Supplementary Data 1

for manuscript Rappez, Stadler et al.

Structural validation of metabolic markers by using LC-MS/MS

Validation of markers detected in co-cultured HeLa and NIH3T3 cells

Summary

Molecular formula	Cell types	METASPACE annotation	Validated using LC-MS/MS as	Validation method	Summary of the MS/MS validation
C40H76NO10P	HeLa and NIH3T3	PS(34:1)	PS(16:0_18:1)	Lipidomics (negative mode)	**SL:** Progenesis *in-silico* Lipid library **HD**: PS **SD**: 18:1 16:0 FA
C42H80NO10P	HeLa and NIH3T3	PS(36:1)	PS(17:0_19:1)	Lipidomics (negative mode)	**SL:** Progenesis *in-silico* Lipid library **HD**: PS **SD**: 17:0 19:1 FA
C18H36O2	HeLa and NIH3T3	Stearic acid, hexyl dodecanoate, 16-methylheptadecanoic acid, hexyl dodecanoate	FA(18:0)	Lipidomics (negative mode)	**Standard:** Stearic acid (Sigma EC10A)
C43H81O13P	HeLa and NIH3T3	PI(34:1)	PI(16:0_18:1)	Lipidomics (negative mode)	**SL:** Progenesis *in-silico* Lipid library **HD:** PI **SD:** 16:0, 18:1 FA
C45H78NO8P	HeLa and NIH3T3	PE(40:6), PC(37:6)	PE(18:0_22:6)	Lipidomics (positive mode) → **HD:** PE Lipidomics (negative mode) → **SD:** 18:0, 22:6 FA	

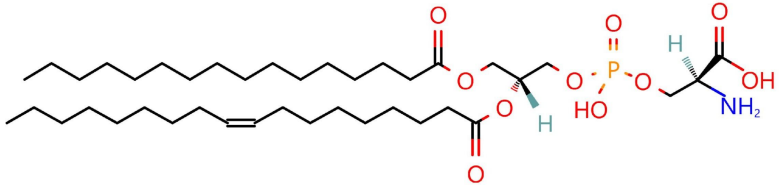
SL: **MS/MS spectral library**
HD: **Head group MS/MS**
SD: **Fatty acid (FA) side chain**

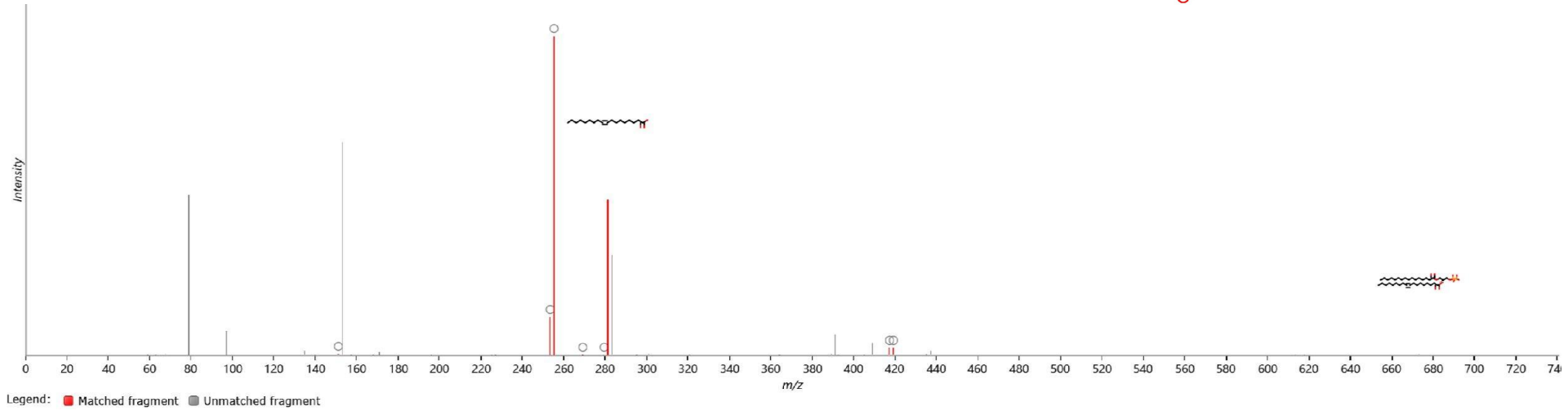
METASPACE annotation: C40H76NO10P, -H, m/z 760.5134, PS(34:1)

Database used : Progenesis in-silico Lipid library

Validated as : **PS(16:0_18:1)** using LC-MS/MS method **Lipidomics** (negative mode)

Progenesis library in silico fragmentation match



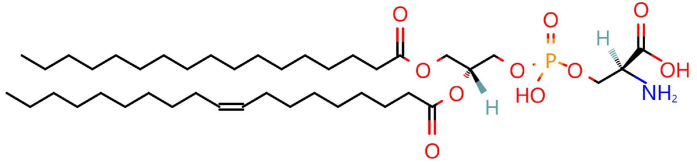


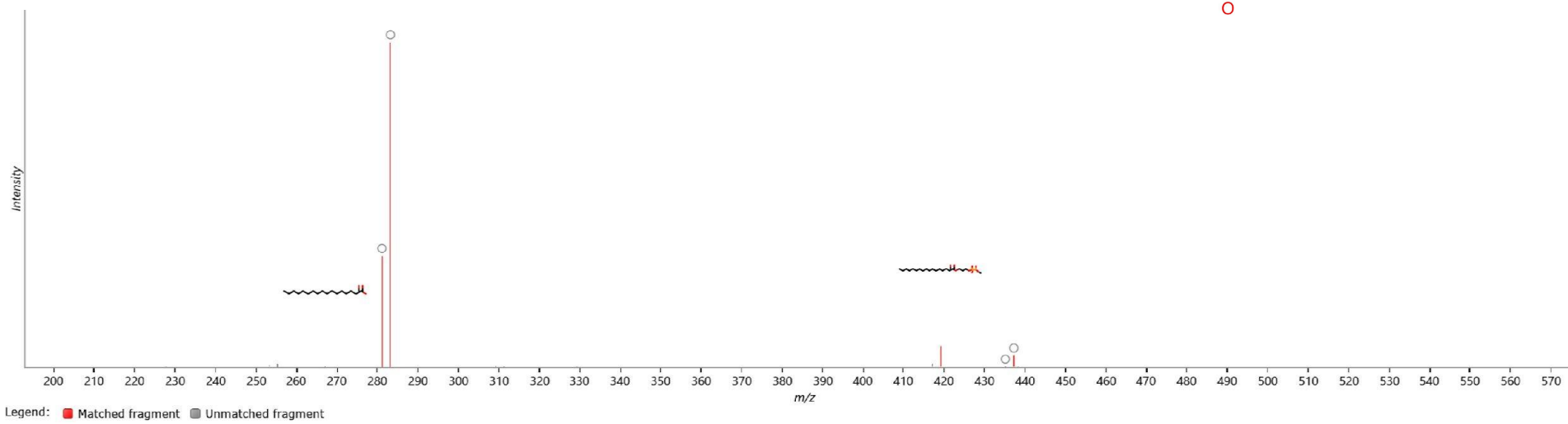
METASPACE annotation: C42H80NO10P, -H, m/z 788.5447, PS(36:1)

Database used : Progenesis in-silico Lipid library

Validated as : **PS(17:0_19:1)** using LC-MS/MS method **Lipidomics** (negative mode)

Progenesis library in silico fragmentation match



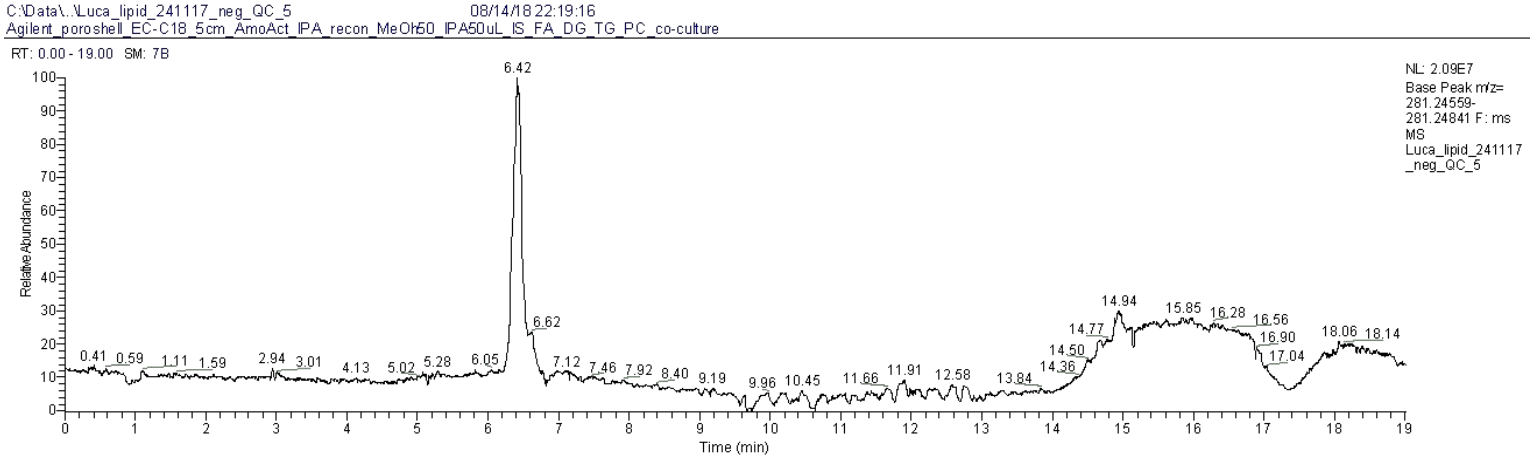


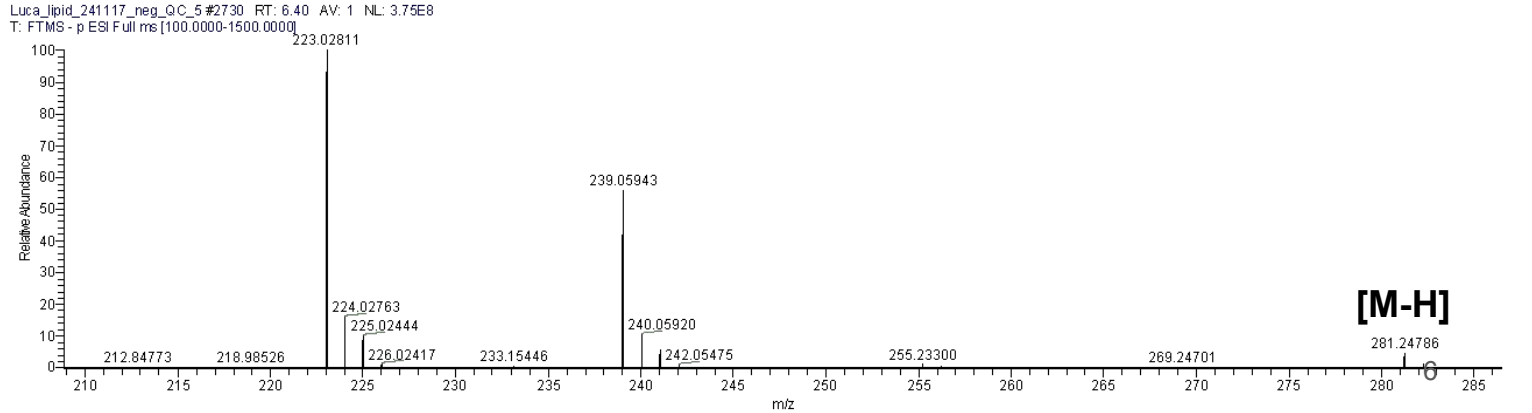
METASPACE annotation: C18H36O2, -H, m/z 283.2642, Stearic acid or another isomeric FA(18:0)

Standard used : **Stearic acid (Sigma EC10A)**

Validated as : **Stearic acid** using LC-MS/MS method **Lipidomics** (negative mode)

RT match with standard



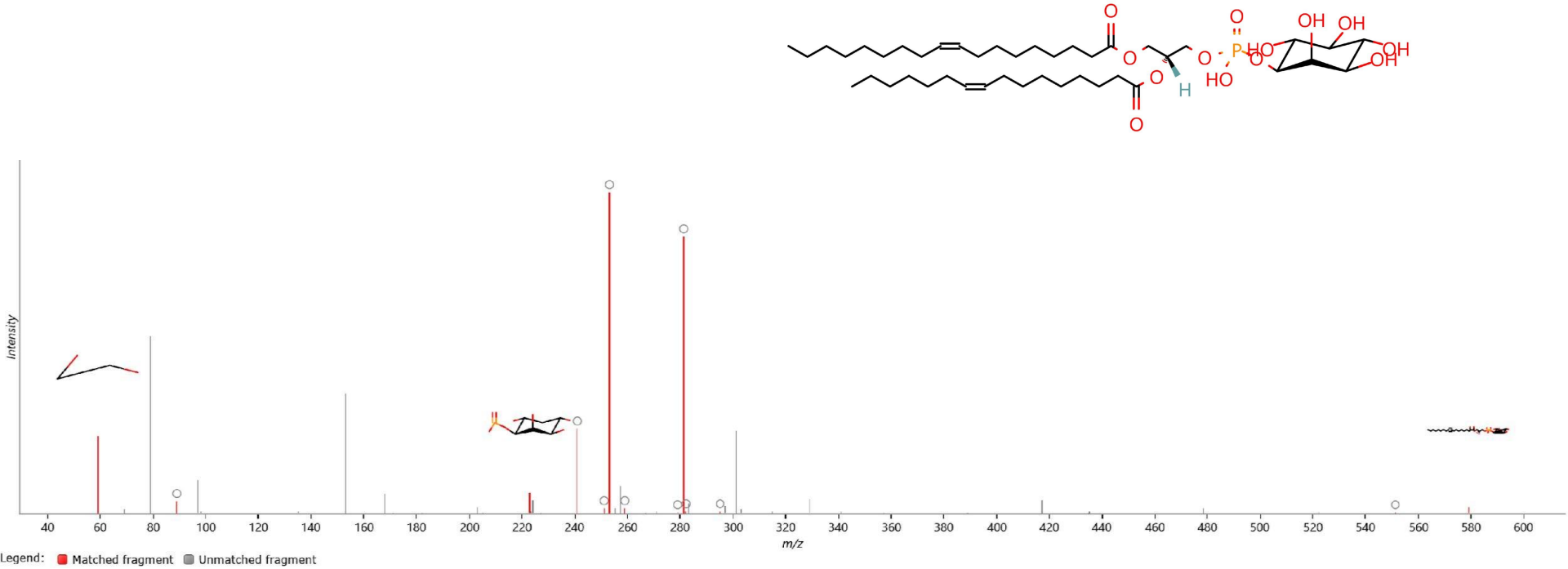


METASPACE annotation: C43H81O13P, -H, m/z 835.5342, PI(34:1)

Database used : Progenesis in-silico Lipid library

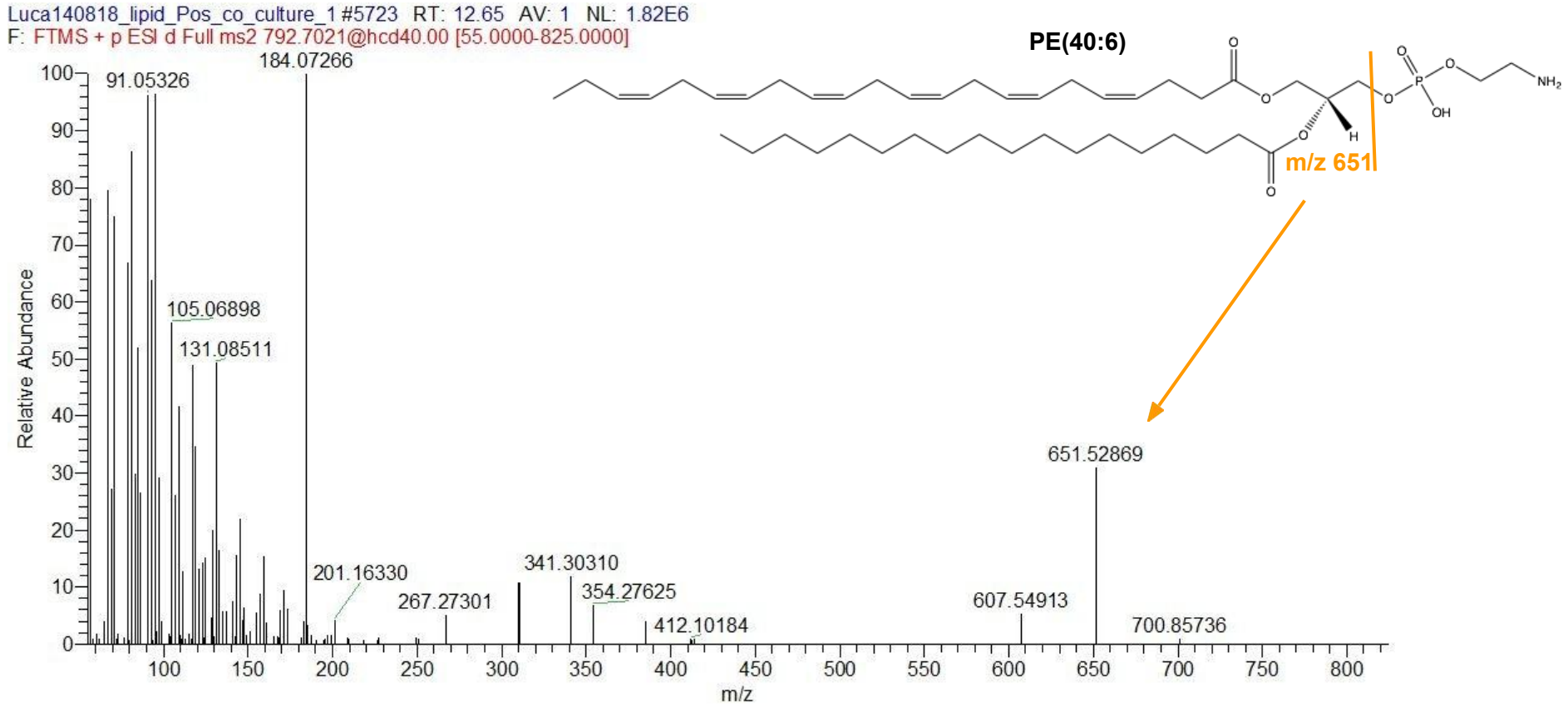
Validated as : **PI(18:1_16:1)** using LC-MS/MS method **Lipidomics** (negative mode)

Progenesis *in silico* Lipid library



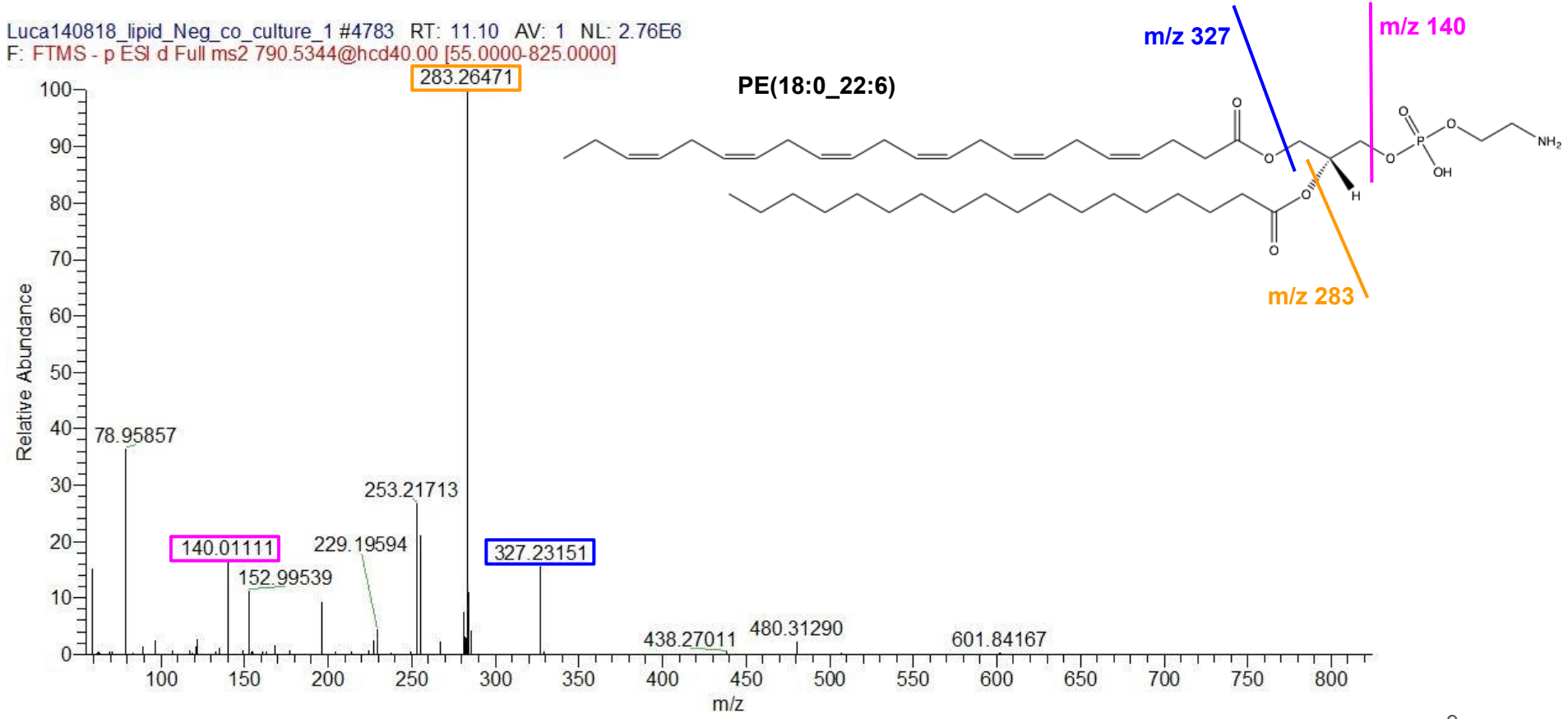
METASPACE annotation: C45H78NO8P, +H, m/z 792.55433, PE(40:6) or PC(37:6)

Validated as : PE(40:6) using LC-MS/MS method **Lipidomics** (positive mode)

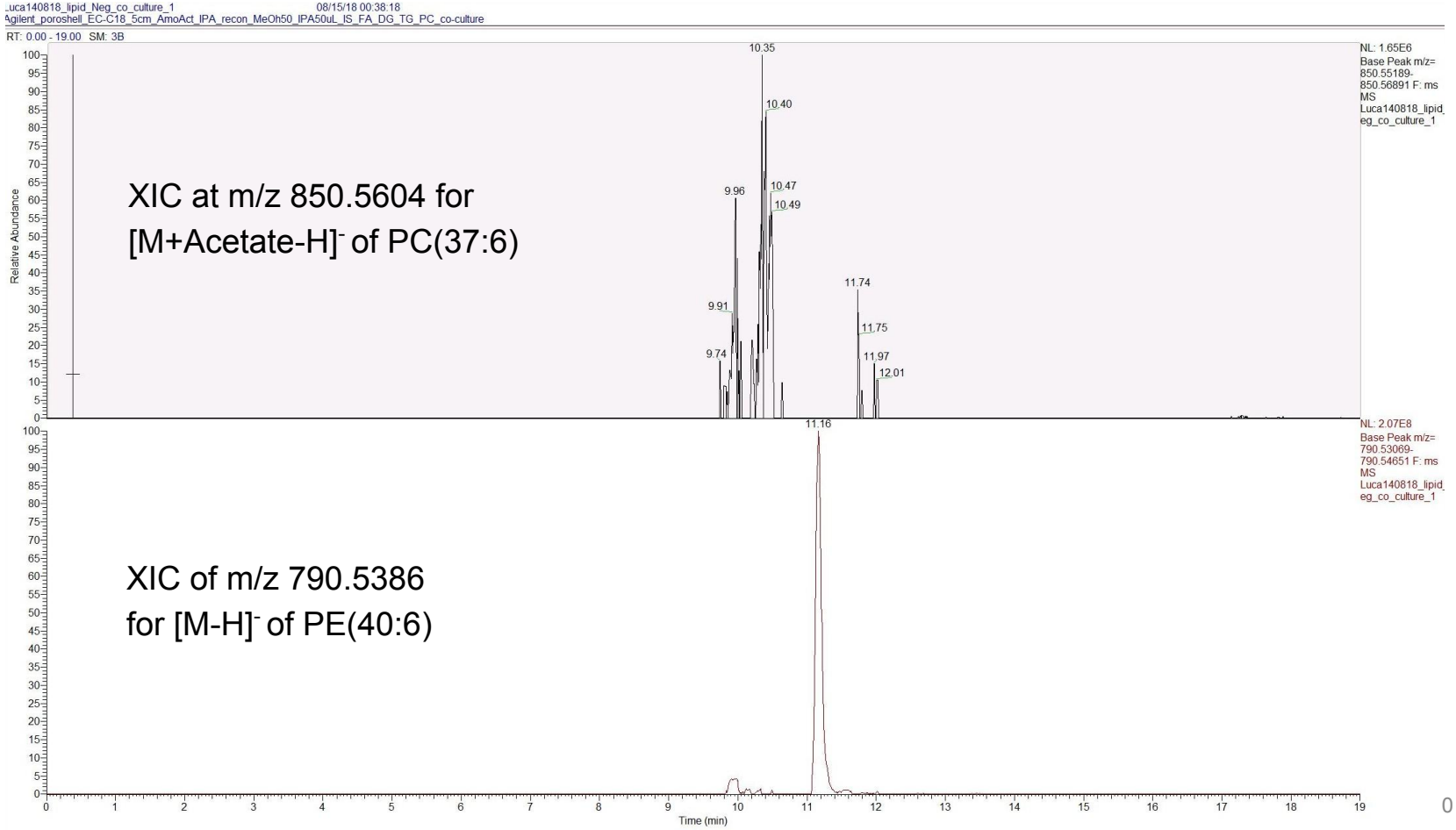


METASPACE annotation: C45H78NO8P, -H, m/z 790.5386, PE(40:6) or PC(37:6)

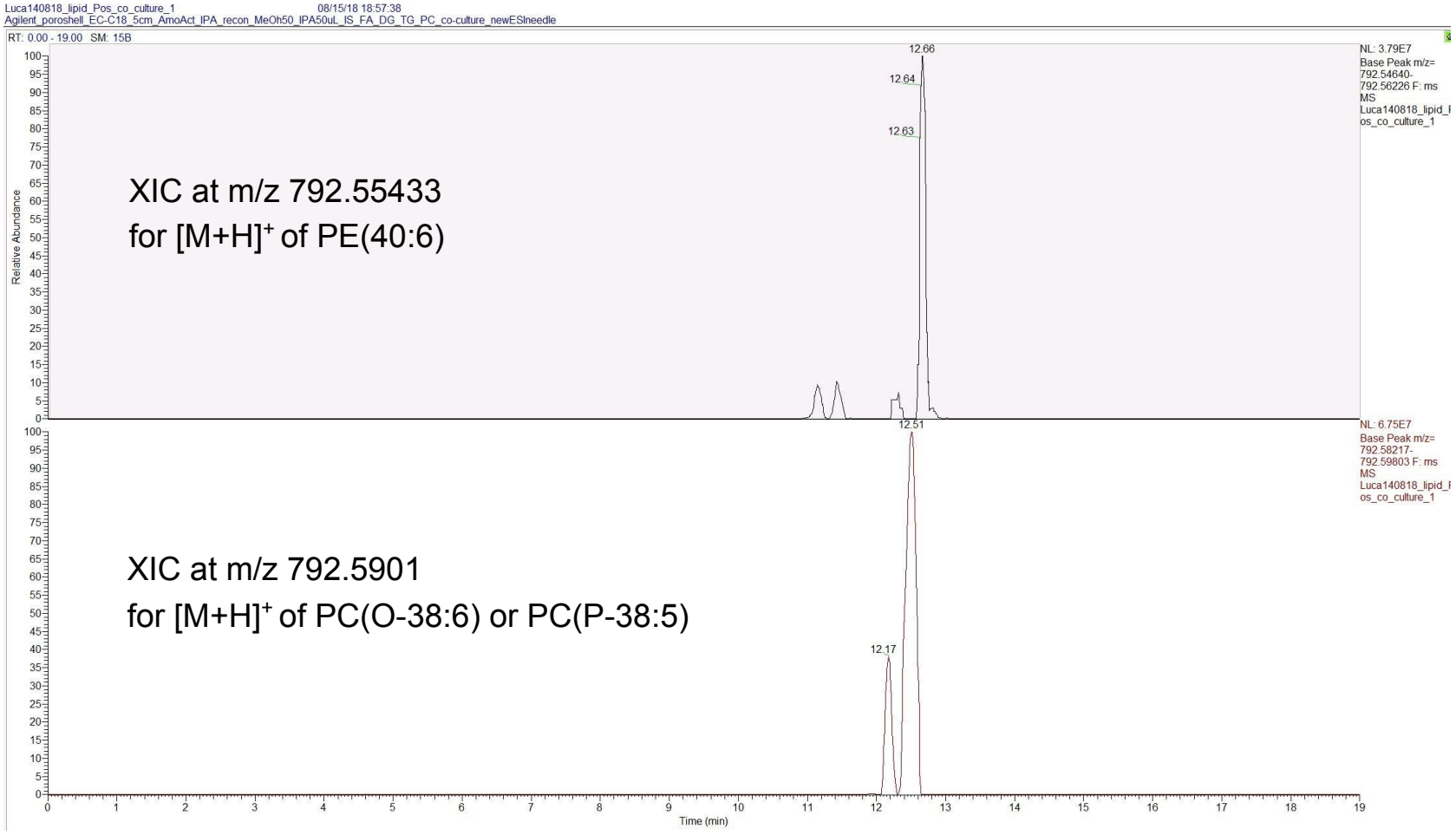
Validated as : PE(18:0_22:6) using LC-MS/MS method **Lipidomics** (negative mode)



Absence of PC(37:6) and detection of PE(40:6) in the negative mode



Possible co-elution of PE(40:6) with a phosphatidylcholine (PC)



Validation of markers detected in dHepaRG hepatocytes

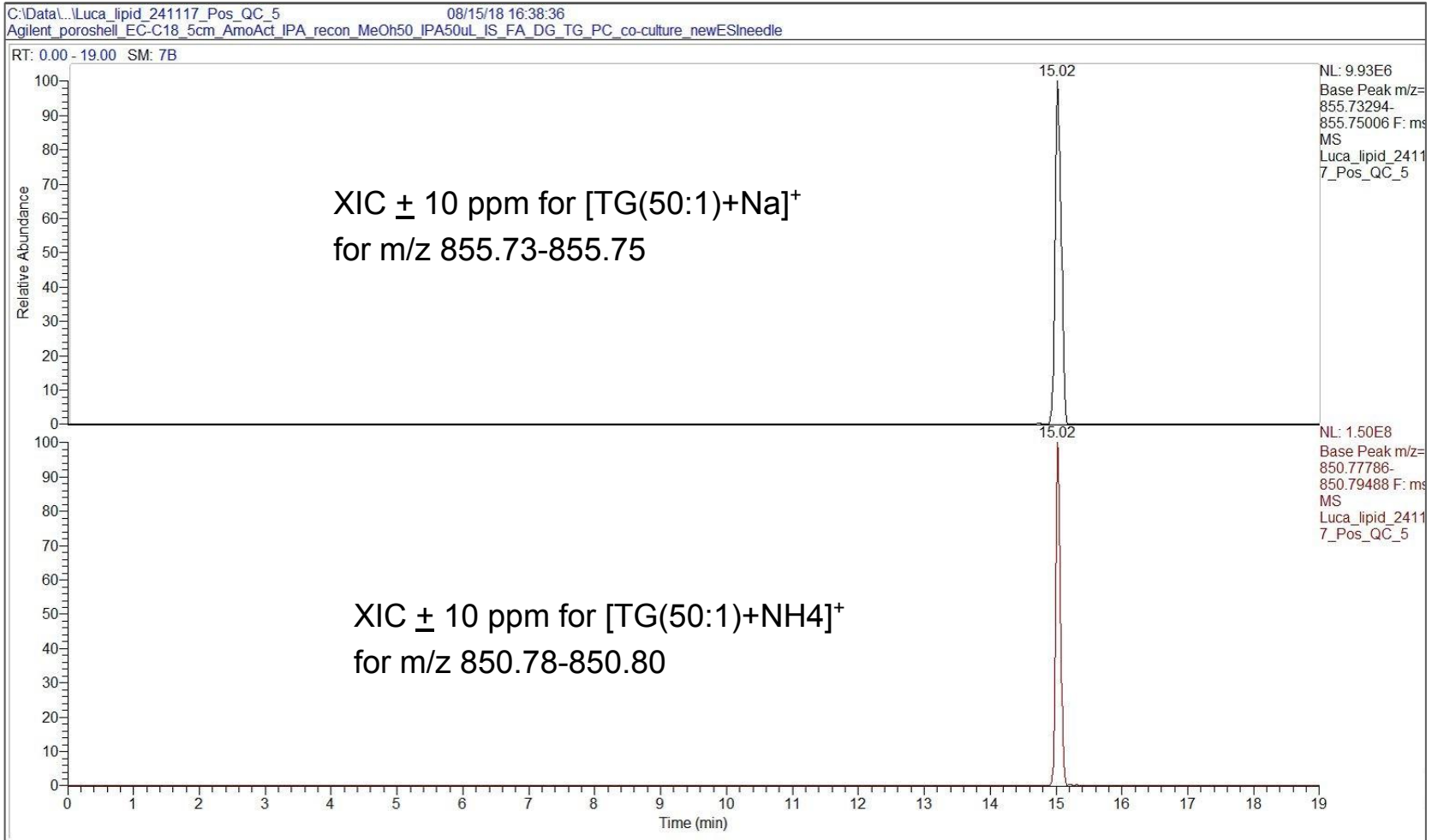
Summary

Molecular formula	Cell type	METASPACE annotation	Validated using LC-MS/MS as	Validation method	Summary of the MS/MS validation
C53H100O6	dHepaRG	TG(50:1)	TG(16:0_18:1_16:0)	Lipidomics (positive mode)	**SL:** Progenesis *in-silico* spectral library **SD:** 16:0, 18:1 FA
C55H102O6	dHepaRG	TG(52:2)	TG(16:0_18:1_18:1)	Lipidomics (positive mode)	**SL:** Progenesis *in-silico* spectral library **SD:** 16:0, 18:1 FA

SL: MS/MS spectral library
SD: Fatty acid (FA) side chain

Validating TG(50:1) as TG(16:0_18:1_16:0)

Validating detection in cell extract by LCMS



Finding the lipid class by exact m/z search on HMDB

Parameters:

- https://hmdb.ca/spectra/ms/search
- Considering the following adducts which are possible in the LCMS positive method:
  - +H, +H-2H2O, +H-H2O, +NH4-H2O, +NH4, +Na, +CH2OH+H
- With 10 ppm tolerance

Results:

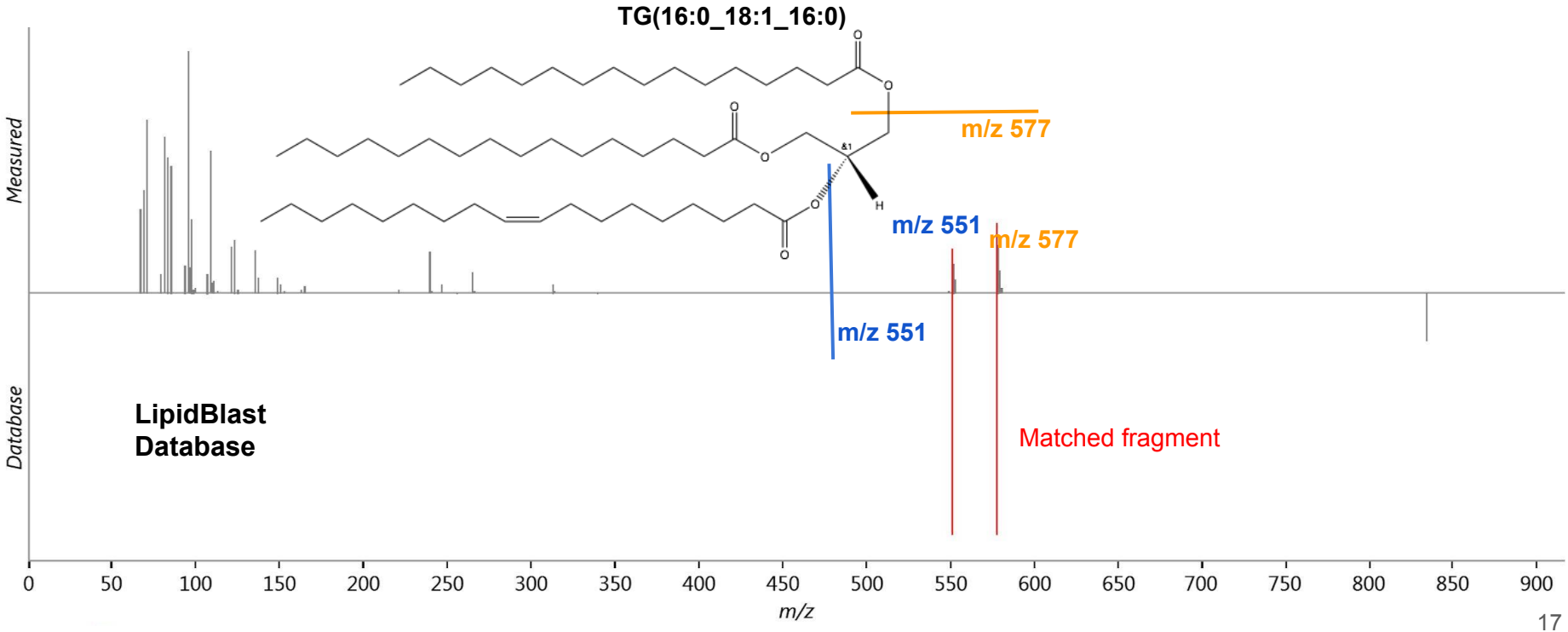
- For 855.74 (corresponding to [TG(50:1)+Na]+): **137 hits, 135 of them TGs**
- For 850.785 (corresponding to [TG(50:1)+NH4]+): **33 hits, all of them TGs, all [TG(50:1)+NH4]+**

Identifying fatty acyl chains of TG(50:1) for +Na

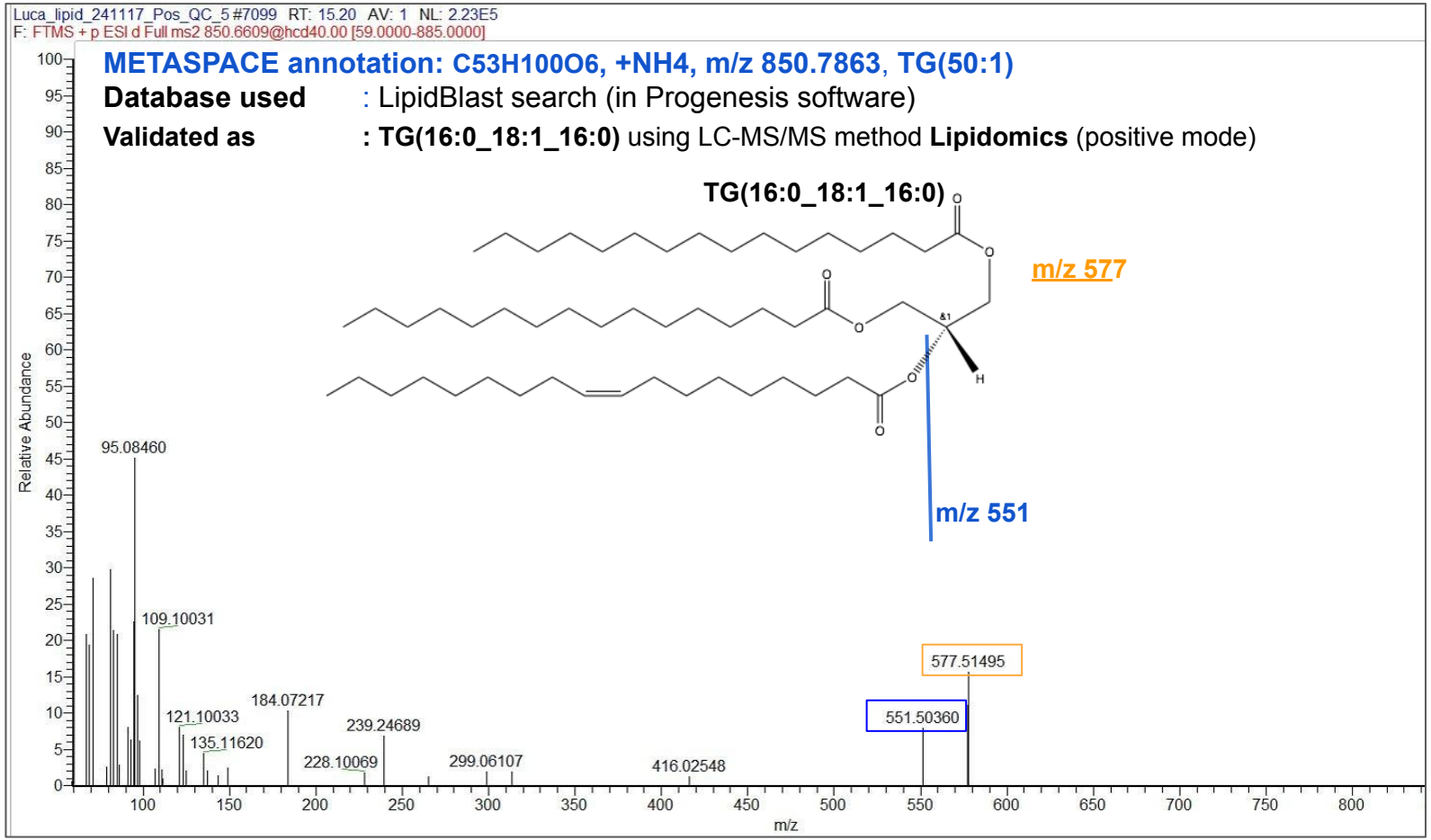
METASPACE annotation: C53H100O6, +Na, m/z 855.7412, TG(50:1)

Database used : LipidBlast search (in Progenesis software)

Validated as **: TG(16:0_18:1_16:0)** using LC-MS/MS method **Lipidomics** (positive mode)

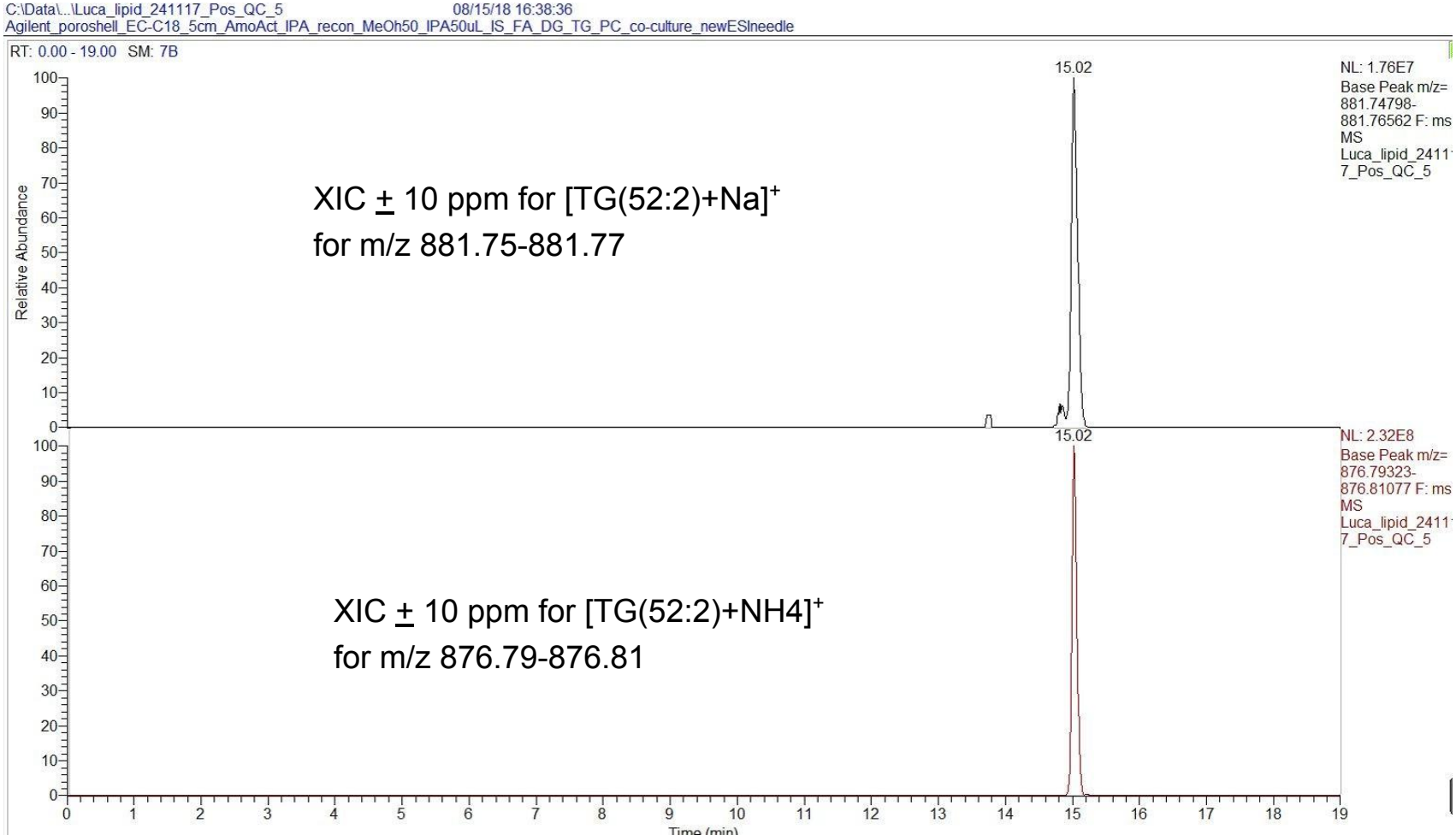


Identifying fatty acyl chains of TG(50:1) for +NH4



Validating TG(52:2) as TG(16:0_18:1_18:1)

Validating detection in cell extract by LCMS



Finding the lipid class by exact m/z search on HMDB

Parameters:

- https://hmdb.ca/spectra/ms/search
- Considering the following adducts which are possible in the LCMS positive method:
  - +H, +H-2H2O, +H-H2O, +NH4-H2O, +NH4, +Na, +CH2OH+H
- With 10 ppm tolerance

Results:

- For 881.76 (corresponding to [TG(52:2)+Na]+): **229 hits, all of them TGs, either +Na or +H (the latter not possible for TGs)**
- For 876.8 (corresponding to [TG(52:2)+NH4]+): **65 hits, all of them TGs, all [TG(52:2)+NH4]+**

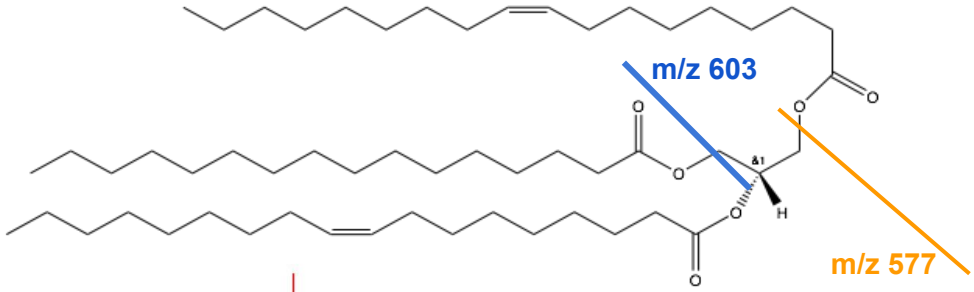
Identifying fatty acyl chains of TG(52:2) for +Na

METASPACE annotation: C55H102O6, +Na, m/z 881.7568, TG(52:2)

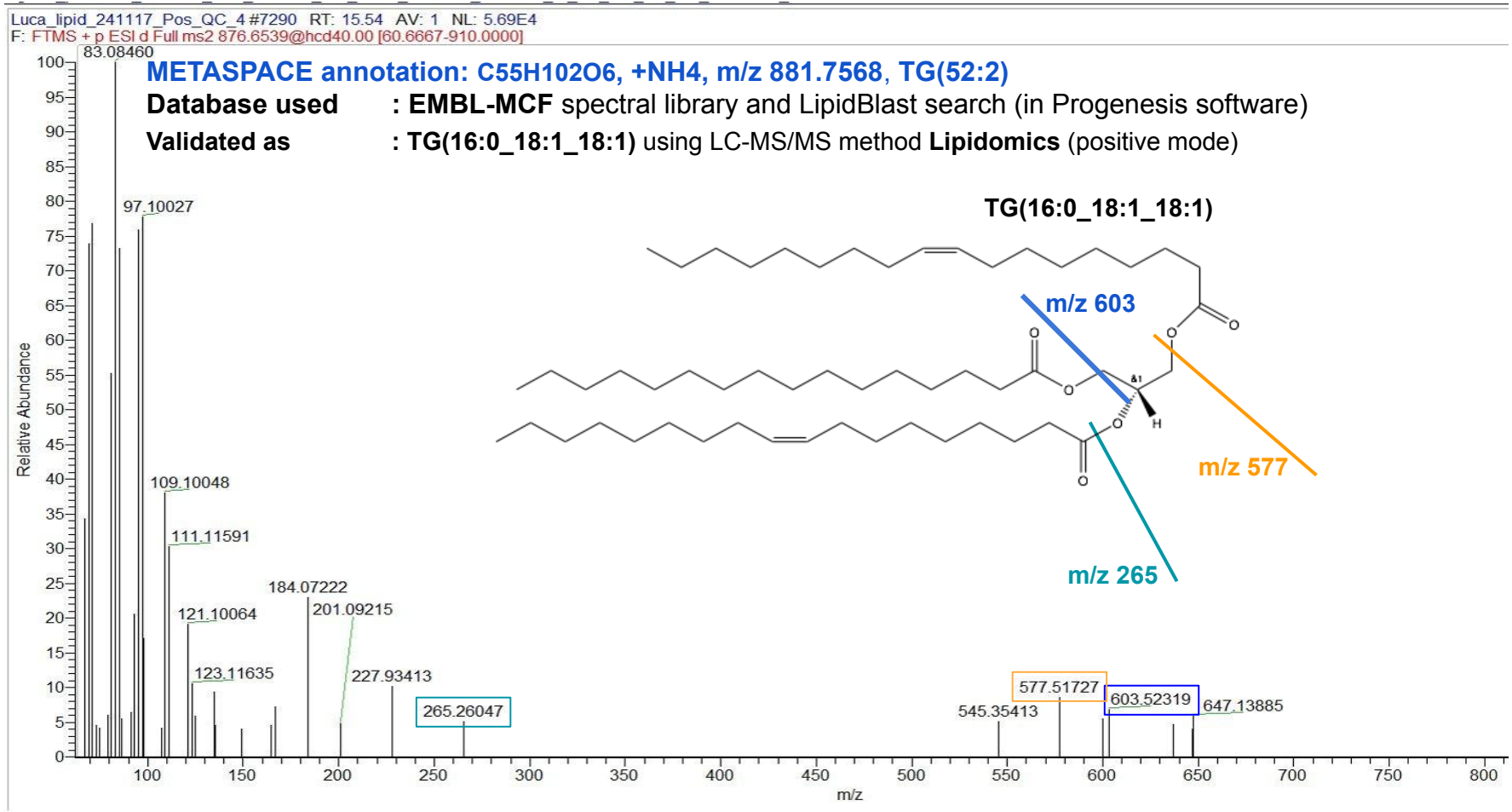
Database used : **EMBL-MCF** spectral library and LipidBlast search (in Progenesis software)

Validated as : **TG(16:0_18:1_18:1)** using LC-MS/MS method **Lipidomics** (positive mode)

TG(16:0_18:1_18:1)



Identifying fatty acyl chains of TG(52:2) for +NH4



Validation of markers detected in *in vivo* murine model of NASH

Molecular formula	Sample type	METASPACE annotation	Validated using LC-MS/MS as	Validation method	Summary of the MS/MS validation
C53H100O6	Mouse liver	TG(50:1)	TG(16:0_18:1_16:0)	Lipidomics (positive mode)	**SL**: Metascope Lipidmaps library **SD**: 16:0, 18:1 FA
C55H102O6	Mouse liver	TG(52:2)	TG(18:1_18:1_16:0)	Lipidomics (positive mode)	**SL**: Metascope Lipidmaps library **SD**: 16:0, 18:1 FA
C35H68O5	Mouse liver	DG(32:0)	DG(16:0/16:0)	Lipidomics (positive mode)	**SL**: Metascope Lipidmaps library **SD**: 16:0 FA
C41H78NO8P	Mouse liver	PE(36:2) / PC(33:2)	PE(18:2_18:0)	Lipidomics (negative mode)	**SL**: Metascope Lipidmaps library **HD**: PE **SD**: 18:2 18:0 FA
C43H80NO8P	Mouse liver	PE(38:3) / PC(35:3)	PE(20:3_18:0)	Lipidomics (negative mode)	**SL**: Metascope Lipidmaps library **HD**: PE **SD**: 20:3 18:0 FA
C42H80NO8P	Mouse liver	PE(37:2) / PC(34:2)	PC(18:2_16:0)	Lipidomics (negative mode)	**SL**: Metascope Lipidmaps library **HD**: PC **SD**: 18:2 16:0 FA
C42H82NO8P	Mouse liver	PE(37:1) / PC(34:1)	PC(18:1_16:0)	Lipidomics (negative mode)	**SL**: Metascope Lipidmaps library **HD**: PC **SD**: 18:1 16:0 FA
C28H50NO7P	Mouse liver	LPE(23:4) / LPC(20:4)	LPC(20:4)	Lipidomics (negative mode)	**SL**: Metascope Lipidmaps library **HD**: PC **SD**: 20:4 FA
C41H83N2O6P	Mouse liver	SM(d36:1)*	SM(d36:1)	Lipidomics (positive mode)	**SL**: Metascope Lipidmaps library

*** indicates ambiguity due to the existence of structural isomers**

SL: MS/MS spectral library
HD: Head group MS/MS
SD: Fatty acid (FA) side chain

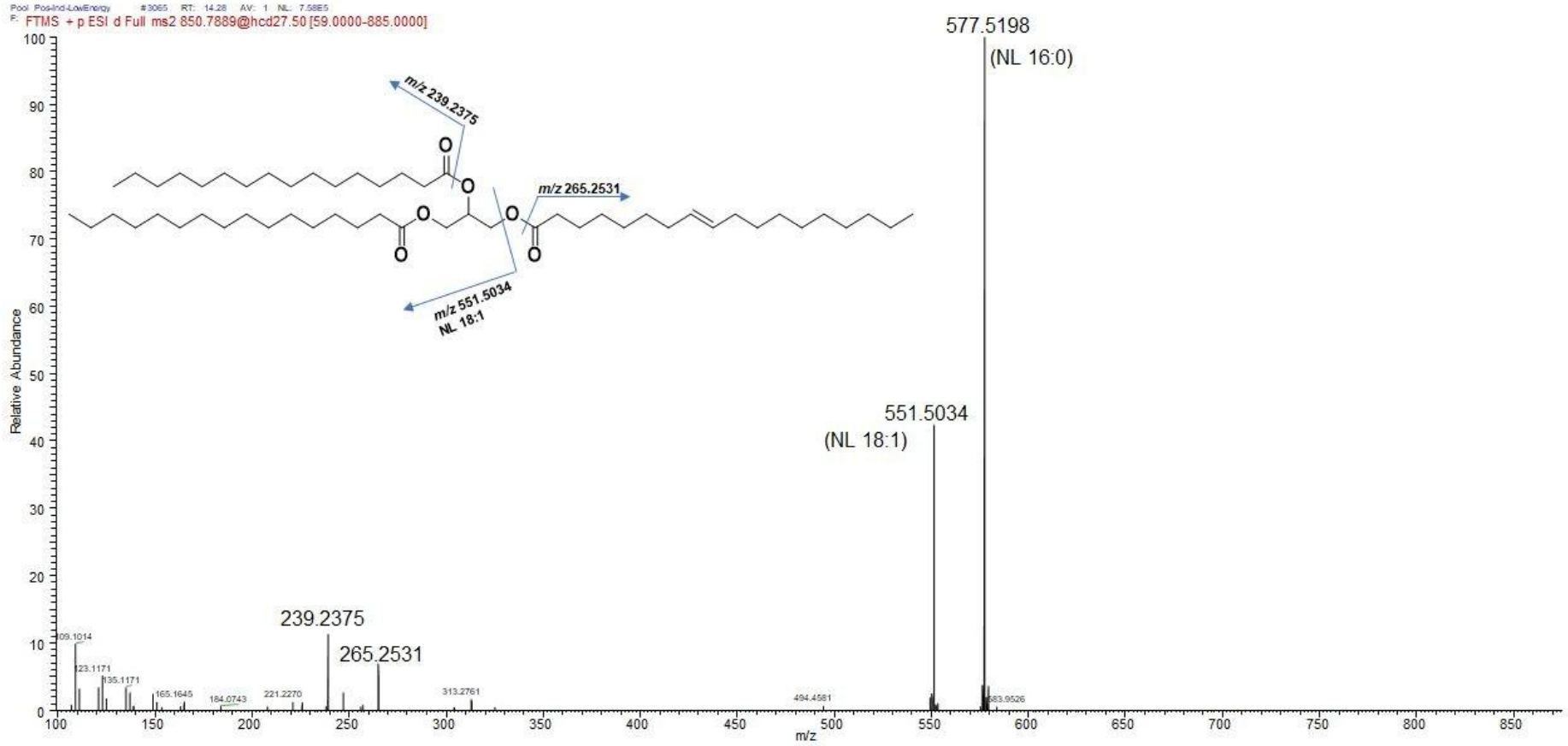
In addition to analyzing the samples using data dependent analysis (DDA). A pooled sample was analyzed using the following experiments to obtain fragmentation data.

1. Fragmentation at low energy of all significant TGs (CE = 27). The TGs were added into an inclusion list
2. Fragmentation at low energy of all significant TGs (CE = 40). The TGs were added into an inclusion list
3. A polarity switching experiment of the pool using positive mode mobile phases (Ammonium Formate + Formic Acid)
4. A polarity switching experiment of the pool using negative mode mobile phases (Ammonium Acetate + Acetic Acid)

METASPACE annotation: C53H100O6, +Na, m/z 855.7412, TG(50:1)

Database used : Metascope Lipidmaps inbuilt library in Progenesis software

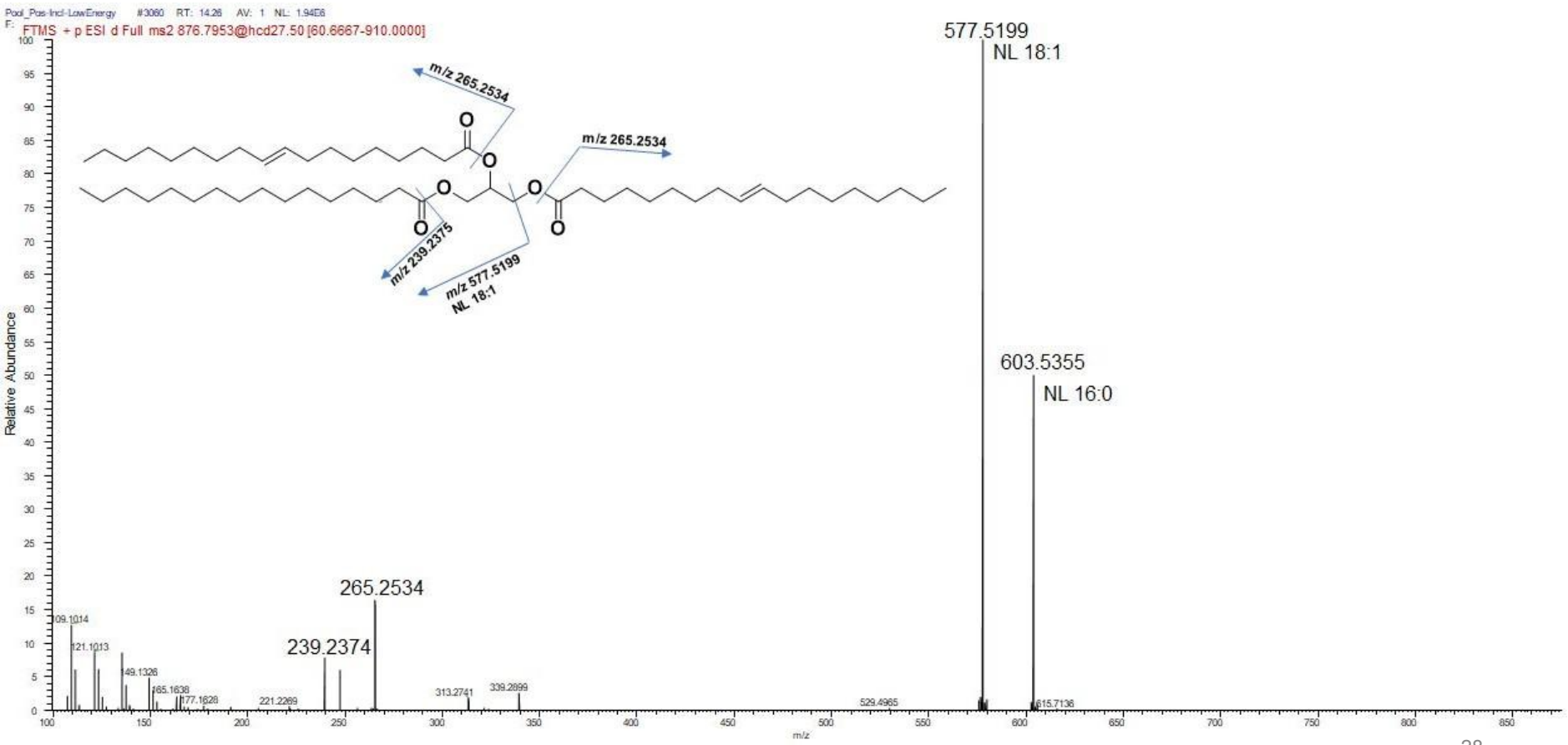
Validated as : **TG(16:0_18:1_16:0)** using LC-MS/MS method **Lipidomics** (positive mode, m/z 850.7889 [M+NH4]+)



METASPACE annotation: C55H102O6, +Na, m/z 881.7568, TG(52:2)

Database used : Metascope Lipidmaps inbuilt library in Progenesis software

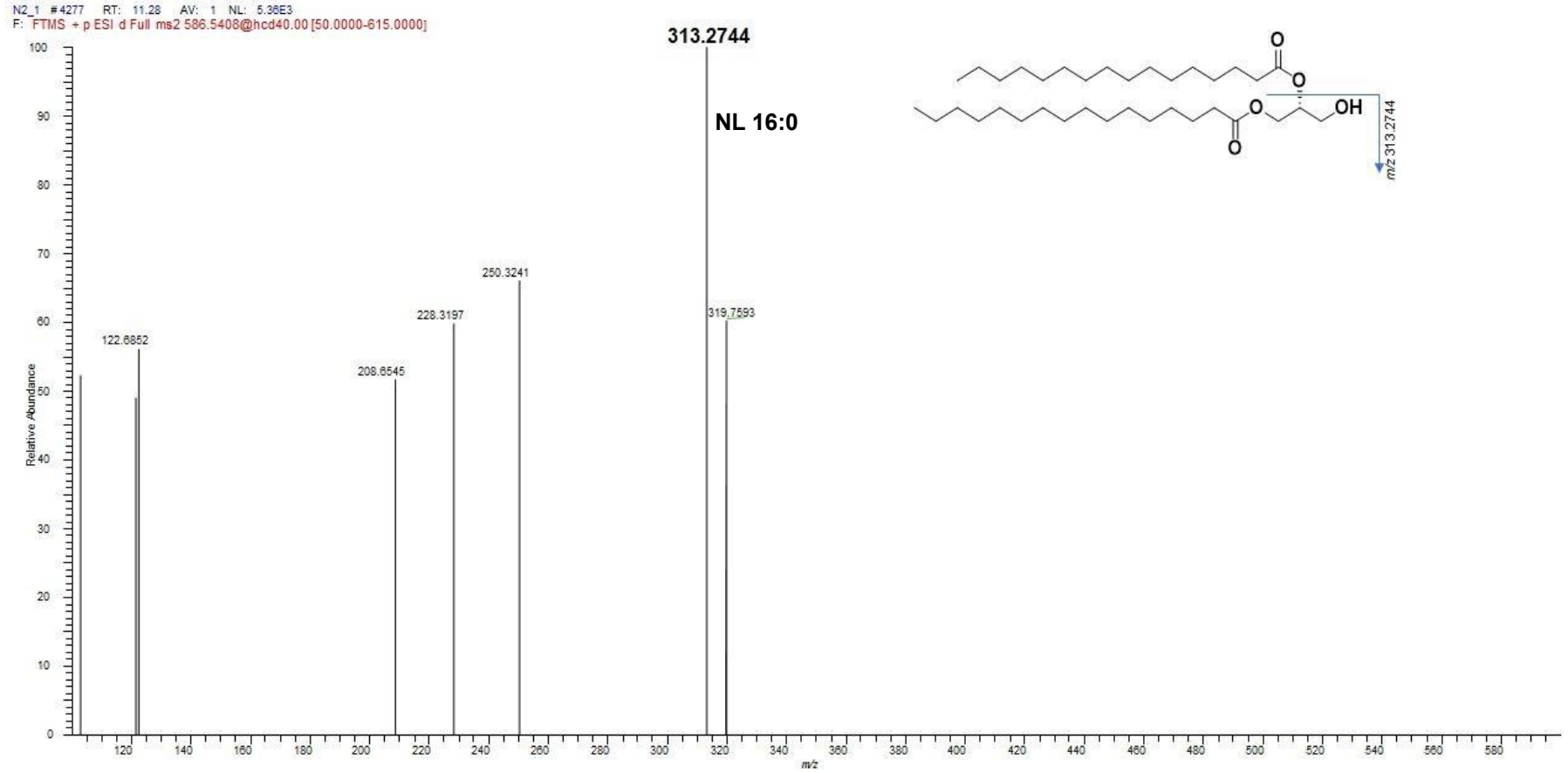
Validated as : **TG(18:1_18:1_16:0)** using LC-MS/MS method **Lipidomics** (positive mode, m/z 876.7953, [M+NH4]+)



METASPACE annotation: C35H68O5, +Na, m/z 591.4959, DG(32:0)

Database used : Metascope Lipidmaps inbuilt library in Progenesis software

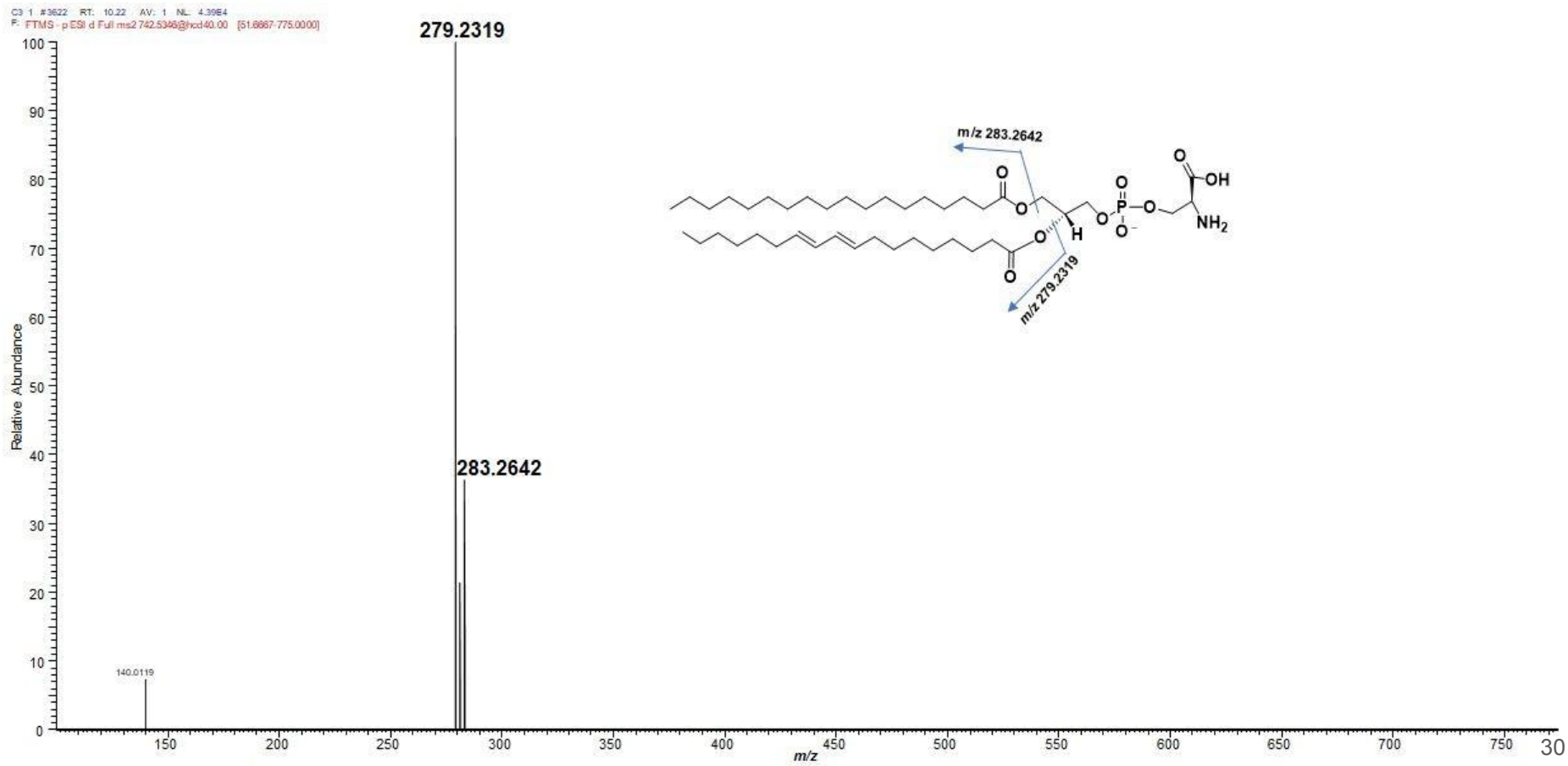
Validated as : **DG(16:0/16:0)** using LC-MS/MS method **Lipidomics** (positive mode, m/z 586.5408, [M+NH4]+)



METASPACE annotation: C41H78NO8P, +H, m/z 744.5537, PE(36:2) or PC(33:2)

Database used : Metascope Lipidmaps inbuilt library in progenesis

Validated as : **PE(18:2_18:0)** using LC-MS/MS method **Lipidomics** (negative mode; m/z 742.5343, [M-H]-)

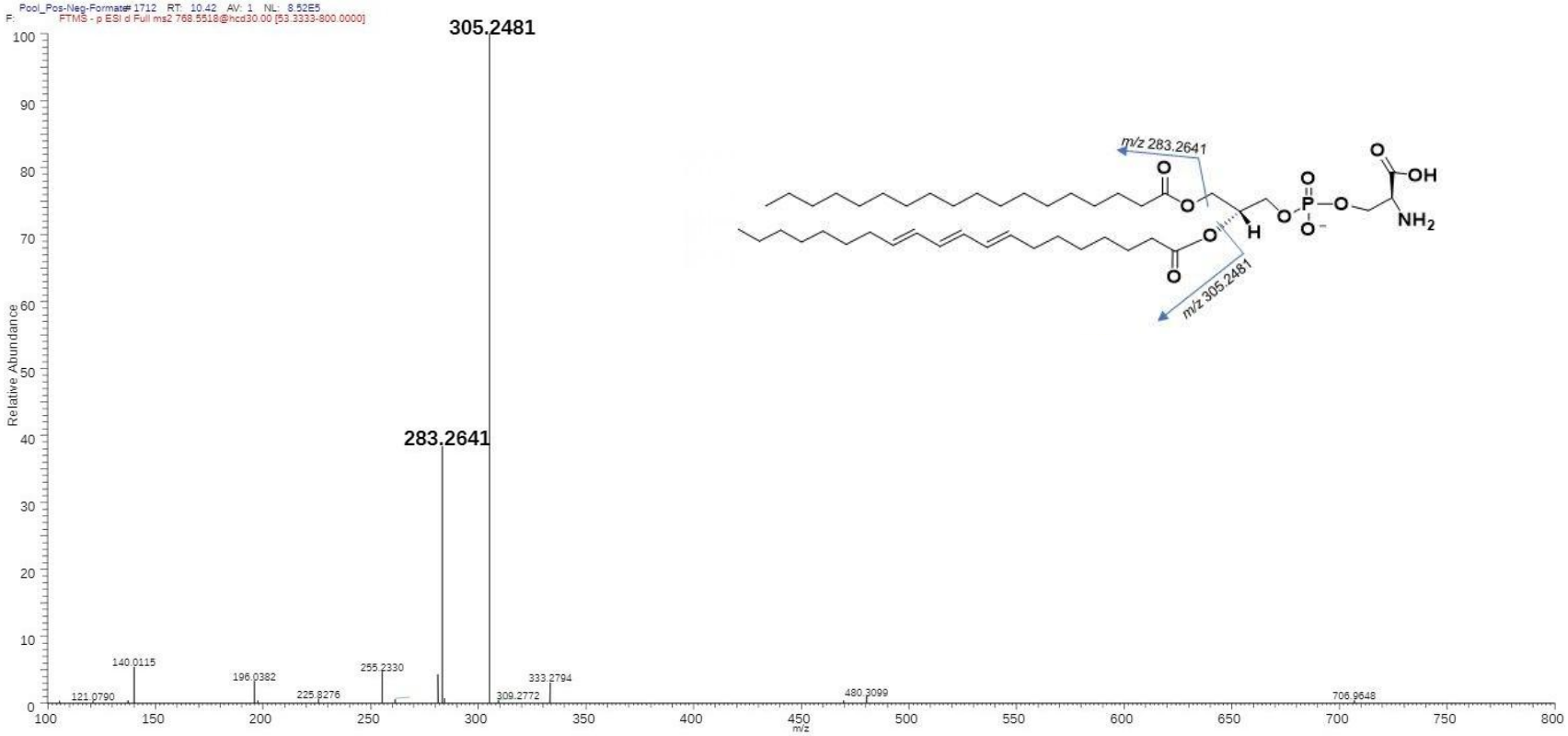


METASPACE annotation: C43H80NO8P, +H, m/z 770.5694, PE(38:3) or PC(35:3)

Database used : Metascope Lipidmaps inbuilt library in Progenesis software

Validated as : **PE(20:3_18:0)** using LC-MS/MS method **Lipidomics** (negative mode, m/z 768.5518, [M-H]-)

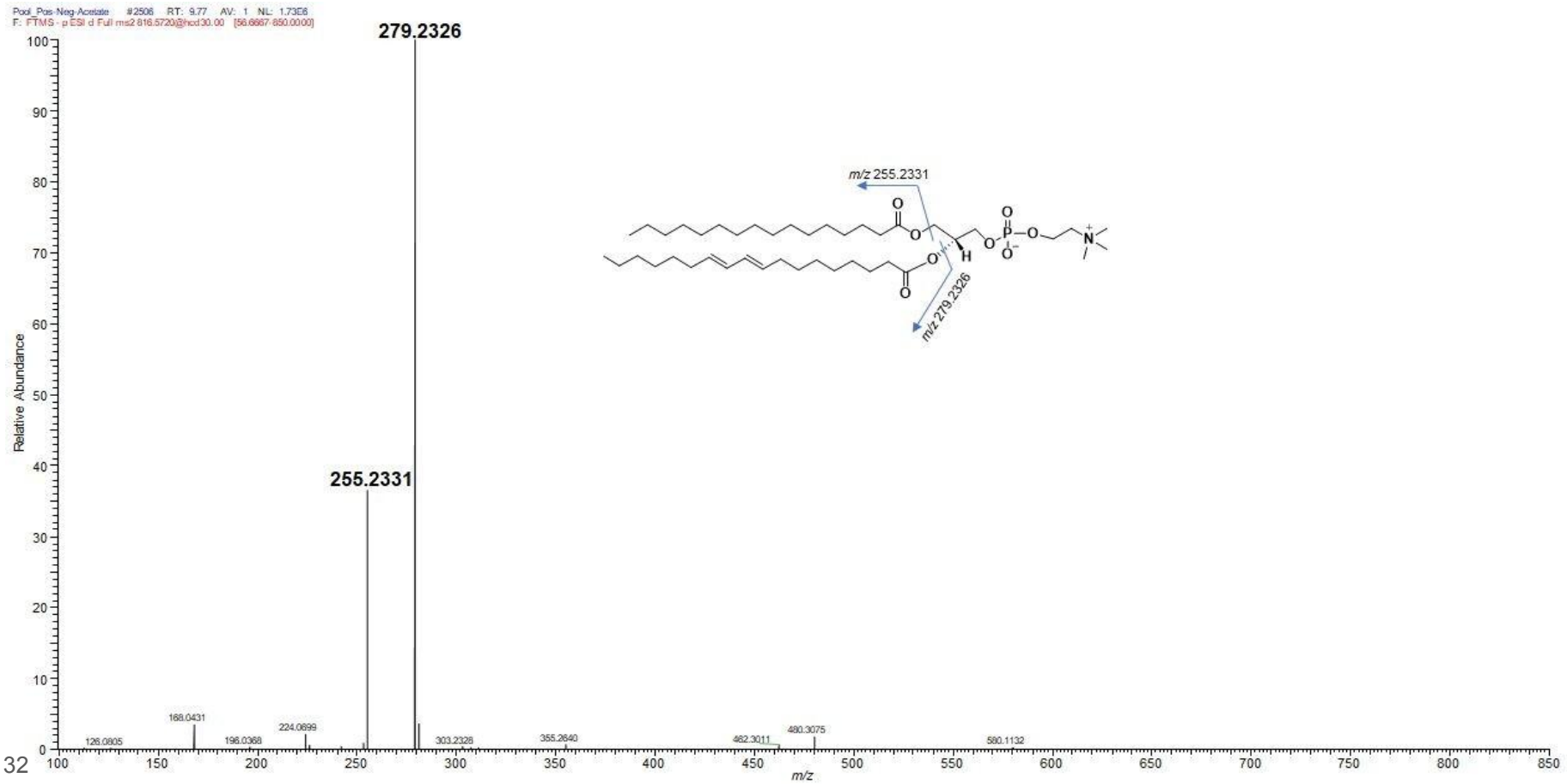
Validated as: PE(20:3_18:0) using LC-MS/MS method Lipidomics (Negative mode 768.5518, [M-H]-)



METASPACE annotation: C42H80NO8P, +Na, m/z 780.5513, PE(37:2) or PC(34:2)

Database used : Metascope Lipidmaps inbuilt library in Progenesis software

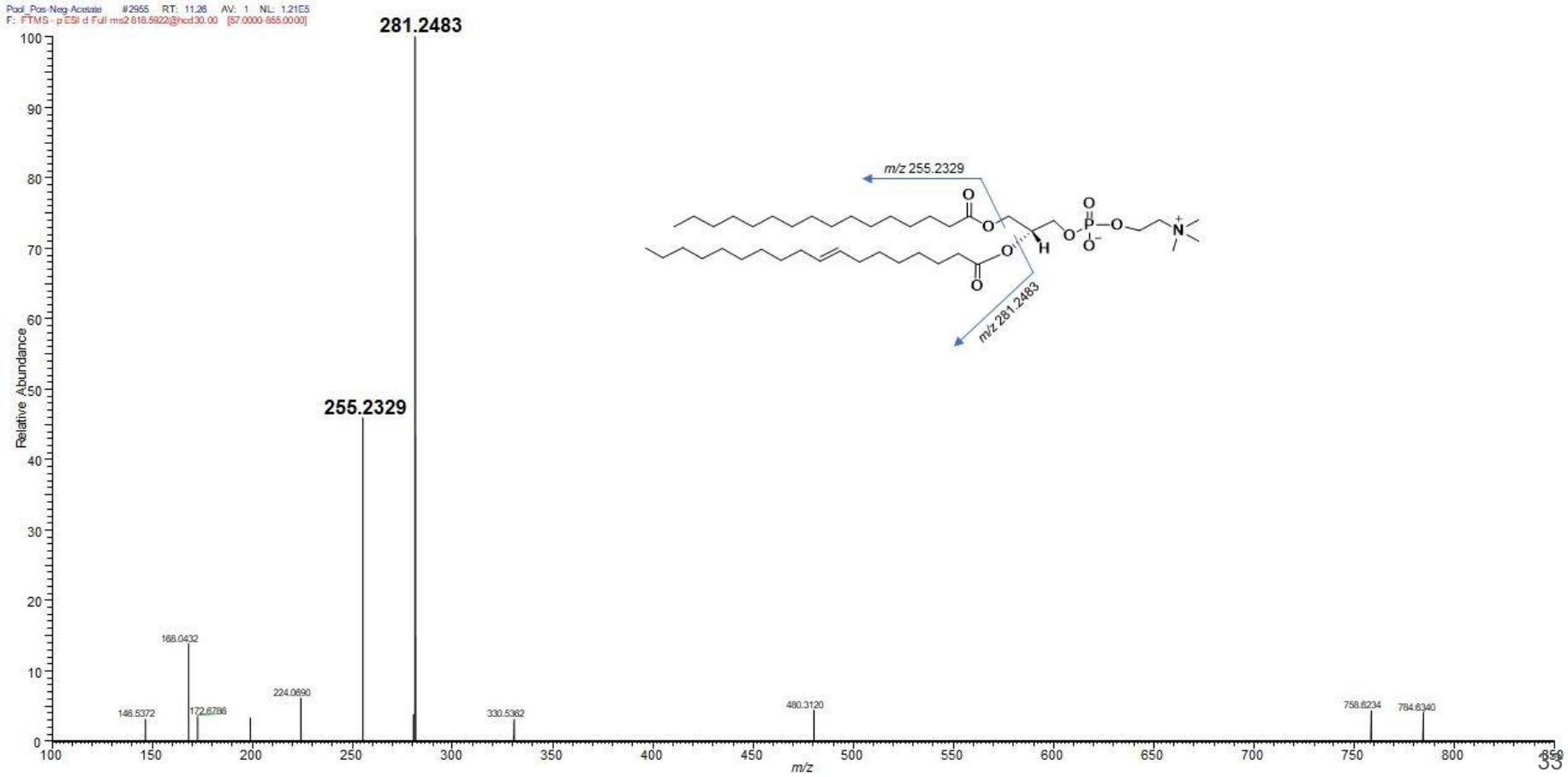
Validated as : **PC(18:2_16:0)** using LC-MS/MS method **Lipidomics** (negative mode, m/z 816.5720, [M+Acetate-H]-)



METASPACE annotation: C42H82NO8P, +H, m/z 760.585, PE(37:1) or PC(34:1)

Database used : Metascope Lipidmaps inbuilt library in Progenesis software

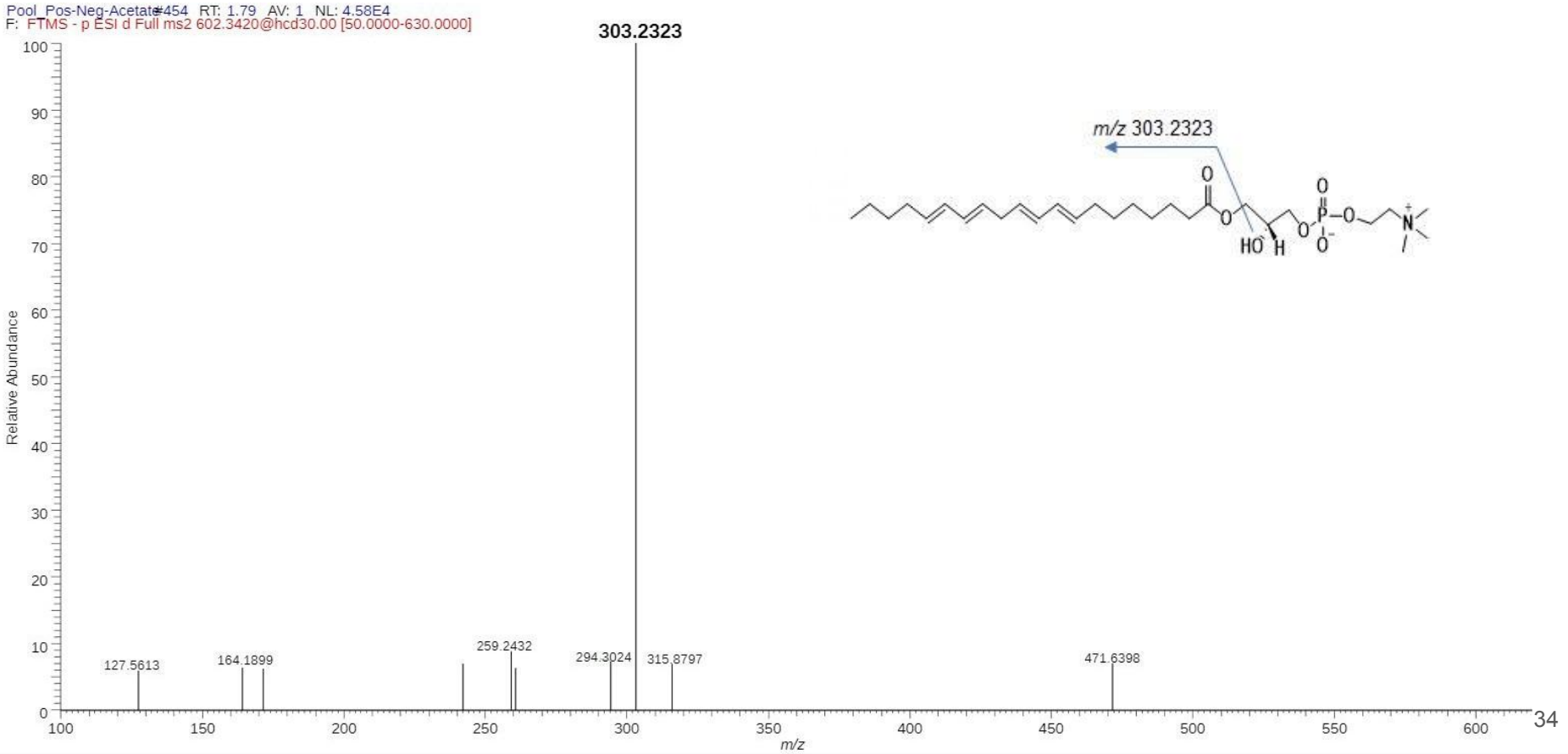
Validated as : **PC(18:1_16:0)** using LC-MS/MS method **Lipidomics** (negative mode, m/z 818.5922, [M+Acetate-H]-)



METASPACE annotation: C28H50NO7P, +H, m/z 544.3397, LPE(23:4) or LPC(20:4)

Database used : Metascope Lipidmaps inbuilt library in Progenesis software

Validated as : **LPC(20:4)** using LC-MS/MS method **Lipidomics** (negative mode, m/z 602.3420, [M+Acetate-H]-)

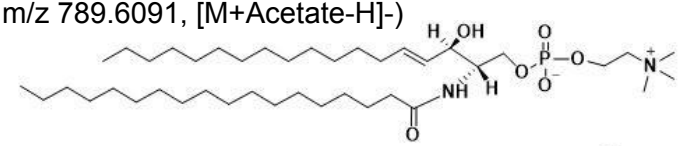


METASPACE annotation: C41H83N2O6P, +Na, m/z 753.5881, SM(d36:1) or PE-Cer(d39:1)

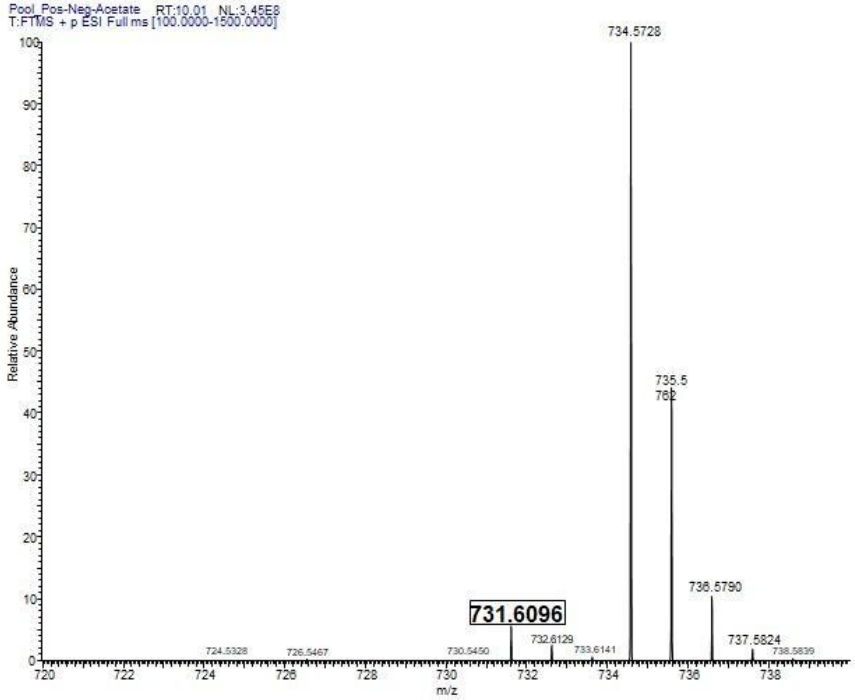
Database used : Metascope Lipidmaps inbuilt library in Progenesis software

Validated as : **SM(d36:1)** using LC-MS/MS method **Lipidomics** (positive mode, m/z 731.6096, [M+H]+ negative mode, m/z 789.6091, [M+Acetate-H]-)

Ambiguities exist due to existence of structural isomers.
Can only confirm C:DB composition i.e SM(d36:1)



Pos Ion Mode; [M+H]+



Neg. Ion Mode; [M+Acetate-H]-