

Table S1: GC-MS information of all standard compounds

| Compounds | Retention Time (RT) | Characteristic fragment ions (ordered by abundance) | Group fragmentions | Quantitative ion |
|--------------|---------------------|---|--------------------|------------------|
| Ala 2TMS | 4.683 | 116/73/147/117/45/74/75/190/118/59 | 116/117/190 | 116 |
| Gly 2TMS | 5.018 | 102/73/147/103/204/45/176/148/75/74 | 102/103/204 | 102 |
| Gly 3TMS | 8.949 | 174/73/147/86/248/175/100/176/45/276 | 174/86/248 | 174 |
| Val 2TMS | 6.880 | 144/73/218/145/147/100/45/75/74/146 | 144/218/145 | 145 |
| Leu 2TMS | 8.145 | 158/73/102/159/147/45/100/75/74 | 158/102/159 | 158 |
| Ile 2TMS | 8.644 | 158/73/218/159/147/100/45/75/74/160 | 158/218/159 | 158 |
| Pro 2TMS | 8.757 | 142/73/143/147/45/216/144/74/75/59 | 142/143/216 | 142 |
| Ser 3TMS | 10.252 | 204/73/218/100/147/205/188/45/219/75 | 204/218/100 | 204 |
| Thr 3TMS | 10.860 | 73/218/219/117/101/57/147/291/129/100 | 218/117/101 | 218 |
| Met 2TMS | 14.070 | 176/128/73/61/147/177/45/75/129/219 | 176/177/219 | 176 |
| Asp 3TMS | 14.138 | 232/73/100/147/233/218/75/188/147/100/74 | 232/233/188 | 232 |
| Cys 3TMS | 14.960 | 73/220/218/100/147/75/221/45/219/132 | 220/218/221 | 220 |
| Orn 3TMS | 16.369 | 142/73/70/74/75/102/143/147/144/115 | 142/70/143 | 142 |
| Glu 3TMS | 16.497 | 246/73/128/147/156/247/75/84/230/45 | 246/128/247 | 246 |
| Pyroglu 2TMS | 14.091 | 156/73/147/157/45/230/258/75/74/232 | 156/157/258 | 156 |
| Phe 2TMS | 16.560 | 73/218/192/100/147/219/45/193/75/74 | 218/192/193 | 218 |
| Asn 3TMS | 17.576 | 73/116/231/132/141/75/188/147/100/74 | 116/231/132 | 116 |
| Gln 3TMS | 19.875 | 156/73/155/75/147/245/157/203/45/74 | 156/155/245 | 156 |
| His 3TMS | 22.835 | 154/73/254/155/75/45/218/100/255/74 | 154/254/155 | 154 |
| Lys 4TMS | 22.950 | 156/73/174/128/230/317/157/147/100/175 | 156/230/317 | 156 |
| Tyr 3TMS | 23.264 | 218/73/100/219/147/280/220/75/45/179 | 218/100/219 | 218 |
| Try 3TMS | 28.371 | 202/73/203/291/45/204/75/74/218/147 | 202/203/291 | 202 |

Table S2: The minimum sample size calculated by power analysis in this study.

| AAs | Effect size | n | AAs | Effect size | n |
|-------------------|-------------|----|---------------|-------------|-----|
| Alanine | 1.58 | 12 | Aspartic acid | 1.76 | 10 |
| Glycine-2TMS | 1.27 | 18 | Ornithine | 0.75 | 47 |
| Valine | 1.35 | 16 | Glutamic acid | 1.73 | 10 |
| Leucine | 1.47 | 14 | Phenylalanine | 1.33 | 16 |
| Isoleucine | 1.34 | 16 | Asparagine | 1.46 | 14 |
| Proline | 1.60 | 12 | Glutamine | 0.86 | 37 |
| Glycine-3TMS | 1.59 | 12 | Histidine | 1.47 | 14 |
| Serine | 1.26 | 18 | Lysine | 1.47 | 14 |
| Threonine | 1.48 | 14 | Tyrosine | 0.33 | 241 |
| Pyroglutamic acid | 1.14 | 21 | Tryptophan | 1.40 | 15 |

The effect size is a critical mean difference.

n is the minimum sample size required for each group.

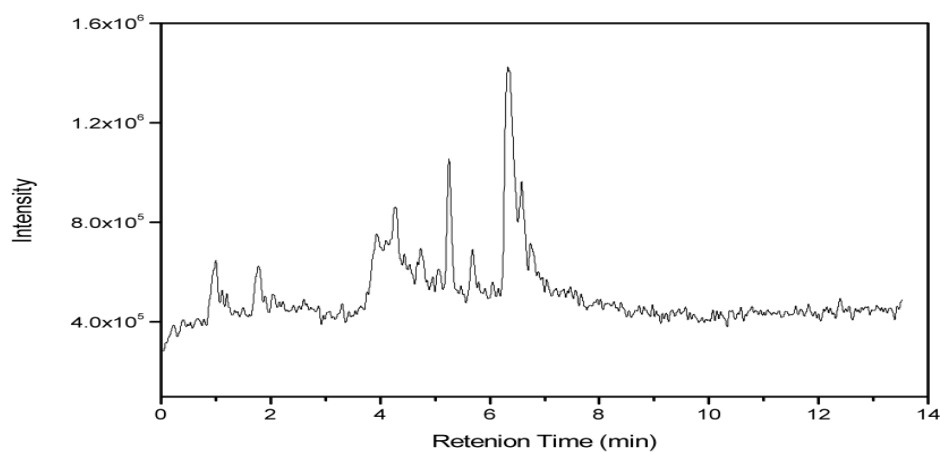


Fig S1 Positive ion mode typical total ion chromatograms of mixed standard solution of 20 amino acids.

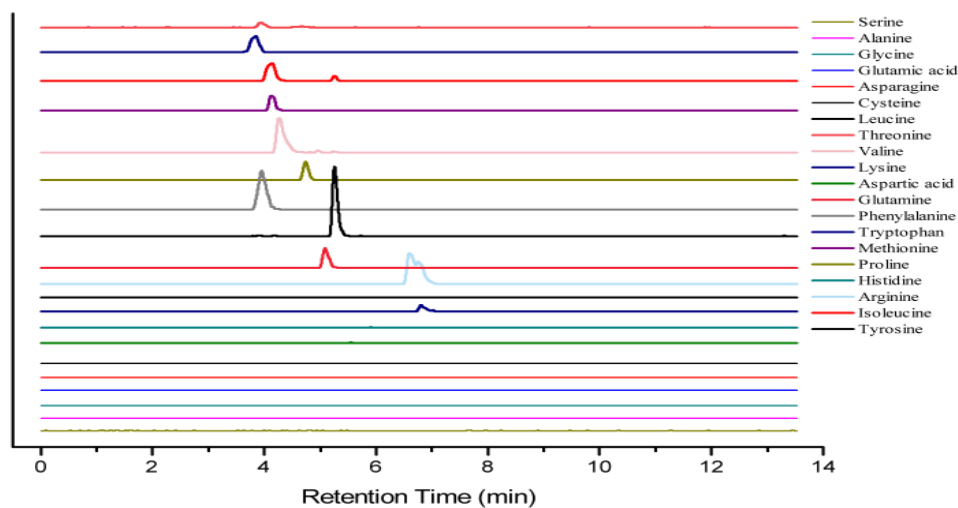


Fig S2 The extracted ion chromatogram of all amino acids.

Table S3 Comparison of amino acid detection based on GC-SIM-MS and UHPLC-IT-TOF-MS method.

| Amino acids | UHPLC-IT-TOF-MS | | GC-SIM-MS | |
|-------------------|-----------------|---------|-----------|---------|
| | LOD (ppm) | RSD (%) | LOD (ppm) | RSD (%) |
| Proline | 0.1130 | 7.85 | 0.1495 | 6.96 |
| Valine | 0.0476 | 6.86 | 0.0437 | 6.69 |
| Threonine | 0.6000 | 10.57 | 0.0199 | 4.18 |
| Leucine | 0.0400 | 0.21 | 0.0331 | 5.05 |
| Isoleucine | 6.6670 | 12.11 | 0.0293 | 4.98 |
| Aspartic acid | 6.0000 | 13.05 | 0.0847 | 4.17 |
| Glutamine | 0.0952 | 9.55 | 0.1732 | 4.68 |
| Lysine | 0.6000 | 7.05 | 0.1927 | 4.58 |
| Methionine | 0.2750 | 15.75 | 0.6601 | 5.37 |
| Histidine | 6.3700 | 11.92 | 0.1639 | 4.58 |
| Phenylalanine | 0.0500 | 11.62 | 0.0279 | 0.77 |
| Tyrosine | 0.0160 | 12.67 | 0.0609 | 3.04 |
| Tryptophan | 0.1020 | 7.52 | 0.0866 | 9.48 |
| Arginine | 0.0645 | 14.43 | / | / |
| Alanine | / | / | 0.0212 | 7.04 |
| Serine | / | / | 0.0367 | 4.59 |
| Cysteine | / | / | 0.0408 | 0.13 |
| Asparagine | / | / | 0.0217 | 5.39 |
| Ornithine | / | / | 2.1429 | 2.20 |
| Pyroglutamic acid | / | / | 0.0872 | 6.55 |
| Glycine 2TMS | / | / | 0.3069 | 5.88 |
| Glycine 3TMS | / | / | 0.0200 | 5.53 |