Electronic Supplementary Material (ESI) for RSC Advances.


# Supplemental information A

## Materials and chemicals

All 100 authentic references used are given as follows. Ginsenosides Rb1, Rb2, Rd, Re, Rg1, Rh1, Rh2, Ro, & F2, pseudoginsenoside F11, notoginsenoside R1, astragalosides A, I & II, protocatechuic aldehyde, protocatechnic A, glycyrrhizic acid, caffeic acid, calycosin, liquiritigenin, isoliquiritigenin, liquiritin, isoliquiritin, liquiritin apioside, formononetin, licochalcone A, ononin, isorhamnetin, glycyrrhetic acid, quercetin, kaempferol, orientin, betulinic acid, oleanolic acid, genistein, genistin, chlorogenic acid, protopanaxatriol (PPT), panaxatriol (PT), and panoxadiol (PD) were supplied by Standard Biotech Co., Ltd. (Shanghai, China). Apigenin-6,8-*di*-*C*-*β*-D-glucopyranoside, liquiritigenin-7,4'-*di*-*O*-*β*-D-glucoside, calycosin-7-*O*-*β*-D-glupyrancoside, and isoliquiritin apioside were kindly provided by Prof. Yong Jiang (Peking University, Beijing, China). Alanine, valine, leucine, isoleucine, phenylalanine, proline, glutamic acid, aspartic acid, gamma amino acid butyric acid (GABA), adenine, guanine, uracil, cytidine, guanosine, uridine, adenosine, inosine, serine, asparagine, threonine, betaine, nicotinamide, oxalic acid, succinic acid, malic acid, adipic acid, maleic acid, nicotinic acid, salicylic acid, galactitol, gallic acid, ferulic acid, vanillic acid, sucrose, arachidonic acid (AA), 15-hydroxy-eicosatetraenoic acid (15-HETE), 12-HETE, 5-HETE, and leukotriene $B_4$ ($LTB_4$) were purchased from Xinjingke Biotechnology Company (Beijing, China) or Sigma-Aldrich (St. Louis, MO, USA). Postaglandin $E_2$ ($PGE_2$), thromboxane $B_2$ ($TXB_2$), and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) were provided by Cayman Chemicals (Ann Arbor, MI, USA). Cholic acid (CA), hyodeoxycholic acid (HDCA), taurohyodeoxycholic acid (THDCA), deoxycholic acid (DCA), cholesterol, estradiol, estrone, methyltestosterone, cortisol, cortisone, and galactitol were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Taurochenodeoxycholic acid (TCDCA), taurocygnocholic acid (TCCA), and testosterone were supplied by the State Key Laboratory of Natural and Biomimetic Drugs, Peking University (Beijing, China). The purities were determined above 98% by HPLC-DAD-IT-TOF-MS (Shimadzu, Tokyo, Japan).

**Table S1** Intra- and inter-day, recovery assay and repeatability, stability results for 37 analytes

| No. | Analyte | Intra-day | | | Inter-day | | | Recovery | | | | | | Stability | Repeatability |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Low | Medium | High | Low | Medium | High | Low | | Medium | | High | | RSD | RSD |
| | | RSD (%) | RSD (%) | RSD (%) | RSD (%) | RSD (%) | RSD (%) | Mean (%) | RSD (%) | Mean (%) | RSD (%) | Mean (%) | RSD (%) | (%) | (%) |
| 1 | uracil | 13.70 | 16.03 | 6.56 | 15.24 | 9.42 | 5.68 | 134.00 | 2.58 | 131.40 | 5.40 | 123.40 | 4.44 | 12.68 | 10.95 |
| 2 | maleic acid | 4.12 | 9.03 | 8.47 | 12.72 | 8.92 | 7.28 | 138.00 | 2.58 | 84.00 | 2.39 | 82.60 | 3.47 | 10.74 | 15.63 |
| 3 | betaine | 8.49 | 4.56 | 3.13 | 8.40 | 7.53 | 4.83 | 110.40 | 2.88 | 84.80 | 6.77 | 88.80 | 14.73 | 9.48 | 9.22 |
| 4 | nicotinic acid | 6.85 | 7.51 | 6.07 | 9.86 | 8.60 | 13.31 | 133.00 | 3.16 | 105.98 | 9.71 | 125.18 | 11.75 | 6.51 | 4.84 |
| 5 | nicotinamide | 8.87 | 2.44 | 5.06 | 6.71 | 7.01 | 6.37 | 114.93 | 2.73 | 110.13 | 6.16 | 87.67 | 10.00 | 8.73 | 7.41 |
| 6 | leucine | 6.99 | 9.01 | 6.31 | 10.31 | 10.05 | 4.86 | 98.47 | 4.67 | 135.23 | 1.82 | 136.83 | 6.14 | 6.66 | 7.58 |
| 7 | isoleucine | 9.95 | 7.72 | 4.98 | 11.66 | 11.98 | 10.71 | 98.00 | 3.22 | 106.00 | 5.53 | 98.38 | 15.90 | 9.89 | 15.54 |
| 8 | adenine | 10.79 | 3.55 | 5.69 | 14.26 | 6.68 | 8.81 | 117.47 | 8.23 | 116.33 | 16.36 | 89.40 | 1.85 | 5.94 | 4.49 |
| 9 | aspartic acid | 11.63 | 12.79 | 9.75 | 15.41 | 17.23 | 10.69 | 129.13 | 12.69 | 86.09 | 16.77 | 116.86 | 2.34 | 13.03 | 2.74 |
| 10 | thymidine | 5.79 | 6.26 | 4.91 | 5.82 | 6.22 | 6.26 | 79.07 | 11.61 | 85.44 | 7.92 | 70.05 | 4.30 | 9.73 | 9.93 |
| 11 | cytidine | 8.91 | 5.53 | 4.68 | 7.63 | 5.56 | 9.33 | 117.55 | 11.67 | 85.71 | 3.30 | 77.31 | 0.37 | 9.90 | 13.50 |
| 12 | uridine | 12.11 | 9.37 | 7.08 | 11.37 | 4.83 | 5.30 | 87.63 | 2.31 | 95.13 | 7.71 | 102.63 | 11.15 | 7.93 | 13.35 |
| 13 | liquiritigenin | 4.91 | 4.65 | 7.97 | 10.79 | 11.50 | 9.32 | 70.69 | 5.44 | 131.52 | 3.88 | 123.37 | 8.65 | 8.93 | 7.36 |
| 14 | isoliquiritigenin | 5.62 | 5.78 | 3.72 | 4.88 | 3.81 | 2.95 | 98.60 | 5.74 | 92.46 | 9.79 | 108.79 | 10.19 | 8.52 | 14.89 |
| 15 | inosine | 14.78 | 12.80 | 3.62 | 13.86 | 13.65 | 7.19 | 131.00 | 9.72 | 133.60 | 2.20 | 127.80 | 0.85 | 8.53 | 7.23 |
| 16 | formononetin | 4.10 | 9.33 | 3.65 | 6.20 | 4.40 | 4.28 | 99.72 | 13.50 | 91.06 | 11.78 | 106.09 | 2.95 | 8.08 | 4.02 |
| 17 | adenosine | 4.71 | 5.61 | 8.50 | 15.58 | 19.05 | 7.74 | 102.07 | 11.50 | 97.16 | 2.62 | 116.00 | 14.10 | 9.81 | 5.21 |
| 18 | calycosin | 4.78 | 3.27 | 5.99 | 5.95 | 8.07 | 8.71 | 135.04 | 0.42 | 135.74 | 2.22 | 135.25 | 4.24 | 9.05 | 11.48 |
| 19 | testosterone | 3.90 | 3.60 | 3.91 | 5.60 | 3.81 | 3.65 | 102.00 | 6.07 | 105.16 | 4.11 | 105.08 | 0.93 | 6.16 | 8.49 |
| 20 | AA | 8.02 | 5.20 | 5.31 | 10.46 | 6.14 | 7.78 | 118.16 | 8.53 | 85.26 | 12.97 | 110.79 | 1.33 | 5.15 | 13.31 |
| 21 | 5-HETE | 7.11 | 7.96 | 2.63 | 11.59 | 9.16 | 3.25 | 123.40 | 5.50 | 139.57 | 3.96 | 136.59 | 5.55 | 14.79 | 11.94 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 22 | licochalcone A | 1.77 | 7.14 | 3.81 | 7.40 | 8.57 | 3.42 | 75.73 | 6.22 | 86.83 | 15.56 | 76.00 | 3.00 | 5.87 | 13.69 |
| 23 | HDCA | 4.30 | 4.24 | 5.05 | 8.71 | 10.43 | 9.14 | 100.92 | 1.75 | 88.59 | 1.15 | 115.21 | 1.84 | 30.89 | 10.47 |
| 24 | CA | 11.26 | 9.41 | 5.34 | 9.21 | 8.01 | 7.30 | 116.32 | 5.14 | 91.21 | 3.76 | 106.18 | 3.08. | 10.37 | 2.09 |
| 25 | isoliquiritin | 6.04 | 16.49 | 8.90 | 10.33 | 7.59 | 7.40 | 130.08 | 3.95 | 72.64 | 4.76 | 132.00 | 2.06 | 8.18 | 7.36 |
| 26 | liquiritin | 7.22 | 7.88 | 6.90 | 6.86 | 8.03 | 8.86 | 108.40 | 3.35 | 126.24 | 2.40 | 125.12 | 6.31 | 8.67 | 4.37 |
| 27 | glycyrrhetic acid | 4.71 | 7.76 | 3.05 | 7.26 | 8.12 | 8.05 | 81.71 | 2.35 | 104.43 | 11.70 | 78.14 | 0.87 | 11.19 | 17.54 |
| 28 | PPT | 15.63 | 5.81 | 6.95 | 10.06 | 10.72 | 8.90 | 121.62 | 5.19 | 81.99 | 1.21 | 99.73 | 5.84 | 12.21 | 0.16 |
| 29 | calycosin-7-*O*-*β*-D-glucoside | 6.22 | 4.93 | 8.81 | 7.91 | 7.29 | 10.32 | 80.64 | 6.55 | 100.48 | 7.28 | 134.00 | 13.40 | 6.68 | 2.78 |
| 30 | THDCA | 6.98 | 4.90 | 9.55 | 5.87 | 7.47 | 5.87 | 127.38 | 4.31 | 131.76 | 5.17 | 126.94 | 0.68 | 8.57 | 7.73 |
| 31 | betulinic acid | 9.72 | 7.51 | 8.52 | 7.76 | 8.87 | 8.62 | 120.18 | 3.32 | 81.26 | 10.38 | 124.37 | 3.97 | 10.90 | 10.90 |
| 32 | isoliquiritin apioside | 8.18 | 6.86 | 5.32 | 7.97 | 5.49 | 8.26 | 85.87 | 6.63 | 119.25 | 5.50 | 131.52 | 12.54 | 7.54 | 5.25 |
| 33 | liquiritin apioside | 7.56 | 7.38 | 5.44 | 5.38 | 4.72 | 9.72 | 83.20 | 8.32 | 101.33 | 2.53 | 119.47 | 9.69 | 5.63 | 3.41 |
| 34 | liquiritigenin-7,4'-di-*O*-*β*-D-glucoside | 8.21 | 10.37 | 5.23 | 9.81 | 8.34 | 9.77 | 88.52 | 2.84 | 105.70 | 6.31 | 106.74 | 1.38 | 8.84 | 8.02 |
| 35 | Rh1 | 9.94 | 4.24 | 11.04 | 12.62 | 9.92 | 14.79 | 87.23 | 4.14 | 108.26 | 3.33 | 103.12 | 2.76 | 7.61 | 6.17 |
| 36 | astragaloside II | 8.33 | 4.54 | 9.71 | 7.91 | 5.55 | 8.56 | 80.53 | 5.98 | 93.71 | 3.85 | 82.47 | 1.93 | 8.66 | 8.13 |
| 37 | notoginsenoside R1 | 8.54 | 12.68 | 5.92 | 13.19 | 6.76 | 9.63 | 103.81 | 2.47 | 142.61 | 10.20 | 106.61 | 7.96 | 8.98 | 2.69 |

# Supplemental information B

**Figure legends**

**Figure S1** Brief schematics of RPLC-HILIC-MS/MS (A), guard column-(HILIC/RPLC)-MS/MS (B), and HILIC-TC-RPLC-MS/MS (C) platforms.

**Figure S2** Extract ion current chromatograms of all analytes on HILIC-TFC-RPLC-MS/MS.

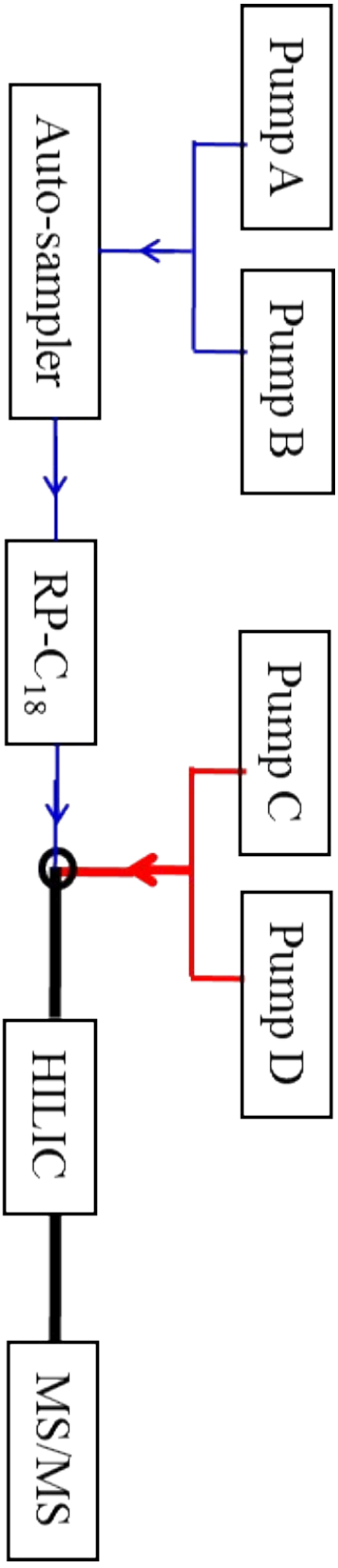
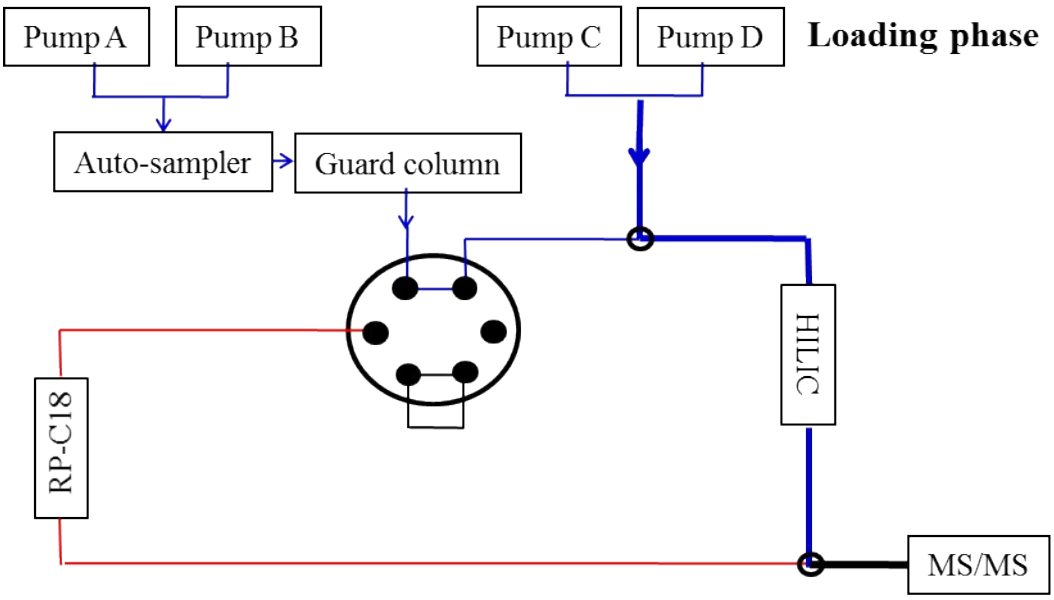



**Fig. S1A**


Pump A
Pump B
Pump C
Pump D
Loading phase
Auto-sampler
Guard column
RP-C18
HILIC
MS/MS

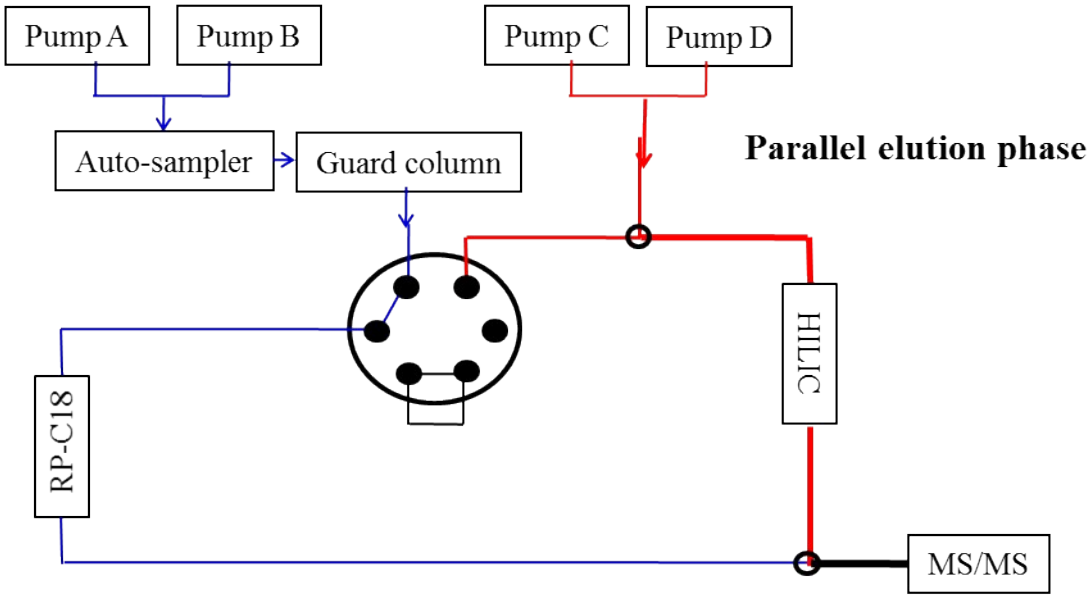
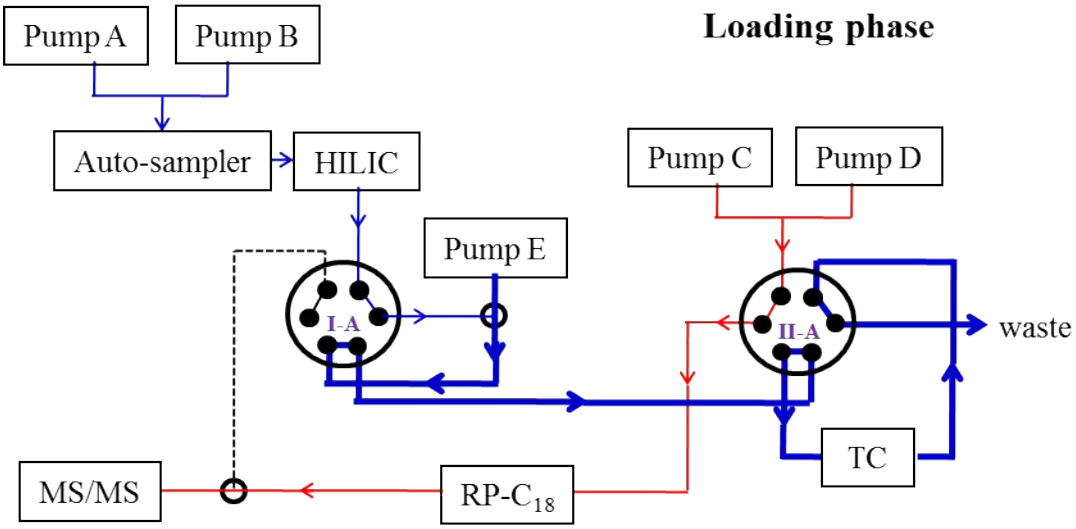

Pump A
Pump B
Pump C
Pump D
Parallel elution phase
Auto-sampler
Guard column
RP-C18
HILIC
MS/MS

**Fig. S1B**


Loading phase
Pump A
Pump B
Auto-sampler
HILIC
Pump C
Pump D
Pump E
I-A
II-A
waste
TC
MS/MS
RP-C18

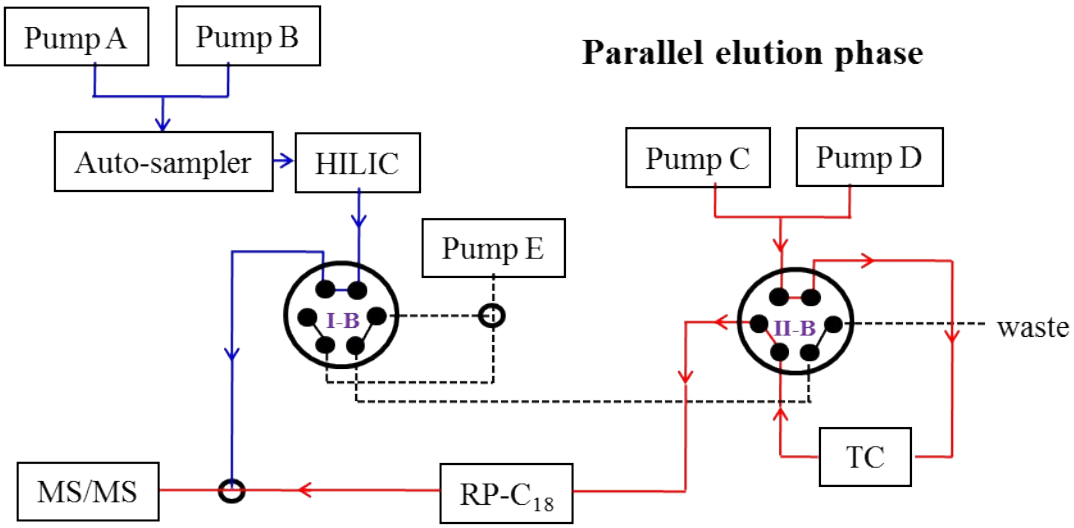

Parallel elution phase
Pump A
Pump B
Auto-sampler
HILIC
Pump C
Pump D
Pump E
I-B
II-B
waste
TC
MS/MS
RP-C18

**Fig. S1C**

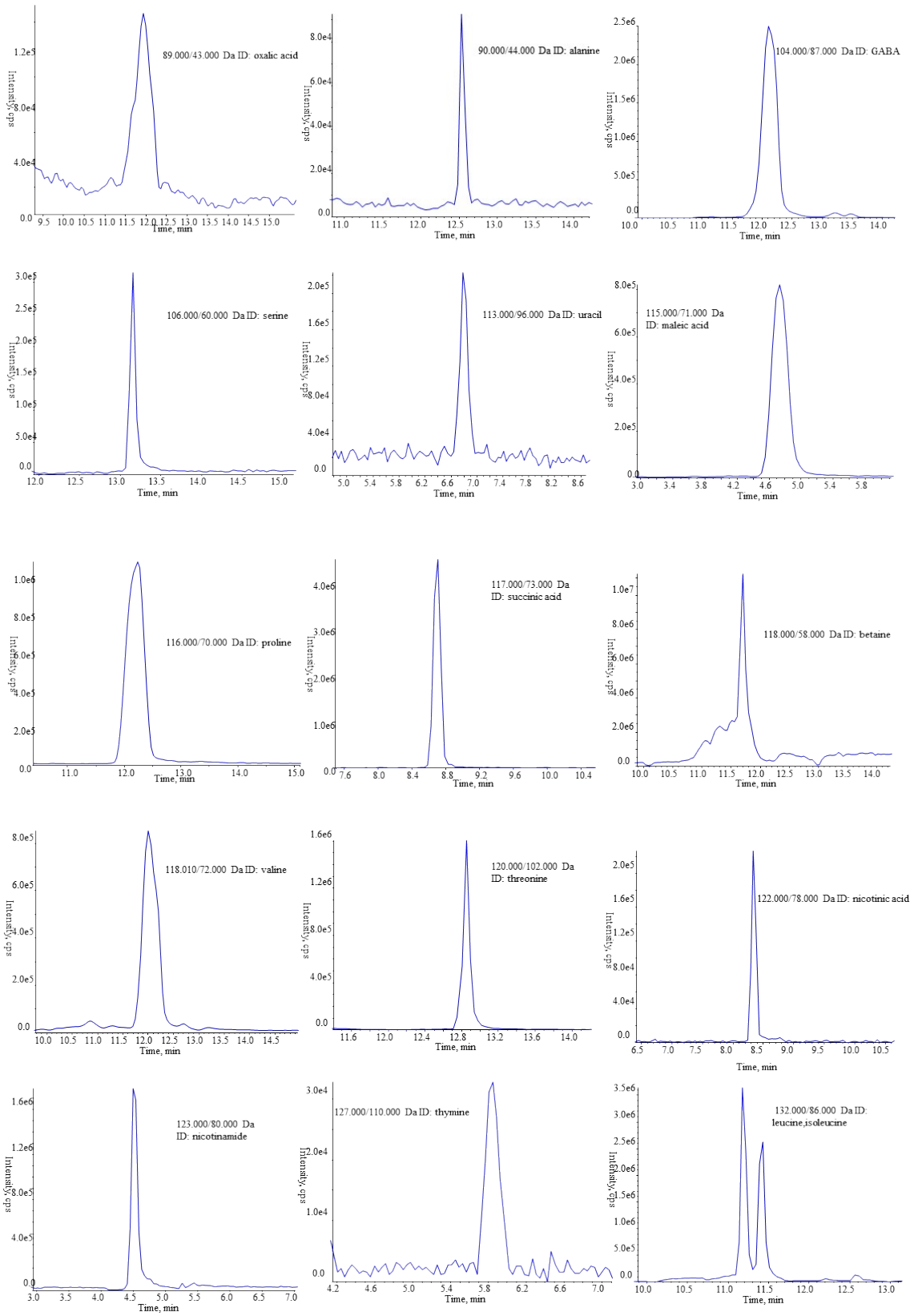

89.000/43.000 Da ID: oxalic acid
90.000/44.000 Da ID: alanine
104.000/87.000 Da ID: GABA
106.000/60.000 Da ID: serine
113.000/96.000 Da ID: uracil
115.000/71.000 Da ID: maleic acid
116.000/70.000 Da ID: proline
117.000/73.000 Da ID: succinic acid
118.000/58.000 Da ID: betaine
118.010/72.000 Da ID: valine
120.000/102.000 Da ID: threonine
122.000/78.000 Da ID: nicotinic acid
123.000/80.000 Da ID: nicotinamide
127.000/110.000 Da ID: thymine
132.000/86.000 Da ID: leucine,isoleucine
Intensity, cps
Time, min

**Fig. S2**

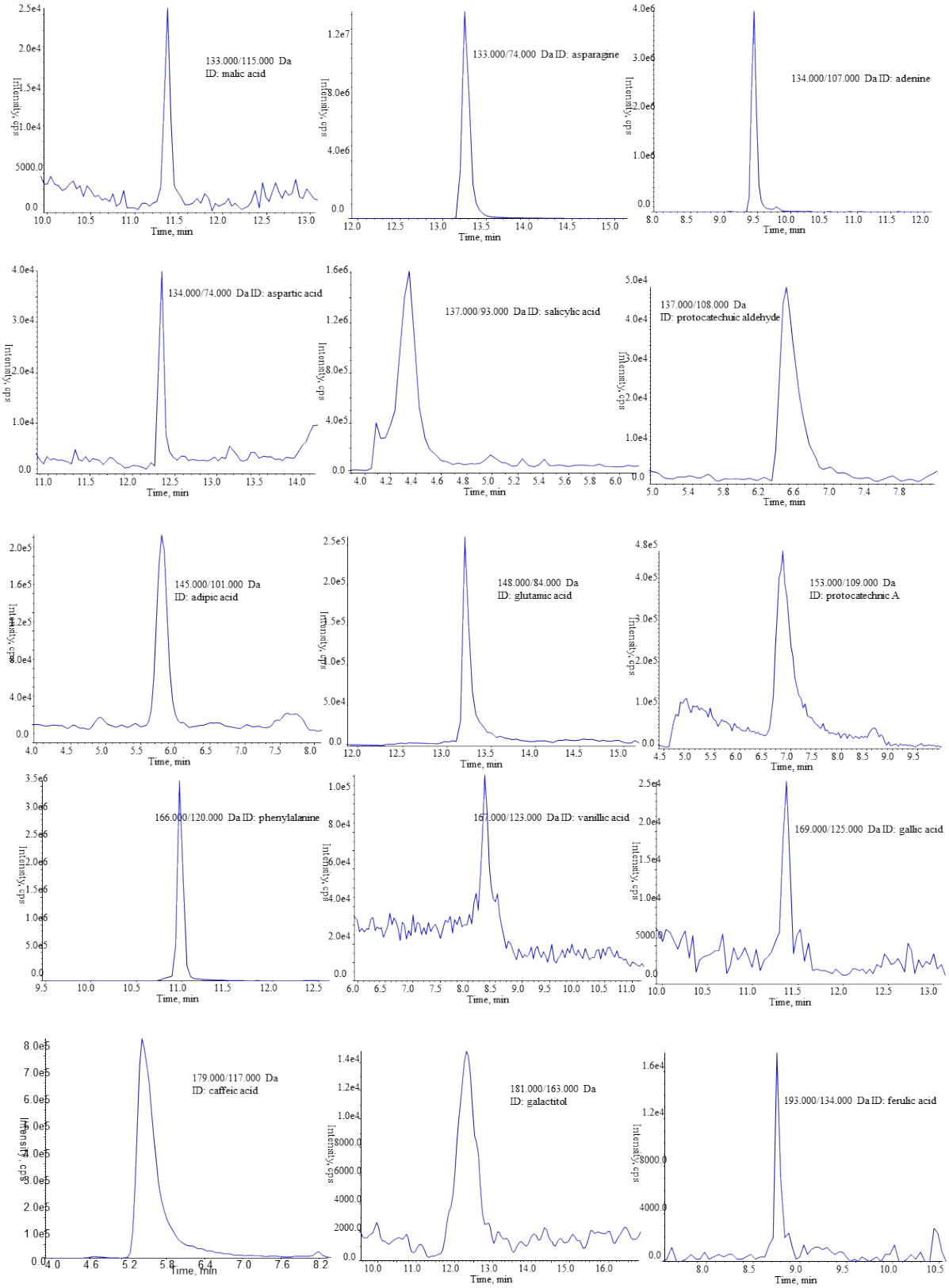



**Fig. S2 (continued)**

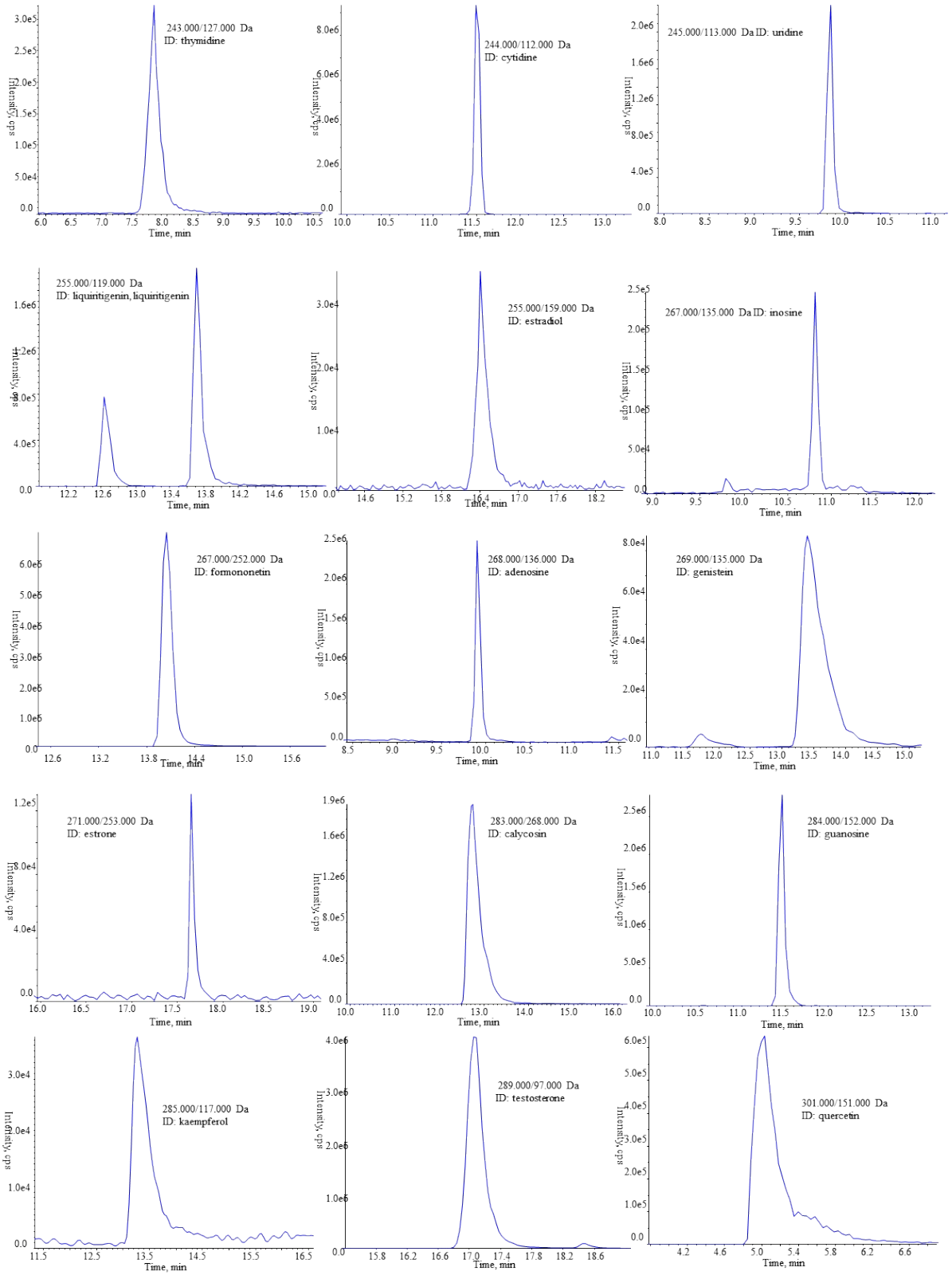



**Fig. S2 (continued)**

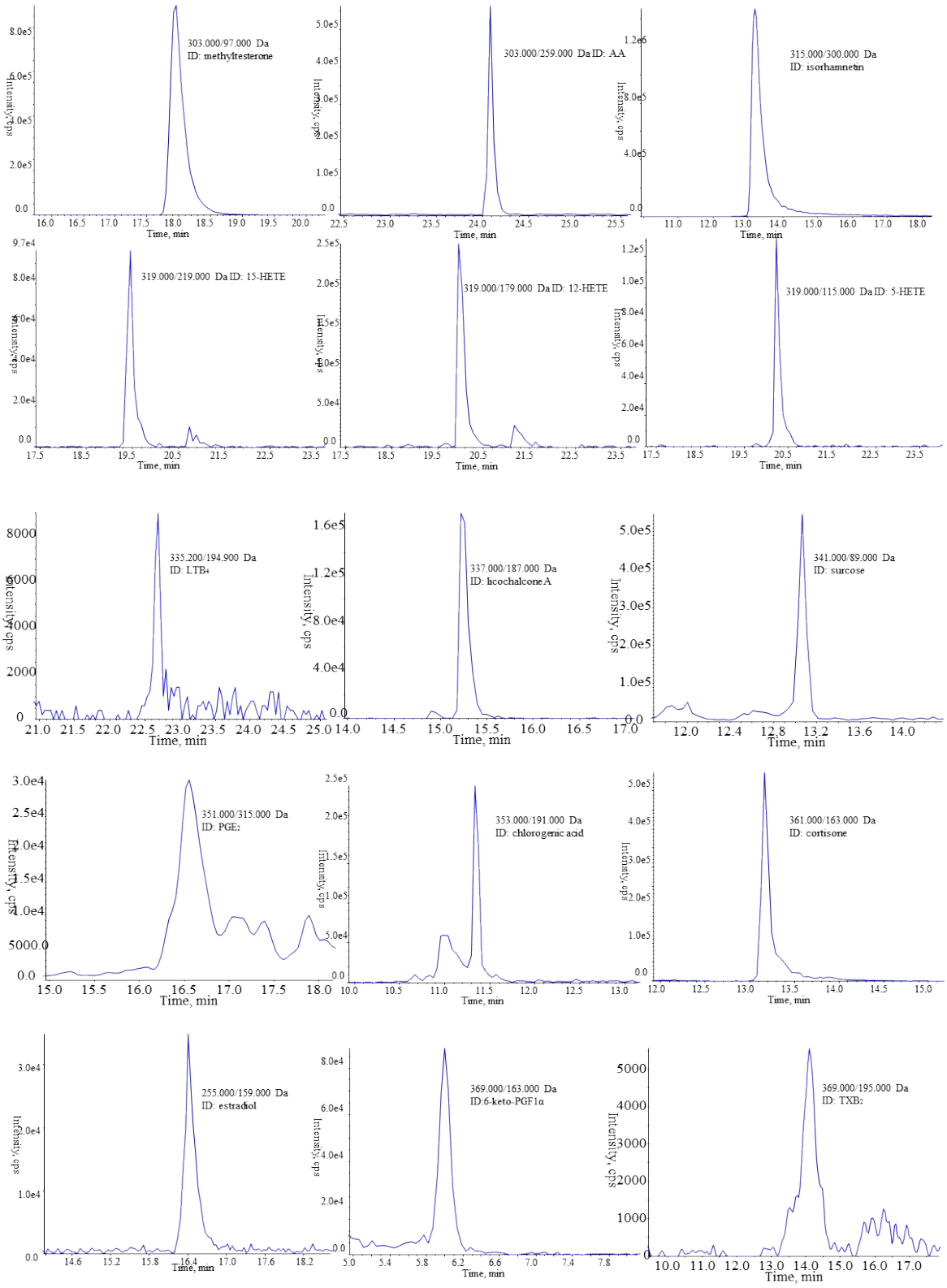



**Fig. S2 (continued)**

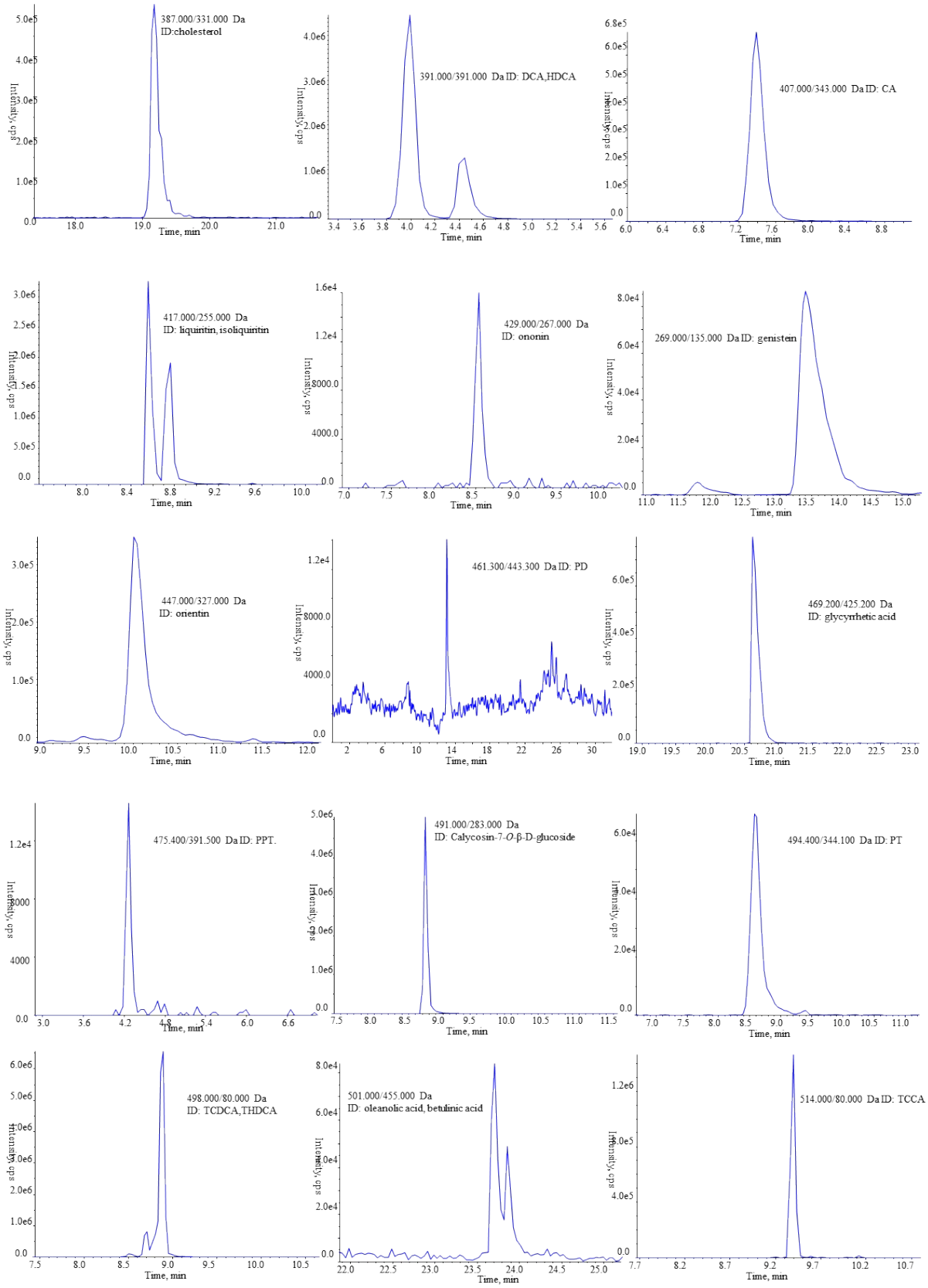



**Fig. S2 (continued)**

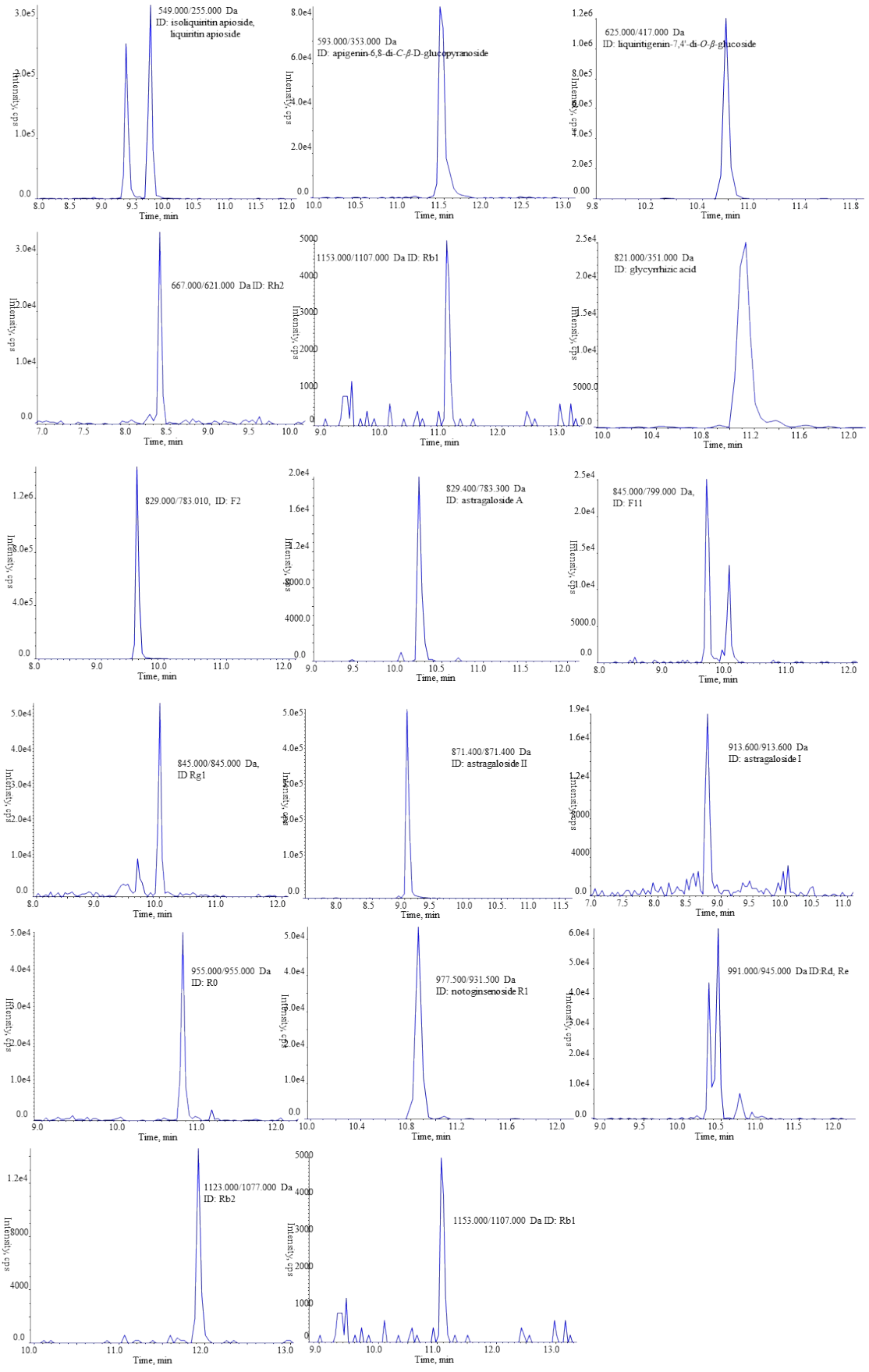



**Fig. S2 (continued)**