobile phases. The mass spectrometer worked with electrospray ionization in positive ion mode and selected reaction monitoring. Our bioanalytical method was successfully validated according to the FDA-EMA guidelines on bioanalytical method validation.

The standard curves were linear ($R^2 \geq 0.9948$) over the concentration ranges (1-10000 ng/mL for PTX and 1-1000 ng/mL for 6α-OH-PTX) and had good back-calculated accuracy and precision. The intra- and inter-day precision and accuracy, determined on three quality control levels for the two analytes, were always <9.9% and within 91.1-114.8%, respectively. Moreover, we evaluated this bioanalytical method by re-analysis of incurred samples as an additional measure of assay reproducibility. This new method was successfully applied to a genotype-guided phase Ib study of weekly PTX in ovarian cancer patients treated with a wide range of drug's dosages.

P36. COMBINING MASS SPECTROMETRY AND PULL-DOWN TECHNIQUES TO IDENTIFY MOLECULAR TARGETS OF AN ANTIMICROBIAL PEPTIDE ACTIVE TOWARDS GRAM-NEGATIVE BACTERIA

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Antimicrobial resistance is an increasingly serious threat to global public health that requires the search for new potent and alternative antibiotics. One of the most important points to exploit a new antimicrobial molecule is the knowledge of its molecular targets, in order to understand the mechanism of action and eventually potentiate its activity. The Pull-Down (PD) assay is an *in vitro* method used to determine a physical interaction between two or more proteins. PD assays allow to detect, and eventually confirm, the existence of a Protein-Protein Interaction (PPI) and may be used as a screening assay for identifying previously unknown PPIs interactions.

In this study the pull-down affinity technique has been coupled to a high resolution mass spectrometry platform to identify the molecular targets of SP-E, a proline-rich antibacterial peptide isolated from swine saliva.

E. coli ATCC 25922 and the *Pseudomonas aeruginosa* ATCC 9027, two gram negative bacterial strains sensible to the SP-E peptide, have been used as model. In order to individuate the potential intracellular molecular targets, a series of PD experiments, challenging immobilized SP-E with bacterial lysates, in coupling with proteomic approaches, were carried out. Two rounds of PD assays were performed: the first with whole bacterial lysates, as a screening assay, followed by the second one with selected fractions obtained by Size Exclusion Chromatography pre-fractionation of the bacterial lysate. The samples isolated from PD assays were run in EF-SDS, the bands cut and digested with trypsin. Digestion products were analyzed by LC-high-resolution MS/MS and the data elaborated with Proteome Discover 2.1. According to the Search Tool for the retrieval of interacting Genes/Proteins (STRING) database, many of the proteins identified are involved in protein biosynthesis, indicating that SP-E, similarly to other PrAMPs, may act by inhibiting both protein translation and folding.

P37. SALIVARY PROTEOMIC ANALYSIS IN THE EVALUATION OF THE EFFICACY OF *LACTOBACILLUS BREVIS* CD2 LOZENGES IN PREVENTING ORAL MUCOSITIS BY HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Treatment-induced Oral Mucositis (OM) is a pathological process characterized by mucosal damage, from mild inflammation to deep ulcerations and affecting one or more parts of the gastrointestinal tract as a consequence of chemotherapy and radiation therapy.

The major complications in haematological malignancies patients (pts) submitted to HSCT are infections, in particular due to *E. coli*, *P. Aeruginosa* and *Candida*. Treatment of OM is only palliative. Several studies showed the successful treatment of the symptoms associated to OM with the strain of *L. brevis*, due to the process of microflora manipulation. Our objective is to test whether the probiotic *L. brevis* CD2 lozenges can reduce the incidence and severity of OM in patients undergoing HSCT. We enrolled 16 pts, affected by multiple myeloma and submitted to autologous HSCT. Eight pts received 6 *L. brevis* CD2 lozenges per day, from 14 days prior chemotherapy and till resolution of OM. Patients. We collected salivary specimens of pts receiving *L. Brevis*, from start of chemotherapy and every week until resolution of OM. For the top-down proteomic analysis, saliva samples were mixed in a 1:1 (v/v) ratio with aqueous 0.2% trifluoroacetic acid solution and centrifuged at 8,000 g at 4°C for 5 min. The soluble acidic fractions were analyzed by HPLC-ESI-MS. Quantification was based on the area of the eXtracted Ion Current (XIC) peaks, which under constant analytical conditions is proportional to the protein concentration. Specifically we investigated the levels of α-defensins 1-4 and some members of S100 family because of their role in inflammation, repair of tissue, damage and in innate and adaptive immunity. Characteristically we found an increased level of α-defensins that correspond to the beginning of the damage of the mucosal barrier, and then a reduction with the recovery from mucositis. No expression of S100 family proteins was found, confirming the strong association between S100 and GVHD, as described in our previous study. There is no influence of *L. brevis* on engraftment and complications post HSCT, but it's necessary to extend study population for a better evaluations of the protective role of *L. brevis* against OM.

P38. TOP-DOWN/BOTTOM-UP LC-MS INTEGRATED PROTEOMIC PLATFORMS FOR MOLECULAR FINGERPRINTING OF ADAMANTINOMATOUS CRANIOPHARYNGIOMA PEDIATRIC BRAIN TUMOR

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Adamantinomatous Craniopharyngioma (ACP) is a rare, slow-growing, intra/suprasellar tumor that represents about 6-10% of all pediatric brain tumors. Although not metastatic and histologically benign, ACP, often associated to a cyst, behaves as an aggressive tumor compromising vital structures, such as hypothalamus, and visual pathway with a high morbidity rate. The pathogenesis of human ACP is not yet clearly understood, therefore attention is now paid on its molecular characterization, including proteomic analysis, in trying to elucidate its cellular mechanisms of onset and progression.

After tissue homogenization an aliquot of the same sample was digested with trypsin for shot-gun proteomic analysis and an aliquot was analysed for characterization of the entire proteome in top-down approach both by nano-LC separation in coupling with Orbitrap Elite mass spectrometry detector. Shot-gun analysis identified a cluster of proteins common to the majority of the analyzed specimens that strongly characterizing ACP tumor tissues. The bioinformatics elaboration of these data by different tools evidenced a complex functional network between the identified proteins and different molecular functions also elucidating possible intracellular pathways involved in disease development and progression. Inside different protein classes found, a relevant part were cytoskeletal proteins and enzymes.

The top-down analysis, mainly focused on characterization of small proteins and peptides, revealed particularly the presence in tumor tissues of α-defensins 1-4 and β-thymosins peptides, supporting previous findings on the ACP intracystic fluid and confirming the role of inflammation in the disease. The analysis of the entire proteome allowed also to characterize naturally occurring protein fragments, proteoforms and PTMs.

The two proteomic strategies resulted complementary in achieving the characterization of ACP proteome in a wide range of molecular weight also accomplished the identification of protein isoforms, PTMs and bioactive peptides. These preliminary results give an interesting contribution in the knowledge of the molecular mechanisms at the basis of ACP onset and progression, also providing hints for future investigations and development of possible targeted therapies.

P39. PROTEOMIC ANALYSIS OF CNF1 IMPURITIES BY NANOFLOW LIQUID-CHROMATOGRAPHY COUPLED WITH AN ORBITRAP-MASS SPECTROMETER

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Certain pathogenic *E. coli* strains produce the Cytotoxic Necrotizing Factor 1 (CNF1) protein toxin that permanently activates proteins belonging to the Rho GTPase family. Recently, CNF1 has been proposed as a novel potential therapeutic tool for a number of central nervous system diseases. In fact, a direct brain injection of CNF1 is able to reverse cognitive impairment and neuroinflammation in Rett syndrome and Alzheimer's disease mouse models.

An intrinsic degree of heterogeneity occurs in recombinant products due to the biosynthetic processes used by living organism to produce them. Consequently, the absolute purity of any biotechnological product is difficult to determine and the results are often method dependent. Liquid-chromatography coupled to mass-spectrometry is the gold standard in proteomic analysis to identify the peptides generated by the proteolysis of biological sample. Consequently, a proteomic analysis of CNF 1, after extraction and purification, has been carried out as following reported.

CNF1-producing392 ISS *E. coli* strain was grown overnight in LB medium with ampicillin. Bacteria were collected by centrifugation and re-suspended in 50 mM sodium phosphate buffer. Bacteria were then disrupted by sonication and the homogenate was centrifuged. The supernatant was precipitated with 50% ammonium sulphate, extensively dialysed against 20 mM Tris buffer (pH 7.2) and finally, chromatographed on a ion-exchange column. CNF1 was eluted, dialysed then precipitated with 50% ammonium sulphate.

The collected fractions were then centrifuged and the pellet re-suspended in buffer. The purity of CNF1 solution was investigated by a standard bottom-up proteomic approach allowing the identification of proteins contained in the sample.

The purified CNF1 sample was treated with TCEP to reduce disulfides then digested overnight with a proteolytic mixture (Trypsin/LysC). Peptide digest was separated by reversed-phase nanoflow liquid chromatography coupled to an Orbitrap (LTQ-OrbitrapTM XL) mass spectrometer operating in data dependent scan mode. Proteins were identified based on a standard peptide matching data analysis using SEQUEST algorithm and a database containing the whole *E. coli* unreviewed proteome database (TrEMBLidentifierECOLX). Trypsin was selected as protease allowing a maximum of 2

missed cleavages. Mass tolerance for precursor and fragment ions were set to 10 ppm and 0.8 Da, respectively; all other parameters were set at default values. Output filters were set to include only proteins giving at least 2 peptide identified with high confidence scores. A number of forty proteins in a 149.2-11.85 score range and 46.87-15,44% coverage range were identified.

P40. NEW LC-MS/MS METHOD FOR THE DETERMINATION OF METHYLMALONIC ACID (MMA) IN DRIED BLOOD SPOT

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Expanded Newborn Screening (NBS) for inherited-metabolic diseases is based on the analysis of amino acids and acylcarnitines in Dried Blood Spot (DBS) by FIA-ESI-MS/MS. Inborn errors of propionate and cobalamine metabolism relies on finding abnormal concentrations of propionylcarnitine. However this analyte is not specific and brings to an high false positive rate due to the overlap of their concentrations in affected and unaffected newborns. Methylmalonic acid (MMA) is a more specific marker for diagnosis of these diseases but this marker is not detected by current NBS methods. The aim of this study was to develop and validate a method for measuring this analyte in DBS by UPLC-ESI-MS/MS.

A 4.6 mm disc was punched from a DBS sample. It was extracted using 200 µl of a solution CH_3CN/H_20 (70/30,v/v) containing 0.5% of HCOOH and 4 µmol/L D3-MMA (internal standard) and it was placed in an ultrasonic bath for 20 min. After, the sample was centrifuged for 5 min. at 4000 g and the supernatant (150µL) was transferred in V bottomed plate and dried. 180 µL 3N HCl in n-butanol were added and then the sample was incubated for 30 min. at 60°C. Subsequently the sample was evaporated and reconstituted with 150 µL of CH_3CN/H_20 (95/5, v/v) containing 0.5% HCOOH. Then the specimen was injected in UPLC-MS/MS system. For quantitative analysis the MRM transitions for the analyte and internal standard were respectively: 231.22→118.83 m/z for MMA and 234.22→121.83 m/z for D_3-MMA. The analysis was performed with an ACQUITY UPLC coupled to Xevo TQ MS (Waters).

The chromatographic separation of MMA was accomplished in 9 minutes. Method validation was performed. The limit of quantitation was 0.086 µmol/L of blood and the response was linear over the range 0-100 µmol/L of blood. The within- and between-day precision was assessed and the CVs were less than 9.8%. To evaluate the sensibility of the method and to define an appropriate cut-off the method was applied to DBSs of 4 patients affected by Mut deficiency, Cbl C deficiency and mother B12 deficiency. Reference ranges for newborns and children were assessed. Prospective application of this second-tier analysis NBS samples led to the identification of 1 affected infants. Application of this assay reduced the false-positive rate and improved the positive predictive value of NBS for conditions associated with abnormal propionylcarnitine concentrations.

P41. ROLE OF ENDOGENOUS FATTY ACID SYNTHESIS IN PERIPHERAL NERVE STRUCTURE AND FUNCTION

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Changes in myelin Fatty Acid (FA) composition have been associated with Peripheral Neuropathy (PN), but the importance of nerve FA synthesis in maintaining myelin functionality is poorly understood. In our study, we explored the extent to which lack of the key regulator of FA synthesis, Sterol Regulatory Element Binding Factor-1c (Srebf1c), could result in the development of PN.

The degree of peripheral neuropathy in Srebf1c null mice was investigated by behavioral tests. Morphological and morphometric analyses along with a transcriptomic approach were used to address the mechanism responsible for the development of PN. In addition, metabolomics analysis was performed by Liquid Chromatography-tandem mass spectrometry (LC)-MS/MS.

Quantitative analysis was performed by means of calibration curves prepared and analyzed daily by Electrospray Ionization (ESI) using an API 4000 triple quadrupole instrument (AB Sciex, USA).

We demonstrated that the neuropathic phenotype observed in Srebf1c knockout mice was due to Remak bundle alterations, hyper-myelinated small-caliber fibers and changes in myelin periodicity. Moreover, these animals displayed, in the peripheral nerves, decreased FA synthesis and glycolytic flux, but increased FA catabolism and mitochondrial function. The mechanism responsible for enhanced FA catabolism in Srebf1c knockout mice restricted to peripheral nerves is due to the accumulation of PC-C16:0/C18:1 and PC-C18:0/C18:1 specifically in the sciatic nerve detected by LC-MS/MS. Increased levels of these two endogenous Pparα ligands activate this transcription factor *in vivo* primarily in Schwann cells, resulting in increased FA catabolism and in the development of peripheral

neuropathy. As evidence of this, treatment of Srebf1c null mice with a Pparα antagonist rescues their neuropathic phenotype.

These data provide new insight into the contribution of endogenous FA synthesis to nerve homeostasis and function.

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P42. HISTOLOGY-DIRECTED HYDROGEL MEDIATED ON-TISSUE QUANTITATIVE PROTEOMICS

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A workflow for multiplexed and spatially localized on-tissue quantitative proteomics was developed. The method is based on the use of an enzyme delivery platform, a polymeric hydrogel disc, allowing for a localized digestion directly onto the tissue surface coupled with an isobaric mass tag strategy for peptides labeling and relative quantification.

Heterogeneous systems for on-tissue digestion in which an enzyme is carried within hydrogels or adsorbed on solid supports (for focused delivery over regions of interest) have recently been developed as a miniaturized method for histology-guided application on biological samples.

This approach was demonstrated to provide histology-directed analysis at resolutions down to 1 mm diameter since gel discs are precisely placed on defined regions, localizing the digestion to a defined area of the tissue. Thus, molecular data are provided from specific areas; specific regions within a single specimen can be targeted as well as those from two different specimens can be compared. The digestion occurs within such hydrogels, followed by peptides solvent extraction and identification by Liquid Chromatography coupled to high-resolution tandem Mass Spectrometry (LC-MS/MS).

Since this is a histology-directed on-tissue analysis, multiple hydrogels were placed onto morphologically and spatially different regions of interest (ROIs) within the tissue surface, e.g. cardiac myxoma tumor vascularized region and the adjacent hypocellular area. After a microwave digestion step (2 min), enzymatically cleaved peptides were labeled using TMT reagents with isobaric mass tags, enabling analysis of multiple samples per experiment. Thus, N=8 hydrogel digested samples from a cardiac myxoma (N=4 from the vascularized ROI, N=4 from the adjacent hypocellular area) were processed and then combined before a single LC-MS/MS analysis. Regulated proteins from both cardiac myxoma regions were assayed in a single experiment.

P43. NATURAL AND MODIFIED BACTERIOCHLOROPHYLLS: FROM PREPARATION TO MALDI-TOF/TOF MASS SPECTRA INTERPRETATION

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Bacteriochlorophylls (BChl) represent an important group of natural chlorophylls and are very common in nature, mainly in photosynthesizing bacteria. The pigments differ by the extent of hydrogenation of the macrocycle and by the nature of the substituents. Several modifications of BChl are known; BChl *a* and *b* have been isolated from purple bacteria, but other modifications there exist. BChl *a* is characterized by the presence of the tetrahydroporphyrin macrocycle and a phytol residue. BChl *a* is usually taken as the initial compound to develop novel photosensitizer for photodynamic therapy (PDT) of cancer and other possible photochemical applications.

The purple bacteria *Rhodobacter sphaeroides* is a good source of Bchl *a*. Our aim was to characterize BChl *a* by Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI MS) and to replace the central metal ion (Mg^{2+}) with Zn^{2+} and Cu^{2+} in order to obtain stable Zn-BChl *a* and Cu-BChl *a*, with spectral characteristics for PDT not inferior to BChl *a* and in some cases even exceeding them. Recently, 1,5-Diaminonaphthalene (DAN) was adopted as an electron-transfer secondary reaction matrix for the identification of intact chlorophylls and their derivatives. DAN was proved to outperform conventional matrices also in the case of BChls, since losses of the central metal ion and phytol-ester cleavage were negligible. Absence of significant fragmentation of both radical cations and anions of BChl *a* at *m/z* 910.55, and Zn/Cu-BChl *a* at *m/z* 950.49 and 947.49, respectively, makes MALDI MS a convenient tool to probe chemical or biochemical insertion of metal ions into porphyrin-based pigments.

P44. DISTINCTION BETWEEN CYANIDIN 3-O-GLUCOSIDE AND CYANIDIN 3-O-GALACTOSIDE BY MASS SPECTROMETRY COMBINED WITH IRMPD SPECTROSCOPY

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Anthocyanins are phytochemicals belonging to the flavonoid group, present in many fruit and plant. These pigments are responsible for the blue, red or purple color of fruit, vegetables and plants, such as blueberry, strawberry, cherry, egg-plant and hydrangea. Due to the intense coloration, water solubility, and non toxicity, these compounds are exploited in the food industry as natural coloring agents. Cyanidin for example is the magenta color named E-163a. Furthermore, anthocyanins have antioxidant properties, reacting as radical scavengers, and recent studies, conducted *in vitro*, have highlighted other potential used of these pigments against cancer, diabetes, neurological and cardiovascular diseases, reducing risk of coronary heart disease, and improving the visual activity and a potential antiviral activity. However, literature reports some controversy over the relative contributions of anthocyanins versus anthocyanidins, the sugar-free aglycones in terms of bioavailability and bioactive potential. It was first assumed that only aglycones could enter the circulation circuit, but now also the absorption and metabolism of anthocyanin glycosides has been reported. It was found that the nature of both the sugar conjugate and the aglycone play an important role in the anthocyanin absorption and excretion in humans and rats.

In this contribution we report on a CID and IRMPD study on cyanidin 3-*O*-β-glucoside (kuromanin) and 3-*O*-β- galactoside (idaein) aimed to find a way to discriminate between the two diastereomers. Unfortunately the first results were not so promising: in the CID experiments both diastereomers give only one fragment corresponding to cyanidin aglycone, by loss of the sugar moiety, and the IRMPD spectra recorded in the O-H stretch region (2800-3900 cm^{-1}) did not reveal substantial differences between the two species. Subsequently, we decided to study kuromanin and idaein aluminium-hydroxy complexes by CID and IRMPD. The results are quite surprising. In the CID experiment, contrary to what was seen for the free anthocyanins, an extensive pattern of fragmentation was observed in both metallic complexes. The first fragment was the loss of a molecule of water for both species, but in the Idaein complexes a fragment at m /z 443, corresponding to the loss of successive loss CH_2O was predominant and distinctive.

Also the IRMPD spectra of kuromanin and idaein aluminium-derived complexes have shown remarkable differences, finding novel means of structural discrimination of potential analytical value.

P45. SYNTHESIS AND MALDI MS/MS CHARACTERIZATION OF A *CIS*-DIAMINOCYCLOHEXYL PT(II)-VITAMIN B_{12} COMPLEX POTENTIALLY USEFUL FOR TARGETED DRUG DELIVERING

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The water-soluble vitamin B_{12} is essential for most organisms despite being produced by only a few bacteria. The demand for vitamin B_{12} is particularly high at sites of enhanced proliferation, such as cancer cells, making it particularly attractive as targeting agent. Indeed, the use of vitamin B_{12} for selectively delivering radioisotopes or various cytotoxic agents into cancer cells has already been actively investigated.

To these purposes vitamin B_{12} needs to be derivatized by the introduction of modifiers, such as ligands or receptor binding molecules. Coordination to the ribose ring and oxidative alkylation are the most common synthetic routes. Interestingly, vitamin B_{12} has been used to form adducts with Tc and Re complexes linked to the axial positions of the cobalt center, demonstrating that its Co(III)-CN moiety tends to bridge a second Metal (M) ion to form a {Co-CN-M} unit.

Cisplatin (*cis*-diammine-dichloro-Pt(II)) is one of the most potent agents against a wide variety of solid tumors, its cytotoxicity being mediated by formation of DNA adducts. Thus the possibility of using vitamin B_{12} as a ligand for cisplatin (and its analogues) would be highly desirable in view of a targeted delivering of anticancer drugs. In this communication, we demonstrate that Co-CN in vitamin B_{12} bridges to Pt(II) center yielding stable complexes with the central structural feature {Co-CN-Pt}. The formation of the *cis*-Pt(II) complex with vitamin B_{12} (*m/z* 1663.647) was investigated by matrix assisted laser desorption/ionization mass spectrometry, MALDI MS in positive ion mode, using 4-chloro-α-cyanocinnamic acid as a matrix.

Structural characterization was accomplished by collision-induced dissociation (ToF/ToF) MS analysis.

P46. GAS PHASE BASICITY AND PROTON AFFINITY OF VITAMIN C

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L-ascorbic acid is an ubiquitous natural component of a wide range of fruits and vegetables and thanks to its reducing power is used to protect foods against oxidation. Many studies have dealt with the precursors of furan products in foods and identified Ascorbic Acid (AA) among one of the major sources. Moreover furan products received increasing attention after the International Agency on Cancer Research (IARC).

In order to investigate the gas phase mechanisms of the acid catalyzed degradation of Ascorbic Acid (AA) to furan molecules and to structurally characterize the gaseous ionic intermediates, we made use of a mass spectrometric techniques (ESI/TQ/MS) and theoretical investigation at the B3LYP/6-31+G(d,p) level of theory.

The gaseous reactant species, the protonated ascorbic acid $[C_6H_80_6]H^+$ ions, were generated by electrospray ionization (ESI) of ascorbic acid solution.

We reported the measurements of the unknown gas phase basicity and proton affinity of Ascorbic Acid (AA) by the extended Cooks's kinetic method and the structural characterization of its protonated form performed by quantum mechanical computational methods.

The kinetic method is based on the formation of proton-bound dimers between the Ascorbic Acid (AA) and a structurally similar reference base (B). The observation of the collisionally induced decomposition of the dimer into the corresponding AAH^+ and BH^+ fragments allows the estimation of the relative affinity of both molecules for the H^+ ion.

The study of the AAH^+ Potential Energy Surface (PES) leads to the characterization of four stable protomers.

Computational results show that the carbonyl oxygen atom of AA is the preferred protonation site and identify the corresponding ionic species as the lowest energy protonated conformer.

From the experimental proton affinity (PA) of 875.0±12 $kJmol^{-1}$ and protonation entropy ΔS_p (108.9±2 $Jmol^{-1}$) a GB value of AA of 842.5±12 $kJmol^{-1}$ at 298K was obtained, which is in agreement with the value issuing from quantum mechanical computations.

P47. GALACTOSAMINE IN THE GAS PHASE. UNVEILING FEATURES OF THE CONFORMERS EXISTING IN A MIXTURE OF THE INTERCONVERTING ANOMERS

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2-Aminosugars are monosaccharide derivatives present in a modified form in several biopolymers (e.g. chitin is an important component of the cell walls of many living organisms). Their further importance lies in the very simple but unique structure that can assume an archetypal behavior by experiencing different conformations.

The conformation of neutral aminosugar arises from the balance of different factors, including the hyperconjugative and the electrostatic effects, as well as the peculiar hydrogen bonding network available to the hydroxyls substituents and the amino group. In the present work the IRMPD spectrum of protonated galactosamine ($GalN{\cdot}H^+$), that is a C4 epimer of glucosamine, has been measured to determine for the first time the IR features of protonated α- and β-anomers present in a methanolic mixture, on the ground of their different absorption bands.

In order to distinguish the signals coming from different anomers, the spectrum of $GalN{\cdot}H^+$ extracted from the same methanol solution of GalN·HCl has been measured at different solution ages, with the purpose of estimating possible modifications in the spectrum profile, which can be safely attributed to the time-evolution of the anomeric composition of the mixture. The experimental data have been compared with a systematic conformational search (B3LYP/6-311++G(d,p)) which clearly indicated the identity of the most populated conformers for both the anomers, which are characterized by a clockwise arrangement of the $N_2H_3^+$-O_3H-O_4H-O_6H hydrogen bond chain and by a $^4C_1$ chair. The gas phase α/β distribution has been determined, and then compared with the condensed phase distribution measured by a NMR analysis carried out on increasing age solutions.

The results indicate a very similar position of the equilibrium measured in the gas phase and in condensed phase, but very different anomerization rates. The orbital occupancies of the structures matching the experiment have been calculated through the natural bond method, and the obtained results firmly indicates that in the α and β anomers of protonated $GalN{\cdot}H^+$ an opposite effect is active: endo anomeric effect is predominant in the first meanwhile in the latter is more important the exo one. Further computational work is in progress to exactly estimate the specific contribute of the electrostatic and hyperconjugative effect to differentially stabilize the anomers of protonated galactosamine.

P48. MASS SPECTROMETRY DISSECTS THE ROLE OF DISULFIDE BONDS IN PROTEIN STRUCTURE AND FUNCTION

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The Disulfide Bond (DB) is a covalent bond derived from two thiol groups. In proteins, Cysteine (Cys) is the only amino acid capable of forming DBs. These bridges are responsible for stabilizing the protein globular structure and therefore have an important role in protein folding and stability. Here, we present two works for the characterization of protein DBs with two different mass spectrometry strategies: the first using a MALDI-TOF instrument, the second with the help of a linear ion-trap (LTQ) coupled with a HPLC with an Electron Transfer Dissociation (ETD) device. The first technique was applied to characterize the 6-Cys-containing lysosomal protein Saposin C (SapC), whose deficiency causes a variant form of Gaucher disease with glucosylceramide accumulation. For this reason, some reported disease-associated SapC proteins carrying mutations in Cys residues were analyzed. To extract information about the structure and the redox state of the Cys residues, all SapC mutants were cleaved by chemical [BrCN] or enzymatic proteolysis [AspN, GluC, trypsin]. These enzymes, were employed alone or in combinations and the resulting peptide mixtures were analyzed by MALDI-TOF. Then, we investigated the role played by the different DBs on SapC susceptibility to the proteolytic activity of the lysosomal enzyme Cathepsin D (Cath D). While wild type SapC is a stable protein, we observed that resistance to proteolytic activity was significantly reduced in all the mutants.

Dithiothreitol (DTT)-mediated disruption of DBs confirmed that the resistance of SapC to proteolysis is to be ascribed largely to its tertiary structure, rather than primary sequence.

LTQ was used for the study of the DB-mediated glutathionylation of hemoglobin (Hb). In response to CO exposure, glutathione concentration (GSH) increases in human red blood cells (RBCs), triggering an antioxidant response. To characterize the glutathionylated residues in Hb, we applied an LC-MS strategy based on a combined-fragmentation approach, coupling ETD-MS^2 and Collision Induced Dissociation (CID)-MS^2. ETD breaks DBs and separates S-S-bound peptides. The resulting product ions are further fragmented by CID to infer their sequences. Using this approach, we found for the first time that Hb β-chain can bind GSH with both Cys93 and Cys112. This CO-dependent effect might represent a new adaptive response to stress conditions in mammalian RBCs.

P49. RESOLVING MIXTURES OF ISOBARIC COMPOUNDS USING ENERGY RESOLVED MS/MS EXPERIMENTS AND THE LEDA POST-PROCESSING ALGORITHM TOOL

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The analysis of isobaric molecules by tandem mass spectrometry, especially if they are isomers, is often complicated by the similarity between their fragmentation patterns. In fact, it is common that the same MS/MS product ions are present in the spectra of all isomers. In this case, an adequate chromatographic separation between compounds should be developed in order to eliminate mutual interferences. In the literature, different approaches were proposed for the quantification of isomers mixtures, also in the case of chromatographically unresolved peaks, but the obtained methods are usually compound-specific. In our work, five positional isomers were studied and, despite the attempts for optimizing the chromatographic separation, an unique LC-MS/MS method suitable to separate all compounds was not achieved. On the other hand, a product ion spectrometry performed on the $[M+H]^+$ species of the different compounds was not always able to characterize the different isomers and, consequently, a series of experiments based on energy resolved MS/MS were carried out. By this approach a clear differentiation among the isomers was obtained but, in order to emphasize such differences, it was necessary to develop a mathematical algorithm that resolved the MS/MS spectra of the components of mixture. This algorithm consists in the application of a matrix of linear regression equations to different experimental data. In our case, the experimental data were the abundance ratios of product and precursor ions selected during MS/MS method set-up. The Linear Equations of Deconvolution Analysis (LEDA) tool was proposed to establish the relative proportions of individual isomers in the sample. Considering the pharmaceutical interest on the compounds under investigation, the LC-MS/MS method developed was tested to be effective at the pharmacological active concentration levels of studied compounds, corresponding to a range between nM to μM (i.e. ng mL^{-1} on processed sample). The performances evaluation of the proposed algorithm (LEDA) confirmed its effectiveness allowing an accurate and precise quantitative analysis of complex mixtures of isomers. It is worth to observe that the LEDA tool is able not only to give the relative quantities of the mixture components but, overall, to distinguish immediately their combination (e.g. binary, ternary, quaternary, ... mixtures) or if the sample is represented by a pure compound.

P50. EVALUATION OF THE POSSIBLE EFFECTS OF SYNTHETIC ISOFLAVONOIDS ON THE ACTIVITY OF CYP19: IMPLICATION IN ANTI-DOPING ANALYSIS

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Flavonoids are 2-phenyl-1, 4-benzopyrone derivatives that are commonly found in vegetables and fruits. They occur in common human diet and have been used as drugs or food supplements. The major activities of flavonoids are anti-oxidants, antiestrogenic, most probably trough the inhibition of aromatase, and androgen promoters. Besides these effects, flavonoids have other several structure-dependent activities like cytostatic, apoptotic, antinflammatory, antiangiogenic, hepatoprotective and chemoprotective. In addition, several flavonoids are modulator of expression and activities of specific cytochrome P450 genes and/or proteins. Isoflavonoids are a group of flavonoids that have a basic structure consisting in a two benzyl rings (A and B) joined by a three-carbon bridge, which may or may not be closed in a pyran ring. They are classified as phytoestrogens and show a weak estrogenic activity. Recently a renewed interest has been paid to the anabolic activity of isoflavonoids because they are potentially abused, as concomitant drugs, during recovering period after the administration of anabolic steroids to increase the natural production of Luteinizing Hormone (LH) and consequently the synthesis of natural androgens. For these reasons, isoflavonoids can be classified as potential confounding factors in the evaluation of the steroid profile and LH data in doping analysis. At present they are not included in the Prohibited List of the World Anti-Doping Agency for the accredited laboratories but, their monitoring might be helpful in routine doping analysis.

In this study we have examined the interaction of two synthetic isoflavonoids, methoxyisoflavone and ipriflavone, with human aromatase (CYP19), enzyme that converts androgens (testosterone and androstenedione) to estrogens (estradiol and estrone) catalyzing three consecutive hydroxylation reaction, by inhibition kinetic analysis monitored by GC-MS. In parallel we have evaluated kinetic plots obtained after the interaction of CYP19 with known aromatase inhibitor drugs (formestane, aminoglutethimide) and natural flavonoids (chrysin, quercetin and daidzein) with the aim to confirm the data present in literature about their potential as inhibitory drugs.

Our *in vitro* assays shows that methoxyisoflavone and ipriflavone could be potential competitive inhibitors of aromatase with respect to the androgen substrate. For these reason the large intake of isoflavonoids could alter the natural homeostasis between androgen and estrogen hormones and then this substances could be classified as possible confounding factors in the evaluation of steroid profile.

P51. EVALUATION OF PHARMACOKINETIC PROFILE OF THE "NEW PSYCHOACTIVE SUBSTANCE" AH-7921 AND ANALYSIS OF ITS *IN VIVO* METABOLISM, BY HIGH RESOLUTION MASS SPECTROMETRY

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AH-7921 is a synthetic opioid added to schedule I of the Single Convention on Narcotic Drugs in 2015. Several metabolites were found in blood and urines of AH-7921 users, as well as in *in vitro* studies. No data are however available for the pharmacokinetic profile of AH-7921, as well as the *in vivo* formation of metabolites.

To obtain these information, rats were treated intraperitoneally with 10 mg/kg AH-7921 and sacrificed at different time-points. Brain and plasma metabolites were separated by high performance liquid chromatography (HPLC) and identified by high-resolution mass spectrometry (HRMS), using a LTQ-Orbitrap XL instrument operating in ESI positive ion mode. A full scan at 70000 resolving power (m/z range 100-1000 u), was done for each sample; MS/MS spectra (resolving power 17500, CID 35eV) were acquired simultaneously, using a data-dependent method which selected the most abundant ions of each MS scan in real time. Determination of drug concentrations, and semi-quantitative analysis of the main metabolites, were obtained by HPLC-ESI-MS/MS using a triple quadrupole operating in positive SRM mode.

Different metabolites (*N*-demethylated, *N*-didemethylated, hydroxylated, *N*-demethylated hydroxylated and *N*,*N*-didemethylated hydroxylated) were identified in plasma and brain samples collected 5 minutes after treatment and at the following time-points. The exact mass of the fragments combined with the chromatographic separation allowed to detect different isomeric forms of the hydroxylated metabolites due to different hydroxylation sites. AH-7921 C_{max} was reached 30 min after treatment, either in plasma (205 ng/mL on average) and brain (2842 ng/g on averaged), with a brain-to-plasma ratio of 16.6. Plasma and brain levels then declined with a similar $t_{1/2}$ of about 3 hours. Semi-quantitative analysis was performed for the main metabolites, *N*-demethylated and *N*,*N*-didemethylated, monitoring the corresponding ion transitions. Time-course profile showed that these metabolites reached, at longer time-points, brain levels comparable or even higher than those of the parent compound. Notably, the *N*,*N*-didemethylated metabolite showed a very slow elimination from brain tissue.

In conclusion, the HPLC-HRMS analysis allowed the identification of several metabolites in plasma and brain deriving from demethylation, hydroxylation and

combination of these reactions. Moreover, quantitative analysis showed that AH-7921, as well as its main metabolites, readily reached the brain with brain-to-plasma ratios of ~15-20. The pharmacokinetic data obtained for the two main metabolites suggests the need of a further characterization of their pharmacological activities and pharmacokinetic studies after repeated treatments.

This research has been funded by the Drug Policies Department, Presidency of the Council of Ministers, Italy (project NPS-RISK ASSESSMENT)

P52. MULTI-CLASS ANALYSIS OF NEW PSYCHOACTIVE SUBSTANCES AND METABOLITES IN HAIR BY PRESSURIZED LIQUID EXTRACTION COUPLED TO HPLC-HRMS

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The use psychoactive substances is common to all ages and cultures, however nowadays a lot of New Psychoactive Substances (NPS) are widespread on the illegal market (more than 500 NPS have been notified in EU since 2005). Due to their rapid spread and the strong variety in chemical composition, it is not an easy task to keep up with the emergence, as NPS are also a heterogeneous group (e.g. phenethylamines, tryptamines, cathinones and synthetic cannabinoids). The identification of these new drugs is a never-ending challenge for forensic toxicologists. Blood and urine are the conventional specimens to determine drug exposure; however, in the last twenty years hair testing has gained increasing attention and recognition as a complement to blood and urine analysis. Hair is a unique material for the retrospective detection of drug consumption, due to its large detection window, and it is easy to collect, store and transport. Consequently, hair analysis of illicit drugs and medicines is currently employed to address a wide range of application, such as drug abuse history, workplace testing, post-mortem toxicology, therapeutic drug monitoring or Drug Facilitated Assault (DFA) investigations.

The extraction of psychoactive substances from the inner of the hair structure is a critical point of the analytical process, different methods have been proposed but most of them include a limited number of analytes. For hair incubation the most used method is the digestion of hair matrix with NaOH. Alternative method consists in the incubation of hair with methanol or ethanol for several hours (4 to 18). Recently, our research group has proposed Pressurized Liquid Extraction (PLE or ASE) for the extraction of illicit drugs from hair demonstrating that this method has an excellent yields in a short time.

In this work a multiclass method for the determination of both traditional drugs of abuse and NPS in hair has been developed. The extraction of drugs is based on PLE extraction while the clean-up is carried on by dispersive-Liquid Liquid Microextraction (dLLME). This miniaturized technique uses a very low amount of extraction solvent resulting in considerable enrichment factors for the tested compounds. The detection was performed by Liquid Chromatography coupled with High Resolution Mass Spectrometry (LC-HRMS). The developed method allows rapid analysis of a large number of substances with quantification limits in the low pg/mg range.

P53. SCREENING OF NOVEL PSYCHOACTIVE SUBSTANCES IN PLASMA BY LC-HRMS AND POST-RUN LIBRARY MATCHING

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Drug abuse is today a growing global problem. Often the consumers are not aware about the type of substances they are using and the correlated risks. In recent years, New Psychoactive Substances (NPS) appeared in the illicit market. These substances are new molecules, natural or synthetic, which are sold in smart shops as incense, bath salts or standard labeled "not for human use". The use of NPS, such as synthetic cathinones, cannabinoids and phenethylamines, which are known to be pharmacologically and toxicologically hazardous, has been frequently reported. More than 500 New Psychoactive Substances (NPS) have been notified in EU since 2005. The report 2016 of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reveals that the number of new substances has not declined in the past year with 100 new substances reported for the first time in 2015.

The aim of this study was the development of a Liquid Chromatography-High-Resolution Mass Spectrometry (LC-HRMS) method for a broad screening of NPS in plasma. Data acquisition was both in MS/MS and full-scan modes; the method was validated as confirmation method for 25 NPS belonging to different chemical classes (Training Set). Quantitative results have been obtained for these analytes with limits of quantification ranging from 0.03 to 0.4 ng/mL.

The method was proven to be suitable for a wide screening of additional substances, included an in-house database containing over 300 NPS and known metabolites. To this aim, a post-run library matching was conducted for every sample with the library, which may be constantly expanded with new drugs, in order to obtain an effective screening of NPS in biological matrices.

P54. EFFECTS OF DIFFERENT ZEOLITE AMENDMENTS ON SOIL MICROBIAL BIOMASS

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Natural zeolite-bearing rocks are known to be a well suitable material for agricultural purposes, however little is known about how they affect soil microbial biomass. In this work we evaluated the short-term effects of different zeolite amendments on soil microbial biomass and activity. A silty-clay agricultural soil was amended in three different ways, including the addition of natural Chabazite zeolites (NZ) in 5 and 15 wt%, respectively, and NH_4^+-enriched Chabazite zeolites (CZ) in 10 wt%. Soil pH, water content, substrate total organic carbon (C) and nitrogen (N), NH_4^+, NO_3^-, NO_2^-, dissolved organic C, total dissolved N, microbial biomass C and N and ergosterol were periodically measured over a time course of 16 days. In order to verify the immobilization of N derived from CZ into microbial biomass, the $\delta^{15}N$ signature of microorganisms was evaluated by the mean of the Extraction-Fumigation-Extraction method followed by EA-IRMS analysis. This latter investigation was possible because zeolites have been enriched with pig-slurry derived NH_4^+, which have a very high ^{15}N natural abundance that allow to trace eventual microbial incorporation.

Microbial biomass C-N immobilization, dissolved organic C and N have not been altered by amendments with both NZ amendments, but a clear increase in ergosterol content was observed for soil amended with 5 wt% of NZ, suggesting that fungal biomass was probably favored. CZ showed strong interactions with microbial biomass, as testified by high dissolved organic C and microbial biomass N. In addition, in CZ amended soil, microbial biomass N was related to nitrate production over time and inversely related to NH_4^+, suggesting high nitrification processes since the second day of incubation. Ergosterol was not altered by CZ amendments, while microbial C/N ratio was very low, suggesting a bacterial prevalence in the substrate. Finally, isotopic measurements confirmed a microbial assimilation of the N adsorbed by CZ since the second day of incubation.

P55. MERCURY SPECIATION: HPLC-ICP-MS METHOD VALIDATION AND PRELIMINARY RESULTS

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Mercury is released into the environment by both natural and anthropogenic sources. Once released, it undergoes a series of complex transformations. For instance in the aquatic environment, mercury is methylated by microbial and abiotic processes giving bioaccumulation and biomagnification phenomena across marine ecosystems. Methylmercury ($MeHg^+$) is the most common form of organic mercury in the food chain, it is highly toxic and its principal target is the nervous system. In humans the exposure occurs predominantly through the consumption of fish and other seafood products. The mercury concentration in fish is strongly related to the age and the trophic level of the species in the food chain (EFSA CONTAM Panel, 2012).

The principal aim of this study was to set up and validate an analytical method to determine organic mercury by HPLC-ICP-MS and to investigate total and organic mercury levels in fish products subjected to official control evaluating the contribution of toxic organic mercury to total mercury.

Total mercury determination was performed using Flow Injection Cold Vapour Atomic Absorption Spectroscopy after mineralization with microwave assisted acid digestion. Organic mercury was measured by HPLC separation coupled to ICP-MS detection following an acidic extraction. The chromatographic speciation method is based on the separation of Hg^{2+} and $MeHg^+$ as cysteine-mercury complexes on a polymer-based cation-exchange column. The method was validated by suitable certified marine biological reference materials: SRM 2976 mussel tissue, DORM-4 fish protein, DOLT-4 dogfish liver and BCR 463 tuna fish. The detection limits obtained were 0.010 mg kg^{-1} for total mercury and 0.016 mg kg^{-1} for organic mercury. The quantification limits established were 0.025 and 0.054 mg kg^{-1} for total and organic mercury respectively. Precision for both of them was <12% in terms of RSD_r. The accuracy of the method was assured by the analysis of certified reference materials and spiked samples.

Twenty fish samples belonging to nine different species collected from January to December 2015 were analysed with the proposed methods. Total and organic mercury concentrations ranged from 0.14 to 1.9 mg kg^{-1} and from 0.11 to 1.5 mg kg^{-1} respectively. The obtained results showed that organic mercury contributes more than 80% to total mercury concentration. The HPLC-ICP-MS method is simple, rapid, cost-effective and it has been successfully applied both to lyophilized and fresh samples. A systematic application of the method would enable the laboratory to collect more data on organic mercury about different fish and seafood of the Adriatic Sea.

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