



Understanding Complex Lipid Metabolism through Quantitative Lipidomics

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CPSA, Metabolomics, Florida. March 2016

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)

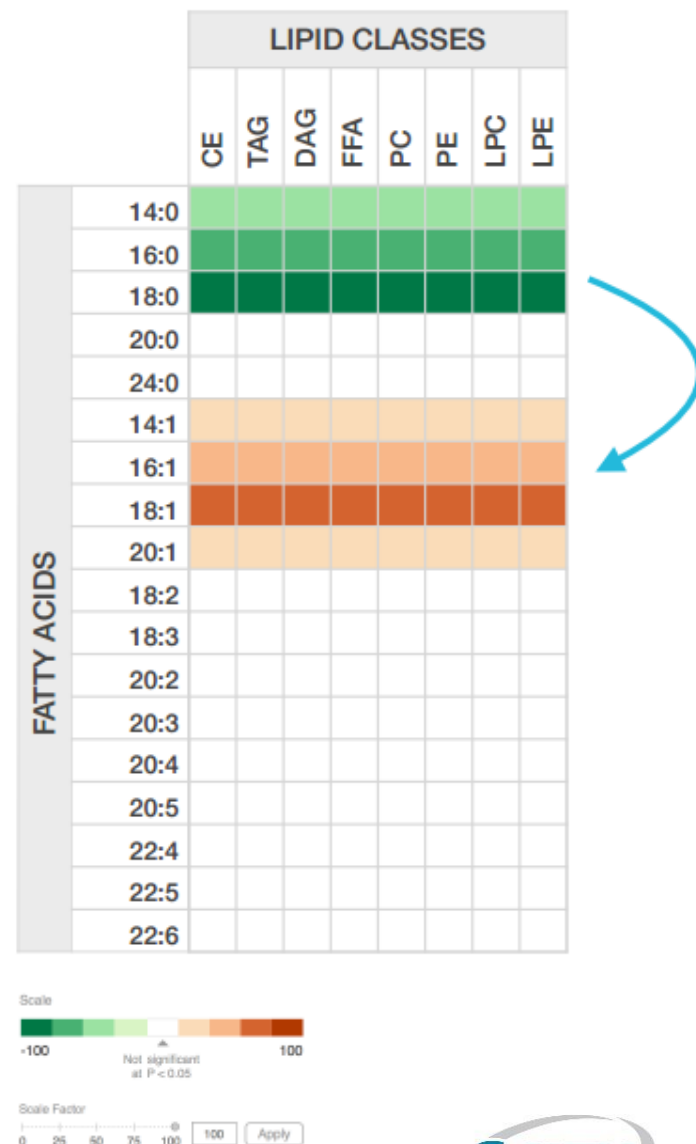
		LIPID CLASSES							
		CE	TAG	DAG	FFA	PC	PE	LPC	LPE
FATTY ACIDS	14:0								
	16:0								
	18:0								
	20:0								
	24:0								
	14:1								
	16:1								
	18:1								
	20:1								
	18:2								
	18:3								
	20:2								
	20:3								
	20:4								
	20:5								
	22:4								
	22:5								
	22:6								

Sum = composition

Sum = concentration

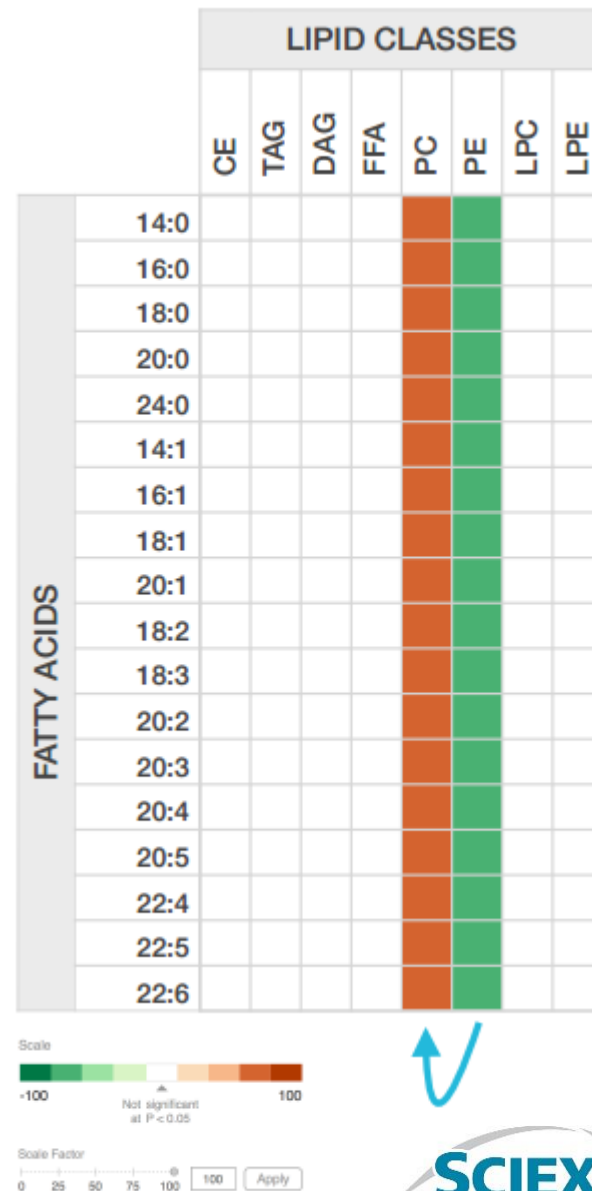
Complex Lipids are like a Matrix

- 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)
- When FA metabolism is altered there is the ability to change FA composition of all classes
 - Steroyl-CoA-Destaurase (SCD1) inserts a double bond into saturated fatty acids
 - 14:0 > 14:1
 - 16:0 > 16:1
 - 18:0 > 18:1



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)
- 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
 - Phosphatidylethanolamine-N-Methyl Transferase (PEMT) converts PE into PC
 - PE > PC



What is Needed from a Lipid Platform

		LIPID CLASSES							
		CE	TAG	DAG	FFA	PC	PE	LPC	LPE
FATTY ACIDS	14:0								
	16:0								
	18:0								
	20:0								
	24:0								
	14:1								
	16:1								
	18:1								
	20:1								
	18:2								
	18:3								
	20:2								
	20:3								
	20:4								
	20:5								
	22:4								
	22:5								
	22:6								

Specificity

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

Quantitation

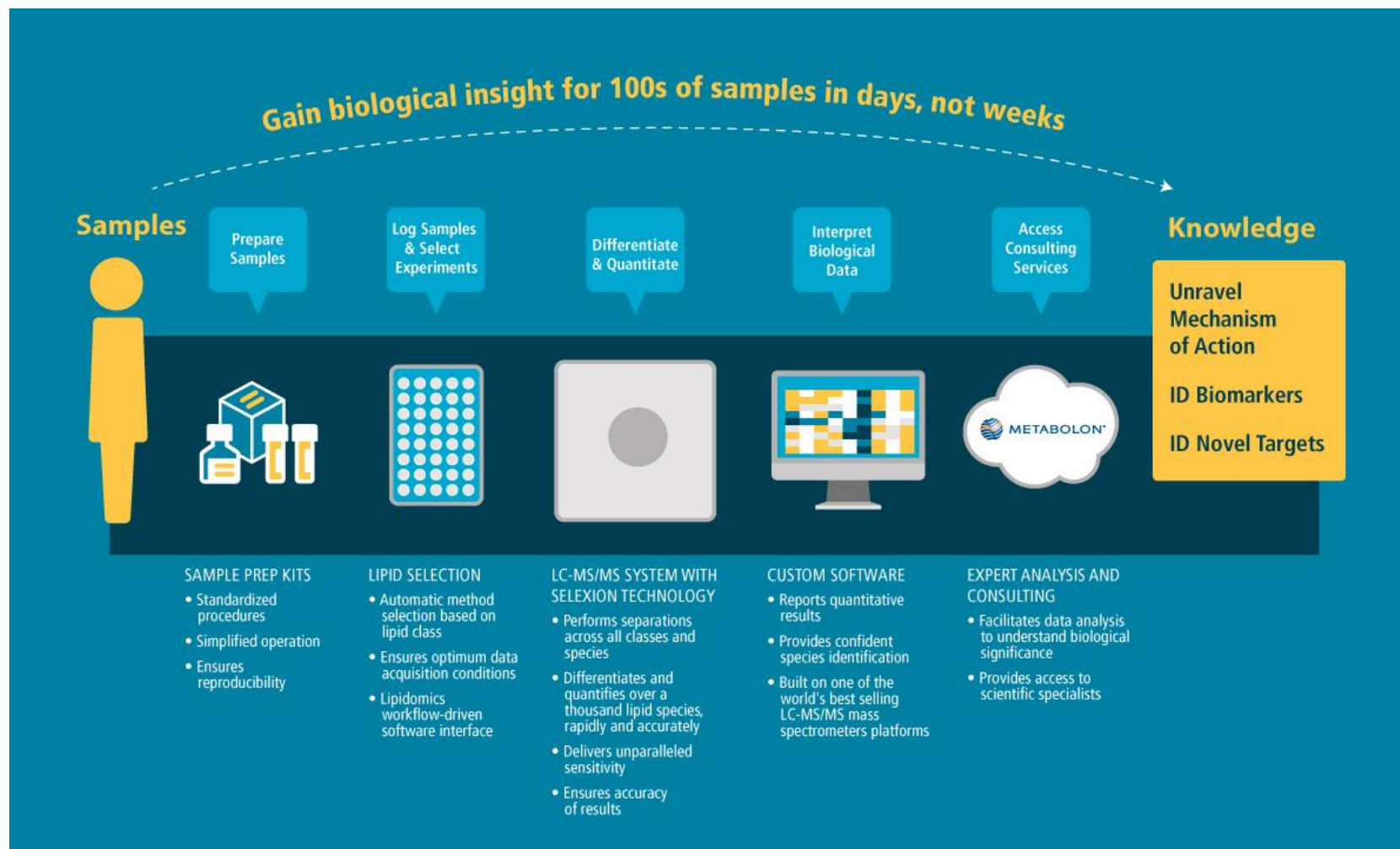
A non-quantitative approach does not allow accurate summing of the rows and columns

Comprehensive Coverage

A partially complete matrix is difficult to interpret

The Lipidzyzer™ Platform - Simplifying the Complexity

Powered by METABOLON®



Full Coverage of Complex Lipid Metabolism

Fraction	Lipid Classes	Number of Species*
Neutral Lipids	Triacylglycerols (TAG)	502
	Diacylglycerols (DAG)	67
	Free Fatty Acids (FFA)	28
	Cholesterol Esters (CE)	34
Polar Lipids	Phosphatidylcholines (PC)	161
	Phosphatidylethanolamines (PE)	233
	Lysophosphatidylcholines (LPC)	28
	Lysophosphatidylethanolamines (LPE)	28
	Sphingomyelins (SM)	16
	Ceramides (CER)	56

*The Ceramides listed above includes the further three classes, DCER, HCER and LCER

Lipidizer™ Enables Ease of Use, Specificity and Quantitative Rigor

Three Key Elements of the Platform:

1. Software: Ease of Use

- Log samples, create batches
- Automated calculation of chemistries, tuning and system tests

2. SelexION™: Specificity

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

3. Internal Standards: Quantitation

- Ensure spray stability
- Minimize carryover
- Neutralize quantitative bias

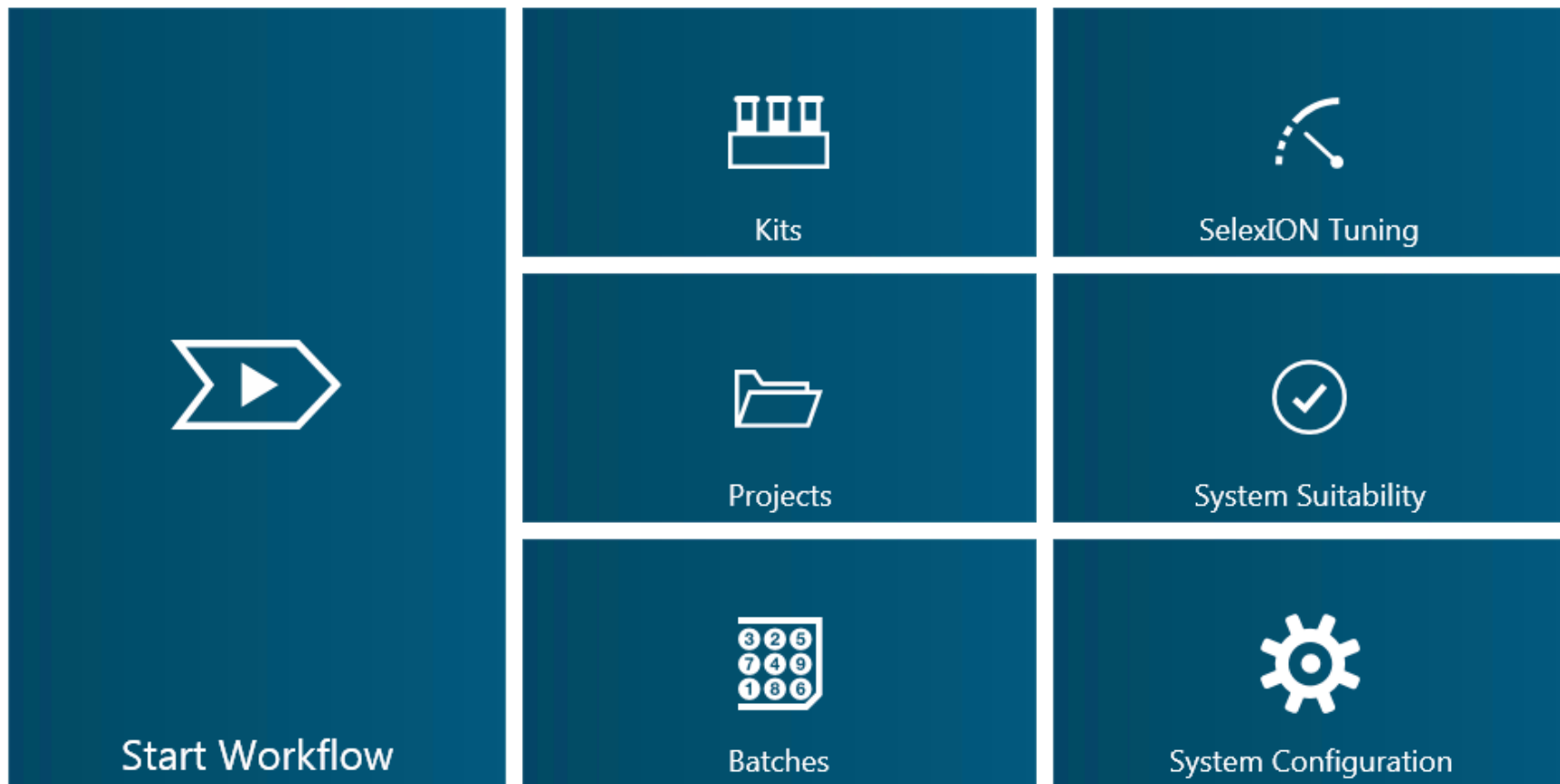


Warning to all mass spectrometerists, the data visualizations that you are about to see are unlike any others that you are used to.

There are NO spectral views!

Lipidomics Workflow Manager: Dashboard

Simple and Guided Workflow



Lipidomics Workflow Manager: Automation

System Suitability Tests and Automated Tuning for SelexION

System Suitability Test Result

Average Intensity Result:

Class	Name	Intensity	Threshold Intensity	Result
CE	CE(16:0)	6433.75	100	PASS
CE	CE(16:1)	8527.25	1000	PASS
CE	CE(18:1)	26758	1000	PASS
CE	CE(18:2)	79762.75	10000	PASS
CE	CE(20:3)	8590.50	1000	PASS
CE	CE(20:4)	9398.50	1000	PASS
CE	CE(20:5)	9735	1000	PASS
CE	CE(22:6)	10165	1000	PASS
CER	CER(16:0)	720090.9650	5000	PASS
DAG	DAG(16:0/16:0)	113207.25	1000	PASS
DAG	DAG(16:0/18:0)	54758.25	1000	PASS
DAG	DAG(16:0/18:1)	81674.75	1000	PASS
DAG	DAG(16:0/18:2)	95766.50	1000	PASS
DAG	DAG(16:0/18:3)	26112	1000	PASS
DAG	DAG(16:0/20:4)	33466.50	1000	PASS
DAG	DAG(16:0/20:5)	30313.50	1000	PASS
DAG	DAG(16:0/22:6)	30900.25	1000	PASS
DCER	DCER(16:0)	162317	10000	PASS
HCER	HCER(16:0)	474741.9950	10000	PASS
LCER	LCER(16:0)	258445.25	10000	PASS
LPC	LPC(16:0)	180609.75	5000	PASS
LPE	LPE(18:0)	108692.50	5000	PASS

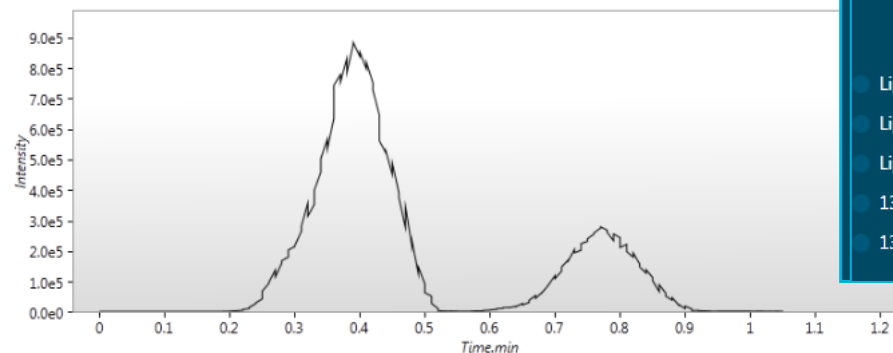
PCT RSD Result:

Class	Name	PCTRSD	Result
DCER	DCER(16:0)	6.54	PASS
HCER	HCER(16:0)	6.73	PASS
LCER	LCER(16:0)	6.20	PASS
LPC	LPC(16:0)	4.76	PASS
LPE	LPE(18:0)	2.97	PASS

Batch Details:

BatchEntryID	Status	Method	DataFile	BatchSampleName
22118	Acquired	Pos Infusion COV 3500 DR10.dam	SelexION Tuning - 20151124152250 - 1.wiff	22118 - BLANK - 201511
22119	Acquired	Pos Infusion COV 3500 DR10.dam	SelexION Tuning - 20151124152250 - 1.wiff	22119 - BLANK - 201511
22120	Acquired	Pos Infusion COV 3500 DR10.dam	SelexION Tuning - 20151124152250 - 1.wiff	22120 - BLANK - 201511
22121	Acquiring	Neg Infusion COV 3500 DR10.dam	SelexION Tuning - 20151124152250 - 2.wiff	22121 - BLANK - 201511
22122	Waiting	Neg Infusion COV 3500 DR10.dam	SelexION Tuning - 20151124152250 - 2.wiff	22122 - BLANK - 201511
22123	Waiting	Neg Infusion COV 3500 DR10.dam	SelexION Tuning - 20151124152250 - 2.wiff	22123 - BLANK - 201511

TIC: from (22121 - BLANK - 20151124152250) of SelexION Tuning - 201511241409



Running 03:53:28

Run Progress

Lipidyzer Batch - 20151114110902

Running: 03:53:28

Method 1.dam



Recent Batches

- Lipidyzer Batch - 20151114110902
- Lipidyzer Batch - 20151114090700
- Lipidyzer Batch - 20151114090425 -
- 13-Nov Ctrl Plasma
- 13-Nov Ctrl Plasma - 1

Lipidomics Workflow Manager: Internal Standard Assembler

Information from Registered Kits

Kit Contents:

Lot Name	Lot Number	Type
Ceramides (CER)	CERISTLPV-100	IS
Cholesterol Ester (CE)	CHEISTLPV-100	IS
Diacylglycerol (DAG)	DAGISTLPV-100	IS
Dihydroceramides (DCER)	DCERISLPV-100	IS
Free Fatty Acids (FFA)	FFAISTLPV-100	IS
Hexosylceramides (HCER)	HCERISLPV-100	IS
Lactosylceramide (LCER)	LCERISLPV-100	IS
Lysophosphatidylcholine (LPC)	LPCISTLPV-100	IS
Lysophosphatidylethanolamine (LPE)	LPEISTLPV-100	IS
Phosphatidylcholine (PC)	PCISTLPV-100	IS
Phosphatidylethanolamine (PE)	PEISTLPV-100	IS
Sphingomyelin (SM)	SMISTLPV-100	IS
Triacylglycerol (TAG)	TAGISTLPV-100	IS

Phosphatidylcholine (PC) Details:

Chemical Name	Concentration
dPC(16:0/16:1)	0.0575
dPC(16:0/18:1)	0.2525
dPC(16:0/18:2)	0.255
dPC(16:0/18:3)	0.065
dPC(16:0/20:3)	0.0725
dPC(16:0/20:4)	0.2775
dPC(16:0/20:5)	0.07
dPC(16:0/22:4)	0.075
dPC(16:0/22:5)	0.0775
dPC(16:0/22:6)	0.145

Lipidomics Workflow Manager: Data Visualization

Project Owner:

LipidyzerExample

Add Export

Project Information

Project Name Description Status Created On Created By Actions

10/21/2015 3:34 PM GCTECH



Heat Map Generate Stats: 10212015

Significance cut-off

0.05

Color range from -2 to 2

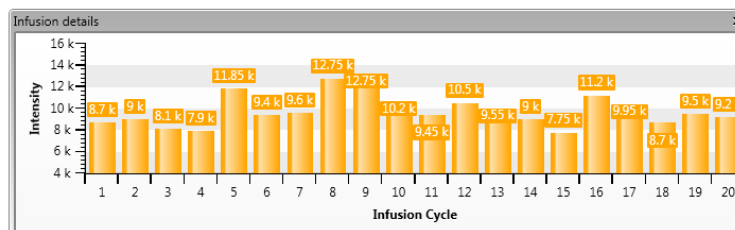
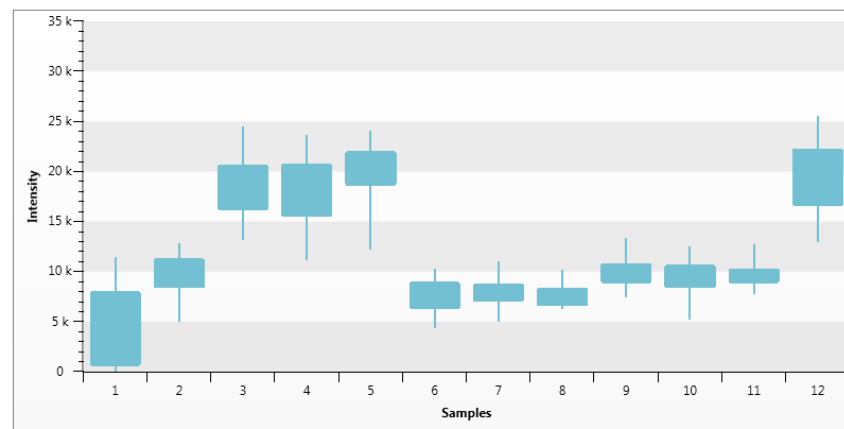
Apply

Choose Export ~ Please Select ~

CLASS	SUB_CLASS	CHEMICAL_NAME	HMDB	KEGG	LIPID_MAPS	HIGH_CE_NORMAL(FOLD)	HIGH_TAG_NORMAL(FOLD)	HIGH_CE_HIGH_TAG(FOLD)
CE		CE(12:0)	HMDB02262		LMST01020001	-0.0469	0.6043	-0.0776
		CE(14:0)	HMDB06725		LMST01020004	1.4199	1.1559	1.2284
		CE(14:1)	HMDB10367		LMST01020021	-3.0216	-0.7978	3.7875
		CE(15:0)	HMDB60057		LMST01020027	1.3759	1.0632	1.2941
		CE(16:0)	HMDB00885	C11251	LMST01020005	1.1187	1.0354	1.0804
		CE(16:1)	HMDB00658		LMST01020006	1.3107	1.1377	1.1521
		CE(17:0)	HMDB60059		LMST01020026	1.3552	1.1253	1.2043
		CE(18:0)	HMDB10368		LMST01020007	1.1659	1.0583	1.1017
		CE(18:1)	HMDB00918		LMST01020003	1.1004	1.0398	1.0582
		CE(18:2)	HMDB00610	C15441	LMST01020008	1.0705	1.0119	1.0579
		CE(18:3)	HMDB10370		LMST01020009	1.3182	1.1823	1.1149
		CE(18:4)	-			-0.8704	-0.3228	2.6962
		CE(20:0)	HMDB06740		LMST01020010	-1.1611	-0.7863	-0.9130
		CE(20:1)	HMDB05193		LMST01020011	0.1312	0.9479	0.1384
		CE(20:2)	-		LMST01020012	3.1452	1.6873	1.8641
		CE(20:3)	HMDB06736		LMST01020013	1.2679	1.1191	1.1329
		CE(20:4)	HMDB06726		LMST01020014	1.1049	1.0261	1.0768
		CE(20:5)	HMDB06731		LMST01020015	1.4038	1.2857	1.0918
		CE(22:0)	HMDB06727		LMST01020016	0.5452	0.6966	0.7827
		CE(22:1)	HMDB10372		LMST01020025	0.6241	0.7762	0.8041
		CE(22:2)	HMDB06737		LMST01020017	0.6587	-0.9859	0.6494
		CE(22:4)	HMDB06729		LMST01020018	0.3507	0.8583	0.4086
		CE(22:5)	HMDB10375		LMST01020031	1.9222	1.3620	1.4113
		CE(22:6)	HMDB06733		LMST01020019	1.2405	1.0706	1.1587
		CE(24:0)	HMDB10376			0.7038		

Raw Data View: CE(14:0)

Select Analyte: CE(14:0)

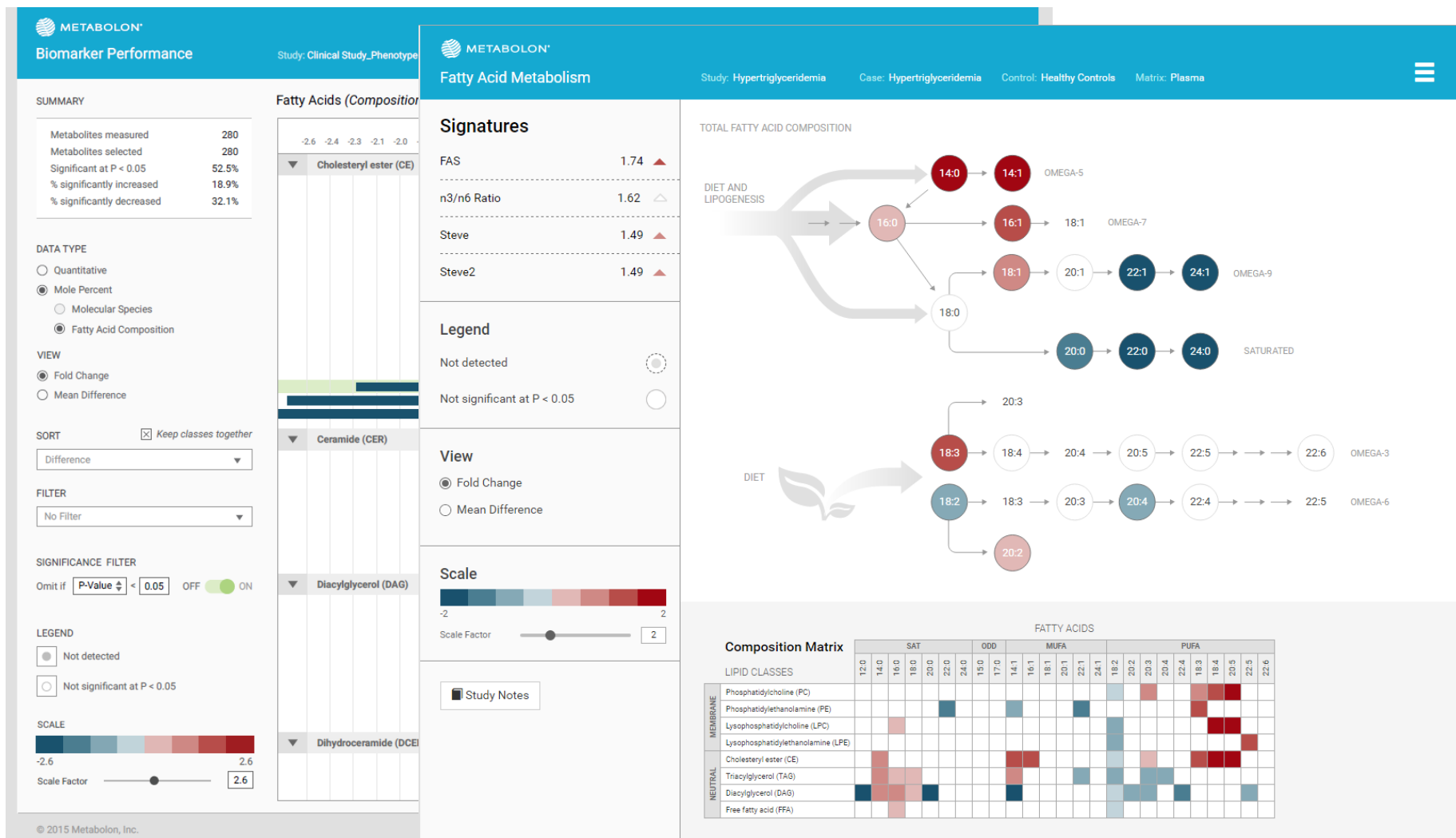


Heat Maps and Statistics

Raw data

Online Pathway Maps and Discovery Tools

Powered by Metabolon



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 ₅ P	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 ₅ P	761.50
PE(20:0/17:0)	1-eicosanoyl-2-heptadecanoyl-glycero-3-phosphoethanolamine	C ₄₂ H ₈₄ NO ₅ P	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 ₅ P	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 ₅ P	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 ₅ P	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 ₅ P	761.50
PE(21:0/16:0)	1-heneicosanoyl-2-hexadecanoyl-glycero-3-phosphoethanolamine	C ₄₂ H ₈₄ NO ₅ P	761.59

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

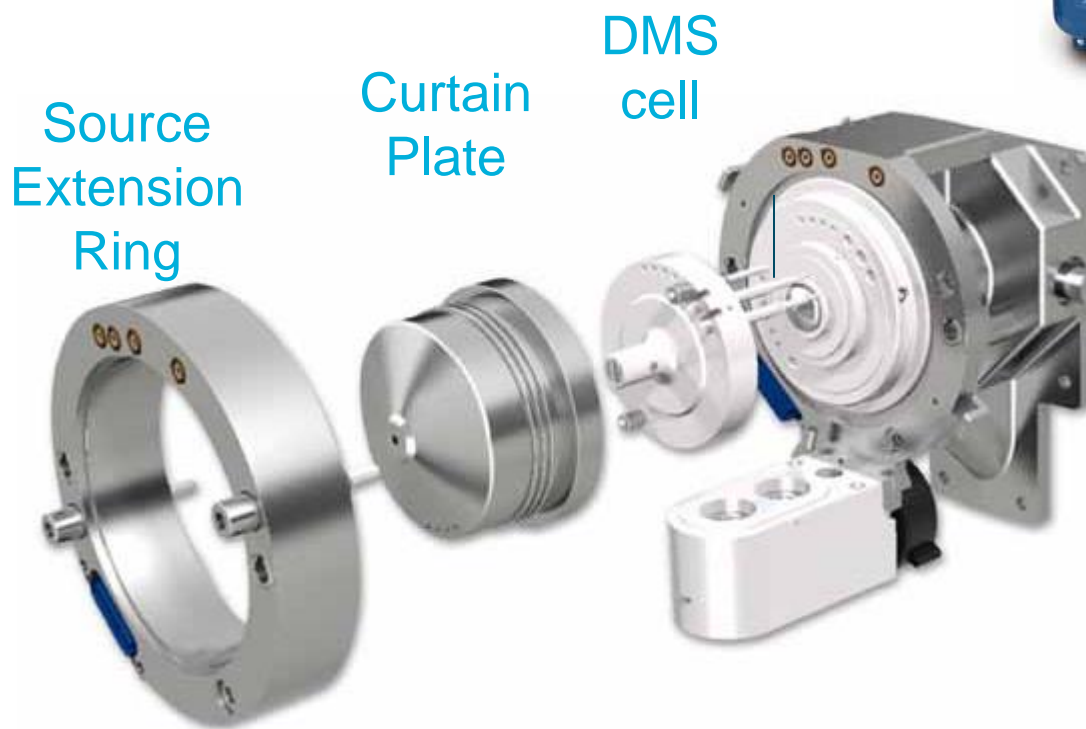
108 Lipids identified

Showing page 1 of 3 Results: [1](#) [2](#) [3](#) [Next](#) Showing results 1 to 50 of 108

Lipidyzer™ Hardware Configuration

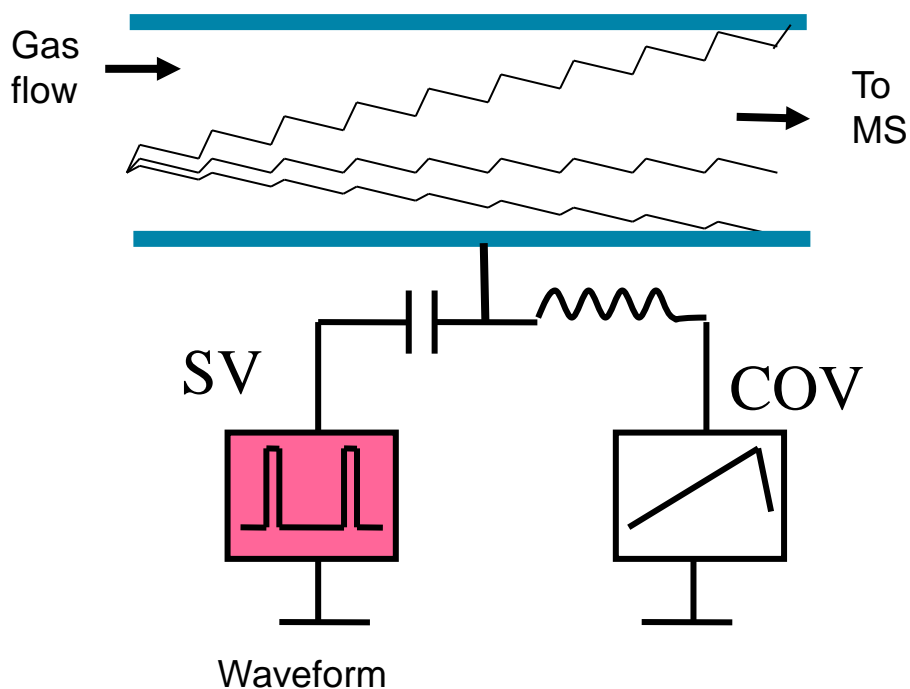
QTRAP® 5500 System with SelexION™ Technology

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



Differential Mobility Spectrometry (DMS)

SelexION™ Technology

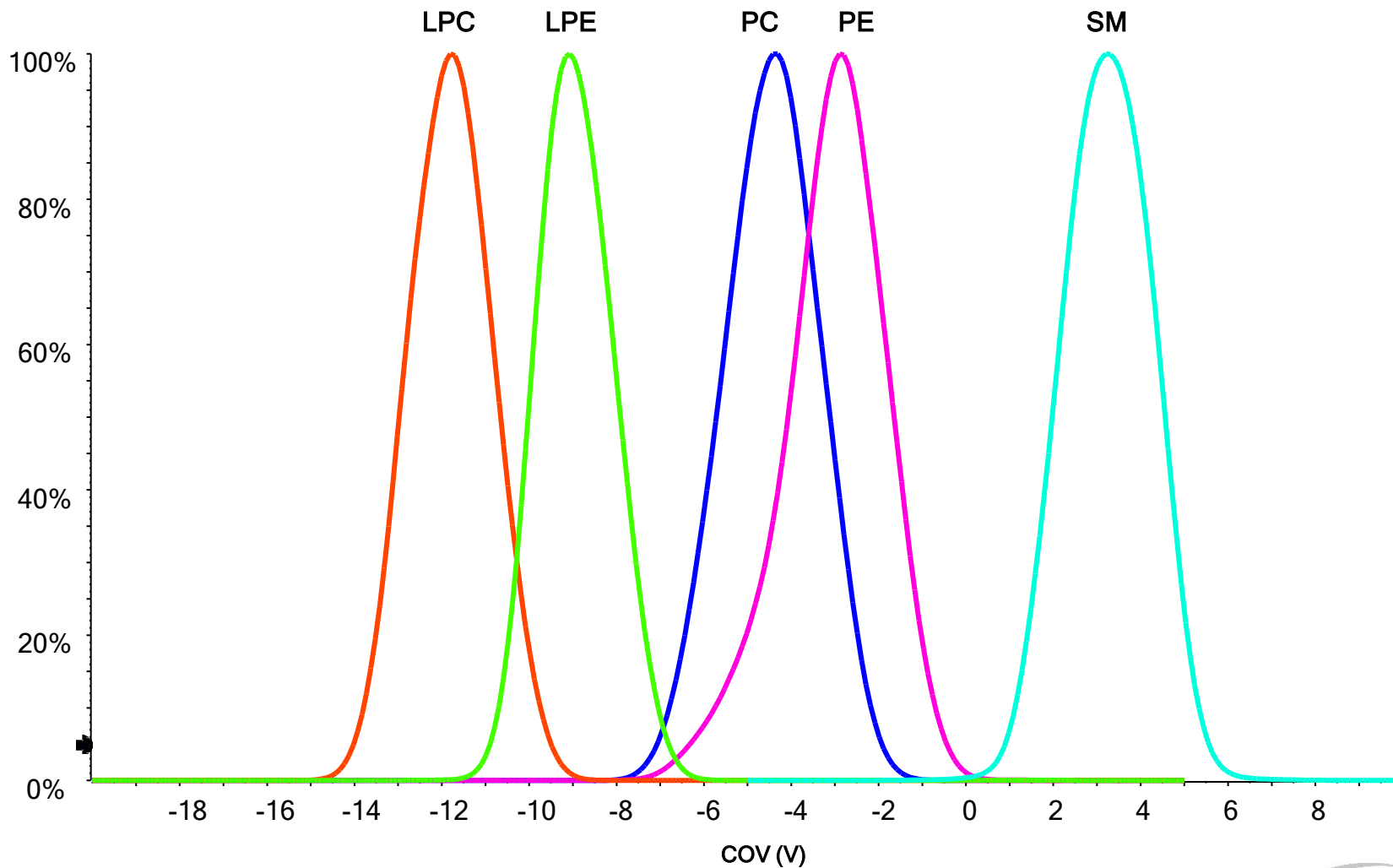


DMS Dimensions
1x10x30 mm

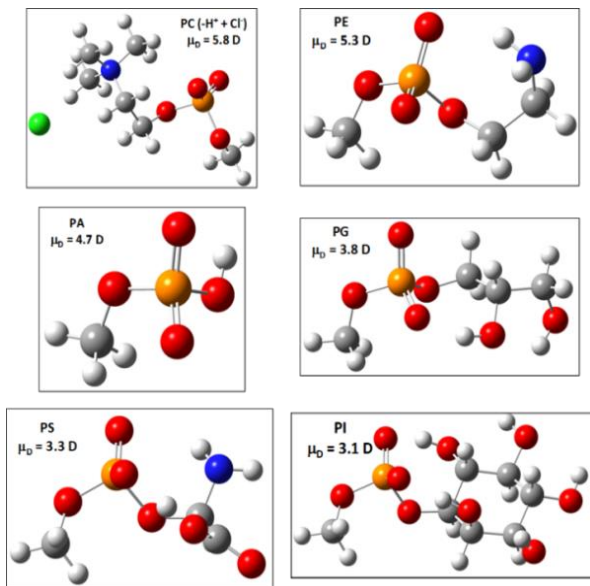
- Planar geometry
- Gas flow towards MS draws ions (transport gas)
- Asymmetric waveform applied which alternates between high field, $K(E)$ and low field, $K(0)$ – separation voltage (SV)
 - Moves charged ion back and forth between plates
 - Ion will have net drift based on its high and low field mobility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

Separation of Lipid Classes Using SelexION™

Negative Ion Mode

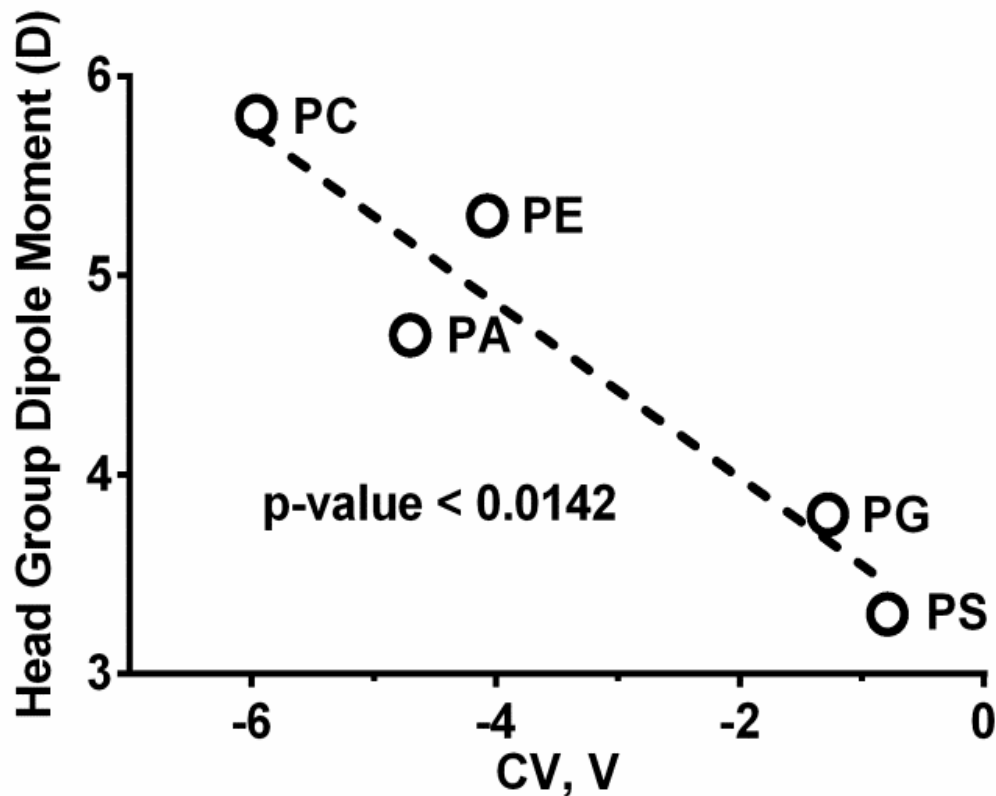


Relationship Between Dipole Moment and COV



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

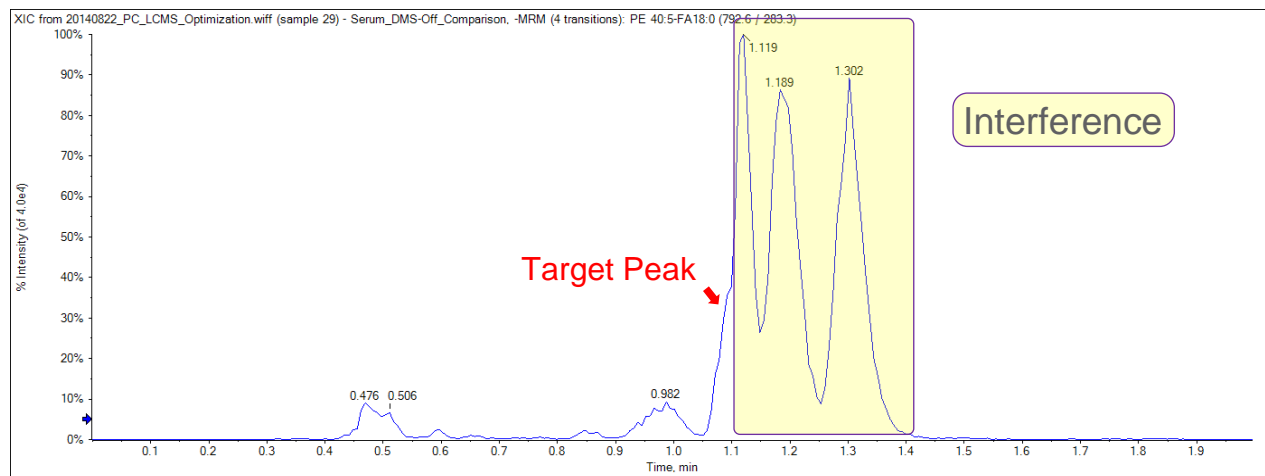


Molecules that have different dipole moments can be separated by DMS

Isobaric Interference in a Complex Sample

Isobaric Interference Makes 'unassisted' MRM Analysis by Infusion Non-Specific.

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



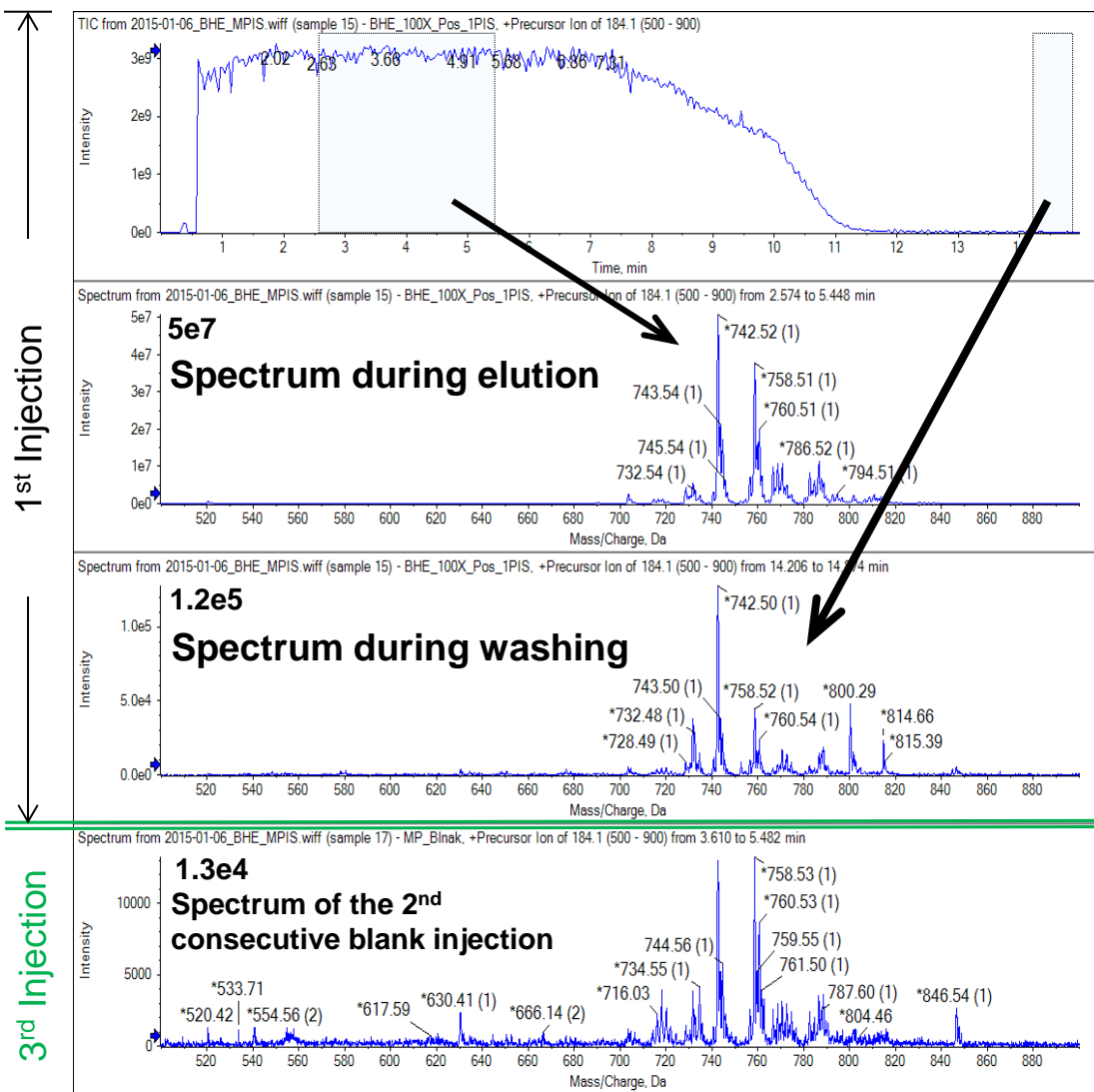
SelexION™ Device Off

Multiple different lipids
have the same MRM
transition:

Isobaric Interference

Sample Carry Over Between Injections

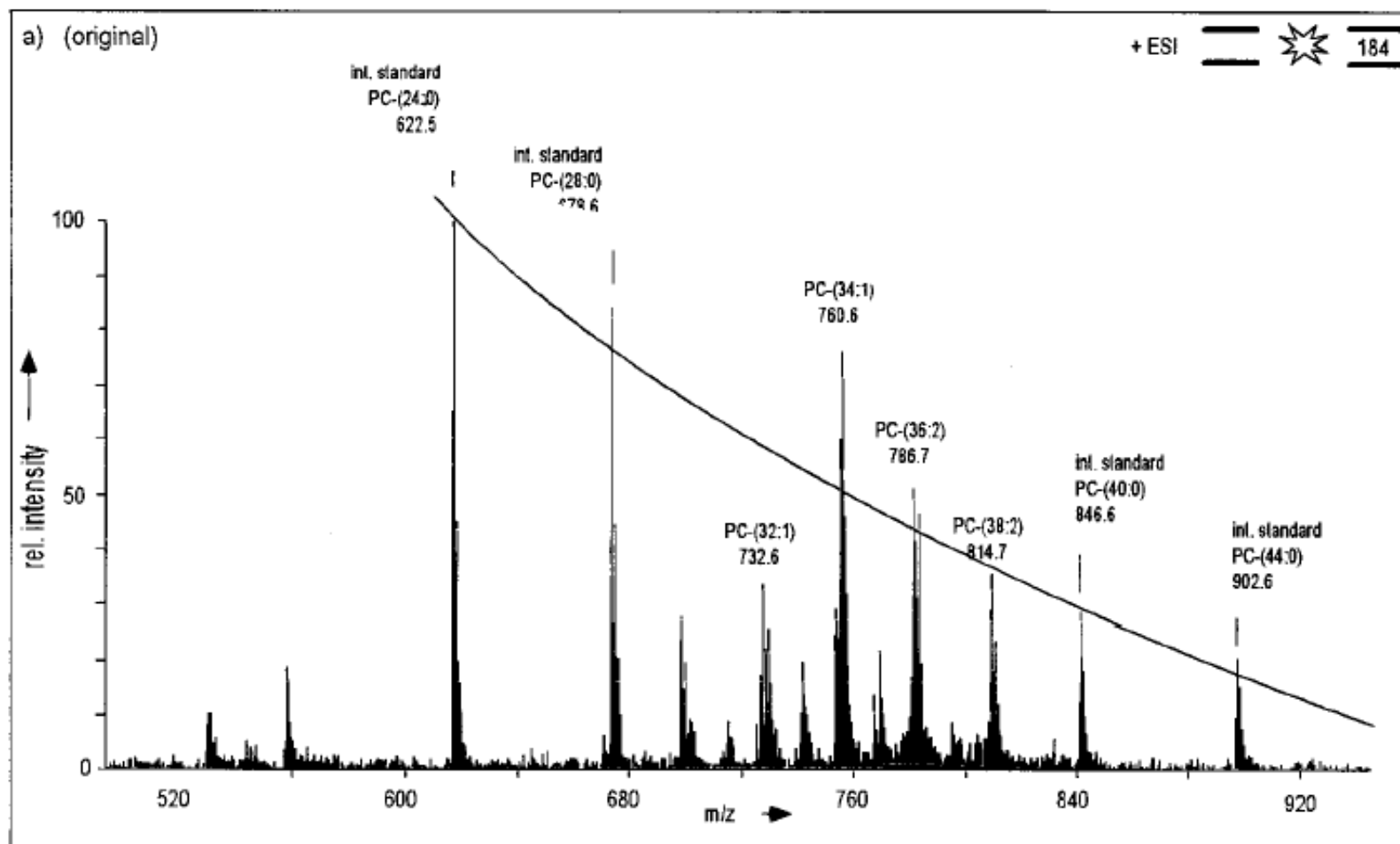
Use of PEEKSIL tubing



- The use of PEEKSIL tubing dramatically reduces carryover compared to regular PEEK tubing
- A wash step at the end of acquisition is enabled into the method by ramping the flow rate up to 30µl/min
- Background level reduced to very low level in the same run

Unequal Fragmentation Efficiency of Lipids

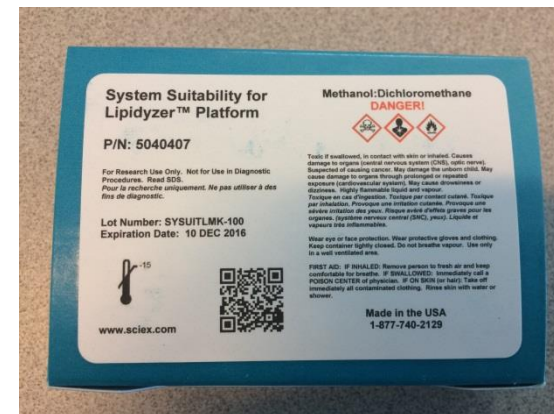
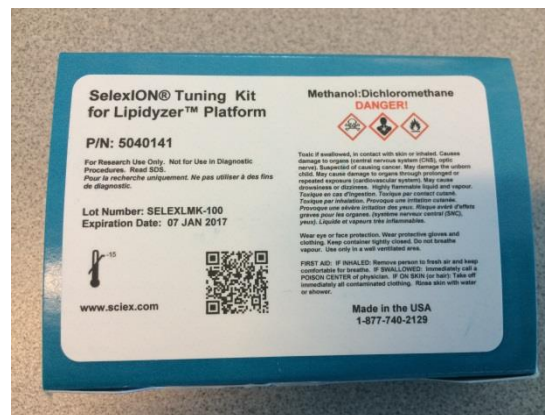
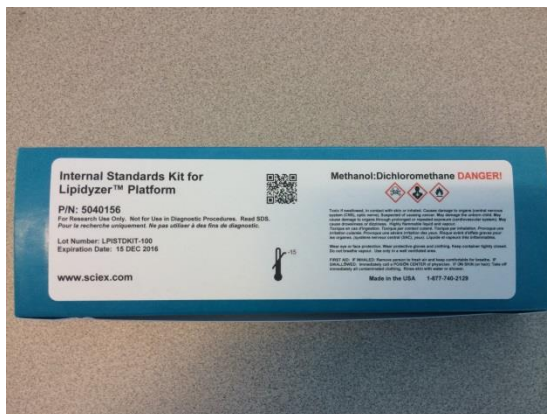
Diversity of Fatty Acid Chain Lengths and Degrees of Unsaturation Result in Differential Fragmentation Efficiency which Impacts Quantitation



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

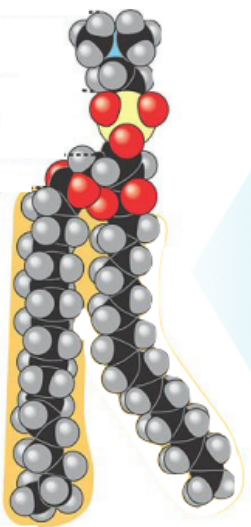
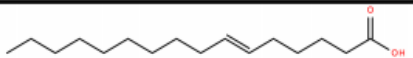
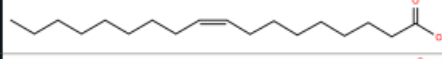


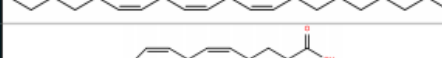

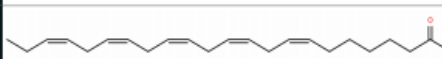
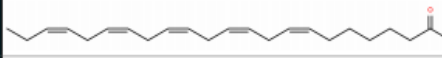
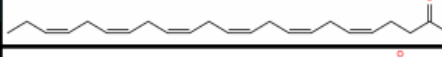
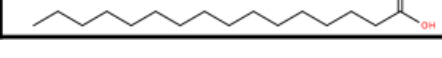
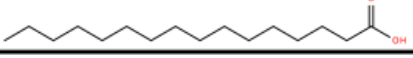
Lipidyzer Kits

Novel Internal Standards



A Broad Range of Internal Standards to Normalize Quantitative Data

Multiple Internal Standards that Reflect the Diversity of Lipid Molecular Species

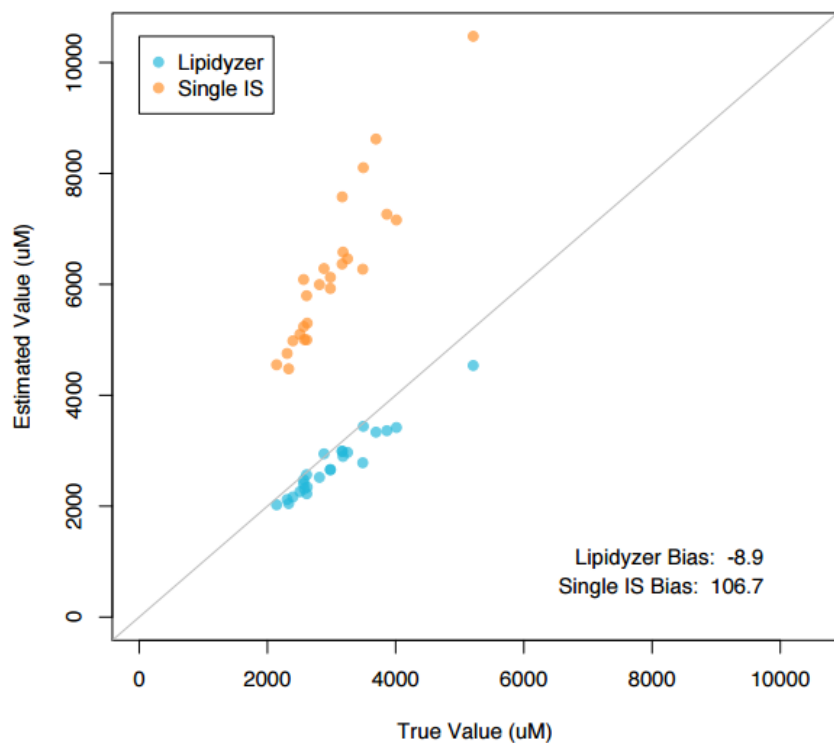
PHOSPHATIDYLCHOLINE (PC) INTERNAL STANDARD MIX				
	STRUCTURE	FATTY ACID	POS	%
		FA16:1 - Palmitoleic acid	sn-2	5
		FA18:1 - Oleic acid	sn-2	20
		FA18:2 - Linoleic acid	sn-2	20
		FA18:3 - α -Linolenic acid	sn-2	5
		FA20:3 - Dihomo- γ -linolenic acid	sn-2	5
		FA20:4 - Arachidonic acid	sn-2	20
		FA20:5 - Eicosapentaenoic acid	sn-2	5
		FA22:4 - Eicosatetraenoic acid	sn-2	5
		FA22:5 - Docosapentaenoic acid	sn-2	5
		FA22:6 - Docosahexaenoic 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

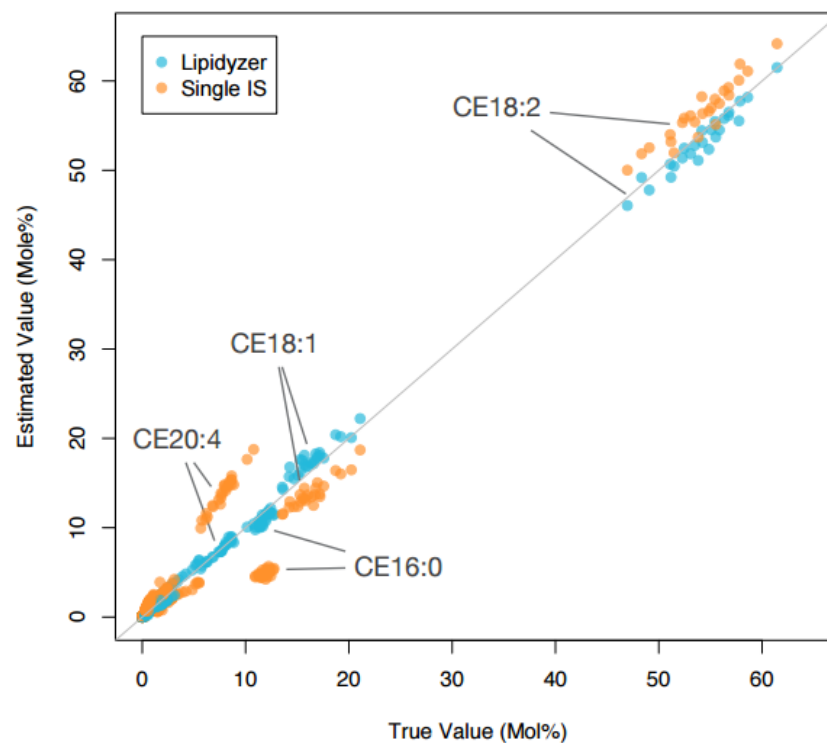
The Lipidizer™ Eliminates Quantitative Bias

Multiple internal standards per class provide accurate quantitation

CHOLESTERYL ESTERS (QUANTITATIVE)



CE FATTY ACID COMPOSITION (MOLE%)



Conclusions

Three Key Elements of the Platform:

1. Software: Ease of Use

- Log samples, create batches
- Automated calculation of chemistries, tuning and system tests

2. SelexION: Specificity

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

3. Internal Standards: Quantitation

- Ensure spray stability
- Minimize carryover
- Neutralize quantitative bias

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