Segmented Flow Strategies for Interfacing Microflow NMR with LC-MS to Identify the Volume and Mass-limited Metabolites and Lipids

Rohit Mahar[1], Jiajun Lei[2], T. J. Garrett[3], R. A. Yost[2], Matthew E. Merritt[1]

[1]Department of Biochemistry & Molecular Biology, [2]Department of Chemistry, [3]Department of Pathology, Immunology & Laboratory Medicine, University of Florida, Gainesville, Florida, USA

The unambiguous identification of known and more importantly unknown analytes in complex mixtures often requires the use of chromatographic separation coupled to detectors that give high structural information.[1] In the hyphenation of LC-MS and NMR, the limitations are derived largely from the low sensitivity of the NMR experiment. A small volume and mass sensitive, capillary-flow probe can play a unique role for the NMR spectroscopist.[2] Microflow NMR combines feature high sensitivity with supreme ease of use and operating at the capillary-scale will save time and money while enhancing your sensitivity. For increased functionality at a remarkable value Microflow NMR is an enabling technology for the analysis of mass- or volume-limited samples.[3]

Lipids, are structurally diverse *e.g.* phospholipids, LPC, PC, LPE, PG, PI, PE and free fatty acids (FFA), contain all the possible isomers such as *cis*, *trans* and *sn* isomers[4]. LC provides very limited separation of isomeric lipids. High resolution MS (exact mass and fragmentation pattern) similarly provides little or no differentiation between lipid isomers, and MS/MS methods can help in limited circumstances. Thus, the addition of NMR to LC/MS and LC/MS/MS workflows may significantly improve our ability to identify unknown lipids or to differentiate isobaric species. The Protasis CapNMR microcoil probe has been used to measure ^{1}H NMR spectra of a series of lipid isomers. Two pairs of phosphatidylcholine (PC) isomers were used to evaluate the potential of microcoil NMR as a means of enhancing LC/MS based lipidomics. This was used for differentiating the (1) cis/trans isomers - PC(16:1/16:1) Δ9 cis and PC(16:1/16:1) Δ9 trans and (2) positional isomers - PC(18:1/18:1) Δ9 cis and PC(18:1/18:1) Δ6 cis. One Diacylglycerol DG(22:1/22:1) *sn* 1,3 was used for measuring the limit of detection (LOD) and it was found to be 0.30 nmol/µL. Since the microcoil active volume is ~2 µl and the concentration is in the nmol range, the microcoil has tremendous advantage for volume- or mass-limited samples. Future studies will be focused on implementing segmented flow to make LC-NMR-MS/MS a complete workflow for unknown metabolite and lipid identification.

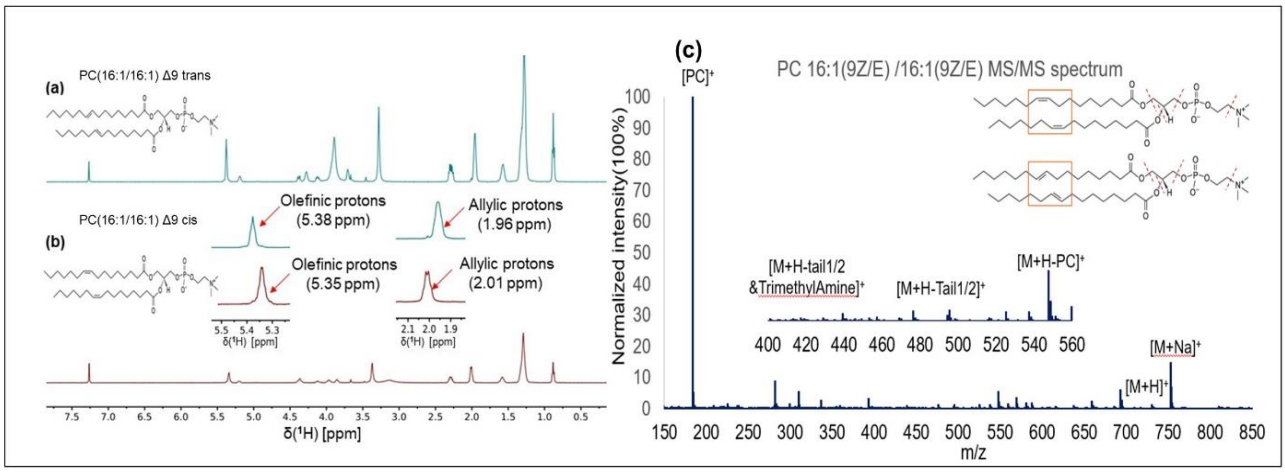

Figure 1. ^{1}H NMR spectra of (a) PC(16:1/16:1) Δ9 trans and (b) PC(16:1/16:1) Δ9 cis showing the chemical shift difference for the olefinic and allylic protons. (c) High resolution MS/MS spectra of two lipid isomer pairs. In the MS/MS spectra above, $[M+H]^+$ and $[M+Na]^+$, ions of PC head groups and neutral loss of tail 1(or tail 2) are also observed.

References:

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