

1 Article

2 CFM-ID 3.0: Significantly Improved ESI-MS/MS 3 Prediction Using a Hybrid In Silico Fragmentation 4 Model with Metadata

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6 1. Compound acquisition

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8 All of the lipid standards were purchased from either Cayman Chemical (Ann Arbor, MI, USA) or
9 Avanti Polar Lipids (Alabaster, AL, USA). More specifically, Glycerol Tridocosahexaenoyl,
10 DL-Palmitoylcarnitine (chloride), 1-Palmitoyl-3-oleoyl-*sn*-glycero-2-PE, Lyso-PC,
11 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate, Cholesteryl heptadecanoate, 2-Linoleoyl Glycerol,
12 1-Stearoyl-2-Arachidonoyl-*sn*-Glycerol, C-16 Ceramide, Palmitoyl Sphingomyelin,
13 1-Palmitoyl-2-linoleoyl PE, and 1-Octadecyl Lysophosphatidic Acid (sodium salt) were purchased
14 from Cayman Chemical; 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (sodium salt),
15 1',3'-bis[1,2-dioleoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (sodium salt), and
16 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine were purchased from Avanti Polar Lipids, Inc.

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19 2. Preparation of the reference solutions

20 15 different lipid reference solutions were prepared. The preparation of each is summarized below:

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22 Glycerol Tridocosahexaenoyl: Glycerol Tridocosahexaenoyl was dissolved in chloroform to 5
23 mg/mL, and further diluted to 5 µg/mL by methanol/water (50/50) containing 0.1% formic acid.

24

25 DL-Palmitoylcarnitine (chloride): DL-Palmitoylcarnitine (chloride) was dissolved in ethanol to
26 5 mg/mL, and further diluted to 5 µg/mL by methanol/water (50/50) containing 0.1% formic acid.

27

28 1-Palmitoyl-3-oleoyl-*sn*-glycero-2-PE: 1-Palmitoyl-3-oleoyl-*sn*-glycero-2-PE was dissolved in
29 chloroform to 2.5 mg/mL, and further diluted to 5 µg/mL by methanol/water (50/50) containing 0.1%
30 formic acid.

31

32 Lyso-PC: Lyso-PC was dissolved in PBS buffer to 1 mg/mL, and further diluted to 5 µg/mL by
33 methanol/water (50/50) containing 0.1% formic acid.

34

35 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate: 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate was
36 dissolved in chloroform to 5 mg/mL, and further diluted to 5 µg/mL by methanol/water (50/50)
37 containing 0.1% formic acid.

38

39 Cholesteryl heptadecanoate: Cholesteryl heptadecanoate was dissolved in chloroform to 5
40 mg/mL, and further diluted to 5 µg/mL by methanol/water (50/50) containing 0.1% formic acid.

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42 2-Linoleoyl Glycerol: 2-Linoleoyl Glycerol was dissolved in acetonitrile to 5 mg/mL, and further
43 diluted to 5 µg/mL by methanol/water (50/50) containing 0.1% formic acid.

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45 1-Stearoyl-2-Arachidonoyl-sn-Glycerol: 1-Stearoyl-2-Arachidonoyl-sn-Glycerol was dissolved
46 in methyl acetate to 10 mg/mL, and further diluted to 5 µg/mL by methanol/water (50/50) containing
47 0.1% formic acid.

48

49 C-16 Ceramide: C-16 Ceramide was dissolved in DMF to 0.1 mg/mL, and further diluted to 5
50 µg/mL by methanol/water (50/50) containing 0.1% formic acid.

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52 Palmitoyl Sphingomyelin: Palmitoyl Sphingomyelin was dissolved in ethanol to 5 mg/mL, and
53 further diluted to 5 µg/mL by methanol/water (50/50) containing 0.1% formic acid.

54

55 1-Palmitoyl-2-linoleoyl PE: 1-Palmitoyl-2-linoleoyl PE was dissolved in chloroform to 10
56 mg/mL, and further diluted to 5 µg/mL by methanol/water (50/50) containing 0.1% formic acid.

57

58 1-Octadecyl Lysophosphatidic Acid (sodium salt): 1-Octadecyl Lysophosphatidic Acid (sodium
59 salt) was dissolved in ethanol to 5 mg/mL, and further diluted to 5 µg/mL by methanol/water (50/50)
60 containing 0.1% formic acid.

61

62 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (sodium salt):
63 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (sodium salt) was dissolved in ethanol to 5
64 mg/mL, and further diluted to 5 µg/mL by methanol/water (50/50) containing 0.1% formic acid.

65

66 1',3'-bis[1,2-dioleoyl-sn-glycero-3-phospho]-sn-glycerol (sodium salt):
67 1',3'-bis[1,2-dioleoyl-sn-glycero-3-phospho]-sn-glycerol (sodium salt) was dissolved in ethanol to 5
68 mg/mL, and further diluted to 5 µg/mL by methanol/water (50/50) containing 0.1% formic acid.

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70 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine:
71 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine was dissolved in chloroform to 5 mg/mL, and
72 further diluted to 5 µg/mL by methanol/water (50/50) containing 0.1% formic acid.

73 3. Instrumentation and Parameterization

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75 An AB Sciex QTrap 4000 mass spectrometer (Framingham, MA, USA) was used to collect all the
76 MS/MS spectra. The samples were introduced via direct infusion with Harvard syringe pump at
77 flow rate of 10 µL/min. Positive & negative instrument mode parameters were set as follows:

78

79 Scan Type: Enhanced Product Ion (EPI)

80 Polarity: Positive

81 Scan Mode: Profile

82 Ion Source: Turbo Spray

83 Resolution of Q1: Unit

84 Scan Rate: 1000 amu/s

85 MR Pause: 5.0070 msec

86 Q0 trapping: No

87 MCA: Yes

88 LIT fill time: 20 msec

89 Dynamic Fill Time: On

90 CUR: 10

91 CAD: High

92 IS: 4500

93 GS1: 20

94 GS2: 0

95 ihe: On

96 DP: 140
97 CES: 0
98 CE: 10/20/30/40 (some lipids were scanned up to 60)
99
100 Scan Type: Enhanced Product Ion (EPI)
101 Polarity: Negative
102 Scan Mode: Profile
103 Ion Source: Turbo Spray
104 Resolution of Q1: Unit
105 Scan Rate: 1000 amu/s
106 MR Pause: 5.0070 msec
107 Q0 trapping: No
108 MCA: Yes
109 LIT fill time: 20 msec
110 Dynamic Fill Time: On
111 CUR: 10
112 CAD: High
113 IS: -4500
114 GS1: 20
115 GS2: 0
116 ihe: On
117 DP: -110
118 CES: 0
119 CE: -10/-20/-30/-40
120

121 4. Measurements and Data Extraction

122 Lipid standard solutions were introduced into the QTrap 4000 mass spectrometer via direct
123 infusion with a Harvard syringe pump at a flow rate of 10 $\mu\text{L}/\text{min}$. For each lipid standard solution,
124 an enhanced MS (EMS) scan was first conducted to identify precursor ions with high abundance
125 (e.g., $\text{M}+\text{H}$, $\text{M}+\text{Na}$, $\text{M}+\text{NH}_4$, $\text{M}-\text{H}$, etc.). Enhanced product ion (EPI) scans for each precursor ion
126 were then conducted to generate the MS/MS spectra with different collision energy (CE) levels.
127 MS/MS spectra for most of the lipids were collected for CE ± 10 to 40 eV, while spectra collected for
128 some lipids were shifted up to CE levels of ± 60 eV depending on the observed fragmentation
129 patterns. For example, if the precursor ion signal in the MS/MS spectrum was still high at a CE level
130 of 40 eV, the next CE level above would then be tested until a CE level was found in which the
131 precursor ion signal was very low or almost zero.

132 MS/MS spectra for each lipid standard were collected for both positive and negative ion modes,
133 with different collision energy (CE) levels, i.e. $+10/+20/+30/+40$, $-10/-20/-30/-40$, eV etc. Each EPI scan
134 was conducted and monitored until a stable signal was observed, then the "Acquire" function in the
135 Sciex Analyst software was applied to collect each MS/MS spectrum for one minute. Molecular
136 masses were first picked via the EMS scan if they showed sufficiently high abundance. For those not
137 seen in the EMS scan, calculations based on the molar mass of the native lipid being analyzed were
138 conducted.

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