Introduction to Lipidomics and the Tools Used to Analyze Lipids

UAB Metabolomics Workshop, July 2018

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Avanti® Polar Lipids, Inc.

Consider the Oh-So-Good-For-You, Healthy, Crunchy Salad

Face it, for most of us, it needs some greasy dressing to make it taste good!

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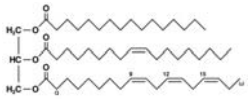
How to Make Salad Dressing

The Basic Recipe

- 1 Part vinegar
- 2 parts oil
- Salt and Pepper

Problem: Shake all you want, it's still vinegar and oil

Primary component of oil: TAG



Primary component of vinegar: Water

The best we can hope for is a brief dispersion between the oil and water; these polar and nonpolar compounds won't mix

We need some help

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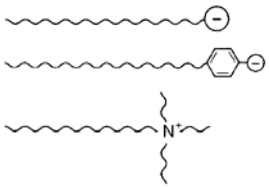
The Key to Salad Dressing Happiness: Emulsifiers

Amphipathic molecules have both polar and non-polar characteristics

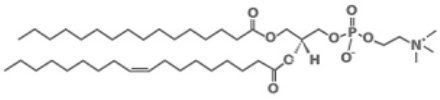
- Detergents

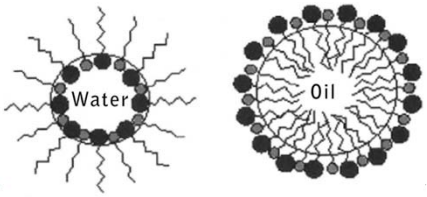
soap
anionic
cationic



We don't really want to eat soap…

- Phospholipids





Emulsifiers can stabilize a mixture of oil and water

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To Make the Dressing Homogenous, Add Egg

Egg yolk is ~ 30% phospholipid (Lethicin)

New Recipe:

- 1 Part vinegar
- 2 parts oil
- Cooked egg yolk
- Salt and Pepper

The phospholipids emulsify the TAGs and the water, creating a homogenous and hopefully tasty salad dressing

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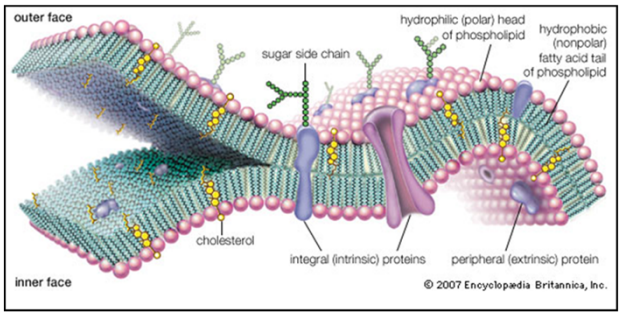
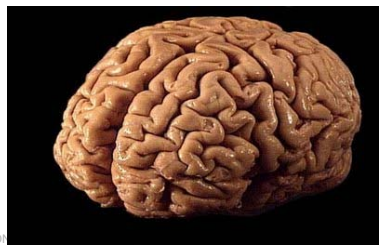
Many Different Lipids with Many Different Roles…

TAGs and DAGs

Phospholipids



Sphingolipids



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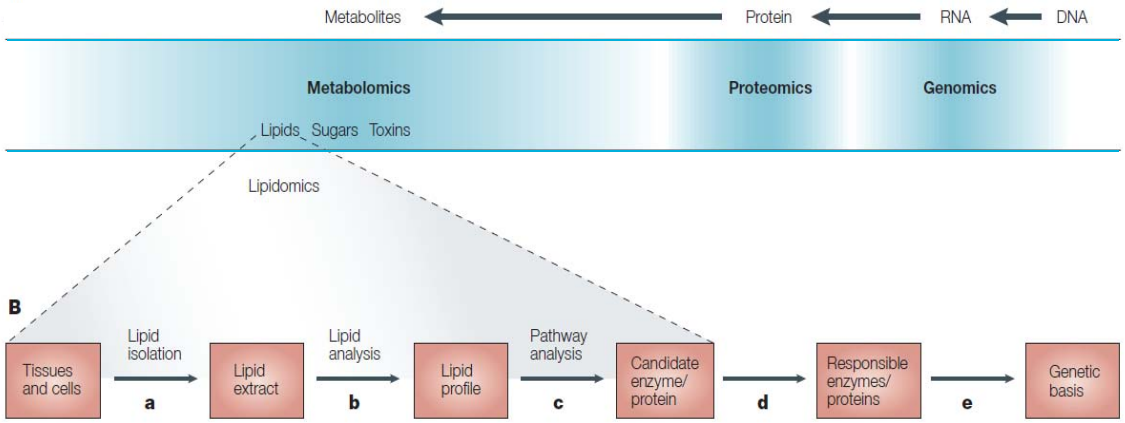
Lipidomics—A Part of the Omics Spectrum

General trend in omics research is to define metabolites and link to genomics



Wenk et al. Nature 2005

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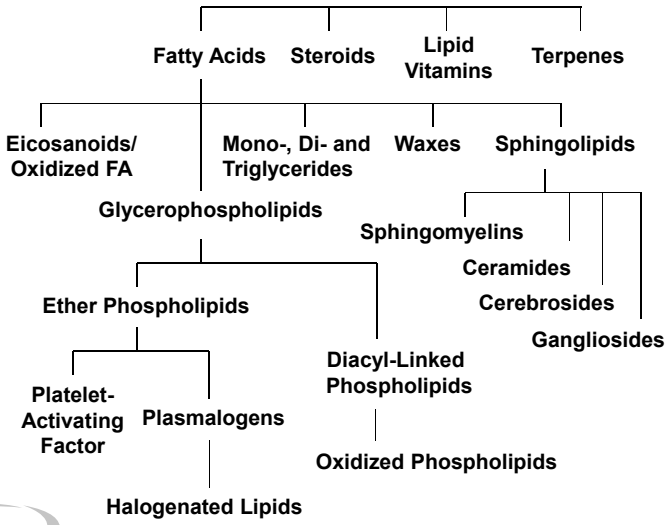
The Lipidome

Comprised of multiple, structurally distinct lipid classes and sub-classes



Lipids play an essential role in human physiology:

- Metabolic homeostasis
- Cell and organelle structure
- Cell signaling

And disease:

- Inflammation
- Cancer
- Cardiovascular disease
- Diabetes
- Inflammatory bowel disease
- Neurological diseases

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The Hierarchy of Specificity

The many layers of the lipidomics onion…

Increasing Specificity

- Lipid Class (PC, SM, TAG, etc…) TLC, Shotgun, NMR
- Sum Composition: PC 34:2 Shotgun
- Fatty acid identification: PC (16:0/18:1 or 18:1/16:0) HILIC, MS/MS^{ALL}, Lipidyzer^{TM}
- Fatty acid position: PC (16:0/18:1) SelexION^{TM}
- Double bond position: PC (16:0/18:1Δ9) EIEIO
- Stereochemisty: PC (16:0/18:1(9Z)) EIEIO

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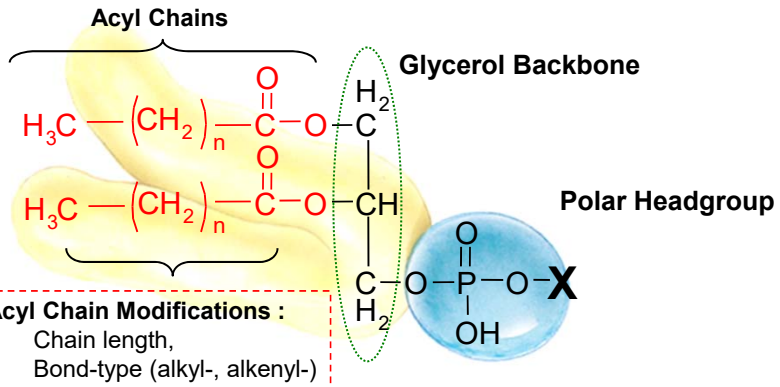
Diversity of Phopholipid Molecular Species



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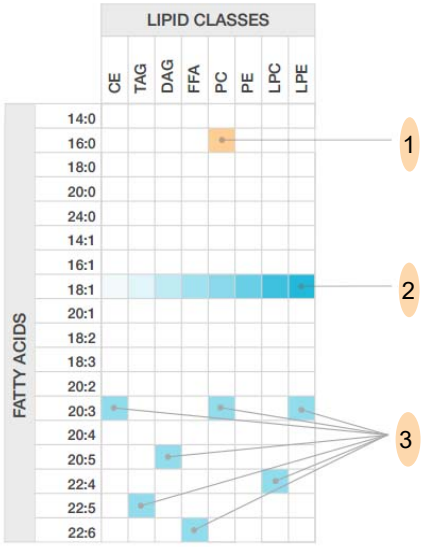
What is needed from a Lipid Platform?



1) Specificity

- Identification of the lipid at the molecular species level (i.e., PC (16:0/18:2) rather than PC 34:2)

2) Quantitative Rigor

- A non-quantitative approach does not allow accurate summing of the rows and columns

3) Comprehensive

- A partially complete matrix is difficult to interpret. E.g., Nat Med 2013 Letter

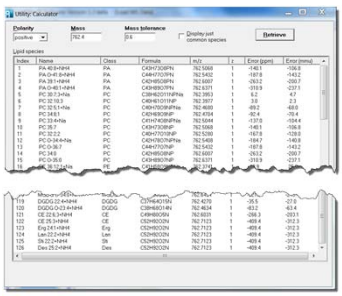
Challenges in Lipidomic Analysis

Fundamental challenge in lipidomics is dealing with isobaric interference.

- There are as many as 180,000 different lipid molecular species that are found in a narrow mass range of ~700 amu.

41 distinct lipid molecular species at *m/z* 762.4 ±0.1 Da.

Problem: The Q1 isolation window during MS/MS is ~1.2 Da, which increases the number of potential isobars.

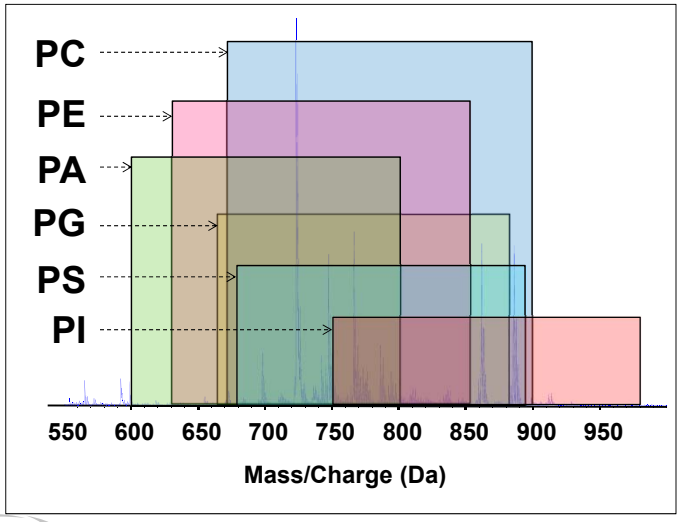


~125 distinct lipid molecular species at *m/z* 762.4 ±0.6 during precursor ion selection during MS/MS.

This occurs on both accurate and nominal mass instruments.

Isobaric Overlap of Phospholipids

Experiment: EMS scan of Bovine Heart Extract (BHE)

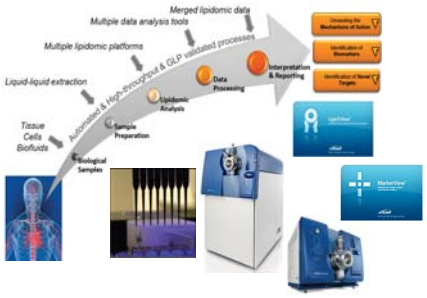


- Lipidomic spectra are incredibly complex
- MS/MS spectra generated on precursors in zones of isobaric overlap will contain product ions from other isobaric species

Current Strategies in Lipidomics

Separation problem:

- SPE extraction
- Fractionation
- Chemical derivatization
- LC chromatography



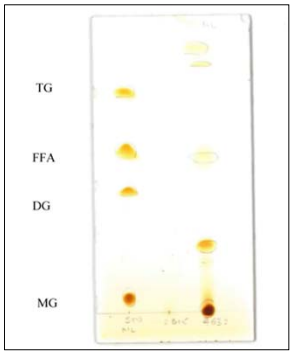
Mass spectrometry approaches:

- Shotgun Lipidomics: dedicated precursor and neutral loss scans (Triple Quadrupole and QTRAP® Systems)
- IDA-based methodologies (TripleTOF® Instruments and QTRAP® Systems)
- MS/MS^{ALL} (TripleTOF® Instruments)
- Differential Ion Mobility Spectrometry (DMS; SelexION™)

What Tools are Needed for Lipidomics?

What are the Tools that People Use for Lipid Analysis?

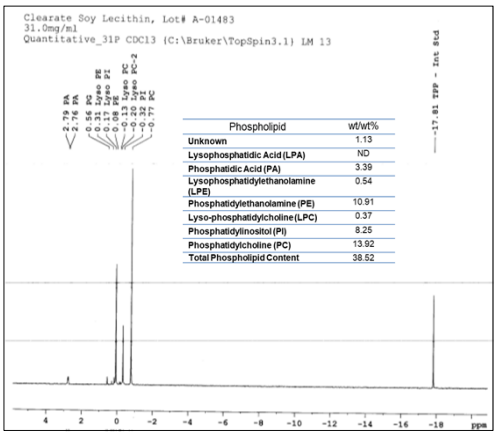
TLC



- Qualitative analysis of lipid classes
- Can be used to assess purity (~99%)
- Relatively cheap
- Good way to take a “snap shot” of what is in your sample

What are the Tools that People Use for Lipid Analysis?

NMR



Phospholipid	wt/wt%
Unknown	1.13
Lysophosphatidic Acid (LPA)	ND
Phosphatidic Acid (PA)	3.39
Lysophosphatidylethanolamine (LPE)	0.54
Phosphatidylethanolamine (PE)	10.91
Lyso-phosphatidylcholine (LPC)	0.37
Phosphatidylinositol (PI)	8.25
Phosphatidylcholine (PC)	13.92
Total Phospholipid Content	38.52

- Quantitative
- Can be used to elucidate and/or confirm structure
- Can be used to detect and quantitate impurities
- Most commonly used at Avanti for quantitative ^{31}P NMR analysis of phospholipid mixtures

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What are the Tools that People Use for Lipid Analysis?

GC FAMEs

- The “Gold Standard” by which fatty acids are analyzed
- Cannot analyze complex lipids
- Samples must be hydrolyzed and derivatized before analysis

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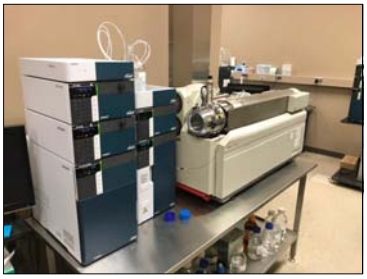
What are the Tools that People Use for Lipid Analysis?

ESI LC-MS/MS

- "Soft" ionization technique allows for intact complex lipid analysis
- MS/MS enables structural elucidation and quantitation
- No derivatization
- Must have a cogent internal standard strategy for quantitation



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Lipid Analysis Using HILIC-Based LC-ESI MS/MS

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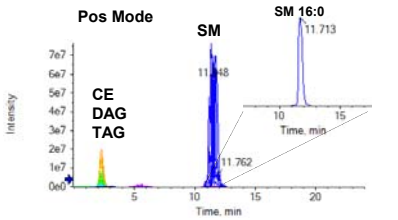
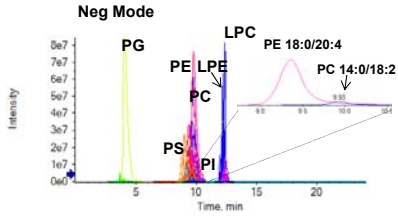
Lipidomics Analysis Using a HILIC LC Strategy with a Broad Target List

Over 1100 lipids analyzed with the capacity for accurate or relative quantitation in 24 minutes





Pos 616 MRMs

	Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	ID	CE (volts)
1	738.663	184.200	11.70	18:1(d9) SM	43.000
2	675.500	184.100	11.86	SM(14:0)	43.000
3	703.600	184.100	11.78	SM(16:0)_STD	43.000
4	731.600	184.100	11.70	SM(18:0)_STD	43.000
5	729.600	184.100	11.65	SM(18:1)_DM	43.000
6	759.600	184.100	11.62	SM(20:0)_STD	43.000
7	757.600	184.100	11.62	SM(20:1)	43.000
8	787.700	184.100	11.55	SM(22:0)_STD	43.000
9	785.700	184.100	11.57	SM(22:1)	43.000
10	815.700	184.100	11.49	SM(24:0)_STD	43.000
11	813.700	184.100	11.50	SM(24:1)_STD	43.000
12	843.700	184.100	11.44	SM(26:0)_STD	43.000
13	841.700	184.100	11.45	SM(26:1)_STD	25.000
14	675.680	369.400	2.27	18:1(d7) Chol	43.000
15	754.700	369.400	2.18	CE(24:0)	25.000
16	714.600	369.400	2.18	CE(22:6)	25.000
17	698.700	369.400	2.26	CE(20:0)	25.000
18	696.700	369.400	2.26	CE(20:1)	25.000

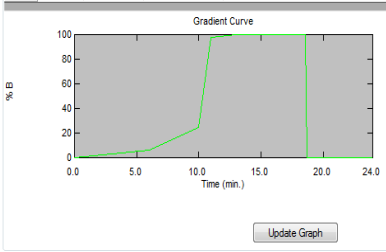
Neg 544 MRMs

	Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	ID	CE (volts)
1	587.409	288.296	12.30	18:1(d7) LPC	-50.000
2	526.317	227.202	12.30	LPC(14:0) +A	-50.000
3	554.346	255.233	12.50	LPC(16:0) +A	-50.000
4	552.331	253.217	12.30	LPC(16:1) +A	-50.000
5	582.378	283.264	12.30	LPC(18:0) +A	-50.000
6	580.362	281.249	12.10	LPC(18:1) +A	-50.000
7	578.346	279.233	12.10	LPC(18:2) +A	-50.000
8	576.331	277.217	12.30	LPC(18:3) +A	-50.000
9	610.409	311.300	12.30	LPC(20:0) +A	-50.000
10	608.393	309.280	12.30	LPC(20:1) +A	-50.000
11	606.378	307.264	12.30	LPC(20:2) +A	-50.000
12	604.362	305.249	12.30	LPC(20:3) +A	-50.000
13	602.346	303.233	12.30	LPC(20:4) +A	-50.000
14	600.331	301.217	12.30	LPC(20:5) +A	-50.000
15	630.364	331.264	11.90	LPC(22:4) +A	-50.000
16	628.362	329.249	12.00	LPC(22:5) +A	-50.000
17	626.346	327.233	12.10	LPC(22:6) +A	-50.000
18	811.625	288.298	10.20	15:0-18:1(d7)	-50.000

LC/MS Acquisition Parameters

Acquisition Parameters		
HPLC System	ExionLC™ System	
MS/MS System	QTRAP® 6500+ System	
Injection Volume	5 µL	
Column Temp	35°C	
Analytical Column	Xbridge Amide 4.6 x 150 mm 3.5 um	
LC Flow Rate	700 µL/min	
Mobile Phase A	5%H2O/95% ACN with 1mM ammonium acetate, pH about 8.2	
Mobile Phase B	50% H2O/50% ACN with 1mM ammonium acetate, pH is 8.2	
Autosampler Wash	IPA	
Source & MS Parameter:	ISV: 5500 V GS1: 50.0 GS2: 60.0 DP: 60 EP: 10.0	CAD: 8 CUR: 30 TEM: 500 CXP: 15

	Time	Module	Event	Parameter
1	0.01	Pumps	Pump B Conc.	0.1
2	6.00	Pumps	Pump B Conc.	6
3	10.00	Pumps	Pump B Conc.	25
4	11.00	Pumps	Pump B Conc.	98
5	13.00	Pumps	Pump B Conc.	100
6	13.40	Pumps	Total Flow	0.7
7	13.50	Pumps	Total Flow	1.5
8	18.60	Pumps	Pump B Conc.	100
9	18.70	Pumps	Pump B Conc.	0.1
10	23.00	Pumps	Total Flow	1.5
11	23.50	Pumps	Total Flow	0.7
12	24.00	Controller	Stop	
13				



Method Development

A comprehensive "long" MRM list was developed to cover most molecular species within each lipid class

Dwell time parameters:

> 3 ms Dwell
> 3 ms pause time
< 5 ms Pos/Neg Switching

PC(18:0/18:1)

Class I.D. based on RT

Mol. Species I.D. based on MRM

Q1	Q2	RT	I.D.
836.55	327.23	6.076	PC(14:0/22:6)+AcO
764.55	227.2	6.074	PC(16:0/14:0)+AcO
792.58	255.23	6.072	PC(16:0/16:0)+AcO
790.56	253.22	6.07	PC(16:0/16:1)+AcO
820.61	283.26	6.068	PC(16:0/18:0)+AcO
818.59	281.25	6.066	PC(16:0/18:1)+AcO
816.58	279.23	6.07	PC(16:0/18:2)+AcO
814.56	277.22	6.02	PC(16:0/18:3)+AcO
846.62	309.28	5.97	PC(16:0/20:1)+AcO
844.61	307.26	5.92	PC(16:0/20:2)+AcO
842.59	305.25	5.87	PC(16:0/20:3)+AcO
840.58	303.23	5.84	PC(16:0/20:4)+AcO
838.56	301.22	5.84	PC(16:0/20:5)+AcO
868.61	331.26	5.84	PC(16:0/22:4)+AcO
866.59	329.25	5.84	PC(16:0/22:5)+AcO
816.58	281.25	5.84	PC(16:1/18:1)+AcO
814.56	253.22	5.84	PC(16:1/18:2)+AcO
864.58	327.23	5.84	PC(16:0/22:6)+AcO
792.58	227.2	5.84	PC(18:0/14:0)+AcO
818.59	253.22	5.84	PC(18:0/16:1)+AcO
846.62	281.25	5.84	PC(18:0/18:1)+AcO
842.59	277.22	5.975	PC(18:0/18:3)+AcO
876.67	283.26	5.97	PC(18:0/20:0)+AcO
874.65	309.28	5.965	PC(18:0/20:1)+AcO
872.64	307.26	5.96	PC(18:0/20:2)+AcO
870.62	305.25	5.955	PC(18:0/20:3)+AcO
868.61	303.23	5.95	PC(18:0/20:4)+AcO
866.59	301.22	5.945	PC(18:0/20:5)+AcO
896.64	331.26	5.94	PC(18:0/22:4)+AcO
894.62	329.25	5.935	PC(18:0/22:5)+AcO
892.61	327.23	5.93	PC(18:0/22:6)+AcO
816.58	281.25	5.925	PC(18:1/16:1)+AcO
844.61	281.25	5.92	PC(18:1/18:1)+AcO

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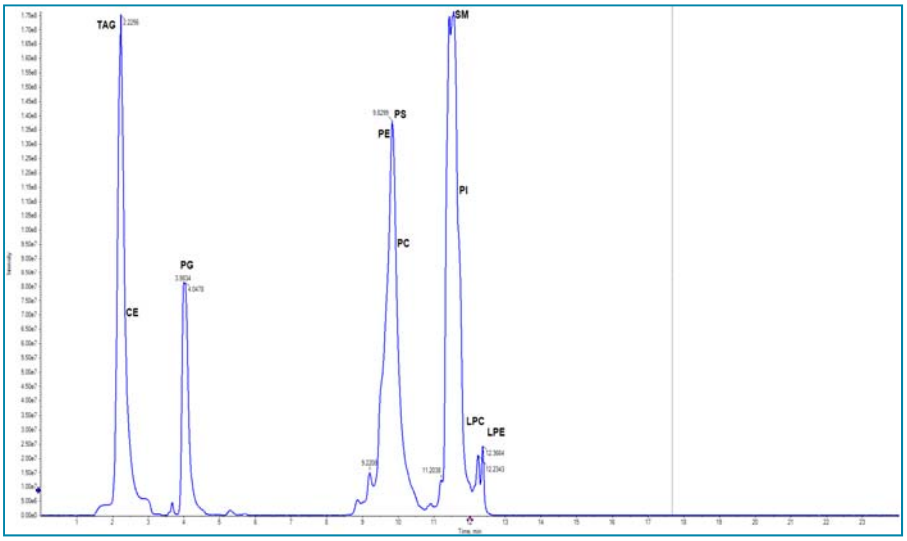
Plasma sample HILIC Method Reproducibility



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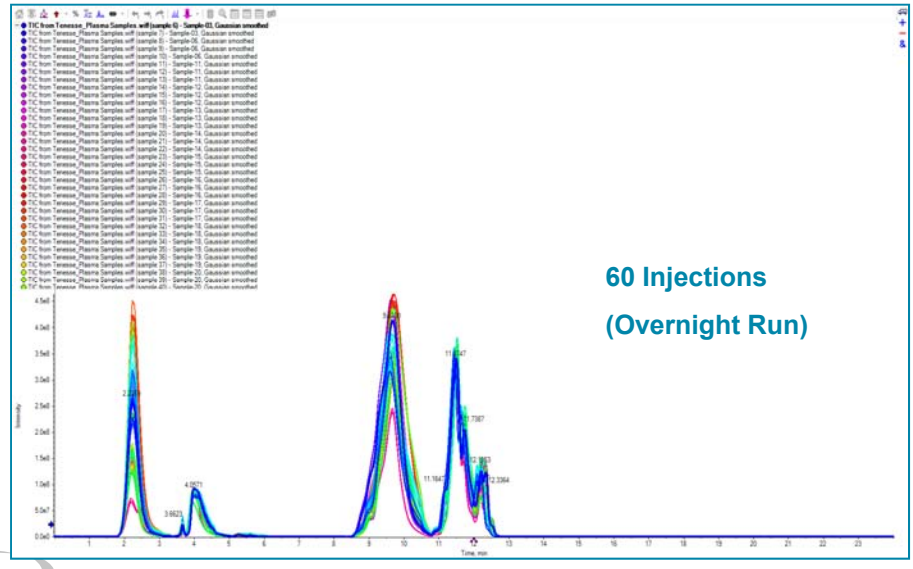
Plasma sample HILIC Method Reproducibility



60 Injections

(Overnight Run)

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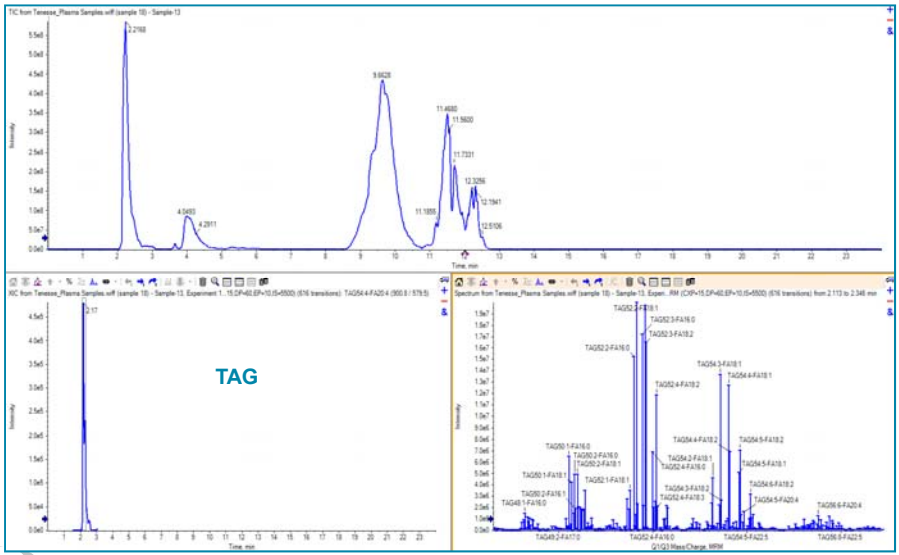
Demo Plasma Samples - TAG's



TAG

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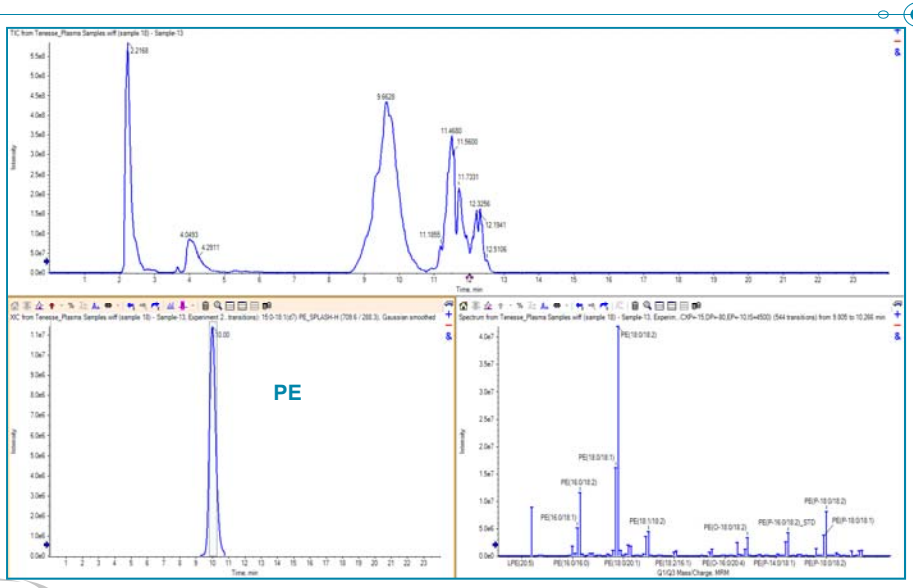
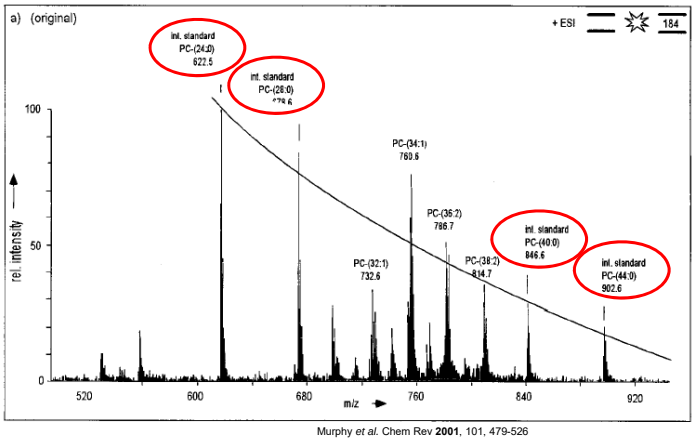
Demo Plasma Samples - PE



Quantitation: The Challenges Inherent to Lipid Analysis

Unequal fragmentation efficiency among lipid molecular species of the same class



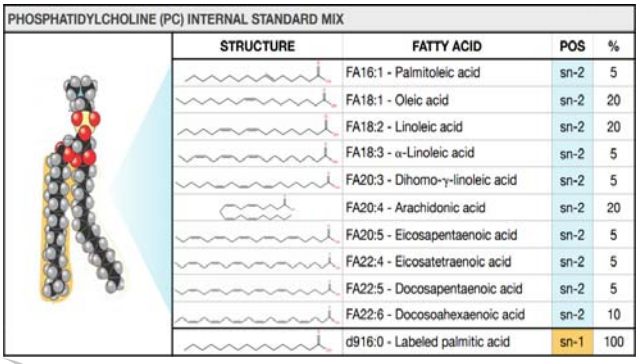
Murphy *et al.* Chem Rev **2001**, 101, 479-526

4 internal standards, all with the same concentration. Chain length and degree of unsaturation affect fragmentation efficency.

Internal Standard Strategies: Lipidyzer Standards

The Lipidyzer standards have multiple labeled molecular species for each lipid class and are used for "accurate quantitation"

- Lipidyzer 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.



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

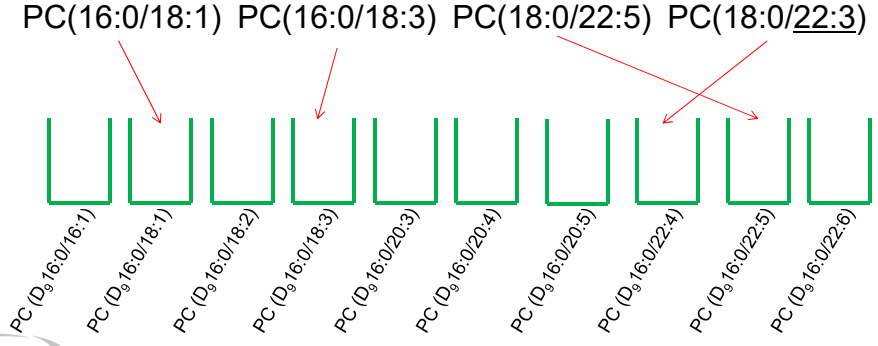
Example of the multiple internal standards available for each the PC class of molecular species

"Accurate quantitation" is defined as accurate within a 10% quantitative bias

Internal Standard Strategy to Provide "Accurate Quantitation"

A way to visualize the IS strategy is to consider each standard in each lipid class (e.g., PC ($D_9$16:0/16:1)) mixture being assigned to a "bucket."

Each analyte is assigned to a "bucket" for quantitation. If the match is not "perfect" the best approximation is used



Avanti Lipidomics: Revolutionary Tools Designed to Usher in a New Framework of Lipid Research

Mass Spectrometry Tools
- LIPID MAPS MS Standards
- LipidoMix® Standards

Protein-Lipid Interaction Tools
- Snoopers
- pacFA

Lipid Binding Antibodies
- E06: Atherosclerosis Research
- WR304: Inositol Phosphate Research
- New Antibodies

Cellular Delivery Tools
- Huzzah™
- Ceramide Delivery System

Imaging Probes Based on Gold Nanoparticles
- Aurora DSG
- Aurora PLC

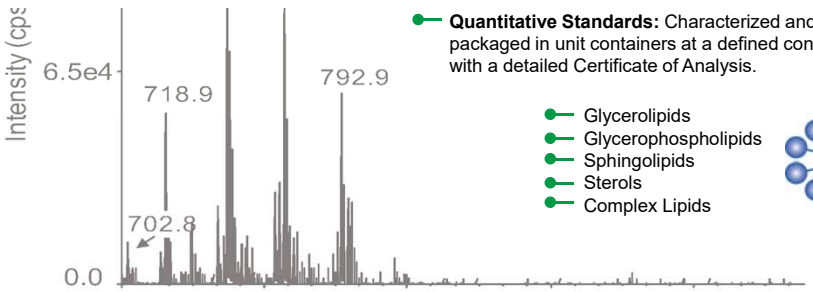
Connecting Lipids, Technology, & Cellular Biology

Avanti® Polar Lipids, Inc.

LIPID MAPS MS Standards: Constructing Meaningful Spectra

In the last decade, Avanti has shipped over 52,000 vials to both LIPIDMAPS™ core groups and mass spectrometry laboratories all over the world.

Over 800 compounds, including the synthesis of over 400 compounds new to Avanti.



- **Quantitative Standards:** Characterized and pre-packaged in unit containers at a defined concentration with a detailed Certificate of Analysis.
  - Glycerolipids
  - Glycerophospholipids
  - Sphingolipids
  - Sterols
  - Complex Lipids

In the final year of the Lipid MAPS initiative, Avanti worked to move towards the development of useful lipid standard mixtures.

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LIPIDOMIX® Standards: Constructing Meaningful Spectra

Single-vial Prepared Lipidomic Analytical Standard for Human plasma lipids

Mixture Component	Target Concentration (µg/mL)
15:0-18:1(d7) PC	160
15:0-18:1(d7) PE	5
15:0-18:1(d7) PS (Na Salt)	5
15:0-18:1(d7) PG (Na Salt)	30
15:0-18:1(d7) PI (NH_4 Salt)	10
15:0-18:1(d7) PA (Na Salt)	7
18:1(d7) Lyso PC	25
18:1(d7) Lyso PE	5
18:1(d7) Chol Ester	350
18:1(d7) MAG	2
15:0-18:1(d7) DAG	10
15:0-18:1(d7)-15:0 TAG	55
d18:1-18:1(d9) SM	30
Cholesterol (d7)	100

Wall VZ, et al. Inflammatory stimuli induce acyl-CoA thioesterase 7 and remodeling of phospholipids containing unsaturated long (≥C20)-acyl chains in macrophages. J Lipid Res. 2017 Jun;58(6):1174-1185.

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LIPIDOMIX® Standards: Constructing Meaningful Spectra

Single-vial Prepared Lipidomic Analytical Standard for Human plasma lipids

Mixture Component	Target Concentration (µg/mL)
15:0-18:1(d7) PC	160
15:0-18:1(d7) PE	5
15:0-18:1(d7) PS (Na Salt)	8
15:0-18:1(d7) PI (NH_4 Salt)	8
18:1(d7) Lyso PC	25
18:1(d7) Lyso PE	0.5
18:1(d7) Chol Ester	350
C18(Plasm)-18:1(d9) PC	8
15:0-18:1(d7) DAG	12
15:0-18:1(d7)-15:0 TAG	55
d18:1-18:1(d9) SM	30
C18(Plasm)-18:1(d9) PE	0.07

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LIPIDOMIX® Standards: Constructing Meaningful Spectra

Single-vial **P**repared **L**ipidomic **A**nalytical **S**tandard for **H**uman plasma lipids

Mixture Component	Target Concentration (µg/mL)
15:0-18:1(d7) PC	75
15:0-18:1(d7) PE	5
15:0-18:1(d7) PS (Na Salt)	15
15:0-18:1(d7) PG (Na Salt)	4
15:0-18:1(d7) PI (NH_4 Salt)	17
15:0-18:1(d7) PA (Na Salt)	7
18:1(d7) Lyso PC	24
18:1(d7) Lyso PE	1
18:1(d7) Chol Ester	165
C18(Plasm)-18:1(d9) PC	16
15:0-18:1(d7) DAG	9
15:0-18:1(d7)-15:0 TAG	28
d18:1-18:1(d9) SM	15
C18(Plasm)-18:1(d9) PE	4

Mouse SPLASH® LIPIDOMIX® Quantitative Mass Spec Internal Standard

Deuterated LIPIDOMIX® standard contains all major lipid classes in ratios relative to **MOUSE** plasma.

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LIPIDOMIX® Standards: Constructing Meaningful Spectra

Odd-Chained LIPIDOMIX® Quantitative Mass Spec Internal Standard

Mixture Component	Target Concentration (µg/mL)
17:1 Lyso PG (Na Salt)	13
17:1 Lyso PA (NH_4 Salt)	15
17:1 Lyso PI (NH_4 Salt)	13
17:1 Lyso PS (Na Salt)	13
17:1 Lyso PC	575
17:1 Lyso PE	12
17:0-17:0 DAG	300
17:0-17:0-17:0 TAG	1500
12:0 SM (d18:1/12:0)	650
17:0-14:1 PC	3775
17:0-14:1 PS (NH_4 Salt)	180
17:0-14:1 PG (NH_4 Salt)	90
17:0-14:1 PA (NH_4 Salt)	15
17:0-14:1 PE	120
17:0-14:1 PI (NH_4 Salt)	200
17:0 Chol Ester	8475

Ceramide LIPIDOMIX® Quantitative Mass Spec Standard

Mixture Component	Target Concentration (µg/mL)
C16 Ceramide (d18:1/16:0)	16.1
C18 Ceramide (d18:1/18:0)	8.5
C24 Ceramide (d18:1/24:0)	48.8
C24:1 Ceramide (d18:1/24:1(15Z))	24.3

Deuterated Ceramide LIPIDOMIX® Quantitative Mass Spec Internal Standard

Mixture Component	Target Concentration (µg/mL)
C16 Ceramide-d7 (d18:1-d7/16:0)	21.8
C18 Ceramide-d7 (d18:1-d7/18:0)	11.5
C24 Ceramide-d7 (d18:1-d7/24:0)	26.3
C24:1 Ceramide-d7 (d18:1-d7/24:1(15Z))	13.1

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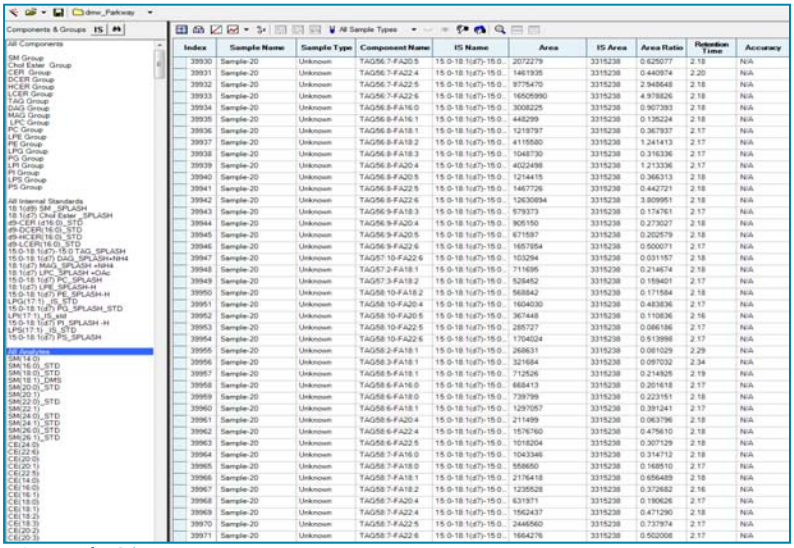
Data Analysis using Multiquant™ Software

Pre-configured MultiQuant method file can rapidly process lipid data



SCIEX
Answers for Science.
Knowledge for Life.™

Data Can Be Directly Exported to Excel

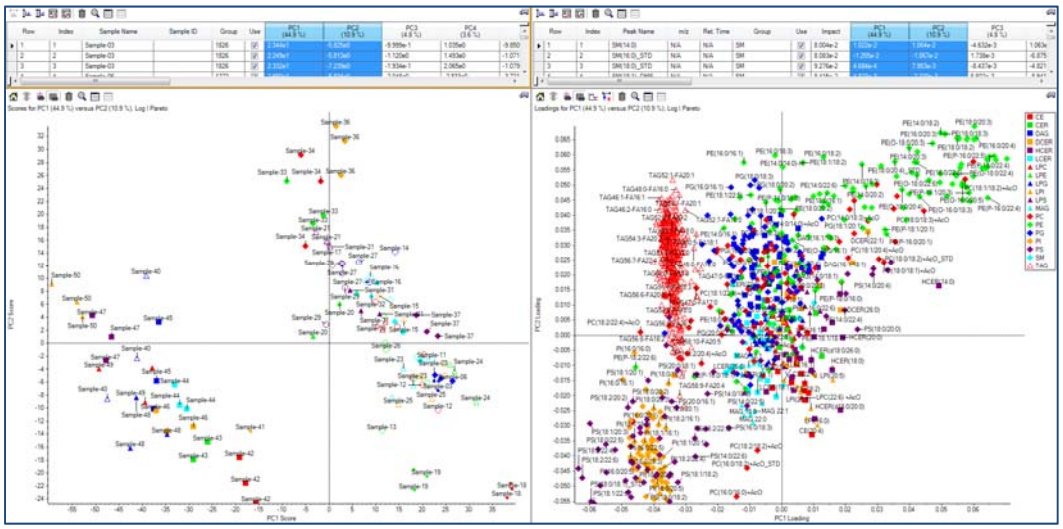
- Data can be exported to Excel and are presented as area ratios with respect to a single IS per class when SPLASH Mix is used

Row	Component Name	Sample Name	Num. Val.	Mean	Standard Deviation	Percent CV	Value #1	Value #2	Value #3
1	SM(14:0)	Blk	1 of 1	3.022e3	N/A	N/A	3.022e3		
2	SM(14:0)	Sample-03	3 of 3	6.094e8	4.669e6	0.77	6.146e8	6.082e8	6.055e8
3	SM(14:0)	Sample-06	3 of 3	5.633e8	1.502e6	0.28	5.634e8	5.617e8	5.649e8
4	SM(14:0)	Sample-11	3 of 3	5.676e8	3.726e6	0.66	5.642e8	5.716e8	5.670e8
5	SM(14:0)	Sample-12	3 of 3	4.522e8	1.107e7	2.45	4.501e8	4.641e8	4.423e8
6	SM(14:0)	Sample-13	3 of 3	5.502e8	6.241e6	1.13	5.517e8	5.434e8	5.556e8
7	SM(14:0)	Sample-14	3 of 3	3.311e8	4.641e6	1.40	3.328e8	3.347e8	3.259e8
8	SM(14:0)	Sample-15	3 of 3	4.419e8	3.258e6	0.74	4.385e8	4.451e8	4.422e8
9	SM(14:0)	Sample-16	3 of 3	5.268e8	1.120e6	0.21	5.269e8	5.246e8	5.260e8
10	SM(14:0)	Sample-17	3 of 3	4.759e8	5.501e5	0.20	4.762e8	4.748e8	4.766e8
11	SM(14:0)	Sample-18	3 of 3	3.979e8	1.808e6	0.45	3.958e8	3.989e8	3.990e8
12	SM(14:0)	Sample-19	3 of 3	5.035e8	6.440e6	1.28	5.104e8	5.023e8	4.977e8
13	SM(14:0)	Sample-20	3 of 3	4.987e8	2.869e6	0.58	4.955e8	5.009e8	4.999e8
14	SM(14:0)	Sample-21	3 of 3	4.976e8	1.881e6	0.38	4.992e8	4.956e8	4.981e8
15	SM(14:0)	Sample-23	3 of 3	4.264e8	1.293e6	0.30	4.257e8	4.256e8	4.278e8
16	SM(14:0)	Sample-24	3 of 3	3.830e8	3.848e6	1.00	3.786e8	3.849e8	3.856e8
17	SM(14:0)	Sample-25	3 of 3	5.122e8	1.656e7	3.23	4.935e8	5.250e8	5.182e8
18	SM(14:0)	Sample-26	3 of 3	4.617e8	1.404e6	0.30	4.630e8	4.602e8	4.618e8
19	SM(14:0)	Sample-27	3 of 3	4.446e8	2.292e7	5.15	4.184e8	4.611e8	4.543e8
20	SM(14:0)	Sample-28	3 of 3	4.510e8	1.007e6	0.22	4.521e8	4.501e8	4.508e8
21	SM(14:0)	Sample-29	3 of 3	4.386e8	2.451e6	0.56	4.414e8	4.372e8	4.372e8
22	SM(14:0)	Sample-30	3 of 3	4.651e8	5.035e6	1.08	4.607e8	4.706e8	4.640e8
23	SM(14:0)	Sample-31	3 of 3	4.304e8	3.373e6	0.78	4.266e8	4.315e8	4.330e8
24	SM(14:0)	Sample-32	3 of 3	4.728e8	4.925e6	1.04	4.684e8	4.718e8	4.781e8
25	SM(14:0)	Sample-33	3 of 3	4.491e8	6.616e6	1.47	4.418e8	4.508e8	4.547e8
26	SM(14:0)	Sample-34	3 of 3	4.579e8	4.090e6	0.89	4.618e8	4.536e8	4.583e8
27	SM(14:0)	Sample-36	3 of 3	4.356e8	9.388e6	2.15	4.248e8	4.405e8	4.416e8
28	SM(14:0)	Sample-37	3 of 3	4.703e8	1.326e7	2.82	4.800e8	4.757e8	4.552e8
29	SM(14:0)	Sample-38	3 of 3	3.107e8	5.472e5	0.18	3.107e8	3.113e8	3.102e8
30	SM(14:0)	Sample-39	3 of 3	2.966e8	2.438e6	0.82	2.947e8	2.959e8	2.994e8

%CV is a function of lipid abundance; low abundance lipids will have higher %CVs

Avanti® Polar Lipids, Inc.

Unsupervised PCA Analysis Reveals Utility of Broad Screening Approach



Data analyzed by MarkerView™ Software Un-Supervised PCA Loading for D1(51.9%) verses D2 (5.9%) Log/Pareto (DA)

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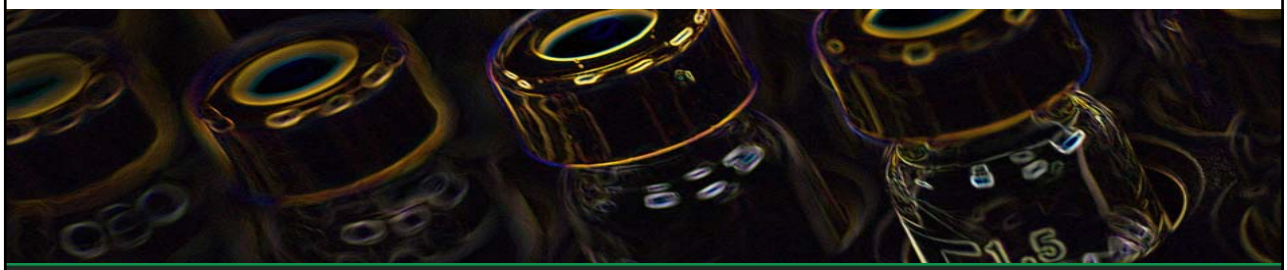

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