Profiling of Urinary Metabolites.

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Overview

Introduction

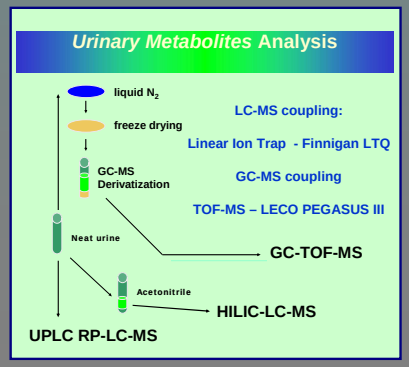
- Measurement of the small molecules in urine samples has been a part of clinical practice and clinical chemistry for more than 100 years. Since patients with metastatic kidney cancer (RCC) frequently have few if any presenting signs or symptoms, a non-invasive urinary assay for this disease, when applied to high-risk patients, has the potential to markedly reduce disease mortality. In this pilot study, we utilized urine samples from 6 patients with clear cell RCC (various stages and grades) and 6 control patients. We approached urinary metabolic profiling as a method of investigation with the techniques developed and validated in our laboratory.

Methods

- Samples have been analyzed by GC-TOF-MS, RP-LC-ESI-MS and HILIC-LC-ESI-MS.
- Multivariate statistics has been applied for data mining.

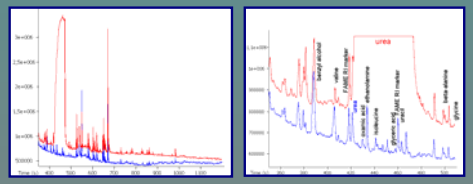
Results

- Results obtained from ANOVA and PLS experiments demonstrated the presence of the important components or metabolites which can be used to discriminate between urine samples from cancer patients and control samples from healthy volunteers based on their urine metabolic profiles.

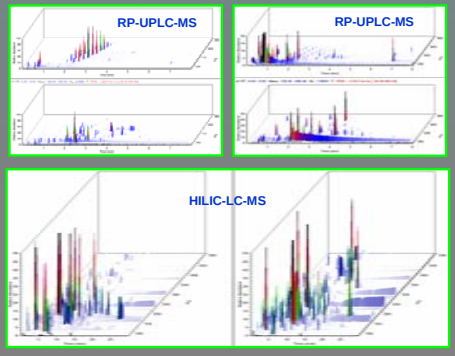


GC-TOF-MS profiling

The Pegasus III TOF mass spectrometer (LECO) offers acquisition of up to 500 spectra per second within mass range ~20 - 600 Da and automated peak deconvolution software for unbiased and high quality peak detection and chromatogram comparisons. We have equipped this instrument with robotic derivatization (GERSTEL) and exchange liner (ALEX) in order to diminish externally induced deviations by reaction times, matrix carryovers and analyte cross contaminations.



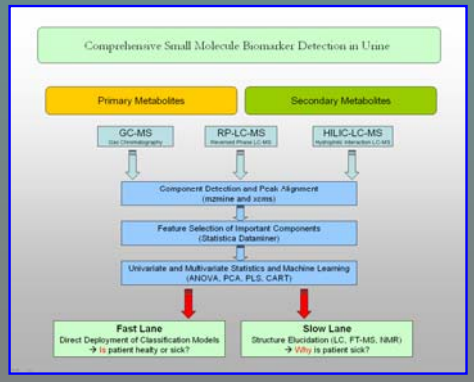
GC-TOF- MS profiles of the identical human urine samples pre-treated with and without urease



LC-MS profiling

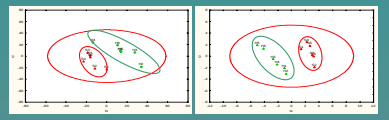
The ACQUITY UPLC System designed to match the performance needs of WATERS column chemistries with robust hardware and easy-to-use software. With UPLC we have the ability to work more efficiently with higher speed, sensitivity and resolution at a much wider range of linear velocities, flow rates and backpressures to obtain superior results.
High speed acquisition offered by FINNIGAN LTQ allows to perform online fragmentation experiments in both positive and negative modes providing detected components with the valuable structural information.

Data Mining

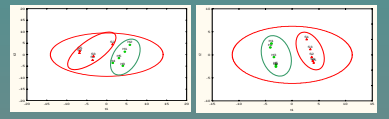


Alignment	Technique	Total features found	Significant features p < 0.5
mzmine	HILIC-LC-MS	3225	12
mzmine	RP-LC-MS	5034	18
mzmine	GC-MS	342	7
xcms	HILIC-LC-MS	1525	13
xcms	RP-LC-MS	136	27
xcms	GC-MS	1894	5

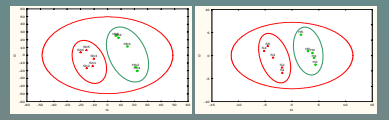
We have tested a number of software packages for sufficient deconvolution, peak picking, and alignment. Here we present the data obtained with the use of XCMS and Mzmine packages. Full scan data have been used to generate results.
PLS score plots for GC-MS urine analysis showing clear separation of **sick** and healthy individuals by 50 important features.
Left panels illustrate data aligned by XCMS, right ones aligned by Mzmine



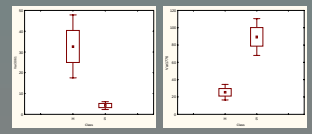
PLS score plots for RP-LC-MS (reversed phase) urine analysis showing clear separation of **sick** and healthy individuals by 50 important features.



PLS score plots for HILIC-LC-MS (hydrophilic interaction) urine analysis showing separation of **sick** and healthy individuals by 50 important features.



Categorized Box & Whisker Plot for HILIC-LC-MS (hydrophilic interaction) components C3061 and C1776. The left plot shows a component which is suppressed in sick individuals. The right plot shows a component which is highly elevated in sick individuals. The plots show the variable mean ± 1.96 times the variable standard deviation (95% confidence interval).



Conclusions

- GC-MS and LC-MS are two complementary methods providing sufficient information for metabolomics studies.
- Three complementary profiling techniques capable of analysis of the polar and non-polar urinary metabolites concluded with the same promising results of possible diagnostics.
- Biomarkers finding procedure can be based and supported with the presented results.