

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 μ m, Waters), a flow rate of 1 mL/min, an injection volume of 10 μ L, 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 acetate⁵².

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 μ m). From the filtered extract, hexane was evaporated using the gentle stream of nitrogen to yield the pure hazelnut oil.

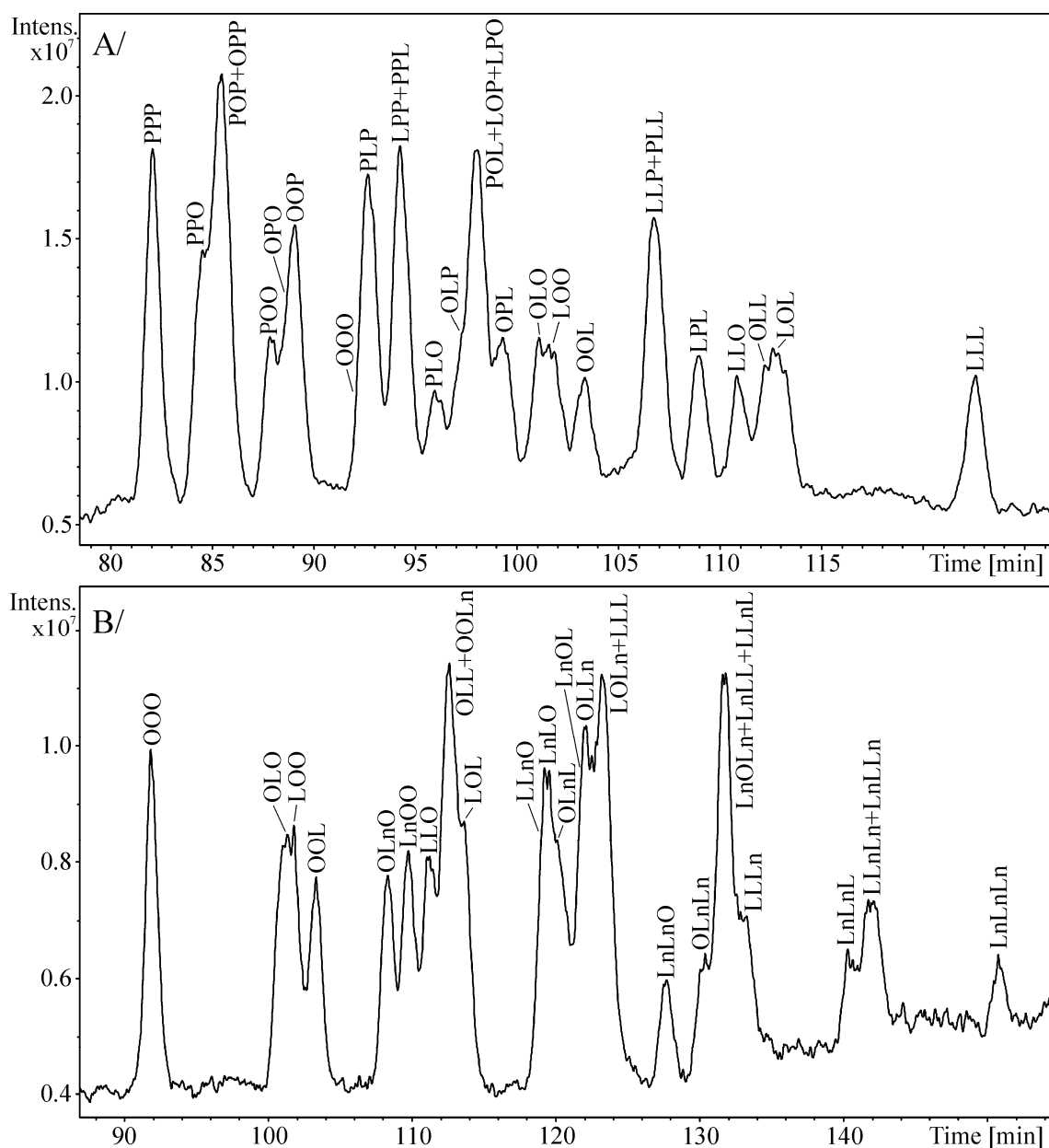


Figure S-1. Chiral HPLC/APCI-MS chromatograms of TG mixtures prepared by the randomization reaction (Method 1) of: A/ OOO, LLL and PPP, and B/ OOO, LLL and LnLnLn. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 μ m, Phenomenex) connected in series, flow rate 1 mL/min, column temperature 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).

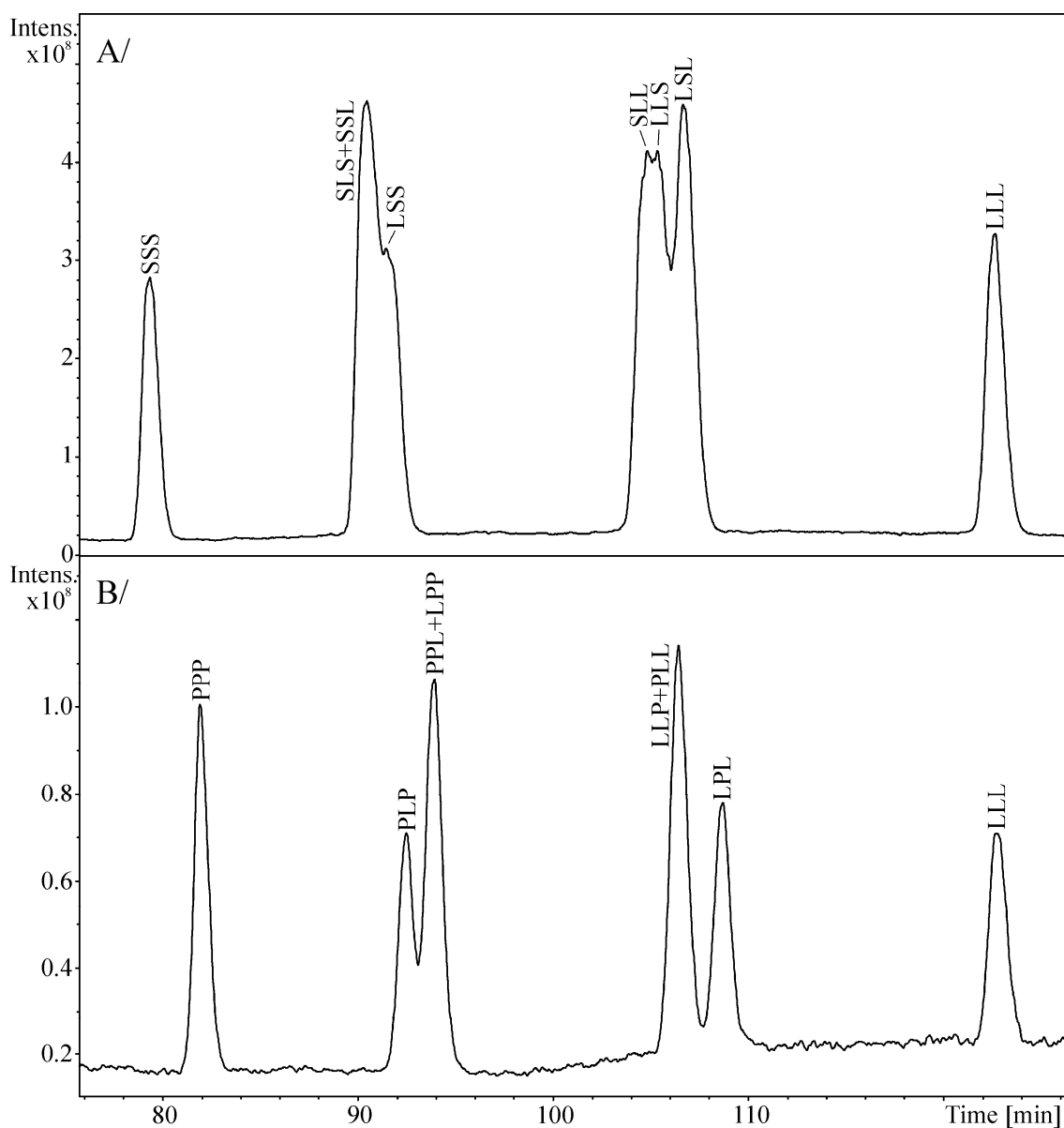


Figure S-2. Chiral HPLC/APCI-MS chromatograms of TG mixtures prepared by the randomization reaction (Method 1) of: A/ SSS and LLL, and B/ PPP and LLL. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 μ m, Phenomenex) connected in series, flow rate 1 mL/min, column temperature 35°C, gradient 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).

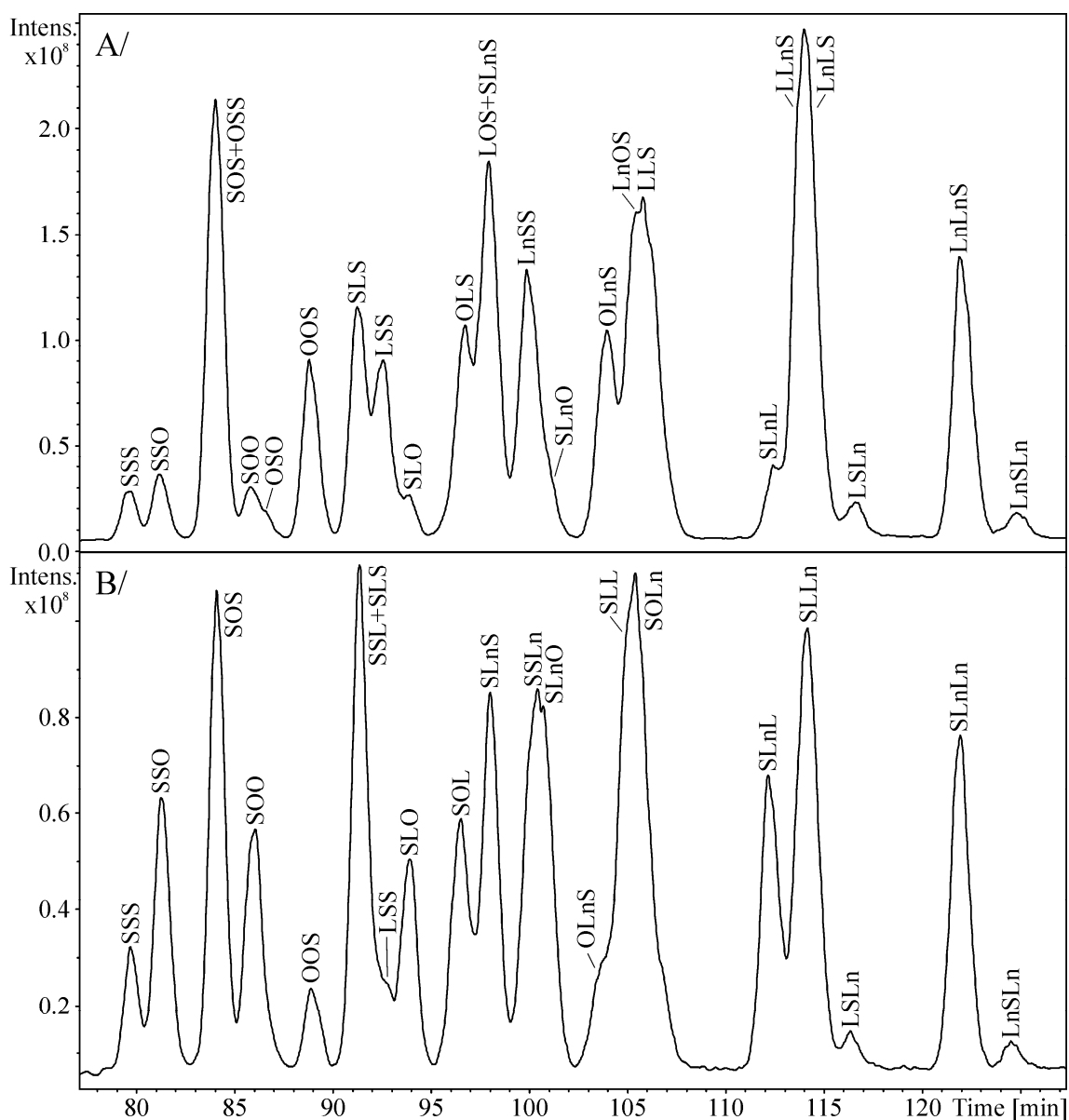


Figure S-3. Chiral HPLC/APCI-MS chromatograms of synthesized mixtures of enantiomeric TGs of R^1R^2S type (A/ Method 5) and SR^2R^3 type (B/ Method 3), where R^i are randomly distributed stearic (S), oleic (O), linoleic (L) and linolenic (Ln) acyls in *sn*-1/2 or *sn*-2/3 positions. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 μ m, 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).

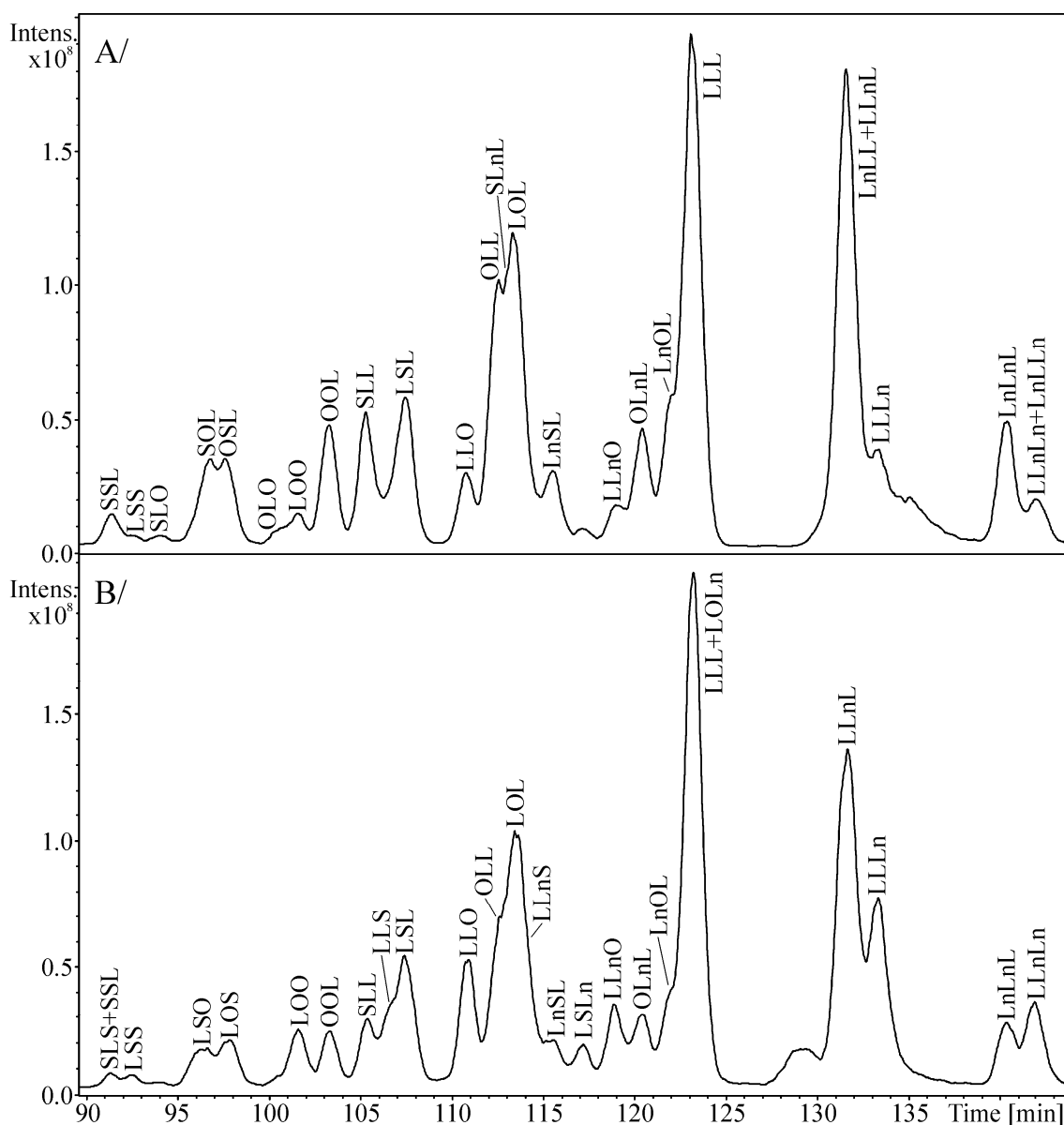


Figure S-4. Chiral HPLC/APCI-MS chromatograms of synthesized mixtures of enantiomeric TGs of R^1R^2L type (A/ Method 5) and LR^2R^3 type (B/ Method 3), where R^i are randomly distributed stearic (S), oleic (O), linoleic (L) and linolenic (Ln) acyls in *sn*-1/2 or *sn*-2/3 positions. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 μ m, 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).

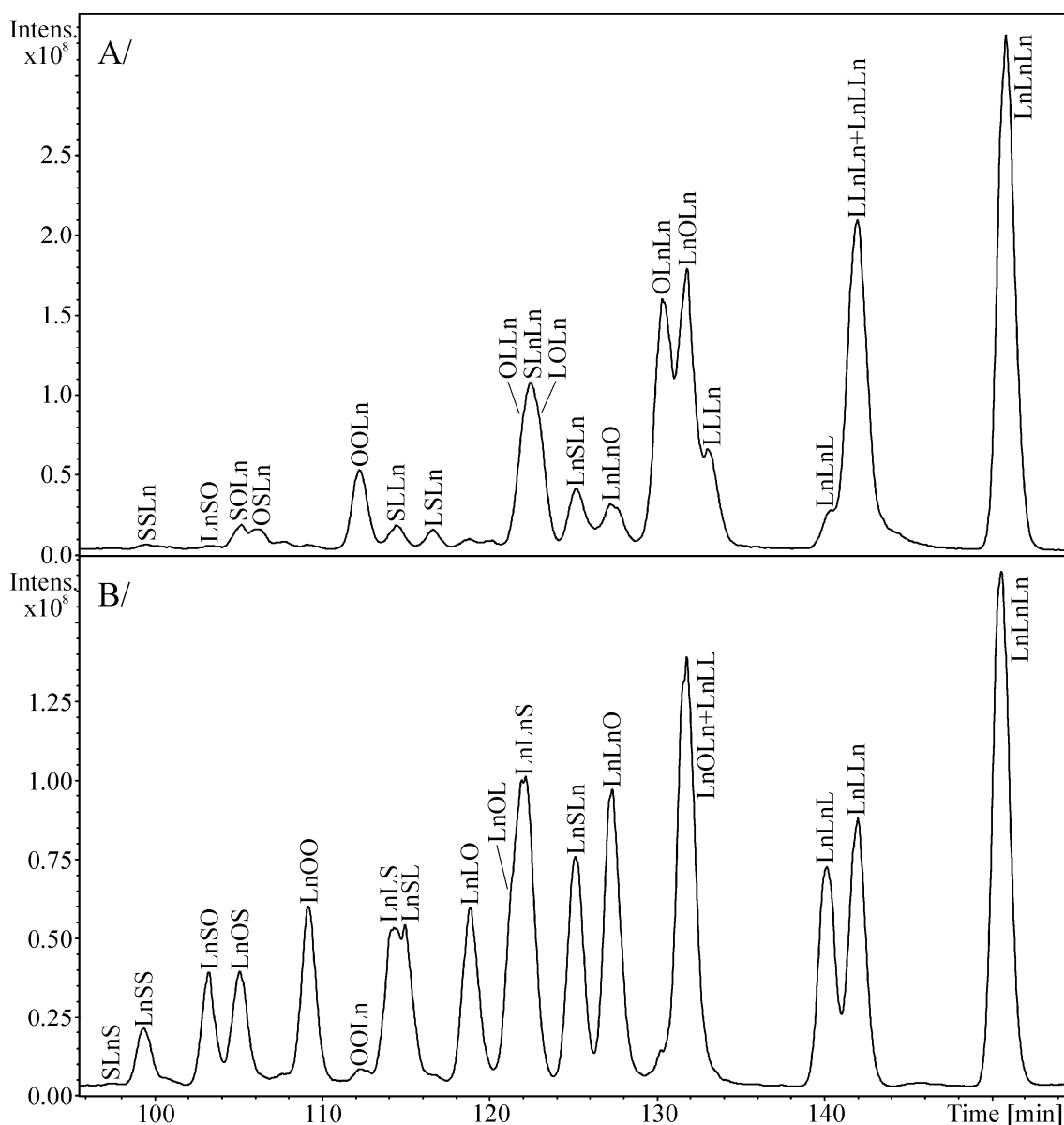


Figure S-5. Chiral HPLC/APCI-MS chromatograms of synthesized mixtures of enantiomeric TGs of R^1R^2Ln type (A/ Method 5) and LnR^2R^3 type (B/ Method 3), where R^i are randomly distributed stearic (S), oleic (O), linoleic (L) and linolenic (Ln) acyls in *sn*-1/2 or *sn*-2/3 positions. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 μ m, 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).

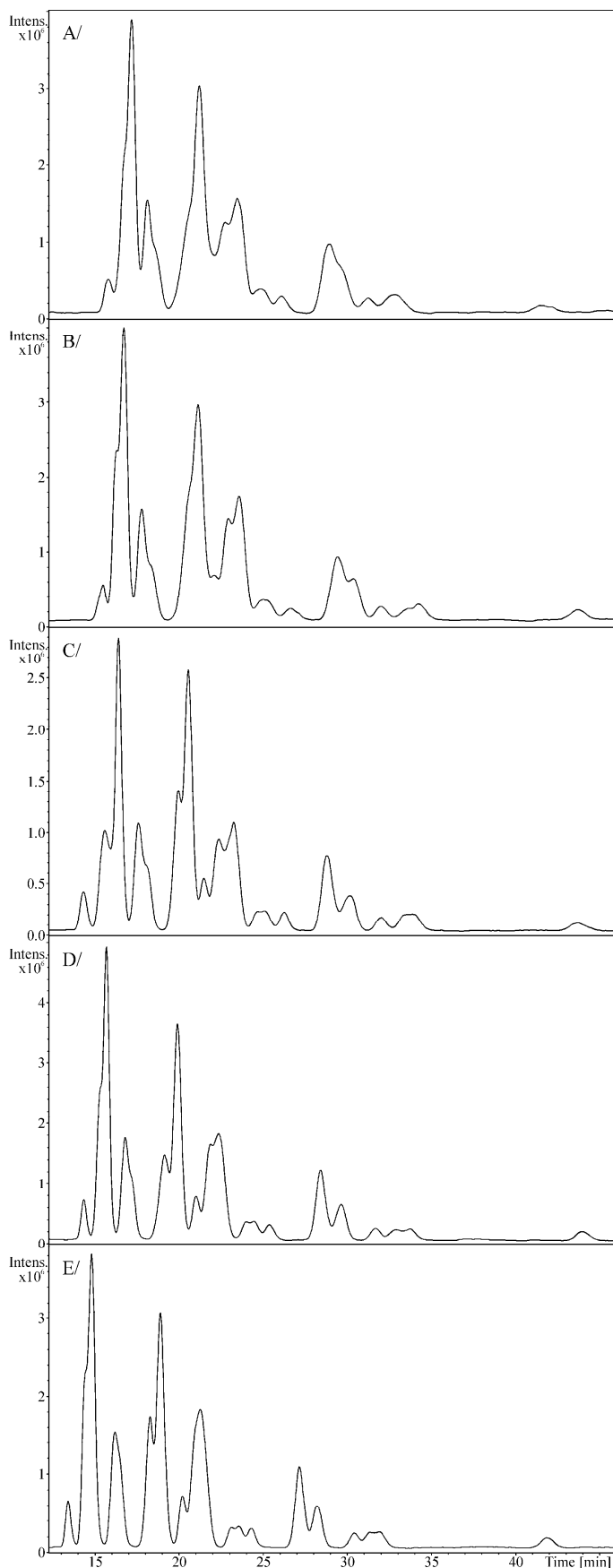


Figure S-6. Effect of separation temperature on the chiral HPLC/APCI-MS analysis of TG isomers prepared by the randomization reaction of AAA, OOO and LnLnLn: A/ 15°C, B/ 25°C, C/ 35°C, D/ 45°C, and E/ 50°C. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 μ m, Phenomenex) connected in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

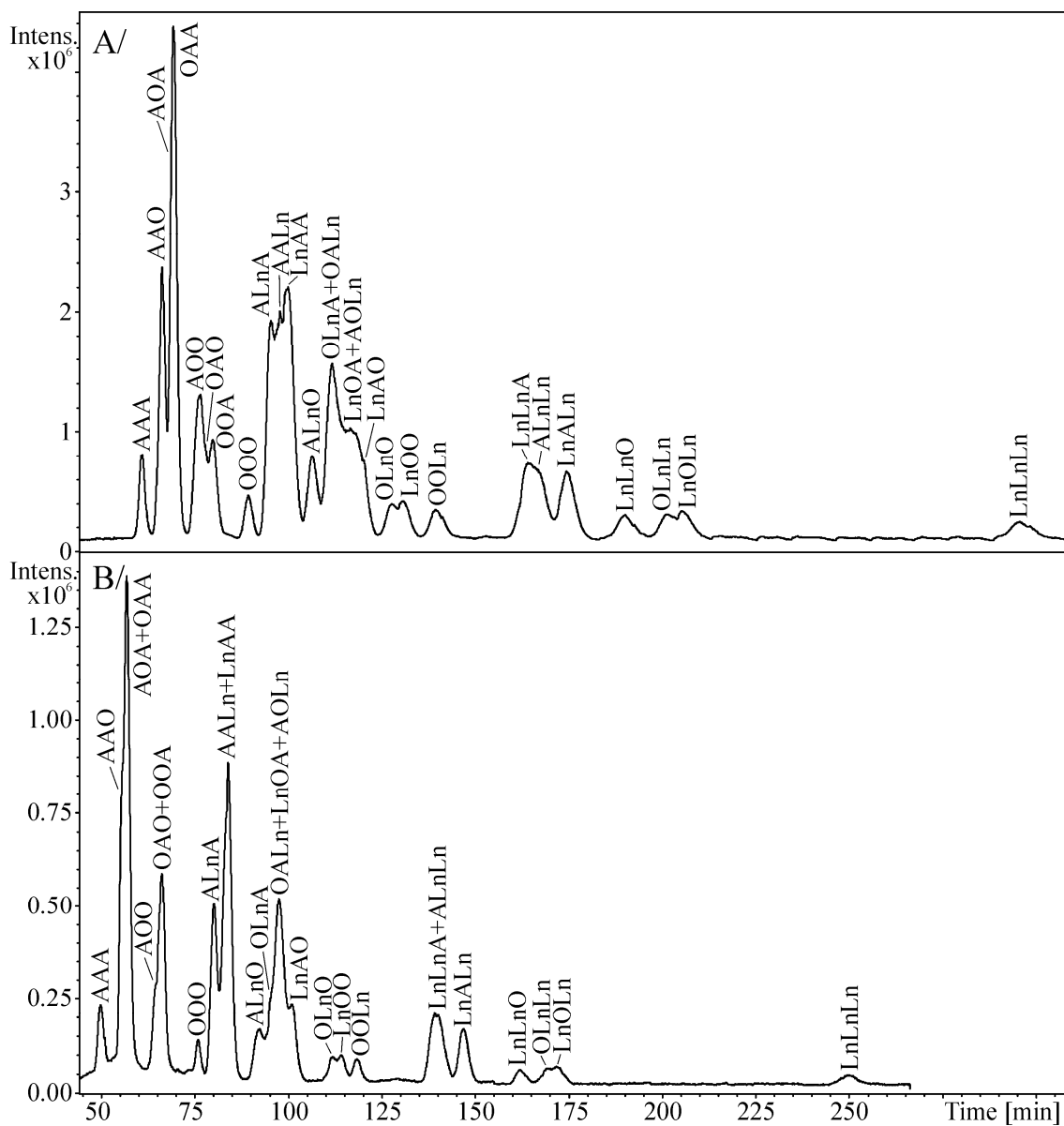


Figure S-7. Effect of mobile phase composition to separation of TG isomers prepared by the randomization reaction of AAA, OOO and LnnLnLn using chiral HPLC/APCI-MS isocratic elution by mobile phase: A/ 99.9% hexane + 0.1% 2-propanol, B/ 99.9% hexane + 0.1% 2-propanol - acetonitrile (1:1, v/v). HPLC conditions: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

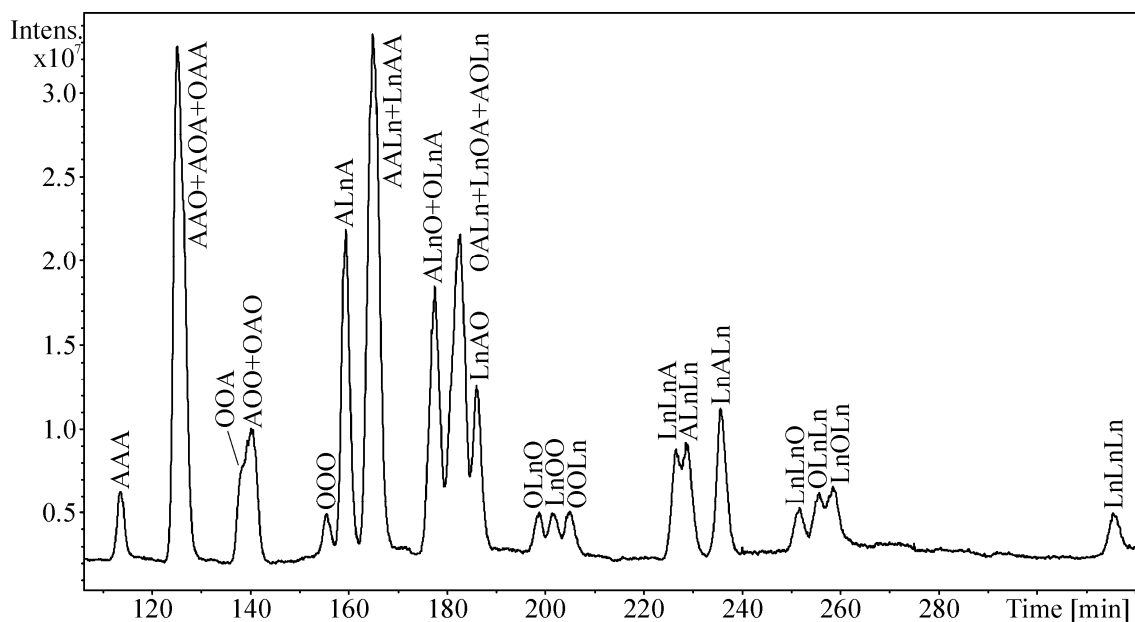


Figure S-8. Effect of hexane - acetonitrile mobile phase on the separation of TG isomers prepared by the randomization reaction of AAA, OOO and LnLnLn using chiral HPLC/APCI-MS. HPLC conditions: two Lux Cellulose-1 columns (250 mm x 4.6 mm, 3 μ m, Phenomenex) connected in series, flow rate 1 mL/min, 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).

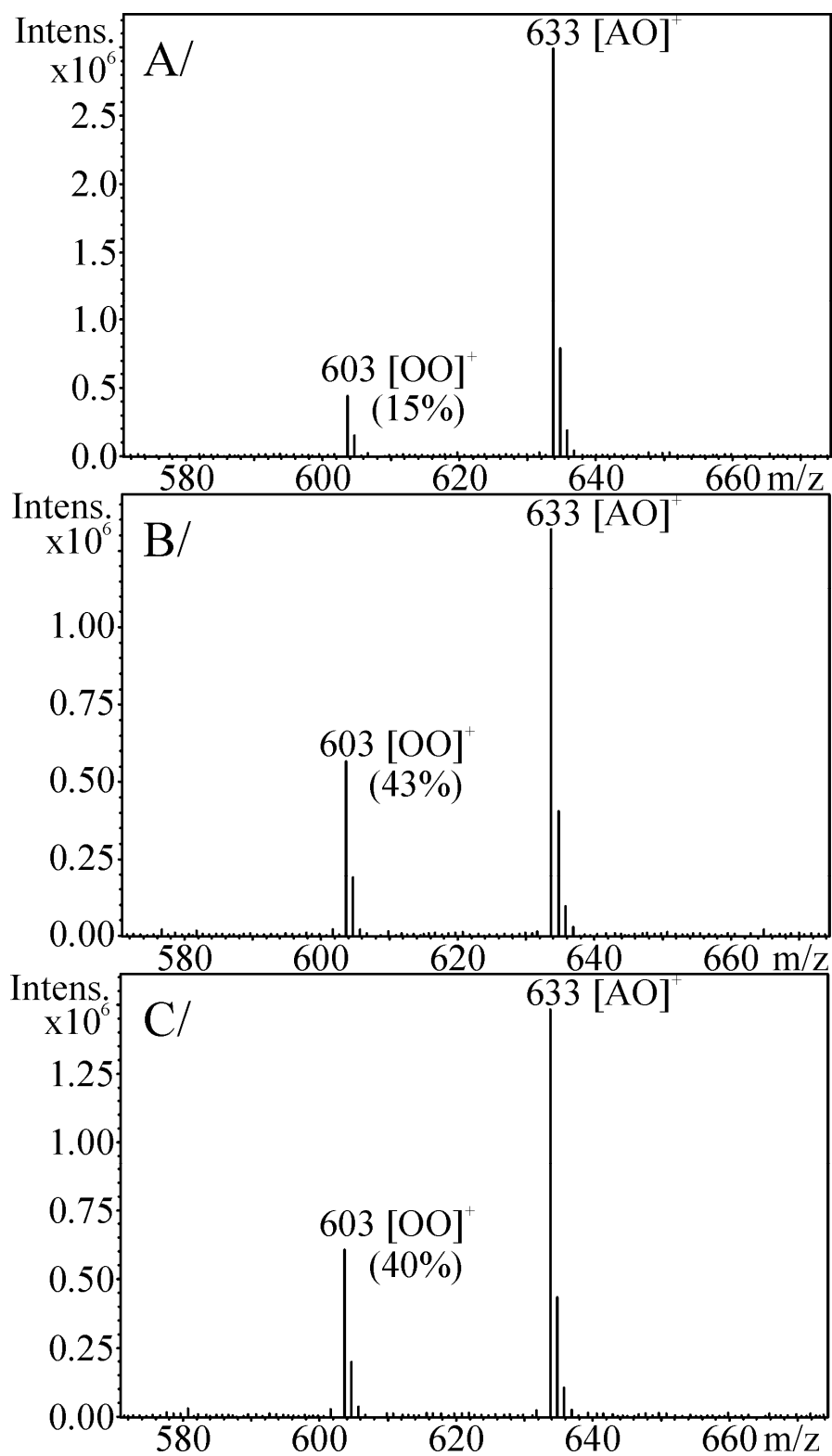


Figure S-9. Comparison of APCI mass spectra of: A/ OAO, B/ AOO, and C/ OOA isomers obtained from the chiral HPLC/APCI-MS analysis of randomization mixture prepared from AAA, OOO and LnLnLn.

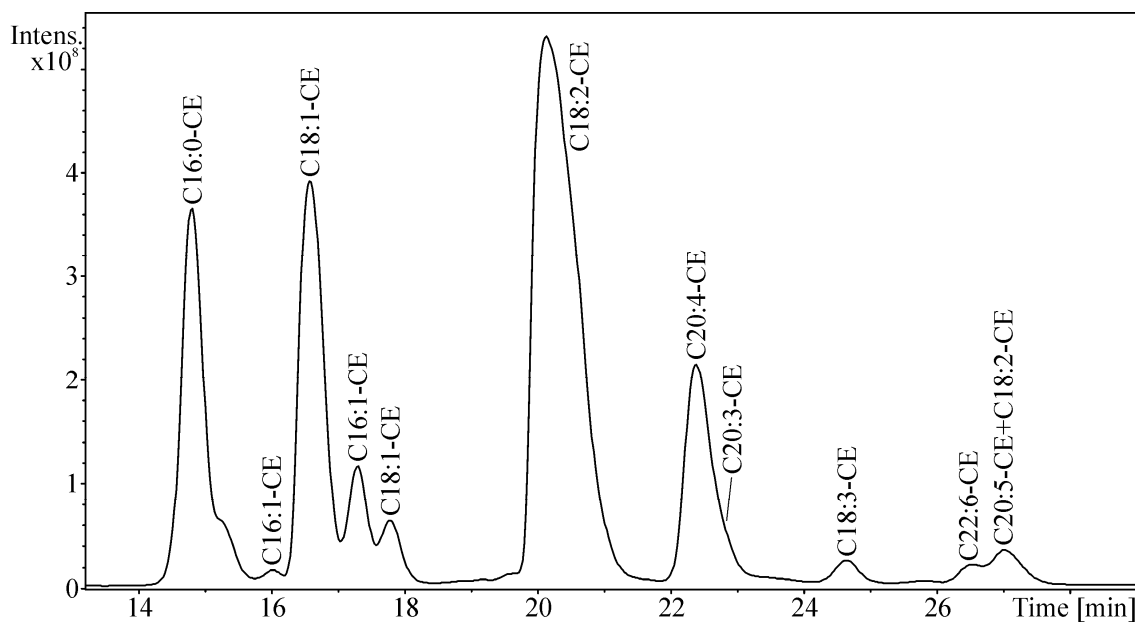


Figure S-10. Chiral HPLC/APCI-MS analysis of cholesteryl esters (CE) from TG fraction of human plasma sample. HPLC conditions: two Lux Cellulose-1 column (250 mm x 4.6 mm, 3 μ m, Phenomenex) connected in series, flow rate 1 mL/min, column temperature 35°C, gradient 0 min 90% A + 10% B, 180 min 60% A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

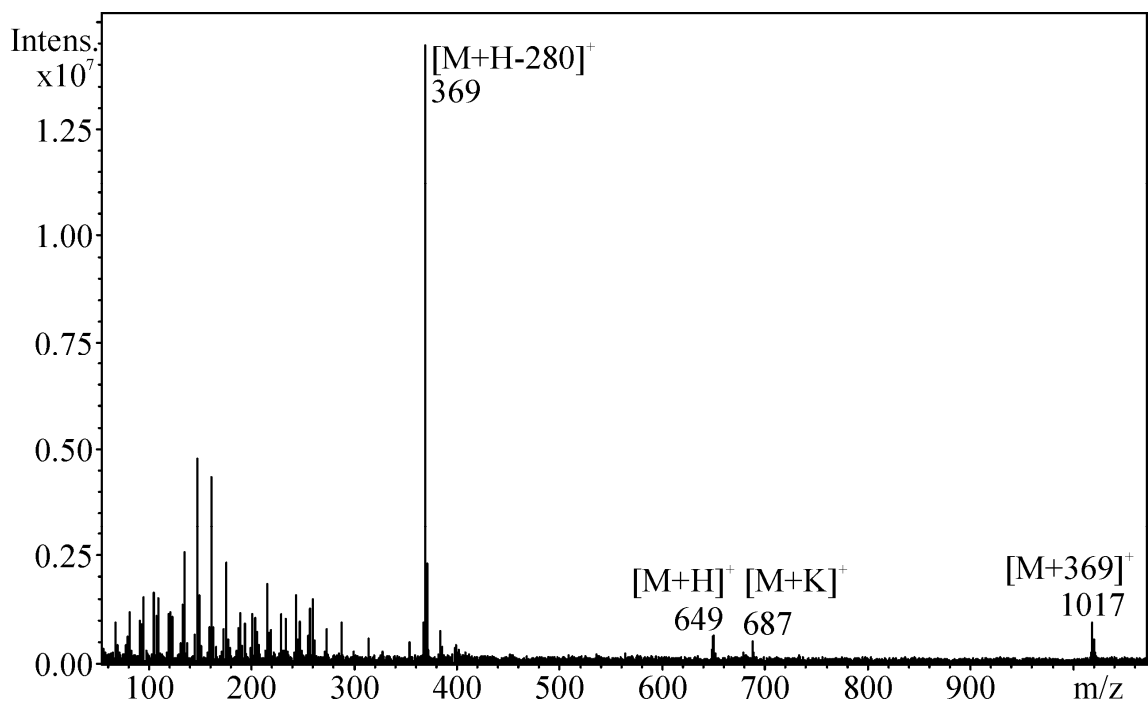


Figure S-11. APCI mass spectrum of C18:2 cholesteryl ester identified in human plasma sample.

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).