Electronic Supplementary Material (ESI) for Analyst.

Supplementary Materials

Simultaneous quantitative LC-MS/MS analysis of 13 apolipoproteins and lipoprotein (a) in human plasma

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Supplemental Table S1. The MRM transitions, collision energy, decluttering potential, and cell exit potential of each peptide and its internal standards.

Peptide	Protein name	Peptide name	Protein MW（Kda）	Peptide MW	Precursor ion	Product ion	DP	CE	SIL
VQPYLDDFQK	apoA1	apoA1-VQPY	30.8	1252.3718	627.1	1025.7 1008.4	44 23	23 24	apoA1-IS
VQPYLDDFQK*	apoA1	apoA1-IS	/	1260.3859	631.1	1033.7 1016.4	46 40	21.3 25.6	/
VSFLSALEEYTK	apoA1	apoA1-VSFL	30.8	1386.5453	694.1	940.3 853.4	72 106	33 30	apoA1-IS
SPELQAEAK	apoA2	apoA2-SPEL	11.2	972.0506	487.1	442.8 659.4	87.3 44	24 26	apoA2-IS
SPELQAEAK*	apoA2	apoA2-IS	/	980.0648	491.1	450.8 667.4	87.3 44	24 26	/
EQLTPLIK	apoA2	apoA2-EQLT	11.2	941.1227	471.5	684.4 571.5	51.4 128.5	20 28	apoA2-IS
VQELQEQLR	apoA5	apoA5-VQEL	41.2	1142.2629	571.9	673.6 915.4	38 40	24 29.6	apoA5-IS
VQELQEQLR*	apoA5	apoA5-IS	/	1152.2711	576.9	683.6 925.4	40 45	30 28	/
FPEVDVLTK	apoB	apoB-FPEV	515.6	1047.2016	524.5	450.5 803.6	149.7 139.4	23 30	apoB-IS1
FPEVDVLTK*	apoB-IS	apoB-IS1	/	1055.2157	528.5	811.6 908.6	139.4 60	30 30	/
TEVIPPLIENR	apoB	apoB-TEVI	515.6	1280.4697	641.1	838.7 741.2	67.4 56	25.2 33	apoB-IS2
TEVIPPLIENR*	apoB-IS	apoB-IS2	/	1290.4780	646.1	848.7 751.2	65.2 27	29 32	/
TGISPLALIK	apoB	apoB-TGIS	515.6	1012.2437	507.1	741.7 654.6	32 25	22 23	apoB-IS

EFGNTLEDK	apoC1	apoC1-EFGN	9.3	1052.0922	527.1	391.3 776.4	41 31	22 24	apoC1-IS
EFGNTLEDK*	apoC1	apoC1-IS	/	1060.1064	531.1	399.3 784.4	41 44	24 22	/
LGPLVEQGR	apoC1	apoC1-LGPL	9.3	968.1083	527	391.3 776.4	41 31	22 24	apoC1-IS
TYLPAVDEK	apoC2	apoC2-TYLP	11.3	1035.1478	518.3	658.3 771.6	60 68	24.2 20.8	apoC2-IS
TYLPAVDEK*	apoC2	apoC2-IS	/	1043.1619	522.3	666.3 779.6	128 86	25 23	/
TAAQNLYEK	apoC2	apoC2-TAAQ	11.3	1037.1240	519.5	865.7 666.5	128 86	25 25	apoC2-IS
GWVTDGFSSLK	apoC3	apoC3	10.9	1196.3085	598.9	854.6 343.2	60 40	27.1 23.6	apoC3-IS
GWVTDGFSSLK*	apoC3	apoC3-IS	/	1204.3226	598.9	854.6 343.2	60 40	27.1 23.6	/
NILTSNNIDVK	apoD	apoD	21.3	1230.3680	616	890.6 789.4	131 146	32 31	apoD-IS
NILTSNNIDVK*	apoD	apoD-IS	/	1238.3821	620	898.6 797.4	144 102	28 35	/
LGPLVEQGR	apoE	apoE-LGPL	36.2	968.1083	484.8	588.5 701.6	60 50	30.6 31.1	apoE-IS
LGPLVEQGR*	apoE-IS	apoE-IS	/	978.1165	489.8	598.5 711.6	60 50	30.8 31.7	/
AATVGSLAGQPLQER	apoE	apoE-AATV	36.2	1497.6524	749.6	625.5 642.3	100 139.1	39 36.1	apoE-IS
ATVVYQGER	apoH	apoH	38.3	1022.1126	512.0	652.4 751.4	66 75	22 15	apoH-IS
ATVVYQGER*	apoH	apoH-IS	/	1032.1209	517.0	662.4 761.4	52 67	33 15	/
VTTVASHTSDSDVPS GVTEVVVK	apoJ	apoJ	51.1	2314.5021	772.4	507.9 650.9	50 45	30.2 28.8	apoE-IS

ALDNLAR	apoL1	apoL1	56	771.8620	386.9	473.4 588.5	53 60	27 30.6	apoL1-IS
ALDNLAR*	apoL1	apoL1-IS	/	781.8703	391.9	483.4 598.5	51 93	23.8 17	/
FLLYNR	apoM	apoM	21.2	824.9660	413.6	565.3 452.3	125 100	20 20	apoM-IS
FLLYNR*	apoM	apoM-IS	21.2	834.9743	418.6	575.3 462.3	119 102	32 32	/
TPENYPNAGLTR	Lp(a)	Lp(a)-TPEN	/	1332.4184	666.9	1005.3 728.2	60 80	37.1 37.4	Lp(a)-TTPEN-IS
TPENYPNAGLTR*	Lp(a)-TPEN-IS	Lp(a)-TPEN-IS	/	1342.4267	672.4	738.4 1015.3	25 65	39.2 28.2	/
GISSTTVTGR	Lp(a)	Lp(a)-GISS	/	978.0585	489.9	808.3 533.3	30 40	21.8 25.2	Lp(a)-GISS-IS
GISSTTVTGR*	Lp(a)-GISS-IS	Lp(a)-GISS-IS	/	988.0668	495.3	543.2 818.2	30 31	22.5 22.8	/

Note: * are the internal standards peptides and the bottom line is the quantitative peptides.

Supplemental Table S2 Effects of different trypsin: plasma protein ratio on measured apolipoproteins and Lp(a).

□	Relative deviation (%)		
Peptide	**1 μg (1:14)**	**2 μg (1:7)**	**4 μg (1:3.5)**
ApoA1	2.43	4.96	-6.46
ApoA2	3.05	5.22	-14.04
ApoA5	13.43	12.62	11.5
ApoB	5.19	10.3	10.82
ApoC1	0.98	1.6	-6.26
ApoC2	2.01	-0.78	-0.48
ApoC3	3.63	4.62	-12.3
ApoD	5.25	4.67	-0.41
ApoE	1.24	-1.43	-0.85
ApoH	1.01	-2.85	-0.39
ApoJ	12.07	-3.16	-2.66
ApoL1	2.09	-11.55	-9.2
ApoM	2.36	-6.47	-4.81
Lp(a)	5.25	-7.51	3.57

Supplementary Table S3. The matrix factor of all measured peptide segments of apolipoproteins and Lp(a) in mice plasma.

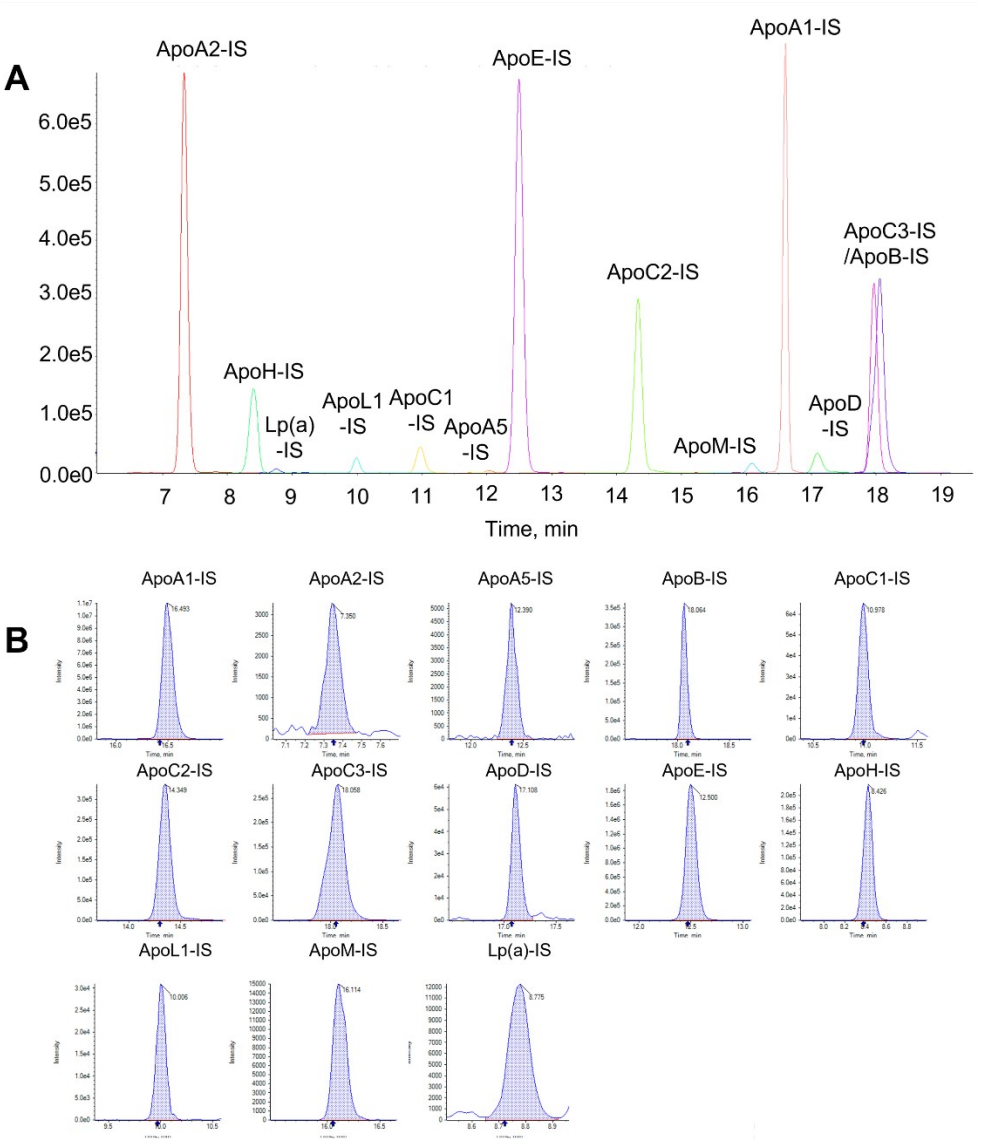
Peptide	LQC		MQC		HQC	
	Con (ng/ml)	% MF	Con (ng/ml)	% MF	Con (ng/ml)	% MF
ApoA1	12500	99.10	25000	104.65	100000	101.03
ApoA2	6250	78.02	12500	87.56	50000	79.19
ApoA5	1.25	303.74	2.5	69.91	10	52.57
ApoB	312.5	99.22	625	94.19	2500	95.88
ApoC1	625	96.36	1250	102.41	5000	97.29
ApoC2	781.25	95.12	1562.5	96.86	6250	90.33
ApoC3	1562.5	97.00	3125	99.40	12500	95.74
ApoD	1562.5	96.23	3125	107.10	12500	100.32
ApoE	156.25	89.51	312.5	99.08	1250	93.16
ApoH	625	99.69	1250	98.84	5000	93.94
ApoJ	1562.5	95.15	3125	70.43	12500	65.18
ApoL1	312.5	86.86	625	72.11	2500	73.21
ApoM	156.25	83.66	312.5	79.92	1250	73.11
Lp(a)	31.25	88.43	62.5	113.24	250	95.62

Supplemental Table S4. Demographical characteristics and distribution of apolipoproteins and Lp(a) concentration in the human plasma from 45 volunteers.

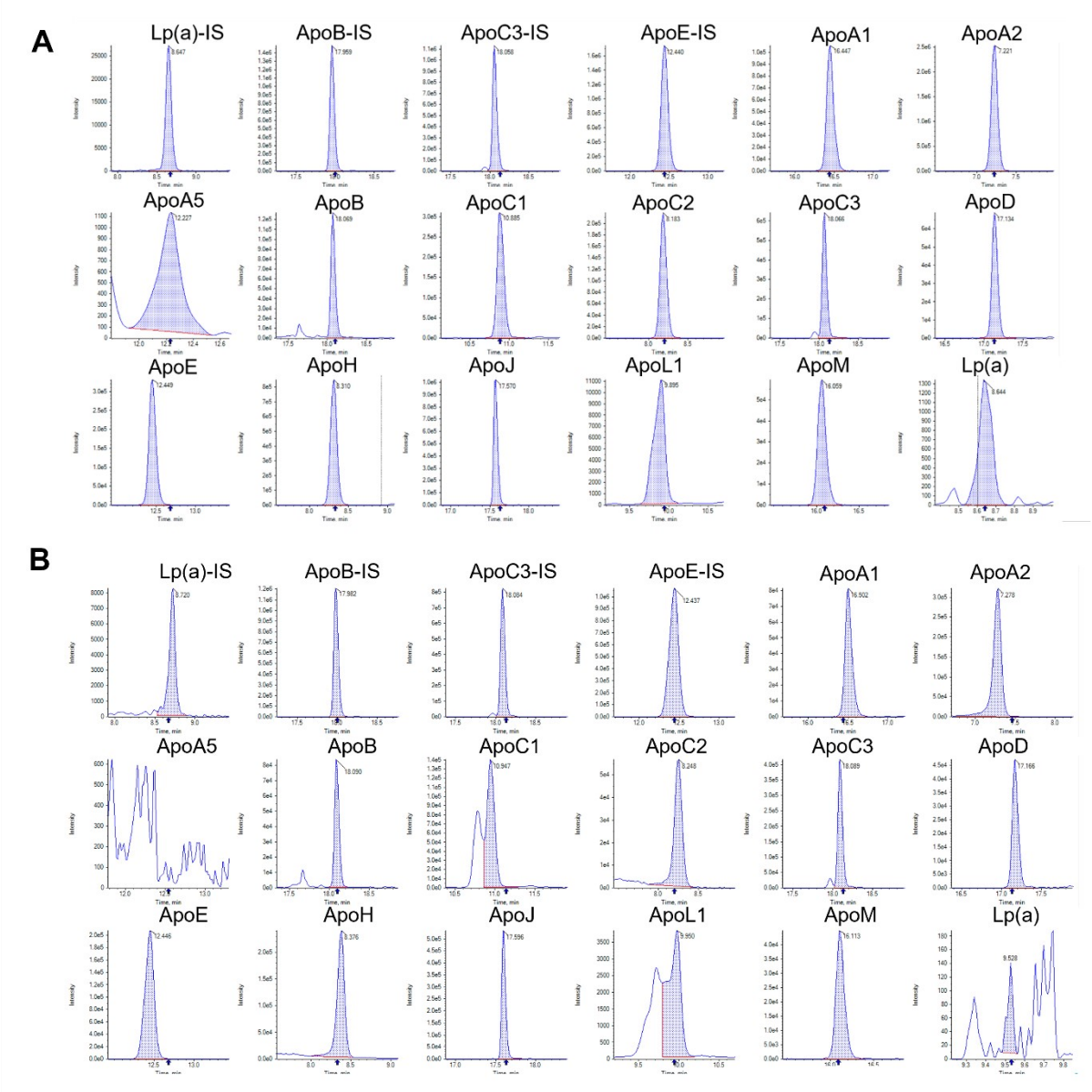
	Volunteers (N=45)
Demographical characteristics	
Age	57.6±14.7
Sex (Male, %)	23 (51.1%)
ALT (U/L)	32.6±35.5
AST (U/L)	29.2±17.1
TC (mmol/L)	4.3±1.4
TG (mmol/L)	1.9±1.6
HDL (mmol/L)	1.1±0.2
LDL (mmol/L)	2.5±1.1
Glucose (mmol/L)	6.4±2.2
Protein concentration	
ApoA1 (g/L)	0.99±0.22
ApoA2 (g/L)	0.19±0.05
ApoA5 (mg/L)	0.24±0.16
ApoB (g/L)	0.62±0.23
ApoC1 (mg/L)	30.31±12.38
ApoC2 (mg/L)	33.89±29.63
ApoC3 (mg/L)	52.48±43.7
ApoD (mg/L)	16.62±6.23
ApoE (mg/L)	55.02±52.29
ApoH (mg/L)	89.32±17.43
ApoJ (mg/L)	204.09±50.91
ApoL1 (mg/L)	30.28±10.15
ApoM (mg/L)	10.26±2.99
Lp(a) (nmol/L)	58.04±68.24

Note: All values are reported in the form of mean ± standard deviation.

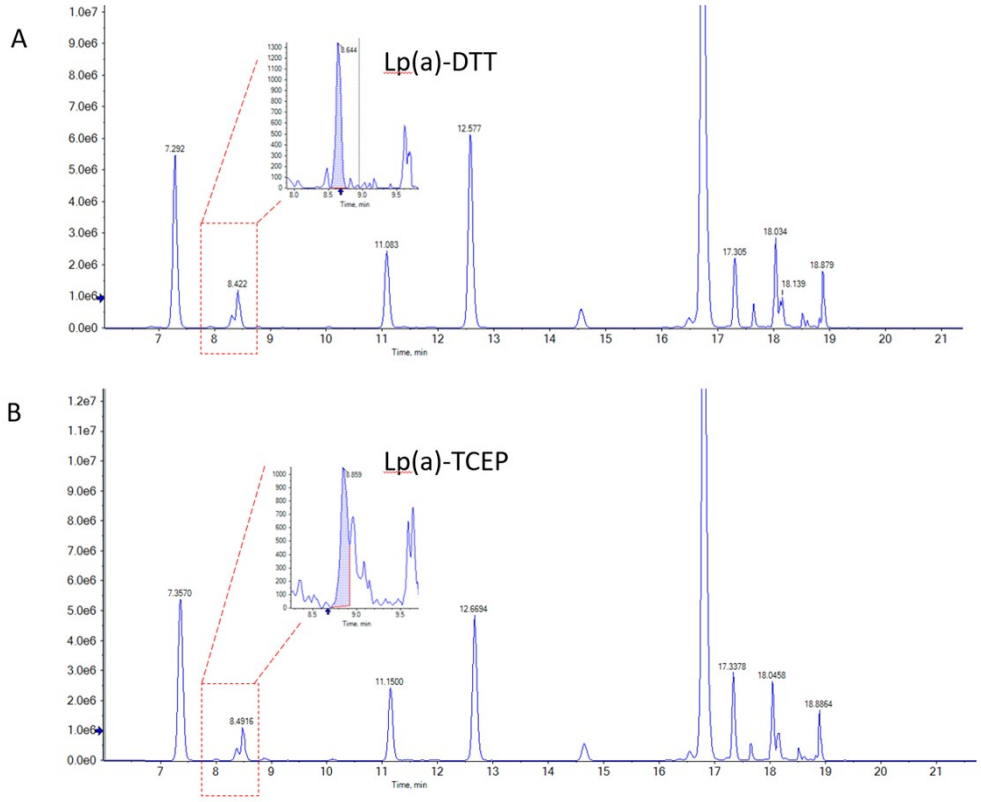
Supplementary Figure S1. The total (A) and dynamic multiple reaction monitoring (B) chromatograms of the internal standards of each peptide in a standard solution mixture.



Supplementary Figure S2. Effects of solid phase extraction on the dynamic multiple reaction monitoring (MRM) chromatograms containing peptide standards and representative internal standards. (A) human plasma without SPE extraction (B) human plasma with HLB SPE extraction.



Supplementary Figure S3. Effects of different reducing agents on the total ion chromatograms containing mixed peptide standards. (A) Extraction by adding reducing agent DTT. (B) Extraction by adding reducing agent TCEP.



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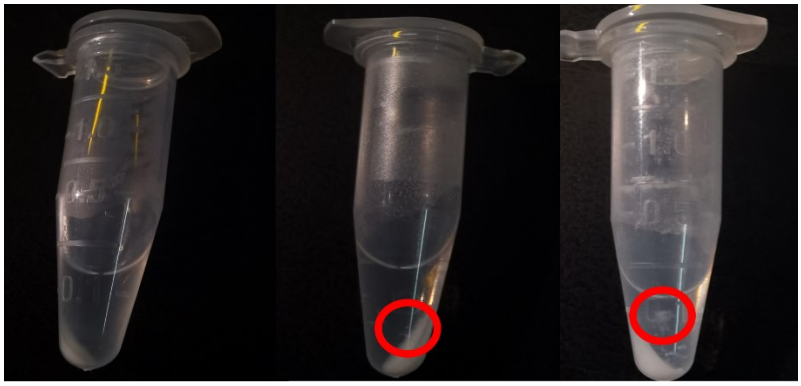

A B C

Supplemental Figure S5. The dynamic multiple reaction monitoring (MRM) chromatograms of different matrix solutions containing representative internal standards. (A) bovine serum albumin, (B) mice plasma.