

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 <i>in-silico</i> 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 <i>in-silico</i> 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 <i>in-silico</i> 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) → Lipidomics (negative mode) →	HD: PE SD: 18:0, 22:6 FA

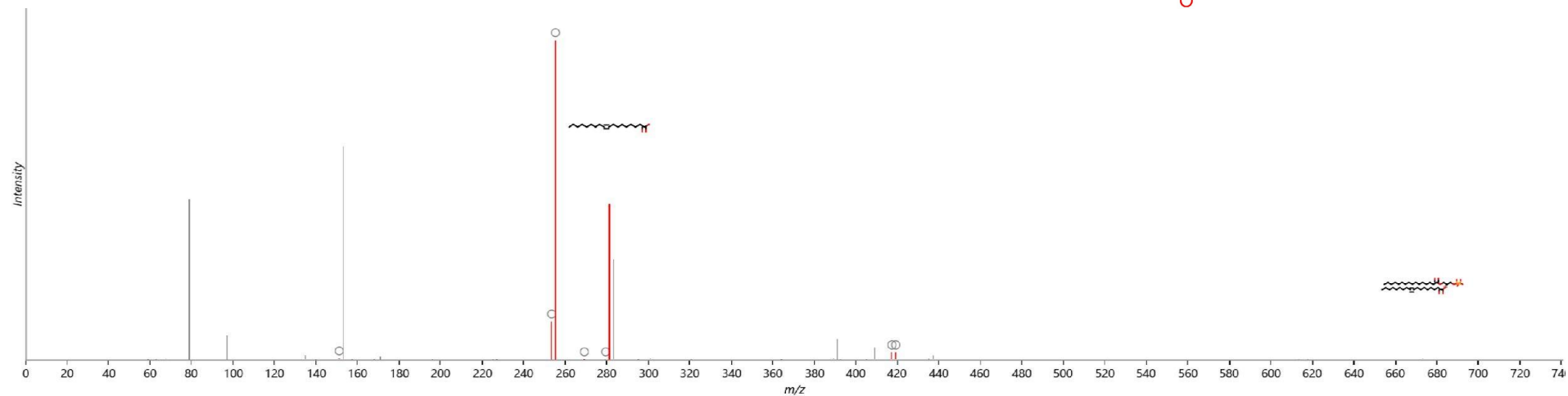
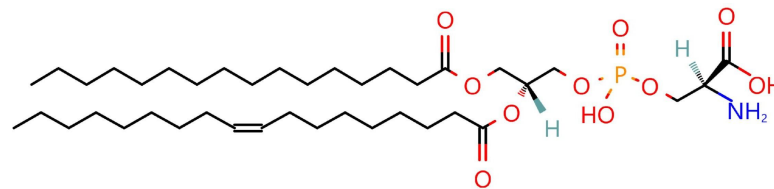
SL: MS/MS spectral library
HD: Head group MS/MS
SD: Fatty acid (FA) side chain

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



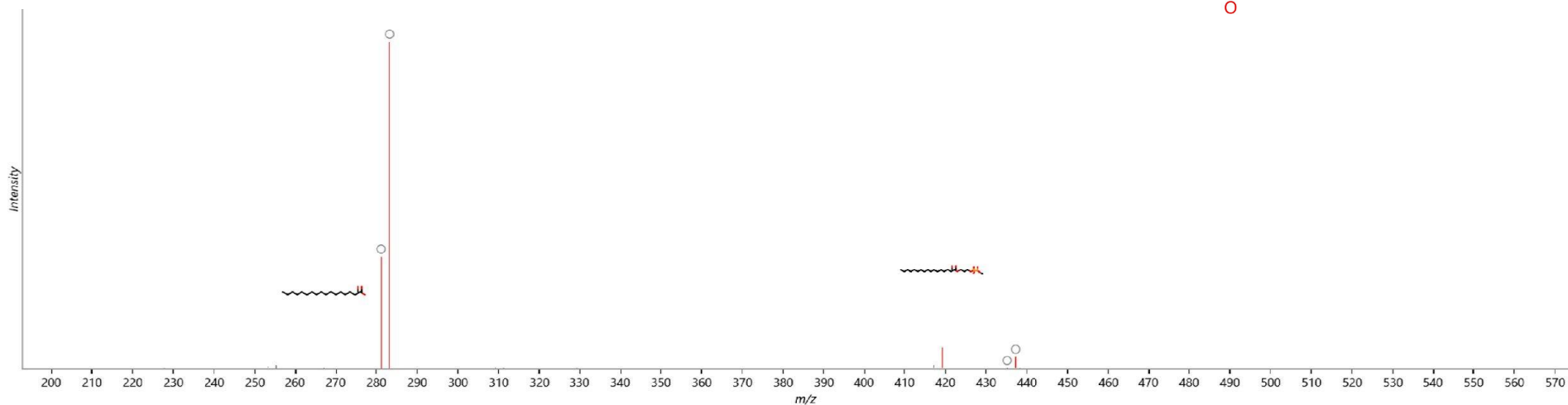
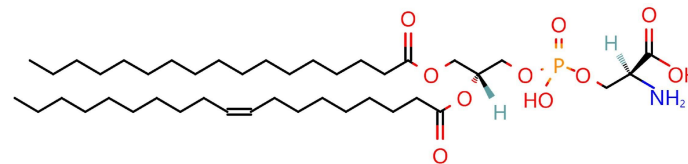
Legend: ■ Matched fragment ■ Unmatched fragment

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

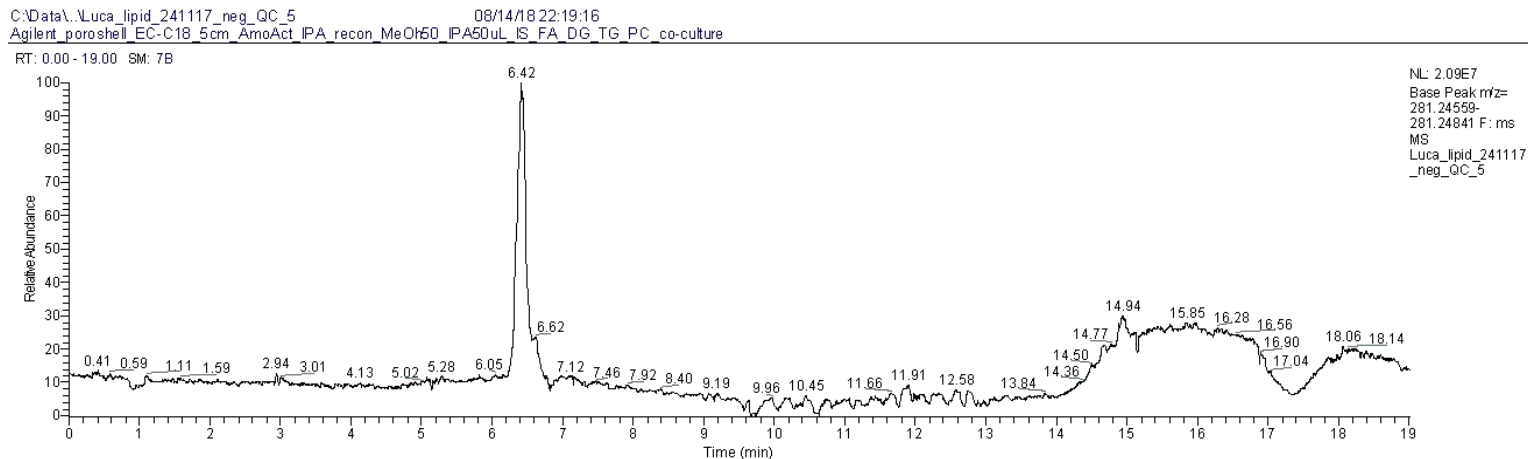


METASPACE annotation: C18H36O2, -H, m/z 283.2642, Stearic acid or another isomeric FA(18:0)

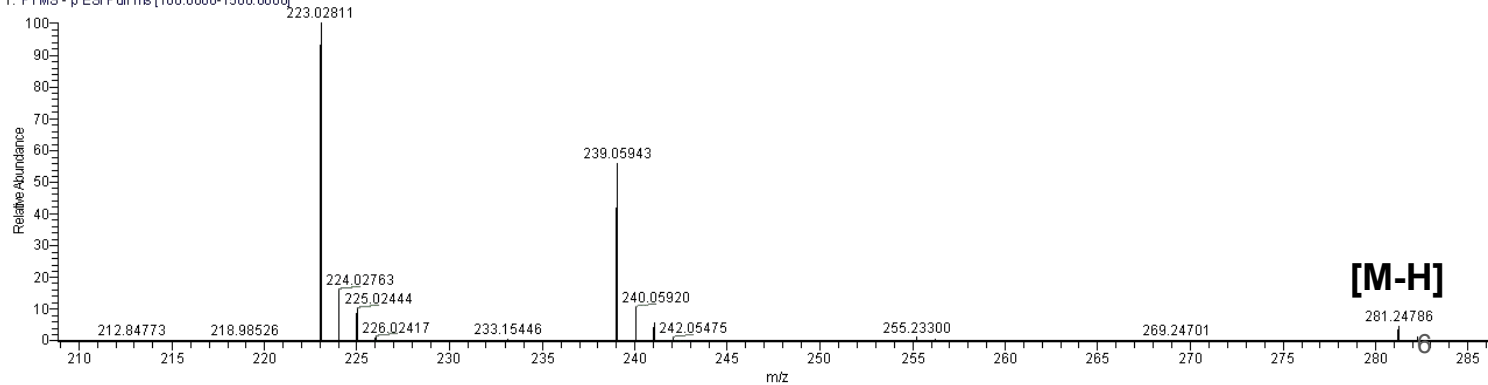
Standard used : Stearic acid (Sigma EC10A)

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

RT match with
standard



Luca_lipid_241117_neg_QC_5 #2730 RT: 6.40 AV: 1 NL: 3.75E8
T: FTMS - p ESI Full ms [100.0000-1500.0000]

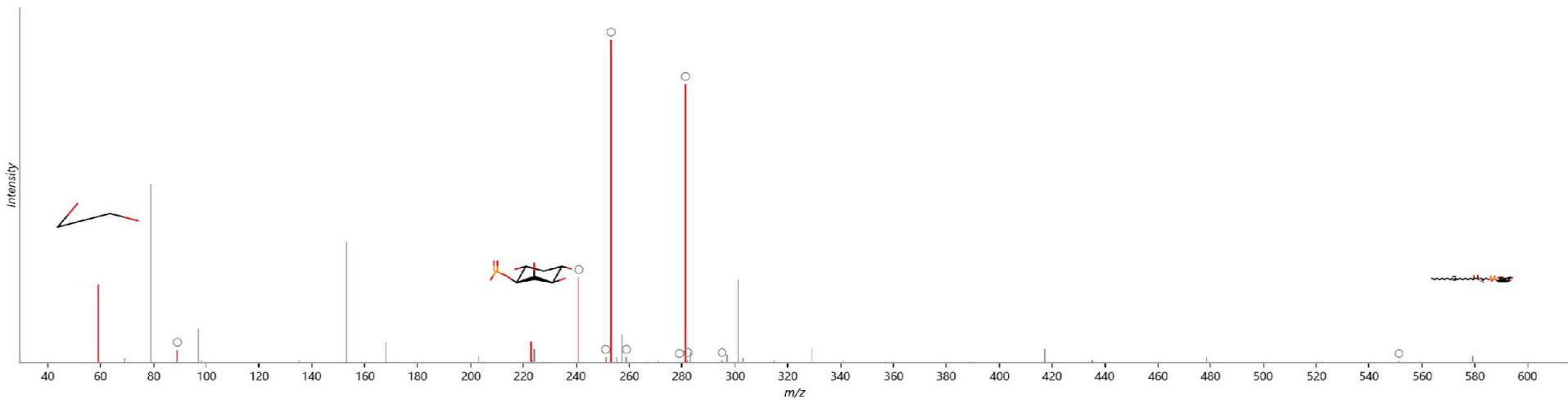
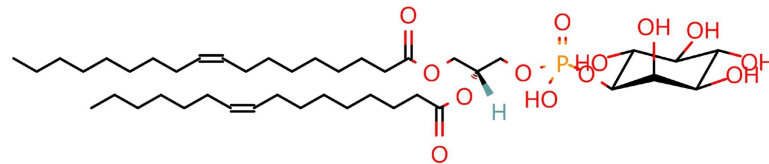


METASPACE annotation: C43H81O13P, -H, m/z 835.5342, PI(34:1)

Database used : Progenesis in-silico Lipid library

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

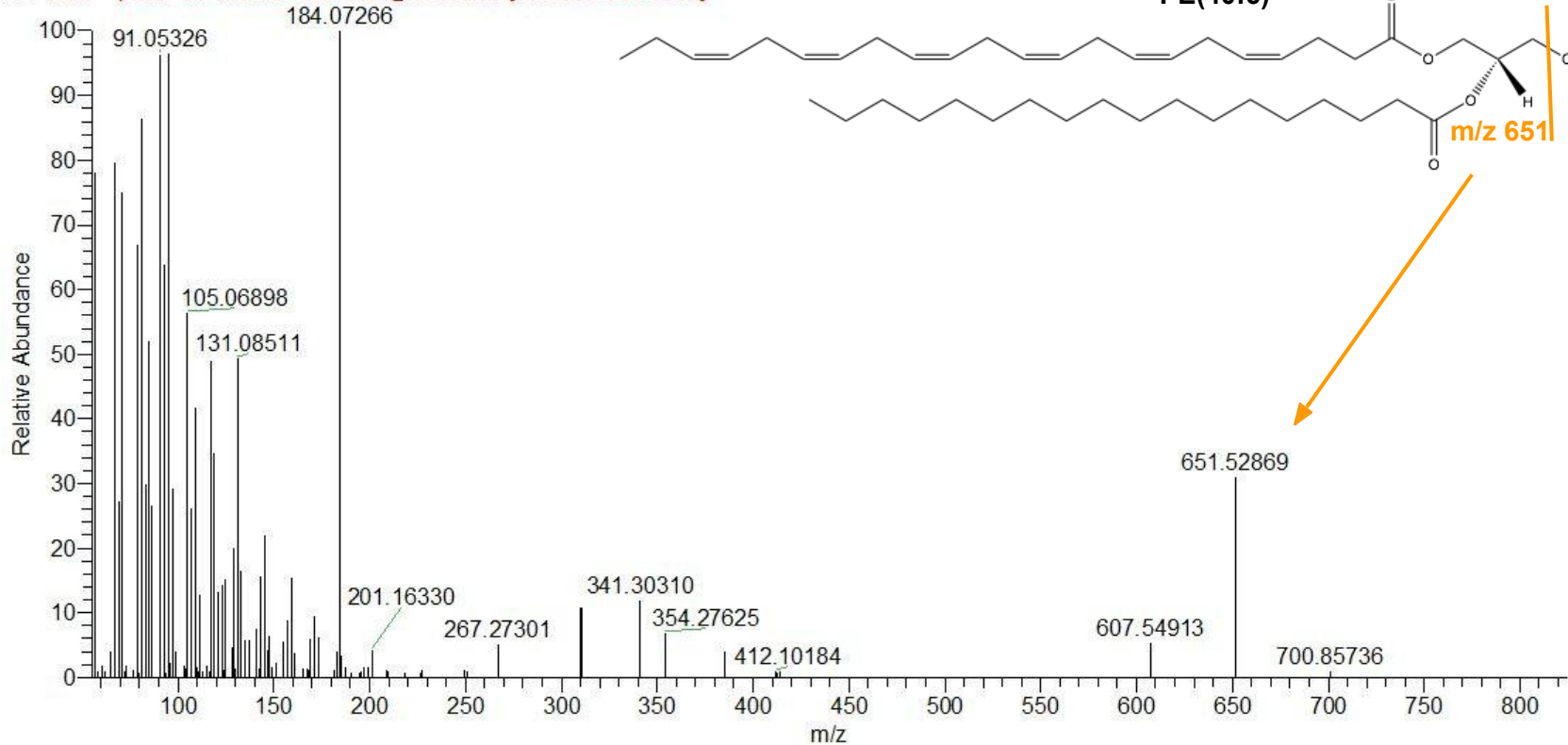
Progenesis *in silico* Lipid library



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)

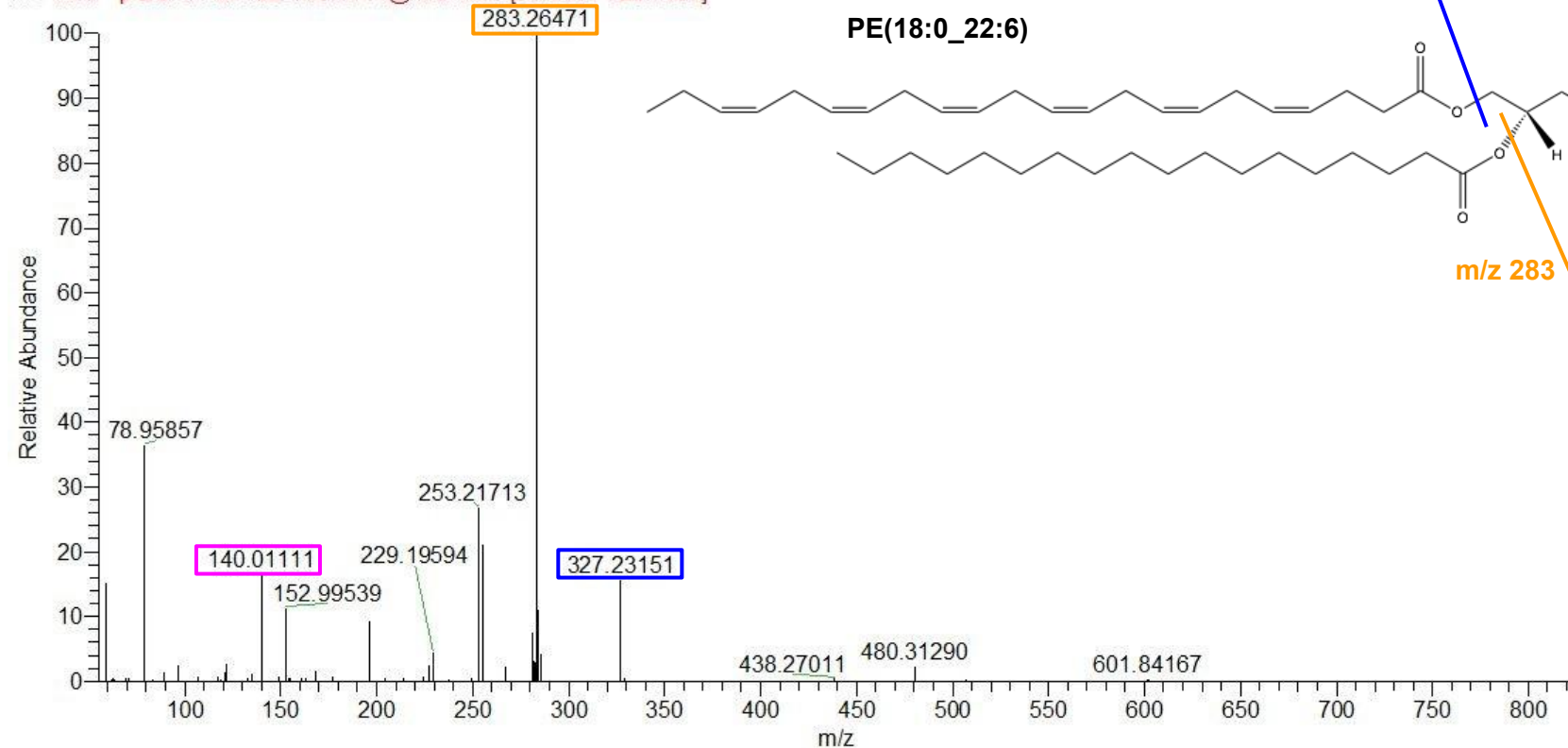
Luca140818_lipid_Pos_co_culture_1 #5723 RT: 12.65 AV: 1 NL: 1.82E6
F: FTMS + p ESI d Full ms2 792.7021@hcd40.00 [55.0000-825.0000]



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

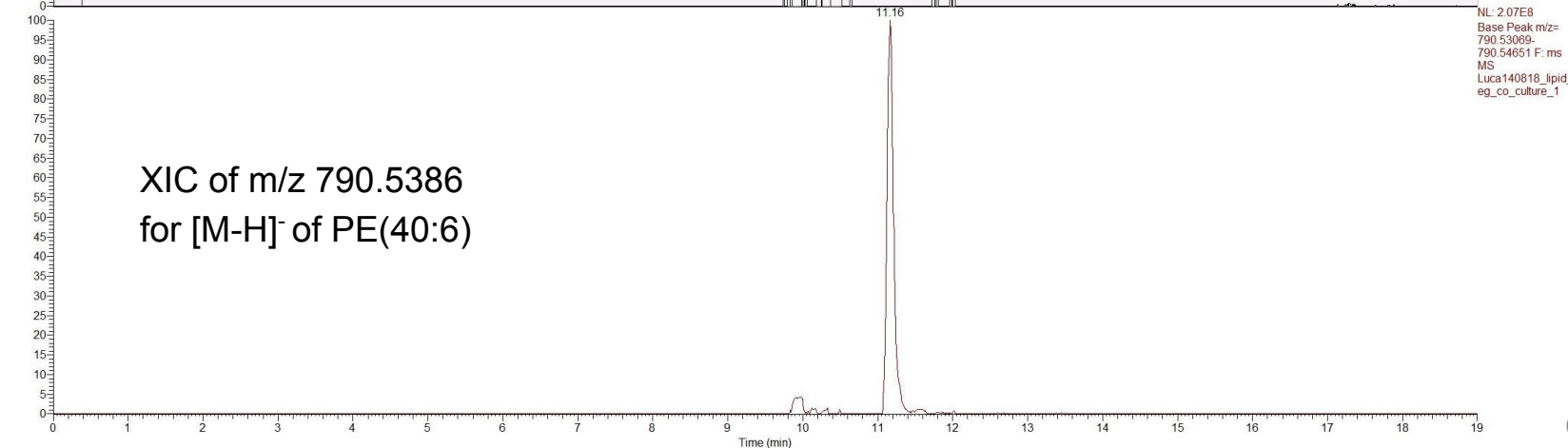
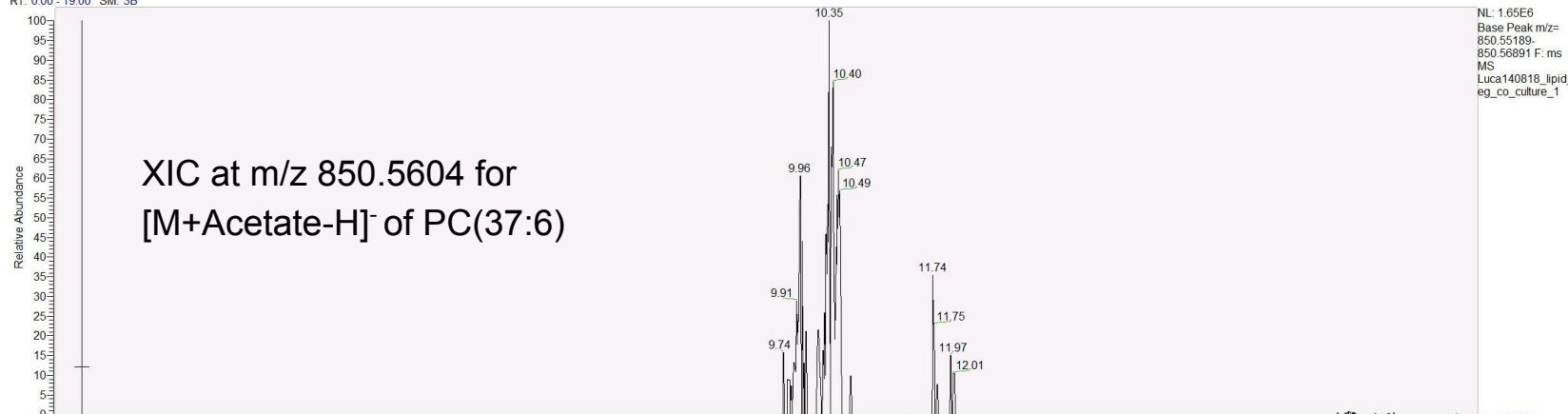
Validated as : PE(18:0_22:6) using LC-MS/MS method Lipidomics (negative mode)

Luca140818_lipid_Neg_co_culture_1 #4783 RT: 11.10 AV: 1 NL: 2.76E6
F: FTMS - p ESI d Full ms2 790.5344@hcd40.00 [55.0000-825.0000]



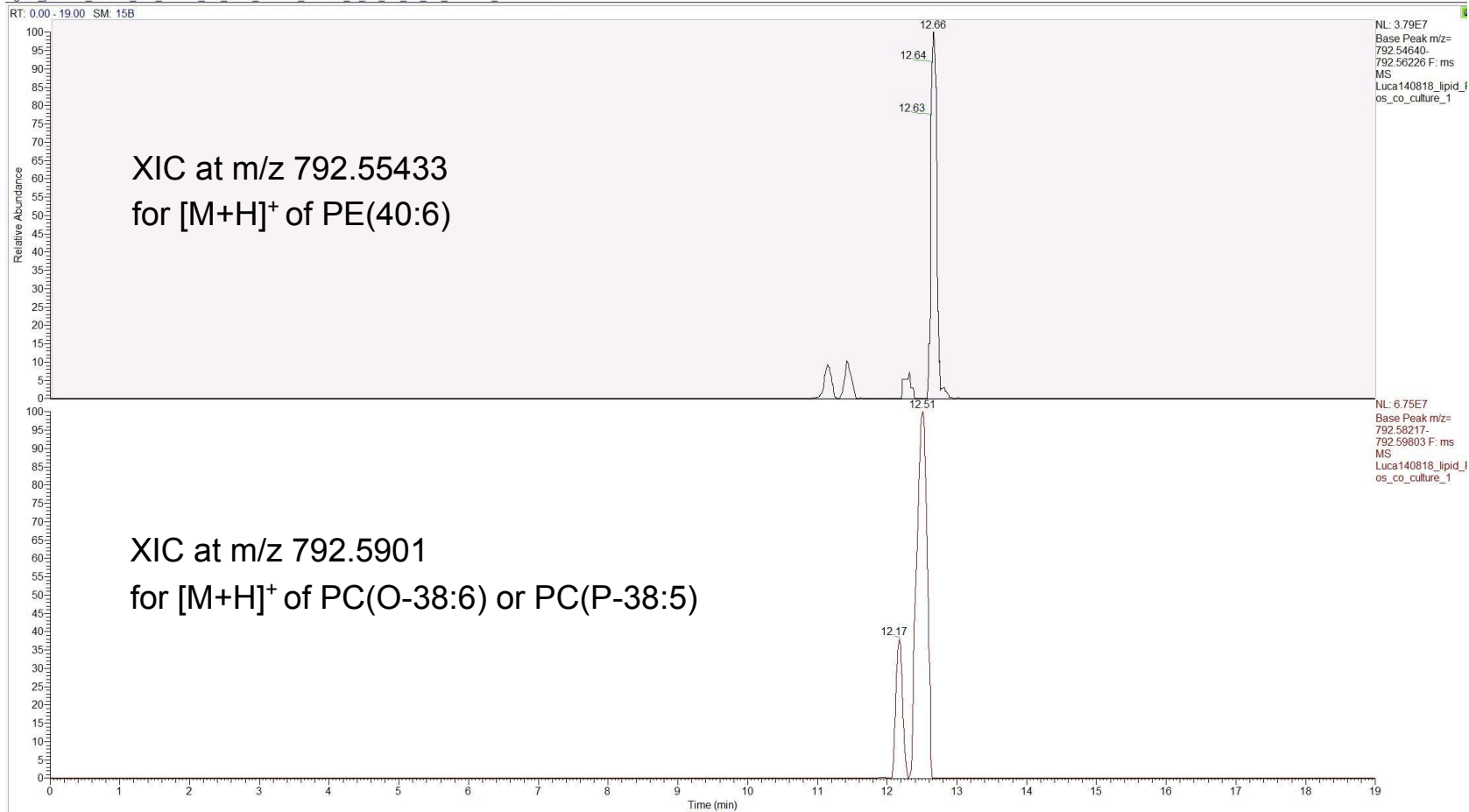
Absence of PC(37:6) and detection of PE(40:6) in the negative mode

Luca140818_lipid_Neg_co_culture_1 08/15/18 00:38:18
Agilent_poroshell_EC-C18_5cm_AmoAct_IPA_recon_MeOH50_IPA50ul_IS_FA_DG_TG_PC_co_culture
RT: 0.00 - 19.00 SM: 3B



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

Luca140818_lipid_Pos_co_culture_1 08/15/18 18:57:38
Agilent_poroshell_EC-C18_5cm_AmoAct_IPA_recon_MeOH50_IPA50uL_IS_FA_DG_TG_PC_co-culture_newESneedle



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 <i>in-silico</i> 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 <i>in-silico</i> spectral library SD: 16:0, 18:1 FA

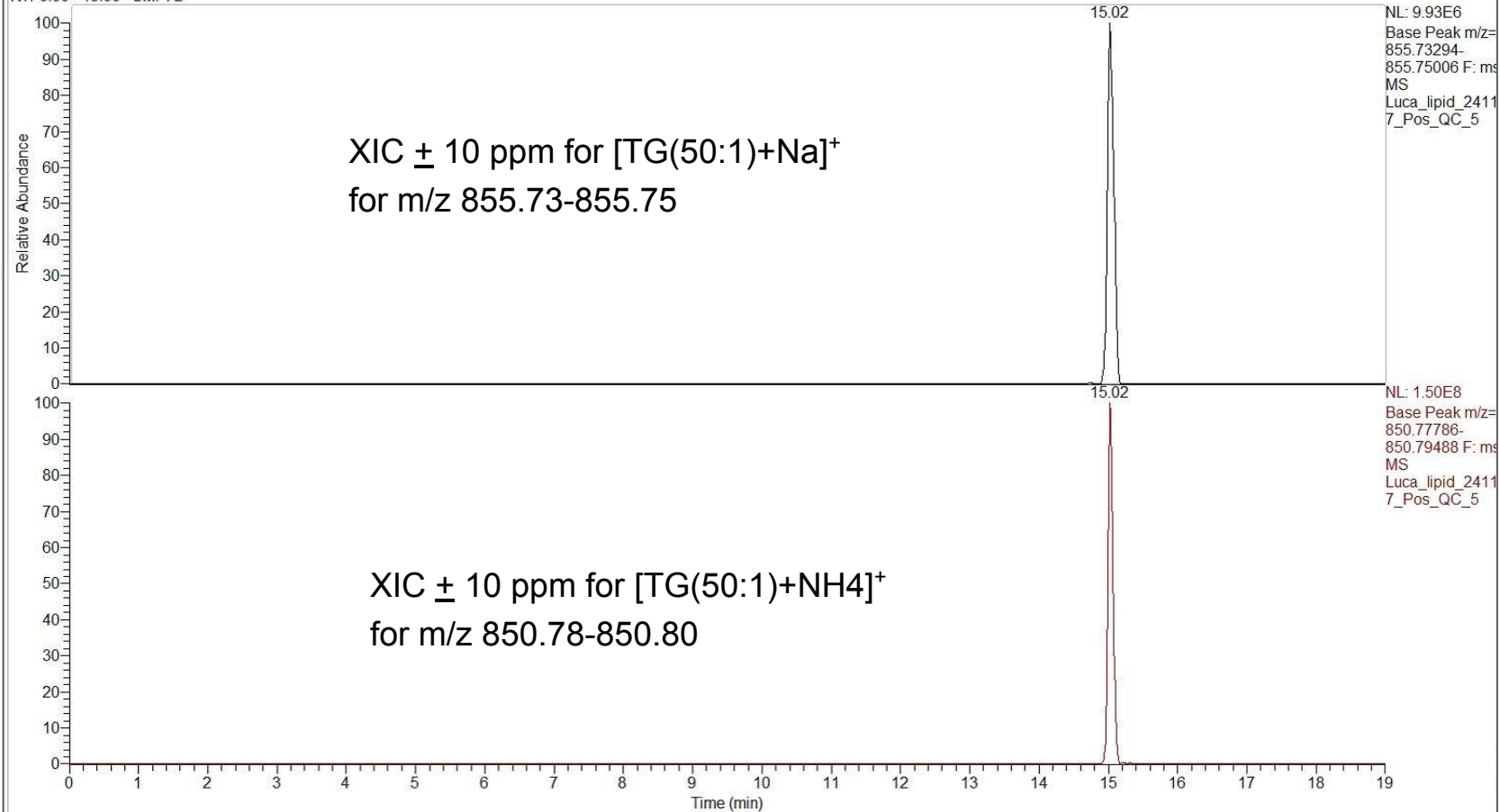
SL: MS/MS spectral library
SD: Fatty acid (FA) side chain

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

Validating detection in cell extract by LCMS

C:\Data\Luca_lipid_241117_Pos_QC_5 08/15/18 16:38:36
Agilent_poroshell_EC-C18_5cm_AmoAct_IPA_recon_MeOH50_IPA50uL_IS_FA_DG_TG_PC_co-culture_newESneedle

RT: 0.00 - 19.00 SM: 7B



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:

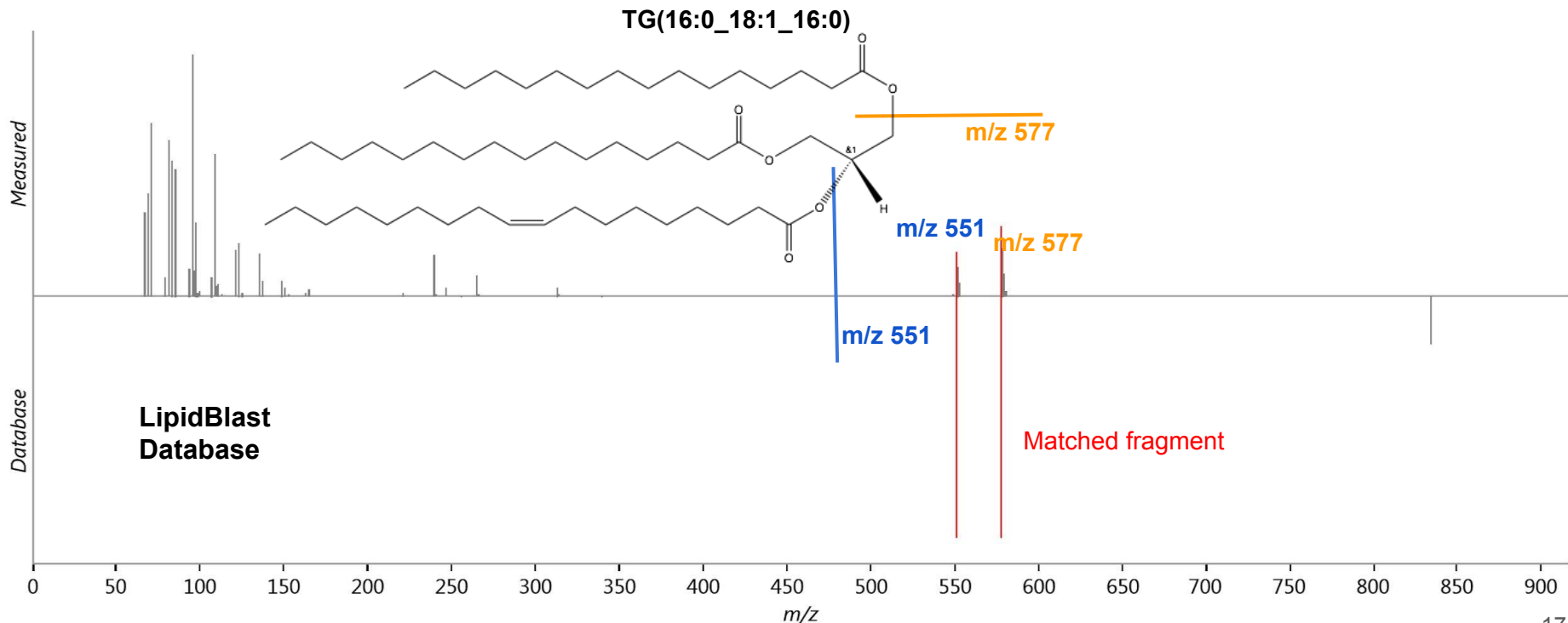
- For 855.74 (corresponding to [TG(50:1)+Na]⁺): **137 hits, 135 of them TGs**
- For 850.785 (corresponding to [TG(50:1)+NH₄]⁺): **33 hits, all of them TGs, all [TG(50:1)+NH₄]⁺**

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)



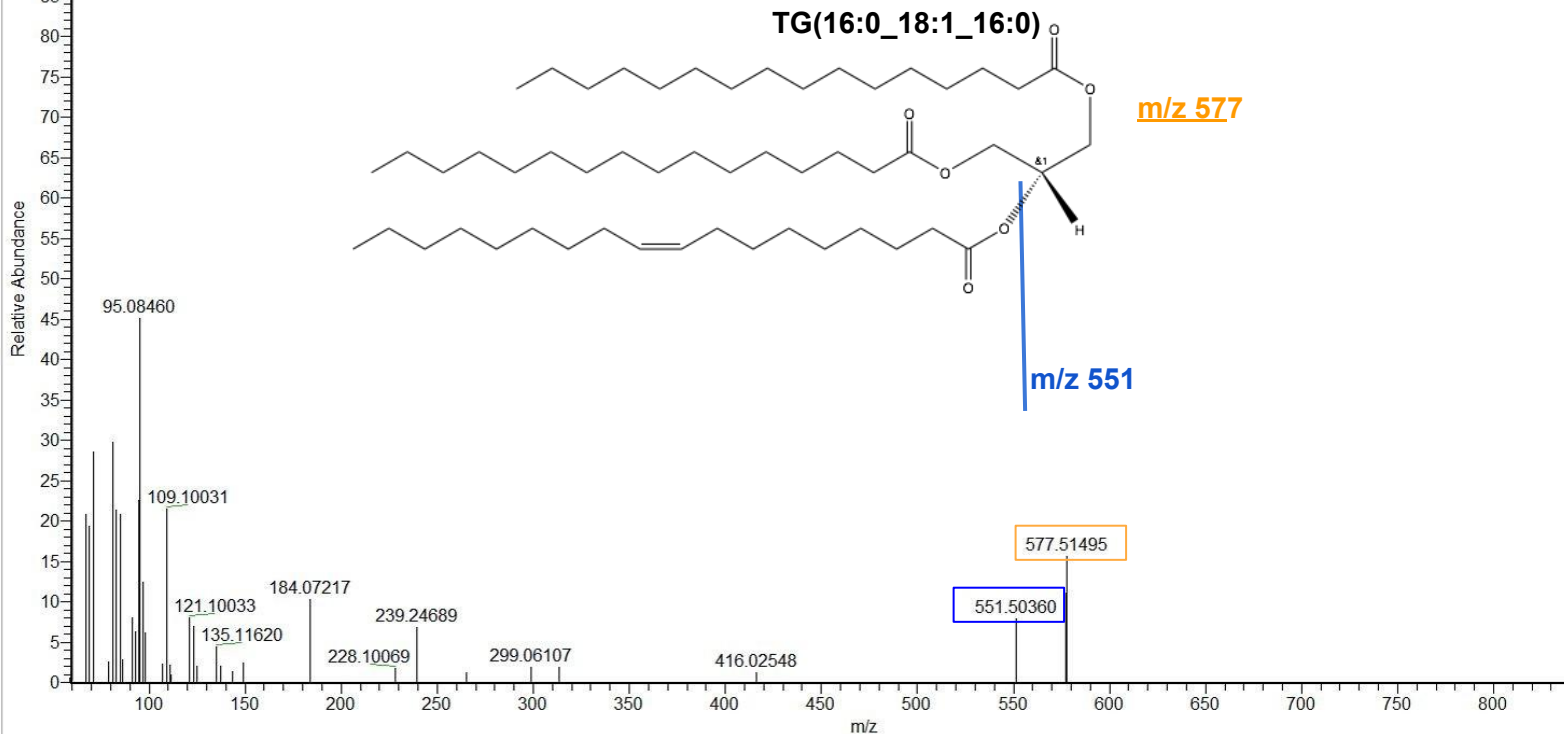
Identifying fatty acyl chains of TG(50:1) for +NH4

Luca_lipid_241117_Pos_QC_5_#7099 RT: 15.20 AV: 1 NL: 2.23E5
F: FTMS + p ESI d Full ms2 850.6609@hcd40.00 [59.0000-885.0000]

METASPACE annotation: C53H100O6, +NH4, m/z 850.7863, 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)

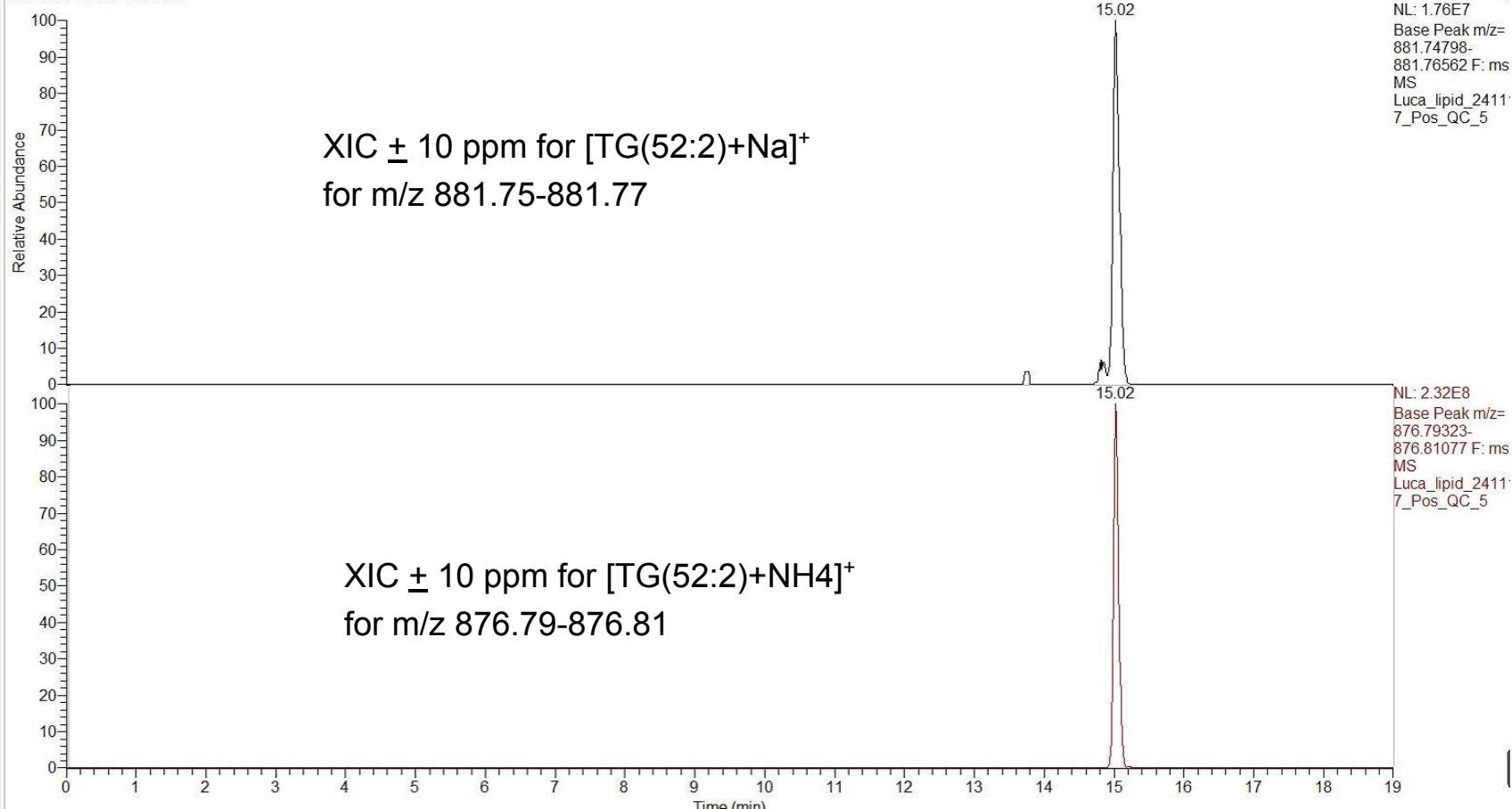


Validating TG(52:2) as TG(16:0_18:1_18:1)

Validating detection in cell extract by LCMS

C:\Data\..Luca_lipid_241117_Pos_QC_5 08/15/18 16:38:36
Agilent_poroshell_EC-C18_5cm_AmoAct_IPA_recon_MeOH50_IPA50uL_IS_FA_DG_TG_PC_co-culture_newESIneedle

RT: 0.00 - 19.00 SM: 7B



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]⁺**

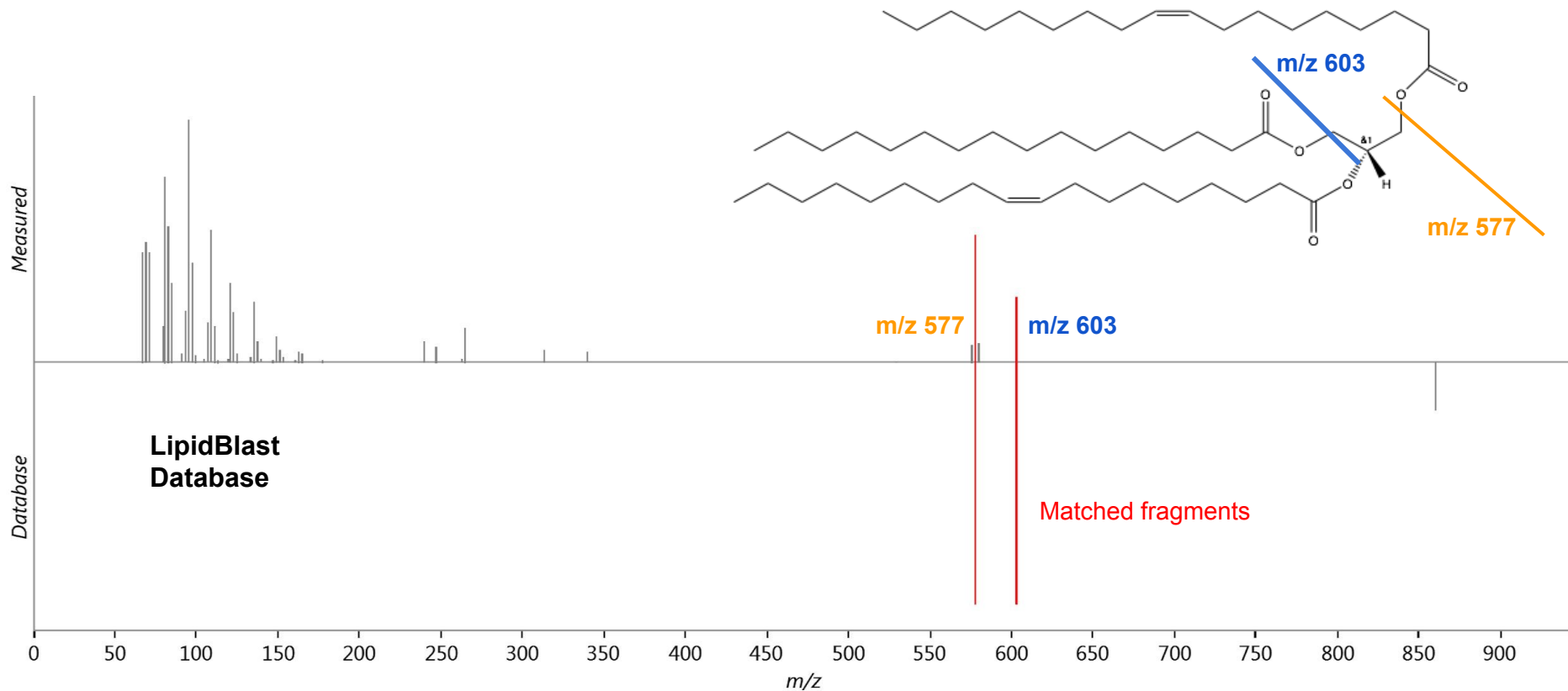
Identifying fatty acyl chains of TG(52:2) for +Na

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

Luca_lipid_241117_Pos_QC_4 #7290 RT: 15.54 AV: 1 NL: 5.69E4

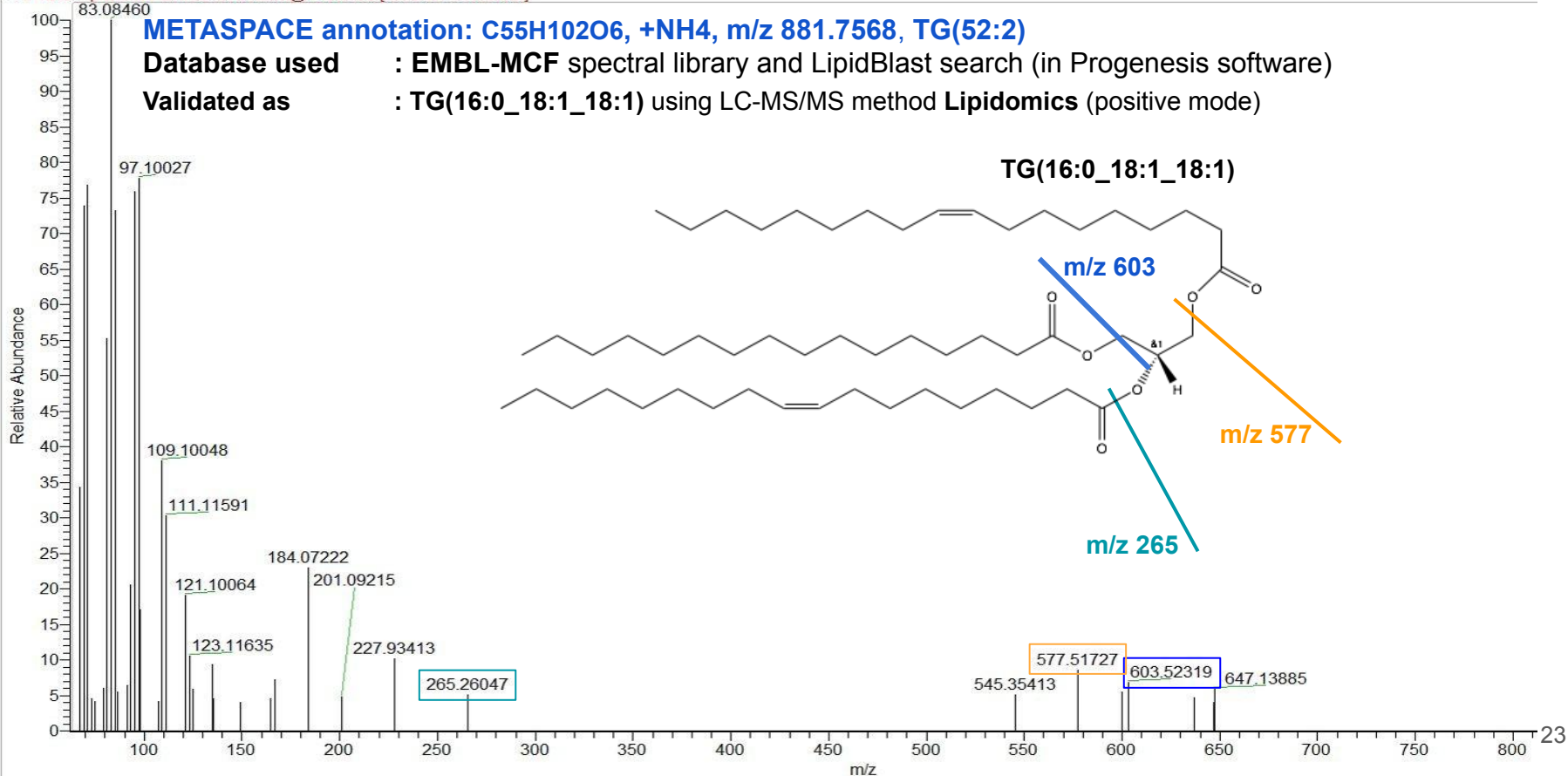
F: FTMS + p ESI d Full ms2 876.6539@hcd40.00 [60.6667-910.0000]

83.08460

METASPACE annotation: C55H102O6, +NH4, 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)



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.

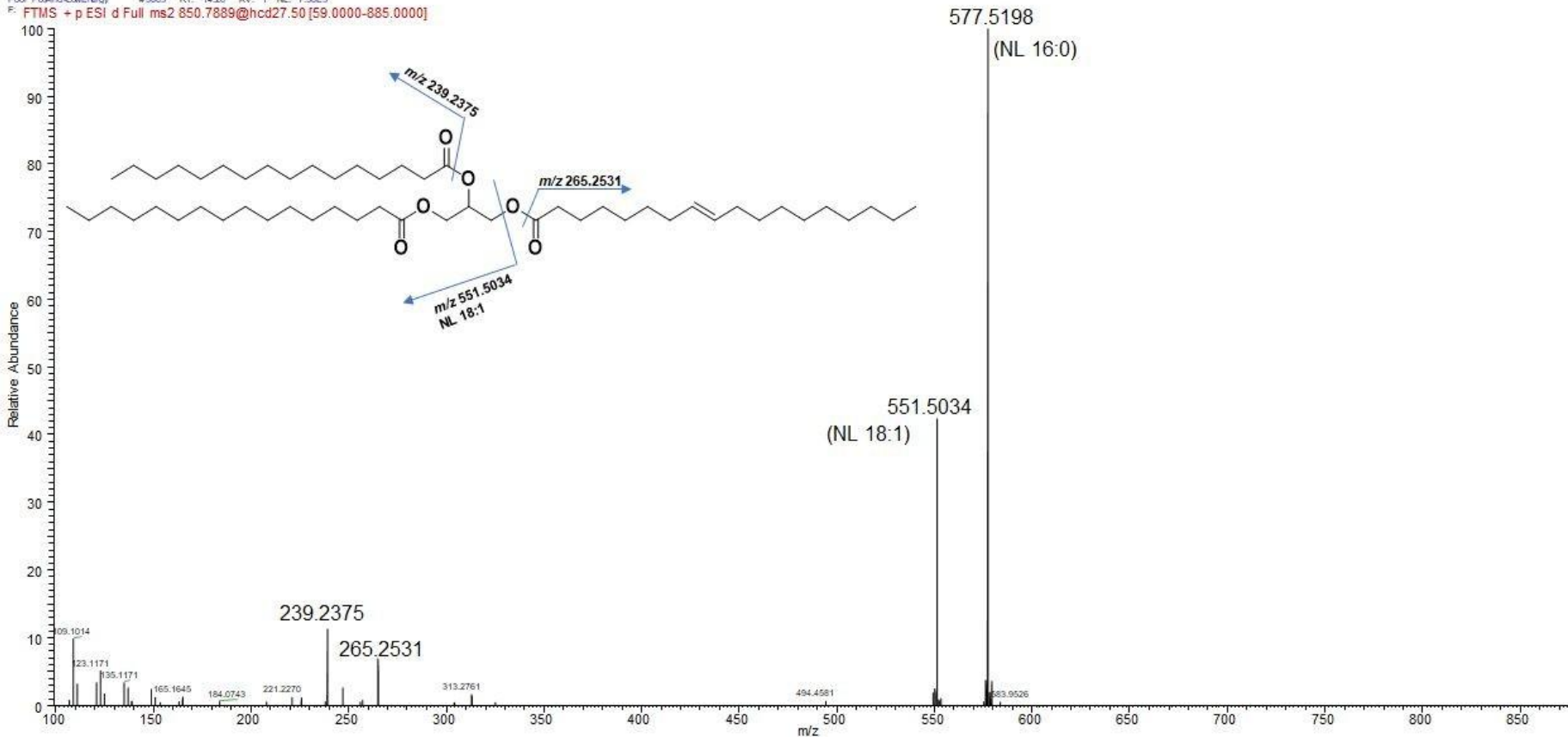
1. Fragmentation at low energy of all significant TGs (CE = 27). The TGs were added into an inclusion list
2. Fragmentation at low energy of all significant TGs (CE = 40). The TGs were added into an inclusion list
3. A polarity switching experiment of the pool using positive mode mobile phases (Ammonium 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 : Metaspace 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]+)

Pool_PosInd:LowEnergy #3065 RT: 14.28 AV: 1 NL: 7.58E5
F: FTMS +p ESI d Full ms2 850.7889@hcd27.50 [59.0000-885.0000]



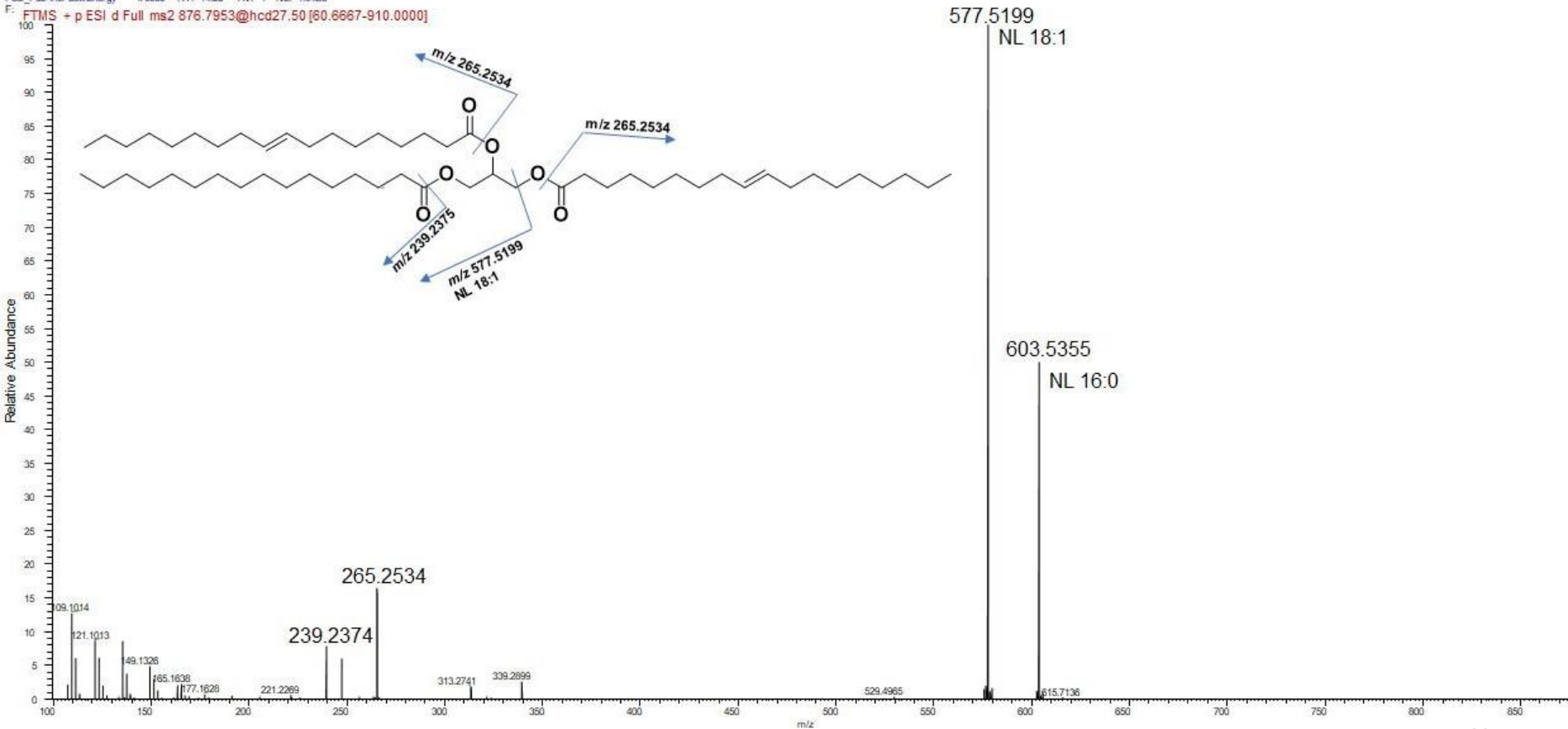
METASPACE annotation: C55H102O6, +Na, m/z 881.7568, TG(52:2)

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]⁺)

Pool_Pos-Incl-LowEnergy #3060 RT: 14.26 AV: 1 NL: 1.94E6

F: FTMS + p ESI d Full ms2 876.7953@hcd27.50 [60.6667-910.0000]

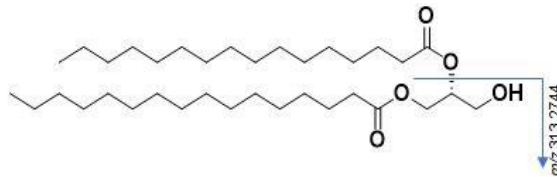
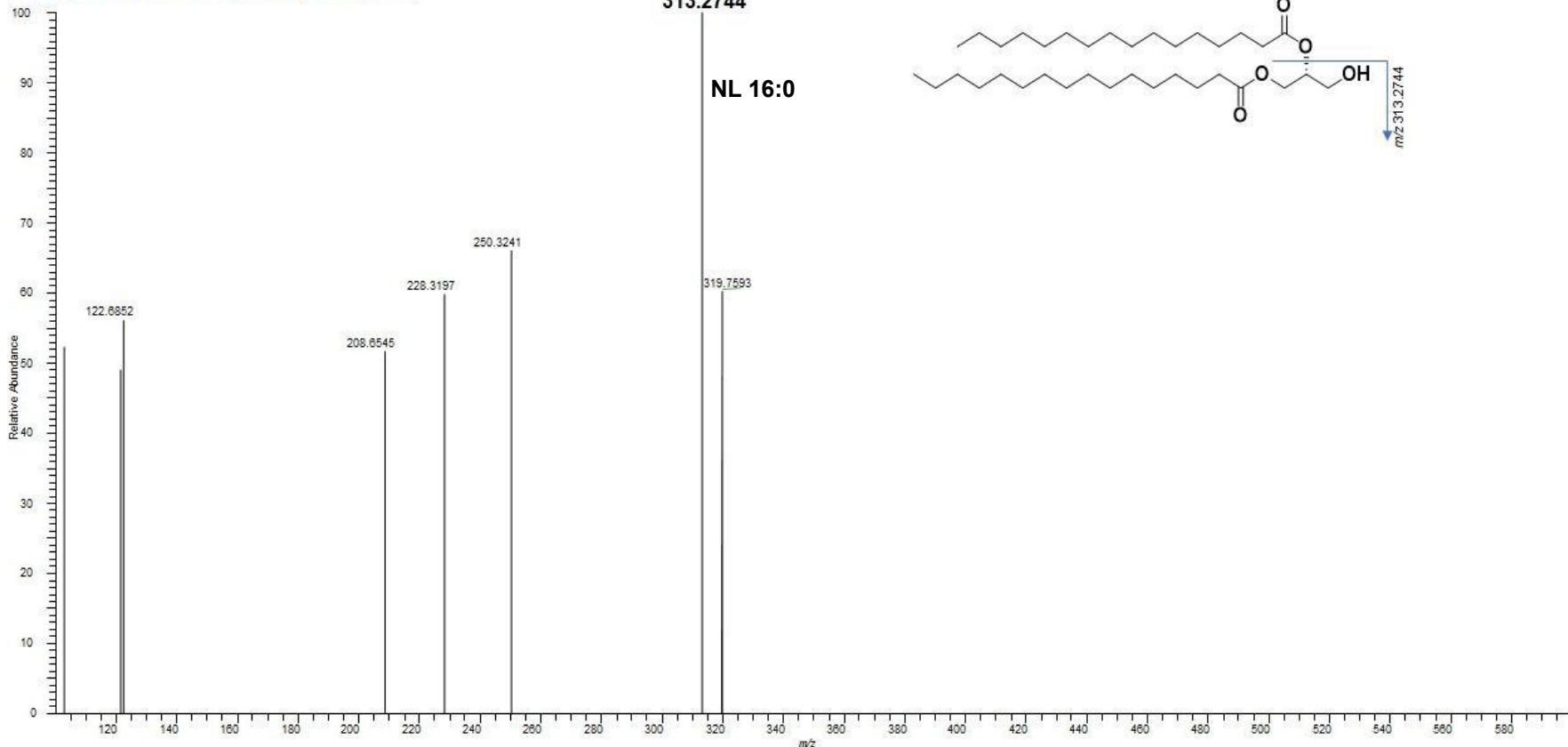


METASPACE annotation: C35H68O5, +Na, m/z 591.4959, DG(32:0)

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]⁺)

NZ_1 #4277 RT: 11.28 AV: 1 NL: 5.38E3
F: FTMS + p ESI d Full ms2 586.5408@hcd40.00 [50.0000-615.0000]

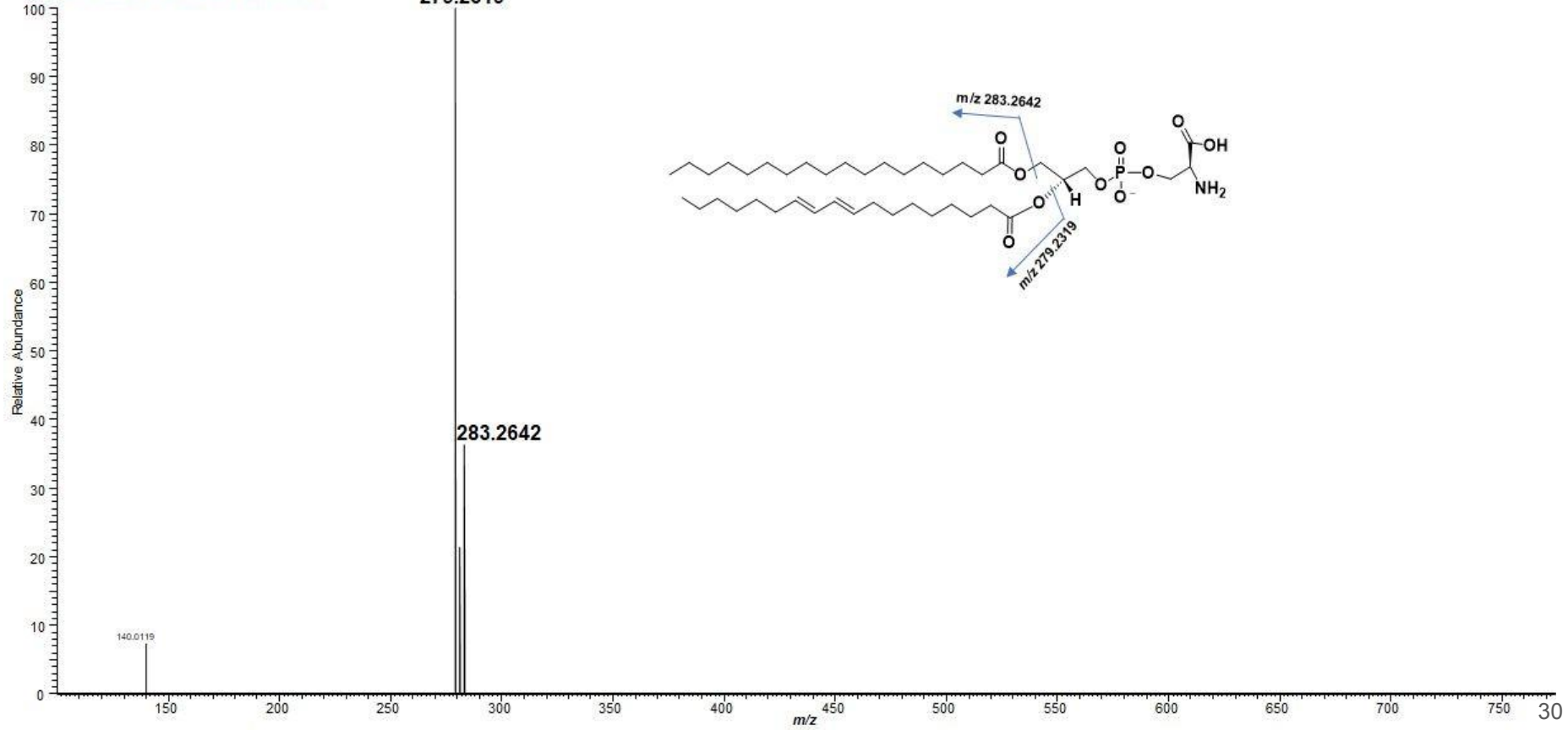


METASPACE annotation: C41H78NO8P, +H, m/z 744.5537, PE(36:2) or PC(33:2)

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]-)

C3 1 #3622 RT: 10.22 AV: 1 NL: 4.39E4
F: FTMS - p ESI d Full ms2 742.5346@hcd40.00 [51.6967:775.0000]

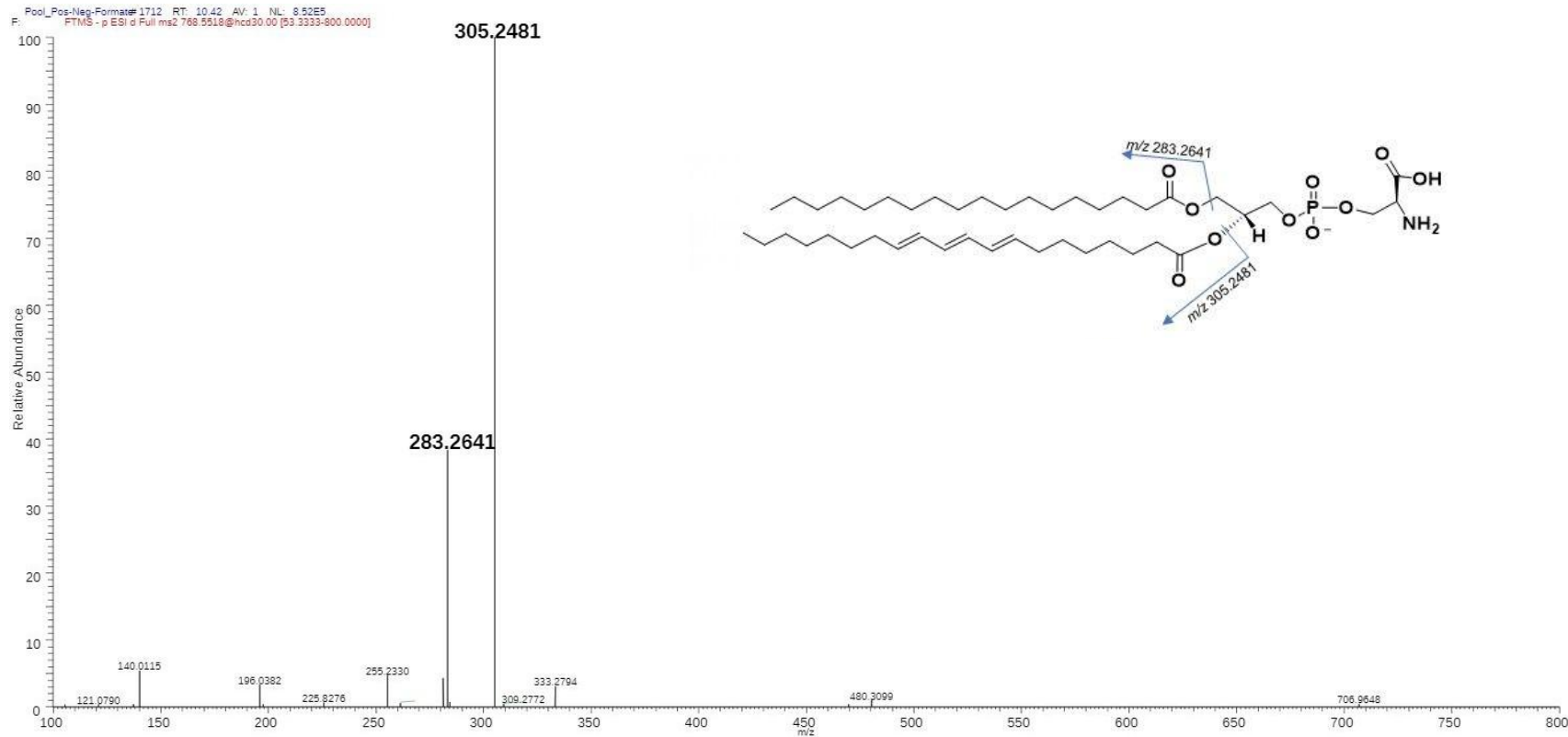


METASPACE annotation: C43H80NO8P, +H, m/z 770.5694, PE(38:3) or PC(35:3)

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]-)

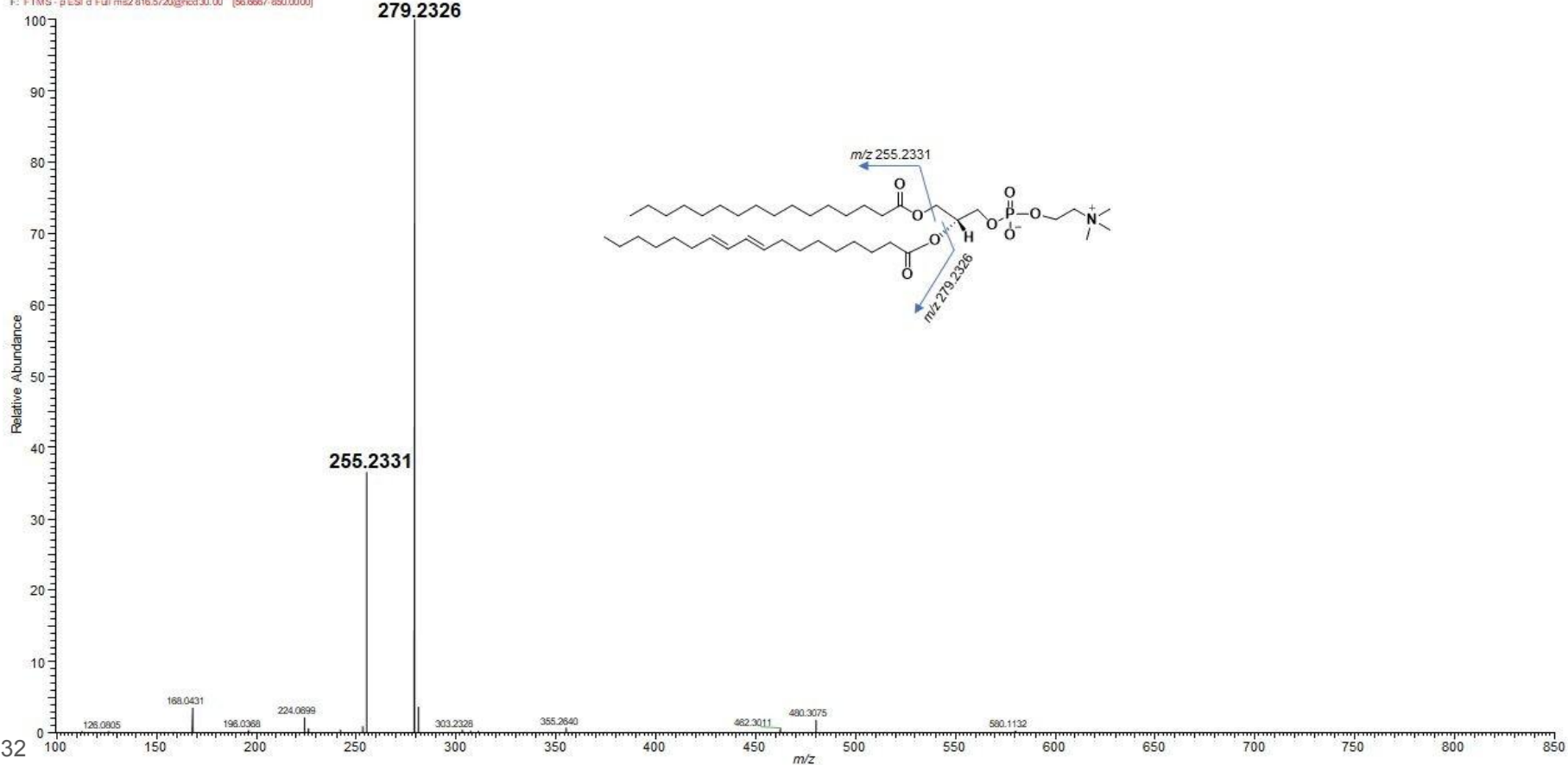


METASPACE annotation: C42H80NO8P, +Na, m/z 780.5513, PE(37:2) or PC(34:2)

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]-)

Pool Pos-Neg-Acetate: #2506 RT: 9.77 AV: 1 NL: 1.73E6
F: FTMS - p ESI d Full ms2 816.5720@hcd30.00 [56.6667-650.0000]

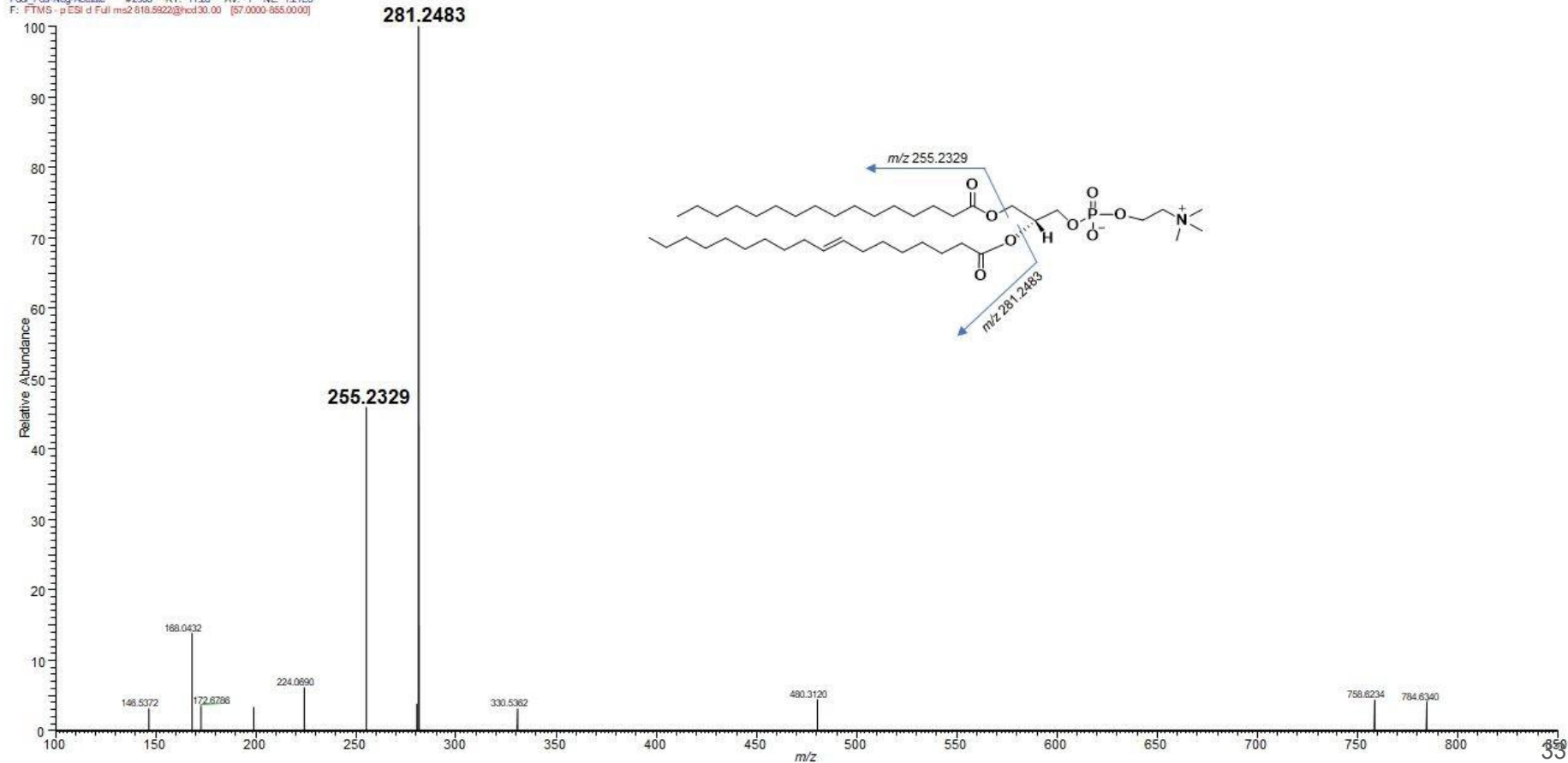


METASPACE annotation: C42H82NO8P, +H, m/z 760.585, PE(37:1) or PC(34:1)

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]-)

Peak Pos Neg Acetate #2955 RT: 11.26 AV: 1 NL: 1.21E5
F: FTMS - p ESI d Full ms2 818.5922@hcd30.00 [57.0000 855.0000]

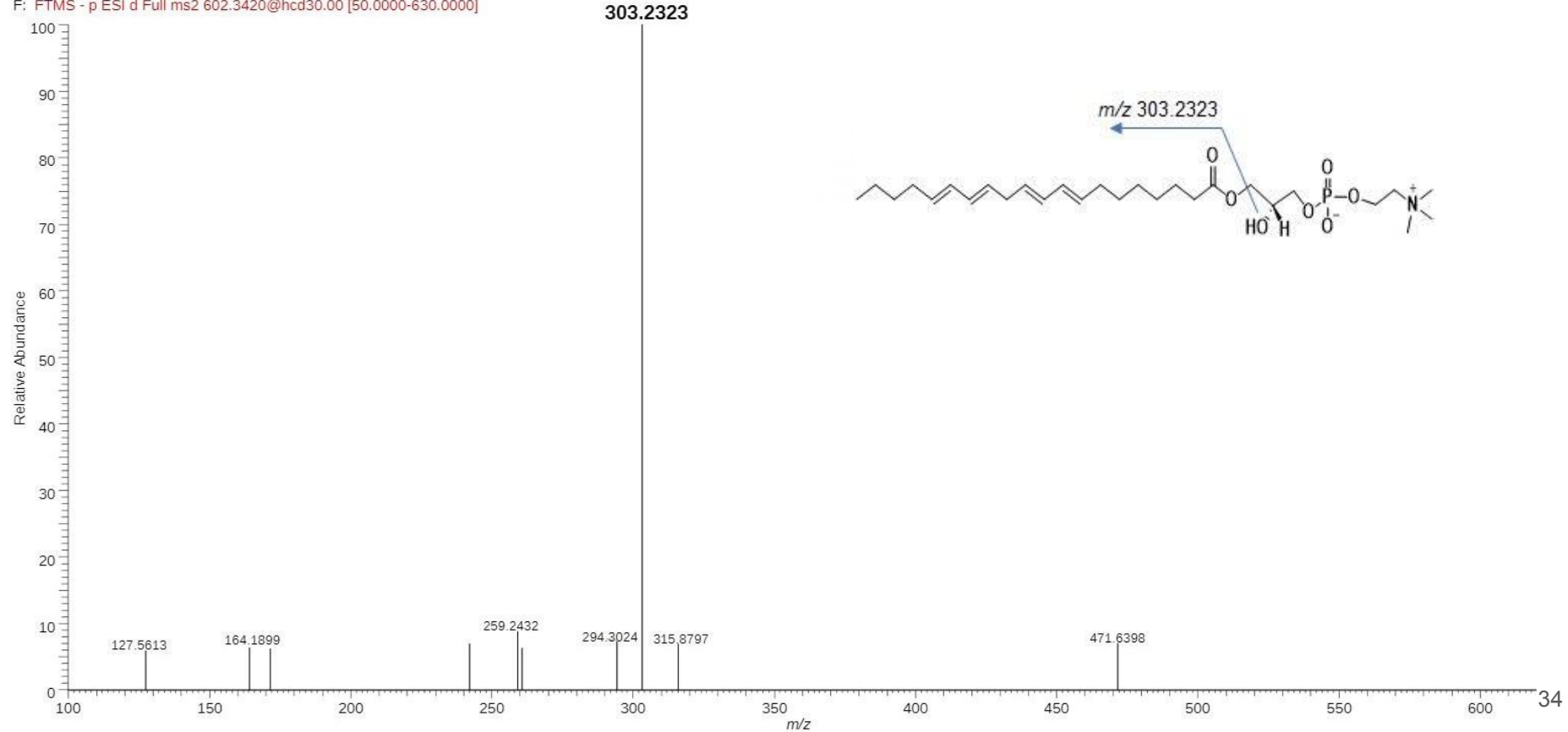


METASPACE annotation: C28H50NO7P, +H, m/z 544.3397, LPE(23:4) or LPC(20:4)

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]-)

Pool_Pos-Neg-Acetate#454 RT: 1.79 AV: 1 NL: 4.58E4
F: FTMS - p ESI d Full ms2 602.3420@hcd30.00 [50.0000-630.0000]



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

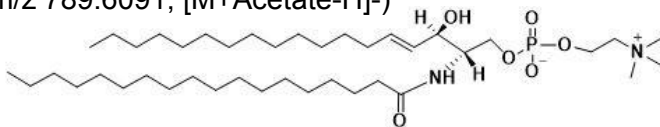
Database used : Metascope Lipidmaps inbuilt library in Progenesis software

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]⁻)

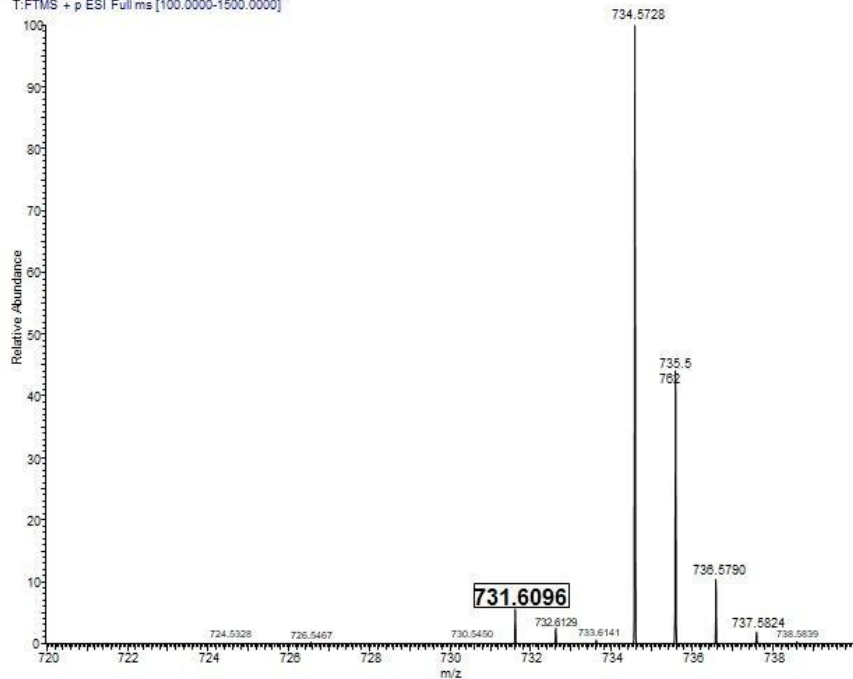
Ambiguities exist due to existence of structural isomers.

Can only confirm C:DB composition i.e SM(d36:1)



Pos Ion Mode; [M+H]⁺

Pool_Pos-Neg-Acetate RT:10.01 NL:3.45E8
T:FTMS - p ESI Full ms [100.0000-1500.0000]



Neg. Ion Mode; [M+Acetate-H]⁻

Pool_Pos-Neg-Acetate RT:10.03 NL:5.75E7
T:FTMS - p ESI Full ms [100.0000-1500.0000]

