Supporting information

Rapid quantification of yeast lipid using microwave-assisted total lipid extraction and HPLC-CAD

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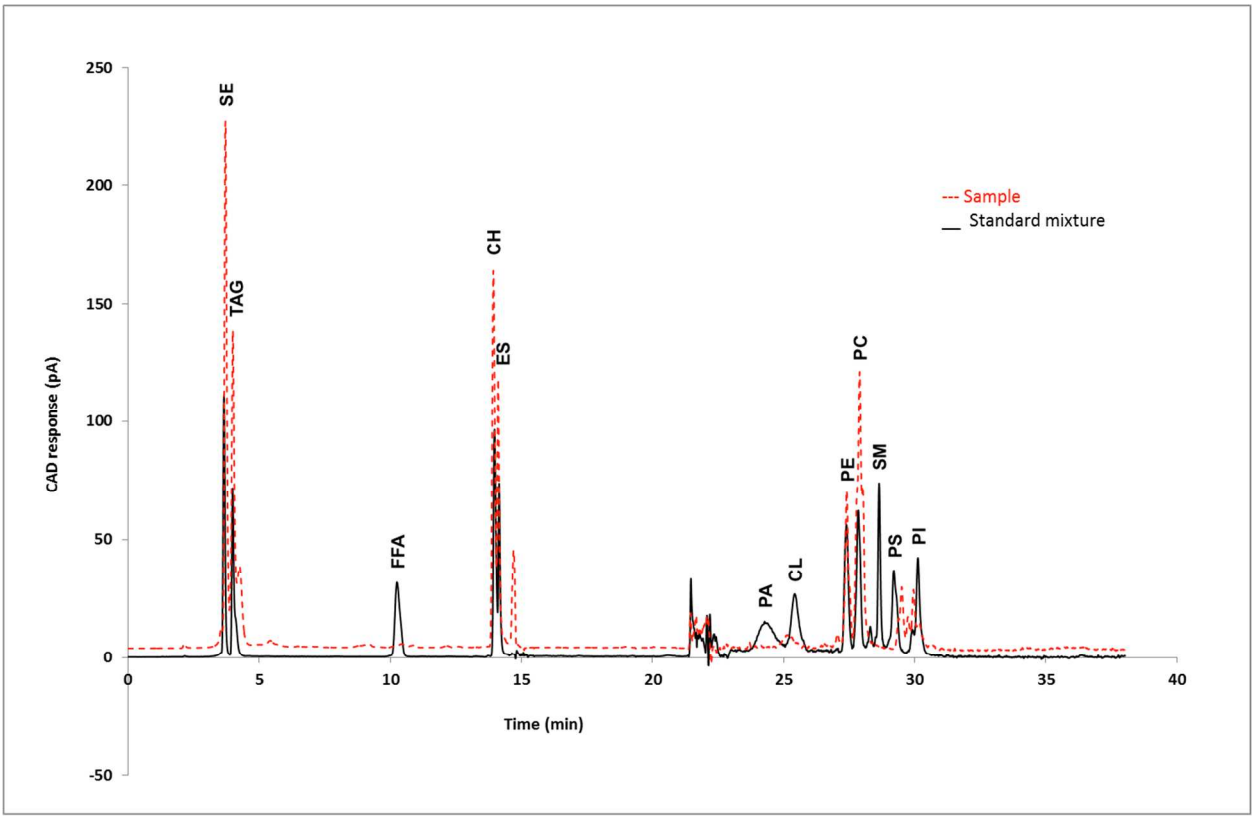
S1 Identification of unknown lipids from yeast samples by comparison of their retention times with known standards under the identical chromatographic condition using HPLC-CAD



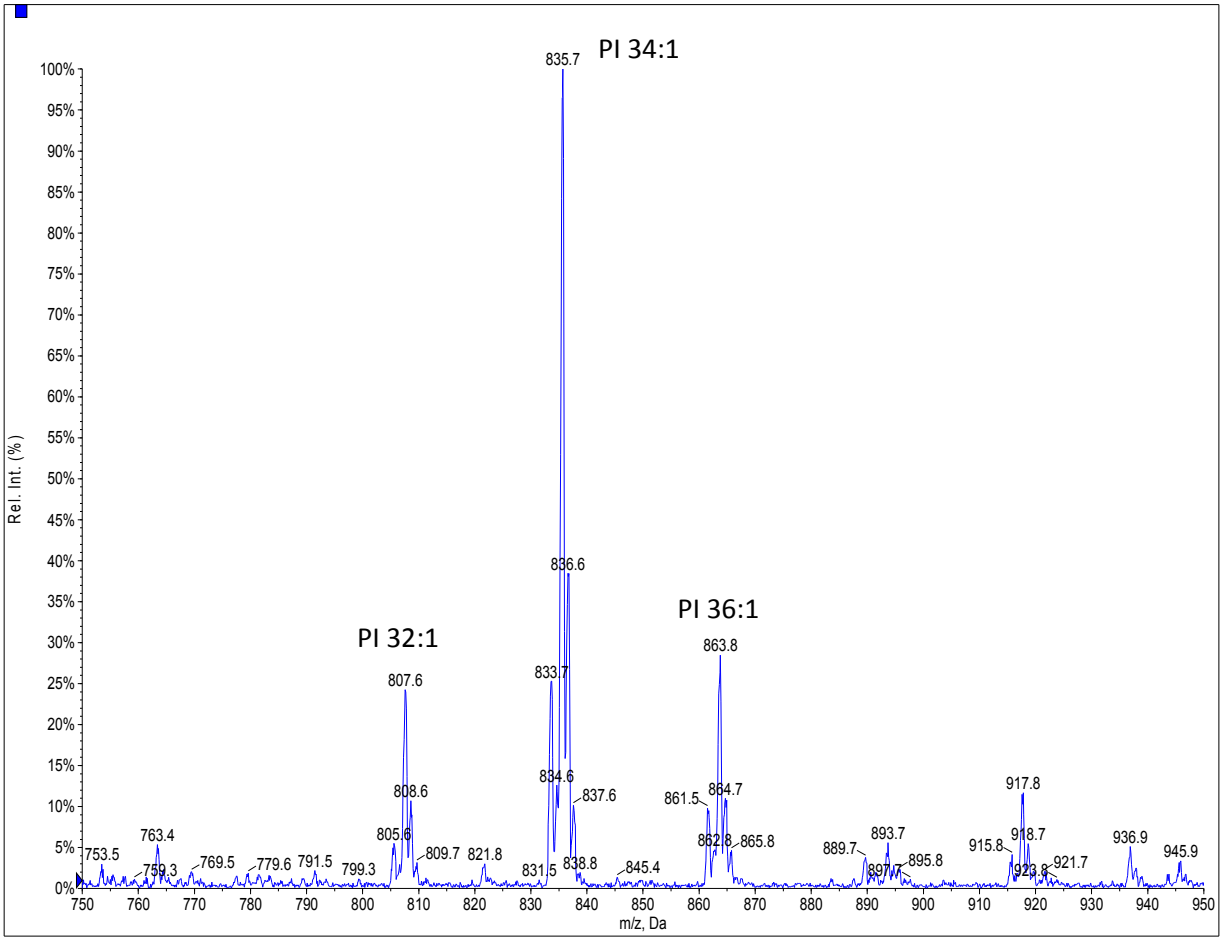
S2 Supplement – Lipid confirmation using mass spectrometry

Initially reference standards were used in order to confirm the identity of the yeast lipids eluting from chromatographic system described in the manuscript. However, as this identification is based on retention time alone, the eluting peaks were also fraction-collected and analyzed using mass spectrometry. Below are the spectra confirming the lipid class identity eluting in the different HPLC fractions.

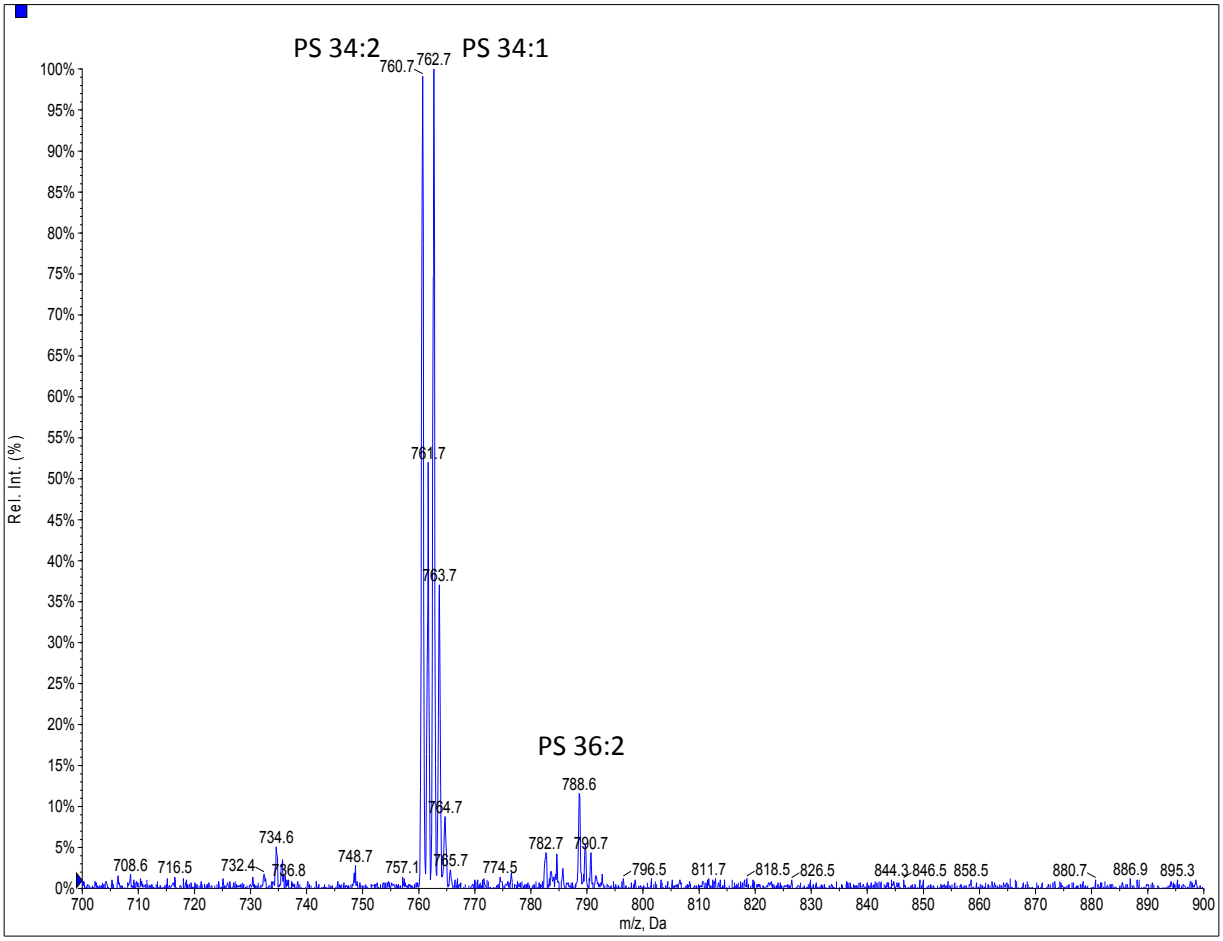
METHODS

The HPLC fractions, collected from a 2µl injection of (~10 mg yeast), were evaporated and reconstituted in 100µl chloroform:methanol [1:2] with 5mM ammonium acetate. The fractions were then infused directly into a QTRAP 5500® mass spectrometer (ABSciex, Toronto, Canada) equipped with a robotic nanoflow ion source NanomateTriversa (Advion Biosciences, Ithaca, NY, USA). The lipid classes were confirmed using lipid class specific scans. In the spectra below the identified lipid species are annotated by their lipid class and the brutto composition of the attached fatty acids (e.g. PC 34:1 refers to a phosphatidylcholine with two fatty acids containing, in total, 34 carbons and 1 double bond).

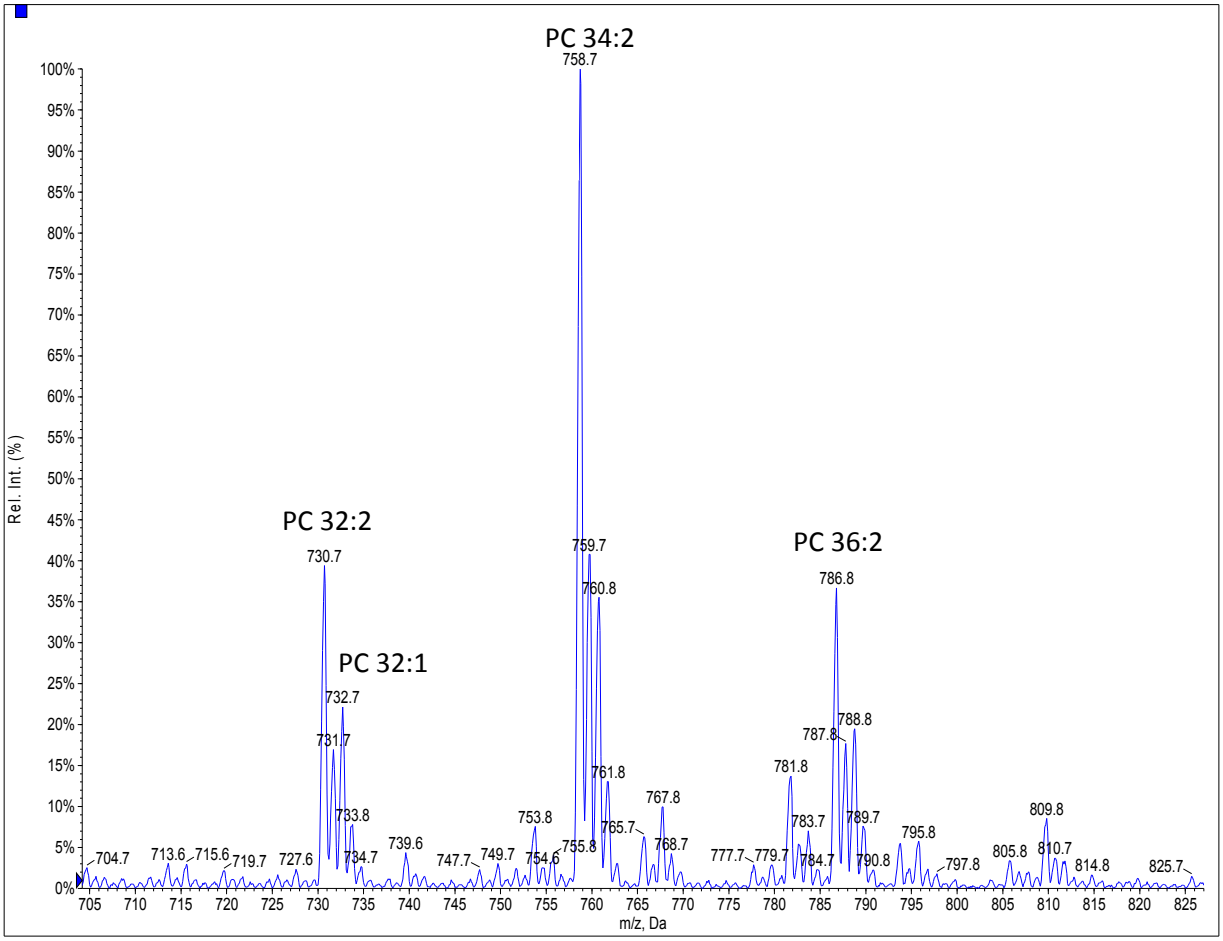
The phosphatidylinositol (PI) was confirmed using precursor ion scanning of *m/z* 241 in negative mode.[1]



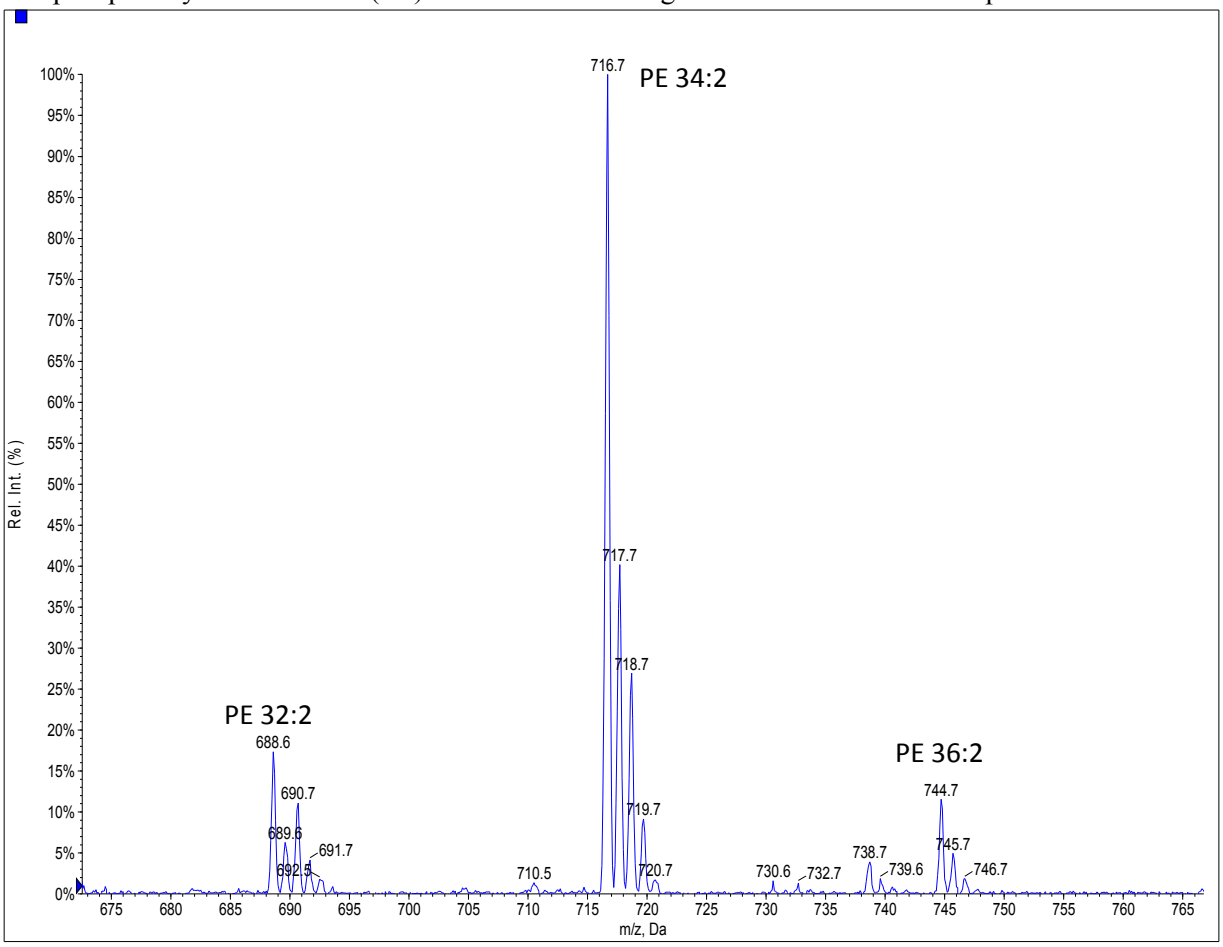
The phosphatidylserine (PS) was confirmed using neutral loss of *m/z* 185 in positive mode.[1]



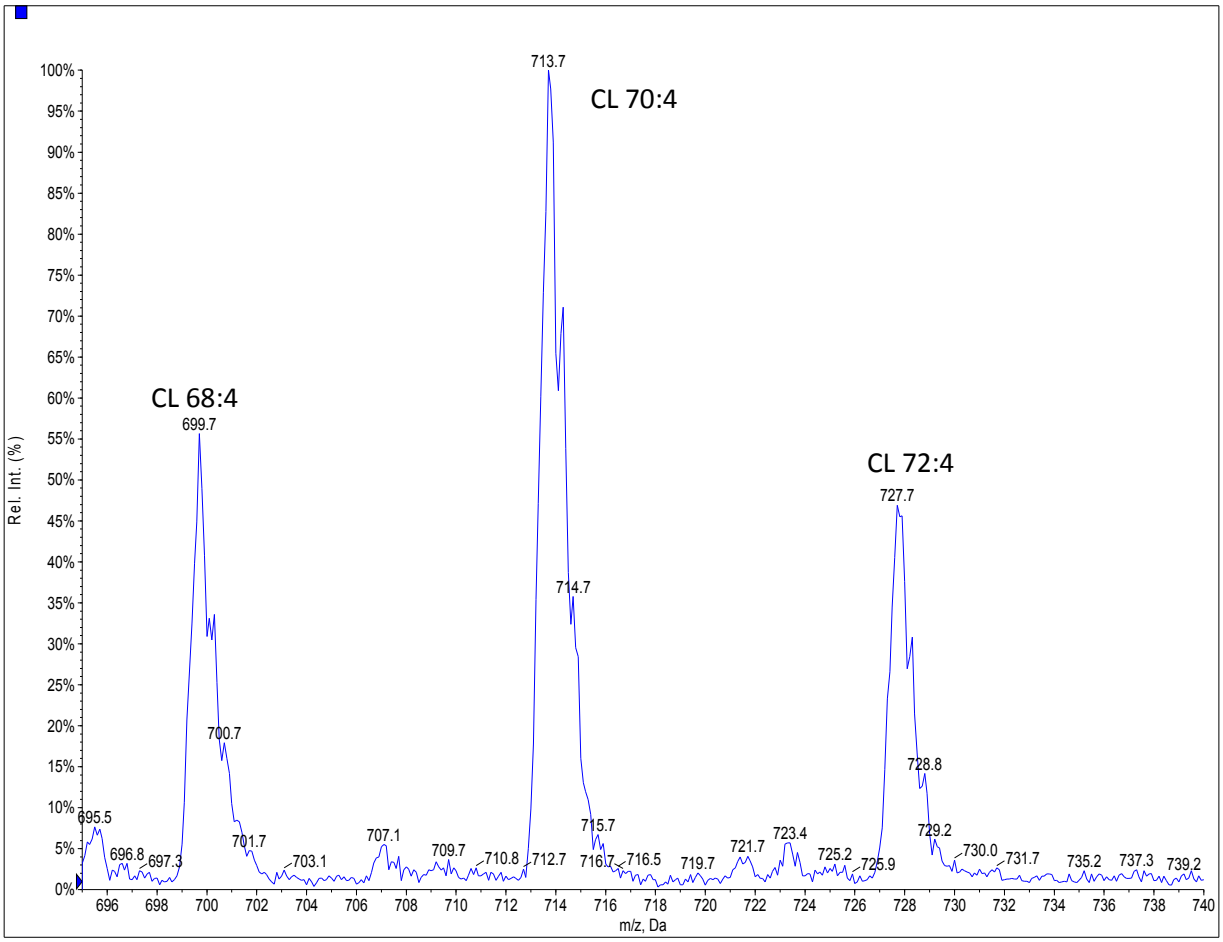
The phosphatidylcholine was identified using precursor ion of *m/z* 184 scanning in positive mode.[1]



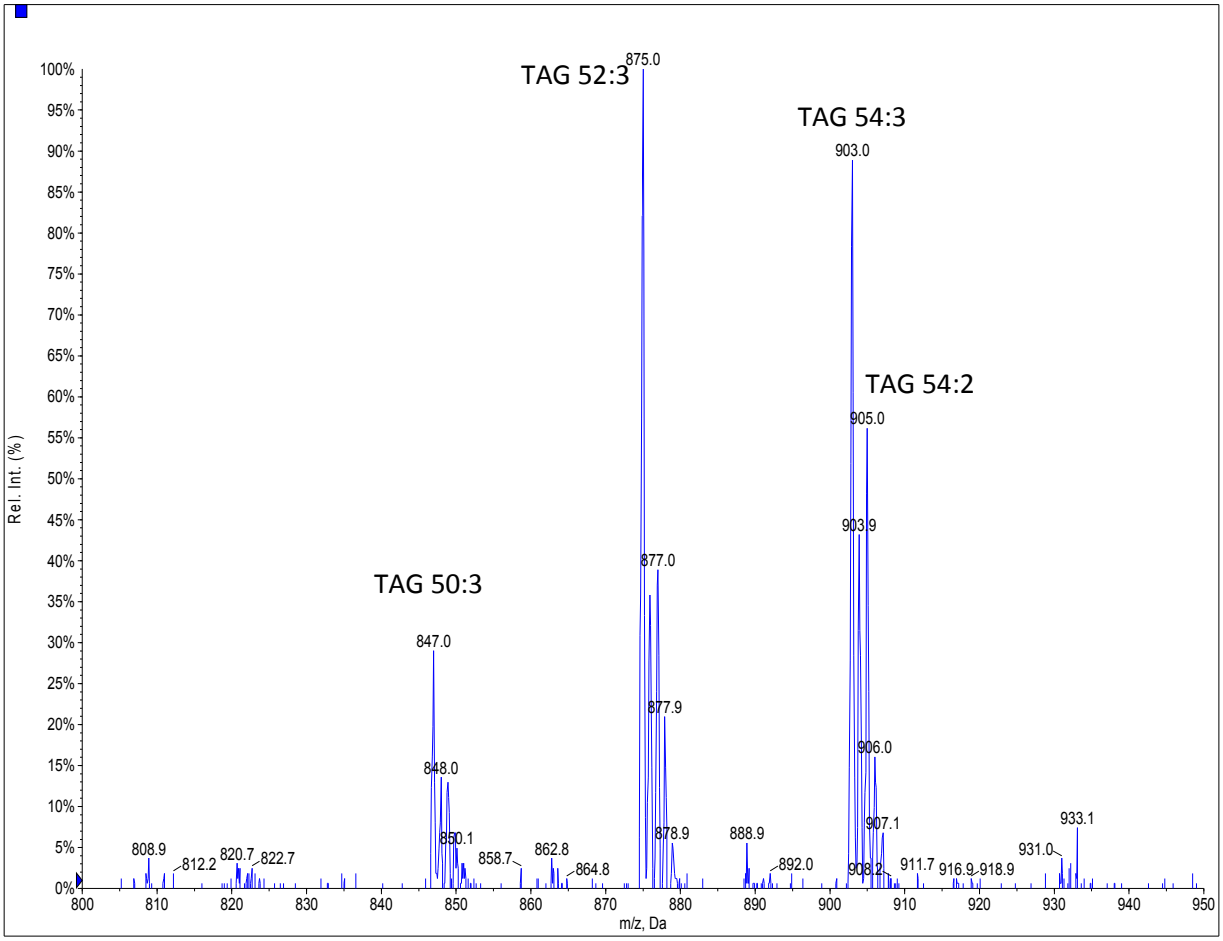
The phosphatidylethanolamine (PE) was confirmed using neutral loss of *m/z* 141 in positive mode.[1]



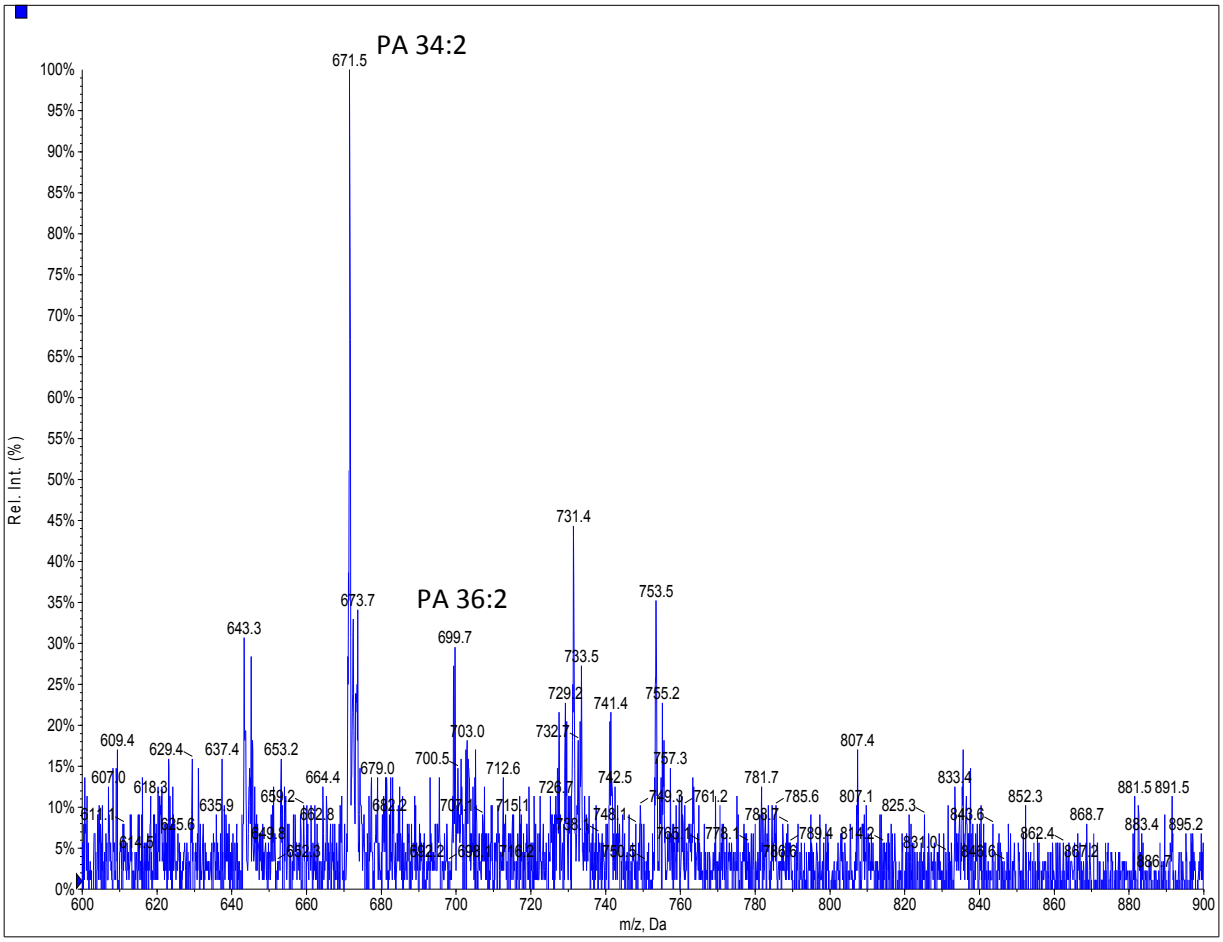
Cardiolipin was identified using precursor ion scanning of *m/z* 279 (oleic acid as fragment ion). Due to the high mass of CL the molecular species were detected as doubly charged.[2 3]



Triacylglycerol (TAG) was identified using neutral loss of m/z 299 (oleic acid).[4]



Phosphatidic acid (PA) was identified using precursor ion scanning of m/z 153.[1] In the sample there are only traces of the low abundant PA lipid class (see chromatogram).



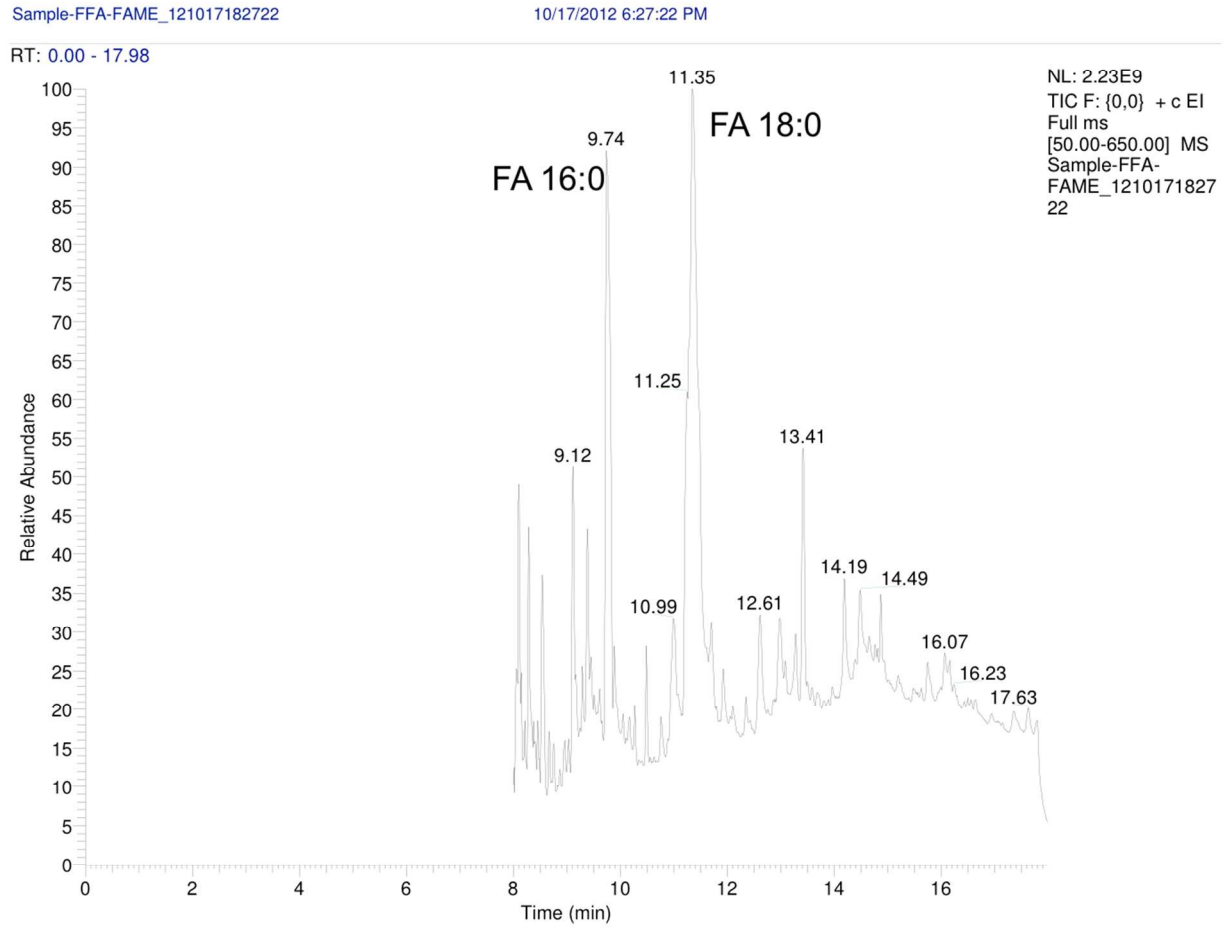
Confirmation of ergosterol

Since sterols are not easily ionized using electrospray, ergosterol and its ester were not analyzed using mass spectrometry. However, the peak in the chromatogram that elutes with the same retention time as the ergosterol reference standard was confirmed using an alternative HPLC method. This method is based on normal-phase HPLC with ELS detection.[5] The unknown peak, which is believed to be ergosterol, was injected (black trace below) and compared to an injection of ergosterol reference standard (blue trace).


1
Yeast peak "ergosterol"
2
Ergosterol 50µg/ml
mV
136
120
110
100
90
80
70
60
50
40
30
20
6
1.3
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.5
min

Confirmation of free fatty acids

Fatty acids (FA) were identified using GC-MS, sample preparation and measurement conditions were performed according to the method from our previous work[6]



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