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Development of automated MS/MS methods on an Orbitrap Fusion™ and a spectral database for in-depth lipidomic analysis of human plasma

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Liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) is the most widely used analytical method for untargeted lipidomic analysis of human biofluids. However, several issues still remain for the comprehensive structural characterization of lipids due to their naturally occurring huge structural diversity with difficult to resolve co-eluting isobaric and isomeric species. In this study, we have evaluated and optimized different new acquisition methods from targeted LC-HRMS/MS (tMS2) with inclusion lists to optimized data dependent (DDA) Top-N and data independent acquisition (DIA) SWATH (Sequential Window Acquisition of all THeoretical fragment ion spectra) workflows using an Orbitrap Fusion™ mass spectrometer (ThermoFisher Scientific) for cataloguing the human lipidome at high confidence.

Several parameters were optimized such as the Orbitrap mass resolution for the collection of MS and MS/MS spectra, the collision energy applied, and the number of windows for the SWATH method. The quality of the resulting spectra were first evaluated by matching to the *in silico* database LipidBlast using the software MS DIAL.

The tMS2 method uses inclusion lists to fragment the most relevant lipid precursor ions and allows the manual annotation of up to 406 unique lipid species in human plasma. The optimized DDA Top 10 method used a resolution of 120k for the MS and 30k for the MS2 scans, using stepped collision energy conditions to generate meaningful MS/MS spectra for the different lipid classes. Under these conditions up to 355 unique lipid species were confidently identified after matching with the LipidBlast database and manual verification.

We also devised a DIA SWATH method with variable overlapping 15 windows to partition the whole set of precursor ions almost equally across the ~350-1000 m/z range, thus yielding 302 unique annotated lipid species following MS-DIAL deconvolution and LipidBlast search.

All the high-quality and manually curated MS/MS spectra of endogenous lipids were then compiled within a single database file for ~450 distinct lipid species. The efficiency of the MS DIAL software was also evaluated for the automatic identification of lipids by matching to both the LipidBlast database and the manually curated database. An in-depth study of the scoring system allowed us to define threshold values for dot and reverse dot product scores, above which MS/MS spectra can be considered as confidently matched. Overall, the generated data and knowledge can be highly useful for the lipidomics community.