Analysis of Lipid Classes using Normal Phase HPLC on Cyanopropyl Coated Silica in Combination with ESI-MS/MS.

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Numerous methods for separation of lipid classes using straight phase liquid chromatography have been developed over the last 40 years. A majority of the methods have been based on stationary phases consisting of either pure silica or diol bonded silica. In 1995, Christie et. al. developed a promising method to separate plant lipid classes using cyanopropyl bonded silica as stationary phase [1]. Unfortunately, despite the fact that excellent separation of several phospholipid and glycolipid classes was achieved and reported, very little work has been done to further characterize the usage of cyanopropyl bonded silica columns for lipid class separation in straight phase liquid chromatography.

From previous work using a cyanopropyl bonded silica column, we have shown that it is possible to get a good separation of a majority of the common lipid classes using non-aqueous mobile phases [2]. In the current work we demonstrate the application of straight phase HPLC with non-aqueous mobile phases in combination with ESI-MS/MS for the analysis of lipids. Here, we separate and elute all investigated lipid classes in 25 minutes on cyanopropyl bonded silica by using a binary gradient consisting of Hexane and Toluene/Methanol (60:40 w/w) +5mM Ammonium Acetate as mobile phases.

With a 3D ion trap system in MS^3 mode we could separate and identify the sodium adducts of phosphatidylethanolamines, phosphatidylcholines and lyso-phosphatidylcholines for quantification of individual lipid species in plant and animal extracts.

1. Christie, W.W. and R.A. Urwin, *SEPARATION OF LIPID CLASSES FROM PLANT-TISSUES BY HIGH-PERFORMANCE LIQUID-CHROMATOGRAPHY ON CHEMICALLY BONDED STATIONARY PHASES.* Hrc-Journal of High Resolution Chromatography, 1995. **18**(2): p. 97-100.
2. Olsson, P., J. Holmbäck, and B. Herslöf, *Separation of Lipid Classes by HPLC on a Cyanopropyl Column.* Lipids, 2012. **47**(1): p. 93-99.