Electronic Supplementary Material (ESI) for Molecular BioSystems.

Electronic supplementary information

Lipidomic alterations of *in* vitro macrophage infection by *L. infantum* and *L. amazonensis*

Fernanda Negrão,[a] Daniel R. Abánades,[b] Caroline F. Jaeeger, [a] Daniele F. O. Rocha,[a] Katia R. A. Belaz,[a] Selma Giorgio,[c] Marcos N. Eberlin [a] and Célio F. F. Angolini [a, *]

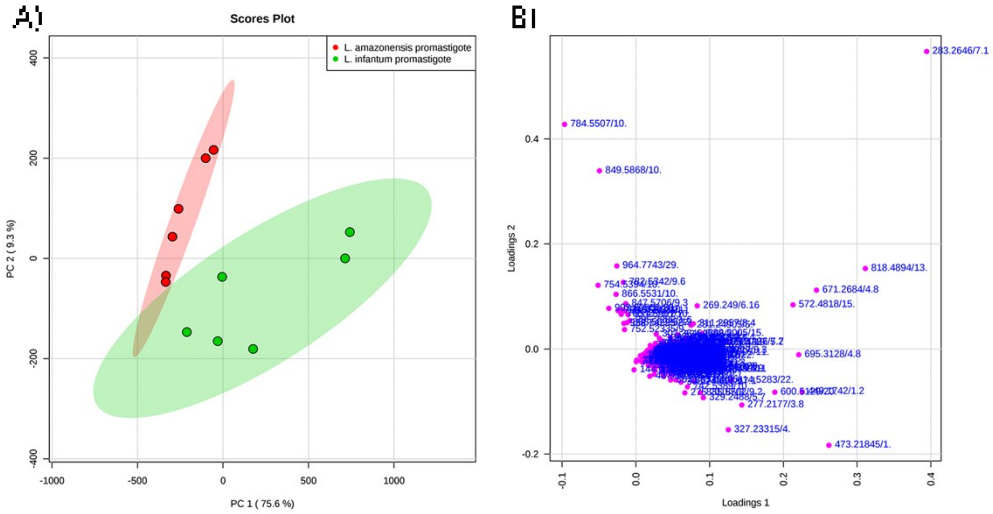

Figure S1 - ***Accessing data quality of lipid data.*** *(A) Principal component analysis of all metabolites identified by LC-MS in cell lipid extracts from L. infantum and L. amazonensis promastigotes; data points corresponds to the analysis of three biological replicate. Principal component 1 and principal component 2 explain 75.6% and 9.3% of the total variance, respectively. (B) The loading plot for each variable as also displayed*

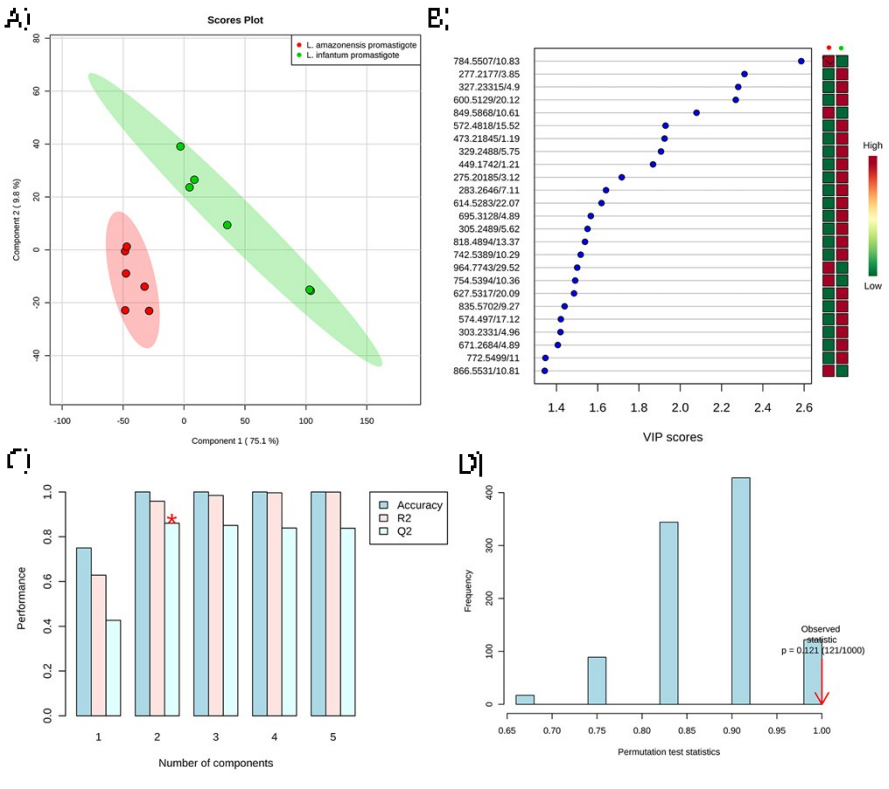

Figure S2 – ***L. amazonensis and L. infantum promastigote lipidomic differences evaluation. (A)*** *PLS-DA score plot of lipid profiles data for the discriminations of* L. amazonensis *promastigote (red) and* L. infantum *promatigote (green). (B) Compounds with higher variable importance in projection values (VIP).(C) PLS-DA cross validation. (D) PLS-DA permutation test (prediction accuracy during training).*

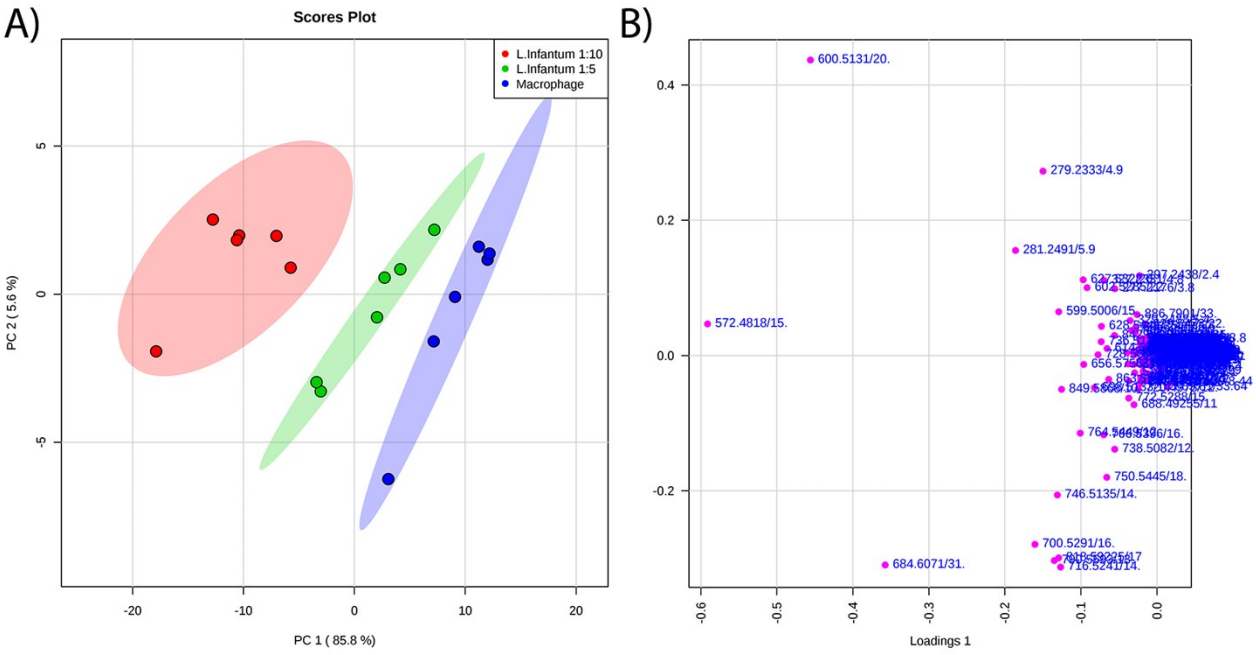

Figure S3 - ***Accessing data quality of lipid data.*** *(A) Principal component analysis of all metabolites identified by LC-MS in cell lipid extracts from macrophage and L. infantum infection experiments; data points corresponds to the analysis of three biological*

replicate. Principal component 1 and principal component 2 explain 85.8% and 5.6% of the total variance, respectively. (B) The loading plot for each variable as also displayed.

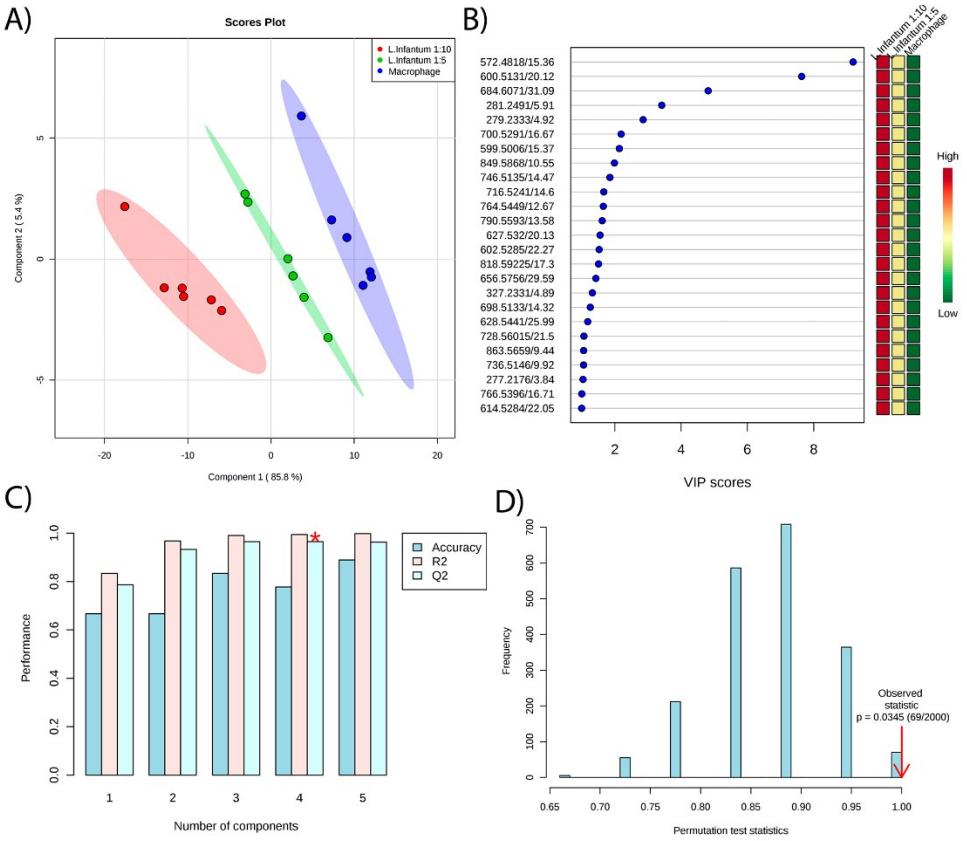

*Figure S4 – **Macrophage vs. viscerotropic promastigote infected macrophages (L. infantum) lipidomic evaluation.** (A) PLS-DA score plot of lipid profiles data for the discriminations of macrophage (blue), and* in vitro *infection 1:5 (green) and 1:10 (red) of* L. infantum. *(B) Compounds with higher variable importance in projection values (VIP).(C) PLS-DA cross validation. (D) PLS-DA permutation test (prediction accuracy during training).*

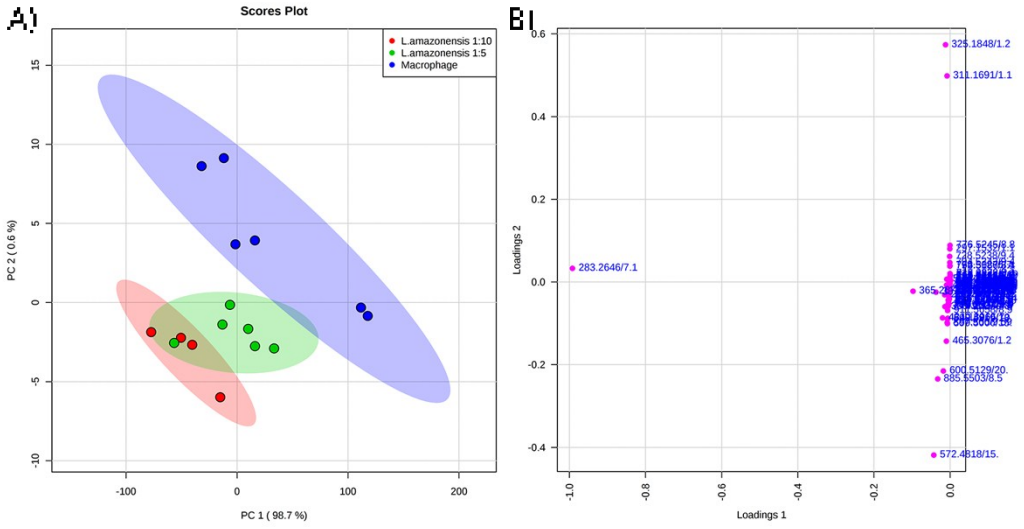

*Figure S5 - **Accessing data quality of lipid data.** (A) Principal component analysis of all metabolites identified by LC-MS in cell lipid extracts from macrophage and L. amazonensis infection experiments; data points corresponds to the analysis of three biological*

replicate. Principal component 1 and principal component 2 explain 98.7% and 0.6% of the total variance, respectively. (B) The loading plot for each variable as also displayed.

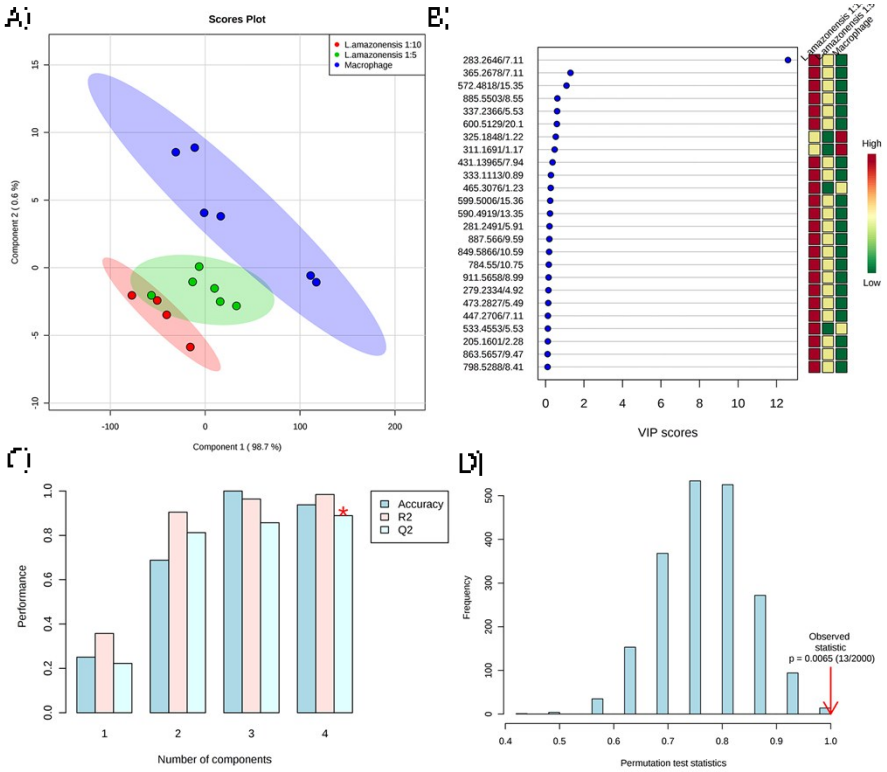

Figure S6 - ***Macrophage vs. cutaneous promastigote infected macrophages (L. amazonensis) lipidomic evaluation.*** *(A) PLS-DA score plot of lipid profiles data for the discriminations of macrophage (blue), and* in vitro *infection 1:5 (green) and 1:10 (red) of* L. amazonensis. *(B) Compounds with higher variable importance in projection values (VIP).(C) PLS-DA cross validation. (D) PLS-DA permutation test (prediction accuracy during training).*

Table S1 - **Identified *phosphatidylethanolamine plasmalogens (PEP) pointed out by statistical analysis.*** *Lipids were identified by their exact mass (3 ppm) and fragmentation pattern.*

m/z	Lipid	Molecular Formula	Normalized abundance Mean ± SD				Lipid Class
			L. infantum				
			Macrophage	Promastigote	1:5	1:10	
698.5133	PE(P-16:0/18:2)	$C_{39}H_{74}NO_7P$	n.d.	0.4 ± 0.2	1.0 ± 0.4	1.8 ± 0.5	Glycerophosphoethanolamines
700.5294	PE(P-16:0/18:1)	$C_{39}H_{76}NO_7P$	n.d.	0.7 ± 0.4	2.2 ± 1.3	3.7 ± 1.0	Glycerophophoethanolamines
728.5601	PE(P18:0/18:1)	$C_{41}H_{80}NO_7P$	n.d.	n.d.	0.4 ± 0.5	1.5 ± 0.8	Glycerophophoethanolamines
746.5135	PE(P-16:0/22:6)	$C_{43}H_{74}NO_7P$	n.d.	n.d.	2.1 ± 0.6	3.1 ± 0.9	Glycerophosphoethanolamines
748.5287	PE(P-16:0/22:5)	$C_{43}H_{72}NO_7P$	n.d.	n.d.	1.9 ± 1.1	2.5 ± 1.4	Glycerophosphoethanolamines
750.5457[a]	PE-P(38:4)	$C_{43}H_{78}NO_7P$	n.d.	n.d.	1.0 ± 0.7	1.36 ± 0.8	Glycerophosphoethanolamines
Total			-	1.1 ± 0.6	8.6 ± 4.6	13.9 ± 4.6	
			L. amazonensis				
698.5131	PE(P-16:0/18:2)	$C_{39}H_{74}NO_7P$	n.d.	0.07 ± 0.004	0.4 ± 0.3	0.72 ± 0.4	Glycerophosphoethanolamines
700.5289	PE(P-16:0/18:1)	$C_{39}H_{76}NO_7P$	n.d.	0.04 ± 0.005	0.7 ± 0.4	1.3 ± 0.3	Glycerophosphoethanolamines
728.5601	PE(P18:0/18:1)	$C_{41}H_{80}NO_7P$	n.d.	n.d.	0.2 ± 0.3	0.65 ± 0.4	Glycerophosphoethanolamines
746.5132	PE(P-16:0/22:6)	$C_{43}H_{74}NO_7P$	n.d.	n.d.	0.9 ± 0.7	1.2 ± 0.3	Glycerophosphoethanolamines
748.5287	PE(P-16:0/22:5)	$C_{43}H_{72}NO_7P$	n.d	n.d.	1.48 ± 0.9	1.7 ± 0.4	Glycerophosphoethanolamines
750.5457[a]	PE-P(38:4)	$C_{43}H_{78}NO_7P$	n.d.	n.d.	0.5 ± 0.4	0.8 ± 0.6	Glycerophosphoethanolamines
Total			-	0.11 ± 0.09	4.1 ± 3	6.4 ± 2.4	

[a] *only identified by their exact mass.* n.d. not detected.

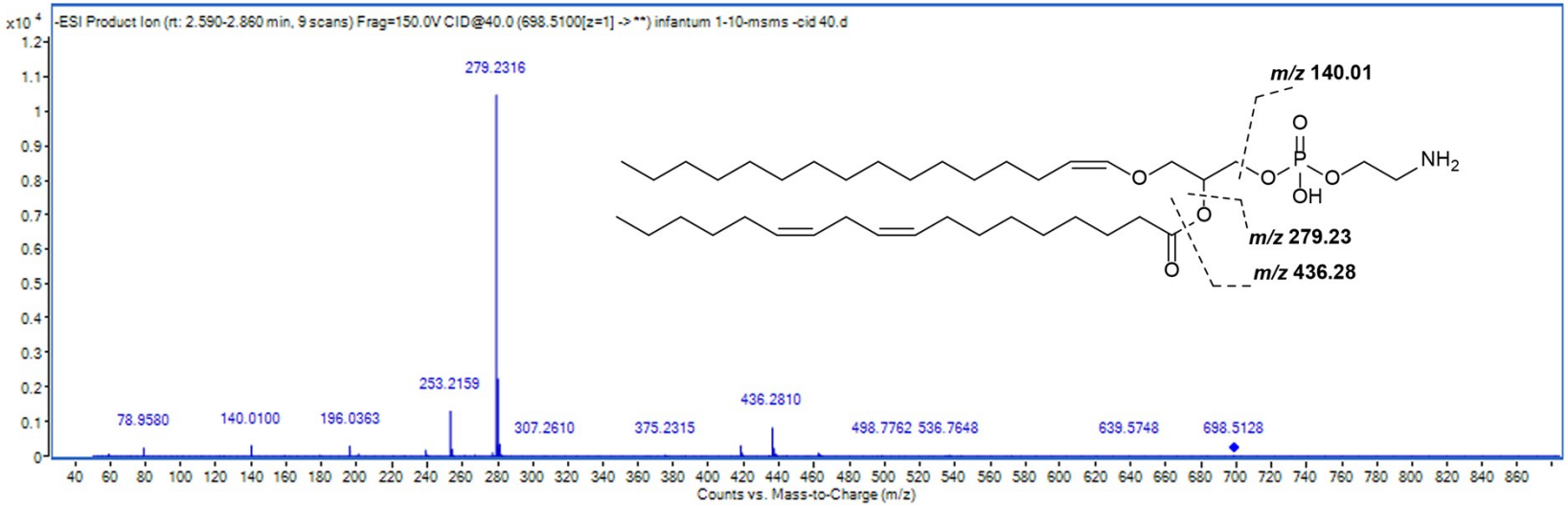

Figure S7 – ***Fragmentation pattern of phosphatidylethanolamine plasmalogens (PEP) class.*** *By analysis of fragmentation pattern is possible elucidate the exact fatty acid chain length and the amount of instauration. However, the exact position of double bond remains unknown.*

Table S2 - **Identified *Phosphatidylserines plasmalogen (PSP) pointed out by statistical analysis.*** *Lipids were identified by their exact mass (3 ppm) and fragmentation pattern.*

m/z	Lipid	Molecular Formula	Normalized abundance Mean ± SD				Lipid Class
			L. infantum				
			Macrophage	Promastigote	1:5	1:10	
784.5507	PS(P-19:0/18:2)	$C_{43}H_{80}NO_9P$	0.1 ± 0.1	0.7 ± 0.4	0.2 ± 0.1	0.2 ± 0.1	Glycerophosphoserine
756.5184[a]	PSP(35:2)	$C_{41}H_{76}NO_9P$	0.2 ± 0.2	0.7 ± 0.2	0.5 ± 0.3	0.2 ± 0.1	Glycerophosphoserine
758.5351[a]	PSP(35:1)	$C_{41}H_{78}NO_9P$	0.7 ± 0.8	0.2 ± 0.08	1.0 ± 0.6	0.5 ± 0.1	Glycerophosphoserine
772.5507[a]	PSP(36:1)	$C_{42}H_{80}NO_9P$	0.2 ± 0.3	0.04 ± 0.01	0.2 ± 0.2	0.2 ± 0.1	Glycerophosphoserine
786.5642[a]	PSP(37:1)	$C_{43}H_{82}NO_9P$	n.d.	2.6 ± 0.8	0.1 ± 0.2	0.3 ± 0.1	Glycerophosphoserine
788.5825[a]	PSP(37:0)	$C_{43}H_{84}NO_9P$	n.d.	n.d.	0.05 ± 0.03	0.06 ± 0.04	Glycerophosphoserine
798.5658[a]	PSP(38:2)	$C_{44}H_{82}NO_9P$	n.d.	0.2 ± 0.1	n.d.	n.d.	Glycerophosphoserine
Total			1.2 ± 1.4	4.4 ± 1.6	2.0 ± 1.4	1.4 ± 0.5	

			L. amazonensis				
784.5507	PS(P-19:0/18:2)	$C_{43}H_{80}NO_9P$	0.1 ± 0.1	9.7 ± 3	0.6 ± 0.2	1.0 ± 0.6	Glycerophosphoserine
756.5184 [a]	PSP(35:2)	$C_{41}H_{76}NO_9P$	0.2 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.3	Glycerophosphoserine
758.5351 [a]	PSP(35:1)	$C_{41}H_{78}NO_9P$	0.7 ± 0.6	1.2 ± 0.8	1.0 ± 0.4	0.8 ± 0.4	Glycerophosphoserine
772.5507 [a]	PSP(36:1)	$C_{42}H_{80}NO_9P$	0.2 ± 0.3	0.3 ± 0.1	0.1 ± 0.07	0.1 ± 0.1	Glycerophosphoserine
786.5642 [a]	PSP(37:1)	$C_{43}H_{82}NO_9P$	n.d.	1.5 ± 1.2	0.2 ± 0.1	0.5 ± 0.3	Glycerophosphoserine
788.5825 [a]	PSP(37:0)	$C_{43}H_{84}NO_9P$	n.d.	n.d.	0.03 ± 0.02	0.02 ± 0.02	Glycerophosphoserine
798.5658 [a]	PSP(38:2)	$C_{44}H_{82}NO_9P$	n.d.	n.d.	n.d.	n.d.	Glycerophosphoserine
Total			1.2 ± 1.2	13.3 ± 5.3	2.4 ± 0.9	2.9 ± 1.7	

[a] *only identified by their exact mass.* n.d. not detected.

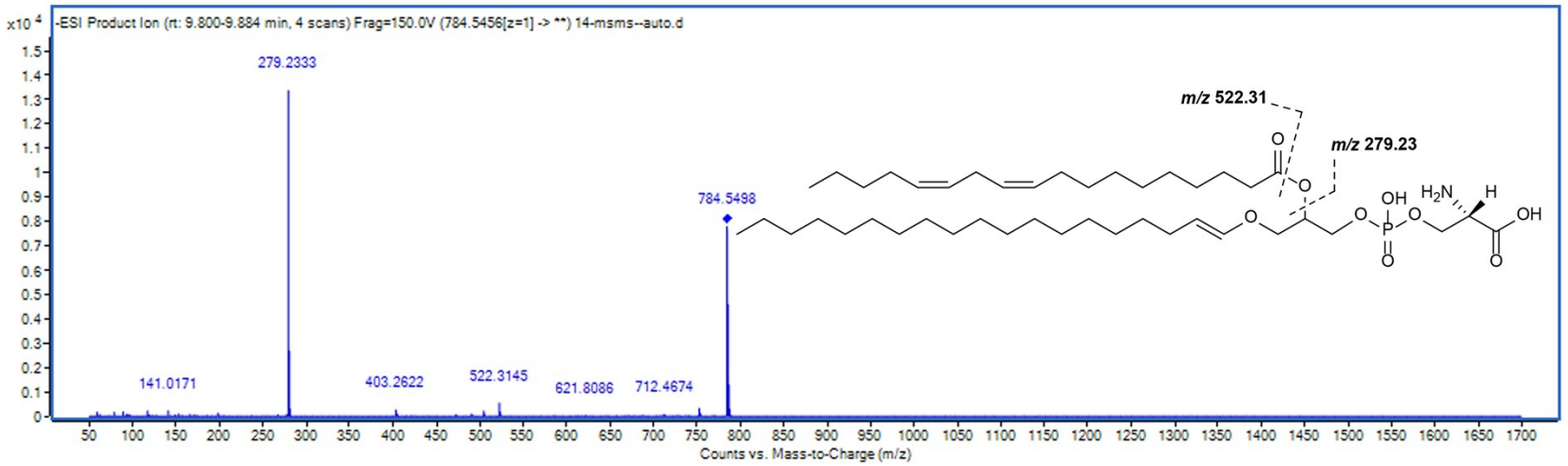

Figure S8 - ***Fragmentation pattern of phosphatidyleserine plasmalogens (PSP) class.*** *By analysis of fragmentation pattern is possible elucidate the exact fatty acid chain length and the amount of instauration. However, the exact position of double bond remains unknown.*

Table S3 - **Identified *shpingolipids (SL) pointed out by statistical analysis.*** *Lipids were identified by their exact mass (3 ppm) and fragmentation pattern.*

m/z	Lipid	Molecular Formula	Normalized abundance Mean ± SD				Lipid Class
			L. infantum				
			Macrophage	Promastigote	1:5	1:10	
684.6075 [a,b]	Cer(d42:1)	$C_{42}H_{83}NO_3$	2.4 ± 1	n.d.	5.1 ± 3	9.6 ± 2	Ceramides
572.4818 [b]	Cer(d16:1/18:0)	$C_{34}H_{67}NO_3$	2.0 ± 1	16.4 6.7	7.4 ± 2.6	15.0 ± 2.2	Ceramides
778.5248 [a]	PI-Cer(d34:1)	$C_{40}H_{78}NO_{11}P$	n.d.	1.5 ± 0.3	n.d.	n.d.	Ceramide phosphoinositols
780.5408 [a]	PI-Cer(d34:0)	$C_{40}H_{80}NO_{11}P$	n.d.	0.3 0.2	0.02 0.02	n.d.	Ceramide phosphoinositols
600.5131 [b]	Cer(d18:1/18:0)	$C_{36}H_{71}NO_3$	0.07 ± 0.08	10.9 ± 5.6	3.5 ± 0.7	9.9 ± 2.3	Ceramides
806.5568 [a]	PI-Cer(d36:1)	$C_{42}H_{82}NO_{11}P$	n.d.	0.5 ± 0.1	0.2 ± 0.2	0.1 ± 0.06	Ceramide phosphoinositols
602.5285 [b]	Cer(d18:0/18:0)	$C_{36}H_{73}NO_3$	n.d.	1.3 ± 0.7	3.5 ± 0.7	1.9 ± 0.3	Ceramides
656.5756 [a,b]	Cer(d40:1)	$C_{40}H_{79}NO_3$	0.6 ± 0.2	n.d.	1.3 ± 0.7	2.7 ± 0.4	Ceramides
628.5441 [a,b]	Cer(d38:1)	$C_{38}H_{75}NO_3$	n.d.	1.45 ± 0.8	0.4 ± 0.4	1.5 ± 0.2	Ceramides
614.5284 [a,b]	Cer(d37:1)	$C_{37}H_{73}NO_3$	n.d.	3.3 ± 2.8	0.4 ± 0.3	1.3 ± 0.8	Ceramides
			L. amazonensis				
684.6075 [a,b]	Cer(d42:1)	$C_{42}H_{83}NO_3$	2.4 ± 1	n.d.	3.8 ± 1.4	3.6 ± 0.4	Ceramides
572.4818 [b]	Cer(d16:1/18:0)	$C_{34}H_{67}NO_3$	2.0 ± 1	7.8 ± 4.9	5.9 ± 1.1	9.7 ± 1.1	Ceramides
778.5248 [a]	PI-Cer(d34:1)	$C_{40}H_{78}NO_{11}P$	n.d.	2.0 ± 1.3	n.d.	n.d.	Ceramide phosphoinositols
780.5408 [a]	PI-Cer(d34:0)	$C_{40}H_{80}NO_{11}P$	n.d.	0.1 0.03	n.d.	n.d.	Ceramide phosphoinositols
600.5131 [b]	Cer(d18:1/18:0)	$C_{36}H_{71}NO_3$	0.07 ± 0.08	2.3 ± 1.0	0.85 ± 0.6	3.6 ± 0.8	Ceramides
806.5568 [a]	PI-Cer(d36:1)	$C_{42}H_{82}NO_{11}P$	n.d.	1.1 ± 0.7	0.01 ± 0.01	0.07 ± 0.04	Ceramide phosphoinositols
602.5285 [b]	Cer(d18:0/18:0)	$C_{36}H_{73}NO_3$	n.d.	0.3 ± 0.1	0.1 ± 0.2	0.4 ± 0.3	Ceramides
656.5756 [a,b]	Cer(d40:1)	$C_{40}H_{79}NO_3$	0.6 ± 0.2	n.d.	1.0 ± 0.6	1.1 ± 0.1	Ceramides
628.5441 [a,b]	Cer(d38:1)	$C_{38}H_{75}NO_3$	n.d.	n.d.	n.d.	n.d.	Ceramides
614.5284 [a,b]	Cer(d37:1)	$C_{37}H_{73}NO_3$	n.d.	n.d	n.d.	n.d.	Ceramides

[a] only identified by their exact mass. [b] detected as $[M+Cl]^-$ n.d. not detected.

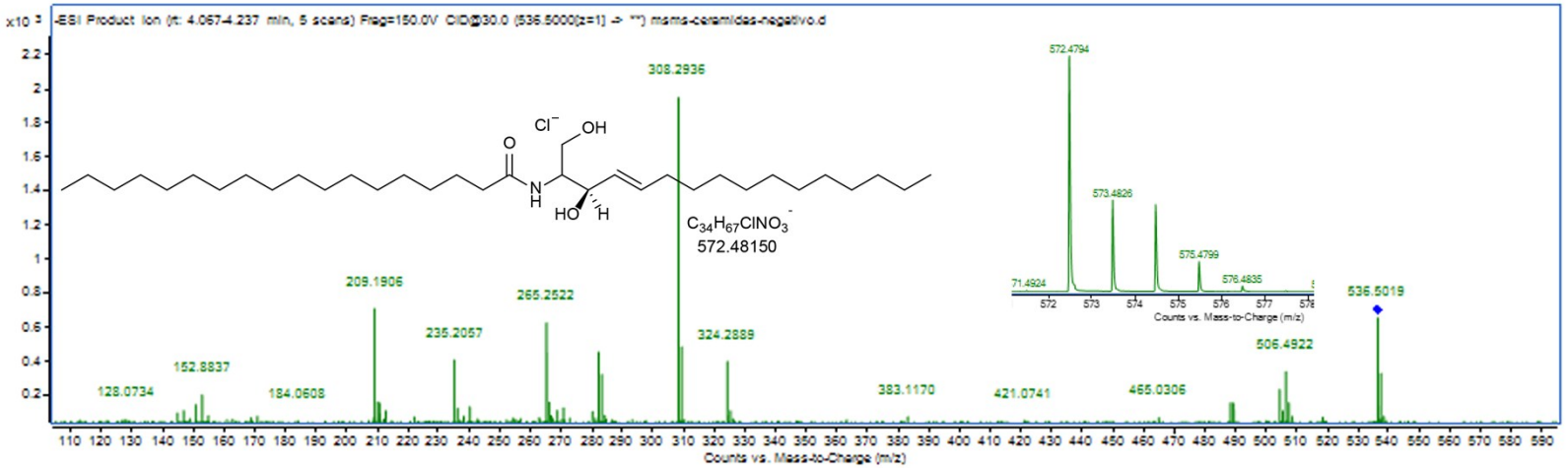

Figure S9 - ***Fragmentation pattern of ceramide (Cer) class.*** *All ceramides were majorly detected as $[M+Cl]^-$, however their MS/MS spectra show no information. The MS/MS pattern used to characterize ceramides was from their $[M-H]^-$ ions. By analysis of fragmentation pattern is possible elucidate the exact fatty acid chain length and the amount of instauration. However, the exact position of double bond remains unknown.*

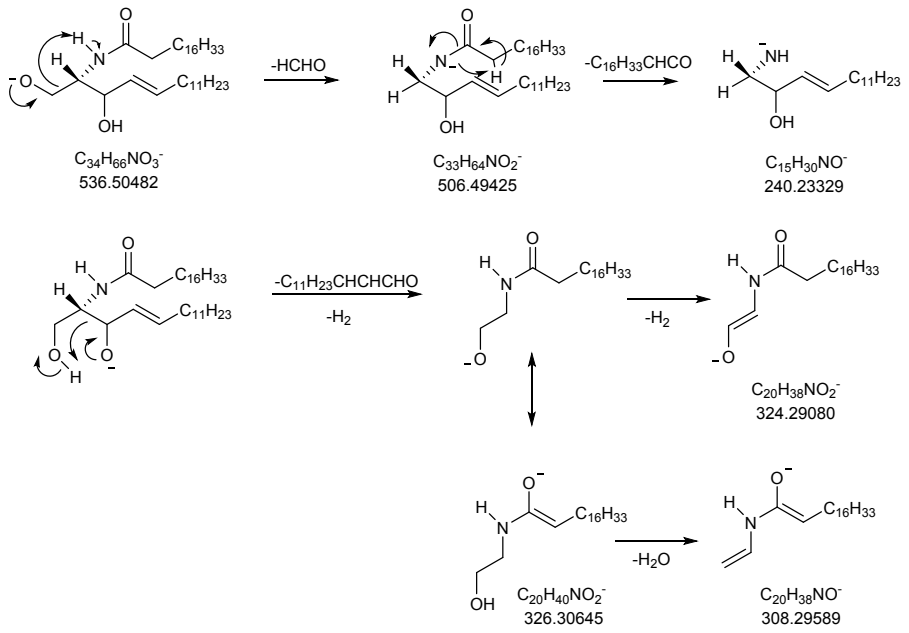

Figure S10 – Scheme of fragmentation route of [M-H]⁻ ceramide ions.[1]

Table S4 - **Identified *glycerophosphoinositols (PSP) pointed out by statistical analysis.*** *Lipids were identified by their exact mass (3 ppm) and fragmentation pattern.*

m/z	Lipid	Molecular Formula	Normalized abundance Mean ± SD				Lipid Class
			L. infantum				
			Macrophage	Promastigote	1:5	1:10	
859.5354 [a]	PI(36:3)	$C_{45}H_{81}O_{13}P$	n.d.	0.5 ± 0.3	0.4 ± 0.4	0.2 ± 0.2	Glycerophosphoinositols
861.5502 [a]	PI(36:2)	$C_{45}H_{83}O_{13}P$	n.d.	1.2 ± 0.8	1.9 ± 1.0	1.2 ± 0.3	Glycerophosphoinositols
821.5533 [a]	PIO(34:1)	$C_{43}H_{83}O_{12}P$	n.d.	0.8 ± 0.5	0.6 ± 0.3	0.8 ± 0.4	Glycerophosphoinositols
849.5856	PI(O-18:0/18:1)	$C_{45}H_{87}O_{12}P$	n.d.	1.0 ± 0.7	1.5 ± 1.1	2.5 ± 1.0	Glycerophosphoinositols
847.5710 [a]	PIO(36:0)	$C_{45}H_{89}O_{12}P$	n.d.	0.2 ± 0.1	0.2 ± 0.3	0.4 ± 0.2	Glycerophosphoinositols
863.5659	PI(18:1/18:0)	$C_{45}H_{85}O_{13}P$	n.d.	9.5 ± 6.7	1.1 ± 0.5	1.5 ± 0.5	Glycerophosphoinositols
			L. amazonensis				

859.5354 [a]	PI(36:3)	$C_{45}H_{81}O_{13}P$	n.d.	0.2 ± 0.07	0.3 ± 0.2	1.0 ± 0.7	Glycerophosphoinositols
861.5502 [a]	PI(36:2)	$C_{45}H_{83}O_{13}P$	n.d.	0.9 ± 0.5	1.5 ± 0.4	1.8 ± 0.7	Glycerophosphoinositols
821.5533 [a]	PIO(34:1)	$C_{43}H_{83}O_{12}P$	n.d.	0.1 ± 0.06	n.d.	n.d.	Glycerophosphoinositols
849.5856	PI(O-18:0/18:1)	$C_{45}H_{87}O_{12}P$	n.d.	5.5 ± 1.9	0.5 ± 0.3	1.1 ± 0.3	Glycerophosphoinositols
847.5710 [a]	PIO(36:0)	$C_{45}H_{89}O_{12}P$	n.d.	0.5 ± 0.2	0.1 ± 0.05	0.2 ± 0.1	Glycerophosphoinositols
863.5659	PI(18:1/18:0)	$C_{45}H_{85}O_{13}P$	n.d.	5.0 ± 1.8	0.4 ± 0.1	0.7 ± 0.2	Glycerophosphoinositols

[a] *only identified by their exact mass.* n.d. not detected.

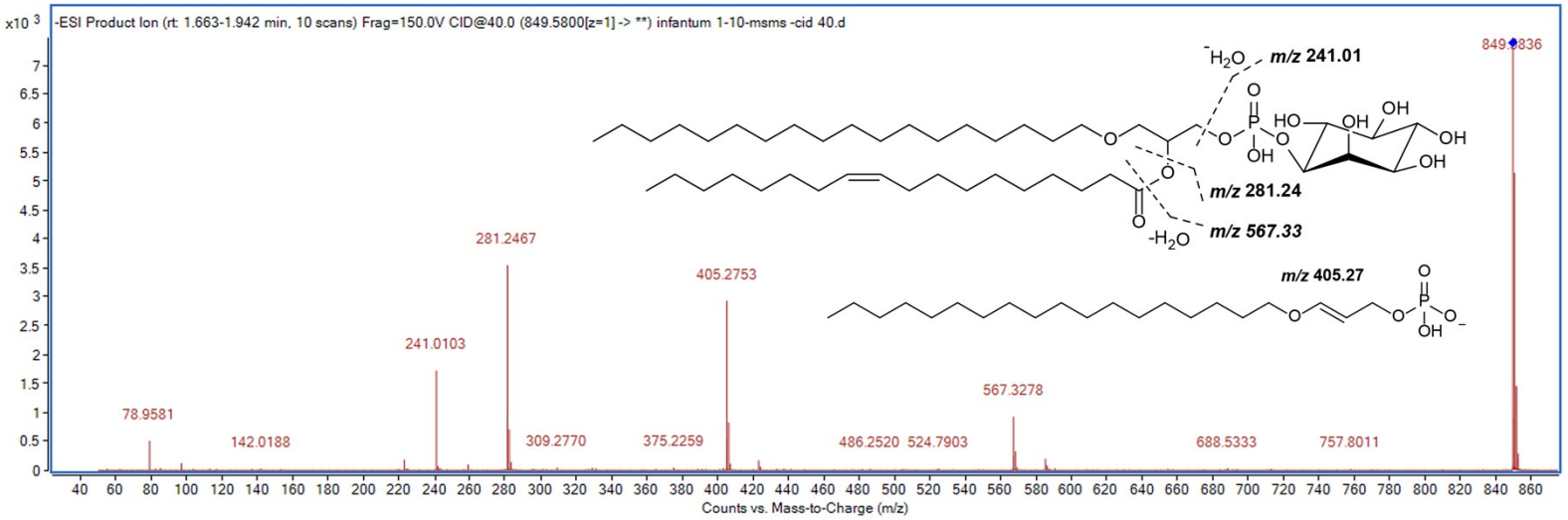

Figure S11 - ***Fragmentation pattern of 1-alkyl,2-acylglycerophosphoinositols (PIO) class.*** *By analysis of fragmentation pattern is possible elucidate the exact fatty acid chain length and the amount of instauration. However, the exact position of double bond remains unknown.*

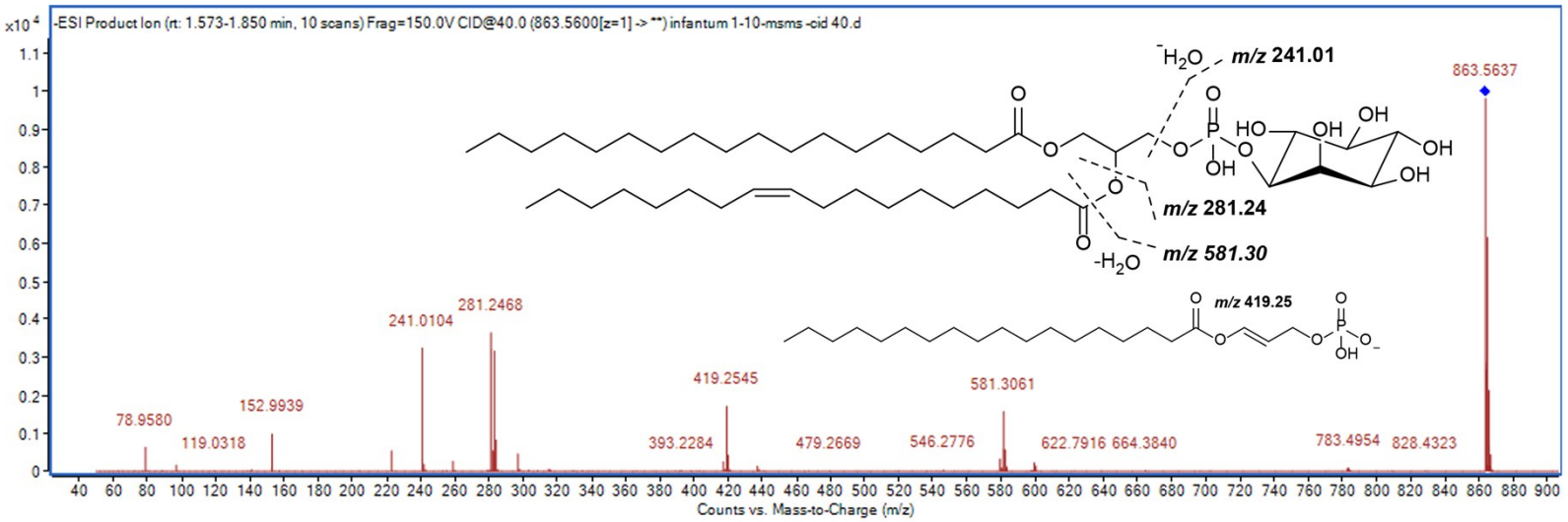

Figure S12 - ***Fragmentation pattern of diacylglycerophosphoinositols (PI) class.*** *By analysis of fragmentation pattern is possible elucidate the exact fatty acid chain length and the amount of instauration. However, the exact position of double bond remains unknown.*

Table S5 - **Identified *glycerophosphoethanolamine and glycerophosphocholine (PE and PC) pointed out by statistical analysis.*** *Lipids were identified by their exact mass (3 ppm) and fragmentation pattern.*

m/z	Lipid	Molecular Formula	Normalized abundance Mean ± SD				Lipid Class
			L. infantum				
			Macrophage	Promastigote	1:5	1:10	
716.5241	PE(16:0/18:1), PE(18:0/16:1)	$C_{39}H_{76}NO_8P$	n.d.	n.d.	2.4 ± 0.8	3.1 ± 0.9	Glycerophosphoethanolamines
764.5255	PE(18:1/20:4) PE(20:4/18:1)	$C_{43}H_{76}NO_8P$	0.08 ± 0.04	n.d.	1.1 ± 0.4	1.2 ± 0.9	Glycerophosphoethanolamines
766.5396	PE(18:0/20:4) PE(20:4/18:0)	$C_{43}H_{78}NO_8P$	n.d.	n.d.	1.2 ± 03	1.6 ± 0.4	Glycerophosphoethanolamines
818.5922[b]	PC(18:1/16:0), PC(16:0/18:1)	$C_{42}H_{82}NO_8P$	1.8 ± 1	0.1 0.1	3.4 ± 1.2	4.5 ± 1.0	Glycerophosphoecholine

764.5449[b]	PC(14:0/16:0) PC(16:0/14:0)	$C_{38}H_{76}NO_8P$	0.5 ± 0.4	n.d.	2.1 ± 0.6	2.8 ± 1.5	Glycerophosphoecholine
			L. amazonensis				
716.5241	PE(16:0/18:1), PE(18:0/16:1)	$C_{39}H_{76}NO_8P$	n.d.	n.d.	1 ± 0.9	1.5 ± 0.4	Glycerophosphoethanolamines
764.5240	PE(18:1/20:4) PE(20:4/18:1)	$C_{43}H_{76}NO_8P$	0.08 ± 0.04	n.d.	0.3 ± 0.5	0.5 ± 0.5	Glycerophosphoethanolamines
766.5395	PE(18:0/20:4) PE(20:4/18:0)	$C_{43}H_{78}NO_8P$	n.d.	n.d.	0.4 ± 0.4	0.4 ± 0.3	Glycerophosphoethanolamines
818.5922[b]	PC(18:1/16:0), PC(16:0/18:1)	$C_{42}H_{82}NO_8P$	1.8 ± 1	0.1 0.02	2.1 ± 1.7	2.8 ± 0.6	Glycerophosphoecholine
764.5449*[b]	PC(14:0/16:0) PC(16:0/14:0)	$C_{38}H_{76}NO_8P$	0.5 ± 0.4	n.d.	1.35 ± 0.7	1.7 ± 0.5	Glycerophosphoecholine

[a] *only identified by their exact mass.* [b] *All PC were detected as* $[M+CH_3COO]^-$ n.d. not detected.

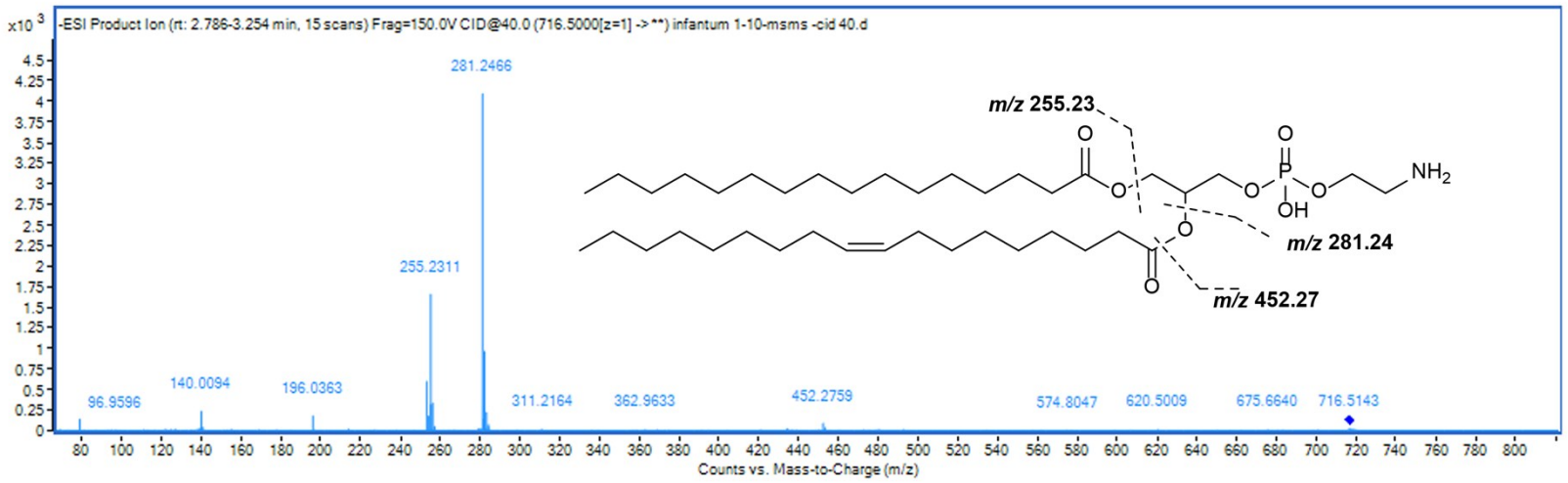

Figure S14 - ***Fragmentation pattern of glycerophosphoethanolamines (PE) class.*** *By analysis of fragmentation pattern is possible elucidate the exact fatty acid chain length and the amount of instauration. However, the exact position of double bond and ester linkage of fatty acid chain remains unknown.*

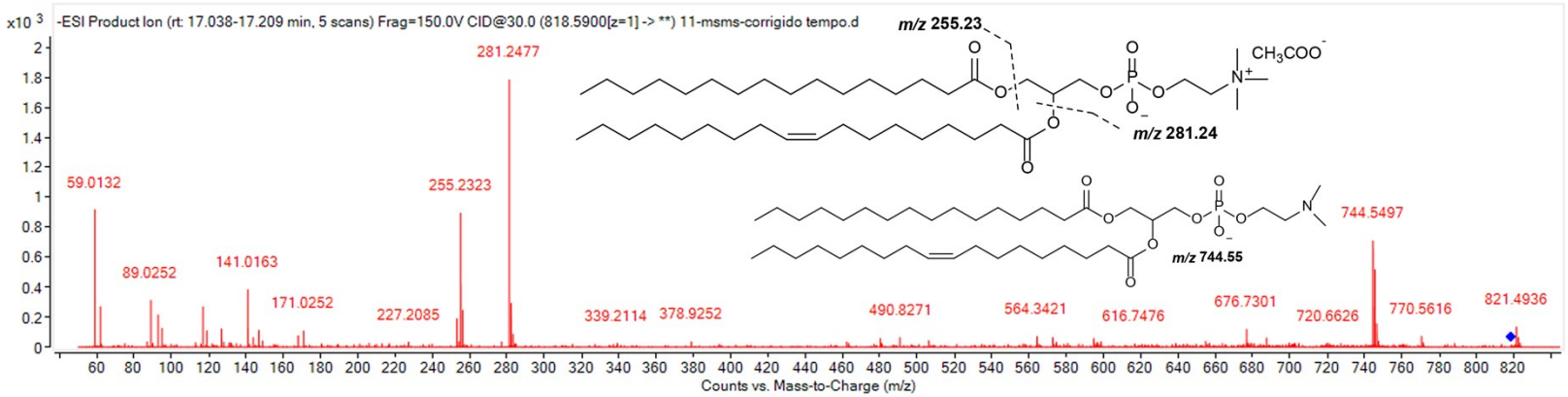

Figure S15 - ***Fragmentation pattern of glycerophosphocholine(PC) class.*** *All PC were detected as $[M+CH_3COO]^-$. By analysis of fragmentation pattern is possible elucidate the exact fatty acid chain length and the amount of instauration. However, the exact position of double bond and ester linkage of fatty acid chain remains unknown.*

Table S6 – Variable p-values and FDR values from Wilcoxon rank-sum test obtained for promastigote species comparison (L. amazonensis vs. L. infantum).

Variable	p-value	FDR	Variable	p-value	FDR	Variable	p-value	FDR
275.20185/3.12	0.0021645	0.033178	768.5551/10.48	0.0036645	0.037195	606.37755/3.36	0.018101	0.098424
277.2177/3.85	0.0021645	0.033178	774.5292/8.98	0.0036645	0.037195	821.4333/6.28	0.018101	0.098424
303.2331/4.96	0.0021645	0.033178	792.47985/6.32	0.0036645	0.037195	521.5301/33.16	0.024992	0.11624
305.2489/5.62	0.0021645	0.033178	798.56525/11.52	0.0036645	0.037195	864.5759/13.95	0.024992	0.11624
327.23315/4.9	0.0021645	0.033178	816.5761/10.57	0.0036645	0.037195	614.3367/6.21	0.025974	0.11624
329.2488/5.75	0.0021645	0.033178	600.5129/20.12	0.004329	0.041268	637.3065/9.32	0.025974	0.11624
331.2645/6.22	0.0021645	0.033178	627.5317/20.09	0.004329	0.041268	740.5234/9.09	0.025974	0.11624
359.2206/3.85	0.0021645	0.033178	335.1759/3.84	0.0043369	0.041268	741.3173/1.15	0.025974	0.11624
437.1325/1.56	0.0021645	0.033178	752.52335/9.29	0.0043369	0.041268	800.5816/14.03	0.026279	0.11624
532.2681/1.19	0.0021645	0.033178	742.5389/10.29	0.0047718	0.044031	938.82825/34.41	0.026279	0.11624
657.523/30.1	0.0021645	0.033178	796.5347/5.66	0.0047718	0.044031	978.7897/29.96	0.027768	0.11624

728.5234/9.47	0.0021645	0.033178	239.12915/1.18	0.0049981	0.044114	249.15005/1.23	0.028441	0.11624
754.5027/7.93	0.0021645	0.033178	411.2509/5.76	0.0049981	0.044114	301.2171/3.9	0.028441	0.11624
758.5342/9.92	0.0021645	0.033178	934.78675/33.4	0.0049981	0.044114	443.2142/6.29	0.028441	0.11624
772.5131/7.98	0.0021645	0.033178	692.4478/20.11	0.0061506	0.05351	530.2521/1.15	0.028441	0.11624
782.5342/9.68	0.0021645	0.033178	802.56/11.15	0.0077958	0.063007	551.03215/0.83	0.028441	0.11624
784.5507/10.83	0.0021645	0.033178	574.497/17.12	0.008127	0.063007	602.345/2.26	0.028441	0.11624
800.5448/9.78	0.0021645	0.033178	602.5282/22.24	0.008127	0.063007	641.5473/22.07	0.028441	0.11624
821.5549/8.56	0.0021645	0.033178	368.242/7.11	0.008658	0.063007	655.5075/28.89	0.028441	0.11624
847.5706/9.38	0.0021645	0.033178	698.2868/4.89	0.008658	0.063007	655.56285/25.97	0.028441	0.11624
849.5868/10.61	0.0021645	0.033178	387.2517/5.62	0.0096217	0.063007	659.2315/5.53	0.028441	0.11624
906.7556/32.9	0.0021645	0.033178	562.2792/1.4	0.0096217	0.063007	681.565/30.1	0.028441	0.11624
910.7435/33.4	0.0021645	0.033178	614.5283/22.07	0.0096217	0.063007	714.5444/18.32	0.028441	0.11624
912.759/33.65	0.0021645	0.033178	630.5596/27.88	0.0096217	0.063007	742.57545/24.59	0.028441	0.11624
936.8025/33.66	0.0021645	0.033178	733.5237/4.9	0.0096217	0.063007	744.55505/18.93	0.028441	0.11624
964.7743/29.52	0.0021645	0.033178	770.4978/7.29	0.0096217	0.063007	784.4906/10.24	0.028441	0.11624
980.8054/30.67	0.0021645	0.033178	786.5291/10.05	0.0096217	0.063007	790.4669/5.87	0.028441	0.11624
295.2282/1.67	0.0027784	0.033178	807.5031/6.53	0.0096217	0.063007	806.76135/33.65	0.028441	0.11624
501.1974/4.9	0.0027784	0.033178	833.5542/8.22	0.0096217	0.063007	814.5601/12.96	0.028441	0.11624
503.21335/5.76	0.0027784	0.033178	860.7277/33.15	0.0096217	0.063007	817.50685/7.06	0.028441	0.11624
628.54405/25.86	0.0027784	0.033178	924.7594/33.55	0.0096217	0.063007	819.52375/7.77	0.028441	0.11624
721.59035/7.53	0.0027784	0.033178	931.5901/10.55	0.0096217	0.063007	831.53995/7.48	0.028441	0.11624
736.49215/10.11	0.0027784	0.033178	936.74225/27.61	0.0096217	0.063007	838.52225/8.72	0.028441	0.11624
742.4653/6.17	0.0027784	0.033178	948.5569/10.81	0.0096217	0.063007	848.54495/9.95	0.028441	0.11624
744.4819/6.7	0.0027784	0.033178	982.7849/29.68	0.0096217	0.063007	878.8149/33.48	0.028441	0.11624
746.4974/7.44	0.0027784	0.033178	1006.9364/34.87	0.0096217	0.063007	885.5633/20.09	0.028441	0.11624
764.4858/6.61	0.0027784	0.033178	1010.8151/30.68	0.0096217	0.063007	926.77525/33.8	0.028441	0.11624
768.5183/8.46	0.0027784	0.033178	1074.8084/30.62	0.0096217	0.063007	932.8666/34.58	0.028441	0.11624
801.4906/5.75	0.0027784	0.033178	447.1709/1.86	0.010065	0.064519	950.81815/33.81	0.028441	0.11624

808.5494/9.81	0.0027784	0.033178	765.4273/7.7	0.010065	0.064519	391.20785/3.61	0.02948	0.11969
820.5132/7.69	0.0027784	0.033178	911.54265/16.96	0.011519	0.073073	363.25175/5.92	0.030348	0.1224
825.60145/33.15	0.0027784	0.033178	423.2263/7.11	0.012436	0.078077	141.0171/0.83	0.034087	0.1348
866.5531/10.81	0.0027784	0.033178	540.2715/6.27	0.015152	0.089585	693.27805/7.11	0.034087	0.1348
868.56925/12.32	0.0027784	0.033178	681.29605/4.88	0.015152	0.089585	950.75805/28.49	0.034087	0.1348
948.7991/33.56	0.0027784	0.033178	794.4156/6.27	0.015152	0.089585	601.5159/17.11	0.037941	0.14812
952.7739/29.48	0.0027784	0.033178	798.5292/8.37	0.015152	0.089585	842.592/15.68	0.037941	0.14812
963.52925/4.89	0.0027784	0.033178	835.5702/9.27	0.015152	0.089585	855.45025/5.53	0.040253	0.15614
966.54755/10.3	0.0027784	0.033178	884.7278/33.2	0.015152	0.089585	578.3462/2.37	0.041126	0.15653
980.7681/28.77	0.0027784	0.033178	937.6034/19.67	0.016059	0.092521	586.4969/17.06	0.041126	0.15653
1008.79995/30.11	0.0027784	0.033178	479.14325/1.07	0.016711	0.092521	626.3463/2.34	0.041126	0.15653
1008.79995/30.11	0.0027784	0.033178	660.3491/2.38	0.016711	0.092521	505.2288/7.11	0.043826	0.16275
357.2039/3.12	0.0036645	0.037195	661.6867/35.2	0.016711	0.092521	794.4977/6.84	0.043826	0.16275
409.2361/4.91	0.0036645	0.037195	662.34185/9.84	0.016711	0.092521	844.5138/7.51	0.043826	0.16275
502.2571/1.17	0.0036645	0.037195	700.5288/16.69	0.016711	0.092521	850.4779/7.11	0.043826	0.16275
714.50705/12.57	0.0036645	0.037195	770.57035/11.94	0.016711	0.092521	365.23635/6.03	0.044951	0.16591

Table S7 - Variable p-values and FDR values from Kruskal Wallis test obtained for macrophage and macrophage infections comparison (L. amazonensis).

Variable	p-value	FDR	Variable	p-value	FDR	Variable	p-value	FDR
279.2334/4.92	0.00074215	0.074644	740.5234/13.37	0.011708	0.14082	793.6569/31.53	0.029766	0.1948
617.0628/2.4	0.00095832	0.074644	717.05645/3.55	0.012119	0.14082	887.6633/5.53	0.029805	0.1948
716.4876/7.19	0.00095832	0.074644	761.53385/9.16	0.012119	0.14082	423.2606/5.53	0.030595	0.19489
817.5028/6.55	0.0010243	0.074644	800.5235/8.77	0.012119	0.14082	779.4644/5.53	0.030595	0.19489
590.4919/13.35	0.0010828	0.074644	555.303/7.1	0.012255	0.14082	495.2251/5.53	0.030997	0.19489
307.2645/6.42	0.0011156	0.074644	729.4794/7.11	0.012255	0.14082	728.5238/9.44	0.031078	0.19489
327.2332/4.89	0.0012026	0.074644	593.18855/1.2	0.012255	0.14082	447.1705/1.86	0.031387	0.19489
769.5025/6.54	0.0012315	0.074644	617.51075/13.35	0.012255	0.14082	407.18735/1.21	0.031962	0.19489
849.5866/10.59	0.0012315	0.074644	391.20785/3.75	0.012481	0.14082	473.2827/5.49	0.032132	0.19489

599.5006/15.36	0.0012643	0.074644	393.2675/7.09	0.012504	0.14082	283.2646/7.11	0.032216	0.19489
815.4867/6.04	0.0014128	0.074644	405.1603/1.88	0.013226	0.14335	313.1918/5.53	0.032216	0.19489
898.5793/14.82	0.0014128	0.074644	675.542/6.03	0.013507	0.14335	932.86525/33.98	0.03279	0.19674
992.8051/30.64	0.0014128	0.074644	277.2177/3.84	0.013512	0.14335	311.2221/1.2	0.033233	0.19747
618.523/17.34	0.0015296	0.074644	534.2834/1.22	0.013512	0.14335	871.6905/25.29	0.033628	0.19747
964.77355/29.53	0.0015296	0.074644	463.1116/0.92	0.014108	0.14753	337.2054/1.22	0.033722	0.19747
806.55535/7.16	0.0022284	0.080688	776.5604/20.04	0.01461	0.15063	615.45815/5.53	0.034617	0.20089
642.5958/31.4	0.0022326	0.080688	605.2593/7.11	0.015108	0.15073	918.8494/34.42	0.034854	0.20089
666.6403/31.41	0.0022644	0.080688	355.1683/1.21	0.015263	0.15073	1099.6665/8.05	0.035324	0.20201
867.5182/6.99	0.0023957	0.080688	281.2491/5.91	0.015393	0.15073	610.3498/15.4	0.036495	0.20207
329.2488/5.48	0.0024085	0.080688	798.5288/8.41	0.015444	0.15073	774.5087/8.43	0.036535	0.20207
586.49715/17.56	0.002517	0.080688	873.70625/29.84	0.016246	0.15346	611.2765/7.11	0.036643	0.20207
305.2488/5.62	0.0025478	0.080688	205.1601/2.28	0.01639	0.15346	501.242/5.53	0.036715	0.20207
331.26435/6.22	0.0026201	0.080688	689.59115/27.71	0.0171	0.15346	533.4553/5.53	0.036715	0.20207
297.1532/1.11	0.0028528	0.080688	752.5605/20.56	0.0171	0.15346	768.496/30.5	0.037984	0.2075
311.1691/1.17	0.0028528	0.080688	886.8129/33.65	0.0171	0.15346	365.2678/7.11	0.038434	0.2084
863.5657/9.47	0.002866	0.080688	437.1326/1.57	0.017213	0.15346	529.3053/1.17	0.038873	0.20923
908.77255/33.2	0.003098	0.082696	419.2392/5.56	0.017394	0.15346	337.2366/5.53	0.03971	0.20934
706.5029/8.41	0.0031632	0.082696	739.50385/5.54	0.0174	0.15346	363.2518/5.91	0.03971	0.20934
572.4818/15.35	0.0033679	0.084268	666.59655/30.75	0.019118	0.1666	774.54455/18.8	0.04036	0.20934
600.5129/20.1	0.0034536	0.084268	431.13965/7.94	0.019862	0.17105	773.4476/5.53	0.040497	0.20934
574.497/17.08	0.004631	0.1042	325.1848/1.22	0.020616	0.17547	433.1553/1.89	0.040602	0.20934
784.55/10.75	0.0048884	0.1042	529.2734/7.11	0.021203	0.17767	829.5221/8.31	0.040877	0.20934
865.5029/6.59	0.004943	0.1042	465.3076/1.23	0.021641	0.17767	368.2421/7.1	0.040896	0.20934
766.50275/7.78	0.0050751	0.1042	340.2038/1.56	0.022298	0.17767	687.5446/11.25	0.044047	0.21386
913.5817/9.95	0.0051052	0.1042	911.5658/8.99	0.022413	0.17767	379.2466/2.45	0.044689	0.21386
561.2139/7.1	0.0051246	0.1042	481.1999/1.25	0.02256	0.17767	452.2785/2.26	0.044689	0.21386
493.4988/32.4	0.0053032	0.1048	771.51785/7.14	0.022863	0.17767	468.17/1.19	0.044689	0.21386

353.20115/1.15	0.0055891	0.1048	870.3568/7.49	0.022995	0.17767	612.2944/1.51	0.044689	0.21386
544.4501/11.75	0.0056892	0.1048	583.24515/5.53	0.023387	0.17767	700.4926/7.82	0.044689	0.21386
794.5343/9.44	0.0059028	0.1048	861.4674/5.53	0.023387	0.17767	748.4953/7.67	0.044689	0.21386
816.5186/8.09	0.0060114	0.1048	447.2706/7.11	0.023556	0.17767	860.5337/7.27	0.044689	0.21386
627.5319/20.12	0.0060668	0.1048	694.672/32.28	0.023556	0.17767	772.5287/15.27	0.044921	0.21386
797.53195/7.63	0.0061933	0.1048	763.59705/12.28	0.023787	0.17767	788.5448/9.98	0.04522	0.21386
887.566/9.59	0.0062993	0.1048	907.5344/7.39	0.024453	0.17957	550.3287/30.5	0.045256	0.21386
668.6123/31.52	0.0067101	0.10915	792.5186/8.13	0.024531	0.17957	805.6603/5.53	0.045284	0.21386
393.17175/1.17	0.0071974	0.11328	409.3106/11.94	0.025392	0.18046	483.2636/1.22	0.047112	0.21682
994.8213/31.14	0.0072736	0.11328	601.5159/17.07	0.025392	0.18046	872.56385/13.55	0.047628	0.21682
476.2782/1.87	0.007987	0.11932	614.5646/30.15	0.025392	0.18046	352.2858/1.22	0.047688	0.21682
532.2686/1.23	0.007987	0.11932	670.6273/32.28	0.027079	0.18883	679.50765/29.11	0.047688	0.21682
587.23225/7.11	0.0081922	0.11993	512.2452/7.11	0.027087	0.18883	789.613/14.58	0.047688	0.21682
842.592/15.67	0.0095921	0.13768	413.2223/5.53	0.028659	0.1948	816.57635/14.79	0.047688	0.21682
834.5658/11.49	0.0098719	0.13897	765.48445/19.82	0.029126	0.1948	885.5503/8.55	0.048291	0.21805
333.1113/0.89	0.011182	0.14082	423.2262/7.11	0.029341	0.1948	707.59465/28.3	0.048716	0.21805
511.147/5.53	0.011368	0.14082	513.2447/7.11	0.029429	0.1948	697.46135/5.53	0.048852	0.21805
786.56555/12.35	0.011428	0.14082	776.5245/8.83	0.029429	0.1948			

Table S8 - Variable p-values and FDR values from Kruskal Wallis test obtained for macrophage and macrophage infections comparison (L. infantum).

Variable	p-value	FDR	Variable	p-value	FDR	Variable	p-value	FDR
552.6002/8.81	0.0016793	0.3885	653.4625/5.72	0.019279	0.3885	764.1283/7.68	0.035185	0.41103
302.3052/1.78	0.0021797	0.3885	675.4762/6.5	0.019279	0.3885	779.5208/13.2	0.035185	0.41103
568.33955/3.89	0.0021797	0.3885	727.2745/7.5	0.019279	0.3885	824.4293/9.78	0.035185	0.41103
633.5966/8.48	0.0021797	0.3885	763.27115/1.47	0.019279	0.3885	121.02875/9.9	0.035253	0.41103
391.3356/12.15	0.0022162	0.3885	799.204/7.5	0.019279	0.3885	692.5221/12.31	0.035992	0.41103
526.292/2.6	0.0030756	0.3885	851.7534/1.47	0.019279	0.3885	901.3209/1.47	0.036033	0.41103
647.5763/10	0.0032861	0.3885	904.84325/10.26	0.019279	0.3885	768.5545/12.15	0.036087	0.41103

842.569/11.87	0.0032861	0.3885	926.30805/1.47	0.019279	0.3885	945.54665/12.04	0.036087	0.41103
798.5669/12.18	0.0034254	0.3885	1044.55245/7.5	0.019279	0.3885	988.4924/7.49	0.037059	0.41103
644.3469/5.46	0.0036512	0.3885	1098.18795/1.47	0.019279	0.3885	261.13085/0.33	0.039109	0.41103
491.45585/8.94	0.0037639	0.3885	352.32175/5.99	0.019459	0.3885	571.43015/10.04	0.039127	0.41103
550.3289/3.78	0.003949	0.3885	844.58565/12.15	0.019659	0.3885	602.4472/2.46	0.039448	0.41103
664.1346/6.11	0.0047794	0.3885	882.2977/7.49	0.01969	0.3885	654.5282/10.05	0.039448	0.41103
512.46465/12.16	0.0050908	0.3885	522.3546/5.81	0.019982	0.3885	399.3939/5.4	0.040414	0.41103
318.2993/0.93	0.0059742	0.3885	664.4911/11.66	0.019982	0.3885	698.4993/11.48	0.040607	0.41103
163.14815/7.73	0.0065737	0.3885	375.321/4.83	0.020358	0.38962	433.15355/6.21	0.040723	0.41103
471.3794/4.72	0.0066424	0.3885	256.26355/6.27	0.020612	0.38962	651.181/9.99	0.042133	0.41103
1025.2646/1.47	0.0067763	0.3885	623.4504/9.8	0.021706	0.40059	330.3367/3.27	0.04343	0.41103
678.03385/12.02	0.0070283	0.3885	394.0965/1.46	0.021781	0.40059	356.3527/3.73	0.04343	0.41103
792.41105/9.77	0.0084846	0.3885	504.34325/4.55	0.022263	0.40282	510.4869/11.88	0.04343	0.41103
273.25345/0.82	0.0085527	0.3885	1087.68655/11.16	0.022494	0.40282	528.3443/4.05	0.04343	0.41103
502.3162/2.86	0.0088391	0.3885	570.354/1.48	0.023321	0.40952	610.5758/12.65	0.04343	0.41103
547.5185/10.69	0.0088475	0.3885	442.7789/1.47	0.023489	0.40952	638.1616/7.49	0.04343	0.41103
688.7485/1.46	0.0089823	0.3885	478.2109/6.22	0.023822	0.40952	670.5695/10.62	0.04343	0.41103
762.20295/1.46	0.0089823	0.3885	411.3829/9.97	0.024097	0.40952	742.5408/12.04	0.04343	0.41103
376.0833/1.46	0.0097687	0.3885	244.1908/7.72	0.024373	0.40952	782.203/7.48	0.04343	0.41103
354.3362/7.07	0.010036	0.3885	670.468/10.61	0.025295	0.41103	794.53555/11.23	0.04343	0.41103
274.27365/0.87	0.010076	0.3885	593.3614/7.05	0.026237	0.41103	868.5824/11.91	0.04343	0.41103
652.3534/12.33	0.010076	0.3885	538.51785/12.34	0.027091	0.41103	919.3873/9.79	0.04343	0.41103
378.2095/7.84	0.010904	0.3885	891.3314/1.48	0.029557	0.41103	1014.9459/7.47	0.04343	0.41103
395.3303/9.02	0.010904	0.3885	494.20645/3.11	0.02994	0.41103	494.2532/1.46	0.04378	0.41103
454.36845/12.07	0.010904	0.3885	284.2947/8.24	0.030169	0.41103	520.50745/11.51	0.04378	0.41103
480.3077/3.71	0.010904	0.3885	246.0214/1.46	0.030486	0.41103	913.3678/1.45	0.04378	0.41103
906.33515/1.45	0.010904	0.3885	192.15885/0.33	0.030902	0.41103	417.1587/4.26	0.044125	0.41103
377.3199/12.07	0.012417	0.3885	468.49805/4.73	0.032118	0.41103	626.3004/6.17	0.044157	0.41103

844.5518/11.4	0.012417	0.3885	377.09095/6.23	0.032156	0.41103	1096.2816/10.75	0.044635	0.41103
503.15625/1.48	0.013326	0.3885	391.2842/9.91	0.032156	0.41103	794.5674/12.08	0.045283	0.41103
635.32595/8.74	0.013725	0.3885	554.2425/6.63	0.032156	0.41103	675.6755/10.12	0.045369	0.41103
1005.4809/10.19	0.013932	0.3885	291.033/1.47	0.032569	0.41103	364.2529/9.19	0.045872	0.41103
894.5638/11.54	0.014295	0.3885	379.2045/6.58	0.032882	0.41103	592.4617/11.93	0.04609	0.41103
775.4896/12.2	0.014456	0.3885	639.4475/4.9	0.033176	0.41103	185.097/1.46	0.047441	0.41103
335.177/6.63	0.014839	0.3885	279.1228/1.46	0.033565	0.41103	623.3258/9.84	0.047653	0.41103
336.3254/5.4	0.015787	0.3885	550.2687/3.12	0.033565	0.41103	752.2547/7.5	0.047653	0.41103
731.9897/1.47	0.016307	0.3885	573.4083/4.92	0.033565	0.41103	135.0804/1.45	0.047972	0.41103
549.3124/11.05	0.016325	0.3885	595.4215/4.91	0.033565	0.41103	787.5009/10.77	0.048155	0.41103
552.3439/4.28	0.016676	0.3885	888.5303/11.44	0.033565	0.41103	535.3985/6.62	0.049427	0.41103
721.1826/1.46	0.019097	0.3885	897.3897/1.46	0.033882	0.41103	644.6208/4.7	0.049481	0.41103
312.3621/6.48	0.019279	0.3885	149.0238/9.91	0.034047	0.41103	608.5093/11.04	0.049673	0.41103
399.2508/3.51	0.019279	0.3885	682.5608/11.5	0.034047	0.41103			
415.4199/1.46	0.019279	0.3885	712.4106/7.01	0.03458	0.41103			
432.67485/1.46	0.019279	0.3885	311.2585/12.52	0.035185	0.41103			
448.4733/5.23	0.019279	0.3885	328.12045/0.82	0.035185	0.41103			
509.8473/4.96	0.019279	0.3885	488.44435/10.91	0.035185	0.41103			
548.54435/16.64	0.019279	0.3885	572.65855/7.18	0.035185	0.41103			
569.1494/7.5	0.019279	0.3885	599.50255/12.3	0.035185	0.41103			

1. F. F. Hsu and J. Turk, *J Am Soc Mass Spectr*, 2002, **13**, 558-570.