Supporting information

Identification of Metabolites of Liquiritin in Rats by UHPLC-Q-TOF-MS/MS: Metabolic Profiling and Pathway Comparison *In Vitro* and *In Vivo*

Xia Zhang, Jintuo Yin, Caijuan Liang, Yupeng Sun，Lantong Zhang*

Department of Pharmaceutical Analysis, School of Pharmacy, Hebei Medical University, Shijiazhuang 050017, P.R. China

* Corresponding author at:

Tel.: +86-311-86266419 E-mail address: zhanglantong@263.net (L. Zhang)

Supplementary identification of *in vivo* phase I metabolites

Oxidation reaction

Metabolites M4, M5 and M6: M4-M6 were observed at retention times of 8.98, 11.42 and 11.94 min with deprotonated molecular ions $[M-H]^-$ at *m/z* 303.0533, 303.0536 and 303.0532, respectively, 114 Da less than the value obtained for deprotonated LQ, which were corresponding to $C_{15}H_{12}O_7$. M4-M6 formed a series of fragment ions at *m/z* 285.1340, 259.1548, 241.1436, 153.0916 and 137.0965 due to the loss of H_2O, CO, O, H_2O and due to *RDA* reaction. Moreover, values of cLog P for M4, M5 and M6 were 1.16623, 1.22684 and 1.28623, respectively. So M4, M5 and M6 were tentatively identified according to cLog P values.

Metabolites M15, M16 and M17: M15-M17 were eluted at retention times of 11.52, 13.66 and 13.88 min, respectively, and the mass spectra showed deprotonated molecular ions $[M-H]^-$ at *m/z* 271.0635, 271.0610 and 271.0605, respectively. The masses of the deprotonated M15-M17 were 16 Da higher than the mass of LQ after the loss of $C_6H_{10}O_5$, which suggested that hydrolyzed LQ had undergone an oxidation reaction. In the MS^2 spectra, the fragment ions at *m/z* 253.1437, 235.1332, 151.0033 and 119.0505 were observed due to the loss of H_2O and H_2O and due to *RDA* reaction. The chemical formula of M15-M17 was deduced to be $C_{15}H_{12}O_5$. The cLog P values of M15, M16 and M17 were 1.93694, 2.05485 and 2.44485, respectively, which was used as the basis for assignment of M15-M17.

Metabolites M21, M22: M21 and M22, eluted at 17.00 and 17.65 min, respectively, showed deprotonated molecular ions $[M-H]^-$ at *m/z* 447.1278 and 447.1273, 30 Da higher than that of M0. The MS/MS spectra showed the typical

neutral loss of glucose (162 Da) fragment ion at *m/z* 285.1150. The other fragment ions at *m/z* 271.0967 [M-$C_6H_{10}O_5$-CH_2-H]$^-$ and 165.0554 (*RDA* reaction) were detected, which suggested that the occurrence of oxidation and methylation reaction. And the chemical formula of M21 and M22 was $C_{22}H_{24}O_{10}$. Moreover, the cLog P values of M21 and M22 were 1.01117 and 1.47117, respectively. Therefore, M21 and M22 were identified according to this information.

Supplementary identification of *in vivo* phase II metabolites

Metabolites M37, M38: The metabolites M37 ([M-H]$^-$ at *m/z* 641.1338) and M38 ([M-H]$^-$ at *m/z* 641.1397) were a pair of isomer with retention times of 9.25 and 12.14 min, respectively. The masses of the deprotonated M37 and M38 were 224 Da higher than that of M0, and showed identical fragment ions at *m/z* 303.0870 [M-$C_6H_8O_6$-$C_6H_{10}O_5$-H]$^-$, 255.0655 [M-$C_6H_8O_6$-$C_6H_{10}O_5$-3O-H]$^-$, 135.0080 (*RDA* reaction) and 119.0504 (*RDA* reaction), which suggested that oxidation and glucuronide conjugation reaction occurred and that the formula was $C_{27}H_{30}O_{18}$. According to ChemDraw 12.0, the cLog P values of M37 and M38 were -2.78222 and -2.64222, respectively. According to these evidences, M37 and M38 were assigned.

Metabolites M39, M40: M39 and M40 were separated at 9.67 and 12.55 min with deprotonated molecular ions [M-H]$^-$ at *m/z* 609.1423 and 609.1430, respectively, corresponding to the molecular formula of $C_{27}H_{30}O_{16}$. The MS/MS spectra showed the typical neutral loss of $C_6H_8O_6$ (176 Da), which implied that M39 and M40 were glucuronide conjugation metabolites. Other fragment ions at *m/z* 433.1146, 255.0812, 151.0395 and 113.0240 were produced due to successive loss of $C_6H_8O_6$, O, $C_6H_{10}O_5$

and due to *RDA* reaction. And they were identified according to the Clog P values of -1.55013 and -1.09013, respectively.

Metabolites M41, M42, M43: M41, M42 and M43, which were eluted at 10.67, 11.45 and 12.00 min, respectively, were characterized as deprotonated molecular ions $[M-H]^-$ at *m/z* 351.0164, 351.0163 and 351.0164, respectively. These values were 66 Da lower than the value obtained for M0. The MS/MS spectra showed a number of representative fragment ions at *m/z* 271.0614, 151.0039 and 119.0507 due to loss of SO_3 and due to *RDA* reaction. It was evident that sulfate conjugation occurred and that the molecular formula of M41-M43 was $C_{15}H_{12}O_8S$. Moreover, M41-M43 were assigned on the basis of cLog P values of 0.361621, 0.821621 and 0.884852, respectively.

Metabolites M48, M49: The mass spectra of M48 and M49 showed deprotonated molecular ions $[M-H]^-$ at *m/z* 513.0667 and 513.0677, respectively, which were 96 Da higher than the value obtained for LQ. M48 and M49 were eluted at 12.54 and 13.24 min, respectively, and generated identical secondary fragment ions at *m/z* 433.0558, 255.0665, 151.0393 and 113.0241 by the loss of SO_3, O, and $C_6H_{10}O_5$ and by *RDA* reaction. These values corresponded to a molecular formula of $C_{21}H_{22}O_{13}S$. M48 and M49 were identified based on the cLog P values of -1.13483 and -0.674831, respectively.

Metabolite M52: The MS/MS spectrum contained a peak for one metabolite at *m/z* 445.1883, which was 28 Da more than the value obtained for M0. The retention time of M52 was 12.53 min, and the formula of M52 was determined to be $C_{21}H_{18}O_{11}$

based on the QTOF-MS data. Moreover, fragment ions at *m/z* 269.0443 [M-$C_6H_8O_6$-H]$^-$ and 113.0239 (*RDA* reaction) were observed, suggesting that M52 was a glucuronide conjugation metabolite.

Supplementary identification of phase *in vitro* I metabolites

Metabolites N5, N6, M7: N5-N7 were eluted at 11.12, 13.60 and 13.83 min and exhibited sharp peaks of deprotonated molecular ions [M-H]$^-$ at *m/z* 271.0596, 271.0595 and 271.0592, which were 16 Da higher than the value obtained for hydrolyzed LQ. This observation suggested the occurrence of oxidation of LQ after the loss of $C_6H_{10}O_5$. The corresponding fragment ions were observed at *m/z* 253.0474, 151.0016 and 135.0439, which were generated by the loss of H_2O and by *RDA* reaction. N5-N7 were isomers and were identified based on their retention times and their cLog P values of 1.93694, 2.05485 and 2.44485, respectively.

Supplementary identification of *in vitro* phase II metabolites

Metabolites N13, N14: On the basis of the deprotonated molecular ions [M-H]$^-$ at *m/z* 431.0989 and 431.0985, the molecular formula of N13 and N14 was determined to be $C_{21}H_{20}O_{10}$.[38] Moreover, N13 and N14 had retention times of 11.19 and 11.93 min, respectively, and the mass/charge ratio was 176 Da more than that of N9, which implied that N9 had undergone a glucuronide conjugation reaction. The representative fragment ions at *m/z* 255.0662, 135.0080 and 113.0242 were produced by the $C_6H_8O_6$ and by *RDA* reaction. N13 and N14 were identified based on the cLog P values of 0.27429 and 0.55864, respectively.

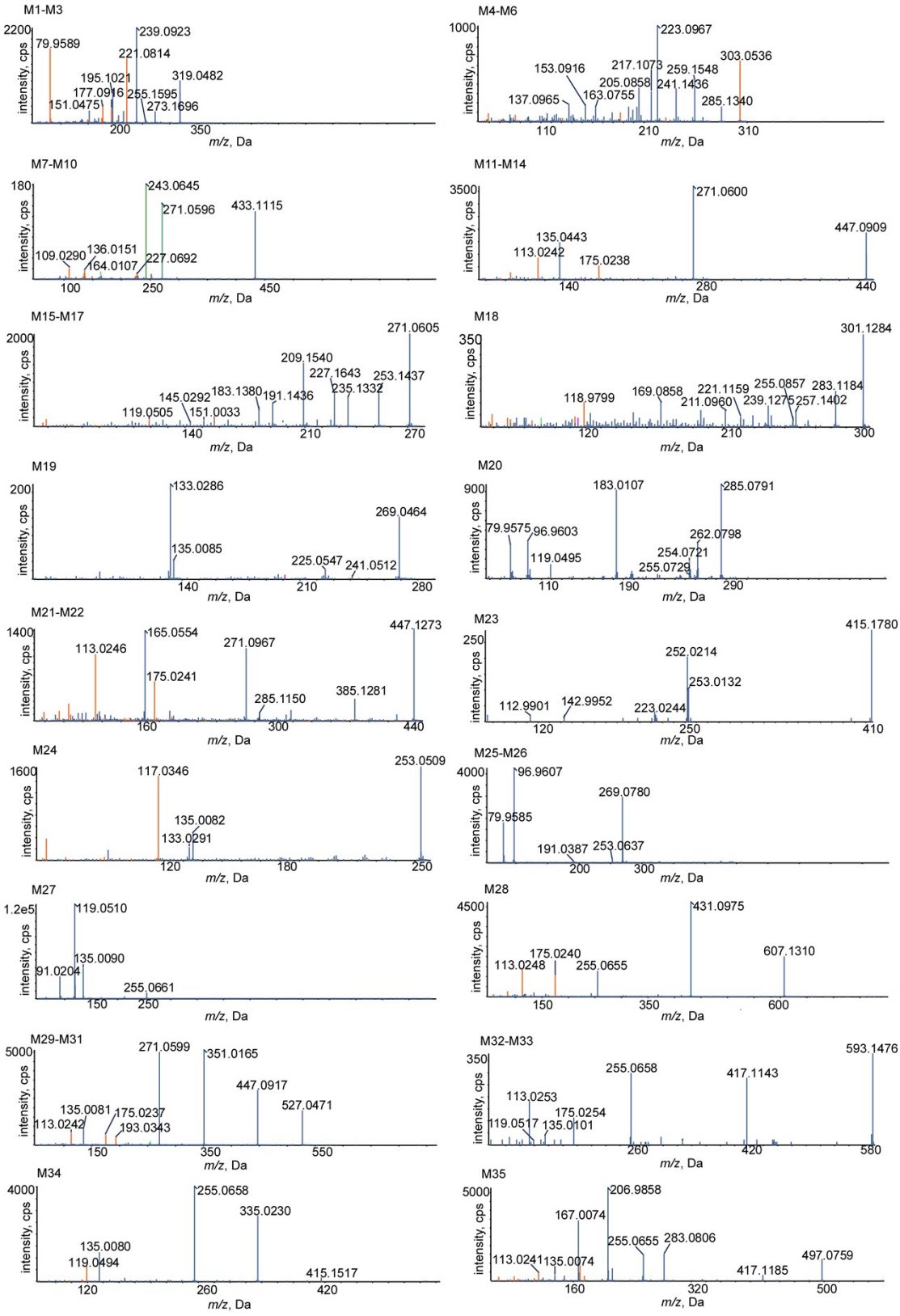
M1-M3
2200
intensity, cps
79.9589
239.0923
221.0814
195.1021
319.0482
177.0916
255.1595
151.0475
273.1696
200
350
m/z, Da
M4-M6
1000
intensity, cps
223.0967
303.0536
153.0916
217.1073
259.1548
205.0858
241.1436
163.0755
137.0965
285.1340
110
210
310
m/z, Da
M7-M10
180
intensity, cps
243.0645
271.0596
433.1115
109.0290
136.0151
164.0107
227.0692
100
250
450
m/z, Da
M11-M14
3500
intensity, cps
271.0600
447.0909
135.0443
113.0242
175.0238
140
280
440
m/z, Da
M15-M17
2000
intensity, cps
271.0605
209.1540
227.1643
253.1437
235.1332
145.0292
183.1380
191.1436
119.0505
151.0033
140
210
270
m/z, Da
M18
350
intensity, cps
301.1284
118.9799
169.0858
221.1159
255.0857
283.1184
211.0960
239.1275
257.1402
120
210
300
m/z, Da
M19
200
intensity, cps
133.0286
269.0464
135.0085
225.0547
241.0512
140
210
280
m/z, Da
M20
900
intensity, cps
183.0107
285.0791
79.9575
96.9603
262.0798
119.0495
254.0721
255.0729
110
190
290
m/z, Da
M21-M22
1400
intensity, cps
447.1273
165.0554
113.0246
271.0967
175.0241
285.1150
385.1281
160
300
440
m/z, Da
M23
250
intensity, cps
415.1780
252.0214
253.0132
112.9901
142.9952
223.0244
120
250
410
m/z, Da
M24
1600
intensity, cps
253.0509
117.0346
135.0082
133.0291
120
180
250
m/z, Da
M25-M26
4000
intensity, cps
96.9607
269.0780
79.9585
191.0387
253.0637
200
300
m/z, Da
M27
1.2e5
intensity, cps
119.0510
135.0090
91.0204
255.0661
150
250
m/z, Da
M28
4500
intensity, cps
431.0975
175.0240
607.1310
113.0248
255.0655
150
350
600
m/z, Da
M29-M31
5000
intensity, cps
271.0599
351.0165
447.0917
527.0471
135.0081
175.0237
113.0242
193.0343
150
350
550
m/z, Da
M32-M33
350
intensity, cps
593.1476
255.0658
417.1143
113.0253
175.0254
119.0517
135.0101
260
420
580
m/z, Da
M34
4000
intensity, cps
255.0658
335.0230
135.0080
119.0494
415.1517
120
260
420
m/z, Da
M35
5000
intensity, cps
206.9858
167.0074
255.0655
283.0806
113.0241
135.0074
497.0759
417.1185
160
320
500
m/z, Da

Fig.S1-1

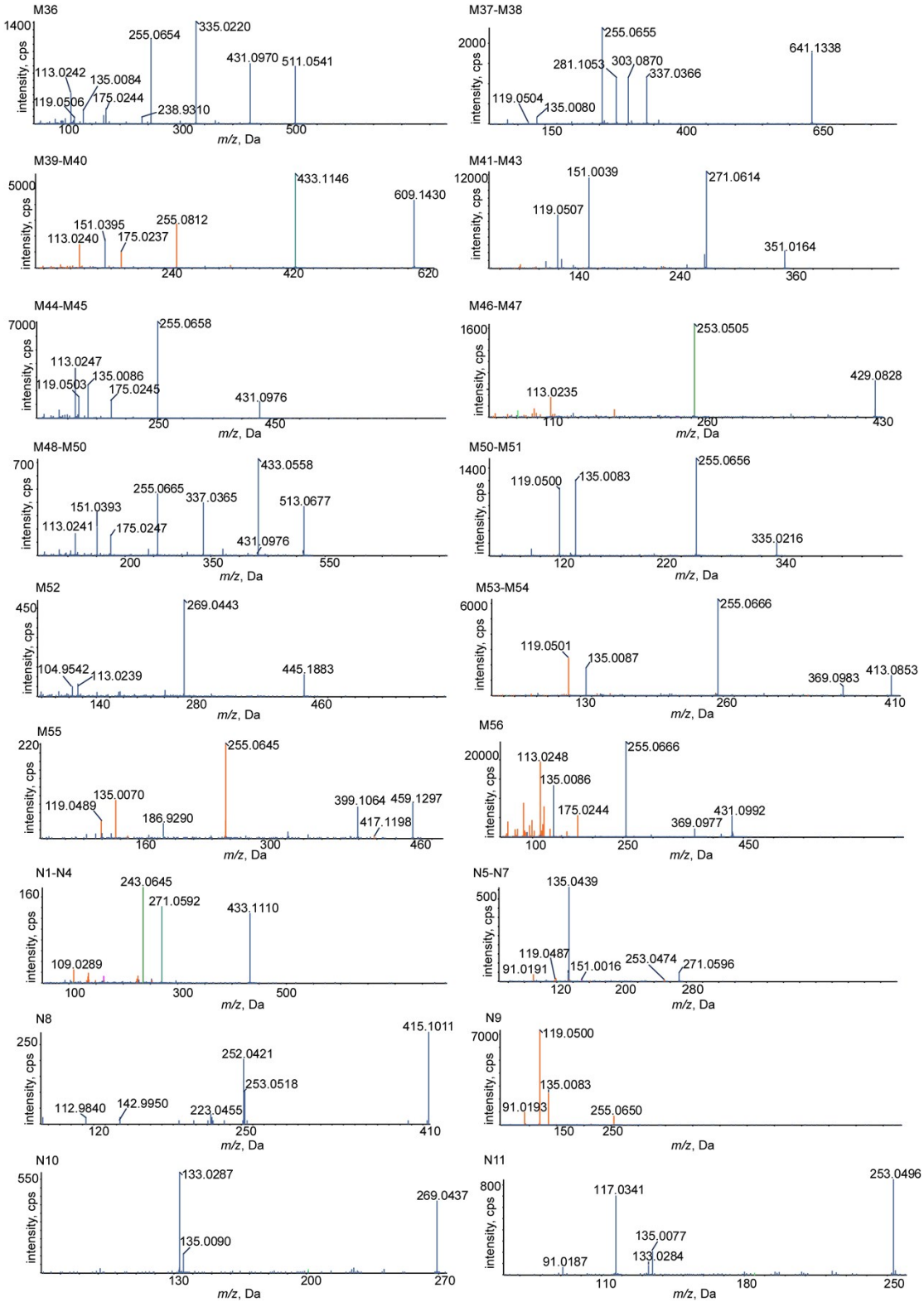
M36
1400
intensity, cps
255.0654
335.0220
431.0970
511.0541
113.0242
135.0084
119.0506
175.0244
238.9310
100
300
500
m/z, Da
M37-M38
2000
intensity, cps
255.0655
641.1338
281.1053
303.0870
337.0366
119.0504
135.0080
150
400
650
M39-M40
5000
intensity, cps
433.1146
609.1430
151.0395
255.0812
113.0240
175.0237
240
420
620
M41-M43
12000
intensity, cps
151.0039
271.0614
119.0507
351.0164
140
240
360
M44-M45
7000
intensity, cps
255.0658
113.0247
135.0086
119.0503
175.0245
431.0976
250
450
m/z, Da
M46-M47
1600
intensity, cps
253.0505
429.0828
113.0235
110
260
430
m/z, Da
M48-M50
700
intensity, cps
433.0558
255.0665
337.0365
513.0677
151.0393
113.0241
175.0247
431.0976
200
350
550
m/z, Da
M50-M51
1400
intensity, cps
255.0656
119.0500
135.0083
335.0216
120
220
340
m/z, Da
M52
450
intensity, cps
269.0443
104.9542
113.0239
445.1883
140
280
460
m/z, Da
M53-M54
6000
intensity, cps
255.0666
119.0501
135.0087
369.0983
413.0853
130
260
410
m/z, Da
M55
220
intensity, cps
255.0645
135.0070
119.0489
399.1064
459.1297
186.9290
417.1198
160
300
460
m/z, Da
M56
20000
intensity, cps
113.0248
255.0666
135.0086
175.0244
431.0992
369.0977
100
250
450
m/z, Da
N1-N4
160
intensity, cps
243.0645
271.0592
433.1110
109.0289
100
300
500
m/z, Da
N5-N7
500
intensity, cps
135.0439
119.0487
91.0191
151.0016
253.0474
271.0596
120
200
280
m/z, Da
N8
250
intensity, cps
415.1011
252.0421
253.0518
112.9840
142.9950
223.0455
120
250
410
m/z, Da
N9
7000
intensity, cps
119.0500
135.0083
91.0193
255.0650
150
250
m/z, Da
N10
550
intensity, cps
133.0287
269.0437
135.0090
130
200
270
m/z, Da
N11
800
intensity, cps
253.0496
117.0341
135.0077
133.0284
91.0187
110
180
250
m/z, Da

Fig.S1-2

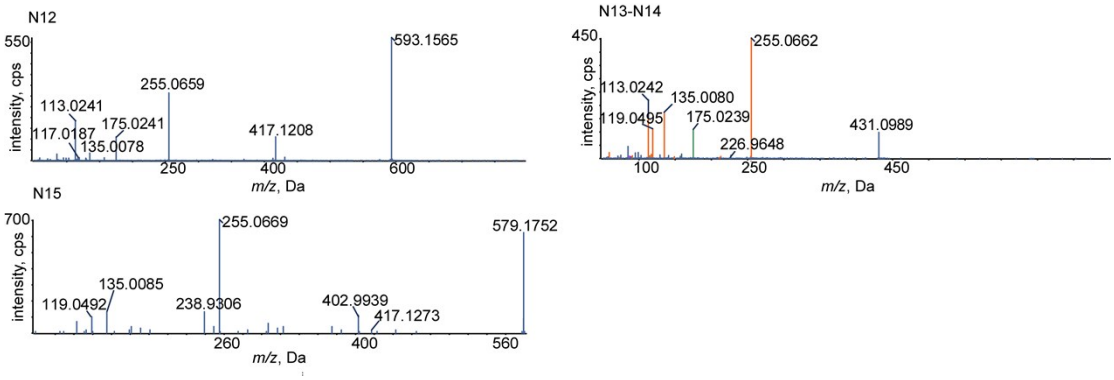
N12
550
intensity, cps
593.1565
255.0659
113.0241
175.0241
117.0187
135.0078
417.1208
250
400
600
m/z, Da
N13-N14
450
intensity, cps
255.0662
113.0242
135.0080
119.0495
175.0239
431.0989
226.9648
100
250
450
m/z, Da
N15
700
intensity, cps
255.0669
579.1752
135.0085
119.0492
238.9306
402.9939
417.1273
260
400
560
m/z, Da

Fig.S1-3