Electronic Supplementary Material (ESI) for Toxicology Research.

Metabolomic Profiling of Emodin-induced Cytotoxicity in Human Liver Cells and the Mechanism Study

Xiaoyan Liu[a,b], Yanqiu Liu[a,c], Yang Qu[a,d], Mengchun Cheng[a], Hongbin Xiao[a,e,]*

[a]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, China

[b]University of Chinese Academy of Sciences, Beijing 100049, China

[c]College (Institute) of Integrative Medicine, Dalian Medical University, Dalian 116044, China

[d]College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian 116600, China

[e]Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China

***Corresponding author:** Prof. Hongbin Xiao, E-mail: hbxiao69@163.com; Tel: ＋86 411 8437 9756; Fax: ＋86 411 8437 9667

Supplementary material

ESM_1 Kinetics of cytotoxicity induced by emodin in L-02 cells. The graph was plotted using GraphPad Prism 5.0

ESM_2 Morphological changes of L-02 cells cultured without emodin or with 10, 20 and 30 μM emodin

ESM_3 Data quality assessment. **a.** The retention time deviation profile generated by XCMS. A positive deviation indicates that the sample was eluting after the median retention time, and a negative deviation indicates that the samples was eluting before the median retention time. **b.** PCA first component for the QC samples versus time analyzed. Raw data of QC sample was analyzed using XCMS online, which follows typical data processing steps, including peak discrimination, peak filtering, peak alignment, noise elimination and the generation of a matrix that consisted of the retention time, m/z value and the peak areas. The data was analyzed using SIMCA-P 11.0 (Umetrics, Sweden) software

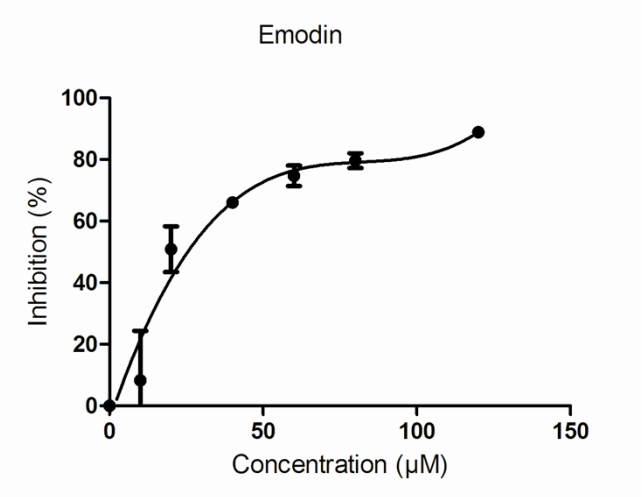
ESM_4 Extracted ion chromatography of identified metabolites listed in Table S1 and the corresponding structures.

ESM_5 Chemical synthesis of emodin-cysteine adduct and structure validation. **a.** MS/MS fragments of emodin-cysteine synthesized through chemical reaction. **b.** Possible mass spectrometric pattern of emodin-cysteine.

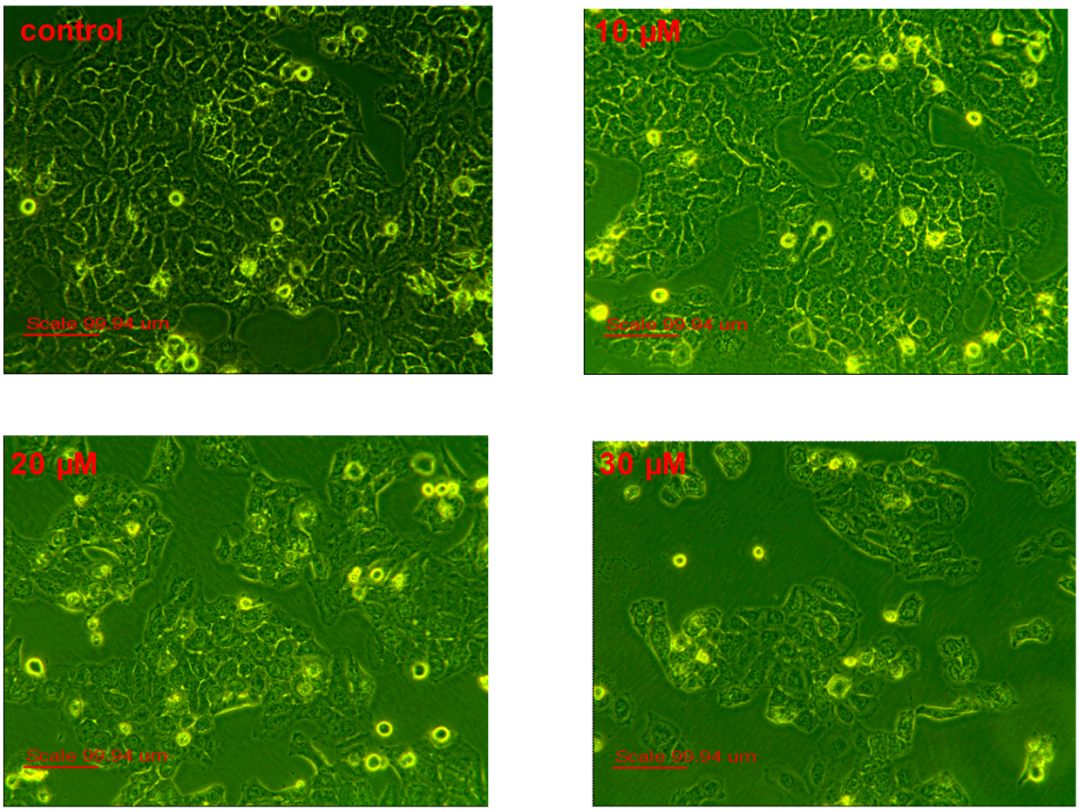
Synthesis of emodin-cysteine adduct: Cysteine (24 mg, 0.2 mmol) was added to 20 ml of sodium carbonate buffer (pH 9.2). The pH was adjusted to 8.0 by adding 200 mM NaOH. A solution of emodin (5 mg) in DMSO (2.5 ml) was added to the cysteine solution, and the mixture was stirred overnight at room temperature. The reaction mixture was detected using analytical method described in “Section 2.4”.

Table S1 Summary of significant biomarkers identified under the positive and negative mode

ESM_1


Emodin
Inhibition (%)
100
80
60
40
20
0
0
50
100
150
Concentration (μM)

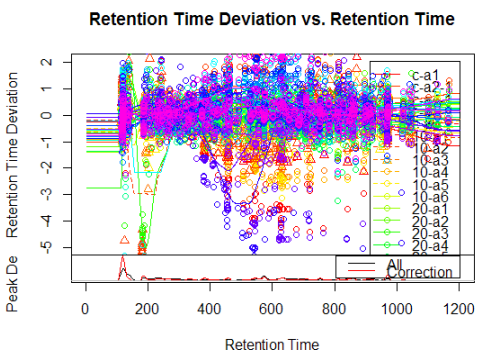
ESM_2


control
Scale 99.94 um
10 µM
Scale 99.94 um
20 µM
Scale 99.94 um
30 µM
Scale 99.94 um

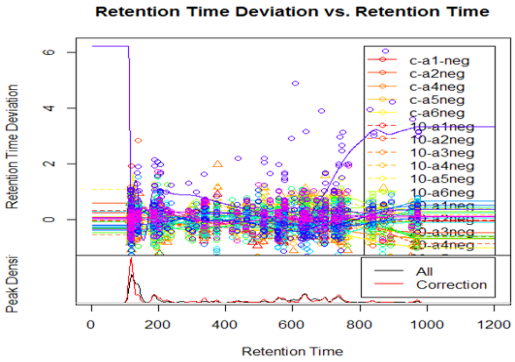
ESM_3

a

Positive mode

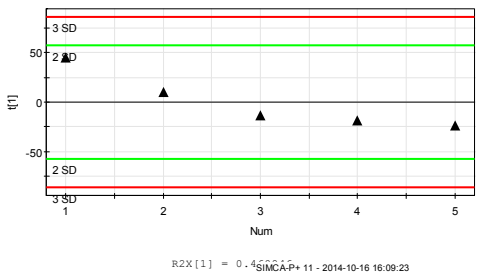


Negative mode

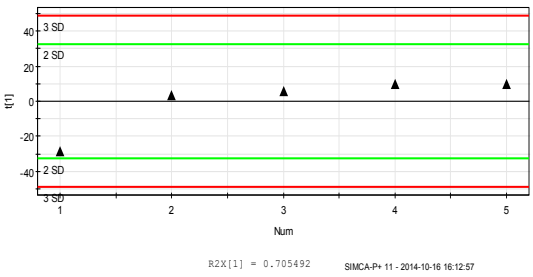


b

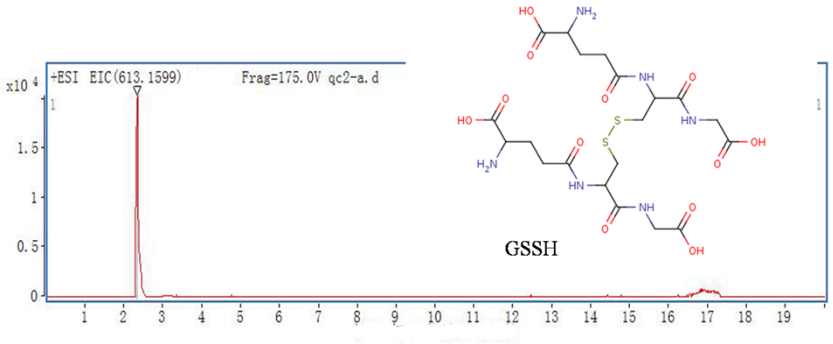
Positive mode



Negative mode



ESM_4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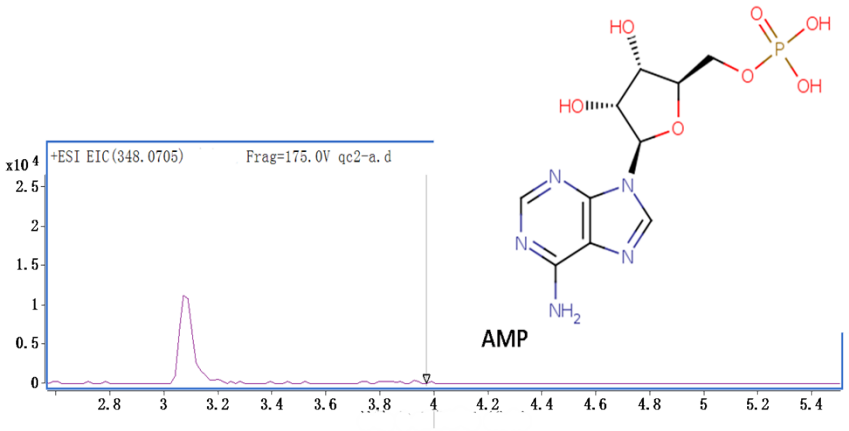
x10 4
GSSH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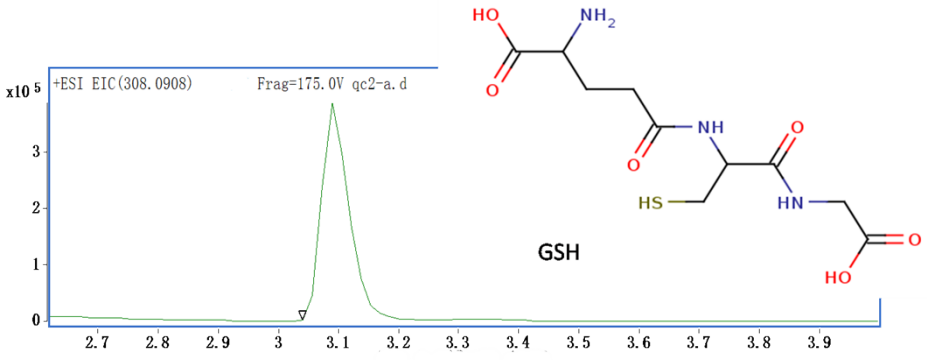
x10 4
AMP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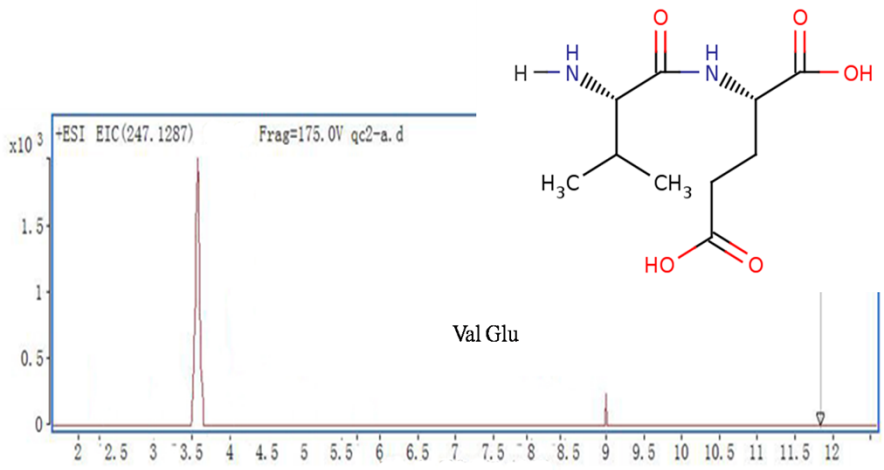
x10 5
GSH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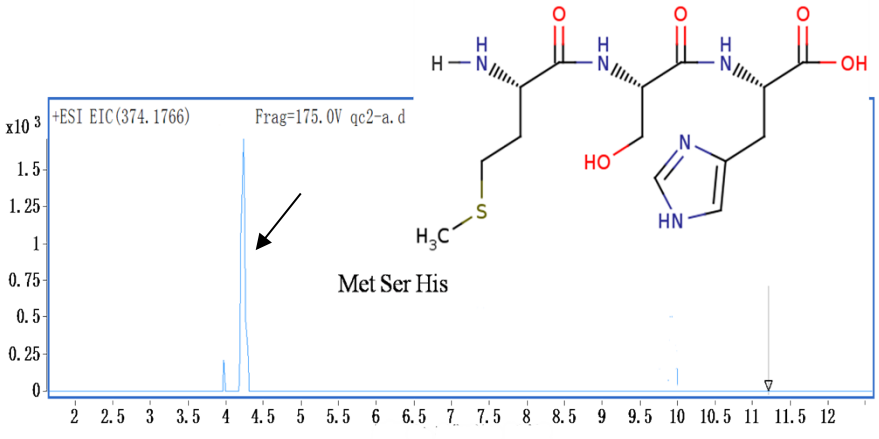
x10 3
Val Glu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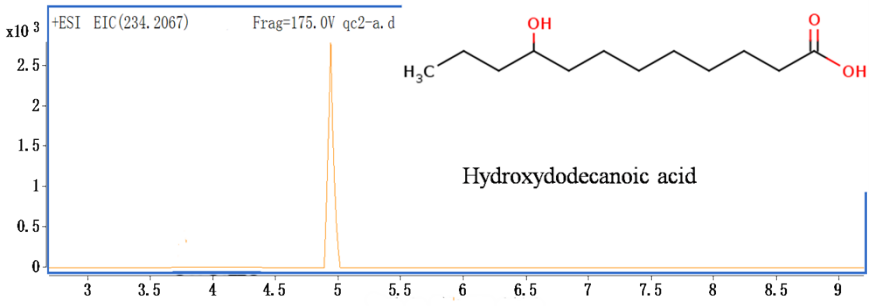
x10 3
Met Ser His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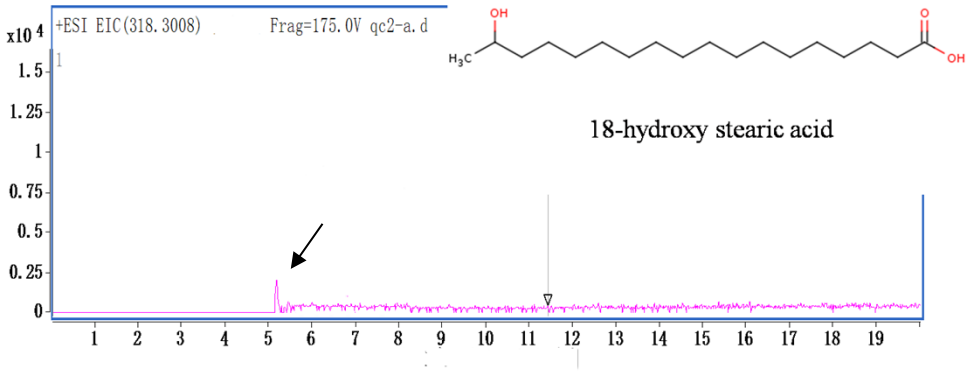
x10 3
Hydroxydodecanoic acid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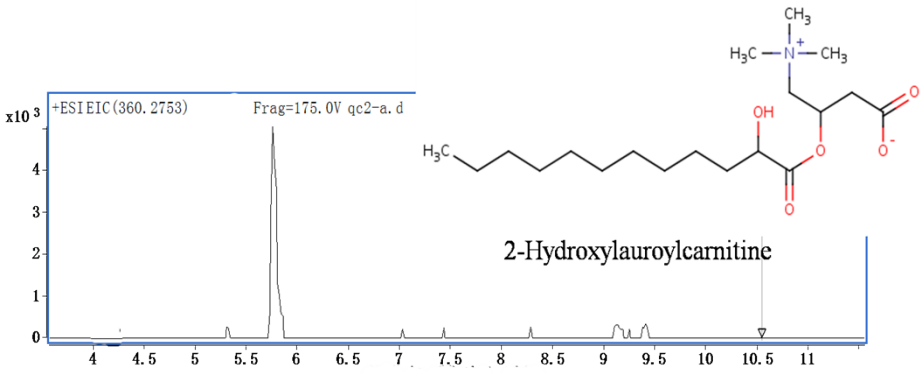
Frag=175.0V qc2-a.d
x10 4
18-hydroxy stearic acid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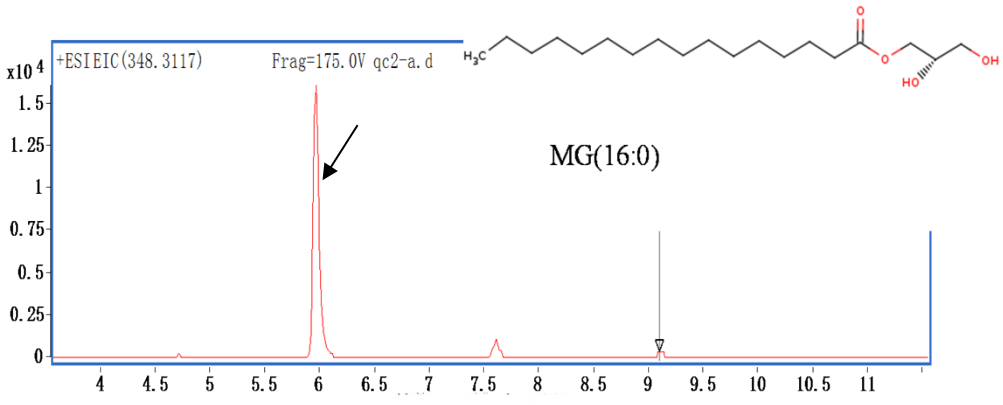
Frag=175.0V qc2-a.d
x10 3
2-Hydroxylauroylcarnitine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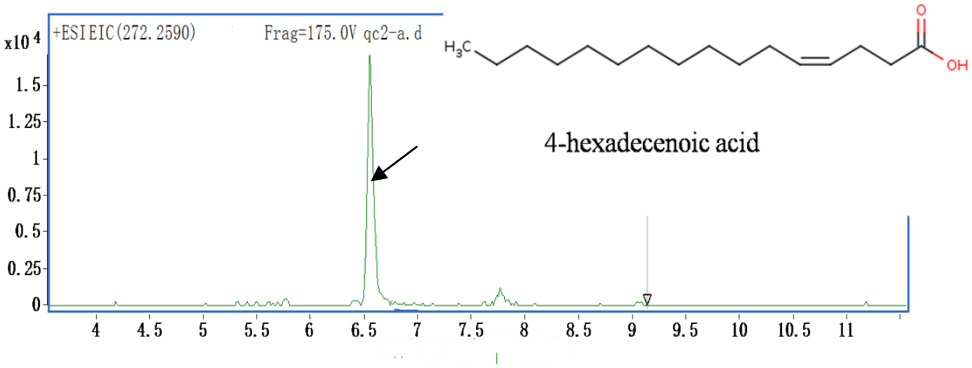
Frag=175.0V qc2-a.d
x10 4
MG(16:0)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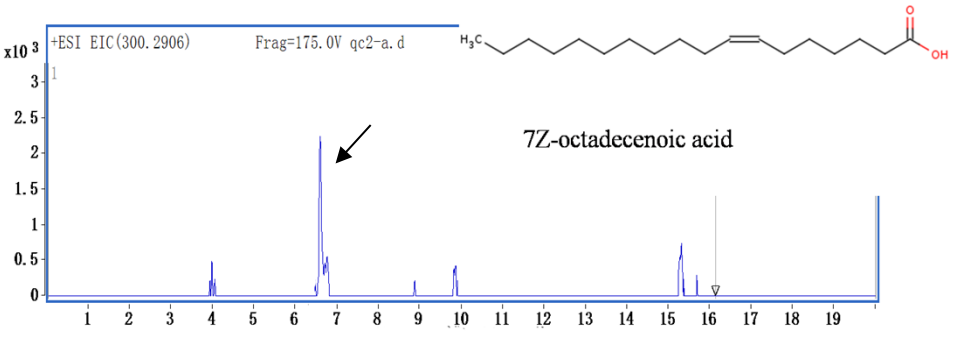
x10 4
4-hexadecenoic acid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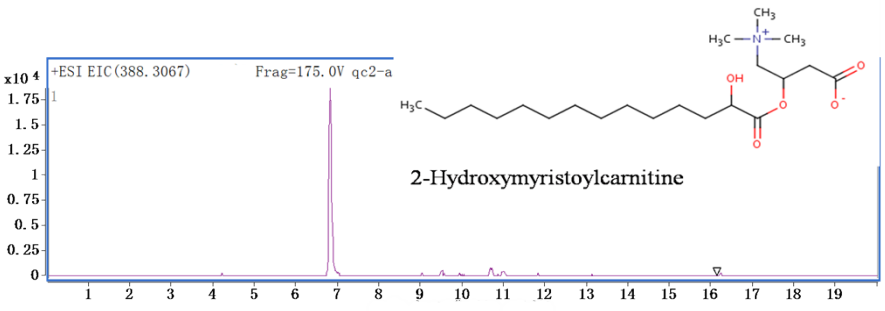
x10 3
7Z-octadecenoic acid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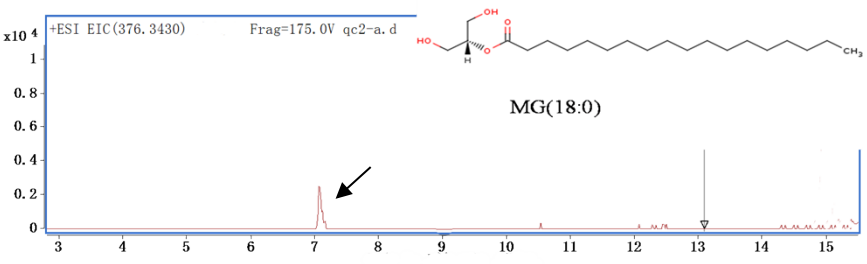
2-Hydroxymyristoylcarnitine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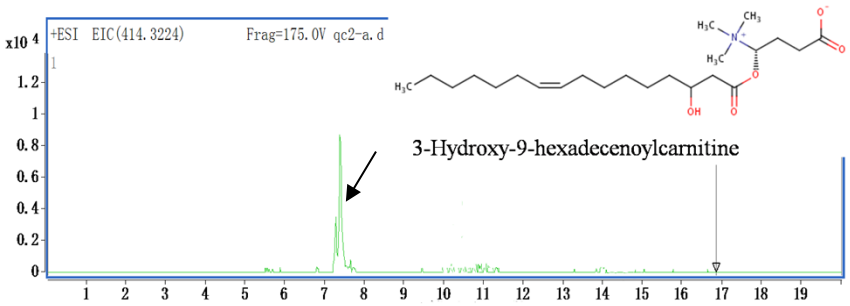
x10 4
MG(18:0)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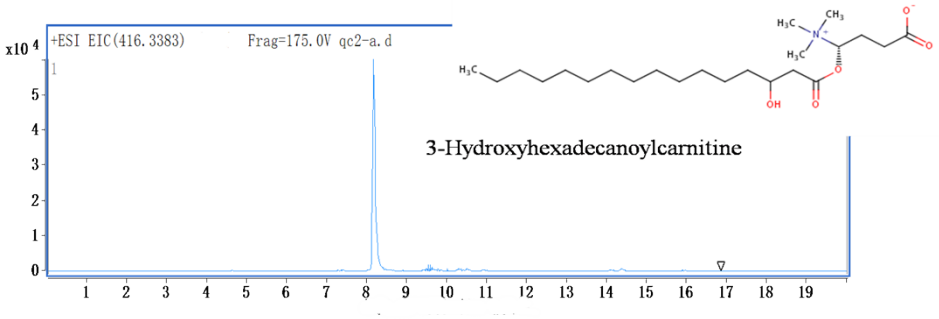
x10 4
3-Hydroxy-9-hexadecenoylcarnitine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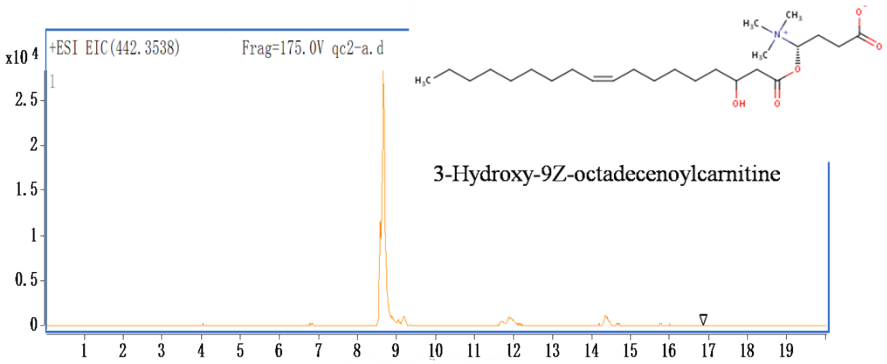
x10 4
3-Hydroxyhexadecanoylcarnitine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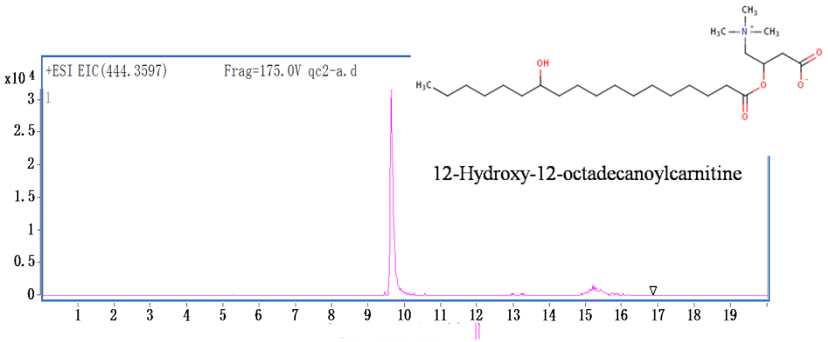
x10 4
3-Hydroxy-9Z-octadecenoylcarnitine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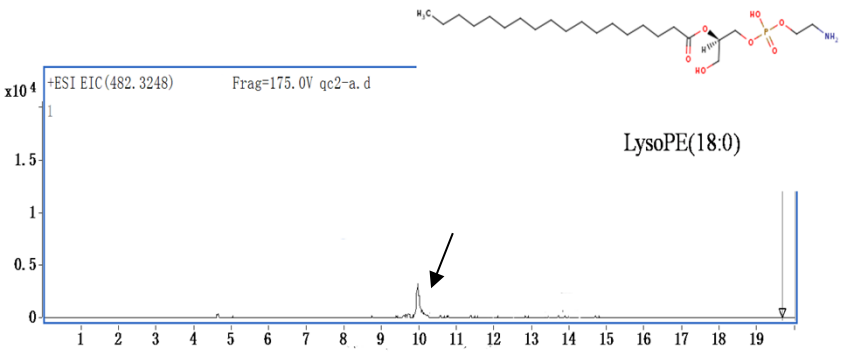
x10 4
12-Hydroxy-12-octadecanoylcarnitine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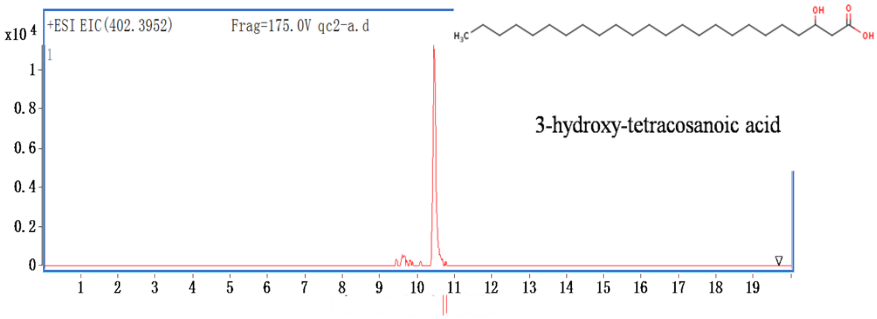
x10 4
LysoPE(18:0)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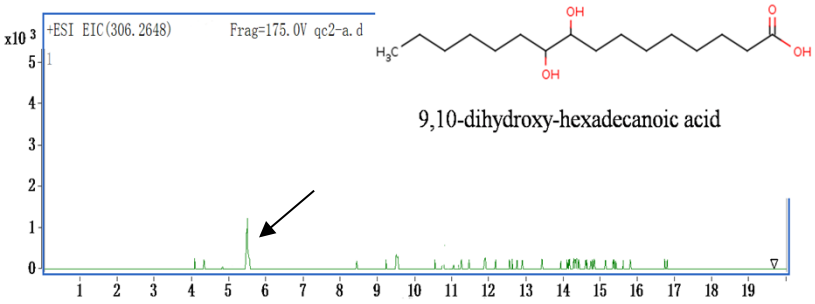
x10 4
3-hydroxy-tetracosanoic acid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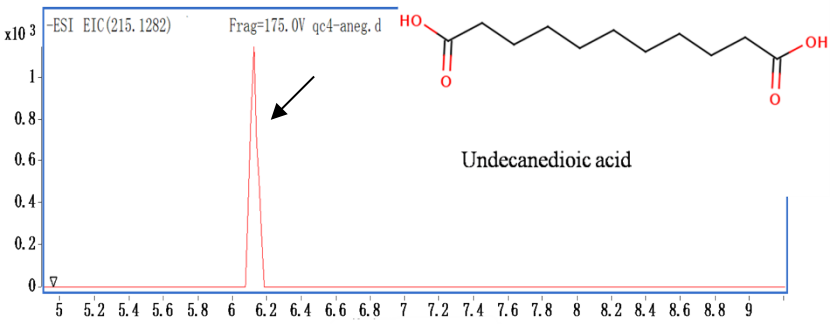
x10 3
9,10-dihydroxy-hexadecanoic acid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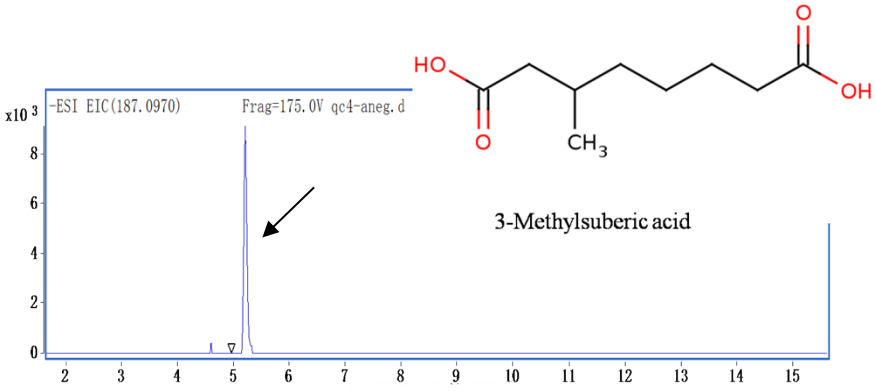
Frag=175.0V qc4-aneg.d
x10 3
HO
OH
O
O
Undecanedioic acid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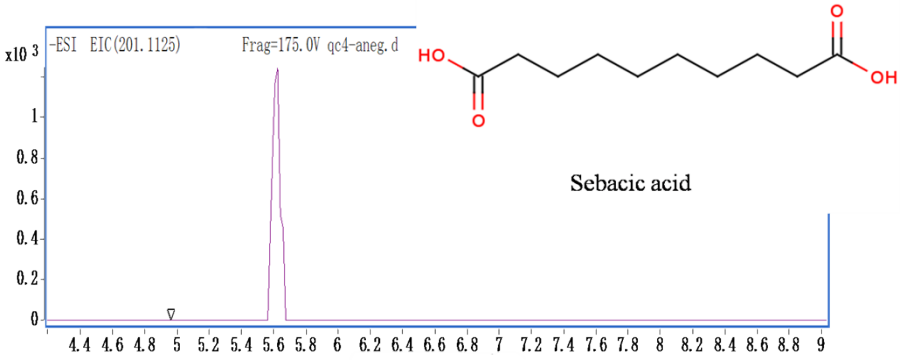
Frag=175.0V qc4-aneg.d
x10 3
HO
OH
O
O
CH3
3-Methylsuberic acid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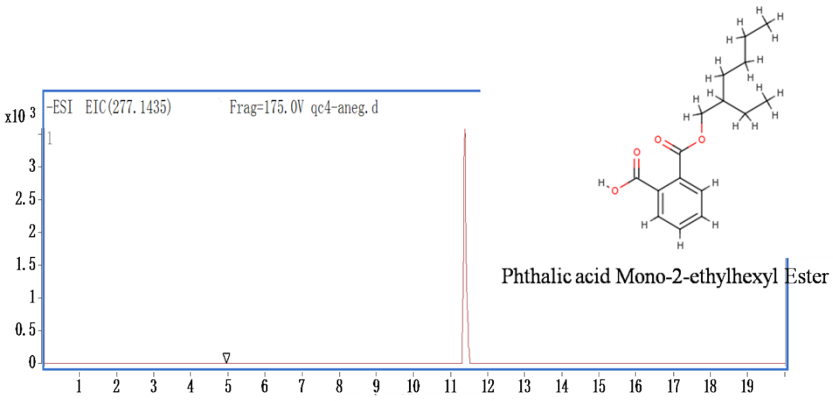
Frag=175.0V qc4-aneg.d
x10 3
HO
OH
O
O
Sebacic acid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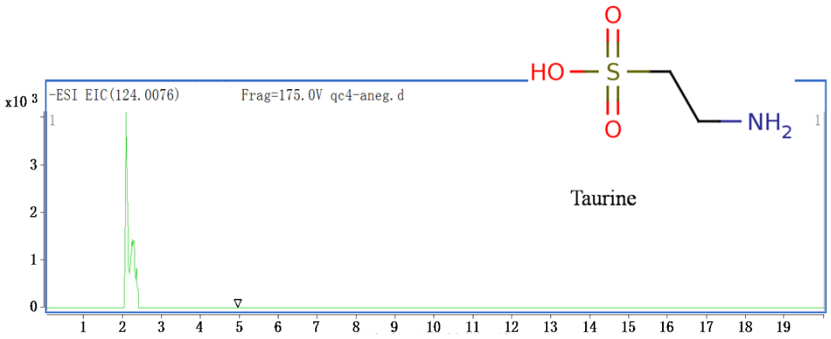
x10 3
Phthalic acid Mono-2-ethylhexyl Ester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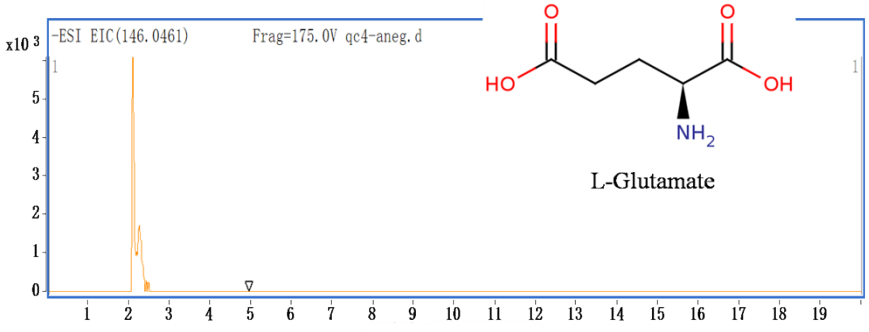
x10 3
HO
S
O
O
NH2
Taurine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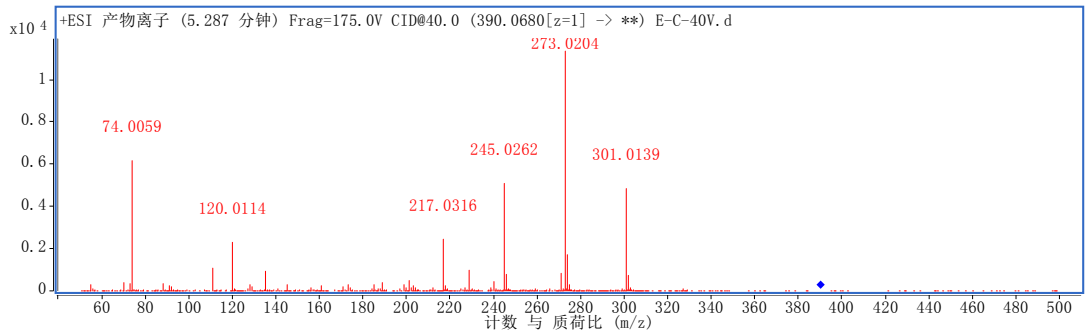
x10 3
HO
OH
NH2
L-Glutamate

ESM_5

a


x10 4
+ESI 产物离子 (5.287 分钟) Frag=175.0V CID@40.0 (390.0680[z=1] -> **) E-C-40V.d
273.0204
74.0059
245.0262
301.0139
120.0114
217.0316
计数 与 质荷比 (m/z)

b

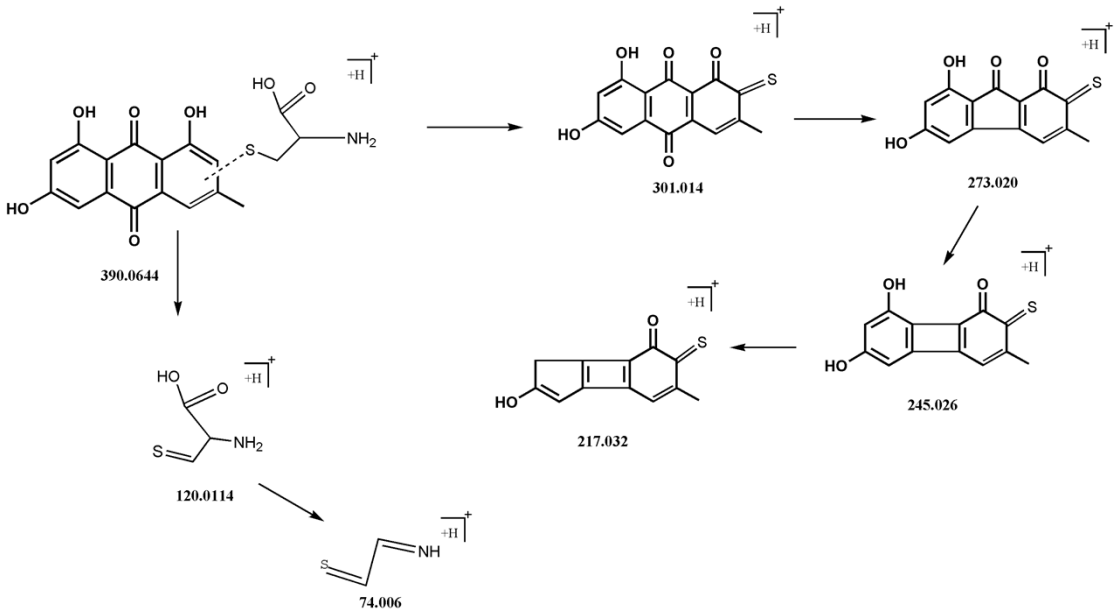

390.0644
301.014
273.020
245.026
217.032
120.0114
74.006

Table S1 Summary of significant biomarkers identified under the positive and negative mode

Num.	m/z	Adduct	Formula	Δppm	Rt (min)	Main MS/MS fragments	Identification	Compound ID	[a]RSD (%)	[b]Up- and down-regulated
[d]1	613.1599	$[M+H]^+$	C20H32N6O12S2	1.08	2.33	538.13,484.12,409.09 355.08,177.03	Glutathione, oxidized	HMDB03337	16.5	↓
[d]2	348.0705	$[M+H]^+$	C10H14N5O7P	-0.4	3.08	136.06,97.03	Adenosine monophosphate	HMDB00045	16.3	↑
[c]3	308.0908	$[M+H]^+$	C10H17N3O6S	0.92	3.1	198.08,152.06	Glutathione	HMDB00125	6.7	↓
4	247.1287	$[M+H]^+$	C10H18N2O5	0.6	3.57	72.08,84.04,156.09, 184.09	Val Glu	MID23976 (Metlin)	21.5	↑
5	374.1466	$[M+H]^+$	C14H23N5O5S1	-6.3	4.23	269.10,243.11,166.06, 110.07	Met Ser His	MID16844	25.9	↑
6	234.2067	$[M+NH4]^+$	C12H24O3	1.41	4.95	55.05,69.07,83.06,107.08 163.15	Hydroxydodecanoic acid	HMDB02059	11.4	↑
7	318.3008	$[M+NH4]^+$	C18H36O3	1.66	5.18	55.05,69.07,99.04,179.97, 240.11	18-hydroxy stearic acid	MID35448	2.7	↑
[e]8	360.2753	$[M+H]^+$	C19H37NO5	-2.36	5.78	282.27,163.15,145.04, 103.04,85.03,60.08	2-Hydroxylauroylcarnitine	HMDB13164	12.7	↑
9	348.3117	$[M+NH4]^+$	C19H38O4	2.48	5.98	256.26,143.10,88.07,70.07	MG(16:0)	HMDB11564 /	4.4	↑

								HMDB11533		
10	272.259	$[M+NH4]^+$	C16H30O2	2.18	6.57	254.25,164.34,86.06, 70.06	4-hexadecenoic acid	MID74430	8.3	↑
11	300.2906	$[M+NH4]^+$	C18H34O2	2.98	6.62	132.10,100.08,84.08,71.08, 57.07	7Z-octadecenoic acid	MID73806	12.8	↑
[e]12	388.3067	$[M+H]^+$	C21H41NO5	2.45	6.83	312.38,145.05,85.03,60.08	2-Hydroxymyristoylcarnitine	HMDB13166	9.5	↑
13	376.343	$[M+NH4]^+$	C21H42O4	-2.3	7.1	284.29,141.11,118.09,88.07	MG(18:0)	HMDB11131 / HMDB11535	7.1	↑
[e]14	414.3224	$[M+H]^+$	C23H43NO5	2.41	7.42	266.14,123.11,85.03,60.08	3-Hydroxy-9-hexadecenoylcarnitine	HMDB13333	21.2	↑
[e]15	416.3383.	$[M+H]^+$	C23H45NO5	3.0	8.2	145.05,85.03,60.08	3-Hydroxyhexadecanoylcarnitine	HMDB13336	9.7	↑
[e]16	442.3538	$[M+H]^+$	C25H47NO5	2.49	8.68	145.05,85.03,60.08	3-Hydroxy-9Z-octadecenoylcarnitine	HMDB13340	9.2	↑
[e]17	444.3597	$[M+H]^+$	C25H49NO5	3.04	9.65	145.05,85.03,60.08	12-Hydroxy-12-octadecanoylcarnitine	HMDB13154	8.0	↑
18	482.3248	$[M+H]^+$	C23H48NO7P	-1.42	9.97	465.43,339.29,258.11, 184.08,104.10	LysoPE(18:0)	HMDB11130 /	17.4	↓

								HMDB11129		
19	402.3952	$[M+NH4]^+$	C24H48O3	2.56	10.48	340.36,102.09,88.07, 70.06,57.07	3-hydroxy-tetracosanoic acid	MID74591	5.0	↑
20	306.2648	$[M+NH4]^+$	C16H32O4	-2.99	5.5	73.08,84.08,99.05, 214.22,226.22,258.24	9,10-dihydroxy-hexadecanoic acid	MID74569	9.2	↑
21	215.1282	$[M-H]^-$	C11H20O4	-3.17	6.12	199.87, 153.14,129.09	Undecanedioic acid	HMDB00888	7.8	↓
[d]22	346.0554	$[M-H]^-$	C10H14N5O7P	-1.18	3.08	261.34,230.88,134.04	Adenosine monophosphate	HMDB00045	18.5	↑
[d]23	187.097	$[M-H]^-$	C9H16O4	-3.11	5.22	169.09,143.10,125.09	3-Methylsuberic acid	HMDB59783	3.6	↑
[d]24	201.1125	$[M-H]^-$	C10H18O4	-3.64	5.61	183.10,139.11,111.08	Sebacic acid	HMDB00792	15.6	↓
25	277.1435	$[M-H]^-$	C16H22O4	-3.73	11.38	233.15,205.16,134.04, 127.11,121.03	Phthalic acid Mono-2-ethylhexyl Ester	HMDB13248	10.2	↓
[c]26	124.0076	$[M-H]^-$	C2H7NO3S	1.71	2.1	79.96	Taurine	HMDB00251	10.6	↓
27	215.1643	$[M-H]^-$	C12H24O3	4.5	4.95	197.15,169.16	Hydroxydodecanoic acid	HMDB02059	20.1	↑
[d]28	146.0461	$[M-H]^-$	C5H9NO4	1.5	2.1	128.04,102.06	L-Glutamate	HMDB00148	5.3	↑

[a] RSD was calculated from QC samples. [b] ↑, up-regulated; ↓, down-regulated. [c]Metabolites identified by standards comparison. [d] Metabolite identified by MS/MS fragments comparison with standard MS/MS database (HMDB, Metlin). [e] Acylcarnitines identified based on the fragmentation patterns of carnitine and the corresponding acyl groups.