

## **Hyphenated Tools for Phospholipidomics**

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The analysis of underivatized lipids within body fluids as well as cell and tissue extracts is still a very challenging task because of its biochemical and clinical relevance. Anomalous lipid concentrations are correlated to neoplastic and neurodegenerative diseases, diabetes etc.. Structural diversity of each lipid or lipid class respectively will have a distinct effect on membrane properties (i.e. fluidity, permeability, oxygen scavenger, etc.).

Therefore, the advantage of recombined use of HPLC, MS and NMR will be shown. Many studies dealt with the analysis of lipids, but to our knowledge nobody used a combinatorial approach so far. A combination of HPLC separation power, MS sensitivity with accurate mass measurement of molecular and fragment ions and NMR structure elucidation power will meet most suitably the challenge. Furthermore, the low NMR sensitivity can be compensated by preceding concentration steps via HPLC and fraction sampling. New HPLC methods for several phospholipid classes (i.e. sphingomyelin, phosphatidylcholine, phosphatidylethanolamine) were developed and the retention times and the detected masses were determined. Location of fatty acids with respect to position sn-1 and sn-2 were identified in negative ion mode by the relative intensity of their [M-H] ions and the neutral loss of the fatty acid ketene. In positive ion mode the polar head group was cleaved off. The molecular formula was generated by matching high mass accuracy and isotopomer pattern. Furthermore, the separated fractions were assigned by means of the 1D- and 2D-NMR-spectra. Saturated, mono unsaturated (MOFA) or polyunsaturated fatty acids (PUFA) show zero, two or four carbon signals between 120 and 130 ppm. The MOFA and PUFA reveal unambiguously different chemical shifts for the olefinic carbons. However, lipids with MOFA's have similar olefinic carbon shifts. Nonetheless, a lipid with two MOFAs is deduced from the intensity ratio of the olefinic protons with respect to the glycerol protons.

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