Implementing Microflow NMR to identify and differentiate the lipid isomers by ^{1}H NMR Spectroscopy

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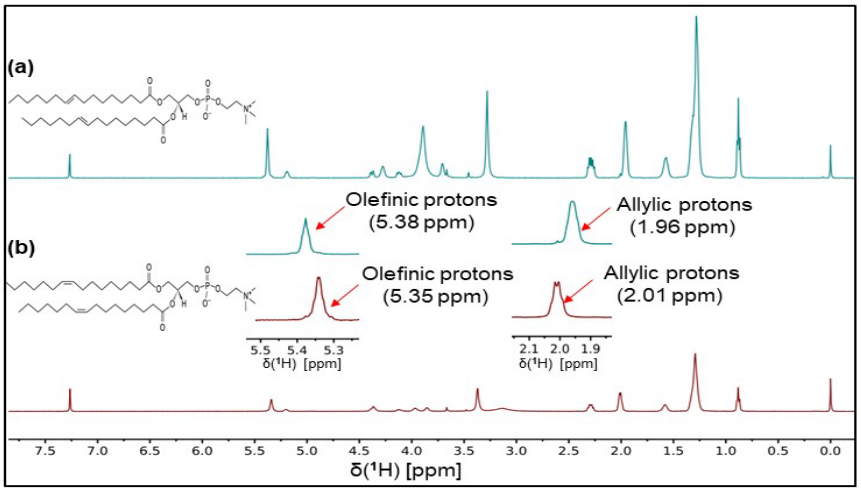
Introduction

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.[1] 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, nothing else compares Microflow NMR as an enabling technology for the analysis of mass- or volume-limited samples.[2] Lipids, are structurally diverse and contain all the possible isomers such as *cis*, *trans* and *sn* isomers of one molecular formula are considered. These isomers cannot be resolved by LC/MS as they have the same molecular weights. Phosphatidylcholine (PC) isomers were used to evaluate the potential of microcoil NMR as a means of enhancing LC/MS based lipidomics.

Experimental

All Lipid samples were prepared in $CDCl_3$ (≥99.5%) of 1.36 mM for Microflow NMR experiments. Protasis CapNMR microcoil probe has been used for recording the ^{1}H NMR spectra of isomers on Bruker Avance III 500 MHz NMR spectrophotometer. The total probe volume is 7µL, with an active RF coil volume of ~2µL and the feedlines are 50 µm (i.d.) fused silica capillary. Samples were manually injected into the inlet feedline port using a syringe. The magnetic field strength is locked on ^{2}H signal. Manual shimming was performed on each of the samples. ^{1}H NMR Parameters were, Relaxation delay = 3.0s; Acquisition time = 2.7s; data points = 16k; pulse width = 13.04 µs and Number of scans = 128.



Results and Discussion

^{1}H NMR spectra helped to easily differentiate the isomeric pairs of the lipids. As shown in **figure 1**, chemical shift of olefinic protons of trans isomer is higher than the cis isomer of PC(16:1/16:1)Δ9, whereas the shifting is reverse for allylic protons. Positional isomeric pair PC(18:1/18:1)Δ9 cis and PC(18:1/18:1)Δ6 cis, were also differentiated by the ^{1}H NMR spectra. One standard of diacylglycerol DG(22:1/22:1) *sn* 1,3 was used for measuring the limit of detection (LOD) and it was found to be 0.27 mM.

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.

Conclusions

Since the microcoil active volume is ~2 µl and the concentration is in the nmol range hence 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 lipidomics.

Acknowledgements

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References

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