Supporting Information

Discovery of Lipidome Alterations Following Traumatic Brain Injury *via* High-Resolution Metabolomics.

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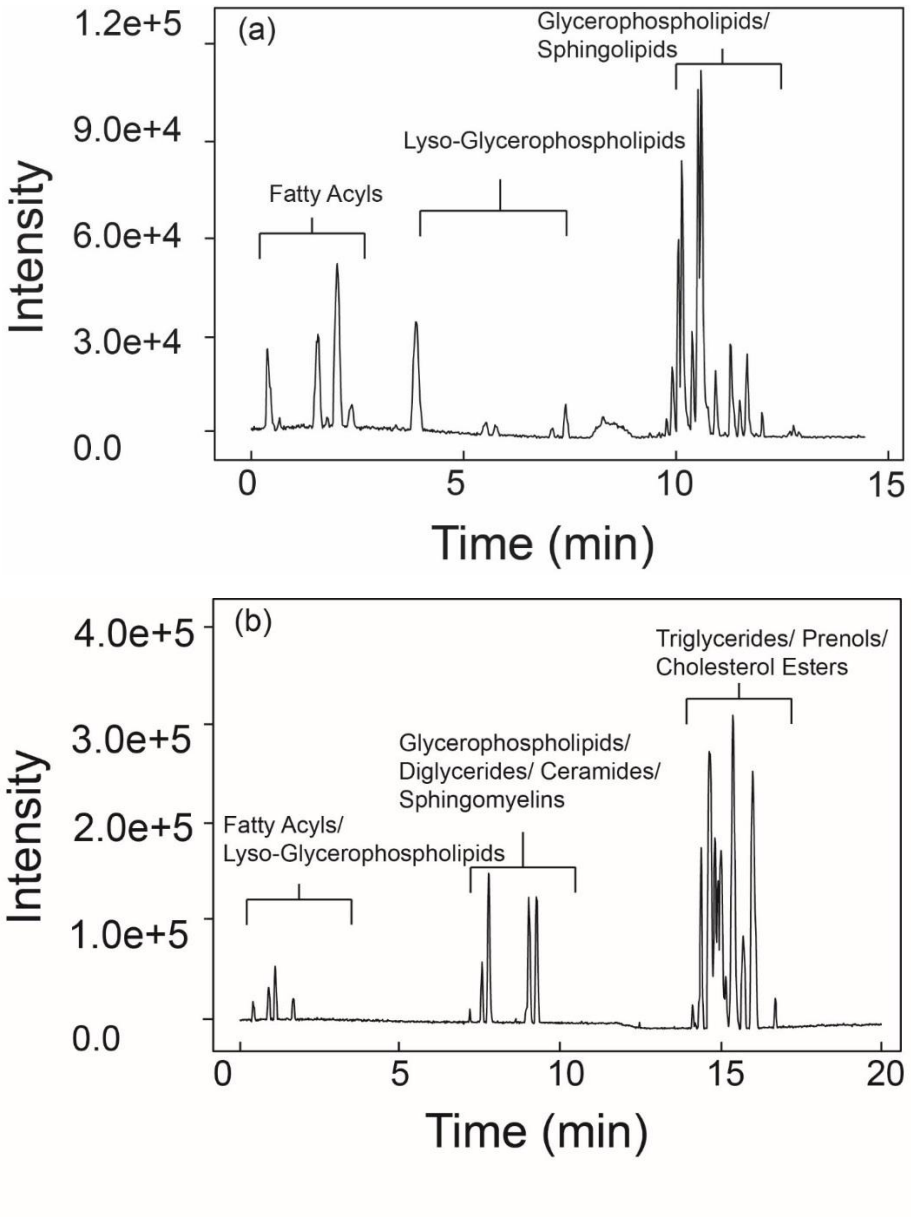
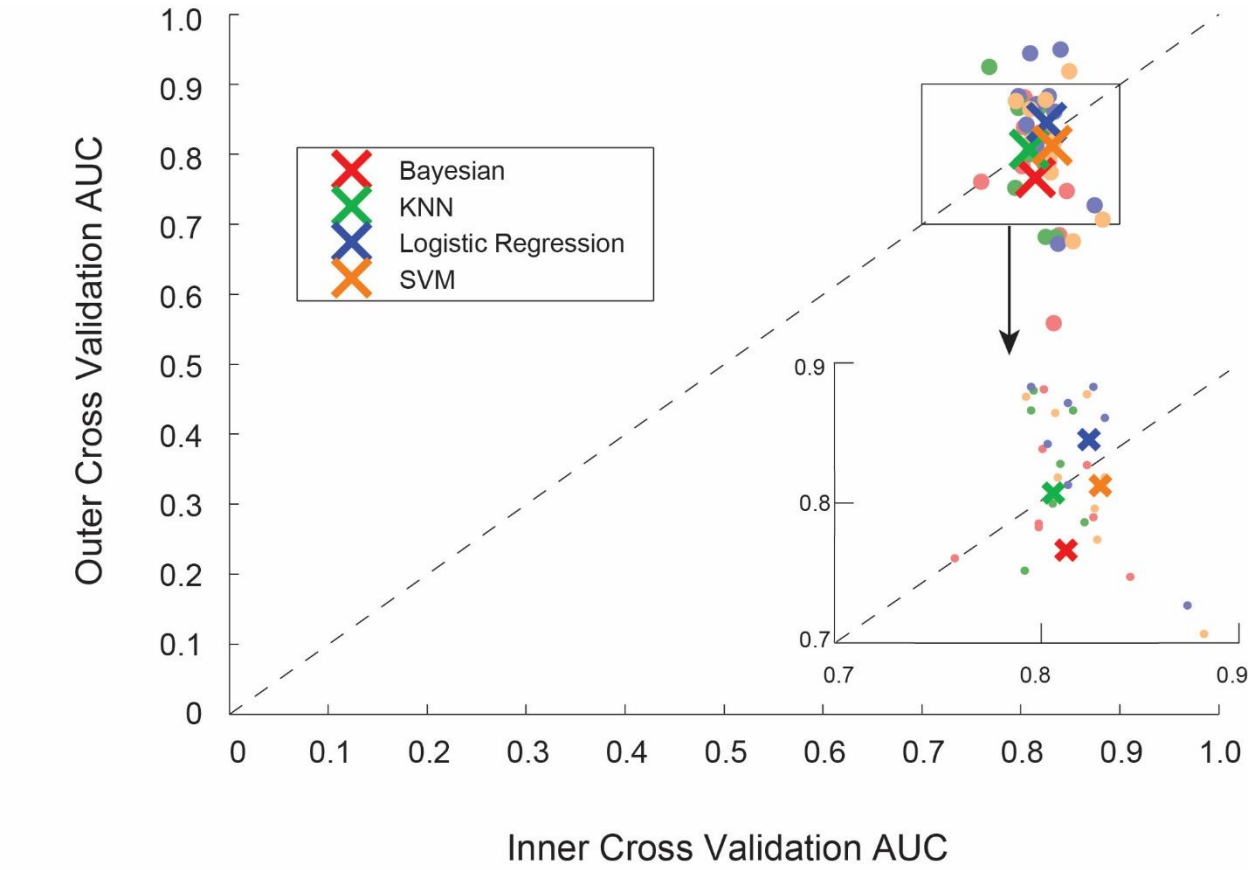
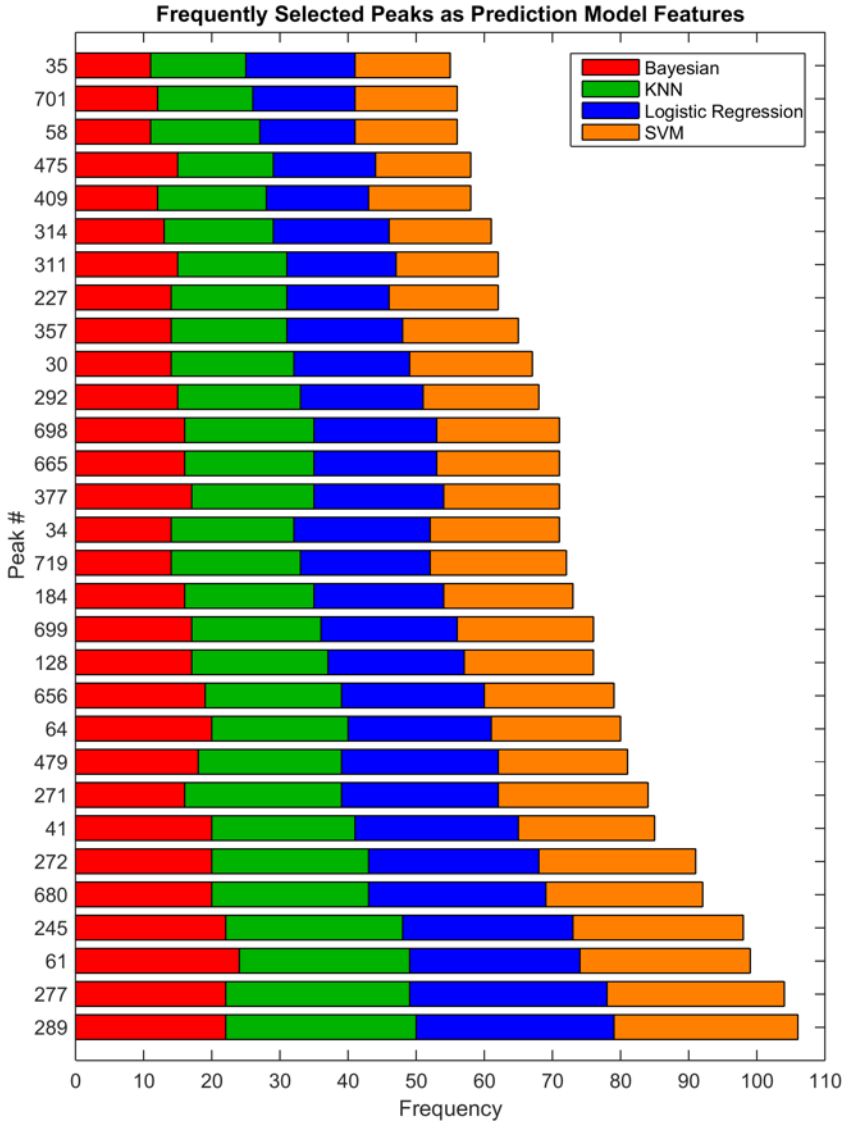


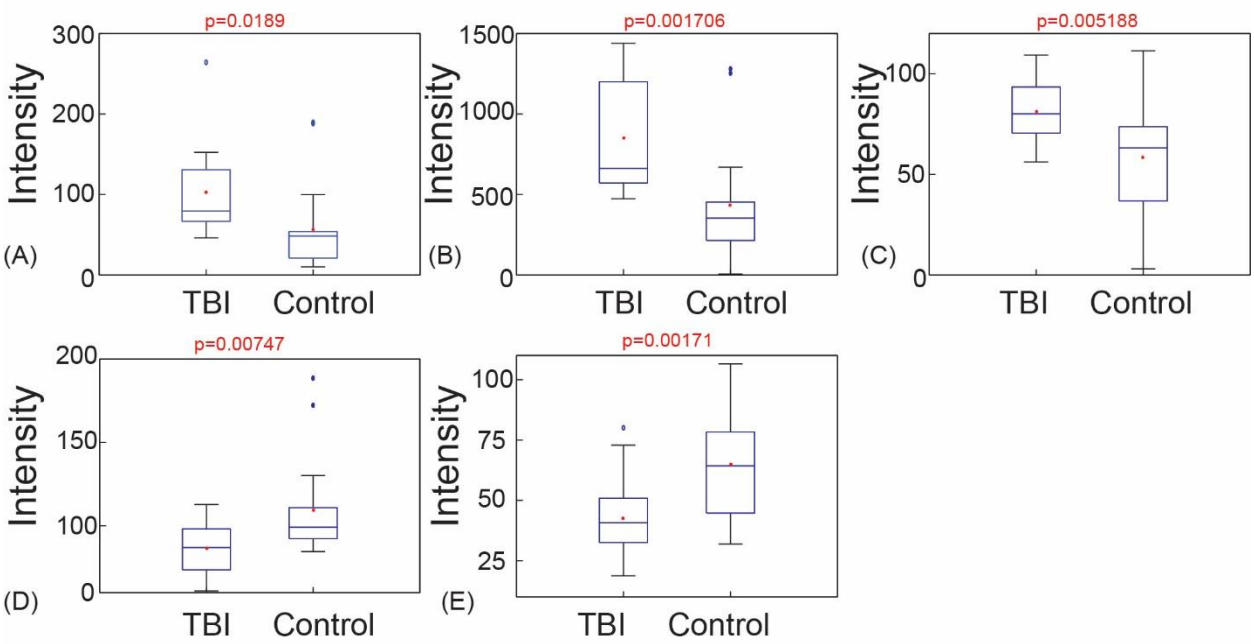
Supplemental Figure S1: Lipid coverage following protein precipitation with isopropyl alcohol. Representative Base Peak Intensity (BPI) chromatograms for pooled serum samples analyzed in a) negative ion mode or b) positive ion mode. Common lipid classes observed are labeled by elution region. Positive ion mode typically favors ionization of phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) which contain an easily ionizable, polar headgroup, while many more classes of lipids can be detected in negative mode.[25]



Supplemental Figure S2: Estimated nested cross validation performance of TBI prediction modeling is 0.8 AUC. Inner cross validation performance is similar to outer cross validation performance, meaning inner cross validation AUC is an unbiased estimate of outer cross validation AUC. Each point represents one iteration of outer cross validation. The large X's represent the average performance of each of the four classifiers. The region around 0.8 AUC is enlarged for clarity.



Supplemental Figure S3: Most commonly selected features sorted by frequency selected to build classification models – 120 maximum. Features were selected to maximize variance between control and injury groups.



Supplemental Figure S4: Box plots depicting features not contained in the 26-feature model, but that were still selected with high frequency by omniClassifier. A) eicosapentaenoic acid, $[M-H]^-$ = 301.217, #34; B) docosahexaenoic acid, $[M-H]^-$ =327.2322, #35; C) LysoPE(20:4), $[M-H]^-$ = 500.278, #227; D) PE(32:0), $[M-H]^-$ = 750.531, #30; E) PC(40:4), $[M+H]^+$ = 842.661, #475.

Table S1. Methods information. S1a) Chromatographic gradients: Mobile Phase A) water: acetonitrile (40:60) and B) 10% acetonitrile in IPA, with 10 mM ammonium formate and 0.1% formic acid additives. S1b) Summary of MS acquisition parameters.

a)

Negative Mode			Positive Mode		
Time (min)	**% A**	**% B**	**Time (min)**	**% A**	**% B**
0	95	5	0	70	30
1	95	5	1	70	30
3	90	10	3	60	40
5	80	20	5	54	46
6	58	42	6	52	48
10	26	74	10	45	55
11	10	90	11	30	70
12	0	100	15	26	74
14	0	100	18	0	100
			20	0	100

b)

	Negative mode	**Positive Mode**
Source capillary voltage	-2.0 kV	3.0 kV
Sampling cone voltage	30 V	40 V
Extraction cone voltage	3 V	4 V
Source temperature	90° C	80° C
Desolvation temperature	250° C	150° C
Desolvation gas flow rate	600 L/h	600 L/h
Cone gas flow rate	50	50

Table S2. Detailed chemical (MS/MS) characteristics of the panel of 26 metabolic features that distinguished TBI and control samples**.** The fragment ions are listed in the table obtained using the corresponding collision energy (CE). The ions selected for fragmentation are underlined. Each metabolite was identified according to the following four rigor levels: 1) identified compounds matched to authentic compound using standards (accurate mass, isotopic abundances, fragmentation spectrum and retention time matched); 2) putatively annotated compounds (accurate mass, isotopic abundances, and fragmentation spectrum matched to databases or consistent with expected fragmentation patterns); 3) putatively characterized compound classes (accurate mass matched to Lipid Maps, Human Metabolome Database (HMDB) or Metlin database entry, and/or fragmentation showing a few matching characteristic fragment ions, such as lipid head group); and 4) unknown compound.

Feature ID	CE (eV), instrument, polarity	Fragment Ion m/z	Relative Intensity	Fragment Annotation	Specific Comments [ID level]
24 PE(20:4_16:0)	40 V Xevo (−)	<u>738.5081</u>, 482.2678, 452.2884, 434.2673, 303.2396, 255.2424, 196.0369, 140.0145, 78.9637	0.10 0.01 0.10 0.02 1.00 0.58 0.04 0.07 0.04	$[M - H]^-$ NL FA 16:0 Loss of FA 20:4 as ketene NL FA 20:4 $[FA\ 20:4 - H]^-$ $[FA\ 16:0 - H]^-$ PE headgroup Ethanolamine phosphate $[PO3]^-$	Consistent with predicted spectrum[1] (Lipid Maps) - [2]
41 Arachidonic acid (AA)	20 V Xevo (−)	<u>303.2325</u>, 285.2262, 259.2401, 231.2073, 205.1944, 177.1663, 59.0131	1.00 0.02 0.14 0.01 0.05 0.01 0.13	$[M - H]^-$ $[M - H - H_2O]^-$ $[M - H - CO_2]^-$ - $[C_{15}H_{25}]^-$ - -	Consistent with Lipid Maps database entry [2]
58 Cer(d18:1_22:0)	HCD 30 QE HF (−)	<u>666.6058</u>, 620.5979, 590.5876, 572.5766, 380.3528 364.3581 339.3264 321.3159 263.2377 237.2223	0.01 1.00 0.10 0.08 0.08 0.50 0.10 0.10 0.10 0.15	$[M + HCO_2]^-$ $[M - H]^-$ $[M - HCHO]^-$ $[M - HCHO - H_2O]^-$ NL 240.2 (sphingosine base) NL 256.2 (sphingosine base) $[FA\ 22:0 - H]^-$ $[FA\ 22:0 - H_2O]^-$ C_{18} sphingosine fragment C_{18} sphingosine fragment	Consistent with predicted spectrum (Lipid Maps) - [2]
61 Cholesterol sulfate (CS)	HCD 30 QE HF (-)	<u>465.3042</u>, 407.2792, 210.8419, 96.9601	1.00 0.15 0.03 0.53	$[M - H]^-$ - - $[HSO_4]^-$	Consistent with spectrum HMDB entry [2]

Feature ID	CE (eV), instrument, polarity	Fragment Ion m/z	Relative Intensity	Fragment Annotation	Specific Comments [ID level]
64 PC(20:2_18:0)	HCD 30 QE HF (−)	858.6259, 798.6026, 727.5843, 554.2817, 508.3405, 391.2258, 307.2643, 283.2643, 224.0694, 168.0431, 152.9959, 78.9588	0.09 1.00 0.01 0.01 0.01 0.02 0.17 0.10 0.02 0.44 0.03 0.53	$[M + HCO_2]^-$ $[M - CH_3]^-$ Loss of Choline & HCO_2 - Loss of FA 20:2 as ketene - $[FA\ 20:2 - H]^-$ $[FA\ 18:0 - H]^-$ $[GPC - CH_3 - H_2O]^-$ Phosphocholine – CH_3 Glycerol-3-phosphate – H_2O $[PO_3]^-$	Consistent with predicted spectrum (Lipid Maps) - [2]
103 PC(16:0_16:0)	HCD 30 QE HF (−)	778.5594, 718.5397, 480.3098, 331.2643, 255.2329, 224.0693, 168.0430, 78.9588	0.03 0.18 0.04 0.10 1.00 0.01 0.03 0.03	$[M + HCO_2]^-$ $[M - CH_3]^-$ Loss of FA 16:0 as ketene - $[FA\ 16:0 - H]^-$ $[GPC - CH_3 - H_2O]^-$ Phosphocholine – CH_3 $[PO_3]^-$	Consistent with predicted spectrum (Lipid Maps) [2][2]
118 PE(18:2_18:0) PC(18:2_16:0)	HCD 30 QE HF (−)	742.5406, 480.3097, 283.2644, 279.2332, 196.0382, 140.0121, 78.9589 742.5397, 480.3095, 462.2993, 279.2329, 255.2330, 224.0694, 168.0430, 152.958, 78.9588	0.10 0.05 0.36 1.00 0.03 0.04 0.03 0.06 0.05 0.01 1.00 0.26 0.02 0.02 0.01 0.03	$[M–H]^-$ Loss of FA 18:2 as ketene $[FA\ 18:0 - H]^-$ $[FA\ 18:2 - H]^-$ PE headgroup Ethanolamine Phosphate $[PO_3]^-$ $[M - CH_3]^-$ Loss of FA 18:2 as ketene NL FA 18:2 – CH_3 $[FA\ 18:2 - H]^-$ $[FA\ 16:0 - H]^-$ $[GPC - CH_3 - H_2O]^-$ Phosphocholine – CH_3 Glycerol-3-phosphate – H_2O $[PO_3]^-$	Consistent with predicted structure(s) (Lipid Maps) - [2][3]
128 DG(22:6_18:1)	30 V Xevo (-)	711.5165 665.6251 268.8004 210.8417 152.8832 92.9277	0.01 0.01 0.25 0.15 0.14 1.00	$[M + HCO_2]^-$ $[M - H]^-$ - - - -	Accurate mass match (Lipid Maps) [2][4],*
129 $C_{43}H_{84}NO_8P$	HCD 30 QE HF (−)	818.5886, 758.5708, 494.3300, 464.3144, 446.3045, 331.2643, 303.2321, 281.2485, 279.2323, 269.2487,	0.01 0.36 0.03 0.05 0.02 0.05 0.36 1.00 0.40 0.38	$[M + HCO_2]^-$ $[M - CH_3]^-$ Loss of FA 18:1 as ketene Loss of FA 20:0 as ketene NL FA 20:0 – CH_3 $[FA\ 22:4 - H]^-$ $[FA\ 20:4 - H]^-$ $[FA\ 18:1 - H]^-$ $[FA\ 18:0 - H]^-$ $[FA\ 17:0 - H]^-$	Consistent with multiple possible PC structures [3]

Feature ID	CE (eV), instrument, polarity	Fragment Ion m/z	Relative Intensity	Fragment Annotation	Specific Comments [ID level]
		255.2330, 224.0692, 168.0430, 78.9588	0.12 0.03 0.04 0.05	[FA 16:0 – H]$^-$ [GPC – CH_3 – H_2O]$^-$ Phosphocholine – CH_3 [PO_3]$^-$	
245 Not identified	HCD 30 QE HF (−)	262.9005, 242.9865, 222.9825, 219.1391, 183.1025	0.01 0.34 0.10 0.48 0.70	- - - - -	---- [4]
271 DG(22:6_18:2)	10 V Xevo (−)	709.5049, 663.6487, 549.5652, 421.0892, 263.0663, 229.0138	1.00 0.05 0.10 0.07 0.04 0.02	[M + HCO_2]$^-$ [M – H]$^-$ - - - -	Accurate mass match (Lipid Maps) [2][5,*]
272 Docosapentaenoic acid (DPA)	20 V Xevo (−)	329.2468, 311.2415, 285.2573, 259.1709, 231.2135	1.00 0.15 0.30 0.05 0.10	[M – H]$^-$ [M – H – H_2O]$^-$ [M – H – CO_2]$^-$ - [$C_{17}H_{27}$]$^-$	Consistent with Lipid Maps database entry [2]
277 DG(20:4_18:1)	10 V Xevo (+)	660.5562, 643.5270, 625.5195, 447.3466, 361.2734, 339.2891, 287.2368, 247.2419	0.05 1.00 0.38 0.10 0.20 0.40 0.05 0.12	[M + NH_4]$^+$ [M + H]$^+$ [M + H – H_2O]$^+$ - NL FA 18:1 + NH_3 NL FA 20:4 + NH_3 FA 20:4 [RC=O]$^+$ FA 18:1 [RC=O]$^+$ -H_2O	Consistent with predicted spectrum (Lipid Maps) - [2]*
289 FFA(18:0)	HCD 30 QE HF (−)	283.2642, 265.1809, 254.9863, 242.9885, 216.9892	1.00 0.03 0.04 0.02 0.05	[M – H]$^-$ [M – H – H_2O]$^-$ - - -	Consistent with spectrum HMDB entry [2]
292 Not identified	HCD 30 QE HF (−)	297.0981, 255.1352, 175.0247, 121.0658, 113.0244, 102.9568, 85.0293, 75.0084, 71.0135, 59.0133	0.18 0.05 0.12 0.71 1.00 0.30 0.67 0.28 0.16 0.25	- - - - - - - - - -	--- [4]
314 FFA(18:2 + 1 O)	HCD 30 QE HF (−)	295.2269, 277.2165, 253.1188, 223.1082, 195.1384, 171.1022, 124.0400, 94.0296	1.00 0.21 0.34 0.02 0.08 0.06 0.19 0.26	[M – H]$^-$ [M – H – H_2O]$^-$ [M – H – CO_2]$^-$ - - - - -	Accurate mass match (Lipid Maps) --- [3]

Feature ID	CE (eV), instrument, polarity	Fragment Ion m/z	Relative Intensity	Fragment Annotation	Specific Comments [ID level]
357 PE(18:0_22:4)	HCD 30 QE HF (−)	794.5697, 528.3086, 510.3400, 331.2639, 303.2324, 283.2639, 259.2428, 224.0691, 196.0369, 168.0429, 152.9957, 78.9597	0.06 0.01 0.08 0.04 1.00 0.70 0.09 0.06 0.02 0.06 0.01 0.06	$[M - H]^-$ Loss of FA 18:0 as ketene NL FA 18:0 $[FA\ 22:4 - H]^-$ $[FA\ 20:4 - H]^-$ $[FA\ 18:0 - H]^-$ - $[GPC - CH_3 - H_2O]^-$ PE headgroup Phosphocholine – CH_3 Glycerol-3-phosphate – H_2O $[PO_3]^-$	PE species matched to predicted spectrum [2], # PC species likely co-selected.
377 SM(d18:1/22:1)	HCD 30 QE HF (-)	829,6448 769.6223, 449.3156, 168.0430, 78.9589	0.01 1.00 0.02 0.49 0.59	$[M + HCO_2]^-$ $[M - CH_3]^-$ Loss of FA 22:1 as ketene Phosphocholine – CH_3 $[PO_3]^-$	Consistent with predicted spectrum (Lipid Maps) - [2], #
409 LysoPC(20:2)	HCD 30 QE HF (-)	592.3619, 532.3398, 307.2637 242.0794 224.0690 168.0426 152.9954 96.9599 78.9586	0.01 0.30 1.00 0.02 0.08 0.01 0.01 0.20 0.05	$[M + HCO_2]^-$ $[M - CH_3]^-$ $[FA\ 20:2 - H]^-$ Loss of FA 20:2 as ketene $[GPC - CH_3 - H_2O]^-$ Phosphocholine – CH_3 Glycerol-3-phosphate – H_2O $[H_2PO_4]^-$ $[PO_3]^-$	Consistent with predicted spectrum (Lipid Maps) - [2], #
479 PS(16:0_20:4)	HCD 30 QE HF (−)	782.4981, 695.4656, 409.2355, 391.2251, 303.2324, 279.2324, 255.2328, 196.0379, 152.9957, 140.0115, 96.9697, 78.9588	0.02 0.15 0.10 0.22 0.14 0.71 1.00 0.02 0.34 0.03 0.04 0.17	[M-H]- Loss of serine Loss of FA 20:4 as ketene NL FA 20:4 $[FA\ 20:4 - H]^-$ $[FA\ 18:2 - H]^-$ $[FA\ 16:0 - H]^-$ - Glycerol-3-phosphate – H_2O - $[H_2PO_4]^-$ $[PO_3]^-$	Consistent with predicted spectrum (Lipid Maps) - [2], #
656 DG(22:6_18:1)	20 V Xevo (+)	684.5526 667.5300 385.2997 339.3150 311.2371 293.2271	0.01 0.05 0.17 1.00 0.08 0.05	$[M + NH_4]^+$ $[M + H]^+$ NL FA 18:1 + NH_3 NL FA 22:6 + NH_3 FA 22:6 $[RC=O]^+$ FA 22:6 $[RC=O]^+ - H_2O$	Consistent with predicted spectrum (Lipid Maps) - [2]
665 PC(18:1_15:0) PC(17:1_16:0)	HCD 30 QE HF (−)	790.5604 730.5388 506.3244 492.3086 480.3083 466.2934 281.2482 267.2326 255.2327	0.01 0.25 0.01 0.01 0.01 0.04 1.00 0.33 0.18	$[M + HCO_2]^-$ $[M - CH_3]^-$ Loss of FA 15:0 as ketene NL FA 18:1 NL FA 17:1 Loss of FA 18:1 as ketene $[FA\ 18:1 - H]^-$ $[FA\ 17:1 - H]^-$ $[FA\ 16:0 - H]^-$	Consistent with multiple possible PC structures [2], #

Feature ID	CE (eV), instrument, polarity	Fragment Ion m/z	Relative Intensity	Fragment Annotation	Specific Comments [ID level]
		241.2170	0.46	[FA 15:0 – H]$^-$	
		224.0691	0.05	[GPC – CH_3 – H_2O]$^-$	
		168.0427	0.05	Phosphocholine – CH_3	
		78.9587	0.05	[PO_3]$^-$	
680 DG(22:6_18:2)	20 V Xevo (+)	682.5402,	0.02	[M + NH_4]$^+$	Consistent with predicted spectrum (Lipid Maps) - [2]
		665.4964,	0.10	[M + H]$^+$	
		647.4899,	0.05	[M + H – H_2O]$^+$	
		385.2837,	0.18	NL FA 18:2 + NH_3	
		337.2716,	1.00	NL FA 22:6 + NH3	
		311.2371,	0.10	FA 22:6 [RC=O]$^+$	
		293.2271,	0.10	FA 22:6 [RC=O]$^+$ – H_2O	
		263.2401,	0.10	FA 18:2 [RC=O]$^+$	
		245.2193	0.05	FA 18:2 [RC=O]$^+$ – H_2O	
698 $C_{47}H_{84}NO_8P$	HCD 30 QE HF (-)	866.5910	0.05	[M + HCO_2]$^-$	Consistent with multiple possible PC structures [3], #
		806.5698	0.40	[M – CH_3]$^-$	
		568.3400	0.01	-	
		520.3397	0.10	Loss of FA 20:4 as ketene	
		494.3244	0.05	-	
		329.2482	0.30	[FA 22:5 – H]$^-$	
		303.2324	1.00	[FA 20:4 – H]$^-$	
		295.2637	0.70	[FA 19:0 – H]$^-$	
		269.2483	0.22	[FA 17:0 – H]$^-$	
		259.2428	0.10	[$C_{19}H_{32}$]$^-$	
		224.0691	0.08	[GPC – CH_3 – H_2O]$^-$	
		168.0428	0.10	Phosphocholine – CH_3	
		78.9587	0.07	[PO_3]$^-$	
699 LysoPC(18:2 + 1 O)	HCD 30 QE HF (−)	582.3412	0.01	[M + HCO_2]$^-$	Consistent with predicted spectrum. Fragment matched to feature #314 [2], #
		522.3202	0.90	[M – CH_3]$^-$	
		420.5263	0.05	-	
		369.4939	0.05	-	
		304.6898	0.41	-	
		297.2235	1.00	[FA 18:2 + 1 O – H]$^-$	
		224.0692	0.12	[GPC – CH_3 – H_2O]$^-$	
		168.0430	0.05	Phosphocholine – CH_3	
719 PC(18:2_22:1)	HCD 30 QE HF (−)	884.6381	0.01	[M + HCO_2]$^-$	Consistent with predicted spectrum (Lipid Maps) - [2], #
		824.6166	0.20	[M – CH_3]$^-$	
		562.3865	0.05	Loss of FA 18:2 as ketene	
		544.3866	0.02	NL FA 18:2	
		337.3108	0.42	[FA 22:1 – H]$^-$	
		279.2325	1.00	[FA 18:2]$^-$	
		224.0690	0.03	[GPC – CH_3 – H_2O]$^-$	
		168.0428	0.03	Phosphocholine – CH_3	
		96.9600	0.06	[H_2PO_4]$^-$	

[1]http://www.lipidmaps.org/tools/structuredrawing/GP_p_form.php

[2]Fragment ions observed were consistent with PC(16:0_16:0), however, fragment ions corresponding to arachidonic acid (20:4) were also observed in the spectrum, suggesting possible co-selection of precursor ions.

[3]The high resolution of the QE HF instrument reveled two precursors under feature # 118 detected with the lower resolution Xevo instrument. Each was examined individually and matched a specific lipid species.

[4]Although this feature could only be assigned a level 3 confidence based on negative ion mode measurements, it is "paired" to another feature in the panel detected in positive ion mode with a higher level of confidence (feature #656).

[5]Although this feature could only be assigned a level 3 confidence based on negative ion mode measurements, it is "paired" to another feature in the panel detected in positive ion mode with a higher level of confidence (feature #680).

[*]Accurate mass matched in negative mode, fragmented in positive mode for FA chain and headgroup info

[#] Accurate mass matched in positive mode, fragmented in negative mode for FA chain and headgroup info