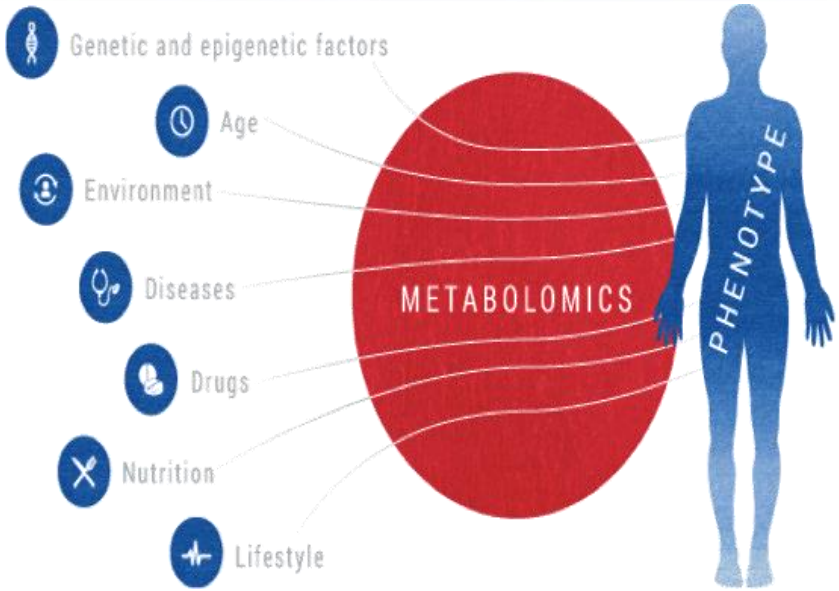
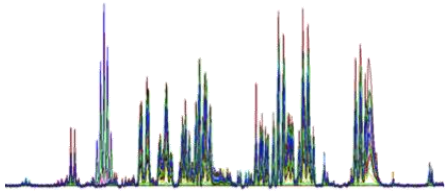




Aristotle University Thessaloniki, Greece
Bioanalysis Network, BIOMIC
http://bioanalysis.web.auth.gr/metabolomics/

SPONSORS

John S. Latsis
Public Benefit Foundation

BRUKER

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

ANALYTICAL INSTRUMENTS S.A.
Dr C.J. VAMVACAS - SCIENTIFIC EQUIPMENT

MALVA S.A.

SCIEX

ANTISEL

HELLAMCO®
SCIENTIFIC EQUIPMENT

ChromatographySpares.com

Aristotle University Thessaloniki, Greece
Bioanalysis Network, BIOMIC
http://bioanalysis.web.auth.gr/metabolomics/

Organizing Committee

Georgios Theodoridis, Aristotle University of Thessaloniki

Helen Gika, Aristotle University of Thessaloniki

Emmanuel Mikros, Athens University

Panos Vorkas, Imperial College London

Evaggelos Gikas, Athens University

Maria Klapa, ICEHT, FORTH, Patras

George Moros, Aristotle University of Thessaloniki (Secretary)

Theodoros Panagoulis, Aristotle University of Thessaloniki (Secretary)

Sunday 17/4		Fundamentals Moderators: Kvalheim/Mougios/Wilson	Speaker	Institution
8:30	9:15	**Registration**		
9:15	9:30	Introduction and Overview	G. Theodoridis	Aristotle Univ.
9:30	10:05	The Path from Profile to Mechanistic Understanding	I.D. Wilson	Imperial College
10:05	10:35	Endogenous Metabolic Personalized Theranostics	T. Lundstedt	Uppsala Univ.
10:35	11:00	MultiOmics for Exposome Analysis	D. Sarigiannis	Aristotle Univ.
11:00	11:30	**Break**		
11:30	12:00	Analysis of Metabolomics Data	O. M. Kvalheim	Bergen Univ.
12:00	12:30	Metaboscape – A Metabolite Profiling Pipeline Driven by Automatic Compound Identification, or How to Link Hram QTOF Plant Metabolomics Data to Biology	A. Barsch	Bruker, Germany
12:30	13:00	A Novel Lipid Screening Platform Allowing a Complete Solution	D. Merkel	Sciex Europe
13:00	14:30	**Lunch/ Poster Session**		
14:30	15:00	The Importance of Human Volatilome	A. Agapiou	Univ. Cyprus
15:00	15:05	Introduction to Seminars	G. Theodoridis	Aristotle Univ.
15:05	16:10	**Seminar 1** Metabolite Identification	M. Witting	Helmholtz Inst. Munich
16:10	16:40	**Break**		
16:40	17:45	**Seminar 2** Data Treatment: Freeware and Commercial Software	P. Franceschi	FEM IASMA Trento
17:45	18:00	**Closure Wrap-Up**		

Monday 18/4		Life Sciences Moderators: Kaklamanos/Raikos/Franceschi	Speaker	Institution
9:00	9:30	Exercise Metabonomics	V. Mougios	Aristotle Univ.
9:30	9:55	Feature Selection Methods for Early Predictive Biomarker Discovery Using Untargeted Metabolomic Data	E. Pujos-Guillot	INRA, France
9:55	10:20	Utilizing an IMS-DIA-MS Workflow to Characterize and Quantify the Lipidome of a Patient Cohort Clinically Diagnosed with Obesity or Diabetes	I. Edwards	Waters UK
10:20	10:40	Mitochondria and Brain Disorders: A Systems Biology Approach	M. Filiou	Max Plank Psychiatry
10:40	11:00	Pharmacometabolomics-Guided Pharmacogenomics in Precision Medicine	T. Katsila	Univ. Patras
11:00	11:30	**Break**		
11:30	12:00	Metabolomics as Part of an Integrated Approach for the Identification of Predictive Markers of Type 2 Diabetes	B. Comte	INRA, France
12:00	12:30	Metabo-Auto, A Novel Platform for Automated Metabolomic GC-MS Profiling	P. Simek	Biology Centre, CZ
12:30	13:00	Metformin treated rats show alterations in the gut microbiome and plasma levels of bile acids, lipids and small molecules	P. Vorkas	Imperial College
13:00	14:30	**Lunch/ Poster Session**		
14:30	14:45	**St1:** Biomarker Discovery in Embryometabolomics Project	C. Virgiliou	Aristotle Univ.
14:45	15:00	**St2:** A Study of Exercise and Ageing Through Metabolomics-based Analysis	O. Deda	Aristotle Univ.
15:00	15:15	**St3:** Neonatal Sepsis Biomarker Discovery	C. Chatziioannou	Aristotle Univ.
15:15	16:15	**Seminar3** Untargeted / SemiTargeted LC-MS Metabolomics	P. Vorkas A. Pechlivanis	Imperial College
16:15	16:40	**Break**		
16:40	17:40	**Seminar 4** Targeted Metabolomics LC-MS/MS	F. Michopoulos C. Virgiliou	AZ UK/ Aristotle Univ.
17:40	17:55	**Closure Wrap-Up**		

Tuesday 19/4		Plant/Food/Nutrition Moderators: Simek/Kalogiannis/ /Klapa	Speaker	Institution
9:00	9:30	Fingerprinting the Salinity Effect on Large-Scale Hydroponic Tomato Cultures Using Integrated GC- and LC-MS Metabolomics	M. Klapa	FORTH Patras
9:30	10:00	Metabolomics in Systems Biology, Integrating Multiomics Data	K. Aliferis	Athens Agricult. Univ.
10:00	10:30	Challenges on analysing double-blind crossover experiments. A nutri-metabolomics example	N. Lemonakis	Athens Univ.
10:30	11:00	Food Analysis and Authentication Using NMR Based Metabolomics	A. Spyros	Univ. of Crete
11:00	11:30	**Break**		
11:30	11:55	Recent Developments in Food Screening by NMR	C. Napoli	Bruker, Italy
11:55	12:20	LC-MS Metabolomics Shows a Smart Way to Reduce Sulfites in Wine	P. Arapitsas	FEM IASMA Trento
12:20	12:40	Bioactive Properties, Mineral Contents, Phenolic Compounds and Fatty Acid Composition of Rose, Blackberry and Redberry Fruits Growing in Belarus	M. M. Özcan	Univ. Selcuk, Turkey
12:40	13:00	Advanced Metabolomics Study of Fungal Metabolism and its Regulation	I. Kalabokis	Athens Agricult. Univ.
13:00	14:30	**Lunch/ Poster Session**		
14:30	15:15	Round Table: Users Needs a Bottom Up Approach	Food Sciences and Regulation Scientists	
15:15	16:00	**Seminar 5:** NMR Based Metabolomics. Tips And Tricks	D. Benaki A. Pechlivanis	Athens Univ./ Imperial College
16:00	16:25	**Break**		
16:25	17:30	**Seminar 6:** GC-MS Metabolomics: Best Practice for Data Acquisition, Normalization and Analysis	G. Tooulakou C. Magka	FORTH Patras
17:30	17:45	**Closure Wrap-Up, Farewell**		

POSTERS

1. **A novel platform for automated GC-MS based metabolomics.** Opekarová I., Moos M., Řimnáčová L., Zahradníčková H., Hušek P., Šimek P.
2. **A sample preparation approach for metabolic profiling of human saliva of smokers and nonsmokers by high performance liquid chromatography.** E. Barda, V.Sakkas, T. Albanis
3. **Assessment of a fast analytical methodology for urinary metabolomic biomarkers by liquid chromatography – mass spectrometry.** S. Vika, V. Sakkas, A.Makis, T. Palianopoulos, T. Albanis
4. **Boron nitrogen coumarin derivatives: Three metabolite building blocks in one small molecule.** K. Theodoridis, H. G. Gika, E. Kotali, P.A. Harris, A. Kotali
5. **Analysis of the vector's bias in the spread of Malaria in the different incidence rates.** Sungchan Kim, Sungchan Kim, Il Hyo Jung
6. **Dynamical behavior of tumor viral therapy with immune response: Deterministic and stochastic analysis.** Kwang Su Kim, Sangil Kim and Il Hyo Jung
7. **The effect of electronic cigarettes smoking cessation: A mathematical modeling approach.** Jae Hun Jung, Il Hyo Jung
8. **Exploring the link between maternal dietary protein intake and the metabolic profile of second trimester amniotic fluid.** Fotiou M., Fotakis Ch., Tsakoumaki F., Kyrkou Ch., Athanasiadou E., Tsiaka T., Dimitropoulou A., Menexes G., Tarlatzis C.B., Biliaderis G. C., Athanasiadis P.A., Zoumpoulakis P., Michaelidou A.M.
9. **LC-MS/MS metabolic profiling study of shikonin's cytotoxicity on Huh-7 cell line.** E.D. Spyrelli, A. Nakas, C. Virgiliou, A.E. Koletti, H. Gika, G. Mossialos, V.P. Papageorgiou, A.N. Assimopoulou
10. **NMR Metabolomics-based study of DSS induced colitis in rats and effect of shikonin.** A. Nakas, O. Deda, I. Taitzoglou, T. Poutahidis, G. Theodoridis, V.P. Papageorgiou, A.N. Assimopoulou, H. Gika
11. **Metabolomics in epidemiologic studies of type II diabetes mellitus: Implications for the exposome.** Xanthi D. Andrianou, Stephanie Gaengler, Konstantinos C. Makris
12. **Determination of tungsten in plant and soil samples by inductively coupled plasma-mass spectrometry.** Ümran Seven Erdemir, Hülya Arslan, Gürcan Güleryuz, Seref Güçer
13. **ICP-MS as a holistic analytical method for the study of phytoaccumulation of heavy metal: A case study of erysimum pulchellum (wild) gay.** Ümran Seven Erdemir, Hülya Arslan, Gürcan Güleryuz, Seref Güçer
14. **QSRR modeling for metabolite standards analyzed by two different chromatographic columns and by using multiple linear regression (MLR).** Ch. Zisi, I. Sampsonidis, A. Pappa-Louisi, P. Nikitas, M. Witting, H. Gika, S. Kalogiannis, G. Theodoridis
15. **NMR-driven identification of biomarkers related with blood neoplastic diseases –Analysis of data acquired in two different magnetic fields (600&700MHz).** S.A. Chasapi, L. Tenori, A. Karela, S.E. Bariamis, K. D. Marousis, A. Spyridonidis, C.Luchinat, G A. Spyroulias
16. **Comprehensive two-dimensional gas chromatography coupled with ToF-MS, a powerful tool for analysis of the volatomes of grapes and wines.** S. Carlin, K. Suklje, F. Mattivi, U. Vrhovsek
17. **"Continuous or Interval Aerobic Training" A comparative H^1 –NMR metabonomics study on athletes.** C.A. Chatziioannou, A. Zafeiridis, H. Sarivasiliou, K. Dipla, I. S. Vrabas, P. Zoumpoulakis, C. Baskakis, A. Pechlivanis, G.Theodoridis
18. **Retention index determination of 94 metabolites with LC-MS/MS: Preliminary Data** O. Begou, H. G. Gika, G. Theodoridis, M. Witting, M. Quilliam.
19. **Assessing validation parameters in multitargeted LC-MS/MS method for metabolite profiling application** E. Tsakelidou, C. Virgiliou, H. Gika, N. Raikos, G. Theodoridis
20. **Untargeted and targeted metabolomics of rat caecum extracts for the discovery of exercise biomarkers.** O. Deda**,** T. Panagoulis, H. Gika, I. Taitzoglou, V. Mougios, G. Theodoridis
21. **Study of derivatization reactions prior to GC-MS metabolomics analysis. Preliminary Results** G. Moros, A.C Chatziioannou, H. Gika, N. Raikos, G.Theodoridis

22. **Development of a Multi–Analyte hydrophilic interaction Liquid Chromatography-Mass Spectrometry (HILIC-UPLC-MS/MS) method for the quantitation of polar metabolites in Bee products** Pina A., Theodorou H., Begou O., Kalogiannis S., Tananaki C., Thrasyvoulou A., Zotou A., Gika H., Theodoridis G.
23. **Application of HILIC-UPLC-MS/MS method for the analysis of polar metabolites in honey and crude royal jelly** Theodorou H., Pina A., Begou O., Kanelis D., Dimou M., Kalogiannis S., Tananaki C., Thrasyvoulou A., Zotou A., Gika H., Theodoridis G.
24. **Differences in metabolomics profile of three exercise modes in individuals with metabolic syndrome** Siopi, A., Deda, O., Manou, V., Kellis, S., Theodoridis, G., Christoulas, K., Mougios, V
25. **Urinary Metabolomics profile of newborns of the western Greece region through high-definition NMR analysis.** S. E. Bariamis, I. Georgakopoulou, S.A. Chasapi, K. Marousis, S. Fouzas, A. Tsidoni, M. Spraul, H. Schäfer, C. Luchinat, A. Varvarigou, G.A. Spyroulias
26. **Understanding the effects of non-saccharomyces yeasts on sauvignon blanc aroma profiles using metabolomics and sensory analysis.** M. E. Beckner Whitener, J. Stanstrup, V. Panzeri, S. Carlin, B. Divol, M. Du Toit, U. Vrhovsek
27. **M-IOLITE: An Integrated suite for the streamlining metabolomic analysis.** C. Maga-Nteve, M.I.Klapa
28. **Metabolomics in brain research; a case study of adult onset hypothyroidism reveals brain regional variation and sex differentiation.** Vasilopoulou C.G., Margarity M., Klapa M.I.
29. **Studying the effects of combined high salinity and elevated CO_2 on tomato development using GC-MS and LC-MS metabolomic analysis** M.E. Papadimitropoulos, G.Tooulakou, I. Giannakos, C. Kittas, P. Kalaitzis, M. I. Klapa
30. **NMR and LC-HRMS based metabolomics for the discovery of novel bio-active small molecules from marine synbionts.** E.Baira, D. Benaki, E. Gikas, E. Mikros, N. Fokialakis
31. **A new data processing procedure of UPLC-ESI(-)-HRMS untargeted metabolomics employing spectral slicing and stitching** Baira E., Siapi E., Zoumpoulakis P ., Deligeorgis S-G., Skaltsounis A-L., Gikas E.
32. **XCMS optimization in high-throughput LC-MS**. C. Beirnaert, M.Cuykx, A.Covaci, K.Laukens.
33. **Exploitation of global microbial biodiversity for the discovery of Novel Cosmeceuticals using LC-HRMS based metabolomics** C. Almeida, N. Lemonakis, Jose Perez del Palacio, N.Tsafantakis, V. González-Menéndez, I. González, F. Reyes E.Gikas, N. Fokialakis, O. Genilloud
34. **How do I analyze a double-blind crossover study? A nutri-metabolomics example.** N. Lemonaki., M. Halabalaki., V. Mougios., E. Mikros ., N. Bliziotis .,A.L. Skaltsounis E. Gikas .
35. **Alteration in the liver metabolome of rats with metabolic syndrome after treatment with hydroxytyrosol. An MS and NMR based metabolomics study.** I.V.Dagla, N. Lemonakis, H. Poudyal, L. Brown, D. Benaki, Al. Skaltsounis, E. Mikros, E. Gikas
36. **A metabolomic study for the discovery of multiple sclerosis biomarkers in cerebrospinal fluid by HRMS and NMR** C. Tsiagkas, D. Benaki, N. Lemonakis, A. Ioannidis' S. Chatzipanagiotou, C. Nikolaou, M. Anagnostouli, E. Mikros, E. Gikas
37. **Could pharmacometabolomics uncover the resistance to clopidogrel?** A.I. Milioni, D. Benaki, M.E. Tsoumani, N. Lemonakis, E. Gikas, A.D. Tselepis, E. Mikros
38. **Integrated NMR and LC-MS plant metabolomics strategies for the investigation of acronychia genus.** E. Kouloura, O. Werz, S. Michel, M. Litaudon, D. Benaki, E. Mikros, L. Skaltsounis, M. Halabalaki.

ABSTRACTS

Oral Presentations

ENDOGENOUS METABOLIC PROFILING AS A FUNDAMENT IN PERSONALIZED THERANOSTICS.

Torbjörn Lundstedt [1,2,7], Hari Shanker Sharma[3], Thomas Moritz[4,7], Frida Troell[5], Kate Bennett[7] Katrin Lundstedt-Enkel[6,7] and Johan Trygg[5,7]
E.mail: torbjorn.lundstedt@acurepharma.com, johan.trygg@chem.umu.se, www.acureomics.com

1, Department of Pharmaceutical Chemistry, Uppsala University, Sweden, 2, AcurePharma, Uppsala, Sweden, 3, Department of Surgical Sciences, Division of Anesthesiology and Intensive Care Medicine, University Hospital, Uppsala University, Sweden, 4, Plant Science Centre, Swedish University of Agricultural Science, Umeå, Sweden. 5, Umeå Research group for Chemometrics, Institute of Chemistry, Umeå University, Sweden. 6,Department of Environmental Toxicology, Uppsala University, Sweden.7. Acureomics AB Umeå

Metabolomics has grown into an established tool in research as;

A. Diagnosis, i.e. classification
B. Identification of biomarkers in relation to e.g. diseases.
C. Dynamic studies i.e. to identify effects from e.g. medical treatment, changes in food intake, environmental or genetically changes to a living species such as human, animal, or plants.

In this presentation the use of metabonomics as a tool in drug discovery and theranostics will be highlighted. In the first part the differences in biochemical profile between healthy volunteers and persons with the diagnosis rheumatoid arthritis (RA) are discussed and identification of novel biochemical pathways for understanding the underlying factors of the disease is discussed.
In the next part a comparison to different animal models is made, in order to identify the most relevant for describing the disease in humans to be used for evaluation of novel treatments, can be drugs nutrition….
In the last part an attempt to understand the origin of the endogenous metabolites we observe in the circulating blood will be discussed.

THE IMPORTANCE OF HUMAN VOLATILOME

Agapios Agapiou

Department of Chemistry, University of Cyprus, P.O. Box 20537, Nicosia 1678, Cyprus
*E-mail: agapiou.agapios@ucy.ac.cy

A pattern of volatile organic compounds (VOCs) is daily produced and released by humans as a result of their daily normal activity. VOCs are emitted from various biological fluids and tissues; exhaled breath, skin emanations, urine, feces and saliva. This aerial chemical fingerprint is of particular importance for the researchers, as it can provide inside medical information for the status of the human body, non-invasively and in real-time. A blend of VOCs can reveal infections, cancer development, metabolic disorders, progression of therapeutic intervention, as well as individual's exposure to environmental pollutants, or toxins. The flagship of human volatilome is exhaled breath, a continues and dynamic source, producing hundreds of VOCs per hour. Various state-of-the-art analytical instruments are currently used to study human VOC biomarkers on-line or off-line (e.g. GC-MS, PTR-MS, SIFT-MS, MCC-IMS, FAIMS and sensor based systems). Nevertheless, the big challenge is the detection of VOCs outside the lab, in the hospital or in the field, by developing personalized monitoring devices. In this context, the latest research on the use of VOCs for identifying alive [1] or deceased [2] individuals after man-made or physical disasters will be presented and discussed, as an alternative method to the use of canines. Without doubt, the particular research updates and enhances crucial aspects of humans' daily routine; that of safety and security fields (search, rescue and emergency applications).

KEYWORDS: Volatile organic compounds (VOCs), odor, scent, search and rescue, medical applications.

REFERENCES:

1. A. Agapiou, A. Amann, P. Mochalski, M. Statheropoulos, C.L.P Thomas, Trends in Analytical Chemistry (2015) 66 158–175.
2. A. Agapiou, E. Zorba, K. Mikedi, L. McGregor, C. Spiliopoulou, M. Statheropoulos, Analytica Chimica Acta (2015) 883 99–108.

FEATURE SELECTION METHODS FOR EARLY PREDICTIVE BIOMARKER DISCOVERY USING UNTARGETED METABOLOMIC DATA

Dhouha Grissa[1], Mélanie Pétéra[2], Marion Brandolini[2], Amedeo Napoli[3], Blandine Comte[1] and Estelle Pujos-Guillot[1,2]*

[1]INRA, UMR1019, UNH-MAPPING, 63000 Clermont-Ferrand, France
[2]INRA, UMR1019, Plateforme d'Exploration du Métabolisme, 63000, Clermont-Ferrand, France
[3]LORIA, B.P. 239, 54506 Vandoeuvre-lès-Nancy, France
*E-mail: estelle.pujos@clermont.inra.fr

Untargeted metabolomics is a powerful phenotyping tool for better understanding biological mechanisms involved in human pathology development and identifying early predictive biomarkers. This approach, based on powerful analytical platforms, such as mass spectrometry, chemometrics and bioinformatics, generates massive and complex data that need appropriate analyses to extract biologically meaningful information [1]. In this context, this work consists in designing a workflow describing the general feature selection process, using knowledge discovery and data mining methodologies to propose advanced solutions for predictive biomarker discovery.

Data were collected from a mass spectrometry-based untargeted metabolomic approach performed on subjects from a case/control study within the GAZEL French population-based cohort. Different feature selection approaches were applied either on the original metabolomic dataset or on reduced subsets. The strategy was focused on evaluating a combination of numeric-symbolic approaches for feature selection with the objective of obtaining the best combination of metabolites, producing an effective and accurate predictive model. Relying first on numerical approaches, and especially on machine learning methods (SVM and RF-based methods) and on univariate statistical analyses (ANOVA), a comparative study was performed on the original metabolomic dataset and reduced subsets. As resampling method, LOOCV was applied to minimize the risk of overfitting. The best k-features obtained with different scores of importance from the combination of these different approaches were compared and allowed determining the variable stabilities using Formal Concept Analysis.

The results revealed the interest of RF-Gini combined with ANOVA for feature selection as these two complementary methods allowed selecting the 48 best candidates for prediction. Using linear logistic regression strategy on this reduced dataset enabled us to obtain the best performances in terms of prediction accuracy and number of false positive with a model including 5 top variables. Therefore, these results highlighted the interest of feature selection methods and the importance of working on reduced datasets for the identification of predictive biomarkers issued from untargeted metabolomics data. Even if they are still not usually applied, these data mining methods are essential tools to deal with massive datasets and contribute to elucidate complex phenomena associated with chronic disease development. With this help, the experts of the scientific field will go deeper in interpretation, attesting the success of the knowledge discovery process.

Project funded by the INRA DID'IT Metaprogramme.

KEYWORDS: feature selection, untargeted metabolomics, biomarker discovery, prediction

REFERENCES:

1. Xi B, Gu H, Baniasadi H, Raftery D. *Methods Mol Biol* (2014) **1198**(1):333-53.2.

MITOCHONDRIA AND BRAIN DISORDERS: A SYSTEMS BIOLOGY APPROACH

Michaela D. Filiou*

Department of Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, Munich, Germany
*mfiliou@psych.mpg.de

Data-driven systemic approaches are pivotal to identify candidate biomarkers for disorders with no fully understood underlying molecular mechanisms. Here, we present a multi-omics platform based on quantitative proteomics, SRM-based metabolomics and *in silico* pathway analysis to identify affected molecular systems in mouse models of brain disorders.

We discuss the application of this platform in two animal models of stress-related pathologies. Hypothesis-free analysis of brain tissue revealed implication of mitochondria in stress-related phenotypes. Selective mitochondrial targeting resulted in attenuation of stress-related symptoms and metabolomics analysis revealed brain and plasma signatures relevant to these stress-relieving effects.

This work highlights the potential of combining hypothesis-free, high throughput metabolomic and proteomic technologies for biomarker discovery and identification of novel therapeutic targets.

KEYWORDS: SRM metabolomics, quantitative proteomics, mitochondria, pharmacological targeting, biomarker discovery, neuropsychiatric disorders

REFERENCES:

1. Lopes S, Teplytska L, Vaz-Silva J, Dioli C, Trindade R, Morais M, Webhofer C, Maccarrone G, Almeida OFX, Turck CW, Sousa N, Sotiropoulos I, Filiou MD Cereb Cortex (2016) in press

2. Nussbaumer M, Asara JM, Teplytska L, Murphy MP, Logan A, Turck CW, Filiou MD Neuropsychopharmacology (2015) Epub Nov 16.

3. Filiou MD, Asara JM, Nussbaumer M, Teplytska L, Landgraf R, Turck CW J Psychiatr Res (2014) 58 115-122.

4. Filiou MD, Zhang Y, Teplytska L, Reckow S, Gormanns P, Maccarrone G, Frank E, Kessler MS, Hambsch B, Nussbaumer M, Bunck M, Ludwig T, Yassouridis A, Holsboer F, Landgraf R, Turck CW Biol Psychiatry (2011) 70 1074-1082.

PHARMACOMETABOLOMICS-GUIDED PHARMACOGENOMICS IN PRECISION MEDICINE

Theodora Katsila and George P. Patrinos
University of Patras, School of Health Sciences, Department of Pharmacy, University Campus, Rion, 26504, Patras, Greece; Email: thkatsila@upatras.gr

Inter-individual variability has been a major hurdle to optimize disease management. Precision medicine holds promise for improving health and healthcare via tailor-made therapeutic strategies. Herein, we outline the paradigm of pharmacometabolomics-guided pharmacogenomics. We envisage merging pharmacometabolomic and pharmacogenomic data (to address the interplay of genomic and environmental influences) with information technologies to facilitate data analysis as well as sense- and decision-making on the basis of synergy between artificial and human intelligence. Humans can detect patterns, which computer algorithms may fail to do so, whereas data-intensive and cognitively complex settings and processes limit human ability. We propose that better-informed, rapid and cost-effective -omics studies need the implementation of holistic and multidisciplinary approaches.

KEYWORDS: PHARMACOGENOMICS, PHARMACOMETABOLOMICS, PRECISION MEDICINE

REFERENCES:

1. Katsila *et al*. EBiomedicine (2016)

METABOLOMICS AS PART OF AN INTEGRATED APPROACH FOR THE IDENTIFICATION OF PREDICTIVE MARKERS OF TYPE 2 DIABETES

E Pujos-Guillot[a,b], M Brandolini[a], D Grissa[b], Y Liu[b], M Pétéra[a], C Joly[a], B Lyan[a], S Czernichow[c,d,e], M Zins[c], M Goldberg[c,f], B Comte[b*]

[a]INRA, UMR1019, Plateforme d'Exploration du Métabolisme, UNH, F-63000 Clermont-Ferrand, France; [b]INRA, UMR1019, UNH-Mapping, F-63000 Clermont-Ferrand; [c]UMS11 Cohortes en population, INSERM/UVSQ, Villejuif, France; [d]Service de Nutrition, Hôpital A. Paré, APHP, Boulogne-Billancourt, France ; [e]Université Versailles Saint Quentin, Versailles, France, [f]Université Paris Descartes, France.

*E-mail: blandine.comte@clermont.inra.fr

The trajectory and underlying mechanisms of human health are determined by a complex interplay between intrinsic and extrinsic factors. Its evolution is a continuum of transitions, involving multifaceted processes at multiple levels and there is an urgent need for integrative biomarkers that can characterize and predict health status evolution. The objective of the present study was to identify accurate and robust multidimensional markers, predictive of type 2 diabetes (T2D). A case-control approach was used within the French population-based cohort GAZEL (n~20,000) [1]. Male overweight subjects (n=112, 25≤BMI<30 kg/m², 52-64 y.o.), free of T2D at baseline, were selected. Cases were defined as having developed T2D at follow-up (5 years later) and were compared for several parameters (clinical, biochemical parameters, and food habits) with Controls matched for BMI, age, and sex. Baseline serum samples were analyzed using mass spectrometry-based untargeted metabolomics [2]. Data mining methods were used to select the best candidate for prediction. Models were built using linear logistic regressions on the resulting reduced dataset and their performances were determined by calculating the area under the receiver operating characteristics curve (AUC), along with their 95% confidence intervals (CI), as well as sensitivity and specificity values. Metabolomic data were integrated with the different parameters from the database in order to determine whether multidimensional models improve prediction. Associations between food habits, clinical parameters and serum metabolites were investigated using correlation networks. Clinical data showed significant differences between Cases and Controls regarding several parameters: body mass index (p=0.046), waist/hip ratio (p=0.005), blood pressures (p=0.005), fasting blood glucose level (p=5E-9), and consumption frequencies of vegetables (p=0.016) and sugar (p=0.0003). Thanks to its broad phenotyping power, the untargeted metabolomics approach allowed the identification of 5 predictive biomarkers. The resulting metabolite-only model showed better performances than the one built with clinical data: a lower misclassification rate (18% *vs* 26%) and a higher AUC (0.82 vs 0.74; CI: [0.748-0.892] *vs* [0.659-0.823]). Integration of metabolomic data with the available clinical and biochemical parameters allowed optimizing the prediction performances (10.8% misclassification, AUC=0.89, CI: [0.833-0.950]). Correlation network analyses contributed to explore the links between metabolic, clinical parameters, and food habits. Therefore, metabolomic markers when combined with clinical characteristics appeared to offer the possibility of better prediction of disease development. These results show the interest of an integrated approach including untargeted metabolomics in the discovery of predictive biomarkers 5 years before T2D occurrence. They should provide new tools to better stratify at-risk populations, as well as additional knowledge on T2D understanding. Project funded by the INRA DID'IT Metaprogramme.

KEYWORDS: metabolomics, biomarkers, prediction, type 2 diabetes, cohort

REFERENCES:

1. Goldberg M, Leclerc A, Zins M. *Int J Epidemiol* (2015) **44**(1):77-g.

2. Pereira H, Martin JF, Joly C, Sebedio JL, Pujos-Guillot E. *Metabolomics* (2010) **6**(2):207-18

MetaboAuto- A Novel Platform for Automated GC-MS and LC-MS Based Metabolomics

P.Šimek[1], M. Moos[1], I. Opekarová[1,2], and H. Zahradníčková[1]
[1]Biology Centre, Czech Academy of Sciences, Ceske Budejovice, Czech Republic
[2]Institute of Chemistry and Technology, Prague
E-mail: psimek@bclab.eu

A novel fully automated platform called MetaboAuto has been developed for comprehensive metabolite profiling. A modular design constructed on the RCT autosampler corpus (CTC, Analytics, Zwingen, Switzerland) enables fully unattended processing of liquid samples by their in-situ treatment with an alkyl chloroformate and concurrent liquid liquid microextraction of the metabolites for GC-MS as well as LC-MS analysis. The in-house written program called Robolab controls about 30 operations involving sample aliquot transfers, addition of internal standards, reagents, vortexing, dilution, mixing, withdrawal of an organic layer, exchange and cleaning of syringes followed by injection of the final sample extract. The designed sample preparation workflow and instrumental analysis proceed simultaneously in less than 20 min, thus enabling unsupervised GC-MS or LC-MS analysis of at least 72 samples per day. The analytical platform performance has been evaluated on profiling of more than 200 metabolites by various MS instruments in biofluids and tissue extracts with the option chiral amino acid analysis.

Financial support: the Czech Technology Agency, project No.TA04011751.

KEYWORDS: metabolomics, automation, GC–MS, LC-MS, sample preparation.

METFORMIN TREATED WISTAR RATS DEMONSTRATE REMARKABLE ALTERATIONS IN THE GUT MICROBIOME AND PLASMA LEVELS OF CONJUGATED BILE ACIDS, OTHER LIPIDS AND SMALL MOLECULES

Panagiotis A Vorkas [A], Perrine Masson [B], Arnaud M Wolfer [A], Erwan Werner [B], Jeremy K Nicholson [A], Bernard Walther [B], Elaine Holmes [A,*]

[A] Biomolecular Medicine, Division of Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London SW7 2AZ, UK

[B] Technologie Servier, 27 Rue Eugène Vignat, Orleans 45000, France

*E-mail: elaine.holmes@imperial.ac.uk

Background: Type 2 diabetes is one of the greatest threats to public health. The beneficial effects of the metformin drug towards mitigating disease manifestation and reducing hyperglycaemia have been connected to gut microbiota and inflammation onset. At the same time metformin has been suggested as an anti-cancer and anti-ageing drug. To our knowledge, this is the first study using comprehensive metabolic profiling in combination with gut microbiota metataxonomic information on metformin treatment.

Materials and Methods: A total of 12 animals were treated with metformin for 5 weeks and 12 were used as controls. Treatment was preceded by a two-week acclimatization period and followed by a two-week recovery period. Plasma and faecal samples were collected every week. Two untargeted UPLC-TOF-MS methods were used for analysing the plasma samples: a reversed-phase lipid profiling method and a polar metabolic phenotyping method using hydrophilic interaction liquid chromatography. A semi-targeted UPLC-TOF-MS method was utilised for bile acid profiling. MiSeq sequencing on faecal DNA was employed for assessing gut microbiome alterations. Data were deconvoluted and analysed by time series statistical analysis.

Results and Discussion: Alterations in the composition of the gut microbiome and conjugated secondary bile acids were observed from the first week of treatment. Small metabolites and lipids demonstrated significant alterations at a later stage (2nd–3rd week). Significantly altered bacterial species were highly correlated to sulfated and tauro-conjugated bile acids. Furthermore, phosphatidylcholines, monoglycerols and free fatty acids were significantly altered and correlated to the gut microbiome changes. In concordance with literature, pro- and anti-inflammatory lipid precursors were reduced in plasma. In general, levels of altered metabolites appeared to recur to control levels after the two week recovery period. This study may provide further insights in the molecular mechanisms (host and microbe-host) underlying metformin action, as well as plasma markers reflecting modifications in gut microbiota.

KEYWORDS: metformin, metabonomics, metabolomics, lipidomics, metataxonomics, gut microbiota, bile acids

EMBRYOMETABOLOMICS

Christina Virgiliou[a], KaterinaChatzimeletiou[b], Leandros Lazaros[c], Apostolos Athanasiadis[b], George Makrydimas[c], Ioannis Georgiou[C], Helen Gika[b], Nikolaos Raikos[b], Basil C. Tarlatzis[b], Georgios Theodoridis[a] Embryometabolomics Consortium[d]

[a]Department of Chemistry, Aristotle University Thessaloniki, 541 24 Thessaloniki, Greece
b Department of Medicine, Aristotle University Thessaloniki, 541 24 Thessaloniki, Greece
c Department of Medicine, University of Ioannina, Greece
d http://www.embryometabolomics.eu/

cvirgiliou@chem.auth.gr

The object of embryometabolomics project is the investigation of the potential of metabolomic profiling as a diagnostic tool for the assessment of embryo viability before implantation in IVF and its growth during pregnancy. In-vitro fertilisation (IVF) represents a major sector of health industry, however success rates remain relatively low. The metabolic content of human embryo culture media could provide information related to embryo viability and biochemical markers that could aid to the selection of embryos with highest implantation potential. Pre-term birth represents an important health problem that can occur for a variety of reasons such as multiple pregnancies, infections and chronic conditions. Mapping different biological fluids, obtained from pregnant women, could allow useful correlations with the fate of the pregnancy. In Embryometabolomics project more than 2000 samples (IVF culture medium, maternal blood/urine, amniotic and coelomic fluid) were analysed using different instrumentation techniques in Holistic and targeted analysis modes. Data was treated with advanced bioinformatics tools in order to find underlying trends in biocemistry and reveal differentiating biomolecules. Multivariate statistical analysis principal component analysis (PCA), Partial least squares discriminant analysis (PLS-DA) and advanced visualisation tools were used for data scrutiny, visualisation and evaluation of findings.

A hydrophilic interaction liquid chromatography (HILIC) MS/MS method was developed in our laboratory for profiling and quantitation of key end- point metabolites in complex matrices. The method provides quantitative data for circa 100 metabolites on MRM mode. The list of analytes included carbohydrates, amino -acids, organic acids and amines. The method was validated in terms of accuracy, precision, repeatability linearity and detection sensitivity. Holistic approach was conducted by the use of UPLC-HRMS-TOF-MS in both RP and HILIC modes for complementary assessment of the metabolic content of biological fluids in untargeted analysis mode. Spent IVF culture media were additionally analysed by applying an untargeted method, specifically developed for that particular matrix, using GC-MS.

Results from both targeted and untargeted analysis reveal trends relating to the metabolic content of the analysed samples with the study hypothesis. Embryo's developmental potential is reflected in the metabolic content of IVF spent culture media. Clear differences were observed in the metabolic content of bio-fluids from women with full versus those with pre-term delivery. Both amniotic fluid and serum proved promising for the identification of biomarkers related to the fate of the pregnancy and embryo growth.

This research has been co-financed by the European Union (European Social Fund- ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) Research Funding Program: Thales II.

Keywords: Metabolomics, IVF, pre-trm delivery, Targeted untargeted metabolomics

A STUDY OF EXERCISE AND AGEING THROUGH METABOLOMICS-BASED ANALYSIS

O. Deda[a*], H. Gika[b], I. Taitzoglou[c], V. Mougios[d], G. Theodoridis[a]

[a] School of Chemistry, [b] School of Medicine,[c] School of Veterinary Medicine,

[d] School of Physical Education and Sport Science,

Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

*oliadmy@gmail.com

Holistic analysis provides new perspectives in metabolic studies and in their application in exercise sciences [1]. The fact that physical exercise modifies both human and animal metabolome has been in the focus of recent research. Urine, plasma, skeletal muscle, liver and other tissues have been analyzed to reveal metabolic changes between exercise and sedentary condition. Recently fecal metabolomics is attracting interest in clinical biochemistry for the investigation of the effect of gut microbiota symbiosis with host. It has also been reported that gut microbiota is altered in exercised rats [2]. For examples exercise in combination with diet intervention reduces secondary bile acids in rat feces and the risk of colorectal cancer [3]. Moreover, the ageing process constitutes another important modifier of the metabolome. Low-intensity swimming presents some benefits in contrast to age-related changes in the metabolome of rat model [4].

The present metabolomics study aimed in the investigation of the alterations of metabolic profiling derived from life-long low-intensity swimming of rats. Longitudinal sampling helped to reveal age-related metabolic changes in both exercise and control groups. Sixty female Wistar rats were divided into 4 groups; life-long training, no-exercise, training until the age of 15th month and training from the 15th until the 21st month of age. The training protocol consisted of swimming, 18-20 min per day, 3-5 days per week, with load of 0-4% of their weight attached to their tail, depending on their age.

To investigate the connection of metabolic pathways of exercise and ageing, targeted HILIC-MS/MS metabolomics-based analysis was performed. Peak tables and quantitative data were analyzed in multivariate statistical analysis. Exercise intervention separated rat metabolic profiles (exercised versus controls) of the tested biosamples (urine, whole blood, fecal samples and gut tissues). Rat feces differed not only due to exercise, but also due to ageing along the 3 sampling time points, showing a clear effect of age on rats' fecal metabolome. The way exercise, ageing and their combination reflect on the metabolome is challenging to clarify. The associated biochemical mechanisms offer promising target for intervention.

Key words: Metabolomics, LC-MS, rats, fecal samples, exercise, ageing

References

1. Bassini A, Cameron LC (2014) Sportomics: building a new concept in metabolic studies and exercise science. Biochem Biophys Res Commun 445:708–716. doi: 10.1016/j.bbrc.2013.12.137

2. Matsumoto M, Inoue R, Tsukahara T, Ushida K, Chiji H, Matsubara N, Hara H (2008) Voluntary running exercise alters microbiota composition and increases n-butyrate concentration in the rat cecum. Biosci Biotechnol Biochem 72:572–576. doi: 10.1271/bbb.70474

3. Hagio M, Matsumoto M, Yajima T, Hara H, Ishizuka S (2010) Voluntary wheel-running exercise and dietary lactose concomitantly reduce the proportion of secondary bile acids in rat feces. J Appl Physiol. doi: 10.1152/japplphysiol.00777.2009

4. Ravi Kiran T, Subramanyam MVV, Asha Devi S (2004) Swim exercise training and adaptations in the antioxidant defense system of myocardium of old rats: relationship to swim intensity and duration. Comp Biochem Physiol B Biochem Mol Biol 137:187–196. doi: 10.1016/j.cbpc.2003.11.002

NEONATAL SEPSIS BIOMARKER DISCOVEY

Chrysovalantou–Anastasia Chatziioannou[1], Kosmas Sarafidis[2], Agathi Thomaidou[2], Helen Gika[3], Aggeliki Kontou[2], Elisavet Diamanti[2], Charalambos Agakidis[2], Vasiliki Drossou[2], Nikolaos Raikos[3], Georgios Theodoridis[1]

1 School of Chemistry, Aristotle University of Thessaloniki, Greece

2 1st Department of Neonatology, School of Medicine, Aristotle University of Thessaloniki, Greece

3 Laboratory of Forensic Medicine and Toxicology, School of Medicine, Aristotle University of Thessaloniki, Greece

Despite advances in neonatal care over the last decades, neonatal sepsis remains a very serious threat for the health and life of neonates. The main difficulty in diagnosing microbial late-onset (LOS) sepsis is the fact that its symptoms are similar to several non-infectious conditions. Most of the known biomarkers evaluated in LOS lack high diagnostic accuracy hindering early and prompt initiation of treatment. Although antibiotics remain the cornerstone of sepsis treatment, their rational use is also very significant, given the adverse effects and antimicrobial resistance. Thus, early and accurate diagnosis of neonatal sepsis is crucial for its management. The aim of our project was to determine significant changes of the metabolites in septic neonates as compared to non-septic ones (controls) hospitalized in the NICU setting using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Development of such profile could possibly enable fast analysis with robust and reliable results assisting thus clinicians in decision making.

Urine samples were collected at the time of initial diagnosis of LOS from 18 neonates with LOS and 18 neonates controls. All metabolites were analysed by UHPLC-MS/MS involving extraction of 50 μL urine with acetonitrile (deproteinization). Chromatographic separation was performed on a Acquity BEH Amide column (150×2.1mm i.d., 1.7 μm) with a flow rate of 0.5 mL/min. Mass spectrometry parameters were optimized for each of the 108 pre-selected analytes (the set included aminocadis, organic acids, sugars, nucleosides, amines and other molecules). Data were processed with multivariate (SIMCA 13) and univariate statistics (ANOVA).

PLS-DA showed clear separation between septic and control neonates. Statistical models were controlled for validity with permutation and other tests. Visualisation plots (S-plots, VIP, box plots) and ANOVA tests verified noteworthy variations in the levels of 24 metabolites.

Results of the metabolomics analysis showed that neonates with LOS show different metabolic profiles from those without sepsis allowing their discrimination with the use of LC-MS/MS-based urine metabolomic analysis. Monitoring and quantitation of specific metabolites could help clinicians in applying the optimal treatment in septic neonates.

Keywords: sepsis, metabonimics, neonatal

Metabolomics in Systems Biology: Integrating Multi-omics Data

Konstantinos A. Aliferis[A,B*], Ioannis Kalambokis[A], Suha Jabaji[B]

A: Laboratory of Pesticide Science, Agricultural University of Athens, Votanikos, 118 55 Athens, Greece
B: Department of Plant Science, McGill University, Montreal, Quebec, H9X 3V9, Canada

*E-mail: konstantinos.aliferis@aua.gr
konstantinos.aliferis@mcgill.ca

The complexity of the metabolism of a biological system necessitates the employment of multi-level approaches and integration of the obtained information for a comprehensive understanding of its regulation and responses to biotic or abiotic stimuli. Such integration requires solid expertise in state-of-the-art statistical methods and/or bioinformatics software. According to the "guilty-by-association" principle, sets of transcripts, proteins, and metabolites, is expected to be under the control of a shared regulatory mechanism. In other words, the discovery of functional links between transcripts, proteins, and metabolites whose levels follow a similar stimulus-specific pattern is foreseen. Based on the central dogma of molecular biology, genomics, transcriptomics, and proteomics provide information on what is expected to happen in a biological system, whereas metabolomics represents the level that best describes what is actually happening being closest to the phenome. Therefore, metabolomics should be a key element in multi-level "omics"-based research. Here, an example on the integration of metabolomics with transcriptomics data sets, which represent the upper and lower ends of "omics" hierarchy, respectively, of a plant-pathogen pathosystem applying orthogonal 2 projection to latent structures (O2PLS), is presented. O2PLS is bidirectional (i.e., X↔Y) enabling high-level data fusion and the discovery of trends between "omics" data sets (1-3). The main objective of such approach is the detection of the joint (or predictive) variation between metabolomics and transcriptomics data sets. This approach reveals structures of metabolite and RNAseq data sets that are possibly under the control of a shared regulatory mechanism. Details on data pre-processing and analyses are presented. Applying a reverse genetics approach, the discovery of the joint systematic variation and the assignment of transcript-to-metabolite links employing powerful bioinformatics tools for the visualization and processing of complex networks, facilitates the in depth understanding and global overview of metabolism and the regulatory networks in the plant-pathogen pathosystem being studied.

KEYWORDS: metabolism regulation, multi-level data integration, O2PLS

REFERENCES

1. Trygg, J. & Wold, S., *J. Chemometrics*, (2003), 17: 53-64.
2. Bylesjö, M., Eriksson, D., Kusano, M., Moritz, T. & Trygg, J., *The Plant Journal*, (2007), 52: 1181-1191.
3. Copley, T.R., Aliferis, K.A., Kliebenstein, D.J. & Jabaji, S., *Plant and Cell Physiology*, (2016), Subm.

FOOD ANALYSIS AND AUTHENTICATION USING NMR BASED METABOLOMICS

M. Amargianitaki, E. Manolopoulou, E. Ralli, A. Kolesnikova, G. Alexakis

and A. Spyros*

NMR Laboratory, Department of Chemistry, University of Crete,

Heraklion Crete, Greece

*E-mail: aspyros@uoc.gr

The excellent analytical capabilities of NMR spectroscopy have transformed it to indespensable tool in food analysis during the last few decades. [1] Apart from the qualitative and quantitative chemical characterization of food ingredients, which is important from a nutritional and quality control point of view, NMR spectroscopy has found increased use by providing spectroscopic input data for multivariate statistical analysis techniques that can be employed to study a broad range of food analysis problems, including authenticity, protected denomination of origin, adulteration, sensory evaluation, etc. [2]

In this contribution we will present some applications of NMR metabolomics currently in progress in our laboratory, including the metabolic profiling, authentication, quality control and sensory evaluation of several foods of special importance for the local Cretan island economy (wine, graviera cheese, olive leaves, tsikoudia and juices).

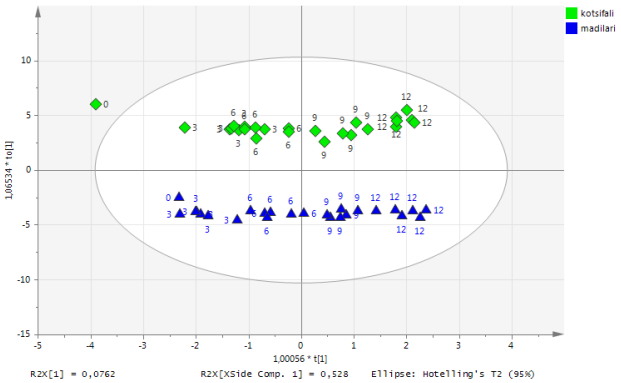


Figure: OPLS modeling of red wine aging by NMR metabolomics

KEYWORDS: NMR spectroscopy, food analysis, multivariate statistical analysis models

REFERENCES

1. Spyros, A., Dais, P. (2013) NMR Spectroscopy in Food Analysis, Royal Society of Chemistry, Cambridge, UK.
2. Mannina, L., Sobolev, A. P. and Viel, S. Progress in Nuclear Magnetic Resonance Spectroscopy (2012) 66, 1-39.

LC-MS METABOLOMICS SHOWS A SMART WAY TO REDUCE SULFITES IN WINE

Panagiotis Arapitsas[A,*], Maurizio Ugliano[B], Daniele Perenzoni[A], Andrea Angeli[A], Paolo Pangrazzi[C], and Fulvio Mattivi[A]

[A]Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy

[B]Department Biotechnology, University of Verona, Verona, Italy

[C]MezzaCorona Winery, Mezzocorona, Italy

*e-mail: panagiotis.arapitsas@fmach.it

What does happen in wine in the presence of oxygen? What is the fate of exogenous antioxidants such as SO_2? A consortium between a winery, a wine stopper producer and a MS metabolomics laboratory, was built to answer the above questions towards an ambitious project. The experimental design included 216 bottles of 12 different white wines produced from 6 different cultivars (Inzolia, Muller Thurgau, Chardonnay, Grillo, Traminer and Pinot gris). Half of them were bottled using the standard industrial process with inert headspace and the other half without inert gas and with extra headspace. After 60 days of storage at room temperature, the wines were analysed using an untargeted LC–MS method [1].

The use of a detailed holistic analysis workflow, with several levels of quality control and marker selection, gave 35 metabolites putatively induced by the different amounts of oxygen. These metabolite markers included ascorbic acid, tartaric acid and various sulfonated compounds observed in wine for the first time (e.g. *S*-sulfonated cysteine, *S*-sulfonated glutathione and *S*-sulfonated pantetheine, sulfonated indole-3-lactic acid hexoside and sulfonated tryptophol). The consumption of SO_2 mediated by these sulfonation reactions was promoted by the presence of higher levels of oxygen on bottling [1].

The reaction between SO_2 and other antioxidants present in wine, like glutathione, results in depleting each other concentration [1]. So instead to have a synergic or additive protection due to the presence of multiple antioxidants, the wine is less protected from oxidations because of the antagonism between the antioxidants. This phenomenon, unknown until today, was pushing often winemakers to increase the added dose of SO_2 without knowing why, and as result to increase sulfites concentration in wine.

KEYWORDS: vitis, chardonnay, antioxidant, glutathione, SO_2, indoles, tryptophan

Reference

1. Arapitsas P, Ugliano M, Perenzoni D, Angeli A, Pangrazzi P, Mattivi F, J Chrom A (2016) 1429 155-165.

Advanced Metabolomics for the Study of Fungal Metabolism and its Regulation: Applications in Crop Protection and Biotechnology

Ioannis Kalambokis[A], George Kapetanakis[B], George Diallinas[B*], Suha Jabaji[C], Konstantinos A. Aliferis[A,C*]

A: Laboratory of Pesticide Science, Agricultural University of Athens, Votanikos, 118 55 Athens, Greece
B: Faculty of Biology, University of Athens, Panepistimioupolis, Athens 15784, Greece
C: Department of Plant Science, McGill University, Montreal, Quebec, H9X 3V9, Canada

*E-mails: konstantinos.aliferis@aua.gr
konstantinos.aliferis@mcgill.ca
diallina@biol.uoa.gr

In the post-genomic era metabolomics have emerged as a powerful bioanalytical tool for the study of the metabolism of biological systems and its fluctuation in response to biotic and abiotic stimuli. Being closer to the phenome compared to other "omics", metabolomics can provide valuable information that could be further exploited towards the unravelling of the missing functional links between the various levels of metabolism, and ultimately, could lead to the understanding of global metabolism regulation. Since its relatively recent emergence, metabolomics has been applied in various scientific disciplines such as, microbiology, food science, crop protection, and plant biotechnology[1,2]. During the last ten years we have been developing cutting-edge metabolomics protocols for applications in environmental sciences and crop protection, by using and integrating data derived by analytical platforms such as, GC/EI/MS/MS, Orbitrap-MS/MS, and NMR. The biological interpretation of the vast amount of the obtained information from such analyses, necessitates the utilization of bioinformatics software for data mining, and metabolite network construction and visualization (e.g., Cytoscape, SIMCA, MATLAB, BIOCYC). Focusing on fungi, we have developed advanced metabolomics protocols for, among others, the study of fungal metabolism and physiology[3], fungal chemotaxonomy[4], and plant-fungal[5,6,7] and fungal-fungal interactions[8]. The discovery of the functional links between genomes and phenomes of mycotoxin producing plant-pathogenic fungi applying metabolomics, represents a largely unexplored field of high importance towards improved crop protection and consumers' health. Currently, we are developing metabolomics protocols for the study of the effects of multidrug resistance of *Aspergillus* species to fungicides on their global metabolism regulation and mycotoxin-biosynthetic capacity. Also, metabolomics is being developed for the study of the effects of point mutations on *Aspergillus* metabolism and the discovery of the links between genomes and phenomes. For this purpose, experiments are being conducted using *Aspergillus nidulans* as the model organism. Resistant strains, obtained by applying molecular biology approaches, such as, knockout and Minos-transposon mutagenesis[9,10,11], will have their metabolomes compared to those of sensitive parental strains, employing metabolomics. The developed protocols will be further adapted in the study of mycotoxin-producing *Aspergillus* species such as *A. flavus*. Results of these studies will provide insights into the effects of fungal resistance to pesticides on their metabolic function and is highly foreseen that will reveal links between gene function and metabolism regulation.

KEYWORDS: *Aspergillus*, Fungal metabolomics, mycotoxins, pesticides, resistance

REFERENCES

4. Aliferis, K.A., & Jabaji, S., *Pesticide Biochemistry and Physiology*, (2011), 100, 105-117
5. Aliferis, K.A., & Chrysayi-Tokousbalides, M., *Metabolomics*, (2011), 7, 35-53
6. Aliferis, K.A., & Jabaji, S., *Metabolomics*, (2010), 6, 96-108
7. Aliferis, K.A., Cubeta, M.A., & Jabaji, S., *Metabolomics*, (2013), S1, 159-169
8. Aliferis, K.A., & Jabaji, S., *PLoS ONE*, (2012), 7(8): e42576
9. Aliferis, K.A., Faubert, D., & Jabaji, S., *PLoS ONE*, (2014), 9(11): e111930
10. Aliferis, K. A., Chamoun, R., & Jabaji, S., *Frontiers in Plant Science*, (2015), 6, 344
11. Chamoun, R., Aliferis, K. A., & Jabaji, S., *Frontiers in Microbiology*, (2015), 6, 353
12. Diallinas, G. (2007), Aspergillus transporters. In "The Aspergilli: Genomics, Medicine, Biotechnology and Research Methods", eds. G. Goldman and S. Osmani, CRC press
13. Diallinas, G., *Frontiers in Pharmacology*, (2014) 5:207
11. Diallinas, G., et.al., *Fungal Genetics and Biology*, (2015), 81, 1-11

POSTERS

A NOVEL PLATFORM FOR AUTOMATED GC-MS BASED METABOLOMICS

I. Opekarová [1,2], L. Řimnáčová[1], M. Moos[1], H. Zahradníčková[1], Petr Hušek[1] and P.Šimek[1*]
[1]Biology Centre, Czech Academy of Sciences, Ceske Budejovice, Czech Republic
[2]University of Chemistry and Technology, Prague, Czech Republic
*E-mail: psimek@bclab.eu

A novel, completely automated analytical method has been developed for GC-MS based metabolomics. The liquid-liquid microextraction (LLME) and alkylchloroformate (RCF) derivatization based sample preparation protocol proceeds unattended on a modified platform (CTC Analytics, Zwingen, Switzerland) which was programmed by an in-house software in 30 consecutive steps involving a sample transfer, addition of internal standards, addition of buffers, reaction and organic media, vortexing after each step, an exchange and cleaning of syringes dedicated separately for sample preparation and GC injection, withdrawal of an organic layer and a final aliquot injection into a gas chromatograph. The derivatized metabolites (esters, N-carbamates and (S,O)-carbonates) are efficiently separated and ionized by EI or isobutane PICI or APCI mass spectrometry and automatically processed by in-house SW platform called Metabolite Mapper which enables automatic metabolite identification against an in-house MS spectral database and quantitative determination of targeted metabolites.

The developed analytical platform was successfully applied to the GC-MS analysis of protic metabolites in human plasma [1-3] and urine [4]. Novel heptafluorobutyl chloroformate (HFBCF) and trifluoroethyl chloroformate (TFECF) based sample preparation protocols have enabled GC-MS analysis of protic plasma metabolites involved in one carbon metabolism [1], 35 amino acid enantiomers in plasma [2], 16 plasma steroids, sterols and tocopherols [3] and 132 amino-carboxylic metabolites in urine [4].

Financial support: the Czech Technology Agency, project No.TA04011751.

KEYWORDS: metabolomics, liquid liquid microextraction, alkyl chloroformate derivatization,

metabolomics, automation, GC–MS

REFERENCES:

1. Šimek P., Hušek P., Zahradníčková H., Anal. Chem. (2008) 80 5776-5782
2. Šimek P., Hušek P., Zahradníčková H. (Alterman M. A., Hunziker P. (eds)) (2012) Amino Acid Analysis: Methods and Protocols, Springer Science & Bussiness Media, New York.
3. Řimnáčová L., Hušek P., Šimek P., J. Chromatogr. A (2014) 1339 154-167.
4. Hušek P., Švagera Z., Hanzlíková D., Řimnáčová L., Zahradníčková H., Opekarová I., Šimek P., J. Chromatogr. A (2016) in press

A SAMPLE PREPARATION APPROACH FOR METABOLOMIC PROFILING OF HUMAN SALIVA OF SMOKERS AND NONSMOKERS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Eleni Barda, Vasilis Sakkas, Triantafyllos Albanis

Ioannina University, Lab of Analytical Chemistry

Email: vsakkas@cc.uoi.gr

Endogenous metabolites such as intermediates and end products of biological processes in humans are most predictable for the phenotype of an organism[1]. However, endogenous alteration of metabolic pathways evoked by smoking are investigated to a much lesser extent. Research on smoking-related changes at the molecular level could contribute to the understanding of the underlying physiological mechanisms and might, therefore, lead to the identification of potential biomarkers of effect. Saliva contains an interesting number of biochemical components that may be useful for diagnosis/monitoring of metabolic disorders, and as markers of cancer or heart disease[2]. In the present study, we demonstrated that saliva, as a noninvasively accessible body fluid, is a suitable biological matrix for the determination of adenine, uridine and adenosine in significantly different levels between smokers and nonsmokers obtained from a strictly diet controlled smoking study. Saliva collected from all the participants was re-aliquoted as 500 μL aliquots into microcentrifuge tubes. Prior to LC analysis, each saliva sample was vortexed for 30 s followed by ultra-sonication for 1 min to breakdown mucous substances in saliva and to improve homogeneity. Protein precipitation was achieved by the addition of 0.5 mL acetonitrile. The mixture was vortexed for 30 s followed by ultra-sonication for 1 min and then subjected to centrifugation at 10,000 g for 10 min at ambient temperature. Precipitated proteins from the sample were removed as a pellet at the bottom of the microcentrifuge tube. The supernatant was transferred to a fresh microcentrifuge tube and evaporated to dryness prior to being reconstituted in 500 μL water.

KEYWORDS: saliva, Liquid chromatography, metabolomics

REFERENCES

1. Fiehn, O. Metabolomics-the link between genotypes and phenotypes. Plant Mol. Biol. 2002, 48 (1−2), 155−171.

2. T. Pfaffe, J. Cooper-White, P. Beyerlein, K. Kostner, C. Punyadeera, Clin. Chem. 57 (2011) 675

ASSESSMENT OF A FAST ANALYTICAL METHODOLOGY FOR URINARY METABOLOMIC BIOMARKERS BY LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY

Sofia Vika[1], Vasilis Sakkas[1], Alexandros Makis[2], Theodoros Palianopoulos[2], Triantafyllos Albanis[1]

[1] Ioannina University, Lab of Analytical Chemistry

[2] Ioannina University, Faculty of Medicine, Child Health Department

Email: vsakkas@cc.uoi.gr

The measurement of metabolite levels and their variation in biofluids may offer many insights into disease processes, drug toxicity as well as response to therapeutic intervention, while at the same time providing information regarding the effects of growth and aging, diurnal variation, nutrition and exercise on metabolism[1]. Urine poses several analytical challenges for metabolic profiling, due to large variations in ionic strength, pH etc especially under conditions of stress. Moreover, urine possesses a wide dynamic range of metabolite concentrations, as well as being subject to variable and unpredictable dilution.

In the present study, an analytical method was developed to efficiently analyze urine samples by liquid chromatography – mass spectrometry (LC/MS) for the determination of adenosine, alanine, proline, valine and arginine. The protocol was applied in patients with beta Thalassemia Disease as well as healthy individuals served as controls. Urine samples were collected, aliquoted on the day of sampling and stored at −80 ˚C until use. Prior to LC/MS analyses, urine samples were diluted 1:10 with acetonitrile (ACN), and centrifuged at 13,000 × g. Precipitated proteins from the sample were removed from the bottom of the microcentrifuge tube and the supernatant was transferred to a fresh microcentrifuge tube.

KEYWORDS: urine, LC/MS, Thalassemia

REFERENCES

1. Elizabeth J Want, Ian D Wilson, Helen Gika, Georgios Theodoridis, Robert S Plumb, John Shockcor,Elaine Holmes & Jeremy K Nicholson Global metabolic profiling procedures for urine using UPLC–MS (2010) 5 1005-1018.

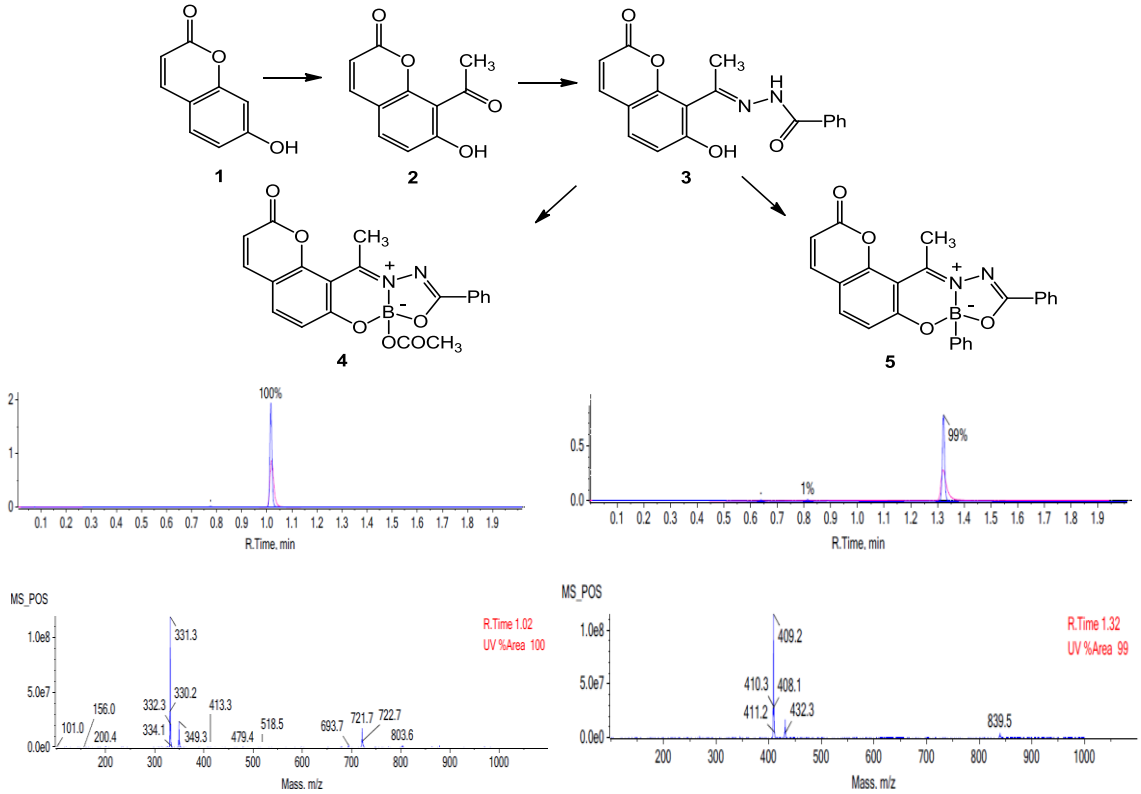
BORON NITROGEN COUMARIN DERIVATIVES: THREE METABOLITE BUILDING BLOCKS IN ONE SMALL MOLECULE

Konstantinos Theodoridis [A], Helen G. Gika [A], Elvira Kotali [B], Philip A. Harris [C], Antigoni Kotali [B,*]

[A] School of Medicine, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece
[B] Department of Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece
[C] GlaxoSmithKleine, 1250 Collegeville Road, Collegeville, PA 19426-0989, USA

*E-mail: kotali@auth.gr

Coumarins are one of the most important secondary metabolites of plants and are known as naturally occurring benzo-α-pyrone derivatives from metabolism of phenylalanine.[1a] Furthermore, the importance of hydrazone derivatives is well known because of their biological properties.[1b] On the other hand, small molecules containing boron have been found to have significant biological properties and recently naturally-occurring boron metabolites have been identified.[1c] On this basis we designed the synthesis of **4** and **5** and produced them following our protocol.[1d] Derivatives **4** and **5** combine the cumarin, hydrazone and boron features and were analysed *via* LCMS.



KEYWORDS: Boron, Coumarins, Hydrazones, Metabolites

REFERENCES:

1. a) Tosum, A. (2012) Biotechnological production of coumarins. In Biotechnological Production of Plant Secondary Metabolites. Bentham e-Books, Sharjah, The United Arab Emirates. b) Rollas, S.; Küçükgüzel, S.G., Molecules (2007) 12, 1910. c) Wolkenstein, K.; Sun, H., Falk, H., Griesinger, C. J. Am. Chem. Soc. (2015) 137, 13460. d) Kotali, A.; Dimoulaki, F.; Kotali, E.; Maniadaki, A.; Harris, P. A.; Różycka-Sokołowska, E.; Bałczewski, P.; Joule, J. A. Tetrahedron (2015) 71, 7245-7249.

ANALYSIS OF THE VECTOR'S BIAS IN THE SPREAD OF MALARIA IN THE DIFFERENT INCIDENCE RATES

Sungchan Kim [A] , Sungchan Kim [A] , Il Hyo Jung* [A]

[A] Pusan National University, Korea

*E-mail: ilhjung@pusan.ac.kr

It was demonstrated that infectious individuals are more attractive to the mosquitos and it is called as the *vector-bias effect*. The aims of this paper are turning out how the vector-bias effect affects i) the changes of dynamics in malaria transmission and ii) the changes of optimal control strategy and its execution costs in the different transmission rates (different areas). The researches of the vector-bias effect, which includes i) and ii) is well-estabilished by Chamchod, Britton and Bruno. But their works are the results in some typical region. In this paper, we paid attention to show differences in between two area: one is high transmission area and the other is low transmission area (as classified by R0). Our work asserts that the dynamics in the two area is different as bias increases and this is because the size of R0 and cost-effectiveness is more and more decreasing when bias is getting high in any region. So our work suggests that considering the vector-bias effect in suitable area helps forecasing the future dynamics and making a well-made policy decision.

KEYWORDS: malaria transmission dynamics; the vector-bias effect; endemic-size; low transmission; high transmission; optimal control; cost-effectivity

REFERENCES:

1. Bruno Buonomo, Cruz Verags-De-Le'on. Mathematicsal Biosciences (2013) 242 (1) 59-67.

2. Bruno Buonomo, Cruz Verags-De-Le'on. Acta Appl Math (2014) 132,127-138.

3. Renaud Lacroix, Wolfgang R. Mukabana, Louis Clement Gouagna, Jacob C. Koella. PLOS Biology (2005) 3 (9) e298.

DYNAMICAL BEHAVIOR OF TUMOR VIRALTHERAPY WITH IMMUNE RESPONSE : DETERMINISTIC AND STOCHASTIC ANALYSIS.

Kwang Su Kim[1], Sangil Kim[2,*] and Il Hyo Jung[1]

[1]Department of Mathematics, Pusan National University, Busan, 609-735, South Korea

[2]WISE Institute, Hankuk University of Foreign Studies, 156-849, South Korea

Email:kwangsu@pusan.ac.kr

Over the past few years, several studies have been made on cancer viral therapy. Recent investigators suggest that cancer can be treated by viral therapy. But, since there are many species of virus, it is important to select virus for cancer viral therapy. So our main problems are " Which virus should we take for more effective treatment?" and “How to avoid failure of viraltherapy?”. Towards solving this problem, we construct deterministic and stochastic model of tumor-viral dynamics. For find conditions of viraltherapy failure, local asymptotic stability and global asymptotic stability of viraltherapy failure equilibrium are studied. By using basic reproductive ratio, we investigate its sensitivity to the parameter values about characteristics of viruses. We derive a system of stochastic differential equations that based on the deterministic model and explore probability of uninfected tumor and infected tumor extinction depends on changing parameter values. The results of this study suggest that we need virus which have high infection rate and optimal cytotoxicity for effective treatment.

KEYWORDS: viraltherapy, stochastic differential equation, cancer, basic reproduct ratio

REFERENCES

[1] Dominik Wodarz 2001, Viruses as Antitumor Weapons : Defining Conditions for tumor Remission, *Cancer Res.*, 2001, **61**: 3501-3507.

[2] David Dingli, Matthew D. Cascino, Kresimir Josic, Stephen J Russell, Zeljko Bajzer 2006. Mathematical modeling of cancer radiovirotherapy. *Mathematical Biosciences*, **199**: 55–78.

[3] Zeljko Bajzer, Thomas Carr, Kresimir Josic, Stephen J. Russell, David Dingli 2008, Modeling of cancer virotherapy with recombinant measles viruses, *J. Theoret. Biol.*, **252** 109-122.

THE EFFECT OF ELECTRONIC CIGARETTES FOR SMOKING CESSATION:

A MATHEMATICAL MODELING APPROACH

Jae Hun Jung [A], Il Hyo Jung [B]

Department of Mathematics, Pusan National University, Busan

*E-mail : ilhjung@pusan.ac.kr

Smoking has a large problem all over the world. Despite overwhelming facts about the risks, smoking is still a bad habit widely spread and is the legalization of drug that kills many of users. Nowadays, smoking has become a matter of common interest of fashionable interpersonally and people fall into temptation of smoking due to peer influence.

Thus, electronic-cigarettes (e-cigarettes) can deliver nicotine and mitigate tobacco withdrawal and are used by many smokers to assist quit attempts. We investigated whether there is an effect on smoking e-cigarettes. In this point of view, we proposed and analyzed a nonlinear mathematical model to study the effect of e-cigarettes on smoking cessation. The equilibrium point of the model obtained and the stability discussed. The analysis shows that on changing parameters of the system. Numerical simulation also supports the analytically obtained results.

KEYWORDS: mathematical modeling, smoking, e-cigarette, cessation

REFERENCES:

1. Anupama Sharma and A.K. Misra, Applied Mathematical Modeling (2015) 39 1087-1098.

2. O. Sharomi, A.B Gumel, Applied Mathematics and Computation (2008) 195 475-499.

3. Gul Zaman, Bulletin of the Malaysian Mathematical Sciences (2011) 34 403-415.

EXPLORING THE LINK BETWEEN MATERNAL DIETARY PROTEIN INTAKE AND THE METABOLOMIC PROFILE OF SECOND TRIMESTER AMNIOTIC FLUID

Fotiou Maria[A], Fotakis Charalambos[B], Tsakoumaki Foteini[A], Kyrkou Charikleia[A], Athanasiadou Elpiniki[C], Tsiaka Thalia[B], Chatziioannou Anastasia - Chrysovalantou[D], Dimitropoulou Aristea[A], Menexes George[E], Theodoridis Georgios[D], Tarlatzis C. Basil[C], Biliaderis G. Costas[A], Athanasiadis P. Apostolos[C], Zoumpoulakis Panagiotis[B*], Michaelidou Alexandra-Maria[A*]

[A]Department of Food Science &Technology, School of Agriculture, Faculty of Agriculture, Forestry & Natural Environment, Aristotle University of Thessaloniki, Greece
[B]Institute of Biology, Medicinal Chemistry & Biotechnology, National Hellenic Research Foundation, Athens, Greece
[C]1st Department of Obstetrics & Gynecology, School of Medicine, Aristotle University of Thessaloniki, Greece
[D]Laboratory of Analytical Chemistry, School of Chemistry, Faculty of Sciences, Aristotle University of
Thessaloniki, Greece
[E]Department of Field Crops & Ecology, School of Agriculture, Faculty of Agriculture, Forestry & Natural Environment, Aristotle University of Thessaloniki, Greece
*E-mail: pzoump@eie.gr, amichail@agro.auth.gr

Aim: The aim of the present study was to examine the link between maternal habitual dietary protein intake and second trimester amniotic fluid (AF) metabolomic profile.
Methodology: Sixty-five singleton pregnant women, undergoing amniocentesis for prenatal diagnosis between 18 and 24 gestation weeks, participated in the study. Dietary assessment was carried out using a semi-quantitative food frequency questionnaire. Hierarchical cluster analysis (HCA) was used to identify homogenous groups of participants on the basis of habitual protein intake (percentage of energy intake). Cluster construction was based on Ward's minimum variance criterion, while the squared Euclidian distance was used as a dissimilarity measure between the participants. The statistical significance of the cluster solution was evaluated with the upper-tailed rule. A holistic metabolomics approach was employed using a 600MHz Varian NMR spectrometer with CPMG pulse sequence to suppress protein signals of the untreated samples. All NMR spectra were phase and baseline corrected, reduced into spectral buckets of 0.0001 ppm and aligned using the MestReNova software. Multivariate data analyses were performed with SIMCA-P 14.0 software. Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) models were extracted on the dietary clusters, in order to explore the differences between them. S-line plots and contribution plots were used to delineate the most important metabolites for sample classification. All models have been validated with the use of external data sets, permutation testing and ROC curves.
Results: A 2-group interpretable and statistically significant clustering of participants was identified and characterized on the basis of protein intake (% of energy intake) from different food groups. A total of 29 women were in cluster 1 (C1) and 36 in cluster 2 (C2). C1 was characterized by a significantly higher energy contribution from sweets and confectionery

proteins, while C2 had a higher energy contribution from meat/meat products, whole milk, and yellow cheeses proteins. The implementation of Chemometrics on the AF NMR spectral data allowed the identification of metabolites associated with different protein intake. Specifically, the holistic NMR metabolomics approach indicated that AF specimens of women in C2 were characterized by increased creatine, histidine, and branched-chain amino acids concentrations.

Conclusion: The data presented in this study suggest that maternal habitual dietary protein intake is reflected in the AF metabolomic profile.

KEYWORDS: amniotic fluid, habitual dietary protein intake, metabolomics

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Thales. Investing in knowledge society through the European Social Fund.

LC-MS/MS METABOLIC PROFILING STUDY OF SHIKONIN'S CYTOTOXICITY ON Huh-7 CELL LINE

E.D. Spyrelli[A], A. Nakas[A], C. Virgiliou[B], A.E. Koletti[A], H. Gika[C], G. Mossialos[D], V.P. Papageorgiou[A], A.N. Assimopoulou[A,*]

[A] Department of Chemical Engineering, Aristotle University of Thessaloniki 54124 Thessaloniki, Greece
[B] Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[C] School of Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[D] Department of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

*E-mail: adreana@eng.auth.gr

Metabolic profiling in cells can provide information on cell's functions, phenotype and response to interventions thus is a promising tool for studying the mechanisms involved under treatment. By this approach changes in the context of the global network of metabolic pathways in a cell can be identified. In this study the inhibitory effect of shikonin on human hepatocellular cell line (Huh-7) was studied by LC-MS/MS profiling method. The aim was to identify primary metabolites affected in the intracellular and cell culture medium and shed light in its mechanism of action. Shikonin, alkannin and their esters deriving mainly from the roots of the Boraginaceae plant family such as *Alkanna tinctoria, Lithospermum erythrorhizon* etc, have been shown to exhibit among others significant cytotoxic effect[1-3] and multiple mechanisms of action involved have been proposed[1-4].

Shikonin exhibited an antiproliferative effect in a dose and time dependant manner with an IC_{50} of 5 μM. Shikonin-treated cell cultures exhibited gradual accumulation of non-viable cells that started 24 h after treatment and reached the maximum value of over 50% at 96 h. A snapshot of the metabolic phenotype of the cells upon treatment (48 h) with IC_{50} was acquired by a LC-MS/MS targeted metabolic profiling method capable to determinate 105 primary metabolites[5]. Extracellular and intracellular material was extracted from Huh-7 cell cultures were analysed by LC-MS/MS. An ACQUITY HILIC, BEH amide column (2.1 × 150 mm, 1.7 μm) was used at 40 ^{o}C, with gradient elution of solvent A (95-5% acetonitrile/water) over solvent B (30-70% acetonitrile/water, 0.01% ammonium formate). Flow rate was 0.5 mL/min and injection volume was 5 μL. Electrospray ionization in both positive and negative mode (polarity switching mode) was applied.

Our results show that the inhibitory effect of shikonin reflects perturbations both in the cell metabolome and in the extracellular medium. Differences of uptake of substrates or excretion of metabolites is been detected in the cell medium e.g. for aminoacids (arginine and proline), while more significant effects are revealed in the intracellular metabolites in response to shikonin intervention.

The study demonstrates the potential of metabolomics to improve the knowledge on the response of hepatocarcinoma cell line to shikonin exposure and could be further used as a roadmap for shikonin, alkannin and other cytotoxic compounds on several cancer cells and *in vivo* experiments.

Acknowledgements: We acknowledge A. Kyriazou for technical assistance on cell culturing.

Keywords: *metabolic profiling, in vitro, shikonin, cytotoxicity, alkannin*

References:

[1] Papageorgiou VP, Assimopoulou AN, Couladouros EA, Hepworth D, Nicolaou KC Angewandte Chemie Int. Ed. (1999) 38 270-300.

[2] Papageorgiou VP, Assimopoulou AN, Samanidou VF, Papadoyannis IN Current Organic Chemistry (2006) 10 2123-2142.
[3] Zhao Q, Assimopoulou AN, Klauck SM, Damianakos H, Chinou I, Kretschmer N, Rios J-L, Papageorgiou VP, Bauer R, Efferth T Oncotarget (2015) 6 38934-38951.
[4] Wiench B, Eichhorn T, Paulsen M, Efferth T Evidence-Based Complementary and Alternative Medicine (2012) 2012 Article ID 726025, 15 p.
[5] Virgiliou C, Sampsonidis I, Gika HG, Raikos N, Theodoridis GA. Electrophoresis (2015) 36 2215-2225.

METABOLOMICS IN EPIDEMIOLOGIC STUDIES OF TYPE II DIABETES MELLITUS: IMPLICATIONS FOR THE EXPOSOME

Xanthi D. Andrianou[A]^, Stephanie Gaengler[A]^, Konstantinos C. Makris[A]*
[A]Water and Health Laboratory, Cyprus International Institute for Environmental and Public Health, Cyprus University of Technology, Limassol, 3041, Cyprus.
^ equal contribution
*E-mail: konstantinos.makris@cut.ac.cy

Investigating the association between environmental and lifestyle factors and non-communicable diseases, such as type II diabetes mellitus (T2D), is gradually shifting away from studying a single disease risk factor to exploring the exposome, i.e., the totality of all human exposures during the whole lifetime, both exposures external to the person, and internally as assessed by biomarkers[1]. Large prospective cohorts with biobanks present the unique opportunity to implement metabolomics methodologies on epidemiologic study designs aiming to assess risk factors of T2D[2].
We conducted a systematic literature review to evaluate the use of metabolomics within the context of population health studies of T2D. The search was conducted using Web of Science, PubMed, and Google Scholar up to March 2016. General population health studies of T2D that incorporated either targeted or untargeted metabolomics protocols were selected. Animal, *in vitro* or studies not referring to the general population (such as case studies or convenience samples) were excluded; conference proceedings, books or book chapters, editorials or commentaries, as well as studies not written in English were also excluded.
In total, about 1300 unique titles and abstracts of the retrieved records were screened. A total of 131 full-text records (including reviews) were retrieved and assessed for relevance. General population studies that applied metabolomics protocols and linked metabolic profiles to the assessment of T2D as the outcome of interest were summarized.
Study designs that allow for the prospective monitoring and follow up of healthy individuals during critical life stages have not yet been widely implemented. The coupling of external environmental and lifestyle exposures with internal exposure metrics and endogenous metabolites would allow for the testing of novel hypotheses of T2D disease process within the framework of the exposome concept.

KEYWORDS: metabolomics, type 2 diabetes, epidemiology, prospective studies

REFERENCES:

1. Wild, C. P. Complementing the genome with an ‘exposome’: the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol. Biomarkers Prev.* **14,** 1847–50 (2005).
2. Drogan, D. *et al.* Untargeted Metabolic Profiling Identifies Altered Serum Metabolites of Type 2 Diabetes Mellitus in a Prospective, Nested Case Control Study. *Clin. Chem.* **61,** 487–497 (2015).

DETERMINATION OF TUNGSTEN IN PLANT AND SOIL SAMPLES BY INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY

Ümran Seven Erdemir[A], Hülya Arslan[B], Gürcan Güleryuz[B], Seref Güçer[A]

[A] Uludag University, Faculty of Arts and Sciences, Department of Chemistry, 16059, Bursa, Turkey

[B] Uludag University, Faculty of Arts and Sciences, Department of Biology, 16059, Bursa, Turkey

E-mail: useven@uludag.edu.tr

Tungsten (W) present at low concentrations in soils and sediments, naturally.(1) However, many anthropogenic activities related to W such as mining, production processes or fertilizers in agricultural fields may significantly increase tungsten concentration at substantial levels in environmental systems that may lead W accessibility from ecosystem to humans.(1-4) So, recent studies indicate that the historical view of tungsten as an "environmentally inert" metal must be reevaluated and environmental regulations of tungsten are needed.(5)

The aim of this work was to propose an analytical approach for leaching the W from plants and soils taken from surroundings of abandoned wolfram mining area in Uludag-Bursa. Samples were digested in Kjeldahl system using acide mixtures. W contents in soil and plant materials were determined by inductively coupled plasma mass spectrometry (ICP-MS) and accuracy was evaluated using recovery studies.

KEYWORDS: Tungsten, mining area, ICP-MS, soil, plant.

ACKNOWLEDGEMENTS:

This work was supported by the Commission of Scientific Research Projects of Uludag University [Project No KUAP(F)-2015/64].

REFERENCES:

1. Strigul N, Koutsospyros A, Arienti P, Christodoulatos C, Dermatas D, Braida W (2005) Chemosphere 61: 248-258.
2. Adamakis IDS, Panteris E, Eleftheriou EP (2010) Plant Biology 12: 114-124
3. Jiang F, Heilmeier H, Hartung W (2007) Plant And Soil 37-49
4. Santos SCPL, Cruz ME, Barroso AME, Fonseca CPS, Guerra M, Carvalho ML, Santos JP (2014) Journal of Soils and Sediments 778-784.
5. Strigul N (2010) Ecotoxicology and Environmental Safety 1099-1113.

ICP-MS AS A HOLISTIC ANALYTICAL METHOD FOR THE STUDY OF PHYTOACCUMULATION OF HEAVY METAL: A CASE STUDY OF *ERYSIMUM PULCHELLUM* (WIILD.) GAY

Ümran Seven Erdemir[A], Hülya Arslan[B], Gürcan Güleryuz[B], Seref Güçer[A]

[A] Uludag University, Faculty of Arts and Sciences, Department of Chemistry, 16059, Bursa, Turkey

[B] Uludag University, Faculty of Arts and Sciences, Department of Biology, 16059, Bursa, Turkey

E-mail: useven@uludag.edu.tr

Industrial activities are the main sources of environmental pollution globally due to the production of increased levels of metals. Plant species which are capable to accumulate metals are taking increased attention for remediation of soils (1). Plants with exceptional metal-accumulating capacity are known as hyperaccumulators. The aim of this study is to evaluate the elemental (B, Cd, Co, Cr, Cu, Fe, Mn, Mo, Pb, W, Zn) composition of *Erysimum pulchellum* spread around the abandoned tungsten mining area of Uludağ Mountain. The plants and their soil samples were analyzed and holistically interpreted by inductively coupled plasma mass spectrometry (ICP-MS) after an acid digestion process. ICP-MS is capable to analyze many elements simultaneously which may show to whole picture of plant systems and their environment. Transport mechanisms can be used for further assessment of phytochelatin ligands and enzyme activities (2).

KEYWORDS: Tungsten mining, heavy metal, phytoremediation, inductively coupled plasma-mass spectrometry.

ACKNOWLEDGEMENTS:

This work was supported by the Commission of Scientific Research Projects of Uludag University [Project No KUAP(F)-2015/64].

REFERENCES:

1. Maestri E, Marmiroli M, Visioli G, Marmiroli N. Environmental and Experimental Botany (2010) 68:1-13.
2. Arslan H, Güleryüz G, Akpınar A, Kırmızı S, Erdemir Seven U, Güçer S. CLEAN-Soil AirWater (2014) 42:824-35.

QSRR modeling for metabolite standards analyzed by two different chromatographic columnS and by using multiple linear regression (MLR)

Ch. Zisi[A]*, I. Sampsonidis[A], A. Pappa-Louisi[A], P. Nikitas[A], M. Witting[B], H. Gika[C], S. Kalogiannis[D], G. Theodoridis[A]

[A] Department of Chemistry, Aristotle University of Thessaloniki, 54124, Greece.
[B] Helmholtz Zentrum München, Research Unit Analytical BioGeoChemistry, Ingolstaedter Landstrasse 1, D-85764 Neuherberg, Germany
[C] Department of Medicine, Aristotle University of Thessaloniki, 54124, Greece.
[D] Alexander Technological Educational Institution of Thessaloniki, Department of Nutrition and Dietetics, Thessaloniki, Greece.
*E-mail: chrizisi@hotmail.com

The use of retention data is of great help for metabolites identification in Metabolomics. In this context, quantitative structure-retention relationship (QSRR) models, based on metabolite molecular descriptors (MDs), are used more frequently lately. In the present study, a 7-parameter alternative QSRR model was developed based on a dataset composed of 94 metabolite standards. This model is differentiated from a common QSRR model by adding an extra variable which is the metabolite retention time measured under the same gradient conditions, but in a different chromatographic column. Multiple linear regression (MLR) was applied to construct the linear QSRR models based on statistical significant molecular descriptors among a variety of 309 theoretical molecular descriptors. The derived regression equations include six descriptors and are formally given bellow

$$t_R(A) = a_1 MD_1 + a_2 MD_2 + \ldots + a_n MD_n + b t_R(B)$$

where $t_R(A)$, $t_R(B)$ are the metabolites retention times measured under the same gradient conditions in two different hydrophilic interaction chromatographic columns, $MD_1,\ldots,MD_n$ are the statistically significant molecular descriptors and a_1, a_2, .., a_n, b are the adjustable parameters. The predictive capability of the developed QSRR models, which relate structural parameters of investigated compounds with their retention data obtained by two different chromatographic columns, is impressively improved in comparison to a classic QSRR model. Moreover, in case the dataset consisting of 94 metabolites was classified into five groups of chemically related compounds and QSRR models were separately developed for each chemically similar metabolite class, the predictive ability of the derived models would be further improved.

KEYWORDS: HILIC retention data, retention data prediction, quantitative structure-retention relationships, metabolite identification

Acknowledgement: Research committee of Aristotle University of Thessaloniki, for a postdoc scholarship “Excellence 2015” to Dr. Ch. Zisi

NMR-DRIVEN IDENTIFICATION OF BIOMARKERS RELATED WITH BLOOD NEOPLASTIC DISEASES –ANALYSIS OF DATA ACQUIRED IN TWO DIFFERENT MAGNETIC FIELDS (600 & 700MHZ).

Styliani A. Chasapi[1], Leonardo Tenori[2,3], Anastasia Karela[4], Stavros E. Bariamis [1], Kostantinos D. Marousis[1], Alexandros Spyridonidis[4] ,Claudio Lucinat[2,5], Georgios A. Spyroulias[1]

[1]Department of Pharmacy, University of Patras, GR-26504, Patras, Greece.

[2]Magnetic Resonance Center (CERM), University of Florence, Via L.Sacconi 6, 50019 Sesto Fiorentino, Italy

[3]Center of Metabolomics, University of Florence, Via L. Sacconi 6, 50019 Sesto Fiorentino, Italy, FiorGen

[4]Hematology Department, University of Patras Medical School, Rio Achaias, TK. 26500, Greece.

[5]Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Italy

**e-mail:* G.A.Spyroulias@upatras.gr

The incidence of neoplastic diseases is increasing every year and since cancer cells are known to possess a highly unique metabolic phenotype, development of specific biomarkers is possible. To date, a metabolic snapshot (single sample) approach is generally used to study correlations between pathologies and the metabolome [1]. Advances in biochemical data obtained from NMR spectra allow us to observe the metabolome in a very accurate manner and thus estimate the complex index of the health state of a human organism.

The aim is to gain insights into the molecular pathogenesis regarding a set of different types of Leukaemia by analysing plasma and serum samples from healthy individuals and patients. Detecting the response of the metabolic profile to malignant transformation during cancer growth, therapy treatment and transplantation along with identification of the metabolites that are uniquely correlated with a specific time point of the pathological state, may contribute to the diagnosis and follow up of transplanted patients. Analysis of the NMR spectra of blood samples (serum and plasma) provide a clear picture for the similarities and differences in metabolites present in each sample (data suggest that serum and plasma of the same individual maintain similar biomarkers' fingerprint) and a correlation between the metabolic fingerprints observed is attempted in order to correlate different biomarkers with blood disease type.

The present study illustrates the comparison of human serum/plasma samples by ^{1}H NMR spectroscopy across two different laboratories and within those, utilizing instruments with RT probe and cryoprobe along with different magnetic fields 600 MHz and 700 MHz, respectively. Data obtained in these two magnetic fields were amenable to comparative analysis through a multivariate statistics approach suggesting a better discrimination among the classes of samples in 700 MHz NMR instrument.

KEYWORDS: neoplastic, metabolomics, biomarkers, multivariate.

1. Bertini, I., Luchinat, C., and Tenori, L. Metabolomics for the future of personalized medicine through information and communication technologies. J.Pers.Med., 9, 133-136, 2012

This work was supported by the EU FP7-REGPOT-2011 "SEE-DRUG" (nr. 285950)

Comprehensive two-dimensional gas chromatography coupled with ToF-MS, a powerful tool for analysis of the volatomes of grapes and wines.

Silvia Carlin[1], Katja Suklje[2] and Fulvio Mattivi[1], , Urska Vrhovsek[1]

[1]Fondazione Edmund Mach, Research and Innovation Centre, Department of Food Quality and Nutrition, San Michele all'Adige (TN), Italy
[2]National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia
Mail: silvia.carlin@fmach.it

Comprehensive two-dimensional gas chromatography has emerged as a powerful analytical technique and it is an excellent tool for unravelling the composition of complex matrices.

This work will present some applications of this technique in the oenological field. The first of these involved comprehensive mapping of volatile compounds in a large sample of 70 sparkling wines, produced by 48 different wineries and from 6 vintages and representative of the two main production areas for premium Italian sparkling wines (Franciacorta (FC) and Trentodoc (TN)), using HS-SPME followed by GCxGC-Tof-MS and multivariate analysis [1]. The results of PCA performed on the biomarkers showing that the two groups of wines are well separated.

A temporal and spatial investigation of Shiraz wines fermented in controlled triplicates from grapes collected from climatically diverse (warm/hot versus cold/temperate) regions of Australia (New South Wales) was made using HS-SPME followed by GCxGC-TOF-MS (LECO Pegasus 4). Wine volatile profiles from warm/hot and cool/temperate climate could be distinguished according to the first two principal components. Wines from cool/temperate climate were characterised by higher levels of several terpenes such as alpha terpineol, linalool oxide, *trans*-hotrienol, citronellol acetate, 1-*p*-menthen-9-al, *cis*-rose oxide) and sesquiterpenes, whereas trend for norisoprenoids was less consistent. Higher TDN levels in wines from warm/hot climate were observed, confirming current literature.

The last application was the use of this technique in association with multivariate analysis to investigate the volatile composition of different wines according to their original grape cultivars and also to find potential markers of these grape cultivars. These results may help the wine industry to develop more effective quality control methods, in order to produce added value wines. Analysis of four types of wines carried out on 18 samples of Müller Thurgau, 48 samples of Pinot Gris, 36 samples of Chardonnay and 18 samples of Gewürztraminer allowed very good separation of the 4 varieties.

Reference

[1] S. Carlin, U. Vrhovsek, P. Franceschi, C. Lotti, L. Bontempo, F. Camin, et al., 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, Food Chem. (n.d.). doi:10.1016/j.foodchem.2016.03.112.

“CONTINUOUS OR INTERVAL AEROBIC TRAINING?”

A COMPARATIVE H1-NMR METABONOMIC STUDY ON ATHLETES

Chrysovalantou Anastasia Chatziioannou[A*1], Andreas Zafeiridis[B*2], Haralampos Sarivasiliou[B], Konstantina Dipla[B], Ioannis S. Vrabas[B], Panagiotis Zoumpoulakis[C], Constantinos Baskakis[C], AlexandrosPechlivanis[D], GeorgiosTheodoridis[A]

[A] School of Chemistry, Aristotle University of Thessaloniki, Greece
[B] Department of Physical Education & Sport Sciences at Serres, Aristotle University Thessaloniki, Greece
[C] Institute of Biology, Medicinal Chemistry & Biotechnology, National Hellenic Research Foundation, Greece
[D] Division of Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, United Kingdom

*E-mail: 1. c.a.chatziioannou@hotmail.com, 2. zafeirid@phed-sr.auth.gr

Continuous and interval aerobic training are the most commonly used training regimes for the development of cardiorespiratory fitness in clinical and sport settings. Both methods are also applicable to the management of chronic diseases and metabolic disorders. Intensity, duration and work to rest transitions may affect metabolic pathways for energy production.
The idea of applying metabolomics in the field of physical exercise in humans came up of the necessity to characterize the metabolomic profile of the two training methods to obtain a comprehensive view of overall metabolic stress and of the individual metabolic pathways involved in each type of training.
Blood plasma was retrieved from healthy men (aged 20.5±2.0 years) that trained in soccer four times per week. Each athlete performed on separate days (within two weeks) in random order three aerobic exercise protocols: heavy continuous, long interval (3-min bouts) and short interval (30-sec bouts). The three protocols were matched for overall effort. The analysis of all samples was accomplished utilizing a 600MHz Varian NMR spectrometer by applying CPMG pulse. PCA and OPLS-DA models were utilized to detect separations between pre- and post-exercise samples, as well as to compare the three different exercise protocols.
All aerobic exercise protocols revealed clear separations of the metabolic profiles before and after exercise. When comparing the exercise protocols in pairs, no statistically robust separation could be achieved. These findings indicate that aerobic exercise protocols matched for overall effort produce comparable overall metabolic stress and may engage similar metabolic pathways for energy production.

KEYWORDS: NMR, metabolomics, physical exercise, blood plasma, training, aerobic, metabolism

RETENTION INDEX DETERMINATION OF 94 METABOLITES WITH LC-MS/MS: PRELIMINARY DATA

O. Begou[1], H. G. Gika[2], G. Theodoridis[1], M. Witting[3], M. Quilliam[4]

1 Laboratory of Analytical chemistry, Department of Chemistry, Aristotle University of Thessaloniki 54124, Thessaloniki Greece
2 Laboratory of Forensic Medicine and Toxicology, Medical School, School of medicine, Aristotle University of Thessaloniki 54124, Thessaloniki Greece
3 Helmholtz Zentrum München, Munich Germany
4 National Research Council Canada, Ottawa
E mail: gkikae@auth.gr

High resolution liquid chromatography separation followed by mass spectrometry (MS^n) detection is extensively used for the determination of different compounds, such as drugs, endogenous metabolites, pesticides etc. The determination of chromatographic retention times (RTs) is of great importance for the confirmation of analytes in complex matrices. The identification of a compound in real samples is usually based on a match of both mass and chromatographic retention time data from the analysis of the sample with those obtained from the analysis of reference standards. However, concerning liquid chromatography (LC), RTs may show variability among different laboratories and instrumentation systems, even between days in the same lab or among the laboratory staff. This problem often requires the analysis of a chemical mixture (reference standard) with each sample for better matching for the identification of RT of each analyte, a fact that increases the cost of the analysis. Thus, in order to report retention data for different analytes, the establishment of a retention time system is needed.

In the present study 94 metabolites (amino acids, organic acids, e.t.c.) were analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS) in both positive and negative ionization (ESI). The metabolites were separated in groups of 10 in concentration level of 10 μg/mL where the reference standard was added, as well, in ratio 9:1. Chromatographic separation was performed on an Aquity BEH C18 column (2.1 x 100 mm, i.d. 1.7 μm) and the mobile phase was consisted of A: Water (+0.1% F.A.) and B: Acetonitrile (+0.1% F.A.). The flow rate was 0.4 mL/min. The selected reference standard included a homologous series of 20 substances based on 1-alkylpyrinium-3-sulfonate (APS) with specific characteristics, as high sensitivity and retention times relative to the metabolites measured. Mass parameters were optimized for each analyte separately (SRM).

Each analyte was measured 6 times and the retention time of the 6 replicates was reported. Retention times for the reference standard were also reported. An interpolation of analyte retention times into a fitted curve of the plot of retention time vs. retention index value for the reference compounds results in a retention index value for each analyte.

KEYWORDS: Retention index, Liquid Chromatography, metabolites

REFERENCES:

1. Quilliam M. A. et al., “A new retention index system for LC-MS/MS” (42nd Symposium of HPLC 2015, Geneva, Switzerland / poster presentation)

Assessing validation parameters in multi-targeted LC-MS/MS method for metabolite profiling application

Elisavet Tsakelidou[1], Christina Virgiliou[1], Helen Gika[2], Nikolaos Raikos[2], Georgios Theodoridis[1]*

[1]Laboratory of Analytical Chemistry, School of Chemistry, Aristotle University of Thessaloniki,Greece
[2]Laboratory of Forensic Medicine and Toxicology, Medical School, Aristotle University of Thessaloniki, Greece

*tsakeliz@hotmail.com

The scope of this study is the validation of a multi-targeted method capable to detect 86 endogenous metabolites (amines, carbohydrates etc) in human urine. Method validation in targeted metabolomics presents limitations due to the absence of analyte free matrix. Complex biofluids including urine contain endogenous metabolites that can decrease or increase the instrumental response (Matrix Effect). Matrix Effects vary and it's difficult to be measured or predicted. Thus, validation of a multi-targeted method for endogenous compounds becomes difficult. In this study, efforts were made to cover most of the common validation parameters including MF, LOD, LOQ, within-run precision and between run precision were determined by applying 3 replicate injections of each calibration standard (9 standards). In order to evaluate between-run precision, standard mixtures were analyzed in between a sample set of real samples (27 urine samples from healthy women). For the estimation of matrix factors pooled samples were fortified with known amount of the standard solutions after the extraction step. The responses were compared to the responses of equally concentrated standard solutions. Only 10 out of 86 detected compounds were found within acceptable limits of matrix factors. Therefore, in order to confirm the results for matrix effect a dilution study was made. Finally, results from this study confirmed that there are analytes with the potential to be quantified using the external calibration curve.

UNTARGETED AND TARGETED METABOLOMICS OF RAT CAECUM EXTRACTS FOR THE DISCOVERY OF EXERCISE BIOMARKERS

O. Deda[a*], T. Panagoulis[a], H. Gika[b], I. Taitzoglou[c], V.Mougios[d], G. Theodoridis[a]

[a] School of Chemistry, [b] School of Medicine, [c] School of Veterinary Medicine,

[d] School of Physical Education and Sport Science,

Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

*oliadmy@gmail.com

Metabolomics has been used to enlighten exercise biochemistry. Urine, plasma, skeletal muscle and liver samples, collected pre- and post-exercise, have been the specimens of strongest interest. The metabolic alteration caused by exercise in gut tissues has not yet been studied. However, there is one study that investigates the alteration of the cecal microbial environment due to exercise which suggests beneficial effects against gastrointestinal disorders [1]. The fact that microbiota composition is different between exercised and sedentary rats is of increasing interest [1]. Rat caecum tissues were analyzed in order to investigate the effect of life-long exercise versus sedentarism. Caecum tissue was chosen over colon because of the presence of microflora that break down food material in the latter and due to the fact that colon has been very commonly analyzed so far. Wistar rats were divided in life-long exercise and control groups. The training protocol consisted of swimming, 18-20 min per day, 3-5 days per week, with a load of 0-4% of body weight attached to the tail, depending on age. Both untargeted and targeted metabolomics analyses were performed. Caecum tissues were homogenized in acetonitrile - 1-propanol - water 3:3:2 (v/v/v) in a 1:20 w/v ratio. After ultra-centrifugation, the supernatants were analyzed immediately using a metabolomics-based HILIC-MS/MS method. For the untargeted GC-MS metabolic profiling methoxyamination and silylation derivatization were conducted prior to analysis. Both metabolomics approaches revealed metabolic changes deriving from exercise. Targeted metabolomics provided data on 35 metabolites, while seven amino acids changed significantly. Untargeted GC-MS data were evaluated using ChemStation and XCMS software. Based on both areas and number of features, the metabolic fingerprint of the exercised group was separated from that of the control group using principal component analysis. Arachidonic acid, urea, lactic acid and inositol seemed to be responsible for this separation. The exact biochemical pathways through which exercise affects the metabolome continue to provide a challenge for further research.

Key words: Caecum, Cecal extracts, Rats, Metabolomics, GC-MS, LC-MS

Reference

1. Matsumoto M, Inoue R, Tsukahara T, Ushida K, Chiji H, Matsubara N, Hara H (2008) Voluntary running exercise alters microbiota composition and increases n-butyrate concentration in the rat cecum. Biosci Biotechnol Biochem 72:572–576. doi: 10.1271/bbb.70474

STUDY OF DERIVATIZATION REACTIONS PRIOR TO GC-MS METABOLOMICS ANALYSIS. PRELIMINARY RESULTS

G. Moros[a*], A.C. Chatziioannou[a], H. Gika[b], N. Raikos[b], G. Theodoridis[a],

a School of Chemistry, Aristotle University of Thessaloniki 54124, Thessaloniki Greece

b Laboratory of Forensic Medicine and Toxicology, School of medicine, Aristotle University of Thessaloniki 54124, Thessaloniki Greece

* E-mail: geomoro@hotmail.gr

GC-MS is restricted to the analysis of thermally stable low molecular weight compounds with high or moderate volatility. Derivatization reactions are used prior to GC in order to alter chemically the analytes to produce compounds with properties, more amenable to chromatographic separation and spectrometric detection. Thus, derivatization is one of the most crucial steps of experimental procedure. In the literature many different methods dealing with the standardization of derivatization protocols have been reported. However there is still lack of a universally accepted protocol. The objective of the present work is to study the derivatisation reaction conditions and suggest a protocol including derivatization agent and conditions for GC-MS metabolomics analysis.

A variety of derivatization agents exist in bibliography. Nevertheless a two-step derivatization procedure, methoxymation followed by silylation, is a quite common method for GC–MS analysis of polar metabolites. In the present study, the most frequently used silylation agents for GC-MS metabolomics analysis (MSTFA, BSTFA with 1% TMCS and MSTFA with 1% TMCS) were tested in plasma samples followed by a standard experimental protocol. The optimization of the selected methoxymation step was examined through different conditions in the same standard extract, consisting of 60 different compounds belonging to several chemical classes which mainly occur in endogenous metabolites. The degree of completion of the methoxymation reaction depends on reaction time and temperature. Both parameters were examined in the most common conditions found in literature. An additional experiment was then conducted in the same standard extract testing the time and the temperature during silylation. Preliminary results extracted with XCMS, AMDIS and Chemstation software from the GC-MS raw data will be presented, followed by extensive statistical analysis and discussion.

KEYWORDS: derivatization, GC-MS, methoxymation, silylation, metabolomics

DEVELOPMENT OF A MULTI-ANALYTE HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY– MASS SPECTROMETRY (HILIC-UPLC-MS/MS) METHOD FOR THE QUANTITATION OF POLAR METABOLITES IN BEE PRODUCTS

Pina A.1, Theodorou H.1, Begou O.1, Kalogiannis S.2, Tananaki C.3, Thrasyvoulou A.3, Zotou A.1, Gika H.4, Theodoridis G.1

1. *Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki*
2. *Department of Nutrition & Dietetics, School of Food Technology and Nutrition, Alexander Technological Educational Institution of Thessaloniki*
3. *Laboratory of Apiculture-Sericulture, School of Agriculture, Aristotle University of Thessaloniki*
4. *Laboratory of Forensic Medicine and Toxicology, School of Medicine, Aristotle University of Thessaloniki*

Honey and royal jelly represent high value food commodity with recognized nutraceutical properties. A primary driver of the value of such products is their authenticity and verification of floral origin. Identification of the floral origin of honey by melissopalynological analysis, sensory and physic-chemical analysis requires a high degree of skill. Further to this, verification of geographical origin and authenticity requires solid data and unambiguous results. Therefore, there is an ongoing need to develop reliable, practical, and information-rich instrumental methods for the analysis of honey products. As such chromatographic methods have been used, but these require derivatisation prior to analysis since the majority of the analytes of interest are small polar molecules. Hydrophilic interaction liquid chromatography (HILIC) has found use in the analysis of polar molecules, and for this reason HILIC was selected as the separation mode for this work.

A HILIC-MS/MS method was developed for the efficient separation and quantification of small polar molecules, mostly primary metabolites. The method was based on an ultrahigh performance liquid chromatography (UHPLC) separation system coupled with Heated Electrospray Ionization (HESI) mass spectrometry on a triple quadrupole mass spectrometer, operating in both positive and negative ionization mode using polarity switching.

The list of target metabolites was comprised by studying the literature; the idea was to develop a method to cover the quantitative analysis of metabolites present in honey and royal jelly. The list included aminoacids, sugars, organic acids, nucleo-bases, amines, vitamins and other molecules.

Chromatography was optimized to achieve separation of 86 selected metabolites over a period of 21 min. Multiple Reaction Monitoring (MRM) MS/MS mode was selected for accurate quantification. Chromatography was performed on an ACQUITY BEH Amide analytical column with a mobile phase of 15mM ammonium formate in water and acetonitrile, at a flow rate of 0.4 mL.

The method was validated by studying the detection (LOD) and quantification limits (LOQ), the linearity ranges and the intraday- and interday-precision of the analysis.

APPLICATION OF A HILIC-UPLC-MS/MS METHOD FOR THE ANALYSIS OF POLAR METABOLITES IN HONEY AND CRUDE ROYAL JELLY

Theodorou H.1, Pina A.1, Begou O.1, Kanelis D.2, Dimou M.2
Kalogiannis S.3, Tananaki C.2, Thrasyvoulou A.2, Zotou A.1, Gika H.4, Theodoridis G.1,
1. *Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki*
2. *Laboratory of Apiculture-Sericulture, School of Agriculture, Aristotle University of Thessaloniki*
3. *Department of Nutrition & Dietetics, School of Food Technology and Nutrition, Alexander Technological Educational Institution of Thessaloniki*
4. *Laboratory of Forensic Medicine and Toxicology, School of Medicine, Aristotle University of Thessaloniki*

Bee products, such as honey and royal jelly, are important functional foods that possess several health promoting properties. Both have been widely used in commercial medical products, healthy foods and cosmetics in many countries. The chemical composition of honey and royal jelly has been studied by several authors, but data available in the literature are highly variable due to the intrinsic variability of those products and the use of different analytical methods, since no reference methods have been established so far. In food science, metabolomics has recently risen as a tool for quality, processing and safety of raw materials and final products

An LC-MS/MS method has been developed for the efficient separation and quantification of small polar molecules. The method was based on an ultrahigh performance liquid chromatography (UHPLC) separation system coupled with Heated Electrospray Ionization (HESI) mass spectrometry on a triple quadrupole mass spectrometer. With the developed method quantitation of 86 compounds belonging in four major classes of polar compounds (sugars, amino-acids, organic acids amines and other compounds) was achieved in a single run of 21 min. The method found application in the analysis of honey and royal jelly samples with the aim to provide additional information on these matrices primary metabolite content.

Focus was given to the sample pre-treatment which is crucial for accurate and reliable analysis. Due to lack of literature information related to sample preparation for both matrices, a simple treatment approach, compatible with the analytical method was tested. Honey samples were diluted with a mix of acetonitrile, methanol and water, centrifuged, and filtered before injection, while crude royal jelly samples were diluted with a mix of methanol and water, centrifuged, and filtered before injection as well.

Due to the extremely high content of some metabolites, such as sugars, in both honey and royal jelly samples, and in order to avoid instrument contamination, special attention was also given to the dilution of real samples.

The results were satisfying and a large number of metabolites were detected in both matrices (57 in honey and 58 in royal jelly). Analysis of such a large number of metabolites allows application of the method for the analysis of bee samples for their characterization: for honey samples with regard to different percentages of pollen and for rude royal jelly samples with regard to feeding experiments.

DIFFERENCES IN THE METABOLIC PROFILE OF THREE EXERCISE MODES IN INDIVIDUALS WITH METABOLIC SYNDROME

Siopi, A., Deda, O., Manou, V., Kellis, S., Theodoridis, G., Christoulas, K., Mougios, V.

Aristotle University of Thessaloniki, Thessaloniki, Greece

*E-mail: aikasiop@phed.auth.gr

Exercise is very important in the prevention and treatment of the metabolic syndrome (MetS), a cluster of cardiometabolic risk factors that raises morbidity. A metabolomics study was performed to investigate whether the response of the human urinary metabolic profile to exercise depends on the presence of MetS or the type of exercise performed. Twenty-three sedentary men were divided into two groups: healthy (n = 14) and MetS (n = 9). All participants completed four trials randomly: rest, high-intensity interval exercise (HIIE), continuous moderate-intensity exercise (CMIE) and resistance exercise (RE). Urine samples were collected pre-exercise and 1, 3 and 24 h post-exercise for targeted metabolomic analysis by liquid chromatography-mass spectrometry (HILIC-UPLC-MS/MS). A 3 (exercise mode) x 4 (time) x 2 (group) repeated-measures ANOVA, followed by post-hoc tests, was performed on the 62 identified metabolites after each metabolite's peak area was normalized to the respective value in the control trial (rest). No significant three-way interactions were observed. The amino acid, citrulline; the monosaccharides, glucose, sucrose and xylose; the nucleic acid components, cytidine and uracil; as well as choline presented significant trial x group interactions. Trimethylamine-*N*-oxide was the only metabolite to exhibit a significant time x group interaction. The amino acids, alanine and homocysteine; the purines, guanine, hypoxanthine, and inosine; the nucleoside, uridine; the carboxylic acids, lactate and pyruvate; the amines, trimethylamine and tryptamine; as well as riboflavin, presented significant trial x time interactions. These interactions were mainly due to differences at first post-exercise time point, where RE caused the highest increases. Overall, glutamine, riboflavin, lysine and betaine were found significantly different between groups, 12 metabolites were significantly different between trials, and 47 metabolites changed significantly over time. The effect of time was predominantly located in the comparison of the first post-exercise sample with all others. Our findings show diverse responses of the urinary metabolic fingerprint to different types of exercise in men with and without MetS. These results indicate changes in several metabolic pathways (such as glycolysis, gluconeogenesis, the urea circle, as well as carbohydrate, protein, purine and pyrimidine metabolism) and support the value of urinary metabolic profiling in the study of exercise metabolism.

KEYWORDS: exercise mode, metabolomics, metabolic syndrome

URINARY METABOLOMICS PROFILE OF NEWBORNS OF THE WESTERN GREECE REGION THROUGH HIGH-DEFINITION NMR ANALYSIS

Stavros E. Bariamis,[A] Ioanna Georgakopoulou,[A] Styliani A. Chasapi,[A] Kostas D. Marousis,[A] Sotirios Fouzas,[B] Asimina Tsidoni,[B] Manfred Spraul,[C] Hartmut Schäfer,[C] Claudio Luchinat,[D,E] Anastasia Varvarigou,[B] Georgios A. Spyroulias[A]

[A] *Department of Pharmacy, University of Patras, GR-26504, Patras, Greece;* [B] *Department of Pediatrics, University General Hospital of Patras, GR-26504, Patras, Greece;* [C] *Bruker BioSpin, Silberstreifen, D-76287 Rheinstetten, Germany;* [D] *Magnetic Resonance Center (CERM), University of Florence, Via L.Sacconi 6, 50019 Sesto Fiorentino, Italy*
[E] *Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Italy*
**E-mail: G.A.Spyroulias@upatras.gr*

Metabolic profiling provides a new opportunity to explore the global metabolic effects of many conditions on complex biological systems. High-resolution proton nuclear magnetic resonance (1H-NMR) spectroscopy is widely used to quantitatively analyze metabolic profiles, due to its reliability and relatively straightforward sample preparation. The metabolites in urine provide a fingerprint for each individual, containing significant information about age, sex, lifestyle, dietary intake, and disease history.[1,2]
The field of pediatric metabolomics is still being pioneered and is important to understand the dynamic metabolic changes, which occurs from the moment that a child is delivered. Through our present study, we goal to gain knowledge into the metabolic profile of term newborns of the Western-Greece region, in order to correlate our identification and quantification findings with metadata like day of collection, delivery mode, mother's diabetic station and pathogenic statements like jaundice and asthma.

This work was supported by the a) EU FP7-REGPOT-2011 "SEE-DRUG" (nr. 285950) and b) Research committee of the University of Patras, Greece, Program "Constantin Carathéodory" (Grant No E068)

KEYWORDS: Urinary metabolomics, NMR spectroscopy, Neonates, Jaundice

REFERENCES:
1. V. Fanos *et al*, Adv. Clin Chem (2012), *58, 193*
2. J. Pinto *et al.* J. Proteome Res., (2016), *15, 311*

UNDERSTANDING THE EFFECTS OF NON-*SACCHAROMYCES* YEASTS ON SAUVIGNON BLANC AROMA PROFILES USING METABOLOMICS AND SENSORY ANALYSIS.

Margaret E. Beckner Whitener[a,b], Jan Stanstrup[a], Valeria Panzeri[c], Silvia Carlin[a], Benoit Divol[b], Maret Du Toit[b], Urska Vrhovsek[a*]

[a] Department of Food Quality and Nutrition, Research and Innovation Center, Fondazione Edmund Mach (FEM), via E. Mach 1, 38010 San Michele all'Adige, Italy

[b] Institute for Wine Biotechnology, Department of Viticulture and Oenology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

[c] Institute for Grape and Wine Sciences (IGWS), Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

* urska.vrhovsek@fmach.it

This study sought to compare untargeted volatile compound profiles from SPME-GCxGC-TOF-MS and sensory analysis data of Sauvignon blanc wine fermented with six different non-*Saccharomyces* yeasts. The yeasts were allowed to ferment to 2% ethanol; *Saccharomyces cerevisiae* (*SC*) was then added to finish the fermentation. The control was *SC* only. Four of the non-*Saccharomyces* yeasts were commercial starter strains, *Torulaspora delbrueckii* (*TD*), *Lachancea thermotolerans* (*LT*), *Pichia kluyveri* (*PK*) and *Metschnikowia pulcherrima* (*MP*), while the other two species, *Candida zemplinina* (*CZ*) and *Kazachstania aerobia* (*KA*), were isolated from wine grape. Each fermentation produced a highly distinct profile both sensorially and chemically. The wines were evaluated for the basic tastes as well as 16 different aromas. Each fermentation was identified as sensorially unique and PCA indicated the dominant flavor and aroma profiles of the yeasts. The *SC* control and *CZ-SC* mixed fermentations were the most distinctly different. *SC* was characterized by guava, grapefruit, banana, and pineapple aromas while *CZ-SC* was driven by fermented apple, dried peach/apricot, and stewed fruit as well as sour flavor. Chemically over 300 unique features were identified as significantly different across the fermentations. When clustered hierarchically all of the biological replicates grouped together displaying strain specific profiles. Variances in esters, alcohols and terpenes were the main drivers of chemical differentiation. The *SC* profile was dominated by esters but all the yeasts had distinct ester profiles likely due to variation in acetyl- and acyltransferase activities between the yeasts. *CZ-SC* displayed the highest number of terpenes and sesquiterpenes of all the fermentations but also produced a large amount of acetic acid. *KA-SC* was second highest in terpenes and showed a number of as yet unidentified analytes. *TD-SC* had few esters but three distinctly higher thiol compounds. The *LT-SC* showed a relatively high number of ketones and acetate esters. The *PK-SC* and *MP-SC* fermentations had high levels of different methylbutyl, methylpropyl, and phenethyl esters. Overall, the sensory and chemistry methods complemented each other well, giving a much more detailed profile of these yeast than anything previously reported.

KEYWORDS: Non-*Saccharomyces*, Sauvignon blanc, *Torulaspora delbrueckii*, *Lachancea thermotolerans*, *Pichia kluyveri*, *Metschnikowia pulcherrima*, *Candida zemplinina*, *Kazachstania aerobia*, SPME-GCxGC-TOF-MS, Sensory analysis

Beckner Whitener, M.E., Stanstrup, J., Panzeri, V., Carlin, S., Divol, B., Du Toit, M., Vrhovsek, U.(2016) Metabolomics, 12 (3), art. no. 53, pp. 1-25.

M-IOLITE: AN INTEGRATED SUITE FOR THE STREAMLINING METABOLOMIC ANALYSIS

C. Maga-Nteve[1,2] & M.I.Klapa[1]
[1] Foundation for Research & Technology, Institute of Chemical Engineering Sciences Patras, Greece
[2] School of Medicine, University of Patras, Greece

The mass spectrometry (MS) metabolomics workflow is a multistep procedure involving analytical and computational parts. As the metabolomic analysis application is expanding to various biological systems and contexts, there is a need for a safely standardized data repository for all types of biological samples. Moreover, MS analysis produces a vast amount of unidentified compound data, so there is a need for unknown peak identification methods. The computational steps of the MS workflow include data normalization, validation and filtering methods, while it is imperative that the acquired metabolic profiles are studied in the context of metabolic network. In the case of Gas Chromatography (GC)-MS metabolomics, the additional metabolite extract derivatization step introduces needs for specialized normalization and data validation methods. In this context, there is a need for the development of software suites that can assist the user to safely deposit data in a standardized way and then carry out all the normalization, analysis and network reconstruction processes. While a number of tools offer new opportunities for data processing, there is no computational platform that emerges as a standardized approach which includes specialized normalization methods for GC-MS and which incorporates the metabolic network analysis into data interpretation. To address these issues, as the datasets obtained from metabolomics experiments still remain extremely large and dense, we designed M-IOLITE.

This study was granted by EC FP7 research project STREPSYNTH (n° 613877).

INVESTIGATING THE METABOLIC PROFILE OF SPECIFIC BRAIN REGIONS UNDER ADULT ONSET HYPOTHYROIDISM IN A MOUSE MODEL USING GC-MS METABOLOMICS

C.G. Vasilopoulou[1,2], M. Margarity[2], M.I. Klapa[1]
[1]Metabolic Engineering & Systems Biology Laboratory, Institute of Chemical Engineering Sciences, Foundation for Research and Technology – Hellas (FORTH/ICE-HT), Patras, GREECE
[2]Human & Animal Physiology Laboratory, Department of Biology, University of Patras, GREECE

The mammalian brain is a highly complex organ that integrates multiple regions with diverse roles, biochemical characteristics and physiological dynamics. Today, there is need to study brain physiology in a systemic and systematic way in the context of brain regional variation. Although brain was considered as metabolically nonresponsive to perturbations in the thyroid hormone (TH) levels, a large number of studies have provided evidence that the mammalian brain is a TH target tissue. The aim of the presented work is to study the metabolic physiology of the brain under AOH based on the integrated analysis of the metabolic profile of five brain regions, i.e. cerebral cortex, cerebellum, midbrain, striatum and hippocampus in both sexes of an AOH Balb/cJ mouse model under perfused or not conditions. AOH is induced chemically by administration of 1% w/v $KClO_4$ in the drinking water of 2 month old mice for 60 days. The metabolic profiles were measured using Gas Chromatography-Mass Spectrometry GC-MS) metabolomics. Our results validated the significant variation in the metabolic profiles between the various brain regions, confirming the need for this variation to be considered in the interpretation of the metabolomic data in the context of AOH; the same holds true for sex differentiation.

Studying the effects of combined high salinity and elevated CO_2 on tomato development using GC-MS and LC-MS metabolomic analysis

Matthaios-Emmanouil Papadimitropoulos [1,5#], *Georgia Tooulakou* [1], *Ilias Giannakos* [2], *Constantinos Kittas* [2,3], *Panagiotis Kalaitzis* [4] *and Maria I. Klapa* [1*]

[1] Metabolic Engineering & Systems Biology Lab, Institute of Chemical Engineering Sciences, Foundation for Research & Technology-Hellas (FORTH/ICE-HT), Patras, GREECE

[2] Institute for Research And Technology, Thessaly, Center for Research & Technology-Hellas (CERTH/IRETETH), Volos, GREECE

[3] Laboratory of Agricultural Constructions and Environmental Control, Department of Agriculture Crop, Production and Rural Environment, University of Thessaly, Volos, GREECE

[4]Horticultural Genetics and Biotechnology Lab, Mediterranean Agronomic Institute of Chania, GREECE

[5] Division of Genetics, Cell and Developmental Biology, Department of Biology, University of Patras, GREECE

High soil or water salinity is a major abiotic stress and a substantial constraint for crop production worldwide. Physiological studies have shown that elevated CO_2 can alleviate the negative effects of salinity stress on plants [1]. Combined metabolomic and transcriptomic analysis on *Arabidopsis thaliana* plants under salt stress has confirmed the alleviating effects of elevated carbon dioxide and has provided insights of the molecular mechanisms related to stress responses [2]. However, so far no relevant omic analyses have been carried out on more complex plants with commercial value.

The aim of our project is to investigate the effects and identify relevant biomarker of high salinity and the alleviating potential of elevated carbon dioxide on hydroponically grown tomato plants in controlled studies in growth chambers and field experiments in green houses, through integrated metabolomic, transcriptomic and leaves reflectance analyses. In the present study, we present the metabolomic analysis results from a time series experiment, in which high salinity and elevated carbon dioxide perturbations were applied for five days continuously either individually or in combination on the salinity resistant *Solanum lycopersicum* cultivar *Elpida* in the controlled environment of a large-scale growth chamber. The metabolic profiles of leaves harvested at the end of each day were measured by Gas Chromatography - Mass Spectrometry (GC-MS) and Liquid Chromatography - Mass Spectrometry (LC-MS) and analyzed by multivariate statistics. The salinity stress is harsher than the elevated CO_2 perturbation and becomes apparent at the molecular level from the first day of treatment, enhancing thus conventional physiological measurements. Furthermore, our results validated the alleviating effect of elevated CO_2 in the growth environment of salinity-stressed plants.

REFERENCES

[1] Takagi M, El-Shemy H, Sasaki S. et al. 2009 *Acta Agr Scand B-S P*. **59**:87-96

NMR and LC-HRMS based metabolomics for the discovery of novel bio-active small molecules from marine synbionts

Eirini Baira [2], Dimitra Benaki [1], Evangelos Gikas [1], Emmanuel Mikros [1], Nikolas Fokialakis[2]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Athens, Panepistimioupolis, Athens, Greece*
[2]*Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimioupolis, Athens, Greece*
[3]*Institut de Chimie des Substances Naturelles (ICSN-CNRS), Gif sur Yvette, France*

**E-mail: fokialakis@pharm.uoa.gr*

TASCMAR is an EU funded project that aspires to develop new tools and strategies in order to overcome existing bottlenecks in the bio-discovery and industrial exploitation of novel marine derived bio-molecules with applications in pharmaceuticals, nutraceuticals and cosmeceuticals as anti-aging ingredients. Exploitation of neglected and underutilized marine invertebrates and symbionts from the under-investigated mesophotic zone, of existing and targeted new collections from global marine biodiversity hotpots has been combined with innovative approaches for the cultivation and extraction of marine organisms from lab to pilot-scale. These novel improvements will ensure sustainable supply of biomass and promote the production of high added value biomolecules. An integrated, holistic technological approach including NMR- and MS-based metabolomics approaches has be applied, in conjunction with bioactivity profiling, as a filtering and a bio-prioritization tool. Moreover, state-of-the-art analytical instrumentation and commercially available, in-house databases and structure identification software will be employed for the dereplication procedures and the extensive characterization of the biomolecules of interest.

In the frame of the project 40 samples generated from the extracts of invertabrates and associated microorganisms collected mainly from the Indian Ocean, have been analysed by a strategy combining UHPLC/Orbitrap-HRMS, in positive and negative ion modes and NMR. For the NMR metabolomics, sample profiles were described by means of noesypr1D and J-resolved spectra recorded under an optimized standard procedure. For the MS-based metabolomics all derived chromatograms and spectra have been analyzed using the XCMS package as implemented in the R statistical language. After the analysis, the data were normalized according to multiple internal standards in order to remove undesired variation. Statistical evaluation of the results was performed by multivariate analysis (MVA) e.g. PCA, PLS-DA, OPLS. The most statistically diversified metabolites recognized were subjected to tentative structural elucidation in order to clarify the biological meaning to the results. The identification pipeline combined the computation of the Pearson's correlation coefficient (PCC) of the covariance of the low energy fragmentation mass spectra and the results by the CAMERA package. Various on-line mass spectral data bases have been used for the final structural assignment of the features identified to chemical structures.

Acknowledgements: This work has been financially supported by EU under the frame of TASCMAR project (H2020-BG-03-2014, Grant agreement: 634674).

Keywords: marine invertebrates, marine microorganisms, metabolomics

A new data processing procedure of UPLC-ESI(-)-HRMS untargeted metabolomics employing spectral slicing and stitching

Baira E[1], Siapi E[2], Zoumpoulakis P[2], Deligeorgis S-G[3], Skaltsounis A-L[4], Gikas E[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Panepistimiopolis, 15771, Athens, Greece
[2]Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48 Vassileos Constantinou, 11635 Athens, Greece
[3]Department of Animal Breeding and Husbandry, Faculty of Animal Science and Aquaculture, Agricultural University of Athens, 75 Iera Odos, 11855, Athens, Greece
[4]Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Panepistimiopolis, 15771, Athens, Greece

In the case of the MS-based metabolomics a fundamental problem is the large number of false positives. The aim of this study is to develop a new strategy, which highlights true positives by applying data processing in multiple narrow mass ranges instead over a wide mass range.
Blood samples from 20 chickens, which were administrated with Naringin in their nutrition and 9 samples from control, were analyzed by UPLC-HRMS (Orbitrap Velos).
Two methodologies have been applied for data processing. In the first one (classical approach), all data i.e. in the 100-900 *m/z* were included in the data processing procedure. To the newly developed methodology, the data were shred in 100 Da slices generating 8 datasets, which has been subjected to the downstream MS data processing. Each dataset was treated as separate and mz/t_R features obtained by either the VIP's or the t-test values were used as input for the construction of the general model. Comparing the two methodologies, 35 true positives according to the t-test and 36 using the VIPs score in the slicing methodology were identified. These features were not detected in the 100-900 *m/z* analysis.
A new workflow for a metabolomic data processing was developed. Slicing the LC/MS area using multiple narrow filters, increases the number of the identified true features since specific parameters were used is for each sliced area.

XCMS OPTIMISATION IN HIGH-THROUGHPUT LC-MS

Charlie Beirnaert[A,B*], Matthias Cuykx[C], Adrian Covaci[C] & Kris Laukens[A,B].

[A] Advanced Database Research and Modeling (ADReM), University of Antwerp; [B] Biomedical Informatics Research Centre Antwerp (biomina); [C] Toxicological Centre, University of Antwerp.

[*]charlie.beirnaert@uantwerpen.be

In the field of metabolomics XCMS[1] is arguably the most common used open-source software for the pre-processing of metabolomics data in R. However, the output of XCMS is highly dependent on the input parameters, a fact that is often ignored. Often the default parameters are chosen or they are adapted to the intuition of the researcher. A few algorithms have been constructed to optimize the parameters by using a dilution series[2] or by using the isotopes of carbon[3]. Nonetheless even with optimized parameters there is still a lot of output which is of no interest. This work aims to optimize the output of XCMS by building further upon the existing algorithms but adding an extra layer of quality control to the output of XCMS by also including the often discarded profile data (through the mzR package[4]). The eventual goal is to aggregate high quality results by performing multiple XCMS runs and keeping relevant data. The discrimination between relevant and irrelevant data is made by combining the centroid data and the profile data to find the isotopic profile of every peak by using wavelets to scan in the m/z dimension and gaussian fits in the RT dimension. With this method we are able to quantify every peak and every isotope not only in retetention time and intensity but also in the m/z dimension (resolving power), something which is not possible with only the centroid data. The first part of this research focusses on the integration between centroid and profile data and the quantification of isotopes. This is compared to the standard way of finding isotopes in an XCMS data analysis workflow namely by using the CAMERA[5] package.

Preliminary results indicate that the results from this workflow are complementary to the standard workflow for isotope identification (XCMS + CAMERA). Whereas in the standard workflow a lot of background signals are identified by XCMS as a collection of unique peaks, resulting in vertical stripes in Fig. 2, it is clear that with the new workflow a lot of these background peaks are automatically filtered out (Fig. 3). The reason for this is that the combined centroid & profile workflow starts with peak quantification in the m/z dimension followed by peak quantification in the RT dimension (whereas in XCMS there is only peak quantification in the RT dimension), and since for a certain background signal there is no such thing as a peak in the RT dimension (just a quasi continuous changing signal) this signal is discarded.

Fig. 2: Number of samples represented in each peak group (an isotope is a separate group in this representation) for the XCMS + CAMERA method.

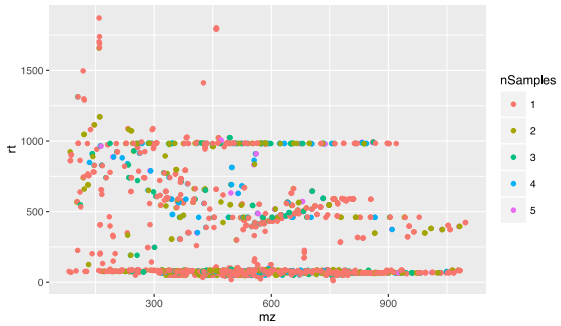


Fig. 3: Number of samples represented in each peak group (an isotope is a separate group in this representation) for the XCMS + profile data analysis method.

Discussion

As can be seen from Figures 2-3 the standard method and the profile data method deliver different results. The profile data method retains less background signals and also more isotopes (877 vs 109). However nearly all peaks found by the standard method are peaks which are present in at least 3 of 5 samples analysed whereas the new method identifies a lot of peaks only present in 1 or 2 samples, which also indicates why more isotopes are found since they are not well grouped and might be larger groups broken up into several smaller groups. Current work focusses on this grouping and better quantification of the differences between the standard method and the method presented

KEYWORDS: xcms, centroid data, profile data, high-throughput lc-ms analysis

REFERENCES:

[1] Smith, Want, et al. Analytical Chemistry (2006) 78.3 779-787

[2] Eliasson, Rännar, et al. Analytical Chemistry (2012) 84.15 6869-6876

[3] Libiseller, Gunnar, et al. BMC Bioinformatics (2015) 16.1 118

[4] Chambers, Matthew, et al. Nature Biotechnology (2012) 30.10 918-920

[5] Kuhl, Tautenhahn, et al. Analytical Chemistry (2011) 84.1 283-289

EXPLOITATION OF GLOBAL MICROBIAL BIODIVERSITY FOR THE DISCOVERY OF NOVEL COSMECEUTICALS USING LC-HRMS BASED METABOLOMICS

Celso Almeida,[1] Nikolaos Lemonakis[2], Jose Perez del Palacio[1], N.Tsafantakis[2], Victor González-Menéndez,[1] Ignácio González,[1] Fernando Reyes,[1] Evagelos Gikas[2], Nikolas Fokialakis,[2] Olga Genilloud [1]

[1] Fundación MEDINA, Av. Del Conocimiento 34, 18016 Armilla, Granada, Spain
[2] Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy - University of Athens, Panepistimioupolis - 15771 - Athens - Greece

MICROSMETICS, is an EU funded project aiming to discover and bring to development innovative products in the area of anti-ageing cosmeceuticals, originating from microbial biodiversity and using emerging and state of the art technologies in the field of biotechnology, natural products chemistry and applied microbiology.

The proprietary microbial collection of Fundación MEDINA (over 116.000 strains) is being exploited by incorporating modern high throughput screening platforms (*in silico* & *in vitro*) for the rational and targeted selection of the most promising strains. Advanced analytical approaches and techniques are being applied for the efficient, accelerated and advantageous isolation and identification of natural constituents, as well as the quality assessment of the lead products. A broad spectrum of bioassays and metabolomics approaches are being incorporated for the evaluation of anti-ageing, more specifically anti-oxidant, skin-protecting, and skin-whitening activity of all derived molecules.

In the frame of MICROSMETICS more than 110 potential candidate strains identified from a Rational Drug Design Tool (using a functional prediction model, virtual screening and similarity search) were selected to be studied. Among them 55 fungi and 55 ascomycetes were cultivated under "nutritional arrays". Approximately 1100 extracts have been generated and evaluated for their biological activity. Among them the top 100 were selected and a strategy combining UHPLC/Orbitrap-HRMS, in positive and negative modes, with multivariate statistical methods was applied. All derived chromatograms have been analyzed and a positive correlation between the profiles of the extracts with the aforementioned bioassays was observed. Thus, the 10 most promising extracts that represent the clusters generated in metabolomics have been forwarded for large-scale cultivation and bioguided isolation of novel molecules. Based on these results, the potentiality of these extracts was discussed.

Acknowledgment: This work has been financially supported by EU under the frame of MICROSMETICS project (FP7-PEOPLE-IAPP 2013, Grant agreement: 612276).

How do I analyze a double-blind crossover study? A nutri-metabolomics example.

Nikolaos Lemonakis[1]; Maria Halabalaki[1]; Vassilios Mougios[2]; Emmanuel Mikros[1]; Nikolaos Bliziotis[1]; Alexios Leandros Skaltsounis[1]; Evangelos Gikas[1]

1University of Athens, Department of Pharmacy, Athens 157 71, Greece;

2Aristotle University of Thessaloniki, Thessaloniki, Greece

Oleuropein is a secoiridoid glycoside that occupies a central role in the diet of the Mediterranean basin, presenting several pharmacological properties. *In vitro* assessments, as well as studies on experimental animal models, have shown that oleuropein possesses among others, hypolipidemic and antioxidant properties. Based on these findings, it is evident that the study of its impact on the metabolome is of paramount importance. In this study, MS nutri-metabolomics has been employed to assess the effect of administering oleuropein to healthy volunteers.

Oleuropein was administered to healthy volunteers and blood and urine were collected by a randomized, balanced, double-blind crossover study. The samples were analyzed by UHPLC-ESI LTQ-Orbitrap in both positive and negative ion mode and the resulting data have been explored by various peak-picking algorithms. The resulting data were subjected to multivariate data analysis employing various algorithms and the corresponding loadings were inserted to on-line databases for the investigation of the metabolites. In addition, possible changes in the lipidemic profile and the blood and urine redox status were investigated.

The impact of the peak peaking algorithms (centwave, centroidPicker and mzmatch) have been investigated giving similar results. The statistical evaluation revealed that the intra-subject variation was large and could obscure the final results; therefore, a multilevel approach using sparse-pls-da has been used in order to analyze the impact of oleuropein supplementation on the metabolism. It has been found that the metabolism is affected in each individual and there is significant discrimination between the groups serving as control and the treatment. Subsequent metabolic pathway analysis of the significant features discovered has revealed that the tryptophan and the acylcarnitines biochemistry have been mostly influenced.

ALTERATION IN THE LIVER METABOLOME OF RATS WITH METABOLIC SYNDROME AFTER TREATMENT WITH HYDROXYTYROSOL. AN MS AND NMR BASED METABOLOMICS STUDY.

I.V.Dagla[1], N. Lemonakis[2], H. Poudyal[3], L. Brown[3], D. Benaki[1], Al. Skaltsounis[2], E. Mikros[1], E. Gikas[1]

[1]Laboratory of Pharmaceutical Analysis, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Panepistimiopolis, 157 71, Athens, Greece

[2]Laboratory of Natural Products Chemistry, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Panepistimiopolis, 157 71, Athens, Greece

[3] Department of Biological and Physical Sciences, University of Southern Queensland 4350, Australia

Metabolic syndrome (MetS) represents a group of abnormalities that raises the risk for cardiovascular disease, diabetes and stroke. The Mediterranean diet seems to be an important dietary pattern which reduces the incidence of MetS. Hydroxytyrosol (HT) - a simple phenol found in olive oil - has received increased attention for its antioxidant activity. In this study, an experimental protocol has been setup including isolated HT administration in a diet induced model of MetS in young Wistar rats in order to find out whether HT can be considered as an active compound that has a protective effect against MetS.

Rats were randomly divided into four groups nurtured by cornstarch (CS) (normal diet), cornstarch + HT (CSHT), high-carbohydrate high-fat (HF) (Met inducing diet) and high-carbohydrate high-fat + HT (HFHT). HT (20 mg/kg/d oral gavage, water vehicle) was administered for 8 weeks on the basal diet (CS or HF). Following the pharmacological data, where the hepatic steatosis was reduced and the inflammatory cells into the liver were infiltrated, extracts of liver tissues were analyzed using UHPLC-HRMS (Orbitrap Discovery) and NMR spectroscopy (Bruker Avance III 600 MHz) followed by multivariate analysis, in order to gain insight on the metabolic effects of HT administration on the liver metabolome. Due to the complexity of the analysis various normalization methodologies i.e. QC-RLSC, Loess normalization etc. have been applied. HFHT rats were clearly distinguished from HF in PLS-DA, showing a difference in the liver metabolome between the groups and specific biomarkers were determined supporting the pharmacological findings.

A METABOLOMIC STUDY FOR THE DISCOVERY OF MULTIPLE SCLEROSIS BIOMARKERS IN CEREBROSPINAL FLUID BY HRMS AND NMR

C. Tsiagkas[1], D. Benaki[1], N. Lemonakis[2], A. Ioannidis[3,4], S. Chatzipanagiotou[4], C. Nikolaou[4], M. Anagnostouli[5,6], E. Mikros[1], E. Gikas[1]

[1]Laboratory of Pharmaceutical Analysis, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Panepistimiopolis, 157 71, Athens, Greece
[2]Laboratory of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Panepistimiopolis, 157 71, Athens, Greece
[3]Department of Nursing, Faculty of Human Movement and Quality of Life Sciences, University of Peloponnese, Greece
[4]Dept of Clinical Microbiology, Aeginition Hospital, University of Athens, Greece
[5]1st Dept of Neurology, Aeginition Hospital, University of Athens, Greece
[6]Immunogenetics Laboratory, 1st Dept of Neurology Aeginition Hospital, University of Athens, Greece

Multiple sclerosis (MS) involves an immune-mediated process where the immune system reacts against myelin as well as the nerve fibers themselves. The symptoms are severe including physical, mental and sometimes psychiatric problems. It can be thought as a disease threatening the general population with a high frequency i.e. by the end of 2008, between 2 and 2.5 million people are affected globally. As there is no clinical diagnostic marker e of the disease, the discovery of such could be of major importance for the correct diagnosis. Thus 65 samples from patients suspect for the disease (as found from IgG levels in cerebrospinal fluid compared to that of blood) have been analyzed by UHPLC – HRMS (Orbitrap Discovery) as well as by NMR (Bruker Avance 600 MHz). The results of measurements by both methodologies have been statistically evaluated using multivariate analysis. Furthermore the results have been compared and we were able to show that a number of metabolites were altered in MS patient samples, among them myo-inositol, glutamine, and tyrosine. Both methodologies converged providing evidence that tyrosine can be considered as a biomarker related to Multiple Sclerosis.

COULD PHARMACONETABOLOMICS UNCOVER THE RESISTANCE TO CLOPIDOGREL?

A.I. Milioni, D. Benaki, M.E. Tsoumani, N. Lemonakis, E. Gikas, A.D. Tselepis, E. Mikros

(1) Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, 15771 Athens, Greece
(2) Laboratory of Biochemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece

Nowadays, it is clear that the concept "the same drug for all patients with the same disease" is going to be replaced due to the different response of each person. It is observed that individuals with the same disease following the same drug therapy respond in a different way on this. For that reason, pharmaceutical science turns the interest to the field of personalized medicine. This term concerns the selection of the appropriate drug treatment according to the special characteristics, such as genotype and phenotype, of a patient in order to improve the efficacy of the administration. Pharmacometabolomics, a recently developed approach, shed light on the personalized response. In this work we present the correlation of the metabolic profile of patients suffering from acute coronary syndrome (ACS) with the resistance developed to clopidogrel treatment and the effort of emerging a potent biomarker to predict the resistance prior to antiplatelet therapy.

The metabolic profile of platelets was exemplified by MS and NMR spectra of aqueous extracts. Specifically, platelets from ACS patients were collected prior and after a 5-day treatment with 75mg clopidogrel. Metabolites were extracted from washed platelets and subsequently lyophilized, reconstituted in proper solvents and MS and NMR spectra were recorded. Aqueous extracts were measured using Ultra Performance Liquid Chromatography –High Resolution Mass Spectrometry (UPLC-HRMS) in (+) and (-) ionization, at 30000 resolution on a UPLC- LTQ Orbitrap. Furthermore, the one-dimensional (1D) noesypr1d pulse sequence and J-Resolved were performed on a Bruker Avance III 600 MHz at 300 K. The statistical analysis of data was performed by supervised multivariate method Partial Least Square-Discriminant Analysis (PLS-DA). Clopidogrel resistance information, determined by VASP phosphorylation (values PRI, platelet reactivity index) of the 5-day platelet samples, was used to create two data sets, i.e. the clopidogrel resistant and the one showing good response to the treatment. The multivariate statistical analysis resulted in a well defined separation of two groups in both treated and non treated data sets. The analysis revealed formate as the major metabolite responsible for the separation between the two groups. Finally, biomarker analysis based on ROC curve was performed in order to elucidate the capability of formate to predict the resistance.

Our results demonstrate that using a UPLC-HRMS and NMR based pharmacometabolomic analysis of platelets provide a valuable tool in the understanding of individual responses to antiplatelet therapy and more importantly in the forecast of resistance.

4th Workshop on Holistic Analytical Methods for Systems Biology Studies

17-19 April 2016
Thessaloniki, Greece

http://bioanalysis.web.auth.gr/metabolomics/index.htm