Metabolite Profiling with Isotopically Encoded Chemical Derivatization

Grace C. Tong, Elizabeth J. Want, Colin A. Smith, Zhouxin Shen, Meng-Lin Tsao, Juncai Meng, Theodore Brandon, William Webb, Gary Siuzdak

Department of Molecular Biology, Department of Chemistry, and The Scripps Center for Mass Spectrometry
The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

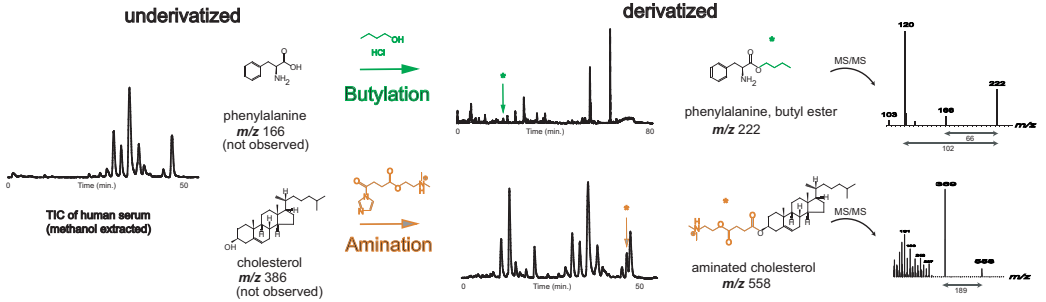
Overview

- A chemical modification platfrom for biomarker discovery through metabolite profiling is described.
- Quantitative analysis of peak intentisy through isotope labeling chemical derivatization is demonstrated.
- Allows for increased throughput through pooling, split pooling, and improved quantitation.

Introduction

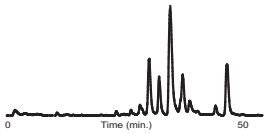
Human plasma contains a complex mixture of metabolites that reflects global changes from system wide catabolism and anabolism, providing a viable resource for biomarker discovery. Recent developments in our laboratory have made it possible to mine large sets of data obtained from LCMS analysis of human plasma metabolites including the development of isotope labeling chemical derivatization, a novel approach to metabolite profiling. This approach applies the same principles as isotope labeling in proteomics to metabolomics, expanding the range of detectable metabolites by the thousands.

Enhanced Ionization Through Chemical Derivatization – Application to Human Plasma Metabolite Profiling

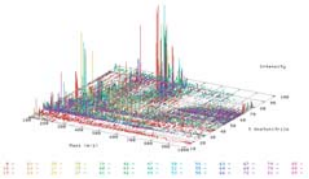


Chemical Derivatization enhances isonization, allowing the detection of a greater number of metabolites, some of which are otherwise undetectable. For example, ions of phenylalanine and cholesterol are absent in underivatized samples, where as the corresponding butyl-ester or aminated forms are readily detected. Further, MSMS fragmentation data varifies that a metabolite has been chemically derivatized.

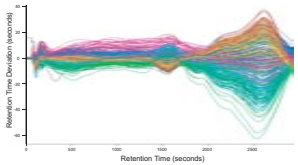
Human Plasma ESI-LCMS Metabolite Profiling



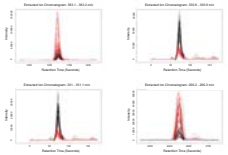
LCMS analysis of methanol extracted human plasma metabolites acquired in the positive ion mode with an Agilent 1100 MSD mass spectrometer. Separation was achieved using a Waters symmetry 2.1mm by 100mm C18 column with gradient conditions of 5 to 95 % acetonitrile.



A METLIN generated 3D plot of features constituting >50 LCMS analyses of plasma metabolites; each analysis contributes >2000 features. METLIN is a webbased database developed for LCMS data viewing and analysis, as well as a resource for unknown metabolite identification. It currently holds 219 LCMS analysis, 3000 metabolites, 70 MS/MS spectra from electrospray, 20 fractions of FTMS data.

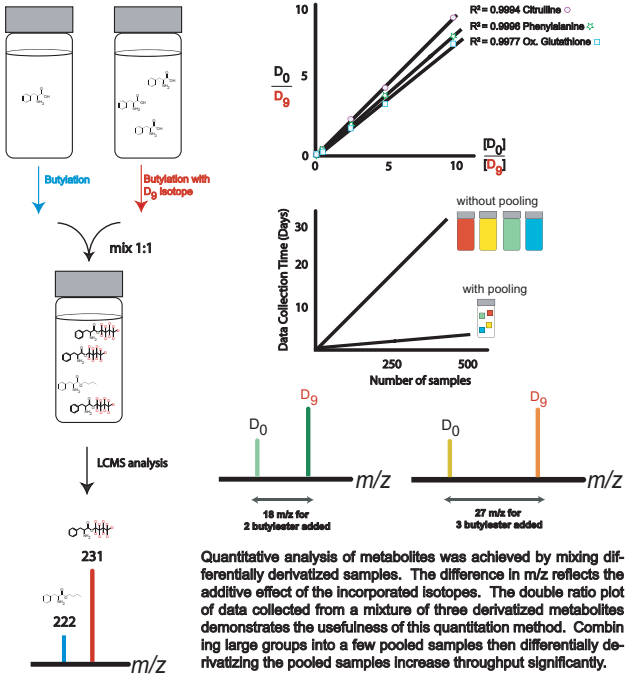


Retention time correction plot generated by XCMS. This plot represents the retention deviation of 500 LCMS analyses of human plasma from 250 different samples separated on one C18 column; total run time was 30 days of consecutive data collection.
XCMS is a software developed for comparative analysis of LCMS data.



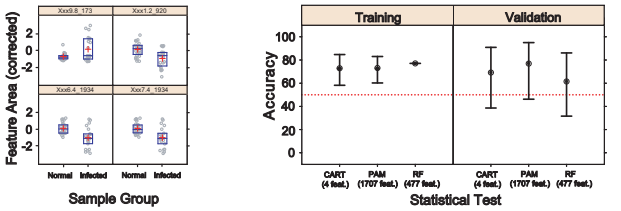
Extracted Ion current chromatograms of retention time corrected features selected by XCMS for direct visual comparison of features intensities among data groups of interest. These peak areas are used in the subsequent statistical analyses for biomarker identification.

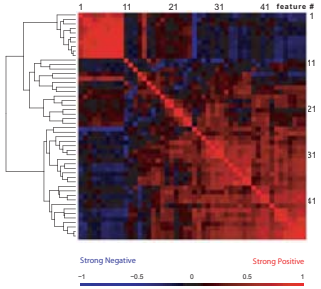
Quantitative Metabolite Profiling Through Isotope Labeling



Quantitative analysis of metabolites was achieved by mixing differentially derivatized samples. The difference in m/z reflects the additive effect of the incorporated isotopes. The double ratio plot of data collected from a mixture of three derivatized metabolites demonstrates the usefulness of this quantitation method. Combining large groups into a few pooled samples then differentially derivatizing the pooled samples increase throughput significantly.

Biomarker Discovery – Results from Data Analysis of 500 Analyses





Feature Correlation Heat Map

Data analysis begins with XCMS and the resulting features were further analyzed to give normalized plots of area sum for each feature detected.
Three independent statistical tests: CART, PAM, and RF were used to evaluate the same data set for possible biomarkers for disease diagnosis. The resulting accuracy were plotted side by side showing an overall 76% chance of correct prediction both in the original dataset (Training) as well as new data (Validation).
Over 10,000 features were detected by XCMS, however, only a fraction of these were selected to be useful for disease diagnosis.
The correlation heat map and histogram illustrates the interrelationship of the top 50 biomarkers chosen. The correlation linkage may represent metabolites produced by the same or similar biochemical pathway.

Conclusion

This study presents an approach for metabolite profiling and biomarker discovery with advantages in enhanced ionization, improved quantitation and increased throughput.