Efficient Separation of Polar and Nonpolar Lipid Classes Utilizing iSPE®-HILIC Material for Solid-Phase Extraction

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Lipids are a large group of biomolecules that play an important role in all organisms. The tasks of lipids are manifold and of great relevance. By the formation of membranes, their main task is the compartmentation of cells where they interact with other biomolecules such as proteins. Furthermore, they are involved in a variety of signalling pathways and some of their representative lipid classes are important for energy storage (1). Lipids differ in their polarity. There are not only completely lipophilic representatives such as triacylglycerols (TAG) or cholesterol ester (CE) but also amphiphilic lipids such as phospholipids (PLs). The analysis of lipids is complex and challenging and is often based on liquid chromatography (LC) hyphenated with mass spectrometric (MS) detection. Due to their amphiphilic character, reversed-phase LC and hydrophilic interaction liquid chromatography (HILIC) are suitable techniques for PLs analysis. While reversed-phase LC enables a separation mainly based on their acyl moieties in lipids, HILIC can separate PL classes according to their specific hydrophilic head group as highlighted in green in Figure 1. Nonpolar lipids are not retarded by the HILIC mechanism and elute earlier from the column (2). Therefore, HILIC enables a separation of polar phospholipids and nonpolar lipids by means of HILIC solid-phase extraction (SPE).

In this application, we demonstrate a fast and efficient separation of representative polar and nonpolar lipid classes by iSPE®-HILIC cartridges. In addition to the recovery studies of eight lipid standards, the polar lipid fraction of a yeast lipid extract was also analyzed by reversed-phase LC–MS after iSPE®-HILIC SPE fractionation.

Experimental

Lipid Standards: Triacylglycerol (TAG 48:0), cholesterol (Chol), cholesteryl ester (CE 18:2), phosphatidylethanolamine (PE 32:0), phosphatidylcholine (PC 32:0), phosphatidylserine (PS 32:0), *lyso*-phosphatidylcholine (LPC 16:0), and cardiolipin (CL 72:8) are respectively from Biomol GmbH and Sigma Aldrich.

Lipids Extraction Protocol with iSPE®-HILIC:

Solvents: a) ammonium acetate buffer (20 mM, pH5.5); b) acetonitrile; c) methanol

Conditioning: 1 mL acetonitrile–buffer (90:10, *v/v*)

Equilibration: 3 mL acetonitrile–buffer (97:3, *v/v*)

Loading: ≤ 200 µL sample (for example, lipid extracts in $CHCl_3$)

Incubation: 1 min

Nonpolar lipids fraction: 4 mL acetonitrile–buffer (95:5, *v/v*)

Polar lipids fraction: 4 mL methanol–buffer (80:20, *v/v*)

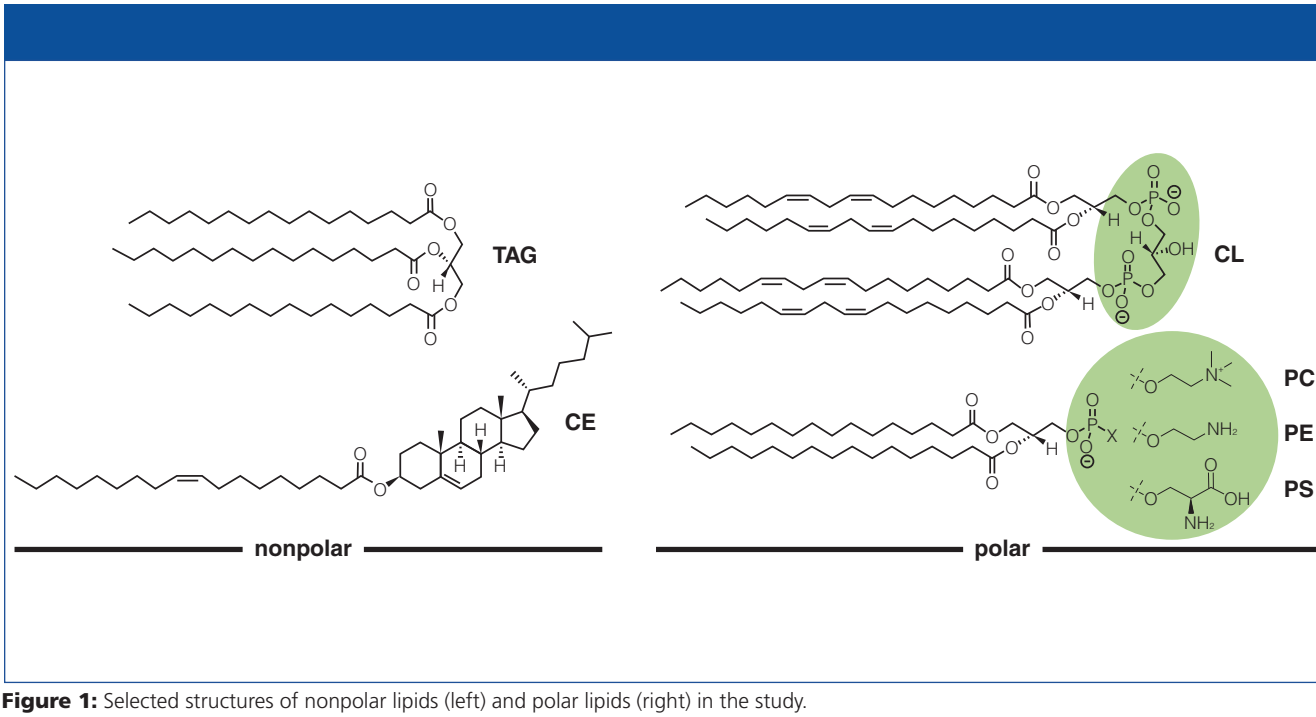

Figure 1: Selected structures of nonpolar lipids (left) and polar lipids (right) in the study.

Sample Preparation and Method Validation: The recovery of three nonpolar lipid species (TAG, Chol, and CE) and five polar phospholipids (PE, PC, PS, LPC, and CL) was determined according to Matuszewski *et al.* (3). In this work, a yeast total lipid extract (*S. cerevisiae*) was utilized as matrix as described by Helmer *et al.* (4).

The samples for the LC–MS analysis were the yeast total lipid extracts (*S. cerevisiae*) that were first cleaned up from nonpolar lipids utilizing iSPE®-HILIC cartridges (1 mL, 100 mg, 50 µm/60 Å, HILICON). After solvent evaporation, the polar lipid fraction was reconstituted in methanol and subjected to the analysis by reversed-phase LC–MS.

LC–MS Setup: A Thermo Scientific Ultimate 3000 UHPLC system was hyphenated to a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer. The ionization was carried out by electrospray ionization in negative ionization mode (4).

Results and Conclusion

An efficient separation of polar and nonpolar lipid representatives was achieved utilizing iSPE®-HILIC material. The lipids were separated according to their polarity and collected into polar and nonpolar lipid fractions for further LC–MS analysis. Figure 2 shows the recoveries of all eight tested lipid standards in the polar and nonpolar fraction. TAG, CE, and Chol were eluted in the nonpolar fraction, while the PLs species were in the polar fraction. Except for minor amounts of PE, no carryover into the other fraction was observed.

By fractionation of a total lipids extract into its polar and nonpolar groups, the interfering influences on chromatographic separation or mass spectrometric detection can be minimized and allows a more tailored analysis. In addition, depending on the lipid composition of tissues, low abundant lipid species such as CL can be enriched with this newly developed SPE method.

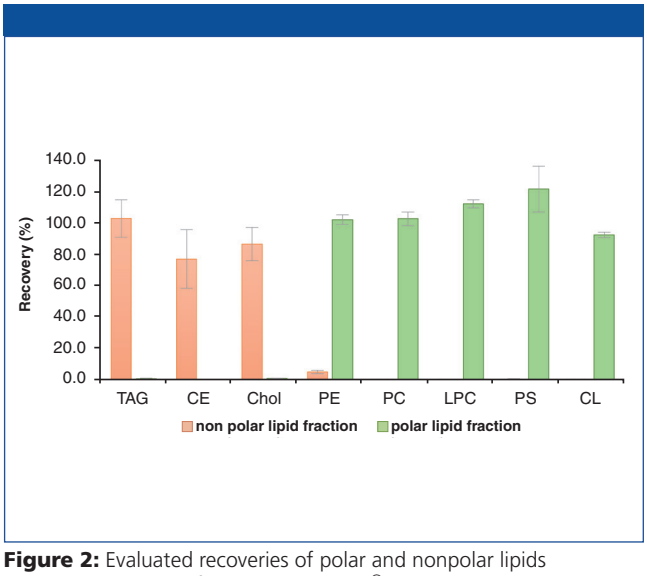

Figure 2: Evaluated recoveries of polar and nonpolar lipids in the respective SPE fractions using iSPE®-HILIC cartridge.

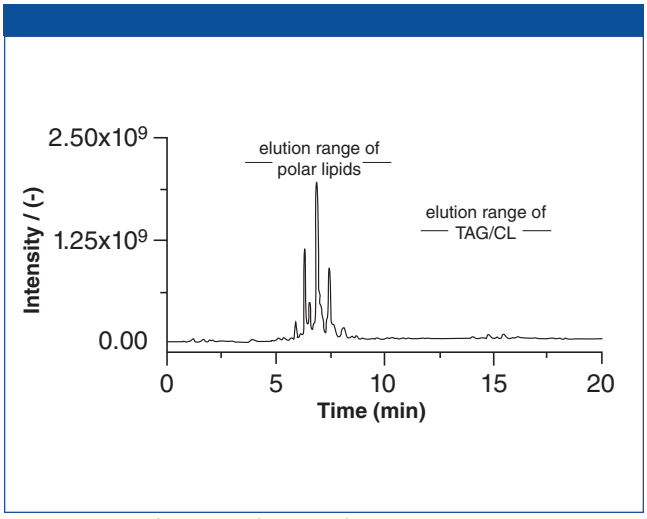

Figure 3: TIC of the polar fraction of a yeast lipid extract utilizing iSPE® HILIC and separation by reversed-phase LC–MS. The separation of polar PLs lipids and the low abundant CL species is illustrated. Interfering TAG species were excluded by cleanup.

Figure 3 shows the total ion chromatogram (TIC) of the polar fraction of a yeast lipid extract by reversed-phase LC–MS after iSPE®-HILIC sample cleanup. Polar lipids were well separated and detected in the TIC, while no interfering TAG was present in the elution range of CL. In comparison to other SPE methods for lipid separation or purification, this method does not require the use of nonpolar volatile solvents, for example, hexane. In summary, the HILIC method with iSPE®-HILIC is a reliable and robust alternative to other SPE separations such as normal-phase liquid chromatography.

References

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