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# Lipidomics Data Analysis Workshop

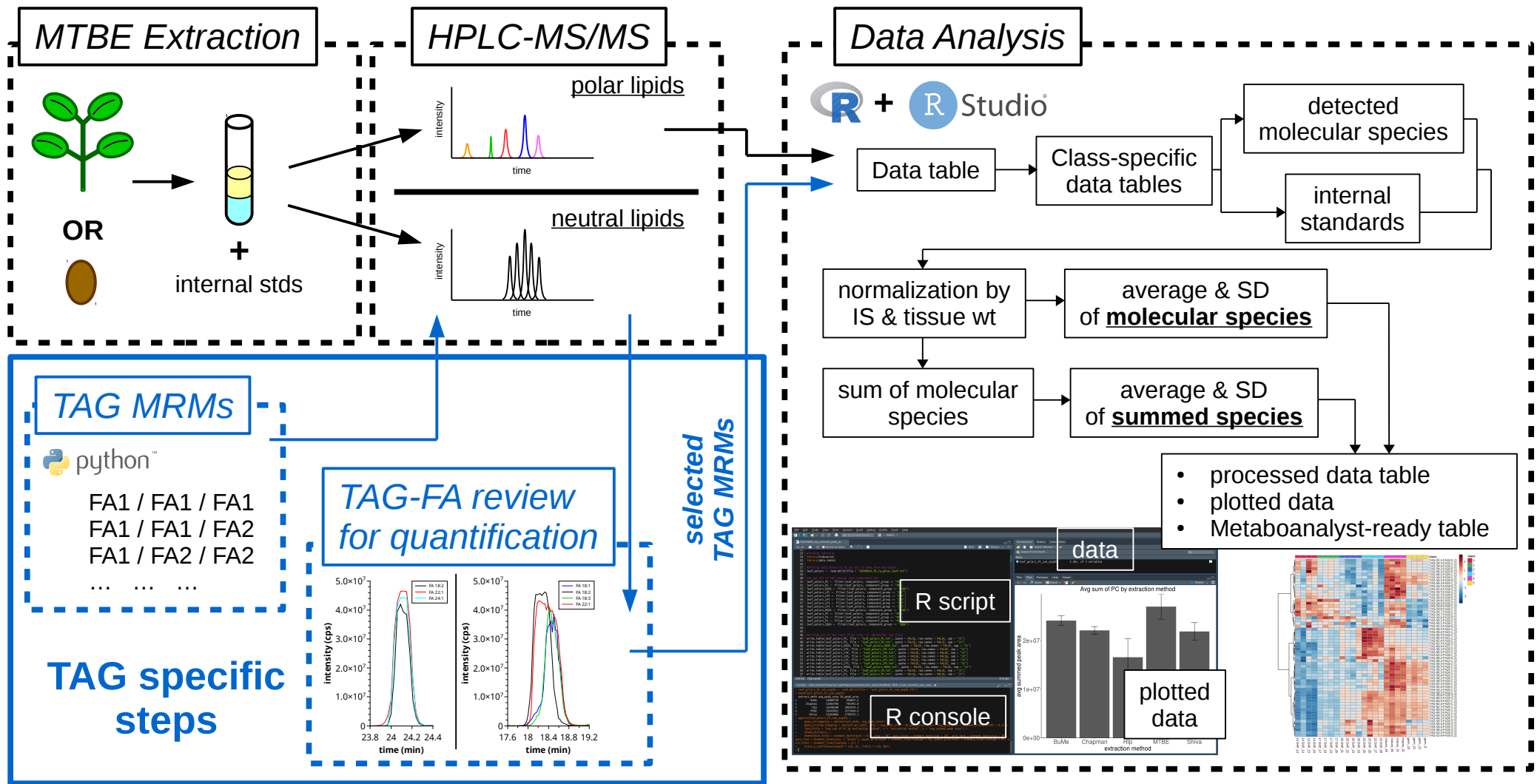
September 23, 2021

# Outline

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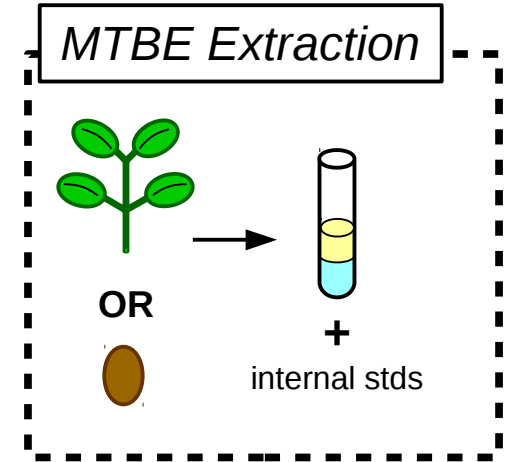
- Overview of lipidomics methods
  - Extraction considerations & internal standards
  - Two different HPLC-MS/MS methods
    - Polar lipids method
    - Neutral lipids method
- Data analysis workflow
  - MRM generation (before analysis)
  - View chromatograms in Analyst
    - Identifying correct TAG-FA MRM transitions
  - Integrating HPLC peaks in Multiquant
  - Formatting data table for R
  - Formatting R script for analysis
  - Troubleshooting

# Lipidomics Workflow — from extraction to analysis



# Extraction considerations & internal standards

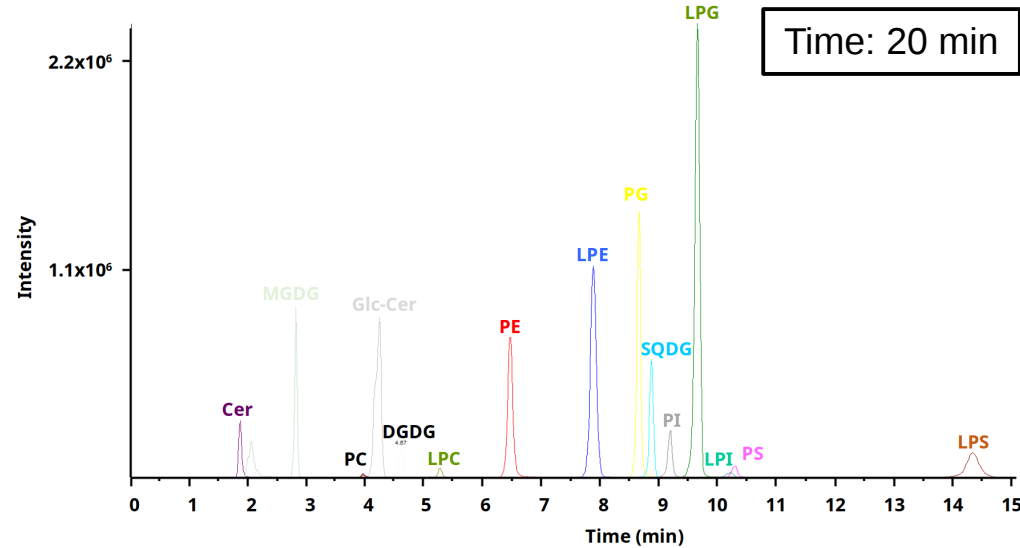
- Which extraction protocol to use?
  - Either the MTBE or IPA/ $\text{CHCl}_3$  (i.e. “Chapman method”)
  - Some considerations:
    - Resuspend in  $\text{CHCl}_3/\text{MeOH}$  (1:1) solvent for Chapman method
    - Extract at room temperature for MTBE method
    - Possibility to normalize by protein concentration with MTBE method(?)
- Which internal standards and how much?
  - UltimateSPLASH ONE (Avanti Polar Lipids, ca. no. 330820)
    - 10  $\mu\text{L}$  per ~10-50 mg tissue extracted
    - Conc. in mix = 25 – 150  $\mu\text{g}/\text{mL}$
    - Amt. added = 0.3 – 1.5 ng (between 0.2 – 2 nmol)
  - If using other internal standards
    - Aim for similar amounts as above for the specific lipid class
    - Use at least 1 per lipid class of interest (ideally 2-3 covering different FA lengths and degrees of unsaturation)



# Two different HPLC-MS/MS methods

## Polar lipids

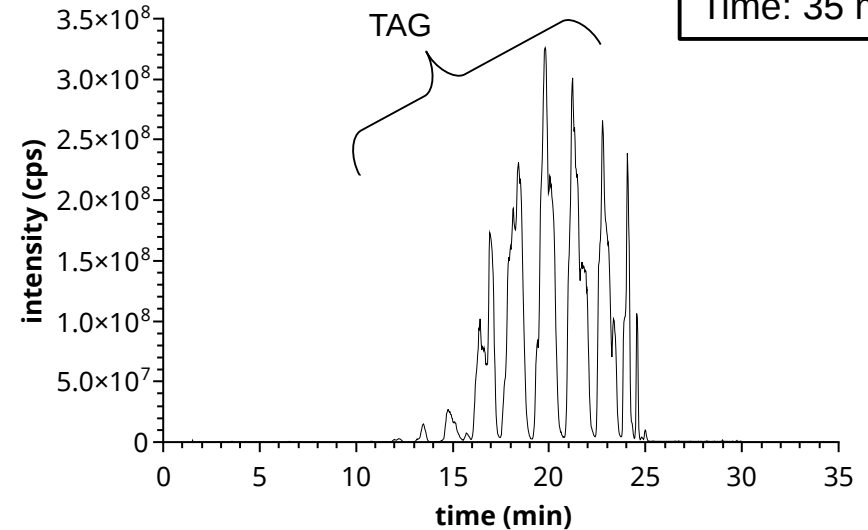
Time: 20 min



- Solvents:
  - A) AcN / H<sub>2</sub>O / Hex (92:6:2) + 2 mM NH<sub>4</sub>Ac
  - B) AcN / H<sub>2</sub>O (50:50) + 2 mM NH<sub>4</sub>Ac
- Column: Phenomenex NH<sub>2</sub> Luna Column (3  $\mu$ m, 100 Å, 150 x 4.6 mm)
- Lipid species: PC, PE, PI, PG, PS, lyso species, MGDG, DGDG, SQDG

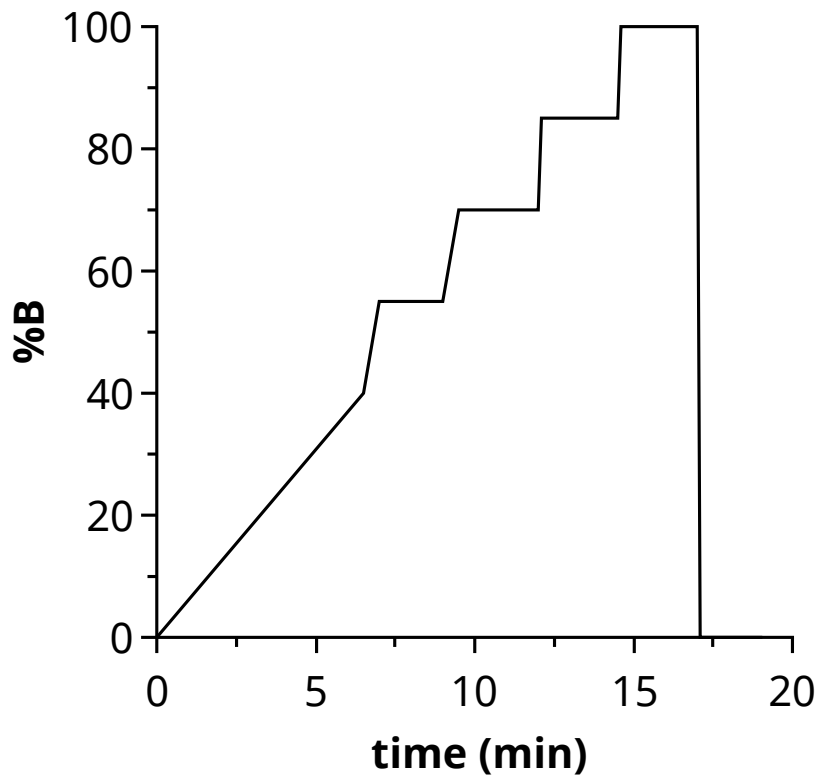
## Neutral lipids

Time: 35 min



- Solvents:
  - A) ACN / H<sub>2</sub>O (60:40) + 10 mM NH<sub>4</sub>COOH + 0.1% CHOOH
  - B) IPA / ACN / H<sub>2</sub>O (90:10:1) + 10 mM NH<sub>4</sub>COOH + 0.1% CHOOH
- Column: ThermoFisher Accucore Core-shell C30 silica (2.6  $\mu$ m, 150 Å, 150 x 2.1 mm)
- Lipid species: MAG, DAG, TAG

# Polar lipids method and parameters



**Solvents:** A) AcN / H<sub>2</sub>O / Hex (92:6:2) + 2mM NH<sub>4</sub>Ac

B) AcN / H<sub>2</sub>O (50:50) + 2mM NH<sub>4</sub>Ac

**Column:** Phenomenex NH<sub>2</sub> Luna Column

**Temp:** 25°C

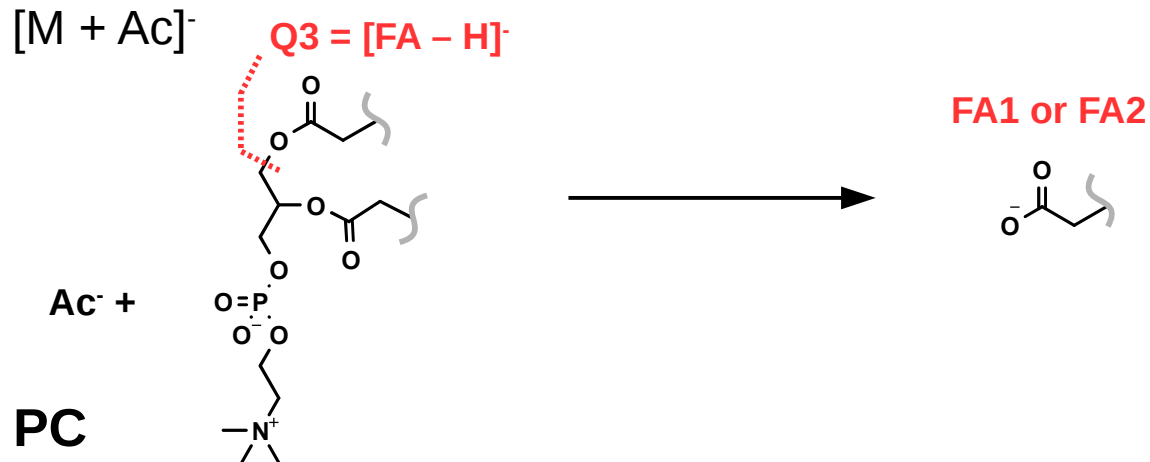
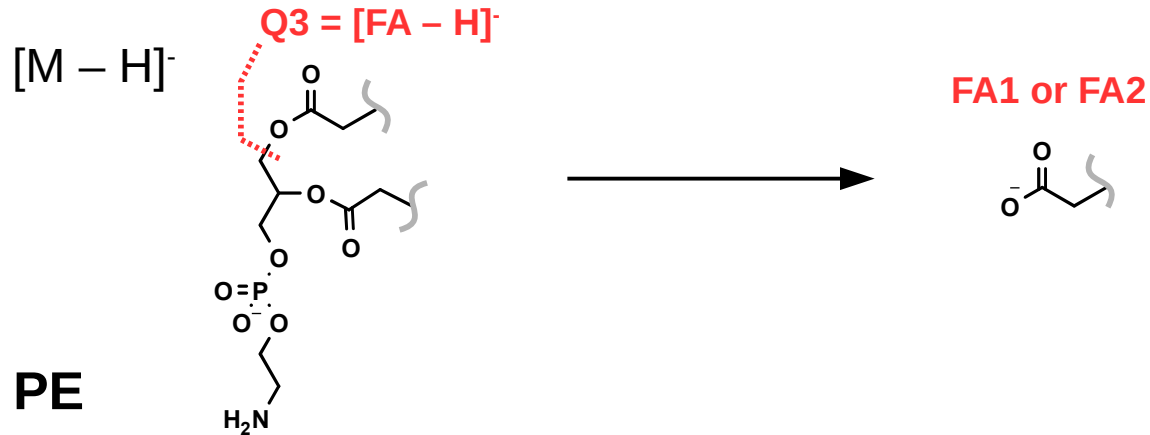
**Flow rate:** 1 mL/min

**Inj. vol.:** 5 µL

**Polarity:** negative

**Scan:** *scheduled* MRM

# Polar lipids general fragmentation and MRM transition

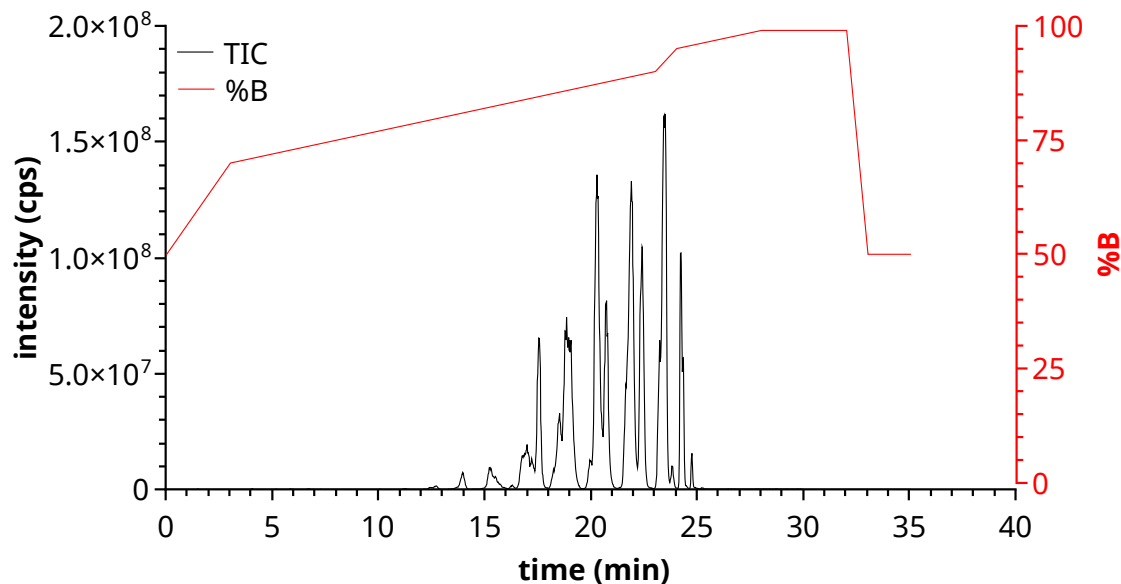


**Parent ion (Q1):**  $[M - H]^-$   
(exception PC and LPC as  $[M + Ac]^-$ )

**Fragment ion (Q3):**  $[FA - H]^-$

Note: Only one of the FAs is detected

# Neutral lipids method and parameters



**Solvents:** A) AcN / H<sub>2</sub>O (60:40)  
+ 10mM NH<sub>4</sub>CHOOH + 0.1% CHOOH

B) IPA / AcN / H<sub>2</sub>O (90:10:1)  
+ 10mM NH<sub>4</sub>CHOOH + 0.1% CHOOH

**Column:** ThermoFisher Accucore C30 Core Shell

**Temp:** 40°C

**Flow rate:** 0.35 mL/min

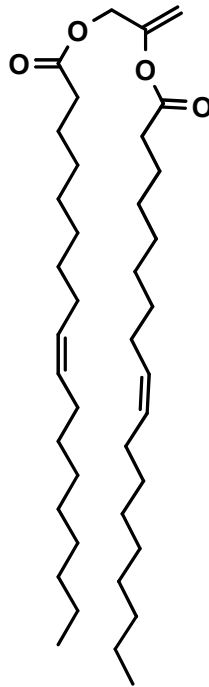
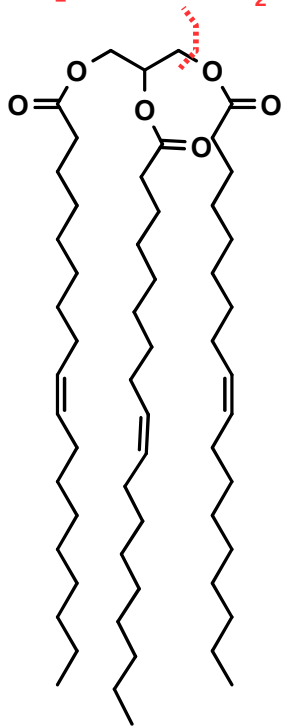
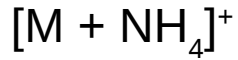
**Inj. vol.:** 5 µL

**Polarity:** positive

**Scan:** MRM



# Neutral lipids general fragmentation and MRM transition



TAG

**Parent ion (Q1):**  $[M + \text{NH}_4]^+$

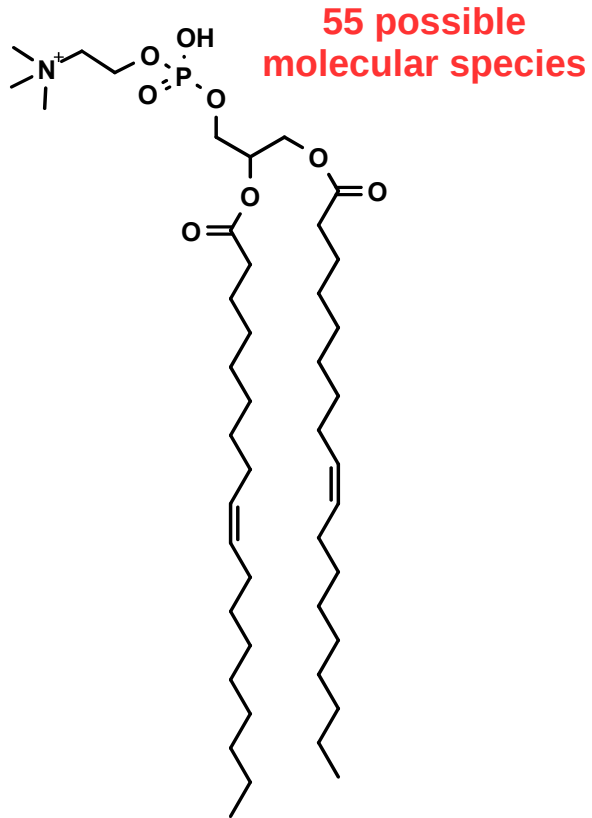
**Fragment ion (Q3):**  $[M - \text{FA} - \text{H}_2\text{O} + \text{H}]^+$   
(exception MAG as  $[\text{FA} - \text{H}_2\text{O} + \text{H}]^+$ )

Note: The loss of each *potential* FA is detected

# MRM generation and the loss of each *potential* FA in TAG

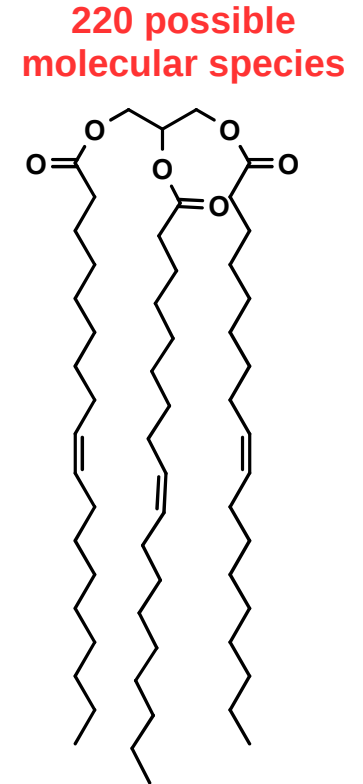
10 fatty acids = [16:0, 18:3, 18:2, 18:1, 18:0, 20:2, 20:1, 20:0, 22:1, 22:0]

*Arabidopsis thaliana*



$$P = \frac{(n+r-1)!}{r!(n-1)!}$$

$P$  = possible combinations  
 $n$  = number of FAs  
 $r$  = number of FAs per molecule



# MRM generation and the loss of each *potential* FA in TAG

My first attempt...

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	AM	AN	AO	AP	
1	Q1/Q3	TAG		Q3 FA		remaining			FA2							des 1						FA1	TAG species	overlap 1	overlap 2	overlap 3	ov
2		C	des	C	des	C	des		16	18	20	22	24	26		0	1	2	3			TAG-50.3 16.3	16.3/16.0/18.0				
3	TAG-50.3-FA16.3	50	3	16	3		34	0	16	16	14	12	10	8		0	0	0	0			TAG-50.3 16.2	16.2/16.0/18.1	16.2/16.1/18.0			
4	TAG-50.3-FA16.2	50	3	16	2		34	1	16	16	14	12	10	8		1	0	0	0			TAG-50.3 16.1	16.1/16.0/18.2	16.1/16.1/18.1	16.1/16.2/18.0		
5	TAG-50.3-FA16.1	50	3	16	1		34	2	16	16	14	12	10	8		2	1	0	0			TAG-50.3 16.0	16.0/16.0/18.3	16.0/16.1/18.2	16.0/16.2/18.1	16.0/16.3/18.0	
6	TAG-50.3-FA16.0	50	3	16	0		34	3	16	16	14	12	10	8		3	2	1	0			TAG-50.3 18.3	18.3/16.0/16.0				
7	TAG-50.3-FA18.3	50	3	18	3		32	0	16	14	12	10	8	6		0	0	0	0			TAG-50.3 18.2	18.2/16.0/16.1				
8	TAG-50.3-FA18.2	50	3	18	2		32	1	16	14	12	10	8	6		1	0	0	0			TAG-50.3 18.1	18.1/16.0/16.2	18.1/16.1/16.1			
9	TAG-50.3-FA18.1	50	3	18	1		32	2	16	14	12	10	8	6		2	1	0	0			TAG-50.3 18.0	18.0/16.0/16.3	18.0/16.1/16.2			
10	TAG-50.3-FA18.0	50	3	18	0		32	3	16	14	12	10	8	6		3	2	1	0			TAG-50.2 16.2	16.2/16.0/18.0				
11	TAG-50.2-FA16.2	50	2	16	2		34	0	16	16	14	12	10	8		0	0	0	0			TAG-50.2 16.1	16.1/16.0/18.1	16.1/16.1/18.0			
12	TAG-50.2-FA16.1	50	2	16	1		34	1	16	16	14	12	10	8		1	0	0	0			TAG-50.2 16.0	16.0/16.0/18.2	16.0/16.1/18.1	16.0/16.2/18.0		
13	TAG-50.2-FA16.0	50	2	16	0		34	2	16	16	14	12	10	8		2	1	0	0			TAG-50.2 18.2	18.2/16.0/16.0				
14	TAG-50.2-FA18.2	50	2	18	2		32	0	16	14	12	10	8	6		0	0	0	0			TAG-50.2 18.1	18.1/16.0/16.1				
15	TAG-50.2-FA18.1	50	2	18	1		32	1	16	14	12	10	8	6		1	0	0	0			TAG-50.2 18.0	18.0/16.0/16.2	18.0/16.1/16.1			
16	TAG-50.2-FA18.0	50	2	18	0		32	2	16	14	12	10	8	6		2	1	0	0			TAG-50.1 16.1	16.1/16.0/18.0				
17	TAG-50.1-FA16.1	50	1	16	1		34	0	16	16	14	12	10	8		0	0	0	0			TAG-50.1 16.0	16.0/16.0/18.1	16.0/16.1/18.0			
18	TAG-50.1-FA16.0	50	1	16	0		34	1	16	16	14	12	10	8		1	0	0	0			TAG-50.1 18.1	18.1/16.0/16.0				
19	TAG-50.1-FA18.1	50	1	18	1		32	0	16	14	12	10	8	6		0	0	0	0			TAG-50.1 18.0	18.0/16.0/16.1				
20	TAG-50.1-FA18.0	50	1	18	0		32	1	16	14	12	10	8	6		1	0	0	0			TAG-52.6 16.3	16.3/16.3/20.0	16.3/18.0/18.3	16.3/16.1/18.2		
21	TAG-52.6-FA16.3	52	6	16	3		36	3	20	18	16	14	12	10		0	0	0	0			TAG-52.6 16.2	16.2/16.3/20.1	16.2/18.1/18.3	16.2/18.2/18.2		
22	TAG-52.6-FA16.2	52	6	16	2		36	4	20	18	16	14	12	10		1	0	0	0			TAG-52.6 16.1	16.1/18.2/18.3				
23	TAG-52.6-FA16.1	52	6	16	1		36	5	20	18	16	14	12	10		2	1	0	0			TAG-52.6 16.0	16.0/18.3/18.3	18.3/16.1/18.2	18.3/16.2/18.1	18.3/16.3/18.0	
24	TAG-52.6-FA16.0	52	6	16	0		36	6	20	18	16	14	12	10		3	2	1	0			TAG-52.6 18.3	18.3/16.0/18.3	18.3/16.1/18.2	18.3/16.2/18.1	18.3/16.3/18.0	
25	TAG-52.6-FA18.3	52	6	18	3		34	3	18	16	14	12	10	8		0	0	0	0			TAG-52.6 18.2	18.2/16.1/18.3	18.2/16.2/18.2	18.2/16.3/18.1		
26	TAG-52.6-FA18.2	52	6	18	2		34	4	18	16	14	12	10	8		1	0	0	0			TAG-52.6 18.1	18.1/16.2/18.3				
27	TAG-52.6-FA18.1	52	6	18	1		34	5	18	16	14	12	10	8		2	1	0	0			TAG-52.6 18.0	18.0/16.3/18.3				
28	TAG-52.6-FA18.0	52	6	18	0		34	6	18	16	14	12	10	8		3	2	1	0								

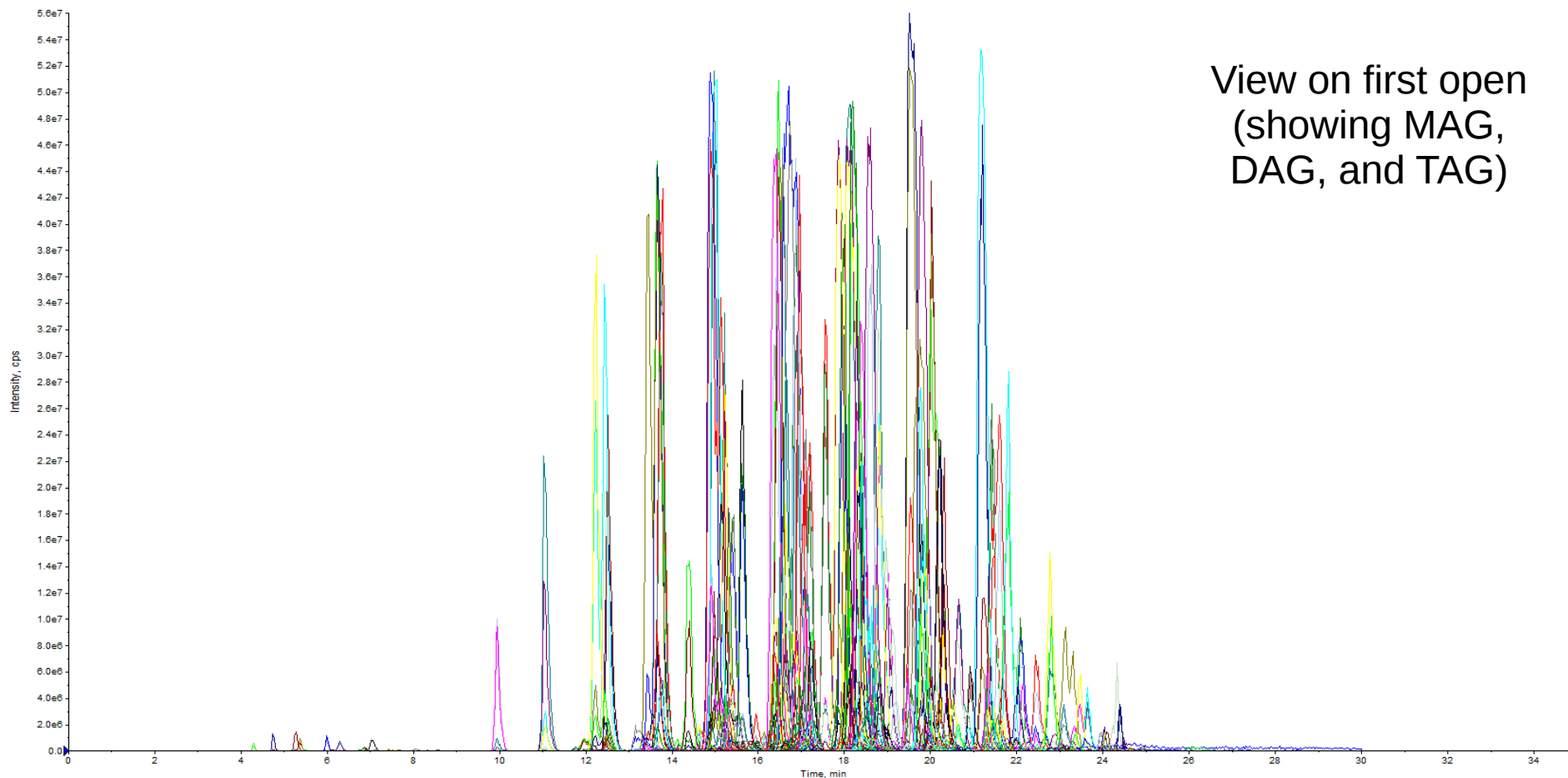
...

# MRM generation and the loss of each *potential* FA in TAG

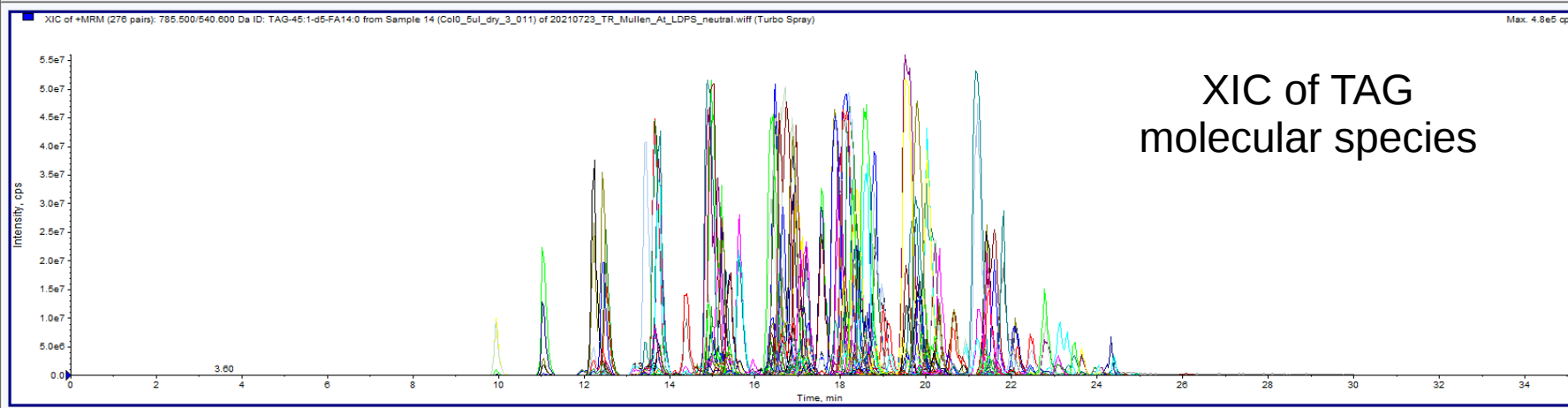
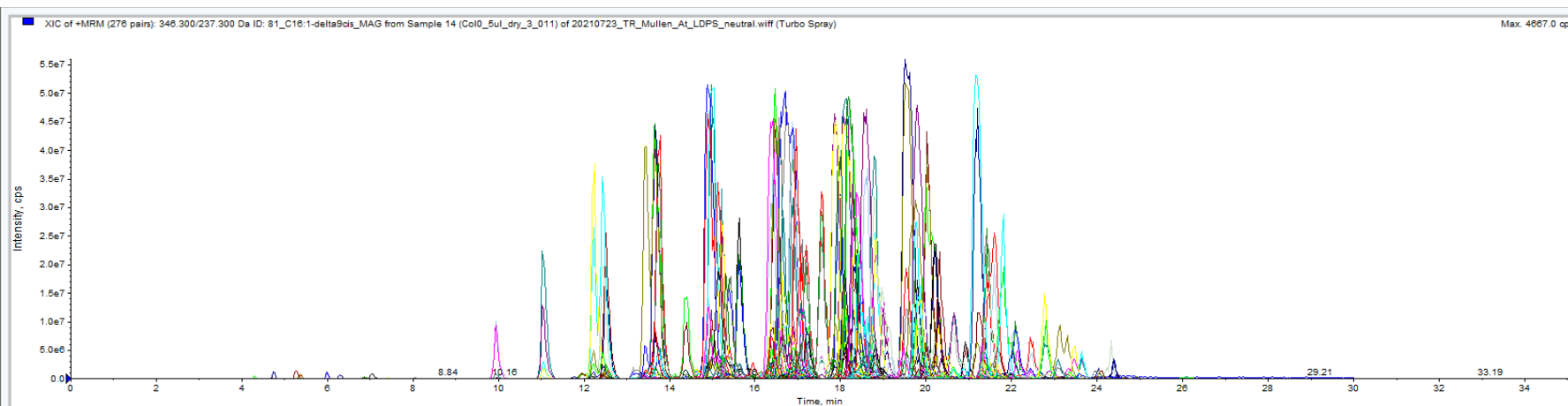
A better approach...

```
1 # TAG-FA MRM generator 2.0
2 import pandas as pd
3
4 fatty_acids = ['16:0', '18:3', '18:2', '18:1', '18:0', '20:2', '20:1', '20:0', '22:1', '22:0'] # Arabidopsis https://plantfadb.org/datasets?plant\_id=17354
5
6
7 def mrm_output(fal):
8
9     carbon = 12.000000
10    hydrogen = 1.007825
11    oxygen = 15.994915
12    ammonium = 18.03382555
13
14    fa_carbon_no = []
15    fa_desat_no = []
16
17    # converting string to int for calculations below
18    for i in fal:
19        fa_carbon_no.append(int(i[:2]))
20        fa_desat_no.append(int(i[-1]))
21
22
23
24    tag_carbon_mass = []
25    tag_desat_mass = []
26    final_tag_carbon = []
27    final_tag_desat = []
28
29    fa_carbon_no_2 = []
30    fa_desat_no_2 = []
31
32    m = len(fa_carbon_no)
33    n = len(fa_desat_no)
34
35    # to get the total fa carbon number
36    while fa_carbon_no != []:
37        for i in fa_carbon_no:
38            tag_carbon_mass += [fa_carbon_no[0] + i]
39
40        fa_carbon_no_2.append(fa_carbon_no[0])
41        fa_carbon_no.remove(fa_carbon_no[0])
42
43
```

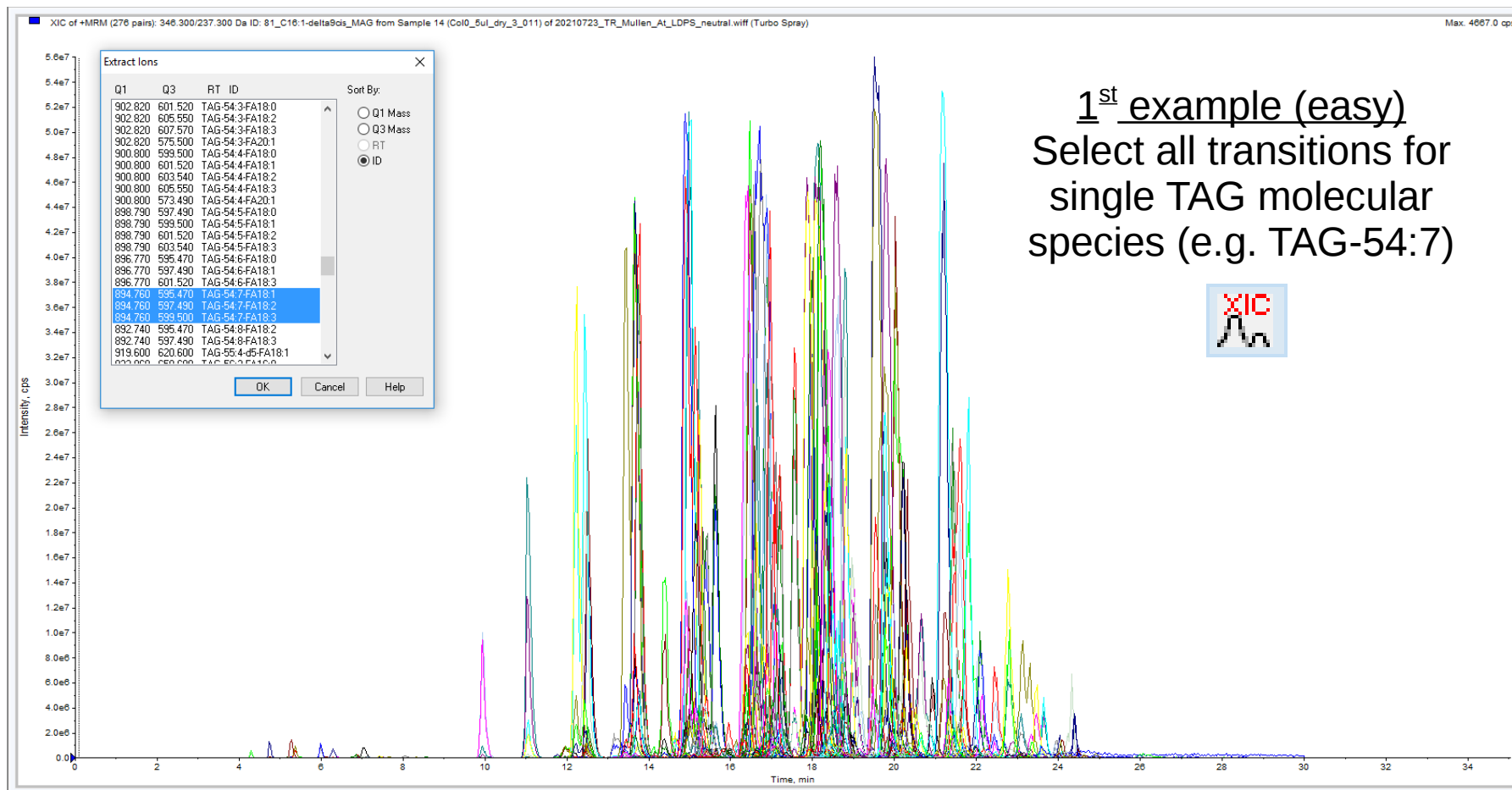
# 1<sup>st</sup> step in TAG analysis: ID'ing which TAG-FA MRM transitions to use for quantification in Analyst



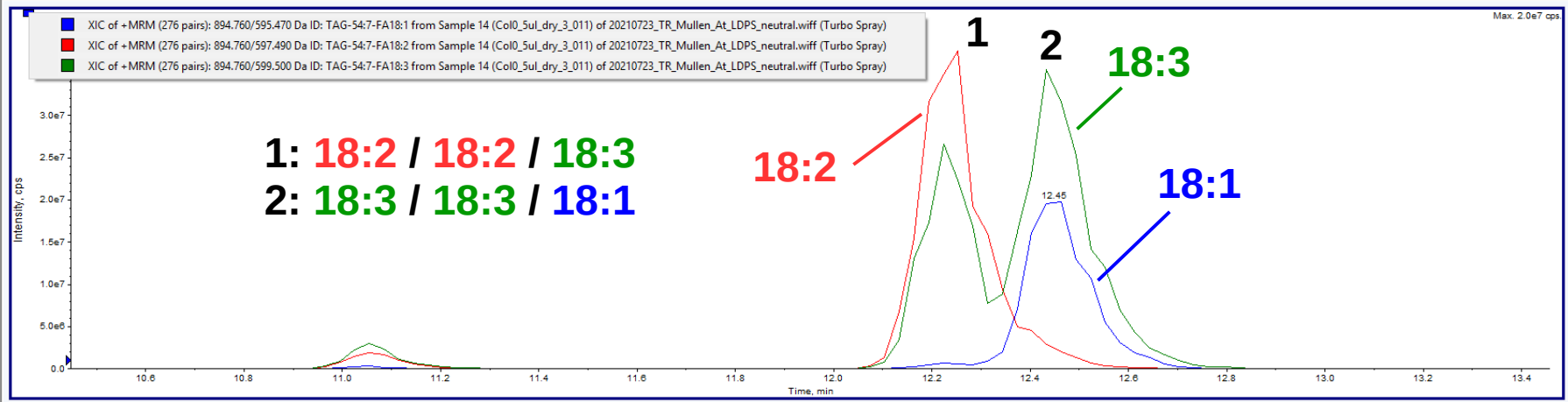
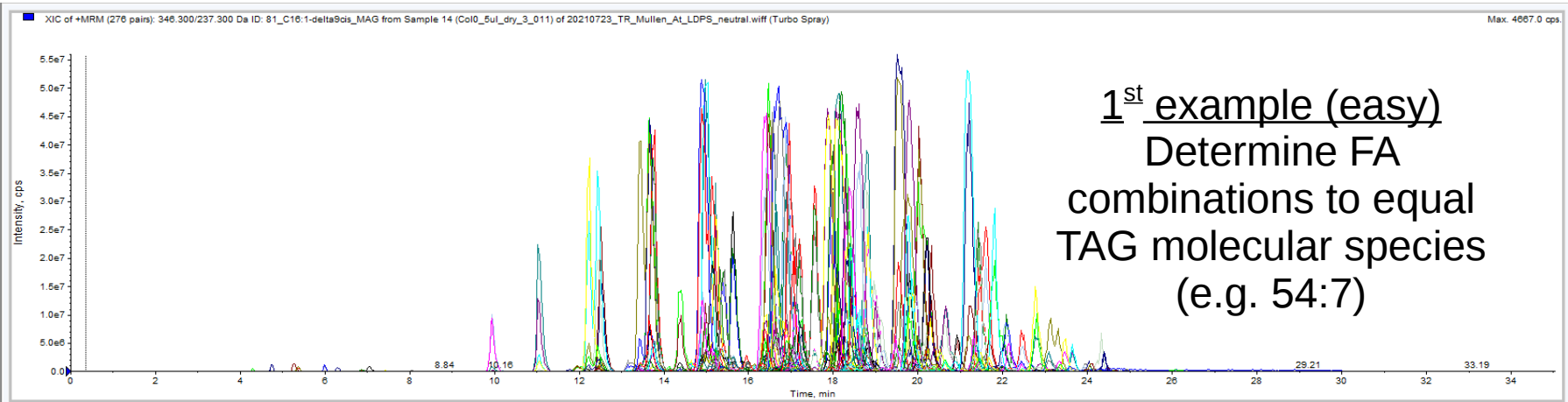
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# 1<sup>st</sup> step in TAG analysis: ID'ing which TAG-FA MRM transitions to use for quantification in Analyst



## TAG 54:7

1: 18:2 / 18:2 / 18:3

2: 18:3 / 18:3 / 18:1

1<sup>st</sup> example (easy)  
Record these TAG  
molecular species and  
FA combinations (RT is  
also good to take down)

	A	B	C	D	E
1	TG molec sp	FA comp	FA for quant	RT	notes
31	tg-54:7	18:2/18:2/18:3	fa-18:2	12.25	/2 ←
32	tg-54:7	18:3/18:3/18:1	fa-18:1	12.44	

Note this value  
will be divided  
by 2

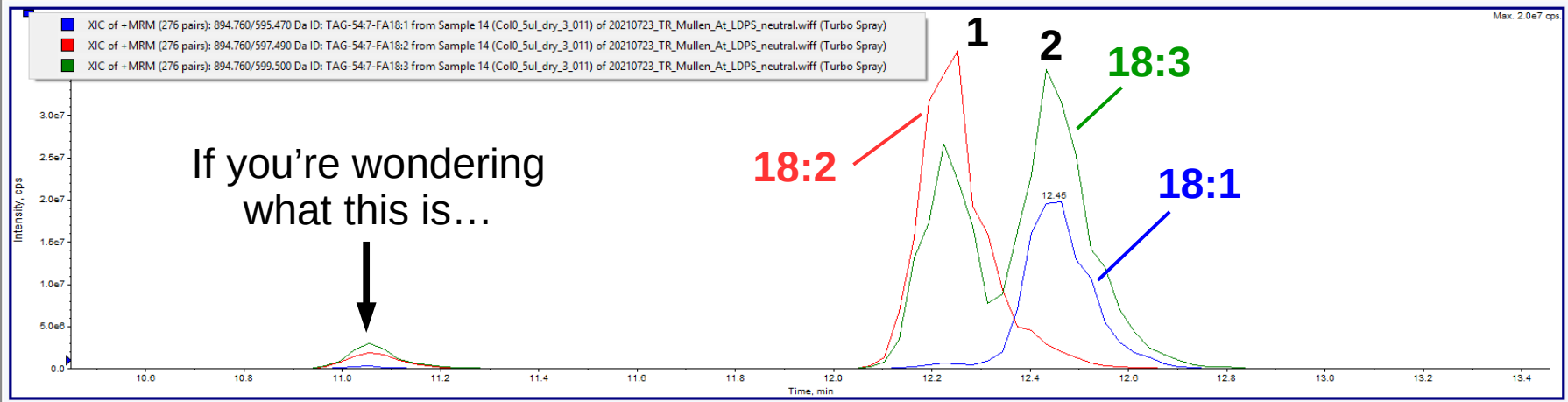
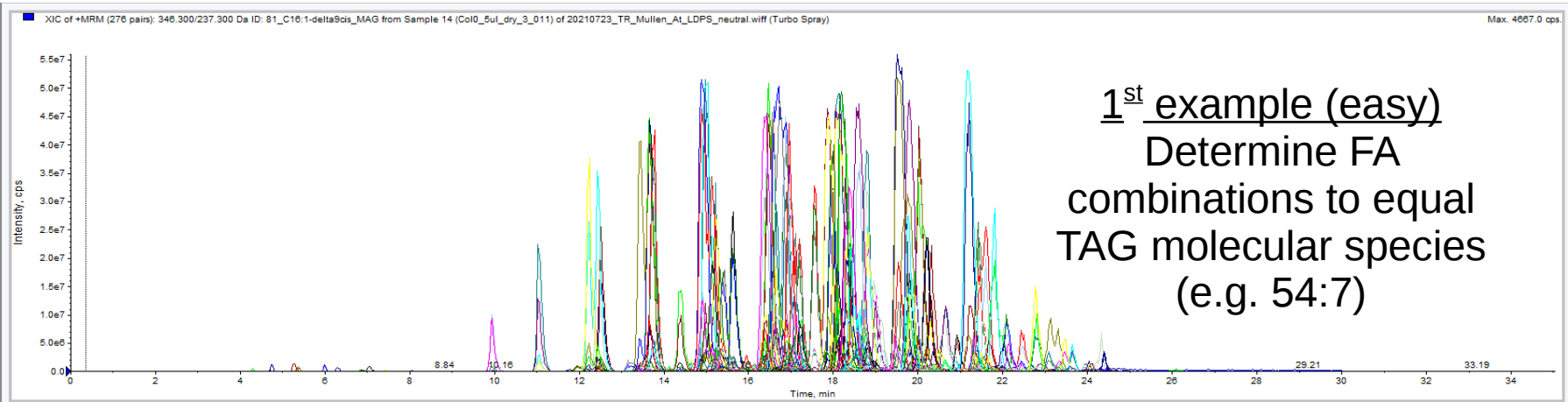
These indicate which MRM transitions to use for quantification:

**TAG-54:7-FA18:2**

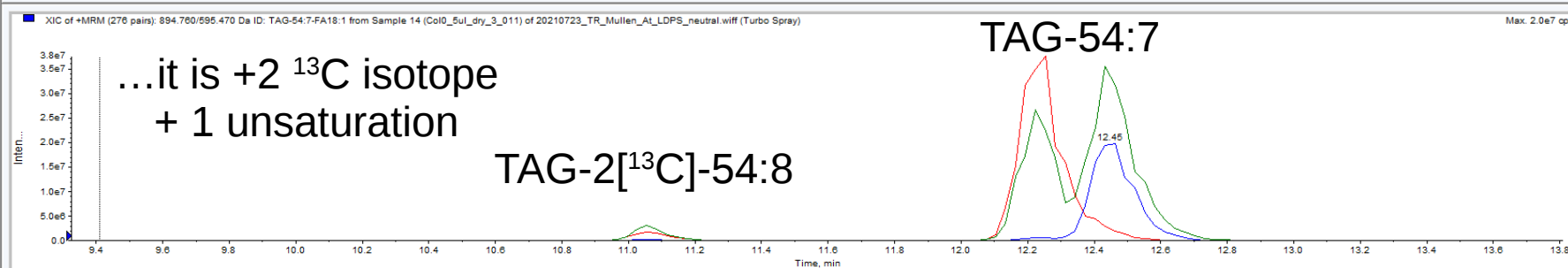
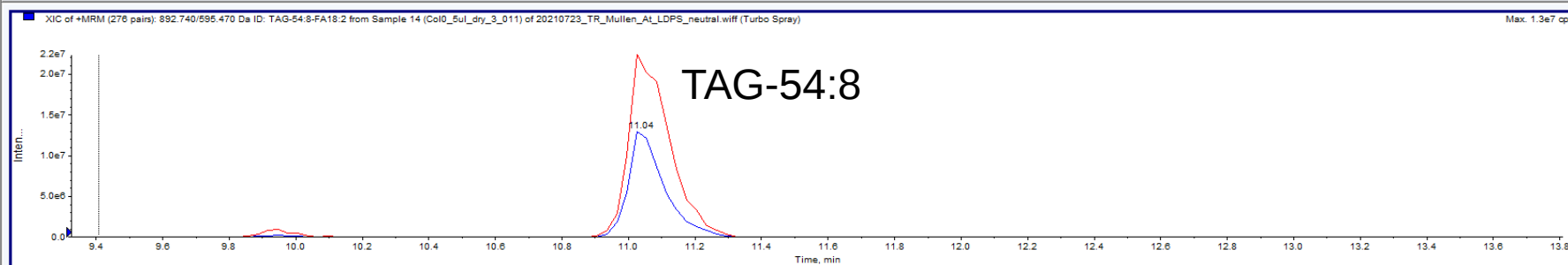
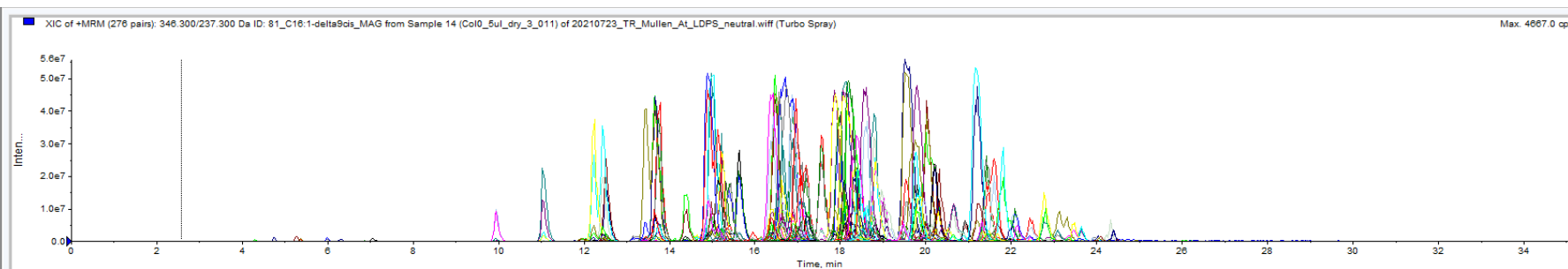
**TAG-54:7-FA18:1**

*TAG-54:7-FA18:3 is not used*

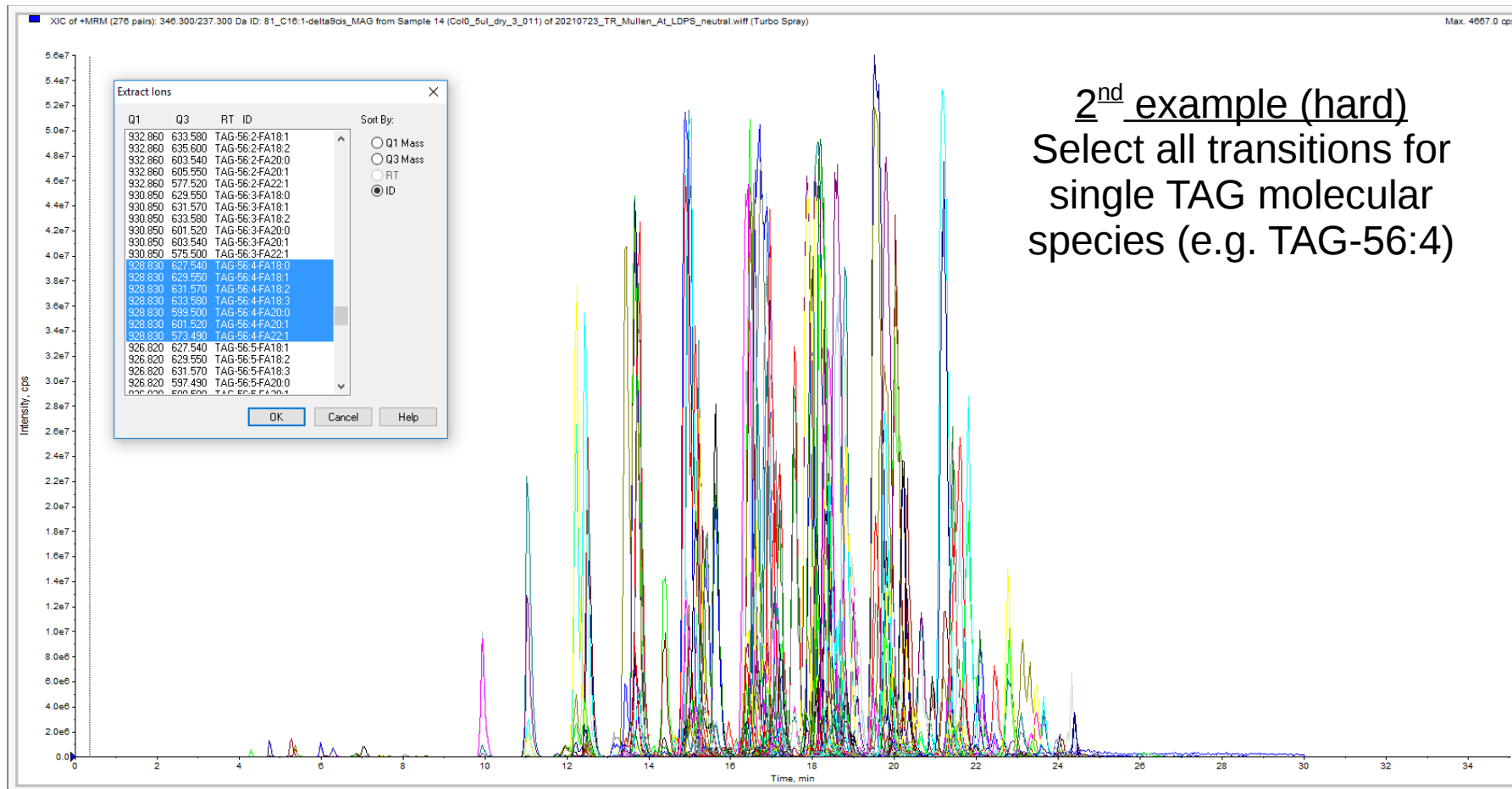
# 1<sup>st</sup> step in TAG analysis: ID'ing which TAG-FA MRM transitions to use for quantification in Analyst



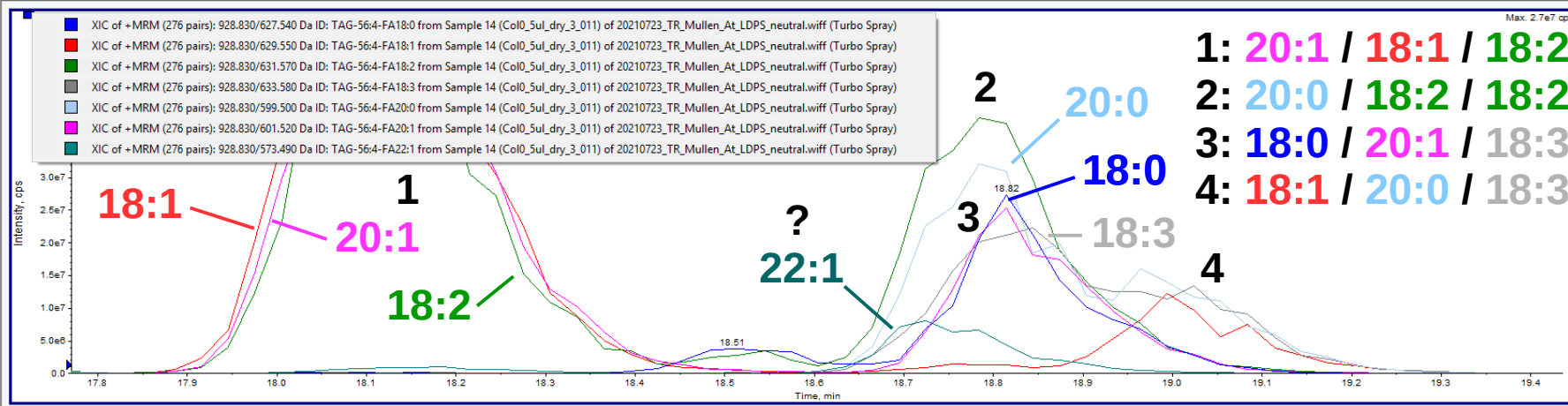
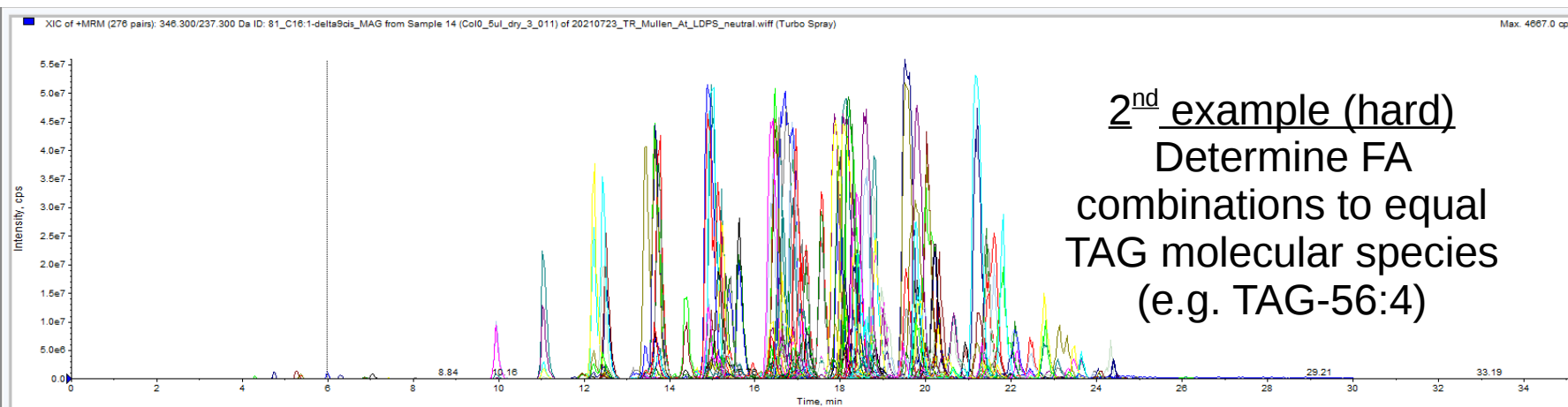
# 1<sup>st</sup> step in TAG analysis: ID'ing which TAG-FA MRM transitions to use for quantification in Analyst



# 1<sup>st</sup> step in TAG analysis: ID'ing which TAG-FA MRM transitions to use for quantification in Analyst



# 1<sup>st</sup> step in TAG analysis: ID'ing which TAG-FA MRM transitions to use for quantification in Analyst



# 1<sup>st</sup> step in TAG analysis: ID'ing which TAG-FA MRM transitions to use for quantification in Analyst



## TAG 56:4

- 1: 20:1 / 18:1 / 18:2
- 2: 20:0 / 18:2 / 18:2
- 3: 18:0 / 20:1 / 18:3
- 4: 18:1 / 20:0 / 18:3

2<sup>nd</sup> example (hard)  
Record these TAG  
molecular species and  
FA combinations (RT is  
also good to take down)

	A	B	C	D	E	F	
1	TG molec sp	FA comp	FA for quant	RT	notes		
41	tg-56:4	20:1/18:1/18:2	fa-18:1	18.19	1st peak		
42	tg-56:4	20:0/18:2/18:2	fa-18:2	18.79	/2; 2nd peak		
43	tg-56:4	18:0/20:1/18:3	fa-18:0	18.84			
44	tg-56:4	18:1/20:0/18:3	fa-20:0	18.99	/2 (second half of peak)		

MRM transitions used for quantification:

**TAG-56:4-FA18:1**  
**TAG-56:4-FA18:2**  
**TAG-56:4-FA18:0**  
**TAG-56:4-FA20:0**

MRM transitions **NOT** used for quantification:

*TAG-56:4-FA18:3*  
*TAG-56:4-FA20:1*  
*TAG-56:4-FA22:1*



# 2<sup>nd</sup> step in TAG analysis: Creating Multiquant method to integrate HPLC peaks

(Also 1<sup>st</sup> step for all the other lipids)

MultiQuant - [IMQ4] 20210727\_TR\_Mullen\_At\_neutral\_lipids.qmethod

File Edit Process Window Help

JCC/Chapman lab

Components & Groups IS MS

Components Integration & Regression Outlier Settings

Experiment: MRM (276 transitions)

Row	IS	Name	
96	<input type="checkbox"/>	TAG-48:3-FA16:1	TG
97	<input type="checkbox"/>	TAG-48:0-FA16:0	TG
98	<input type="checkbox"/>	TAG-50:3-FA18:3	TG
99	<input type="checkbox"/>	TAG-50:2-FA18:2	TG
100	<input type="checkbox"/>	TAG-50:2-FA18:1	TG
101	<input type="checkbox"/>	TAG-50:1-FA18:1	TG
102	<input type="checkbox"/>	TAG-52:6-FA16:0	TG
103	<input type="checkbox"/>	TAG-52:5-FA16:0	TG
104	<input type="checkbox"/>	TAG-52:4-FA18:2	TG
105	<input type="checkbox"/>	TAG-52:4-FA18:1	TG
106	<input type="checkbox"/>	TAG-52:3-FA18:3	TG
107	<input type="checkbox"/>	TAG-52:3-FA18:1	TG
108	<input type="checkbox"/>	TAG-52:2-FA18:2	TG
109	<input type="checkbox"/>	TAG-52:2-FA18:1	TG
110	<input type="checkbox"/>	TAG-54:9-FA18:3	TG
111	<input type="checkbox"/>	TAG-54:8-FA18:2	TG
112	<input type="checkbox"/>	TAG-54:7-FA18:2	TG
113	<input type="checkbox"/>	TAG-54:7-FA18:1	TG
114	<input type="checkbox"/>	TAG-54:6-FA18:1	TG
115	<input type="checkbox"/>	TAG-54:6-FA18:0	TG
116	<input type="checkbox"/>	TAG-54:6-FA18:2	TG
117	<input type="checkbox"/>	TAG-54:5-FA18:3	TG
118	<input type="checkbox"/>	TAG-54:5-FA18:2	TG
119	<input type="checkbox"/>	TAG-54:5-FA18:0	TG
120	<input type="checkbox"/>	TAG-54:4-FA18:3	TG
121	<input type="checkbox"/>	TAG-54:4-FA18:2	TG
122	<input type="checkbox"/>	TAG-54:4-FA18:0	TG
123	<input type="checkbox"/>	TAG-54:3-FA18:1	TG
124	<input type="checkbox"/>	TAG-54:3-FA18:3	TG
125	<input type="checkbox"/>	TAG-54:3-FA18:0	TG
126	<input type="checkbox"/>	TAG-54:2-FA18:2	TG
127	<input type="checkbox"/>	TAG-54:2-FA18:1	TG
128	<input type="checkbox"/>	TAG-56:5-FA20:0	TG
129	<input type="checkbox"/>	TAG-56:5-FA18:3	TG
130	<input type="checkbox"/>	TAG-56:5-FA18:2	TG
131	<input type="checkbox"/>	TAG-56:4-FA18:2	TG
132	<input type="checkbox"/>	TAG-56:4-FA18:1	TG
133	<input type="checkbox"/>	TAG-56:4-FA18:0	TG
134	<input type="checkbox"/>	TAG-56:4-FA20:0	TG

Nothing really needs to change for polar lipids or MAG or DAG

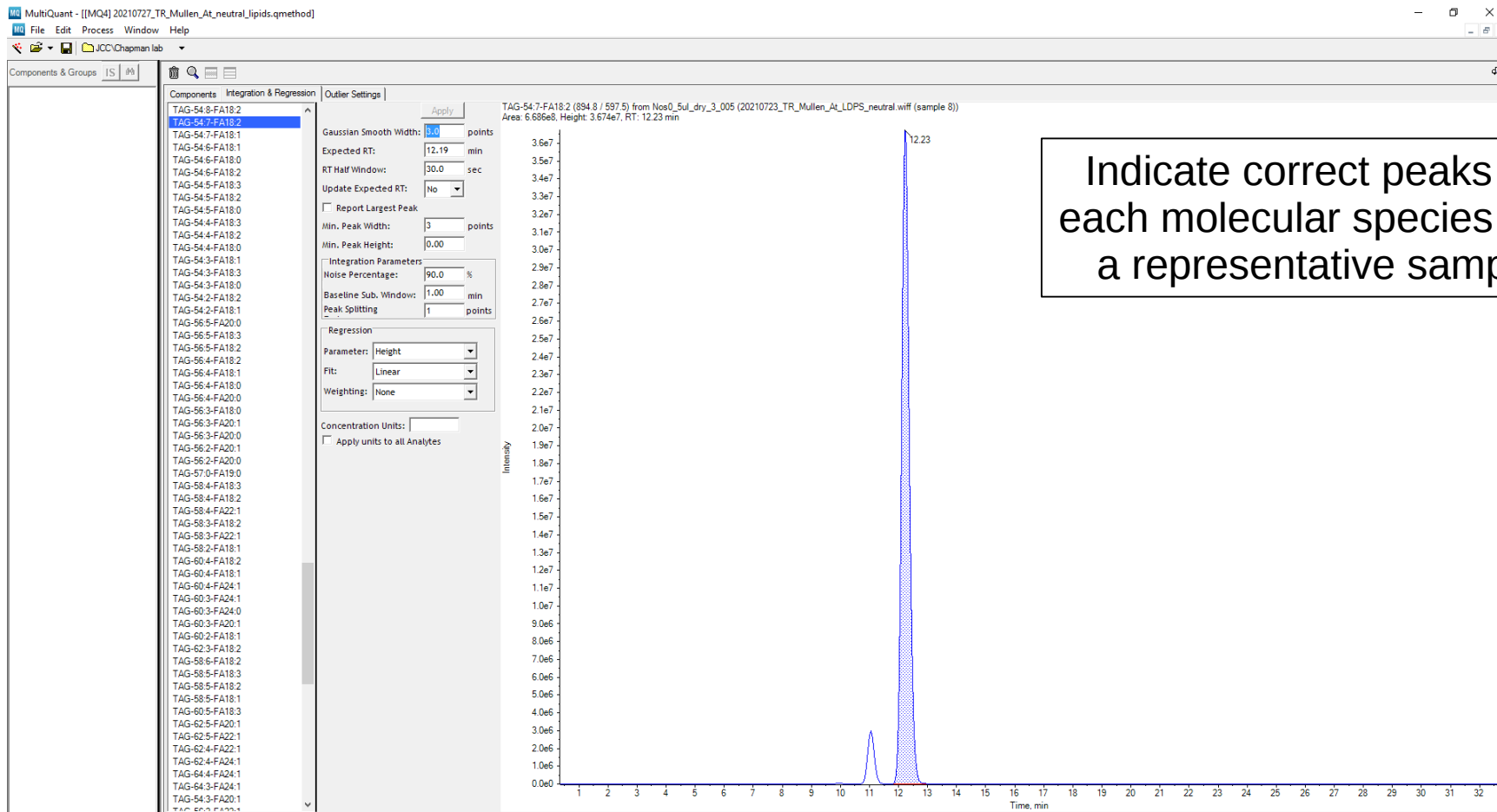
For TAG, delete all the MRM transitions that were not selected in Step 1

Q1/Q3
818.7 / 547.4
824.7 / 551.5
846.8 / 551.5
848.8 / 551.5
848.8 / 549.5
850.8 / 551.5
868.7 / 595.5
870.8 / 597.5
872.8 / 575.5
872.8 / 573.5
874.8 / 579.5
874.8 / 575.5
876.8 / 579.5
876.8 / 577.5
890.8 / 595.6
892.7 / 595.5
894.8 / 597.5
894.8 / 595.5
896.8 / 597.5
896.8 / 595.5
896.8 / 599.6
898.8 / 603.5
898.8 / 601.5
898.8 / 597.5
900.8 / 605.6
900.8 / 603.5
900.8 / 599.5
902.7 / 603.5
902.8 / 607.6
902.8 / 601.5
904.8 / 607.6
904.8 / 605.6
926.8 / 597.5
926.8 / 631.6
926.8 / 629.6
928.8 / 631.6
928.8 / 629.6
928.8 / 627.5
928.8 / 599.5



# 2<sup>nd</sup> step in TAG analysis: Creating Multiquant method to integrate HPLC peaks

(Also 1<sup>st</sup> step for all the other lipids)

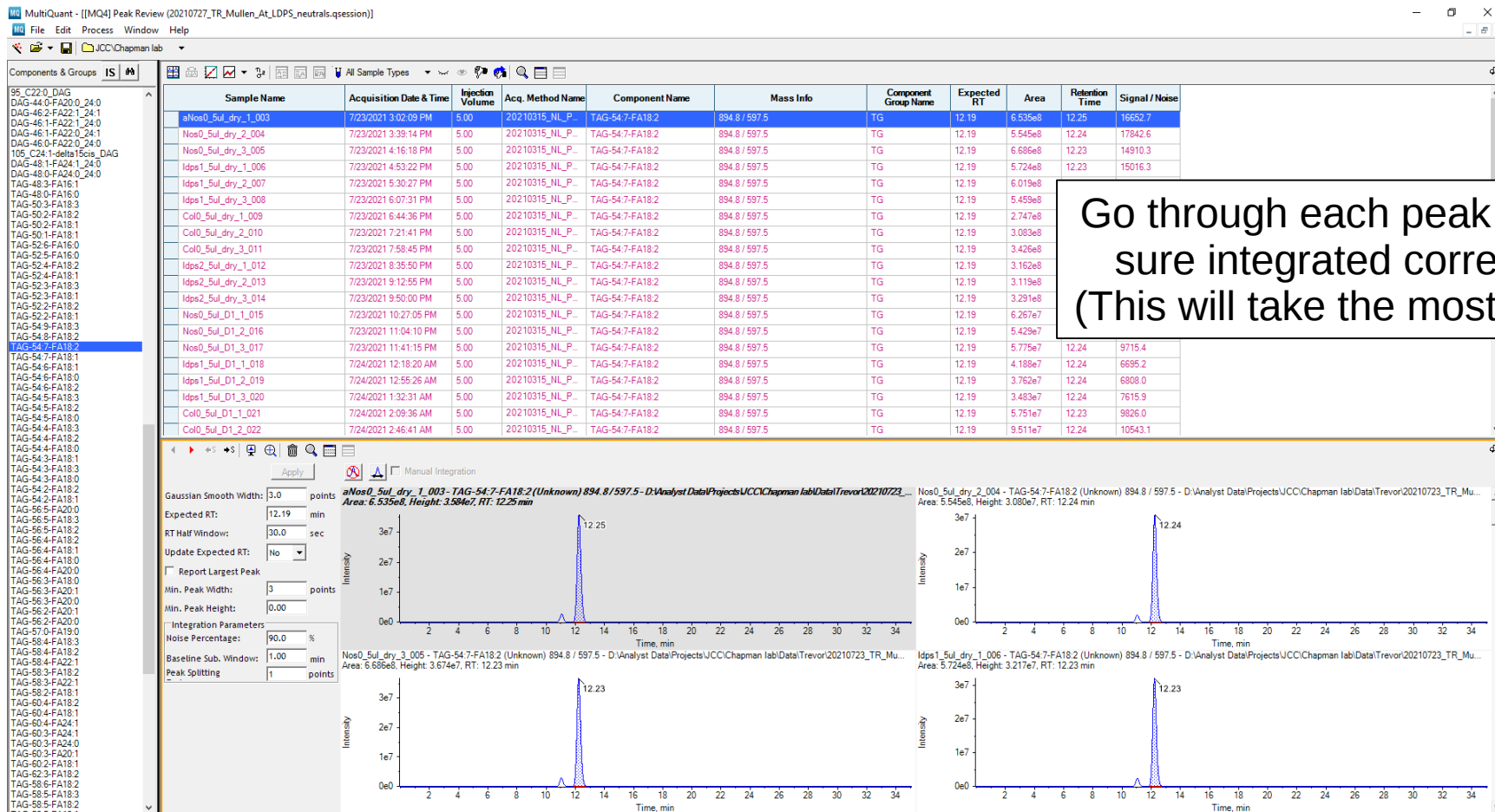






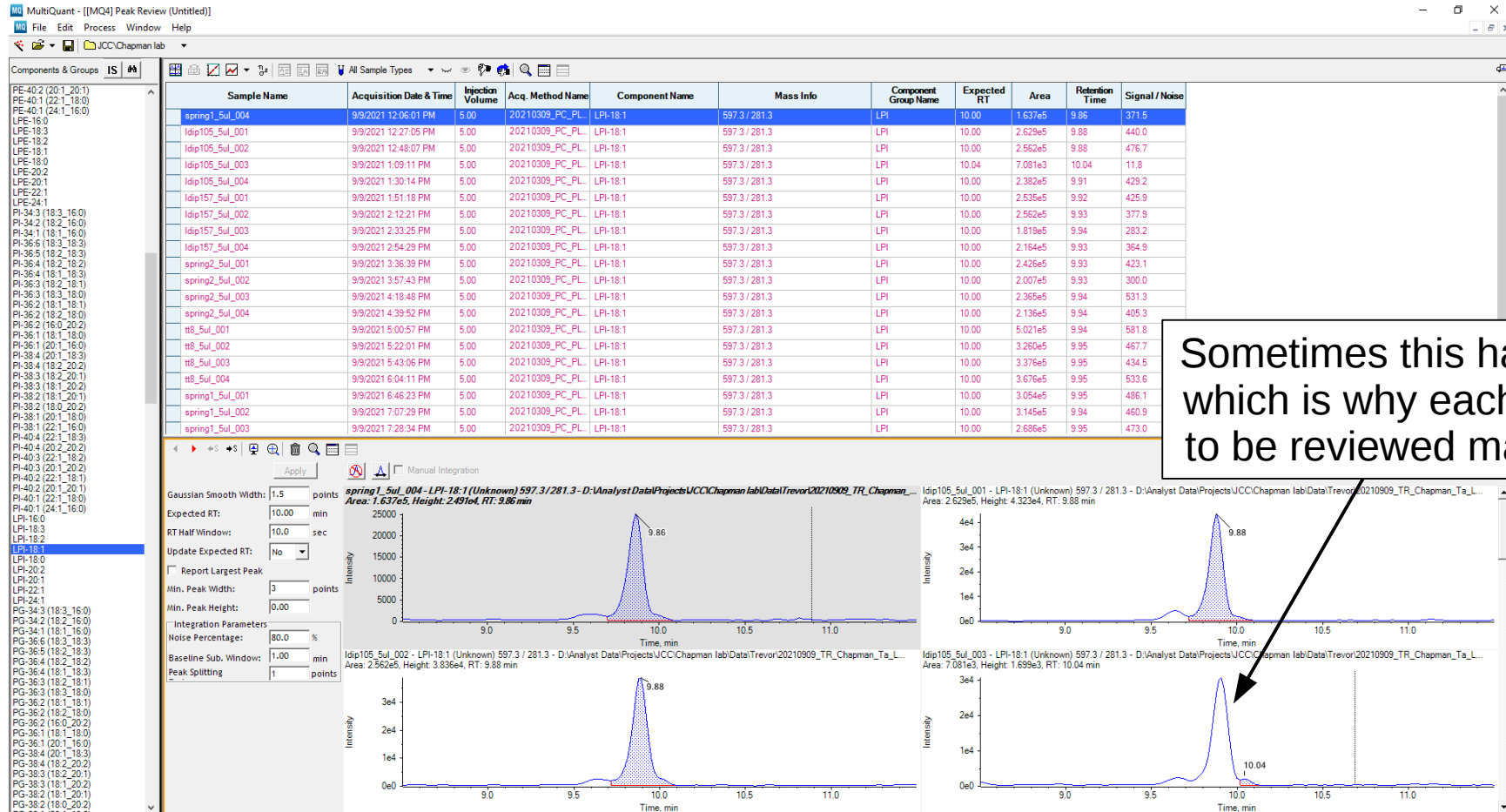
# 2<sup>nd</sup> step in TAG analysis: Creating Multiquant method to integrate HPLC peaks

(Also 1<sup>st</sup> step for all the other lipids)

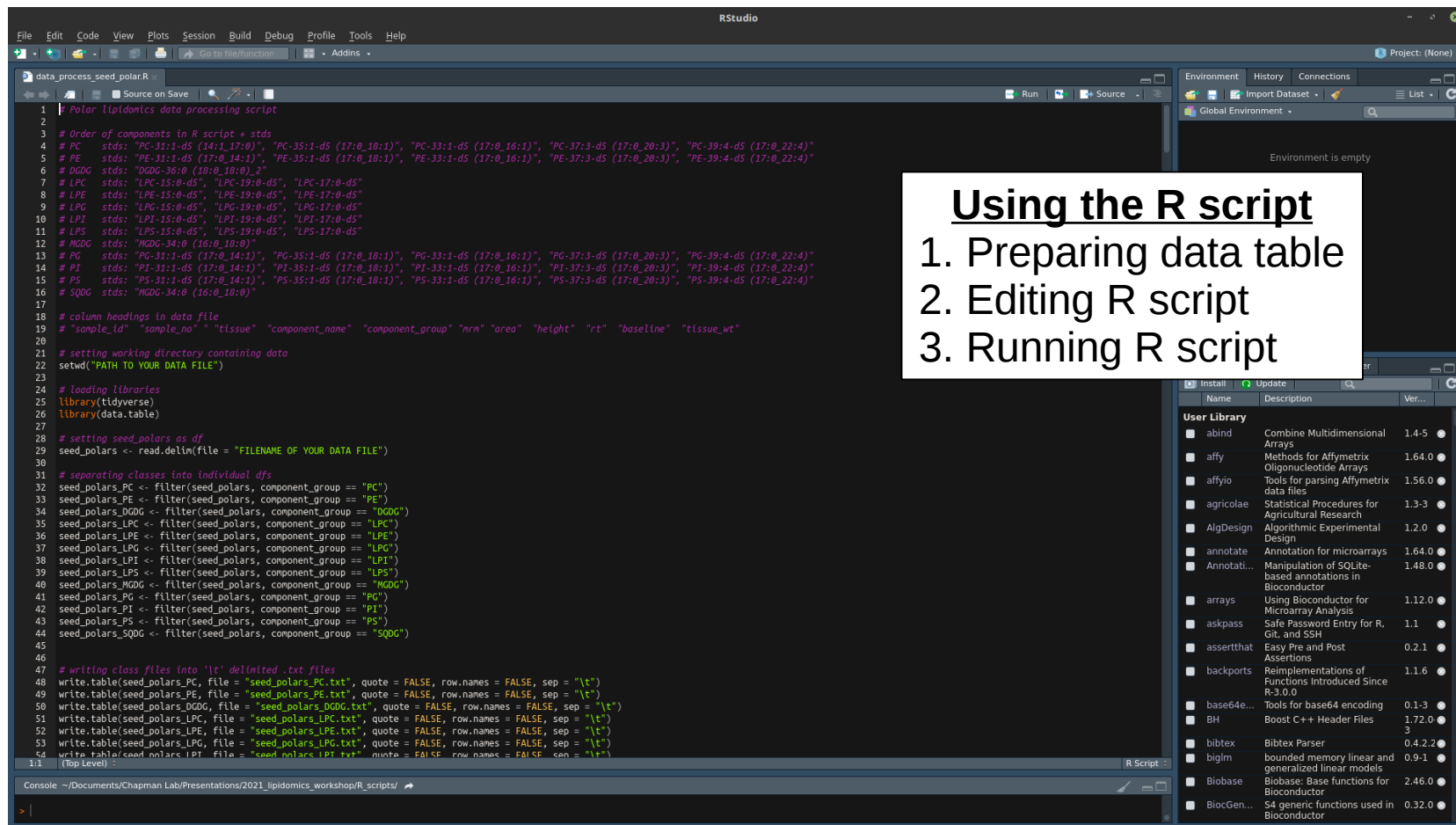


# 2<sup>nd</sup> step in TAG analysis: Creating Multiquant method to integrate HPLC peaks

(Also 1<sup>st</sup> step for all the other lipids)



# 3<sup>rd</sup> step in data analysis: Using R/RStudio to quantify integrated HPLC peaks



**Using the R script**

1. Preparing data table
2. Editing R script
3. Running R script

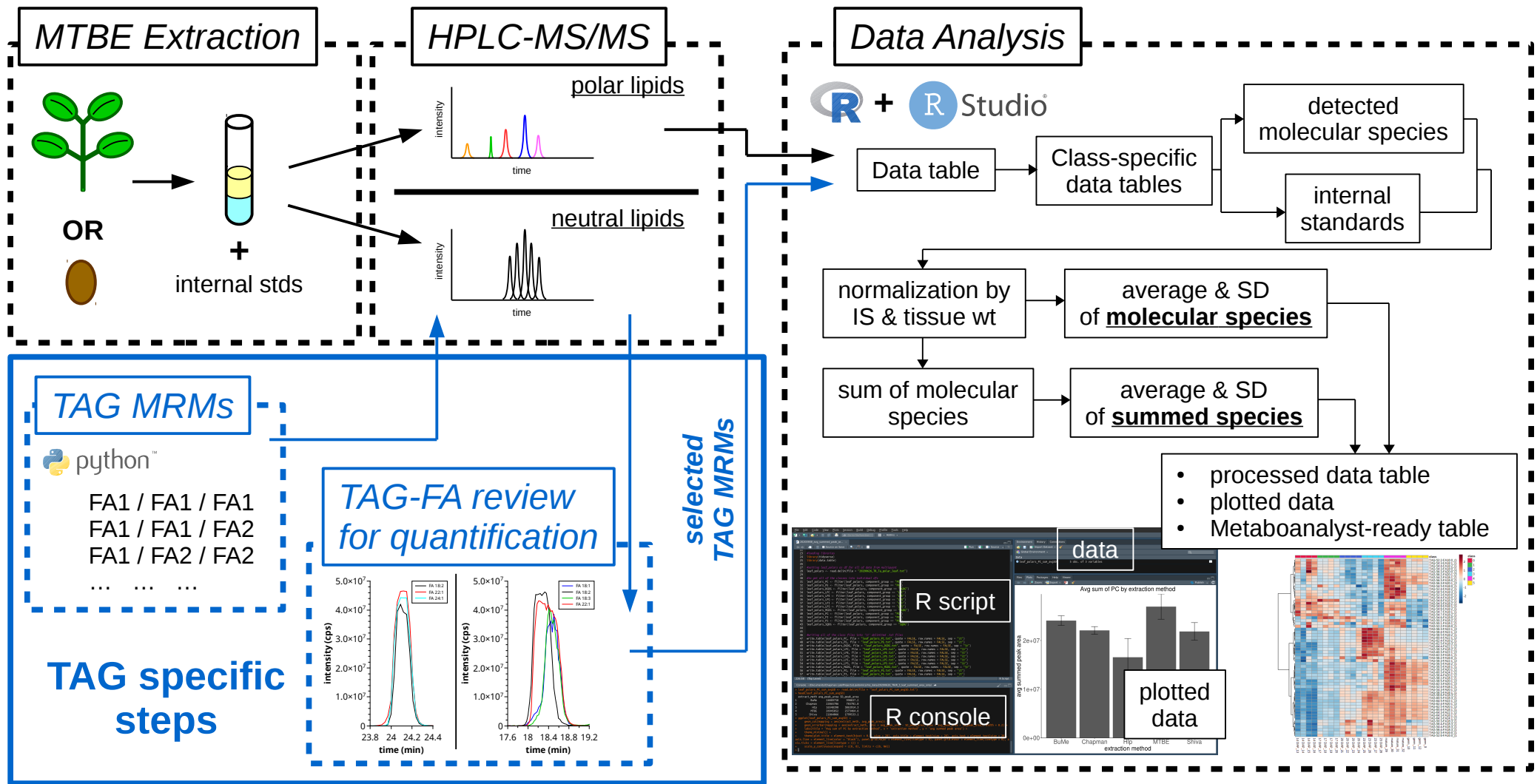
```
1 # Polar lipidomics data processing script
2
3 # Order of components in R script + stds
4 # PC stds: "PC-31:1-ds (14:1 17:0)", "PC-35:1-ds (17:0 18:1)", "PC-33:1-ds (17:0 16:1)", "PC-37:3-ds (17:0 20:3)", "PC-39:4-ds (17:0 22:4)"
5 # PE stds: "PE-31:1-ds (17:0 14:1)", "PE-35:1-ds (17:0 18:1)", "PE-33:1-ds (17:0 16:1)", "PE-37:3-ds (17:0 20:3)", "PE-39:4-ds (17:0 22:4)"
6 # DGDG stds: "DGDG-36:0 (18:0 18:0)", "DGDG-36:0 (18:0 18:0)", "DGDG-36:0 (18:0 18:0)"
7 # LPC stds: "LPC-15:0-ds", "LPC-19:0-ds", "LPC-17:0-ds"
8 # LPE stds: "LPE-15:0-ds", "LPE-19:0-ds", "LPE-17:0-ds"
9 # LPG stds: "LPG-15:0-ds", "LPG-19:0-ds", "LPG-17:0-ds"
10 # LPI stds: "LPI-15:0-ds", "LPI-19:0-ds", "LPI-17:0-ds"
11 # LPS stds: "LPS-15:0-ds", "LPS-19:0-ds", "LPS-17:0-ds"
12 # MDG stds: "MDG-34:0 (16:0 18:0)"
13 # PG stds: "PG-31:1-ds (17:0 14:1)", "PG-35:1-ds (17:0 18:1)", "PG-33:1-ds (17:0 16:1)", "PG-37:3-ds (17:0 20:3)", "PG-39:4-ds (17:0 22:4)"
14 # PI stds: "PI-31:1-ds (17:0 14:1)", "PI-35:1-ds (17:0 18:1)", "PI-33:1-ds (17:0 16:1)", "PI-37:3-ds (17:0 20:3)", "PI-39:4-ds (17:0 22:4)"
15 # PS stds: "PS-31:1-ds (17:0 14:1)", "PS-35:1-ds (17:0 18:1)", "PS-33:1-ds (17:0 16:1)", "PS-37:3-ds (17:0 20:3)", "PS-39:4-ds (17:0 22:4)"
16 # SQDG stds: "SQDG-34:0 (16:0 18:0)"
17
18 # column headings in data file
19 # 'sample_id' 'sample_no' 'tissue' 'component_name' 'component_group' 'nmr' 'area' 'height' 'rt' 'baseline' 'tissue_wt'
20
21 # setting working directory containing data
22 setwd("PATH TO YOUR DATA FILE")
23
24 # loading libraries
25 library(tidyverse)
26 library(data.table)
27
28 # setting seed_polars as df
29 seed_polars <- read.delim(file = "FILENAME OF YOUR DATA FILE")
30
31 # separating classes into individual dfs
32 seed_polars_PC <- filter(seed_polars, component_group == "PC")
33 seed_polars_PE <- filter(seed_polars, component_group == "PE")
34 seed_polars_DGDG <- filter(seed_polars, component_group == "DGDG")
35 seed_polars_LPC <- filter(seed_polars, component_group == "LPC")
36 seed_polars_LPE <- filter(seed_polars, component_group == "LPE")
37 seed_polars_LPG <- filter(seed_polars, component_group == "LPG")
38 seed_polars_LPI <- filter(seed_polars, component_group == "LPI")
39 seed_polars_LPS <- filter(seed_polars, component_group == "LPS")
40 seed_polars_MDG <- filter(seed_polars, component_group == "MDG")
41 seed_polars_PG <- filter(seed_polars, component_group == "PG")
42 seed_polars_PI <- filter(seed_polars, component_group == "PI")
43 seed_polars_PS <- filter(seed_polars, component_group == "PS")
44 seed_polars_SQDG <- filter(seed_polars, component_group == "SQDG")
45
46
47 # writing class files into 'lt' delimited .txt files
48 write.table(seed_polars_PC, file = "seed_polars_PC.txt", quote = FALSE, row.names = FALSE, sep = "\t")
49 write.table(seed_polars_PE, file = "seed_polars_PE.txt", quote = FALSE, row.names = FALSE, sep = "\t")
50 write.table(seed_polars_DGDG, file = "seed_polars_DGDG.txt", quote = FALSE, row.names = FALSE, sep = "\t")
51 write.table(seed_polars_LPC, file = "seed_polars_LPC.txt", quote = FALSE, row.names = FALSE, sep = "\t")
52 write.table(seed_polars_LPE, file = "seed_polars_LPE.txt", quote = FALSE, row.names = FALSE, sep = "\t")
53 write.table(seed_polars_LPG, file = "seed_polars_LPG.txt", quote = FALSE, row.names = FALSE, sep = "\t")
54 write.table(seed_polars_LPI, file = "seed_polars_LPI.txt", quote = FALSE, row.names = FALSE, sep = "\t")
55 write.table(seed_polars_LPS, file = "seed_polars_LPS.txt", quote = FALSE, row.names = FALSE, sep = "\t")
56 write.table(seed_polars_MDG, file = "seed_polars_MDG.txt", quote = FALSE, row.names = FALSE, sep = "\t")
57 write.table(seed_polars_PG, file = "seed_polars_PG.txt", quote = FALSE, row.names = FALSE, sep = "\t")
58 write.table(seed_polars_PI, file = "seed_polars_PI.txt", quote = FALSE, row.names = FALSE, sep = "\t")
59 write.table(seed_polars_PS, file = "seed_polars_PS.txt", quote = FALSE, row.names = FALSE, sep = "\t")
60 write.table(seed_polars_SQDG, file = "seed_polars_SQDG.txt", quote = FALSE, row.names = FALSE, sep = "\t")
```

# **3<sup>rd</sup> step in data analysis: Using R/RStudio to quantify integrated HPLC peaks**

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(in-person R/RStudio demonstration here)

# Lipidomics Workflow — from extraction to analysis



# Other Resources

## R resources

R: <https://www.r-project.org/>

RStudio: <https://www.rstudio.com/products/rstudio/>

R for Data Science (online ebook): <https://r4ds.had.co.nz/index.html>

Tidyverse library: <https://www.tidyverse.org/>

Data.Table library: <https://cran.r-project.org/web/packages/data.table/vignettes/datatable-intro.html>

W3: <https://www.w3schools.com/r/default.asp>

Anaconda: <https://www.anaconda.com/>

Visual Studio Code: <https://code.visualstudio.com/>

Python Data Science Handbook (online ebook):  
<https://jakevdp.github.io/PythonDataScienceHandbook/>

Numpy (included with Anaconda): <https://numpy.org/>

Pandas (included with Anaconda): <https://pandas.pydata.org/>

Matplotlib (included with Anaconda): <https://matplotlib.org/>

W3: <https://www.w3schools.com/python/default.asp>

## Python resources

## Lipidomics tools

mMass (viewing spectra/chromatogram from .txt file): <http://www.mmass.org/>

MarvinSketch (chemical drawing):  
<https://chemaxon.com/products/marvin>

Metaboanalyst (statistical analysis):  
<https://www.metaboanalyst.ca/>

AOCS Lipid Library: <https://lipidlibrary.aocs.org/>

ARALIP: The Arabidopsis Acyl-Lipid Metabolism  
Website:  
<http://aralip.plantbiology.msu.edu/about>

Acyl-Lipid Metabolism:  
<https://doi.org/10.1199/tab.0133>

LIPID MAPS: <https://www.lipidmaps.org/>

## Lipid metabolism