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➢ Divisione di Spettrometria di Massa

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Programma Scientifico

Divisione Spettrometria di Massa

Lunedì 11 Settembre 2017

	Sala Cassandra
Sessione Metabolomica e Lipidomica	
Chairperson: Donatella Caruso	
9:00 – 9:30	*Apertura lavori divisione*
9:30-10:10	MAS PL01: Nico Mitro, Matteo Audano, Silvia Pedretti, Maurizio Crestani, Enrique Saez, Emma De Fabiani and Donatella Caruso *Metabolomic approaches to unravel the role of a novel mitochondrial regulator*
10:10-10:30	MAS OR01: Laura Goracci, Sara Tortorella, Paolo Tiberi, Roberto Maria Pellegrino, Alessandra Di Veroli, Aurora Valeri, Lucia Cesarini, Gabriele Cruciani *Lipostar, a novel platform-neutral cheminformatics tool for untargeted and targeted lipidomics*
10:30 – 11:00	Coffee Break
Sessione Metabolomica e Lipidomica	
Chairperson: Donatella Caruso	
11:00 – 11:20	MAS OR02: Gianluca Giorgi *Metabolic fingerprinting of plants and wines*
11:20 – 11:40	MAS OR03: Simona Scarpella, Arthur Moseley, Chris Hughes, Erik J. Soderblom, Keith Richardson, Will Thompson, Johannes PC Vissers, Jason Wildgoose, James Langridge *Qualitative and quantitative characterization of a novel DIA method for omics analysis*
11:40 – 12:00	MAS OR04: Calogera Monastero, Angela Cuttitta, Antonella Maggio, Antonio Mazzola, Santino Orecchio, Bernardo Patti *Qualitative and quantitative analysis of fatty acids extracted from pelagic species of the Sicilian Channel, comparison with endogenous variables*
12:00 – 12:20	MAS OR05: Gilda D'Urso, Cosimo Pizza, Sonia Piacente, Paola Montoro *LC-MS based metabolomics and evaluation of the antioxidant activity of Fragaria vesca leaves*
12:20 – 12:40	MAS OR06: Luisa Mattoli, Cangi Francesca, Burico Michela, Anna Gaetano, Lorenzo Tafini, Fodaroni Giada, Stella Bedont, Sara Tamimi, Denise De Carli, Simona Propersi, Veronica Ercolani, Valentina Fiordelli, Enrico Flamini, Luca Grigi *Mass Spectrometry and natural complex products metabolomic analysis*
12:40 – 13:00	MAS OR07: Mariateresa Maldini *-OMICS world: take it easy! Solutions to Advance your Metabolomics Research*
13:00 – 14:00	Intervallo Pranzo

	Sala Cassandra
Sessione Congiunta Chimica Analitica Spettrometria di Massa	
Chairperson: Tommaso Cataldi	
15:00 – 15:30	ANA/MAS KN01: Cosima Damiana Calvano, Marco Glaciale, Sara Granafei, Anna Maria Sardanelli, Luana Bellanova, Antonella Mastrorocco, Francesco Palmisano, Tommaso R.I Cataldi *Advanced mass spectrometric techniques for the untargeted lipidome characterization of fibroblasts in early on-set Parkinson's disease patients*
15:30 – 15:50	ANA/MAS OR01: Simone Nicolardi, Yuri E.M. van der Burgt, Jeroen D.C. Codée, Manfred Wuhrer, Cornelis, H. Hokke, Fabrizio Chiodo *Structural characterization of bio-functionalized gold nanoparticles by ultrahigh resolution mass spectrometry.*

15:50 – 16:10	ANA/MAS OR02: Lucia Cenci, Graziano Guella, Alessandra Maria Bossi *Molecularly imprinted materials coupled to MALDI-TOF Mass Spectrometry for the targeted analysis of peptides and proteins.*
16:10 – 16:30	ANA/MAS OR03: Rossana Scarpone, Roberta Rosato, Federico Bacá, Manuel Sergi, Dario Compagnone *Unknown and non-target analysis to determine pesticides in fruit and vegetables by means of UHPLC-HRMS (Orbitrap)*
16:30 – 17:00	Coffee Break
Sessione Congiunta Chimica Analitica Spettrometria di Massa	
Chairperson: Paola Montoro	
17:00 – 17:30	ANA/MAS KN02: Danilo Sciarrone, Antonino Schepis, Luigi Mondello *Advanced analytical capabilities exploiting isotope ratio mass spectrometry and quadrupole mass spectrometry coupled to multidimensional gas chromatography*
17:30 – 17:50	ANA/MAS OR04: Andreina Ricci, Paola Cimino, Anna Troiani, Federico Pepi, Stefania Garzoli, Chiara Salvitti and Vincenzo Barone *From ascorbic acid to furan molecules: a theoretical and experimental study on the gas phase acid catalyzed degradation of vitamin C*
17:50 – 18:10	ANA/MAS OR05: Veronica Termopoli, Pierangela Palma, Giorgio Famiglini, Maurizio Piergiovanni, Achille Cappiello *Liquid-EI (LEI) Atmospheric Pressure Mechanism for the introduction of liquid streams into an unmodified electron ionization source of a mass spectrometer.*
18:10 – 18:30	ANA/MAS OR06: Chiara Salvitti, Andreina Ricci, Federico Pepi, Stefania Garzoli, Anna Troiani, Giulia De Petris, Marzio Rosi *Selective gas-phase conversion of D-fructose to 5-hydroxymethylfuraldehyde through a base-assisted dehydration process*

Martedì 12 Settembre 2017

	Sala Cassandra
Sessione Isotopi stabili	
Chairperson: Gianluca Giorgi	
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9:40 – 10:00	MAS OR08: Antonella Macrì, Paola Iacumin *Stable isotopes in fossil remains and environmental reconstruction*
10:00 – 10:20	MAS OR09: Alessandro Pratesi, Tiziano Marzo, Damiano Cirri, Luigi Messori *Mass spectrometry and metallomics: a powerful technique to delineate the mode-of-action of anticancer metallodrugs. The case of Oxaliplatin and its analogues*
10:20 – 10:40	MAS OR10: Lionnel Mounier, Luca Simonotti, Andreas Hilkert *Chromatography-based EA-IRMS: redesigning the elemental analyzer around modern chromatographic principles to match the challenges of today's and tomorrow's applications*
10:40 – 11:00	Coffee Break
Sessione Life Sciences	
Chairperson: Nico Mitro	

11:00 – 11:40	MAS PL03: Violette Gautier, Linsey Raaijmakers, Christian K. Frese, Renske Penning, Charlotte A.G.H. van Gelder, Thierry Schmidlin, Gianluca Maddalo, Kristel Kemper, Oscar Krijgsman, Marina Mikhaylova, Riccardo Stucchi, Qingyang Liu, Harm Post, Markus Brockmann, Vincent A. Blomen, Joppe Nieuwenhuis, Elmer Stickel, Matthijs Raaben, Onno B. Bleijerveld, Lucas T. Jae, Thijn R. Brummelkamp, Shabaz Mohammed, Albert J. R. Heck, Casper C. Hoogenraad, Daniel S. Peeper and A.F. Maarten Altelaar *High-resolution proteomics, integrative phosphoproteomics and targeted mass spectrometry to unravel complex biology*
11:40 – 12:00	MAS OR11: Maurizio Piergiovanni, Achille Cappiello, Giorgio Famiglini, Veronica Termopoli, Pierangela Palma *Determination of benzodiazepines in beverages using green extraction methods and HPLC-UV detection*
12:00 – 12:20	MAS OR12: Marcello Manfredi, Eleonora Conte, Elisa Robotti, Elettra Barberis, Fabio Gosetti, Eleonora Mazzucco, Valeria Caneparo, Ester Vanni, Santo Landolfo, Marisa Gariglio, Marco De Andrea, Emilio Marengo *Quantification of Plasma Proteins with micro-LC SWATH®-MS for Biomarker Discovery in Inflammatory Bowel Disease*
12:20 –12:40	MAS OR13: Ilaria Santoro, Giovanni Sindona, Monica Nardi, Cinzia Benincasa *Improvements of extraction and identification methodologies of PUFA from algae*
12:40 –13:00	MAS OR14: Roberto Spezzano, Gaia Cermenati, Mariateresa Maldini, Silvia Pedretti, Matteo Audano, Silvia Giatti, Marzia Pesaresi, Roberto Cosimo Melcangi, Nico Mitro, Donatella Caruso *Lack of sterol regulatory element binding protein-1c induces alteration of neuroactive steroid levels in sciatic nerve*

13:00 – 14:00	Intervallo Pranzo

	Sala Paestum B
14:00 – 15:00	*Sessione Poster 1 (MAS PO01 – MAS PO05)*

Conferenze Plenarie

- MAS PL01: Nico Mitro, Università degli Studi di Milano
- MAS PL02: Federica Camin, Fondazione Edmund Mach
- MAS PL03: A.F. Maarten Altelaar, Utrecht Institute for Pharmaceutical Sciences

Metabolomic Approaches to Unravel the Role of a Novel Mitochondrial Regulator

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Metabolomics is a powerful tool to gain new insights contributing to the identification of complex molecular mechanisms in human and animal cells. Our aim was to identify novel factors regulating mitochondria with the final goal to gain further knowledge on the links between energy metabolism and key cell processes. We isolated Zinc Finger CCCH-Type Containing 10 (Zc3h10), a gene not yet linked to mitochondria, as a novel mitochondrial regulator. We found that Zc3h10 is essential for mitochondrial activity. However, its role in metabolism is still unknown. We performed transcriptomic analysis in proliferating myoblasts treated with scramble or shRNA against Zc3h10 to down-regulate Zc3h10 expression. Gene Set Enrichment Analysis (GSEA) of transcriptomic data revealed that Zc3h10 positively associated with mitochondria-related metabolic pathway (i.e. α-Ketoglutarate metabolic process). Next, we also evaluated proteomic profile in Zc3h10 knock-down myoblasts. This experiment identified a total of 3755 proteins of which 137 up- and 170 down-regulated by the silencing of Zc3h10. Gene Ontology (GO) analyses detected statistically enrichment of biological processes only for down-regulated proteins among which electron transport chain (ETC).

To gain further insights into metabolic phenotype driven by Zc3h10 knock-down in myoblasts, we used targeted metabolomic analysis. Steady-state metabolomics indicated that Zc3h10 silencing increased AMP and ADP and concomitantly decreased ATP and NADH levels. These latter data are in line with proteomic experiment showing reduced levels of some subunits of the ETC. Furthermore, we also observed reduced levels of pyruvate, α-ketoglutarate (α-KG), fumarate, malate and oxaloacetate (OAA) and increased levels of succinate. Next, we interrogated MetaboAnalyst 3.0 (1, 2) to integrate, independently, data from transcriptomic or proteomic profile with metabolomics. Both integrated analyses revealed that tricarboxylic acid (TCA) cycle is the most affected pathway.

Based on these evidences, to gain more detailed insights into mitochondrial substrate utilization we cultured myoblasts in the presence of [U-$^{13}C_6$]glucose or [U-$^{13}C_{16}$]palmitate or [U-$^{13}C_5$]glutamine. These analyses indicated that palmitate and glucose utilization, based on fully labeled and M2 acetyl-CoA levels, were not affected in Zc3h10 silenced cells. However, fully labeled glucose-derived α-KG and palmitate-derived fumarate and OAA were decreased relative to scramble controls. In addition, we observed higher levels of fully labeled glutamine-derived α-KG indicating increased anaplerosis. Isotopic enrichment was used to provide additional insight into TCA cycle activity. We observed reduced levels of glucose-derived M2 α-KG and M4 succinyl-CoA, of palmitate-derived M3 fumarate and M3 OAA suggesting a slower TCA cycle function in Zc3h10 depleted cells compared to scramble control. In addition, glutamine oxidative metabolism was also decreased as evidence from M4 malate and M4 citrate.

Taken together, these data demonstrate that Zc3h10 silencing primarily leads to altered ETC activity, which in turn, negatively impacts TCA cycle function.

Acknowledgements. Supported by the European Foundation for the Study of Diabetes (EFSD).

References: 1. Xia, J., Sinelnikov, I.V., Han, B., and Wishart, D.S. (2015). MetaboAnalyst 3.0--making metabolomics more meaningful. Nucleic Acids Res *43*, W251-257. 2. Xia, J., and Wishart, D.S. (2010). MetPA: a web-based metabolomics tool for pathway analysis and visualization. Bioinformatics *26*, 2342-2344.

Stable Isotope Ratios for Food Authentication and Traceability

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Determining the authenticity of foods means uncovering non-compliance with the established legislative standards, substitution with cheaper but similar ingredients, extension of food using adulterants, misdescription of origin (e.g. geographical), species or production method (e.g. organic vs conventional). Nowadays, the objective assessment of food authenticity is of paramount importance as consumers come into daily contact with a wide variety of foods. Traceability has thus become a cornerstone of the EU's food safety policy.

Methods for testing authenticity and providing analytical data on traceability require robust analytical techniques that can be utilised by the various regulatory authorities. Of the many techniques available, one of the most widely-used is isotope ratio mass spectrometry (IRMS), applied since around 1975 to detect adulteration of products like wine, honey, fruit juice, maple syrup, vinegar with cheaper extenders, such as sugar, or simply water. Those "traditional" applications of stable isotopes in food control rely on the analysis of the isotopic ratios of only one or two elements ($^{13}C/^{12}C$ and/or $^{18}O/^{16}O$, $^{13}C/^{12}C$ and $^{2}H/^{1}H$) and several of these methods have been officially validated and acknowledged as AOAC, CEN, EU or OIV methods. More recently multi-isotope ratio analysis ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{2}H/^{1}H$, $^{34}S/^{32}S$) sometimes combined with $^{87}Sr/^{86}Sr$ and elemental profiling and GC- or HPLC- IRMS, have been successfully applied for verifying geographical origin and organic production of food (e.g. cereal, tomato, meat, cheese) and for identifying the natural origin of flavours (e.g. vanillin) and bioactive molecules (e.g. Monacolin K).

High-Resolution Proteomics, Integrative Phosphoproteomics and Targeted Mass Spectrometry to Unravel Complex Biology

Violette Gautier[a], Linsey Raaijmakers[a], Christian K. Frese[a], Renske Penning[a], Charlotte A.G.H. van Gelder[a], Thierry Schmidlin[a], Gianluca Maddalo[a], Kristel Kemper[b], Oscar Krijgsman[b], Marina Mikhaylova[c], Riccardo Stucchi[c], Qingyang Liu[c], Harm Post[a], Markus Brockmann[b], Vincent A. Blomen[b], Joppe Nieuwenhuis[b], Elmer Stickel[b], Matthijs Raaben[b], Onno B. Bleijerveld[b], Lucas T. Jae[b], Thijn R. Brummelkamp[b], Shabaz Mohammed[a], Albert J. R. Heck[a], Casper C. Hoogenraad[c], Daniel S. Peeper[b] and A.F. Maarten Altelaar[a]

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Society is dealing with great challenges regarding the health of its population, reflected in the increase in incidences of diseases like cancer and neurological disorders. Many of these diseases have underlying DNA mutations; however, the molecular basis maintaining and expanding the disease can be found at the level of the cellular proteome. Here, expression, degradation, interactions, or localization determine protein function, and alteration in protein function can be associated with disease. The regulation of protein function is largely achieved by post-translational modifications (PTMs) on these proteins, which influence their behavior to a large extent. In particular protein phosphorylation is well known for its essential role in virtually all biological processes. Protein phosphorylation is a fast and reversible process allowing rapid signal transduction and coordinates many cellular processes through regulation of protein activity, localization, interactions, etc. The ability to rapidly add and remove phosphate groups via, respectively, kinases and phosphatases makes phosphorylation highly dynamic. Current optimized proteomics technologies allow the identification of thousands of phosphorylation sites, which can be used to infer system wide regulatory parameters. However, the high complexity of phosphorylation data in combination with the lack of functional knowledge of most phosphorylation events, severely limit the ability to understand protein activity. Therefore, alternative strategies utilizing targeted MS are gaining momentum to address specific cellular signaling events, known to be (de)regulated in disease.
Here, I will present several applications of high-resolution (integrative) proteomics as well as targeted proteomics approaches to unravel the molecular mechanisms underlying complex (disease) biology. (1-6)

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Keynote e Conferenze su Invito

- ANA/MAS KN 01: Cosima Damiana Calvano, Università degli Studi di Bari Aldo Moro
- ANA/MAS-KN02: Danilo Sciarrone, Università degli Studi di Messina

Advanced mass spectrometric techniques for the untargeted lipidome characterization of fibroblasts in early on-set Parkinson's disease patients

Cosima Damiana Calvano[a,b], *Marco Glaciale*[a], *Sara Granafei*[a], *Anna Maria Sardanelli*[c,d], *Luana Bellanova*[c], *Antonella Mastrorocco*[c], *Francesco Palmisano*[a,b], *Tommaso R.I. Cataldi*[a,b]

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Parkinson's disease (PD) is a progressive neurodegenerative disease involving the nigrostriatal pathway, where patients manifest dysfunction in motor symptoms when more than 50% of neurons are lost [1]. Although it is well recognized that alterations of lipid signaling and metabolism plays a significant role in many human diseases [2], little is known about the role of lipids associated with this specific disease. Recently, it has been reported that altered lipid pathways in the primary visual cortex and the anterior cingulate are possible in neurological disorders such as PD by analyzing post-mortem tissues from patients in advanced neuronal degeneration stage [3]. Such an approach, however, hinders the identification of the first neuronal changes. Thus, understanding the mechanisms of PD and identifying neuronal changes in the early phase of PD, by recurring to samples alternative to post-mortem biopsies, represents an urgent and challenging task.

According to their polygenic predisposition and environmental etiopathology [4], skin fibroblasts are now widely recognized as a useful model of primary human cells, capable of reflecting the chronological and biological aging of the patients. A lipidomics study of easily accessible primary human fibroblasts is presented here based on hydrophilic interaction liquid chromatography coupled to electrospray ionization-Fourier transform mass spectrometry, using both positive and negative polarities [5]. After testing different extraction protocols, the Bligh-Dyer method was shown to provide the largest number of recovered lipids. Thus, phospholipids (PL) from dermal fibroblasts of two unrelated PD patients with different parkin mutations and two controls were characterized by recurring to single and tandem MS measurements on a hybrid quadrupole-Orbitrap mass spectrometer. This untargeted approach enabled the identification of various PL classes as phosphatidylcholines (PC), phosphatidylethanolamines (PE), lysoPC, lysoPE, phosphatidylinositols, phosphatidylserines, sphingomyelins, mono-, di- and tri-hexosylceramides and ganglioside GM1, GM2 and GM3. To identify the main lipids and/or lipid classes involved in the pathological condition of PD, lipidomics data on a higher number of samples need to be collected and processed by multivariate statistical analyses. In this communication, an interesting set of preliminary findings will be reported and discussed.

Acknowledgments

This work was supported by *Fondazione Puglia* into the framework of the project "*Sviluppo ed uso di tecniche avanzate di spettrometria di massa per la caratterizzazione del profilo lipidomico cellulare e mitocondriale in fibroblasti controllo e di pazienti affetti da morbo di Parkinson*" PARLIAMS (Parkinson lipidome by advanced mass spectrometry).

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Advanced Analytical Capabilities Exploiting 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 provide information 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-IRMS, 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 (^{12}C, ^{1}H and ^{16}O) 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.

Comunicazioni Orali

Lipostar, a Novel Platform-Neutral Cheminformatics Tool for Untargeted and Targeted Lipidomics

Laura Goracci[a]; *Sara Tortorella*[a]; *Paolo Tiberi*[b]; *Roberto Maria Pellegrino*[a]; *Alessandra Di Veroli*[a]; *Aurora Valeri*[a]; *Lucia Cesarini*[a]; *Gabriele Cruciani*[a]

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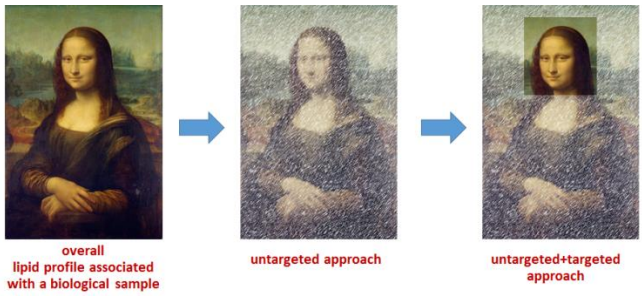
Lipid impairment can occur in the pathophysiology of diseases including diabetes, obesity, heart diseases, or neurodegenerative diseases.(1,2) Consequently, lipidomics represents an emerging field with the aim of unravelling diagnostic biomarkers, new drug targets, and of rationalizing toxicity effects. Mass spectrometry (MS), due to its sensitivity and selectivity, is the elected method for qualitative and quantitative lipidomics analysis. In addition, the recent improvements in MS technologies have moved interest from targeted to untargeted approaches. Nowadays, untargeted lipidomics is still suffering for the lack of adequate computational and cheminformatics tools that are able to support the LC/MS analysis of complex lipid mixtures from biological samples. Indeed, an *in silico* aid for untargeted lipidomics must assist peak detection from raw files, data mining, statistical analysis (including prediction), and lipid identification. To address these issues, we recently developed Lipostar, a vendor-neutral high-throughput software to support targeted and untargeted LC-MS lipidomics.(3) The major innovative points in the Lipostar algorithms are the matrix-based procedure for isotopes and adducts handling, and the use of two lipid identification approaches. The first one is database-based, and searches for matches on databases of fragmented lipids. Customized databases can be generated using a specific module in the software, and trained based on the experimental results. The second identification approach is database-independent and it mimics the manual interpretation of the experimental MS/MS spectra searching for fragments that are lipid-class-specific. When Lipostar is used for pharmaceutical and medical applications, it can be also connected with the Mass-MetaSite software for the automatic identification of drug metabolites possibly extracted with lipids. A case study on drug safety assessment will be presented to describe the general Lipostar workflow.



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Metabolic Fingerprinting of Plants and Wines

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Metabolic fingerprinting is a high-throughput technology which mostly uses advanced methods to characterize and quantify hundreds to thousands of low molecular weight analytes simultaneously, using targeted or untargeted approaches.
In metabolomics studies, mass spectrometry (MS) is widely used being a very effective methodology for identifying, characterizing and quantifying unknowns. High sensitivity, high selectivity and high specificity are some of its main features (1).
Modern MS offers an array of technologies that differ in operational principles and performance, ranging from a wide range of ionization techniques, to high resolution and many methods for inducing dissociations of ions.
A typical strategy in metabolomics is coupling MS with chromatography. So GC-MS and LC-MS are used for studying mixtures of volatile or polar compounds, respectively.
Another approach consists of direct MS analyses of sample crude mixtures without chromatographic separations. This approach, even if less informative, provides a high throughput screening tool and allows a direct comparison between different samples.
Direct MS applicability in metabolomics is broadened by advanced instrumentation capable of high resolution, accurate mass measurements, and tandem mass spectrometry methods.
In this communication, a direct MS strategy for obtaining metabolic fingerprints of plants and wines is presented. Electrospray and paper spray ionizations have been used together with tandem MS and high resolution measurements. For identification of unknowns databases have been also used.
Different classes of compounds have been identified and interesting analogies as well as differences have been observed among homogeneous classes of samples.
A correct interpretation of the data is mandatory for avoiding errors in assigning structures.

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Qualitative and Quantitative Characterization of a Novel DIA Method for Omics Analysis

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Intro
In typical data independent acquisition (DIA) methods on Q-TOF instruments, the quadrupole mass filter or ion trap either operates in wide pass mode or in stepped mode with typical transmission windows in the range of 1-20Da. Here we describe a mode of DIA operation whereby a resolving quadrupole is scanned repetitively over alternating low and elevated energy scans

Methods
The m/z range of the quadrupole was continuously and repetitively scanned with data acquired using a ToF acquisition system capable of delivering 2000 ToF spectra / s. Alternate scans contain low energy data for precursor and high energy data for fragment ions. The resulting 2D data format can be processed using both commercial and open source software for identification / quantitative results

Preliminary Data
The effect of the scanning quadrupole transmission window has been investigated to assess qualitative performance. Tryptic digest standards were injected onto a LC system and separated using a 90 minute gradient. It was found that quadrupole transmission windows of 20-30 Da provided optimum protein identifications. Over 1,000 proteins were identified from a cytosolic E.coli digest standard (4% FDR). Additional evaluations of this methodology for qualitative and quantitative proteomic analyses will be made via the analyses of two disparate sample cohorts - characterization of synaptic proteomes as part of a study of developmental brain disorders, and differential analyses of protein:protein complexes of calcineurin (Aspergillus fumigatus) as a function of mechanism-of-action of several antifungal drugs.

Novel Aspect
DIA method incorporating scanning quadrupole provides greater specificity in 'Omics experiments compared with default DIA acquisition method

Qualitative and Quantitative Analysis of Fatty Acids Extracted from Pelagic Species of the Sicilian Channel, Comparison with Endogenous Variables

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Fatty acids, especially ones in fish lipids, are very important nutritional elements for human health especially n-3 fatty acids (FAs) (1) (1.2). The interest of this research has been to acquire more in-depth knowledge to evaluate the profile ω3/ω6 (GC/MS) as function of a number of endogenous variables of anchovy (*Engraulis encrasicolus)* from Sicily Channel area, and recent literature doesn't provide any results regarding this site that is a large basin economic and social importance.

Figure 1

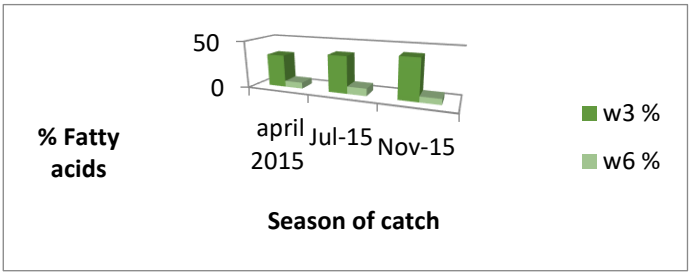

Figure 1 shows the variation of the main W3 and W6 in the muscle as a function of the season. The composition of fatty acids variability, depending on the season (2) (2.3).The values of W3 is significantly higher ($p<0.05$) than W6 independently of the period.
Figure 2 (FAs in April- maximum spawning period) (3) (3.4). The % of palmitic acid C16:0 is lower in 160-169 mm individuals (same sex) than smaller size individuals. The most important w3 shows same trend, the DHA 22:6 and EPA are higher in class 160-169 mm for same sex. This study detects a fatty acid dependence versus endogenous variables

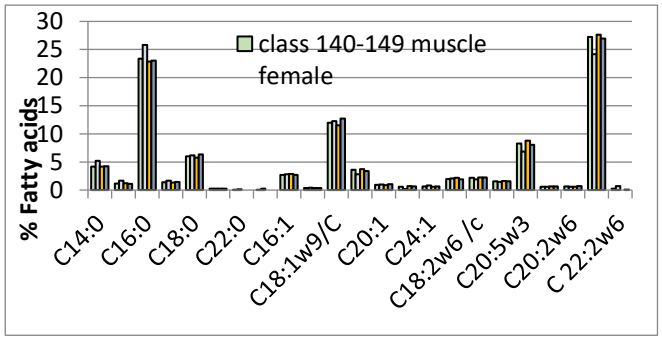

Figure 2

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LC-MS Based Metabolomics and Evaluation of the Antioxidant Activity of Fragaria vesca Leaves

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Wild strawberries (*Fragaria vesca*), one of the best dietary source of bioactive compounds, are well appreciated by consumers and represent an important economic source. For the strong antioxidant activity, they are of great interest also for nutritionists (1, 2). Southern Italy is an important producer of *F. vesca* berries, in fact in the Campania Region, mainly in the "Alburni" and the "Alto Sele" areas, they are recognized as a traditional food product under the name "Fragolina degli Alburni" (3).
In the recent years, also leaves of strawberry have received a lot of attention as potential source of bioactive metabolites that can be used for the formulation of pharmaceutical products (4).
In the present work, secondary metabolites of *F. vesca* leaves coming from populations spontaneously growing in the underwood (spontaneous) and in crop (cultivated) and from autochthonous and non-autochthonous germplasm of Campania region (Italy) were investigated, following an approach based on untargeted and targeted metabolomics by using the combination of LC-ESI-FT-MS analysis coupled to chemometrics data analysis like PCA (Principal Component Analysis) and PLS (Partial Least Square).
Moreover LC-MS metabolomics data were also combined with the antioxidant activity of each extract determined by TEAC assay. By this approach it was possible to classify the samples on the basis of their genetic factors and environmental conditions.
Leaves of *F. vesca* with autochthonous germplasm of Campania region showed higher antioxidant activity compared to the samples with non-autochthonous germplasm. Thus they represent a rich source of bioactive metabolites that can be used in specific food supplements and in cosmetic formulations.

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Mass Spectrometry and Natural Complex Products Metabolomic Analysis

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Mass Spectrometry plays a relevant role as by using HRLC-MS, GC-MS, IRGC-MS, ICP-MS it is possible to characterize globally natural complex products. Untargeted metabolomic analysis by means of ESI-MS methods with multivariate statistical analysis can be an effective tool to check batch compliance, assuring constancy on the therapeutic effect. Targeted metabolomic analysis, by using a "in house" compound library (Aboca was able to build up a library of about 1000 standards) through HRLC-MS and GC-MS methods is useful to identify and quantify as much compounds as possible, achieving the correct compositional knowledge of complex natural products. We should not forget metallomic analysis by ICP-MS (also coupled with HPLC or ionic chromatograph), as inorganic salts or organometallic complexes are naturally presents and contribute to give the characteristic bioavailability to natural complex products.

Today it is mandatory to assure efficacy and safety of natural complex products through standardized processes (following Good Manufacturing Practices, GMPs) from the raw material to the formulated products. It is obvious that together with rigorous process controls, only an adequate analytical policy can help to ensure the production chain's quality.

As it is known that all the compounds present in natural complex products contributes to their multi-targeted action and consequently to their specific effect, here it is presented how a metabolomic approach get a comprehensive panorama of natural complex product's composition, useful in routine quality control (eg.: identification test and batch release, stability monitoring program, check of production process robustness).

-OMICS world: take it easy! Solutions to Advance your Metabolomics Research

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-OMICS is a general term for a broad discipline of science and engineering for analyzing the interactions of biological information objects in various -omes. Metabolomics, as a methodology for measuring small-molecule metabolite profiles and fluxes in biological matrices, following genetic modification or exogenous challenges, has become an important component of systems biology. Because of the comprehensive nature of metabolite measurement and the capacity to detect subtle changes in a large dataset, Metabolomics has found broad application. One of the many goals of researchers in the field of metabolomics is to analyse a large number of samples and obtain the most information in shortest times and with a little or no sample preparation time. The recent progress and developments of analysis techniques are going to satisfy this demand.
The TripleTOF® and X500R QTOF systems can collect high resolution MS/MS spectra at high MS/MS acquisition rates and have excellent low mass sensitivity, making the ideal instruments for metabolomics workflows.
In addition, improved, easy to use software, methods and libraries custom-designed for targeted customer applications are available. The breadth of data acquisition capabilities is been improved by SWATH™ Acquisition, MRMHR acquisition, information dependent high-resolution MS acquisition (IDA), and high speed MS/MS scanning.

Stable Isotopes in Fossil Remains and Environmental Reconstruction

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Molluscs precipitate their shells by adding carbonate layers in isotopic equilibrium with surrounding water solutions (1). This means that the patterns and differences in the stable isotopic composition of the carbonate shells are related to molluscs habitat and lifestyle. Here we discuss the freshwater system, focusing on the identification of some variables which influence the oxygen and carbon in the carbonate of the aquatic gastropods. $\delta^{18}O_{carb}$ mainly reflects the $\delta^{18}O$ of the water and its temperature whereas $\delta^{13}C$ is a function of dissolved inorganic carbon (DIC). Malacofauna found in archaeological context provides proxy materials for environmental and climatic reconstructions (2). The occurrence of shells in stratigraphic sequence allows us to perform a diachronic analysis of long environmental history. In order to evaluate seasonal and intraspecific variations, we present a methodological investigation and a preliminary study of bulk-shells and local sample-spot of fresh-water recent and fossil molluscs from Sudan. From this study it will be possible to see how the drying process has evolved over time along the Nilotic area.

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Mass Spectrometry and Metallomics: a Powerful Technique to Delineate the Mode of-Action of Anticancer Metallodrugs. the Case of Oxaliplatin and its Analogues

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Since the discovery of the antitumor properties of cisplatin during the sixties, metal based drugs have been playing a major role in anticancer chemotherapeutic strategies. There is today a general consensus on the necessity to elucidate the mechanism of action of metal based drugs at the molecular level in such a way to rationally design novel and better anticancer metallodrugs through the so called "mechanism oriented" approach. In general, DNA is considered as the primary target for cisplatin and its close analogues (1,2) while proteins appear to play crucial roles in the transport, uptake, excretion, biodistribution, toxicity profile and resistance phenomena related to Pt drugs themselves. Even more interesting, proteins are involved in crucial aspects of the mode of action of various non-platinum anticancer agents, like ruthenium or gold complexes (3).
Metallomics is mainly concerned with the identification and characterization of all chemical species, present in a certain biological sample (a cell, a tissue or an organism), that contain the metal of interest, e.g. Pt, Au or Ru. MS represents today a fast, sensitive, specific and high-throughput tool for the analysis of biomolecules; in particular, electrospray ionization mass spectrometry (ESI-MS) potentially provides a wealth of structural and functional information mainly due to its non-destructive nature that even preserves non-covalent interactions (4). Yet, the stability of metal-protein coordination bonds may be a critical issue. This led us to build up a general protocol to test metallodrug-protein adduct stability under the typical conditions of the filter-aided sample preparation (FASP)/bottom-up procedure, ranging from the analysis of solutions containing metal-protein adducts to tandem mass spectrometry experiments. More in detail, we identified nine critical situations, either during the sample manipulations or instrumental, as a potential source of metal-protein bond impairment when using FASP operative conditions and a nanoLC-nanoESI-LTQ-Orbitrap mass spectrometer system (5).
With this experimental protocol, we successfully described the mode-of-action of some important metallodrugs. One interesting case is represented by oxaliplatin and its halido-derivatives: at variance with oxaliplatin, PtX_2(DACH) were poorly reactive toward some model proteins (Lysozyme and Ribonuclease A) while retaining a significant affinity for a representative DNA molecule (oligonucleotide). These experimental evidences, obtained through ESI-MS measurements, led us to hypothesize a structurally-related mode-of-action for these metal complexes and clearly emerged the key-role of the bidentate oxalate ligand during in recognizing the protein binding site (6).

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Chromatography-Based EA-IRMS: Redesigning the Elemental Analyzer Around Modern Chromatographic Principles to Match the Challenges of Today's and Tomorrow's Applications

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The elemental analyzer (EA) was invented by Justus Liebig in 1830 and is deeply rooted in analytical chemistry, but the steps to make it an analytical tool for biology and geochemistry came in 1968, when Carlo Erba company replaced trapping with isothermal gas chromatography using a packed GC column, and 1980, when Tom Preston put a Carlo Erba EA onto an IRMS, inventing "continuous flow-IRMS". The technique was rapidly adopted and the work flow was extended from N to C and then S as well as to H and O. In 2016, Thermo Scientific introduced the EA-IsoLink, a revolutionary change to the elemental analyzer, where every component has been examined and either optimized or redesigned, from the auto-sampler though to the TCD. The Dumas combustion products are resolved on a GC column using variable helium flow rates and temperature ramping, and for the first time, chromatographic terms (e.g. baseline and resolution) are rigorously defined. The result is improved chromatographic performance which leads to improved isotope ratio precision for every mode of measurement and for every sample size, while at the same time improving throughput and greatly reducing helium consumption. Concrete applications of EA-IRMS will be presented.

Determination of Benzodiazepines in Beverages Using Green Extraction Methods and HPLC-UV Detection

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Microextraction by packed sorbent (MEPS) and Dispersive Liquid–Liquid Micro Extraction (DLLME) with and without ultrasound assistance (UA-DLLME) were used as "green" extraction methods for the determination of benzodiazepines (BDZ) in beverages followed by HPLC-UV detection. BDZ are pharmaceutical compounds usually employed for their tranquilizing and anti-depressive effect. However, their simple availability and reduced cost make them attractive for criminal intent (1). The very low amount of sample usually available for the analyses makes the determination suitable for micro–scale extraction techniques. MEPS and DLLME are emerging techniques based on different principles, and they can be considered "green" thanks to low solvent consumption, reduced execution time and good recovery values (2,3).
MEPS, DLLME, UA-DLLME were used for the extraction of 8 BDZ (chlordiazepoxide, oxazepam, lorazepam, bromazepam, flurazepam, flumitrazepam, clobazam, and clonazepam) in three common beverages (tonic water, Spritz and red fruit juice). MEPS extraction was optimized testing various elution mixtures of solvents to yield the maximum recovery percentage. Several parameters influencing DLLME, such as type and dispersive solvent volumes, type and extraction solvent volumes, and ionic strengths were investigated and optimized to yield the highest recoveries.
The analyses were performed with a Agilent series 1100 capillary pump system with a Thermo Scientific DionexUltiMate 3400 Variable Wavelenght UV detector, 45 nL flow cell, 100 nL injection volume and an Agilent Zorbax XDB C18 (3,5 µm x 300 µm x 150 mm) column. The chromatographic separation was performed with a 4 µL/min multi-step gradient with H_2O (0,1% HCOOH) and Acetonitrile (0,1% HCOOH) and the detection was carried out at 254 nm; capillary HPLC separation with UV-detection was the analytical technique of choice because of its simplicity, robustness and wide diffusion.
The DLLME extraction was performed mixing centrifuged matrix (5500 rpm, 5 minutes), Acetone (dispersive solvent) and CH_2Cl_2 (extraction solvent) for 1 minute; then the mixture was centrifuged (5500 rpm, 5 minutes), the extraction phase was recovered, evaporated and the analyte was re-dissolved in H_2O (0,1% HCOOH). The detailed description of the method, its quantitative performance and a comparison with the MEPS results are presented. The MEPS extraction was carried out with a SGE eVol XR digital analytical syringe aspiring directly from the matrix and eluting with an Acetonitrile:H_2O 90:10 both acidified with 0,1% HCOOH. The method was validated in terms of linearity, precision, accuracy and recovery, LOD, and LOQ. Good linearity was obtained both with DLLME and MEPS with correlation coefficients (R^2) spanning from 0.996 to 0.9999. The limits of detection (LODs) of all analytes ranged from 1 ng/µL to 2.5 ng/µL. The recoveries in spiked beverages spanned from 31.7% to 69.7% for DLLME and from 62.3 % to 98.8 % for MEPS in all matrices spiked at the concentration of 20 ng/µL.

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Quantification of Plasma Proteins with Micro-LC SWATH®-MS for Biomarker Discovery in Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is a chronic inflammatory condition of unknown aetiology that can affect any portion of the digestive tract, but most frequently the terminal ileum and/or the colon. Crohn's disease (CD) and ulcerative colitis (UC) are the most common form of IBD. Genetic factors, an abnormal immune response to microbial infections and unbalance in the gut-microbiota are thought to be involved in disease pathogenesis. The diagnosis and management of IBD still presents a number of challenges: the presence of intestinal inflammation is a primary criterion for diagnosis and differentiation from other diseases. Moreover, no definitive diagnostic test exists as a gold standard, which is made on the basis of history and physical examination, supplemented with objective findings from laboratory, radiological, endoscopic and histological studies.
The interest for the quantitation of large proteomes across multiple samples has rapidly increased during the last years, especially stimulated by the development of new instruments and tools. Low-abundant human plasma proteins are considered the most promising biomarkers for disease diagnosis and therapeutic monitoring. In this research we will present the use SWATH-MS for the reliable and fast quantitation of low-abundant plasma proteins for biomarker discovery in IBD. All the plasma samples were depleted of the 14 most abundant proteins, digested and then analyzed with SWATH-MS in order to obtain a proteomic fingerprint of each patient. Through the use of chemometric and bioinformatic tools we were able to identify several new biomarkers for the early and non-invasive diagnosis of IBD, and for the discrimination of the two subclasses of Crohn's disease and ulcerative colitis. Moreover, the application of multivariate analysis identified proteins correlations with the inflammatory index and the localization of the inflammation in the intestine. The validation and the assessment of the diagnostic power of the MS-identified biomarkers were performed on an external cohort of patients using ELISA assays.
In conclusion, we demonstrated that shotgun proteomics could have a great impact on the discovery of new biomarkers.

Improvements of Extraction and Identification Methodologies of PUFA from Algae

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Marine algae are an important source of bioactive compounds like PUFA (polyunsaturated fatty acids) (2). The role of PUFA in human nutrition and disease prevention has been scientifically recognized and described. (1).
The aim of this work is the evaluation of different methodologies, suitable to be used in food and pharmaceutical industries, to properly extract lipids from algae, considering that the composition of fatty acids varies in the different algae strains.
The algae strain used in this work, Schizochytrium sp., was chosen for the high content of total polyunsaturated fatty acid in dry weight cellular.
It was shown that the highest yield of oil can be obtained by hexane/ethanol (2:8) in a soxhlet apparatus, and by CO_2 supercritical fluid extraction.
The composition of fatty acids in algae oil was evaluated by tandem mass spectrometry by ESI ionization system (3, 4).
Algae is a green sea plant used as a food and additive in many marine countries. Moreover, algae biofuels may provide a viable alternative to fossil fuels. It is becoming important in both vital application, to device modern and scientifically effective methodology to clearly identify each important component and to device suitable separation methods. The results presented here and others available from the literature indicate that it is possible to device new protocols in this important field.

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Lack of Sterol Regulatory Element Binding Protein-1c Induces Alteration of Neuroactive Steroid Levels in Sciatic Nerve

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Sterol regulatory element-binding factor-1c (Srebf-1c) is a transcription factor that controls the synthesis of fatty acids (FAs) and triglycerids. Fatty acids and cholesterol represent the most abundant myelin lipids of the peripheral nervous system (PNS). We recently described that a genetic model of reduced fatty acid synthesis, the sterol regulatory binding factor-1c knock-out mice (Srebf-1cKO), developed peripheral neuropathy over time (1). Indeed, Srebf-1cKO mice, at two months of age, displayed a neuropathic phenotype characterized by impairment of thermal and mechanical nociceptive threshold and subtle abnormalities of small unmyelinated C fibers (1). At ten months of age, we found that Srebf-1cKO sciatic nerves showed an apparent hypermyelination of small-caliber fibers due to changes in myelin periodicity resulting in myelin instability and evident Remak bundle degeneration (1). In this contest, the role of neuroactive steroids synthesis plays an important role since it's well know that the levels of neuroactive steroids are altered in various neurodegenerative diseases, including different experimental models of peripheral neuropathy (2, 3). Previously we showed that dihydroprogesterone and 3-alpha-diol are protective agents against diabetic peripheral neuropathy by regulating the *de novo* lipogenesis pathway, which positively influences myelin fatty acid profile and consequently improved myelin structure and function (4). Based on our previous observations, to prove that neuroactive steroids and fatty acid synthesis are two metabolic pathways sensitive to each other, we decide to evaluate, for the first time, neuroactive steroids levels in sterol Srebf-1cKO male mice and compared with observations in wild type animals. Neuroactive steroids levels have been evaluated by liquid chromatography tandem mass spectrometry in plasma and sciatic nerve at two and ten months of age (5). These analyses were complemented by the gene expression profile of crucial steroidogenic enzymes in Srebf-1cKO sciatic nerve of Srebf-1cKO and relative littermate control mice. Data obtained at two months of age showed an increase of pregnenolone in sciatic nerve associated with a decrease of its first metabolite, progesterone, and further metabolites (i.e., dihydroprogesterone and isopregnanolone). High levels of testosterone and 17-β estradiol were also observed. At ten months of age, the neuroactive steroid profile showed further differences. Indeed, in addition to the changes observed at two months of age, lower levels of pregnenolone and high levels of dihydroprogesterone, tetrahydroprogesterone and isopregnanolone were detected. Furthermore, the levels of testosterone and its metabolites (i.e., dihydrotestosterone, 3α -diol and 3β -diol) were significantly decreased. These results were further corroborated by gene expression analysis, which follows the same changes. Interestingly, the levels of pregnenolone and progesterone were unmodified in plasma, suggesting a specific effect of SREBF-1c on neurosteroidogenesis. Since this peripheral neuropathy is due to altered fatty acid biosynthesis, data here reported support the concept that the cross-talk between fatty acid synthesis and neuroactive steroids, may represent a possible therapeutic strategy for peripheral neuropathy. This work was supported from the Fondazione Cariplo to R.C.M. (grant number 2012-0547) and to N.M. (grant number 2014-0991)

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Structural characterization of bio-functionalized gold nanoparticlesby ultrahigh resolution mass spectrometry

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Bio-functionalized gold nanoparticles (AuNPs) have a vast field of applications. The unique properties of AuNPs functionalized with biomolecules such as peptides, proteins, lipids and carbohydrates enable innovative translational research and development in biomedicine. Current research focuses for example on the development of AuNPs for imaging, photothermal therapy, vaccination strategies and drug delivery. AuNPs in the 2-100 nm size range are typically synthesized in solution by redox reactions and can be functionalized by introducing molecules containing a thiol group to form a strong nanoparticle-sulfur bond. The structural characterization of functionalized AuNPs is challenging and requires the combination of multiple analytical techniques. Mass spectrometry (MS) has been successfully used to analyze AuNPs functionalized with small synthetic ligands with molecular mass smaller than 1000 Da. Laser desorption/ionization (LDI) and matrix-assisted LDI (MALDI) have been used in combination with time-of-flight (TOF) MS to analyzed ligands directly detached from the surface of AuNPs during the ionization process. However, TOF MS provides limited performance in terms of mass resolution and MS/MS possibilities. Thus, the analysis of AuNPs ligands has been limited to the determination of molecular mass only. To overcome these limitations, we designed an new strategy for the analysis of AuNPs based on ultrahigh resolution Fourier transform ion cyclotron resonance (FTICR) MS and a combination of LDI and MALDI. Following this strategy, we comprehensively characterized the surface chemistry of AuNPs conjugated via a thiol-ending linker to either the ovalbumin peptide (OVA 323-339), the Lewis X antigen (Galβ1-4[Fucα1-3]GlcNAcβ1) trisaccharide, the tetramannoside Manα1-2Manα1-2Manα1-3Manα1, or a mixture of both carbohydrates. We analyzed all bio-functionalized AuNPs by 15T LDI/MALDI-FTICR MS (Bruker) using 1,5-diaminonaphthalene (1,5-DAN) as a MALDI matrix. We used collision-induced dissociation (CID) to characterize the structure of pseudo-molecular ions generated by LDI/MALDI, in-depth. These included $[M+H]^+$ and $[M+Na]^+$, and importantly also $[M+Au]^+$ and $[M+2Au-H]^+$ ions which provide direct evidence for the Au-conjugation of ligands. In addition, we used our strategy to monitor proteolytic cleavage of peptides conjugated to the AuNP surface.

This study presents a novel application of ultrahigh resolution LDI/MALDI-(CID)-FTICR MS for the characterization of bio-functionalized AuNPs.

Molecularly Imprinted Materials Coupled to MALDI-TOF Mass Spectrometry for the Targeted Analysis of Peptides and Proteins

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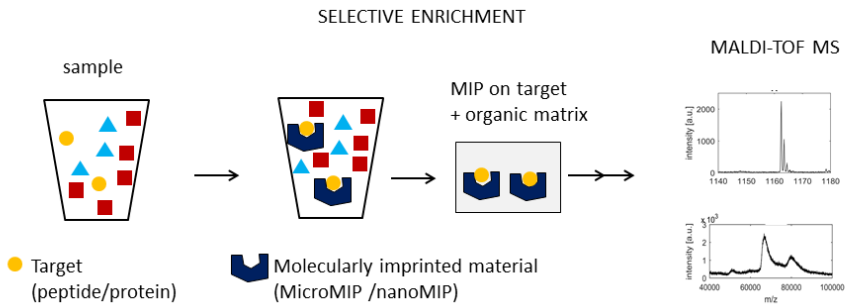
Molecularly imprinted polymers (MIPs) are a class of tailor-made biomimetic materials, prepared by template assisted synthesis: the monomers and the crosslinker are polymerized in the presence of the target analyte, called the template, thus printing onto the growing polymeric chains both the stereo and the chemical complementarity for the template. MIPs exhibit exceptional recognition properties for the template (being this a small molecule, a peptide or even a protein) with reported affinities and selectivity of the par of natural antibodies[1-3]

With the aim at improving the analytical methodologies meant for targeted proteomics and clinical applications, we propose the development of a flexible analytical platform based on the integration of MIPs to MALDI-TOF mass spectrometry (MS) for targeted protein analysis and characterized by selective enrichment,[4] high sensitivity and selectivity and short analysis times.

Libraries of micro and nano-MIPs addressed at the recognition of peptides and proteins, including responsive MIP-materials, were synthesized by radical polymerization of acrylamido-based monomers. The prepared materials were characterized physico-chemically, showing ~2 µm microMIPs and ~50 nm nanoMIPs. The MIP compositions were confirmed by XPS analysis. The binding isotherms demonstrated nanomolar affinities for the templates. At last, the micro- and nanoMIP materials were coupled to MALDI-TOF-MS.

The analytical performance of the MIP/MALDI-TOF-MS was studied by challenging the system with selected peptides and proteins, at concentrations spanning from the nano- to the pico-molar; in model solution and in real biological specimens.[5,6]

The results demonstrated the ability of the MIP/MALDI-TOF-MS hyphenation to detect in short times (few minutes) pico- to femto-moles of the target analyte straight from serum samples, with minimal sample handling, hence proving the strength of coupling micro- and nano-MIP materials to MALDI-TOF-MS, opening up innovative analytical perspectives.



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Unknown and non-target analysis to determine pesticides in fruit and vegetables by means of UHPLC-HRMS (Orbitrap)

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In the last years, high-resolution techniques have enhanced the number of pesticides and pesticide-related metabolites that can be detected in food. Conventionally, the detection of pesticides in food by means of both non-target and unknown screening methods has been accomplished by either GC-HRMS or LC-HRMS followed by data processing with specific, but limited, compound databases developed by companies working in the field (1). Empirical formulas of the molecules under investigation can be generated through a combination of parameters such as mass accuracy, isotopic clusters and ion fragments in order to be used for researches on online databases (e.g. ChemSpider).
This work reports a chromatographic-alignment mass-spectrometry-based approach to detect and identify pesticides and pesticide-related metabolites by comparison of matrix blank chromatograms ("CONTROL") with unknown sample chromatograms ("SAMPLE").
An UHPLC/Orbitrap system was used to carry out five chromatographic runs of both blank matrices (CONTROL) and sample matrices (SAMPLE) and a single FullScan-ddSM^2 chromatographic run.
The software calculated a SAMPLE to CONTROL *ratio* taking the average intensity of signals originated from unknown sample chromatograms and the signals originated from matrix blank chromatograms. SAMPLE/CONTROL ratios and p-values calculated on the SAMPLE signals were used to establish a threshold to filter out non-significant values. The unknown pesticides were then identified referring to online databases such as ChemSpider/Pesticides common Names, EPA Toxcast and FDA.
A specific software was used to confirm suggested compound identities and structures based on observed fragmentation patterns. All metabolites and/or degradation products of pesticides that can possibly be found in the samples can be investigated after identification through the method described above.
Quality control approach to test this method was made using SANTE/11945/2015 document as reference. A team of research unrelated to this experiment spiked samples of stone fruits with 36 different pesticides showing a wide range of physical-chemical properties thereby ensuring all compound classes detectable by LC-MS to be represented.
This method was compared to both a screening target method that uses a 350 compounds homemade database with 350 compounds and non-target method that uses a database with 650 pesticides from. The results provided an unambiguous identification and structural characterization of the compounds based on accurate mass measurement and informative fragmentation spectra resulting in no false positive and no false negative data. Moreover, this method allowed to detect and identify two metabolites undetected by both target and non-target approach.

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From Ascorbic Acid to Furan Molecules: A Theoretical and Experimental Study on the Gas Phase Acid Catalyzed Degradation of Vitamin C

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Degradation of L-ascorbic acid (L-AA) occurs into two types of reactions, named the non-oxidative and the oxidative. The main difference between these two pathways is that furfural is more easily produced through the former. It should be noted that the expression non-oxidative refers solely to the nature of the initial step, since subsequent transformations may involve various oxidation steps. Indeed, the oxidative pathway describes the reaction which involves as an initial step the oxidation of L-ascorbic acid to dehydro-L-ascorbic acid. Likewise, the non-oxidative pathway relates to the direct decomposition of L-ascorbic acid with exclusion of dehydro-L-ascorbic acid as an intermediate structure. In1995, furan and its derivatives were classified by the International Agency on Cancer Research (IARC) in the group 2B, as possibly carcinogenic to humans.

Since the first report in 1933, the formation of furfural from L-AA in strong acid media has been confirmed by many workers and some reaction mechanisms for the formation of furfural from L-AA have been proposed. However, none of them seems to be acceptable as the mechanism taking place in ordinary food stuffs.

Here we report on the gas-phase investigation performed by a joined mass spectrometric and theoretical approach on the acid catalysed mechanism for the formation of furan compounds in the non-oxidative degradation of L-AA. According to this approach, gaseous protonated ascorbic acid ions, $[C_6H_8O_6]H^+$, at m/z 177, were generated by Electrospray Ionization Mass Spectrometry of an ascorbic acid solution. The $[C_6H_8O_6]H^+$ ionic reactants at m/z 177 were previously structurally characterized as the ascorbic acid molecule protonated at the O2 carbonyl oxygen atom.(1)

They were subjected to collisionally activated decomposition (CAD) in order to induce the gas phase unimolecular degradation pathway of protonated ascorbic acid.

The degradation pathway emerging from the CAD mass spectrum of the precursor ion at m/z 177 shows a twofold dehydration step, 177→159 and 159→141 followed by the elimination of an $HCOOH/CO_2$ moiety, 141→95/97, leading to the formation of furanic products.

Energy Resolved CAD mass spectra allowed to obtain informations on the relative energies of degradations steps. Experimental results were compared with those of a theoretical investigation performed at B3LYP/6-31+G(d,p) level of theory highlighting the most favourable decomposition pathway. The mechanism leading to furan compounds involves dehydration, hydrolysis of the lactone ring followed by decarboxylation.

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Liquid-EI (LEI) Atmospheric Pressure Mechanism for the Introduction of Liquid Streams into an Unmodified Electron Ionization Source of a Mass Spectrometer

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We have combined, for the first time, an atmospheric pressure gas-phase conversion mechanism with new ceramic coatings to create an innovative interface, called Liquid-EI (LEI) (1). LEI is based on electron ionization (EI) but differs from previous attempts; the vaporization of solutes and mobile phase takes place at atmospheric pressure into a specifically designed region, called the "vaporization micro-channel", before entering the high-vacuum ion source. The interface is completely independent from the rest of the instrumentation, and can be adapted to any gas chromatography-mass spectrometry (GC-MS) system, as an add-on for a rapid LC-MS conversion. A ceramic liner, placed inside the vaporization micro-channel, acts as an inert, 'non-stick' vaporization surface, speeding up the gas-phase conversion of large molecules while lessening possible memory effects.
EI is an unparalleled, well-established tool for the identification of unknown gas-phase molecules. Its extension to a liquid phase, without the drawbacks and limitations that troubled this hybrid combination to date, provide the same unique advantages (library searchable mass spectra, robustness, negligible matrix effects) to LC amenable compounds, opening the door to new, challenging LC-MS applications. Deactivated silica coatings help to release the heaviest compounds to the gas-phase, improving vaporization efficiency and reducing high-temperature contact time for the most labile substances, bridging the gap between the world of classic LC-MS and GC-MS.

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Selective Gas-Phase Conversion of D-Fructose to 5-Hydroxymethylfuraldehyde Through a Base-Assisted Dehydration Process

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5-hydroxymethylfuraldehyde (5-HMF) is the main product of the thermal acid-catalysed dehydration of monosaccharides and together with other furan compounds is considered a platform molecule for the production of chemicals and fuels. Identifying alternative catalytic strategies to synthesize 5-HMF represents a key-step to increase reaction selectivity and reduce degradation-by-product yields. In this regard, mass spectrometry has proved to be an useful tool for studying reaction mechanisms in absence of solvent molecules. This approach has already been employed to investigate the acid-catalysed D-glucose and D-fructose dehydration mechanism, highlighting the formation in the gas-phase of a 5-HMF protomers and isomers mixed population (1,2). In this work the effect produced by nitrogen-containing bases on the D-fructose dehydration reaction has been evaluated using tandem mass spectrometry. Ionic complexes formed by the protonated sugar and a nitrogen base were allowed to undergo collision-induced dissociation (CID) in an ion trap mass spectrometer. The dehydration process was followed step-by-step by isolating in turn the resulting ionic intermediates that still retain the attached bases depending on their proton affinity values. The sequential fragmentation leads to the formation of $[C_6H_6O_3]H^+$ ions corresponding to a pure protonated 5-HMF population when the base loss occurs as the last reaction event (Figure 1). This evidence demonstrates the existence of a selective and effective base-assisted mechanism. Theoretical calculations are in progress in order to: i) elucidate the structures of the starting reactant ion and of the intermediates ii) validate a feasible reaction mechanism.

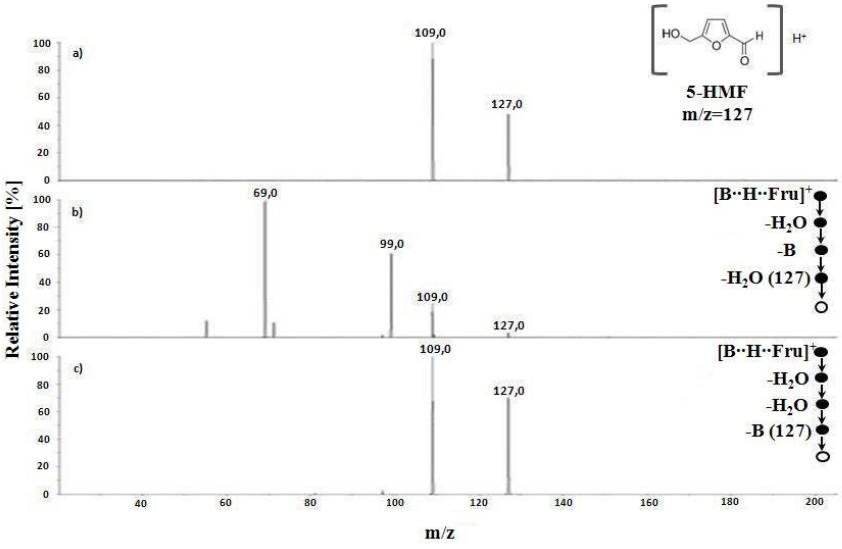

Fig. 1 CID mass spectra of a) protonated standard 5-HMF, b) ions at m/z 127 obtained after a premature base loss and c) ions at m/z 127 arising when the base loss occurs as the last reaction event.

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Comunicazioni Poster

Metabolic profiling of Sicilian Opuntia ficus indica Mill. flowers

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Opuntia ficus indica Mill. (Cactaceae) is a succulent plant native of Mexico and widely spread all over the world thanks to its capability to adapt itself to almost all types of climates. It is constituted by a modified stem made up of cladodes covered by thorns, with yellow flowers and juicy fruits of different colors (1). Even if spontaneous, it is largely cultivated for the production of its fruits, consummed fresh or dried. In Sicily, to increase the production of the fruits and also to enhance their quality, the flowers appearing during the first blooming are chopped off (a process called "scozzolatura"), in order to induce a second blooming, leading to the growth of bigger and more numerous fruits (2). Since the disposal of the discarded flowers represents a further expense to the production costs, this study is aimed at evaluating the metabolic profile of the polar fraction of the Sicilian *O. ficus indica* flowers, in order to exploit such by-products as a source of phenolics with anti-oxidant activity to employ in nutraceutical and cosmetic fields.
The hydroalcholic extract of the flowers was firstly submitted to LC-MS experiments to obtain a complete profile of the secondary metabolites and successively purified by size-exclusion chromatography and by RP-HPLC. The structures of the isolated compounds were elucidated by NMR experiments and confirmed by MS experiments. Moreover, the quantitative analysis of the major compounds was carried out by UHPLC-ESI-QqQ-MS/MS experiments using the Multiple Reaction Monitoring (MRM) approach. Finally, the total phenolic content of the extract was evaluated by Folin-Ciocalteu assay, as well as the radical scavenging activity by DPPH and TEAC assays.
The obtained results suggested that the Sicilian *O. ficus indica* flowers are a rich source of phenolic compounds with anti-oxidant activity, to be employed in the production of cosmetic formulations rich in polyphenols and in the manifacture of dietary supplements.

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Multiple MS approaches for the identification of new psychoactive substances, a case report: identification of deschloroketamine in seized sample from Genova and Torino

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The spread of new psychoactive substances is facilitated by new technologies: a large range of social networks, blogs and fora are being used to share NPS-related opinions, information, links, and experiences; moreover electronic currencies and anonymous transaction infrastructures were developed (1). This phenomenon is constantly monitored by law enforcement agencies and their resulting investigation activities require timely and qualified technical support for the repressive action. This work presents the analytical approach that led to the identification of deschloroketamine. This molecule, not directly classified as illicit drug by the Italian law, has similar hallucinogenic effects of ketamine, though potency seems lower and effects varied enormously between users (2). Deschloroketamine, along with other alkylarylcyclohexylamines (i.e. methoxetamine), is among the substances of interest in drugs abusers and vendors, as confirmed by a recent analysis of trends on cryptomarket fora (3).
In the same period, two cases from Genova and Torino required technical support for the analysis of similar white powder samples. Investigation activities suggested samples were drug of abuse, but the first GC-MS analysis did not allow any identification and the same analytical response was recorded for both samples. The mass spectrum obtained in full scan mode showed intense peaks at m/z 146, 175 and weak signal at m/z 203, so nominal mass was difficult to attribute. The same analytical method was exported to GC-QTOF instrumentation which provided accurate masses of the previously recorded peaks: in particular m/z 203.1306 at relatively low intensity. Only through the LC-HRMS analysis molecular weight of the unknown substance was attributed with greater certainty: an intense signal at m / z 204.1391 was detected by modulating suitably ionization and collision energy. Mass spectrum allowed to generate the empirical formula $C_{13}H_{17}NO$. Subsequent MS/MS fragmentation studies both in GC and LC-HRMS enabled to characterize the unknown molecule. Molecular Structure Correlator (MSC) was used: the software attempt to explain each observed fragment ion into a proposed structure using a "systematic bond-breaking" approach.
Identification of deschloroketamine in both cases was performed also through the information exchange network "National Early Warning System" activated by Department for Antidrug Policies-Presidency of the Council of Ministers. Data was compared with similar cases presented in Puglia and Liguria in 2015; recent studies have been made relating to further cases presented in Veneto (4). The essential features of the analytical approach presented were speed of the analysis and relative ease and suitability of data interpretation using MSC software and available databases: this allowed to promptly provide required technical support for the prosecution of investigations.

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Mass Spectrometry-Based Lipidomics in Different Grape Varieties

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Lipids are a large class of biomolecules with a wide range of biological activities. In plant cells, they act as storing energy, signalling molecules and as structural components of cell membranes. Furthermore, are also widely known their beneficial health effects since they prevent cardiovascular diseases (1,2).
Corvina, Raboso Piave, and Glera are grape varieties cultivated in Veneto and used to produce important Italian wines, such as Amarone and Recioto, Raboso and Raboso Passito, Prosecco.
To the best of our knowledge, reports on lipidomic profiles of these grapes are very poor, and the objective of this study was to characterize the lipid pattern, namely phytosterols (PSs), fatty acids (FAs) and phospholipids (PLs), in skin and seed extracts of these grape varieties. The study was performed by using different liquid chromatography-mass spectrometry approaches.
For phytosterols analysis, a HPLC-APCI-IT-MS/MS method was developed for determination of campesterol and stigmasterol. Fatty acids and phospholipids were analysed by using HPLC-ESI-QqQ-MS/MS instrument in SIM and MRM mode, respectively.
Different contents of PSs, FAs, and PLs were found in skin and seeds. In general, higher PSs were found in seeds. The varieties showed different profiles: major FA content was found in Glera and Raboso Piave seeds, and in Corvina and Glera skins; the highest PSs were found in Glera seeds and skin. These preliminary results indicate Glera is a grape variety richer in lipids.
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Insert references in brackets as follows: (1) (1,2,3) and add reference list at the bottom of the abstract using justified Times New Roman 10, with line-spacing 1. As indicated below:

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LC-MS/MS Analysis of a Water Cherry (*Prunus Avium* L.) Extract with Promising Radiomodulating Effects

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Human exposure to ionizing radiation is ubiquitous because of its natural occurrence and widespread use in diagnostics and therapy. Therefore, developing radioprotectors and radiorecovery drugs is of great importance in view of their potential application during both planned radiation exposure (e.g. radiotherapy) and unplanned radiation exposure (e.g. in the nuclear industry, natural background radiation emanating from the earth or other sources) (1). To this purpose, several natural plant products have been investigated (2) and antioxidant plant extracts, as well as their fractions and isolated constituents, have been shown to display important radioprotective properties. On the other hand, certain types of cancers show an inherent resilience to radiation therapy, due to pleiotropic genetic control, stem cell niches and oxygen-depleted necrotic regions (3). This has prompted a long-standing quest for radiosensitizing drugs, including many based on plant-derived polyphenols (4). Based on our previous data, showing that high doses of cherry polyphenol extracts (> 200 μg/mL) were able to target *in vitro* redox mitochondrial activity of human neuroblastoma cells (5), using different extraction conditions, involving ultrasound assisted maceration (UAM), the formulation of an aqueous extract (PaDRw) from fruits of *Prunus avium* cv. Della Recca was achieved. PaDRw exerted promising radiomodulatory properties towards neuroblastoma SH-SY5Y cell line irradiated with four graded x-ray doses (0, 0.5, 2, and 4 Gy). In fact, it was able to act as radioprotector at lower tested doses (25 and 50 μg/mL), and radiosensitizer at 400 and 500 μg/mL dose levels. To comprehensively identify the metabolites responsible of PaDRw capability, the extract underwent a simple fractionation protocol, based on the use of the Amberlite XAD-4 non-ionic polymeric resin. The simplification of the complex sample in two fractions, coupled to LC-UV-MS/MS techniques, proved to be efficient also in the disclosure of lower constituents. Quantitative analysis demonstrated that about 63% of the whole PaDRw extract was constituted by hexitol, followed by ~22.8% of fructose and ~10.7% of glucose. Phenol compounds, mainly chlorogenic acids and flavonoids, which accounted only for about 2.2%, were hypothesized to be the main actors in PaDRw-induced radiomodulation.

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LC-ESI/LTQOrbitrap/MS/MS^n analysis of the polar lipids of *Corylus avellana* (cultivar "Tonda di Giffoni") hazelnut kernel

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Corylus avellana L. (Betullaceae) is the most famous hazelnut tree. The hazelnut world production accounts for about 800,000 tons, with Turkey being the leading producer (64%), followed by Italy (13%) (1). Hazelnuts are typically consumed as whole nuts (fresh or roasted) or as ingredient for a great variety of bakery, candy and chocolate products. The kernel of the hazelnut seed is edible and has a thin, dark brown skin, which sometimes is removed before cooking.
Hazelnuts contain a great number of bioactives and health-promoting components. They are highly nutritious and contain macronutrients (lipids, proteins and carbohydrates), micronutrients (minerals and vitamins), and various phytochemicals (2). Thereby, due to their nutritional and nutraceutical properties, Food and Drug Administration (FDA) has recognized hazelnuts as "heart-healthy" foods, and several research groups reported the benefits of inclusion of hazelnuts in the human diet (3). In particular, the oil extracted from hazelnuts has proved to be able to decrease cholesterol levels in blood and to control adverse effects of hypertension. This may be due to the favourable hazelnut oil lipid profile, highly rich in MUFA (primarily oleic acid), PUFA (primarily linoleic acid), tocopherols and sterols (4). In Italy there are two hazelnut cultivars registered with the mark of Protected Geographical Indication (PGI), "Nocciola del Piemonte" and "Tonda di Giffoni (TG)". The latter is a cultivar of the Campania region, which contributes largely to the production of national hazelnut, of which it accounts for about a third.
Considering that until now no comprehensive analysis is available about polar lipids of 'TG' *C. avellana* hazelnut kernel, in the present work a detailed characterization of the lipids present in the *n*-butanol extract of fresh 'TG' hazelnut kernels (without skin) was performed by using an analytical approach based on high-performance liquid chromatography coupled to multiple-stage linear ion-trap and orbitrap high-resolution mass spectrometry (LC-ESI/LTQOrbitrap/MS/MS^n). Considering the remarkable structural diversity of lipid classes, differing in their ionization capacity and producing polarity-dependent forms of molecular anions and cations, both negative and positive electrospray ionization were used. This methodological approach enabled the analysis of a wide range of compounds from oxylipins to intact high molecular weight lipids, such as phospholipids, sphingolipids, no- and diglycosylated monoglycerides, and sulfoquinovosyldiacylglycerols.

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