SUPPORTING INFORMATION

Characterization of Triacylglycerol Enantiomers Using Chiral HPLC/APCI-MS and Synthesis of Enantiomeric Triacylglycerols

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Experimental conditions, figures: 11, tables: 2

EXPERIMENTAL CONDITIONS

Preparation of TG Fraction from Plasma Sample

0.5 mL of plasma sample was homogenized with 10 mL of chloroform - methanol (2:1, v/v) mixture and the homogenate was filtered using a coarse filter paper. Then, 2 mL of 1 mol/L NaCl was added and the mixture was centrifuged at 3000 rpm for 5 min at room temperature. The chloroform (bottom) layer containing lipids was evaporated by a gentle stream of nitrogen and redissolved in 2-propanol - water (1:1, v/v) mixture for the HILIC analysis. The fractionation of total lipid extract into lipid classes was carried out on Spherisorb Si column (250 x 4.6 mm, 5 μm, Waters), a flow rate of 1 mL/min, an injection volume of 10 μL, separation temperature of 40°C and the mobile phase gradient: 0 min - 94% A + 6% B, 60 min - 77% A + 23% B, where A is acetonitrile and B is 5 mM aqueous ammonium acetate[52].

Preparation of Hazelnut Oil

10 g of seeds were carefully crushed in a mortar to yield fine particles. Then, 15 mL of hexane was added and this mixture was stirred occasionally for 15 min. Solid particles were filtered out using a coarse filter paper and the extract was filtered again using the fine filter (0.45 μm). From the filtered extract, hexane was evaporated using the gentle stream of nitrogen to yield the pure hazelnut oil.

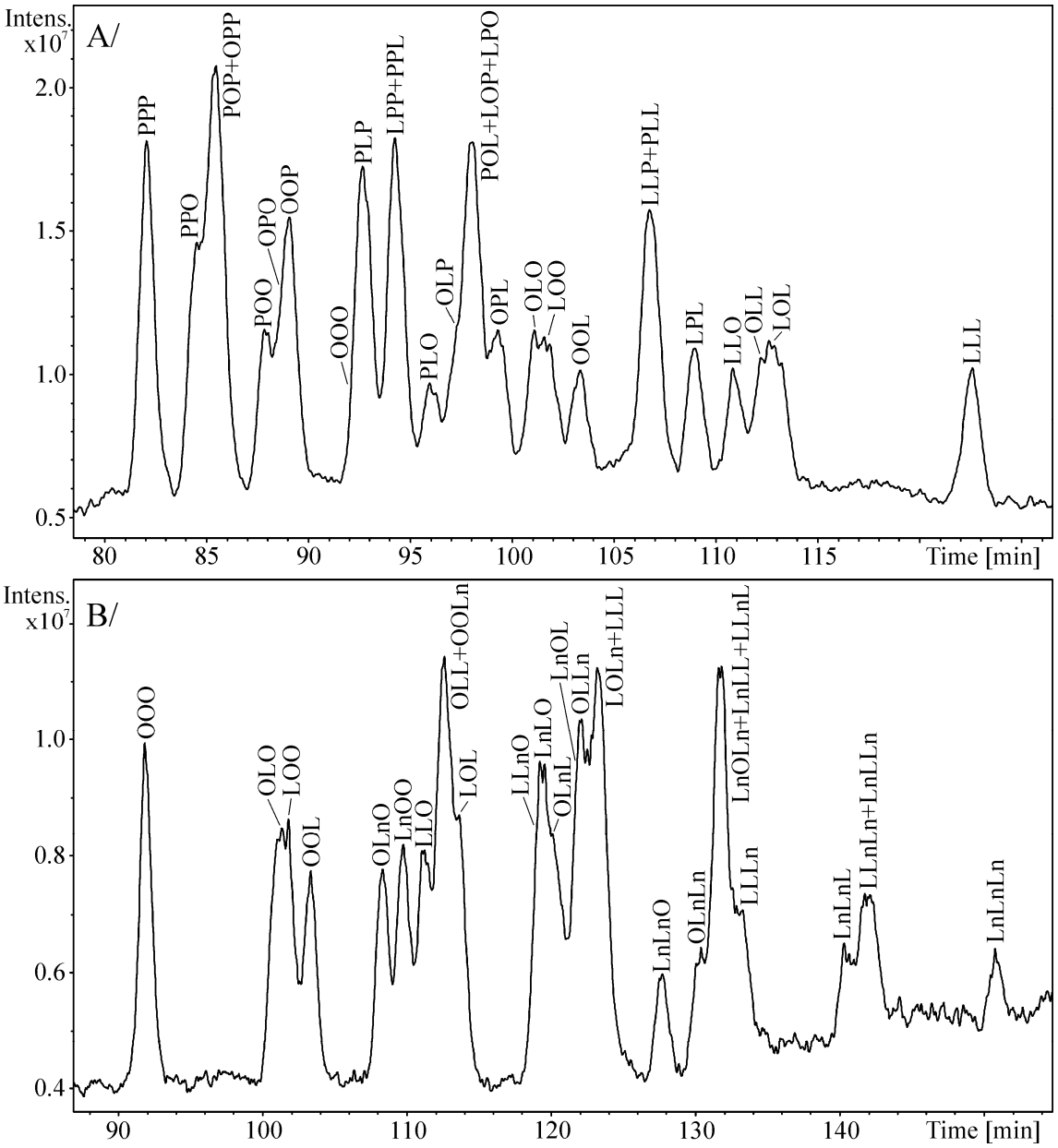

Figure S-1. Chiral HPLC/APCI-MS chromatograms of TG mixtures prepared by the randomization reaction (Method 1) of: A/ OOO, LLL and PPP, and B/ OOO, LLL and LnLnLn. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 μm, Phenomenex) connected in series, flow rate 1 mL/min, column temperature 35°C, gradient 0 min – 90% A + 10% B, 180 min 60% A + 40% B, where A is hexane and B is the mixture of hexane - 2-propanol (99:1, v/v).

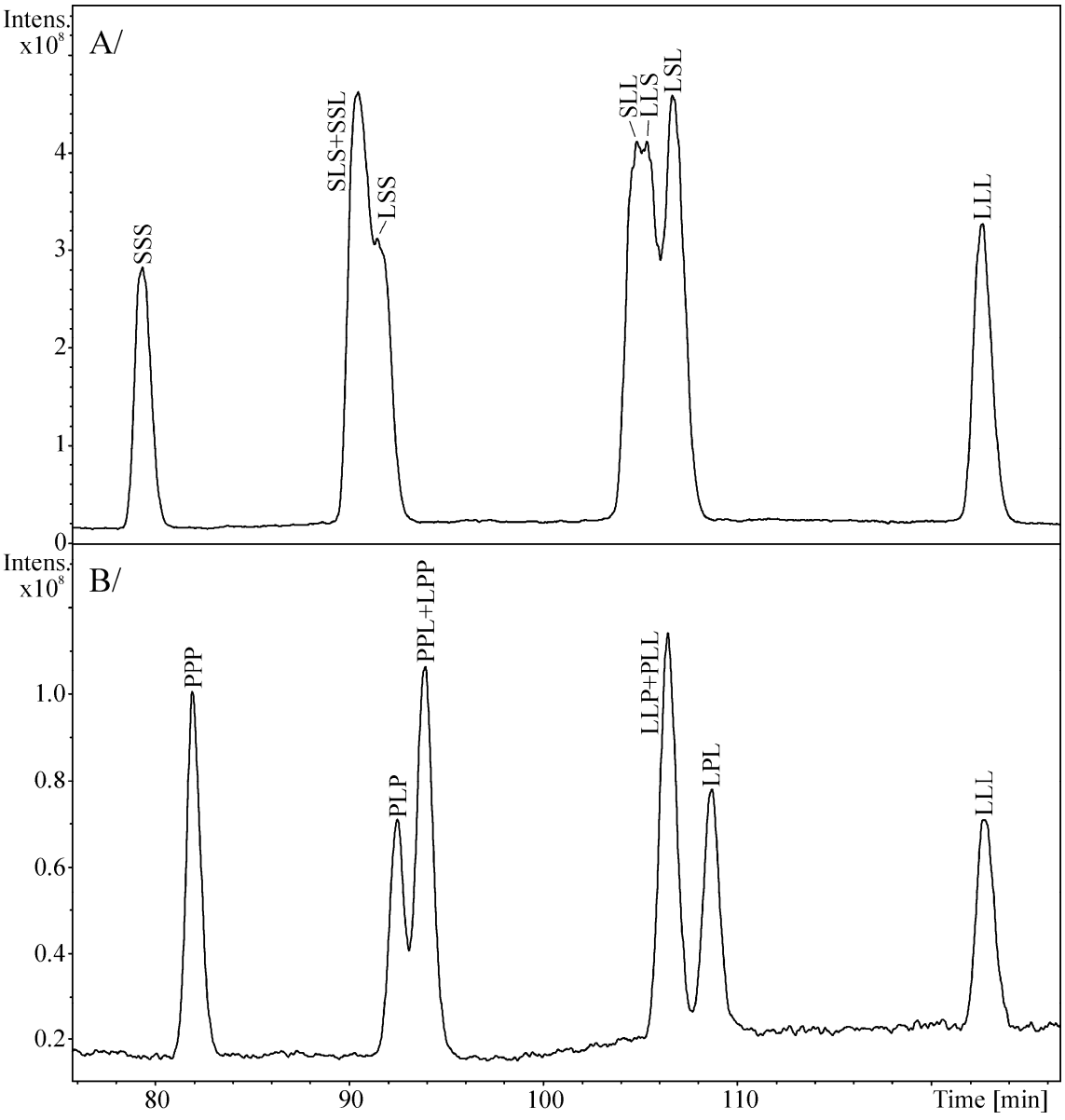

Figure S-2. Chiral HPLC/APCI-MS chromatograms of TG mixtures prepared by the randomization reaction (Method 1) of: A/ SSS and LLL, and B/ PPP and LLL. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 μm, Phenomenex) connected in series, flow rate 1 mL/min, column temperature 35°C, gradient 0 min – 90% A + 10% B, 180 min 60% A + 40% B, where A is hexane and B is the mixture of hexane - 2-propanol (99:1, v/v).

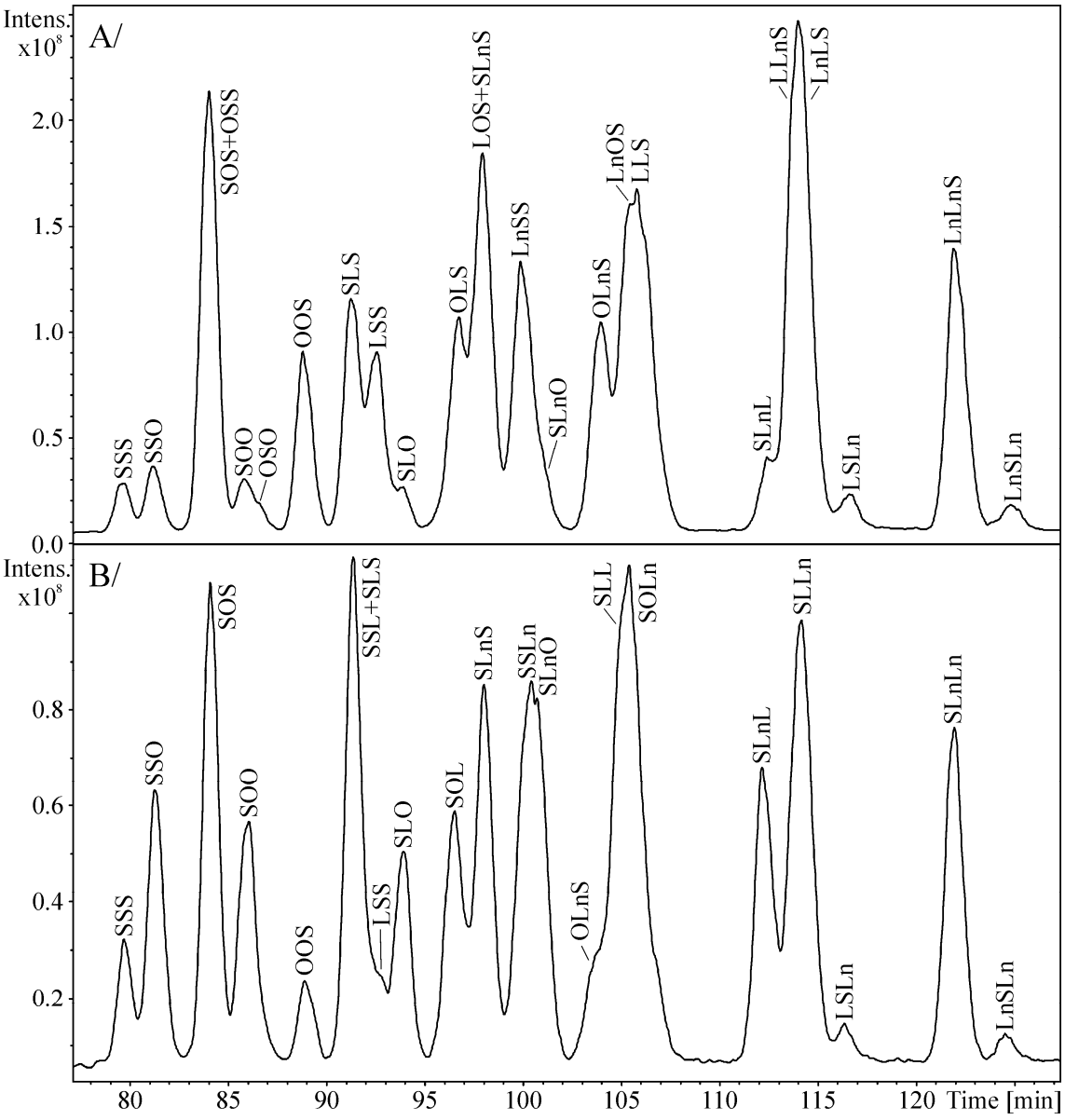

Figure S-3. Chiral HPLC/APCI-MS chromatograms of synthesized mixtures of enantiomeric TGs of R^1R^2S type (A/ Method 5) and SR^2R^3 type (B/ Method 3), where R^i are randomly distributed stearic (S), oleic (O), linoleic (L) and linolenic (Ln) acyls in *sn*-1/2 or *sn*-2/3 positions. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 µm, Phenomenex) connected in series, flow rate 1 mL/min, column temperature 35°C, gradient 0 min – 90% A + 10% B, 180 min 60% A + 40% B, where A is hexane and B is the mixture of hexane - 2-propanol (99:1, v/v).

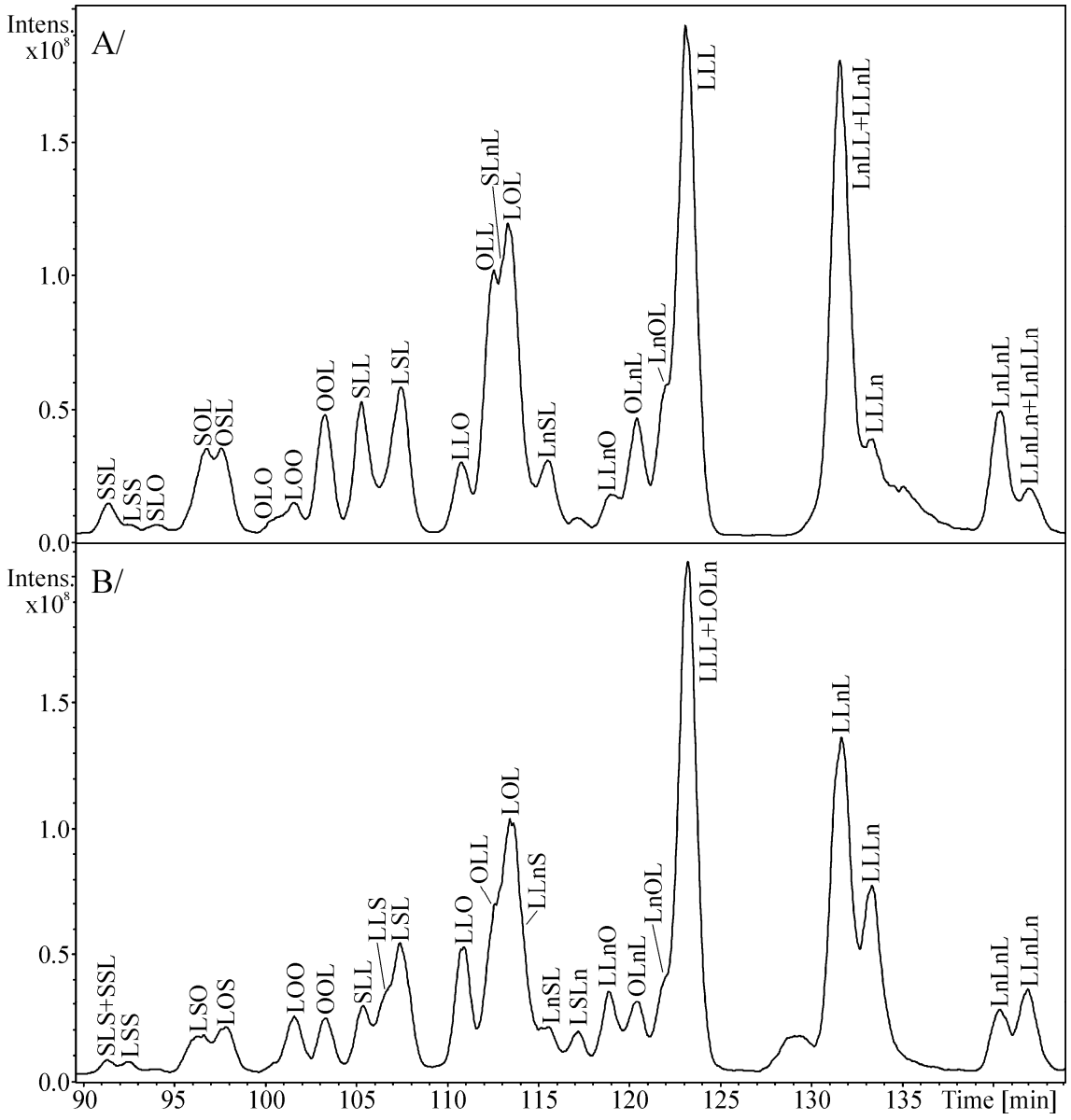

Figure S-4. Chiral HPLC/APCI-MS chromatograms of synthesized mixtures of enantiomeric TGs of R^1R^2L type (A/ Method 5) and LR^2R^3 type (B/ Method 3), where R^i are randomly distributed stearic (S), oleic (O), linoleic (L) and linolenic (Ln) acyls in *sn*-1/2 or *sn*-2/3 positions. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 µm, Phenomenex) connected in series, flow rate 1 mL/min, column temperature 35°C, gradient 0 min – 90% A + 10% B, 180 min 60% A + 40% B, where A is hexane and B is the mixture of hexane - 2-propanol (99:1, v/v).

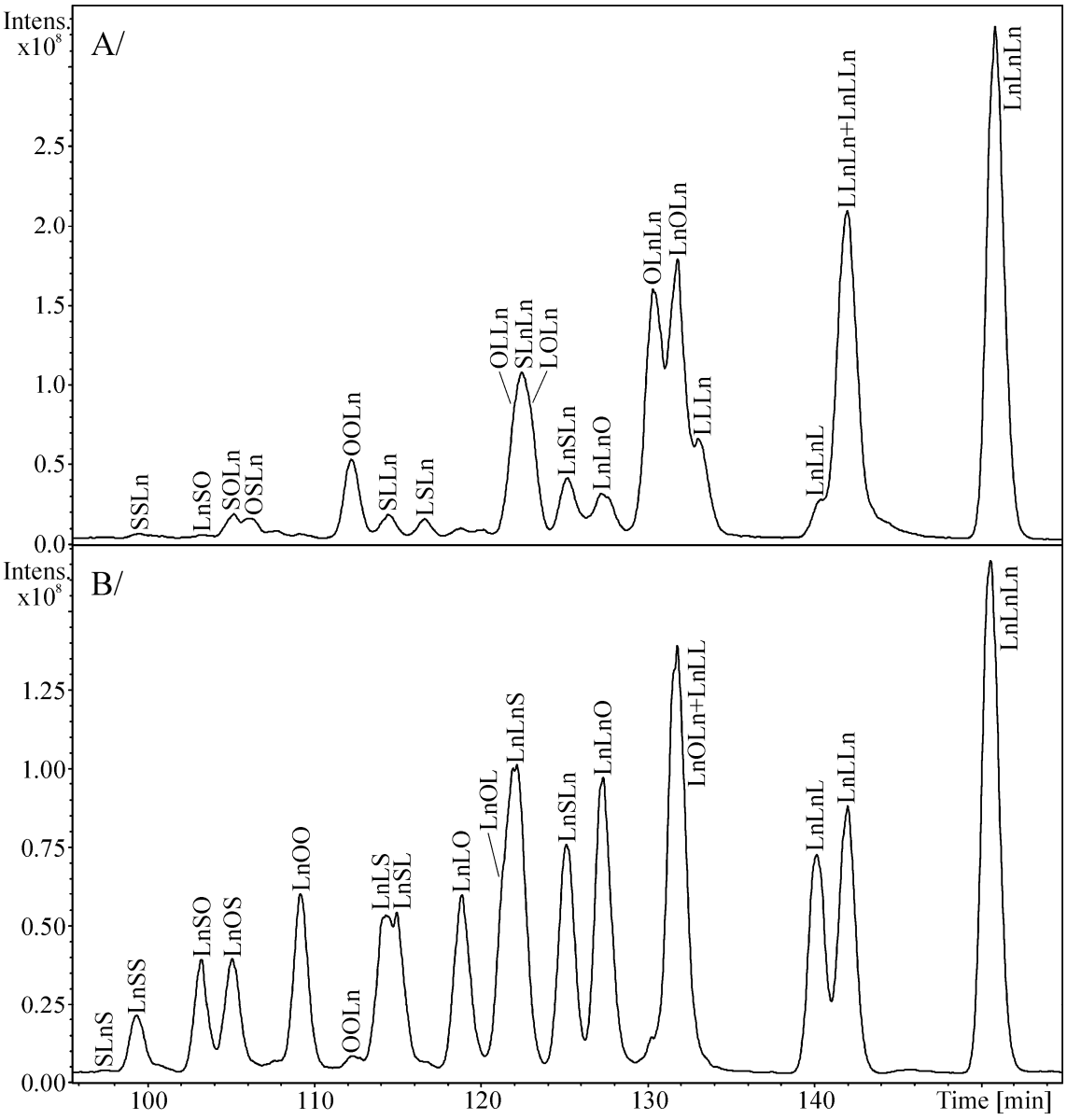

Figure S-5. Chiral HPLC/APCI-MS chromatograms of synthesized mixtures of enantiomeric TGs of R^1R^2Ln type (A/ Method 5) and LnR^2R^3 type (B/ Method 3), where R^i are randomly distributed stearic (S), oleic (O), linoleic (L) and linolenic (Ln) acyls in *sn*-1/2 or *sn*-2/3 positions. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 µm, Phenomenex) connected in series, flow rate 1 mL/min, column temperature 35°C, gradient 0 min – 90% A + 10% B, 180 min 60% A + 40% B, where A is hexane and B is the mixture of hexane - 2-propanol (99:1, v/v).

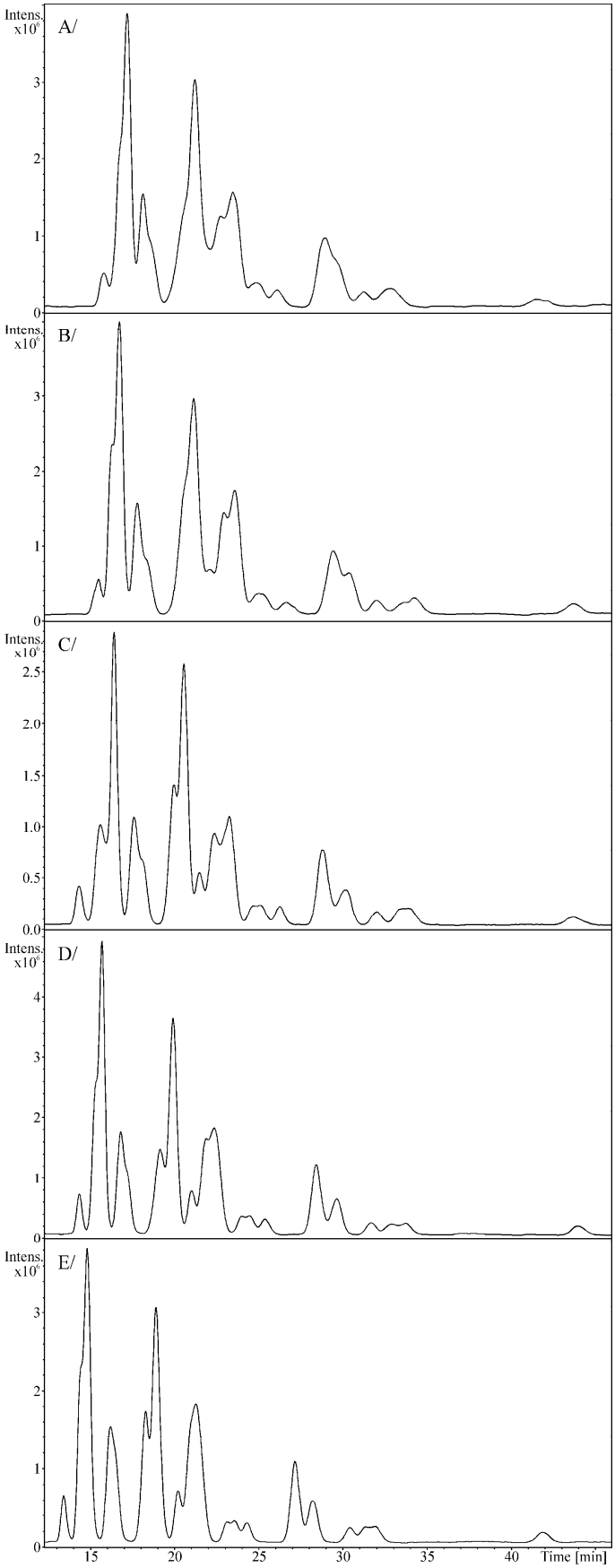

Figure S-6. Effect of separation temperature on the chiral HPLC/APCI-MS analysis of TG isomers prepared by the randomization reaction of AAA, OOO and LnLnLn: A/ 15°C, B/ 25°C, C/ 35°C, D/ 45°C, and E/ 50°C. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 μm, Phenomenex) connected in series, flow rate 1 mL/min, isocratic elution using 99.7% hexane and 0.3% 2-propanol.

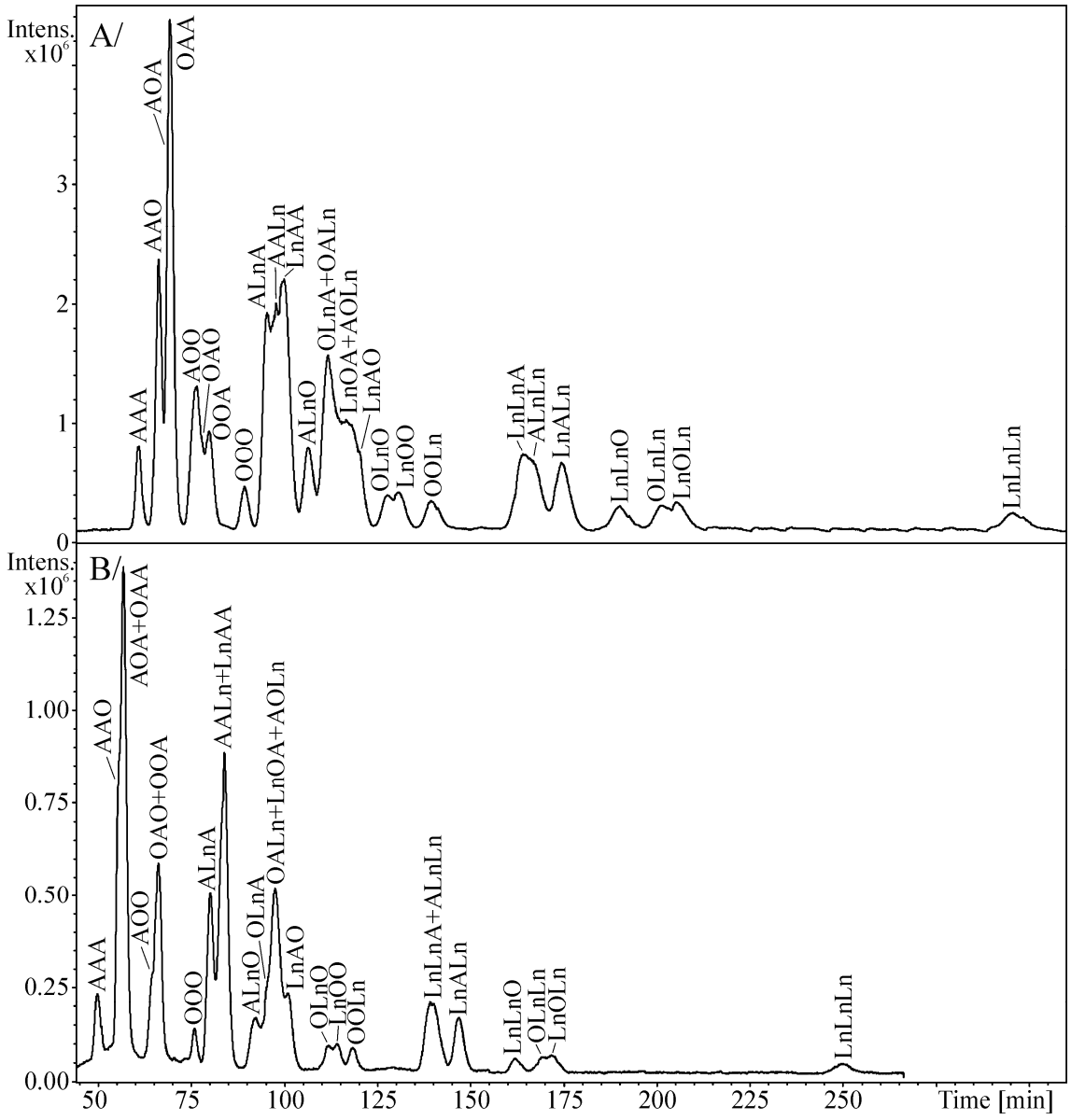

Figure S-7. Effect of mobile phase composition to separation of TG isomers prepared by the randomization reaction of AAA, OOO and LnLnLn using chiral HPLC/APCI-MS isocratic elution by mobile phase: A/ 99.9% hexane + 0.1% 2-propanol, B/ 99.9% hexane + 0.1% 2-propanol - acetonitrile (1:1, v/v). HPLC conditions: Lux Cellulose-1 column (250 mm x 4.6 mm, 3 μm, Phenomenex), flow rate 1 mL/min, column temperature 35°C, isocratic elution.

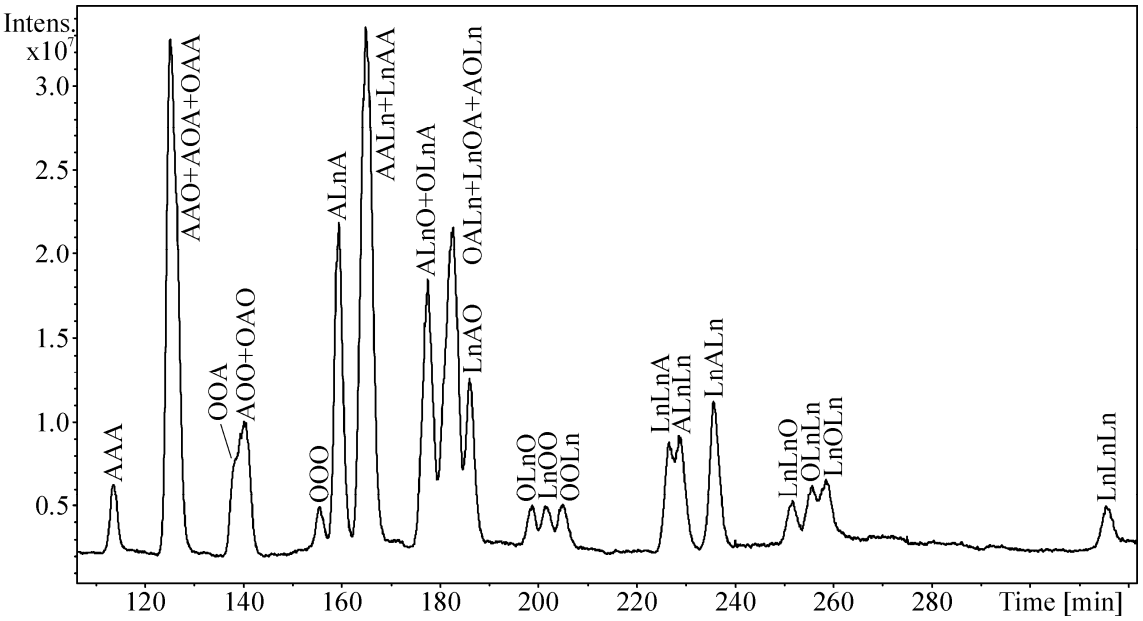

Figure S-8. Effect of hexane - acetonitrile mobile phase on the separation of TG isomers prepared by the randomization reaction of AAA, OOO and LnLnLn using chiral HPLC/APCI-MS. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 µm, Phenomenex) connected in series, flow rate 1 mL/min, column temperature 35°C, gradient 0 min 90% A + 10% B, 360 min 30% A + 70% B, where A is hexane and B is the mixture of hexane - acetonitrile (99.5:0.5, v/v).

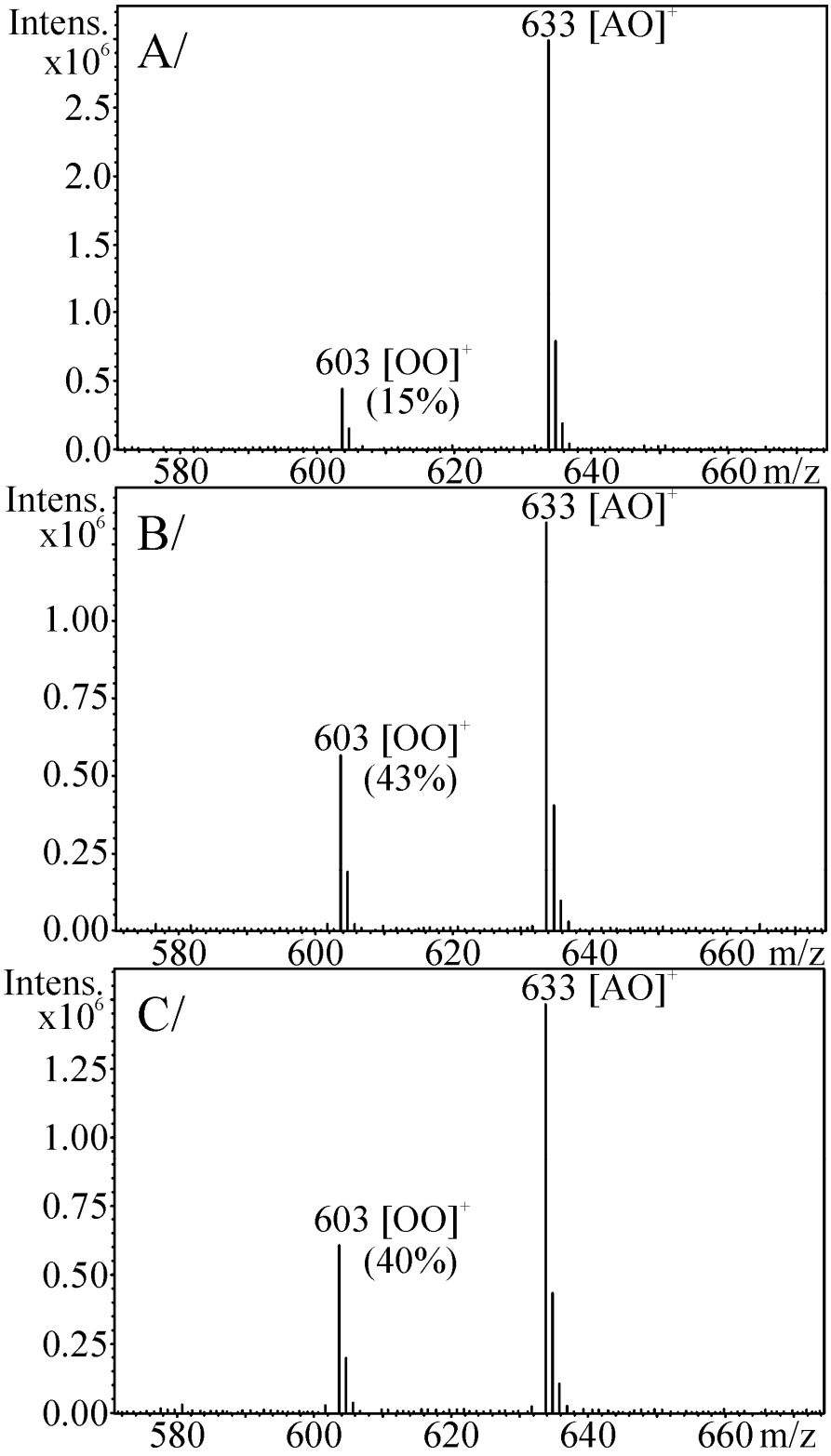

Figure S-9. Comparison of APCI mass spectra of: A/ OAO, B/ AOO, and C/ OOA isomers obtained from the chiral HPLC/APCI-MS analysis of randomization mixture prepared from AAA, OOO and LnLnLn.

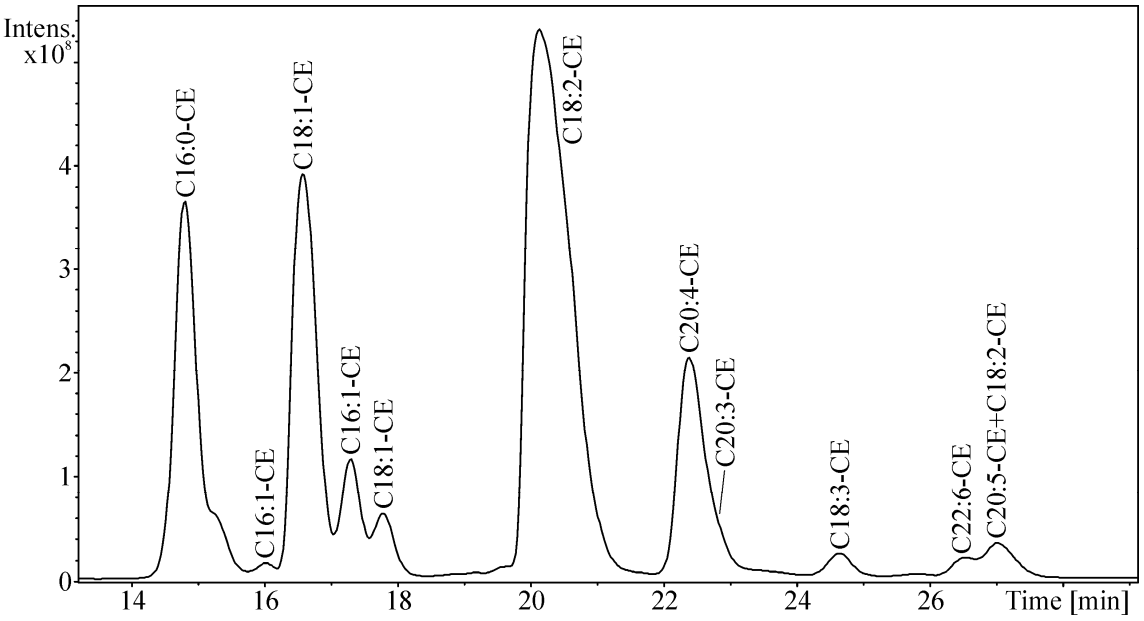

Figure S-10. Chiral HPLC/APCI-MS analysis of cholesteryl esters (CE) from TG fraction of human plasma sample. HPLC conditions: two Lux Cellulose-1 column (250 mm x 4.6 mm, 3 μm, Phenomenex) connected in series, flow rate 1 mL/min, column temperature 35°C, gradient 0 min 90% A + 10% B, 180 min 60% A + 40% B, where A is hexane and B is the mixture of hexane – 2-propanol (99:1, v/v).

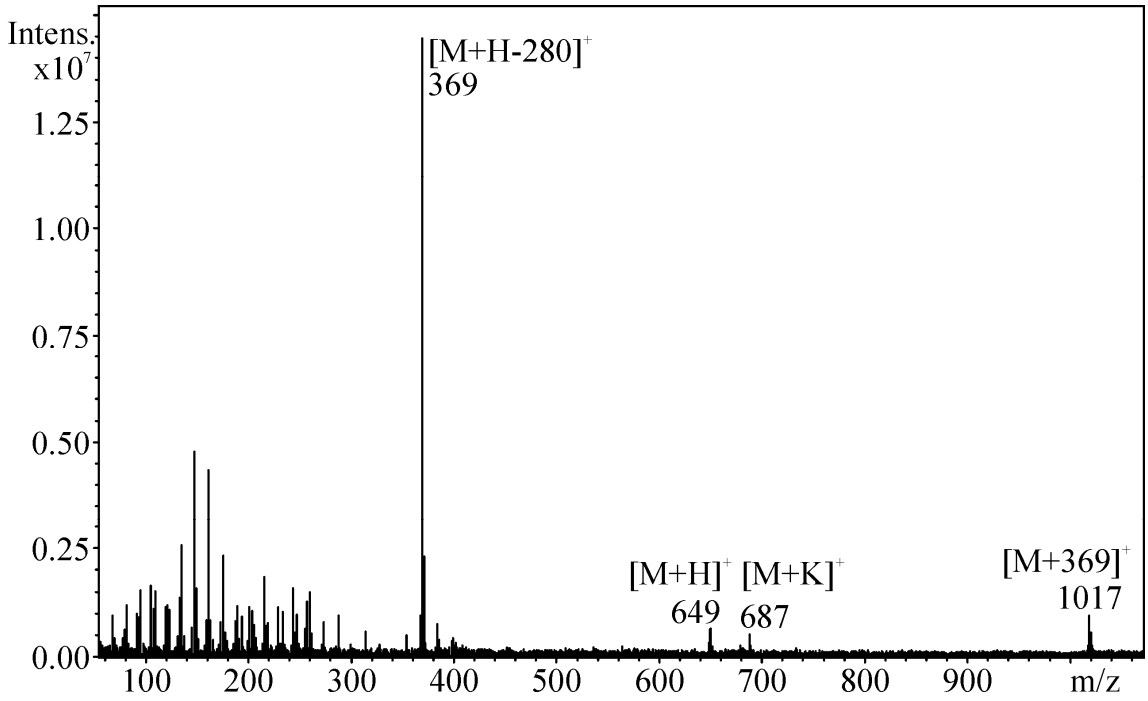

Figure S-11. APCI mass spectrum of C18:2 cholesteryl ester identified in human plasma sample.

Table S-1 Retention Times and Number of Double Bonds (DB) of Identified TGs Using Chiral HPLC/APCI-MS.

TG	DB	t_R (min)	TG	DB	t_R (min)
BBB	0	72.0	OOS	2	88.8
C21:0C21:0C21:0	0	73.6	OPO	2	89.3
AAA	0	75.3	EEE	3	90.2
C19:0C19:0C19:0	0	77.3	OOP	2	90.5
AAO	1	78.6	OPPo	2	90.5
SSS	0	79.6	SSL	2	91.2
AOA	1	80.1	SLS	2	91.2
OAA	1	80.3	OPoP	2	91.4
PSP	0	80.7	C8:0C8:0C8:0	0	91.4
SSO	1	81.2	OOO	3	91.7
MaMaMa	0	81.4	C12:0C12:0C12:0	0	91.8
PPP	0	82.1	PLP	2	92.0
SPP	0	82.1	LSS	2	92.5
OSP	1	82.7	ALnA	3	93.1
SPO	1	82.7	LPP	2	93.7
C15:0C15:0C15:0	0	83.2	PPL	2	93.7
SOS	1	84.0	SLO	3	93.8
OSS	1	84.0	C11:0C11:0C11:0	0	94.7
SOP	1	84.0	C9:0C9:0C9:0	0	94.7
AOO	2	84.3	AALn	3	95.3
PPO	1	84.3	LnAA	3	95.3
MMM	0	85.0	PLO	3	95.3
POP	1	85.0	LSO	3	95.7
OPP	1	85.0	C10:0C10:0C10:0	0	96.3
OAO	2	85.3	SOL	3	96.4
SOO	2	85.8	PePePe	3	96.5
OOA	2	86.5	OLS	3	96.7
OSO	2	86.7	OLP	3	96.7
POO	2	87.4	POL	3	97.2
C13:0C13:0C13:0	0	87.7	LOP	3	97.2

TG	DB	t_R (min)	TG	DB	t_R (min)
LPO	3	97.2	OLL	5	112.5
OSL	3	97.3	OOLn	5	112.5
LOS	3	97.9	LOL	5	113.0
SLnS	3	97.9	LLnS	5	113.6
OPL	3	98.6	SLLn	5	114.0
ALnO	4	98.9	LnLS	5	114.3
cVacVacVa	3	99.6	LnSL	5	115.1
LnSS	3	99.8	LSLn	5	116.6
SSLn	3	99.8	LLnO	6	118.5
OLO	4	100.3	LnLO	6	118.9
SLnO	4	100.9	LnLnA	6	119.5
OLnA	4	101.0	ALnLn	6	119.5
LOO	4	101.1	OLnL	6	119.9
LnAO	4	101.2	LnOL	6	121.6
LnOA	4	102.8	OLLn	6	122.1
AOLn	4	102.8	LnLnS	6	122.3
OOL	4	103.1	SLnLn	6	122.3
LnSO	4	103.3	LnALn	6	122.3
OLnS	4	103.9	LOLn	6	123.2
OALn	4	104.3	LLL	6	123.2
SLL	4	104.8	LnSLn	6	124.8
LnOS	4	105.4	LnLnO	7	127.1
SOLn	4	105.4	OLnLn	7	130.1
LLS	4	106.1	LnOLn	7	131.5
LLP	4	106.1	LnLL	7	131.5
PLL	4	106.1	LLnL	7	131.5
OSLn	4	106.8	γLnγLnγLn	9	131.8
LSL	4	106.9	LLLn	7	132.7
OLnO	5	107.9	LnLnL	8	139.9
LPL	4	108.5	LLnLn	8	141.6
LnOO	5	109.3	LnLLn	8	141.6
LLO	5	110.3	LnLnLn	9	150.5
SLnL	5	112.0			

Table S-2 Retention Times of CEs and Cholesterol Identified in TG Fraction from Total Lipid Extract of Human Plasma Sample Using Chiral HPLC/APCI-MS.

CEs [a]	**t_R (min)**	**CEs**	**t_R (min)**
C16:0-CE	14.8	C20:3-CE	22.8
C16:1-CE	16.0	C18:3-CE	24.6
C18:1-CE	16.6	C22:6-CE	26.5
C16:1-CE	17.3	C20:5-CE	27.0
C18:1-CE	17.8	C18:2-CE	27.0
C18:2-CE	20.1	Cholesterol	92.2
C20:4-CE	22.4		

[a] CEs with identical CN:DB composition are isomers without the identification of the isomerism (cholesterol enantiomers or positional isomer of DBs).