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**Πρόγραμμα Θαλής-**«Αξιοποίηση Φυσικών Αντιοξειδωτικών στην Εκτροφή των Αγροτικών Ζώων για Παραγωγή Προϊόντων Ποιότητας»

# Αζιοποίηση Φυσικών Αντιοζειδωτικών στην Εκτροφή των Αγροτικών Ζώων για Παραγωγή Προϊόντων Ποιότητας

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A comparison study in the processing procedure of data from untargeted metabolomic approaches by UPLC-ESI(-)-HRMS

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## **ABSTRACT**

# A comparison study in the processing procedure of data from untargeted metabolomic

## approaches by UPLC-ESI(-)-HRMS

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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 90-900 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<sub>R</sub> 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, 34 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 90-900 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.

#### A comparison study in the processing procedure of data from untargeted metabolomic approaches by UPLC-ESI(-)-HRMS HELLENIC REPUBLIO National and Kapodistrian Baira E1, Siapi E2, Zoumpoulakis P2, Deligeorgis S-G3, Skaltsounis A-L4, Gikas E1 University of Athens Department of Pharmaceutical Chemistry, Faculty of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Panepistimiopolis, 15771, Athens, Greece Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48 Vassileos Constantinou, 11635 Athens, Greece Department of Animal Breeding and Husbandry, Faculty of Animal Science and Aquaculture, Agricultural University of Athens, 75 lera Odos, 11855, Athens, Greece Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Panepistimiopolis, 15771, Athens, Greece Introduction Materials and Methods Samples: Blood samples from 20 chickens, which were Metabolomics is a new scientific field that studies the level of all administrated with Naringin in their nutrition and 9 samples from metabolites of a biological system giving the chemical fingerprint of the metabolism. One of the most commonly used technique Sample Preparation: For protein precipitation of plasma proteins, thereof, is mass spectrometry coupled to a separation technique cold acetone was added to reach a final ratio of 1/8 eg. LC-MS. The collected data are analyzed using a wide mass range which inevitably results in a large number of false positives. plasma/acetone (v/v). Instrumentation: ESI-LTQ-Orbitrap Velos connected to an Aim of the study UHPLC system. The development of a new metabolomics data processing Data Analysis: The data were analyzed by the open source strategy, that highlights the true positive features obtained software package MZmine 2.14.2. Multivariate analysis (MVDA) employing data processing over multiple narrow mass was performed using Simca-P+11.5. ranges vs a wide mass range Data processing chromatogram baseline correction deconvolution. Classical peak detection normalization PLS-DA R24 Q2 PLS-DA based Crop-Filter on Vips "cut" the chromatogram to 100 Da slice 100-200 200-300 300-400 400-500 500-600 600-700 700-800 800-900 m/z m/z m/z | m/z | m/z m/z PLS-DA based on Vips R2Y=0.96 PLS-DA based on t-test 40 ■ Vips 20 90-900 m/z crop-filter Fig.1 Comparison of the true positive features that are detected using either the Vips or the t-Comparing the two methodologies, 39 true positives according to the t-test test which are obtained after the data processing and 36 using the VIPs score in the slicing methodology were identified. These in multiple narrow mass ranges than over a wide features were not detected in the 90-900 m/z analysis mass range Conclusion 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 for each sliced area.

Η Επιτροπή Πιστοποίησης Παραδοτέων

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