SCIEX

Answers for Science.
Knowledge for Life.™

Obtaining Answers to Biological Questions – Sample Prep to Data Analysis

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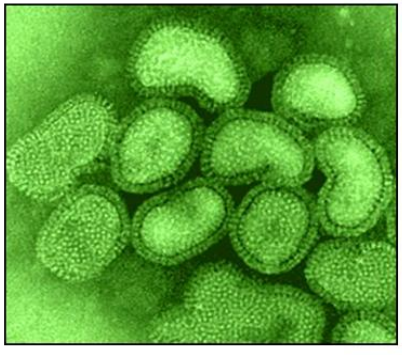
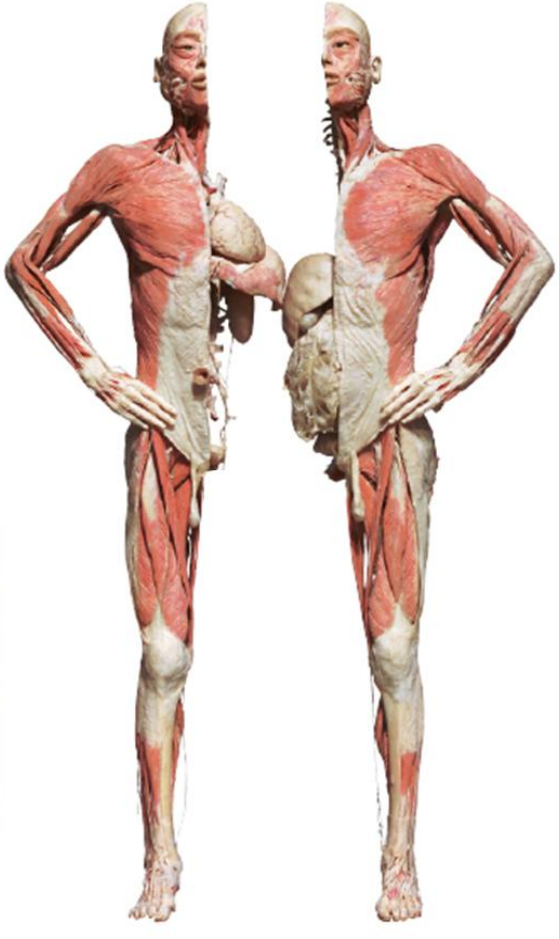
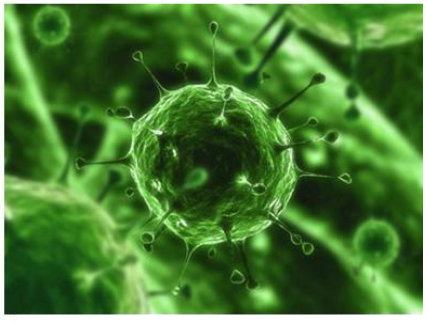
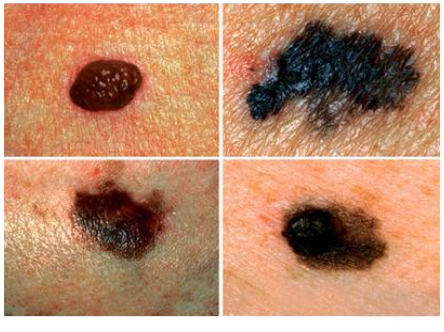
Advanced Workflow Specialist in OMICS

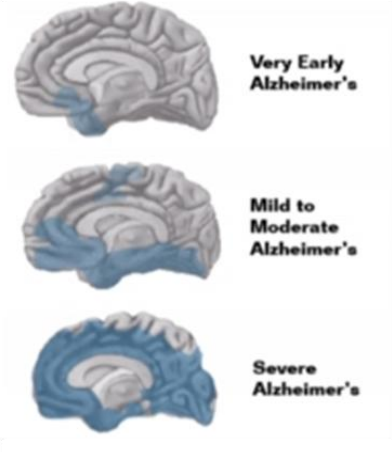
OMICS Research











http://www.luxor.com/entertainment/bodies.aspx

Bodies Exhibit

SCIEX Metabolomics \ Lipidomics Workflows

Discovery | **Quantitative Targeted Profiling** | **Clinical Utilization**

TripleTOF®Platforms

AL LV MV

IDA MSMS

SWATH™ Global Discovery

(MS/MS^{ALL}) for Lipids

XCMS plus

QTRAP® Platforms

AL LV MV

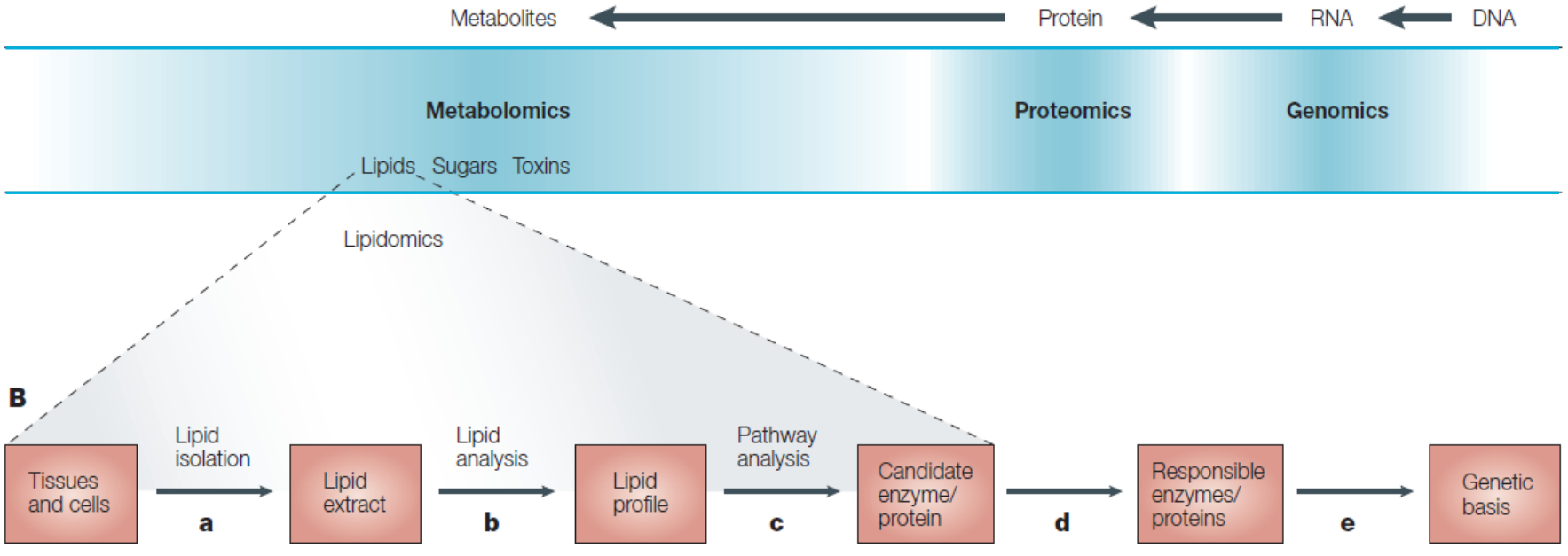
Global Discovery (MPIS)

The Lipidizer™ Platform

(Full Workflow)

Today's Story – Lipidomics

Lipidomics — A Part of the Omics Spectrum

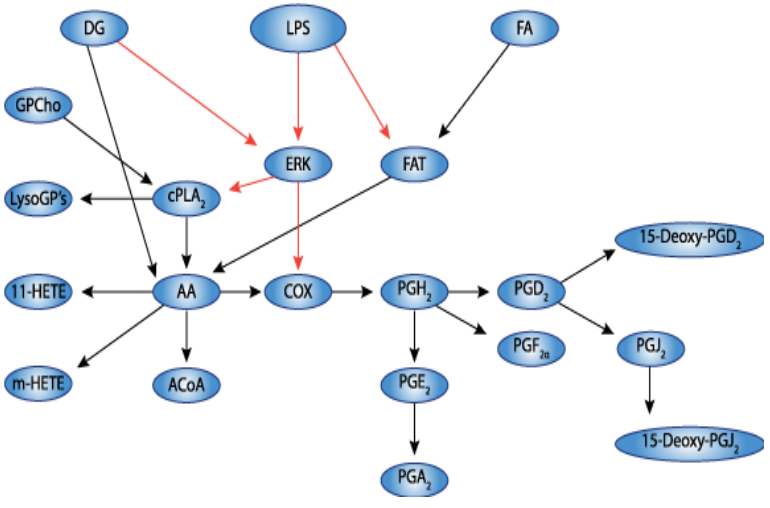


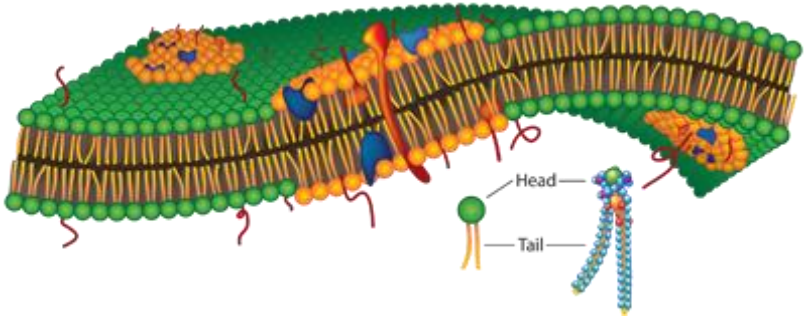
Wenk et al. Nature 2005

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





Why's That Potato Crisp So Tasty?

Nutrition Facts

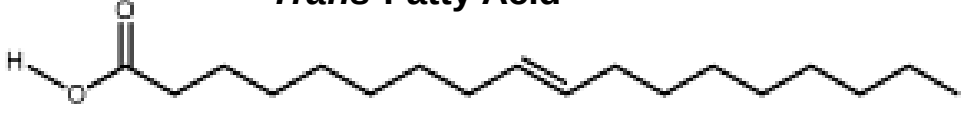
Serving Size 1 oz (28g/About 15 chips)

Amount Per Serving	
Calories 160	Calories from Fat 90
	% Daily Value*
Total Fat 10g	**16%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 170mg	**7%**
Potassium 350mg	**10%**
Total Carbohydrate 15g	**5%**
Dietary Fiber 1g	**5%**
Sugars less than 1g	
Protein 2g	

Total Fat 10g	**16%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	

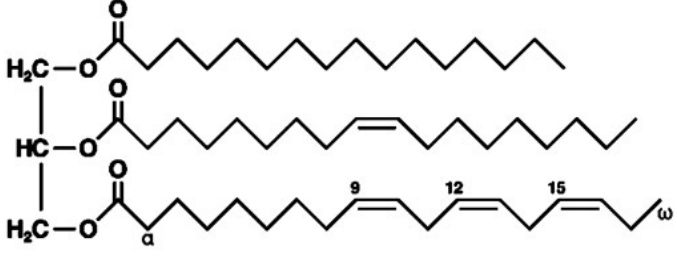
Saturated Fatty Acid

***Trans*-Fatty Acid**



Raise the level of LDL ('bad' cholesterol) and promote CVD

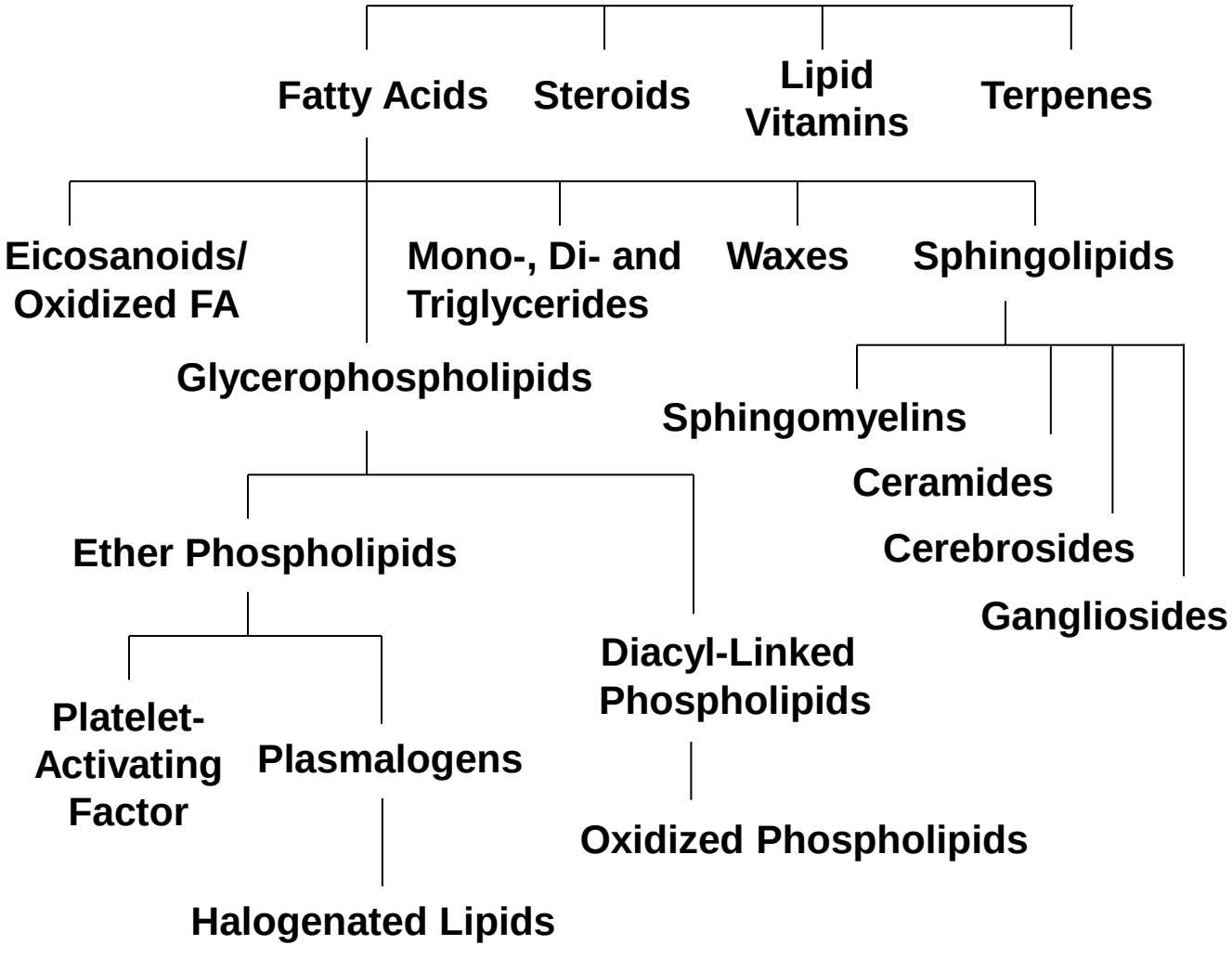
Triglyceride



Clinical studies have shown that the type and position of the fatty acyl substituents of TAGs play an essential role in lipid digestion, absorption and metabolism

Lipidomics

- Comprised of multiple, distinct structural lipid classes



Lipids play an essential role in human physiology:

- Metabolic homeostasis
- Cell and organelle structure
- Cell signaling

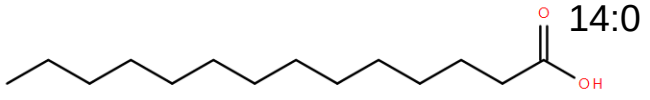
And disease:

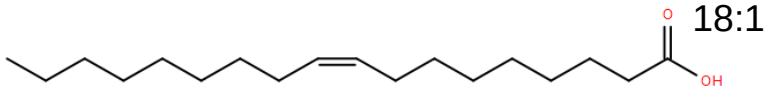
- Inflammation
- Cancer
- Cardiovascular disease
- Diabetes
- Inflammatory bowel disease
- Neurological diseases

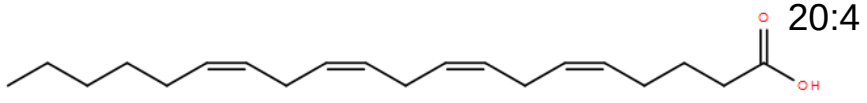
Short List of Must Know Common Fatty Acids

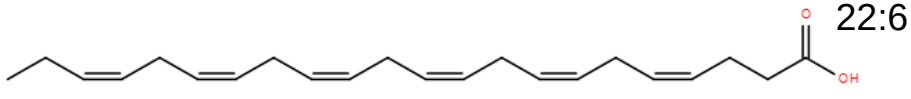
Common Name	Carbons: Double Bonds	ES(-) m/z
Myristic Acid	14:0	227.2
Palmitic Acid	16:0	255.2
Stearic Acid	18:0	283.2
Oleic Acid	18:1	281.2
Linoleic Acid	18:2	279.2
Linolenic Acid	18:3	277.2
Arachidonic Acid	20:4	303.2
Eicosapentenoic Acid	20:5	301.2
Docosapentenoic Acid	22:5	329.3
Docosahexenoic Acid	22:6	327.3

Structural Examples



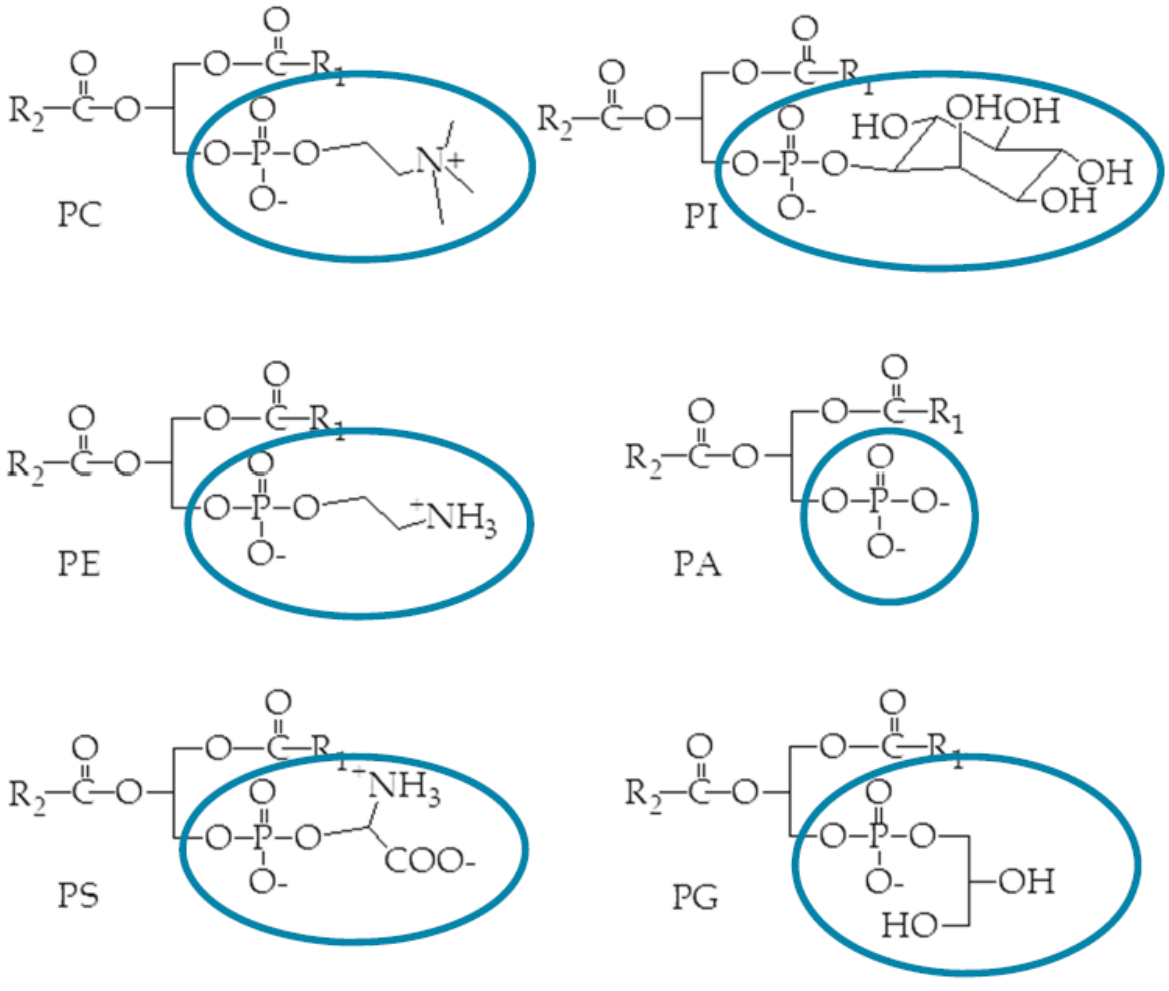




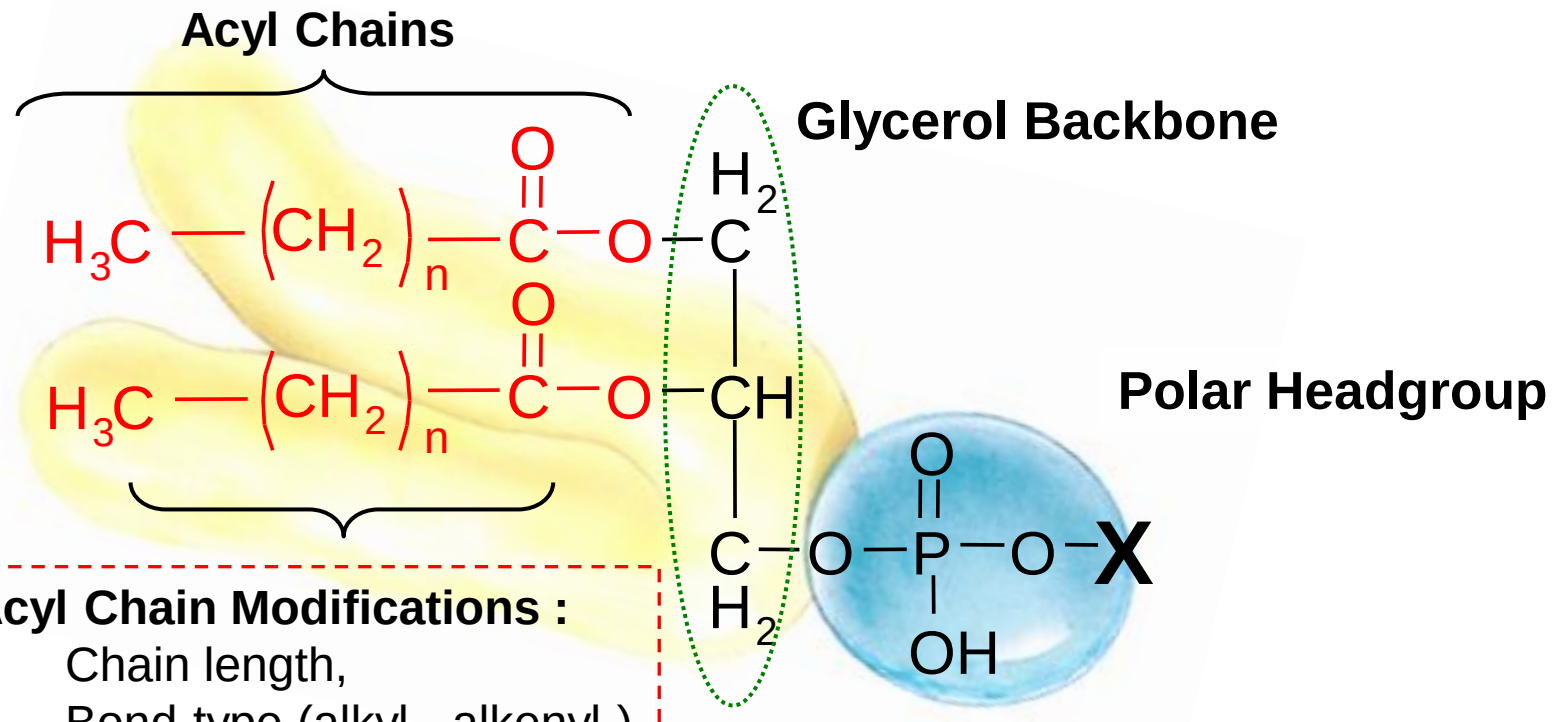


Common Phospholipids

Phospholipid (PL) Sub-Classes



Diversity of Phopholipid Molecular Species



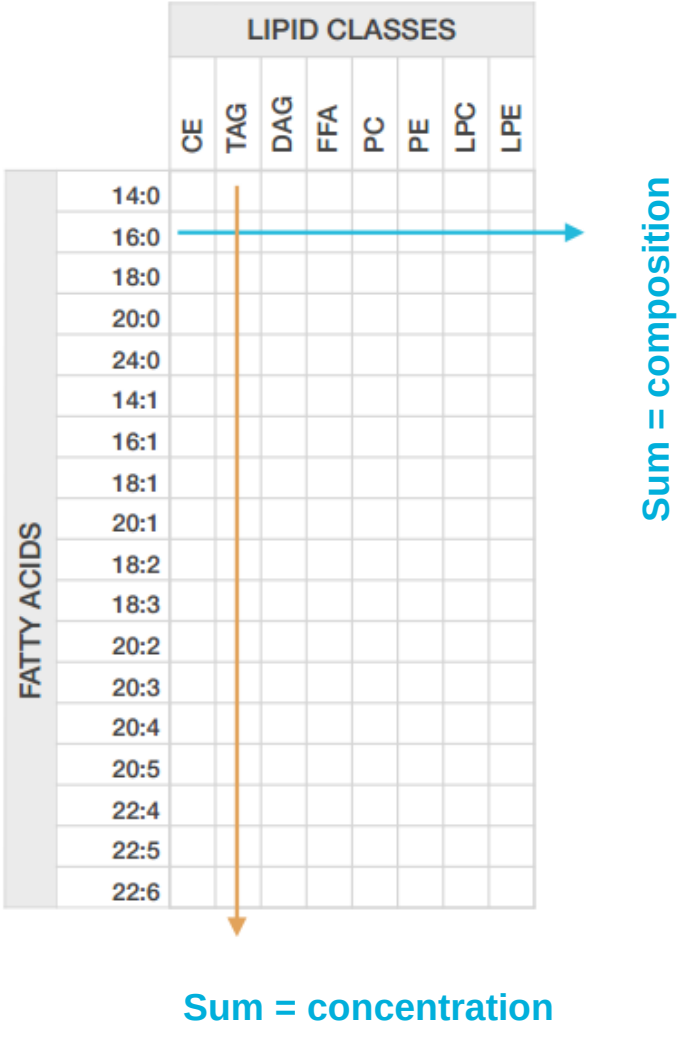
Acyl Chain Modifications :

- Chain length,
- Bond-type (alkyl-, alkenyl-)
- Double bonds,
- Methylation,
- Hydroxylation,
- Epoxidation
- Cyclation
- Conjugation

X=PA, PG, PE, PC, PS, PI, PIP, PIP_2...

Complex Lipids are like a Matrix

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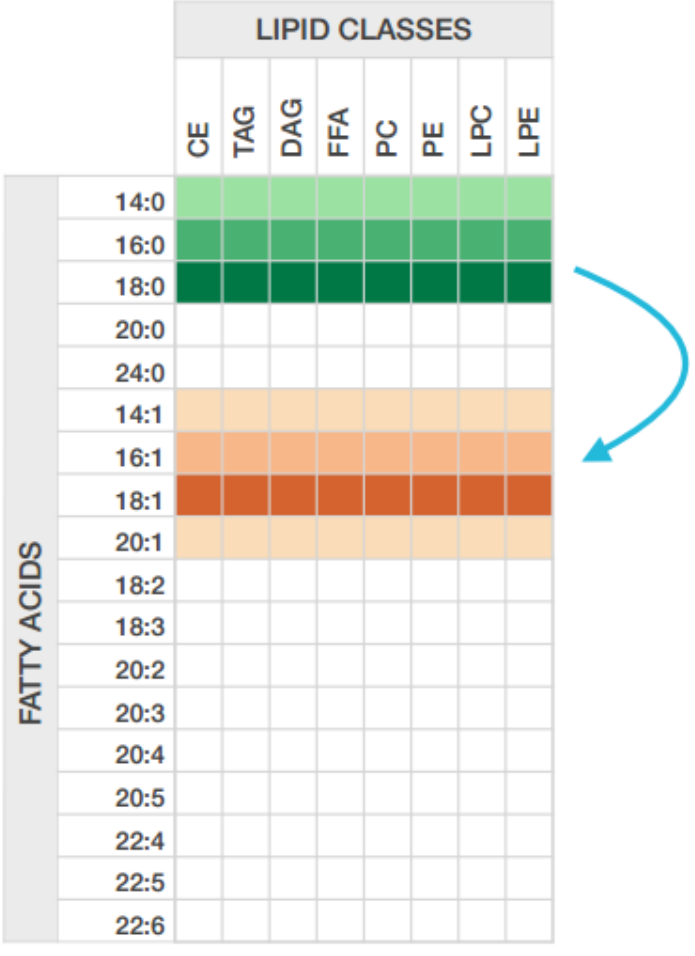
- When FA metabolism is altered there is the ability to change FA composition of all classes



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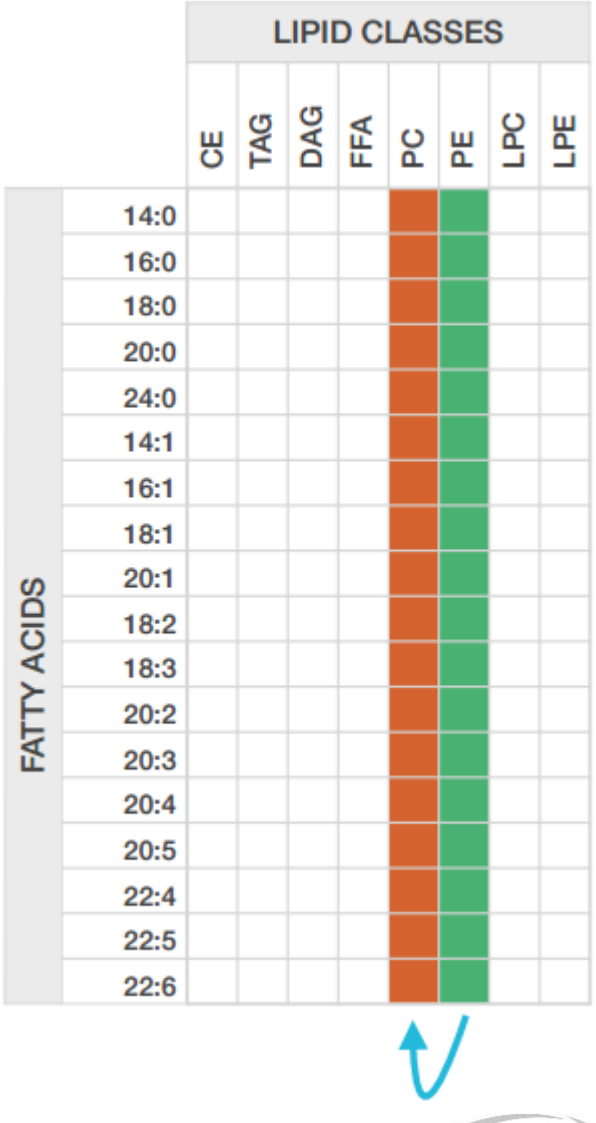
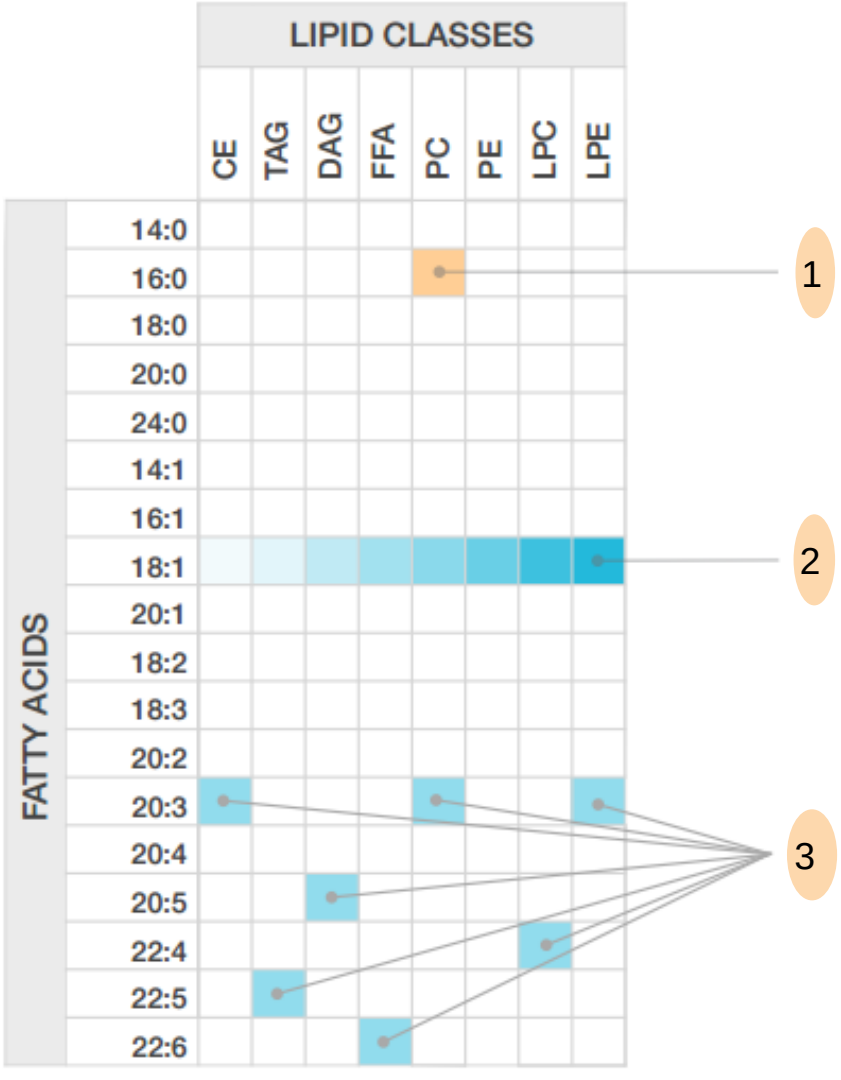
  - Concentration = sum of the FAs for any given class (column)
  - Composition = relative abundances of each FA (or species) across many classes (rows)
- When FA metabolism is altered there is the ability to change FA composition of all classes
- When lipid class metabolism is altered there is the ability to change all members of the class



What is needed from a Quantitative 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

Putting it All Together
Sample Prep to Answers

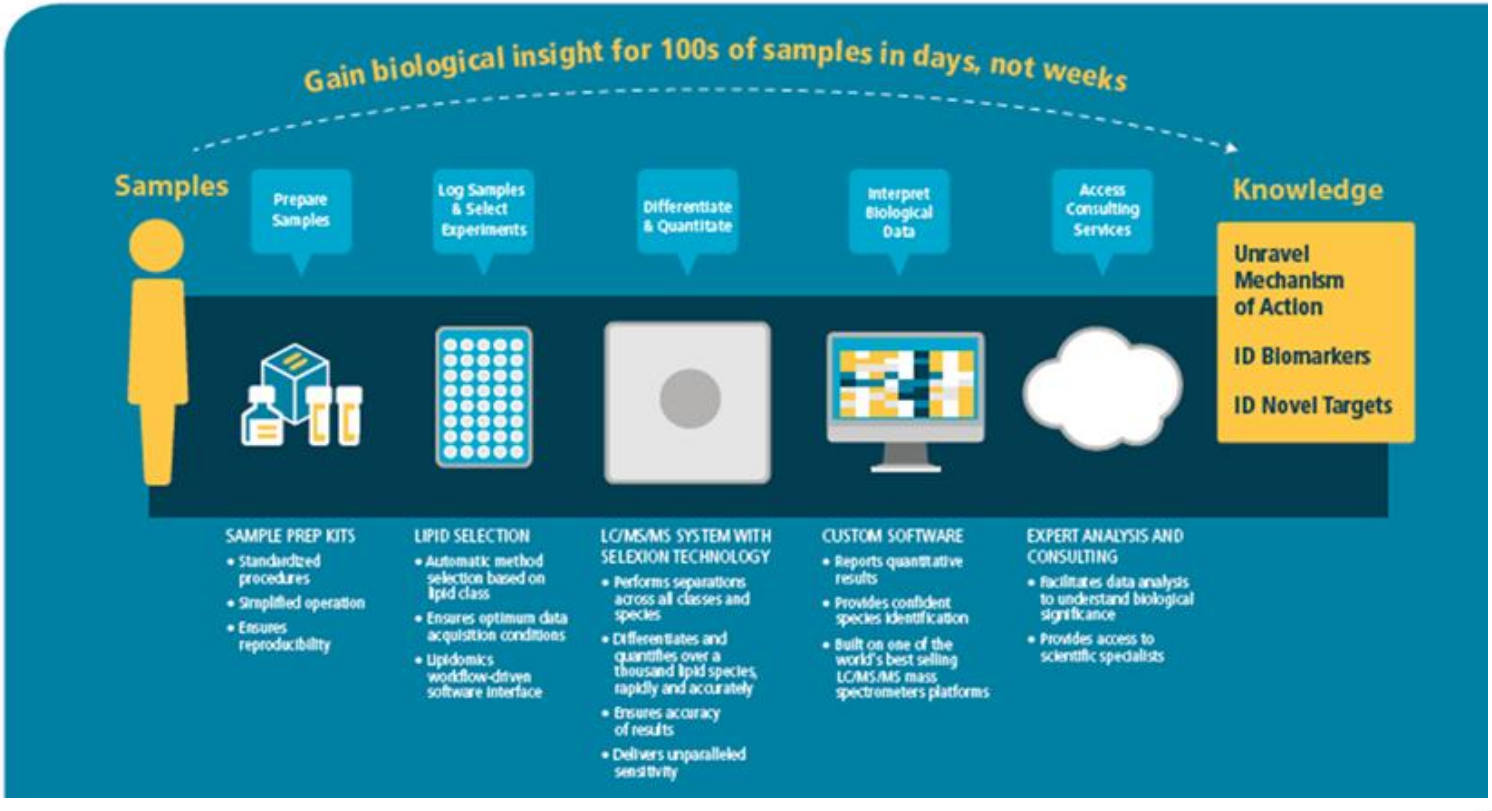
Simplifying the Complexity of Lipidomics and Multiple Steps from Sample Preparation to Knowledge

Powered by METABOLON®

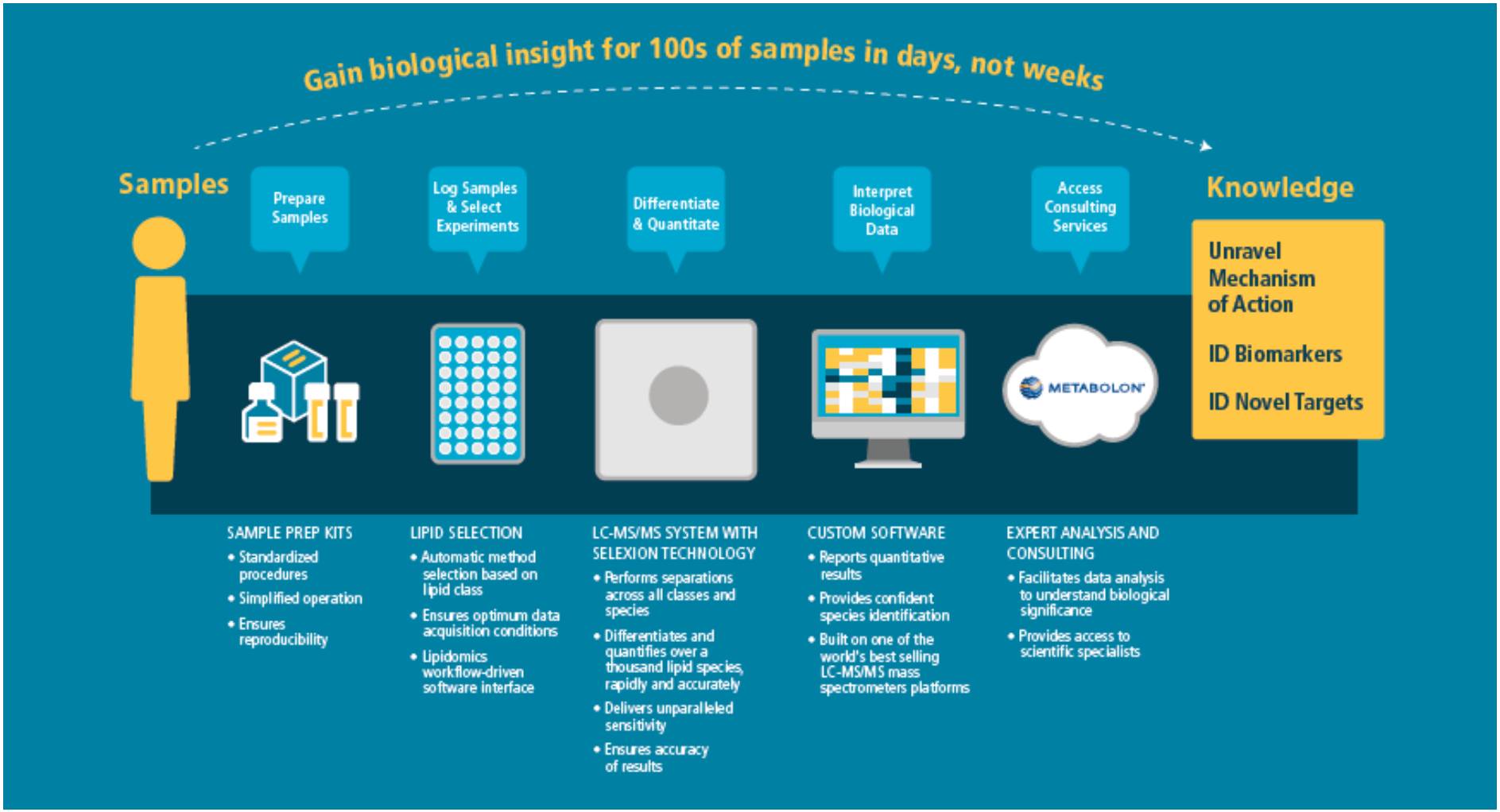
One Streamlined Workflow for Peak Performance

Work faster and smarter

Lipidyzer Platform makes lipid analysis easy and seamless. The simple, integrated workflow allows you access to comprehensive data, quickly and confidently, while expert analysis services provide you with the the assurance to gain accurate biological insight.



Simplifying the Complexity



Analytical Challenges in Lipidomic Analysis

The Lipidyzer™ developed to address specificity and quantitative rigor

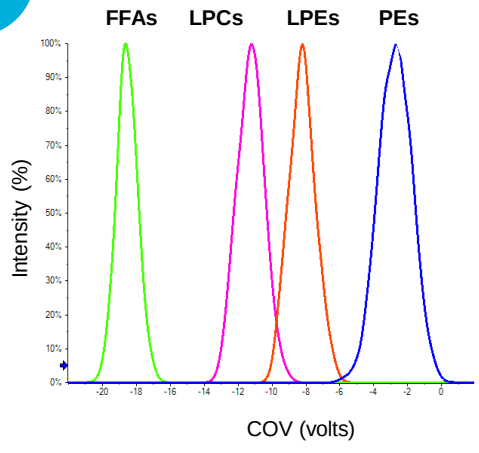
- Specificity:
  - Resolve isobaric interference between different lipid classes
  - Determine lipid class and molecular species composition in a single run

- Quantitation:
  - Ensure spray stability
  - Minimize carryover
  - Neutralize quantitative bias

Benefits of the Lipidyzer™ Platform

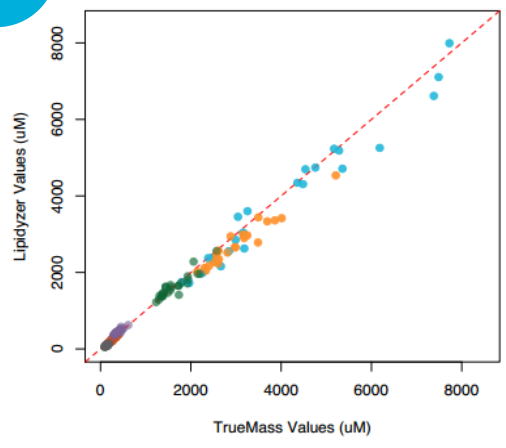
Powered by METABOLON®

1



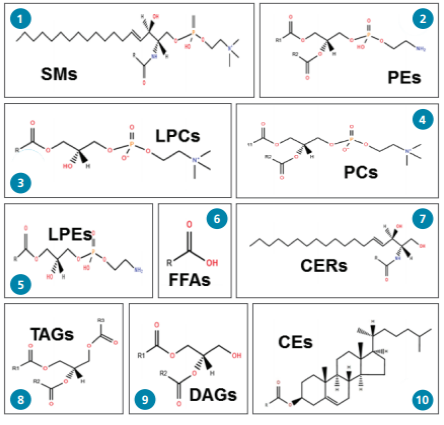
Specificity

2



Quantitation

3

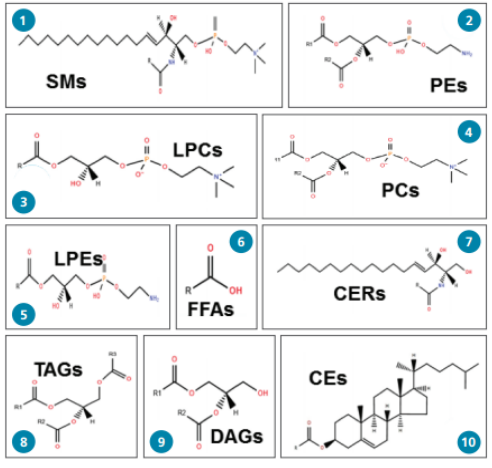


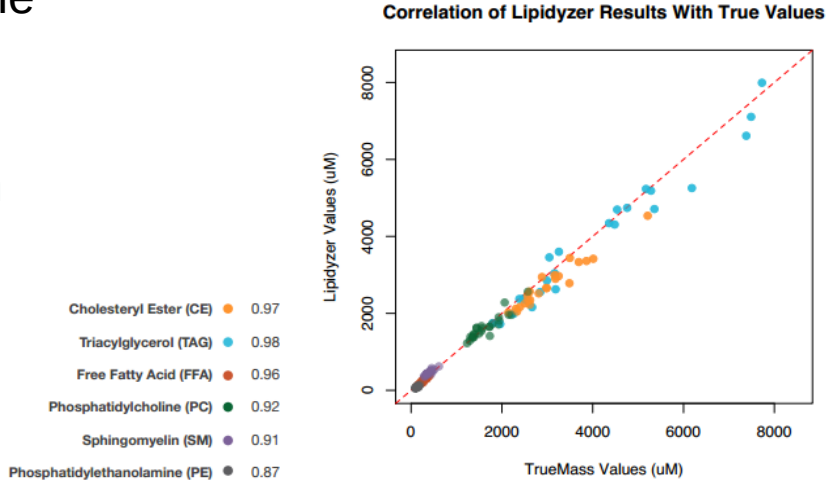
Coverage

Why the Lipidyzer™ Platform? – Standards

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!**





Sample Prep: Lipid Extraction Methods

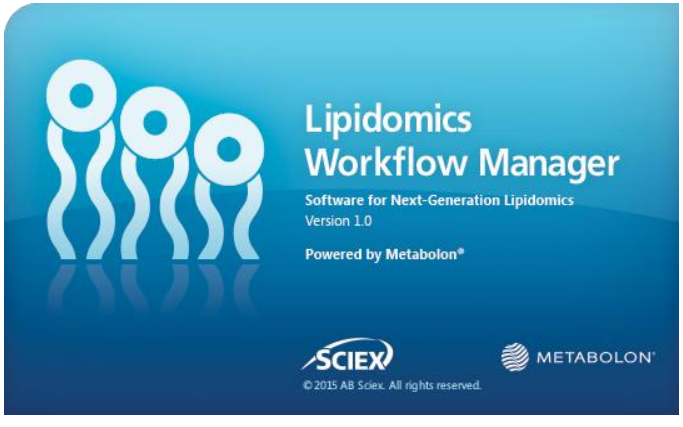
- Primary source of error in lipid analysis
  - Internal standards help, but manual process can contributes to a high %CV
- Classic techniques: Methods of Bligh and Dyer and Folch
  - **Bligh and Dyer**: 1 Part aqueous (sample), 2 parts MeOH, 0.9 part CH_2Cl_2; Vortex (except plasma and brain—gently invert sealed test tube to avoid emulsion); Add 1 part H_2O, 1 part CH_2Cl_2; 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 MeOH/CH_2Cl_2; Vortex; Add 4 parts H_2O (or 0.9% NaCl); Vortex; Centrifuge (1200 rpm x 10 min); Take lower layer and evaporate solvent; Re-suspend in appropriate solvent for injection

CH_2Cl_2 will extract plasticizers; always use glass

Why the Lipidyzer™ Platform? – Software

Lipidomics Workflow Manager

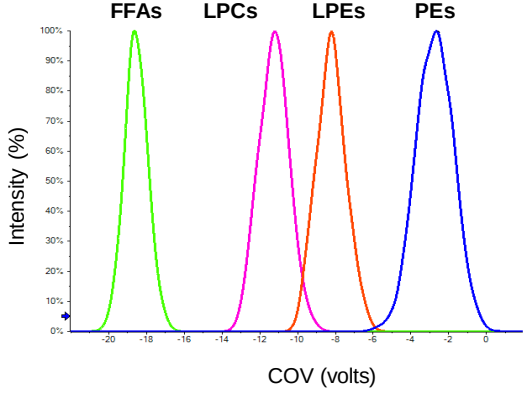
- Sample login and metadata entry
- Selection of lipid class-specific methods
- Fully automated experimental design
  - Internal standard assembler allows automated calculation of volumes to add for your analysis
  - Automated templates of samples batches to ensure statistical distribution
  - Automated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



Why the Lipidyzer™ Platform? – Measurement

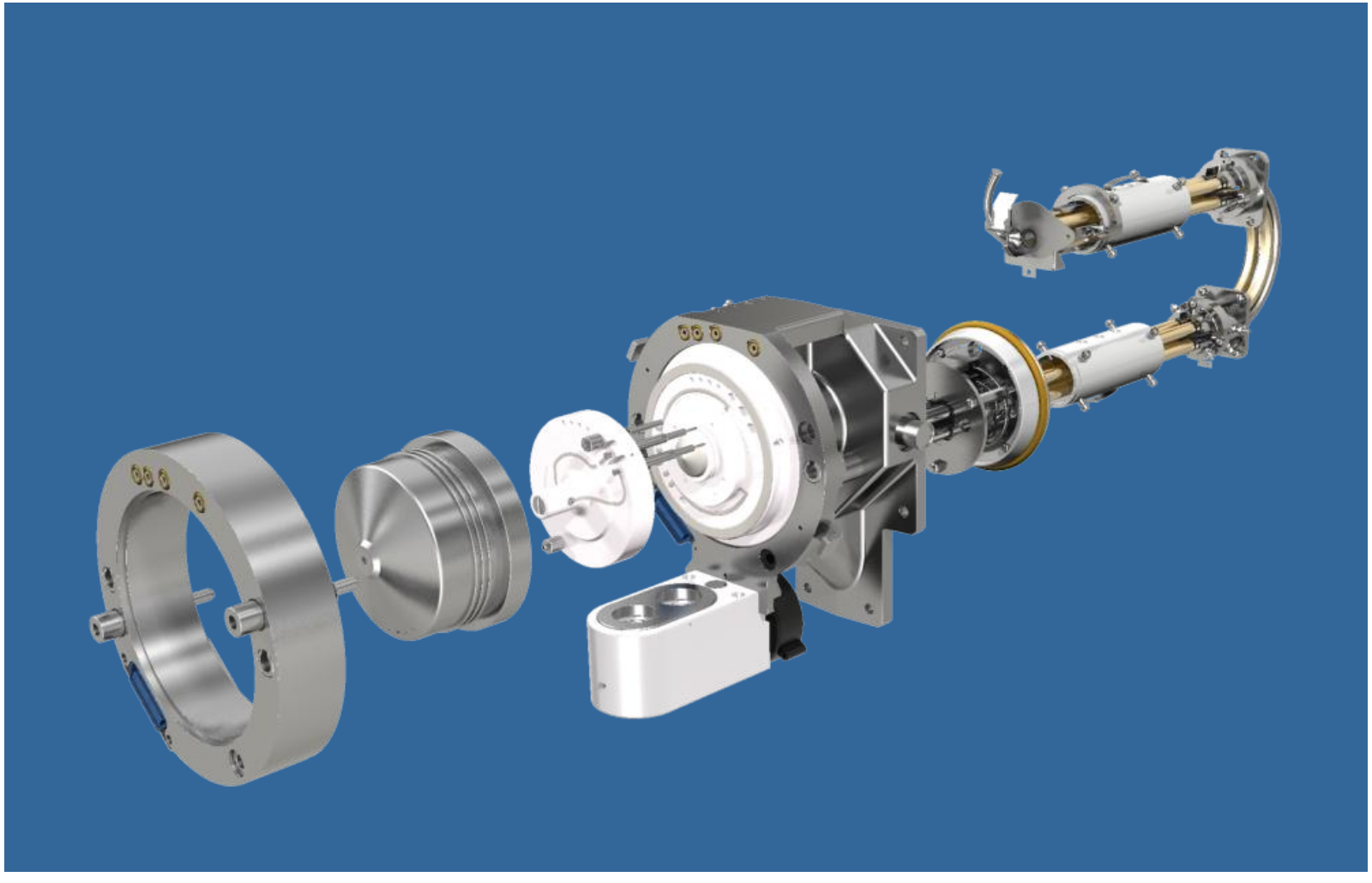
QTRAP® 5500 & SelexION™ Technology

- A complete package for lipidomics analysis
- Robust and trusted quantitative mass spectrometry platform the 5500 QTRAP®
- Ensure the highest level of data reproducibility using the new SCIEX branded ExionLC System.
- Perform most confident separation of isobaric lipid species using **SelexION™ Differential Mobility Separation Technology**



Lipidyzer™ Hardware Configuration

5500 QTRAP® System with SelexION™ Technology



Challenges in Lipidomic Analysis: Isobaric Overlap

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

PE(18:3(9Z,12Z,15Z)/20:4(5Z,8Z,11Z,14Z))	1-(9Z,12Z,15Z-octadecatrienoyl)-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-glycero-3-phosphoethanolamine	$C_{43}H_{72}NO_8P$	761.50
PE(18:4(6Z,9Z,12Z,15Z)/20:3(8Z,11Z,14Z))	1-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-2-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3-phosphoethanolamine	$C_{43}H_{72}NO_8P$	761.50
PE(20:0/17:0)	1-eicosanoyl-2-heptadecanoyl-glycero-3-phosphoethanolamine	$C_{42}H_{84}NO_8P$	761.59
PE(20:3(8Z,11Z,14Z)/18:4(6Z,9Z,12Z,15Z))	1-(8Z,11Z,14Z-eicosatrienoyl)-2-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-glycero-3-phosphoethanolamine	$C_{43}H_{72}NO_8P$	761.50
PE(20:4(5Z,8Z,11Z,14Z)/18:3(6Z,9Z,12Z))	1-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-2-(6Z,9Z,12Z-octadecatrienoyl)-glycero-3-phosphoethanolamine	$C_{43}H_{72}NO_8P$	761.50
PE(20:4(5Z,8Z,11Z,14Z)/18:3(9Z,12Z,15Z))	1-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-2-(9Z,12Z,15Z-octadecatrienoyl)-glycero-3-phosphoethanolamine	$C_{43}H_{72}NO_8P$	761.50
PE(20:5(5Z,8Z,11Z,14Z,17Z)/18:2(9Z,12Z))	1-(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl)-2-(9Z,12Z-octadecadienoyl)-glycero-3-phosphoethanolamine	$C_{43}H_{72}NO_8P$	761.50
PE(21:0/16:0)	1-heneicosanoyl-2-hexadecanoyl-glycero-3-phosphoethanolamine	$C_{42}H_{84}NO_8P$	761.59

Showing page 1 of 3 **Results:** 1 2 3 Next Showing results 1 to 50 of 108

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

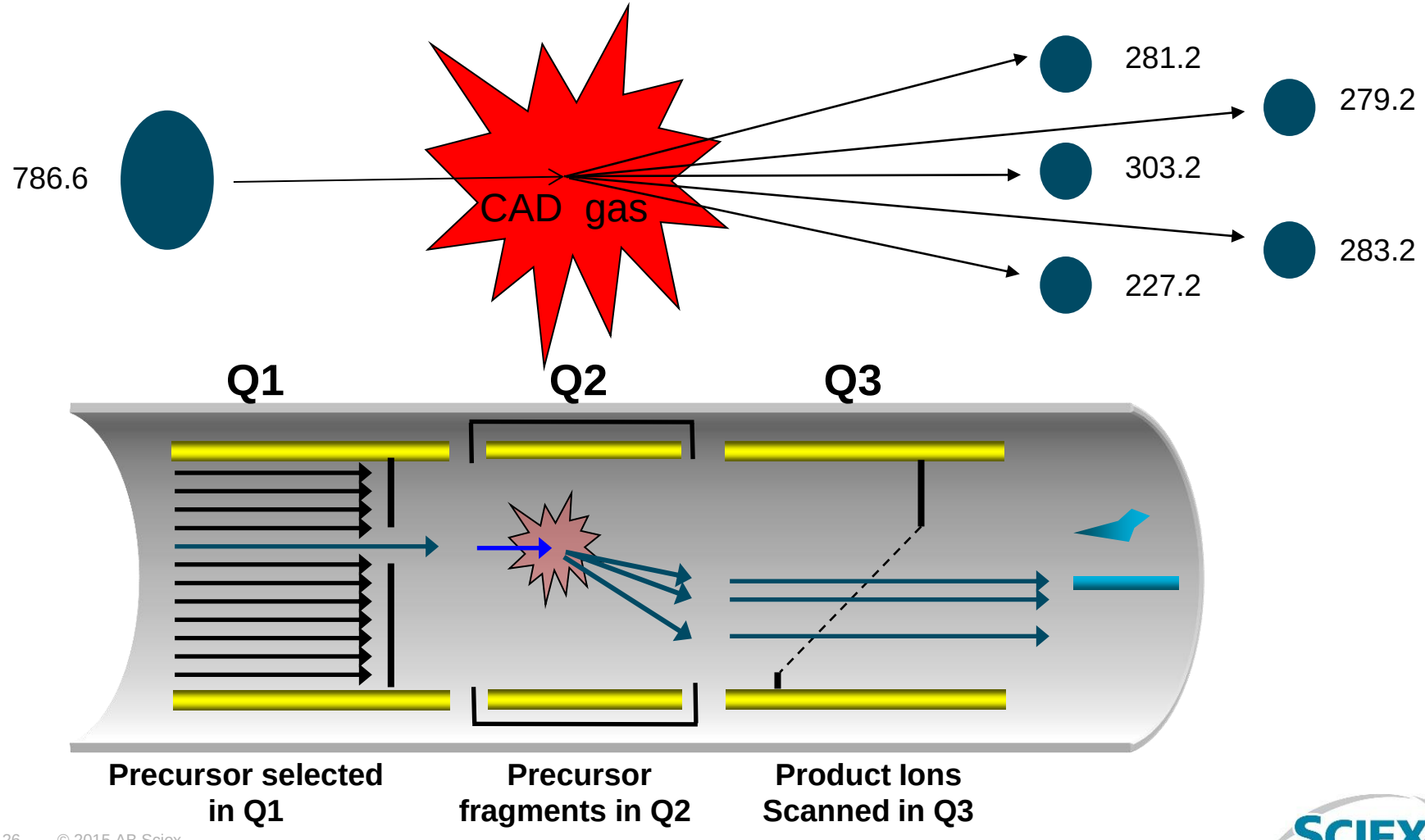
LIPIDMAPS Calculator exercise:
Select mass of 762.4 with a tolerance
of 1.0 amu

108 Lipids identified

The ambiguous data make it very difficult to use MSMS spectra to positively identify a particular molecular species and makes it nearly impossible to accurately quantitate that molecule

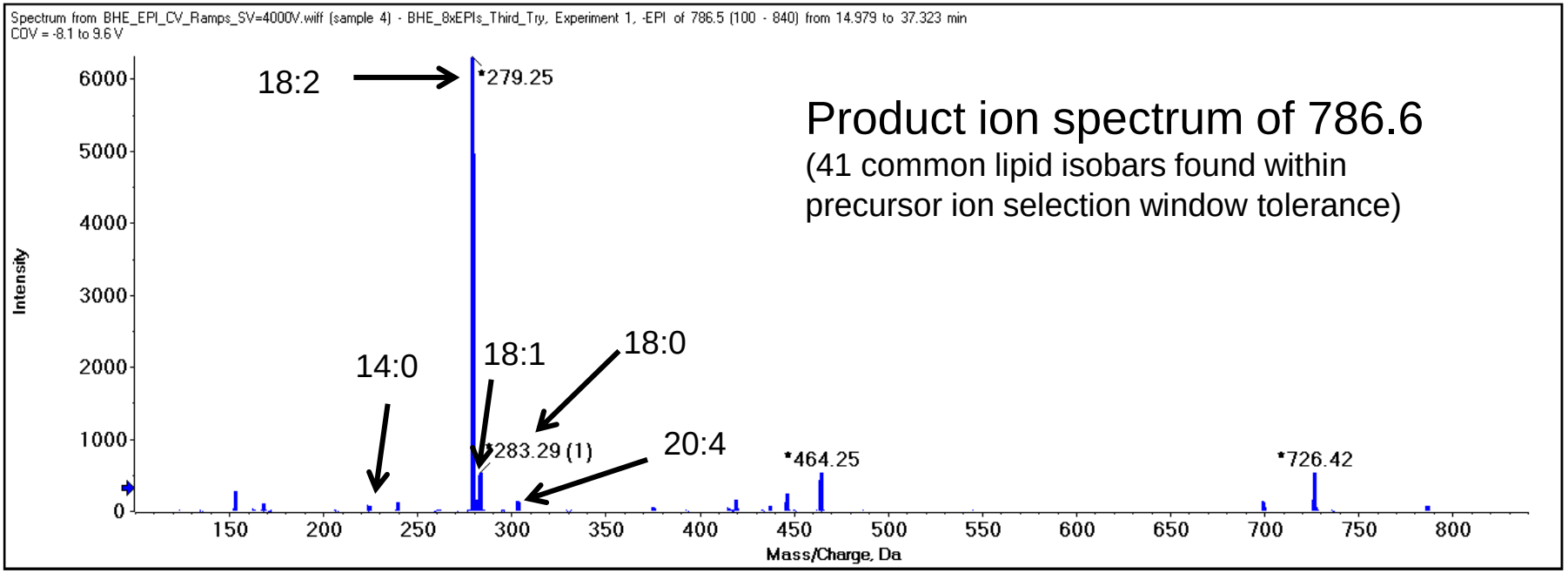
Qualitative Analysis of Lipids – Measurement

Negative ion mode MS/MS fragmentation pattern denotes fatty acid composition of complex lipids



Product Ion Analysis of Lipids

Experiment: Product ion spectrum (EPI) of bovine heart extract (m/z 786.5); no DMS

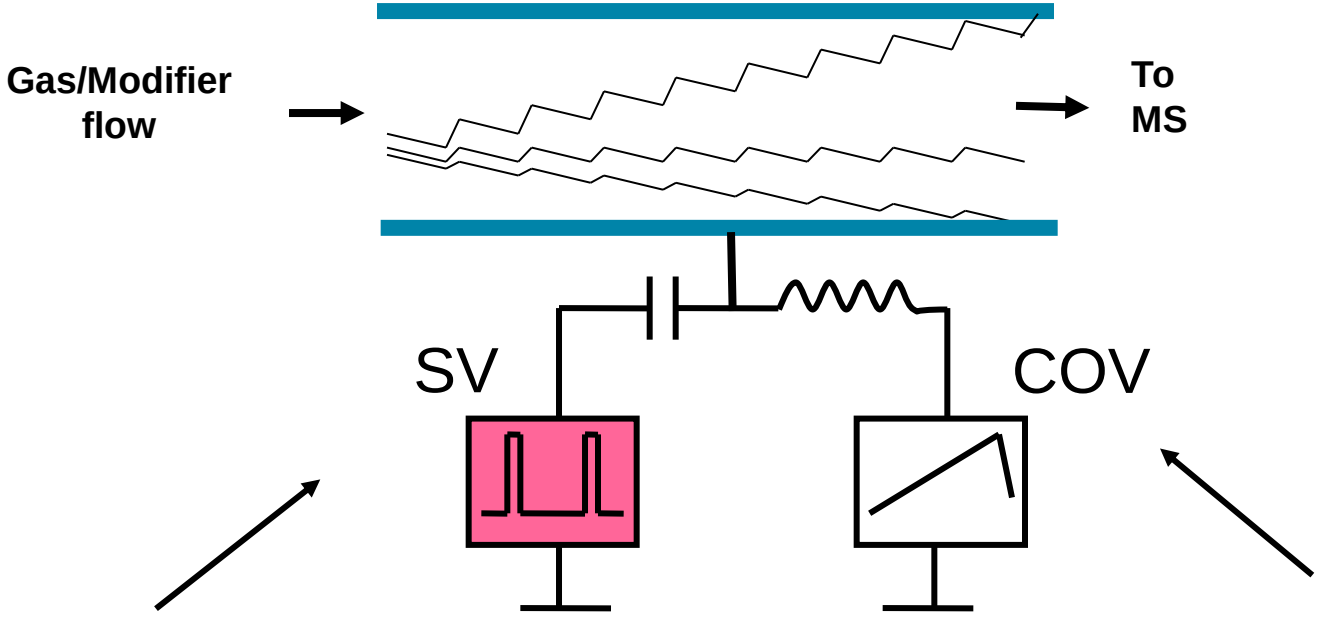


Multiple fatty acid peaks are visible in the spectrum; no clearly identified precursor molecule is apparent from this spectrum

Many lipid isobars share the same fragment ions

How Does SelexION™ Technology Separate Ions?

Differential Mobility Spectrometry (DMS) separates molecules using planar geometry

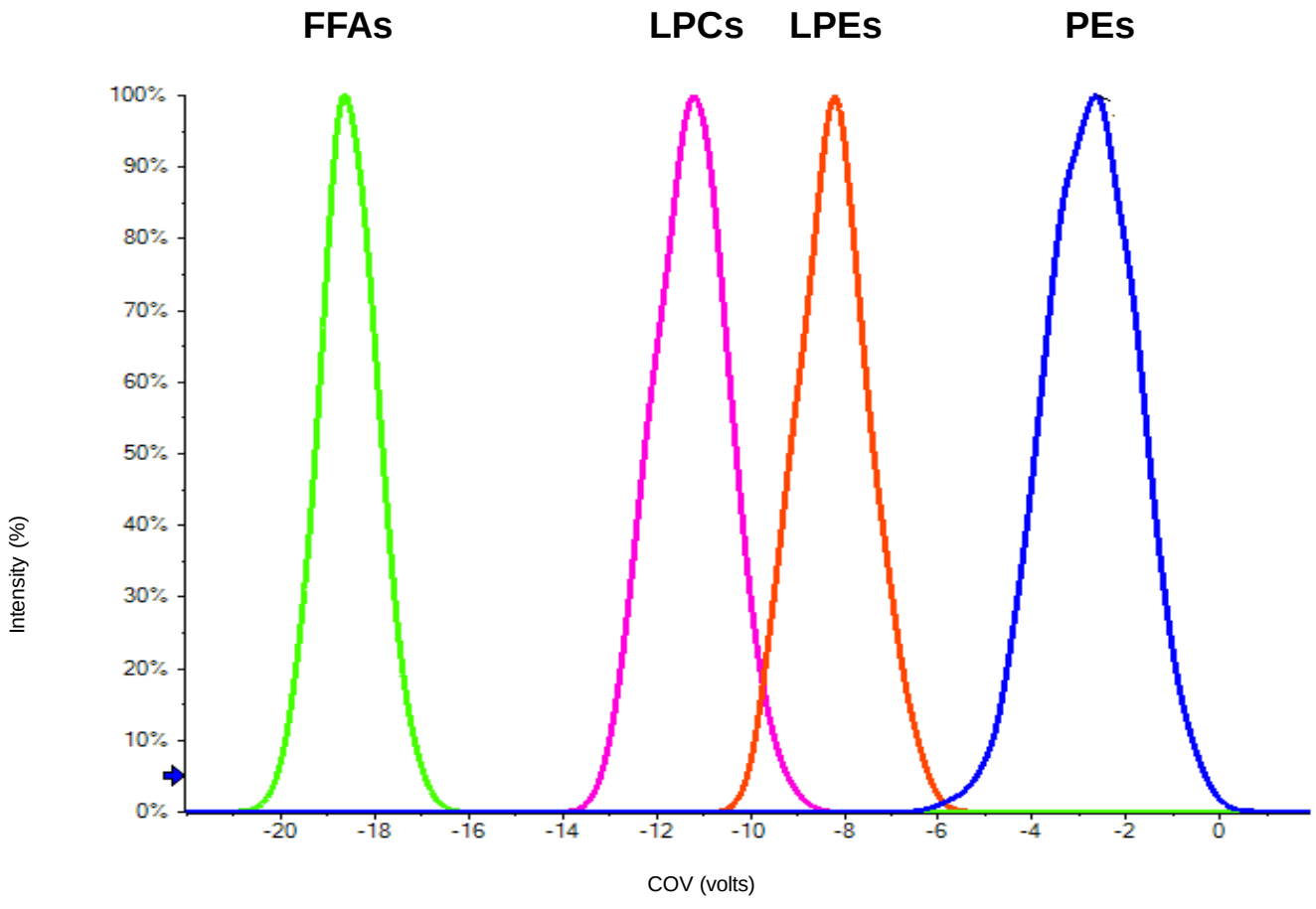


Separation waveform (SV): radially displaces ions towards one or the other electrode, depending upon high and low mobility characteristics

Compensation voltage (COV): restores the trajectory for a given ion or range of ions to allow them to transmit through the DMS device and enter the mass spectrometer

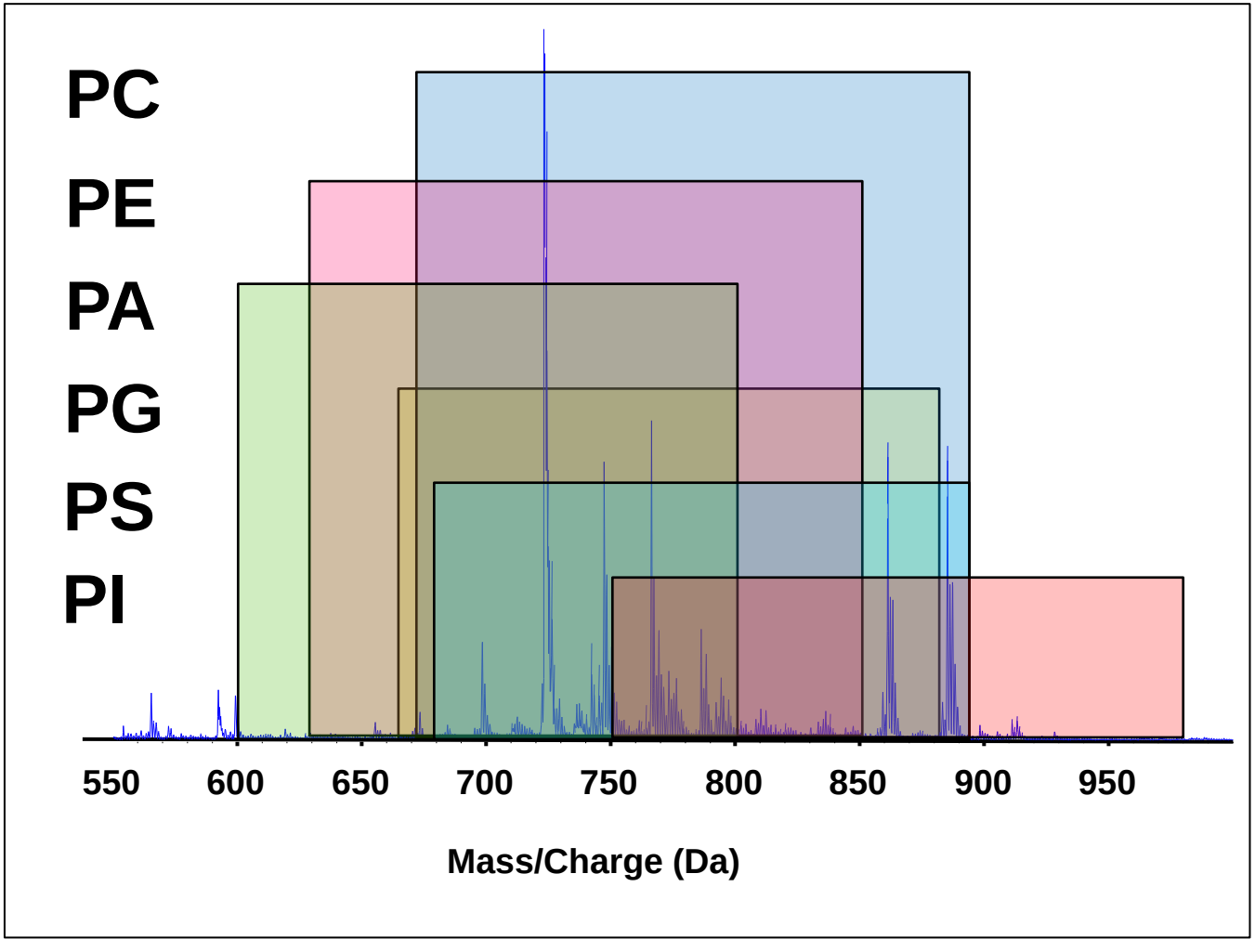
Separation of Lipid Classes Using SelexION™ Technology

Negative Ion Mode



*subset of lipid classes that can be separated

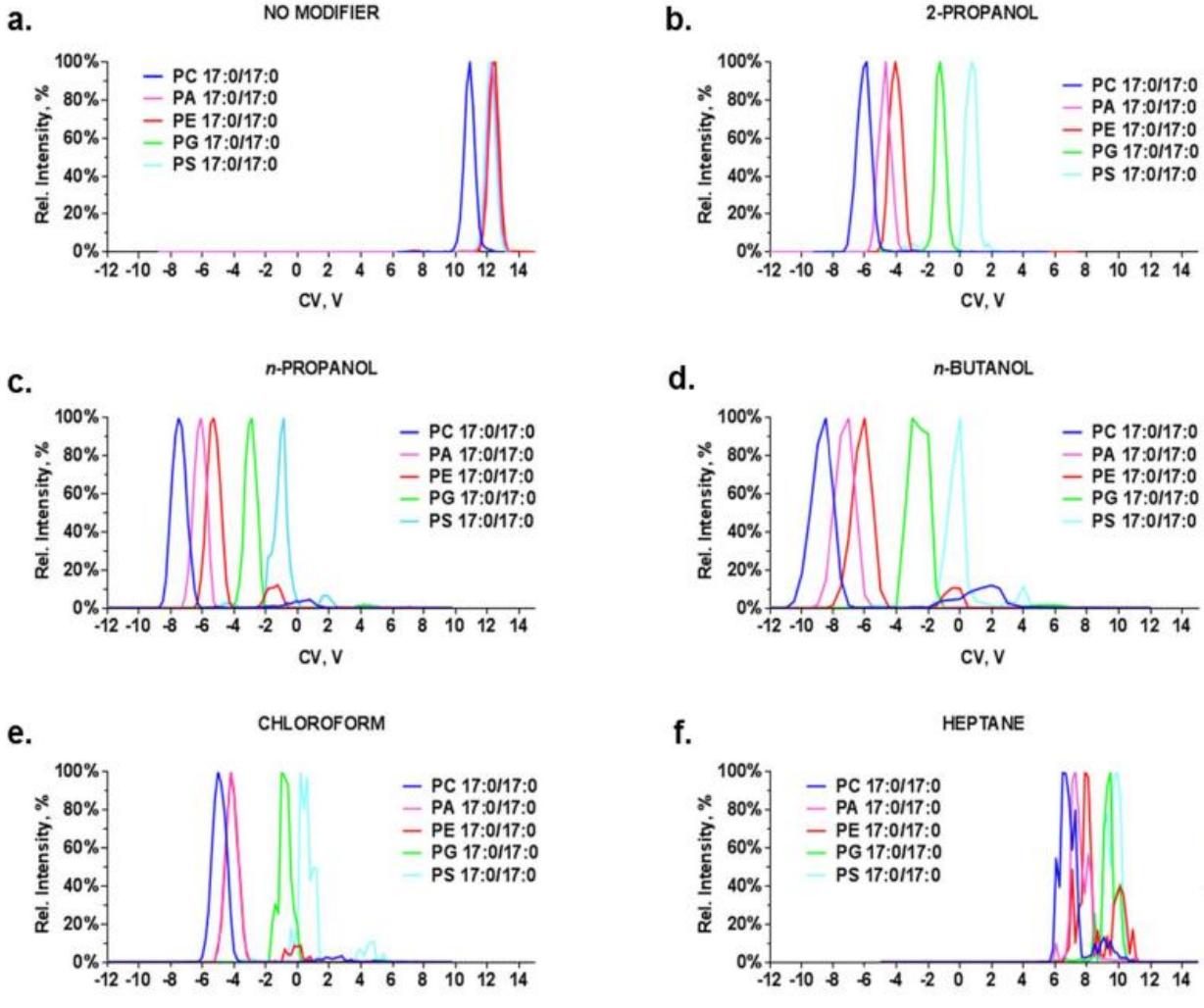
Isobaric Overlap of Phospholipids



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

Separation of Lipid Classes Using SelexION™ Technology

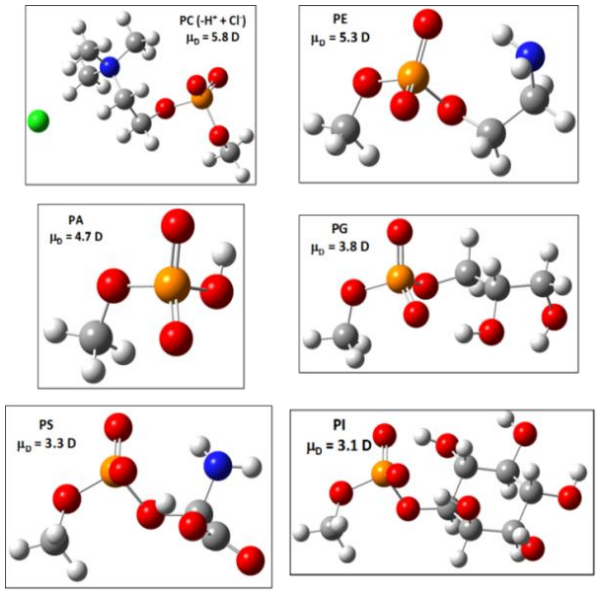
Effects of Modifier on COV Values



Differential Mobility Spectrometry-Driven Shotgun Lipidomics

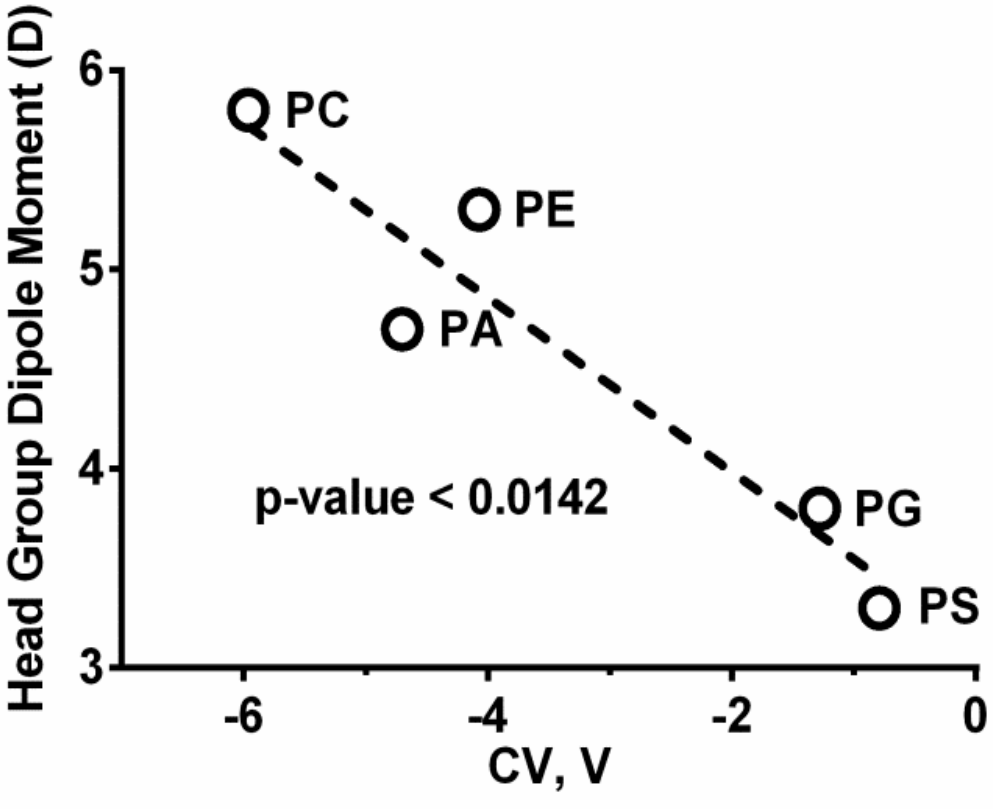
Anal. Chem., In Press **DOI:** 10.1021/ac5021744

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

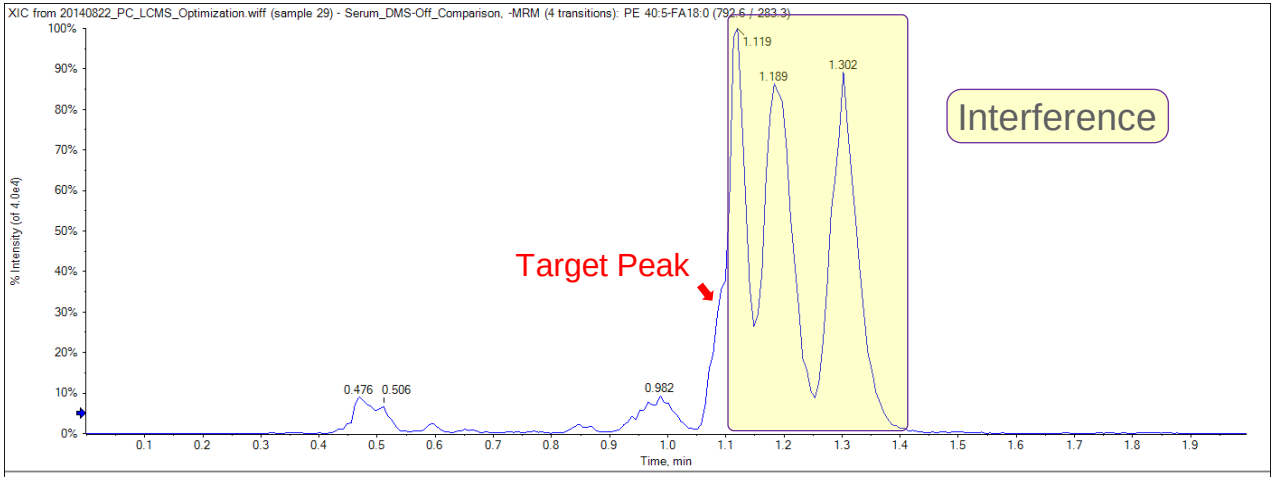


Molecules that have different dipole moments can be separated by DMS

Isobaric Interference Among Different Lipid Molecular Species

Isobaric interference makes ‘unassisted’ MRM analysis by infusion non-specific. SelexION™ Technology makes it possible

Experiment: mrm analysis of CSF (PE 40:5; 792.6/283.2)



SelexION™ Device Off

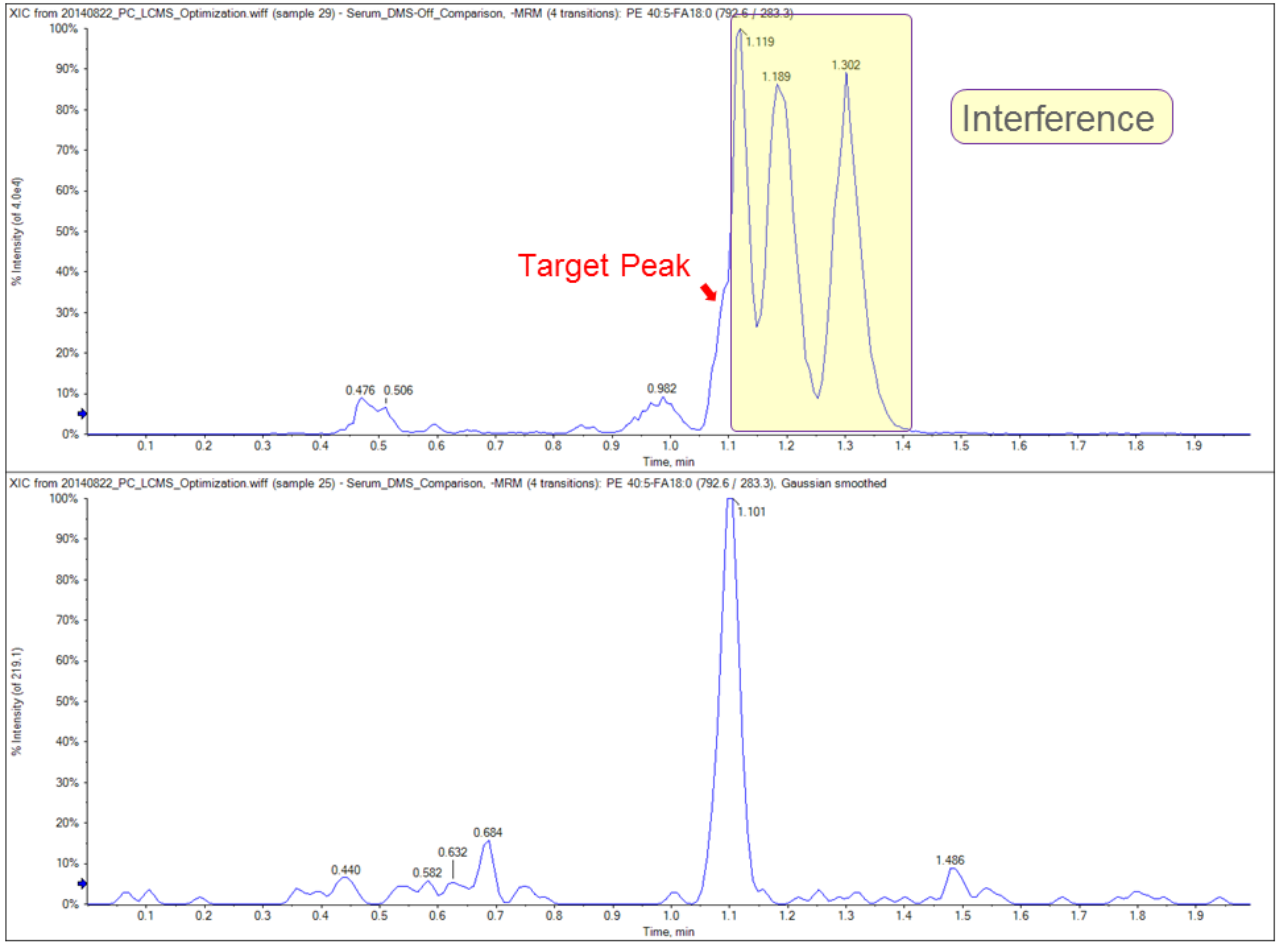
Multiple different lipids have the same MRM transition:

Isobaric Interference

Isobaric Interference Among Different Lipid Molecular Species

Isobaric interference makes 'unassisted' MRM analysis by infusion non-specific. SelexION™ Technology makes it possible

Experiment: mrm analysis of CSF (PE 40:5; 792.6/283.2)



SelexION™ Device Off

Multiple different lipids have the same MRM transition:

Isobaric Interference

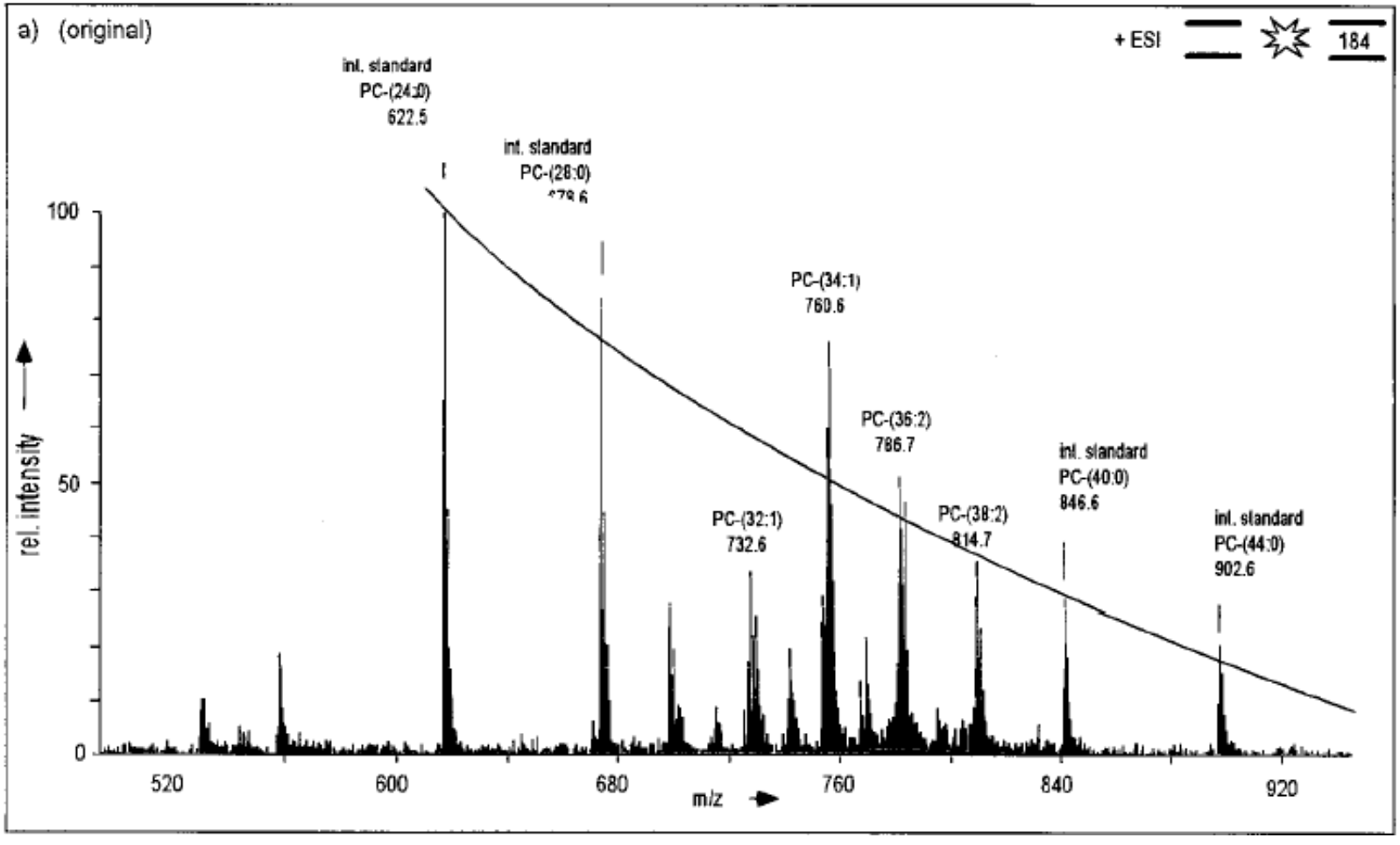
SelexION™ Device On

PE specific CoV=-3.8

Isobaric interference removed making quantitation possible

Challenge of Lipid Quantitation: Unequal Fragmentation Efficiency of Lipids within the Same Class

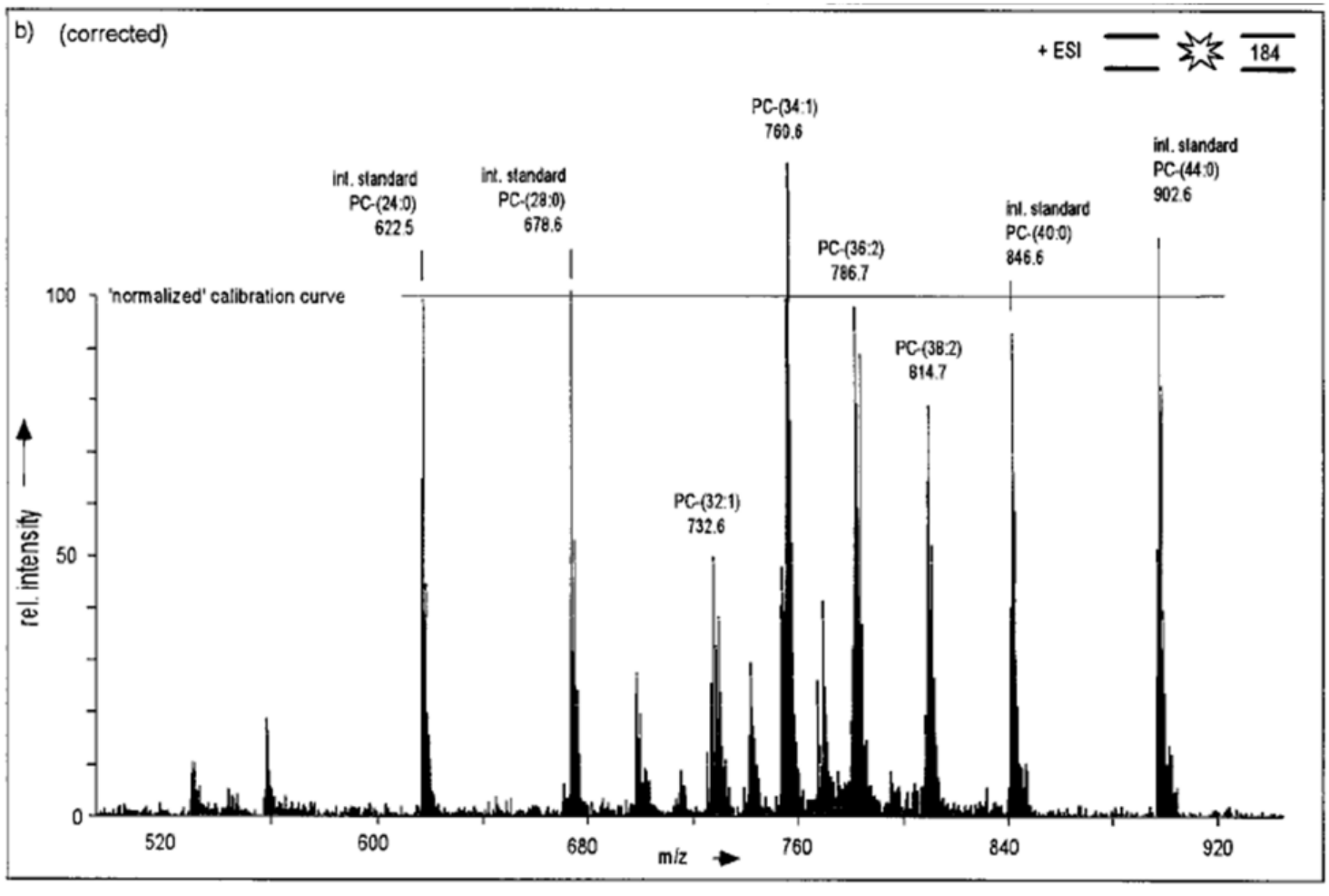
Diversity of fatty acid chain lengths and degrees of unsaturation result in differential fragmentation efficiency



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

Challenge of Lipid Quantitation: Unequal Fragmentation Efficiency of Lipids within the Same Class

Corrected Peak Intensities

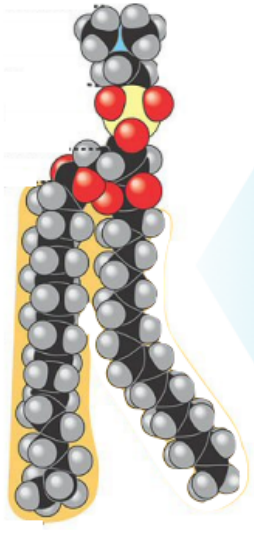


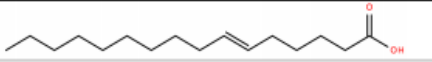
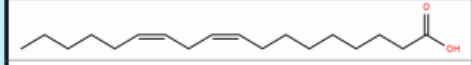
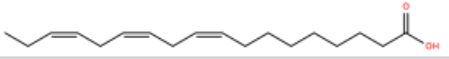
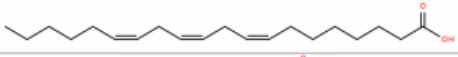
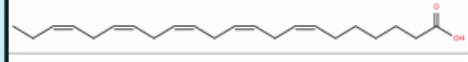
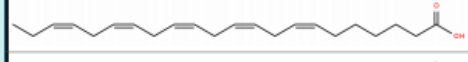
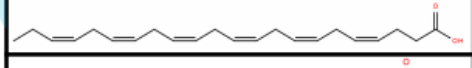
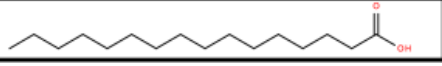
Murphy *et al.* Chem Rev **2001**, 101, 479-526

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

Multiple internal standards that reflect the diversity of lipid molecular species within a lipid class “unwarps” quantitative data

PHOSPHATIDYLCHOLINE (PC) INTERNAL STANDARD MIX

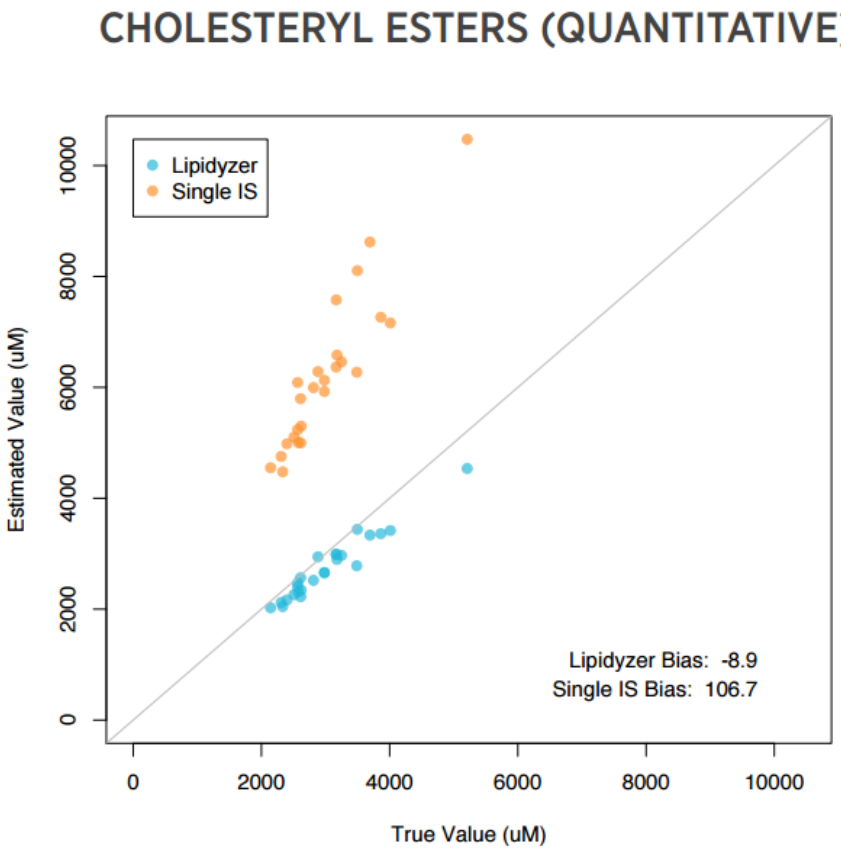


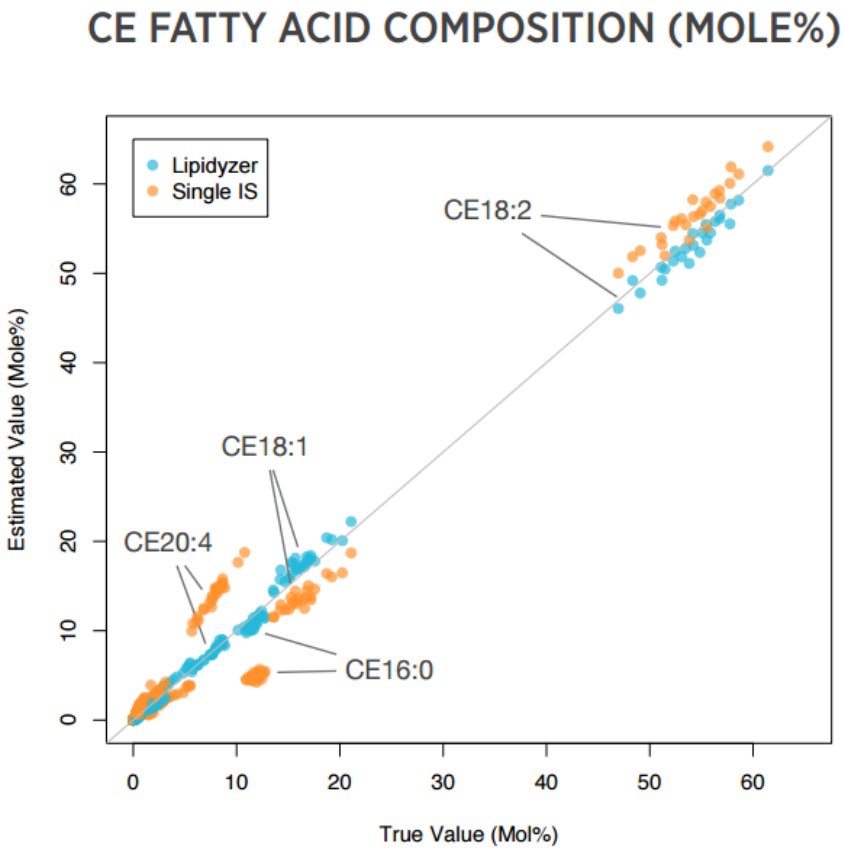
STRUCTURE	FATTY ACID	POS	%
	FA16:1 - Palmitoleic acid	sn-2	5
	FA18:1 - Oleic acid	sn-2	20
	FA18:2 - Linoleic acid	sn-2	20
	FA18:3 - α-Linoleic acid	sn-2	5
	FA20:3 - Dihomo-γ-linoleic acid	sn-2	5
	FA20:4 - Arachidonic acid	sn-2	20
	FA20:5 - Eicosapentaenoic acid	sn-2	5
	FA22:4 - Eicosatetraenoic acid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

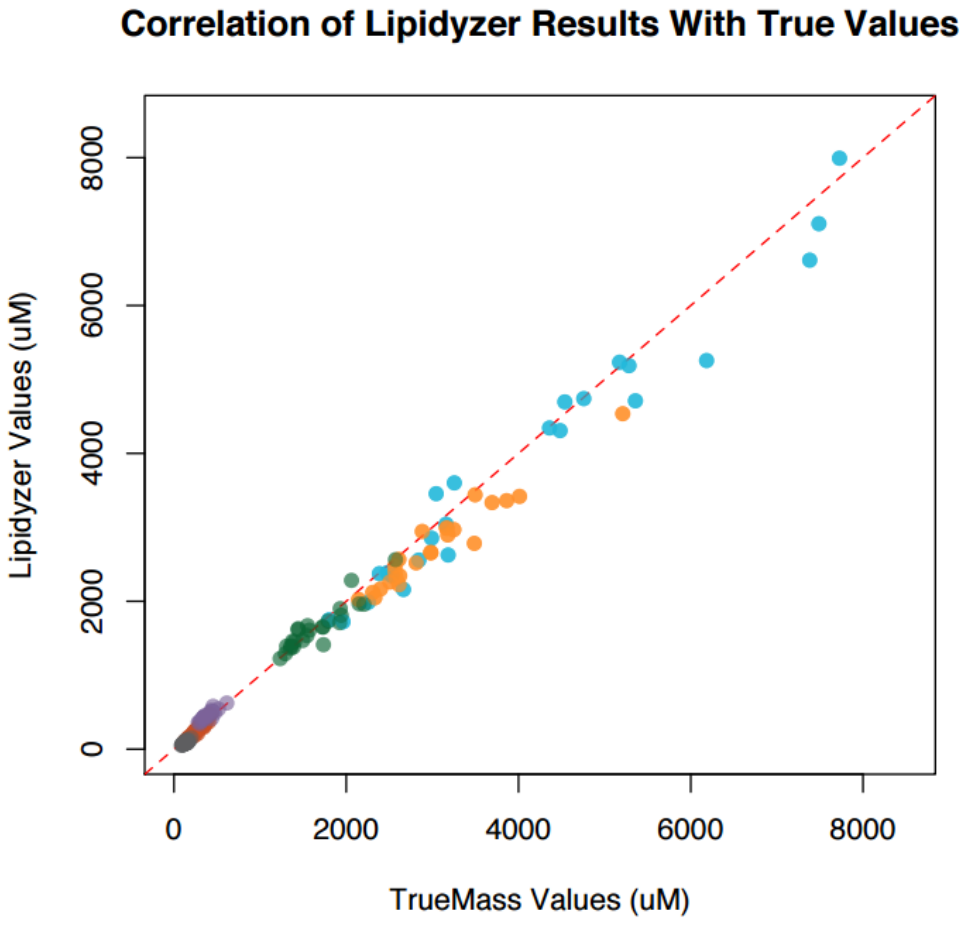




*Each dot represents one patient serum sample. We measure 25 serum samples from 25 patients

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

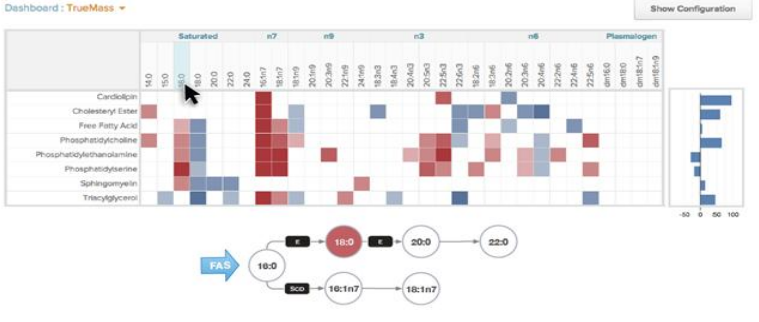
*TrueMass = GC-FID, gold standard

Why the Lipidyzer™ Platform? – Data Analysis

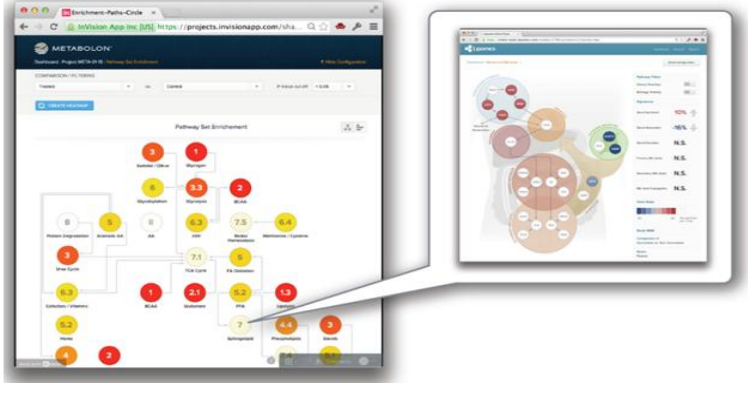
Automated Output of Results

- 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



Why the Lipidyzer™ Platform? – Expertise

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**

analytical chemistry

Article

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Autonomous Metabolomics for Rapid Metabolite Identification in Global Profiling

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OMICS – Continued Education

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Targeted Metabolic Profiling Using a High-Resolution Accurate Mass Database to Identify and Confirm Potential Biomarkers in Rose and Sunflower Plant Extracts

Jeffrey D. Miller[1], Cyrus Papan[2], Dr. Jens Pfannstiel[3], Iris Klaiber[3], Baljit K. Ubhi[4], Fadi Abdi[1], Gerard Hopfgartner[5], Emmanuel Varesio[5], Tobias Bruderer[5].
[1]SCIEX, Framingham, MA 01701, [2]SCIEX, Darmstadt, Germany, [3]Universität Hohenheim, Serviceeinheit des LSC, August von Hartmann Str. 3, 70599 Stuttgart, Germany. [4]SCIEX, Redwood Shores, CA, [5]University of Geneva, Switzerland.

INTRODUCTION

Leaves and petals of plants such as rose and sunflowers have long been used for medicinal and aesthetic purposes around the world. Recently, investigators found that phenolic antioxidants especially those levels present in rose petal extracts of deep color (intense red to mauve) may be responsible for the activity or inhibitions, and thus justifying their use in traditional medicines.[1] In addition, sunflower leaf tea is used in traditional medicine to reduce high fevers and has astringent properties. Sunflower leaf poultice may be used on snakebites and insect bites. The leaves are diuretic and expectorant, as are the seeds, so determining what compounds are contributing to the activity and involvement in metabolic pathways is of interest to many scientists. Here we undertook a study to see if three separate rose leaf extracts and sunflowers would produce variances in their profiles in comparison to one another and identify compounds associated with these changes perhaps leading to metabolic pathways for further investigation.

ABSTRACT

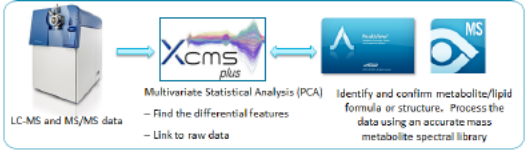
High resolution, accurate mass/spectrometers are the instruments of choice for global metabolomics applications as well as targeted metabolic profiling as they employ a high degree of selectivity over nominal mass systems. Unbiased targeted profiling is a technique applied on QTOF instruments which allow for the collection of MS and MSMS data in a single-injection. This discovery data can be searched with a targeted list of metabolites, from many chemical classes, pathways and species. Metabolite identification with a high resolution accurate mass MSMS library ensures increased confidence in assignment and purity scores of unknowns from a discovery experiment. Recently, a metabolite library has been developed to facilitate this process.[2] Here we present some recent results that illustrate this powerful technique in examining a study of plant extracts from different lots of rose petal and sunflowers and employing an accurate mass library for confirmation.

MATERIALS AND METHODS

Sample Preparation:
Extractions of rose petals from 3 different lots and an extraction of sunflower leaves were provided by Dr. Pfannstiel, University of Hohenheim, Stuttgart, Germany. All extracts were combined into another "pooled" lot as a quality control. The five different sample groups were then injected in triplicate or duplicate for analysis.

LCMS Method:
Mass Spectrometer: SCIEX TripleTOF® 6600 System. Data were acquired in both positive and negative ion mode using SWATH™ acquisition and TOF-IDA experiments for comparison of the MSMS quality of spectra. HPLC gradient method used an Agilent 1290 (pump, column oven, autosampler). Column: Phenomenex Kinetex® XB-C18 2.6µm, 2.1 x 100mm. Flow rate: 300 µL/min. Injection volume: 5 µL. Oven temperature: 40°C. Mobile phase: A: H_2O with 5mM NH_4OAc. B: Acetonitrile with 5mM NH_4OAc.

Work flow for metabolomic profiling



RESULTS

Step 1: Examination of the sample batch to ensure reproducibility and integrity injection to injection (Figure 1).
Step 2: "Did the experiment work?", i.e. did multivariate statistical analysis of the different lots produce unique differences between the groups? Here one can easily see differences between the three rose lot extracts, pooled QC's and sunflowers. The "pooled" QC samples are shown at the center of the Scores plot as expected since they are a composite of all samples (Figure 2).

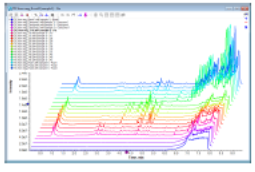


Figure 1. Sample batch of negative mode data, showing injection to injection reproducibility and TIC stacked profiles.

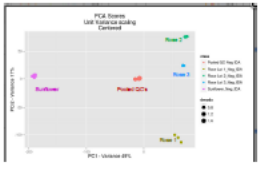


Figure 2. Multivariate statistical analysis of the different lots produced unique differences, the pooled QC samples end up at the center of the Scores Plot.

Step 3: XCMS[plus] software[3] was used for multi-variant statistical analysis to generate the non-targeted list of candidates expressing the lowest *p*-values. Clicking on an line entry, the Selected Ion Chromatogram appears on the right hand side. The list was exported into MasterView™ Software for processing along with the targeted *Accurate Mass Metabolite Spectral Library* (Figure 3).

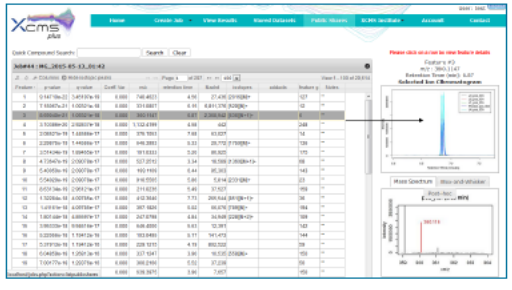


Figure 3. The Results Table from XCMS[plus] software showing the top 19 candidates with the lowest *p*-values from the positive mode IDA data. Selecting an entry line such as #3 shown links the overlaid XIC's from all samples for visual comparison. The light blue traces shown were the sunflower extracts, dark blue were the "pooled" sample traces.

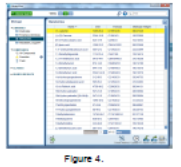


Figure 4.

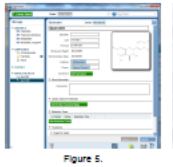


Figure 5.

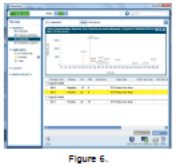


Figure 6.

Figures 4-6, shown above are screen captures of the *Accurate Mass Metabolite Spectral Library* in LibraryView™ Software. Figure 4, at left, is the list of compounds (570 entries) and libraries used in LibraryView™ Software. Figure 5, center, is the individual entry (quercetin) for editing, and Figure 6 is the spectral information input window.

Step 4: Importing the non-targeted results from the multivariate statistical analysis and subsequently processing in MasterView™ Software along with the *Accurate Mass Metabolite Spectral Library* confirmed the presence and relative amounts (in relation to other groups) of catechin, quercetin, kaempherol and phloretin as well as several endogenous organic acids (Figure 7). The top pane shows the XIC of the line chosen (in this case catechin), the center pane contains the report information and formulas and the bottom two panes show the library matches of MS and MSMS spectra.

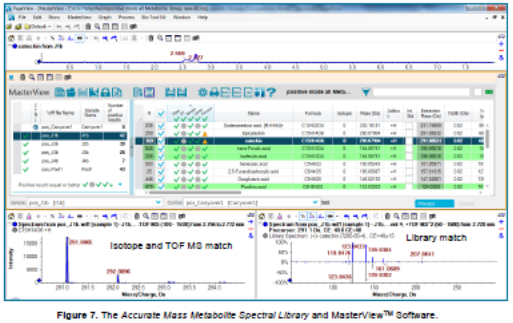
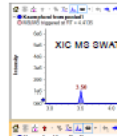


Figure 7. The *Accurate Mass Metabolite Spectral Library* and MasterView™ Software.

Figure 8, above right, shows the positive mode SWATH™ acquisition compared to the IDA data. XIC of the MS spectra was basically the same (left panes), whereas the MSMS XIC of fragment 153 (kaempferol) shows the SWATH™ acquisition MSMS is much better than IDA-acquired providing more data points across the XIC peak chromatograms.



	Metabolite library compound name
1	Citric acid
2	L-Glutamic acid
3	L-Phenylalanine
4	catechin
5	genistein
6	kaempferol
7	phloretin
8	quercetin
10	quercitrin/astragalin

Table I. Summary of so

CONCLUSIONS

The *Accurate Mass Metab* and endogenous metabolit these compounds were ea of kaempherol, quercetin, flavonoid known to be pres rose leaf extracts at differe not identified in this sample low levels in the rose extra IDA methods, which is usef

REFERENCES

1. Cunja, V.; Mikulic-Petko *Species and Cultivars: an* 2. Metabolite accurate ma University of Geneva, Swit 3. Developed by Gary Si

TRADEMARKS/

OMICS – Continued Education

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Targeted Metabolic Profiling Using a High-Resolution Accurate Mass Database to Identify and Confirm Potential Bioma

Jeffrey D. M
[1]SCIEX, Fra

INTRODU
Leaves and pet
purposes aroun
present in rose
inhibitions, and
traditional medi
on snakebites a
compounds are
scientists. Here
variances in the
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of metabolites,
resolution accur
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process.[2] Here
plant extracts fr
confirmation.

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LCMS Method:
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mode using SW
HPLC gradient
Kinetex® XB-C1
Mobile phase: A

LC-MS and MS/MS

Biomarkers and Omics

SCIEX

Quantitative and Qualitative Metabolomics for the Investigation of Intracellular Metabolism

Targeted Analysis on the QTRAP® 5500 System and Reverse-Phase Ion-Pairing Chromatography

Douglas McCloskey[1] and Baljit K. Ubhi[2]
[1]Department of Bioengineering, University of California, San Diego, CA, USA, [2]SCIEX, USA

Liquid chromatography-mass spectrometry (LC-MS) provides a powerful analytical tool for understanding and monitoring intracellular metabolism by measuring the metabolome. The study of intracellular metabolism of model organisms, such as *E. coli*, is vital to further our biochemical knowledge[1], to develop new pharmaceuticals that target harmful pathogens[2], and to improve industrial applications that aim to metabolically engineer bacteria in order to produce commodity chemicals from renewable resources[3]. Paramount to these endeavors is the ability to reliably and accurately measure the intracellular metabolome. For microorganisms, the compounds of most interest comprise intermediates of high flux pathways such as

SCIEX

Acknowledgements

SCIEX

- Baljit Ubhi
- Paul Baker
- Eva Duchoslav
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- Yves LeBlanc
- Leo Wang
- Pauline Vollmerhaus
- Fadi Abdi
- Aaron Hudson

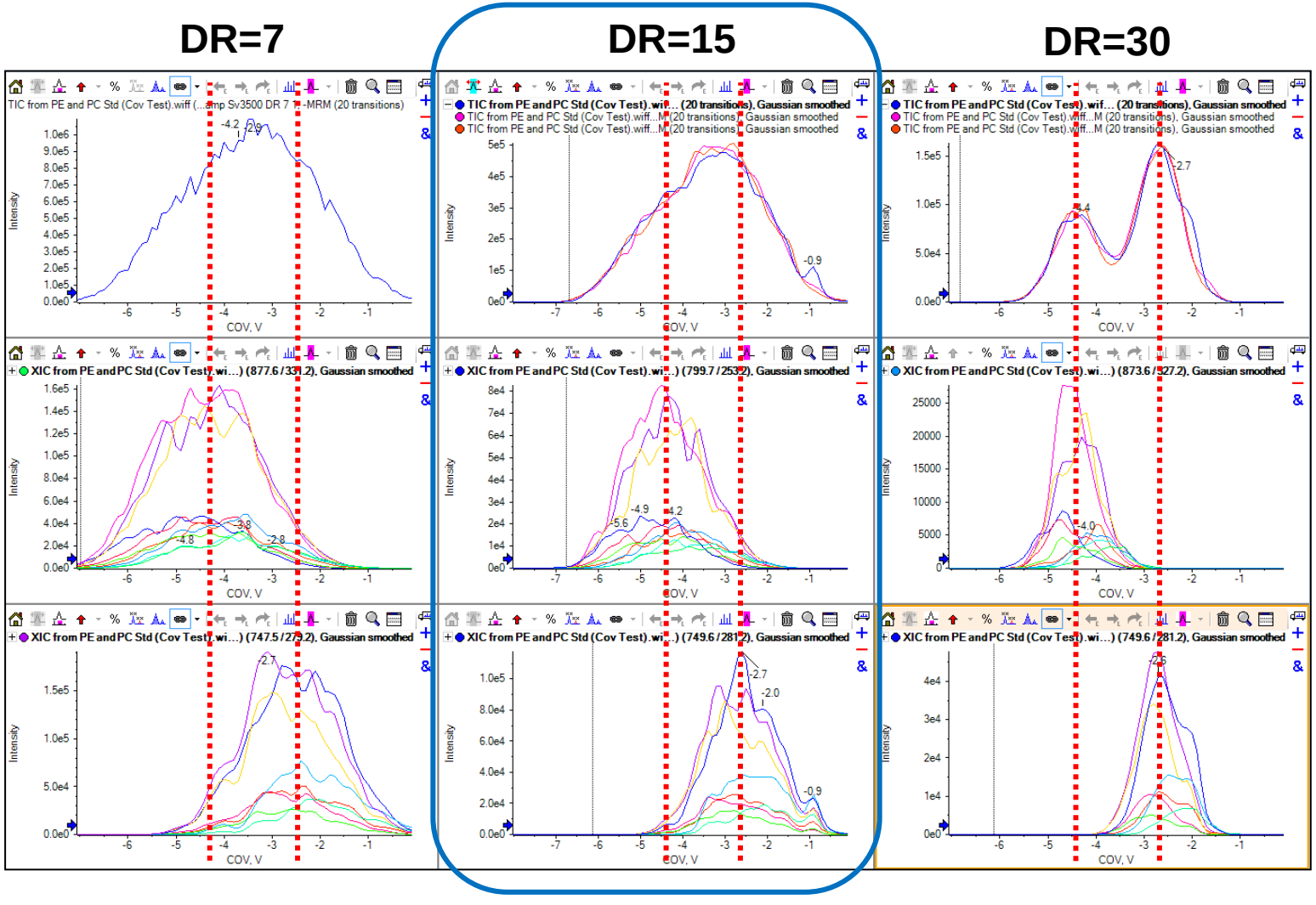
METABOLON

- Alex Conner
- Steve Watkins
- Annie Evans
- Richard Robinson
- Luke Miller
- Sarada Tanikella
- Corey DeHaven

APPENDIX

PC/PE Standard Mixture Resolution

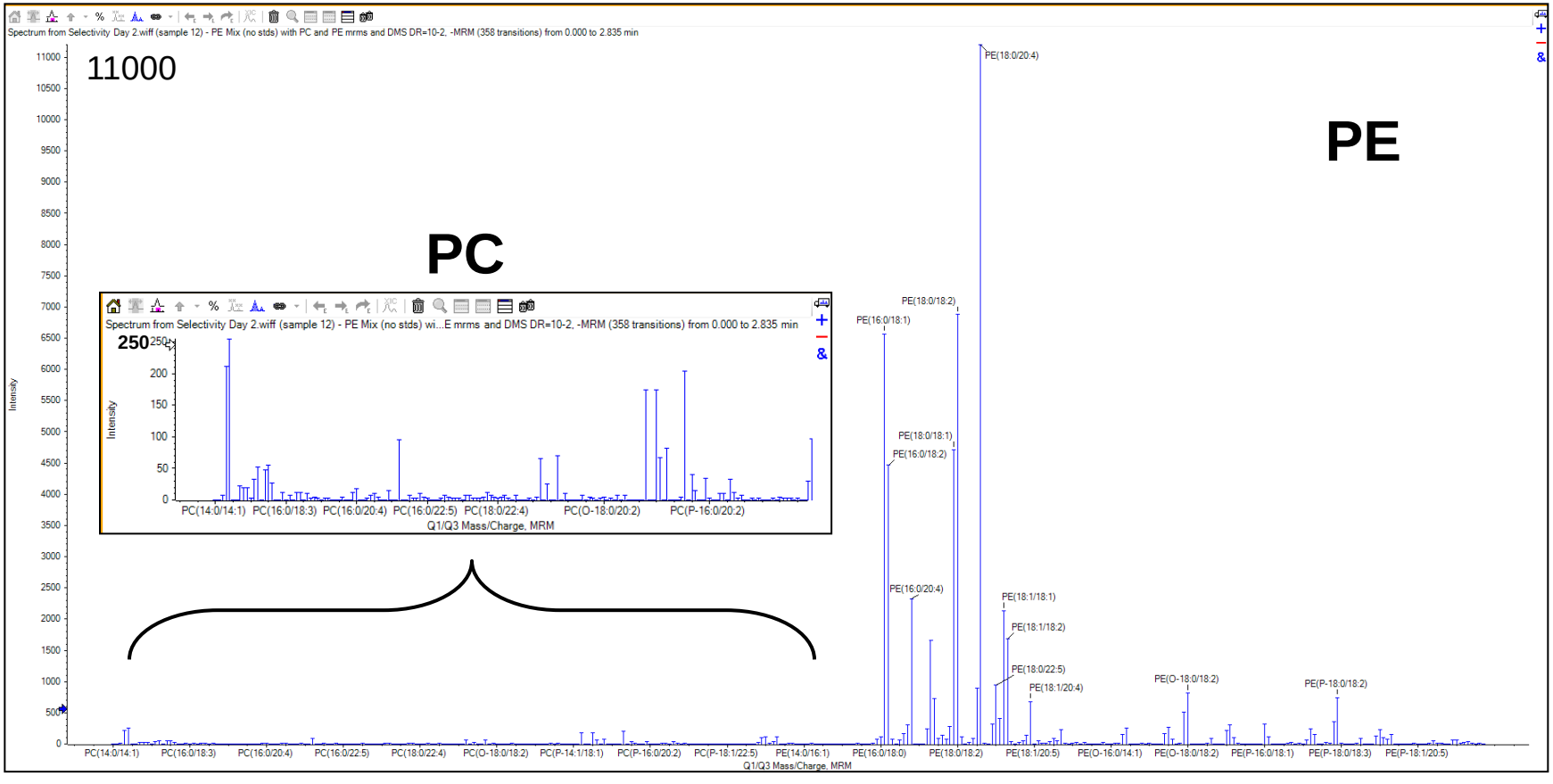
SelexION™ Technology effectively resolves different lipid classes



Resolution gas (DR) slows down ions in the DMS cell to enhance resolution

Cross lipid Class Contamination

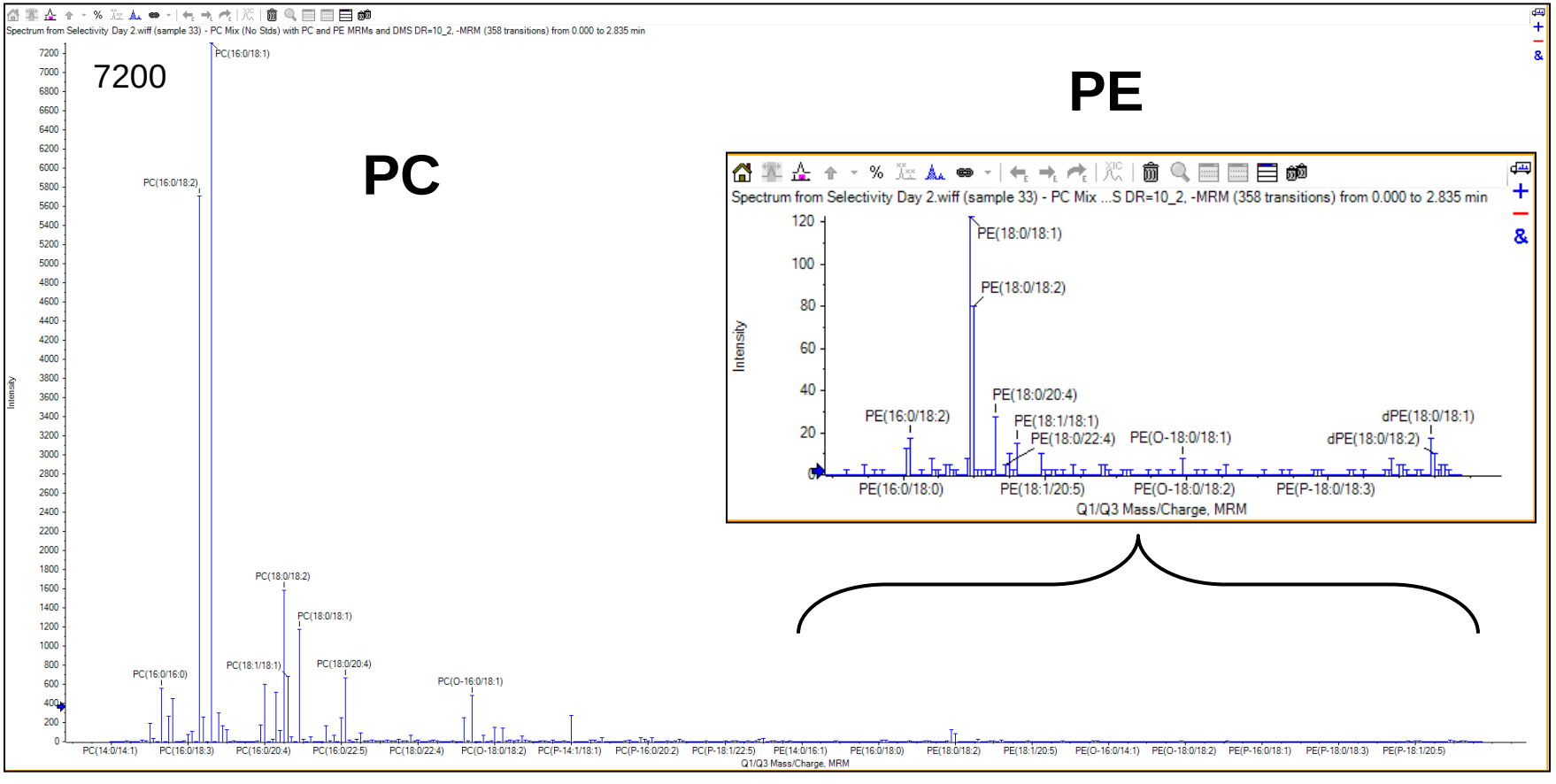
PE Mixture analyzed with both PE and PC MRM transitions



Cross over estimation: $\frac{\text{Base peak intensity (PC)}}{\text{Base peak intensity (PE)}}$ **X 100 = (250/11000)* 100 =** **2.3%**

Cross lipid Class Contamination

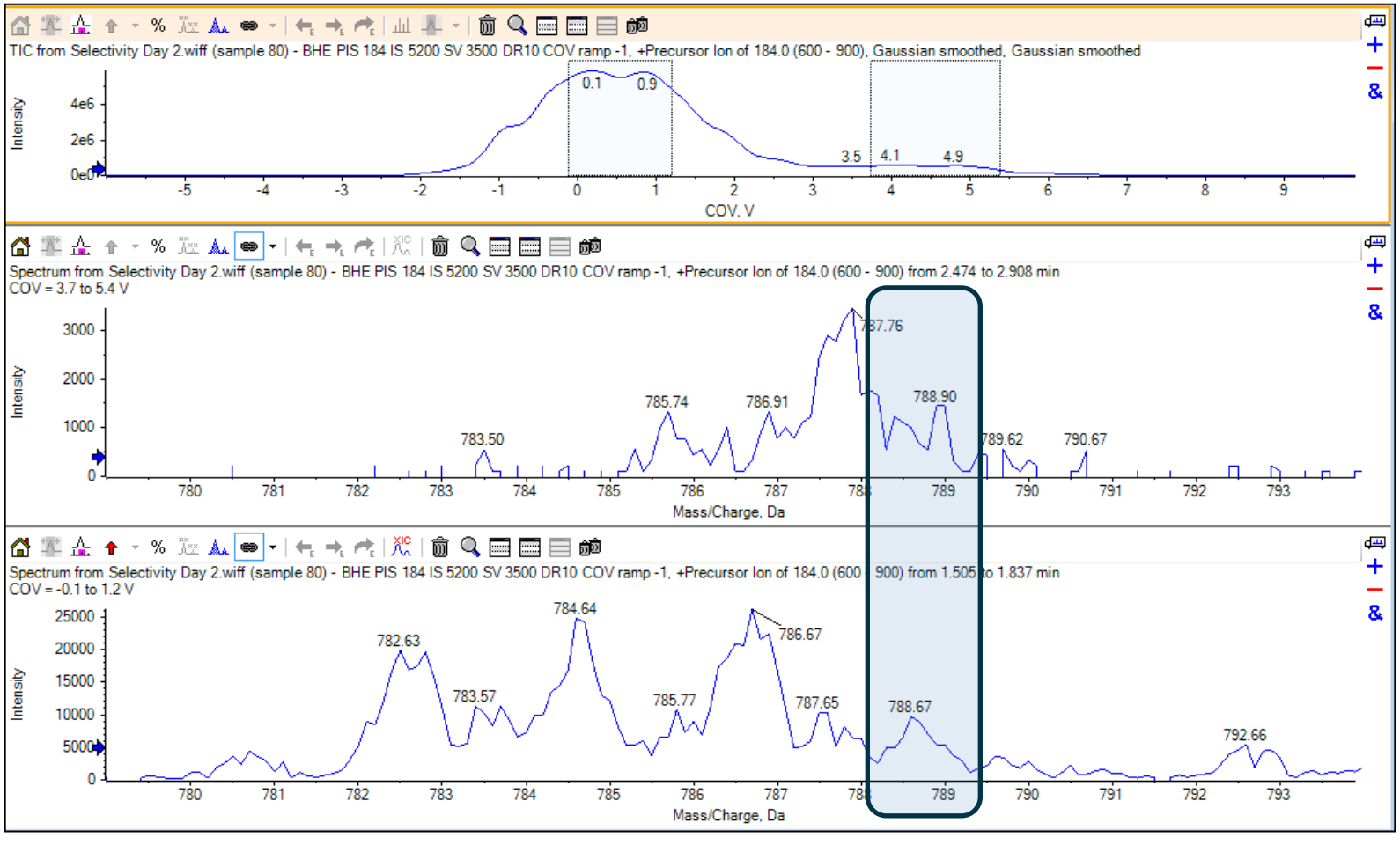
PC Mixture analyzed with both PE and PC MRM transitions



Cross over estimation: $\frac{\text{Base peak intensity (PC)}}{\text{Base peak intensity (PE)}}$ **X 100 = (120/7200)* 100 =** **1.7%**

Challenge in the Analysis of PC and SM

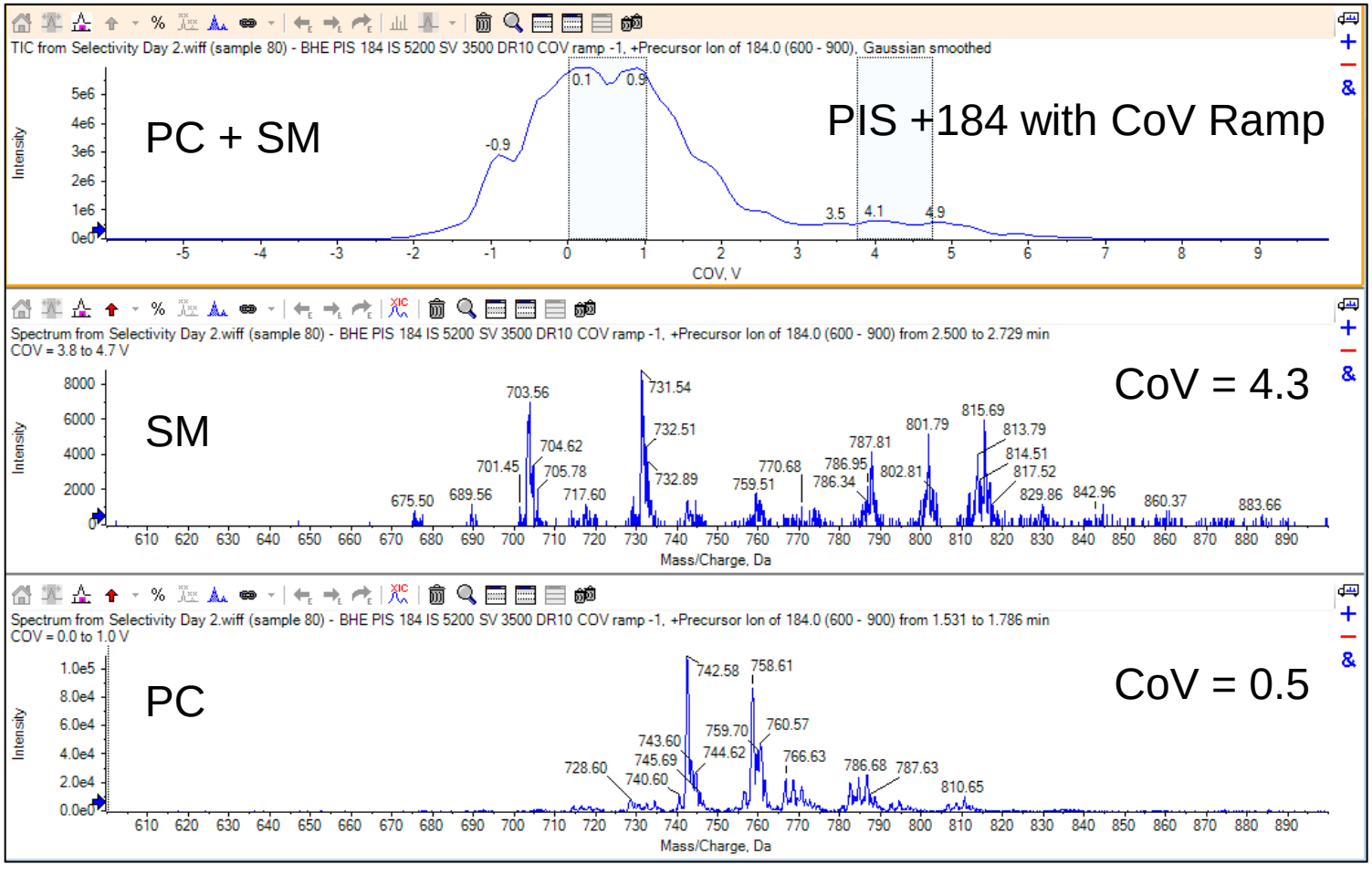
Both Phosphatidylcholine and sphingomyelin share the same fragment mass: +184



The peak intensity of 787/184, without resolution, is attributable to both SM 40:1;2 and the n+1 isotope of PC 36:2

SelexION™ Technology Resolves PC and SM Lipid Classes

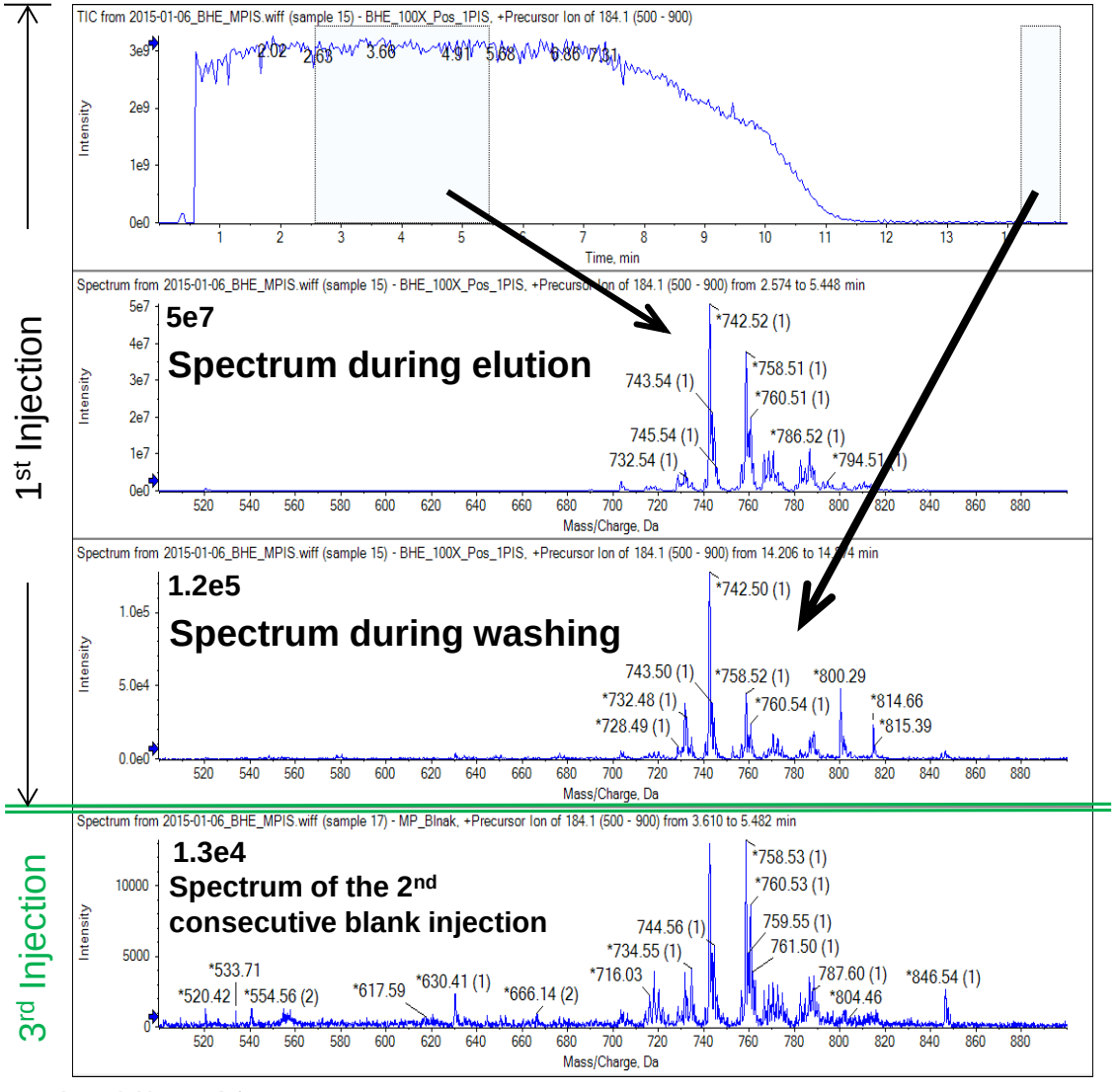
Despite sharing a common, identifying fragment, PC and SM can easily be differentiated



MRMs associated with a class-dependent CoV are specific and selective

Key Concern #2 : Sample Carry-Over Between Injections

The solution is the use of PeakSil tubing

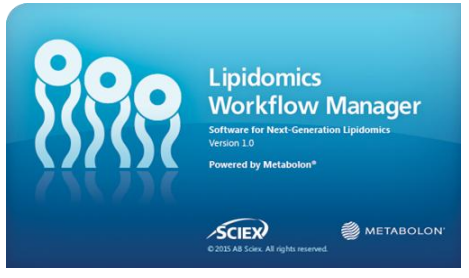


- The Use of PeakSIL tubing dramatically reduces carryover, as compared to regular Peak tubing
- Background level reduced to very low level in the same run
- Background level further reduced to after two blank injections

The Lipidyzer™ Platform Products

- **The Lipidyzer™ platform**
  - Components:
    - Exion LC, SelexION, 5500 QTRAP®,
    - Lipidomics Workflow Manager software
    - Platform Getting Started Kits
    - PN: 504900

- **Lipidomics Workflow Manager: Standalone**
  - Lipidomics Workflow Manager Software
  - Getting started kits
  - PN:5041390



- **The Lipidyzer Internal Standard Kits**
  - SelexION Tuning Kit the Lipidyzer™ Platform
  - Getting Started Kit for the Lipidyzer™ Platform
  - System Suitability Kit for the Lipidyzer™ Platform
  - Internal Standards Kits for Lipidyzer™ Platform