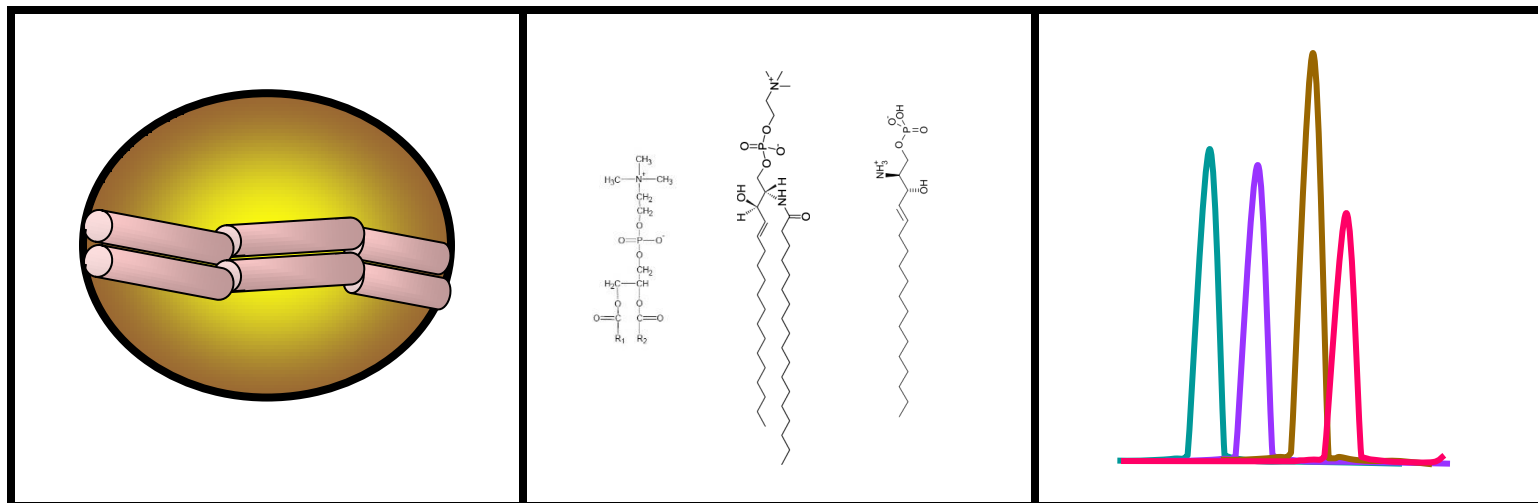


Using Skyline for Targeted Lipidomics

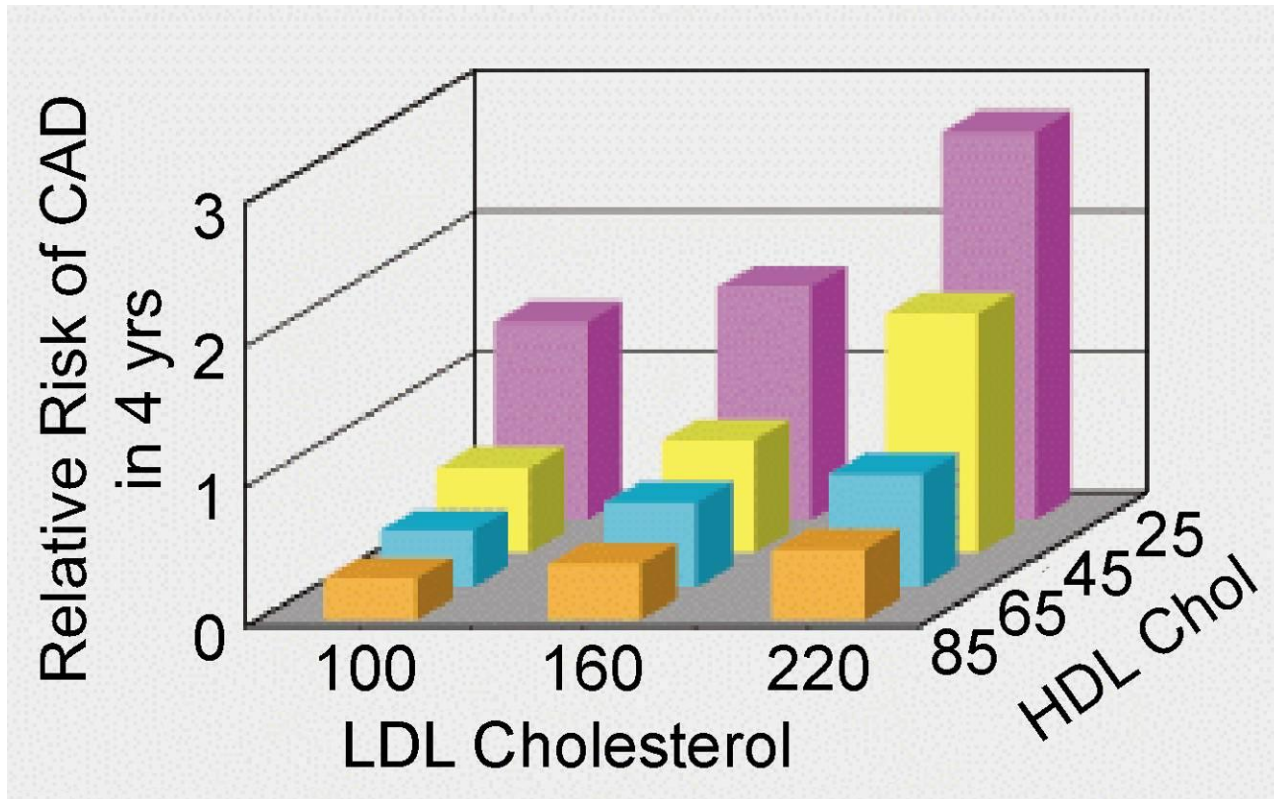


User's Group Meeting
June 9, 2013

Hari Nair, PhD
Andy Hoofnagle, MD PhD
University of Washington

HDL is the Good Cholesterol

The Framingham Heart Study



HDL Cholesterol is Not the Whole Story

Torcetrapib (ILLUMINATE)

60% increase in HDL cholesterol
40% increase in poor outcomes

Dalcetrapib (dal-OUTCOMES)

35% increase in HDL cholesterol
No improvement in outcomes

Niacin (AIM-HIGH)

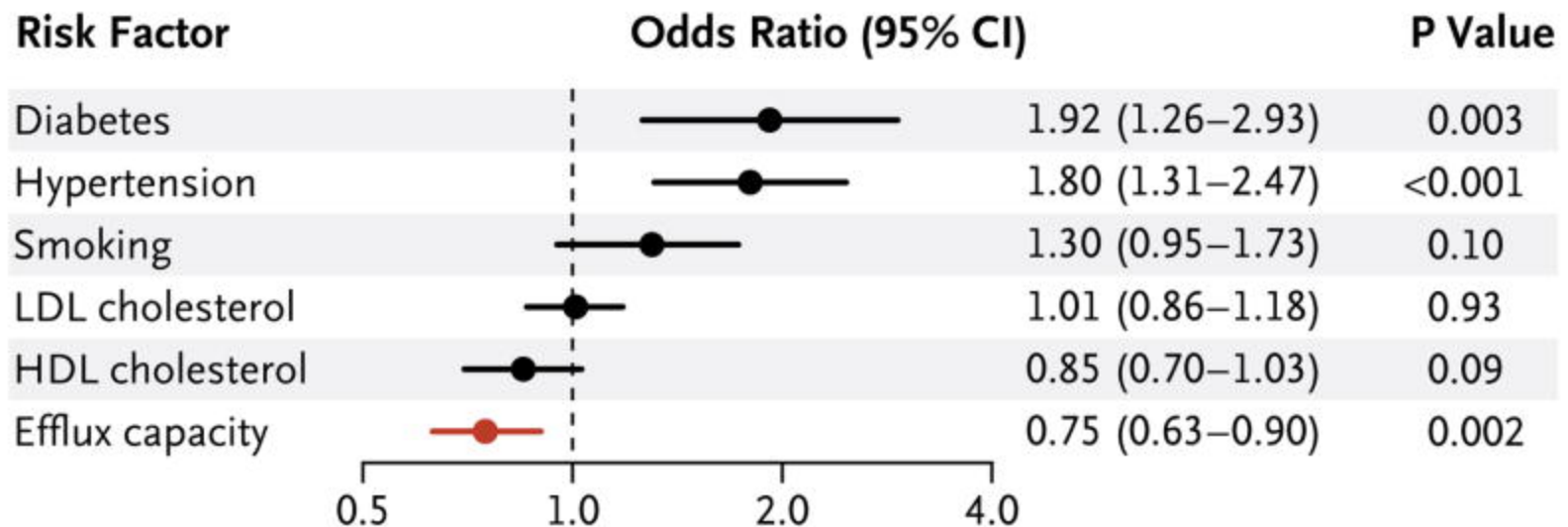
30% increase in HDL cholesterol
Twice as many strokes

Three HDL-C raising studies terminated early

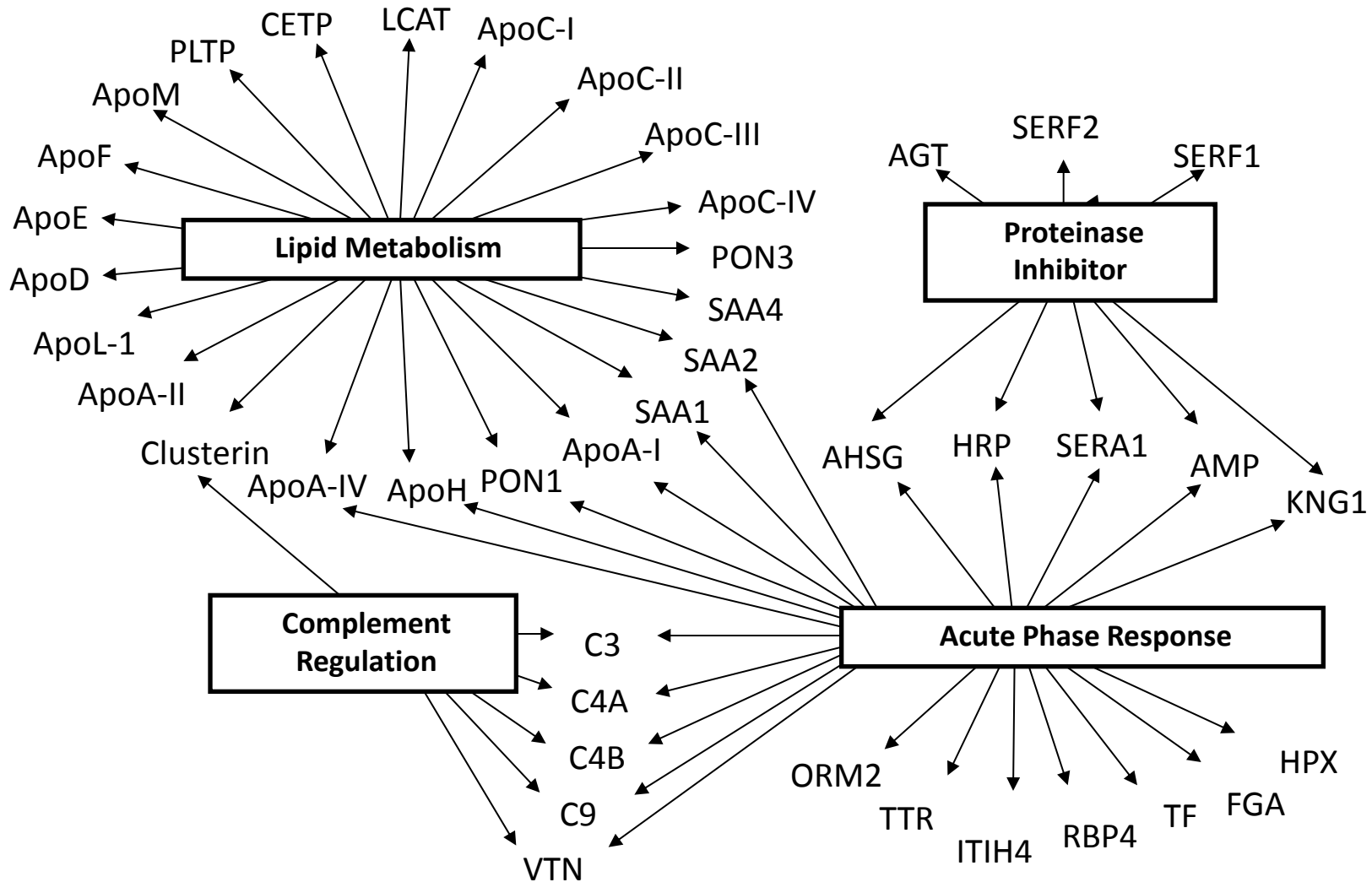
HDL Does More than Just Carry Lipids

Functional Assays of HDL

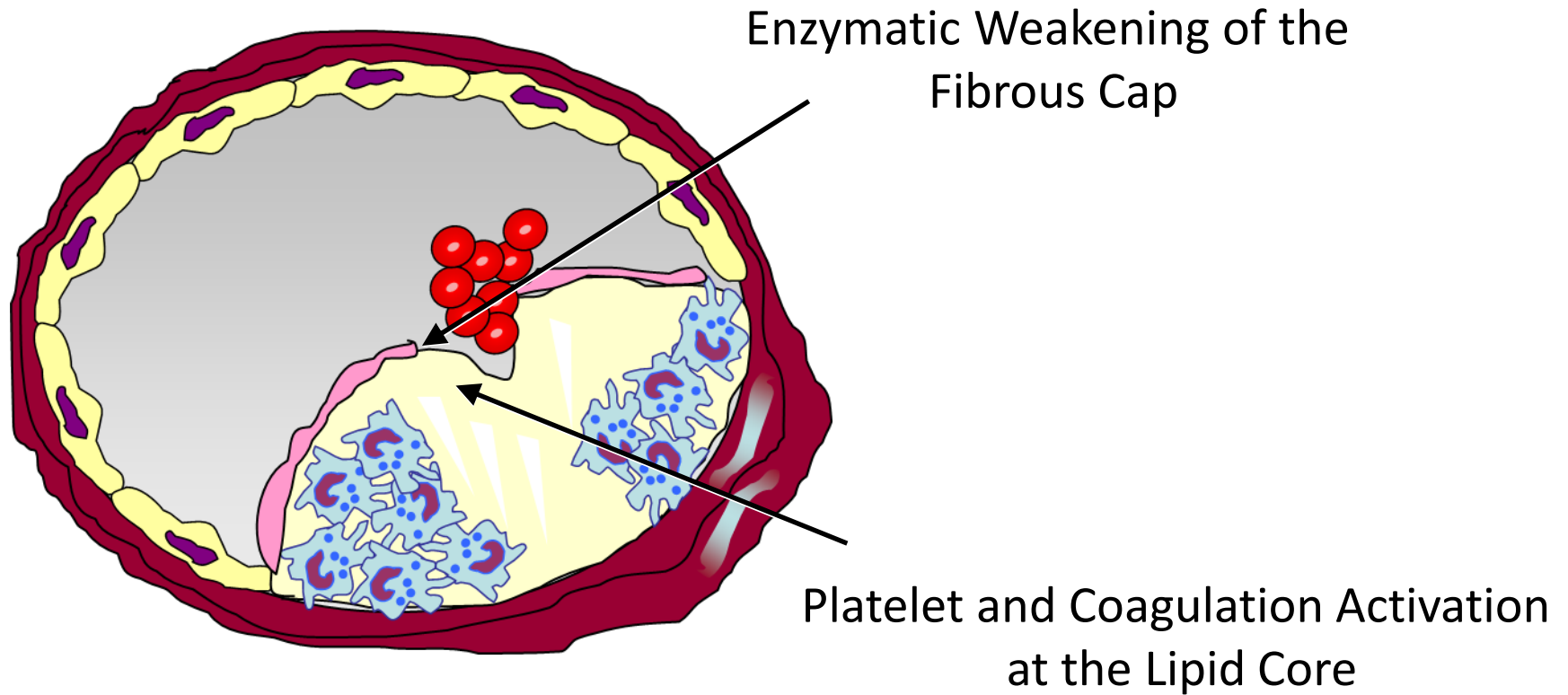
Particles remove lipids from lipid-laden macrophages
HDL activity in vitro is predictive of coronary artery disease



The HDL Proteome is Vast



Thrombotic Occlusion of Coronary Artery



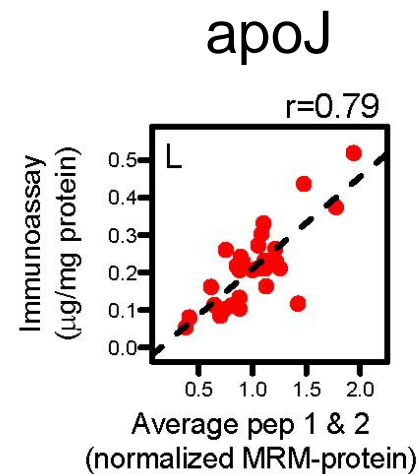
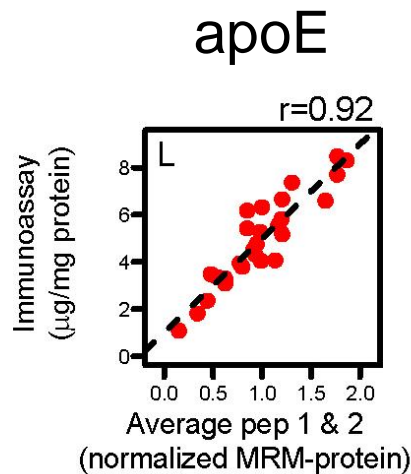
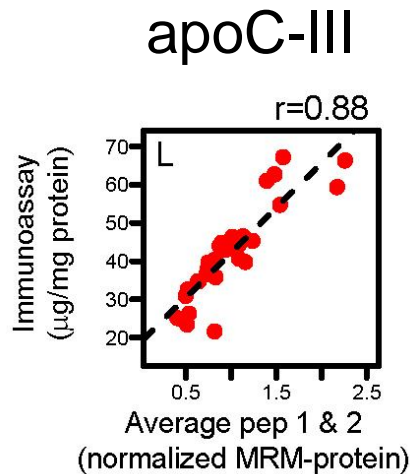
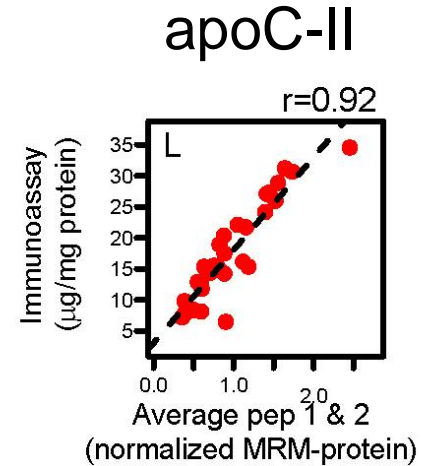
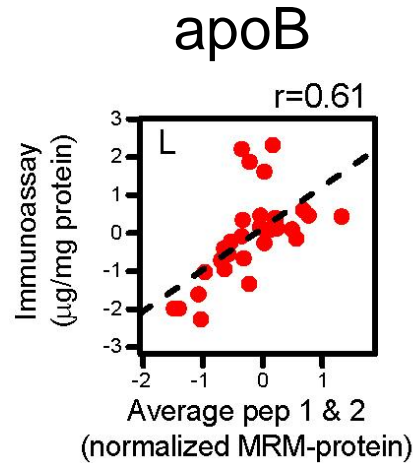
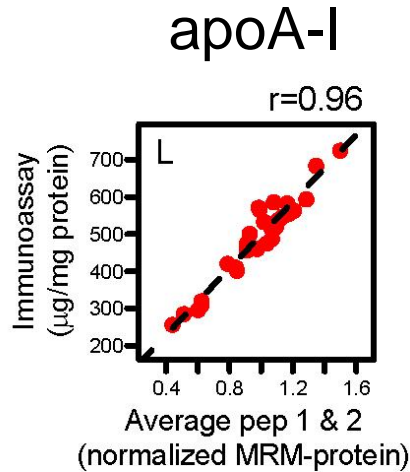
Acute Coronary Syndrome and Sudden Death

Proteolysis

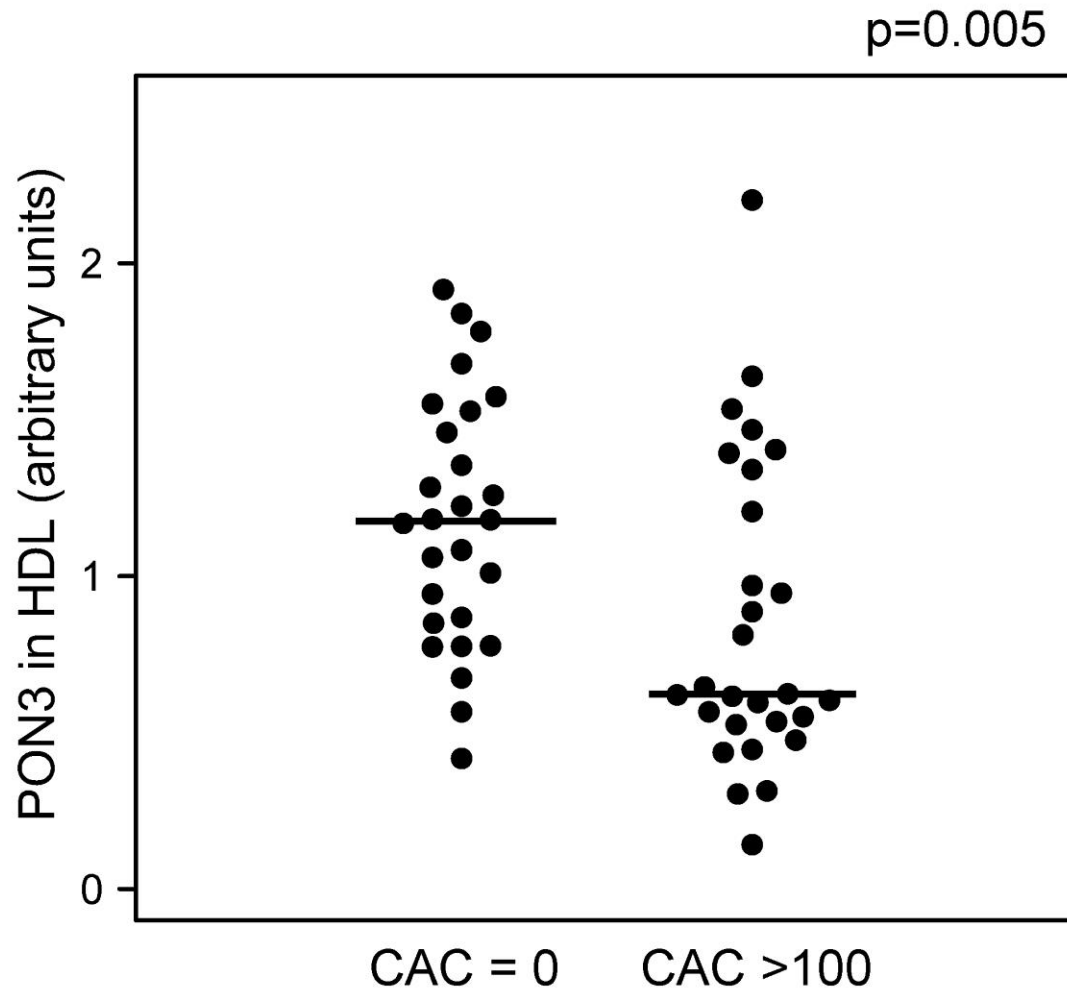
Complement Activation

Targeted Proteomics for HDL

Internal Standard Protein

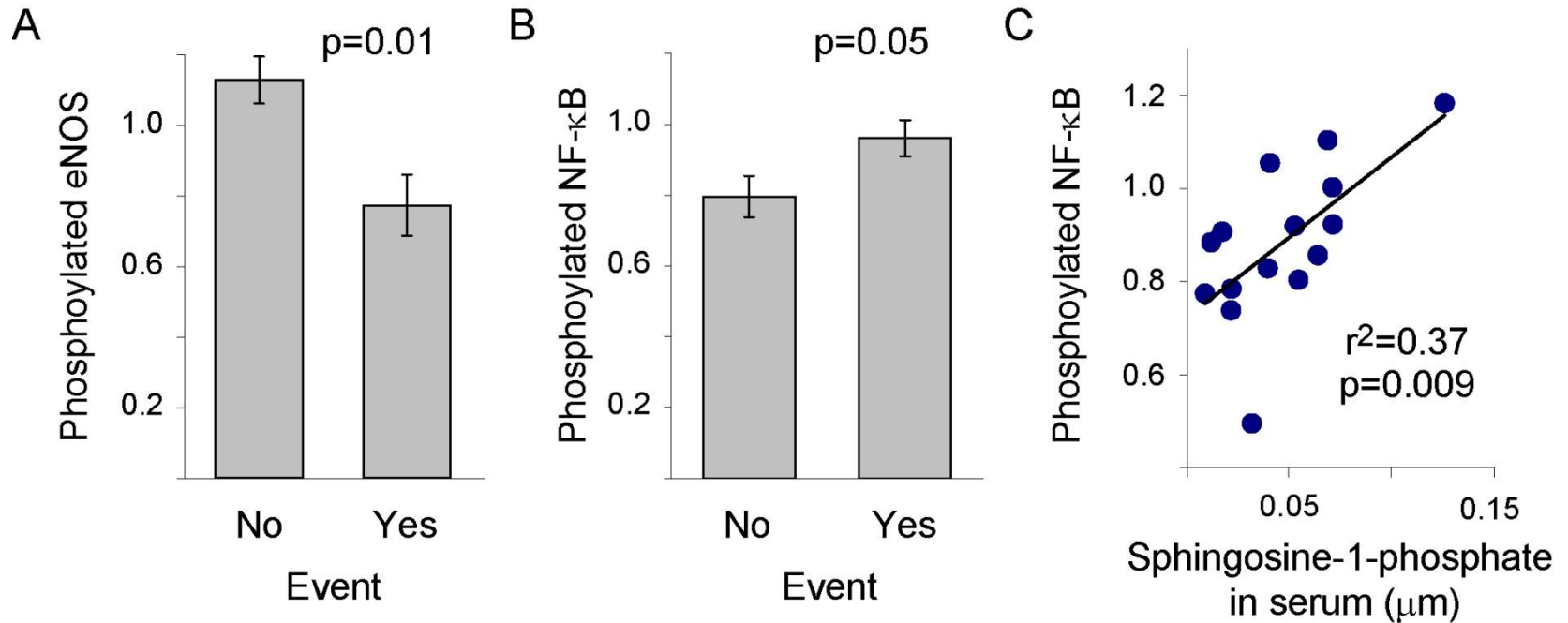


Paraoxonase 3 is Depleted in Patients with Type 1 Diabetes and Artery Calcification



Endothelial Cell Function

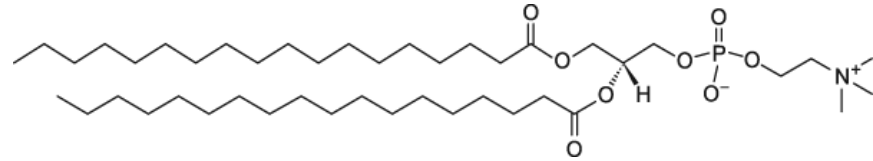
Implicating HDL Lipids



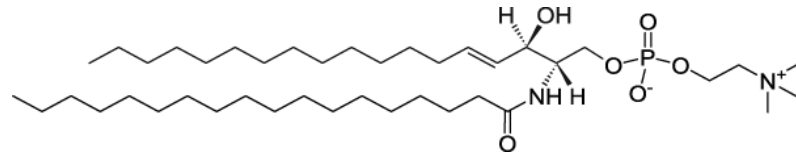
Thinking Beyond Cholesterol

Phospholipids and sphingolipids

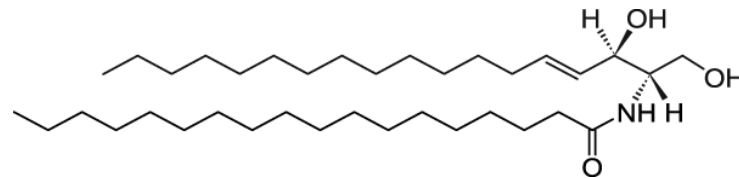
Phosphotidylcholine



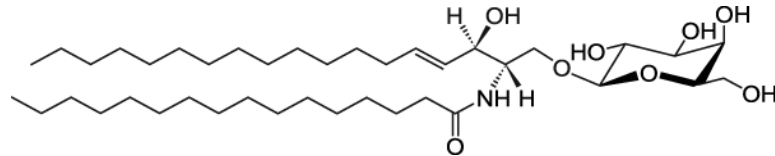
Sphingomyelin



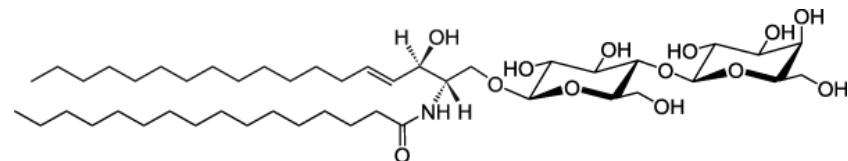
Ceramide



Glucosylceramide

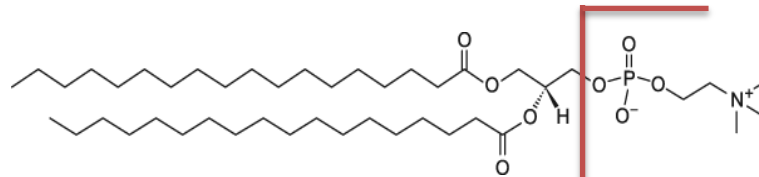


Lactosylceramide



Common Fragment Ions

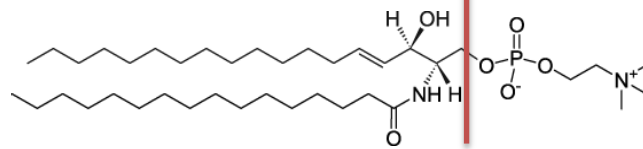
Phosphatidylcholine



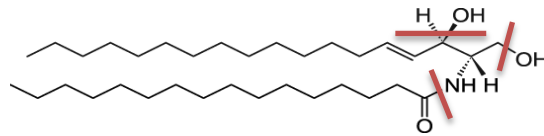
Common Head Group
Fragment (m/z)

184

Sphingomyelin

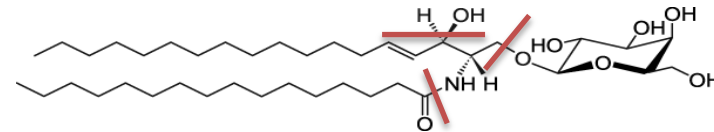


Ceramide



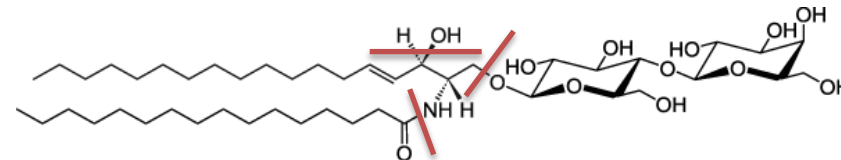
Common Backbone
Fragment (m/z)

Glucosylceramide

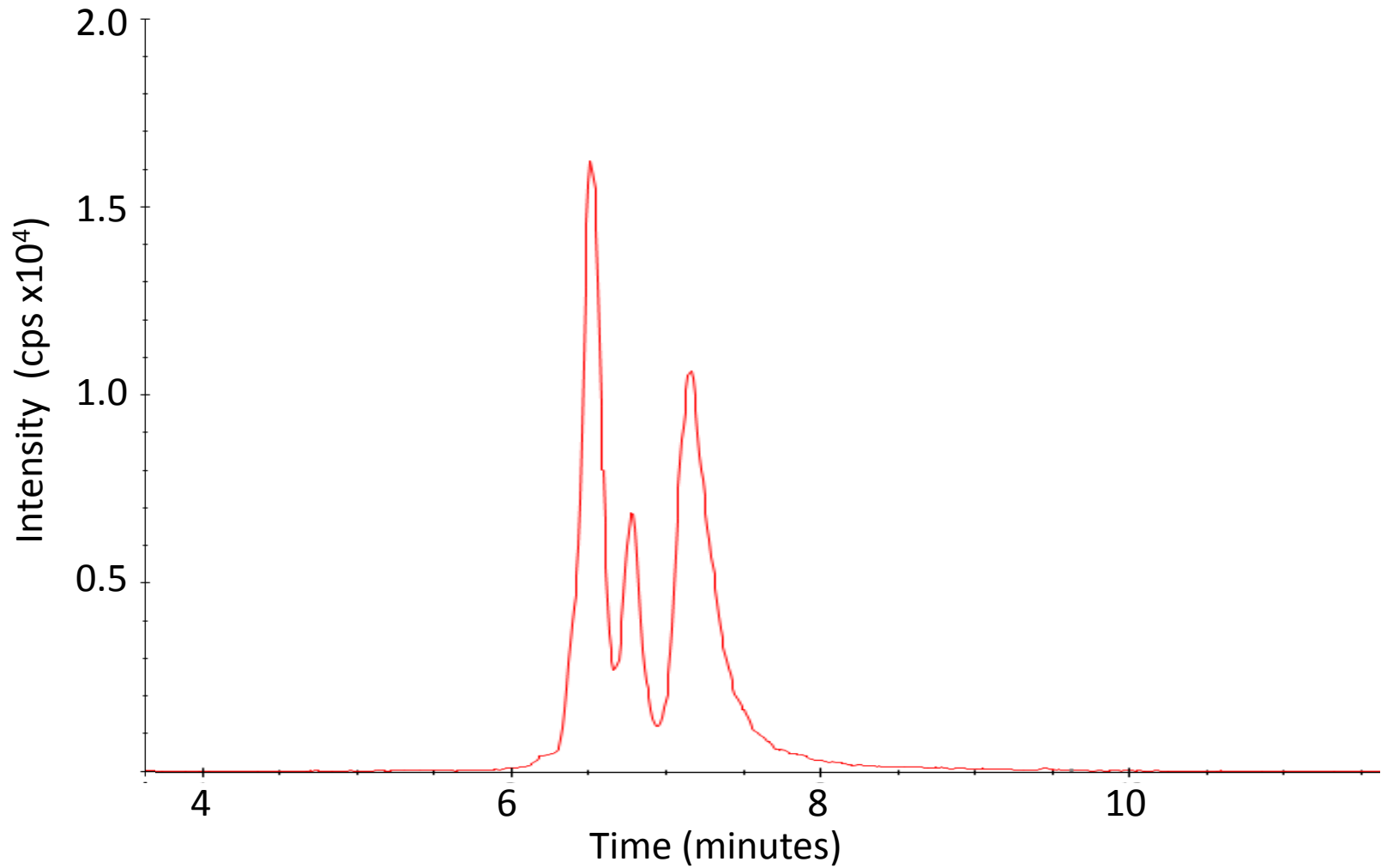


264

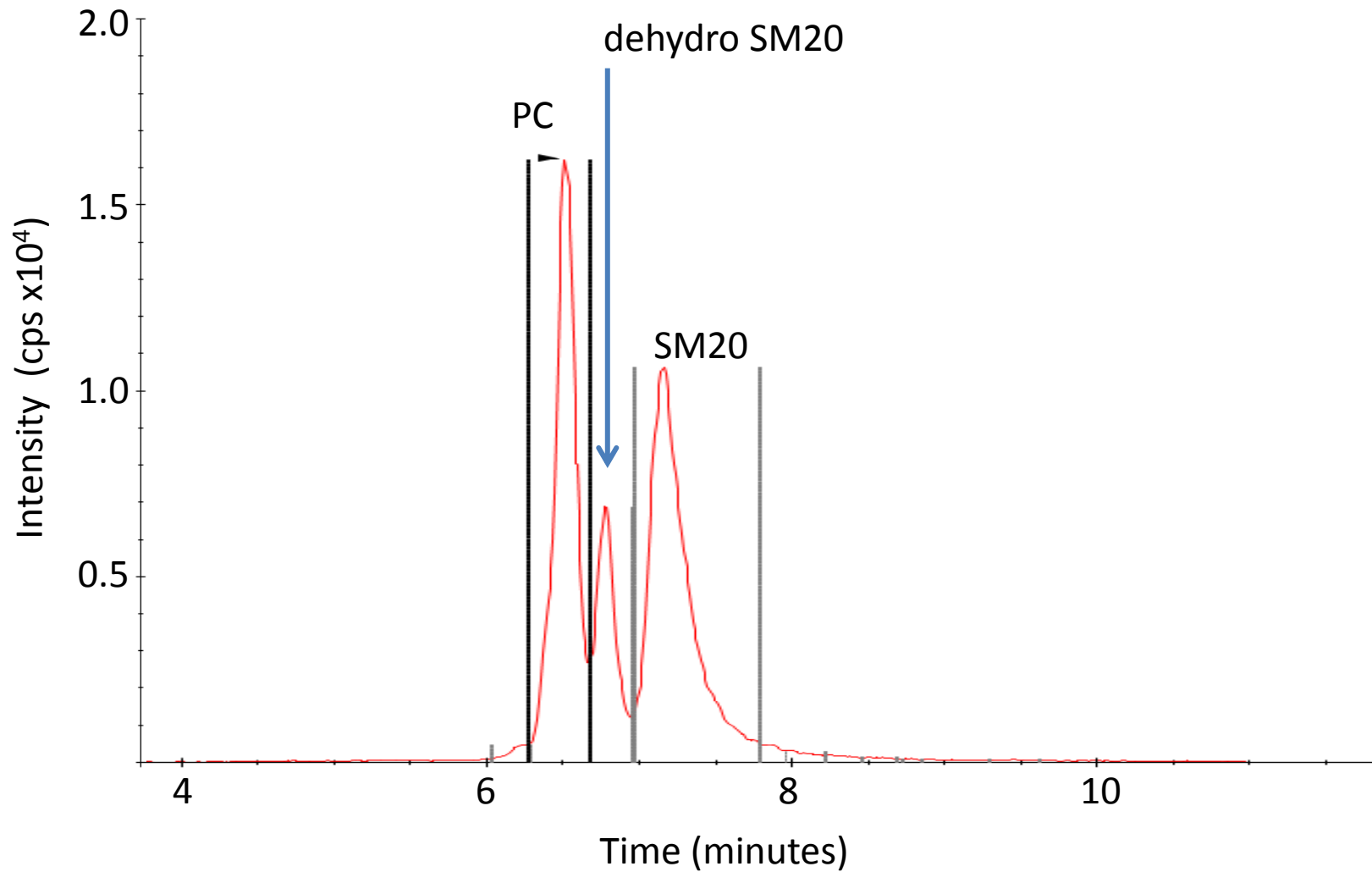
Lactosylceramide



Typical Chromatogram



Biological Complexity



Step 1: Design Data Processing Method

File View Zoom Options GoTo Help

Identification Detection Calibration Levels System Suitability Peak Purity

Name: <New> Retention time: Expected (min): 1.00 Window (sec): 30.00

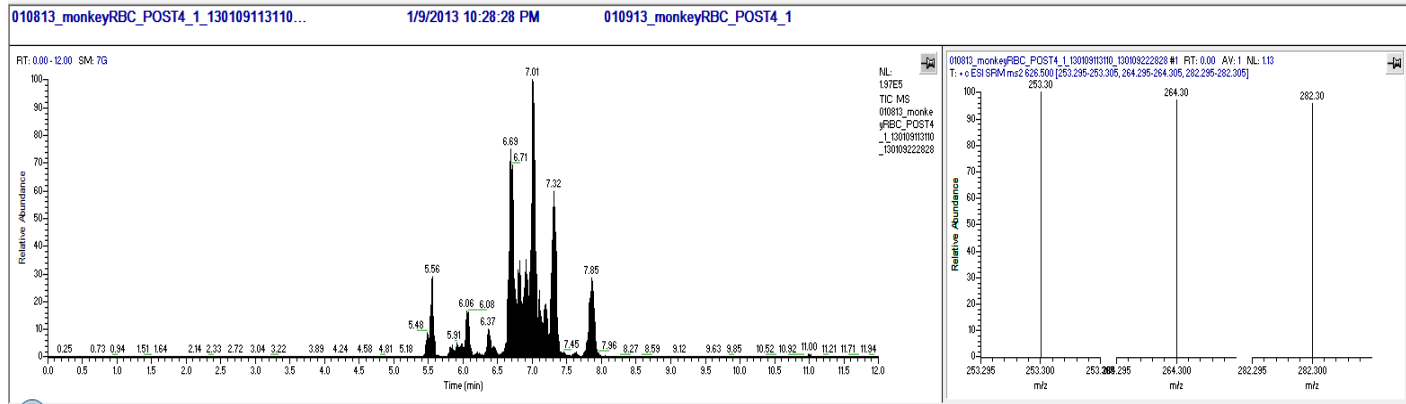
Detector type: MS Peak Detect: ICIS Use as RT reference View width (min): 5.00

Filter: 253.305, 264.295-264.305, 282.295-282.305

Trace: + c ESI SRM ms2 626.500 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 682.600 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 708.600 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 710.600 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 738.600 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 766.700 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 788.600 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 792.700 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 794.700 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 808.700 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 820.700 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 822.700 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 844.600 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 870.600 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 872.600 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 900.700 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 928.700 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 954.700 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 956.700 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 970.800 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 982.800 [253.295-253.305, 264.295-264.305, 282.295-282.305]
+ c ESI SRM ms2 984.800 [253.295-253.305, 264.295-264.305, 282.295-282.305]

OK

Assign transitions



Step 2: Design Data Processing Method

File View Zoom Options GoTo Help

Identification Detection Calibration Levels System Suitability Peak Purity

Name: LACCEP24

Retention time
Expected (min): 6.99 Window (sec): 30.00

Detector type: MS Peak Detect: ICIS

Filter: + c ESI SRM ms2 956.700 [253.295-253.305]

Trace: TIC

Mass (m/z):

Use as RT reference View width (min): 5.00

Adjust using:

Keys:

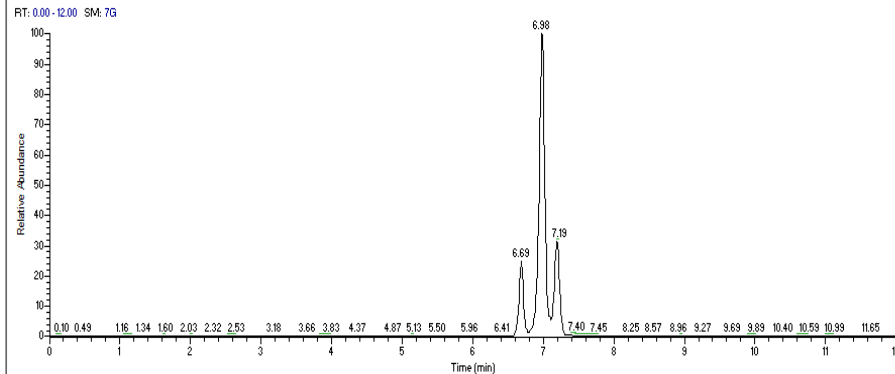
OK Cancel Save As Default Help

Set RT and Integration window

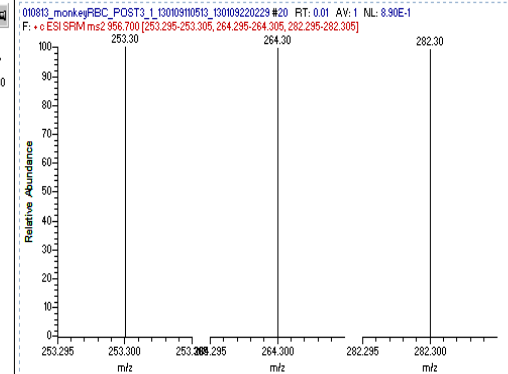
010813_monkeyRBC_POST3_1_130109110513...

1/9/2013 10:02:29 PM

010913_monkeyRBC_POST3_1



NL: 147E5
TIC F: + c ESI SRM ms2 956.700
[253.295-253.305, 264.295-264.305,
282.295-282.305] MS
010813_monkeyRBC_POST3_1_130
10910512_130109220229



Step 3: Design Data Processing Method

File View Zoom Options GoTo Help

ICIS Peak Integration

Smoothing points: 7

Baseline window: 80

Area noise factor: 5

Peak noise factor: 10

Constrain peak width

Peak height (S/N): 5.0

Tailing factor: 1.0

ICIS Peak Detection

Highest peak

Nearest RT

Minimum peak height (S/N): 3.0

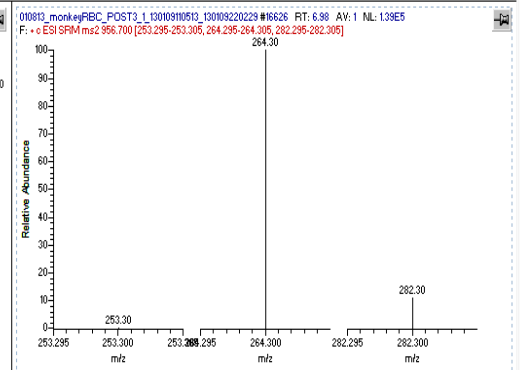
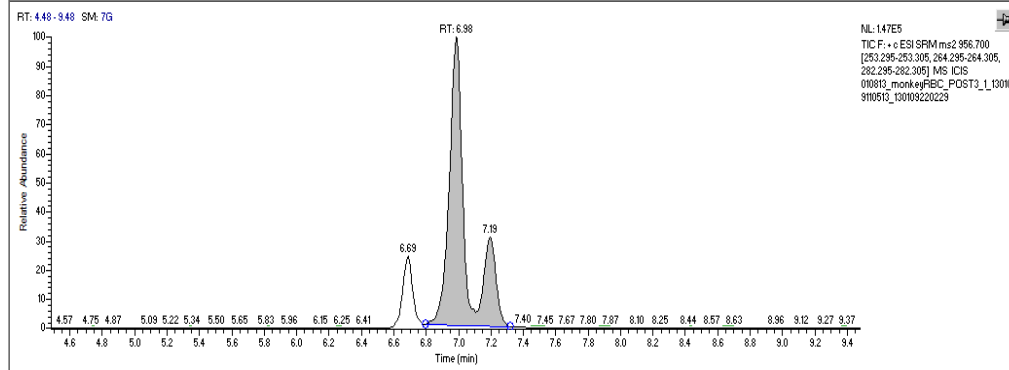
OK Cancel Save As Default Advanced... Flags... Help

Assign smoothing parameters

010813_monkeyRBC_POST3_1_130109110513...

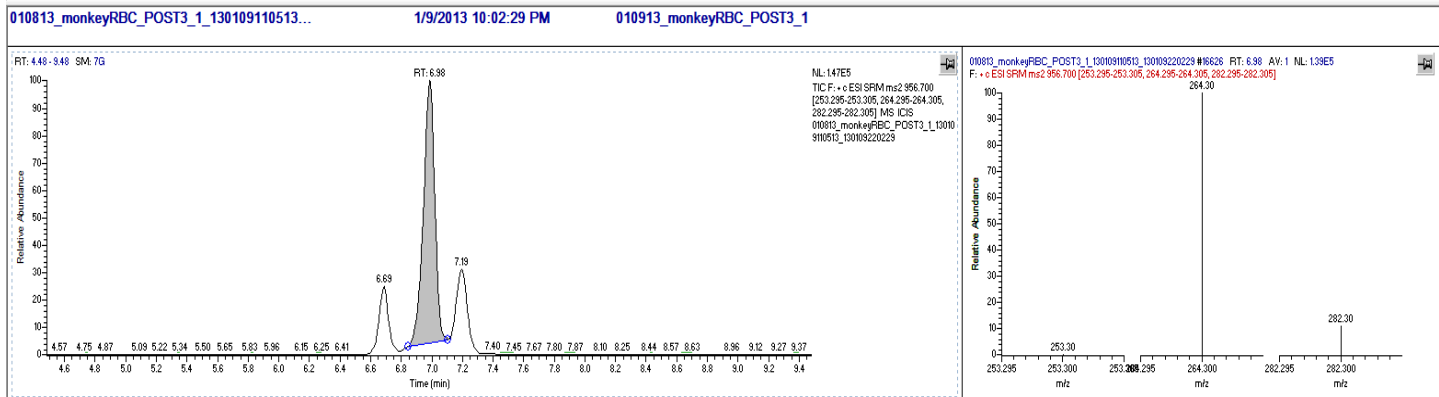
1/9/2013 10:02:29 PM

010913_monkeyRBC_POST3_1



Step 4: Design Data Processing Method

The screenshot shows the 'Detection' tab of a software interface. It contains two main sections: 'ICIS Peak Integration' and 'ICIS Peak Detection'.
ICIS Peak Integration:
Smoothing points: 7
Baseline window: 20
Area noise factor: 5
Peak noise factor: 10
 Constrain peak width
Peak height: 5.0
Tailing factor: 1.0
ICIS Peak Detection:
 Highest peak
 Nearest RT
Minimum peak height (S/N): 3.0
At the bottom of the settings are buttons for 'OK', 'Cancel', 'Save As Default', 'Advanced...', 'Flags...', and 'Help'.
A large red text overlay 'Manually pick peak' is positioned over the right side of the interface.



Step 5: Process the Data

File Edit Change Actions View GoTo Help



Status Acquisition Queue

Run Manager

- Waiting For Devices
- Sequence:
- Sample Name:
- Working On:
- Position:
- Raw File:
- Inst. Method:
- Accelea Open AS
 - Ready to Download
 - Accelea 1250 Pump
 - Stand By
 - TSQ Vantage
 - Stand By

	SampleName	File Name	Position	Inj Vol	Path	Inst Meth	Proc Meth
1	010813_monkeyRBC_PRE1_1	010813_monkeyRBC_PRE1_1	CSik1-01:1	5.000	C:\Xcalibur\LIPID\VERIFICATION	C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
2	010813_monkeyRBC_PRE1_2	010813_monkeyRBC_PRE1_2	CSik1-01:1	5.000	C:\Xcalibur\LIPID\VERIFICATION	C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313b	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
3	010813_monkeyRBC_PRE1_3	010813_monkeyRBC_PRE1_3	CSik1-01:1	5.000	C:\Xcalibur\LIPID\VERIFICATION	C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_050313C	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
4	010813_monkeyRBC_POST1_1	010813_monkeyRBC_POST1_1	CSik1-01:2	5.000	C:\Xcalibur\LIPID\VERIFICATION	C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
5	010813_monkeyRBC_POST1_2	010813_monkeyRBC_POST1_2	CSik1-01:2	5.000	C:\Xcalibur\LIPID\VERIFICATION	C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313b	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
6	010813_monkeyRBC_POST1_3	010813_monkeyRBC_POST1_3	CSik1-01:2	5.000	C:\Xcalibur\LIPID\VERIFICATION	C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_050313C	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
7	010813_monkeyRBC_PRE2_1	010813_monkeyRBC_PRE2_1	CSik1-01:3	5.000	C:\Xcalibur\LIPID\VERIFICATION	C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
8	010813_monkeyRBC_PRE2_2	010813_monkeyRBC_PRE2_2	CSik1-01:3	5.000	C:\Xcalibur\LIPID\VERIFICATION	C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313b	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
9					C:\Xcalibur\LIPID\VERIFICATION	C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_050313C	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
10					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
11					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313b	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
12					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_050313C	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
13					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
14					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313b	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
15					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_050313C	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
16					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
17					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313b	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
18					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_050313C	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
19					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
20					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313b	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
21					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_050313C	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
22					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
23					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313b	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
24					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_050313C	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
25					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
26					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313b	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
27					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_050313C	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
28					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
29					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_042313b	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
30					C:\Xcalibur\Lipid paper\CBAL\amidec18_NEW\stdlist_BC_050313C	C:\Xcalibur\Lipid paper\paper_amideC18_rbc	C:\Xcalibur\Lipid paper\paper_amideC18_rbc
*							

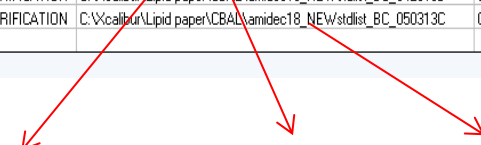
Batch Reprocess Setup

Processing Actions

- iQuari Process Rows: 1-30
 - Peak Detection & Integration
 - Calibration
 - Quantitation
- Qual
 - Peak Detection & Integration
 - Spectrum Enhancement
 - Library Search
- Reports
 - Print Sample Reports
 - Print Summary Reports
- Programs
 - Create Quan Summary Spreadsheet
- Advanced Options
 - Replace Sample Info

OK Cancel Help

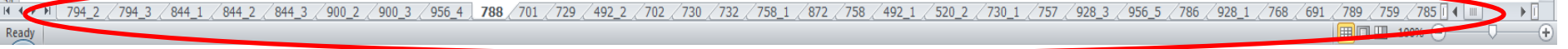
Method 1 Method 2 Method 3



Step 5: Export the Data

Component Name	Curve Index	Weighting Index	Origin Index	Equation	Area	Height	ISTD Area	ISTD									
788				NA													
Filename	Sample Type	Sample Name	Sample ID	Exp Amnt	Calc Amnt	Units	%Diff	Level	%RSD-RESP	Peak Status	Response Ratio	Response Type	Equation	Area	Height	ISTD Area	ISTD
010813_monkeyRBC_PRE1_1	Unknown Sample	010813_monkeyRBC_PRE1_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	136487.59	28560.30	??	??
010813_monkeyRBC_PRE1_2	Unknown Sample	010813_monkeyRBC_PRE1_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	145206.09	30314.25	??	??
010813_monkeyRBC_PRE1_3	Unknown Sample	010813_monkeyRBC_PRE1_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	149180.09	30249.19	??	??
010813_monkeyRBC_POST1_1	Unknown Sample	010813_monkeyRBC_POST1_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	182658.50	38818.23	??	??
010813_monkeyRBC_POST1_2	Unknown Sample	010813_monkeyRBC_POST1_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	190733.55	39764.02	??	??
010813_monkeyRBC_POST1_3	Unknown Sample	010813_monkeyRBC_POST1_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	173889.14	34070.94	??	??
010813_monkeyRBC_PRE2_1	Unknown Sample	010813_monkeyRBC_PRE2_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	189164.62	40319.93	??	??
010813_monkeyRBC_PRE2_2	Unknown Sample	010813_monkeyRBC_PRE2_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	167379.77	34061.77	??	??
010813_monkeyRBC_PRE2_3	Unknown Sample	010813_monkeyRBC_PRE2_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	193727.86	39262.33	??	??
010813_monkeyRBC_POST2_1	Unknown Sample	010813_monkeyRBC_POST2_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	168495.02	31550.61	??	??
010813_monkeyRBC_POST2_2	Unknown Sample	010813_monkeyRBC_POST2_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	188380.69	36724.43	??	??
010813_monkeyRBC_POST2_3	Unknown Sample	010813_monkeyRBC_POST2_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	170135.61	33313.56	??	??
010813_monkeyRBC_PRE3_1	Unknown Sample	010813_monkeyRBC_PRE3_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	135639.70	29214.99	??	??
010813_monkeyRBC_PRE3_2	Unknown Sample	010813_monkeyRBC_PRE3_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	134814.65	28652.55	??	??
010813_monkeyRBC_PRE3_3	Unknown Sample	010813_monkeyRBC_PRE3_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	138835.41	27577.20	??	??
010813_monkeyRBC_POST3_1	Unknown Sample	010813_monkeyRBC_POST3_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	187401.10	35500.49	??	??
010813_monkeyRBC_POST3_2	Unknown Sample	010813_monkeyRBC_POST3_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	175078.66	34355.31	??	??
010813_monkeyRBC_POST3_3	Unknown Sample	010813_monkeyRBC_POST3_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	181405.21	36857.98	??	??
010813_monkeyRBC_PRE4_1	Unknown Sample	010813_monkeyRBC_PRE4_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	160985.25	32890.93	??	??
010813_monkeyRBC_PRE4_2	Unknown Sample	010813_monkeyRBC_PRE4_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	182843.09	40270.93	??	??
010813_monkeyRBC_PRE4_3	Unknown Sample	010813_monkeyRBC_PRE4_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	171569.68	34277.97	??	??
010813_monkeyRBC_POST4_1	Unknown Sample	010813_monkeyRBC_POST4_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	192123.97	38070.61	??	??
010813_monkeyRBC_POST4_2	Unknown Sample	010813_monkeyRBC_POST4_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	169116.74	34466.66	??	??
010813_monkeyRBC_POST4_3	Unknown Sample	010813_monkeyRBC_POST4_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	167021.19	33967.62	??	??
010813_monkeyRBC_PRE5_1	Unknown Sample	010813_monkeyRBC_PRE5_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	148311.81	28999.64	??	??
010813_monkeyRBC_PRE5_2	Unknown Sample	010813_monkeyRBC_PRE5_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	149206.67	28003.17	??	??
010813_monkeyRBC_PRE5_3	Unknown Sample	010813_monkeyRBC_PRE5_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	156327.86	32923.56	??	??
010813_monkeyRBC_POST5_1	Unknown Sample	010813_monkeyRBC_POST5_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	188079.90	38208.70	??	??
010813_monkeyRBC_POST5_2	Unknown Sample	010813_monkeyRBC_POST5_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	177395.34	38309.32	??	??
010813_monkeyRBC_POST5_3	Unknown Sample	010813_monkeyRBC_POST5_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	218723.46	43443.40	??	??

An Excel spreadsheet with a single transition per tab



Step 6: Manual Integration & Re-process

Component Name	Curve Index	Weighting Index	Origin Index	Equation	Area	Height	ISTD Area	ISTD									
788				NA													
Filename	Sample Type	Sample Name	Sample ID	Exp Amnt	Calc Amnt	Units	%Diff	Level	%RSD-RESP	Peak Status	Response Ratio	Response Type	Equation	Area	Height	ISTD Area	ISTD
010813_monkeyRBC_PRE1_1	Unknown Sample	010813_monkeyRBC_PRE1_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	136487.59	28560.30	??	??
010813_monkeyRBC_PRE1_2	Unknown Sample	010813_monkeyRBC_PRE1_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	145206.09	30314.25	??	??
010813_monkeyRBC_PRE1_3	Unknown Sample	010813_monkeyRBC_PRE1_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	149180.09	30249.19	??	??
010813_monkeyRBC_POST1_1	Unknown Sample	010813_monkeyRBC_POST1_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	182658.50	38818.23	??	??
010813_monkeyRBC_POST1_2	Unknown Sample	010813_monkeyRBC_POST1_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	190733.55	39764.02	??	??
010813_monkeyRBC_POST1_3	Unknown Sample	010813_monkeyRBC_POST1_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	173889.14	34070.94	??	??
010813_monkeyRBC_PRE2_1	Unknown Sample	010813_monkeyRBC_PRE2_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	189164.62	40319.93	??	??
010813_monkeyRBC_PRE2_2	Unknown Sample	010813_monkeyRBC_PRE2_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	167379.77	34061.77	??	??
010813_monkeyRBC_PRE2_3	Unknown Sample	010813_monkeyRBC_PRE2_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	193727.86	39262.33	??	??
010813_monkeyRBC_POST2_1	Unknown Sample	010813_monkeyRBC_POST2_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	168495.02	31550.61	??	??
010813_monkeyRBC_POST2_2	Unknown Sample	010813_monkeyRBC_POST2_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	188380.69	36724.43	??	??
010813_monkeyRBC_POST2_3	Unknown Sample	010813_monkeyRBC_POST2_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	170135.61	33313.56	??	??
010813_monkeyRBC_PRE3_1	Unknown Sample	010813_monkeyRBC_PRE3_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	135639.70	29214.99	??	??
010813_monkeyRBC_PRE3_2	Unknown Sample	010813_monkeyRBC_PRE3_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	134814.65	28652.55	??	??
010813_monkeyRBC_PRE3_3	Unknown Sample	010813_monkeyRBC_PRE3_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	138835.41	27577.20	??	??
010813_monkeyRBC_POST3_1	Unknown Sample	010813_monkeyRBC_POST3_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	187401.10	35500.49	??	??
010813_monkeyRBC_POST3_2	Unknown Sample	010813_monkeyRBC_POST3_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	175078.66	34355.31	??	??
010813_monkeyRBC_POST3_3	Unknown Sample	010813_monkeyRBC_POST3_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	181405.21	36857.98	??	??
010813_monkeyRBC_PRE4_1	Unknown Sample	010813_monkeyRBC_PRE4_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	160985.25	32890.93	??	??
010813_monkeyRBC_PRE4_2	Unknown Sample	010813_monkeyRBC_PRE4_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	182843.09	40270.93	??	??
010813_monkeyRBC_PRE4_3	Unknown Sample	010813_monkeyRBC_PRE4_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	171569.68	34277.97	??	??
010813_monkeyRBC_POST4_1	Unknown Sample	010813_monkeyRBC_POST4_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	192123.97	38070.61	??	??
010813_monkeyRBC_POST4_2	Unknown Sample	010813_monkeyRBC_POST4_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	169116.74	34466.66	??	??
010813_monkeyRBC_POST4_3	Unknown Sample	010813_monkeyRBC_POST4_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	167021.19	33967.62	??	??
010813_monkeyRBC_PRE5_1	Unknown Sample	010813_monkeyRBC_PRE5_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	148311.81	28999.64	??	??
010813_monkeyRBC_PRE5_2	Unknown Sample	010813_monkeyRBC_PRE5_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	149206.67	28003.17	??	??
010813_monkeyRBC_PRE5_3	Unknown Sample	010813_monkeyRBC_PRE5_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	156327.86	32923.56	??	??
010813_monkeyRBC_POST5_1	Unknown Sample	010813_monkeyRBC_POST5_1	1	NA	NA		NA	NA	NA	NF	Area	NA	NA	188079.90	38208.70	??	??
010813_monkeyRBC_POST5_2	Unknown Sample	010813_monkeyRBC_POST5_2	2	NA	NA		NA	NA	NA	NF	Area	NA	NA	177395.34	38309.32	??	??
010813_monkeyRBC_POST5_3	Unknown Sample	010813_monkeyRBC_POST5_3	3	NA	NA		NA	NA	NA	NF	Area	NA	NA	218723.46	43443.40	??	??

Identify samples with wrong retention time, etc.
 Fix processing parameters, reprocess
 There are 100 tabs here!



Step 7: Compile Data: A Single Spreadsheet

	3	4	5	6	7	8	9	10	11	12	13	14
1	Day	Patient	Injection	788	758	626	492	464	759	647	520	65
2				PC(18:0/18:1(9Z) 12Z)	PC(16:0/18:2(10E, 12Z))	GluCer(d18:1/12:0)	Cer(d18:1/14:0)	Cer(d18:1/12:0)	SM(d18:1/20:0)	SM(d18:1/12:0)	Cer(d18:0/16:0)	PC(10:0/16:0)
3	1	P14	1	0.29	134.16	0.60	0.01	0.59	1.22	1.28	0.12	0.03
4	1	P14	2	0.33	135.95	0.72	0.01	0.61	1.29	1.20	0.14	0.03
5	1	P14	3	0.27	124.54	0.62	0.01	0.54	1.10	1.15	0.11	0.03
6	1	P16	1	0.26	41.82	0.61	0.00	0.53	0.46	1.11	0.10	0.03
7	1	P16	2	0.32	46.51	0.68	0.01	0.63	0.53	1.24	0.16	0.03
8	1	P16	3	0.28	46.05	0.64	0.01	0.65	0.40	1.15	0.14	0.03
9	1	P21	1	0.34	63.23	0.72	0.00	0.60	0.55	1.27	0.14	0.03
10	1	P21	2	0.29	62.54	0.72	0.01	0.63	0.63	1.20	0.11	0.03
11	1	P21	3	0.27	58.79	0.68	0.00	0.57	0.52	1.21	0.14	0.03
12	1	P26	1	0.28	54.43	0.60	0.01	0.52	0.52	1.02	0.13	0.03
13	1	P26	2	0.33	58.25	0.67	0.02	0.53	0.55	1.15	0.13	0.03
14	1	P26	3	0.31	58.01	0.70	0.01	0.61	0.44	1.11	0.13	0.03
15	1	P33	1	0.28	67.67	0.64	0.01	0.58	0.61	1.16	0.12	0.03
16	1	P33	2	0.27	64.86	0.61	0.01	0.47	0.53	1.16	0.18	0.03
17	1	P33	3	0.28	63.04	0.67	0.00	0.57	0.50	1.09	0.12	0.03
18	1	P34	1	0.32	57.93	0.68	0.00	0.59	0.53	1.09	0.14	0.03

Workflow Complexity

Three different LC-MS runs

Create a processing method for each transition

Using a previously acquired data file:

- Assign RT, peak width, smoothing parameters.

Process data using this processing method

Adjust processing parameters as needed

Export data:

- One worksheet in Excel per transition

- Random order of worksheets in Excel

- Compile data into a single file

Perform QC:

- Manually ensure proper integration (retention time, peak shape)

- Export data

- Perform QC

Lather, Rinse, Repeat...

Possible Solution: Skyline

Easy to manipulate large data sets

Chromatographic alignment

Automated peak integration

Simple report configuration

Simple data export

Possible Solution: Skyline

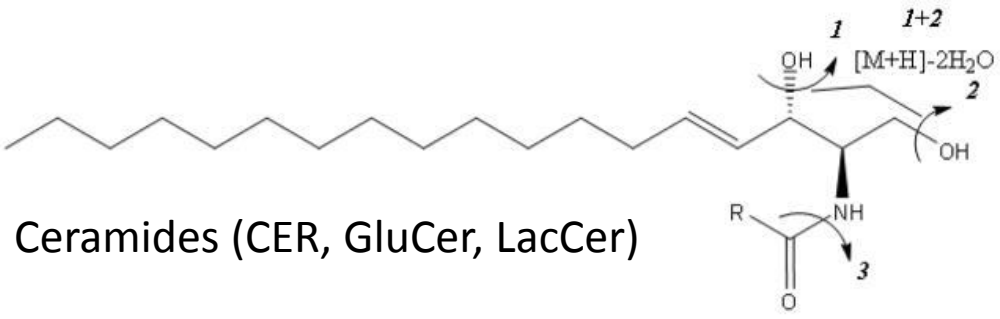
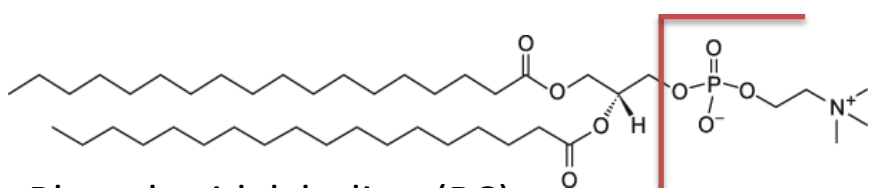
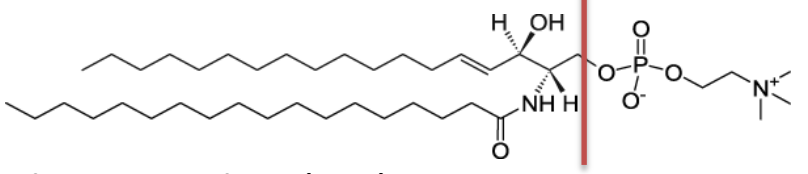
Tricking Skyline

Set one amino acid to be the fragment for the class of lipids

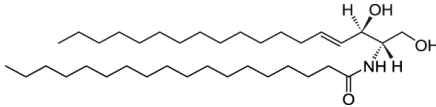
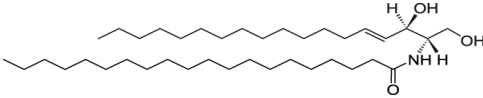
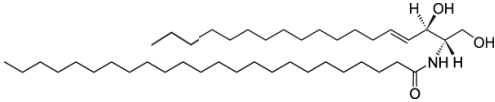
Set another amino acid to add up to the right precursor mass

Pretend the two amino acids are a peptide

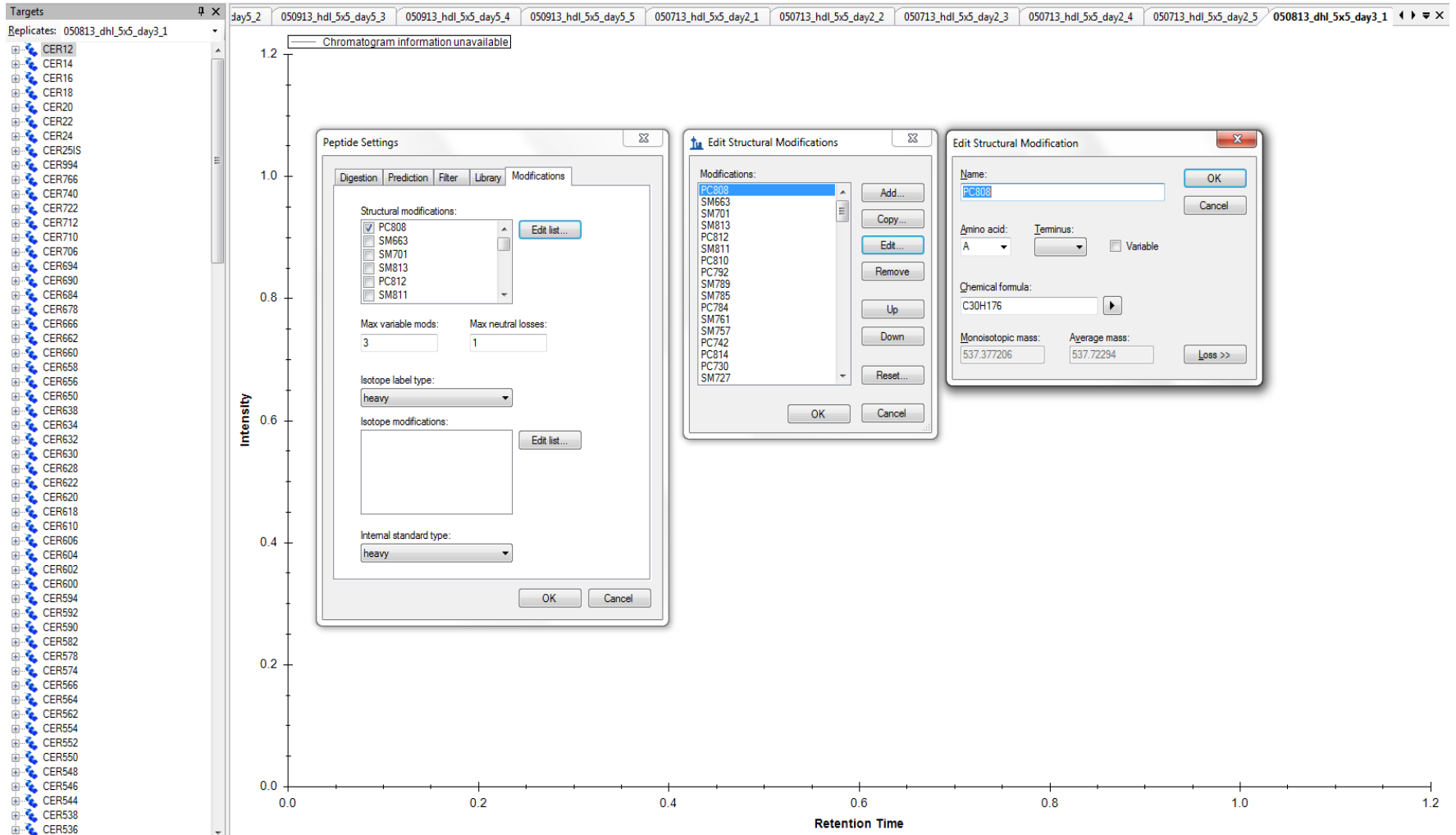
Inventing Amino Acids: The Constant Part

	M+H ⁺	Modified Glycine Chemical Formula
 <p>Ceramides (CER, GluCer, LacCer)</p>	264	C ₁₅ H ₂₄
 <p>Phosphatidylcholine (PC)</p>	184	C ₁₀ H ₄
 <p>Sphingomyelins (SM)</p>		

Inventing Amino Acids: The Variable Part

	M+H ⁺	Modified Alanine Chemical Formula
C18 Ceramide (d18:1/18:0) 	548	C ₁₃ H ₁₃
C20 Ceramide (d18:1/20:0) 	576	C ₂₀ H ₁₃
C22 Ceramide (d18:1/22:0) 	604	C ₁₇ H ₂₁

Step 1: Invent Modified Amino Acids



Step 2: Invent New "Proteins" and "Peptides"

The screenshot shows the Skyline software interface. On the left, a 'Targets' list contains various protein and peptide entries, each with a blue 'AG' icon. A large blue arrow points from the 'AG' icon of the SM16 entry in the list to the 'AG' entry in the 'Protein List' table of the 'Insert' dialog box.

The 'Insert' dialog box is open, showing a 'Protein List' table with the following data:

Name	Description	Sequence
AG	SM12	
AG	SM14	
AG	SM16	
AG	SM18	
AG	SM20	
AG	SM22	
AG	SM24	
*		

At the bottom of the dialog box, there are three buttons: 'Check for Errors', 'Insert', and 'Cancel'.

Step 3: Modify the Amino Acids on the "Peptides"

The screenshot shows the Skyline software interface. On the left, the 'Targets' list is visible, containing various lipid and peptide targets such as CER20, SM12, LACCER12, and GLUCER12. A blue arrow points to the target 'SM20' in this list. An 'Edit Modifications' dialog box is open in the foreground, showing a dropdown menu for 'Structural' with 'SMFRAG' selected. The 'Isotope heavy' section has two dropdown menus, one for 'A' and one for 'G'. The dialog box also includes 'OK', 'Cancel', 'Reset', and 'Create copy' buttons.

Targets

- CER20
- CER22
- CER24
- SM12
- SM14
- SM16
- SM18
- SM20
- SM22
- SM24
- LACCER12
- LACCER14
- LACCER16
- LACCER18
- LACCER20
- LACCER22
- LACCER24
- GLUCER12
- GLUCER14
- GLUCER16

759.5459+
G [z1] - 11

Edit Modifications

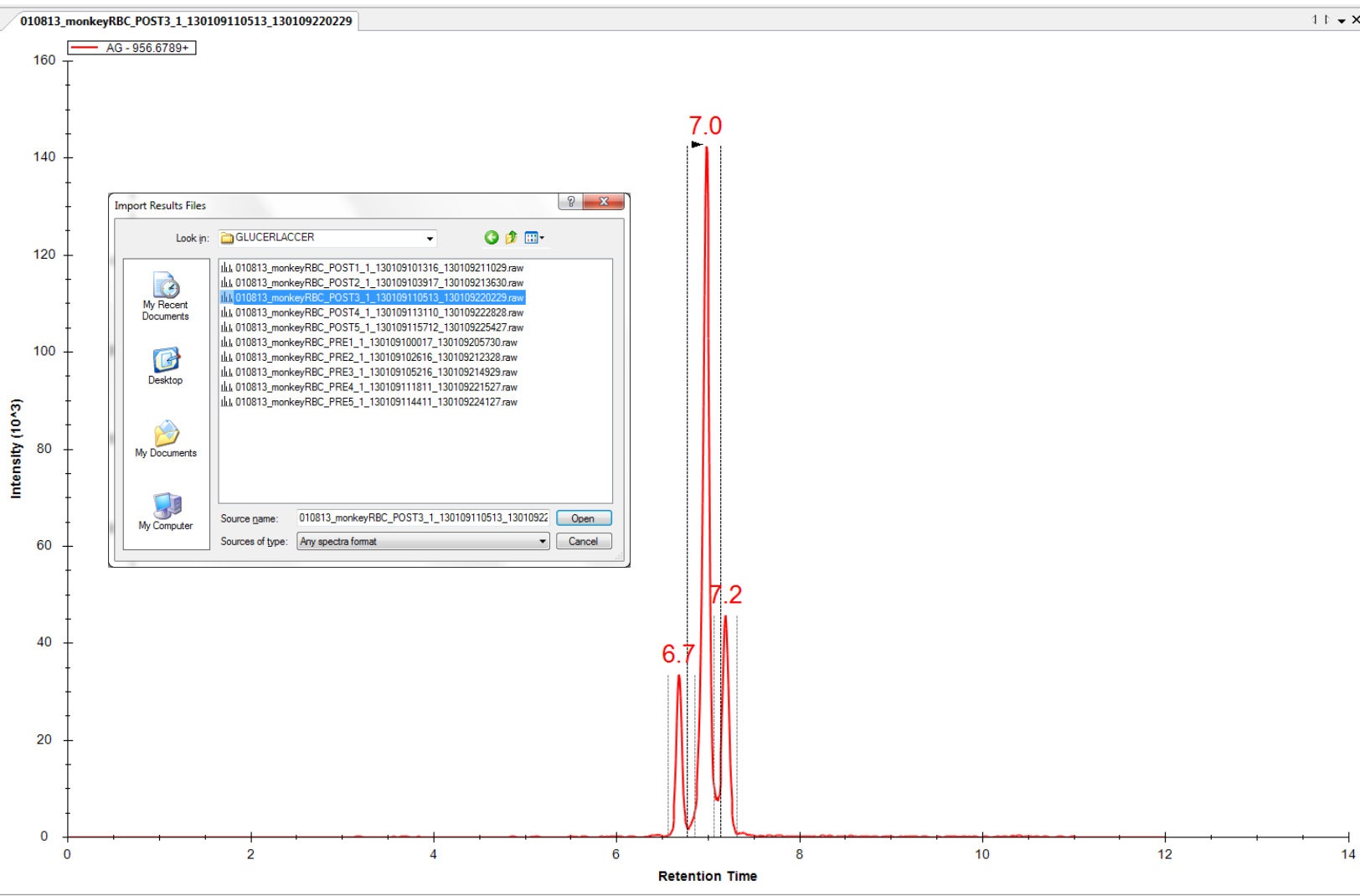
Structural: SM20
SMFRAG
PCFRAGMENT
SMFRAG
CERFRAG
<Add...>
<Edit list...>

Isotope heavy:
A
G

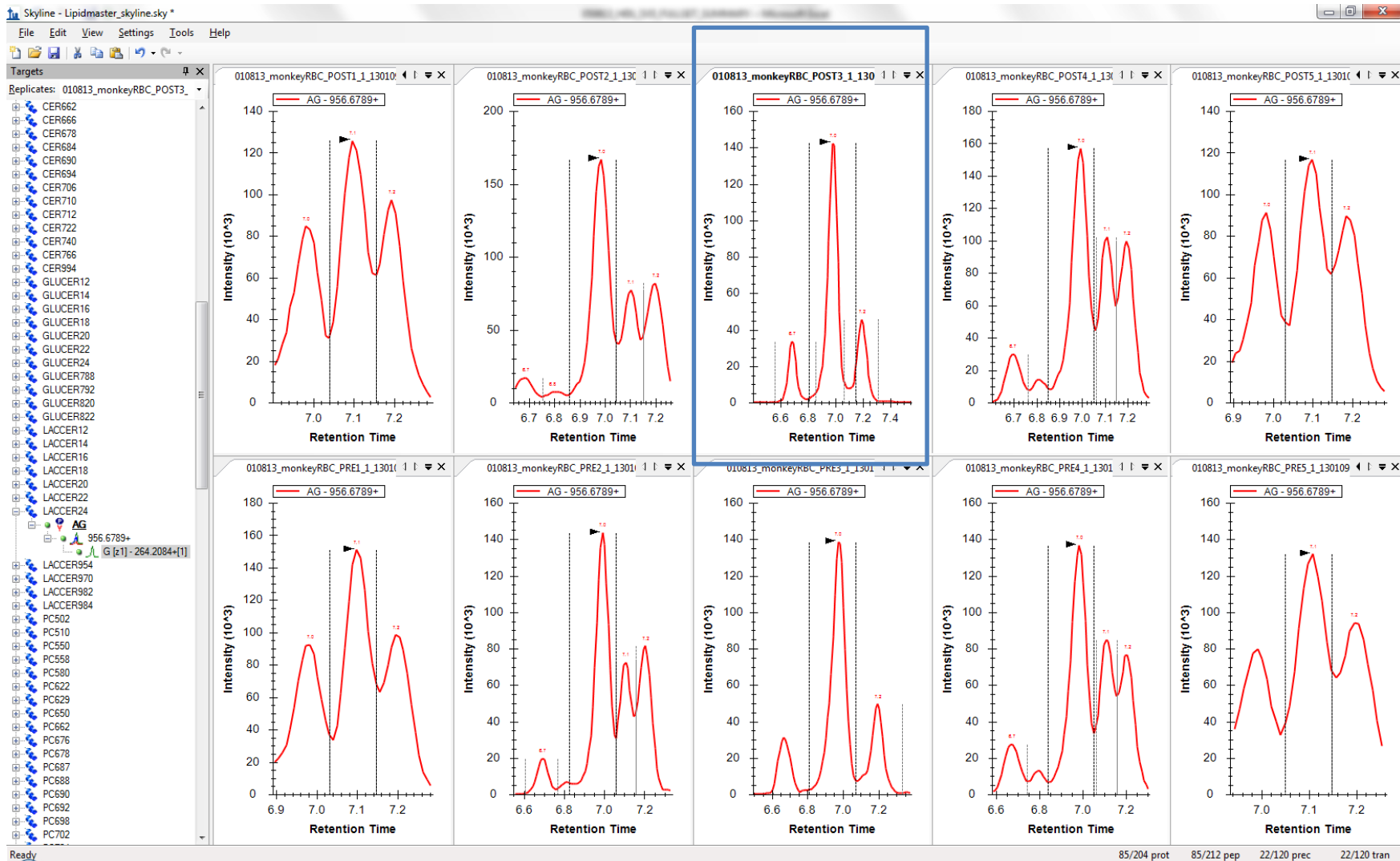
OK
Cancel
 Create copy
Reset

Step 4: Import Data

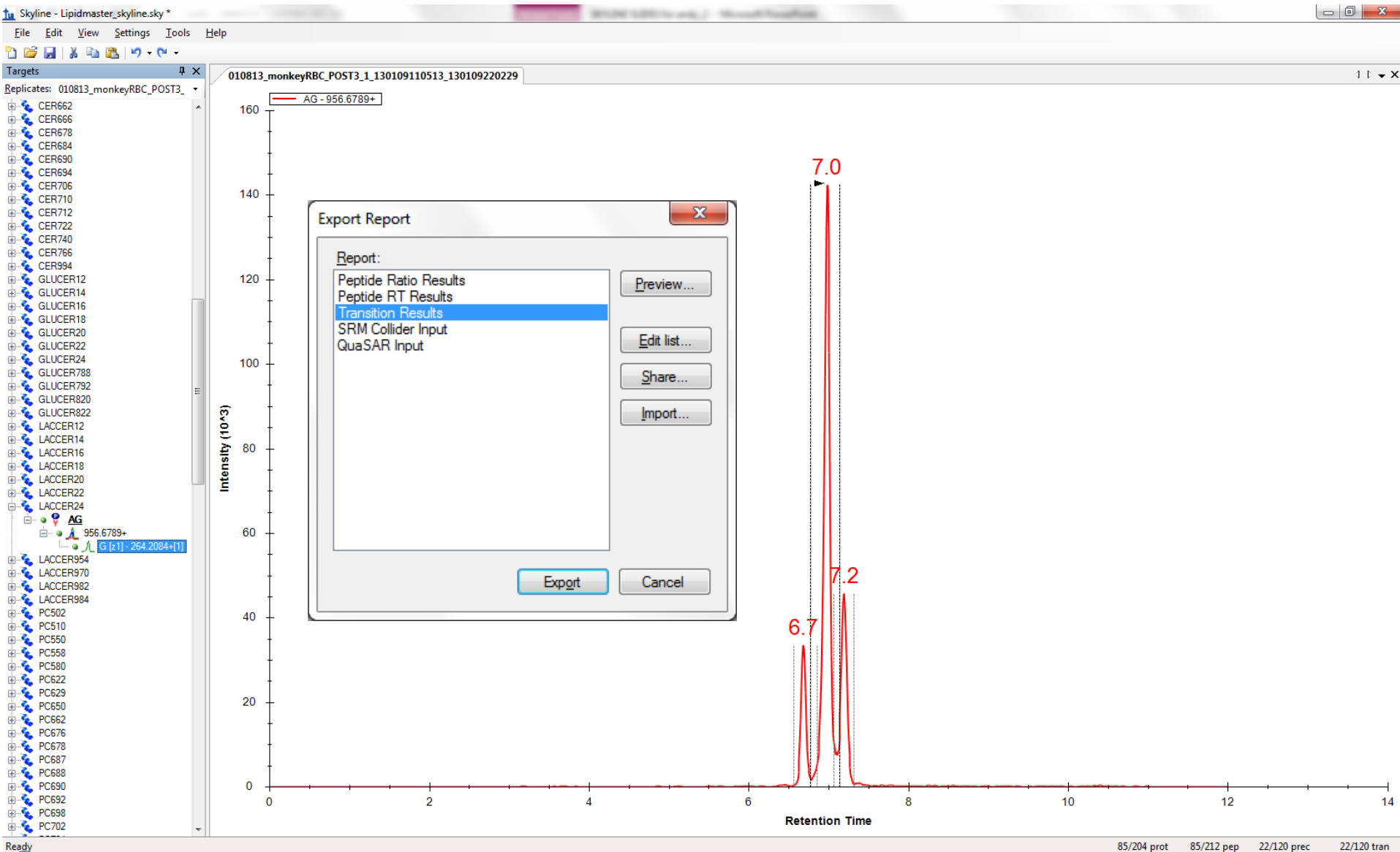
- Targets
- Replicates: 010813_monkeyRBC_POST3_1
- CER662
 - CER666
 - CER678
 - CER684
 - CER690
 - CER694
 - CER706
 - CER710
 - CER712
 - CER722
 - CER740
 - CER766
 - CER994
 - GLUCER12
 - GLUCER14
 - GLUCER16
 - GLUCER18
 - GLUCER20
 - GLUCER22
 - GLUCER24
 - GLUCER788
 - GLUCER792
 - GLUCER820
 - GLUCER822
 - LACCR12
 - LACCR14
 - LACCR16
 - LACCR18
 - LACCR20
 - LACCR22
 - LACCR24
 - AG 956.6789+
 - G [z1] - 264.2084+11
 - LACCR954
 - LACCR970
 - LACCR982
 - LACCR984
 - PC502
 - PC510
 - PC550
 - PC558
 - PC580
 - PC622
 - PC629
 - PC650
 - PC662
 - PC676
 - PC678
 - PC687
 - PC688
 - PC690
 - PC692
 - PC698
 - PC702



Step 5: Manually Pick Peaks if Needed



Step 6: Export the Data



A Deliverable: One Tab on One Spreadsheet

Skylines_integration_results - Microsoft Excel

	A	B	C	D	E	F	G	H	I	J	K	L
1	PeptideSe	ProteinName	ReplicateName	PrecursorI	PrecursorS	ProductM	ProductCh	FragmentI	Retention	Area	Background	PeakRank
2	AG	CER25IS	010813_monkeyRBC_POST1_1_130109101316_130109211029	646.507	1	264.208	1	Z1	7.86	775240	8642	1
3	AG	CER25IS	010813_monkeyRBC_POST2_1_130109103917_130109213630	646.507	1	264.208	1	Z1	7.87	762913	22507	1
4	AG	CER25IS	010813_monkeyRBC_POST3_1_130109110513_130109220229	646.507	1	264.208	1	Z1	7.84	866426	11862	1
5	AG	CER25IS	010813_monkeyRBC_POST4_1_130109113110_130109222828	646.507	1	264.208	1	Z1	7.85	818839	16619	1
6	AG	CER25IS	010813_monkeyRBC_POST5_1_130109115712_130109225427	646.507	1	264.208	1	Z1	7.86	762376	13313	1
7	AG	CER25IS	010813_monkeyRBC_PRE1_1_130109100017_130109205730	646.507	1	264.208	1	Z1	7.87	782732	7317	1
8	AG	CER25IS	010813_monkeyRBC_PRE2_1_130109102616_130109212328	646.507	1	264.208	1	Z1	7.87	755580	8150	1
9	AG	CER25IS	010813_monkeyRBC_PRE3_1_130109105216_130109214929	646.507	1	264.208	1	Z1	7.85	840945	16718	1
10	AG	CER25IS	010813_monkeyRBC_PRE4_1_130109111811_130109221527	646.507	1	264.208	1	Z1	7.89	685160	19827	1
11	AG	CER25IS	010813_monkeyRBC_PRE5_1_130109114411_130109224127	646.507	1	264.208	1	Z1	7.85	819965	3158	1
12	AG	GLUCER12	010813_monkeyRBC_POST1_1_130109101316_130109211029	626.444	1	264.208	1	Z1	5.54	443129	11675	1
13	AG	GLUCER12	010813_monkeyRBC_POST2_1_130109103917_130109213630	626.444	1	264.208	1	Z1	5.55	466824	4500	1
14	AG	GLUCER12	010813_monkeyRBC_POST3_1_130109110513_130109220229	626.444	1	264.208	1	Z1	5.54	499538	4857	1
15	AG	GLUCER12	010813_monkeyRBC_POST4_1_130109113110_130109222828	626.444	1	264.208	1	Z1	5.56	485051	9259	1
16	AG	GLUCER12	010813_monkeyRBC_POST5_1_130109115712_130109225427	626.444	1	264.208	1	Z1	5.54	456098	3641	1
17	AG	GLUCER12	010813_monkeyRBC_PRE1_1_130109100017_130109205730	626.444	1	264.208	1	Z1	5.55	453919	2366	1
18	AG	GLUCER12	010813_monkeyRBC_PRE2_1_130109102616_130109212328	626.444	1	264.208	1	Z1	5.56	468158	2116	1
19	AG	GLUCER12	010813_monkeyRBC_PRE3_1_130109105216_130109214929	626.444	1	264.208	1	Z1	5.55	439384	10424	1
20	AG	GLUCER12	010813_monkeyRBC_PRE4_1_130109111811_130109221527	626.444	1	264.208	1	Z1	5.56	418712	11232	1
21	AG	GLUCER12	010813_monkeyRBC_PRE5_1_130109114411_130109224127	626.444	1	264.208	1	Z1	5.56	489066	3833	1
22	AG	GLUCER16	010813_monkeyRBC_POST1_1_130109101316_130109211029	682.507	1	264.208	1	Z1	6.06	157560	881	1
23	AG	GLUCER16	010813_monkeyRBC_POST2_1_130109103917_130109213630	682.507	1	264.208	1	Z1	6.98	32491	999	1
24	AG	GLUCER16	010813_monkeyRBC_POST3_1_130109110513_130109220229	682.507	1	264.208	1	Z1	6.99	27784	782	1
25	AG	GLUCER16	010813_monkeyRBC_POST4_1_130109113110_130109222828	682.507	1	264.208	1	Z1	6.06	378191	361	1
26	AG	GLUCER16	010813_monkeyRBC_POST5_1_130109115712_130109225427	682.507	1	264.208	1	Z1	6.06	159261	5806	1
27	AG	GLUCER16	010813_monkeyRBC_PRE1_1_130109100017_130109205730	682.507	1	264.208	1	Z1	6.98	24018	13	1
28	AG	GLUCER16	010813_monkeyRBC_PRE2_1_130109102616_130109212328	682.507	1	264.208	1	Z1	6.06	208145	1014	1

Ready | Skylines_integration_results | 140%

An Experiment: The Data First

Lipid in HDL extract	Within Day CV	Between Day CV	Lipid in HDL extract	Within Day CV	Between Day CV
Cer(d18:1/12:0)	9%	9%	PC(18:0/20:3)	12%	15%
Cer(d18:1/24:0)	12%	11%	PC(18:0/22:5)	11%	16%
Glucosylceramide (d18:1/12:0)	8%	9%	PC(18:0/22:6)	10%	15%
PC(10:0/16:0)	12%	30%	PC(18:1/22:6)	8%	14%
PC(10:0/18:1)	10%	19%	PC(20:5/16:0)	13%	15%
PC(12:0/17:2)	11%	35%	SM(d18:0/16:0)	9%	14%
PC(12:0/18:1)	9%	14%	SM(d18:0/18:0)	10%	17%
PC(12:0/18:2)	10%	14%	SM(d18:0/20:0)	11%	15%
PC(13:0/20:3)	11%	14%	SM(d18:0/22:0)	9%	15%
PC(14:0/18:1)	11%	13%	SM(d18:1/12:0)	8%	14%
PC(14:0/18:2)	9%	14%	SM(d18:1/12:0)-18	11%	22%
PC(14:0/18:3)	13%	11%	SM(d18:1/16:0)	10%	13%
PC(15:0/20:5)	11%	14%	SM(d18:1/16:1)	11%	15%
PC(15:0/22:6)	11%	15%	SM(d18:1/18:1)	11%	13%
PC(16:0/18:1)	11%	15%	SM(d18:1/20:0)	11%	14%
PC(16:0/18:2)	10%	13%	SM(d18:1/22:0)	11%	14%
PC(16:0/20:3)	10%	14%	SM(d18:1/22:1)	10%	14%
PC(16:0/20:4)	10%	13%	SM(d18:1/24:0)	10%	16%
PC(16:0/22:4)	11%	15%	SM(d18:1/24:1)	8%	15%
PC(16:0/22:5)	10%	14%	SM(d18:2/18:1)	13%	14%
PC(18:0/11:1)	14%	17%	SM(d18:2/22:1)	11%	13%
PC(18:0/18:2)	11%	15%	SM(d18:2/24:1)	10%	17%
PC(18:0/20:2)	10%	17%			

How long would you be willing to spend to get this amount of data?

Vendor Software: 15 hours, Skyline: 4 hours

Actively collaborating with the author of the software: Priceless