A workflow for high resolution ESI-MS lipidomics data processing

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Lipids comprise a diverse group of biomolecules organized in height major categories with a considerable range of structural features and chemical properties [1]. Great attention has been recently devoted to lipidomics since it has been observed that dysregulation in lipid profile may be related to several human diseases, such as , e.g. Parkinson disease [2], tumorigenesis [3], atherosclerosis [4] and diabetes [5] etc.. Typically, individual species are identified and quantified with mass spectrometry (MS)-based platforms using sample preparation protocols and synthetic lipid standards if a targeted approach is followed. Alternatively, lipids can also be profiled with untargeted analysis; in this case, lipidomics data requires automation with the support of dedicated software. In the last few years, several efforts have been shaped with the aim to develop software to analyze HR-MS data, such as XCMS [6] or Alex^{123} [7]. Note, however, that the only matching of *m/z* values without further data processing and validation can represent a major source of error. Here, a workflow applied to lipidomics analysis of complex real samples (*i.e.* human plasma, lymphocytes and fibroblasts or food samples) is described; hydrophilic interaction liquid chromatography (HILIC) coupled with electrospray ionization and Fourier-transform mass spectrometry (ESI-FTMS) enabled us to collecting data and Alex^{123} software was employed to obtain data matrix. Orthogonal information provided by lipid class elution time, along with specific databases created for searching specific adducts (i.e. demethylated adducts for choline-containing lipids) corroborate putative attributions; the subsequent regiochemical assignment was accomplished upon careful examination of each CID-MS/MS spectrum obtained using ESI-LIT-MS instrumentation, interpreted with the auxiliary of a home-made database that, in silico, simulate MS/MS profiles.

References

1. E. Fahy *et al.*, Update of the LIPID MAPS comprehensive classification system for lipids, *Journal of Lipid Research*, 2009, pp. S9–S14.
2. Calvano *et al.*, Searching for Potential Lipid Biomarkers of Parkinson's Disease in Parkin-Mutant Human Skin Fibroblasts by HILIC-ESI-MS/MS: Preliminary Findings, *nternational Journal of Molecular Sciences*, 2019, p. 3341.
3. C. R. Santos and A. Schulze, Lipid metabolism in cancer, *FEBS Journal*, 2012, pp. 2610–2623.
4. M. F. Linton *et al.*, *The Role of Lipids and Lipoproteins in Atherosclerosis*, *Endotext*, 2000.
5. R. W. Gross and X. Han, "Lipidomics in Diabetes and the Metabolic Syndrome," 2007, pp. 73–90.
6. N. America and S. Nature, "Systems biology guided by XCMS Online metabolomics Addressing reproducibility in single- laboratory phenotyping experiments," *Nature Publishing Group*, 2017, Nature Publishing Group, pp. 461–462.
7. P. Husen *et al.*, Analysis of lipid experiments (ALEX): A software framework for analysis of high-resolution shotgun lipidomics data, *PLoS ONE*, 2013, pp. 1–13.