Supporting Information

An automated high-throughput sample preparation method using double-filtration for serum metabolite LC-MS analysis

Minjoong Joo,[#a] Jong-Moon Park,[#a] Van-An Duong,[a] Dami Kwon,[a] Jongho Jeon,[a] Miso Han,[a] Byung-Kwan Cho,[b] Hyung-Kyoon Choi,[c] Choul-Gyun Lee,[d] Hee-Gyoo Kang,[e] and Hookeun Lee*[a]

[a.] *College of Pharmacy, Gachon University, Incheon 21936, Republic of Korea*

[b.] *Department of Biological Sciences, KAIST, Daejeon 305-701, Republic of Korea*

[c.] *College of Pharmacy, Chung-Ang University, Seoul 156-756, Republic of Korea*

[d.] *Institute of Industrial Biotechnology, Department of Biological Engineering, Inha University, Incheon 402-751, Republic of Korea*

[e.] *Department of Biomedical Laboratory Science, College of Health Sciences, Eulji University, Seongnam-si, Gyeonggi-do 13135, Republic of Korea*

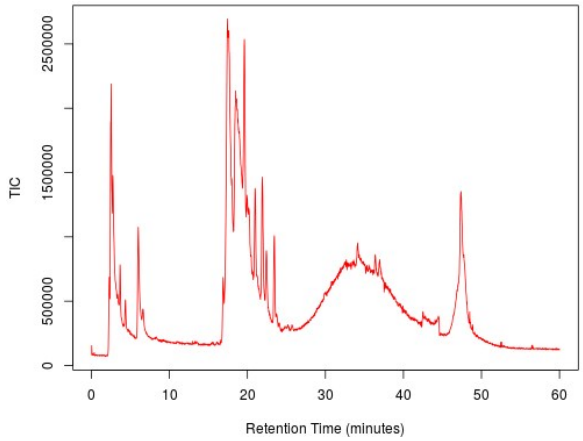
[#] These authors contributed equally

*Corresponding Author

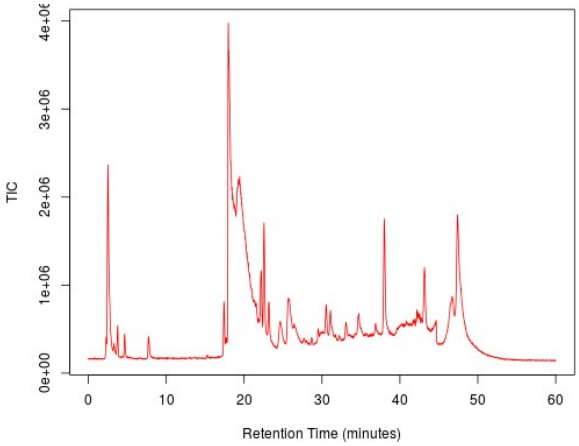
Telephone: + 82-32-820-4927. Email: hklee@gachon.ac.kr

Table of Content

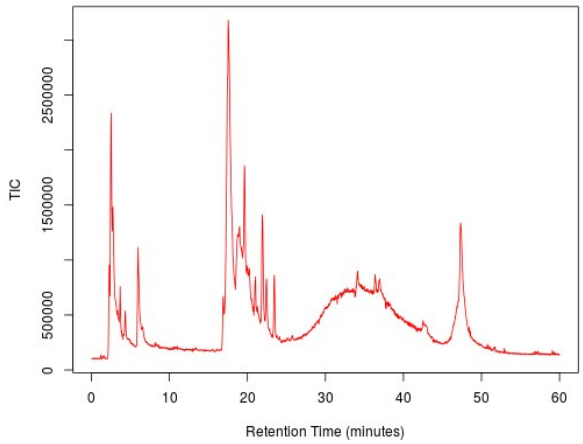
A-1



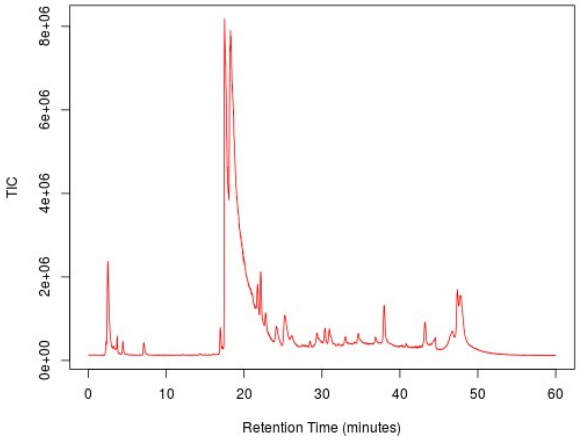
B-1



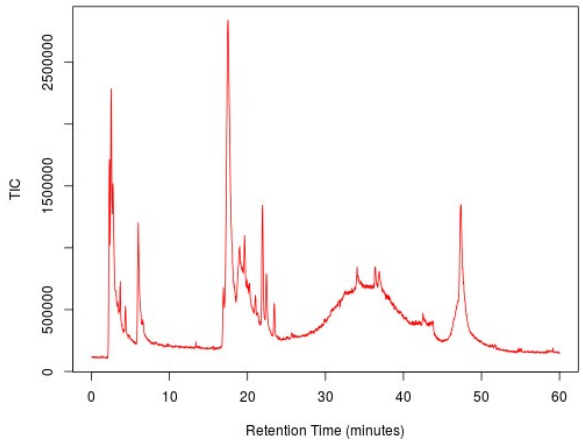
A-2



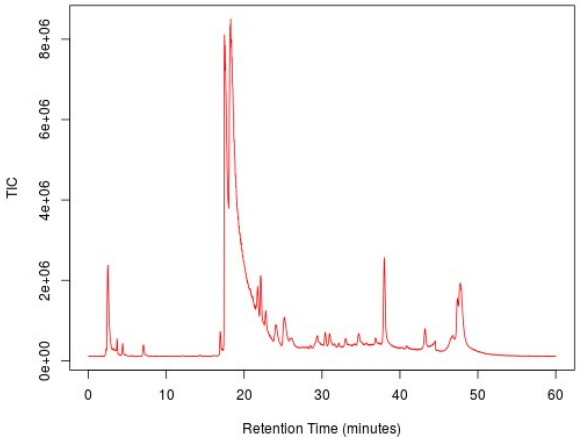
B-2



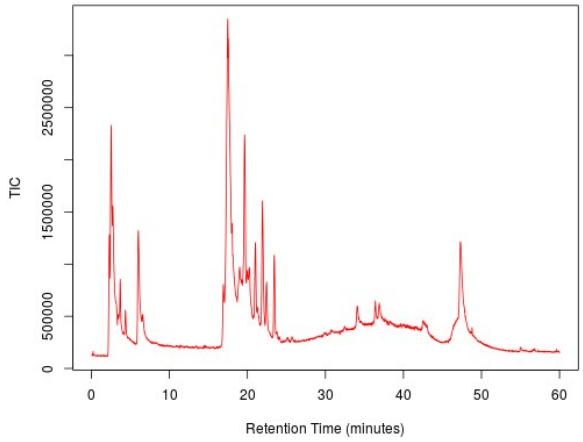
A-3



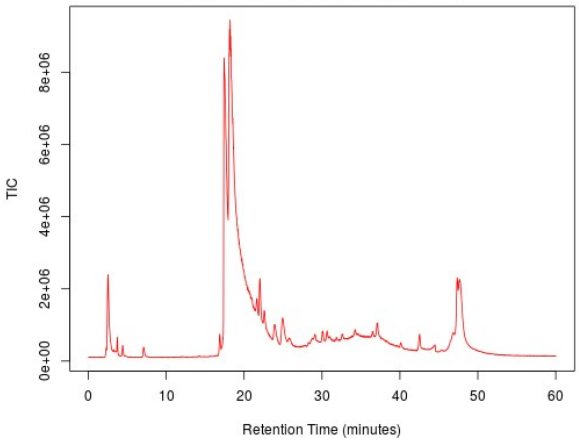
B-3



A-4



B-4



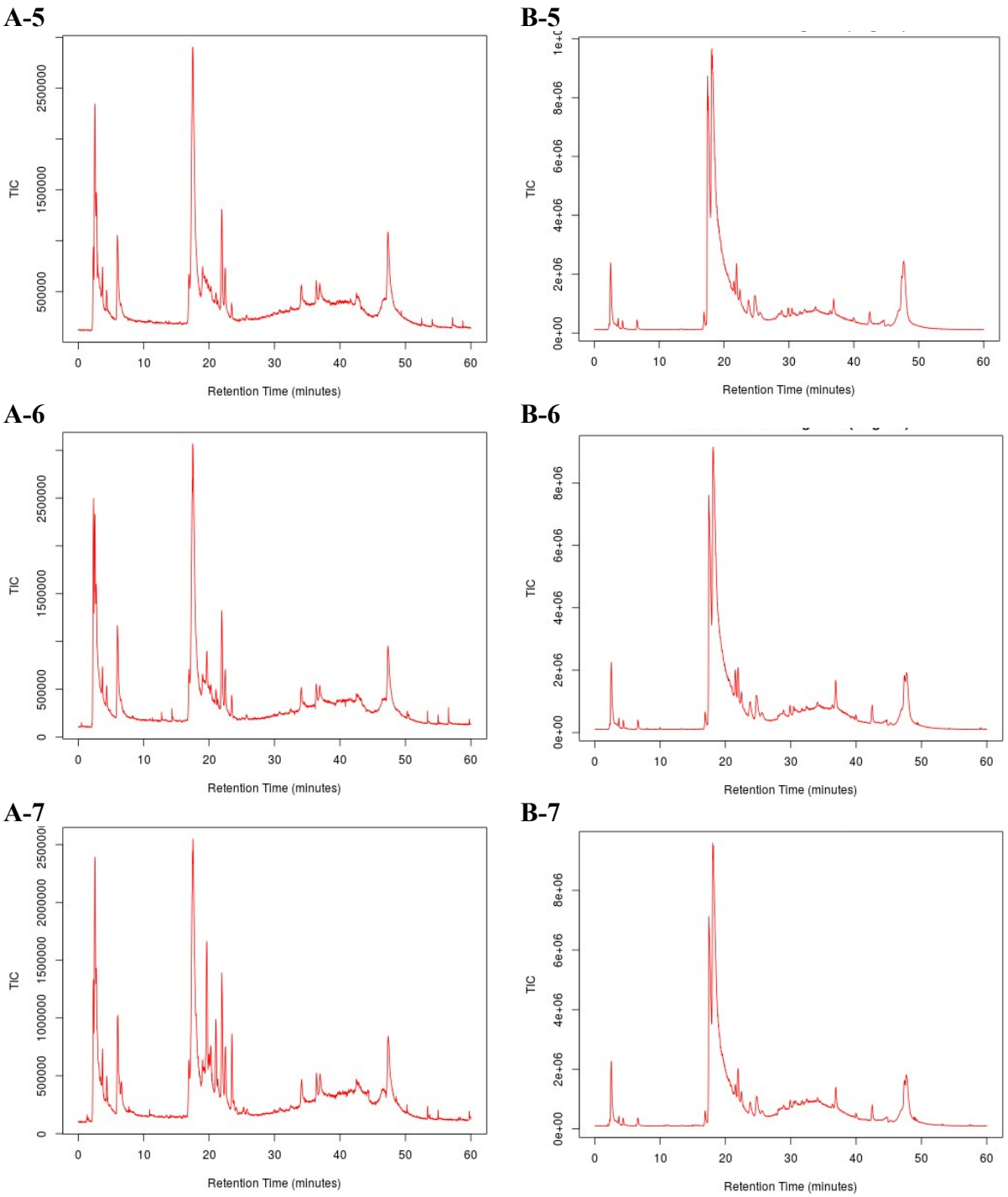
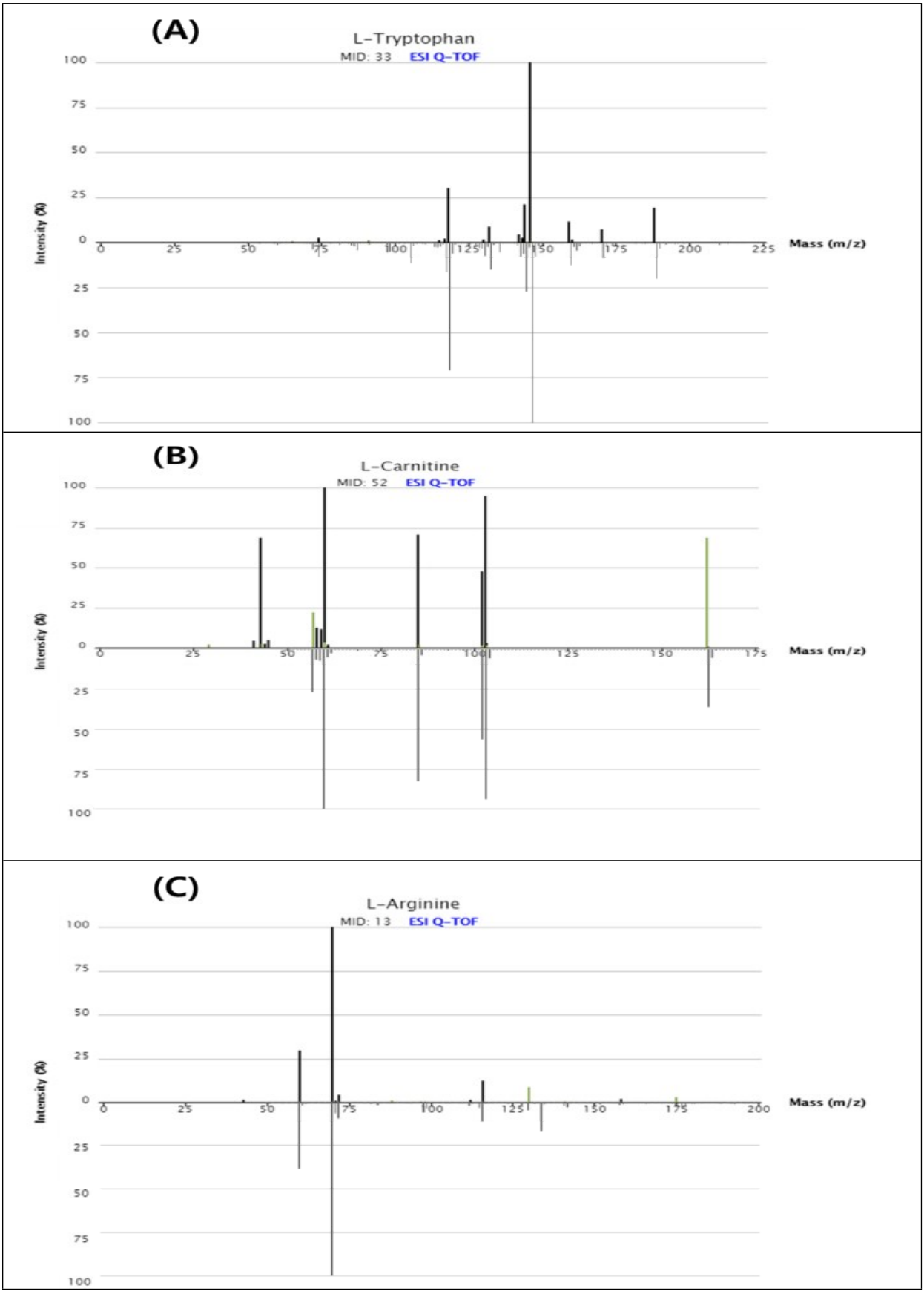
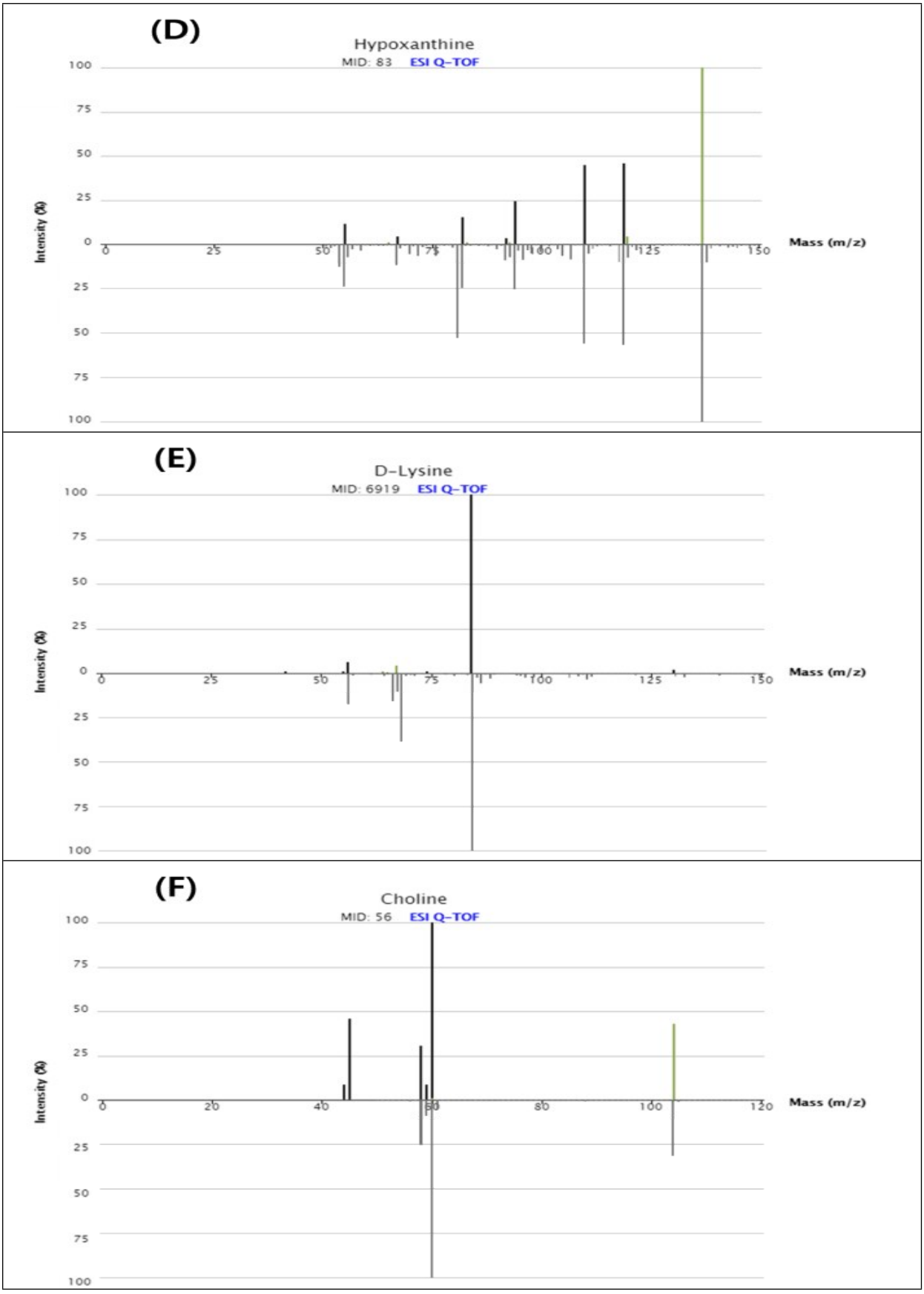


Fig. S1. Total ion current (TIC) chromatograms of seven samples prepared by (A) automated double-filtration method and (B) manual centrifugation-based method


(A)
L-Tryptophan
MID: 33
ESI Q-TOF
Intensity (%)
Mass (m/z)
(B)
L-Carnitine
MID: 52
ESI Q-TOF
Intensity (%)
Mass (m/z)
(C)
L-Arginine
MID: 13
ESI Q-TOF
Intensity (%)
Mass (m/z)


(D)
Hypoxanthine
MID: 83
ESI Q-TOF
Intensity (%)
Mass (m/z)
(E)
D-Lysine
MID: 6919
ESI Q-TOF
Intensity (%)
Mass (m/z)
(F)
Choline
MID: 56
ESI Q-TOF
Intensity (%)
Mass (m/z)

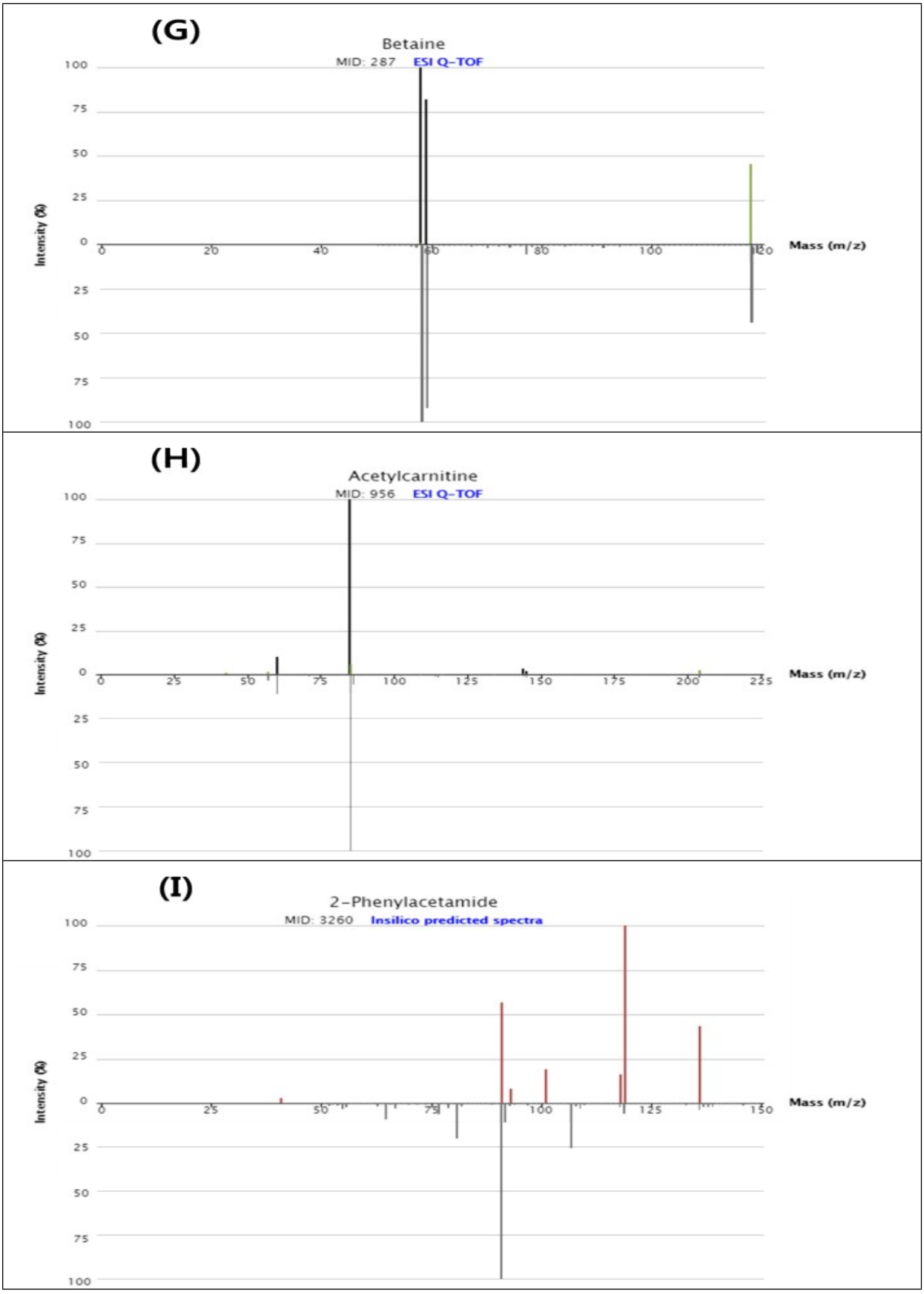
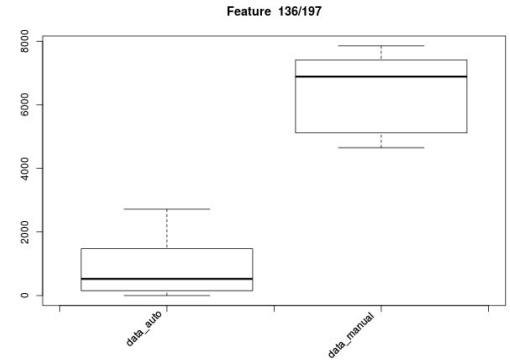
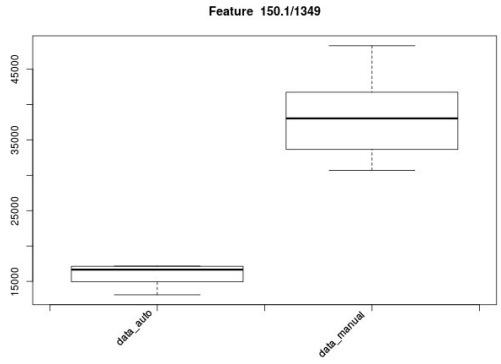


Fig. S2. MS/MS match of 9 endogenous metabolites between the data obtained from the HPLC/QTOF-MS analysis (up) and the data in Metlin database (down)

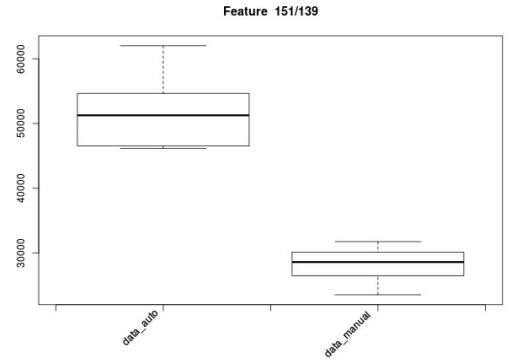
(1) Xanthine



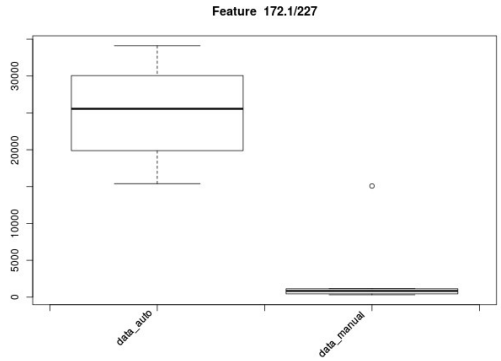
(2) L-phenylalanine



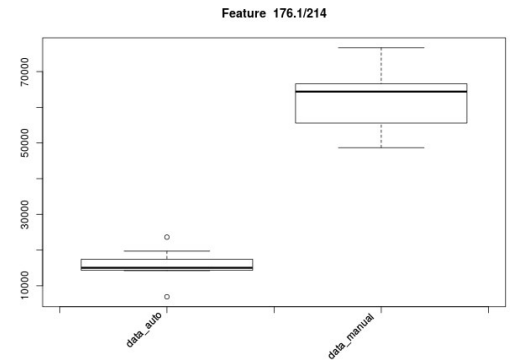
(3) 3,4-dihydroxyphenylacetate/ pyridoxal



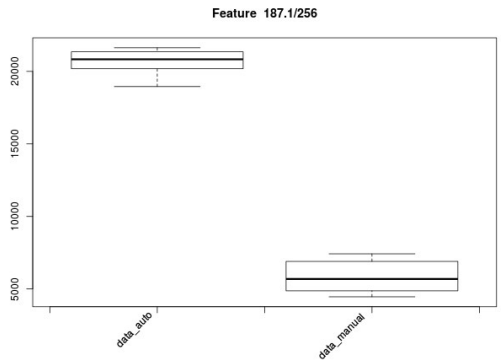
(4) 5'-α-carboxylmethylbutylhydroxychroman



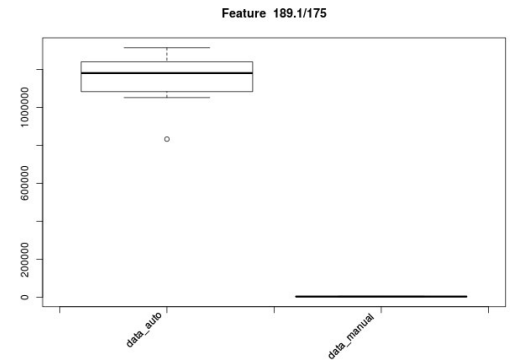
(5) 5-hydroxyindole acetaldehyde/ indole-3-acetate



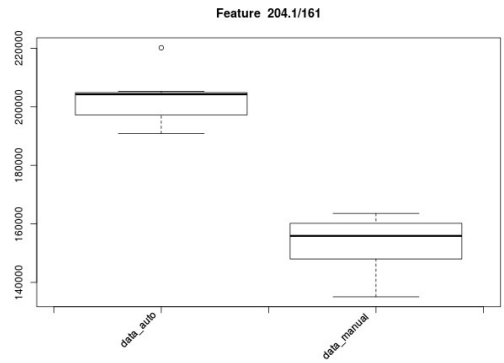
(6) 7-O-acetylsalutaridinol



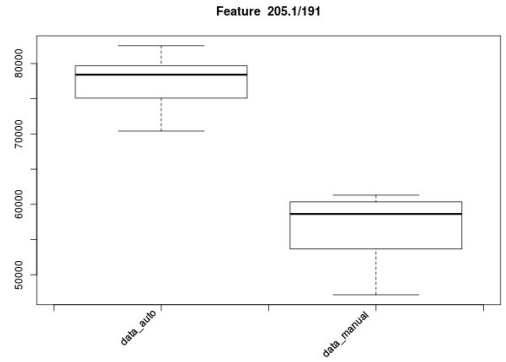
(7) L-tryptophan



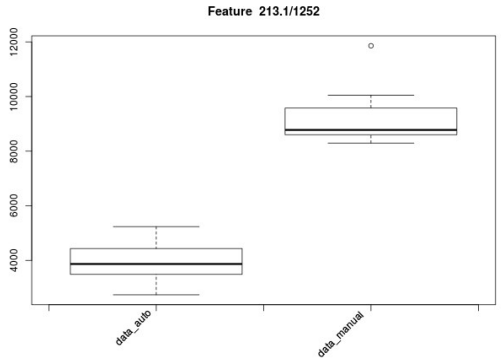
(8) 4-hydroxy-4-(3-pyridyl)-butanoate



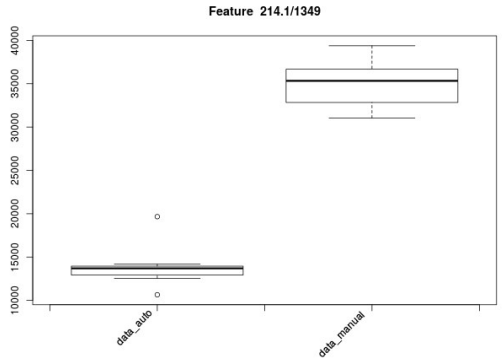
(9) O-acetylcarnitine



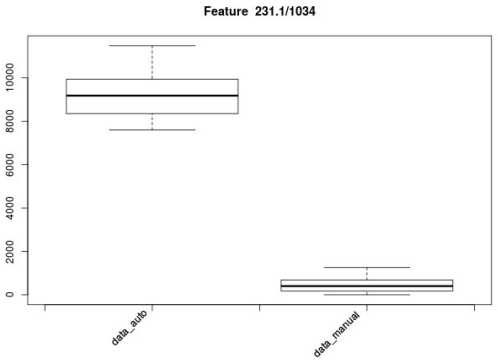
(10) 3-methoxy-4-hydroxyphenyllactate



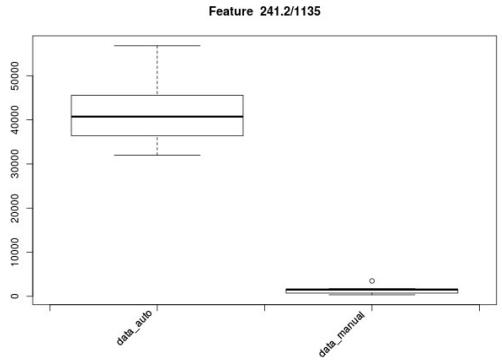
(11) 5-methoxytryptophol



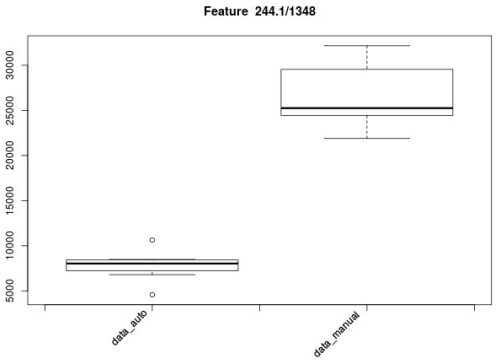
(12) Thyronamine



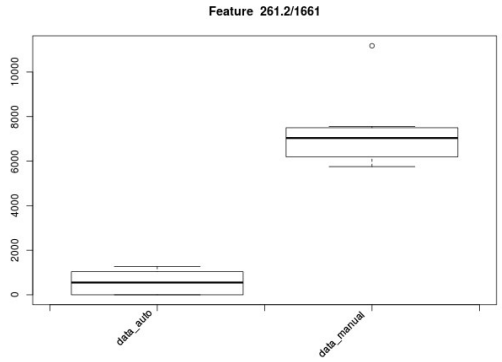
(13) Spermine



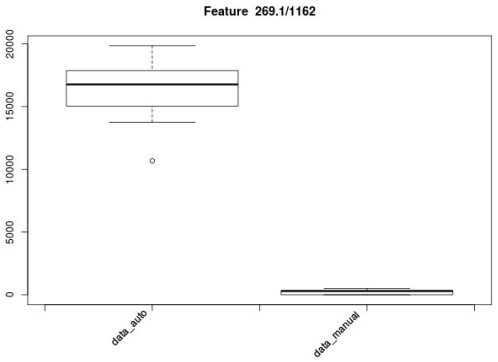
(14) 4-hydroxy-2-nonenal-glutathione conjugate



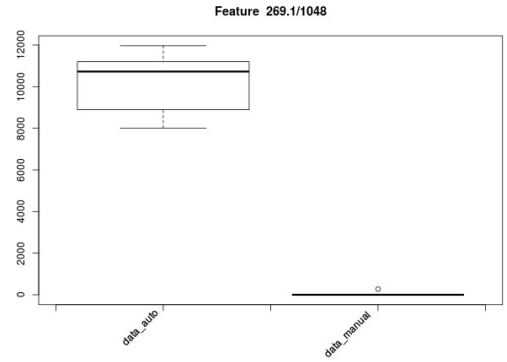
(15) 2-trans-hexadecenal



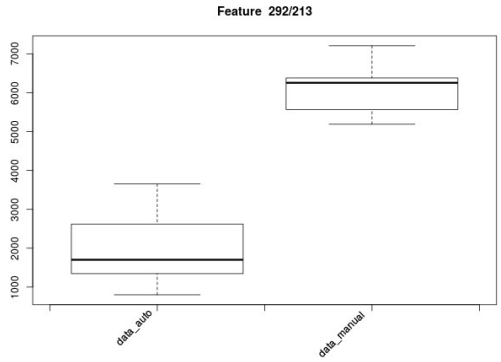
(16) Inosine



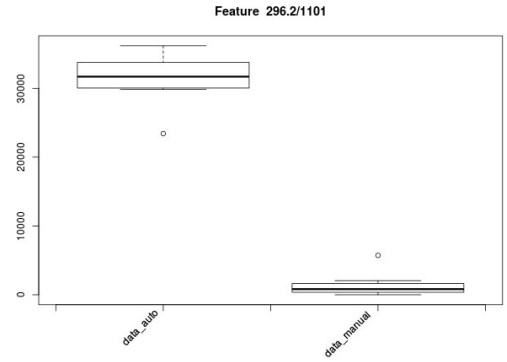
(17) γ-L-glutamyl-L-cysteine



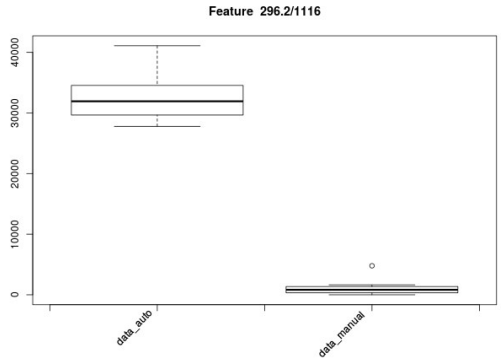
(18) (R)-mevalonatediphosphate



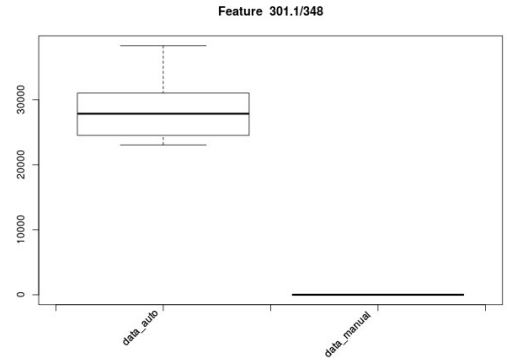
(19) Protoporphyrinogen IX



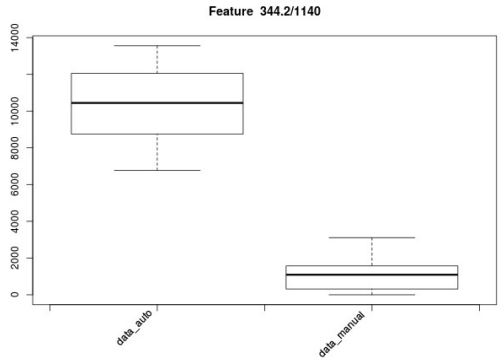
(20) Protoporphyrinogen IX[2]



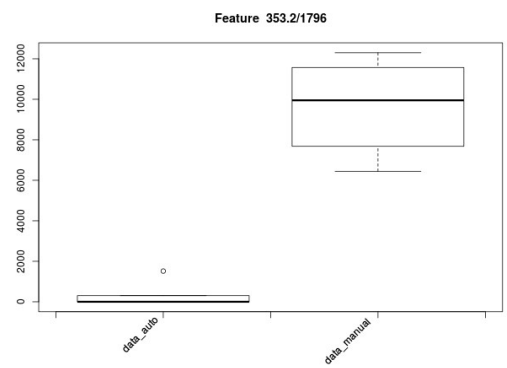
(21) 4-hydroxy-2-nonenal-[L-Cys] conjugate



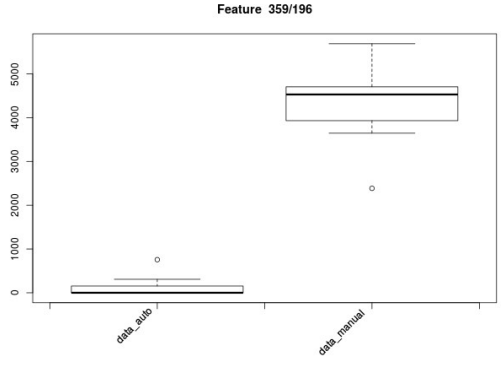
(22) Aldosterone



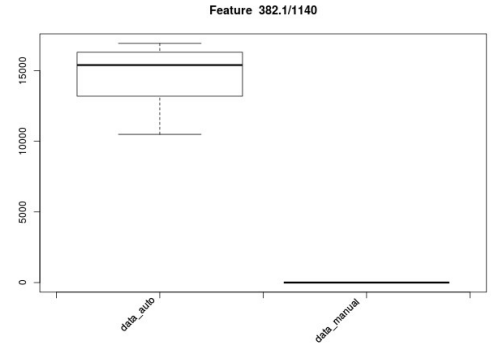
(23) Prostaglandin E2/ thromboxane A2/ lipoxin A4/ prostaglandin-H2



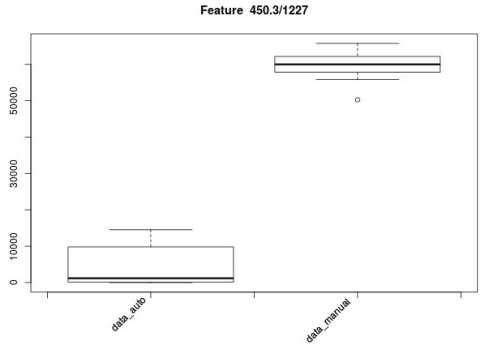
(24) β-nicotinate D-ribonucleotide



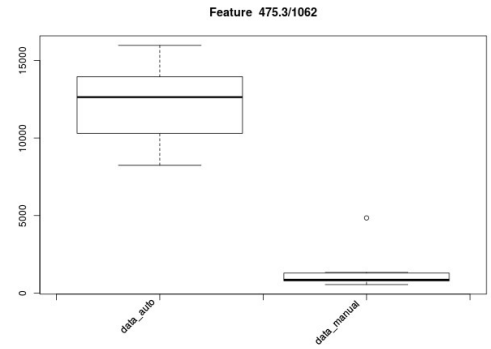
(25) S-adenosyl-L-methionine



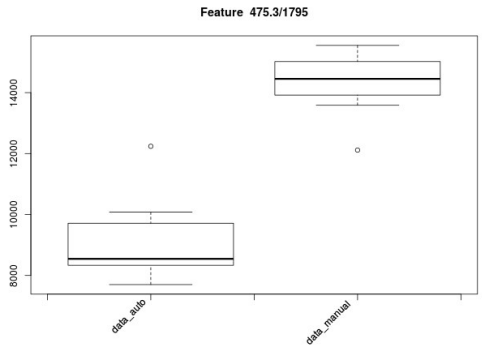
(26) Sphingosylphosphorylcholine



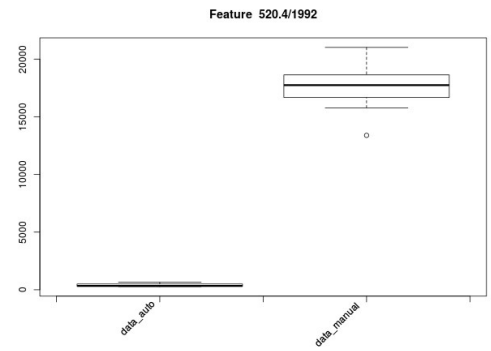
(27) (25R)-5β-cholestane-3α,7α,12α,26-tetraol



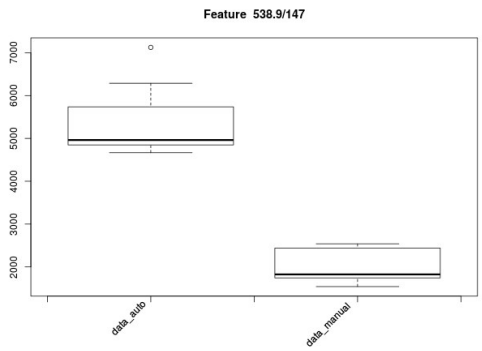
(28) (25R)-5β-cholestane-3α,7α,12α,26-tetrao[2]



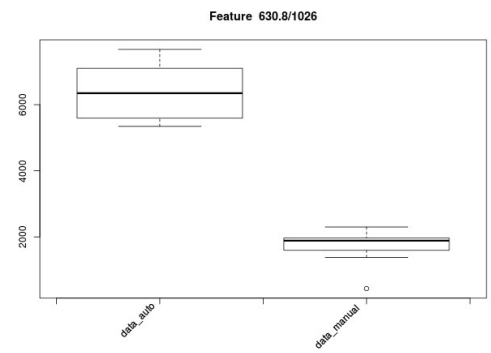
(29) All-trans-β-carotene



(30) D-myo-inositol (3,4,5,6)-tetrakisphosphate



(31) 3,3',5-triiodothyronamine



(32) 1D-myo-inositol 1,2,3,4,5,6-hexakisphosphate

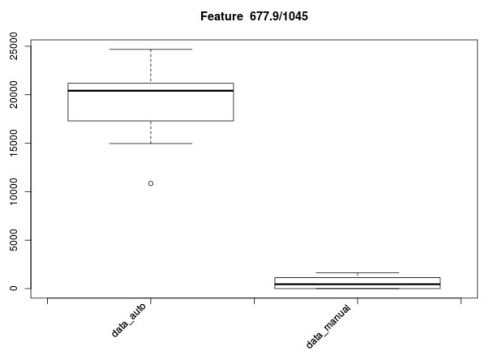


Fig. S3. Box plots of peak areas of 32 metabolites in automated method (left) and manual method (right)

Table S1. Number of features detected in two sample preparation methods by XCMS

Method	Sample code	Number of features	Average	SD
Automated method	1	4769	4674	385
	2	4707		
	3	4586		
	4	5392		
	5	4093		
	6	4595		
	7	4573		
Manual method	1	3702	4150	221
	2	4119		
	3	4202		
	4	4424		
	5	4146		
	6	4241		
	7	4217		

Table S2. Statistical values of RSDs of peak areas obtained from XCMS for automated method and manual method

	Automated method	Manual method
Upper whisker	72.11	155.70
3rd quartile	39.82	73.21
Median	26.18	32.10
1st quartile	18.14	17.73
Lower whisker	2.43	2.39
Number of data points	2355	2418

Table S3. Formula, *m/z*, and retention time of 9 serum metabolites identified by the MS/MS match with the Metlin database

Metabolite	Formula	m/z ($[M+H]^+$)	R_t (min)
L-Tryptophan	$C_{11}H_{12}N_2O_2$	205.0972	6.5973
L-Carnitine	$C_7H_{16}NO_3$	162.1125	2.6960
L-Arginine	$C_6H_{14}N_4O_2$	175.1190	2.5662
Hypoxanthine	$C_5H_4N_4O$	137.0458	4.0912
D-Lysine	$C_6H_{14}N_2O_2$	147.1128	2.3944
Choline	$C_5H_{14}NO$	104.1070	2.6740
Betaine	$C_5H_{12}NO_2$	118.0863	2.8432
Acetylcarnitine	$C_9H_{18}NO_4$	204.1230	3.1851
2-Phenylacetamide	C_8H_9NO	136.0757	3.6725

Table S4. Peak areas of 9 serum metabolites identified by the MS/MS match with the Metlin database

Method	Metabolite	Peak area						
		1	2	3	4	5	6	7
Automated method	L-Tryptophan	575943	268079	261108	538398	155429	224629	629008
	L-Carnitine	1177348	1211202	1140874	1203456	1182163	1130998	1267629
	L-Arginine	163428	159341	210816	174526	152180	192632	158952
	Hypoxanthine	197574	139039	134047	134102	180523	137254	189515
	D-Lysine	66791	66683	56318	64305	64372	44511	71740
	Choline	166722	162521	163450	174874	161463	166616	182557
	Betaine	782676	859949	736650	807616	879324	857151	879582
	Acetylcarnitine	735911	702035	658883	712061	662052	689641	756964
	2-Phenylacetamide	148955	115481	128231	146143	147102	118634	139354
Manual method	L-Tryptophan	355	1204763	1165012	1047988	1177229	1055711	1242902
	L-Carnitine	940872	1145361	1148257	925791	1184293	1124987	1102187
	L-Arginine	136307	145478	183041	218912	159162	189060	131104
	Hypoxanthine	81905	199606	74507	76431	165094	161119	97748
	D-Lysine	72798	83397	86696	85282	83572	80051	70174
	Choline	219173	253916	240200	603079	223235	208561	200339
	Betaine	771065	851315	809080	920873	799670	778480	706442
	Acetylcarnitine	464427	550148	546004	539558	540560	506227	471068
	2-Phenylacetamide	149901	166463	168207	188174	133942	123493	123236

Table S5. The mean, SD, and RSD of peak areas of 9 serum metabolites identified by the MS/MS match with the Metlin database

Metabolite	Automated method			Manual method		
	Mean	SD	RSD	Mean	SD	RSD
L-Tryptophan	378942	194386	51	984851	440200	45
L-Carnitine	1187667	46070	4	1081678	104436	10
L-Arginine	173125	21318	12	166152	32117	19
Hypoxanthine	158865	28856	18	122344	51538	42
D-Lysine	62103	9025	15	80281	6389	8
Choline	168315	7697	5	278358	144336	52
Betaine	828993	54731	7	805275	67311	8
Acetylcarnitine	702507	36236	5	516856	36500	7
2-Phenylacetamide	134843	14017	10	150488	24950	17