SOFTWARE TOOLS FOR UPLC-MS METABOLOMICS

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1 MOTIVATION

MDRenal has developed three PC software tools to help the processing and analysis of mass spectrometry data sets in three different phases/aspects of a metabolomics study: **URINE-MS**, a tool designed for the optimization of the experimental conditions for metabolite detection over a group of target metabolites; **TOTALMATCH**, the software solution included in a kit for the absolute quantification of metabolites; **LIVERTOX**, a software that applies a predictive model for liver damage on a set of samples.

GENERAL FEATURES

1. Based on metabolite's signal detection in UPLC-MS spectra.
2. Independent of the mass spectrometry platform used (input spectra in the .mzXML open data format for mass spectrometry).
3. Background or noise subtraction (using blank solvent runs).
4. Data normalization using internal standards and bias corrections with calibration samples for systematic error reduction.
5. Visualization of chromatograms and spectra.
6. Export of intensities and signal-to-noise ratio values and report generation.

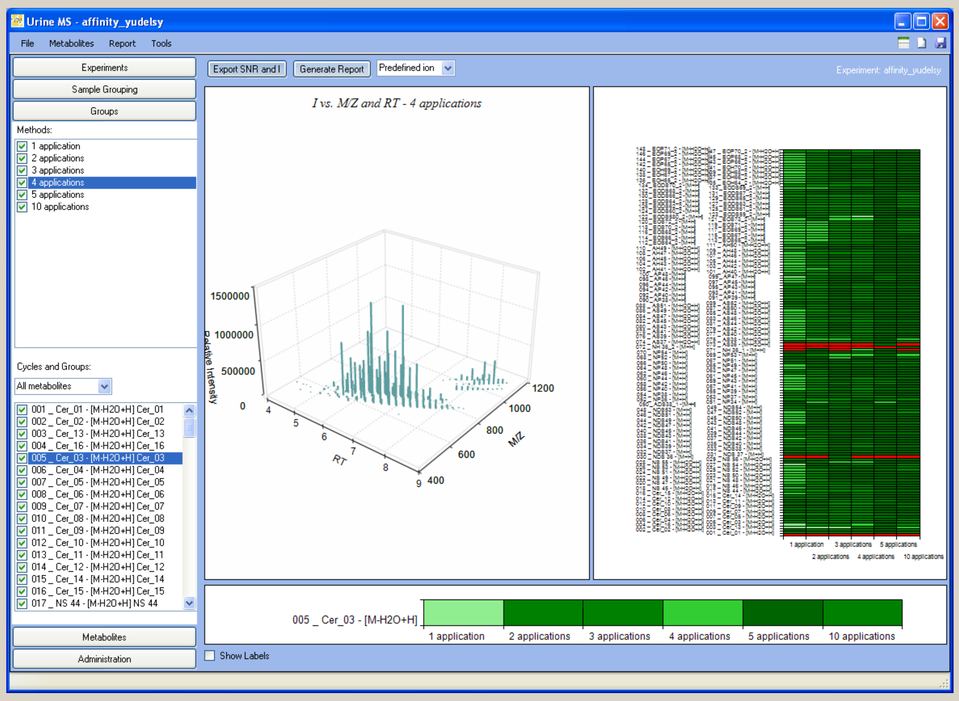
2 URINE-MS

URINE-MS is a targeted metabolomic data processing tool for the optimization of the experimental conditions in UPLC-MS studies.

The software processes the dataset for samples and analyzes intensity and retention time values for different ions/adducts of the metabolites, displaying the results on plots, tables and statistics, helping the selection of the optimal ion for the analysis.

URINE-MS enables an overall comparison of the intensities and retention time values among groups of samples corresponding to different experimental conditions, allowing the selection of the best method for the detection of the metabolites of interest.

Sampling for ceramide (CER) profiling in stratum corneum



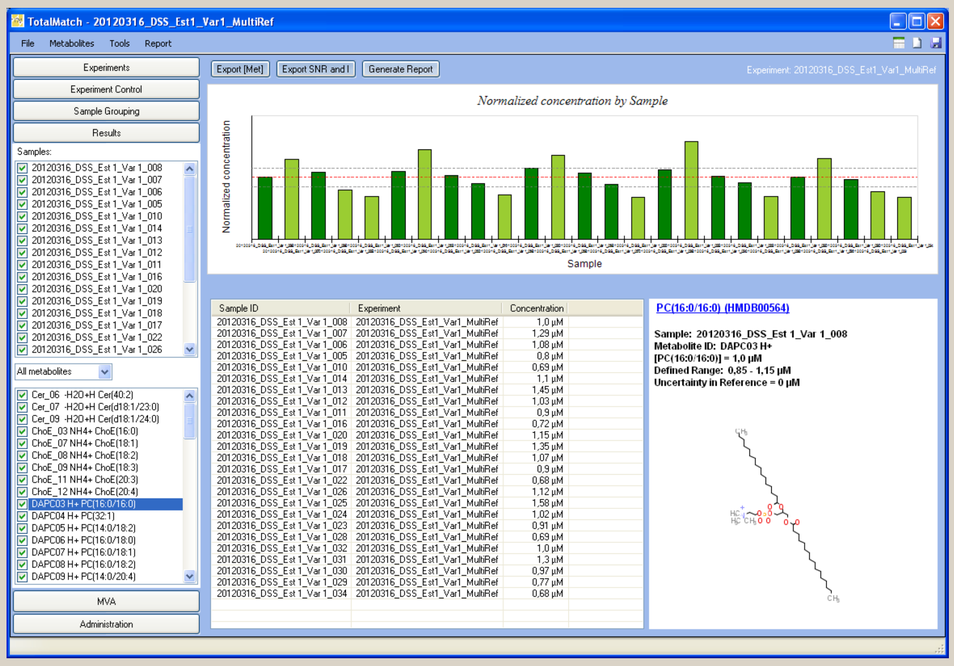
Sampling for ceramide (CER) profiling in stratum corneum: As part of method development for CER profiling it was necessary to establish the most adequate sampling procedure (number of applications of an adhesive strip) in order to obtain the highest amount of targeted compounds. Six adhesive strips of 5 cm length were applied 1, 2, 3, 4, 5 and 10 times over the same skin area. As shown, the software generates an intensity heat map that allowed the selection of the 5 applications-method as the most suitable one. This software-based tool reduced considerably the required time for method development.

3 TOTALMATCH

TOTALMATCH is the software solution for the determination of the concentration of metabolites on an UPLC-MS based platform by comparison with a previously quantified reference sample. Besides the software, the kit also consists on reference samples and the hardware accessories required for the analysis.

It can also be used in a "generic mode", setting to unit the concentration values of the reference sample. In such a case, the software can handle most kinds of metabolomic data offering diverse options for data normalization, chromatogram visualization, and multivariate statistical analysis.

Lipidomic profiling in Dried Serum Spots (DSS)

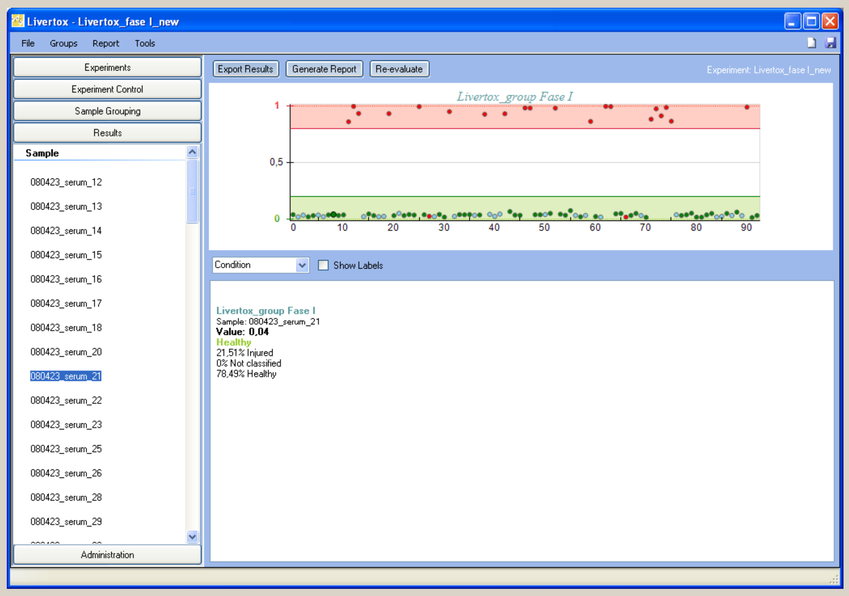


Lipidomic profiling in Dried Serum Spots (DSS): Aliquots of 100uL serum were spotted onto Agilent DMS cards. Punches of 3mm were drawn out from cards at times: 0, 24, 48, 72 and 96h. Additionally a sample of an "end point" (5040 h) was included in this study. The first sample, assuming time 0 when serum was already dried, was used as reference sample (RS). A wide variety of lipids were analyzed and their intensities compared with the RS. The software shows a user-friendly visualization of the results. For most metabolites the maximum intensity was reached at 48h and it is also shown the good reproducibility between injection replicates (n=5).

4 LIVERTOX

LIVERTOX applies a predictive model for hepatotoxicity over the set of samples to predict liver damage. The algorithm integrates the signal of the selected biomarkers in patients' samples and evaluates model's expression for these values to infer a diagnose.

Liver damage caused by galactosamine (GalN)



Liver damage caused by galactosamine (GalN): Blood serum samples of 24 mice, obtained pre- and post-treatment with saline solution and GaIN, were analyzed in an UPLC-MS based metabolomic platform. After data analysis a reduced group of metabolites were selected as putative biomarkers of liver damage and a predictive model was established. The software displays a plot with the predicted values and the established acceptance ranges (0.2 from maximum/minimum values). As shown, most of the samples were adequately assigned by the software as treated, control and non-treated animals.