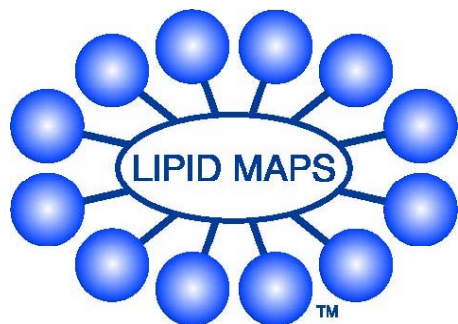




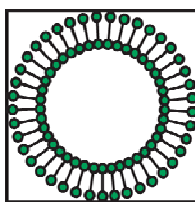
www.lipidmaps.org

LIPID MAPS Lipidomics Workshop

April 28, 2007

Internal Standards for Lipidomic Analysis

Walt Shaw



Avanti®
POLAR LIPIDS, INC.

LIPID MAPS: Core F(a)
Lipid Standards and Production

members:

Mass spectrometry

Synthesis

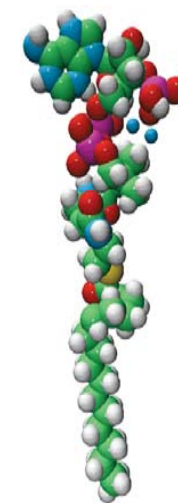
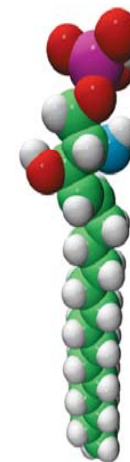
Walt Shaw

Bill Caufield

Shengrong Li

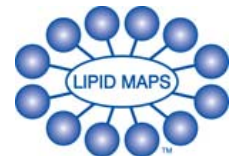
Jeff Moore

Dale Smith



Discussion Points

- Production of MS standards
 - Synthesis
 - Packaging
- Why bad things happen to good lipids
 - Surfaces
 - Reactive chemicals
- Available standards

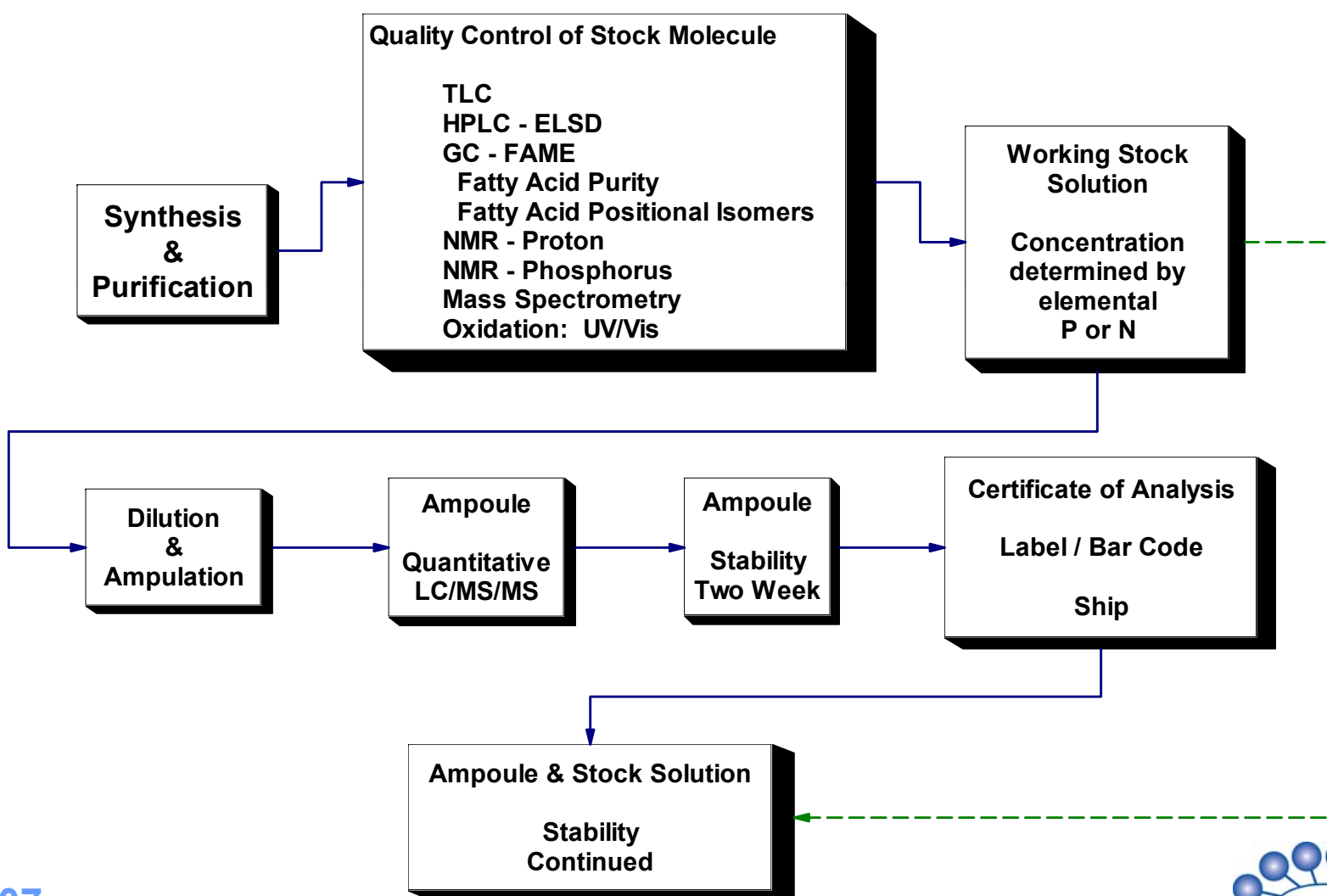


Synthesis of Lipid Standards

- Two tenets that govern synthesis of Avanti's molecules
 - It is not possible to analyze **quality** into a product!
 - **Quality** must be synthesized into a product!
 - Quality is defined as
 - (1) a molecule of defined purity
 - (2) ability to reproduce the molecule batch after batch after batch

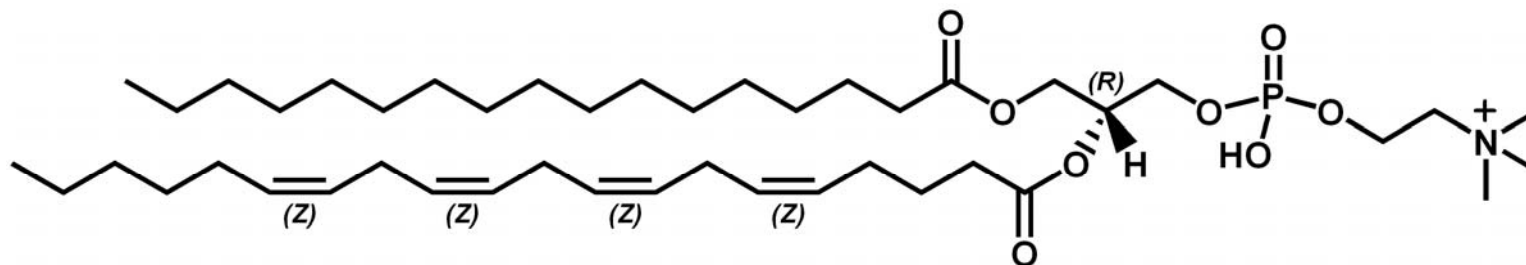


Production of MS Standards



Product Example

17:0-20:4 GPCho



LM_ID: LMGP01010003

SYS_NAME: 1-heptadecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-*sn*-glycero-3-phosphocholine

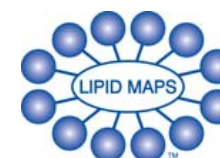
Exact Mass: 796.59

FORMULA: C₄₅H₈₃NO₈P

CATEGORY: Glycerophospholipids

MAIN_CLASS: Glycerophosphocholines [01]

SUB_CLASS: Diacylglycerophosphocholines





Lipid MAPS Product Release Specification

Page 1 of 1

Mixed Acyl Phosphatidylcholine

Origination Date: January 28, 2004
Revision Date: June 28, 2004

Lipid MAPS ID No.: LMGP01010001 – LMGP01019999

Avanti Product No.: LM-1000 to LM-1099

Specification No.: LMP-001a

QC Sample Quantity: Not less than 200 mg

ANALYSIS	SPECIFICATION
TLC	Ninhydrin spray, negative Iodine, 1 major spot Phosphorus, positive Charring, positive (unsaturated species) negative (saturated species) Water dip, 1 major spot Rf consistent with structure
HPLC	Not less than 95% (AUC)
FAME (GC/FID)	Not less than 98% (AUC) components of interest
NMR (proton)	Consistent with theoretical structure
NMR (phosphorus)	Not less than 97% of total integrated area for compound of interest
Mass Spectrometry	Consistent with theoretical structure
Verification of sn-1 and sn-2 fatty acid structure (GC)	Not less than 90% of theoretical sn-1, sn-2 structure
Analysis to be performed on polyunsaturated samples only	
UV/VIS Oxidation	$\Sigma\%$ Oxidation at 234, 268, 278nm = not more than 5%

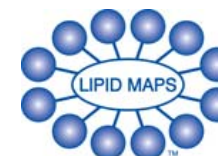
Approved By: _____ **Date:** _____

Avanti Polar Lipids Representative

Approved By: _____ **Date:** _____

Lipid Maps Core Representative

5/07

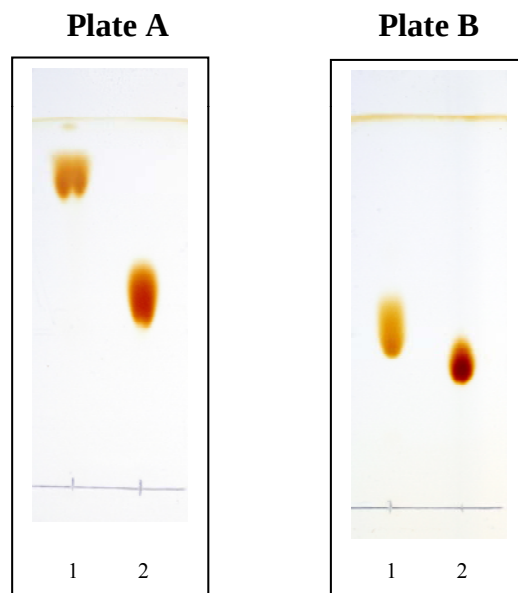


Thin layer Chromatography

Conditions: Plate A Mobile Phase: 65:25:4 CHCl₃:MeOH:H₂O
Plate B Mobile Phase: 65:25:4 CHCl₃:MeOH:NH₄OH

TLC Plate: Whatman Partisil K6: 10-12 µm silica, pore size 60Å (1 inch X 3 inch)

Data:



Lane 1: Suitability (99% 18:1 PE + 1% oleic acid)
Lane 2 (17:0-20:4 PC)

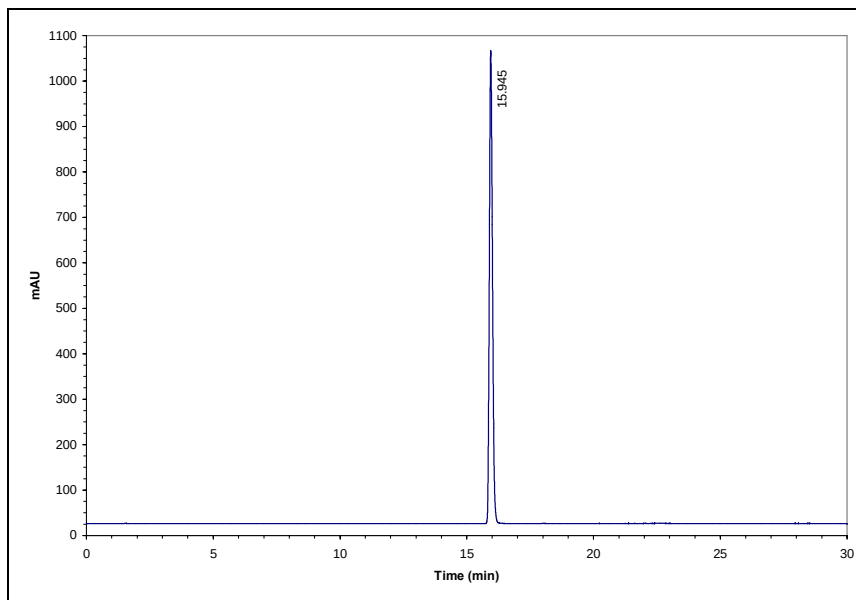
Interpretation: One spot in iodine, ninhydrin (-), phosphorus (+), char (+) and One Spot after water dip



Reversed Phase HPLC-ELSD

Conditions: Mobile Phase A: 1:1 MeOH:H₂O + 20 mM NH₄OAC
Mobile Phase B: 100 % MeOH + 20 mM NH₄OAC
Gradient: (Linear) 100% A to 100% B over 20 minutes
Column: C8, 4.6 mm i.d X 150 mm, 5µm
Flow rate: 1 ml/min
Detector: Sedex 75, 40°C, 3.5 bar, gain of 5
Injection: 10 µl @ 1 µg/µl
Volume:

Data:



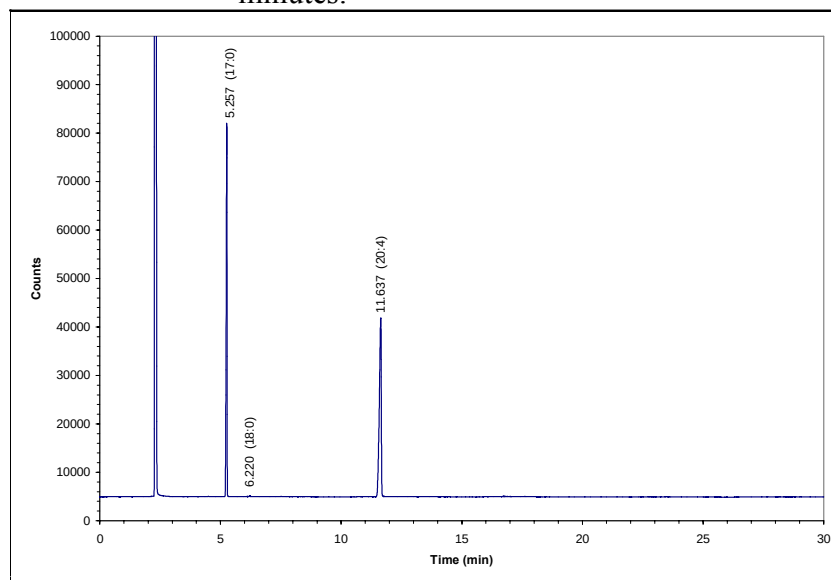
Interpret: Purity = 100% AUC



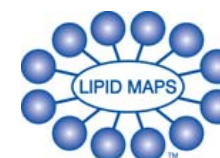
Fatty acid Methyl Esters by GC

Conditions: Column: DB-225, 30M X 0.25 mm, i.d.
Temperatures: Isocratic Oven Temp. = 220° C
Injection = 220° C,
Detector = 250° C
Carrier: Grade 5 Helium at 1 ml/min
Injection: 1 µl @ 1:20 split
Sample: 5 mg/ml in hexane after reaction with 25%
sodium methoxide at room temperature for 5
minutes.

Data:



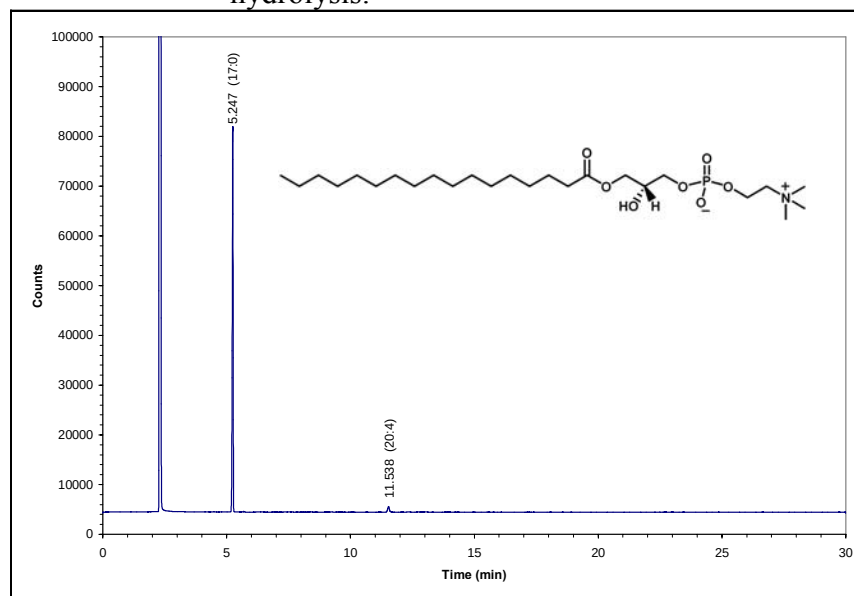
Interpret: Purity = 47.1% 17:0 FAME, 52.8% 20:4, 0.1% 18:0 FAME
(AUC)



Positional Isomeric Purity by GC

Conditions: Column: DB-225, 30M X 0.25 mm, i.d.
Temperatures: Oven = 220°C isocratic
Injection = 250°C,
Detector = 250°C
Carrier: Grade 5 Helium at 1 ml/min
Injection: 1 µl @ 1:20 split
Sample: Fatty acid methyl ester analysis with GC/FID of
lyso PC fraction after Phospholipase A2
hydrolysis.

Data:

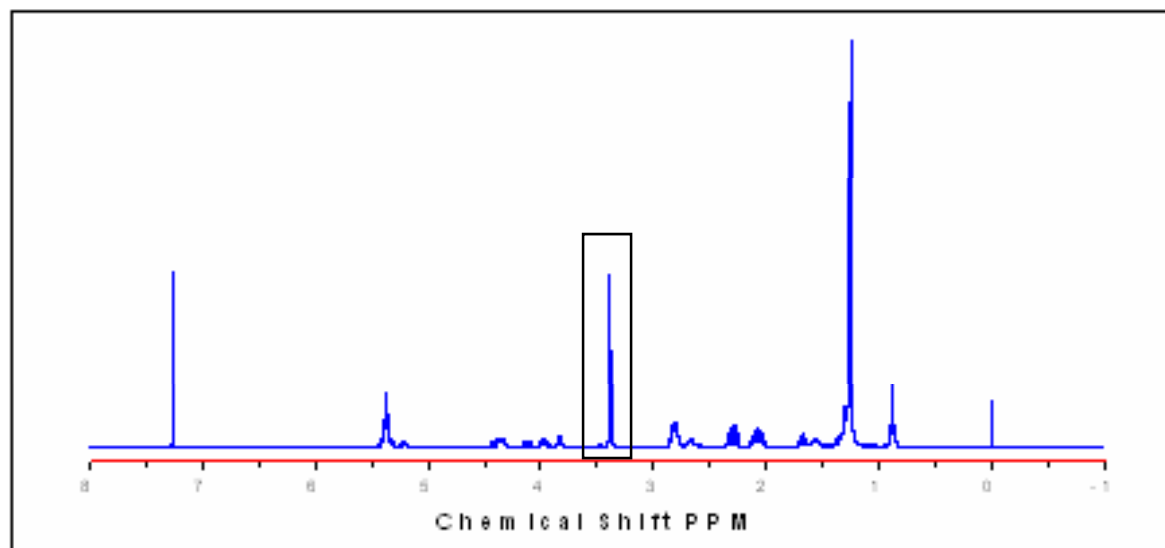


Interpretation: 97.1% sn-1, 17:0: 2.9% sn-1, 20:4 PC

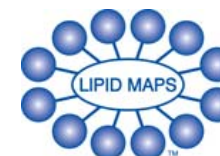
Proton NMR

Conditions:

Sample Conc:	5 mg/ml in CDCl_3 + TMS (shift reference)
Temp:	300 K
Field Strength:	300 M Hz
Pulse:	30" C for 5.24 sec.
Relaxation time:	5 sec
# of scans:	32
Line Broadening	0.00 Hz
Shift standard:	TMS - 0.00 ppm



Interpretation:	Shift (ppm)	Actual Protons	Theoretical Protons	Assignment
	5.20954 - 5.38665	9.107	9	definic protons + (proton on the 2 nd carbon of the glycerol backbone)
	4.33978 - 4.43004	2.954	3	(protons near the O on the choline) + (proton on the 1 st carbon of
	4.09507 - 4.15866	1	1	(proton on the 1 st carbon of the glycerol backbone)
	3.94257 - 3.99892	2.008	2	(protons on the 3 rd carbon of the glycerol backbone)
	3.83241	1.983	2	(protons near the N on the choline)
	3.38289	9.111	9	(3 methyl groups on the N)
	2.81104 - 2.85339	6.433	6	("interior" allylic protons)
	~2.65	2.197	0	water
	2.4920 - 2.34770	4.282	4	alpha protons
	2.04214 - 2.11303	4.401	4	allylic protons on both "ends"
	1.54489 - 1.69769	4.776	4	beta protons
	1.2511	34.621	32	remaining -CH ₂ on the fatty acid chains
	0.85563 - 0.91054	6.682	6	methyls on the fatty acids

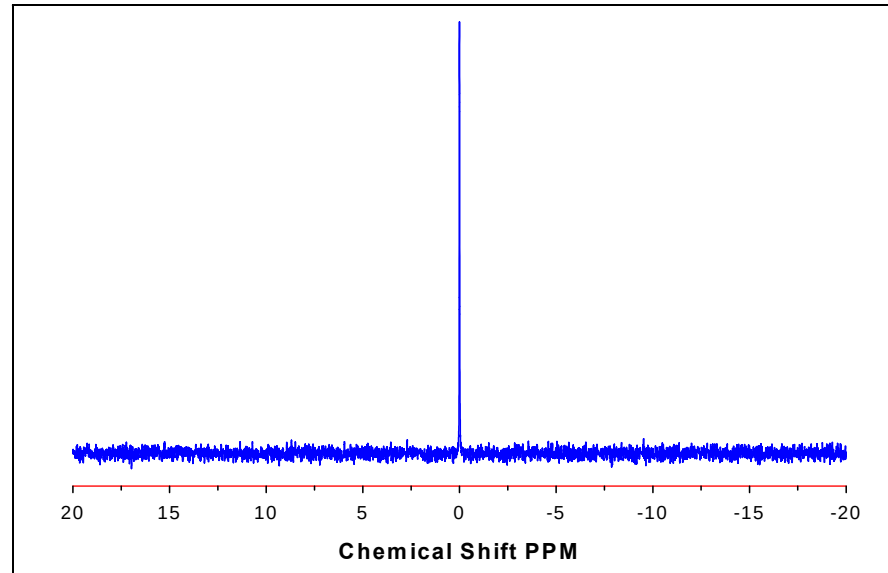


Phosphorus NMR

Conditions:

Sample conc.:	5 mg/ml in CDCl_3
Temp.:	300 K
Field Strength:	121.5 MHz
Pulse:	30° for 0.79 sec.
Relaxation time:	2 sec.
# of scans:	64
Line	1.0 Hz
Broadening:	

Data:



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Interpret: One shift under described conditions

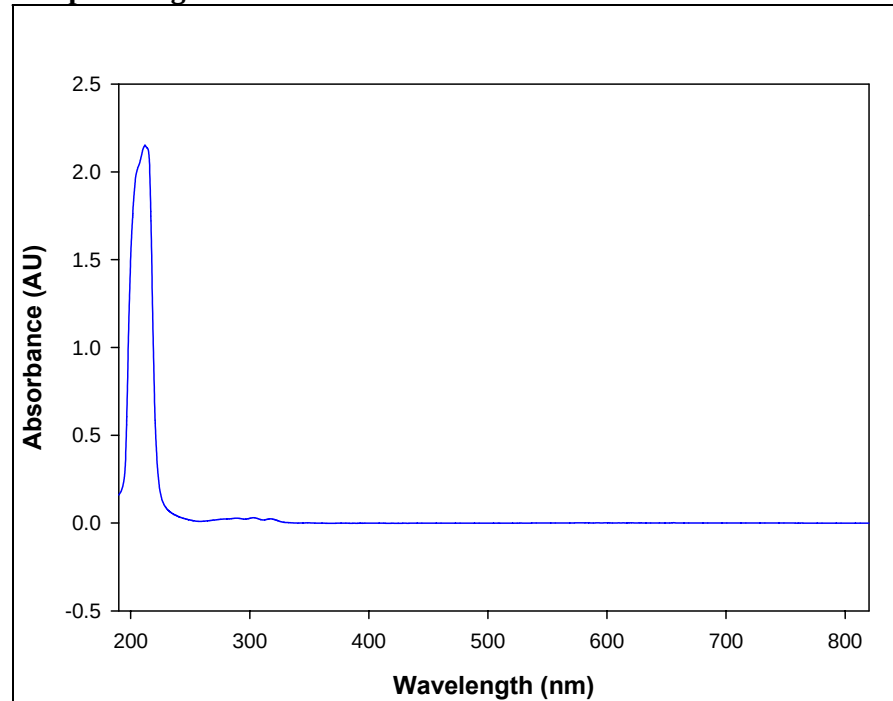


Oxidation by UV/Visible Spectrophotometry

Conditions: Agilent Model 8453 UV Visible Detector

Sample: 1 mg/ml in ethanol

Data:



Interpretation: Peroxide @ 234 nm = 0.04%
Conjugated Dienes @ 268 nm = 0.03%
Conjugated Dienes @ 278 nm = 0.05%



Mass Spectrometry (Q1 scan)

Qualitative
Conditions:

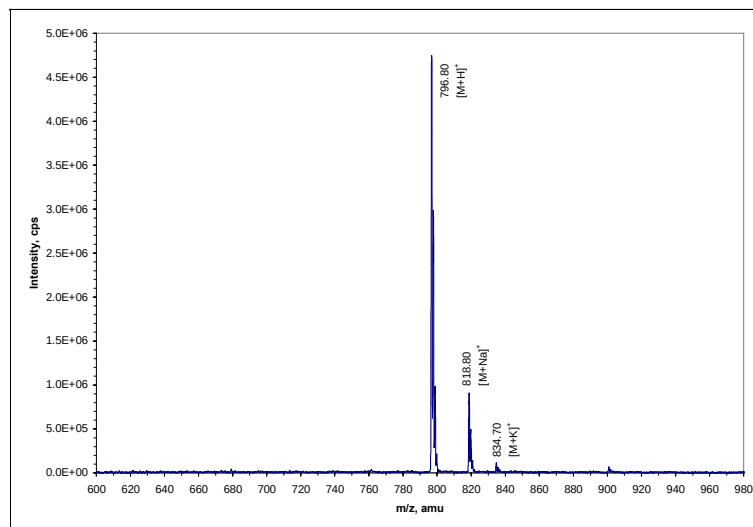
Flow infusion Scan

Period 1 Experiment 1:

Instrument: Applied Biosystems 4000 Q Trap
Scan Type: Q1 MS (Q1)
Polarity: Positive
Scan Mode: Profile
Ion Source: Turbo Spray

Start (amu)	Stop (amu)	Time (sec)
596.00	996.00	1.00

Data:



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Interpretation: +Q1: [M+H]⁺ = 796.80 u, [M+Na]⁺ = 818.80 u, [M+K]⁺ = 834.70 u.



Mass Spectrometry (Ion Trap)

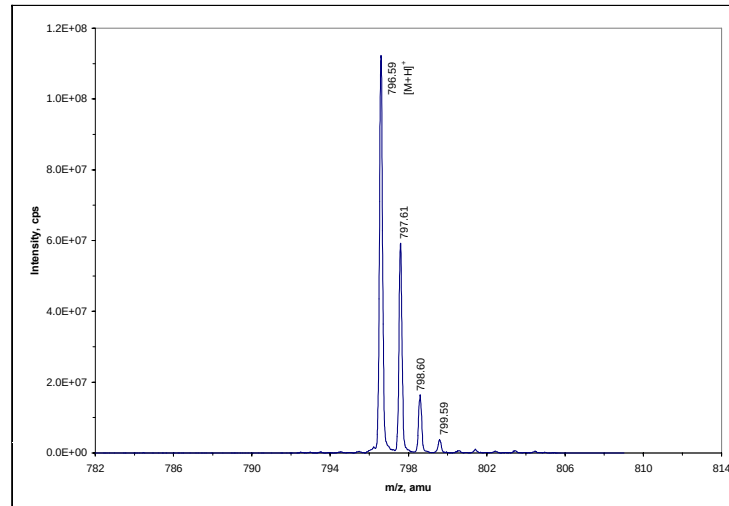
Qualitative Conditions: Flow infusion Q Trap

Period 1 Experiment 2:

Instrument: Applied Biosystems 4000 Q Trap
Scan Type: Enhanced Resolution (ER)
Polarity: Positive
Scan Profile
Mode:
Ion Source: Turbo Spray

Start (amu)	Stop (amu)	Time (sec)	Param	Start	Stop
781.80	811.80	0.1201	AF3	0.30	0.31
			EXB	-151.49	-151.33

Data:



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Interpret: +ER: [M+H]⁺ = 796.59 u.



LC/MS/MS

17:0-20:4 GPCho Ampoules

Quantitative Conditions:

HPLC/MS/MS Acquisition Info

Comment: Mercury Luna C18 2x20mm 3 micron
 Mobile Phase A: 9:1 H₂O:MeOH + 10 mM NH₄OAc
 Mobile Phase B: 100% MeOH + 10 mM NH₄OAc
 Gradient: (Linear) 100% A to 100% B over 3.5 min

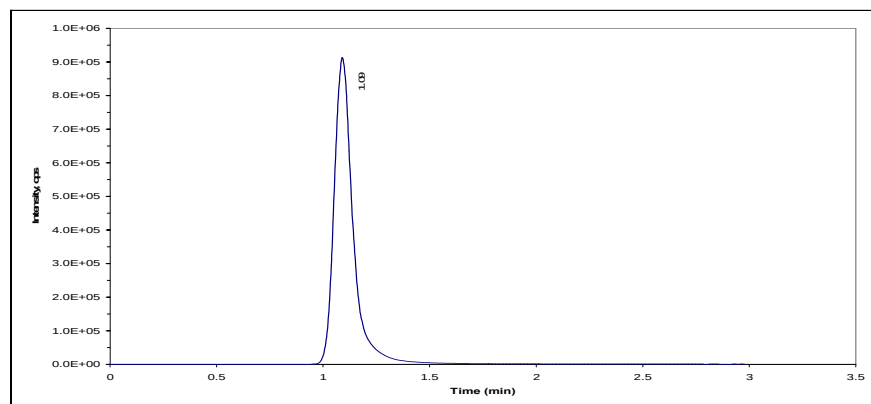
MRM Transition

Q1 Mass (amu)	Q3 Mass (amu)	Dwell(msec)
796.70	184.30	1000.00

Step Table

Step	Total Time (min)	Flow Rate (μl/min)	A (%)	B (%)
0	0	1000	5	95
1	3	1000	5	95

Data:

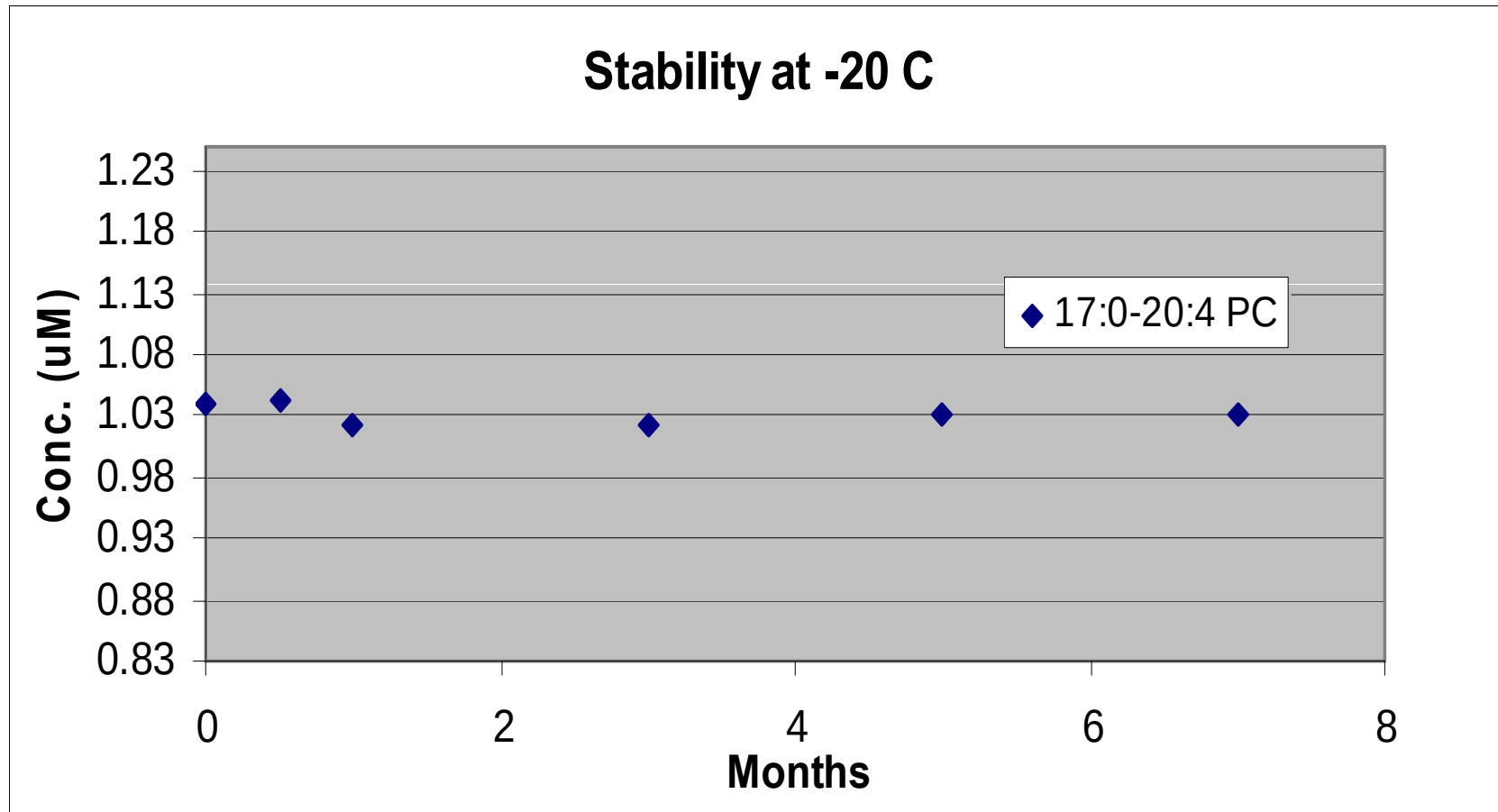


Interpret:

Total 17:0-20:4 PC = 1.041 μM

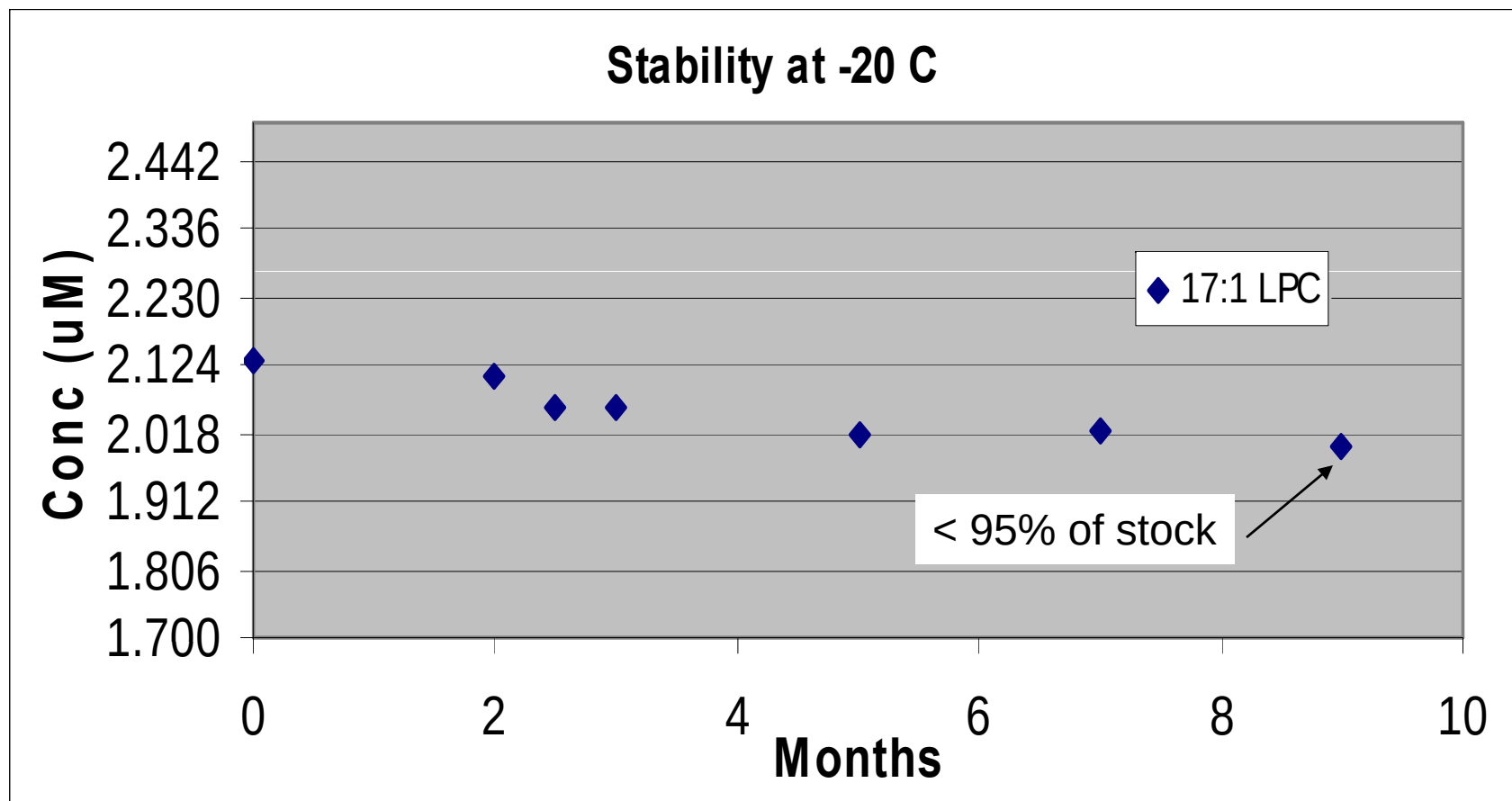


Stability in Ampoules



Stability in Ampoules

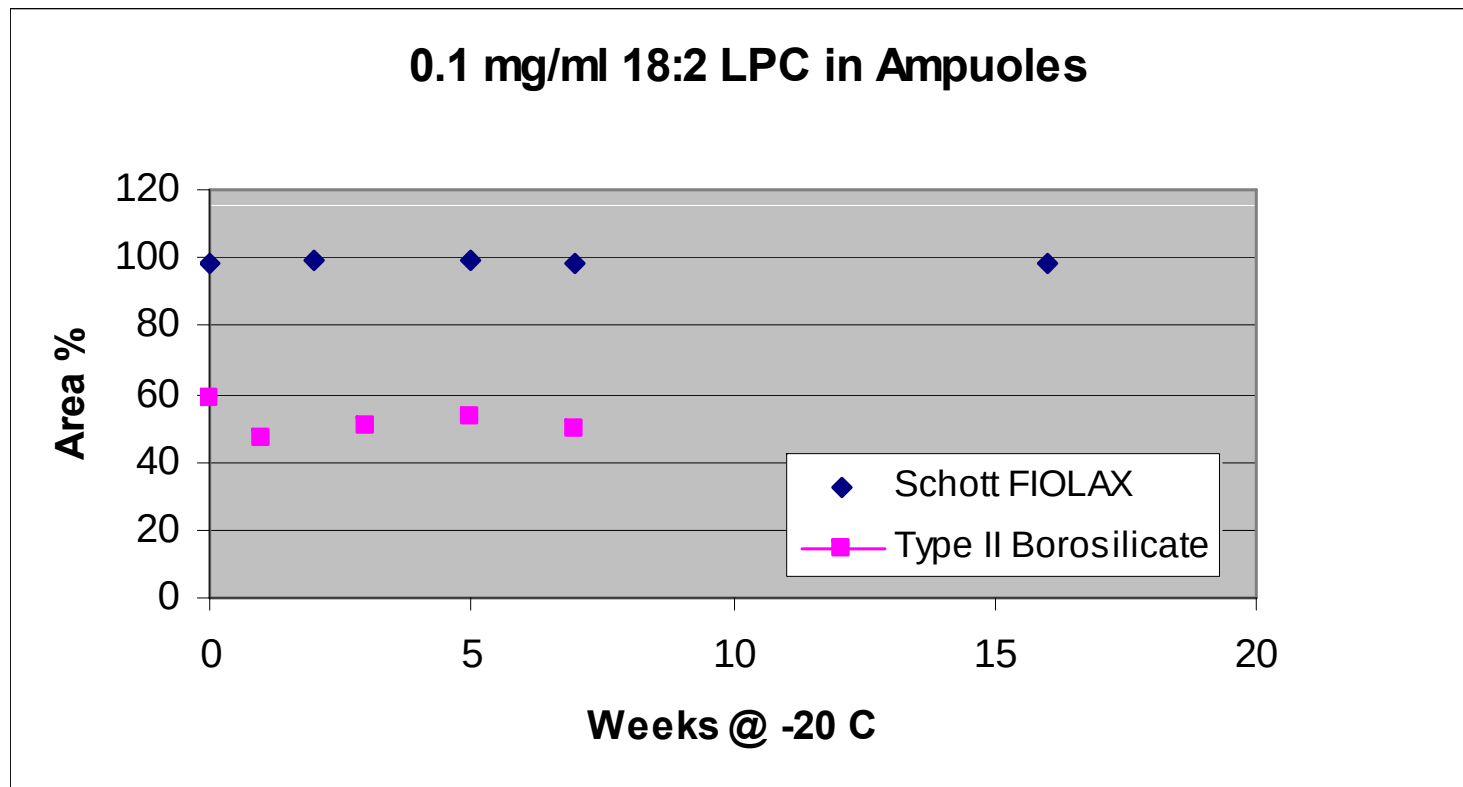
Failed



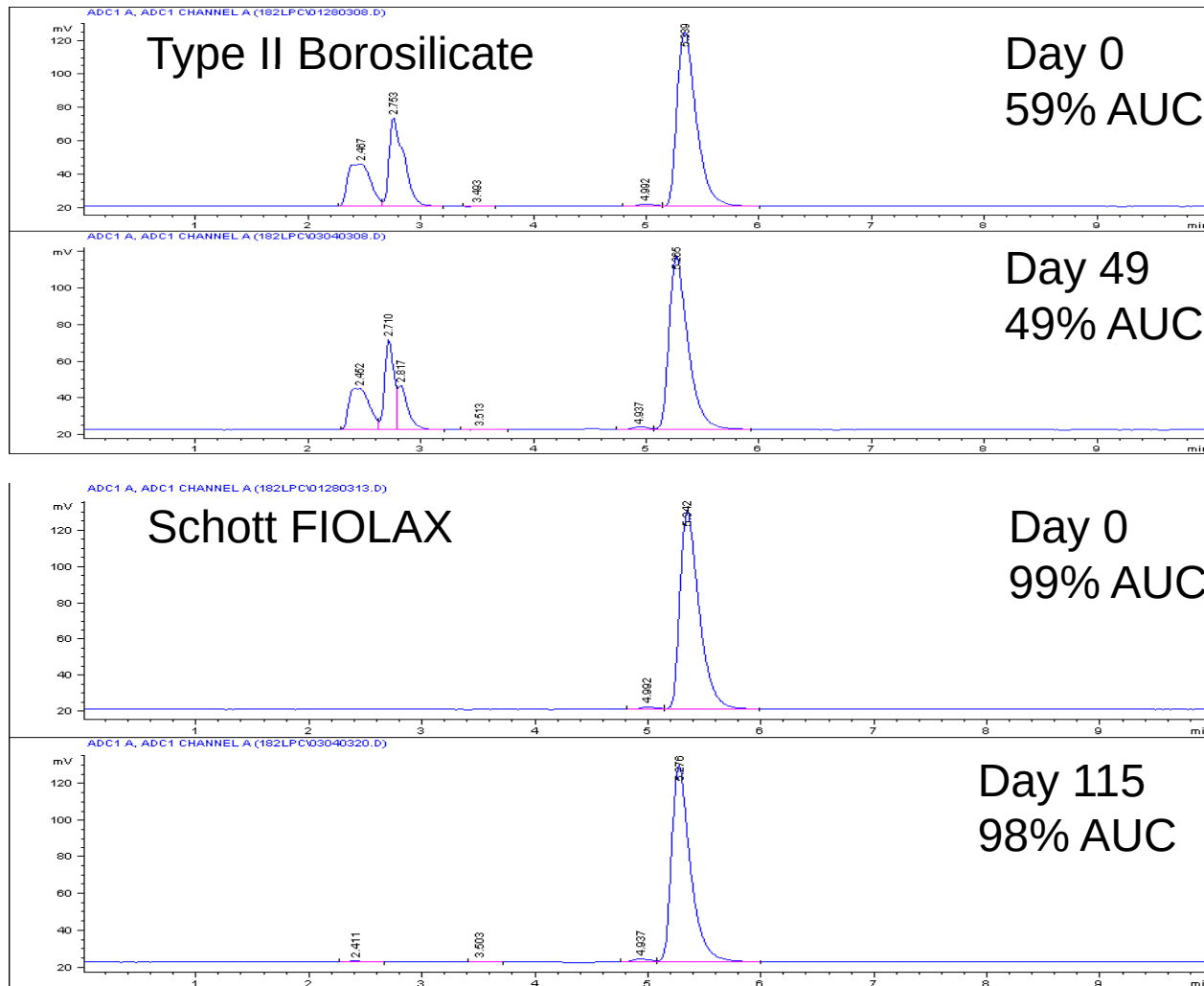


*why bad
things happen
to good lipids!*

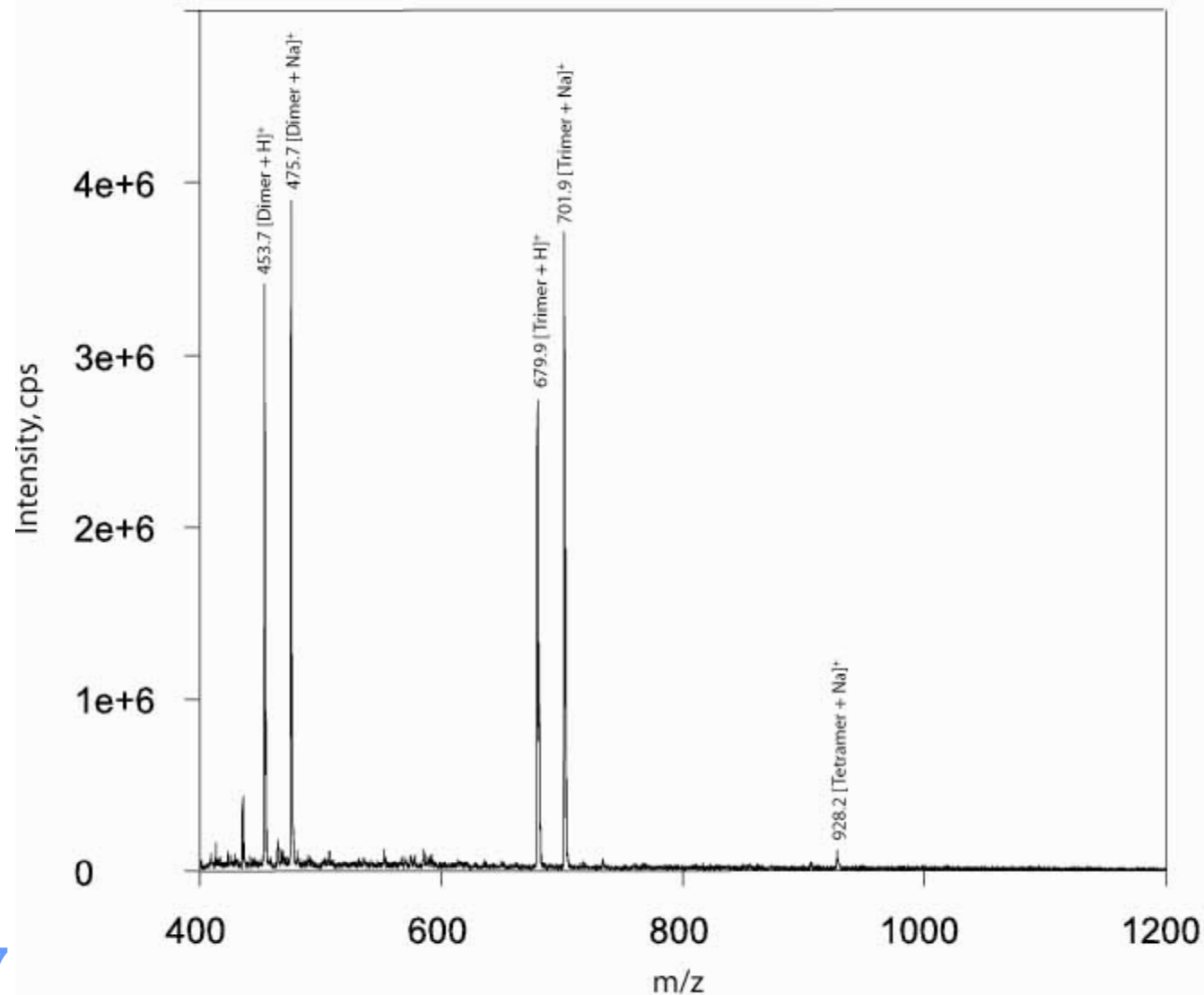
Packaging Glass Matters !



Packaging Glass Matters !

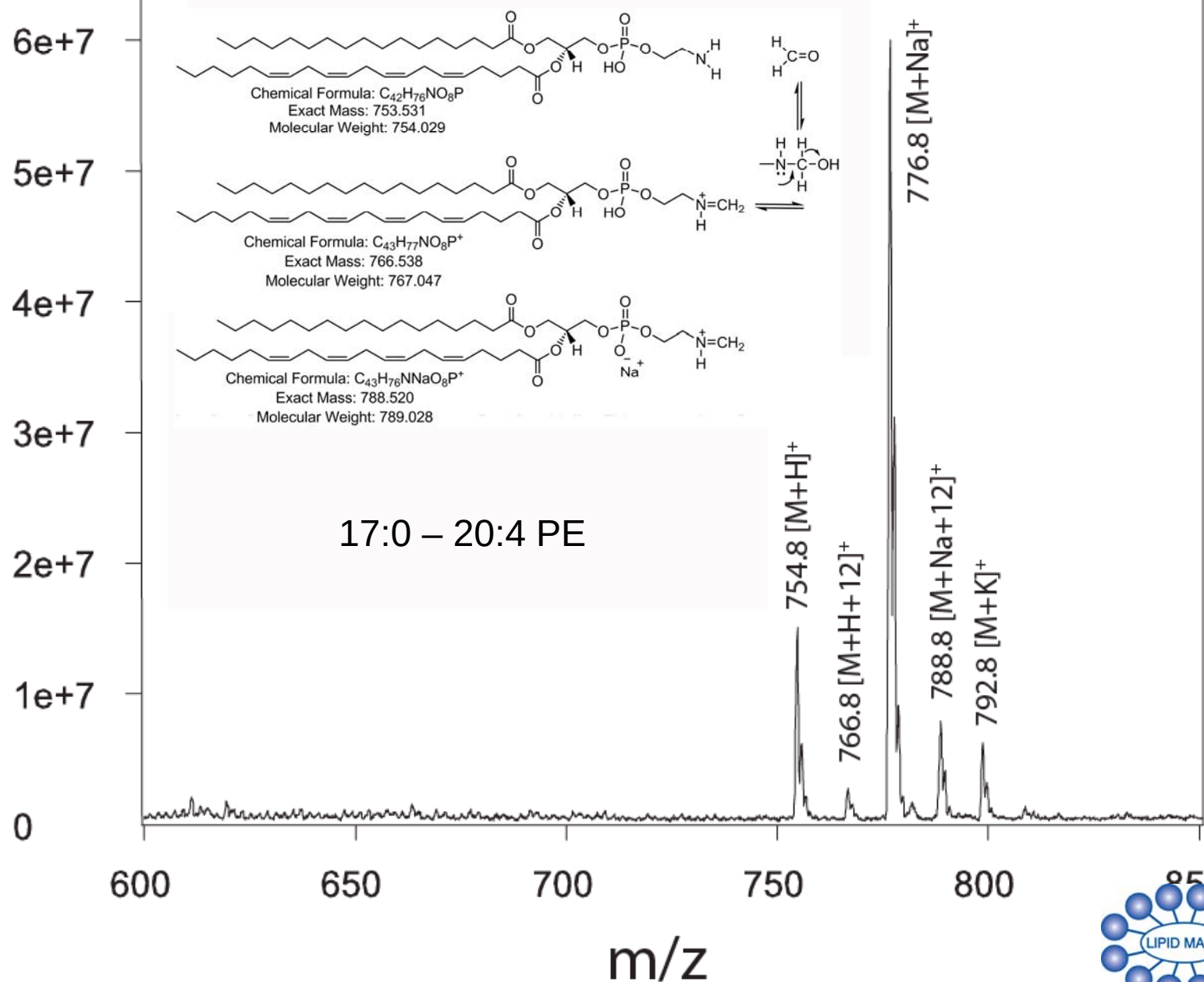


Nylon Filter Additives/ Solvent Methanol

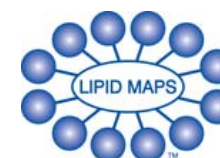
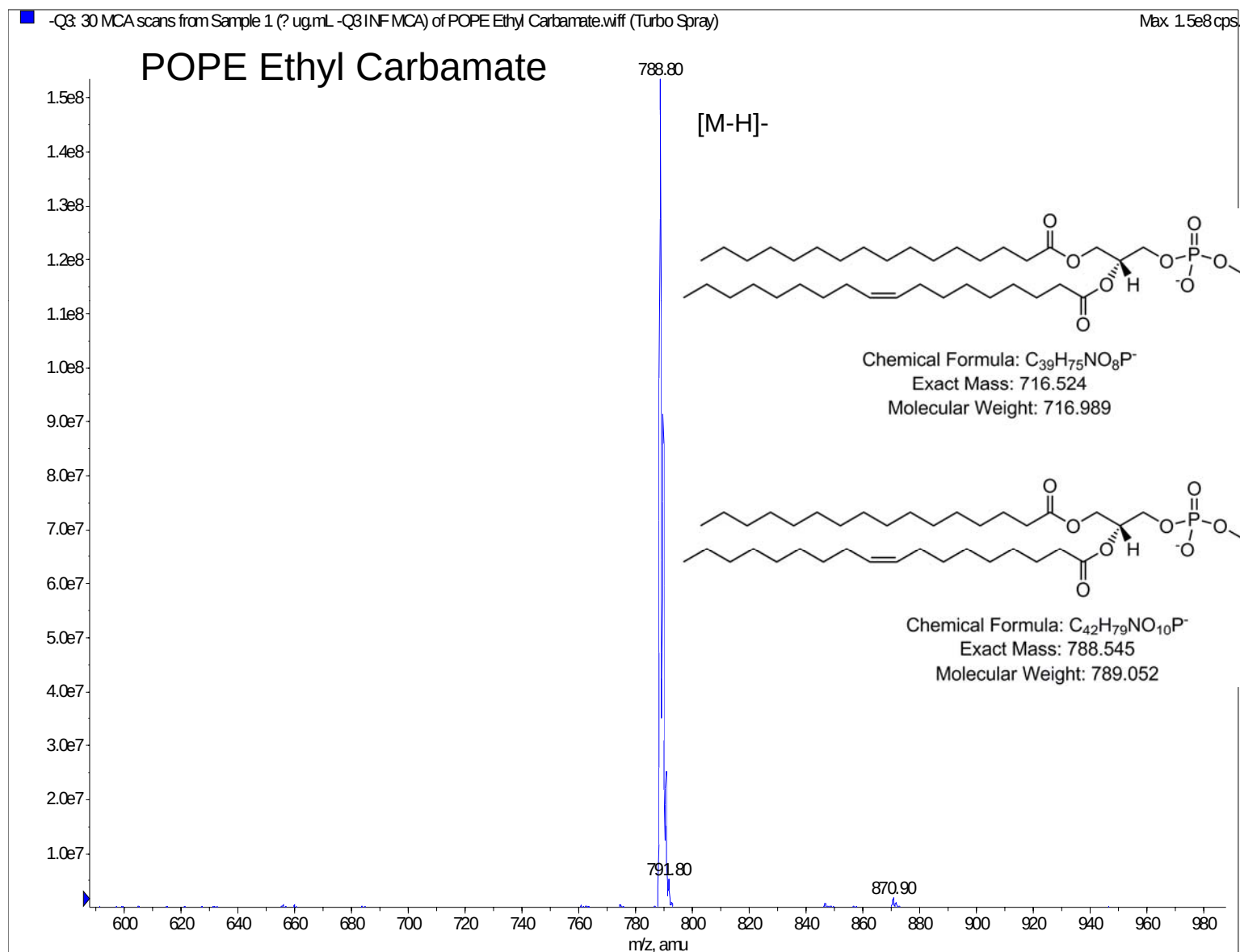


Methanol with Formaldehyde – Schiff Base

Abundance

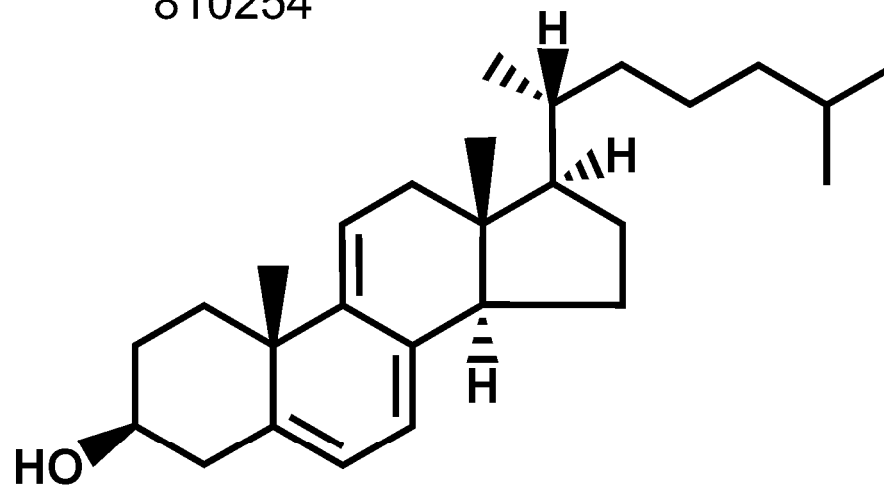


Phosgene from Chloroform

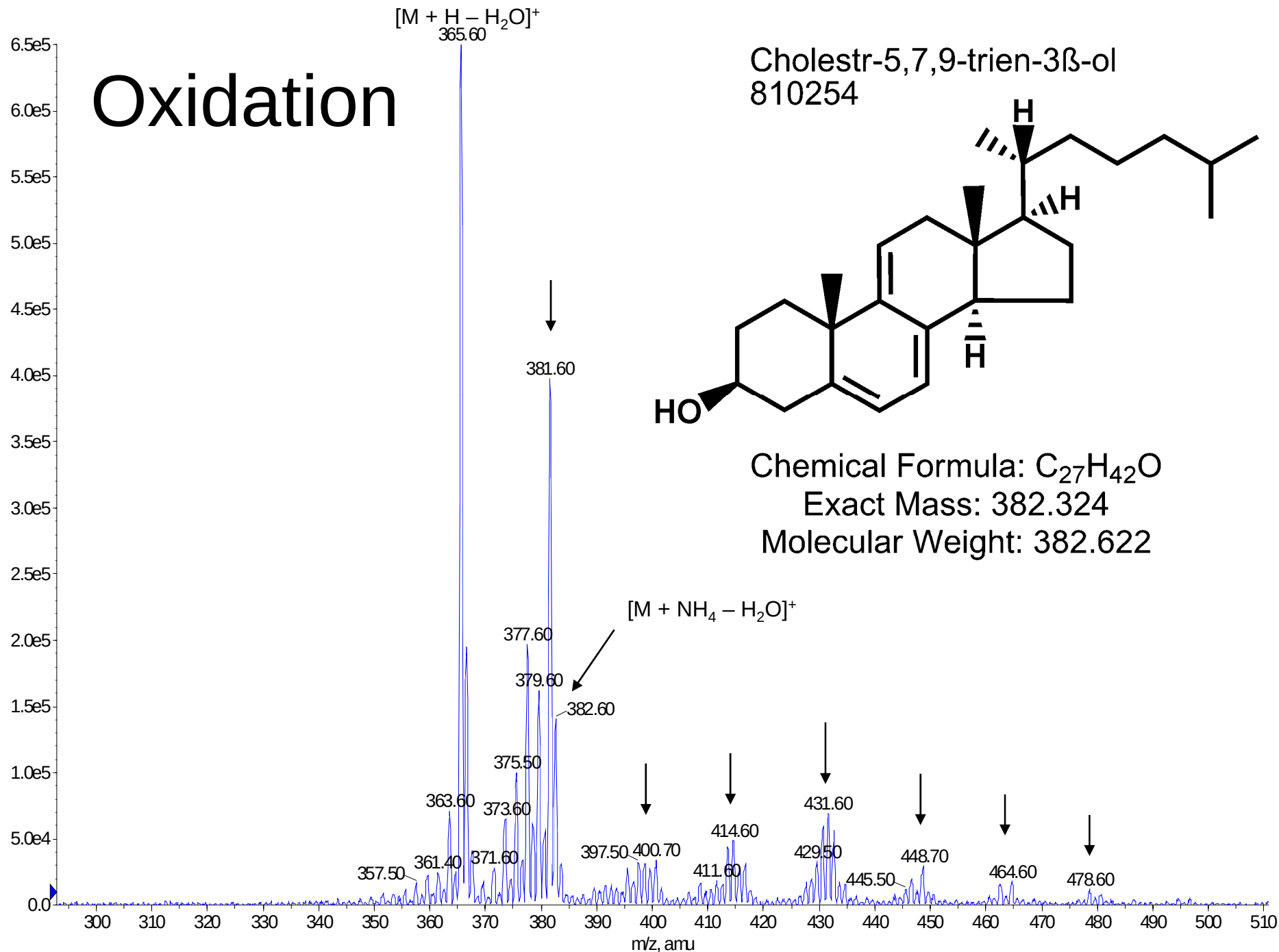


Oxidation

Cholestr-5,7,9-trien-3 β -ol
810254



Chemical Formula: C₂₇H₄₂O
Exact Mass: 382.324
Molecular Weight: 382.622



Available Standards



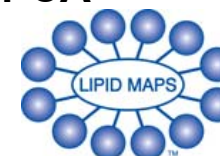
Sphingolipid Mixture, LM-6002

- C17 Sphingosine
- C17 Sphinganine
- C17 Sphingosine-1-phosphate
- C17 Sphinganine-1-phosphate
- C12 Sphingomyelin
- C12 Ceramide
- C12 Glucosyl Ceramide
- C12 Lactosyl Ceramide
- C12 Ceramide-1-phosphate
- C25 Ceramide



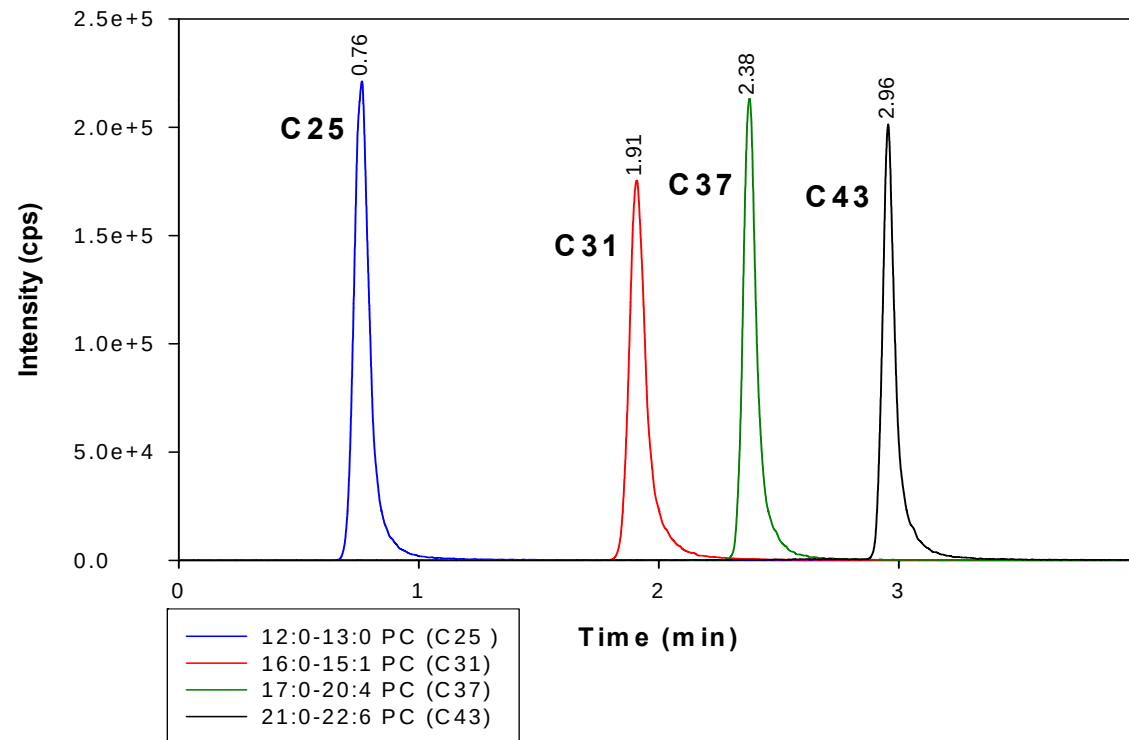
Glycerophospholipids

• LM-1000	12:0-13:0 PC	624	• LM-1400	12:0-13:0 PA
• LM-1004	17:0-14:1 PC	+ 82	• LM-1404	17:0-14:1 PA
• LM-1002	17:0-20:4 PC	+160	• LM-1402	17:0-20:4 PA
• LM-1003	21:0-22:6 PC	+240	• LM-1403	21:0-22:6 PA
• LM-1100	12:0-13:0 PE		• LM-1500	12:0-13:0 PI
• LM-1104	17:0-14:1 PE		• LM-1504	17:0-14:1 PI
• LM-1102	17:0-20:4 PE		• LM-1502	17:0-20:4 PI
• LM-1103	21:0-22:6 PE		• LM-1503	21:0-22:6 PI
• LM-1200	12:0-13:0 PG		• LM-1600	13:0 LPC
• LM-1204	17:0-14:1 PG		• LM-1601	17:1 LPC
• LM-1202	17:0-20:4 PG		• LM-1700	13:0 LPA
• LM-1203	21:0-22:6 PG		• LM-1701	17:1 LPA
• LM-1300	12:0-13:0 PS		•	
• LM-1304	17:0-14:1 PS		• LM-1802	14:1(3)-15:1 CA
• LM-1302	17:0-20:4 PS		• LM-1803	15:0(3)-16:1 CA
• LM-1303	21:0-22:6 PS		• LM-1804	22:1(3)-14:1 CA
			• LM-1801	24:1(3)-14:1 CA



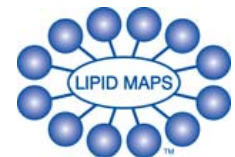
Application: Mixture of GPCCho ISTDs

1,2 diacyl-*sn*-glycero-3-phosphocholine species separation



PIP's

- **LM-1900** **17:0-20:4 PI(3)P**
- **LM-1901** **17:0-20:4 PI(4)P**
- **LM-1902** **17:0-20:4 PI(5)P**
- **LM-1903** **17:0-20:4 PI(3,4)P2**
- **LM-1905** **17:0-20:4 PI(3,5)P2**
- **LM-1904** **17:0-20:4 PI(4,5)P2**
- **LM-1906** **17:0-20:4 PI(3,4,5)P3**



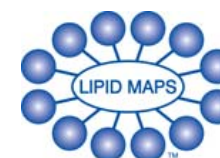
Sterols

- LM-4100 cholest-5-en-3 β -ol(d7)
- LM-4101 cholest-5-en-3 β ,25-diol(d3)
- LM-4102 cholest-5-en-3 β ,4 β -diol(d7)
- LM-4103 cholest-5-en-3 β ,7 α -diol(d7)
- LM-4104 cholest-5-en-3 β ,7 β -diol(d7)
- LM-4105 5,6 α -epoxy-5 α -cholestan-3 β -ol(d7)
- LM-4106 5 α -cholestan-3 β ,6 α -diol(d7)
- LM-4107 7-oxo-cholest-5-en-3 β -ol(d7)
- LM-4108 cholest-5,24-dien-3 β -ol(d6)
- LM-4109 24,25-epoxy-cholest-5-en-3 β -ol(d6)
- LM-4110 cholest-5-en-3 β ,24-diol(d6)



Neutral Lipids (DG, TG)

- | | | | |
|-----------|----------------|-----------|----------------------|
| • LM-3107 | 1,3-20:5 DG-d5 | • LM-3207 | 20:5-22:6-20:5 TG-d5 |
| • LM-3108 | 1,3-14:0 DG-d5 | • LM-3208 | 14:0-16:1-14:0 TG-d5 |
| • LM-3109 | 1,3-15:0 DG-d5 | • LM-3209 | 15:0-18:1-15:0 TG-d5 |
| • LM-3110 | 1,3-16:0 DG-d5 | • LM-3210 | 16:0-18:0-16:0 TG-d5 |
| • LM-3111 | 1,3-17:0 DG-d5 | • LM-3211 | 17:0-17:1-17:0 TG-d5 |
| • LM-3112 | 1,3-19:0 DG-d5 | • LM-3212 | 19:0-12:0-19:0 TG-d5 |
| • LM-3113 | 1,3-20:0 DG-d5 | • LM-3213 | 20:0-20:1-20:0 TG-d5 |
| • LM-3114 | 1,3-20:2 DG-d5 | • LM-3214 | 20:2-18:3-20:2 TG-d5 |
| • LM-3115 | 1,3-20:4 DG-d5 | • LM-3215 | 20:4-18:2-20:4 TG-d5 |
| • LM-3116 | 1,3-16:1 DG-d5 | | |
| • LM-3117 | 1,3-18:0 DG-d5 | | |
| • LM-3118 | 1,3-18:1 DG-d5 | | |
| • LM-3119 | 1,3-18:2 DG-d5 | | |



Packaging



LIPID MAPS Product Labels



1g LM-0001 STORE IN FREEZER -20°C M.W. 999.99
1-Lauroyl-2-Hydroxy-*sn*-Glycero-3-Phosphocholine
Lot #: LM1-xxxxxxxxxx 20mg/ml Chloroform 1g



- PDF417 2D Barcode Information:
 - a) LIPID MAPS Reagent ID
 - b) Avanti Lot Number

