

Acquiring MS/MS data, data processing and normalization

MS/MS spectra for lipids were acquired using an iterative approach in the MassHunter Acquisition Software (Version 10.1.48, Agilent Technologies) on an Agilent 6545 QTOF. Source settings for MS1 data acquisition were used. MS/MS spectra were acquired at a scan rate of 3 spectra/s with different intensity thresholds and collision energies of 10, 20, and 40 V to increase identification rates. For the ID-X Orbitrap, data were acquired in data dependent acquisition (DDA) by using the built-in deep scan option (AcquireX) with a mass range of 67-900 m/z. The MS/MS scans were acquired at 15K resolution and cross referenced to a library generated from NIST SRM 1950 plasma sample in both positive and negative ion mode with different collision energies in the range of 20 NCE to 50 NCE for HCD and 30 NCE for CID to maximize identifications.

Lipid iterative MS/MS data were annotated with the Agilent Lipid Annotator software. All data files were then analyzed in Skyline-daily (Version 22.2.1.256) to obtain peak areas, m/z values of the metabolite and lipid target lists, obtained from the metabolite identification workflow, which had at least an MS/MS match to an online library, were extracted under consideration of retention times.