Discovery of unexpected sphingolipids in almonds and pistachios with an innovative use of triple quadrupole tandem mass spectrometry

Supplementary information

Figure S1. High-resolution mass spectrometry analysis for the confirmation of the unexpected ceramide species in the extract of a pistachio cultivar. A1 (left) Molecular ion region of protonated A1 and (right) fragment ions spectrum (CE 30 eV). A2 (left) Molecular ion region of protonated A2 and (right) fragment ions spectrum (CE 30 eV).

Table S1 High-resolution measurement of fragment ion spectra of the four main ceramide species in two almond and two pistachio extracts[a].

Figure S2. High-resolution molecular (a, c, e) and fragment ion spectra (b, d, f) of triglycerides as ammonium adducts. (a, b) TG 54:5 (m/z 898.7771). (c,d) TG 54:4 (m/z 900.7925). (e,f) TG 54:3 (m/z 902.8055).

Figure S3. Chromatographic traces of the Par263 and Par264 scans for a representative pistachio extract.

Figure S4. Formation of protonated diglyceride and acylium fatty acid fragments from ammoniated triglycerides by Collision Activated Decomposition.

Figure S5. Plot of the relationship of chromatographic retention vs. molecular size for the series of ceramides with d18:1D4 sphingosine long-chain base (LCB) and saturated straight-chain even-Carbon fatty acids (FA).

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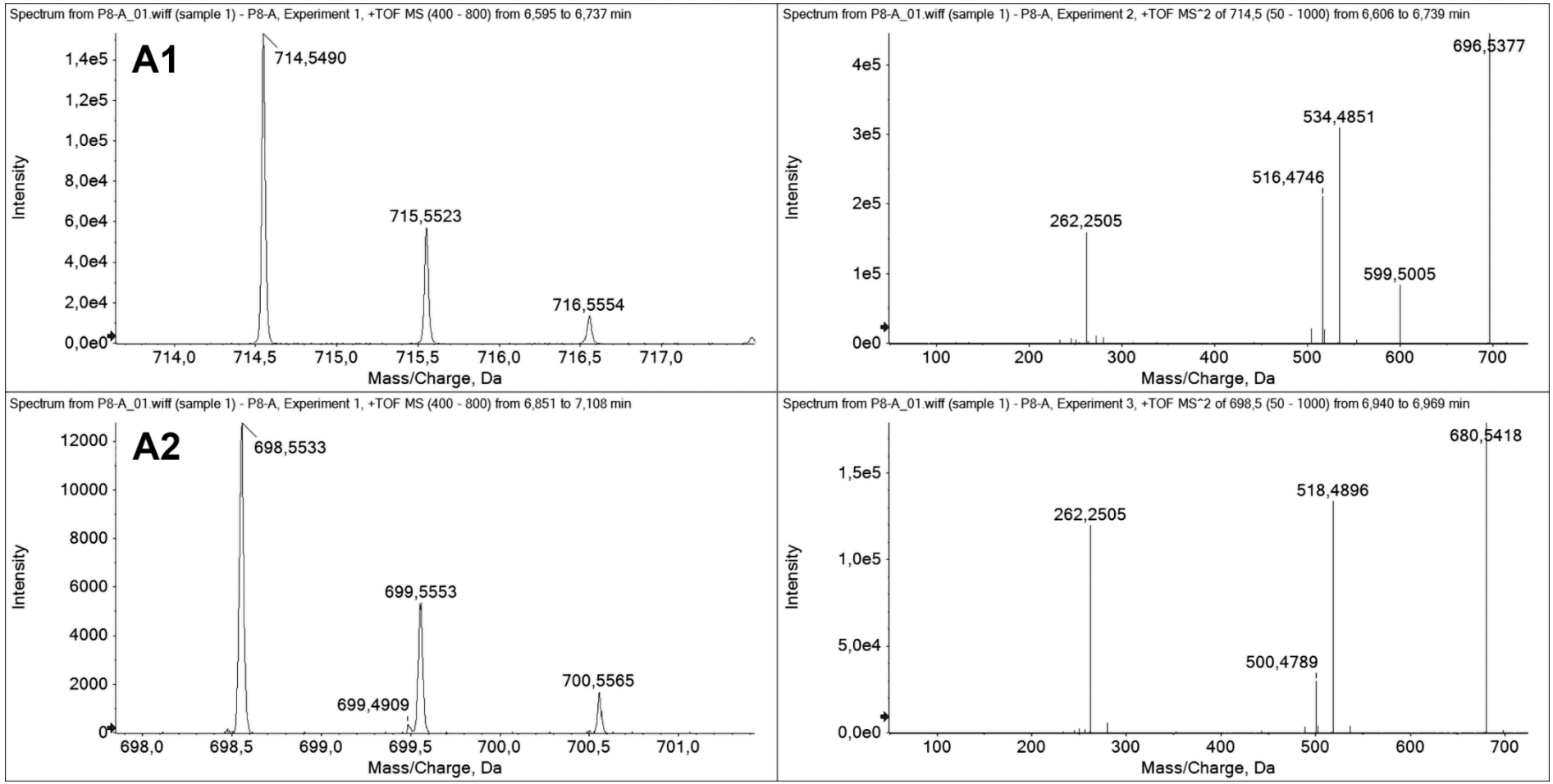


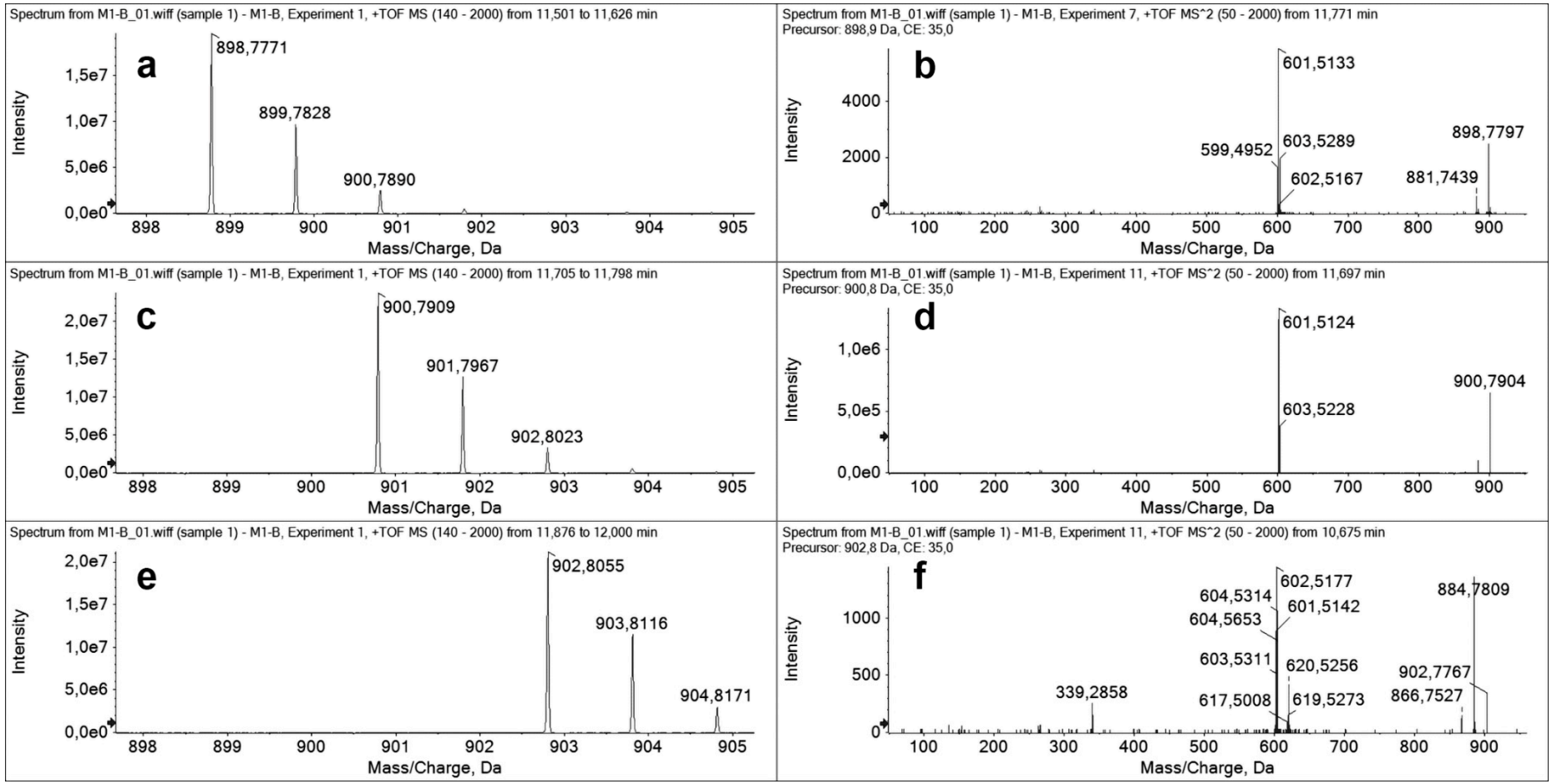
TOF-MS analysis (250 ms accumulation time, DP 50 eV) and TOF-MS/MS analysis (100 ms accumulation time, DP 50 eV, CE 30 eV).

Table S1. High-resolution measurement of fragment ion spectra of the four main ceramide species in two almond and two pistachio extracts.

cpd	*ion_id*	*calc for*	*mz*	*M1 mz*	*M2 mz*	*P8 mz*	*P9 mz*	*mean mz*	*M1 ppm*	*M2 ppm*	*P8 ppm*	*P9 ppm*
A1 [a]	*MH+*	*C40 H76 N1 O9*	***714.5520***	***714.5436***	***714.5464***	***714.5490***	***714.5450***	***714.5460***	*-11.8*	*-7.8*	*-4.2*	*-9.8*
A1	*-H2O*	*C40 H74 N1 O8*	***696.5414***	*696.5329*	*696.5359*	*696.5376*	*696.5347*	*696.5353*	*-12.3*	*-8.0*	*-5.5*	*-9.7*
A1	*-C6H12O6*	*C34 H64 N1 O3*	***534.4886***	*534.4824*	*534.4846*	*534.4821*	*534.4833*	*534.4831*	*-11.6*	*-7.5*	*-12.2*	*-10.0*
A1	*-H2O-C6H12O6*	*C34 H62 N1 O2*	***516.4781***		*516.4717*	*516.4746*	*516.4722*	*516.4728*		*-12.3*	*-6.7*	*-11.3*
A1	*-C6H12O6-H2CO*	*C33 H62 N1 O2*	***504.4781***	*504.4718*	*504.4771*	*504.4748*	*504.4726*	*504.4741*	*-12.4*	*-1.9*	*-6.5*	*-10.8*
A1	*O'' B18d:2 - 2 H2O*	*C18 H32 N1 O0*	***262.2535***	*262.2505*	*262.2519*	*262.2505*	*262.2504*	*262.2508*	*-11.3*	*-6.0*	*-11.3*	*-11.7*
A2 [a]	*MH+*	*C40 H76 N1 O8*	***698.5571***	***698.5485***	***698.5475***	***698.5535***	***698.5516***	*698.5503*	*-12.3*	*-13.7*	*-5.1*	*-7.9*
A2	*-H2O*	*C40 H74 N1 O7*	***680.5465***	*680.5381*	*680.5391*	*680.5420*	*680.5412*	*680.5401*	*-12.4*	*-10.9*	*-6.7*	*-7.8*
A2	*-C6H12O6*	*C34 H64 N1 O2*	***518.4937***	*518.4864*	*518.4881*	*518.4895*	*518.4889*	*518.4882*	*-14.1*	*-10.8*	*-8.1*	*-9.3*
A2	*-H2O-C6H12O6*	*C34 H62 N1 O1*	***500.4831***	*500.4767*	*500.4769*	*500.4788*	*500.4784*	*500.4777*	*-12.9*	*-12.5*	*-8.7*	*-9.5*
A2	*O' B18d:2 - H2O*	*C18 H34 N1 O1*	***280.2640***		*280.2605*			*280.2605*		*-12.6*		
A2	*O'' B18d:2 - 2 H2O*	*C18 H32 N1 O0*	***262.2535***	*262.2497*	*262.2510*	*262.2504*	*262.2508*	*262.2505*	*-14.4*	*-9.4*	*-11.7*	*-10.2*
Cer12: [a] ***0***	*MH+*	*C30 H60 N1 O3*	***482.4573***	*482.4513*	*482.4521*	*482.4540*	*482.4514*	*482.4522*	*-12.5*	*-10.8*	*-6.9*	*-12.3*
Cer12:0	*- H2O*	*C30 H58 N1 O2*	***464.4468***	*464.4411*	*464.4423*	*464.4426*	*464.4415*	*464.4419*	*-12.2*	*-9.6*	*-8.9*	*-11.3*
Cer12:0	*O' B18d:1 - H2O*	*C18 H36 N1 O1*	***282.2797***	*282.2760*	*282.2772*	*282.2766*	*282.2757*	*282.2764*	*-13.1*	*-8.8*	*-10.9*	*-14.1*
Cer12:0	*O'' B18d:1 - 2 H2O*	*C18 H34 N1 O0*	***264.2691***	*264.2662*	*264.2671*	*264.2659*	*264.2656*	*264.2662*	*-11.1*	*-7.7*	*-12.2*	*-13.3*
Cer12:0	*O''' B18d:1 - H2O -H2CO*	*C17 H34 N1 O0*	***252.2691***	*252.2658*	*252.2669*	*252.2665*	*252.2653*	*252.2661*	*-13.2*	*-8.8*	*-10.4*	*-15.2*
HexCer12:0 [a]	*MH+*	*C36 H70 N1 O8*	***644.5101***	*644.4935*	*644.5023*	*644.5071*	*644.5046*	*644.5019*	*-25.8*	*-12.2*	*-4.7*	*-8.6*
HexCer12:0	*MH+ -H2O*	*C36 H68 N1 O7*	***626.4996***	*626.4924*	*626.4945*	*626.4957*	*626.4927*	*626.4938*	*-11.5*	*-8.1*	*-6.2*	*-11.0*
HexCer12:0	*MH+ Cer12:0*	*C30 H60 N1 O3*	***482.4573***	*482.4502*				*482.4502*	*-14.8*			
HexCer12:0	*-C6H10O5*	*C30 H58 N1 O2*	***464.4468***	*464.4416*	*464.4431*	*464.4431*	*464.4414*	*464.4423*	*-11.1*	*-7.9*	*-7.9*	*-11.5*
HexCer12:0	*-C6H10O5 - H2O*	*C30 H56 N1 O1*	***446.4362***	*446.4313*	*446.4323*	*446.4325*	*446.4313*	*446.4319*	*-11.0*	*-8.7*	*-8.3*	*-11.0*
HexCer12:0	*MH+ -Glc - H2CO*	*C29 H56 N1 O1*	***434.4362***	*434.4306*				*434.4306*	*-12.9*			
HexCer12:0	*O' B18d:1 - H2O*	*C18 H36 N1 O1*	***282.2797***	*282.2756*		*282.2768*	*282.2753*	*282.2759*	*-14.5*		*-10.2*	*-15.6*
HexCer12:0	*O'' B18d:1 - 2 H2O*	*C18 H34 N1 O0*	***264.2691***	*264.2661*	*264.2671*	*264.2661*	*264.2656*	*264.2662*	*-11.4*	*-7.7*	*-11.4*	*-13.3*
HexCer12:0	*O''' B18d:1 - H2O -H2CO*	*C17 H34 N1 O0*	***252.2691***	*252.2649*			*252.2649*	*252.2649*	*-16.7*			*-16.7*

[a]**:** A1 and A2 are the natural components of the extracts; Cer12:0 and HexCer12:0 (Hex = Glucose) are the two added internal standards.

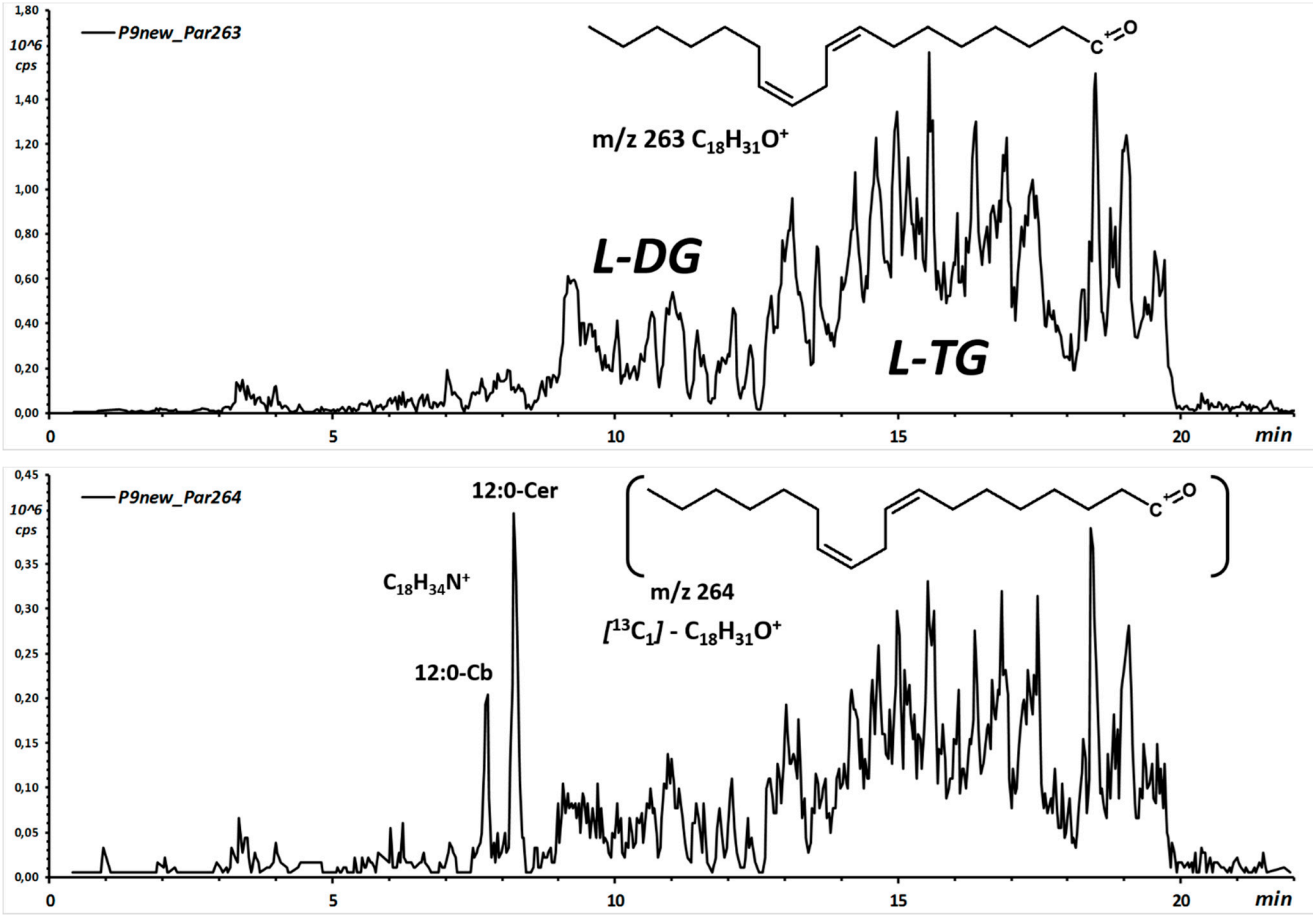
Figure S2. High-resolution molecular (a, c, e) and fragment ion spectra (b, d, f) corresponding to triglycerides as ammonium adducts. (a, b) TG 54:5 (m/z 898.7771). (c,d) TG 54:4 (m/z 900.7925). (e,f) TG 54:3 (m/z 902.8055).



TOF-MS analysis (250 ms accumulation time, DP 50 eV) and TOF-MS/MS analysis (100 ms accumulation time, DP 50 eV, CE 35 eV).

In the fragment ion spectrum of m/z 900, observed are the (protonated dehydrated) diglyceride fragments (*m/z 601* and 603, 3:1 ratio) due to the alternative competitive loss of oleic and linoleic acid as ammoniated neutral species.

Figure S3. Chromatographic traces of the Par263 and Par264 scans for a representative pistachio extract.



From 13 to 20 min the Par263 scan detects triglycerides that contain *all*-^{12}C linoleic acid (L-TG) by formal loss of the corresponding ammoniated fatty acid. In the same time frame, the Par264 scan detects triglycerides that contain $^{13}C_1$ linoleic acid isotopomers. As apparent from the values of the vertical scale, the signals are in an *approx.* 4:1 intensity ratio that is expected for the occurrence of the ^{13}C isotopomer in the isotope cluster of ammoniated triglyceride precursors that detach an ammoniated fatty acid.

Figure S4. General scheme of the formation of protonated diglyceride and acylium fatty acid fragments from ammoniated triglycerides by Collision Activated Decomposition.

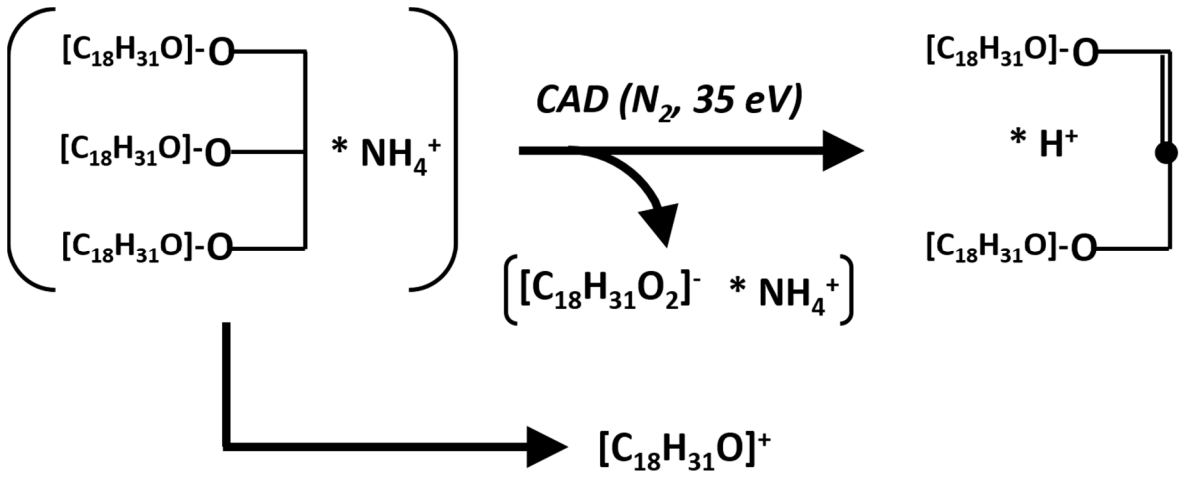
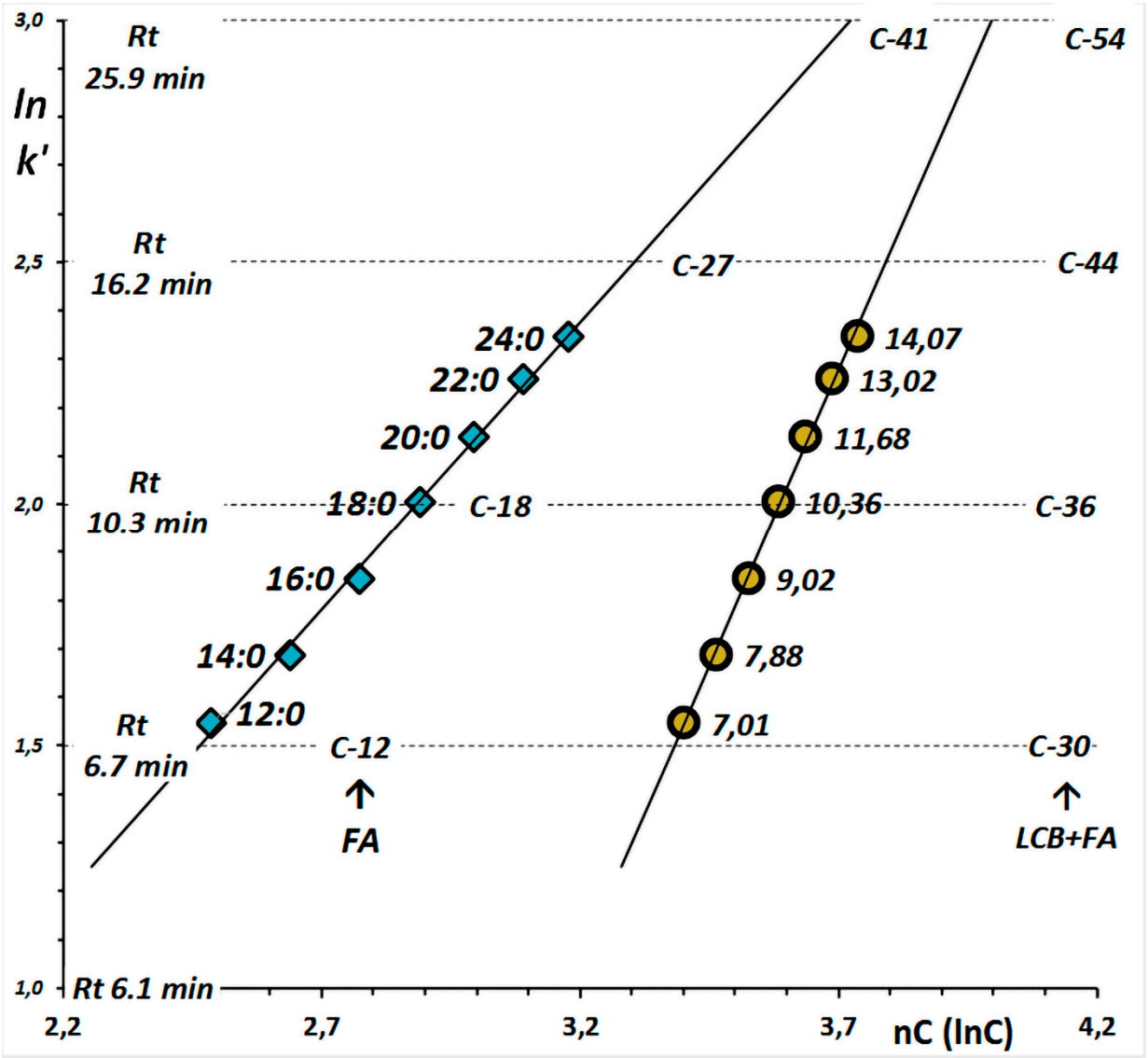


Figure S5. Plot of the relationship of chromatographic retention vs. molecular size for the series of ceramides with d18:1D4 sphingosine long-chain base (LCB) and saturated straight-chain even-Carbon fatty acids (FA).



Retention (y axis) is calculated as the capacity factor (k') and reported as its natural logarithm, ln(k'). Molecular size (x axis) is reported as the natural logarithm of both the sole number of Carbons in the FA (blue ◇), and the total number of Carbon in the ceramide (18 carbons of the LCB + n Carbons of the FA; yellow ○). On y axis are also reported for some ln(k') the corresponding retention time (min). Linear regression equations are y=1,1921x-1,4391 R^2=0,9969 and y=2,4496x-6,787 R^2=0,9974 for the number of fatty acid Carbon and for the total number of Carbons, respectively.