SCIEX

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Knowledge for Life.™

A Novel Lipid Screening Platform Allowing a Complete Solution for Lipidomics Research

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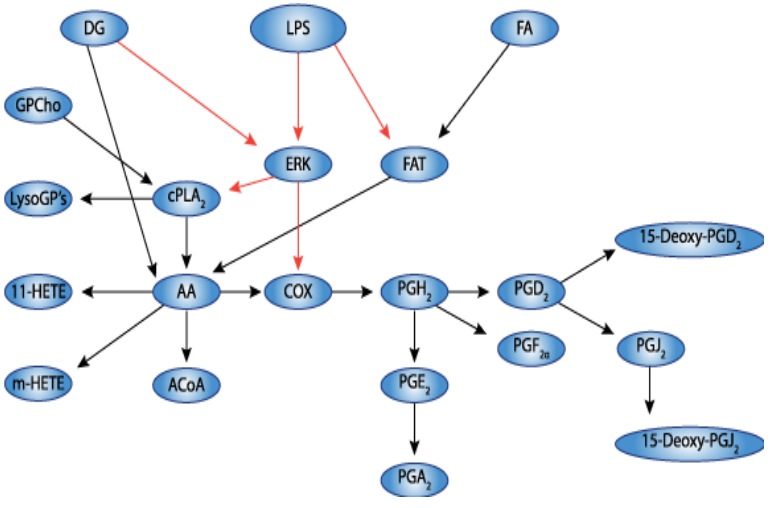
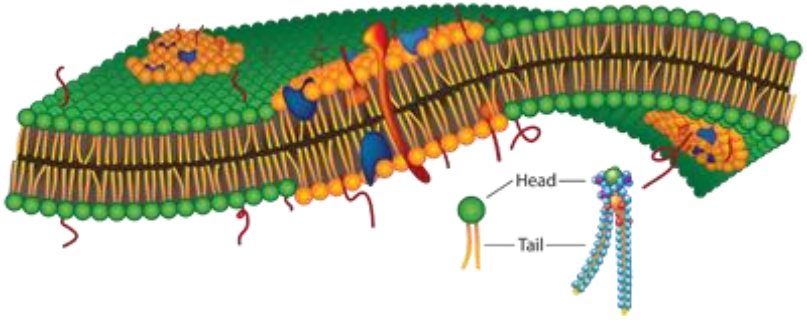
Lipidomics

A Subset of the Metabolome

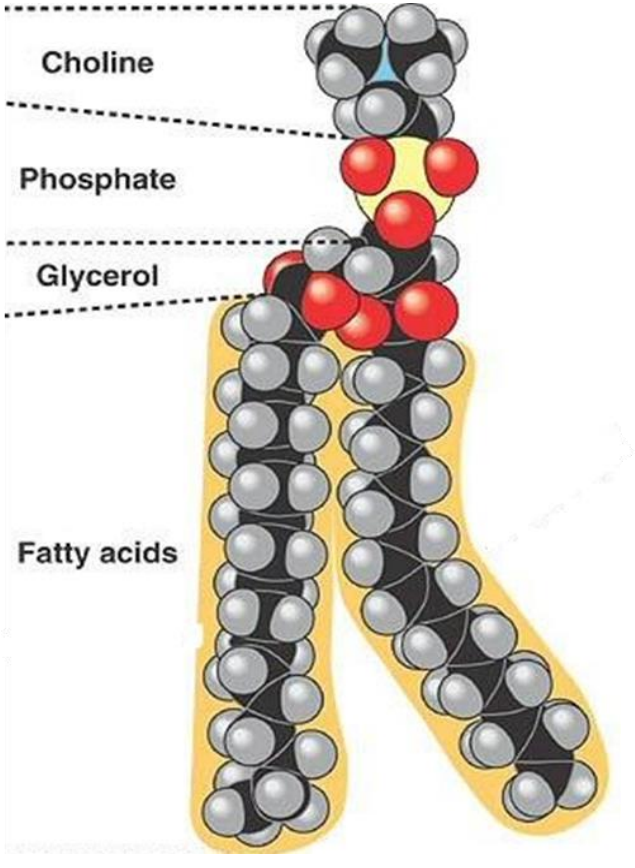
- The study of pathways and networks of cellular lipids in biological systems.
- The ‘lipidome’ describes the complete lipid profile within a cell, tissue or organism and is a subset of the ‘metabolome’
- The metabolome is the total number of metabolites present within an organism, cell, or tissue





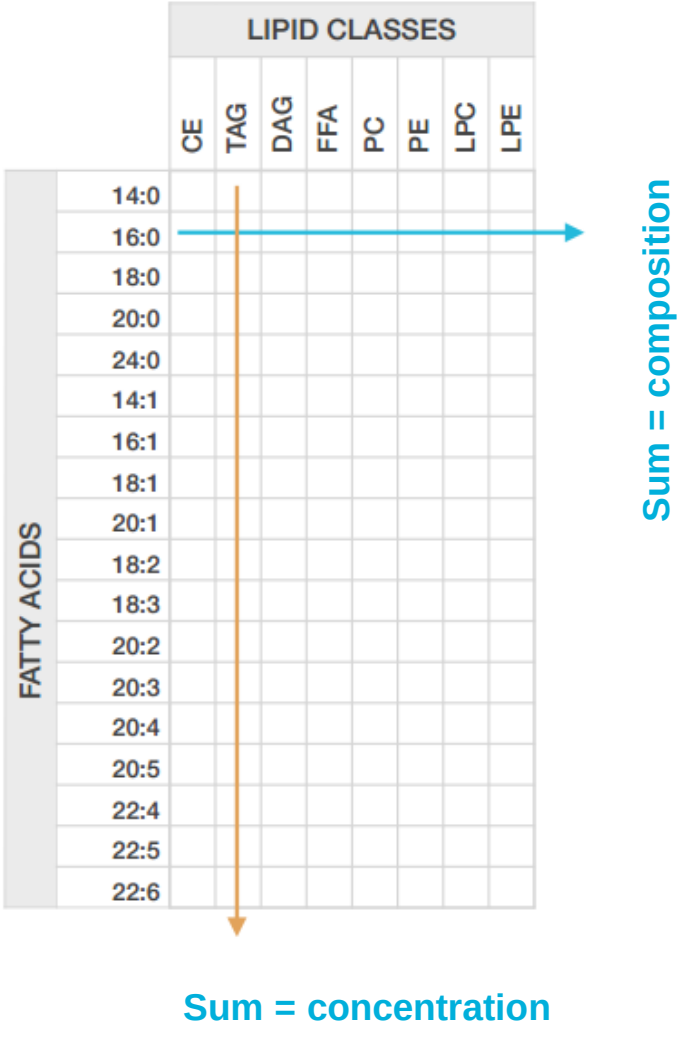
The Challenge in Lipidomics Research

- Lipids are polymeric structures and their individual elements have their own pathways
- Breakdown into intermediary metabolites (FAs)
- Mapping lipids requires mapping to the right level



Complex Lipids are like a Matrix

- Lipids are present in classes that have concentrations and compositions (important for level of metabolism)
  - Concentration = sum of the FAs for any given class (column)
  - Composition = relative abundances of each FA (or species) across many classes (rows)



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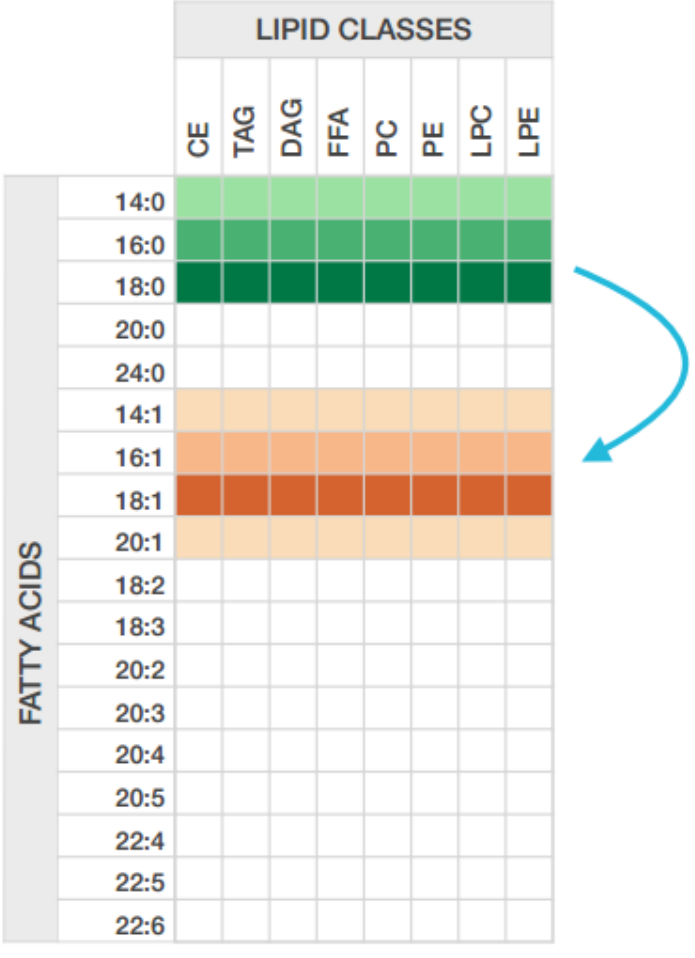
- When FA metabolism is altered there is the ability to change FA composition of all classes



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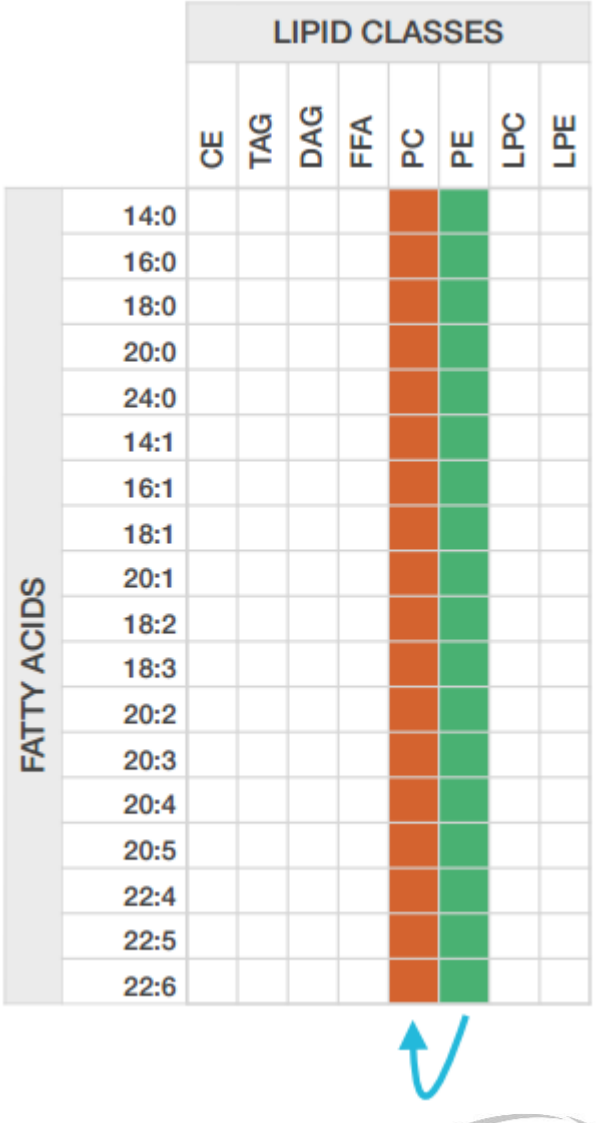
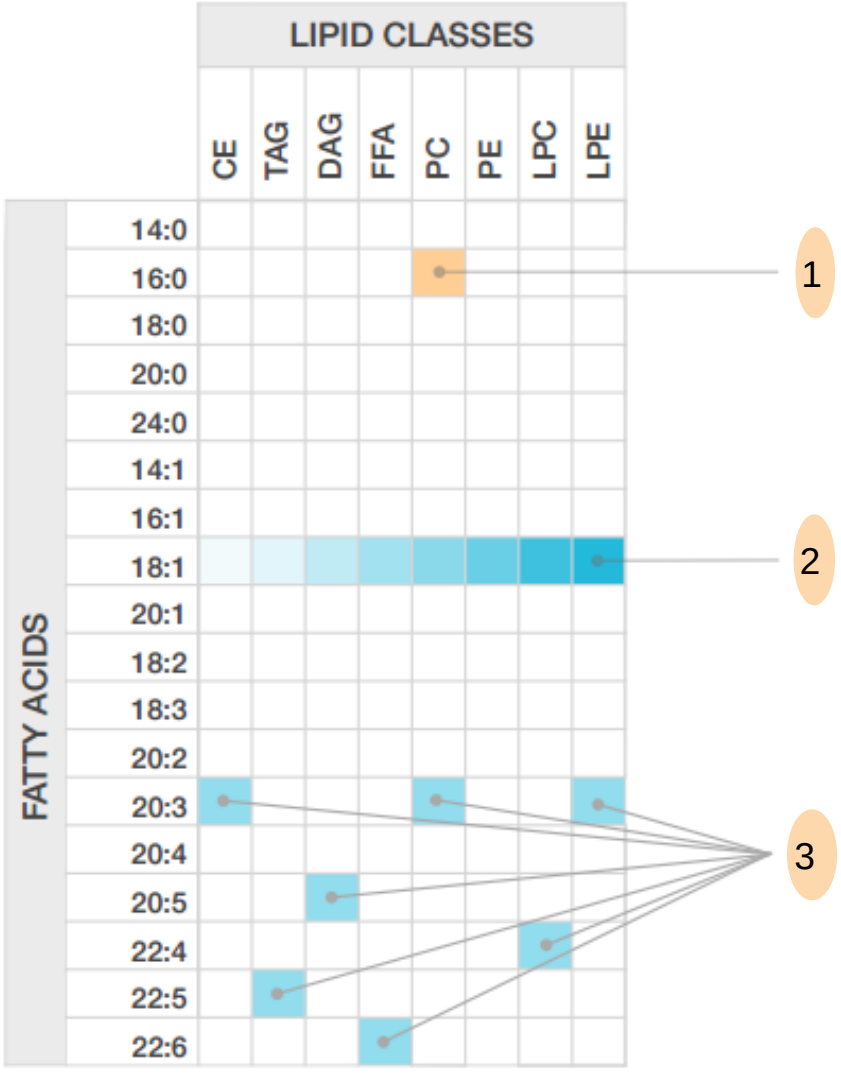
- When lipid class metabolism is altered there is the ability to change all members of the class



What is needed from a Lipid Platform



1) Specificity

- A non-specific method (e.g. PC 36:2) does not allow mapping to the elements of the matrix

2) Quantitation

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

3) Comprehensive Coverage

- A partially complete matrix is difficult to interpret

Metabolon®

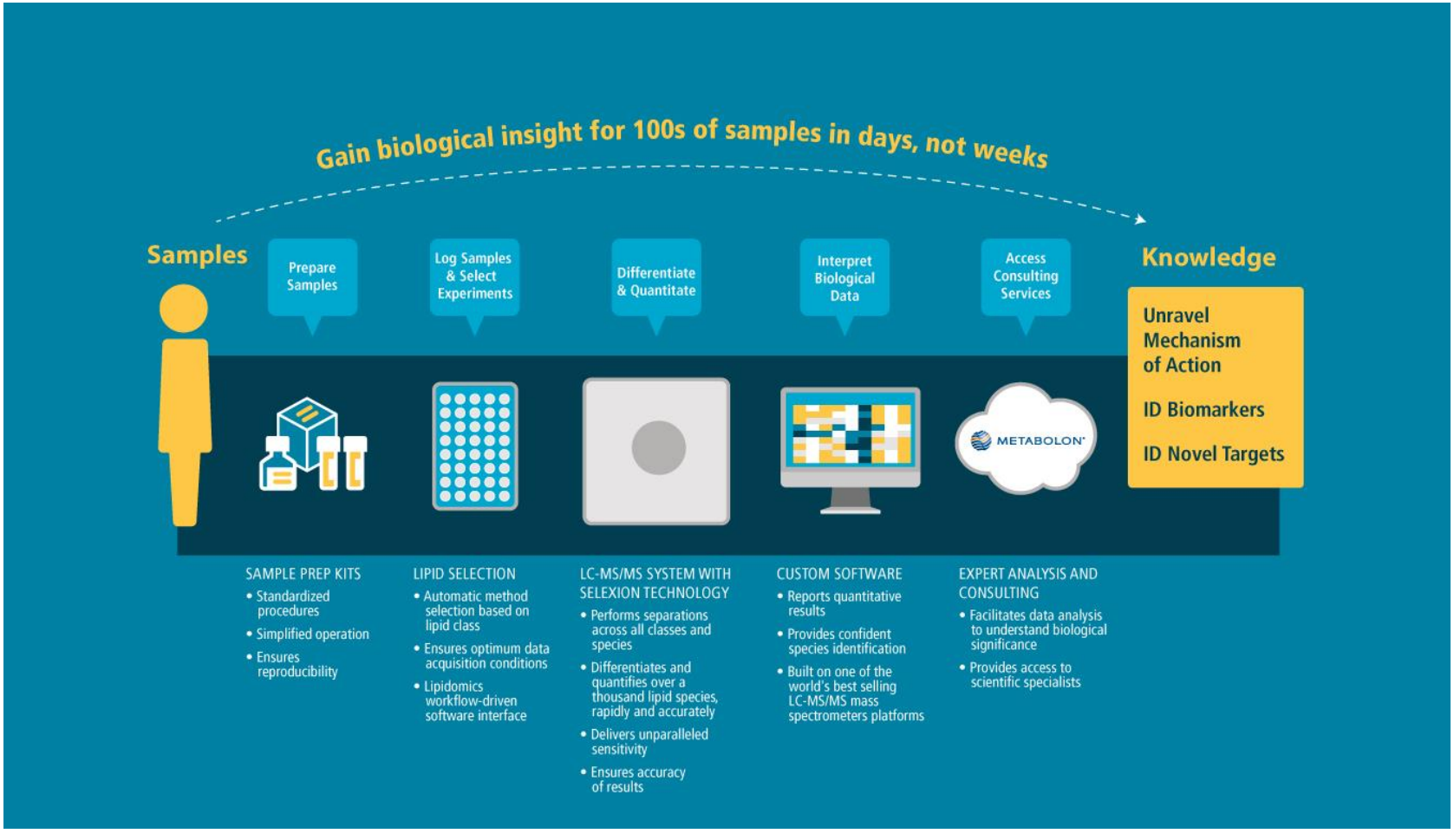
Global Leader in Metabolomics Applications

- Over 10 years of continuous leadership in metabolomics technology development
- Core business is metabolomics services and diagnostic development
- 500 publications, many in top tier journals (Nature, Science, Cell)
- Over 4000 projects conducted with hundreds of clients
- Acquired Lipomics in 2012, a lipidomics company founded in 2000

Partnered with SCIEX to build Next-Gen Lipidomic capabilities

The SCIEX Lipidyzer™ Platform - Simplifying the Complexity

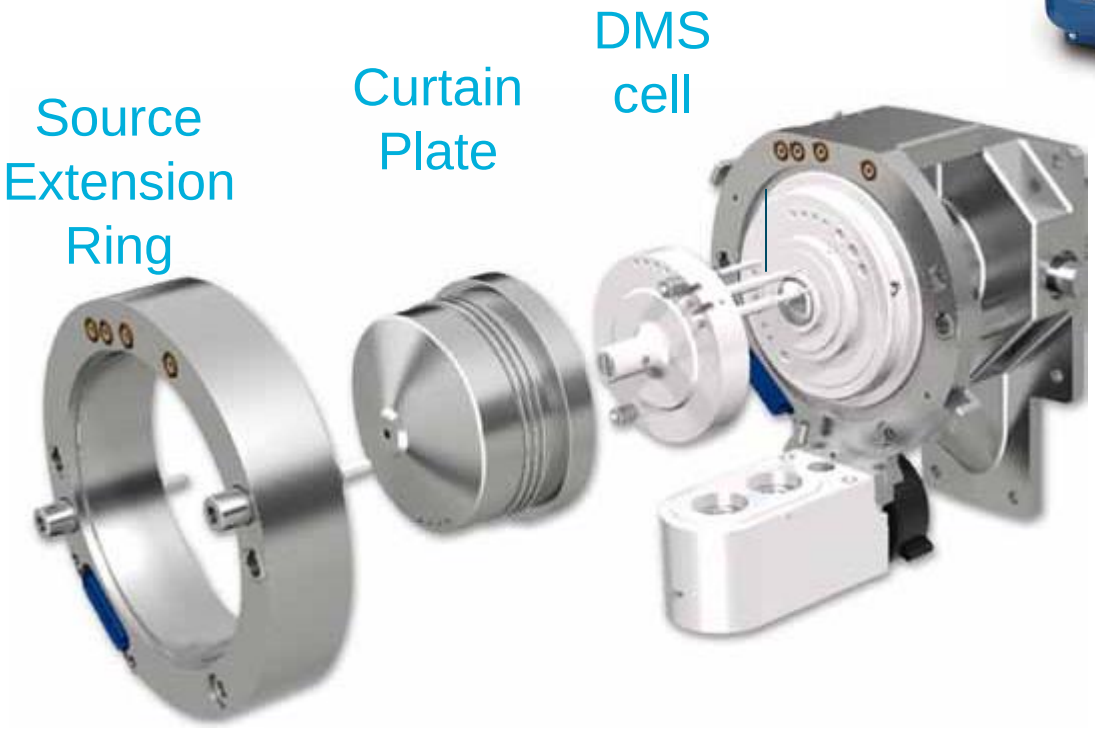
Powered by METABOLON®



Lipidyzer™ Hardware Configuration

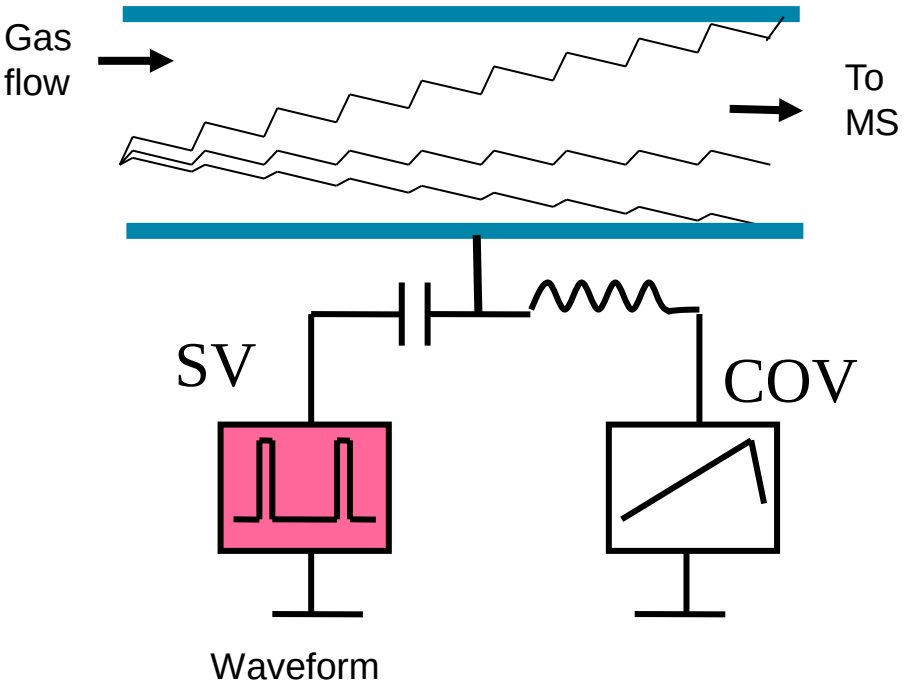
QTRAP® 5500 System with SelexION™ Technology

- Differential Mobility Spectrometry (DMS)
- Installation / removal of DMS in < 2mins – no tools required



Differential Mobility Spectrometry (DMS)

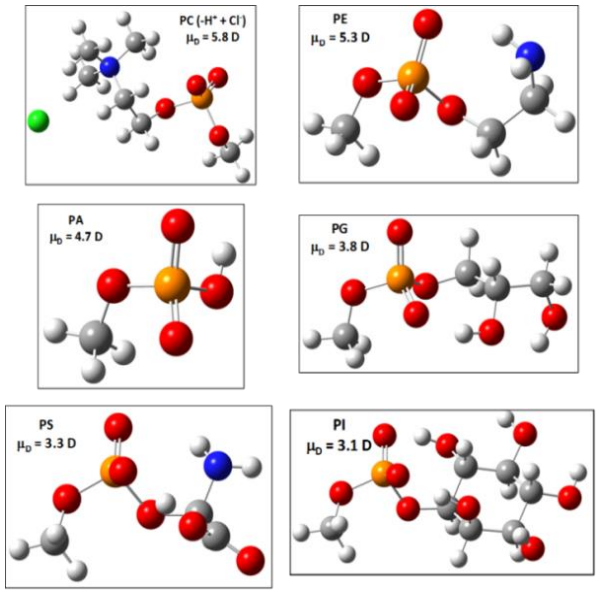
SelexION™ Technology



DMS Dimensions
1x10x30 mm

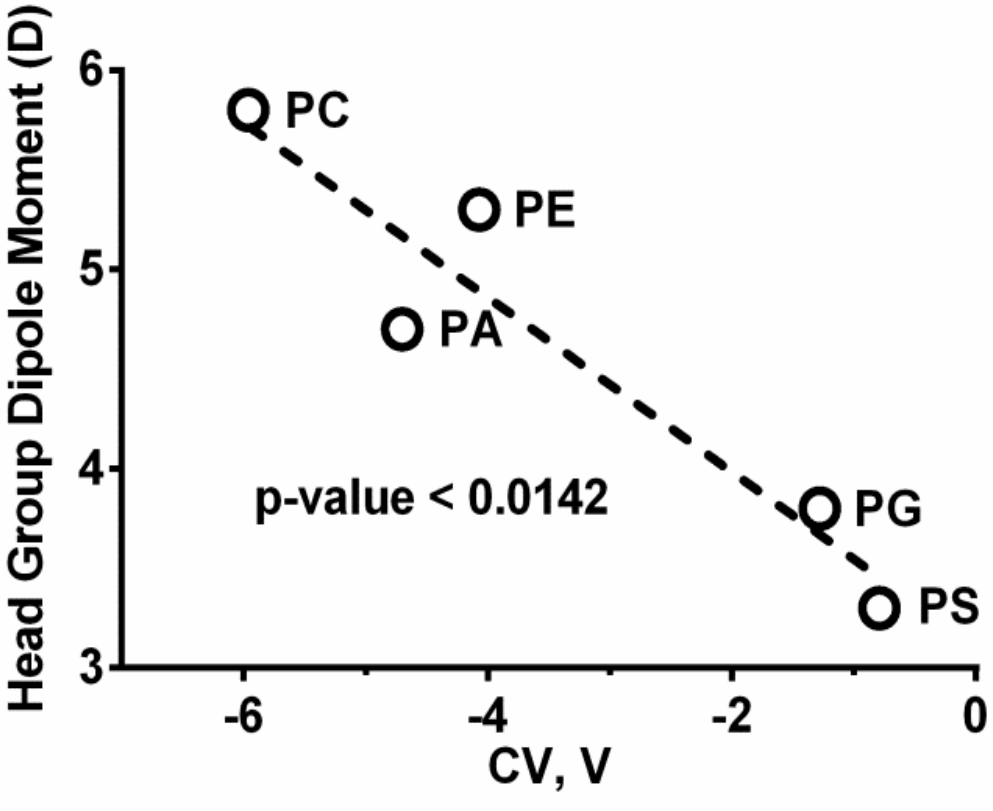
- Planar geometry
- Gas flow towards MS draws ions (transport gas)
- Asymmetric waveform applied which alternates between high field, K(E) and low field, K(0) – separation voltage (SV)
  - Moves charged ion back and forth between plates
  - Ion will have net drift base on its high and low field mobility
- Compensation voltage (COV) is small DC offset between the plates – filtering voltage

Relationship Between Dipole Moment and COV



Differential Mobility Spectrometry-Driven Shotgun Lipidomics
Anal. Chem. 2014. 86. 9662-9669
10.1021/ac5021744

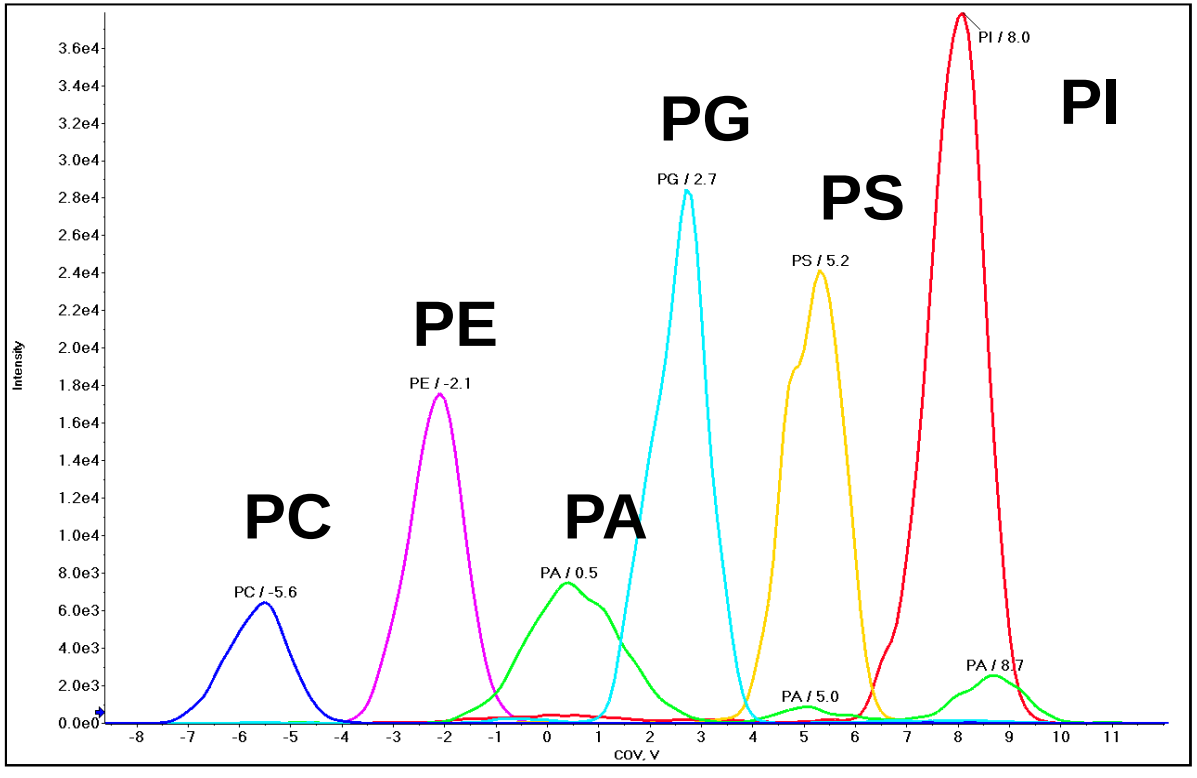
Theoretical dipole moments were calculated using isopropanol as a modifying solvent



Molecules that have different dipole moments can be separated by DMS

SelexION™ Technology Separates Phospholipid Sub-Classes

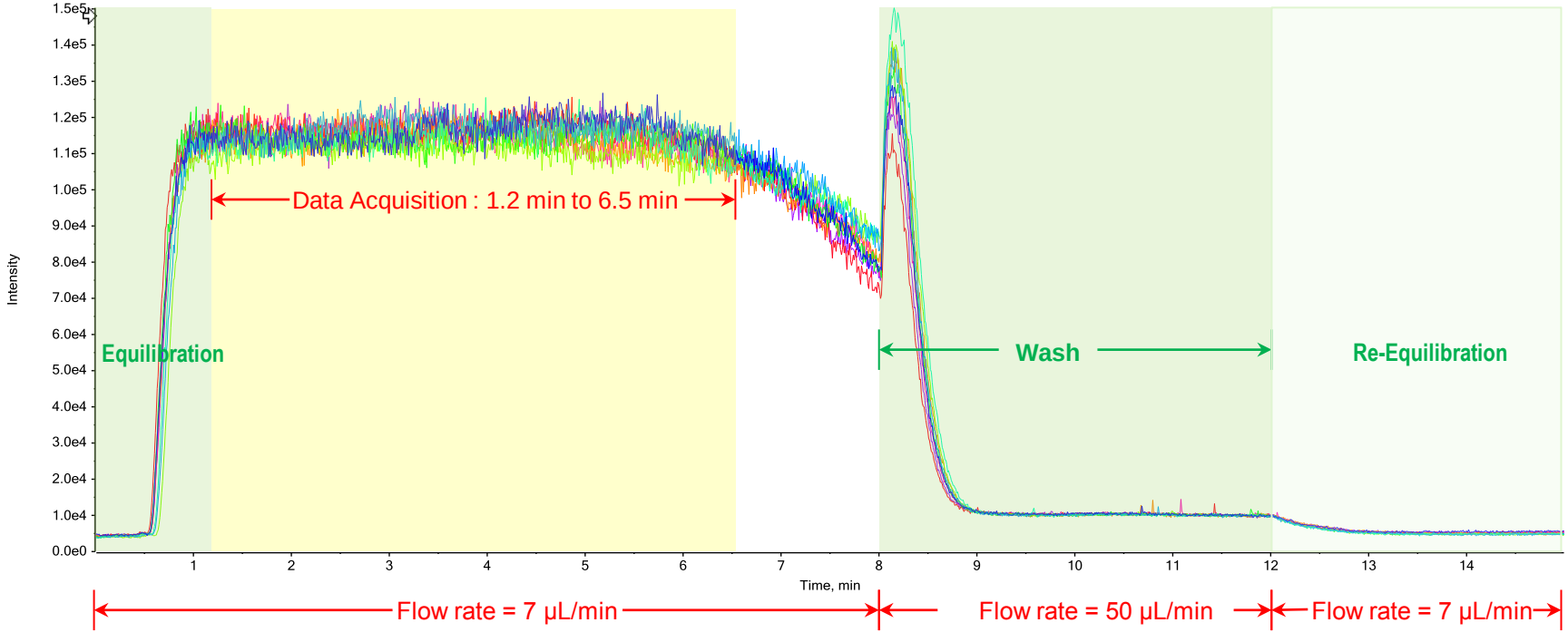
Experiment: MRM scan of 6 phospholipid standards with COV ramp



- **Using DMS alone, a mixture of lipids can be separated into it's individual components**
- **Baseline separation can be achieved, which abrogates**
- **isobaric interference**

Proof of concept: DMS separates different lipid classes
Implications: High degree of lipid class specificity without LC

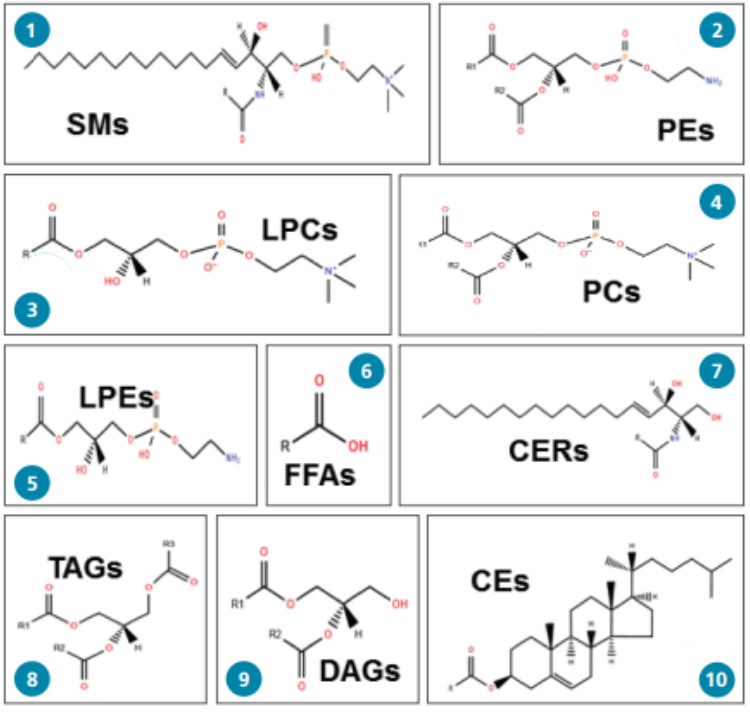
Eluting Profile and Data Acquisition Window



Why the Lipidyzer™ Platform?

Standardization of Sample Preparation

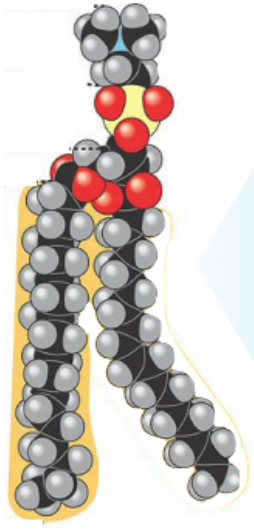
- Novel internal standard kits and methods designed exclusively for the Lipidizer™.
- Built on Metabolon's "know-how" of commercial lipid analysis platforms and standard procedures
- Provides user with confident, reproducible quantitation
- **Over 90 internal standards across ten lipid classes – a complete unique strategy!**



The Lipidyzer™ Uses a Broad Array of Internal Standards to Normalize Quantitative Data

Multiple Internal Standards that Reflect the Diversity of Lipid Molecular Species

PHOSPHATIDYLCHOLINE (PC) INTERNAL STANDARD MIX

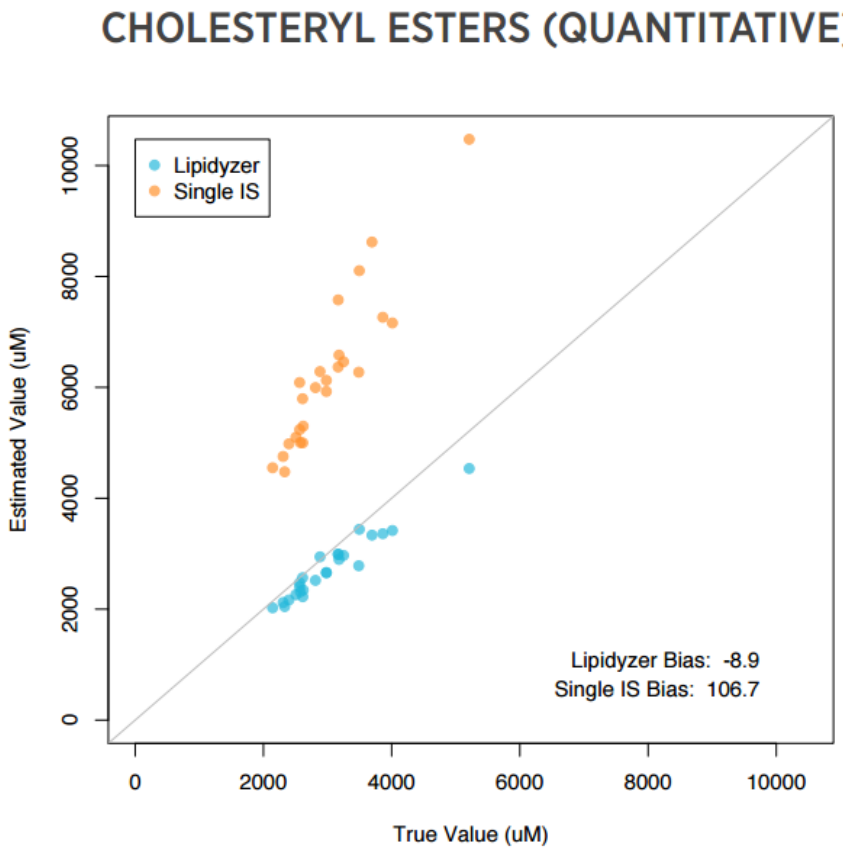


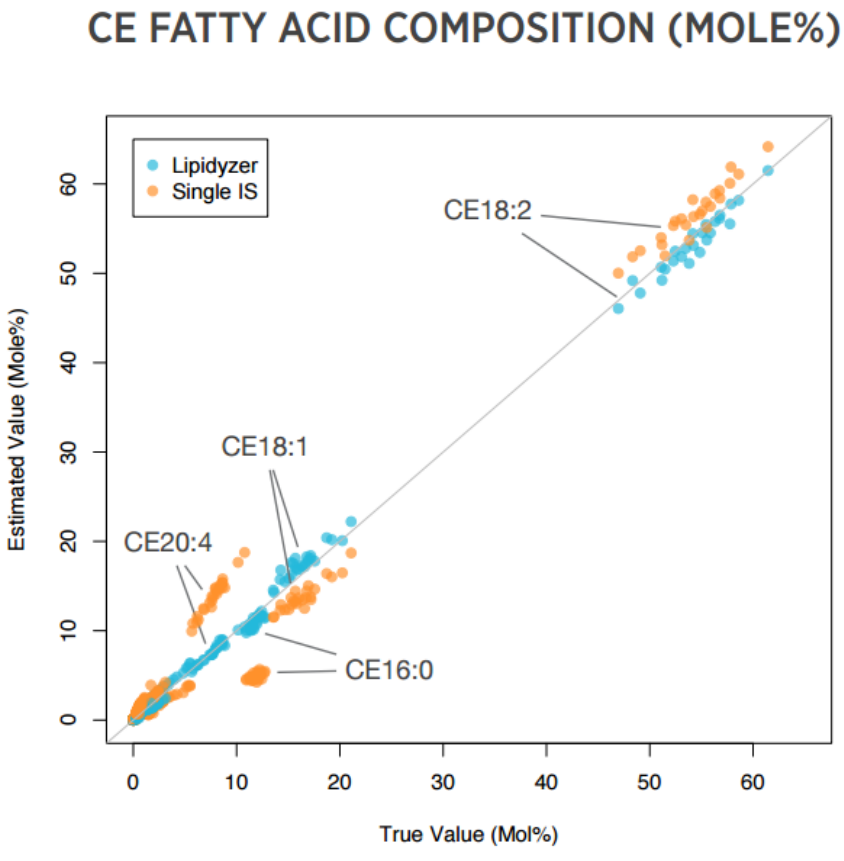
STRUCTURE	FATTY ACID	POS	%
	FA16:1 - Palmitoleic acid	sn-2	5
	FA18:1 - Oleic acid	sn-2	20
	FA18:2 - Linoleic acid	sn-2	20
	FA18:3 - α-Linoleic acid	sn-2	5
	FA20:3 - Dihomo-γ-linoleic acid	sn-2	5
	FA20:4 - Arachidonic acid	sn-2	20
	FA20:5 - Eicosapentaenoic acid	sn-2	5
	FA22:4 - Eicosatetraenoic acid	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

Each lipid class has multiple internal standards at concentrations that reflect those found in biology

The Lipidyzer™ Eliminates Quantitative Bias

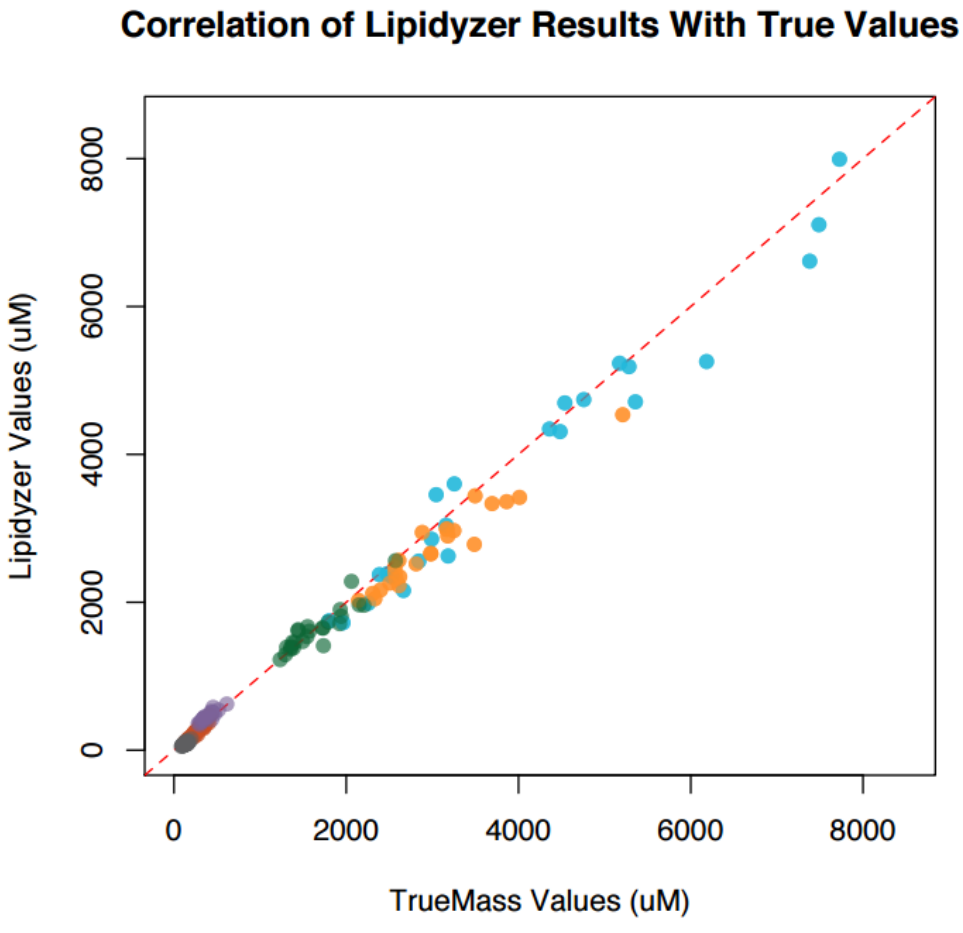
Multiple internal standards per class provide accurate quantitation





The Lipidyzer™ Generates Accurate Lipid Class Quantitation

Quantitative data with < 10% bias and ~ 5% RSD for lipid classes

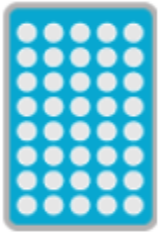


CORRELATION WITH TRUEMASS DATA

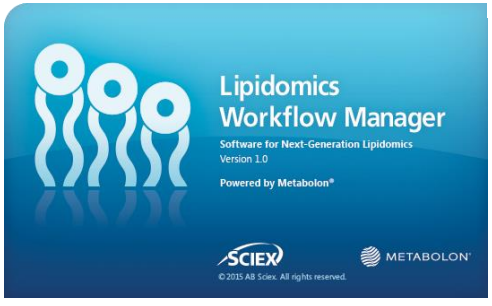
Cholesteryl Ester (CE)	0.97
Triacylglycerol (TAG)	0.98
Free Fatty Acid (FFA)	0.96
Phosphatidylcholine (PC)	0.92
Sphingomyelin (SM)	0.91
Phosphatidylethanolamine (PE)	0.87

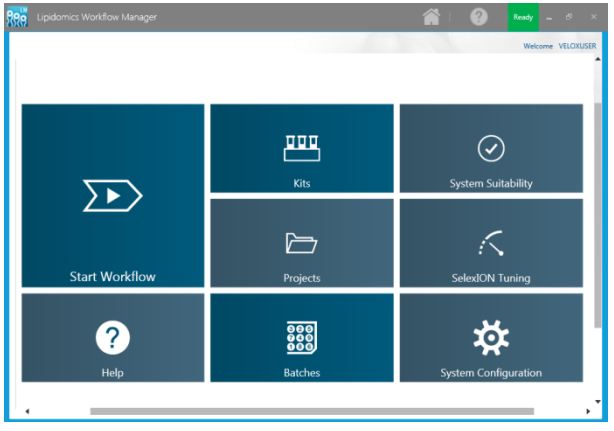
Why the Lipidyzer™ Platform?

Lipidomics Workflow Manager



- Sample login and metadata entry
- Selection of lipid class-specific methods
- Fully automated experimental design
  - Internal standard assembler allows automated calculation of volumes to add for your analysis
  - Automated templates of samples batches to ensure statistical distribution
  - Automated SelexION™ tuning and system suitability tests.
- **Controls your entire workflow**



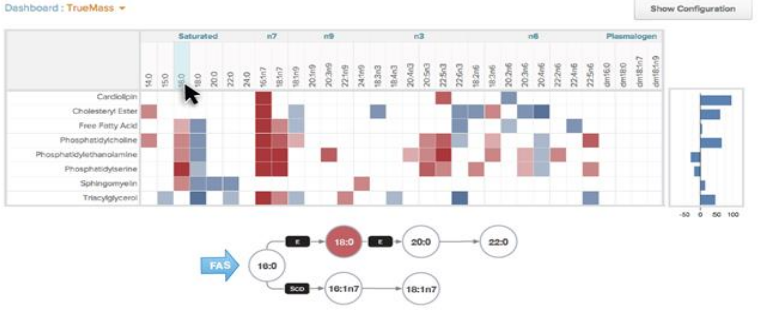


Why the Lipidyzer™ Platform?

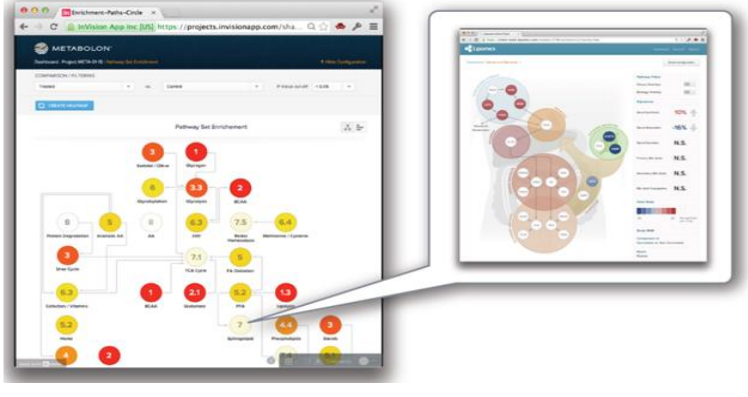
Automated Output of Results

- Data Visualization including pathway mapping, heat maps, QC charts and quantitative data tables
- Figure resolution allows direct use for publication
- Easy publishing to the cloud portal for expert data interpretation
- **True biological insights**

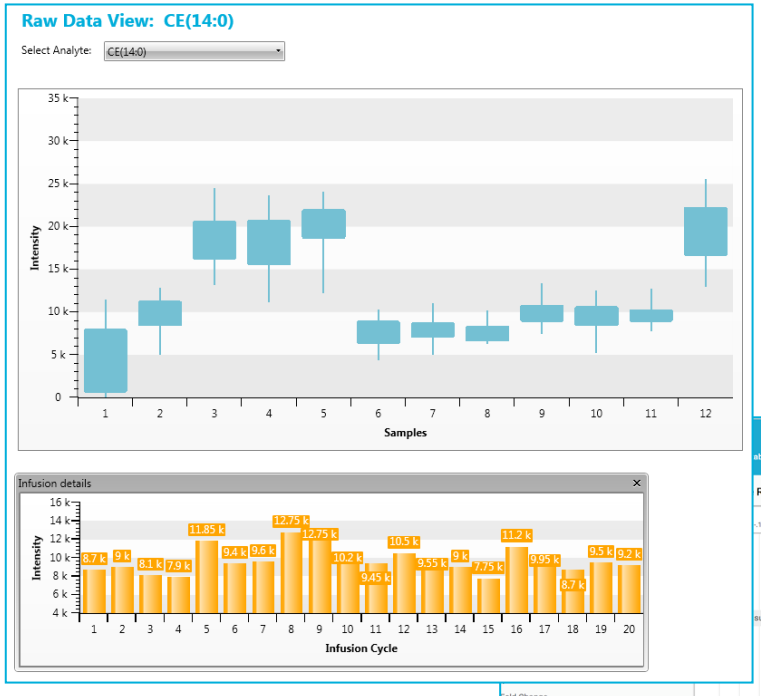
Callout plots to display contribution of species to composition



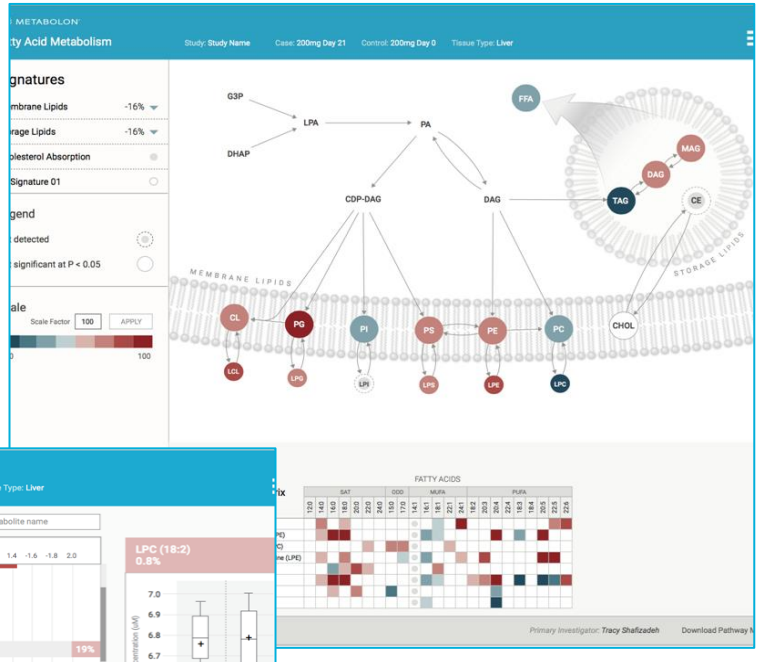
Identifying pathways contributing to effect



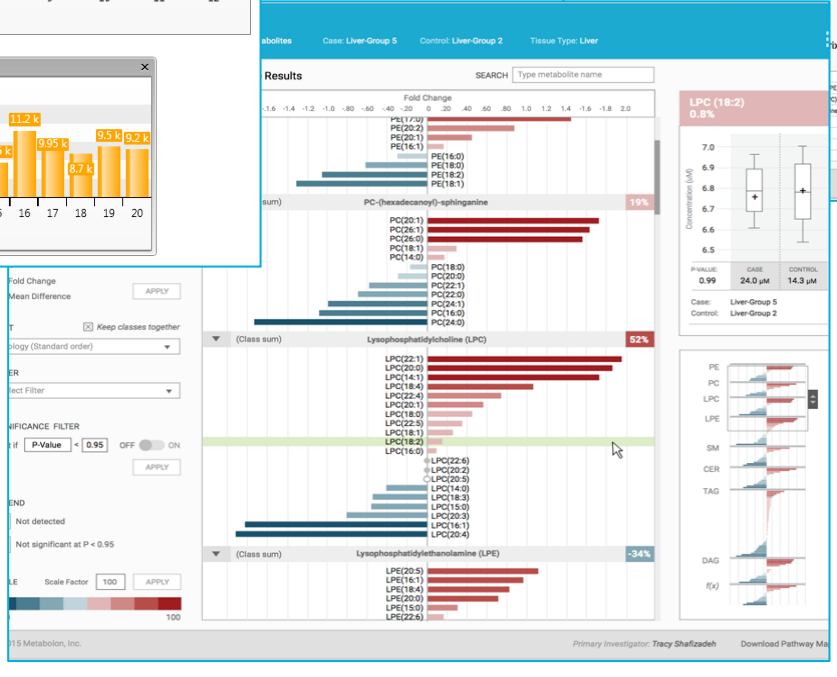
Lipidomics Workflow Manager: Data Visualization



Raw data



Pathway Mapping



Quantitative Data

Why the Lipidyzer™ Platform?

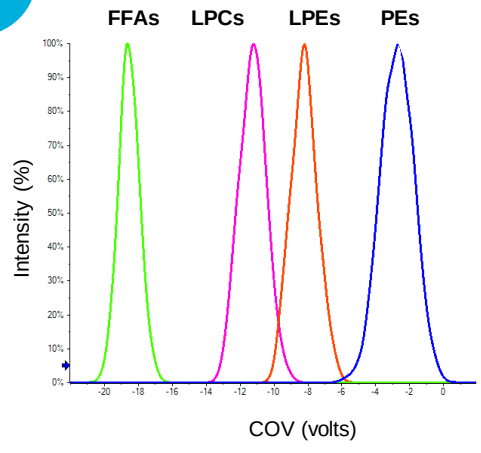
Access to Metabolon's Consulting Services

- Cloud enabled data processing and sharing
- Consulting services and study design for in-depth biological data interpretation and disease relevance.
- Expert advice on alternative matrices and sample preparation
- **Expertise at your fingertips**

Benefits of the Lipidyzer™ Platform

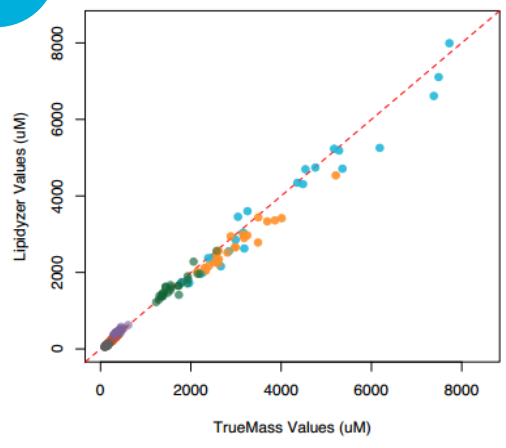
Powered by METABOLON®

1



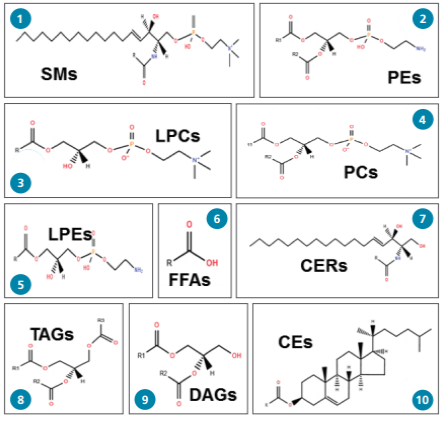
Specificity

2



Quantitation

3



Coverage

Acknowledgements

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Legal Acknowledgements:

Appendix