

## SUPPLEMENTARY INFORMATION

### **Retention dependences support highly confident identification of lipid species in human plasma by reversed-phase UHPLC/MS**

Zuzana Vaňková, Ondřej Peterka, Michaela Chocholoušková, Denise Wolrab, Robert Jirásko, Michal Holčapek\*

*University of Pardubice, Faculty of Chemical Technology, Department of Analytical*

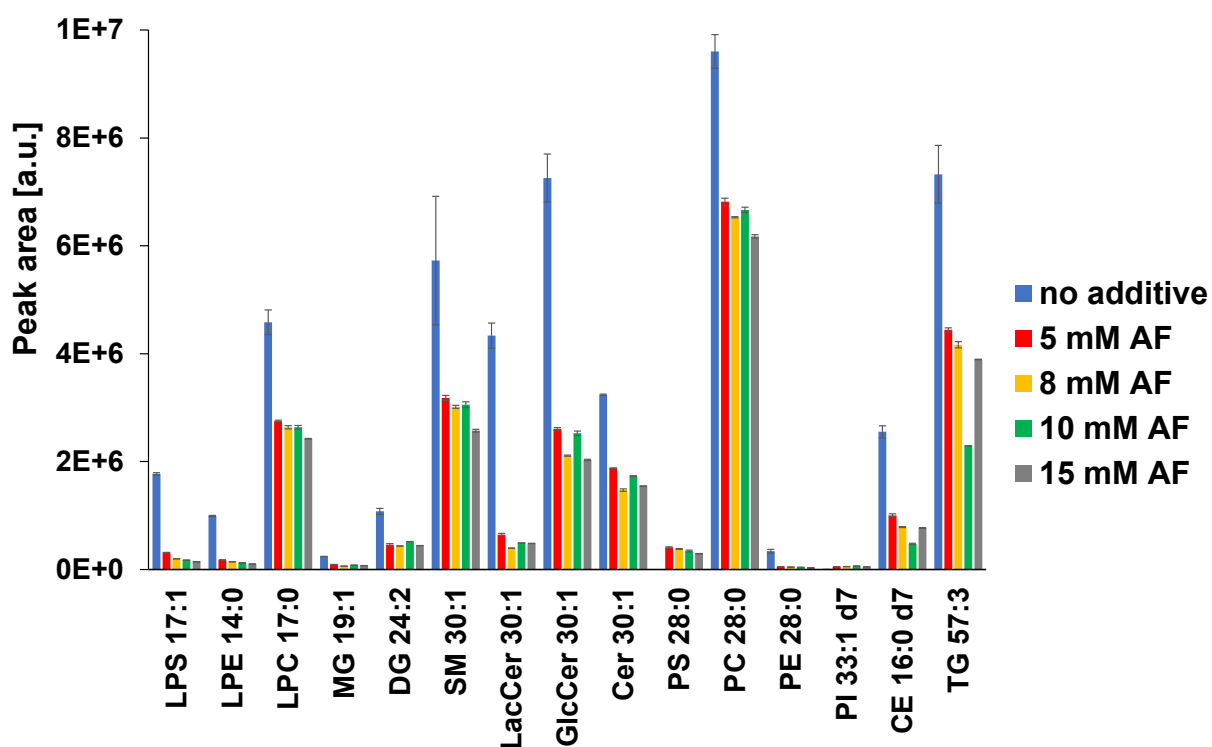
*Chemistry Studentská 573, 53210 Pardubice, Czech Republic*

\* **Corresponding author:** Michal Holčapek, Tel.: +420466037087; Fax: +420466037068;

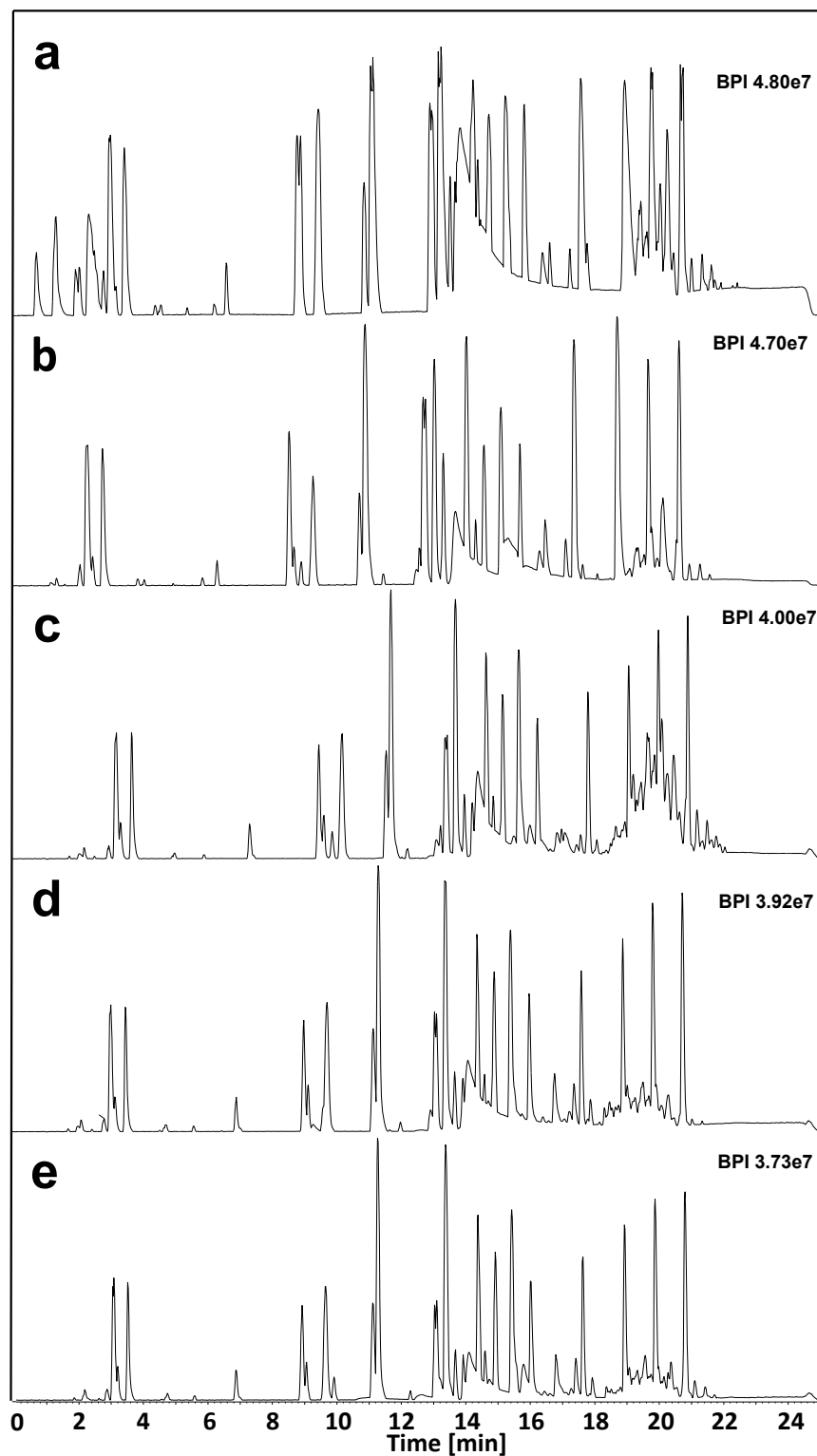
Email: Michal.Holcapek@upce.cz

**Figures: 7**

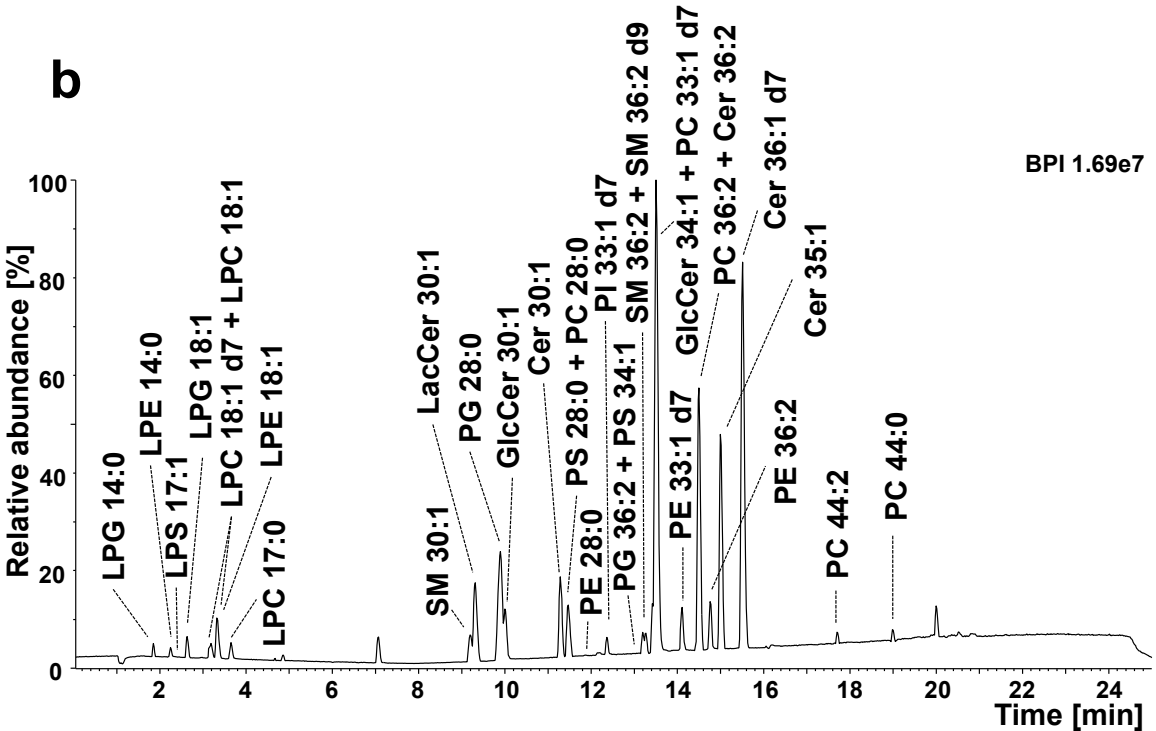
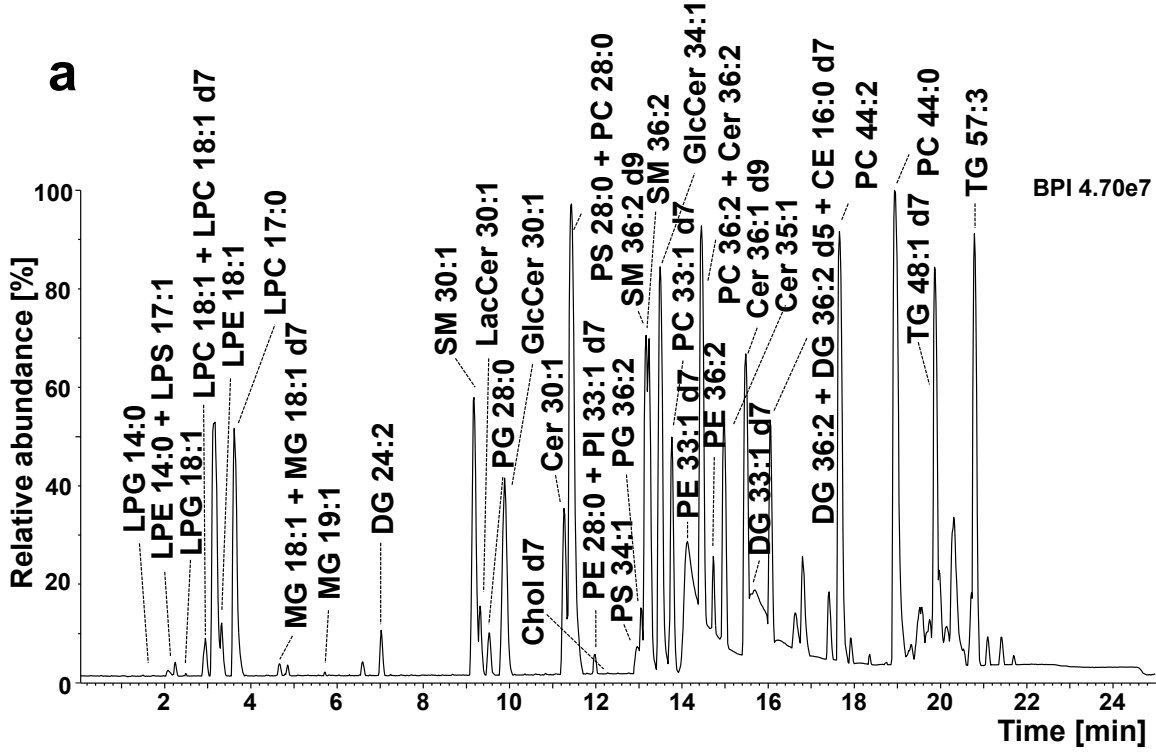
**Fig. S-1** Comparison of peak areas *vs.* molar concentrations of ammonium formate (AF) for some selected deuterated and short fatty acyl chain lipid standards, whereas other standards follow the same trends. PI 33:1 d7 and PS 28:0 are not ionized in the mobile phase without additives. Data are presented as the mean value  $\pm$  standard deviation (SD) from three measurements.



**Fig. S-2** Influence of molar concentration of ammonium formate (AF) on RP-UHPLC separation of lipid standards: **(a)** 0 mM of AF, **(b)** 5 mM AF, **(c)** 8 mM AF, **(d)** 10 mM AF, and **(e)** 15 mM AF in mobile phase. The conditions of analysis are reported in Materials and methods.

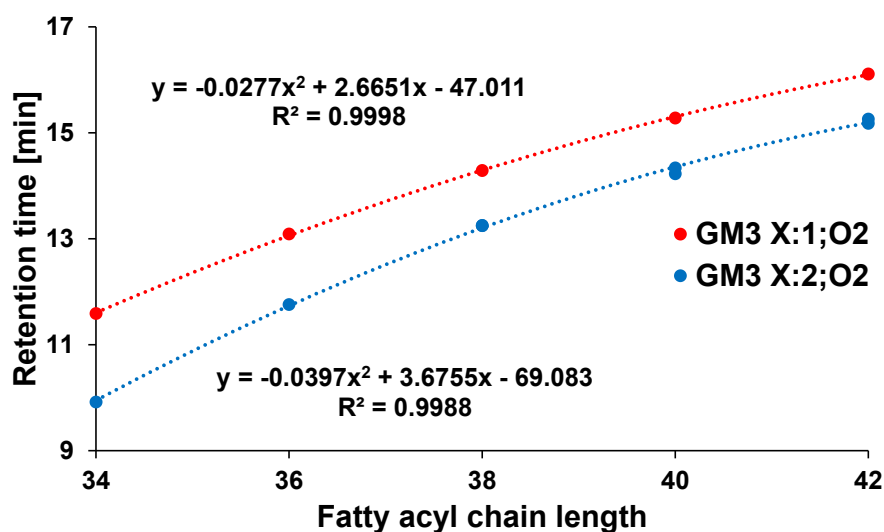


**Fig. S-3** Base peak chromatograms of the lipid standard mixture measured by RP-UHPLC/MS in: (a) positive-ion mode and (b) negative-ion mode. The conditions of analysis are reported in Materials and methods.



**Fig. S-4** Retention behavior of various lipid species within lipid class shows the polynomial dependences of retention times on the length of fatty acyl chains: (a) GM3 X:1;O2 and GM3 X:2;O2, (b) PC X:3 and PC X:4, (c) TG X:1 and TG X:3, where X represents carbon number and number of present double bonds is written behind colon.

**a**



**b**

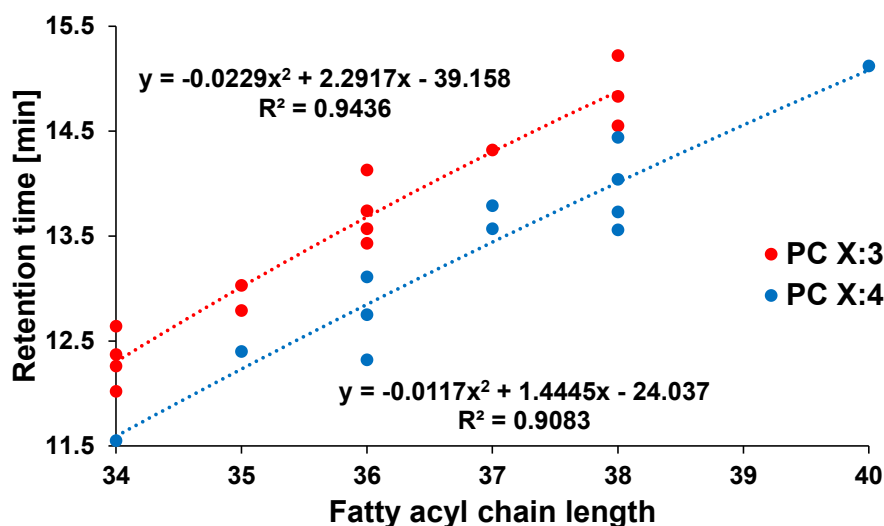
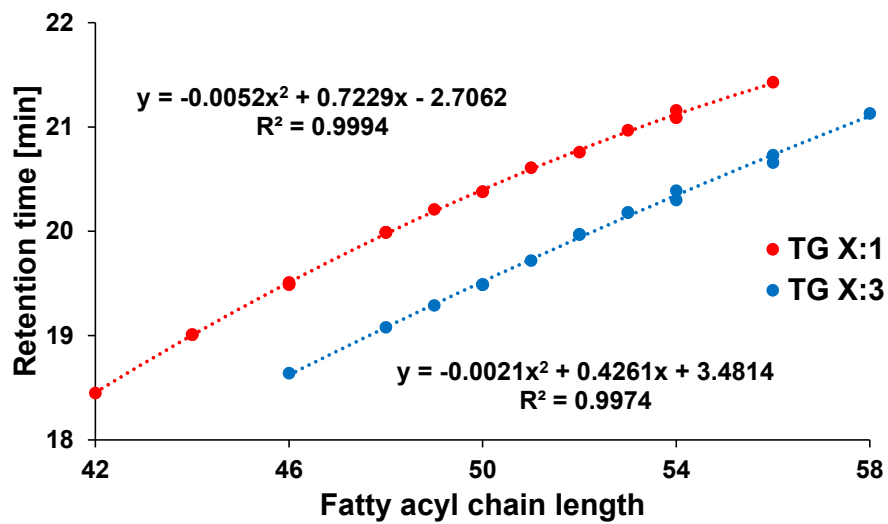


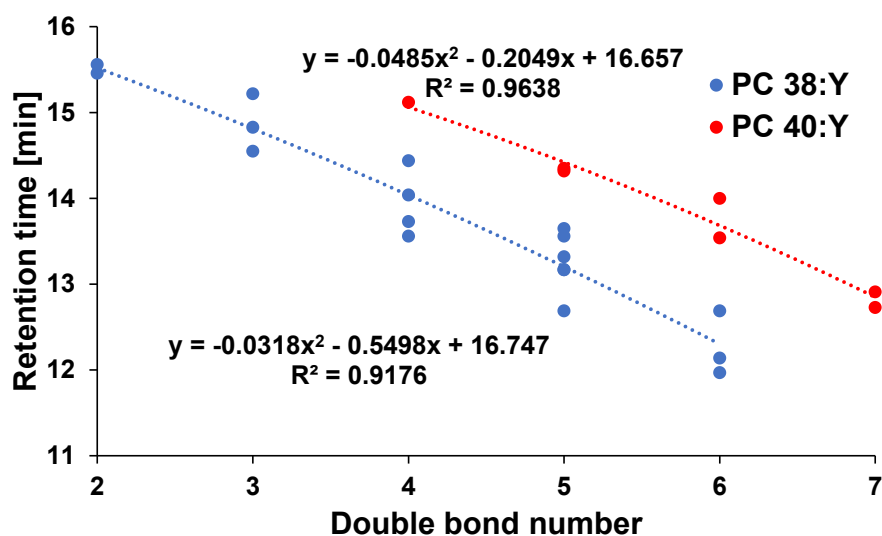
Fig. S-4 (Continuation)

**C**



**Fig. S-5** Retention behavior of various lipid species within lipid class shows the polynomial dependences of retention times on the number of double bonds: **(a)** PC 38:Y and PC 40:Y, **(b)** PE 36:Y, PE 38:Y, and PE 40:Y, **(c)** PI 36:Y and PI 38:Y, **(d)** CE 18:Y and CE 20:Y, **(e)** TG 46:X, TG 48:X, and TG 49:X, **(f)** TG 50:Y and TG 51:Y, **(g)** TG 56:Y, where Y represents number of double bond(s) and carbon number is written before colon.

**a**



**b**

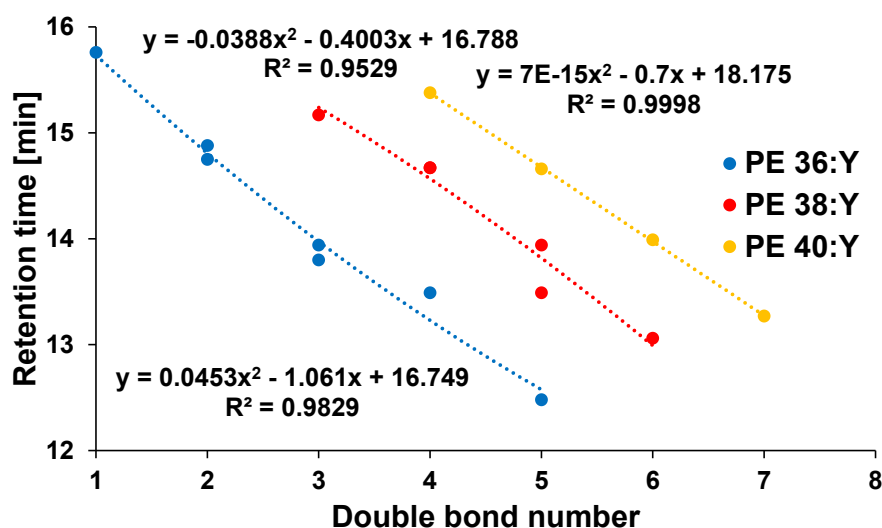
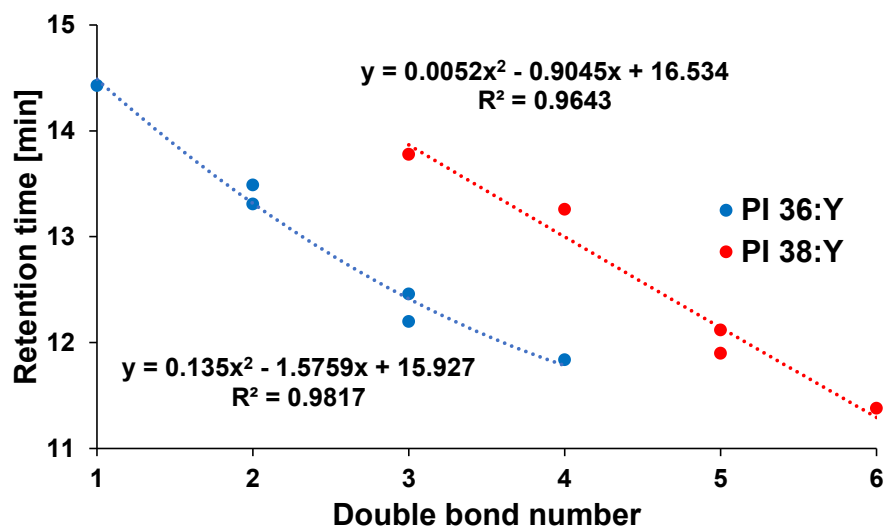


Fig. S-5 (Continuation)

**C**



**d**

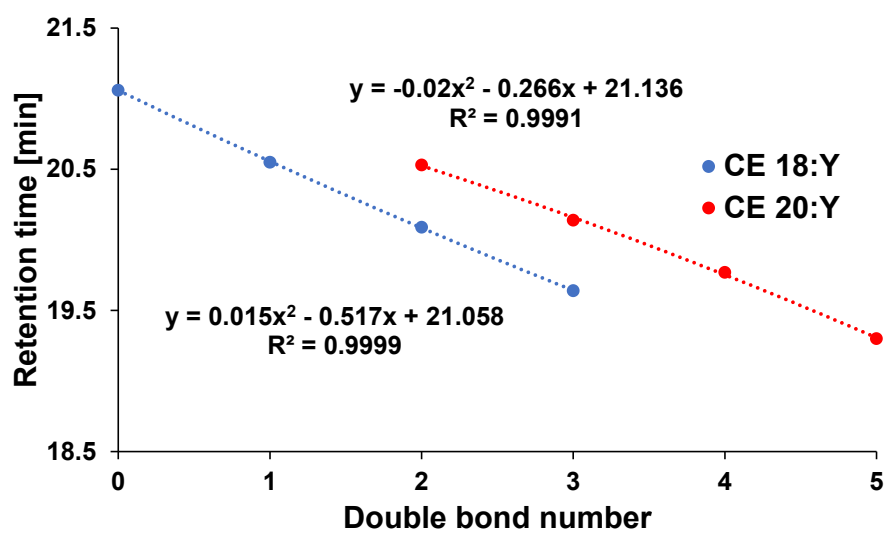
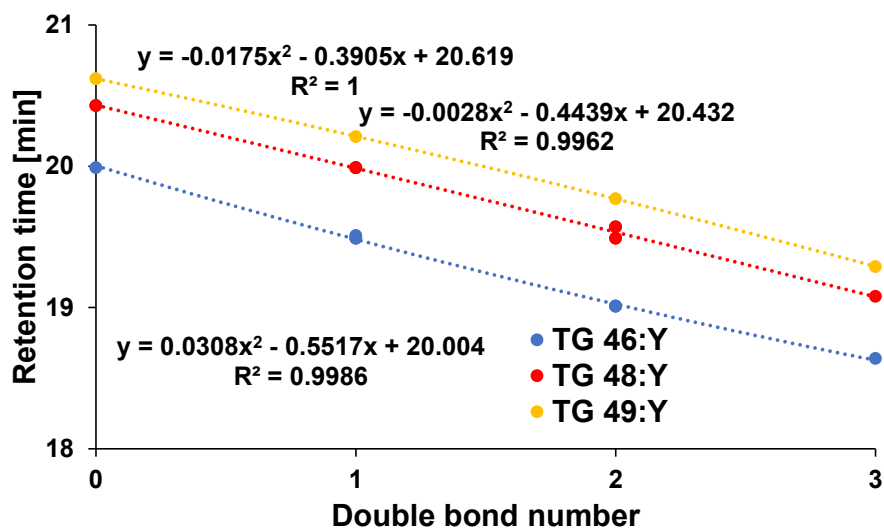


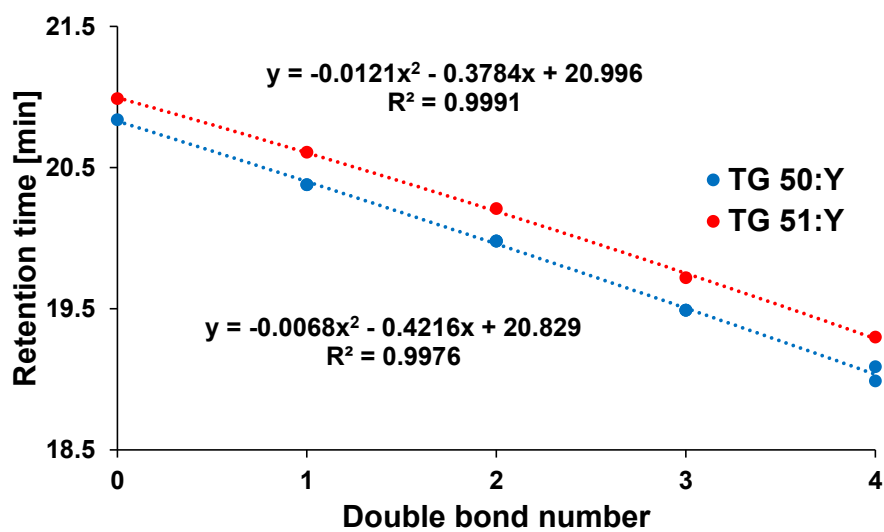


Fig. S-5 (Continuation)

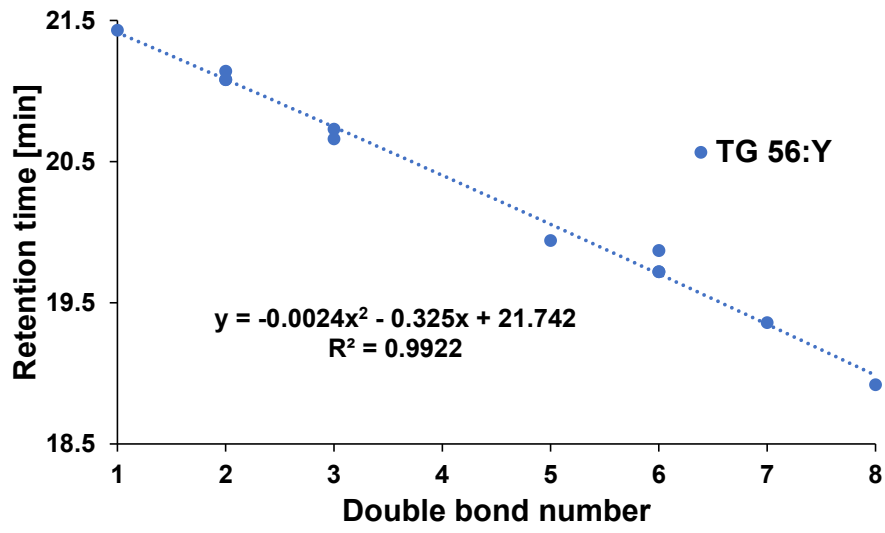
e



f



9



**Fig. S-6** Retention behavior of various lipid species bonding the fatty acyl on the defined *sn*-position or containing the characteristic sphingoid backbone shows the polynomial dependences of retention times on the length of other fatty acyl chains: (a) LPC 0:0/X:1, (b) PC X:0/20:3 and PC X:0/20:4, (c) Cer 18:1;O2/X:0 and Cer 18:2;O2/X:0, (d) Cer 17:1;O2/X:0 and Cer 19:1;O2/X:0, (e) HexCer 18:1;O2/X:0, Hex2Cer 18:1;O2/X:0, and Hex3Cer 18:1;O2/X:0, (f) SM 18:1;O2/X:0 and SM 18:2;O2/X:0, where X represents carbon number and number of present double bonds is written behind colon.

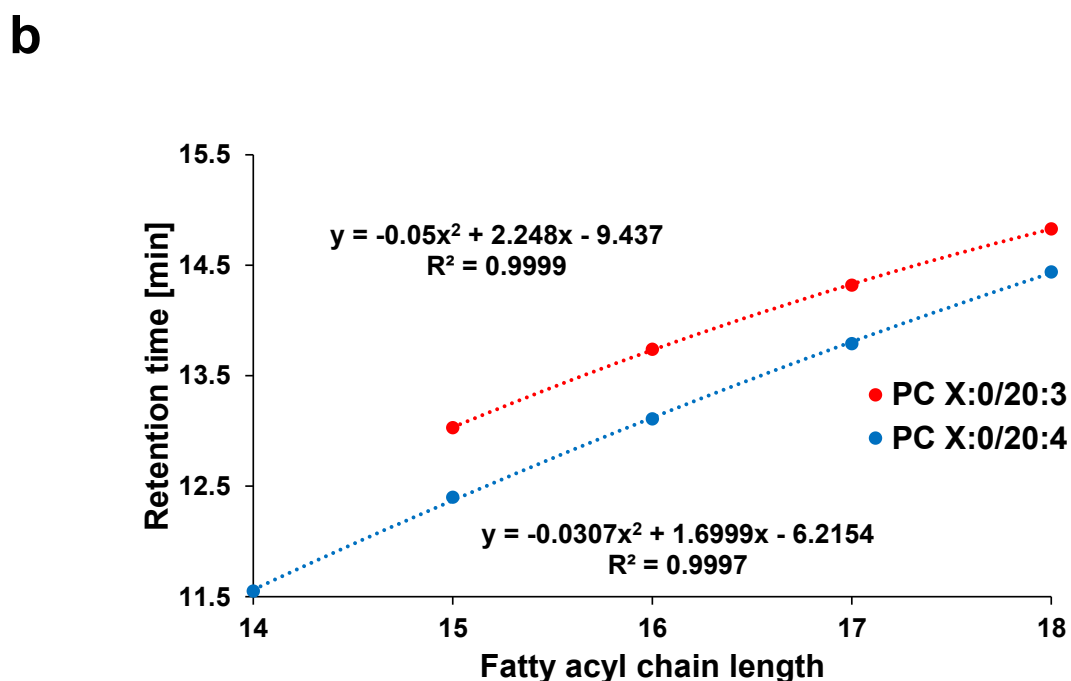
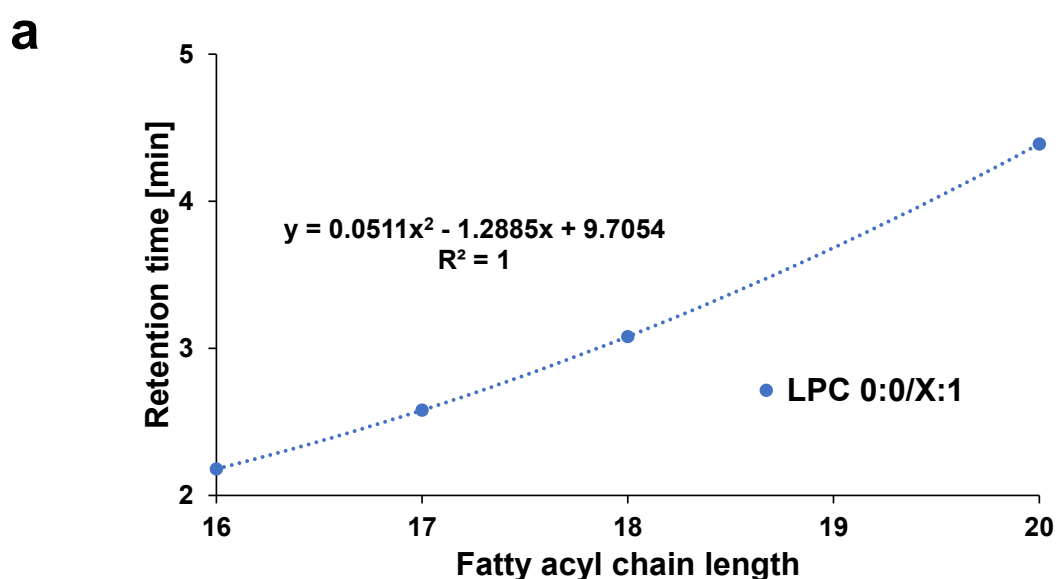
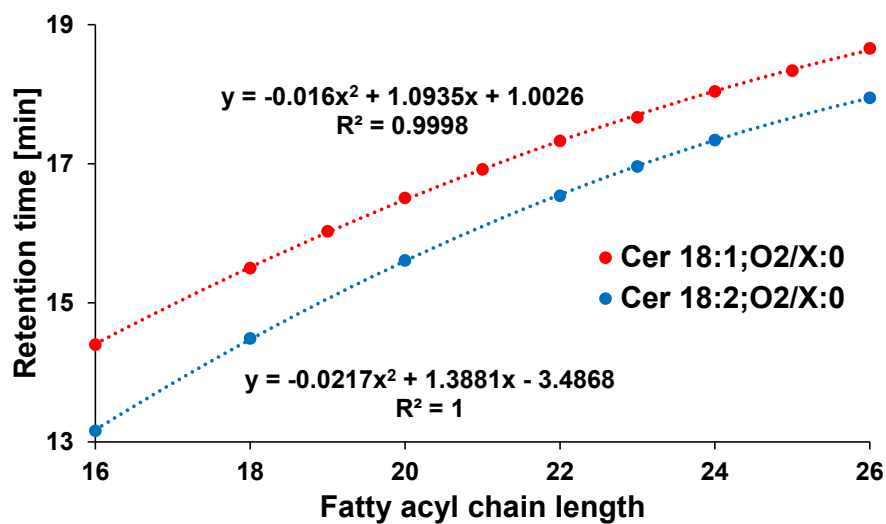


Fig. S-6 (Continuation)

**C**



**d**

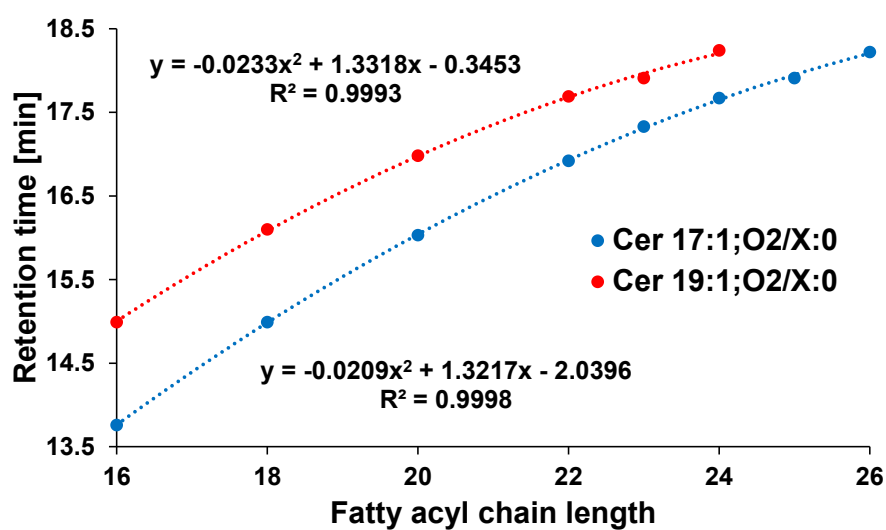
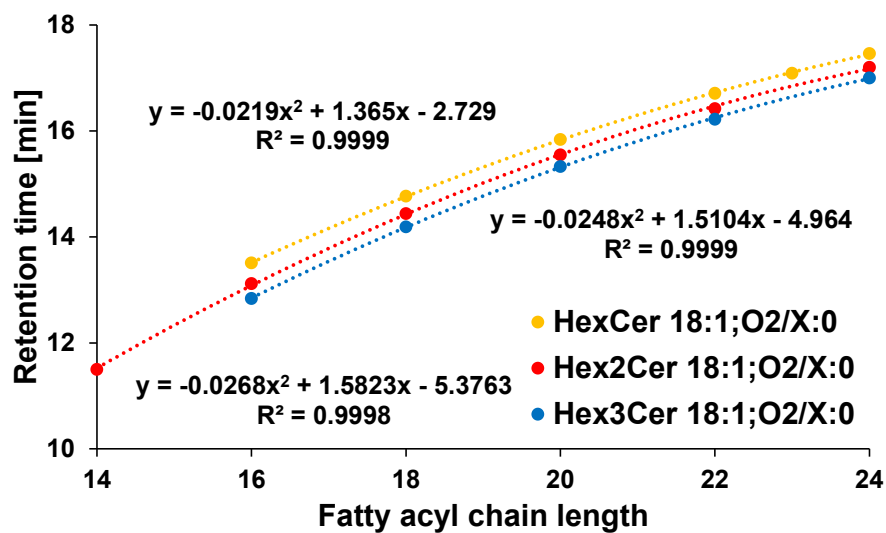
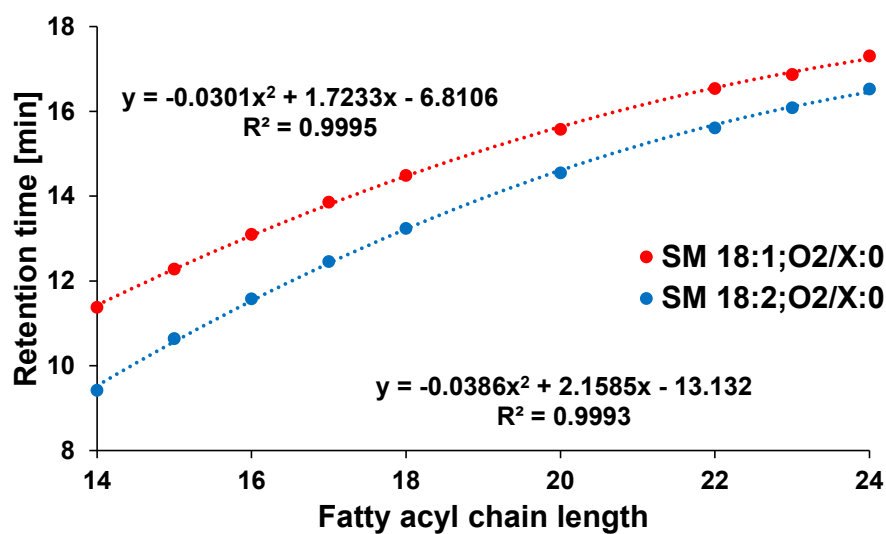


Fig. S-6 (Continuation)

**e**

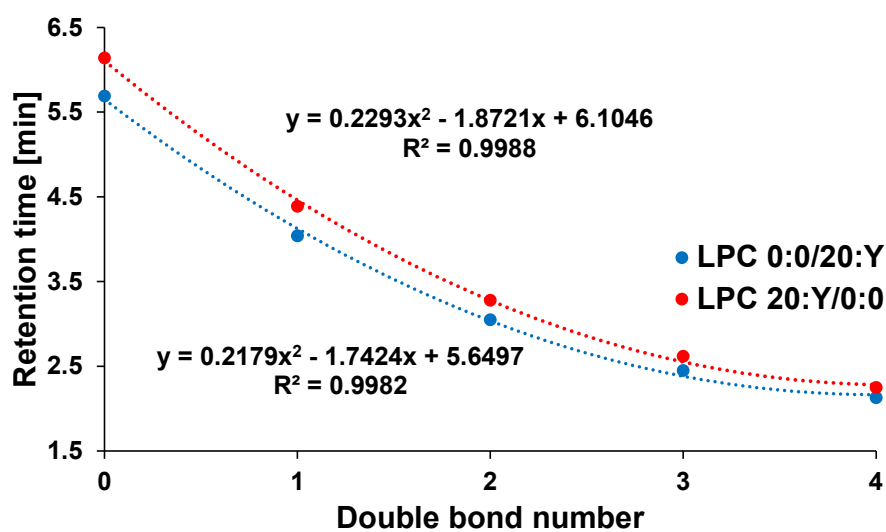


**f**



**Fig. S-7** Retention behavior of various lipid species bonding the fatty acyl on the defined *sn*-position shows the polynomial dependences of retention times on the number of double bonds: (a) LPC 0:0/20:Y and LPC 20:Y/0:0, (b) PC 16:0/20:Y and PC 18:0/20:Y, where Y represents number of double bond(s) and carbon number is written before colon.

**a**



**b**

