

Supporting information

High-Performance Thin-Layer Chromatography-Densitometry-Tandem ESI-MS to evaluate phospholipid content in exosomes of cancer cells

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Figure S1.- HPTLC chromatographic development of standards and front of elution (m.d. ≥ 30 mm). Structure of standards (see Materials and Methods): 1, SM; 2, PC; 3, PS; 4, PI; 5, PE; 6, PA; 7, HexCer; 8, Chol

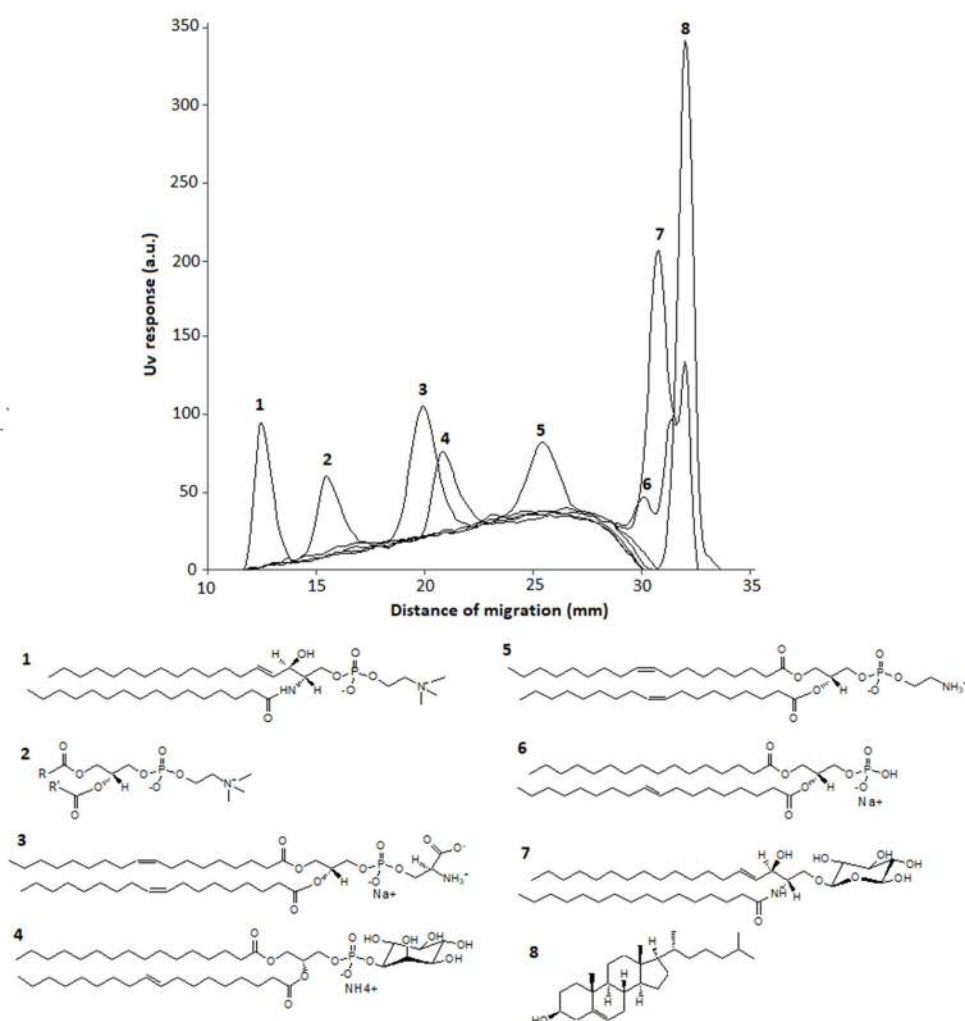


Figure S2.- Intra- and inter-plate HPTLC repeatability results for the standards (A) and PL classes of the B16-F1-EXOs (B), B16-F10-EXOs (C), and NIH-3T3-EXOs (D) including the RSD%, \pm Area (for a 95% confidence interval, CI).

A	Intra-plate Repeatability						Inter-plate Repeatability		
	Plate 1		Plate 2		Plate 3		Average area	%RSD	CI (95%)
	Average peak area	%RSD	Middle pic Area	%RSD	Average peak area	%RSD			
SM	1071.3	2.5	1067.5	2.5	1111.0	5.6	1083.2	3.9	289.1
PC	735.4	0.4	745.8	1.0	803.3	4.3	761.5	4.8	251.4
PS	1406.2	2.9	1297.2	1.0	1208.6	3.2	1304.0	6.9	626.3
PE	1039.2	0.1	992.4	7.9	835.6	2.4	955.7	10.5	697.2

B	Inter-plate Repeatability				
	Peak area Plate 1	Peak area Plate 2	Peak area Plate 3	Average area	%RSD
SM	1173.0	1088.9	828.8	1030.2	17.4
PC	1931.1	1923.6	1723.1	1859.3	6.3
PS	15.9	50.0	59.8	41.9	55.0
PE	3857.7	3686.4	3884.3	3809.5	2.8

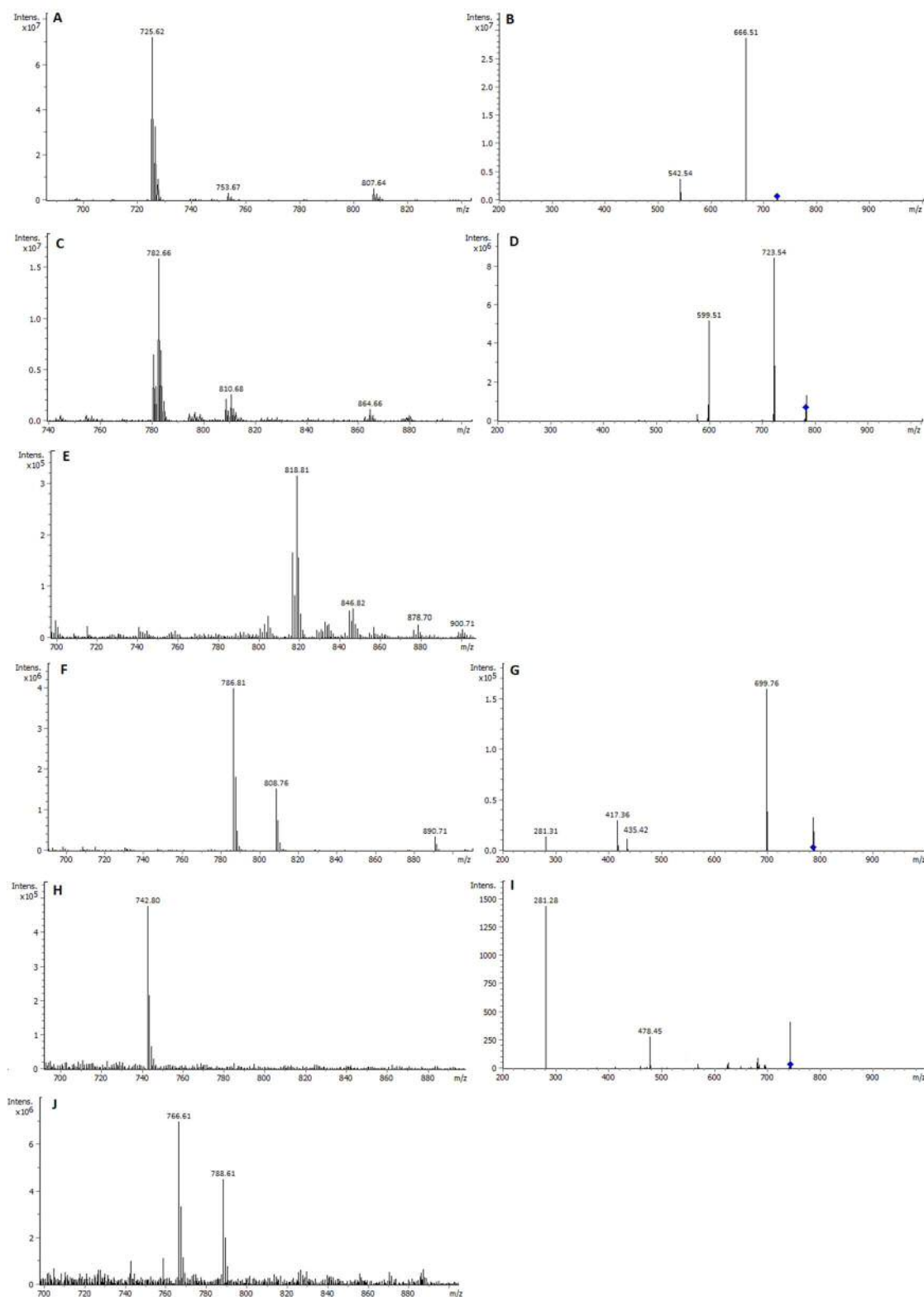
C	Inter-plate Repeatability				
	Peak area Plate 1	Peak area Plate 2	Peak area Plate 3	Average area	%RSD
SM	1736.6	1716.1	1485.9	1646.2	8.5
PC	2382.5	2484.9	2367.7	2411.7	2.6
PS	98.3	224.4	289.1	203.9	47.6
PE	7112.8	6940.1	7151.8	7068.2	1.6

D	Intra-plate Repeatability	
	Average peak area Plate 1	%RSD
SM	1089.1	18.0
PC	1205.7	15.8
PS	206.5	11.8
PE	382.9	8.0

Figure S3.- HPTLC-ESI-MS spectra of standards

S3-1.- SM, PC, PS and PE standards

- A) HPTLC-ESI⁺-MS spectrum of SM standard
B) HPTLC-ESI⁺-MS/MS spectrum of ion precursor at m/z 725.6 (SM standard)
C) HPTLC-ESI⁺-MS spectrum of PC standard
D) HPTLC-ESI⁺-MS/MS spectrum of ion precursor at m/z 782.6 (PC standard)
E) HPTLC-ESI-MS spectrum of PC standard
F) HPTLC-ESI-MS spectrum of PS standard
G) HPTLC-ESI-MS/MS spectrum of ion precursor at m/z 786.8 (PS standard)
H) HPTLC-ESI-MS spectrum of PE standard
I) HPTLC-ESI-MS/MS spectrum of ion precursor at m/z 742.8 (PE standard)
J) HPTLC-ESI⁺-MS spectrum of PE standard



S3-2.- phosphatidylinositol (PI), phosphatidic acid (PA) and glucosyl ceramide (GlcCer) standards

A) HPTLC-ESI-MS spectrum of PI standard

B) HPTLC-ESI-MS/MS spectrum of ion precursor at m/z 835.8 (PI standard)

C) HPTLC-ESI-MS spectrum of PA standard

D) HPTLC-ESI-MS/MS spectrum of ion precursor at m/z 673.9 (PA standard)

E) HPTLC-ESI⁺-MS spectrum of GlcCer standard

F) HPTLC-ESI⁺-MS/MS spectrum of ion precursor at m/z 722.6 (GlcCer standard)

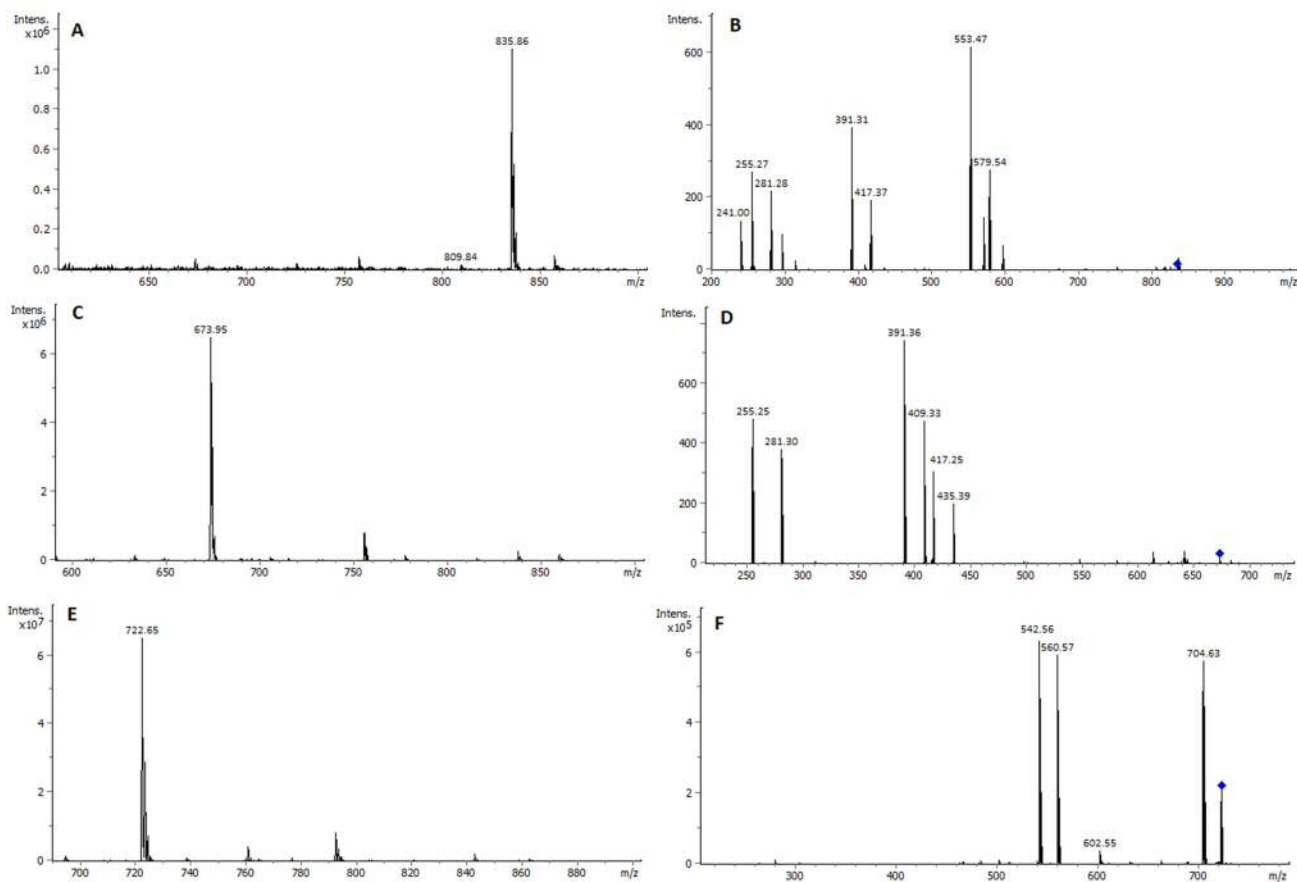


Figure S4.-HPTLC-ESI-MS spectra of SM, PC, PS and PI from NIH-3T3-EXOs, B16-F1-EXOs and B16-F10-EXOs.

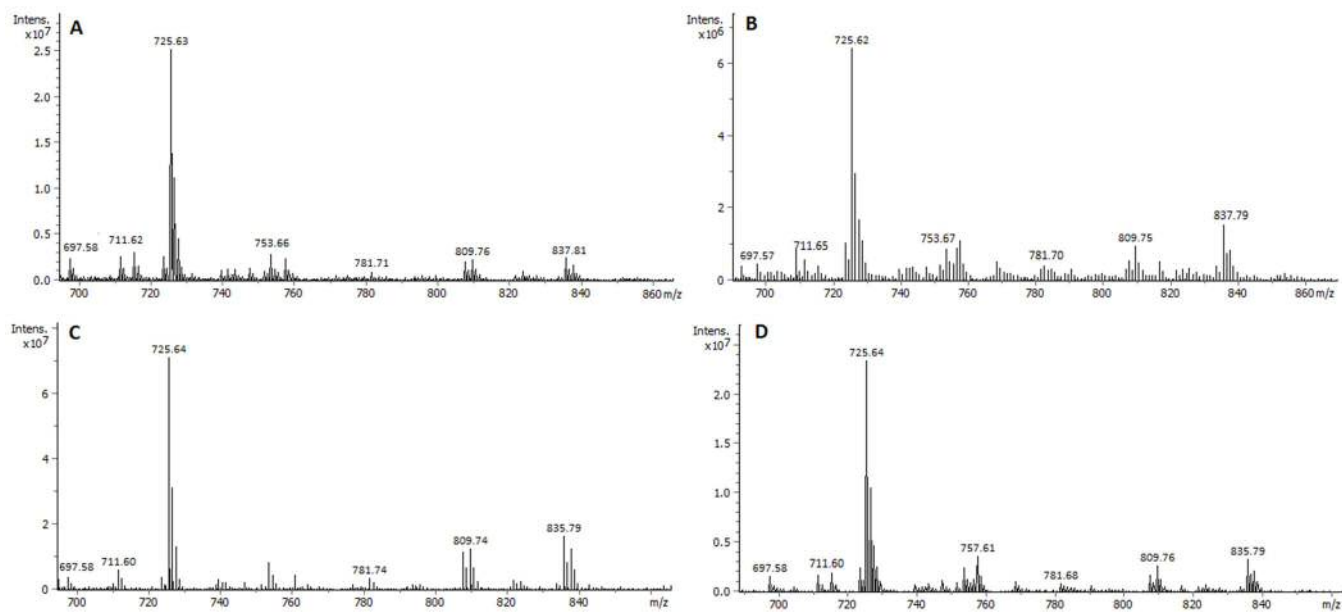
S4-1.- Example of repeatability of SM from different exosome batches (1 and 2)

A) HPTLC-ESI⁺-MS spectrum of B16-F1-EXOs (batch 1; peak at 14.6 mm-m.d.)

B) HPTLC-ESI⁺-MS spectrum of B16-F1-EXOs (batch 2; peak at 14.6 mm-m.d.)

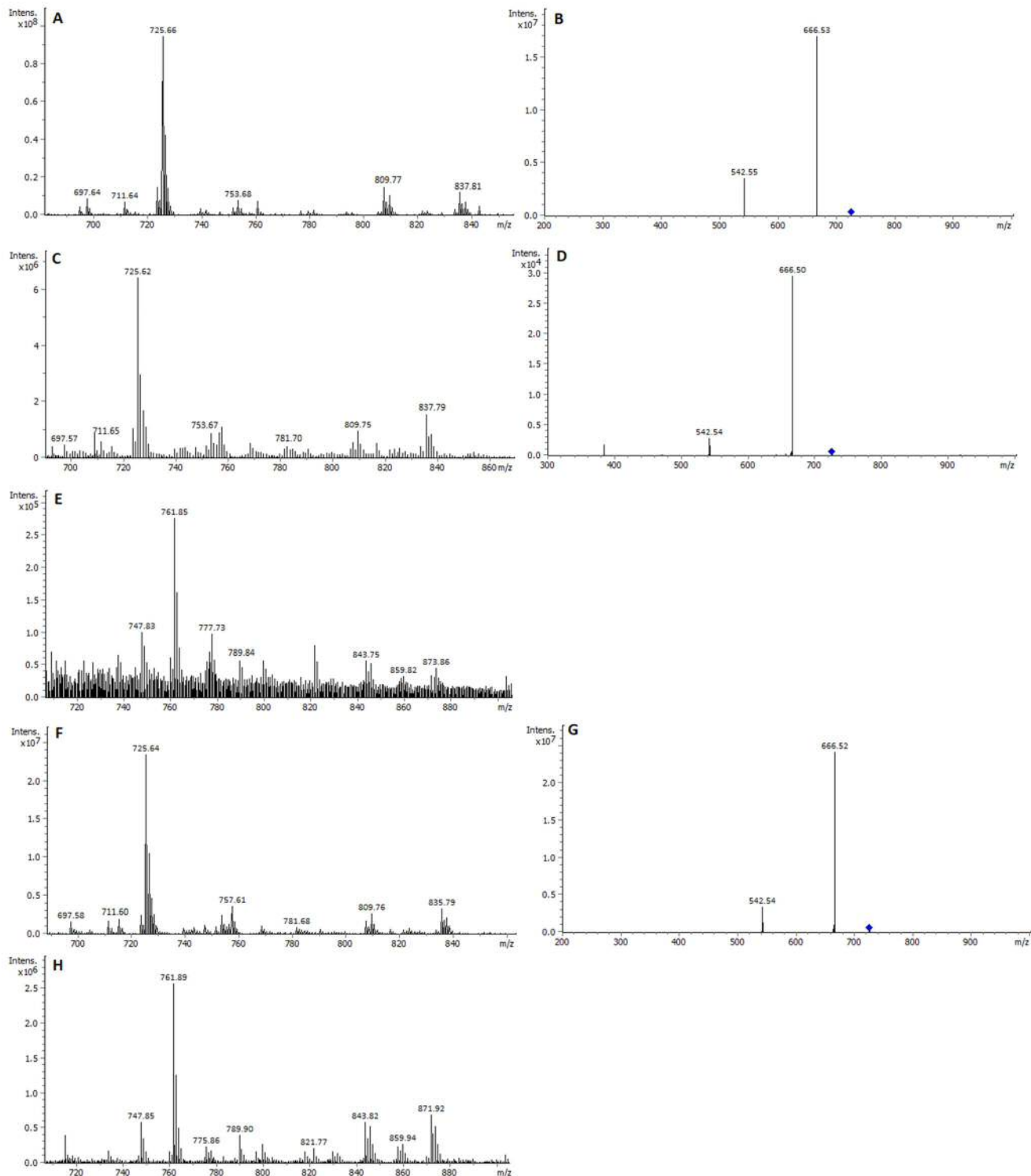
C) HPTLC-ESI⁺-MS spectrum of B16-F10-EXOs (batch 1; peak at 14.5 mm-m.d.)

D) HPTLC-ESI⁺-MS spectrum of B16-F10-EXOs (batch 2; peak at 14.5 mm-m.d.)



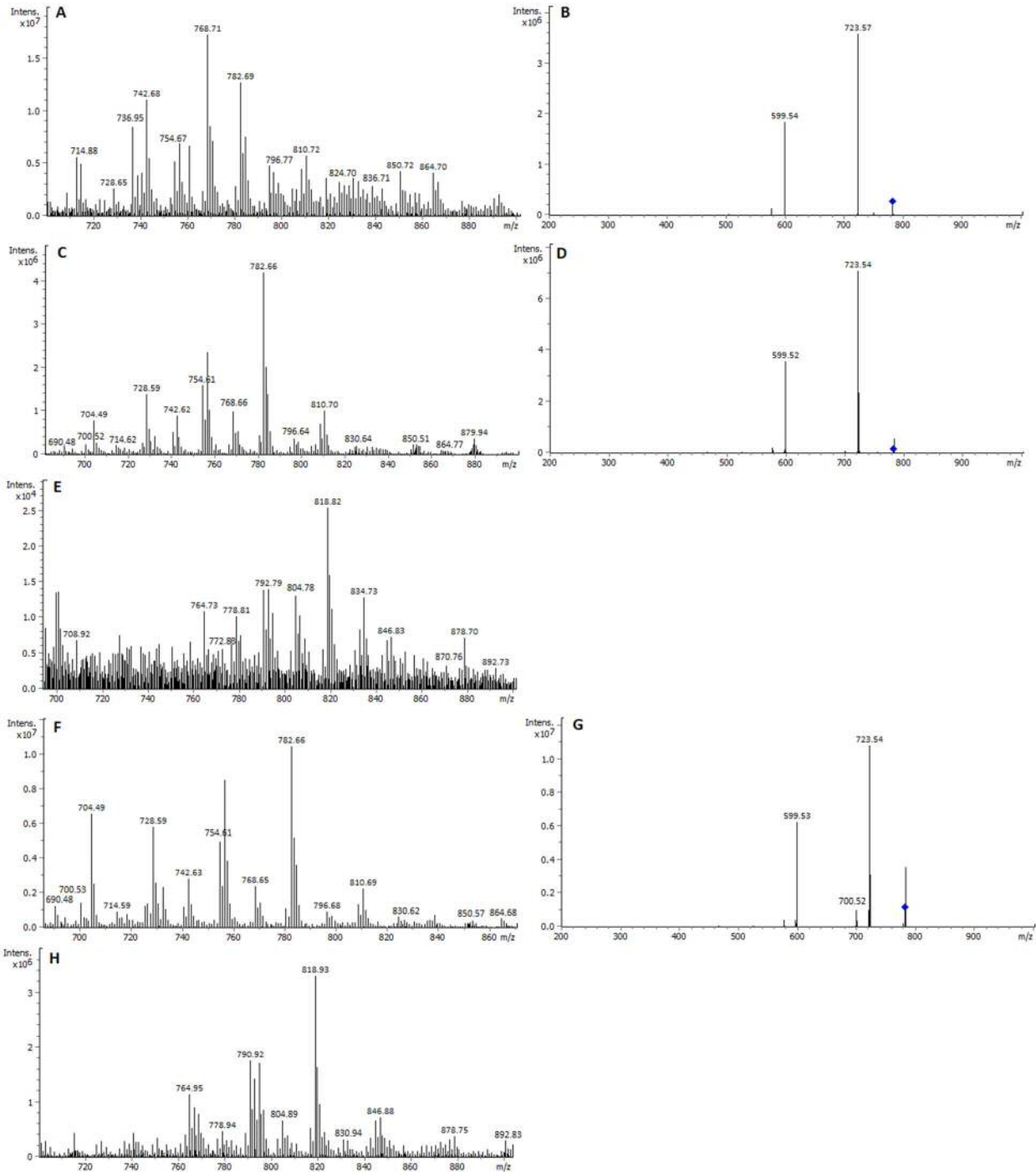
S4-2.- Sphingomyelins (SM)

- A) HPTLC-ESI⁺-MS spectrum of NIH-3T3-EXOs (peak at 14.0 mm-m.d.)
B) HPTLC-ESI⁺-MS/MS spectrum of ion precursor at m/z 725.6 (NIH-3T3-EXOs)
C) HPTLC-ESI⁺-MS spectrum of B16-F1-EXOs (peak at 14.6 mm-m.d.)
D) HPTLC-ESI⁺-MS/MS spectrum of ion precursor at m/z 725.6 (B16-F1-EXOs)
E) HPTLC-ESI-MS spectrum of B16-F1-EXOs (peak at 14.6 mm-m.d.)
F) HPTLC-ESI⁺-MS spectrum of B16-F10-EXOs (peak at 14.5 mm-m.d.)
G) HPTLC-ESI⁺-MS/MS spectrum of ion precursor at m/z 725.6 (B16-F10-EXOs)
H) HPTLC-ESI-MS spectrum of B16-F10-EXOs (peak at 14.5 mm-m.d.)



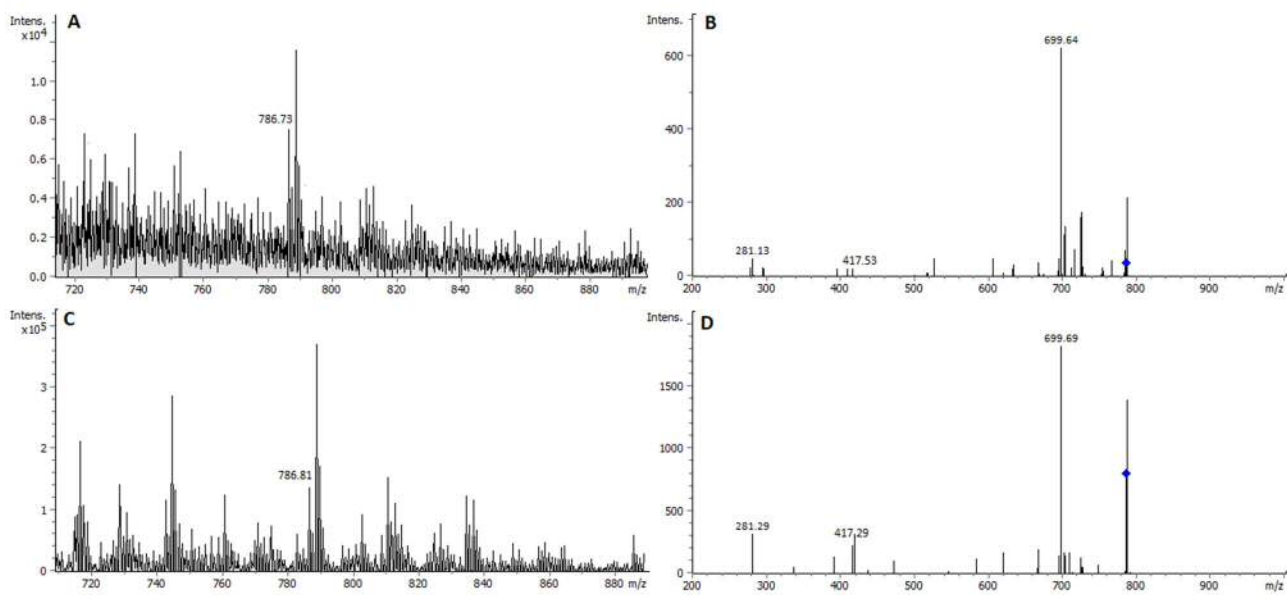
S4-3.- Phosphatidylcholines (PC)

- A) HPTLC-ESI⁺-MS spectrum of NIH-3T3-EXOs (peak at 19.1 mm-m.d.)
B) HPTLC-ESI⁺-MS/MS spectrum of precursor ion at m/z 768.7 (NIH-3T3-EXOs)
C) HPTLC-ESI⁺-MS spectrum of B16-F1-EXOs (peak at 20.3 mm-m.d.)
D) HPTLC-ESI⁺-MS/MS spectrum of precursor ion at m/z 782.6 (B16-F1-EXOs)
E) HPTLC-ESI-MS spectrum of B16-F1-EXOs (peak at 20.3 mm-m.d.)
F) HPTLC-ESI⁺-MS spectrum of B16-F10-EXOs (peak at 18.0 mm-m.d.)
G) HPTLC-ESI⁺-MS/MS spectrum of precursor ion at m/z 782.6 (B16-F10-EXOs)
H) HPTLC-ESI-MS spectrum of B16-F10-EXOs (peak at 18.0 mm-m.d.)



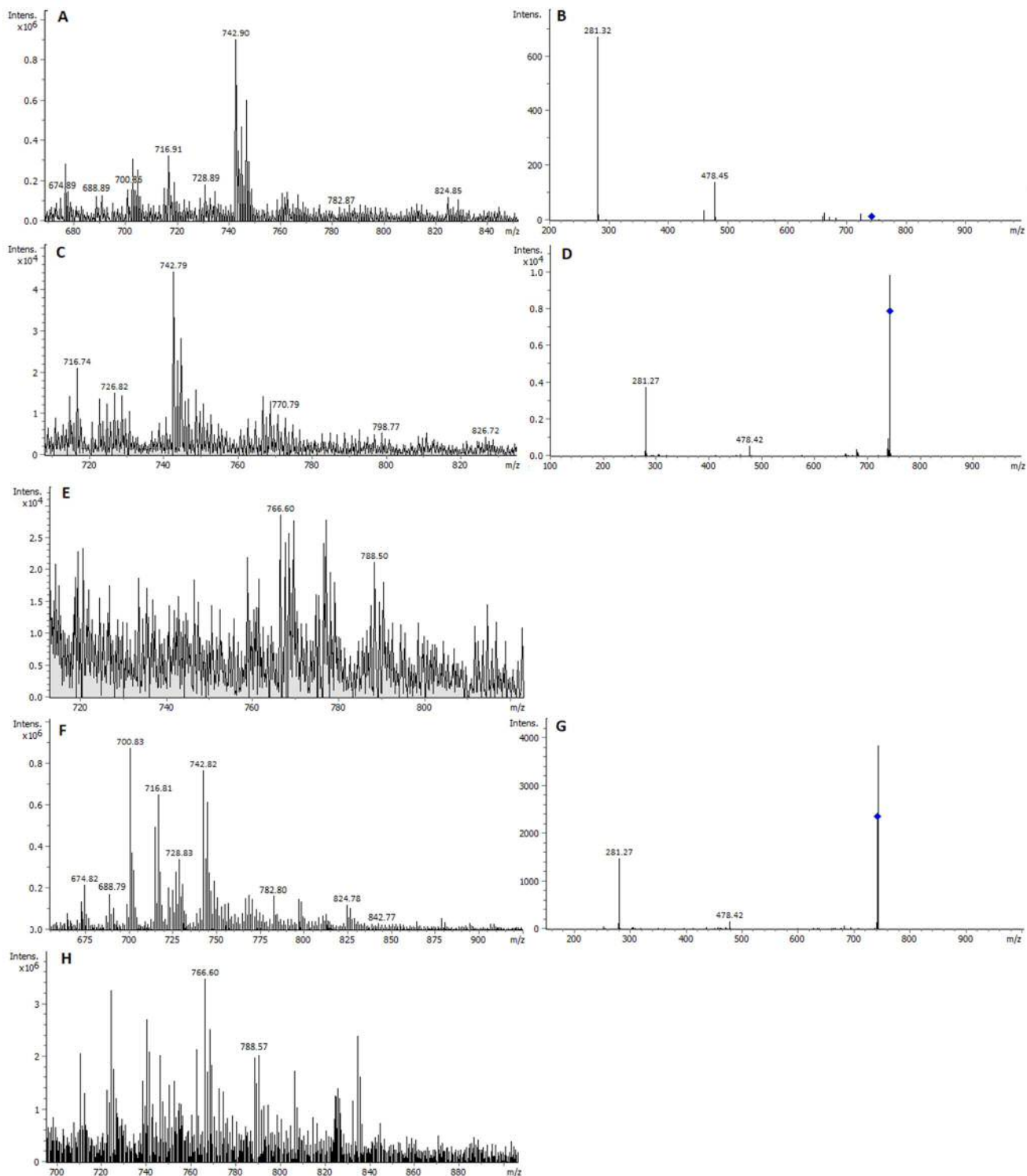
S4-4.- Phosphatidylserines (PS)

- A) HPTLC-ESI-MS spectrum of B16-F1-EXOs (peak at 23.8 mm-m.d.)
B) HPTLC- ESI-MS/MS spectrum of precursor ion at 786.7 m/z (B16-F1-EXOs)
C) HPTLC-ESI-MS spectrum of B16-F10-EXOs (peak at 22.6 mm-m.d.)
D) HPTLC- ESI-MS/MS spectrum of precursor ion at m/z 786.8 (B16-F10-EXOs)



S4-5.- Phosphatidylethanolamines (PE)

- A) HPTLC-ESI-MS spectrum of NIH-3T3-EXOs (peak at 24.8 mm-m.d.)
B) HPTLC-ESI-MS/MS spectrum of precursor ion at m/z 742.9 (NIH-3T3-EXOs)
C) HPTLC-ESI-MS spectrum of B16-F1-EXOs (peak at 25.9 mm-m.d.)
D) HPTLC-ESI-MS/MS spectrum of precursor ion at m/z 742.7 (B16-F1-EXOs)
E) HPTLC-ESI⁺-MS spectrum of B16-F1-EXOs (peak at 25.9 mm-m.d.)
F) HPTLC-ESI-MS spectrum of B16-F10-EXOs (peak at 22.6 mm-m.d.)
G) HPTLC-ESI-MS/MS spectrum of precursor ion at m/z 742.8 (B16-F10-EXOs)
H) HPTLC-ESI⁺-MS spectrum of B16-F10-EXOs (peak at 22.6 mm-m.d.)



S4-6.- Front of elution (band at 30 mm-m.d.)

A) HPTLC-ESI-MS spectrum of B16-F1-EXOs

B) HPTLC-ESI-MS spectrum of B16-F1-EXOs

C) HPTLC-ESI-MS spectrum of B16-F10-EXOs

D) HPTLC-ESI-MS spectrum of B16-F10-EXOs

