

Sphingolipidome quantification by liquid chromatography- high resolution mass spectrometry:

whole blood vs. plasma

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Table of Contents

Figure S1-S16

Table S1-S2.

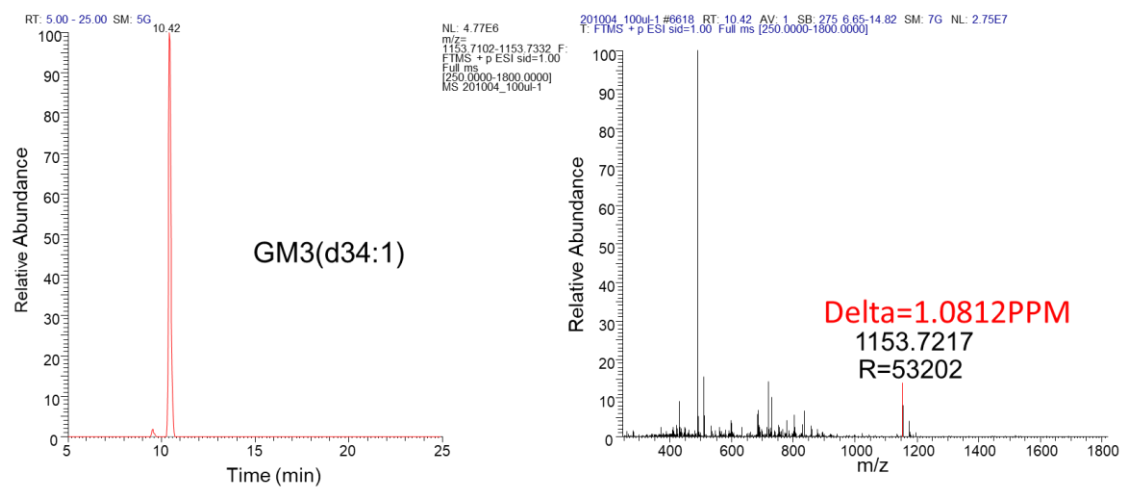


Figure S1. The chromatogram and MS spectra of GM3(d34:1). Accuracy was under 2 ppm and the resolution was 53, 202.

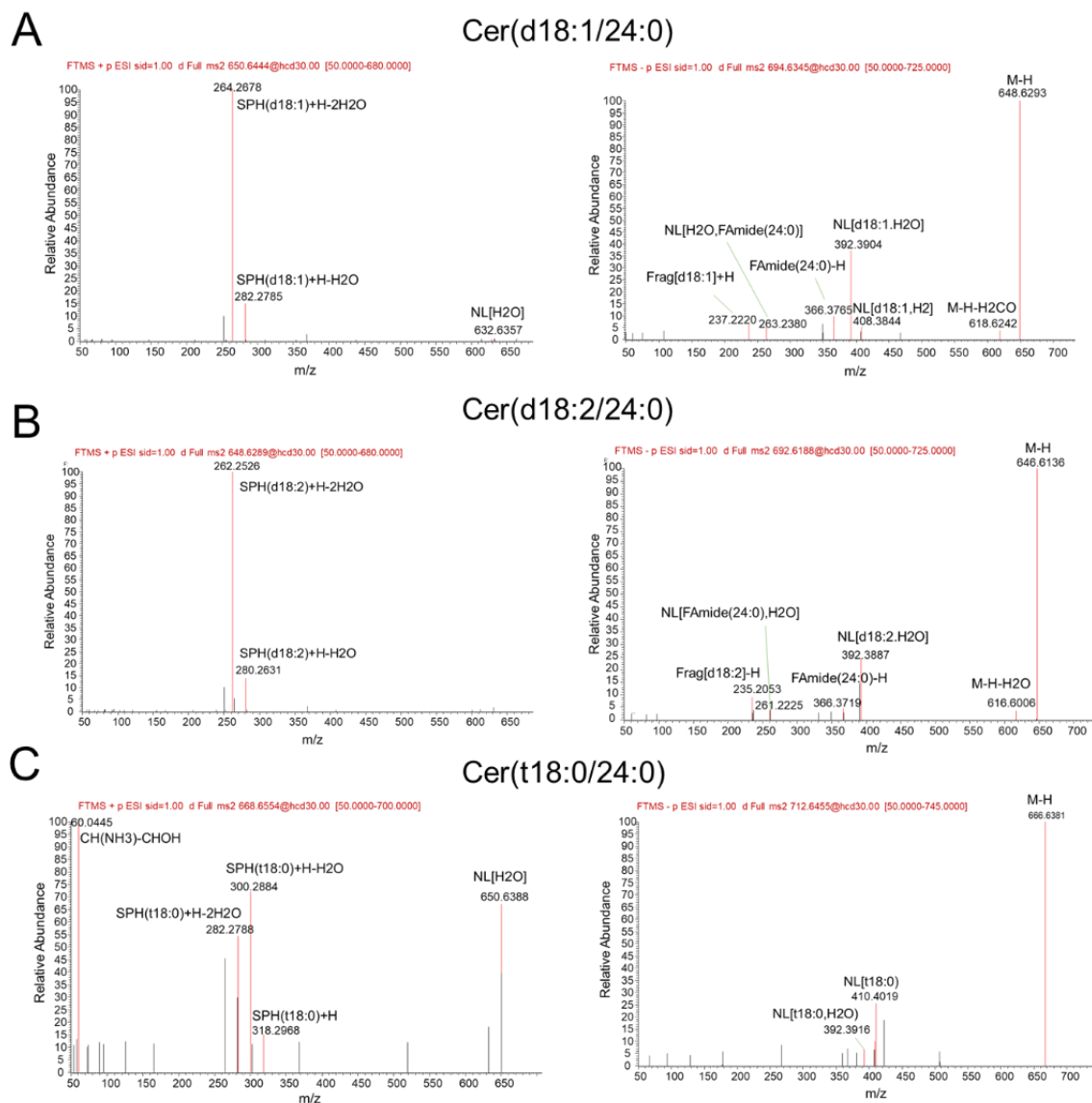


Figure S2. Representative MS2 spectra of Cer(d18:1/24:0) (A), Cer(d18:2/24:0) (B), Cer(t18:0/24:0) (C) in both positive (left) and negative (right) ion mode.

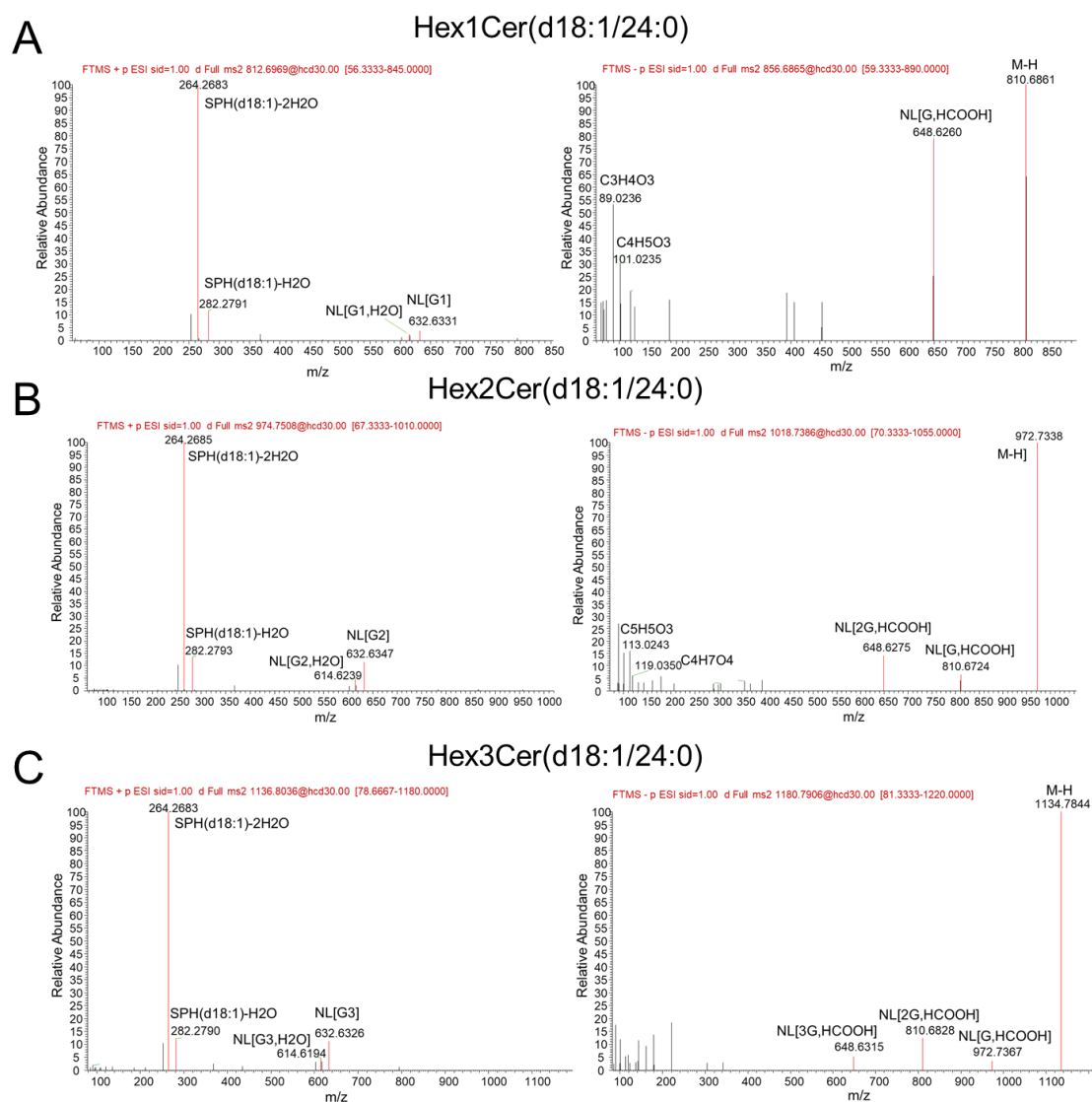


Figure S3. Representative MS2 spectra of Hex1Cer(d18:1/24:0) (A), Hex2Cer(d18:1/24:0) (B), Hex3Cer(d18:1/24:0) (C) in both positive (left) and negative (right) ion mode.

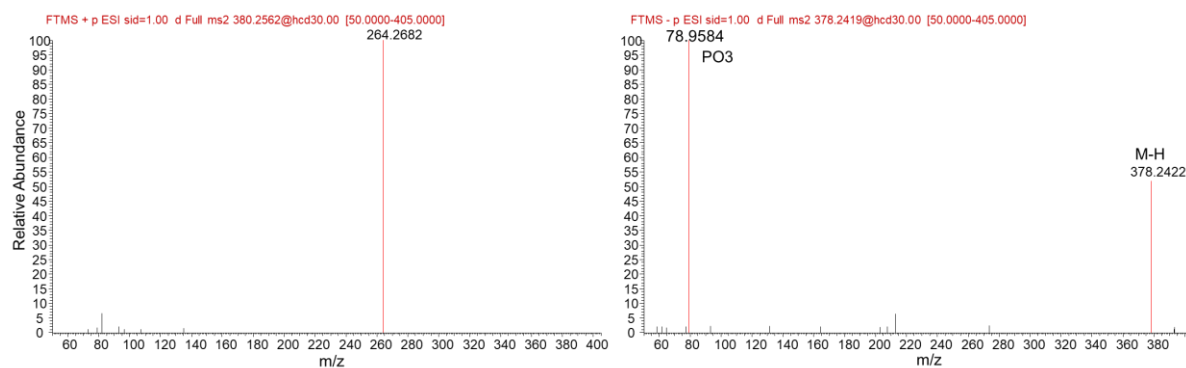


Figure S4. Representative MS2 spectra of sphingosine-1-P in both positive (left) and negative (right) ion mode.

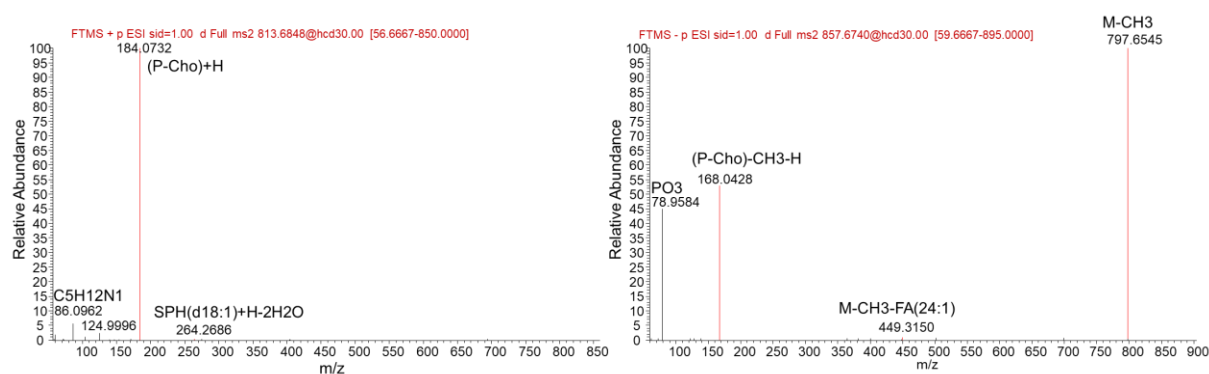


Figure S5. Representative MS2 spectra of SM(d18:1/24:1) in both positive (left) and negative (right) ion mode.

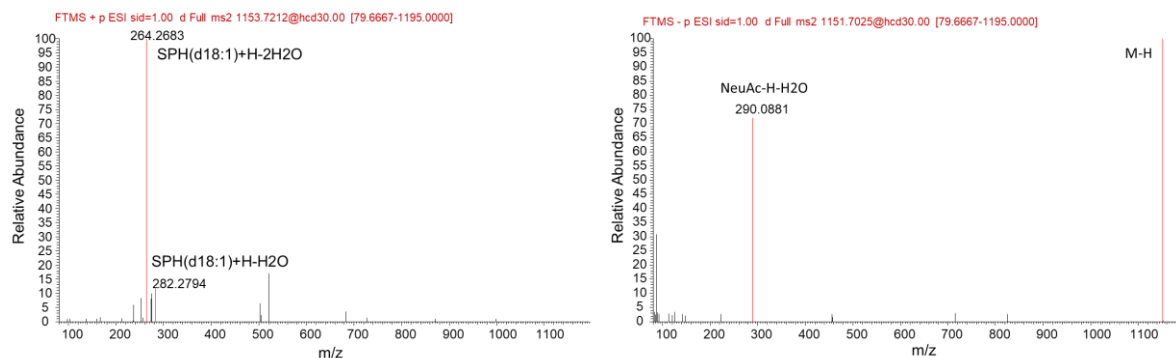


Figure S6. Representative MS2 spectra of GM3(d34:1) in both positive (left) and negative (right) ion mode.

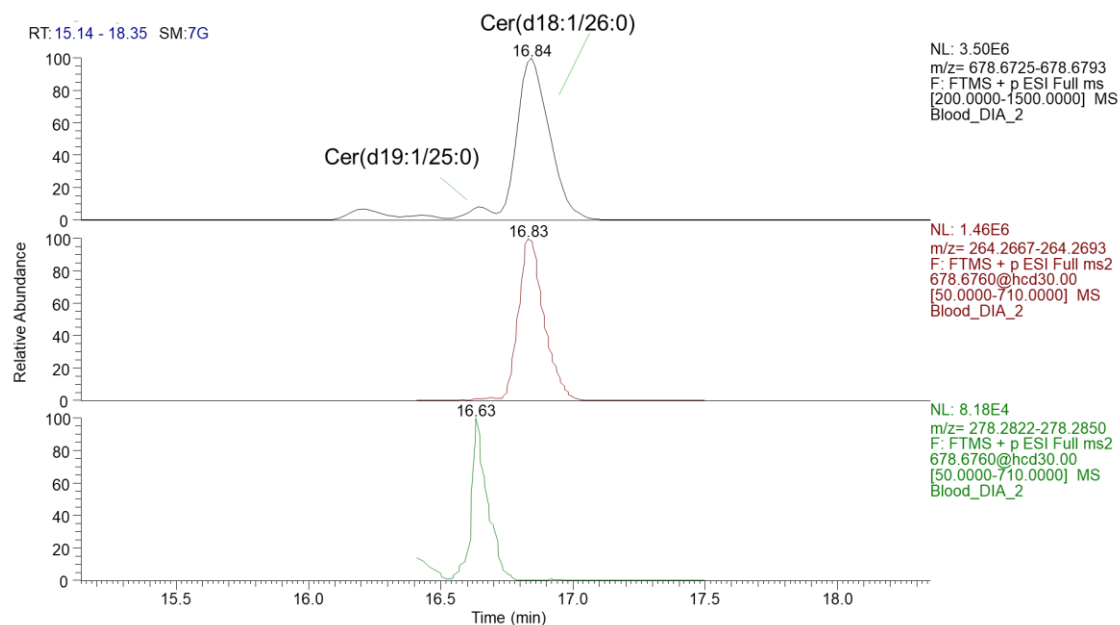


Figure S7. The MS2 product extracted chromatograms of Cer(d19:1/25:0) (lower channel, RT 16.63 min) and Cer(d18:1/26:0) (middle channel, RT 16.83 min) in data independent acquisition (DIA) mode.

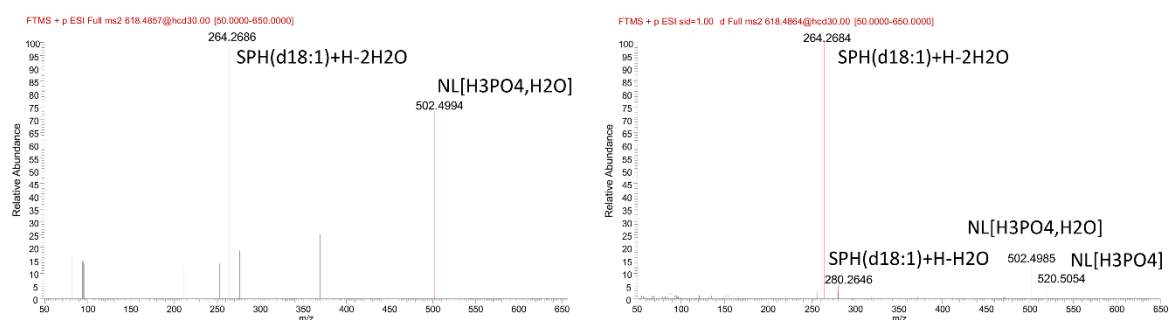


Figure S8. Representative MS2 spectra of CerP(d18:1/16:0) acquired from whole blood samples (left panel) or standard solution (right panel) in positive ion mode.

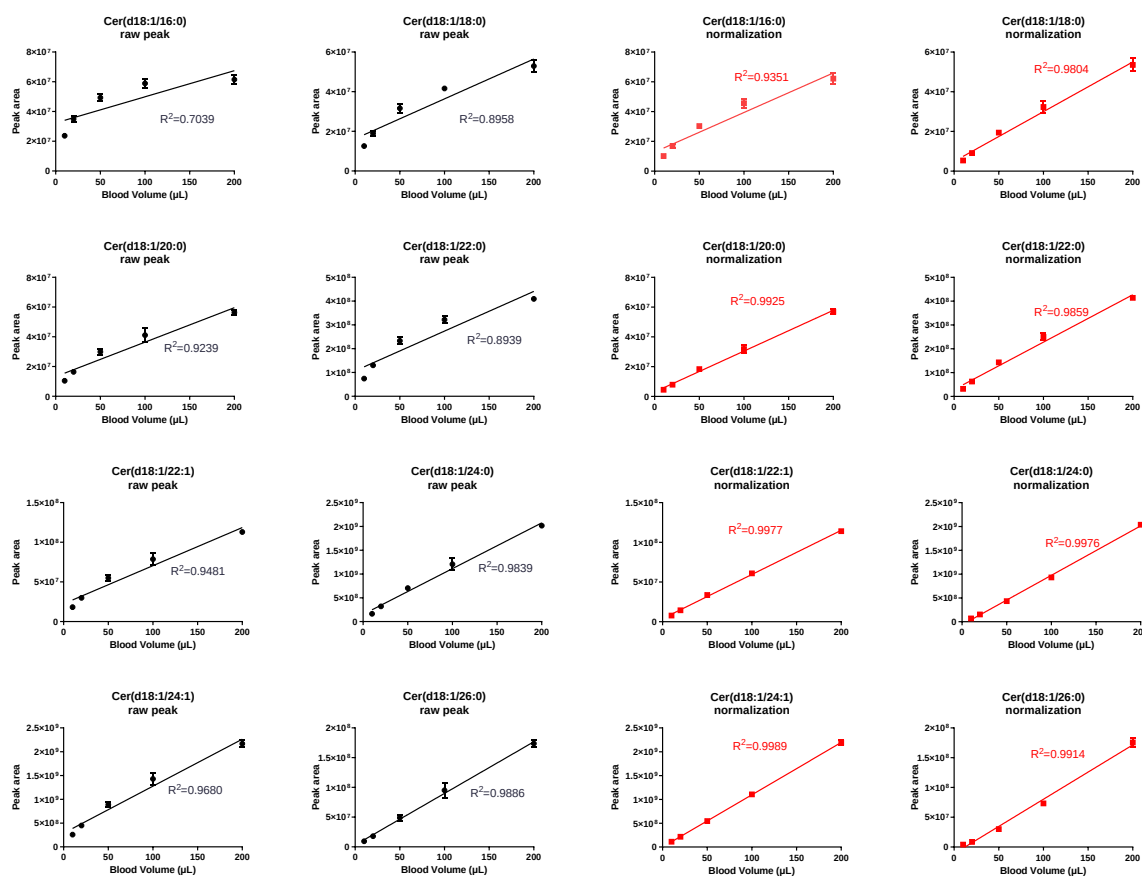


Figure S9. Comparison of the raw peak intensity or normalized intensity (internal standard 12:0 ceramide) of ceramides, and their linear response vs different volume of blood. Linear regression was used with equal weighting and each point had three technical replicates. SD is showed on the graphs.

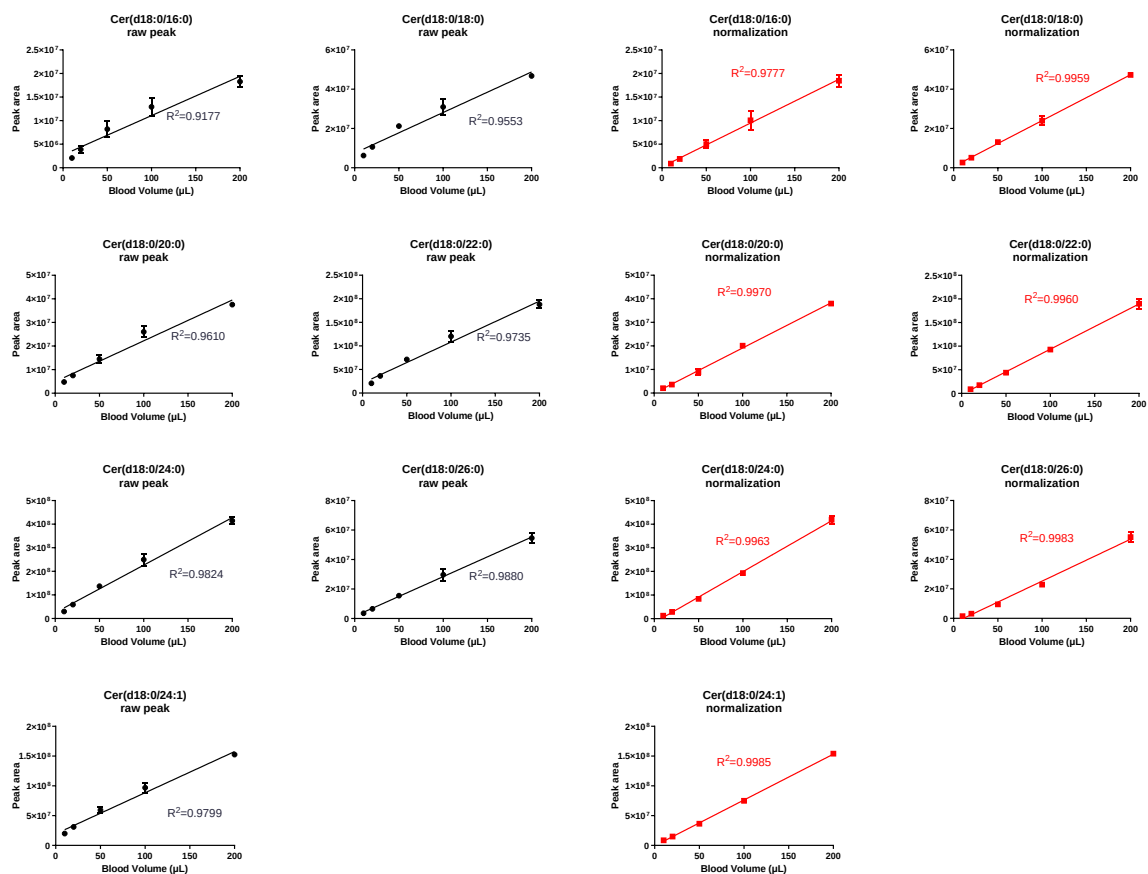


Figure S10. Comparison of the raw peak intensity or normalized intensity (internal standard 12:0 ceramide) of dihydroceramides, and their linear response vs different volume of blood. Linear regression was used with equal weighting and each point had three technical replicates. SD is showed on the graphs.

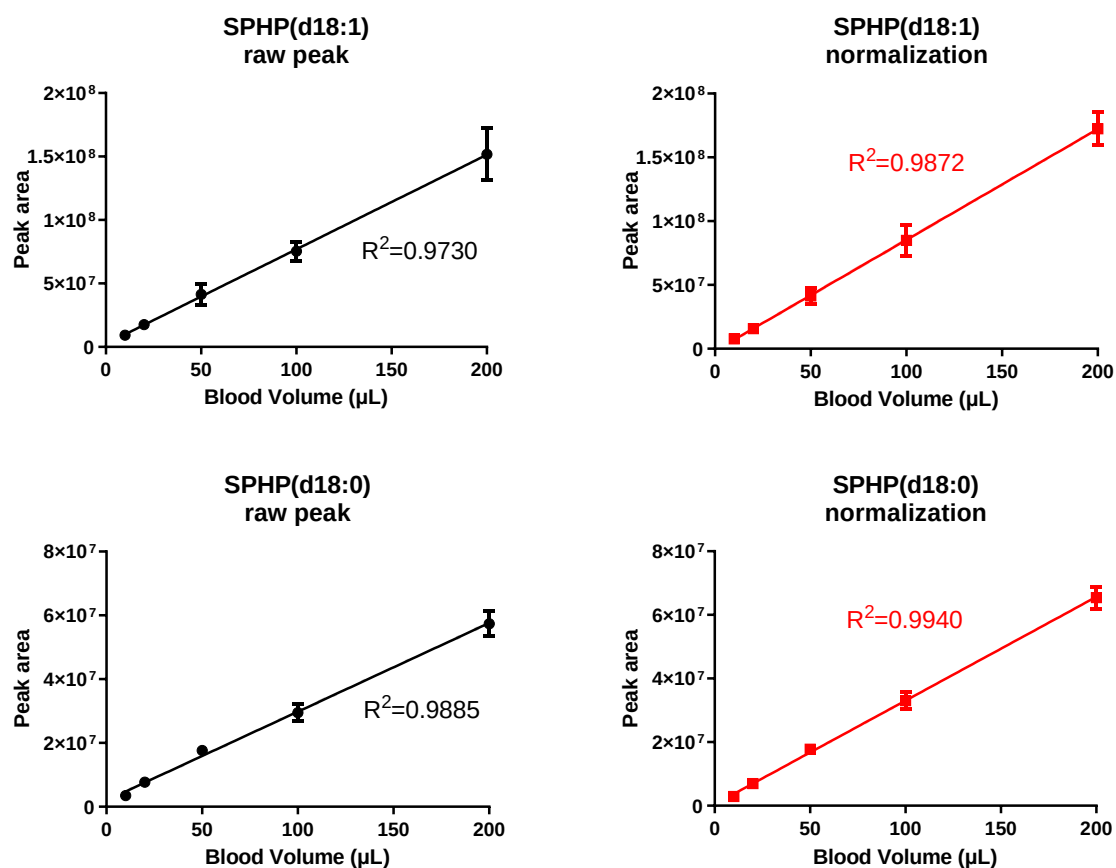


Figure S11. Comparison of the raw peak intensity or normalized intensity (internal standard C17 base SPHP) of SPHP, and their linear response vs different volume of blood. Linear regression was used with equal weighting and each point had three technical replicates. SD is showed on the graphs.

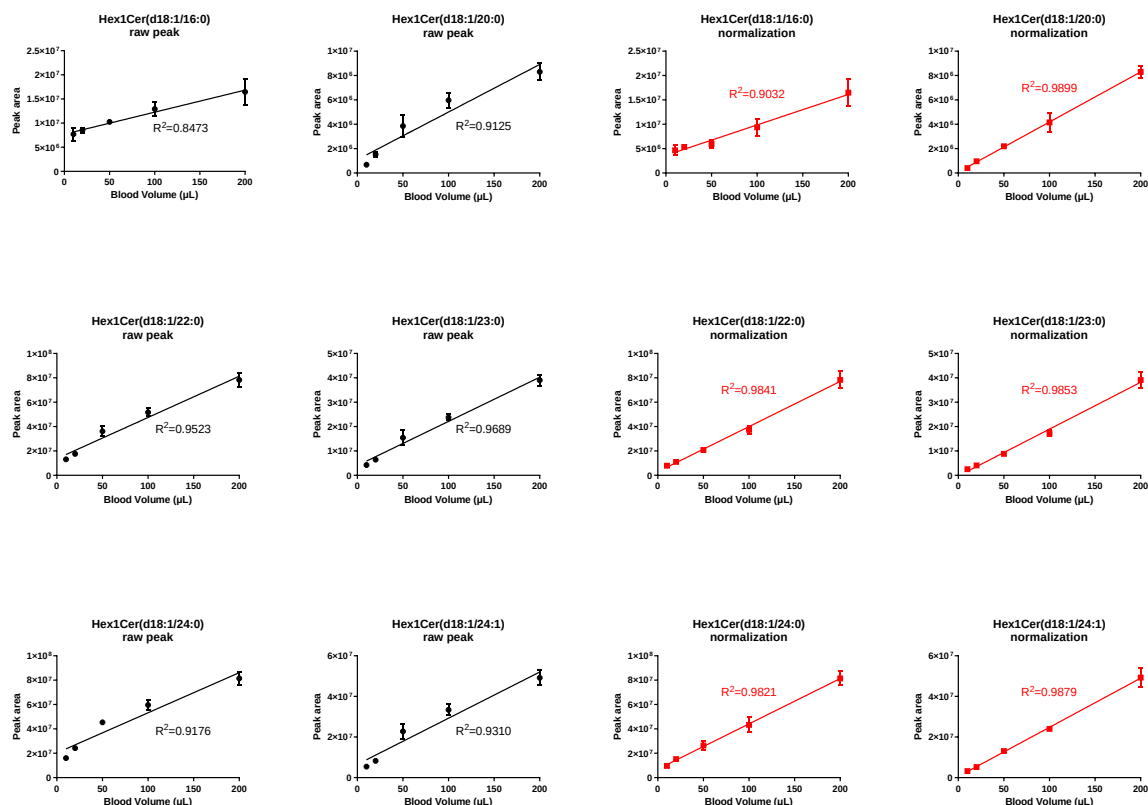


Figure S12. Comparison of the raw peak intensity or normalized intensity (internal standard C15 Hex1Cer-D7) of Hex1Cer, and their linear response vs different volume of blood. Linear regression was used with equal weighting and each point had three technical replicates. SD is showed on the graphs.

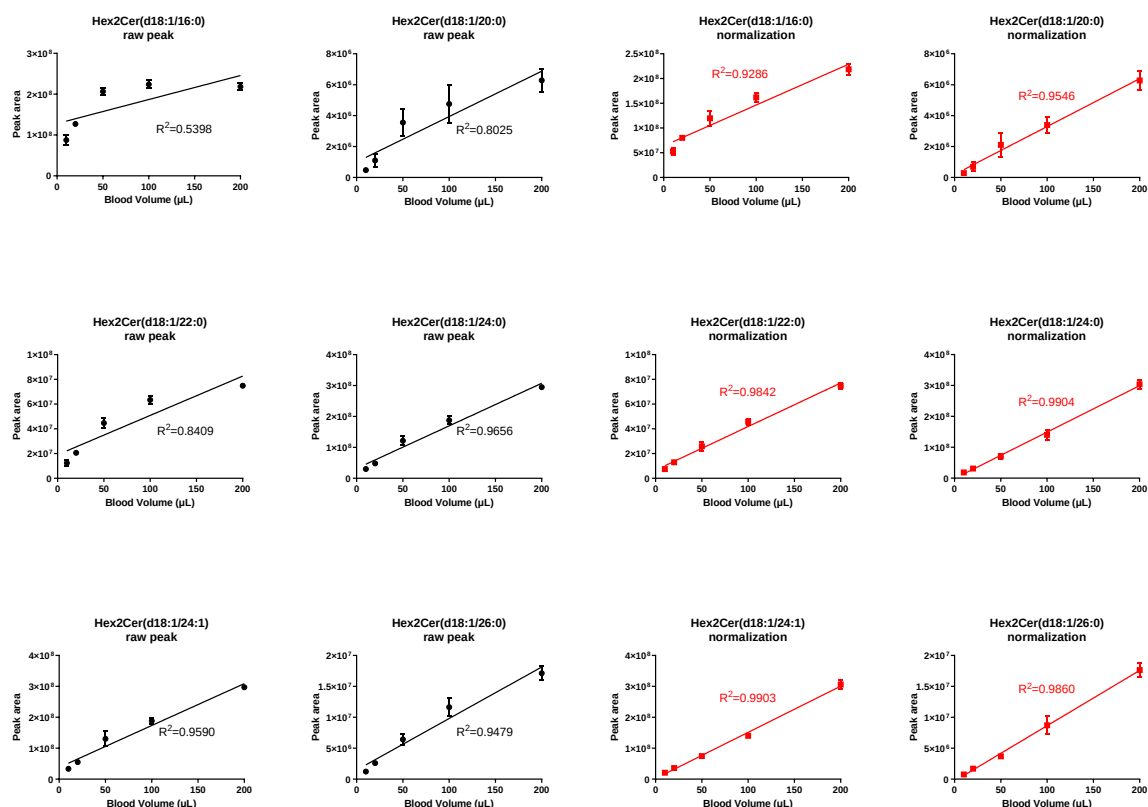


Figure S13. Comparison of the raw peak intensity or normalized intensity (internal standard C15 Hex1Cer-D7) of Hex2Cer, and their linear response vs different volume of blood. Linear regression was used with equal weighting and each point had three technical replicates. SD is showed on the graphs.

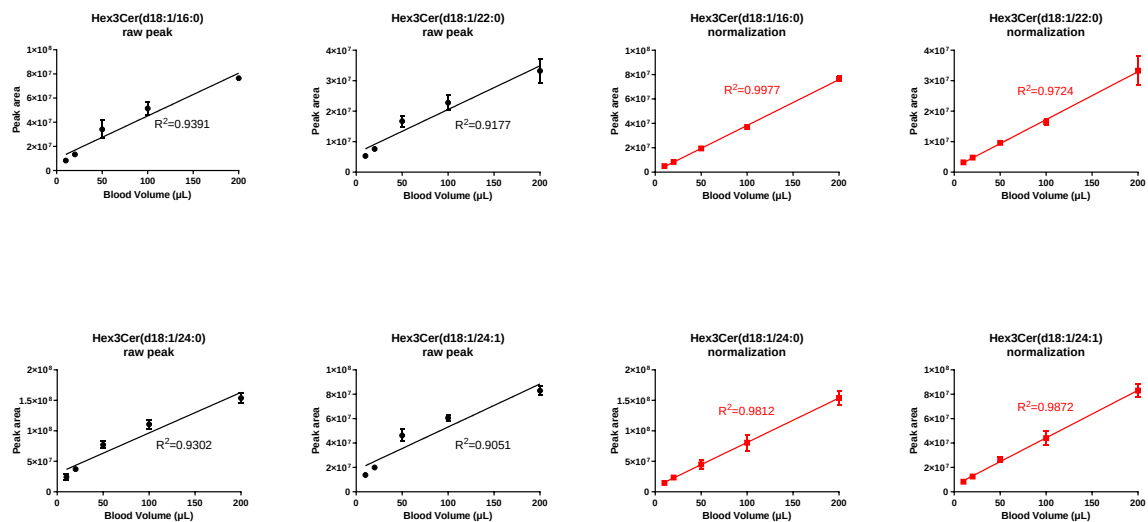


Figure S14. Comparison of the raw peak intensity or normalized intensity (internal standard C15 Hex1Cer-D7) of Hex3Cer, and their linear response vs different volume of blood. Linear regression was used with equal weighting and each point had three technical replicates. SD is showed on the graphs.

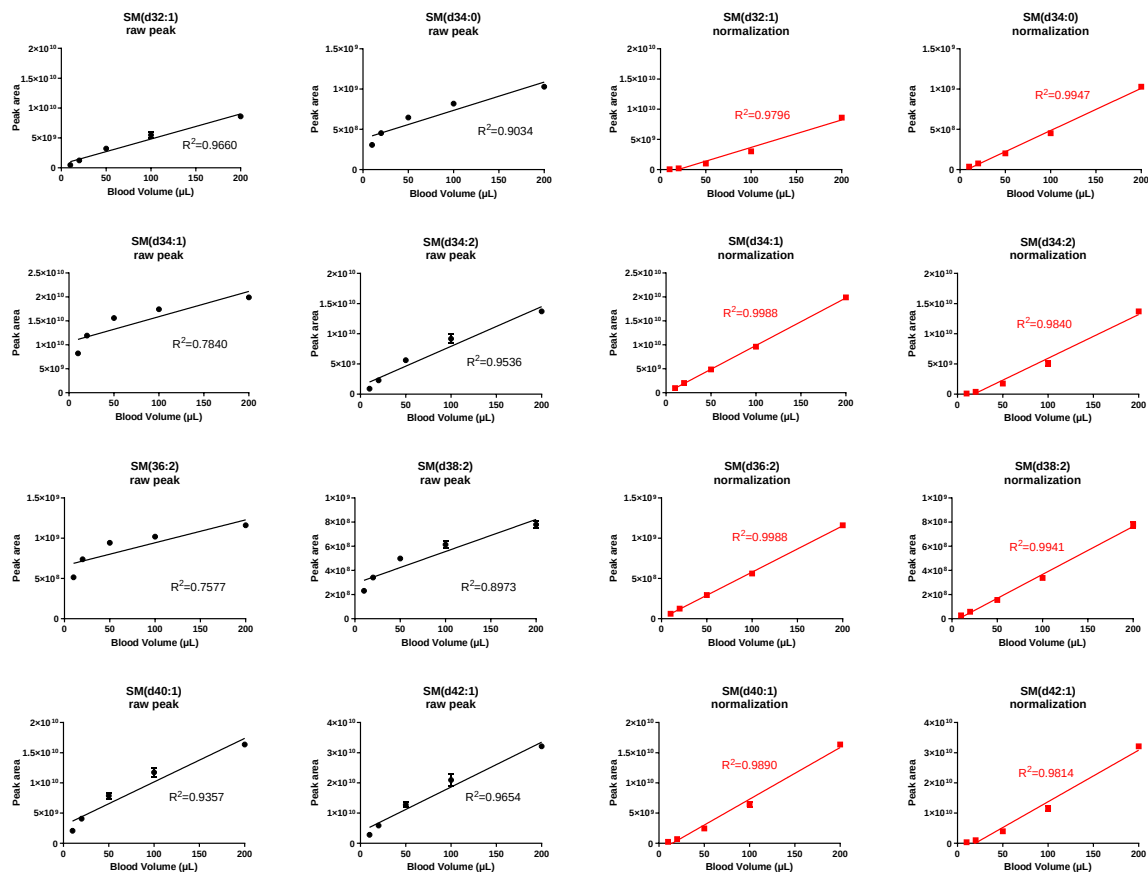


Figure S15. Comparison of the raw peak intensity or normalized intensity (internal standard 18:1 d7 SM) of SM, and their linear response vs different volume of blood. Linear regression was used with equal weighting and each point had three technical replicates. SD is showed on the graphs.

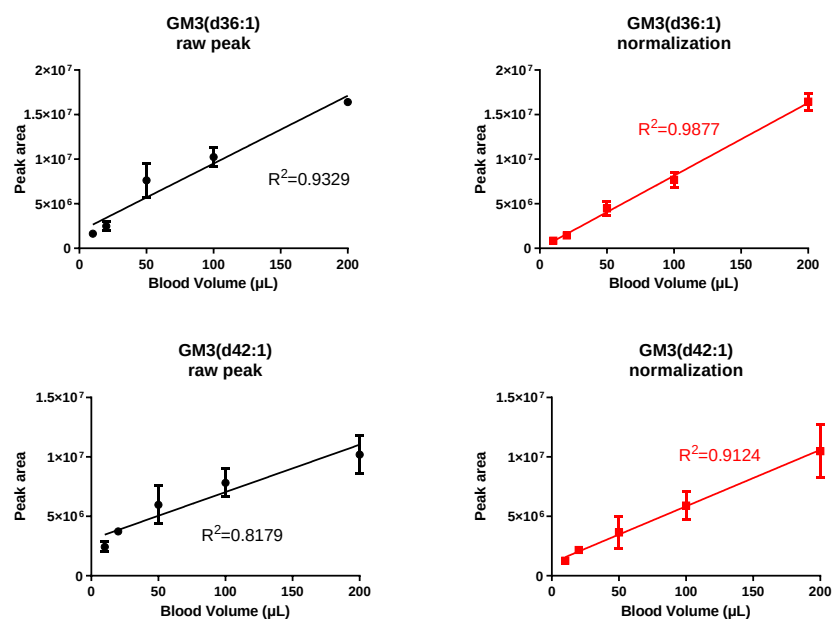


Figure S16. Comparison of the raw peak intensity or normalized intensity (internal standard C18:0 GM3-d5) of GM3, and their linear response vs different volume of blood. Linear regression was used with equal weighting and each point had three technical replicates. SD is showed on the graphs.

Table S1. Ceramide/Sphingoid Internal Standard Mixture I.

ISTD	Formula	m/z Positive	Adduct	Stock conc	Mol weight
C17 Sphingosine	C17H35NO2	286.274	M+H	12.5 μM	285.27
C17 Sphinganine	C17H37NO2	288.2897	M+H	12.5 μM	287.28
C17 Sphingosine-1-P	C17H36NO5P	366.2401	M+H	12.5 μM	365.23
C17 Sphinganine-1-P	C17H38NO5P	368.256	M+H	12.5 μM	367.25
C12 SM	C35H71N2O6P	647.5118	M+H	12.5 μM	646.51
C12 Ceramide	C30H59NO3	482.4571	M+H	12.5 μM	481.45
C12 Glucosyl Ceramide	C36H69NO8	644.509	M+H	12.5 μM	643.5
C12 Lactosyl Ceramide	C42H79NO13	806.5615	M+H	12.5 μM	805.56
C12 Ceramide-1-P	C30H60NO6P	562.4232	M+H	12.5 μM	561.42
C25 Ceramide	C43H85NO3	664.6602	M+H	12.5 μM	663.65

Table S2. Inclusion list for PRM experiments.

Lipidlon	RT	Monoisotopic mass	IonFormula	CalcMz	Scan start	Scan end
Cer(d18:0_16:0)	13.1	539.5277	C34H69O3N1	540.535	12.6	13.6
Cer(d18:0_18:0)	14	567.559	C36H73O3N1	568.5663	13.5	14.5
Cer(d18:0_20:0)	14.9	595.5903	C38H77O3N1	596.5976	14.4	15.4
Cer(d18:0_22:0)	15.7	623.6216	C40H81O3N1	624.6289	15.2	16.2
Cer(d18:0_23:0)	16.1	637.6373	C41H83O3N1	638.6446	15.6	16.6
Cer(d18:0_24:0)	16.5	651.6529	C42H85O3N1	652.6602	16	17
Cer(d18:0_24:1)	15.5	649.6373	C42H83O3N1	650.6446	15	16
Cer(d18:0_24:2)	14.8	647.6216	C42H81O3N1	648.6289	14.3	15.3
Cer(d18:0_25:0)	16.9	665.6686	C43H87O3N1	666.6759	16.4	17.4
Cer(d18:0_26:0)	17.2	679.6842	C44H89O3N1	680.6915	16.7	17.7
Cer(d18:0_26:2)	15.6	675.6529	C44H85O3N1	676.6602	15.1	16.1
Cer(d18:1_16:0)	12.7	537.5121	C34H67O3N1	538.5194	12.2	13.2
Cer(d18:1_18:0)	13.7	565.5434	C36H71O3N1	566.5507	13.2	14.2
Cer(d18:1_20:0)	14.5	593.5747	C38H75O3N1	594.582	14	15
Cer(d18:1_22:0)	15.4	621.606	C40H79O3N1	622.6133	14.9	15.9
Cer(d18:1_22:1)	14.3	619.5903	C40H77O3N1	620.5976	13.8	14.8
Cer(d18:1_23:0)	15.8	635.6216	C41H81O3N1	636.6289	15.3	16.3
Cer(d18:1_23:1)	14.7	633.606	C41H79O3N1	634.6133	14.2	15.2
Cer(d18:1_24:0)	16.2	649.6373	C42H83O3N1	650.6446	15.7	16.7
Cer(d18:1_24:0+O)	14.8	665.6322	C42H83O4N1	666.6395	14.3	15.3
Cer(d18:1_24:1)	15.2	647.6216	C42H81O3N1	648.6289	14.7	15.7
Cer(d18:1_24:2)/Cer(d18:2_24:1)	14.4	645.606	C42H79O3N1	646.6133	13.9	14.9
Cer(d18:1_25:1)	15.5	661.6373	C43H83O3N1	662.6646	15	16
Cer(d18:1_26:0)	16.9	677.6686	C44H87O3N1	678.6759	16.4	17.4
Cer(d18:1_26:1)	16	675.6529	C44H85O3N1	676.6602	15.5	16.5
Cer(d18:1_26:2)/Cer(d18:2_26:1)	15.2	673.6373	C44H84O3N1	674.6446	14.7	15.7
Cer(d18:2_20:0)	13.7	591.559	C38H73O3N1	592.5663	13.2	14.2

Cer(d18:2_22:0)	14.6	619.5903	C40H77O3N1	620.5976	14.1	15.1
Cer(d18:2_23:0)	15	633.606	C41H79O3N1	634.6133	14.5	15.5
Cer(d18:2_24:0)	15.4	647.6216	C42H81O3N1	648.6289	14.9	15.9
Cer(d18:2_25:0)	15.8	661.6373	C43H83O3N1	662.6446	15.3	16.3
Cer(d18:2_26:0)	16.1	675.6529	C44H85O3N1	676.6602	15.6	16.6
Cer(d19:1_22:0)	15.6	635.6216	C41H81O3N1	636.6289	15.1	16.1
Cer(d19:1_23:0)	15.9	649.6373	C42H83O3N1	650.6446	15.4	16.4
Cer(d19:1_24:0)	16.4	663.6529	C43H85O3N1	664.661	15.9	16.9
Cer(d19:1_24:1)	15.4	661.6373	C43H83O3N1	662.6446	14.9	15.9
Cer(d19:1_25:0)	16.7	677.6686	C44H87O3N1	678.6759	16.2	17.2
Cer(d20:1_24:0)	16.9	677.6686	C44H87O3N1	678.676	16.4	17.4
Cer(m18:0_22:0)	16.1	607.6267	C40H81O2N1	608.6341	15.6	16.6
Cer(m18:0_24:0)	16.9	635.658	C42H85O2N1	636.6654	16.4	17.4
Cer(m18:0_24:1)	15.9	633.6424	C42H83O2N1	634.6497	15.4	16.4
Cer(m18:0_26:0)	17.6	663.6893	C44H89O2N1	664.6968	17.1	18.1
Cer(m18:1_22:0)	15.3	605.6111	C40H79O2N1	606.618	14.8	15.8
Cer(m18:1_24:0)	16.1	633.6424	C42H83O2N1	634.6498	15.6	16.6
Cer(m18:1_24:0+O)	15.5	649.6373	C42H83O3N1	650.6446	15	16
Cer(m18:1_26:0)	16.7	661.6737	C44H87O2N1	662.6812	16.2	17.2
Cer(m19:0_24:0)	17	649.6737	C43H87O2N1	650.6812	16.5	17.5
Cer(t18:0_16:0)	12.3	555.5227	C34H69O4N1	556.5302	11.8	12.8
Cer(t18:0_22:0)	15	639.6166	C40H81O4N1	640.6238	14.5	15.5
Cer(t18:0_23:0)	15.4	653.6322	C41H83O4N1	654.6395	14.9	15.9
Cer(t18:0_24:0)	15.8	667.6479	C42H85O4N1	668.6551	15.3	16.3
Cer(t18:0_26:0)	16.5	695.6792	C44H89O4N1	696.6864	16	17
CerP(d18:1_16:0)	11.1	617.4784	C34H68O6N1P1	618.4857	10.6	11.6
CerP(d18:1_22:0)	13.7	701.5723	C40H80O6N1P1	702.5796	13.2	14.2
CerP(d18:1_24:0)	14.5	729.6036	C42H84N1O6P1	730.6109	14	15
CerP(d18:1_24:1)	13.5	727.5879	C42H82N1O6P1	728.5952	13	14

GD3(d34:1)	10.2	1443.809	C68H121O29N3	1442.801	9.7	10.7
GM1(d40:1)	12.8	1601.939	C77H139O31N3	1600.932	12.3	13.3
GM1(d42:1)	13.5	1629.971	C79H143O31N3	1628.961	13	14
GM1(d42:2)	12.7	1627.955	C79H141O31N3	1626.947	12.2	13.2
GM3(d32:1)	9.4	1124.682	C55H100O21N2	1125.69	8.9	9.9
GM3(d34:1)	10.4	1152.713	C57H104O21N2	1153.721	9.9	10.9
GM3(d34:1+O)	10.2	1168.708	C57H104O22N2	1169.716	9.7	10.7
GM3(d34:2)	9.5	1150.698	C57H102O21N2	1151.705	9	10
GM3(d36:1)	11.4	1180.745	C59H108O21N2	1181.753	10.9	11.9
GM3(d36:2)	10.6	1178.729	C61H106O21N2	1179.737	10.1	11.1
GM3(d40:1)	12.9	1236.807	C63H116O21N2	1237.815	12.4	13.4
GM3(d40:2)	12.3	1234.791	C63H114O21N2	1235.799	11.8	12.8
GM3(d42:1)	13.6	1264.838	C65H120O21N2	1265.848	13.1	14.1
GM3(d42:2)	12.8	1262.823	C65H118O21N2	1263.831	12.3	13.3
Hex1Cer(d18:1_16:0)	12	699.5649	C40H77O8N1	700.5722	11.5	12.5
Hex1Cer(d18:1_16:0+O)	10.4	715.5598	C40H77O9N1	716.5671	9.9	10.9
Hex1Cer(d18:1_18:0)	12.9	727.5962	C42H81O8N1	728.6035	12.4	13.4
Hex1Cer(d18:1_20:0)	13.7	755.6275	C44H85O8N1	756.6346	13.2	14.2
Hex1Cer(d18:1_22:0)	14.6	783.6588	C46H89O8N1	784.6661	14.1	15.1
Hex1Cer(d18:1_24:0)	15.4	811.6901	C48H93O8N1	812.6974	14.9	15.9
Hex1Cer(d18:1_24:1)	14.4	809.6745	C48H91O8N1	810.6817	13.9	14.9
Hex1Cer(d18:1_24:2)/Hex1Cer(d18:2/24:1)	13.7	807.6588	C48H89O8N1	808.6661	13.2	14.2
Hex1Cer(d18:2_24:0)	14.6	809.6745	C48H91O8N1	810.6818	14.1	15.1
Hex2Cer(d18:0_16:0)	12	863.6334	C46H89O13N1	864.6406	11.5	12.5
Hex2Cer(d18:1_16:0)	11.6	861.6177	C46H87O13N1	862.625	11.1	12.1
Hex2Cer(d18:1_18:0)	12.6	889.649	C48H91O13N1	890.6563	12.1	13.1
Hex2Cer(d18:1_20:0)	13.4	917.6803	C50H95O13N1	918.6876	12.9	13.9
Hex2Cer(d18:1_22:0)	14.2	945.7116	C52H99O13N1	946.7189	13.7	14.7
Hex2Cer(d18:1_24:0)	15	973.7429	C54H103O13N1	974.7502	14.5	15.5

Hex2Cer(d18:1_24:1)	14.1	971.7273	C54H101O13N1	972.7346	13.6	14.6
Hex2Cer(d18:1_26:0)	15.8	1001.774	C56H107O13N1	1002.783	15.3	16.3
Hex2Cer(d18:1_26:1)	14.8	999.7586	C56H105O13N1	1000.766	14.3	15.3
Hex2Cer(d18:2_16:0)	10.7	859.6021	C46H85O13N1	860.6094	10.2	11.2
Hex3Cer(d18:1_16:0)	11.4	1023.671	C52H97O18N1	1024.679	10.9	11.9
Hex3Cer(d18:1_18:0)	12.3	1051.702	C54H101O18N1	1052.71	11.8	12.8
Hex3Cer(d18:1_22:0)	14	1107.765	C58H109O18N1	1108.773	13.5	14.5
Hex3Cer(d18:1_24:0)	14.8	1135.796	C60H113O18N1	1136.803	14.3	15.3
Hex3Cer(d18:1_24:1)	13.8	1133.78	C60H111O18N1	1134.789	13.3	14.3
Hex3Cer(d18:1_26:0)	15.5	1163.827	C62H117O18N1	1164.835	15	16
SPH(d18:1)	3.4	299.2824	C18H37O2N1	300.2895	2.9	3.9
SPH(d18:0)	3.9	301.2981	C18H39O2N1	302.3054	3.4	4.4
SPHP(d18:0)	3.2	381.2644	C18H40O5N1P1	382.2717	2.7	3.7
SPHP(d18:1)	3.1	379.2488	C18H38O5N1P1	380.256	2.6	3.6
SPHP(d18:2)	1.5	377.2331	C18H36O5N1P1	378.2404	1	2
SM(d30:0)	9.9	648.5206	C35H73O6N2P1	649.5291	9.4	10.4
SM(d18:1_12:0)	9.4	646.505	C35H71O6N2P1	647.5124	8.9	9.9
SM(d30:2)	8.2	644.4893	C35H69O6N2P1	645.4967	7.7	8.7
SM(d31:1)	10	660.5206	C36H73O6N2P1	661.5283	9.5	10.5
SM(d32:0)	11.1	676.5519	C37H77O6N2P1	677.5598	10.6	11.6
SM(d32:1)	10.6	674.5363	C37H75O6N2P1	675.544	10.1	11.1
SM(d32:2)	9.6	672.5206	C37H73O6N2P1	673.5279	9.1	10.1
SM(d33:2)	10.3	688.5519	C38H77O6N2P1	689.5596	9.8	10.8
SM(d18:0_16:0)	12.2	704.5832	C39H81O6N2P1	705.5904	11.7	12.7
SM(d18:1_16:0)	11.7	702.5676	C39H79O6N2P1	703.5751	11.2	12.2
SM(d18:1_16:1)	10.8	700.5519	C39H77O6N2P1	701.5594	10.3	11.3
SM(d34:4)	10.6	696.5206	C39H73O6N2P1	697.5257	10.1	11.1
SM(d35:4)	11.2	710.5363	C40H75O6N2P1	711.5414	10.7	11.7
SM(d36:0)	13.2	732.6145	C41H85O6N2P1	733.6198	12.7	13.7

SM(d36:1)	12.8	730.5989	C41H83O6N2P1	731.6066	12.3	13.3
SM(d18:1_18:1)	11.9	728.5832	C41H81O6N2P1	729.5905	11.4	12.4
SM(d18:1_18:2)	11.1	726.5676	C41H79O6N2P1	727.575	10.6	11.6
SM(d36:5)	10.8	722.5363	C41H75O6N2P1	723.5413	10.3	11.3
SM(d37:1)	13.3	744.6145	C42H85O6N2P1	745.6222	12.8	13.8
SM(d37:2)	12.4	742.5989	C42H83O6N2P1	743.6063	11.9	12.9
SM(d38:0)	14.2	760.6458	C43H89O6N2P1	761.6519	13.7	14.7
SM(d18:1_20:0)	13.7	758.6302	C43H87O6N2P1	759.6378	13.2	14.2
SM(d18:1_20:1)	12.9	756.6145	C43H85O6N2P1	757.6216	12.4	13.4
SM(d38:3)	12	754.5989	C43H83O6N2P1	755.606	11.5	12.5
SM(d20:0_18:4)	12.8	752.5832	C43H81O6N2P1	753.5884	12.3	13.3
SM(d38:5)	11.9	750.5676	C43H79O6N2P1	751.5726	11.4	12.4
SM(d38:6)	11.1	748.5519	C43H77O6N2P1	749.558	10.6	11.6
SM(d39:1)	14.2	772.6458	C44H89O6N2P1	773.6534	13.7	14.7
SM(d18:1_21:1)	13.4	770.6302	C44H87O6N2P1	771.6378	12.9	13.9
SM(d39:4)	13.3	766.5989	C44H83O6N2P1	767.6037	12.8	13.8
SM(d40:0)	15.1	788.6771	C45H93O6N2P1	789.6839	14.6	15.6
SM(d40:1)	14.6	786.6615	C45H91O6N2P1	787.669	14.1	15.1
SM(d18:1_22:1)	13.8	784.6458	C45H89O6N2P1	785.6531	13.3	14.3
SM(d40:3)	12.8	782.6302	C45H87O6N2P1	783.6377	12.3	13.3
SM(d20:0_20:4)	13.7	780.6145	C45H85O6N2P1	781.6196	13.2	14.2
SM(d40:7)	10.7	774.5676	C45H79O6N2P1	775.5751	10.2	11.2
SM(d41:1)	15.2	800.6771	C46H93O6N2P1	801.6846	14.7	15.7
SM(d18:1_23:1)	14.3	798.6615	C46H91O6N2P1	799.6692	13.8	14.8
SM(d41:2)	14	798.6615	C46H91O6N2P1	799.6689	13.5	14.5
SM(d41:3)	13.3	796.6458	C46H89O6N2P1	797.6531	12.8	13.8
SM(d18:2_23:2)	14.2	794.6302	C46H87O6N2P1	795.6367	13.7	14.7
SM(d18:1_24:0)	15.5	814.6928	C47H95O6N2P1	815.7003	15	16
SM(d18:1_24:1)	14.4	812.6771	C47H93O6N2P1	813.6842	13.9	14.9

SM(d18:1_24:2)	13.6	810.6615	C47H91O6N2P1	811.6687	13.1	14.1
SM(d18:2_24:2)	12.9	808.6458	C47H89O6N2P1	809.6507	12.4	13.4
SM(d18:1_24:4)	13.5	806.6302	C47H87O6N2P1	807.6356	13	14
SM(d43:1)	16.1	828.7084	C48H97O6N2P1	829.7162	15.6	16.6
SM(d43:2)	14.9	826.6928	C48H95O6N2P1	827.7	14.4	15.4
SM(d43:3)	14.1	824.6771	C48H93O6N2P1	825.6842	13.6	14.6
SM(d43:5)	14	820.6458	C48H89O6N2P1	821.6507	13.5	14.5
SM(d43:6)	13.3	818.6302	C48H87O6N2P1	819.6358	12.8	13.8
SM(d44:1)	16.6	842.7241	C49H99O6N2P1	843.7318	16.1	17.1
SM(d44:2)	15.4	840.7084	C49H97O6N2P1	841.7163	14.9	15.9
SM(d44:3)	14.5	838.6928	C49H95O6N2P1	839.7	14	15
SM(d44:4)	13.8	836.6771	C49H93O6N2P1	837.6845	13.3	14.3
SM(d44:7)	12.9	830.6302	C49H87O6N2P1	831.6359	12.4	13.4