

# Analysis of Phospholipid Classes by iHILIC®-Fusion

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Phospholipids (PLs) are the major components of cellular membranes. They are important for the functionality of membrane proteins or serve as precursors for second messengers. Several studies reveal the role of PL alterations in various diseases such as cancer (1). Therefore, it's crucial to identify and quantify PLs in complex biological samples for the lipidomic studies and clinical researches.

The PL classification is based on their different polar head groups. The diversity within each PL class is generated by combination of different fatty acids (FA) with variation of chain length and the number of double bonds. Nowadays, tandem mass spectrometry (MS/MS) is the most important technique for the identification and quantification of PL species. By hyphenation with liquid chromatography (LC), LC-MS/MS provides prodigious power for the separation of isobaric or isomeric lipids. Since the amphiphilic properties of PLs, both reversed phase liquid chromatography (RPLC) and hydrophilic interaction liquid chromatography (HILIC) are applied for the separations. However, HILIC enables the separation of PL classes according to their polar head groups and minimizes ion suppression effects between PLs. The HILIC-MS/MS approach makes it possible to quantify lipid species based on similar retention times with internal standards of each lipid class (2) and also their characteristic fragments. Therefore, the assignment of lipid species to a

specific lipid class is straightforward.

In this application, 10 PL classes are separated by HILIC using an iHILIC®-Fusion column. The applicability of this method for potential clinical application is demonstrated by the analysis of PL species in a lipid extract of a MCF-7 breast cancer cell culture (3).

## Experimental

**LC-MS System:** Thermo Scientific Ultimate 3000 system coupled to a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer, equipped with HESI II source and operated in negative ionization mode (ESI-).

**Column:** 150 × 2.1 mm, 3.5 μm, 100Å, iHILIC® Fusion (P/N 110.152.0310, HILICON, Sweden).

**Eluent:** A) Ammonium acetate solution (35 mM, pH 5.75) and acetonitrile (95:5, v/v); B) Acetonitrile.

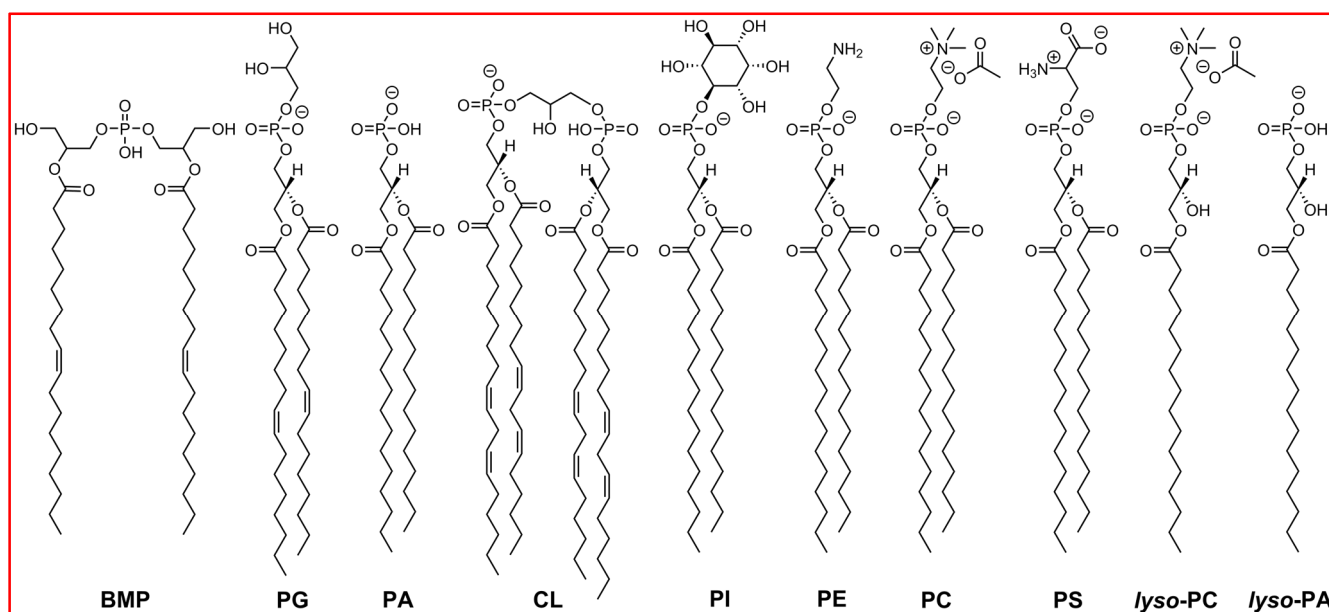
**Gradient elution:** 0-0.5 min, 97% B; 0.5-26.5 min, from 97% to 75% B; 27-33 min, 60% B; 35-45 min 97% B.

**Flow rate:** 0.3 mL/min

**Column temperature:** 40 °C

**Injection volume:** 10 μL

**Phospholipid standards:** Bis(monoacylglycerol)phosphate (BMP 36:2), phosphatidylglycerol (PG 36:2), phosphatidylcholine (PC 32:0), phosphatidylethanolamine (PE 32:0), phosphatidylserine (PS 32:0),



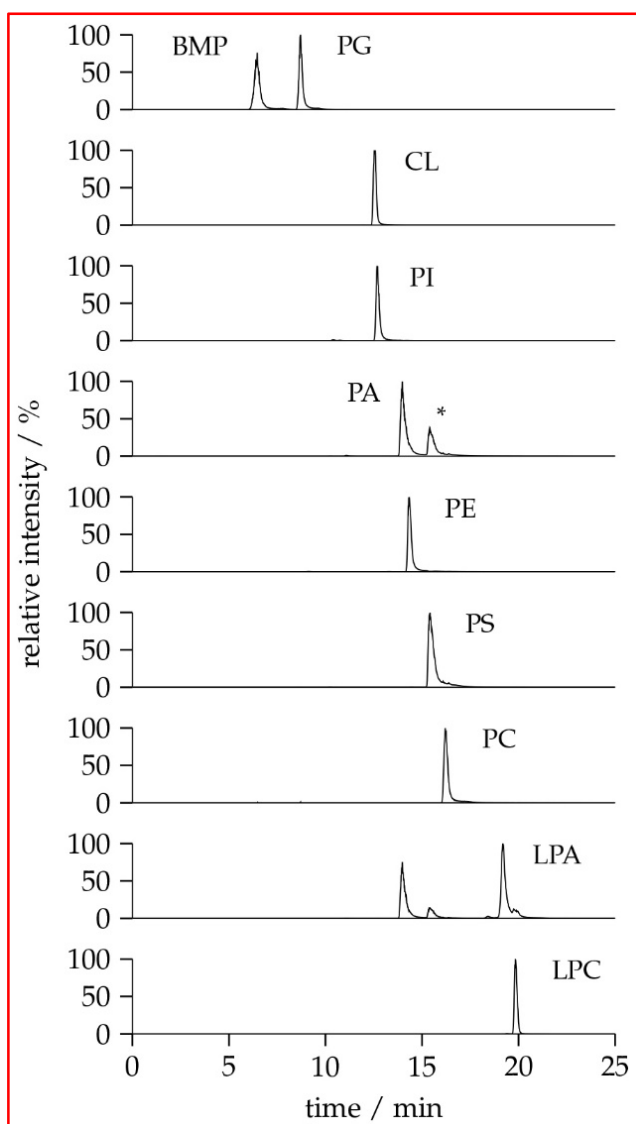
**Figure 1:** Chemical structures of phospholipids used in the study.

phosphatidylinositol (PI 32:0), lyso-phosphatidylcholine (LPC 16:0), phosphatidic acid (PA 16:0), lyso-phosphatidic acid (LPA), and cardiolipin (CL 72:8). The chemical structures are shown in Figure 1.

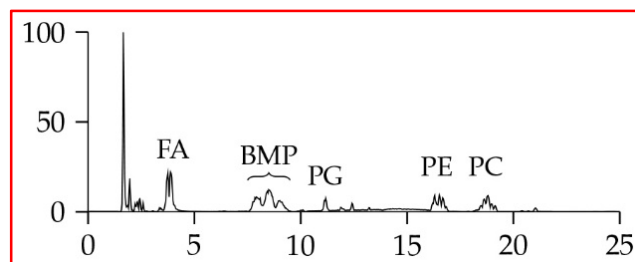
**MCF-7 breast cancer cells:** The extract of MCF-7 cell sample was provided by the working group of Dr. Cristina Cadenas (Leibnitz Research Centre for Working Environment and Human Factors, Dortmund, Germany).

## Results and conclusion

Figure 2 shows the separation of 10 PL standards according to their polar head groups and different chain length using an iHILIC®-Fusion column. Importantly, the constitutional isomers BMP and PG were baseline separated. It was found that the other PL classes were also well separated. This is very important for the separation between PA and PS due to the potential in-source fragmentation of the PS head group leading to a mass-to-charge ratio ( $m/z$ ) of the



**Figure 2:** Separation of PL standards in ascending retention order with iHILIC®-Fusion. \* Double peak of PA is caused by in-source fragmentation of the PS head group resulting in corresponding PA species.



**Figure 3:** Chromatogram of PL class separation of MCF-7 cell extract with iHILIC®-Fusion.

corresponding PA species. In short, the iHILIC®-Fusion provides good peak shapes and narrow peaks for all PL classes. The buffer concentration has turned out to be the most crucial factor for method development that affected both separation efficiency and elution order. Figure 3 shows the base peak chromatogram of a separation of MCF-7 cell extract. Besides our focused BMP and PG class, further PL classes such as PE or PC could also be detected.

The combination of HILIC and tandem MS is a powerful tool for analysis of PL classes and PL species. The iHILIC®-Fusion provides an efficient separation of PL classes, which is mandatory for isomers such as PG and BMP(3). This facilitates a quantification approach based on single class-specific internal standards.

## References

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