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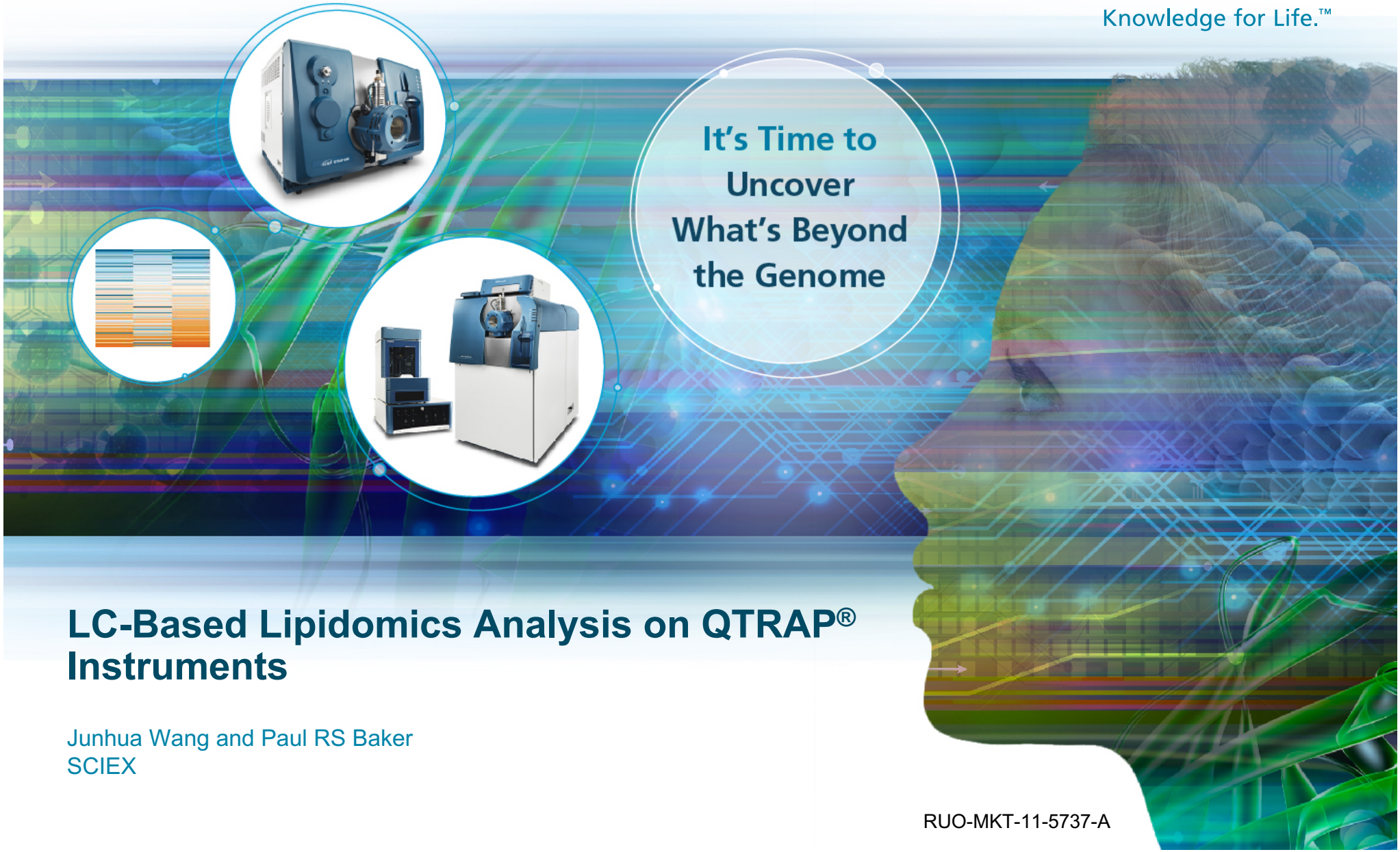

LC-Based Lipidomics Analysis on QTRAP® Instruments

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SCIEX

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LC-Based Lipidomics Analysis

Topics Covered

- Lipid extraction techniques
- Hydrophilic Interaction Chromatography (HILIC) LC method for lipid class separation
- Targeted MRM list for lipids
- Data processing by MultiQuant™ Software
  - Relative quantitation
  - Accurate quantitation
- Links to helpful tools/references

This presentation is intended to provide LC and MS conditions that will enable targeted yet broad coverage of multiple lipid classes present in biological samples. It has not been validated and is intended to be a starting point for further method development . The technique can be adapted to include additional lipid classes as long as care is taken to ensure sufficient points across a peak are generated for quantitative purposes

Lipid Extraction Techniques

For a consistent, broad-based lipid extraction, use either of these two methods

Bligh and Dyer:

1 Part aqueous (sample), 2 parts methanol, 0.9 part dichloromethane; Vortex (except plasma and brain—gently invert sealed test tube to avoid emulsion); Add 1 part water, 1 part dichloromethane; Vortex; Centrifuge (1200 rpm x 10 min); Take lower layer and evaporate solvent; Re-suspend in appropriate solvent for injection.

Folch:

1 Part aqueous (sample), 19 parts 50:50 methanol/ dichloromethane; Vortex; Add 4 parts water (or 0.9% sodium chloride); Vortex; Centrifuge (1200 rpm x 10 min); Take lower layer and evaporate solvent; Re-suspend in appropriate solvent for injection.

NOTE: Dichloromethane will extract plasticizers; always use glass

Lipid Extraction Techniques

Example protocol for plasma extraction

1. Use 13 x 100 mm new glass screw capped tubes. Do not use washed tubes as you may extract detergent residue.
2. To 25 µl plasma, add 975 µl water; let sit on ice for 10 min
3. Add 2.0 mL methanol
4. Add 0.9 ml dichloromethane
5. Vortex
6. Make sure you have a mono-phase at this stage. If you see two distinct phases, add 50 µl methanol and vortex, check to see if solution is a single phase. If not repeat addition of 50 µl methanol and vortex
7. Add Internal standard, vortex and let mixture sit for 30 min
8. Add 1 ml water
9. Add 0.9 ml dichloromethane
10. Invert tubes 10 times. DO NOT VORTEX or you will form an emulsion
11. Centrifuge at 1200 rpm for 10 min
12. Collect lower layer and put into a fresh glass tube
13. Add 2 mL dichloromethane to remains in extraction tube
14. Mix, centrifuge, collect lower layer and add to first extract
15. Evaporate solvent under a stream of nitrogen
16. Re-suspend lipids in 50:50 LC solvents A and B

Adapted from the method of Bligh and Dyer (Can J Biochem Phys, Vol 31, 912-917, 1959)

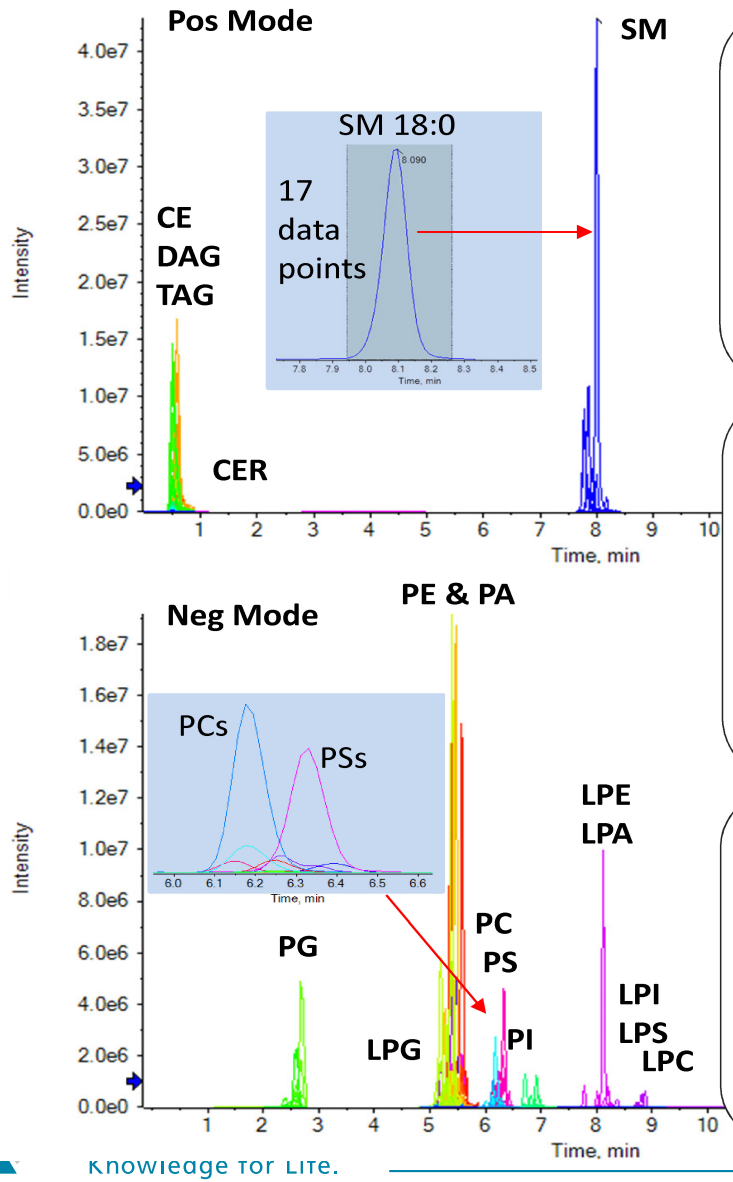
Lipid Analysis by LC

Important things to consider:

- Lipids are very sticky and will contaminate the MS. Some lipids are more tenacious than others—especially phosphorylated lipids such as sphingosine-1-phosphate. Always dilute a test sample to determine the optimal sample concentration.
- Use a relatively high curtain (CUR) gas setting—25 to 40 is ideal—to minimize lipid contamination.
- The LC rinse solvent should be a strong organic such as 100% IPA. The addition of 0.1% phosphoric acid reduces carry over of some lipid species such as phosphatidic acid (PA) and phosphatidylserine (PS).
- HILIC columns are very pH-sensitive. Carefully adjust the pH of your LC solvents to the proscribed levels to ensure reproducible chromatography. Additionally, HILIC columns generally require longer equilibration times. In this method, the column is equilibrated for 5 min at high flow.
- There is a certain amount of non-specific lipid binding to LC columns—especially with new columns. Run 4-5 test runs with a lipid extract to condition the column before using for analysis.

Lipidomics Analysis Using a HILIC LC Strategy

Over 1300 lipids analyzed with the capacity for accurate or relative quantitation in 15 minutes



Pos 665 sMRMs

	Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	ID
1	738.670	184.100	8.10	18:1(d9) SM _SPLA
2	703.600	184.100	8.10	SM(16:0) Bovine +H
3	731.600	184.100	8.10	SM(18:0)Bovine +H
4	729.600	184.100	8.10	SM(18:1) Bovine+H
5	759.600	184.100	8.10	SM(20:0)Bovine +H
6	757.600	184.100	8.10	SM(20:1)Bovine +H
7	787.700	184.100	8.10	SM(22:0)Bovine+H
8	465.500	184.050	9.0	LSM 18:1 +H
9	675.160	369.400	0.70	18:1(d7) CE _SPLA
10	684.700	369.400	0.70	CE 19:0_QC
11	376.200	376.200	0.70	(d7) Cholesterol _SP
12	369.300	369.300	0.70	Cholesterol (Std)

Extracted 1300 MRM transitions

Neg 635 sMRMs

	Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	ID
2	666.520	288.300	5.50	15:0-18:1(d7) PA_SPLA
3	647.466	255.233	5.50	PA(16:0/16:0) -H
4	777.544	329.249	5.50	PA(20:0/22:5) -H
5	587.410	288.300	8.90	18:1(d7) LPC_SPLASH
6	580.300	281.200	8.90	LPC 18:1_QC+AcO
7	578.346	279.233	8.90	LPC(18:2) +AcO
8	811.630	288.300	6.50	15:0-18:1(d7) PC_SPLA
9	816.576	279.233	6.50	PC(16:0/18:2)+AcO
10	840.576	303.233	6.50	PC(16:0/20:4)+AcO
11	480.310	283.264	8.20	LPE(18:0)-H
12	485.340	288.300	8.20	18:1(d7) LPE SPLASH-

LC-MS Parameters for HILIC-Based Lipid Class Separation

LC Parameters

Mobile Phases	**(A)**: water/acetonitrile (5:95, v/v) with 10 mM ammonium acetate; pH = 8.0 (pH adjustment critical) **(B)**: water/acetonitrile (50:50, v/v) with 10 mM ammonium acetate; pH = 8.0 (pH adjustment usually not needed)
Flow Rate	0.5 mL/min
HPLC Column	Waters Acquity UPLC BEH HILIC , 1.7µm, 2.1 x 100 mm *(Part #186003461, Waters [Milford, MA 01757])*
Column Temperature	35°C *(* see note below)*
Autosampler Temp.	4°C
Injection Volume	5 µL
Needle Wash	IPA
LC Program	Gradient with column diversion (to minimize contamination)

Preparation of Mobile Phase A (1 L)

- Fill a 1L flask with 950 mL water/acetonitrile (5:95, v/v)
- Prepare 1 M stock solution of **ammonium acetate** in above solvent
- Add 10 ml ammonium acetate stock solution and mix well
- Using a pH meter, carefully adjust pH to 8.0
- Mix well, bring up to 1 L volume with water/acetonitrile (5:95, v/v)
- Store at 20°C for up to 6 months

Preparation of Mobile Phase B (1 L)

- Fill a 1L flask with 950 mL water/acetonitrile (50:50, v/v)
- Prepare 1 M stock solution of **ammonium acetate** in above solvent
- Add 10 ml ammonium acetate stock solution and mix well
- Using a pH meter, carefully adjust pH to 8.0, if needed
- Mix well, bring up to 1 L volume with water/acetonitrile (50:50, v/v)
- Store at 20°C for up to 6 months

LC Gradient

Time (min)	% Mobile Phase B
0.0	0.1
10	20
11	98
13	98
13.1	0.1
16	Stop

MS Parameters

Parameter	Value
CUR	35
GS1*	50
GS2*	60
IS	5200/-4500
TEM	500

* Zero-grade air should be used as the nebulizing gas to avoid corona discharge.

Method Development

To develop a scheduled MRM method, a short list of IS and major lipid molecular species typically found in most biological samples was used to identify retention times of all major lipid species

Period Summary

Duration: 15.997 (min) Delay Time: 0 (sec)

Cycles: 1054 Cycle: 0.9106 (sec)

	Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)	ID	CE (volts)
23	773.600	305.200	5.0	dPE(18:0d5/20:3)	-43.000
24	771.500	303.200	5.0	dPE(18:0d5/20:4)	-43.000
25	769.500	301.200	5.0	dPE(18:0d5/20:5)	-43.000
26	797.600	329.200	5.0	dPE(18:0d5/22:5)	-43.000
27	795.500	327.200	5.0	dPE(18:0d5/22:6)	-43.000
28	578.346	279.233	5.0	LPC(18:2) +AcO	-50.000
29	480.310	283.264	5.0	LPE(18:0)	-40.000
30	500.278	303.233	5.0	LPE(20:4)	-40.000
31	816.576	279.233	5.0	PC(16:0/18:2)+A	-50.000
32	840.576	303.233	5.0	PC(16:0/20:4)+A	-50.000
33	844.607	279.233	5.0	PC(18:0/18:2)+A	-50.000
34	816.576	281.249	5.0	PC(18:1/16:1)+A	-50.000
35	814.560	253.217	5.0	PC(18:2/16:1)+A	-50.000
36	864.576	303.233	5.0	PC(18:2/20:4)+A	-50.000
37	766.539	303.233	5.0	PE(18:0/20:4)	-50.000
38	712.492	279.233	5.0	PE(18:2/16:1)	-50.000
39	724.529	303.233	5.0	PE(O-16:0/20:4)	-50.000
40	698.500	279.233	5.0	PE(P-16:0/18:2)	-50.000
41	722.500	303.233	5.0	PE(P-16:0/20:4)	-50.000
42	748.500	301.217	5.0	PE(P-18:0/20:5)	-50.000
43	749.534	283.264	5.0	PG(16:0/18:0)	-50.000
44	769.503	303.233	5.0	PG(16:0/20:4)	-50.000
45	747.518	253.217	5.0	PG(18:0/16:1)	-50.000
46	773.534	279.233	5.0	PG(18:0/18:2)	-50.000
47	797.534	303.233	5.0	PG(18:0/20:4)	-50.000
48	769.503	279.233	5.0	PG(18:2/18:2)	-50.000
49	807.503	281.249	5.0	PI(14:0/18:1)	-50.000
50	805.487	279.233	5.0	PI(14:0/18:2)	-50.000
51	829.487	303.233	5.0	PI(14:0/20:4)	-50.000
52	814.560	277.217	5.0	PI(16:0/18:3)	-50.000
53	865.581	283.264	5.0	PI(18:0/18:0)	-50.000
54	861.550	279.233	5.0	PI(18:0/18:2)	-50.000
55	831.503	279.233	5.0	PI(18:2/16:1)	-50.000
56	788.545	281.249	5.0	PS(18:0/18:1)	-50.000
57	786.529	279.233	5.0	PS(18:0/18:2)	-50.000
58	838.560	303.233	5.0	PS(20:0/20:4)	-50.000
59					

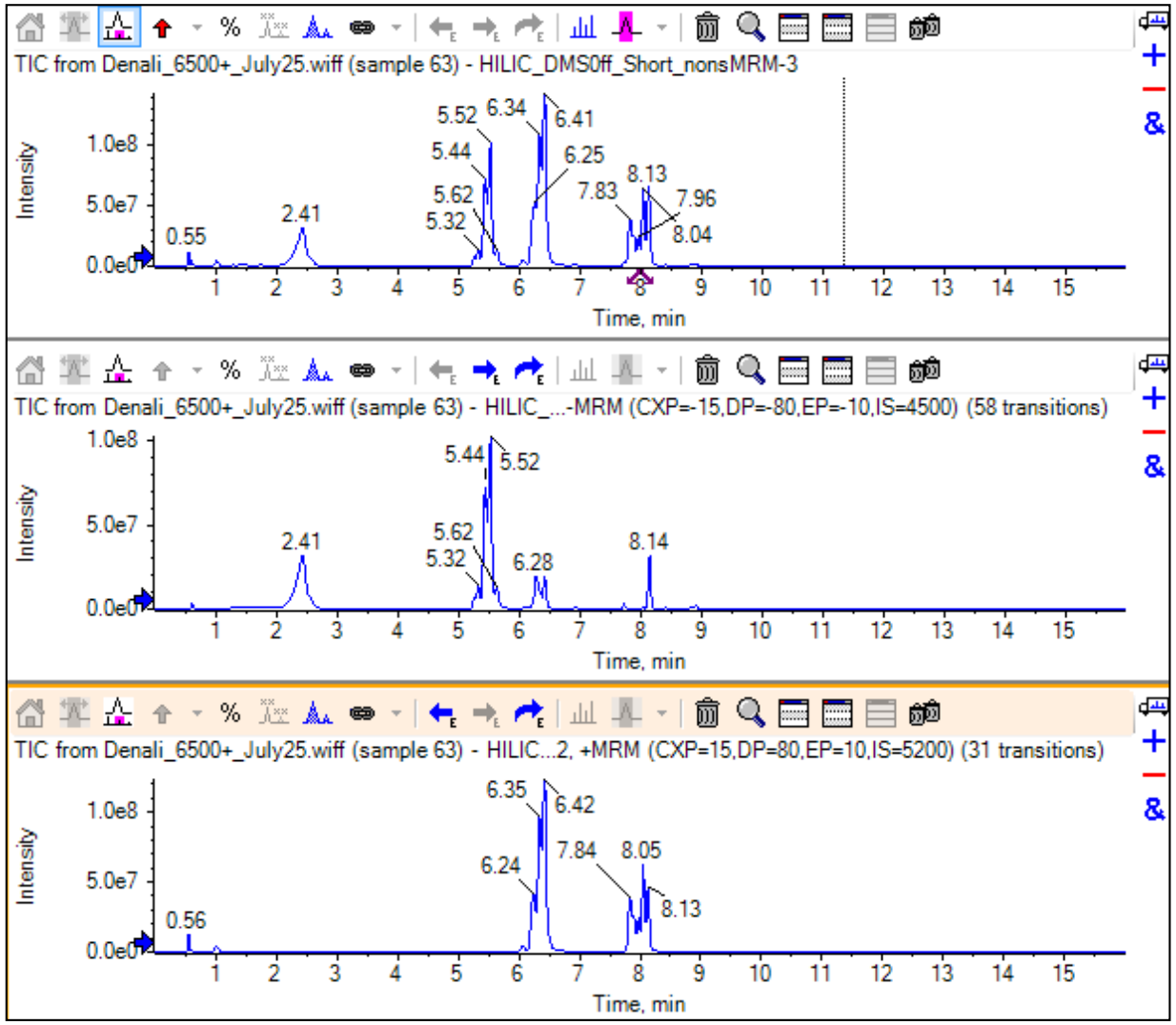
Period Summary

Duration: 15.997 (min) Delay Time: 0 (sec)

Cycles: 1054 Cycle: 0.9106 (sec)

	Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)	ID	CE (volts)
1	703.600	184.100	5.0	SM(16:0)	43.000
2	731.600	184.100	5.0	SM(18:0)	43.000
3	729.600	184.100	5.0	SM(18:1)	43.000
4	759.600	184.100	5.0	SM(20:0)	43.000
5	757.600	184.100	5.0	SM(20:1)	43.000
6	787.700	184.100	5.0	SM(22:0)	43.000
7	785.700	184.100	5.0	SM(22:1)	43.000
8	815.700	184.100	5.0	SM(24:0)	43.000
9	813.700	184.100	5.0	SM(24:1)	43.000
10	843.700	184.100	5.0	SM(26:0)	43.000
11	841.700	184.100	5.0	SM(26:1)	43.000
12	710.600	184.200	5.0	dSM(16:0)	43.000
13	736.600	184.200	5.0	dSM(18:1)	43.000
14	822.700	184.200	5.0	dSM(24:0)	43.000
15	820.700	184.200	5.0	dSM(24:1)	43.000
16	649.600	376.500	5.0	dCE(16:0)	22.000
17	647.600	376.500	5.0	dCE(16:1)	22.000
18	675.600	376.500	5.0	dCE(18:1)	22.000
19	673.600	376.500	5.0	dCE(18:2)	22.000
20	699.600	376.500	5.0	dCE(20:3)	22.000
21	697.600	376.500	5.0	dCE(20:4)	22.000
22	695.600	376.500	5.0	dCE(20:5)	22.000
23	721.600	376.500	5.0	dCE(22:6)	22.000
24	482.600	264.400	5.0	dCER(12:0)	43.000
25	547.600	264.400	5.0	dCER(d16:0)	43.000
26	484.700	266.400	5.0	dDCER(d12:0)	43.000
27	549.600	266.400	5.0	dDCER(d16:0)	43.000
28	806.800	264.400	5.0	dLCER(12:0)	43.000
29	871.900	264.400	5.0	dLCER(16:0)	43.000
30	644.600	264.400	5.0	dHCER(12:0)	43.000
31	709.700	264.400	5.0	dHCER(16:0)	43.000
32					
33					
34					
35					
36					
37					
38					

HILIC method with Short MRM List



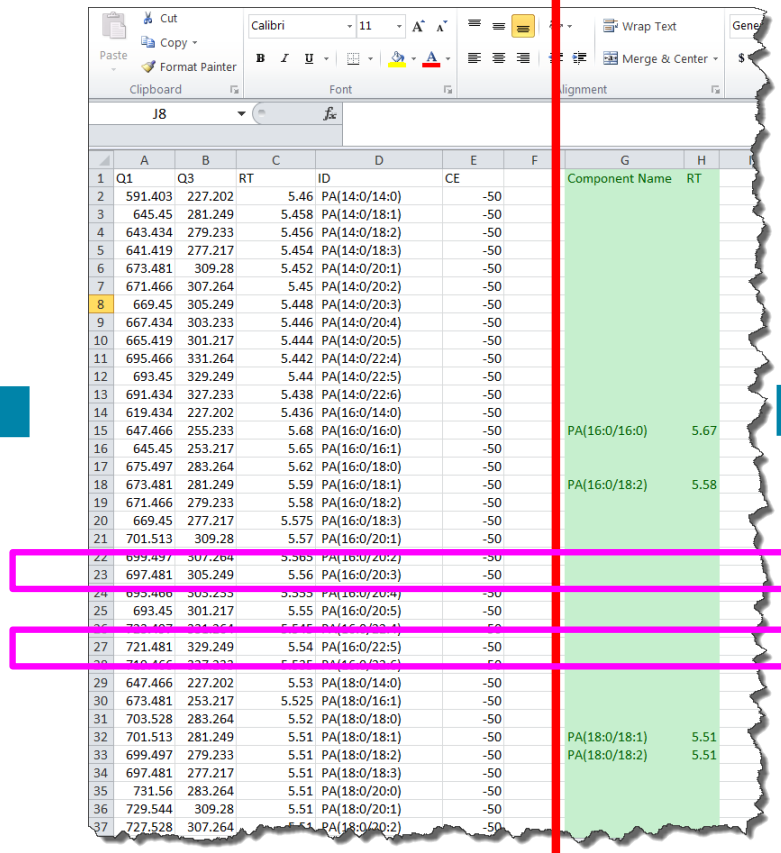
Retention Time Alignment Using Selected Lipids and IS

A similar strategy can be used to add compounds to target list

sMRM list of all

648 neg

595 pos



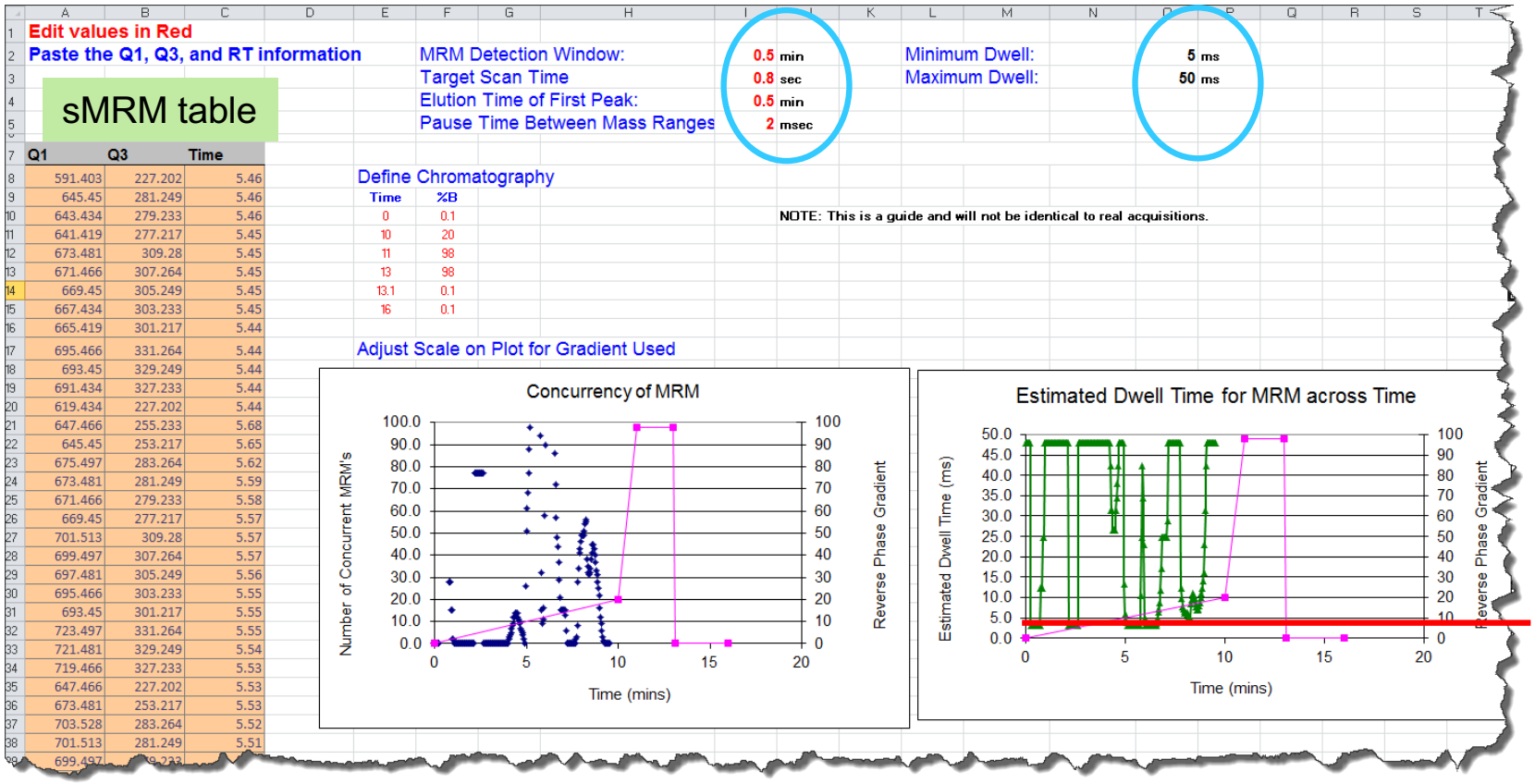
	A	B	C	D	E	F	G	H
1	Q1	Q3	RT	ID	CE		Component Name	RT
2	591.403	227.202	5.46	PA(14:0/14:0)	-50			
3	645.45	281.249	5.458	PA(14:0/18:1)	-50			
4	643.434	279.233	5.456	PA(14:0/18:2)	-50			
5	641.419	277.217	5.454	PA(14:0/18:3)	-50			
6	673.481	309.28	5.452	PA(14:0/20:1)	-50			
7	671.466	307.264	5.45	PA(14:0/20:2)	-50			
8	669.45	305.249	5.448	PA(14:0/20:3)	-50			
9	667.434	303.233	5.446	PA(14:0/20:4)	-50			
10	665.419	301.217	5.444	PA(14:0/20:5)	-50			
11	695.466	331.264	5.442	PA(14:0/22:4)	-50			
12	693.45	329.249	5.44	PA(14:0/22:5)	-50			
13	691.434	327.233	5.438	PA(14:0/22:6)	-50			
14	619.434	227.202	5.436	PA(16:0/14:0)	-50			
15	647.466	255.233	5.68	PA(16:0/16:0)	-50		PA(16:0/16:0)	5.67
16	645.45	253.217	5.65	PA(16:0/16:1)	-50			
17	675.497	283.264	5.62	PA(16:0/18:0)	-50			
18	673.481	281.249	5.59	PA(16:0/18:1)	-50		PA(16:0/18:2)	5.58
19	671.466	279.233	5.58	PA(16:0/18:2)	-50			
20	669.45	277.217	5.575	PA(16:0/18:3)	-50			
21	701.513	309.28	5.57	PA(16:0/20:1)	-50			
22	699.497	307.264	5.565	PA(16:0/20:2)	-50			
23	697.481	305.249	5.56	PA(16:0/20:3)	-50			
24	695.466	303.233	5.555	PA(16:0/20:4)	-50			
25	693.45	301.217	5.55	PA(16:0/20:5)	-50			
26	723.497	331.264	5.545	PA(16:0/22:4)	-50			
27	721.481	329.249	5.54	PA(16:0/22:5)	-50			
28	719.466	327.233	5.535	PA(16:0/22:6)	-50			
29	647.466	227.202	5.53	PA(18:0/14:0)	-50			
30	673.481	253.217	5.525	PA(18:0/16:1)	-50			
31	703.528	283.264	5.52	PA(18:0/18:0)	-50			
32	701.513	281.249	5.51	PA(18:0/18:1)	-50		PA(18:0/18:1)	5.51
33	699.497	279.233	5.51	PA(18:0/18:2)	-50		PA(18:0/18:2)	5.51
34	697.481	277.217	5.51	PA(18:0/18:3)	-50			
35	731.56	283.264	5.51	PA(18:0/20:0)	-50			
36	729.544	309.28	5.51	PA(18:0/20:1)	-50			
37	727.528	307.264	5.51	PA(18:0/20:2)	-50			

Short MRM list of selected 89 lipids, including:

1. Most abundant endogenous lipids
2. labeled lipids from Lipidyzer IS kit

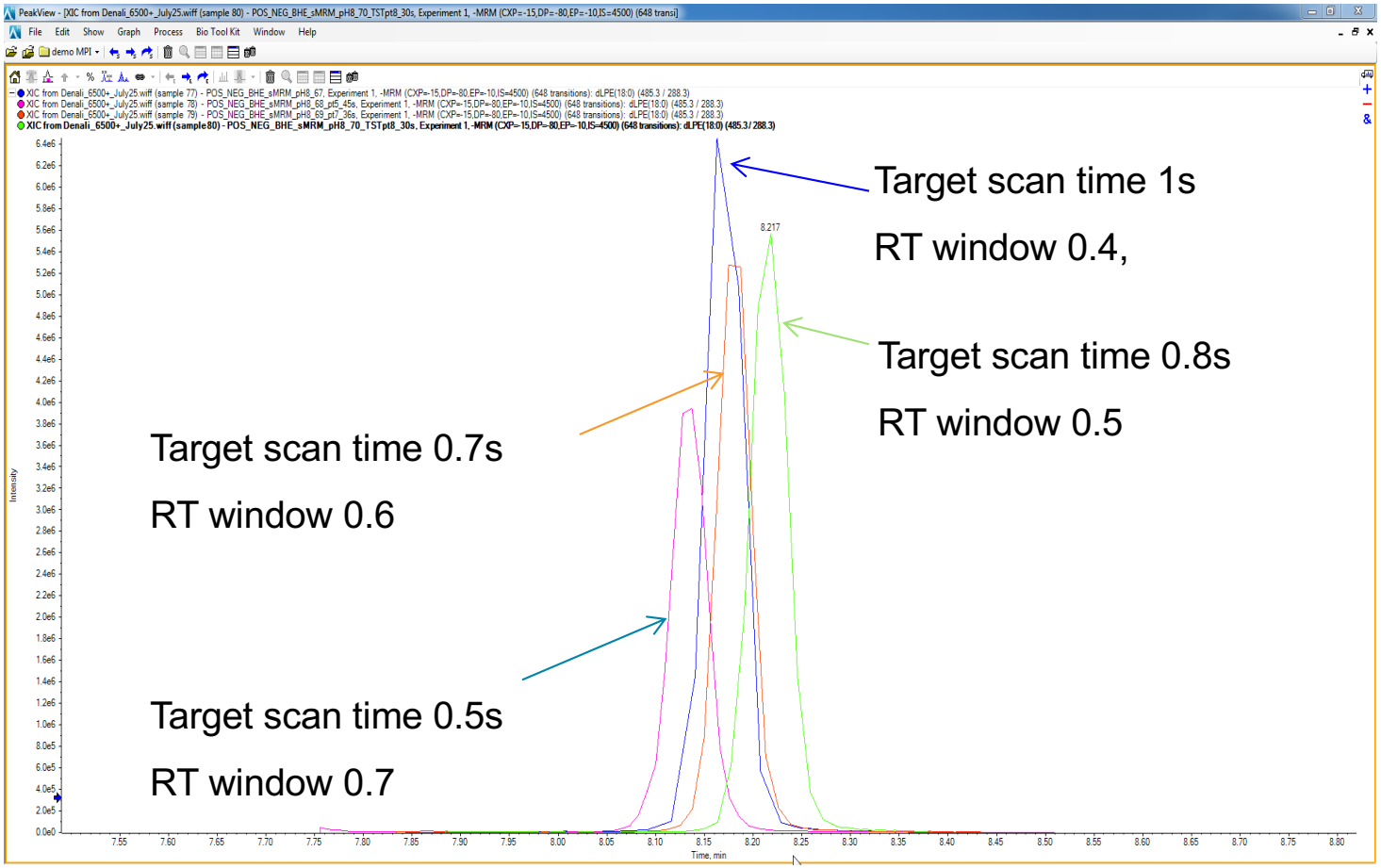
Scheduled MRM transitions Optimization

An excel macro is available to help optimize instrument parameter settings to obtain the best signal for each analyte



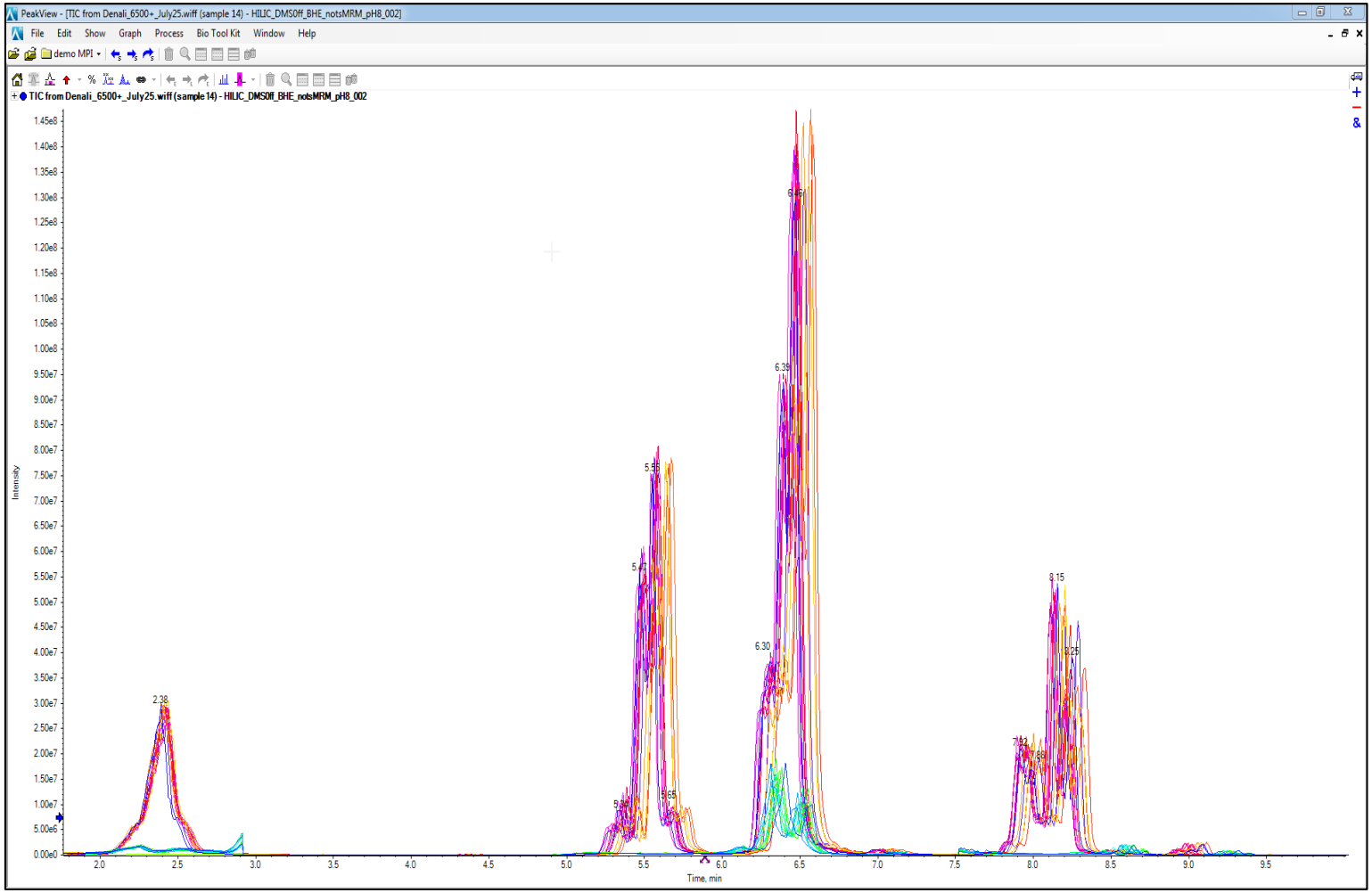
Scheduled MRM Optimization

Monitoring a single MRM transition, sMRM settings can be optimized based on suggestions from sMRM macro



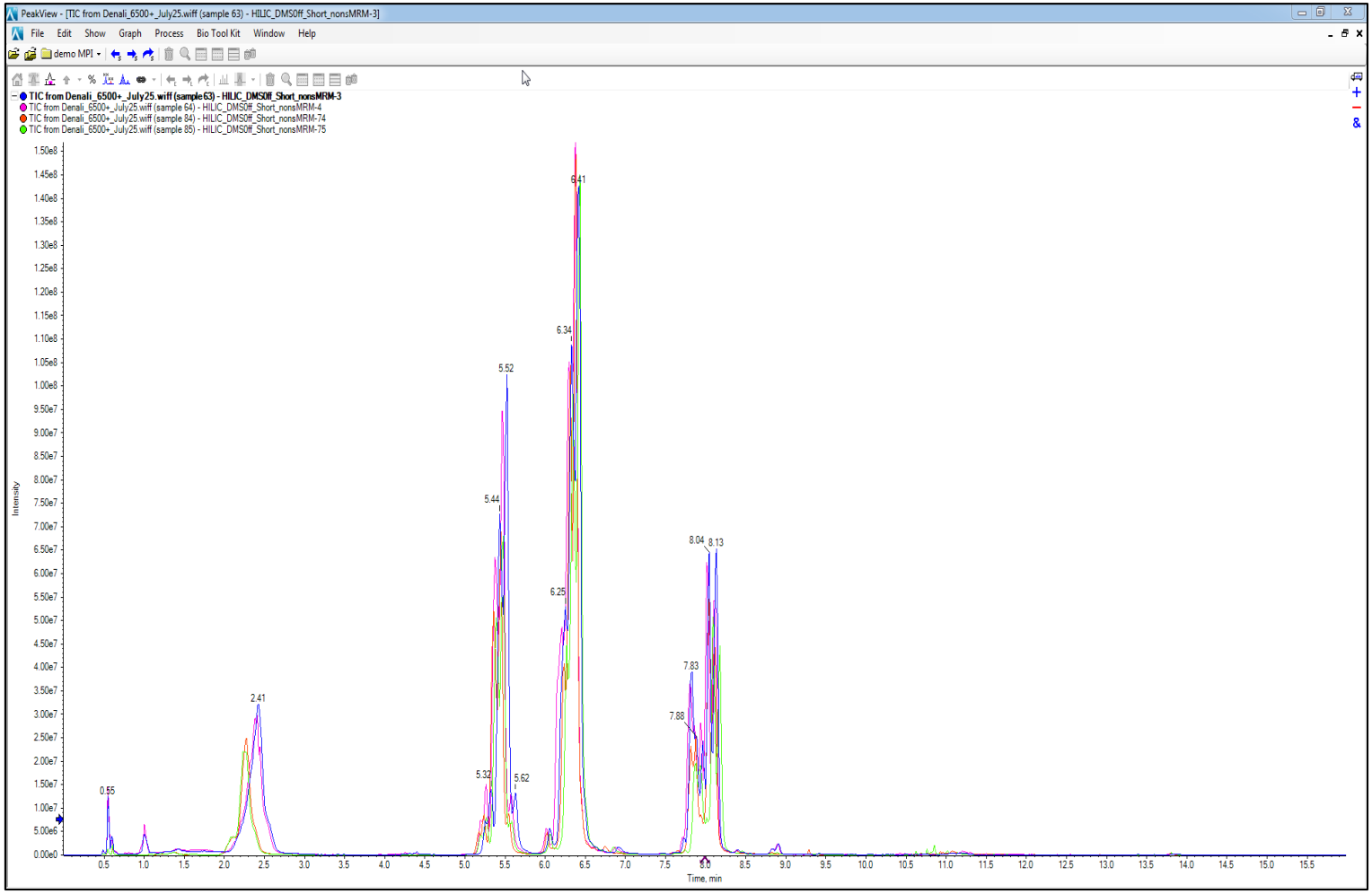
HILIC method: Reproducibility

Multiple injections over the course of 1 day



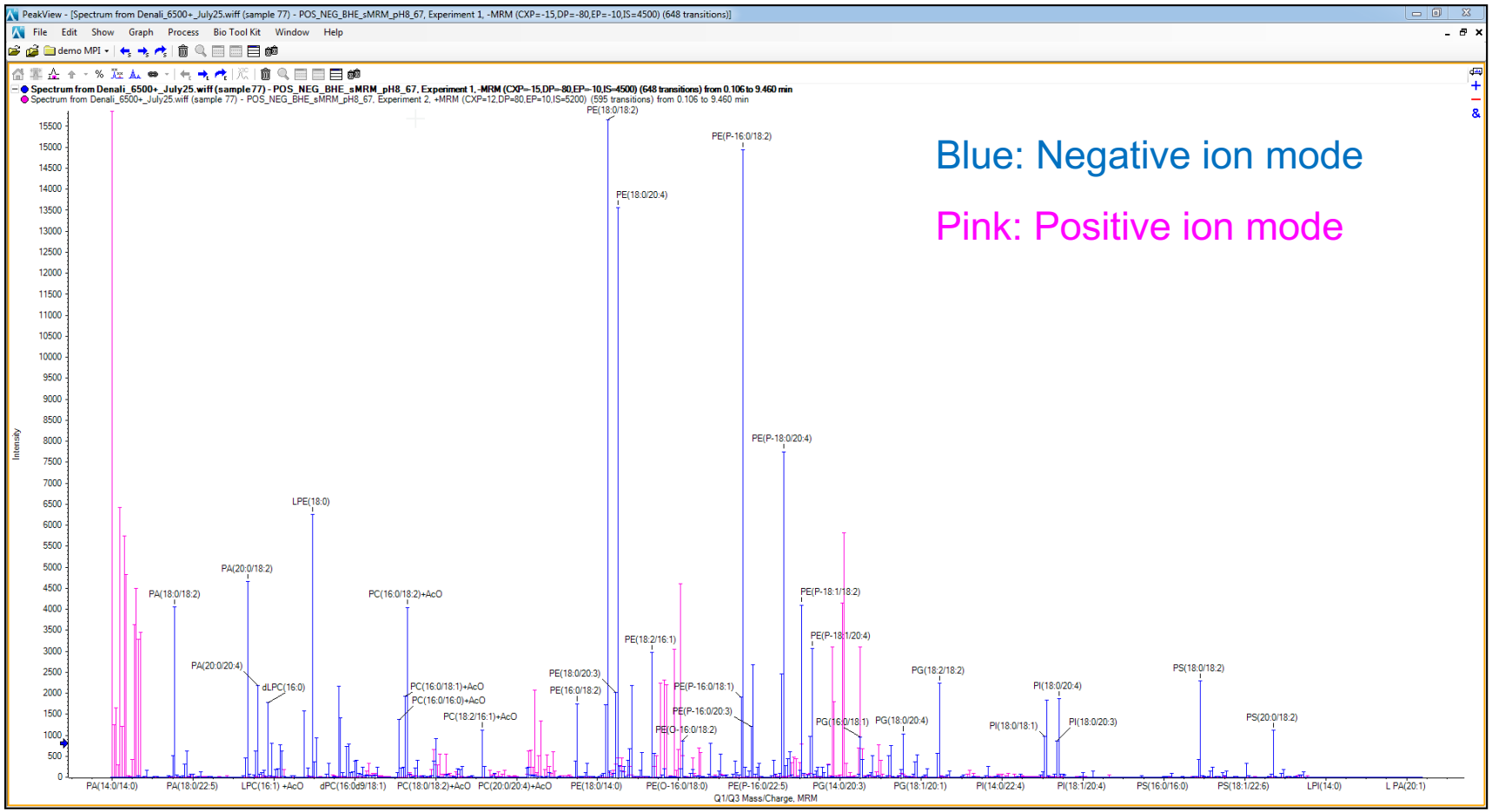
HILIC method: Reproducibility

Multiple injections over the course of 4 days



Extracted MRM transitions from TIC

~1300 lipid molecular species monitored

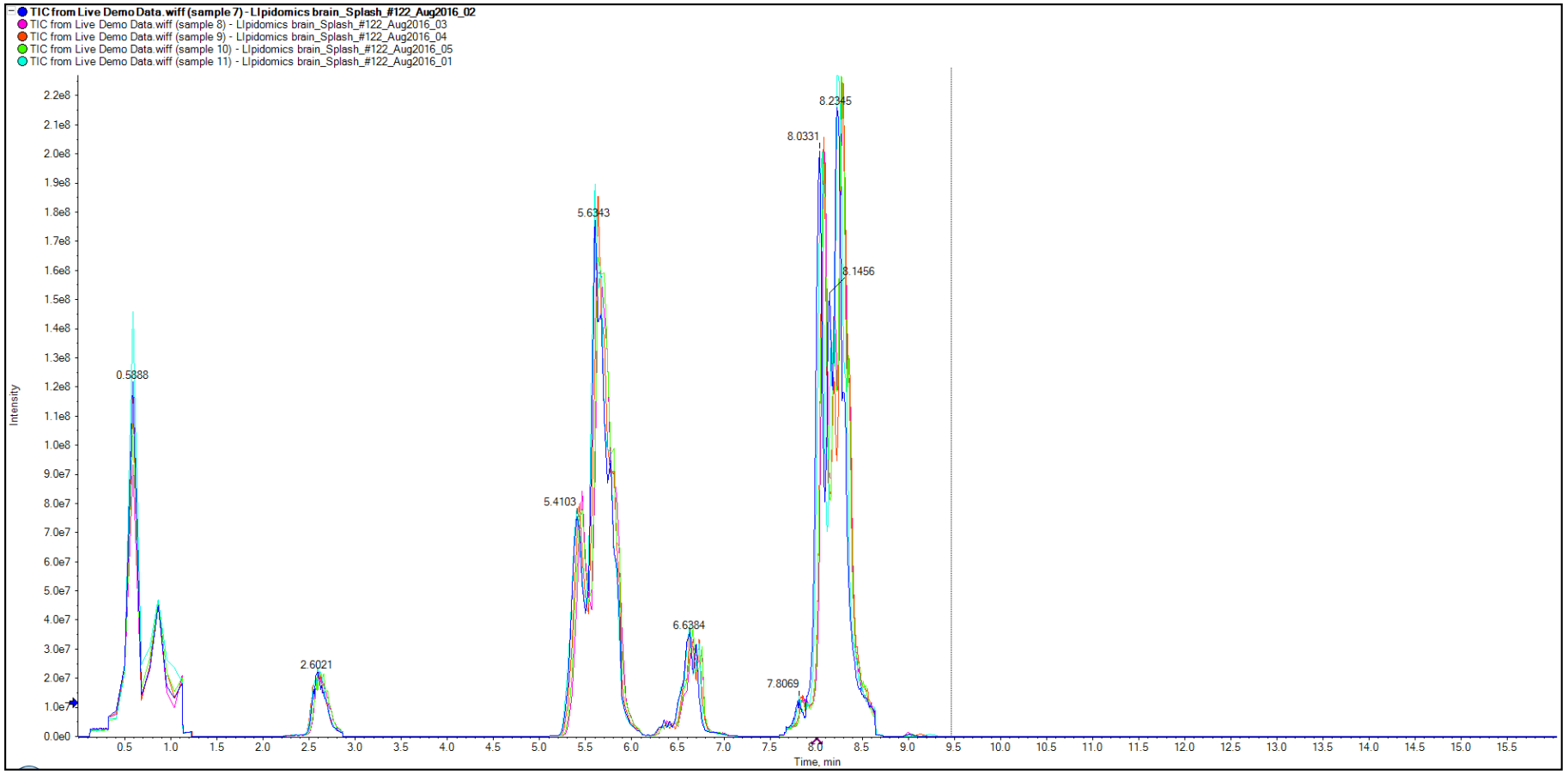


Blue: Negative ion mode

Pink: Positive ion mode

Biological Example: Brain Lipidome

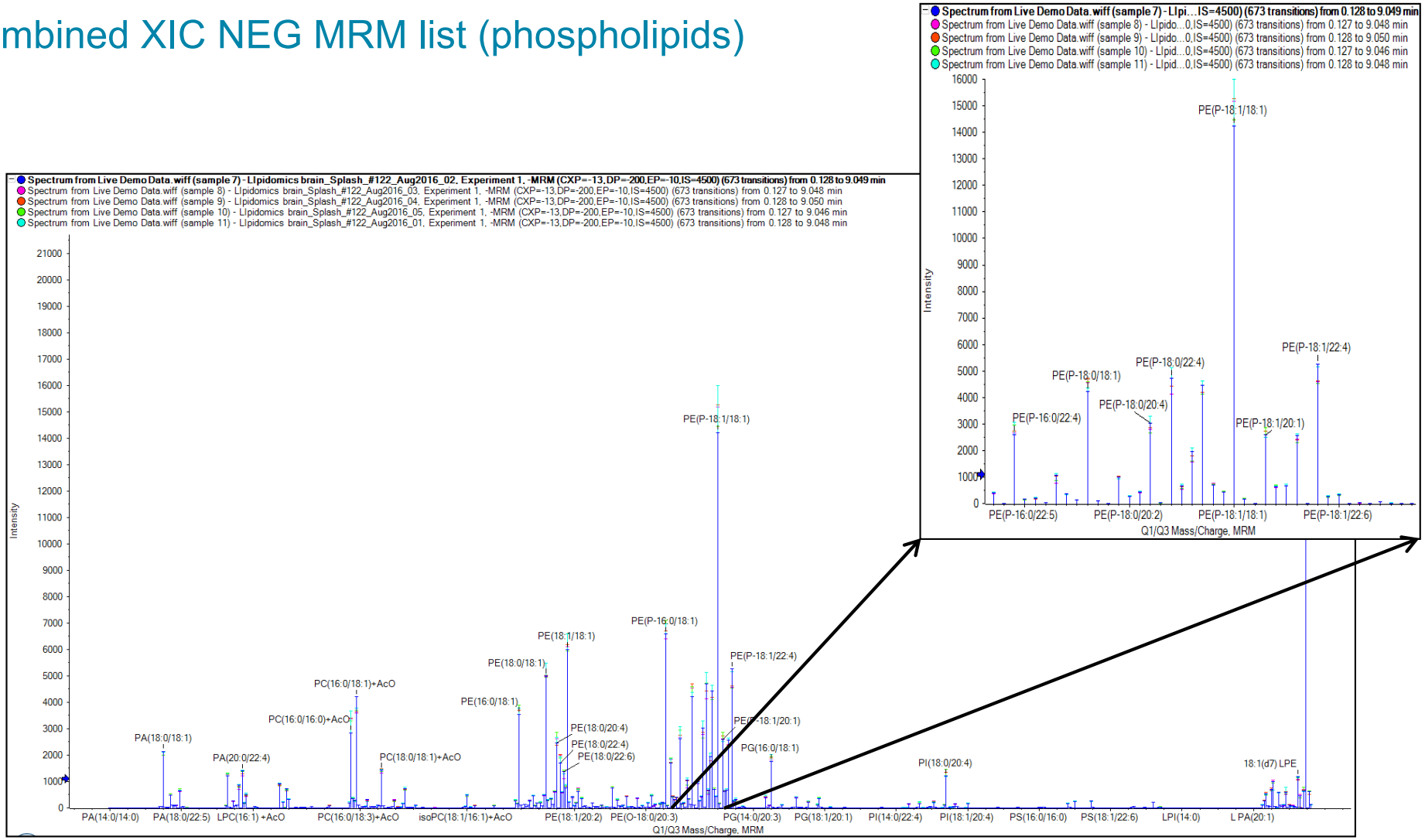
Brain lipid extract (5 technical replicates)



100 mg brain tissue extracted via Bligh and Dyer (SPLASH and Lipidyzer IS included)

Biological Example: Brain Lipidome

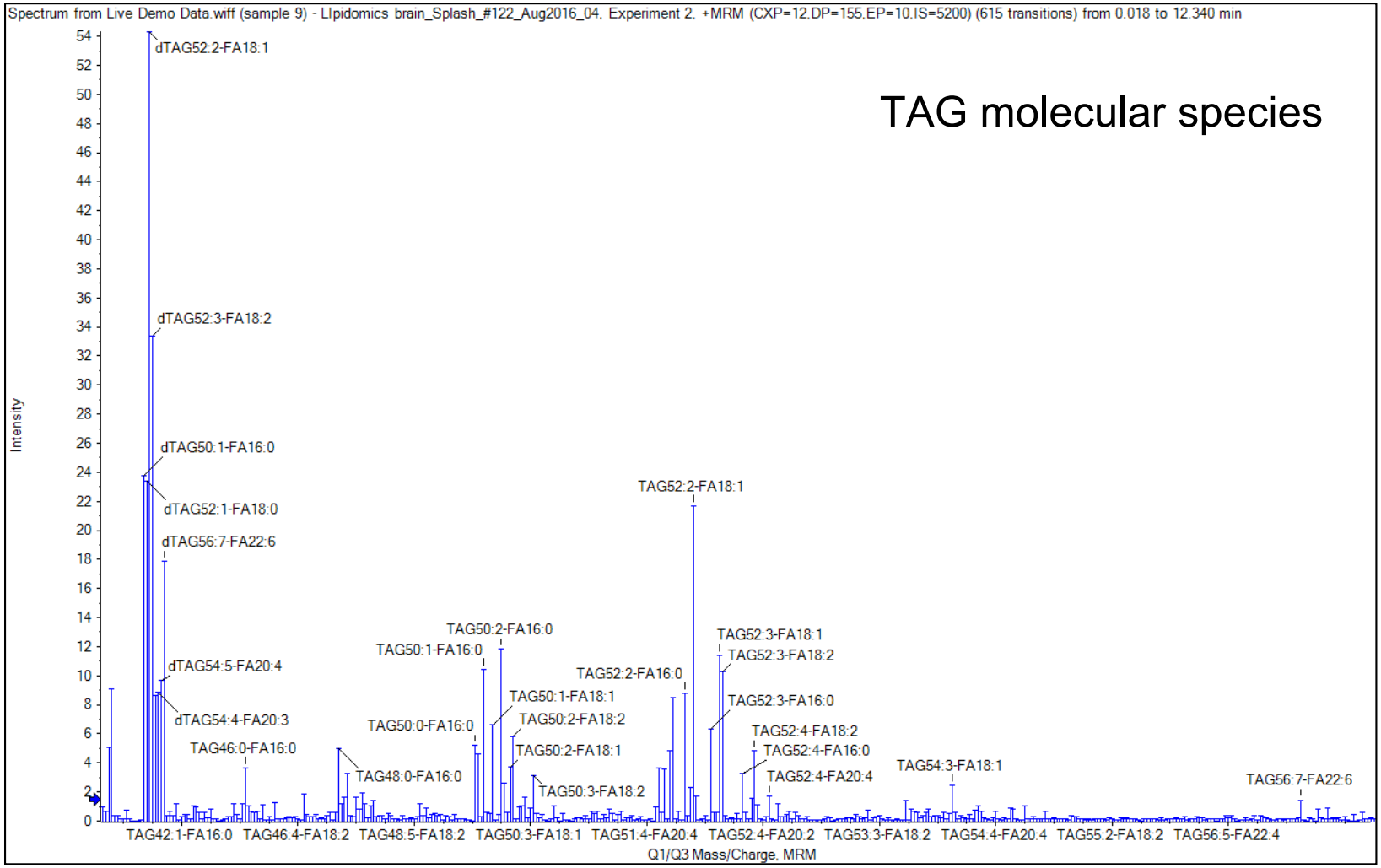
Combined XIC NEG MRM list (phospholipids)



100 mg brain tissue extracted via Bligh and Dyer (SPLASH and Lipidyzer IS included)

Biological Example: Brain Lipidome

XIC of triglyceride molecular species in brain extract



Internal Standard Strategies: SPLASH Mix

Two potential internal standard kits can be used for quantitative purposes: SPLASH mix and the Lipidyzer™ Platform internal standards

SPLASH Mix is used for relative quantitation

Mixture Components	Target Conc. (µg/ml)
15:0-18:1(d7) PC 15:0-	160
18:1(d7) PE 15:0-18:1(d7)	5
PS	5
15:0-18:1(d7) PG	30
15:0-18:1(d7) PI	10
15:0-18:1(d7) PA	7
18:1(d7) LPC	25
18:1(d7) LPE	5
18:1(d7) Chol Ester	350
18:1(d7) MG	2
15:0-18:1(d7) DG	10
15:0-18:1(d7)-15:0 TG	55
18:1(d9) SM	30
Cholesterol (d7)	100

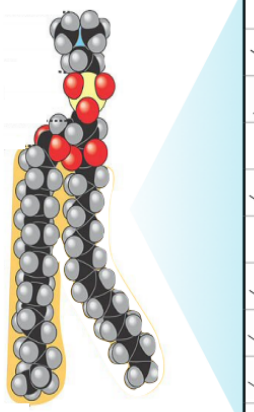
Using one standard / lipid class enables relative quantitation. The standards correct for extraction efficiency and ionization efficiency only.

Internal Standard Strategies: Lipidyzer™ Platform Standards

Two potential internal standard kits can be used for quantitative purposes: SPLASH mix and the Lipidyzer™ Platform internal standards

Lipidyzer™ Platform standards correct for extraction and ionization efficiencies, but also correct for differential fragmentation efficiencies due to fatty acid chain length and number of double bonds within the fragmenting fatty acid chain. This enables accurate quantitation; however, phospholipid classes PS, PA, PI and PG are not included.

PHOSPHATIDYLCHOLINE (PC) INTERNAL STANDARD MIX

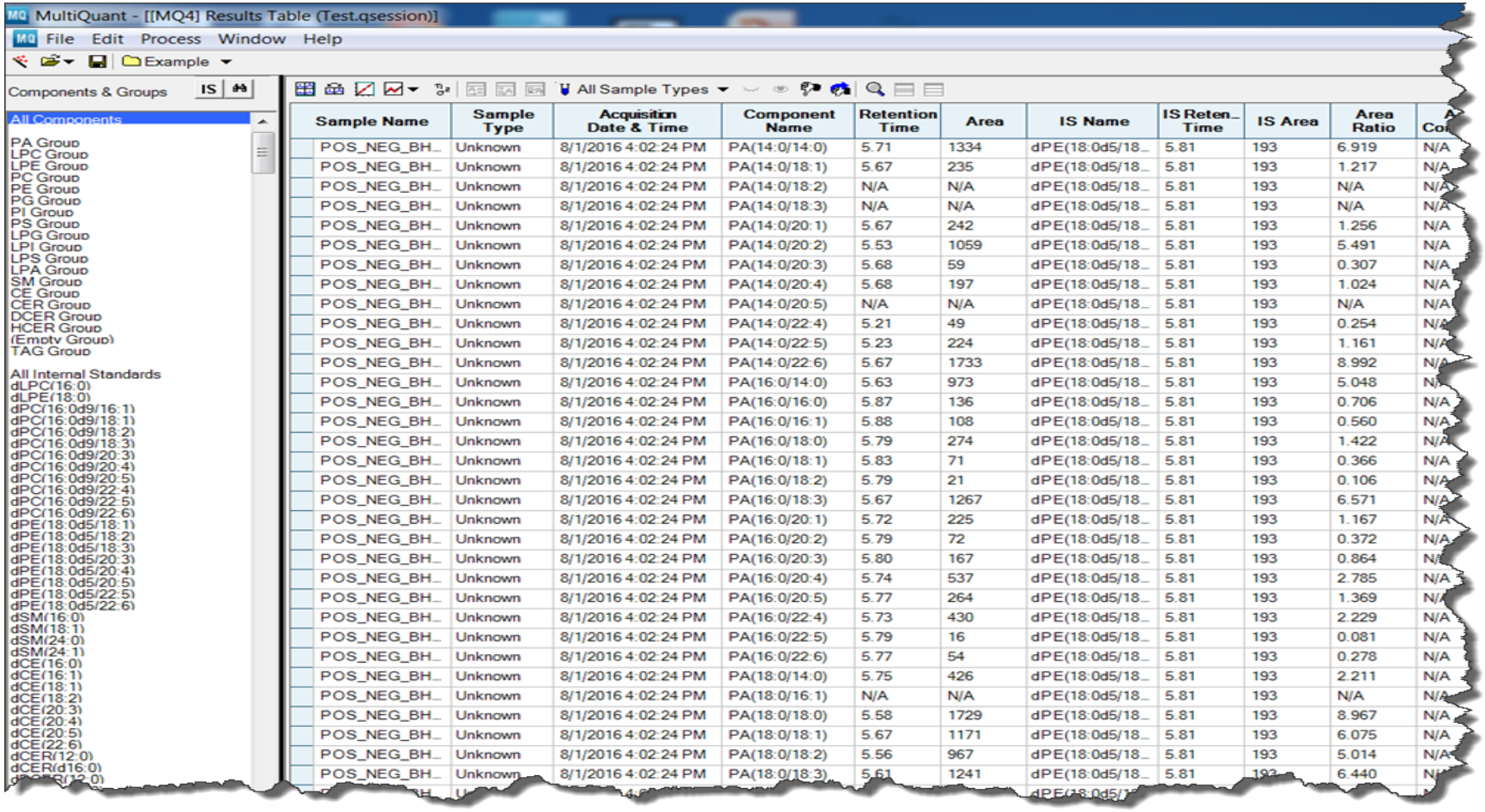


STRUCTURE	FATTY ACID	POS	%
	FA16:1 - Palmitoleic acid	sn-2	5
	FA18:1 - Oleic acid	sn-2	20
	FA18:2 - Linoleic acid	sn-2	20
	FA18:3 - α-Linoleic acid	sn-2	5
	FA20:3 - Dihomo-γ-linoleic acid	sn-2	5
	FA20:4 - Arachidonic acid	sn-2	20
	FA20:5 - Eicosapentaenoic acid	sn-2	5
	FA22:4 - Eicosatetraenoic acid	sn-2	5
	FA22:5 - Docosapentaenoic acid	sn-2	5
	FA22:6 - Docosoahexaenoic acid	sn-2	10
	d916:0 - Labeled palmitic acid	sn-1	100

Example of the multiple internal standards available for each lipid class. Each class IS mix contains multiple different fatty acids that enables normalization at the molecular species level.

Data Analysis using MultiQuant™ Software

Pre-configured MultiQuant™ Software method file can rapidly process lipid data



Sample Name	Sample Type	Acquisition Date & Time	Component Name	Retention Time	Area	IS Name	IS Reten_ Time	IS Area	Area Ratio	A Co
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/14:0)	5.71	1334	dPE(18:0d5/18_	5.81	193	6.919	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/18:1)	5.67	235	dPE(18:0d5/18_	5.81	193	1.217	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/18:2)	N/A	N/A	dPE(18:0d5/18_	5.81	193	N/A	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/18:3)	N/A	N/A	dPE(18:0d5/18_	5.81	193	N/A	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/20:1)	5.67	242	dPE(18:0d5/18_	5.81	193	1.256	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/20:2)	5.53	1059	dPE(18:0d5/18_	5.81	193	5.491	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/20:3)	5.68	59	dPE(18:0d5/18_	5.81	193	0.307	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/20:4)	5.68	197	dPE(18:0d5/18_	5.81	193	1.024	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/20:5)	N/A	N/A	dPE(18:0d5/18_	5.81	193	N/A	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/22:4)	5.21	49	dPE(18:0d5/18_	5.81	193	0.254	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/22:5)	5.23	224	dPE(18:0d5/18_	5.81	193	1.161	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(14:0/22:6)	5.67	1733	dPE(18:0d5/18_	5.81	193	8.992	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/14:0)	5.63	973	dPE(18:0d5/18_	5.81	193	5.048	N/
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/16:0)	5.87	136	dPE(18:0d5/18_	5.81	193	0.706	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/16:1)	5.88	108	dPE(18:0d5/18_	5.81	193	0.560	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/18:0)	5.79	274	dPE(18:0d5/18_	5.81	193	1.422	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/18:1)	5.83	71	dPE(18:0d5/18_	5.81	193	0.366	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/18:2)	5.79	21	dPE(18:0d5/18_	5.81	193	0.106	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/18:3)	5.67	1267	dPE(18:0d5/18_	5.81	193	6.571	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/20:1)	5.72	225	dPE(18:0d5/18_	5.81	193	1.167	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/20:2)	5.79	72	dPE(18:0d5/18_	5.81	193	0.372	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/20:3)	5.80	167	dPE(18:0d5/18_	5.81	193	0.864	N/
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/20:4)	5.74	537	dPE(18:0d5/18_	5.81	193	2.785	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/20:5)	5.77	264	dPE(18:0d5/18_	5.81	193	1.369	N/
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/22:4)	5.73	430	dPE(18:0d5/18_	5.81	193	2.229	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/22:5)	5.79	16	dPE(18:0d5/18_	5.81	193	0.081	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(16:0/22:6)	5.77	54	dPE(18:0d5/18_	5.81	193	0.278	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(18:0/14:0)	5.75	426	dPE(18:0d5/18_	5.81	193	2.211	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(18:0/16:1)	N/A	N/A	dPE(18:0d5/18_	5.81	193	N/A	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(18:0/18:0)	5.58	1729	dPE(18:0d5/18_	5.81	193	8.967	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(18:0/18:1)	5.67	1171	dPE(18:0d5/18_	5.81	193	6.075	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(18:0/18:2)	5.56	967	dPE(18:0d5/18_	5.81	193	5.014	N/A
POS_NEG_BH_	Unknown	8/1/2016 4:02:24 PM	PA(18:0/18:3)	5.61	1241	dPE(18:0d5/18_	5.81	193	6.440	N/

Data Analysis using MultiQuant™ Software

Two different approaches to quantitation can be used

- **Relative quantitation** utilizes a single internal standard for each lipid class. The lipid-class IS is assigned for all MRM transitions within its specific lipid class. The data output is an area ratio of analyte to IS that can be used to compare relative changes between samples. However, these data cannot be used to measure relative concentrations within the same sample.
- **Accurate quantitation** utilizes the Lipidyzer™ Platform standards that are comprised of multiple molecular species of each lipid class. A specific IS within the mixture must be assigned to each analyte according to the fatty acid chain lost during CID. This accommodates the differential fragmentation efficiency due to chain length and number of double bonds. The quantitative bias using these standards is <10%.

Appendix – Web tools and References

Web-Based Resources

- LIPIDMAPS
  - Extensive, searchable lipid database with structural and chemical information
  - Library of LC and MS methods
- Avanti Polar Lipids
  - Commercial site that has comprehensive offering of high quality lipid standards
  - Technical application notes of lipid handling methods
- AOCS Lipid Library
- Lipidweb
- *Lipidomics: Technologies and Applications,* by Kim Ekroos (IMBD 978-3-527-33098-0)

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