

# Separation of Phospholipids Derived from Biological Extracts Using a Solid Core Reversed Phase HPLC Column

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## Key Words

Accucore RP-MS, lipidomics, lipid profiling, phospholipids

## Abstract

In this application a Thermo Scientific Accucore RP-MS LC column was used to separate a range of lipids derived from biological sources. Excellent chromatographic performance was achieved at backpressures comparable with conventional HPLC instrumentation.

The Accucore™ RP-MS HPLC column is an ideal phase for lipid analysis due to the balance of speed and efficiency. It utilizes core enhanced technology™ with optimized bonding for reversed phase HPLC and LC-MS analysis.

## Introduction

The role of membrane lipids on cell surfaces is known to be of key importance to cell function and inter-cellular communication. Interest in lipidomics (the large-scale study of pathways and networks of cellular lipids in biological systems<sup>1</sup>) is growing rapidly, and understanding which lipids are present within cells is an important aspect of biological studies. The separation, detection and classification of lipids by conventional LC-MS methods can be a challenging application. Lipids comprise a range of different classes and generally include a polar head group together with at least one attached hydrocarbon chain (Figure 1). Their analysis is complicated by the wide variation in composition and structures present in any biological extract combined with high retention on C18 reversed phases. Solid-core stationary phases have been shown to be able to yield UHPLC-levels of resolution due to the minimized resistance to mass transfer by the diffusional path of analytes being limited by the depth of the porous outer layer. The optimized packing results in more uniform paths through the LC column. These can be attained without the unwanted side effect of elevated backpressure which is associated with smaller particle sizes. In this application note a separation protocol for the analysis of lipids by LC-MS using a solid-core stationary phase is presented.

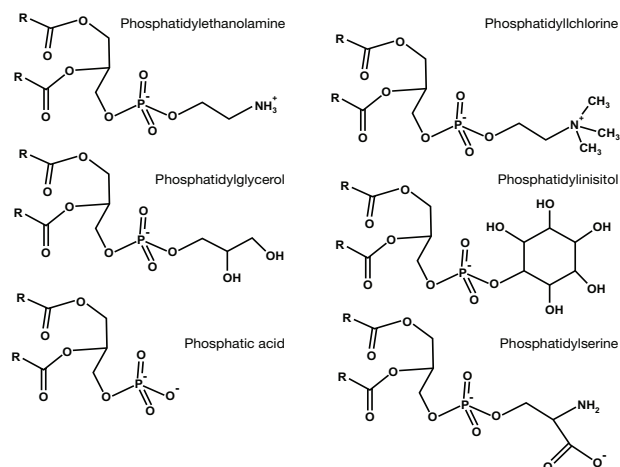
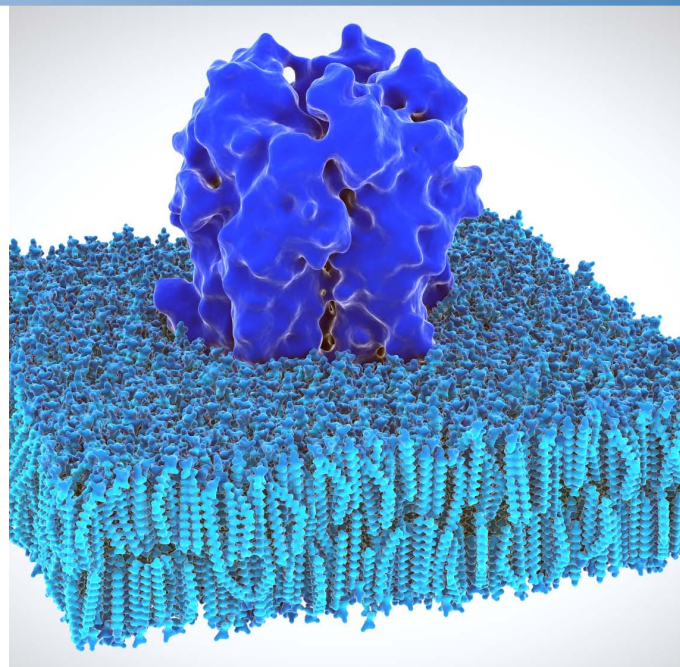


Figure 1: Polar head group structures for selected phospholipids

## Experimental Details

Consumables		Part Number
Fisher chemical methanol LC MS grade		M/4062/17
Fisher chemical water LC MS grade		W/0112/17
Fisher chemical ammonium acetate		A/3440/50
Liquid handling hardware	eVol Dispensing System	66002-020
Thermo Scientific vials and closures	Mass Spec Certified Vials Kit	MSCERT4000-34W

### Sample Preparation

Samples:	Escherichia coli ( <i>E.coli</i> ) lipid extract and yeast lipid extract prepared in chloroform then resuspended in mobile phase A (final concentration 1 µg/mL)
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Separation Conditions		Part Number	
Instrumentation:	Thermo Scientific Accela 1000 HPLC		
Column:	Accucore RP-MS 2.1 x 100 mm, 2.6 µm particle size	17626-102130	
Backpressure:	< 400 bar at the start conditions		
Mobile phase:	A: 25 mM ammonium acetate (aq)/methanol, 30/70 (v/v) B: methanol		
Gradient:	Time / minutes	% A	% B
	0	70	30
	2	70	30
	7	30	70
	26	0	100
	31	0	100
	32	70	30
	45	70	30
Flow rate:	0.15 mL/min		
Run time:	45 minutes		
Injection volume:	4 µL		
Injection wash solvent:	propan-2-ol		

### MS Conditions

Instrumentation:	Thermo Scientific LCQ Deca XP MS
Ionization conditions:	negative ion electrospray
Scan ranges:	500 – 850 m/z

### Data Processing

Software:	Thermo Scientific Xcalibur 2.0
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## Results

### Analysis of *E. coli* Phospholipids

The composition of phospholipids varies greatly within biological samples. The lipids in *E. coli* consist largely of two phospholipid types: phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) lipids. Even with the reduced number of lipid classes there is still a high level of complexity arising from the many different possible alkyl chain lengths and double-bond configurations. This can be seen in Figure 2, which shows the chromatogram for the lipids extracted from *E. coli* and analyzed using an Accucore RP-MS HPLC column. In this chromatogram the peaks are narrow and there is a reasonable spread of peaks across the gradient window. Identification of lipids is best achieved using MS, as fragmentation within the mass spectrometer allows for unambiguous assignment of the lipids in most cases. In these experiments lipids were identified using their intact masses plus their elution order and comparing these with literature data<sup>2</sup>.

Chromatographic separation is essential for optimal detection and quantification as it reduces the effect of co-elution impacting the distribution of charges, ionization efficiency and therefore the lipid detection.

The nature of the head group of the phospholipid is the major controlling factor on the retention on the Accucore RP-MS HPLC column. Figure 3 shows the base peak chromatogram corresponding to lipids with PG and PE head groups respectively. It can be clearly seen that the PG lipids elute as a class earlier than the PE lipids and that these two lipid classes account for the majority of the features in the chromatogram.

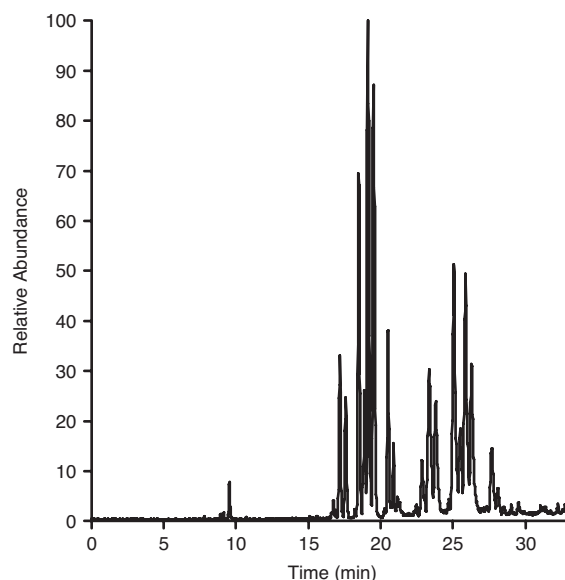


Figure 2: *E. coli* lipid extract base peak chromatogram obtained using Accucore RP-MS HPLC column

Note: Method designed so there was a window at the beginning of the gradient where peptides could elute.

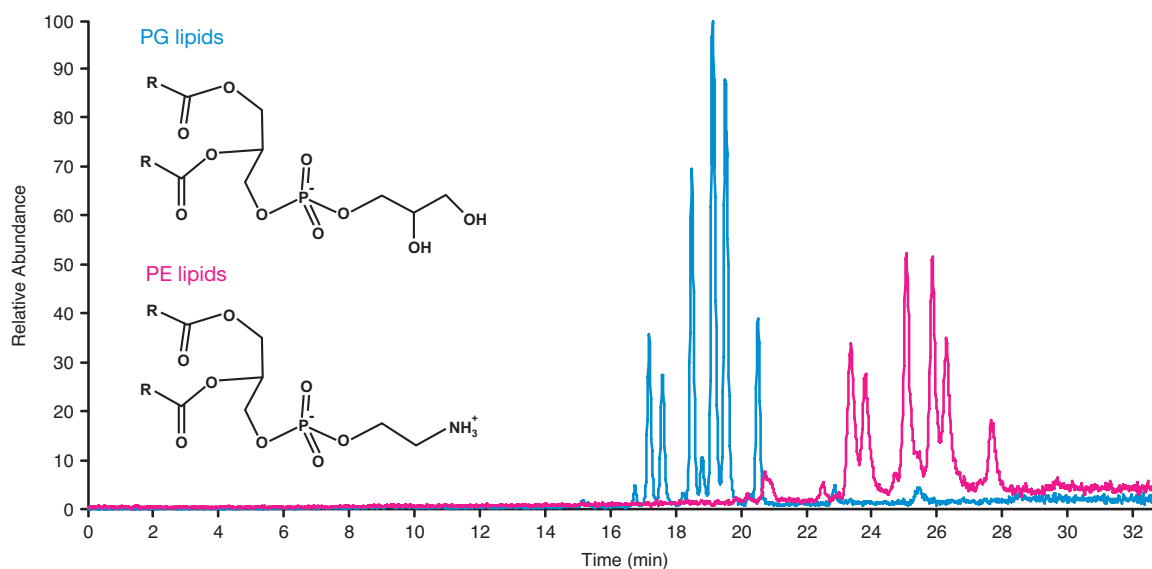


Figure 3: *E. coli* lipid extracted ion chromatograms overlaid for phosphatidylglycerol (PG, blue) and phosphatidylethanolamine (PE, pink) lipids

In Figure 4 the effect of increasing saturation and fatty acid chain length is illustrated. The impact of these changes is smaller than that for varying the polar head group. Optimization of the bonding on the Accucore RP-MS HPLC column ensures that secondary interactions are minimized, yielding highly symmetrical peak shapes. In this example the retention is defined predominantly by the polar head group, followed by hydrophobic chain length and fatty acid chain unsaturation (saturated chains are more retained than unsaturated chains).

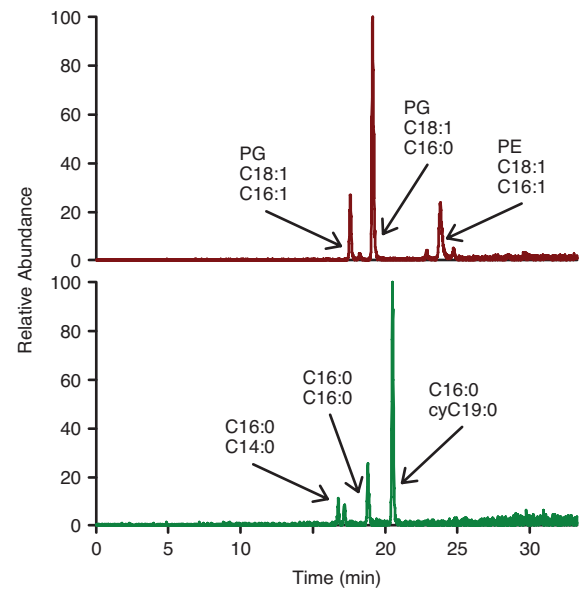


Figure 4: Extracted ion chromatograms for selected *E.coli* lipid ions to demonstrate the impact on retention of fatty acid unsaturation, chain length and lipid head group

### Analysis of Yeast Phospholipids

*E.coli* lipid membranes are predominantly composed of two phospholipid types and in order to confirm the separation of other lipid classes a yeast lipid extract was analyzed. Yeast membranes contain a range of lipid types including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine with smaller amounts of phosphatidic acid and phosphatidylglycerol lipids. The chromatography of the yeast lipid extract is shown in Figure 5. Some of these lipids are better detected in positive ion mode MS and others in negative ion mode, but the data shown in Figure 5 are from negative ion mode MS detection. Whilst no lipid identification was carried out on this sample the narrow peaks observed in the chromatography and good separation window would be ideal for identification or quantification if required.

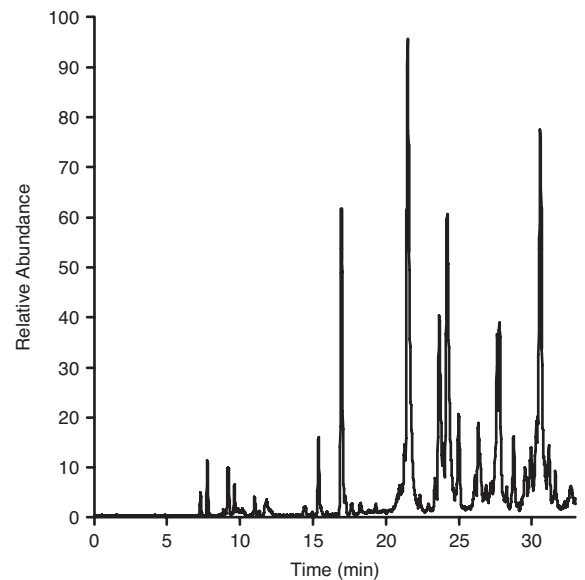


Figure 5: Yeast lipid extract base peak chromatogram from an Accucore RP-MS HPLC column

## Conclusion

- Accucore RP-MS HPLC columns show excellent separation and peak shapes for a range of lipid families from biological extracts
- Analysis can be carried out in MS compatible conditions
- Separation of lipid class was observed between PE and PG lipids
- Optimized chromatographic separation of lipids enables improved MS detection, analysis and quantification
- Accucore solid core HPLC columns generate backpressures comparable with standard HPLC instrumentation

## References

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2. Oursel, D. et al., Lipid composition of membranes of Escherichia coli by liquid chromatography/tandem mass spectrometry using negative electrospray ionization, Rapid Comm. Mass Spectrom. (2007), 21, 1721-1728

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