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Lipidomic Profiling Using Sub-2μm Particle CO_2 Based Supercritical Chromatography Mass Spectrometry

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The typical chromatographic methods for analyzing lipids include gas chromatography/mass spectroscopy (GC/MS) and liquid chromatography-tandem mass spectrometry (LC/MS/MS). However, there are shortcomings associated with each of these methods. For example, GC methods require derivatization of the fatty acids to hydrolyze and convert to methyl esters, which is time-consuming and risks re-arrangement of the fatty acids during derivatization, leaving doubt as to whether the esters formed are from free fatty acids (FFA) or intact complex lipids. In LC/MS methods, although no sample derivatization is required, the runs typically involve labor-intensive and time-consuming sample preparation, and utilize toxic organic solvents, which are expensive to purchase and dispose. In a typical reversed phase (RP) LC/MS analysis, the organic extracts containing all the lipids have to be evaporated and re-constituted in a more compatible injection solvent. Here, we present a rapid, high-throughput and efficient sub-2μm particle CO_2 based supercritical chromatographic method for the separation and analysis of FFA and Neutral lipids (MG, DG, TG and CE) using UltraPerformance Convergence Chromatography (UPCC, or UPC^2) with mass spectrometry. The organic phase extract containing all the lipids can be injected directly into the system, which results in significant savings in sample preparation and analysis time, solvent costs, and solvent waste disposal. The analysis of complex biological extract produced a very good separation of the FFA and neutral lipids in less than 5 minutes. The separation mechanism is according to both chain-length and degree of unsaturation. Another benefit of the method is the ability to separate between lipid isomers. Lipids can have different biological functions based on the double bond position (e.g., omega-3 and omega-6). The separation of 20:3 (Δ8,11,14) and 20:3 (Δ11,14,17) isomers based on the position of the double bond have been observed. A novel software tool will be used to provides automatic peak detection followed a quantitative comparison and statistical analysis to differentiate those features that are significantly changing and finally identify those features from the mass spectrometry data. Preliminary results showed there is a group separation between the different biological samples. Some potential lipid biomarkers that contribute to the group difference have been identified.

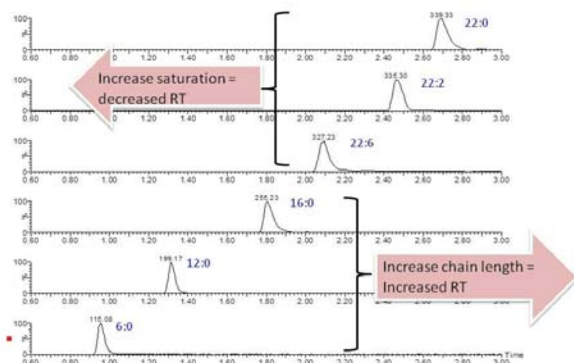

Figure 1