Correlation of analytical data obtained by NMR and LC-MS/MS in metabolomics studies

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In metabolomics a multitude of methods is typically used: NMR, LC-MS and GC-MS but data is only partially correlated usually by manual comparison of findings (e.g. comparing signals for a few selected metabolites). At present there is no technology in linking such complex multi-dimensional data, to combine them into one table of metabolites. Generation of such tools will advance the process and the success rate of discovery work.

The holistic NMR analysis was accomplished utilizing a 600 MHz Varian NMR spectrometer by applying CPMG pulse sequence and using sodium maleate as internal standard. Sample pretreatment of HAF samples included freeze—drying to increase signal intensities. The LC—MS—MS analysis was achieved by the ACQUITY Ultra Performance Liquid Chromatography System Xevo TQD MS System (Waters). An ACQUITY HILIC, 2.1x150 mm, 1.7 um, BEH amide column together with a ACQUITY UPLC BEH Amide 1.7 um VanGuard pre—column was used under 500 uL/min. Gradient elution employed a ramp of water vs ACN both buffered with formic acid and ammonia (10 mM). 100 MRM channels were set in time windows of ca.1—3 min for total analysis time of 40 min (Virgiliou et al, Electrophoresis, Submitted).

Venn diagrams for amniotic fluid compounds identified with each technique. This diagram was applied to specify the metabolites identified with both analytical techniques (common metabolites). 17 metabolites out of the 51 and 37 metabolites identified in HAF samples with LC—MS/MS and NMR respectively were common. In serum samples the common metabolites were 17 out of the 55 and 27 metabolites identified with LC-MS/MS and NMR respectively.

Correlation of the areas of the metabolites Val, Ala, Crtn identified with LC-MS/MS and NMR in blood serum samples.

DISCUSSION

Combination of LC-MS/MS and NMR data is not possible for the HAF samples, as the correlation of the common metabolites is low. This could result from the sample preparation procedure. On the contrary, high correlation is observed on the common metabolites found in serum. This observation leads us to the next step, the fusion of the two datasets reached with the different analytical techniques. A major obstacle in this direction is the large difference in signal counts (3 orders of magnitude) and the nature of signal response (analyte specific in MS).