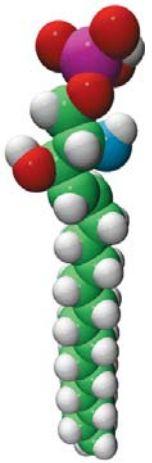
www.lipidmaps.org

LIPID MAPS Lipidomics Workshop

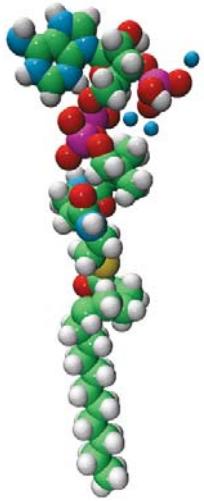
April 18, 2009



Solvents & Surfaces – Lipid's Bane

Walter A. Shaw

Avanti® Polar Lipids, Inc.



LIPID MAPS Core F(A): Lipid Standards

Synthesis	Analytical
Avanti's Synthesis Group	Avanti's Analytical Group

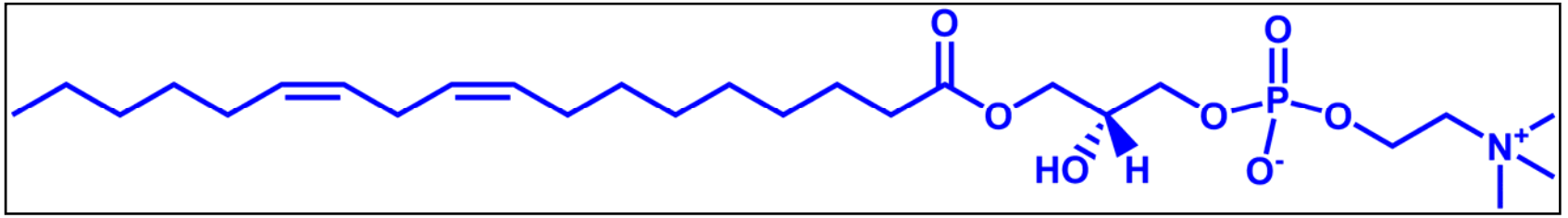
Outline

- Glass
- Glass plus
- Solvents
- Surfaces
- Equipment Geometry

Glass

NOT EXACTLY CHEMICALLY INERT !

Glass Surface Interaction of 18:2 LPC in Flame Sealed Ampoules



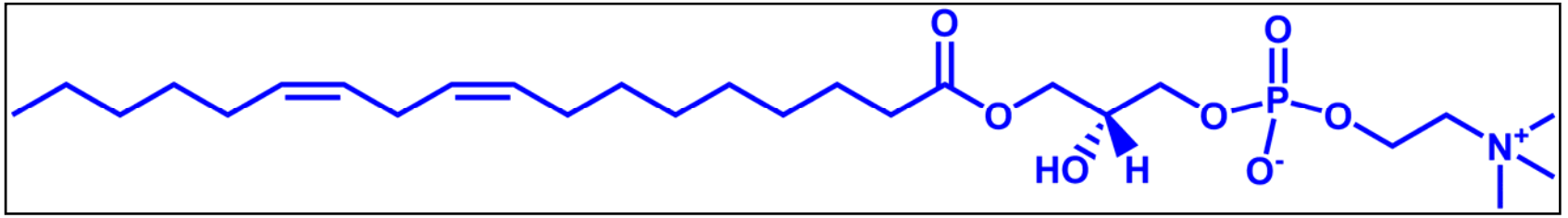
HPLC results of 100µg/ml 18:2 LPC in 0.2 ml of methanol (Incubated at room temperature for 1 hour)

Ampoule	Description	% Purity (sealed)	% Purity (un-sealed)
Kimble #12010S	C, B, U	61.5	80.1
Kimble #12050G-1	C, B, T1	77.1	82.9
Kimble #12040U-1	A, B	64.8	75.8
Schott #12201026	C, B, USP, T1	49.7	67.6
Schott #61430300	A, Fiolax®	97.7	98.5

C = clear, B = borosilicate, U = untreated, T1 = type 1, A = amber, USP = US Pharmacopea

Product degrades according to glass chemistry surface interaction and flame sealing

Glass Surface Interaction of 18:2 LPC in Flame Sealed Ampoules



HPLC results of 100 µg/ml 18:2 LPC in Schott Fiolax® ampoules (Incubated at room temperature for 1 hour)

Volume	% recovery (sealed)	% Purity (sealed)
Stock =(6 injections)	101 4	97.8 1
0.10 ml (0.10 mg)	97.0	93.1
0.25 ml (0.25 mg)	95.8	97.9
0.50 ml (0.50 mg)	98.7	98.0
1.00 ml (1.00 mg)	96.1	98.0

Fiolax™ 70% SiO_3, 7% B_2O_3, 6% Al_2O_3, 7% Na_2O, 1% K_2O, 5% TiO_2 and 1% $Fe2O_3$

Minimal degradation according to volume surface interaction

Packaging

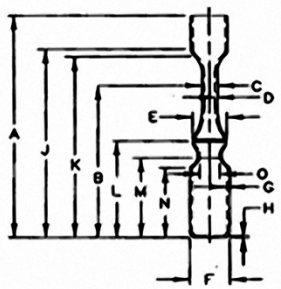
- Container type is a 2 ml, flame sealable amber ampoule
  - Has a funnel top opening constructed to USP/EP type I specifications
  - The glass material is known as FIOLAX® 8414 amber
  - Readily available from Avanti®, Product number 600050
  - Provides chemical and concentration stability superior to Type II borosilicate ampoules

SCHOTT FIOLAX®

Special Glass Tubes for the Production of Pharmaceutical Primary Packaging

SCHOTT
glass made of ideas

SYMBOL + NOMENCLATURE		INCHES	MILLIMETERS
A	OVERALL HEIGHT	2.874±.060	73.00±1.50
B	SEALING HEIGHT	1.969±.060	50.00±1.50
C	STEM DIAMETER	.169±.016	4.30± .40
D	STEM WALL THKNS.	.015±.004	.40± .10
E	BULB O.D.	.375±.020	9.50± .50
F	BODY DIAMETER	.443±.008	11.25± .20
G	BODY WALL THKNS.	.022±.002	.55± .05
H	BOTTOM WALL THKNS.	.018 MIN.	.45 MIN.
J	CHIN HEIGHT	2.441±.040	62.00±1.00
K	HANGING HEIGHT	2.343±.060	59.50±1.50
L	STRIPE HEIGHT	1.232±.060	31.30±1.50
M	CONSTRICTION HEIGHT	1.063±.060	27.00±1.50
N	BODY HEIGHT	.950 MIN.	24.15 MIN.
O	CONSTRICTION I.D.	.190±.020	4.85± .50



REVISIONS

REV.	DATE	DESCRIPTION	ORI.	APP'D

REFERENCE DWG'S 11101001-0

APPROVALS	DATE
DRAWN C. EDRIS	20 MAY 02
SPECIFICATION H. FRANZ	20 MAY 02
QUALITY D. COFFMAN	20 MAY 02
MANUFACTURING L. CASSERT	20 MAY 02
CUSTOMER SEE CUST. FILE	13 SEPT 02

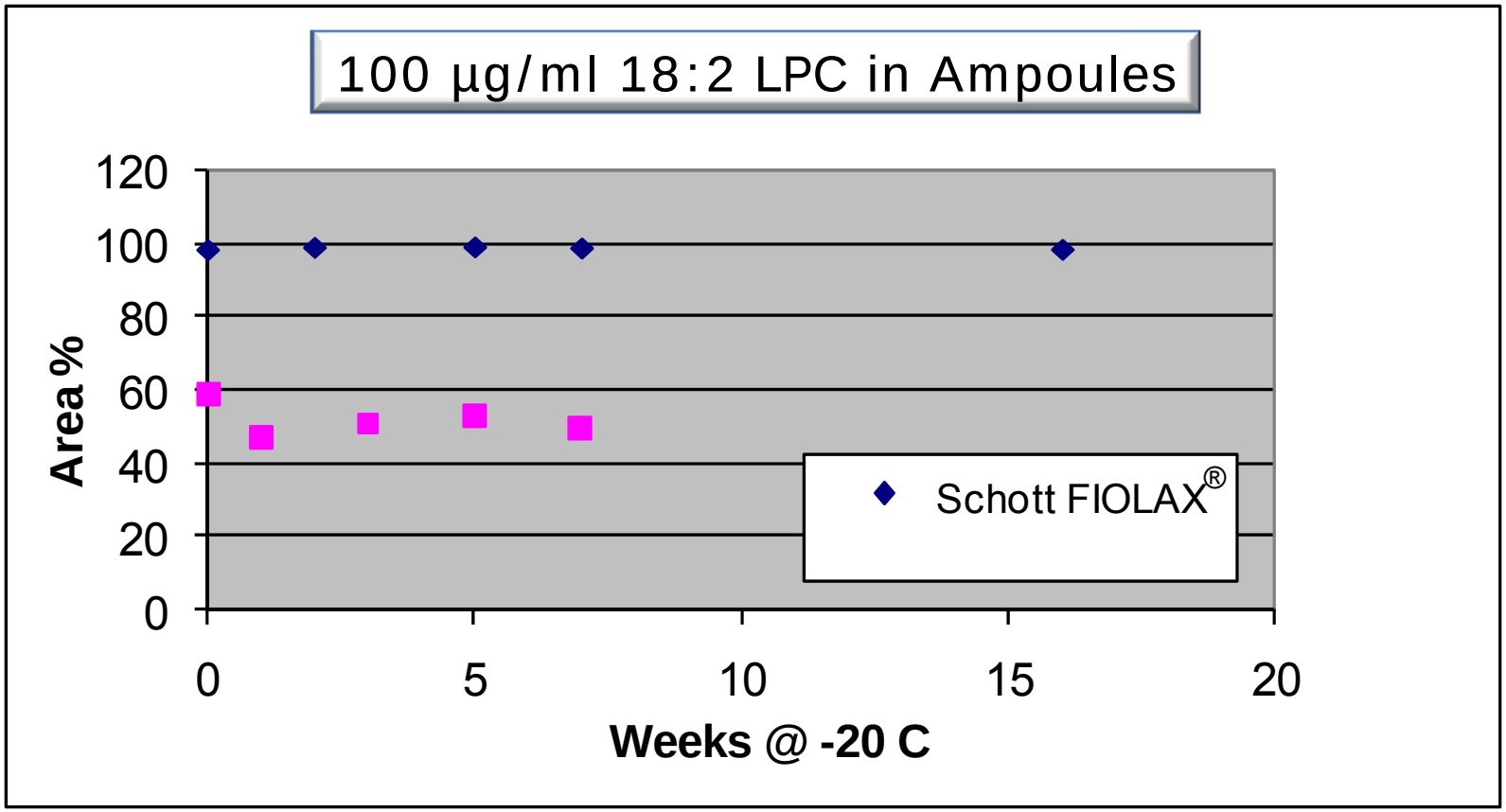
SCHOTT
Schott Pharmaceutical Packaging, Inc. Oconomowoc, WI

TITLE: 1ml FUNNEL TOP AMPUL
10 x 73 mm

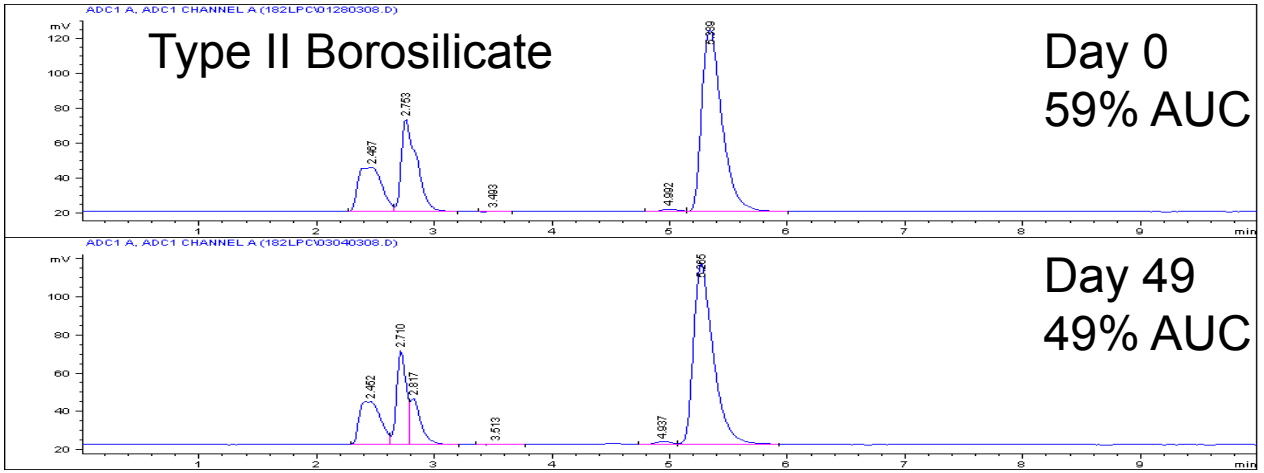
SCALE: 1 : 1
MATL: USP/EP TYPE I GLASS

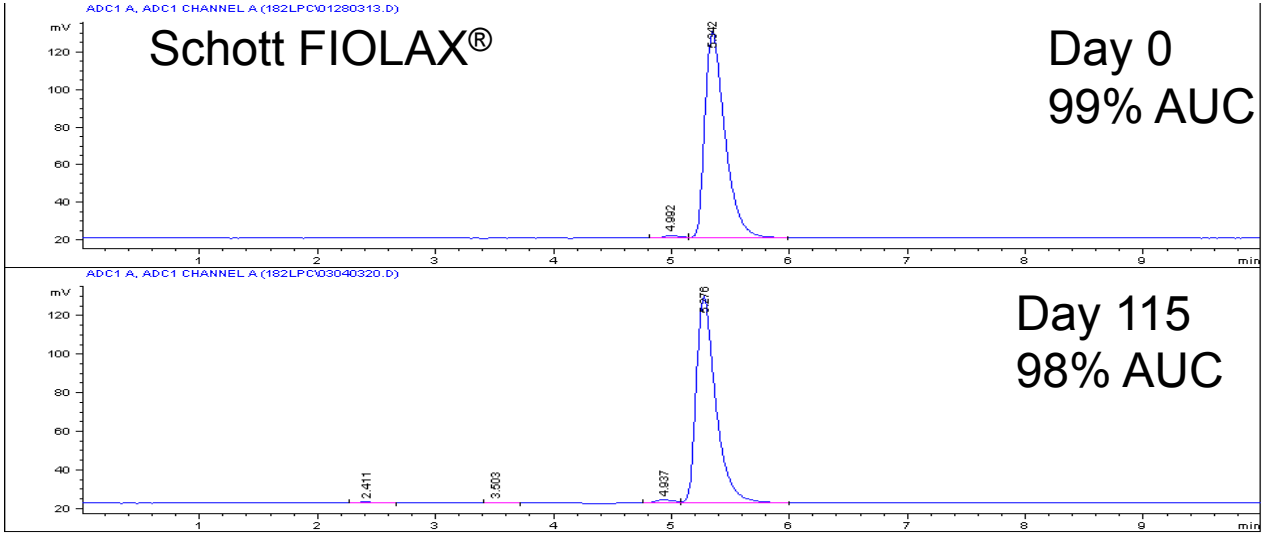
DRAWING NO. 11201007
REV. —

Stability of 18:2 LPC in FIOLAX®



HPLC results for 18:2 LPC stored in Borosilicate or FIOLAX®

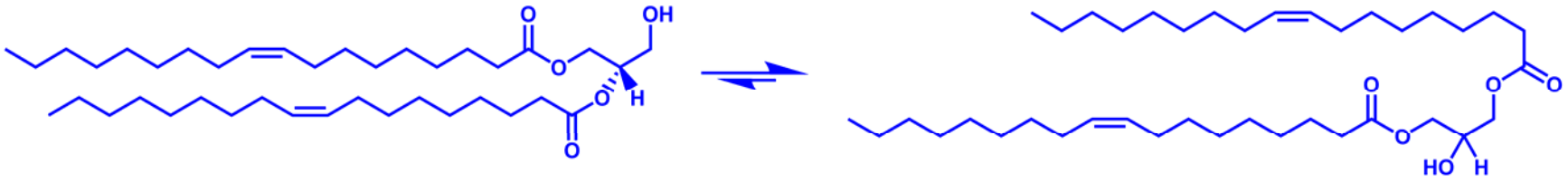




Acyl Migration

GLASS PLUS MULTIPLE FACTORS COME INTO PLAY

Acyl Migration of 1,2-Diacylglycerols



1,2 DAG

1,3 DAG

- Solutions and lyophilized solids of 1,2-diacylglycerol compounds demonstrate acyl migration to form 1,3-diacylglycerols upon packaging in (pulled) borosilicate glass ampoules and Schott Fiolax® glass ampoules.

- Experiments were performed with variations of our packaging protocols to prevent or diminish this migration.

Experiment Design - Drying

- All packaging protocols were performed in amber (molded) glass containers (non-acid washed) using 25mg of 1,2-diacylglycerol
- Thin-layer chromatography on silica plates can separate the 1,2 and 1,3 diacylglycerols when developed in Toluene:Chloroform:Methanol 85:15:5
- Each sample was plated at ~ 25mg/ml prepared in the mobile phase solvent.
- Proton NMR was performed on original stock materials to confirm absence of 1,3-isomers.
- Proton NMR was performed on processed materials to confirm presence of 1,3-isomers.

Thin-layer Chromatography Results

Compound	Conditions	1,3-DG	1,2-DG
1,2-8:0-DG	Stock solution	-	+
	BD + **No solvent** + Lyo	+	+
	BD + **Cyc** + Lyo	+	+
	BD + **TB** + Lyo	+	+
1,2-12:0 DG	Stock solution	-	+
	BD + **TB** + Lyo	+	-
	BD + **No solvent** + Lyo	+	+
	BD + **Etoh** + **BD(no Lyo)**	+	+
	BD + **Cyc** + **BD(no Lyo)**	+	+
1,2-18:1 DG	Stock Solution	-	+
	BD + Cyc + **Fz** + Lyo	+	+
	BD + Cyc + **SFz** + Lyo	+	+
	BD + Cyc + **CO2** + Lyo	+	+

BD = blown dry under nitrogen at room temperature.
Lyo = solution lyophilized
Cyc = cylohexane
Etoh = ethanol
TB = t-Butanol
Fz = stored @ -20 C until frozen
SFz = shell frozen with dry ice in acetone bath
CO2 = block frozen with dry ice

Experimental Design - Concentration

- Solutions of 1,2-diacylglycerol were filled into 30 ml amber glass bottles to contain 10, 25, 50, 100, 500 and 1000 mg. They were blown down with nitrogen at room temperature and resuspended in cyclohexane in equivalent volumes, shell frozen in dry ice/acetone bath and then lyopholized overnight.

Results

- Lyopholized amber bottles containing > 500 mg demonstrated minimal acyl migration by thin-layer chromatography plated at 25mg/ml.

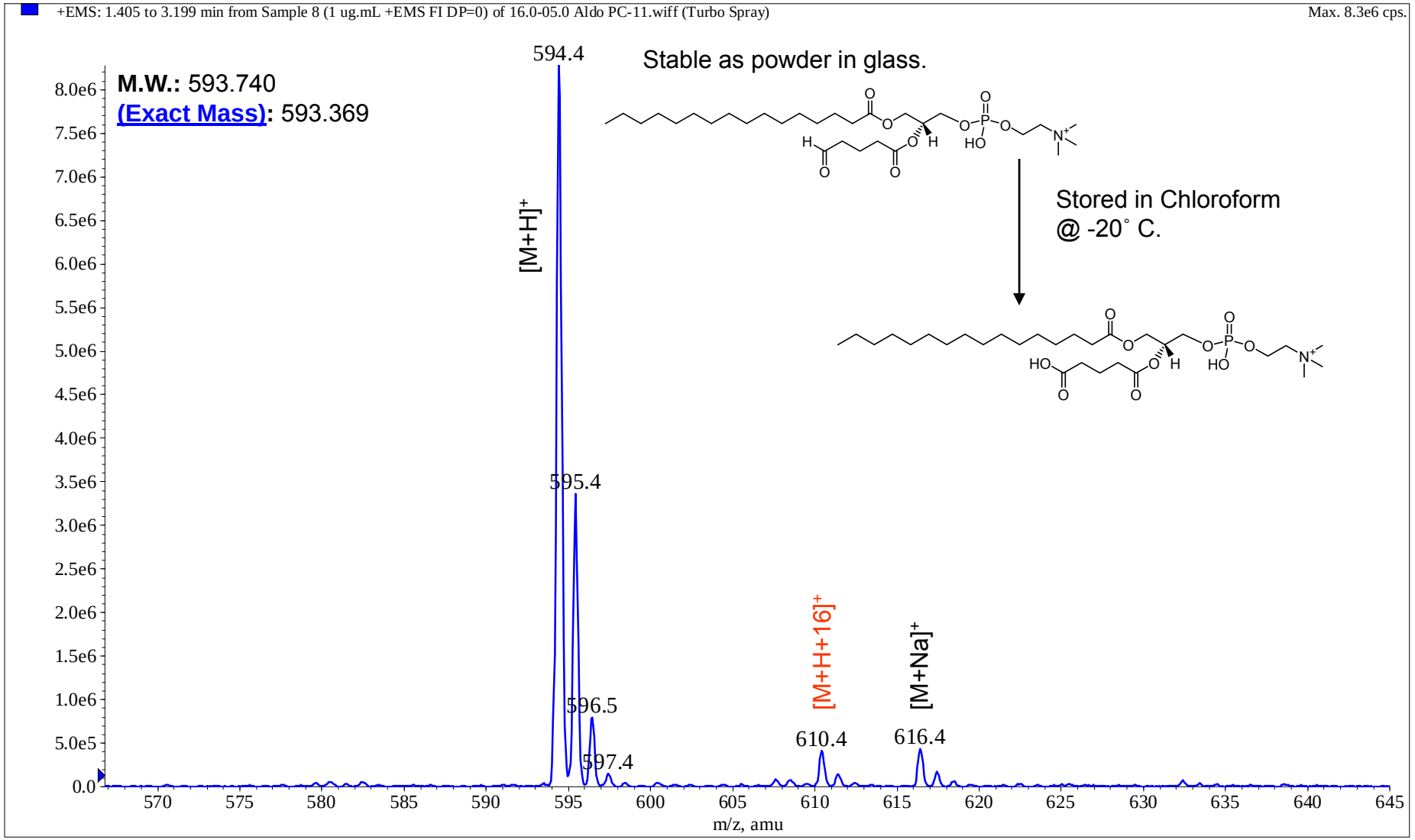
- Samples lyopholized in Teflon® containers demonstrated no acyl migration by thin-layer chromatography.

Conclusion

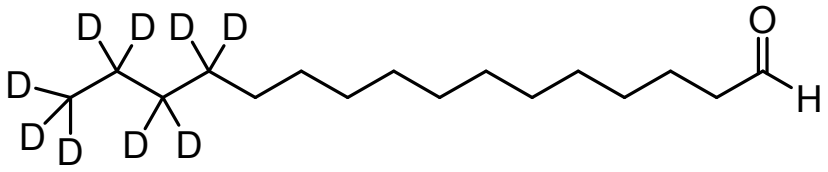
- Acyl migration of pure 1,2-diacylglycerols to their 1,3-isomers is through some as yet unidentified mechanism with glass surfaces.
- Increased ratio of 1,2-diacylglycerols mass to glass surface diminished the extent of migration.
- No migration was observed when materials were processed in Teflon® containers.
- Avanti® ships 1,2-diacylglycerol oils in quantities of 100mg or less in Teflon® containers.
  - 1,2-diacylglycerol species that form powders at room temperature are stable to migration in glass.

ALDEHYDE STABILITY

16:0-05:0 (ALDO) PC (POVPC)
1-Palmitoyl-2-(5'-oxo-Valeroyl)-*sn*-Glycero-3-Phosphocholine

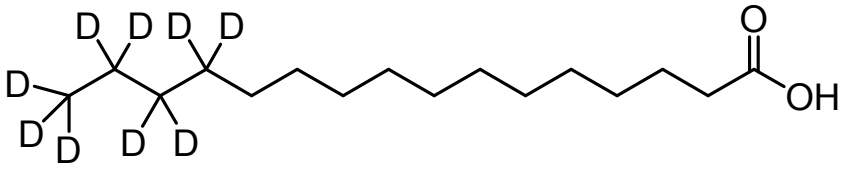


Hexadecanal-d9



Chemical Formula: $C_{16}H_{23}D_9O$
Exact Mass: 249.3
Molecular Weight: 249.48

Stable as dichloromethane solution (5 mg/ml) stored in Teflon®.



Chemical Formula: $C_{16}H_{23}D_9O_2$
Exact Mass: 265.3
Molecular Weight: 265.48

Produced upon exposure to air, acidic and basic conditions, on glass surfaces and in solvents other than dichloromethane.

Solvents

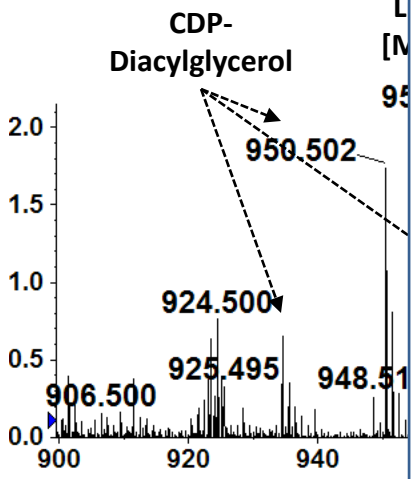
BEWARE:

WHAT LURKS IN SOLVENTS?

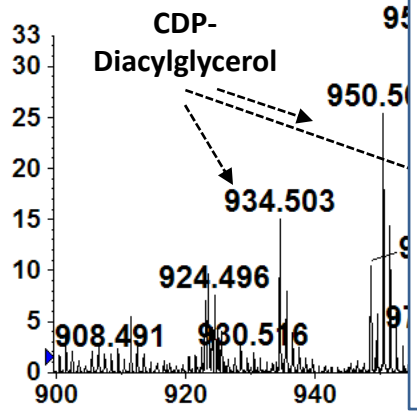
PHOSGENE

Phosgene Artifacts During $CHCl_3$ Extraction: Glutathione-conjugated PE in *E. coli* Lipid Extract

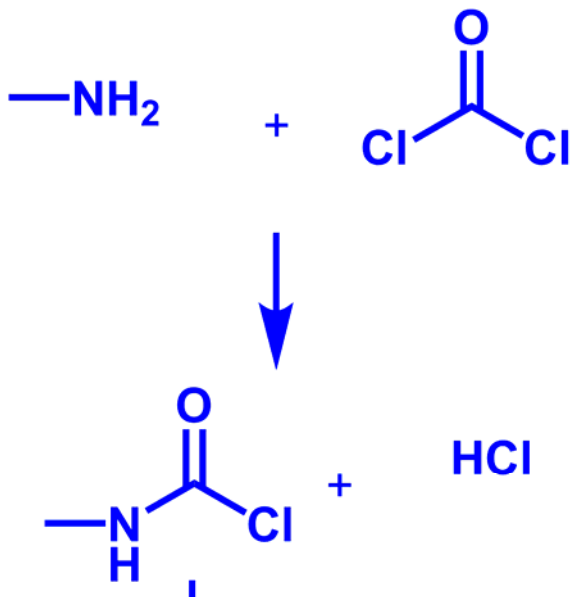
1) $CHCl_3$ Extraction

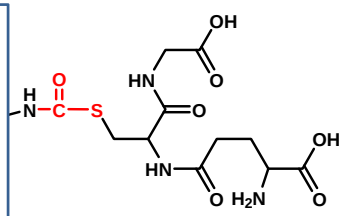


2) CH_2Cl_2 Extraction



Phosgene in Chloroform



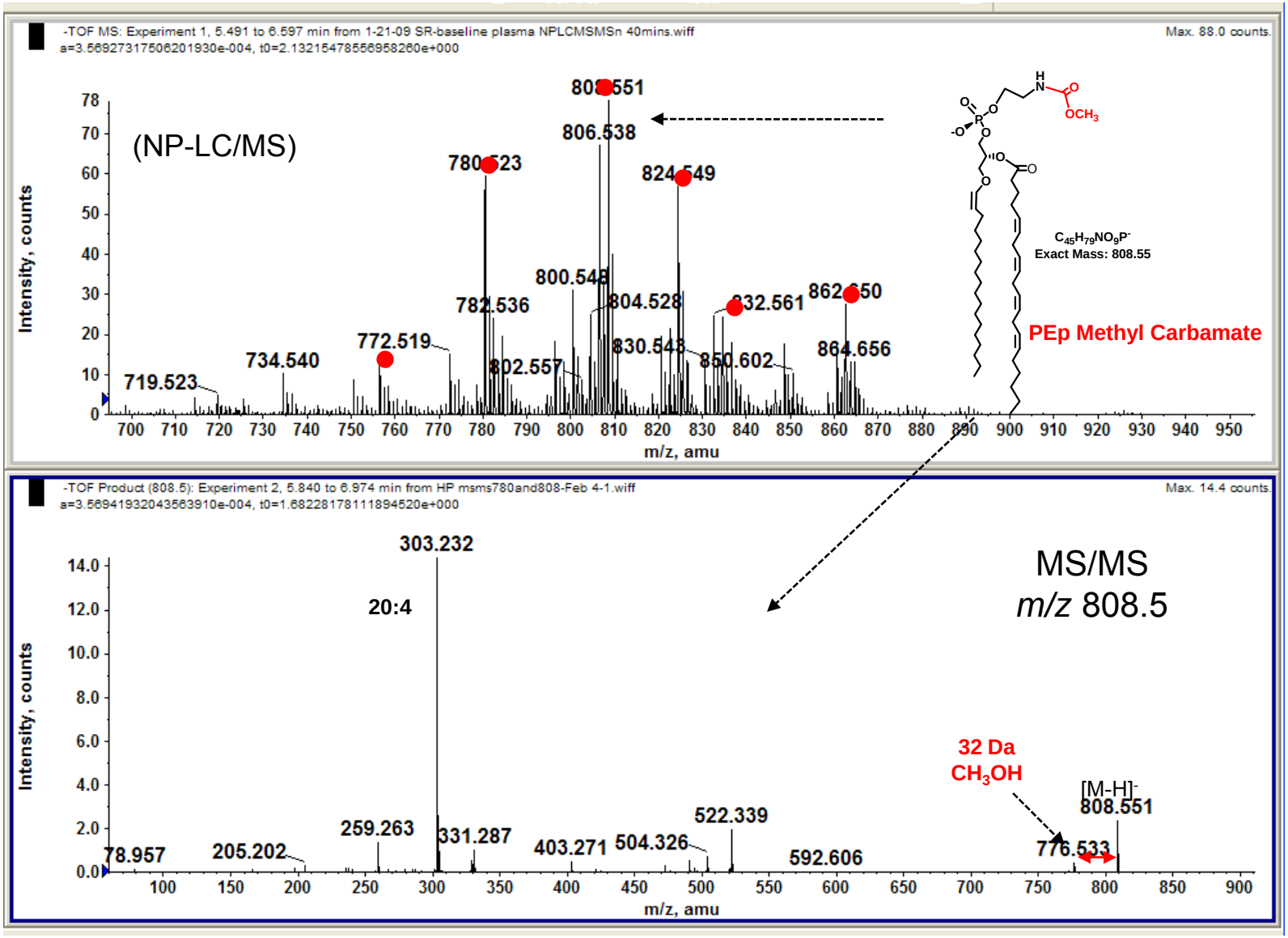


$C_{48}H_{86}N_4O_{15}PS$
Exact Mass: 1021.5548

m/z, amu

Raetz Laboratory, Duke University

Phosgene Artifacts During $CHCl_3$ Extraction: PEp and PE-Methyl Carbamates in Human Plasma Lipid Extract



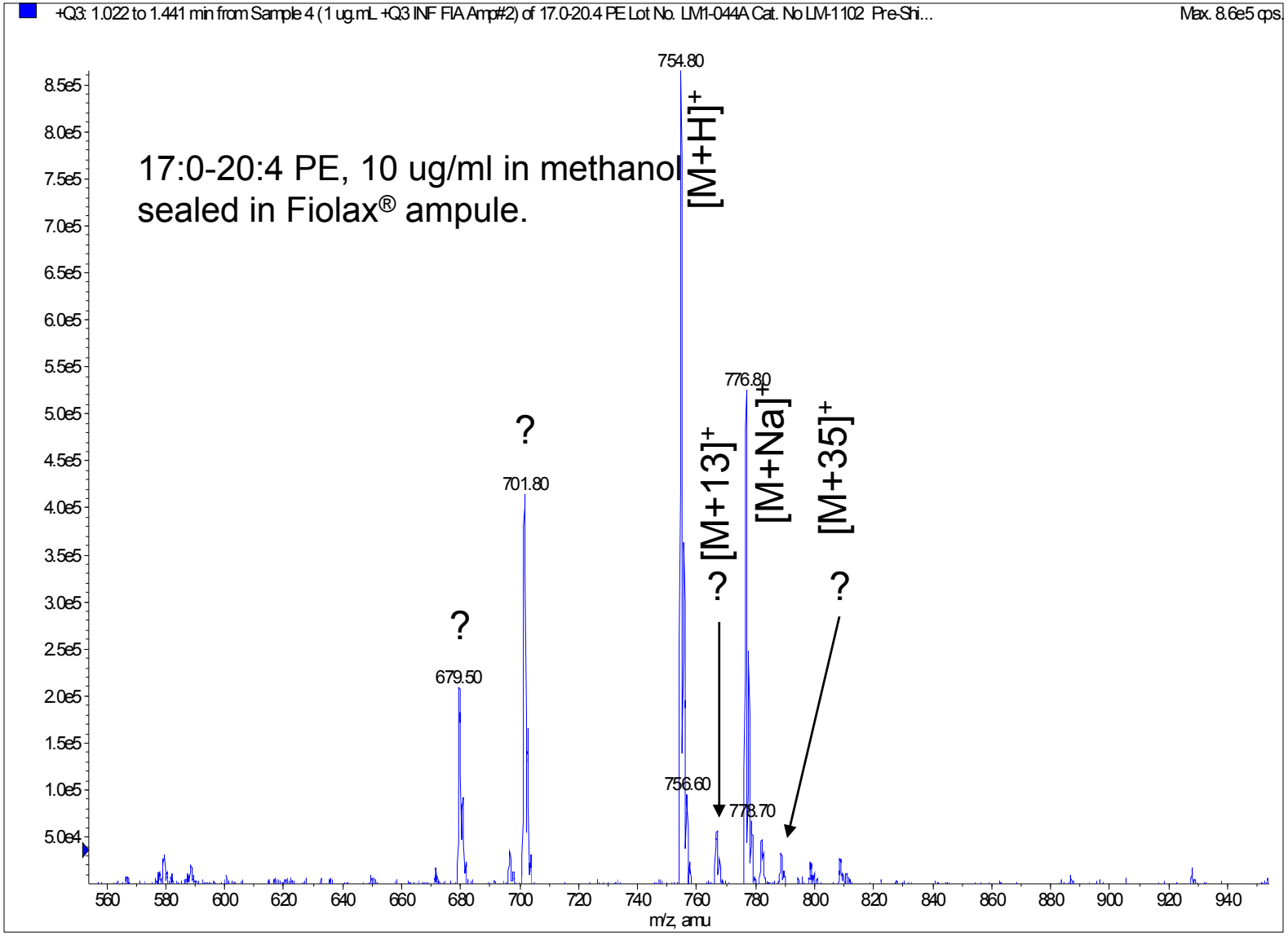
Raetz Laboratory, Duke University

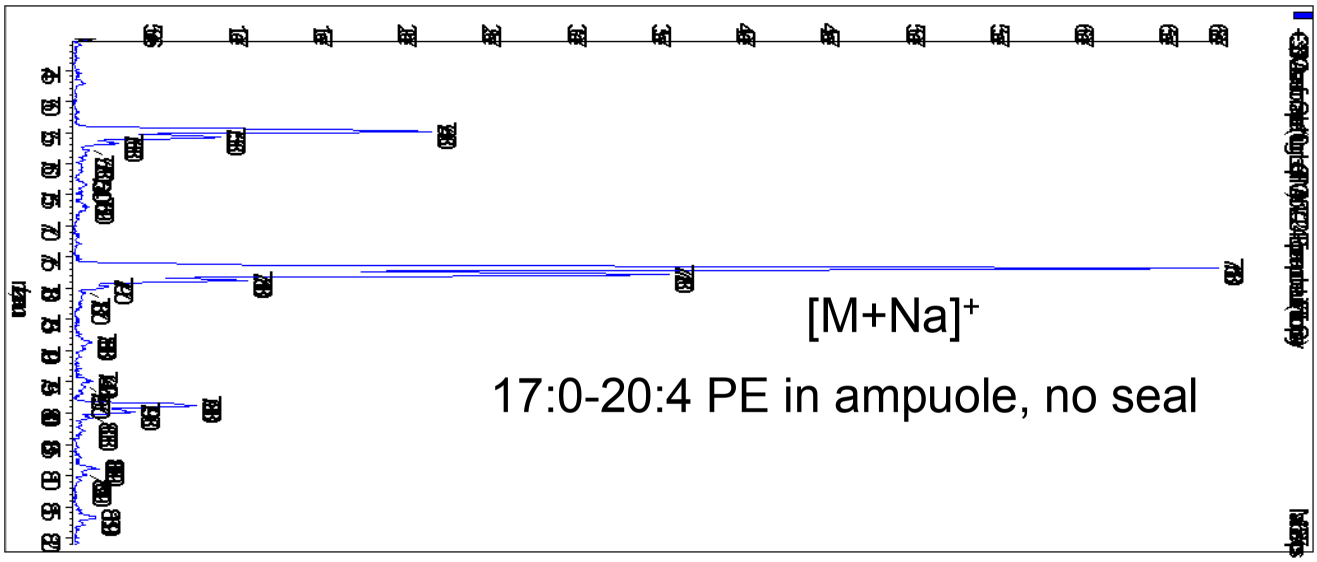
CARBONYL COMPOUNDS

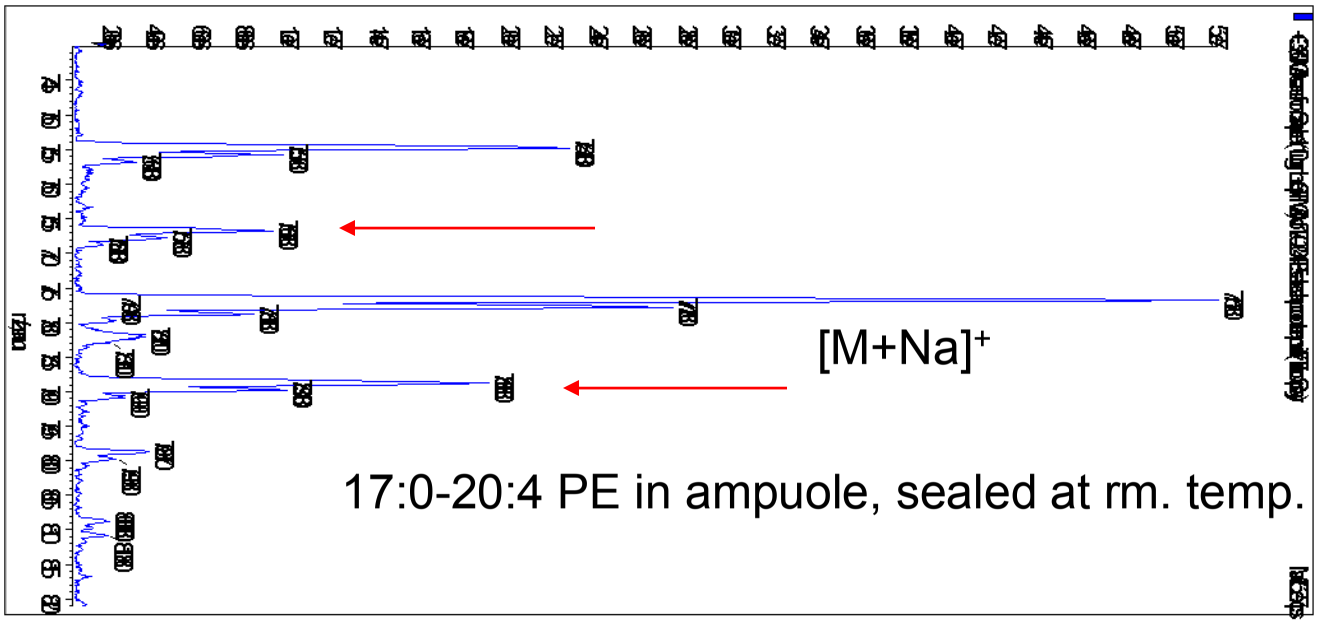
Methanol and Sealing Ampuoles

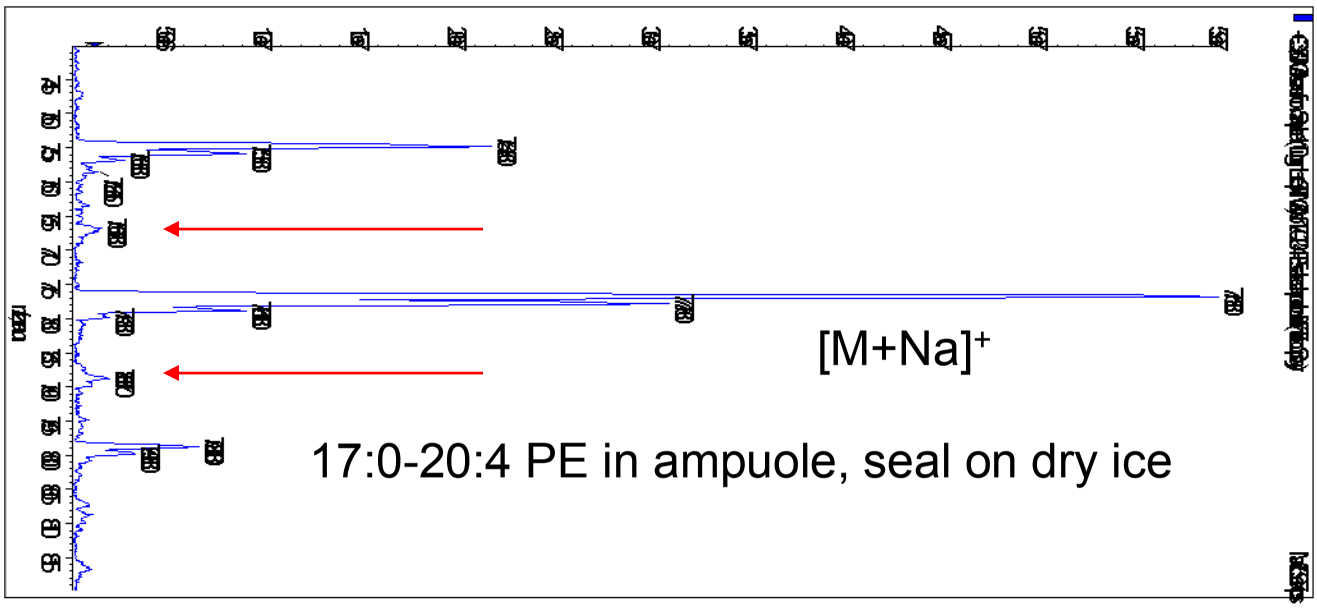
- Fisher brand HPLC grade methanol (cat# A452-4) contains carbonyl compounds which vary from lot to lot.
  - 046913 = 0.004%
  - 050351 = 0.002%
  - 051772 = 0.001%
- No Schiff base formation of 10 µg/ml PE solutions in ampoule. (Not Sealed)
- Significant reaction upon flame sealing of room temperature solutions in ampules.
- Minimal reaction upon flame sealing at -80 C.

Mystery Peaks in PE

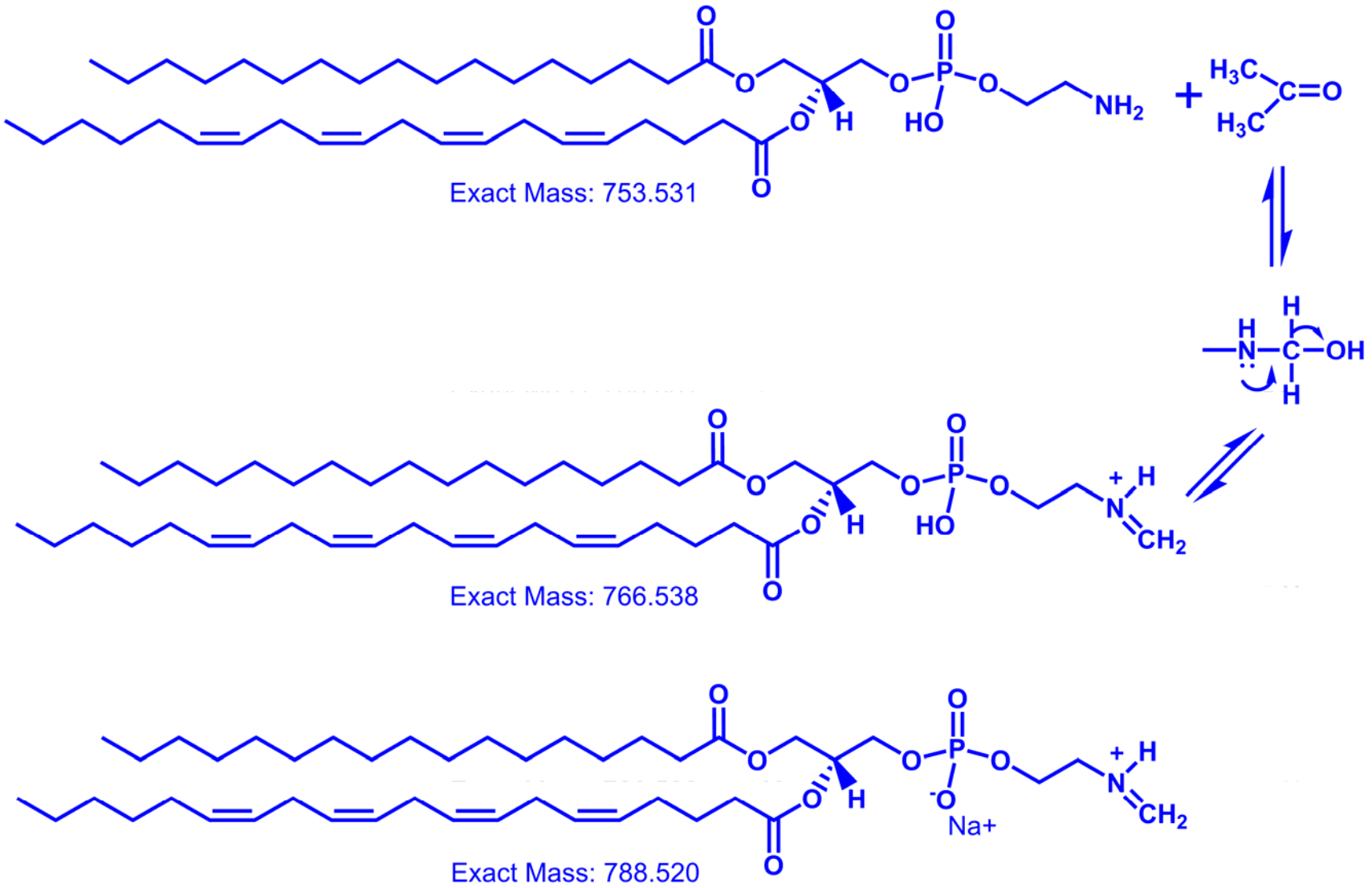



[M+Na]+
17:0-20:4 PE in ampuole, no seal


[M+Na]+
17:0-20:4 PE in ampuole, sealed at rm. temp.


[M+Na]+
17:0-20:4 PE in ampuole, seal on dry ice

Schiff Base Reaction of PE

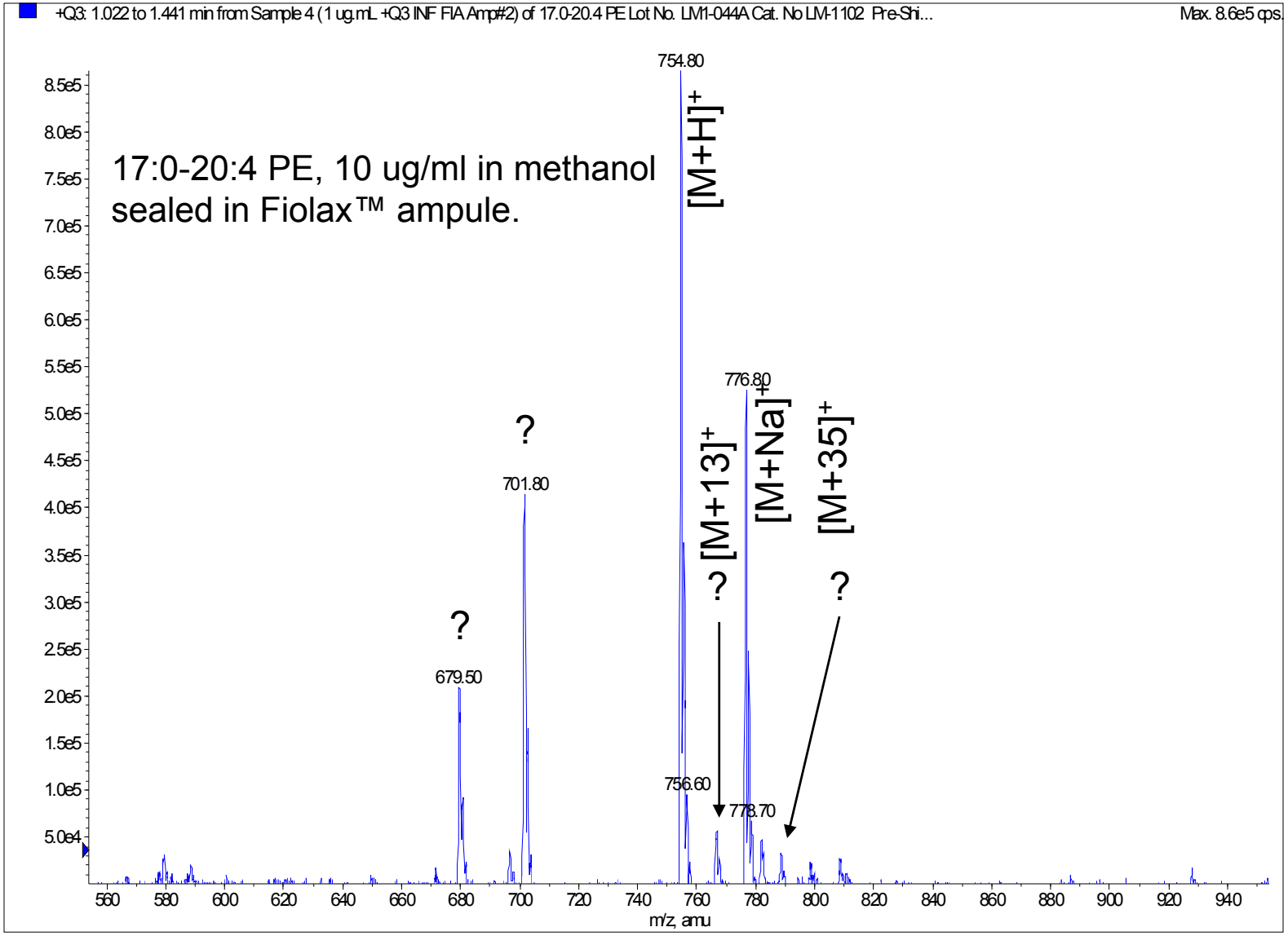


Organic Extractables, Leachables from surfaces

IF IT AIN'T GLASS, TEFLON® OR STAINLESS STEEL DON'T USE IT

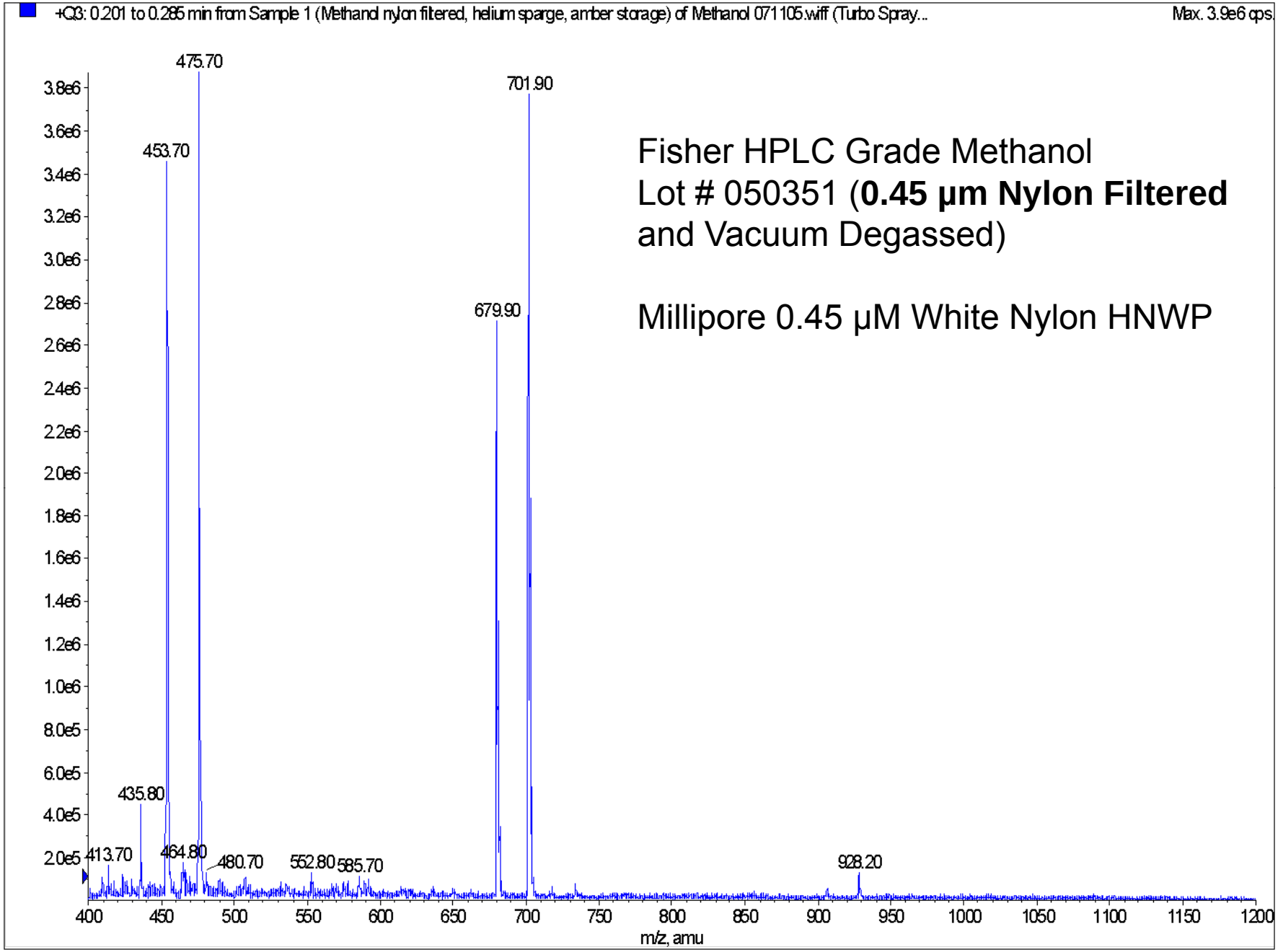
NYLON FILTERS

Mystery Peaks in PE



Mystery Peaks

- 679 and 701 u present in ampoules prepared within 1 week of 17:0-20:4 PE packaging.
  - 17:0-20:4 PG (+ve mode only)
  - 21:0-22:6 PG (+ve mode only)
  - 21:0-22:6 PE
- Database search yields 20-40 possible phospholipid candidates.
- +PI of 679 and 701 not phospholipids.
- $[M+13]^+$ and $[M+35]^+$ in PE ampules only.



Fisher HPLC Grade Methanol
Lot # 050351 (**0.45 µm Nylon Filtered** and Vacuum Degassed)

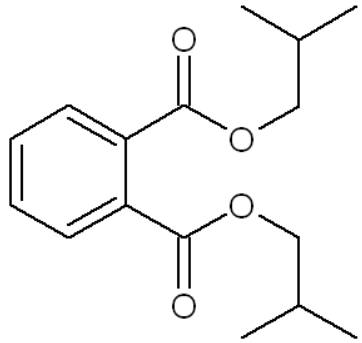
Millipore 0.45 µM White Nylon HNWP

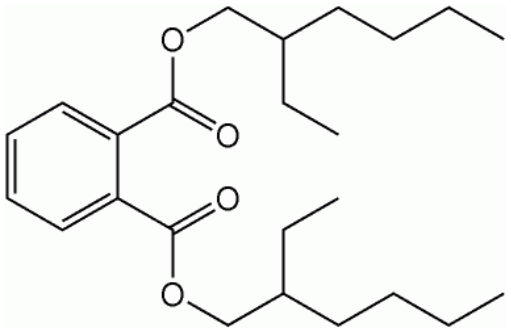
PLASTICIZER CONTAMINANTS EXTRACTED FROM COMMON POLYPROPYLENE LABORATORY PLASTICWARE USING 1:1 CHLOROFORM:METHANOL

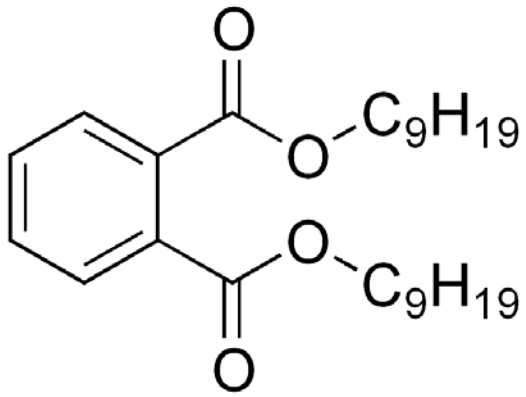
Phthalates

Table of the most common phthalates

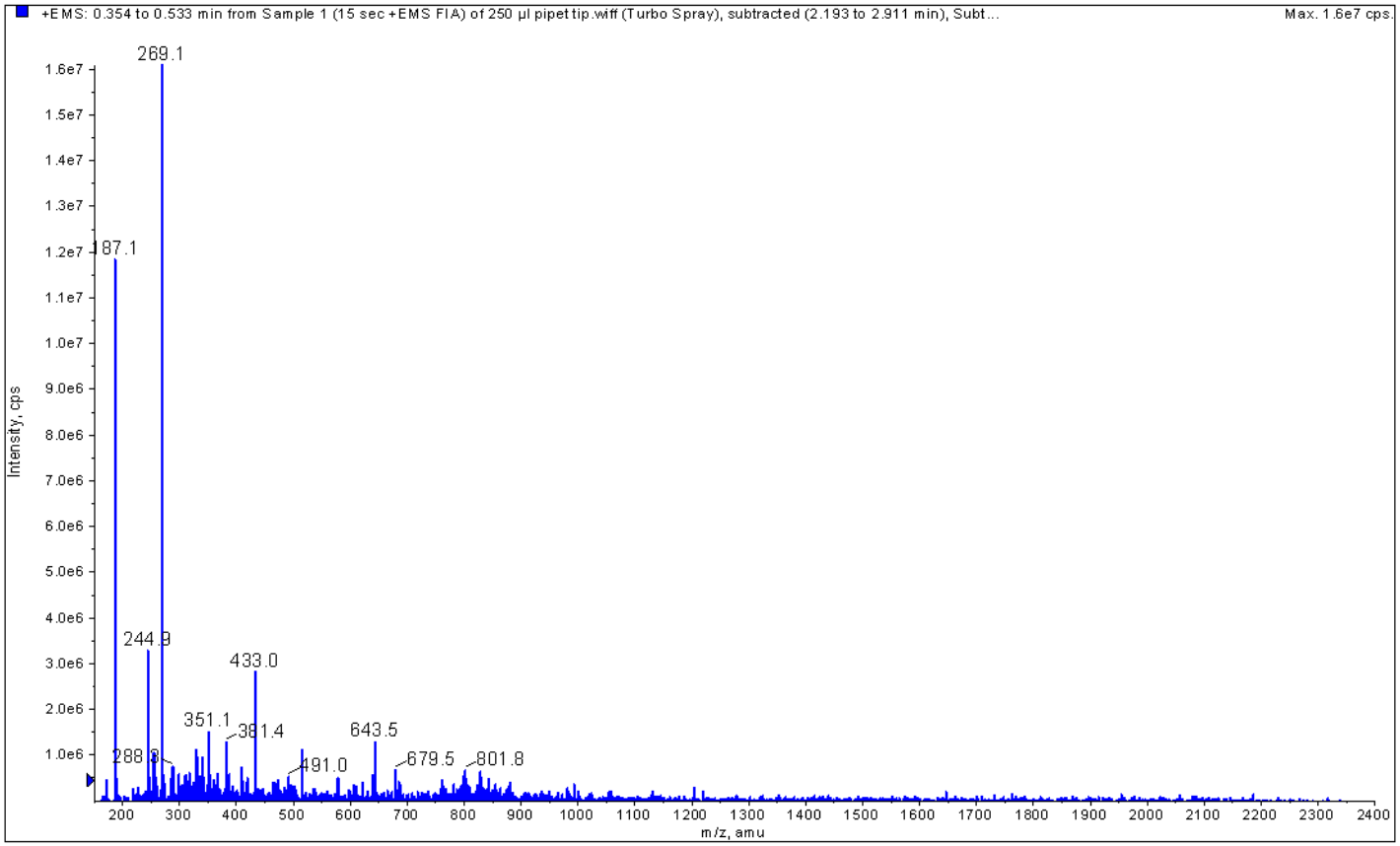
Name	Acronym	Structural formula	CAS No.
Dimethyl phthalate	DMP	$C_6H_4(COOCH_3)_2$	131-11-3
Diethyl phthalate	DEP	$C_6H_4(COOC_2H_5)_2$	84-66-2
Diallyl phthalate	DAP	$C_6H_4(COOCH_2CH{=}CH_2)_2$	131-17-9
Di-n-propyl phthalate	DPP	$C_6H_4[COO(CH_2)_2CH_3]_2$	131-16-8
Di-n-butyl phthalate	DBP	$C_6H_4[COO(CH_2)_3CH_3]_2$	84-74-2
Diisobutyl phthalate	DIBP	$C_6H_4[COOCH_2CH(CH_3)_2]_2$	84-69-5
Butyl cyclohexyl phthalate	BCP	$CH_3(CH_2)_3OOCC_6H_4COOC_6H_{11}$	84-64-0
Di-n-pentyl phthalate	DNPP	$C_6H_4[COO(CH_2)_4CH_3]_2$	131-18-0
Dicyclohexyl phthalate	DCP	$C_6H_4[COOC_6H_{11}]_2$	84-61-7
Butyl benzyl phthalate	BBP	$CH_3(CH_2)_3OOCC_6H_4COOCH_2C_6H_5$	85-68-7
Di-n-hexyl phthalate	DNHP	$C_6H_4[COO(CH_2)_5CH_3]_2$	84-75-3
Diisohexyl phthalate	DIHxP	$C_6H_4[COO(CH_2)_3CH(CH_3)_2]_2$	146-50-9
Diisoheptyl phthalate	DIHpP	$C_6H_4[COO(CH_2)_4CH(CH_3)_2]_2$	41451-28-9
Butyl decyl phthalate	BDP	$CH_3(CH_2)_3OOCC_6H_4COO(CH_2)_9CH_3$	89-19-0
Di(2-ethylhexyl) phthalate	DEHP, DOP	$C_6H_4[COOCH_2CH(C_2H_5)(CH_2)_3CH_3]_2$	117-81-7
Di(n-octyl) phthalate	DNOP	$C_6H_4[COO(CH_2)_7CH_3]_2$	117-84-0
Diisooctyl phthalate	DIOP	$C_6H_4[COO(CH_2)_5CH(CH_3)_2]_2$	27554-26-3
n-Octyl n-decyl phthalate	ODP	$CH_3(CH_2)_7OOCC_6H_4COO(CH_2)_9CH_3$	119-07-3
Diisononyl phthalate	DINP	$C_6H_4[COO(CH_2)_6CH(CH_3)_2]_2$	28553-12-0
Diisodecyl phthalate	DIDP	$C_6H_4[COO(CH_2)_7CH(CH_3)_2]_2$	26761-40-0
Diundecyl phthalate	DUP	$C_6H_4[COO(CH_2)_{10}CH_3]_2$	3648-20-2
Diisoundecyl phthalate	DIUP	$C_6H_4[COO(CH_2)_8CH(CH_3)_2]_2$	85507-79-5
Ditridecyl phthalate	DTDP	$C_6H_4[COO(CH_2)_{12}CH_3]_2$	119-06-2
Diisotridecyl phthalate	DIUP	$C_6H_4[COO(CH_2)_{10}CH(CH_3)_2]_2$	68515-47-9



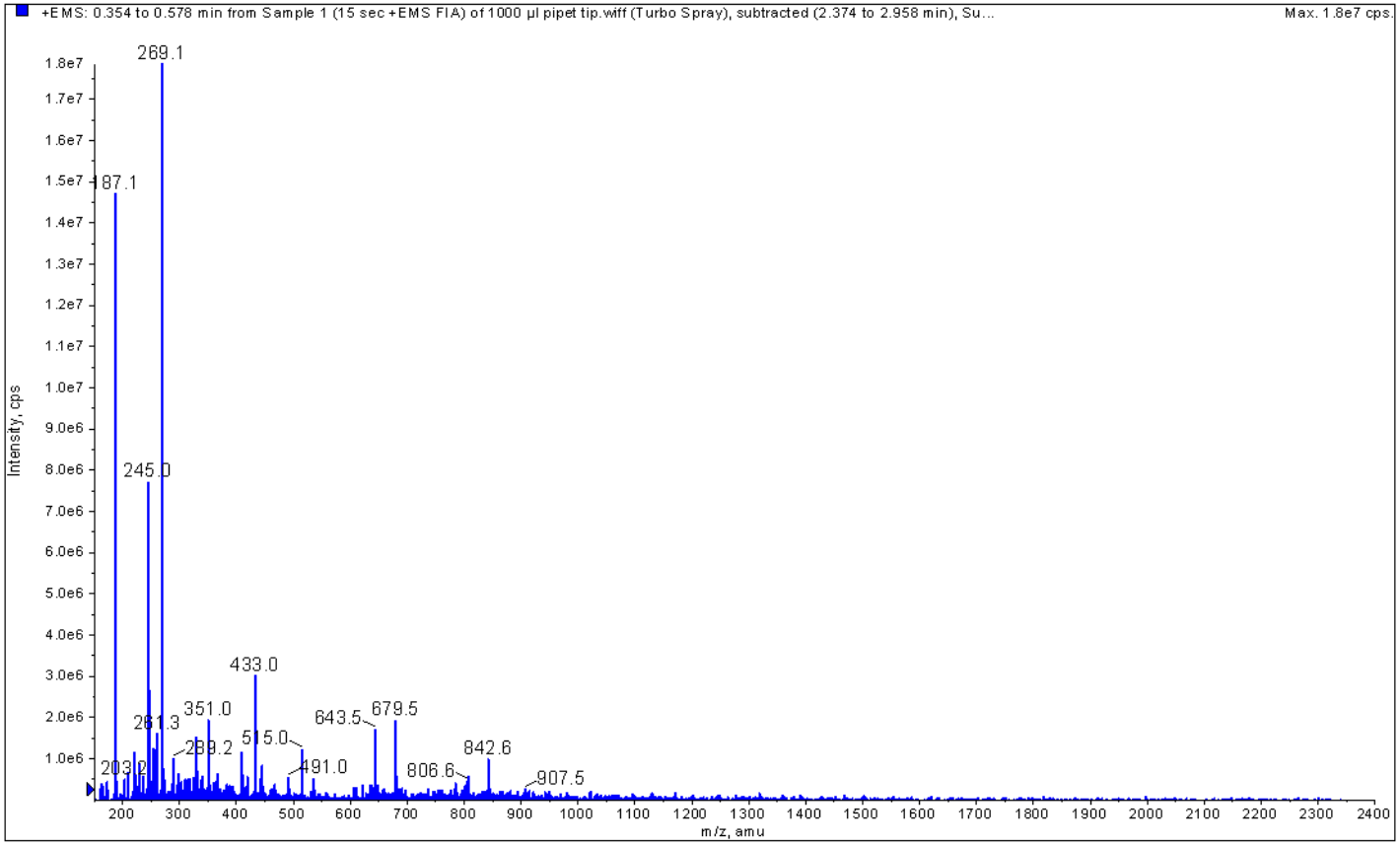




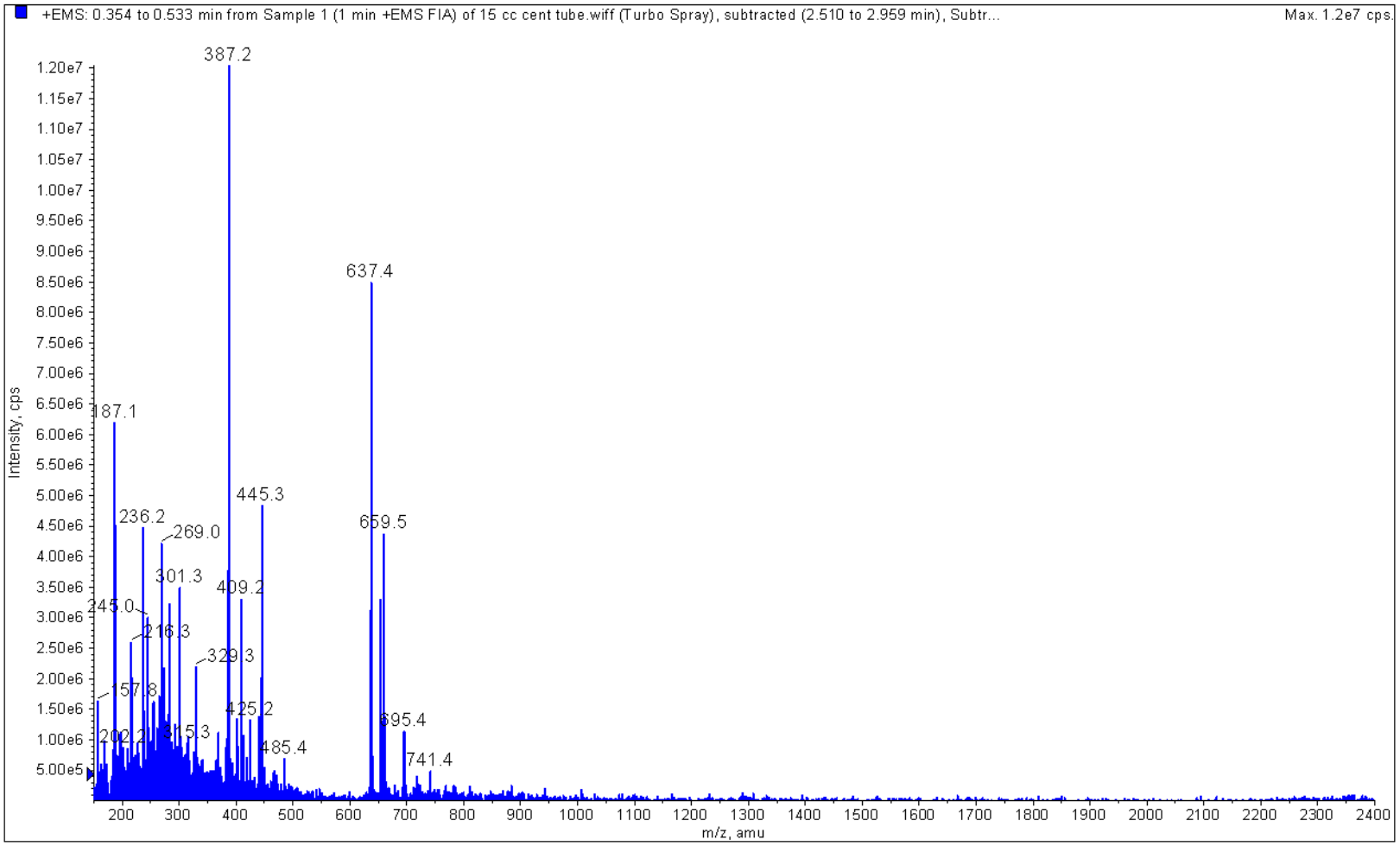
Manufacturer: Rainin
Product: Bioclean 250 µl Precision Pipette Tips
P/N: RT-20
Exposure Time: 15 seconds



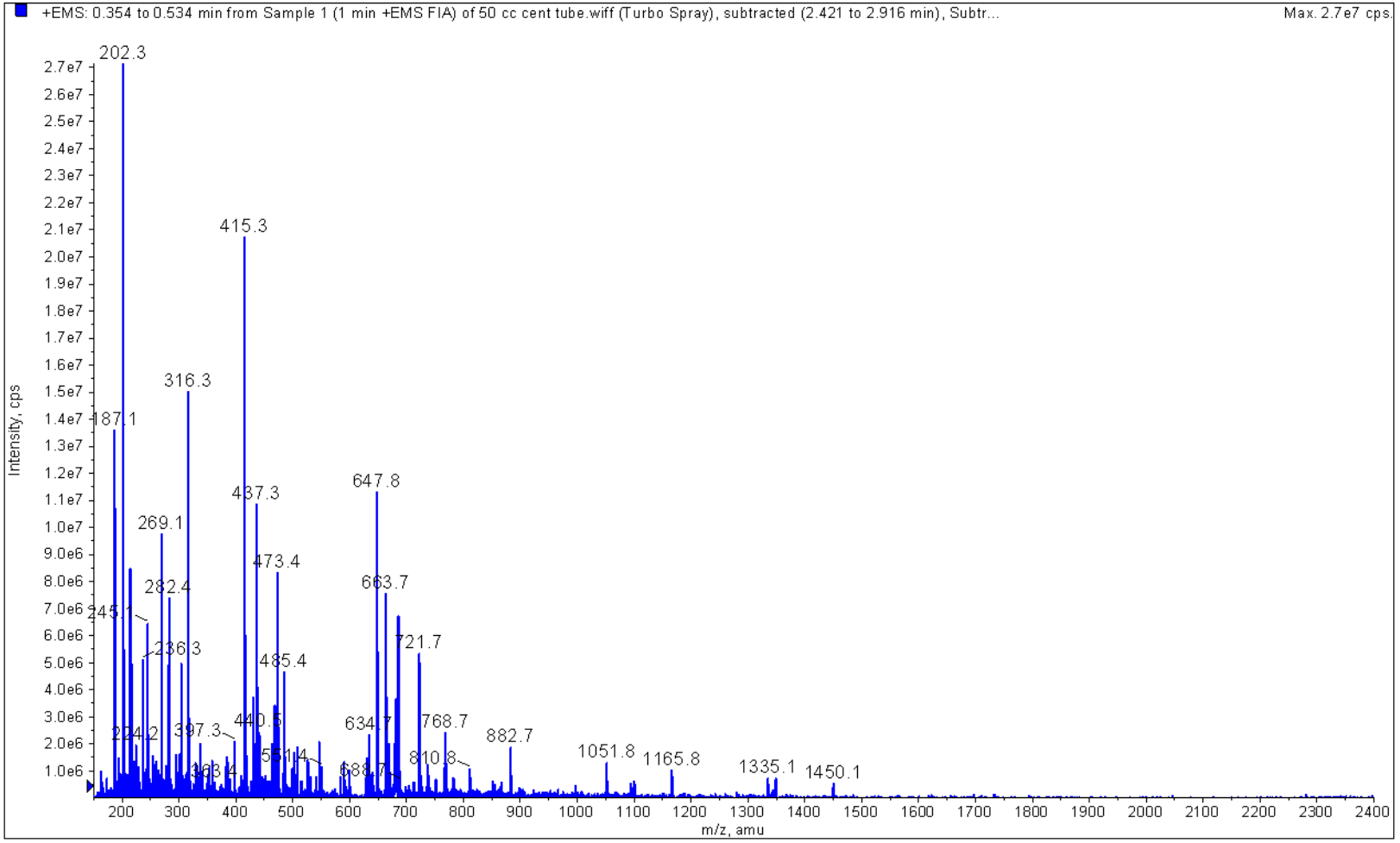
Manufacturer: Rainin
Product: Bioclean 1000 µl Precision Pipette Tips
P/N: RT-1000
Exposure Time: 15 seconds



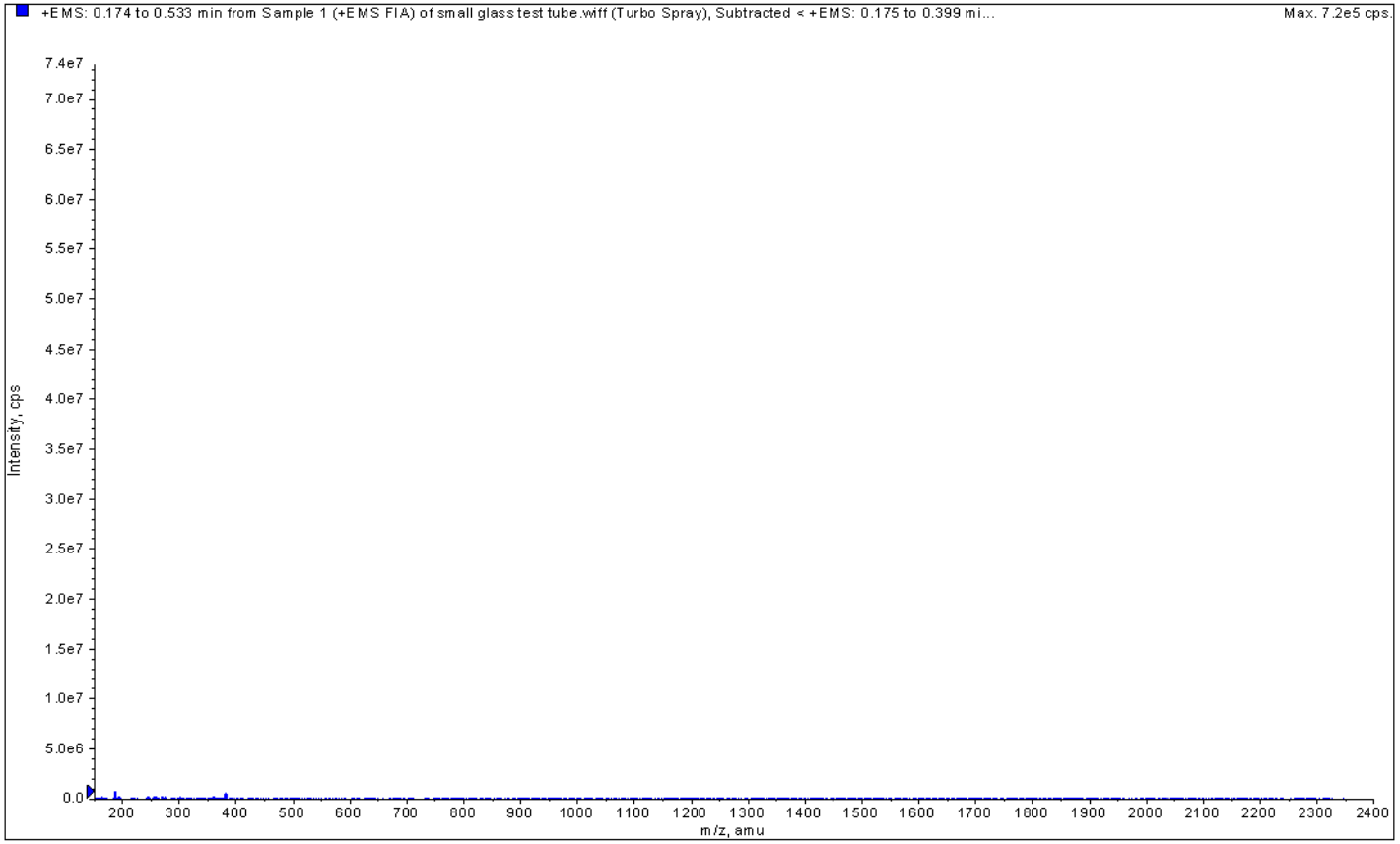
Manufacturer: Falcon (Becton Dickinson)
Product: BlueMax Jr. 15 ml PP Conical Tube
P/N: 352097
Exposure Time: 1 minute



Manufacturer: Sarstedt
Product: 50 ml Skirted PP Centrifuge Tube
P/N: 101093-818
Exposure Time: 1 minute

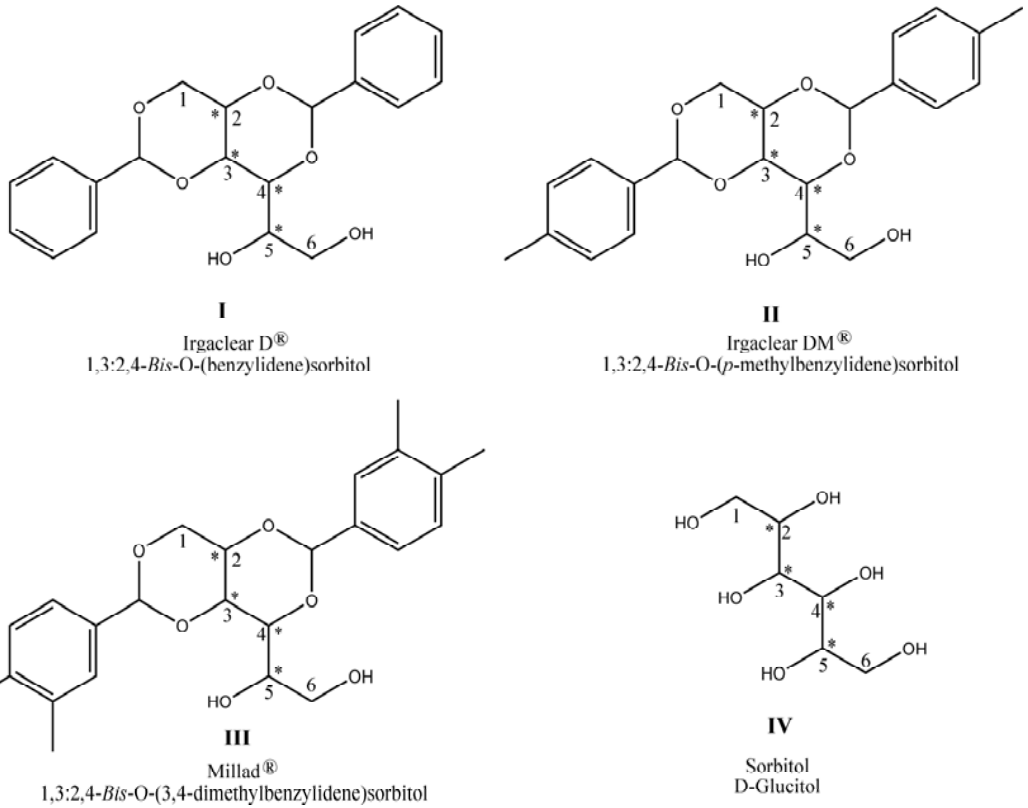


Manufacturer: Kimble Glass, Inc.
Product: Disposable Culture Tube with Glass Screw Thread (16x100 mm)
P/N: 73770-16100
Exposure Time: 15 minutes



Sorbitol-Based Nuclear Clarifying Agents

Extracted from Common Laboratory and Consumer Plasticware Made of Polypropylene



Structures and trade names of sorbitol-based nuclear clarifying agents and sorbitol. Stereocenters are indicated with an asterisk (*).

Published in: Jeffrey G. McDonald; Carolyn L. Cummins; Robert M. Barkley; Bonne M. Thompson; Holly A. Lincoln; *Anal. Chem.* **2008,** 80, 5532-5541.
DOI: 10.1021/ac8005632

Table 1. Summary of Useful Information for Sorbitol-Based Nuclear Clarifying Agents

	Irgaclear D (I)	Irgaclear DM (II)	Millad (III)
systematic name	1,3:2,4-bis-*O*-(benzylidene) sorbitol	1,3:2,4-bis-*O*-(*p*-methylbenzylidene) sorbitol	1,3:2,4-bis-*O*-(3,4-dimethylbenzylidene) sorbitol
CAS number	32647−67−9	81541−12−0	135861−56−2
molecular weight	358.14	386.44	414.2
solubility in EtOH	−1.2 mg/mL	−0.25 mg/mL	−0.14 mg/mL
LOD (LC/MS)	10 pg	20 pg	2 pg
retention time			
LC/MS	10 min	11 min	11.5 min
GC/MS	16 min/15.5 min	17.5 min/16.5 min	19 min/18 min

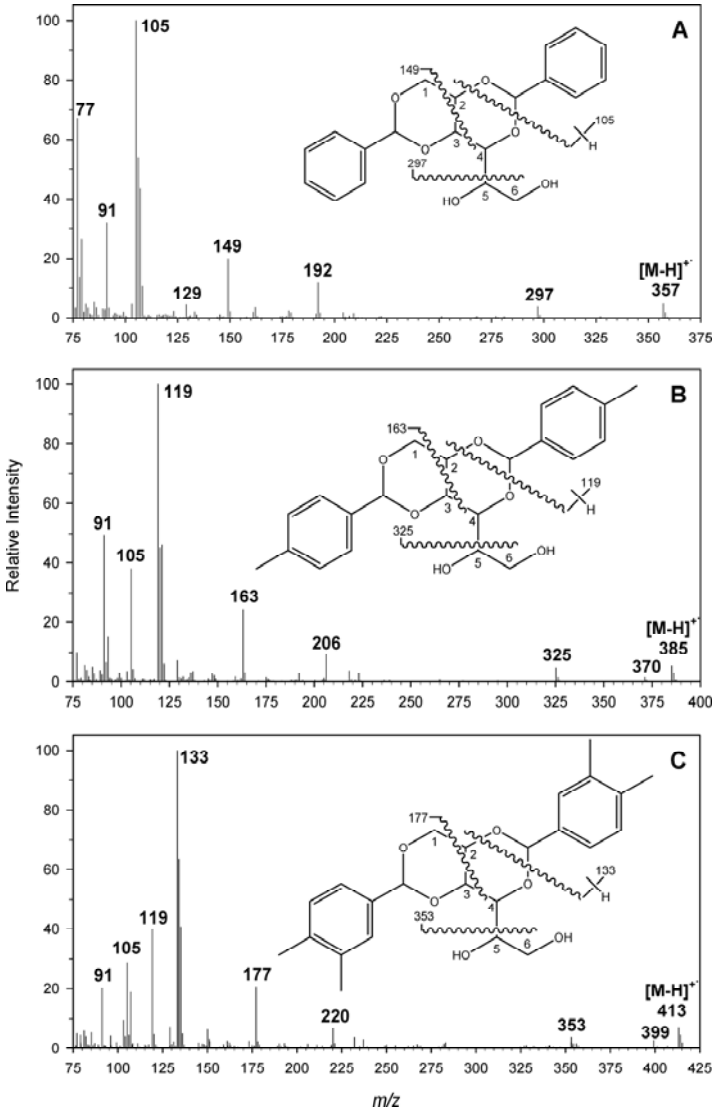
a Determined gravimetrically using a saturated solution in EtOH.

b Mass on column, defined as at least 2 × s/n for I and II, and 2 × average background level for III.

c Retention times are based on the instrumental methods described in the Experimental Section. Approximations are provided because a defined retention index system (e.g., methylene retention index) was not used here. Retention times are provided as an aid to the analyst wishing to analyze for these compounds.

d Underivatized/trimethyl silyl ether derivative.

Published in: Jeffrey G. McDonald; Carolyn L. Cummins; Robert M. Barkley; Bonne M. Thompson; Holly A. Lincoln; *Anal. Chem.* **2008,** 80, 5532-5541.
DOI: 10.1021/ac8005632



Electron ionization mass spectra of sorbitol-based nuclear clarifying agents (A−C). Characteristic fragments are shown with the structure in each panel.

Published in: Jeffrey G. McDonald; Carolyn L. Cummins; Robert M. Barkley; Bonne M. Thompson; Holly A. Lincoln; *Anal. Chem.* **2008,** 80, 5532-5541.
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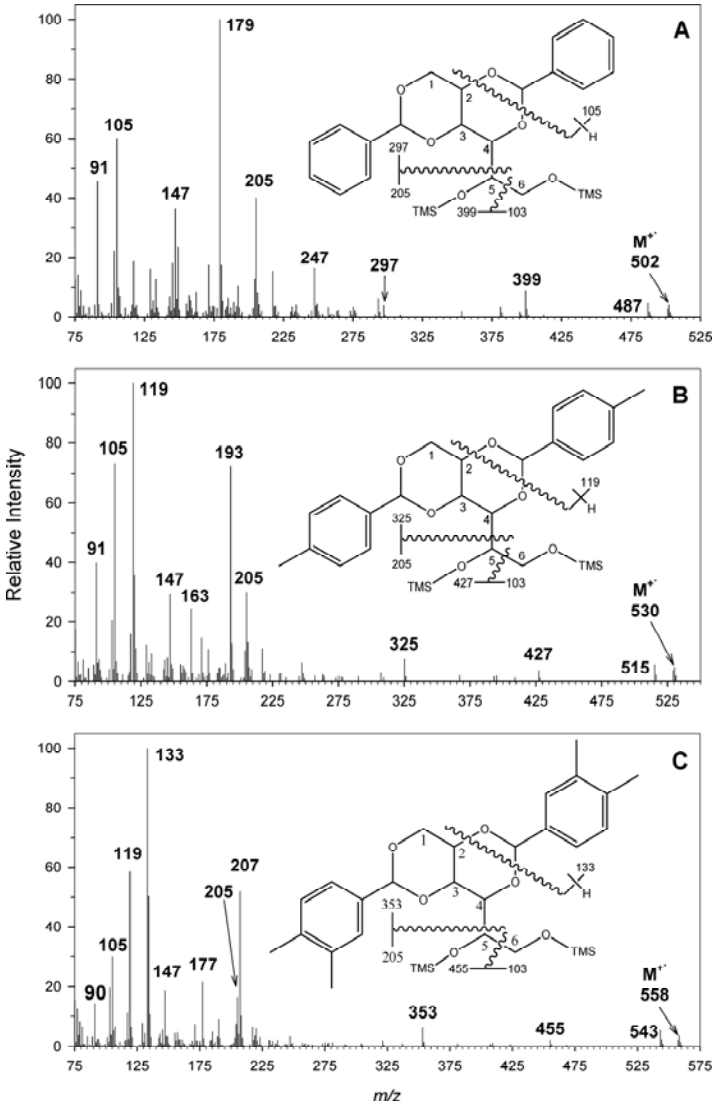
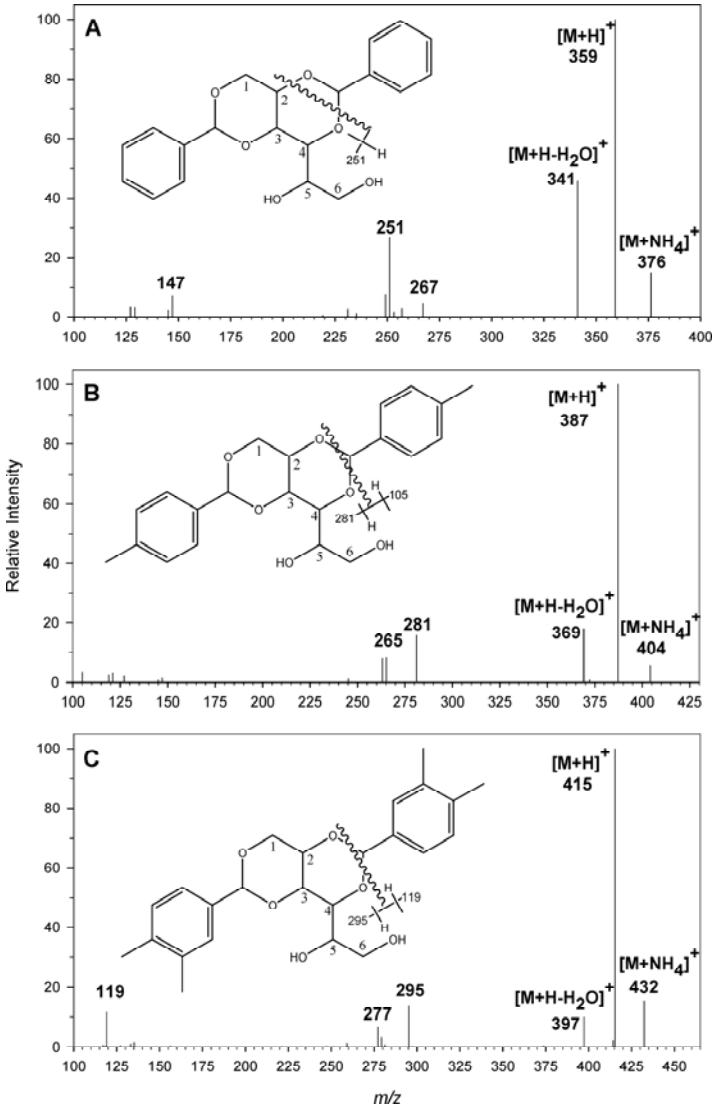


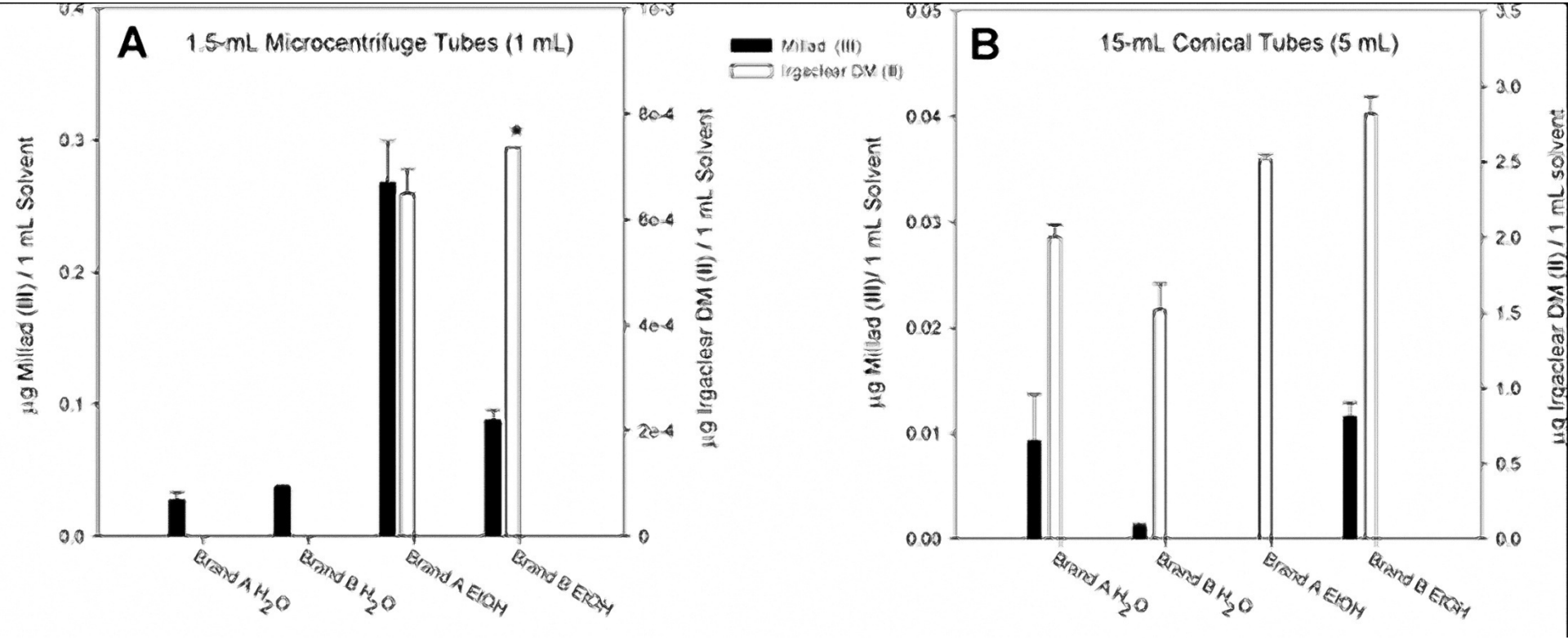
Electron ionization mass spectra of trimethylsilyl derivatives of sorbitol-based nuclear clarifying agents (A−C). Characteristic fragments are shown with the structure in each panel.

Published in: Jeffrey G. McDonald; Carolyn L. Cummins; Robert M. Barkley; Bonne M. Thompson; Holly A. Lincoln; *Anal. Chem.* **2008,** 80, 5532-5541.
DOI: 10.1021/ac8005632



CID mass spectra of sorbitol-based nuclear clarifying agents. Characteristic fragment ions are shown with the structure in each panel.

Published in: Jeffrey G. McDonald; Carolyn L. Cummins; Robert M. Barkley; Bonne M. Thompson; Holly A. Lincoln; *Anal. Chem.* **2008,** 80, 5532-5541.
DOI: 10.1021/ac8005632



Quantitative data for sorbitol-based nuclear clarifying agents extracted from common laboratory plasticware and household food containers. Error bars represent the standard error (n = 3). Note the use of left and right axes in panels A and B. The absence of data for a specific compound indicates it was not detected at the detection limits given in Table 1. For panels C and D, an "A" in the label indicates the first extraction and the "B" label indicates the second extraction. The data are reported in µg/mL and the total volume extracted in each experiment is given in parentheses following the title in each figure. The data for brand A washed is shown at a 100-fold increase to place it on a similar scale with other data. Actual concentration is ~0.002 µg/mL. * n = 2.

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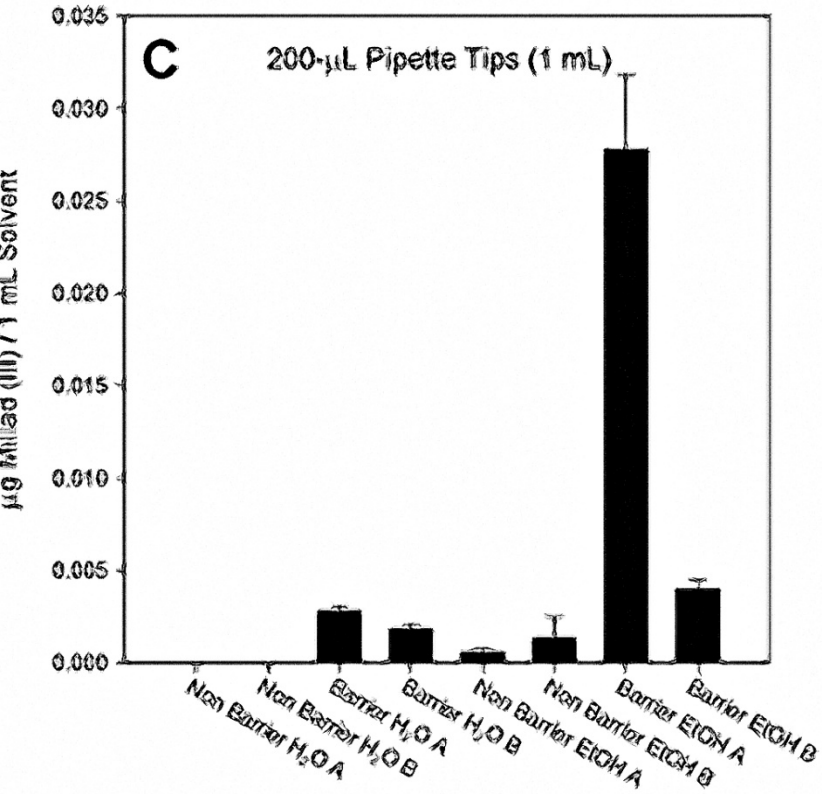
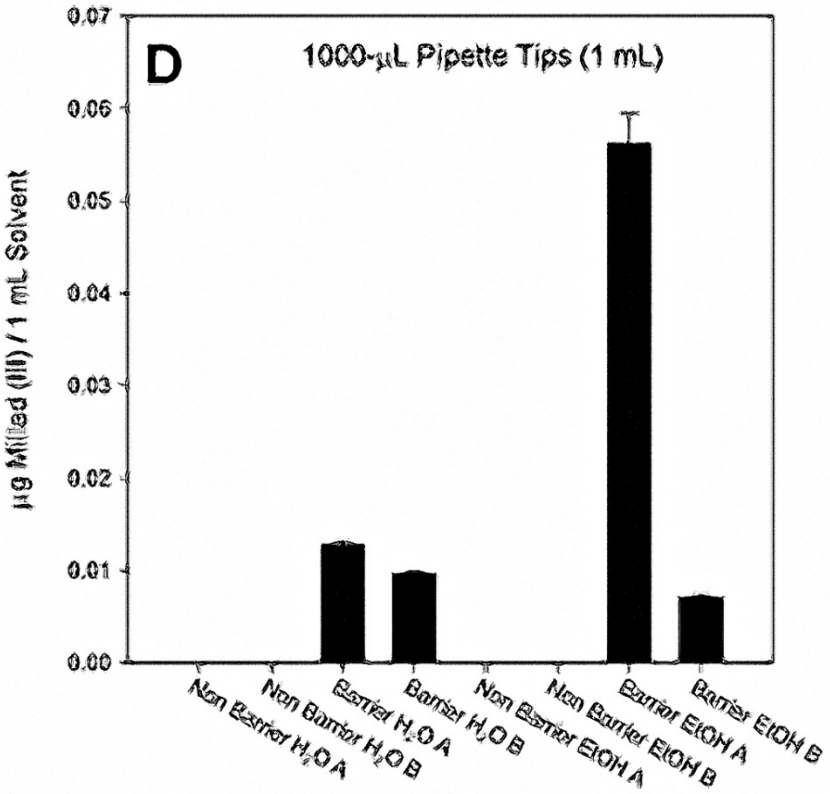
DOI: 10.1021/ac8005632





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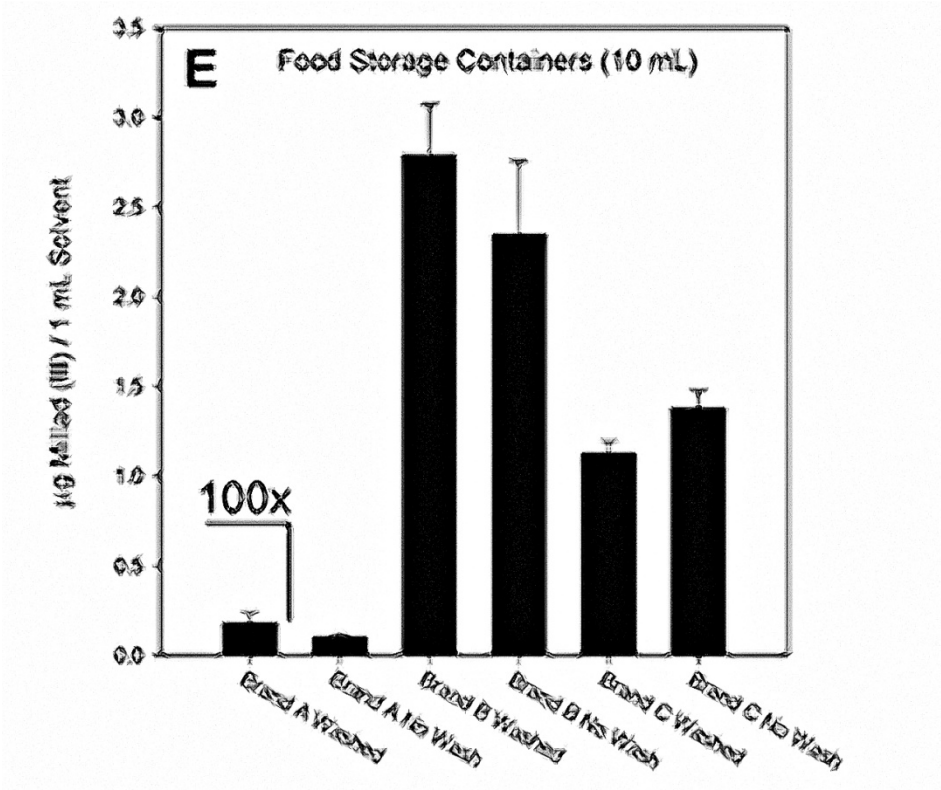

Quantitative data for sorbitol-based nuclear clarifying agents extracted from common laboratory plasticware and household food containers. Error bars represent the standard error (n = 3). Note the use of left and right axes in panels A and B. The absence of data for a specific compound indicates it was not detected at the detection limits given in Table 1. For panels C and D, an "A" in the label indicates the first extraction and the "B" label indicates the second extraction. The data are reported in μg/mL and the total volume extracted in each experiment is given in parentheses following the title in each figure. The data for brand A washed is shown at a 100-fold increase to place it on a similar scale with other data. Actual concentration is ~0.002 μg/mL. * n = 2.

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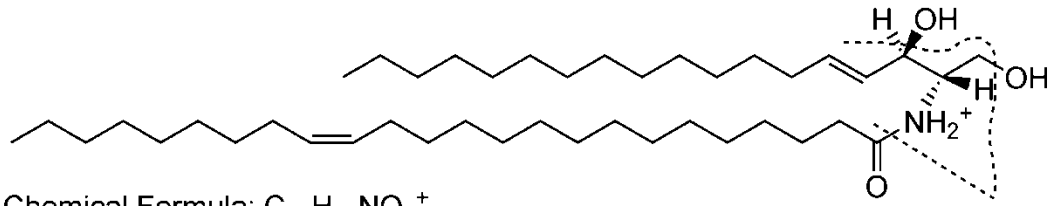
DOI: 10.1021/ac8005632

LC-MS/MS

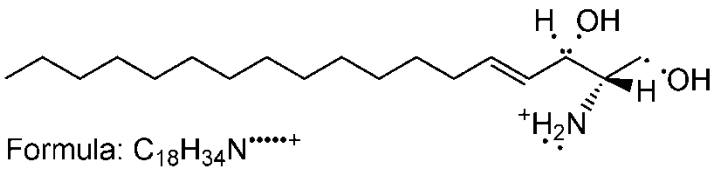
DIFFERENT GEOMETRIES - DIFFERENT RESULTS

QQQ

LCMS of Internal Standard



Chemical Formula: $C_{42}H_{82}NO_3^+$
Exact Mass: 648.6



Chemical Formula: $C_{18}H_{34}N^{\bullet\bullet\bullet\bullet\bullet+}$
Exact Mass: 264.3

C24:1 Ceramide MRM

Relative Intensity

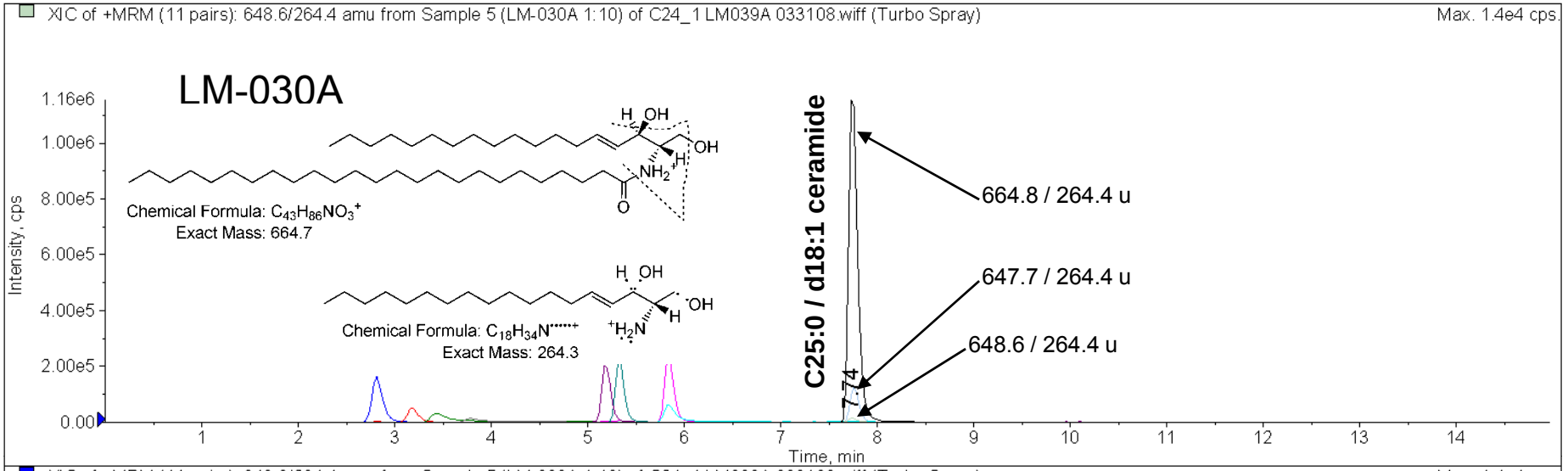
482.6/264.4 538.7/264.4 540.7/266.4 566.7/264.4 594.7/264.4 622.8/264.4 648.9/264.4 650.9/264.4 676.9/264.4 678.9/264.4

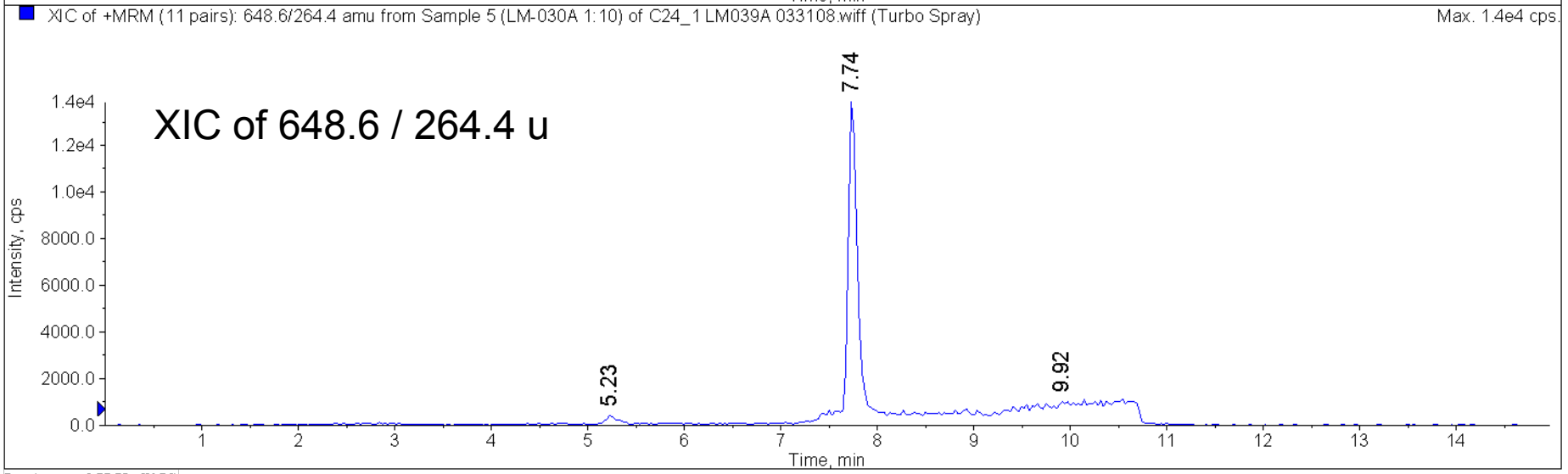
Q1/Q3 Masses, amu

Qtrap

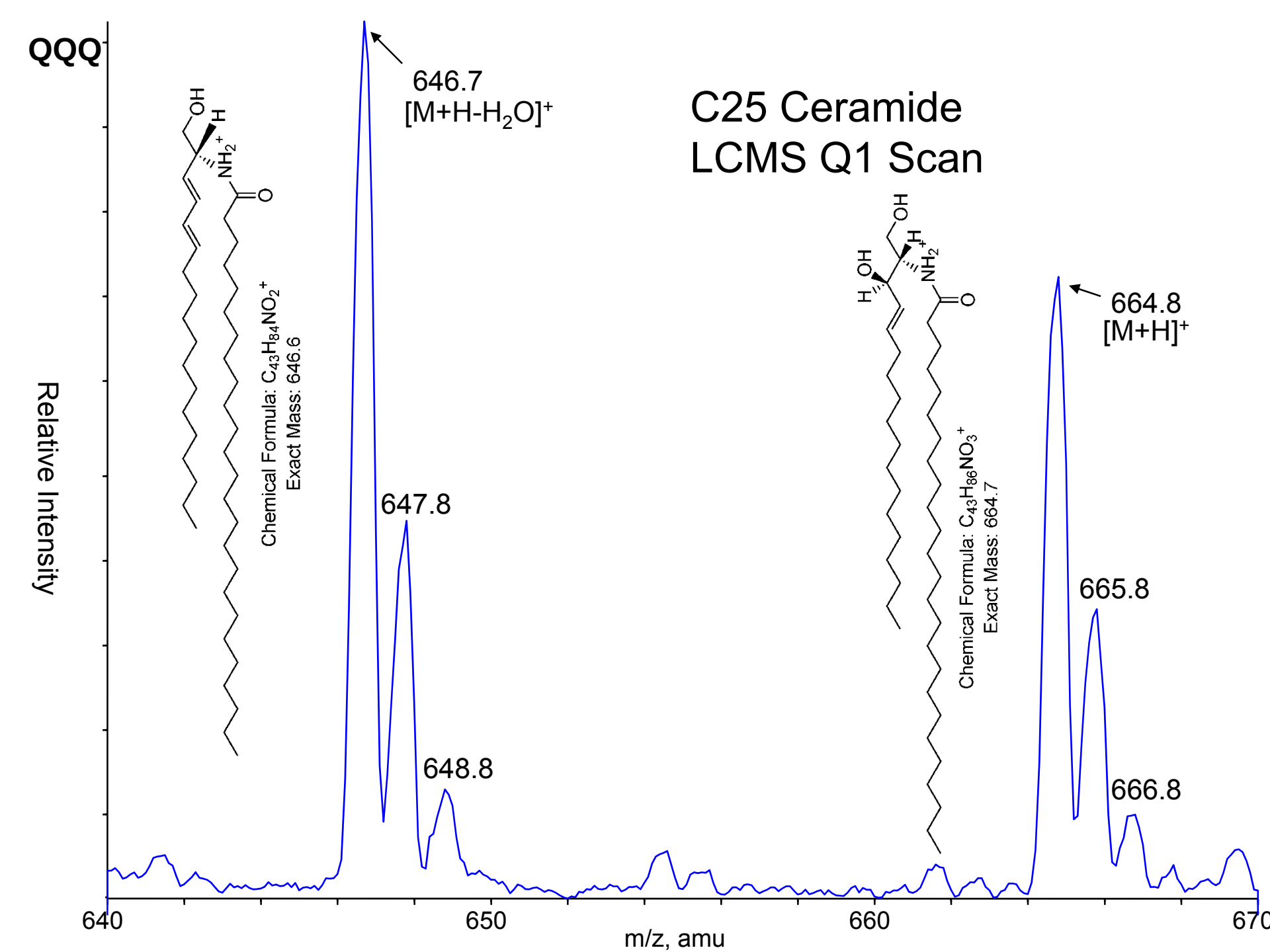
Sample ID:
Sample Name: LM-030A 1:10
Sample Comment:

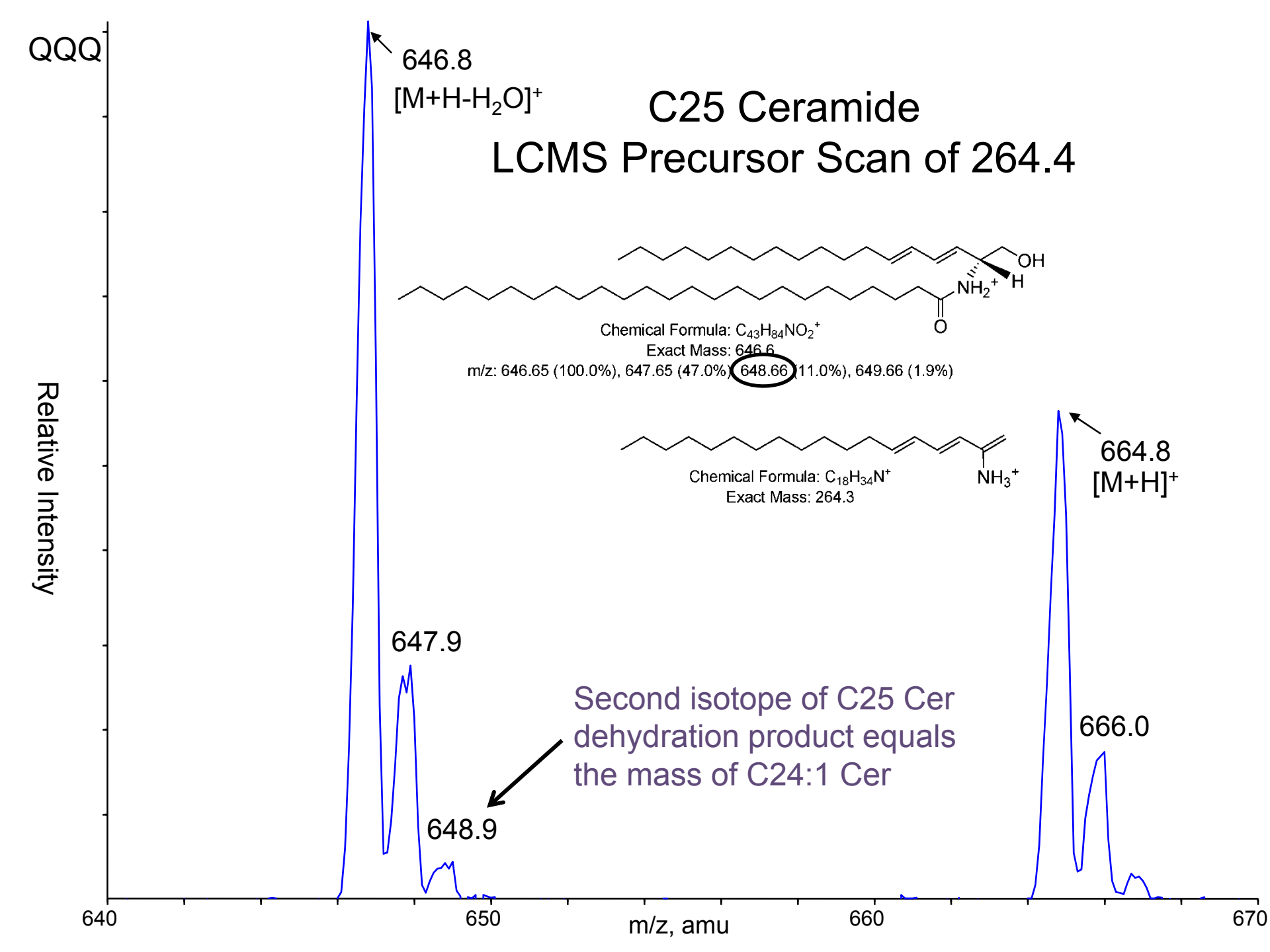
Acq. Date: Monday, March 31, 2008
Acq. Time: 12:30





Project: LIPID MAPS


QQQ
646.7
[M+H-H2O]+
C25 Ceramide
LCMS Q1 Scan
Chemical Formula: C43H84NO2+
Exact Mass: 646.6
647.8
648.8
664.8
[M+H]+
Chemical Formula: C43H86NO3+
Exact Mass: 664.7
665.8
666.8
Relative Intensity
640
650
660
670
m/z, amu


QQQ
646.8
[M+H-H2O]+
C25 Ceramide
LCMS Precursor Scan of 264.4
OH
NH2+
H
O
Chemical Formula: C43H84NO2+
Exact Mass: 646.6
m/z: 646.65 (100.0%), 647.65 (47.0%), 648.66 (11.0%), 649.66 (1.9%)
Chemical Formula: C18H34N+
Exact Mass: 264.3
NH3+
664.8
[M+H]+
647.9
648.9
Second isotope of C25 Cer dehydration product equals the mass of C24:1 Cer
666.0
Relative Intensity
640
650
660
670
m/z, amu

Cleaning Validation

- Manufacturing process controls mandate cleaning validation to document limited cross contamination to < 0.05% on:
  - Glassware surfaces
  - Reactors and mixers
  - Reusable utensils
- Contaminants that effect purity and function
  - **Residual lipid** cross contaminants
  - **Ions** (process chemicals and heavy metals)
  - **Cleaning detergents**

Moral of the Story

BE

VIGILANT