

Supplementary Material

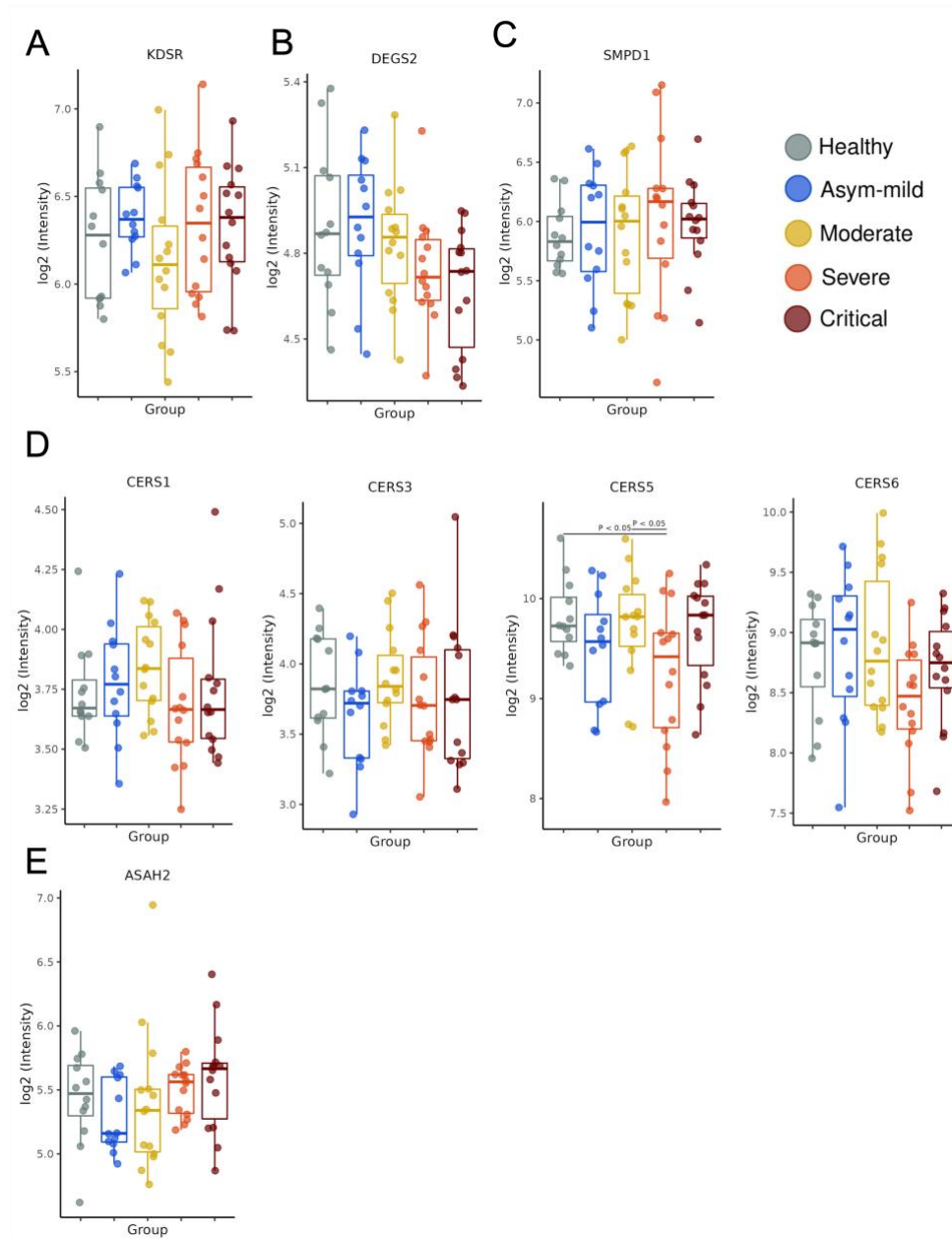
Plasma sphingomyelin disturbances: unveiling its dual role as a crucial imunopathological factor and a severity prognostic biomarker in COVID-19

Diana M. Toro^{1,2,†}, Pedro V. da Silva-Neto^{1,2,†}, Jonatan C. S. de Carvalho^{1,3,†}, Carlos A. Fuzo^{1,†}, Malena M. Pérez¹, Vinícius E. Pimentel^{1,4}, Thais F. C. Fraga-Silva⁴, Camilla N. S. Oliveira^{1,4}, Glaucia R. Caruso¹, Adriana F. L. Vilela³, Pedro N. Azevedo^{3,4}, Thiago V. D. Felipe³, Jamille G. M. Argolo⁵, Augusto M. Degiovani⁶, Fátima M. Ostini⁶, Marley R. Feitosa^{7,11}, Rogerio S. Parra^{7,11}, Fernando C. Vilar^{8,11}, Gilberto G. Gaspar⁸, José J. R. da Rocha⁷, Omar Feres^{7,11}, Gabriel Peinado Costa¹⁰, Sandra R. C. Maruyama⁹, Elisa M. S. Russo¹, Ana P. M. Fernandes⁵, Isabel K. F. M. Santos⁴, Adriana Malheiro², Ruxana T. Sadikot¹², Vânia L. D. Bonato⁴, Cristina R. B. Cardoso¹, Marcelo Dias-Baruffi¹, Átila A. Trapé^{10,‡}, Lúcia H. Faccioli^{1,‡} and Carlos A. Sorgi^{2,3,4,‡*} and ImmunoCovid Consortium Group.

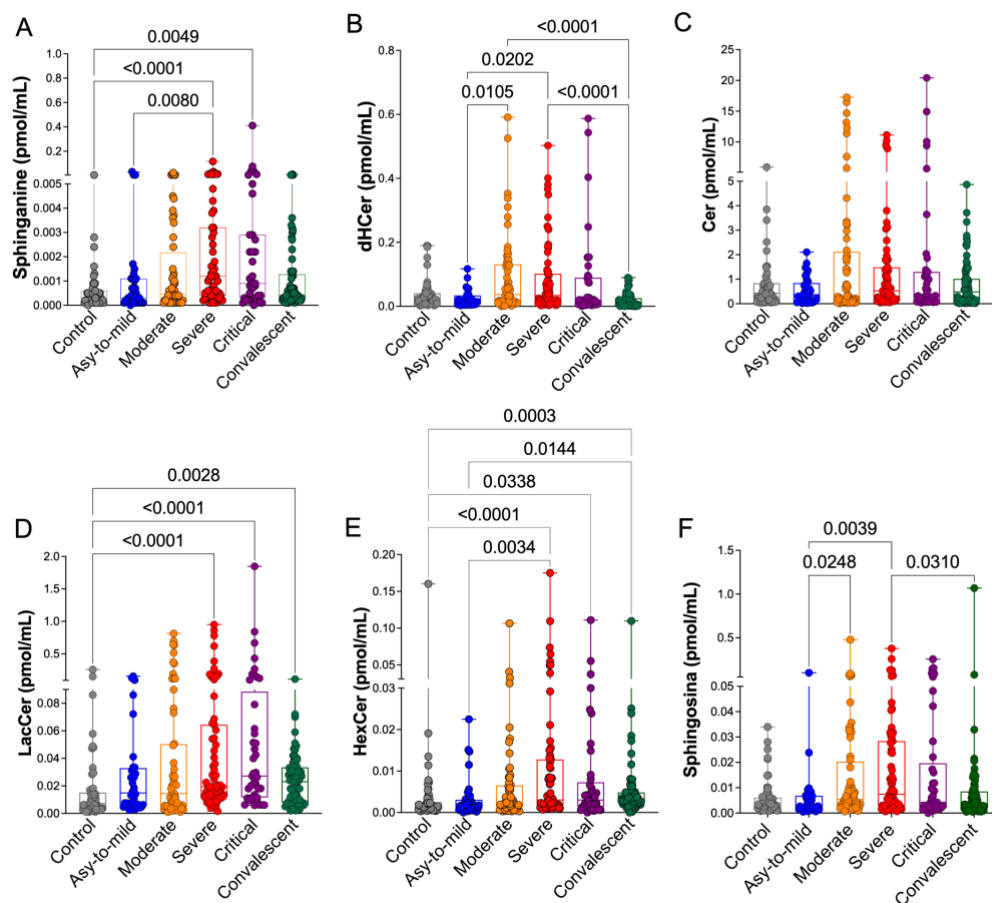
*** Correspondence:** Carlos A. Sorgi - carlos.sorgi@usp.br +55 16 3315-9176

† These authors contributed equally to this work and share first authorship

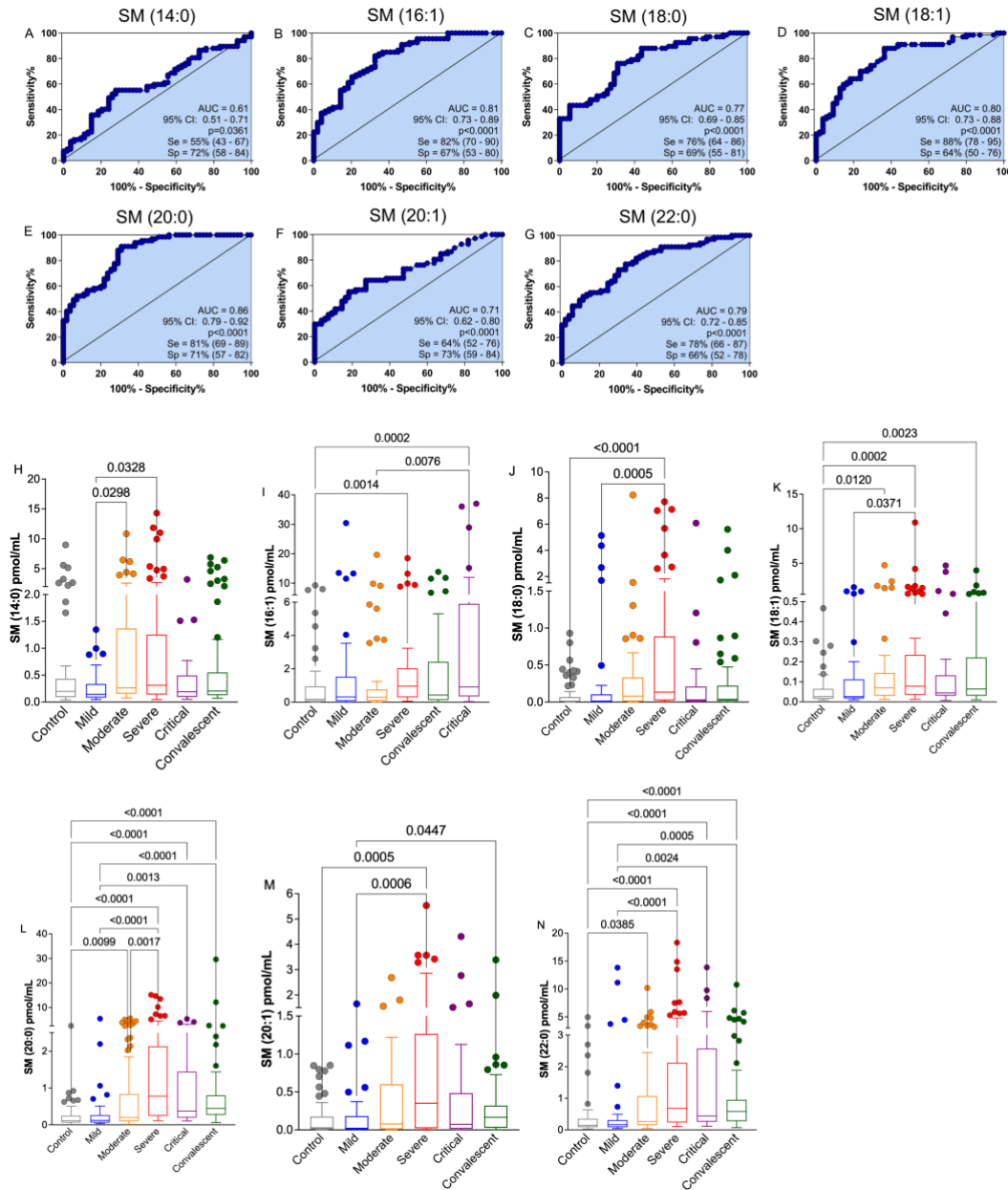
These authors contributed equally to this work and share senior authorship



Supplementary Figure 1. Expression of sphingolipid metabolic enzymes in patients with COVID-19 according to severity. (A) 3-ketodihydrosphingosine reductase (*KDSR*), (B) Sphingolipid delta (4)-desaturase DES2 (*DEGS2*), (C) Sphingomyelin phosphodiesterase (*SMPD 1*), (D) Ceramide synthase 1, 3, 4, 5 (*CERS1/3/5/6*), (E) N-acylsphingosine amidohydrolase 2 (*ASAH2*), in: Control (n=12), mild (n=12), moderate (n=14), severe (n=14) and critical (n=14). The log₂ of normalized gene expression profiles for analyzed groups are shown as boxplots. Significant differences in transcript expression correspond to Benjamini and Hockberg adjusted p-values obtained from whole transcriptome differential expression analysis considering a threshold of < 0.05 in at least one pair of clinical groups. Details of enzyme nomenclatures Supplementary Table 4.



Supplementary Figure 2. Sphingolipids abundance profile in patients with COVID-19. LC-MS/MS measurements for (A) Sphinganine (18:0), (B) dihydroceramide (dHCer), (C) Ceramide (Cer), (D) Lactosylceramide 16:0 (LacCer), (E) Hexaglycosylceramide (HexCer) and (F) Sphingosine. Statistical analyzes were performed using the Kruskal-Wallis multiple comparison test (non-parametric), followed by the Dunn post-test for pairwise comparison. Data are expressed as median in boxplot graphics. Significance levels shown are based on statistically significant p-values between groups with p-value < 0.05.



Supplementary Figure 3. ROC curves and Analysis of serum abundance sphingomyelin (SM) species (14:0), (16:1), (18:0), (18:1), (20:0), (20:1) and (22:0) in COVID-19. (A-G) ROC curves for sphingomyelin species (SM) in patients with severe forms of Covid-19. The curves compare the Severe patients groups (n= 67) with the control (n=55). AUC: area under the curve; Se: sensitivity; Sp: specificity; CI: 95% confidence interval. (H-N) sphingomyelin class profile in control subjects compared to patients with COVID-19 and convalescents in: mild (n=36), moderate (n=60), severe (n=67), critical (n=41) and Convalescent individuals (n=77). Statistical analyzes were performed using the Kruskal-Wallis multiple comparison test (non-parametric), followed by the Dunn post-test for pairwise comparison. Data are expressed as median in boxplot graphics with minimum and maximum values. Significance levels shown are based on statistically significant p-values between groups with p-value < 0.05.

$$(I) AUC_{n(k)total} = AUC_{n(k)} (1 + 0.0109n + \frac{0.0109^2 n(n-1)}{2})$$

$$(II) C_{lipid} = \frac{AUC_{lipid}}{AUC_{IS}} * C_{IS}$$

Supplementary Figure 4. Lipid quantification using internal standard and monoisotopic correction. (I) Monoisotopic correction (1): $AUC_{n(k)total}$ = corrected analyte area under the curve, $AUC_{n(k)}$ = quantified analyte monoisotopic mass area under the curve, n = number of C atoms, k = number of double bonds, **(II)** Lipid quantification based on the internal standard (1): C_{lipid} = analyte concentration (pmol/mL), AUC_{lipid} = monoisotopically corrected analyte area under the curve by Equation I ($AUC_{n(k)total}$), AUC_{IS} = area under the curve of internal standard corresponding to the analyte family, C_{IS} = internal standard concentration according to the Certificate of Analysis (Lot Number: LM6004-LM40-112A, Avanti Polar Lipids).

Supplementary Table 1: Sphingolipid subclasses and species detected in our cohort.

Subclasses	Number of species	Species Description
Ceramide	9	Cer (16:0), Cer (18:0), Cer (18:1), Cer (20:0), Cer (22:0), Cer (23:0), Cer (24:0), Cer (24:1), Cer (25:0)
Sphingosine	2	Sphingosine (14:0), Sphingosine (18:0)
Sphinganine	1	Sphinganine (18:0)
Sphingomyelin (SM)	17	SM (14:0), SM (16:0), SM (16:1), SM (18:0), SM (18:1), SM (20:0), SM (20:1), SM (22:0), SM (22:1), SM (23:0), SM (23:1), SM (24:0), SM (24:1), SM (25:0), SM (25:1), SM (26:0), SM (26:1).
Hexaglycosylceramide (HexCer)	2	HexCer (16:0), HexCer (24:0)
Lactosylceramide (LacCer)	2	LacCer (16:0), LacCer(d18:1/14:0)
Dihydroceramide (dHCer)	5	dHCer(d18:0/16:0), dHCer(d18:0/18:0), dHCer(d18:0/24:0), dHCer(d20:0/16:0), dHCer(d20:0/22:0)
Total	38	

Supplementary Table 2. Target high-resolution mass spectrometry parameters for SL species.

Experiment	Precursor ion (m/z)	Analyte	Internal Standard	Fragment ion (m/z)
1	100-2000	TOF-MS (untargeted)	-	-
2	552,5	Ceramide (d18:1/17:0)	IS	534.5237-534.5297
3	647,5	SM (d18:1/12:0)	IS Mix II (Avanti)	184.0703-184.0753
4	286,2	Sphingosine C17:0	IS Mix II (Avanti)	268.2623-268.2663

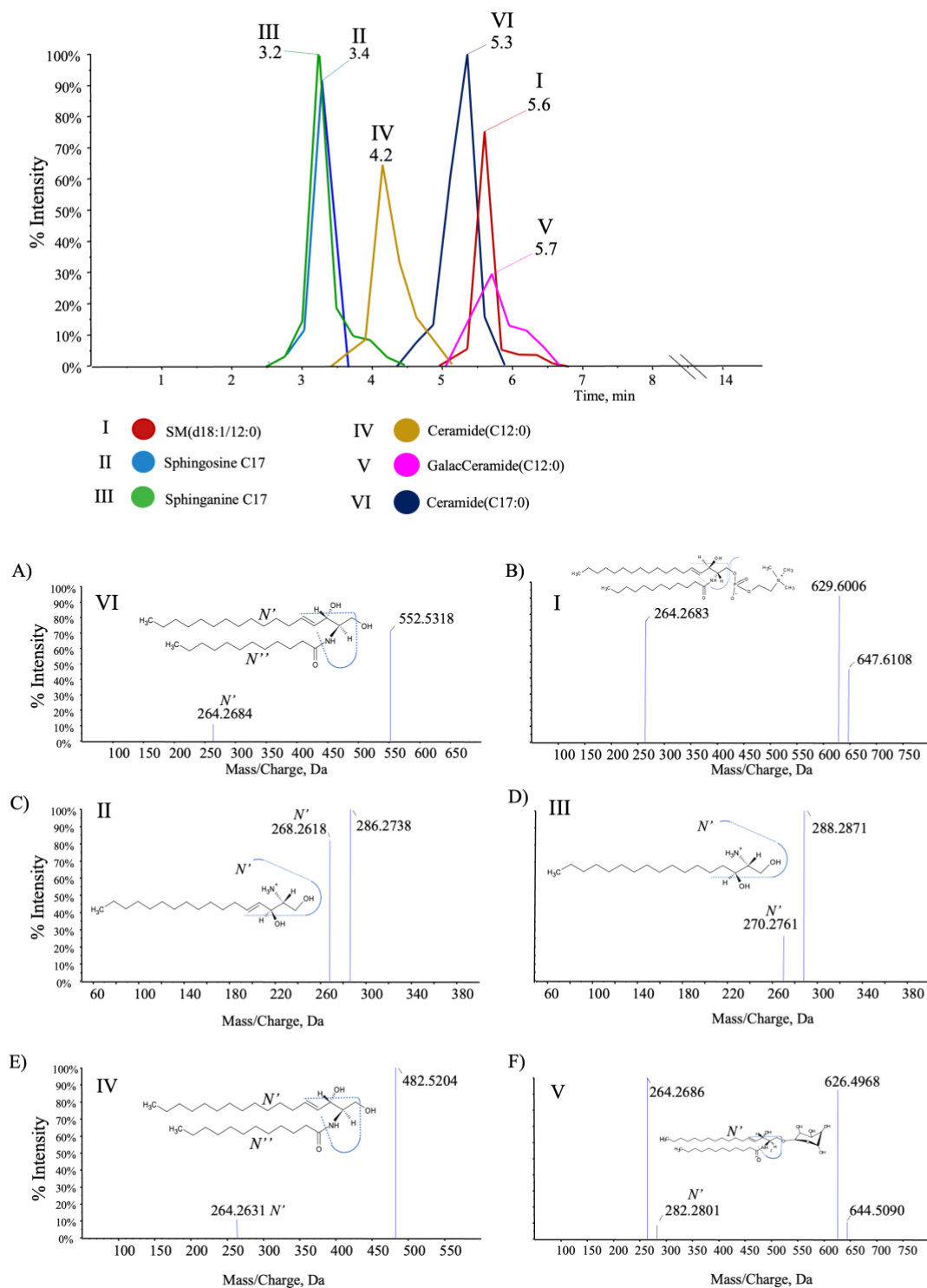
5	288,2	Sphinganine C17:0	<i>IS Mix II (Avanti)</i>	270.2750-270.2810
6	368,2	Sphinganine-1-P C17:0	<i>IS Mix II (Avanti)</i>	270.2750-270.2830
7	482,4	Ceramide (d18:1/12:0)	<i>IS Mix II (Avanti)</i>	264.2653-264.2703
8	644,5	GlcCer (d18:1/12:0)	<i>IS Mix II (Avanti)</i>	264.2652-264.26702
9	806,5	LacCer (d18:1/12:0)	<i>IS Mix II (Avanti)</i>	264.2652-264.26702
10	538,5	Ceramide (d18:1/16:0)	<i>Ceramide (d18:1/12:0)</i>	264.2652 - 264.26710
11	566,5	Ceramide (d18:1/18:0)	<i>Ceramide (d18:1/12:0)</i>	264.2652 - 264.26704
12	648,6	Ceramide (d18:1/24:1)	<i>Ceramide (d18:1/12:0)</i>	264.2652 - 264.26802
13	650,6	Ceramide (d18:1/24:0)	<i>Ceramide (d18:1/12:0)</i>	264.2652 - 264.26702
14	664,4	Ceramide (d18:1/25:0)	<i>Ceramide (d18:1/12:0)</i>	264.2652 - 264.26702
15	700,5	GlcCer (d18:1/16:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26702
16	728,6	GlcCer (d18:1/18:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26702
17	810,6	GlcCer (d18:1/24:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26702
18	731,6	SM (d18:1/18:0)	<i>SM (d18:1/12:0)</i>	184.0703 - 184.0753
19	815,7	SM (d18:1/24:0)	<i>SM (d18:1/12:0)</i>	184.0703 - 184.0753
20	862,6	LacCer (d18:1/16:0)	<i>LacCer (d18:1/12:0)</i>	264.2652 - 264.26702
21	974,7	LacCer (d18:1/24:0)	<i>LacCer (d18:1/12:0)</i>	264.2652 - 264.26702
22	300,3	Sphingosine C18:0	<i>Sphingosine C17:0</i>	282.2480 - 282.2530
23	272,2	Sphingosine C16:0	<i>Sphingosine C17:0</i>	254.2164 - 254.2214
24	244,2	Sphingosine C14:0	<i>Sphingosine C17:0</i>	226.1765 - 226.1815
25	302,2	Sphinganine C18:0	<i>Sphinganine C17:0</i>	284.2912 - 284.2952
26	274,2	Sphinganine C16:0	<i>Sphinganine C17:0</i>	256.2612 - 256.2652
27	382,2	Sphinganine-1-P C18:0	<i>Sphinganine-1-P C17:0</i>	364.2120 - 364.2160
28	354,2	Sphinganine-1-P C16:0	<i>Sphinganine-1-P C17:0</i>	336.2298 - 336.2338
29	356,35	Sphingosine C22:1	<i>Sphingosine C17:0</i>	338.3500 - 338.3540
30	328,3	Sphingosine C20:1	<i>Sphingosine C17:0</i>	310.3000 - 310.3040
31	330,3	Sphinganine C20:0	<i>Sphinganine C17:0</i>	214.3000 - 214.3040
32	410,3	Sphinganine-1-P C20:0	<i>Sphinganine C17:0</i>	392.3000 - 392.3040
33	464,4	Ceramide (d18:1/18:1)	<i>Ceramide (d18:1/12:0)</i>	264.2694 - 264.2714
34	594,6	Ceramide (d18:1/20:0)	<i>Ceramide (d18:1/12:0)</i>	264.2671 - 264.2701
35	622,7	Ceramide (d18:1/22:0)	<i>Ceramide (d18:1/12:0)</i>	264.2671 - 264.2701
36	634,7	Ceramide (d18:1/23:1)	<i>Ceramide (d18:1/12:0)</i>	264.2671 - 264.2701
37	636,7	Ceramide (d18:1/23:0)	<i>Ceramide (d18:1/12:0)</i>	264.2671 - 264.2701
38	662,8	Ceramide (d18:1/25:1)	<i>Ceramide (d18:1/12:0)</i>	264.2671 - 264.2701
39	676,8	Ceramide (d18:1/26:1)	<i>Ceramide (d18:1/12:0)</i>	264.2671 - 264.2701
40	678,8	Ceramide (d18:1/26:0)	<i>Ceramide (d18:1/12:0)</i>	264.2671 - 264.2701

41	675,5	SM (d18:1/14:0)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
42	701,6	SM (d18:1/16:0)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
43	703,57	SM (d18:1/16:0)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
44	729,6	SM (d18:1/18:1)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
45	757,6	SM (d18:1/20:1)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
46	759,6	SM (d18:1/20:0)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
47	785,591	SM (d18:1/22:1)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
48	787,6	SM (d18:1/22:0)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
49	799,6	SM (d18:1/23:1)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
50	801,6	SM (d18:1/23:0)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
51	813,7	SM (d18:1/24:1)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
52	827,7	SM (d18:1/25:1)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
53	829,7	SM (d18:1/25:0)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
54	841,7	SM (d18:1/26:1)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
55	843,7	SM (d18:1/26:0)	<i>SM (d18:1/12:0)</i>	183.9758 - 184.0758
56	726,58	HexCer (d18:1/18:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26702
57	754,7	HexCer (d18:1/20:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26703
58	756,7	HexCer (d18:1/20:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26704
59	782,7	HexCer (d18:1/22:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26705
60	784,66	HexCer (d18:1/22:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26706
61	796,7	HexCer (d18:1/23:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26707
62	798,7	HexCer (d18:1/23:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26708
63	808,6	HexCer (d18:1/24:2)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26709
64	812,7	HexCer (d18:1/24:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26710
65	824,6	HexCer (d18:1/25:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26711
66	826,8	HexCer (d18:1/25:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26712
67	836,6	HexCer (d18:1/26:2)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26713
68	838,8	HexCer (d18:1/26:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26714
69	840,8	HexCer (d18:1/26:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26715
70	716,7	HexCer OH (d18:1/16:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26716
71	744,7	HexCer OH (d18:1/18:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26717
72	772,7	HexCer OH (d18:1/20:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26718
73	798,7	HexCer OH (d18:1/22:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26719
74	800,7	HexCer OH (d18:1/22:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26720
75	812,7	HexCer OH (d18:1/23:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26721
76	814,7	HexCer OH (d18:1/23:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26722
77	826,8	HexCer OH (d18:1/24:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26723

78	828,8	HexCer OH (d18:1/24:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26724
79	840,8	HexCer OH (d18:1/25:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26725
80	842,8	HexCer OH (d18:1/25:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26726
81	854,8	HexCer OH (d18:1/26:1)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26727
82	856,8	HexCer OH (d18:1/26:0)	<i>GlcCer (d18:1/12:0)</i>	264.2652 - 264.26728
83	974,7502	LacCer (d18:0/24:1)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2727
84	972,7346	LacCer (d18:1/24:1(15Z))	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2728
85	1002,7815	LacCer (d18:0/26:1)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2729
86	1000,7659	LacCer (d18:1/26:1(17Z))	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2730
87	886,6407	LacCer (d18:1/18:1(9Z))	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2731
88	889,6563	LacCer (d18:0/18:1)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2732
89	948,7346	LacCer (d18:0/22:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2733
90	946,7189	LacCer (d18:1/22:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2734
91	920,7033	LacCer (d18:0/20:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2735
92	918,6876	LacCer (d18:1/20:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2736
93	1004,7972	LacCer (d18:0/26:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2737
94	1002,7815	LacCer(d18:1/26:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2738
95	864,6407	LacCer (d18:0/16:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2739
96	806,5624	LacCer (d14:1/16:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2740
97	892,672	LacCer (d18:0/18:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2741
98	836,6094	LacCer (d14:0/18:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2742
99	890,6563	LacCer (d18:1/18:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2743
100	976,7659	LacCer (d18:0/24:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2744
101	974,7502	LacCer (d18:1/24:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2745
102	836,6094	LacCer (d18:0/14:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2746
103	834,5937	LacCer (d18:1/14:0)	<i>LacCer (d18:1/12:0)</i>	264.2687 - 264.2747
104	484,465	dHCer (d18:0/12:0)	<i>Ceramide (d18:1/12:0)</i>	264.2687 - 264.2747
105	540,535	dHCer (d18:0/16:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
106	568,563	dHCer (d18:0/18:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
107	596,5976	dHCer (d18:0/20:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
108	624,6289	dHCer (d18:0/22:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
109	650,6446	dHCer (d18:0/24:1(15Z))	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
110	652,6602	dHCer (d18:0/24:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
111	678,6759	dHCer (d18:0/26:1(17Z))	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
112	680,6915	dHCer (d18:0/26:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
113	566,5507	dHCer (d18:0/18:1)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983

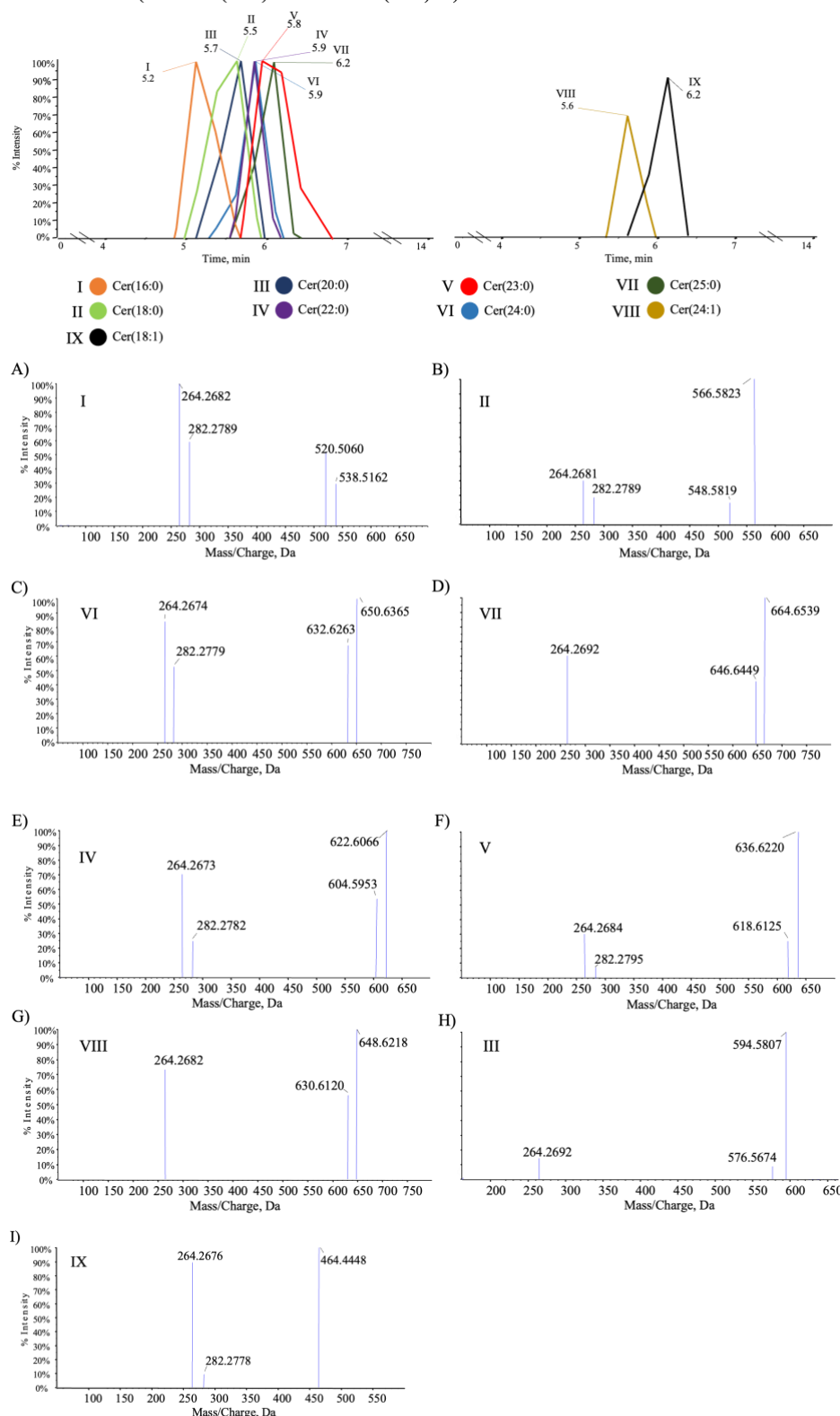
114	512,5037	dHCer (d18:0/14:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
115	668,6551	dHCer (d18:0/h24:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
116	696,6864	dHCer (d18:0/h26:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
117	570,5456	dHCer (d18:0/h17:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
118	498,4881	dHCer (d18:0/13:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
119	526,5194	dHCer (d18:0/15:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
120	554,5507	dHCer (d18:0/17:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
121	568,3566	dHCer (d20:0/16:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
122	596,5976	dHCer (d20:0/18:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2983
123	624,6289	dHCer (d20:0/20:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2984
124	652,6602	dHCer (d20:0/22:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2985
125	680,6915	dHCer (d20:0/24:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2986
126	708,7228	dHCer (d20:0/26:0)	<i>Ceramide (d18:1/12:0)</i>	284.2943 - 284.2987

Internal standards (IS)



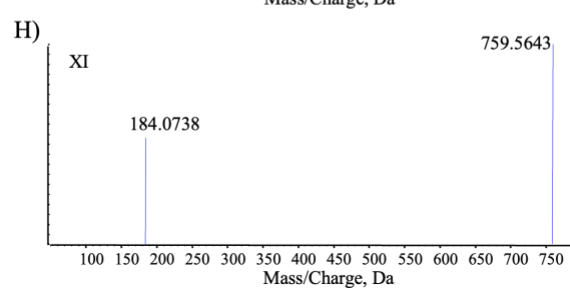
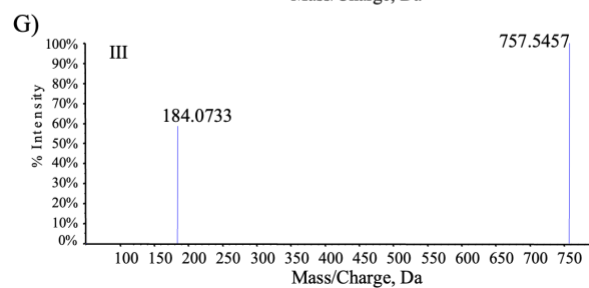
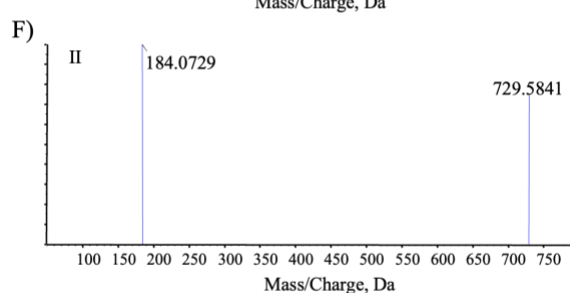
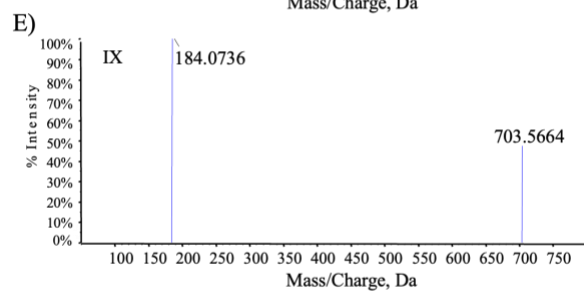
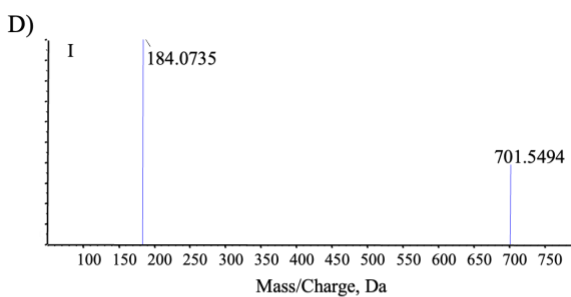
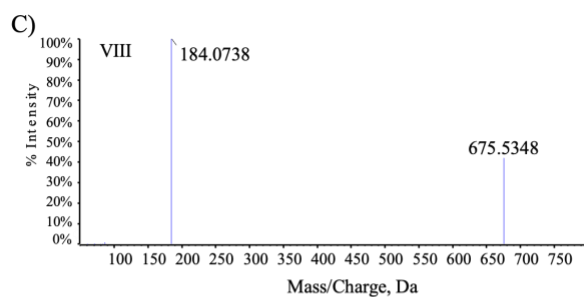
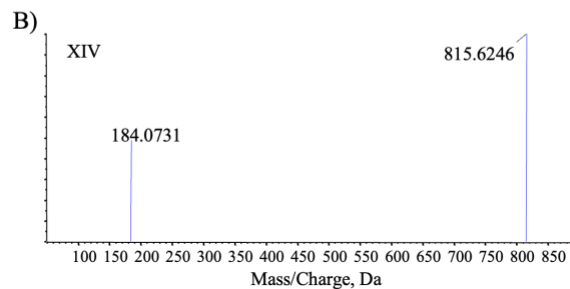
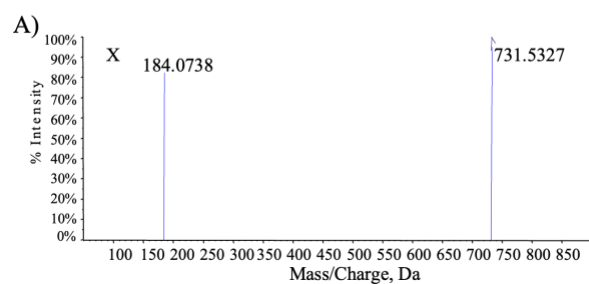
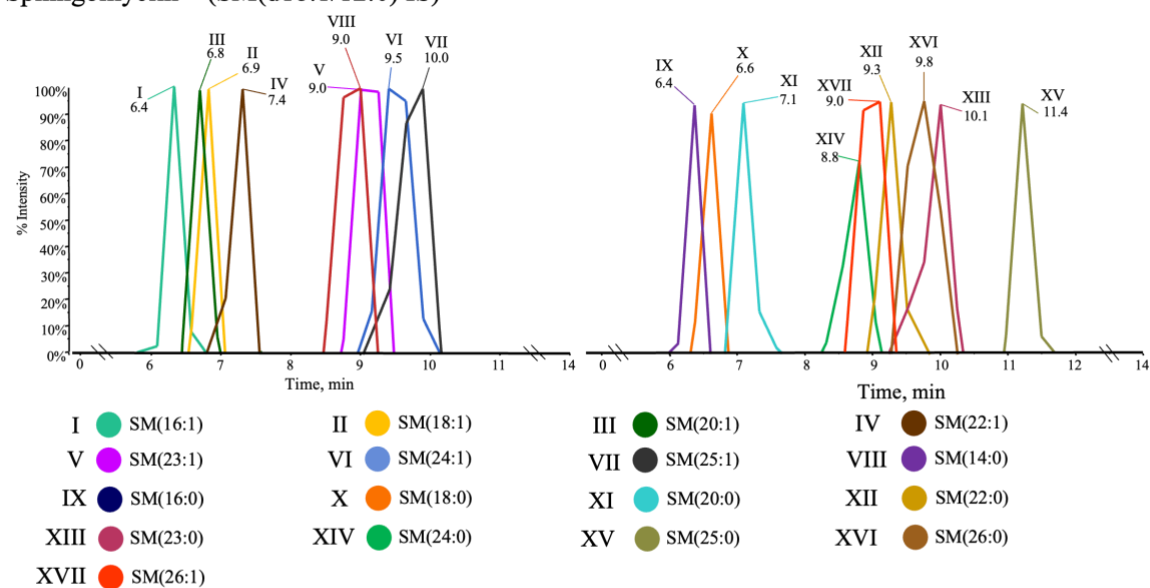
Supplementary Figure 5. Chromatographic profile and mass spectra of standards used as reference for analysis. (I) Sphingomyelin (d18:0/12:0). (II) Sphingosine (17:0). (III) Sphinganine (17:0). (IV) Ceramide (12:0). (V) Galactosylceramide (12:0). (VI) Ceramide (17:0).

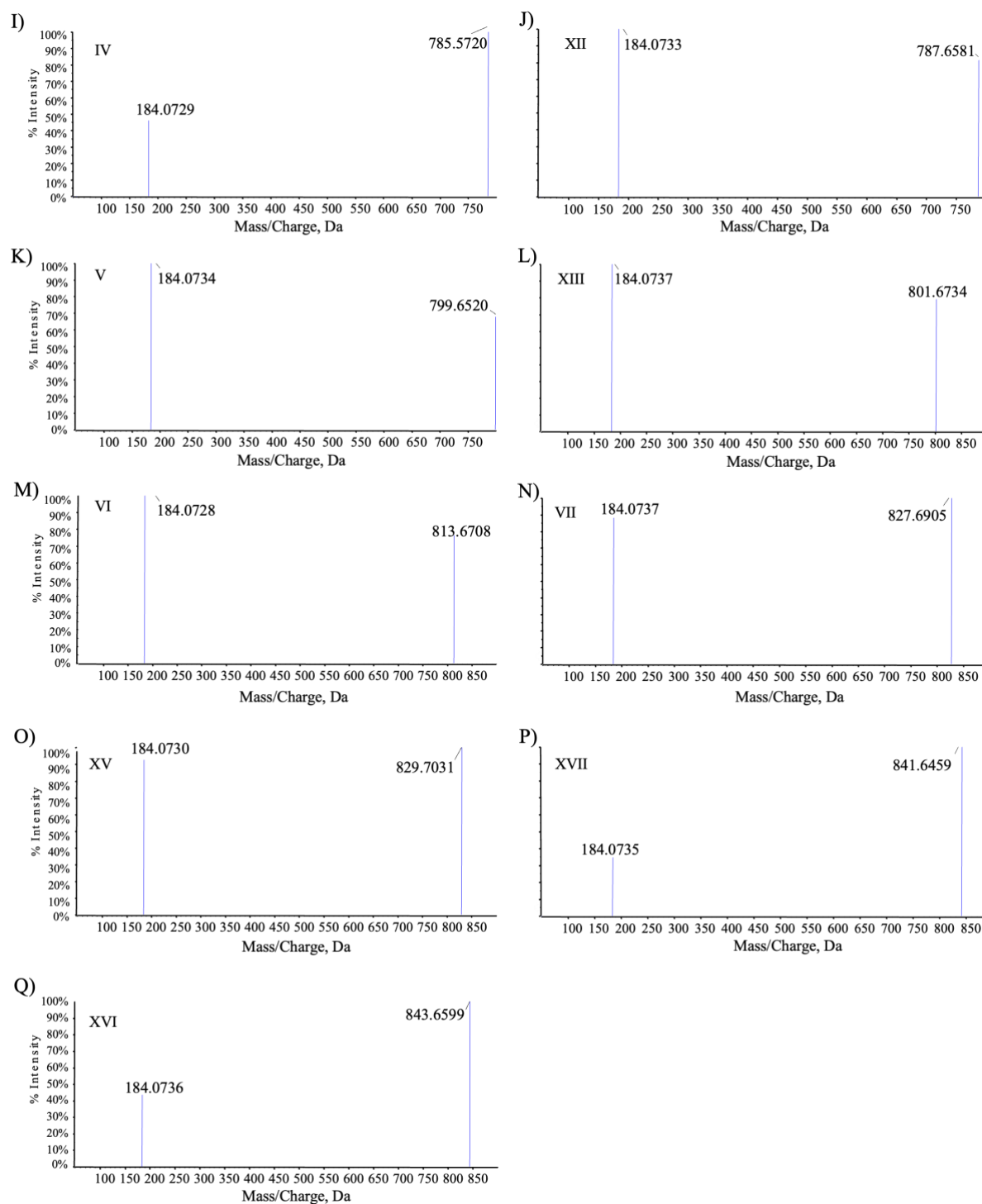
Ceramides – (Ceramide(12:0) and Ceramide(17:0) IS)



Supplementary Figure 6. Chromatographic profile and mass spectra of detected ceramides. (I) Ceramide (16:0). (II) Ceramide (18:0). (III) Ceramide (20:0). (IV) Ceramide (22:0). (V) Ceramide (23:0). (VI) Ceramide (24:0). (VII) Ceramide (25:0). (VIII) Ceramide (24:1). (IX) Ceramide (18:1).

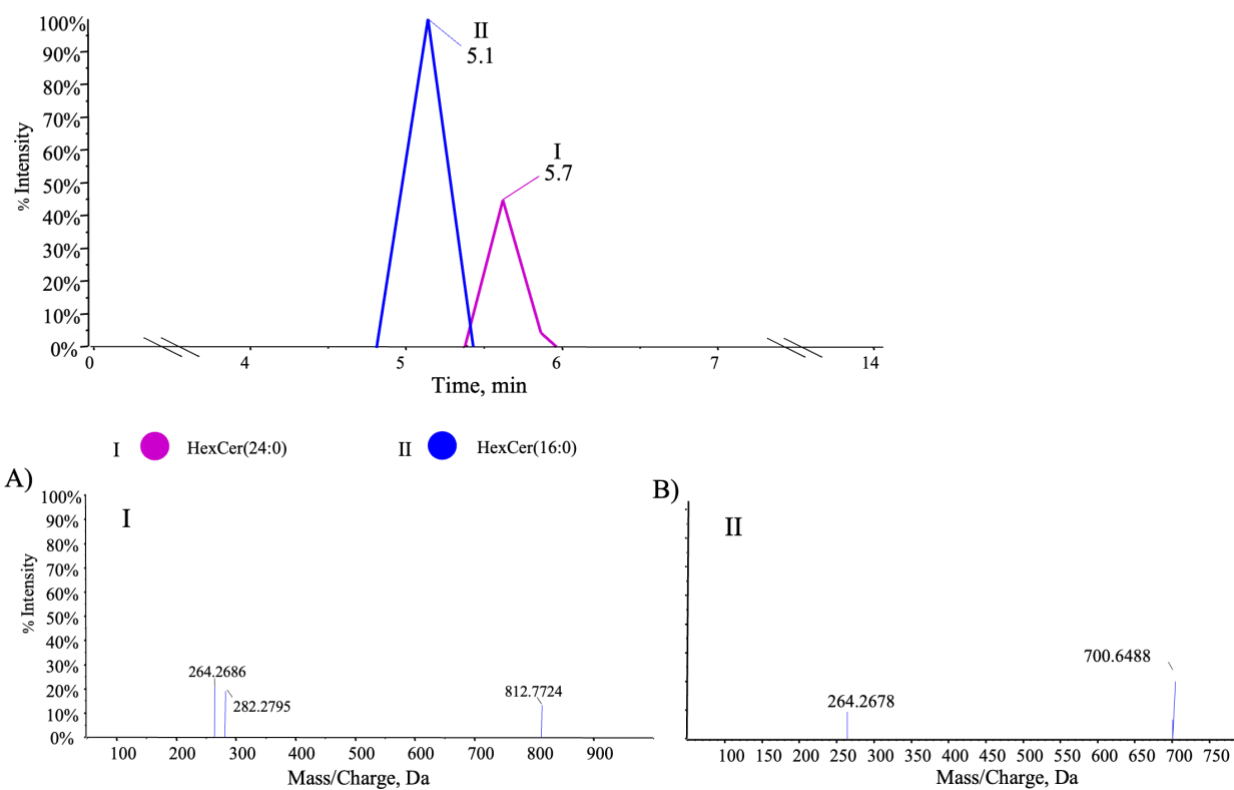
Sphingomyelin – (SM(d18:1/12:0) IS)





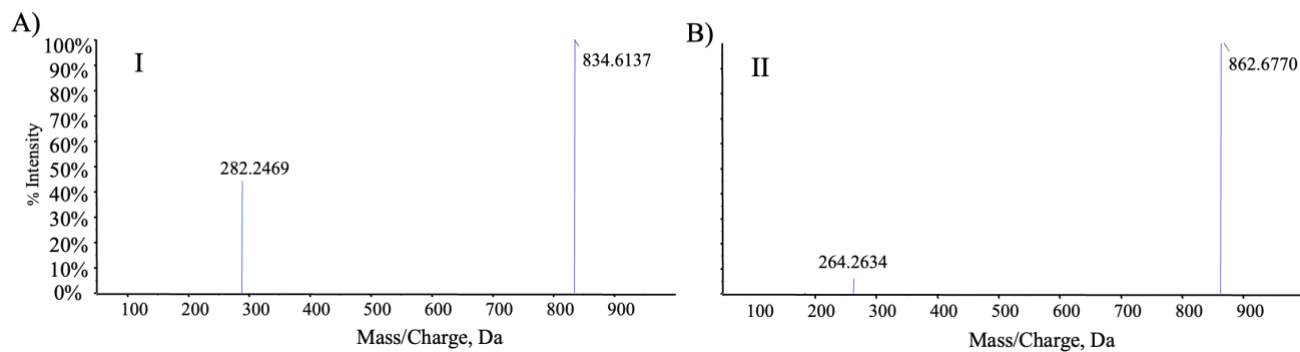
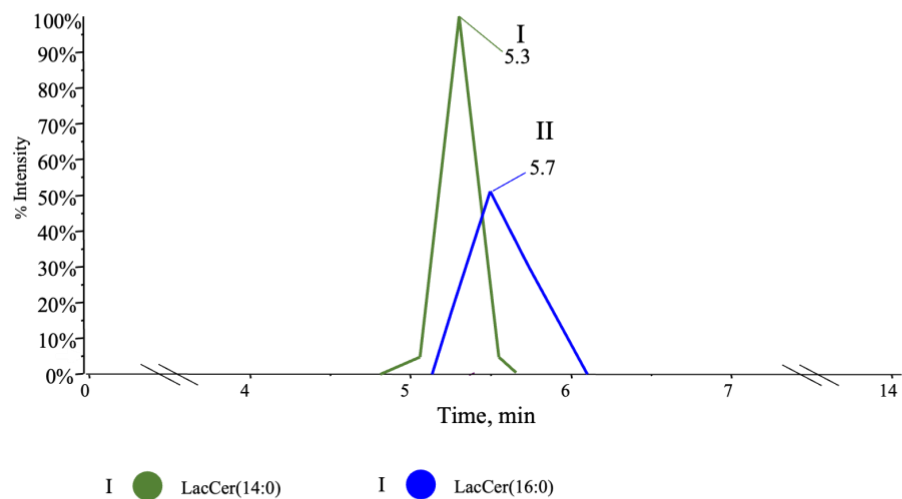
Supplementary Figure 7. Chromatographic profile and mass spectra of detected sphingomyelins. (I) Sphingomyelin (16:1). (II) Sphingomyelin (18:1). (III) Sphingomyelin (20:1). (IV) Sphingomyelin (22:1). (V) Sphingomyelin (23:1). (VI) Sphingomyelin (24:1). (VII) Sphingomyelin (25:1). (VIII) Sphingomyelin (14:0). (IX) Sphingomyelin (16:0). (X) Sphingomyelin (18:0). (XI) Sphingomyelin (20:0). (XII) Sphingomyelin (22:0). (XIII) Sphingomyelin (23:0). (XIV) Sphingomyelin (24:0). (XV) Sphingomyelin (25:0). (XVI) Sphingomyelin (26:0). (XVII) Sphingomyelin (26:1).

Hexosylceramides - Cer(12:0) IS



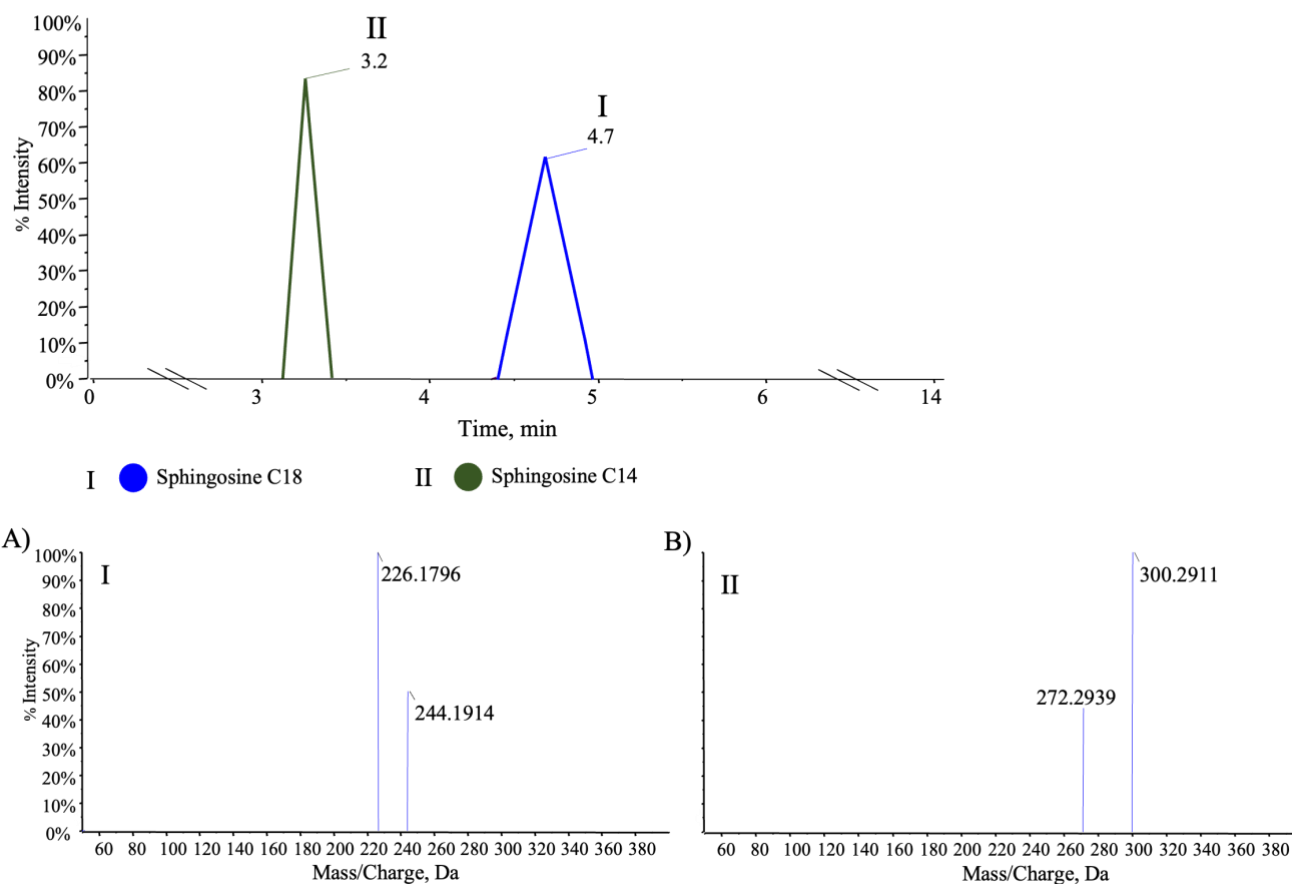
Supplementary Figure 8. Chromatographic profile and mass spectra of detected hexosylceramides. (I) HexosylCeramide (24:0). (II) HexosylCeramide (16:0).

LacCeramides - LacCer(12:0) IS



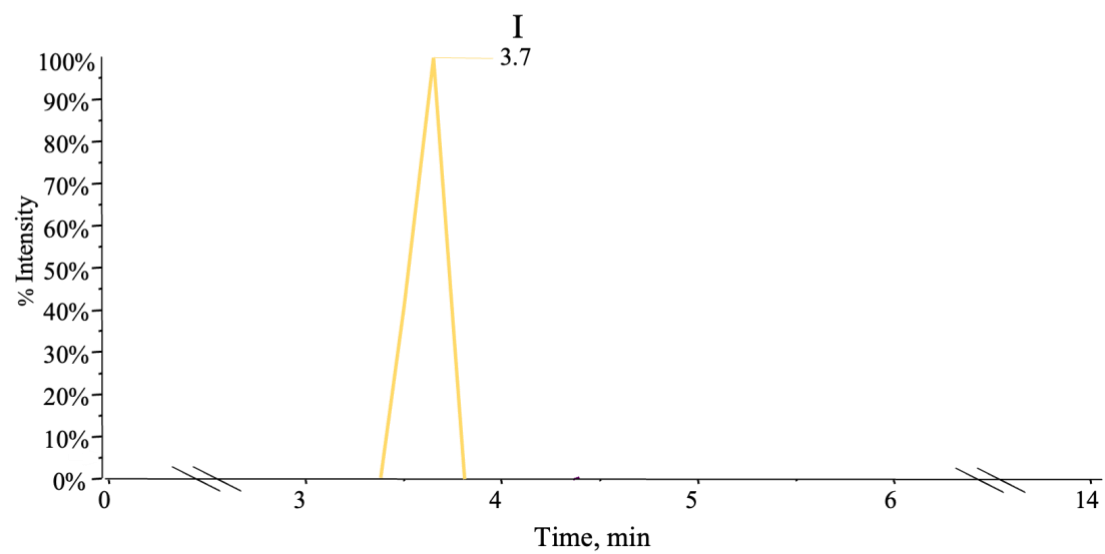
Supplementary Figure 9. Chromatographic profile and mass spectra of detected lactosylceramides. (I) LacCer (14:0). (II) LacCer (16:0).

Sphingosine - (Sphingosine C17:0 IS)

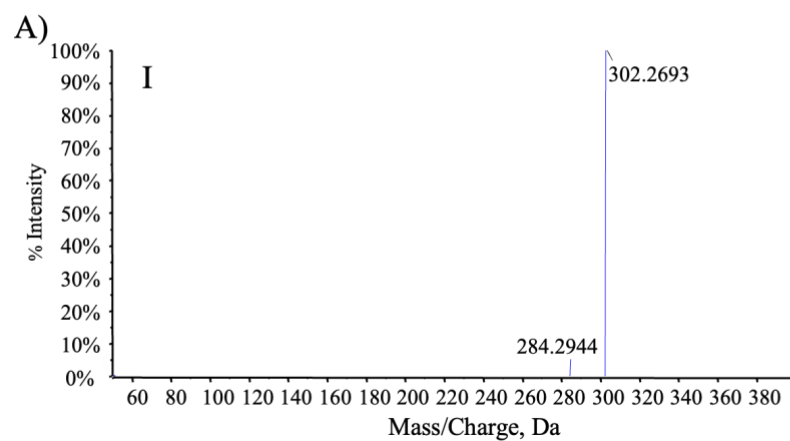


Supplementary Figure 10. Chromatographic profile and mass spectra of detected sphingosines. (I) Sphingosine (18:0). (II) Sphingosine (14:0).

Sphinganine – (Sphingosine C17:0 IS)

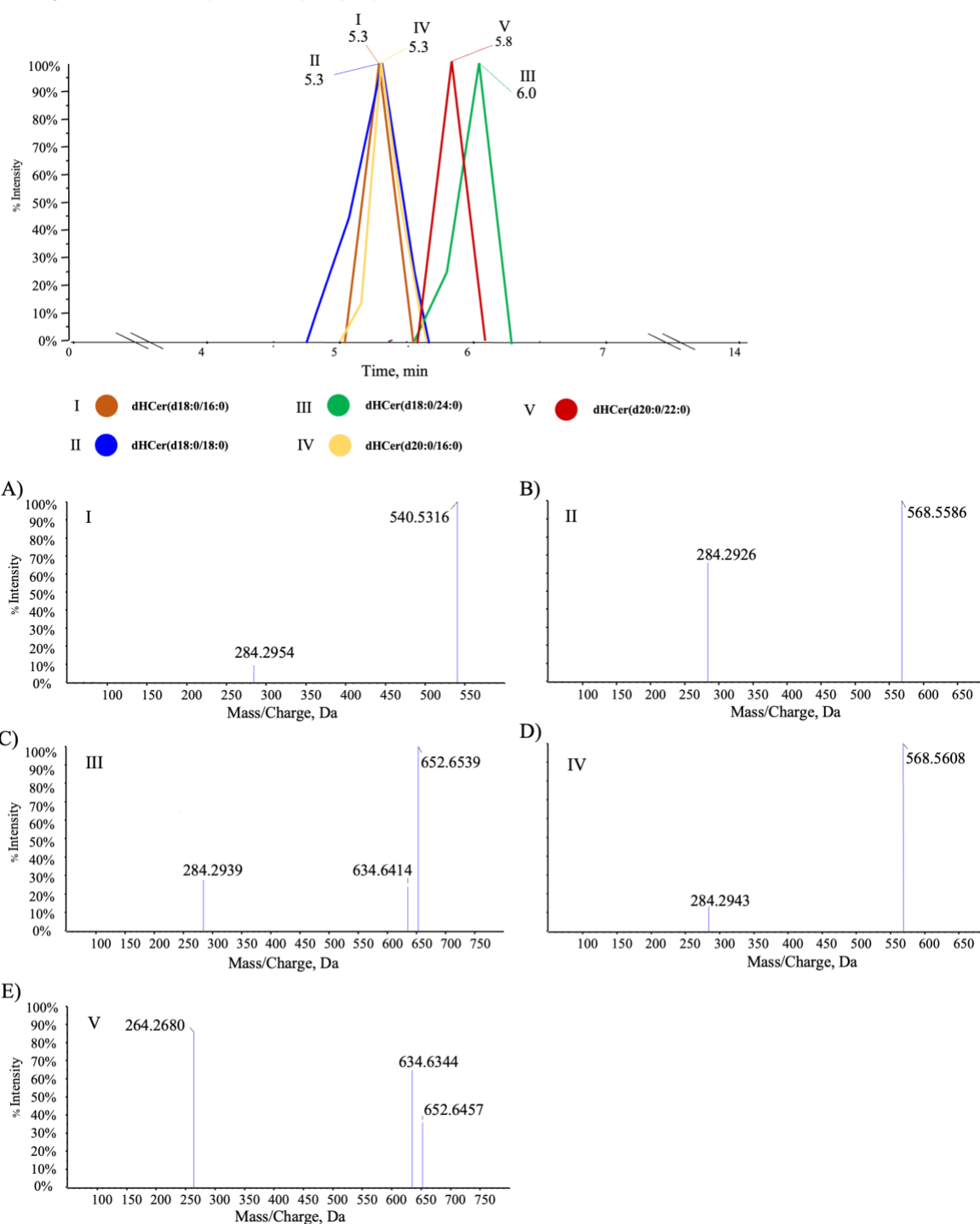


I ● Sphinganine(18:0)



Supplementary Figure 11. Chromatographic profile and mass spectra of detected sphinganine. (I) Sphinganine (18:0).

Dihydroceramides – (Ceramide(12:0) IS)



Supplementary Figure 12. Chromatographic profile and mass spectra of detected dihydroceramides. (I) dHCer(d18:0/16:0). (II) dHCer(d18:0/18:0). (III) dHCer(d18:0/24:0). (IV) dHCer(d20:0/16:0). (V) dHCer(d20:0/22:0).

Supplementary Table 3. List of sphingolipid pathway enzymes and their respective acronyms and ID number.

Enzyme name	Acronym enzyme	Acronym Gene	HGNC ID	NCBI ID
Serine palmitoyltransferase long chain base subunit 1	SPT	SPTLC1	11277	10558
Serine palmitoyltransferase long chain base subunit 2	SPT	SPTLC2	11278	9517
Serine palmitoyltransferase long chain base subunit 3	SPT	SPTLC3	16253	55304
3-ketodihydrosphingosine reductase	KDR	KDSR	4021	2531
Ceramide synthase 1	CerS	CERS1	14253	10715
Ceramide synthase 2	CerS	CERS2	14076	29956
Ceramide synthase 3	CerS	CERS3	23752	204219
Ceramide synthase 4	CerS	CERS4	23747	79603
Ceramide synthase 5	CerS	CERS5	23749	91012
Ceramide synthase 6	CerS	CERS6	23826	253782
Sphingomyelin synthase 1	SMS	SGMS1	29799	259230
Sphingomyelin synthase 2	SMS	SGMS2	28395	166929
Sphingomyelin phosphodiesterase 1	SMase	SMPD1	11120	6609
Sphingomyelin phosphodiesterase 2	SMase	SMPD2	11121	6610
Sphingomyelin phosphodiesterase 3	SMase	SMPD3	14240	55512
Sphingomyelin phosphodiesterase 4	SMase	SMPD4	32949	55627
N-acylsphingosine amidohydrolase (acid ceramidase)	Csase	ASAH1	735	427
N-acylsphingosine amidohydrolase 2 (Neutral ceramidase/non-lysosomal ceramidase)	Csase	ASAH2	18860	56624
Ceramide synthase 1	CerS	CERS1	14253	10715
Ceramide synthase 2	CerS	CERS2	14076	29956
Ceramide synthase 3	CerS	CERS3	23752	204219
Ceramide synthase 4	CerS	CERS4	23747	79603
Ceramide synthase 5	CerS	CERS5	23749	91012
Ceramide synthase 6	CerS	CERS6	23826	253782

References

1. Fedorova, M, Lange, M: Evaluation of lipid quantification accuracy using HILIC and RPLC MS on the example of NIST[®] SRM[®] 1950 metabolites in human plasma. *Anal Bioanal Chem* 412, 3573–3584 (2020). <https://doi.org/10.1007/s00216-020-02576-x>