

2013

Book of Abstracts of the 1st International Symposium on Profiling

2nd - 4th
September 2013
Caparica - Portugal



**Book of Abstracts of the 1st International
Symposium on Profiling 2013
ISPROF 2013 - 1st**

Caparica - Almada, Portugal

2nd – 4th SEPTEMBER 2013

Book of Abstracts of the 1st International Symposium on Profiling 2013
ISPROF 2013 - 1st

Cover design: Hugo Santos

Organization of the Book of Abstracts: José Luís Capelo, Mário Diniz, Carlos Lodeiro, Hugo Santos, Elisabete Oliveira, Eduardo Araujo

ISBN: 978-989-98415-5-0 (pdf version)

ISBN: 978-989-98415-4-3 (paper version)

Printed by Proteomass (Portugal)

Printage: 200 copies (CD-ROM); 30 copies (paper)

Caparica, Portugal, 2013

Table of contents

Welcome	ix
Preface.....	xi
Plenary Lectures.....	19
PL1. Issues in Profiling: How to identify valid proteomic biomarkers and classifiers	21
PL2. Mining the urine proteome: approaches and challenges	22
PL3. Profiling Renal Cancer Using High Throughput Targeted Sequencing for Discovery, Diagnosis and Therapy.....	23
PL4. Fuzzy Optimal Associate Memories for Modeling Chemical Profiles: Authentication of Foods and Nutraceuticals.....	24
PL5. Latest development for the profiling and dereplication of natural products in complex biological matrices: evolution of revolution?.....	25
Shot-Gun Presentations	27
S1. Proteome Profiling of primary human multiple myeloma cells in comparison to the established multiple myeloma cell line RPMI-8226	29
S2. Quantitative proteomics of the chemokine IL-8 applying orbitrap and triple quadrupole mass spectrometer	30
S3. Metabolite profiling of cancer preventive polyphenols in a <i>Terminalia chebula</i> Retzius extract	31
S4. Assessment of drug effects exemplified by activated PBMCs treated with Aspirin and Dexamethasone, respectively	32
S5. Introducing microwave-assisted digestion protocol in top-down mass spectrometric protein analysis	33
S6. Bioactive molecules profile of two <i>Lactarius</i> species from Serbia	34
S7. Metabolite profiling of propolis polyphenols by microwave-assisted extraction combined with high-performance liquid chromatography using the fused-core technology	35
S8. Titanium dioxide nanoparticles inhibits <i>Saccharomyces cerevisiae</i> BY4741 proliferation, modifying the profile of antioxidant response.....	36
S9. Application of a high-resolution mass spectrometry for identification and quantification of endothelium biomarkers.....	37
S10. Optimized chromatographic analysis of ergosterol in wild and cultivated mushrooms.....	38
S11. Comprehensive two-dimensional liquid chromatography coupled to a multichannel detector: potentials and limitations for non-target analysis of complex samples	39
S12. Enzyme activity profile of peroxidases and polyphenoloxidases of <i>Malus domestica</i> Borkh varieties from Portuguese orchards during cold storage.....	40

P9. Time profiles of cypermethrin metabolites in orally exposed volunteers	126
P10. Profiling of thyroid hormone related gene expression to access the neonatal effects of endocrine disruptors	127
P11. Comparative analysis of the exoproteomes of <i>Listeria monocytogenes</i> strains grown at low temperatures.....	128
P12. Epidermal growth factor receptor inhibitory activity of new potential antitumor di(hetero)arylethers and di(hetero)arylamines in the thieno[3,2- <i>b</i>]pyridine series.	129
P13. Nanoencapsulation of aqueous extracts and essential oils from aromatic plants to use in food systems.....	130
P14. Sample preparation with aminothiols derivatization for metabolic characterization of endothelial dysfunction.....	131
P15. 2D-DIGE of the soluble fraction of sickle cells collected under steady-state and vaso-occlusive crisis reveals candidate transition pathway	132
P16. Gold Nanoparticles and Profiling: Human Serum@Chemical Depletion@AuNPs assisted Protein Separation.....	133
Sponsors Talks.....	135
Multiplexed label-free bio-affinity measurement using Surface Plasmon Resonance imaging ...	137

S10. Optimized chromatographic analysis of ergosterol in wild and cultivated mushrooms

João C.M. Barreira^{1,2}, Isabel C.F.R. Ferreira^{1*}

¹CIMO-ESA, Instituto Politécnico de Bragança, Apartado 1172, 5301-855 Bragança, Portugal

²REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

*iferreira@ipb.pt

Abstract

Sterols belong to the unsaponifiable fraction of several matrices, where they can be found as free or conjugated structures. In the latter, the 3 β -hydroxyl group is esterified with a fatty acid or a hydroxycinnamic acid, or glycosylated with a hexose (usually glucose) or a 6-fatty acyl hexose [1]. Ergosterol (an important vitamin D₂ precursor), is clearly the main sterol in mushrooms. Typically, the analysis of individual sterols includes the extraction of lipids, saponification (which might include a previous acid hydrolysis), extraction of unsaponifiable matter and separation/partial purification of sterols. The subsequent separation step might be performed by high performance liquid chromatography (HPLC), which is faster than gas chromatography analysis and operates under milder column temperatures and non-destructive detection conditions [2]. Herein, an analytical method for ergosterol determination in cultivated and wild mushrooms was developed using HPLC coupled to ultraviolet detection. The chromatographic separation was achieved in a Inertsil 100A ODS-3 reverse phase column using an isocratic elution with acetonitrile:methanol (70:30, v:v) at a flow rate of 1 mL/min. Different extraction methodologies were tested, using *n*-hexane, methanol:dichloromethane (75:25, v:v) or chloroform:methanol (20:10, v:v).

After studying the linearity (11 levels) for ergosterol ($t_R = 13.0 \pm 0.1$ min; coefficient of variation, CV = 0.65%), a seven-level calibration curve ($y = 0.6566x + 0.01098$; $R^2 = 0.9996$) was made using the peak/area ratio versus concentration of the standard (in $\mu\text{g/mL}$). The average of triplicate determinations for each level was used. The limit of detection (LOD), calculated as the concentration corresponding to 3.3 times the standard error of the calibration curve divided by the slope, was 0.3498 $\mu\text{g/mL}$; the limit of quantification (LOQ), calculated using the concentration corresponding to ten times the standard error of the calibration curve divided by the slope, was 1.060 $\mu\text{g/mL}$. The precision of the equipment was evaluated injecting seven consecutive times the sterols extract of the same mushroom. The optimized method proved to be precise (CV = 1.25%). Repeatability was assessed by applying the extraction procedure three times to the same dried mushroom powder. The method proved also to be repeatable (CV = 1.88%). The method accuracy was evaluated by the standard addition procedure (percentage of recovery). The standard mixture was added to the samples in three concentration levels (25, 50 and 100 % of the peak/area concentration, each one in triplicate) before the extraction. The method showed good recovery (above 90%).

Overall, a reproducible and accurate HPLC-UV technique was fully optimized. Chromatographic signals presented good resolution, indicating the suitability of this methodology to assess sterol profiles in future studies. Moreover, the chosen extraction and saponification steps do not require complex conditions, being suitable for routine analysis.

References

- [1] R.A. Moreau; B.D. Whitaker; K.B. Hicks. 2002. *Prog. Lipid Res.*; 41; 457-500.
- [2] A. Villares; A. García-Lafuente; E. Guillamón; Á. Ramos. 2012. *J. Food Compos. Anal.*; 26; 177-182.
- [3] J.C. Bada; M.L. Camacho; M. Prieto; L. Alonso. 2004; *Eur. J. Lipid Sci. Tech.*; 106; 294-300.

Acknowledgements

The authors thank to Fundação para a Ciência e a Tecnologia (FCT, Portugal) and COMPETE/QREN/EU for the financial support of this work (research project PTDC/AGRALI/110062/2009) and to CIMO (strategic project PEst-OE/AGR/UI0690/2011). J.C.M. Barreira also thanks to FCT, POPH-QREN and FSE for his grant (SFRH/BPD/72802/2010).