Boosting annotation confidence in untargeted lipidomics experiments by the use of complementary chemical properties

Mar Garcia-Aloy, Domenico Masuero, Graziano Guella, Urska Vrhovsek, Pietro Franceschi

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Italian Metabolomics Network General Meeting 2023 | https://github.com/mar-garcia/IMN2023

Introduction:

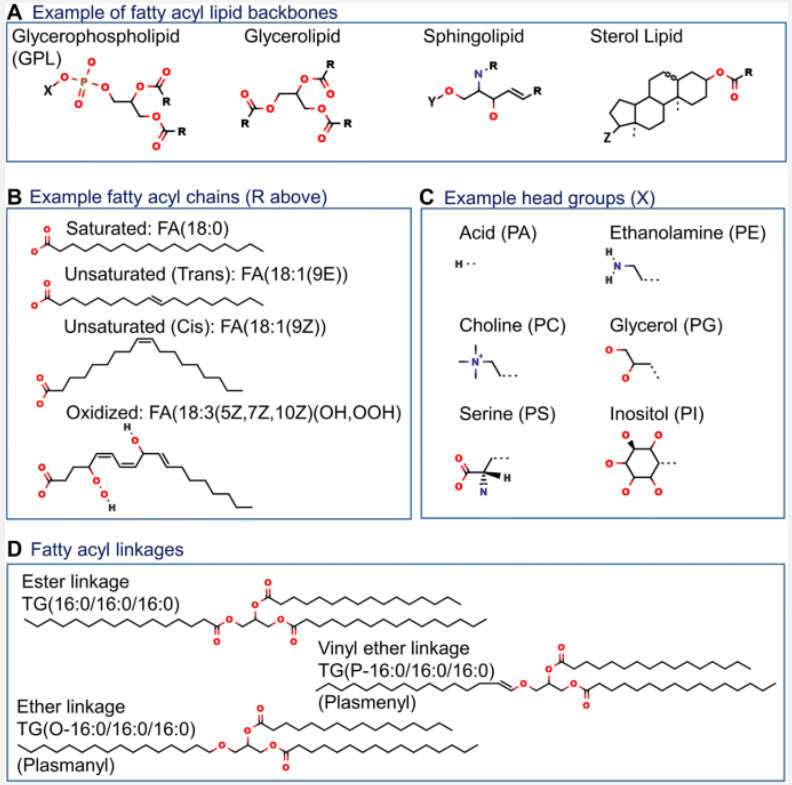
Lipids are typically composed of various building blocks:

a) the lipid backbone

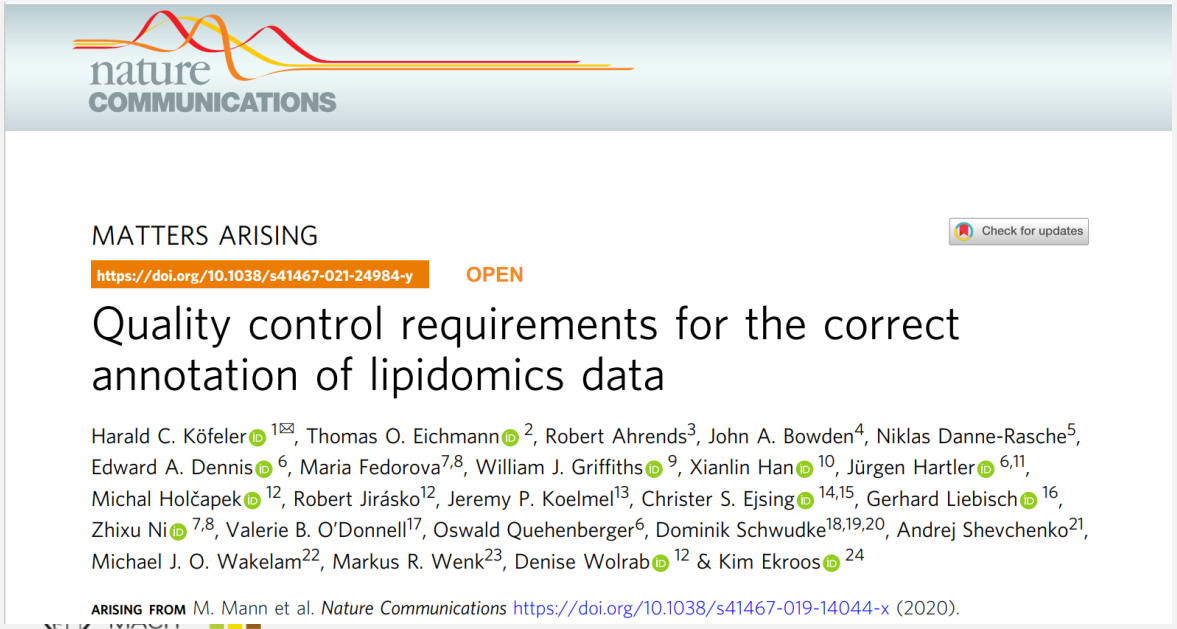
b) fatty acyl chains

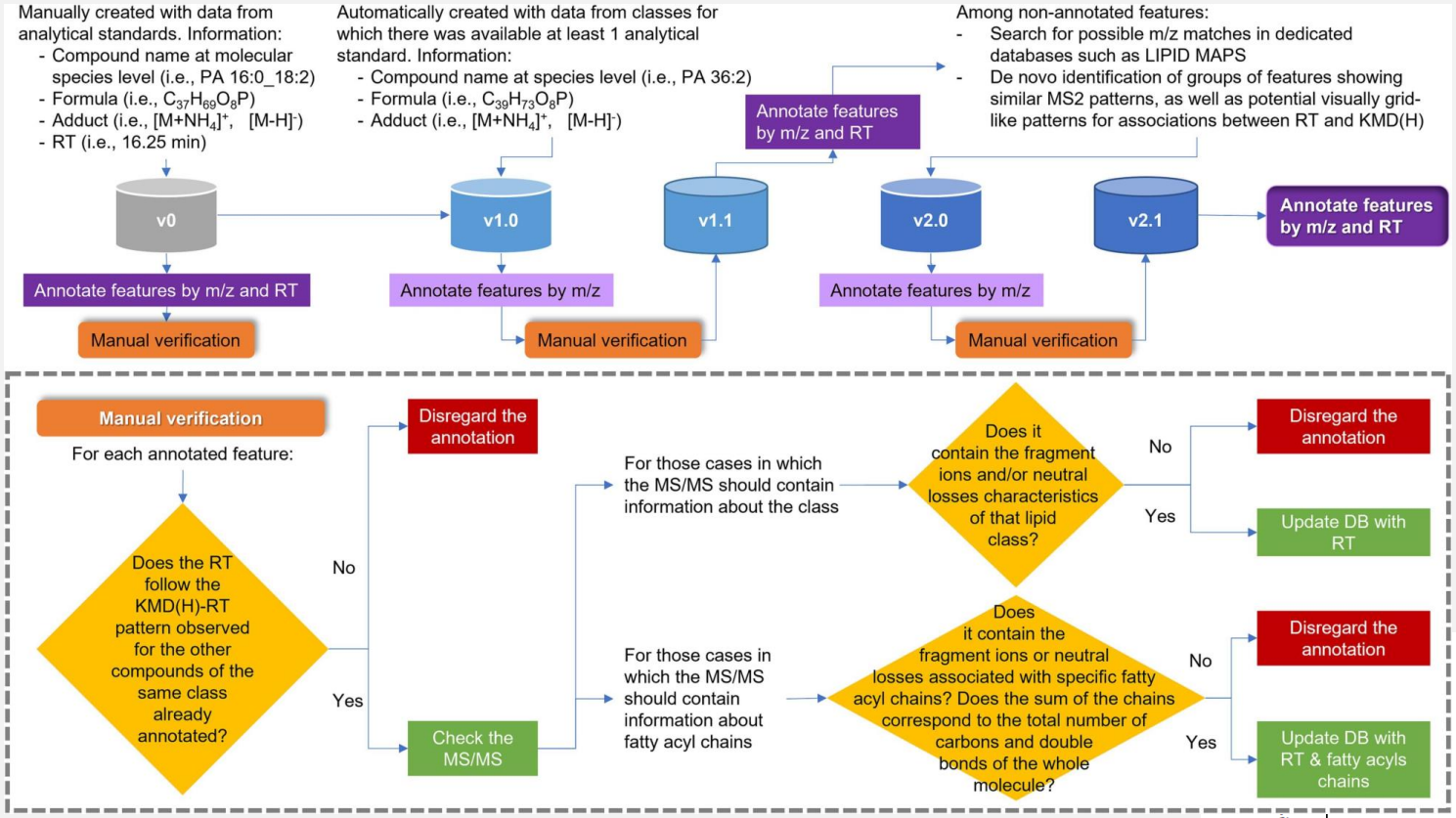
c) head groups

d) various linkages between backbone and fatty acyl constituents



Koelmel, J.P., Napolitano, M.P., Ulmer, C.Z. et al. Metabolomics 16, 56 (2020). https://doi.org/10.1007/s11306-020-01665-3





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Untargeted lipidomic profiling of grapes highlights the importance of modified lipid species beyond the traditional compound classes

Mar Garcia-Aloy [a,*], Domenico Masuero [a], Giulia Chitarrini [a], Domen Škrab [a,b], Paolo Sivilotti [b], Graziano Guella [c], Urska Vrhovsek [a], Pietro Franceschi [d]

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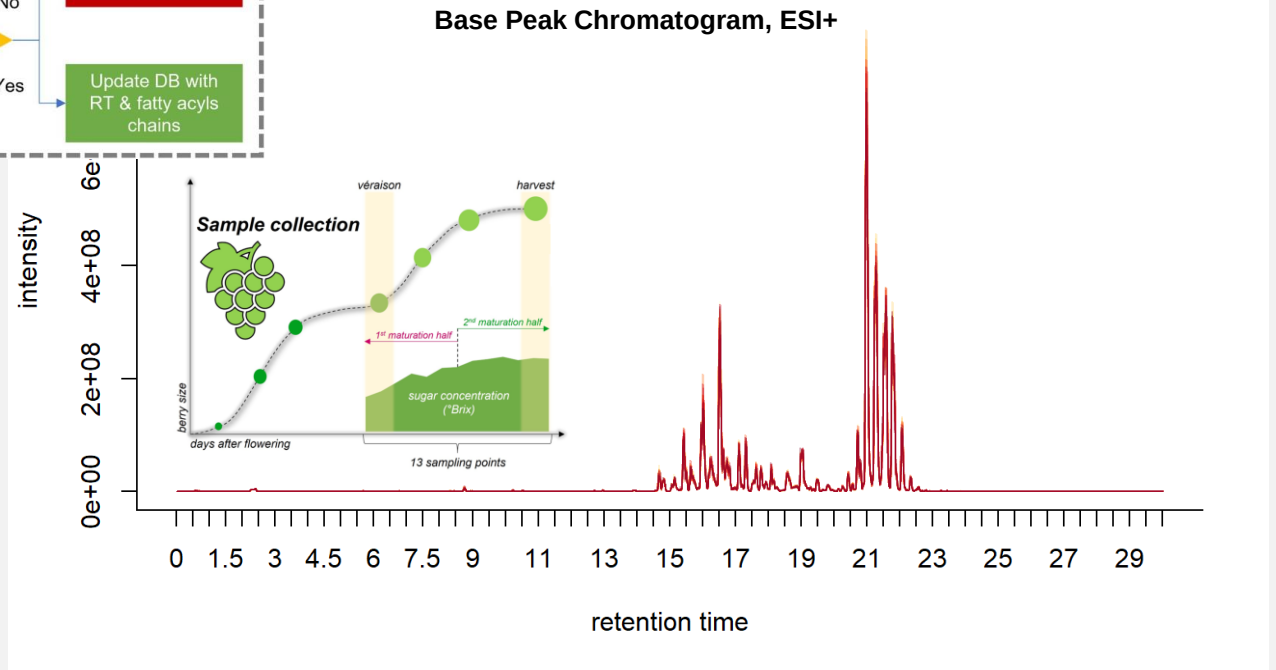
[b] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, 33100 Udine, Italy
[c] Bioorganic Chemistry Laboratory, Department of Physics, University of Trento, 38123 Trento, Italy
[d] Unit of Computational Biology, Research and Innovation Centre, Fondazione Edmund Mach, 38098 San Michele all'Adige, Italy



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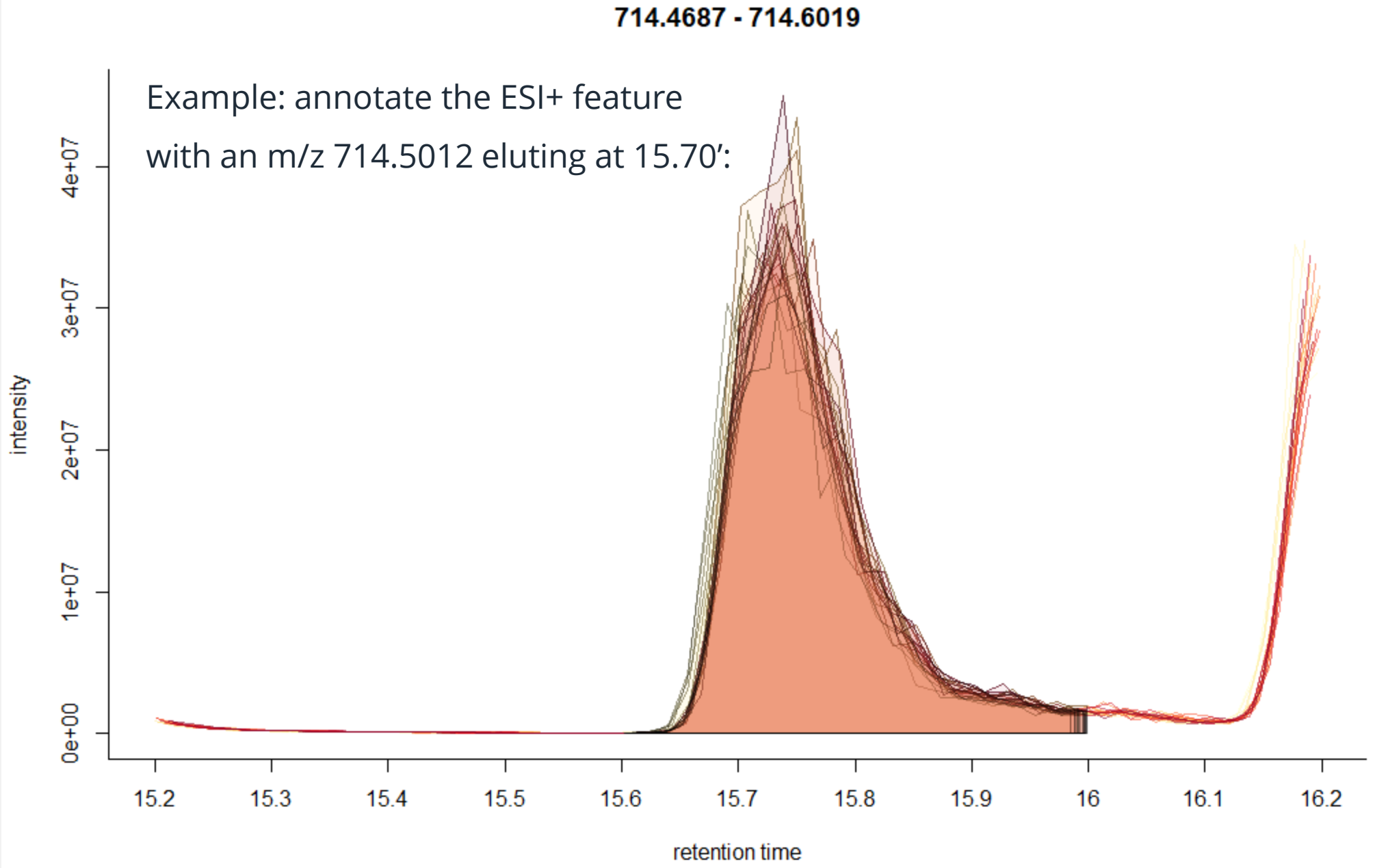
CENTRO RICERCA e INNOVAZIONE

Example: annotate the ESI+ feature with an m/z 714.5012 eluting at 15.70':



Isomeric and isobaric overlap:

	Lipid	Adduct	Formula	m/z	ppm
1	PA 36:4	[M+NH4]+	C39H69O8P+NH4$^+$	714.5068	7.84
2	PE 34:3	[M+H]+	C39H72NO8P+H	714.5068	7.84
3	PC 31:3	[M+H]+	C39H72NO8P+H	714.5068	7.84
4	MGDG 30:3	[M+NH4]+	C39H68O10	714.5151	19.45
5	PA 36:5	2(13C)[M+NH4]+	C39H67O8P	714.4975	5.18
6	PE 34:4	2(13C)[M+H]+	C39H70NO8P	714.4975	5.18
7	PC 31:4	2(13C)[M+H]+	C39H70NO8P	714.4975	5.18
8	MGDG 30:4	2(13C)[M+NH4]+	C39H66O10	714.5057	6.30
9	PA 37:3	13C[M+H]+	C40H73O8P	714.5150	19.31
10	PC 30:5	13C[M+NH4]+	C38H66NO8P	714.4897	16.10
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Type II overlap:

	Lipid	Adduct	Formula	m/z	ppm
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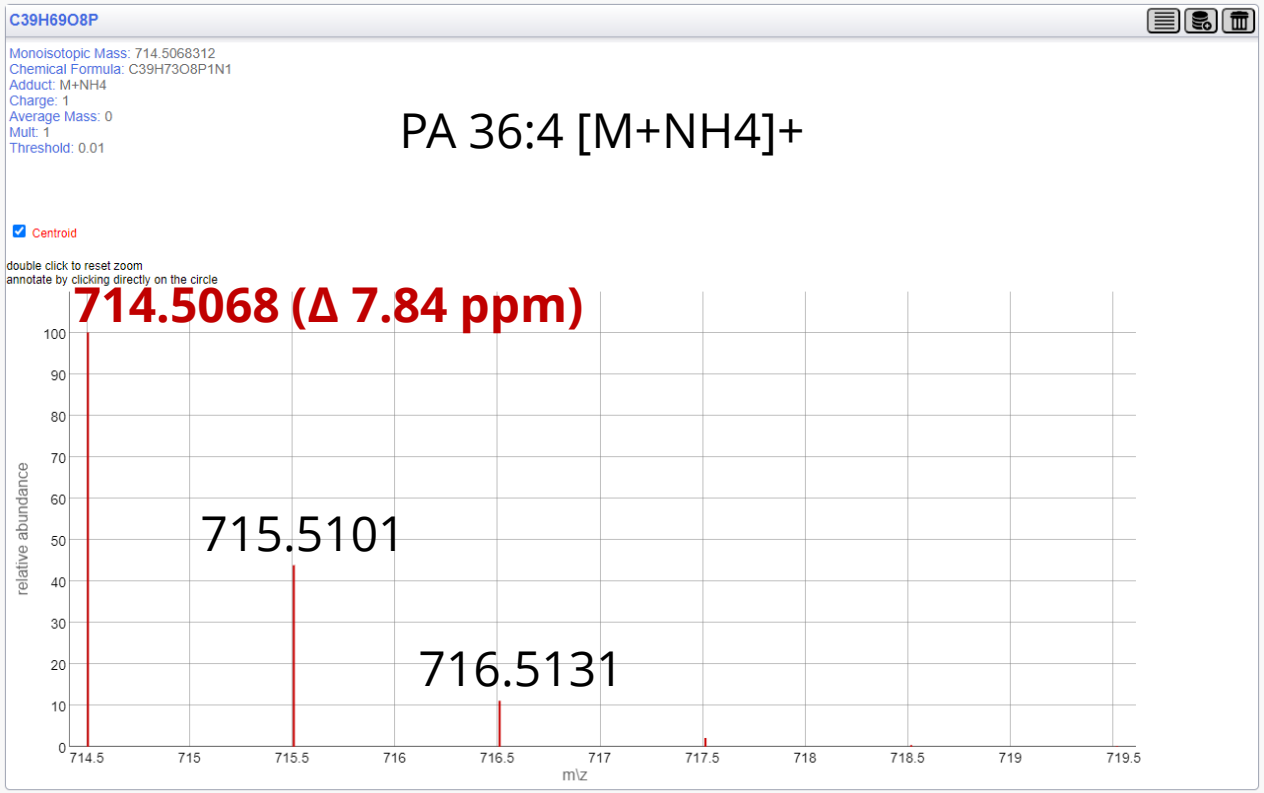

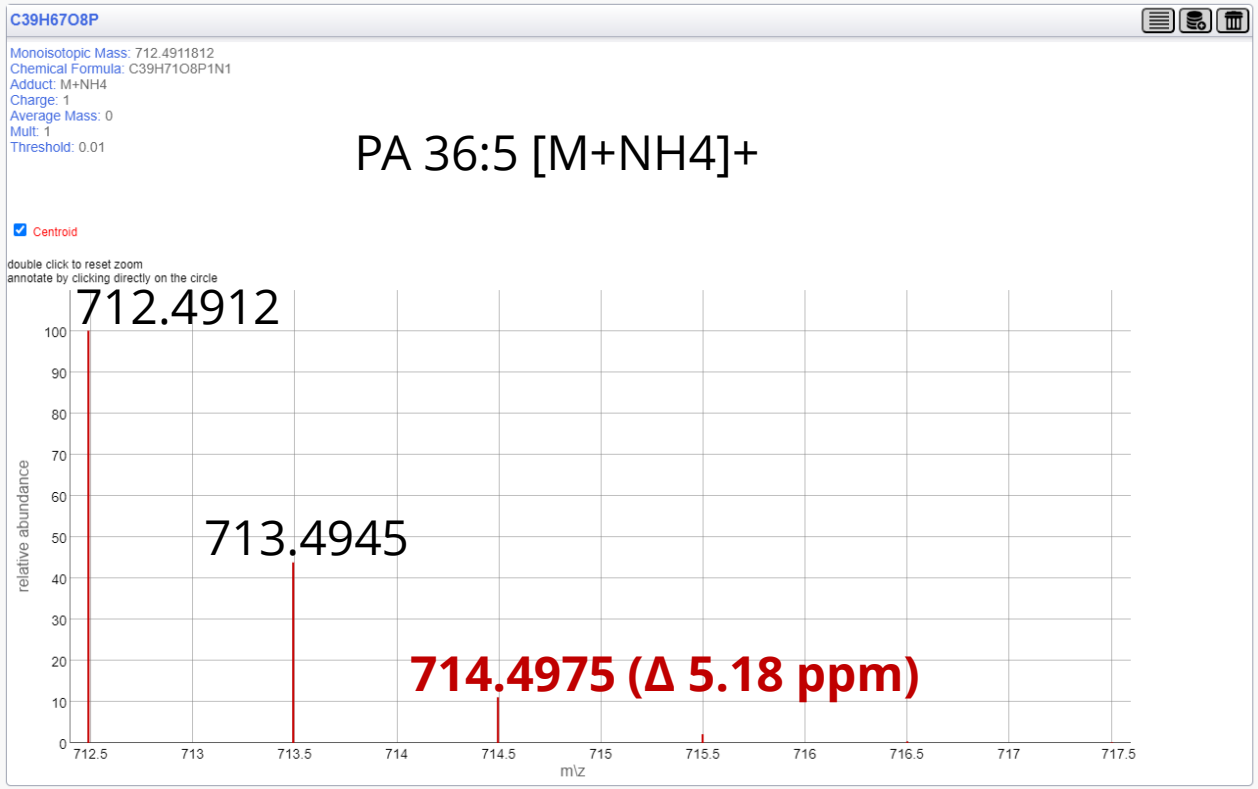

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1. Main ion species for each lipid class
2. Isotopic pattern
3. Complementarity of main ion species for each lipid class in each polarity mode
4. Fragmentation pattern
5. Retention time dependences

1. Main ion species for each lipid class

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9	PA 37:3	13C[M+H]+
10	PC 30:5	13C[M+NH4]+
11	PE 33:5	13C[M+NH4]+

Lipid class	Positive
Fatty acid, FA	–
(Lyso)Phosphatidic acid, (L)PA	[M+NH4]+
(Lyso)Phosphatidylcholine, (L)PC	[M+H]+
(Lyso)Phosphatidylethanolamine, (L)PE	[M+H]+
(Lyso)Phosphatidylglycerol, (L)PG	[M+NH4]+
(Lyso)Phosphatidylinositol, (L)PI	[M+NH4]+
(Lyso)Phosphatidylserine, (L)PS	[M+H]+
Monogalactosyldiacylglycerol, MGDG	[M+NH4]+
Digalactosyldiacylglycerol, DGDG	[M+NH4]+
Diacylglycerol, DG	[M+NH4]+
Triacylglycerol, TG	[M+NH4]+

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Digalactosyldiacylglycerol, DGDG	[M+NH4]+
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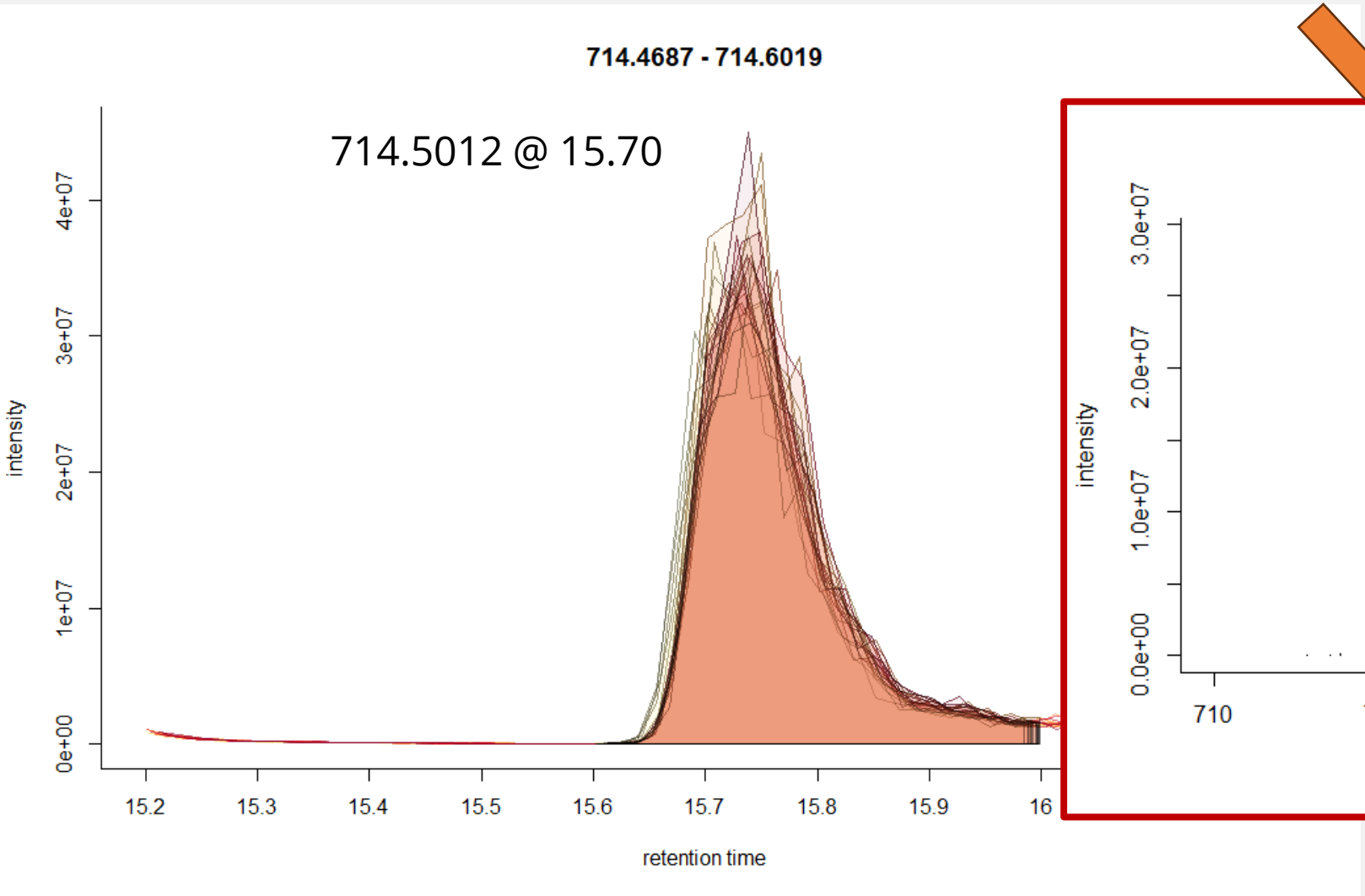
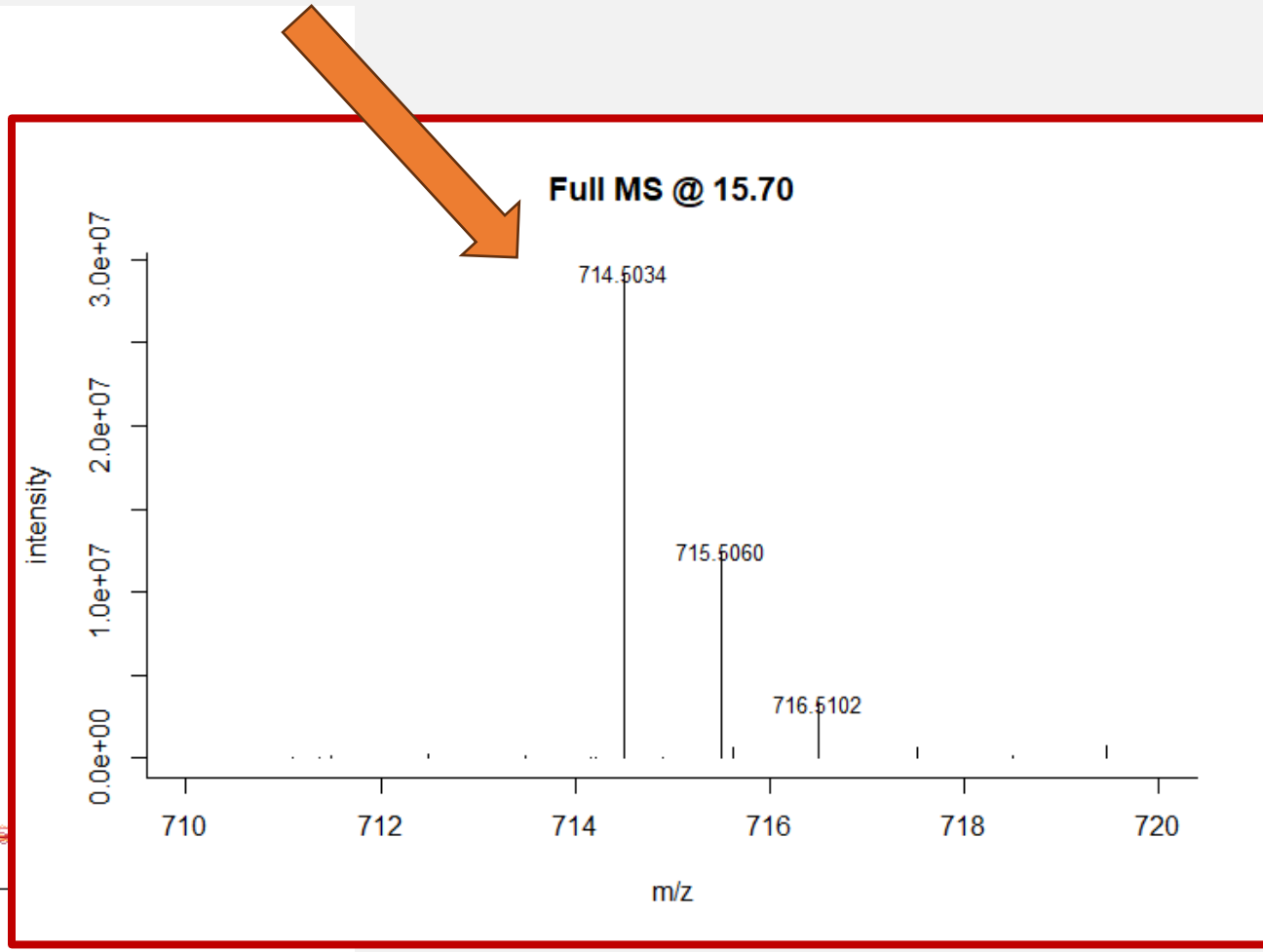
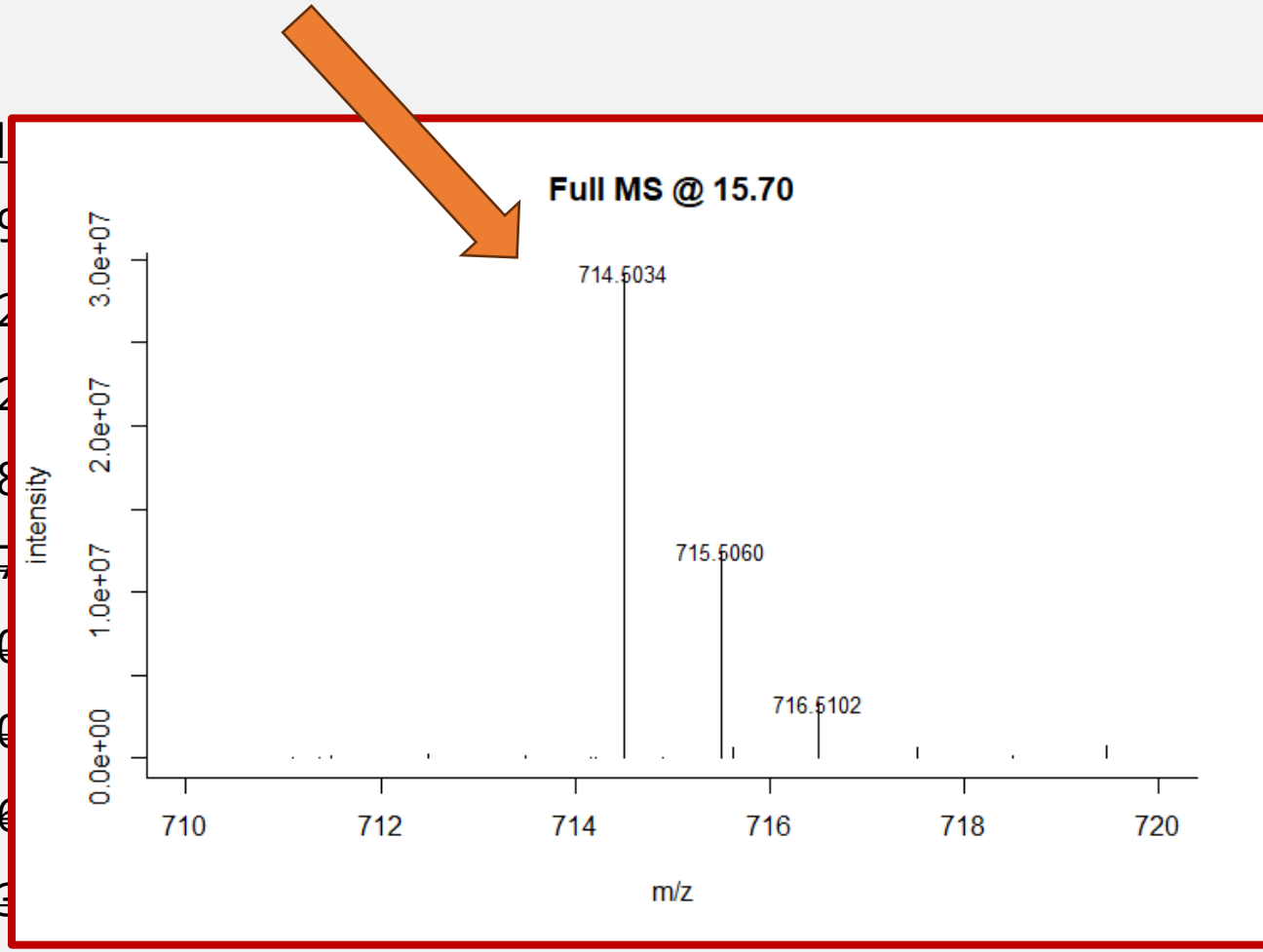
2. Isotopic pattern





2. Isotopic pattern

	Lipid	Adduct	Formul		
1	PA 36:4	[M+NH4]+	C39H69		
2	PE 34:3	[M+H]+	C39H72		
3	PC 31:3	[M+H]+	C39H72		
4	MGDG 30:3	[M+NH4]+	C39H68		
~~5~~	~~PA 36:5~~	~~2(13C)[M+NH4]+~~	~~C39H67~~		
~~6~~	~~PE 34:4~~	~~2(13C)[M+H]+~~	~~C39H70~~		
~~7~~	~~PC 31:4~~	~~2(13C)[M+H]+~~	~~C39H70~~		
~~8~~	~~MGDG 30:4~~	~~2(13C)[M+NH4]+~~	~~C39H66~~		
~~9~~	~~PA 37:3~~	~~13C[M+H]+~~	~~C40H73~~		
~~10~~	~~PC 30:5~~	~~13C[M+NH4]+~~	~~C38H66NO8P~~	~~714.4897~~	~~16.10~~
~~11~~	~~PE 33:5~~	~~13C[M+NH4]+~~	~~C38H66NO8P~~	~~714.4897~~	~~16.10~~



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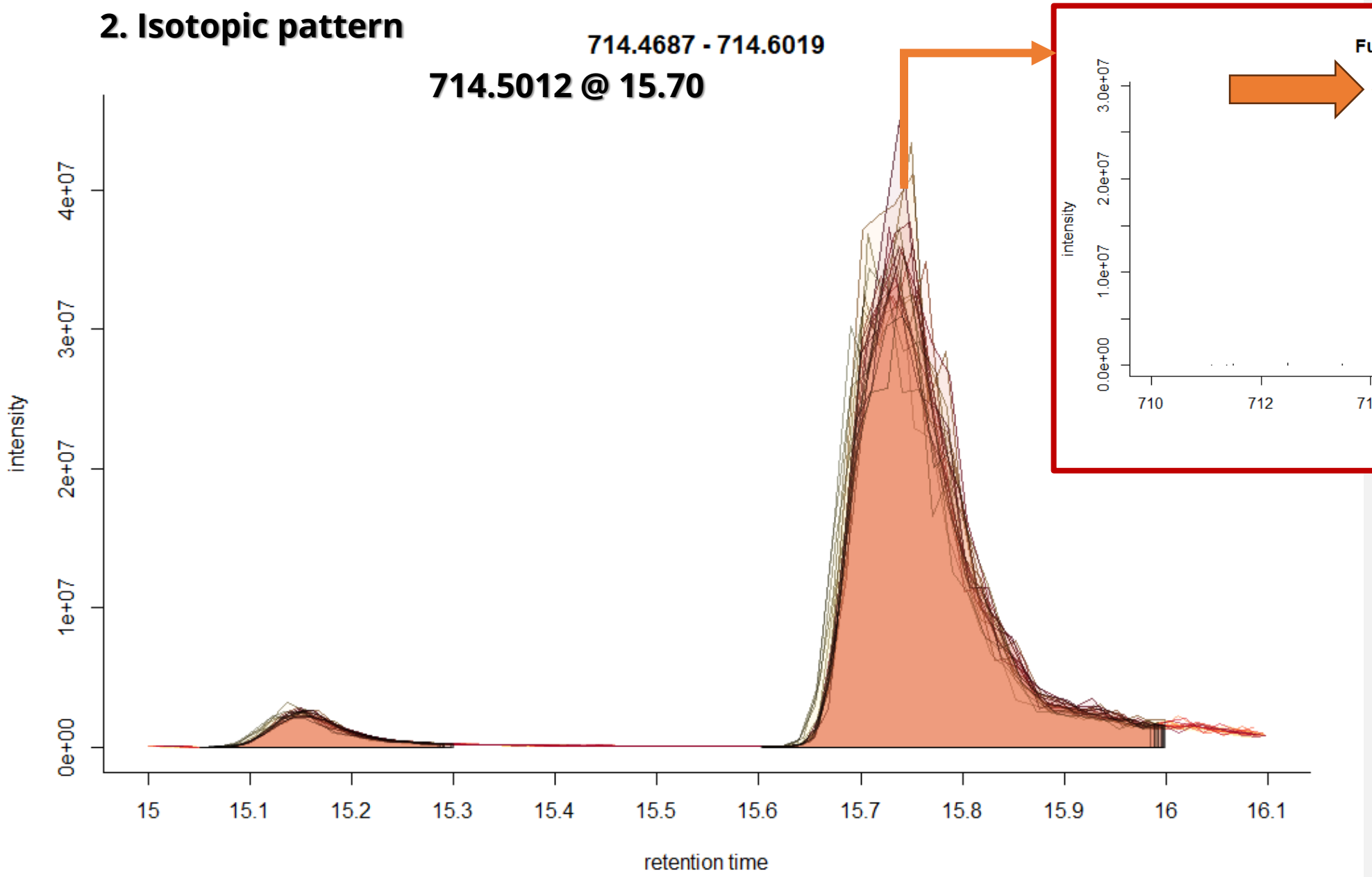

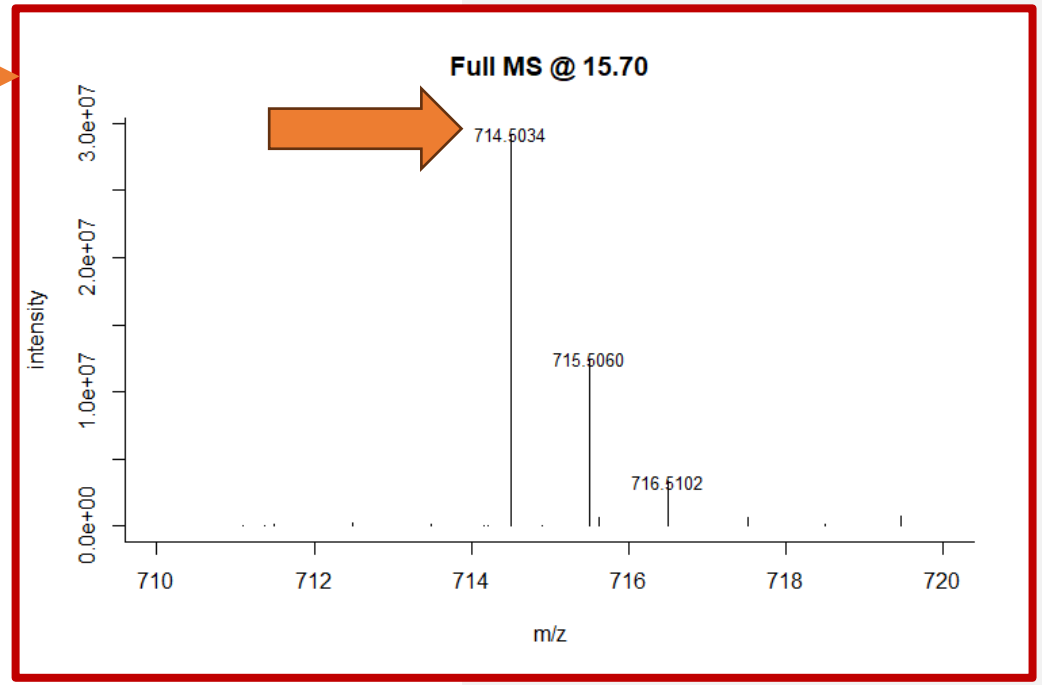

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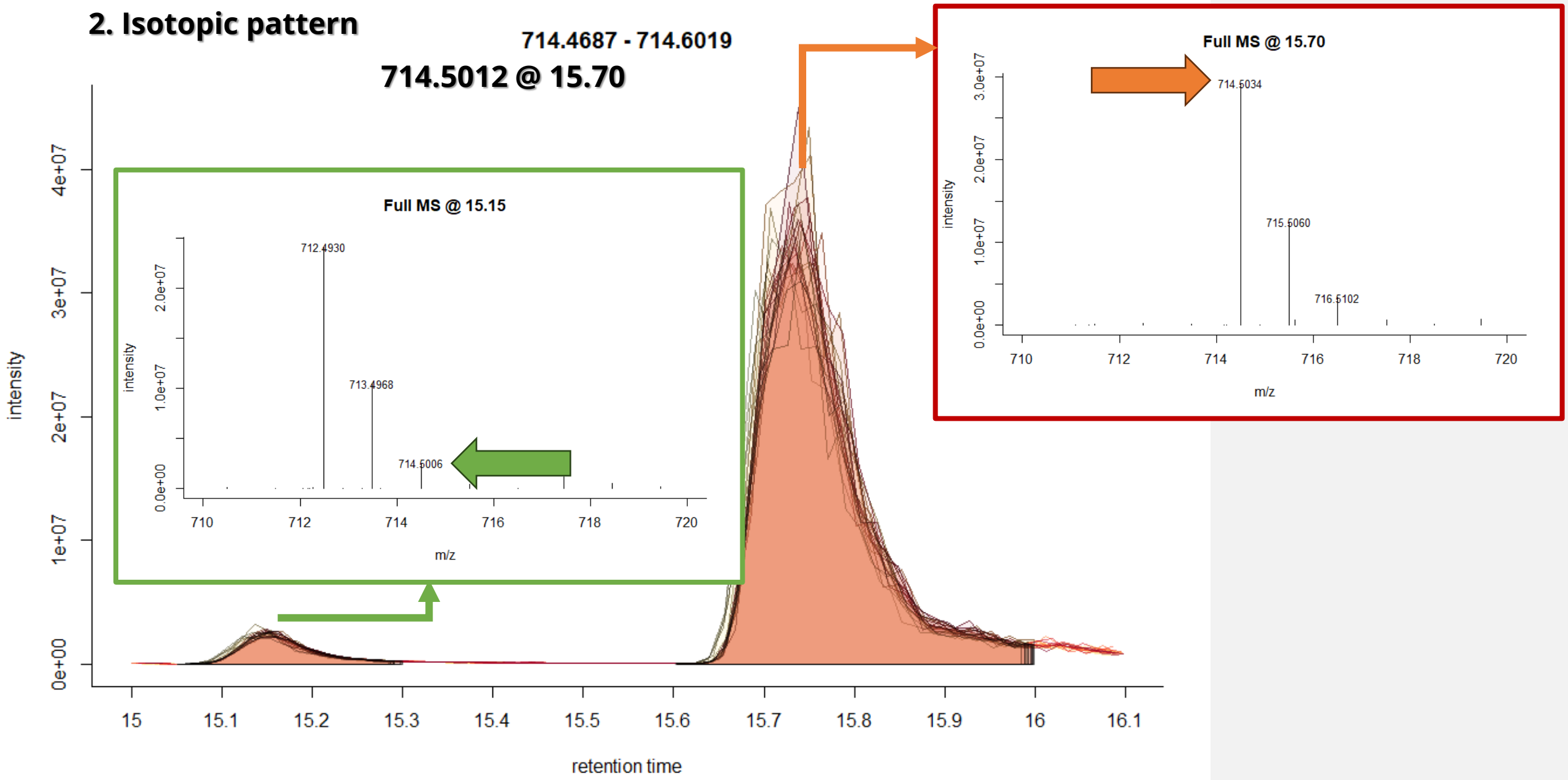

3. Complementarity of main ion species for each lipid class in each polarity mode

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Lipid class	Positive	Negative
Fatty acid, FA	–	[M-H]-
(Lyso)Phosphatidic acid, (L)PA	[M+NH4]+	[M-H]-
(Lyso)Phosphatidylcholine, (L)PC	[M+H]+	[M+CHO2]-
(Lyso)Phosphatidylethanolamine, (L)PE	[M+H]+	[M-H]-
(Lyso)Phosphatidylglycerol, (L)PG	[M+NH4]+	[M-H]-
(Lyso)Phosphatidylinositol, (L)PI	[M+NH4]+	[M-H]-
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Digalactosyldiacylglycerol, DGDG	[M+NH4]+	[M+CHO2]-
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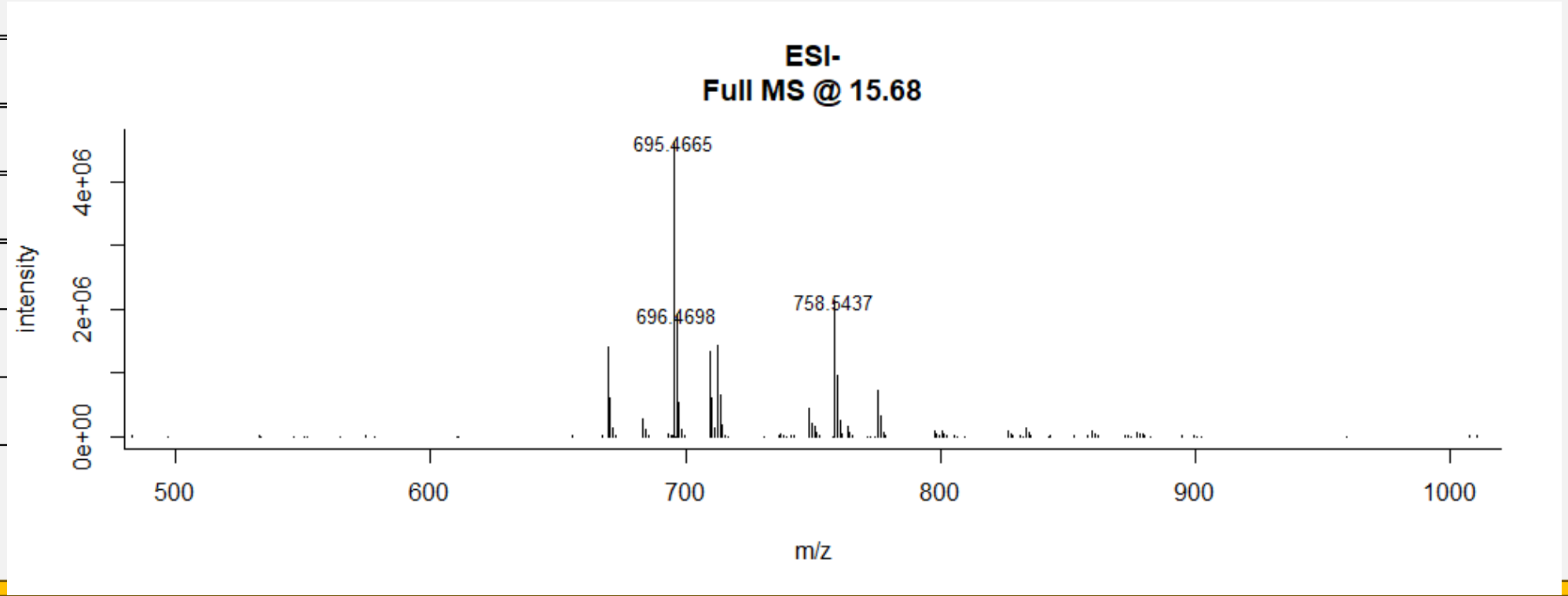

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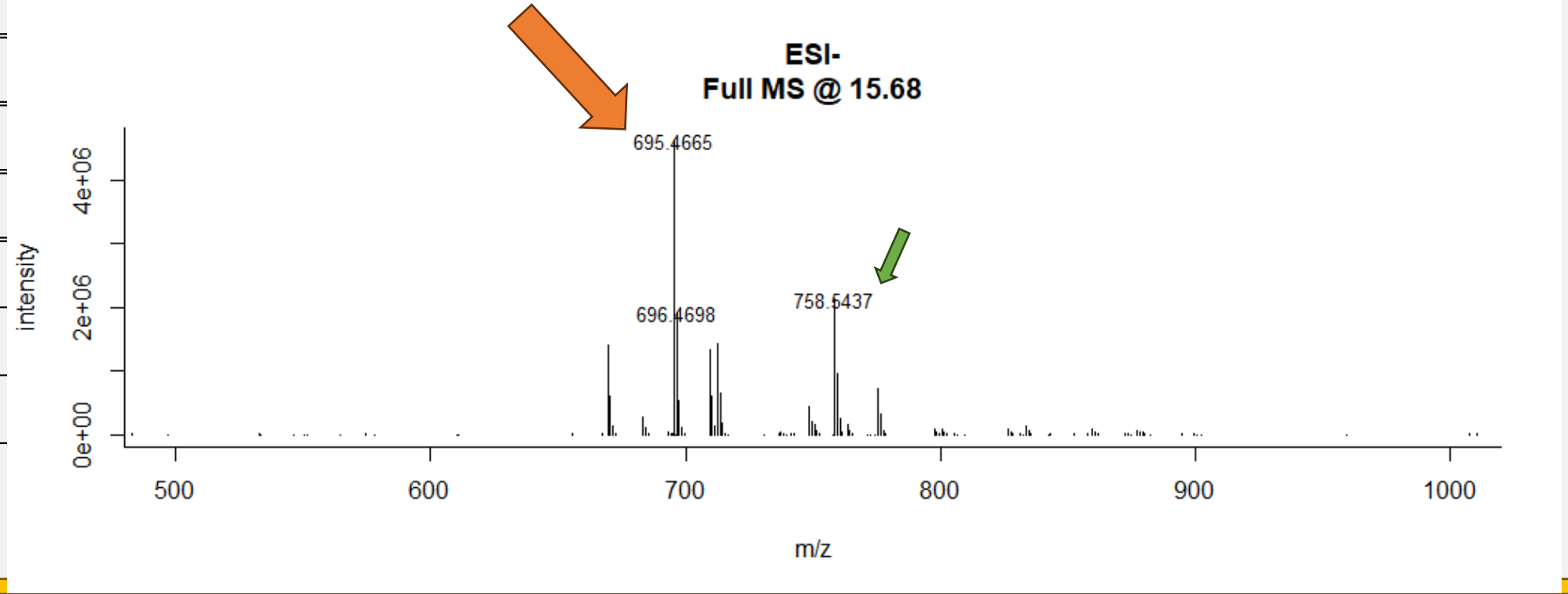

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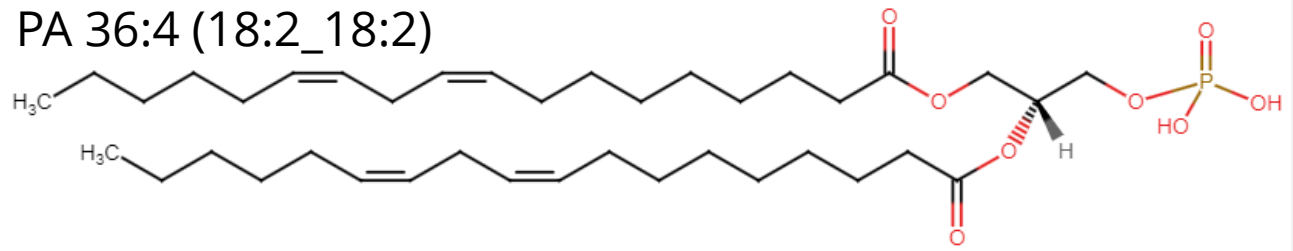
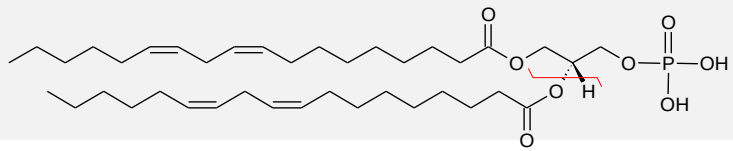
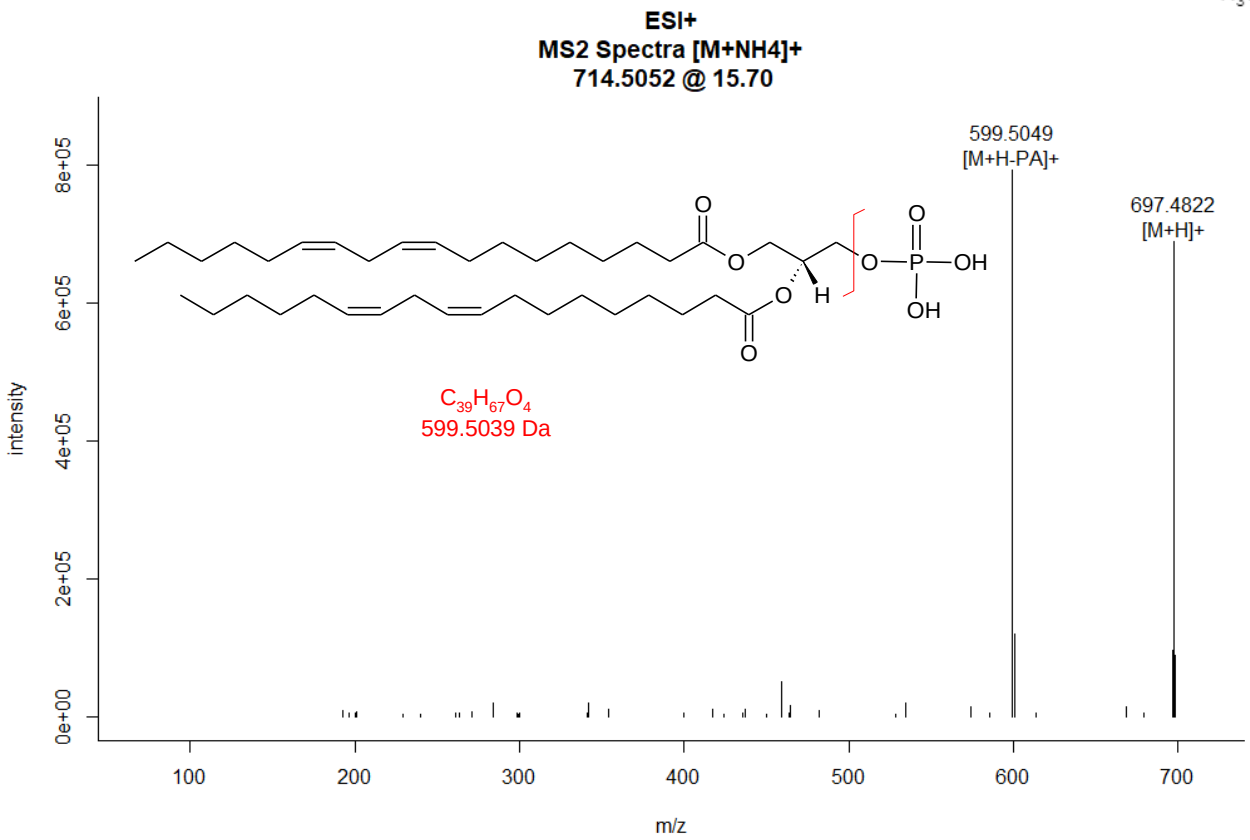
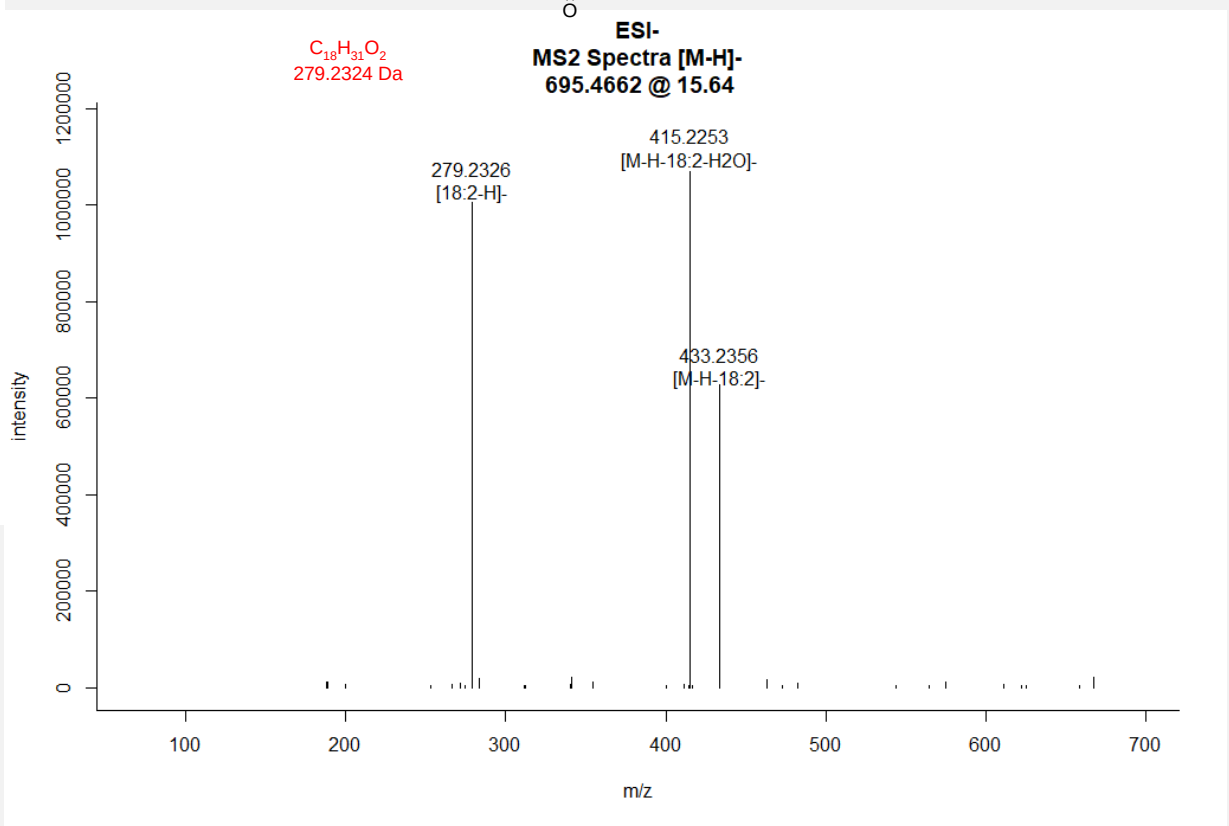
4. Fragmentation pattern

PA 36:4 (18:2_18:2)









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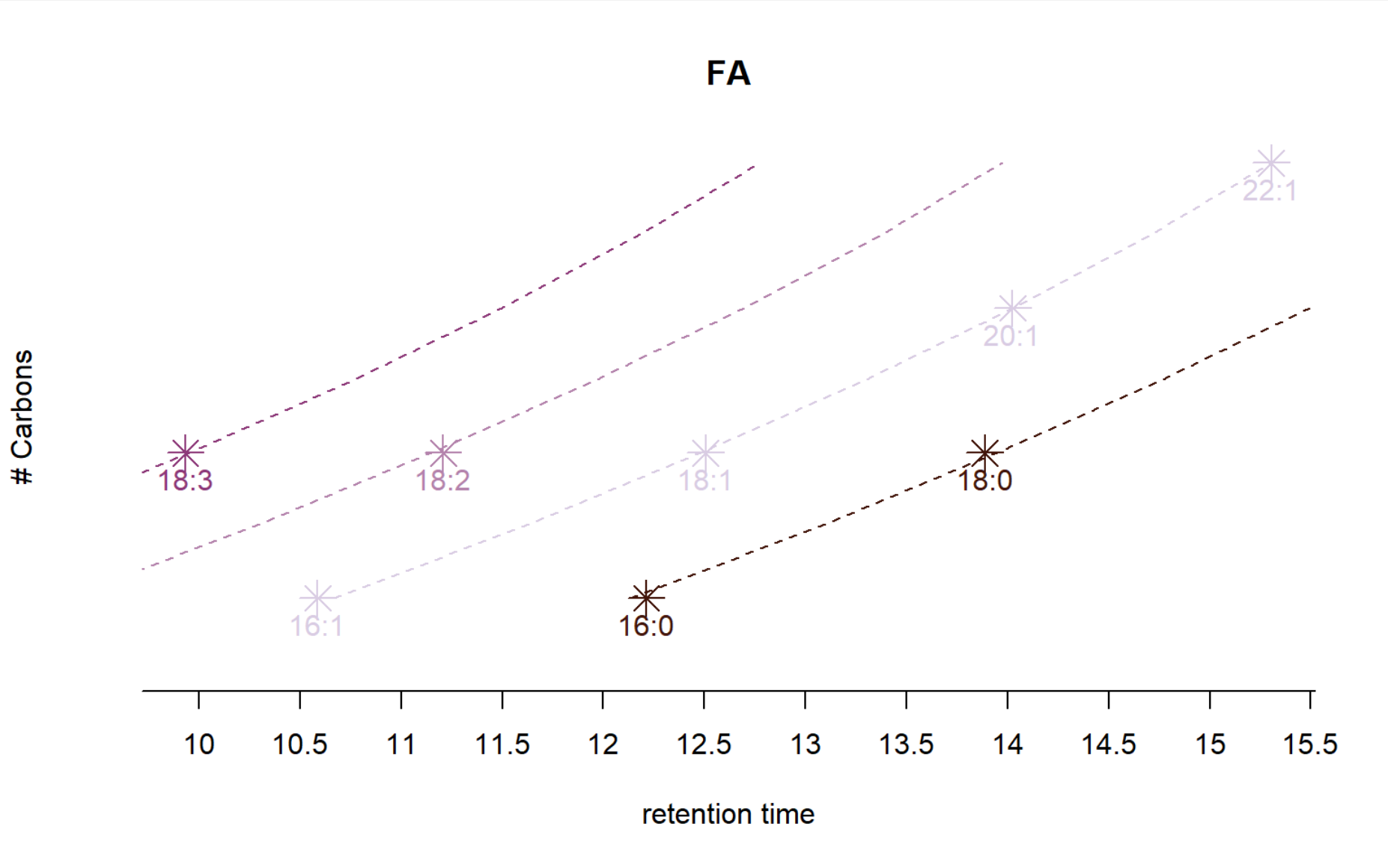
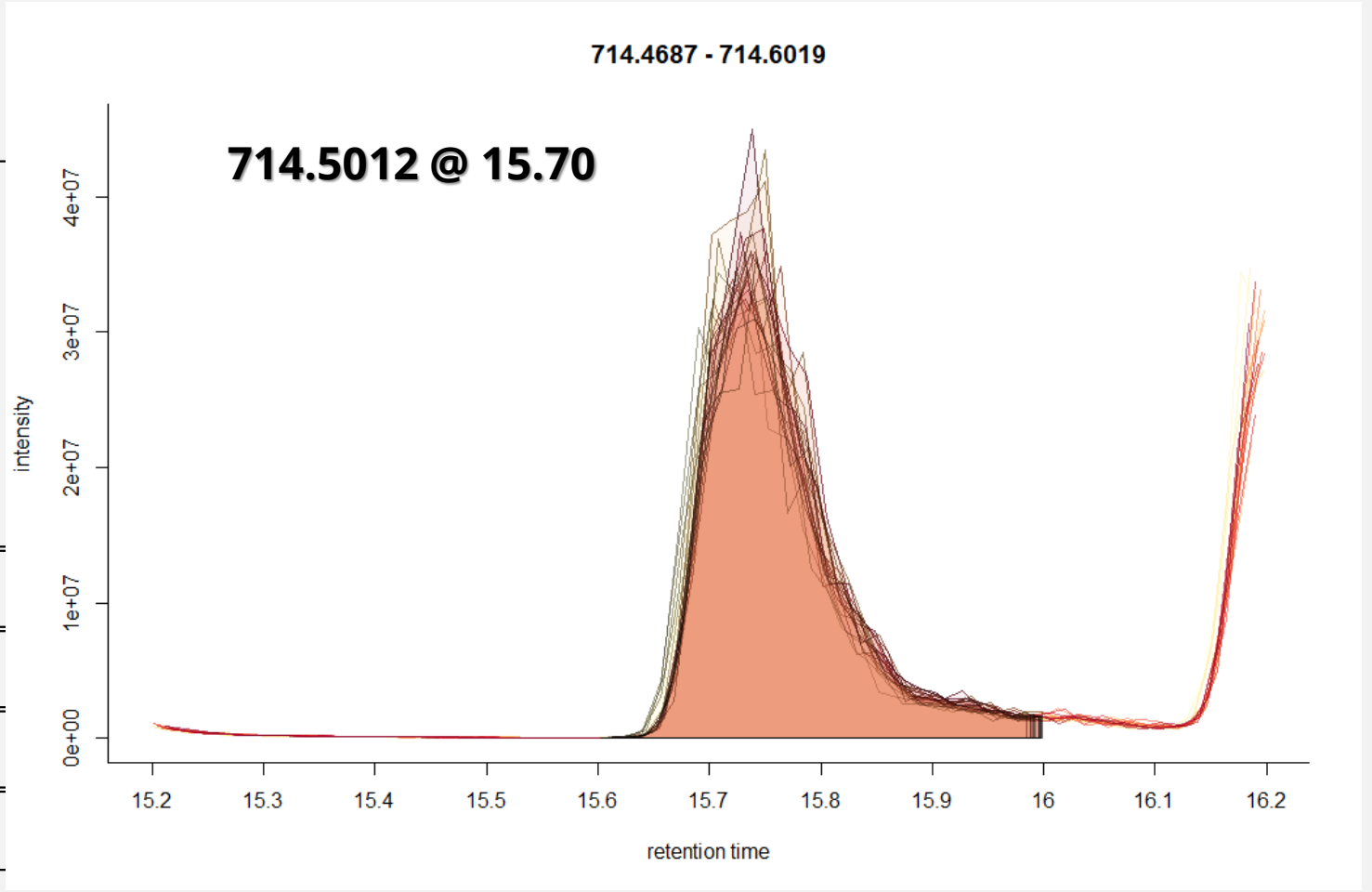
CENTRO RICERCA e INNOVAZIONE

5. Retention time dependences



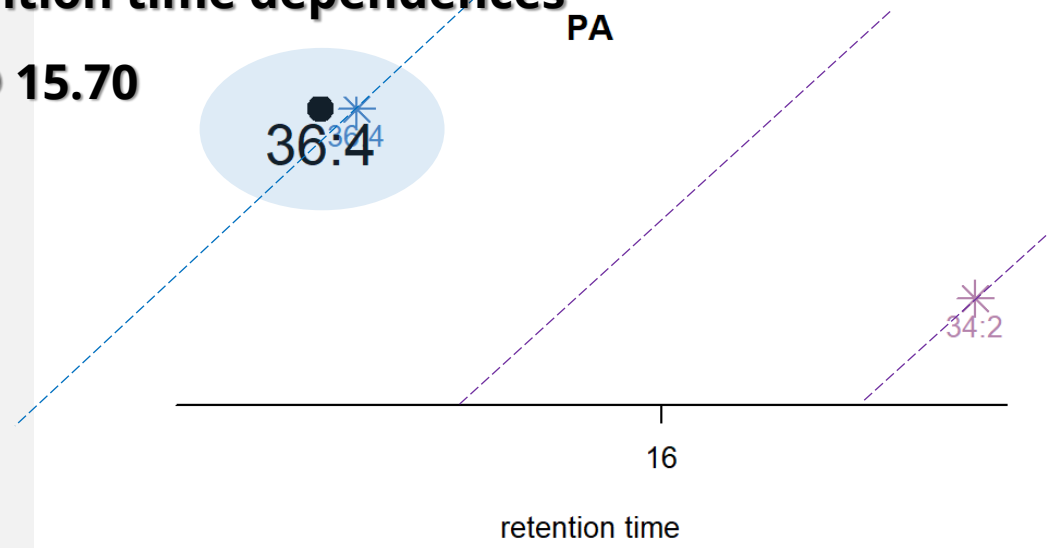
5. Retention time dependences

	Lipid	Adduct			
1	PA 36:4	[M+NH4]+			
2	PE 34:3	[M+H]+			
3	PC 31:3	[M+H]+			
4	MGDG 30:3	[M+NH4]+			
~~5~~	~~PA 36:5~~	~~2(13C)[M+NH4]+~~			
~~6~~	~~PE 34:4~~	~~2(13C)[M+H]+~~			
~~7~~	~~PC 31:4~~	~~2(13C)[M+H]+~~			
~~8~~	~~MGDG 30:4~~	~~2(13C)[M+NH4]+~~			
~~9~~	~~PA 37:3~~	~~13C[M+H]+~~			
~~10~~	~~PC 30:5~~	~~13C[M+NH4]+~~	~~C38H66NO8P~~	~~714.4897~~	~~16.10~~
~~11~~	~~PE 33:5~~	~~13C[M+NH4]+~~	~~C38H66NO8P~~	~~714.4897~~	~~16.10~~



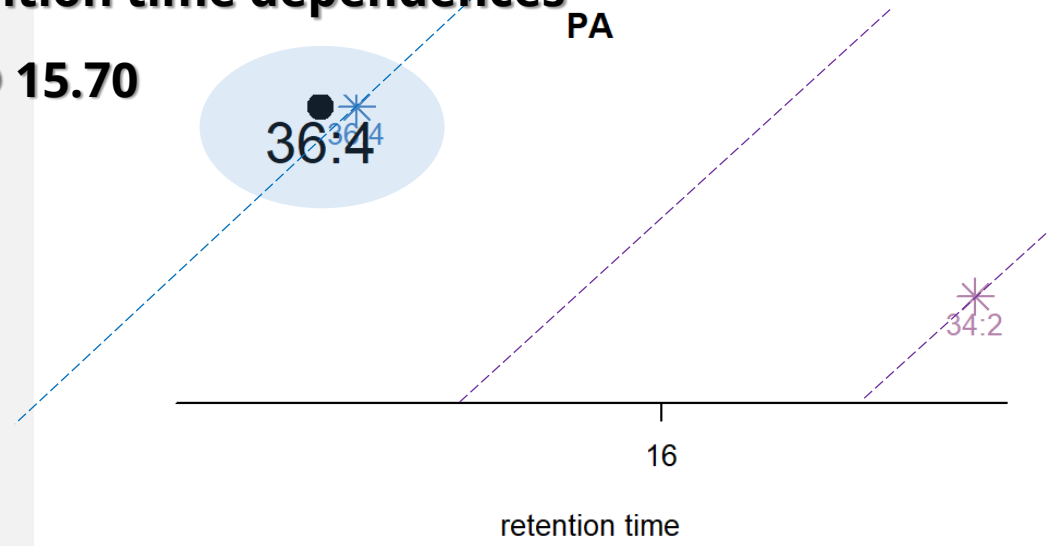
5. Retention time dependences

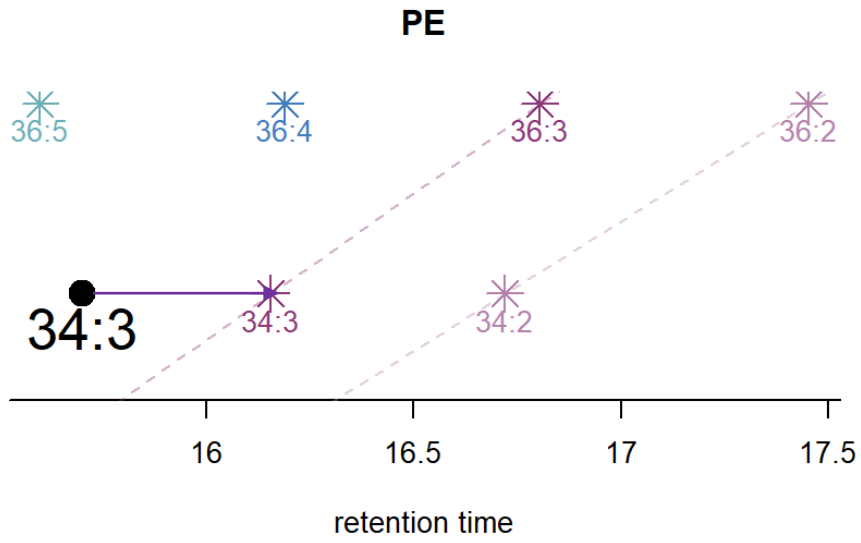
714.5012 @ 15.70



5. Retention time dependences

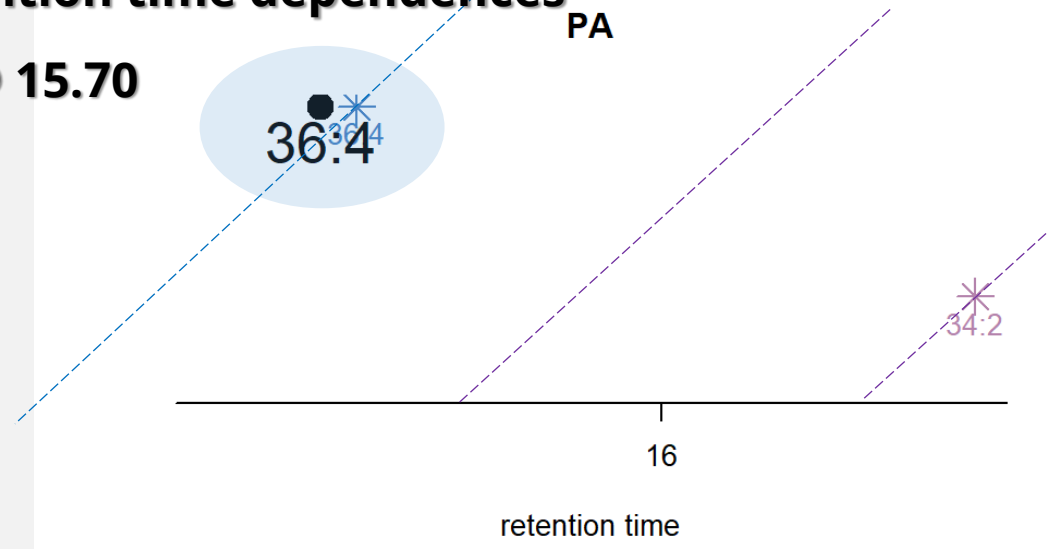
714.5012 @ 15.70

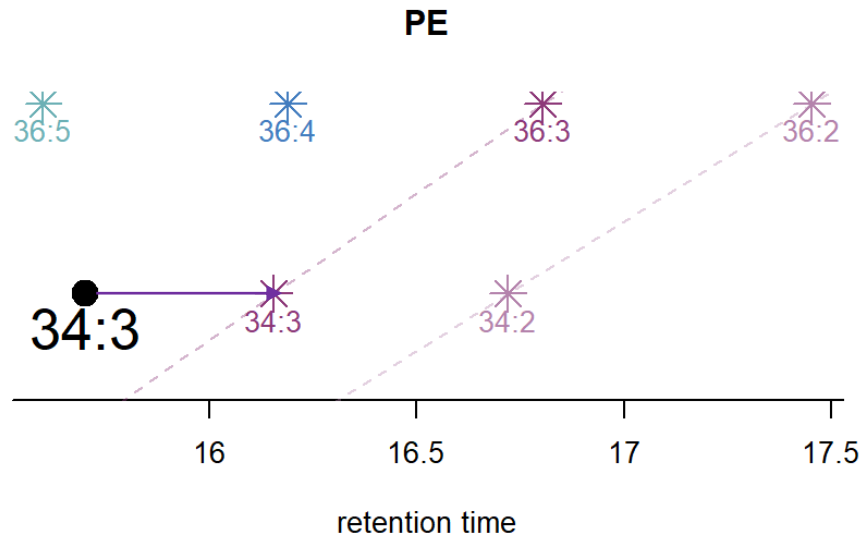


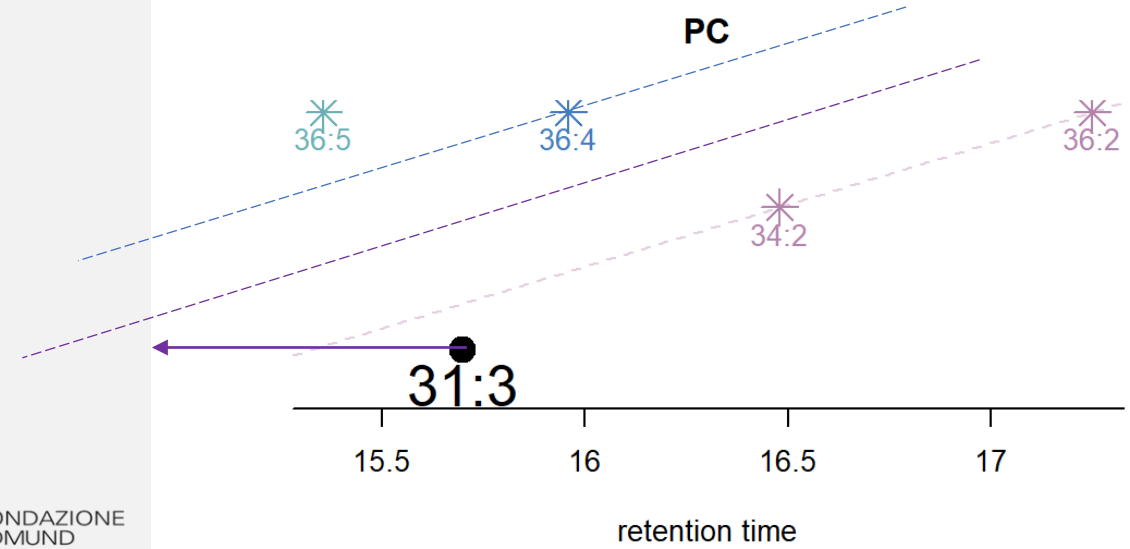


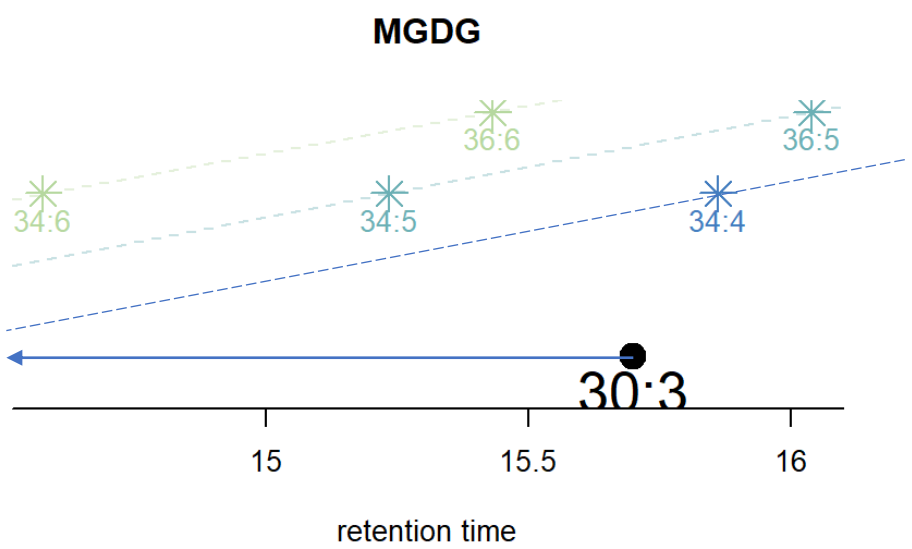
5. Retention time dependences

714.5012 @ 15.70









Designed pipeline :

Contents lists available at ScienceDirect

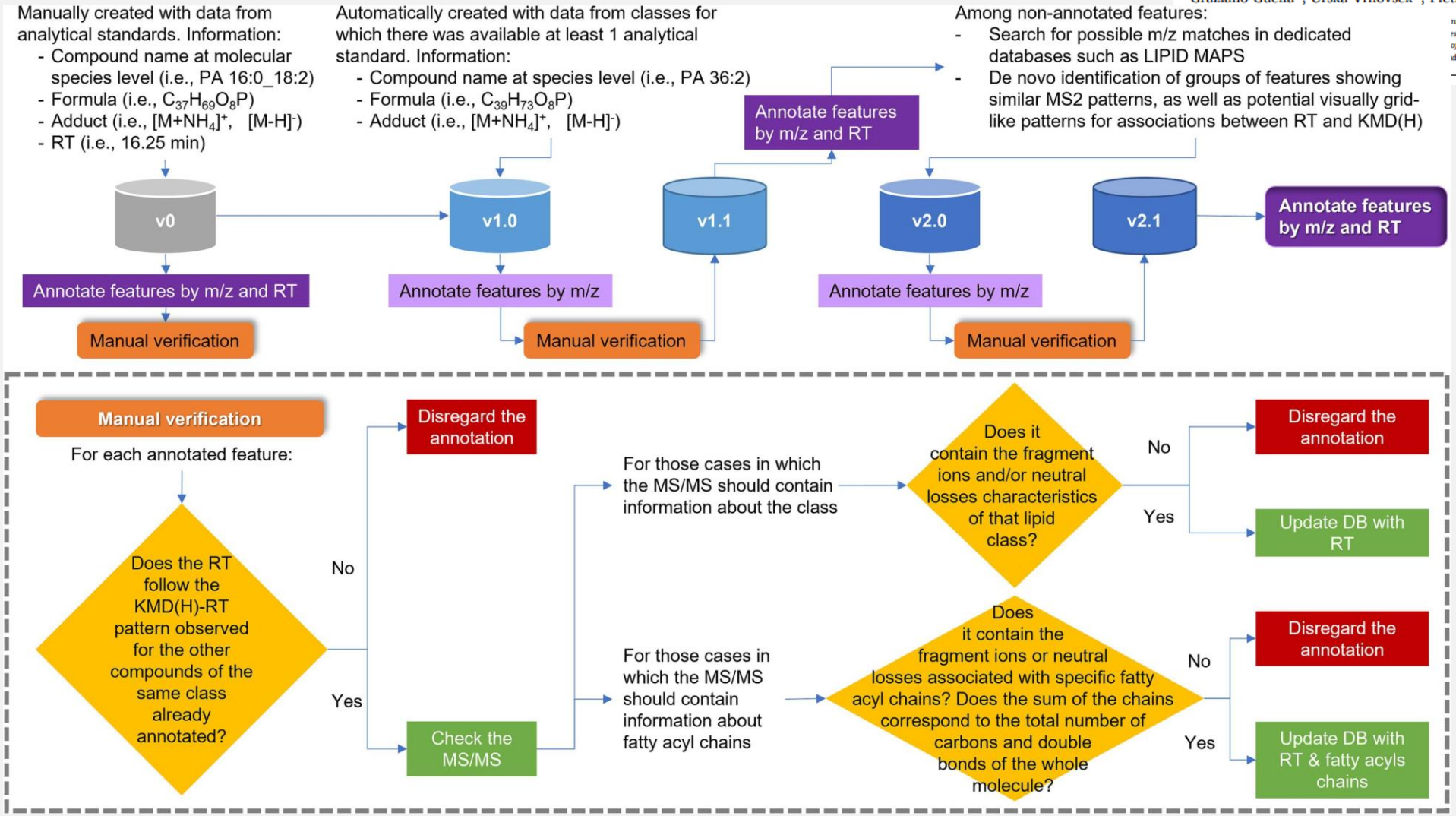
Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

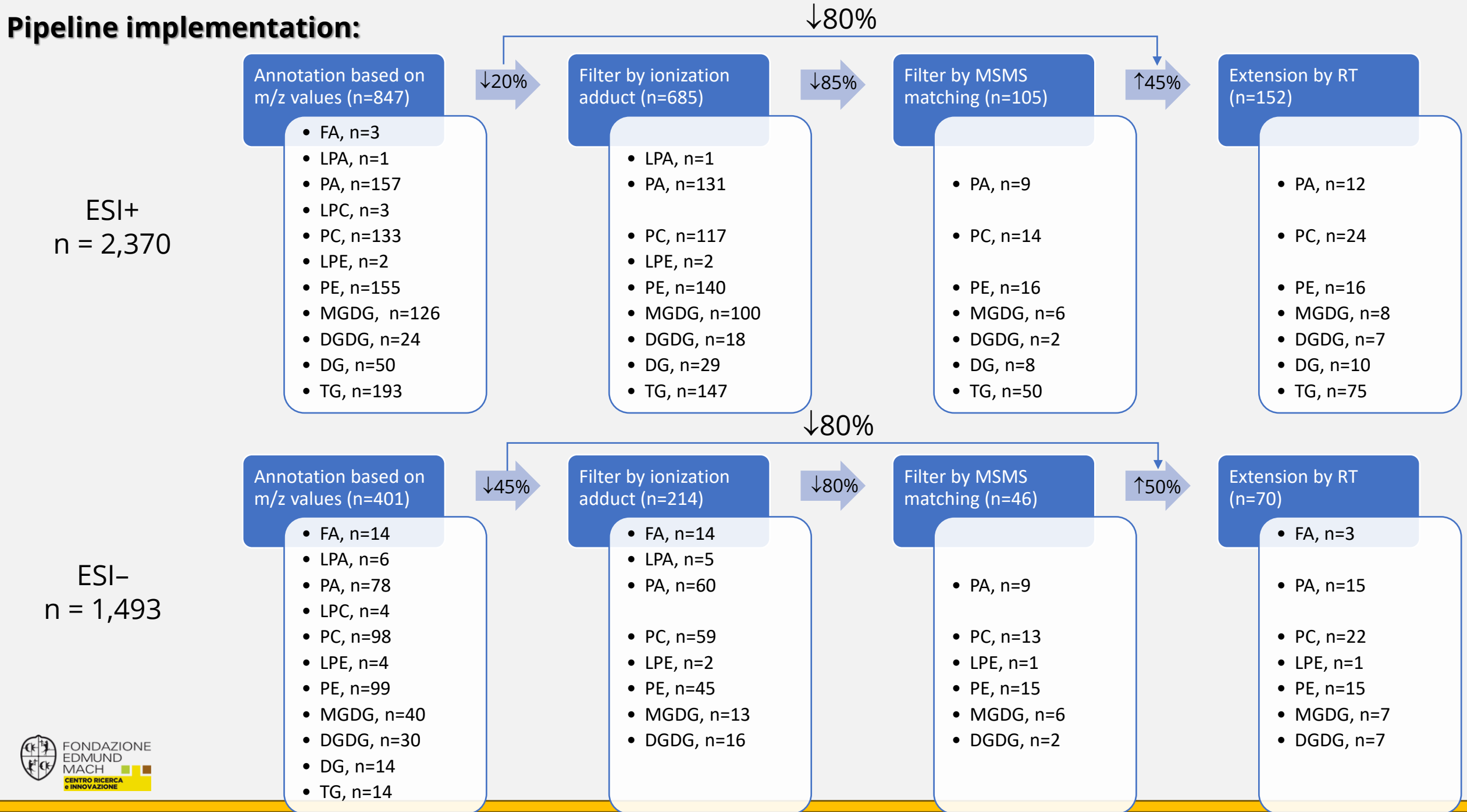
Untargeted lipidomic profiling of grapes highlights the importance of modified lipid species beyond the traditional compound classes

Mar Garcia-Aloy [a,*], Domenico Masuero [a], Giulia Chitarrini [a], Domen Škrab [a,b], Paolo Sivilotti [b], Graziano Guella [c], Urska Vrhovsek [a], Pietro Franceschi [d]

...mund Mach, 38098 San Michele all'Adige, Italy
...s, University of Udine, 33100 Udine, Italy
...of Trento, 38123 Trento, Italy
...dazione Edmund Mach, 38098 San Michele all'Adige, Italy



Pipeline implementation:



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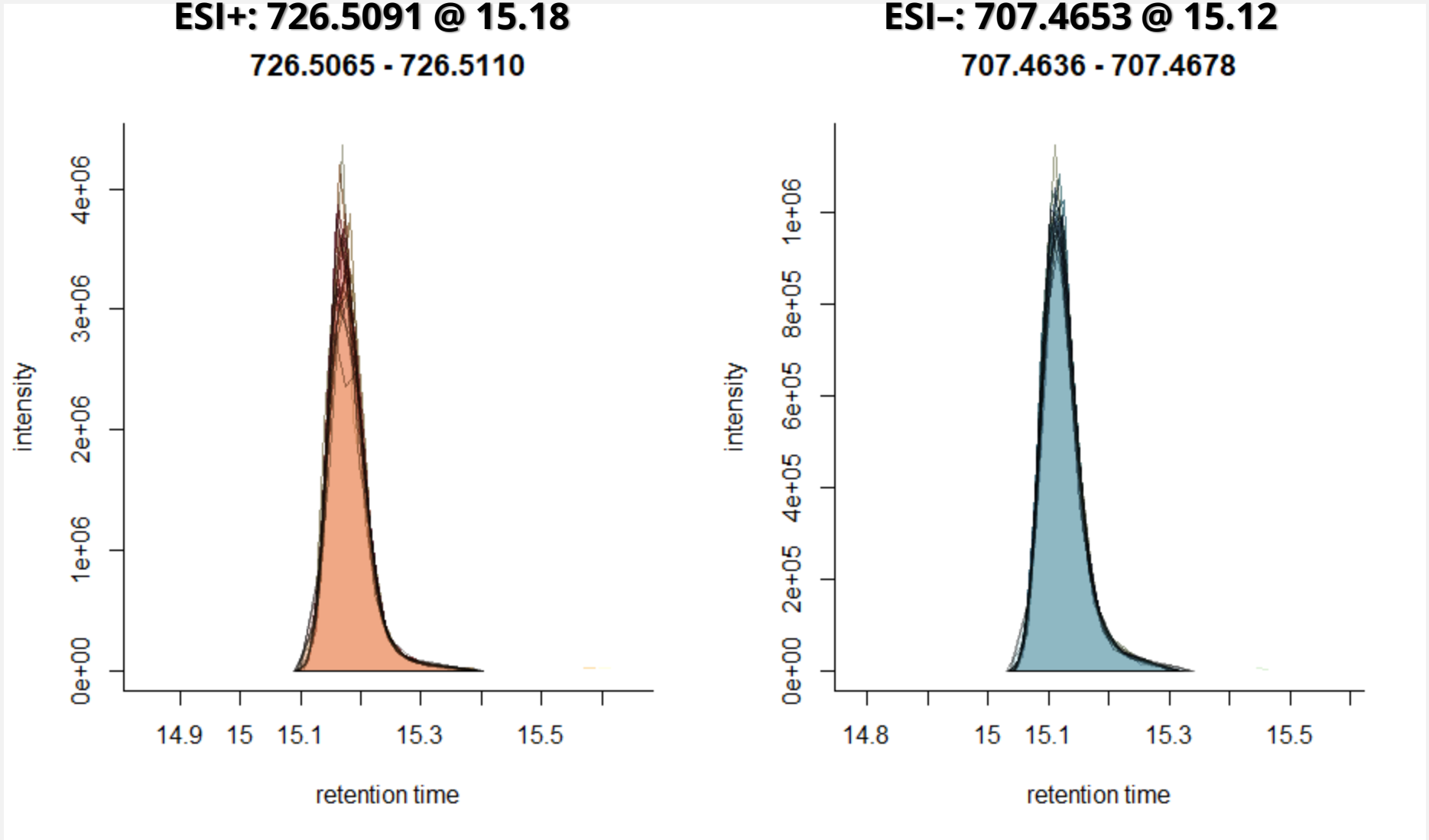
Annotation of less common lipid classes:

https://garciamar.shinyapps.io/lipidomics_tool/



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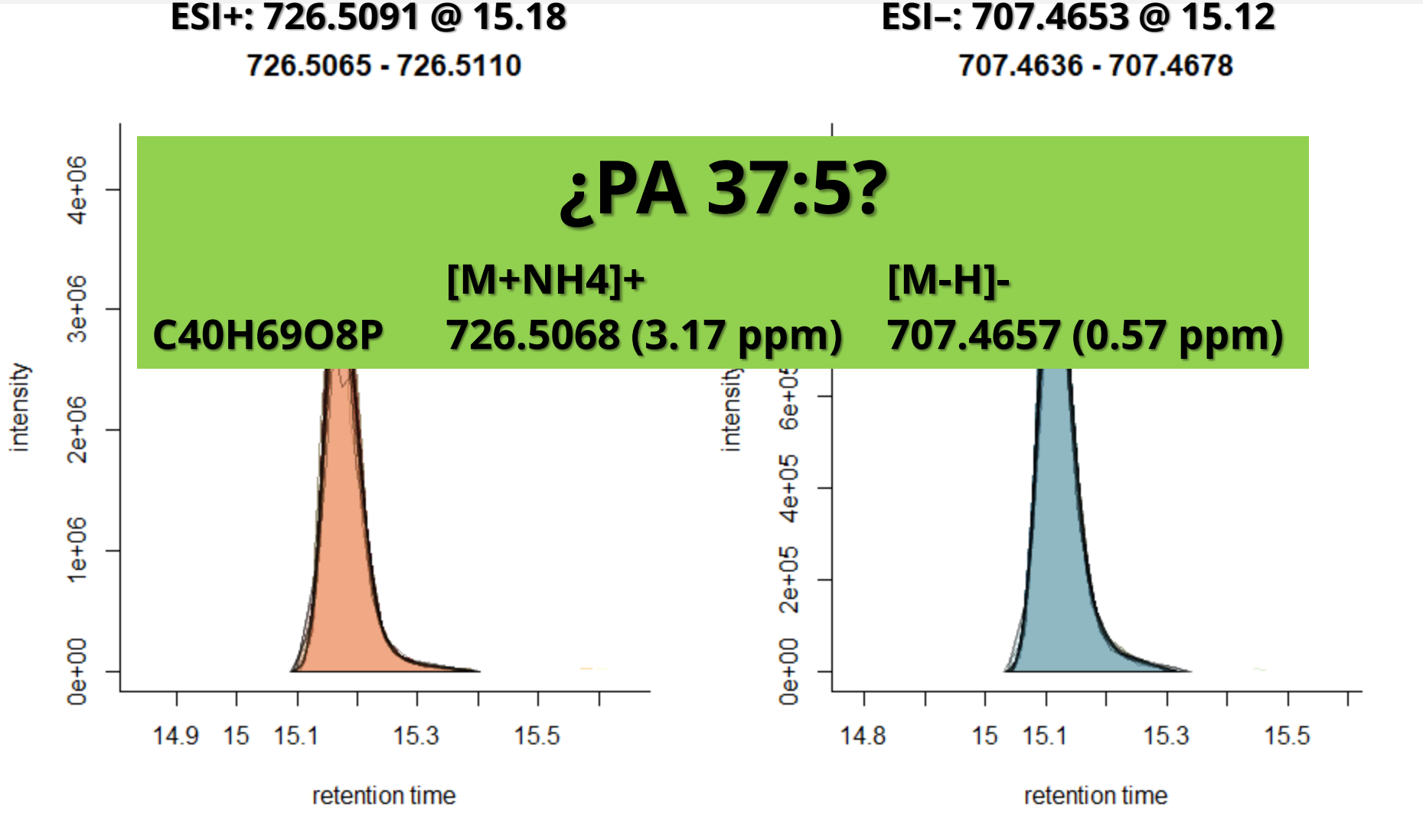

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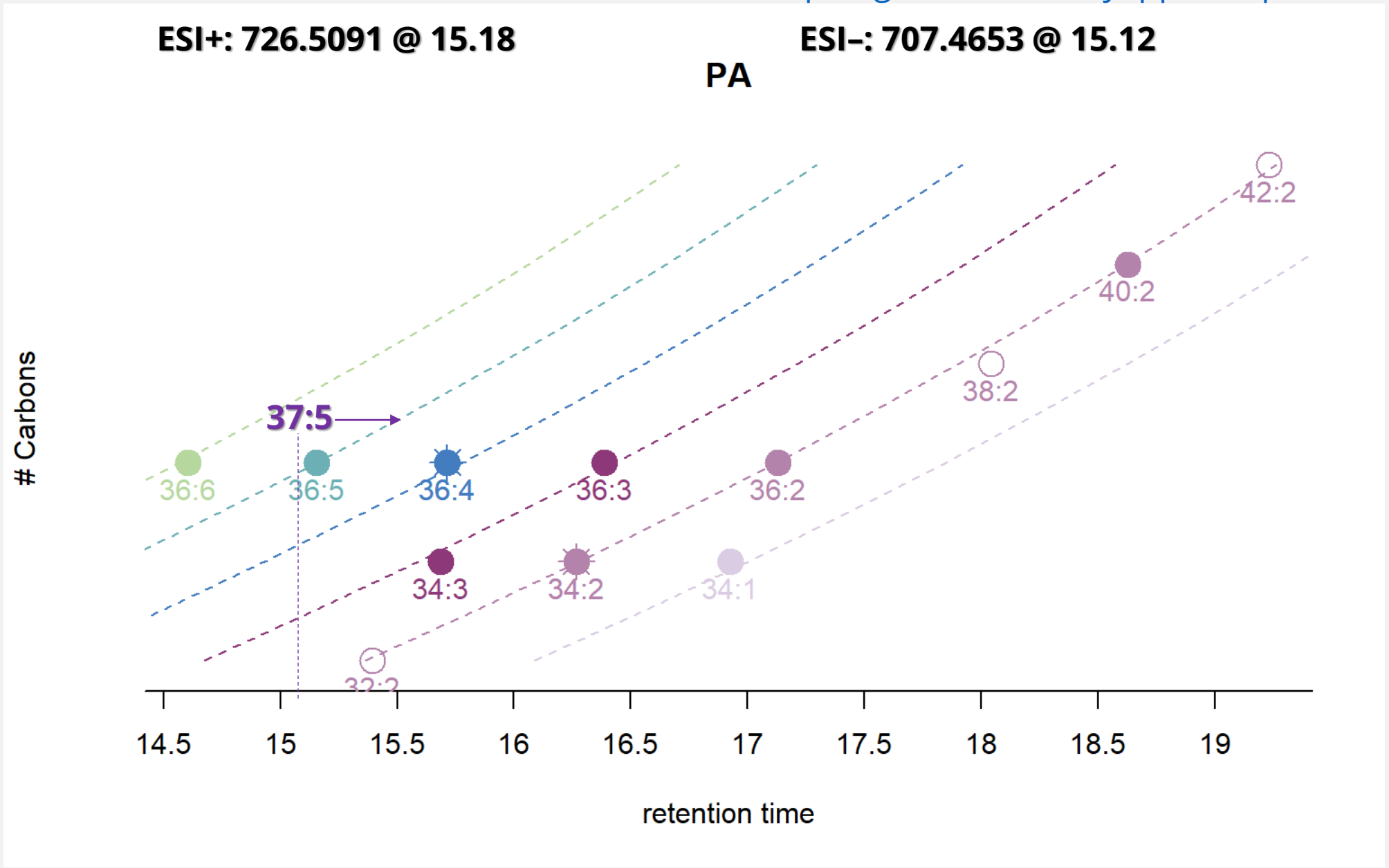

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Annotation of less common lipid classes:

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Lipidomics | Main Panel | MS2

Formula

Lipid class: PA

C: 37

db: 5

[1] "C40H69O8P"

Major adducts

	[M+NH4]+	[M-H]-
C40H69O8P	726.5068	707.4657

Compound Prediction

m/z: 726.5091

[1] "PA 37:5" "PC 32:4" "PI

Polarity:

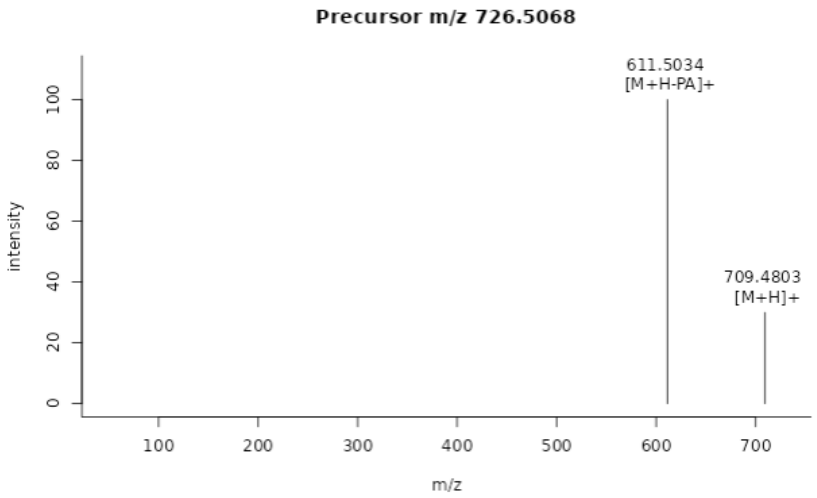
- (•) ESI+
- ( ) ESI-

ppm: 5

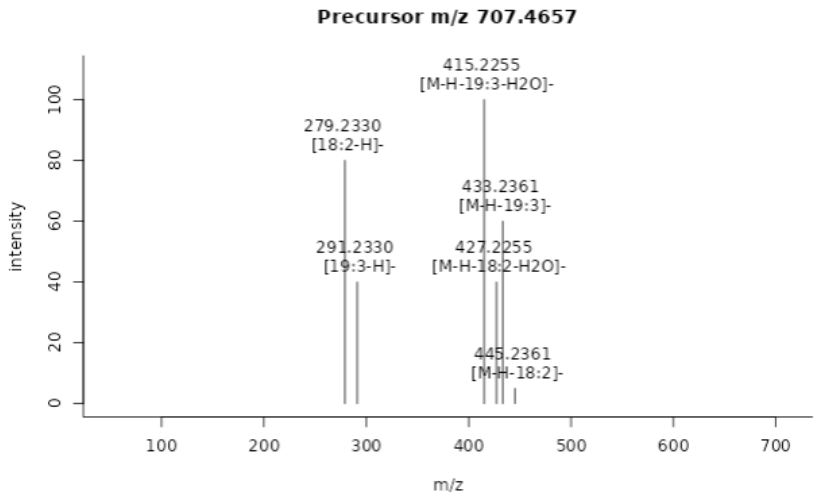
Theoretical MS2

sn1: 18:2 **sn2:** 19:3 **sn3:** 18:0

ESI+



ESI-



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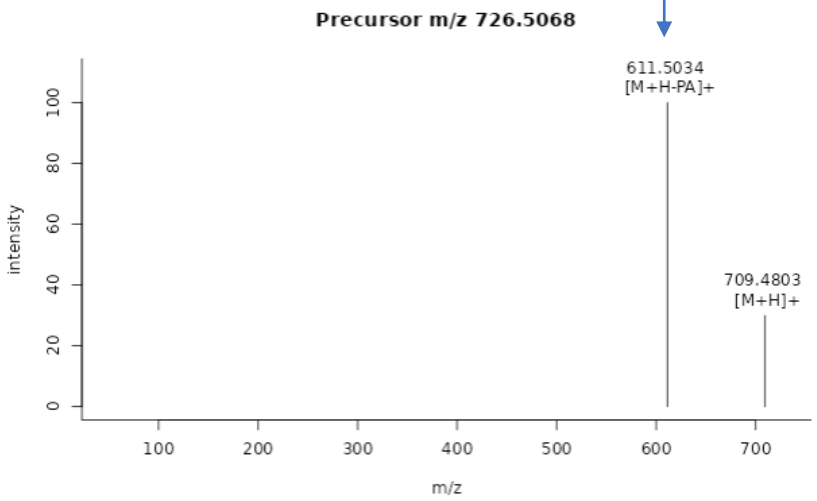
ESI+



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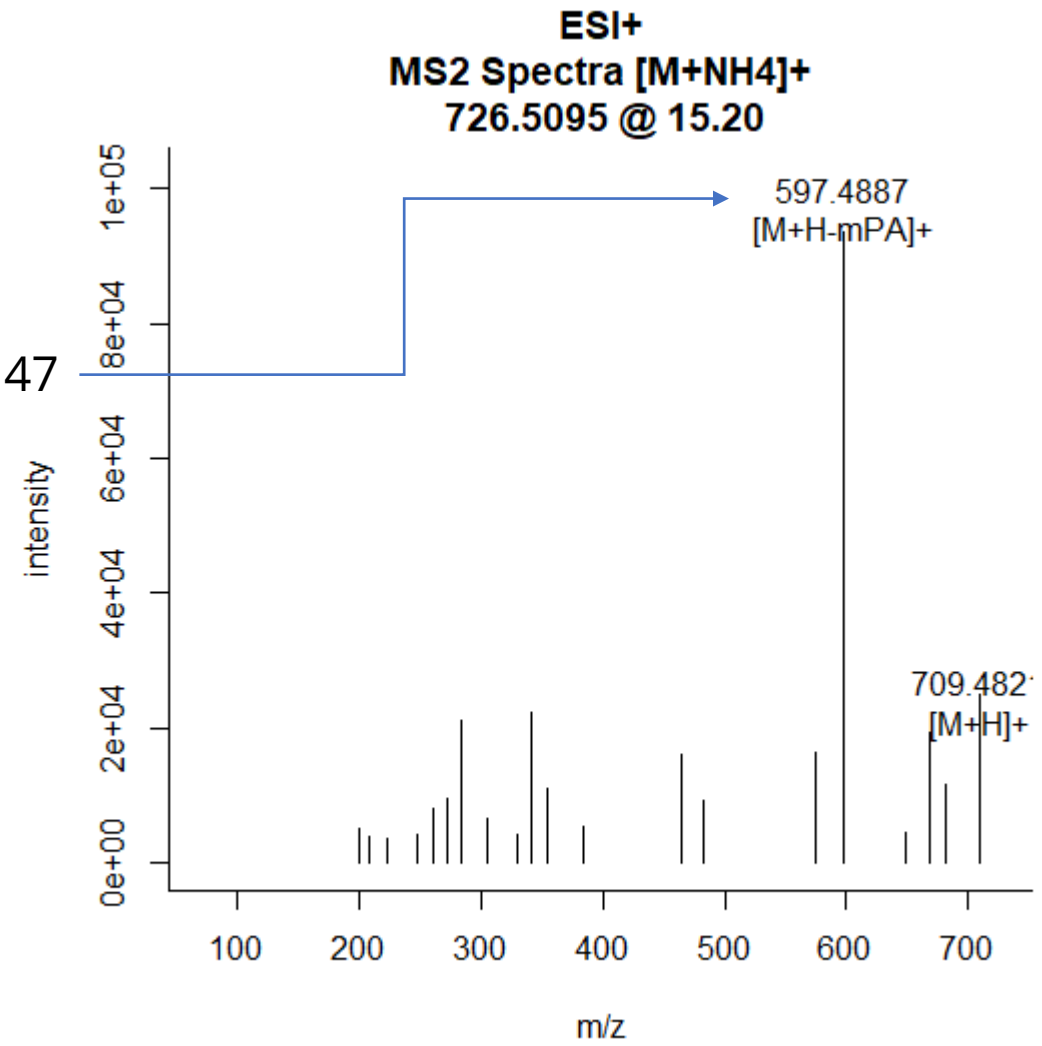
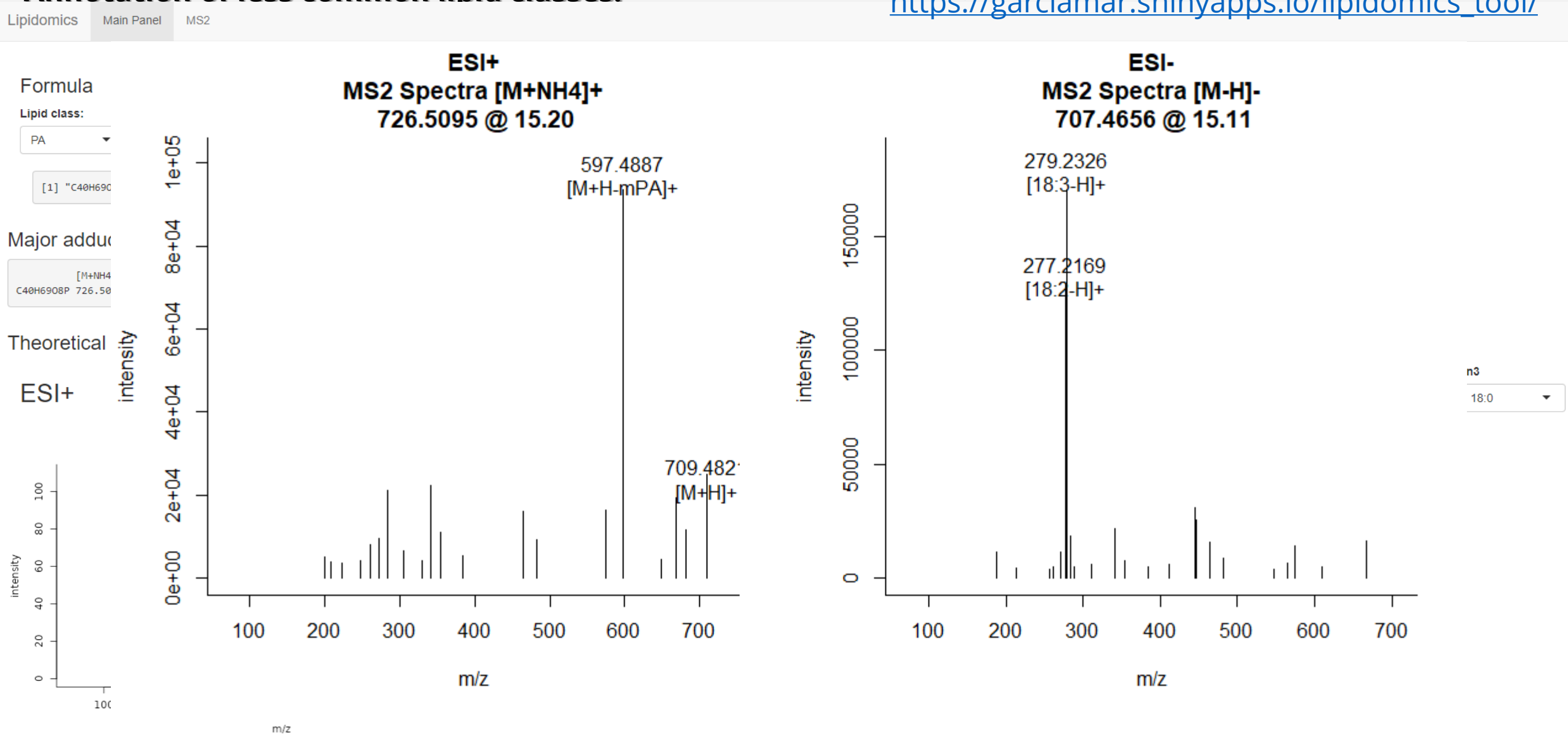


n2: 19:3 | sn3: 18:0

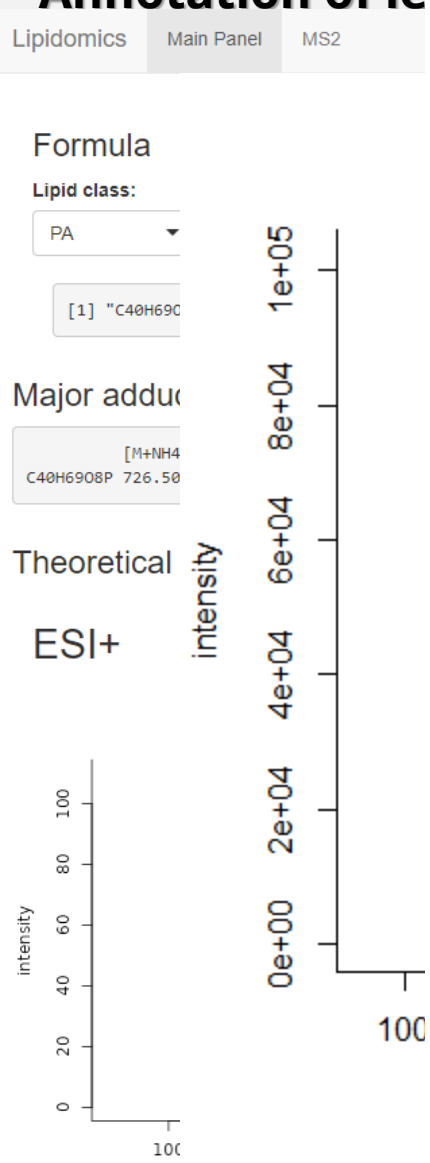
Annotation of less common lipid classes:

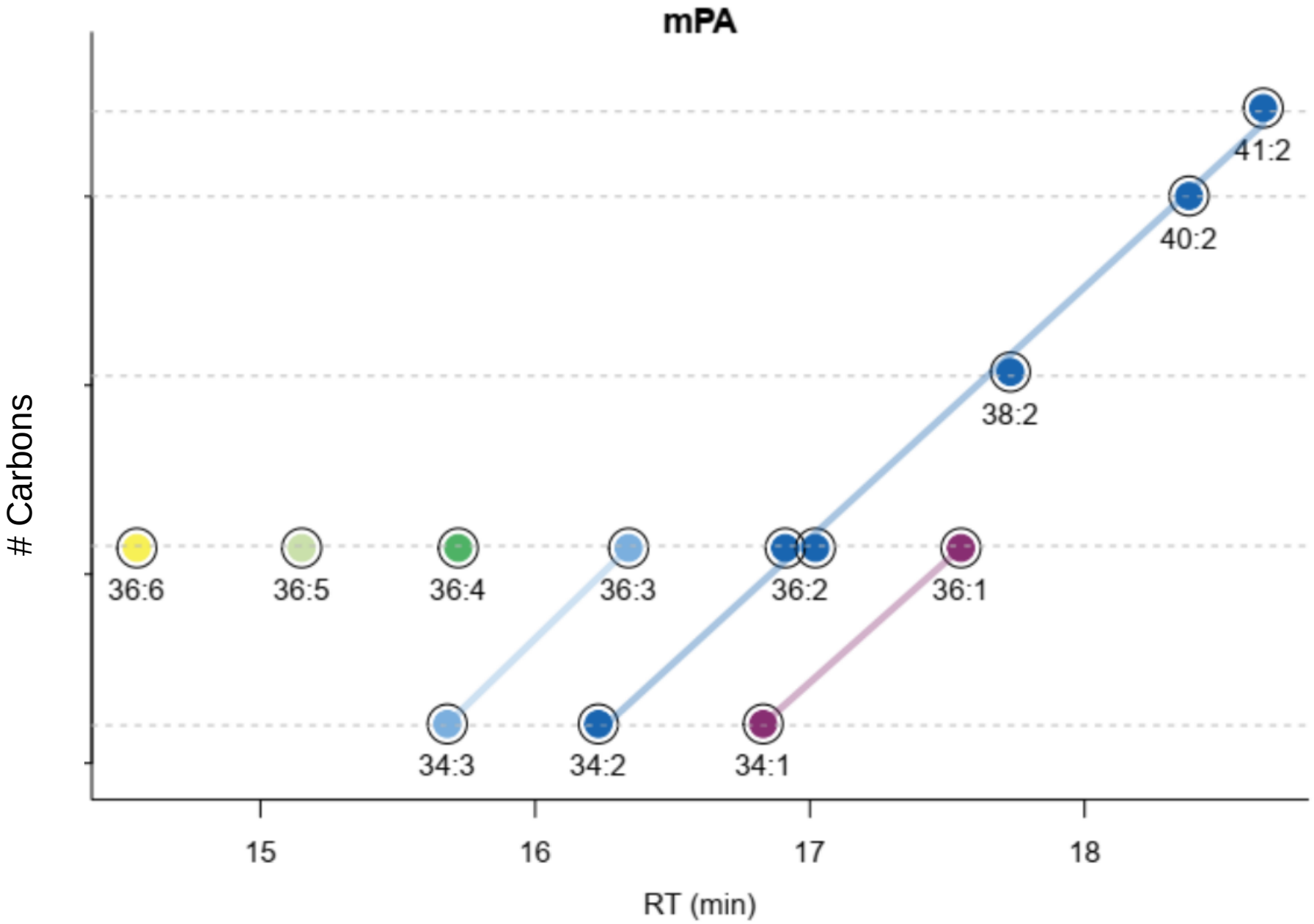
https://garciamar.shinyapps.io/lipidomics_tool/



Annotation of le

s_tool/





n3
18:0

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Thank you for your attention!

mar.garcia@fmach.it – github: mar-garcia

Italian Metabolomics Network General Meeting 2023| https://github.com/mar-garcia/IMN2023