

ANALYTICAL SERVICES DIVISION

## **Experiment Report**

### **Lipidomic Analysis of Lipid Droplet Extracts from Ovarian Steroidogenic Cells by Liquid Chromatography / Mass Spectrometry.**

Dr. John Davis  
University of Nebraska Medical Center  
Obstetrics and Gynecology  
Omaha, Nebraska

May 31, 2013

Jeff D. Moore, Ph.D.  
Director, Analytical Technologies  
Avanti Polar Lipids, Inc.  
Analytical Services Division

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)  
FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)

## Introduction

Three (3) samples of lipid droplet extracts were received as dried residues in glass 4 mL vials on October 24, 2012. These were immediately stored at -80° C until time of analysis. The samples were provided for lipidomic profiling by mass spectrometry techniques specifically for free sterols, cholesterol esters, triacyl- and diacylglycerols, phospholipids and sphingolipids. The molecular species within each class were to be identified, quantified and summed to report relative difference between the 3 samples.

## Principle

Lipids among the various classes are highly numerous and heterogeneous according to their molecular structures and corresponding molecular weights. A single class can be comprised on 50-100 molecular species detectable by mass spectrometry within a molecular weight range of 50-200 Da. Diversity of saturated to polyunsaturated species in the molecular structures would suggest a possible detectable compound every 2 Da, many of which are isobaric. To provide resolution and quantitative ability beyond the mass resolution of the tandem quadrupole mass spectrometers employed, molecular species were resolved by reversed phase liquid chromatography in the presence of class and sub-class specific internal standard compounds added to each sample. The compounds were detected in tandem mass spectrometry (MS/MS) for mass specific fragment ions according to lipid class and molecular weight of compound, known as multiple reaction monitoring (MRM). Selectivity was further enhanced by scheduling the detection of each compound according to its elution from the HPLC column, known as scheduled MRM (sMRM). The semi-quantization was calculated using the integrated area of each analyte sMRM peak, divided by the appropriate internal standard peak area, multiplied by its known concentration. Quantization of cholesterol and cholesterol esters were directly calculated with standards and internal standards from calibration response curves.

## Samples

The samples were described as extracts of steroidogenic ovarian cells prepared using a standard Bligh and Dyer extraction procedure on 3 samples of 250 µL each. Initial protein

ANALYTICAL SERVICES DIVISION

concentrations were provided.

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)  
FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)

## ANALYTICAL SERVICES DIVISION

Label	Name	Protein (ug/uL)	Protein (ug/sample)
bCL-LD1	2012.09.25	0.316	79
bCL-LD2	2012.09.27	0.335	84
bCL-LD3	2012.10.03	2.004	501

Prior to analysis, each extracted residue was dissolved in 1 milliliter of 8:2 (v/v) chloroform : methanol to provide a working stock for each LC/sMRM method.

## LC/MS/MS

A Waters, Acquity™ Ultra-performance liquid chromatography system coupled with an AB Sciex 5500 tandem quadrupole mass spectrometer was the instrumentation platform. The general description of the LC/sMRM methods are summarized below.

Method	MS Mode	MS Detection	Column
DAG / TAG	positive	MI M+NH <sub>4</sub>	Waters Acquity BEH™ C18, 50 X 1.2 mm, 1.7 um
LPC / PC	positive	sMRM Prec 184 u	Agilent Eclipse XDB™-C8, 50 X 4.6 mm, 1.8 um
LPE / PE	positive	sMRM NL 141 u	Agilent Eclipse XDB™-C8, 50 X 4.6 mm, 1.8 um
LPI / PI	negative	sMRM Prec 241 u	Agilent Eclipse XDB™-C8, 50 X 4.6 mm, 1.8 um
LPG / PG	positive	sMRM NL 172 u	Agilent Eclipse XDB™-C8, 50 X 4.6 mm, 1.8 um
LPS / PS	negative	sMRM NL 87 u	Agilent Eclipse XDB™-C8, 50 X 4.6 mm, 1.8 um
SM	positive	sMRM Prec 184 u (KOH)	Agilent Eclipse XDB™-C8, 50 X 4.6 mm, 1.8 um
SB / CER	positive	sMRM Prec SB frag.	Thermo Hypersil Gold™C-18, 50 X 1.2 mm, 1.7 um
CE	positive	sMRM Prec 369 u	Agilent Eclipse XDB-PLUS™ C18, 50 X 1.2 mm, 1.8 um
ST	positive	sMRM M+NH <sub>4</sub> mass frag.	Waters Acquity CSH™ C18, 50 X 1.2 mm, 1.7 um

The working internal standard solutions used for measurement of lipid classes are provided below.

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) \$ E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)

FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)

## ANALYTICAL SERVICES DIVISION

**Phospholipid Working Internal Standards**

Compound	Lot #	M.W.	Stock [uM]	dilution	Working [nM]
17:0 LPC	LM1-009D	507.64	1.000	10	100
12:0-13:0 PC	LM1-086A	635.85	1.000	10	100
17:0-14:1 PC	LM1-054C	718.00	1.000	10	100
17:0-20:4 PC	LM1-152C	796.11	1.000	10	100
21:0-22:6 PC	LM1-058A	876.24	1.000	10	100
17:1 LPE	171LPE-10	465.65	1.000	20	50
12:0-13:0 PE	LM1-013C	593.77	1.000	20	50
17:0-14:1 PE	LM27-001B	675.92	1.000	20	50
17:0-20:4 PE	LM27-002B	754.03	1.000	20	50
21:0-22:6 PE	LM27-004A	834.16	1.000	20	50
17:1 LPI	171LPI-10	583.32	1.000	100	10
12:0-13:0 PI	LM7-130B	712.42	1.000	100	10
17:0-14:1 PI	LM7-112B	794.49	1.000	100	10
17:0-20:4 PI	LM39-102A	873.13	1.000	100	10
21:0-22:6 PI	LM7-111B	952.60	1.000	100	10
17:1 LPS	171LPS-10A	513.26	1.000	100	10
12:0-13:0 PS	LM1-023D	637.78	1.000	100	10
17:0-14:1 PS	LM1-059B	719.93	1.000	100	10
17:0-20:4 PS	LM27027B	798.04	1.000	100	10
21:0-22:6 PS	LM27-053A	878.17	1.000	100	10
17:1 LPG	171LPG-10	496.55	1.000	100	10
12:0-13:0 PG	LM1-024C	624.78	1.000	100	10
17:0-14:1 PG	LM1-059A	706.93	1.000	100	10
17:0-20:4 PG	LM27-007B	785.04	1.000	100	10
21:0-22:6 PG	LM27-065A	865.17	1.000	100	10

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)

FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA &amp; CANADA) OR 205-663-2494 (INT)

ANALYTICAL SERVICES DIVISION

**Sphingoid Base / Ceramide Working  
Internal Standards**

Compound	Lot #	exact M.W.	(uM)	dilution	Working [nM]
C17So	LM15-085	285.47	2.490	100	24.9
C17Sa	LM15-086	287.48	2.500	100	25
C12 SM	LM15-090	646.92	2.500	100	25
C12 Cer	LM15-088	481.79	2.510	100	25.1
C12 GlucCer	LM15-091	643.93	2.510	100	25.1
C12 LacCer	LM25-056	806.08	2.540	100	25.4

**Sterol Working Internal  
Standards**

Compound	lot #	Exact Mass	Stock [uM]	Dilution	Working [uM]
Cholesterol-d7	LM39-093A	393.40	1262.91	50.00	25.26
Cholestanol-d5	Chanol(D5)-10	393.40	86.55	50.00	1.73
Lanosterol-d6	Lan(D6)-10	432.42	112.43	50.00	2.25
Zymosterol-d5	Zym(D5)-10	389.37	147.96	50.00	2.96

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)

FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA &amp; CANADA) OR 205-663-2494 (INT)

ANALYTICAL SERVICES DIVISION

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)  
FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)

## ANALYTICAL SERVICES DIVISION

**TAG and DAG Internal Standards**

Compounds	CC:DB	Lot #	Exact Mass	Stock [uM]
14:0-16:1-14:0 TG-d5	C44:1	LM39-095A	753.69	0.40
15:0-18:1-15:0 TG-d5	C48:1	LM39-095A	809.75	0.40
16:0-18:0-16:0 TG-d5	C50:0	LM39-095A	839.80	0.39
17:0-17:1-17:0 TG-d5	C51:1	LM39-095A	851.80	0.39
19:0-12:0-19:0 TG-d5	C50:0	LM39-095A	839.80	0.40
20:0-20:1-20:0 TG-d5	C60:1	LM39-095A	977.94	0.40
20:2-18:3-20:2 TG-d5	C58:7	LM39-095A	937.81	0.40
20:4-18:2-20:4 TG-d5	C58:10	LM39-095A	931.77	0.40
20:5-22:6-20:5 TG-d5	C62:16	LM39-095A	975.74	0.39
1,3-14:0 DG-d5	C28:0	LM39-132A	517.48	0.40
1,3-15:0 DG-d5	C30:0	LM39-132A	545.51	0.41
1,3-16:0 DG-d5	C32:0	LM39-132A	573.54	0.40
1,3-17:0 DG-d5	C34:0	LM39-132A	601.57	0.40
1,3-19:0 DG-d5	C38:0	LM39-132A	657.63	0.40
1,3-20:0 DG-d5	C40:0	LM39-132A	685.66	0.40
1,3-20:2 DG-d5	C40:4	LM39-132A	677.60	0.40
1,3-20:4 DG-d5	C40:8	LM39-132A	669.54	0.40
1,3-20:5 DG-d5	C40:10	LM39-132A	665.51	0.40

**Sample Preparation and LC/MS/MS****Phospholipids**

The sample solutions in 8:2 (v/v) chloroform : methanol were diluted 1:1 into the phospholipid working internal standard solution by adding 100 uL of sample to 100 uL of internal standard solution. This sample was assayed for the 5 phospholipid sub-classes on separate methods for PC, PE, PI, PS, PG molecular species.

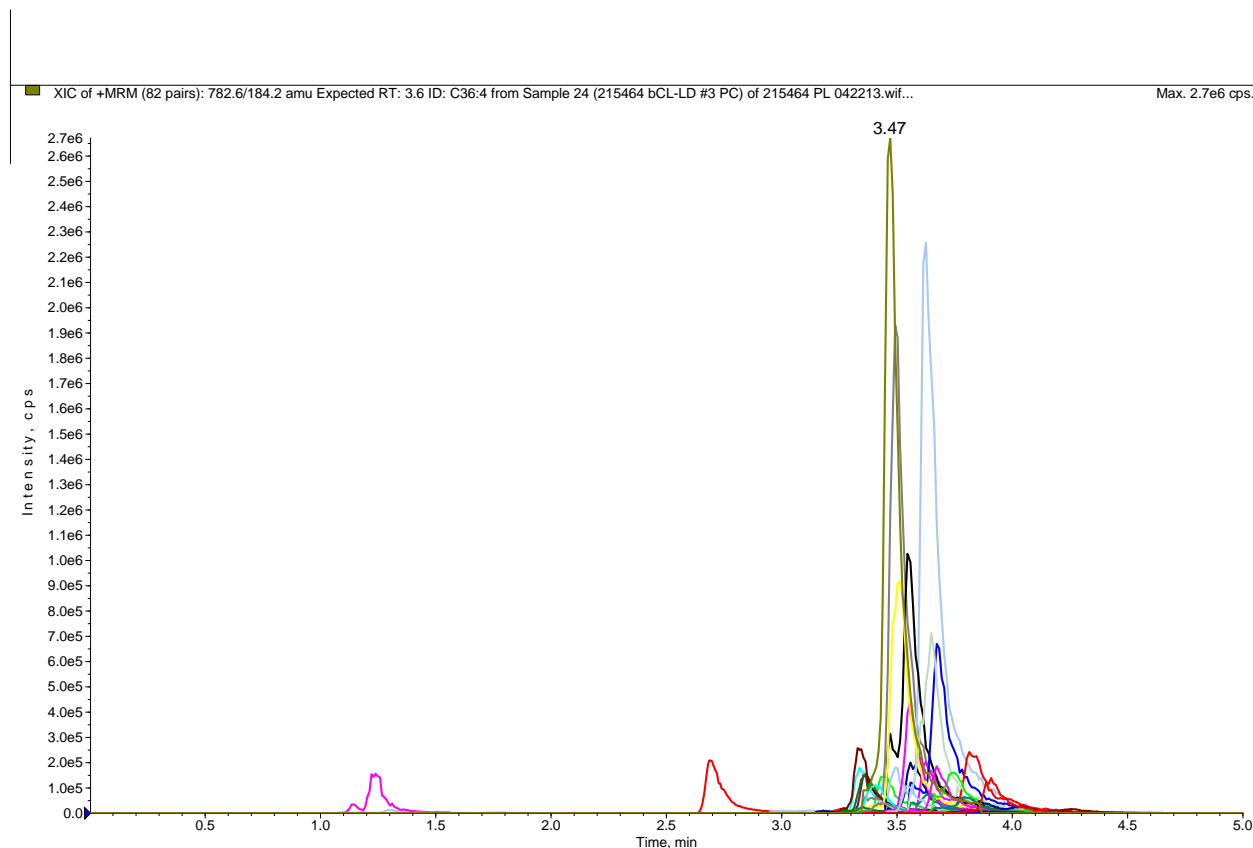
700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)

FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA &amp; CANADA) OR 205-663-2494 (INT)



ANALYTICAL SERVICES DIVISION

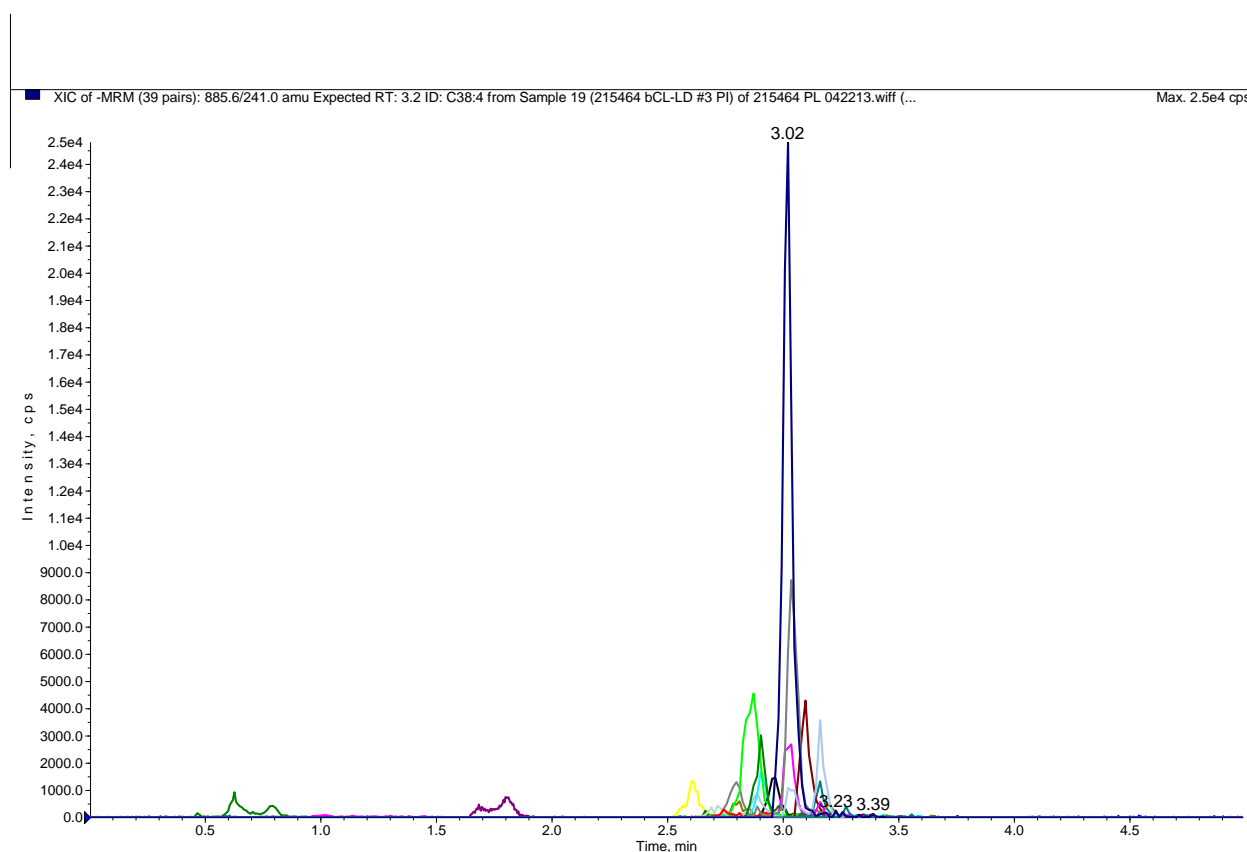


**Figure 1:** UPLC/sMRM of phosphatidylcholine species in bCL-LD3. Peak at 3.47 minutes is C36:4 PC.

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)  
FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)

ANALYTICAL SERVICES DIVISION



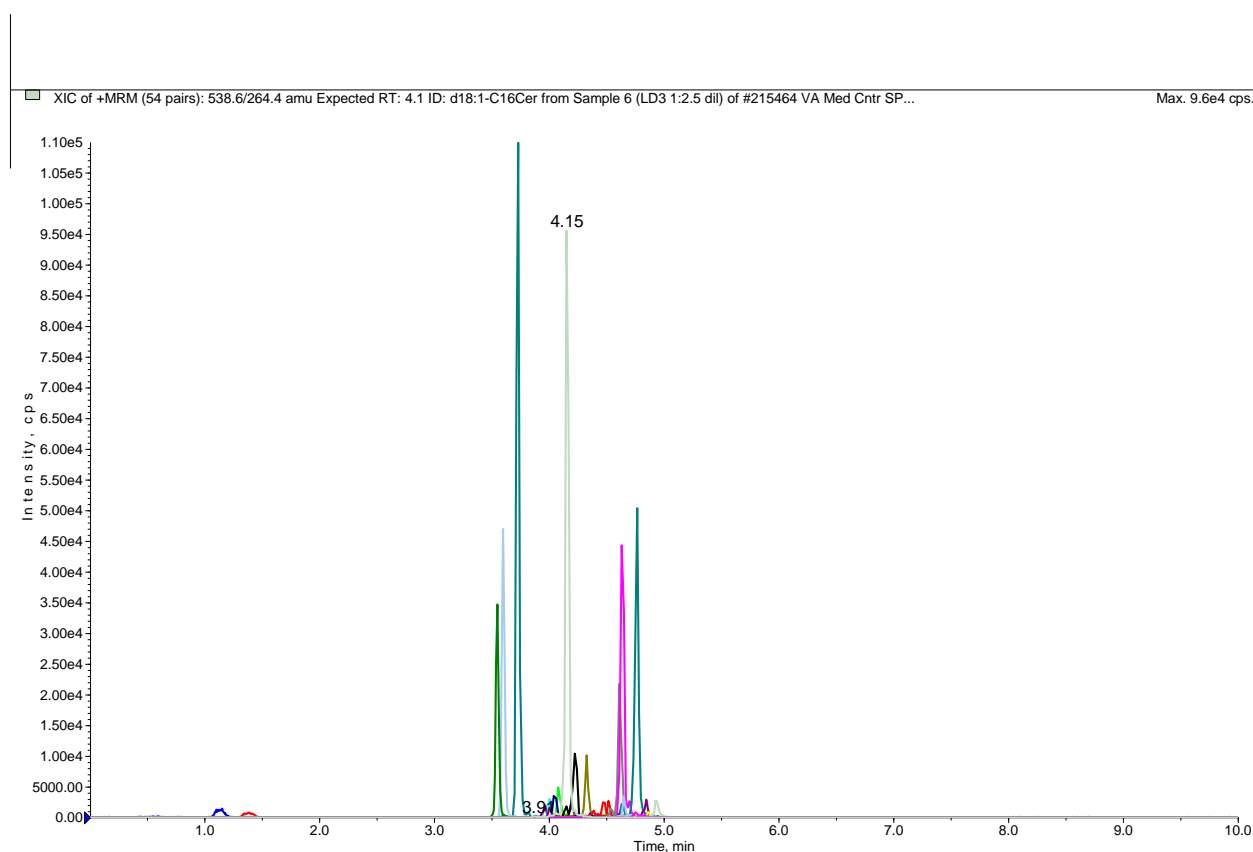
**Figure 2:** UPLC/sMRM of phosphatidylinositol in bCL-LD3. Peak at 3.02 minutes is C38:4 PI.

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) \$ E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)  
FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)

## Sphingoid Bases and Sphingolipids

The samples solutions were diluted into the working sphingoid base / ceramide internal standard solution by adding 100  $\mu$ L of sample to 100  $\mu$ L of internal standard and mixed. This solution was evaporated under nitrogen gas at room temperature. The residue was re-dissolved in 200  $\mu$ L of HPLC and 50  $\mu$ L of 1M KOH in methanol. The sample was incubated for 1 hour at 70° C and injected.



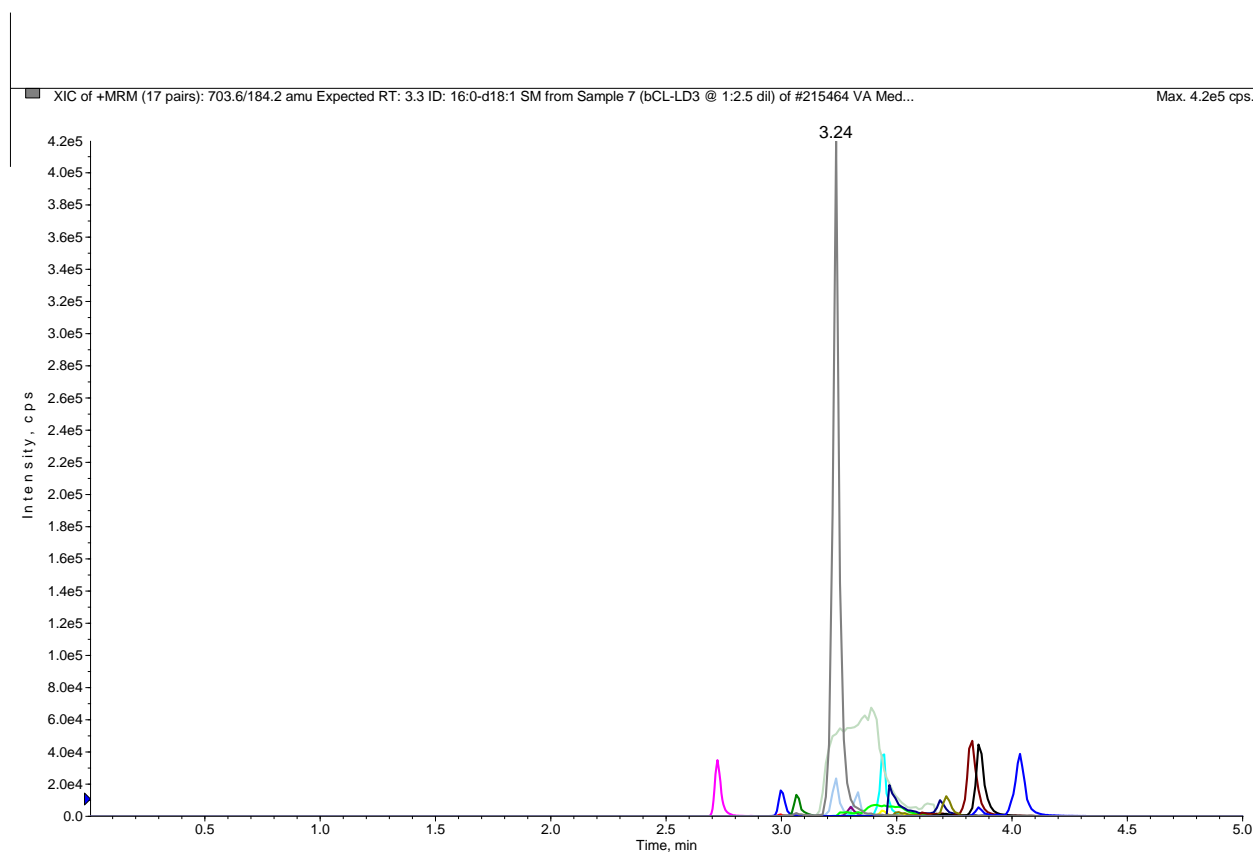
**Figure 3:** UPLC/sMRM of sphingoid bases and ceramides in bCL-LD3. The peak at 4.15 minutes in d18:1-16:0 ceramide.

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)

FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)

ANALYTICAL SERVICES DIVISION



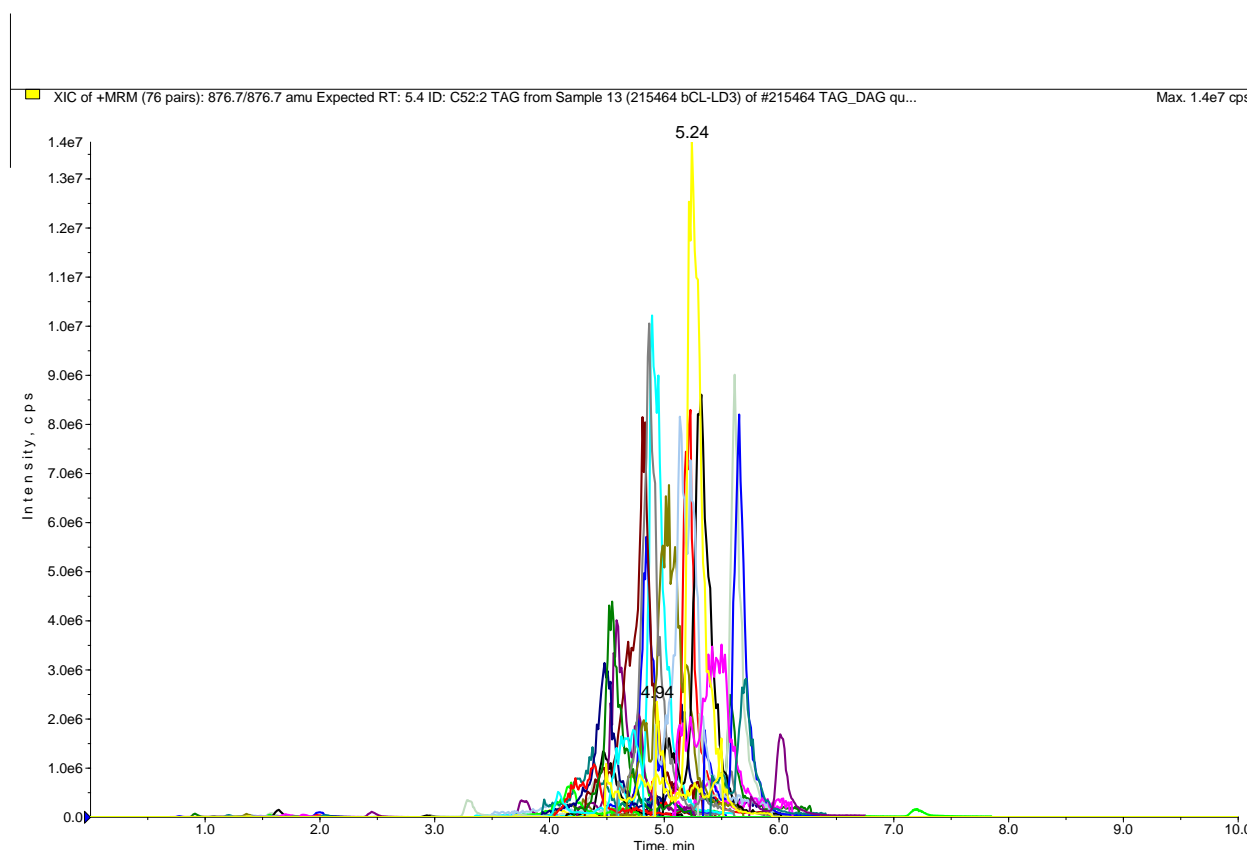
**Figure 4:** UPLC/sMRM of sphingomyelins in bCL-LD3. The peak at 3.24 minutes is d18:1-16:0 SM.

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)  
FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)

## TAGs and DAGs

The stock internal standard mixtures for TAG-d5 (50 uL) and DAG d-5 (25 uL) were added to 100 uL of the sample extracts. These solutions were mixed and evaporated under nitrogen gas at room temperature then re-dissolved in 200 uL of 9:1 (v/v) methanol : dicholormethane for injection onto the UPLC/sMRM method.



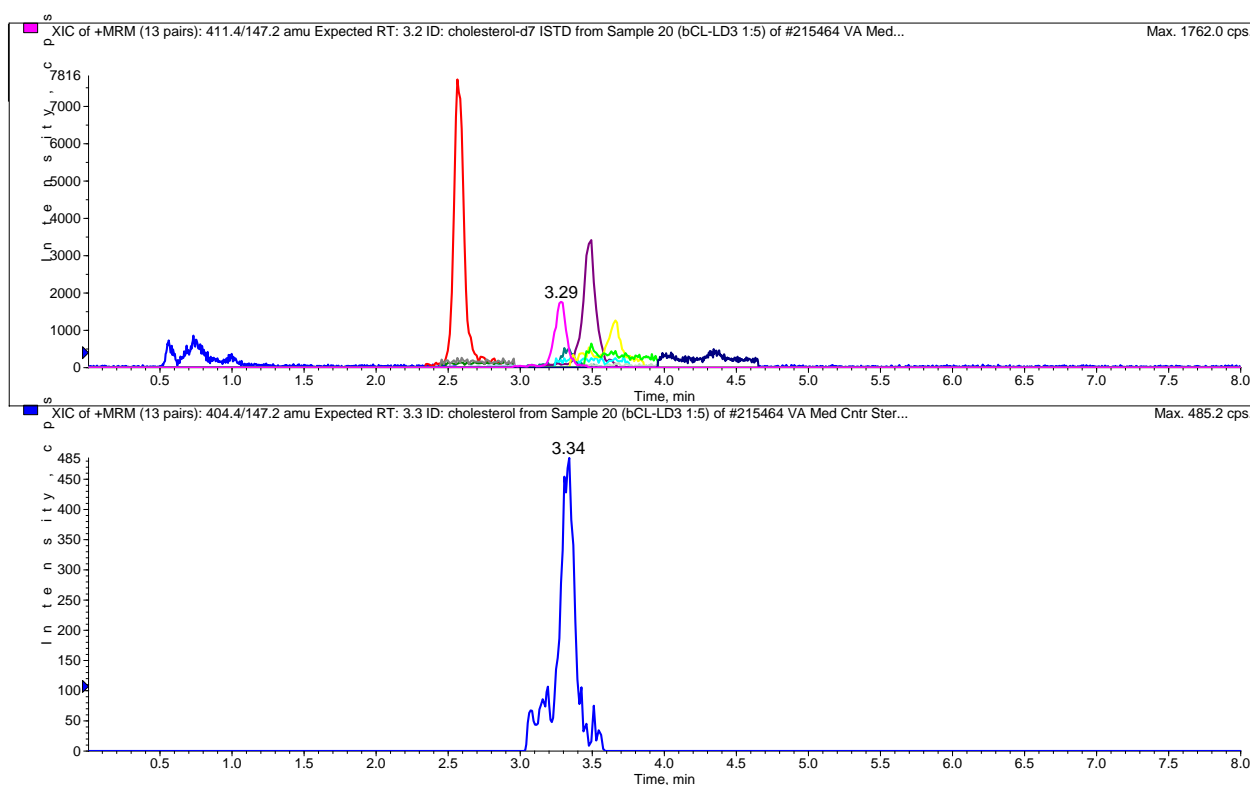
**Figure 5:** UPLC/sMRM of DAGs and TAGs of bCL-LD3. The peak at 5.24 minutes is 52:2 TAG

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) \$ E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)  
FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)

## Sterols

The sample extracts were added to 13 X 100 mm test tubes at 10, 50 and 100 uL and dried under nitrogen gas at room temperature. To each tube 500 uL of the working sterol internal standard mixture was added and mixed. Each sterol compound was assayed against an ISTD/Std calibration curve within a range of ~ 0.3 – 10 uM.



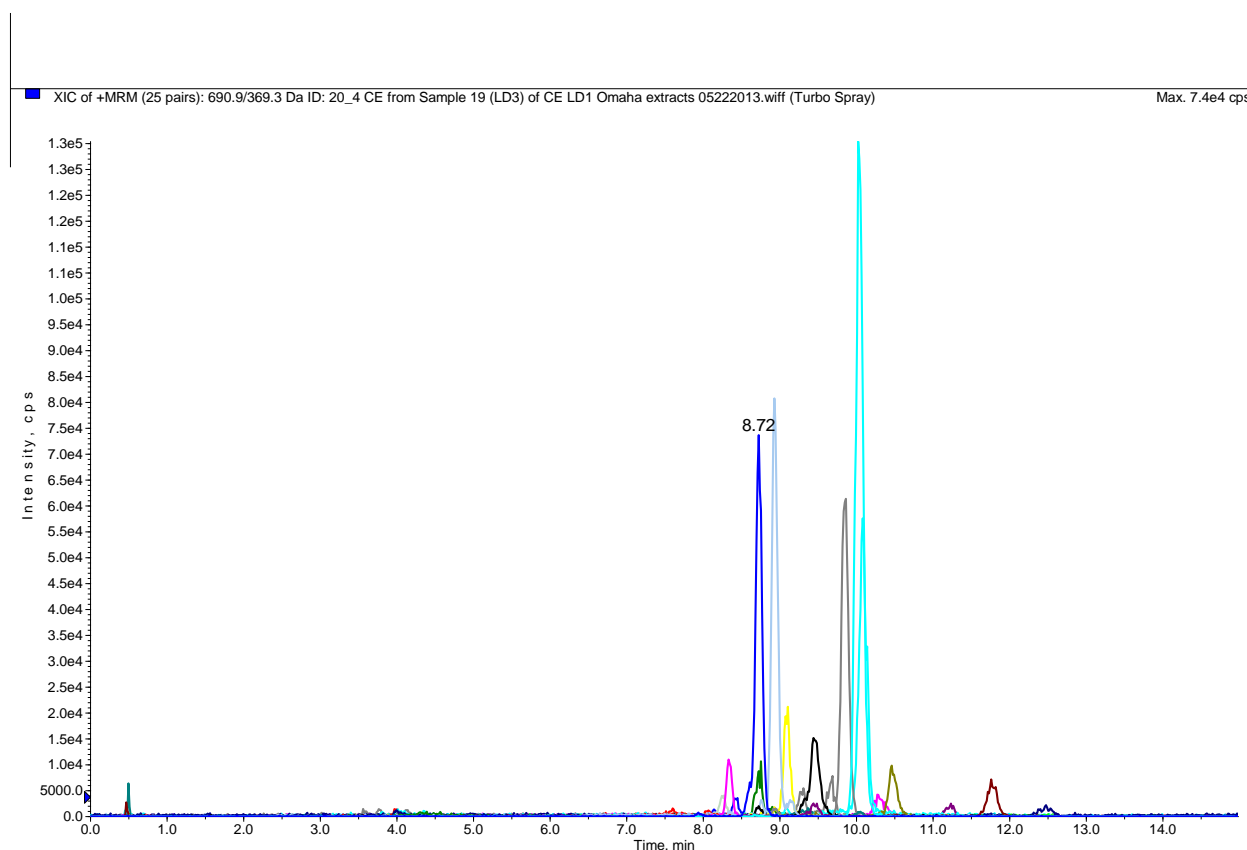
**Figure 6:** UPLC/sMRM chromatogram of deuterated sterol ISTDs, cholesterol-d7 at 3.29 minutes (TOP). Extracted ion chromatogram of cholesterol from bCL-LD3 @ 1:5 dilution (BOTTOM).

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)  
FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)

## Cholesterol esters

The internal standard at 10 ug/mL of 18:1-d5 cholesterol ( 5 ul) was added to 100 uL of the sample extracts. This solution was evaporated under nitrogen at room temperature and re-dissolved in 200 uL of methanol for analysis by the UPLC/sMRM method.



**Figure 7:** UPLC/sMRM of cholesterol esters of bCL-LD3. The peak at 8.72 minutes is 20:2 cholesterol ester.

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) \$ E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)  
FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)

## ANALYTICAL SERVICES DIVISION

## Results

The individual molecular species within each lipid class was semi-quantified by the described methods. The comparison for each among the three samples is provided in the accompanying Excel™ spreadsheet. For comparison of total classes, the individuals were summed within class and reported below as nM (pmol per sample) and normalized to the total protein measured in the original 250 uL droplet.

**nM (pmol per sample)**

Lipid Class	bCL-LD #1	bCL-LD #2	bCL-LD #3
DAG	775.0	319.8	1617.6
TAG	19314.0	8358.8	80612.5
LPC	2.6	1.2	9.1
PC	686.3	379.3	1994.7
LPE	2.5	1.3	3.3
PE	165.5	106.4	395.6
LPI	8.4	0.0	2.3
PI	220.1	135.0	333.5
LPG	8.4	4.2	5.6
PG	6.2	8.2	29.3
LPS	1.1	0.8	3.0
PS	47.3	60.5	320.6
SM	247.9	181.4	1369.8
SB	0.0	0.1	1.1
Cer	31.2	18.6	164.2
GlucCer	3.3	3.2	17.6
LacCer	1.2	0.5	2.7
CE	292.6	118.0	1614.8
ST	ND	ND	1913.9
<b>TOTAL</b>	<b>21813.5</b>	<b>9697.4</b>	<b>88497.3</b>

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)

FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA &amp; CANADA) OR 205-663-2494 (INT)



## ANALYTICAL SERVICES DIVISION

**pmol / ug protein**

ug/protein	79	83.75	501
Lipid Class	bCL-LD #1	bCL-LD #2	bCL-LD #3
DAG	9.81	3.82	3.23
TAG	244.48	99.81	160.90
LPC	0.03	0.01	0.02
PC	8.69	4.53	3.98
LPE	0.03	0.02	0.01
PE	2.09	1.27	0.79
LPI	0.11	0.00	0.00
PI	2.79	1.61	0.67
LPG	0.11	0.05	0.01
PG	0.08	0.10	0.06
LPS	0.01	0.01	0.01
PS	0.60	0.72	0.64
SM	3.14	2.17	2.73
SB	0.00	0.00	0.00
Cer	0.40	0.22	0.33
GlucCer	0.04	0.04	0.04
LacCer	0.01	0.01	0.01
CE	3.70	1.41	3.22
ST	ND	ND	3.82
<b>TOTAL</b>	<b>276.1</b>	<b>115.8</b>	<b>176.6</b>

## Conclusions

The UPLC/sMRM methods employed provide selective semi-quantitative comparison of molecular species and lipid classes within the 3 samples. The use of internal standard applied after extraction does not correct for recovery within the samples, but does allow direct calculation of relative concentrations inter and intra sample(s). The original droplet volume for each sample was stated to be 250 uL. From the data, sample bCL-LD3 would be the most concentrated with 4-

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)

FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA &amp; CANADA) OR 205-663-2494 (INT)

## ANALYTICAL SERVICES DIVISION

10 times more total lipid and protein. When normalized for protein, sample bCL-LD1 contains approximately twice that of samples 2 and 3. Curiously, there were very little free sterols detected in any of the samples. The values reported for bCL-LD3 were just below the limit of quantization for the assay, but reported for comparative purposes.

Jeff D. Moore, Ph.D.  
Director, Analytical Technologies  
Avanti Polar Lipids, Inc.

700 INDUSTRIAL PARK DRIVE, ALABASTER, AL 35007-9105

FAX 800-229-1004 (USA & CANADA) 205-663-0756 (INT) § E-MAIL [INFO@AVANTILIPIDS.COM](mailto:INFO@AVANTILIPIDS.COM) OR VISIT OUR WEB SITE [WWW.AVANTILIPIDS.COM](http://WWW.AVANTILIPIDS.COM)  
FOR INFORMATION PLEASE CONTACT OUR PRODUCT HOTLINE 800-227-0651 (USA & CANADA) OR 205-663-2494 (INT)