SUPPLEMENTARY METHODS – JLR/2013/037622

Figure legends for supplementary methods

Figure 1 – Contribution of isobars – Ten PC species [(10 ng of PC(16:0/18:0), PC(16:0/18:1), PC(16:0/18:2), PC(18:0/16:0), PC(16:0/16:0), PC(18:0/18:0), PC(20:0/20:0), PC(16:0/20:4), PC(16:0/20:5), PC(16:0/22:6)] were individually injected and analyzed as described in the Methods Section. For each specie, a spectrum was obtained as shown for PC(16:0/20:4) (Figure 1A). The relative abundance of the positive ions $[M+H]^+$ was obtained for m/z plus 1, 2, 3, 4, and 5 ions. Figure 1B shows the mean ± SEM of the 10 PC species. These data show that isobaric interference is 60.3 ± 3.4 (*m/z* +1), 21.3 ± 1.9 (*m/z* +2), 7.4 ± 1 (*m/z* + 3), 4.2 ± 0.8 (*m/z* +4) and 3.5 ± 0.5 (*m/z* ± 5). The progressive decrease in relative abundance from *m/z* suggests that any *m/z*+2 ions that are higher than the *m/z*+1 represents an authentic PC specie.

Figure 2 – Collision energy and tube lens optimization – A mixture of PC standards in HPLC solvent A at different concentrations (25 ng/ml, 50 ng/ml, and 100 ng/ml) was directly infused into the MS. The collision energy curves for SRM transitions were obtained (Figure 2A). Figure 2B is the optimized collision energy (mean ± SD, n=4) of 8 PC species. Figure 2C is the Q1MS tube lens optimization of the same 8 PC species. These data show that the SRM collision energies and optimized tube lens voltage are similar for different PC species.

Supplementary Methods

Figure 1A - Relative abundance of PC(16:0/20:4) at *m/z* 782 showing isobars

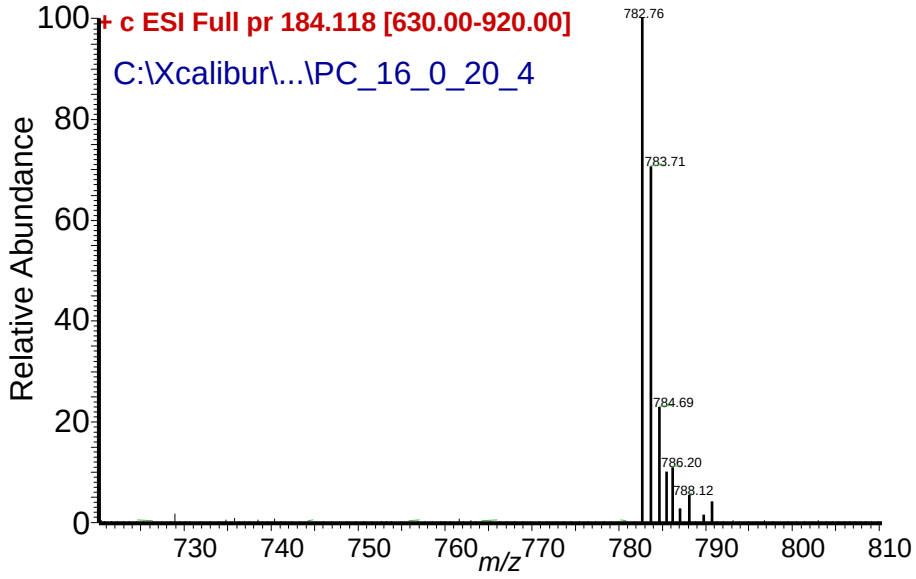


Figure 1B - Mean (+/- SEM) Abundance of 10 PC Species compared with parent *m/z*

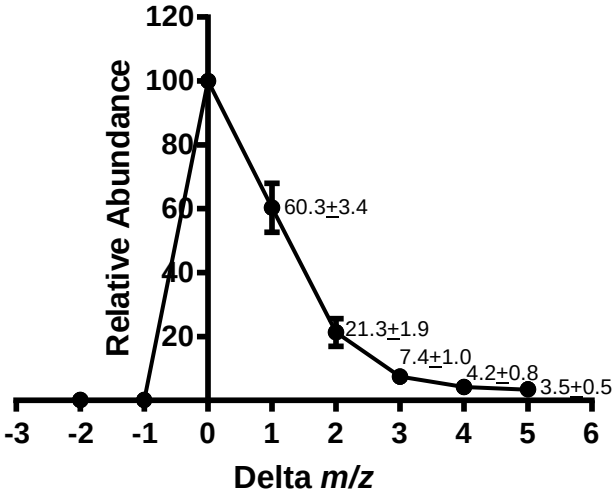


Figure 2A -Collision Energy Curves of SRM Transitions

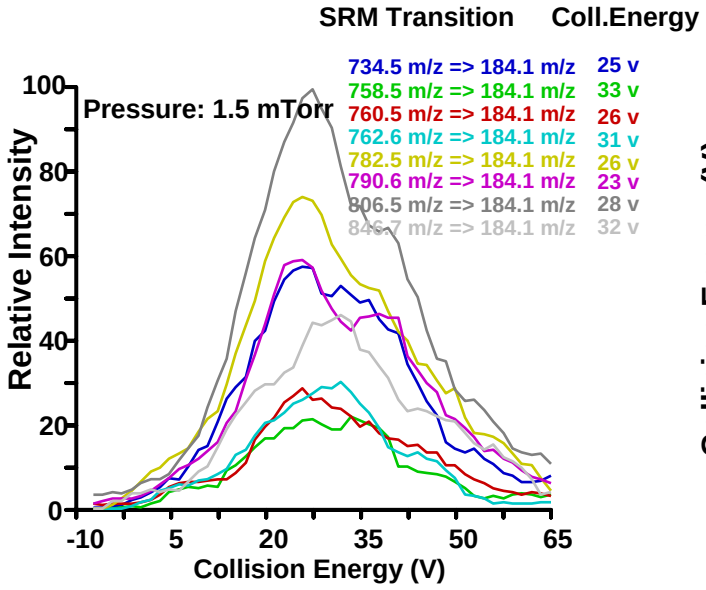


Figure 2B - Average Collision Energy for SRM Transitions of 8 PC species

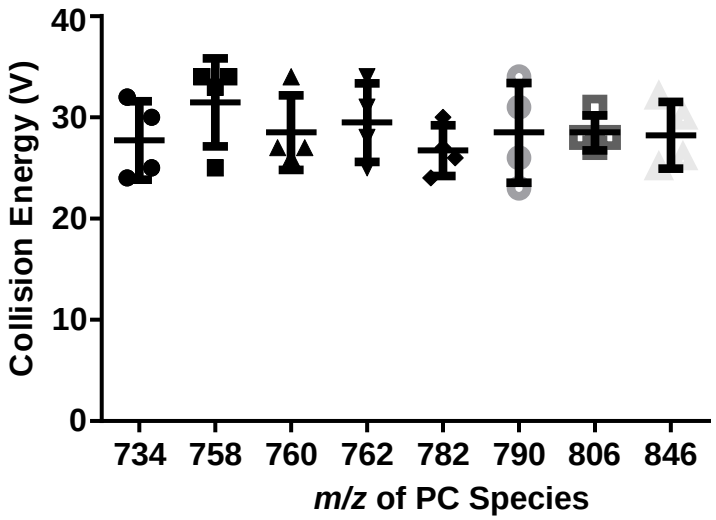
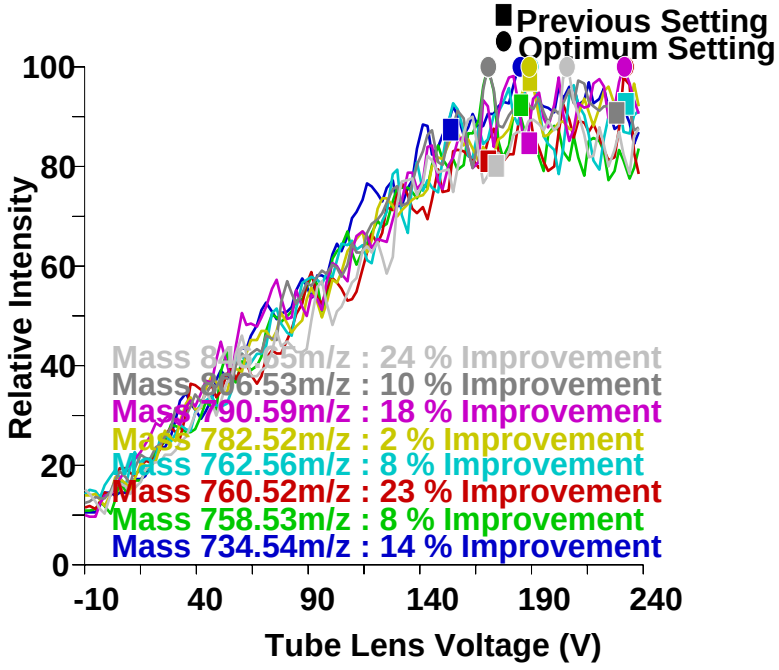


Figure 3C – Q1MS Tube lens Optimization for 8 PC species



LC-MS/MS of GPs - GPs from HILIC were analyzed using positive ion ESI MS/MS divided into 3 scan segments with several scan events. For Segment 1, neutral loss scans, parent ion scans or MRM was used to quantify PE, xPE, PA, and NAPE. For Segment 2, PS, PC, PI, PC(11:0/11:0), and PC species were analyzed; PAF-LL and LPC were quantified in Segment 3. The m/z mass ranges, peak width (PW), collision energies (CE) and scan specific instrument setups are shown for each Segment. PC species have acyl-, ether-, or plasmalogen bonds at the *sn-1* position of the glycerol moiety. Oxidized PC species include 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (Paz-PC), 1-palmitoyl-2-(9'-oxo-nonanoyl)-*sn*-glycero-3-phosphocholine ((ALDO) PC), and 1-palmitoyl-2-(5'-oxo-valeroyl)-*sn*-glycero-3-phosphocholine (Pov-PC).

TSQ Quantum Instrument Method	
Creator: ANF	
TSQ MS Method Settings:	
Method Type: Regular Method	
MS Run Time (min): 35.00	
Segment 1 2 3	
Duration (min) 6.00 8.50 20.50	
Scan Events 6 6 6.0	
Segment 1:	
Tune Method C:\Xcalibur\methods\Lipids_Road_Map_Sept2012.TSQTune	
Chrom filter: Not used	
Q2 Gas Pressure: 1.0	
1: + c Full Neutral Loss Scan, N.Loss 141.01, CE 20	Glycerophosphoethanolamine (PE
Scan Time 0.08, Q1 PW 0.70, Q3 PW 0.70, [660.00-860.00]	and xPE)
2: + c Full Parent Scan, Product Mass 120.84, CE 35	Glycerophosphates (PA)
Scan Time 0.09, Q1 PW 0.70, Q3 PW 0.70, [480.00-800.00]	
3: + c Full Parent Scan, Product Mass 62.00, CE 15	Arachidonoyl ethanolamine (AEA) Species
Scan Time 0.04, Q1 PW 0.70, Q3 PW 0.70, [295.00-415.00]	
4: + c Full Parent Scan, Product Mass 287.31, CE 8	AEA Species (AA loss)

Scan Time 0.04, Q1 PW 0.70, Q3 PW 0.70, [340.00-490.00]	
5: + c SRM Skimmer Offset 15, Micro Scans 1,	
Parent Center Width Time CE Q1 PW Q3 PW Tube Lens	
979.00 330.26 1.000 0.005 22 1.00 0.70 201	N-arachidonoyl phosphatidylethanolamine (NAPE1)
979.00 551.54 1.000 0.005 22 1.00 0.70 201	NAPE2
995.66 330.34 1.000 0.005 25 1.00 0.70 146	NAPE1-NH_4^+
995.66 551.60 1.000 0.005 16 1.00 0.70 146	NAPE2-NH_4^+
6: + c Full Parent Scan, Product Mass 551.54, CE 18	NAPE Species
Scan Time 0.04, Q1 PW 0.70, Q3 PW 0.70, [900.00-1050.00]	
Segment 2:	
Tune Method C:\Xcalibur\methods\Lipids_Road_Map_Sept2012.TSQTune	
Chrom filter: Not used	
Q2 Gas Pressure: 1.0	
Syringe Pump: Off	
Scan Events:	
1: + c Full Neutral Loss Scan, N.Loss 185.12, CE 25	Glycerophosphoserine (PS)
Scan Time 0.10, Q1 PW 0.70, Q3 PW 0.70, [645.00-900.00]	
2: + c Full Parent Scan, Product Mass 184.12, CE 22	Glycerophosphocholine (PC)
Scan Time 0.58, Q1 PW 0.10, Q3 PW 0.10, [630.00-920.00]	
3: + c Full Neutral Loss Scan, N.Loss 277.00, CE 20	Glycerophosphoinositol (PI)
Scan Time 0.08, Q1 PW 0.70, Q3 PW 0.70, [720.00-1000.00]	
4: + c Full Neutral Loss Scan, N.Loss 189.01, CE 12	Glycerophosphoglycerol (PG)
Scan Time 0.08, Q1 PW 0.70, Q3 PW 0.70, [635.00-900.00]	
5: + p SRM Skimmer Offset 15, Micro Scans 1,	PC Molecular Species
Parent Center Width Time CE Q1 PW Q3 PW Tube Lens	
m/z 184.12 1.000 0.001 19 0.70 0.70 145	PC species identified in CSF
m/z 184.12 1.000 0.001 19 0.70 0.70 145	Oxidized PC species from published data
6: + c Full Parent Scan, Product Mass 184.118, CE 22,	PC(11:0/11:0)
Scan Time 0.00, Q1 PW 0.70, Q3 PW 0.70, [590.00-600.00]	
Segment 3:	
Tune Method C:\Xcalibur\methods\Lipids_Road_Map_Sept2012.TSQTune	
Chrom filter: Not used	

Q2 Gas Pressure: 1.0	
Syringe Pump: Off	
Scan Events:	
1: + p SRM Skimmer Offset 15, Micro Scans 1,	
Parent Center Width Time CE Q1 PW Q3 PW Tube Lens	Lysophosphatidylcholine (LPC) or Short Chain PC Species
m/z 184.12 1.000 0.001 18 0.70 0.70 145	PAF, PAF_LL, Short chain PC species identified in CSF or from published data
2: + c Full Parent Scan, Product Mass 184.12, CE 18,	Low molecular weight PC
Scan Time 0.04, Q1 PW 0.70, Q3 PW 0.70, [440.00-580.00]	
3: + c Full Parent Scan, Product Mass 184.118, CE 18	LPC(C11:0)
Scan Time 0.05, Q1 PW 0.70, Q3 PW 0.70, [420.00-430.00]	
4: + c Full Parent Scan, Product Mass 104.089, CE 16	LPC species
Scan Time 0.04, Q1 PW 0.70, Q3 PW 0.70, [440.00-580.00]	
5: + c Full Parent Scan, Product Mass 104.089, CE 16,	LPC(C11:0)
Scan Time 0.04, Q1 PW 0.70, Q3 PW 0.70, [420.00-430.00]	
6: + p SRM Skimmer Offset 15, Micro Scans 1,	LPC Species
Parent Center Width Time CE Q1 PW Q3 PW Tube Lens	
m/z 104.09 1.000 0.001 16 0.70 0.70 145	LPC species identified in CSF or from published data
Hamilton Syringe Settings:	
Flow Rate (µL/min) : 5.00 Volume (µL) : 500.00	
Stop Syringe Pump at End of Run : Yes	
Divert Valve: in use during run	
Divert Time (min) Valve State	
==============================	
0.00 Inject \ Waste	
1.00 Load \ Detector	