A multi-omics approach to analyze serum using a liquid chromatography/tandem mass spectrometry (LC-MS/MS) platform.

Mass Spectrometry

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Blood serum is an important biological fluid carrying vital information in forms of hormones, proteins, and other molecules reflecting the health and disease states of an individual. The dynamic range and complexity of these biomolecules presents challenges in analytical reproducibility and sensitivity. Using a multi-omics LC-MS/MS approach, this study aims to globally profile proteins as well as polar and nonpolar metabolites from a single serum sample.

Samples were prepared in parallel using a modified Folch metabolite extraction protocol on 0, 10, 50 and 100µl of human serum (Sigma). Briefly, samples were mixed with 1-iso-propanol and water, vortexed and incubated in liquid nitrogen with 3-cycle of thaw-freeze-thaw prior to a 10-minute sonication. Chloroform was added to induce a bi-phasic separation and three sample fractions, A-aqueous, O-organic, and NR-non-extractable residue, were collected after centrifugation. Fractions “A” and “O” were analyzed using a LC-MS/MS metabolite method over 60 minutes on an Eclipse™ in triplicates. Fractions “NR” was enzymatically digested and analyzed using RP-C18 HPLC over a 120-min gradient, on a QE-plus™ in duplicates.

A total of 119 proteins were observed in eight “NR” samples. Albumin and IgG accounted for ~40% of the entire protein content. The top 10 most abundant proteins such as serotransferrin, transthyretin and apolipoproteins constituted ~45% with the remaining ~15% of low abundance proteins and common contaminants. Sixty-seven proteins were observed from the “10µl” datasets. For the “O” and “A” fractions, a total of 32 samples were analyzed using Compound Discoverer 3.1.0.305. Without filtering, >3500 features were observed in each fraction. With stringent filtering criteria, 1610 and 1227 compounds remained in the “O” and “A” datasets, respectively. Taken together, this multi-omics LC-MS/MS approach worked for as little as 10µl of serum profiling 119 proteins and >3500 metabolites globally. Further method development is needed for robust, reliable, and reproducible data interpretations.